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# *Steinernema riobravis* n. sp. (Rhabditida : Steinernematidae) from Texas

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**Summary** – *Steinernema riobravis* n. sp. is a new entomopathogenic nematode species discovered in the Lower Rio Grande Valley of Texas. Morphological, hybridization, and DNA examinations indicated the distinctness of *S. riobravis* n. sp. from *S. carpocapsae, S. feltiae, S. glaseri,* and *S. intermedia.* Diagnostic characters include the length of the infective-stage juveniles, the color and shape of the spicules and gubernaculum, and lack of a tail projection in the male. The ratio E (distance from anterior end to excretory pore divided by tail length) of the infective juveniles separates this new species from previously described steinernematids. *S. riobravis* n. sp. did not hybridize with other *Steinernema* species. DNA analysis showed that the 304 base pair region of the 26 S ribosomal subunit examined in *S. riobravis* n. sp. is significantly divergent from the same region in *S. carpocapsae, S. feltiae, S. glaseri* and *S. serratum.* It appears to be naturally selected for the subtropical semi-arid environment where it serves as a biological control agent for corn earworm, *Helicoverpa zea,* and fall armyworm, *Spodoptera frugiperda,* (Lepidoptera : Noctuidae), at high temperatures.

**Résumé – Steinernema riobravis *n. sp. (Rhabditida : Steinernematidae) provenant du Texas* –** *Steinernema riobravis,* nouvelle espèce de nématode entomopathogène, a été découvert dans la basse vallée du Rio Grande, au Texas. Sa morphologie, ses séquences d'ADN et l'impossibilité du croisement le sépare de *S. carpocapsae, S. feltiae, S. glaseri* et de *S. intermedia.* Les caractères diagnostiques incluent la taille des juvéniles infestants, la couleur et la forme des spicules et du gubernaculum ainsi que l'absence d'appendice sur la queue du mâle. Le quotient E (distance de l'extrémité antérieure au pore excréteur divisée par la longueur de la queue) des juvéniles infestants sépare cette nouvelle espèce des Steinernematidae déjà décrits. *S. riobravis* n. sp. ne se croise pas avec d'autres espèces de *Steinernema.* L'analyse du DNA de *S. riobravis* a montré qu'une région de 304 paires de bases de la sous-unité ribosomale 26S est très différente de la même région chez *S. carpocapsae, S. feltiae, S. glaseri* et *S. serratum.* Il apparaît que cette nouvelle espèce est adaptée aux températures élevées des régions semi-arides subtropicales où elle constitue un agent de contrôle biologique de la chenille des épis du maïs, *Helicoverpa zea,* et de la noctuelle méditerranéenne, *Spodoptera frugiperda* (Lepidoptera : Noctuidae).

**Key-words :** Entomopathogenic nematodes, *Steinernema riobravis,* taxonomy, description, corn earworm, fall armyworm, biocontrol.

Nematode species of the family Steinernematidae include some of the most promising agents for biological control of agricultural and horticultural insect pests. The demand for biopesticides as an alternative to chemical pesticides is increasing due to concern about pesticide residues in crops and hazards to the environment. An indigenous steinernematid nematode has been found naturally suppressing insect pest populations in a subtropical region. As part of the corn earworm and fall armyworm long-range migration research, Raulston *et al.* (1992) observed that some of the dead prepupae and pupae of corn earworm, *Helicoverpa (= Heliothis) zea* (Boddie), and fall armyworm *(Spodoptera frugiperda)* (J. E. Smith) were parasitized by a *Steinernema* nematode. In July 25, 1990, soil material was collected to isolate the nematode and to determine its pathogenicity on corn earworm prepupae (Cabanillas & Raulston, unpubl.). This entomopathogenic nematode was determined to be a new species of *Steinernema.*

For a species to be considered new, morphological, physiological and ecological differences, and reproductive isolation, should be detectable when compared with other closely related species. Although morphological characters help in the identification of nematode species, it is evident that for species descriptions, examinations on interspecific variability, hybridization and DNA analysis are needed to confirm distinctness. The purpose of this investigation was to describe *Steinernema riobravis* n. sp. based on morphological, hybridization, and DNA examinations.

## Materials and methods

*Steinernema riobravis* n. sp. was isolated from soil samples in corn fields after harvest at the U.S. Department

of Agriculture (USDA), South Farm near Weslaco, Texas, U.S.A. in July 1990. A modified Bedding and Akhurst (1975) baiting technique for detecting steinernematid nematodes in soil was used; *H. zea* prepupae were used as a trap host, instead of wax moth (*Galleria mellonella* L.) larvae. Infective juveniles were collected and stored in sterile distilled water at 10 °C. This nematode population has been maintained through rearing *in vivo* using *H. zea* prepupae as a preferred host.

Crosses between *S. riobravis* n. sp. with *S. carpocapsae* Weiser (All strain), *S. feltiae* Filipjev (SN strain), *S. glaseri* Steiner, and *S. intermedia* Poinar, were conducted using the hanging blood drop method. This technique involves placing infective stage juveniles (surface sterilized with a 1 % solution Hyamine 10X) in separate hanging drops of wax moth *(Galleria mellonella)* blood at 24 °C. These crosses were divided in two groups : the first consisted of placing one nematode in each of ten separate blood drops; the second group included two nematodes per blood drop, one *S. riobravis* n. sp. crossed with one nematode of each *Steinernema* species, in ten separate blood drops. After reaching the pre-adult stage, males and females of the same and different species were placed together in separate blood drops (first group) or kept undisturbed (second group) in their original blood drops. Controls consisted of crosses of the same species. Evaluation of these controlled matings were conducted over a period of ten days. These crosses were repeated three times.

DNA sequence analysis of *S. riobravis* n. sp. was conducted by John Curran (CSIRO Division of Entomology, Canberra ACT 2601, Australia). Total DNA was extracted from infective-stage juveniles using standard procedures (Curran *et al.,* 1985). A variable region of the 26 S ribosomal subunit was amplified using polymerase chain reaction, cloned into pUC 119 and sequenced using the United States Biochemical Corporation Sequenase v. 2 kit. Three clones were sequenced. The DNA sequence of this new species was compared to those of *S. carpocapsae, S. feltiae, S. glaseri,* and *S. serratum* Liu.

The symbiotic bacterium was isolated from *S. riobravis* n. sp. infective-stage juveniles using the blood drop method. Surface sterilized (with a 1 % solution Hyamine 10X) infective-stage juveniles were transferred to a hanging drop of corn earworm blood and incubated at 24 °C for 24 h. The bacteria were then aseptically transferred to plates containing nutrient agar (Difco), Mac Conkey agar (Difco), or nutrient bromothymol blue – triphenyl tetrazolium chloride agar (NBTA) (Woodring & Kaya, 1988). Identification of the symbiotic bacterium was based on its morphology and the characteristic coloration of the primary form's adsorption of neutral red from Mac Conkey agar (Woodring & Kaya, 1988). *S. riobravis* infective juveniles were also sent to Ray J. Akhurst (CSIRO Division of Entomology, Canberra ACT 2601, Australia) for bacterium identification.

For morphological examinations and measurements, *S. riobravis* n. sp. was reared on wax moth larvae, *Galleria mellonella,* under laboratory conditions. This was accomplished by inoculating about 40 third-stage infective juveniles per insect and incubating them at 21 ± 1 °C. Adults and infective juveniles were heat killed (60 °C), fixed in 3.5 % TAF and processed to glycerin. Coverglass supports were used to prevent flattening specimens. Microscopic examinations were conducted with a Leitz Orthoplan microscope fitted for differential interference contrast.

## Results

Attempts to cross hybridize *S. riobravis* n. sp. with *S. carpocapsae, S. feltiae, S. glaseri,* and *S. intermedia* yielded no progeny. However, all intra-specific crossings using the hanging drop method resulted in offspring (Table 1).

***Table 1.*** *Results of hybridization experiments between* Steinernema riobravis *n. sp. and other* Steinernema *species.*

| Species : | *carpocapsae* (All) | *feltiae* (SN) | *glaseri* | *intermedia* | *riobravis* (TX) |
|---|---|---|---|---|---|
| *carpocapsae* (All) | + | – | – | – | – |
| *feltiae* (SN) | – | + | – | – | – |
| *glaseri* | – | – | + | – | – |
| *intermedia* | – | – | – | + | – |
| *riobravis* (TX) | – | – | – | – | + |

+ presence of progeny
– absence of progeny

The DNA analysis of the 304 base pair region of the 26 *S. ribosomal* subunit examined in *S. riobravis* n. sp. was significantly divergent from the same region in *S. carpocapsae, S. feltiae, S. glaseri,* and *S. serratum* (John Curran, pers. comm.).

These results, together with the following morphological studies, indicated *S. riobravis* is a new species and its description follows.

### *Steinernema riobravis** n. sp.

(Figs 1 - 3)

MEASUREMENTS

Third-stage infective juveniles, males and females : see Tables 2, 3, respectively.

***Table 2.*** *Measurements (in μm) of the third-stage juvenile of* Steinernema riobravis *n. sp. (n = 20).*

| Character/ratio | Mean ± SD (Range) |
|---|---|
| Body length (L) | 622.0 ± 39.5 (561.0-701.0) |
| Greatest width (W) | 27.6 ± 1.7 (25.6-30.0) |
| Ant. end to excret. pore (EP) | 56.2 ± 3.2 (51.2-63.7) |
| Ant. end. to nerve ring | 87.2 ± 1.4 (83.7-88.7) |
| Oesoph. length (ES) | 113.5 ± 2.1 (108.7-116.2) |
| Tail length | 53.5 ± 3.5 (46.2-58.7) |
| Anal body width | 16.0 ± 0.4 (15.0-16.5) |
| Ratio A (L/W) | 22.5 ± 1.1 (19.9-23.5) |
| Ratio B (L/ES) | 5.4 ± 0.3 (4.9-6.0) |
| Ratio C (L/Tail) | 11.6 ± 0.7 (10.1-12.4) |
| Ratio D (EP/ES) | 0.49 ± 0.02 (0.45-0.55) |
| Ratio E (EP/Tail) | 1.05 ± 0.05 (0.93-1.11) |

DESCRIPTION

*Adults :* Cuticle smooth; head rounded, continuous with body. Ten sensory papillae : six labial, four cephalic. Six distinct lips each with one papilla. Four cephalic papillae located further back on the head in sub-medial positions. Amphids small, located behind lateral labial papillae. Stoma shallow, partially collapsed; pharyngeal collar absent, pharynx extending near to mouth opening. Cheilorhabdions located beneath the lips and represented by a thick ring of sclerotized material. Posterior to cheilorhabdions, another sclerotized ring representing the prorhabdions. Oesophagus muscular with a cylindrical procorpus, a slightly swollen nonvalvate metacorpus, a narrow isthmus, and a basal bulb with a distinct small valve. Nerve ring surrounding isthmus. Oesophago - intestinal valve almost bilaterally symmetric which projects into the intestine. Excretory pore opening usually anterior to nerve ring, and its location variable.

Location of excretory pore opening in relation to the oesophagus length (ratio D) 49 % and 56% in the first and second generation females, respectively. Ratio D 71 % and 61 % in the first and second - generation males, respectively. Lateral fields and phasmids inconspicuous. Reproduction by amphimixis.

*Female :* Body robust, assuming shape of letter C when relaxed by heat or coiling when active. First generation females larger (average length : 6.5 mm) than the second generation females (average length : 1.7 mm). Gonads didelphic, amphidelphic with reflexed ovaries. Vulva a transverse slit, usually protruding slightly from the body surface. Vagina short, leading into paired uteri. Eggs deposited initially, but later hatching inside the female bodies and the juveniles boring their way out (endotokia matricida). First generation tail usually wide with a rounded wedge-shaped projection on the tip. In contrast, second generation females tail straight, pointed V-shaped, with a prominent semicircle postanal swelling and pronounced concavity ventrally. Tail pointed, larger in older females than in young ones. Pigmy forms occurring in some instances.

*Male :* Body slender, smaller than females. First generation males 3.8 times smaller than the first generation females; the second generation males 1.9 times smaller than the first generation females. First generation males (average length : 1.7 mm) larger than second generation males (average length : 0.9 mm). Males J-shaped when heat relaxed. Gonad monorchid; testis usually reflexed one time. Testis reflexion length of the first-generation males (226 μm) about 1.5 times larger than the second-generation males (154 μm) (Table 3). Ratio T (testis reflexion length divided by body length) smaller in the first generation males (mean 0.13, range 0.12 - 0.14) than in the second generation males (mean 0.17, range 0.13 - 0.17). Spicules paired, arcuate, separate but contiguous distally, with a distinct golden dark yellow coloration. Spicule head (capitulum, manubrium) elongated, broad, somewhat angular-shaped; shaft (calomus) with a prominent ventral arch. Shaft and blade (lamina) angle averaging 100° (range 90 - 100°), blade tapering smoothly toward the distal portion. Each spicule provided with two internal ribs with termination points variable. Gubernaculum 0.7 times as long as spicules, boat

* The specific name is derived from the Rio Bravo River (the Mexican name for the Rio Grande River).

***Table 3.*** *Comparative measurements of first and second generation males and females of* Steinernema riobravis *n. sp. (n = 10); all measurements in µm, except L in mm).*

| Character/ratio | Males | | Females | |
|---|---|---|---|---|
| | First generation Mean ± SD (range) | Second generation Mean ± SD (range) | First generation Mean ± SD (range) | Second generation Mean ± SD (range) |
| Body Length (L) | 1.7 ± 0.09 (1.5-1.9) | 0.9 ± 0.1 (0.8-1.1) | 6.5 ± 1.6 (3.7-8.3) | 1.7 ± 0.2 (1.4-2.0) |
| Greatest width | 133.0 ± 13.5 (116.2-159.6) | 71.5 ± 11.8 (56.6-87.5) | 274.8 ± 53.7 (199.5-390.1) | 151.5 ± 18.9 (125.0-191.0) |
| Stoma length | 3.5 ± 0.3 (3.1-3.8) | 2.1 ± 0.3 (1.8-2.5) | 5.7 ± 0.5 (4.3-6.3) | 4.2 ± 0.4 (3.7-5.0) |
| Stoma width | 5.8 ± 0.6 (5.0-6.9) | 3.7 ± 0.3 (3.1-4.3) | 8.0 ± 0.6 (7.1-8.8) | 6.4 ± 0.2 (6.2-6.8) |
| Ant. end to Excret. Pore (EP) | 103.2 ± 4.8 (93.7-111.1) | 87.9 ± 10.1 (76.2-93.7) | 95.5-12.7 (79.8-117.5) | 94.3 ± 3.3 (90.0-97.5) |
| Ant. end to Nerve Ring | 102.8 ± 7.6 (106.3-134.0) | 113.2 ± 11.7 (93.7-126.2) | 146.8 ± 13.3 (131.1-168.1) | 123.7 ± 5.6 (112.5-131.2) |
| Oesoph. length (ES) | 144.0 ± 7.9 (128.2-154.0) | 145.5 ± 11.5 (118.7-156.2) | 192.5 ± 13.9 (171.0-210.9) | 167.6 ± 9.5 (156.2-188.7) |
| Testis reflexion | 226.0 ± 26.5 (185.2-256.5) | 154.0 ± 36.8 (100.0-182.4) | | |
| Tail length | 31.1 ± 1.7 (28.5-35.0) | 26.0 ± 2.7 (21.2-31.2) | 45.1 ± 3.5 (41.3-50.0) | 51.6 ± 4.3 (43.7-58.7) |
| Anal body width | 58.9 ± 4.7 (50.0-63.8) | 46.7 ± 8.1 (35.0-62.5) | 92.7 ± 17.6 (62.5-115.0) | 54.3 ± 6.7 (45.0-62.5) |
| Spicule length | 66.9 ± 4.2 (62.5-75.0) | 54.3 ± 4.0 (48.7-62.5) | | |
| Spicule width | 12.4 ± 0.7 (11.2-13.7) | 10.0 ± 1.3 (8.7-12.5) | | |
| Gubernaculum length | 50.9 ± 2.6 (47.5-56.2) | 38.2 ± 2.2 (35.0-42.5) | | |
| Gubernaculum width | 8.1 ± 0.6 (7.1-8.7) | 6.3 ± 0.2 (6.2-6.8) | | |
| Vulva % | | | 51.9 ± 3.3 (48.8-56.2) | 59.0 ± 3.2 (55.3-63.3) |
| Ratio D (EP/ES) | 0.71 ± 0.83 (0.6-0.8) | 0.61 ± 0.07 (0.53-0.67) | 0.49 ± 0.06 (0.42-0.62) | 0.56 ± 0.02 (0.53-0.59) |

shaped in lateral view, and ventrally curved, with a proximal knob or hook. Bursa absent. Eleven pairs of genital papillae, plus one single genital papilla located in the posterior portion of the male body. First five pairs located above the cloaca in a preanal subventral position; one single genital papilla positioned ventrally just above the cloaca. Four pairs of genital papillae in a postanal subventral position; one pair in the region posterior to the gubernaculum; and one pair in the caudal subdorsal location. Tail ventral portion usually straight. Thin flap of sclerotized cuticle occuring over the cloaca, closing the cloacal opening when spicules in a resting position. Cloacal opening slit like with, on both sides, two small lateral papillae - like structures.

*Infective-stage juvenile* (third-stage juvenile often enclosed in a second-stage cuticle). Body slender, gradually tapering posteriorly. Lip region continuous. Mouth and anus closed. Excretory pore anterior to nerve ring. Oesophagus long and slender, apparently nonfunctional. Basal bulb weak and less prominent than in adults. In the anterior portion of the intestine occurs a pouch containing live cells of the symbiotic bacteria *Xenorhabdus*. Tail pointed, usually curved ventrally when relaxed forming an angle *ca.* 110 degrees with body.

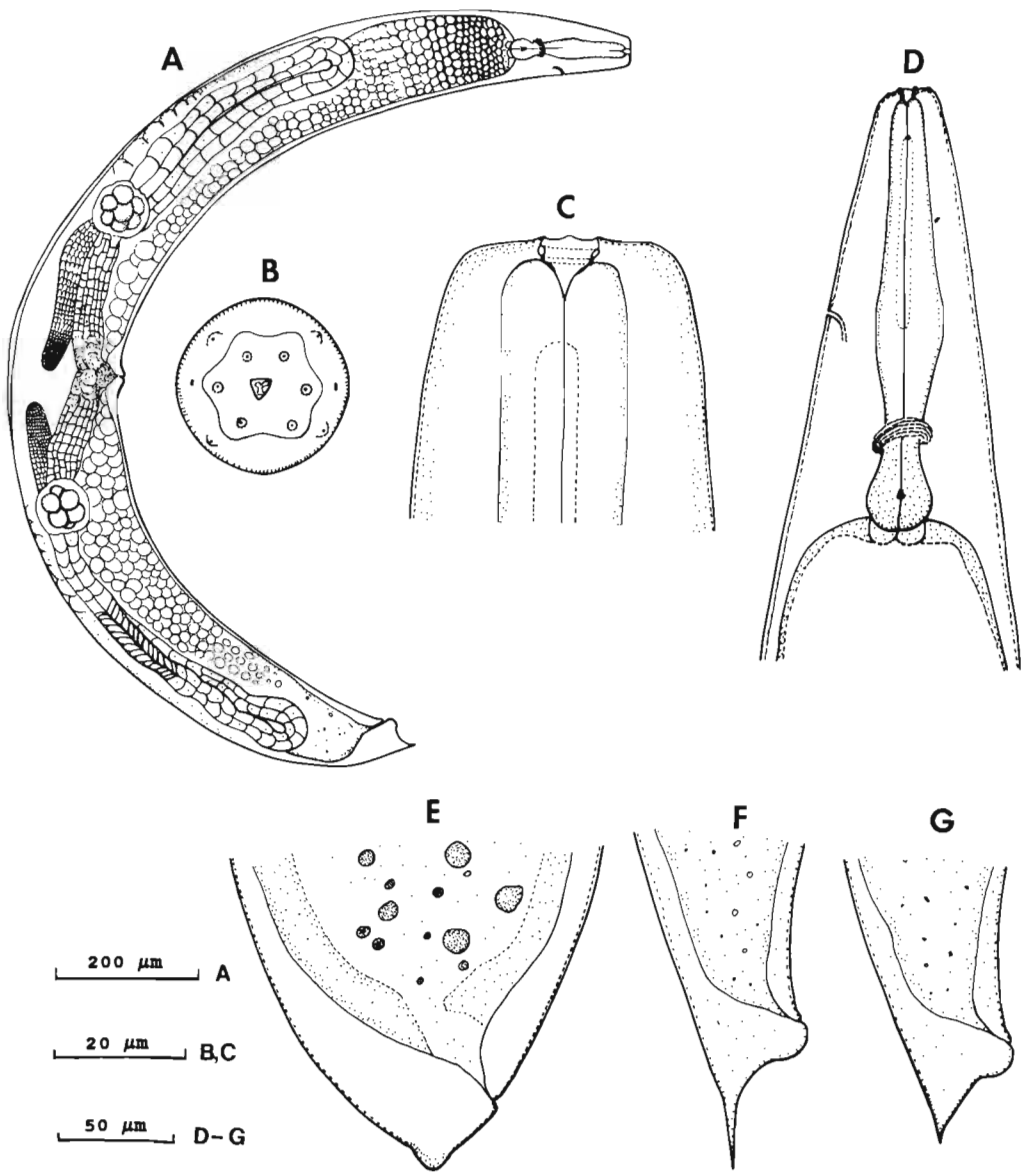


***Fig. 1.*** Steinernema riobravis *n. sp.-Female. A : Second-generation female, entire body; B :* En face *view of anterior end of first-generation female; C : Head (ventrad), first-generation female; D : Pharyngeal region of first-generation female; E : Tail, first generation female; F-G : Variation in tails of second-generation females.*

TYPE HOST AND LOCALITY

Corn earworm, *Helicoverpa (= Heliothis) zea* (Boddie) (Lepidoptera : Noctuidae) was used as a trap insect to isolate *S. riobravis* n. sp. from a corn field soil at the United States Department of Agriculture South Farm, in the Lower Rio Grande Valley near Weslaco, Texas, U.S.A. The location where soil samples were taken was recorded by using a portable Global Positioning System (GPS) receiver. The GPS coordinates are latitude 26 ° 08.155'N, longitude 97 ° 57.366'W, and altitude 21.7 m above mean sea level.

TYPE SPECIMENS

Holotype (male, first-generation) and allotype (female, first generation) deposited in the Nematode Collection at the University of California, Davis, California (UCDNC) (Holotype slide no. UCDNC 3227, allotype slide no. UCDNC 3228). Paratypes (Slide : one first generation male, one first generation female. Vial : ten first generation males, ten first generation females, 25 third-stage juveniles) deposited in the United States Department of Agriculture Nematode Collection, Beltsville, Maryland (Slide no. T-4392 p. Vial no. T-344 p),

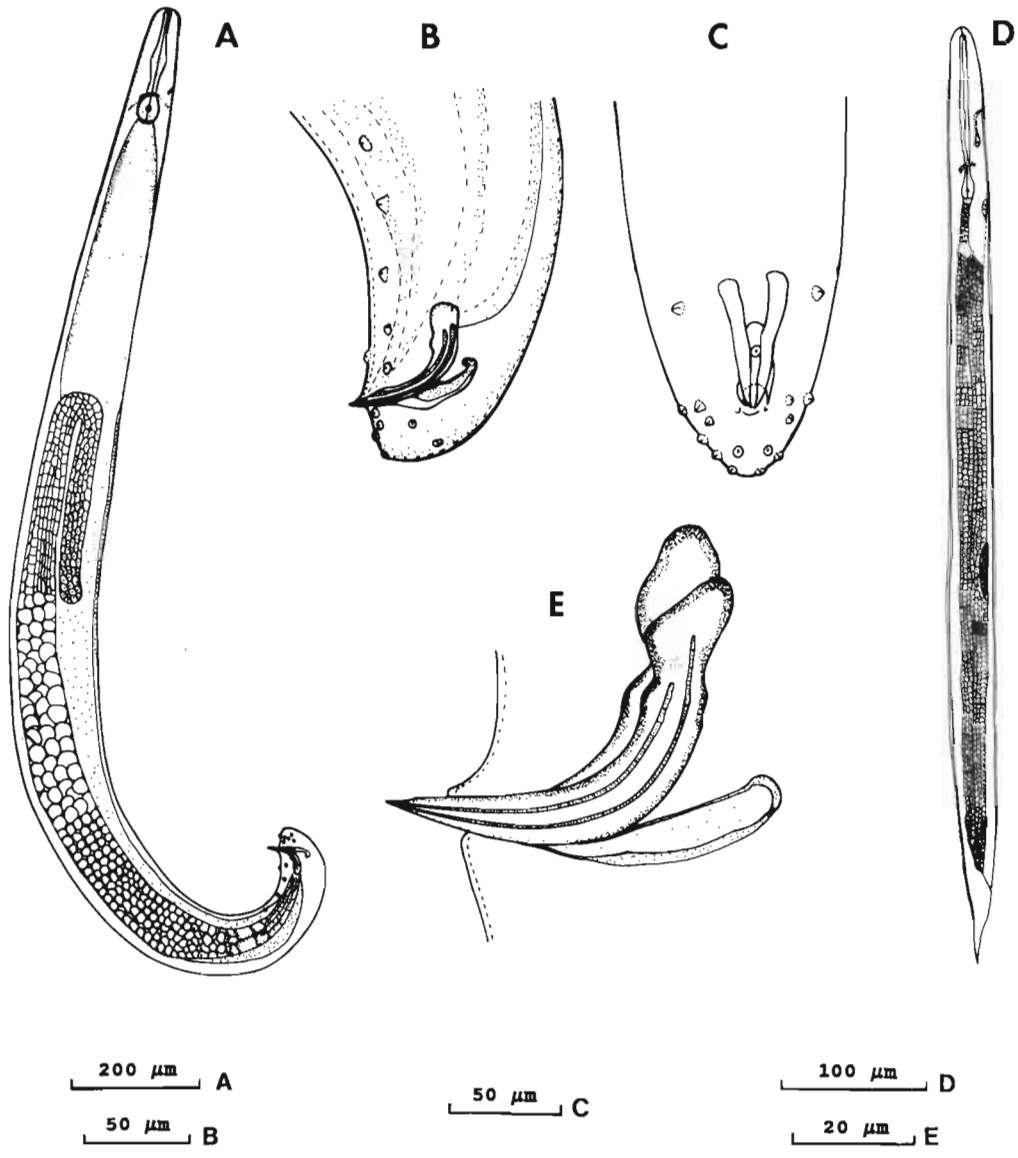


***Fig. 2.*** Steinernema riobravis *n. sp. – First-generation male and third-stage infective juvenile. A : Male, entire body; B : Male tail region (lateral view), C : Male tail region (ventral view); D : Third-stage infective juvenile, entire body; E : Spicules (lateral view).*

in the Nematode Collection at the University of California, Davis (Vial no. UCDNC 3229), and in the laboratoire des Vers, Muséum National d'Histoire Naturelle, Paris, France (Slide no. 648 WA). Furthermore, *S. riobravis* n. sp. was entered into the international entomopathogenic nematode database compiled by Dr. Ray J. Akhurst (Reference number 0307. CSIRO, Division of Entomology, Canberra ACT 2601, Australia).

### Diagnosis and relationship

*Steinernema riobravis* n. sp. can be separated from *S. carpocapsae, S. feltiae, S. glaseri,* and *S. intermedia* by morphological, DNA, and hybridization characters.

Morphologically, the average length of *S. riobravis* n. sp. (622 µm) infective juveniles is different from those of *S. carpocapsae* (558 µm), *S. intermedia* (671 µm), *S. feltiae* (849 µm), and *S. glaseri* (1130 µm) (Table 4). However, the length range of *S. riobravis* infective juveniles (561 - 701 µm) somewhat overlaps with those of *S. carpocapsae* (438 - 650 µm) and *S. intermedia* (608 - 800 µm); but no overlapping occurs with those of *S. feltiae* (736 - 950 µm), and *S. glaseri* (864 - 1448 µm). The ratio E (distance from the head to the excretory pore divided by the tail length) of the infective juveniles of *S. riobravis* (0.93 - 1.11) does not overlap with those of *S. carpocapsae* (0.54 - 0.66), *S. feltiae* (0.69 - 0.86), and *S. glaseri* (1.22 - 1.38), but it somewhat overlaps with those of *S. intermedia* (0.89 - 1.08) (Table 4).

***Table 4.*** *Comparison of morphometric characters (in μm) of third-stage infective juveniles of* Steinernema *species.*

| Species[a] | Body Length | EP | Ratio D | Ratio E |
|---|---|---|---|---|
| *carpocapsae* | 558 (438-650) | 38 (30-56) | 0.26 (0.23-0.28) | 0.60 (0.54-0.66) |
| *feltiae* | 849 (736-950) | 62 (53-67) | 0.45 (0.42-0.51) | 0.78 (0.69-0.86) |
| *glaseri* | 1130 (864-1148) | 102 (87-110) | 0.65 (0.58-0.71) | 1.31 (1.22-1.38) |
| *intermedia* | 671 (608-800) | 65 (59-69) | 0.51 (0.48-0.58) | 0.96 (0.89-1.08) |
| *riobravis* | 622 (561-701) | 56 (51-64) | 0.49 (0.45-0.55) | 1.05 (0.93-1.11) |

[a] Reference for all species but *riobravis* : Poinar, 1990.
Abbreviations (EP, Ratio D, Ratio E) as in table 2.

Males can be separated from other *Steinernema* species by the absence of a terminal tail mucro, strongly curved spicules and the golden dark yellow spicules. The lack of a terminal tail mucro distinguishes *S. riobravis* n. sp. from both *S. carpocapsae* and *S. feltiae* (Poinar, 1990). The golden dark yellow color of *S. riobravis* n. sp. spicules separates from the colorless (clear) spicules of *S. intermedia* (Poinar, 1985), and their pointed spicules separate them from the hooked tip spicules of *S. glaseri*. Furthermore, the ratio T of the first-generation males (mean 0.13; range 0.12 - 0.14) of *S. riobravis* n. sp. is only 50 % of the T ratio of *S. intermedia* (mean 0.26; range 0.17 - 0.34) (Poinar, 1985), which separates them morphologically as different *Steinernema* species.

The DNA sequence of *S. riobravis* n. sp. 304 base pair region of the 26 S ribosomal subunit is significantly different from those of *S. carpocapsae, S. feltiae, S. glaseri* and *S. serratum*.

*S. riobravis* n. sp. is reproductively isolated from *S. carpocapsae, S. feltiae, S. glaseri,* and *S. intermedia* as indicated by the negative results in the cross-breeding tests.

The diagnostic morphological characters of the third-stage infective juveniles and males of the present nematode does not fit the descriptions of currently recognized species of steinernematid nematodes (Poinar, 1990; Nguyen & Smart, 1992). Also, *S. riobravis* n. sp. is morphologically different from recently described *Steinernema* species such as *S. longicaudum* Shen, 1991; *S. serratum* Liu, 1992; and *S. neocurtillis* Nguyen & Smart, 1992 relative to one or more of the following characteristics : 1) tail length of the third-stage infective juvenile, 2) average length of third-stage infective juvenile, 3) the distance from the anterior end to the excretory pore of third-stage infective juvenile, 4) shape of terminal projection of first-generation female, and 5) the ratio D (the distance from the anterior end to the excretory pore divided by the oesophagus length) of the first-generation male.

Our study separates this nematode as a new species on morphological as well as on other evident characteristics. Hybridization and DNA examinations indicated the distinctness of *S. riobravis* n. sp. in comparison with populations of *S. carpocapsae, S. feltiae, S. glaseri* and *S. intermedia.*

## Biology and ecology

### *Symbiotic bacteria*

The mutualistic bacteria associated with *S. riobravis* n. sp. isolated on nutrient agar produced a brownish cream color. The primary form of this bacterium was characterized by its adsorption of bromothymol blue from NBTA, and adsorption of neutral red from Mac Conkey Agar (red colonies). These characteristics do not differ from the description of *Xenorhabdus nematophilus* (Akhurst, 1983; Woodring & Kaya, 1988). Similar results were obtained by R. J. Akhurst (pers. comm.).

Each steinernematid species has its own particular symbiotic bacterial " strain " or subspecies (Akhurst, 1983). Further studies on symbiotic bacterium, *Xenorhabdus* sp., associated with *S. riobravis* n. sp. will provide detailed identification and its role as a bioinsecticide.

### *Life history*

Life history studies indicates that *S. riobravis* n. sp. has a life cycle comparable to that of existing species of *Steinernema.* It includes the egg, four juvenile stages (separated by molts) and the adult. The third-stage infective juveniles enter the hemocoel of insects, deliver their associated bacteria, complete usually two generations, and then emerge from the insect cadaver as infective juveniles. Infective juveniles transferred in insect blood drops (*Galleria mellonella* and *Helicoverpa zea*) reached the adult stage in 48 h and produced eggs in 72 h at 24 °C. In Petri dish assays, *S. riobravis* n. sp. reached the preadult and/or adult stages in 48 h. in fall

armyworm larvae (9 day-old) exposed to infective juveniles at 29.5 °C (Cabanillas & Raulston, unpubl.). This indicates that *S. riobravis* n. sp. development is markedly influenced by temperature. It appears that the optimum temperature for growth and development of *S. riobravis* n. sp. is about 30 °C. Further studies will elucidate the effect of temperature on *S. riobravis* n. sp. viability and pathogenicity.

*Behaviour of infective juveniles*

A characteristic behaviour of the infective-juveniles is their high motility and tendency to nictate, with their body sometimes upright, standing up on their tail. We observed infective juvenile movement by bridging, leaping, crawling and climbing. In bridging, the nematode adopts an erect position and waves its anterior end until it comes into contact with a substrate, as described by Reed (1965). If no " bridge " occurs, the nematode may remain in its vertical position, attached by its bent tail (bottom of Petri dish with water; insect part e.g. seta) and the waving motion may continue provided optimum relative humidity or stop if relative humidity is restricted (e.g. taking the lid off the Petri dish). The nematode was observed forming loops by bending its anterior end to contact its posterior end. Also, the " leaping " movement described by Reed (1965) associated with this bending was observed. Crawling and climbing behaviour were observed when infective juveniles contacted corn earworm prepupa or pupa. The nematodes moved slowly in a sinusoidal wave across the insect body or insect part (e.g. seta, suture, spiracle).

*Ecology (habitat)*

This is a semi-arid subtropical region that receives about 600-700 mm of annual rainfall. Corn fields receive irrigation during the corn-growing season (February-June) and are exposed to dry periods for the remainder of the year. Fruiting usually begins in early to mid-May, then mature corn earworm and fall armyworm larvae exit the corn to pupate in late May and early June. Limited or no pesticides are used to control the corn earworm and fall armyworm larvae that infest the corn crop. The soil type where this nematode was isolated is a Hidalgo sandy clay loam (47.9 % sand, 35.6 % clay, 16.5 % silt, 1.1 % organic matter, pH 8.3, 39.95 meq/100 g CEC). Daytime soil temperatures at 5 cm. deep during the corn growing season are about 35 ± 4 °C.

Its ability to survive and persist in the soil after extended dry periods indicates that *S. riobravis* n. sp. is well adapted to this semiarid region. For example, Cabanillas and Raulston (unpubl.) were able to collect viable nematodes in November 1991 from fallow field soil near Weslaco, Texas, U.S.A., where corn had been planted the preceding spring. Furthermore, studies on the vertical and horizontal spatial patterns of *S. riobravis* in its natural habitat indicated that this nematode moves upwards and downwards throughout the soil profile (Cabanillas & Raulston, unpubl.). Since most infective juveniles were in the upper 20 cm. of soil and decreased with deeper soil layers, it may indicate that active vertical migration occurs and can serve as a means of survival for this nematode (Cabanillas & Raulston, unpubl.). It appears to be naturally selected for biological control of Lepidoptera pests at high temperatures. Indeed, field evaluations on the efficacy of *S. riobravis* n. sp. and *S. carpocapsae* (All strain) applied to soil to control corn earworm prepupae and pupae resulted in a higher rate of insect mortality (89 % - 100 %) due to *S. riobravis* compared to the failure of parasitism due to *S. carpocapsae*. The soil temperature, 35 ± 4 °C at 5 cm.-deep, appears to be a significant factor for the success or failure of these entomopathogenic nematodes (Cabanillas & Raulston, unpubl.).

Finally, the uniqueness of *S. riobravis* n. sp. is further demonstrated by its high motility and host finding capabilities. This new species is a naturally selected entomopathogenic nematode that exhibits insect finding strategies of both " cruiser " and " ambusher " types. All these attributes indicate that *S. riobravis* n. sp. (TX) is a unique potential biocontrol agent for insect pests. The strain of *S. riobravis* described here is called the TX strain after Texas. A patent application has been submitted covering the use of this nematode for suppressing insect populations in field crops.

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