

Morphological and molecular characterisation of an isolate of *Steinernema diaprepesi* Nguyen & Duncan, 2002 (Rhabditida: Steinernematidae) from Argentina and identification of its bacterial symbiont

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Abstract Entomopathogenic nematodes of the families Heterorhabditidae Poinar, 1976 and Steinernematidae Chitwood & Chitwood, 1937 are used for biological control of insect pests. An isolate of *Steinernema diaprepesi* Nguyen & Duncan, 2002 was recovered from a carrot field in the locality of Santa Rosa de Calchines (Santa Fe Province, Argentina). These nematodes were characterised based on morphological, morphometric and molecular studies. Their symbiotic bacterium was identified as *Xenorhabdus doucetiae* Tailliez, Pagès, Ginibre & Boemare, 2006 by sequencing the 16S rRNA gene. The isolate of *S. diaprepesi* studied exhibits some morphometric differences with the original description, especially in the first generation adults. This is the first description of the species in Argentina.

Introduction

Entomopathogenic nematodes of the families Heterorhabditidae Poinar, 1976 and Steinernematidae Chitwood & Chitwood, 1937 infect and kill insects and present a high potential as biological control agents (Grewal et al., 2005). Their association with symbiotic bacteria of the genera *Photorhabdus* Boemare, Akhurst & Mourant, 1993 (for heterorhabditids) and *Xenorhabdus* Thomas & Poinar, 1979 (for steinernematids) makes them highly virulent (Boemare, 2002). Steinernematidae includes two genera, *Steinernema* Travassos, 1927 with 65 described species (Kepenekci, 2014), and *Neosteinernema* Nguyen & Smart, 1994 with only one species (Nguyen & Smart, 1994).

Species of *Steinernema* have been isolated from all continents (except Antarctica) and almost all regions of the world (Hominick, 2002). In Argentina, six species of this genus have been reported: *S. ritteri* Doucet & Doucet, 1990 (see Doucet & Doucet, 1990); *S. scapterisci* Nguyen & Smart, 1990 (see Stock, 1992); *S. feltiae* Filipjev, 1934 (see Stock, 1993); *S. carpocapsae* Weiser, 1955 (see Agüera de Doucet, 1995); *S. rarum* Doucet, 1986 (see Doucet & Giayetto, 1998); and *S. glaseri* Steiner, 1929 (see Doucet et al., 2001). An isolate of *S. diaprepesi* Nguyen & Duncan, 2002 was recently detected in Santa Fe Province (Lax et al., 2011).

Steinernema diaprepesi was first detected in larvae of *Diaprepes abbreviatus* Linnaeus (Coleoptera:

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Curculionidae) that were buried in cages beneath citrus trees in Polk County, Florida, USA (Nguyen & Duncan, 2002). This nematode was also found in Venezuela (Spiridonov et al., 2004), Mexico (Molina-Ochoa et al., 2009) and the Caribbean islands Martinique and Guadeloupe (Tailliez et al., 2006). At present, the only morphological and morphometric data of this species are from the original description (Nguyen & Duncan, 2002). The aim of this work was to present morphological and molecular data of the Argentinian isolate and to identify its symbiotic bacterium.

Materials and methods

Nematode isolate and symbiotic bacterium culture

The isolate of *S. diaprepesi* SRC was collected in a carrot field with sandy soil in the locality of Santa Rosa de Calchines (31°25'00"S, 60°20'00"W; Garay Department, Santa Fe Province, Argentina) by the insect baiting method (Bedding & Akhurst, 1975); *Galleria mellonella* Linnaeus (Lepidoptera: Noctuidae) was used as bait insect. Nematodes were maintained in laboratory on *G. mellonella* larvae (Kaya & Stock, 1997). For morphological studies, the insects were individually placed in Petri dishes (35 mm in diameter) lined with two filter papers and inoculated with 100 infective juveniles (IJs). The infected larvae were dissected in Ringer's solution to obtain first and second generation adults 2–4 and 5–7 days, respectively, after the insects died (Nguyen & Duncan, 2002). The IJs were collected from White traps (White, 1927) 7 days posterior to larvae death.

One hundred IJs were surface-sterilised in 5% NaClO for 3 min and washed with sterile water (Ensign et al., 2012). Externally sterilised nematodes were homogenised with a stick to release the symbiotic bacteria. A drop of the homogenate was streaked on to plates with brain-heart infusion agar as growth medium. Colonies were isolated after 48 h of incubation at 28°C.

Morphological and morphometric studies

The different stages of *S. diaprepesi* were killed and fixed in formaldehyde-acetic acid solution (FA 4:1) at 60°C. Fixed nematodes were slowly dehydrated and processed to anhydrous glycerin (Seinhorst, 1962) and mounted in pure glycerin using glass fragments as

support for the cover slides. Observations and measurements were made using a Carl Zeiss Axiolab microscope equipped with a camera lucida (Carl Zeiss Jena) and a digital camera Canon Power Shot A640. For studies, 20 males and females of each generation and IJs were examined. For direct observations to confirm the morphology or the variations of specific structures, the nematodes were either examined live or after being killed with gentle heat in a drop of water. Measurements are in micrometres and are provided as the range followed by the mean and standard deviation in parentheses.

Molecular characterisation

(i) *Nematode isolate*. Nematode DNA was extracted from single IJ as described by Lax et al. (2007). Partial ribosomal RNA (rRNA) gene (part of 18S small subunit RNA gene, internal transcribed spacer 1, 5.8S rRNA gene, internal transcribed spacer 2, part of 28S large subunit rRNA gene) was amplified by PCR using the forward 18S (5'-TTG ATT ACG TCC CTG CCC TTT-3') and the reverse 28S (5'-TTT CAC TCG CCG TTA CTA AGG-3') primers (Vrain et al., 1992). The D2-D3 expansion segments of 28S rRNA were also amplified using the forward D2A (5'-ACA AGT ACC GTG AGG GAA AGT TG-3') and reverse D3B (5'-TCG GAA GGA ACC AGC TAC TA-3') primers (Al-Banna et al., 1997).

Protocols for PCR reactions were performed following Lax et al. (2007). PCR amplifications for both primers were performed in a Mastercycler Eppendorf thermal cycler programmed for an initial denaturation at 94°C for 3 min, followed by 39 cycles of 30 s at 93°C, 90 s at 48°C, 1 min at 72°C, and a final extension of 10 min at 72°C. Negative controls were added in all assays to check for possible contamination. Amplification products were separated by electrophoresis on 1% agarose gel in 0.5× TBE buffer. A molecular weight marker of 100-bp DNA Ladder (Promega, Madison, USA) was used. Gels were stained with ethidium bromide and photographed with a Kodak-DC digital camera under a UV transilluminator. PCR amplifications were purified and sequenced in both directions by Macrogen Korea Inc. using the PCR primers.

(ii) *Symbiotic bacteria*. Liquid cultures with brain-heart broth were made from each individual colony for extraction of total DNA with the commercial kit QIAamp DNA mini kit (Qiagen, Hilden, Germany).

The almost complete 16S rRNA gene was amplified by PCR using primers 16SP1 (forward: 5'-GAA GAG TTT GAT CAT GGC TC-3') and 16SP2 (reverse: 5'-AAG GAG GTG ATC CAG CCG CA-3') and PCR and cycling conditions as described by Tailliez et al. (2006). PCR products were purified and sequenced by MacroGen Korea Inc.; sequences overlapping the 16S rRNA gene were obtained using three sequencing primers (SP1: 5'-ACC GCG GCT GCT GGC ACG-3', position 514 reverse; SP2: 5'-CTC GTT GCG GGA CTT AAC-3', position 1089 reverse; and 16SP2).

Phylogenetic analyses

Sequence data were compared with those available in the GenBank database by means of the Basic Local Alignment Search Tool (BLAST) of the National Centre for Biotechnology Information (NCBI). The ITS and D2-D3 sequences of *S. diaprepesi*. and corresponding nucleotide sequences of other *Steinernema* spp. of the “glaseri group” available in GenBank were aligned using ClustalX 2.0 (Larkin et al., 2007) with default parameters. Sequence alignments were manually edited using BioEdit (Hall, 1999). The 16S rRNA sequence of the symbiotic bacteria was aligned to corresponding sequences of *Xenorhabdus* spp.; a sequence of *Proteus vulgaris* Hauser, 1885 was considered as outgroup. Phylogenetic analyses were performed with Maximum Likelihood based on the Tamura-Nei model (Tamura et al., 2004) using the Molecular Evolutionary Genetics Analysis 5 (MEGA 5) software (Tamura et al., 2011). The estimation of the support for each node was assessed by bootstrap analysis with 1,000 replicates. The newly obtained sequences were submitted to the NCBI GenBank database under accession numbers indicated in bold on the phylogenetic trees.

Family Steinernematidae Chitwood & Chitwood, 1937

Genus *Steinernema* Travassos, 1927

Steinernema diaprepesi Nguyen & Duncan, 2002

Description (Figs. 1, 2)

Male, first generation [Morphometric data in Table 1.] Body J-shaped when heat-killed. Lateral fields and phasmids indistinct. Head rounded, continuous with body. Lips not distinguished. Stoma broad,

cheilorhabdions present. Oesophagus with cylindrical procorpus; metacarpus absent (Fig. 1A). Isthmus distinct, surrounded by nerve-ring; basal bulb present. Excretory pore situated anterior to nerve-ring, located on average at 72% of oesophagus length. Oesophago-intestinal valve present. Testis single, reflexion testis length variable. Spicules paired, symmetrical, with brown coloration, curved (Fig. 1C–H). Length of spicule manubrium greater than width, calomus very short, lamina arcuate, broad, narrowing posteriorly, end rounded. Velum present; spicule with two internal ribs. Gubernaculum boat-shaped in lateral view, *c.*70% of spicule length; proximal part slightly curved ventrally or with 2 ventral projections of different shapes and lengths (Fig. 1C–G); distal part ending in a bifurcate projection (Fig. 1I). Genital papillae 11 pairs (1 lateral pre-anal pair; a row of 5 pairs anterior to anal opening in ventro-lateral position; 2 post-anal ventral pairs; 1 post-anal dorsal pair; 2 adanal pairs) plus single ventral pre-anal papilla. Tail short (on average *c.*61% of anal body width), conical, with rounded terminus, without mucron.

Male, second generation [Morphometric data in Table 1.] Similar to first generation (Fig. 1B), differing by shorter and thinner body and shorter testis reflexion. Tail length *c.*75% of anal body width. Mucron on tail terminus absent.

Female, first generation [Morphometric data in Table 2.] Body length variable, C-shaped when heat-killed. Lateral fields and phasmids not observed. Head rounded, continuous with body. Lips undistinguished. Stoma length shorter than width. Cheilorhabdions distinct and sclerotised. Oesophagus with cylindrical procorpus and slightly swollen metacarpus (Fig. 2A). Isthmus distinct, basal bulb valvate. Nerve-ring situated anterior to basal bulb or in some cases surrounding its first portion. Excretory pore located at 71% of oesophagus length. Oesophago-intestinal valve distinct. Reproductive system amphidelphic, ovaries reflexed. Vulva a transverse slit at 52% of body length. Vulval lips protuberant or not; anterior lip bigger than posterior (Fig. 2D). Epiptygma double-flapped, pointing to the anterior region (Fig. 2E, F). Tail length *c.*56% of anal body width; tail shape variable: rounded, conical, or ending in digitate terminus (Fig. 2G–J). Post-anal swelling absent.

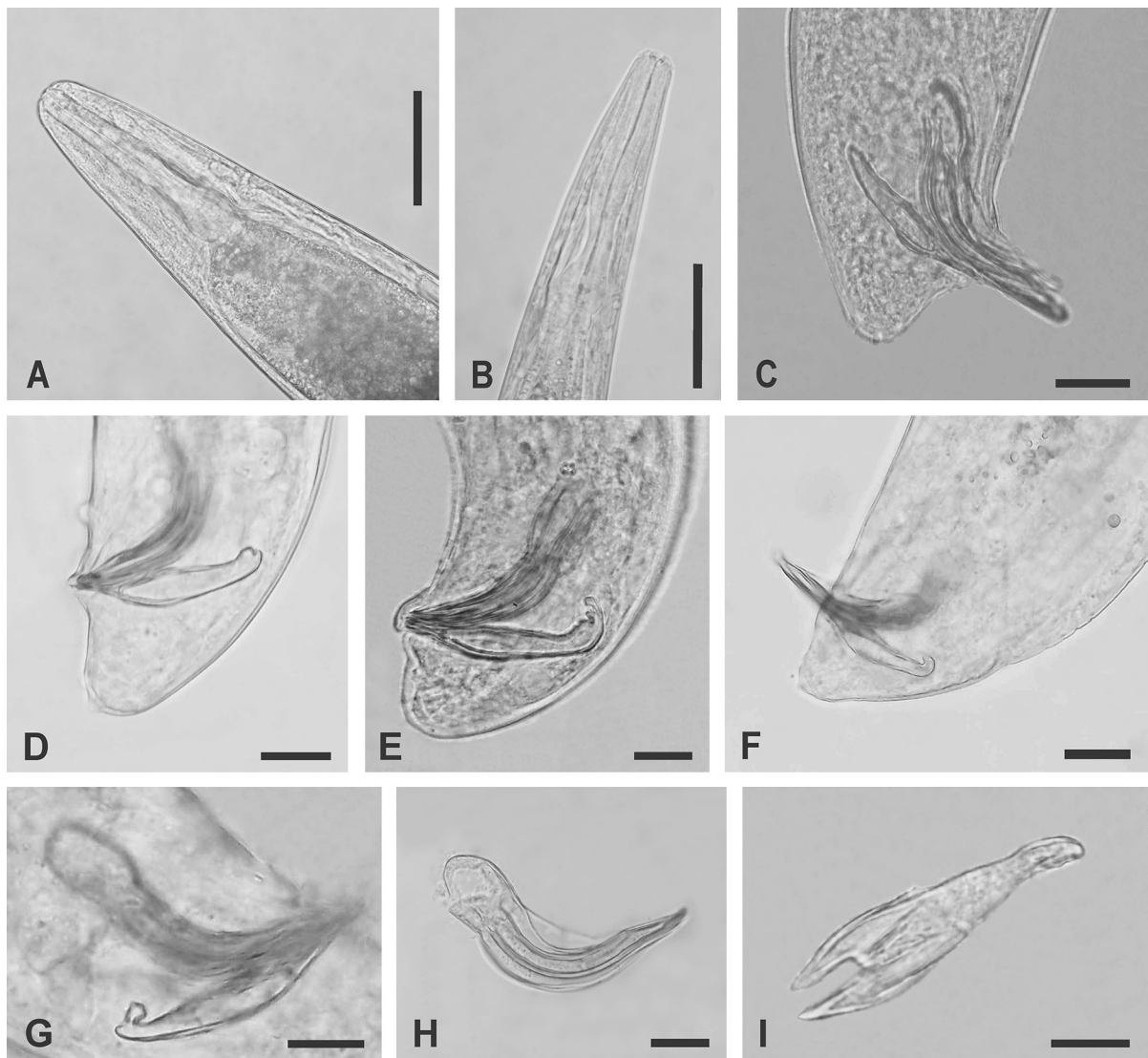


Fig. 1 *Steinernema diaprepesi* Nguyen & Duncan, 2002 from Argentina. A, First generation male, anterior region; B, Second generation male, anterior region; C–I, First generation males; C–G, Posterior region, detail of spicules and gubernaculum; H, Spicule, lateral view; I, Gubernaculum, ventral view. Scale-bars: A, B, 70 μ m; C–H, 20 μ m; I, 10 μ m

Papilla-like structures on tail tip observed in some specimens.

Female, second generation [Morphometric data in Table 2.] Similar to first generation (Fig. 2B, C) but shorter and thinner and excretory pore more anterior (at 68% of oesophagus length). Vulva less protuberant than in first generation. Tail longer, about length of anal body width, with sharp pointy end. Ventral post-anal swelling present (Fig. 2K, L).

Infective juvenile [Morphometric data in Table 3.] Body thin, elongate. Second-stage cuticle present, with prominent transverse striations. Lateral field with 8 longitudinal ridges at midbody. Labial region continuous with body. Oesophagus slender, basal bulb present, valvate. Excretory pore situated half-way between anterior region and basal bulb. Nerve-ring just anterior to basal bulb. Anus distinct, tail conoid, tapering gradually from anus to pointed terminus. Hyaline portion occupying *c.*70% of tail.

Table 1 Comparative morphometric data for males of *Steinernema diaprepesi* Nguyen & Duncan, 2002 from Argentina and USA

Source	Argentinian isolate		USA isolate	
	Present study		Nguyen & Duncan (2002)	
Character	First generation (n = 25)	Second generation (n = 20)	First generation (n = 20)	Second generation (n = 20)
Body length	1,001–2,074 (1,397 ± 256)	1,102–1,494 (1,296 ± 106)	1,506–2,078 (1,735 ± 156)	1,036–1,343 (1,176 ± 117)
Body width	61–170 (101 ± 26)	55–78 (69 ± 6)	90–145 (113 ± 15)	54–78 (64 ± 7)
ES	128–173 (147 ± 11)	145–160 (154 ± 4)	136–162 (150 ± 7)	129–168 (138 ± 12)
EP	83–138 (106 ± 13)	88–115 (103 ± 7)	100–130 (115 ± 9)	71–108 (83 ± 8)
NR	103–133 (118 ± 9)	123–140 (130 ± 5)	109–129 (119 ± 6)	100–136 (110 ± 8)
Testis reflexion	164–663 (308 ± 102)	203–319 (262 ± 32)	241–542 (391 ± 68)	187–428 (264 ± 16)
T	18–40 (28 ± 5)	28–35 (31 ± 2)	20–32 (25 ± 3)	18–30 (24 ± 5)
ABW	31–65 (46 ± 9)	35–48 (42 ± 3)	36–50 (42 ± 11)	29–42 (34 ± 6)
Gubernaculum length	34–61 (46 ± 8)	33–50 (41 ± 5)	45–61 (54 ± 5)	30–53 (40 ± 6)
Gubernaculum width	6–10 (8.0 ± 0.8)	6–10 (8 ± 1)	6–12 (8.7 ± 0.4)	5–9 (6.7 ± 1.4)
Spicule length	60–87 (74 ± 7)	59–74 (66 ± 4)	71–90 (79 ± 5)	61–76 (69 ± 8)
Spicule width	10–19 (14 ± 2)	10–15 (12 ± 1)	14–20 (16 ± 2)	11–15 (12.5 ± 3)
SW	1.2–2.3 (1.6 ± 0.3)	1.4–1.9 (1.6 ± 0.2)	1.5–2.0 (1.8 ± 1.3)	1.66–2.33 (2.0 ± 0.2)
D = EP/ES (%)	55–84 (72 ± 7)	57–77 (67 ± 5)	68–86 (80 ± 6)	54–67 (60 ± 8)
GS	0.5–1.0 (0.6 ± 0.1)	0.5–0.8 (0.6 ± 0.1)	0.59–0.79 (0.69 ± 0.1)	0.46–0.73 (0.59 ± 0.07)

Abbreviations: ES, distance from anterior end to end of oesophagus; EP, distance from anterior end to excretory pore; NR, distance from anterior end to nerve-ring; T, tail length; ABW, anal body width; SW, spicule length/ABW; GS, gubernaculum length/spicule length

Molecular characterisation

The sequences of the D2-D3 domains of the Argentinian isolate of *S. diaprepesi* were tested in BLAST against data deposited in GenBank, showing a 99% similarity with sequences of the same species. The

phylogenetic analysis showed a well supported group (100% bootstrap) that comprised the studied isolate and the known sequences of the species; this group had a close relationship with *S. brazilense* Nguyen, Ginarte, Leite, Santos & Harakava, 2010 and *S. australe* Edgington, Buddie, Tymo, Hunt, Nguyen,

France, Merino & Moore, 2009 (Fig. 3). The same grouping was observed with the ITS rRNA region (Fig. 4).

A similarity value of 99% placed the 16S rRNA gene sequence of the symbiotic bacteria among those of *X. doucetiae* Tailliez, Pagès, Ginibre & Boemare, 2006 from Martinique and Guadeloupe deposited in the GenBank. This species formed a well-supported group including *X. magdalenensis* Tailliez, Pagès, Edgington, Tymo & Buddie, 2012, the symbiotic bacteria associated with *S. australe* (Fig. 5).

Discussion

In this study, despite using the same host (*G. mellonella*) as in the original description (Nguyen & Duncan, 2002), morphological and morphometric

differences were detected. In second generation males, mucron was absent whereas in the USA isolate this was occasionally present. In contrast with the observations made by Nguyen & Duncan (2002), a post-anal swelling was observed only in second generation females. In first generation males, mean body length, testis reflexion and gubernaculum length were smaller than in the original isolate (1,397 vs 1,735 μm ; 308 vs 391 μm ; and 46 vs 54 μm , respectively) while in the second generation, Argentinian males were longer (1,296 vs 1,176 μm). Mean body length was also shorter for first and second generation females (3,839 vs 6,508 μm and 2,043 vs 2,433 μm , respectively). In first and second generation females, the tail was shorter in the Argentinian isolate (39 vs 52 μm and 53 vs 78 μm , respectively). In infective juveniles of the Argentinian isolate, excretory pore and the nerve ring were more anterior (63 vs 74 μm and 90 vs 102 μm ,

Table 2 Comparative morphometric data for females of *Steinernema diaprepsi* Nguyen & Duncan, 2002 from Argentina and USA

Source	Argentinian isolate		USA isolate	
	Present study		Nguyen & Duncan (2002)	
Character	First generation (n = 25)	Second generation (n = 20)	First generation (n = 20)	Second generation (n = 20)
Body length	1,864–9,478 (3,839 \pm 2,089)	1,182–3,682 (2,043 \pm 711)	4,061–9,878 (6,508 \pm 1,512)	2,030–3,030 (2,433 \pm 260)
Body width	153–337 (244 \pm 66)	92–275 (149 \pm 45)	190–343 (272 \pm 44)	137–180 (158 \pm 12)
Stoma length	4–15 (9 \pm 3)	8–15 (10 \pm 1)	8–14 (10 \pm 2)	9–12 (11 \pm 1)
Stoma width	5–15 (11 \pm 3)	9–15 (10 \pm 1)	9–18 (12 \pm 3)	9–14 (10 \pm 2)
ES	150–238 (177 \pm 24)	153–218 (168 \pm 17)	119–232 (200 \pm 25)	161–211 (182 \pm 11)
EP	100–160 (124 \pm 18)	90–145 (114 \pm 15)	98–214 (156 \pm 39)	94–139 (116 \pm 15)
NR	130–213 (152 \pm 21)	120–155 (132 \pm 11)	105–179 (150 \pm 21)	120–153 (144 \pm 12)
T	20–92 (39 \pm 15)	30–86 (53 \pm 16)	38–68 (52 \pm 10)	67–91 (78 \pm 6)
ABW	33–150 (69 \pm 25)	35–79 (53 \pm 10)	59–138 (94 \pm 22)	39–59 (50 \pm 7)
D = EP/ES (%)	53–89 (71 \pm 10)	55–95 (68 \pm 9)	46–105 (80 \pm 19)	47–74 (64 \pm 8)

Abbreviations: ES, distance from anterior end to end of oesophagus; EP, distance from anterior end to excretory pore; NR, distance from anterior end to nerve-ring; T, tail length; ABW, anal body width; a, body length/body width; b, body length/ES; c, body length/T

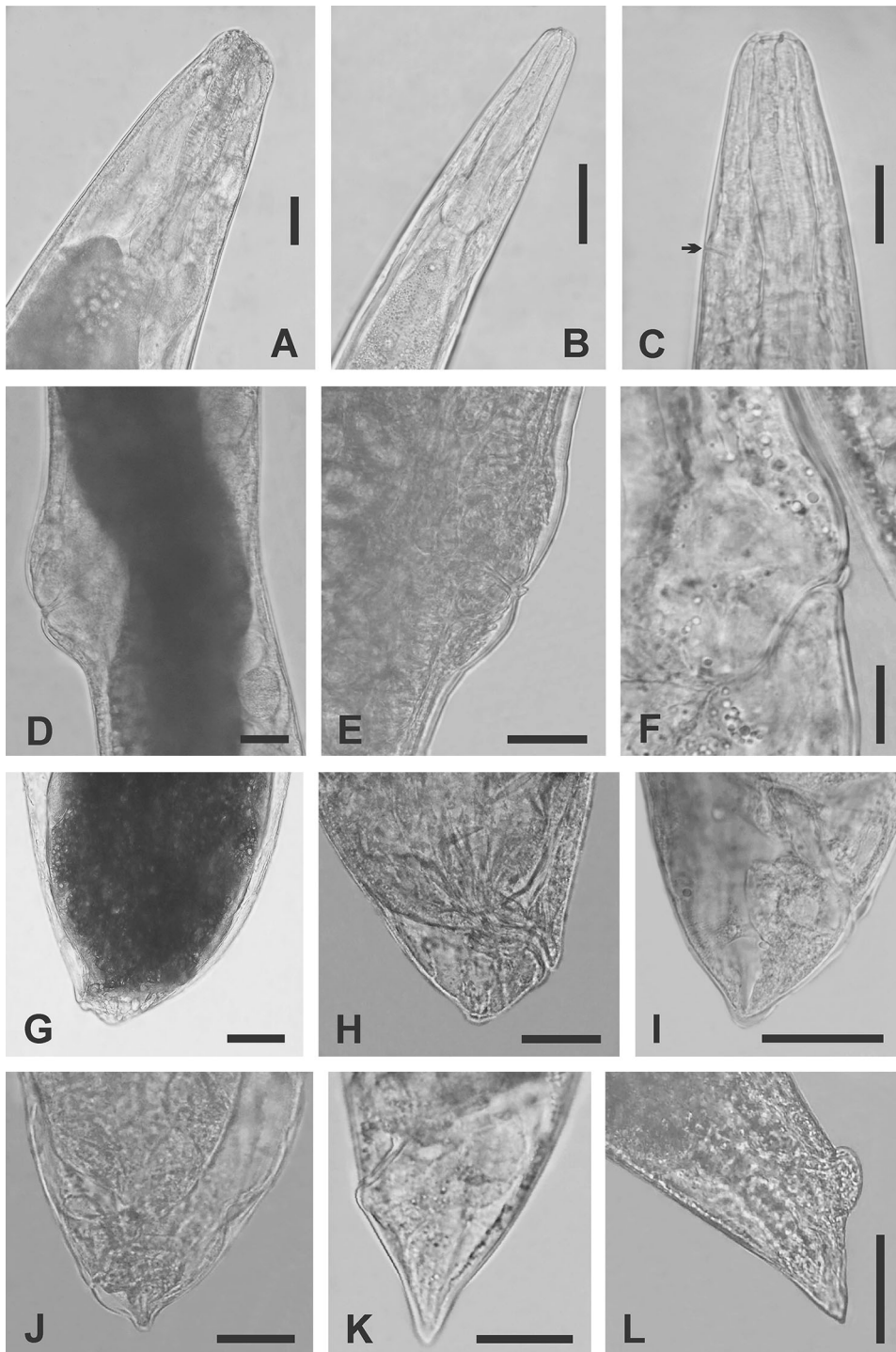


Fig. 2 *Steinernema diaprepsi* Nguyen & Duncan, 2002 from Argentina. A, First generation female, anterior region; B, C, Second generation female; B, Anterior region; C, Anterior region, detail of excretory pore (arrow); D–J, First generation females; D, E, Vulval region; F, Vulval region, detail of double-flapped epiptygma; G–J, Variability of posterior region; K, L, Second generation females, posterior region. Scale-bars: 50 μ m

Table 3 Comparative morphometric data for infective juveniles of *Steinernema diaprepesi* Nguyen & Duncan, 2002 from Argentina and USA

Source	Argentinian isolate		USA isolate
	Present study (n = 20)		Nguyen & Duncan (2002) (n = 20)
Body length	768–1,102 (987 ± 69)		880–1,133 (1,002 ± 53)
Body width	27–48 (33 ± 4)		30–42 (34 ± 4)
ES	93–153 (131 ± 18)		111–152 (138 ± 7)
EP	52–74 (63 ± 5)		66–83 (74 ± 6)
NR	63–98 (90 ± 8)		74–109 (102 ± 6)
T	51–77 (71 ± 5)		65–91 (83 ± 5)
ABW	18–23 (21 ± 1)		21–27 (23 ± 2)
a	23–34 (29.9 ± 3.3)		23–35 (30.0 ± 3.6)
b	6.1–10.1 (7.6 ± 0.9)		6.5–8.3 (7.3 ± 0.5)
c	12.9–15.5 (13.8 ± 0.7)		10.4–13.2 (12.1 ± 0.7)
D = EP/ES (%)	36–62 (49 ± 6)		30–70 (54 ± 5)
E = EP/T (%)	78–114 (89 ± 7)		78–114 (90 ± 7)

Abbreviations: EP, distance from anterior end to excretory pore; NR, distance from anterior end to nerve-ring; ES, distance from anterior end to end of oesophagus; ABW, anal body width; T, tail length; a, body length/width; b, body length/ES; c, body length/T

respectively) and the tail was shorter (71 vs 83 μm). Nguyen & Smart (1995) suggested that, when possible, *Steinernema* spp. should be reared in *G. mellonella* for nematode identification. However, the differences observed in the present study regarding the original isolate (both multiplied in *G. mellonella*) indicate a particular intraspecific variability. Also, Nguyen & Duncan (2002) observed morphometric differences between first generation males and IJs from different hosts (*G. mellonella* and *D. abbreviatus*). Morphological and morphometric differences between populations could be attributed to their geographical origin (Stock et al., 2000), and also to different environmental conditions and host

interactions (Poinar, 1992). Hence the importance of characterising new isolates of the species.

Until present, the natural hosts of the Argentinian isolate remain unknown. Regarding the ecological features of this isolate, previous studies evaluated its life-cycle and pathogenicity to different arthropod species. The nematode showed high virulence to lepidopteran larvae (Caccia et al., 2014; Del Valle et al., 2014), being lower in some species from the orders Coleoptera, Blattodea and Diptera, and nil for Isopoda and Orthoptera (Del Valle et al., 2014). The original isolate of *S. diaprepesi* showed a lethal effect on the coleopteran *D. abbreviatus* (see El-Borai et al., 2012; Nguyen & Duncan, 2002). Furthermore, a

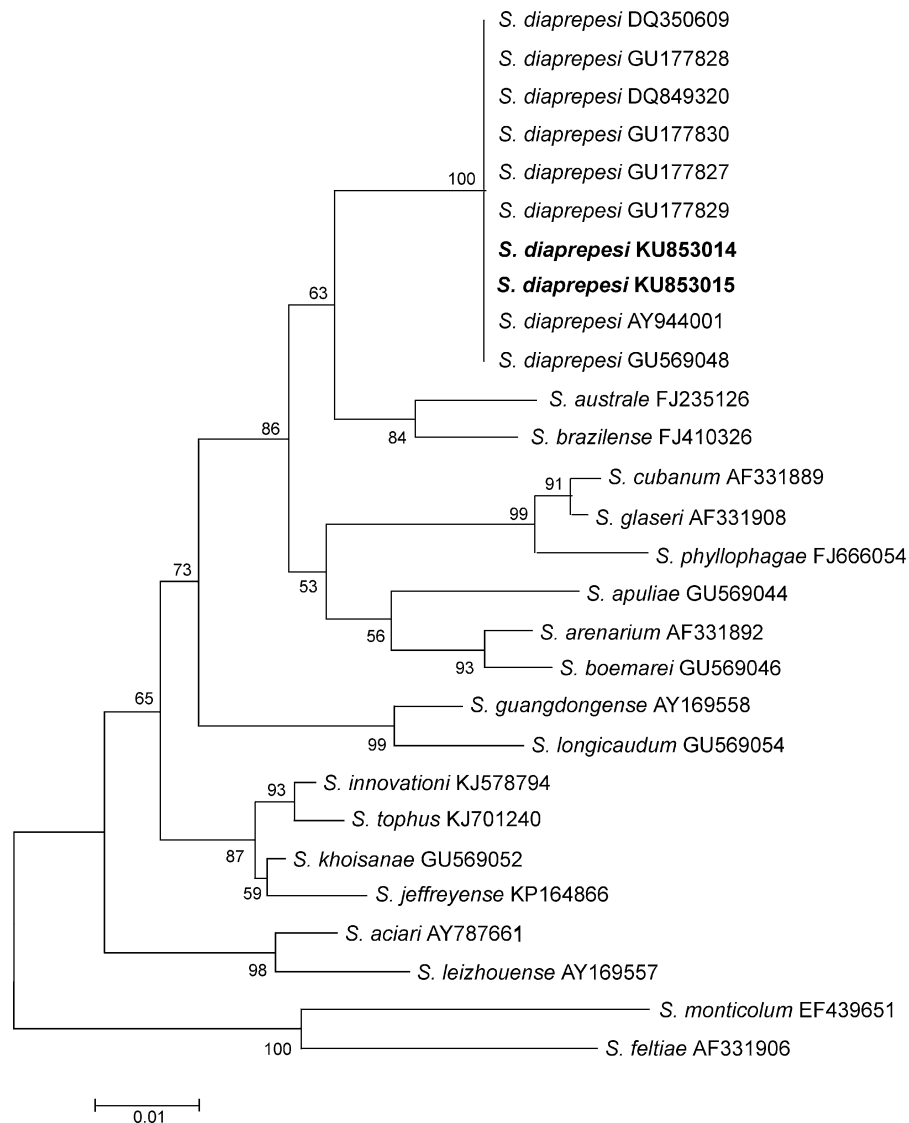


Fig. 3 Phylogenetic relationships derived from an analysis of the D2-D3 expansion segments of the 28S rRNA gene sequences of *Steinermea* spp. based on Maximum-Likelihood analysis using Tamura-Nei model. Bootstrap support values of 1,000 replications are given in the nodes. Sequences of the Argentinian isolate in bold type

Mexican isolate of this species showed virulence when tested in *Boophilus microplus* Canestrini (Acari: Ixodidae) (Molina-Ochoa et al., 2009). The efficacy of the Argentinian isolate was found to be favored by sandy soils and high temperatures; IJs were infective to larvae of *G. mellonella* at 40°C (Del Valle et al., 2014). This tolerance may be attributed to its bacterial symbiont, *X. doucetiae*, that has the ability to grow at temperatures above 35°C (Taillez et al., 2006).

The phylogenetic analysis of D2-D3 and ITS rRNA gene sequences showed a clade grouping *S. diaprepesi*, *S. braziliense* and *S. australe*. The phylogenetic analysis based on 16S rRNA gene grouped *X. doucetiae* and *X. magdalenensis*, the respective symbiotic bacteria of *S. diaprepesi* and *S. australe*, also sharing a common ancestor (Taillez et al., 2012). So far, the bacterial symbiont of *S. braziliense* is not yet characterised. It has been mentioned that these three

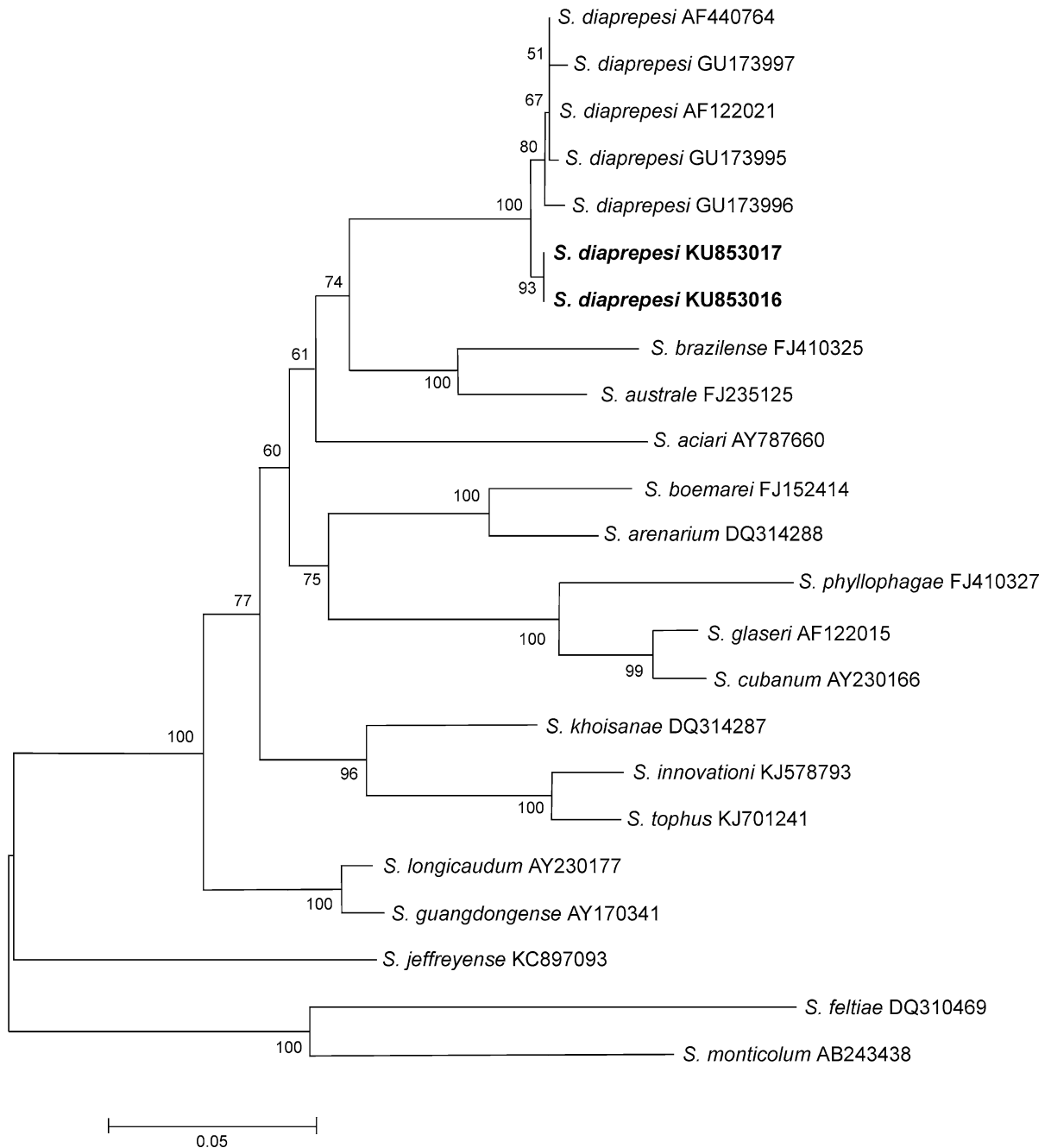


Fig. 4 Phylogenetic relationships derived from analysis of the ITS rRNA gene sequences of *Steinerinema* spp. based on Maximum-Likelihood analysis using Tamura-Nei model. Bootstrap support values of 1,000 replications are given in the nodes. Sequences of the Argentinian isolate in bold type

Steinerinema spp. may probably be endemic to the American continent (Tailliez et al., 2012). Up to now, in South America, *S. diaprepesi* has been found in

Venezuela and Argentina. The detection of this species in Argentina increases the knowledge of the diversity of the genus in the country.

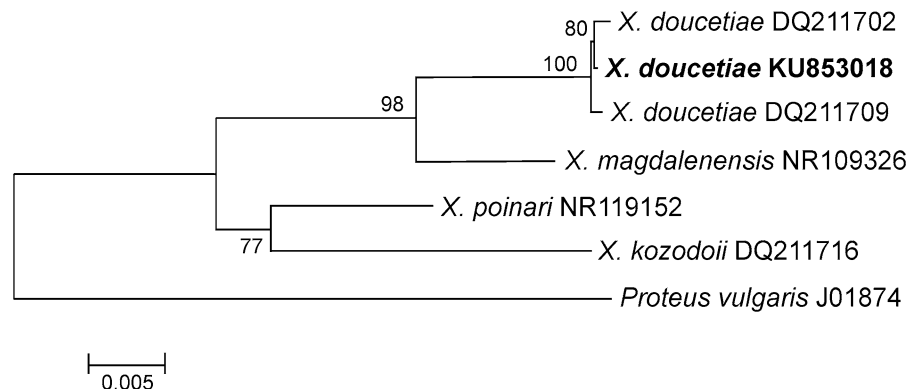


Fig. 5 Phylogenetic relationships derived from analysis of the 16S rRNA gene of *Xenorhabdus* spp. based on Maximum-Likelihood analysis using Tamura-Nei model. Bootstrap support values of 1,000 replications are given in the nodes. Sequence of the Argentinian isolate in bold type

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable institutional, national and international guidelines for the care and use of animals were followed.

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