Journal of Parasitology 2024 110(1) 22–39

Published 9 February 2024

DOI: 10.1645/23-60

Contents and archives available through www.bioone.org or www.jstor.org

Journal of Parasitology

journal homepage: www.journalofparasitology.org

STEINERNEMA ADAMSI N. SP. (RHABDITIDA: STEINERNEMATIDAE), A NEW ENTOMOPATHOGENIC NEMATODE FROM THAILAND

Anil Baniya[1], Chanakan Subkrasae[2], Jiranun Ardpairin[2], Kyle Anesko[1], Apichat Vitta[2,3], and Adler R. Dillman[1]

[1] Department of Nematology, University of California, Riverside, California 92521.
[2] Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, Phitsanulok 65000, Thailand.
[3] Centre of Excellence for Biodiversity, Faculty of Sciences, Naresuan University, Phitsanulok 65000, Thailand.
Correspondence should be sent to Adler R. Dillman (https://orcid.org/0000-0001-7171-4332) at: *adlerd@ucr.edu*

KEY WORDS

Steinernema adamsi
Steinernematidae
Entomopathogenic nematode
Morphometrics
New species

ABSTRACT

A new species of entomopathogenic nematode, *Steinernema adamsi* n. sp., was recovered from the soil of a longan tree (*Dimocarpus* sp.) in Mueang Lamphun District, Thailand, using baiting techniques. Upon analysis of the nematode's morphological traits, we found it to be a new species of *Steinernema* and a member of the *Longicaudatum* clade. Molecular analyses of the ITS rDNA and D2D3 of 28S rDNA sequences further confirmed that *S. adamsi* n. sp. is a new species of the *Longicaudatum* clade, which is closely related to *Steinernema guangdongense* and *Steinernema longicaudam*. Using morphometric analysis, the infective juveniles measure between 774.69 and 956.96 μm, males have a size range of 905.44 to 1,281.98 μm, and females are within the range of 1,628.21 to 2,803.64 μm. We also identified the symbiotic bacteria associated with the nematode based on 16S sequences as *Xenorhabdus* spp. closely related to*Xenorhabdus griffiniae*. Furthermore, we have successfully assessed a cryopreservation method for the long-term preservation of *S. adamsi* n. sp. Successful cryopreservation of this new species will allow for the longer preservation of its traits and will be valuable for its future use. The discovery of this new species has significant implications for the development of effective biological control agents in Thailand, and our work contributes to our understanding of the diversity and evolution of entomopathogenic nematodes.

Entomopathogenic nematodes (EPNs) are a group of nematodes that specifically infect and parasitize insects and are distributed worldwide (Adams et al., 2006; Dillman et al., 2012). Broadly, most described species of EPNs are classified into 2 families: Steinernematidae and Heterorhabditidae, and they have a symbiotic relationship with bacteria from the genera *Xenorhabdus* and *Photorhabdus*, respectively (Stock and Blair, 2008). These nematodes are effective in controlling and killing insects; hence they have great potential in biological control and integrated pest management. Some species of EPNs are commercially produced and are readily available for use (Shapiro-Ilan et al., 2023).

Pest control in commercial agriculture is largely based on chemical pesticides, which are detrimental to the environment and human health (Rani et al., 2021). The growing awareness of these issues has sparked a rise in the popularity of biological control—including classical, augmentation, and conservation methods (Stuart et al., 2006, 2008). The success of EPNs in controlling some important insect pests has sparked research into various aspects of their biology, allowing researchers to identify effective strains of EPNs that can be used as biological control agents (Parkman and Smart, 1996; Nguyen and Duncan, 2002; Stock et al., 2018). Although EPNs have great potential in biological control, understanding is limited as to their diversity, behavior, and factors affecting their distribution (Stuart et al., 2006). One hundred species of Steinernematidae have been recovered and described from soils around the world, and the number of newly described species is increasing (Koppenhöfer et al., 2020; Lis et al., 2021; Tian et al., 2022). According to phylogenetic analyses, nematodes within Steinernematidae are divided into 12 multispecies clades: *Glaseri*, *Karii*, *Longicaudatum*, *Khoisanae*, *Costaricense*, *Cameroonense*, *Feltiae*, *Kushidai*, *Monticolum*, *Carpocapsae*, *Bicornutum*, and *Affine*, and 9 of these clades can be combined into 3 superclades (Spiridonov and Subbotin, 2016).

Discovering new EPN species within Steinernematidae and using them for agricultural pest management may result in a sustainable solution because few nematodes within Steinernematidae seem to have strong host specificity, making the newly identified species of *Steinernema* a potential source of effective biological

Version of Record, first published online with fixed content and layout, in compliance with ICZN Arts. 8.1.3.2, 8.5, and 21.8.2 as amended, 2012. ZooBank publication registration: urn:lsid:zoobank.org:pub:60C8B5F6-F8E1-4A8E-B9B1-09273695D4A2.

Table I. *Steinernema* spp. and GenBank accession numbers considered for the phylogenetic studies.

Nematode species	GenBank accession number		
	ITS	*28S*	Concatenated
Steinernema abbasi	AY230158	GU569041	*
Steinernema aciari	AY787660	GU395637	*
Steinernema adamsi	This study	This study	*
Steinernema affine	AY171296	GU569042	*
Steinernema akhursti	DQ375757	KF289902	*
Steinernema apuliae	HQ416968	GU569044	*
Steinernema arasbaranense	FJ860039	—	—
Steinernema arenarium	AY230160	AF331892	*
Steinernema ashiunense	DQ354694	FJ165550	*
Steinernema asiaticum	AY230184	—	—
Steinernema asonense	AY487919	—	—
Steinernema australe	FJ235125	FJ235126	*
Steinernema backanense	AY487918	—	—
Steinernema balochiense	JX135547	JX068821	*
Steinernema beddingi	AY603397	AY603396	*
Steinernema bicornutum	AY171279	GU569045	*
Steinernema boemarei	FJ152414	GU569046	*
Steinernema brazilense	FJ410325	FJ410326	*
Steinernema cameroonense	—	JX985265	—
Steinernema carpocapsae	AF121049	KJ950292	*
Steinernema ceratophorum	AF440765	AF331888	*
Steinernema changbaiense	JN865168	—	—
Steinernema cholashanense	EF431959	—	—
Steinernema citrae	FJ235074	—	—
Steinernema costaricense	AY171276	—	—
Steinernema cubanum	AY230166	AF331889	—
Steinernema cumgarense	AY487920	—	—
Steinernema diaprepesi	GU173996	GU569048	*
Steinernema eapokense	AY487921	—	—
Steinernema ethiopiense	JN651413	—	—
Steinernema feltiae	AF121050	AF331906	*
Steinernema glaseri	AY171288	AF331908	*
Steinernema guangdongense	AY170341	AY169558	*
Steinernema hebeiense	DQ105794	DQ399664	*
Steinernema hermaphroditum	MF663703	AY598358	*
Steinernema huense	KF857581	—	—
Steinernema ichnusae	EU421129	EU421130	*
Steinernema innovationi	KJ578793	KJ578794	*
Steinernema intermedium	AY171290	AF331909	*
Steinernema jeffreyense	KC897093	—	—
Steinernema jollieti	AY171265	GU569051	*
Steinernema karii	AY230173	AF331902	*
Steinernema khoisanae	DQ314287	GU569052	*
Steinernema khoungi	GU174002	—	—
Steinernema kraussei	AY171264	GU569053	*
Steinernema kushidai	AB243440	AF331897	*
Steinernema lamjungense	HM000101	HM000102	*
Steinernema leizhouense	AY170340	—	—
Steinernema litorale	AB243441	—	—
Steinernema loci	GQ497740	—	—
Steinernema longicaudum	AY230177	GU569054	*
Steinernema minutum	GU647156	—	—
Steinernema monticolum	AF122017	GU395647	*
Steinernema neocurtillae	AF122018	FJ263674	*
Steinernema nepalense	HQ190044	HQ190045	*
Steinernema nyetense	—	JX985264	—
Steinernema oregonense	AF122019	AF331891	*
Steinernema pakistanense	AY230181	—	—
Steinernema papillatum		KJ913708	—
Steinernema phyllophagae	FJ410327	FJ666054	*
Steinernema poinari	KF145173	KF241749	*
Steinernema puertoricense	—	AF331903	—
Steinernema pui	GU395618	—	—
Steinernema rarum	DQ221116	AF331905	*
Steinernema riobrave	DQ835613	—	—
Steinernema riobravis	—	AF331893	—
Steinernema robustispiculum	AY355442	—	—
Steinernema sacchari	—	KC633096	—
Steinernema sangi	AY355441	—	—
Steinernema scapterisci	AY230183	GU395646	*
Steinernema scarabaei	FJ263673	—	—
Steinernema schliemanni	HM778112	—	—
Steinernema siamkayai	AF331917	—	—
Steinernema sichuanense	DQ884965	DQ884966	*
Steinernema silvaticum	AY171255	—	—
Steinernema surkhetense	HQ190042	HQ190043	*
Steinernema tami	AY171280	—	—
Steinernema texanum	EF152568	EF152569	*
Steinernema thanhi	AY355444	—	—
Steinernema thesami	AY171291	—	—
Steinernema tielingense	GU994201	—	—
Steinernema tophus	KJ701241	KJ701240	*
Steinernema unicornum	GQ497167	—	—
Steinernema vulcanicum	MF491477	—	—
Steinernema weiseri	KJ696685	GU569059	*
Steinernema xinbinense	JN171593	—	—
Steinernema xueshanense	FJ666052	—	—
Steinernema yirgalemense	AY748450	AY748451	*
Caenorhabditis elegans	X03680	NR_000055	*
Caenorhabditis latens	N636079	—	—
Caenorhabditis nigoni	JN636060	—	—
Caenorhabditis sinica	—	KF732844	—
Caenorhabditis wallacei	—	JN636137	—

* Accession used for concatenated phylogeny.

control (Lis et al., 2021). A new species of *Steinernema* was discovered during an EPN survey of agricultural fields in Thailand. Here we describe the morphological, morphometric, and molecular characterization of the newly discovered species, *Steinernema adamsi* n. sp., from Thailand.

MATERIALS AND METHODS

Nematode isolation and maintenance

The nematode was first recovered from a soil sample from the rhizosphere of a longan tree (*Dimocarpus* sp.) in the Mueangjee Sub-District, Mueang Lamphun District (18°28′05.1″N, 98°58′17.7″E), Lamphun Province, Thailand, during an EPN survey between October 2017 and October 2019. Approximately 500 g of collected soil was placed in a plastic bag and transported to the laboratory at the Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, Phitsanulok Province, Thailand. To isolate nematodes, 5 waxworm (*Galleria mellonella*) larvae were used as bait as previously described (Bedding and Akhurst, 1975). The dead *G. mellonella* were washed, and infective juveniles (IJs) were collected using a modified White trap (White, 1927; Kaya and Stock, 1997). The new isolate of *Steinernema* was noted as *Steinernema* eALN11.5_TH and sent to the Department of Nematology, University of California Riverside (UCR) for further characterization. Newly obtained IJs were washed using M9 buffer (6 g Na_2HPO_4, 3 g KH_2PO_4, and 5 g NaCl dissolved in 900 ml distilled deionized [DI] water and autoclaved) and transferred to a lawn of its symbiotic bacteria on nematode growth media (NGM) plates (20 g agar, 3 g NaCl, 10 ml Uracil [2 g/L], 2.5 g Peptone, 975 ml DI water), autoclaved at 121 C at 15 psi for 15 min. After autoclaving,

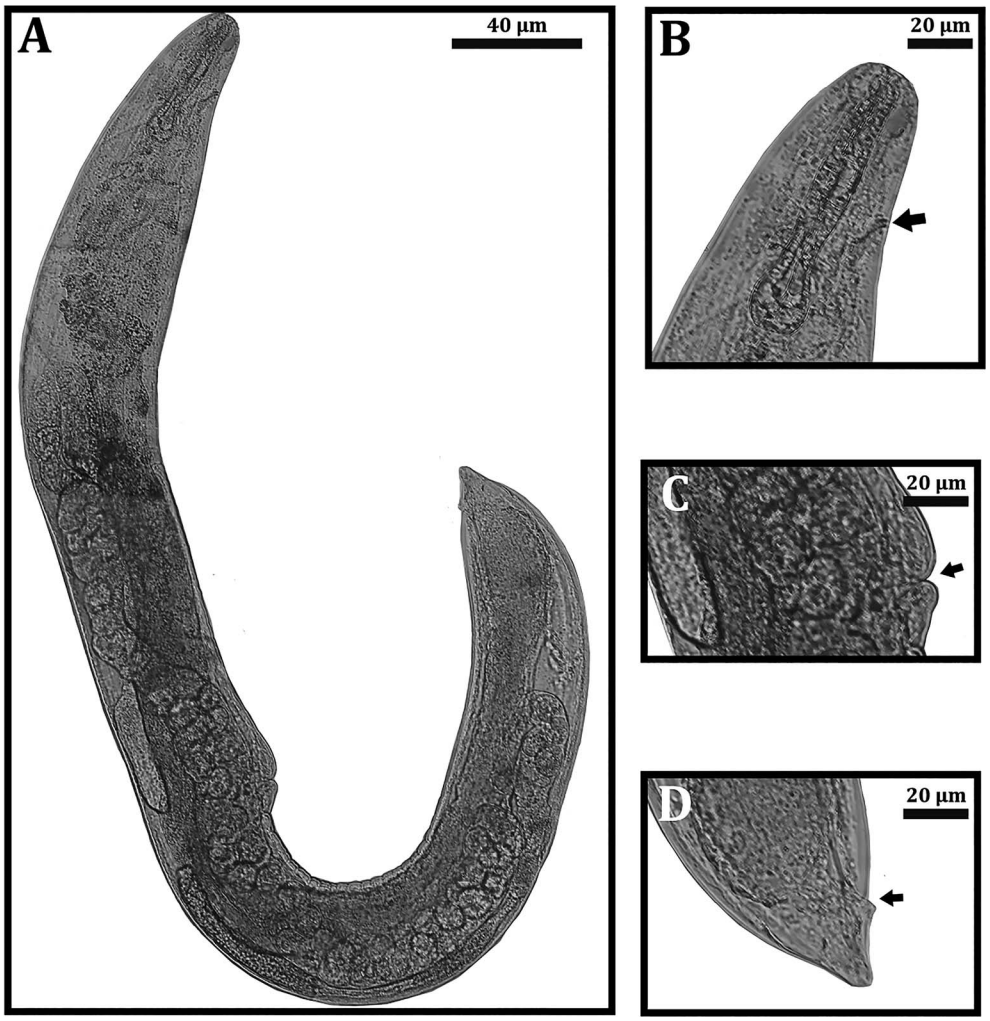

Figure 1. *Steinernema adamsi* n. sp., light microscopy photographs. Female morphology (**A**) in toto, lateral view; (**B**) anterior end showing location of excretory pore (arrow), lateral view; (**C**) vulva (arrow), lateral view; (**D**) tail, and anus (arrow), lateral view.

1 ml cholesterol (5 mg/ml in ethanol), 1 ml 1 M $MgSO_4$, 1 ml 1 M $CaCl_2$, and 25 ml 1 M KPO_4 (pH 6.0) were added, and plates were incubated at 27 C. Nematodes used for morphological and morphometric studies were maintained on an NGM medium inoculated with the symbiotic bacteria and were relocated to fresh plates whenever necessary.

Morphological archiving and measurements

For light microscopy, infective juveniles (IJs) were collected from the infected cadavers using modified White traps. Males and females were also collected from the NGM plates. All nematodes were collected in M9 buffer and prepared for fixation in a hot water bath at 60 C for 2 min. An equal volume of double-strength formalin-glycerol solution (4:1) was heated with the nematode solution, mixed, and incubated at room temperature for 2 days. Formalin-preserved nematodes were processed to anhydrous glycerol using modified Seinhorst's rapid method to glycerol (Seinhorst, 1959). The specimens were mounted on glass slides for morphometric measurement using Volocity image analysis software (Quorum Technologies, Puslinch, Ontario, Canada). The images of analyzed specimens were recorded on a Nikon Eclipse E600 (Nikon, Melville, New York) and Keyence (BZ-X800 Series) (Keyence Corporation, Osaka, Japan). Using Adobe Photoshop, line drawings of all life stages were created by tracing over images captured with the Keyenece and Nikon Eclipse E600 microscope.

DNA extraction, amplification, and sequencing

Approximately 10 freshly emerged IJs were used for DNA extraction. The surface sterilized IJs were pipetted into a 0.2 ml microcentrifuge polymerase chain reaction (PCR) tube mixed with 9 µl of 10 mM Tris, 1 mm EDTA, 0.5 µl of 2% Triton X, and 0.5 µl of Proteinase K (20 mg/ml, New England Biolabs, Ipswich, Massachusetts). The nematodes' cuticle was disrupted by freezing and thawing the sample 3 times using liquid nitrogen, and then the nematode was incubated overnight at −20 C. The next day the frozen lysate was incubated at 56 C for 1 hr followed by 95 C for 10 min for DNA extraction. PCR reaction was performed with a total volume of 50 µl, which included 4 µl of genomic DNA as the template, 25 µl of EconoTaq PLUS 2X Master Mix (Lucigen, LGC Biosearch Technologies, Madison, Wisconsin), 2.5 µl each of forward and reverse primers at a concentration of 10 µM, and 16 µl of nuclease-free water. The primer 18S: 5-TTGATTACGTCCCTGCCCTTT-3 (forward) and 26R: 5-TTTCACTCGCCGTTACTAAGG-3 (reverse) was used to amplify the *ITS1–5.8S–ITS2* region of rDNA gene (Vrain et al., 1992). Similarly, the *28S* rDNA gene was amplified

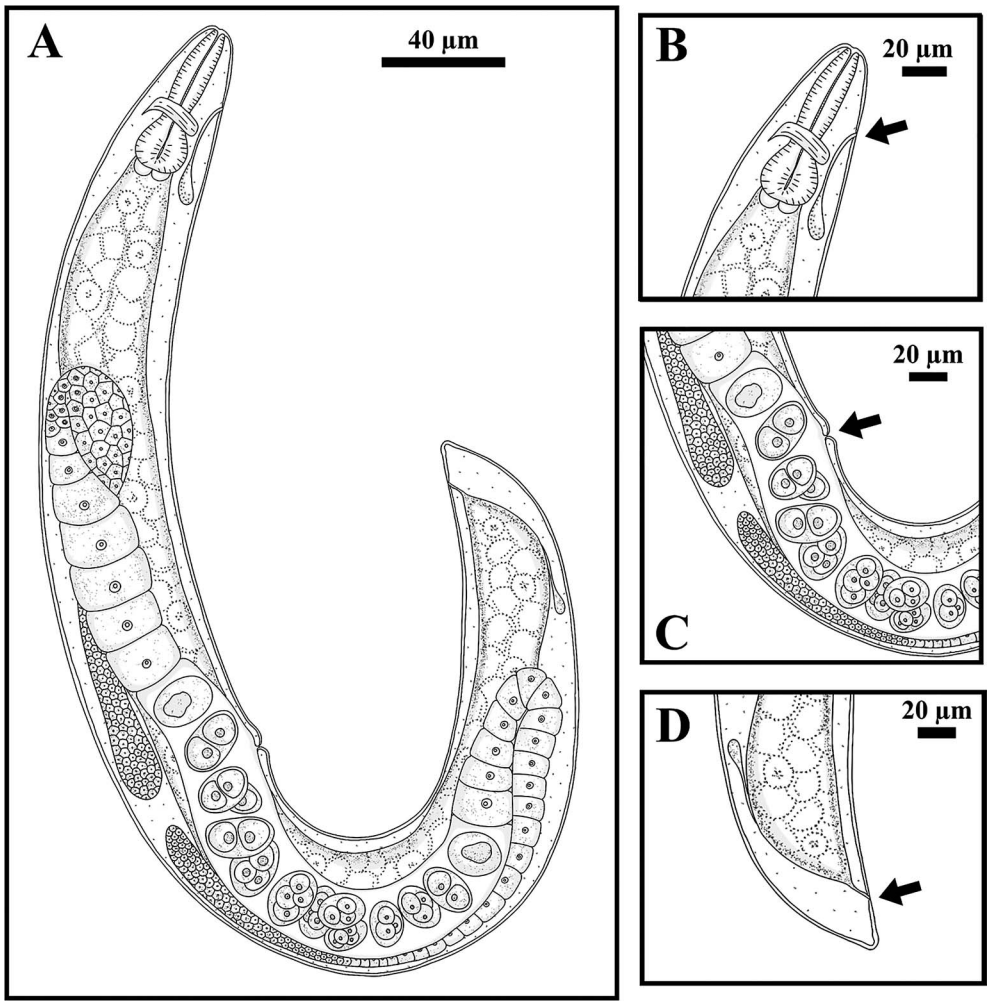

Figure 2. Line drawings *of Steinernema adamsi* n. sp. Female morphology (**A**) full body, lateral view; (**B**) anterior end (lateral view), showing execratory pore (arrow), nerve ring, and stoma region; (**C**) vulva (arrow), lateral view; (**D**) tail region and anus (arrow), (lateral view).

using D2F: 5-CCTTAGTAACGGCGAGTGAAA-3 (forward) and 536: 5-CAGCTATCCTGAGGAAAC-3 (reverse) primers (Nguyen et al., 2006). Similar conditions were used for the PCR amplification of both loci: initial denaturation at 94 C for 5 min, then 35 cycles at 94 C for 1 min, annealing at 57 C for 90 sec, extension at 72 C for 2 min, and a final extension step at 72 C for 10 min. PCR results were visualized on a 1% agarose gel stained with 0.0003% ethidium bromide and a 1-kb plus DNA ladder (New England Biolabs). PCR products were purified using the QIAquick® PCR Purification Kit (Qiagen, Germantown, Maryland) following the manufacturer's protocol. Purified PCR products were Sanger sequenced from the forward and reverse strands at the UCR Core Instrumentation Facility according to the manufacturer's protocol. Chromatogram sequence ambiguities of both forward and reverse sequences of each locus were visually checked and assembled using SeqManII software (DNASTAR, Madison, Wisconsin). Closely related species were identified in the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST). The sequences generated as part of this investigation have been deposited in NCBI GenBank under the accession numbers OR050811 for ITS sequences and OR050810 for D2-D3 rDNA sequences.

Sequence alignment and phylogenetic analyses

The ITS and 28S sequences of *S. adamsi* n. sp. and their respective best hits from NCBI (Table I) were aligned using Clustal W in MEGA 11 with the default parameters, and some alignment inconsistencies were corrected manually (Tamura et al., 2021). The aligned files were used to infer the phylogenetic relationships using the BEAST v. 1.10.4 program (Drummond and Rambaut, 2007) which used Bayesian analyses using the GTR + I + G model of DNA evolution as analyzed by Spiridonov and Subbotin (2016). The BEAST package uses a family of Markov chain Monte Carlo (MCMC) algorithms for Bayesian phylogenetic inference. To construct the concatenated phylogenetic tree of ITS and 28S, all aligned sequences of the isolates that are represented in both ITS and 28S phylogenetic trees were concatenated with a Bayesian analysis using the GTR + I + G model of DNA evolution as analyzed by Spiridonov and Subbotin (2016). The characteristics of the BEAST file were determined using BEAUti, which included the best substitution model from a previously determined model, clock type strict, MCMC chain length of 10,000,000, and all other parameters were at the default setting. The resulting tree was visualized using TreeAnnotator v. 1.10.4, and a burn-in of 10,000 states was specified. The phylogenetic trees were visualized using Figtree v. 1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/).

Table II. Morphometrics of *Steinernema adamsi* n. sp. All measurements are in micrometers. Ranges, means, and standard deviation are also provided.

Character	Male		Female		Infective juvenile
	Holotype	Paratype	Allotype	Paratype	Paratype
Total body length [TBL]	1,138.63	1,109.66 ± 104.37 (905.44–1,281.98)	2,458.55	2,089.25 ± 293.29 (1,628.21–2,803.64)	879.66 ± 49.36 (774.69–956.96)
Maximum body width [MBW]	67.21	69.97 ± 8.34 (56.08–89.64)	167.5	164.37 ± 28.25 (95.23–227.87)	34.96 ± 2.54 (31.58–39.85)
Anterior end to excretory pore [EP]	81.59	88.94 ± 7.26 (78.03–102.56)	100.02	99.87 ± 6.82 (88.69–113.43)	69.84 ± 3.14 (63.85–75.23)
Anterior end to nerve ring [NR]	102.05	100.93 ± 8.13 (84.21–112.41)	116.5	119.22 ± 9.33 (102.59–135.81)	82.84 ± 4.47 (75.02–92.31)
Anterior end to esophagus [ES]	132.48	130.74 ± 10.61 (97.69–146.64)	159.24	154.31 ± 7.75 (139.55–172.19)	103.17 ± 5.51 (90.31–111.73)
Testis reflection [TR]	128.77	92.91 ± 29.22 (57.51–170.03)	—*	—	—
Tail length [TL]	54.29	54.32 ± 5.21 (39.66–63.71)	56.49	55.78 ± 7.58 (32.98–69.66)	72.91 ± 5.39 (61.62–81.19)
Anal body diameter [ABW]	49.29	48.61 ± 2.39 (43.47–52.34)	37.25	47.9 ± 10.56 (36.79–79.56)	20.36 ± 1.71 (17.06–23.16)
Spicule length [SL]	65.85	61.53 ± 5.94 (44.15–68.86)	—	—	—
Gubernaculum length [GL]	36.87	37.01 ± 7.21 (27.85–59.35)	—	—	—
Hyaline Length [H]	—	—	—	—	33.62 ± 3.18 (28.9–38.84)
a [TBL/MBW]	16.94	15.93 ± 1.11 (13.22–17.77)	14.66	12.84 ± 1.23 (10.93–17.10)	25.25 ± 1.92 (21.61–28.52)
b [TBL/ES]	8.59	8.55 ± 1.14 (6.66–12.23)	15.44	13.51 ± 1.59 (10.98–16.58)	8.54 ± 0.64 (7.70–10.00)
c [LBL/TL]	20.97	20.58 ± 2.5 (16.88–26.08)	43.52	38.38 ± 8.52 (26.92–65.64)	12.08 ± 0.52 (11.25–13.65)
Hyaline% [(H/T) × 100]	—	—	—	—	46.12 ± 2.69 (41.04–50.82)
D% [(EP/ES) × 100]	61.59	68.23 ± 4.95 (61.28–79.88)	62.81	64.79 ± 4.16 (54.96–73.50)	67.87 ± 4.59 (61.47–78.74)
E% [(EP/TL) × 100]	150.29	165.64 ± 24.39 (134.84–235.55)	177.06	182.88 ± 30.09 (139.87–288.48)	96.24 ± 7.34 (83.89–111.36)
Anterior to vulva	—	—	1,310.7	1,129.71 ± 150.82 (894.07–1,471.63)	—
Vulval diameter	—	—	158.75	153.56 ± 24.03 (99.55–205.25)	—
SW% [(SL/ABW) × 100]	133.6	126.79 ± 12.84 (92.66–143.13)	—		—
GS% [(GL/SL) × 100]	55.99	60.91 ± 14.34 (42.99–100.0)	—		—
V% [(Vulva – anterior end/L) × 100]	—	—	53.51	54.15 ± 1.87 (50.81–57.45)	—

* — = Character absent.

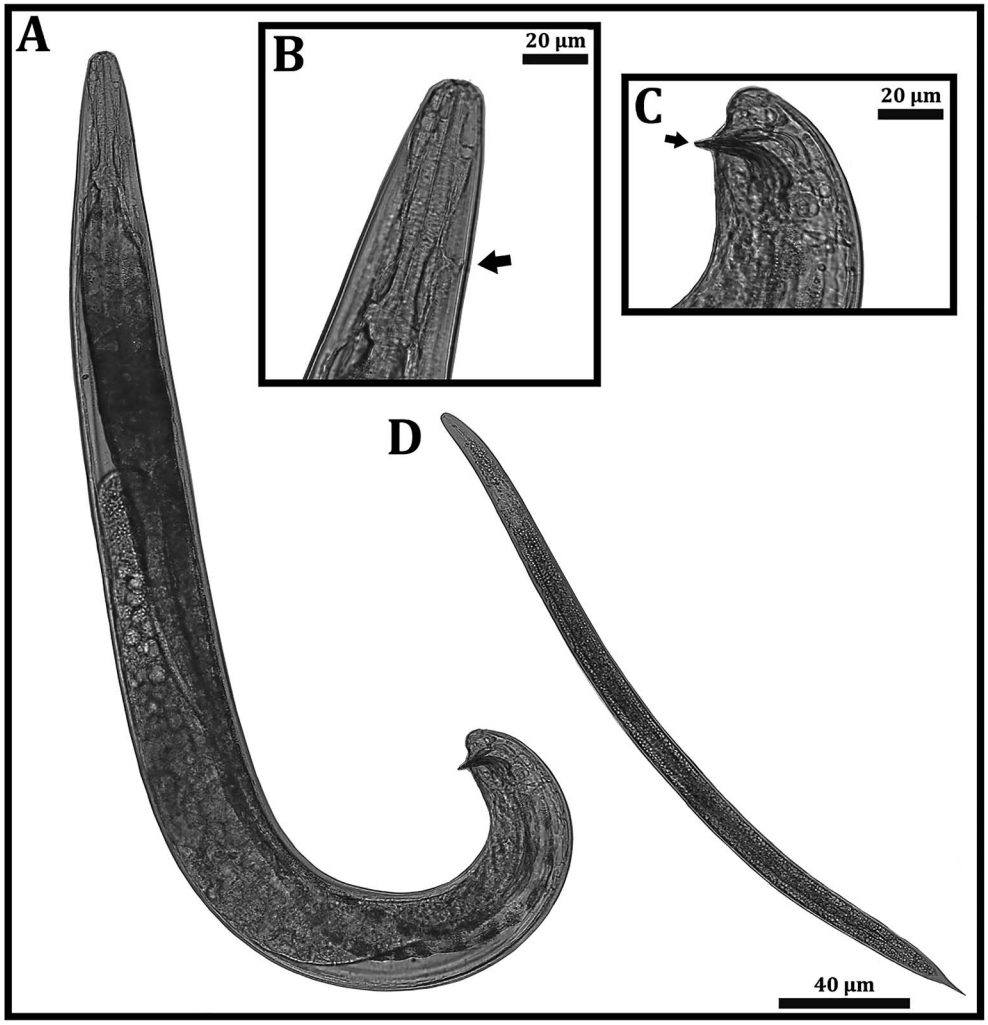

Figure 3. Light microscopy of *Steinernema adamsi* n. sp. male and infective juvenile morphology (**A**) full body morphology of males, lateral view; (**B**) anterior end showing location of excretory pore (arrow), lateral view; (**C**) tail region and spicule (arrow), lateral view; (**D**) full body morphology of infective juvenile.

Cross-hybridization

To examine the reproductive compatibility of *S. adamsi* n. sp. with other close relative species within the genus *Steinernema* a cross-hybridization assay was conducted in NGM plates. Two EPN species *Steinernema glaseri* and *Steinernema diaprepesi* were used to test the reproductive compatibility. There were 10 replicates per cross; in the first 5 plates, individual females of test species were crossed with 2 males of *S. adamsi* n. sp.; in the remaining 5 plates, 2 males of test species were crossed with 1 female of *S. adamsi* n. sp. The experiment was conducted twice with controls consisting of 2 male and 1 female nematodes of the same species.

Identification of symbiotic bacteria

To extract the symbiotic bacteria associated with *S. adamsi* n. sp., *G. mellonella* larvae were infected with IJs. After 48 hr of infection, the insect cadaver was surface sterilized by dipping it into 75% ethanol for 1 minute and placed in a sterile Petri dish to dry. With sterile forceps, the third segment from the head of *G. mellonella* was opened, and its hemolymph was excessed using a sterile loop. The loop was then streaked on nutrient agar plates supplemented with bromothymol blue and triphenyl-2,3,5-tetrazolium chloride (NBTA; Sigma-Aldrich, St. Louis, Missouri). Normally colonies of *Xenorhabdus* on NBTA are blue or brown, but we have observed that the symbionts of *S. adamsi* n. sp. are greenish. The green colonies of the *Xenorhabdus* bacteria were observed after 5 days of incubation in a dark condition at 28 C. After 5 days a single colony of the *Xenorhabdus* bacteria was transferred to 5 ml of Luria-Bertani (LB) broth and incubated at 28 C overnight with shaking at 180 rpm. To identify the symbiotic bacteria, genomic DNA was extracted from bacterial pellets using the Proteinase K method as described above. The PCR amplification of the *16S* rRNA gene used EconoTaq PLUS 2X Master Mix (Lucigen). The primers used for PCR reaction are F8: 5-CAGGCATCCAGACTTTGATYMTGGCTCAG-3 (forward) and R1512: 5-GTGAAGCTTACGGYTAGCTTGTTACGACTT-3 (reverse) as previously described (Dreyer et al., 2018). The PCR conditions were the same as above except for the annealing temperature, which was set at 55 C. The PCR product was gel purified and Sanger sequenced as previously described. A partial *16S* rDNA gene sequence was compared with existing gene sequences in the NCBI database using BlastN (Agarwala et al., 2017). Based on the Blast result, different *Xenorhabdus* species were used to construct the phylogeny. The 16S sequences of

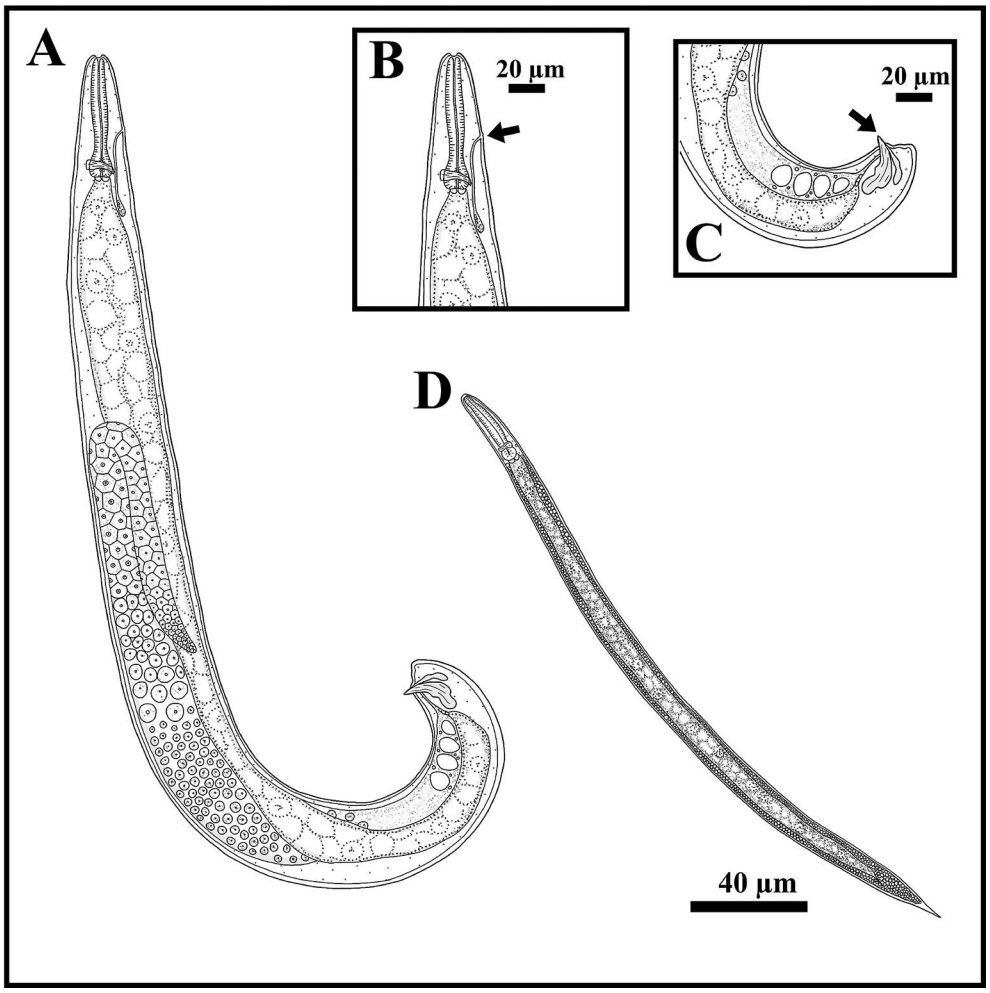

Figure 4. Line drawings of *Steinernema adamsi* n. sp. male and infective juvenile: (**A**) Full body of the male, lateral view; (**B**) anterior end, showing stoma region, nerve ring and execratory pore (arrow), lateral view; (**C**) tail region, spicule (arrow) and gubernaculum (lateral view); (**D**) line drawing of full third-stage infective juvenile (lateral view).

Morganella morganii (AJ301681) were used as an outgroup. All the sequences were aligned using Clustal W in MEGA 11 with the default parameters, and some alignment inconsistencies were corrected manually (Tamura et al., 2021). The program IQ-Tree (Nguyen et al., 2015) was used to select the best-fitting model for the 16S data set using the "find best model" function and to construct a maximum-likelihood (ML) phylogenetic tree, utilizing ultrafast bootstrap branch support with 1,000 replicates.

Cryopreservation of *S. adamsi* n. sp.

The cryopreservation for *Steinernema adamsi* n. sp. was optimized by modifying a trehalose-DMSO freezing protocol for *C. elegans* (Poullet and Braendle, 2015). IJs freshly emerged from wax worms were placed on the NGM plates seeded with symbionts of *S. adamsi* n. sp. The NGM plates were incubated at 28 C until the bacteria on the NGM plates were completely depleted. At this stage, the plates are full of the mixed stage nematodes with most of the eggs freshly hatched, and females are full of J1 stage nematodes that are internally hatched. Nematodes from the several plates were collected by washing nematodes using M9 buffer and concentrated using centrifugation for 1 min at ~1,000 *g* at room temperature in a 15 ml tube. Once the nematodes were concentrated the pellet was incubated in 5 ml of trehalose-DMSO solution for more than 30 min (1 L of trehalose-DMSO solution is prepared with 30.2 gm D-Trehalose anhydrous [Thermo Fisher Scientific, Waltham, Massachusetts]), 35.4 ml dimethylsulfoxide [DMSO, Fisher Scientific], volume adjusted to 1 L using M9, buffer and is filter sterilized). The nematodes were transferred into cryotubes to slowly reduce the temperature, and cryotubes were placed inside Styrofoam boxes and placed in a −80 C freezer overnight. After incubation cryotubes were taken out of the Styrofoam box and transferred to a cardboard box in a −80 C freezer for long-term storage.

DESCRIPTION

Order Rhabditida Chitwood, 1933 Family Steinernematidae Filipjev, 1934 Genus *Steinernema*; Travassos, 1927 *Steinernema adamsi* n. sp.

Adult female diagnosis: Heat-killed and relaxed females are C-shaped (Figs. 1, 2), 628–2,803 µm long (Table II). Head round and cuticle smooth under light microscopy. An excretory pore opening is present anterior to the nerve ring. Stoma reduced (cheilo-, gymno- and stegostom vestigial), short and wide funnel shaped, inconspicuous sclerotized walls. The nerve ring surrounds the isthmus just anterior to the basal bulb. The esophagus is

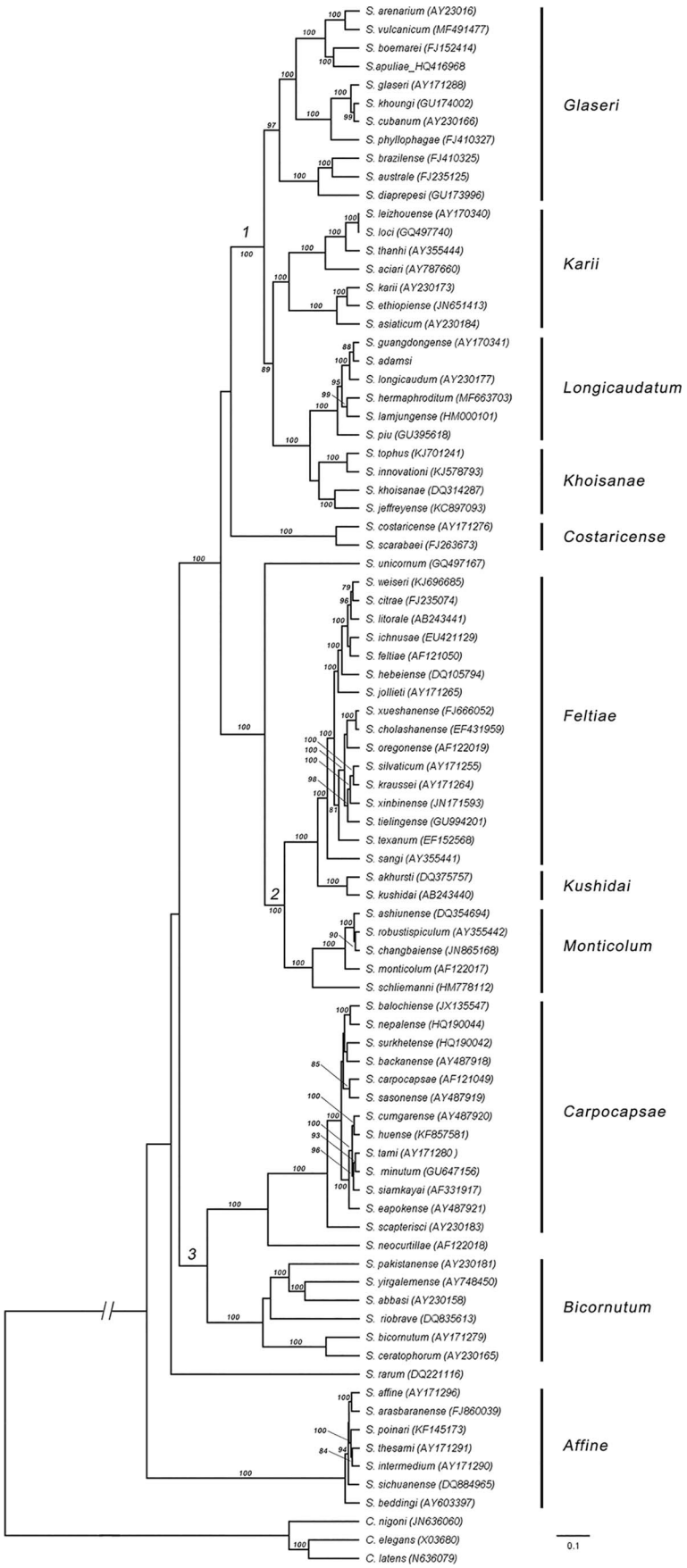

Figure 5. The phylogenetic relationships among different species of *Steinernema* as inferred from Bayesian analysis of the *ITS* rRNA gene sequences using the GTR+I+G model of DNA evolution. Clades with posterior probability values greater than 75% are labeled with appropriate numbers. Number 1 corresponds to the superclade of Glaseri-Karii-Longicaudatum-Khoisanae. Number 2 represents Feltiae-Kushidai-Monticolum superclade, and Number 3 denotes the Carpocapsae-Biocornutum superclade.

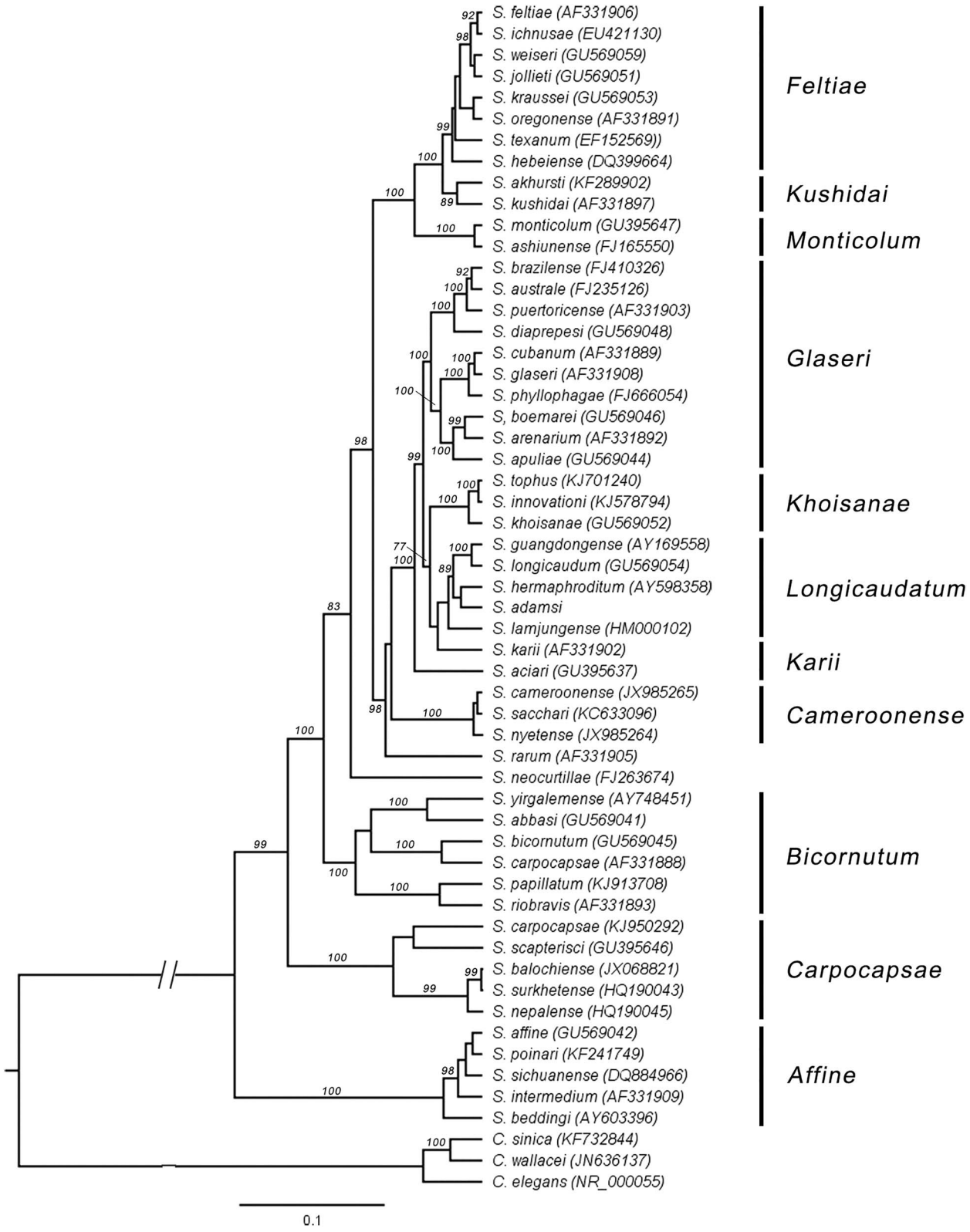

Figure 6. The phylogenetic relationships among different species of *Steinernema* as inferred from Bayesian analysis of the D2-D3 region of *28S* rRNA gene sequences using the GTR+I+G model of DNA evolution. Clades with posterior probability values greater than 75% are labeled with appropriate numbers.

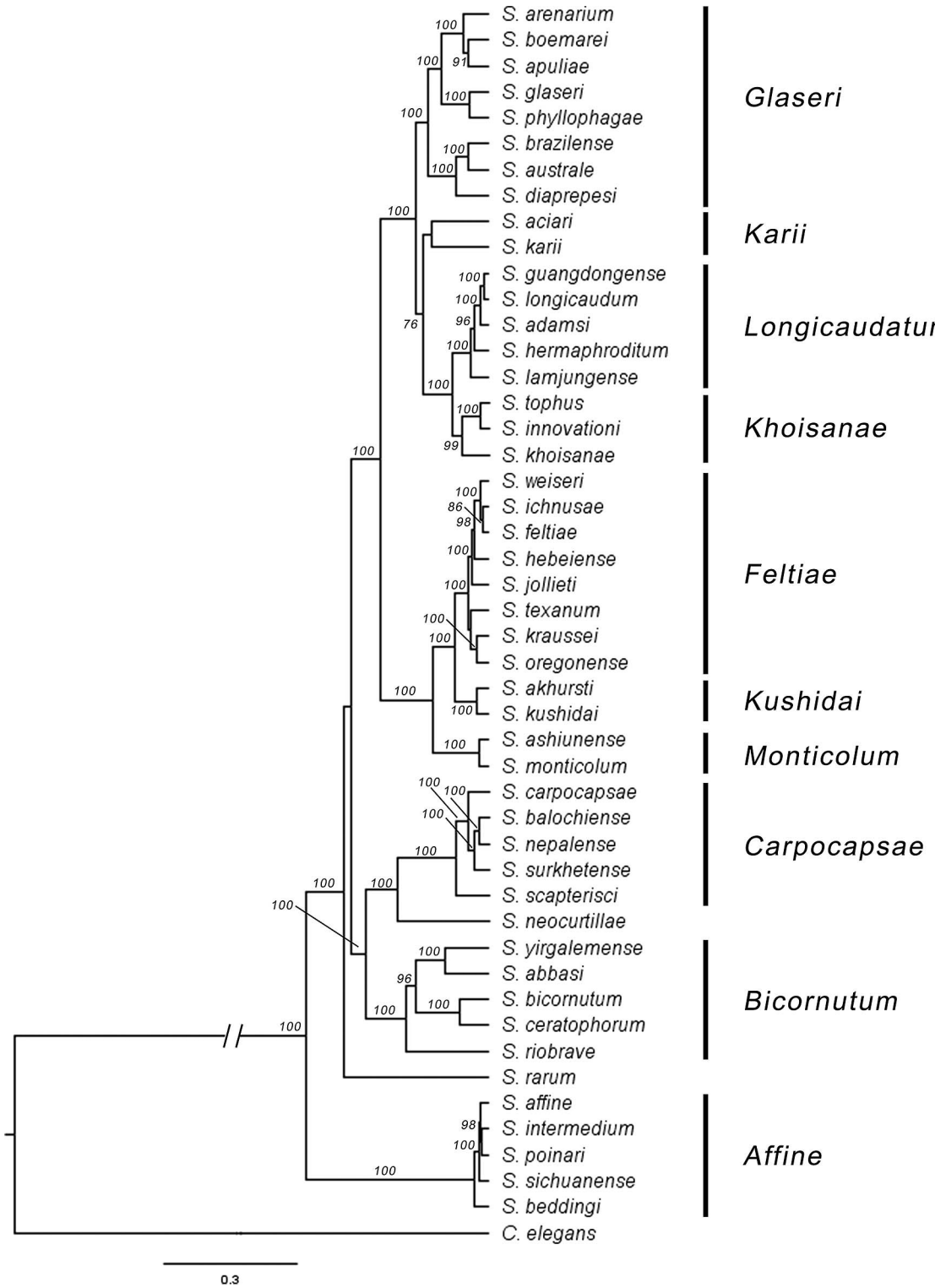

Figure 7. The phylogenetic relationships among different species of *Steinernema* as inferred from Bayesian analysis of the ITS and D2-D3 region of *28S* rRNA gene sequences using the GTR+I+G model of DNA evolution. Clades with posterior probability values greater than 75% are labeled with appropriate numbers.

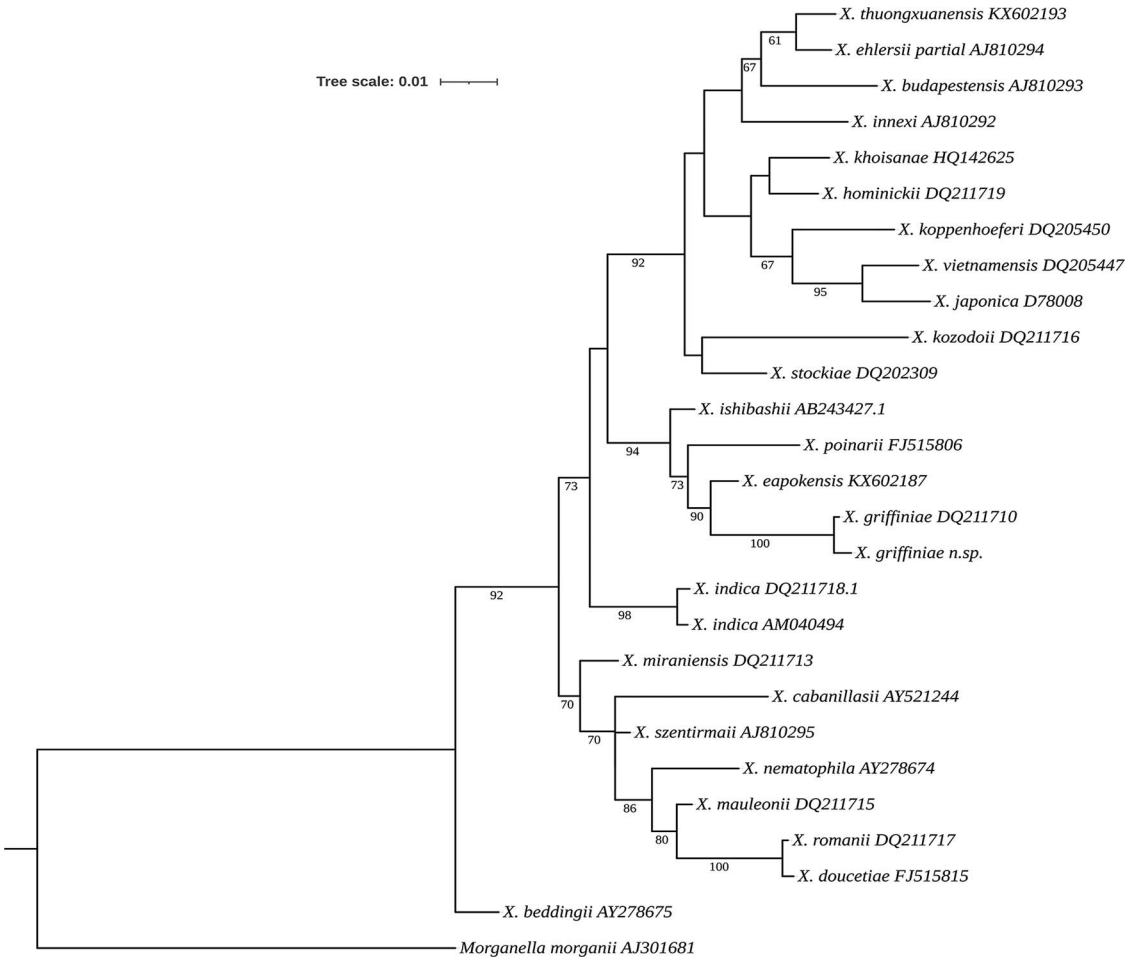

Figure 8. Maximum likelihood phylogenetic tree inferred from published *Xenorhabdus* sequences and newly sequenced *Xenorhabdus griffinae* based on the *16S* rRNA gene. The analysis was run with a bootstrap value of 1,000. Numbers at the branches represent bootstrap support values for each node.

muscular with a cylindrical procorpus, followed by a slightly swollen non-valvated metacorpus, isthmus, and basal bulb. Gonads are didelphic-amphidelphic. Vulva is located at the mid-portion of the body. The vagina is short and connected to paired uteri. Vulva a transverse slit varying from slightly protruding to significantly protruding from the body surface, with a thick flap. Females deposit the eggs internally; subsequently, juveniles hatch inside the female and bore their way out. Tail blunt, conoid. Post-anal lips non-protruding or slightly protruding.

Adult male diagnosis: Heat-killed specimens have J-shaped slender body and are ventrally curved posteriorly (Figs. 3, 4). Cuticle of the male is smooth, inconspicuous lateral field and phasmids under light microscopy. The head is truncated to somewhat round, and it is continuous with the body. Stoma like that of the female. Esophagus muscular; procorpus cylindrical; metacorpus slightly swollen, non-valvate; isthmus distinct Nerve ring just anterior to the basal bulb. Excretory pores at the middle of the esophagus. The reproductive system (testis) is monorchic and ventrally reflexed. The spicules average 65.85 µm long and are moderately curved and golden brown with a sharp tip (Table II). Gubernaculum fusiform, approximately about one-half of the length of spicules. Tail conoid and non-mucronate, and bursa absent.

Infective juveniles: Body straight or slightly curved, tapering gradually towards the anterior end from the base of esophagus and towards the distal end when infective juveniles are heat killed and relaxed (Figs. 3, 4). The mouth and the anus are closed, and the esophagus and intestine are collapsed. Excretory pores are located approximately around the mid-corpus region. The nerve ring is located at isthmus level slightly above basal bulb. The lateral field patterns and lateral lines were not observed in light microscopy. The intestine lumen is narrow and is filled with fat globules. Rectum is long, straight, and anus is distinct. The hyaline occupies c. 46% of the tail length (Table II). The tail is conoid with pointed terminus.

Taxonomic summary

Type locality: The rhizosphere of the longan tree (*Dimocarpus* sp.) in Lamphun Province, Thailand (18°28′05.1″N, 98°58′17.7″E).

Table III. Comparison of morphometric traits (mean and range) of infective juveniles of *Steinernema adamsi* n. sp. with other steinernematids. All measurements are in micrometers and in the form "mean (range)."

Steinernema spp.	TBL*	MBW*	EP*	TL*	C*	D%*	E%*	Reference
S. arenarium	1,134 (724–1,408)	46 (28–77)	83 (76–86)	75 (64–84)	15.1 (12.2–17.9)	55 (52–59)	119 (106–130)	Artyukhovsky et al., 1997
S. boemarei	1,101 (1,005–1,323)	39 (34–46)	91 (82–111)	86 (77–109)	13 (12–14)	63 (56–68)	106 (94–122)	Lee et al., 2009
S. glaseri	1,130 (864–1,448)	29 (25–32	65 (59–69)	66 (53–74)	14.7 (13.6–15.7)	65 (58–71)	131 (122–138)	Poinar, 1990
S. khuongi	1,066 (985–1,195)	31 (24–39)	80.5 (71–96)	65 (55–76)	16 .4 (15.7–17.9)	60.5 (49–71)	126 (104.5–150)	Stock et al., 2018
S. cubanum	1,283 (1,149–1,508)	37 (33–46)	106 (101–114)	67 (61–77)	19 NA	70 NA	160 NA	Mráček et al., 1994
S. phyllophagae	1,251 (1,017–1,446)	39 (16–41)	100 (86–107)	85 (72–96)	14.7 (12.1–15.9)	73 (61–83)	115 (103–130)	Nguyen and Buss, 2011
S. brazilense	1,157 (1,023–1,284)	42 (39–49)	94 (87–102)	88 (80–104)	13 (12–14.5)	63 (58–70)	106 (95–118)	Nguyen et al., 2010
S. diaprepesi	1,002 (880–1,133)	34 (30–42)	74 (66–83)	83 (65–91)	12.1 (10.4–13.2)	54 (30–70)	90 (78–114)	Nguyen and Duncan, 2002
S. aciari	1,113 (975–1,250)	47 (42–53)	95 (87–100)	78 (68–88)	14.4 (14–16)	65 (60–70)	12 (113–134)	Qiu et al., 2005
S. karii	932 (876–982)	33 (31–35)	74 (68–80)	74 (64–80)	12.6 NA	57 NA	96 NA	Waturu et al., 1997
S. ethiopense	898 (768–1,010)	34 (32–43)	78 (65–84)	73 (64–80)	12 (11–14)	56 (51–58)	107 (91–116)	Tamiru et al., 2012
S. guangdonense	1,055 (987–1,145)	42 (30–48)	80 (71–85)	91 (82–103)	11.6 (10.2–12.9)	59 (54–65)	88 (74–100)	Qiu et al., 2004
S. adamsi n. sp.	879.66 (774.69– 956.96)	34.96 (31.58–39.85)	69.84 (63.85–75.23)	72.91 (61.62–81.19)	12.08 (11.25–13.65)	67.87 (61.47–78.74)	96.24 (83.89–111.36)	This study
S. longicaudum	1,043 (929–1,170)	37 (26–31)	82 (74–92)	94 (79–105)	11.1 (10.5–12.1)	57 (52–63)	87 (76–104)	Stock et al., 2001
S. hermaphroditum	929 (700–950)	29 (25–32.5)	65 (62.5–82.5)	77 (65–82.5)	10.6 (9.3–11.7)	50 (47–55)	85 (76–100)	Stock et al., 2004
S. lamjungense	832 (690–950)	27 (23–31)	68 (61–73)	88 (67–103)	9.6 (7.7–10.8)	54 (48–63)	79 (69–97)	Khatri-Chhetri et al., 2011
S. innovationi	1,055 (1,000–1,103)	37 (31–50)	88 (82–91)	76 (66–80)	NA	58 (54–63)	115 (104–137)	Çimen et al., 2015
S. khoisanae	1,062 (904–1,214)	31 (27–39)	95 (84–100)	85 (69–98)	12.5 (10.6–13.8)	68 (115–147)	112 (95–123)	Nguyen et al., 2006
S. jeffreyense	926 (784–1,041)	35 (23–43)	87 (78–107)	81 (50–96)	12 (9.7–19)	66 (57–85)	109 (86–169)	Malan et al., 2016
S. costaricense	1,696 (1,600–1,773)	39 (23–45.5)	77 (75–82)	63.5 (54.5–68.5)	10.2 (8.9–11.4)	53 (45–60)	85 (82–91.5)	Uribe-Lorío et al., 2007
S. scarabaei	918 (890–959)	31 (25–37)	77 (72–81.5)	76 (71–80)	12 (11–13)	60 (50–75)	100 (90–110)	Stock and Koppenhöfer, 2003

* Abbreviations: TBL: total body length; MBW: maximum body width; EP: anterior end to excretory pore; TL: tail length; C: ratio of body length to tail length; D%: percentage of ratio between anterior ends to excretory pore to anterior end to esophagus; E%: percentage of ratio between anterior ends to excretory pore to tail length.

Table IV. Comparison of morphometric traits (mean and range) of males of *Steinernema adamsi* n. sp. with other steinernematids. All measurements are in micrometers and in the form "mean (range)."

Steinernema spp.	SL*	GL*	SW%*	GS%*	D%*	References
S. aciari	86 75–95	56 48–65	204 180–240	65 57–77	76 69–88	Qiu et al., 2005
S. adamsi n. sp.	61.53 (44.15–68.86)	37.01 (27.85–59.35)	126.79 (92.66–143.13)	60.91 (42.99–100.0)	68.23 (61.28–79.88)	This study
S. arenarium	84 (81–91)	55 (49–60)	210 NA	65 (60–66)	93 (88–102)	Artyukhovsky et al., 1997
S. boemarei	79 (64–96)	52 (43–65)	170 (120–240)	70 (50–90)	85 (68–99)	Lee et al., 2009
S. brazilense	83 75–89	47 41–56	192 158–208	56 48–65	68 57–80	Nguyen et al., 2010
S. costaricense	92 (81–101)	46 (40.5–50.5)	160 (150–1.07)	49 (45–55)	56 (50.5–66)	Uribe-Lorío et al., 2007
S. cubanum	58 (50–67)	39 (37–42)	140	70	70	Mráček et al., 1994
S. diaprepesi	79 (71–90)	54 (45–61)	180 (150–200)	69 (59–79)	80 (68–86)	Nguyen and Duncan, 2002
S. ethiopense	73 69–77	49 46–57	164 154–175	67 63–70	57 54–61	Tamiru et al., 2012
S. glaseri	77 (62–90)	46 (40–50)	183	60	91	Poinar, 1990
S. guangdonense	86 (80–94)	64 (47–73)	175 (152–216)	75 (59–82)	70 (67–78)	Qiu et al., 2004
S. hermaphroditum	68 (65–70)	48 (47–50)	150 (140–160)	71 (70–75)	48 (44–50)	Stock et al., 2004
S. innovationi	81 (74–91)	58 (54–63)	140 (110–190)	70 (50–100)	82 (67–95)	Çimen et al., 2015
S. jeffreyense	88 79–95	57 51–61	215 171–295	65 61–171	47 34–68	Malan et al., 2016
S. karii	83 (73–91)	57 (42–64)	151 N/A	70 N/A	70 (50–80)	Waturu et al., 1997
S. khoisanae	85 (70–88)	56 (50–63)	203 (167–2.7)	70 (60–80)	85 (71–99)	Nguyen et al., 2006
S. khuongi	78.5 (64–99)	55 (50–62)	120 (100–150)	60 (50–70)	68 (60–82.5)	Stock et al., 2018
S. lamjuyense	87 81–94	57 50–66	173 151–193	65 56–77	61 44–91	Khatri-Chhetri et al., 2011
S. longicaudum	77	48	160	62	62	Stock et al., 2001
S. phyllophagae	72 65–77	51 46–56	187 136–223	71 61–77	75 68–80	Nguyen and Buss, 2011
S. *scarabaei*	75	44	170	60	66	Stock and Koppenhöfer, 2003

* Abbreviations: SL: spicule length; GL: gubernaculum length; SW%: percentage of ratio between spicule length to anal body diameter; GS%: percentage of ratio between gubernaculum length to spicule length; D%: percentage of ratio between anterior ends to excretory pore to anterior end to esophagus.

Type host: The natural host of this novel species is unknown.

Specimens deposited: Six females (allotype and paratypes), 6 males (holotype and paratypes), and 6 infective juveniles (IJs) were deposited in the nematode collection at the University of California–Davis, Davis, California. The accession number for the holotype male and allotype female is UCDNC 5260, for the paratype male is UCDNC 5261, for the paratype female is UCDNC 5262, and for the infective juvenile is UCDNC 5263.

Sequences deposited: ITS rDNA genes (OR050811) and D2/D3 domain of the 28S (OR050810) are deposited in GenBank.

ZooBank registration: urn:lsid:zoobank.org:act:B9B5622C-D2F9-4DA8-8249-1BD1336BBB8D.

Etymology: This species is named in honor of Dr. Byron J. Adams, acknowledging his extensive contributions to the study of the ecology and biology of entomopathogenic nematodes and our understanding of species.

Cross-hybridization results

Cross-hybridization assays between males and females of *S. adamsi* with *S. glaseri* and *S. diaprepesi* yielded no progeny. However, offspring were produced in all self-crossed species.

Molecular characterization and phylogenetic analysis of nematode

Sequences obtained from the *ITS* rDNA genes (OR050811) and D2/D3 domain of the 28S (OR050810) are deposited in GenBank. Based on the BLAST analysis in NCBI of ITS sequences of *S. adamsi*, we selected 81 different *Steinernema* species. In addition to the 82 steinernematids (81 plus *S. adamsi*), 3 outgroup species were included to construct phylogenetic trees. The phylogenetic tree inferred from the Bayesian analysis of ITS datasets is given in Figure 5. Based on the ITS phylogeny all the *Steinernema* nematodes were differentiated into 11 clades: '*Glaseri*' (11 species), '*Karii*' (7), '*Longicaudatum*' (6), '*Khoisanae*' (4), '*Costaricense*' (2), '*Feltiae*' (16), '*Kushidai*' (2), '*Monticolum*' (5), '*Carpocapsae*' (13), '*Bicornutum*' (6), '*Affine*' (7), and 3 monospecies clades for *Steinernema unicornum*, *Steinernema neocurtillae*, and *Steinernema rarum*. The phylogenetic tree inferred from the Bayesian analysis of 28S data sets is represented in Figure 6. All the *Steinernema* species were divided into 11 clades similar to those of the ITS phylogeny except for 1 additional *Cameroonense* clade (3 species) instead of the *Costaricense* clade. The placement of the new species was consistent with the ITS phylogeny in the *Longicaudatum* clade with the posterior possibility (77%) (Fig. 6). For the concatenated phylogeny, we used species represented in both ITS and D2D3 phylogenies. Bayesian analysis of the concatenated dataset of 48 species yielded a similar topology to that of the ITS and D2D3 phylogenies, placing *S. adamsi* firmly in *Longicaudatum* clade, with a very high posterior probability (100%) (Fig. 7).

Cryopreservation of *S. adamsi* n. sp.

The efficacy and survival of the frozen nematode stocks were tested on NGM plates 2 wk and 2 mo after the cryopreservation at −80 C. We were able to recover mixed-stage nematodes from both tubes, and these nematodes reproduced stably downstream.

Molecular characterization and phylogenetic analysis of symbiotic bacteria

The sequence obtained from the *16S* rDNA gene with accession number OR047834 is deposited in GenBank. The BLAST analysis of *16S* rDNA gene sequences revealed that the symbiotic bacterium is 99.7% similar to *Xenorhabdus griffiniae*. Based on the BLAST result, 25 different *Xenorhabdus* species were used to construct the phylogeny. The IQ-Tree program selected TIM2+F+I+G4 as the best-fitting model to construct a maximum-likelihood (ML) phylogenetic tree. Subsequently, a maximum likelihood phylogenetic tree for *Xenorhabdus* spp. isolated from *S. adamsi* n. sp. was generated based on a partial *16S* rDNA sequence (1,286 bp), in combination with *Xenorhabdus* strains from GenBank. *Morganella morganii* was used as an outgroup. The bootstrap values were computed based on 1,000 replicates, and the numbers displayed on the branches represent the support values for Maximum Likelihood clades above the 50% threshold (Fig. 8).

Diagnosis and relationships

Based on molecular, morphological, and morphometric traits, *S. adamsi* is a member of the '*Longicaudatum*-clade.' Nematodes of this group are characterized by having third-stage IJs between 832 mm to 1,055 mm long. *Steinernema adamsi*, a member of the '*Longicaudatum*-clade,' presents several traits common to this group. Morphologically, the IJs of *S. adamsi* can be recognized by having a medium body length of 879.66 (range: 774.69–956.96) μm, compared to other members of the same clade, *Steinernema lamjungense* 832 (690–950) μm being the smallest and *Steinernema guangdongense* 1,055 (987–1,145) μm being the largest. A similar pattern was observed for the body diameter with 34.96 (31.58–39.85) μm, with other members *S. lamjungense* 29 (23–31) μm being the smallest and *S. guangdongense* 42 (30–48) μm as the largest. Within the *Longicaudatum* clade, the new species shares some of the hallmark morphological traits. However, the new species can be differentiated from closely related taxa by several morphometric and morphological differences between the IJs and males (Tables III, IV). Based on the ITS phylogeny, the novel species is closely related to *S. guangdongense*. However, morphologically the third-stage juveniles of new species are much shorter (average 879.66 μm vs. 1,055 μm). Based on the 28S phylogeny, the new species is placed together with *Steinernema hermaphroditum*. Morphologically, *S. hermaphroditum* IJs are larger compared to the new species (average 1,043 μm vs. 879.66 μm). Other morphological traits such as excretory pores of *S. adamsi* are more anteriorly located than that of *S. guangdongense* and posteriorly located compared to that of *S. hermaphroditum* (average 69.84 μm vs. average 80 μm vs. average: 65 μm). The closely related species also differ in the values of 'c' (ratio of total body length to the tail length) with an average of 12.08 in *S. adamsi*, 11.6 in *S. guangdongense*, and 10.6 in *S. hermaphroditum*. Similarly, other morphometric values for IJs also differed between closely related species such as 'D%' (the proportion of the length from the anterior end to the excretory pore to the length from the anterior end to the esophagus; 67.87 in *S. adamsi* vs. 59 *S. guangdongense* and 50 in *S. hermaphroditum*) and 'E%' (the proportion of the length from the anterior end to the excretory pore to the tail length; 96.24 in *S. adamsi* vs. 88 *S. guangdongense* and 85 in

S. hermaphroditum). Additionally, IJs of the novel species differ from *S. guangdongense*, *S. hermaphroditum*, *S. longicaudum*, and *S. lamjungense* by having a shorter tail (average: 72.91 μm). The new species can be recognized further by the morphological characteristics of males. The spicules of *S. adamsi* are shorter, i.e., an average of 61.53 μm vs. an average of 68 μm in *S. hermaphroditum* and an average of 86 μm in *S. guangdongense*. Similarly, gubernaculum length also varied between 3 closely relative species (37.01μm in *S. adamsi* vs. 64 μm in *S. guangdongense*. vs. 48 μm in *S. hermaphroditum*), the values of the 'SW%' (the proportion of spicule length to the anal body diameter) (average: 126.79 in *S. adamsi* vs. 150 in *S. hermaphroditum* and 175 in *S. guangdongense*), and 'D%' (the proportion of the length from the anterior end to the excretory pore to the length from the anterior end to the esophagus) were different 68.23 in *S. adamsi* vs. 48 in *S. hermaphroditum* vs. 70 in *S. guangdongense*. *Steinernema adamsi* can be distinguished from other related species by morphometrical characteristics listed in Tables III and IV.

In this paper we focus more on the molecular characterization of the nematodes than morphological and morphometric characterization. The addition of detailed morphological features may provide more support for the classification of steinernematid species into different clades; however, support for the distinction between 2 closely related nematodes inferred from the molecular data does not significantly differ from morphological and morphometric characteristics (Spiridonov et al., 2004). Some morphological features are shared between nematodes of different clades; for example, most nematodes within the clades *Bicornutum, Glaseri, Khoisane*, and *Longicaudatum* have a lateral field with 8 ridges of equal height and width, which might create confusion during the species differentiation between these congeners (Spiridonov and Subbotin, 2016). Another morphological feature that is used to differentiate males of steinernematids is the number and distribution of the genital papillae. This morphological feature varies even between specimens of the same species, whereas, most of the time, steinernematids share a uniform number of papillae, i.e., 11 pairs of papillae, and 1 midventral precloacal papilla. Thus, the number of papillae (22 + 1) is a shared characteristic of most steinernematids, providing little help in differentiating species between different clades (Spiridonov and Subbotin, 2016).

In this study we identified the native symbiont of *S. adamsi*. Based on the 16S sequence results; the bacteria are identified as *Xenorhabdus* spp. highly similar to *Xenorhabdus griffiniae*. Bacterium *X. griffiniae* is also found in a symbiotic relationship with other species, including *S. hermaphroditum* and *Steinernema khoisanae* (Mothupi et al., 2015; Cao et al., 2022). Despite having high similarity to the species *X. griffiniae*, *Xenorhabdus* spp. isolated from *S. adamsi* grown on NGM have a greenish hue, which is not true of other *X. griffiniae* isolates. In this study, we did not investigate this further, but it seems to be a novel characteristic of this strain of bacteria that warrants further investigation.

Entomopathogenic nematodes parasitize insects and are used in biological control programs against insect pests in agriculture. The use of chemical pesticides for insect control has negative impacts on both human health and the environment. Using EPNs in pest management selectively targets insects, reducing harmful effects on human health, non-target creatures, and the environment (Piedra-Buena et al., 2015). Some species of EPNs have a broad host range such as *Steinernema carpocapsae*, which can infect approximately 200 insects across 10 orders, and some species have a specific host range such as *Steinernema scapterisci*, which is adapted to infect insects within the order Orthoptera (Koppenhöfer et al., 2020). However, the host range of the majority of EPNs is largely unknown (Peters, 1996; Shapiro-Ilan et al., 2017). In laboratory assays, nematodes may have a wider than normal host range that does not translate to the field. However, species that are isolated from the natural environment are well adapted to those environmental conditions and are effective against existing pests (Campos-Herrera et al., 2016). The host range of *S. adamsi* has not been explored yet, so future studies should focus on studying the host range of this novel species to identify the potential host to improve the efficacy of EPNs in biological control. Most of the EPNs that are isolated from the environment are reared in the waxworm *Galleria mellonella*, as the waxworm is highly susceptible to EPNs, is commercially available, and is easy to maintain. However, it has been reported that continuous lab rearing results in the trait deterioration for many valuable traits of EPNs such as infectivity, virulence, longevity, reproductive capacity, heat tolerance, and sex ratio (Gaugler and Campbell, 1991; Hopper et al., 1993; Shapiro et al., 1996; Bai et al., 2005; Bilgrami et al., 2006; Chaston et al., 2011; Stuart and Gaugler, 2011). Though deterioration of biocontrol traits can occur in a laboratory or commercial conditions due to repeated culturing, strains can be stabilized through cryopreservation or by establishing single or multiple purebred homozygous lines (Bai et al., 2005; Anbesse et al., 2013). In this study we were able to successfully modify the cryopreservation technique for novel species and conserve their inherent pathogenic trait.

To summarize, the results obtained from molecular analyses using ITS rDNA, D2D3 of the *28S* rDNA gene sequences, and morphological and morphometric analyses provide evidence supporting the designation of *S. adamsi* as a new species of *Steinernema* based on the morphological, phylogenetic, and evolutionary species concept (Adams, 1998). This species possesses distinctive characteristics and is well suited to the environmental conditions of Thailand, where it was isolated. However, additional research is necessary to assess the host range of this newly identified species and enhance the efficiency of EPNs in biological control.

ACKNOWLEDGMENTS

We are grateful to members of the Vitta lab who helped isolate and rear this nematode. This research was supported by the United States Department of Agriculture, National Institute of Food and Agriculture (USDA NIFA) Multistate project W5185. This work was partially supported by the Royal Golden Jubilee Ph.D. Program (PHD/0086/2561), through the National Research Council of Thailand (formerly the Thailand Research Fund).

LITERATURE CITED

Adams, B. J. 1998. Species concepts and the evolutionary paradigm in modem nematology. Journal of Nematology 30: 1–21.

Adams, B. J., A. Fodor, H. S. Koppenhöfer, E. Stackebrandt, S. P. Stock, and M. G. Klein. 2006. Biodiversity and

systematics of nematode-bacterium entomopathogens. Biological Control 37: 32–49. doi:10.1016/j.biocontrol.2005.11.008.

Agarwala, R., T. Barrett, J. Beck, D. A. Benson, C. Bollin, E. Bolton, D. Bourexis, J. R. Brister, S. H. Bryant, K. Canese, et al. 2017. Database resources of the National Center for Biotechnology Information. Nucleic Acids Research 45: D12–D17. doi:10.1093/nar/gkw1071.

Anbesse, S., N. H. Sumaya, A. V. Dörfler, O. Strauch, and R. U. Ehlers. 2013. Stabilisation of heat tolerance traits in *Heterorhabditis bacteriophora* through selective breeding and creation of inbred lines in liquid culture. BioControl 58: 85–93. doi:10.1007/s10526-012-9467-x.

Artyukhovsky, A. K., E. M. Kozodoi, A. P. Reid, and S. E. Spiridonov. 1997. Redescription of *Steinernema arenarium* (Artyukhovsky, 1967) topotypes from Central Russia and a proposal for *S. anomalae* (Kozodoi, 1984) as a junior. Russian Journal of Nematology 5: 31–37.

Bai, C., D. I. Shapiro-Ilan, R. Gaugler, and K. R. Hopper. 2005. Stabilization of beneficial traits in *Heterorhabditis bacteriophora* through creation of inbred lines. Biological Control 32: 220–227. doi:10.1016/j.biocontrol.2004.09.011.

Bedding, R., and R. Akhurst. 1975. A simple technique for the detection of insect parasitic Rhabditid nematodes in soil. Nematologica 21: 109–110. doi:10.1163/187529275X00419.

Bilgrami, A. L., R. Gaugler, D. I. Shapiro-Ilan, and B. J. Adams. 2006. Source of trait deterioration in entomopathogenic nematodes *Heterorhabditis bacteriophora* and *Steinernema carpocapsae* during *in vivo* culture. Nematology 8: 397–409. doi:10.1163/156854106778493394.

Campos-Herrera, R., F. E. El-Borai, J. A. Rodríguez Martín, and L. W. Duncan. 2016. Entomopathogenic nematode food web assemblages in Florida natural areas. Soil Biology and Biochemistry 93: 105–114. doi:10.1016/j.soilbio.2015.10.022.

Cao, M., H. T. Schwartz, C. H. Tan, and P. W. Sternberg. 2022. The entomopathogenic nematode *Steinernema hermaphroditum* is a self-fertilizing hermaphrodite and a genetically tractable system for the study of parasitic and mutualistic symbiosis. Genetics 220: 1. doi:10.1093/GENETICS/IYAB170.

Chaston, J. M., A. R. Dillman, D. I. Shapiro-Ilan, A. L. Bilgrami, R. Gaugler, K. R. Hopper, and B. J. Adams. 2011. Outcrossing and crossbreeding recovers deteriorated traits in laboratory cultured *Steinernema carpocapsae* nematodes. International Journal for Parasitology 41: 801–809. doi:10.1016/j.ijpara.2011.02.005.

Cimen, H., M. Lee, J. Hatting, and S. Hazir. 2015. *Steinernema innovationi* n. sp. (Panagrolaimomorpha: Steinernematidae), a new entomopathogenic nematode species from South Africa. Journal of Helminthology 89: 415–427. doi:10.1017/s0022149X14000182.

Dillman, A. R., J. M. Chaston, B. J. Adams, T. A. Ciche, H. Goodrich-Blair, S. P. Stock, and P. W. Sternberg. 2012. An entomopathogenic nematode by any other name. PLoS Pathogens 8: e1002527. doi:10.1371/journal.ppat.1002527.

Dreyer, J., A. P. Malan, and L. M. T. Dicks. 2018. First report of a symbiotic relationship between *Xenorhabdus griffiniae* and an unknown *Steinernema* from South Africa. Archives of Microbiology 200: 349–353. doi:10.1007/s00203-017-1452-4.

Drummond, A. J., and A. Rambaut. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7: 214. doi:10.1186/1471-2148-7-214.

Gaugler, R., and J. F. Campbell. 1991. Selection for enhanced host-finding of scarab larvae (Coleoptera: Scarabaeidae) in an entomopathogenic nematode. Environmental Entomology 20: 700–706. doi:10.1093/ee/20.2.700.

Hopper, K. R., R. T. Roush, and W. Powell. 1993. Management of genetics of biological-control introductions. Annual Review of Entomology 38: 27–51. doi:10.1146/annurev.en.38.010193.000331.

Kaya, H., and S. Stock. 1997. Techniques in insect nematology. *In* Manual of Techniques in Insect Pathology, L. A. Lacey (ed.). Academic Press, San Diego, California, p. 281–394.

Khatri-Chhetri, H. B., L. Waeyenberge, S. Spiridonov, H. K. Manandhar, and M. Moens. 2011. *Steinernema lamjungense* n. sp. (Rhabditida: Steinernematidae), a new species of entomopathogenic nematode from Lamjung district, Nepal. Nematology 13: 589–605. doi:10.1163/138855410X533644.

Koppenhöfer, A. M., D. I. Shapiro-Ilan, and I. Hiltpold. 2020. Entomopathogenic nematodes in sustainable food production. Frontiers in Sustainable Food Systems 4: 125. doi:10.3389/fsufs.2020.00125.

Lee, M. M., M. Sicard, M. Skeie, and S. P. Stock. 2009. *Steinernema boemarei* n. sp. (Nematoda: Steinernematidae), a new entomopathogenic nematode from southern France. Systematic Parasitology 72: 127–141. doi:10.1007/s11230-008-9166-2.

Lis, M., E. Sajnaga, M. Skowronek, A. Wiater, K. Rachwal, and W. Kazimierczak. 2021. *Steinernema sandneri* n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from Poland. Journal of Nematology 53: 1–24. doi:10.21307/JOFNEM-2021-051.

Malan, A., R. Knoetze, and L. Tiedt. 2016. *Steinernema jeffreyense* n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from South Africa. Journal of Helminthology 90: 262–278. doi:10.1017/S0022149X15000097.

Mothupi, B., J. Featherston, and V. Gray. 2015. Draft whole-genome sequence and annotation of *Xenorhabdus griffiniae* strain BMMCB associated with the south African entomopathogenic nematode *Steinernema khoisanae* strain BMMCB. Genome Announcements 3: e00785-15. doi:10.1128/genomea.00785-15.

Mráček, Z., E. Hernández, and N. B. Pathology. 1994. *Steinernema cubana* sp. n. (Nematoda: Rhabditida: Steinernematidae) and the preliminary characterization of its associated bacterium. Journal of Invertebrate Pathology 64: 123–129. doi:10.1006/jipa.1994.1080.

Nguyen, K., and E. Buss. 2011. *Steinernema phyllophagae* n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from Florida, USA. Nematology 13: 425–442. doi:10.1163/138855410X520927.

Nguyen, K. B., and L. W. Duncan. 2002. *Steinernema diaprepesi* n. sp. (Rhabditida: Steinernematidae), a parasite of the citrus root weevil *Diaprepes abbreviatus* (L) (Coleoptera: Curculionidae). Journal of Nematology 34: 159–170.

Nguyen, K. B., C. M. A. Ginarte, and L. Leite. 2010. *Steinernema brazilense* n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from Mato Grosso, Brazil. Journal of Invertebrate Pathology 103: 8–20.

Nguyen, K. B., A. P. Malan, and U. Gozel. 2006. *Steinernema khoisanae* n. sp. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from South Africa. Nematology 8: 157–175. doi:10.1163/156854106777998728.

Nguyen, L. T., H. A. Schmidt, A. von Haeseler, and B. Q. Minh. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Molecular Biology and Evolution 32: 268–274. doi:10.1093/molbev/msu300.

Parkman, J. P., and G. C. Smart. 1996. Entomopathogenic nematodes, a case study: Introduction of *Steinernema scapterisci* in Florida. Biocontrol Science and Technology 6: 413–420. doi:10.1080/09583159631389.

Peters, A. 1996. The natural host range of *Steinernema* and *Heterorhabditis* spp. and their impact on insect populations. Biocontrol Science and Technology 6: 389–402. doi:10.1080/09583159631361.

Piedra-Buena, A., J. López-Cepero, and R. Campos-Herrera. 2015. Entomopathogenic nematode production and application: Regulation, ecological impact and non–target effects. *In* Nematode Pathogenesis of Insects and Other Pests: Ecology and Applied Technologies for Sustainable Plant and Crop Protection, R. Campos-Herrera (ed.). Springer International Publishing, New York, New York, p. 255–282. doi:10.1007/978-3-319-18266-7_10.

Poinar, G. O. 1990. Biology and taxonomy of Steinernematidae and Heterorhabditidae. *In* Entomopathogenic Nematodes in Biological Control, R. Gaugler and H. K. Kaya (eds.). CRC Press, Boca Raton, Florida, p. 23–31.

Poullet, N., and C. Braendle. 2015. Sampling and isolation of *C. elegans* from the natural habitat. *In C. elegans* Methods in Molecular Biology, D. Biron, and G. Haspel (eds.). Humana Press, Totowa, New Jersey, p. 221–229. doi:10.1007/978-1-4939-2842-2_16.

Qiu, L., Y. Fang, Y. Zhou, Y. Pang, and K. B. Nguyen. 2004. *Steinernema guangdongense* sp. n. (Nematoda: Steinernematidae), a new entomopathogenic nematode from southern China with a note on *S. serratum* (nomen nudum). Zootaxa 704: 1–20. doi:10.11646/zootaxa.704.1.1.

Qiu, L., X. Yan, Y. Zhou, K. Nguyen, and Y. Pang. 2005. *Steinernema aciari* sp. n. (Nematoda: Steinernematidae), a new entomopathogenic nematode from Guangdong, China. Journal of Invertebrate Pathology 88: 58–69.

Rani, L., K. Thapa, N. Kanojia, N. Sharma, S. Singh, A. S. Grewal, A. L. Srivastav, and J. Kaushal. 2021. An extensive review on the consequences of chemical pesticides on human health and environment. Journal of Cleaner Production 283: 124657. doi:10.1016/j.jclepro.2020.124657.

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

Shapiro, D. I., I. Glazer, and D. Segal. 1996. Trait stability and fitness of the heat tolerant entomopathogenic nematode *Heterorhabditis bacteriophora* IS5 strain. Biological Control 6: 238–244. doi:10.1006/bcon.1996.0030.

Shapiro-Ilan, D. I., I. Hiltpold, and E. E. Lewis. 2017. Nematodes. *In* Ecology of Invertebrate Diseases, A. E. Hajek, and D. I. Shapiro-Ilan (eds.). John Wiley & Sons, Chichester, U.K., p. 415–440. doi:10.1002/9781119256106.CH11.

Shapiro-Ilan, D. I., L. G. Leite, and R. Han. 2023. Production of entomopathogenic nematodes. *In* Mass Production of Beneficial Organisms: Invertebrates and Entomopathogens, J. Morales-Ramos, M. G. Rojas, and D. Shapiro-Ilan (eds.). Academic Press, London, U.K., p. 293–315. doi:10.1016/B978-0-12-822106-8.00005-1.

Spiridonov, S. E., A. P. Reid, K. Podrucka, S. A. Subbotin, and M. Moens. 2004. Phylogenetic relationships within the genus *Steinernema* (Nematoda: Rhabditida) as inferred from analyses of sequences of the ITS1-5.8S-ITS2 region of rDNA and morphological features. Nematology 6: 547–566. doi:10.1163/1568541042665304.

Spiridonov, S. E., and S. A. Subbotin. 2016. Phylogeny and phylogeography of *Heterorhabditis* and *Steinernema*. *In* Advances in Entomopathogenic Nematode Taxonomy and Phylogeny, D. J. Hunt and K. B. Nguyen (eds.). Brill, Leiden, The Netherlands, p. 413–427. doi:10.1163/9789004285347.

Stock, S. P., and H. G. Blair. 2008. Entomopathogenic nematodes and their bacterial symbionts: The inside out of a mutualistic association. Symbiosis 46: 65–75.

Stock, S. P., R. Campos-Herrera, F. E. El-Borai, and L. W. Duncan. 2018. *Steinernema khuongi* n. sp. (Panagrolaimomorpha, Steinernematidae), a new entomopathogenic nematode species from Florida, USA. Journal of Helminthology 93: 226–241. doi:10.1017/S0022149X18000081.

Stock, S. P., C. T. Griffin, and R. Chaerani. 2004. Morphological and molecular characterisation of *Steinernema hermaphroditum* n. sp. (Nematoda: Steinernematidae), an entomopathogenic nematode from Indonesia. Nematology 6: 401–412. doi:10.1163/1568541042360555.

Stock, S. P., J. Heng, D. J. Hunt, A. P. Reid, X. Shen, and H. Y. Choo. 2001. Redescription of *Steinernema longicaudum* Shen & Wang (Nematoda: Steinernematidae); geographic distribution and phenotypic variation between allopatric populations. Journal of Helminthology 75: 81–92. doi:10.1079/joh200036.

Stock, S. P., and A. Koppenhöfer. 2003. *Steinernema scarabaei* n. sp. (Rhabditida: Steinernematidae), a natural pathogen of scarab beetle larvae (Coleoptera: Scarabaeidae) from New Jersey, USA. Nematology 5: 191–204.

Stuart, R. J., M. E. Barbercheck, P. S. Grewal, R. A. J. Taylor, and C. W. Hoy. 2006. Population biology of entomopathogenic nematodes: Concepts, issues, and models. Biological Control 38: 80–102. doi:10.1016/j.biocontrol.2005.09.019.

Stuart, R. J., F. E. El-Borai, and L. W. Duncan. 2008. From augmentation to conservation of entomopathogenic nematodes: Trophic cascades, habitat manipulation and enhanced biological control of *Diaprepes abbreviatus* root weevils in Florida citrus groves. Journal of Nematology 40: 73–84.

Stuart, R. J., and R. Gaugler. 2011. Genetic adaptation and founder effect in laboratory populations of the entomopathogenic nematode *Steinernema glaseri*. Canadian Journal of Zoology 74: 164–170. doi:10.1139/z96-021.

Tamiru, T., L. Waeyenberge, T. Hailu, R. U. Ehlers, and V. Půža. 2012. *Steinernema ethiopiense* sp. n. (Rhabditida: Steinernematidae), a new entomopathogenic nematode from Ethiopia. Nematology 14: 741–757.

Tamura, K., G. Stecher, and S. Kumar. 2021. MEGA11: Molecular evolutionary genetics analysis Version 11. Molecular Biology and Evolution 38: 3022–3027. doi:10.1093/molbev/msab120.

Tian, C. L., F. Zhu, X. Y. Li, J. H. Zhang, V. Půža, D. Shapiro-Ilan, D. Zhao, J. W. Liu, J. J. Zhou, Y. Ding, et al. 2022. *Steinernema populi* n. sp. (Panagrolaimomorpha, Steinernematidae), a new entomopathogenic nematode species from China. Journal of Helminthology 96: e57. doi:10.1017/S0022149X22000426.

Uribe-Lorío, L., M. Mora, and S. P. Stock. 2007. *Steinernema costaricense* n. sp. and *S. puntauvense* n. sp. (Rhabditida: Steinernematidae), two new entomopathogenic nematodes from Costa Rica. Systematic Parasitology 68: 167–182. doi:10.1007/S11230-007-9098-2.

Vrain, T. C., D. A. Wakarchuk, A. C. Lévesque, and R. I. Hamilton. 1992. Intraspecific rDNA restriction fragment length polymorphism in the *Xiphinema americanum* group. Fundamental and Applied Nematology 15: 563–573.

Waturu, C. N., D. J. Hunt, and A. P. Reid. 1997. *Steinernema karii* sp. n. (Nematoda: Steinernematidae), a new entomopathogenic nematode from Kenya. International Journal of Nematology 7: 68–75. https://www.cabdirect.org/cabdirect/abstract/19971701143.

White, G. F. 1927. A method for obtaining infective nematode larvae from cultures. Science 66: 302–303. doi:10.1126/science.66.1709.302.b.