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Xenorhabdus innovationi sp. nov., associated with the entomopathogenic nematode *Steinernema innovationi* from South Africa

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Summary – The symbiotic bacterium, referred to as *Xenorhabdus innovationi* sp. nov. strain SGI-60 (synonymous to S.inno), was isolated from *Steinernema innovationi* SGI-60, found in grain-field soil samples from Bethlehem, South Africa. Identification was done *via* whole-genome phylogenomic analyses, core genome sequence identity metrics, and detailed phenotypic traits. Strain SGI-60 is Gram-negative, rod-shaped, with an average size of 1.34 × 0.38 μm, produces lecithinase and lipase, and lacks catalase and oxidase activity. Phylogenetic reconstructions based on 16S rRNA gene sequences and whole-genome sequences showed that strain SGI-60 is closely related to *X. bakwenae* SF857^{T}, *X. griffiniae* Kalro, *X. ehlersii* DSM 16337^{T}, *X. thailandensis* CCN3.3^{T} and *X. taiwanensis* TCT-1^{T}. Digital DNA-DNA hybridisation (dDDH) and average nucleotide identity (ANI) values shared between strain SGI-60 and closely related species are below the prokaryotic species delineation threshold of 70 and 95%, respectively. Strain SGI-60 was identified as a member of the genus *Xenorhabdus* and was classified as *X. innovationi* sp. nov. Juveniles of *S. innovationi* cultured *in vitro* with strain SGI-60, and used to infect last-instar false codling moth larvae (*Thaumatotibia leucotreta*), killed 64% of the larvae within 48 h. This suggests that the *S. innovationi*–*X. innovationi* SGI-60 complex may be used as a biological control agent in citrus and grapevine. *X. innovationi* is a novel species within the genus *Xenorhabdus* of the family Morganellaceae, class Gammaproteobacteria. Only one isolate with these phenotypic and genotypic characteristics was isolated and strain SGI-60 is proposed as the type.

Keywords – *Steinernema innovationi*, symbiotic bacteria, virulence, whole-genome sequencing, *Xenorhabdus innovationi* sp. nov.

Species of the genus *Xenorhabdus*, classified within the family Morganellaceae and order Enterobacterales, engage in a facultative to obligate symbiotic association with entomopathogenic nematodes (EPN) of the *Steinernema* genus. The *Steinernema*-*Xenorhabdus* association is vital for the EPN virulence and reproductive fitness, as these soil-dwelling nematodes rely on their bacterial symbiont to help kill and pre-digest insects and other small arthropods (San-Blas *et al.*, 2024). Cells of *Xenorhabdus* are housed in a specialised intracellular structure within the nematode, referred to as the bacterial receptacle, a specialised region of the anterior intestine located just below the esophagointestinal junction. The receptacle is not considered an intracellular structure, but rather a specialised luminal pouch or vesicle in the lumen. The bacteria are released into the hemocoel of the insect upon infection. Following release, both the nematode and its bacterial symbiont produce a diverse array of secondary metabo-

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lites with antimicrobial and insecticidal properties, resulting in host mortality through septicaemia (Lu *et al.*, 2017; Dreyer *et al.*, 2018, 2019). These bioactive compounds also serve to protect the insect cadaver by preventing colonisation by competing microorganisms or secondary insect invaders. The resulting insect biomass is enzymatically degraded and utilised as a nutrient source to support nematode and bacterial proliferation. Upon depletion of the host-derived nutrients, newly developed infective juveniles (IJ) act as vectors for the bacterial symbionts which are dispersed into the soil to infect new insect hosts (Stock *et al.*, 2017).

Steinernema was first identified in 1923 as *Aplectana krausei* (now *Steinernema kraussei*), with the genus name *Steinernema* being proposed by Travassos in 1927 (Steiner, 1923; Travassos, 1927). The genus initially consisted of five species "groups" which were based on body length of the IJ (Nguyen *et al.*, 2007), and were subdivided into 12 multispecies clades: '*Affine*', '*Bicornutum*', '*Cameroonense*', '*Carpocapsae*', '*Costaricense*', '*Feltiae*', '*Glaseri*', '*Karii*', '*Khoisanae*', '*Kushidai*', '*Longicaudum*' and '*Monticola*', and three monospecies clades: *Steinernema neocurtillae*, *Steinernema unicornum* and *Steinernema rarum* (Spiridonov & Subbotin, 2016; Půža *et al.*, 2025). By the end of 2024, 114 valid *Steinernema* spp. were described, many from African soil. *Steinernema innovationi* Çimen, Lee, Hatting, Hazir & Stock, 2014 (isolate SGI-60) from South Africa belongs to the *Khoisanae*-clade, referred to as the all-African clade, as all the members of the clade were isolated from Africa, specifically South Africa (Çimen *et al.*, 2015; Půža *et al.*, 2025).

Thomas & Poinar (1979) proposed the genus *Xenorhabdus*, which is currently comprised of 38 species and one subspecies, *X. bovienii* subsp. *africana* (National Center for Biotechnology Information (NCBI), Taxonomy: *Xenorhabdus*, 1996; Machado *et al.*, 2023). Advanced techniques used in whole-genome sequencing clearly separated species within the genus and identified strains with low genomic relatedness. Phylogenomic analyses are typically based on core-genome sequences using various software tools (Půža *et al.*, 2024). Of the 38 species, 33 have a valid publication, following the guidelines of the International Journal of Systematic and Evolutionary Microbiology (IJEM), and therefore effectively published names. *Xenorhabdus bakwenae* (Ritter *et al.*, 2023), *X. anantnagensis* (Machado *et al.*, 2023), *X. littoralis*, *X. santafensis* (Palma *et al.*, 2024) and *X. taiwanensis* (Tseng *et al.*, 2024) are effectively published and in the validation process (Oren and Göker, 2024; Oren and Göker, 2025).

EPN are successfully used to control native and invasive agricultural pests such as false codling moth and codling moth (Malan & Moore, 2016; Lacey *et al.*, 2024). The bacterial symbionts of these EPN are well-known for their antimicrobial, insecticidal, and nematocidal properties. Moreover, peptides produced by *Xenorhabdus* spp. displayed antitumor activity (Zhang *et al.*, 2024). As such, research describing new *Xenorhabdus* species and evaluating their biological activities is important for addressing current challenges, including antimicrobial resistance and the need for effective biocontrol agents (Sajnaga *et al.*, 2024).

Strain SGI-60, isolated from *S. innovationi*, belongs to a new species, *X. innovation* sp. nov. strain SGI-60 is proposed as the type. *Xenorhabdus innovation* sp. nov. SGI-60 may be used to control false codling moth (*Thaumatotibia leucotreta*).

Materials and methods

Origin of nematodes

Steinernema innovationi SGI-60 was isolated from grain-field soil samples at the campus of the Agricultural Research Council-Small Grain (28°9′55.12″S, 28°18′32.97″E) in Bethlehem, Free State province, South Africa (Hatting *et al.*, 2009; Çimen *et al.*, 2015). *Steinernema jeffreyense* Malan, Knoetze & Tiedt, 2015 (isolate J194) was isolated from soil underneath a guava tree on the coastline of Jeffreys Bay, South Africa (Malan *et al.*, 2016), and *Steinernema khoisanae* Nguyen, Malan & Gozel, 2006 (isolate I10) from soil sampled in the JS Marais Park, Stellenbosch, South Africa (Nthenga *et al.*, 2014).

Isolation of bacteria

Galleria mellonella L. (Lepidoptera: Pyralidae) larvae were infected with IJ of *S. innovationi* (Ritter *et al.*, 2023). After 18 h, while the larvae were still alive, they were sprayed with 99% ethanol, dried in a laminar flow cabinet, and a drop of haemolymph, obtained by puncturing a proleg of the insect, and streaked onto NBTA plates (8.0 g nutrient broth; 15.0 g agar; 0.25 g bromothymol blue; 1 l distilled H_2O and 0.04 g triphenyl tretrazolium chloride) and incubated for 24 h at 28°C. A single blue bacterial colony, representing the genus *Xenorhabdus*,

was selected and inoculated into 30 ml Tryptic Soy Broth (TSB). Incubation was at 28°C for 48 h on an orbital shaker (140 rpm). Cells were streaked to purity on TSB agar and labelled as strain SGI-60. Cells were preserved in TSB, supplemented with glycerol (15%, v/v, final concentration) and stored at −80°C.

Pathogenic bacterial cultures

Listeria monocytogenes ATCC 7644, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 were obtained from the Dicks' laboratory (Stellenbosch University, Stellenbosch, South Africa) glycerol stocks. The bacteria were incubated at 37°C. All the bacteria were cultured on Brain Heart Infusion (BHI) agar or broth (Biolab Diagnostics, Gauteng, South Africa). For the agar-well diffusion assay, a single pure colony was inoculated into a 5 ml BHI test tube and incubated at 37°C overnight. To inoculate the BHI agar used for the assay, 1% of overnight culture was used (Dreyer *et al.*, 2019).

Production of cell-free supernatant

Single colonies of strain SGI-60 were streaked onto NBTA plates, consisting of nutrient agar supplemented with 0.025% (w/v) bromothymol blue and 0.004% (w/v) triphenyl tetrazolium chloride (TTC) and incubated for a minimum of 48 h at 30°C. After incubation, a single blue colony, representing secondary metabolite production, was isolated, and inoculated into 5 ml TSB. Inoculated test tubes were placed on an orbital wheel (100 rpm) for 48 h at 30°C. To obtain the cell-free supernatant (CFS), the bacterial culture was transferred to multiple 50-ml Falcon tubes (ISOLAB, Germany) and centrifuged at 11 000 g for 20 min at 4°C. The supernatant was transferred to new 50-ml Falcon tubes, leaving the cell pellet behind to be discarded. The tubes were centrifuged again at 11 000 g for 20 min at 4°C. The centrifuged supernatant was filtered through a 0.22 μm cellulose acetate membrane filter placed on a vacuum filter. An aliquot of the filtrated CFS was streaked onto NBTA agar plates to verify the absence of bacterial cells.

Genomic and phylogenetic characterisation

Genomic DNA was extracted from strain SGI-60, using the ZymoBIOMICS™ DNA Miniprep Kit (Zymo Research) according to the manufacturer's instructions. The Rapid Sequencing DNA V14 (SQK-RBK114.24) barcoding kit (Oxford Nanopore Technologies, ONT) was used to construct a DNA library. DNA sequencing was performed using the ONT MinION Mk1B (MN40899), R9.4.1 Flongle flow cell, Flongle Sequencing Expansion Kit (EXP-FSE001) and MinKNOW v22.12.7 software. High-accuracy base calling was performed by Guppy v6.4.6 (ONT). Adapter sequences were removed with Porechop 0.2.4 (https://github.com/rrwick/Porechop). Short reads with a low-quality score length were removed by using Nanofilt (De Coster *et al.*, 2018). The resulting reads were assembled into a genome using the Bacterial Genomes pipeline v1.4.0 on EPI2ME v5.2.1.0. *De-novo* assembly was performed with Flye 2.9.5-b1801 (Kolmogorov *et al.*, 2020), errors were corrected using Medaka 2.0.0, and annotation was done using Prokka 1.14.5 (Seemann, 2014). A phylogenetic tree was constructed from genome sequences (Fig. 1). Accession numbers of *Xenorhabdus* spp. included in this study are listed in Supplementary Table S1.

A 16S rRNA phylogenetic tree was constructed using MEGA12 (Kumar *et al.*, 2024). The 16S rRNA sequences of strain SGI-60 and available type strains of *Xenorhabdus* spp. (Supplementary Table S1) were aligned using MUSCLE (Edgar, 2004). The phylogeny was inferred using the Maximum Likelihood method and General Time Reversible model (Nei & Kumar, 2000) of nucleotide substitutions, using the tree with the highest log-likelihood. Type strains from the species sharing the highest 16S rDNA sequence relatedness to strain SGI-60 (Supplementary Fig. S1 in the Apendix) were subjected to a nucleotide Basic Local Alignment Search Tool (BLAST; Altschul *et al.*, 1990).

The whole genome of strain SGI-60 and the reference genomes of *Xenorhabdus* species (Supplementary Table S1) were uploaded to the Type (Strain) Genome Server (TYGS, https://tygs.dsmz.de), for a whole genome-based taxonomic analysis (Meier-Kolthoff & Göker, 2019; Meier-Kolthoff *et al.*, 2022). The genome phylogenetic tree was inferred with FastME 2.1.6.1 (Lefort *et al.*, 2015) from the Genome BLAST Distance Phylogeny (GBDP) distances calculated from genome sequences. The branch lengths are scaled in terms of GBDP distance formula d_5. The numbers above the branches are GBDP pseudo-bootstrap support values $>$ 70% from 100 replications, with an average branch support of 93.0%. The tree was rooted at the midpoint (Farris, 1972). All phylogenetic trees were viewed and edited with Figtree v 1.4.4 (Rambaut, 2009).

The whole genome of strain SGI-60 was compared to genomes of reference strains using digital DNA-

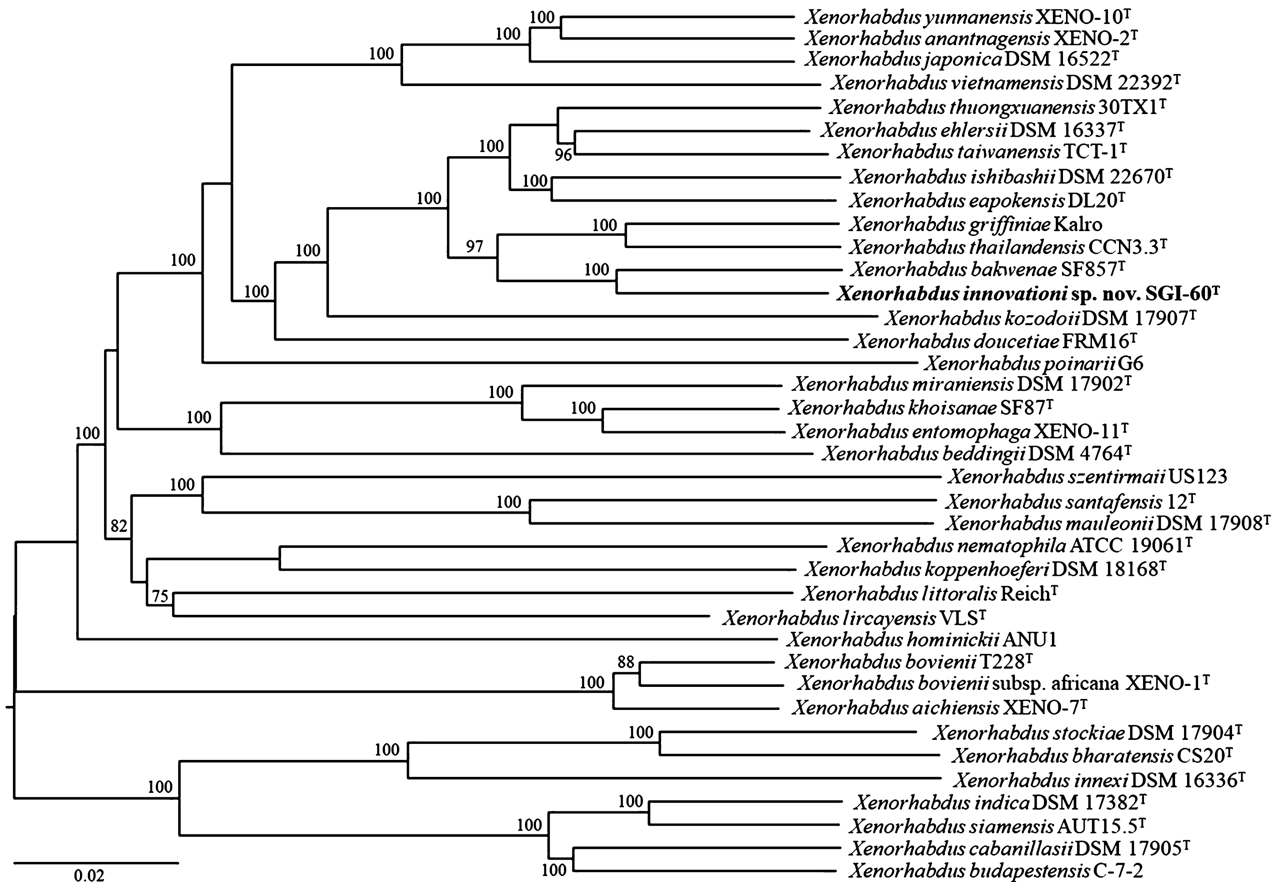

Fig. 1. Phylogenetic placement of *Xenorhabdus innovationi* sp. nov. SGI-60^T within the genus *Xenorhabdus* based on the genome sequence. The tree was inferred with FastME 2.1.6.1 (Lefort *et al.*, 2015) from GBDP distances calculated from genome sequences. The branch lengths are scaled in terms of GBDP distance formula d_5. The numbers above branches are GBDP pseudo-bootstrap support values > 70% from 100 replications, with an average branch support of 93.0%. The tree was rooted at the midpoint (Farris, 1972). The analysis involved 38 genomes.

DNA hybridisation (dDDH; Auch *et al.*, 2010). Data were analysed using the Genome-to-Genome Distance Calculator 3.0 (Meier-Kolthoff *et al.*, 2022) and formula 2 from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ; http://ggdc.dsmz.de). The Genome-to-Genome Distance Calculator uses an algorithm to efficiently determine high-scoring segment pairs or maximally unique matches to infer intergenomic distances. Complementary analysis was done using the orthologous average nucleotide identity (OrthoANI) tool (https://www.ezbiocloud.net/tools/ani; Lee *et al.*, 2016). The antibiotics and secondary metabolite analysis shell (antiSMASH; Blin *et al.*, 2025) database was used for the identification, annotation, and analysis of secondary metabolite biosynthesis gene clusters (BGCs) in strain SGI-60 and closely related *Xenorhabdus* spp. The ClusterCompare algorithm was used to compare each proto cluster (including neighbourhood) in the query region to the entire reference region. Similarity scores above 0.5 were considered present in the genome; below the thresholds, BGCs were considered absent or non-functional.

Phenotypic characterisation

The culturing and phenotypic properties of strain SGI-60 were determined in TSB and nutrient agar (NA), using phase I cells. Cell size and shape were determined after 48 h of incubation at 30°C on NA plates. Bacterial cell size was measured using the Nikon E400 light microscope (Nikon Instruments) equipped with a camera, com-

puter, and digital image software. Colony pigmentation was observed on NBTA and MacConkey Agar (Biolab) after 48 h at 30°C. Isolates were streaked onto the plates and incubated for 48 h at 30°C. The optimal incubation temperature was assessed at 25, 30 and 37°C after 24 h in TSB, with changes in growth measured spectrophotometrically at 600 nm. A growth curve was generated using the Tecan Spark®, a 96-well plate with TSB was inoculated and incubated for 48 h at 30°C, with shaking at 180 rpm. Optical density (OD) was measured every 20 min at 600 nm.

Several phenotypic characteristics were determined, including Gram-, oxidase-, catalase- lecithinase-, and lipase reactions (Bartholomew & Mittwer, 1952; Steel, 1961; Reiner, 2010). Lecithinase activity was determined by streaking a 48-h-old bacterial culture on NA plates supplemented with 0.9% (w/v) NaCl and 10% (v/v) egg yolk emulsion (Ferreira *et al.*, 2013a,b). Lipase activity was determined with Tween-20, Tween-40, Tween-60 and Tween-80 as described by Sierra (1957). Biochemical tests were performed, at 30°C, to assess acid production from sugars and carbon source utilisation using the API 50 CH and API 20 NE kits (bioMérieux, France) according to the manufacturer's instructions. Carbohydrate fermentation reactions were recorded 48 h after inoculation. To verify the reliability of the results, API strips were observed again after 10 days of incubation.

The CFS of strain SGI-60 was tested for antimicrobial activity against *L. monocytogenes* ATCC 7644, *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853, using the agar-well diffusion assay (Dreyer *et al*., 2019). Physiological and biochemical tests were done in triplicate.

Virulence screening

Five flat-bottom 24-well bioassay plates (Nunc™, Cat. No. 144530) were used as experimental arenas for each treatment group. To ensure uniform spatial distribution, circular filter papers (2.5 mm diam. Whatman No. 1 Labotec) were placed in alternating wells, resulting in 12 usable wells per plate and a total of five plates per treatment. Each plate received 12 last-instar false codling moth larvae, totalling 60 larvae per treatment group, with an equal number of larvae ($n = 60$) assigned to control plates treated with water only. For each EPN treatment of *S. innovationi*, *S. khoisanae*, and *S. jeffreyense*, 50 μl of distilled water containing 50 IJ was applied directly onto the filter paper in each well. Control wells received only water. One larva was placed into each prepared well, which was then sealed using a glass pane secured with a rubber band to prevent insect larval escape. The plates were incubated within a moisture chamber, in a growth chamber maintained at 25 ± 2°C for 48 h. Larval mortality was assessed at 48 h post-exposure. Nematode infection was confirmed through dissection using a stereomicroscope. The entire bioassay procedure was replicated on a different date using freshly prepared nematode suspensions.

Statistical analysis

Statistical analyses were conducted using Statistica version 13.0 (StatSoft, 2008). No significant interaction was detected between test date and treatment and data from both experimental runs were combined and subjected to a one-way ANOVA, followed by *post hoc* mean comparisons. In cases where residuals deviated from normality, bootstrap-based multiple comparison procedures were applied in accordance with the methodology outlined by Efron & Tibshirani (1993).

Results and discussion

Phylogenetic characterisation

Phylogenetic analysis of 16S rRNA gene sequences (1539 bp) placed strain SGI-60 in a clade with *X. bakwenae* SF857^T, *X. taiwanensis* TCT-1^T, *X. griffiniae* ID10^T, and *X. thailandensis* CCN3.3^T (Supplementary Fig. S1). Nucleotide BLAST results comparing the 16S rRNA region of strain SGI-60 to that of *X. bakwenae* SF857^T, *X. taiwanensis* TCT-1^T, *X. griffiniae* ID10^T, and *X. thailandensis* CCN3.3^T were 98.18, 98.05, 97.69 and 97.66%, respectively. All values were below the 98.7% cut-off for strains grouped into the same species (Chun *et al.*, 2018).

The whole genome of strain SGI-60 consists of 1 contig with a 44.18% GC content, 3998 protein count, and 4 193 757 bp total length. The genome phylogenetic tree was similar to the 16S rRNA phylogenetic tree, with strain SGI-60 forming a clade with *X. bakwenae* SF857^T, *X. griffiniae* ID10^T, and *X. thailandensis* CCN3.3^T (Fig. 1). However, phylogenetic analysis of the whole genome placed *X. taiwanensis* TCT-1^T in a different clade than observed for the 16S rRNA phylogenetic tree. The dDDH scores (Fig. 2) showed that the top five closest species to strain SGI-60 are *X. bak-*

Pairwise digital DNA-DNA hybridisation score (%) comparisons from red (lowest) to green (highest)	***X. innovationi* sp. nov. SGI-60^T**	*X. aichiensis* XENO-7^T	*X. anantnagensis* XENO-2^T	*X. bakwenae* sp. nov. SF857^T	*X. beddingii* DSM 4764^T	*X. bharatensis* CS20^T	*X. bovienii* subsp. *africana* XENO-1^T	*X. bovienii* T228	*X. budapestensis* C-7-2	*X. cabanillasii* DSM 17905	*X. doucetiae* FRM16^T	*X. eapokensis* DL20^T	*X. ehlersii* DSM 16337^T	*X. entomophaga* XENO-11^T	*X. griffiniae* Kalro	*X. hominickii* ANU1	*X. indica* DSM 17382	*X. innexi* DSM 16336^T	*X. ishibashii* DSM 22670	*X. japonica* DSM 16522	*X. khoisanae* SF87^T	*X. koppenhoeferi* DSM 18168	*X. kozodoii* DSM 17907	*X. lircayensis* VLS^T	*X. littoralis* Reich^T	*X. mauleonii* DSM 17908	*X. miraniensis* DSM 17902	*X. nematophila* ATCC 19061	*X. poinarii* G6	*X. santafensis* 12^T	*X. stockiae* DSM 17904	*X. szentirmaii* US123	*X. taiwanensis* TCT-1^T	*X. thailandensis* CCN3.3^T	*X. thuongxuanensis* 30TX1^T	*X. vietnamensis* DSM 22392	*X. yunnanensis* XENO-10^T
***X. innovationi* sp. nov. SGI-60^T**	ID	32	27	53	24	23	24	24	24	24	32	41	47	28	53	26.3	24	22.3	40	31.8	28	26.4	34	28.7	26	24.8	27.3	25.8	27.4	24.7	22.7	24.2	44.3	44.6	43.3	30.7	31.6
X. aichiensis XENO-7^T	32	ID	25	24	25	23	64	25	25	24	24	24	25	28	24	25.5	24.3	23.1	24.4	24.7	25	24.9	24	25.8	25	23.3	25.2	24.4	23.2	23.5	23.2	23.1	32.2	31.8	24.1	24.4	25.1
X. anantnagensis XENO-2^T	27	25	ID	31	27	23	25	25	25	24	30	32	33	31	31	27.2	24.2	22.5	32	51.8	29	27.2	30	29.4	27	24.9	28.4	26.3	29.1	25	23.5	24.3	27	27	32.4	40	56.4
X. bakwenae sp. nov. SF857^T	53	24	31	ID	27	23	24	24	24	24	39	39	44	27	41	26	23.5	22.5	37.8	31.2	27	26.7	33	28.2	26	24.7	27.1	26	27.1	24.8	22.8	24.4	41.4	42	40.2	30	30.5
X. beddingii DSM 4764^T	24	25	27	27	ID	23	25	71	23	23	27	27	27	25	27	26.3	23.3	22.5	26.9	27.3	32	27	26	29.6	27	24.9	31.8	26.2	25.2	24.9	22.9	24.8	24.1	24.3	26.7	27	27.2
X. bharatensis CS20^T	23	23	23	23	23	ID	23	23	27	27	22	23	23	23	23	23.4	26.6	33.2	22.5	22.7	23	22.9	22	22.8	22	22.1	22.9	22.5	22.3	22.1	53	22.2	22.7	22.7	22.3	22.8	23.3
X. bovienii subsp. *africana* XENO-1^T	24	64	25	24	25		ID	100	24	24	24	24	25	25	25	25.5	24.3	23.1	24.3	24.5	25	24.8	24	25.7	25	23.2	25	24.2	23	23.3	23.6	23.2	24.2	24.2	24.1	24.5	24.8
X. bovienii T228	24	25	25	24	71	23	100	ID	25	24	24	24	25	25	25	25.9	24.1	23.3	24.5	24.6	25	25.1	24	25.8	25	23.2	25.1	24.7	23.3	23.5	23.6	23.3	24.2	24.2	24.2	24.7	24.8
X. budapestensis C-7-2	24	25	25	24	23	27	24	25	ID	53	23	24	25	24	24	24.7	50.8	26.5	24.1	24.2	25	24	23	23.9	24	22.9	24.5	23.9	22.9	22.9	27.3	23.1	24.3	24.1	24	24	24.5
X. cabanillasii DSM 17905	24	24	24	24	23	27	24	24	53	ID	24	24	24	24	24	23.9	49.9	27.2	24	23.8	24	24.6	23	24.3	24	24	24.1	25.2	23.4	23.5	26.4	23.7	24.1	23.7	23.6	23.5	23.7
X. doucetiae FRM16^T	32	24	30	39	27	22	24	24	23	24	ID	32	32	27	31	25.8	23	22.8	31.8	30.6	27	26.9	31	28.3	26	24.8	26.8	26.3	27.5	24.9	22.6	24.6	31.9	31.8	32.1	29.3	30.2
X. eapokensis DL20^T	41	24	32	39	27	23	24	24	24	24	32	ID	48	28	40	26.4	23.8	22.4	51.2	31.9	28	26.4	34	28.7	26	24.7	27.8	25.8	27.4	24.8	22.9	24.1	47.9	40.7	51.9	31.3	32
X. ehlersii DSM 16337^T	47	25	33	44	27	23	25	25	25	24	32	48	ID	28	46	26.8	24.3	22.5	46.4	32.7	28	26.7	35	29.3	27	24.8	28	26.1	27.8	24.8	23.5	24.2	55.5	45.5	53.5	31.8	32.5
X. entomophaga XENO-11^T	28	28	31	27	25	23	25	25	24	24	27	28	28	ID	28	27	24.8	22.8	27.8	28.2	64	27.7	27	30.3	27	25	53.8	26.8	25.4	25	23.2	24.8	27.7	27.7	27.7	27.6	28
X. griffiniae Kalro	53	24	31	41	27	23	25	25	24	24	31	40	46	28	ID	26.3	24	22.1	39	31.2	28	26.4	33	28.5	26	24.8	27.4	25.8	27.5	24.6	22.9	24.3	43.8	58.6	42.5	30.2	30.9
X. hominickii ANU1	26	26	27	26	26	23	26	26	25	24	26	26	27	27	26	ID	24	22.6	26.4	27	27	26.8	26	28.8	26	24.3	27.3	26.3	25	24.5	23.3	24.3	26.4	26.4	26.2	26.9	27.4
X. indica DSM 17382	24	24	24	24	23	27	24	24	51	50	23	24	24	25	24	24	ID	26.2	24.1	24	24	23.8	24	23.6	23	22.7	24.7	23.3	22.9	22.8	27.1	22.4	24	24.2	23.7	23.9	24.2
X. innexi DSM 16336^T	22	23	23	23	23	33	23	23	27	27	23	22	23	23	22	22.6	26.2	ID	22.2	22.5	23	23.2	22	23.1	23	23.1	22.6	23.5	22.5	22.7	34.2	22.9	22.4	22.2	22.3	22.7	22.8
X. ishibashii DSM 22670	40	24	32	38	27	23	24	25	24	24	32	51	46	28	39	26.4	24.1	22.2	ID	32	28	26.5	33	28.8	27	24.7	27.8	26.3	27.6	24.8	22.9	24.8	45.4	39.9	48.3	31.3	32
X. japonica DSM 16522	32	25	52	31	27	23	25	25	24	24	31	32	33	28	31	27	24	22.5	32	ID	28	27	30	29.6	27	24.9	28.5	26.4	29.1	24.9	23.4	24.4	32.1	31.3	32.3	41.6	53.4
X. khoisanae SF87^T	28	25	29	27	32	23	25	25	25	24	27	28	28	64	28	27.1	24.3	22.6	27.6	28	ID	27.7	27	30.5	27	25.2	53.3	26.7	25.2	25.1	23.2	24.6	27.8	27.5	27.8	27.6	28.3
X. koppenhoeferi DSM 18168	26	25	27	27	27	23	25	25	24	25	27	26	27	28	26	26.8	23.8	23.2	26.5	27	28	ID	28	30.5	29	26.5	27.5	34.2	25.3	26.4	23.2	25.7	26.4	26.4	26.3	27	27.6
X. kozodoii DSM 17907	34	24	30	33	26	22	24	24	23	23	31	34	35	27	33	25.5	23.6	22	33.3	30	27	27.7	ID	27.5	26	24.3	27	25.3	26.7	24.3	22.3	23.9	33.6	33.3	34.1	29.4	30.2
X. lircayensis VLS^T	29	26	29	28	30	23	26	26	24	24	28	29	29	30	29	28.8	23.6	23.1	28.8	29.6	31	30.5	28	ID	31	26.6	30.3	29.1	26.5	26.1	22.9	26.3	28.8	28.7	29.1	28.7	29.3
X. littoralis Reich^T	26	25	27	26	27	22	25	25	24	24	26	26	27	27	26	26.4	23.1	23.3	26.5	27	27	28.6	26	31.4	ID	25.9	27.4	29.3	25.2	25.9	22.6	27.1	26.4	26.3	26.5	26.6	27
X. mauleonii DSM 17908	25	23	25	25	25	22	23	23	23	24	25	25	25	25	25	24.3	22.7	23.1	24.7	24.9	25	26.5	24	26.6	26	ID	25.2	26.3	24.3	40.8	22.2	26.4	24.8	25	24.7	24.6	25
X. miraniensis DSM 17902	27	25	28	27	32	23	25	25	25	24	27	28	28	54	27	27.3	24.7	22.6	27.8	28.5	53	27.5	27	30.3	27	25.2	ID	26.8	25.5	25.3	23.6	24.7	27.9	27.6	27.9	27.9	28.2
X. nematophila ATCC 19061	26	24	26	26	26	23	24	25	24	25	26	26	26	27	26	26.3	23.3	23.5	26.3	26.4	27	34.2	25	29.1	29	26.3	26.8	ID	25.6	26.2	22.8	26.3	25.9	25.8	25.7	26	26.4
X. poinarii G6	27	23	29	27	25	22	23	23	23	23	28	27	28	25	28	25	22.9	22.5	27.6	29.1	25	25.3	27	26.5	25	24.3	25.5	25.6	ID	24.1	22.5	24.2	27.5	27.5	27.7	28.8	29.2
X. santafensis 12^T	25	24	25	25	25	22	23	24	23	24	25	25	25	25	25	24.5	22.8	22.7	24.8	24.9	25	26.4	24	26.1	26	40.8	25.3	26.2	24.1	ID	22.2	25.9	24.7	24.8	24.7	24.7	24.8
X. stockiae DSM 17904	23	23	24	23	23	53	24	24	27	26	23	23	24	23	23	23.3	27.1	34.2	22.9	23.4	23	23.2	22	22.9	23	22.2	23.6	22.8	22.5	22.2	ID	22.3	23.3	22.8	22.5	23.5	23.5
X. szentirmaii US123	24	23	24	24	25	22	23	23	23	24	25	24	24	25	24	24.3	22.4	22.9	24.8	24.4	25	25.7	24	26.3	27	26.4	24.7	26.3	24.2	25.9	22.3	ID	24.2	24.5	24.3	24.2	24.5
X. taiwanensis TCT-1^T	44	32	27	41	24	23	24	24	24	24	32	48	56	28	44	26.4	24	22.4	45.4	32.1	28	26.4	34	28.8	26	24.8	27.9	25.9	27.5	24.7	23.3	24.2	ID	43.9	53.3	31.3	32.1
X. thailandensis CCN3.3^T	45	32	27	42	24	23	24	24	24	24	32	41	46	28	59	26.4	24.2	22.2	39.9	31.3	28	26.4	33	28.7	26	25	27.6	25.8	27.5	24.8	22.8	24.5	43.9	ID	43.2	30.9	31.5
X. thuongxuanensis 30TX1^T	43	24	32	40	27	22	24	24	24	24	32	52	54	28	43	26.2	23.7	22.3	48.3	32.3	28	26.3	34	29.1	27	24.7	27.9	25.7	27.7	24.7	22.5	24.3	53.3	43.2	ID	31.7	32.5
X. vietnamensis DSM 22392	31	24	40	30	27	23	25	25	24	24	29	31	32	28	30	26.9	23.9	22.7	31.3	41.6	28	27	29	28.7	27	24.6	27.9	26	28.8	24.7	23.5	24.2	31.3	30.9	31.7	ID	40.4
X. yunnanensis XENO-10^T	32	25	56	31	27	23	25	25	25	24	30	32	33	28	31	27.4	24.2	22.8	32	53.4	28	27.6	30	29.3	27	25	28.2	26.4	29.2	24.8	23.5	24.5	32.1	31.5	32.5	40.4	ID

Fig. 2. Pairwise nucleotide comparisons (%) of the dDDH scores between members of the genus *Xenorhabdus* including strain *X. innovationi* sp. nov.

Table 1. Predicted compounds produced by *Xenorhabdus innovationi* sp. nov. SGI-60^T, *X. bakwenae* SF857^T, *X. griffiniae* Kalro, *X. thailandensis* CCN3.3^T, and *X. taiwanensis* TCT-1^T.

Compound(S)	*X. innovationi* sp. nov. SGI-60^T	*X. bakwenae* SF857^T	*X. griffiniae* Kalro	*X. thailandensis* CCN3.3^T	*X. taiwanensis* TCT-1^T
1-Heptadecene	−	−	−	−	+
1-Nonadecene, (14Z)-1,14-Nonadecadiene	−	+	−	−	−
Aryl polyenes	+	+	+	−	+
Asperlactone	+	−	+	−	−
Bicornutin A1 And A2	−	+	−	+	+
Bovienimide A	+	+	+	−	+
Dimethylcoprogen	−	+	+	−	−
Epichloenin A	−	+	−	−	−
Gamexpeptide C	+	+	−	−	+
Griseoluteic acid, Demethoxygriseoluteic acid, Phenaszentine A-D	−	+	−	−	−
Kolossin	−	+	−	−	−
Nostovalerolactone, 9-Dehydronostovalerolactone	+	−	+	−	−
Pyreudione A-E	+	−	+	+	+
Rhizomide A-C	+	−	−	−	−
Sodorifen	+	−	+	+	+
Trifolitoxin	+	−	+	+	−
Tobramycin	−	−	−	−	+
Xefoampeptides A-G	−	−	+	+	−
Xenematide	+	−	+	+	+
Xenotetrapeptide	+	−	+	−	−
Yersiniabactin	−	+	−	+	−

+: produced; −: not produced.

wenae SF857^T (53.1%), *X. griffiniae* Kalro (53.0%), *X. ehlersii* DSM 16337^T (46.6%), *X. thailandensis* CCN3.3^T (44.6%), and *X. taiwanensis* TCT-1^T (44.3%). Overall, the dDDH scores ranged from 22.3 to 53.1% (Fig. 2), which is lower than the species threshold of 70% (Chun *et al.*, 2018). To confirm these results, the OrthoANI tool was used, comparing strain SGI-60 to *X. bakwenae* SF857^T (94.76%), *X. griffiniae* Kalro (93.62%), *X. ehlersii* DSM 16337^T (92.21%), *X. thailandensis* CCN3.3^T (91.46%) and *X. taiwanensis* TCT-1^T (91.58%). Similarity values were below 95%, the proposed cut-off limit for species delineation (Chun *et al.*, 2018). The phylogenetic results indicate that strain SGI-60 represents a new species within the genus *Xenorhabdus*.

In silico analysis revealed that strain SGI-60 and closely related species *X. bakwenae* SF857^T, *X. griffiniae* Kalro, *X. thailandensis* CCN3.3^T, and *X. taiwanensis* TCT-1^T contain BGCs to potentially produce *Xenorhabdus* metabolites and metabolites from other microorganisms including 1-heptadecene, 1-nonadecene, (14Z)-1,14-nonadecadiene, aryl polyenes, asperlactone, bicornutins, bovienimide A, dimethylcoprogen, epichloenin A, gamexpeptide C, griseoluteic acid, demethoxygriseoluteic acid, phenaszentines, kolossin, nostovalerolactone, 9-dehydronostovalerolactone, pyreudiones, rhizomides, sodorifen, tobramycin, trifolitoxin, xefoampeptides, xenematide, xenotetrapeptide, yersiniabactin (Table 1). Most of these metabolites are produced by non-ribosomal peptide synthetases (NRPS) (Table S2 in the Appendix). Strain SGI-60 BGC profile was similar to *X. griffiniae* Kalro and least similar to that of *X. bakwenae* SF857^T and *X. taiwanensis* TCT-1^T. Observing the unique profiles, only strain SGI-60 contained the machinery for rhizomide production; only *X. bakwenae* SF857^T had the BGCs present to produce 1-nonadecene, (14Z)-1,14-nonadecadiene, epichloenin A, griseoluteic acid, demethoxygriseoluteic acid, phenaszentines, and kolossin; and only *X. taiwanensis* TCT-1^T had BGCs to produce 1-heptadecene and tobramycin.

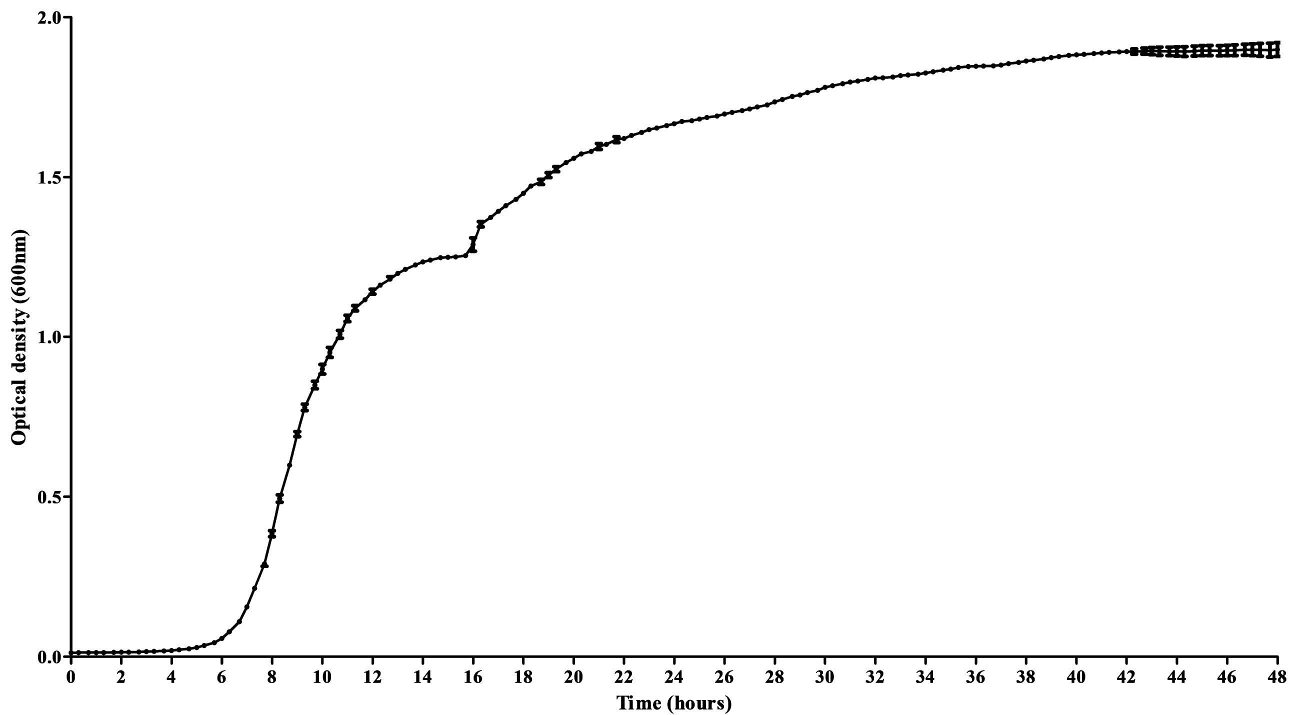

Fig. 3. The mean optical density (measured at 600 nm) of *Xenorhabdus innovationi* sp. nov. in TSB over 48 h at 30°C. Error bars represent standard error.

PHENOTYPIC CHARACTERISTICS

Strain SGI-60 is an aerobic motile, Gram-negative rod-shaped bacterium with an average cell size of 1.34 × 0.38 μm. Lipase activity was observed on Tween 80, Tween 60, and Tween 20 media, but not on plates containing Tween 40. Strain SGI-60 is catalase and oxidase negative and has lecithinase activity. Colonies appear blue on NBTA and red with pink outer layer on MacConkey Agar. Growth was observed between 26 and 37°C, with optimal growth recorded at 30°C and stationary phase reached after 40 h incubation (Fig. 3). Table 2 shows the acid production and assimilation of strain SGI-60 compared to the closely related type strain, *X. bakwenae* SF857^T. According to the API 50 CH system, strain SGI-60 showed acidification with erythritol, D-glucose, D-mannose, maltose, trehalose, N-acetylglucosamine and weakly exhibited acidification with D-fructose. Results from API 20 NE showed assimilation occurred with glucose, mannose, maltose, and N-acetylglucosaminemalate. Hydrolysation of gelatine (bovine origin) was also observed. No antimicrobial activity was observed against the tested microorganisms. Based on phylogenetic and phenotypic data, strain SGI-60 belongs to a new species within the genus *Xenorhabdus*. The species name *Xenorhabdus innovationi* sp. nov. is proposed, with strain SGI-60 as the type strain.

DESCRIPTION OF *X. INNOVATIONI* SP. NOV.

Cells are aerobic, motile, Gram-negative, and rod-shaped with an average cell size of 1.34 × 0.38 μm. Colonies appear blue on NBTA plates and red with a pink outer layer on MacConkey Agar. Optimal growth occurs at 30°C in TSB. Catalase- and oxidase negative with lecithinase activity. Lipase activity is observed on Tween-20, Tween-60, and Tween-80 media, but not Tween-40. Strain *X. innovationi* sp. nov. produces acid from erythritol, D-glucose, D-mannose, maltose, trehalose, N-acetylglucosamine and weakly exhibited acidification D-fructose. Assimilates glucose, mannose, maltose, and N-acetylglucosamine and can hydrolyses gelatin (bovine origin). The whole genome *X. innovationi* sp. nov. SGI-60^T consists of 1 contig with a 44.18% GC content, 3998 protein count, and 4 193 757 bp total length. The whole genome sequence is available in the NCBI data bank under the accession number CP196860. This study provides enough phylogenetic and phenotypic evidence to

Table 2. Biochemical results for *Xenorhabdus innovationi* sp. nov. SGI-60^T compared to *X. bakwenae* SF857^T.

Characteristics	*X. innovationi* sp. nov. SGI-60^T	*X. bakwenae* SF857^T
Acid production from		
Ribose	−	−
Glucose	+	+
Fructose	+	+
Mannose	w	+
Inositol	−	−
Sorbitol	−	−
N-Acetylglucosamine	+	+
Aesculin	−	−
Maltose	+	w
Trehalose	+	w
Erythritol	+	−
Gluconate	−	−
Assimilation of:		
Glucose	+	+
D-Mannose	+	+
N-Acetylglucosamine	+	+
Aesculin	−	−
Maltose	+	+
Trisodium citrate	−	+
Malate	−	+
D-Gluconate	−	−

w: weakly positive; +: positive; −: negative. All the biochemical tests were carried out at 30°C, the results were recorded after 48 h of incubation.

regard *X. innovationi* sp. nov. as a new species in the genus *Xenorhabdus*, with *X. innovationi* sp. nov. SGI-60 ^{T} as the type strain. *X. innovationi* sp. nov. SGI-60^T was isolated from *S. innovationi* SGI-60.

Etymology

Xenorhabdus innovationi sp. nov. (N.L. gen. n. ’in·no·va·tsee·OH·nee) epithet pertains to the “Innovation Fund” administered by the National Research Foundation (NRF) of South Africa.

Virulence screening

As there were no significant interactions between the main effects of date and treatment, data from the two test dates were pooled and analysed using a one-way ANOVA ($F_{(2,27)} = 40.375$, $P < 0.001$). A significant difference ($P < 0.05$) in the mortality of false codling moth larvae after 48 h exposure was found between the three EPN species. *Steinernema jeffreyense* was highly virulent with

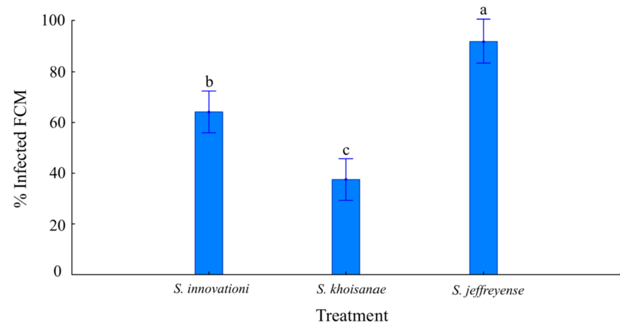

Fig. 4. Mean percentage mortality (=infection) (95% confidence intervals) of last-instar false codling moth larvae, inoculated with 50 infective juveniles of *Steinernema innovationi* (SGI-60), *S. jeffreyense* (J196) and *S. khoisanae* (I10) 48 h after treatment (one-way ANOVA: ($F_{(2,27)} = 40.375$, $P < 0.001$). Different letters above the vertical bars indicate significant differences ($P < 0.05$) between nematode species and infection.

a mortality of 91.67 ± 9.62%, followed by *S. innovationi* with a mortality of 64.17 ± 25.14% and *S. khoisanae* (37.50 ± 12.56%) (Fig. 4).

The three nematode species used in the virulence screening belong to the all-African group, the *Khoisanae*-clade with large IJ of ≈1000 μm. Both *S. jeffreyense* and *S. khoisanae* share the same mutualistic bacterium *X. khoisanae* (Spiridonov *et al.*, 2016; Malan *et al.*, 2023). However, in terms of virulence, *S. jeffreyense* outperformed both *S. khoisanae* and *S. innovationi* with the latter being the second-best performer. In a previous study *S. bakwena*e also outperformed *S. khoisanae* (Ritter *et al.*, 2023) in virulence to codling moth larvae. This study indicated *S. innovationi* can be used against false codling moth, as was previously shown to be effective against a range of different hosts (Ramakuwela *et al.*, 2018).

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Supplementary Table S1. *Xenorhabdus* spp. used in the study.

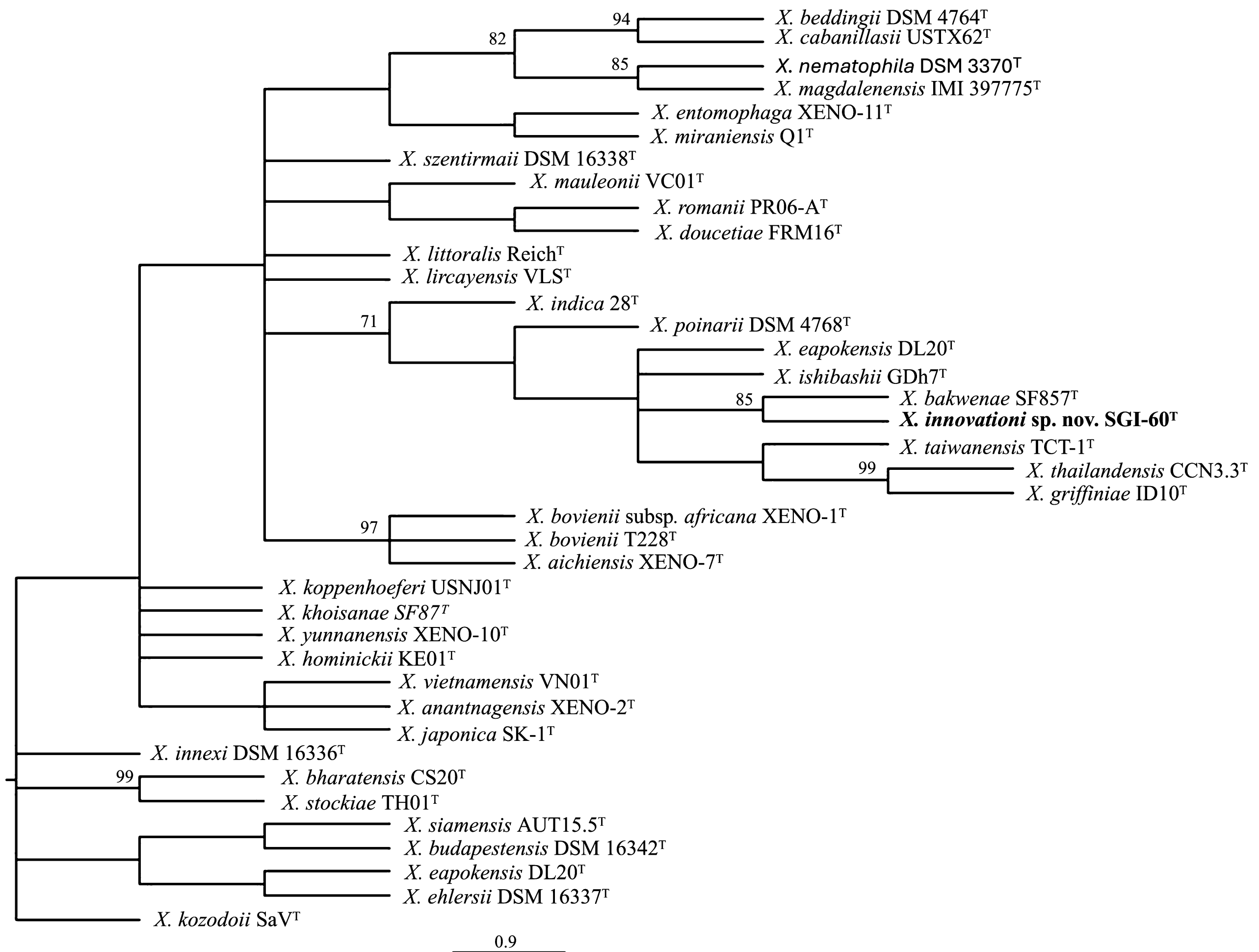
Type strain	16S rRNA accession number	Reference genome	Genome accession number
X. aichiensis XENO-7^{T}	OQ439939.1	*X. aichiensis* XENO-7^{T}	GCF_028598775.1
X. anantnagensis XENO-2^{T}	Extracted from genome	*X. anantnagensis* XENO-2^{T}	GCF_028598555.1
X. bakwenae	Extracted from genome	*X. bakwenae* SF857^{T}	GCF_030272115.1
X. beddingii DSM 4764^{T}	NR_042822.1	*X. beddingii* DSM 4764^{T}	GCF_002127545.1
X. bharatensis CS20^{T}	Extracted from genome	*X. bharatensis* CS20^{T}	GCF_037996705.1
X. bovienii subsp. *africana* XENO-1^{T}	Extracted from genome	*X. bovienii* subsp. *africana* XENO-1^{T}	GCF_024316195.1
X. bovienii T228^{T}	NR_042820.1	*X. bovienii* T228^{T}	GCF_024721015.1
X. budapestensis DSM 16342^{T}	NR_042326.1	*X. budapestensis* C-7-2	GCF_017743015.1
X. cabanillasii USTX62^{T}	NR_042945.1	*X. cabanillasii* DSM 17905^{T}	GCF_003386665.1
X. doucetiae FRM16^{T}	NR_043642.1_	*X. doucetiae* FRM16^{T}	GCF_000968195.1
X. eapokensis DL20^{T}	NR_156925.1	*X. eapokensis* DL20^{T}	GCF_001908105.1
X. ehlersii DSM 16337^{T}	NR_042327.1	*X. ehlersii* DSM 16337^{T}	GCF_003610465.1
X. entomophaga XENO-11^{T}	Extracted from genome	*X. entomophaga* XENO-11^{T}	GCF_037996725.1
X. griffiniae ID10^{T}	NR_043643.1	*X. griffiniae* Kalro	GCF_030016155.2
X. hominickii KE01^{T}	NR_043648.1	*X. hominickii* ANU1	GCF_001721185.1
X. indica 28^{T}	NR_114965.1	*X. indica* DSM 17382^{T}	GCF_014467235.1
X. innexi DSM 16336^{T}	NR_042325.1	*X. innexi* DSM 16336^{T}	GCF_002632485.1
***X. innovationi* sp. nov. SGI-60^{T}**	Extracted from genome	***X. innovationi* sp. nov. SGI-60^{T}**	
X. ishibashii GDh7^{T}	NR_117216.1	*X. ishibashii* DSM 22670^{T}	GCF_002632755.1
X. japonica SK-1^{T}	D78008.1	*X. japonica* DSM 16522^{T}	GCF_900115195.1
X. khoisanae SF87^{T}	NR_117921.1	*X. khoisanae* SF87^{T}	GCF_028598485.1
X. koppenhoeferi USNJ01^{T}	NR_043637.1	*X. koppenhoeferi* DSM 18168^{T}	GCF_900116635.1
X. kozodoii SaV^{T}	NR_043646.1	*X. kozodoii* DSM 17907^{T}	GCF_002632875.1
X. lircayensis VLS^{T}	MT466535.1	*X. lircayensis* VLS^{T}	GCF_016306625.1
X. littoralis Reich^{T}	MK634327.1	*X. littoralis* Reich^{T}	GCF_033949345.1
X. magdalenensis IMI 397775^{T}	NR_109326.1	NO GENOME	
X. mauleonii VC01^{T}	NR_043645.1	*X. mauleonii* DSM 17908^{T}	GCF_900113945.1
X. miraniensis Q1^{T}	NR_043644.1	*X. miraniensis* DSM 17902^{T}	GCF_002632615.1
X. nematophila DSM 3370^{T}	NR_042821.1	*X. nematophila* ATCC 19061^{T}	GCF_000252955.1
X. poinarii DSM 4768^{T}	NR_119152.1	*X. poinarii* G6	GCF_000968175.1
X. romanii PR06-A^{T}	NR_043647.1	NO GENOME	
X. santafensis 12^{T}	Extracted from genome	*X. santafensis* 12^{T}	GCF_033949405.1
X. siamensis AUT15.5^{T}	Extracted from genome	*X. siamensis* AUT15.5^{T}	GCF_037996745.1
X. stockiae TH01^{T}	NR_043634.1_	*X. stockiae* DSM 17904^{T}	GCF_002632825.1
X. szentirmaii DSM 16338^{T}	NR_042328.1	*X. szentirmaii* US123	GCF_002632595.1
X. taiwanensis TCT-1^{T}	Extracted from genome	*X. taiwanensis* TCT-1^{T}	GCF_036512875.1
X. thailandensis CCN3.3^{T}	Extracted from genome	*X. thailandensis* CCN3.3^{T}	GCF_037996785.1
X. thuongxuanensis 30TX1^{T}	NR_156924.1	*X. thuongxuanensis* 30TX1^{T}	GCF_001908095.1
X. vietnamensis VN01^{T}	NR_115713.1_	*X. vietnamensis* DSM 22392^{T}	GCF_002127535.1
X. yunnanensis XENO-10^{T}	Extracted from genome	*X. yunnanensis* XENO-10^{T}	GCF_028598805.1

Supplementary Table S2. Predicted secondary metabolites produced by *Xenorhabdus innovationi* sp. nov. SGI-60^T and closely related species *X. bakwenae* SF857^T, *X. griffiniae* Kalro, *X. thailandensis* CCN3.3^T, and *X. taiwanensis* TCT-1^T.

Species	Region	Similarity score	Type	Compound(s)
X. innovationi sp. nov. SGI-60^T	1	0.57	NRPS	gamexpeptide C
	2	0.91	other (other)	aryl polyenes
	3	0.52	PKS	nostovalerolactone, 9-dehydronostovalerolactone
	4	0.81	NRPS	bovienimide A
	5	0.83	NRPS	bovienimide A
	6	1.5	NRPS	xenotetrapeptide
	7	1.41	NRPS	xenematide
	8	1.04	NRPS	bovienimide A
	9	0.32	other (fatty acid)	pseudopyronine A, pseudopyronine B
	10	0.65	PKS	asperlactone
	11	0.55	ribosomal	trifolitoxin
	12	0.58	NRPS	rhizomide A, rhizomide B, rhizomide C
	13	0.5	terpene	sodorifen
	14	0.9	NRPS	pyreudione A, pyreudione B, pyreudione C, pyreudione D, pyreudione E
	15	0.43	other (other)	ketomemicin B3, ketomemicin B4
X. bakwenae SF857^T	1	0.8	NRPS	bovienimide A
	2	0.66	NRPS, other (phenazine)	griseoluteic acid, demethoxygriseoluteic acid, phenaszentine A, phenaszentine C, phenaszenketide C, phenaszenketide D
	3	1.09	NRPS	yersiniabactin
	4	0.72	NRPS	epichloenin A
	5	0.82	NRPS	bovienimide A
	6	0.59	NRPS	kolossin
	7	0.32	other (fatty acid)	pseudopyronine A, pseudopyronine B
	8	0.83	NRPS	bovienimide A
	9	0.43	NRPS	kleboxymycin
	10	0.55	PKS	1-nonadecene, (14Z)-1,14-nonadecadiene
	11	0.87	other (other)	aryl polyenes
	12	0.33	other (other)	rhizoferrin
	13	0.63	NRPS	bicornutin A1, bicornutin A2
	14	0.86	NRPS	gamexpeptide C
	15	0.31	other (other)	amphotericin B
	16	0.67	NRPS	bicornutin A1, bicornutin A2
	17	0.89	NRPS	dimethylcoprogen
X. griffiniae Kalro	1	0.84	NRPS	dimethylcoprogen
	2	0.87	other (other)	aryl polyenes
	3	0.5	PKS	nostovalerolactone, 9-dehydronostovalerolactone
	4	0.8	NRPS	bovienimide A
	5	0.83	NRPS	bovienimide A
	6	1.44	NRPS	xenotetrapeptide
	7	1.41	NRPS	xenematide
	8	0.81	NRPS	bovienimide A
	9	0.32	other (fatty acid)	pseudopyronine A, pseudopyronine B
	10	0.65	PKS	asperlactone

Supplementary Table S2. (Continued.)

Species	Region	Similarity score	Type	Compound(s)
	11	0.55	ribosomal	trifolitoxin
	12	0.54	NRPS	xefoampeptides A-G
	13	0.5	terpene	sodorifen
	14	0.9	NRPS	pyreudione A, pyreudione B, pyreudione C, pyreudione D, pyreudione E
	15	0.43	other (other)	ketomemicin B3, ketomemicin B4
X. thailandensis CCN3.3^{T}	1	0.74	NRPS	xenematide
	2	0.55	ribosomal	trifolitoxin
	3	0.5	terpene	sodorifen
	4	0.54	NRPS	xefoampeptides A-G
	5	1.06	NRPS	bovienimide A
	6	0.4	PKS	oryzanaphthopyran A, oryzanaphthopyran B, oryzanaphthopyran C, oryzanthrone A, oryzanthrone B, chlororyzanthrone A, chlororyzanthrone B
	7	0.79	NRPS	bovienimide A
	8	0.85	NRPS	bovienimide A
	9	0.69	NRPS	bovienimide A
	10	0.64	NRPS	xenotetrapeptide
	11	0.43	PKS	nostovalerolactone, 9-dehydronostovalerolactone
	12	0.91	NRPS	pyreudione A, pyreudione B, pyreudione C, pyreudione D, pyreudione E
	13	0.42	ribosomal	methanobactin
	14	0.32	other (fatty acid)	pseudopyronine A, pseudopyronine B
	15	0.67	NRPS	yersiniabactin
	16	0.67	NRPS	bicornutin A1, bicornutin A2
	17	0.64	NRPS	bicornutin A1, bicornutin A2
	18	0.58	NRPS	bovienimide A
X. taiwanensis TCT-1^{T}	1	0.27	NRPS, PKS	mutanocyclin
	2	0.85	NRPS	gamexpeptide C
	3	0.88	other (other)	aryl polyenes
	4	0.9	NRPS	pyreudione A, pyreudione B, pyreudione C, pyreudione D, pyreudione E
	5	0.67	NRPS	bicornutin A1, bicornutin A2
	6	0.5	terpene	sodorifen
	7	0.51	saccharide	tobramycin
	8	0.65	PKS	1-heptadecene
	9	0.24	PKS	pyxidicycline A, pyxidicycline B
	10	0.32	other (fatty acid)	pseudopyronine A, pseudopyronine B
	11	0.4	ribosomal	michiganin A
	12	0.81	NRPS	bovienimide A
	13	0.96	NRPS	bicornutin A1, bicornutin A2
	14	1.36	NRPS	xenematide
	15	0.83	NRPS	bovienimide A
	16	0.8	NRPS	bovienimide A
	17	0.48	other (other)	marinacarboline A, marinacarboline B, marinacarboline C, marinacarboline D



Supplementary Fig. S1. Phylogenetic placement of strain SGI-60 within the genus *Xenorhabdus* based on 16S rRNA gene sequence (1539 bp). The phylogeny was inferred using the Maximum Likelihood method and General Time Reversible model (Nei and Kumar, 2000) of nucleotide substitutions and the tree with the highest log likelihood (−5905.55) is shown. The percentage of replicate trees in which the associated taxa clustered together (1000 replicates) is shown above the branches (Felsenstein, 1985). The initial tree for the heuristic search was selected by choosing the tree with the superior log-likelihood between a Neighbor-Joining (NJ) tree (Saitou and Nei, 1987) and a Maximum Parsimony (MP) tree. The NJ tree was generated using a matrix of pairwise distances computed using the General Time Reversible model (Nei and Kumar, 2000). The MP tree had the shortest length among 10 MP tree searches; each performed with a randomly generated starting tree. The evolutionary rate differences among sites were modelled using a discrete Gamma distribution across 5 categories (+G, parameter = 0.3568), with 77.10% of sites deemed evolutionarily invariant (+I). The analytical procedure encompassed 39 nucleotide sequences with 1550 positions in the final dataset. Evolutionary analyses were conducted in MEGA12 (Kumar *et al.*, 2024) utilizing up to 4 parallel computing threads.