

-SUPPLEMENTARY MATERIAL-

Xenorhabdus bharatensis sp. nov., *Xenorhabdus entomophaga* sp. nov., *Xenorhabdus siamensis* sp. nov., and *Xenorhabdus thailandensis* sp. nov. isolated from *Steinernema* entomopathogenic nematodes

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-SUPPLEMENTARY FIGURES-

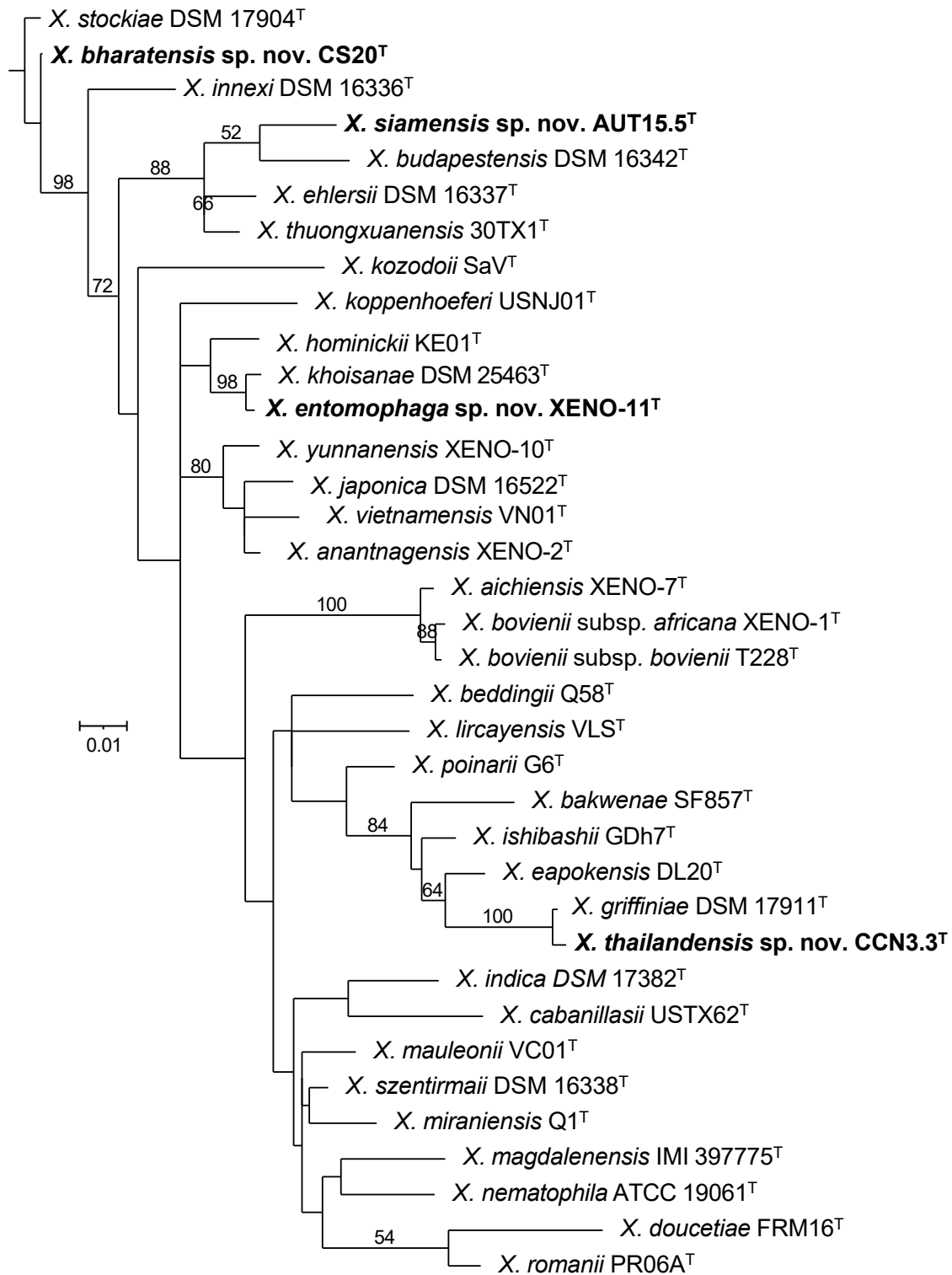


Figure S1. Maximum-likelihood phylogenetic tree reconstructed from the 16S rRNA gene sequences of the type strains of all *Xenorhabdus* species with validly published names. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model. The tree with the highest log likelihood is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. There were a total of 1334 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 based on 100 replications. Accession numbers of used gene sequences are shown in Table S1.

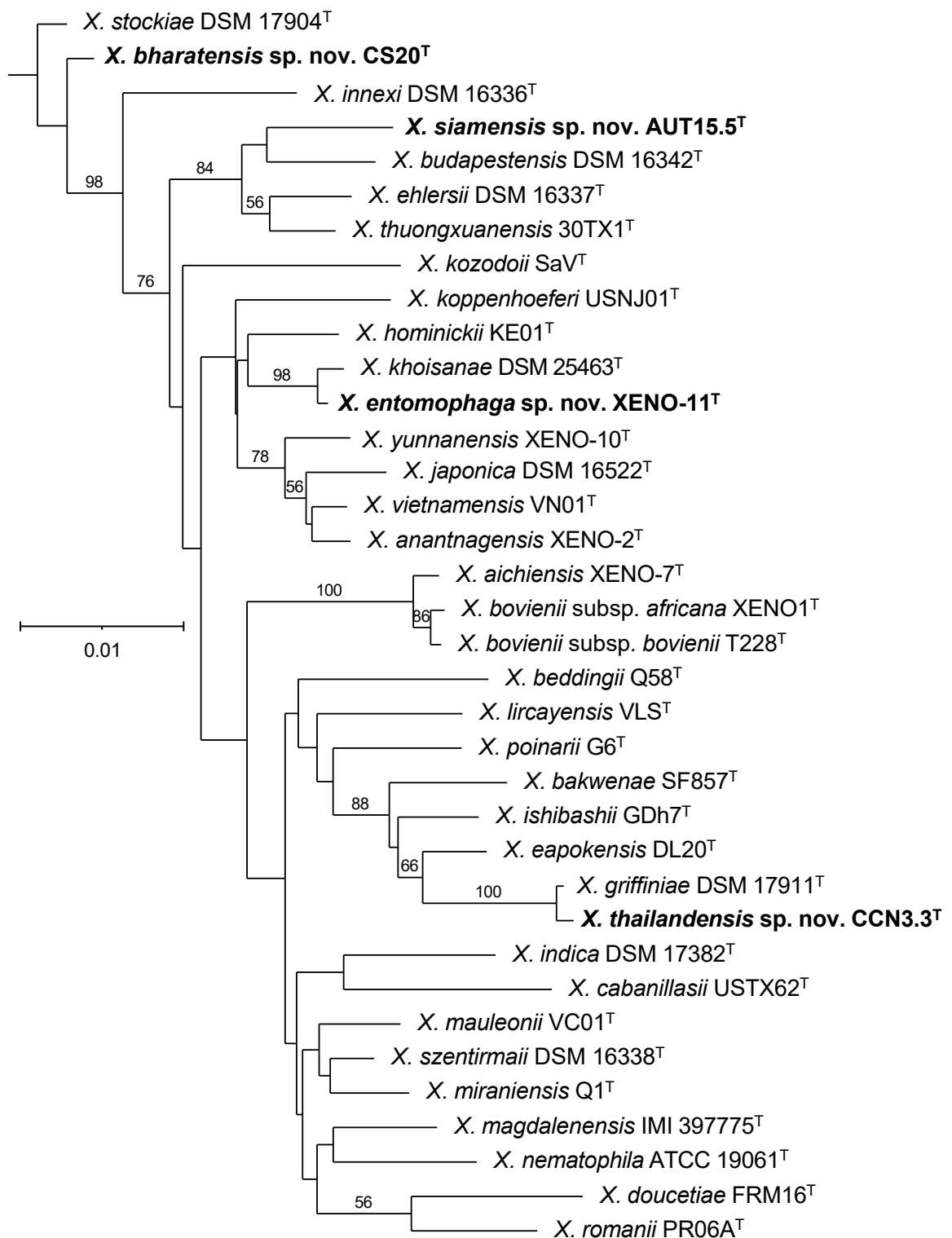


Figure S2. Neighbor-Joining phylogenetic tree reconstructed from the 16S rRNA gene sequences of the type strains of all *Xenorhabdus* species with validly published names. The evolutionary distances were computed using the Kimura 2-parameter model. The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. There were a total of 1334 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 based on 100 replications. Accession numbers of used gene sequences are shown in Table S1.

Figure S3. Pairwise comparisons of the nucleotide similarity values (%) of the 16S rRNA gene sequences of the type strains of all *Xenorhabdus* species with validly published names. A total of 1334 nucleotide positions were analyzed. Accession numbers of used gene sequences are shown in Table S1.

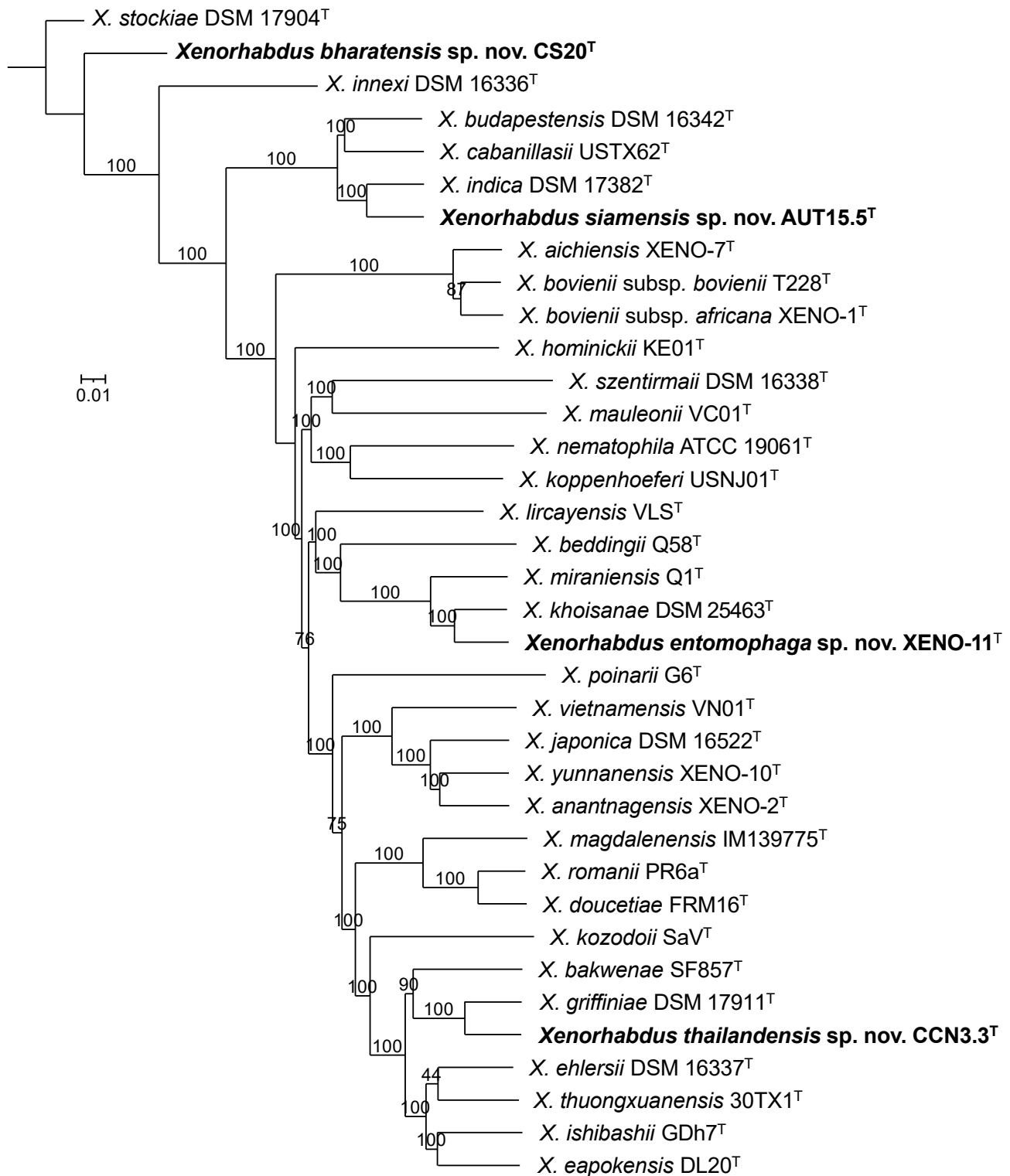


Figure S4. Phylogenetic reconstruction based on whole genome sequences of the type strains of all *Xenorhabdus* species with validly published names. Trees were inferred with FastME 2.1.6.1 from GBDP distances calculated from genome sequences. The branch lengths are scaled in terms of GBDP distance formula d_5 . The numbers above branches are GBDP pseudo-bootstrap support values from 100 replications. NCBI accession numbers of the sequences used for the analyses are shown in Table S1.

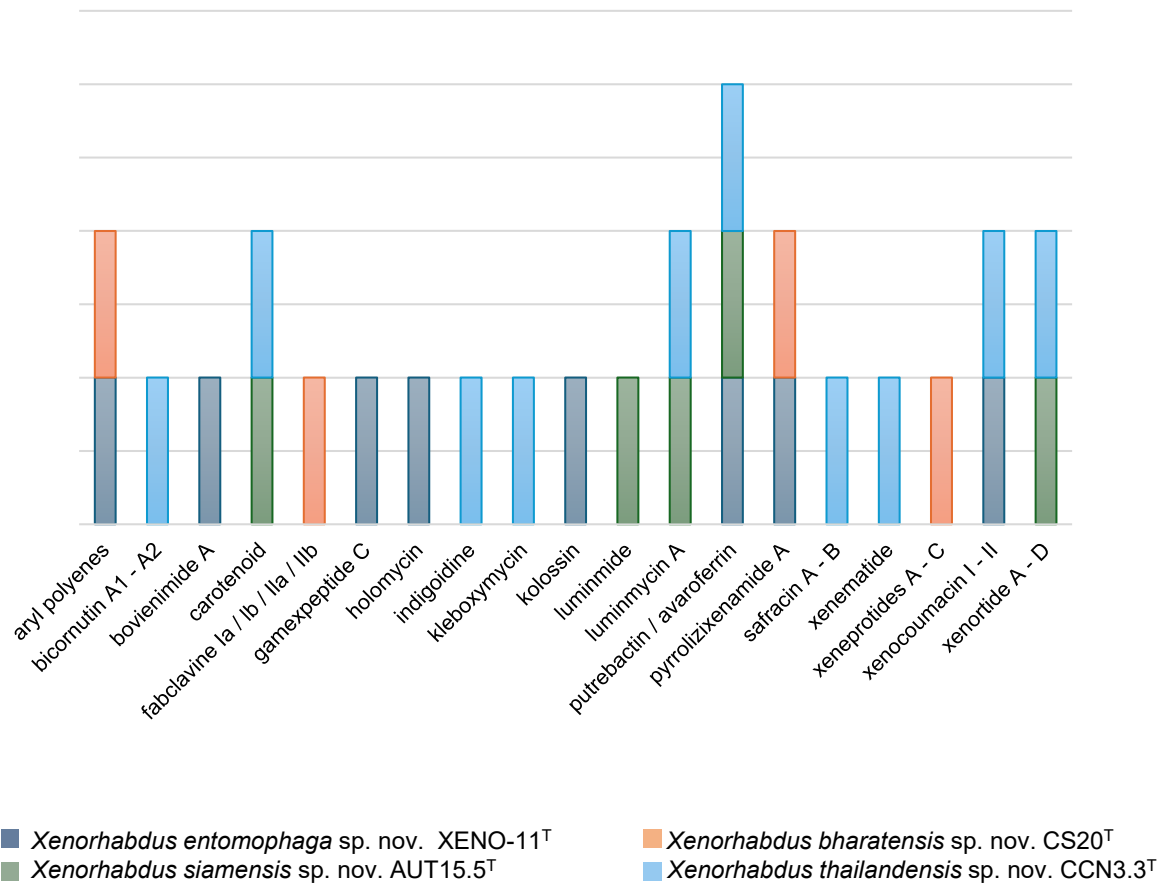


Figure S6. Predicted secondary metabolites produced by *Xenorhabdus bharatensis* sp. nov. CS20^T, *X. entomophaga* sp. nov. XENO-11^T, *X. siamensis* sp. nov. AUT15.5^T, and *X. thailandensis* sp. nov. CCN3.3^T.

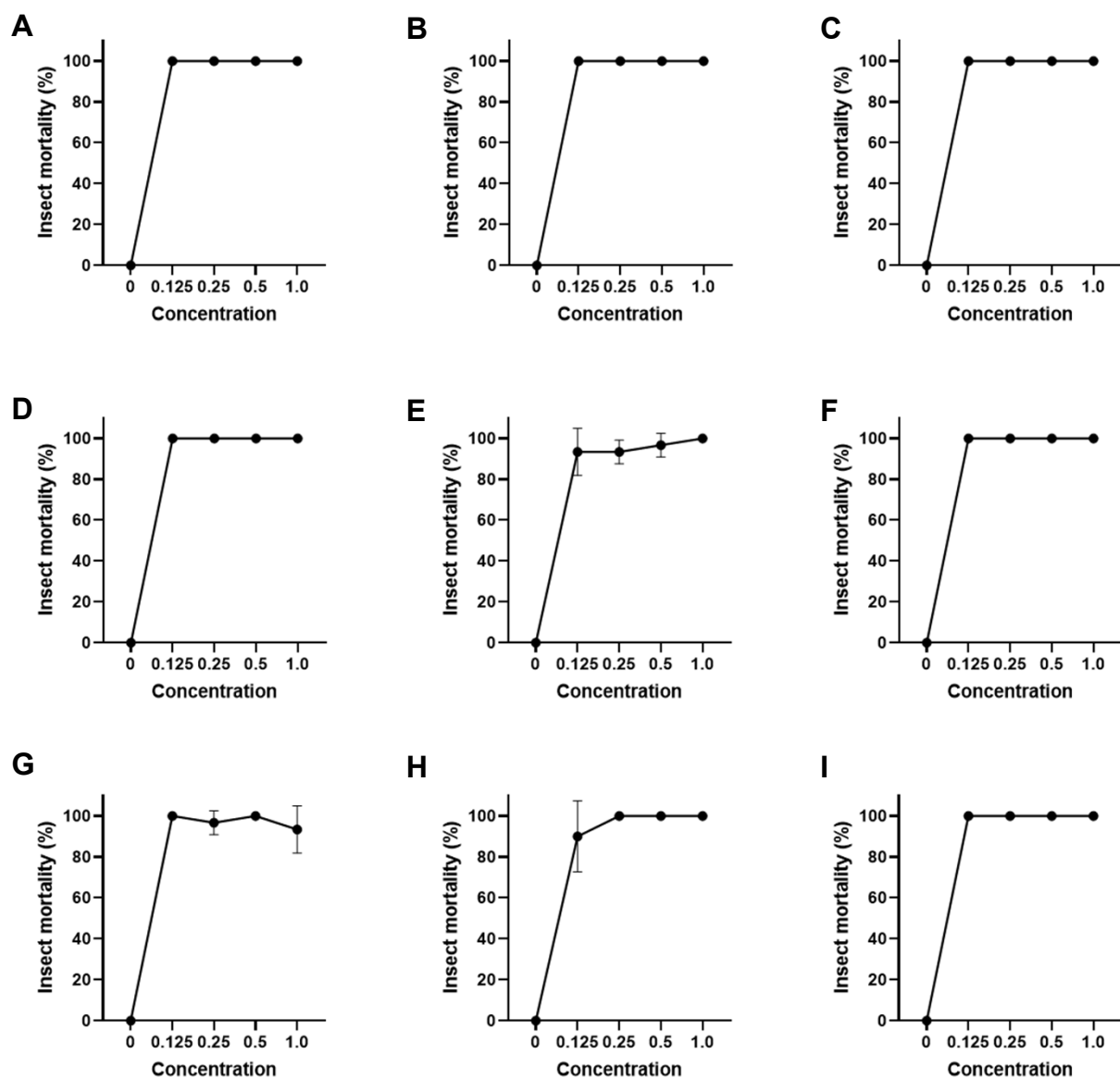


Figure S7. Mortality rate (%) of waxworm larvae 24h after injecting different *Xenorhabdus* strains at various cell concentrations (OD₆₀₀ = 0.125, 0.25, 0.5 and 1.0). A) *X. bharatensis* sp. nov. CS20^T B) *X. entomophaga* sp. nov. XENO-11^T; C) *X. griffiniae* DSM 17911^T; D) *X. indica* DSM 17382^T; E) *X. innexi* DSM 16336^T; F) *X. khoisanae* DSM 25463^T; G) *X. siamensis* sp. nov. AUT15.5^T; H) *X. stockiae* DSM 17904^T; I) *X. thailandensis* sp. nov. CCN3.3^T.

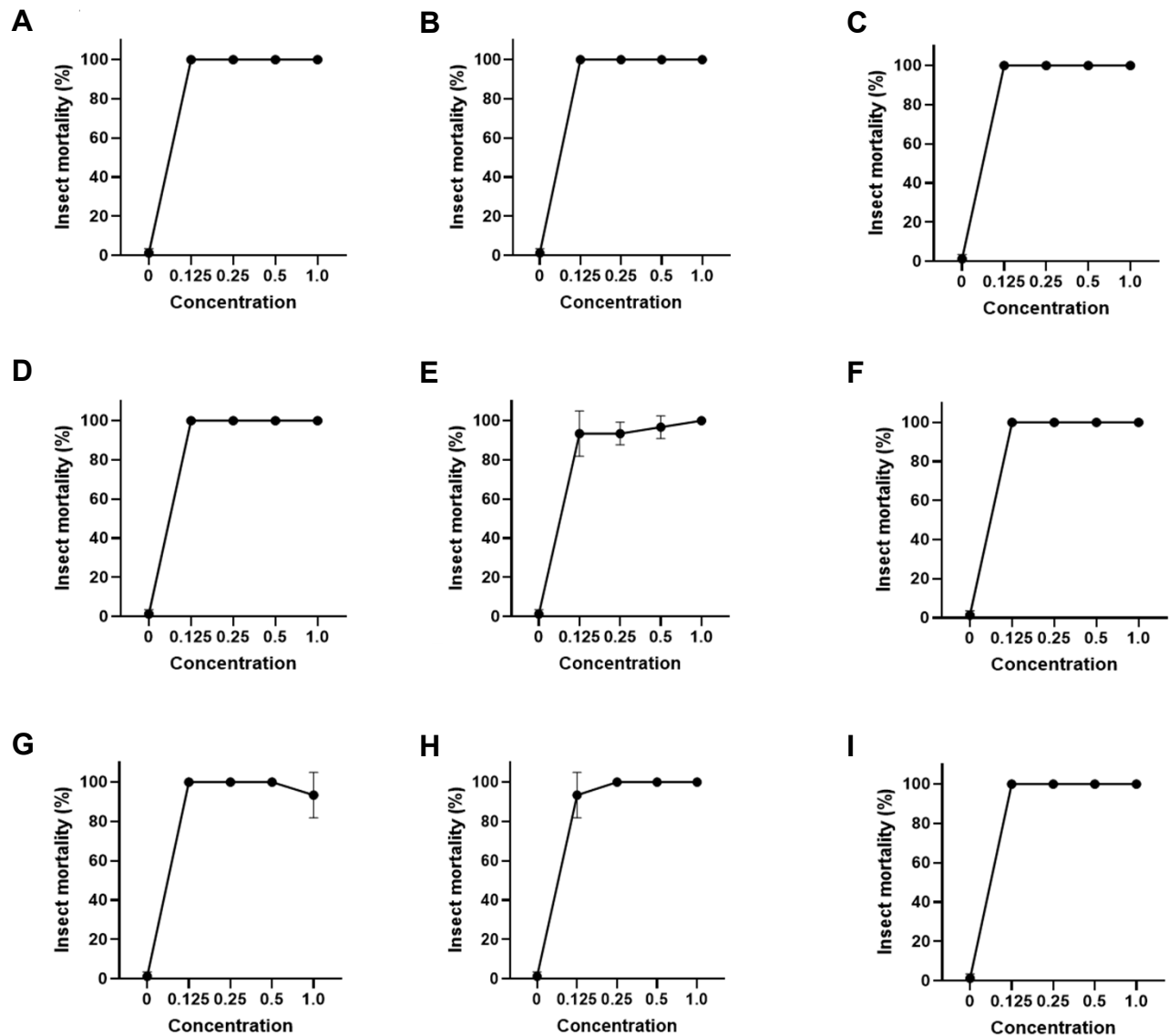


Figure S8. Mortality rate (%) of waxworm larvae 48h after injecting different *Xenorhabdus* strains at various cell concentrations ($OD_{600} = 0.125, 0.25, 0.5$ and 1.0). A) *X. bharatensis* sp. nov. CS20^T B) *X. entomophaga* sp. nov. XENO-11^T; C) *X. griffinae* DSM 17911^T; D) *X. indica* DSM 17382^T; E) *X. innexi* DSM 16336^T; F) *X. khoisanae* DSM 25463^T; G) *X. siamensis* sp. nov. AUT15.5^T; H) *X. stockiae* DSM 17904^T; I) *X. thailandensis* sp. nov. CCN3.3^T.

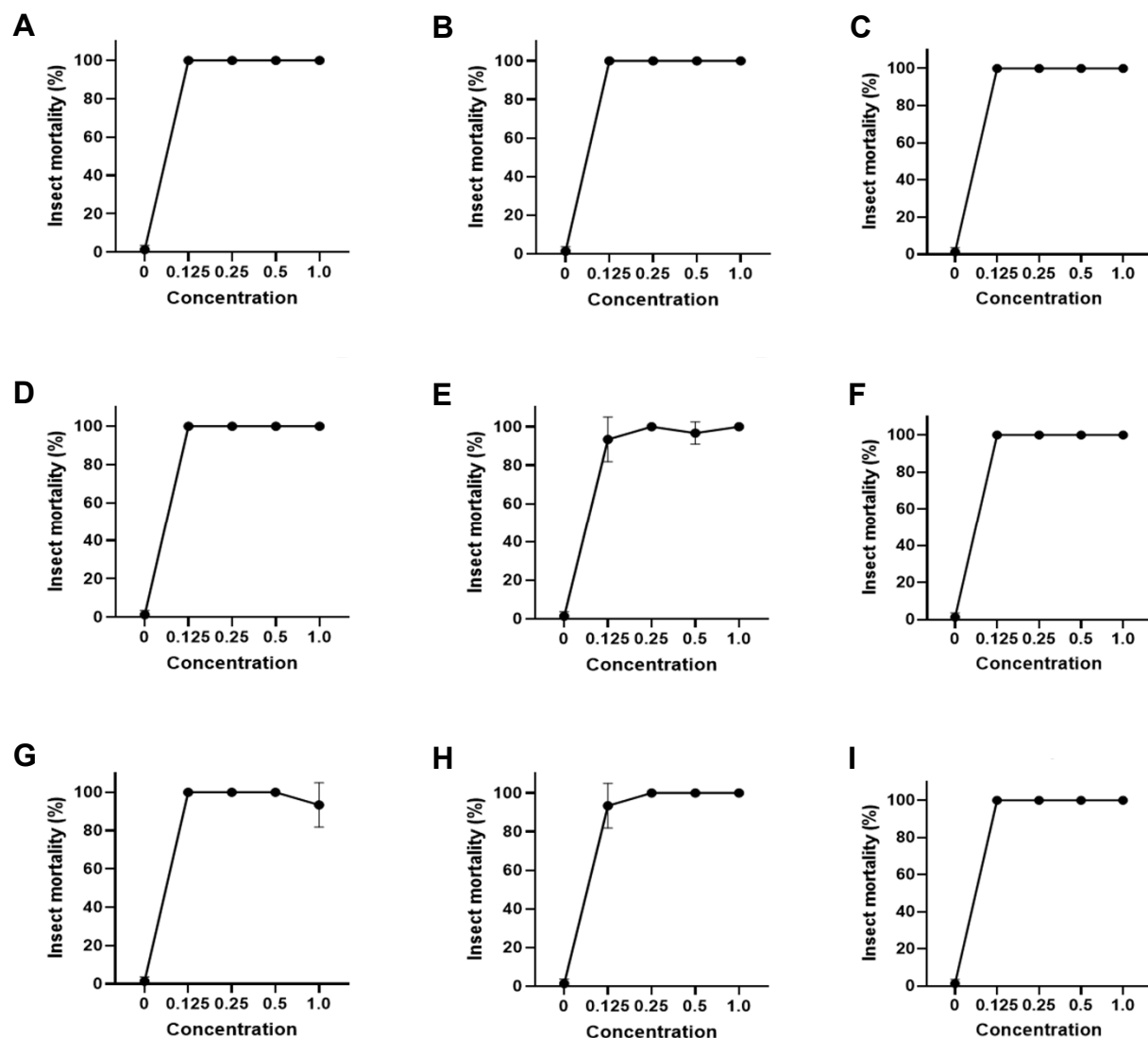


Figure S9. Mortality rate (%) of waxworm larvae 72h after injecting different *Xenorhabdus* strains at various cell concentrations (OD_{600} = 0.125, 0.25, 0.5 and 1.0). A) *X. bharatensis* sp. nov. CS20^T B) *X. entomophaga* sp. nov. XENO-11^T; C) *X. griffiniae* DSM 17911^T; D) *X. indica* DSM 17382^T; E) *X. innexi* DSM 16336^T; F) *X. khoisanensis* DSM 25463^T; G) *X. siamensis* sp. nov. AUT15.5^T; H) *X. stockiae* DSM 17904^T; I) *X. thailandensis* sp. nov. CCN3.3^T.

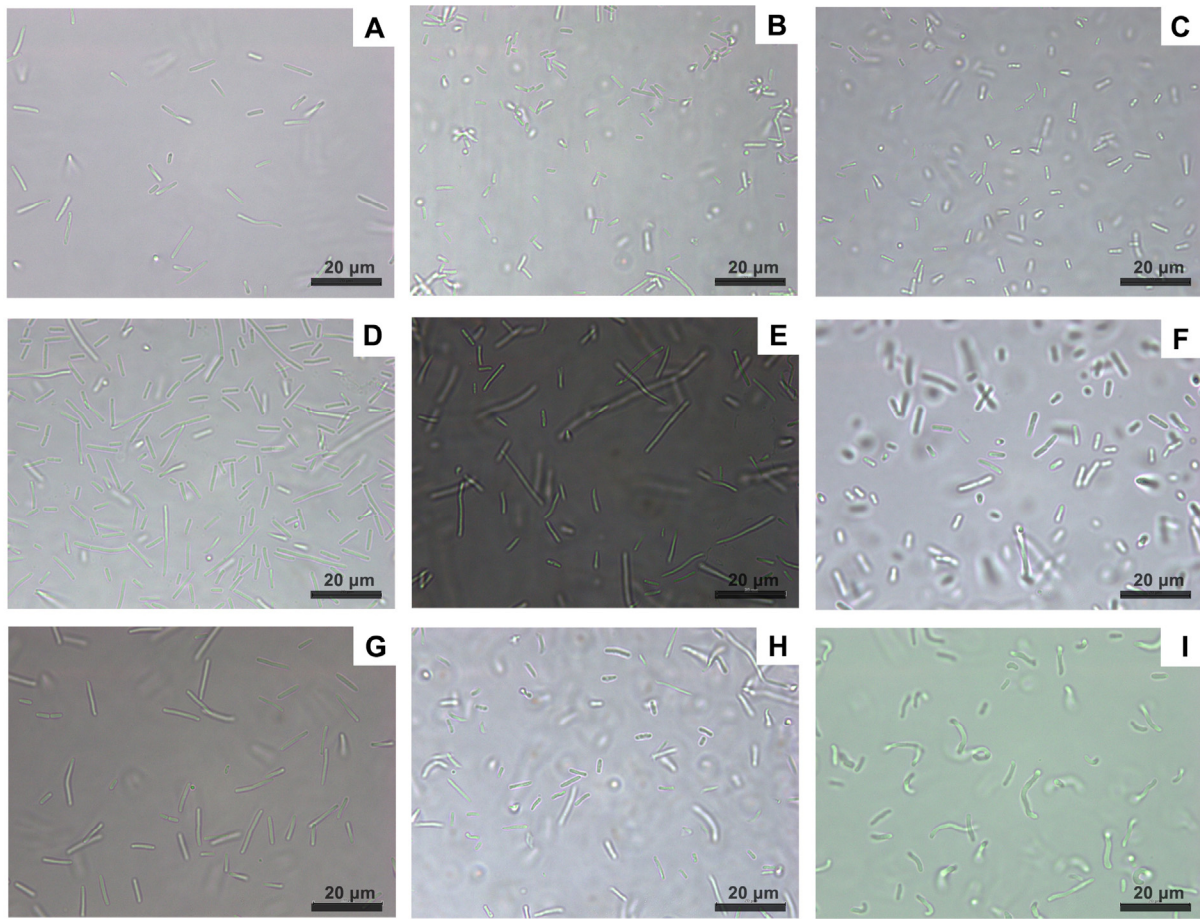


Figure S10. Light microscopy (LM) photographs of the newly described *Xenorhabdus* species and their more closely related species. A) *X. bharatensis* sp. nov. CS20^T; B) *X. entomophaga* sp. nov. XENO-11^T; C) *X. griffiniae* DSM 17911^T; D) *X. indica* DSM 17382^T; E) *X. innexi* DSM 16336^T; F) *X. khoisanae* DSM 25463^T; G) *X. siamensis* sp. nov. AUT15.5^T; H) *X. stockiae* DSM 17904^T; I) *X. thailandensis* sp. nov. CCN3.3^T. Bars correspond to 20 µm.

-SUPPLEMENTARY TABLES-

Table S1. National Center for Biotechnology Information (NCBI) accession numbers of the bacterial sequences used in this study. Sequences generated in this study are shown in bold.

Strain	16S	Genome
<i>X. aichiensis</i> XENO-7 ^T	OQ439939	JAQRFO01
<i>X. anantnagensis</i> XENO-2 ^T	OQ439938	JAQRFN01
<i>X. bakwenae</i> SF857 ^T	OQ418033	CP119194.1
<i>X. beddingii</i> Q58 ^T	AY278675	MUBK01
<i>X. bharatensis</i> sp. nov. CS20^T	PP544787	JBBMXG01
<i>X. bovienii</i> subsp. <i>africana</i> XENO-1 ^T	ON497255	JAMGSK01
<i>X. bovienii</i> subsp. <i>bovienii</i> T228 ^T	AY278673	JANAIF01
<i>X. budapestensis</i> DSM 16342 ^T	AJ810293	NIBS01
<i>X. cabanillasii</i> USTX62 ^T	AY521244	QTUB01
<i>X. doucetiae</i> FRM16 ^T	DQ211709	FO704550
<i>X. eapokensis</i> DL20 ^T	KX602187	MKGQ01
<i>X. ehlersii</i> DSM 16337 ^T	AJ810294	NIBT01
<i>X. entomophaga</i> sp. nov. XENO-11^T	PP544789	JBBMXH01
<i>X. griffiniae</i> DSM 17911 ^T	DQ211710	JAQRFM01
<i>X. hominickii</i> KE01 ^T	DQ211719	NJAI01
<i>X. indica</i> DSM 17382 ^T	AM040494	NKHP01
<i>X. innexi</i> DSM 16336 ^T	AJ810292	NIBU01
<i>X. ishibashii</i> GDh7 ^T	GQ149086	NJAK01
<i>X. japonica</i> DSM 16522 ^T	D78008	FOVO01
<i>X. khoisanae</i> DSM 25463 ^T	HQ142625	JAQRFL01
<i>X. koppenhoeferi</i> USNJ01 ^T	DQ205450	FPBJ01
<i>X. kozodoii</i> SaV ^T	DQ211716	NJCX01
<i>X. lircayensis</i> VLS ^T	MT466535	JACOI01
<i>X. magdalenensis</i> IMI 397775 ^T	HQ877464	JAQRFK01
<i>X. mauleonii</i> VC01 ^T	DQ211715	NITY01
<i>X. miraniensis</i> Q1 ^T	DQ211713	NITZ01
<i>X. nematophila</i> ATCC 19061 ^T	AY278674	FN667742
<i>X. poinarii</i> G6 ^T	D78010	FO704551
<i>X. romanii</i> PR06-A ^T	DQ211717	JAQRFJ01
<i>X. siamensis</i> sp. nov. AUT15.5^T	PP544788	JBBMXE01
<i>X. stockiae</i> DSM 17904 ^T	DQ202309	NJAJ01
<i>X. szentirmaii</i> DSM 16338 ^T	AJ810295	NIBV01
<i>X. thailandensis</i> sp. nov. CCN3.3^T	PP544790	JBBMXF01
<i>X. thuongxuanensis</i> 30TX1 ^T	KX602193	MKGR01
<i>X. vietnamensis</i> VN01 ^T	DQ205447	MUBJ01
<i>X. yunnanensis</i> XENO-10 ^T	OQ439939	JAQRFI01

Table S2. Features of the *Xenorhabdus* genomes used in this study.

Species	Basepairs	Percent G+C	No. proteins
<i>X. aichiensis</i> XENO-7 ^T	4 699 893	44.63	4196
<i>X. ananthnagensis</i> XENO-2 ^T	4 318 764	42.88	3905
<i>X. bakwenae</i> SF857 ^T	4 030 269	44.78	3830
<i>X. beddingii</i> Q58 ^T	4 096 354	45.19	3633
<i>X. bharatensis</i> sp. nov. CS20^T	4 776 084	43.45	4228
<i>X. bovienii</i> subsp. <i>africana</i> XENO-1 ^T	4 674 198	44.73	4217
<i>X. bovienii</i> subsp. <i>bovienii</i> T228 ^T	4 567 727	44.73	4395
<i>X. budapestensis</i> DSM 16342 ^T	4 311 148	43.13	3686
<i>X. cabanillasii</i> USTX62 ^T	4 335 622	42.90	3812
<i>X. doucetiae</i> FRM16 ^T	4 195 202	45.71	3645
<i>X. eapokensis</i> DL20 ^T	4 242 650	43.49	3757
<i>X. ehlersii</i> DSM 16337 ^T	4 058 264	43.78	3807
<i>X. entomophaga</i> sp. nov. XENO-11^T	4 963 997	43.88	4815
<i>X. griffiniae</i> DSM 17911 ^T	4 557 634	43.67	4187
<i>X. hominickii</i> KE01 ^T	5 335 857	43.39	4871
<i>X. indica</i> DSM 17382 ^T	4 501 483	42.86	3800
<i>X. innexi</i> DSM 16336 ^T	4 573 808	43.67	4019
<i>X. ishibashii</i> GDh7 ^T	3 859 346	42.94	3554
<i>X. japonica</i> DSM 16522 ^T	3 560 854	42.70	3214
<i>X. khoisanæ</i> DSM 25463 ^T	4 805 416	43.81	4322
<i>X. koppenhoeferi</i> USNJ01 ^T	3 181 650	43.06	2763
<i>X. kozodoii</i> SaV ^T	4 127 775	44.70	3726
<i>X. lircayensis</i> VLS ^T	4 279 293	44.15	3626
<i>X. magdalenensis</i> IM139775 ^T	3 710 083	45.59	3407
<i>X. mauleonii</i> VC01 ^T	5 117 864	43.90	4428
<i>X. miraniensis</i> Q1 ^T	4 992 355	43.67	4252
<i>X. nematophila</i> ATCC 19061 ^T	4 587 837	44.21	4549
<i>X. poinarii</i> G6 ^T	3 659 523	44.55	3371
<i>X. romanii</i> PR6a ^T	3 879 794	45.05	3631
<i>X. siamensis</i> sp. nov. AUT15.5^T	4 425 965	42.77	3912
<i>X. stockiae</i> DSM 17904 ^T	4 667 712	43.52	4021
<i>X. szentirmaii</i> DSM 16338 ^T	4 824 775	43.98	4566
<i>X. thailandensis</i> sp. nov. CCN3.3^T	3 794 703	43.23	3508
<i>X. thuongxuanensis</i> 30TX1 ^T	3 935 399	43.05	3474
<i>X. vietnamensis</i> VN01 ^T	4 663 685	42.99	3909
<i>X. yunnanensis</i> XENO-10 ^T	4 667 994	43.14	4534

Table S3. Features of the genomes of *X. bharatensis* sp. nov. CS20^T, *X. entomophaga* sp. nov. XENO-11^T, *X. siamensis* sp. nov. AUT15.5^T, and *X. thailandensis* sp. nov. CCN3.3^T generated in this study.

	CS20 ^T	XENO-11 ^T	AUT15.5 ^T	CCN3.3 ^T
Scaffold L50	9.00	42	7	3
Scaffold N50	199653	34174	272423	304672
Scaffold L90	25	146	16	11
Scaffold N90	49219	7008	118374	92447
Scaffold len_max	409348	111868	483853	1146418
Scaffold len_min	208	201	18239	3155
Scaffold len_mean	35910	8342	201180	180700
Scaffold len_median	520	704	184451	92447
Scaffold len_std	79715	16925	120236	265911
Scaffold num_A	1350859	1388244	1271733	1077099
Scaffold num_T	1349817	1397561	1261433	1077322
Scaffold num_C	1045411	1082467	961748	812529
Scaffold num_G	1029997	1095725	931051	827753
Scaffold num_N	0	0	0	0
Scaffold num_bp	4776084	4963997	4425965	3794703
Scaffold num_bp_not_N	4776084	4963997	4425965	3794703
Scaffold num_seq	133	595	22	21
Scaffold GC content overall	43.45	43.88	42.77	43.23

Table S4. Completeness (%) and contamination (%) of the bacterial genomes generated in this study assessed by checkM.

Strain	Completeness (%)	Contamination (%)
<i>X. bharatensis</i> sp. nov. CS20 ^T	100	0.54
<i>X. entomophaga</i> sp. nov. XENO-11 ^T	100	0.0
<i>X. siamensis</i> sp. nov. AUT15.5 ^T	100	1.08
<i>X. thailandensis</i> sp. nov. CCN3.3 ^T	100	0.0

Table S5. Antibiotic-resistance conferring genes of *X. bharatensis* sp. nov. CS20^T, *X. entomophaga* sp. nov. XENO-11^T, *X. siamensis* sp. nov. AUT15.5^T, and *X. thailandensis* sp. nov. CCN3.3^T. (+): present; (-): absent or non-functional.

Gene	Resistance Mechanism	AMR Gene Family	Drug Class	<i>X. bharatensis</i> sp. nov. CS20 ^T	<i>X. entomophaga</i> sp. nov. XENO-11 ^T	<i>X. siamensis</i> sp. nov. AUT15.5 ^T	<i>X. thailandensis</i> sp. nov. CCN3.3 ^T
KpnH	Antibiotic efflux	Major facilitator superfamily (MFS) antibiotic efflux pump	Macrolide antibiotic, fluoroquinolone antibiotic, aminoglycoside antibiotic, carbapenem, cephalosporin, penam, peptide antibiotic	+	+	+	+
rsmA		Resistance-Nodulation-Cell Division (RND) Antibiotic Efflux Pump	Fluoroquinolone antibiotic, diaminopyrimidine antibiotic, phenicol antibiotic	+	+	+	+
CRP			Macrolide antibiotic, fluoroquinolone antibiotic, penam	+	+	+	+
adeF			fluoroquinolone antibiotic, tetracycline antibiotic	+	-	-	-
KpnF		Small Multidrug Resistance (SMR) Antibiotic Efflux Pump	Macrolide antibiotic, aminoglycoside antibiotic, cephalosporin, tetracycline antibiotic, peptide antibiotic, rifamycin antibiotic, disinfecting agents and antiseptics	-	+	+	+
qacJ			Disinfecting agents and antiseptics	-	-	+	+
vanT	Antibiotic target alteration	Glycopeptide Resistance Gene Cluster	Glycopeptide antibiotic	+	+	+	+
EF-Tu		Elfamycin resistant EF-Tu	Elfamycin antibiotic	-	+	+	+
PBP3		Penicillin-Binding Protein Mutations Conferring Resistance to Beta-Lactam Antibiotics	Cephalosporin, cephamycin, penam	-	-	+	+
ArnT		Phosphoethanolamine Transferase	Peptide antibiotic	+	+	+	+
FosG	Antibiotic inactivation	Fosfomycin Thiol Transferase	Phosphonic acid antibiotic	-	-	-	+
FosA8		Fosfomycin Thiol Transferase		-	-	-	+