

**Supplementary materials for Journal of Wildlife Diseases DOI: 10.7589/JWD-D-21-00148: Irina Goodrich, Clifton McKee, Gabriele Margos, and Michael Kosoy. Molecular characterization of a novel relapsing fever *Borrelia* species from the desert cottontail (*Sylvilagus audubonii*) in New Mexico, USA.**

**Supplementary Table 1.** PCR primers used for characterization of relapsing fever *Borrelia* species. Details on the PCR primers and cycling conditions for housekeeping genes (*clpX*, *pepX*, *recG*, and *rplB*) can be found from documentation on the PubMLST website ([https://pubmlst.org/static/organisms/borrelia-spp/Borrelia\\_MLSA\\_database\\_Organism\\_Info.pdf](https://pubmlst.org/static/organisms/borrelia-spp/Borrelia_MLSA_database_Organism_Info.pdf)).

Gene	Product	Primer
<i>flaB</i>	flagellin	Fla-ans5': 5'-TGTGATATCCTTTTAAAGAGACAAATGG-3' Fla-alt3': 5'-TCTAAGCAATGACAATACATATTGAGG-3'
<i>clpX</i>	clp protease subunit X	clpXF109: 5'-GCYATTTGTTTTGAATGTTCTAAAATATG-3' clpXR1277: 5'-TAAAGTTCCTTTTGCCCAAGG-3'
<i>pepX</i>	dipeptidyl aminopeptidase	pepXF361: 5'-AGAGAYTTAAGYTTAKCAGG-3' pepXR1207: 5'-CYATAGTTTCTCTTAAAGAYTGC-3'
<i>recG</i>	ATP-dependent DNA helicase	BmrecF908*: 5'-CTAGYATTCTTYTAATTGAGGC-3' BmrecR1779*: 5'-TTCRGTTAAAGGTTCTTATAAAG-3' BmrecF909-seq: 5'-TAGCATTCTTTAGTTGAGGC-3' Bmrec1671R-seq: 5'-CTCAGCATGCTCAACTACC-3'
<i>rplB</i>	50S ribosomal protein L2	BmrplF18*: 5'-ATTAAGACTTATARGCCAAAAAC-3' BmrplR761*: 5'-GGCTGNCCCCAAGGWGAT-3' BmrplF23-seq: 5'-GACTTATAGGCCAAAAACTTC-3' Bmrpl759R-seq: 5'-GATACAGGATGACGACCACC-3'

**Supplementary Table 2.** *Borrelia* isolates used for phylogenetic analysis of concatenated housekeeping genes. GenBank accession numbers are provided for each gene unless they were retrieved from the PubMLST database.

Species	Isolate	clpX	pepX	recG	rplB
<i>B. afzelii</i>	HLJ01	NC_018887	PubMLST	NC_018887	NC_018887
<i>B. americana</i>	SCW-41	PubMLST	PubMLST	PubMLST	PubMLST
<i>B. andersonii</i>	21123	AB526116	AB526118	AB526120	AB526121
<i>B. anserina</i>	BA2	CP005829	PubMLST	CP005829	CP005829
<i>B. bavariensis</i>	Pbi	CP000013	CP000013	CP000013	CP000013
<i>B. bissettii</i>	DN127	NC_015921	PubMLST	NC_015921	NC_015921
<i>B. burgdorferi</i>	B31	NC_001318	PubMLST	NC_001318	NC_001318
<i>B. californiensis</i>	CA446	KT709518	KT709520	KT709522	KT709523
<i>B. carolinensis</i>	SCW-22	PubMLST	PubMLST	PubMLST	PubMLST
<i>B. chilensis</i>	VA1	PubMLST	PubMLST	CP009910	CP009910
<i>B. coriaceae</i>	Co53	NZ_CP005745	PubMLST	NZ_CP005745	NZ_CP00574
<i>B. crocidurae</i>	Achema	CP003426	PubMLST	PubMLST	CP003426
<i>B. duttonii</i>	Ly	NC_011229	PubMLST	NC_011229	NC_011229
<i>B. finlandensis</i>	SV1	NZ_ABJZ02000005		NZ_ABJZ02000005	NZ_ABJZ02000005
<i>B. garinii</i>	20047	CP018744	PubMLST	CP018744	CP018744
<i>B. hermsii</i>	DAH	NC_010673	PubMLST	NC_010673	NC_010673
<i>B. hispanica</i>	CR1	PubMLST	PubMLST	PubMLST	PubMLST
<i>B. japonica</i>	HO14	AB526148	AB526150	AB526152	AB526153
<i>B. kurtenbachii</i>	25015	PubMLST	PubMLST	PubMLST	PubMLST
<i>B. lanei</i>	CA28-91	PubMLST	PubMLST	PubMLST	PubMLST
<i>B. lusitaniae</i>	PotiB2	PubMLST	PubMLST	PubMLST	PubMLST
<i>B. maritima</i>	CA690	KF939518	KF939520	KF939522	KF939523
<i>B. mayonii</i>	MN14-1539	CP015796	PubMLST	CP015796	PubMLST
<i>B. miyamotoi</i>	HT31	AB900801	AB900803	AB900805	AB900806
<i>B. parkeri</i>	SLO	CP005851	PubMLST	CP005851	CP005851
<i>B. persica</i>	LMU-C01	KP826797	KP826798	KP826800	KP826801
<i>B. recurrentis</i>	A1	NC_011244	PubMLST	NC_011244	NC_011244
<i>B. sinica</i>	CMN3	AB526132	AB526134	AB526136	AB526137
<i>B. spielmanii</i>	A14S	PubMLST	PubMLST	PubMLST	PubMLST
<i>B. tanukii</i>	HK501	PubMLST	PubMLST	PubMLST	PubMLST
<i>B. turcica</i>	IST7	CP028884	PubMLST	CP028884	CP028884
<i>B. turdi</i>	T1990A	NZ_QBLN01000001	PubMLST	NZ_QBLN01000001	NZ_QBLN01000001
<i>B. turicatae</i>	91E135	NC_008710	PubMLST	NC_008710	NC_008710
<i>B. valaisiana</i>	Tom4006	NZ_CP009117	PubMLST	NZ_CP009117	NZ_CP009117
<i>B. yangtzensis</i>	Okinawa-CW62	AB526069	AB526071	AB526073	AB526074
<i>Borrelia</i> sp.	tHM16w	LC171371	LC171373	LC171375	LC171376
<i>Candidatus B. fainii</i>	Qtaro	LC365920	LC365922	LC365923	LC365924
<i>Candidatus B. ibitipoquensis</i>	IP37	PubMLST	PubMLST	PubMLST	PubMLST
<i>Candidatus B. johnsonii</i>	15-3581	MF062076	MF062078	MF062080	MF062081
<i>Candidatus B. kalaharica</i>	15-0335452	KY560341	KY560343	KY560345	KY560346
<i>Candidatus B. mahuryensis</i>	A-FGy1	PubMLST	PubMLST	PubMLST	PubMLST
<i>Candidatus B. tachyglossi</i>	Bc-F10-1268	CP025785		CP025785	CP025785
<i>Candidatus Borrelia</i> sp.	cottontail	new	new	new	new

**Supplementary Table 3.** BLAST search results for amplified gene sequences from the *Borrelia* sp. detected in desert cottontail (*Syvilagus audubonii*) from New Mexico, USA.

Gene	Identity (matching bp)	Accession number	Species	Source (reference)	Location
<i>flaB</i>	96.60% (596/617)	LC170031	<i>Borrelia</i> sp. tHM16w	<i>H. megaspinosa</i> from wild boar (Furuno et al. 2017)	Japan
	96.33% (472/490)	MN958349	<i>Borrelia</i> sp. Ir-Maz173	<i>R. sanguineus</i> from sheep (Naddaf et al. 2020)	Iran
	96.12% (471/490)	LC229594	<i>Borrelia</i> sp. B269_1-AP2017	<i>R. sanguineus</i> from sheep (Pereira et al. 2018)	Portugal
	95.84% (461/481)	KR677090	<i>Borrelia</i> sp. RSF2354	<i>R. sanguineus</i> (Nunes et al. 2016)	Portugal
	95.81% (366/382)	AB897888	<i>Borrelia</i> sp. 130707_13_HJF_flaB	<i>H. japonica</i> from Sika deer (Lee et al. 2014)	Japan
	95.29% (364/382)	LC170019	<i>Borrelia</i> sp. HFW-501	<i>H. flava</i> (Furuno et al. 2017)	Japan
	95.29% (364/382)	LC170021	<i>Borrelia</i> sp. HKL-29	<i>H. kitaokai</i> (Furuno et al. 2017)	Japan
	94.98% (265/279)	AB897886	<i>Borrelia</i> sp. 120217D6	Sika deer (Lee et al. 2014)	Japan
	94.90% (465/490)	KT364297	<i>Borrelia</i> sp. 2366	<i>R. sanguineus</i> (Nunes et al. 2016)	Portugal
	94.39% (454/481)	KR677087	<i>Borrelia</i> sp. HaeL2374	<i>R. sanguineus</i> (Nunes et al. 2016)	Portugal
	94.27% (263/279)	LC278445	<i>Borrelia</i> sp. Deer_1043	Sika deer (Kumagai et al. 2018)	Japan
	94.27% (263/279)	LC278447	<i>Borrelia</i> sp. Deer_1100	Sika deer (Kumagai et al. 2018)	Japan
	93.91% (262/279)	LC278446	<i>Borrelia</i> sp. Deer_1291	Sika deer (Kumagai et al. 2018)	Japan
	93.88% (414/441)	KR677086	<i>Borrelia</i> sp. HaeL418	<i>R. sanguineus</i> (Nunes et al. 2016)	Portugal
	93.55% (261/279)	LC278443	<i>Borrelia</i> sp. Deer_2490	Sika deer (Kumagai et al. 2018)	Japan
	95.46% (589/617)	KF569936	<i>B. theileri</i>	<i>R. geigy</i> from cattle	Mali
	95.46% (589/617)	U26705	<i>B. lonestari</i>	<i>A. americanum</i>	USA
89.72% (567/632)	CP004217	<i>B. miyamotoi</i>	<i>Apodemus argenteus</i>	Japan	
<i>clpX</i>	95.99% (598/623)	LC171371	<i>Borrelia</i> sp. tHM16w	<i>H. megaspinosa</i> from wild boar	Japan
	91.49% (570/623)	CP037471	<i>B. miyamotoi</i>	human	Russia
<i>pepX</i>	94.21% (537/570)	LC171373	<i>Borrelia</i> sp. tHM16w	<i>H. megaspinosa</i> from wild boar	Japan
	87.46% (495/566)	LC164103	<i>B. miyamotoi</i>	<i>I. persulcatus</i>	Japan
<i>recG</i>	95.24% (620/651)	LC171375	<i>Borrelia</i> sp. tHM16w	<i>H. megaspinosa</i> from wild boar	Japan
	88.7% (573/646)	CP017126	<i>B. miyamotoi</i>	<i>I. scapularis</i>	USA
<i>rplB</i>	96.63% (602/623)	LC171376	<i>Borrelia</i> sp. tHM16w	<i>H. megaspinosa</i> from wild boar	Japan
	91.99% (574/624)	CP046389	<i>B. miyamotoi</i>	<i>I. ricinus</i>	Czech Republic

bp – base pairs, *A.* – *Amblyomma*, *H.* – *Haemaphysalis*, *I.* – *Ixodes*, *R.* – *Rhipicephalus*.

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