

Additional file 1

Table S1. Summary of Australian goanna tick samples tested for *Borrelia*

Goanna species	Goanna ID	Tick species	Tick life stage	Number	Positive for <i>Borrelia</i> (%)	
<i>Varanus varius</i> (NSW)	1-11	<i>B. undatum</i>	Female	4	1 (25%)*	
		<i>B. undatum</i>	Male	11	2 (18.18%)*	
	2-11	<i>B. undatum</i>	Female	4	1 (25%)*	
		<i>B. undatum</i>	Male	14	1 (7.14%)*	
	4-11	<i>B. undatum</i>	Female	5	0	
		<i>B. undatum</i>	Male	3	0	
	5-11	<i>B. undatum</i>	Female	5	0	
		<i>B. undatum</i>	Male	17	0	
	7-11	<i>B. undatum</i>	Female	1	0	
		<i>B. undatum</i>	Male	5	0	
	9-11	<i>B. undatum</i>	Male	1	0	
		<i>B. undatum</i>	Nymph	7	0	
	10-11	<i>B. undatum</i>	Female	4	0	
		<i>B. undatum</i>	Male	1	0	
			Total	84	5 (5.95%)	
	<i>Varanus giganteus</i> (NT)	P9	<i>A. calabyi</i>	Female	15	4 (26.67%)*
			<i>A. calabyi</i>	Male	10	2 (20%)
		<i>A. limbatum</i>	Nymph	2	0	
P11		<i>A. limbatum</i>	Nymph	2	0	
P12		<i>A. calabyi</i>	Male	2	1 (50%)*	
P14		<i>A. calabyi</i>	Female	1	1 (100%)*	
P20		<i>A. calabyi</i>	Female	1	0	
P22		<i>A. calabyi</i>	Female	2	0	
		<i>A. fimbriatum</i>	Female	4	2 (50%)*	
P26		<i>A. calabyi</i>	Female	3	0	
		<i>A. fimbriatum</i>	Female	1	1 (100%)*	
		<i>A. limbatum</i>	Nymph	12	4 (33.33%)*	
P27		<i>A. calabyi</i>	Female	1	1 (100%)	
P28		<i>A. calabyi</i>	Female	1	1 (100%)	
		<i>A. fimbriatum</i>	Female	7	5 (71.43%)*	
		<i>A. fimbriatum</i>	Male	12	11 (91.67%)*	
P31		<i>A. calabyi</i>	Female	1	1 (100%)	
		<i>A. calabyi</i>	Male	5	2 (40%)	
P34		<i>A. calabyi</i>	Female	2	2 (100%)*	
		<i>A. fimbriatum</i>	Female	2	1 (50%)	
P36	<i>A. calabyi</i>	Female	2	1 (50%)*		
P37	<i>A. calabyi</i>	Male	1	0		
P38	<i>A. limbatum</i>	Nymph	2	0		
		Total	91	40 (43.95%)		
<i>V. acanthurus</i> (WA)	VA01	<i>A. limbatum</i>	Male	7	0	
		<i>A. limbatum</i>	Female	1	0	
	VA02	<i>A. limbatum</i>	Male	7	0	
	VA03	<i>A. limbatum</i>	Male	5	0	
	VA04	<i>A. limbatum</i>	Male	5	0	
	VA05	<i>A. limbatum</i>	Male	2	0	
	VA06	<i>A. limbatum</i>	Male	2	0	
	VA07	<i>A. limbatum</i>	Male	7	0	
	VA08	<i>A. limbatum</i>	Male	3	0	
	VA09	<i>A. limbatum</i>	Male	3	0	
	VA11	<i>A. limbatum</i>	Male	2	0	
	VA12	<i>A. limbatum</i>	Male	3	0	
		<i>A. limbatum</i>	Female	1	0	
VA15	<i>A. limbatum</i>	Male	7	0		
		Total	55	0		

* Indicates samples for which *flaB* and 16S rRNA amplicons were sequenced.

Table S2. Sequencing statistics for metagenomic sequencing of *Borrelia*

Tick ID	Tick species	Host ID	Host species	Sequencing platform	Read length	Reads passing QC	<i>Borrelia</i> reads	% <i>Borrelia</i> reads
AG58	<i>Bot. undatum</i>	2-11	<i>V. varius</i>	Illumina NextSeq	2 x 150 bp	614,334,544	822,237	0.13%
P9F1	<i>A. calabyi</i>	P9	<i>V. giganteus</i>	Illumina MiSeq	2 x 300 bp	37,758,726	925,977	2.45%

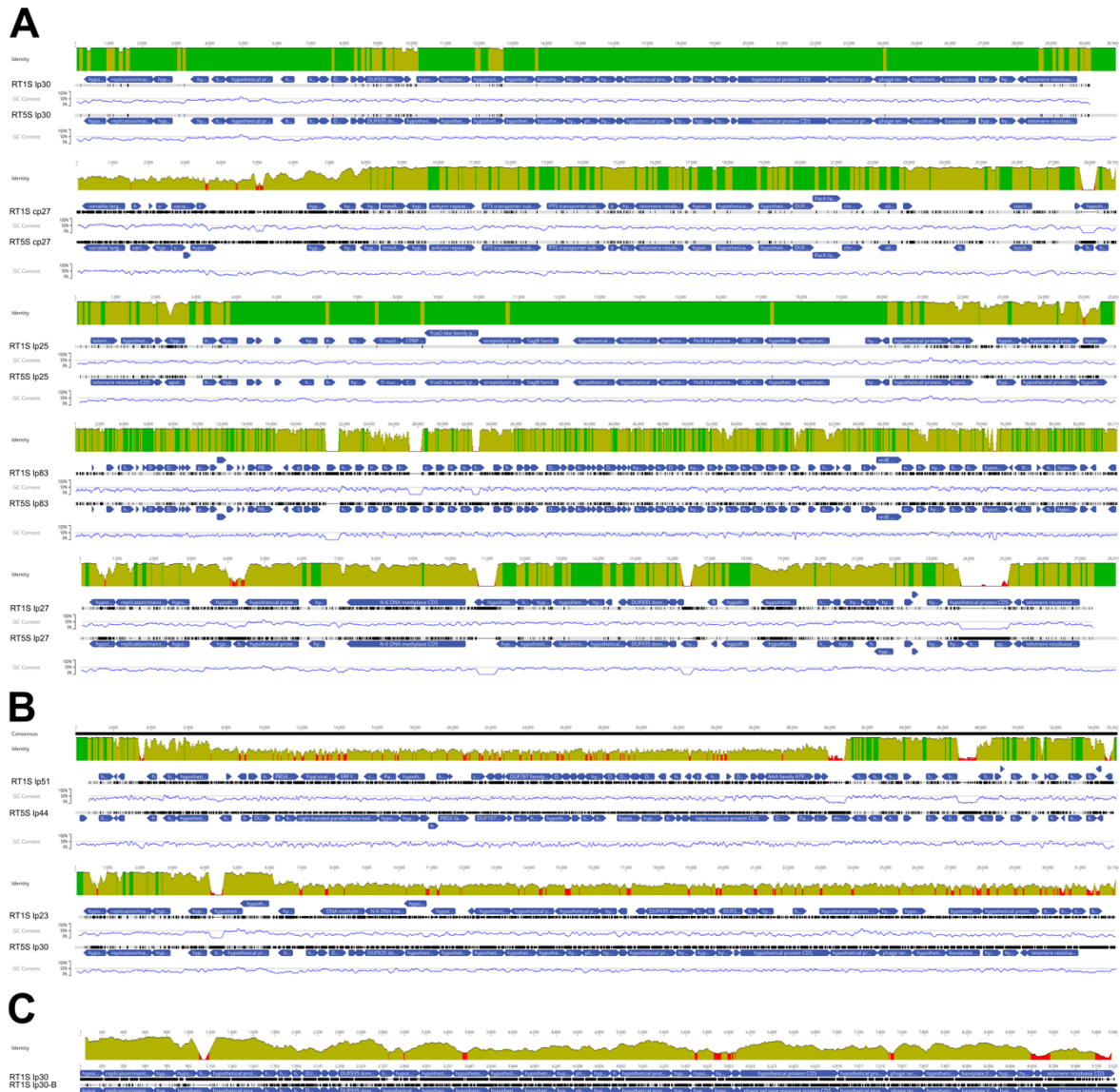


Figure S1 Pairwise alignment plots of *Borrelia salvatorii* strains RT1S and RT5S core plasmids (A), accessory plasmids (B), and duplicated plasmids (C). Pairwise identity plots (log scale, 99 nt sliding window) are shown above each alignment, protein coding sequences are annotated in purple, and GC% plots shown below each alignment.

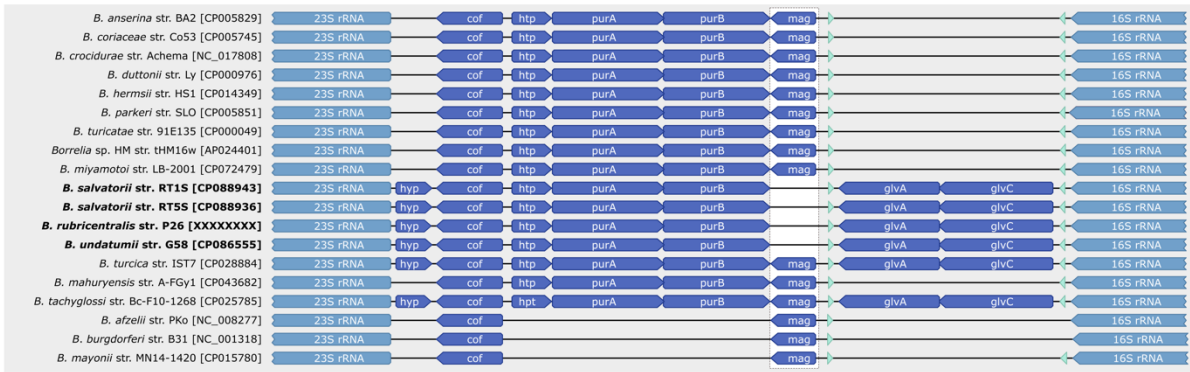


Figure S2 Schematic alignment of the ribosomal RNA operon of RF, REP, and LB *Borrelia* highlighting the deletion of the *mag* gene, which is synaptomorphic for Varanid-associated *Borrelia* spp.

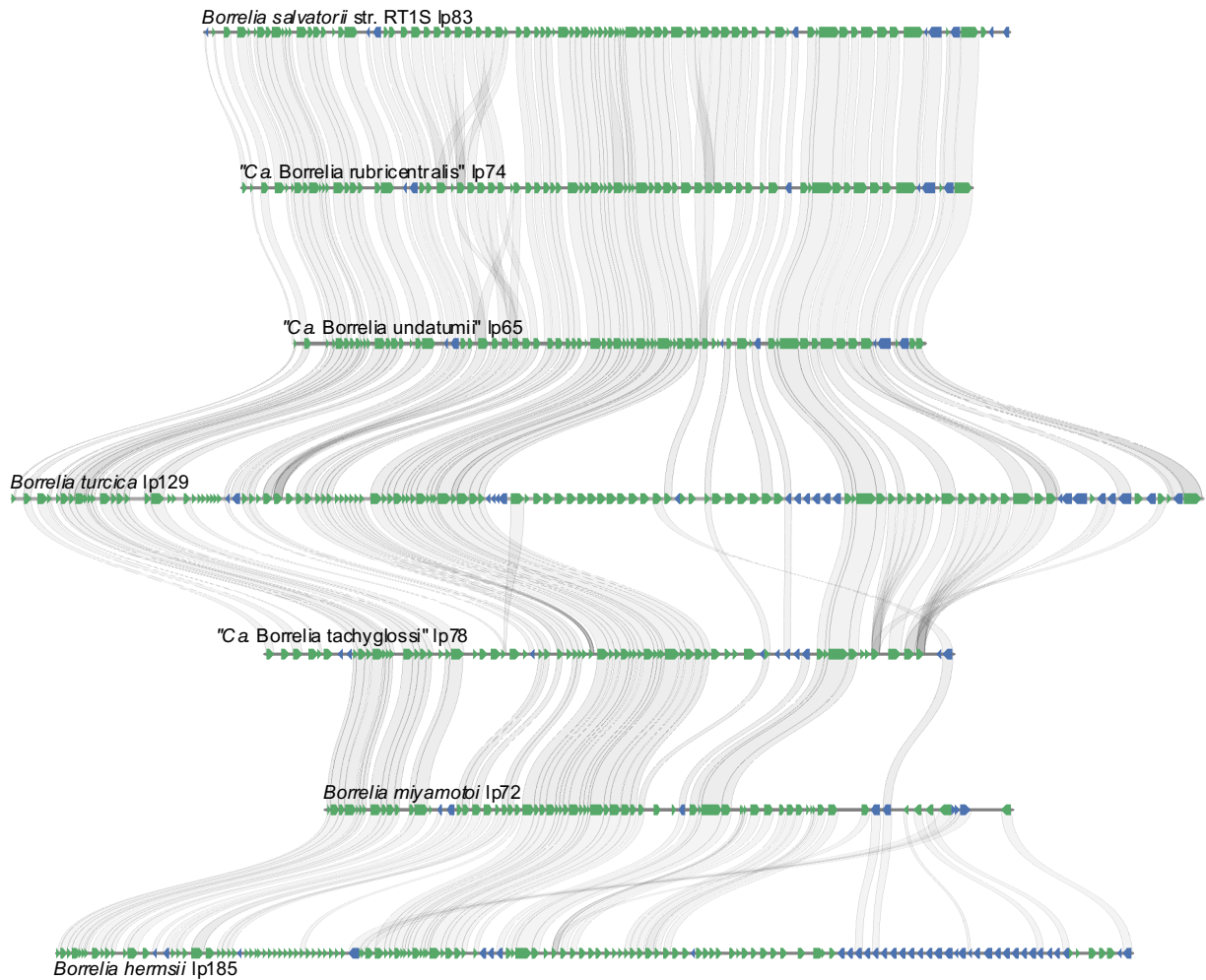


Figure S3. MCscan plot showing orthologous protein coding genes between relapsing fever and reptile-associated *Borrelia* linear 'megaplasms'.

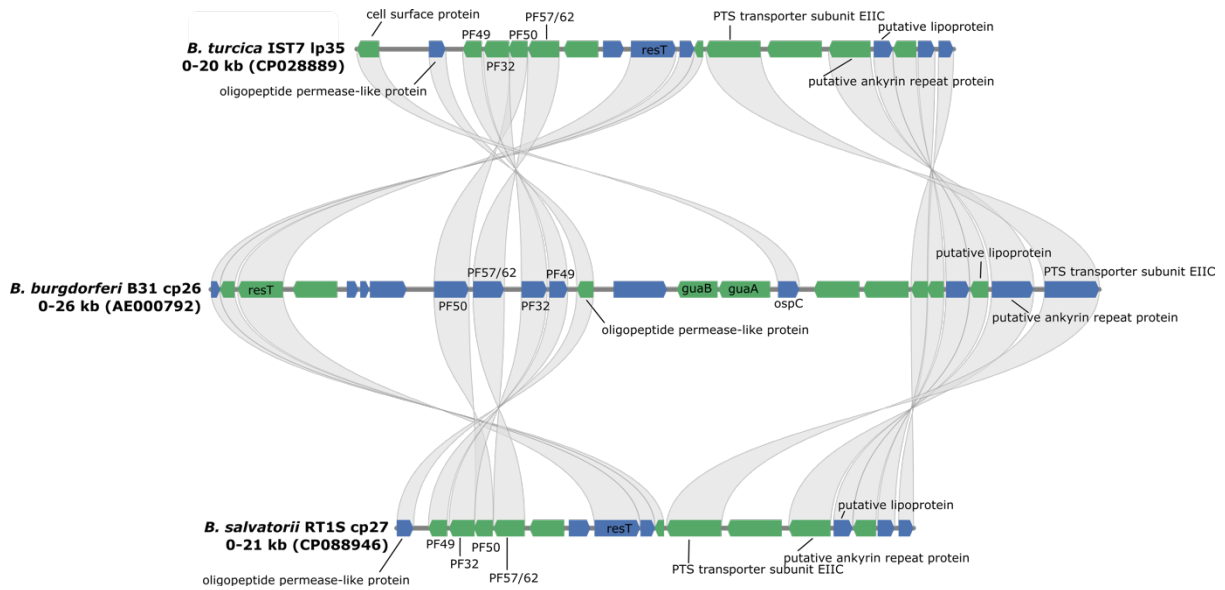


Figure S4. MCscan plot showing orthologous proteins coding genes between *B. burgdorferi* B31 plasmid cp26 and *B. turcica* IST7 plasmid lp25 and *B. salvatorii* RT1S cp27.

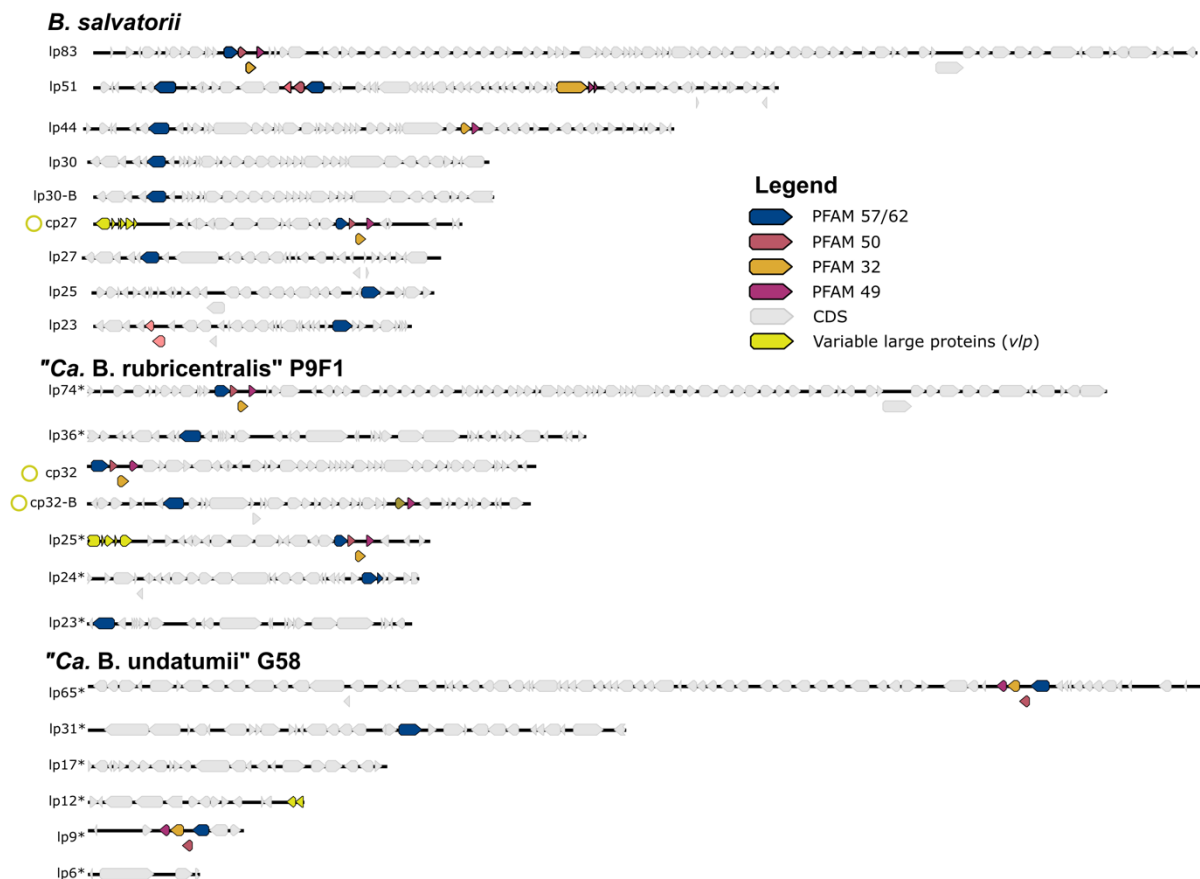


Figure S5 Schematic of Varanid-associated *Borrelia* spp. and the approximate locations of putative plasmid partitioning genes, and variable large proteins.

Extended caption for Fig. 5

The isolate, ORF#, and plasmid is given for each sequence. isolate prefixes: Bsal-RT1S, *B. salvatorii* RT1S; Bsal-RT5S, *B. salvatorii* RT5S; Brub, *B. rubricentralis*; Bund, *B. undatum*; Btur, *B. turcica* IST7; BmahAFGy1, *B. mahuryensis* AFGy1; BtachBcF101268, *B. tachyglossi* BcF101268; baBA2, *B. anserina* BA2; bhDAH, *B. hermsii* DAH; bhYOR, *B. hermsii* YOR; bcCo53, *B. coriaceae* Co53; bpuSUM, *B. puertoricensis* n. sp. SUM; bpSLO, *B. parkeri* SLO; bvRMA01, *B. venezuelensis* RMA01; bt91E135, *B. turicatae* 91E135; btBTE5EL, *B. turicatae* BTE5EL; DB299, *B. bavarensis* Pbi; BBIDN127, *B. bissettii* DN12; BB, *B. burgdorferi* B31; BBUJD1, *B. burgdorferi* JD1; Bmayo, *B. mayonii* MN14-1420; AXH25, *B. miyamotoi* CT13-2396. Reference PF32 and PF57/62 sequences were sourced from [31].