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GENOME SEQUENCES

Complete Genome Sequence of *Rickettsia parkeri* Strain Black Gap

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ABSTRACT A unique genotype of *Rickettsia parkeri*, designated *R. parkeri* strain Black Gap, has thus far been associated exclusively with the North American tick, *Dermacentor parumapertus*. The compete genome consists of a single circular chromosome with 1,329,522 bp and a G+C content of 32.5%.

Rickettsia parkeri, a pathogen of the family *Rickettsiales*, is transmitted predominantly by various *Amblyomma* spp. in several countries of the Western Hemisphere (1). Investigators have identified strains isolated from or detected in ticks and humans that are distinct from *R. parkeri sensu stricto* strains associated with the *Amblyomma maculatum* tick group (2–7). We report the complete genome of a unique genotype of *R. parkeri* that was isolated from a *Dermacentor parumapertus* tick that had been removed from a black-tailed jackrabbit (*Lepus californicus*) in Brewster County, Texas, in 2015 (6). To date, this genotype has been associated exclusively with *D. parumapertus* ticks collected in the western United States and northern Mexico (6, 8).

The Black Gap strain of *R. parkeri* was propagated in Vero E6 (*Chlorocebus aethiops*) cells incubated at 32°C in a 5% CO_2-in-air atmosphere (6) and fed with minimal essential medium supplemented with 0.1 mM nonessential amino acids, 10 mM HEPES buffer, 2 mM L-glutamine, 10 mM sodium pyruvate, and 5% heat-inactivated fetal bovine serum. Genomic DNA was extracted and purified from the contents of a 150-cm^2 tissue culture flask containing the eighth passage of *R. parkeri* using a Gentra PureGene kit (Qiagen, Germantown, MD), as described previously (9). The same DNA preparation was subsequently used for both Pacific Biosciences (PacBio) and Illumina sequencing. Purified DNA was concentrated using AMPure XP paramagnetic beads (Beckman Coulter, Indianapolis, IN), and a SMRTbell Express library preparation kit (v2.0) was used to prepare the library for PacBio sequencing; DNA was not selected for size. Libraries were run on a PacBio Sequel instrument at 10 pM in continuous long-read mode for 10 h using the Sequel Binding kit v3.0 to produce 191,717 reads (N_{50}, 4,621 reads), which were processed subsequently by SmrtLink v8.0 (PacBio, Menlo Park, CA) (with default settings) and converted to fastq files using bam2fastq (SmrtLink v8.0, with default settings). The reads were assembled using two different parameter sets in Flye v2.6 (10) (--meta -g 100m –pacbio-raw and --meta -g 10m –pacbio-raw), producing 3 and 8 contigs, respectively. BLAST+ v2.8 was used to classify the contigs. After removal of primate DNA contigs (Vero E6 cells), a single circularized rickettsial contig was identified in each assembly, with mean coverage of 260× (lengths of 1,329,509 and 1,329,504 bp). The subassemblies option of Flye v2.6 (--g 1.35m –subassemblies) was used to resolve the differences between the two assemblies, resulting in a single circularized contig of 1,329,464 bp with mean coverage of 260×. The contig was polished using Arrow (SmrtLink v8.0, with default settings), and the final contig contained 1,329,522 bp, with a G+C content of 32.5%. The genome was rotated to have a zero site consistent with those of *R. parkeri* strains Portsmouth and Atlantic Rainforest.

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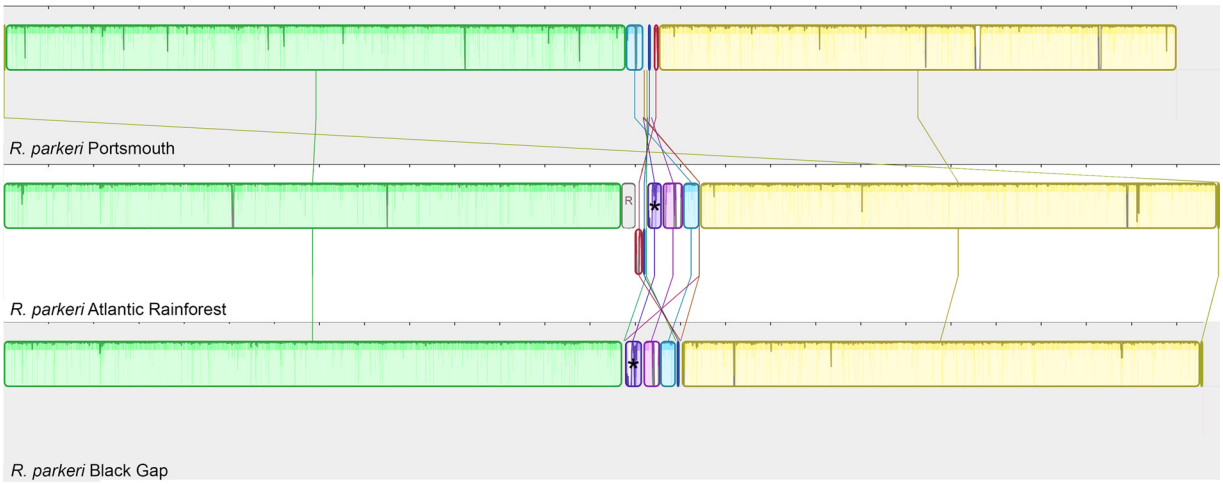

FIG 1 Mauve alignment of complete genomes from *Rickettsia parkeri* strains Portsmouth, Atlantic Rainforest, and Black Gap. Dark purple locally colinear blocks (asterisks) in *R. parkeri* Atlantic Rainforest and *R. parkeri* Black Gap contain a transfer operon that includes *traB* (partial in Black Gap), *traC* (partial in Black Gap), *traW*, *traU*, *trbC*, *traN*, and *traF*. The gray block (R) was added to the Mauve output figure and contains a larger transfer operon found only in Atlantic Rainforest, which includes *traB* to *traF*, *traH*, *traG*, a tetratricopeptide repeat-containing protein, the Flp pilus assembly complex, and ATPase components *tadA*, *traA*, and *traD*. The transfer operons are absent in *R. parkeri* Portsmouth.

Purified DNA was additionally shotgun sequenced using an Illumina MiSeq sequencer (2 × 250-bp paired-end reads) with a Nextera XT library preparation kit (Illumina, Foster City, CA). Reference alignment to the PacBio-assembled genome indicated mean coverage of 15× after removal of reads from contaminating primate (Vero E6 cell) DNA. Pilon v1.23 (11) (--genome --fix all --changes --frags --threads 14 --output) was used to polish the genome using the PacBio and Illumina reads; no changes to the PacBio assembly were indicated by polishing. The polished genome was annotated using Prokka v1.14.5 (12).

Assembly completeness was assessed by using Benchmarking Universal Single-Copy Orthologs (BUSCO) with a *Rickettsiales* Odb10 data set (v2019-04-20) in genome mode (13). Of 364 ortholog groups, only 1 was missing in the assembly. That gene (JRD95_00724; glutamine synthase) was present as a complete open reading frame (ORF) but was not annotated by Prokka because of a 2-bp overlap with an upstream ORF. This gene was manually annotated in the deposited genome.

The Black Gap genome was compared with those of *R. parkeri* strain Portsmouth (GenBank accession number CP003341.1) and *R. parkeri* strain Atlantic Rainforest (GenBank accession number CP040325.1) using progressiveMauve v1.1.1 (14). The three genomes are syntenic, with the differences in genome size (Atlantic Rainforest, 1,348,030 bp; Black Gap, 1,329,522 bp; Portsmouth, 1,300,386 bp) being predominantly due to the presence or absence of bacterial conjugation genes (7) (Fig. 1).

Data availability. The *R. parkeri* strain Black Gap genome sequence was deposited in GenBank with the accession number CP069388. The raw sequencing reads were deposited in the NCBI SRA database; both the PacBio and Illumina raw reads may be found under the BioProject accession number PRJNA699590.

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