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Complete Circularized Genome Sequences of Four Strains of *Elizabethkingia anophelis*, Including Two Novel Strains Isolated from Wild-Caught *Anopheles sinensis*

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ABSTRACT We provide complete circularized genome sequences of two mosquito-derived *Elizabethkingia anophelis* strains with draft sequences currently in the public domain (R26 and Ag1), and two novel *E. anophelis* strains derived from a different mosquito species, *Anopheles sinensis* (AR4-6 and AR6-8). The genetic similarity of all four mosquito-derived strains is remarkable.

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Elizabethkingia anophelis was described based on type strain R26, obtained from the midgut of an *Anopheles gambiae* malaria vector mosquito, G3 strain (1, 2). The bacterium is often found in association with *Anopheles* and *Aedes* mosquitoes (2–7). Previously, we provided draft genomes of strain R26, from the I. Faye lab at Stockholm University in Sweden, and strain Ag1, which was isolated from an *A. gambiae* G3 strain in the J. Xu lab at New Mexico State University, USA (8).

Here we report the genome sequences of two strains, AR4-6 and AR6-8, which were isolated from two female individuals of *Anopheles sinensis* mosquitoes that were caught wild in Sichuan, China, in July 2015. The midgut content of individual mosquito specimens was cultured on LB plates at 29°C; species identity was determined by 16S rRNA gene sequencing. At CDC, isolates were grown on heart infusion agar with 5% rabbit blood at 35°C. Genomic DNA was extracted via the Joint Genome Institute (JGI) bacterial DNA isolation cetyltrimethylammonium bromide (CTAB) protocol (9). Libraries were prepared using the NEBNext Ultra DNA library prep kit (New England Biolabs, Inc., Ipswich, MA, USA), and sequence reads were generated on the Illumina MiSeq instrument (Illumina, Inc., San Diego, CA, USA). Using CLC Genomics Workbench v8.5, reads were trimmed with a quality limit of 0.2, and then *de novo* assembled using default parameters. The resulting contigs were ordered and oriented based on NcoI whole-genome optical maps with MapSolver v3.2 (Opgen, Inc.), and joined based on read sequence alignments using the sequence editing tool BioEdit v7.1.9 (10). Strains R26 and AR6-8 were also sequenced using PacBio. Genomic DNA was extracted using the MasterPure DNA purification kit (Epicentre, Madison, WI, USA) and quality assessed with a Qubit fluorometer (Invitrogen, Carlsbad, CA, USA). Libraries of 20 kb were generated with the SMRTbell template prep kit 1.0 (Pacific Biosciences, Menlo Park, CA) and then size selected with the Blue Pippin (Sage Science, Beverly, MA). Libraries were bound to

polymerase using the DNA/polymerase binding kit P6v2 (Pacific Biosciences) and were then loaded on one SMRTcell (Pacific Biosciences) and sequenced with C4v2 chemistry (Pacific Biosciences) for 360-min movies on the RSII instrument (Pacific Biosciences). The reads were *de novo* assembled using PacBio's hierarchical genome assembly process (HGAP3 and SMRT Analysis 2.3.0). The resulting contigs were examined for overlap and circularized using Circlator (v1.2.1) (11). Illumina reads of strains Ag1 and AR4-6 were mapped to these PacBio assemblies using CLC genomics workbench, and a consensus sequence was extracted. Assemblies for all four strains were compared with the Illumina/Opgen hybrid assemblies, and minor discrepancies were resolved. The complete circularized genomes were annotated by using the NCBI Prokaryotic Genome Annotation Pipeline v4.2.

The genomes of AR4-6 and AR6-8 are identical, with a genome size of 4,093,688 bp. The core genomes of all four mosquito-derived strains are closely related, forming a sublineage in lineage A of *E. anophelis* strains (12). The genomes of Ag1, AR4-6, and AR6-8 contain an ~35.3-kb phage insertion that is absent in R26.

Accession number(s). The genomes of the strains have been deposited at DDBJ/ENA/GenBank with the accession numbers CP023401 (R26), CP023402 (Ag1), CP023403 (AR6-8), and CP023404 (AR4-6).

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