

# Whole-Genome Sequence of “*Candidatus Rickettsia asemboensis*” Strain NMRCii, Isolated from Fleas of Western Kenya

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Herein we present the draft genome sequence and annotation of “*Candidatus Rickettsia asemboensis*” strain NMRCii. “*Ca. Rickettsia asemboensis*” is phylogenetically related to but distinct from the flea-borne spotted fever pathogen *Rickettsia felis*. “*Ca. Rickettsia asemboensis*” was initially identified in and subsequently isolated from *Ctenocephalides* cat and dog fleas from Kenya.

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Rickettsial diseases are endemic worldwide and they can be severe and fatal if diagnosis and antibiotic treatment are delayed (1–3). The causative agents are vectored to humans and animals by various ticks, mites, lice, and fleas. *Rickettsia* spp. are obligate intracellular Gram-negative bacteria requiring biosafety level 3 procedures and laboratories to work with, which makes it impracticable to use cell culture for routine diagnostics. Serological assays with paired samples and rapid quantitative real-time PCR tests are used for confirmatory diagnosis (4).

*Rickettsia felis*, a flea-borne spotted fever pathogen, was first identified in the United States and subsequently found in many other countries (5, 6). A number of *R. felis* and *R. felis*-like organisms have been detected from a wide range of invertebrate hosts (7) and recently from arthropods in the Asembo division, Siaya County, western Kenya (8). Sequences of *rrs*, *gltA*, *ompA*, *ompB*, *sca4*, and the 17 kDa antigen genes from flea DNA preparations suggested the presence of two rickettsial genotypes, one belonging to *R. felis* and a new genotype related to *R. felis* but distinct from it. The differences between this new *Rickettsia* genotype and other established *Rickettsia* species satisfied the gene sequence-based criteria to classify it as a new species, designated “*Candidatus Rickettsia asemboensis*” (8). Prevalence of “*Ca. Rickettsia asemboensis*” in domestic fleas from Asembo was about nine times that of *R. felis*. Interestingly, all clinical rickettsial infections examined in the area were associated with *R. felis* and not “*Ca. Rickettsia asemboensis*” (8, 9).

The genome of “*Ca. Rickettsia asemboensis*” strain NMRCii was sequenced by using the MiSeq sequencer (Illumina, San Diego, CA, USA) with the TruSeq DNA PCR-Free shotgun library and paired-end sequencing with the MiSeq Reagent Kit version 3 (600-cycle). A total of 1,976,742 quality-filtered reads, 600 Mb of sequence data, were subjected to *de novo* assembly with the software DeconSeq (10) and the Roche GS *De Novo* assembler (Newbler) version 2.8 followed by contig scaffolding (11). The sequences of rickettsial culture host, *Aedes albopictus* C6/36 cell line,

were detected based on coverage quantitation and BLAST search and removed from the analysis. The draft genome sequence consisted of 88 contigs with sizes ranging from 207 to 86,066 bp and an average sequence alignment coverage of 346-fold. The estimated genome size and G+C content were 1.44 Mb and 32.2%, respectively.

The IGS Annotation Engine was used for whole-genome structural and functional annotation (12). The “*Ca. Rickettsia asemboensis*” genome has 1,147 predicted protein-coding genes, 33 tRNA genes, and 3 *rrn* operons. The features agree with the genome of *R. felis* (NC\_007109), which is 1.49 Mb in size and contains 1,400 protein-coding genes, 33 tRNA genes, and 3 *rrn* operons (7). Of the predicted *R. felis* proteins, 1,157 (83%) have homologs in “*Ca. Rickettsia asemboensis*.” Further comparative analysis will shed light on the pathogenicity of *R. felis* and the probability of human infection by “*Ca. Rickettsia asemboensis*.”

**Nucleotide sequence accession numbers.** This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under the accession number [JWSW000000000](https://www.ncbi.nlm.nih.gov/nuccore/JWSW000000000). The version described in this paper is version [JWSW010000000](https://www.ncbi.nlm.nih.gov/nuccore/JWSW010000000).

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We declare no conflicts of interest.

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## REFERENCES

- Parola P, Paddock CD, Socolovschi C, Labruna MB, Mediannikov O, Kernif T, Abdad MY, Stenos J, Bitam I, Fournier PE, Raoult D. 2013. Update on tick-borne rickettsioses around the world: a geographic approach. *Clin Microbiol Rev* 26:657–702. <http://dx.doi.org/10.1128/CMR.00032-13>.
- Centers for Disease Control and Prevention. 2004. Fatal cases of Rocky

- Mountain spotted fever in family clusters—three states, 2003. *MMWR. Morb Mortal Wkly Rep* 53:407–410.
3. Centers for Disease Control and Prevention. 2000. From the Centers for Disease Control and Prevention. Consequences of delayed diagnosis of Rocky Mountain spotted fever in children—West Virginia, Michigan, Tennessee, and Oklahoma, May–July 2000. *JAMA* 284:2049–2050.
  4. Luce-Fedrow A, Mullins K, Kostik AP, St John HK, Jiang J, Richards AL. 2015. Strategies for detecting rickettsiae and diagnosing rickettsial diseases. *Future Microbiol*. In press.
  5. Parola P. 2011. *Rickettsia felis*: from a rare disease in the USA to a common cause of fever in sub-Saharan Africa. *Clin Microbiol Infect* 17:996–1000. <http://dx.doi.org/10.1111/j.1469-0691.2011.03516.x>.
  6. Edouard S, Bhengsi S, Dowell SF, Watt G, Parola P, Raoult D. 2014. Two human cases of *Rickettsia felis* infection, Thailand. *Emerg Infect Dis* 20:1780–1781. <http://dx.doi.org/10.3201/eid2010.140905>.
  7. Ogata H, Renesto P, Audic S, Robert C, Blanc G, Fournier PE, Parinello H, Claverie JM, Raoult D. 2005. The genome sequence of *Rickettsia felis* identifies the first putative conjugative plasmid in an obligate intracellular parasite. *PLoS Biol* 3:e248. <http://dx.doi.org/10.1371/journal.pbio.0030248>.
  8. Jiang J, Maina AN, Knobel DL, Cleaveland S, LaDisoit A, Wamburu K, Ogola E, Parola P, Breiman RF, Njenga MK, Richards AL. 2013. Molecular detection of *Rickettsia felis* and *Candidatus Rickettsia asemboensis* in fleas from human habitats, Asembo, Kenya. *Vector Borne Zoonotic Dis* 13:550–558. <http://dx.doi.org/10.1089/vbz.2012.1123>.
  9. Maina AN, Knobel DL, Jiang J, Halliday J, Feikin DR, Cleaveland S, Ng'ang'a Z, Junghae M, Breiman RF, Richards AL, Njenga MK. 2012. *Rickettsia felis* infection in febrile patients, western Kenya, 2007–2010. *Emerg Infect Dis* 18:328–331. <http://dx.doi.org/10.3201/eid1802.111372>.
  10. Schmieder R, Edwards R. 2011. Fast identification and removal of sequence contamination from genomic and metagenomic datasets. *PLoS One* 6:e17288. <http://dx.doi.org/10.1371/journal.pone.0017288>.
  11. Onmus-Leone F, Hang J, Clifford RJ, Yang Y, Riley MC, Kuschner RA, Waterman PE, Lesho EP. 2013. Enhanced *de novo* assembly of high throughput pyrosequencing data using whole genome mapping. *PLoS One* 8:e61762. <http://dx.doi.org/10.1371/journal.pone.0061762>.
  12. Galens K, Orvis J, Daugherty S, Creasy HH, Angiuoli S, White O, Wortman J, Mahurkar A, Giglio MG. 2011. The IGS standard operating procedure for automated prokaryotic annotation. *Stand Genomic Sci* 4:244–251. <http://dx.doi.org/10.4056/sigs.1223234>.