- 1 Complete Coding Genome Sequence for a Novel Multicomponent Kindia Tick Virus
- 2 Detected from Ticks Collected in Guinea
- 4 Running title: Genome Sequence for Kindia Tick Virus from Guinea
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24 25 ABSTRACT 26 Kindia tick virus (KITV) is a novel multicomponent virus first detected by direct sequencing of 27 Rhipicephalus geigyi ticks in Guinea in 2017. Here, we present a complete coding genome 28 sequence for all four segments of KITV/2017/1. This virus appears to be evolutionarily related to 29 unclassified flaviviruses, such as Alongshan virus. 30 31 ANNOUNCEMENT 32 The multicomponent (segmented) tick flaviviruses evolutionarily related to the unsegmented viruses of the genus *Flavivirus* have been recognized since only 2014 (1-4). This group of 33 34 unclassified segmented tick-borne flaviviruses now includes Alongshan virus, Jingmen tick virus 35 (JMTV) and Mogiana tick virus (MGTV), which have been detected in Asia, Europe and South 36 America, respectively. These diverse and globally distributed viruses are capable of infecting a 37 wide range of hosts, such as ticks, animals and humans (1–8). However, only a few complete 38 genome sequences have been reported for JMTV (Kosovo and China), Alongshan virus (China) and MGTV (Brasilia). 39 40 Here, we report the complete coding genome sequences for the first African isolate, named Kindia tick virus (KITV), from *Rhipicephalus geigyi* collected from domestic cattle (*Bos taurus*) 41 42 in Kindia, Guinea, West Africa. Twenty-six pools of 5 ticks each were frozen in liquid nitrogen, 43 crushed by plastic pestles, homogenized in phosphate-buffered saline and used for RNA isolation 44 with TRIzol reagent (Invitrogen Co., USA). Total RNA was quantified with a Qubit RNA Assay Kit (Invitrogen Co., USA) following the manufacturer's instructions. RNA-seq libraries were 45 46 constructed with an NEBNext Ultra RNA Library Prep Kit for Illumina (New England Biolabs). Sequencing was performed using a MiSeq Reagent Kit v3 for 600 cycles. Cutadapt (version 47 48 1.18) and SAMtools (version 0.1.18) were used to remove the Illumina adaptors and duplicate

reads. After removing adapters, the read length was 108-118 bases, with the numbers of reads

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50 per pool ranging from 151,282 to 540,929. The contigs were assembled de novo using the MIRA 51 assembler with default parameters (version 4.9.6). In five pools, we found Mogiana-like 52 fragments using BLASTN. These fragments were aligned to the reference genome segments of 53 Mogiana tick virus isolate MGTV/V4/11 (4). The average coverage of the four segments was 35, 18, 52, and 30, respectively. The complete sequences for KITV/2017/1 were verified by Sanger 54 55 sequencing of Seg 2 (2 short fragments), Seg 3 (1 sf) and Seg 4 (2 sf), with gaps or low 56 coverage, as previously described (9). Total RNA from tick pools positive for KITV/2017/1 was 57 used for first-strand DNA synthesis using a Reverta-L Kit (Interlabservice, Russia). The 58 KITV/2017/1 genetic material was amplified by PCR with specific primers designed based on 59 draft NGS sequences (available on request), with subsequent PCR fragment isolation and sequencing. The sequences for two detected KITVs (KITV/2017/1 and KITV/2017/2) were also 60 61 deposited in GenBank. The nucleotide identity between KITV/2017/1 and KITV/2017/2 was 62 99.7% (Seg 1), 99.4% (Seg 2), 98.1% (Seg 3), and 99.3% (Seg 4). KITV contains putative open reading frames (ORFs) congruent with JMTV and MGTV, namely, nonstructural protein 1 (Seg 63 64 1), VP1 (Seg 2), NSP2 (Seg 3), VP2 and VP3 (Seg 4). The sizes of the sequenced segments 65 (ORFs) are 2968 (2743), 2805 (2262), 2667 (2427) and 2725 (2351) bases, with CG contents of 66 52.3%, 54.9%, 54.4% and 54.2% for each segment, respectively. The lengths of the 5' UTR and 67 3' UTR were different for each segment and were 97-156 and 121-387 bases, respectively. The 68 5' UTR conservative motif GCAAGTGCA typical for JMTV was found in four segments, and 69 the 3` UTR conservative motifs GGCAAGTGC and CAAGTG were also found in Seg 2 and Seg 70 4 of KITV. The divergence between KITV and MGTV/JMTV based on nucleotide sequence was 7–28% (Seg 1), 7.7–20.6% (Seg 2), 2.3–29.3% (Seg 3), and 0.4–22% (Seg 4), and based on 71 72 amino acid sequences, it was 3.1–21.8% (Seg 1), 3.4–20.3% (Seg 2), 1.3–20.8% (Seg 3), and 0.2–15% (Seg 4), as evaluated by BLAST. Given these similarities, we suggest that KITV is a 73 74 new member of the multicomponent (segmented) tick-borne flavivirus group and possibly 75 represents a new species together with MGTV.

- Data availability. GenBank accession numbers for the viral sequences: MH678723 and
- 77 MH678727 (Seg 1), MH678724 and MH678728 (Seg 2), MH678725 and MH678729 (Seg 3),
- 78 and MH678726 and MH678730 (Seg 4); complete sequences MK673133 (Seg 1), MK673134
- 79 (Seg 2), MK673135 (Seg 3), and MK673136 (Seg 4); and Sequence Read Archive accessions
- 80 SRX5930668 to SRX5930671 under project PRJNA545394. The annotations have also been
- 81 deposited into GenBank for these sequences.
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- 88 REFERENCES
- 1. Qin XC, Shi M, Tian JH, Lin XD, Gao DY, He JR, Wang JB, Li CX, Kang YJ, Yu B,
- 90 Zhou DJ, Xu J, Plyusnin A, Holmes EC, Zhang YZ. 2014. A tick-borne segmented RNA
- 91 virus contains genome segments derived from unsegmented viral ancestors. Proc Natl
- 92 Acad Sci U S A 111:6744–6749. https://doi.org/10.1073/pnas.1324194111.
- 93 2. Shi M, Lin XD, Vasilakis N, Tian JH, Li CX, Chen LJ, Eastwood G, Diao XN, Chen
- 94 MH, Chen X, Qin XC, Widen SG, Wood TG, Tesh RB, Xu J, Holmes EC, Zhang YZ.
- 95 2015. Divergent viruses discovered in arthropods and vertebrates revise the evolutionary
- history of the *Flaviviridae* and related viruses. J Virol 90:659–669.
- 97 <u>https://doi.org/10.1128/JVI.02036-15.</u>
- 98 3. Ladner JT, Wiley MR, Beitze lB, Auguste AJ, Dupuis AP, Jr, Lindquist ME, Sibley
- 99 SD, Kota KP, Fetterer D, Eastwood G, Kimmel D, Prieto K, Guzman H, Aliota MT,
- 100 Reyes D, Brueggemann EE, StJohn L, Hyeroba D, Lauck M, Friedrich TC, O'Connor
- DH, Gestole MC, Cazares LH, Popov VL, Castro-Llanos F, Kochel TJ, Kenny T,

- White B, Ward MD, Loaiza JR, Goldberg TL, Weaver SC, Kramer LD, Tesh RB,
- Palacios G. 2016. A multicomponent animal virus isolated from mosquitoes. Cell Host
- 104 Microbe 20:357–367. https://doi.org/10.1016/j.chom.2016.07.011.
- 4. Villa EC, Maruyama SR, de Miranda-Santos IKF, Palacios G, Ladner JT. 2017.
- 106 Complete Coding Genome Sequence for Mogiana Tick Virus, a Jingmenvirus Isolated
- from Ticks in Brazil. Genome Announc 5(18). pii: e00232-17.
- https://doi.org/10.1128/genomeA.00232-17.
- 5. Shi M, Lin XD, Vasilakis N, Tian JH, Li CX, Chen LJ, Eastwood G, Diao XN, Chen
- MH, Chen X, Qin XC, Widen SG, Wood TG, Tesh RB, Xu J, Holmes EC, Zhang YZ.
- 2015. Divergent viruses discovered in arthropods and vertebrates revise the
- evolutionary history of the *Flaviviridae* and related viruses. J Virol 90:659–669.
- https://doi.org/10.1128/JVI.02036-15
- 6. Meng F, Ding M, Tan Z, Zhao Z, Xu L, Wu J, He B, Tu C. 2019. Virome analysis of tick-
- borne viruses in Heilongjiang Province, China. Ticks Tick Borne Dis 10(2):412-420.
- https://doi.org/10.1016/j.ttbdis.2018.12.002.
- 7. Emmerich P, Jakupi X, von Possel R, Berisha L, Halili B, Günther S, Cadar D, Ahmeti S,
- Schmidt-Chanasit J. 2018. Viral metagenomics, genetic and evolutionary characteristics
- of Crimean-Congo hemorrhagic fever orthonairovirus in humans, Kosovo. Infect Genet
- Evol 65:6-11. https://doi.org/10.1016/j.meegid.2018.07.010.
- 8. Wang ZD, Wang B, Wei F, Han SZ, Zhang L, Yang ZT, Yan Y, Lv XL, Li L, Wang SC,
- Song MX, Zhang HJ, Huang SJ, Chen J, Huang FQ, Li S, Liu HH, Hong J, Jin YL, Wang
- W, Zhou JY, Liu Q.A. 2019. New Segmented Virus Associated with Human Febrile
- 124 Illness in China. N Engl J Med 380(22):2116-2125.
- https://doi.org/10.1056/NEJMoa1805068.
- 9. Ponomareva EP, Ternovoi VA, Mikryukova TP, Protopopova EV, Gladysheva AV,
- Shvalov AN, Konovalova SN, Chausov EV, Loktev VB. 2017. Adaptation of tick-borne

encephalitis virus from human brain to different cell cultures induces multiple genomic substitutions. Arch Virol 162(10):3151-3156. https://doi.org/10.1007/s00705-017-3442-x