Title: Oropouche virus detection in saliva and urine.

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Abstract

Oropouche virus (OROV) is an arthropod-borne virus of the *Peribunyaviridae* family, transmitted to humans primarily by *Culicoides paraensis*. It is one of the main arboviruses that infect humans in Brazil, mainly in the Amazon region. We report the OROV detection in the saliva and urine five days after the symptom's onset. Results were further confirmed by nucleotide sequencing and phylogenetic analysis. To our knowledge, this is the first report that OROV may be detected in the saliva and urine of infected patients. In addition, our results may contribute to the current knowledge regarding the natural history of Oropouche fever.

Key words: Arboviruses; Orthobunyavirus; Oropouche virus; Saliva; Urine; Real-Time Polymerase Chain Reaction

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Text

The Oropouche virus (OROV) is an arthropod-borne virus, with a triple-segmented negative-stranded RNA linear genome. Each segment is designated according to its size as L (large), M (medium), and S (small). This arbovirus belongs of the *Peribunyaviridae* family, genus *Orthobunyavirus, species Oropouche orthobunyavirus* (https://talk.ictvonline.org/taxonomy/), and two invertebrate vectors have been associated with the urban transmission cycle, *Culicoides paraensis* (Ceratopogonidae), which is considered the primary vector, and *Culex quinquefasciatus* (Culicidae) [(1)]. Recently, one study reinforced the potential role of *Culex sp.* mosquitoes in the OROV transmission [(2)].

The infection with Oropouche virus can result in an acute febrile and exanthematous illness, with symptoms frequently related to other viral infections such as dengue. Oropouche fever cases were reported in several Brazilian states including Amazonas, Acre, Pará, and Mato Grosso, as well as in other South America countries [(2-9)].

The Oropouche fever is usually confirmed by detecting OROV genome in the plasma or serum of acutely infected patients, or by specific IgM serology during convalescence [(1,10,11)]. Nevertheless, recent studies showed arboviruses detection using other body fluids, such as saliva and urine. This was demonstrated to different viral species such as *Chikungunya virus* (CHIKV, family *Togaviridae*, *Alphavirus* genus) [(12,13)]; *Dengue virus* (DENV) [(14)], *West Nile virus* (WNV) [(15)], and *Zika virus* (ZIKV) [(16-18)], all members of the *Flaviviridae* family, *Flavivirus* genus. On the other hand, the detection of an Orthobunyavirus in the saliva or urine was not reported to date. Therefore, this study aimed to investigate the presence of OROV in these biological specimens, during the acute phase of the illness.

Between February and June 2016, a total of 352 acute-phase specimens, collected amid 0 to 5 days after symptom's onset, were sent to Instituto Leônidas e Maria Deane – Fiocruz (ILMD), a research unit of the Brazilian Ministry of Health that was responsible for the laboratory diagnosis of ZIKV during its emergence in the Amazonas State, Brazil. Initially, plasma samples were submitted to RNA extraction using QIAamp Viral RNA Mini Kit (Qiagen), according to the manufacturer's instructions. Posteriorly, we tested all samples for ZIKV [19]; CHIKV [20], and DENV [21], by reverse transcription real-time polymerase chain reaction (RT-qPCR). A multiplex RT-qPCR assay further tested negative samples for Mayaro virus (MAYV) and OROV [10].

We evaluated the saliva and the urine of five OROV positive patients in plasma, using the same protocol. Subsequently, the OROV positive samples were submitted to conventional RT-PCR targeting L, M, and S segments using a protocol developed by our group. Initially, we performed the reverse transcription reaction using SuperScript IV reverse transcriptase and random primers (Thermo Fisher Scientific). The cDNA was amplified in a reaction using 1.5mM of Mg^{2+}, 0.2 mM of dNTPs, 1U of Platinum Taq DNA Polymerase (Thermo Fisher Scientific) and 0.3 µM of specific primers for S and L segments, and 0.5 µM for M segment (Table).

The nucleotide sequencing reaction was carried out on an ABI3130 genetic analyzer at the ILMD genomics platform. The data were analyzed using the Geneious software v10.2.6 [22] for quality check, trimming, and contig assembly. The genome segments sequenced in this study were analyzed together with three different datasets, one for each segment, containing 75 Orthobunyavirus species recognized by the International Committee on Taxonomy of Viruses (ICTV - Virus Metadata Repository: version June 1, 2019; MSL34 - https://talk.ictvonline.org/taxonomy/vmr/m/vmr-file-

repository/8287/download), with the full genome records available in GenBank at 01-Jun-2019. All datasets were aligned with MUSCLE (codons) embedded in MEGA X software [23]. Species confirmation was performed using phylogenetic reconstruction by Bayesian Inference (BI) with MrBayes 3.2.6 with two runs and 20 million Markov chain Monte Carlo (MCMC) generations [24] at CIPRES Science Gateway V. 3.3 (https://www.phylo.org) and maximum-likelihood (ML) with PhyML 3.0 [25] with Smart Model Selection (SMS) [26] (http://www.atgc-montpellier.fr/phyml/). All procedures of this study were according to the Ethics Committee of the State University of Amazonas (CAAE: 56,745,116.6.0000.5016).

Among the tested plasma samples, 202 were positive for ZIKV, one for CHIKV, three for DENV, and five to OROV. All OROV positive patients in plasma had saliva and urine further evaluated. One fifty-one years-old female patient (BR_AM_ILMD_0240AOS_2016), living in Manaus, Amazonas State, Brazil, whose samples were collected at 2016-04-11, five days after the symptom's onset, presented positivity for OROV both in saliva and urine, with Cts values of 31 and 26, respectively. According to the medical records, the patient presents fever, rash, myalgia, pruritus, headache, arthralgia, lymphadenopathy, diarrhea, and vomit during illness.

Partial CDS sequencing was successful for both the L (396bp), M (648bp), and S (555bp) segments and these sequences were used for phylogenetic reconstruction, using a dataset of ICTV recognized Orthobunyavirus species. Both BI and ML phylogeny were evaluated using the nucleotide substitution model GTR+G+I, as selected by the SMS approach. All Bayesian runs reached convergence with an average standard deviation of split frequencies lower than 0.009 and ESS values > 200. For the three genome segments, the sample BR_AM_ILMD_0240AOS_2016 clustered with the

OROV RefSeq with high (1.0) posterior probability support (Figure). The same topology was observed in the ML tree (data not shown).

Several reports have shown that different arboviruses like ZIKV and CHIKV can be detected testing unusual body fluids, such as saliva and urine. Two studies with samples collected from patients infected with ZIKV showed that some individuals were positive only in saliva and not in serum [17,27]. Interesting, our group found similar results during the ZIKV emergence in the Amazonas State, Brazil (unpublished observations). Other reports showed that saliva might be an alternative specimen for CHIKV detection during the acute phase of illness, with positivity raging from 58.3 to 77% [12,13]. However, no previous study reported the detection of a member of the *Orthobunyavirus* genus in these biological fluids.

Therefore, we decided to investigate if OROV, an endemic arbovirus in the Amazon region, could also be identified using the same biological specimens. In the present study, OROV was detected by RT-qPCR in saliva and urine of one patient, whose specimens were collected five days after the symptom's onset. This result was further confirmed by conventional RT-PCR, followed by nucleotide sequencing and phylogenetic analysis using the ICTV reference database for orthobunyaviruses, which clustered all the three genomic segments sequences obtained in this work with the OROV RefSeqs.

It was beyond the scope of the present study to assess the best human specimen for OROV detection, but it was interesting that we found the higher viral load in urine, when compared with saliva or plasma. Prior studies have also reported the detection of arboviruses in urine. One study with WNV, an arbovirus of the *Flaviviridae* family, reported a higher viral load in urine than in plasma, during the acute phase of illness [15]. On the other hand, two different studies with CHIKV and DENV showed a significantly

lower rate of detection when urine was tested during the first days after symptom's onset, in comparison with samples collected during the second week of illness [13,14]. Altogether, these results suggest that urine may be used as a specimen for different arbovirus detection. Albeit, longitudinal studies, with a more significant number of patients, are necessary to evaluate the potential use of different body fluids for OROV detection.

To our knowledge, this is the first report that OROV may be detected in the saliva and urine of infected patients, showing that these specimens can be employed as alternative sources for the detection of OROV and, perhaps, for other members in the *Peribunyaviridae* family. Moreover, the detection of OROV in urine and saliva strongly suggests that this virus sheds into additional body fluids than blood and Cerebrospinal fluid, as previously reported [28]. Therefore, our results may further contribute to the current knowledge regarding the natural history of Oropouche fever.

Nucleotide sequence accession number:

The partial CDS sequences of OROV isolate BR_AM_ILMD_0240AOS_2016 are available in GenBank, under the accession numbers MN419356 (L); MN419357 (M); MN419358 (S).

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AUTHOR'S CONTRIBUTION:

VAN, LFA, JHAS, and FGN conceived the study. VAN, LFA, JHAS, and FGN designed the study protocol. VAN, DCSM, KPP, AJLC, VCS, and FGN performed the molecular tests and the analysis and interpretation of these data. LFA and JHAS collected clinical information. VAN and FGN wrote the manuscript. FGN financed the study. VAN, LFA, and FGN critically revised the manuscript for intellectual content. All authors read and approved the final manuscript.

REFERENCES

1. Travassos da Rosa JF, de Souza WM, Pinheiro F de P, Figueiredo ML, Cardoso JF, Acrani GO, et al. Oropouche Virus: Clinical, Epidemiological, and Molecular Aspects of a Neglected Orthobunyavirus. Am J Trop Med Hyg 2017;96(5):1019–30.

2. Cardoso BF, Serra OP, Heinen LBDS, Zuchi N, Souza VC de, Naveca FG, et al. Detection of Oropouche virus segment S in patients and inCulex quinquefasciatus in the state of Mato Grosso, Brazil. Mem Inst Oswaldo Cruz 2015;110(6):745–54.

3. Mourão MPG, Bastos MS, Gimaqu JBL, Mota BR, Souza GS, Grimmer GHN, et al. Oropouche fever outbreak, Manaus, Brazil, 2007-2008. Emerging Infect Dis 2009;15(12):2063–4.

4. Azevedo RSDS, Nunes MRT, Chiang JO, Bensabath G, Vasconcelos HB, Pinto AYDN, et al. Reemergence of Oropouche fever, northern Brazil. Emerging Infect Dis 2007;13(6):912–5.

5. Bernardes-Terzian AC, de-Moraes-Bronzoni RV, Drumond BP, Da Silva-Nunes M, da-Silva NS, Urbano-Ferreira M, et al. Sporadic oropouche virus infection, acre, Brazil. Emerging Infect Dis 2009;15(2):348–50.

6. Naveca FG, Nascimento VA, Souza VC, de Figueiredo RMP. Human Orthobunyavirus Infections, Tefé, Amazonas, Brazil. PLoS Curr 2018;10.

7. Romero-Alvarez D, Escobar LE. Oropouche fever, an emergent disease from the Americas. Microbes Infect 2018;20(3):135–46.

8. Wise EL, Pullan ST, Márquez S, Paz V, Mosquera JD, Zapata S, et al. Isolation of Oropouche Virus from Febrile Patient, Ecuador. Emerging Infect Dis 2018;24(5):935–7.

9. Alva-Urcia C, Aguilar-Luis MA, Palomares-Reyes C, Silva-Caso W, Suarez-Ognio L, Weilg P, et al. Emerging and reemerging arboviruses: A new threat in Eastern Peru. PLoS ONE 2017;12(11):e0187897.

10. Naveca FG, Nascimento VAD, Souza VC de, Nunes BTD, Rodrigues DSG, Vasconcelos PFDC. Multiplexed reverse transcription real-time polymerase chain reaction for simultaneous detection of Mayaro, Oropouche, and Oropouche-like viruses. Mem Inst Oswaldo Cruz 2017;112(7):510–3.

11. Nunes MRT, de Souza WM, Savji N, Figueiredo ML, Cardoso JF, da Silva SP, et al. Oropouche orthobunyavirus: Genetic characterization of full-length genomes and development of molecular methods to discriminate natural reassortments. Infect Genet Evol 2019;68:16–22.

12. Gardner J, Rudd PA, Prow NA, Belarbi E, Roques P, Larcher T, et al. Infectious Chikungunya Virus in the Saliva of Mice, Monkeys and Humans. PLoS ONE 2015;10(10):e0139481.

13. Musso D, Teissier A, Rouault E, Teururai S, de Pina J-J, Nhan T-X. Detection of chikungunya virus in saliva and urine. Virol J 2016;13(1):102.

14. Hirayama T, Mizuno Y, Takeshita N, Kotaki A, Tajima S, Omatsu T, et al. Detection of dengue virus genome in urine by real-time reverse transcriptase PCR: a laboratory diagnostic method useful after disappearance of the genome in serum. J Clin Microbiol 2012;50(6):2047–52.

15. Barzon L, Pacenti M, Franchin E, Pagni S, Martello T, Cattai M, et al. Excretion of West Nile virus in urine during acute infection. J Infect Dis 2013;208(7):1086–92.

16. Barzon L, Pacenti M, Berto A, Sinigaglia A, Franchin E, Lavezzo E, et al. Isolation of infectious Zika virus from saliva and prolonged viral RNA shedding in a traveller returning from the Dominican Republic to Italy, January 2016. Euro Surveill 2016;21(10):30159.

17. Musso D, Roche C, Nhan T-X, Robin E, Teissier A, Cao-Lormeau V-M. Detection of Zika virus in saliva. J Clin Virol 2015;68:53–5.

18. Jeong YE, Cha G-W, Cho JE, Lee EJ, Jee Y, Lee W-J. Viral and serological kinetics in Zika virus-infected patients in South Korea. Virol J 2017;14(1):70–6.

19. Lanciotti RS, Kosoy OL, Laven JJ, Velez JO, Lambert AJ, Johnson AJ, et al. Genetic and Serologic Properties of Zika Virus Associated with an Epidemic, Yap State, Micronesia, 2007. Emerging Infect Dis 2008;14(8):1232–9.

20. Lanciotti RS, Kosoy OL, Laven JJ, Panella AJ, Velez JO, Lambert AJ, et al. Chikungunya virus in US travelers returning from India, 2006. Emerging Infect Dis 2007;13(5):764–7.

21. Gurukumar KR, Priyadarshini D, Patil JA, Bhagat A, Singh A, Shah PS, et al. Development of real time PCR for detection and quantitation of Dengue Viruses. Virol J 2009;6(1):10.

22. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 2012;28(12):1647–9.

23. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol Biol Evol 2018;35(6):1547–9.

24. Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, et al. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 2012;61(3):539–42.

25. Guindon S, Lethiec F, Duroux P, Gascuel O. PHYML Online--a web server for fast maximum likelihood-based phylogenetic inference. Nucleic Acids Res 2005;33(Web Server issue):W557–9.

26. Lefort V, Longueville J-E, Gascuel O. SMS: Smart Model Selection in PhyML. Mol Biol Evol 2017;34(9):2422–4.

27. Abdalla LF, Santos JHA, Barreto RTJ, Souza EME, D'Assunção FF, Borges MA, et al. Atrial fibrillation in a patient with Zika virus infection. Virol J 2018;15(1):23.

28. Bastos M de S, Figueiredo LTM, Naveca FG, Monte RL, Lessa N, Pinto de Figueiredo RM, et al. Identification of Oropouche Orthobunyavirus in the cerebrospinal fluid of three patients in the Amazonas, Brazil. Am J Trop Med Hyg 2012;86(4):732–5.

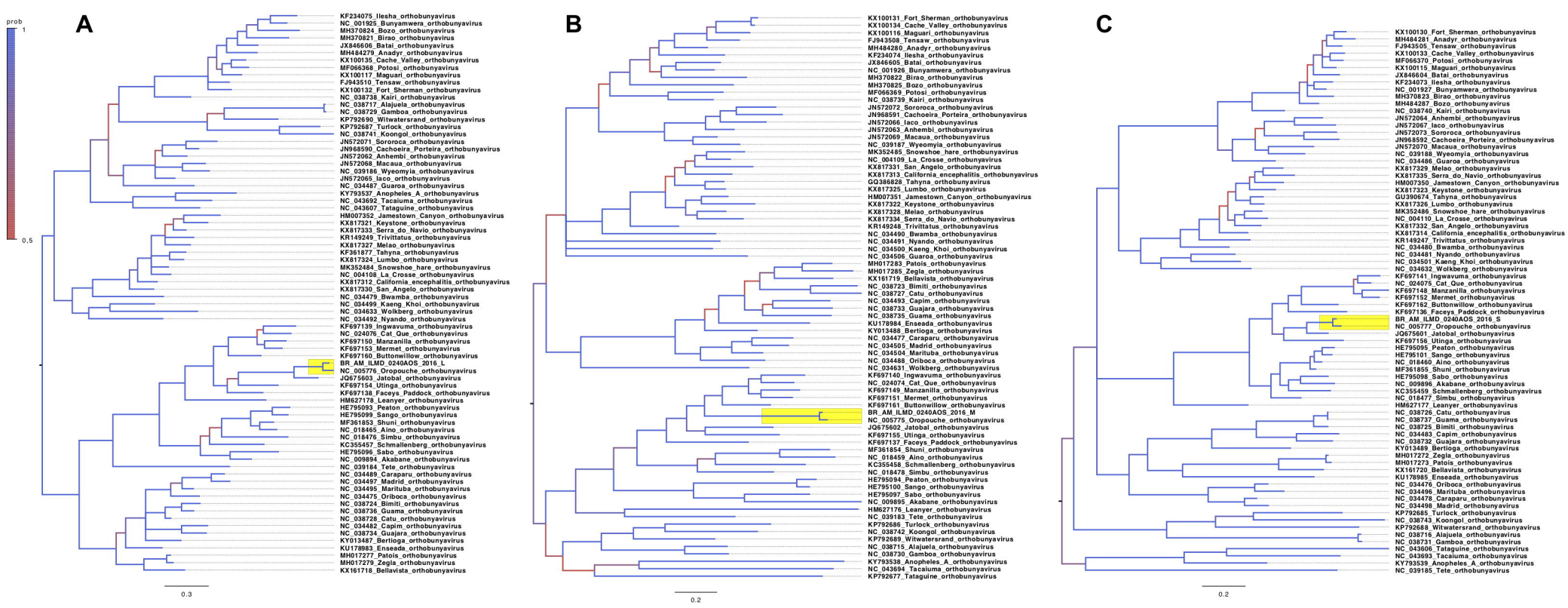
Table. Oligonucleotides designed and used in this study.

Oligo	Sequence (5'- 3')	Start	Stop
OROV_L_56_FNF	TTGCTCAACCARTATCGRAATAGGAT	56	81
OROV_L_174_FNF	CTGCAAAYCTTGAGTAYAGAAATGATG	174	200
OROV_L_621_FNR	TCAATCCATGGCAATGTCATTGT	621	599
OROV_M_2185_FNF	TCCCAAATCTAATCCTTTTACYGAT	2185	2209
OROV_M_2864_FNF	AGTATAGATGTACAAGGTACAGAATC	2864	2889
OROV_M_3564_FNR	TTCCTTCTCATAGCATGGCAT	3564	3544
OROV_S_6_FNF	TGTACTCCACAATTCAAAACAT	6	27
OROV_S_133_FNF	ACGGACAAGTGCTCAATGCT	133	152
OROV_S_728_FNR	TCCGAATTGGCGCAAGAAGT	728	709

Assay	Primer pairs	Size (bp)
L - 1st PCR (55°C)	OROV_L_56_FNF + OROV_L_621_FNR	566
L – semi-nested (50°C)	OROV_L_174_FNF + OROV_L_621_FNR	448
M - 1st PCR (55°C)	OROV_M_2185_FNF + OROV_M_3564_FNR	1380
M - semi-nested (55°C)	OROV_M_2864_FNF + OROV_M_3564_FNR	701
S - 1st PCR (58°C)	OROV_S_6_FNF + OROV_S_728_FNR	723
S - semi-nested (58°C)	OROV_S_133_FNF + OROV_S_728_FNR	596

Start/Stop positions refers to the nucleotide position of OROV GenBank reference sequence NC_005776.1 (segment L); NC_005775.1 (segment M); NC_005777.1 (segment S). To increase sensitivity, we developed semi-nested reactions for each genome segment. All 1st PCR reactions followed the same program: 94°C for 2 minutes for enzyme activation; 35 X (94°C for 30 seconds, 55 or 58°C for 30 seconds, and 72°C during 1 minute / Kb), a final step at 72°C for 5 minutes. For semi-nested reactions: 94°C for 2 minutes for enzyme activation; 30 X (94°C for 30 seconds, 50, 55 or 58°C for 30 seconds, and 72°C during 1 minute / Kb), a final step at 72°C for 5 minutes. All primers used in this study were synthesized by IDT DNA Technology, USA.

Figure. **Phylogenetic tree of Orthobunyavirus species**. Three mid-rooted Bayesian trees, one for each genome segment, were constructed with MrBayes software v3.2.6 and 76 taxa (the 75 Orthobunyavirus species recognized by the International Committee on Taxonomy of Viruses (ICTV) with complete genome records available in GenBank at 01-Jun-2019 and the sample BR_AM_ILMD_0240AOS_2016 reported in this study). Phylogenetic trees were set mid-rooted, with increased node order in FigTree 1.4.4 for clarity. A color-key represents the posterior probability values of each branch. The clade containing the sequence described in this study is highlighted in yellow, clustered with the Oropouche virus Refseq. The scale bar represents nucleotide substitutions per site. A = L segment tree; B = M segment tree; C = S segment tree.


A
prob
1
0.5
KF234075_Ilesha_orthobunyavirus
NC_001925_Bunyamwera_orthobunyavirus
MH370824_Bozo_orthobunyavirus
MH370821_Birao_orthobunyavirus
JX846606_Batai_orthobunyavirus
MH484279_Anadyr_orthobunyavirus
KX100135_Cache_Valley_orthobunyavirus
MF066368_Potosi_orthobunyavirus
KX100117_Maguari_orthobunyavirus
FJ943510_Tensaw_orthobunyavirus
KX100132_Fort_Sherman_orthobunyavirus
NC_038738_Kairi_orthobunyavirus
NC_038717_Alajuela_orthobunyavirus
NC_038729_Gamboa_orthobunyavirus
KP792690_Witwatersrand_orthobunyavirus
KP792687_Turlock_orthobunyavirus
NC_038741_Koongol_orthobunyavirus
JN572071_Sororoca_orthobunyavirus
JN968590_Cachoeira_Porteira_orthobunyavirus
JN572062_Anhembi_orthobunyavirus
JN572068_Macaua_orthobunyavirus
NC_039186_Wyeomyia_orthobunyavirus
JN572065_Iaco_orthobunyavirus
NC_034487_Guaroa_orthobunyavirus
KY793537_Anopheles_A_orthobunyavirus
NC_043692_Tacaiuma_orthobunyavirus
NC_043607_Tataguine_orthobunyavirus
HM007352_Jamestown_Canyon_orthobunyavirus
KX817321_Keystone_orthobunyavirus
KX817333_Serra_do_Navio_orthobunyavirus
KR149249_Trivittatus_orthobunyavirus
KX817327_Melao_orthobunyavirus
KF361877_Tahyna_orthobunyavirus
KX817324_Lumbo_orthobunyavirus
MK352484_Snowshoe_hare_orthobunyavirus
NC_004108_La_Crosse_orthobunyavirus
KX817312_California_encephalitis_orthobunyavirus
KX817330_San_Angelo_orthobunyavirus
NC_034479_Bwamba_orthobunyavirus
NC_034499_Kaeng_Khoi_orthobunyavirus
NC_034633_Wolkberg_orthobunyavirus
NC_034492_Nyando_orthobunyavirus
KF697139_Ingwavuma_orthobunyavirus
NC_024076_Cat_Que_orthobunyavirus
KF697150_Manzanilla_orthobunyavirus
KF697153_Mermet_orthobunyavirus
KF697160_Buttonwillow_orthobunyavirus
BR_AM_ILMD_0240AOS_2016_L
NC_005776_Oropouche_orthobunyavirus
JQ675603_Jatobal_orthobunyavirus
KF697154_Utinga_orthobunyavirus
KF697138_Faceys_Paddock_orthobunyavirus
HM627178_Leanyer_orthobunyavirus
HE795093_Peaton_orthobunyavirus
HE795099_Sango_orthobunyavirus
MF361853_Shuni_orthobunyavirus
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NC_018476_Simbu_orthobunyavirus
KC355457_Schmallenberg_orthobunyavirus
HE795096_Sabo_orthobunyavirus
NC_009894_Akabane_orthobunyavirus
NC_039184_Tete_orthobunyavirus
NC_034489_Caraparu_orthobunyavirus
NC_034497_Madrid_orthobunyavirus
NC_034495_Marituba_orthobunyavirus
NC_034475_Oriboca_orthobunyavirus
NC_038724_Bimiti_orthobunyavirus
NC_038736_Guama_orthobunyavirus
NC_038728_Catu_orthobunyavirus
NC_034482_Capim_orthobunyavirus
NC_038734_Guajara_orthobunyavirus
KY013487_Bertioga_orthobunyavirus
KU178983_Enseada_orthobunyavirus
MH017277_Patois_orthobunyavirus
MH017279_Zegla_orthobunyavirus
KX161718_Bellavista_orthobunyavirus
0.3
B
KX100131_Fort_Sherman_orthobunyavirus
KX100134_Cache_Valley_orthobunyavirus
KX100116_Maguari_orthobunyavirus
FJ943508_Tensaw_orthobunyavirus
MH484280_Anadyr_orthobunyavirus
KF234074_Ilesha_orthobunyavirus
JX846605_Batai_orthobunyavirus
NC_001926_Bunyamwera_orthobunyavirus
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MH370825_Bozo_orthobunyavirus
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JN968591_Cachoeira_Porteira_orthobunyavirus
JN572066_Iaco_orthobunyavirus
JN572063_Anhembi_orthobunyavirus
JN572069_Macaua_orthobunyavirus
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KX817313_California_encephalitis_orthobunyavirus
GQ386828_Tahyna_orthobunyavirus
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HM007351_Jamestown_Canyon_orthobunyavirus
KX817322_Keystone_orthobunyavirus
KX817328_Melao_orthobunyavirus
KX817338_Serra_do_Navio_orthobunyavirus
KR149248_Trivittatus_orthobunyavirus
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NC_034491_Nyando_orthobunyavirus
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KF697161_Buttonwillow_orthobunyavirus
BR_AM_ILMD_0240AOS_2016_M
NC_005775_Oropouche_orthobunyavirus
JQ675602_Jatobal_orthobunyavirus
KF697155_Utinga_orthobunyavirus
KF697137_Faceys_Paddock_orthobunyavirus
MF361854_Shuni_orthobunyavirus
NC_018459_Aino_orthobunyavirus
KC355458_Schmallenberg_orthobunyavirus
NC_018478_Simbu_orthobunyavirus
HE795094_Peaton_orthobunyavirus
HE795100_Sango_orthobunyavirus
HE795097_Sabo_orthobunyavirus
NC_009895_Akabane_orthobunyavirus
HM627176_Leanyer_orthobunyavirus
NC_039183_Tete_orthobunyavirus
KP792686_Turlock_orthobunyavirus
NC_038742_Koongol_orthobunyavirus
KP792689_Witwatersrand_orthobunyavirus
NC_038715_Alajuela_orthobunyavirus
NC_038730_Gamboa_orthobunyavirus
KY793538_Anopheles_A_orthobunyavirus
NC_043694_Tacaiuma_orthobunyavirus
KP792677_Tataguine_orthobunyavirus
0.2
C
KX100130_Fort_Sherman_orthobunyavirus
MH484281_Anadyr_orthobunyavirus
FJ943505_Tensaw_orthobunyavirus
KX100133_Cache_Valley_orthobunyavirus
MF066370_Potosi_orthobunyavirus
KX100115_Maguari_orthobunyavirus
JX846604_Batai_orthobunyavirus
KF234073_Ilesha_orthobunyavirus
NC_001927_Bunyamwera_orthobunyavirus
MH370823_Birao_orthobunyavirus
MH484287_Bozo_orthobunyavirus
NC_038740_Kairi_orthobunyavirus
JN572064_Anhembi_orthobunyavirus
JN572067_Iaco_orthobunyavirus
JN572073_Sororoca_orthobunyavirus
JN968592_Cachoeira_Porteira_orthobunyavirus
JN572070_Macaua_orthobunyavirus
NC_039188_Wyeomyia_orthobunyavirus
NC_034486_Guaroa_orthobunyavirus
KX817329_Melao_orthobunyavirus
KX817335_Serra_do_Navio_orthobunyavirus
HM007350_Jamestown_Canyon_orthobunyavirus
KX817323_Keystone_orthobunyavirus
GU390674_Tahyna_orthobunyavirus
KX817326_Lumbo_orthobunyavirus
MK352486_Snowshoe_hare_orthobunyavirus
NC_004110_La_Crosse_orthobunyavirus
KX817332_San_Angelo_orthobunyavirus
KX817314_California_encephalitis_orthobunyavirus
KR149247_Trivittatus_orthobunyavirus
NC_034480_Bwamba_orthobunyavirus
NC_034481_Nyando_orthobunyavirus
NC_034501_Kaeng_Khoi_orthobunyavirus
NC_034632_Wolkberg_orthobunyavirus
KF697141_Ingwavuma_orthobunyavirus
NC_024075_Cat_Que_orthobunyavirus
KF697148_Manzanilla_orthobunyavirus
KF697152_Mermet_orthobunyavirus
KF697162_Buttonwillow_orthobunyavirus
KF697136_Faceys_Paddock_orthobunyavirus
BR_AM_ILMD_0240AOS_2016_S
NC_005777_Oropouche_orthobunyavirus
JQ675601_Jatobal_orthobunyavirus
KF697156_Utinga_orthobunyavirus
HE795095_Peaton_orthobunyavirus
HE795101_Sango_orthobunyavirus
NC_018460_Aino_orthobunyavirus
MF361855_Shuni_orthobunyavirus
HE795098_Sabo_orthobunyavirus
NC_009896_Akabane_orthobunyavirus
KC355459_Schmallenberg_orthobunyavirus
NC_018477_Simbu_orthobunyavirus
HM627177_Leanyer_orthobunyavirus
NC_038726_Catu_orthobunyavirus
NC_038737_Guama_orthobunyavirus
NC_038725_Bimiti_orthobunyavirus
NC_034483_Capim_orthobunyavirus
NC_038732_Guajara_orthobunyavirus
KY013489_Bertioga_orthobunyavirus
MH017272_Zegla_orthobunyavirus
MH017273_Patois_orthobunyavirus
KX161720_Bellavista_orthobunyavirus
KU178985_Enseada_orthobunyavirus
NC_034476_Oriboca_orthobunyavirus
NC_034496_Marituba_orthobunyavirus
NC_034478_Caraparu_orthobunyavirus
NC_034498_Madrid_orthobunyavirus
KP792685_Turlock_orthobunyavirus
NC_038743_Koongol_orthobunyavirus
KP792688_Witwatersrand_orthobunyavirus
NC_038716_Alajuela_orthobunyavirus
NC_038731_Gamboa_orthobunyavirus
NC_043606_Tataguine_orthobunyavirus
NC_043693_Tacaiuma_orthobunyavirus
KY793539_Anopheles_A_orthobunyavirus
NC_039185_Tete_orthobunyavirus
0.2