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Investigating the Role of Easter Island in Migration of Zika Virus from South Pacific to Americas

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The role of Easter Island in the dissemination of Zika virus from the Pacific islands into the Americas remains unclear. We analyzed new Zika virus sequences from Eastern Island and found that Zika virus was independently disseminated from French Polynesia into the Americas and Easter Island at around the same time.

Zika virus is a mosquitoborne flavivirus associated with several recent outbreaks in human populations in the Pacific region and the Americas. Phylogeographic studies indicate that Zika virus strains circulating in South Pacific islands and Latin America comprise a single lineage ($ZIKV_{SP\text{-}AM}$) that arose because of sequential single viral disseminations from Southeast Asia into French Polynesia and from the South Pacific into Latin America (*1*–*5*). However, whether the ancestral Zika virus strain introduced into the Americas arose directly from French Polynesia or from another South Pacific island is unclear.

In early 2014, a Zika virus outbreak occurred in Easter Island (*6*), months before the first identification of Zika virus in Brazil (*7*). Easter Island is located at the southeastern edge of the Polynesian Triangle, roughly equidistant from French Polynesia and the South America mainland. Geographic position and intense touristic activity make Easter Island a potential staging post in the spread of Zika virus from French Polynesia to continental America. This hypothesis was suggested previously (*4*), but the study used a limited sequence dataset. We tested this hypothesis by using a more comprehensive dataset of Zika virus sequences from the South Pacific.

Blood samples from suspected Zika virus–infected human patients who visited the emergency unit of Hanga Roa Hospital on Easter Island during January–May 2014 were sent to the Public Health Institute of Chile for characterization, according to Ministry of Health of Chile guidelines for surveillance of transmissible diseases. The complete E and partial NS5 genes of 7 Zika virus strains were obtained as previously described (*6*). The concatenated fragments were aligned with Zika virus Asian genotype sequences available in GenBank and used for spatiotemporal viral diffusion

reconstruction (online Technical Appendix, https://wwwnc.cdc.gov/EID/article/24/11/18-0586-Techapp1.pdf).

The overall timescale of the phylogenetic tree estimated for Zika virus Asian genotype strains was fully consistent with previous reports (*1*–*5*; online Technical Appendix Table 2). Asymmetric (online Technical Appendix Figure 1) and symmetric (online Technical Appendix Figure 2) phylogeographic models placed the most recent common ancestor of the Asian genotype epidemic strains in Southeast Asia (posterior state probability [PSP] ≥0.96) at 1999 (Bayesian credible interval [BCI] 1996–2002) and support 2 independent disseminations from Southeast Asia into the Pacific region—the first in Micronesia around 2007, with no further spread, and the second originating the $ZIKV_{SP-AM}$ lineage that fueled epidemics during 2013–2016 in the South Pacific and the Americas.

The origin of the $ZIKV_{SP-AM}$ lineage was traced to French Polynesia (PSP ≥0.98) at 2013.3 (BCI 2012.9–2013.6). This lineage exhibits strong geographic subdivision; several highly supported island-specific monophyletic subclades nested among basal strains from French Polynesia (*5*), besides the $ZIKV_{AM}$ subclade (online Technical Appendix Figures 1, 2). We found 2 major clades within the $ZIKV_{SP-AM}$; clade I (posterior probability [PP] 0.97) contains strains from French Polynesia, the Americas, New Caledonia, and Vanuatu, whereas clade II (PP ≥0.94) encloses strains from Easter Island and the Cook Islands. Zika virus strains from Fiji, American Samoa, Samoa, Tonga, and the Solomon Islands branched together in a large but not well-supported (PP ≤0.57) monophyletic group.

French Polynesia was the most probable source location of the Zika virus clade I strains introduced into the Americas (PSP 1), Vanuatu (PSP 1), and New Caledonia (PSP ≥0.98), as well as of the clade II strain introduced into Easter Island (PSP ≥0.77), refuting the hypothesis of introduction of Zika virus into the Americas through Easter Island. The source location of the clade II strain of the Cook Islands was traced to French Polynesia (PSP >0.34) or Easter Island (PSP ≥0.37). Our analyses indicate that Zika virus was introduced into Easter Island, New Caledonia, the Cook Islands, and the Americas at around the same time (BCI 2013.6–2014.8), supporting a much longer period of undetected Zika virus transmission in the Americas (≈15 months) (*7*,*8*) than in those South Pacific islands (<1 month) (*8*). Zika virus also spread from French Polynesia into Samoa, Fiji, Tonga, and American Samoa from late 2015 to early 2016, probably following a stepping-stone process (online Technical Appendix Figures 1, 2). Migratory routes between those islands, however, were difficult to resolve (inconsistency between phylogeographic models) or do not fully agree with epidemiologic data (e.g. the dissemination from Tonga to Fiji) (*8*).

Our results indicate that French Polynesia was the main hub of dissemination of the $ZIKV_{SP-AM}$ lineage and seeded independent outbreaks in several South Pacific islands (including Easter Island) and the Americas from late 2013 to mid-2014, coinciding with a peak in the number of suspected Zika cases in French Polynesia. The long period of cryptic circulation of Zika virus in the Americas, the early detection of Zika virus in the Caribbean in December 2014 (*4*), and the reported dissemination of dengue (*9*) and chikungunya (*10*) viruses from French Caribbean territories into French Polynesia during 2013–2014 support the hypothesis that Zika virus might have been introduced to and circulated in the Caribbean region for several months before its detection in Brazil in 2015. Human movement between overseas French territories might create an epidemiologic link for arboviral transmissions between the South Pacific and the Caribbean region.

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Novel Multidrug-Resistant *Cronobacter sakazakii* Causing Meningitis in Neonate, China, 2015

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We report a case of meningitis in a neonate in China, which was caused by a novel multidrug-resistant *Cronobacter sakazakii* strain, sequence type 256, capsular profile K1:CA1. We identified genetic factors associated with bacterial pathogenicity and antimicrobial drug resistance in the genome and plasmids. Enhanced surveillance of this organism is warranted.

Cronobacter sakazakii is a foodborne pathogen associated with outbreaks of life-threatening necrotizing enterocolitis, meningitis, and sepsis in neonates and infants. Although the incidence of *C. sakazakii* infection is low, fatality rates range from 40% to 80% (*1*). Infections are usually limited to specific sequence types (STs) and complex clonal complexes (*2–4*). *C. sakazakii* ST4 is predominantly associated with meningitis in neonates; *C. sakazakii* ST12, with necrotizing enterocolitis in neonates (*2*,*3*). *C. sakazakii* is usually resistant to cephalothin and penicillin but is more sensitive to antimicrobial drugs than are other members of the family *Enterobacteriaceae*. Few reports describe drug-resistance patterns in *C. sakazakii* isolates (*4–6*). We report 1 multidrug-resistant (MDR) *C. sakazakii* ST256 strain that caused meningitis in a neonate in China.

On September 29, 2015, a 26-day-old boy, who was born after 38 weeks' gestation and had abdominal distention, fever, and jaundice, was hospitalized in a children's hospital in Guangzhou, China. He was fed breast milk; however, it could not be determined whether he had been exclusively breast-fed or whether the breast milk had been expressed by use of a pump. His cerebrospinal fluid contained numerous leukocytes, and his cerebrum contained abscesses. After a series of symptomatic treatments, including initial intravenous ceftazidime followed by meropenem, his clinical signs gradually improved. However, when discharged from the hospital after 3 weeks, his mental and physical development were remarkably impaired.

Cronobacter, isolated from brain abscess fluid, was identified by using an automated VITEK 2 Compact system (bioMérieux, Marcy l'Etoile, France). An isolate, GZcsf-1, was determined to be *C. sakazakii* ST256 with serotype O1. The *Cronobacter* PubMLST (https://pubmlst.org/cronobacter/) contains 2 ST256 isolates: MOD1-Ls15 g, isolated from the alimentary canal of the green bottle fly, *Lucilia sericata*, in the United States; and 2061 (no source information), detected in France. This ST had not been reported to cause meningitis in neonates. Susceptibility to 15 antimicrobials was tested by using the broth dilution method; MICs are shown in the online Technical Appendix (https://wwwnc.cdc.gov/EID/article/24/11/18-0718-Techapp1.pdf). GZcsf-1 was resistant to 8 antimicrobials: ampicillin, cefazolin, ceftriaxone, aztreonam, gentamicin, tetracycline, chloramphenicol, and trimethoprim/sulfamethoxazole.

The Beijing Genomics Institute performed genomic and plasmid DNA sequencing by using the PacBio RS II (Pacific Biosciences, Menlo Park, CA, USA) and HiSeq (Illumina, San Diego, CA, USA) platforms. The annotations were performed as previously described (*7*). *C. sakazakii* GZcsf-1 had 1 circular chromosome, 4.43 Mb long, containing 56.87% GC and 2 plasmids (denoted pGW1, 340,723 bp, 57.2% GC; pGW2, 135,306 bp, 54.0% GC) (GenBank accession nos. CP028974–6). On the basis of the characteristics of K antigen and colanic acid biosynthesis encoding genes in GZcsf-1, we determined its capsular profile to be K1:CA1, which differs from the capsular profile K2:CA2, proposed to be strongly associated with *C. sakazakii* isolated from neonates with severe infection (*3*). The subtype I-E

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Investigating the Role of Easter Island in Migration of Zika Virus from South Pacific to Americas

Technical Appendix

Methods

Zika Virus Sequencing

Blood samples were collected from symptomatic patients following the epidemiologic surveillance guidelines proposed by the Ministry of Health of Chile (Documents B51/833–2015 and 158/04 for surveillance of transmissible disease). These guidelines include the obligatory notification of suspected cases and require the sending of samples to the Public Health Institute of Chile for diagnostic and characterization of viral agents.

Viral RNA was extracted from serum using an easyMAG extraction system (bioMerieux) and the complete E and partial NS5 genes were amplified by nested RT-PCR in two fragments (PCR primers are described in Technical Appendix Table 1) and sequenced bidirectionally by using Sanger Sequencing, as previously described (*2*). Nucleotide sequences were assembled and edited using the SeqMan program (DNASTAR, Madison, WI).

Sequence Dataset and Phylogeographic Analysis

Concatenated E and NS5 fragments of seven Easter Island ZIKV strains were aligned with ZIKV Asian genotype complete coding sequences (CDS) and E sequences available on GenBank by December 2017 with information about country of infection and date of isolation from: Southeast Asia (CDS sequences), Pacific Islands (CDS and E sequences) and the Americas (CDS sequences) (alignment available upon request). Representative subsets of sequences from the Americas and Singapore were selected using a previously described strategy (*3*).

The spatiotemporal viral diffusion pattern was reconstructed using the Markov chain Monte Carlo (MCMC) algorithm implemented in the BEAST v1.8 package (*4*) with a relaxed uncorrelated lognormal molecular clock model, the GTR+Γ4 nucleotide substitution model, a Bayesian Skyline tree coalescent model and both reversible (symmetric) and nonreversible (asymmetric) discrete phylogeographic models. MCMC were run sufficiently long (100 million MCMC steps) to ensure stationary and convergence of all parameters (Effective Sample Size >200), through inspection with Tracer v1.6 (http://tree.bio.ed.ac.uk/software/tracer/). After discarding the 10% burn-in, Maximum Clade Credibility (MCC) trees were generated with TreeAnnotator (BEAST v1.8) and visualized with FigTree v1.4 (http://tree.bio.ed.ac.uk/software/figtree/). Results are presented in Technical Appendix Table 2.

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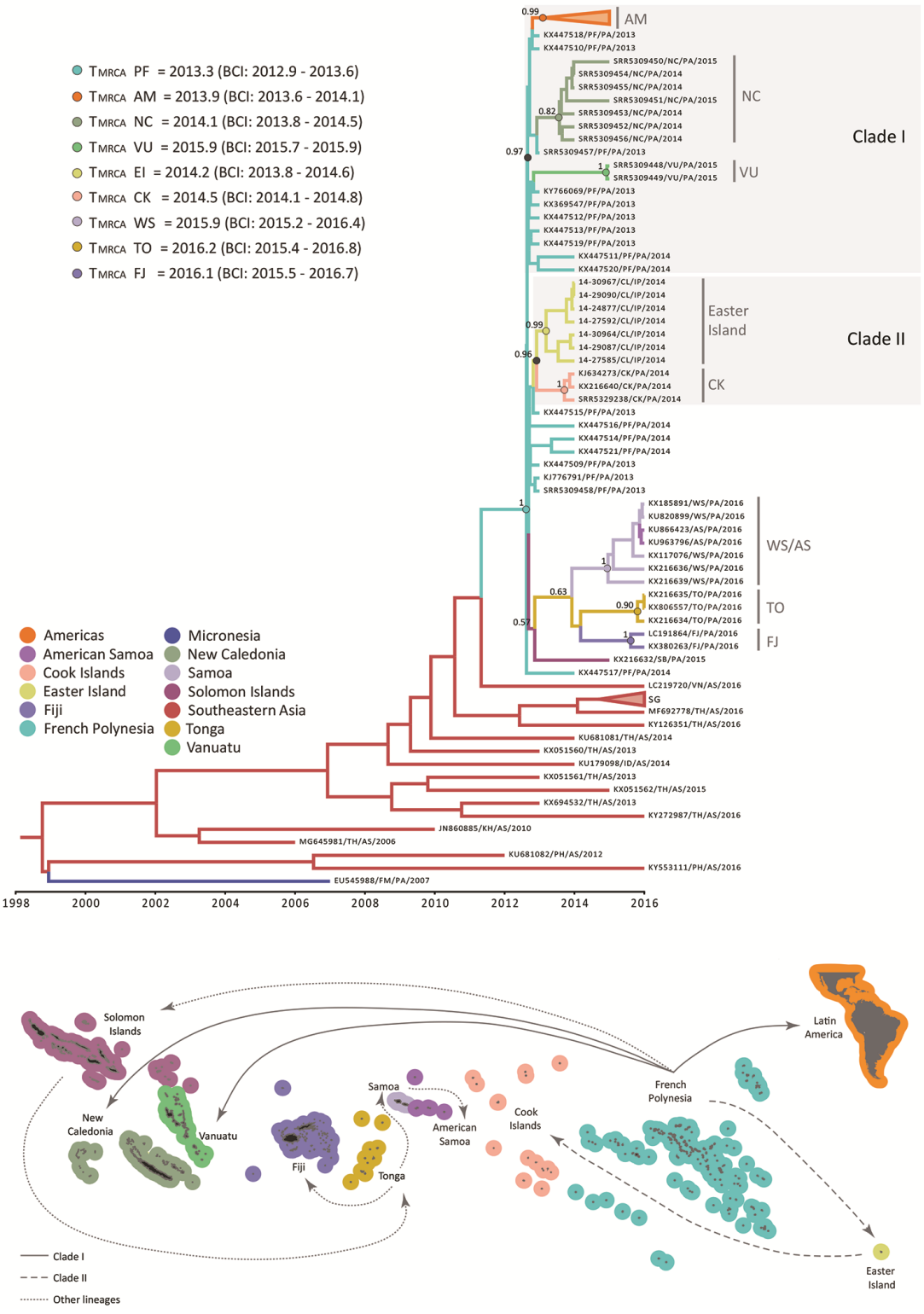
Technical Appendix Table 1. Primers used in this study

Target	Reference	Start pos	Stop pos	Size (bp)	Primer name	Sequence (5′ → 3′)
NS5	KJ776791	8167	8189	1040	400_28_out_L*	GGTGGGGGATTGGCTTGAAAAA
		9184	9206		400_30_out_R*	TAATCCCAGCCCTTCAACACCA
E Fragment 1		979	1000	416	400_4_out_L*	TCAGGTGCATAGGAGTCAGCAA
		1415	1394		400_4_out_R*	GGAGCCATGAACTGACAGCATT
E Fragment 2		1257	1278	731	400_5_out_L*	AGAACGTTAGTGGACAGAGGCT
		1966	1987		400_6_out_R*	CCATCTGTCCCTGCGTACTGTA
E Fragment 3		1876	1897	668	400_7_out_L*	TGAAGGGCGTGTCATACTCCTT
		2524	2543		E3_out_R	CCCTGTACCGCATCTCGTCT

*Primers described in (*1*).

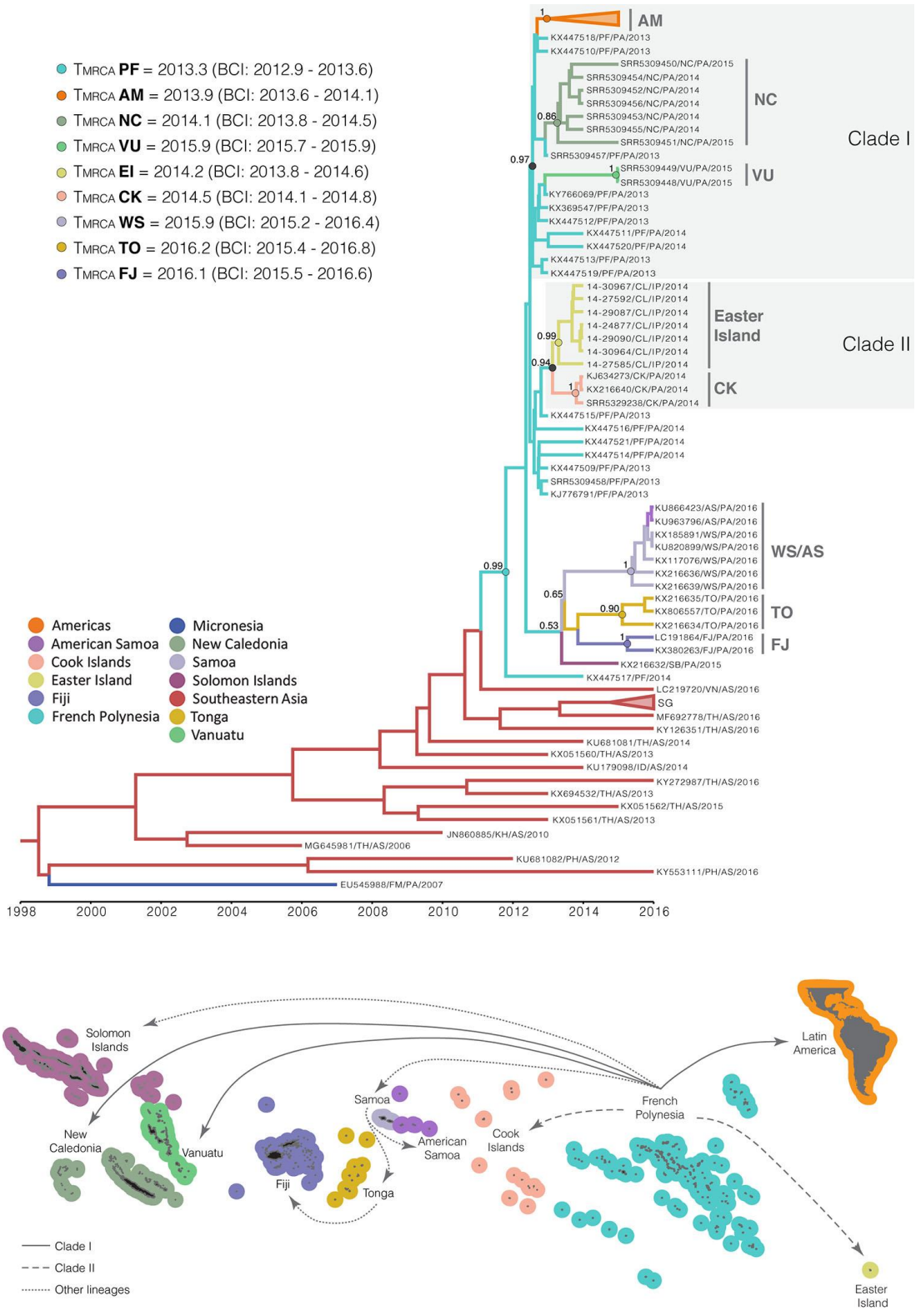
Technical Appendix Table 2. Posterior estimates of evolutionary parameters of ZIKV Asian genotype

Study	Phylogeographic model	Evolutionary rate (95% BCI)	TMRCA (95% BCI)		
			Pandemic Asian clade	Southern Pacific clade	American clade
Faria et al. 2016 (*5*)	–	1.1×10^{-3} (0.9×10^{-3}–1.3×10^{-3})	–	–	2014.0 (2013.6–2014.3)
Faria et al. 2017 (*6*)	Asymmetric	1.1×10^{-3} (1.0×10^{-3}–1.3×10^{-3})	–	–	2014.2 (2013.9–2014.4)
Metsky et al. 2017 (*7*)	–	1.2×10^{-3} (1.0×10^{-3}–1.3×10^{-3})	–	–	2014.1 (2013.6–2014.6)
Pettersson et al. 2016 (*8*)	–	1.2×10^{-3} (1.1×10^{-3}–1.3×10^{-3})	2001.7 (2000.6–2002.7)	2013.3 (2012.8–2013.4)	2013.9 (2013.8–2014.2)
This study	Symmetric	0.9×10^{-3} (0.8×10^{-3}–1.1×10^{-3})	1999.5 (1996.4–2002.2)	2013.3 (2012.9–2013.6)	2013.9 (2013.6–2014.1)
	Asymmetric	0.9×10^{-3} (0.8×10^{-3}–1.1×10^{-3})	1999.4 (1996.3–2002.4)	2013.3 (2012.8–2013.6)	2013.9 (2013.6–2014.1)



Technical Appendix Figure 1. Geographic dissemination of Zika virus Asian genotype, South Pacific and the Americas. Bayesian time-scaled maximum clade credibility phylogeny estimated from 110 Zika virus Asian genotype sequences with the asymmetric model. The branches' colors represent the most

probable location state (geographic region) of their ancestral node. The times to the most recent common ancestor of key nodes (colored circles) are indicated. Numbers at selected nodes indicate the clade posterior probabilities. The shaded areas highlight the Zika virus clades we have described. All horizontal branch lengths are drawn to a scale of years. Arrows between locations represent the estimated viral migration events and its line's pattern discriminate the viral flux of each Zika virus clade. The sequences of the Eastern Island isolates were submitted to GenBank under accession numbers MG982560–MG982573. AM, Americas; AS, American Samoa; CK, Cook Islands; EI, Easter Island; FJ, Fiji; NC, New Caledonia; PF, French Polynesia; TMRCA, time to the most recent common ancestor; TO, Tonga; VU, Vanuatu; WS, Samoa.



Technical Appendix Figure 2. Geographic dissemination of Zika virus Asian genotype, South Pacific and the Americas. Bayesian time-scaled maximum clade credibility phylogeny estimated from 110 Zika virus Asian genotype sequences with the symmetric model. The branches' colors represent the most

probable location state (geographic region) of their ancestral node. The times to the most recent common ancestor of key nodes (colored circles) are indicated. Numbers at selected nodes indicate the clade posterior probabilities. The shaded areas highlight the Zika virus clades we have described. All horizontal branch lengths are drawn to a scale of years. Arrows between locations represent the estimated viral migration events and its line's pattern discriminate the viral flux of each Zika virus clade. The sequences of the Eastern Island isolates were submitted to GenBank under accession numbers MG982560–MG982573. AM, Americas; AS, American Samoa; CK, Cook Islands; EI, Easter Island; FJ, Fiji; NC, New Caledonia; PF, French Polynesia; TO, Tonga; VU, Vanuatu; WS, Samoa; TMRCA, time to the most recent common ancestor.