

1 **Chikungunya virus outbreak in the Amazon region: replacement of**
2 **the Asian genotype by an ECSA lineage?**

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53 **Abstract**

54 *Background*

55 Since its first detection in the Caribbean in late 2013, chikungunya virus (CHIKV) has
56 affected 51 countries in the Americas. The CHIKV epidemic in the Americas was
57 caused by the CHIKV-Asian genotype. In August 2014, local transmission of the
58 CHIKV-Asian genotype was detected in the Brazilian Amazon region. However, a
59 distinct lineage, the CHIKV-East-Central-South-America (ECSA)-genotype, was
60 detected nearly simultaneously in Feira de Santana, Bahia state, northeast Brazil. The
61 genomic diversity and the dynamics of CHIKV in the Brazilian Amazon region
62 remains poorly understood despite its importance to better understand the
63 epidemiological spread and public health impact of CHIKV in the country.

64

65 *Methodology/Principal Findings*

66 We report a large CHIKV outbreak (5,928 notified cases between August 2014 and
67 August 2018) in Boa Vista municipality, capital city of Roraima's state, located in the
68 Brazilian Amazon region. In just 48 hours, we generated 20 novel CHIKV-ECSA
69 genomes from the Brazilian Amazon region using MinION portable genome
70 sequencing. Phylogenetic analyses revealed that despite an early introduction of the
71 Asian genotype in 2015 in Roraima, the large CHIKV outbreak in 2017 in Boa Vista
72 was caused by an ECSA-lineage most likely introduced from northeastern Brazil.
73 Epidemiological analyses suggest a basic reproductive number of R_0 of 1.66, which
74 translates in an estimated 39 (95% CI: 36 to 45) % of Roraima's population infected
75 with CHIKV-ECSA. Finally, we find a strong association between Google search
76 activity and the local laboratory-confirmed CHIKV cases in Roraima.

77

78 *Conclusions/Significance*

79 This study highlights the potential of combining traditional surveillance with portable
80 genome sequencing technologies and digital epidemiology to inform public health
81 surveillance in the Amazon region. Our data reveal a large CHIKV-ECSA outbreak in
82 Boa Vista, limited potential for future CHIKV outbreaks, and indicate a replacement
83 of the Asian genotype by the ECSA genotype in the Amazon region.

84

85

86 **Author Summary**

87 Until the end of 2017, Brazil notified the highest number of infections caused by
88 chikungunya virus (CHIKV) in the Americas. We investigated a large CHIKV
89 outbreak in Boa vista municipality in the Brazilian Amazon region. Rapid portable
90 genome sequencing of 20 novel isolates and subsequent genetic analysis revealed that
91 ECSA lineage was introduced from northeastern Brazil to Roraima around July 2016.
92 Epidemiological analyses suggest a basic reproductive number of R_0 of 1.66, which
93 suggests that approximately 39% of Roraima's population was infected with CHIKV-
94 ECSA. Given the dominance of the CHIKV-Asian genotype in the Americas, our data
95 highlights the rapid spread of a less understood and poorly characterized CHIKV-
96 ECSA genotype in Brazil. Investigations on potential associations between public
97 health impact of CHIKV and genetic diversity of circulating strains are warranted to
98 better evaluate its impact in Brazil and beyond.

99

100 **Keywords**

101 Chikungunya, East-Central-South-African, surveillance, Amazon region, MinION
102 genome sequencing, traditional epidemiology, genomic epidemiology, digital
103 epidemiology, phylodynamics.

104 **Introduction**

105 In August 2014, local transmission of chikungunya virus (CHIKV) was detected in
106 Brazil for the first time, with cases being reported nearly simultaneously in Oiapoque
107 (Amapá state, north Brazil) and Feira de Santana (Bahia state, northeast Brazil), two
108 municipalities separated by >2000 km distance. Genetic analysis confirmed the co-
109 circulation of distinct virus lineages in Brazil: the Asian genotype (CHIKV-Asian)
110 was introduced to Oiapoque possibly from neighbouring French Guiana, while the
111 East-Central-South-African genotype (CHIKV-ECSA) was introduced to Feira de
112 Santana from a traveller returning from Angola [1].

113 Since 2014 and until the end of September 2018, a total of 697,564 CHIKV
114 cases have been notified in Brazil (including 94,672 laboratory-confirmed cases). This
115 is the largest number recorded in any of the 51 countries or territories reporting local
116 CHIKV transmission in the Americas [2]. The virus has been circulating in the
117 Americas since 2013 where approximately 260 million people live in areas at-risk of
118 transmission [2-4]. Similar to the recent Zika virus epidemic [5], the rapid spread of
119 CHIKV in the Americas, including in Brazil, results from several factors, including
120 the establishment and abundance of competent *Aedes* spp. vectors, lack of population
121 immunity, and increased mobility of vectors and humans between regions reporting
122 current presence of the virus [6].

123 Chikungunya virus is an enveloped, non-segmented, single-stranded positive
124 polarity RNA alphavirus that is a member of the *Togaviridae* family and is
125 transmitted predominately by the *Aedes aegypti* and *Aedes albopictus* vectors, which
126 are widespread in Brazil [7]. There are four main genotypes: (i) the West African
127 genotype is maintained in an enzootic cycle in Africa, (ii) the Asian genotype, which
128 is endemic in Asia, (iii) the East-Central-South-African genotype, endemic to Africa,
129 and (iv) the Indian Ocean Lineage (IOL) genotype, an epidemic lineage that emerged
130 from the ECSA genotype around 2004 and swept through the Indian Ocean region
131 causing a series of explosive outbreaks [8].

132 The first symptoms of CHIKV infection are a rapid increase in temperature
133 (>38.9°C), followed by severe, often debilitating polyarthralgia. Serological data
134 from La Reunion, Philippines and the Indian Ocean island of Mayotte suggest that
135 75-97% of persons infected with CHIKV develop symptomatic infections [9].
136 Seroprevalence data from Brazil suggests that 45.7 to 57.1% Riachão do Jacuípe

137 and of Feira de Santana, both located in Bahia state, were exposed to CHIKV in
138 2015, with a total of 32.7% to 41.2% of the population reporting symptoms [10].

139 Throughout Asia and the Americas, chikungunya virus outbreaks have been
140 associated with unique clinical features [11], including long-lasting symptoms [12],
141 and high mortality resulting from complications associated with CHIKV infection
142 [13, 14]. In Brazil, a striking proportion of 68.1 to 75% of the population with
143 positive serological results reporting symptoms contracted a chronic form of the
144 disease [12, 15]. However, the epidemiological features, genomic diversity, and
145 transmission dynamics of recent CHIKV outbreaks in this country remain poorly
146 understood. Inferences that are based only on clinical-epidemiological notifications
147 are complicated by underreporting of cases by the national reporting system [16],
148 mostly due to the co-circulation and co-infection with viruses that cause overlapping
149 symptoms, such as Zika and dengue viruses [17-19]. Moreover, CHIKV serological
150 tests may cross-react with other alphaviruses, such as Mayaro virus, that circulate in
151 the north and centre-west regions of Brazil [20, 21]. In this context, it is challenging
152 to use only clinical-epidemiological and serological data to evaluate the true extent of
153 the disease. Moreover, accurate incidence data is critical to forecast and provide
154 prediction of the course of epidemics [22].

155 Until the end of 2016, 83.3% of the cases in Brazil were reported in northeast
156 region of the country [23]. However, in 2017, Roraima state, located in the Amazon
157 basin in the north of Brazil, reported its first large CHIKV outbreak. Roraima is the
158 northernmost state of Brazil, lies in the Amazon basin, borders Venezuela and French
159 Guiana to the north, and Amazonas and Pará states to the south, and its equatorial
160 climate favours year round transmission of mosquito-borne viruses [24]. Within
161 Brazil's northern states, Roraima has been implicated as a stepping-stone to virus
162 introductions from other Latin American regions, such as dengue [25], and yellow
163 fever virus in the past [26]. Moreover, the Amazon region has recently been
164 highlighted as a region with high transmission potential of vector-borne diseases [4]
165 and, more generally, a region with high potential for virus zoonoses and emergence
166 [27].

167 Due to its connectivity and potential impact on global epidemiology of vector-
168 borne and zoonotic virus from the Amazon basin, it is important to improve genomic
169 pathogen surveillance in Roraima. By August 2018, the public health laboratory of
170 Boa Vista (capital city of Roraima state) had reported 5,928 CHIKV cases, 3,795 of

171 which were laboratory-confirmed. Here we use a combination of on-site portable
172 virus genome sequencing, and epidemiological analysis of case count and web search
173 data to describe the circulation, genetic diversity, epidemic potential and attack rates
174 of a large CHIKV outbreak in Boa Vista.
175

176 **Methods**

177 **Connectivity in study area**

178 Roraima is the northernmost of Brazil's 27 federal units (**Figure 1a**) and has an
179 estimated population of 450,479, of whom 284,313 live in the capital city of Boa
180 Vista (ibge.gov.br/). Despite being Brazil's least populated federal unit, Roraima is
181 one of the best-connected Brazilian states in the Amazon basin [28]. Within Brazil,
182 Roraima is connected to Amazonas state in the south via the road BR-174. This
183 road also connects Roraima's capital city, Boa Vista, to the states of Bolivar and
184 Amazonas in Venezuela in the north. Further, the road BR-401 links Boa Vista to
185 Guyana in the east. There are four daily flights connecting Boa Vista with Brasília,
186 capital of Brazil, as well as six weekly flights to Manaus, the capital city of
187 Amazonas state and the biggest city in the north of the country, with connecting
188 daily nonstop flights to all other Brazilian states/regions and international
189 destinations, including important international airport hubs in Panamá City and
190 Miami, USA. There are also less-commonly used seasonal fluvial networks that
191 connect Boa Vista and Manaus via the Amazonas river.

192

193 **[Figure 1 around here]**

194

195 **Chikungunya virus case count time series**

196 The Roraima State Central Laboratory (LACEN-RR) is responsible for the differential
197 diagnosis of suspected arbovirus cases presenting to Roraima's public health units.
198 Between Jan 2014 and September 2018, LACEN-RR notified 5,928 CHIKV cases in
199 Boa Vista alone, 3,795 of these laboratory-confirmed, to the National Reportable
200 Disease Information System (SINAN). Case count time series are available from
201 Github (<http://github.com/arbospread>). We follow the Brazilian Ministry of Health's
202 guidelines and define a notified CHIKV case as a suspected case characterized by (i)
203 acute onset of fever $>38.5^{\circ}\text{C}$, (ii) severe arthralgia and/or arthritis not explained by
204 other medical conditions, and (iii) residing or having visited epidemic areas within 15
205 days before onset of symptoms. A laboratory-confirmed case is a suspected case
206 confirmed by laboratory methods such as (i) virus isolation in cell culture, (ii)
207 detection of viral RNA, (iii) detection of virus-specific IgM antibodies in a single
208 serum sample collected in the acute or convalescent stage of infection; or (iv) a four-

209 fold rise of IgG titres in samples collected during the acute phase, in comparison with
210 a sample collected in the convalescent period.

211

212 **Nucleic acid isolation and RT-qPCR**

213 Residual anonymized clinical diagnostic samples were sent to Instituto Leônidas e
214 Maria Deane, FIOCRUZ Manaus, Amazonas, Brazil, for molecular diagnostics as
215 part of the ZiBRA-2 project. The ZiBRA-2 project was approved by the Pan
216 American Health Organization Ethics Review Committee (PAHOERC) n° PAHO-
217 2016-08-0029. Total RNA extraction was performed with QIAmp Viral RNA Mini kit
218 (Qiagen), following manufacturer's recommendations. Samples were first tested
219 using a multiplexed qRT-PCR protocol against CHIKV, dengue virus (DENV1-4),
220 yellow fever virus, Zika virus, Oropouche virus and Mayaro virus [29]. All qRT-PCR
221 results were corroborated using a second protocol [30]; comparable Ct values were
222 obtained with the two protocols. CHIKV positive samples tested negative for all other
223 arboviruses tested. Samples were selected for sequencing based on Ct-value <30 (to
224 maximize genome coverage of clinical samples by nanopore sequencing [31]), and
225 based on the availability of epidemiological metadata, such as date of onset of
226 symptoms, date of sample collection, gender, municipality of residence, and
227 symptoms (**Table 1**). We included 13 samples from Roraima state plus 5 additional
228 samples from patients visiting the LACEN-Amazonas in Manaus, under the auspices
229 of the ZiBRA project (<http://www.zibraproject.org/>). All samples were processed in
230 accordance with the terms of Resolution 510/2016 of CONEP (National Ethical
231 Committee for Research, Brazilian Ministry of Health).

232

233 **Complete genome MinION nanopore sequencing**

234 Between the 1st and 7th June 2017, we attempted sequencing at Instituto Leônidas e
235 Maria Deane, FIOCRUZ Manaus on all selected samples with Ct-value <30. We used
236 an Oxford Nanopore MinION device with protocol chemistry R9.4, as previously
237 described [32]. Sequencing statistics can be found in **Table S1 (Julien)**. In brief, we
238 employed a protocol with cDNA synthesis using random primers followed by strain-
239 specific multiplex PCR [32]. Extracted RNA was converted to cDNA using the
240 Protoscript II First Strand cDNA synthesis Kit (New England Biolabs, Hitchin,
241 UK) and random hexamer priming. CHIKV genome amplification by multiplex
242 PCR was attempted using the CHIKAsianECSA primer scheme and 35 cycles of

243 PCR using Q5 High-Fidelity DNA polymerase (NEB) as described in [32]. PCR
244 products were cleaned up using AmpureXP purification beads (Beckman Coulter,
245 High Wycombe, UK) and quantified using fluorimetry with the Qubit dsDNA High
246 Sensitivity assay on the Qubit 3.0 instrument (Life Technologies). PCR products
247 for samples yielding sufficient material were barcoded and pooled in an equimolar
248 fashion using the Native Barcoding Kit (Oxford Nanopore Technologies, Oxford,
249 UK). Sequencing libraries were generated from the barcoded products using the
250 Genomic DNA Sequencing Kit SQK-MAP007/SQK-LSK108 (Oxford Nanopore
251 Technologies). Libraries were loaded onto a R9/R9.4 flow cell and sequencing data
252 were collected for up to 48 hr. Consensus genome sequences were produced by
253 alignment of two-direction reads to a CHIKV virus reference genome (GenBank
254 Accession number: N11602) as previously described in [32]. Positions with $\geq 20\times$
255 genome coverage were used to produce consensus alleles, while regions with lower
256 coverage, and those in primer-binding regions were masked with N characters.
257 Validation of the sequencing protocol was previously performed in [32].

258

259 **Collation of CHIKV-ECSA complete genome datasets**

260 Genotyping was first conducted using the phylogenetic arbovirus subtyping tool
261 available at <http://www.krisp.org.za/tools.php>. Complete and near complete
262 sequences were retrieved from GenBank on June 2017 [33]. Two complete or near-
263 complete CHIKV genome datasets were generated. Dataset 1 included ECSA-
264 PreAm (ECSA sampled outside the Americas) and ECSA-Br (ECSA sequences
265 sampled in the Americas) sequences. This dataset contained 36 complete genomes
266 from the ECSA genotype, including 7 from East and Central Africa (HM045823
267 from Angola 1962; HM045784 from Central African Republic 1984; HM045812
268 from Uganda 1982; KY038947 from Central African Republic 1983; HM045793
269 from Central African Republic 1986; HM045822 from Central African Republic
270 1978; and KY038946 from Central African Republic 1975). Dataset 1 also included
271 29 sequences from Brazil, including the new 18 genomes reported here from the
272 ECSA lineage and 3 genomes from the outbreak caused by the ECSA lineage in
273 June 2016 in Maceió, Alagoas states, northeast Brazil (**Figure 1a**) [34]. Dataset 2
274 (ECSA-Br) included only the 29 Brazilian genome sequences. Using a robust
275 nonparametric test [35], no evidence of recombination was found in both datasets.
276

277

278

279 **Maximum likelihood analysis and temporal signal estimation**

280 Maximum likelihood (ML) phylogenetic analyses were performed for each dataset
281 using RAxML v8 [36]. We used a GTR nucleotide substitution model with 4
282 gamma categories (GTR+4 Γ). In order to investigate the evolutionary temporal
283 signal in each dataset, we regressed root-to-tip genetic distances against sample
284 collection dates using TempEst [37]. For both datasets we obtained a strong linear
285 correlation (dataset 1: $r^2=0.93$; dataset 2: $r^2=0.84$) suggesting these alignments
286 contain sufficient temporal information to justify a molecular clock approach.
287 However, for dataset 1, the Angola/M2022/1962 strain was positioned substantially
288 above the regression line. Previous investigations have suggested this strain may
289 have been the result of contamination or high passage in cell culture [8], so this
290 sequence was removed from subsequent analyses.

291

292 **Molecular clock phylogenetic analysis**

293 To estimate time-calibrated phylogenies we used the BEAST v.1.10.1 software
294 package [38]. To infer historical trends in effective population size from the
295 genealogy we used several different coalescent models. Because preliminary
296 analysis indicated oscillations in epidemic size through time (as also expected from
297 national case report data), we used three flexible, non-parametric models: a) the
298 standard Bayesian skyline plot (BSP; 10 groups) [39], b) the Bayesian skyride plot
299 [40], and c) the Bayesian skygrid model [41], with 45 grid points equally spaced
300 between the estimated TMRCA of the CHIKV-ECSA genotype in Brazil and the
301 date of the earliest available isolate, collected in 18 March 2017 [41]. For
302 comparison, we also used a constant population size coalescent model. We tested
303 two molecular clock models: a) the strict molecular clock model, which assumes a
304 single rate across all phylogeny branches, and b) the more flexible uncorrelated
305 relaxed molecular clock model with a lognormal rate distribution (UCLN) [42].
306 Because the marginal posterior distribution of the coefficient of variation of the
307 UCLN model did not exclude zero (most likely due to the small alignment size), we
308 used a strict molecular model in all analyses. For each coalescent model, Markov

309 Chain Monte Carlo analyses were run in duplicate for 10 million steps using a ML
310 starting tree, and the GTR+4 Γ codon partition (CP)1+2,3 model [42].

311

312 **Epidemiological analysis**

313 The epidemic basic reproductive number (R_0) was estimated from monthly
314 confirmed cases, as previously described [31, 43]. Because (i) the Asian genotype
315 was circulating in the north region of Brazil since 2014 [1], and (ii) we observed a
316 relatively small number of cases both in the notified and confirmed time series, we
317 assume cases from June 2014 and December 2016 did not represent autochthonous
318 transmission of CHIVK-ECSA. We assume a mean generation time of 14 days, as
319 previously reported elsewhere [44]. We report R_0 estimates for different values of
320 the generation time (g) parameter, along with corresponding estimates of the
321 epidemic exponential growth rate, per month (r).

322

323 **Web search query data**

324 Available in near-real time, disease-related Internet search activity has been shown
325 to track disease activity (a) in seasonal mosquito-borne disease outbreaks, such as
326 those caused by dengue [42, 82], and (b) in unexpected and emerging mosquito-
327 borne disease outbreaks such as the 2015-2016 Latin American Zika outbreak [45].
328 Here, we investigated whether we could find a meaningful relationship between
329 Internet search activity and the local chikungunya outbreak in Roraima. Indeed,
330 novel Internet-based data sources have the potential to complement traditional
331 surveillance by capturing early increases in disease-related search activity that may
332 signal an increase in the public's perception of a given public health threat and may
333 additionally capture underlying increases in disease activity. Internet searches may
334 be particularly important and indicative of changes in disease transmission early
335 during an outbreak, when ongoing information on the virus transmission is
336 obfuscated by a lack of medical surveillance. In addition, Internet search trends
337 may also help track disease activity in populations that may not seek formal
338 medical care. We used the Google Trends (GT) tool [45] to compile the monthly
339 fraction of online searches for the term "Chikungunya", that originated from Boa
340 Vista municipality (Roraima state), between January 2014 and July 2018. For
341 comparison, GT search activity for the term "Chikungunya" was collected for the

342 same time period for Manaus municipality (Amazonas state). The synchronicity of
343 GT time series and notified and confirmed case counts from Boa Vista and Manaus
344 was assessed using the Spearman's rank correlation test in the R software [46].

345

346 **Data availability**

347 XML files and datasets analysed in this study are available in the GitHub
348 repository (<http://github.com/arbospread>). New sequences have been deposited in
349 GenBank under accession numbers MK121891-MK121908 (CHIKV-ECSA) and
350 MK134712-MK134713 (CHIKV-Asian).

351 **Results**

352 Although most CHIKV notified cases in Brazil were reported in 2016
353 (**Figure 1**), in Roraima, the majority of notified and confirmed cases in Roraima
354 state were reported in 2017 (5,027 notified cases and 3,720 laboratory-confirmed
355 infections). The number of cases in Roraima started increasing exponentially in
356 January 2017, and the outbreak peaked in July 2017.

357

358 **Figure 1 around here**

359

360 We attempted on-site portable nanopore sequencing of isolates collected
361 during the early phase of the outbreak (February to March 2017). We selected 15
362 RT-qPCR+ virus isolates from autochthonous cases in Roraima state (11 from Boa
363 Vista, 1 from Bonfim, and 1 from Iracema municipalities) (**Table 1**) with a cycle
364 threshold (Ct) ≤ 30 (mean 20.3, range 13.7 – 27.41). We included two isolates from
365 two infected travellers returning to Roraima in December 2014, and an additional five
366 isolates from Amazonas state (all from Manaus municipality), sampled between July
367 2015 and March 2017. In less than 48 hours genome sequence data was obtained for
368 all selected isolates and in less than 72 hours preliminary results were shared with
369 local public health officials and the Brazilian Ministry of Health. A mean genome
370 coverage of 86% (20x) per base pair was obtained for the sequenced data; mean
371 coverage increased to 90% when focusing on samples with Ct < 26 (**Figure 2a**).
372 Coverage of individual sequences and epidemiological information for each
373 sequenced isolate can be found in **Table 1**.

374

375 **Table 1 around here**

376

377 Manual and automated phylogenetic analysis identified the ECSA genotype as
378 the dominant genotype circulating in both Roraima and Manaus between 2015 and
379 2017. However, two cases from late 2014 returning from Venezuela to Roraima
380 (AMA294 and AMA295) were classified as Asian genotype, the dominant lineage
381 circulating in Latin America. Regression analysis of genetic divergence and sampling
382 dates shows accumulation of temporal signal in the ECSA-Br dataset ($r^2 = 0.84$)
383 (**Figure 2b**).

384 We estimated the evolutionary time-scale of the ECSA-Br lineage using
385 several well-established molecular clock coalescent methods. Our substitution rate
386 estimates indicate that the ECSA-Br lineage is evolving at 7.15×10^{-4} substitutions
387 per site per year (s/s/y; 95% Bayesian credible interval: $5.04 - 9.55 \times 10^{-4}$). This
388 estimated rate is higher than that estimated for endemic lineages, and is similar to the
389 evolutionary rates estimated for the epidemic lineage circulating in the Indian Ocean
390 region (**Figure 2c**). A closer inspection of amino acid mutations indicate that the
391 ECSA-Br strains lack both the A226V (E1 protein) and the L210Q (E2 protein)
392 mutations that has been reported to increase virus transmissibility and persistence
393 in *Ae. albopictus* populations in the Indian Ocean [47].

394 ML and Bayesian phylogenetic analyses reveal that the ECSA sequences from
395 Brazil (hereafter named ECSA-Br lineage) form a single well-supported clade
396 (bootstrap support = 100) (**Figure 3**). This is consistent with the establishment of the
397 ECSA genotype in Brazil following the introduction of a single strain to the Americas
398 [1]. The two isolates collected in late 2014 in Roraima cluster together and fall as
399 expected within the diversity of other Asian genotype sequences from the Americas.
400 Our phylogenetic reconstruction suggests at least five separate introductions of the
401 Asian genotype strain Brazil (**Figure S1**), in contrast to a single introduction of the
402 ECSA genotype followed by onward transmission. Moreover, all 13 ECSA isolates
403 sampled in Roraima (*node C*) cluster together with maximum phylogenetic support
404 (bootstrap support = 100; posterior probability = 1.00) (**Figure 3**). We consistently
405 estimate the date of the most recent common ancestor of ECSA-Br Roraima clade to
406 be mid-July 2016 (95% BCI: late March to late October 2016) (**Figure 3**); similar
407 dating estimates under different coalescent models (**Figure S2**). In contrast to the
408 Roraima strains, sequences from Manaus were found to be interspersed with isolates
409 from Bahia and Pernambuco (**Figure 3**), indicating separate introductions of the
410 CHIKV-ECSA lineage, some in early 2015 (*node B*), possibly from the northeast
411 region of Brazil. Interestingly, according to travel history reports, the first
412 autochthonous transmission of CHIKV in Manaus was linked to an index patient
413 who reported spending holidays in Feira de Santana (Bahia state) in early 2015,
414 during a period when this city was experiencing a large CHIKV outbreak [5]. The
415 date of *node A* was estimated to be around mid-July 2014 (95% BCI: early Jul – late
416 Aug 2014), shortly after the arrival of the presumed index case in Feira de Santana,
417 Bahia [5]. This is in line with a single introduction to Bahia (*node A*), followed by

418 subsequent waves of transmission across the northeast and southeast regions of Brazil
419 [5, 48, 49]. Our demographic reconstructions indicate that the outbreak in Roraima
420 2017 probably represents the third epidemic wave spreading across Brazil (**Figure**
421 **S3**).

422 Next, we used notified case counts to estimate the basic reproductive
423 number, R_0 , of the epidemic. R_0 is the average number of secondary cases caused by
424 an infected individual and can be estimated from epidemic growth rates during its
425 early exponential phase [43]. We find that $R_0 \approx 1.66$ (95% CI: 1.51 – 1.83), in line
426 with previous reports from other settings [50-52]. A sensitivity analysis considering
427 different exponential growth phase periods resulted in a lower bound for R_0 of
428 around 1.23 (**Figure S4**). To gain insights into the possible magnitude of the
429 outbreak and local surveillance capacity we used the equilibrium end state of a
430 simple susceptible-infected-recovered (SIR) model: $N = S + I + R$, $S \sim 1/R_0$, $I \sim 0$,
431 with N being the total population size of Roraima. Using this simple mathematical
432 approach, we obtain an attack rate (R) of 0.39 (95% CI: 0.36 – 0.45), slightly lower
433 than elsewhere in Brazil [12, 15]. This corresponds to an estimated 110,882 (95%
434 CI: 102,352 – 127,940) infected individuals, and a case detection rate of 5.34%
435 (95% CI: 4.63 – 5.79). This implies that approximately 1 case was notified for
436 every 19 infections. If we assume 32.7 – 41.2% of the estimated infections are
437 symptomatic, as previously reported in Bahia and Sergipe [53], then we estimate
438 that the local observation success of symptomatic cases was between 12.8 –
439 16.1%. However, if we assume that 75 – 97% of people infected with CHIKV will
440 develop symptomatic infections, as reported for the Indian Ocean lineage [10, 54,
441 55], then the chances of reported a symptomatic CHIKV case decrease to 5 – 7%
442 [9]. Case reports suggest that the beginning of the exponential phase of the
443 outbreak was in December 2016 (**Figure S4**), while genetic data suggests that the
444 outbreak clade emerged around July 2016. However, between August 2014 and
445 June 2016, 612 CHIKV notified cases and 40 confirmed cases were reported by the
446 LACEN-RR. It is therefore likely that prior to Jan 2017, low but non-neglectable
447 transmission of the Asian genotype occurred in Roraima.

448 We investigated the public's awareness of the chikungunya outbreak by
449 retrospectively monitoring Google searches of the search term “chikungunya” in
450 Roraima state from January 2014 to July 2018 (**Figure 4**). As a comparison, we

451 performed a similar search focusing on the neighbouring state of Amazonas. We
452 found that web search activity and CHIKV cases counts in Roraima are highly
453 correlated (notified cases: $r = 0.89$; confirmed cases: $r = 0.92$, **Figure 4d – e**).
454 Additionally, the timing of the peak of Google searches corresponds to that of
455 notified and confirmed cases with a peak in July 2017 (**Figure 4a and c, Figure 4b**
456 **and f**). It is important to note that web search activity was available weeks or
457 months before the final number of confirmed (and suspected) cases were made
458 publicly available. This fact highlights the potential utility of monitoring disease-
459 related searches during the outbreak. Interestingly, we find some web-search
460 activity in Roraima before June 2016, particularly in September 2014, March 2015
461 and March 2016 (**Figure 4f**). These patterns are distinct to those in the Amazonas
462 neighbouring state (notified cases: $r = 0.65$; confirmed cases: $r = 0.15$), which
463 shows an early peak in November 2014, soon after the estimated age of *node B*
464 (**Figure 3b**), followed by a peak in February 2016 and another in March 2017
465 (**Figure 4c**). These multiple peaks in internet search queries are consistent with the
466 timing of at least 3 introductions detected in our phylogenetic analyses (**Figure**
467 **3b**), each possibly resulting in small epidemic waves of CHIKV in Manaus and
468 Amazonas states.

469 **Discussion**

470 We describe a genomic epidemiological study which used genetic,
471 epidemiological, and digital search data to investigate an outbreak caused by CHIKV
472 in Boa Vista city, Roraima state, northern Brazil, in 2017. Using a combination of
473 genetic, laboratory-confirmed and -suspected, and digital search data from 2014 to
474 2018, we find evidence for the replacement of the Asian lineage by the ECSA lineage
475 in the north of Brazil. Moreover, we find that ECSA lineage was introduced in
476 Roraima around July 2016, six months before the beginning of the exponential
477 increase in case numbers. Using simple epidemiological modes, we find that on
478 average 1 in 17 (95% CI: 14 – 20) symptomatic CHIKV cases, a fraction of the
479 110,882 (95% CI: 102,352 – 127,940) estimated number of infections, sought
480 medical care during the outbreak of CHIKV ECSA in Roraima. Finally, we find that
481 Google search activity data shows a strong association with CHIKV notified cases in
482 Roraima. Moreover, although the nanopore-based sequencing protocol for CHIKV
483 utilized in this study has been described and validated previously [32], this study
484 represent to our knowledge the first effort to generate on-site complete CHIKV
485 genome sequences. Our results provide evidence of lineage replacement in Brazil, and
486 to the best of our knowledge, deliver a description of the largest outbreak ever
487 reported in north Brazil, revealing the circulation of the ECSA lineage in the Amazon
488 region.

489 We estimate that 39% (95% CI: 36 – 45)% of Roraima's population was
490 infected with CHIKV-ECSA-Br during the outbreak in 2017. Our estimates are higher
491 than the 20% seropositive observed in a rural community in Bahia [10], and slightly
492 lower than the 45.7 – 57.1% observed in two serosurveys conducted in the same state
493 [12], where the ECSA lineage also seems to predominate. The observed differences
494 in terms of the proportion of the population exposed to CHIKV in Roraima
495 compared to previous estimates from the northeast region could result from partial
496 protection resulting from low-level transmission of the CHIKV-Asian genotype
497 during 2014 – 2016 in the north region. Alternatively, some level of cross-
498 protection could have been conferred by previous exposure to Mayaro virus
499 (MAYV); Mayaro is an antigenically-related alphavirus that may provide some
500 level of cross-reactivity [56, 57] and is associated with *Haemagogus* spp. vectors
501 [58], but has also been identified in *Culex quinquefasciatus* and *Aedes aegypti*
502 mosquitoes [59]. MAYV has been detected in the north [60-64] and centre-west

503 [21, 59, 65-68] regions of Brazil. Moderate to high prevalence of MYV IgM have
504 been found in urban northern areas [60], which could explain the limited spread of
505 CHIKV in Manaus compared to Roraima.

506 Different CHIKV circulating lineages may have remarkably different public
507 health consequences. Lineage-specific clinical presentations have been recently
508 highlighted by a recent index cluster study which showed that 82% of CHIKV
509 infections caused by the ECSA lineage are symptomatic, in comparison to only 52%
510 of symptomatic infections caused by the Asian genotype [54]. While the Asian
511 lineage seems to have circulated cryptically for 9 months before its first detection in
512 the Caribbean [3], the faster detection of the ECSA lineage in Brazil could at least in
513 part be a consequence of a higher rate of symptomatic to asymptomatic infections of
514 the ECSA lineage circulating in Brazil. The time lag between the phylogenetic
515 estimate of the date of introduction of a virus lineage and the date of the first
516 confirmed case in a given region, enables us to identify surveillance gaps between the
517 arrival and discovery of a virus in that region [69].

518 We used genomic data collected over a 3-year period to estimate the genetic
519 history of the CHIKV-ECSA-Br lineage. We estimate that the CHIKV-ECSA-Br
520 lineage arrived in Roraima around July 2016, whilst the first confirmed CHIKV cases
521 in Roraima occurred earlier, in August 2014. That the discovery date anticipates the
522 estimated date of introduction can be explained by initial introduction(s) of the Asian
523 lineage (from the north of Brazil or from other south American regions) resulting in
524 only limited onwards transmission, followed by the replacement of the Asian lineages
525 by an epidemiological successful ECSA lineage. Transmission of the Asian genotype
526 during this period is in line with an increase in notified and confirmed cases, as well
527 internet search query data between August 2014 and June 2016. Nationwide
528 molecular and seroprevalence studies combined with epidemiological modelling [70]
529 will help to determine the proportion of cases caused by the ECSA compared to the
530 Asian lineage in different geographic settings, and to identify which populations are
531 still at risk of infection in Brazil.

532 We estimated high rates of nucleotide substitution for this lineage, which
533 equates to around 8 (95% BCI: 6 – 11) nucleotide substitutions per year across the
534 virus genome. Such rates are similar to the evolutionary rates estimated for the IOL
535 lineage; these are typical of urban and epidemic transmission cycles in locations with
536 an abundance of suitable hosts and lack of herd immunity [8]. None of the mutations

537 associated previously with increased transmissibility of the IOL lineage in *Ae.*
538 *albopictus* mosquitos in the Indian Ocean region were identified in this study.
539 However, it is currently unclear whether we should expect the same mutations to be
540 linked with increased transmission in *Aedes* spp. populations both from Brazil and
541 from Southeast Asia. Further, it is possible that CHIKV in Brazil is vectored mainly
542 by the *Ae. aegypti* vector that is abundant throughout Brazil [71]. In line with this,
543 CHIKV-ECSA was recently detected in *Aedes aegypti* from Maranhão [72] and Rio
544 de Janeiro states [73].

545 The past dengue serotype 4 genotype II outbreak in Brazil ignited in the north
546 of the country, and is inferred to have been introduced from Venezuela to Roraima,
547 before spreading to the northeast and southeast region of Brazil [74]. Our genetic
548 analysis reveals at least four instances of ECSA-Br virus lineage migration in the
549 opposite direction, i.e., from northeastern to northern Brazil. Such a pattern may not
550 be surprising due to the year-round persistence of *Aedes aegypti* mosquitos in the
551 northeast and the north areas [31]. Within-country transmission will be dictated by
552 human mobility, climatic synchrony, and levels of population immunity. Moreover,
553 international spread of the ECSA-Br lineage is expected to regions linked to Brazil.
554 Previous analyses of dengue virus serotypes has identified a strong connectivity
555 between north Brazil and Venezuela [25, 75], and northeast Brazil and Haiti [31, 76].
556 In addition, Angola and Brazil are linked by human mobility and synchronous
557 climates that have facilitated the migration of CHIKV-ECSA [1] and Zika virus
558 (<http://virological.org/t/circulation-of-the-asian-lineage-zika-virus-in-angola/248>).

559 Improving surveillance in the Amazon region may help anticipate
560 transmission of vector-borne diseases and also spillover from wild mammals of
561 zoonotic viruses of particular concern [27]. Genomic portable sequencing of vector-
562 borne viral infections in the Amazon may be particularly important in the context of
563 early identification of circulation of strains newly (re)-introduced from wildlife. For
564 example, yellow fever strains collected in Roraima seem to be at the source of the
565 2016-2018 yellow fever virus outbreak in southeast Brazil, which has affected large
566 urban centres in Minas Gerais, São Paulo and Rio de Janeiro [26]. In the near future,
567 the increasing rapidity and decreasing cost of genome sequencing in poorly sampled
568 areas, combined with emerging theoretical approaches [77], will facilitate the
569 investigation of possible associations between arbovirus lineage diversity, mosquito
570 vectors, reservoir species, and transmission potential.

571 Finally, the reported synchronicities between notified chikungunya case
572 counts in Roraima and the chikungunya-related Internet searches originated in the
573 region highlight the potential complementarity that Internet search activity may offer
574 in future disease outbreaks. Specifically, given that disease-related search activity can
575 be monitored in near-real time, early signals of increases in disease activity may be
576 spotted weeks or months before lab-confirmed case counts may be available in an
577 unfolding outbreak.

578

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584 additional flowcells and corresponding reagents, and also thank QIAGEN for
585 donation of consumables.

586

587 **Figure Legends**

588 **Figure 1. Context of this study.** A. Map showing municipalities of Roraima state,
589 including Boa Vista, bordering countries (Venezuela and French Guiana) and
590 bordering Brazilian federal states (Amazonas and Pará). B. Map of Brazilian states,
591 showing the states from which CHIKV sequence data in this study was analysed
592 (Bahia, Alagoas, Pernambuco, Paraíba, Amazonas and Roraima). C. Barplot showing
593 the annual number of notified CHIKV cases in selected states of Brazil (data obtained
594 from the Brazilian Ministry of Health). Map was made with Natural Earth. Free
595 vector and raster map data at naturalearthdata.com.

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598 **Fig. 2. Sequencing statistics, temporal signal and evolutionary rates of the**
599 **CHIKV-ECSA lineage.** A. Genome coverage plotted against RT-qPCR CT-values
600 for the newly generated sequence data. B. Genetic divergence regressed against dates
601 of sample collection for dataset 2 (CHIKV-ECSA-Br lineage). C. Evolutionary rate
602 estimates for the CHIKV-ECSA-Br lineage obtained by this study (circle number 1)
603 compared to published evolutionary rates obtained for other lineages. Circles
604 numbered 2 to 8 represent point estimates reported in [1, 8, 78]. Horizontal bars
605 represent 95% highest posterior density credible intervals for associated evolutionary
606 rates.

607

608 **Figure 3. Genetic analysis of the CHIKV-ECSA genotype.** A. Maximum likelihood
609 phylogeny depicting the monophyletic clade containing all the Brazilian ECSA
610 isolates (ECSA-Br lineage). B. Time-calibrated phylogeny of all available CHIKV-
611 ECSA whole genome sequences from Brazil, including 18 novel genomes from
612 Roraima and Amazonas states. Colours correspond to state of sample collection.
613 Violin plots show 95% Bayesian credible intervals for associated node heights [38].

614

615 **Figure 4. Digital surveillance of chikungunya disease in northern Brazil.** A and B
616 show respectively the number of notified CHIKV cases in LACEN-RR and LACEN-
617 AM between Jan 2014 and Sep 2018. Panels C and F show Google Trends activity for
618 the term “chikungunya” in Amazonas (C) and Roraima (F) from Jan 2016 and Sep
619 2018. Panels D and G show the correlation between Google Trends activity and
620 confirmed cases in Amazonas (D) and Roraima (G), while panels E and H show the

621 correlation between Google Trends activity and notified cases in Amazonas (E) and
622 Roraima (H).

623 **Table 1.** Epidemiological data for virus isolates from Roraima (RR) and Amazonas
624 (AM). CT=cycle threshold, *d*=days from onset of symptoms to sample collection.
625 Corresponding sequencing statistics are available in **Table S1**. Isolates were
626 collected around 2.3 (range: 0 – 5) days after onset of symptoms. Acc. Number =
627 GenBank accession number.

628

Isolate	State, Municipality	Acc. Number	Ct RT- qPCR	Coverage (%)	Age, Sex	Collection date	<i>d</i>
AMA290	AM, Manaus	MK121891	NA	90.2	76, F	15/07/2015	5
AMA291	AM, Manaus	MK121892	NA	80.7	48, F	15/07/2015	4
AMA292	AM, Manaus	MK121893	NA	90.2	50, M	15/07/2015	0
AMA293	AM, Manaus	MK121894	NA	84.4	42, M	31/01/2016	4
AMA294	RR, Boa Vista	MK134712	NA	90.2	45, F	01/12/2014	2
AMA295	RR, Unknown	MK134713	NA	90.2	9, F	11/11/2014	1
AMA74	AM, Manaus	MK121895	15	90.2	32, F	20/03/2017	2
AMA346	RR, Boa Vista	MK121896	13.7	90.2	30, F	03/03/2017	1
AMA350	RR, Bonfim	MK121897	27.15	54.7	32, F	20/02/2017	1
AMA352	RR, Boa Vista	MK121898	17.33	88.6	3, F	22/02/2017	1
AMA354	RR, Boa Vista	MK121899	23.36	86.9	19, F	17/03/2017	1
AMA362	RR, Iracema	MK121900	18.63	88.6	31, F	17/03/2017	1
AMA364	RR, Boa Vista	MK121901	25.93	83.3	19, F	17/03/2017	2
AMA366	RR, Boa Vista	MK121902	19.87	90.0	36, F	17/03/2017	2
AMA368	RR, Boa Vista	MK121903	25.91	93.1	26, F	15/03/2017	2
AMA369	RR, Boa Vista	MK121904	21.55	95.6	52, M	02/03/2017	3
AMA374	RR, Boa Vista	MK121905	27.41	71.4	64, F	02/03/2017	4
AMA379	RR, Boa Vista	MK121906	17.5	96.1	38, F	27/02/2017	4
AMA381	RR, Boa Vista	MK121907	16.66	97.7	31, F	27/02/2017	4
AMA382	RR, Boa Vista	MK121908	14.58	76.6	30, F	05/03/2017	1

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636 **Supplementary Figure Legends**

637 **Figure S1. Maximum likelihood phylogenetic tree of the CHIKV Asian genotype.**

638 Includes isolates from Southeast Asia, Americas and Brazil. Isolates represented by
639 blue tips were sampled in Roraima, while isolates shown in red represent other strains
640 sampled in Brazil.

641

642 **Figure S2. Dating estimates obtained under different coalescent models.**

643 Estimates for node A (time of the most recent common ancestor, in dark red, see
644 Figure 3b), node B (main Amazonas clade, in green), and node C (Roraima clade, in
645 purple) are shown for different non-parametric models (Bayesian skygrid, skyride,
646 skyline) and for a simple constant population size model.

647

648 **Figure S3. Demographic dynamics of CHIKV ECSA-Brazilian lineage in Brazil.**

649 Fluctuation of effective population size over time as inferred through a Bayesian
650 skygrid coalescent model.

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652 **Figure S4. Exponential Period of the CHIKV epidemic in Boa Vista**

653 **municipality, Roraima state.** Log number of notified cases per month are plotted
654 against number of months since January 2015.

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670 **Supplementary Table**

671 **Table S1. Minion sequencing statistics**

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Isolate	Mapped reads	Average depth coverage	Bases covered >10x	Bases covered > 25x	Reference covered (%)
AMA290	13624	767	10276	10258	90.2
AMA291	60261	2047	9489	9280	80.7
AMA292	68090	2746	10402	10223	90.2
AMA293	64953	2096	9745	9701	84.4
AMA294	21361	701	10252	10022	90.2
AMA295	16370	531	10188	10077	90.2
AMA74	42276	1951	10396	10195	90.2
AMA346	31210	1225	10243	10208	90.2
AMA350	63672	1673	7522	7168	54.7
AMA352	13530	536	10219	10184	88.6
AMA354	22214	752	10082	9985	86.9
AMA362	9938	398	10237	10128	88.6
AMA364	28494	1079	9813	9577	83.3
AMA366	38228	1441	10264	10224	90.0
AMA368	12968	503	11122	10825	93.1
AMA369	7280	311	11225	11149	95.6
AMA374	7030	305	10225	8805	71.4
AMA379	7970	348	11226	11092	96.1
AMA381	7522	327	11214	11208	97.7
AMA382	14040	411	9915	9424	76.6

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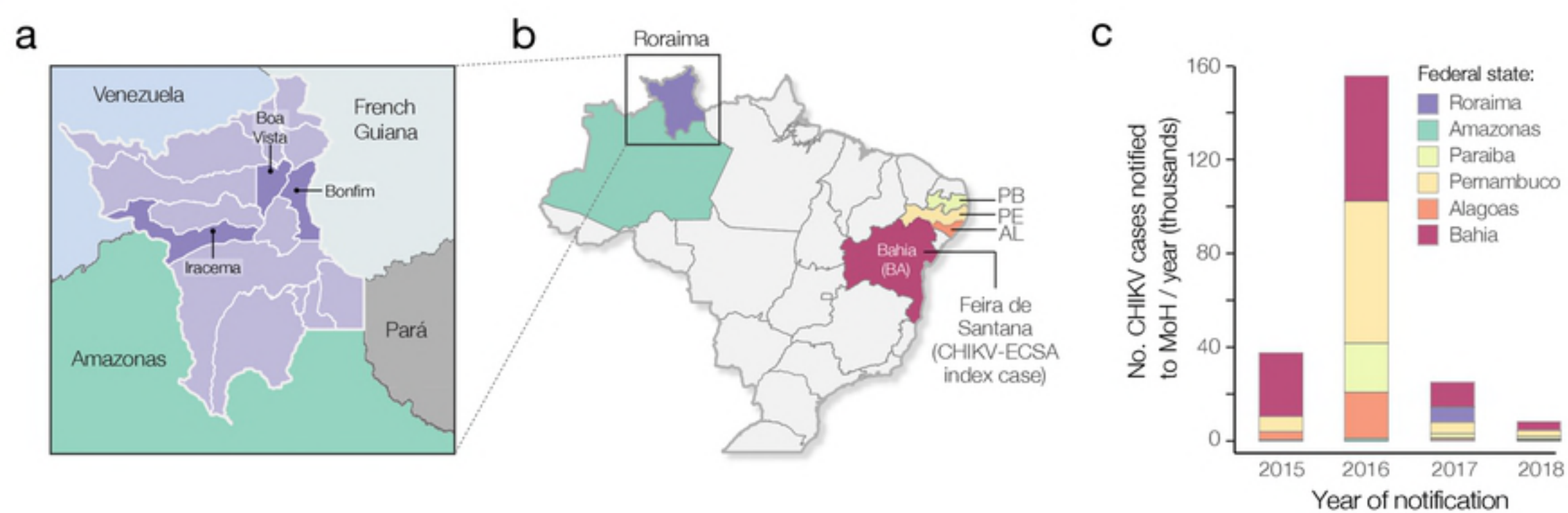


Figure 1

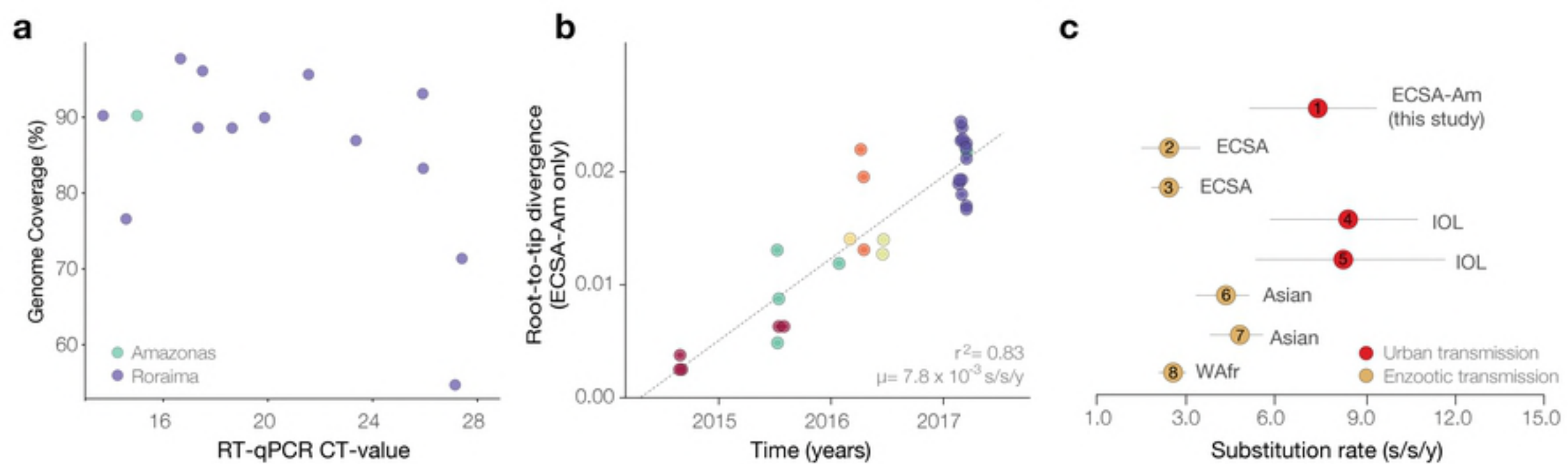
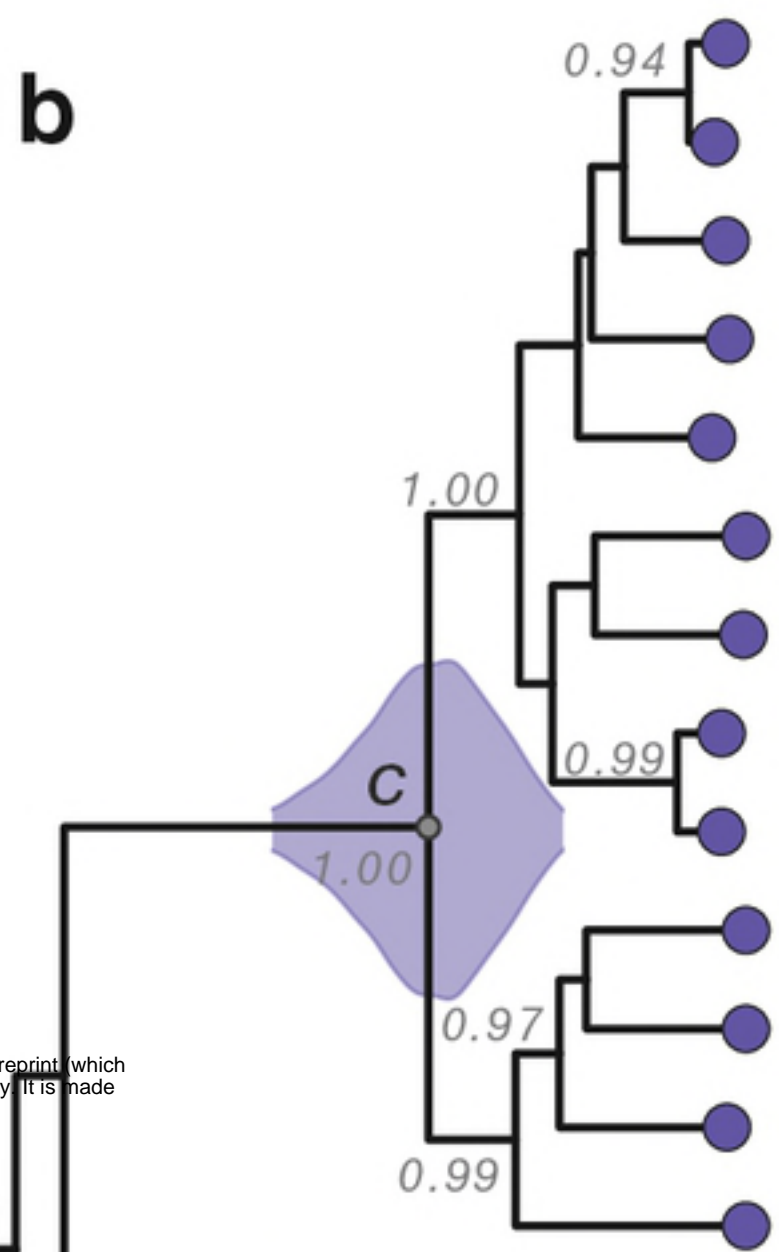
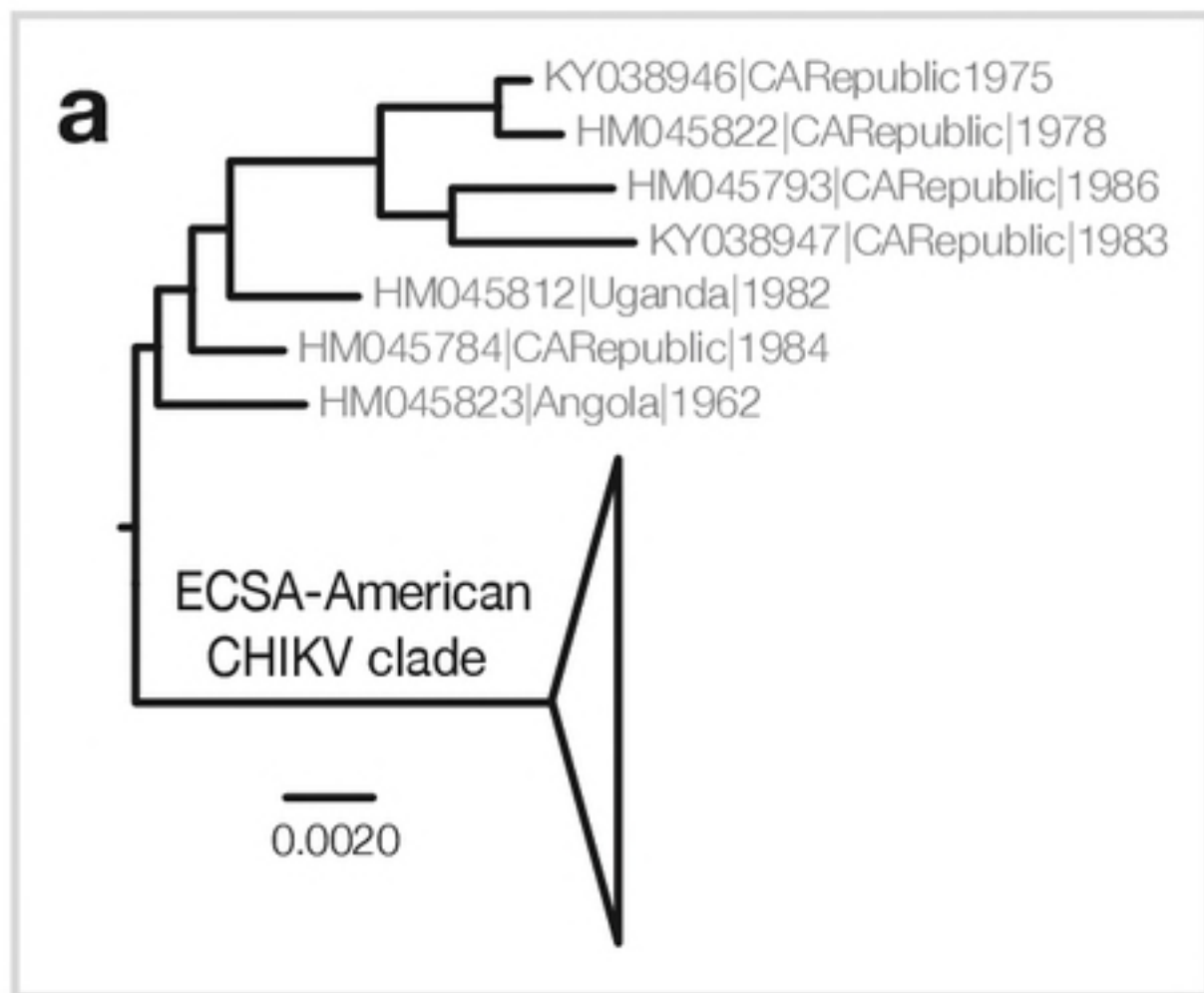


Figure 2



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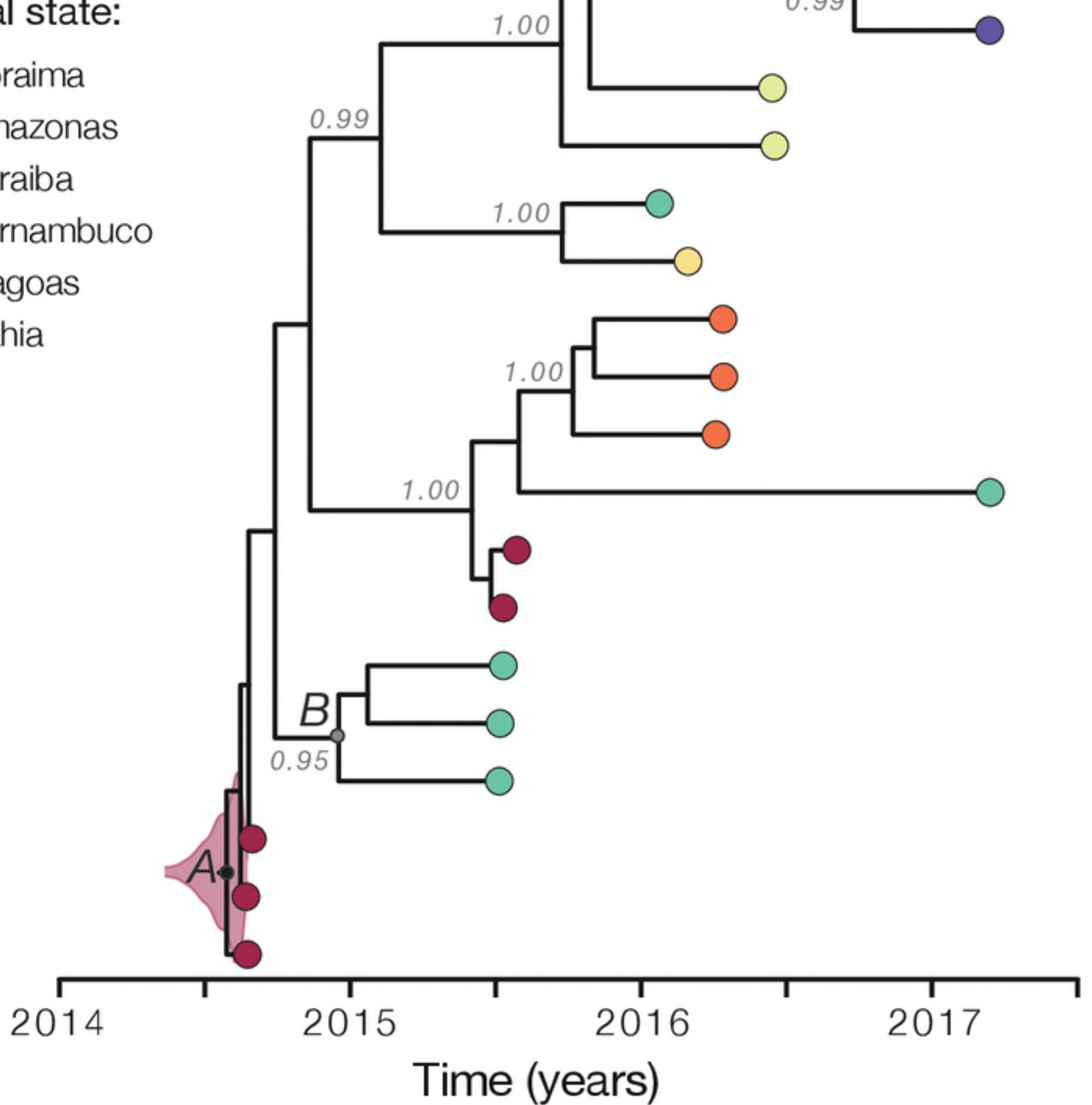


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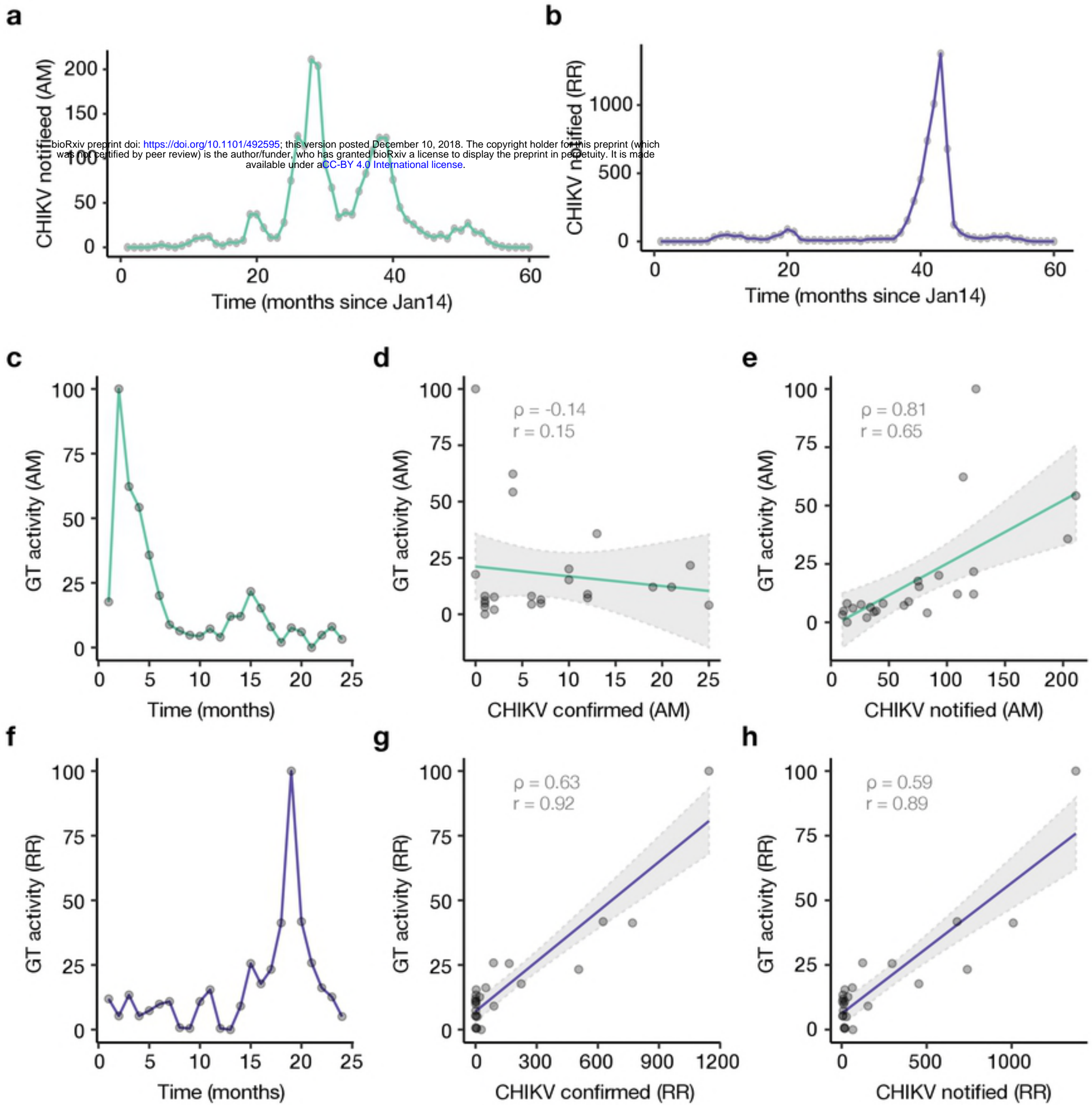


Figure 4