

1 **Dynamics and ecology of a multi-stage expansion of Oropouche virus in Brazil.**

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44

## 45 **Abstract**

46 In March 2024, the Pan American Health Organization (PAHO) issued an alert in response to a rapid  
47 increase in Oropouche fever cases across South America. Brazil has been particularly affected, reporting a  
48 novel reassortant lineage of the Oropouche virus (OROV) and expansion to previously non-endemic areas  
49 beyond the Amazon Basin. Utilising phylogeographic approaches, we reveal a multi-scale expansion  
50 process with both short and long-distance dispersal events, and diffusion velocities in line with human-  
51 mediated jumps. We identify forest cover, banana and cocoa cultivation, temperature, and human  
52 population density as key environmental factors associated with OROV range expansion. Using  
53 ecological niche modelling, we show that OROV circulated in areas of enhanced ecological suitability  
54 immediately preceding its explosive epidemic expansion in the Amazon. This likely resulted from the  
55 virus being introduced into simultaneously densely populated and environmentally favourable regions in  
56 the Amazon, such as Manaus, leading to an amplified epidemic and spread beyond the Amazon. Our  
57 study provides valuable insights into the dispersal and ecological dynamics of OROV, highlighting the  
58 role of human mobility in colonisation of new areas, and raising concern over high viral suitability along  
59 the Brazilian coast.

60

61 **Keywords:** Oropouche virus; phylodynamics; phylogeography; ecological niche modelling.

62

## 63 **Main text**

64 Oropouche virus (OROV; *Oropouche orthobunyavirus*) is an arthropod-borne virus first identified in  
65 1955 in Oropouche, a village in Trinidad and Tobago (1). OROV typically causes a febrile illness with  
66 symptoms such as high fever, headache, myalgia, arthralgia, photophobia, nausea, vomiting, and  
67 dizziness (2). In some cases, the illness can progress to severe neurological complications, including  
68 meningo-encephalitis (1). This re-emerging virus circulates primarily among wildlife such as non-human  
69 primates, rodents, sloths, and birds. It has caused around 30 documented human outbreaks in the Amazon  
70 region in recent years (3, 4). The midge *Culicoides paraensis* serves as the primary vector for human  
71 transmission, but other secondary vectors include *Culex quinquefasciatus*, *Coquillettidia venezuelensis*,  
72 and *Aedes (Ochlerotatus) serratus* (1, 5).

73 In March 2024, the Pan American Health Organization (PAHO) issued an alert in response to a rapid  
74 increase in Oropouche fever cases across several countries, including Brazil, Cuba, Bolivia, Colombia,  
75 and Peru (6, 7). By October 6, 2024, a total of 10,275 confirmed cases of Oropouche had been reported  
76 across nine countries in the Americas, as well as the first two deaths (6, 8). Brazil has been particularly

77 affected, reporting not only the highest number of cases, but also severe complications linked to  
78 Oropouche virus infection (9). Recent epidemiological data and genomic investigations in Brazil (10, 11)  
79 have described the recent expansion of OROV into previously non-endemic regions. These studies have  
80 identified reassortment events in the virus genome that may have contributed to its changing  
81 epidemiology. While the exact role of reassortment in the adaptation of OROV to novel environments  
82 remains to be fully understood, it has possibly impacted its spread into new ecological niches (10, 11).

83  
84 As with other arboviruses (12), recent changes in ecological context, such as deforestation, urbanisation,  
85 human mobility, and climate change, have possibly contributed to the emergence of OROV in new  
86 regions (13). In particular, environmental disruption pushes non-human mammal reservoirs and vectors  
87 into closer contact with human populations, facilitating viral spread (14). Additionally, human activities  
88 (15, 16) like urban expansion and altered land use increase the risk of transmission of OROV in peri-  
89 urban and urban settings, where vectors such as *Culicoides paraensis* and *Culex quinquefasciatus* thrive  
90 (17–19). Despite these insights, significant gaps remain in quantifying the precise impact of these factors  
91 on recent OROV transmission dynamics. In addition, much remains unknown about the broader disease  
92 ecology of OROV, particularly concerning environmental correlates of local circulation and of its recent  
93 expansion. This lack of comprehensive understanding hampers effective risk assessment and preparedness  
94 efforts, both within Brazil and across the Americas.

95  
96 This study aims to formally test key epidemiological hypotheses regarding OROV disease ecology and its  
97 range expansion. We integrate spatially explicit pathogen genomes and epidemiological data with  
98 geospatial data in a phylodynamic and ecological niche modelling framework to (i) reconstruct the  
99 dispersal history of OROV lineages across Brazil and analyse dispersal statistics in the context of a range  
100 expansion, (ii) evaluate the environmental factors associated with OROV transmission during distinct  
101 transmission phases, and (iii) map the ecological niche of OROV transmission to identify covariates of  
102 circulation suitability in the context of the expansion, and to pinpoint surveillance blind spots.

## 103 104 **Results**

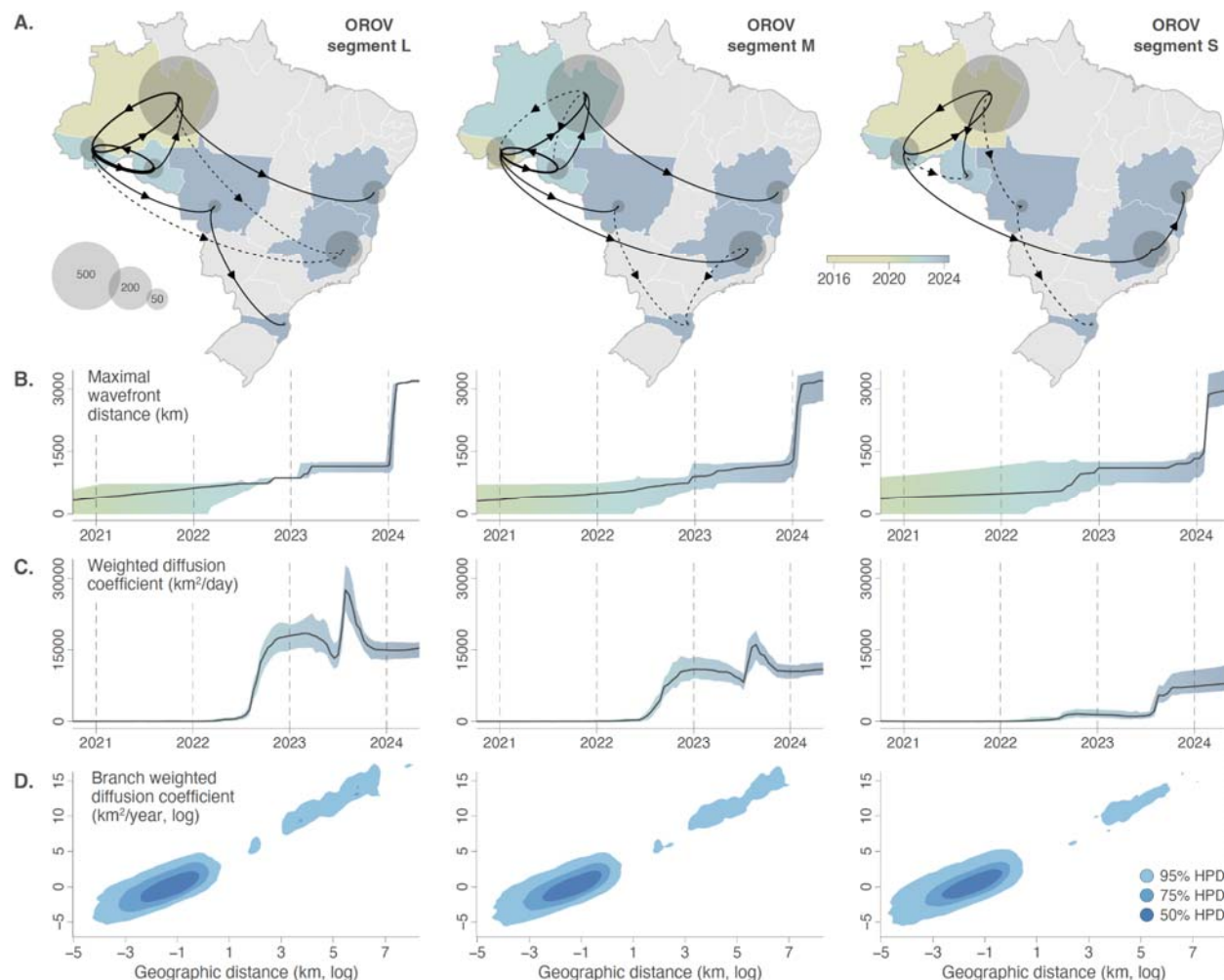
### 105 106 **Dispersal history and dynamics of OROV in Brazil**

107 The epidemiological dynamics of OROV expansion in Brazil in late 2023 and 2024 show a two-stage  
108 process: a rapid rise in cases in Amazonian states, particularly Manaus, followed by widespread  
109 circulation in other parts of the country (Supplementary Figure S1). To further investigate, we applied a  
110 continuous phylogeographic approach using over ~500 genomes sampled between 2022 and 2024 (10,  
111 11). Building on prior knowledge of reassortment events across genome segments (10, 11), we conducted  
112 separate phylogeographic reconstructions for segments L, M, and S. Our analysis extracted  
113 spatiotemporal data from 100 annotated trees subsampled from post burn-in posterior distributions,  
114 revealing new insights into transition events between sampled regions (**Figure 1A**).

115  
116 The earliest lineage dispersal events were restricted to the Amazon basin and inferred to be before 2020,  
117 with the virus gradually spreading to other Brazilian states in a southeasterly direction in 2023 and 2024.  
118 By further examining the spatial dissemination of OROV lineages across Brazil, we found that the virus  
119 reached a maximal wavefront distance of over 3,000 km from its epidemic origin through the entire  
120 dissemination period (**Figure 1B**). This reconstruction further captured two distinct expansion phases

121 during which the wavefront distance increased rapidly, indicating the invasion of new areas >2024  
122 (**Figure 1B**). The rapid expansion of wavefront distance in 2024 corresponds to the increase in cases in  
123 areas outside the Amazon (**Supplementary Figure S1**). Furthermore, diffusion coefficient estimates have  
124 evolved through time, with increases in mid-2022 and mid-2023 consistently detected in all three OROV  
125 segments (**Figure 1C**). As a result, the weighted diffusion coefficient estimated for the overall period was  
126 also notably high: 582 km<sup>2</sup>/day (95% HPD = [477, 672]) for segment L, 574 km<sup>2</sup>/day (95% HPD = [464,  
127 677]) for segment M, and 515 km<sup>2</sup>/day (95% HPD = [435, 675]) for segment S. These values are  
128 significantly higher than those reported for key viral dispersal events, such as for West Nile virus spread  
129 via birds in North America (20), reflecting a substantial dispersal capacity. In 2024, a large number of  
130 dispersal events occurred within the states of Mato Grosso, Minas Gerais and Bahia (midwest, southeast  
131 and northeast Brazil, respectively), which were previously thought to be non-endemic for OROV  
132 transmission.

133  
134 With respect to dispersal distance and diffusion velocity, we observe a large group of phylogenetic  
135 branches associated with relatively short dispersal distance (<20km) and slow diffusion (<4km<sup>2</sup>/day;  
136 **Figure 1D**), and a subsequent group of branches that correspond to faster long-distance dispersal events  
137 (**Figure 1D**). This supports a multi-scale expansion process with a combination of short-distance  
138 diffusive movement and fast long-distance jumps, with some of the latter likely reflecting human-  
139 mediated virus movements. We also identified substantial isolation-by-distance (IBD) patterns, with  
140 Pearson correlations between patristic and log-transformed geographic distances between samples close  
141 or greater than 0.5 for all three segments (0.470 (95% HPD = [0.360, 0.599]) for segment L, 0.575 (95%  
142 HPD = [0.286, 0.607]) for segment M, and 0.684 (95% HPD = [0.326, 0.710]) for segment S). Overall,  
143 these dispersal metrics provide a clear indication of rapid long-distance dispersal events during the recent  
144 OROV expansion in 2024 beyond the Amazon Basin, likely human-mediated, followed by more localised  
145 viral circulation. This view is supported by an examination of air travel data from Brazil, with  
146 considerable human mobility between the Amazon region and other parts of the country, primarily  
147 through the airport in Manaus (**Supplementary Figure S2**). Air travel data also support the inferred long-  
148 distance dispersal routes from the state of Acre to the east coast of Brazil, and also highlight the  
149 intermediate role played by the state of Mato Grosso in the virus' migration out of the Amazon region.  
150



151  
152 **Figure 1. Dispersal history and dynamics of OROV lineages in Brazil.** (A) Dispersal history of OROV lineages  
153 inferred through continuous phylogeographic reconstructions. Lineage dispersal events between Brazilian states  
154 with a posterior probability  $\geq 0.95$  are displayed by solid arrows, and dispersal events with a posterior probability  
155  $< 0.95$  are displayed by dashed arrows. Additionally, the location of the different areas is represented by transparent  
156 grey dots whose surface is proportional to the number of local lineage dispersal events, i.e. phylogenetic branches  
157 inferred as remaining in that state. Brazilian states are coloured according to the estimated date of the first invasion  
158 event (median date computed from the 100 trees sampled from the posterior distribution). (B) Evolution through  
159 time of the spatial wavefront distance, representing the maximal distance from the epidemic origin over time. (C)  
160 Evolution through time of the weighted diffusion coefficient, a dispersal metric that measures the dispersal capacity  
161 of viral lineages. (D) Kernel density plots with the branch-weighted diffusion coefficient against the geographic  
162 distance travelled by each branch (both axes being log-transformed).

### 163 164 **Ecological factors associated with the transmission of OROV in Brazil**

165 To elucidate the ecological factors associated with the spatial expansion of OROV in Brazil, we analysed  
166 virus dispersal history in relation to 28 environmental factors (including land-use, climatic, and  
167 demographic variables) (**Supplementary Figure S3**). In the absence of established species distribution  
168 models for *Culicoides paraensis*, the presumed primary vector for OROV, we also considered the  
169 ecological suitability of *Ae. aegypti* as a covariate, as this species could act as a proxy for urban  
170 anthropophilic vectors, or as a potential secondary vector for OROV (21, 22). *Aedes (Ae.) aegypti* is a

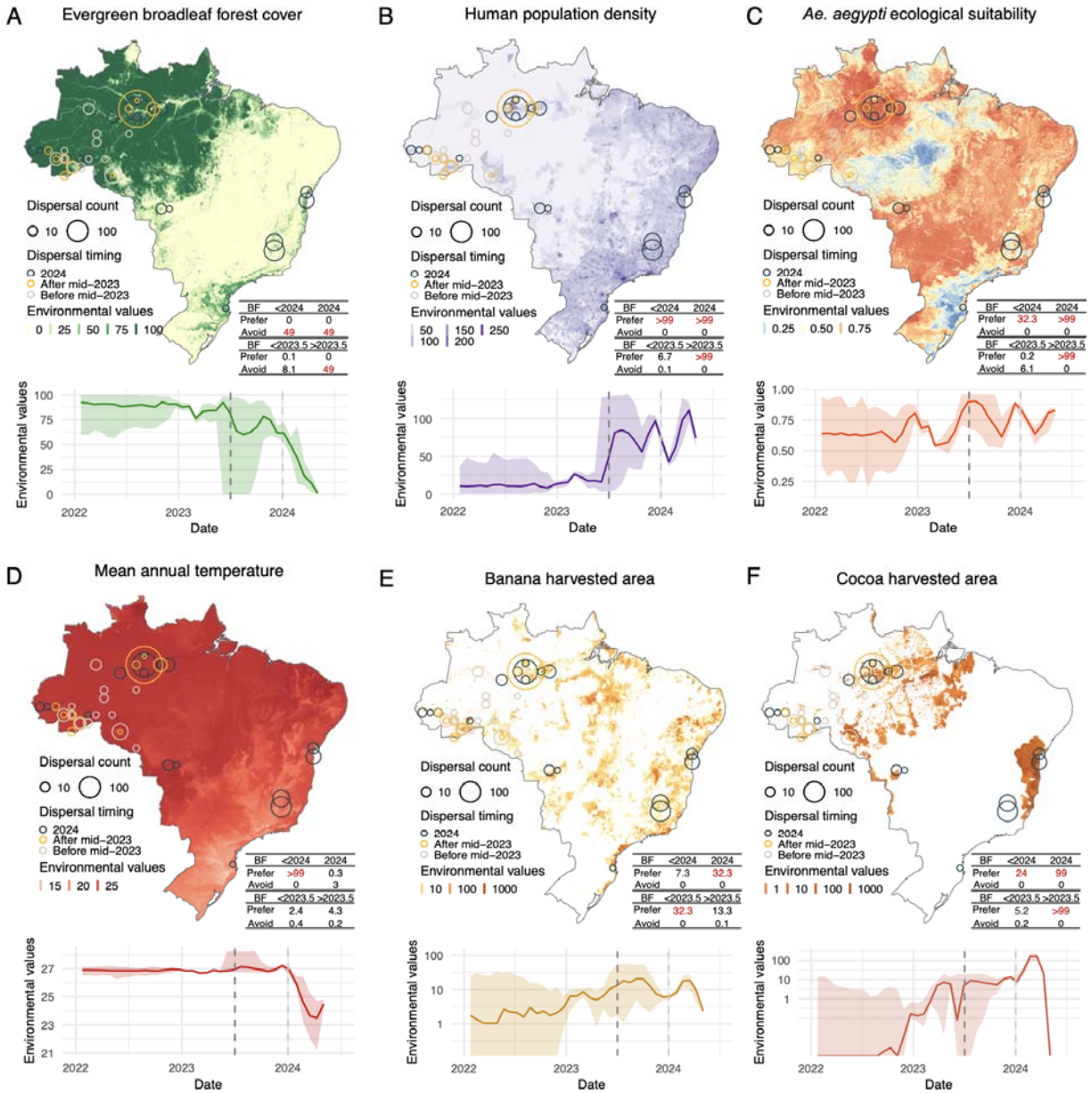


171 well-established vector for several arboviruses, including dengue, Zika, and chikungunya, and its wide  
172 distribution across urban areas in Brazil (**Supplementary Figure S3**) may facilitate the spread of OROV,  
173 especially in densely populated regions with favourable breeding conditions. While so far *Ae. aegypti* has  
174 not been demonstrated as a viable vector, the situation with the novel reassortant lineage could be  
175 different (21).

176  
177 Using high-resolution environmental rasters for each covariate (**Supplementary Figure S3**), we extracted  
178 environmental values at the geographic location of each phylogenetic node. These correspond to inferred  
179 positions through continuous phylogeographic analysis for internal nodes, and to sampling locations  
180 (down to municipality level) for tip nodes (**Supplementary Figure S4**). Our analyses revealed that  
181 several covariates were temporally associated with OROV dispersal events, highlighting distinct viral  
182 circulation environments over time. We assessed shifts in dispersal environments relative to three specific  
183 time points: (1) prior to the re-emergence and epidemic expansion in the Amazon (<mid-2023), (2) during  
184 the Amazon-restricted transmission phase (<2024), and (3) during the national expansion phase (>2024).  
185 When comparing dispersal events before and after 2023-2024, different environmental conditions for  
186 OROV circulation become apparent (**Figure 2**). Certain dispersal environments seem to shift at the mid-  
187 2023 time point, while others at the 2024 time point (**Figure 2, Supplementary Figure S4**). For instance,  
188 prior to 2024, dispersal events occurred on average in areas with relatively higher evergreen broadleaf  
189 forest cover, higher precipitation and temperature, but lower population density and cocoa cultivation  
190 areas (**Figure 2, Supplementary Figure S4**). Interestingly, most lineage dispersal locations before 2024  
191 were associated with a mean temperature of  $\sim 27^{\circ}\text{C}$ , which may indicate an optimal viral replication  
192 environment for OROV in its vector and is supported by preliminary thermal biology studies on biting  
193 midges (23). However, dispersal environments were already shifting towards areas with high population  
194 density, increased urbanisation, and larger cocoa cultivation areas around mid-2023 (**Figure 2,**  
195 **Supplementary Figure S4**). With strong Bayes factor support (24), our analyses demonstrated that these  
196 trends in dispersal environments were consistent across the posterior distribution of trees obtained  
197 through continuous phylogeographic inference.

198  
199 To statistically test the association of environmental conditions with the spread of OROV lineages, we  
200 employed a landscape phylogeographic approach to analyse the environmental values extracted at the tree  
201 node positions (25). Specifically, for the three distinct time periods mentioned above, we tested whether  
202 inferred OROV lineages tended to preferentially circulate in or avoid certain environmental conditions.  
203 Statistical support (Bayes factors [BF]) was obtained by comparing the results from phylogeographic  
204 reconstructions with a null dispersal model, in which a new continuous diffusion process was randomly  
205 simulated along the same tree topologies. During both transmission phases divided by the 2024 cut-off,  
206 our results reveal strong support (BF >20 for at least two out of three segments) for preferential  
207 circulation of inferred OROV lineages in areas with higher population density and urbanisation, lower  
208 evergreen broadleaf forest cover, and areas associated with cocoa cultivation (**Figure 2, Supplementary**  
209 **Table S1**). We also highlight a preferential circulation of inferred OROV lineages in areas associated  
210 with banana cultivation in the expansion phase (>2024), as well as in the pre-expansion phase (<2024) for  
211 segment L (**Supplementary Figure S6**). These results are consistent with existing knowledge about *C.*  
212 *paraensis* larvae developing in microhabitats of decaying debris from banana and cacao plantations (26–  
213 28). Specific to the Amazon-only transmission phase (<2024), our results also indicate preferred  
214 circulation in areas with higher mean annual temperatures (**Figure 2D**). Additionally, our analyses

215 indicate strong support for preferential circulation of inferred OROV lineages in areas associated with  
216 higher *Ae. aegypti* ecological suitability, either in both phases (segment M) or in the only expansion phase  
217 (segments S and L; **Figure 2B, Supplementary Figure S5, S6 (Supplementary Table S1)**), although  
218 this could be an indirect association with human human density. These results indicate similarities in  
219 environmental conditions associated with OROV lineage circulation before and after the 2024 cut-off,  
220 suggesting that the expansion of the virus to areas outside the Amazon was not associated with drastically  
221 different environmental conditions. However, an examination of the differences in dispersal locations  
222 between transmission phases prior to and after mid-2023 reveals compelling differences: for viral lineages  
223 inferred prior to mid-2023, there was not strong support for a preferential circulation in densely populated  
224 areas, areas associated with a lower evergreen broadleaf forest coverage, and those more ecologically  
225 suitable for *Ae. aegypti*, although urbanised areas were still preferred (**Supplementary Table S1**). This  
226 suggests ecological differences underlying endemic OROV circulation within the Amazon before the re-  
227 emergence and rapid epidemic expansion at the end of 2023. Overall, our findings reveal similar trends  
228 across the three segments analysed (**Supplementary Figure S5, S6, Supplementary Table S1**).



229  
 230 **Figure 2. Environmental conditions associated with OROV lineage dispersal locations over time (for segment**  
 231 **M).** Figure panels show the spatial distribution of six main environmental factors (units specified): evergreen  
 232 broadleaf forest cover (%) (A), human population density (normalised between 0 and 255 per km<sup>2</sup> for visual clarity)  
 233 (B), *Ae. aegypti* ecological suitability (probability of occurrence) (C), mean annual temperature (°C) (D), banana  
 234 harvested area (in hectares, log-transformed) (E), and cocoa harvested area (in hectares, log-transformed) (F) in the  
 235 top rows. Circles on the map depict the end node of dispersal locations inferred by continuous phylogeography,  
 236 sized by the number of dispersal events in an area, and coloured by the timing of the event. Bottom rows of each  
 237 figure panel are line graphs depicting the environmental covariates associated with the locations of OROV lineage  
 238 dispersal events in Brazil. Each plot illustrates how specific ecological conditions have changed over time (2022-  
 239 2024) at the sites of viral lineage dispersal. The embedded tables show the association between environmental  
 240 conditions and the dispersal location of inferred OROV lineages. Based on the analysis of 100 posterior trees  
 241 obtained from continuous phylogeographic inference, the table reports Bayes factor (BF) supports for association



242 between environmental raster values and tree node locations. Following the scale of interpretation of Kass and  
243 Raftery (24), we highlight BF values >20 considered as strong supports.

244

### 245 **Mapping ecological niches for OROV transmission and range expansion in Brazil**

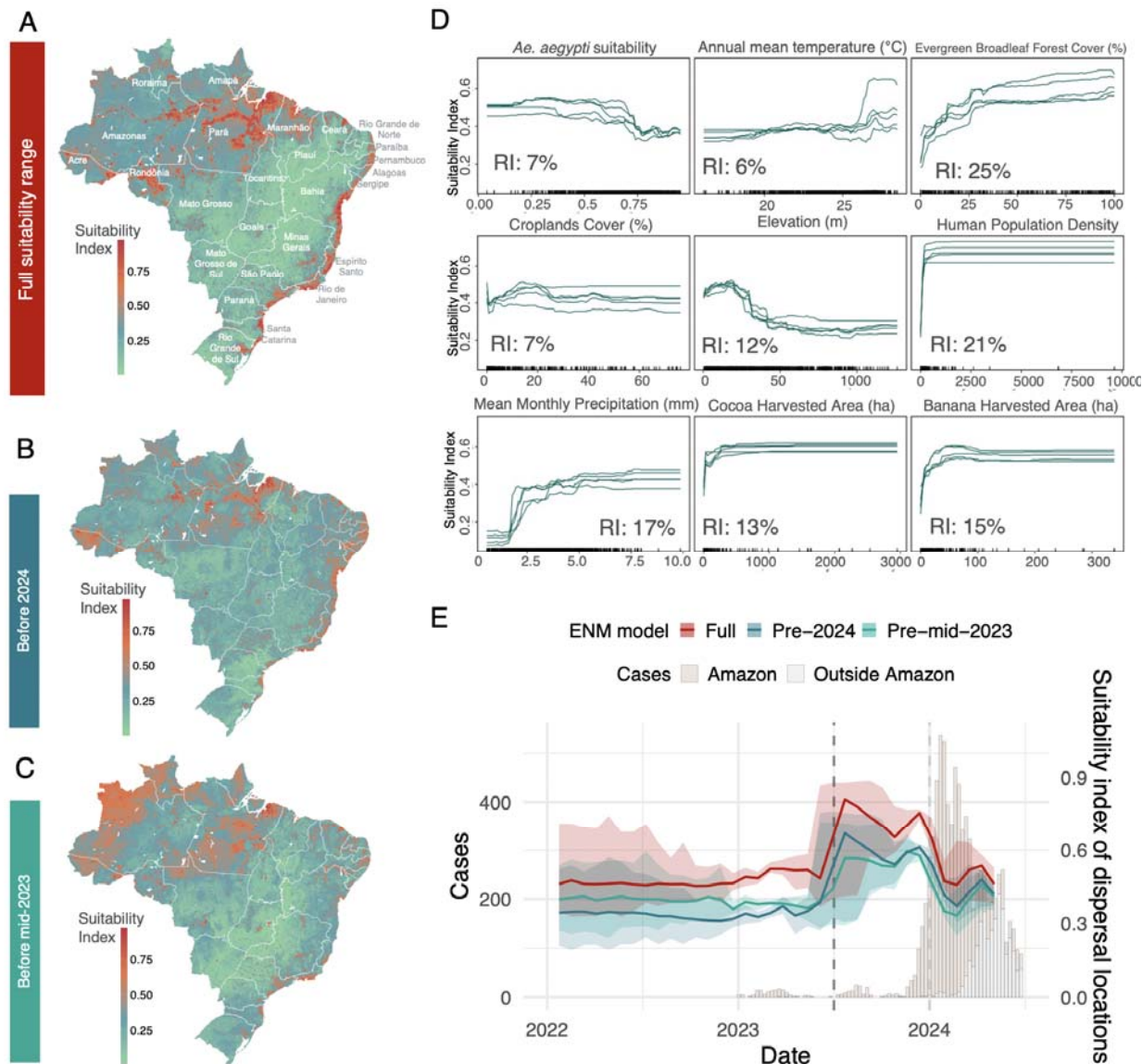
246 The increased detection of OROV cases in humans also provides an opportunity to apply modelling to  
247 examine areas ecologically suitable for local circulation of the virus, leading to human infections. We  
248 used an ensemble modelling approach to reveal OROV transmission suitability across Brazil. The  
249 suitability index ranges from 0 (unsuitable conditions) and 1 (highly suitable conditions) and illustrates  
250 the potential geographic areas where environmental conditions are most favourable for OROV  
251 transmission (**Figure 3A**). The disease presence points used as model input, represent OROV circulation  
252 leading to human cases from 450 geocoded sampling locations in Brazil (based on molecular testing and  
253 sequencing records) from 1957 to 2024 (~80% corresponding to 2023-2024). Given this large time span,  
254 climatic variables were matched to the corresponding decade of the occurrence point. In the context of  
255 presence-only ecological niche modelling, where true disease absence data is unavailable, we sampled  
256 pseudo-absence points. Pseudo-absence points sampling was informed by a kernel density estimate of  
257 human population density to reflect surveillance efforts which we assumed is proportional to human  
258 population density, with an exclusion radius around presence points (**Supplementary Figure S7**). The  
259 model incorporated the same environmental covariates used in the landscape phylogeography analyses  
260 (**Figure S1**), while testing for overfitting along with model performance (**Supplementary Table S3**). A  
261 principal component analysis (PCA) was performed to assess multicollinearity between the environmental  
262 covariates (**Supplementary Figure S8**), resulting in a final selection of nine variables: *Ae. aegypti*  
263 ecological suitability, annual mean temperature, evergreen broadleaf forest cover, croplands cover,  
264 elevation, human population density, annual mean monthly precipitation, as well as cocoa and banana  
265 harvested area coverages. We also report variability among our model predictions as a measure of  
266 uncertainty (**Supplementary Figure S9**). Uncertainty is higher in the central regions of Brazil  
267 (**Supplementary Figure S9**), where lower population density and limited sampling reduce the accuracy  
268 of our predictions. Overall, our findings indicate that the highest ecological suitability for local OROV  
269 circulation (index >0.7) is concentrated in the northern regions (**Figure 3A**), particularly within the  
270 Amazon Basin, which aligns with previous studies identifying this area as a significant epicentre for the  
271 virus (10, 11). Additionally, moderate to high suitability areas extend toward the northeast and central-  
272 western parts of Brazil, particularly in states such as Para, Maranhao, Bahia, and Mato Grosso, suggesting  
273 an expansion into previously non-endemic regions. Our model also highlights the potential for OROV  
274 transmission to move beyond traditional sylvatic cycles, affecting urban and peri-urban areas, particularly  
275 in the northeast (**Figure 3A**), where human interaction with vectors may amplify transmission risks. This  
276 trend is especially notable for highly suitable areas around the coast of Brazil, where over half of the 200  
277 million people in Brazil reside (**Figure 2B**).

278

279 To determine the individual contributions of each environmental factor to our suitability prediction, we  
280 further calculated their relative importance (RI) in the resulting ecological niche models. We found that  
281 evergreen broadleaf forest cover and human population density contributed the most to the model  
282 predictions, followed by precipitation, and banana and cocoa agricultural lands (**Figure 3D**). This aligns  
283 with factors identified in the independent landscape phylogeographic analyses, and provides important  
284 insights into the disease ecology of OROV. We plotted response curves to assess the relationship between  
285 the environmental factors and OROV transmission suitability. These curves illustrate how ecological

286 suitability varies with changes in one factor while all others are kept constant at their mean. We observe a  
287 clear tipping point in the suitability index with mean monthly precipitation, where environments with  
288 <2.5 mm mean monthly precipitation appear unsuitable for OROV transmission (ecological suitability  
289 ~0) whereas suitability increases considerably above this threshold (ecological suitability >0.4) (**Figure**  
290 **3D**). For temperature, there is a clear increase in OROV suitability in environments with annual mean  
291 temperatures above 25°C (**Figure 3D**). OROV transmission suitability decreases considerably at high  
292 elevations, while higher human population density, evergreen broadleaf forest cover, and higher areas of  
293 banana and cocoa agriculture appear to favour transmission (**Figure 3D**).

294  
295 To investigate whether the expansion of OROV in Brazil in late 2023 and 2024 was associated with an  
296 expansion of its ecological niche, we compared ecological niche models trained on pre-mid-2023, pre-  
297 2024, and all available occurrence records. Specifically, we compared both the resulting maps of OROV  
298 ecological suitability and the capacity of each category of models to predict the distribution of most recent  
299 occurrence data. The suitability range obtained with the pre-2024 models (**Figure 3B**) highlights a similar  
300 spatial distribution of highly suitable regions compared to that obtained with the full models (**Figure 3A**).  
301 While the full suitability estimates show a slight expansion of suitable areas, the pre-2024 model was able  
302 to predict the 2024 occurrence points with a relatively high performance (TSS = 0.6, AUC = 0.862;  
303 **Supplementary Table S2**). However, the pre-mid-2023 suitability range (**Figure 3C**) shows clear  
304 differences from the full model, particularly in the landscape of ecological suitability within the Amazon;  
305 and the models had a lower predictive ability for occurrence points sampled after mid-2023 (TSS = 0.4,  
306 AUC = 0.55; **Supplementary Table S2**). This indicates a shift in ecological suitability after mid-2023,  
307 associated with OROV amplification within the Amazon, prior to circulation in other parts of the country.  
308 Given that this shift did not occur in 2024, viral circulation outside the Amazon was likely associated  
309 with OROV lineages recently reaching areas that were already ecologically suitable for local OROV  
310 transmission. This is supported by our earlier findings of long-distance and rapid dispersal events from  
311 continuous phylogeography.



312  
 313 **Figure 3. Ecological niche prediction for OROV local circulation in Brazil.** A) Predicted ecological suitability  
 314 for OROV transmission across Brazil utilising all disease occurrence points. Suitability predictions range from  
 315 unsuitable (0) to highly suitable (1). B) Ecological suitability prediction using input disease occurrence points  
 316 sampled before 2024. C) Ecological suitability prediction using input disease occurrence points sampled before mid-  
 317 2023. D) Response curves and relative importance (RI) for individual environmental factors obtained from the  
 318 random forest (RF) suitability prediction model. These response curves (five iterations) depict the relationship  
 319 between the environmental factors and the response (the ecological suitability of OROV transmission). E)  
 320 Ecological suitability values of OROV dispersal locations (for segment M) overlaid on weekly recorded OROV  
 321 cases in Brazil. Suitability values are estimated from three ecological niche models (ENM), as described in the text.  
 322

323 Integrating results from the ecological niche modelling with the inferred dispersal histories, we can  
 324 further assess the estimated ecological suitability values at each dispersal location associated with OROV  
 325 circulation across space and time (**Figure 3E**). Examining dispersal locations occurring prior to mid-  
 326 2023, after mid-2023, and in 2024, it becomes evident that for most of its dispersal history, OROV

327 circulated in regions of moderate ecological suitability (~0.4-0.5), consistent across the three temporal  
328 models we estimated. This was then followed by a peak in ecological suitability values, reaching ~0.6-0.8  
329 associated with OROV dispersal from mid to late 2023, coinciding with unprecedented epidemic  
330 expansion within the Amazon (**Figure 3E, Supplementary Figure S1**). This peak in ecological  
331 suitability was likely due to the virus being introduced to areas of the Amazon that had a combination of  
332 favourable environments and higher population densities, such as Manaus, leading to an amplification of  
333 transmission. This expansion in a highly suitable and densely populated environment, in turn, likely  
334 facilitated the pathogen's spread beyond its usual transmission range. Additionally, this amplification  
335 environment was more connected via human mobility to the rest of the country (**Supplementary Figure**  
336 **S2**), compared to more remote areas of the Amazon, where the virus had previously circulated. This  
337 further emphasises the need for improved surveillance in blind spot regions where transmission suitability  
338 is high, but where cases may be underreported or genomic data may be scarce. In such regions,  
339 introductions could rapidly lead to amplified outbreaks. A subsequent drop in ecological suitability  
340 associated with dispersal locations after 2024 is likely due to the virus colonising areas with less  
341 favourable environmental conditions (e.g., lower mean annual temperatures - **Figure 2D**, higher  
342 elevation, and lower precipitation - **Supplementary Figure S4**), despite high population densities.

343

#### 344 **Discussion, limitations and conclusions**

345 The recent emergence and expansion of OROV into previously non-endemic regions underscores the  
346 critical need for a deeper understanding of the factors associated with its spread. In response to the 2023-  
347 2024 outbreaks, recent studies have described the emergence of a reassortant lineage (10, 11),  
348 investigated spatiotemporal movement dynamics at a smaller scale (6, 7, 10, 11), reported severe clinical  
349 outcomes (9), and documented biological differences in the novel lineage (29). However, none had  
350 investigated the ecological mechanisms associated with the amplification of OROV within the Amazon  
351 and in other parts of the country (30). Here, we integrated phylogeographic and ecological modelling  
352 approaches to explore potential correlates of OROV transmission and range expansion. Our  
353 phylogeographic reconstructions provide initial evidence for the underlying dynamics governing OROV's  
354 amplification and spread in Brazil. By analysing environmental phylogeographic relationships and the  
355 ecological niche associated with viral transmission during distinct phases, we found that OROV circulated  
356 in areas of enhanced ecological suitability immediately preceding its explosive expansion in the Amazon.  
357 Our phylogeographic reconstructions also demonstrate that this expansion occurred with a considerable  
358 diffusion capacity, characterised by a series of rapid long-distance dispersal, most likely human-mediated  
359 through air travel across the country.

360

361 Environmental conditions play a pivotal role in shaping OROV transmission dynamics (31). Zones  
362 undergoing land-cover transitions, particularly those involving deforestation and agricultural activities,  
363 have emerged as critical hotspots for virus spread (32, 33). As these areas transition from sylvatic (forest)  
364 environments to more urbanised or agricultural landscapes, the resulting habitat changes bring vectors and  
365 reservoir hosts into closer contact with human populations, creating new opportunities for OROV  
366 transmission, and these must be studied using an integrated approach (34). Through the landscape  
367 phylogeographic and ecological niche modelling analyses conducted in this study, we propose a  
368 mechanism for the expansion of OROV, first within and then beyond the Amazon. We detect both a shift  
369 in estimated ecological suitability and differences in the environmental space visited by dispersal events  
370 prior to epidemic amplification within the Amazon. However, this was not concurrent with the emerging



371 circulation in regions outside the Amazon, where the virus has not widely circulated before. This  
372 consistently supports a two-step expansion process, in which circulation within a highly suitable  
373 environment led to an explosive outbreak in the Amazon, which then facilitated OROV's spread beyond  
374 its usual transmission range. These findings are supported by independent reconstructions of all three  
375 genomic segments of the virus. While previous studies have identified the Amazon region as the primary  
376 source of OROV emergence in Brazil (10, 11), the re-emergence or introduction of the virus in the  
377 vicinity of Manaus, the capital of the vast state of the Amazon, would have exposed a large number of  
378 naive human hosts and contributed to an amplified outbreak. Higher suitability in the amplification zone  
379 within the Amazon could therefore be attributed to a combination of favourable environmental conditions  
380 and densely populated urban areas. Our results also suggest that the novel circulation of OROV in non-  
381 endemic parts of Brazil, albeit concerning, could be self-limiting, particularly in areas with less  
382 favourable environments.

383  
384 Our study illustrates the power of integrating phylodynamics, landscape phylogeography and ecological  
385 niche modelling in elucidating complex eco-epidemiological dynamics of re-emerging arboviruses and  
386 urban amplification. These findings have several implications. First, by using complementary analytical  
387 methods and a comprehensive genomic data set, we identify key environmental factors important for  
388 virus transmission. Results from both approaches implicate human population density, banana and cocoa  
389 cultivation, and temperature as factors associated with favourable transmission environments. Such  
390 information can directly inform public health planning and mitigation measures, such as vector control  
391 activities around banana and cocoa plantations, particularly near urban areas. A recent study also  
392 implicated banana and cocoa as important crop types in epidemic locations (35). Second, while not a  
393 confirmation, our work provides a robust initial indication that OROV expanded opportunistically by  
394 finding more favourable environments for transmission, which were also more connected to the rest of the  
395 country. Other hypotheses have explored the idea that higher viral replication in mammalian cells could  
396 allow more efficient transmission to vector and onwards, or that the immune escape ability of the new  
397 reassortant lineage may play a role (29). Synthesising recent work with our findings identifies a proposed  
398 mechanism for this emerging outbreak. It is possible that genetic reassortment resulted in a viral genotype  
399 characterised by more efficient infection and transmission between humans and the vector, resulting in  
400 higher viremia, in turn explaining the epidemic expansion in favourable urban environments detected in  
401 our study. The approach we propose is highly adaptable to risk mapping and the characterisation of  
402 disease ecology for other arboviruses and zoonotic infections. Finally, the concentration of high-  
403 suitability areas in Brazil's coastal regions is concerning, as this is where most of the population resides  
404 and where multiple arboviruses already circulate. Multiple introduction events into these areas could lead  
405 to further amplification of this pathogen, particularly given the presence of immunologically naive  
406 populations, or the circulation of a variant that can evade prior immunity (29). Additionally, several  
407 suitable areas, especially in the northeastern and central-west regions, remain surveillance blindspots for  
408 OROV. These areas may harbour undetected transmission due to limited sampling. Prioritising these  
409 blind spots for active surveillance is essential for timely viral detection and early interventions to prevent  
410 outbreaks.

411  
412 This study needs to be interpreted in light of certain limitations. Our landscape phylogeographic approach  
413 is affected by the pattern of the sampling effort, as approximately half of the node locations are sequenced  
414 tips, making them prone to sampling biases (25). This means that this approach cannot currently test the



415 true drivers of transmission but rather test the strength of associations of dispersal environments.  
416 Additionally, we lack a species distribution model for the actual vector, *Culicoides paraensis*, and  
417 vertebrate host species that typically compose OROV's natural reservoir, which limits our ability to fully  
418 understand host-vector-specific environmental suitability. To mitigate this bias, we test associations  
419 against a null dispersal model generated through simulations, and only consider factors with the strongest  
420 Bayes factor support (>20). Additionally, this study was designed only to examine the ecological factors  
421 associated with epidemic expansion in Brazil. The potentially critical role of reassortants in viral  
422 adaptation and fitness has not been addressed here. Our phylogeographic analyses focused solely on the  
423 reassortant lineage that emerged in Brazil. To elucidate the role of these evolutionary processes in the  
424 virus' ability to adapt to new niches, hosts or vectors, our approach could be extended to analyse  
425 evolutionarily distinct lineages of OROV that previously circulated in Brazil or neighbouring regions. The  
426 role of vectors is central to the expansion of OROV, with the primary vector *Culicoides paraensis* likely  
427 adapting to new landscapes. The interaction between the virus, its vectors and its reservoir hosts is crucial  
428 for both the colonisation of new transmission zones and the maintenance of transmission in established  
429 areas. Our analysis also revealed increased exposure of OROV to urban and peri-urban vectors such as  
430 *Ae. aegypti*, which could facilitate its spread in densely populated regions. However, our approach cannot  
431 definitively support that this vector was directly involved in epidemic expansion. While the virus has  
432 previously been detected in *Ae. aegypti* mosquitoes (21), further vector competence studies, particularly  
433 against this novel reassortant lineage, are required to understand the risks posed by increased exposure of  
434 this virus to secondary urban vectors.

435  
436 The geographic expansion of OROV into new regions highlights the pressing need for integrating  
437 environmental monitoring into public health frameworks. To effectively predict and mitigate the risks  
438 posed by OROV and other arboviruses, surveillance efforts must account for the complex interaction  
439 between environmental changes, vector ecology and human behaviour. As OROV continues to adapt to  
440 new ecological niches, driven by combinations of genetic evolution and both natural and anthropogenic  
441 factors, a deep understanding of these dynamics will be essential for developing targeted intervention  
442 strategies to control its spread and minimise its public health impact.

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609



## 610 **Supplementary Materials**

611 Materials and Methods

612 Supplementary Figures S1 to S10

613 Supplementary Tables S1 to S3

614 References (35–51)

615 CLIMADE Consortium Contributing Authors

616

## 617 **Materials and Methods**

### 618 **Oropouche Virus genomic data**

619 Complete genome sequences of the S, M, and L segments of the Oropouche virus (OROV), obtained from  
620 the first extra-Amazon OROV cases reported in the states of Bahia (Northeast Brazil), Minas Gerais  
621 (Southeast Brazil), Mato Grosso (Midwest Brazil), and Paraná (South Brazil), were combined with the  
622 corresponding segments of recently published full-length OROV sequences belonging to the Brazilian  
623 2022-2024 sublineage (10). The OROV sequences used in this study correspond to the Genbank  
624 accession IDs: PQ168520-PQ247806 and PP153945-PQ065491. Sequence alignment for each segment  
625 (n=545) was performed using MAFFT (36, 37) and subsequently curated manually to remove artefacts  
626 using AliView (38). Genomic regions identified by RDP5 to have likely been acquired by recombination  
627 and genomic segments identified by RDP5 to have been acquired by reassortment, were stripped from the  
628 full genome data sets by replacing these regions with gap characters (“-”) in the alignment file, thereby a  
629 yielding a free full genome alignment free of recombination and reassortment as previously described  
630 (10). Sequences with recombination and reassortment signals along the majority of the genome were  
631 completely discarded (n=43 for segment S and n=1 for segment L).

632

### 633 **Disease occurrence data**

634 Disease occurrence data was compiled from multiple sources. Epidemiological data on Oropouche virus  
635 (OROV) cases were retrieved from the Brazilian Ministry of Health, accessible at  
636 <https://www.gov.br/saude/pt-br/assuntos/saude-de-a-a-z/o/oropouche>. The data set includes confirmed  
637 case reports from all Brazilian states where OROV cases have been notified, including but not limited to  
638 Acre (AC), Alagoas (AL), Amazonas (AM), Bahia (BA), Ceará (CE), Minas Gerais (MG), Pará (PA), Rio  
639 de Janeiro (RJ), and São Paulo (SP). The data cover the years 2023 and 2024, organised by  
640 epidemiological week, and include key variables such as the municipality, state, year of occurrence, and  
641 the corresponding epidemiological week of reporting. This data was geocoded at the municipality level  
642 and occurrence deduplicated by month. Additional OROV occurrence data was gathered and geocoded  
643 from all records on the GBIF (years: 1957-present) and Genbank databases (years: 2015-present). After  
644 deduplicating all occurrence records, we obtained a total of 450 unique sampling location points covering  
645 the years 1957 to 2024, with a majority (~85%) sampled in 2023-2024.

646

### 647 **Geospatial data**

648 We tested several environmental factors both as associations with viral dispersal locations and as  
649 covariates in our ecological niche model. These factors included human population density, main land  
650 cover and climatic variables within the study area (Brazil). Each environmental factor was described by a  
651 raster that defines its spatial heterogeneity. **Supplementary Table S3** details the source and resolution of  
652 each original raster file. Each raster was cropped to match our study area (Brazil) by using Brazil  
653 shapefiles from the “rnaturalearth” package in R.

654

### 655 **Air travel data**

656 We retrieved domestic flight data from Brazil's Civil Aviation Agency (ANAC) using the "flightsbr" R  
657 package (Version 0.1.0). This dataset contains information on flight routes, departure and arrival times,  
658 airlines, and airport locations. With flight data only available through 2020, we chose to use 2019 as it  
659 represents the most recent complete year before the COVID-19 pandemic. It provides a reliable baseline,  
660 with reports confirming the recovery of air travel to pre-pandemic levels in 2023 (39). We merged the  
661 flight data with airport location information, filtering specifically for public airports across Brazil. We  
662 focused on creating a network of departing flights, visualised spatially by airport, to highlight regional  
663 connectivity patterns. All data processing and visualisations were conducted using R, with geospatial  
664 mapping performed using the ggplot2 and sp packages.

665

### 666 **Phylogeographic reconstruction and dispersal statistics**

667 To model the spatiotemporal spread of OROV using spatially-explicit phylogeographic reconstruction  
668 using the continuous diffusion model implemented in the software package BEAST 1.10 (40), we utilised  
669 three different data subsets containing the 2022-2024 OROV sequences from various regions of Brazil of  
670 the S ( $n = 501$ ), M ( $n = 545$ ), and L ( $n = 544$ ) segments. Before conducting the phylogeographic analyses,  
671 we assessed the strength of the molecular clock signal in each data subset using the root-to-tip regression  
672 method available in TempEst v1.5.3 (41). Preliminary BEAST reconstructions revealed an outgroup of  
673 sequences which diverged from the main clade ~60 years ago for each segment; these were subsequently  
674 discarded from our analyses. Temporal structure was accepted for all datasets as the correlation  
675 coefficients were all close to or above 0.5 (S: 0.4972, M: 0.635, L: 0.5123). We reconstructed the spread  
676 of OROV lineages within Brazil by using a flexible relaxed random walk diffusion model (42), which  
677 accommodates branch-specific variation in dispersal rates, with a Cauchy distribution and a jitter window  
678 size of 0.01 (43). The latitude and longitude coordinates of each sample were used in this analysis.  
679 MCMC analyses were run in BEAST v1.10.4, with chains of up to 1 billion iterations each, sampling  
680 every 100,000 steps in the chain. The chains were stopped when convergence was reached following the  
681 removal of burn-in states. Convergence of each run was assessed using Tracer v1.7.1, ensuring that the  
682 effective sample size (ESS) for all relevant model parameters was  $>200$  (44). Maximum clade credibility  
683 trees were summarised using TreeAnnotator after discarding burn-in samples, the number of which was  
684 also determined in Tracer. Finally, the R package "seraphim" (45) was employed to extract and map the  
685 spatiotemporal information embedded in the posterior trees. We further used "seraphim" to estimate three  
686 dispersal statistics from these movement vectors for each segment: maximal wavefront distances,  
687 weighted diffusion coefficients (46), measuring the dispersal capacity of viral lineages, and an isolation-  
688 by-distance (IBD) signal measured as the Pearson correlation between the patristic and log-transformed  
689 geographic distances computed for each pair of tip nodes (20).

690

### 691 **Landscape phylogeographic analyses**

692 To test the association between environmental conditions (**Supplementary Figure S2**) and dispersal  
693 locations of inferred OROV lineages, we employed a landscape phylogeographic approach (25). We first  
694 extracted and visualised the environmental values explored by phylogenetic branches using the  
695 "spreadValues" function implemented in the R package "seraphim". For these analyses, we sampled 100  
696 posterior trees obtained from the continuous phylogeographic inference. For each posterior tree sampled  
697 during the phylogeographic analysis, this function extracts then averages the environmental values at the

698 tree node positions. For each analysed environmental factor, we then obtained a posterior distribution of  
699 mean environmental values at tree node positions for each segment data set. To assess the tendency of  
700 inferred viral lineages to preferentially circulate within or avoid circulating in specific environmental  
701 conditions, we compared the distribution of mean environmental values extracted at node positions in  
702 inferred trees ( $E_{\text{estimated}}$ ) with those extracted at node positions in trees whose dispersal history had been  
703 re-simulated under a null dispersal model ( $E_{\text{simulated}}$ ). To generate such a null dispersal model, a RRW  
704 diffusion process was simulated along each tree topology used for the phylogeographic analyses. These  
705 RRW simulations were performed using the “simulatorRRW1” function of the R package “seraphim”  
706 from the sampled precision matrix parameters estimated by the phylogeographic analyses. Therefore,  
707 from these simulations, values at node positions ( $E_{\text{simulated}}$ ) constitute the distribution of mean  
708 environmental values explored under a dispersal scenario that is not impacted by any underlying  
709 environmental condition. For each environmental factor and segment-specific phylogeographic  
710 reconstruction, we then compared the distribution of  $E_{\text{estimated}}$  values computed from posterior trees with  
711 the distribution of  $E_{\text{simulated}}$  values retrieved from the same tree topologies along which a RRW diffusion  
712 process had been re-simulated. Specifically, we approximated a Bayes factor (BF) support equal to  $(p_e/(1-$   
713  $p_e))/(0.5/(1-0.5))$ . To test if viral lineages tended to avoid circulating within a particular environmental  
714 factor  $e$ ,  $p_e$  was defined as the frequency at which  $E_{\text{estimated}} < E_{\text{simulated}}$ ; and to test if viral lineages tended to  
715 preferentially circulate within a particular environmental factor  $e$ ,  $p_e$  was defined as the frequency at  
716 which  $E_{\text{simulated}} < E_{\text{estimated}}$ . Following the scale of interpretation of Kass and Raftery (24), we here  
717 highlight BF values  $>20$  considered as strong supports.

718

### 719 **Ecological Niche Modelling**

720 Ecological niche models (ENMs) are built using a variety of statistical methods, each varying in  
721 complexity and underlying assumptions about the interaction between species occurrences and  
722 environmental factors (47). Recent studies have shown that disparities among different model structures  
723 can be very large, making model selection difficult (48). An alternative is to use an ensemble of models to  
724 avoid selecting one single best model but instead to use a group of methods for inference. In other words,  
725 the presence of a species might be well classified by some models and misclassified by others, such that  
726 making use of an ensemble model can reduce the predictive uncertainty of a single model by combining  
727 predictions (49). In this study, we applied this approach to model the distribution of OROV transmission  
728 by creating a suitability map based on the occurrence of OROV disease in Brazil and relevant  
729 environmental variables (see Geospatial Data section). For this analysis, we aggregate all raster maps to  
730 the lowest resolution available ( $\sim 27\text{km}^2$ ). We used an ensemble of seven statistical, machine learning, and  
731 envelope models: Generalised Linear Model (GLM), Generalised Additive Model (GAM), Boosted  
732 Regression Trees (BRT), Random Forest (RF), Classification Tree Analysis (CTA), Surface Range  
733 Envelope (SRE) and Maximum Entropy Model (MAXENT). To assess the potential expansion of  
734 OROV's ecological niche in Brazil during late 2023 and 2024, we computed and compared ecological  
735 niche models (ENMs) using occurrence data from different time periods. Specifically, we created three  
736 ensemble models using pre-mid-2023 (89 occurrences), pre-2024 (133 occurrences), and all available  
737 occurrence records (450 occurrences).

738

739 To assess the models' performance we use block cross validation (50). This is a spatially explicit method  
740 used to assess model performance by dividing the study area into geographic blocks. Instead of randomly  
741 splitting data, this technique ensures that training and testing data are spatially independent, reducing the

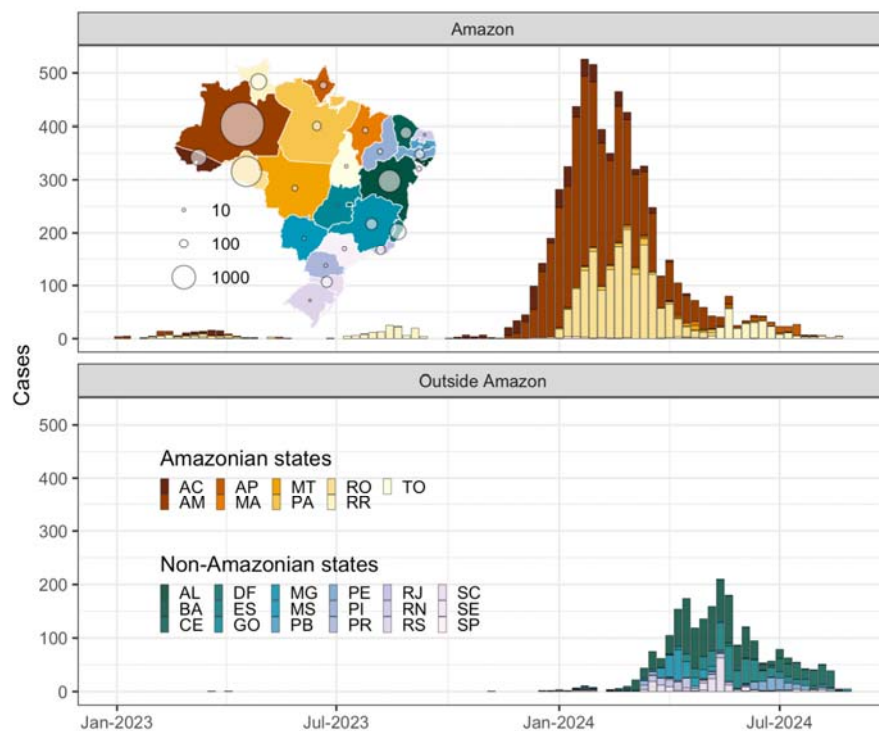
742 risk of spatial autocorrelation. True Skills Statistics (TSS) and receiver-operating characteristic (ROC)  
743 curve (area under curve - AUC) are then used to evaluate the predictive performance of the models based  
744 on the test (validation) dataset. TSS is equivalent to sensitivity+specificity-1 and ranges from -1 to 1;  
745 value of 1 indicates perfect classification, 0 means the model is no better than random guessing, and  
746 negative TSS indicates the model performs worse than random guessing. AUC ranges from 0 to 1; AUC  
747 of 1 indicates perfect model performance, 0.5 indicates no discrimination (i.e., the model is no better than  
748 random guessing), and <0.5 indicates the model performs worse than random guessing. We only retained  
749 models with a TSS score of >0.7 to build the ensemble model (**Supplementary Figure S10**). The mean  
750 probabilities from each model were then computed, and we weighted the predictions of each model  
751 according to its performance during training, giving more weight to better-performing models. The  
752 weights ensure that higher-quality models contribute more to the final ensemble prediction. The different  
753 resulting ‘suitability indexes’ are then combined to get a single value per site. Once the ensemble  
754 predictions are generated, the ensemble model itself is evaluated using the same metrics applied to the  
755 individual models; TSS and AUC.

756  
757 Disease presence points used as input represent OROV circulation occurrence from 450 unique sampling  
758 locations in Brazil (molecular testing and sequencing records) from the years 1957 to 2024. Occurrence  
759 points with available collection dates were matched to corresponding climatic variables by month.  
760 Specifically, temperature and precipitation data for each point were extracted from the monthly climate  
761 layers matching the collection month. The sampling of pseudo-absences was done at a 1:1 ratio with  
762 presence points and based on those distribution of presence points and a human population density kernel  
763 density estimate. Our aim was to sample absences in proportion to the rate of presence points while giving  
764 higher priority to areas with greater population density, ensuring more focused sampling in regions where  
765 human populations are denser. This approach helps us account for disease testing biases in more  
766 urbanized areas in the pseudo-absence distribution. Pseudo-absences were also selected within a  
767 perimeter of 50-300 km around presence points, ensuring that absence points are neither too close to  
768 presence points (to avoid the same niche) or too far (to promote localised sampling strategy).

769  
770 To determine the independent contributions of each variable to our suitability prediction, we further  
771 calculated their relative importance (RI). In the case of random forest (RF) models, RI is computed by  
772 assessing how frequently a variable is selected for splitting at tree nodes, weighted by the squared  
773 improvement in model performance resulting from each split, and averaged across all trees (51). Higher  
774 RI values indicate greater relative contribution of that variable to the predictive performance of the model.  
775 We also produced response curves to visualise the effect of each variable on suitability predictions in the  
776 RF models. These response curves allow us to observe how changes in a single variable influence the  
777 predicted outcome, while other variables are held constant (at their mean). By examining these  
778 relationships, we gain insights into how each variable individually contributes to the model’s overall  
779 predictions.

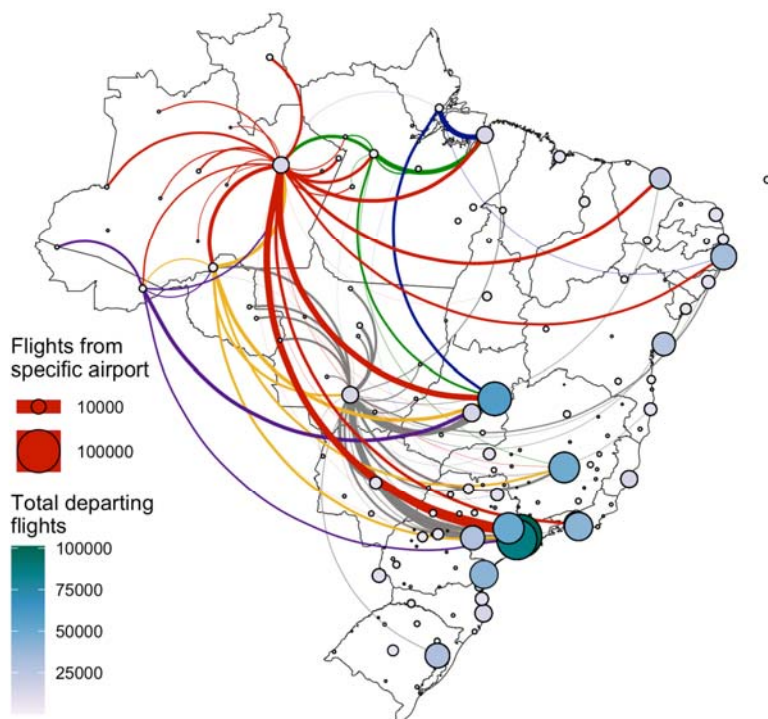
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786 **Supplementary Figures**

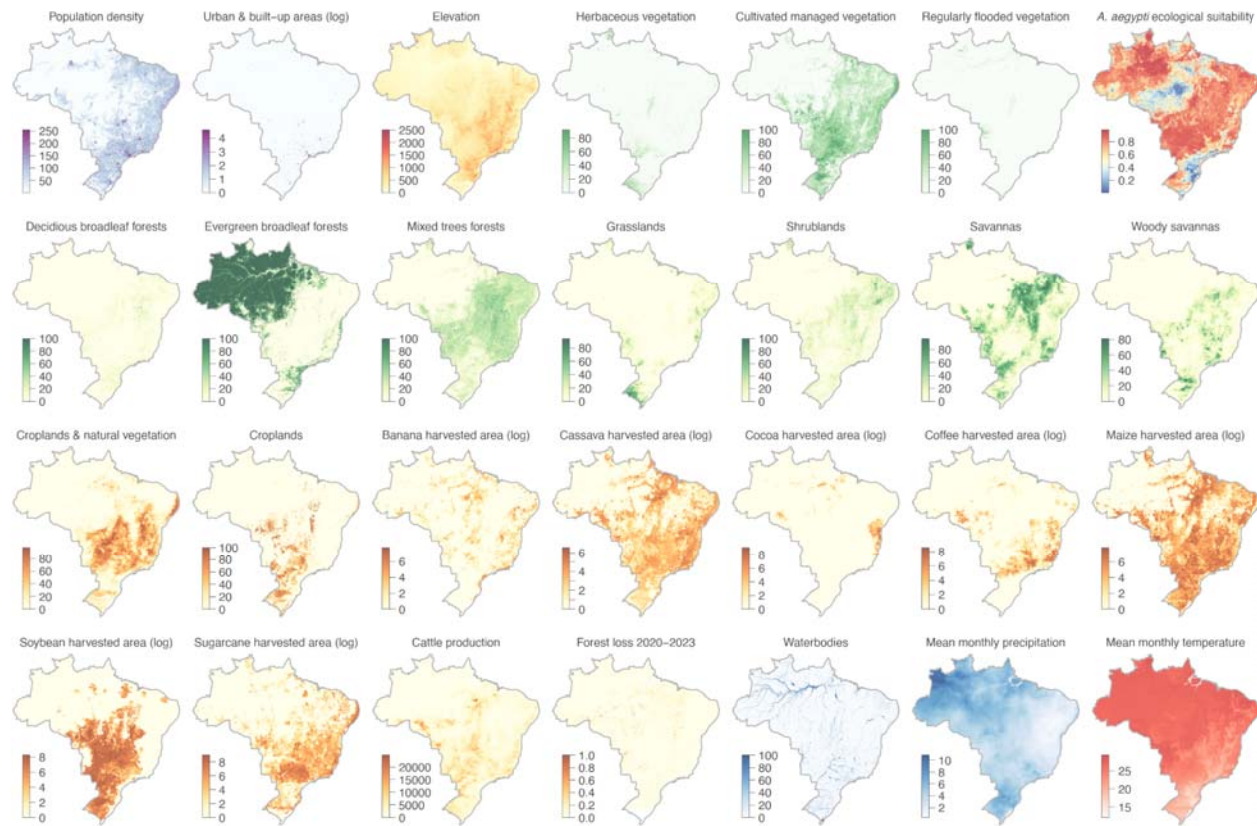


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788 **Figure S1. Epidemiological curve of OROV cases in Brazil.** Weekly cases are shown for 2023 and 2024 divided  
789 into two epidemiological curves, one for states in the Amazon region, and one for states outside the Amazon region.  
790 The inset map is coloured by the specific state, and the circles represent the total number of recorded OROV cases in  
791 that state.  
792

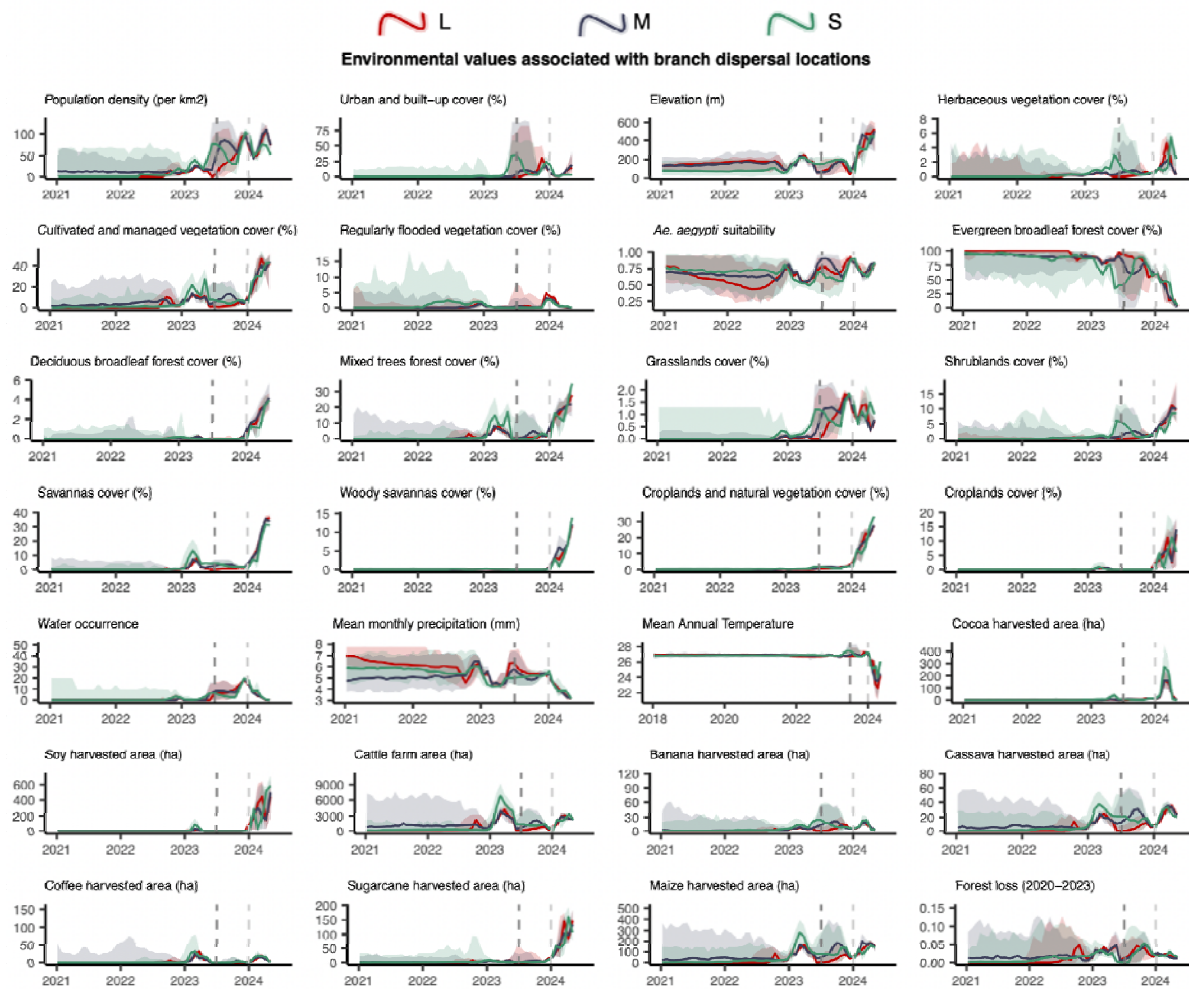




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794 **Figure S2. Human mobility through air travel in Brazil.** The figure captures air travel data in Brazil in 2019. The  
795 map shows the total number of departing flights from all airports in Brazil. Circles are both coloured and sized by  
796 the number of flights departing from an airport origin location. The coloured curves show the number and network  
797 of flights from airports of specific municipalities, namely Manaus in state of Amazonas (red), Santarém in the state  
798 of Pará (green), Cuiabá in the state of Mato Grosso (grey), Porto Velho in the state of Rondônia (yellow), Rio  
799 Branco in the state of Acre (purple), and Macapá in the state of Amapá (blue).  
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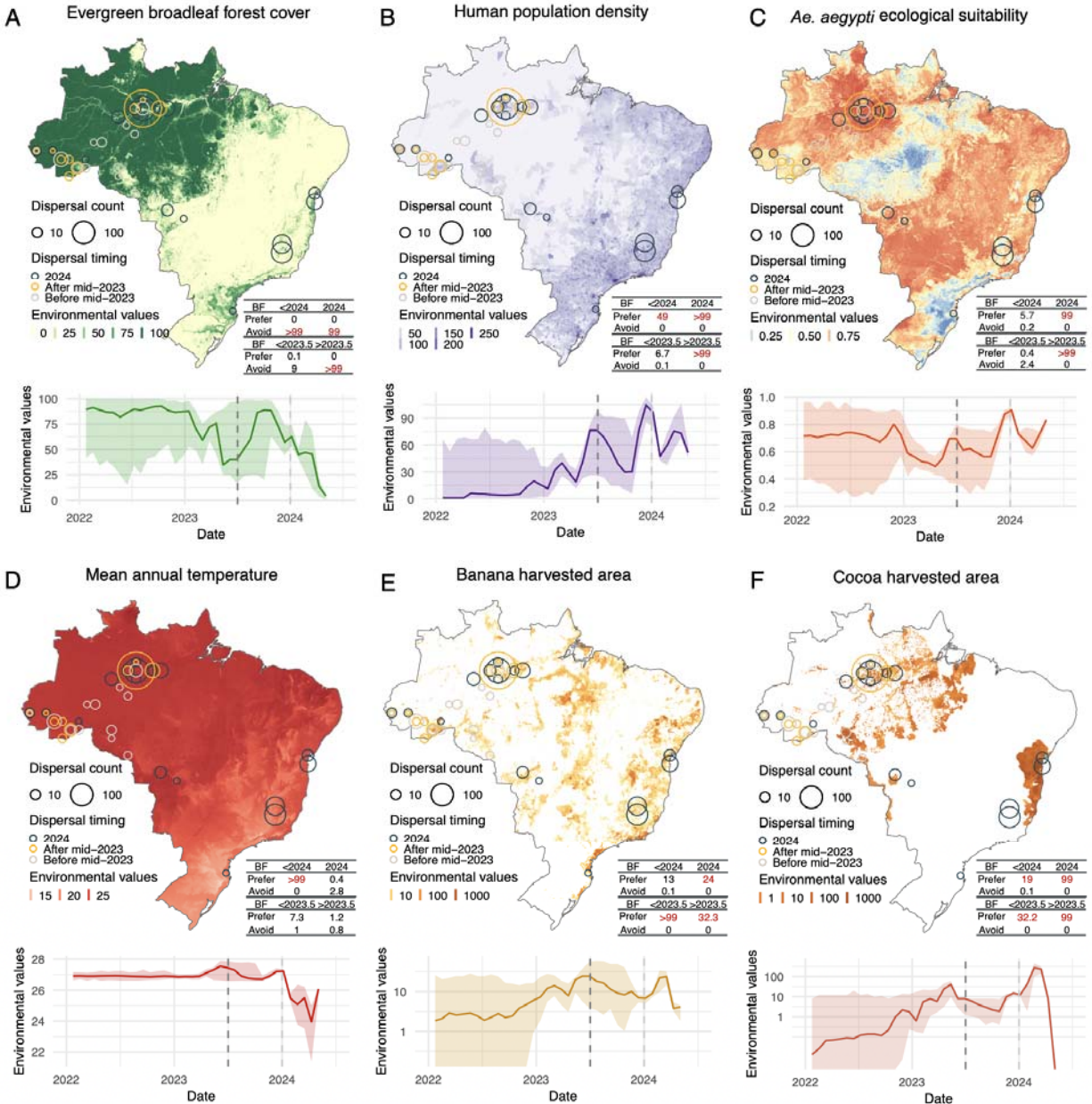
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802 **Figure S3. Environmental covariates analyzed in the context of the range expansion of OROV in Brazil.**  
803 Various environmental rasters, such as demographic, land-use, and climatic covariates, were analyzed in the study to  
804 investigate their association with the spread of the OROV in Brazil. Demographic variables encompass population  
805 density and urban areas, while land-use patterns focus on the presence of croplands, water bodies, and regions  
806 impacted by deforestation linked to specific agricultural activities, such as cocoa, soy, and banana crops.  
807



808  
 809 **Figure S4. Environmental values associated with OROV branch dispersal locations over time.** Line graphs  
 810 depicting the environmental covariates associated with the locations of OROV lineage dispersal events in Brazil.  
 811 Each plot illustrates how specific ecological conditions have changed over time (2021–2024) at the sites of viral  
 812 lineage dispersal. This is shown for segment L (in red), segment M (in blue), and segment S (in green).

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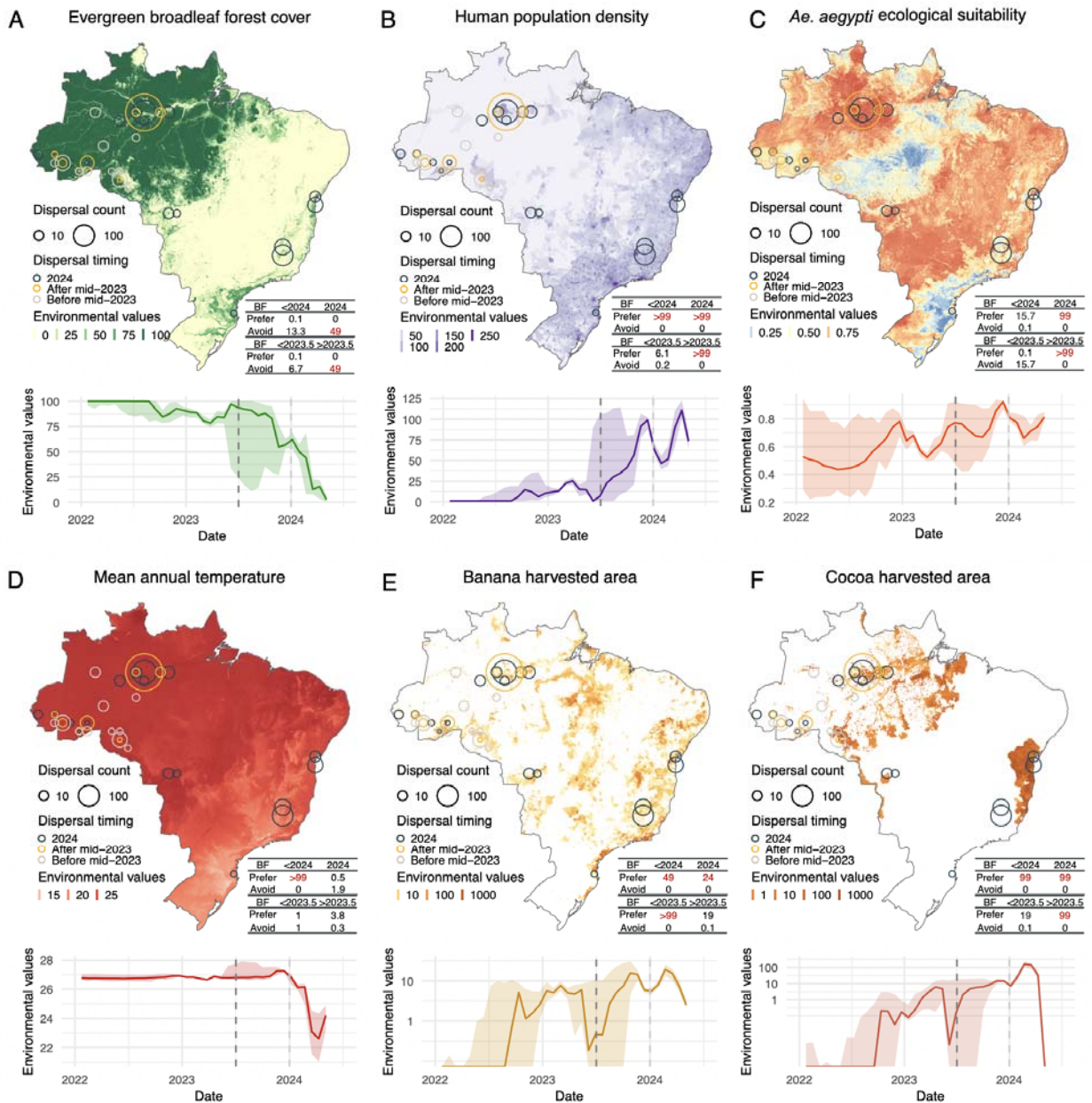


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815 **Figure S5. Environmental conditions associated with OROV lineage dispersal locations over time (for**  
 816 **segment S).** Figure panels show the spatial distribution of six main environmental factors (units specified):  
 817 evergreen broadleaf forest cover (%) (A), human population density (normalised between 0 and 255 per km<sup>2</sup> for  
 818 visual clarity) (B), *Ae. aegypti* ecological suitability (probability of occurrence) (C), mean annual temperature (°C)  
 819 (D), banana harvested area (hectares - log) (E), and cocoa harvested area (hectares - log) (F) in the top rows. Circles  
 820 on the map depict the end node of dispersal locations inferred by continuous phylogeography, sized by the number  
 821 of dispersal events in an area, and coloured by the timing of the event. Bottom rows of each figure panel are line  
 822 graphs depicting the environmental covariates associated with the locations of OROV lineage dispersal events in  
 823 Brazil. Each plot illustrates how specific ecological conditions have changed over time (2022-2024) at the sites of  
 824 viral lineage dispersal. The embedded tables show the association between environmental conditions and the  
 825 dispersal location of inferred OROV lineages. Based on the analysis of 100 posterior trees obtained from continuous  
 826 phylogeographic inference, the table reports Bayes factor (BF) supports for association between environmental

827 raster values and tree node locations. Following the scale of interpretation of Kass and Raftery (24), we highlight BF  
 828 values >20 considered as strong supports.

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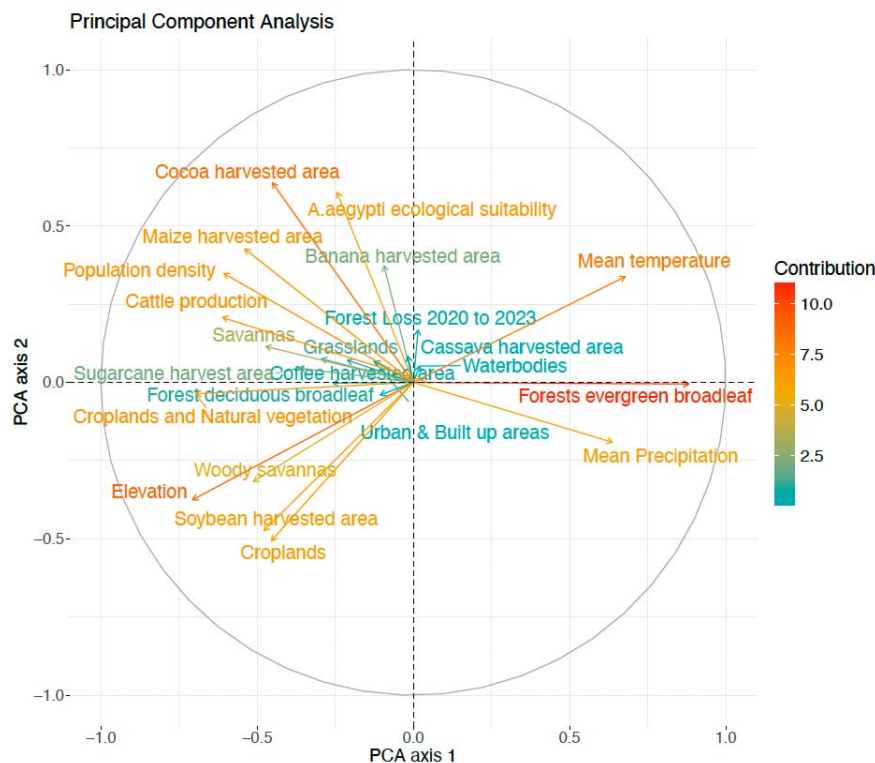
832 **Figure S6. Environmental conditions associated with OROV lineage dispersal locations over time (for**  
 833 **segment L).** Figure panels show the spatial distribution of six main environmental factors (units specified):  
 834 evergreen broadleaf forest cover (%) (A), human population density (normalised between 0 and 255 per km<sup>2</sup> for  
 835 visual clarity) (B), *Ae. aegypti* ecological suitability (probability of occurrence) (C), mean annual temperature (°C)  
 836 (D), banana harvested area (hectares - log) (E), and cocoa harvested area (hectares - log) (F) in the top rows. Circles  
 837 on the map depict the end node of dispersal locations inferred by continuous phylogeography, sized by the number  
 838 of dispersal events in an area, and coloured by the timing of the event. Bottom rows of each figure panel are line  
 839 graphs depicting the environmental covariates associated with the locations of OROV lineage dispersal events in



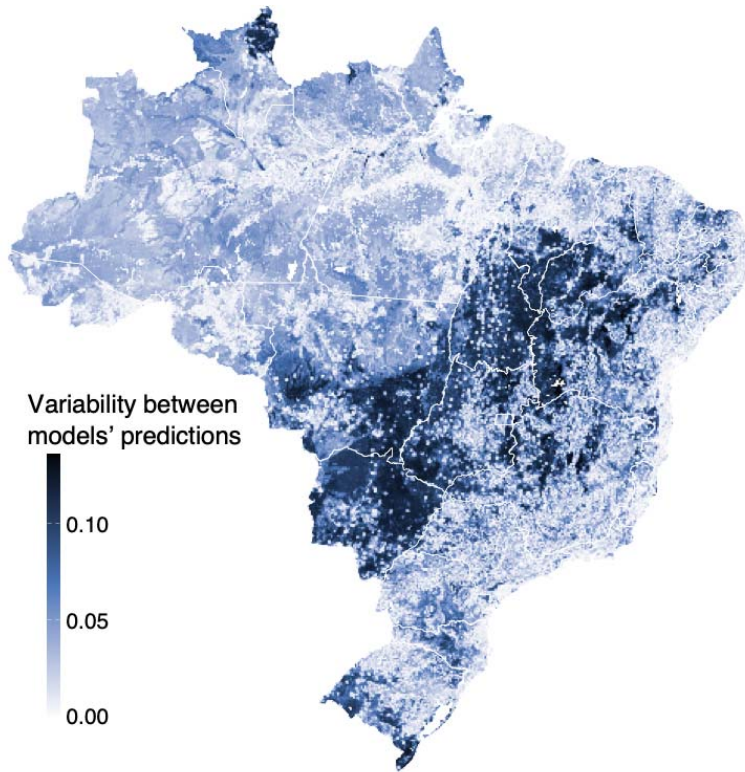
840 Brazil. Each plot illustrates how specific ecological conditions have changed over time (2022-2024) at the sites of  
841 viral lineage dispersal. The embedded tables show the association between environmental conditions and the  
842 dispersal location of inferred OROV lineages. Based on the analysis of 100 posterior trees obtained from continuous  
843 phylogeographic inference, the table reports Bayes factor (BF) supports for association between environmental  
844 raster values and tree node locations. Following the scale of interpretation of Kass and Raftery (24), we highlight BF  
845 values >20 considered as strong supports.  
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849 **Figure S7. Distribution of disease presence and pseudo-absence points.** We generated pseudo-absence  
850 points at a 1:1 ratio with presence points by sampling from the distribution of presence points and the  
851 kernel density estimate of human population density.  
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857 **Figure S8. Principal Component Analysis (PCA) plot illustrating the relationships between**  
858 **variables.** Arrows that lie within the same quadrant or are positioned close to each other indicate a higher  
859 correlation among the corresponding variables. Furthermore, longer arrows signify a greater contribution  
860 of those variables to the principal components, highlighting their discrimination in the overall dataset.  
861



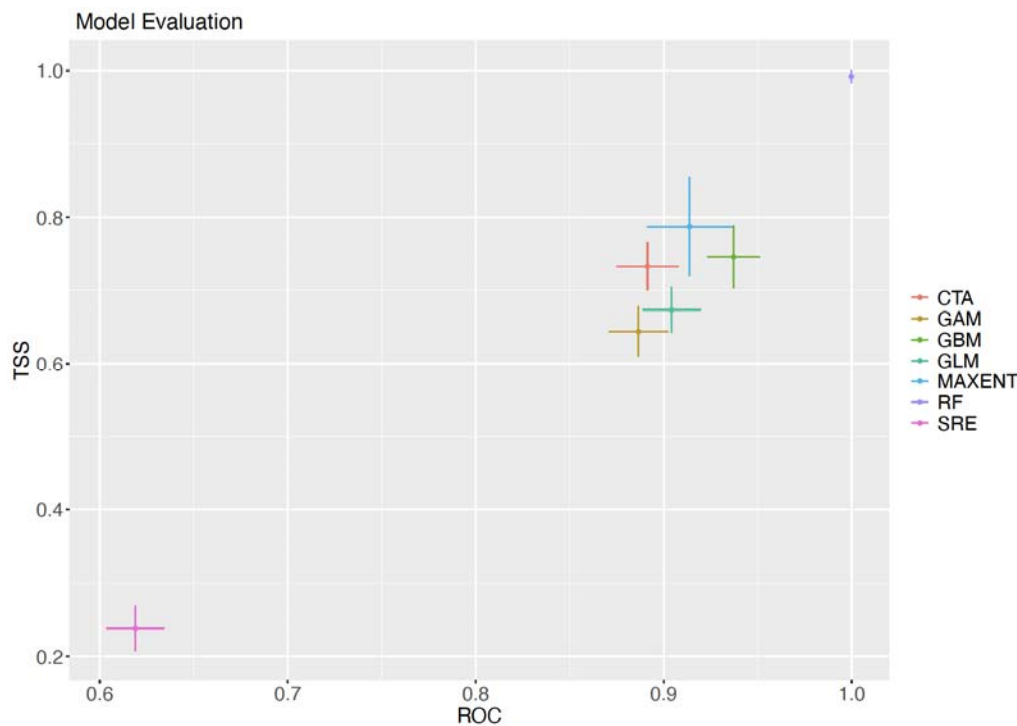
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863 **Figure S9. Ecological niche models variability.** The degree of variability in suitability prediction values  
864 among the models in our ensemble, highlighting areas where different models either converge or diverge  
865 in their predictions.

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871 **Figure S10. Ecological niche models evaluation results.** Results from block cross-validation of the  
872 individual environmental niche models of the full model (using all data points). The x- and y-axis show  
873 the True Skill Statistic (TSS) and area under the Receiver Operating Characteristic (ROC) curve,  
874 respectively.

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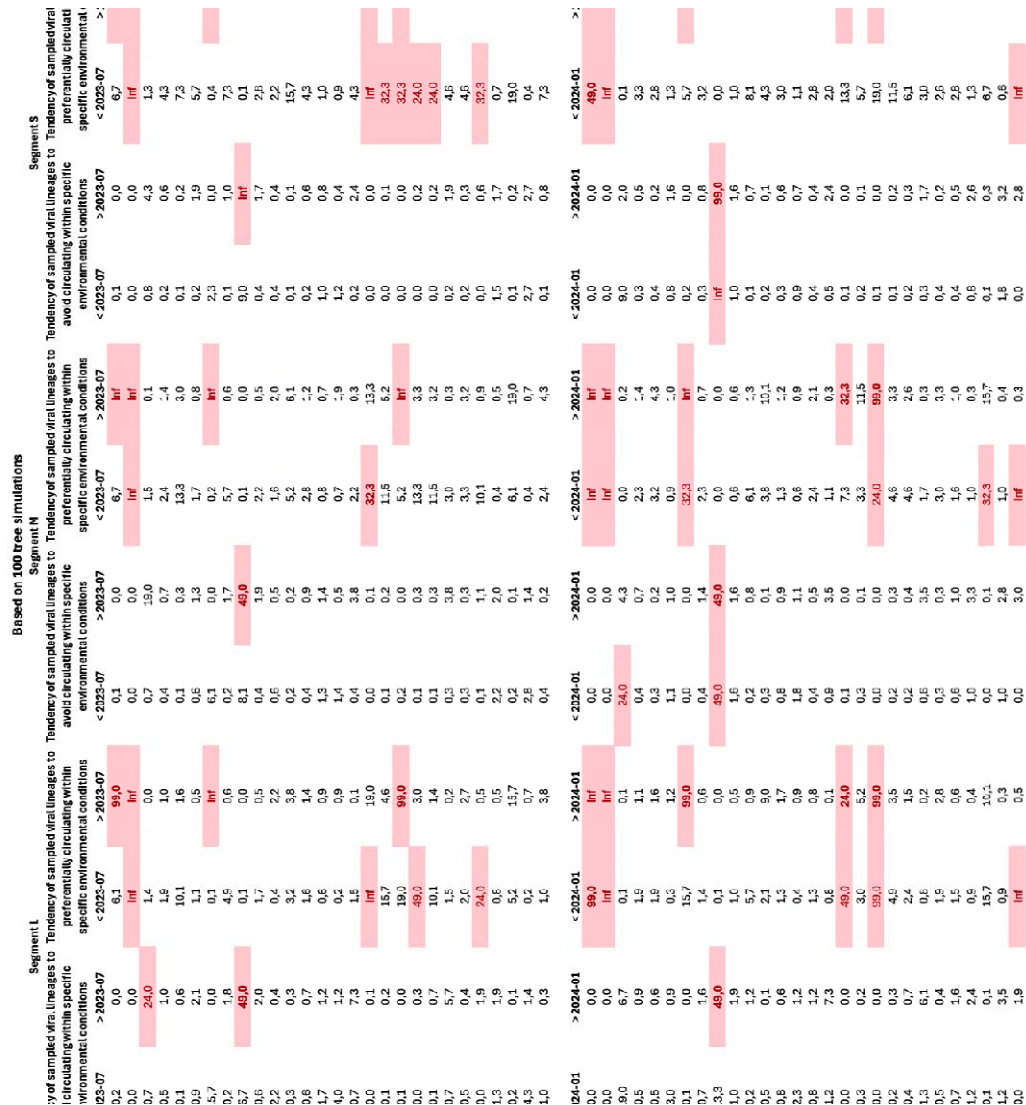
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895 **Supplementary Table S1:**



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897 **Supplementary Table S2: Model evaluation metrics (TSS and ROC) for the ensemble models, with**  
898 **results from testing on both the training/calibration datasets (left) and an independent dataset from 2024**  
899 **cases (right). The models were evaluated in three stages: points collected before mid-2023, points**  
900 **collected before 2024, and all available points combined.**

Model performance	Occurrence Points	Testing on calibration dataset		Testing on independent data (Year = 2024 cases)	
		TSS	AUC	TSS	AUC
<u>Pre-mid-2023</u>	89	0.77	0.885	0.4	0.55



<u>Pre-2024</u>	133	0.801	0.957	0.6	0.862
<u>Full</u>	450	0.835	0.974	0.785	0.95

901  
 902 **Supplementary Table S3:** Environmental variables used in the study, with respective resolutions and  
 903 data sources.

<b>Environmental Variables</b>	<b>Resolution (degrees ~ km)</b>	<b>Source</b>
Population density	0.0083 ~ 1 km	WorldPop, Global Human Settlement Layer (GHSL)
Urban & built up areas cover	0.0083 ~ 1 km	GHSL, World Urbanization Prospects
Annual mean temperature	0.0090 ~ 1 km	WorldClim
Elevation	0.0083 ~ 1 km	USGS
Herbaceous vegetation cover	0.0083 ~ 1 km	MODIS Land Cover, Global Land Cover (GLC)
Cultivated managed vegetation cover	0.0083 ~ 1 km	EarthEnv - <a href="https://www.earthenv.org/landcover">https://www.earthenv.org/landcover</a>
Regularly flooded vegetation cover	0.0083 ~ 1 km	EarthEnv - <a href="https://www.earthenv.org/landcover">https://www.earthenv.org/landcover</a>
<i>Ae. aegypti</i> ecological suitability	0.0146 ~ 1.62 km	DOI: <a href="https://doi.org/10.7554/eLife.08347">https://doi.org/10.7554/eLife.08347</a>
Deciduous broadleaf forest cover	0.0083 ~ 1 km	EarthEnv - <a href="https://www.earthenv.org/landcover">https://www.earthenv.org/landcover</a>
Evergreen broadleaf forest cover	0.0083 ~ 1 km	EarthEnv - <a href="https://www.earthenv.org/landcover">https://www.earthenv.org/landcover</a>
Mixed trees forest cover	0.0083 ~ 1 km	EarthEnv - <a href="https://www.earthenv.org/landcover">https://www.earthenv.org/landcover</a>
Grasslands cover	0.25 ~ 27.75 km	EarthEnv -

		<a href="https://www.earthenv.org/landcover">https://www.earthenv.org/landcover</a>
Shrublands cover	0.0083 ~ 1 km	EarthEnv - <a href="https://www.earthenv.org/landcover">https://www.earthenv.org/landcover</a>
Savannas cover	0.25 ~ 27.75 km	EarthEnv - <a href="https://www.earthenv.org/landcover">https://www.earthenv.org/landcover</a>
Woody savannas cover	0.25 ~ 27.75 km	EarthEnv - <a href="https://www.earthenv.org/landcover">https://www.earthenv.org/landcover</a>
Croplands & natural vegetation cover	0.25 ~ 27.75 km	EarthEnv - <a href="https://www.earthenv.org/landcover">https://www.earthenv.org/landcover</a>
Croplands cover	0.25 ~ 27.75 km	EarthEnv - <a href="https://www.earthenv.org/landcover">https://www.earthenv.org/landcover</a>
Banana harvested area	0.0833 ~10 km	EarthEnv - <a href="https://www.earthenv.org/landcover">https://www.earthenv.org/landcover</a>
Cassava harvested area	0.0833 ~10 km	EarthEnv - <a href="https://www.earthenv.org/landcover">https://www.earthenv.org/landcover</a>
Cocoa harvested area	0.0833 ~10 km	EarthEnv - <a href="https://www.earthenv.org/landcover">https://www.earthenv.org/landcover</a>
Coffee harvested area	0.0833 ~10 km	EarthEnv - <a href="https://www.earthenv.org/landcover">https://www.earthenv.org/landcover</a>
Maize harvested area	0.0833 ~10 km	EarthEnv - <a href="https://www.earthenv.org/landcover">https://www.earthenv.org/landcover</a>
Soybean harvested area	0.0833 ~10 km	EarthEnv - <a href="https://www.earthenv.org/landcover">https://www.earthenv.org/landcover</a>

Sugarcane harvested area	0.0833 ~10 km	EarthEnv - <a href="https://www.earthenv.org/landcover">https://www.earthenv.org/landcover</a>
Cattle cultivation area	0.0833 ~10 km	EarthEnv - <a href="https://www.earthenv.org/landcover">https://www.earthenv.org/landcover</a>
Forest loss (2020-2023)	0.025 ~ 2.78 km	<a href="https://glad.earthengine.app/view/global-forest-change">https://glad.earthengine.app/view/global-forest-change</a>
Water occurrence	0.0833 ~ 10 km	Global surface water explorer
Annual mean precipitation	0.05 ~ 5.55 km	WorldClim

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