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Genomic and transmission dynamics of the 2024 Marburg Virus Outbreak in Rwanda

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Genomic analyses from the ongoing outbreak of Marburg virus disease in Rwanda point to a single zoonotic origin of the outbreak.

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1. Extended Data

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Extended Data Fig. 1	Extended Data Figure 1	**Extended_data_figure1.pdf**	**A)** Time resolved maximum clade credibility tree of global MARV dataset, with closest human (KP985768) and zoonotic (JX458855) sequence to the Rwandan outbreak lineage annotated. The tMRCAs of the Rwandan lineage and KP985768 and JX458855 respectively are highlighted in red and orange
Extended Data Fig. 2	Extended Data Figure 2	**Extended_Figure_2.pdf**	*A) Mining cave entrance with human activity; B) Trapping fruit bats with net; C) Sampling bats for Marburg virus.*
Extended Data Fig. 3	Extended Data Figure 3	**Extended_data_figure3.pdf**	Temporal regression of the global MARV dataset

2. Supplementary Information:

A. PDF Files

Item	**Present?**	**Filename** Whole original file name including extension. i.e.: Smith_SI.pdf. The extension must be .pdf	**A brief, numerical description of file contents.** i.e.: *Supplementary Figures 1-4, Supplementary Discussion, and Supplementary Tables 1-4.*
Supplementary Information	No		

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Genomic and transmission dynamics of the 2024 Marburg Virus Outbreak in Rwanda

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The ongoing outbreak of Marburg virus disease (MVD) in Rwanda marks the third largest historically, though it has exhibited the lowest fatality rate. Genomic analysis of samples from 18 cases identified a lineage with limited internal diversity, closely related to a 2014 Ugandan case. Our findings suggest that the Rwandan lineage diverged decades ago from a common ancestor shared with diversity sampled from bats in Uganda. Our genomic data reveals limited genetic variation, consistent with single zoonotic transmission event and limited human-to-human transmission. Investigations including contact tracing, clinical assessments, sequencing and serology, linked the index case to a mining cave inhabited by *Rousettus aegyptiacus*. Serology tests identified three individuals seropositive for IgG and IgM, further supporting the zoonotic origin of the outbreak through human-animal interactions.

Since its discovery in 1967, the Marburg virus disease (MVD) has emerged as a significant global health threat, resulting in several outbreaks characterized by alarmingly high case fatality rates ranging from 22% to 90%[1]. The first reported instances of MVD occurred in simultaneous outbreaks in Marburg and Frankfurt, Germany, as well as in Belgrade, Yugoslavia (now Serbia)[2]. To date, there have been 19 recorded outbreaks, 563 confirmed cases, and 428 deaths worldwide[3]. On September 27, 2024, Rwanda reported its first ever cases of MVD[4]. The ongoing outbreak in Rwanda has confirmed 66 cases, with a case fatality rate of approximately 23%[4].

MVD has two distinct variants: Marburg virus (MARV) and Ravn virus (RAVV), with MARV being the more prevalent variant, responsible for the majority of sporadic MVD outbreaks worldwide. MARV is a negative-sense single-stranded RNA virus from the Filoviridae family, similar to Ebola and possesses inverse-complementary 3′ and 5′ termini[3]. MVD is predominantly a zoonosis, transmitting to humans from either the bat natural reservoir or through intermediate hosts, including non-human primates. The mechanisms by which bats regulate viral replication and maintain complex immunity in contrast to humans remain unclear due to a lack of bat-specific research tools necessary for comparative studies, such as antibodies for flow cytometry, genomics, and transcriptomics[1,2]. Studies confirm that MARV's natural reservoir is the Egyptian fruit bat

(*Rousettus aegyptiacus*)[5]. The virus can cause viral hemorrhagic fever in both humans and primates. However, the precise transmission dynamics between the natural reservoir and humans or primates are insufficiently understood, though exposure to contaminated excretions from fruit bats is likely a contributing factor[5]. The virus is transmitted from human to human, particularly through direct contact with body fluids like blood, saliva, semen, and other body fluids from infected individuals[6].

The Marburg viral genome consists of 19,114 nucleotides encoding for seven proteins, including glycoprotein (GP), nucleoprotein (NP) virion protein 30 (VP30), VP35, VP24, VP40 and large viral polymerase[3]. Genomic characterization of MARV strains has revealed substantial genetic diversity across its global phylogeny, but exhibits limited genomic variation within outbreaks[7]. Sequencing of the Marburg genome has provided deeper insights into viral evolution and identified critical mutation hotspots during adaptation to new hosts, particularly in the VP40 protein and the NP-VP35 intergenic region[8]. These mutations can influence the virus's ability to evade the immune system, therefore enhancing its pathogenicity.

In this study, we used a near real-time genomics sequencing approach to characterize the Marburg virus in blood samples obtained from patients in the ongoing outbreak in Rwanda. Our aim was to use the genomic sequencing data to identify and characterize the virus and understand its evolution and transmission dynamics during the current outbreak. This research underscores the importance of continuous genomic surveillance which not only facilitates outbreak tracking but also informs public health strategies for effectively controlling this deadly pathogen.

Results

Rwanda effectively contained the spread of MARV from further reaching the broader community by prompt isolation of positive cases and treatment with monoclonal antibodies and antivirals. Over the span of the outbreak, the case fatality rate was approximately 23%. We generated genomes from 18 early cases to investigate the zoonotic origin and transmission dynamics of MARV in the early epidemic. Our phylogenetic analyses support that the outbreak resulted from a single zoonotic transmission event with limited human-to-human transmission rather than multiple independent zoonotic transmission events (Figure 1A, B). Multiple independent zoonotic transmissions would have resulted in a set of more diverged sequences introduced from the genetically diverse viral population in the reservoir[9]. The outbreak lineage is most closely related to a sequence sampled in Kampala, Uganda in September 2014 from a healthcare worker (HCW), with no secondary cases observed (KP985768). The source of the zoonotic transmission in 2014 was never identified[10]. However, the Rwanda-Uganda sister lineages are significantly diverged from one another, separated by 82 nucleotide substitutions (Figure 1A, B). In Bayesian phylogenetic reconstructions, we estimate that the two lineages diverged from a common ancestor that circulated in the animal reservoir in November 2008 [95% highest posterior density, or HPD: May 2007 and June 2010] (Extended Data Figure 1).

The outbreak lineage in Rwanda is nested within a larger clade that includes diversity sampled from bats in southwestern Uganda from 2007 onwards, along with two human cases identified in Uganda in 2017 (Figure 1A, B). The closest bat virus (JX458855) was sampled from a juvenile Egyptian fruit bat (*Rousettus Aegyptiacus)* in 2009 from Python Cave in Queen Elizabeth National Park, a popular tourist attraction in south western Uganda (Figure 1B)[5]. The lineage in Rwanda is

significantly diverged from JX458855, sharing a common ancestor that likely circulated in the reservoir as early as June 2006 (95% HPD: December 2004 to November 2007) (Extended Data Figure 1 A-B). *Rousettus aegyptiacus* are a cave dwelling species, with spillover events and limited outbreaks frequently associated with mining activities. This includes outbreaks and sporadic cases linked to Python cave and the Kitaka mine that is only 50 km away in 2007 and 2008, which both hosts *Rousettus aegyptiacus* colonies of over 50 000 bats[5,9,11]. The divergent relationship of the Rwandan Marburg virus outbreak lineage indicates that the virus's dispersal through host networks involved larger scale animal movement over a long period of time, rather than a direct ancestral connection to the *Rousettus aegyptiacus* colony in southwestern Uganda.

The index patient's close relative was admitted to Hospital 1 (H1) at the end of August 2024 but succumbed to the illness prior to the definitive diagnosis of MVD. It is highly probable that this patient infected healthcare workers (HCWs) at H1, where MVD was subsequently confirmed at the end of September 2024. From 22-28 September, 26 cases were reported in HCWs at H1 and H2, respectively. The limited genetic variation among the outbreak sequences was consistent with a very recent common ancestor, the short sampling period, and previous observations from the Angola MARV outbreak (Figure 2A)[11]. A cluster of eight identical sequences was sampled, spanning the full sampling period (C1 in Figure 2A, B). The majority of the C1 sequences were isolated from healthcare workers (HCWs) at H1 from 22-28 September, with reported symptom onset as early as mid-September (Figure 2B). A C1 sequence was sampled from a HCW at H2 at the end of September, suggesting infection from an unsampled C1 case in H2 or dissemination between the two healthcare facilities. There are recorded cases of healthcare workers moving between healthcare settings H1 and H2. This is further supported by cluster C2, consisting of two

healthcare workers from H1 and H2 respectively that share the synonymous SNP C11070T relative to C1. Both C2 cases report symptom onset and were sampled in the later weeks of the epidemic. There are a further seven identical sequences (C4) sampled between H1 and H2 across the full sampling period that are one synonymous SNP (T10031C) away from C1. C002, a healthcare worker employed at both H1 and H2, is believed to have acquired the infection at H1. This individual subsequently became the index case at H2, leading to a nosocomial cluster at H2 and infections at H1. Overall, there were only three synonymous SNPs observed in the outbreak clade: A5856G (n=1 sequence), T10031C (n=7), and C11070T (n=2). A5856G and T10031C as well as C11070T are outside of the CDS of the *GP* and *VP24* genes, respectively. There is therefore no evidence that post-emergence substitutions enhanced human transmission.

We conducted an exhaustive epidemiological investigation to identify the index case, including reviews of clinical records, travel history, antibody serology testing results, and contact tracing information. In this investigation, we identified a group of individuals linked to a mining cave and screened them for MARV. All individuals were PCR negative, but serological testing revealed that three individuals had antibodies indicating prior exposure to Marburg virus. We found that the probable index case of this group was a man in his 20's with an occupational exposure to fruit bats (*Rousettus aegyptiacus*) in a mining cave environment. The individual exhibited the earliest symptoms, which were highly consisted with the classic clinical presentation of MVD. Additionally, serological testing of three contacts of the index case exhibited MARV antibodies, further supporting epidemiological tracing of the index case. However, no sequence data were obtained from these cases. In response to these findings, the Rwanda Ministry of Health through a

multisectoral collaboration initiated a surveillance investigation e aimed at screening fruit bats, primarily cave dwellers, for Marburg virus (Extended Data Figure 2 A, B and C).

Discussion

The Rwanda MVD outbreak primarily involved limited onward human-to-human transmission between cases and healthcare personnel, as is often observed with viral hemorrhagic fever (VHF) outbreaks closely associated with nosocomial and occupational infections[12]. This underscores the ongoing need to enhance healthcare personnel's knowledge and attitudes regarding VHF case management. In our phylogenetic analyses, we observed that the early outbreak sequences represented limited genomic variation, indicating that the outbreak originated from a single zoonotic transmission event. The Rwandan lineage shared a common ancestor with sequences originating from diversity sampled in bats in southwestern Uganda, thought the lineages had diverged over decades in the animal reservoir. It is likely that enhanced zoonotic surveillance will reveal many unsampled intermediates that can clarify the ecological pathways of transmission between bat colonies in Uganda and Rwanda. Previous research indicates that bats can harbor MARV for extended periods, with active mining areas in Rwanda providing ideal habitats for bats and increasing the likelihood of zoonotic transmission[13].

We observed three synonymous single nucleotide polymorphisms (SNPs) outside the coding regions of the *GP* and *VP24* genes, respectively. Studies have shown that the Marburg virus *VP24* protein interacts with the nucleoprotein (NP) and other cellular membranes, facilitating the release of new virions from infected cells[14]. Additionally, research highlights that MARV *VP24* directly interacts with the human and bat Keap1 proteins, which modulate antioxidant responses to support

viral replication, a strategy likely critical for viral persistence and host adaptation[15]. Genomic sequencing of MARV in Rwanda has yielded critical insights into viral circulation dynamics. However, as these SNPs fall outside of coding regions, it is unlikely that they functionally contribute to enhanced viral fitness. Our results should be interpreted within the context of our smaller sample size, which only covers the first two weeks of the outbreak.

This research underscores the importance of continuous genomic surveillance which not only facilitates outbreak tracking but also informs public health strategies for effectively controlling this deadly pathogen. In addition, previous research indicates that bats can harbor MARV for extended periods, with active mining areas in Rwanda providing ideal habitats for bats and increasing the likelihood of zoonotic transmission. Furthermore, Rwanda's location in a region of consistent turbulent outbreaks, including Ebola, Rift Valley fever, COVID-19, dengue, and Marburg, underscores the importance of regional and international collaboration to enhance outbreak preparedness and responses for global health security.

Our study provides valuable insights into the evolutionary dynamics of MARV. However, our findings should be interpreted within the context of our limited sample. Our sample did not encompass all positive cases from the outbreak due to the rapid timeline required for an urgent response. Additionally, the availability of historical genomic data for comparison was limited, including MARV samples from animals, as gaps in sequencing capacity restricted the inclusion of sequences from recent outbreaks.

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Author contributions

Conceptualization: Y.B, L.M, S.N, C.H, C.M.M, Methodology: Y.B, L.M, A.A, E.U, R.M, E.P, P.S, C.H, C.M.M Investigation: I.M, E.R, E.K, E.R, N.G Sampling: R.S, P.G, P.S, J.C.M PCR Experiment: E.K, F.U, V.M, G.I, H.M Sequencing: Y.B, L.M, E.U, L.I, C.M, A.U, I.U, R.N, A.U, J.K, J.P.M Analysis: E.P, R.M, J.P.M, P.S ,N.G, J.R, A.O, C.H, Data Visualization: E.P. Lab Enablement: S.T.K, A.A, O.O, O.A, A.H, C.M.M Writing- Original Draft: Y.B, L.M, E.P., C.M, R.M, J.P.M, P.S, Writing Review and Editing: Y.B, L.M, E.P., E.E.S.F, P.S,E.R, C.M, M.S, M.N, T.T, D.T, E.S, M.G, J.P.R, J.N, A.R, F.K, G.M, A.A, S.N, C.H, C.M.M.

Competing interests: The authors declare no conflicts of interest.

Figure legends:

Figure 1: A) Maximum likelihood phylogeny of the global MARV dataset. Tips are annotated by country of isolation. This study's tips are relatively enlarged. B) The Rwandan outbreak clade nested in diversity sampled from sporadic human cases and fruit bats respectively in Uganda C) Map of Uganda and Rwanda. The city of patients in this study (Kigali) is annotated in dark red. Python Cave and Kitaka Mine in Ibanda district in southwestern Uganda are annotated in light blue and yellow, respectively.

Figure 2: A) Maximum likelihood phylogeny of the outbreak clade, with the Kampala outgroup. Sequences are colored with associated hospital. SNPs reconstructed relative to the common ancestor are annotated in text, as are the clusters defined by the SNP presence. The coordinates are relative to NC_001608 B) Epidemiological timeline of the sampled sequences in Figure 2A, as annotated on the y-axis. The first black marker, if present, indicates the week of symptom onset if distinct from week of sampling, with the second indicating the week of sampling. Sequences are colored by their associated hospital (H1, H2), and partitioned by SNP presence into clusters, as annotated in text. Red dashed line indicates the contact of the index case admitted to H1.

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Methods

Ethics declaration

The study was approved by the Rwanda National Ethics Committee (FWA Assurance No. 00001973 IRB 00001497 of IORG0001100-Protocol approval notice: N° 121/RNEC/2024). All necessary patient/participant consent has been obtained and the appropriate institutional forms have been archived. Patient/participant/sample identifiers included were not known to anyone (*e.g., hospital staff, patients or participants themselves*) outside the research group so cannot be used to identify individuals. Under the circumstances of the emergency of the outbreak verbal consent was obtained.

Patient sample collection

We obtained whole blood samples from suspected patients presented with clinical symptoms (high fever, severe headaches, muscle aches, fatigue, nausea, vomiting, and diarrhea) of MDV. Sample testing was performed at Rwanda Biomedical Centre/National Reference Laboratory whereby samples were kept in a cold chain prior to plasma separation and analysis.

Nucleic acid extraction

Viral RNA was extracted from 140µl plasma using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany), following the manufacturer's instructions adapted to the in-house standard operating procedures with an elution volume of 60µl. The extracted RNA was quantified using a Qubit fluorometer, and the samples were stored at -80°C until further analysis.

RT-PCR and genomic sequencing

For the detection and amplification of the target regions of the MVD, we employed the use of RealStar® Filovirus Screen RT-PCR Kit 1.0 (Altona Diagnostics, Hamburg, Germany), which is based on real-time PCR technology, for the qualitative detection and differentiation of Ebola and

Marburg virus specific RNA in human EDTA plasma. The RT-qPCR was conducted following manufacturer's user guide and performed on CFX96 BIORAD machine. The amplification followed an initial denaturation at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 30 seconds, annealing at 55°C for 30 seconds, and extension at 72°C for 1 minute, concluding with a final extension at 72°C for 5 minutes. The amplified products were analyzed via 2% agarose gel electrophoresis to confirm successful amplification and appropriate product size.

Following amplification, library construction was performed on 30 samples using the Illumina RNA Prep with Enrichment (L) Tagmentation (IRPE) workflow and the lllumina Viral Surveillance Panel v2 kit (VSP v2 kit). Total RNA extracts were converted to cDNA, tagmented and thereafter amplified. Genomic region of interest was captured using a hybrid capture method. Probes were isolated using magnetic pulldown which are selectively enriched for the desired regions. Enriched libraries were quantified using dsDNA HS assay and Qubit fluorometer. Libraries were thereafter denatured and normalized at a final loading concentration of 0.8pM. Paired-end sequencing was performed using a NextSeq 550 with a 300-cycle mid-output cartridge, with the sequencing depth aimed at a minimum coverage of 100x to ensure robust variant detection.

Bioinformatics Analysis

Genome Assembly

We employed a reference-based genome assembly pipeline to analyze the sequencing data generated using the Illumina Viral Surveillance Panel (VSP) v2 Kit (described above). This workflow integrates several steps including; quality control, host genome filtering, and viral genome assembly to accurately reconstruct viral sequences from targeted sequencing. To ensure that only high-quality data were processed downstream, raw sequencing reads were first processed

with *fastp v. 0.23.24*[16] for trimming adapters and filtering out low-quality bases. Following this, human host genome sequences were filtered out by aligning the cleaned reads to the human genome (hg38) using *Bowtie2 v.2.5.4*[17]. Unmapped reads, presumed to be of viral origin, were retained for downstream analysis. These de-hosted reads were then aligned to a Marburg virus reference genome (NC_001608.3) using *Minimap2 v.2.28*[18], the sequences had 70-97% genomic coverage. We manually masked two mutations in respective consensus sequences that were adjacent to potential misalignment.

Variant Calling and Consensus Generation

Variant calling was performed using both *ivar 1.4.3*[19] and *LoFreq v.2.1.5*[20] for comparison. A depth coverage threshold of 50 reads per nucleotide position was applied to ensure robust and reliable variant detection. This threshold was also applied to the generation of consensus sequences which was also done using *ivar 1.4.3*.,

Phylogenetic analyses

We combined our eighteen higher quality sequences (coverage >= 70%) with all publicly available MARV sequences available on Genbank (N=81). To root the tree, we included 8 Ravn virus sequences included as an outgroup, which were pruned from the tree and excluded in all subsequent analyses. We aligned the sequences using *Mafft v 7.52*[21] and reconstructed a phylogenetic tree using *IQTree 2.2.5*[22] under modelfinder plus[23], with ultrafast bootstrapping[24]. We performed the initial ancestral state reconstruction using *Treetime v0.9.3*[25].

Bayesian phylogenetic reconstruction

The current genomic data does not provide sufficient temporal signal to estimate the rate of evolution in the outbreak clade. However, we were not interested in estimating the time to the most recent common ancestor (tMRCA) of the outbreak clade, as determining the emergence timing of

the outbreak clade will more likely depend on the epidemiological and contact history of the index case. We wanted to estimate tMRCA of the outbreak clade and its closest human and zoonotic outgroups to better understand its potential zoonotic emergence pathway. We therefore reconstructed the time-resolved phylogeny under a fixed local clock with the BEAST software package. We assumed a fixed value of 1e-3 substitutions per site per year as a plausible rate of evolution for an RNA virus in the early stage of an outbreak for the defined outbreak clade. We allowed the remainder of the tree to evolve under a lognormal prior centered on 5e-4[26] We used a two-phase coalescent model: the tree from the MRCA (Rwandan lineage) onward was modeled with an exponential growth model, with the earlier phase modeled as a constant-population size coalescent model. We ran two independent chains of 100 million states to ensure convergence, discarding the initial 10% of each chain as burn-in. The chains were then combined with LogCombiner. For all subsequent analyses, we assessed convergence using Tracer, and constructed a maximum clade credibility (MCC) tree in TreeAnnotator 1.10[27]. See Extended Data Figure 3 for a plot of the temporal regression.

Data availability

All the sequences are available on NCBI - GenBank under Accession Number PQ552725-PQ552742 (https://linkmix.co/31343096). All analyses code and data are available at: https://github.com/rbc-bioinformatics/vsp-genomic-assembly-pipeline/ and https://github.com/EdythParker/MARV_transmission_dyanmics_in_Rwanda_outbreak, excluding shapefiles, which are available on request owing to size. All shapes files were obtained from the FAO geoNetwork. All pipelines are publicly available, and any issues or inquiries can be addressed through the respective GitHub pages, where they will be promptly

attended to within 24 hours. For assistance with the genome assembly pipeline, please contact bfx@rbc.gov.rw, and for phylogenetic analyses, please reach out to edythp@run.edu.ng.

Code availability

All code to run the analyses and make figures are available in https://github.com/EdythParker/MARV_transmission_dyanmics_in_Rwanda_outbreak. Code used for genome assembly available at https://github.com/rbc-bioinformatics/vsp-genomic-assembly-pipeline/ .

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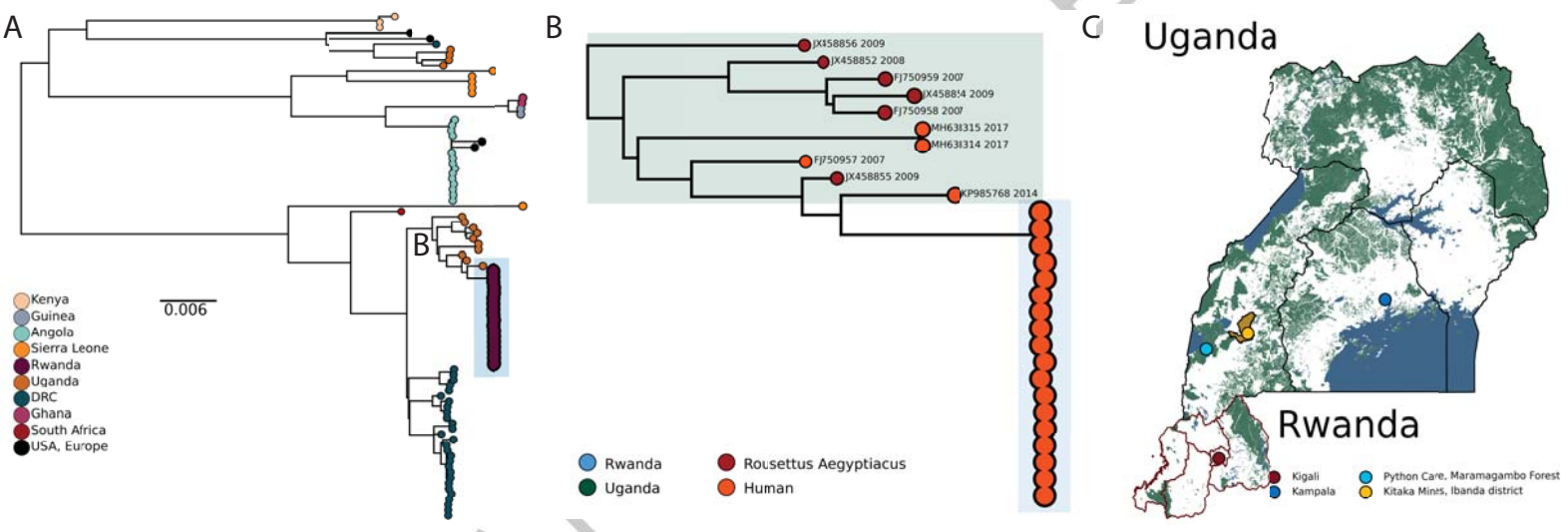

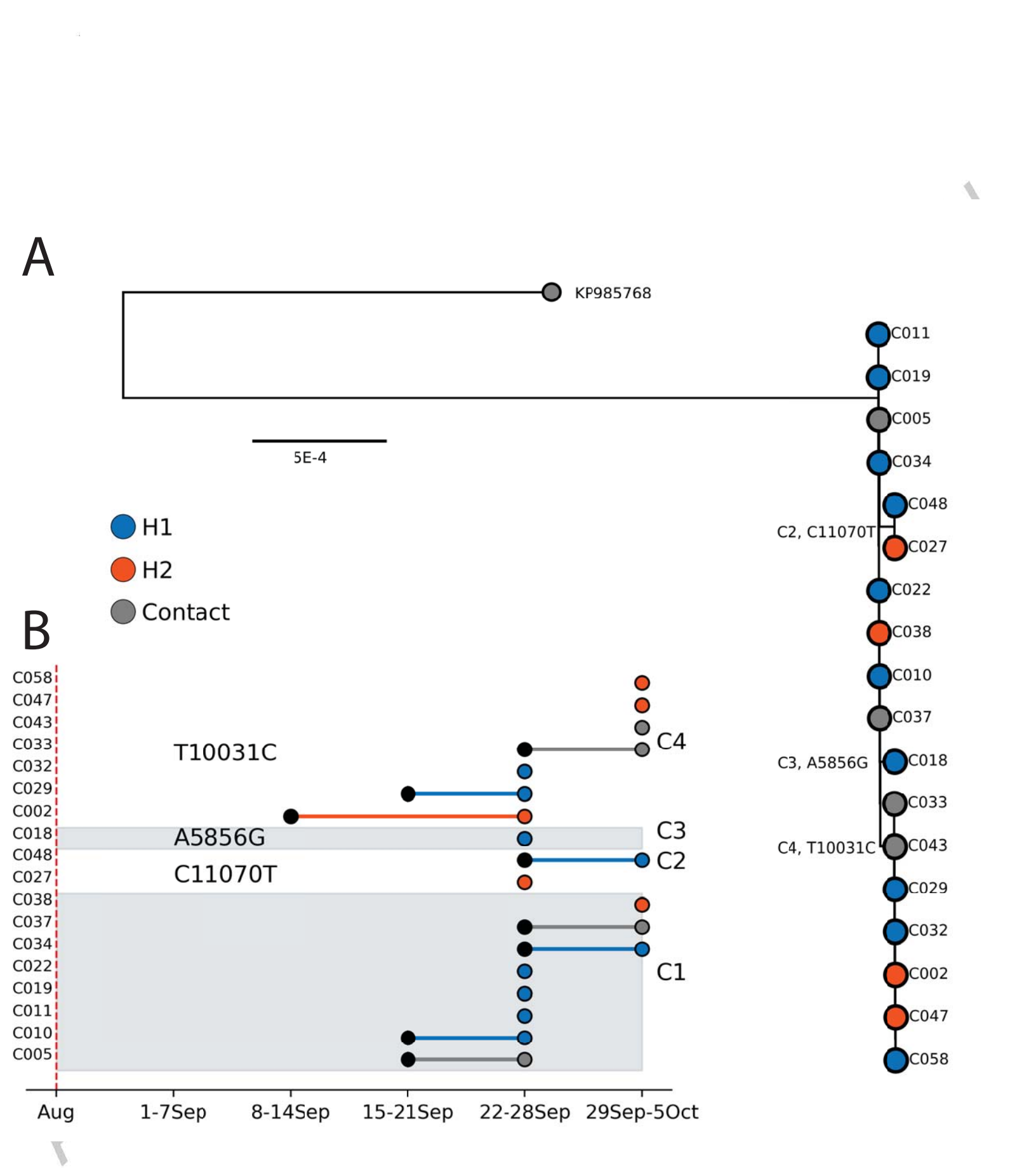
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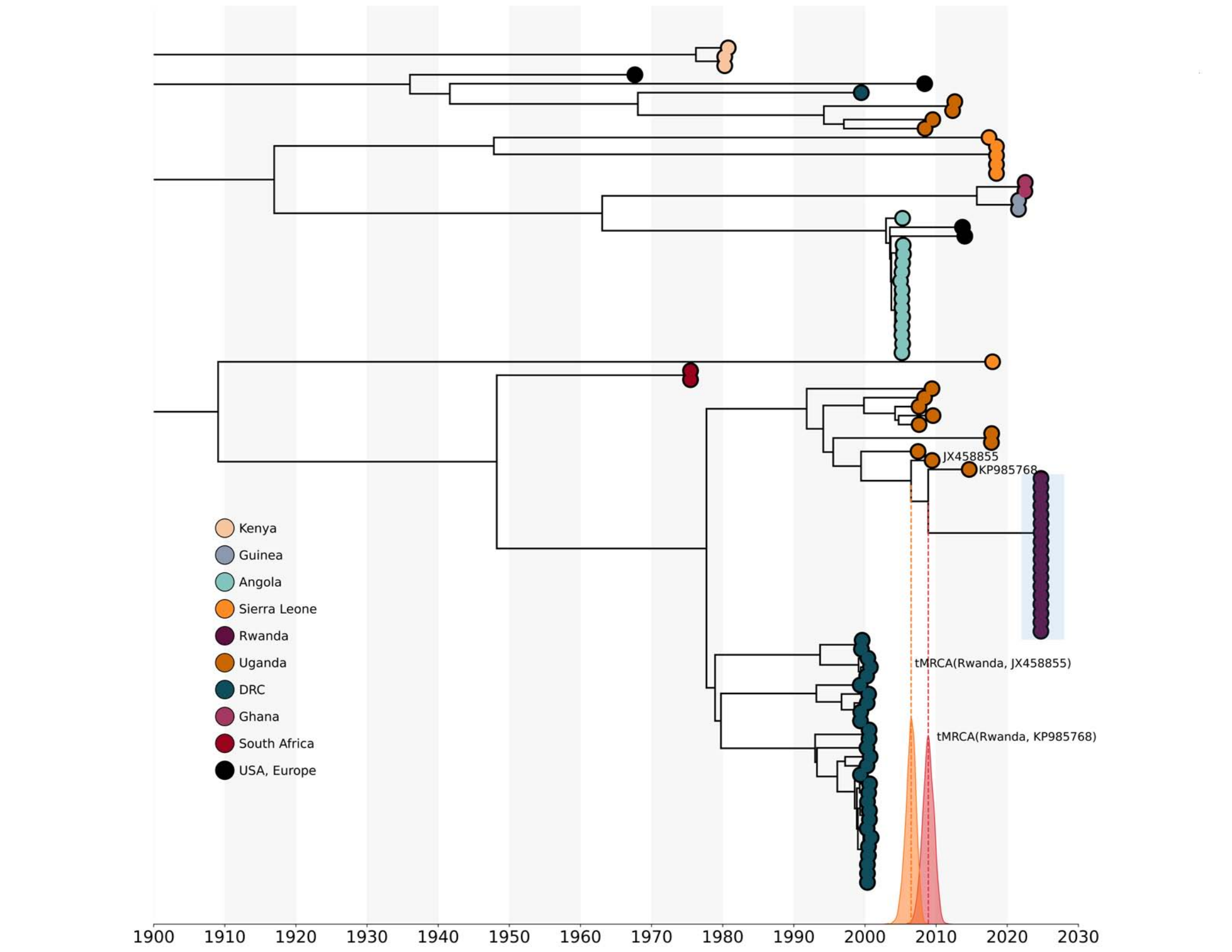
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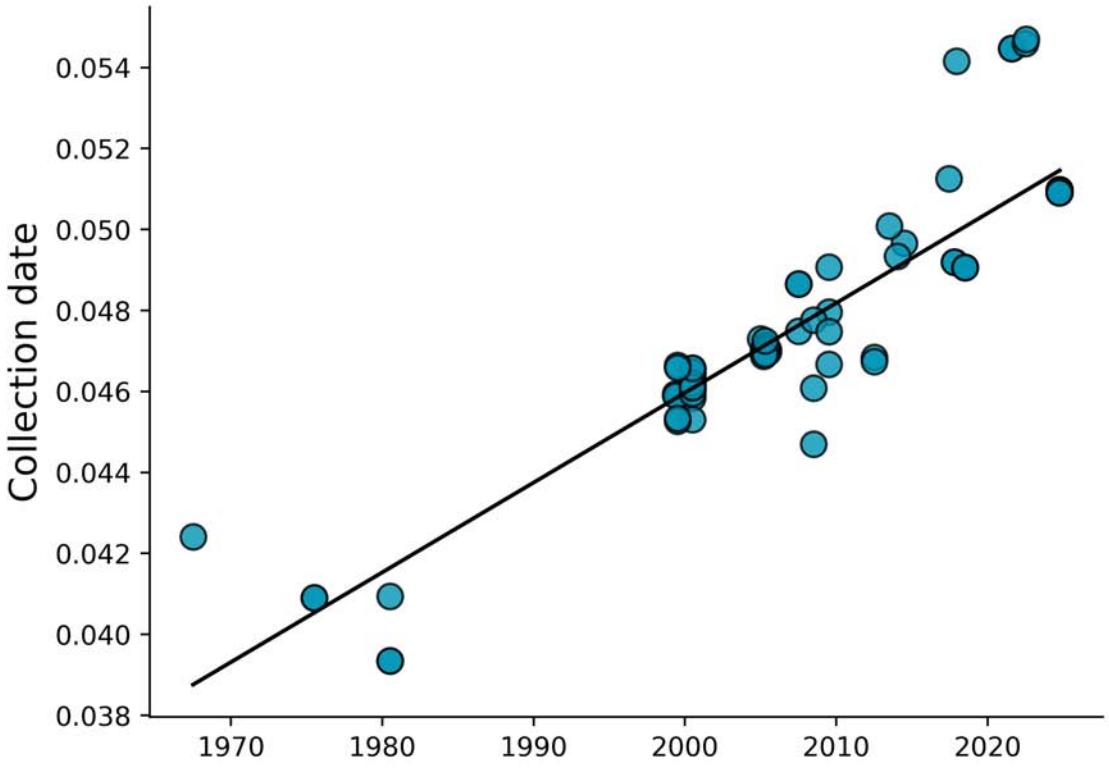
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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☒	☐ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☒	☐ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐ A description of all covariates tested
☒	☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☒	☐ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☐	☒ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Code for genome assembly is available via github as listed in the code availability statement. This includes ivar v1.4.3, LoFreq v.2.1.5 : https://github.com/rbc-bioinformatics/vsp-genomic-assembly-pipeline/
Data analysis	IQtree v 2.2.5, Mafft v7.5.2 and Treetime v.0.9.3 are publically available. BEAST v.1.10.5 XMLs available via associated github as stated in code availability staement: https://github.com/EdythParker/MARV_transmission_dyanmics_in_Rwanda_outbreak.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All the sequences are available on NCBI - GenBank under Accession Number PQ552725- PQ552742 (https://linkmix.co/31343096). All analyses code and data are available at: https://github.com/rbc-bioinformatics/vsp-genomic-assembly-pipeline/ and https://github.com/EdythParker/

MARV_transmission_dyanmics_in_Rwanda_outbreak, excluding shapefiles, which are available on request owing to size. All shapes files were obtained from the FAO geoNetwork. All pipelines are publicly available, and any issues or inquiries can be addressed through the respective GitHub pages, where they will be promptly attended to within 24 hours. For assistance with the genome assembly pipeline, please contact bfx@rbc.gov.rw, and for phylogenetic analyses, please reach out to edythp@run.edu.ng.
We combined our eighteen higher quality sequences (coverage >= 70%) with all publicly available MARV sequences available on Genbank (N=81). The full alignment with all accensions is available on the Github via the XML file.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	No sample criteria was selected
Reporting on race, ethnicity, or other socially relevant groupings	No sample criteria was selected
Population characteristics	Marburg Disease Patients. There are no covariate relevant charactaristics as we do not do statistical tests on demographic factors. The only related statistic is the CFR, and that is a descriptive statistic for the population over the full epidemic.
Recruitment	Any patient that presented at a hospital in Kigali with suspected Marburg disease. No selection performed. Access to healthcare biases may affect the sample.
Ethics oversight	The study was approved by the Rwanda National Ethics Committee (FWA Assurance No. 00001973 IRB 00001497 of IORG0001100-Protocol approval notice: N° 121/RNEC/2024). All necessary patient/participant consent has been obtained and the appropriate institutional forms have been archived. Patient/participant/sample identifiers included were not known to anyone (e.g., hospital staff, patients or participants themselves) outside the research group so cannot be used to identify individuals. Under the circumstances of the emergency of the outbreak verbal consent was obtained.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☐ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No sample size was selected. As we sought to investigate the early epidemic, we selected all cases in the first two weeks. All sequences generated above 70% genome coverage were included to ensure phylogenetic robustness. The sample size is appropriate as it all high quality sequences from the early epidemic, which is relevant to investigating the zoonotic origin.
Data exclusions	All sequences below 70% genome threshold, as there phylogenetic placement can be uncertain.
Replication	We're investigating an epidemic during its early stages. All sequences represent individual time points, no replication required. For the Bayesian analyses, we ran tqwo independent chains and they converged.
Randomization	Nothing to randomise.
Blinding	The data needs to be associated with metadata for the epidemiolgical investigation.

Behavioural & social sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	*Briefly describe the study type including whether data are quantitative, qualitative, or mixed-methods (e.g. qualitative cross-sectional, quantitative experimental, mixed-methods case study).*
Research sample	*State the research sample (e.g. Harvard university undergraduates, villagers in rural India) and provide relevant demographic information (e.g. age, sex) and indicate whether the sample is representative. Provide a rationale for the study sample chosen. For studies involving existing datasets, please describe the dataset and source.*
Sampling strategy	*Describe the sampling procedure (e.g. random, snowball, stratified, convenience). Describe the statistical methods that were used to*

Sampling strategy	*predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient. For qualitative data, please indicate whether data saturation was considered, and what criteria were used to decide that no further sampling was needed.*
Data collection	*Provide details about the data collection procedure, including the instruments or devices used to record the data (e.g. pen and paper, computer, eye tracker, video or audio equipment) whether anyone was present besides the participant(s) and the researcher, and whether the researcher was blind to experimental condition and/or the study hypothesis during data collection.*
Timing	*Indicate the start and stop dates of data collection. If there is a gap between collection periods, state the dates for each sample cohort.*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, provide the exact number of exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Non-participation	*State how many participants dropped out/declined participation and the reason(s) given OR provide response rate OR state that no participants dropped out/declined participation.*
Randomization	*If participants were not allocated into experimental groups, state so OR describe how participants were allocated to groups, and if allocation was not random, describe how covariates were controlled.*

Ecological, evolutionary & environmental sciences study design

All studies must disclose on these points even when the disclosure is negative.

Study description	*Briefly describe the study. For quantitative data include treatment factors and interactions, design structure (e.g. factorial, nested, hierarchical), nature and number of experimental units and replicates.*
Research sample	*Describe the research sample (e.g. a group of tagged Passer domesticus, all Stenocereus thurberi within Organ Pipe Cactus National Monument), and provide a rationale for the sample choice. When relevant, describe the organism taxa, source, sex, age range and any manipulations. State what population the sample is meant to represent when applicable. For studies involving existing datasets, describe the data and its source.*
Sampling strategy	*Note the sampling procedure. Describe the statistical methods that were used to predetermine sample size OR if no sample-size calculation was performed, describe how sample sizes were chosen and provide a rationale for why these sample sizes are sufficient.*
Data collection	*Describe the data collection procedure, including who recorded the data and how.*
Timing and spatial scale	*Indicate the start and stop dates of data collection, noting the frequency and periodicity of sampling and providing a rationale for these choices. If there is a gap between collection periods, state the dates for each sample cohort. Specify the spatial scale from which the data are taken*
Data exclusions	*If no data were excluded from the analyses, state so OR if data were excluded, describe the exclusions and the rationale behind them, indicating whether exclusion criteria were pre-established.*
Reproducibility	*Describe the measures taken to verify the reproducibility of experimental findings. For each experiment, note whether any attempts to repeat the experiment failed OR state that all attempts to repeat the experiment were successful.*
Randomization	*Describe how samples/organisms/participants were allocated into groups. If allocation was not random, describe how covariates were controlled. If this is not relevant to your study, explain why.*
Blinding	*Describe the extent of blinding used during data acquisition and analysis. If blinding was not possible, describe why OR explain why blinding was not relevant to your study.*

Did the study involve field work? ☐ Yes ☐ No

Field work, collection and transport

Field conditions	*Describe the study conditions for field work, providing relevant parameters (e.g. temperature, rainfall).*
Location	*State the location of the sampling or experiment, providing relevant parameters (e.g. latitude and longitude, elevation, water depth).*
Access & import/export	*Describe the efforts you have made to access habitats and to collect and import/export your samples in a responsible manner and in compliance with local, national and international laws, noting any permits that were obtained (give the name of the issuing authority, the date of issue, and any identifying information).*
Disturbance	*Describe any disturbance caused by the study and how it was minimized.*

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We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☐	☐ MRI-based neuroimaging

Magnetic resonance imaging

Experimental design

Design type	*Indicate task or resting state; event-related or block design.*
Design specifications	*Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials.*
Behavioral performance measures	*State number and/or type of variables recorded (e.g. correct button press, response time) and what statistics were used to establish that the subjects were performing the task as expected (e.g. mean, range, and/or standard deviation across subjects).*

Acquisition

Imaging type(s)	*Specify: functional, structural, diffusion, perfusion.*
Field strength	*Specify in Tesla*
Sequence & imaging parameters	*Specify the pulse sequence type (gradient echo, spin echo, etc.), imaging type (EPI, spiral, etc.), field of view, matrix size, slice thickness, orientation and TE/TR/flip angle.*
Area of acquisition	*State whether a whole brain scan was used OR define the area of acquisition, describing how the region was determined.*

Diffusion MRI ☐ Used ☐ Not used

Preprocessing

Preprocessing software	*Provide detail on software version and revision number and on specific parameters (model/functions, brain extraction, segmentation, smoothing kernel size, etc.).*
Normalization	*If data were normalized/standardized, describe the approach(es): specify linear or non-linear and define image types used for transformation OR indicate that data were not normalized and explain rationale for lack of normalization.*
Normalization template	*Describe the template used for normalization/transformation, specifying subject space or group standardized space (e.g. original Talairach, MNI305, ICBM152) OR indicate that the data were not normalized.*
Noise and artifact removal	*Describe your procedure(s) for artifact and structured noise removal, specifying motion parameters, tissue signals and physiological signals (heart rate, respiration).*
Volume censoring	*Define your software and/or method and criteria for volume censoring, and state the extent of such censoring.*

Statistical modeling & inference

Model type and settings	*Specify type (mass univariate, multivariate, RSA, predictive, etc.) and describe essential details of the model at the first and second levels (e.g. fixed, random or mixed effects; drift or auto-correlation).*
Effect(s) tested	*Define precise effect in terms of the task or stimulus conditions instead of psychological concepts and indicate whether ANOVA or factorial designs were used.*

Specify type of analysis: ☐ Whole brain ☐ ROI-based ☐ Both

Statistic type for inference (See Eklund et al. 2016)

Specify voxel-wise or cluster-wise and report all relevant parameters for cluster-wise methods.

Correction

Describe the type of correction and how it is obtained for multiple comparisons (e.g. FWE, FDR, permutation or Monte Carlo).

Models & analysis

n/a	Involved in the study
☐	☐ Functional and/or effective connectivity
☐	☐ Graph analysis
☐	☐ Multivariate modeling or predictive analysis

Functional and/or effective connectivity

Report the measures of dependence used and the model details (e.g. Pearson correlation, partial correlation, mutual information).

Graph analysis

Report the dependent variable and connectivity measure, specifying weighted graph or binarized graph, subject- or group-level, and the global and/or node summaries used (e.g. clustering coefficient, efficiency, etc.).

Multivariate modeling and predictive analysis

Specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.