

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☐ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☐	☒ 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 <i>d</i> , Pearson's <i>r</i>), 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	DATA COLLECTION Primal Scheme (v1.4.1); Primer3 (modified version embedded in Geneious Prime); Geneious Prime 2023.2.1; Miseq Control Software v2.6.2.1; NextSeq 1000 Control Software Suite v1.2.0; BaseSpace (Illumina’s cloud-based application); BBDuk, Dedupe, and BBMap v38.84 (embedded in Geneious Prime 2023.2.1)
Data analysis	JModelTest 2.1.10; MrBayes 3.2.7a; Tracer v1.7; Treeio v3.1.7; ggtree v3.2.1; TempEst v1.5.3; BEAST 1.10 with BEAGLE library v3; BEAST2; RDP5 using nine different detection algorithms (RDP, Geneconv, Bootscan, Maxchi, Chimaera, SiScan, PhylPro, LARD, and 3Seq. For all theses algorithms: version embedded in RDP5); BEAST 1.10.4; TreeAnnotator v1/10; FigTree v1.4.4; IcyTree (web version - https://icytree.org); SPREAD v1.0.7; ggplot2; sf; goober; GraphPad v9.0 (Prism Software); R v4.4.1; RStudio v2024.04.2 Code: all input files (Fasta, Nexus, and XML) and scripts utilized in this study are publicly available at: https://github.com/larboh-ioc/orov_br .

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 OROV genomes generated and analyzed in this study were deposited at GenBank under accession numbers PP153945 to PP154172 and PQ064571 to PQ065491.

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

Findings apply to both sexes. Sex and gender was not considered in the study design. We include information of clinical symptoms from 2,272 Oropouche fever patients from the Amazonas state, 1,085 females and 1,187 males that answered the medical form in 2024 and were collected by the Amazonas State Health Surveillance Foundation - Dr Rosemary Costa Pinto (FVS-RCP).

Reporting on race, ethnicity, or other socially relevant groupings

We did not use race, ethnicity or other socially relevant categorization variable in our study.

Population characteristics

We did not consider sex or gender in the study design and we did not perform a priori analyses for sex or gender. We received and analyzed samples for sequencing characterization based only on a positive OROV result, i.e., detected in real-time PCR and with a Ct value lower than 30 to ensure complete sequencing, regardless of prior knowledge of any other information, except the location and collection data for those samples. We did not use further variables to select samples for sequencing. We tried to include as many municipalities as possible from different Brazilian states in the western Amazon region. However, we analyzed information about sex related to clinical symptoms for 2,272 Oropouche fever patients from the Amazonas state, 1,085 females, and 1,187 males who answered the medical form in 2024. The sex information (biological attribute) was acquired during the sample collection by the health professionals from the Amazonas State Health Surveillance Foundation—Dr. Rosemary Costa Pinto (FVS-RCP), who had contact with patients based on the patient's self-report. Since we analyzed this data for all cases with a medical record (more than 2,000 patients), we consider that there is no sex bias, and we had a sufficient sample size for comparisons, as supported by Two-tailed Fisher exact test results. All age groups were included in this study. On average, participants had 35.7 years (Standard Deviation = 17.0). All individuals included were diagnosed with Oropouche fever, with Fever (96.7%), headache (92.3%), myalgia (77.8%), nausea (41.9%), retro-orbital pain (33.9%), arthralgia (28.4%) and vomiting (25.6%) reported as the more frequently symptoms. No information about specific treatment was collected, since those are not relevant to this study.

Recruitment

Patients were not directly recruited since we work with samples that were detected as positive for OROV in the routine surveillance conducted by the state Central Laboratories from the Ministry of Health and the Fiocruz-Amazonas. Sampled were selected based on a RT-PCR Ct value <30 and in order to include as many municipalities as possible from different Brazilian states in western Amazon region.

Ethics oversight

Ethics Committee of Amazonas State University

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

The sample size for this study was not determined through a priori calculation but rather through convenience sampling. We tested nearly all samples submitted with suspected arbovirus infections during the study period for OROV. The selection for genome sequencing was constrained by available resources and guided by a positive RT-PCR result with a Ct value of <30. The laboratory at Fiocruz-Amazonas and Fiocruz Rondônia in collaboration with the state Central Laboratories of Acre, Roraima, and Rondônia, conducted the diagnostic testing. All positive samples that met the inclusion criteria were submitted for whole genome sequencing. Through this approach, between late 2022 and early 2024, the state of Amazonas (AM) reported the highest number of cases, with 3,685 notified cases, followed by Rondônia (RO) with

1,756 cases, Acre (AC) with 441 cases, and Roraima (RR) with 344 cases. From these, we sequenced full-length OROV genomes from 382 patients residing in 37 municipalities across these states, collected between 30th August 2022 and 25th February 2024.

Data exclusions	We undertook a thorough process to identify and merge duplicate entries in the line-list, which involved cross-checking records to eliminate redundancy and ensure each case was uniquely represented. Additionally, we reconciled discrepancies between the onset date and the notification date to correct any inconsistencies.
Replication	Due to time and funding limitations, we did not replicate the sequencing and real-time PCR data. However, to ensure reliable consensus sequences, we employed a strategy focused on achieving a high mean depth coverage. As a result, the newly generated genomes had a mean depth coverage of approximately 2000x, providing a high level of confidence in the accuracy of the sequences.
Randomization	In our study, convenience sampling was employed, and as such, no randomization was implemented. All eligible samples identified for sequencing were directed accordingly, without the application of random selection methods. Given that this is a descriptive epidemiological study, the absence of randomization does not significantly impact the validity of our results. The primary objective was to provide a comprehensive overview of the Oropouche virus (OROV) genomic landscape and its epidemiological distribution.
Blinding	We were blinded to sex and age during data collection and to symptoms and disease outcome during both data collection and analysis.

Reporting for specific materials, systems and methods

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

Clinical data

Policy information about [clinical studies](#)

All manuscripts should comply with the ICMJE [guidelines for publication of clinical research](#) and a completed [CONSORT checklist](#) must be included with all submissions.

Clinical trial registration	<i>Provide the trial registration number from ClinicalTrials.gov or an equivalent agency.</i>
Study protocol	<i>Note where the full trial protocol can be accessed OR if not available, explain why.</i>
Data collection	<i>Describe the settings and locales of data collection, noting the time periods of recruitment and data collection.</i>
Outcomes	<i>Describe how you pre-defined primary and secondary outcome measures and how you assessed these measures.</i>

Plants

Seed stocks	<i>Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.</i>
Novel plant genotypes	<i>Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.</i>
Authentication	<i>Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosaicism, off-target gene editing) were examined.</i>