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Short Communication

Genomic evidence of active circulation of *Orthobunyavirus* in Ecuador

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ABSTRACT

Background: Between 2023 and 2025, the largest Oropouche fever epidemic recorded in history unfolded across Brazil seeding cases throughout Latin America. In 2024, three cases of Oropouche fever were reported in Ecuador.
Methods: An Oropouche fever case detected in Bolívar Province in June 2024 was preliminarily diagnosed as Oropouche virus (OROV) through RT-qPCR and was further processed using next-generation sequencing.
Results: Segments L and S of this virus forms a monophyly with another sequence circulating in Ecuador in April 2024 (i.e. PQ863772.1 isolate Ecuador traveler), with an uncertain province origin. Both sequences differ from previous OROV Ecuadorian sequences detected in 2016 and from the OROV strain driving the 2023-2025 epidemic in Brazil.
Conclusions: *Orthobunyavirus oropoucheense* has an endemic circulation in Ecuador. Genomic surveillance of *Orthobunyavirus* in Ecuador and other regions should be actively pursued—independent of epidemics—to anticipate potential zoonotic outbreaks.
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Introduction

Orthobunyavirus oropoucheense is a single-stranded, negative-sense RNA virus species complex (Baltimore V) with three segments (L, M, and S), a member of the Simbu serogroup [1]. Five viruses are recognized as part of *O. oropoucheense*: Oropouche virus (OROV), Iquitos virus (IQTV), Madre de Dios virus (MDDV), Perdões virus (PEDV), and Pintupo virus (PINTV) [1]. OROV infection in humans causes Oropouche fever, an undifferentiated febrile illness endemic to Central and South America. Since its discovery in Trinidad and Tobago in 1955, it has caused significant human outbreaks, mainly, in Brazil and Peru [2]. OROV has also been isolated from different mammals, including sloths (*Bradypus tridactylus*), non-human primates (*Alouatta caraya, Sapajus apella*, etc.), and serologically detected in birds [2].

IQTV and MDDV were discovered as pathogens causing human outbreaks in Iquitos and Madre de Dios, Peru in 1999 and 2007, respectively. Moreover, MDDV and PEDV have been isolated from a capuchin monkey (*Cebus olivaceus*) in Venezuela in 2010 and a marmoset (*Callithrix penicillata*) in Brazil in 2012, respectively. The last member of the *O. oropoucheense* complex, PINTV, was isolated from *Bradypus variegatus* (sloth) and *Culicoides diabolicus*-complex

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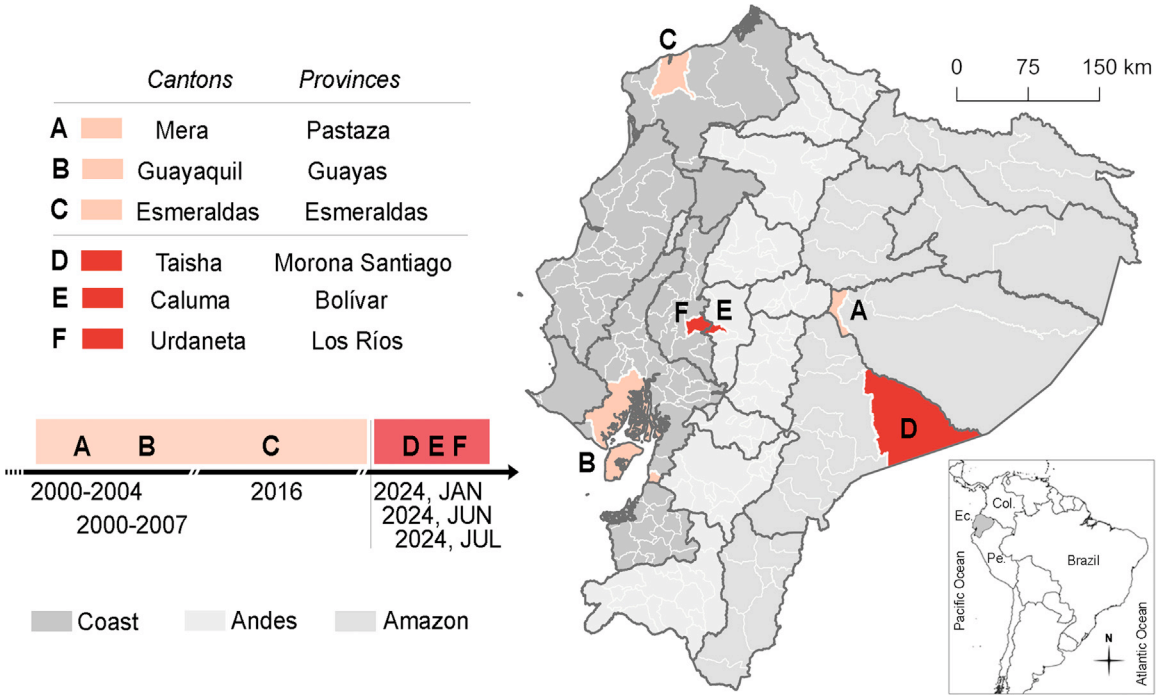

Figure 1. Oropouche fever cases diagnosed in Ecuador. Diagnoses of Oropouche fever in Ecuador were done using serological tests starting in the 2000s (A and B; orange). The first molecular detection of Oropouche virus occurred in Esmeraldas province in 2016 (C; orange) from a sample obtained from a febrile patient. Three more patients infected with the Oropouche virus were detected during 2024 (red) in the provinces of Morona Santiago (in January; D), Bolívar (in June; E), and Los Ríos (in July; F). Isolation of genomic sequences was possible for sample E from Caluma, Bolívar Province at the Andes region. In the inset: Ec. = Ecuador, Col. = Colombia, Pe. = Peru.

midges in Panama between the 70s and 80s. Up to 2025, genomic sequences for PINTV were unavailable [1,3].

In Ecuador, Oropouche fever due to OROV was first reported via immunoglobulin M seroconversion and immunofluorescence assay in one of 304 serum samples collected between 2001 and 2004 in the Amazon region, Pastaza Province [4] (Figure 1). A similar study detected further serological evidence of OROV in Guayaquil City, Guayas Province in the Coast region of the country between 2000 and 2007 [5]. In 2016, a febrile patient from the province of Esmeraldas was diagnosed as OROV-positive, with the molecular detection of the virus via quantitative reverse transcription–polymerase chain reaction (RT-qPCR). The patient never traveled outside the province. Further analysis of 258 stored serum samples at the same health center detected five more OROV-positive cases in 2016 from Esmeraldas [6] (Figure 1).

Starting October 2023 and throughout 2024, the more significant epidemic of OROV has been recorded in Latin America, with 16,239 cases for the end of that year [7], affecting countries in which OROV has never been detected, including Bolivia and Cuba [7]. As of the writing of this manuscript, the last Pan American Health Organization (PAHO) report in February 2025 for the Americas informed 3765 confirmed cases [7]. Strikingly, for the first time, deaths attributable to Oropouche fever plus evidence of potential vertical transmission were recorded during the epidemic [7]. Three Oropouche fever cases were reported in Ecuador in 2024 across different provinces. We recovered genomic segments from one of these cases and used phylogenetic inference to derive conclusions regarding the circulation of *Orthobunyavirus* in Ecuador.

Methods

Active surveillance of the disease due to Oropouche fever epidemic awareness has allowed the detection of cases in newer areas. In Ecuador, the National Institute for Research on Public Health (INSPI) detected OROV through RT-qPCR for the S segment in three dengue-negative cases examined in 2024. Ecuador has tree continental regions, the Coast at the west, the Andean region transversed by the homonym mountain ridge, and the Amazon at the east. As reported by the PAHO, one case was detected in Taisha, Morona Santiago at the Amazon (male, 45 years old, January 2024); the other in Caluma, Bolívar at the Andean region (male, 62 years old, June 2024); and one at Urdaneta, Los Ríos at the Coast region (female, 36 years old, July 2024). None of these patients have a history of traveling outside their province of residence [7] (Figure 1). The serum sample from Bolívar Province had a threshold cycle value less than 30 (threshold cycle = 29.45), which theoretically allows whole genome sequencing; accordingly, it was sent to the National Reference Center for Genomics, Sequencing, and Bioinformatics in INSPI for processing. No additional samples were submitted for genomic analyses.

After RNA extraction, we used the CovidSeq Test kit (Illumina, CA, USA), adapted with two pools of specific primers for library preparation, which allows the sequencing of the three OROV viral segments, following Naveca et al. [8] (Supplementary Table 1), because Ecuadorian cases potentially were exported from the Brazilian epidemic. Sequencing was performed on a MiSeq platform (Illumina, CA, USA). Segments L and S were available for further analysis. Despite multiple attempts, the M segment was never recovered using this experiment. We built phylogenetic trees of each genome using maximum parsimony and Bayesian inference models [8] (Supplementary Material).

Results and discussion

Only *Othobunyavirus* was recovered during library preparation (Supplementary Material). Preliminarily, segments L and S recovered were labeled as OROV and obtained with a depth of 9540× for L and 45,398× for S (Global Initiative on Sharing All Influenza

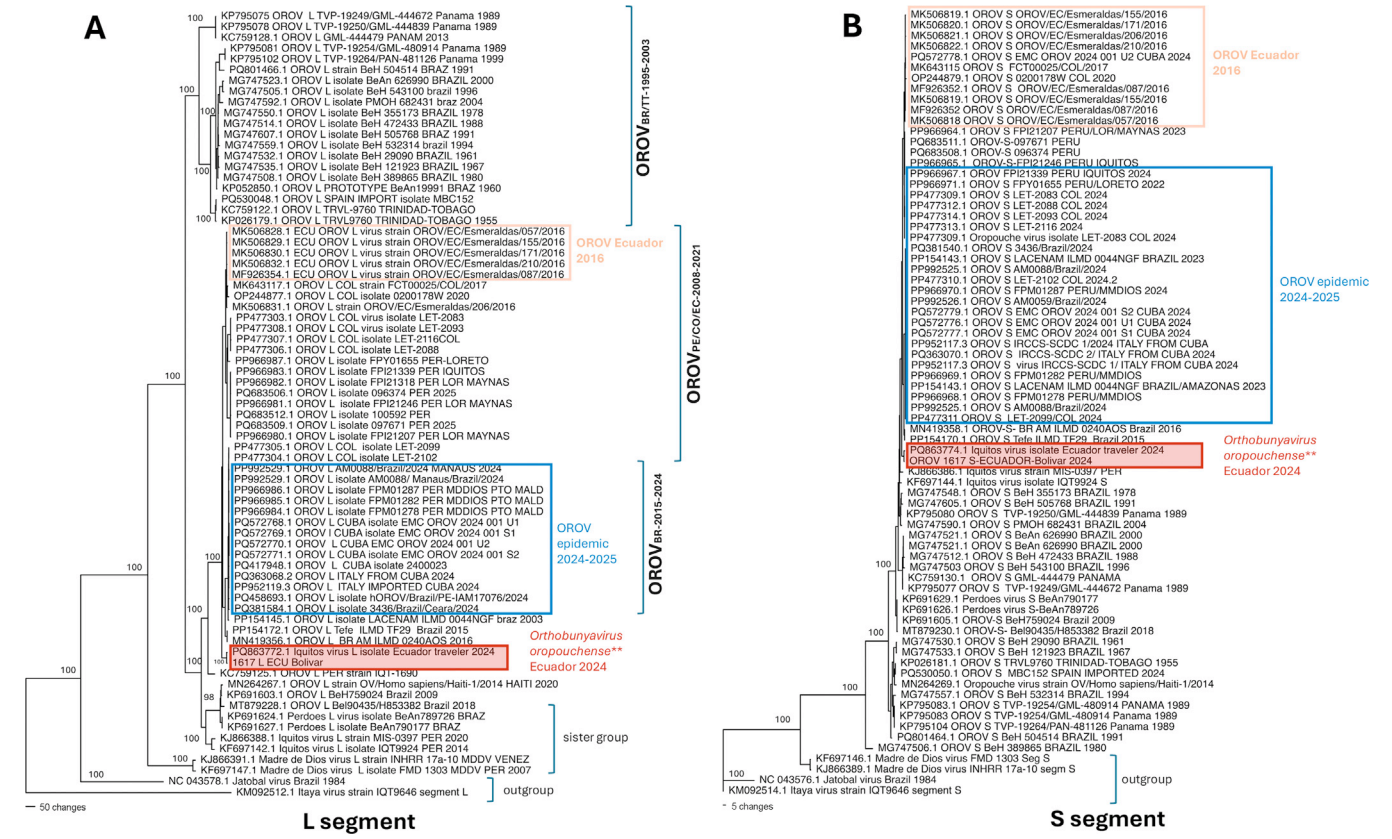

Figure 2. Phylogenetic tree of segments L and S of *Orthobunyavirus* in Latin America. Using a maximum parsimony framework, we developed phylogenetic trees with representative genomes of *Orthobunyavirus* from different outbreaks through time (n = 73). The sequence from Bolívar Province, Ecuador, clusters with PQ863772.1 Ecuador traveler 2024 sequence, which was isolated from a foreigner visiting different provinces of the country in 2024. Phylogenetic trees using Bayesian inference recovered the same topology as shown in the Supplementary Material. ***Reddish box, *Orthobunyavirus oropoucheense* monophyly detected in Ecuador in 2024. OROV = Oropouche virus.

Data, accession number: EPI_ISL_20052988; Supplementary Figure 1 and Material). Phylogenetic trees for both segments developed with different methodologies clustered the recovered genomes from Bolívar Province with PQ863772.1 isolate sequence, which was obtained from a traveler who visited many provinces of Ecuador during April 2024 [9] (Figure 2). The traveler case was initially diagnosed as Oropouche fever by OROV but further analysis suggested that the M segment clustered with the corresponding segment of IQTV. Accordingly, the authors labeled this sequence as IQTV and suggested its circulation in Ecuador. This case never visited the provinces where the other 2024 Ecuadorian cases have been reported [9].

Our trees show that the sequence from Bolívar Province (i.e. 1617 EC) potentially belongs to the same lineage of OROV circulating in Colombia, Peru, and Ecuador ($OROV_{PE/CO/EC-2008-2021}$ following [8]) but clusters in a monophyletic clade with the PQ863772.1 sequence (Figure 2 and Supplementary Figure 2). This finding has important implications regarding OROV in Ecuador. By sharing a common ancestor with the mentioned sequence, we demonstrate that the L and S segments belong to a different lineage to the OROV strain driving the current epidemic in Brazil ($OROV_{BR-2015-2024}$ following [8]; Figures 1 and 2, and Supplementary Figure 2), which implies an endemic *Orthobunyavirus* circulation.

Without the M segment of the virus, we face two scenarios. First, because we used standardized primers to detect OROV sequences that are currently circulating in South America, the absence of recovery suggests the presence of a potential reassortment. Second, even if the M segment is similar to that of the OROV strain of the Brazilian epidemic, the fact that the L and S segments cluster with the PQ863772.1 sequence suggests that both belong to a local circulating *Orthobunyavirus* (Figures 1 and 2). Nevertheless, Oropouche fever cases exported from the Brazilian epidemic can co-occur in Ecuador, as has been evidenced in Colombia [10] and, potentially, in Panama (Figure 2).

Our data also prompts us to suggest that the IQTV identity suggested for the traveler sequence should be reconsidered [9]. First, the Ecuador MF926354_2016 and the PQ863772.1 sequence, although closely related in the S and L segment trees, do not form a monophyletic group in S (see Panel A, highlighted in yellow in [9]). Moreover, Ecuador MF926354_2016 sequence and the PQ863772.1 sequence from the traveler case form a polytomy in segment L, with an unresolved relationship between them (Panel B, highlighted in yellow in [9]). Second, the phylogenetic tree of the PQ863772.1 Ecuador traveler 2024 sequence lacks genomes from IQTV for comparison in the L and S segments and is named as such because of its sister location in the tree with the M segment [9]. Our results in segments L and S (Figure 2 and Supplementary Figure 2) demonstrate that the PQ863772.1 sequence clusters with the 1617 Bolívar sequence within the monophyletic Oropouche group, whereas IQTV is basal to OROV and distant from both sequences (Figure 2).

Many of the interpretations derived from the L and S segments recovered here are constrained to current published data on *Orthobunyavirus* in Ecuador, which are scarce. As such, the hypotheses presented should be further explored in future studies. The lack of the M segment in the current isolate renders our conclusions exploratory. However, our study allows the confirmation of

Orthobunyavirus in Ecuador as a strain unrelated to the Brazilian OROV epidemic and, therefore, genomic evidence of endemic virus circulation.

Without dedicated arbovirus genomic surveillance guidelines in the country and due to the lack of systematic *Orthobunyavirus* surveillance, the evidence of active circulation of potential reassortants independent of OROV epidemic strains should highlight the importance of including Ecuadorian data in integrative surveillance programs. Genomic surveillance for arbovirus and other pathogens should be routinely pursued, independent of epidemics, to actively prevent and control unpleasant outbreaks.

Declaration of competing interest

The authors have no competing interests to declare.

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Ethical approval statement

Ethical approval was waived because the study was conducted as part of public health surveillance by official authorities.

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Author contributions

D.R.A., J.C.N., and A.C.M. conceptualized the study. J.C. obtained funding. D.G.P., G.E.G., C.L.C., B.F.F., and G.G.D. performed the laboratory work. D.R.A., J.C.N., G.E.G., C.L.C., and A.C.M. analyzed the data. D.R.A., J.C.N., D.G.P., G.L.W., J.C., and A.C.M. drafted the manuscript. D.R.A., J.C.N., D.G.P., G.L.W., J.C., and A.C.M. edited the manuscript. All authors read and approved the final version of the manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ijid.2025.108000.

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