

The evolutionary dynamics of Oropouche Virus (OROV) in South America

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Supplementary Figures

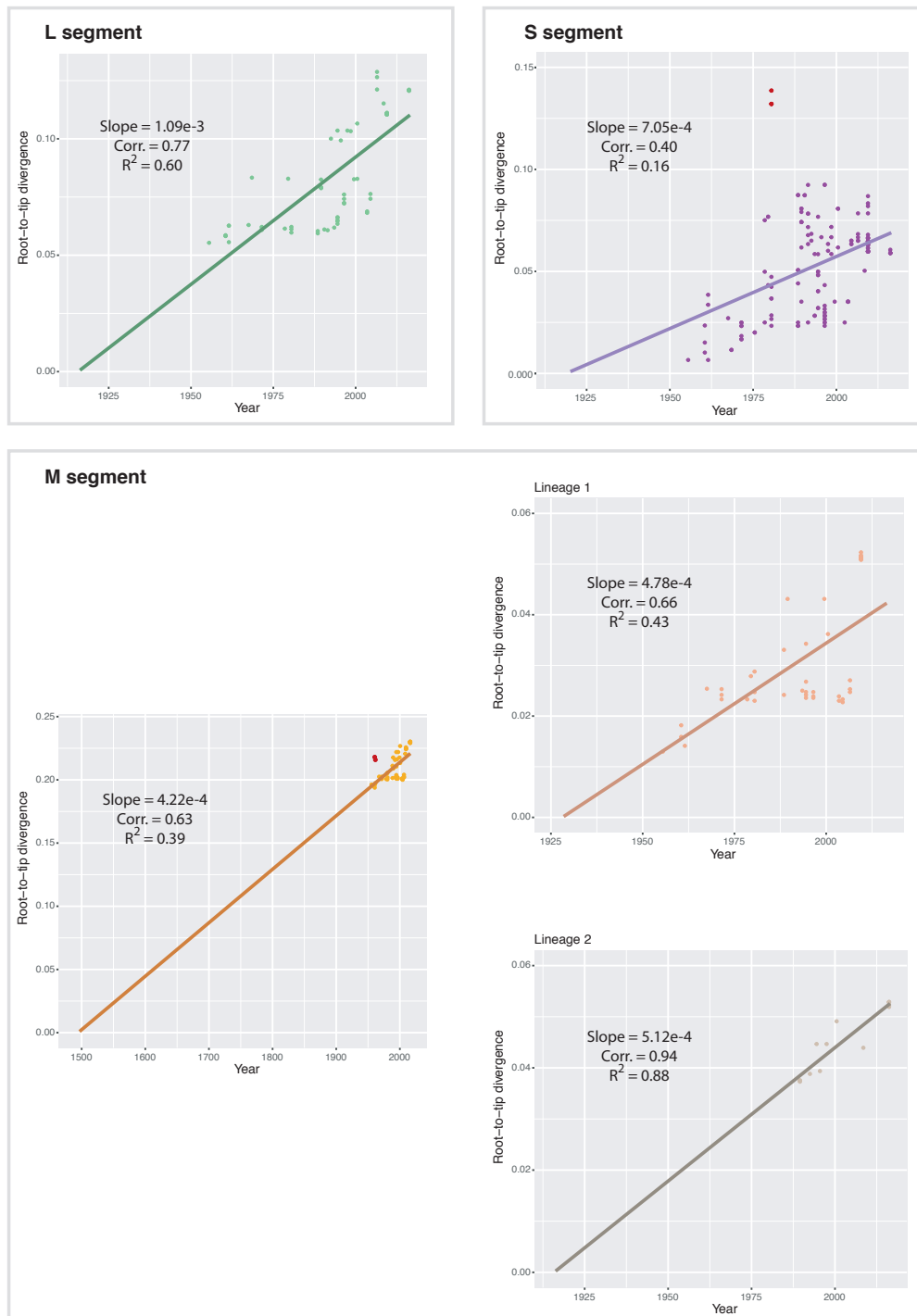


Figure S1 (above). Correlation plots between tip-to-root distances versus sampling estimated in TempEst for the three OROV genome segments. Outliers (determined through visual inspection) are highlighted in red. Sequences corresponding to the points highlighted in red (sequences PA01 and PA03 for the M segment; sequences AM01, AM03 and BeH389865 for the S segment) were excluded as outliers prior to the estimation of the individual plots for each M lineage and all further molecular clock analyses. Regression slope (interpreted as rough estimates of the mean evolutionary rate), correlation coefficients and R^2 values are shown for each plot.

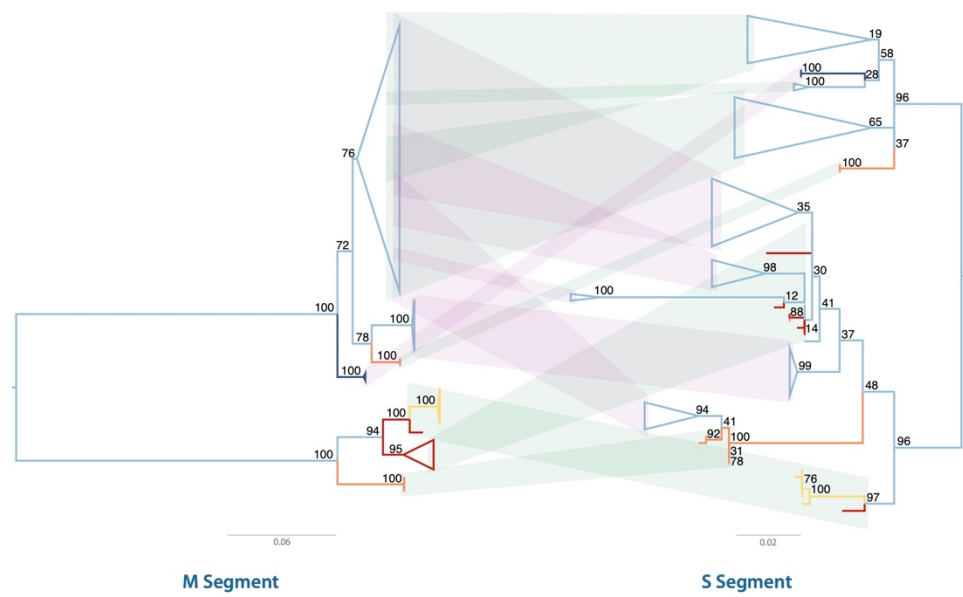
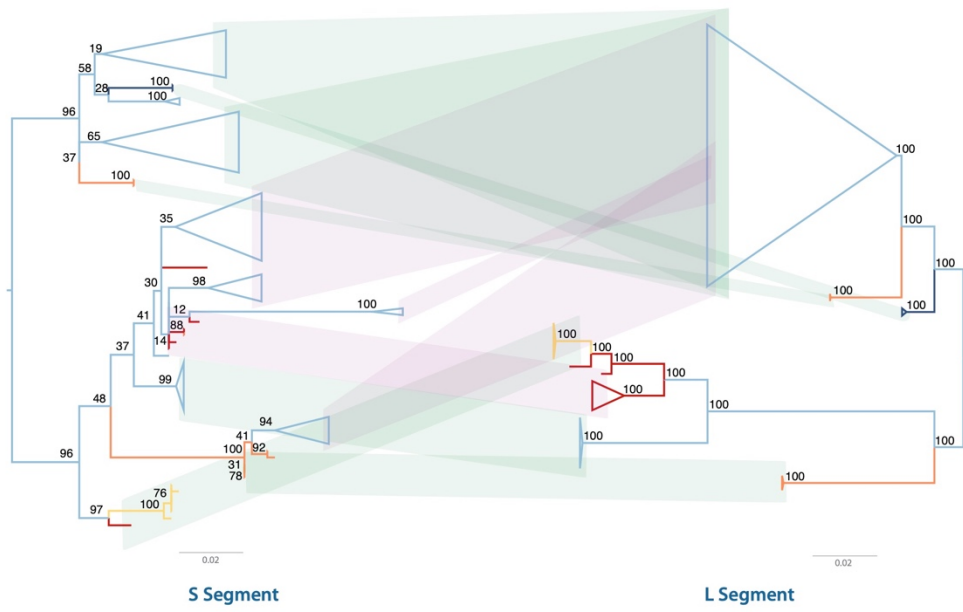
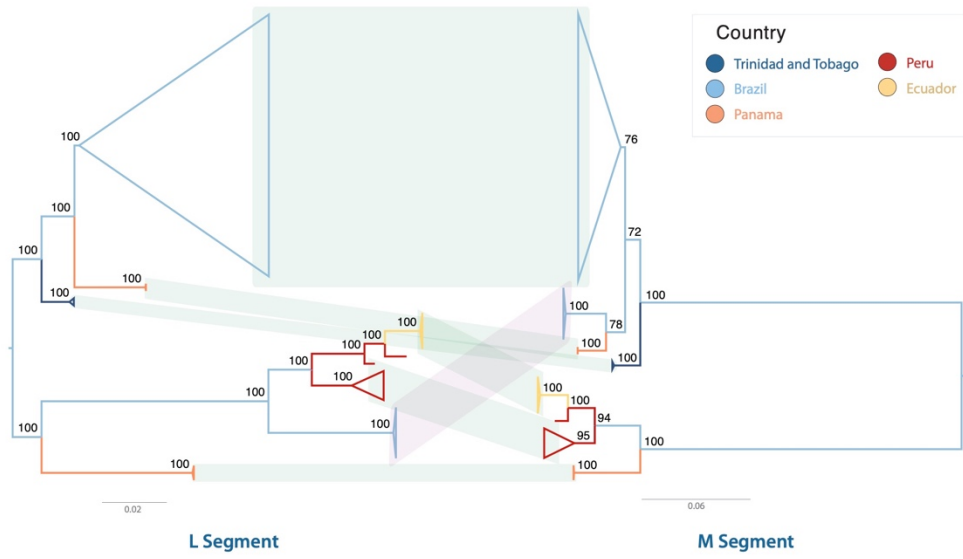


Figure S2 (above). Pairwise comparison of maximum-likelihood phylogenetic trees of the three OROV genome segments suggest widespread reassortment. The L and S trees are midpoint rooted, and the M tree was re-rooted to maximise its topological congruency with its closest match, the L tree. Node support representing 100 bootstraps is shown on the tree nodes, where important monophyletic clades are collapsed in all trees (most collapsed nodes have high node support). All branches are coloured according to the most probable location using on a parsimony approach; colours match those used Fig. 1. Topologically similar clusters are highlighted in green, while potentially reassortant clusters are highlighted in purple.

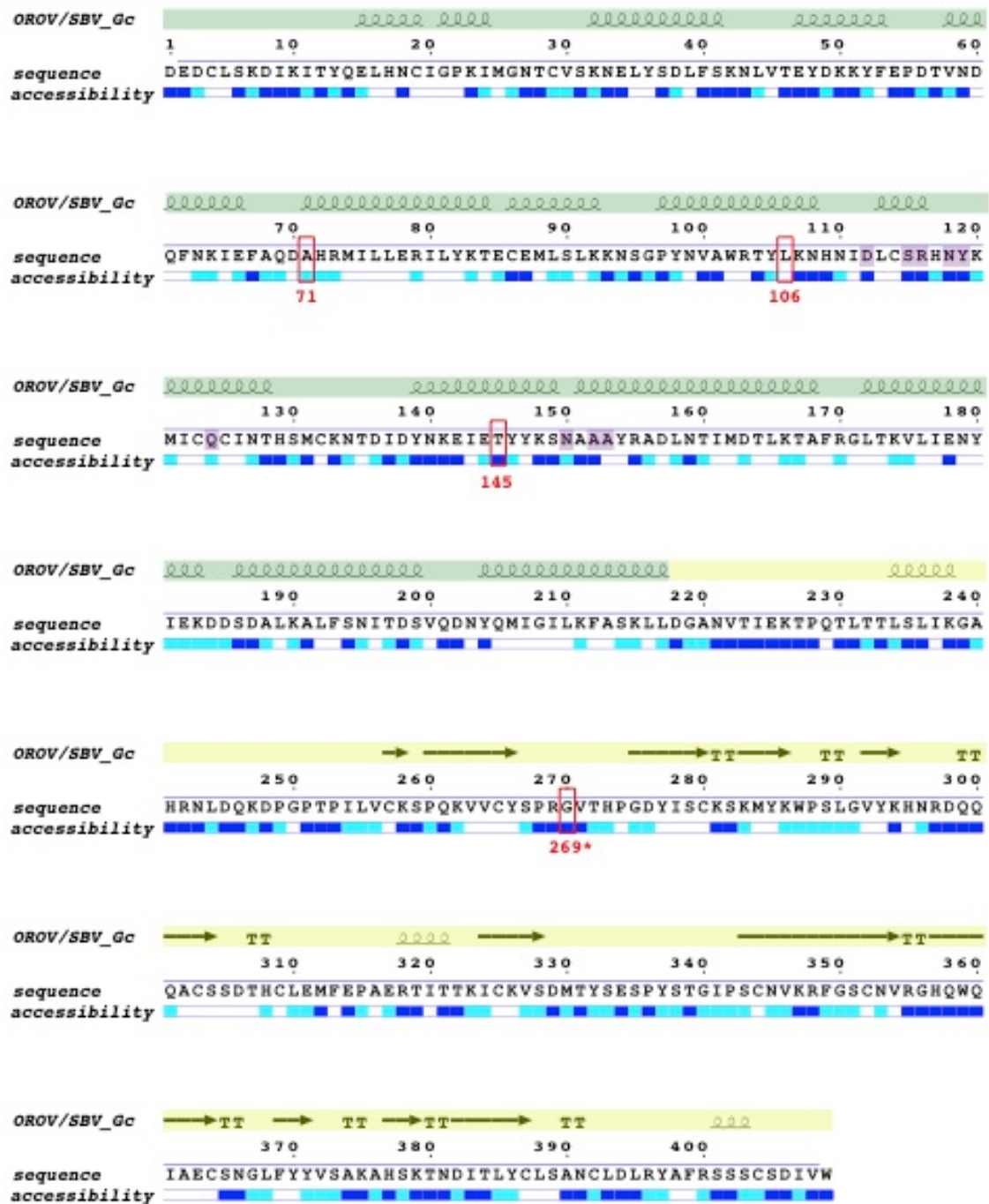


Figure S3. Relative residue accessibility for the OROV/SBV composite model of the Gc protein, based on its atomic structure and amino acid sequence, using the ESPrpt server. Accessibility is scored as buried residues (white), intermediate residues (cyan) and exposed residues (blue). Red boxes show sites identified to be evolving under positive selection, with

the red numbering highlighting the equivalent numbering at the OROV Gc sequence. An asterisk represents the site mapped onto the SBV component of the composite model, hence the numbering discrepancy. Secondary structures are shown above the sequence and highlighted based on the virus from which each component of the sequence was used to construct the composite model (green for OROV, yellow for SBV). Residues that directly play a role in the trimerisation interface of the head spike architecture, as identified in the PDBePISA server, are highlighted in purple.

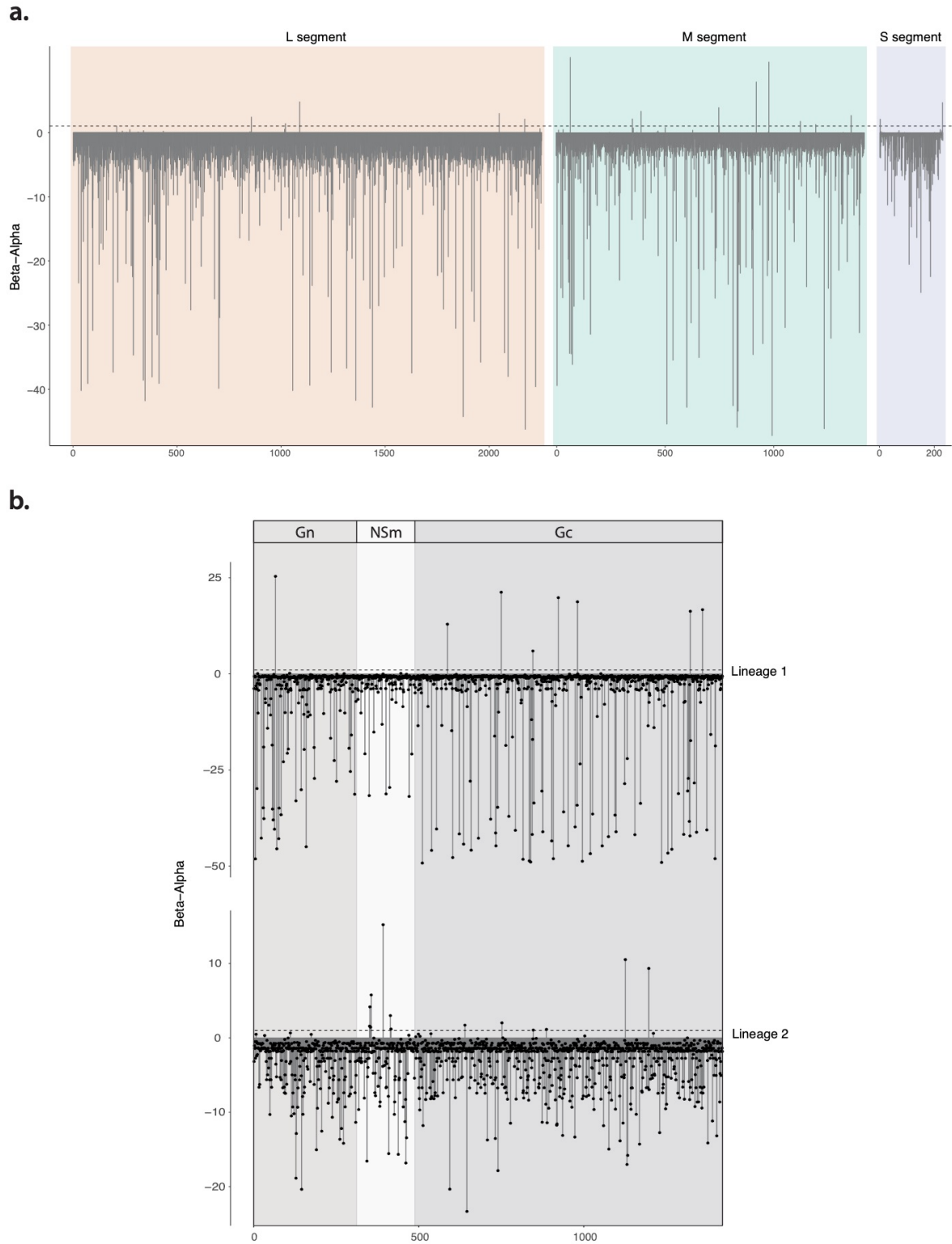


Figure S4. Site-wise differences between the non-synonymous (β) and synonymous (α) substitution rates in the OROV genome. The three genome segments (a) show a limited number of sites that evolve at a higher non-synonymous substitution rate (>1 , marked by the

dotted line), suggestive of adaptive selection. Within the M segment (b), different codons (mostly on different protein reading frames) show evidence for adaptive selection.

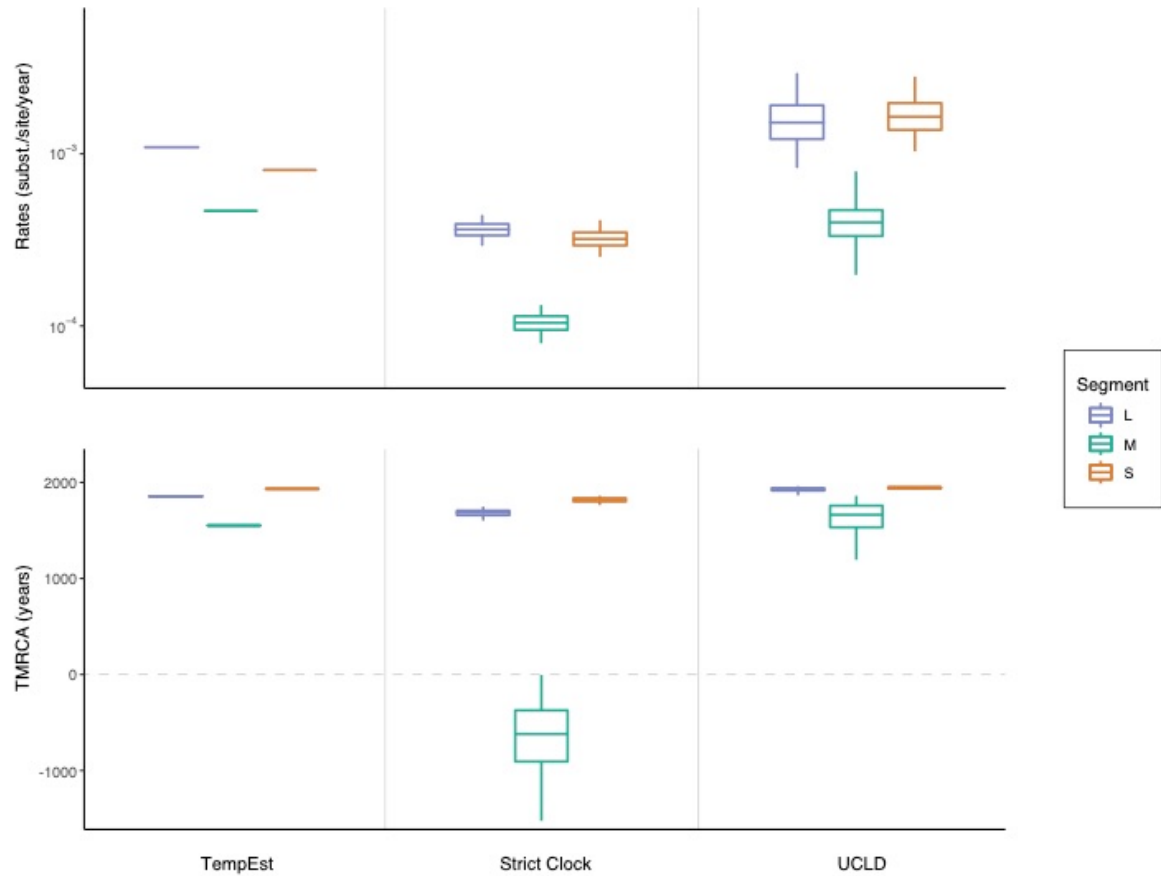


Figure S5. Comparison of evolutionary rate estimate (upper panel) and times to the most recent common ancestor estimates (lower panel) for the three OROV genome segments estimated through different methods and clock models: a root-to-tip regression slope (TempEst), and a phylogenetically co-inferred rate using a strict clock model and a relaxed clock model (UCLD).

Supplementary Tables

Table S1. Summary of all isolates included in this study, with accession numbers for each genome segment (including six new sequences from Ecuador).

ID	Host	City	Province/State	Country	Year	S	M	L
AC02	Homo sapiens	Xapuri	Acre	Brazil	1996	MG747503	MG747504	MG747505
AM01	Homo sapiens	Manaus	Amazonas (BR)	Brazil	1980	MG747506	MG747507	MG747508
AM03	Homo sapiens	Manaus	Amazonas (BR)	Brazil	1980	MG747509	MG747510	MG747511
MA02	Homo sapiens	Porto Franco	Maranhao	Brazil	1988	MG747512	MG747513	MG747514
MA03	Homo sapiens	Porto Franco	Maranhao	Brazil	1988	MG747515	MG747516	MG747517
MA06	Homo sapiens	Barra da Corda	Maranhao	Brazil	1993	MG747518	MG747519	MG747520
MG01	Callithrix sp.	Arino	Minas Gerais	Brazil	2000	MG747521	MG747522	MG747523
01-812-98	Homo sapiens	Iquitos	Loreto	Peru	1998	AF164553	---	---
D-057/057v	Homo sapiens	Esmeraldas	Esmeraldas	Ecuador	2016	MK506818	MK506823	MK506828
D-087/087	Homo sapiens	Esmeraldas	Esmeraldas	Ecuador	2016	MF926352	MF926353	MF926354
D-155/155v	Homo sapiens	Esmeraldas	Esmeraldas	Ecuador	2016	MK506819	MK506824	MK506829
D-171/171v	Homo sapiens	Esmeraldas	Esmeraldas	Ecuador	2016	MK506820	MK506825	MK506830
D-206/206v	Homo sapiens	Esmeraldas	Esmeraldas	Ecuador	2016	MK506821	MK506826	MK506831
D-210/210v	Homo sapiens	Esmeraldas	Esmeraldas	Ecuador	2016	MK506822	MK506827	MK506832
BeAn19991	Bradypus trydactylus	Sao Miguel do Guama	Para	Brazil	1960	KP052852	KP052851	KP052850
BeAn19991	Bradypus trydactylus	Sao Miguel do Guama	Para	Brazil	1960	AF164532	AF441119	KP052850
BeAn206119	Bradypus trydactylus	Maracana	Para	Brazil	1971	HQ830378	---	---
BeAn208402	Bradypus trydactylus	Maracana	Para	Brazil	1971	HQ830379	---	---
BeAn208819	Bradypus trydactylus	Maracana	Para	Brazil	1971	HQ830380	---	---
BeAn208823	Bradypus trydactylus	Maracana	Para	Brazil	1971	AY993912	---	---
BeAn626990	Callithrix sp.	Arino	Minas Gerais	Brazil	2000	AY117135	---	---
BeAr136921	Culex quinquefasciatus	Belem	Para	Brazil	1968	HM470111	---	---
BeAr366927	Culicoides paraensis	Belem	Para	Brazil	1979	HQ830448	---	---
BeAr473358	Culicoides paraensis	Porto Franco	Maranhao	Brazil	1988	AF164539	---	---
BeH244576	Homo sapiens	Belem	Para	Brazil	2009	HQ830444	---	---
BeH271078	Homo sapiens	Santarem	Para	Brazil	1975	HQ830445	---	---
BeH271815	Homo sapiens	Santarem	Para	Brazil	1975	AF164533	---	---
BeH29086	Homo sapiens	Belem	Para	Brazil	1961	HM470108	---	---
BeH355186	Homo sapiens	Tome-Acu	Para	Brazil	1978	HQ830446	---	---
BeH356898	Homo sapiens	Belem	Para	Brazil	1978	HQ830447	---	---
BeH366781	Homo sapiens	Belem	Para	Brazil	1979	HQ830449	---	---
BeH379693	Homo sapiens	Castanhal	Para	Brazil	1980	AF164534	---	---

Table S1. (Continued)

ID	Host	City	Province/State	Country	Year	S	M	L
BeH381114	Homo sapiens	Belem	Para	Brazil	1980	AF164535	---	---
BeH384192	Homo sapiens	Portel	Para	Brazil	1980	HQ830450	---	---
BeH384193	Homo sapiens	Portel	Para	Brazil	1980	HQ830451	---	---
BeH385591	Homo sapiens	Belem	Para	Brazil	1980	HQ830452	---	---
BeH389865	Homo sapiens	Manaus	Amazonas (BR)	Brazil	1980	HQ830453	---	---
BeH390233	Homo sapiens	Manaus	Amazonas (BR)	Brazil	1980	AF164536	---	---
BeH472200	Homo sapiens	Porto Franco	Maranhao	Brazil	1988	AF164537	---	---
BeH472204	Homo sapiens	Porto Franco	Maranhao	Brazil	1988	AF164538	---	---
BeH472433	Homo sapiens	Porto Franco	Maranhao	Brazil	1988	HQ830455	---	---
BeH472435	Homo sapiens	Porto Franco	Maranhao	Brazil	1988	HQ830456	---	---
BeH475248	Homo sapiens	Tucuruí	Para	Brazil	1988	AF164540	---	---
BeH498913	Homo sapiens	Machadinho D'Oeste	Rondonia	Brazil	1990	HQ830457	---	---
BeH504514	Homo sapiens	Americano	Para	Brazil	1991	AF164541	---	---
BeH505442	Homo sapiens	Ouro Preto do Oeste	Rondonia	Brazil	1991	AF164542	---	---
BeH505663	Homo sapiens	Ariquemes	Rondonia	Brazil	1991	AF164543	---	---
BeH505764	Homo sapiens	Ariquemes	Rondonia	Brazil	1991	HQ830458	---	---
BeH505768	Homo sapiens	Ariquemes	Rondonia	Brazil	1991	HQ830459	---	---
BeH505805	Homo sapiens	Ariquemes	Rondonia	Brazil	1991	HQ830460	---	---
BeH521086	Homo sapiens	Barra da Corda	Maranhao	Brazil	1993	AY704559	---	---
BeH532314	Homo sapiens	Serra Pelada	Para	Brazil	1994	HQ830461	---	---
BeH532422	Homo sapiens	Serra Pelada	Para	Brazil	1994	HQ830462	---	---
BeH532490	Homo sapiens	Serra Pelada	Para	Brazil	1994	HQ830463	---	---
BeH532500	Homo sapiens	Serra Pelada	Para	Brazil	1994	HQ830464	---	---
BeH541863	Homo sapiens	Altamira	Para	Brazil	1996	AF164544	---	---
BeH543033	Homo sapiens	Oriximina	Para	Brazil	1996	AF164545	---	---
BeH543087	Homo sapiens	Xapuri	Acre	Brazil	1996	AF164547	---	---
BeH543091	Homo sapiens	Xapuri	Acre	Brazil	1996	HQ830465	---	---
BeH543100	Homo sapiens	Xapuri	Acre	Brazil	1996	HQ830466	---	---
BeH543618	Homo sapiens	Oriximina	Para	Brazil	1996	AF164548	---	---
BeH543639	Homo sapiens	Oriximina	Para	Brazil	1996	AY704562	---	---
BeH543733	Homo sapiens	Oriximina	Para	Brazil	1996	AY704560	---	---
BeH543760	Homo sapiens	Oriximina	Para	Brazil	1996	AY704568	---	---
BeH543857	Homo sapiens	Oriximina	Para	Brazil	1996	AY704566	---	---
BeH543880	Homo sapiens	Oriximina	Para	Brazil	1996	AY704565	---	---
BeH544552	Homo sapiens	Altamira	Para	Brazil	1996	AF164546	---	---
BeH622544	Homo sapiens	Parana	Tocantins	Brazil	2002	EF467368	---	---
BeH669314	Homo sapiens	Parauapebas	Para	Brazil	2003	EF467370	---	---
BeH669315	Homo sapiens	Parauapebas	Para	Brazil	2003	EF467369	---	---

Table S1. (Continued)

ID	Host	City	Province/State	Country	Year	S	M	L
BeH682426	Homo sapiens	Porto de Moz	Para	Brazil	2004	EF467371	---	---
BeH682431	Homo sapiens	Porto de Moz	Para	Brazil	2004	EF467372	---	---
BeH706890	Homo sapiens	Igarape Acu	Para	Brazil	2009	HQ830473	---	---
BeH706893	Homo sapiens	Igarape Acu	Para	Brazil	2009	HQ830474	---	---
BeH707157	Homo sapiens	Maracana	Para	Brazil	2006	HQ830476	---	---
BeH708139	Homo sapiens	Magalhaes Barata	Para	Brazil	2006	HQ830475	---	---
BeH708717	Homo sapiens	Maracana	Para	Brazil	2009	HQ830477	---	---
BeH758669	Homo sapiens	Mazagao	Amapa	Brazil	2009	HQ830479	---	---
BeH758687	Homo sapiens	Mazagao	Amapa	Brazil	2009	HQ830478	---	---
BeH759018	Homo sapiens	Mazagao	Amapa	Brazil	2009	HQ830486	---	---
BeH759021	Homo sapiens	Mazagao	Amapa	Brazil	2009	KP691608	KP691607	KP691606
BeH759022	Homo sapiens	Mazagao	Amapa	Brazil	2009	KP691611	KP691610	KP691609
BeH759023	Homo sapiens	Mazagao	Amapa	Brazil	2009	HQ830487	---	---
BeH759024	Homo sapiens	Mazagao	Amapa	Brazil	2009	KP691605	KP691604	KP691603
BeH759025	Homo sapiens	Mazagao	Amapa	Brazil	2009	KP691614	KP691613	KP691612
BeH759038	Homo sapiens	Mazagao	Amapa	Brazil	2009	HQ830484	---	---
BeH759040	Homo sapiens	Mazagao	Amapa	Brazil	2009	KP691617	KP691616	KP691615
BeH759041	Homo sapiens	Mazagao	Amapa	Brazil	2009	HQ830488	---	---
BeH759042	Homo sapiens	Mazagao	Amapa	Brazil	2009	HQ830489	---	---
BeH759043	Homo sapiens	Mazagao	Amapa	Brazil	2009	HQ830490	---	---
BeH759044	Homo sapiens	Mazagao	Amapa	Brazil	2009	HQ830491	---	---
BeH759146	Homo sapiens	Mazagao	Amapa	Brazil	2009	KP691632	---	---
BeH759483	Homo sapiens	Mazagao	Amapa	Brazil	2009	HQ830492	---	---
BeH759525	Homo sapiens	Mazagao	Amapa	Brazil	2009	HQ830480	---	---
BeH759529	Homo sapiens	Mazagao	Amapa	Brazil	2009	KP691620	KP691619	KP691618
BeH759531	Homo sapiens	Mazagao	Amapa	Brazil	2009	HQ830482	---	---
BeH759541	Homo sapiens	Mazagao	Amapa	Brazil	2009	HQ830481	---	---
BeH759558	Homo sapiens	Mazagao	Amapa	Brazil	2009	HQ830483	---	---
BeH759562	Homo sapiens	Mazagao	Amapa	Brazil	2009	HQ830485	---	---
BeH759620	Homo sapiens	Mazagao	Amapa	Brazil	2009	KP691623	KP691622	KP691621
DEI216	Homo sapiens	Iquitos	Loreto	Peru	1992	KP795074	KP795073	KP795072
GML444477	Homo sapiens	Chame	Panama	Panama	1989	AF164555	---	---
GML444479	NA	NA	Panama	Panama	1989	KC759130	KC759129	KC759128
GML444672	Homo sapiens	San Miguelito	Panama	Panama	1989	KP795077	KP795076	KP795075
GML444839	Homo sapiens	Bejuco	Panama	Panama	1989	KP795080	KP795079	KP795078
GML444911	Homo sapiens	Chame	Panama	Panama	1989	AF164556	---	---
GML445252	Homo sapiens	San Miguelito	Panama	Panama	1989	AF164557	---	---

Table S1. (Continued)

ID	Host	City	Province/State	Country	Year	S	M	L
GML450093	Homo sapiens	Chilibre	Panama	Panama	1989	AF164558	---	---
GML480914	Homo sapiens	NA	Panama	Panama	1989	KP795083	KP795082	KP795081
IQE7894	Homo sapiens	Iquitos	Loreto	Peru	2008	KP795086	KP795085	KP795084
IQT1690	Homo sapiens	Iquitos	Loreto	Peru	1992	KC759127	KP795088	KC759125
IQT1690*	Homo sapiens	Iquitos	Loreto	Peru	1995	KP795089	---	KP795087
IQT4083	Homo sapiens	Iquitos	Loreto	Peru	1997	KP795092	KP795091	KP795090
IQT4083	Homo sapiens	Iquitos	Loreto	Peru	1997	AF164552	---	---
IQT7085	Homo sapiens	Iquitos	Loreto	Peru	1998	KP795095	---	KP795093
IQT7085	Homo sapiens	Iquitos	Loreto	Peru	1998	AF164554	---	---
MD023	Homo sapiens	Madre de Dios	Madre de Dios	Peru	1993	AF164550	KP795097	KP795096
OBS9478	Homo sapiens	Bagua	Amazonas (PE)	Peru	2000	KP795101	KP795100	KP795099
PAN481126	Homo sapiens	Colon	Colon	Panama	1999	KP795104	KP795103	KP795102
TRVL9760	Homo sapiens	Vega de Oropouche	Trinidad	Trinidad	1955	KP026181	KP026180	KP026179
PA01	Ochlerotatus serratus	Ipixuna	Para	Brazil	1960	MG747524	MG7475215	MG747526
PA03	Homo sapiens	Belem	Para	Brazil	1961	MG747527	MG747528	MG747529
PA04	Homo sapiens	Belem	Para	Brazil	1961	MG747530	MG747531	MG747532
PA05	Homo sapiens	Braganca	Para	Brazil	1967	MG747533	MG747534	MG747535
PA06	Culex quinquefasciatus	Belem	Para	Brazil	1968	MG747536	MG747537	MG747538
PA07	Bradypus trydactylus	Maracana	Para	Brazil	1971	MG747539	MG747540	MG747541
PA08	Bradypus trydactylus	Maracana	Para	Brazil	1971	MG747542	MG747543	MG747544
PA09	Bradypus trydactylus	Maracana	Para	Brazil	1971	MG747545	MG747546	MG747547
PA14	Homo sapiens	Ananindeua	Para	Brazil	1978	MG747548	MG747549	MG747550
PA17	Culicoides paraensis	Belem	Para	Brazil	1979	MG747551	MG747552	MG747553
PA22	Homo sapiens	Belem	Para	Brazil	1980	MG747554	MG747555	MG747556
PA25	Homo sapiens	Serra Pelada	Para	Brazil	1994	MG747557	MG747558	MG747559
PA26	Homo sapiens	Serra Pelada	Para	Brazil	1994	MG747560	MG747561	MG747562
PA27	Homo sapiens	Serra Pelada	Para	Brazil	1994	MG747563	MG747564	MG747565
PA28	Homo sapiens	Serra Pelada	Para	Brazil	1994	MG747566	MG747567	MG747568
PA29	Homo sapiens	Altamira	Para	Brazil	1994	MG747569	MG747570	MG747571
PA34	Homo sapiens	Oriximina	Para	Brazil	1996	MG747572	MG747573	MG747574
PA38	Homo sapiens	Oriximina	Para	Brazil	1996	MG747575	MG747576	MG747577
PA39	Homo sapiens	Oriximina	Para	Brazil	1996	MG747578	MG747579	MG747580
PA41	Homo sapiens	Parauapebas	Para	Brazil	2003	MG747581	MG747582	MG747583
PA42	Homo sapiens	Parauapebas	Para	Brazil	2003	MG747584	MG747585	MG747586
PA43	Homo sapiens	Porto de Moz	Para	Brazil	2004	MG747587	MG747588	MG747589

Table S1. (Continued)

ID	Host	City	Province/State	Country	Year	S	M	L
PA43	Homo sapiens	Porto de Moz	Para	Brazil	2004	MG747587	MG747588	MG747589
PA44	Homo sapiens	Porto de Moz	Para	Brazil	2004	MG747590	MG747591	MG747592
PA47	Homo sapiens	Magalhaes Barata	Para	Brazil	2006	MG747593	MG747594	MG747595
PA49	Homo sapiens	Magalhaes Barata	Para	Brazil	2006	MG747596	MG747597	MG747598
PA50	Homo sapiens	Maracana	Para	Brazil	2006	MG747599	MG747600	MG747601
RO01	Homo sapiens	Machadinho D'Oeste	Rondonia	Brazil	1990	MG747602	MG747603	MG747604
RO05	Homo sapiens	Ariquemes	Rondonia	Brazil	1991	MG747605	MG747606	MG747607
Trinidad1961	Homo sapiens	Vega de Oropouche	Trinidad	Trinidad	1961	---	MF620128	MF620129

*June 1995 sequence excluded from analysis.

Table S2. Links to the data exploration tool Microreact for the three OROV genome segments analysed in this study.

OROV segment	Microreact link
L	https://microreact.org/project/OROV_America_L?lu=y
M	https://microreact.org/project/OROV_America_M?lu=y
S	https://microreact.org/project/OROV_America_S?lu=y

Table S3. Allele frequencies for codons under adaptive selection in the OROV genome.

Protein	Codon	Identity	Frequency
Gn	66	K	0.444
		R	0.333
		A	0.190
		T	0.032
NSm	86	R	0.651
		N	0.206
		S	0.143
Gc	269	D	0.656
		G	0.344
	442	T	0.734
		S	0.188
		I	0.078