Amplification and sequencing of Lassa virus genome fragments. The 20-µl assay (QIAGEN OneStep RT-PCR Kit) contained 2 µl RNA, 0.5 µM forward and reverse primers in various combinations, 0.4 mM dNTP, 1x RT-PCR buffer, and 0.8 µl enzyme mix. The reaction was performed in a Primus25advanced thermocycler (PeqLab, Erlangen, Germany) using the following temperature profile: 50°C for 30 min, 95°C for 15 min followed by 45 cycles of 95°C for 20 s, 50–55°C for 20 s and 72°C for 1 min. PCR fragments were purified and sequenced on both strands using the PCR primers. No specific reaction conditions were applied for amplification and sequencing the intergenic region.

Old World arenavirus consensus primers used for PCR and sequencing:

S RNA primers

OWS0001-fwd GCGCACCGGGGATCCTAGGC
OWS0330-rev TAGTGATGGGWGTTGTTCTYTGWGCA
OWS0700-fwd TGATTATTCAGAAYACAWCCTGGGA
OWS0730-rev TGATCYTCCCAGGWTGTRTTCTGAAT
OWS0980-fwd CATGATGARGARTTCTGTGACATG
OWS1000-rev AGCATGTCACAGAAYTCYTCATCATG
OWS1210-fwd AATGGNTCATACCTAAATGARAC
OWS1240-rev TGGGTYTCATTTAGGTATGANCCATT
OWS1400-fwd TCAAAATWCCAACACATAGRCACAT
OWS1430-rev CCTWYTATGTGYCTATGTGTTGG
OWS1900-fwd GAGTCAAGRAGYTTTCTGATRTCATC
OWS1930-rev GATGACATCAGAAARCTYCTDGA
OWS2120-fwd GGTCTCCCTTCAATGTCMATCCA
OWS2150-rev CCAAATGCTAANACNTGGATGGACAT
OWS2165A-fwd TCTTCAGGTCTCCCTTCWATGTCNATCCANGT
OWS2165B-fwd TCTTCAGGTCTCCCTTCWATGTCNATCCA
OWS2170-fwd CTCCCTTCWATGTCNATCCANGT
OWS2330-fwd GATGTTCTWGATGCWATGTAWGGCCA
OWS2380A-fwd GATGTYCTTGATGCWATGTATGGCCANCC
OWS2380B-fwd GATGTYCTTGATGCWATGTATGGCCA
OWS2410-rev CCAGGKGAAAGRAACCCTTATGA
OWS2770-fwd CTTGGCATKGTCCCAAAYTGRTTGTT
OWS2800-rev AACAATCARTTTGGGACMATGCCAAG
OWS2805-fwd GTCAGGCTTGGCATTGTCCCAAACTGRTTRTT
OWS2810-fwd CTTGGCATTGTCCCAAACTGRTTRTT
OWS2840A-rev AAYAAYCAGTTTGGGACNATGCCAAG
OWS2840B-rev AAYAAYCAGTTTGGGACNATGCC
OWS3160A-fwd TGAACATTGGCAACYTCAGAGAAGTC
OWS3160B-fwd TGAACATTGCTGACYTCAGAGAAGTC
OWS3200-rev CATGGGCTKGACTTCTCTGARGT
OWS3200-rev CATGGGCTKGACTTCTCTGARGT
OWS3400A-rev CGCACAGTGGATCCTAGGCTATTKGATTGCGC
OWS3400-rev GCGCACAGTGGATCCTAGGC

L RNA primers

OWL1800-fwd TGTTCATTYTATGCAGATCCTAAAAG
OWL1830-rev TAYCTTTTAGGATCTGCATARAATGA
OWL2250-fwd ACAGATCAGATWAARTGYTTTGAGAAGT
OWL2270-rev ACTTCTCAAARCAYTTWATCTGATCTGT
OWL2570-fwd AGCAACAAAAGTGTKGTTGTGAAYAA
OWL2590-rev TTRTTCACAACMACACTTTTGTTGCT
OWL3359D_Y-fwd AGAATCAGTGAAAGGGAAAGCAAYTC
OWL3359G_Y-fwd AGAATTAGTGAAAGGGAGAGTAAYTC
OWL3754A_R-rev CACATCATTGGTCCCCATTTACTATGRTC
OWL3754D_R-rev CACATCATTGGTCCCCATTTACTGTGRTC
OWL4540-fwd GATGTTARAGAYTGGGTNGATGG
OWL4560-rev CCATCNACCCARTCTYTAACATC
OWL5020-fwd TGCARGACACTRGGTAGCAARTGTGT
OWL5330-fwd CCATGTGATTATTTCCCHATRAAGCC
OWL5360-rev GGCTTYATDGGGAAATAATCACATGG
OWL540-fwd TTTGAAGAATCWGAGTAYWCTAGRCTTTGTGA
OWL5500-fwd AGGATWAARTTTCTTGATCTYTGTGT
OWL5510-rev AACTTAATYCTRGGTGTCCACYTCAT
OWL570-rev TCACAAAGTCTAGWRTACTCWGAYTCTTCAAA

Supplementary Table 1. Technical sequencing parameters

Strain	S RNA			L RNA		
	No. of sequence reads	Sequence coverage reads/base	Ambiguities	No. of sequence reads	Sequence coverage reads/base	Ambiguities
Nig08-A18	21	3.4	1	39	3.1	0
Nig08-A19	21	3.1	0	38	3.2	0
Nig08-A37	23	3,7	0	39	3.2	1
Nig08-A41	23	3.3	0	38	3.1	0
Nig08-A47	20	3.2	0	39	3.2	0
Nig08-04	20	3.2	0	35	2.9	0

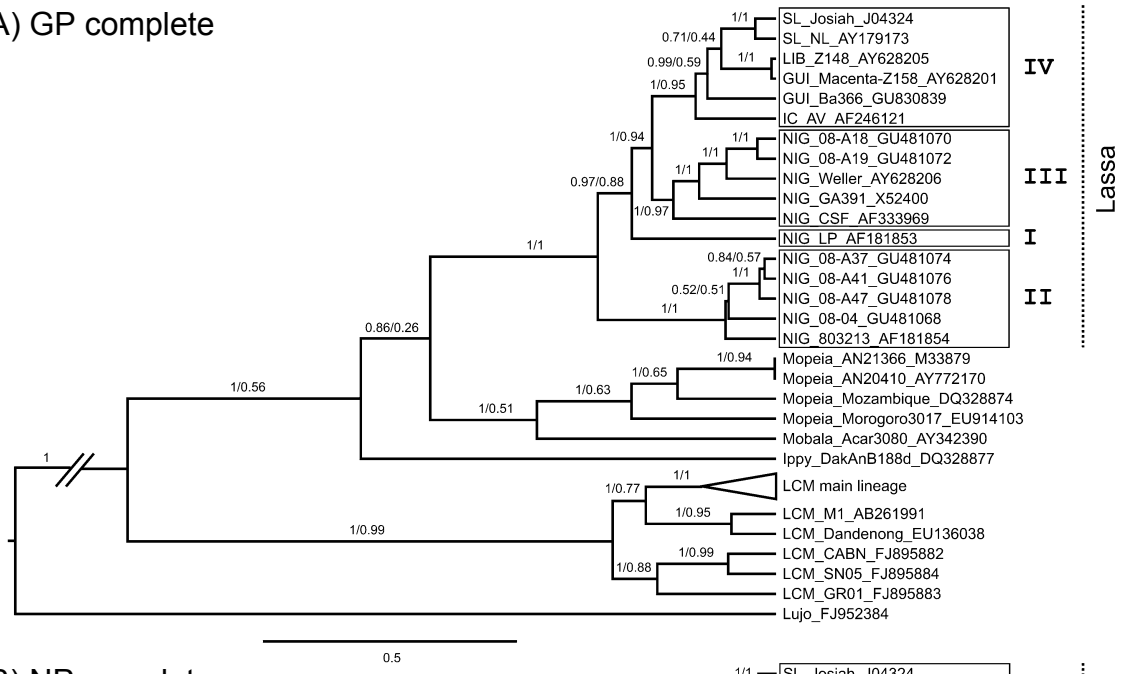
Supplementary Table 2. Basic sequence features of Lassa virus strains examined in this study in comparison to known sequences

Strain	GP[a]	NP[a]	L[a]	Z[a]	Potential N-glycosylation sites in GP1[b]	GP1/GP2 cleavage site SRRLL/GT	Late domains in Z PTAP-PPPY	Polymerase motif in L D-SSDD	Endo-nuclease motif in L PD-E-K
Josiah	491	569	2218	99	79, 99, 109, 119, 167, 224	...../..	....-....	.-....	..-.-.
NL	491	569	2220	99	79, 99, 109, 119, 167, 224	...../..	....-....	.-....	..-.-.
Z148	491	569	2219	99	79, 99, 109, 119, 167, 224	...../..	....-....	.-....	..-.-.
Macenta	491	569	2219	99	79, 99, 109, 119, 167, 224	...../..	....-....	.-....	..-.-.
Ba366	491	569	2217	99	79, 99, 109, 119, 167, 224	...../..	....-....	.-....	..-.-.
AV	491	569	2220	99	79, 99, 109, 119, 167, 224	...../..	....-....	.-....	..-.-.
CSF	490	569	2217	99	78, 98 ,108, 118, 166, 223	...../..	....-....	.-....	..-.-.
Nig08-A18	490	569	2222	99	78, 98 ,108, 118, 166, 223	...../..	....-....	.-....	..-.-.
Nig08-A19	490	569	2222	99	78, 98 ,108, 118, 166, 223	...../..	....-....	.-....	..-.-.
Nig08-A37	490	569	2220	99	78, 98 ,108, 118, 166, 223	...../..	.S..-....	.-....	..-.-.
Nig08-A41	490	569	2220	99	78, 98 ,108, 118, 166, 223	...../..	.S..-....	.-....	..-.-.
Nig08-A47	490	569	2220	99	78, 98 ,108, 118, 166, 223	...../..	.S..-....	.-....	..-.-.
Nig08-04	490	569	2220	99	78, 98 ,108, 118, 166, 223	...../..	.S..-....	.-....	..-.-.

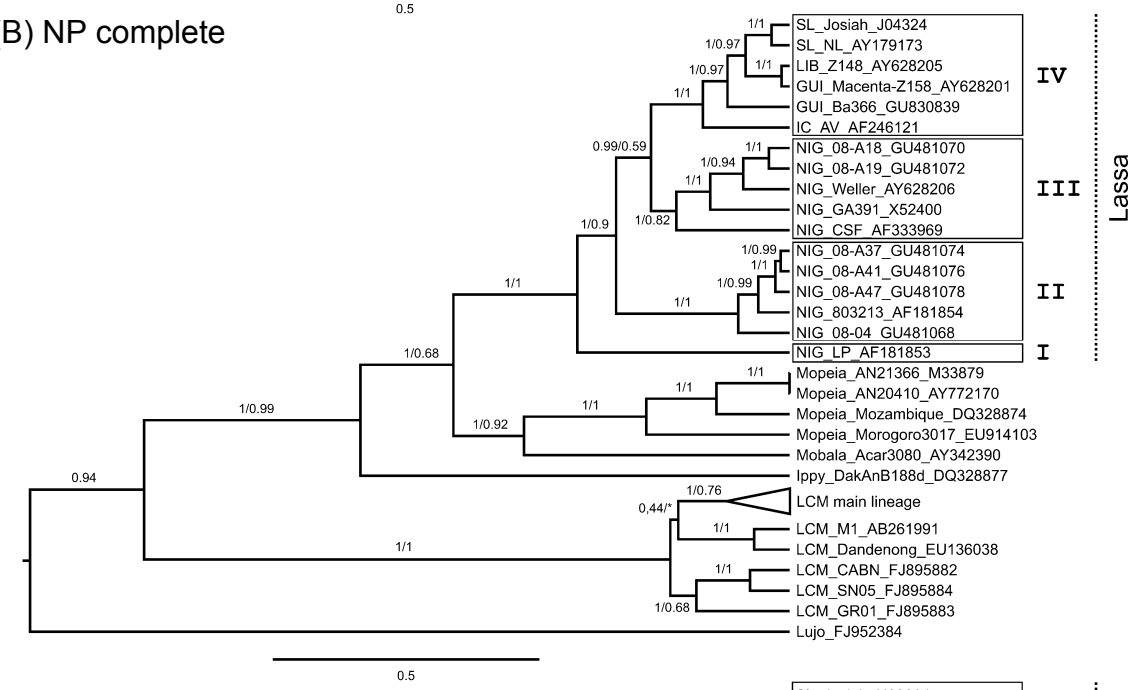
[a] Number of amino acid residues.
[b] Residue position predicted with NetNGlyc 1.0 Server at http://www.cbs.dtu.dk/services/NetNGlyc/

Supplementary Figure 1 (page 3). Phylogenetic analysis of Old World arenavirus complex using complete nucleotide sequences of GP (1473 nucleotides), NP (1707 nucleotides), and L gene (6654 nucleotides). Trees were inferred by the Bayesian Markov Chain Monte Carlo method implemented in BEAST software with the following parameters: general time reversible (GTR) model with gamma-distributed sites; relaxed lognormal clock with mean substitution rate fixed at 1; 10^7 steps with sampling every 10^5th step; and two independent runs combined (effective sampling size >200 for all parameters). The tree topology was confirmed by using a maximum likelihood approach implemented in PhyML software with the following parameters: GTR model with gamma-distributed sites and consensus of 100 bootstrap trees (trees not shown). BEAST and PhyML support values are indicated on the branches (posterior/bootstrap). The GenBank accession numbers are shown with the strains. The origin of Lassa virus strains is indicated by a prefix: SL, Sierra Leone; LIB, Liberia; GUI, Guinea; IC, Ivory Coast; NIG, Nigeria. The lymphocytic choriomeningitis virus (LCM) main lineage in tress based on complete sequences includes strains CH-5692, Armstrong, WE, Traub, MX, Pasteur, and Marseille 12.

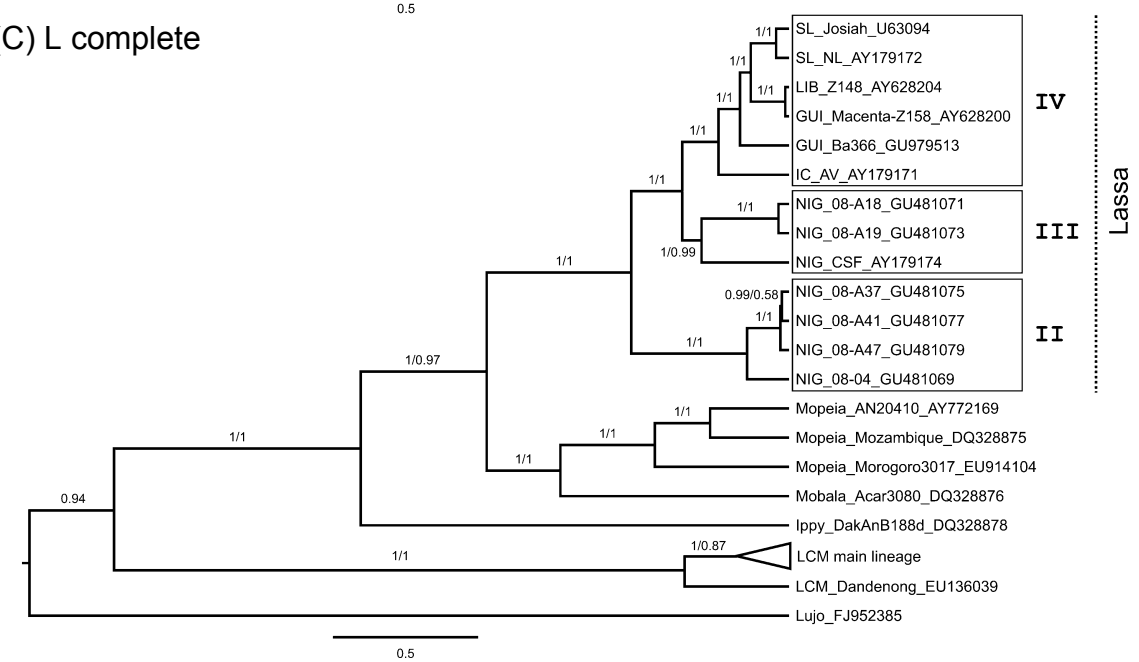
(A) GP complete



(B) NP complete

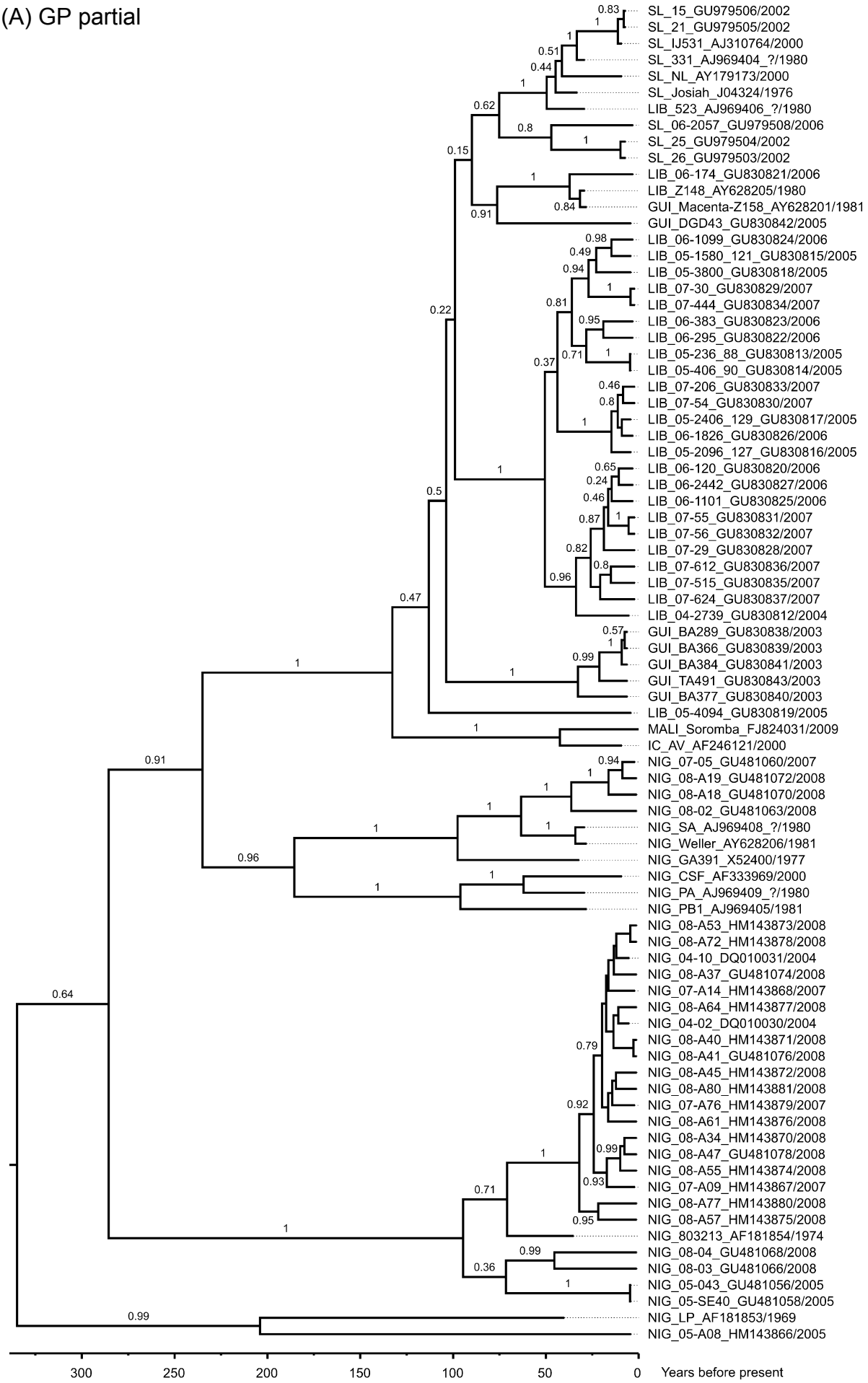


(C) L complete



Supplementary Figure 2 (pages 5-7). Phylogenetic analysis of the Lassa virus clade with estimation of substitution rate and age of most recent common ancestors using partial nucleotide sequences of GP (237 nucleotides), NP (631 nucleotides), and L gene (342 nucleotides). Phylogenies were inferred by using BEAST software with the following parameters: general time reversible model with gamma-distributed sites; strict clock with estimation of substitution rates and node ages from the isolation dates of the sequences; 10^7 steps with sampling every 10^5th step; and 2 independent runs combined (effective sampling size >200 for all parameters). The trees shown were generated with an underlying Bayesian skyline demographic model. Trees obtained with an exponential growth model were essentially identical (not shown). Posterior values are not shown on distal or poorly supported branches. GenBank accession number and year of isolation are given with the strains. The origin of Lassa virus strains is indicated by a prefix: SL, Sierra Leone; LIB, Liberia; GUI, Guinea; IC, Ivory Coast; NIG, Nigeria.

(A) GP partial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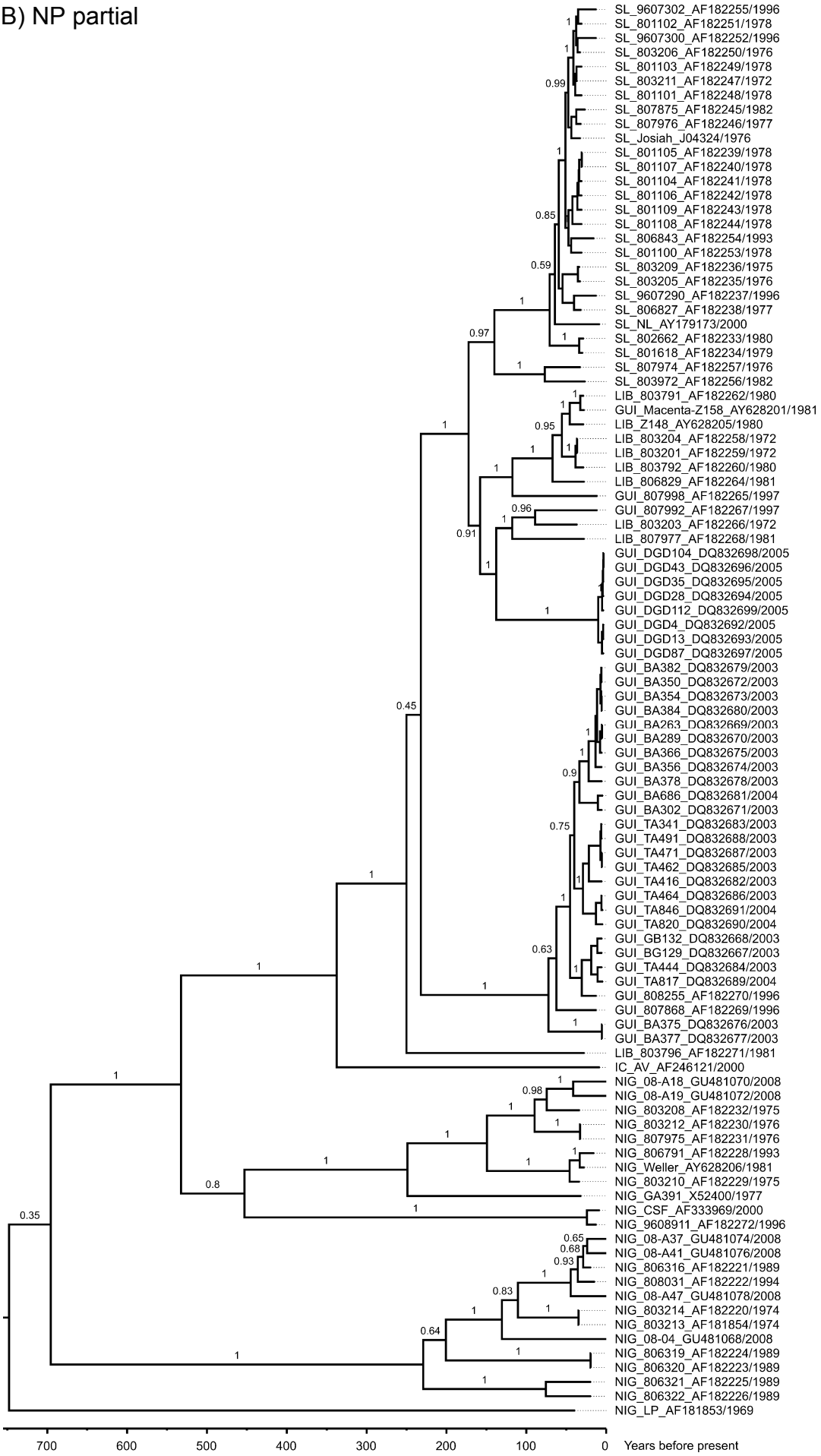
Years before present

(B) NP partial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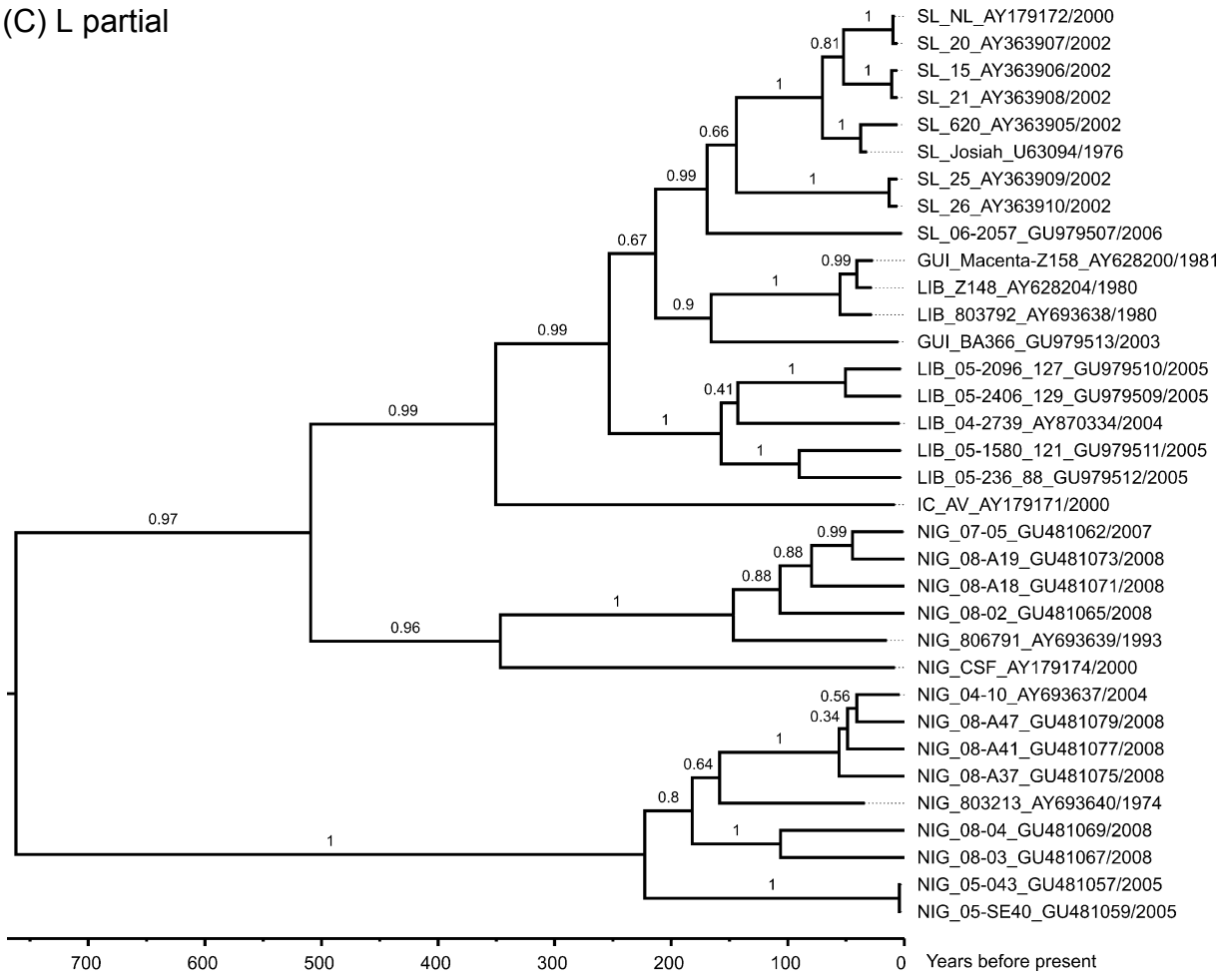
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