New HIV-1 Genomes From the DRC Expand Viral Diversity and Revise Subtype Emergence Dating

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BACKGROUND

- Kinshasa in DRC is a reservoir of highly diverse and ancestral HIV-1 Group M lineages that may pose a challenge for diagnostic tests.
- Genomic data from the post-2003 period in Kinshasa are limited despite high HIV prevalence.[1]
- We sequenced recent (2017-2019) viremic cases to define circulating diversity and assess implications for diagnostics and evolutionary dating.

METHODS

- Plasma specimens (N=197) were collected between 2017–2019 from 31 health facilities in/around Kinshasa.
- Sequences were generated by Illumina NGS using MSSPE metagenomics[2] and/or xGen target enrichment[3]. Consensus sequences were assembled with an automated pipeline.
- Subtyping was inferred with maximum likelihood phylogenetic trees (IQ-TREE) and recombination analysis was conducted with SimPlot.
- Temporal analysis was conducted using Nextstrain root-to-tip regression and BEAST Bayesian inference (UCLN clock; Skyride coalescent tree prior).
- Diagnostic performance was assessed using Abbott ARCHITECT HIV Ag/Ab Combo, Alinity i HIV Ag/Ab Combo Next, RealTi*m*e HIV-1 VL, Alinity m HIV VL, and Determine HIV-1/2 Ag/Ab Combo rapid antigen assays.

Figure 1. Distribution of HIV-1 Subtypes/CRFs Across 31 Collection Sites in and Around Kinshasa

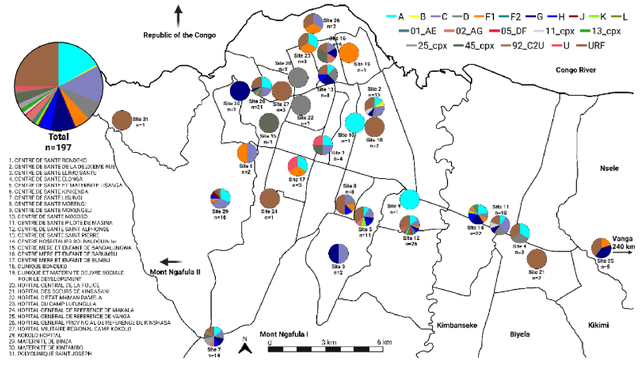


Table 1. Summary of Sequenced Subtype Classifications and Diagnostic Test Results

Subtype or CRF/URF	No. Seq.	ARCHITECT Ag/Ab Tested/Detected (%)	RealTime VL Tested/Detected (%)	Alinity i Ag/Ab Next Tested/Detected (%)	Alinity m VL Tested/Detected (%)a	Determine Ag/Ab RDT Tested/Detected (%)
A	33	33/33 (100)	33/33 (100)	29/29 (100)	29/29 (100)	29/29 (100)
B	1	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)
C	27	27/27 (100)	27/27 (100)	19/19 (100)	19/19 (100)	19/19 (100)
D	14	14/14 (100)	14/14 (100)	14/14 (100)	14/14 (100)	14/14 (100)
F1	11	11/11 (100)	11/11 (100)	10/10 (100)	10/10 (100)	10/10 (100)
G	18	18/18 (100)	18/18 (100)	15/15 (100)	15/15 (100)	15/15 (100)
H	9	9/9 (100)	9/9 (100)	3/3 (100)	3/3 (100)	3/3 (100)
J	1	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)
K	1	1/1 (100)	1/1 (100)	N/A	N/A	N/A
CRF01_AE	3	3/3 (100)	3/3 (100)	3/3 (100)	3/3 (100)	3/3 (100)
CRF02_AG	6	6/6 (100)	6/6 (100)	6/6 (100)	6/6 (100)	6/6 (100)
CRF05_DF	1	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)	1/1 (100)
CRF11_cpx	2	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)
CRF13_cpx	3	3/3 (100)	3/3 (100)	3/3 (100)	3/3 (100)	3/3 (100)
CRF25_cpx	4	4/4 (100)	4/4 (100)	4/4 (100)	4/4 (100)	4/4 (100)
CRF45_cpx	8	8/8 (100)	8/8 (100)	8/8 (100)	8/8 (100)	8/8 (100)
CRF92_C2U	2	2/2 (100)	2/2 (100)	1/1 (100)	1/1 (100)	1/1 (100)
U	3	3/3 (100)	3/3 (100)	2/2 (100)	2/2 (100)	2/2 (100)
URF	50	50/50 (100)	50/50 (100)	39/39 (100)	39/39 (100)	39/39 (100)
Total	197	197/197 (100)	197/197 (100)	161/161 (100)	161/161 (100)	161/161 (100)

Whole-genome sequencing of 197 HIV-1 samples from Kinshasa (2017–2019) reveals ongoing circulation of rare and basal Group M lineages.

Including these new sequences shifted Bayesian tMRCA estimates for HIV-1 Group M and the rare subtypes G/H/J by 3-7 years each.

RESULTS

- Broad subtype diversity was observed including pure subtypes (A, B, C, D, F1, G, H, J, K), multiple CRFs, URFs, and unclassifiable genomes (Figure 1, Figure 2).
- All evaluated diagnostic assays detected all tested samples with 100% sensitivity (Table 1).
- Many sequences branch basally within or outside subtype clades, consistent with ongoing circulation of lineages whose origins pre-date the emergence of the canonical modern subtypes (Figure 2, Figure 3).
- Rare subtypes G/H/J/K comprised 14.7% (29/197), including nine subtype H genomes which nearly doubles the number of known genomes of this subtype (Figure 3, Table 1).
- Bayesian phylodynamic analysis shifts tMRCA earlier by 3-7 years for Group M and rare subtypes G/H/J when these new sequences were added (Figure 4).

Figure 2. Maximum-likelihood Trees of new DRC sequences and Subtype References Curated from GenBank

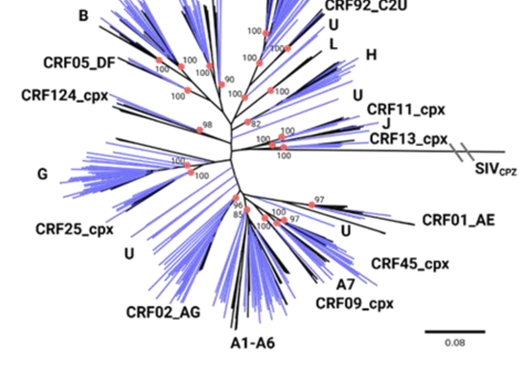


Figure 3. Maximum-likelihood Tree of Subtype H Sequences and Recombination Analysis of 146_drc

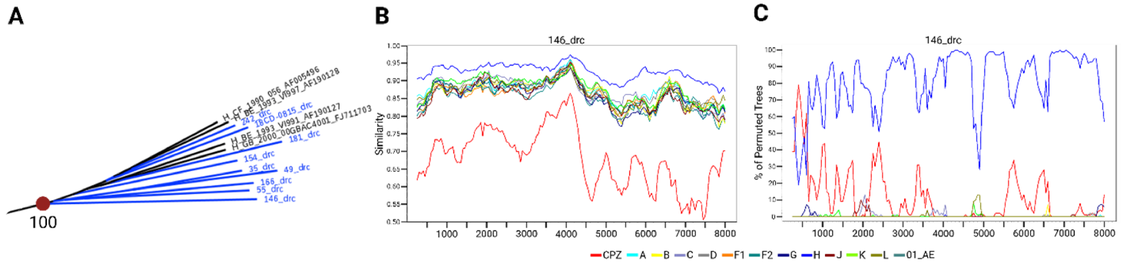
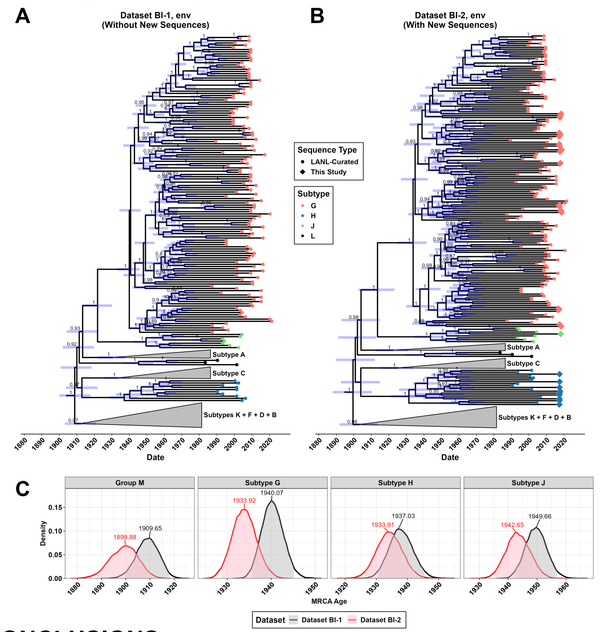


Figure 4. Bayesian Phylogenetic Inference (*env* gene) Showing Earlier tMRCA Estimates when Adding New G/H/J Sequences.



CONCLUSIONS

- Kinshasa in DRC continues to harbor highly diverse HIV-1 lineages, including rare and basal Group M subtypes.
- Addition of these new sequences to tMRCA analyses suggest that rare subtypes such as G, H, and J emerged earlier than previous estimates.
- Current and next generation Abbott diagnostic assays detected the totality of the HIV-1 Group M diversity observed, ensuring that even highly diverse HIV-1 strains are detectable.
- This study highlights the continued importance and relevance of HIV surveillance in the DRC and should spur further HIV monitoring in this region.

References

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2. Deng, X, *et al.* Metagenomic sequencing with spiked primer enrichment for viral diagnostics and genomic surveillance. *Nat Microbiol* **2020,** 5, (3), 443-454.
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ADDITIONAL INFORMATION

- Disclosures: MA, GSO, VH, AO, BJH, MGB, GA, and MAR completed this work as employees/shareholders of Abbott Laboratories.
- Ethical Approval: Université Protestante au Congo IRB (CEUPC-027)
- Acknowledgments: The authors would like to acknowledge the contributions of the late Carole McArthur, MD, PhD to this work.

PLAIN LANGUAGE SUMMARY

The area in and around Kinshasa continues to harbor many rare and highly diverse HIV-1 strains which can still be detected with current and next generation diagnostic assays.

These new genomes suggest HIV-1 began diversifying 3-7 years earlier than previously estimated.