Infection, Genetics and Evolution xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

# On the contribution of Angola to the initial spread of HIV-1

Andrea-Clemencia Pineda-Peña [a,b], Jorge Varanda [a,c,d], João Dinis Sousa [a,e], Kristof Theys [e], Inês Bártolo [f], Thomas Leitner [g], Nuno Taveira [f,h], Anne-Mieke Vandamme [a,e], Ana B. Abecasis [a,e,*]

[a] Global Health and Tropical Medicine, GHTM, Instituto de Higiene e Medicina Tropical, IHMT, Universidade Nova de Lisboa, UNL, Lisboa, Portugal
[b] Molecular Biology and Immunology Department, Fundación Instituto de Inmunología de Colombia (FIDIC), Basic Sciences Department, Universidad del Rosario, Bogotá, Colombia
[c] Department of Life Sciences, University of Coimbra, Coimbra, Portugal
[d] CRIA-UC, Centre for Research in Anthropology, University of Coimbra, Coimbra, Portugal
[e] KU Leuven - University of Leuven, Department of Microbiology and Immunology, Rega Institute for Medical Research, Clinical and Epidemiological Virology, B-3000 Leuven, Belgium
[f] Instituto de Investigação do Medicamento (iMed.ULisboa), Faculdade de Farmácia da Universidade de Lisboa, Lisbon, Portugal
[g] Theoretical Biology and Biophysics Group, Los Alamos National Laboratory, Los Alamos, NM 87545, USA
[h] Centro de Investigação Interdisciplinar Egas Moniz (CiiEM), Instituto Superior de Ciências da Saúde Egas Moniz, Monte de Caparica, Portugal

ARTICLE INFO

*Article history:*
Received 10 May 2016
Received in revised form 5 August 2016
Accepted 9 August 2016
Available online xxxx

*Keywords:*
Angola
HIV-1
Origin
Group M
Phylogeography

ABSTRACT

Angola borders and has long-term links with Democratic Republic of Congo (DRC) as well as high levels of Human Immunodeficiency Virus (HIV) genetic diversity, indicating a potential role in the initial spread of the HIV-1 pandemic. Herein, we analyze 564 C2V3 and 354 *pol* publicly available sequences from DRC, Republic of Congo (RC) and Angola to better understand the initial spread of the virus in this region. Phylogeographic analyses were performed with the BEAST software. While our results pinpoint the origin of the pandemic to Kinshasa (DRC) around 1906, the introduction of HIV-1 to Angola could have occurred early between the 1910s and 1940s. Furthermore, most of the HIV-1 migrations out of Kinshasa were directed not only to Lubumbashi and Mbuji-Mayi (DRC), but also to Luanda and Brazzaville. Kinshasa census records corroborate these findings, indicating that the early exportation of the virus to Angola might be related to the high number of Angolans in Kinshasa at that time, originated mostly from the North of Angola.
In summary, our results place Angola at the epicenter of the early HIV dissemination, together with DRC and RC.


## 1. Introduction

At the end of 2014, the Human Immunodeficiency Virus (HIV) had caused approximately 78 million infections and 39 million deaths (UNAIDS, 2014). HIV is classified in types 1 and 2, but most infections are caused by HIV-1 group M. This group M epidemic started in Kinshasa (Democratic Republic of Congo, DRC), and soon spread across the Congo river to Brazzaville located in Republic of Congo (RC), and further to Lubumbashi and Mbuji-Mayi (DRC), around 1937 (Faria et al., 2014; Rambaut et al., 2001; Worobey et al., 2008). The HIV-1 epidemic in Angola displays levels of genetic diversity comparable to DRC, which is consistent with an early viral introduction into this country (Bartolo et al., 2009; Bartolo et al., 2016). Nonetheless, the contribution of Angola to the early dispersal of HIV was never investigated.

*Abbreviations:* AEF, *Afrique Équatoriale Française*; BCI, Bayesian Credible Interval; BF, Bayes factor; CPP, Clade Posterior Probability; DRC, Democratic Republic of Congo; HIV, Human Immunodeficiency Virus; MCC, maximum clade credibility; MCMC, Bayesian Markov Chain Monte Carlo; MRCA, most recent common ancestor; RC, Republic of Congo; SPP, State Posterior Probability.

* Corresponding author at: Rua da Junqueira 100, 1349-008 Lisboa, Portugal.
*E-mail address:* ana.abecasis@ihmt.unl.pt (A.B. Abecasis).

## 2. Methods

All sequences for *env* C2V3 and *pol* (HXB2 positions 7044–7347 and 2319–3302) sampled in Angola, DRC and RC were downloaded from the LANL (http://www.hiv.lanl.gov/). Duplicates and sequences that did not meet LANL quality control parameters were deleted. City locations were retrieved from the original publications (Afonso et al., 2012a; Bartolo et al., 2005; Bartolo et al., 2009; Djoko et al., 2011; Gao et al., 2001; Guimaraes et al., 2009; Vergne et al., 2000; Vidal et al., 2006). Sequences with unknown year, country or city of sampling were excluded from the analyses. The resulting datasets had 564 C2V3 and 354 *pol* sequences (Table S1). The *env* and *pol* datasets contained 349 and 190 sequences from DRC, 118 and 50 from RC, 97 and 115 from Angola, respectively. To investigate how sampling affects the results, two *env* and *pol* datasets were randomly constructed using Phylogenetic Diversity Analyzer (http://www.cibiv.at/software/pda/), with a similar number of sequences from the most sampled cities and maintaining the temporal span and viral diversity. The resulting *env* and *pol* datasets included each time 265 and 230 sequences, respectively (Table S1).

Sequences were aligned using Muscle (Edgar, 2004), and edited in SeaView (Gouy et al., 2010). The temporal signal of all datasets was evaluated with Path-O-Gen (http://tree.bio.ed.ac.uk/software/

http://dx.doi.org/10.1016/j.meegid.2016.08.009
1567-1348/

pathogen/). To estimate the most recent common ancestor (MRCA), the Bayesian Markov Chain Monte Carlo (MCMC) inference implemented in BEAST v1.7.5 was used (Drummond et al., 2012) with the GTR model, a relaxed uncorrelated Lognormal molecular clock model (Drummond et al., 2006) and a Skygrid coalescent tree prior (Gill et al., 2013). To evaluate the early dispersal and the most important migrations, a discrete phylogeographic model was applied using Bayesian Stochastic Search Variable Selection (BSSVS) analyses and the robust counting approach (Edwards et al., 2011; Lemey et al., 2009; Minin et al., 2008; O'Brien et al., 2009; Talbi et al., 2010). The MCMC chains were run for 100 million generations at least three times with a burn-in of 10%. Convergence was evaluated using Tracer (http://beast.bio.ed.ac.uk/Tracer). The

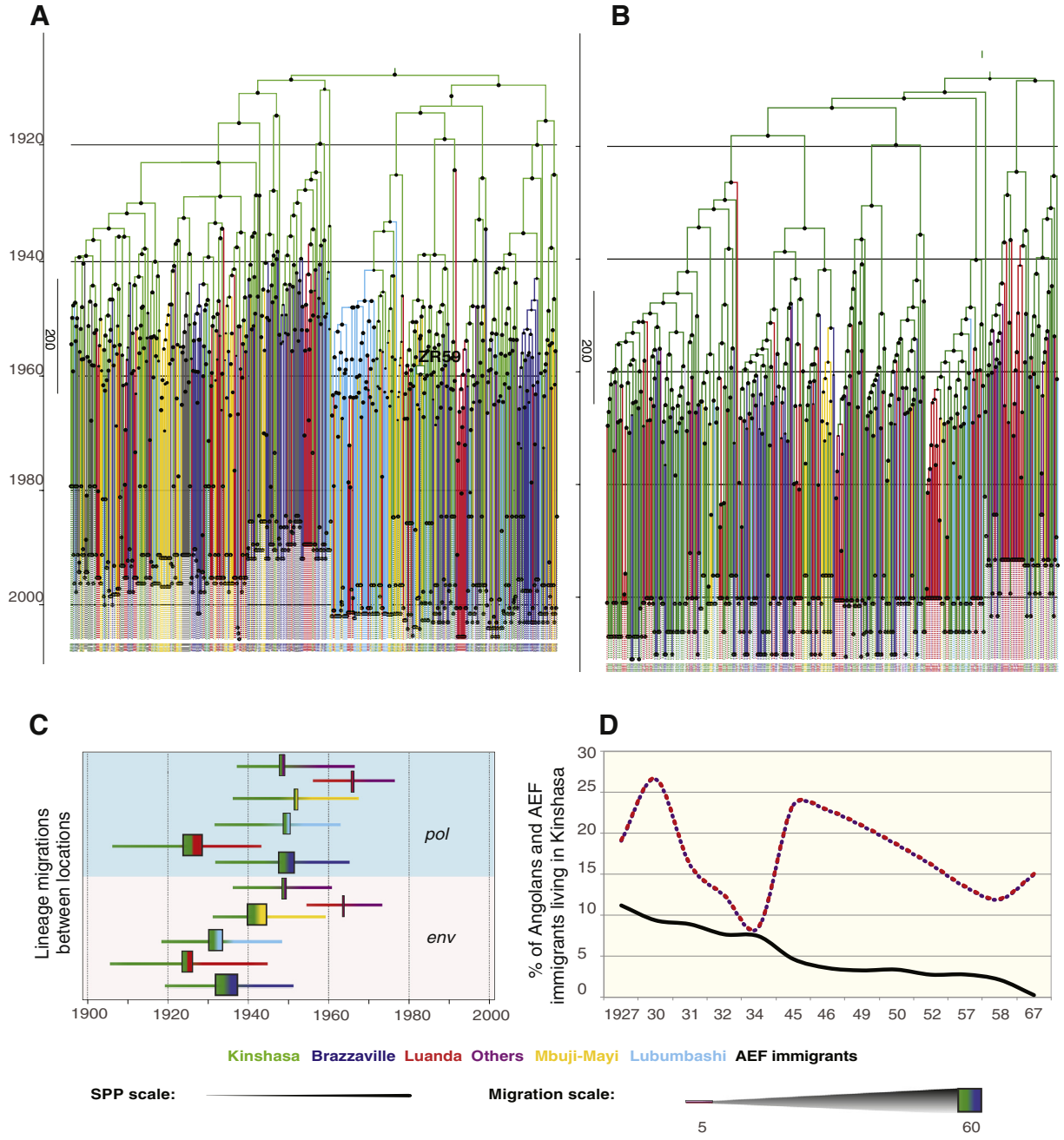


**Fig. 1.** Phylogeographic analysis of the early spread of HIV-1. The MCC trees of the C2V3 (A) and *pol* (B) datasets are shown. The most likely location of the parental node is represented with colors that are explained in the lower panel. The oldest sequence available sampled in DRC in 1959 is represented with a square (ZR59). The C2V3 and *pol* datasets included the following number of sequences per location (C2V3/pol): Angola: Cabinda (12/19), Cuanza Norte (1/1), Luanda (78/82), Lunda Norte (2/2), Malanje (2/8), Zaire province (2/2); Democratic Republic of Congo: Bwamanda (33/1), Kimpese (0/1), Kinshasa (97/142), Kisangani (23/8), Likasi (24/0), Lubumbashi (76/20), Mbuji-Mayi (96/18); Congo: Brazzaville (97/50), Pointe Noire (21/0). Regions of Angola with low number of sequences such as Cabinda, Cuanza Norte, Malanje, Lunda Norte, and Zaire were grouped into 'Others'. Similarly, regions of DRC such as Bwamanda, Kimpese and Kinsangani were grouped into DC others for the *pol* dataset (color no shown). The State Posterior Probability (SPP) is pictured as a circle in the nodes, the size reflects the scale of this probability. (C) The earliest dates of significant lineage migrations in DRC, RC and Angola in *env* C2V3 and *pol* are represented by colors as indicated. The rectangle represents the median of the earliest introduction from the exporter (left) to the importer (right) according to the direction of the migration, whereas the horizontal size of the rectangle is proportional to the number of migrations in each transition. Lines represent the Bayesian Credible Intervals. (D) The percentage of Angolans (mainly from the North region, red and violet dashed line) and people from *Afrique Équatoriale Française* (AEF, black line) living in Leopoldville/Kinshasa according to different censuses of the population (Affaires Indigènes et Main d'Oeuvre (AIMO), 1927; Comissariat de Police de Léopoldville, 1931; Congo Belge, 1930, 1932, 1934, 1949, 1957; Institut National de la Statistique, 1969; Service d'Administration de la Population Noire, 1945, 1946, 1950, 1952; Spitaels, 1959). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Please cite this article as: Pineda-Peña, A.-C., et al., On the contribution of Angola to the initial spread of HIV-1, Infection, Genetics and Evolution (2016), http://dx.doi.org/10.1016/j.meegid.2016.08.009

maximum clade credibility (MCC) tree was summarized with TreeAnnotator after removal of the burn-in, and visualized with FigTree v1.4.2 (http://tree.bio.ed.ac.uk).

## 3. Results

The MRCA of group M was in 1906 [Bayesian Credible Interval (BCI): 1892–1921] using *env* and 1906 [1878–1930] using *pol*. Kinshasa was indicated as the origin of this pandemic for both genomic regions [State Posterior Probability (SPP) and Clade Posterior Probability (CPP) = 1] (Fig. 1A–B).

Exportations of HIV-1 from DRC to Luanda (Angola) were observed soon after, in 1924 [1905–1944] using C2V3 and 1927 [1906–1943] using *pol*. These exportations were supported by high SPP values (>0.9), yet with lower CPP (<0.5). These virus migrations preceded others, including to Brazzaville and Lubumbashi. The introduction to Brazzaville was around 1935 [1919–1952] using C2V3 and 1949 [1933–1965] using *pol*, whereas the introduction to Lubumbashi was estimated in 1933 [1918–1948] using C2V3 and 1949 [1931–1963] using *pol*; respectively (Fig. 1C, Table S2). The earliest exportations of the virus out of Kinshasa to Angola and Brazzaville were almost simultaneous when high CPP values were considered. The introduction to Angola was estimated around 1946 [1932–1960] using C2V3 and 1945 [1929–1960] using *pol*, whereas the introduction to Brazzaville was around 1941 [1928–1955] and 1951 [1934–1965], respectively (Fig. 1C, Table S2).

Luanda and Brazzaville were not only the earliest but also the most substantial targets for HIV-1 migrations out of Kinshasa (Fig. 1C; Bayes factor (BF) > 1000; Luanda: 29 and 52 transitions using C2V3 and *pol*, respectively [20–37 and 47–59]; Brazzaville: 60 and 43 transitions in C2V3 and *pol*, respectively [52–70 and 36–47]). Within DRC, Lubumbashi and Mbuji-Mayi were the cities with the highest number of migrations from Kinshasa (BF > 1000; Lubumbashi: 38 and 19 transitions using C2V3 and *pol*, respectively [27–47 and 15–21]; Mbuji-Mayi: 52 and 10 transitions using C2V3 and *pol*, respectively [40–61 and 7–14]).

A significant migration link with limited transitions was also found between Kinshasa and other regions of Angola around the late-1940s, but this is not surprising given the scarcity of sequences from other regions than Luanda (Table S2). Similarly, few migrations were estimated between Luanda and other cities of Angola (BF > 1000, 5 [3–7] and 8 [5–11] in *env* and *pol*, respectively). However, the ongoing historical population movement between different areas of the country would suggest that our sampling most likely reflects a broader geographic region of the country. When the two smaller *env* and *pol* random datasets were analyzed (Tables S1–S2), the results were mostly consistent with the main analyses. However, the number of migrations out of Kinshasa to Angola and to Brazzaville was comparable and slightly different from the main analyses, indicating a potential effect of the sampling in the quantification of migrations (Table S2).

Analysis of archival data about Kinshasa immigration records in that region in the early 20th century indicated that Angolan immigrants constituted an important percentage of the population in Kinshasa (Fig. 1D). The number of Angolans living in Kinshasa reached 26% of the city population in 1930 and 23% in the 1940s. Specifically, these Angolans, in the 1930s–40s, originated mostly from the area of Maquela do Zombo, which lies in the Uíge province of Angola (Congo Belge, 1930–1957). Despite the distance, Angolans were present in higher numbers than individuals from Afrique Équatoriale Française (Fig. 1D).

## 4. Discussion

Herein we analyze for the first time the role of Angola in the early dissemination of the HIV-1 group M epidemic. Our results indicate that the earliest estimated exportations of the virus out of Kinshasa occurred quasi concurrently to Angola and Brazzaville in the early 20th century, around the same time as to other locations in DRC. The exportation of HIV-1 to Angola accounted for a major part of HIV-1 migrations out of Kinshasa, as much as to Brazzaville or other cities of DRC. The large proportion of Angolans living in Kinshasa at that time could explain these findings, at least partly.

Together, our results indicate an important role of Angola in the early dispersal of the epidemic. These findings are consistent with the similar levels of genetic diversity found in Kinshasa and Luanda (Bartolo et al., 2009), and with their profound historical connectivity. Intense native population movements across the borders of colonial Angola and DRC are related with shared ethnicities and became divided by colonial borders (Vansina, 1966). The export of HIV to Angola coincided with a time frame where a large proportion of Kinshasa's population consisted of Angolan immigrants, likely related to labor migration movements as a result of the late 19th–early 20th century expansion of infrastructures, agriculture and mining industries in both Angola and DRC (Henriques, 2004; Varanda, 2011).

Although Angola contributed to the early ignition of the epidemic, its role seems to have been limited for the spread of the worldwide predominant subtypes B and C (data no shown). While for subtype B this is expected, for subtype C seems to have been mostly imported to Angola from the 1960s onwards (Afonso et al., 2012b). More comprehensive sampling of HIV-1 patients in the North of Angola would be important to better understand the early epidemic of HIV-1 and the spread of different subtypes.

In conclusion, herein we show for the first time that Angola played an important role in the early dissemination of HIV-1 group M. Including its sequences in future analyses is therefore crucial to better understand the origin of HIV or its spread through population movements during colonial times.

## Role of funding source

This study was supported by European Funds through grant 'Bio-Molecular and Epidemiological Surveillance of HIV Transmitted Drug Resistance, Hepatitis Co-Infections and Ongoing Transmission Patterns in Europe - BEST HOPE - (project funded through HIVERA: Harmonizing Integrating Vitalizing European Research on HIV/Aids, grant 249697)'; by L'Oréal Portugal Medals of Honor for Women in Science 2012 (financed through L'Oréal Portugal, Comissão Nacional da Unesco and Fundação para a Ciência e Tecnologia (FCT - http://www.fct.pt)); by FCT for funds to GHTM-UID/Multi/04413/2013; by the Fonds voor Wetenschappelijk Onderzoek – Flanders (FWO) grant G.0692.14 and G.0611.09N; by a National Institutes of Health (NIH) grant AI087520; by FCT (grants PTDC/SAU-EPI/122400/2010, VIH/SAU/0029/2011 and PTDC/AFR/100646/2008); and by CRIA/ANT/04038/2013; and by National Endowment for the Humanities Collaborative Research Grant No. RZ5152313, "An International Collaboration on the Political, Social, and Cultural History of the Emergence of HIV/AIDS." The computational resources and services used in this work were provided by the Hercules Foundation and the Flemish Government – department EWI-FWO Krediet aan Navorsers (Theys, KAN2012 1.5.249.12.). I.B. is supported by a post-doc fellowship (SFRH/BPD/76225/2011) from FCT. K.T. is supported by a postdoctoral grant from FWO.

## Authors' contributions

APP: data analysis, data interpretation, figures, writing; JV: data analysis, literature review, writing; JS: data collection, writing; KT: writing; IB: data collection, writing; TL: writing; NT: data collection, writing; AMV: data interpretation, writing; AA: study design, data analysis, data interpretation, figures, writing.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.meegid.2016.08.009.

Please cite this article as: Pineda-Peña, A.-C., et al., On the contribution of Angola to the initial spread of HIV-1, Infection, Genetics and Evolution (2016), http://dx.doi.org/10.1016/j.meegid.2016.08.009

## References


Affaires Indigènes et Main d'Oeuvre (AIMO), 1927. Enquête sur la Main d'œuvre, District Urbain de Léopoldville. Léopoldville, Belgian Congo: AIMO, Province du Congo-Kasai. Afrika Archief (FO-BZBHO, Brussels), Series GG, Folder GG 16186.

Afonso, J.M., Bello, G., Guimaraes, M.L., Sojka, M., Morgado, M.G., 2012a. HIV-1 genetic diversity and transmitted drug resistance mutations among patients from the North, Central and South regions of Angola. PLoS One 7, e42996.

Afonso, J.M., Morgado, M.G., Bello, G., 2012b. Evidence of multiple introductions of HIV-1 subtype C in Angola. Infect. Genet. Evol. 12, 1458–1465.

Bartolo, I., Epalanga, M., Bartolomeu, J., Fonseca, M., Mendes, A., Gama, A., Taveira, N., 2005. High genetic diversity of human immunodeficiency virus type 1 in Angola. AIDS Res. Hum. Retrovir. 21, 306–310.

Bartolo, I., Rocha, C., Bartolomeu, J., Gama, A., Marcelino, R., Fonseca, M., Mendes, A., Epalanga, M., Silva, P.C., Taveira, N., 2009. Highly divergent subtypes and new recombinant forms prevail in the HIV/AIDS epidemic in Angola: new insights into the origins of the AIDS pandemic. Infect. Genet. Evol. 9, 672–682.

Bartolo, I., Calado, R., Borrego, P., Leitner, T., Taveira, N., 2016. Rare HIV-1 subtype J genomes and a new H/U/CRF02_AG recombinant genome suggests an ancient origin of HIV-1 in Angola. AIDS Res. Hum. Retrovir. 32, 822–828.

Comissariat de Police de Léopoldville, 1931. Récensement de la Cité Indigène de Léopoldville. Est. Afrika Archief (FO-BZBHO, Brussels), Inventory A39, Box RA/CB GG 239.

Congo Belge, 1930. Census of Léopoldville, discriminating regions of origin of inhabitants. Afrika Archief: Federale Overheidsdients – Buitenlandse Zaken Buitenlandse Handel en Ontwikkelingssamenwerking (FO-BZBHO), Series GG Folder GG 5408, Brussels 1934, 1949, 1957.

Congo Belge, 1932. Récensement de Léopoldville-Est. Afrika Archief: Federale Overheidsdients – Buitenlandse Zaken Buitenlandse Handel en Ontwikkelingssamenwerking (FO-BZBHO), Inventory A39, Box RA/MED 46, Brussels.

Djoko, C.F., Rimoin, A.W., Vidal, N., Tamoufe, U., Wolfe, N.D., Butel, C., LeBreton, M., Tshala, F.M., Kayembe, P.K., Muyembe, J.J., Edidi-Basepeo, S., Pike, B.L., Fair, J.N., Mbacham, W.F., Saylors, K.E., Mpoudi-Ngole, E., Delaporte, E., Grillo, M., Peeters, M., 2011. High HIV type 1 group M pol diversity and low rate of antiretroviral resistance mutations among the uniformed services in Kinshasa, Democratic Republic of the Congo. AIDS Res. Hum. Retrovir. 27, 323–329.

Drummond, A.J., Ho, S.Y., Phillips, M.J., Rambaut, A., 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. 4, e88.

Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A., 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol. Biol. Evol. 29, 1969–1973.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797.

Edwards, C.J., Suchard, M.A., Lemey, P., Welch, J.J., Barnes, I., Fulton, T.L., Barnett, R., O'Connell, T.C., Coxon, P., Monaghan, N., Valdiosera, C.E., Lorenzen, E.D., Willerslev, E., Baryshnikov, G.F., Rambaut, A., Thomas, M.G., Bradley, D.G., Shapiro, B., 2011. Ancient hybridization and an Irish origin for the modern polar bear matriline. Curr. Biol. 21, 1251–1258.

Faria, N.R., Rambaut, A., Suchard, M.A., Baele, G., Bedford, T., Ward, M.J., Tatem, A.J., Sousa, J.D., Arinaminpathy, N., Pepin, J., Posada, D., Peeters, M., Pybus, O.G., Lemey, P., 2014. HIV epidemiology. The early spread and epidemic ignition of HIV-1 in human populations. Science 346, 56–61.

Gao, F., Vidal, N., Li, Y., Trask, S.A., Chen, Y., Kostrikis, L.G., Ho, D.D., Kim, J., Oh, M.D., Choe, K., Salminen, M., Robertson, D.L., Shaw, G.M., Hahn, B.H., Peeters, M., 2001. Evidence of two distinct subsubtypes within the HIV-1 subtype A radiation. AIDS Res. Hum. Retrovir. 17, 675–688.

Gill, M.S., Lemey, P., Faria, N.R., Rambaut, A., Shapiro, B., Suchard, M.A., 2013. Improving Bayesian population dynamics inference: a coalescent-based model for multiple loci. Mol. Biol. Evol. 30, 713–724.

Gouy, M., Guindon, S., Gascuel, O., 2010. SeaView version 4: a multiplatform graphical user interface for sequence alignment and phylogenetic tree building. Mol. Biol. Evol. 27, 221–224.

Guimaraes, M.L., Vicente, A.C., Otsuki, K., da Silva, R.F., Francisco, M., da Silva, F.G., Serrano, D., Morgado, M.G., Bello, G., 2009. Close phylogenetic relationship between Angolan and Romanian HIV-1 subtype F1 isolates. Retrovirology 6, 39.

Henriques, I.C., 2004. Os pilares da diferença: relações Portugal-África: séculos XV-XX.

Institut National de la Statistique, 1969. Étude Socio-Démographique de Kinshasa 1967. Kinshasa, DRC: Ministère d'État Chargé du Plan, de la Recherche Scientifique, de l'Amenagement du Territoire et de la Coordination de la Planification. Office National de la Recherche et du Dévéloppement.

Lemey, P., Rambaut, A., Drummond, A.J., Suchard, M.A., 2009. Bayesian phylogeography finds its roots. PLoS Comput. Biol. 5, e1000520.

Minin, V.N., Bloomquist, E.W., Suchard, M.A., 2008. Smooth skyride through a rough skyline: Bayesian coalescent-based inference of population dynamics. Mol. Biol. Evol. 25, 1459–1471.

O'Brien, J.D., Minin, V.N., Suchard, M.A., 2009. Learning to count: robust estimates for labeled distances between molecular sequences. Mol. Biol. Evol. 26, 801–814.

Rambaut, A., Robertson, D.L., Pybus, O.G., Peeters, M., Holmes, E.C., 2001. Human immunodeficiency virus. Phylogeny and the origin of HIV-1. Nature 410, 1047–1048.

Service d'Administration de la Population Noire, 1945. Récensement par origine des habitants des 7 quartiers de Léo-Est. Afrika. Archief (FO-BZBHO, Brussels), Inventory A39, Box RA/AIMO 120 annexe 1946, 1950, 1952.

Spitaels, G., 1959. Letter to the Centre de Recherches et d'Informations Socio-Politiques (Brussels). Archives of Afrika Museum, Tervuren, Belgium.

Talbi, C., Lemey, P., Suchard, M.A., Abdelatif, E., Elharrak, M., Nourlil, J., Faouzi, A., Echevarria, J.E., Vazquez Moron, S., Rambaut, A., Campiz, N., Tatem, A.J., Holmes, E.C., Bourhy, H., 2010. Phylodynamics and human-mediated dispersal of a zoonotic virus. PLoS Pathog. 6, e1001166.

UNAIDS, 2014. World AIDS Day 2014 Report-Fact Sheet, Ending the AIDS Epidemic. Joint United Nations Programme on HIV/AIDS, Geneva, Switzerland.

Vansina, J., 1966. Kingdoms of the Savanna. University of Wisconsin Press, Madison.

Varanda, J., 2011. A asa protectora de Outros: As relações transcoloniais do Serviço de Saúde da Diamang. In: Bastos, C., Barreto, R. (Eds.), Circulação do Conhecimento Médico. ICS, Lisbon, pp. 287–314.

Vergne, L., Peeters, M., Mpoudi-Ngole, E., Bourgeois, A., Liegeois, F., Toure-Kane, C., Mboup, S., Mulanga-Kabeya, C., Saman, E., Jourdan, J., Reynes, J., Delaporte, E., 2000. Genetic diversity of protease and reverse transcriptase sequences in non-subtype-B human immunodeficiency virus type 1 strains: evidence of many minor drug resistance mutations in treatment-naive patients. J. Clin. Microbiol. 38, 3919–3925.

Vidal, N., Mulanga, C., Bazepeo, S.E., Mwamba, J.K., Tshimpaka, J., Kashi, M., Mama, N., Valea, D., Delaporte, E., Lepira, F., Peeters, M., 2006. HIV type 1 pol gene diversity and antiretroviral drug resistance mutations in the Democratic Republic of Congo (DRC). AIDS Res. Hum. Retrovir. 22, 202–206.

Worobey, M., Gemmel, M., Teuwen, D.E., Haselkorn, T., Kunstman, K., Bunce, M., Muyembe, J.J., Kabongo, J.M., Kalengayi, R.M., Van Marck, E., Gilbert, M.T., Wolinsky, S.M., 2008. Direct evidence of extensive diversity of HIV-1 in Kinshasa by 1960. Nature 455, 661–664.


Please cite this article as: Pineda-Peña, A.-C., et al., On the contribution of Angola to the initial spread of HIV-1, Infection, Genetics and Evolution (2016), http://dx.doi.org/10.1016/j.meegid.2016.08.009