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Short Communication: Reassessing the Origin of the HIV-1 CRF02_AG Lineages Circulating in Brazil

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Abstract

HIV-1 CRF02_AG is responsible for at least 8% of the HIV-1 infections worldwide and is distributed mainly in West Africa. CRF02_AG has recently been reported in countries where it is not native, including Brazil. In a previous study including 10 CRF02_AG Brazilian samples, we found at least four independent introductions and two autochthonous transmission networks of this clade in Brazil. As more CRF02_AG samples have been identified in Brazil, we performed a new phylogeographic analysis using a larger dataset than before. A total of 20 Brazilian (18 from Rio de Janeiro and two from São Paulo) and 1,485 African HIV-1 CRF02_AG *pol* sequences were analyzed using maximum likelihood (ML). The ML tree showed that the Brazilian sequences were distributed in five different lineages. The Bayesian phylogeographic analysis of the Brazilian and their most closely related African sequences ($n = 212$) placed the origin of all Brazilian lineages in West Africa, probably Ghana, Senegal, and Nigeria. Two monophyletic clades were identified, comprising only sequences from Rio de Janeiro, and their date of origin was estimated at around 1985 (95% highest posterior density: 1979–1992). These results support the existence of at least five independent introductions of the CRF02_AG lineage from West Africa into Brazil and further indicate that at least two of these lineages have been locally disseminated in the Rio de Janeiro state over the past 30 years.

The human immunodeficiency virus type 1 (HIV-1) is classified into four groups (M, N, O, and P), each one representing independent zoonotic transmission events to humans.[1] The M group is responsible for the worldwide pandemic, having evolved and diversified into different clades,[2] characterized by an extensive genetic diversity and further subdivided into pure subtypes (A–D, F–H, J, and K), subsubtypes (A1–A4, F1–F2), 72 circulating recombinant forms (CRFs) and multiple unique recombinant forms (URFs) (Los Alamos HIV Sequence Database—www.hiv.lanl.gov/).

Among the CRFs, the CRF02_AG is the most prevalent worldwide and is responsible for at least 8% of the HIV-1 global infections.[3] This recombinant is distributed mainly in West Africa and, to a lesser extent, in the Middle East and North Africa. In recent years, an increase in the number of CRF02_AG-infected patients has been observed in Europe,[4–6] North America,[7,8] and South America,[9–12] probably due to migrations from African endemic regions. In Brazil, HIV-1 molecular epidemiology studies based in the *pol* gene have identified a high prevalence of HIV-1 subtype B (70–90%), followed by subsubtype F1 (5–15%) and subtype C (1–10%) in almost the entire country, with the exception of the southern region, where subtype C reaches high prevalences (25–66%).[13–20]

Despite the predominance of subtypes B, F1, and C and recombinants between them in the Brazilian HIV-1 epidemic, clade CRF02_AG has already been described in the states of Rio de Janeiro,[10–12,15,21] São Paulo,[22,23] Pará,[16] and Bahia,[24] with prevalences between 0.2% and 1.9%, indicating a limited spread of this CRF in the country. However, these studies were conducted with convenience samples that might not represent the prevalence of this subtype in Brazil.

In a previous attempt to trace the origin of some of those sequences, we have found that at least four introductions of this clade occurred in Brazil and that at least two CRF02_AG Brazilian lineages were successful in getting established and disseminated throughout the Rio de Janeiro state. The precise time and country of origin of those CRF02_AG lineages introduced in Brazil, however, remain unknown.[10] As more CRF02_AG samples have been identified in Brazil,[12,22] we

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aimed to improve our knowledge about the spread of this CRF infection through the country by using robust Bayesian phylogeographic analysis to estimate the country of origin and date of introduction of Brazilian CRF02_AG lineages.

In this study, all available HIV-1 CRF02_AG *pol* sequences previously reported in Brazil (n = 19, 17 from Rio de Janeiro and two from São Paulo)[10,12,21,25,26] were combined with one new CRF02_AG sequence obtained from a sample collected from one antiretroviral (ARV)-naive pregnant woman recruited at the Hospital Geral de Nova Iguaçu (HGNI), Rio de Janeiro. This sample was amplified and sequenced as described elsewhere.[27] Brazilian sequences were combined with all HIV-1 CRF02_AG *pol* sequences from Sub-Saharan Africa available in the Los Alamos HIV Sequence Database (www.hiv.lanl.gov) that covered the entire protease and partial reverse transcriptase (PR/RT) regions (nt positions 2253–3272 relative to the HIV-1 HXB2 clone) and had a known sampling year. Sub-Saharan Africa has an estimated CRF02_AG prevalence of >7% and as the prevalence of this clade outside this region is very low,[3] sequences from other countries were not included in the study. The subtype assignment of all sequences was confirmed by the REGA HIV subtyping tool v.2,[28] neighbor-joining (NJ) phylogenetic analysis, and bootscanning analysis. The NJ phylogeny with HIV-1 group M subtype reference sequences was constructed with MEGA 5.0 software[29] under the Tamura–Nei nucleotide substitution model.

Bootscanning analyses were performed with the Kimura two-parameter model, within a 250 bp window moving in steps of 10 bases, using SimPlot 3.5.1 software.[30] Sequences with an incorrect CRF02_AG classification and multiple sequences from the same individual were removed, as well as sequences with 100% of identity recognized with the CD-HIT suite online web server.[31] These procedures resulted in a final dataset composed by 1,505 HIV-1 CRF02_AG *pol* sequences (AFR-BR-I) sampled from 17 African countries and Brazil (Table 1).

The phylogenetic signal of all datasets was evaluated by substitution saturation analysis, plotting the observed number of transitions and transversions against genetic distance for each pairwise comparison using DAMBE 5.3 software[32] and with the likelihood mapping method[33] by analyzing 10,000 random quartets using TREE-PUZZLE 5.2 software[34] in the web platform Mobyle@Pasteur (http://mobyle.pasteur.fr/). Maximum likelihood (ML) phylogenetic tree of the AFR-BR-I dataset was inferred under the $GTR + I + \Gamma_4$ nucleotide substitution model, selected using the jModeltest program.[35] The ML tree was reconstructed with PhyML 3.0 software[36] using an online web server[37] and visualized in FigTree 1.4 software.[38] A heuristic tree search was performed using the SPR branch-swapping algorithm and the reliability of the obtained topology was estimated with the approximate likelihood ratio test (aLRT)[39] based on the Shimodaira–Hasegawa-like procedure.

A second alignment (AFR-BR-II) was used for spatio-temporal reconstruction (Table 1). This alignment included all Brazilian sequences and the African sequences more closely related to the Brazilian ones, branching with high support (aLRT > 0.8) up to the second ancestral node in the ML phylogenetic tree. The evolutionary rate (μ, nucleotide substitutions per site per year, subst./site/year), the time of the most recent common ancestor (T_{MRCA}, years), and the geographic transitions of the AFR-BR-II dataset were jointly estimated using the Bayesian Markov Chain Monte Carlo (MCMC) approach as implemented in BEAST 1.8[40,41] with BEAGLE to increase computational speed.[42] Analyses were performed using the $GTR + I + \Gamma_4$ nucleotide substitution model, the uncorrelated relaxed lognormal molecular clock model,[43] and the nonparametric Bayesian Skyline coalescent tree prior as a coalescent demographic model.[44] Migration

Table 1. HIV-1 CRF02_AG *pol* Sequences Dataset Composition

		AFR-BR-I (ML)		*AFR-BR-II (Bayesian)*		
Region	*Country*	N	*%*	N	*Sampling date*	*%*
South America	Brazil	20	—	19[a]	1998–2012	—
Central Africa	Angola	1	0.1	0	—	0.0
	DRC[b]	8	0.5	0	—	0.0
West-Central Africa	Cameroon	538	36.2	22	1998–2009	10.4
	Equatorial Guinea	21	1.4	1	2008	0.5
	Gabon	57	3.8	2	2008	0.9
West Africa	Benin	93	6.3	10	2004–2009	4.7
	Burkina Faso	79	5.3	7	2003–2006	3.3
	Cape Verde	41	2.8	16	2010–2011	7.5
	Côte d'Ivoire	2	0.1	0	—	0.0
	Gambia	1	0.1	0	—	0.0
	Ghana	184	12.4	43	1993–2007	20.3
	Guinea	1	0.1	1	2007	0.5
	Guinea-Bissau	5	0.3	2	2004–2005	0.9
	Liberia	1	0.1	0	—	0.0
	Nigeria	165	11.1	38	1999–2009	17.9
	Senegal	233	15.7	61	1998–2011	28.8
	Togo	55	3.7	9	2006–2008	4.2

[a]The shortest sequence (KF922174) was excluded to avoid compromising the Bayesian estimates.
[b]The Democratic Republic of Congo.

events throughout the phylogenetic history were identified using a reversible discrete Bayesian phylogeographic model[45] with a continuous-time Markov chain rate reference prior.[46] MCMC chains were run for 2.5×10^8 generations and adequate chain mixing was checked by calculating the effective sample size (ESS) after excluding 10% burn-in using the TRACER 1.6 program.[47] Maximum clade credibility (MCC) trees were summarized from the posterior set of trees with TreeAnnotator 1.8[40] and visualized with FigTree 1.4 software.[38]

Transitions and transversions versus divergence analysis and likelihood mapping indicated that both AFR-BR-I and AFR-BR-II datasets retained enough phylogenetic signals for consistent phylogenetic and molecular clock inferences (Supplementary Fig. S1; Supplementary Data are available online at www.liebertpub.com/aid). The ML phylogenetic analysis of the AFR-BR-I dataset revealed that the 20 Brazilian CRF02_AG sequences formed two monophyletic clusters, consisting of 11 (BR-I) and six (BR-II) sequences. The remaining (three sequences) were sparsely distributed in the ML tree, intermixed among sequences of African origin (Fig. 1). We detected at least three independent introductions of the CRF02_AG clade in Rio de Janeiro (BR-I, BR-II and 06BRRJ34) and two in São Paulo (BR09SP371 and SP3686), but only the introductions into Rio de Janeiro (BR-I and BR-II) seem to have been successfully disseminated locally.

The lineage BR-I formed a highly supported clade (aLRT = 0.93) composed by 11 sequences sampled between 2004 and 2012 from patients living in the state of Rio de Janeiro. This lineage was described in our previous work as being composed of three sequences isolated between 2004 and 2010,[10] thus representing a four-times size increase of this lineage size since our previous work. It is noteworthy that seven of the new sequences were isolated between 2005 and 2007 from patients classified as recent HIV-1 seroconverters,[12] whereas the remaining one was isolated in 2012 from a 29-year-old pregnant woman diagnosed in the same year. The presence of HIV-1 recent seroconverters indicates the occurrence of the local spread of this CRF02_AG lineage in the Rio de Janeiro metropolitan region, at least in the past decade, probably through heterosexual contacts.

The lineage BR-II was composed of six sequences, all isolated from patients from Rio de Janeiro state that clustered together with high support (aLRT = 0.85). This CRF02_AG lineage was also described in our previous work as being composed of five sequences isolated between 2006 and 2011.[10] The new sequence identified within this cluster was isolated in 2002 from a patient attending the Army Health Service in Rio de Janeiro[21] and does not represent a recent transmission event. The remaining CRF02-AG Brazilian sequences (06BRRJ34, BR09SP371, and SP3686) did not form clusters. The sequences 06BRRJ34 and BR09SP371 were already described in our previous work[10] whereas sequence SP3686 corresponds to an CRF02_AG transmission event previously unidentified.

Sequence BR09SP371 was isolated in 2009 from an ARV-naive adult patient newly diagnosed in the state of São Paulo,[26] sequence 06BRRJ34 was isolated in 2006 from a woman from the city of Rio de Janeiro,[10] and sequence SP3686 was isolated in 1998 from a blood donor at the Blood Center of São Paulo.[25]

The Brazilian HIV-1 CRF02_AG sequences were combined with their most closely related African sequences in the ML phylogenetic tree. The lineages BR-I and BR-II and sequence 06BRRJ34 were nested within a highly supported CRF02_AG monophyletic clade (aLRT = 0.92), mainly composed of sequences from Senegal (31.8%), Ghana (23.5%), and Cameroon (11.7%). Sequence BR09SP371 was nested within a cluster with high support (aLRT = 0.87) composed mostly of sequences from Cape Verde (57.1%). Sequence SP3686 was located inside a relatively well-supported cluster (aLRT = 0.88) mostly composed of sequences from Nigeria (82.1%) (Fig. 1).

Comparing the frequencies of each African country in the complete (AFR-BR-I) and Brazilian-related (AFR-BR-II) datasets, an increase in the frequency of some West African countries including Ghana (from 12.4% to 20.3%), Nigeria (from 11.1% to 17.9%), Senegal (from 23.3% to 28.8%), and Cape Verde (from 2.8% to 7.4%) was noted as well as a reduction in the frequency of some West-Central African countries, mainly Cameroon (from 36.2% to 10.4%) and Gabon (from 3.8% to 0.9%) (Table 1).

The geographic origin and time scale of Brazilian HIV-1 CRF02_AG strains were inferred from the AFR-BR-II dataset using a Bayesian phylogeographic framework. The median evolutionary rate of the AFR-BR-II *pol* dataset, estimated under a chronological time scale employing the dates of the sequences, was 1.9×10^{-3} [95% highest posterior density (HPD): $1.4 \times 10^{-3} - 2.4 \times 10^{-3}$] substitutions/site/year, consistent with previous estimations made from this CRF.[48] The estimated coefficient of rate variation in this dataset was 0.34 (HPD: 0.28–0.39), thus supporting a significant variation of substitution rate among branches and the use of a relaxed molecular clock model. The BR-I and BR-II lineages formed highly supported monophyletic clades [posterior probability (PP) = 1] in the reconstructed Bayesian phylogeny and appeared to have originated in Ghana [posterior state probability (PSP) = 1 and PSP = 0.94, respectively] at around 1985 (HPD: 1979–1992). The sequence 06BRRJ34 probably originated in Ghana (PSP = 0.67) or Benin (PSP = 0.27), the sequence BR09SP371 in Senegal (PSP = 0.62) or Guinea-Bissau (PSP = 0.23), and the sequence SP3686in Nigeria (PSP = 1) (Fig. 2).

These results confirm that HIV-1 CRF02_AG strains circulating in Brazil are closely related to those circulating in western African countries from the Bight of Benin (Ghana, Nigeria, and Benin), Senegal, and Guinea-Bissau, where this CRF is highly prevalent.[3] Economic and cultural relationships between these African countries and Brazil are quite restricted, with the exceptions of Nigeria, for which Brazil is an important trading partner (DESA/UNSD, 2013), and Guinea-Bissau, which shares the Portuguese colonization and official language with Brazil.

West African countries have a long history of population migration, internally and externally, traditionally following old colonial linkages.[49] In Ghana and Nigeria, the emigration rate intensified in the 1970s and 1980s, as economic and political conditions worsened,[50] which may have contributed to the spread of HIV-1 CRF02_AG outside these countries, possibly also to Brazil. Thus, the introduction of CRF02_AG strains into Brazil seems to be the result of sporadic events, rather than the consequence of the continuous mobility of people between Brazil and West

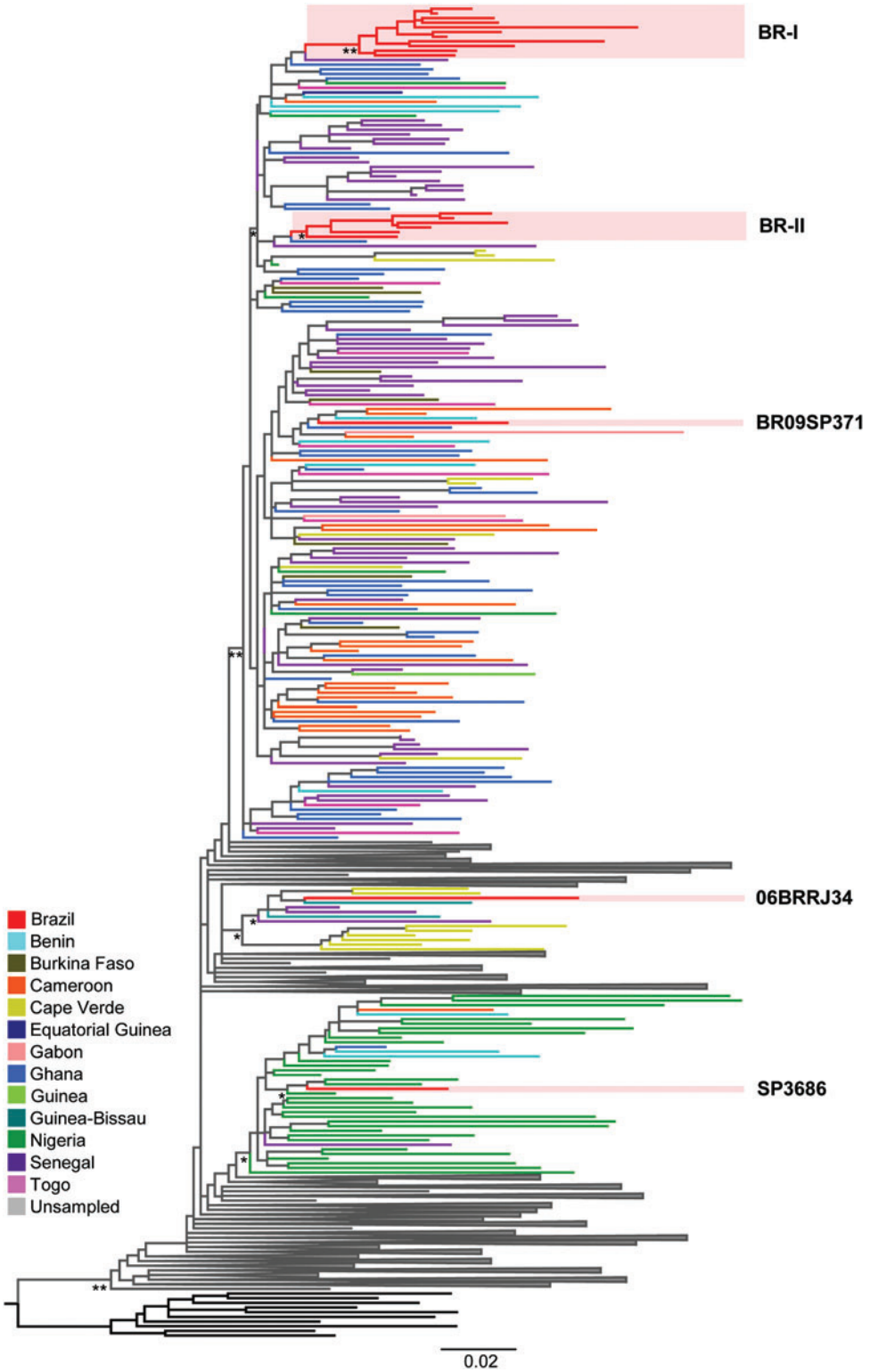

FIG. 1. Maximum likelihood (ML) tree of HIV-1 CRF02_AG *pol* (~1,000 pb) sequences from Brazil ($n = 20$) and African countries ($n = 1,485$). The tree was rooted using HIV-1 subtype G sequences (*black branches*). The branch colors represent the geographic origin of the sequences, according to the legend. The *red boxes* highlight the position of the Brazilian CRF02_AG sequences. For visual clarity, some African clades were collapsed in triangles and only the clades most closely related to the Brazilian lineages and used in the subsequent analysis are shown. The sequences not used in the phylogeographic analysis are in *gray*. *Asterisks* point to key nodes with aLTR support values between 0.80 and 0.9 (*) and >0.9 (**). Horizontal branch lengths are drawn to scale with the bar at the bottom indicating nucleotide substitutions per site. Color images available online at www.liebertpub.com/aid

Africa. However, it is important to note that immigration of Africans to Brazil has grown in recent years, mainly from Central and West African countries.[51] These countries have HIV-1 prevalence rates among adults ranging from 1% to 5%[52] and this migration increase can contribute to the future introduction and establishment of African HIV-1 variants in Brazil.

It is necessary to consider the sampling bias caused by the unequal number of sequences and countries used in the phylogeographic reconstructions. Here, we included only sequences from Sub-Saharan Africa, a region that has a consolidated HIV-1 CRF02_AG epidemic, particularly Western Africa, where the prevalence of this clade is ~50%.[3] Outside this continent, reports of CRF02_AG are anecdotal,[3]

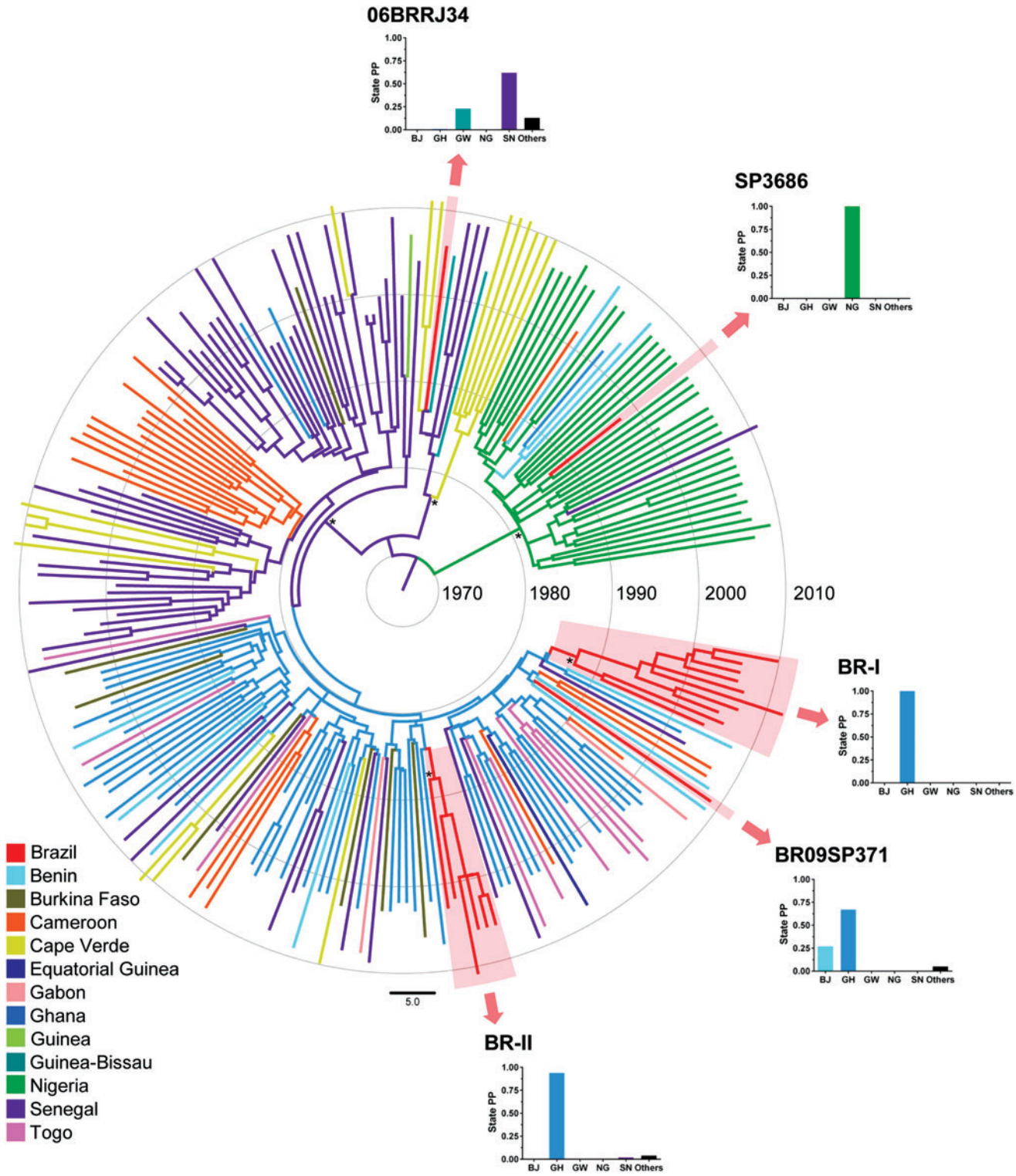

FIG. 2. Time-scaled Bayesian maximum clade credibility (MCC) tree of the HIV-1 CRF02_AG Brazilian strains and the most closely related African sequences ($n = 212$). Branches are colored according to the most probable location state of their descendent nodes as indicated in the legend. The *red boxes* highlight the position of the Brazilian HIV-1 CRF02_AG strains. *Asterisks* point to key nodes with high posterior probability support ($PP > 0.9$). The tree was automatically rooted under the assumption of a relaxed molecular clock and the branch lengths are drawn to scale with dates indicated in the concentric circles. The scale bar at the bottom indicates years. The posterior state probability (*PSP*) distributions at the first ancestral nodes of the Brazilian CRF02_AG strains at the Bayesian MCC tree are indicated in the graphics. Countries represented are BJ (Benin), GH (Ghana), GW (Guinea-Bissau), NG (Nigeria), SN (Senegal), and ''others'' for countries with minor contributions. Color images available online at www.liebertpub.com/aid

except in Europe, where an increasing prevalence of CRF02_AG among newly diagnosed HIV-1 infections was reported.[53] However, the estimated prevalence is <5% and this proportion is mostly due to immigrants originating from Africa.[53] This study clearly demonstrates the dissemination of CRF02_AG lineages of Western African countries to Brazil; however, the exact route of migration of these lineages remains unclear. It is not possible to exclude the hypothesis of Europe acting as a staging post in the dissemination of CRF02_AG to Brazil; however, the low prevalence of this HIV-1 clade in Europe makes this scenario unlikely.

Dated phylogeny made it possible to estimate 1985 (HPD: 1979–1992) as the T_{MRCA} of both BR-I and BR-II lineages, thus suggesting that the HIV-1 CRF02_AG strains started to circulate in Brazil around 10 years later than the other more prevalent HIV-1 subtypes C and F1.[54–56] Although the conditions of introduction of subtypes C, F1, and CRF02_AG in Brazil may have been similar, involving a single or a few related strains of African origin,[54,57–59] the relatively late spread of these CRF02_AG lineages may have limited the dissemination of this viral clade, possibly because it was introduced when the HIV-1 epidemic in Brazil started to stabilize.[60] Variation in the spread of different HIV-1 clades may also be explained by differences in the efficiency of transmission networks that promoted the initial dissemination of these lineages.

HIV-1 subtypes C and F1 may have been introduced and initially disseminated through highly connected networks of injection drug users.[61–63] The CRF02_AG clade, however, seems to have been introduced and mostly disseminated in poorly connected networks primarily involving sexual transmissions, thus resulting in a limited propagation.

The results presented here add a body of evidence to support the existence of at least five independent introductions of the CRF02_AG lineage from West Africa into Brazil and further indicate that at least two of these lineages have been disseminated in the Rio de Janeiro state for about 30 years, reinforcing the important role of this state in the introduction of new HIV-1 strains of African origin. Previous studies have also described the presence of HIV-1 lineages of African origin in the Rio de Janeiro state, such as HIV-1 subtype D variants, probably originating from South Africa,[64] and five HIV-1 subtype C lineages, probably imported from different eastern and southern African countries.[54] In addition, the periodic isolation of typical African HIV-1 subtypes A, D, and CRF02_AG in Rio de Janeiro reinforces the hypothesis that these lineages are being introduced and becoming established in this state, albeit in a minority form.[10,11,65–68] Altogether, the continuous surveillance of HIV-1 genetic diversity is warranted and fundamental to the early detection of the introduction and dissemination of newly emerging viral clades in the Brazilian epidemic, which may expose unknown transmission networks.

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Author Disclosure Statement

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