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Molecular epidemiology and phylogeography of a HIV-1 subtype F cluster recently expanded among men who have sex with men in Spain, deriving from a variant widely circulating in Western Europe

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Background

We recently reported an HIV-1 subtype F outbreak among men who have sex with men (MSM) in Galicia, Northwest Spain, comprising 41 viruses grouping in a monophyletic cluster related to the Brazilian F1 subsubtype variant*. The published report analyzed samples collected from 2009, when the first case was diagnosed, to April 2011. Here we update this outbreak with samples collected up to December 2013, analyze near full-length genomes, determine phylogenetic relationships, and estimate its geographic and temporal origin.

*Thomson MM, Fernández-García A, Delgado E, et al. Rapid expansion of a HIV-1 subtype F cluster of recent origin among men who have sex with men in Galicia, Spain. *J Acquir Immune Defic Syndr*. 2012;59:e49-e51.

Methods

HIV-1 segments in protease-reverse transcriptase (PR-RT) and env V3 region were amplified from plasma RNA by RT-PCR and nested PCR. Near full-length genomes were amplified in four overlapping fragments from RNA extracted either from plasma or from culture supernatants of primary isolates. Phylogenetic analyses were performed via maximum likelihood with RAxML applying the GTR+G+I substitution model. Recombination was analyzed by bootscanning with SimPlot. Relationships with database sequences were examined by BLAST searches followed by phylogenetic analyses. Most probable locations at nodes and times of most recent common ancestors (tMRCA) were estimated using PR-RT sequences with a Bayesian method implemented in BEAST, with results summarized in a maximum clade credibility tree.

Results

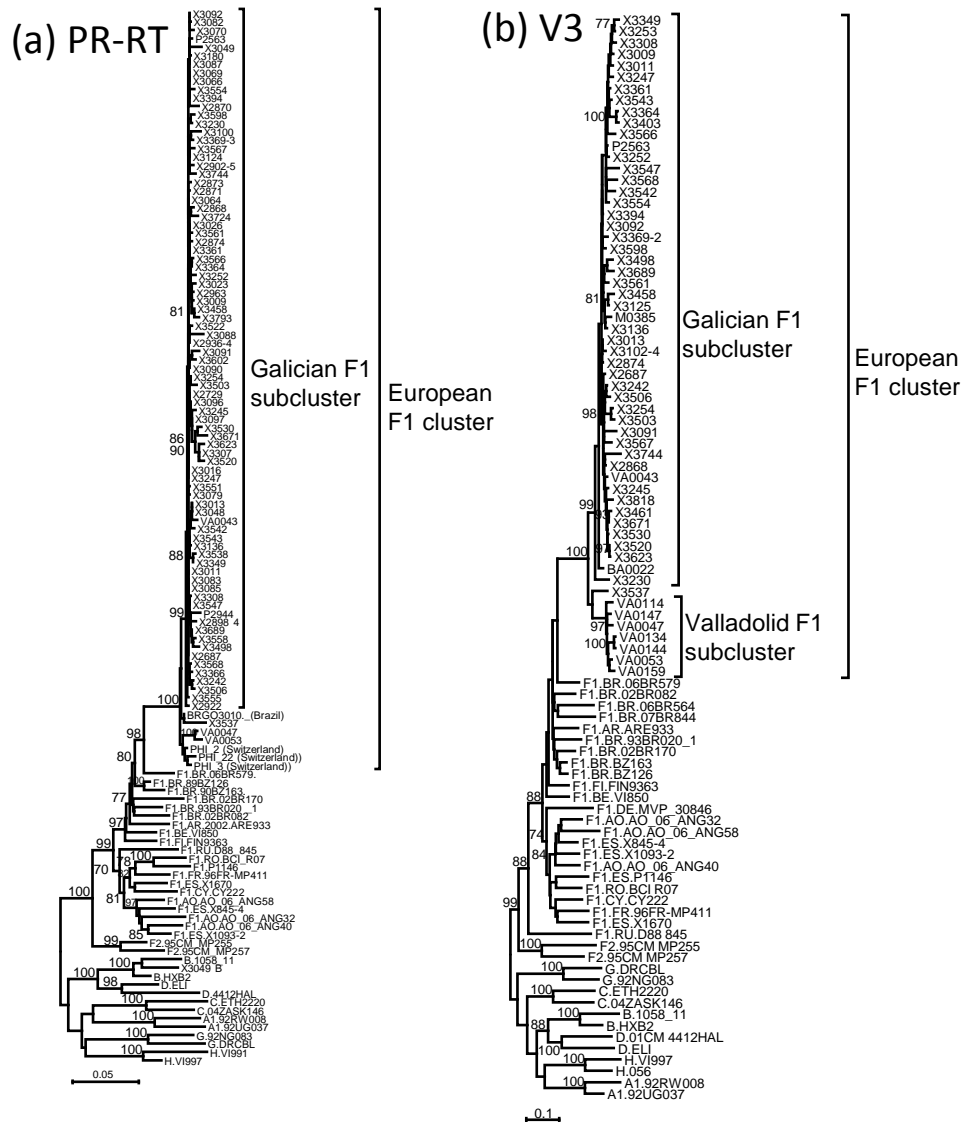


Fig. 1. Maximum likelihood trees of PR-RT (a) and V3 (b) segments of viruses of the subtype F cluster from Spain. The PR-RT tree also includes 3 sequences from Lausanne, Switzerland (PHI_2, PHI_3, PHI_22) and 1 from Goiania, Brazil (BRGO3010) previously identified by us to be closely related to the Spanish viruses of the subtype F cluster. Both trees also include references of F1 subsubtype. Country of sample collection of these viruses is indicated with the ISO two-letter country code. Among viruses of this study collected in Spain, those with names starting with X, P, VA, BA, and M are from the region of Galicia and the Spanish cities of Bilbao, Valladolid, Badajoz, and Madrid, respectively. Only bootstrap values $\geq 70\%$ are shown.

92 viruses group in the Galician F1 subcluster, most of them from Galicia, but also from Bilbao (n=2), Madrid (n=1), Valladolid, (n=1) and Badajoz (n=1). 7 viruses from Valladolid (Central Spain) group in another subcluster. Both subclusters form part of what we term the European F1 cluster, also comprising viruses from Switzerland, 1 from Brazil and several from other Western European countries (see below). In total, 100 viruses collected in Spain in 2009-2013, sequenced in PR-RT and/or the V3 region, were identified as belonging to the European subtype F cluster.

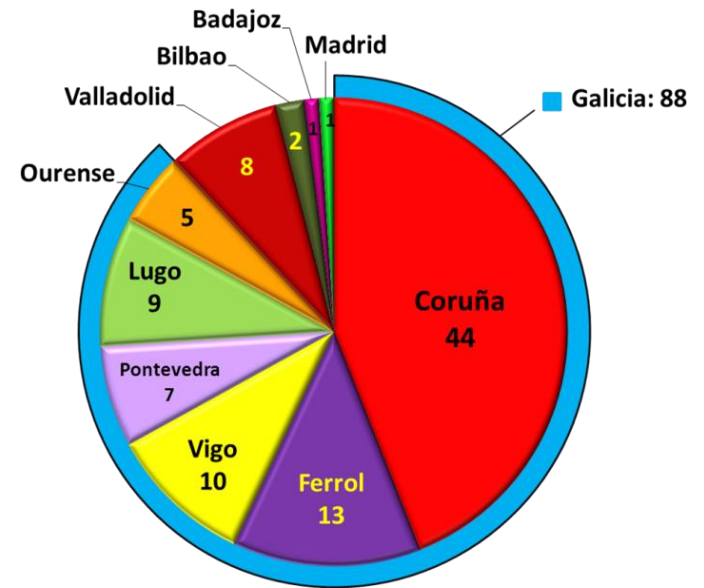


Fig. 2. Cities of sample collection of viruses of the subtype F cluster, shown in a map of Spain and as a pie chart. 88 viruses were from Galicia (44 from A Coruña) and 12 from four other regions. All individuals harboring them were men, and all 94 with reported transmission routes were infected sexually. Of these, at least 71 were MSM. At least 12 infections correspond to recent seroconversions (<1 year from last seronegative to first seropositive samples).

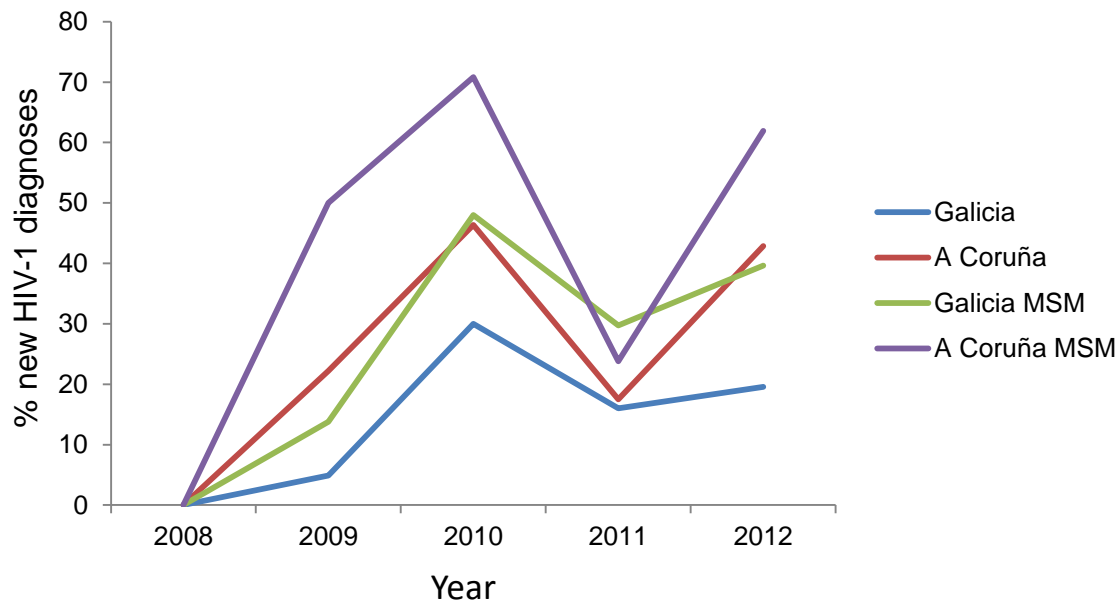


Fig. 3. Prevalence of infections with viruses of the subtype F cluster in 2009-2012 in the region of Galicia and in the Galician city of A Coruña, among all new HIV-1 diagnoses and among new HIV-1 diagnoses in MSM in each corresponding year.

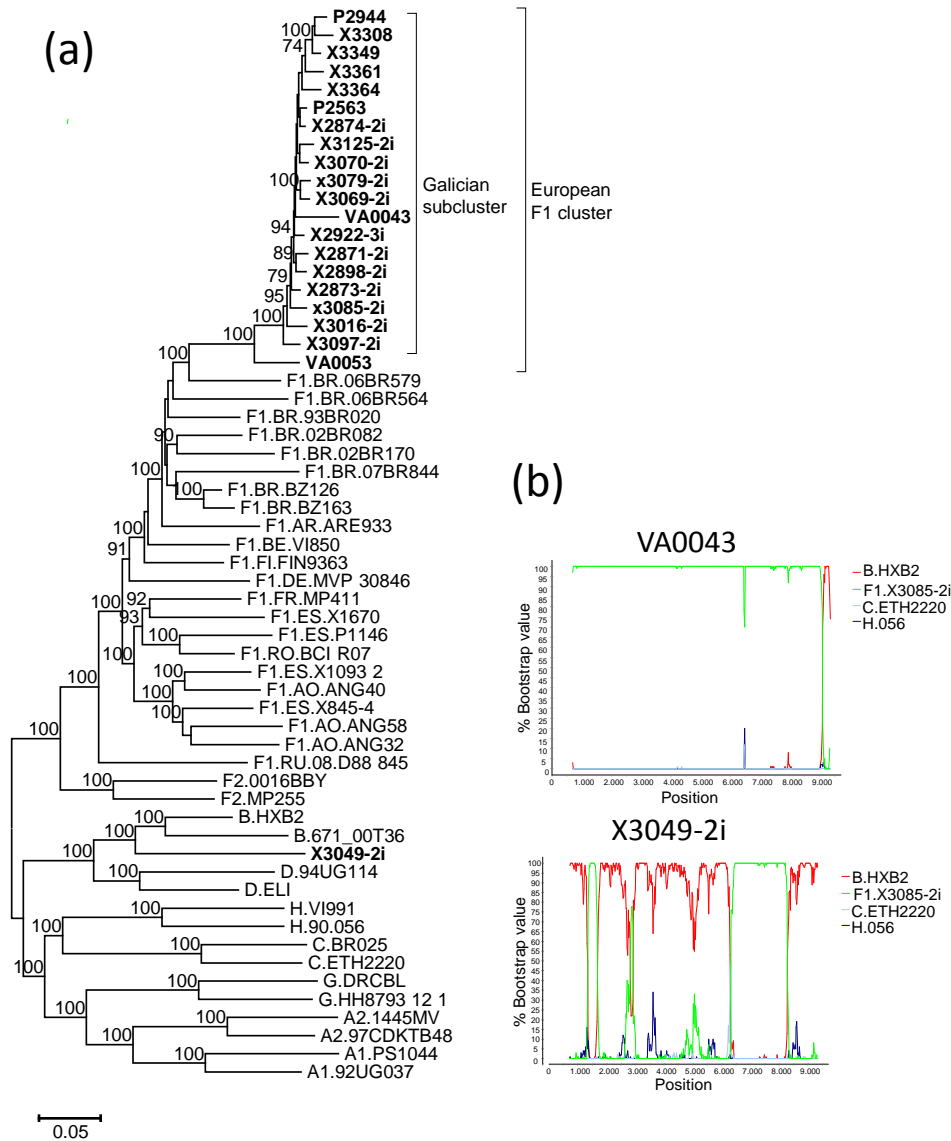


Fig. 4. Analysis of near full-length genomes of the subtype F cluster (≥ 8 kb). 21 were obtained, 13 from primary isolates (labeled with “i”) and 8 from plasma. (a) Phylogenetic tree. 20 sequences group in a cluster, with VA0053 branching in a basal position. One sequence (X3049-2i) branches with subtype B (this isolate derives from a dual infection, as identified in the PR-RT sequence, where numerous mixed positions with nucleotides characteristic of subtypes B and F1 were observed). (b) Bootscan analyses. Most viruses of the F1 cluster were uniformly of F1 subspecies (not shown), except VA0043, which had a short subtype B segment in the nef-coding region. X3049-2i was also BF1 recombinant, with most of its genome deriving from subtype B

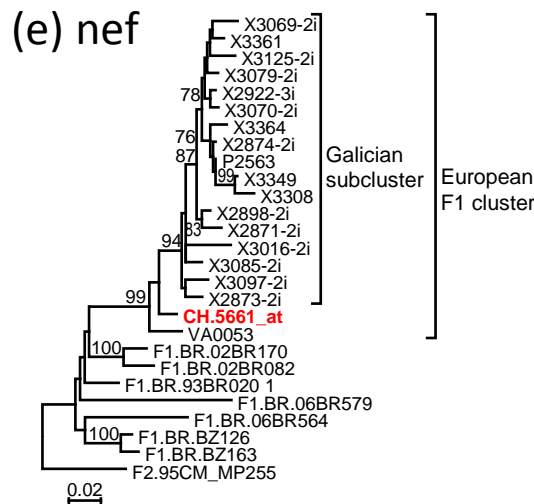
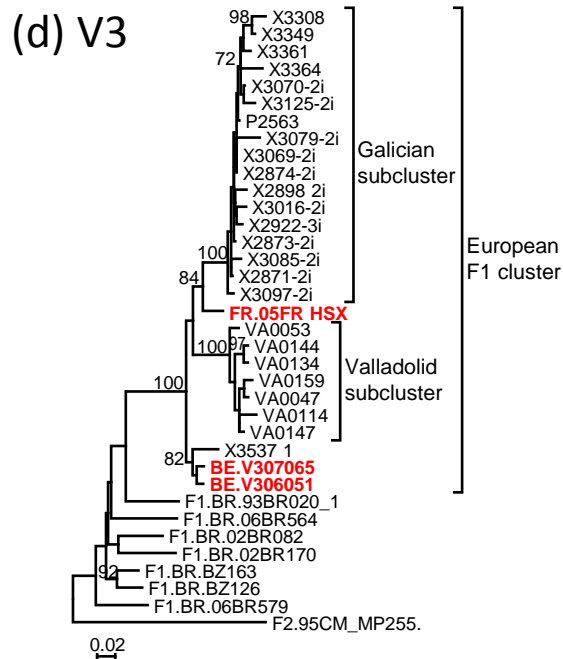
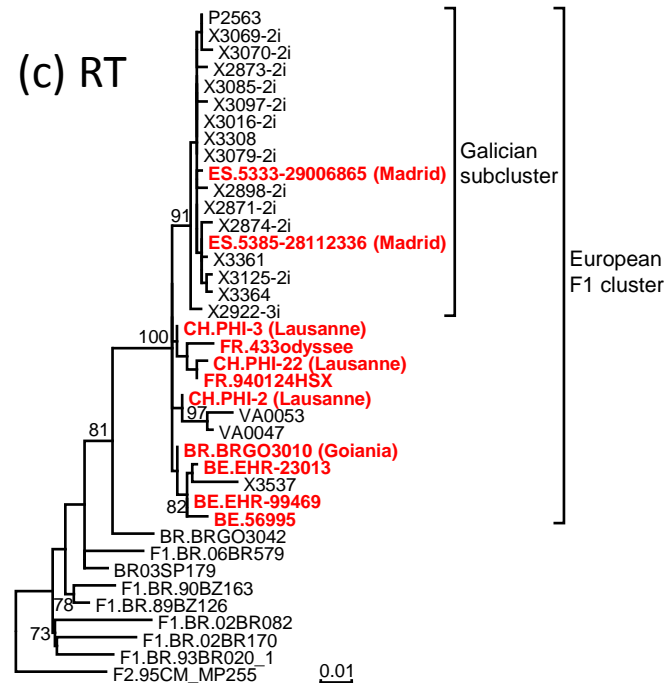
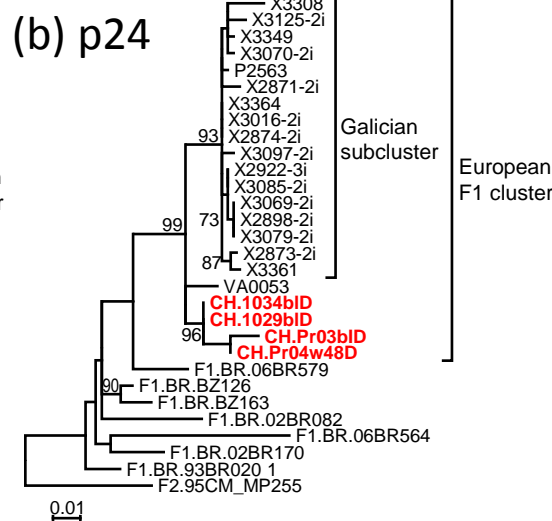
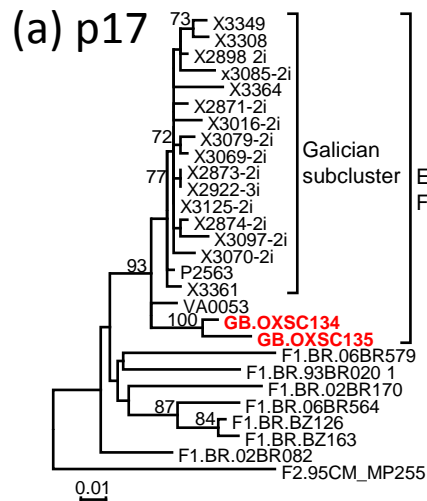


Fig. 5. Viruses of the subtype F cluster from databases. 8 viruses from Switzerland (CH), 6 from Belgium (BE), 3 from France (FR), 2 from the United Kingdom (GB), 1 from Goiania, Brazil, and 2 from Madrid, Spain, (shown in red) branched in the European subtype F cluster. Except those from Madrid, viruses branched outside of both Galician and Valladolid subclusters. Viruses from Switzerland and Belgium grouped in local subclusters. One virus from Galicia (X3537) branched with Belgian viruses, suggesting a Belgian ancestry.

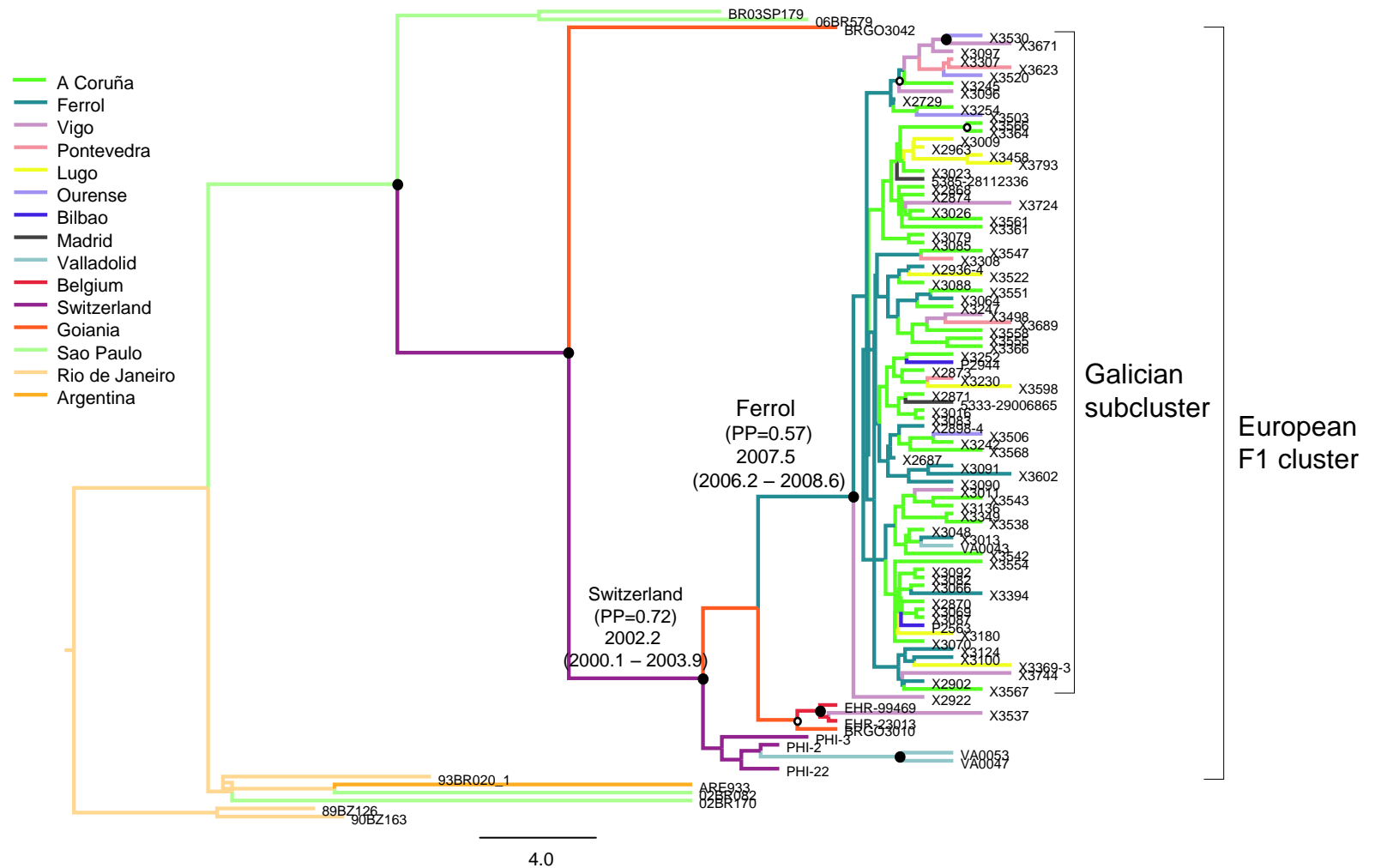


Fig. 6. Maximum clade credibility tree of PR-RT sequences of the subtype F European cluster and Galician subcluster. Nodes supported by PP=1 and PP=0.95-0.99 are marked with filled and unfilled circles, respectively. Colors of terminal and internal branches represent, respectively, sampling locations and most probable locations of the corresponding nodes, according to the legend on the left. For the nodes corresponding to the Galician subcluster and the European cluster, the posterior probabilities for the most probable locations and the tMRCAs, with 95% HPD intervals in parentheses, are indicated above the subtending branches.

Conclusions

- A recently expanded HIV-1 subtype F cluster originated in Galicia, Northwest Spain, continues to circulate widely in this region among MSM and has spread to other Spanish regions.
- This cluster derives from a larger subtype F cluster circulating in at least four other Western European countries: Switzerland, France, Belgium and the United Kingdom.
- Viruses of the subtype F cluster group in local subclusters in Valladolid (Central Spain), Switzerland, and Belgium, in addition to Galicia, indicating local spread of the viruses in multiple distant geographical areas.
- Phylogeographic analyses indicate that the most probable origin of the Galician subtype F subcluster is the city of Ferrol, around 2007, and that of the European cluster, Switzerland, around 2002.
- The subtype F cluster here described is the largest non-subtype B cluster reported in Western Europe.
- Its rapid expansion and its spread throughout multiple local networks suggest some inherent biological feature of high sexual transmissibility.

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