Journal of Medical Virology

WILEY

JOURNAL OF MEDICAL VIROLOGY

RESEARCH ARTICLE

Identification and Molecular Characterization of 10 Novel Recombinant Variants of Hepatitis C Virus (HCV) in Cameroon Using Oxford Nanopore Sequencing and Phylogenomic Analysis

Aristide Mounchili-Njifon[1,2] | Vireak Heang[3] | Leakhena Pum[3] | Loique Landry E. Messanga[1] | Abdou Fatawou Modiyinji[1] | Moise Henri Moumbeket-Yifomnjou[1] | Herman Philipe Nfombouot-Njitoyap[1] | Simon Frederic Lissock[1] | Pretty Rosereine Mbouyap[1] | Jean Paul Assam Assam[2] | Erik A. Karlsson[3] | Janin Nouhin[3] | Richard Njouom[1]

[1]Department of Virology, Centre Pasteur of Cameroon, Yaoundé, Cameroon | [2]Department of Microbiology, Faculty of Sciences, University of Yaounde I, Yaound, Cameroon | [3]Virology Unit, Institut Pasteur du Cambodge, Pasteur Network, Phnom Penh, Cambodia

Correspondence: Richard Njouom (njouom@pasteur-yaounde.org)

Received: 23 February 2026 | **Revised:** 24 July 2026 | **Accepted:** 10 August 2026

Funding: Institut Pasteur in Paris

Keywords: Cameroon | hepatitis C virus (HCV) | inter-genotypic recombination | Oxford nanopore technology sequencing (ONT)

ABSTRACT

Hepatitis C virus (HCV) remains a major global public health concern due to the absence of an effective vaccine and its diversity, which complicate diagnosis, genotyping, and clinical management despite the widespread use of direct-acting antivirals (DAAs). In regions where multiple HCV genotypes co-circulate, inter-genotypic recombination represents an additional challenge for accurate classification and molecular surveillance. In this study, we investigate the presence and characterize inter-genotypic recombinant strains of HCV in Cameroon. Among 512 chronically infected patients initially genotyped by Sanger sequencing of Core and NS5B regions, 10 samples (1.9%) showed genotypic discordance, suggesting putative recombination. Whole-genome sequencing using Oxford Nanopore technology, combined with phylogenomic and recombinant analysis, confirmed the recombinant nature of all 10 isolates. Most recombinant (7/10) shared a unique breakpoint in the NS2/NS3 region, primarily displaying a genotype 2/1 genomic profile, while one group had a breakpoint in the E2/p7 region (genotype 4/1 genomic profile). One isolate exhibited a more complex structure with two successive breakpoints (4/2 followed by 2/1). Genotypes 1 and 2 were the most frequently involved parental lineages. These findings demonstrate the ongoing circulation of diverse natural HCV recombinant forms in Cameroon and identify genomic regions that appear preferentially involved in recombination events. Our results highlight the limitations of genotypic approaches based on partial genomes and underscore the importance of comprehensive genomic characterization to improve HCV genotype classification, surveillance, and therapeutic strategies in Central Africa.

Abbreviations: CI, confidence interval; CPC, Centre Pasteur du Cameroun; DAA, direct-anting antiviral; E2, envelope protein 2; EASL, European Association for the Study of the Liver; HCV, hepatitis C virus; NGS, next-generation sequencing; NS2, non-structural protein 2; NS3, non-structural protein 3; NS5A, non-structural protein 5A; NS5B, non-structural protein 5B; P7, protein 7; RNA, ribonucleic acid; SISPA, sequence-independent single-primer amplification; WHO, World Health Organization.

Janin Nouhin and Richard Njouom are Joint senior authors.

1 | Introduction

Chronic infection with the hepatitis C virus (HCV) remains a major global public health challenge, affecting an estimated 50 million people worldwide and accounting for approximately 242 000 deaths in 2022, primarily due to cirrhosis or hepatocellular carcinoma [1]. Although direct-acting antivirals (DAAs) achieve cure rates exceeding 95%, access to diagnosis and treatment remains limited in many low- and middle-income countries, and the absence of an effective vaccine continues to hinder global elimination efforts [2].

HCV is a positive-sense single-stranded RNA virus belonging to the Flaviviridae family [3]. Its approximately 9.6 kb genome encodes a single polyprotein that is cleaved by viral and cellular proteases into three structural proteins (capsid and envelope glycoproteins E1 and E2), as well as seven non-structural proteins (p7, NS2, NS3, NS4A and NS4B, and NS5A and NS5B), which are essential for viral replication and assembly [4].

The high genetic diversity of the HCV has led to the emergence of eight genotypes and more than 90 subtypes, many of which display geographically restricted distributions [5]. With the exception of genotypes 5 and 7, all genotypes are further subdivided into multiple subtypes, with intra-subtypic nucleotide divergence generally less than 15%. Despite this great genetic diversity, recombination has historically been considered a relatively rare evolutionary mechanism in HCV with other RNA viruses such as HIV-1 [6].

Viral recombination results in the generation of chimeric genomes through the exchange of genetic material between distinct viral lineages and requires co-infection of the same host by at least two viral variants. In HCV, natural recombination events are rare, likely because infected hepatocytes are less permissive to the replication of a secondary infecting virus, making it improbable for a single cell to be infected by multiple HCV strains [7, 8]. Nonetheless, several types of recombination have been reported over the years and classified into three categories: inter-genotypic, inter-subtypic, and intra-patient/intra-subtypic recombination [9]. To date, 11 types of inter-genotypic recombination (2k/1b, 2i/6p, 2b/1b, 2/5, 2b/6w, 4/1, 3a/1b, 3a/1a, 2b/1a, 2a/1a, and 6a/3a) and seven types of inter-subtypic recombination (1b/1a, 1a/1c, 6a/6o, 6e/6o, 6e/6h, 6n/6o, and 4a/4d) have been described based on complete or partial genomic sequences [10–17]. Intra-patient recombination events have also been reported, although they are more difficult to detect due to the genetic relatedness of viral sequences [10, 18].

Recombination breakpoints have been identified throughout the HCV genome, including both non-structural and structural regions [9, 19–21]. Although the full biological and clinical significance of the recombination remains to be determined, this process may accelerate viral adaptation by linking together multiple advantageous mutations that can confer drug resistance or escape mechanisms, potentially affecting diagnosis, therapy, and epidemiology [22]. The identification of new recombinant HCV strains and positive selection sites is crucial for the development of antiviral intervention measures [23].

Advances in next-generation sequencing (NGS) technology have substantially improved the characterization of viral genetic diversity. Compared with traditional Sanger sequencing, NGS enables comprehensive analysis of viral populations, facilitating the detection of mixed infections and the identification of recombinant variants, including those present at low frequencies [24–27].

In Cameroon, HCV infection is considered slightly endemic, with a prevalence below < 2% in the general population, where genotypes 1, 2, and 4 are the most frequently reported [28]. To date, only two studies have previously reported HCV recombinants: one based on partial genomic regions using Sanger sequencing and another identifying recombination breakpoints from full-length genomes generated using by Illumina NGS [20, 29]. In our previous study, we identified 10 HCV-positive samples, collected between 2013 and 2023, with discordant genotype between the core and non-structural protein 5B (NS5B) using Sanger sequencing, suggesting the presence of putative recombinant forms of HCV [30]. However, a systematic and comprehensive mapping of inter-genotypic recombination points across the complete HCV genome from Cameroonian strains remains nonexistent. In the present study, we characterized these 10 putative inter-genotypic recombinant HCV strains using whole-genome sequencing. We provide new insights into the diversity, breakpoint distribution, and epidemiological relevance of recombinant HCV strains; the adaptation of anti-HCV treatments in response to recombinants and associated resistances; and the design of vaccines in the context of high viral diversity.

2 | Materials and Methods

2.1 | Study Population and Samples

This cross-sectional retrospective study was conducted at the Centre Pasteur du Cameroun (the national reference laboratory) between January 2013 and October 2023. From an initial cohort of 512 plasma samples positive for HCV RNA, genotyping by Sanger sequencing of the *Core* and *NS5B* regions was routinely performed. Ten samples that showed discordant genotyping results between these two regions, as previously reported [30], were selected for whole-genome sequencing using NGS (Nanopore) technology to confirm the recombination events and determine their precise breakpoints.

2.2 | Ethical Statement

The study protocol was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki, and was approved by the Regional Center's Human Health Research Ethics Committee (CRERSH/C) under approval number: E00571/CERSH/C/2023.

Given the retrospective nature of the study and the use of anonymized samples obtained through routine diagnostic procedures, the requirement for informed consent was waived by the ethics committee. No additional biological samples or clinical data were collected specifically for this study.

2.3 | Viral RNA Extraction

Total RNA nucleic acid extraction from 200 μL of plasma was performed using the Quick-DNA/RNA Pathogen Miniprep kit

(Zymo Research, reference R1053) according to the manufacturer's instructions with some modifications. Briefly, 200 μL of plasma was mixed with 200 μL of DNA/RNA Shield (Zymo Research) and 4 μL of Proteinase K. After incubation at room temperature for 5 min, 800 μL of Pathogen DNA/RNA Buffer was added. The mixture was then transferred to a Zymo-Spin IICR column and centrifuged at 12 000*g* for 1 min. The flow-through was discarded, and the step was repeated if necessary. The column was washed with 500 μL of Pathogen DNA/RNA Wash Buffer by centrifuging at 12 000*g* for 1 min, and this washing step was repeated once. An additional wash with 500 μL of 100% ethanol was performed. The column was dried by centrifugation at 12 000*g* for 1 min. Nucleic acids were eluted with 50 μL of DNase/RNase-free water (ZymoBiOMICS DNase/RNase-Free Water) and stored at −80°C until further analysis.

2.4 | HCV Whole-Genome Sequencing

To confirm the recombination events and determine the precise recombination breakpoints initially suggested by Sanger sequencing, whole-genome sequencing was performed on all 10 HCV samples (15054469, 15099836, 15130451, 16068628, 16144336, 16658500, 17118155, 18064594, 18079447, and 14029187) that were previously identified as putative recombinants. Sequencing was conducted using ONT sequencing at the Virology Unit, Institut Pasteur du Cambodge, using a sequence-independent single-primer amplification (SISPA) metagenomic approach as previously described [31, 32]. Briefly, extracted viral RNA was treated with DNase prior to reverse transcription. Double-stranded complementary DNA (cDNA) was generated using random primers and then amplified by PCR with a high-fidelity polymerase. Purified amplicons were used for barcoded libraries preparation using the ONT ligation kit (kit SQK-NBD114.24), followed by adapter ligation. Libraries were loaded onto a flow cell (R10.4.1) and sequenced on the GridION sequencer. Raw electrical signals were converted into FAST5 files and then basecalled into FASTQ format, and sequence reads were demultiplexed based on barcode sequences.

2.5 | Genome Assembly and Analysis

Raw FASTQ data were assessed for quality using FastQC, trimmed for adapters and primers using Porechop, and filtered (length > 500 bp, quality > Q10) with NanoFilt. De novo assembly was performed using MEGAHIT, followed by taxonomic classification of the contigs via Kraken2. The contigs assigned to HCV were extracted using a custom script, aligned against reference sequences with Minimap2, and the majority consensus sequences were generated with samtools. The complete genome was then subtyped utilizing a phylogenetic boot scanning approach implemented via SimPlot++ version 1.3 [33] and SimPlot version 3.5.1 [34], with a sliding window of 100 bp, a step size of 10 bp, and a distance model optimized by GTR (or Kimura two-parameter with Ts/Tv = 10 for Bootscan). DIVEIN was used to calculate the maximum likelihood pairwise genetic distances of the genomic regions, confirming the parental subtypes (1l_KC248193, 1e_KC248194, 2a_AB047639, 4f_EF589161, 4p_FJ462431). Sequencing depth for the 10 samples ranged from 150× to 1200×, with a minimum of 50× at all genomic positions. Consensus sequences were generated by applying a minimum coverage threshold of 20×, which allowed us to achieve > 99% identity. Following quality filtering, the average read length was between 850 and 1200 bp.

2.6 | In-Depth Analysis of Recombination

To complement phylogenetic boot scanning of consensus genomes, an informative yet limited approach that cannot fully leverage NGS data or conclusively rule out minority genotype co-infections, we performed a supplementary similarity and boot scanning analysis using SimPlot++. This method enabled recombination detection directly from long-read data. An alignment was constructed using the consensus genome together with two putative parental reference genomes. Using positional information from the aligned reads, the number of nucleotide differences between each read and the reference sequences was calculated and normalized into relative proportions. These values were then plotted against genomic position to generate a relative similarity plot for the assessment of recombination breakpoints. The outcomes of this analysis are documented in Tables 1 and 2.

All analyses were performed with a sliding window of 100 base pairs and a step size of 10 bp. For the Simplot++ analyses, an optimized GTR substitution model was used. For the Bootscans, the Kimura two-parameter model was employed with a transitions/transversions (Ts/Tv) ratio fixed at 10. The concordance between the two methods confirmed the natural origin of the 10 intergenotypic recombination events.

2.7 | Accession Numbers of Nucleotide Sequences

The consensus of the HCV whole-genome sequence obtained by ONT sequencing of 10 recombinant cases has been submitted to GenBank and is accessible under the following accession numbers: PX361034, PX446960, PX446221, PX445002, PX376056, PX376057, PX445001, PX446959, PX376058, and PX376059.

3 | Results

3.1 | Characterization of Complete Recombinant Genomes of HCV

Whole-genome Nanopore sequencing confirmed the recombinant nature of the 10 suspected isolates (15054469, 15099836, 15130451, 16068628, 16144336, 16658500, 17118155, 18064594, 18079447, and 14029187), generating 10 high-fidelity consensus sequences covering approximately 9.6 kb with an identity exceeding 99%. These sequences revealed phylogenetic discordances between the Capsid and NS5B regions, indicating natural recombination events [30]. The main demographic and virological characteristics of the 10 patients (age, sex, place of residence, and viral load) are presented in Table 1.

The analysis of these 10 genomes through Nanopore sequencing, along with similarity analyses and bootscans (via SimPlot++), confirmed the hypothesis of putative recombinants previously revealed by Sanger sequencing by identifying precise breakpoints,

TABLE 1 | Demographic and virological characteristics of the study population.

Code	Age	Sex	Region of Cameroon	Viral load (IU/mL)
15054469	64	Female	Center	3 972 095
15099836	63	Female	Center	4 044 350
15130451	70	Male	Center	16 261
16068628	76	Female	Center	3 361 632
16144336	73	Male	Center	3 613 772
16658500	66	Male	Center	264 892
17118155	72	Male	Center	120 541
18064594	59	Male	Center	84 334
18079447	48	Female	Center	571 847
14029187	56	Female	Center	451 435

primarily in the NS2/NS3 regions (7 out of 10 cases) and E2/p7 (3 cases), primarily involving genotypes 1 and 2, with one complex case having two breakpoints (4/2 then 2/1). The observed recombinant profiles included 2/1 (six cases), 4/1 (three cases), and 4/2/1 (one case), suggesting that NS2/NS3 serves as a hotspot for recombination of HCV in this population. These 10 genomes have been deposited in GenBank under the access numbers PX361034, PX446960, PX446221, PX445002, PX376056, PX376057, PX445001, PX446959, PX376058, and PX376059 for accessibility to the scientific community. These results highlight "hotspots" of recombination and emphasize the predominant involvement of genotypes 1 and 2 (Figure 1).

Regarding Figure 1, for each isolate (Panels 1 to 10), the Simplot ++ analysis (left plot, A) displays the percentage of nucleotide identity along the HCV genome, referenced according to the numbering of the genotype 1a H77 strain (positions ~1 to 9600). The corresponding Bootscan analysis (right plot, B) shows the percentage of bootstrap trees supporting the query sequence's affiliation to each of the putative parental genotypes within a sliding window.

The analyzed query sequences correspond to the confirmed recombinant isolates: 15054469, 15099836, 15130451, 16068628, 16144336, 16658500, 17118155, 18064594, 18079447, and 14029187. The reference sequences of the suspected parental genotypes are indicated by distinct colors: genotype 1l (KC248193) in red, 1e (KC248194) in orange, 2a (AB047639) in blue, 4f (EF589161) in green, and 4p (FJ462431) in purple.

A genomic schematic below each Bootscan analysis illustrates the inferred recombinant architecture, with a color change indicating the breakpoint(s). The analyses revealed that six cases (1, 3, 5, 6, 9, 10) exhibited a 2/1 recombination profile with a single breakpoint in the NS2/NS3 region. Three cases (2, 4, 7) showed a 4/1 profile with a breakpoint in E2/p7. One complex case (8) presented two breakpoints, suggesting successive 4/2 recombination in E2/p7 followed by 2/1 recombination in NS2/NS3.

The main recombinant cases of HCV described in our study, the most frequently observed breakpoint/recombination regions, and the approximate sizes of the major genes and proteins are presented in Table 2 below, according to the H77 reference strain (genotype 1a, total length ≈ 9646 nucleotides).

The positions are approximate and based on alignment with the reference strain H77 (genotype 1a, NC_038882).

The case in Panel 8 featured two recombination breakpoints, involving first a recombination between genotypes 4 and 2 in the E2/p7 region, followed by a recombination between genotypes 2 and 1 in the NS2/NS3 region. All other cases showed a single recombination point. The majority of recombinations (7/10) occurred in the NS2/NS3 region, and genotypes 1 and 2 were the most frequently involved. These results suggest that certain genomic regions, such as NS2/NS3, may be recombination hotspots for HCV, and that genotypes 1 and 2 are particularly prone to these events in our study population. It is important to note that, although these parental subtypes have already been described in the literature, the recombinant forms themselves are novel, as no identical recombinant structure has been reported to date.

4 | Discussion

This study employed a combined Sanger and long-read NGS strategy to identify and characterize 10 inter-genotypic recombinant HCV strains (1.9% prevalence) within a cohort of 512 chronically infected patients in Cameroon. The majority of recombination breakpoints (7/10) clustered at the NS2/NS3 junction, reinforcing the role of this genomic region as a hotspot for natural HCV recombination, a finding of particular relevance in the Central African context where genotypes 1, 2, and 4 co-circulate [35–38].

This prevalence of 1.90% for putative recombinant strains exceeds the sporadic estimates previously reported in Cameroon [39] and contributes to the growing body of evidence highlighting the underappreciated diversity of HCV in Central Africa. While mixed infections involving multiple HCV genotypes have been documented in 5%−10% of HCV-seropositive individuals in the general population and up to 14%−39% among people who inject drugs [40, 41], the true prevalence remains uncertain and is strongly biased by the sensitivity and resolution of the genotyping methods employed.

Conventional genotyping approaches based on partial genome sequencing may underestimate both mixed infections and recombinant forms, particularly when minority variants are present or when low-variability genomic regions are targeted [42–45]. In the present study, the absence of consistent secondary peaks in Sanger chromatograms, together with the reproducible phylogenetic discordance between Core and NS5B, favored the hypothesis of true recombination rather than co-infection. This was subsequently confirmed by full-genome sequencing, underscoring the added value of long-read NGS for studying viral genetic diversity. These advanced approaches provide a comprehensive view of viral populations, facilitating the detection of mixed infections [20, 46] and the identification of recombinant forms, even when present as

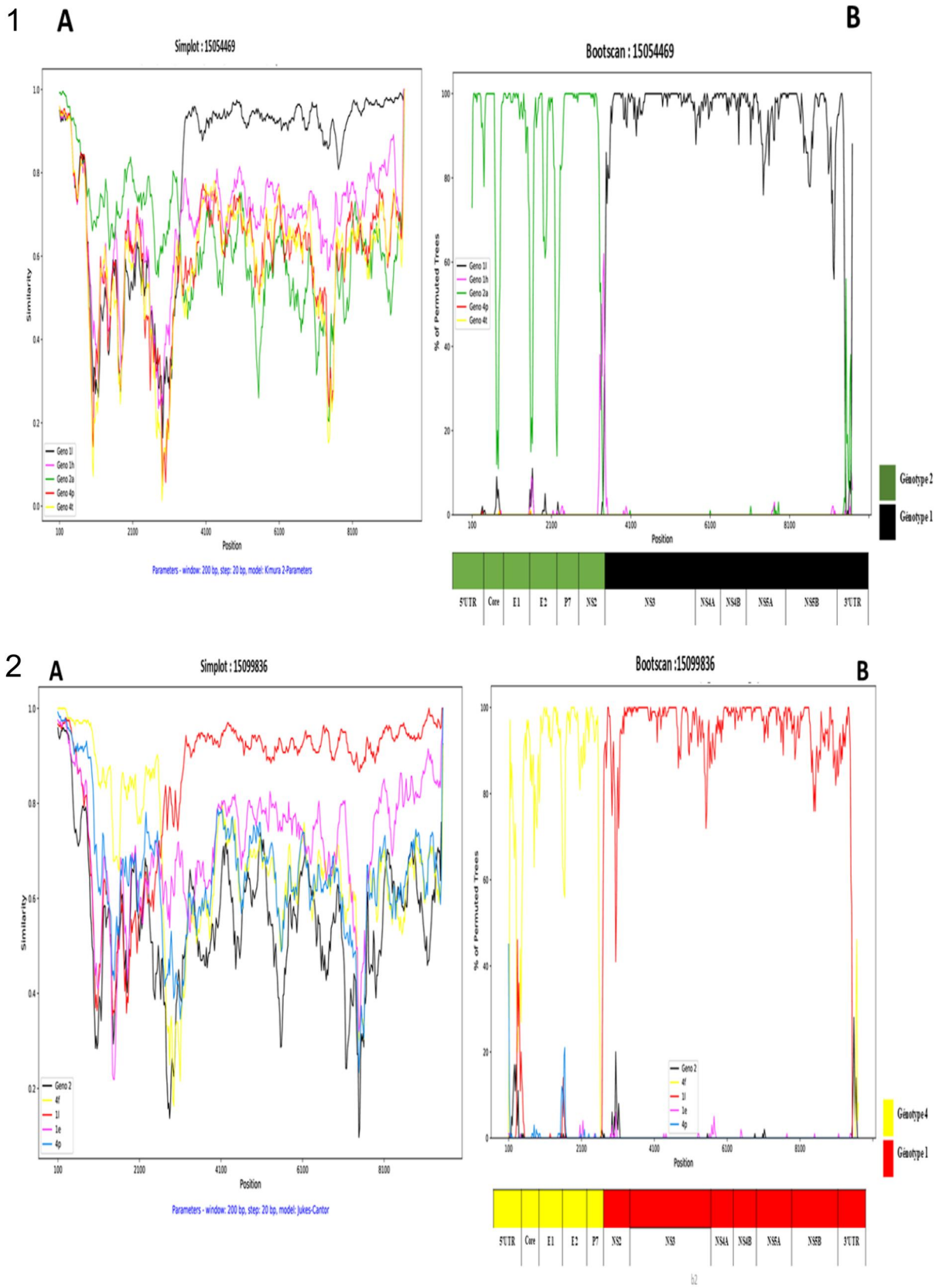

FIGURE 1 | Similarity plots (Simplot++) and Bootscan analyses of the 10 HCV intergenotypic recombinants were generated from the complete genomic sequences, using the Simplot++ V1.3 and Simplot version 3.5.1 software.

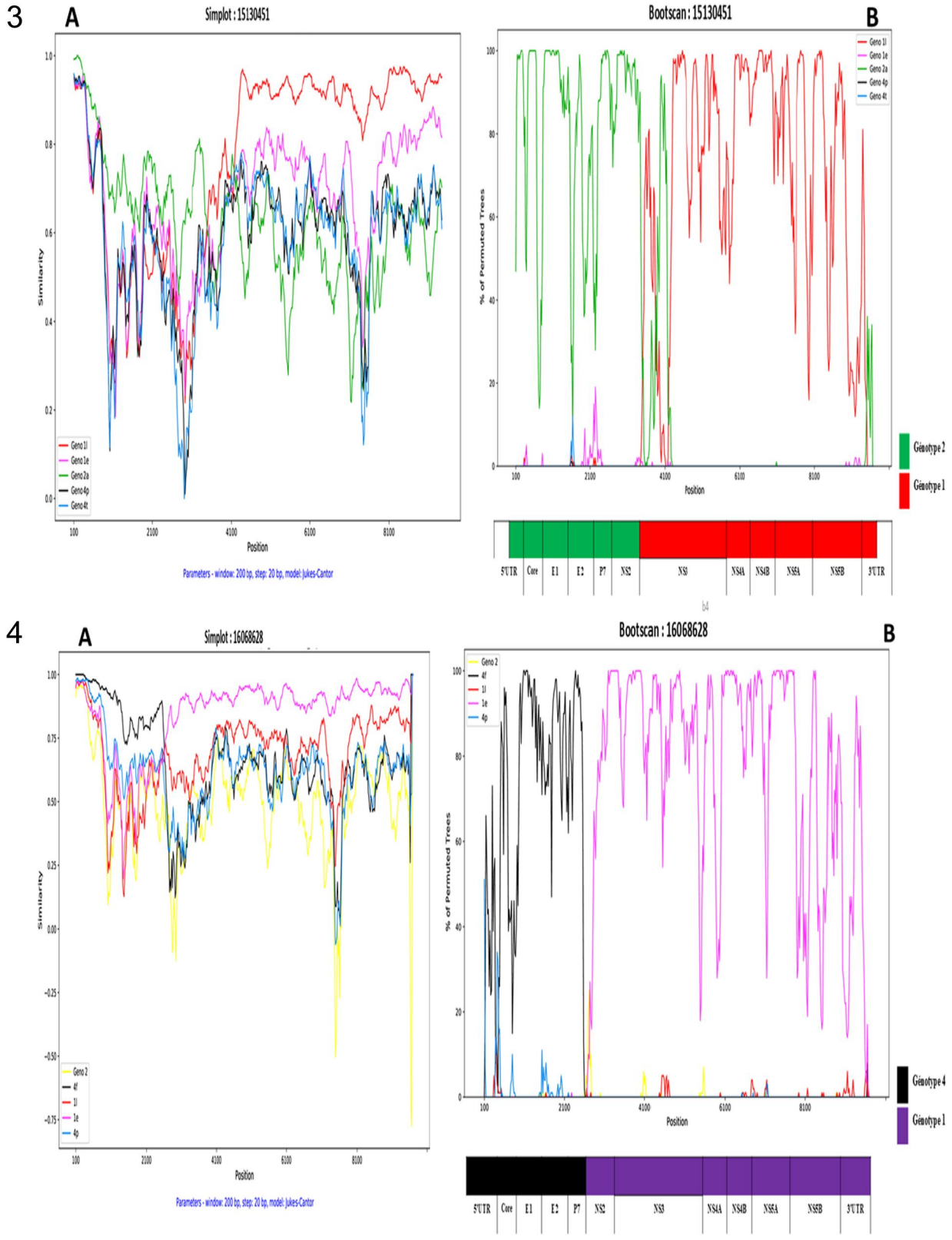

FIGURE 1 | (Continued)

minority variants. Although NGS sequencing enables detection of mixed viral variants, interpretation must remain cautious, as low-abundance reads alone do not necessarily constitute evidence of mixed infection [47].

These results are therefore consistent with the hypothesis of inter-genotypic recombination for these 10 isolates, justifying their selection for full genomic characterization by Nanopore sequencing (GridION + SISPA) to confirm recombination and

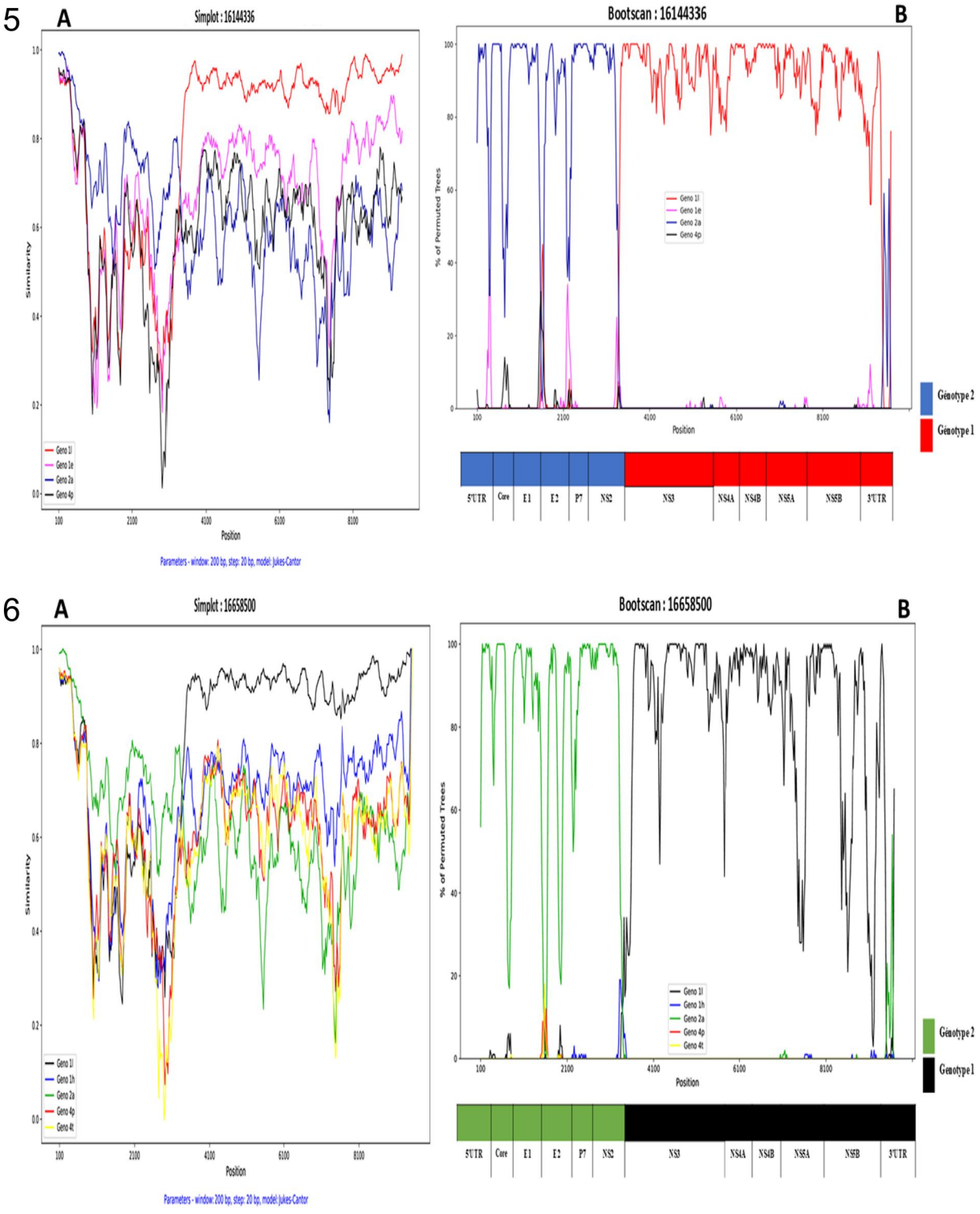

FIGURE 1 | (Continued)

precisely map the crossover breakpoints. The breakpoint distribution observed in this study is consistent with a previous report showing that inter-genotypic HCV recombination preferentially occurs at the boundary between the structural and non-structural genes of HCV, particularly within or near NS2 and NS3 [48]. Seven of the 10 recombinants identified here exhibited a single breakpoint in the NS2/NS3 region, while two cases showed a breakpoint in the E2/p7 region. One isolate displayed a more complex mosaic structure involving two successive recombination events, suggesting sequential superinfections and highlighting the evolutionary complexity that may arise in settings of high viral diversity. Similar mosaic patterns have been described in other geographic regions, including Europe and Asia [13]. The preferential localization of recombination breakpoints near NS2/NS3 may reflect structural constraints within the

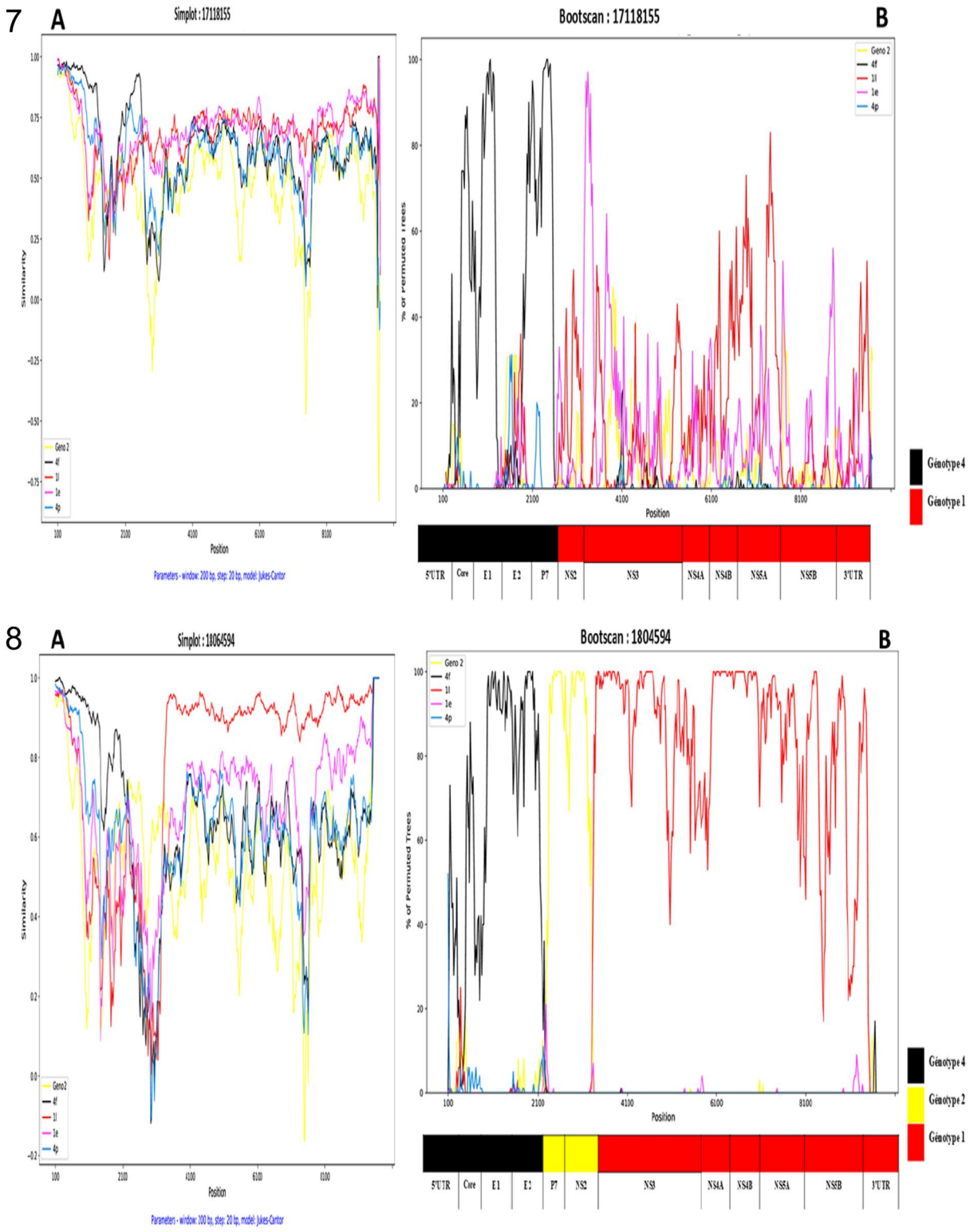

FIGURE 1 | (Continued)

HCV genome, as RNA secondary structures and protein processing requirements likely limit the viability of recombinants arising elsewhere [49]. However, the precise molecular mechanisms governing breakpoint selection remain poorly understood and warrant further investigation using experimental in vitro systems as already demonstrated for HIV [50].

Our findings underscore the clinical and public health importance of comprehensive genomic surveillance for HCV, as partial typing based solely on Core or NS5B regions can miss inter-genotypic recombinants, potentially leading to diagnostic inaccuracies or therapeutic errors, such as resistance to DAAs in cases involving NS5A/NS5B recombination. The relevance of screening for mixed infections and recombinants has evolved

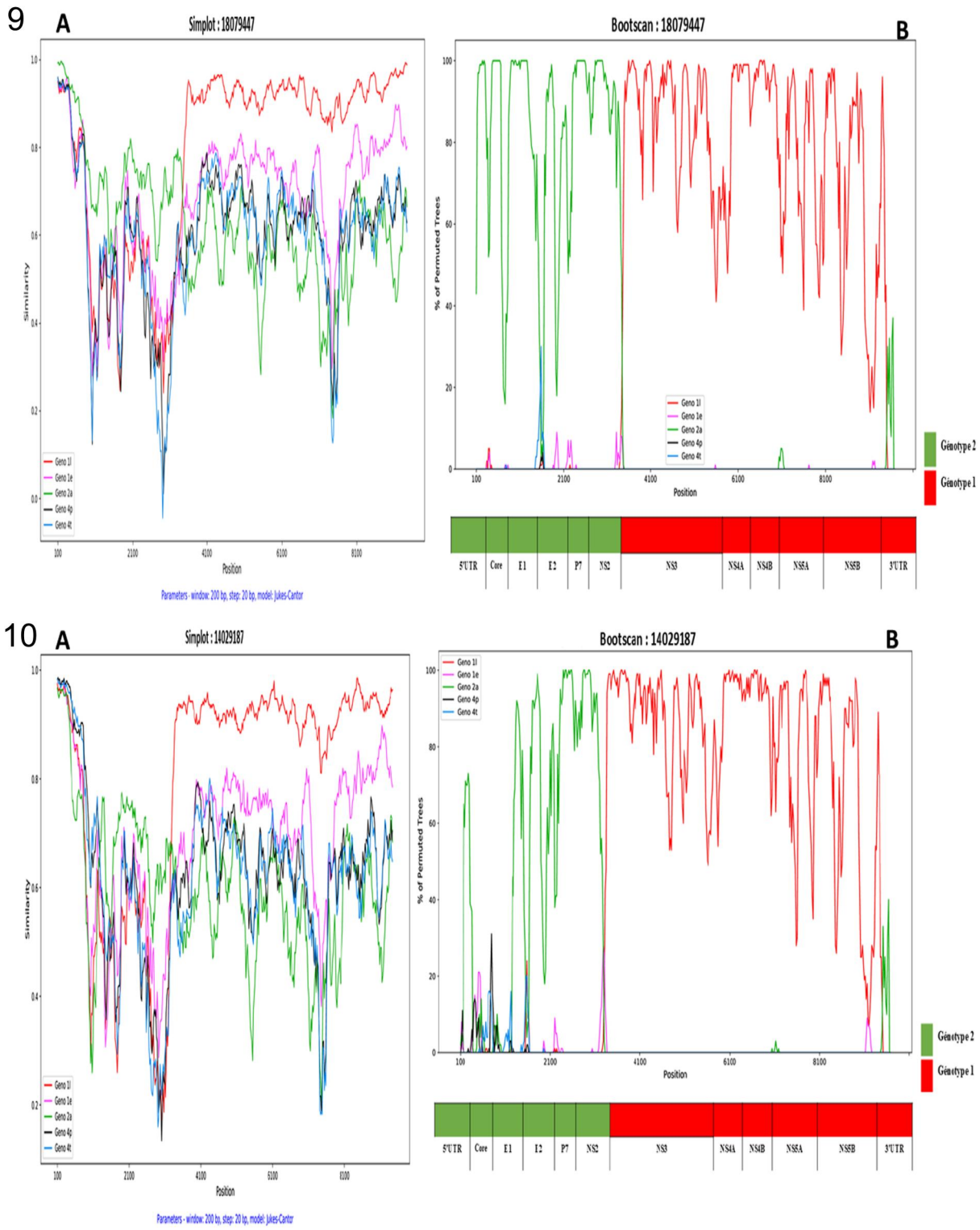

FIGURE 1 | (Continued)

alongside antiviral therapy advancements: early treatments with pegylated interferon alpha plus ribavirin or first-generation genotype-specific DAAs were prone to failure in mixed infections, where elimination of the dominant genotype allowed the minority one to emerge [24, 25]. With the advent of highly effective pan-genotypic DAA regimens, the emphasis on routine genotyping and mixed-infection detection has decreased, as real-world studies report high sustained virologic response rates even in the presence of mixed genotypes [51–53]. Although inter-genotypic recombination appears infrequent in nature despite documented homologous and non-homologous events in vitro [54], multiple natural recombinants have been described since 2002 [11, 13, 16, 17], and our study identifies 10 novel forms involving genotypes 1, 2, and 4 in Cameroon. While pan-genotypic DAAs have reduced the need for systematic genotyping in treatment-naïve patients, precise characterization of

TABLE 2 | Architecture of HCV recombinants identified by Simplot/Bootscan analysis.

Case	Involved genotypes	Genome region	Breakpoint range (nt)	Observations
1	2a/1l	NS2/NS3	3350−3450	Unique recombination point.
2	4f/1l	E2/p7	2550−2650	Unique point.
3	2a/1l	NS2/NS3	3450−3550	Unique point.
4	4f/1e	E2/p7	2350−2450	Unique point.
5	2a/1l	NS2/NS3	3150−3250	Unique point.
6	2a/1l	NS2/NS3	3450−3550	Unique point.
7	4f/1l	E2/p7	2400−2500	Unique point.
8	4f/2a then 2a/1l	E2/p7 then NS2/NS3	2150−2250 (1st); 3350−3450 (2nd)	Two recombination points.
9	2a/1l	NS2/NS3	3550−3650	Unique point.
10	2a/1l	NS2/NS3	3350−3450	Unique point.

recombinants or minority variants remains essential in challenging contexts such as treatment failure, cirrhosis, prior treatment experience, or genetically diverse regions like Central Africa, where it can guide adaptations like adding ribavirin or extending therapy [24, 25]. Active surveillance for recombinants and resistant strains in high-variance settings aligns with EASL recommendations [55, 56] and, combined with NGS, enhances our understanding of HCV evolution and supports tailored public health strategies in understudied populations.

Recombination may serve as a key driver of evolution and genetic diversity in RNA viruses [56–58]. In contrast to other RNA viruses, the occurrence of natural recombination events within HCV is rare [59]. This is corroborated by the observation of superinfection exclusion, where an established viral infection prevents or disrupts subsequent infection by a second virus [7, 8]

The use of long-read NGS (Nanopore) was crucial for accurately mapping the recombination breakpoints, confirming the NS2/NS3 junction as a predominant and globally recognized site for genetic exchange in HCV [46]. This region between structural and non-structural proteins appears to tolerate recombination without compromising viral viability, as supported by extensive literature [12, 13, 60–62]. The identification of a double recombination event in our study underscores the evolutionary complexity in high-endemicity settings, likely resulting from successive reinfections. Such complex mosaic forms could evade diagnosis based on single-region sequencing. However, the precise molecular mechanisms favoring recombination in this genomic hotspot remain unclear and warrant further investigation using robust cell culture systems [63, 64].

In conclusion, this study reports the identification of 10 intergenotypic HCV recombinant strains circulating in Cameroon, confirmed by ONT NGS, with a clear predominance of breakpoints in the NS2/NS3 region. The predominance of breakpoints at the NS2/NS3 junction reinforces the role of this region as a major recombination hotspot and contributes to a better understanding of HCV evolution in sub-Saharan Africa. These findings support the integration of NGS into epidemiological surveillance and diagnostic/therapeutic strategies in settings of high genetic diversity.

Author Contributions

All authors have read and agreed to the published version of the manuscript.

Acknowledgments

We sincerely thank the patients who participated in this study and the staff of the Centre Pasteur du Cameroun's virology laboratory for their essential contributions. We also acknowledge our collaborators at the Institut Pasteur du Cambodge. This study was conducted with financial support from a Calmette and Yersin scholarship awarded by the Institut Pasteur in Paris. This funding covered the costs associated with transporting samples from the Centre Pasteur du Cameroun, as well as the travel and accommodation expenses for the scholarship recipient, MOUNCHILI NJIFON Aristide, PhD student, at the Virology Unit of the Institut Pasteur du Cambodge, where the decisive analyses for this work were carried out.

Consent

The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data sets used during the current study are available from the corresponding author upon reasonable request.

References

1. WHO, 2025. https://www.who.int/fr/news-room/fact-sheets/detail/hepatitis-c.

2. N. Echeverría, F. Gámbaro, S. Beaucourt, et al., "Mixed Infections Unravel Novel HCV Inter-Genotypic Recombinant Forms Within the Conserved IRES Region," *Viruses* 16, no. 4 (April 2024): 560.

3. J. Bukh, "The History of Hepatitis C Virus (HCV): Basic Research Reveals Unique Features in Phylogeny, Evolution and the Viral Life Cycle With New Perspectives for Epidemic Control," supplement, *Journal of Hepatology* 65, no. S1 (October 2016): S2–S21.

4. S. M. Borgia, C. Hedskog, B. Parhy, et al., "Identification of a Novel Hepatitis C Virus Genotype From Punjab, India: Expanding Classification of Hepatitis C Virus Into 8 Genotypes," *Journal of Infectious Diseases* 218, no. 11 (October 2018): 1722–1729.

5. E. Vo-Quang and J. M. Pawlotsky, "'Unusual' HCV Genotype Subtypes: Origin, Distribution, Sensitivity to Direct-Acting Antiviral Drugs and Behaviour on Antiviral Treatment and Retreatment," *Gut* 73, no. 9 (August 2024): 1570–1582.

6. A. Galli and J. Bukh, "Comparative Analysis of the Molecular Mechanisms of Recombination in Hepatitis C Virus," *Trends in Microbiology* 22, no. 6 (2014): 354–364.

7. D. M. Tscherne, M. J. Evans, T. von Hahn, et al., "Superinfection Exclusion in Cells Infected With Hepatitis C Virus," *Journal of Virology* 81, no. 8 (April 2007): 3693–3703.

8. B. Webster, S. Wissing, E. Herker, M. Ott, and W. C. Greene, "Rapid Intracellular Competition Between Hepatitis C Viral Genomes as a Result of Mitosis," *Journal of Virology* 87, no. 1 (January 2013): 581–596.

9. F. González-Candelas, F. X. López-Labrador, and M. A. Bracho, "Recombination in Hepatitis C Virus," *Viruses* 3, no. 10 (October 2011): 2006–2024.

10. R. Colina, D. Casane, S. Vasquez, et al., "Evidence of Intratypic Recombination in Natural Populations of Hepatitis C Virus," *Journal of General Virology* 85, no. pt. 1 (January 2004): 31–37.

11. D. Gupta, K. Saha, A. Biswas, R. Firdaus, M. Ghosh, and P. C. Sadhukhan, "Recombination in Hepatitis C Virus Is Not Uncommon Among People Who Inject Drugs in Kolkata, India," *Infection, Genetics and Evolution* 48 (2017): 156–163.

12. S. Kageyama, D. M. Agdamag, E. T. Alesna, et al., "A Natural Inter-Genotypic (2b/1b) Recombinant of Hepatitis C Virus in the Philippines," *Journal of Medical Virology* 78, no. 11 (November 2006): 1423–1428.

13. O. Kalinina, H. Norder, S. Mukomolov, and L. O. Magnius, "A Natural Intergenotypic Recombinant of Hepatitis C Virus Identified in St. Petersburg," *Journal of Virology* 76, no. 8 (April 2002): 4034–4043.

14. I. Moreau, S. Hegarty, J. Levis, et al., "Serendipitous Identification of Natural Intergenotypic Recombinants of Hepatitis C in Ireland," *Virology Journal* 3 (November 2006): 95.

15. S. Nopporpanth, T. X. Lien, Y. Poovorawan, S. L. Smits, A. D. M. E. Osterhaus, and B. L. Haagmans, "Identification of a Naturally Occurring Recombinant Genotype 2/6 Hepatitis C Virus," *Journal of Virology* 80, no. 15 (August 2006): 7569–7577.

16. W. Shi, I. T. Freitas, C. Zhu, W. Zheng, W. W. Hall, and D. G. Higgins, "Recombination in Hepatitis C Virus: Identification of Four Novel Naturally Occurring Inter-Subtype Recombinants," *PLoS One* 7, no. 7 (2012): e41997.

17. K. Yokoyama, M. Takahashi, T. Nishizawa, et al., "Identification and Characterization of a Natural Inter-Genotypic (2b/1b) Recombinant Hepatitis C Virus in Japan," *Archives of Virology* 156, no. 9 (September 2011): 1591–1601.

18. V. Sentandreu, N. Jiménez-Hernández, M. Torres-Puente, et al., "Evidence of Recombination in Intrapatient Populations of Hepatitis C Virus," *PLoS One* 3, no. 9 (September 2008): e3239.

19. D. Bhattacharya, M. A. Accola, I. H. Ansari, R. Striker, and W. M. Rehrauer, "Naturally Occurring Genotype 2b/1a Hepatitis C Virus in the United States," *Virology Journal* 8 (October 2011): 458.

20. J. C. Iles, R. Njouom, Y. Foupouapouognigni, et al., "Characterization of Hepatitis C Virus Recombination in Cameroon by Use of Non-specific Next-Generation Sequencing," *Journal of Clinical Microbiology* 53, no. 10 (October 2015): 3155–3164.

21. R. S. Ross, J. Verbeeck, S. Viazov, P. Lemey, M. Van Ranst, and M. Roggendorf, "Evidence for a Complex Mosaic Genome Pattern in a Full-Length Hepatitis C Virus Sequence," *Evol Bioinforma Online* 4 (October 2008): 249–254.

22. V. Morel, C. Fournier, C. François, et al., "Genetic Recombination of the Hepatitis C Virus: Clinical Implications," *Journal of Viral Hepatitis* 18, no. 2 (February 2011): 77–83.

23. M. Qi, M. Yang, L. Xu, et al., "Complete Genome Analysis Identifies Recombinant Events and Positive Selection Sites of Hepatitis C Virus From Mainland China During 2010-2019," *Virus Research* 296 (April 2021): 198354.

24. S. Bagaglio, C. Uberti-Foppa, C. Di Serio, et al., "Dynamic of Mixed HCV Infection in Plasma and PBMC of HIV/HCV Patients Under Treatment With Peg-IFN/Ribavirin," *Medicine* 94, no. 43 (October 2015): e1876.

25. J. A. Del Campo, M. Parra-Sánchez, B. Figueruela, et al., "Hepatitis C Virus Deep Sequencing for Sub-Genotype Identification in Mixed Infections: A Real-Life Experience," *International Journal of Infectious Diseases* 67 (2018): 114–117.

26. B. Fernández-Caso, J. Á. Fernández-Caballero, N. Chueca, et al., "Infection With Multiple Hepatitis C Virus Genotypes Detected Using Commercial Tests Should Be Confirmed Using Next Generation Sequencing," *Scientific Reports* 9, no. 1 (June 2019): 9264.

27. A. D. Olmstead, V. Montoya, C. K. Chui, et al., "A Systematic, Deep Sequencing-Based Methodology for Identification of Mixed-Genotype Hepatitis C Virus Infections," *Infection, Genetics and Evolution* 69 (2019): 76–84.

28. F. Levoa Eteme, N. Mafopa Goumkwa, A. L. Otam, et al., "Highly Genetically Diverse Variants of Hepatitis C Virus Still Predominate in Cameroon but With Low Frequency of Mutations Associated to Resistance of NS5B Polymerase-Targeted Antivirals," *JAC-Antimicrob Resist* 7, no. 4 (July 2025): dlaf114.

29. P. A. Tagnouokam-Ngoupo, M. N. Ngoufack, S. Kenmoe, et al., "Hepatitis C Virus Genotyping Based on Core and NS5B Regions in Cameroonian Patients," *Virology Journal* 16, no. 1 (August 2019): 101.

30. A. M. Njifon, A. F. Modiyinji, C. G. Monamele, et al., "A Decade-Long Retrospective Study of Hepatitis C Virus Genetic Diversity in Cameroon, 2013-2023: Presence of a High Proportion of Unsubtypable and Putative Recombinant HCV Strains," *Archives of Virology* 169, no. 10 (September 2024): 197.

31. A. Brinkmann, S. Uddin, E. Krause, et al., "Utility of a Sequence-Independent, Single-Primer-Amplification (SISPA) and Nanopore Sequencing Approach for Detection and Characterization of Tick-Borne Viral Pathogens," *Viruses* 13, no. 2 (January 2021): 203.

32. K. Chrzastek, D. Lee, D. Smith, et al., "Use of Sequence-Independent, Single-Primer-Amplification (SISPA) for Rapid Detection, Identification, and Characterization of Avian RNA Viruses," *Virology* 509 (September 2017): 159–166.

33. K. S. Lole, R. C. Bollinger, R. S. Paranjape, et al., "Full-Length Human Immunodeficiency Virus Type 1 Genomes From Subtype C-Infected Seroconverters in India, With Evidence of Intersubtype Recombination," *Journal of Virology* 73, no. 1 (January 1999): 152–160.

34. S. Samson, É. Lord, and V. Makarenkov, "SimPlot++: A Python Application for Representing Sequence Similarity and Detecting Recombination," *Bioinformatics* 38 (March 2022): 3118–3120, https://doi.org/10.1093/bioinformatics/btac287.

35. S. M. Borgia, C. Hedskog, B. Parhy, et al., "Identification of a Novel Hepatitis C Virus Genotype From Punjab, India: Expanding Classification of Hepatitis C Virus Into 8 Genotypes," *Journal of Infectious Diseases* 218, no. 11 (October 2018): 1722–1729.

36. M. A. Rodgers, V. Holzmayer, A. Vallari, et al., "Hepatitis C Virus Surveillance and Identification of Human Pegivirus 2 in a Large Cameroonian Cohort," *Journal of Viral Hepatitis* 26, no. 1 (January 2019): 30–37, https://onlinelibrary.wiley.com/doi/abs/10.1111/jvh.12996.

37. P. A. Tagnouokam-Ngoupo, M. N. Ngoufack, S. Kenmoe, et al., "Hepatitis C Virus Genotyping Based on Core and NS5B Regions in Cameroonian Patients," *Virology Journal* 16, no. 1 (August 2019): 101.

38. B. R. Tietcheu Galani, R. Njouom, and P. F. Moundipa, "Hepatitis C in Cameroon: What Is the Progress From 2001 to 2016?," *Journal of Translational Internal Medicine* 4, no. 4 (December 2016): 162–169, https://pmc.ncbi.nlm.nih.gov/articles/PMC5290894/.

39. J. C. Iles, R. Njouom, Y. Foupouapouognigni, et al., "Characterization of Hepatitis C Virus Recombination in Cameroon by Use of Non-specific Next-Generation Sequencing," *Journal of Clinical Microbiology* 53, no. 10 (October 2015): 3155–3164, https://pmc.ncbi.nlm.nih.gov/articles/PMC4572555/.

40. P. Ferenci, "Impact and Management of Patients With Multiple Hepatitis C Virus Genotypes," in *Clinical Dilemmas in Viral Liver Disease [Internet]* (John Wiley & Sons, Ltd, 2020), 74–77. https://onlinelibrary.wiley.com/doi/abs/10.1002/9781119533481.ch12.

41. E. B. Cunningham, T. L. Applegate, A. R. Lloyd, G. J. Dore, and J. Grebely, "Mixed HCV Infection and Reinfection in People Who Inject Drugs—Impact on Therapy," *Nature Reviews Gastroenterology & Hepatology* 12, no. 4 (April 2015): 218–230.

42. J. T. Blackard and K. E. Sherman, "Hepatitis C Virus Coinfection and Superinfection," *Journal of Infectious Diseases* 195, no. 4 (February 2007): 519–524.

43. S. Bowden, R. McCaw, P. A. White, N. Crofts, and C. K. Aitken, "Detection of Multiple Hepatitis C Virus Genotypes in a Cohort of Injecting Drug Users," *Journal of Viral Hepatitis* 12, no. 3 (May 2005): 322–324.

44. B. Fernández-Caso, J. Á. Fernández-Caballero, N. Chueca, et al., "Infection With Multiple Hepatitis C Virus Genotypes Detected Using Commercial Tests Should Be Confirmed Using Next Generation Sequencing," *Scientific Reports* 9, no. 1 (June 2019): 9264.

45. C. Minosse, E. Giombini, B. Bartolini, M. Capobianchi, and A. Garbuglia, "Ultra-Deep Sequencing Characterization of HCV Samples With Equivocal Typing Results Determined With a Commercial Assay," *International Journal of Molecular Sciences* 17, no. 10 (October 2016): 1679.

46. A. L. McNaughton, V. B. Sreenu, G. Wilkie, R. Gunson, K. Templeton, and E. C. M. Leitch, "Prevalence of Mixed Genotype Hepatitis C Virus Infections in the UK as Determined by Genotype-Specific PCR and Deep Sequencing," *Journal of Viral Hepatitis* 25, no. 5 (May 2018): 524–534.

47. A. D. Olmstead, V. Montoya, C. K. Chui, et al., "A Systematic, Deep Sequencing-Based Methodology for Identification of Mixed-Genotype Hepatitis C Virus Infections," *Infection, Genetics and Evolution* 69 (2019): 76–84.

48. N. Echeverría, "Hepatitis C Virus Genetic Variability and Evolution," *World Journal of Hepatology* 7, no. 6 (April 2015): 831–845.

49. P. Simmonds, A. Tuplin, and D. J. Evans, "Detection of Genome-Scale Ordered RNA Structure (GORS) in Genomes of Positive-Stranded RNA Viruses: Implications for Virus Evolution and Host Persistence," *RNA* 10, no. 9 (September 2004): 1337–1351.

50. B. C. Ramirez, E. Simon-Loriere, R. Galetto, and M. Negroni, "Implications of Recombination for HIV Diversity," *Virus Research* 134, no. 1–2 (June 2008): 64–73.

51. W. N. Chiu, C. H. Hung, S. N. Lu, et al., "Real-World Effectiveness of Glecaprevir/Pibrentasvir and Ledipasvir/Sofosbuvir for Mixed Genotype Hepatitis C Infection: A Multicenter Pooled Analysis in Taiwan," *Journal of Viral Hepatitis* 27, no. 9 (September 2020): 866–872.

52. Y. J. Ding, C. K. Lu, W. M. Chen, et al., "Pangenotypic Direct-Acting Antiviral Agents for Mixed Genotype Hepatitis C Infection: A Real-World Effectiveness Analysis," *Journal of Gastroenterology and Hepatology* 36, no. 10 (October 2021): 2911–2916.

53. H. H. Yen, Y. Y. Chen, J. H. Lai, et al., "Pan-Genotypic Direct-Acting Antiviral Agents for Undetermined or Mixed-Genotype Hepatitis C Infection: A Real-World Multi-Center Effectiveness Analysis," *Journal of Clinical Medicine* 11, no. 7 (March 2022): 1853.

54. T. K. H. Scheel, A. Galli, Y. P. Li, L. S. Mikkelsen, J. M. Gottwein, and J. Bukh, "Productive Homologous and Non-Homologous Recombination of Hepatitis C Virus in Cell Culture," *PLoS Pathogens* 9, no. 3 (March 2013): e1003228.

55. D. Bhattacharya, A. Aronsohn, J. Price, and V. I. I. I. Lo Re, "Hepatitis C Guidance 2023 Update: American Association for the Study of Liver Diseases–Infectious Diseases Society of America Recommendations for Testing, Managing, and Treating Hepatitis C Virus Infection," *Clinical Infectious Diseases [Internet]* (May 2023): ciad319, https://doi.org/10.1093/cid/ciad319.

56. C. Q. He, Z. X. Xie, G. Z. Han, et al., "Homologous Recombination as an Evolutionary Force in the Avian Influenza A Virus," *Molecular Biology and Evolution* 26, no. 1 (January 2008): 177–187.

57. E. Simon-Loriere and E. C. Holmes, "Why Do RNA Viruses Recombine?," *Nature Reviews Microbiology* 9, no. 8 (July 2011): 617–626.

58. C. Yuan, W. Liu, Y. Wang, J. Hou, L. Zhang, and G. Wang, "Homologous Recombination Is a Force in the Evolution of Canine Distemper Virus," *PLoS One* 12, no. 4 (2017): e0175416.

59. J. Raghwani, C. H. Wu, C. K. Y. Ho, et al., "High-Resolution Evolutionary Analysis of Within-Host Hepatitis C Virus Infection," *Journal of Infectious Diseases* 219, no. 11 (May 2019): 1722–1729.

60. Y. M. Lee, H. J. Lin, Y. J. Chen, et al., "Molecular Epidemiology of HCV Genotypes Among Injection Drug Users in Taiwan: Full-Length Sequences of Two New Subtype 6w Strains and a Recombinant Form_2b6w," *Journal of Medical Virology* 82, no. 1 (January 2010): 57–68.

61. F. Legrand-Abravanel, J. Claudinon, F. Nicot, et al., "New Natural Intergenotypic (2/5) Recombinant of Hepatitis C Virus," *Journal of Virology* 81, no. 8 (April 2007): 4357–4362.

62. S. Noppornpanth, T. X. Lien, Y. Poovorawan, S. L. Smits, A. D. M. E. Osterhaus, and B. L. Haagmans, "Identification of a Naturally Occurring Recombinant Genotype 2/6 Hepatitis C Virus," *Journal of Virology* 80, no. 15 (August 2006): 7569–7577.

63. J. Reiter, G. Pérez-Vilaró, N. Scheller, L. B. Mina, J. Díez, and A. Meyerhans, "Hepatitis C Virus RNA Recombination in Cell Culture," *Journal of Hepatology* 55, no. 4 (October 2011): 777–783.

64. T. Wakita, T. Pietschmann, T. Kato, et al., "Production of Infectious Hepatitis C Virus in Tissue Culture From a Cloned Viral Genome," *Nature Medicine* 11, no. 7 (July 2005): 791–796.