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Research Article

Historical drivers of HCV Subtypes 1b and 3a in Thailand and 6f in Phetchabun, an HCV endemic area of the country

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Abstract

The World Health Organization has set a target to eliminate viral hepatitis as a public threat by 2030. In pursuit of this goal, Thailand initiated a hepatitis C virus (HCV) microelimination project targeting Phetchabun province, a well-recognized high-burden HCV endemic area. However, the historical transmission dynamics of HCV in Phetchabun, and in Thailand in general, remain unclear. This study investigates the epidemic histories of HCV in Phetchabun, focusing on Subtypes 1b, 3a, and 6f, and their relationship with HCV in other regions of Thailand, using molecular phylogenetic analyses. Our results reveal nationwide the presence of Subtypes 1b and 3a, while Subtype 6f is mainly confined to Phetchabun. The initial spread of Subtype 1b was inferred to coincide with World War II and the period of suboptimal medical and hygienic standards in Thai blood transfusion services, suggesting a correlation between the two. The early expansion of Subtype 3a was, on the other hand, found to correlate with the epidemic of intravenous drug use in Thailand during the time of Vietnam War. The early expansion of Subtype 6f, in contrast, appears to coincide with the period of severe regional political conflict and social and economic instability. All these findings suggest the complex interplay between social determinants of health and HCV transmission. Post the mid-1990s/early 2000s, all subtypes showed significantly reduced population growth rates, aligning with improvements in blood transfusion safety standards, the nationwide "War on Drugs" policy, and enhanced accessibility to public healthcare and HCV treatments. These combined efforts likely have contributed to curbing the spread of HCV in Thailand. Nevertheless, our analyses reveal that the prevalence of HCV in Thailand remains high overall, emphasizing the need for further research and a nationwide approach to more effectively reduce the HCV burden in Thailand.

Keywords: HCV; epidemic history; epidemiological dynamics; historical factor; HCV microelimination program; phylogenetic analysis.

1. Introduction

Hepatitis C virus (HCV) poses a significant global health threat, affecting ~58 million people with chronic infections and, in some cases, severe liver-related diseases (Polaris Observatory HCV Collaborators 2022). HCV is currently classified into Genotypes 1–8 (Smith et al. 2014, Borgia et al. 2018), each associated with distinct geographical distributions and epidemiological patterns. Genotypes 1, 2, and 3 are common worldwide, while Genotype 4 is most prevalent in the Middle East and North Africa, and Genotype 5 is frequently reported in South Africa. Genotypes 6, 7, and 8 are, on the other hand, restricted mainly to Southeast Asia, Central Africa, and South Asia, respectively (Gower et al. 2014, Smith et al. 2014, Murphy et al. 2015, Borgia et al. 2018). HCV transmission primarily occurs through significant or repeated direct percutaneous exposures to infected blood. Common transmission routes include

blood transfusion, needle-sharing among drug users, and unsafe medical practices; however, these routes can vary across HCV genotypes and subtypes (Pybus et al. 2005).

In 2016, the World Health Organization launched a campaign to eliminate viral hepatitis as a public health problem by 2030 (World Health Organization 2016). Thailand is one of the countries significantly affected by HCV and has actively aligned itself with this global goal. The HCV viremia rate in the general Thai population has been estimated to be about 0.5–0.8%, with ∼343 000–571 000 viremic cases nationwide (Polaris Observatory HCV Collaborators 2022). In Thailand, the most common HCV genotypes, in order of abundance, are Genotypes 3, 6, and 1. Genotype 3 can be found nationwide, affecting ∼48% of HCV patients in the country (Wasitthankasem et al. 2016), while Genotype 6 is most commonly found in Northern and Northeastern Thailand (Wasitthankasem et al. 2015), diagnosed in ∼35% of the patients (Wasitthankasem et al. 2016). Genotype 1 is also widespread but less common than Genotype 3, accounting for ∼17% of the total cases (Wasitthankasem et al. 2016).

Phetchabun, a province in northern Thailand, stands out for its high burden of HCV infection. Between 2003 and 2006, the National Notifiable Disease Passive Surveillance reported a rapid and continuous increase in HCV cases in Thailand, with the majority of cases being from Phetchabun, most commonly infected with Genotype 6, followed by Genotypes 3 and 1 (Buathong et al. 2013). A nationwide survey in 2014 then revealed a significant decrease in the overall HCV seroprevalence rate in Thailand, dropping from 2.15% in 2004 to 0.94% in 2014 (Wasitthankasem et al. 2016). However, the burden remained relatively high in the northern region, particularly among individuals aged 41–50 years, and rather worryingly, the frequency of Genotype 6 was noted to increase from 8.9% in 2004 to 34.8% in 2014 (Wasitthankasem et al. 2016). These findings led to a series of investigations into the HCV burden in Phetchabun. A 2015 study focusing on individuals aged 30–64 years living in two northern districts of Phetchabun revealed a seroprevalence rate of 15.5%, much higher than the 3.6% rate in the adjacent province of Khon Kaen (Wasitthankasem et al. 2017). A follow-up study in 2017, including individuals aged 18–30 years confirmed this trend and showed that HCV seroprevalence rate was indeed higher among individuals older than 35 years compared to those younger than 35 years (Wasitthankasem et al. 2018). A subsequent systematic province-wide survey in 2018 among individuals aged 35–64 years from all districts estimated an overall HCV seroprevalence rate of 6.9% in Phetchabun (Wasitthankasem et al. 2020), far greater than the nationwide rate of 0.94% (Wasitthankasem et al. 2016). All these findings highlight HCV as a critical and unique public health issue in Phetchabun and established it as an HCV endemic area in Thailand. HCV Genotype 6, particularly Subtype 6f, was consistently reported to be the dominant genotype in Phetchabun, followed by Subtypes 3a and 1a (Wasitthankasem et al. 2017, 2020). Furthermore, in addition to older age, male gender, a history of intravenous drug use (IVDU), having tattoos, receiving blood transfusions, limited education (less than high school), and working in agriculture-related occupations, all had been consistently identified as risk factors for HCV infection in Phetchabun (Buathong et al. 2013, Wasitthankasem et al. 2017, 2018, 2020).

Due to the high burden of HCV infection, Phetchabun has been a target area for the HCV microelimination program in Thailand since 2015 (Wasitthankasem et al. 2017, 2018, 2020, 2023, Pratedrat et al. 2023), which has been demonstrated to be feasible and achievable in various countries (Mangia et al. 2021). Beginning in 2019, village health volunteers and nurses in health-promoting hospitals have been continuously enrolling interested residents aged over 30 years into this program for anti-HCV serological screening using a simple rapid diagnostic test with whole blood from a finger prick (Wasitthankasem et al. 2023). All individuals who test positive for both anti-HCV and HCV RNA are provided with the necessary treatment to reduce the chances of further transmission and the overall HCV-related health problems. As of 4 July 2024, 281 434 individuals (86.62% of the 324 916 target population) had been enrolled in the program and 13 989 (∼5%) were found to be anti-HCV positive. Among the 11 600 anti-HCV confirmed cases, 8 423 (∼73%) tested positive for HCV RNA and 7 546 (89.59%) had received treatments (Phetchabun Provincial Public Health Office 2024).

Despite having the highest HCV burden in the country and being the focus of extensive HCV surveillance for quite some time, factors that drove the early spread of the virus in Phetchabun remain largely uncharacterized. To better understand the origin of HCV in this endemic area, and in Thailand in general, this study analyzes HCV samples collected during the microelimination project between 2020 and 2021, supplemented with publicly available data, to investigate the molecular epidemiology of the virus in Phetchabun and how it relates to HCV in other regions of the country. Our results provide new insights into historical events that likely influenced the virus's past transmission dynamics and shaped its current distribution. Future directions to better curb HCV transmission in Thailand are discussed.

2. Materials and methods

The study protocol was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University (Institutional Review Board number 028/63), and written informed consent was obtained from all participants.

2.1. HCV Core and NS5B coding region sequencing

This study sequenced the HCV Core and NS5B coding regions from 600 plasma samples that tested positive for anti-HCV antibodies and RNA. All samples were collected during a previous HCV microelimination project conducted in Phetchabun, Thailand, in 2021 (Pratedrat et al. 2023). Viral RNA was extracted from 140 μl of plasma using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) and was reverse-transcribed using random hexaprimers and the ImProm-II Reverse Transcription System (Promega, Madison, WI), following the manufacturer's protocol. The HCV Core and NS5B coding regions were amplified using the AccuStart II GelTrack PCR Super Mix (QuantaBio, Beverly, MA), under the polymerase chain reaction (PCR) conditions described in Wasitthankasem et al. (2015). Primer sequences used are listed in Supplementary Table S1.

The PCR products of the Core (∼400 base pairs) and NS5B (∼660 base pairs) coding regions were visualized using 2% agarose gel electrophoresis, purified by using the QIAquick® Gel Extraction Kit (Qiagen, Hilden, Germany), and subjected to Sanger sequencing (1st BASE Laboratories, Selangor, Malaysia). Nucleotide sequences were inspected using Chromas LITE (V.2.6.6) and edited using SeqMan Ultra, Lasergene v.17 (DNASTAR, Madison, WI).

2.2. HCV genotyping

The obtained Core and NS5B sequences were initially classified into the three major HCV genotypes (1, 3, and 6) using BLASTN and Viral Genotyping Tool: Hepatitis C virus (https://www.ncbi.nlm.nih.gov/projects/genotyping/formpage.cgi). For finer scale genotyping, separate Core and NS5B neighbor-joining trees were

constructed using MEGA v.11 (Supplementary Figs S1 and S2, respectively), supplemented with a total of 61 HCV reference sequences (Supplementary Table S2) and 413 additional Thai HCV sequences retrieved from the National Center for Biotechnology Information (NCBI) GenBank database (Supplementary Table S3). The viruses were then genotyped based on the genotype of their closest reference genome. Multiple sequence alignments (MSAs) of the Core and NS5B sequences were generated by using ClustalX v.2.1 and were manually curated in BioEdit v.7.2.5 (Ibis Therapeutics, Carlsbad, CA). Pairwise distances were estimated using the Kimura's two-parameter nucleotide substitution model, and clade support values were computed based on 1000 bootstrap replicates. The distribution of HCV genotypes and subtypes in Phetchabun and other provinces in Thailand is presented in Supplementary Table S4.

2.3. Maximum-likelihood phylogenetic analysis

To investigate the overall genetic diversity of HCV in Thailand, we performed a maximum-likelihood (ML) phylogenetic analysis on a dataset of 852 concatenated Core and NS5B sequences, generated using ClustalX v.2.1. This dataset comprised 439 sequences generated in this study and 413 publicly available Thai HCV sequences. Collection dates and sampling locations were known for all sequences except for 16, of which sampling locations were unknown (Supplementary Table S3). Nucleotide positions 545–556 (relative to the reference strain H77, accession number AF009606) were excluded from the analysis due to excessive gaps. Recombination analysis was performed using the pairwise homoplasy index test implemented in Recombination Detection Program 4. The analysis indicated no significant evidence of recombination ($P = .996$), thus justifying the use of the concatenated Core-NS5B MSA. ML phylogenetic inference was conducted using IQ-TREE (Minh et al. 2020). The general time-reversible (GTR) nucleotide subsitution model with rates varying among sites according to the invariable sites (+I) and discrete gamma distribution with 4 categories (+Γ4) (also known as GTR + I + Γ4) was identified as the best-fitting model under the Bayesian Information Criterion by ModelFinder (Kalyaanamoorthy et al. 2017) implemented in IQ-TREE, and was used for the tree reconstruction. Branch support values were calculated using 1000 bootstrap replicates.

2.4. Temporal signal assessment

The ML tree was first separated into individual subtype trees, and a root-to-tip linear regression analysis (i.e. regression of root-to-tip genetic distance on the sampling date) was performed to assess the temporal signal within each subtype's sequence data using the lm function in R v.4.0.2. For sequences with known sampling years only, their sampling dates were set to their sampling year plus 0.5. Three subtypes, including 6c, 6m, and 6v, were excluded from the analysis since our dataset contained only one sequence for each of these subtypes (Supplementary Table S5 and Figs S3 and S4).

2.5. Molecular dating and population dynamic estimations

Analyses were conducted only for subtypes 1b, 3a, and 6f as they were the only subtypes with sufficient temporal signals (see Section 3). For each HCV subtype, a concatenated Core-NS5B MSA was created, and BEAST v1.10.4 was used to perform tip-dating and population dynamics analysis, employing the best-fit nucleotide substitution model determined under the Bayesian Information Criterion by ModelFinder (Kalyaanamoorthy et al. 2017) implemented in IQ-TREE (Minh et al. 2020) (Supplementary Table S6). All analyses employed the Bayesian time-aware Gaussian Markov random field (GMRF) Skyride coalescence tree prior, and two molecular clock models were considered: the uncorrelated log-normal relaxed clock and the random local relaxed clock. Posterior distributions were estimated using the Markov chain Monte Carlo (MCMC) sampling method, with chain lengths ranging from 300 000 000 to 800 000 000 steps. Parameter values were logged every 30 000th to 80 000th step, depending on the chain lengths, to obtain 10 000 MCMC sampled evolutionary parameter datasets, and the first 10% were discarded as burn-in. Marginal likelihoods of the evolutionary models considered were estimated using the path sampling and stepping-stone sampling methods, and they were compared to compute Bayes factors to identify the best-fit model (Supplementary Table S6). Tracer 1.7.2 was used to inspect the posterior distributions of estimated evolutionary model parameters, and all were found to be adequately sampled by the MCMC sampling chains (effective sample size values > 200 for all parameters). The summary phylogeny was then generated from the best-fit model results using Tree annotator v1.10.4 with at least 10% MCMC burn-in, and node heights were computed based on sequence common ancestor heights. Tracer 1.7.2 was also used to compute the median and 95% highest posterior density (HPD) for past effective population size.

3. Results and discussion

3.1. Overview of the HCV sequence data analyzed in this study

This study sequenced 600 plasma samples which tested positive for both anti-HCV and RNA, previously collected during an HCV microelimination project conducted in Phetchabun, Thailand, between September 2020 and December 2021 (Pratedrat et al. 2023). From this, we successfully recovered 576 HCV Core coding sequences and 490 NS5B coding sequences. We further supplemented our dataset with 413 publicly available Core and NS5B coding sequences of Thai HCV isolates from the NCBI GenBank database for downstream statistical and phylogenetic analyses. These supplemented sequences represented samples from 24 provinces across all six major geographical regions of the country (Central, East, North, Northeast, South, and West) collected between 2007 and 2017. Notably, 100 of these publicly available sequences (24.21%) were reported to originate from Phetchabun (Supplementary Table S3). In total, 854 samples had both Core and NS5B coding sequences available, and these formed the basis of our analyses.

The genotypes of these 854 samples were determined through phylogenetic analyses (Supplementary Figs S1 and S2). Analyses of Core and NS5B sequences gave consistent genotypes for all samples, except for two (0.23%). Several scenarios could explain these conflicting genotyping results, including coinfection, or sample contamination with multiple virus genotypes, or that the viruses were genuine recombinants. While the exact reasons for these results were unclear, the differing genotypes of the Core and NS5B sequences indicated that the generated sequences contained conflicting evolutionary signals, which can bias the standard evolutionary analysis if analyzed together as single "mosaic" sequences, i.e. sequences with different parts having different evolutionary histories (Aiewsakun 2024). They were therefore excluded from further downstream analyses.

Excluding the two samples with conflicting Core and NS5B genotypes, consistent with previous results (Wasitthankasem et al. 2015, 2016), phylogenetic analysis of the concatenated Core and NS5B coding regions revealed the presence of three major HCV genotypes (and 11 subtypes) in this dataset: Genotype 1 (Subtypes 1a and 1b), Genotype 3 (Subtypes 3a and 3b), and Genotype

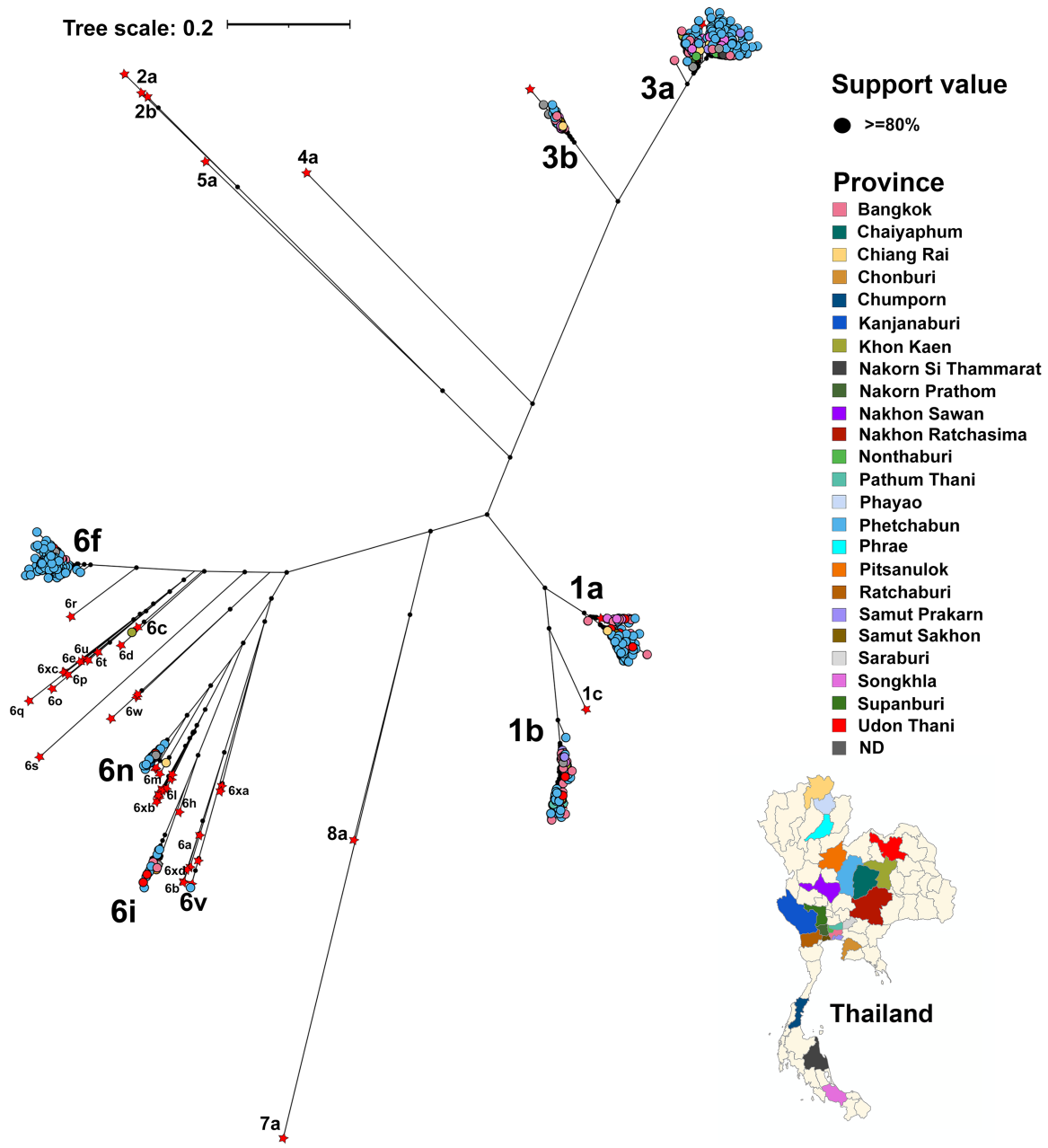

Figure 1. ML phylogeny depicting the diversity of HCV analyzed in this study ($n = 852$). The tree was constructed from a concatenated alignment of Core and NS5B coding regions. Among these sequences, 539 sequences are from Phetchabun, while the remaining are from 24 provinces across all six major geographical regions in Thailand. Asterisks indicate HCV reference samples with known genotypes. Labels next to the tree indicate HCV subtypes. The geographical origins of the sequences at the provincial level are also indicated on the tree. The map shows the location of each province in Thailand. Nodes with bootstrap clade support values of ≥80% are indicated by black circles. The scale bar is in the units of substitutions per site.

6 (Subtypes 6c, 6 f, 6i, 6j, 6m, 6n, and 6v) (Fig. 1). No novel subtypes or genotypes were found.

3.2. HCV genotype and subtype distributions in Phetchabun compared to other provinces in Thailand

Considering samples with known sampling locations and consistent Core and NS5B genotypes ($n = 836/852$, 98.12%), we found that, overall, the distribution of HCV genotypes in the Phetchabun dataset (n = 539/836, 64.47%) was significantly different from that of other provinces ($n = 297/836$, 35.53%) (multinomial regression analysis with Type III analysis of deviance: $df = 2$; $\chi^2 = 46.435$, $P = 8.257 \times 10^{-11}$; Fig. 2a and b). Similar results were observed at the subtype level ($df = 5$; $\chi^2 = 99.180$, $P = 7.869 \times 10^{-20}$; Fig. 2c and d). In particular, Genotype 6 was significantly enriched in the Phetchabun dataset [% difference = +21.69%, standard error (SE) = 2.99%, $df = 4$, $t = 7.259$, $P = .0019$], especially Subtype 6f (% difference = +26.29%, SE = 2.49%, $df = 10$, $t = 10.544$, $P = 9.76 \times 10^{-7}$). Conversely, Genotype 3 was significantly more enriched in the sequence dataset from the rest of the country (% difference = +15.17%, SE = 3.55%, $df = 4$, $t = 4.274$, $P = .0129$), and this trend could be observed for both Subtypes 3a (% difference = + 9.13%, SE = 3.50%, $df = 10$, $t = 2.612$, $P = .0260$) and 3b (% difference = +6.04%, SE = 1.69%, $df = 10$, $t = 3.565$, $P = .0051$). The percentage of Genotype 1 was slightly lower in the Phetchabun dataset, but this difference was not statistically significant overall

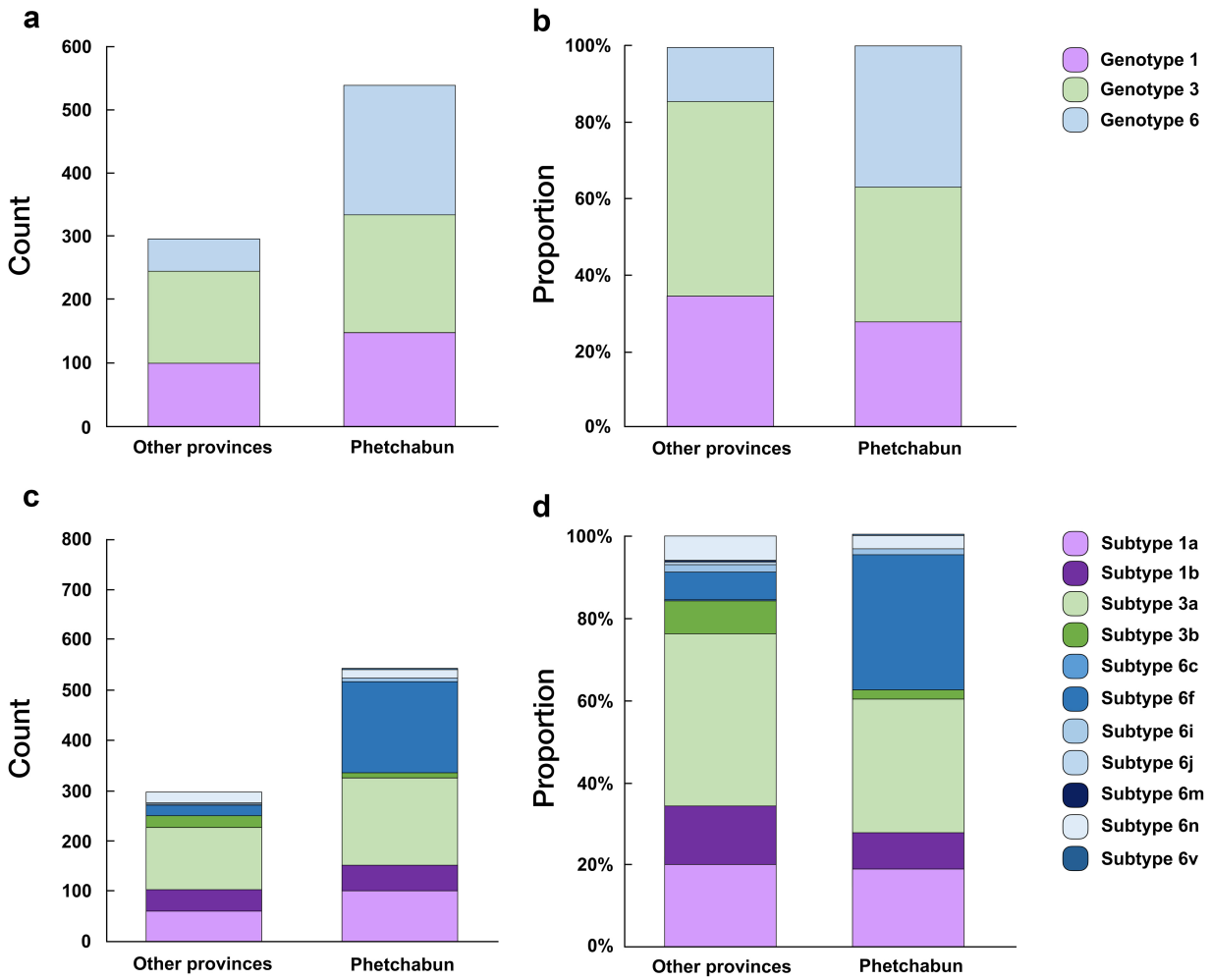

Figure 2. Distribution of HCV genotypes (a and b) and subtypes (c and d) in Phetchabun ($n = 539$) and other provinces ($n = 297$) in Thailand. The plots are presented in terms of count (a and c) and proportion (b and d). See Supplementary Table S4 for the estimated genotype and subtype proportions in both datasets and their pairwise % comparisons.

(% difference = –6.51%, SE = 3.36%, $df = 4$, $t = -1.936$, $P = .125$). These results highlight the differential geographical distributions of various HCV genotypes and subtypes. The proportions of all genotypes and subtypes in the two datasets are presented in Supplementary Table S4.

3.3. Population dynamics analyses

Root-to-tip regression analyses (Supplementary Fig. S3) suggested significantly different evolutionary rates among different HCV subtypes (i.e. having statistically different slopes; analysis of variance: $F = 7.2758$, $df = 7$, $P = 1.745 \times 10^{-8}$). Further examination revealed that only Subtypes 1b, 3a, and 6f had sufficient temporal signals (Supplementary Table S5). Further root-to-tip regression analysis of these three subtypes (Supplementary Fig. S4) confirmed that they had significantly different evolutionary rates (analysis of variance: $F = 6.6026$, $df = 2$, $P = 1.459 \times 10^{-3}$). Consequently, molecular dating and population dynamics analyses were performed separately for each of these HCV subtypes using their recombination-free concatenated Core-NS5B coding sequences (Fig. 3).

3.3.1 *Subtype 1b*

The basal diversification date of Subtype 1b was estimated to be ~1713.87 (95% HPD: 1253.63–1975.32), making it the oldest subtype in our dataset (Table 1). This subtype exhibited the lowest rate of evolution among the three subtypes investigated, at 3.72×10^{-4} (95% HPD: 2.31×10^{-6} to 7.44×10^{-4}) substitutions/site/year (s/n/y) (Table 1). The virus phylogenetic tree showed two major deep clades (Fig. 3a). One contains sequences from multiple provinces, including Phetchabun, suggesting a nationwide spread of this sublineage. The other clade comprises mainly sequences from Phetchabun, with a few sequences from other provinces, indicating local expansion of this sublineage in Phetchabun with occasional cross-province transmissions. Regarding its population dynamics, the virus population size was inferred to remain relatively stable for more than two centuries, from its basal diversification in the 1710s until the mid-1940s, when a rapid exponential expansion started. The expansion continued until the mid-1990s/early 2000s, after which the population growth slowed substantially and appeared to stabilize.

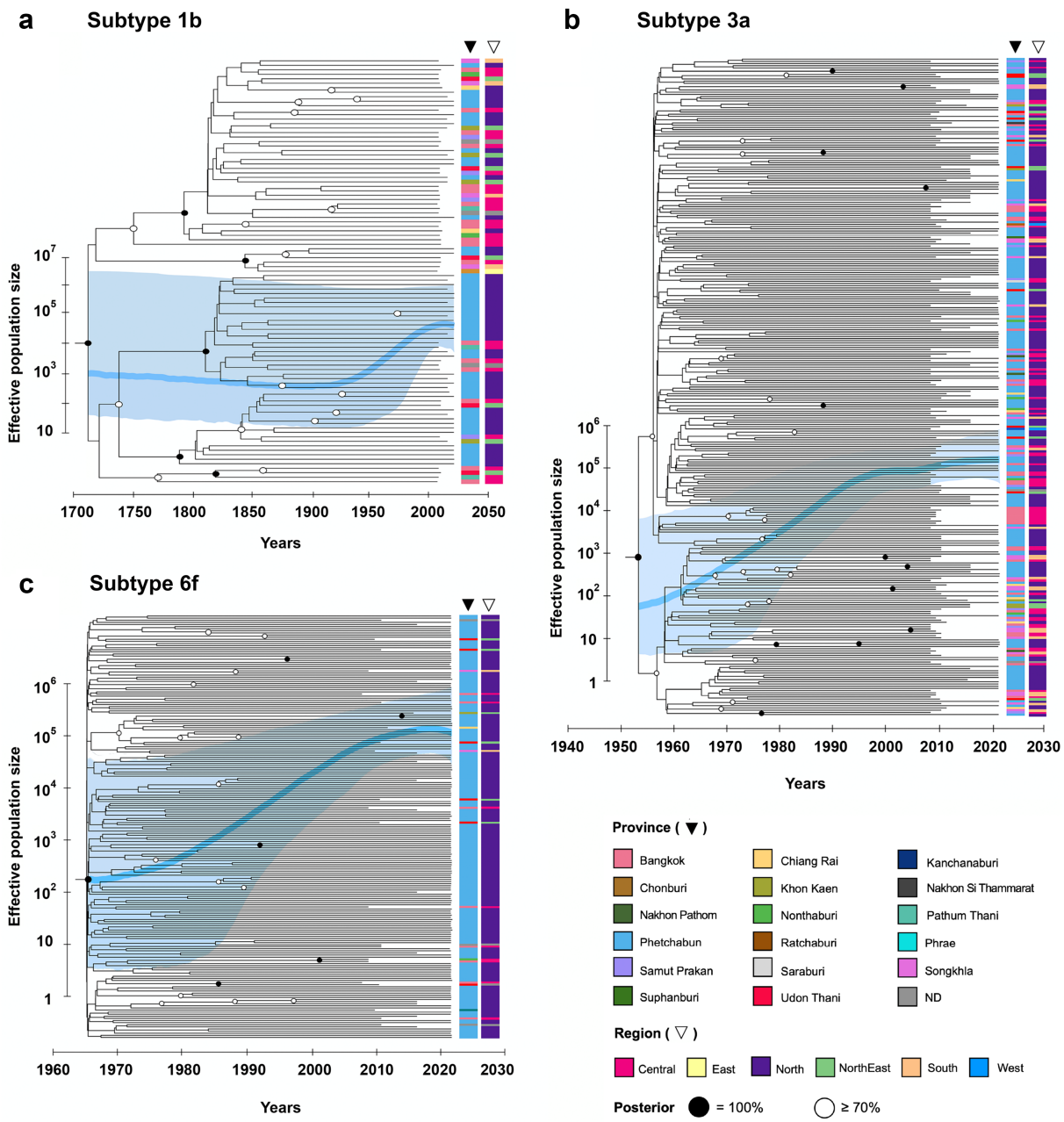

Figure 3. Molecular phylodynamic analyses of (a) HCV Subtypes 1b, (b) 3a, and (c) 6f. The time-calibrated phylogenies were inferred using a Bayesian phylogenetic framework implemented in BEAST 1.10.4. Substitution models used were the TIM2 + F + I + Γ4, TVMe + I + Γ4, and TIM2e + I + Γ4 for Subtypes 1b, 3a, and 6f, respectively, along with the Bayesian time-aware GMRF Skyride coalescent tree prior and the uncorrelated log-normal relaxed clock model. Nodes with 100% clade support are labeled with black solid circles, while those with >70% clade support are labeled with open circles. Bars on the right indicate HCV-isolated locations at the provincial level (filled triangle) or at the major region level (open triangle). The inferred past population dynamics are also shown (thick blue line = median effective population size; light blue region = 95% HPD).

3.3.2 *Subtype 3a*

The most recent common ancestor of Subtype 3a in our dataset was estimated to be around 1953.30 (95% HPD: 1927.13–1974.85), consistent with a previous study (Akkarathamrongsin et al. 2013), with an estimated evolutionary rate of 1.00×10^{-3} (95% HPD: 6.11×10^{-4} to 1.39×10^{-3}) s/n/y (Table 1). Phylogenetic analysis revealed two large clades of Subtype 3a, both containing sequences from multiple provinces, suggesting a widespread and unstructured geographic distribution of this subtype in Thailand (Fig. 3b). The population dynamics were inferred to show an exponential expansion starting around the 1960s to the mid-1990s/early 2000s. After this period, the virus population size continued to grow but at a markedly slower rate.

3.3.3. *Subtype 6f*

Subtype 6f, the predominant strain in the Phetchabun dataset, was estimated to share the most recent common ancestor around 1964.68 (95% HPD: 1931.03–1987.72). The evolutionary rate of Subtype 6f was comparable to that of Subtype 3a, estimated at 1.00×10^{-3} (95% HPD: 5.04×10^{-4} to 1.50×10^{-3}) s/n/y (Table 1). The sequences formed one large unstructured clade, with most samples from Phetchabun, and a few sequences from other provinces

Table 1. Bayesian evolutionary parameters estimated for HCV Subtypes 1b, 3a, and 6f.

Parameters	Subtype 1b	Subtype 3a	Subtype 6f
MCMC (million states)	300	800	700
Burn-in (million states)	30	220	70
Mean root height (years) (95% HPD)	307.59 (46.14, 767.81)	68.16 (46.61, 94.33)	56.76 (33.72, 90.41)
Mean root age (Common Era) (95% HPD)	1713.87 (1253.63, 1975.32)	1953.3 (1927.13, 1974.85)	1964.68 (1931.03, 1987.72)
Uncorrelated log-normal relaxed clock model mean rate (s/n/y) (95% HPD)	3.72×10^{-4} (2.31×10^{-6}, 7.44×10^{-4})	1.00×10^{-3} (6.11×10^{-4}, 1.39×10^{-3})	1.00×10^{-3} (5.04×10^{-4}, 1.50×10^{-3})
Coefficient of rate variation (95% HPD)	0.5 (0.38,0.63)	0.34 (0.29,0.39)	0.32 (0.26, 0.39)

Parameters were inferred under the best-fitting model by using BEAST v. 1.10.4 as indicated in Supplementary Table S6.

interspersed across the tree (Fig. 3c). This supports that this subtype is a locally predominant strain in Phetchabun, with occasional spillover to other provinces. The population size was estimated to have exponentially increased from the mid-1970s, peaking in the late 2010s and then stabilizing in recent years.

3.4. Potential historical drivers of early HCV transmission in Thailand

By mapping key historical events onto the estimated virus population trajectories, we attempted to identify potential drivers of early HCV spread in Thailand (Fig. 4).

The estimated early expansion of Subtype 1b, beginning around the mid-1940s, coincided with World War II (WWII; 1939–1945) (U.S. Army Europe and Africa), suggesting a possible link between the two. Shortly after WWII, the Thai Red Cross Society introduced blood donation and transfusion services in 1952 (Pikulsod and Nuchprayoon 1999). Although this was a significant advancement in healthcare in Thailand, these services were initially carried out with suboptimal hygiene standards and medical practices. At the time, for example, protocols for blood-borne pathogen screening were lacking (Pikulsod and Nuchprayoon 1999, Chimparlee et al. 2011), potentially exacerbating the early spread of the virus. Paralleling this, previous studies have implicated both WWI and WWII in the global dissemination of HCV, particularly in North America and Australia, during which many significant advancements in medical practices for major trauma management occurred, such as blood transfusion, subcutaneous administration, and routine use of morphine injection for medical treatment (Rodrigo et al. 2016). The initial expansion of Subtype 1b in Portugal has also been estimated to have occurred around the same time period, between the 1930s and the 1960s (Palladino et al. 2018), with unsafe injection practices and blood transfusions being proposed as primary transmission routes, similar to ours. Moreover, similar transmission routes have been implicated for Subtype 1b in China, particularly during the Chinese Cultural Revolution in the 1960s (Nakano et al. 2006). All these convergent results highlight this as a potential common global phenomenon.

For Subtype 3a, its early expansion was estimated to start in the 1960s, coinciding with the Vietnam War (1955–75) (Vietnam War Commemoration 2024), a period marked by widespread malpractice and illicit drug abuse, including a nationwide heroin epidemic in Thailand from the 1960s to the 1980s (Reid and Costigan 2002, Janos 2018). This finding supports a possible link between the early spread of this subtype with high-risk behaviors associated with IVDUs, such as needle sharing. Studies have reported extremely high HCV infection rates among IVDUs in Thailand, reaching 70%–93% (Hansurabhanon et al. 2002, Verachai et al. 2002, Vongchak et al. 2005), while infection rates among noninjecting drug addicts were reported to be ~12%–21% (Verachai et al. 2002), markedly lower than the rates among IVDUs. Indeed, needle sharing has been identified as a high-risk factor for HCV infections (Hansurabhanon et al. 2002, Verachai et al. 2002), supporting the plausibility of this hypothesis. Similar patterns linking HCV transmission to drug injection and/or needle sharing have been proposed in several other regions, including the transmission of Subtype 3a in China (Liu et al. 2011), Subtype 2a in Japan (Tanaka et al. 2005), and Subtype 4a in Egypt (Pybus et al. 2003). These results collectively highlight the global significance of this route for HCV transmission.

The early expansion of Subtype 6f, on the other hand, was dated to coincide with a period of severe political conflict in Phetchabun between the Thai government military force and an armed civilian resistance group between 1968 and 1982 (Fig. 4) (Kositanont 2018), suggesting a potential connection between the two. The battles mainly occurred in the northern part of the province, and access to medical and healthcare resources was limited during that time, likely worsen by the mountainous terrain of the northern districts. This unique historical event might have contributed to the early spread of the virus in Phetchabun and offered a plausible explanation for its high HCV prevalence relative to other provinces. The mountainous terrain itself might have also limited virus movement beyond provincial boundaries (Wasitthankasem et al. 2017, 2020), potentially explaining the localization of Subtype 6f in Phetchabun.

This hypothesis is corroborated well by that northern districts in Phetchabun showed both higher seroprevalence rates (6.7%–15.5%) and active/chronic infection rates (inferred from HCV RNA positive rates, 3.37%–12.26%) compared to southern districts (0.9%–4.6% and 0.29%–3.09%, respectively) (Wasitthankasem et al. 2020). In addition, previous studies reported a higher prevalence of HCV infection among males of working age with limited education residing in northern areas and employed in agriculture (Wasitthankasem et al. 2017, 2018, 2020)—the main demographic group involved in the political fight—further corroborating the hypothesis. These demographic factors also often correlate with lower socioeconomic status and reduced access to healthcare, which could have increased vulnerability to infectious diseases. Other risk behaviors, such as IVDU, blood transfusion, and tattooing, have also been associated with HCV infection in this population (Buathong et al. 2013, Wasitthankasem et al. 2017, 2018), highlighting the complex interplay of multiple factors influencing HCV transmission.

3.5. Potential impacts of public health initiatives on HCV population dynamics in more recent years

Remarkably, the three HCV subtypes were independently estimated to have expanded markedly slower at around the same time, starting from the mid-1990s/early 2000s (Fig. 4). In line with this are the observed substantial reduction in HCV antibody

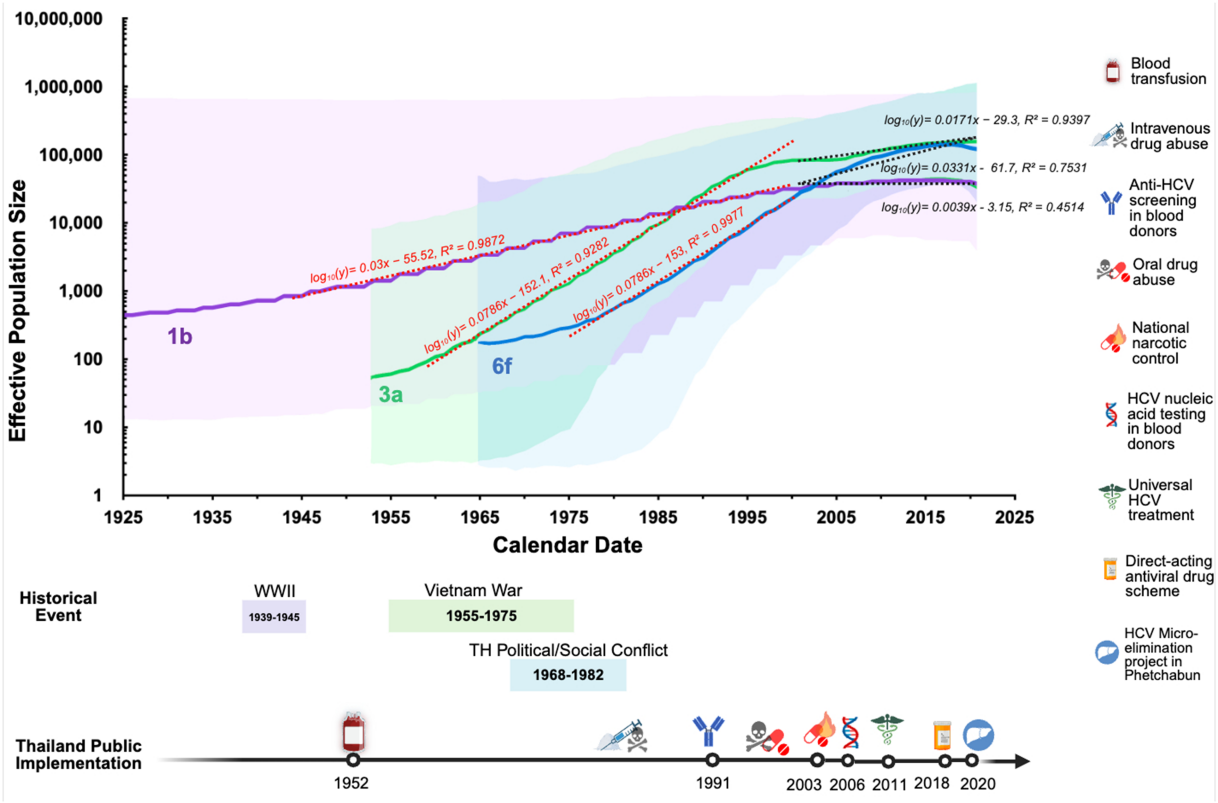

Figure 4. Estimated population dynamics of HCV Subtypes 1b, 3a, and 6f. Solid lines and filled areas depict the median effective population size and associated estimation uncertainty, respectively, on a log scale as estimated using BEAST 1.10.4. Dotted lines depict linear regression models of the HCV population growth before the 2000s (red) and after the 2000s (black). The timings of major global and local historical events, as well as social and public health implementations in Thailand that might be associated with the HCV population dynamics, are shown at the bottom using different icons. Refer to the keys located to the right for interpretation. The figure created in BioRender.com.

prevalence among blood donors and a decline in overall national prevalence, staring from 1995 onward (Pikulsod and Nuchprayoon 1999, Chimparlee et al. 2011, Wasitthankasem et al. 2016, Chiewsilp 2019).

During this time and thereafter, Thailand saw several improvements in public healthcare procedures related to blood transfusion services. In 1991, the Thai Red Cross Society introduced mandatory HCV serological screening of all blood donors. This was followed by viral nucleic acid testing of seronegative blood units in 2006, a mini pool of six blood units in 2008, and all individual blood units in 2016 (Pikulsod and Nuchprayoon 1999, Chimparlee et al. 2011, Chiewsilp 2019). Additionally, the Thai government launched the Universal Health Coverage program in 2002, which expanded access to public healthcare across all socioeconomic classes nationwide. Universal HCV treatment was then introduced in 2011, with the availability of effective direct-acting antiviral (DAA) in 2015, which subsequently became a standard regimen for HCV treatment in 2018 (Rattanavipapong et al. 2018, Wasitthankasem et al. 2020). Moreover, in 2003, the Thai government announced the "War on Drugs" campaign, aiming to curb narcotic drug use nationwide. This campaign reportedly led to an 85% reduction in IVDU (Vongchak et al. 2005), a major high-risk factor for HCV infections (Hansurabhanon et al. 2002, Verachai et al. 2002, Bangkok Tenofovir Study Group 2019). These concerted efforts likely contributed to the inferred overall reduction in the spread of the virus in recent years.

4. Conclusions and final remarks

This study provides new insights into the epidemic histories of HCV circulating in Thailand, with a particular focus on Phetchabun, the first province targeted for HCV elimination. To the best of our knowledge, this is the first study of HCV in Thailand to explore plausible interactions between social events, public health policies, and population dynamics of different HCV genotypes inferred using molecular phylodynamic analysis. Our findings reveal distinct epidemiological patterns and early historical drivers for Subtypes 1b, 3a, and 6f. Specifically, our analyses dated the initial expansion of Subtype 1b back to the period round WWII and the early years of blood transfusion services in Thailand, which were carried out with suboptimal hygienic and medical standards. In contrast, the early spread of Subtype 3a correlated with widespread IVDU in the country, dating back to around the time of Vietnam War. The early expansion of Subtype 6f, predominantly found in Phetchabun, was linked to the severe political conflict and social and economic upheaval in the province between the 1960s and 1980s.

Remarkably, all subtypes independently showed a substantial decline in population growth around the mid-1990s/early 2000s, with rates remaining relatively low thereafter. Starting from this period, several significant improvements in blood transfusion safety standards were introduced, and the accessibility to public healthcare and HCV treatment was greatly enhanced in the country, likely contributing to this decline. The Thai government's "War

on Drugs" campaign, announced in 2003, also likely played a role in reducing HCV transmission through by curbing IVDU, a significant risk factor for HCV infection (Hansurabhanon et al. 2002, Verachai et al. 2002, Vongchak et al. 2005, Bangkok Tenofovir Study Group 2019).

This study is limited by the availability of data, primarily consisting of sequences from Phetchabun province. While our results clearly indicate that different HCV subtypes show different geographical distributions and that the virus can spread across provinces, further in-depth subtype enrichment and phylogeographic analyses are needed to better understand how the virus spreads throughout Thailand, but this requires additional sequence and epidemiological data systematically collected across the country. Such data would also enable more detailed reconstruction of the virus population and evolutionary trajectories, allowing for a more precise identification of factors driving its spread in recent times. This information would be highly valuable for designing more effective disease control measures, highlighting the importance of expanding HCV surveillance to other regions, and underscoring the need for a well-planned and systematic nationwide HCV surveillance program in the future.

Lastly, while our results support that significant progress has been made in curbing HCV transmission in Thailand, our analyses suggest that, overall, the prevalence of HCV in Thailand remains high, with no clear declining trend yet evident (Fig. 4). This includes Subtype 6f, which is primarily confined to Phetchabun, the focal point of the HCV microelimination program in Thailand. However, this could be because our sequence data only included samples up until 2021, just 3 years after the full-scale implementation of the test-to-treat campaign in 2019 (Wasitthankasem et al. 2023). The operation also faced difficulties due to the coronavirus disease 2019 pandemic, which resulted in a prolonged lack of sufficient human resources, budget allocation, and antiviral treatment supply, causing delays in screening and treatment initiation (Wasitthankasem et al. 2023). Therefore, the impact of the program might not yet be fully realized. Further research is warranted to fully assess the long-term effectiveness of the HCV microelimination efforts.

Indeed, our findings suggest that continued research and a multipronged approach are still needed to more effectively reduce the burden of HCV in Thailand. Studies have consistently identified IVDUs as a major high-risk population for HCV infection (Hansurabhanon et al. 2002, Verachai et al. 2002, Wasitthankasem et al. 2017, 2018, Bangkok Tenofovir Study Group 2019), highlighting the importance of continuous HCV surveillance among this group and the necessity for more effective strategies to reduce drug abuse and associated risks across various drug use behaviors to further curb the virus transmission. Moreover, our analyses revealed that HCV can readily spread across provincial boundaries, emphasizing the need for coordinated efforts nationwide to control the disease most effectively. In general, strengthening blood transfusion safety protocols, broadening access to DAAs, intensifying HCV surveillance programs, implementing more effective drug control programs and targeted interventions for IVDUs, and expanding microelimination efforts while respecting regional differences are all essential steps toward achieving the goal of eliminating HCV.

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Author contributions

Rujipat Wasitthankasem (Conceptualization, Investigation, Resources, Formal analysis, Data curation, Visualization, Project administration, Funding acquisition, Writing—original draft, Writing—review & editing), Pakorn Aiewsakun (Data curation, Formal analysis, Investigation, Methodology, Visualization, Supervision, Validation, Writing—original draft, Writing—review & editing), Sutthinee Lapchai and Maneerat Raksayot (Investigation and Data curation), Chantisa Keeratipusana (Investigation), Pakawat Jarupund and Vorthunju Nakhonsri (Visualization), Napaporn Pimsing (Resources and Project administration), Sissades Tongsima (Resources, Visualization, Supervision, Writing—review & editing), and Yong Poovorawan (Conceptualization, Resources, Funding acquisition, Writing—review & editing). All authors read and approved of the manuscript.

Supplementary data

Supplementary data is available at *Virus Evolution* online.

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Data availability

The nucleotide sequences of HCV generated in this study have been deposited in GenBank under accession numbers PP163805–PP164380 and PP164397–PP164886. Additional data that support the findings of this study are available in the Supplementary material. Further inquiries regarding the data can be directed to the corresponding author.

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