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Short communication

Genotypic persistence of dengue virus in the Philippines

Ma. Jowina Galarion[a,*], Brian Schwem[a], Coleen Pangilinan[a], Angelo dela Tonga[a], Joy Ann Petronio-Santos[d], Erlinda delos Reyes[b], Raul Destura[a,b,c]

[a] National Institute of Molecular Biology and Biotechnology, National Institutes of Health, University of the Philippines Manila, Ermita, Manila, Philippines
[b] The Medical City Hospital and Infectious Disease Practice and Innovations, Pasig City, Philippines
[c] Philippine Genome Center, University of the Philippines System, Diliman, Quezon City, Philippines
[d] Biological Sciences Research and Services Laboratory, Natural Sciences Research Institute, University of the Philippines Diliman, Diliman, Quezon City, Philippines

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ABSTRACT

The Philippines is known to have one of the world's highest prevalences of dengue infection. The disease has been endemic in the country since 1956 and the severe form was first reported during an outbreak in Manila in 1954. Among all of the countries in the world, the Philippines had the highest case fatality rate from 2008 to 2012. With the increasing rate of international travel, the country is also considered one of the primary sources of imported dengue cases in non-endemic areas in Asia, Australia, and Europe. Despite this high prevalence, there is a dearth of literature describing the circulating strains in the Philippines at the genotype level. Using data from sequence databases, this study aimed to characterize all available Philippine sequences, at the molecular level. Capsid/pre-membrane (C/prM) junction gene and envelope (E) gene sequences of dengue serotypes 1, 2, 3 and 4 from 1956 to 2016 were used for phylogenetic analysis and genotypic identification. All four serotypes co-circulate in the country over the last 50 years with conspicuous genotypic characteristics. DENV-1 exhibited an apparent persistence of a single genotype since 1974. DENV-2 showed strong evidence of genotypic shift in 1999–2002 accompanied by a genotypic persistence thereafter. DENV-3 and DENV-4 displayed a temporal domination of a single genotype, with evidence of a minor co-circulating genotypic population. The persistence and pre-domination of specific DENV genotypes warrant continuous molecular surveillance for signs of genotypic shifts that can cause local outbreak events or an increased risk for severity.

1. Introduction

Dengue is the most prevalent arthropod-borne viral (arboviral) disease in humans. It caused an estimated 400 million infections globally in 2010, with over 500,000 developing the severe forms every year (Bhatt et al., 2013; Whitehorn and Simmons, 2011). According to the World Health Organization, the Philippines has recurring DHF epidemics every 2-3-years (WHO, 2001). Compared to other countries in Asia, the Philippines had the highest dengue case fatality rates (CFR) ranging from 0.49–0.94% based on a five-year WHO report from 2008 to 2012 (Arima et al., 2015; Arima et al., 2013; Arima and Matsui, 2011).

Since 1995, dengue virus (DENV) serotypes 1, 2 and 3 have predominantly circulated during recorded local outbreaks with sporadic cases of DENV-4 (Salda et al., 2005; ter Meulen et al., 2000). There has been co-circulation of at least two serotypes and hyperendemicity of dengue in the country for more than two decades. In 2014, at least 113,000 dengue-suspected cases nationwide were reported, followed by a 60% increase in both 2015 and 2016 (DOH Epidemiology Bureau Public Health Surveillance Division, 2016, 2015, 2014).

The Philippines is also identified as one of the significant geographic origins of imported cases of DENV to non-endemic countries based on surveillance studies in Taiwan, China, Japan, South Korea, Australia and Germany (Chang et al., 2016; Go et al., 2015; Huang et al., 2007; Ito et al., 2007; Jeong et al., 2011; Shihada et al., 2017; Shu et al., 2009; Sun et al., 2016; Warrilow et al., 2012).

Apart from a few clinical and serotypic studies of local strains, no recent studies describe the circulating dengue viruses in the Philippines at the molecular level, despite its endemicity (Salda et al., 2005; ter Meulen et al., 2000). Given the country's morbidity rates, relatively high CFRs, and its potential role in global expansion through importation into non-endemic countries, it is imperative to continuously characterize the local strains and compare these with globally circulating strains. This study presents the phylogenetic analysis of available published DENV sequences from the Philippines isolated in 1956–2016.

* Corresponding author at: G/F Rm. 108, National Institutes of Health Bldg., U.P. Manila, 623 Pedro Gil St., Ermita, Manila 1000, Philippines.
E-mail address: mhgalarion@up.edu.ph (M.J. Galarion).

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2. Materials and methods

2.1. Nucleotide sequences

This study is a secondary data analysis of all available Philippine DENV sequences in the National Center for Biotechnology Information (NCBI) (https://www.ncbi.nlm.nih.gov/) from 1956 to 2016. From the NCBI's Nucleotide database, sequences were queried using the Boolean search keywords “dengue virus AND Philippines”. A total of 607 dengue virus sequences were associated with the Philippines. Both FASTA format and full GenBank files for each accession were downloaded. Sequence metadata was also obtained, such as isolate/strain name, GenBank accession number, collection date, serotype, country, sequence length, and protein product.

2.2. Pre-processing of sequences

Of the 607 sequences, 593 unique isolates/strains were collected from the Philippines or from patients who recently traveled from the Philippines. Fourteen samples were automatically excluded, as they are either duplicate strains or synthetic constructs. Considering that a majority of the available sequences code for the capsid/pre-membrane (C/prM) junction or the envelope (E) gene, only sequences that cover these gene regions were included for further analysis (total of 556 sequences). The number of sequences was further trimmed down to 318 after removing entries with greater than 95.5% similarity using an in-house Python script.

2.3. Phylogenetic analysis

Genotyping was based on methods by Villabona-Arenas et al. for DENV-1 or by Klungthong et al. for DENV-2, 3 and 4 (Klungthong et al., 2008; Villabona-Arenas and Zanotto, 2013). Genotypic analysis was performed by reconstructing rooted maximum likelihood (ML) trees from the 318 Philippine sequences (125 envelope genes and 193C/prM junction genes), 89 reference sequences, and 208 global DENV sequences. Global strains were selected based on geographic representativeness of available complete genome sequences. No outgroups were used in the analysis. Multiple sequence alignment and phylogenetic tree reconstruction were performed using Clustal v.1.2.4 and RAxML v.2.0.6, respectively. ML trees for each gene region were constructed using the general time reversible (GTR) evolutionary model with

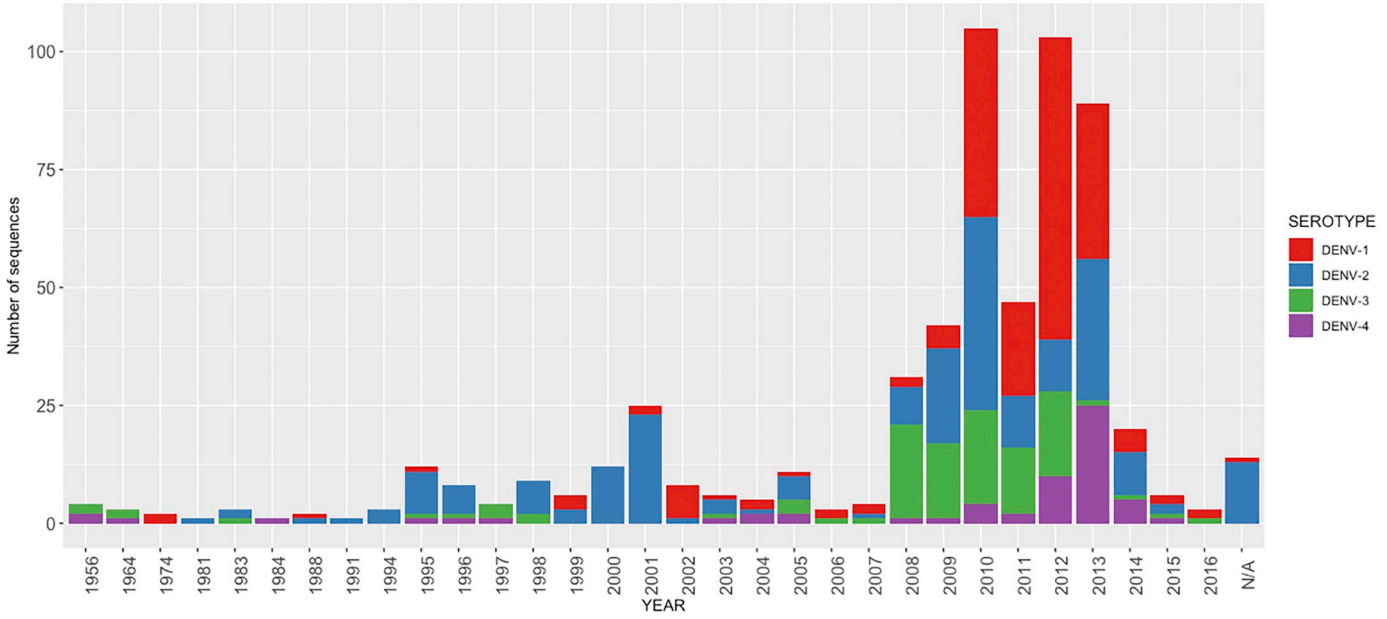


Fig. 1. Sequence serotype. Serotype distribution of all available NCBI DENV sequences associated with the Philippines (593 total) from 1956 to 2016. N/A indicates accessions without any information on isolation date.

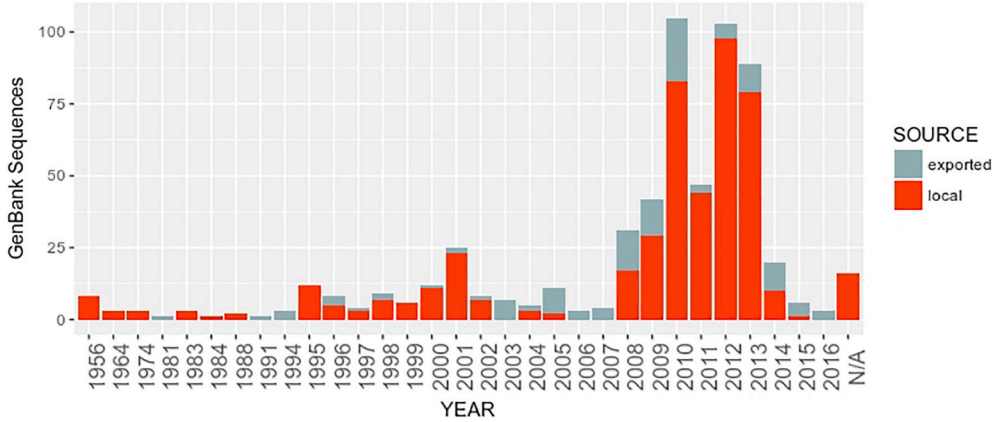
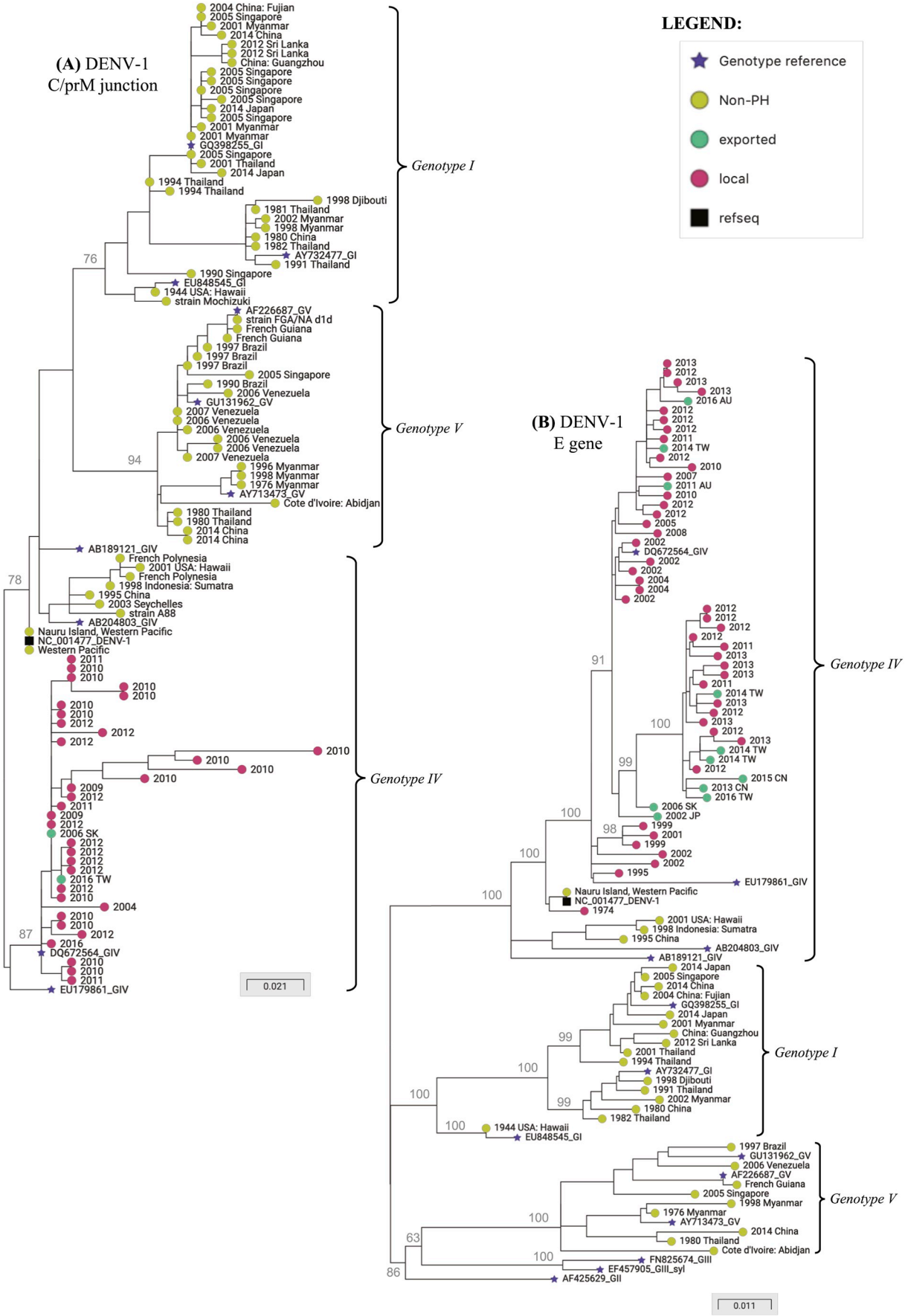


Fig. 2. Sequence source. Source (locally isolated or exported to other countries) of all available NCBI DENV sequences associated with the Philippines (593 total) from 1956 to 2016. N/A indicates accessions without any information on isolation date.



(caption on next page)

Fig. 3. Phylogenetic trees of C/prM junction and E gene sequences. ML trees were created using the GTR nucleotide substitution model with gamma distribution at 1000 bootstrap replicates. Trees are rooted midpoint in increasing node order. Distance scale represents nucleotide substitution per site. Isolates are labeled by collection year. Seven local sequences in Fig. 3D published in August 2000 have missing collection/isolation dates, and were instead labeled as the year they were published. Reference sequences (blue star) are labeled by their NCBI accession numbers and the corresponding genotype it represents. (Abbr.: TW-Taiwan, DE-Germany, CN-China, JP-Japan, AU-Australia, SK-South Korea, G-genotype, AsI-Asian I, AsII-Asian II, AsAm-Asian/American, Cos-Cosmopolitan, Ame-American). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

gamma distribution, at 1000 bootstrap replicates. Trees were viewed and edited using FigTree v.1.4.2 and Microreact (https://microreact.org), accordingly.

2.4. Accession numbers

The full list of sequence accession numbers used in this study is found in Supplementary Table 1. All reference sequences used for genotypic analysis are found in Supplementary Table 2.

3. Results and discussion

3.1. Serotype distribution

Serotype distribution of the 593 unique Philippine dengue sequences shows the presence of all three serotypes DENV-1 (198 sequences), DENV-2 (224 sequences) and DENV-3 (110 sequences), with an intermittent isolation of DENV-4 (61 sequences) between 1956 and 2016 (Fig. 1). Based on surveillance, all four serotypes have been circulating in the Philippines for the last 50 years (Tayag, 1998; WHO, 2001). Previously recorded epidemic events include a major outbreak in 1998 and a substantial increase in the number of cases in 2010 (Bravo et al., 2014). The majority of the downloaded sequences were collected in 2010, due to the increased cases. However, the apparent surge of sequence distribution over time can also be partly due to an improved dengue surveillance system in the Philippines. DENV-2 was the most isolated and characterized serotype from 1995 to 2010 (Petronio et al., 2014; Salda et al., 2005). No further genotype data of Philippine sequences have been available since that time.

Of the available sequences associated with the Philippines, approximately 21% (124 total) were GenBank submissions by other countries, 119 of which were imported cases by Taiwan, Japan, South Korea, China, Australia, Germany and some unspecified European countries (Fig. 2) (Huang et al., 2012; Go et al., 2015; Moore et al., 2017; Shihada et al., 2017; Jeong et al., 2011; Fortuna et al., 2017; Huang et al., 2007; Chang et al., 2016; Shu et al., 2009; Ito et al., 2007; Warrilow et al., 2012). The relatively large proportion of exported isolates suggests the Philippines' role in the expansion of the disease not only within neighboring Asian countries but also to other continents.

3.2. Genotypic and clade analysis

3.2.1. DENV-1

Since 1974, circulating sequences of DENV-1 in the Philippines belong to a single genotype (IV), as seen in both C/prM junction and E gene trees (Fig. 3A and Fig. 3B). Genotype IV is generally found in but not limited to Asia and the Pacific, and the study by Villabona-Arenas strongly considered the Philippines as its “ancestral area” and the probable source of its introduction to the Pacific and the Americas (Villabona-Arenas and Zanotto, 2013). Within the genotype IV lineage in the E tree, there are at least two independent clusters of local sequences with bootstrap support of 91 (Fig. 3B). One cluster that includes most of the older E gene sequences (2002 − 2010) are 98% identical to HawO663 strain (genotype reference DQ672564) isolated from a DF patient traveling from Samoa in 2001. The other cluster with bootstrap support of 100, mostly includes relatively recent sequences from 2011 to 2016, which may have evolved locally from the older group. Separated from mainland Asia, the Philippine topography may have modulated the re-introduction of new strains, thus supporting the theory of local evolution among its genotype IV viruses.

Some published sequences from South Korea, Taiwan, China, Australia, and Japan, which are grouped with local Philippine sequences, were previously isolated from patients traveling from the country in 2002–2015 (Fig. 3A and Fig. 3B) (Huang et al., 2012; Jeong et al., 2011; Shu et al., 2009; Ito et al., 2007; Moore et al., 2017). These reports suggest active importation and continuing introduction of the virus to neighboring countries. Based on the surveillance studies from 2003 to 2010, genotype IV is the second most frequently imported DENV-1 in Asia. In fact, the Philippines is being reported by nearby countries (Taiwan and South Korea) as the only source of this genotype (Huang et al., 2012; Jeong et al., 2011; Shu et al., 2009).

Unlike in the Philippines, co-circulation of at least two genotypes (I and II) has been observed in neighboring Asian countries (A-Nuegoonpipat et al., 2004; Goncalvez et al., 2002; Huang et al., 2012; Osman et al., 2009). The persistence of genotype IV in the country may be due to the archipelagic topography, which provides a natural barrier to the introduction of other genotypes. However, increased rates of travel and migration to and from other dengue-endemic countries may lead to the introduction of other genotypes, thereby causing epidemic events (Yamanaka et al., 2011).

3.2.2. DENV-2

An analysis of the C/prM junction and E gene sequences from 1995 to 2015 (Fig. 3C and Fig. 3D) showed the persistence of the DENV-2 Cosmopolitan genotype for over 15 years in the Philippines. In Fig. 3D however, earlier isolates from 1983 to 2005 are Asian II genotype signifying a DENV-2 genotype shift in the country (Salda et al., 2005). The study by Salda et al. in 2005 first showed evidence of a genotypic shift from Asian I to Cosmopolitan around 1999 to 2002. The paper specifically mentioned that the Cosmopolitan strains from their study are closely related to older strains from Australia (1991, 1997), Singapore (1991) and Thailand (1998) implying that these countries may have subsequently introduced this genotype. Most C/prM sequences were recent isolates and are of the Cosmopolitan genotype (Fig. 3C), suggesting its persistence (Petronio et al., 2014).

A study by Holmes and Twiddy in 2003 mentioned that most of the global DENV-2 Asian II strains have originated from the Philippines, providing further evidence that the probable ancestral genotype has been recently replaced in the country by the Cosmopolitan (Holmes and Twiddy, 2003).

The shift from Asian II to Cosmopolitan can be accounted for due to the latter's strong positive selection associated with its virulence and dispersal ability (Twiddy et al., 2002). The study by Twiddy et al. in 2002 mentioned evidence of a positive selection in position E390 of the Cosmopolitan genotype (with Serine residue), affecting both virulence and cell tropism. Additional amino acid changes (E71, 129, 149, 164 and 462) occurring on the tree branch leading to the Cosmopolitan genotype are located within B-cell epitopes of the virus thereby also implicated in virulence. These changes in the E gene are present in all Philippine DENV-2 Cosmopolitan strains.

Based on available data, the last recorded Asian II genotype from the Philippines was in 2005, and studies published between 2008 and 2010 have specifically mentioned that no other Asian II strains have since been isolated from any Southeast Asian countries (Huang et al., 2012; Shu et al., 2009).

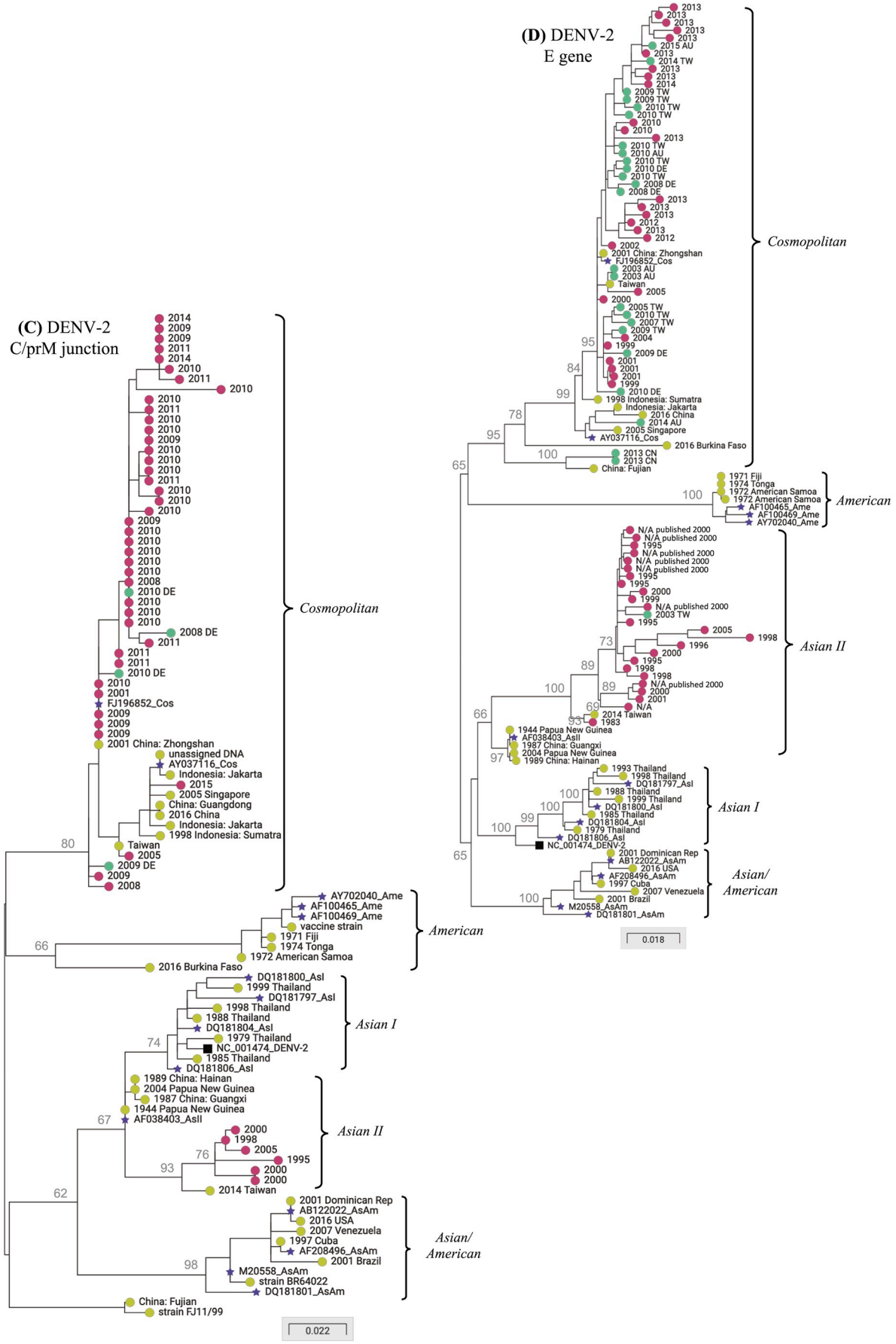


Fig. 3. (*continued*)

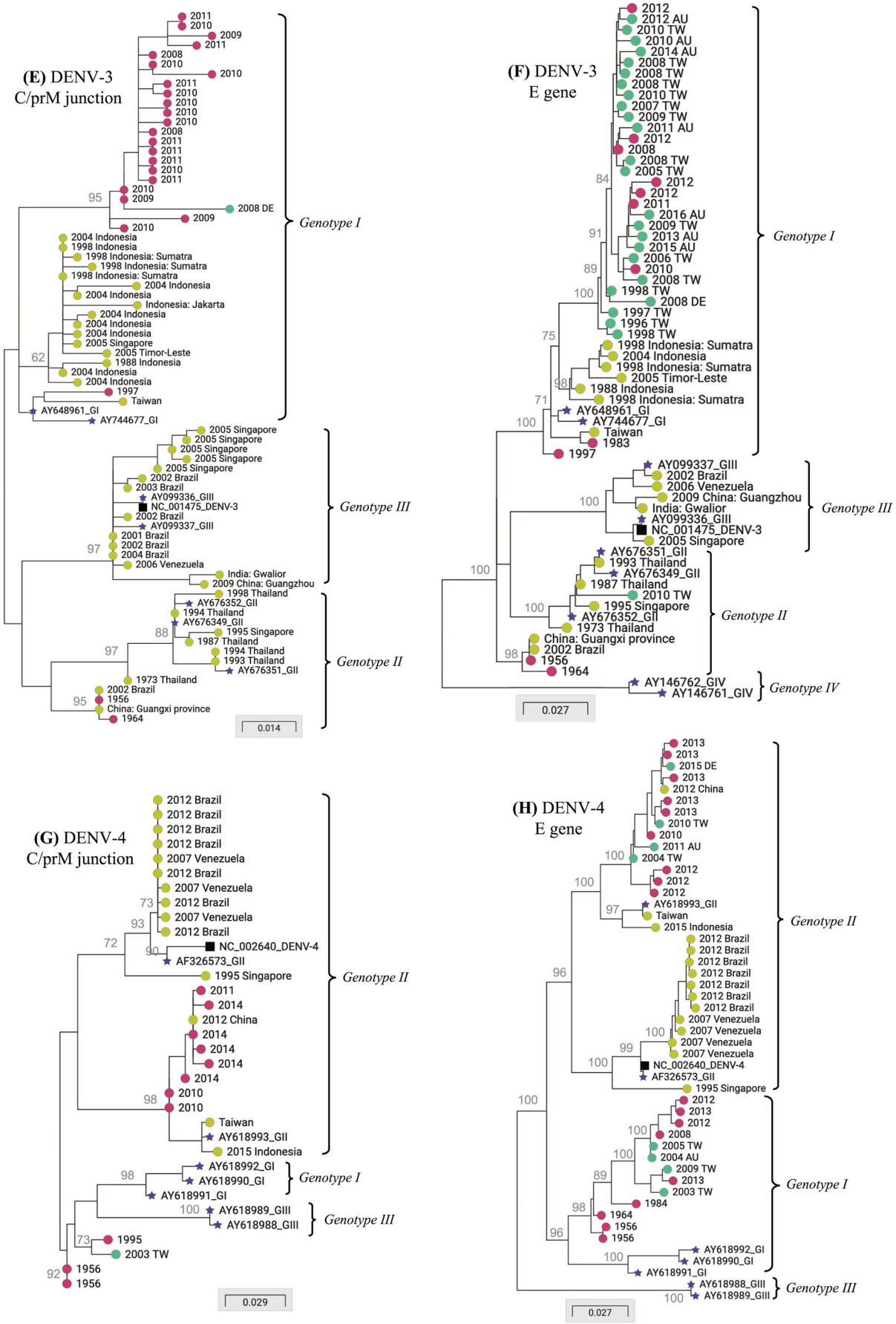


Fig. 3. (*continued*)

3.2.3. DENV-3

At least two genotypes (I and II) of DENV-3 have been found to be circulating in the country since 1983, with an apparent pre-domination of I over II. Most DENV-3 Philippines sequences from 1983 to 2012 are genotype I which implies its pre-domination for more than 25 years (Fig. 3E and Fig. 3F). An old local Philippine sequence, strain H87 from 1983, which clades separately (bootstrap support of 100) from older isolates 1956 and 1964 (Fig. 3F), suggests the presence of the DENV-3 genotype I lineage in the country since the early 1980s.

In addition, surveillance studies from 2002 to 2010 of other countries (Fig. 3F) as well as local C/prM sequences (Fig. 3E) have shown consistent isolation of genotype I strains from dengue patients coming from the Philippines, implying that its persistence remains unchanged (Shu et al., 2009). Whereas many of the DENV-3 sequences are of a single genotype, one strain belonging to genotype II was exported to Taiwan in 2010, which shows a possible co-circulation in the country, with genotype II being a minor population (Fig. 3F).

3.2.4. DENV-4

Although DENV-4 is the least frequently isolated dengue virus in Asia, the isolation of the 1956 strain H-241 prototype (FJ439174) and BID-V3361 (GQ868594) suggests the presence of DENV-4 in the Philippines since the earliest reports of an outbreak in Manila in 1954. Its apparent absence in 1998–2002 and in 2006–2007 (seen in Fig. 1) may be due to its low isolation rates or a possible re-introduction to the country. These “re-introduced” DENV-4 strains form a separate group within genotype II lineage and are 97% identical to viruses isolated in Guangzhou, China (Fig. 3H).

The E tree shows older sequences grouped with genotype I, together with newer sequences from 2003 to 2009 (Fig. 3H). However in the C/prM trees, the same isolates from 1956 and a recent exported strain in Taiwan do not clade with genotype I lineage (Fig. 3G). Although it is noteworthy that the 1956 Philippine sequences have already undergone series of laboratory manipulations prior to its submission in 2009 and 2016, its 98% similarity with the exported 2003 Taiwan isolate from serum discounts the issue of low sequence fidelity. Since there are only four local DENV-4 complete genomes published, it is difficult to conclude the difference between the E and C/prM trees. This warrants further investigation in the whole genomic characteristics of locally circulating DENV-4 in the Philippines.

Except for a 2004 strain from a dengue patient in Taiwan imported from the Philippines, new C/prM sequences from 2010 to 2014 clade with genotype II (Fig. 3G) (Shu et al., 2009). These results show that at least two different DENV-4 genotypes may have co-circulated in the Philippines during the last ten years. Similarly, a co-circulation of two genotypes of DENV-4 is apparent within the same period (Fig. 3H). Genotype I appears to be the older circulating genotype based on the ML trees.

3.3. Conclusion

Reports have shown that the introduction and subsequent genotypic replacements among specific serotypes are correlated with the occurrence of severe forms of dengue in the Americas and Sri Lanka (Messer et al., 2003; Rico-Hesse et al., 1997). On a larger scale and more importantly, Philippine strains can contribute to the introduction and re-introduction of the virus to neighboring countries through international travel, as seen among travelers from Taiwan, Japan, South Korea, China, Germany, Australia, Italy and some parts of Europe (Huang et al., 2012; Ito et al., 2007; Jeong et al., 2011; Shu et al., 2009).

To address the limitations of this study, the analysis can be further expanded to include all available DENV sequences. The Philippine data can also be improved by the continued submission of complete genome sequence data to international databases. The continuation of viral molecular surveillance in the country, including close monitoring of circulating genotypic strains, is necessary to alert the public health community of the replacement of older genotypes with the more recent ones, which may cause outbreaks or be predictors of disease severity.

Conflict of interest

We declare no conflicts of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.meegid.2019.01.020.

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