

## Original Article

# Surveillance and Molecular Characterization on Dengue Viruses in Taiwan, 2013

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### Abstract

We present our surveillance results on imported and indigenous dengue cases in Taiwan in 2013. A total of 264 imported dengue patients were identified. Among them, 113 (42.8%) cases were identified by fever screening at airports. The travelers were infected in 16 countries, including India, China, Brazil, and those located in Southeast Asia and South Pacific islands. Most of these imported cases were infected in Indonesia, next in Thailand, the Philippines, and Malaysia. The main dengue virus serotype was DENV-1 in all countries, with the exception of the Philippines and India in which DENV-4 and DENV-2 were the main serotypes, respectively. Molecular epidemiologic study showed that six different strains of DENV were co-circulated in southern Taiwan.

The first dengue outbreak occurred in Chunri Township, Pingtung County and was caused by a strain of DENV-1 which was also responsible for epidemics in Tainan City in 2011 and 2012. The second outbreak occurred in Xinyuan Township, Pingtung County and was caused by a strain of DENV-3 imported from Indonesia. The third outbreak which caused by a strain of DENV-2 imported from Indonesia began in Chaozhou Township, Pingtung County and later some sporadic cases were reported in Xinpi Township and Zhutian Township. The fourth outbreak caused by a different strain of DENV-2 also imported from Indonesia. This large-scale outbreak began in Pingtung City and later spread to Pingtung County, Kaohsiung City, and Tainan City. In addition, a different strain of DENV-1 which likely introduced from Malaysia caused few sporadic cases in Xinpi Township, Pingtung County, Taipei City, and New Taipei City. Finally, a DENV-2 strain most closely related to Singapore strain caused only one case in North District, Tainan City. These results provide an updated view of geographic distributions and dynamic transmission of DENV strains circulating in Taiwan and in Southeast Asian countries.

**Keywords:** Dengue virus, imported, epidemic, phylogenetic analysis

## Introduction

Dengue is currently considered the most important mosquito-borne viral disease in the world by the World Health Organization (WHO) [1]. The incidence of dengue has grown dramatically in recent years. Approximately 3.97 billion people living in 128 countries are now at risk for dengue [2], and it was estimated there are 390 million dengue infections worldwide annually [3]. The disease symptoms are ranging from asymptomatic infection to moderate febrile disease, classic dengue fever (DF), to severe and fatal dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). The disease causative agent is dengue virus (DENV) which belongs to the genus *Flavivirus* in the family *Flaviviridae* and consists of four genetically and antigenically distinct serotypes named DENV-1, -2, -3, and -4, originally classified based on their serological characteristics [4]. DENV is an enveloped virus with single-stranded positive-sense RNA, which is about 10,700 nucleotides and encodes a single long open reading frame (ORF), flanked by highly structured 5' and 3' untranslated regions (UTRs). The N-terminal of the polyprotein contains three structural proteins (capsid [C], precursor membrane [prM], and envelope [E]), followed by seven non-structural (NS) proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) [5].

The DENVs are transmitted to humans through the bite of infective female *Aedes* mosquitoes; predominate of *Aedes aegypti*, and to a lesser extent *Ae. albopictus* [1]. *Aedes albopictus* is found throughout Taiwan at elevations below 1500m, whereas *Ae. aegypti* is distributed only in the south of the Tropic of Cancer (23.5° N) [6]. Dengue is not considered endemic in Taiwan; however, the frequent importation of multiple DENVs from the neighboring Southeast Asian countries through foreign workers, immigrants, and international tourists is responsible for the local outbreaks each year [7-10]. To reduce the introduction of DENV strains and prevent their local outbreaks, fever screening at international airports in Taiwan had been demonstrated to be an effective means of early identifying imported dengue cases [11]. Dengue surveillance system plays critical roles on dengue prevention and control program because it detects epidemics rapidly, monitors trends in the distribution and spread of dengue over time and geographically, prevents further virus transmission.

In this study, we present the geographic distributions and current dynamics of DENV strains isolated from imported and indigenous dengue cases in Taiwan in 2013.

## Materials and Methods

### A. Human serum samples

Dengue fever and dengue hemorrhagic fever are category 2 reportable infectious diseases in Taiwan and suspected cases must be reported within 24 hours of clinical diagnosis. To provide effective surveillance, both passive (hospital-based reporting system) and active (such as fever screening at airports, self-reporting, and expanded screening for contacts of confirmed cases) surveillance systems were established by central and local health departments in Taiwan. Human serum samples of suspected dengue cases were

submitted to 3 laboratories for confirmation of DENV infection. Two central dengue laboratories, the Kun-Yang Laboratory in Taipei City and the Southern Laboratory in Kaohsiung City, and the dengue laboratory in Kaohsiung Medicine University Hospital (approved by Taiwan CDC) were set up for routine dengue diagnosis. Human serum samples used in this study were derived from confirmed dengue cases between January 1, 2013 and February 12, 2014. Serum samples collected 1–7 days after the onset of symptoms were referred to as acute-phase samples. Serum samples collected between 8 and 30 days after the onset of symptoms were referred to as convalescent-phase samples.

### **B. Laboratory diagnosis**

The DENV infection was confirmed by the detection of DENV specific IgM and IgG antibodies, isolation of DENV, or detection of DENV RNA by reverse transcription–polymerase chain reaction (RT-PCR). A one-step SYBR Green I-based real-time RT-PCR was performed in the Mx3000P quantitative PCR system (Agilent Technologies) to detect and differentiate DENV serotypes in acute-phase serum samples as previously described [12]. For the detection of DENV-specific IgM and IgG antibodies, envelope/membrane–specific capture IgM and IgG enzyme-linked immunosorbent assays were used to detect and differentiate primary and secondary DENV infections in the acute-phase and convalescent-phase serum samples as previously described [13]. Isolation of DENV was performed using a mosquito cell line (clone C6/36 of *Ae. albopictus* cells) as previously described [10]. The viruses were passaged in C6/36 cells and harvested for nucleotide sequencing after the first or second passage. Viruses were identified using the nomenclature of serotype/country/strain/year of isolation.

### **C. Preparation of viral RNA, RT-PCR amplification, and nucleotide sequencing**

Viral RNA was extracted from either acute-phase serum samples or DENV infected culture supernatants using the QIAamp Viral RNA Mini kit (Qiagen). The DENV-1 partial E gene (534 bp) was amplified by RT-PCR with the forward primer D1-1546F: 5'-ATC ATG GCT TGT CCA CAA AC-3' and reverse primer D1-2123R: 5'-TGC TTC CYT TCT TGA ACC AGC-3'. The DENV-2 partial E gene (534 bp) was amplified by RT-PCR with the forward primer D2-1541F: 5'-ARA YAA AGC TTG GCT GGT GCA-3' and reverse primer D2-2162R: 5'-GCT CCY CTC ATT GTT GTC TC-3'. The DENV-3 partial E gene (540 bp) was amplified by RT-PCR with the forward primer D3-1568F: 5'-TTT GAC CTA CCY CTA CCA TGG-3' and reverse primer D3-2149R: 5'-TCT GGC AGT GGC CTC GAA C-3'. The DENV-4 partial E gene (530 bp) was amplified by RT-PCR with the forward primer D4-1543F: 5'-AAA AGA AAA CRT GGC TTG TGC-3' and reverse primer D4-2114R: 5'-GAA CCA ATG GAG TGT TAA TGC-3'. The Super Script III One-step RT-PCR Platinum Taq HiFi system (Invitrogen) was used for RT-PCR. RT reaction was carried out at 50°C for 30 minutes, followed by PCR at 94°C for 2 minutes, 45 cycles of 94°C for 15 seconds, 50°C for 30 seconds, and 68°C for 1 minute, and a prolonged elongation at 68°C for 5 minutes. The PCR products were submitted to Mission Biotech Co.,

Ltd for sequencing. Sequence reads were assembled and edited with the Lasergene software package (DNASTAR Inc., Madison, WI). The partial E gene sequences used for phylogenetic analysis are from position 634 to 1167 of DENV-1 E gene (534 bp), position 628 to 1161 of DENV-2 E gene (534 bp), position 656 to 1195 of DENV-3 E gene (540 bp) and position 626 to 1155 of DENV-4 E gene (530 bp).

#### D. Phylogenetic analysis

The nucleotide sequences were aligned using ClustalW software [14]. Phylogenetic analysis was performed using MEGA version 6 (<http://www.megasoftware.net/>) [15]. Phylogenetic trees were constructed using the neighbor-joining method [16]. The reliability of the analysis was evaluated by a bootstrap test with 1,000 replications. For genotype classification, we grouped the isolate sequences with the relevant reference sequences based on classification by A-Nuegoonpipat et al. [17], Twiddy et al. [18], Lanciotti et al. [19], and Klunthong et al. [20] for the DENV-1, -2, -3 and -4, respectively.

## Results

### Imported dengue cases in Taiwan, 2013

A total of 264 laboratory confirmed imported dengue cases were identified in Taiwan in 2013. Among them, 113 (42.8%) cases were identified by fever screening at airports. Table 1 summarizes the countries of origin and DENV serotypes of these imported cases. The travelers were infected in 16 countries, including India, China, Brazil, and those located in Southeast Asia as well as in South Pacific islands. Most cases were introduced from Southeast Asian countries. Among these, Indonesia (71 cases), Thailand (63 cases), the Philippines (38 cases), Malaysia (26 cases), Vietnam (17 cases), and Singapore (10 cases) were the most frequent importing countries. In addition, cases were also imported from China, Indian subcontinent (India and Sri Lanka), South Pacific region (Fiji and Solomon Islands), and Latin America (Brazil and Saint Lucia).

**Table 1. Serotype distributions of DENV strains from imported dengue cases in Taiwan, 2013**

Country origin	Case	Fever screening	DENV-1	DENV-2	DENV-3	DENV-4	Unknown
Indonesia	71	30	16	11	12	0	32
Thailand	63	20	12	12	2	3	34
Philippines	38	14	6	3	1	10	18
Malaysia	26	19	13	4	1	2	6
Vietnam	17	10	4	3	3	3	4
Singapore	10	7	6	4	0	0	0
India	10	7	2	6	1	0	1
Myanmar	9	3	4	2	0	0	3
Cambodia	7	1	2	0	0	0	5
Sri Lanka	4	0	1	0	0	0	3
China	3	0	1	0	0	0	2
Lao	2	0	0	0	1	0	1
Brazil	1	1	0	0	0	1	0
Fiji	1	0	0	0	0	0	1
Saint Lucia	1	1	0	0	0	1	0
Solomon Islands	1	0	0	0	1	0	0
Total	264	113	67	45	22	20	110

### Serotype and genotype distributions of imported DENV strains in 2013

From the 264 imported dengue cases, 67, 45, 22, and 20 cases were determined to be infected with the DENV-1, DENV-2, DENV-3, and DENV-4 strains, respectively (Table 1). The main DENV serotype was DENV-1 in all countries, with the exception of the Philippines and India in which DENV-4 and DENV-2 was the main serotype, respectively. Among them, 136 DENV strains were isolated from the acute-phase serum samples of patients infected in 15 countries. Phylogenetic analyses of the partial E-gene sequences of all DENV strains isolated from imported cases were conducted to determine the genetic relationship of these viral strains. The designation of DENV genotypes are based on the classification of A-Nuegoonpipat and others [17], Twiddy and others [18], Lanciotti and others [19], and Klunthong and others [20] for the DENV-1, DENV-2, DENV-3, and DENV-4 strains, respectively. Table 2 summarizes the serotype and genotype distributions of 136 DENV isolates in each of these countries.

**Table 2. Summary of genotype distributions of DENV strains isolated from imported cases in Taiwan, 2013**

Serotype	DENV-1			DENV-2		DENV-3			DENV-4		Total
	I	II	III	Cosmopolitan	Asian 1	I	II	III	I	II	
Indonesia	8	4	0	10	0	9	0	0	0	0	31
Thailand	12	0	0	1	9	0	1	1	3	0	27
Malaysia	9	1	3	4	0	0	0	1	0	2	20
Philippines	1	4	0	2	0	1	0	0	3	6	17
Vietnam	3	0	1	1	2	0	2	1	2	0	12
India	0	0	2	6	0	0	0	1	0	0	9
Singapore	0	0	4	3	0	0	0	0	0	0	7
Myanmar	3	0	0	0	2	0	0	0	0	0	5
Cambodia	2	0	0	0	0	0	0	0	0	0	2
China	1	0	0	0	0	0	0	0	0	0	1
SriLanka	1	0	0	0	0	0	0	0	0	0	1
Lao	0	0	0	0	0	0	1	0	0	0	1
Solomon Islands	0	0	0	0	0	1	0	0	0	0	1
Brazil	0	0	0	0	0	0	0	0	0	1	1
Saint Lucia	0	0	0	0	0	0	0	0	0	1	1
Total	40	9	10	27	13	11	4	4	8	10	136

### Phylogenetic analysis of DENV-1 isolates

DENV-1 viruses isolated from imported cases in 2013 fell into three genotypes (Table 2 and Figure 1A). Genotype I was the most dominant genotype of DENV-1 in Southeast Asian countries except in the Philippines and Singapore. Genotype I contains viruses from Thailand, Malaysia, Indonesia, Vietnam, Myanmar, Cambodia, the Philippines, China and Sri Lanka. Genotype II comprises viruses from Indonesia, the Philippines, and Malaysia. Genotype III has a wide geographic distribution including Asia, the Pacific Islands, the Americas, and parts of Africa. All DENV-1 viruses isolated from India and Singapore belong to Genotype III. Three strains from Malaysia and one strain from Vietnam also belong to this genotype.

### **Phylogenetic analysis of DENV-2 isolates**

DENV-2 viruses isolated from imported cases in 2013 fell into two genotypes (Table 2 and Figure 1B). The Cosmopolitan genotype is composed of viruses from diverse geographical localities, which include Asia, Australia, Africa, and Latin America. Twenty-seven strains isolated from imported cases from Indonesia, India, Malaysia, Singapore, the Philippines, Thailand, and Vietnam belonged to this genotype. The Asian genotype 1 contains viruses from Southeast Asia, including 13 strains of viruses imported from Thailand, Vietnam, and Myanmar. No Asian genotype 2 and Asian/ American genotype strains were found from imported cases in 2013.

### **Phylogenetic analysis of DENV-3 isolates**

DENV-3 viruses isolated from imported cases in 2013 fell into three genotypes (Table 2 and Figure 1C). Genotype I contains viruses from Southeast Asia and the Pacific islands, including 11 viral strains imported from Indonesia, the Philippines and Solomon Islands. Genotype II is composed of viral strains from Southeast Asia, including 4 viral strains imported from Thailand, Vietnam, and Laos. Genotype III contains viruses from the Indian subcontinent, Africa, and Latin America. Notably, this genotype also contains virus strains from Thailand, Malaysia, and Vietnam.

### **Phylogenetic analysis of DENV-4 isolates**

DENV-4 is less abundant in Asia and the isolates from imported cases in 2013 can be grouped into two genotypes (Table 2 and Figure 1D). Genotype I contains viruses from Southeast Asia, including 8 viral strains imported from Thailand, the Philippines and Vietnam. Genotype II is composed of viral strains from Southeast Asia, the South Pacific, and the Americas, including 10 viral strains imported from the Philippines, Malaysia, Brazil and Saint Lucia.

### **Multiple dengue epidemics in southern Taiwan during 2013 to early 2014**

A total of 595 laboratory confirmed indigenous dengue cases were recorded in Taiwan during 2013. There were 14 DHF cases and no deaths. Among them, 16 cases were infected with either DENV-1 or DENV-2 between January and March. These cases represented the last wave of the 2012 outbreak in the regions of Tainan City and Kaohsiung City. The remaining 579 cases were infected with DENV-1, DENV-2 or DENV-3 between April and December 2013. Molecular epidemiologic study showed that six different strains of DENV, two DENV-1 (D1/Taiwan/942PT1304a/2013 and D1/Taiwan/925PT1308a/2013), three DENV-2 (D2/Taiwan/920PT1306a/2013, D2/Taiwan/900PT1307a/2013, and D2/Taiwan/704TN1310a/2013), and one DENV-3 (D3/Taiwan/932PT1305a/2013) were co-circulated in the regions of Pingtung City/County, Kaohsiung City, and Tainan City. The major outbreak of DENV-2 caused by D2/Taiwan/900PT1307a/2013 DENV strain was lasted to February 6, 2014 and additional 15 cases were infected.



### Transmission dynamics of the six DENV strains

Table 3 summarizes the transmission dynamics, areas infected, and the total cases estimated for each of the six DENV strains. The first dengue outbreak occurred in Chunri Township, Pingtung County on April 10 and was caused by a strain of DENV-1 (D1/Taiwan/942PT1304a/2013). There were 36 confirmed cases and the outbreak ended in July. The second outbreak caused by a strain of DENV-3 (D3/Taiwan/932PT1305a/2013), occurred on May 26 in Xinyuan Township, Pingtung County and ended in July. This outbreak was small scale with only 11 confirmed cases. The third outbreak caused by a strain of DENV-2 (D2/Taiwan/920PT1306a/2013) and began in Chaozhou Township, Pingtung County on June 9 and later some sporadic cases were reported in Xinpi Township and Zhutian Township. There were 36 confirmed cases and the outbreak ended in November 6. The fourth outbreak caused by a different strain of DENV-2 (D2/Taiwan/900PT1307a/2013), began in Pingtung City on July 23 and later spread to Pingtung County on September 9, Kaohsiung City on October 28, and Tainan City on November 4. It is a large-scale outbreak with about 290, 112, 66, and 32 confirmed cases in Pingtung City, Pingtung County, Kaohsiung City, and Tainan City, respectively, and ended in February in 2014. In addition, a different strain of DENV-1 (D1/Taiwan/925PT1308a/2013) caused few sporadic cases in Xinpi Township, Pingtung County, Taipei City, and New Taipei City from August 8 to September 9. Finally, a DENV-2 strain (D2/Taiwan/704TN1310a/2013) caused only one case in North District, Tainan City in October, 2014.

**Table 3. Summary of the dengue epidemics in southern Taiwan during April 2013 to February 2014**

Dengue virus strain	Serotype Genotype	Epidemic area infected	First case	Last case	Total cases
D1/Taiwan/942PT1304a/2013	DENV-1 GenotypeIII	Chunri Township, Pingtung County	April 10	July 2	36
D3/Taiwan/932PT1305a/2013	DENV-3 GenotypeI	Xinyuan Township, Pingtung County	May 26	July 5	11
D2/Taiwan/920PT1306a/2013	DENV-2 Cosmopolitan	Chaozhou Township, Pingtung County	June 9	November 6	33
		Xinpi Township, Pingtung County	September 8	September 24	2
		Zhutian Township, Pingtung County	October 8	October 8	1
D2/Taiwan/900PT1307a/2013	DENV-2 Cosmopolitan	Pingtung City	July 23	February 1, 2014	290
		Pingtung County	September 9	January 13, 2014	112
		Kaohsiung City	October 28	January 15, 2014	66
		Tainan City	November 4	February 6, 2014	32
D1/Taiwan/925PT1308a/2013	DENV-1 GenotypeI	Xinpi Township, Pingtung County	August 8	August 29	2
		Taipei City	August 14	August 23	7
		New Taipei City	September 9	September 9	1
D2/Taiwan/704TN1310a/2013	DENV-2 Cosmopolitan	North District, Tainan City	October 22	October 22	1

### Molecular epidemiologic study of DENV in Taiwan, 2013

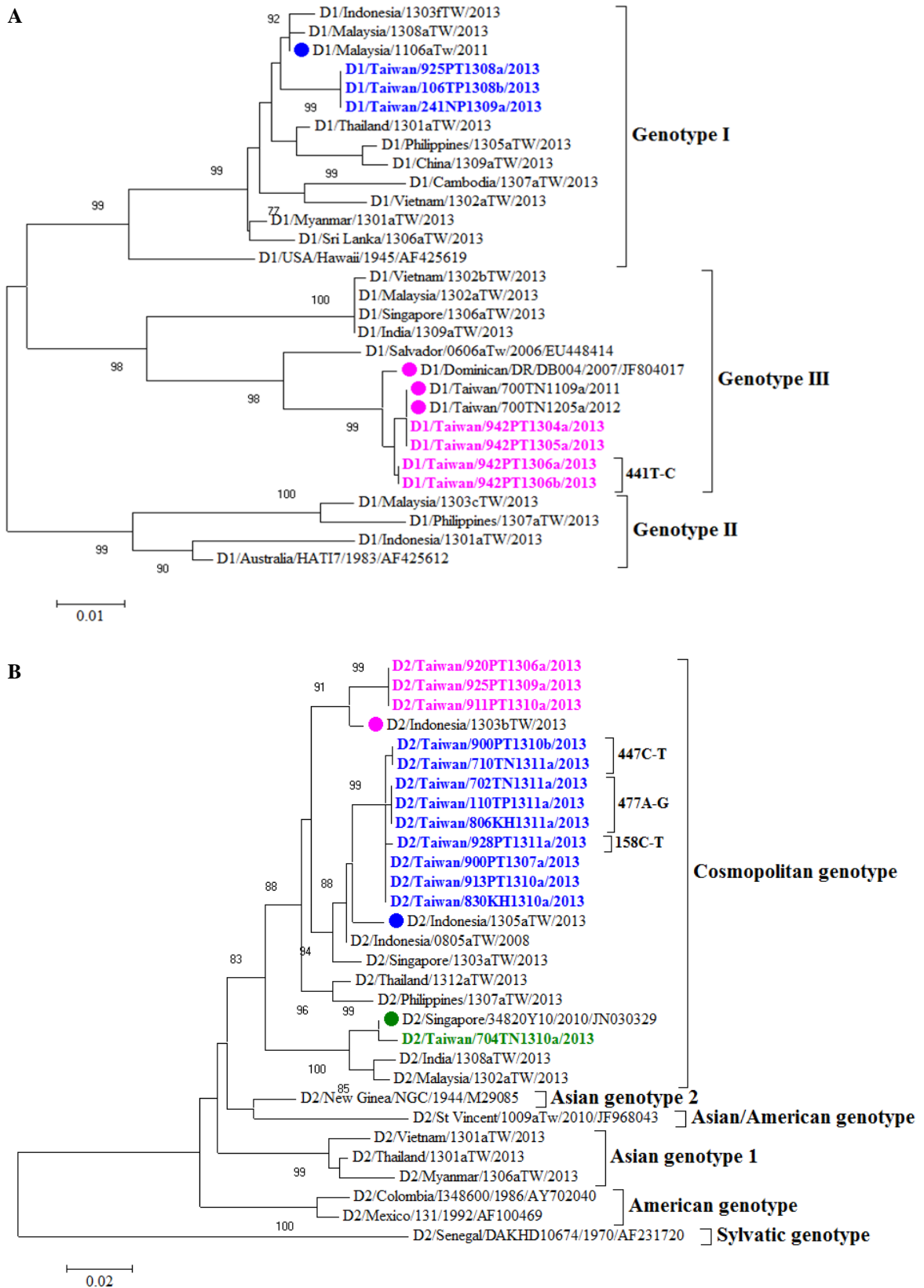
The partial E gene sequences of six distinct DENV isolates (D1/Taiwan/925PT1308a/2013, D1/Taiwan/942PT1304a/2013, D2/Taiwan/920PT1306a/2013, D2/Taiwan/900PT1307a/2013, D2/Taiwan/704TN1310a/2013, D3/Taiwan/932PT1305a/2013) from indigenous index cases were determined and compared with the sequences available from GenBank and the dengue database of the Taiwan CDC.

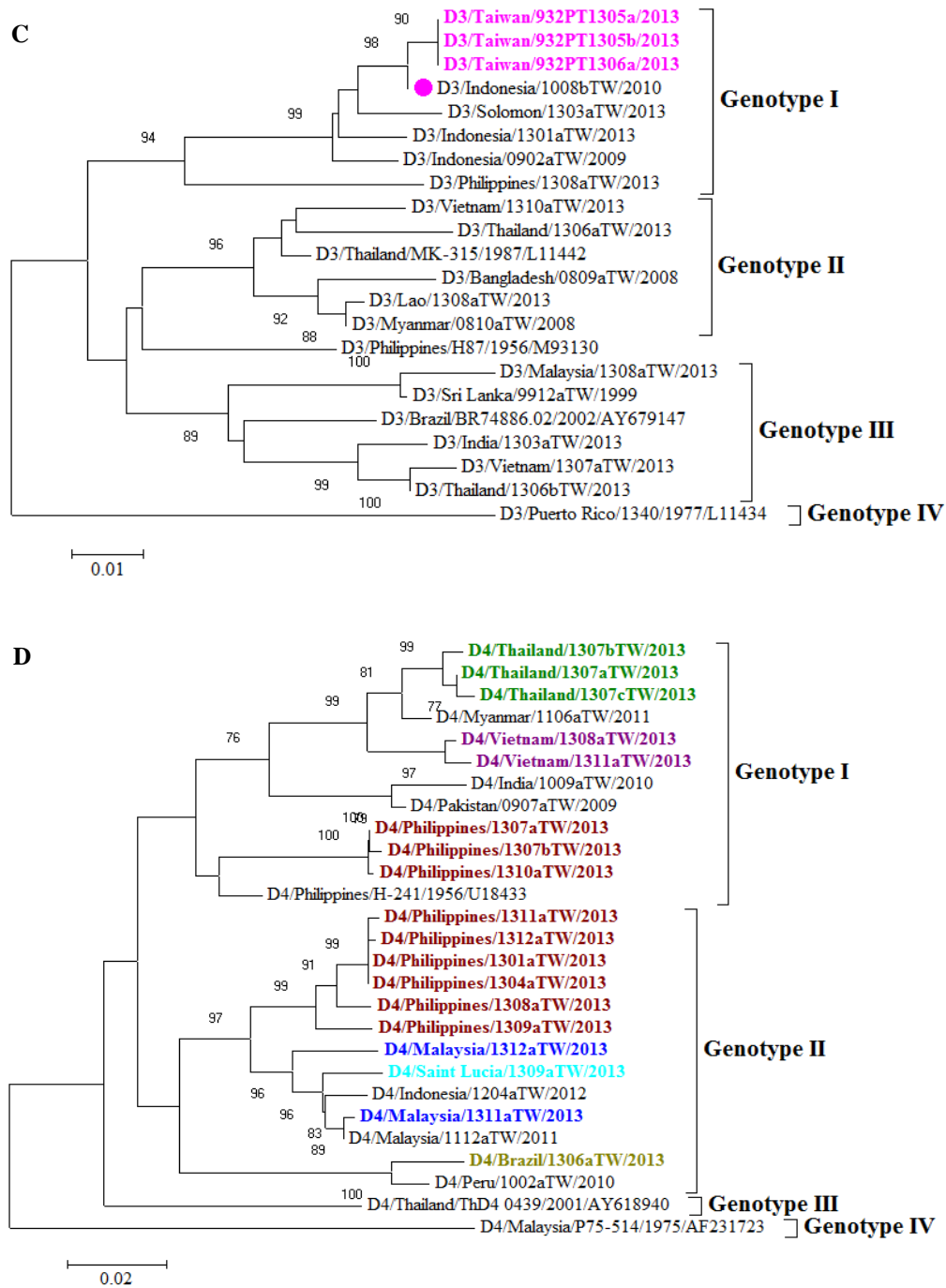
Figure 1A shows the phylogenetic tree of partial E gene sequences (534bp) of DENV-1. The index strain, D1/Taiwan/925PT1308a/2013, belongs to Genotype I and is most closely related to the isolate D1/Malaysia/1106aTw/2011 and was likely introduced from Malaysia. The DENV-1 strains, D1/Taiwan/106TP1308b/2013 and D1/Taiwan/241NP1309a/2013, caused a few sporadic cases in Taipei City and New Taipei City and are the same strain as the index strain D1/Taiwan/925PT1308a/2013. Another index strain D1/Taiwan/942PT1304a/2013 belongs to Genotype III and its partial E gene sequence is the same as the sequences of D1/Taiwan/700TN1109a/2011 and D1/Taiwan/700TN1205a/2012 strains that caused epidemics in Tainan City in 2011 and 2012. This strain is most closely related to the American virus strain of D1/Dominican/DR/DB004/2007. The index strain D1/Taiwan/942PT1304a/2013 was responsible for the dengue outbreak which began on April 10 in Chunri Township, Pingtung County and then a nucleotide changed from T to C at position 1074 of E gene in strains D1/Taiwan/942PT1306a/2013 and D1/Taiwan/942PT1306b/2013.

Figure 1B shows the phylogenetic tree of partial E gene sequences (534bp) of DENV-2. The index strain, D2/Taiwan/920PT1306a/2013, belongs to the Cosmopolitan genotype and is most closely related to the virus isolate D2/Indonesia/1303bTW/2013, and was likely introduced from Indonesia. D2/Taiwan/925PT1309a/2013 and D2/Taiwan/911PT1310a/2013 strains that caused a few sporadic cases in Xinpi and Zhutian Township, Pingtung County are the same as the index strain D2/Taiwan/920PT1306a/2013. Another index strain, D2/Taiwan/900PT1307a/2013, also belongs to Cosmopolitan genotype, is most closely related to the virus isolate D2/Indonesia/1305aTW/2013, and was likely introduced from Indonesia. The index strain D2/Taiwan/900PT1307a/2013 was responsible for the major dengue outbreaks which occurred in July in Pingtung City/County, Kaohsiung City, and Tainan City. In October and November, 3 phylogenetic clusters derived from the original index virus were identified. One cluster (D2/Taiwan/900PT1310b/2013, D2/Taiwan/710TN1311a/2013) had a nucleotide change from C to T at position 1074 of E gene, another cluster (D2/Taiwan/702TN1311a/2013, D2/Taiwan/110TP1311a/2013, and D2/Taiwan/806KH1311a/2013) had a nucleotide change from A to G at position 1104 of E gene, and the other (D2/Taiwan/928PT1311a/2013) had a change from C to T at position 785 of E gene. Finally, a DENV-2 strain D2/Taiwan/704TN1310a/2013, which caused only one case in North District, Tainan City in October, belongs to Cosmopolitan genotype and is most closely related to D2/Singapore/34820Y10/2010/JN030329.



Figure 1C shows the phylogenetic tree of partial E gene sequences (540bp) of DENV-3. The index strain, D3/Taiwan/932PT1305a/2013, belongs to Genotype I and is most closely related to the isolate D3/Indonesia/1008bTW/2010, and was likely introduced from Indonesia. No nucleotide change was observed on the partial E gene sequence during the outbreak from May 26 to July 5.





**Figure 1.** Phylogenetic trees based on the partial E gene sequences of A, 30 strains of DENV-1 and B, 30 strains of DENV-2 and C, 22 strains of DENV-3 and D, 27 strains of DENV-4. The partial E gene sequences used for phylogenetic analysis are from position 634 to 1167 of DENV-1 E gene (534 bp), position 628 to 1161 of DENV-2 E gene (534 bp), position 656 to 1195 of DENV-3 E gene (540 bp) and position 626 to 1155 of DENV-4 E gene (530 bp). The trees were constructed using the neighbor-joining method [16]. The reliability of the analysis was evaluated by a bootstrap test with 1,000 replications. Bootstrap support values greater than 75 are shown. A, B, and C, the epidemic strains isolated from major dengue outbreaks in Taiwan are designated in bold and color. The strains most closely related to the epidemic strains are marked with colored circle. D, the imported strains isolated from 2013 are designated in bold and color. Viruses are identified using the nomenclature of serotype/country/strain/year of isolation and if a GenBank accession number available is shown at the end. The scale bars on the left indicate substitutions per site.

## Discussion

A total of 264 imported dengue cases were identified in 2013, this number is higher than the numbers in 2011 (157 cases) and 2012 (207 cases). Among them, 113 (42.8%) cases were identified by fever screening at airports. The travelers were infected in 16 countries; most of these imported cases were infected in Indonesia (71 cases), next in Thailand (63 case), the Philippines (38 cases), Malaysia (26 cases), and Vietnam (17 cases). Interestingly, Vietnam was the top one or two list of the dengue importing country during 2004-2011 in Taiwan, however, the numbers of imported dengue cases from Vietnam had decreased significantly in 2012 and 2013. According to the data from Western Pacific Regional Office of WHO (WPSAR Vol 4, No 2, 2013 and [http://www.wpro.who.int/emerging\\_diseases/Dengue\\_SituationUpdates/en/](http://www.wpro.who.int/emerging_diseases/Dengue_SituationUpdates/en/)), the numbers of dengue cases in Vietnam were 69680, 86026, and 66140 in 2011, 2012, and 2013, respectively. No significant decreasing trend of dengue cases was observed in Vietnam in the last 3 years. In addition, more and more people traveled between Vietnam and Taiwan in recent years (<http://admin.taiwan.net.tw/statistics/year.aspx?no=134>). The reason why the numbers of imported dengue cases from Vietnam has declined in 2012 and 2013 is still uncertain. However, among the six different strains of DENV co-circulated in southern Taiwan last year, three strains were imported from Indonesia that was consistent with the fact that most imported cases were infected in Indonesia in 2013. These results provide evidence to support that multiple dengue epidemics that occurred in Taiwan may be caused by continuous introductions of multiple DENVs from the neighboring Southeast Asian countries. Nevertheless, there is an exception that the virus strain D1/Taiwan/942PT1304a/2013 which was responsible for the dengue outbreak began on April 10, 2013 in Chunri Township, Pingtung County, is the same virus strain that caused dengue outbreaks in Tainan City during 2011 and 2012. The results indicated that silent transmission of DENV occurred during inter-epidemic periods in southern Taiwan although the mosquito densities were low during winter season (inter-epidemic period). This DENV-1 strain originated from Dominican Republic has caused dengue epidemics in southern Taiwan for three consecutive years. Although the 2013 outbreak was small and has ended on July 2013, we still should strengthen mosquito prevention and control, to prevent dengue from becoming endemic in Taiwan.

The main serotype of DENV was DENV-1 in imported cases from all countries, with the exception of the Philippines and India in which DENV-4 and DENV-2 were the main serotypes, respectively. Among the top five frequently importing countries, all four DENV serotypes were identified except that no DENV-3 strains were identified from imported cases from Indonesia. Our previous studies showed that only 7 strains of DENV-1 Genotype III have been isolated from imported cases between 2003 and 2010 [9], however, in 2013, there were 10 DENV-1 genotype III strains isolated from imported cases from Singapore, Malaysia, India and Vietnam. The DENV-3 genotype III strains have seldom been found in Southeast Asia before 2007; however, more and more DENV-3 genotype III strains have been

identified from imported dengue cases from Thailand, Malaysia, and Vietnam in recent years. These data indicate that the DENV-1 genotype III and DENV-3 genotype III strains had expanded their geographic distribution in Southeast Asia countries. In addition, a DENV-3 genotype I strain from Solomon Islands, a DENV-4 genotype II strain from Brazil, and a DENV-4 genotype II strain from Saint Lucia were identified in this study.

Molecular epidemiologic study showed that six different strains of DENV (two DENV-1s, three DENV-2s, and one DENV-3) were co-circulated in southern Taiwan. The index strain D2/Taiwan/900PT1307a/2013 was responsible for the major dengue outbreaks began in July in Pingtung City/County, Kaohsiung City, and Tainan City and then three different clusters each with a nucleotide changed from C to T at position 1074 of E gene (D2/Taiwan/900PT1310b/2013, D2/Taiwan/710TN1311a/2013), A to G at position 1104 of E gene (D2/Taiwan/702TN1311a/2013, D2/Taiwan/110TP1311a/2013, D2/Taiwan/806KH1311a/2013), and C to T at position 785 of E gene (D2/Taiwan/928PT1311a/2013) were observed when epidemics lasted until November. The nucleotide variations at position 1074 (C1074T) and 1104 (A1104G) of the E gene are synonymous mutations, while the other nucleotide variation at position 785 (C785T) results in a nonsynonymous mutation. The index strain D1/Taiwan/942PT1304a/2013, which was responsible for the dengue outbreak in 2011-2012, caused dengue epidemics in April 2013 in Chunri Township, Pingtung County and then a nucleotide changed from T to C at position 1074 of E gene (D1/Taiwan/942PT1306a/2013) was observed when epidemics lasted until June. The nucleotide variation at position 1074 (T1074C) of the E gene is a synonymous mutation.

Due to convenience for international transportation and climate change, dengue is spreading rapidly throughout the world and the situation is worsening and increases the threat to human health. It is important to establish a complete set of dengue disease and vector surveillance system. In this study, we established DENV genomic database and used molecular epidemiology methods to monitor the introduction and geographic distribution of DENV strains. These results provide essential information for understanding the origins and dynamics of the epidemic DENV strains and are valuable information for effective epidemic prevention and control.

Vaccine development should be the most effective way to control dengue, unfortunately until now still no approved dengue vaccine is available. Because *Aedes* mosquitoes, the dengue vectors, have proven to be very difficult to control, in addition, constant importation of multiple DENVs from the neighboring Southeast Asian countries was responsible for yearly local outbreaks in Taiwan, therefore, early detection of imported and indigenous dengue cases and rapid response to outbreak are critical to prevent dengue transmission. Currently fever screening at airports implemented by Taiwan CDC can test out 40%-50% of the total number of imported dengue cases; therefore, it is one of the effective surveillance systems to reduce the introduction of foreign DENVs.

Since there are no specific treatments or vaccines for DENV infection, well-organized dengue disease surveillance and vector surveillance systems are the most straightforward strategies for its prevention. Taiwan CDC has implemented various active and passive surveillance systems to detect imported and indigenous dengue cases at an early stage and through early response activities to save resources, and reduce the impact of outbreaks on individuals, health systems and economies.

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