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Research paper

The distinct distribution and phylogenetic characteristics of dengue virus serotypes/genotypes during the 2013 outbreak in Yunnan, China Phylogenetic characteristics of 2013 dengue outbreak in Yunnan, China

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ABSTRACT

Since 2000, sporadic imported cases of dengue fever were documented almost every year in Yunnan Province, China. Unexpectedly, a large-scale outbreak of dengue virus (DENV) infection occurred from August to December 2013, with 1538 documented cases. In the current study, 81 dengue-positive patient samples were collected from Xishuangbanna, the southernmost prefecture of the Yunnan province, and 23 from Dehong, the westernmost prefecture of the Yunnan province. The full-length envelope genes were amplified and sequenced. Phylogenetic analysis revealed that nine strains (39.1%) and 14 strains (60.9%) from the Dehong prefecture were classified as genotype I of DENV-1 and Asian I genotype of DENV-2, respectively. All strains from Xishuangbanna were identified as genotype II of DENV-3. Bayesian coalescent analysis indicates that the outbreak originated from bordering southeastern Asian countries. These three epidemic genotypes were predicted to originate in Thailand and then migrate into Yunnan through different routes.

1. Introduction

Dengue fever (DF) is one of the most serious health threats in tropical and subtropical regions of the world, caused by infection with any of the four dengue virus (DENV) serotypes: DENV-1, DENV-2, DENV-3, and DENV-4. The virus is transmitted by *Aedes aegypti* and *Aedes albopictus* mosquitoes, which are present in most parts of the tropical and sub-tropical regions of the world. Moreover, globalization, ease of transportation, and the lack of effective vaccines have aggravated its global prevalence. In the past 50 years, DENV infection has increased 30-fold with 50–100 million new infections being reported each year (WHO, 2012). The rapid increase in the incidence of dengue fever (DF) in recent years has become a serious public health threat to nearly half of the world's population. The outbreaks of dengue have been proposed to have begun in Asia, where the greatest number of dengue cases is currently found. Almost all countries in Southeastern Asia (e.g., Indonesia, Cambodia, Laos, Myanmar, Malaysia, Philippines, Thailand, and Vietnam) have documented epidemic dengue outbreaks (Dorji et al., 2009; Gubler, 1998; Holmes et al., 2009; Jarman et al., 2008; Schreiber et al., 2009; Teoh et al., 2010).

DENV is a member of the *Flavivirus* genus belonging to the Flaviviridae family, with a single stranded, non-segmented, positive-sense RNA genome of approximately 10.7 kb that encodes a single open reading frame for three structural proteins (C, capsid; prM/M, precursor of membrane; and E, envelope) and seven non-structural proteins (NS1, NS2a, NS2b, NS3, NS4a, NS4b, and NS5) (Chambers et al., 1990). Great genetic diversity is a major characteristic of DENV, due to the lack of proofreading capacity of RNA-dependent RNA polymerases. Based on an intergenotypic divergence of at least 6% in the E gene sequence, each serotype of DENV can be classified into several genotypes (Rico-Hesse, 1990). Epidemiological and phylogenic reports have demonstrated a wide range of diversity within each of the four DENV serotypes, which is used for further differentiation of viral genotypes (Rico-Hesse, 1990; Kyle and Harris, 2008; Lanciotti et al., 1997; Twiddy et al., 2002a).

DENV serotypes and genotypes have different epidemic potentials for various geographic locations. As the most widespread serotype, DENV-1 is further classified into five genotypes (I–IV) based on entire or partial sequence of the E gene (Rico-Hesse, 1990; Villabona-Arenas et al., 2013). Phylogeographically, genotype I is prevalent in Southeast

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Asian countries (Vietnam, Laos, and Myanmar), East Asian countries (China, Japan), and East Africa; genotype II is common in Thailand; genotype III is primarily found in Malaysia; strains of genotype IV are primarily found in Australia, Indonesia, and the Philippines; and all genotype V viruses were isolated in the United States. DENV-2 is classified into six genotypes: Asian I, Asian II, Sylvatic, American/Asian, American, and Cosmopolitan. Except for the sylvatic genotype, the other five genotypes of DENV-2 present unique geographical distributions. Among them, the Cosmopolitan genotype has a wide distribution across the tropical and subtropical regions (Twiddy et al., 2002b); both Asian I and II are currently circulating in Southeast Asia; and both the American and American/Asian genotypes have only been reported in epidemics in America, though the latter is considered to have originated in Southeast Asia (Rico-Hesse et al., 1997). Since the first reported outbreak in the Philippines in 1956 (Hammon et al., 1960), DENV-3 infections have been described worldwide. Of the five genotypes (I to V) of DENV-3, genotypes I, II, and III are the major genotypes circulating in Southeast Asia, the Indian subcontinent, the South Pacific, East Africa, and the Americas (Lanciotti et al., 1994). For DENV-4, three epidemic genotypes (I–III) and a sylvatic genotype have been described. The sylvatic genotype has been isolated from sentinel monkeys in Malaysia, whereas genotypes I–III are circulating in South Asia and Southeast Asia (Chen and Vasilakis, 2011).

Since the first outbreak in Guangdong Province in 1978, dengue epidemics have been reported annually in southern China in regions such as the Guangdong, Guangxi, Fujian, Yunnan, and Hainan provinces and the Special Administrative Regions Macao and Hong Kong (Yi et al., 2003). Except for two large-scale dengue outbreaks in 1980 and 1986 with 452,674 and 118,881 infected cases, respectively, the majority of DENV occurrence was sporadic (Xiong and Chen, 2014). DENV strains of all four serotypes have been identified in China. Surprisingly, the DENV infection has become more serious in recent years, with 44,896 DENV infections being reported during the 2014 dengue outbreak in Guangdong. Moreover, imported cases of DENV infection from Southeast Asian countries were also reported to have increased in the past 30 years because of the rapid increase in trade and travel between these countries and China (Ren et al., 2003).

Geographically, the Yunnan province is located in southeastern China, and neighbors the dengue endemic Southeast Asian countries Laos, Vietnam, and Myanmar (Fukunaga et al., 1983; Thu et al., 2004). In the past decade, only sporadic cases of dengue infection have been typically reported in Yunnan. Unfortunately, a large-scale dengue outbreak occurred in 2013 with 1538 confirmed DENV infections, accounting for 33% of the total reported numbers nationwide. This dengue outbreak was primarily centered in the Xishuangbanna and Dehong prefectures, and both harmed the health of the local population and a resulting panic caused a dramatic reduction in tourism. However, the phylogenetic characteristics of this DENV outbreak and its origins are so far unclear. In current study, using phylogenetic analysis and Bayesian coalescent analysis, we described the molecular epidemiological characteristics of DENV during this dengue outbreak in the Yunnan province, and attempted to illuminate DENV transmission models and even the cause of this outbreak.

2. Materials and methods

2.1. Detection of DENV infection and sample collection

During the dengue outbreak in Yunnan province in 2013, serum samples from suspected clinical cases (fever with a rash and swollen lymph nodes) were subjected to DENV NS1 antigen detection with the One-Step Dengue NS1 RapiDip™ InstaTest kit (Cortez Diagnostics, Inc., Calabasas, CA) and a self-established reverse transcription polymerase chain reaction (RT-PCR) detection protocol. In total, 1538 serum samples have been detected positive, their demographic data were collected for further statistical analysis. Serum samples were collected during the first 5 days of illness and stored at −80 °C.

2.2. DENV E gene amplification and nucleotide sequencing

104 serum samples were randomly selected for phylogenetic and evolutionary analysis from antibody positive serums, including 81 (accounted for 6.1% of total infection) from diagnosed dengue patients at the local hospital of Xishuangbanna, the southernmost prefecture of the Yunnan province, and 23 (accounted for 12.1% of total infection) from the local hospital in Dehong, the westernmost prefecture of the Yunnan province. Their RNA was extracted from 100-μL serum samples using a High Pure Viral RNA kit (Roche, Shanghai, China), according to the manufacturer's instructions. The entire E gene was amplified by a one-step reverse transcription polymerase chain reaction, using the method previous reported (Wang et al., 2015). Serotype was also determined by RT-PCR using these serotype-specific primers reported previously (Foster et al., 2000). PCR products were confirmed by electrophoresis and purified using an Agarose Gel DNA Extraction kit (TaKaRa). Sequencing was then performed by the Invitrogen Company (Beijing, China). All raw sequences obtained were analyzed using the Chromas program (https://www.chroma.com/). DNA fragments encoding the full-length E protein of DENV were submitted to GenBank (accession numbers KJ9394385 to KJ939407 and KR347358 to KR347438).

2.3. Phylogenetic and evolutionary analysis

Sequences of E gene were aligned using ClustalX program (Larkin et al., 2007) and compared with retrieved sequences in the GenBank database. Genotypes were determined by the maximum likelihood (ML) tree which was constructed using the method we previous reported (Wang et al., 2015). The nodal reliability of the ML tree was assessed by bootstrap (BS) with 1000 pseudo-replicates.

Bayesian coalescent analysis was performed to estimate the mean evolutionary rate of the E gene and time of the most recent common ancestor (TMRCA) for the most prevalent group using a BEAST v1.6.1 software package (Drummond and Rambaut, 2007). The data were analyzed using the general time reversible GTR + G4 + I model and a relaxed uncorrelated lognormal molecular clock. Each MCMC analysis was run for at least 50 million generations and sampled every 10,000 generations. Convergence of the MCMC sample on the posterior distribution was defined at an effective sample size (ESS) value >200, which was calculated by the Tracer v.1.4 program (available at http://beast.bio.ed.ac.uk/Tracer). The maximum clade credibility (MCC) tree was constructed using TreeAnnotator v.1.4.8 and then visualized using FigTree v.1.3.1 (available at http://tree.bio.ed.ac.uk/software/figtree/).

2.4. Ethical statement

All the participants were informed at study enrolment and written informed consent was received before sample collection. The research was approved by the Institutional Ethical Committee of Kunming University of science and technology.

3. Results

3.1. The dengue outbreak in 2013 in Yunnan

During the dengue outbreak from July to December of 2013, 1538 cases were confirmed positive in Yunnan. The majority of cases of DENV infection (93.04%; 1431/1538) occurred during 3 months: August, September, and October. The presence of dengue was reported in eight prefectures. Among them, Xishuangbanna, the southernmost prefecture bordering Laos, and Dehong, the westernmost prefecture bordering Myanmar, were the most seriously afflicted regions, with 1320 and 190 cases of DENV infection, respectively. The remaining 28

Table 1
Demographic characteristics of the DENV-infected individuals during the 2013 dengue outbreak in Yunnan.

Characteristics	Xishuangbanna (n = 1320)	Dehong (n = 190)	Other prefectures[a] (n = 28)	Total (n = 1538)
Gender (male/female)	624/724	97/93	15/13	721/817
Years (mean, range)	37.27 (0.5–89)	33.00 (0.75–80)	33.29 (7–60)	36.53 (0.5–89)
<20	220 (16.7%)	35 (18.4%)	4 (14.3%)	259 (16.8%)
21–40	569 (43.1%)	94 (49.5%)	17 (60.7%)	680 (44.2%)
41–60	383 (29.0%)	51 (26.8%)	7 (25.0%)	441 (28.7%)
>60	148 (11.2%)	10 (5.3%)	0 (0%)	158(10.3%)
Occupations				
Unemployed[b]	377 (28.6%)	18 (9.5%)	1 (3.6%)	396 (25.7%)
Businessman	238 (18.0%)	63 (33.2%)	3 (10.7%)	304 (19.8%)
Services[c]	122 (9.2%)	17 (8.9%)	2 (7.1%)	141 (9.2%)
Industrial	149 (11.3%)	11 (5.8%)	5 (18.0%)	165 (10.7%)
Office clerk	105 (8.0%)	4 (2.1%)	3 (10.7%)	112 (7.3%)
Farmer	81 (6.1%)	33 (17.4%)	2 (7.1%)	116 (7.5%)
Student	143 (10.8%)	23 (12.1%)	4 (14.3%)	170 (11.1%)
Teacher	22 (1.7%)	8 (4.2%)	2 (7.1%)	32(2.1%)
Others[d]	83 (6.3%)	13 (6.8%)	6 (21.4%)	102 (6.6%)

[a] Includes 21 cases from Kunming city, two from Baoshan, one from Dali, one from Lijiang, one from Puer, one from Yuxi, and one from Honghe prefectures.
[b] Mainly includes housewives and retired elderly men.
[c] Guides, medical staff, drivers, security guards, restaurant waiters, etc. are included.
[d] Includes several soldiers and the individuals for whom we lack such information.

dengue cases were from Kunming (the capital of Yunnan province) and the other southern and western prefectures of Yunnan, including Baoshan, Dali, Lijiang, Puer, Yuxi, and Honghe.

The population of DENV infected individuals consisted of 721 males and 817 females (sex ratio of 1:1.1) and ranged from 13 to 43 years of age (median, 36.53 years). The age ranges of 21 to 40 and 41 to 60 accounted for 44.2% and 28.7% of all cases, respectively (Table 1). More than eight occupations were recorded for the infected individuals; there is no obvious occupational preference for the DENV infections.

3.2. Distinct distribution of DENV serotypes/genotypes between Dehong and Xishuangbanna

The E gene from each of the 104 randomly selected samples was amplified and sequenced successfully. Phylogenetic analysis was performed based on the sequences obtained and reference sequences from the NCBI GenBank. We classified nine strains (39.1%) from Dehong prefecture as genotype I of DENV-1 (Fig. 1a), which was the most predominant genotype circulating in Southeast Asian countries. Of these strains, the greatest number was closely related to viruses from Thailand and Guangdong, whereas strain D1-019 clustered with viruses from Myanmar. The other 14 strains (60.9%) from Dehong were identified as the Asian I genotype of DENV-2 (Fig. 1b). Within the Asian I genotype were two clades; one cluster contained strains from southeastern Asian countries (Laos, Myanmar, Kampuchea, Thailand, and Vietnam) and 13 DENV-2 strains from this study, the other cluster included the D2-030 local strain and strains from Myanmar and the other regions of China. Comparatively, all the strains from Xishuangbanna formed a tight cluster in the ML tree (Fig. 2) classified as genotype II of DENV-3, which were closely related to viruses from Thailand, Myanmar, Vietnam, and Bangladesh.

3.3. Bayesian phylogeographic profile of the predominant DENV genotypes

To further characterize the origin of the predominant DENV genotypes in this outbreak, Bayesian phylogeography analysis was performed to these 104 strains. The MCC tree in the relaxed exponential clock model revealed that DENV-1 could be delineated into five genotypes, designated I–V, approximately 109 years ago (1906; Fig. 3). It was estimated that the most recent common ancestor of genotype I strains originated in ca. 1964, and the strains from Dehong diverged 10.9 years ago. Notably, the local D1-019 cluster and strains from Myanmar had diverged a little earlier (1997).

The Bayesian phylogenetic analysis also revealed the existence of at least six genotypes within DENV-2 (Fig. 4). The predominant Asian I genotype, including 14 strains from Dehong, was a relatively young genotype with an estimated diverge time of 45.7 years ago (1969). However, the cluster representing most of the local strains from Dehong was calculated to have diverged 13 years ago, except for D2-030, which had diverged earlier than the main cluster of Asian I genotype isolates from Dehong (2004).

More samples were collected from Xishuangbanna, because of the comparatively more serious DENV prevalence. All of the 81 strains tested were classified as genotype II of DENV-3. Using representative sequences from GenBank, Bayesian coalescent analysis revealed that DENV-3 originated approximately 122 years ago from an ancestral lineage (Fig. 5) earlier than that of DENV-1 and DENV-2. Subsequently,

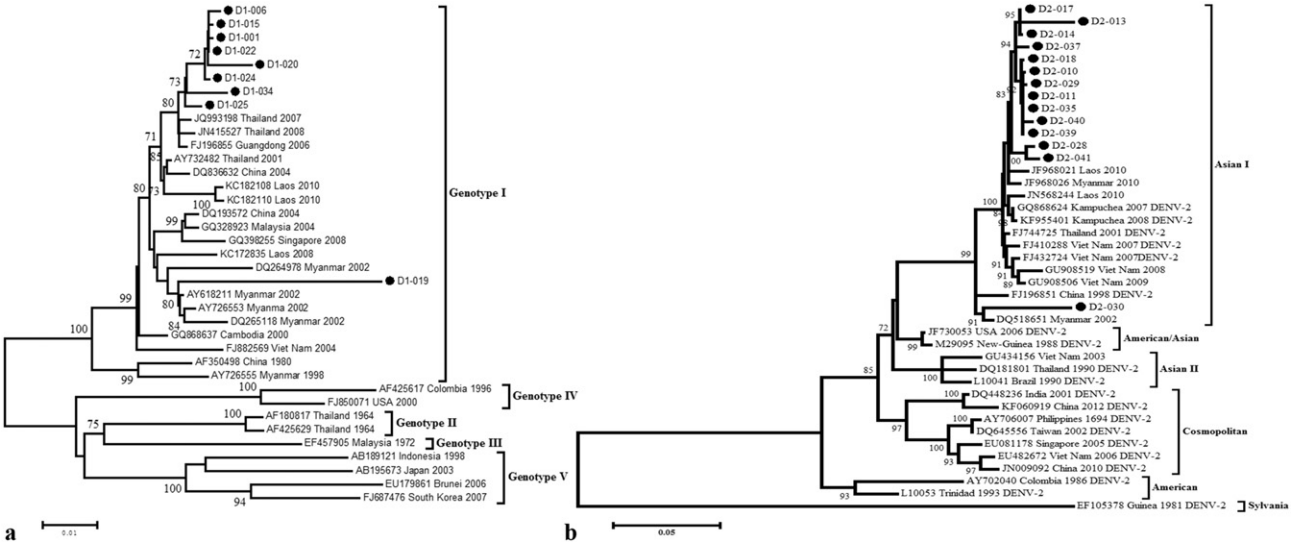

Fig. 1. Phylogenetic Tree of DENV-1 (a) and DENV-2 (b) Strains from the Dehong Prefecture. The phylogenetic tree was constructed using the maximum likelihood method with a Kimura 2 parameter model in MEGA 5.0 software. Bootstrap values were set for 1000 repetitions. The black dots denote endemic strains from local patients. Representative strains of all DENV-1 genotypes were retrieved from GenBank (http://www.ncbi.nlm.nih.gov/genbank/).

DENV-3 diverged into five separate genotypes (I–V). The genotype II cluster containing the Xishuangbanna strains also included viruses from Southeast Asian countries like Thailand, Vietnam, Myanmar, and Cambodia. As a tight cluster, the MRCA of the strains from Xishuangbanna was estimated to have diverged in 2007, while the MRCA of all genotype II strains was estimated to have existed in 1973.

Considering the phylogeographic profiles of the three predominant genotypes together suggested that the dengue virus in the 2013 outbreak was probably imported from Southeast Asian countries. DENV in Xishuangbanna were likely to have a single source, having been imported from Myanmar. However, the strains of DENV-1 and DENV-2 were probably introduced into Dehong from multiple locations, including Southeast Asian countries and other provinces in southern China.

4. Discussion

Southeast Asian countries bordering China to the southwest are considered the origin of all four serotypes of DENV, and are sites of endemic DENV infection (Guzman and Harris, 2015). DENV infection has led to serious public health problems in these countries owing to the appropriate tropical to subtropical climate and the presence of the vector mosquitoes, especially in Thailand, Myanmar, Vietnam, and Cambodia (Dorji et al., 2009; Jarman et al., 2008; Hammon et al., 1960; Fukunaga et al., 1983). Owing to the more frequent interactions between the populations of Southeast Asian countries and China, imported dengue epidemics have been documented in southern China. Over the past 20 years, DENV infection was detected in southern China nearly every year, fortunately with only low rates of infection. All four serotypes of DENV have circulated in Mainland China; however, as they have been described as imported infections from Southeast Asian countries, they have been characterized as being endemic in these regions (Jiang et al., 2014).

Yunnan province is located in the southwestern China, bordering Vietnam, Laos, and Myanmar. Only sporadic cases and minor outbreaks of DF had been documented in Yunnan Province before the large-scale outbreak in 2013 with thousands of DENV infections. This outbreak was characterized by its rapid progression. The peak of infection was between August and October, consistent with a previous outbreak in Guangzhou, China (Jiang et al., 2013). According to the records from the Center of Disease Control of Yunnan province, 1538 DENV infections were confirmed during this outbreak, with patients from eight

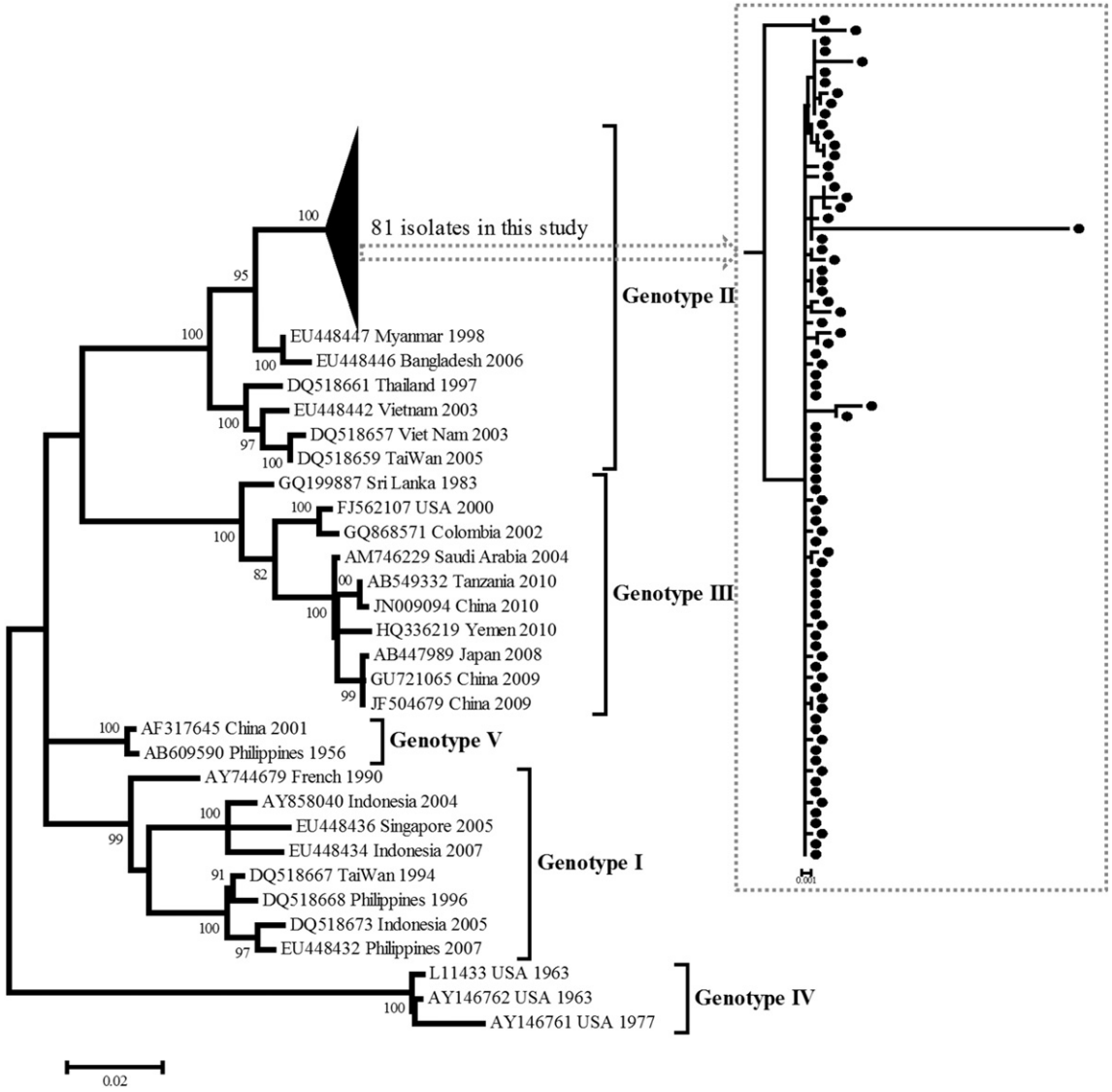

Fig. 2. Phylogenetic Tree of DENV-3 Strains from the Xishuangbanna Prefecture. The phylogenetic tree was constructed using the maximum likelihood method with a Kimura 2 parameter model in MEGA 5.0 software. Bootstrap values were set for 1000 repetitions. All 81 strains were classified as genotype II of DENV-3. Representative strains of all DENV-3 genotypes were retrieved from GenBank (http://www.ncbi.nlm.nih.gov/genbank/).

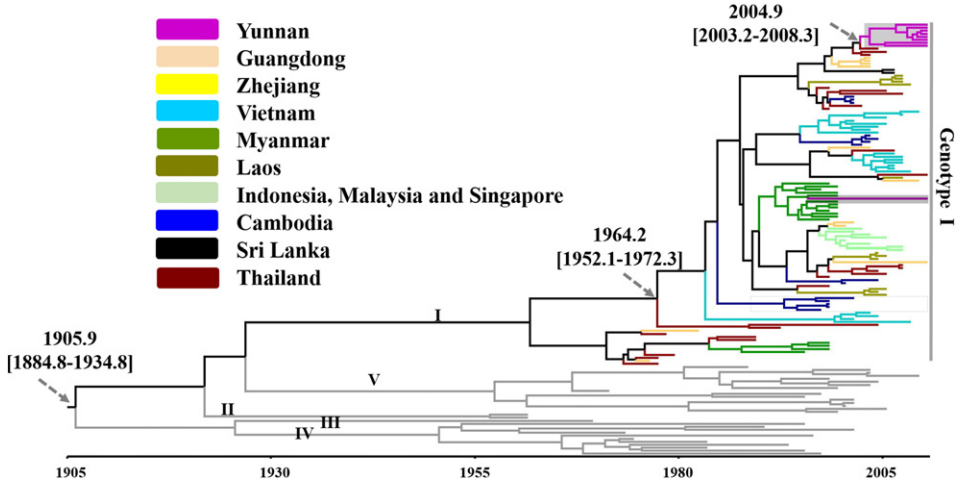

Fig. 3. Maximum clade credibility tree of the E-coding region of DENV-1. A time-scaled maximum clade credibility (MCC) tree was constructed based on the envelope gene sequences (1485 nt) of 9 DENV-1 strains from the present study together with representative sequences derived from GenBank, with sampling time and geographic localities. The genotype I strains from different regions were marked in indicated colors. Estimates for the age of some relevant nodes on the tree (pointed by arrows) are also highlighted. The strains in present study are placed in gray box.

prefectures. Among them, Xishuangbanna and Dehong were the two prefectures with extensive borders shared with Southeast Asian countries. These two prefectures contained the greatest extent of DENV infections, possibly due to the increase in cross-border transportation and exchange of travelers.

To characterize the distribution of DENV genotypes, 23 dengue-positive patient samples from Dehong and 81 from Xishuangbanna were selected at random. There were obvious differences in demographic parameters between these two populations: both DENV-1 genotype I and the DENV-2 Asian I genotype strains were collected in Dehong whereas all strains from Xishuangbanna were determined DENV-3 genotype II. These serotypes/genotypes were also the predominant circulating serotypes/genotypes in the neighboring Southeastern Asian countries. However, the distinct distribution of DENV genotypes in Dehong and Xishuangbanna suggested distinct events of transmission of DENV into China. Xishuangbanna is the southernmost prefecture of the Yunnan province, Dehong is the westernmost prefecture of the Yunnan province. They are geographically isolated, 600 km away. The geographic separation of these two prefectures also suggests the independence of these epidemic DENV outbreaks.

In a GTR model with a discretized gamma distribution across-site rate variation (GTR + Γ4) substitution model and a relaxed (uncorrelated lognormal) molecular clock model, the Bayesian coalescent analysis of E gene sequences estimated the TMRCAs were 1906 and 1794 for DENV-1 and DENV-2, respectively, which is consistent with previous estimations (Teoh et al., 2013; Weaver & Vasilakis, 2009; Sun and Meng, 2013). The TMRCA of the DENV-3 genotype II was calculated to be 42 years ago, much more recent than for DENV-1 and DENV-2. Considering these, and previously published data, we deduced migration patterns of DENV genotypes based on the genetic relationships between local strains and circulating strains in Southeast Asian countries (Fig. 6). Genotype I of DENV-1 and Asian I genotype of DENV-2 are predicted to have originated in Thailand and then spread to neighboring countries (Fig. 6a and b); Genotype II DENV-1 is also predicted to have originated in Thailand, but spread only to local regions, such as Myanmar, Vietnam, and Cambodia. In comparison, the DENV-1 genotype I outbreak was more widespread; it was detected in Japan, Djibouti, Singapore, and Taiwan (Villabona-Arenas and Zanotto, 2013). By contrast, the DENV-2 Asian I genotype epidemic was limited to Asia (Walimbe et al., 2014), with few reports in other continents. The epidemic of genotype

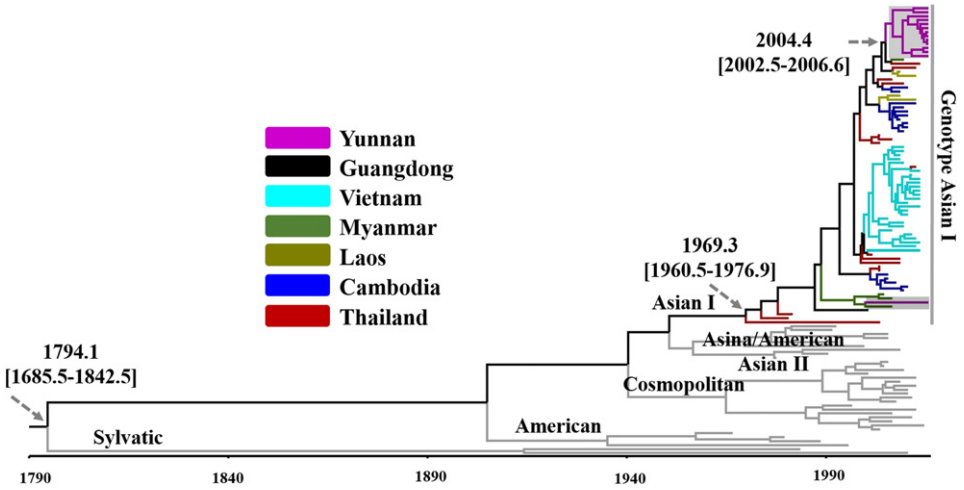

Fig. 4. Maximum clade credibility tree of the E-coding region of DENV-2. A time-scaled maximum clade credibility (MCC) tree was constructed based on the envelope gene sequences (1485 nt) of 14 DENV-2 strains from the present study together with representative sequences derived from GenBank, with sampling time and geographic localities. The Asian I genotype strains from different regions were marked in indicated colors. Estimates for the age of some relevant nodes on the tree (pointed by arrows) are also highlighted. The strains in present study are placed in gray box.

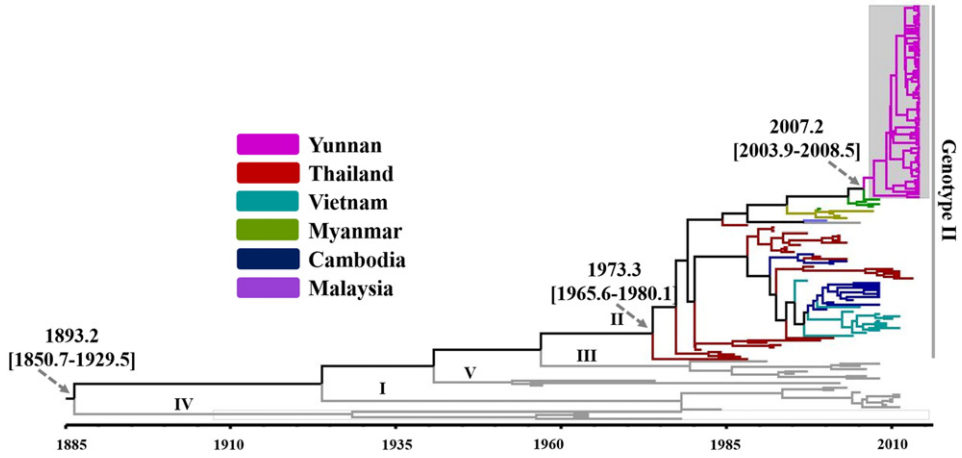

Fig. 5. Maximum clade credibility tree of the E-coding region of Dengue Virus (DENV)-3. A time-scaled maximum clade credibility (MCC) tree was constructed based on 81 envelope gene sequences (1485 nt) of DENV-3 strains in this study, together with representative sequences from GenBank, with sampling time and geographic localities. The genotype II strains from different regions were marked in indicated colors. Estimates for the age of some relevant nodes on the tree (pointed by arrows) are also highlighted. The strains in present study are placed in gray box.

II of DENV-3 showed a distinct pattern, limited to Thailand, Cambodia, and Vietnam, and appeared to have been transmitted into Malaysia, Indonesia, and Bangladesh (Araújo et al., 2009).

Interestingly, a completely different genotype distribution was observed in Dehong and Xishuangbanna: all strains from Xishuangbanna were identified as DENV-3 genotype II, whereas two genotypes (DENV-1 genotype I and DENV-2 Asian I genotype) were identified in Dehong. In an ML tree and an MCC tree, multiple DENV-1 and DENV-2 reference strains with strong similarities to the strains from this study indicated a variety of sources for these two genotypes. These strains may have been imported from Myanmar, Laos, and other regions of China (Fig. 6a & b). Myanmar was considered as the only source of the predominant DENV-3 (Fig. 6c). Different routes of transmission are likely to have led to such clear differences in these two regions during this dengue outbreak (Fig. 6d).

In the past, neither sporadic cases nor outbreaks of DENV infection in China were considered to be endemic, as most researchers attributed the presence of DF to imported cases (Ren et al., 2003; Li et al., 2013; Zheng et al., 2009). We hypothesized that the 2013 dengue outbreak was caused by imported DENV strains from surrounding countries. The development of this hypothesis was described in detail previously (Wang et al., 2015), and was based upon comparison of genetic diversity between imported cases and local cases. Moreover, this dengue outbreak was characterized by its sudden, rapid progression and single circulating genotype in Xishuangbanna Prefecture. These characteristics strongly suggest a local outbreak. Because global climate change has

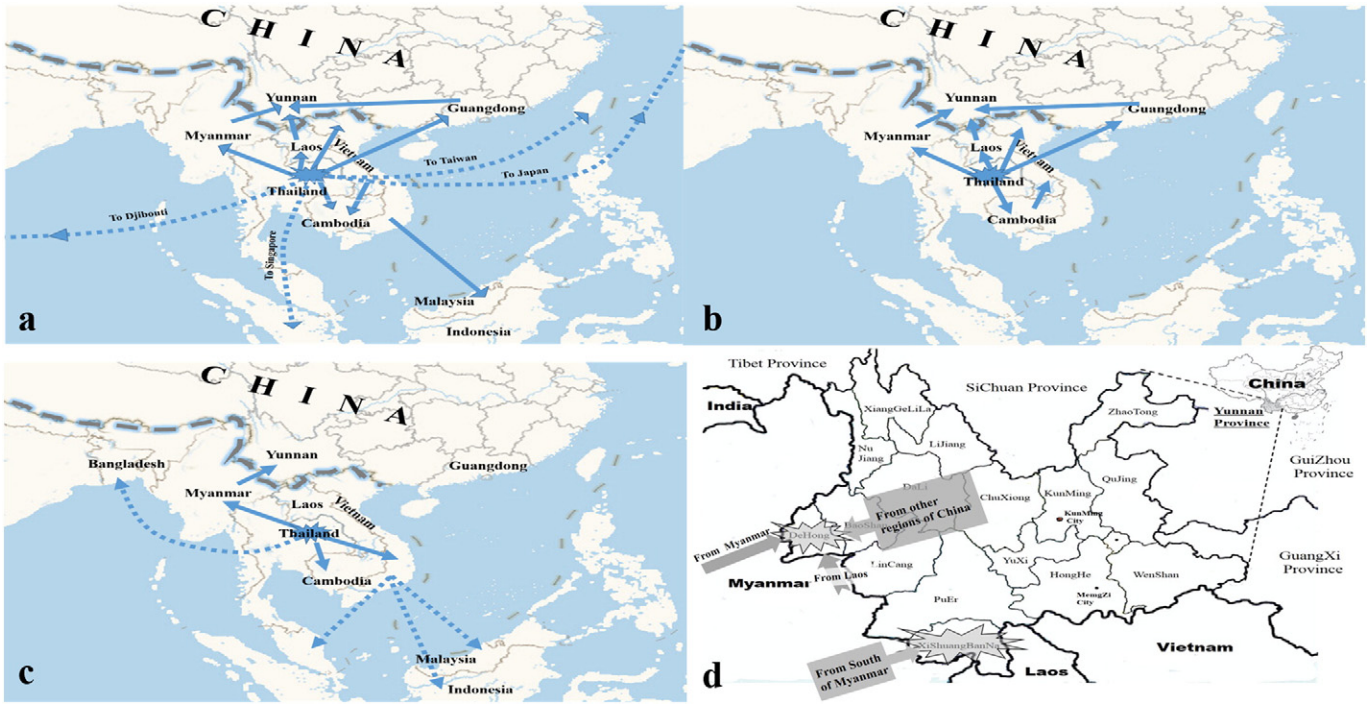

Fig. 6. Proposed migration patterns of dengue virus (DENV) into Yunnan region. The migration patterns of DENV-1 genotype I (a) DENV-2 genotype Asian I (b), and DENV-3 genotype II (c) based on all available data. The solid lines represent the DENV migration routes deduced from the maximum clade credibility tree from this study, whereas the dotted lines represent the routes documented in other studies. The possible imported route of the DENV strains of this outbreak was integrated (d).

created more tropical and sub-tropical areas in China, there are increased possibilities for local DF outbreaks in China, which will require increased surveillance.

In conclusion, our findings described the epidemiological characteristics of the 2013 dengue outbreak in Yunnan. The only DENV genotype in Xishuangbanna was DENV-3 genotype II, while DENV-1 genotype I and DENV-2 Asian I genotype circulated in Dehong. Using phylogenetic and Bayesian coalescent analysis, we hypothesize that the 2013 outbreak in China was due to imported DENV infections from Southeast Asian countries. Furthermore, we calculated the origin time of these three predominant DENV genotypes. The three DENV genotypes were deduced to have originated in Thailand, and were then transmitted into Yunnan in different migration patterns. Our findings will provide a better understanding of this dengue outbreak in Yunnan Province, and can improve the strategies for prevention and control of dengue in this region.

Unfortunately, small scale epidemic is still occurring until today in Yunnan province. We'll pay constant attention to its epidemic and cross-bored transmission, and the latest information of dengue is collecting and analyzing. Dynamically surveillance and further description on epidemiological characteristics of dengue virus are worthy of putting more public investment in this area.

Acknowledgments

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