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Molecular characterization of the envelope gene of dengue virus type 3 newly isolated in Guangzhou, China, during 2009–2010

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SUMMARY

Background: After an absence of 29 years, dengue virus type 3 (DENV-3) re-emerged in Guangzhou in 2009 and again in 2010. However, the geographical route by which the virus entered the city, and how it has changed genetically, remain unclear. Therefore, we carried out a comprehensive investigation into the molecular characteristics of the DENV-3 involved.

Methods: The envelope (*E*) genes of viruses isolated from dengue patients during the 2009–2010 epidemics were sequenced and compared with previously published *E* gene sequences of global representative DENV-3 strains available in GenBank, including isolates circulating in other provinces of China.

Results: A total of 13 isolates (seven from 2009 and six from 2010) were obtained from human serum samples. Phylogenetic analysis revealed that the isolates were grouped into three genotypes (I, III, and V) and then two clades within genotype III (genotype I from Indonesia, genotype III clade A from Côte d'Ivoire, genotype III clade B from Tanzania, and genotype V from Philippines). In addition, there were 1.3–9.0% and 0.5–3.9% differences in the nucleic and deduced amino acid sequences between the 2009 and 2010 strains, respectively.

Conclusions: The DENV-3 viruses from the period 2009–2010 were not from the continuous spread of an epidemic strain or the re-emergence of the 2009 strains in the 2-year period. The introduction of different DENV-3 genotypes following more than one geographical route was an important contributing factor to the 2009–2010 dengue epidemics in Guangzhou.

1. Introduction

The causative agents of dengue disease are four antigenically distinct viruses designated dengue virus types 1–4 (DENV 1–4), belonging to the genus *Flavivirus*, family *Flaviviridae*. The DENV genome consists of a single-stranded, non-segmented, positive-sense RNA molecule roughly 10.7 kb in size.[1] Infection with one of the four DENV serotypes can be asymptomatic or trigger a wide spectrum of clinical manifestations, ranging from mild acute febrile illness to classical dengue fever (DF) and to severe dengue hemorrhagic fever (DHF)/dengue shock syndrome (DSS).[2]

During the last two decades, the increase in incidence and the geographical expansion of dengue have been facilitated by the rapid movement of viruses and vectors through international air travel and trade, uncontrolled urbanization, modern transportation, and population flow.[3–7] Dengue is currently the most important arthropod-borne viral disease in the world. Some 2.5 billion people living in tropical and sub-tropical regions are at risk of dengue infection, which equates to about 40% of humanity.[8,9] An estimated 100 million infections occur annually, with 500 000 DHF/DSS cases and more than 25 000 deaths, mostly among children.[10]

In China, historical epidemics of dengue-like illness were documented before 1950. However, etiological and epidemiological investigations were not carried out during these epidemics. After a 28-year period without dengue cases between 1950 and 1978, a DENV-4 outbreak occurred in the city of Foshan (contiguous with Guangzhou) in 1978,[11] which started the epidemic of dengue in southern China.[12] In August 1978, dengue infections first spread to Guangzhou from Foshan. Since then, highly localized and relatively sporadic yearly outbreaks have been reported in Guangzhou. Until now, a cumulative total of nearly 20 000 dengue cases have been identified in Guangzhou, accounting for 67% of all reported cases in Guangdong Province and 56% in mainland China.[13]

Serotypes DENV-1, DENV-2, DENV-3, and DENV-4 circulated sequentially in Guangzhou between 1978 and 2008.[13] Beginning in 2000, outbreaks of DENV in Guangzhou were due to DENV-1 only,

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but in 2009, DENV-3 circulation was detected for the first time since 1980.[14] In 2010, DENV-3 was isolated again in Guangzhou, producing a larger outbreak that affected five districts of the city of Guangzhou (Total of 12 districts). As the origin of these outbreaks was not determined through epidemiological investigation, the question arose again as to whether the dengue had become endemic in Guangzhou. To understand the molecular epidemiology of DENV-3 in Guangzhou, and also particularly to determine whether the 2009 and 2010 outbreaks were caused by the same viral genotype, the complete envelope (*E*) gene sequences of isolates from both years were determined.

2. Materials and methods

The following is a brief outline of the methods employed in the present study. Additional details are provided by Luo et al.,[13] Jing et al.,[15] and Zheng et al.[16]

2.1. Patients and samples

A total of 55 clinically diagnosed cases were identified from passive surveillance or recognized by active searches during the 2009–2010 dengue epidemics/outbreaks in Guangzhou. In each case, 8 ml of peripheral venous blood was collected in plasma separator gel tubes during the acute phrase and taken to the laboratory for analysis within 6 h of collection. The present study was approved by the Ethics Committee of the Guangzhou Center for Disease Control and Prevention. Written informed consent was obtained from all patients upon seeking medical services or before entry into the study.

2.2. Virus strains

In brief, 17 serum samples were found to be positive for DENV RNA by real-time PCR (RT-PCR)[16] and 13 isolates were identified as DENV-3. These strains were inoculated into C6/36 *Aedes albopictus* mosquito cells[17] for passaging, and cell supernatants were preserved at −80 °C until RNA extraction.

2.3. RNA extraction and reverse transcriptase PCR

Viral RNA was extracted from 200 μl of DENV-3-infected C6/C36 culture supernatant using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) in accordance with the manufacturer's instructions. Amplification of the *E* gene was then performed using the One-Step Reverse Transcriptase-PCR Kit (TaKaRa, Shiga, Japan) and three manually designed oligonucleotide primer pairs (Table 1 for details) in order to produce three overlapping fragments covering the complete *E* gene. Briefly, the reverse transcriptase PCR program consisted of a reverse transcription step at 50 °C for 30 min and the denaturation of reverse transcriptase enzyme at 95 °C for 10 min, followed by the PCR reaction performed for three cycles at 94 °C for 1 min, 60 °C for 40 s, 72 °C for 1 min, 35 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 40 s, and a final 72 °C for 3 min.

2.4. Sequencing and phylogenetic analysis

Amplified cDNA products of DENV-3 were sequenced directly after purification using a QIAquick PCR Purification Kit (Qiagen, Germany). Double-stranded sequencing of the *E* gene was performed on an ABI PRISM 3700 Genetic Analyzer (Applied Biosystems) using an ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA) in accordance with the manufacturer's protocol. DENV primers used for sequencing were designed on the basis of published DENV sequences and are presented in Table 1. The assemblies of sequences were created with SeqMan II software (DNASTAR, Inc., Madison, WI, USA).[15] Percent nucleotide and amino acid sequence identities were calculated using the BLAST engine (http://www.ncbi.nlm.nih.gov/Entrez/).[18] Multiple sequence alignment was carried out using Clustal X v.1.83 software.[16] Phylogenetic trees were constructed by the neighbor-joining and maximum likelihood methods with a Kimura two-parameter model using MEGA 4.0 software (Molecular Evolutionary Genetics Analysis, version 4.0; http://www.megasoftware.net/mega4/mega.html).[15] One thousand bootstrap repetitions were calculated for placing confidence values on groupings within trees. DENV-1 strain D1/USA/Hawaii/1945 (GenBank accession number **AF425619**) was used as out-group to root the trees.

Table 1
Primers used for amplification and sequencing of the complete DENV-3 envelope gene

Reaction	Primer name	Sequence (from 5′ to 3′)	Position
Amplification	D3E1F	AGACATTGACTGGTGGTGC	622–640
Amplification	D3E1R	TGATGACGGTGTATTTGAGG	1336–1355
Amplification	D3E2F	CGGTTGTGGTTTGTTTGG	1243–1260
Amplification	D3E2R	CTGCGTTTCTGAGACTTCTTTC	1855–1876
Amplification	D3E3F	TACCGCACTGACAGGAGC	1711–1728
Amplification	D3E3R	CCACAACTACCGTTAATTTGAT	2663–2684
Sequencing	D3-Seq1S	GCTGGTTACCCCATCCA	912–930
Sequencing	D3-Seq2S	AGGAGCAGGACCAGAAC	1185–1202
Sequencing	D3-Seq3S	CTGAATATGGAACCCTCG	1455–1473
Sequencing	D3-Seq5S	GTGACCAAGAAGGAGGA	2000–2017
Sequencing	D3-Seq2C	GCCATCTTGGGAGACA	2165–2181
Sequencing	D3-Seq6C	CAGCTGGCGACCCTAA	1088–1104
Sequencing	D3rI	GGCAACAGCCATTGCAGGC	2539–2558

DENV-3, dengue virus type 3.

3. Results

We determined the complete *E* gene nucleotide sequences of the 13 DENV-3 strains (seven from 2009 and six from 2010) from Guangzhou. The sequences of all the strains reported in this paper have been deposited in GenBank under the accession numbers listed in Table 2.

3.1. Comparison of nucleic and amino acid sequences

The percentages of nucleotide and amino acid identities of the complete *E* gene among the 13 different DENV-3 strains are shown in Table 3. With the exception of 10/GZ/10549, the five other DENV-3 isolates from the 2010 epidemic area displayed high similarity, with 99.5–100% and 98.7–100% sequence identity in nucleotide and amino acid sequences, respectively. Three DENV-3 isolates (09/GZ/1483, 09/GZ/10806, and 09/GZ/1081) from the

Table 2
DENV-3 strains newly isolated in Guangzhou during 2009–2010

Strain	Date of occurrence	GenBank accession number	District
09/GZ/1081	2/28/2009	**Hm466962**	Yuexiu
09/GZ/1483	3/18/2009	**Hm466963**	Haizhu
09/GZ/10806	7/22/2009	**Hm466965**	Haizhu
09/GZ/10616	7/25/2009	**Hm466964**	Yuexiu
09/GZ/11144	8/6/2009	**Hm466966**	Yuexiu
09/GZ/11194	8/6/2009	**Hm466967**	Yuexiu
09/GZ/13105	9/8/2009	**Hm466968**	Haizhu
10/GZ/4898	4/25/2010	**JN009093**	Yuexiu
10/GZ/5536	5/12/2010	**JN009094**	Nansha
10/GZ/9119	8/05/2010	**JN009095**	Baiyun
10/GZ/9534	8/12/2010	**JN009096**	Baiyun
10/GZ/9721	8/22/2010	**JN009097**	Baiyun
10/GZ/10549	9/11/2010	**JN009098**	Panyu

DENV-3, dengue virus type 3.

Table 3
Percentage identity within the complete *E* genomic sequences of 13 different DENV-3 strains obtained from Guangzhou, during 2009–2010

	Pairwise amino acid identity (%)												
No.	Strain	1	2	3	4	5	6	7	8	9	10	11	12	13
1	10/GZ/4898	-	1	0.987	1	1	0.967	0.969	0.967	0.995	0.969	0.993	0.993	0.985
2	10/GZ/5536	1	-	0.987	1	1	0.967	0.969	0.967	0.995	0.969	0.993	0.993	0.985
3	10/GZ/9119	0.995	0.995	-	0.987	0.987	0.955	0.957	0.957	0.983	0.957	0.981	0.981	0.973
4	10/GZ/9534	1	1	0.995	-	1	0.967	0.969	0.967	0.995	0.969	0.993	0.993	0.985
5	10/GZ/9721	1	1	0.995	1	-	0.967	0.969	0.967	0.995	0.969	0.993	0.993	0.985
6	10/GZ/10549	0.911	0.911	0.907	0.911	0.911	-	0.973	0.971	0.971	0.973	0.969	0.969	0.961
7	09/GZ/1081	0.934	0.934	0.930	0.934	0.934	0.936	-	0.997	0.973	1	0.971	0.971	0.963
8	09/GZ/1483	0.933	0.933	0.930	0.933	0.933	0.935	0.998	-	0.971	0.997	0.969	0.969	0.961
9	09/GZ/10616	0.987	0.987	0.983	0.987	0.987	0.916	0.938	0.937	-	0.973	0.997	0.997	0.989
10	09/GZ/10806	0.934	0.934	0.930	0.934	0.934	0.936	1	0.998	0.938	-	0.971	0.971	0.963
11	09/GZ/11144	0.985	0.985	0.981	0.985	0.985	0.913	0.937	0.935	0.997	0.937	-	1	0.991
12	09/GZ/11194	0.985	0.985	0.981	0.985	0.985	0.914	0.937	0.936	0.997	0.937	0.999	-	0.991
13	09/GZ/13105	0.981	0.981	0.977	0.981	0.981	0.910	0.933	0.932	0.993	0.933	0.995	0.995	-
	Pairwise nucleotide identity (%)													

DENV-3, dengue virus type 3.

2009 epidemic area showed the highest similarity, with more than 99.7% sequence identity in both nucleotide and amino acid sequences. Three other 2009 DENV-3 isolates, including 09/GZ/10616, 09/GZ/11144, and 09/GZ/11194 strains, showed relatively lower sequence identity compared with the 09/GZ/13105 isolate (99.3–99.5% nucleotide and 98.9–99.1% amino acid sequence identity). However, differences in the nucleotide and deduced amino acid sequences between the 2009 and 2010 strains were found to be 1.3–9.0% and 0.5–3.9%, respectively, which suggested that the 13 isolates might have originated from different regions. Further phylogenetic analysis revealed that these viruses belong to different genotypes (see below for details).

3.2. Phylogenetic analysis

Sequences available in GenBank and corresponding to strains belonging to all major branches of previously published DENV-3 phylogenies (see Table 4 for details) were included in this analysis and selected to maximize the representation of genotypes, regions, and years of isolation. However, isolates that showed higher nucleotide sequence homology but were adequately represented were removed from the analysis. Hence, the final tree only included representative local samples for each genotype. The trees derived from the neighbor-joining method and the maximum likelihood method were very similar to each other. Thus, only the tree from the neighbor-joining method based on the complete *E* gene sequences is shown.

Five genetic subtypes, each representing a distinct genotype, could be distinguished, as shown in Figure 1: one containing virus isolated in Indonesia, Malaysia, Singapore, Philippines, and the South Pacific (genotype I); the second including viruses collected from Thailand, Myanmar, Cambodia, and China (genotype II); the third including a large array of viruses sampled from Southeast Asia, Central and South America, Africa, and the Middle East (genotype III); the fourth, the most divergent group, containing virus from Puerto Rico from the 1960s/1970s (genotype IV); and the fifth represented by the oldest strain, isolated in the Philippines in 1956 (genotype V).

Phylogenetically, as demonstrated in Figure 1, 13 newly identified DENV-3 isolates were characterized into genotypes I, III, and V. Only one DENV-3 strain (10/GZ/10549) from 2010 belonged to genotype I and clustered closely with strains isolated from 2004 to 2007 in Indonesia. Three 2009 isolates (09/GZ/1081, 09/GZ/1483, and 09/GZ/10806) clustered together as genotype V, which was closely related to the strain from Guangxi China in 1980.

Within Guangzhou genotype III strains, two clusters (A and B) of nine isolates were observed. Clade A contained four 2009 strains (09/GZ/11144, 09/GZ/11194, 09/GZ/10616, and 09/GZ/13105). Five other DENV-3 isolates (10/GZ/4898, 10/GZ/5536, 10/GZ/9119, 10/GZ/9534, and 10/GZ/9721) from 2010 outbreaks clustered together and fell into clade B. They resided in the phylogenetic tree much closer to the isolate from Tanzania in 2010.

4. Discussion

DENV-3 was initially isolated during an outbreak in 1956 in Philippines,[19] and since then the size of the geographic range and the incidence of this serotype have increased substantially worldwide.[11,20–23] To date, DENV-3 has been subdivided into four to five genotypes.[24,25] Genotypes I–III are responsible for most DENV-3 infections and have been associated with DF/DHF epidemics in Southeast Asia, the Indian subcontinent, East Africa, and the Americas. In contrast, genotypes IV and V are only found in a few older strains.[20,26] Genotype IV, representing the earlier strains from Puerto Rico, has not been isolated for about 40 years. Genotype V, represented by the H87 strain from Philippines in 1956 and other early isolates from Asia, has also rarely been associated with dengue epidemics.

The recent emergence of DENV-3 in Guangzhou occurred after a decade of active DENV-1 circulation, about 29 years after the previous report of DENV-3 in the region.[13] In order to complement the epidemiological data and arrive at a better understanding of the geographical route that allowed the virus entry and spread in Guangzhou, we conducted a phylogenetic analysis on the complete *E* gene of DENV-3 strains newly isolated during the 2009–2010 epidemics/outbreaks.

The resulting phylogenetic tree shows that all 13 DENV-3 strains were grouped into three genotypes (I, III, and V) (Figure 1). As previously described,[27] two genotypes (III and V) of DENV-3 were co-circulating in Guangzhou in 2009. However, 2009 and 2010 genotype III strains were segregated into two different clades (clade A and clade B). With regard to clade A strains (09/GZ/11144, 09/GZ/11194, 09/GZ/10616, and 09/GZ/13105) isolated from Guangzhou in 2009, it was previously deemed that they were clustered with epidemic strains in India in 2008.[27] However we deduced that they were more closely related to a previously reported strain from Côte d'Ivoire isolated in 2008.[28] Inconsistent results may be related to the biased conclusion resulting from the smaller number of strains included in the previous study. Five 2010 strains (10/GZ/4898, 10/GZ/5536, 10/GZ/9119, 10/GZ/9534, and 10/GZ/9721) are nearly identical and located in clade B formed by **AB549332** from Tanzania in 2010. Sequence comparison of strains

Table 4
DENV-3 strains from GenBank used in the phylogenetic analysis described in this study

Strain	Country	Year	GenBank	Strain	Country	Year	GenBank
HIID02	Indonesia	2005	**AB219138**	0707aTw	Myanmar	2007	**EU448443**
NIID48	Japan	2008	**AB447989**	0607aTw	Myanmar	2006	**EU448444**
00510	Ivory Coast	2008	**FM213456**	0711aTw	Bangladesh	2007	**EU448445**
Hu/08/NIID	Tanzania	2010	**AB549332**	0611aTw	Bangladesh	2006	**EU448446**
Hawaii	USA	1945	**AF425619**	9809aTw	Myanmar	1998	**EU448447**
6805	Saudi Arabia	2004	**AM746229**	BID-V1736	Puerto Rico	1999	**FJ390376**
6475	Saudi Arabia	2004	**AM746232**	SV0837_07	Bhutan	2007	**FJ606708**
BR74886.02	Brazil	2002	**AY679147**	ND143	India	2007	**FJ644564**
Cuba580	Cuba	2001	**AY702030**	BID-V2328	Thailand	2001	**FJ744739**
Cuba21	Cuba	2002	**AY702031**	BID-V2329	Thailand	2001	**FJ744740**
Cuba116	Cuba	2000	**AY702032**	BID-V1605	Puerto Rico	2004	**FJ850055**
PF903050	French Polynesia	1990	**AY744679**	BID-V2412	Sri Lanka	1989	**FJ882572**
PF922986	Tahiti	1992	**AY744683**	BID-V2413	Sri Lanka	1993	**FJ882573**
FW01	Indonesia	2004	**AY858040**	BID-V2978	Colombia	2001	**GQ199891**
KJ30i	Indonesia	2004	**AY858042**	DTID-ZJU04	China	2009	**GU189648**
KJ71	Indonesia	2004	**AY858044**	zhengjiang2709	China	2009	**GU721068**
TB16	Indonesia	2004	**AY858047**	GZ1D3	China	2009	**GU363549**
TB55i	Indonesia	2004	**AY858048**	Zhejiang/08/09	China	2009	**GU721065**
0409aTw	Vietnam	2004	**DQ518656**	Zhejiang/15/09	China	2009	**GU721066**
0507aTw	Vietnam	2005	**DQ518658**	Zhejiang/17/09	China	2009	**GU721067**
0308aTw	Thailand	2003	**DQ518660**	Zhejiang/31/09	China	2009	**GU721069**
9807aTw	Thailand	1998	**DQ518663**	Alto Lucero/5	Mexico	2006	**HM171538**
0211aTw	Thailand	2002	**DQ518664**	Tlaltizapan/3	Mexico	2006	**HM171540**
0108aTw	Bangladesh	2001	**DQ518665**	ARU99.1	Aruba	1999	**HM348812**
0508aTw	Myanmar	2005	**DQ518666**	H50978	Venezuela	2000	**HM348813**
0508aTw	Philippines	2005	**DQ518673**	H63263	Venezuela	2001	**HM348815**
Indo9804aTw	Indonesia	1998	**DQ518676**	L6700	Venezuela	2001	**HM348817**
0312aTw	Indonesia	2003	**DQ518677**	H63788	Venezuela	2001	**HM348823**
0508aTw	Indonesia	2005	**DQ518678**	H64276	Venezuela	2001	**HM348824**
9912aTw	Sri Lanka	1999	**DQ518679**	H220034	Venezuela	2003	**HM348825**
0707aTw	Philippines	2007	**EU448432**	H41302058	Venezuela	2005	**HM348828**
0608aTw	Philippines	2006	**EU448433**	H413302054	Venezuela	2005	**HM348829**
0702aTw	Indonesia	2007	**EU448434**	H297222	Venezuela	2007	**HM348830**
0603aTw	Indonesia	2006	**EU448435**	H298650	Venezuela	2007	**HM348831**
0508aTw	Singapore	2005	**EU448436**	Lam Dong	Vietnam	2010	**HQ141582**
0302aTw	Indonesia	2003	**EU448437**	BID-V4743	Nicaragua	2009	**HQ166034**
0710aTw	Malaysia	2007	**EU448438**	BID-V4762	Nicaragua	2009	**HQ541797**
0610aTw	Malaysia	2006	**EU448439**	DID-V4838	Nicaragua	2009	**HQ705619**
0609aTw	Cambodia	2006	**EU448440**	BID-V4307	Cambodia	2007	**JF295012**
0604aTw	Vietnam	2006	**EU448441**	1340	Puerto Rico	1977	**L11434**
0310aTw	Vietnam	2003	**EU448442**	1339	Puerto Rico	1977	**AY146761**
80-2	China	1980	**AF317645**	BS- PRico63	Puerto Rico	1963	**AY146762**
07CHLS001	China	2007	**EU367962**	2783	Sri Lanka	1991	**L11438**
GZ2D3	China	2009	**JN662391**	MK-315	Thailand	1987	**L11442**
ZJYW2009	China	2009	**JF540679**	H-87	Philippines	1956	**M93130**

DENV-3, dengue virus type 3.

from clade A and clade B showed lower nucleotide identity (97.7–98.7%) (Table 3), which suggested that they might have originated from different countries.

All these results suggest that: (1) the re-emergence of DENV-3 in Guangzhou during 2009–2010 may have been initiated by imported cases; (2) DENV-3 from 2009–2010 were not from the continuous spread of the same epidemic strain or re-emergence of the 2009 strains in the 2010 period; (3) there may be more than one plausible route of entrance of DENV-3 into Guangzhou, i.e., three strains from Baiyun District (10/GZ/9119, 10/GZ/9534, and 10/GZ/9721), one strain form Nansha District (10/GZ/5536), and one strain from Yuexiu District (10/GZ/4898) in 2010 were closely related to the strain isolated in 2010 from Tanzania; the 2010 strain from Panyu District (10/GZ/10549) was similar to those from Indonesia (Figure 2). These conclusions are supported by the recent research findings of Moi et al.[28] and King et al.[20]

Sequence comparison also revealed that the outbreaks in Guangzhou and Zhejiang Province (more than 1400 km apart) in 2009 were caused by the same genotype of DENV-3 (Figure 1). According to the survey results described previously, partial sequences of the envelope gene of DENV-3 isolated from Zhejiang Province in 2009 had 99% similarity to that of **AM746229**, which was detected in Jeddah, Saudi Arabia, in 2004.[29] However, in our opinion, the isolates collected from both Guangzhou and Zhejiang Province in 2009 were more closely related to two Côte d'Ivoire isolates in 2008, one of which was isolated from a Japanese traveler to Côte d'Ivoire (Japan/NIID48/2008/AB447989). Furthermore, the 2010 DENV-3 genotype III isolates (IIIB clade) share genotype and clade not only with the Tanzania strain but also with the Saudi Arabia strain (Figure 1). That is to say, both clades (genotype III clade A and genotype III clade B) share a common ancestral lineage with the 2004 Saudi Arabia isolate. Clearly, these two clades evolved separately from a common ancestor originating in Saudi Arabia. These findings suggest that DENV-3 subtype III is easily transmitted among humans and mosquitoes and can adapt efficiently to new areas. Thus, other regions where the climate is similar to that of Guangzhou and Zhejiang Province (subtropical monsoon) should be aware of the risk for expansion of these strains.[29]

In summary, all these findings suggest that the repeated introductions of different genotypes of DENV-3 following more than one long-distance route through the continent into Guangzhou was an important cause of the 2009–2010 dengue epidemics, and that DENV-3 has not become endemic in

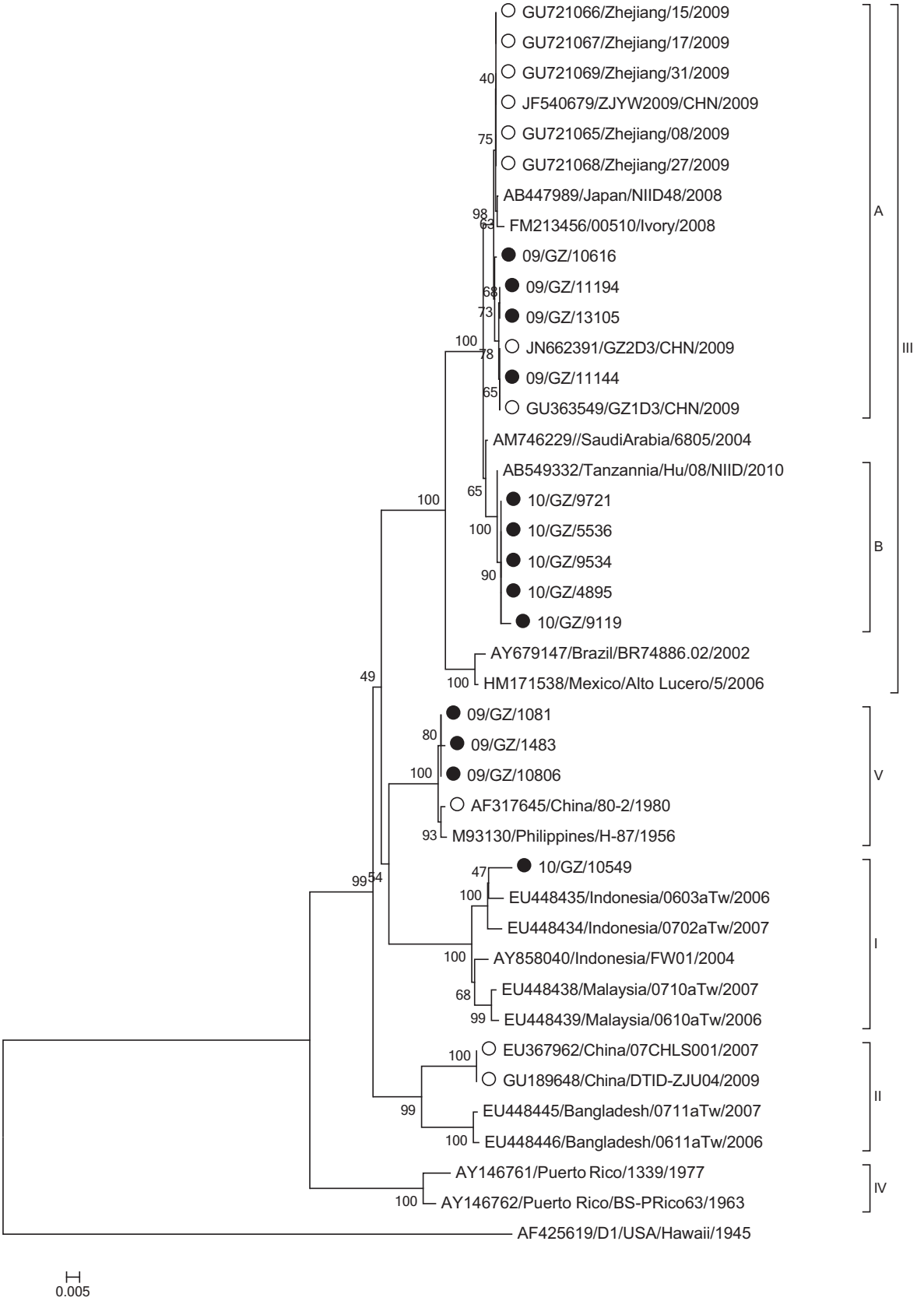

Figure 1. Phylogenetic tree based on the complete envelope gene from 90 DENV-3 strains sampled globally. Isolates that showed higher nucleotide sequence homology but were adequately represented were removed from the analysis. Hence, the final tree only includes 41 representative samples. The evolutionary history was inferred using the neighbor-joining method with MEGA 4.0 software. Taxon names correspond to 'GenBank accession number/country/strain/year of isolation'. Main bootstrap values are shown on the key nodes of the tree. The tree was rooted using DENV-1 strain **AF425619**. The newly isolated DENV-3 strains in the study are marked with black dots and the other Chinese DENV-3 isolates taken for comparison are marked with white dots.

Figure 2. The origin of DENV-3 strains isolated in Guangzhou during 2009–2010. The maps in the boxes represent the Southeast Asia, East Asia, and Africa sub-regions. The map on the right is an enlargement of Guangzhou City. The seven dots denote DENV-3 strains isolated in 2009; the six stars denote DENV-3 strains isolated in 2010.

Guangzhou. However, DENV-3 genotypes, particularly genotype III, have demonstrated higher epidemic potential with regards to severe DHF epidemics.[23,30] Once the transmission cycle is established locally, genotype III will soon result in dengue epidemics, even a trans-national dengue pandemic.[25,31,32] Thus, it will be necessary to initiate a high level of airport fever screening to identify imported dengue cases and to strengthen dengue virological surveillance to monitor the emergence of DENV strains with higher epidemic potential.

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