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Original Article

Distribution of Mosquitoes and Mosquito-Borne Viruses along the China-Myanmar Border in Yunnan Province

Yun Feng[1,2], Shihong Fu[1], Hailin Zhang[2], Minghua Li[1], Tao Zhou[2], Jinglin Wang[1], Yuzhen Zhang[2], Huanyu Wang[1], Qing Tang[1], and Guodong Liang[1]*

[1]*State Key Laboratory for Infectious Disease Prevention and Control, Department of Viral Encephalitis, Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing; and*
[2]*Yunnan Institute of Endemic Diseases Control and Prevention, Yunnan, China*

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SUMMARY: A total of 54,673 mosquitoes were collected at 11 sites located near the China-Myanmar border in the western part of Yunnan Province during July and August 2007. There were 29 species in 4 genera identified from the collections, including 12 species of *Culex*, 12 species of *Anopheles*, 3 species of *Aedes*, and 2 species of *Armigeres*. *Culex tritaeniorhynchus* Giles (67.9%, 37,119/54,673) and *Anopheles sinensis* Wiedemann (25.9%, 14,170/54,673) were the most abundant species in this investigation. Virus was isolated using BHK-21 and C6/36 cells from 22 of 510 mosquito pools. Isolates included Japanese encephalitis virus (JEV) and Getah virus (GETV), which were identified by serological and molecular methods. Twenty JEV strains were isolated from *Cx. tritaeniorhynchus* (15 isolates), *An. sinensis* (3 isolates), and *Armigeres subalbatus* Coquillett (2 isolates), and 2 GETV strains were isolated from *Culex pseudovishnui* Colless and *Cx. tritaeniorhynchus*. This study suggests that *Ar. subalbatus* is a potentially important local vector because of the high JEV infection ratio found in this species. Enzootic JEV transmission persists in this area and therefore, surveillance for human disease caused by JEV and GETV should be conducted in the region.

INTRODUCTION

Yunnan Province, located in southwestern China, shares a 1,997 km border with Myanmar. This border has a long history of being an important trade and tourism area and is becoming a key region as far as geopolitics is concerned. Previous studies have shown the presence of Japanese encephalitis (JE) and dengue in the southern part of Yunnan Province (1). Surveys have also detected antibodies against JE virus (JEV), dengue virus, Chikungunya virus, Sindbis virus, and Batai virus in human and animal sera collected from the border area (2,3). The increase in development and trade along the border has also increased the risk of infectious diseases, particularly those borne by mosquitoes, which occur and thrive in the region due in part to the subtropical climate and abundant rainfall (4,5). However, information regarding the mosquitoes associated with these viruses in the western part of Yunnan Province is lacking. In this study, we report a survey of the mosquitoes and associated viruses found in this area during the summer of 2007.

*Corresponding author: Mailing address: State Key Laboratory for Infectious Disease Prevention and Control, Department of Viral Encephalitis, Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, 155 Chang Bai Street, Chang Ping District, Beijing 102206, People's Republic of China. Tel: +86 10 58900838, Fax: +86 10 58900839, E-mail: gdliang@hotmail.com

MATERIALS AND METHODS

Field collection methods: During July and August 2007, mosquitoes were collected at 11 different locations in Tengchong (N98°51′, E25°01′), Lianghe (N98°30′, E24°78′), and Longchuan (N97°96′, E24°33′) counties and Ruili City (N97°83′, E24°00′) in Yunnan Province (Fig. 1).

Mosquitoes were collected using UV light traps (12 V, 300 mA; Wuhan Lucky Star Environmental Protection Tech Co., Hubei, China) and human landing collections in the vicinity of residential structures, including livestock sheds and pigpens. Collecting was conducted from 21:00 to 06:00 for one night at each site. Human landing collections were conducted from 20:00 to 22:00 for one night. After freezing and sacrifice at −20°C for at least 40 min, female mosquitoes were identified to the species level using morphologic characteristics and subsequently stored in liquid nitrogen. The male mosquitoes were discarded.

Virus isolation and identification: All mosquitoes collected from each site were tested for viruses. The identified mosquitoes were removed from liquid nitrogen, grouped into pools by location and species (*Culex tritaeniorhynchus* had a maximum of 150 specimens per pool, other mosquito species had less than 100 specimens per pool), immediately homogenized in minimal essential medium, and centrifuged as reported previously (6,7). The supernatant was inoculated into confluent monolayers of BHK-21 and C6/36 cells and incubated at 37°C and 28°C, respectively, in a 5% CO_2 incubator. Specimens were considered to be positive for virus if

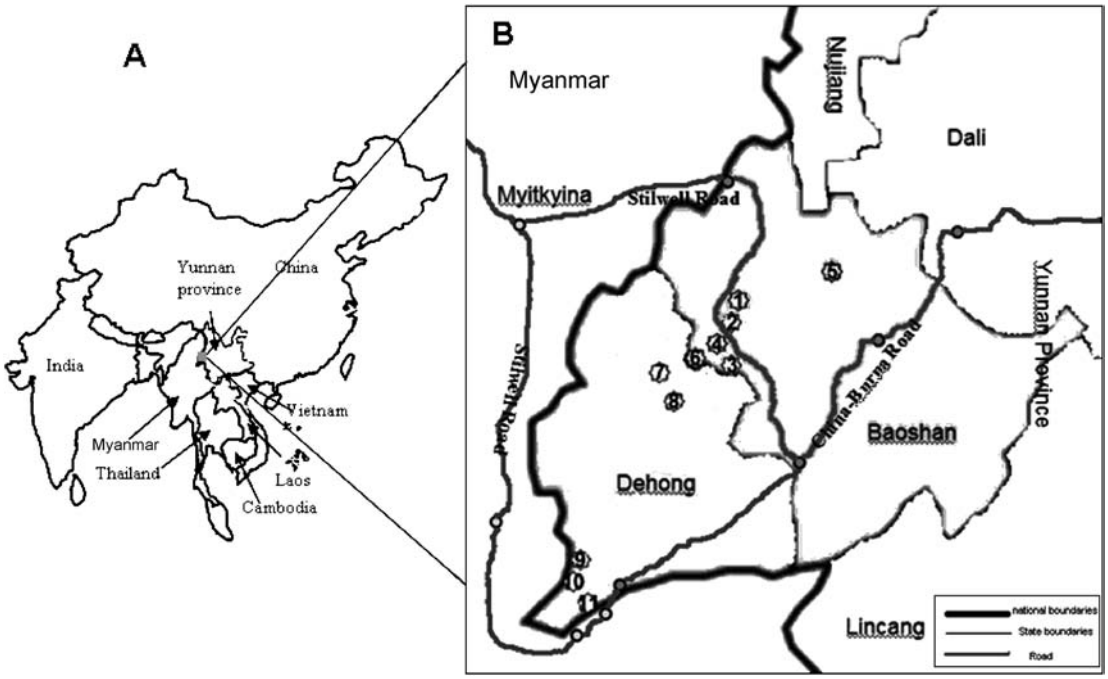

Fig. 1. (A) Map showing location of Yunnan Province in China and counties where mosquitoes have been collected (shown in square). (B) Location of mosquito-collecting sites along the border areas between China and Myanmar. Mosquito-collecting sites: 1, Youdeng village; 2, Dazhuang village; 3, Yongle village; 4, Shilangba village; 5, Hongmu village; 6, Mingtuan village; 7, Hexi village; 8, Zhedao village; 9, Mangbang village; 10, Diesa village; 11, Mengmao village (1–6 sites belong to Tengchong County; 7 and 8 belong to Lianghe County; 9 and 10 belong to Longchuan County; 11 site belong to Ruili City).

Table 1. Identification and sequence of the primers used in this study

Primer	Amplity region	Sequence data (5′→3′)	Site in genome	Size	Reference
Flaviridae					
FU1	NS5	TACCACATGATGGGAAAGAGAGAGAA	8969–8993	310	10
cFD2		GTGTCCCAGCCGGCGGTGTCATCAGC	9258–9282		
JEV					
JE-955F	E	TGYTGGTCGCTCCGGCTTA	955–973	1581	11
JE-2536R		AAGATGCCACTTCCACAYCTC	2516–2536		
Alphavirus					
M2W	NS1	YAGAGCDTTTTCGCAYSTRGCHW	164–186	434	12
cM3W		ACATRAANKGNGTNGTRTCRAANCCDAYCC	568–597		
M2W2		TGYCCNVTGMDNWSYVCNGARGAYCC	288–313		
GETV					
GETVE2F	E2	GTAACAATAGTGCACGCCACC	8479–8517	1400	13
GETVE2R		GGCAGCAGCAAAGCAGGTTC	9899–9918		

F means forward primer; R means reverse primer. M, C/A; W, A/T; Y, C/T; K, G/T; R, G/A; V, G/A/C; D, T/A/G.

they showed a cytopathic effect (CPE) in 3 successive cell passages.

Antigenic testing and reverse-transcription polymerase chain reaction (RT-PCR) were performed to identify the isolates. Immunofluorescence assay (IFA) of infected cells was performed using the following antibodies: flavivirus group specific, alphavirus group specific, bunyavirus group specific; JEV specific, and Getah virus (GETV) specific. All antibodies were prepared in our laboratory (8,9).

Total RNA was extracted from 140 μl of cell culture supernatants using a QIAamp Viral RNA Mini Kit (Qiagen, Valencia, Calif., USA) and first strand cDNA was generated using Ready-To-Go You-Prime First-Strand Beads (Amersham Pharmacia Biotech, Piscataway, N.J., USA). PCR was employed for molecular identification of flavivirus, E fragments of JEV, alphavirus, and E2 fragments of GETV. The primers used for amplification and sequencing are listed in Table 1 (10–13). Sequencing was performed by the genome institute in Beijing.

The sequences referenced in this study were submitted to GenBank and analyzed using Clustal1.8X and MEGA4 in order to carry out multiple sequence alignment and phylogenetic analyses. Phylogenetic trees were constructed a cladogram using the neighbor-joining algorithm with 500 bootstrap replicates.

Minimum infection rate (MIR): The MIR (number of

Table 2. Mosquitoes collected in the China-Myanmar border in the western part of Yunnan Province in China

Mosquito	Location											Total no. (%)
	1 no. (%)	2 no. (%)	3 no. (%)	4 no. (%)	5 no. (%)	6 no. (%)	7 no. (%)	8 no. (%)	9 no. (%)	10 no. (%)	11 no. (%)	
Culex tritaeniorhynchus Giles, 1901	8,600 (70.4)	2,882 (64.9)	26 (10.2)	1,730 (29.7)	871 (52.5)	780 (33.8)	2,900 (70.1)	4,020 (66.3)	4,250 (83.1)	7,360 (91.0)	3,700 (81.0)	37,119 (67.9)
Culex annulus Theobald, 1901	75 (0.6)	69 (1.6)			36 (2.2)	20 (0.9)	118 (2.9)	108 (1.8)	55 (1.1)	272 (3.4)	144 (3.2)	897 (1.6)
Culex pseudovishnui Colless, 1957		102 (2.3)	2 (0.8)	64 (1.1)	38 (2.3)	16 (0.7)		32 (0.5)	13 (0.3)	24 (0.3)		291 (0.5)
Culex theileri Theobald, 1903	25 (0.2)	13 (0.3)	6 (2.4)	235 (4.0)	2 (0.1)							281 (0.5)
Culex fuscanus Wiedemann, 1820	1 (0.0)			1 (0.0)		5 (0.2)	1 (0.0)	3 (0.1)	1 (0.0)			12 (0.0)
Culex bitaeniorhynchus Giles, 1901	7 (0.1)		9 (3.5)	6 (0.1)	6 (0.4)	11 (0.5)	1 (0.0)	1 (0.0)	1 (0.0)	2 (0.0)	5 (0.1)	49 (0.0)
Culex fuscocephala Theobald, 1907		2 (0.1)		1 (0.0)	3 (0.2)	6 (0.3)		6 (0.1)	65 (1.3)	12 (0.2)	143 (3.1)	238 (0.4)
Culex pallidothorax Theobald, 1905			6 (2.4)					1 (0.0)	5 (0.1)			12 (0.0)
Culex halifaxia Theobald, 1903			2 (0.8)			3 (0.1)					3 (0.1)	8 (0.0)
Culex pipiens quinquefasciatus Say, 1832				28 (0.5)	14 (0.8)	15 (0.7)	58 (1.4)	1 (0.0)	7 (0.1)	2 (0.0)	6 (0.1)	131 (0.2)
Culex whitmorei Giles, 1904						2 (0.1)	1 (0.0)				5 (0.1)	8 (0.0)
Culex gelidus Theobald, 1901						2 (0.1)			2 (0.0)		61 (1.3)	65 (0.1)
Anopheles sinensis Wiedemann, 1828	3,500 (28.7)	1,293 (29.1)	180 (70.9)	3,576 (61.4)	639 (38.5)	1,420 (61.5)	1,024 (24.8)	1,876 (30.9)	315 (6.2)	342 (4.2)	5 (0.1)	14,170 (25.9)
Anopheles annularis Van der Wulp, 1884									321 (6.3)			321 (0.6)
Anopheles maculatus Theobald, 1901			2 (0.8)	2 (0.0)					5 (0.1)			9 (0.0)
Anopheles kunmingensis Dong and Wang, 1995			1 (0.4)	39 (0.7)	1 (0.1)							41 (0.1)
Anopheles splendidus Koidzumi, 1920					25 (1.5)				14 (0.3)			39 (0.1)
Anopheles barbirostris Van der Wulp, 1884											23 (0.5)	23 (0.0)
Anopheles tessellatus Theobald, 1901						3 (0.1)	7 (0.2)	1 (0.0)	13 (0.3)	57 (0.7)	10 (0.2)	91 (0.2)
Anopheles minimus Theobald, 1901						3 (0.1)	2 (0.1)		8 (0.2)			13 (0.0)
Anopheles vagus Donitz, 1902								1 (0.0)	13 (0.3)		73 (1.6)	87 (0.2)
Anopheles hyrcanus Pallus, 1771									4 (0.1)			4 (0.0)
Anopheles peditaeniatus Leicester, 1908			2 (0.8)								139 (3.0)	141 (0.3)
Anopheles crawfordi Reid, 1953	2 (0.0)											2 (0.0)
Aedes harveyi Barraud, 1923						1 (0.0)						1 (0.0)
Aedes vexans Meigen, 1830	6 (0.1)	42 (1.0)	3 (1.2)	130 (2.2)	23 (1.4)	2 (0.1)				1 (0.0)	9 (0.2)	216 (0.4)
Aedes albolateralis Theobald, 1910						2 (0.1)						2 (0.0)
Armigeres subalbatus Coquillett, 1898		41 (0.9)	9 (3.5)	10 (0.2)		17 (0.7)	24 (0.6)	14 (0.2)	21 (0.4)	15 (0.2)	243 (5.3)	394 (0.7)
Armigeres durhami Edwards, 1917			6 (2.4)				2 (0.1)					8 (0.0)
Total	12,216 (100.0)	4,444 (100.0)	254 (100.0)	5,822 (100.0)	1,658 (100.0)	2,308 (100.0)	4,138 (100.0)	6,064 (100.0)	5,113 (100.0)	8,087 (100.0)	4,569 (100.0)	54,673 (100.0)
Percent of the sites	(22.3)	(8.1)	(0.5)	(10.7)	(3.0)	(4.2)	(7.6)	(11.1)	(9.4)	(14.8)	(8.4)	(100.0)

The male mosquito was not including.
Mosquito-collecting sites: 1, Youdeng village; 2, Dazhuang village; 3, Yongle village; 4, Shilangba village; 5, Hongmu village; 6, Mingtuan village; 7, Hexi village; 8, Zhedao village; 9, Mangbang village; 10, Diesa village; 11, Mengmao village.

positive pools/total specimens tested $\times 1{,}000$) was calculated for each mosquito species and virus collected over the duration of the project. The MIR is expressed as the number of positive mosquitoes per 1,000 tested and assumes that a positive pool contains only 1 infected mosquito.

RESULTS

Mosquito collection: A total of 54,673 mosquitoes representing 4 genera and 29 species were collected, including 12 species of *Culex*, 12 species of *Anopheles*, 3 species of *Aedes*, and 2 species of *Armigeres*. The predominant species were *Cx. tritaeniorhynchus* (67.9% of the total; 37,119/54,673), and *Anopheles sinensis* (25.9%; 14,170/54,673). *Culex annulus* Theobald comprised 1.6% (897/54,673) of the total. None of the other 26 species evaluated comprised more than 1% of the total collected. The pattern of species distribution was similar in all of the areas sampled (Table 2).

Virus isolation and identification: A total of 22 pools produced CPE in 3 successive cell culture passages. Most of the isolates, which were subsequently identified as JEV, produced CPE after 72 h to 96 h in both BHK-21 and C6/36 cells, as characterized by cell shrinking and shedding. The other 2 viruses (TC07180 and LH07012, which were subsequently identified as GETV) caused shrinking and shedding 24 h to 48 h post infection in BHK-21 cells and shedding in C6/36 cells 24 h post infection.

IFA results showed that 20 of the isolates were JEV (Table 3). None of the isolates reacted with bunyavirus specific antibodies. Phylogenetic analyses comparing 1,500 nucleotides from the JEV E gene with several other strains (Table 1) revealed that the 20 strains of JEV belonged to genotype 1 (Fig. 2, Table 4). The isolates came from *Cx. tritaeniorhynchus* (15 strains), *An. sinensis* (3 strains), and *Armigeres subalbatus* (2 strains) (Table 3).

IFA results also indicated that isolates TC07180 and LH07012 reacted strongly with alphavirus and GETV antibodies. For these 2 isolates, 1,400 nucleoties of the GETV E2 gene were obtained. When compared with several other GETV isolates, phylogenetic analyses of the nucleotides from the GETV E2 gene (Table 5) showed that the 2 strains of newly isolated GETV were closely related to YN0542 and YN0540, which were obtained in China in 2005 (Fig. 3, Table 5). The 2 isolates came from *Culex pseudovishnui* and *Cx. tritaeniorhynchus* (Table 3).

MIR: The MIR of JEV in *Cx. tritaeniorhynchus*, *An. sinensis*, and *Ar. subalbatus* was 0.40/1,000, 0.21/1,000, and 5.08/1,000, respectively. The MIR of GETV in *Cx. tritaeniorhynchus* and *Cx. pseudovishnui* was 0.03/1,000 and 3.44/1,000, respectively (Table 6).

DISCUSSION

Previous surveys on mosquitoes in the southern part of Yunnan Province have shown that *Cx. tritaeniorhynchus*, *An. sinensis*, and *Cx. pseudovishnui* are the primary species found in association with human habitats and livestock pens across the region (14). These mosquitoes feed primarily at night and predominantly on humans, pigs, cattle, and other livestock (14,15). The results of the current study, in which 67.9% of the mosquitoes collected were *Cx. tritaeniorhynchus* and 25.9% were *An. sinensis*, are mostly consistent with the previous observations. However, in our collections, *Cx. pseudovishnui* accounted for less than 1% of the total.

Of the 20 JEV isolates, 15 were recovered from pools of *Cx. tritaeniorhynchus*, which was expected given that this species has long been recognized as the primary vector of JEV in this region (14,15). Our observations ex-

Table 3. Source and identification of the viruses isolated from mosquitoes in this study

Isolate	Species	Location	Collection site	Manner of collecting	Virus isolate
TC07008	*Culex tritaeniorhynchus*	Youdeng village	Pig and cattle pen	UV Light Trap	JEV
TC07011	*Culex tritaeniorhynchus*	Youdeng village	Pig and cattle pen	UV Light Trap	JEV
TC07012	*Culex tritaeniorhynchus*	Youdeng village	Pig and cattle pen	UV Light Trap	JEV
TC07018	*Culex tritaeniorhynchus*	Youdeng village	Pig and cattle pen	UV Light Trap	JEV
TC07020	*Culex tritaeniorhynchus*	Youdeng village	Pig and cattle pen	UV Light Trap	JEV
TC07028	*Culex tritaeniorhynchus*	Youdeng village	Pig and cattle pen	UV Light Trap	JEV
TC07046	*Culex tritaeniorhynchus*	Youdeng village	Pig and cattle pen	UV Light Trap	JEV
TC07099	*Culex tritaeniorhynchus*	Youdeng village	Pig and cattle pen	UV Light Trap	JEV
TC07101	*Culex tritaeniorhynchus*	Youdeng village	Pig and cattle pen	UV Light Trap	JEV
TC07109	*Culex tritaeniorhynchus*	Youdeng village	Pig and cattle pen	UV Light Trap	JEV
TC07111	*Culex tritaeniorhynchus*	Youdeng village	Pig and cattle pen	UV Light Trap	JEV
TC07172	*Culex tritaeniorhynchus*	Dazhuang village	Pig pen	Landing Collection	JEV
TC07177	*Armigeres subalbatus*	Dazhuang village	Pig pen	Landing Collection	JEV
TC07255	*Anopheles sinensis*	Shilangba village	Garden	UV Light Trap	JEV
TC07257	*Armigeres subalbatus*	Shilangba village	Garden	UV Light Trap	JEV
TC07259	*Anopheles sinensis*	Shilangba village	Garden	UV Light Trap	JEV
TC07273	*Culex tritaeniorhynchus*	Hongmu village	Pig and cattle pen	Landing Collection	JEV
TC07290	*Culex tritaeniorhynchus*	Mingtuan village	Pig and cattle pen	UV Light Trap	JEV
TC07292	*Culex tritaeniorhynchus*	Mingtuan village	Pig and cattle pen	UV Light Trap	JEV
TC07295	*Anopheles sinensis*	Mingtuan village	Pig and cattle pen	UV Light Trap	JEV
TC07180	*Culex pseudovishnui*	Dazhuang village	Pig and cattle pen	Landing Collection	GETV
LH07012	*Culex tritaeniorhynchus*	Hexi village	Pig and cattle pen	UV Light Trap	GETV

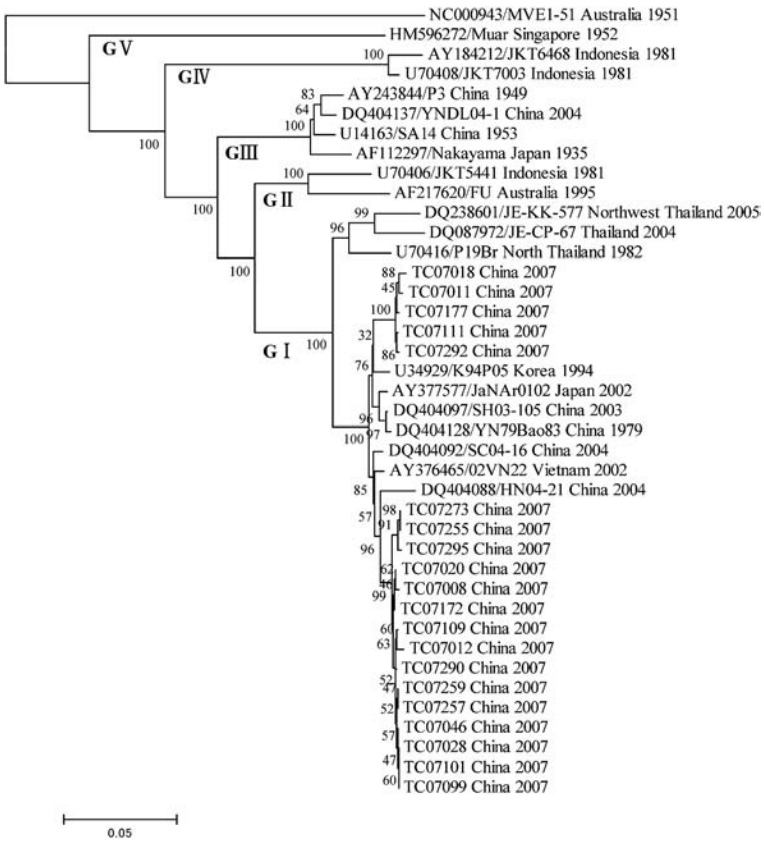

Fig. 2. Phylogenetic analysis of JEV isolates based on E gene sequence. Distances and groupings were determined by the p-distance algorithm and neighbor-joining method with MEGA version 4 software (www.megasoftware.net). Bootstrap values are indicated and correspond to 500 replications. The tree was rooted by using MVE1-51 as the outgroup virus. Scale bars indicate a genetic distance of 0.05-nt substitutions per position.

Table 4. JEV strains used in the phylogenetic analysis

Virus isolate	GenBank accession no.	Year	Location	Source	Genotype
JaNAr0102	AY377577	2002	Japan	Pig blood	I
K94P05	U34929	1994	Korea	Mosquito	I
P19Br	U70416	1982	North Thailand	Human brain	I
JE-KK-577	DQ238601	2005	Northwest Thailand	Pig	I
JE-CP-67	DQ087972	2004	Thailand	Pig	I
02VN22	AY376465	2002	Vietnam	Pig blood	I
HN04-21	DQ404088	2004	China	*Culex*	I
SH03-105	DQ404097	2003	China	*Culex tritaeniorhynchus*	I
SC04-16	DQ404092	2004	China	*Armigeres*	I
YN79-Bao83	DQ404128	1979	China	*Culex tritaeniorhynchus*	I
FU	AF217620	1995	Australia	Human serum	II
JKT5441	U70406	1981	Indonesia	Mosquito	II
P3	AY243844	1949	China	Mosquito	III
Nakayama	AF112297	1935	Japan	Human brain	III
SA14	U14163	1953	China	*Culex pipiens*	III
YNDL04-1	DQ404137	2004	China	*Culex tritaeniorhynchus*	III
JKT7003	U70408	1981	Indonesia	Mosquito	IV
JKT6468	AY184212	1981	Indonesia	*Culex tritaeniorhynchus*	IV
Muar	HM596272	1952	Singapore	Human brain	V
MVE1-51	NC-000943	1951	Australia	Human brain	—

pand the distribution of JEV association with *An. sinensis*, which is the predominant species in Tengchong, southern Yunnan Province. Interestingly, we isolated JEV from 2 pools of *Ar. subalbatus*, despite this species representing only approximately 1% of the total mosquito collection. Previously, 2 strains of JEV were isolated from *Ar. subalbatus* in Eryuan County, Dali City, China. However, numerous other attempts to isolate virus from mosquitoes in the region over 6-year period have been unsuccessful in detecting JEV in this

Table 5. GETV strains used in the phylogenetic analysis

Virus isolates	GenBank accession no.	Year	Location	Source
LEIV-16275-Mag	EF631998	2000	Russia	*Aedes* spp.
LEIV-17741-MPR	EF631999	2000	Mongolia	*Culex* spp.
GETV-MM2021	AF339484	1955	Malaysia	*Culex gelidus*
GETV-South Korea	AY702913	2004	South Korea	Swine
Sagiyama-virus	AF339483	1956	Japan	Mosquito
strain-M1	EU015061	1964	China	Mosquito
HB0234	EU015062	2002	China	*Culex tritaeniorhynchus*
HB0215-3	EU015065	2002	China	*Culex tritaeniorhynchus*
YN0540	EU015063	2005	China	*Armigeres subalbatus*
YN0542	EU015064	2005	China	*Armigeres subalbatus*
SH05-6	EU015066	2005	China	*Culex tritaeniorhynchus*
SH05-15	EU015067	2005	China	*Culex tritaeniorhynchus*
SH05-16	EU015068	2005	China	*Culex tritaeniorhynchus*
SH05-17	EU015069	2005	China	*Culex tritaeniorhynchus*
GS10-2	EU015070	2006	China	*Armigeres subalbatus*
Chikungunya	GU562830	2009	India	*Aedes albopictus*

Table 6. Minimum infection rate of JEV and GETV in mosquitoes in this study

	JEV			GETV		
	Specimen	Positive pool	MIR[1)]	Specimen	Positive pool	MIR[1)]
Culex tritaeniorhynchus	37119	15	0.40/1000	37119	1	0.03/1000
Culex pseudovishnui	—	—	—	291	1	3.44/1000
Anopheles sinensis	14170	3	0.21/1000	—	—	—
Armigeres subalbatus	394	2	5.08/1000	—	—	—

[1)]: Minimum infection rate (MIR) expressed as number infected/1,000 tested.

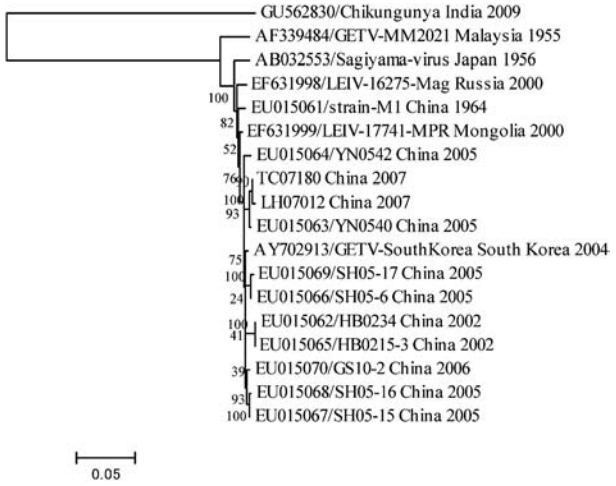

Fig. 3. Phylogenetic analysis of GETV isolates based on E2 gene sequence. Distances and groupings were determined by the p-distance algorithm and neighbor-joining method with MEGA version 4 software (www. megasoftware.net). Bootstrap values are indicated and correspond to 500 replications. The tree was rooted by using Chikungunya as the outgroup virus. Scale bars indicate a genetic distance of 0.05-nt substitutions per position.

species (15). JEV has also been isolated from *Ar. subalbatus* in Taiwan, in a study that also verified the competence of this species to serve as a JE vector (16). Our observation represents the first isolation of JEV from *Ar. subulbatus* in southern Yunnan Province, China.

This area is characterized by abundant rainfall and perennial rice planting. These conditions provide a suitable habitat for mosquito breeding. In addition, nearly every family resides near their paddy fields and keeps livestock, such as pigs and cattle, close to their lodging. We found a relatively high abundance of JEV-infected *Cx. tritaeniorhynchus*, which is not unexpected in this area. We also noted the presence of a high JEV infection rate in *Ar. subalbatus*, suggesting that it may play a role in local JEV transmission. Economic development, an increasing acreage of irrigated rice, and extensive pig rearing have combined to create a serious threat to public health.

GETV was first isolated from *Culex* mosquitoes collected in Malaysia and is widely distributed in Southeast and East Asia. It can cause disease in livestock but there are no reports indicating that GETV is associated with human diseases (17–20). GETV has been isolated from mosquitoes collected in the southern, northern, southwestern, and northwestern parts of China in recent years, demonstrating that the virus is widespread in China (4,13,21,22). In 2005, GETV was isolated from *An. sinensis* and *Ar. subalbatus* collected from the northwestern part of Yunnan Province (4,13). In the current investigation, GETV was isolated from *Cx. tritaeniorhynchus* and *Cx. pseudovishnui*, suggesting that these species may play a role in transmitting GETV in the western part of Yunnan Province.

In summary, this is the first study to report results from mosquito collections and arbovirus assays conducted in the China-Myanmar border areas of Yunnan Province. The results indicate that important vector spe-

cies such as *Cx. tritaeniorhynchus* are common across the area, and that JEV is frequently found in these mosquitoes. The isolation of GETV in human-biting mosquitoes suggests that *Cx. tritaeniorhynchus* may be transmitting this virus to humans in the region.

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Conflict of interest None to declare.

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