

## Molecular Detection of *Rickettsia felis* and *Rickettsia bellii* in Mosquitoes

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### Abstract

To add to the limited information on *Rickettsia* in mosquitoes in China, we carried out a PCR survey on convenience samples of 3051 mosquitoes collected with hand nets in and around domestic dwellings in 25 provinces. Five species of mosquitoes were identified: *Culex pipiens pallens* ( $n = 1620$ ), *Aedes albopictus* (806), *Armigeres subalbatus* (377), *Anopheles sinensis* (168), and *Culex tritaeniorhynchus* (80). A *Rickettsia* nested-PCR targeting the variable domain of *gltA* showed *Rickettsia felis* in four mosquito species of 16 provinces *Cx. pipiens pallens* (1.8%, 29/1620); *Ae. albopictus* (1.2%, 10/806); *An. sinensis* (1.2%, 2/168); and *Ar. subalbatus* (2.1%, 8/377). *Rickettsia bellii* was also widespread, occurring in 12 provinces and 2 species: *Cx. pipiens pallens* (4.3%, 69/1620) and *An. sinensis* (0.6%, 1/168). *R. felis* and *R. bellii* were found in almost similar numbers in female [1.5% (27/1809) and 1.2% (21/1809), respectively] as in male mosquitoes [1.8% (22/1242) and 4.0% (49/1242), respectively]. Our results indicated that mosquitoes in China are widely infected with *R. felis*, the agent of human flea-borne spotted fever, and that *R. bellii* can also occur outside of the Americas and its usual tick hosts.

**Keywords:** *Rickettsia felis*, *Rickettsia bellii*, PCR, mosquito, China

### Introduction

THE GENUS *RICKETTSIA* comprises gram-negative obligate intracellular  $\alpha$ -proteobacteria that have been divided into four clades or groups based on phylogenetic analysis (Gillespie et al. 2007, 2008). The spotted fever group and typhus groups are important human pathogens worldwide that are principally transmitted by ixodid ticks, and fleas and lice, respectively. The major clinical signs of infection include fever, rash, headache, chills, myalgia, and malaise (Blanton and Walker 2017, Carvalho et al. 2017). Some *Rickettsia*, for example, *Rickettsia africae*, cause mild or subclinical disease while other species, such as *Rickettsia prowazekii* and *Rickettsia rickettsii*, can cause severe disease and high mortality (Mediannikov et al. 2013, Blanton and Walker 2017). The *Rickettsia* have been divided into four clades or groups based on phylogenetic analysis (Gillespie et al. 2007, 2008). The

transitional group, represented by *Rickettsia felis*, and the ancestral group, represented by *Rickettsia bellii*, are most widely reported to be transmitted by fleas and a wide range of tick species in the Americas, respectively (Krawczak et al. 2018).

Since 1982, at least 10 recognized and uncharacterized species of spotted fever group rickettsiae have been identified in China (Fang et al. 2015). One of the more recently described, *R. felis*, is the agent of flea-borne spotted fever in people. Although originally associated with the cat flea (*Ctenocephalides felis*) (Blanton and Walker 2017), it has now been found to occur in a variety of mosquitoes: *Aedes* spp., *Anopheles* spp., and *Mansonia uniformis* in Africa (Socolovschi et al. 2012a, 2012b, Mediannikov et al. 2013) and *Anopheles sinensis* and *Culex pipiens* in China (Zhang et al. 2014). Furthermore, it has been shown that *Anopheles gambiae* can be infected with *R. felis* and can transmit the

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organism during feeding, indicating it to be a potential vector (Dieme et al. 2015). Finally, novel *Rickettsia* spp. have been found in *An. gambiae* and *Anopheles melas* in Africa (Socolovschi et al. 2012a), *Cx. pipiens* (Zhang et al. 2014) and *An. sinensis* and *Culex tritaeniorhynchus* (Guo et al. 2016) in China, and *M. uniformis*, *C. pipiens*, and *Aedes esoensis* in Korea (Maina et al. 2017).

In previous studies, we have reported *Rickettsia* sp. in mosquitoes from Yangzhou, China (Zhang et al. 2014, 2016). To add to the available data on *Rickettsia* in different mosquito species in China, we collected samples of mosquitoes from 25 provinces and tested them for *Rickettsia* spp. by PCR.

## Materials and Methods

### Sample collection

Between July and September 2014, student volunteers from Yangzhou University used hand nets to collect convenience samples of mosquitoes in their primary homes located in 26 cities in 25 provinces or municipalities in China. Mosquitoes were immediately placed individually in sterile tubes containing 400  $\mu$ L DNA/RNA stabilization reagent for blood/bone marrow (Roche Molecular Biochemicals, Indianapolis, IN). Then, samples were transported to Yangzhou University College of Veterinary Medicine at room temperature, where they were stored at  $-80^{\circ}\text{C}$  until DNA extraction.

### DNA extraction

Each mosquito was thawed at room temperature and homogenized individually in a shaker (Bertin Technologies, France) with four 3.0 mm ceramic beads for two short durations of 15 s (3160 g with a 15-s break in between) each. The High-Pure PCR Template Preparation Kit (Roche Molecular Biochemicals) was used to extract total nucleic acids in 100  $\mu$ L elution buffer according to the manufacturer's instructions and as described before (Wei et al. 2014, Zhang et al. 2014).

### Mosquito identification

The species and gender of the mosquitoes were determined using standard morphological criteria (Harbach 2013). Species identification was further confirmed with PCR and gene sequencing by using the primers (LCO1490: 5'-GGTCAA CAAATCATAAAGATATTGG-3', and HCO2198: 5'-TAAA CTTCAGGGTGACCAAAAAATCA-3') targeting the mitochondrial cytochrome c oxidase subunit I gene (*COI*) as described earlier (Folmer et al. 1994, Wang et al. 2012).

### Identification of *Rickettsia* spp. in mosquitoes

A previously validated *gltA*-based FRET-qPCR (Zhang et al. 2014) was used to detect *Rickettsia* spp. in the individual mosquitoes. Samples positive for *Rickettsia* spp. based on the FRET-qPCR and high-resolution melting curve analysis were further amplified with primers of a nested-PCR targeting the *gltA* gene as described previously (Zhang et al. 2014) and subsequent sequencing of amplicons (BGI, Shanghai, China). Identification of *Rickettsia* spp. using nested-PCR targeting the *gltA* has been shown to be as reliable as identification based on MLST (*gltA*, *ompA*, *ompB*, and *sca4* genes) (Zhang et al. 2014, 2016). The PCR amplicon of

*R. felis* identified previously (Zhang et al. 2014) served as the positive control, while sterile water was used as the negative control.

### Phylogenetic analysis

The retrieved *Rickettsia* sequences of the present study and the reference sequences from GenBank were aligned using the MEGA 6.0 software (Fig. 2). Based on these alignments, phylogenetic trees were constructed by the neighbor-joining method using the Kimura 2-parameter model with MEGA 6.0. Bootstrap values were calculated using 500 replicates (Zhang et al. 2018).

### Statistical analysis

Chi-squared test was used to compare the prevalence of *Rickettsia* spp. between different mosquito species and in different provinces of China. A value of  $p < 0.05$  was considered to be significant.

## Results

### Mosquito species identified

Of the 3051 mosquitoes captured (Fig. 1), 59.3% (1809/3051) were female and 40.7% (1242/3051) were male (Table 1). Five mosquito species were identified using morphological criteria and PCR followed by DNA sequencing: *Aedes albopictus* ( $n=806$ , 26.4%), *An. sinensis* ( $n=168$ , 5.5%), *Armigeres subalbatus* ( $n=377$ , 12.4%), *Culex pipiens pallens* ( $n=1620$ , 53.1%), and *Culex tritaeniorhynchus* ( $n=80$ , 2.6%). The *Culex* spp. ( $n=1700$ ) were found in all 26 cities studied, except for Zhuzhou in Hunan province (Table 1 and Fig. 1). *Culex tritaeniorhynchus* was only identified in Guizhou province in the southwest of China (Fig. 1).

### *Rickettsia* spp. in mosquitoes

Overall, 3.9% (119/3051) of the mosquitoes were found positive in the *Rickettsia* FRET-qPCR. Positive mosquitoes were found in all but four provinces (21/25), including Hebei, Liaoning, Jiangxi, and Fujian. The prevalences of *Rickettsia* spp. in mosquitoes from Heilongjiang (24.6%, 27/110), Shanghai (15.8%, 19/120), and Beijing (16.8%, 20/119) were significantly higher than the other provinces (0% of 0/451 to 5.2% of 6/116;  $p < 0.01$ ). Significantly higher numbers of *Cx. pipiens pallens* (6.1%, 98/1620) were found positive when compared with the other species: *Ae. albopictus* (1.2%, 10/806), *An. sinensis* (1.8%, 3/168), *Ar. subalbatus* (2.1%, 8/377), and *Cx. tritaeniorhynchus* (0%, 0/80) ( $p < 0.05$ ). Also, significantly more male (5.7%, 71/1242) than female (2.7%, 48/1809) mosquitoes were positive for *Rickettsia* spp. (Table 1) ( $p < 0.01$ ).

Sequencing of the amplicons of the PCR targeting the variable domain of the *gltA* indicated that there were two *Rickettsia* spp. in the mosquitoes: *R. felis* and *R. bellii*. The *gltA* sequences of all the *R. felis* species were identical to one another (given the GenBank accession number MK061744) and a reference *R. felis* strain from Brazil (KX446943). There was only one nucleotide mismatch with the *R. felis* previously identified from China (KJ440519) (Fig. 2). Similarly, all the *R. bellii* sequences were identical to one another (given the



**FIG. 1.** Mosquitoes collected in and around domestic dwellings in China. The mosquito samples ( $n = 3051$ ) were collected from 25 provinces/municipalities in northern, southern, and eastern China. Five species of mosquito were collected, mainly *Anopheles sinensis*, *Culex pipiens pallens*, *Aedes albopictus*, *Armigeres subalbatus*, and *Culex tritaeniorhynchus*. Color images are available online.

GenBank accession number MK697586) and to reference strains in GenBank from China and South Korea (KU586331 and KY799071).

The *R. bellii* species were found more commonly (2.3%, 70/3051) than *R. felis* (1.6%, 49/3051). In fact, *R. felis* was found in four mosquito species (*Cx. pipiens pallens*, 1.8%, 29/1620; *Ae. albopictus*, 1.2%, 10/806; *An. sinensis*, 1.2%, 2/168; and *Ar. subalbatus*, 2.1%, 8/377), while *R. bellii* was only detected in *Cx. pipiens pallens* (4.3%, 69/1620) and *An. sinensis* (0.6%, 1/168). *R. felis* appeared more widespread, being present in 16 provinces, while *R. bellii* was identified in only 12 provinces. Similar numbers of female (*R. felis*: 1.5%, 27/1809; *R. bellii*: 1.2%, 21/1809) and male mosquitoes (*R. felis*: 1.8%, 22/1242; *R. bellii*: 4.0%, 49/1242) were positive for each organism. Any concurrent infections were not detected.

## Discussion

There are around 41 genera and 3500 species and subspecies of mosquitoes worldwide with at least 390 species

occurring in China and a few new species still not being identified (Wang et al. 2012, Guo et al. 2018). All five mosquito species collected in our study feed on humans. Some of the mosquitoes also feed on other animals, such as *Cx. pipiens pallens* on birds, *An. Sinensis* on cattle and buffalo, and *Cx. Tritaeniorhynchus* on pigs and cattle (Wang et al. 2012). Their capture in our study, then, might have been as a result of livestock being kept in the vicinity of the students' houses. The species most commonly collected, *Cx. pipiens pallens* followed by *Ae. albopictus*, were also found in most of the provinces studied, which confirms previous reports on these species' high prevalence and widespread distribution in China (Cao et al. 2011, Wang et al. 2011, Feng et al. 2012, Guo et al. 2014, Huang et al. 2015).

Previously, we described *R. felis* in mosquitoes from Jiangsu Province in China (Zhang et al. 2014). In our study, we show that the organism is very widespread in China, occurring in 16 of 25 provinces. To date, *R. felis* has been recovered from *An. sinensis* and *Cx. pipiens pallens* in China (Zhang et al. 2014) and *An. gambiae* (Socolovschi et al.

TABLE 1. POSITIVITY OF RICKETTSIA IN MOSQUITOES COLLECTED IN THIS STUDY

Province	City	Species	Gender	No.	Total	<i>Rickettsia felis</i>	<i>Rickettsia bellii</i>
Zhejiang	Wenzhou	<i>An. sinensis</i>	F	24			
			M	70	1		
		<i>Cx. p. pallens</i>	F	3			
			M	11			1
			<i>Ae. albopictus</i>	F	8		
M	4						
Shanghai	Shanghai	<i>Cx. p. pallens</i>	F	80	11	6	5
			M	37	7	5	2
		<i>An. sinensis</i>	F	3	1	1	
Anhui	Fuyang	<i>Cx. p. pallens</i>	F	54			
			M	63	1		1
		<i>Ae. albopictus</i>	F	2			
Hebei	Shijiazhuang	<i>Cx. p. pallens</i>	F	6			
			M	2			
		<i>Ae. albopictus</i>	F	57			
			M	61			
Yunnan	Yiwei	<i>Cx. p. pallens</i>	F	27			
			M	94	4		4
		<i>An. sinensis</i>	F	63	8		8
M	54		12	1	11		
Guangdong	Zhanjiang	<i>Ae. albopictus</i>	F	10			
			M	3			
		<i>Cx. p. pallens</i>	F	68			
			M	3			
			<i>An. sinensis</i>	F	5	1	1
Henan	Anyang	<i>Cx. p. pallens</i>	F	35			
			M	50	2	2	
Liaoning	Jinzhou	<i>Ae. albopictus</i>	F	29	1	1	
			M	4			
		<i>An. sinensis</i>	F	7			
			M	4			
			F	6			
		<i>Cx. p. pallens</i>	F	3			
			M	29			
F	34						
M	36						
Gansu	Jingyuan	<i>Cx. p. pallens</i>	F	23	3	3	
			M	59			
Sichuan	Chengdu	<i>Ae. albopictus</i>	F	14			
			M	57			
		<i>Cx. p. pallens</i>	F	58	2	2	
Shandong	Heze	<i>Ae. albopictus</i>	F	4			
			M	55			
		<i>Cx. p. pallens</i>	F	58			
			M	6			
	Liaocheng	<i>Cx. p. pallens</i>	F	2			
			M	50	2		2
			<i>Ae. albopictus</i>	F	63	3	2
Jilin	Changchun	<i>Ae. albopictus</i>	F	4			
			M	1			
		<i>Cx. p. pallens</i>	F	3			
			M	115	2		2
<i>Ae. albopictus</i>	F	1					
	M	5					

(continued)

TABLE 1. (CONTINUED)

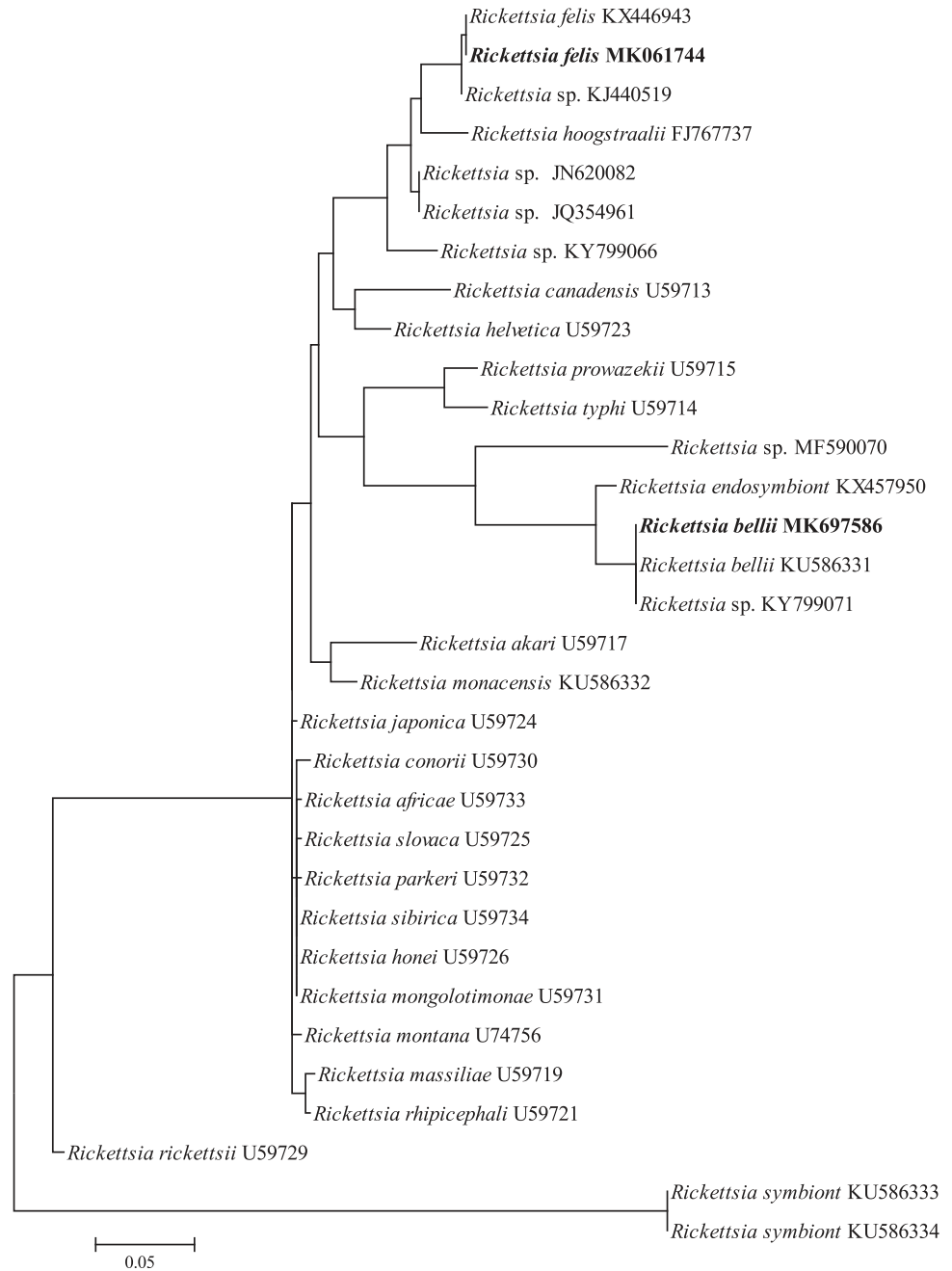
Province	City	Species	Gender	No.	Total	<i>Rickettsia felis</i>	<i>Rickettsia bellii</i>
Jiangxi	Xingan	<i>Ae. albopictus</i>	F	23			
			M	29			
		<i>Cx. p. pallens</i>	F	13			
			M	20			
			<i>An. sinensis</i>	F	7		
		M	10				
Chongqing	Chongqing	<i>Ar. subalbatus</i>	F	42	2	2	
			M	65	3	3	
		<i>Ae. albopictus</i>	F	10			
			M	3			
Hunan	Zhuzhou	<i>Ae. albopictus</i>	F	115	2	2	
			M	2			
		<i>Ar. subalbatus</i>	F	2			
Hubei	Wuhan	<i>Ar. subalbatus</i>	F	34			
			M	20			
		<i>Cx. p. pallens</i>	F	21			
			M	21	3	1	2
			<i>Ae. albopictus</i>	F	10		
		M	3				
Shaanxi	Changzhi	<i>Cx. p. pallens</i>	F	99	1	1	
			M	9			
		<i>Ae. albopictus</i>	F	3			
Guangxi	Nanning	<i>Ae. albopictus</i>	F	90	3	3	
			M	18	2	2	
		<i>Cx. p. pallens</i>	F	10			
			M	2			
Tianjin	Tianjin	<i>Cx. p. pallens</i>	F	83	6	5	1
			M	6			
		<i>Ae. albopictus</i>	F	20			
			M	7			
Shanxi	Yanan	<i>Cx. p. pallens</i>	F	17	1	1	
			M	97	2		2
		<i>Ae. albopictus</i>	F	1			
Fujian	Longyan	<i>Ar. subalbatus</i>	F	77			
			M	38			
		<i>Cx. p. pallens</i>	F	1			
			M	1			
			<i>Ae. albopictus</i>	F	1		
		<i>An. sinensis</i>	F	2			
Guizhou	Liupanshui	<i>Cx. tritaeniorhynchus</i>	F	70			
			M	10			
		<i>Ar. subalbatus</i>	F	18			
			M	9	3	3	
		<i>An. sinensis</i>	F	5			
Heilongjiang	Haerbin	<i>Cx. p. pallens</i>	F	45	2		2
			M	62	25		25
		<i>An. sinensis</i>	F	3			
Jiangsu	Yangzhou	<i>Cx. p. pallens</i>	F	51	2	1	1
			M	40	1	1	
		<i>Ae. albopictus</i>	F	27			
			M	20			

*Cx. p. pallens*: *Culex pipiens pallens*; *Cx. tritaeniorhynchus*: *Culex tritaeniorhynchus*; *An. sinensis*: *Anopheles sinensis*; *Ar. subalbatus*: *Aedes albopictus*; *Ar. subalbatus*: *Armigeres subalbatus*.

2012a) and *Ae. albopictus* in Africa (Socolovschi et al. 2012b). Our study has confirmed the presence of *R. felis* in *Cx. pipiens pallens*, *An. Sinensis*, and *Ae. albopictus* and has shown that the organism can also occur in *Ar. subalbatus*, a species found widely in China, Japan, and Southeast Asia

(Chaves et al. 2015). The females feed primarily on people and are the natural vectors of filarial worms (Muslim et al. 2013). Our findings that *R. felis* occurs widely in China in an increasing number of mosquito species that are known to feed on people should alert health workers to the possibility of

**FIG. 2.** Molecular phylogenetic analysis of *Rickettsia felis* and *Rickettsia bellii* identified in this study. Distances and groupings of *R. felis* and *R. bellii* detected from the mosquitoes in this study (*R. felis* MK061744 from China; *R. bellii* MK 697586 from China) (**bold font**) and reference *Rickettsia* sequences from NCBI (*R. felis* KX446943 from Caratinga of Brazil; *R. bellii* KU586331 from Beijing of China; *Rickettsia* sp. KY799071 from Gyeonggi of South Korea) were determined by applying the neighbor-joining method to a matrix of pairwise distances estimated using the MCL approach with MEGA version 6 software based on the *gltA* gene (443 bp). Scale bar indicates a genetic distance of 0.05-nt substitutions per position. MCL, maximum composite likelihood.



mosquito-transmitted infections in their patients. However, there are only limited data on the transmission of *R. felis* by mosquitoes (Dieme et al. 2015, Legendre and Macaluso 2017),

Previously, evidence has been provided for vertical transmission of *R. felis* in mosquitoes: the organism was recovered in the ovaries of experimentally infected *An. gambiae* (Dieme et al. 2015) as well as in a male *An. arabiensis* (Mediannikov et al. 2013). In each mosquito species found to be infected, similar numbers of males and females were positive for *R. felis*, overall 1.5% (27/1809) and 1.8% (22/1242), respectively. As male mosquitoes do not take blood meals (Nikbakhtzadeh et al. 2016), they most likely become infected by vertical transmission. Our data then provide further support for transovarial transmission of *R. felis* in mosquitoes (Dieme et al. 2015) and indicate this occurs

across a wide range of mosquito species. In vivo infection studies are required to determine the roles of different mosquito species and hosts in the epidemiology of *R. felis* in Asia and its transmission to people.

Until recently, *R. bellii* has been considered to occur in a basal group of rickettsiae found in a wide range of hard and soft ticks in the Americas (Krawczak et al. 2018). Recent studies, however, have identified *R. bellii* or closely related organisms in ticks from Thailand (Sumrandee et al. 2016) and China (Song et al. 2018), *An. sinensis* in Zhejiang, China (Guo et al. 2016), and pools of *Cx. pipiens* from Korea (Maina et al. 2017). Our finding of *R. bellii* in mosquitoes from 10 provinces in China indicates that the organisms are probably widespread in mosquitoes in the region. As with *R. felis*, we found almost similar numbers of male and female *C. pipiens pallens* infected with *R. bellii* (1.2%, 21/1809;

4.0%, 48/1242, respectively), and hence, this organism might also be transmitted transovarially. Further studies are indicated to more precisely determine the epidemiology of *R. bellii* in Asia and its significance in terms of animal and human health.

### Conclusions

The present study revealed the presence of five common mosquito species, *Culex pipiens pallens*, *Aedes albopictus*, *Armigeres subalbatus*, *Anopheles sinensis*, and *Culex tritaeniorhynchus*, in and around domestic dwellings in 25 provinces of China. Both male and female mosquitoes from multiple provinces were found infected with *R. felis* or *R. bellii* indicating that the organisms are likely to be transmitted transovarially.

### Acknowledgments

This project was supported by a grant from the National Natural Science Foundation of China (no: 31472225) and the Priority Academic Program Development of Jiangsu Higher Education Institutions, Yangzhou, Jiangsu, P. R. China, and by the Alabama Agricultural Experimental Station and the USDA National Institute of Food and Agriculture, Hatch project (ALA052-1-17026).

### Authors' Contribution Statement

C.W. and J.Z. designed the research. J.Z. and G.L. collected the samples. J.Z., J.L., M.L., J.W., K.H., H.Q., J.Y., R.Z., Y.W., and Y.Z. performed the research. J.Z. and C.W. analyzed the data. J.Z., C.W., P.K., and H.W. prepared the article.

### Author Disclosure Statement

No competing financial interests exist.

### References

- Blanton LS, Walker DH. Flea-Borne Rickettsioses and Rickettsiae. *Am J Trop Med Hyg* 2017; 96:53–56.
- Cao Y, Fu S, Tian Z, Lu Z, et al. Distribution of mosquitoes and mosquito-borne arboviruses in Inner Mongolia, China. *Vector Borne Zoonotic Dis* 2011; 11:1577–1581.
- Carvalho R, Vazquez D, Silveira P, Lencastre L. Rickettsiosis: A rare challenge in ICU. *Intensive Care Med* 2017; 43:1514–1515.
- Chaves LF, Imanishi N, Hoshi T. Population dynamics of *Armigeres subalbatus* (Diptera: Culicidae) across a temperate altitudinal gradient. *Bull Entomol Res* 2015; 105: 589–597.
- Dieme C, Bechah Y, Socolovschi C, Audoly G, et al. Transmission potential of *Rickettsia felis* infection by *Anopheles gambiae* mosquitoes. *Proc Natl Acad Sci U S A* 2015; 112: 8088–8093.
- Fang LQ, Liu K, Li XL, Liang S, et al. Emerging tick-borne infections in mainland China: An increasing public health threat. *Lancet Infect Dis* 2015; 15:1467–1479.
- Feng Y, Fu S, Zhang H, Li M, et al. Distribution of mosquitoes and mosquito-borne viruses along the China-Myanmar border in Yunnan Province. *Jpn J Infect Dis* 2012; 65:215–221.
- Folmer O, Black M, Hoeh W, Lutz R, et al. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol* 1994; 3:294–299.
- Gillespie JJ, Beier MS, Rahman MS, Ammerman NC, et al. Plasmids and rickettsial evolution: Insight from *Rickettsia felis*. *PLoS One* 2007; 2:e266.
- Gillespie JJ, Williams K, Shukla M, Snyder EE, et al. *Rickettsia* phylogenomics: Unwinding the intricacies of obligate intracellular life. *PLoS One* 2008; 3:e2018.
- Guo WP, Tian JH, Lin XD, Ni XB, et al. Extensive genetic diversity of *Rickettsiales* bacteria in multiple mosquito species. *Sci Rep* 2016; 6:38770.
- Guo XX, Li CX, Wang G, Zheng Z, et al. Host feeding patterns of mosquitoes in a rural malaria-endemic region in hainan island, china. *J Am Mosq Control Assoc* 2014; 30:309–311.
- Guo Y, Song Z, Luo L, Wang Q, et al. Molecular evidence for new sympatric cryptic species of *Aedes albopictus* (Diptera: Culicidae) in China: A new threat from *Aedes albopictus* subgroup? *Parasit Vectors* 2018; 11:228.
- Harbach, RE. Mosquito Taxonomic Inventory. 2013. <http://mosquito-taxonomic-inventory.info/> Accessed February 12, 2019.
- Huang JX, Xia ZG, Zhou SS, Pu XJ, et al. Spatio-temporal analysis of malaria vectors in national malaria surveillance sites in China. *Parasit Vectors* 2015; 8:146.
- Krawczak FS, Labruna MB, Hecht JA, Paddock CD, et al. Genotypic Characterization of *Rickettsia bellii* reveals distinct lineages in the United States and South America. *Biomed Res Int* 2018; 2018:8505483.
- Legendre KP, Macaluso KR. *Rickettsia felis*: A Review of Transmission mechanisms of an emerging Pathogen. *Trop Med Infect Dis* 2017; 2:64.
- Maina AN, Klein TA, Kim HC, Chong ST, et al. Molecular characterization of novel mosquito-borne *Rickettsia* spp. from mosquitoes collected at the Demilitarized Zone of the Republic of Korea. *PLoS One* 2017; 12:e0188327.
- Mediannikov O, Socolovschi C, Edouard S, Fenollar F, et al. Common epidemiology of *Rickettsia felis* infection and malaria, Africa. *Emerg Infect Dis* 2013; 19:1775–1783.
- Muslim A, Fong MY, Mahmud R, Lau YL, et al. *Armigeres subalbatus* incriminated as a vector of zoonotic *Brugia pahangi* filariasis in suburban Kuala Lumpur, Peninsular Malaysia. *Parasit Vectors* 2013; 6:219.
- Nikbakhtzadeh MR, Buss GK, Leal WS. Toxic effect of blood feeding in male mosquitoes. *Front Physiol* 2016; 7:4.
- Socolovschi C, Pages F, Ndiath MO, Ratmanov P, et al. *Rickettsia* species in African *Anopheles* mosquitoes. *PLoS One* 2012a; 7:e48254.
- Socolovschi C, Pages F, Raoult D. *Rickettsia felis* in *Aedes albopictus* mosquitoes, Libreville, Gabon. *Emerg Infect Dis* 2012b; 18:1687–1689.
- Song S, Chen C, Yang M, Zhao S, et al. Diversity of *Rickettsia* species in border regions of northwestern China. *Parasit Vectors* 2018; 11:634.
- Sumrandee C, Baimai V, Trinachartvanit W, Ahantarig A. Molecular detection of *Rickettsia*, *Anaplasma*, *Coxiella* and *Francisella* bacteria in ticks collected from *Artiodactyla* in Thailand. *Ticks Tick Borne Dis* 2016; 7:678–689.
- Wang G, Li C, Guo X, Xing D, et al. Identifying the main mosquito species in China based on DNA barcoding. *PLoS One* 2012; 7:e47051.
- Wang J, Zhang H, Sun X, Fu S, et al. Distribution of mosquitoes and mosquito-borne arboviruses in Yunnan Province near the

- China-Myanmar-Laos border. *Am J Trop Med Hyg* 2011; 84: 738–746.
- Wei L, Kelly P, Zhang J, Yang Y, et al. Use of a universal hydroxymethylbilane synthase (HMBS)-based PCR as an endogenous internal control and to enable typing of mammalian DNAs. *Appl Microbiol Biotechnol* 2014; 98:5579–5587.
- Zhang J, John Kelly P, Lu G, Cruz-Martinez L, et al. *Rickettsia* in mosquitoes, Yangzhou, China. *Emerg Microbes Infect* 2016; 5:e108.
- Zhang J, Lu G, Kelly P, Zhang Z, et al. First report of *Rickettsia felis* in China. *BMC Infect Dis* 2014; 14:682.
- Zhang J, Wang J, Chen L, Yassin AK, et al. Housefly (*Musca domestica*) and Blow Fly (*Protophormia terraenovae*) as vectors of bacteria carrying colistin resistance genes. *Appl Environ Microbiol* 2018; 84:e01736-17.

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