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The double-mutation (M918I + L1014F) *kdr* allele is fixed in *Cimex hemipterus* populations in Guangxi, China

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Research Paper

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Abbreviations:
COI: cytochrome c oxidase subunit I; *kdr*: knock down resistance; PCR: polymerase chain reaction; VGSC: voltage-gated sodium channel

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Abstract

Four putative knockdown resistance (*kdr*) mutations have been documented in the voltage-gated sodium channel (*VGSC*) gene of *Cimex hemipterus* from several countries. However, no information regarding *kdr* mutations in any Chinese tropical bed bug population is available to date. In this study, a double-mutation (M918I + L1014F) *kdr* allele was identified in six *C. hemipterus* populations across Guangxi Zhuang Autonomous Region of China. The frequency of this allele was 100% in all the six examined populations. In addition, only two cytochrome c oxidase I (*COI*) gene haplotypes, with one synonymous nucleotide variation, were identified in a total of 48 individuals from six locations. The fixation and broad geographic distribution of this resistant allele questions the continued use of pyrethroids in the treatment of tropical bed bug infestations. The very low genetic diversity within and among these populations indicates that these bed bugs may have a single origin.

Introduction

The tropical bed bug *Cimex hemipterus* (Hemiptera: Cimicidae) is distributed mainly in tropical and subtropical regions (Doggett *et al.*, 2018*a*). This bloodsucking pest of significant health importance feeds on humans and birds. Bites by bed bugs may cause skin reactions including the formation of papular lesions with associated itch (Doggett *et al.*, 2018*b*), and the resulting scratching may further lead to secondary infections including cellulitis, ecthyma, impetigo and lymphangitis (Lai *et al.*, 2016; Doggett *et al.*, 2012, 2018*a*, 2018*b*). In addition to the direct effects of bites and secondary infections, bed bug infestations impose substantial social, mental, and financial distresses (Yoon *et al.*, 2008).

Over the last 15–20 years, bed bugs have been undergoing a dramatic global resurgence with many infestation reports around the world (Davies *et al.*, 2012; Dang *et al.*, 2017; Naylor *et al.*, 2018). The reappearance or introduction of the tropical bed bug has been documented in tropical and subtropical regions (Davies *et al.*, 2012; Campbell *et al.*, 2016; Naylor *et al.*, 2018). Although several factors, such as an increase in global travel and trade, have been suspected to be attributable to the resurgence of bed bugs, the evolution of insecticide resistance has largely been incriminated as the main factor for the resurgence of this pest (Romero *et al.*, 2007; Lai *et al.*, 2016; Dang *et al.*, 2017).

With the extensive use of pyrethroids, resistance to this chemical group has been documented in *C. hemipterus* (Myamba *et al.*, 2002; Karunaratne *et al.*, 2007; Tawatsin *et al.*, 2011; Dang *et al.*, 2015). Knockdown resistance, which is caused by non-synonymous mutations within the voltage-gated sodium channel (*VGSC*) gene, is an important mechanism of pyrethroid and DDT in the bed bugs. So far, four mutations (L899V, M918I, D953G, and L1014F) were identified in the *VGSC* gene of *C. hemipterus* collected from multiple countries (Dang *et al.*, 2015). Similar to observations from *Musca domestica*, the M918I mutation was always found together with the L1014F mutation (Dang *et al.*, 2015). Strains of *C. hemipterus* carrying the two mutations (M918I and L1014F) were reported to be associated with higher resistance to d-allethrin than that those with just the L1014F mutation (Dang *et al.*, 2015). A causal link between one or both of the mutations at sites 918 and 1014 with pyrethroid resistance was reported in several other insects (Williamson *et al.*, 1996; Vais *et al.*, 2003; Usherwood *et al.*, 2007; Sonoda, 2010; Sonoda *et al.*, 2012; Dong *et al.*, 2014). However, the role of the conserved mutations L899V and D953G within the *VGSC* gene in insecticide resistance has yet to be evaluated (Dang *et al.*, 2015).

Although many studies addressing *kdr* mechanisms have been conducted on common bed bugs (*C. lectularius*) (Zhu *et al.*, 2010; Dang *et al.*, 2017), few reports on the tropical bed bug are available. The presence of possible *kdr* mutations has been investigated in tropical bed bugs collected from Australia, India, Malaysia, Kenya, and Thailand (Dang *et al.*, 2015). However, to

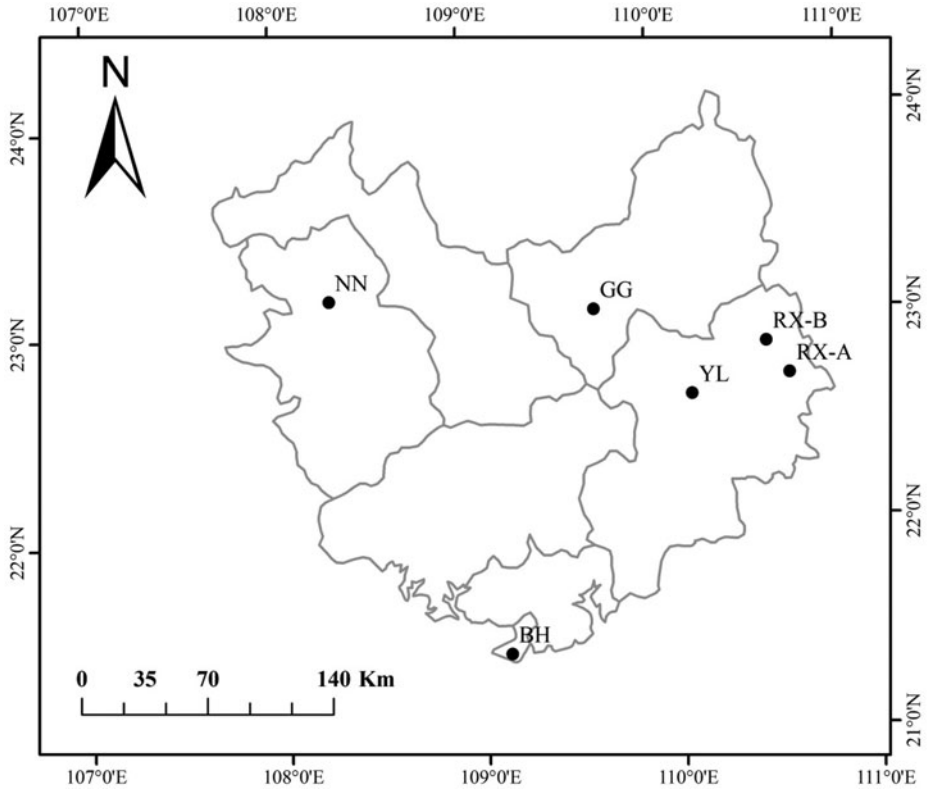

Figure 1. Map of the sampling locations for *Cimex hemipterus*, Guangxi Zhuang Autonomous Region, China. NN, Nanning; GG, Guigang; YL, Yulin; RX, Rongxian; BH, Beihai.

Table 1. *COI* haplotypes identified in the tropical bed bugs from Guangxi, China

Populations	Locations	Structure	Individuals examined	Frequency (%) H1	H2
RX-A	Yangmei, Rongxian	School dormitory	10	100	0
RX-B	Rongzhou, Rongxian	School dormitory	10	20	80
YL	Yulin	Hotel	6	0	100
GG	Guigang	School dormitory	10	0	100
BH	Beihai	Residency	6	0	100
NN	Nanning	Hospital	6	0	100

Note: The GenBank accession numbers are MK883766 and MK883767 for H1 and H2, respectively

our knowledge, no study on *kdr* mutation in Chinese tropical bed bug populations has been reported.

Guangxi is located in a subtropical monsoon climate zone with high average annual temperatures, which is suitable for bed bug occurrence. Moreover, control failure of bed bugs using pyrethroids in the field was reported by local pest control operators. In this context, we attempted to investigate the presence, distribution, and frequency of *kdr* mutations of tropical bed bugs in Guangxi of China, through the application of DNA sequencing. Such information will be useful for monitoring and managing insecticide resistance.

Materials and methods

Sample collection

The bed bugs were collected from six different locations in Guangxi, China (fig. 1, table 1). RXA and RXB samples were caught in 2014, and the others were collected in 2018. The bed bugs were placed in 95% ethanol and stored at −20°C until use.

DNA extraction and gene sequencing

The genomic DNA from individual bed bugs was extracted by the method of Rinkevich *et al.* (2006), and stored at −18°C until use.

A fragment (~550 bp) of the cytochrome c oxidase I (COI) gene was amplified using primers CHCOI-F (5′-TATTCGG-AATGTGGGCAGGG-3′) and CHCOI-R (5′-CACCCGCTAA TACAGGTAGGA-3). The PCR mixture in a total volume of 25 µl, containing 12.5 µl of 2 × Taq MASTER MIX (Tiangen Co., Beijing), 0.2 µM each primer, and 100–200 ng of DNA as a template. The thermal cycling profile was: an initial step of denaturation at 95°C for 2 min, followed by 34 cycles of 94°C for 30 s, 52°C for 30 s, 72°C for 50 s, and a final extension step

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at 72°C for 5 min. The PCR products were gel-purified and directly sequenced using the forward primer CHCOI-F.

Similarly, a fragment of the *VGSC* gene, encoding for the domain IIS4–IIS6 region and encompassing five putative *kdr* mutation sites (M918, L925, T929, L932, and L1014), was amplified using primers CHVG-SC-F and CHVG-SC-R (Dang *et al.*, 2015). The PCR products were sequenced using the reverse primer CHVG-SC-R.

Bioinformatics analysis

Data from DNA sequencing were checked and cleaned manually. All confirmed DNA sequences were aligned by using Muscle 3.8 (Edgar, 2004), and variable sites were identified. NCBI resources (https://www.ncbi.nlm.nih.gov/) including databases and software indicated in the following text were used for bioinformatics analysis.

Results

COI *haplotypes*

PCR products of the *COI* gene fragment were amplified from 48 individuals collected from six locations. Sequence alignment (472 bp in length) identified two haplotypes (H1 and H2, table 1). Chromatograms revealed only one haplotype within an individual, and only one synonymous nucleotide variation was observed between the two haplotypes (fig. 2). H1 was detected in two populations, while H2 was widely distributed in five of the six populations (table 1). Blast search revealed that H2 was shorter than, but 100% identical to the corresponding region of several *COI* sequences of tropical bed bugs from other Asian countries, such as MH607404 (Bangladesh), MG770889 (Iran), and KF018754 (Malaysia.).

kdr *mutations*

PCR products of the *VGSC* gene from each bed bug with a size of around 800 bp were sequenced. The DNA sequences excluding ambiguous terminal regions (with a length of 776 bp) were processed for further analysis. Sequence alignment indicated that all the *VGSC* sequences were identical and occurred in the homozygous form in the 44 examined individuals. This fragment (GenBank accession: MK883768) included three introns and four exons, with a sequence identity of 95.26% to the corresponding region of the common bed bug *VGSC* gene (GenBank No. JRLE01000129.1) (fig. 3). We notice that the length of exon 19 was 188 bp, rather than 190 bp as described in Dang *et al.* (2015). The result was confirmed by re-sequencing two randomly selected samples using the forward primer CHVG-SC-F.

To identify possible mutations within these exon regions, the deduced protein sequence was compared with those from the common bed bug *C. lectularius*, housefly *M. domestica*, and German cockroach *Blattella germanica* (fig. 4). Two non-synonymous variations, respectively, at sites 918 (ATA) and 1014 (TTC) were observed, resulting in M918I (ATG to ATA) and L1014F (CTG to TTC) substitutions. The previously reported L899V and D953G (Dang *et al.*, 2015) were not detected in our samples.

Discussion

This study is the first detection to date of the putative *kdr* mutations in Chinese tropical bed bug populations. We found that all the investigated bed bugs carried both M918I and L1014F mutations (table 2). Based on the findings of Dang *et al.* and others (Williamson *et al.*, 1996; Vais *et al.*, 2003; Usherwood *et al.*, 2007; Sonoda, 2010; Sonoda *et al.*, 2012; Dong *et al.*, 2014; Dang *et al.*, 2015), we predict that these bed bug populations in Guangxi exhibit a high level of resistance to pyrethroids. This result provides an explanation for the control failure of bed bugs reported by local pest control operators in the field. This situation also calls for a decrease in reliance on pyrethroids, and highlights the need of alternative insecticides with different modes of action or alternative approaches such as heat treatments or fumigation in order to make bed bug control more effective.

Notably, all these examined bed bugs that were collected in different locations share an identical *VGSC* haplotype. Moreover, *VGSC* exists in homozygous forms in these samples. In addition, while two *COI* haplotypes exist, they differ by only one synonymous nucleotide variation. These data reveal a very low genetic diversity within and among these populations. The limited diversity within each population may be explained by observations that bed bug populations are often highly inbred. The low genetic diversity among these six populations, which were collected from different locations (fig. 3), probably is a consequence of strong anthropogenic selection by synthetic insecticides and followed by a founder effect. It is very likely that these populations in this study have a single origin, then establish through the introduction of singly mated female or a small group of extremely related individuals carrying the M918I + L1014F allele, and spread due to human-mediated movement. Although the M918I + L1014F alleles were found in populations from Australia, India, and Thailand (Dang *et al.*, 2015), we are not sure if these alleles have a common origin, because the corresponding genomic DNA sequences of the *VGSC* gene in these samples used by Dang *et al.* (2015) are not publicly available.

The two haplotypes (H1 and H2) of COI identified from a total of 48 individuals differ from each other at a single nucleotide (fig. 2). Interestingly, H2 is distributed in five of the six locations, while only sites RX-A and RX-B contain individuals of H1, and RX-B is the only location sampled which contains both H1 and H2 (table 1). A Blast search using H2 against the GenBank nucleotide collection (non-redundant) database (on 14 November 2019) yielded 12 entries with 100% identity and 100% coverage from *C. hemipterus* individuals sampled from Bangladesh, Indonesia, Iran, and Malaysia, demonstrating a wide distribution of H2. By contrast, no hit that is 100% identical to H1 was retrieved. This observation makes us propose that H1 may be a newly evolved haplotype. Further studies are required in order to clarify this hypothesis *via* investigating more samples from different locations. The coexistence of both H1 and H2 in RX-B implies that the bed bugs carrying H1 are spreading from RX-A to nearby RX-B. Taking action to limit the potential migration of bed bugs across locations is recommended.

This work focused on the investigation of *kdr* mutations. Based on lessons from studies on the related common bed bug *C. lectularius*, in which multiple resistance mechanisms have been identified (Yoon *et al.*, 2008; Mamidala *et al.*, 2012; Zhu *et al.*, 2013), it is possible that other resistance mechanisms such as increased

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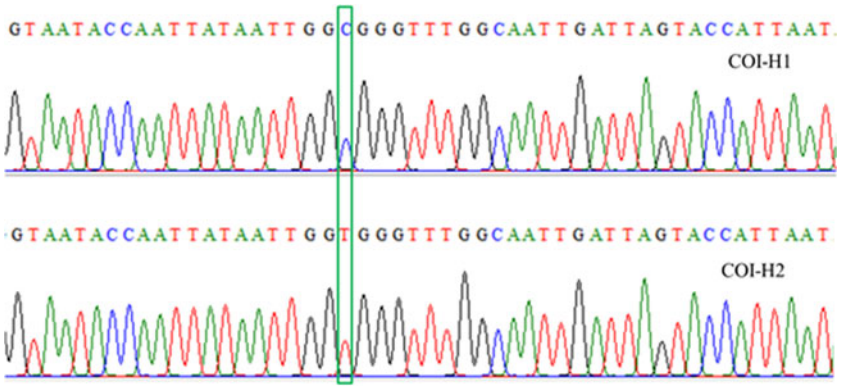

Figure 2. Chromatograms showing the nucleotide variation in the *COI* gene detected in the tropical bed bugs from Guangxi, China. The base variation is boxed.

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Chem-vgsc-GX      ATGAAGTTGATTGCAATGAGTCCAAAATATTACTTTCAAGAAGGCTGGAATATATTTGAT
Clec-vgsc-ref     ATGAAGTTGATAGCAATGAGTCCAAAATATTACTTTCAAGAAGGCTGGAATATATTTGAT
                  ********** **************************************************
Chem-vgsc-GX      TTTATCATAGTCGCTCTATCGTTATTAGAACTTAGTCTTGAAGGAATTCAAGGTCTATCA
Clec-vgsc-ref     TTTATCATAGTCGCTCTATCGTTATTAGAACTTAGTCTTGAAGGAATTCAAGGTCTATCA
                  ************************************************************
Chem-vgsc-GX      GTTTTGAGGTCATTCAGATTGGTATGTTTATTAACTAAAATTTAATCCAACTTACCTTCT
Clec-vgsc-ref     GTTTTGAGGTCATTCAGATTGGTATGTTTATTAACTGAAAATTAATCCAACTTACCTTCT
                  ********************************** *** ********************
Chem-vgsc-GX      TATTAATAATTAATAAACTAATAATAATTTCAGCTCAGGGTGTTTAAGCTGGCTAAGTCA
Clec-vgsc-ref     TACTAATAACTAATAAACTAATAATAATTTCAGCTCAGGGTGTTTAAGCTGGCTAAGTCA
                  ** ****** **************************************************
Chem-vgsc-GX      TGGCCAACACTCAATCTTCTCATTTCCATTATAGGCAGAACAGTGGGTGCCCTCGGTAAT
Clec-vgsc-ref     TGGCCAACACTCAATCTTCTCATTTCCATTATGGGCAGAACAGTGGGTGCCCTTGGTAAT
                  ********************************** ********************* ******
Chem-vgsc-GX      TTAACTTTTGTGTTGTGCATTATTATATTCATCTTTGCTGTGATGGGAATGCAGTTGTTT
Clec-vgsc-ref     TTAACTTTTGTGTTGTGCATTATTATTTTCATCTTTGCTGTGATGGGAATGCAGTTGTTT
                  ************************** **********************************
Chem-vgsc-GX      GGAAAAAATTATATCGGTAATTTTTTACAATGAATTGTATAATTTTTGACACGTGTACAA
Clec-vgsc-ref     GGAAAGAACTATATCGGTAATTTTTTG-AATGAATTGTCTAATTTTTGACATGTGTACAA
                  ***** ** ***************  ********** ************ ********
Chem-vgsc-GX      ATTGATTTATATACTTACAGATAACATGGATAGATTCCCTGATGGAGAACTCCCGAGGTG
Clec-vgsc-ref     ATTGATTTATATTCT---AGATAATATGGATAGATTCCCTGATGGCGAACTCCCGAGGTG
                  ************ **   ****** ******************** ***************
Chem-vgsc-GX      GAATTTTACAGATTTCATGCATTCGTTTATGATAGTGTTTAGGGTCCTGTGTGGAGAGTG
Clec-vgsc-ref     GAATTTTACAGATTTCATGCATTCGTTTATGATAGTGTTTAGGGTCCTGTGTGGAGAGTG
                  ************************************************************
Chem-vgsc-GX      GATAGAATCGATGTGGGATTGTATGCACGTGGGAGATGTTTCTTGCATTCCATTTTTTCT
Clec-vgsc-ref     GATAGAATCGATGTGGGATTGTATGCATGTGGGAGATGTTTCTTGCATTCCATTTTTTCT
                  ***************************** ********************************
Chem-vgsc-GX      AGCGACAGTAGTAATTGGGAATTTCGTTGTAAGTGCATTTTTTATTAATTAAAAATCAGT
Clec-vgsc-ref     AGCGACAGTAGTAATTGGGAATCTGGTTGTAAGTGCATTTTTTACTAATTAAAAATCAGT
                  ********************** * ******************** ***************
Chem-vgsc-GX      TGTTT--TTAAACAGTGTTTTTGTTTAACTGTTTTCGATGATATTTTTTTAGGTTCTCAA
Clec-vgsc-ref     TGTTTTATTTAACCTTTTTTGGGTTTAACTGTTTTGGATA----TTTTTTAGGTTCTCAA
                  *****  ** ***  * ***  ************ ***     ****************
Chem-vgsc-GX      CCTTTTCTTAGCCCTGTTGCTCAGTAATTTTGGCTCGTCGAGTCTTTCAGCTCCGACAG
Clec-vgsc-ref     CCTTTTCTTAGCCCTGTTGCTCAGTAATTTTGGCTCGTCGAGTCTTTCAGCTCCGACAG
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Figure 3. Exon–intron structure of the amplified genomic fragment of the *VGSC* genes from *C. hemipterus*, aligned with the corresponding region from the common bed bug *C. lectularius* (JRLE01000129.1). The two codons for M918I (ATA) and L1014F (TTC) mutations were indicated in bold. The introns are underlined.

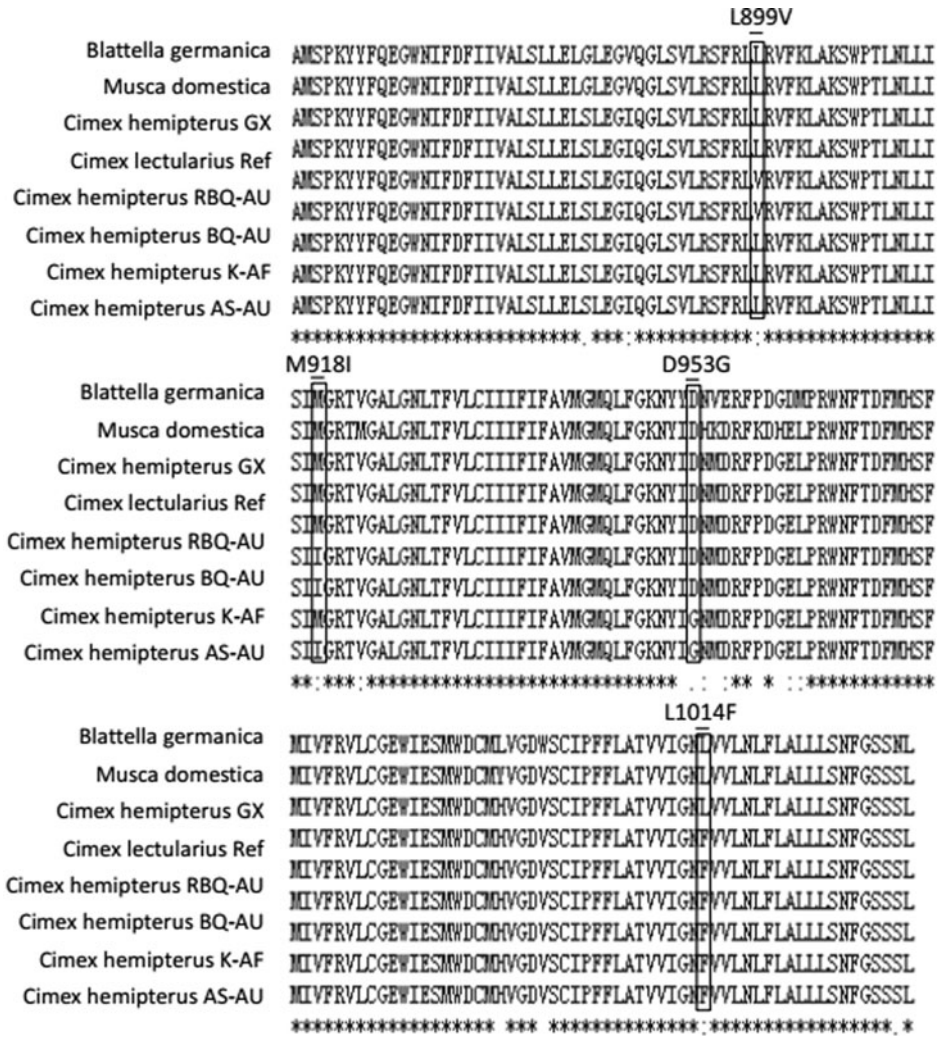

Figure 4. Alignments of the partial protein sequence of the *VGSC* gene from *C. hemipterus* (this study and those in Dang *et al*., 2015) with those from the common bed bug *C. lectularius* Ref (FJ031996), housefly *Musca domestica* (U38813), and German cockroach *Blattella germanica* (U71083). Asterisks (*), colons (:), and black dots (·) indicate identical, conserved, and weakly conserved sites, respectively.

Table 2. *kdr* mutations and their frequencies detected in the tropical bed bug populations of Guangxi, China

Populations	*n*	Codon 918	Frequency (%)	Codon 1014	Frequency (%)
RX-A	10	ATA	100	TTC	100
RX-B	10	ATA	100	TTC	100
YL	6	ATA	100	TTC	100
GG	6	ATA	100	TTC	100
BH	6	ATA	100	TTC	100
NN	6	ATA	100	TTC	100

metabolism or reduced penetration are also present in *C. hemipterus*. Further work is required to gain a full understanding of the genetics of insecticide resistance in *C. hemipterus* populations.

Conclusion

This study represents the first effort to investigate the *kdr*-type resistance in the tropical bed bugs in China. Two putative and conserved *kdr* point mutations (M918I and L1014F) were detected and this double-mutation allele was found to be fixed in the six examined *C. hemipterus* populations across Guangxi of China. Our results strongly question the efficacy of pyrethroids for *C. hemipterus* control. Therefore, employing alternative tactics such as physical treatment (e.g. heat, cold, vacuuming), or using insecticides with an alternative mode of action are recommended in these regions.

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Author contribution. Conceived and designed the experiments: XHQ and XYF. Performed the experiments: YHZ and ML. Analyzed the data: XHQ and YHZ. Contributed reagents/materials/analysis tool: ML and XYF. Wrote the paper: XHQ and YHZ. All authors read and approved the final manuscript.

Conflict of interest. The author(s) declare that they have no competing interests.

References

Campbell BE, Koehler PG, Buss LJ and Baldwin RW (2016) Recent documentation of the tropical bed bug (Hemiptera: Cimicidae) in Florida since the common bed bug resurgence. *Florida Entomologist* **99**, 549–551.

Dang K, Toi CS, Lilly DG, Lee CY, Naylor R, Tawatsin A, Thavara T, Bu W and Doggett SL (2015) Identification of putative *Kdr* mutations in the tropical bed bug, *Cimex hemipterus* (Hemiptera: Cimicidae). *Pest Management Science* **71**, 1015–1020.

Dang K, Doggett SL, Singham BV and Lee CY (2017) Insecticide resistance and resistance mechanisms in bed bugs, *Cimex* Spp. (Hemiptera: Cimicidae). *Parasites & Vectors* **10**(1), 318.

Davies TGE, Field LM and Williamson MS (2012) The re-emergence of the bed bug as a nuisance pest: implications of resistance to the pyrethroid insecticides. *Medical and Veterinary Entomology* **26**, 241–254.

Doggett SL, Dwyer DE, Peñas PF and Russell RC (2012) Bed bugs: clinical relevance and control options. *Clinical Microbiology Reviews* **25**, 164–192.

Doggett SL, Miller DM and Lee CY (2018a) Introduction. In Doggett SL, Miller DM and Lee CY (eds), *Advances in the Biology and Management of Modern Bed Bugs*. Oxford, United Kingdom: Wiley Blackwell, pp. 1–5.

Doggett SL, Miller DM and Lee CY (eds) (2018b) *Advances in the Biology and Management of Modern Bed Bugs*. Oxford, United Kingdom: Wiley Blackwell, 472pp.

Dong K, Du Y, Rinkevich F, Nomura Y, Xu P, Wang L, Silver K and Zhorov BS (2014) Molecular biology of insect sodium channels and pyrethroid resistance. *Insect Biochemistry and Molecular Biology* **50**, 1–17.

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* **32**, 1792–1797.

Karunaratne SHPP, Damayanthi BT, Fareena MHJ, Imbuldeniya V and Hemingway J (2007) Insecticide resistance in the tropical bed bug *Cimex hemipterus*. *Pesticide Biochemistry and Physiology* **88**, 102–107.

Lai O, Ho D, Glick S and Jagdeo J (2016) Bed bugs and possible transmission of human pathogens: a systematic review. *Archives of Dermatological Research* **308**, 531–538.

Mamidala P, Wijeratne AJ, Wijeratne S, Kornacker K, Sudhamalla B, RiveraVega LJ, Haelmer A, Meulia T, Jones SC and Mittapalli O (2012) RNA-Seq and molecular docking reveal multi-level pesticide resistance in the bed bug. *BMC Genomics* **13**, 6.

Myamba J, Maxwel CA, Asidi A and Curtis CF (2002) Pyrethroid resistance in tropical bed bugs, *Cimex Hemipterus*, associated with use of treated bednets. *Medical and Veterinary Entomology* **16**, 448–451.

Naylor R, Balvín O, Delaunay P and Akhoundi PM (2018) The bed bug resurgence in Europe and Russia. In Doggett SL, Miller DM and Lee C-Y (eds), *Advances in the Biology and Management of Modern Bed Bugs*. Oxford, United Kingdom: John Wiley & Sons, pp. 59–65.

Rinkevich FD, Zhang L, Hamm RL, Brady SG, Lazzaro BP and Scott JG (2006) Frequencies of the pyrethroid resistance alleles of Vssc1 and CYP6D1 in house flies from the eastern United States. *Insect Molecular Biology* **15**, 157–167.

Romero A, Potter MF, Potter DA and Haynes KF (2007) Insecticide resistance in the bed bug: a factor in the pest's sudden resurgence? *Journal of Medical Entomology* **44**, 175–178.

Sonoda S (2010) Molecular analysis of pyrethroid resistance conferred by target insensitivity and increased metabolic detoxification in *Plutella xylostella*. *Pest Management Science* **66**, 572–575.

Sonoda S, Shi X, Song D, Zhang Y, Li J, Wu G, Liu Y, Li M, Liang P, Wari D, Matsumura M, Miakuchi C, Tanaka T, Miyata T and Gao X (2012) Frequencies of the M918I mutation in the sodium channel of the diamondback moth in China, Thailand and Japan and its association with pyrethroid resistance. *Pesticide Biochemistry and Physiology* **102**, 142–145.

Tawatsin A, Thavara U, Chompoosri J, Phusup Y, Jonjang N, Khumsawads C, Bhakdeenuan P, Sawanpanyalert P, Asavadachanukorn P, Mullar MS, Siriyasatien P and Debboun M (2011) Insecticide resistance in bed bugs in Thailand and laboratory evaluation of insecticides for the control of *Cimex Hemipterus* and *Cimex lectularius* (Hemiptera: Cimidae). *Journal of Medical Entomology* **48**, 1023–1030.

Usherwood PNR, Davies TGE, Mellor IR, O'Reilly AO, Peng F, Vais H, Khambay BPS, Field LM and Williamson MS (2007) Mutations in DII5 and the DIIS4–S5 linker of *Drosophila Melanogaster* sodium channel define binding domains for pyrethroids and DDT. *FEBS Letters* **581**, 5485–5492.

Vais H, Atkinson S, Pluteanu F, Goodson SJ, Devonshire AL, Williamson MS and Usherwood PNR (2003) Mutations of the para sodium channel of Drosophila melanogaster identify putative binding sites for pyrethroids. *Molecular Pharmacology* **64**, 914–922.

Williamson MS, Martines-Torres D, Hick CA and Devonshire AL (1996) Identification of mutations in the housefly para-type sodium channel gene associated with knock down resistance (*kdr*) to pyrethroid insecticides. *Molecular and General Genetics* **252**, 51–60.

Yoon KS, Kwon DH, Strycharz JP, Hollingsworth CS, Lee SH and Clark JM (2008) Biochemical and molecular analysis of deltamethrin resistance in the common bed bug (Hemiptera: Cimicidae). *Journal of Medical Entomology* **45**, 1092–1101.

Zhu F, Wigginton J, Romero A, Moore A, Ferguson K, Palli R, Potter MF, Haynes KF and Palli SR (2010) Widespread distribution of knockdown resistance mutations in the bed bug, *Cimex lectularius* (Hemiptera: Cimicidae), populations in the United States. *Archives of Insect Biochemistry and Physiology* **73**, 245–257.

Zhu F, Gujar H, Gordon JR, Haynes KF, Potter MF and Palli SR (2013) Bed bugs evolved unique adaptive strategy to resist pyrethroid insecticides. *Scientific Reports* **3**, 1456.