Detection of pyrethroid resistance mutations and intron variants in the voltage-gated sodium channel of Aedes (Stegomyia) aegypti and Aedes (Stegomyia) albopictus mosquitoes from Lao People’s Democratic Republic

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Abstract
In Lao People’s Democratic Republic, Aedes aegypti (Linnaeus 1762) and Aedes albopictus (Skuse 1894) mosquitoes (Diptera: Culicidae) are vectors of arboviral diseases such as dengue. As the treatment for these diseases is limited, control of the vectors with the use of pyrethroid insecticides is still essential. However, mutations in the voltage-gated sodium channel (vgsc) gene giving rise to pyrethroid resistance are threatening vector control programs. Here, we analysed both Ae. aegypti and Ae. albopictus mosquitoes, which were collected in different districts of Laos (Kaysone Phomvihane, Vangvieng, Saysettha and Xaythany), for vgsc mutations commonly found throughout Asia (S989P, V1016G and F1534C). Sequences of the vgsc gene showed that the F1534C mutation was prevalent in both Aedes species. S989P and V1016G mutations were detected in Ae. aegypti from each site and were always found together. In addition, the mutation T1520I was seen in Ae. albopictus mosquitoes from Saysettha district as well as in all Ae. aegypti samples. Thus, mutations in the vgsc gene of Ae. aegypti are prevalent in the four districts studied indicating growing insecticide resistance throughout Laos. Constant monitoring programmes and alternative strategies for controlling Aedes should be utilized in order to prolong the effectiveness of pyrethroids thereby maximizing vector control.

KEYWORDS
Aedes, deltamethrin, insecticide resistance, Kdr, permethrin, pyrethroid, vector control
**INTRODUCTION**

*Aedes aegypti* and *Aedes albopictus* mosquitoes are vectors of various viral diseases such as yellow fever, Zika, chikungunya and dengue that have colonized almost all continents and are still expanding geographically (Lwande et al., 2020). As a consequence, the global incidence of dengue has grown dramatically in recent decades and about half of the world’s population is now at risk (World Health Organization, 2022). In the absence of drugs and vaccines, managing these diseases is heavily reliant on vector control, such as the use of insecticides (Wilson et al., 2020). However, overreliance on these insecticides has resulted in the emergence of resistance in many countries throughout the world, which threatens to undermine efforts to control dengue and other viruses carried by *Aedes* mosquitoes (Smith et al., 2016).

Understanding and monitoring the extent of insecticide resistance is crucial in informing the optimal use of vector control strategies thereby minimizing disease outbreaks.

In Lao People’s Democratic Republic (PDR; hereafter referred to as Laos), a land-locked country located in the middle of the Indochinese peninsula, dengue fever is a major national health problem where outbreaks have been regularly declared (Calvez et al., 2020). Since the early 2000s, pyrethroid insecticides such as deltamethrin and permethrin have been used in Laos for the control of adult mosquitoes. In 2019, Marcombe et al. reported that several populations of adult *Ae. aegypti* taken from different regions of Laos were highly resistant to permethrin and populations from Attapeu and Vientiane Capital were also resistant to deltamethrin (Marcombe et al., 2019). In line with this, resistant mosquitoes possessed V1016G and F1534C mutations (amino acid numbering used throughout is according to the *Musca domestica* sodium channel protein) in the voltage-gated sodium channel (*vgsc*) gene that are associated with target-site resistance (Du et al., 2016; Marcombe et al., 2019; Smith et al., 2016). The V1016G and F1534C mutations, as well as S989P, have been commonly observed in pyrethroid-resistant *Aedes* mosquitoes throughout Asian countries (Fan et al., 2020) including China (Li et al., 2015), Indonesia (Wulandari et al., 2015), Malaysia (Leong et al., 2019), Myanmar (Kawada et al., 2014), Saudi Arabia (Al Nazawi et al., 2017), Sri Lanka (Fernando et al., 2018), Taiwan (Chung et al., 2019), Thailand (Yanola et al., 2011) and Vietnam (Kasai et al., 2019).

Recently, S989P + V1016G or S989P + V1016G + F1534C mutations were found to be significantly associated with resistance to deltamethrin in *Ae. aegypti* from Xaythany located in Vientiane Capital (Shimono et al., 2021). Analysis of *Ae. albopictus* taken from either Luang Prabang province or Vientiane Capital found that these mosquitoes were not resistant to deltamethrin nor permethrin with one exception being a group of mosquitoes taken from Kao-nhot village, in Vientiane Capital, which showed suspected resistance to permethrin (Tangena et al., 2018). The presence of *vgsc* target site mutations associated with pyrethroid resistance in *Ae. albopictus* from Laos has yet to be measured. The S989P and V1016G mutations are located in domain II of the VGSC protein whilst F1534C is present in domain III (Du et al., 2016). In this study, sequences of domains II and III were examined to further investigate the prevalence of *vgsc* mutations in *Ae. aegypti* from Savannakhet, Vientiane and Vientiane Capital provinces as well as in *Ae. albopictus* from Vientiane province and Vientiane Capital.

**MATERIALS AND METHODS**

**Collection of mosquito samples**

Mosquito collections were carried out in three provinces of Laos (Vientiane Capital, Vientiane Province and Savannakhet; Table 1 and Figure 1). *Aedes* sp. mosquitoes at larval and pupal stages were collected in rural and urban areas in different sampling containers (buckets, cups, fridges, jars, tires, toilets, vases, etc.). All samples were brought back to the laboratory at the Institut Pasteur du Laos and maintained under controlled conditions for rearing until adults (F1 generation) following previously described standardized techniques (Marcombe et al., 2019). After adult identification using morphological keys (Rattanarithikul et al., 2006), mosquitoes were separated by species and were kept for breeding. Female mosquitoes from each population were stored in desiccated tubes at −80°C and sent to Oxford Brookes University for molecular analyses.

**Detection of mutations in the voltage-gated sodium channel**

Genomic DNA was extracted from individual mosquitoes (191 *Ae. aegypti* and 81 *Ae. albopictus*) using 250 μl Trizol (Fisher Scientific, Loughborough, UK) following the manufacturer’s protocol. With 2 μl of extracted DNA as template, DNA encoding for transmembrane region 6 (TM6) in domain I, transmembrane region TM6 in domain II or TM6 of domain III from the *Ae. aegypti* or *Ae. albopictus* *vgsc* gene was amplified by polymerase chain reaction (PCR) using the QS® High-Fidelity PCR Kit (New England Biolabs, Ipswich, MA, U.S.A.). We designed primers to amplify TM6 in domain I, which were 5'-TCTTCGTTGTTGTAAGACAC-3' (forward) and 5'-TTCCGCCTCAACGGCCTC-3' (reverse) with resulting amplification products being sequenced with the 5'-TCTTCGTTGTTGTAAGACAC-3' (forward) and 5'-TTCCGCCTCAACGGCCTC-3' (reverse) with resulting amplification products being sequenced with either sense (5’-ACGAGATCATTCCGGATGTG-3’) or antisense (5’-CTTGGTTCCGTTGTCTTGG-3’) oligonucleotides. For TM6 in domain II, the primers used were 5’-AGACAAATGTGGATCGCTTTC-3’ (forward) and 5’-GATATCCCTCTCCAGACACCAAG-3’ (forward) with resulting amplification products being sequenced with either sense (5’-ACGAGATCATTCCGGATGTG-3’) or antisense (5’-CTTGGTTCCGTTGTCTTGG-3’) oligonucleotides. For TM6 in domain III, the primers used were 5’-AGACAAATGTGGATCGCTTTC-3’ (forward) and 5’-CCCTAGGGCGCTGAATAGC-3’ (reverse) with amplification products being sequenced using either sense (5’-ACGAGATCATTCCGGATGTG-3’) or antisense (5’-TTCCGCCTCAACGGCCTC-3’) oligonucleotides. The PCR products were purified using the Monarch® PCR & DNA Cleanup Kit (New England Biolabs, Ipswich, MA, U.S.A.) and then sequenced at SourceBioscience (https://www.soucrebiomience.com/). Sequence chromatograms were visualized using Chromas (available online: https://technelysis.com.au/wp/chromas/).
RESULTS

Detection of mutations and intron variants in the voltage-gated sodium channel

Genomic DNA encoding for TM6 in domains II or III of the vgsc gene from individual female *Ae. aegypti* mosquitoes taken from the districts of Kaisone Phomvihane (Savannakhet province), Vangvieng (Vientiane province), Saysetha (Vientiane Capital) or Xaythany (Vientiane Capital) were amplified and analysed for mutations associated with insecticide resistance (Du et al., 2016). Also, vgsc sequences from *Ae. albopictus* mosquitoes taken from either Vangvieng or Saysettha were analysed. Sequence chromatograms showed no mutation at L982 (Brengues et al., 2003) in the 127 *Ae. aegypti* or 23 *Ae. albopictus*
### TABLE 2

Frequencies of mutations in the \textit{vgsc} gene of \textit{Aedes aegypti} and \textit{Aedes albopictus} collected from Kaisone Phomvihane (Savannakhet province, SVKS), Vangvieng (Vientiane province, VTVV), Saysettha (Vientiane capital, VTESVV) or Xaythany (Vientiane capital, VTESVL)

| District | Aedes species | V410L | L982W | S989P | I1011M | V1016G | T1520I | I1532T | F1534C |
|----------|--------------|-------|-------|-------|--------|--------|--------|--------|--------|
|          |              | VV    | LL    | SS    | SP     | PP     | VV     | VG     | GG     |
| SVKS     | aegypti      | 12    | 56    | 42    | 12     | 2      | 56     | 42     | 12     |
|          | Total        | 12    | 56    | 56    | 56     | 68     | 68     | 68     |
| Mutation frequency | 0% | 0% | 14% | 0% | 14% | 24% | 0% | 76% |
| VTVV     | aegypti      | -     | 21    | 7     | 19     | 7      | 33     | 7      | 19     |
|          | Total        | -     | 21    | 33    | 33     | 33     | 44     | 44     |
| Mutation frequency | - | 0% | 50% | 0% | 50% | 14% | 0% | 58% |
| VTESVV   | aegypti      | -     | 32    | 24    | 9      | 3      | 36     | 24     | 9      |
|          | Total        | -     | 32    | 36    | 36     | 36     | 34     | 34     |
| Mutation frequency | - | 0% | 21% | 0% | 21% | 16% | 0% | 68% |
| VTESVL   | aegypti      | -     | 18    | 9     | 6      | 9      | 24     | 9      | 6      |
|          | Total        | -     | 18    | 24    | 24     | 24     | 30     | 30     |
| Mutation frequency | - | 0% | 50% | 0% | 50% | 2% | 0% | 52% |
| VTVV     | albopictus   | -     | 11    | 32    | 0      | 0      | 32     | 28     | 0      |
|          | Total        | -     | 11    | 32    | 32     | 28     | 26     | 26     |
| Mutation frequency | - | 0% | 0% | 0% | 0% | 0% | 0% | 54% |
| VTESVV   | albopictus   | -     | 12    | 31    | 0      | 0      | 31     | 31     | 0      |
|          | Total        | -     | 12    | 31    | 31     | 31     | 33     | 33     |
| Mutation frequency | - | 0% | 0% | 0% | 0% | 5% | 0% | 21% |

### FIGURE 2

Sequence chromatograms showing heterozygous and homozygous mutations in \textit{Aedes} sp. collected in Lao PDR. The amino acid position of the voltage-gated sodium channel (\textit{Musca domestica} numbering) is shown.
mosquitoes analysed (Table 2). Likewise, no mutations were detected at I1011 (Rajatileka et al., 2008) or I1532 (Wei et al., 2021) in all Ae. aegypti or Ae. albopictus mosquitoes studied. In addition, no mutations were detected at V410 (Haddi et al., 2017) in domain I of the voltage gate sodium channel (vgsc) gene in 12 Ae. aegypti mosquitoes from Kaisone Phomvihane. However, the mutation F1534C (TTC to TGC; Figure 2), was observed in all groups of mosquitoes with frequencies ranging from 21% to 76% (Table 2). The mutation T1520I (ACC to ATC; Figure 2) was seen in Ae. albopictus mosquitoes taken from the Saysettha district (with a frequency of 5%) as well as in Ae. aegypti from all four districts (frequencies ranging from 2% to 24%; Table 2). The S989P (TCC to CCC) and V1016G (GTA to GGA) mutations were not detected in Ae. albopictus (Table 2) but were found in Ae. aegypti from each group (mutation frequencies ranging from 14% to 50%; Table 2 and Figure 2).

In Ae. aegypti, 13 different genotypes for VGSC mutations were observed (Table 3). With the exception of one mosquito from Kaisone Phomvihane, all Ae. aegypti analysed had at least one mutation. The S989P and V1016G mutations always occurred together, whether heterozygous (mutant and wild-type) or homozygous, and the heterozygous triple mutant, S989P + V1016G + F1534C, was most commonly observed being in 31 of the total of 133 Ae. aegypti analysed across the 4 districts. This heterozygous triple mutation was predominant in Saysettha and Vangvieng. For the Kaisone Phomvihane district, the most abundant genotype was the homozygous F1534C mutation whilst this was also the most common genotype seen for mosquitoes from Xaythany along with the homozygous S989P + V1016G double mutant. Interestingly, this homozygous double mutant was not seen at all in mosquitoes from Kaysone Phomvihane, highlighting that mosquitoes from different districts may have varying complements of genotypes. The homozygous S989P + V1016G + F1534C triple mutation was only detected in Ae. aegypti from Saysettha at a low frequency of 3%. The T1520I mutation (heterozygous or homozygous) always occurred with at least an F1534C mutation and the heterozygous S989P + V1016G + T1520I + F1534C quadruple mutation was observed in mosquitoes from Kaysone Phomvihane and Vangvieng.

Sequences with the S989P and V1016G mutations always had the intervening intron 20 consisting of 250 bp (Figure 3), which has been previously described as group A, whereas the intron in

| Species          | 989 | 1016 | 1520 | 1534 | I29 | SVKS | VTESVV | VTVV | VTESVL |
|------------------|-----|------|------|------|-----|------|--------|------|--------|
| aegypti          |     |      |      |      |     |      |        |      |        |
| SP VG            | TT  | FC   | ++   |      | 13% (6/48) | 28% (9/32) | 40% (12/30) | 18% (4/23) |
| SS VV            | TT  | CC   | ++   |      | 30% (14/48) | 16% (5/32) | 7% (2/30) | 26% (6/23) |
| SS VV            | TI  | CC   | ++   |      | 25% (12/48) | 16% (5/32) | 13% (4/30) | 4% (1/23) |
| SS VV            | TT  | FC   | ++   |      | 8% (4/48) | 22% (7/32) | 0% (0/30) | 4% (1/23) |
| PP GG            | TT  | FF   | ++   |      | 0% (0/48) | 3% (1/32) | 10% (3/30) | 26% (6/23) |
| PP GG            | TT  | FC   | ++   |      | 2% (1/48) | 3% (1/32) | 10% (3/30) | 14% (3/23) |
| SP VG            | TI  | FC   | ++   |      | 6% (3/48) | 0% (0/32) | 13% (4/30) | 0% (0/23) |
| SS VV            | TI  | FC   | ++   |      | 4% (2/48) | 6% (2/32) | 3% (1/30) | 0% (0/23) |
| SP VG            | TT  | FF   | ++   |      | 4% (2/48) | 0% (0/32) | 3% (1/30) | 4% (1/23) |
| SS VV            | TI  | CC   | ++   |      | 4% (2/48) | 3% (1/32) | 0% (0/30) | 0% (0/23) |
| SP VG            | TT  | CC   | ++   |      | 2% (1/48) | 0% (0/32) | 0% (0/30) | 4% (1/23) |
| PP GG            | TT  | CC   | ++   |      | 0% (0/48) | 3% (1/32) | 0% (0/30) | 0% (0/23) |
| SS VV            | TT  | FF   | ++   |      | 2% (1/48) | 0% (0/32) | 0% (0/30) | 0% (0/23) |
| albopictus       |     |      |      |      |     |      |        |      |        |
| SS VV            | TT  | FF   | --   |      | 48% (14/29) | 15% (3/20) | - | - |
| SS VV            | TT  | FC   | --   |      | 24% (7/29) | 50% (10/20) | - | - |
| SS VV            | TT  | CC   | ++   |      | 0% (0/29) | 25% (5/20) | - | - |
| SS VV            | TT  | FC   | ++   |      | 7% (2/29) | 10% (2/20) | - | - |
| SS VV            | TI  | FC   | ++   |      | 7% (2/29) | 0% (0/20) | - | - |
| SS VV            | TI  | FC   | ++   |      | 3.5% (1/29) | 0% (0/20) | - | - |
| SS VV            | TT  | FF   | --   |      | 3.5% (1/29) | 0% (0/20) | - | - |
| SS VV            | TT  | FF   | ++   |      | 3.5% (1/29) | 0% (0/20) | - | - |

Note: I29 marks intron 29 where ‘+’ denotes the 69 bp intron whilst ‘-’ indicates 1 of the 5 intron 29 variants (Figure 4). Grey shading indicates a heterozygous mutation, black shading indicates a homozygous mutation whilst no shading indicates homozygous wild-type. Highest frequencies in each district are underlined.
FIGURE 3  Alignment of variable intron 20 sequences and parts of flanking exons found in the vgsc gene of *Aedes aegypti* or *Aedes albopictus* collected in Laos. Genomic DNA sequences from *Ae. albopictus* are intron20Var81a (accession no. OM513683), intron20Var81b (OM513684), intron20Var84 (OM513685) and intron20Var90 (OM513686). Sequences from *Ae. aegypti* with S989P + V1016G mutations (group A, OM513681) and without these mutations (group B, OM513682) are shown. Also included in the alignment are group A and group B intron 20 sequences of *Ae. aegypti* from Brazil (Martins et al., 2009), Ghana (Kawada et al., 2016), Saudi Arabia (Fang et al., 2021) and Taiwan (Chung et al., 2019). Black and grey shading indicates degree of conservation. Amino acid regions corresponding to coding regions are shown at the top of the alignment whilst mutated amino acids (989P, 1012M, 1016I and 1016G) found in group A are shown at the bottom. Splice donor and acceptor sites are marked by asterisks. The alignment was constructed using Clustal X2 (Thompson et al., 1997) using default settings and viewed using Genedoc (http://nrbsc.org/gfx/genedoc/index.html).

FIGURE 4  Alignment of intron 29 and parts of flanking exons of the vgsc gene from *Aedes aegypti* and the variable sequences found for intron 29 found in the vgsc gene from *Aedes albopictus* collected in Laos. The alignment includes the only sequence detected in *Ae. aegypti* consisting of 69 bp (accession number MN413379) along with a 69 bp intron in *Ae. albopictus* (MF774494.1) as well as intron29Var68 (M622708), intron29Var70 (M622709), intron29Var83 (M622710), intron29Var83b (M622711) and intron29Var83c (M622712). Black and grey shading indicates degree of conservation. The amino acid residues encoded by the flanking exons are shown at the top. Splice donor and acceptor sites are marked by asterisks. The alignment was constructed using Clustal X2 (Thompson et al., 1997) using default settings and viewed using Genedoc (http://nrbsc.org/gfx/genedoc/index.html).
sequences without this double mutation was group B consisting of 234 bp (Chung et al., 2019; Fang et al., 2021; Martins et al., 2009). Four different intron 20 sequences were observed in the *Ae. albopictus* vgsc gene, which are considerably shorter than the *Ae. aegypti* intron 20 sequences, consisting of 81, 81, 84 and 90 bp (Figure 3). They have been denoted here as intron20Var81a (Accession no. OM513683), intron20Var81b (OM513684), intron20Var84 (OM513685) and intron20Var90 (OM513686), respectively. No link between any of the *Ae. albopictus* intron 20 variants and mutations were detected.

For *Ae. albopictus*, nine genotypes for vgsc were observed (Table 3). For mosquitoes from Vangvieng, the heterozygous F1534C mutant was most abundant. The homozygous F1534C mutation was only seen in mosquitoes from Vangvieng whilst the heterozygous T1520I mutation was found only in mosquitoes from Saysettha always together with the heterozygous F1534C mutant.

Six different intron 29 (Chang et al., 2009) sequences were detected in the *Ae. albopictus* vgsc whereas only one was seen for *Ae. aegypti* (Table 3, Figure 4). Both *Ae. aegypti* and *Ae. albopictus* possess an intron consisting of 69 bp with the same sequence whilst the remaining five *Ae. albopictus* introns are novel, consisting of 68, 70 bp and three with 83 bp. They have been denoted here as intron29Var68 (accession number MZ622708), intron29Var70 (MZ622709), intron29Var83 (MZ622710), intron29Var83b (MZ622711) and intron29Var83c (MZ622712), respectively. Synonymous mutations were detected in domain III of *Ae. albopictus* vgsc (Figure 4). D1505D (GAC or GAT), G1513G (GGA or GGC) and F1528F (TTT or TTC) have been previously observed in *Ae. albopictus* from West Bengal, India (Chatterjee et al., 2018), and we found a fourth, P1516P (CCG or CCA). These synonymous mutations appear to be linked to the intron 29 variant, for example, D1505 (GAC) was found in the 69 bp sequence whilst D1505 (GAT) was found in the other variants, and P1516 (CCA) was observed in intron29Var83b and intron29Var83c whereas P1516 (CCG) was present in the other variants.

**DISCUSSION**

In the present study, sequences of domains II and III of the vgsc gene from individual adult female *Ae. aegypti* and *Ae. albopictus* mosquitoes from Laos were analysed for the prevalence of mutations associated with pyrethroid resistance. As with previous reports studying *Ae. aegypti* from Laos (Marcombe et al., 2019; Shimono et al., 2021), the F1534C mutation was detected at a high frequency (over 50%, Table 2) in *Ae. aegypti* collected from all four sites, one of which is in Savannakhet (Kaisone district), a province that has not been previously investigated. Indeed, the homozygous F1534C mutation was the most frequent genotype detected for mosquitoes from Kaisone (Table 3). Notable incidence of the F1534C mutation appears to be a consistent trend in *Ae. aegypti* from the neighbouring countries China (average allele frequency 50%; Li et al., 2015), Cambodia (100%; Saingamsook et al., 2017), Myanmar (40%; Naw et al., 2020) and Thailand (63%; Stenhouse et al., 2013 and 62%; Plermsub, Saingamsook, Yanola, Lumjuan, Tippawangkosol, Walton, & Somboon, 2016) as well as other countries such as Costa Rica (100%; Zarkoohi et al., 2020), Cameroon (average 50%; Djiaji-Tchamen et al., 2021, 2022), Sri Lanka (average 37%; Ranathunge et al., 2021) and Saudi Arabia (55%; Fang et al., 2021). See Chen et al. (2020) and Fan et al. (2020) for comprehensive summaries of vgsc mutations found in *Ae. aegypti* by country (Chen et al., 2020; Fan et al., 2020). The F1534C mutation has been associated with resistance to type I pyrethroids as shown by bioassays where *Ae. aegypti* females surviving permethrin exposure harboured the F1534C mutation but not S989P and V1016G mutations that underlie resistance to type I and type II pyrethroids (Yanola et al., 2011). Heterologous expression of *Ae. aegypti* vgsc in Xenopus laevis oocytes also shows that the F1534C mutation alone reduces sensitivity to permethrin but not the type II pyrethroid, deltamethrin (Du et al., 2013).

We observed the T1520I mutation in all *Ae. aegypti* populations tested (Table 2), which never occurred by itself and was always present with at least the F1534C mutation (Table 3). The co-occurrence of T1520I and F1534C mutations has also been reported in *Ae. aegypti* from India (Kushwah et al., 2015) and Myanmar (Naw et al., 2020). Expression of mutant *Ae. aegypti* vgsc in Xenopus oocytes showed that alone the T1520I mutation did not alter sensitivity to permethrin but that the double T1520I + F1534C mutant was more resistant to permethrin than just F1534C whilst remaining sensitive to deltamethrin (Chen et al., 2019). This suggests that the addition of T1520I to F1534C may heighten the tolerance of mosquitoes to type I pyrethroids.

The S989P and V1016G mutations were found in all the *Ae. aegypti* populations studied (Table 2). Both mutations were always found together and both were either heterozygous or homozygous (Table 3). This confirms the previous report that S989P and V1016G mutations are present in Laos where the homozygous S989P + V1016G double mutant, which is found mainly in *Ae. aegypti* from Xaythany, was associated with deltamethrin resistance (Shimono et al., 2021 and Table 3). The co-occurrence of both mutations, which confers resistance to pyrethroids (Du et al., 2016), has also been observed in *Ae. aegypti* from several other countries such as Thailand (Stenhouse et al., 2013), Myanmar (Kawada et al., 2014), Singapore (Kasai et al., 2014), China (Li et al., 2015), Indonesia (Wulandari et al., 2015), Sri Lanka (Fernando et al., 2018), Malaysia (Leong et al., 2019), Taiwan (Chung et al., 2019) and Saudi Arabia (Fang et al., 2021).

In *Ae. aegypti* mosquitoes from Saysettha and Vangvieng, the most prevalent genotype observed was heterozygous for the triple S989P + V1016G + F1534C mutant (Table 3). This genotype was also found in mosquitoes from Kaisone, Xaythany, Pakkading (Borlikhamxay province) and Khounkham (Khammouane Province) but not Thakhek (Khammouane Province; Table 3 and Shimono et al., 2021), demonstrating that it has reached several districts throughout Laos. Crossing experiments to generate *Ae. aegypti* with known vgsc genotypes showed that resistance to deltamethrin in heterozygous S989P + V1016G + F1534C mosquitoes were 28 fold when compared to the homozygous S989 + V1016 + F1534
susceptible strain (Plernsub, Saingamsook, Yanola, Lumjuan, Tippawangkosol, Sukontason, et al., 2016). Mosquitoes with the S989P + V1016G double mutation showed 4-fold resistance to deltamethrin demonstrating that the addition of the F1534C mutation heightens pyrethroid resistance (Plernsub, Saingamsook, Yanola, Lumjuan, Tippawangkosol, Sukontason, et al., 2016). In line with this, *Ae. aegypti* vgsc with the S989P + V1016G + F1534C triple mutation expressed in Xenopus oocytes was considerably less sensitive to permethrin or deltamethrin than the S989P + V1016G double mutant (Hirata et al., 2014). Therefore, our findings of heterozygous S989P + V1016G + F1534C in *Ae. aegypti* in four districts (Table 3) indicate resistance to pyrethroid types I and II in Laos. We found only one *Ae. aegypti*, from Saysettha, to harbour the homozygous S989P + V1016G + F1534C triple mutant (Table 3) and only one mosquito with this genotype was observed from Pakkading and Khounkham (Shimono et al., 2021). *Ae. aegypti* with the homozygous triple mutant were found to be nearly two-fold more resistant to deltamethrin than the heterozygous triple mutant (Plernsub, Saingamsook, Yanola, Lumjuan, Tippawangkosol, Sukontason, et al., 2016). Thus, it is prudent to continue monitoring for the increase in the prevalence of the homozygous S989P + V1016G + F1534C triple mutant, which would signal further selection for pyrethroid resistance.

For the first time, the presence of resistance mutations in the vgsc gene in *Ae. albopictus* from Laos was surveyed. S989P and V1016G mutations were not detected, however, F1534C were observed in mosquitoes from both Saysettha and Vangvieng whilst T1520I was only seen in mosquitoes from Saysettha (Table 2). The most prevalent genotype for *Ae. albopictus* from Saysettha was homozygous wild-type with no mutations whilst the second most common genotype was the heterozygous F1534C mutation (Table 3). For Vangvieng, the F1534C heterozygote was the most frequent genotype whilst the second most common genotype was the homozygous F1534C mutation, which may indicate an emerging resistance to type I pyrethroids in this district (Pichler et al., 2019; Yanola et al., 2011). In line with this, *Ae. albopictus* taken from Kao-gnot, a village near our study sites in the Vientiane Capital, showed suspected resistance to permethrin (Tangena et al., 2018). In agreement with bioassays performed on *Ae. albopictus* (Tangena et al., 2018), the lack of S989P and V1016G mutations indicates that *Ae. albopictus* in Laos remain susceptible to deltamethrin. However, the detection of the V1016G mutation in *Ae. albopictus* from Italy and Vietnam (Kasai et al., 2019; Pichler et al., 2019) suggests that these mutations may be selected for with continued use of pyrethroids.

Our study focused on domains II and III of the vgsc gene. However, mutations associated with pyrethroid resistance have been found in other domains, such as V410L in domain I of *Ae. aegypti* from America (Granada et al., 2018; Haddi et al., 2017; Saavedra-Rodriguez et al., 2018) and D1763Y in domain IV of *Ae. aegypti* from Taiwan (Chang et al., 2009). An initial analysis did not detect a mutation at 410 in 12 *Ae. aegypti* mosquitoes (Table 2) but it is prudent to analyse sequences of all domains in the future in order to understand more comprehensively the prevalence of target-site resistance in Laos.

We observed intron variants in the vgsc genes in both *Ae. aegypti* and *Ae. albopictus*. For *Ae. aegypti* we detected two different sequences for intron 20 with lengths of 250 and 234 bp, respectively, denoted as groups A and B (Martins et al., 2009). It has been noted that there is a link between intron length and the presence of resistance mutations, where the I1011M and the V1016I mutations were found only when there was the longer group A intron in *Ae. aegypti* from Brazil (Martins et al., 2009). We observed the group A intron co-occurring with the S989P and V1016G mutations (Figure 3), and that the F1534C mutation, when without S989P and V1016G, was found with the group B intron, which was also seen in *Ae. aegypti* from Taiwan and Saudi Arabia (Chung et al., 2019; Fang et al., 2021). However, F1534C was found with the group A intron in *Ae. albopictus* from Ghana (Kawada et al., 2016), which may reflect different histories of insecticide usage and mutation events in Asia and Africa (Cosme et al., 2020; Fang et al., 2021).

We found four novel variants for intron 20 in *Ae. albopictus* vgsc (Figure 3), which has a low sequence identity (around 30%) to Groups A and B of *Ae. aegypti*. Also, six different sequences ranging in length from 68 to 83 bp were detected for *Ae. albopictus* intron 29, which precedes F1534 (Figure 4). All of the *Ae. albopictus* intron 29 variants occurred in the wild-type vgsc lacking mutations in domains II and III and where T1520I and F1534C mutations were detected there did not appear to be an association of particular variants of either intron 20 or intron 29 with resistance mutations. Interestingly, the majority of vgsc sequences lacking resistance mutations did not possess the 69 bp intron 29 sequence (Table 3). Further analysis of *Ae. albopictus* vgsc sequences should be conducted to determine whether there are links between intron 20 and 29 sequences and resistance mutations or lack thereof.

Our detection of mutations in the vgsc gene of *Ae. aegypti* from the four districts studied highlights previous findings that insecticide resistance in Laos is of concern and is threatening efforts to maintain effective vector control (Marcombe et al., 2019; Shimono et al., 2021). Therefore, integrated vector control approaches and continuous insecticide resistance monitoring programmes are of prime importance in order to control diseases caused by arboviruses in this country. The Lao government recently (2019) adopted a new strategy to deploy alternative larvicides with different modes of action to overcome the spread of temephos resistance in *Ae. aegypti* larvae (Marcombe et al., 2018). Repurposing insecticides originally used in agriculture may provide further vector control tools. For example, the neonicotinoid clothianidin, used in conjunction with deltamethrin, has the potential to prolong the control of the malaria mosquito, *An. gambiense*, which are showing resistance to pyrethroids (Ngufor et al., 2017). A similar strategy could be used for insecticides that act on γ-aminobutyric acid (GABA) receptors, such as fipronil, especially considering that it has been shown that Aedes mosquitoes in Laos lack a mutation at A296 in the GABA receptor, resistance to dieldrin (RDL), which underlies insecticide resistance (Marcombe et al., 2020). A combination of fipronil and permethrin has been shown to provide highly effective inhibition of feeding as well as considerable insecticidal efficacy against *Aedes* mosquitoes on dogs for at least four weeks, which may aid in the control of mosquitoes in the vicinity of treated domesticated animals (Fankhauser et al., 2015).
Based on bioassays (Tangen et al., 2018) and the present study, the status of pyrethroid resistance in Ae. albopictus from Laos is not of serious concern when compared to that of Ae. aegypti. However, there are signs of emerging resistance to type I pyrethroids, in particular in the Vientiane province. A similar situation appears to be occurring on a more global scale, with an increase in vgsc mutations observed in Ae. albopictus samples that were taken between 2011 and 2018, most notably in China, Greece and Italy (Tancredi et al., 2020).

Thus, as with Ae. aegypti, constant monitoring programmes and alternative strategies for controlling Ae. albopictus should be utilized in order to prolong the effectiveness of pyrethroids against this species thereby maximizing vector control.

**AUTHOR CONTRIBUTIONS**
Andrew K. Jones and Sebastien Marcombe conceived the idea for the project. Sebastien Marcombe, Phonesavanh Luangamath, Somphat Nilakay, Vacky Vungkkyly, Phoutmany Thammavong and Paul T. Brey supervised mosquito collections in Laos, rearing and laboratory tests. Sebastien Marcombe and Phoutmany Thammavong analysed insecticide resistance tests and contributed to writing the manuscript. Katherine Shimell, Rachel Savage, Edward Howlett, Anne Baby, Mathew King, Josie Clarke, Chloe Jeffries, Josna Jojo, Emily Lacey, Farris Bhatty, Dadirayi Mabika, Andrea Dela Cruz, Cerys Fisher, Milca Mbadu and Iasonas Despinias extracted DNA from mosquitoes, amplified domains II and III of vgsc and analysed sequence data. Andrew K. Jones analysed sequence data and contributed to writing the manuscript. All authors read and approved the final manuscript.

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**CONFLICT OF INTEREST**
The authors declare that they have no competing interests.

**DATA AVAILABILITY STATEMENT**
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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**REFERENCES**
Al Nazawi, A.M., Aqili, J., Alzahrani, M., McCall, P.J. & Weetman, D. (2017) Combined target site (kdr) mutations play a primary role in highly pyrethroid resistant phenotypes of Aedes aegypti from Saudi Arabia. Parasites & Vectors, 10, 161.

Brengues, C., Hawkes, N.J., Chandre, F., McCarroll, L., Duchon, S., Guillet, P. et al. (2003) Pyrethroid and DDT cross-resistance in Aedes aegypti is correlated with novel mutations in the voltage-gated sodium channel gene. Medical and Veterinary Entomology, 17, 87–94.

Calvez, E., Pommelet, V., Somlor, S., Pompon, J., Viengphouthong, S., Bounmany, P. et al. (2020) Trends of the dengue Serotype-4 circulation with epidemiological, phylogenetic, and entomological insights in Lao PDR between 2015 and 2019. Pathogens, 9, 728.

Chang, C., Shen, W.K., Wang, T.T., Lin, Y.H., Hsu, E.L. & Dai, S.M. (2009) A novel amino acid substitution in a voltage-gated sodium channel is associated with knockdown resistance to permethrin in Aedes aegypti. Insect Biochemistry and Molecular Biology, 39, 272–278.

Chatterjee, M., Ballav, S., Maji, A.K., Basu, N., Sarkar, B.C. & Saha, P. (2018) Polymorphisms in voltage-gated sodium channel gene and susceptibility of Aedes albopictus to insecticides in three districts of northern West Bengal, India. PLoS Neglected Tropical Diseases, 12, e0006192.

Chen, M., Du, Y., Nomura, Y., Zhorov, B.S. & Dong, K. (2020) Chronology of sodium channel mutations associated with pyrethroid resistance in Aedes aegypti. Archives of Insect Biochemistry and Physiology, 104, e21686.

Chen, M., Du, Y., Wu, S., Nomura, Y., Zhu, G., Zhorov, B.S. et al. (2019) Molecular evidence of sequential evolution of DDT- and pyrethroid-resistant sodium channel in Aedes aegypti. PLoS Neglected Tropical Diseases, 13, e0007432.

Chung, H.H., Cheng, I.C., Lin, C., Tomita, T. & Teng, H.J. (2019) Voltage-gated sodium channel intron polymorphism and four mutations comprise six haplotypes in an Aedes aegypti population in Taiwan. PLoS Neglected Tropical Diseases, 13, e0007291.

Cosme, L.V., Gloria-Soria, A., Caccone, A., Powell, J.R. & Martins, A.J. (2020) Evolution of kdr haplotypes in worldwide populations of Aedes aegypti: independent origins of the F1534C kdr mutation. PLoS Neglected Tropical Diseases, 14, e0008219.

Djappi-Tchamen, B., Nana-Ndjangwo, M.S., Mavridis, K., Talipouo, A., Nchoutpouen, E., Makoudjou, I. et al. (2021) Analyses of insecticide resistance genes in Aedes aegypti and Aedes albopictus mosquito populations from Cameroon. Genes, 12, 828.

Du, Y., Nomura, Y., Satar, G., Hu, Z., Nauen, R., He, S.Y. et al. (2013) Molecular evidence for dual pyrethroid-receptor sites on a mosquito sodium channel. Proceedings of the National Academy of Sciences of the United States of America, 110, 11785–11790.

Du, Y., Nomura, Y., Zhorov, B.S. & Dong, K. (2016) Sodium channel mutations and pyrethroid resistance in Aedes aegypti. Insects, 7, 60.

Fan, Y., O’Grady, P., Yoshimizu, M., Ponlawat, A., Kaufman, P.E. & Scott, J. G. (2020) Evidence for both sequential mutations and recombination in the evolution of kdr alleles in Aedes aegypti. PLoS Neglected Tropical Diseases, 14, e0008154.

Fang, Y., Tambo, E., Xue, J.B., Zhang, Y., Zhou, X.N. & Khater, E.I.M. (2021) Molecular analysis of targeted insecticide resistance gene mutations in field-caught mosquitoes of medical importance from Saudi Arabia. Journal of Medical Entomology, 58, 1839–1848.

Fankhauser, B., Dumont, P., Hunter, J.S., 3rd, McCall, J.W., Kaufmann, C., Mathis, A. et al. (2015) Repellent and insecticidal efficacy of a new combination of fipronil and permethrin against three mosquito species (Aedes albopictus, Aedes aegypti and Culex pipiens) on dogs. Parasites & Vectors, 8, 64.

Fernando, S.D., Alapuga, M., Perera, R., Saavedra-Rodriguez, K., Black, W.C. & De Silva, N.K. (2018) First report of V1016G and S989P knockdown resistant (kdr) mutations in pyrethroid-resistant Sri Lankan Aedes aegypti mosquitoes. Parasites & Vectors, 11, 526.

Granada, Y., Mejia-Jaramillo, A.M., Strode, C. & Triana-Chavez, O. (2018) A point mutation V419L in the sodium channel gene from natural populations of Aedes aegypti is involved in resistance to lambda-Cyhalothrin in Colombia. Insects, 9, 23.
Haddi, K., Tone, H.V.V., Du, Y., Valbon, W.R., Nomura, Y., Martins, G.F. et al. (2017) Detection of a new pyrethroid resistance mutation (V410L) in the sodium channel of Aedes aegypti: a potential challenge for mosquito control. Scientific Reports, 7, 46549.

Hirata, K., Komagata, O., Itoikawa, K., Yamamoto, A., Tomita, T. & Kasai, S. (2014) A single crossing-over event in voltage-sensitive Na+ channel genes may cause critical failure of dengue mosquito control by insecticides. PLoS Neglected Tropical Diseases, 8, e3085.

Kasai, S., Caputo, B., Tsunoda, T., Cuong, T.C., Maekawa, Y., Lam-Phua, S.G. et al. (2019) First detection of a Vssc allele V1016G conferring a high level of insecticide resistance in Aedes albopictus collected from Europe (Italy) and Asia (Vietnam), 2016: a new emerging threat to controlling arboviral diseases. Euro Surveill, 24, 1700847.

Kasai, S., Komagata, O., Itoikawa, K., Shono, T., Ng, L.C., Kobayashi, M. et al. (2014) Mechanisms of pyrethroid resistance in the dengue mosquito vector, Aedes aegypti: target site insensitivity, penetration, and metabolism. PLoS Neglected Tropical Diseases, 8, e2948.

Kawada, H., Higa, Y., Futami, K., Muranami, Y., Kawashima, E., Osei, J.H. et al. (2016) Discovery of point mutations in the voltage-gated Sodium Channel from African Aedes aegypti populations: potential phylogenetic reasons for gene introgression. PLoS Neglected Tropical Diseases, 10, e0004780.

Kawada, H., Oo, S.Z., Thaug, S., Kawashima, E., Maung, Y.N., Thu, H.M. et al. (2014) Co-occurrence of point mutations in the voltage-gated sodium channel of pyrethroid-resistant Aedes aegypti populations in Myanmar. PLoS Neglected Tropical Diseases, 8, e3032.

Kushwah, R.B., Dykes, C.L., Kapoor, N., Adak, T. & Singh, O.P. (2015) Pyrethroid-resistance and presence of two knockdown resistance (kdr) mutations, F1534C and a novel mutation T1520I, in Indian Aedes aegypti. PLoS Neglected Tropical Diseases, 9, e3332.

Leong, C.S., Vythilingam, I., Liew, J.W., Wong, M.L., Wan-Yusuf, W.S. & Lau, Y.L. (2019) Enzymatic and molecular characterization of insecticide resistance mechanisms in field populations of Aedes aegypti from Selangor, Malaysia. Parasit Vectors, 12, 236.

Li, C.X., Kaufman, P.E., Xue, R.D., Zhao, M.H., Wang, G., Yan, T. et al. (2015) Relationship between insecticide resistance and kdr mutations in the dengue vector Aedes aegypti in southern China. Parasites & Vectors, 8, 325.

Lwande, O.W., Obanda, V., Lindstrom, A., Ahlm, C., Evander, M., Naslund, J. et al. (2020) Globe-trotting Aedes aegypti and Aedes albopictus: risk factors for arbovirus pandemics. Vector Borne and Zoonotic Diseases, 20, 71–81.

Marcombe, S., Chonephetsarath, S., Thammavong, P. & Brey, P.T. (2018) Alternative insecticides for larval control of the dengue vector Aedes aegypti in Lao PDR: insecticide resistance and semi-field trial study. Parasites & Vectors, 11, 616.

Marcombe, S., Fustec, B., Cattel, J., Chonephetsarath, S., Thammavong, P., Phommavanh, N. et al. (2019) Distribution of insecticide resistance and mechanisms involved in the arbovirus vector Aedes aegypti in Laos and implication for vector control. PLoS Neglected Tropical Diseases, 13, e0007852.

Marcombe, S., Thammavong, P., Luangamath, P., Chonephetsarath, S., Phommavanh, N., Lakeomany, K. et al. (2020) Malaria and dengue mosquito vectors from Lao PDR show a lack of the rdl mutant allele responsible for Cycloidiene insecticide resistance. Journal of Medical Entomology, 57, 815–823.

Martins, A.J., Lins, R.M., Lins, J.G., Peixoto, A.A. & Valle, D. (2009) Voltage-gated sodium channel polymorphism and metabolic resistance in pyrethroid-resistant Aedes aegypti from Brazil. The American Journal of Tropical Medicine and Hygiene, 81, 108–115.

Naw, H., Su, M.N.C., Vo, T.C., Le, H.G., Kang, J.M., Jun, H. et al. (2020) Overall prevalence and distribution of knockdown resistance (kdr) mutations in Aedes aegypti from Mandalay region, Myanmar. The Korean Journal of Parasitology, 58, 709–714.

Ngufor, C., Fongnikin, A., Rowland, M. & N’Guessan, R. (2017) Indoor residual spraying with a mixture of clothianidin (a neonicotinoid insecticide) and deltamethrin provides improved control and long residual activity against pyrethroid resistant Anopheles gambiae s.s in southern Benin. PLoS One, 12, e0189575.

Pichler, V., Malandruccolo, C., Serini, P., Bellini, R., Severini, F., Toma, L. et al. (2019) Phenotypic and genotypic pyrethroid resistance of Aedes albopictus, with focus on the 2017 chikungunya outbreak in Italy. Pest Management Science, 75, 2642–2651.

Plermsub, S., Saingamsook, J., Yanola, J., Lumjuan, N., Tippawangkosol, P., Sukontason, K. et al. (2016) Additive effect of knockdown resistance mutations, S989P, V1016G and F1534C, in a heterozygous genotype conferring pyrethroid resistance in Aedes aegypti in Thailand. Parasites & Vectors, 9, 417.

Plermsub, S., Saingamsook, J., Yanola, J., Lumjuan, N., Tippawangkosol, P., Walton, C. et al. (2016) Spatial and temporal frequency of knockdown resistance mutations, F1534C and V1016G, in Aedes aegypti in Chiang Mai city, Thailand and the impact of the mutations on the efficiency of thermal fogging spray with pyrethroids. Acta Tropica, 162, 125–132.

Rajatieleka, S., Black, W.C.T., Saavedra-Rodriguez, K., Trongtokit, Y., Apiwathananorn, C., McCall, P.J. et al. (2008) Development and application of a simple colorimetric assay reveals widespread distribution of sodium channel mutations in Thai populations of Aedes aegypti. Acta Tropica, 108, 54–57.

Ranathunge, T., Udayangala, L., Sarasija, S., Karunathilaka, S., Nawaratne, K., Rathnaraajah, H. et al. (2021) Voltage-gated sodium channel (Vgsc) mutation-based pyrethroid resistance in Aedes aegypti populations of three endemic dengue risk areas of Sri Lanka. BioMed Research International, 2021, 8874092.

Rattanarithikul, R., Harrison, B.A., Harbach, R.E., Panthushiri, P., Coleman, R.E. & Panthushiri, P. (2006) Illustrated keys to the mosquitoes of Thailand. IV. Anopheles. The Southeast Asian Journal of Tropical Medicine and Public Health, 37 Suppl 2, 1–128.

Saavedra-Rodriguez, K., Maloof, F.V., Campbell, C.L., Garcia-Rejon, J., Lenhart, A., Penilla, P. et al. (2018) Parallel evolution of vgs mutation at domains I56, I56 and I56 in pyrethroid resistant Aedes aegypti from Mexico. Scientific Reports, 8, 6747.

Saingamsook, J., Saeung, A., Yanola, J., Lumjuan, N., Walton, C. & Somboon, P. (2017) A multiplex PCR for detection of knockdown resistance mutations, V1016G and F1534C, in pyrethroid-resistant Aedes aegypti. Parasites & Vectors, 10, 465.

Shimono, T., Kanda, S., Lamaningao, P., Murakami, Y., Darcy, A.W., Mishima, N. et al. (2021) Phenotypic and haplotypic profiles of insecticide resistance in populations of Aedes aegypti larvae (Diptera: Culicidae) from central Lao PDR. Trop Med Health, 49, 32.

Smith, L.B., Kasai, S. & Scott, J.G. (2016) Pyrethroid resistance in Aedes aegypti and Aedes albopictus: important mosquito vectors of human diseases. Pesticide Biochemistry and Physiology, 133, 1–12.

Stenhouse, S.A., Plermsub, S., Yanola, J., Lumjuan, N., Dantrakool, A., Choochote, W. et al. (2013) Detection of the V1016G mutation in the voltage-gated sodium channel gene of Aedes aegypti (Diptera: Culicidae) by allele-specific PCR assay, and its distribution and effect on deltamethrin resistance in Thailand. Parasites & Vectors, 6, 253.

Tancredi, A., Papandrea, D., Marconcini, M., Carballar-Lejarazu, R., Casas-Martinez, M., Lo, E. et al. (2020) Tracing temporal and geographic distribution of resistance to pyrethroids in the arboviral vector Aedes albopictus. PLoS Neglected Tropical Diseases, 14, e0008350.

Tagana, J.A., Marcombe, S., Thammavong, P., Chonephetsarath, S., Somphong, B. & Sayteng, K. et al. (2018) Bioinformatics and insecticide resistance of the arboviral vector Aedes albopictus in northern Lao PDR. PLoS One, 13, e0206387.

Thompson, J.D., Gibson, T.J., Plewistik, F., Jeammoign, F. & Higgins, D.G. (1997) The CLUSTAL_X windows interface: flexible strategies for
multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, 25, 4876–4882.

Wei, Y., Zheng, X., He, S., Xin, X., Zhang, J., Hu, K. et al. (2021) Insecticide susceptibility status and knockdown resistance (kdr) mutation in *Aedes albopictus* in China. *Parasites & Vectors*, 14, 609.

Wilson, A.L., Courtenay, O., Kelly-Hope, L.A., Scott, T.W., Takken, W., Torr, S.J. et al. (2020) The importance of vector control for the control and elimination of vector-borne diseases. *PLoS Neglected Tropical Diseases*, 14, e0007831.

World Health Organization. (2022) Dengue and severe dengue.

Wuliandari, J.R., Lee, S.F., White, V.L., Tantowijoyo, W., Hoffmann, A.A. & Endersby-Harshman, N.M. (2015) Association between three mutations, F1565C, V1023G and S996P, in the voltage-sensitive sodium channel gene and knockdown resistance in *Aedes aegypti* from Yogjakarta, Indonesia. *Insects*, 6, 658–685.

Yanola, J., Somboon, P., Walton, C., Nachaiwieng, W., Somwang, P. & Prapanthadara, L.A. (2011) High-throughput assays for detection of the F1534C mutation in the voltage-gated sodium channel gene in permethrin-resistant *Aedes aegypti* and the distribution of this mutation throughout Thailand. *Tropical Medicine & International Health*, 16, 501–509.

Zardkoohi, A., Castaneda, D., Lol, J.C., Castillo, C., Lopez, F., Marin Rodriguez, R. et al. (2020) Co-occurrence of kdr mutations V1016I and F1534C and its association with phenotypic resistance to Pyrethroids in *Aedes aegypti* (Diptera: Culicidae) populations from Costa Rica. *Journal of Medical Entomology*, 57, 830–836.

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