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Chronology of sodium channel mutations associated with pyrethroid resistance in *Aedes aegypti*

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Abstract

Aedes aegypti is the primary mosquito vector of dengue, yellow fever, Zika and chikungunya. Current strategies to control *Ae. aegypti* rely heavily on insecticide interventions. Pyrethroids are a major class of insecticides used for mosquito control because of their fast acting, highly insecticidal activities and low mammalian toxicity. However, *Ae aegypti* populations around the world have begun to develop resistance to pyrethroids. So far, more than a dozen mutations in the sodium channel gene have been reported to be associated with pyrethroid resistance in *Ae. aegypti*. Co-occurrence of resistance-associated mutations is common in pyrethroid-resistant *Ae aegypti* populations. As global use of pyrethroids in mosquito control continues, new pyrethroid-resistant mutations keep emerging. In this microreview, we compile pyrethroid resistance-associated mutations in *Ae. aegypti* in a chronological order, as they were reported, and summarize findings from functional evaluation of these mutations in an *in vitro* sodium channel expression system. We hope that the information will be useful for tracing possible evolution of pyrethroid resistance in this important human disease vector, in addition to the development of methods for global monitoring and management of pyrethroid resistance in *Ae. aegypti*.

Voltage-gated sodium channels are critical for electrical signaling in the nervous system. Pyrethroids prolong the opening of insect sodium channels, resulting in disruption of the function of the nervous system (Narahashi, 1988; Soderlund, 2005; Dong et al., 2014; Silver et al., 2014; Field, Emyr Davies, O'Reilly, Williamson, & Wallace, 2017). Pyrethroids are used worldwide in the control of human disease-transmitting insect vectors, including mosquitoes. Various commercial products of pyrethroids, such as mosquito aerosol sprays and mosquito coils, are widely used in control of adult mosquitoes. Unfortunately, spreading

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mosquito resistance to pyrethroids has become a serious global threat for effective mosquito control to curtail transmission of devastating human diseases including dengue, yellow fever, Zika and chikungunya. One major form of pyrethroid resistance, known as knockdown resistance (kdr), is caused by mutations in insect sodium channels, which result in reduced sodium channel and neuronal sensitivity to pyrethroids (Dong et al., 2014; Field, Emyr Davies, O'Reilly, Williamson, & Wallace, 2017; Rinkevich, Du, & Dong, 2013; Soderlund, 2005; Soderlund & Bloomquist, 1990). This type of resistance mechanism has been documented globally in all major arthropod pests and disease vectors, including *Aedes* mosquitoes (Amelia-Yap et al., 2019; Du, Nomura, Zhorov, & Dong, 2016; Moyes et al., 2017; Smith, Kasai, & Scott, 2016). Besides target-site insensitivity, other mechanisms of resistance, particularly 450-mediated metabolic detoxification, also play very important roles in conferring pyrethroid resistance in many mosquito populations around the world. Furthermore, multiple mechanisms may co-selected in the same population and they could potentially produce a synergistic effect on resistance. Comprehensive reviews on pyrethroid resistance in *Aedes* mosquitoes can be found in Smith et al., (2016) and Moyes et al., (2017). This microreview is intended to chronicle sodium channel mutations that are associated with pyrethroid resistance and summarize the effects of these mutations on sodium channel response to pyrethroids.

Like their mammalian orthologs, the pore-forming α -subunit of an insect sodium channel folds from a single polypeptide chain of four homologous repeats (I-IV), each having six transmembrane segments (S1-S6) (Fig. 1). Segments S1-S4 in each repeat form a voltage-sensing domain (VSD), whereas S5s, S6s, and the membrane reentrant extracellular loops (called the P-region with two helices P1 and P2) between S5s and S6s form the pore domain (PD). Unlike mammals, which have nine sodium channel genes, however, only one sodium channel gene is found in *Ae. aegypti* and most of other insects (Dong et al., 2014, Silva and Scott, 2019). Figure 1 and Table 1 depict thirteen sodium channel mutations that have been reported, to date, to be associated with pyrethroid-resistance in *Ae. aegypti* populations around the world. Most of molecular and toxicological analyses of *Ae. aegypti* field populations for pyrethroid resistance used permethrin, a type I pyrethroid, and deltamethrin, a type II pyrethroid. Two additional type II pyrethroids, cyfluthrin and lambda-cyhalothrin, were used in some studies (Aponte, Penilla, Rodríguez, & Ocampo, 2019; Granada, Mejía-Jaramillo, Strode, & Triana-Chavez, 2018; Leong et al., 2019; Lien, Ngoc, Hien, Hoang, & Binh, 2018; Maestre-Serrano, Pareja-Loaiza, Gomez Camargo, Ponce-Garcia, & Flores, 2019; Pinto et al., 2019; Sayono et al., 2016; Seixas et al., 2017).

Several aspects of pyrethroid resistance-associated mutations in *Ae. aegypti* are notable. First, most studies so far focused on targeted sequence analysis of the genomic regions encoding IIS6 and/or IIS6 because earlier studies from other arthropod pests identified several kdr mutations in these regions (Dong et al., 2014; Rinkevich et al., 2013). Interestingly, kdr mutations which were detected in earlier studies from other species (Rinkevich et al., 2013), such as L1014F in IIS6 and F1538I in IIS6, are not found in pyrethroid resistant *Ae. aegypti* mosquitoes. Second, the V1016G mutation in IIS6, the first kdr mutation discovered in *Ae. aegypti*, was first reported in Thailand in 2002 (Bregues et al., 2003; Prapanthadara et al., 2002). Since then, this mutation has remained prevalent and exclusive to pyrethroid-resistant populations in Asia, except for a recent report of its

detection in a single mosquito in Panama (Murcia et al., 2019). Another mutation, S989P, was found concurrent with V1016G in Asian populations. In a recent study, S989P alone (without V1016G or F1534C) was detected in some *Ae. aegypti* individuals, but the association of S989P with pyrethroid resistance is uncertain because this mutation is found in pyrethroid-susceptible individuals (Leong et al., 2019). V1016I, another substitution of V1016 in IIS6, was first reported in 2007 (Saavedra-Rodriguez et al., 2007) and subsequently in many other subsequent studies (Table 1). This mutation is detected mainly in Latin America and more recently in the US populations with one exception of detecting this mutation in Vietnam (Table 1). Third, the I1011M mutation was first reported in 2003 together with another mutation, G923V, but was later detected alone (i.e., without the G923V mutation) in several studies or, more recently, in combination with the V1016G mutation in one individual mosquito in Panama (Murcia et al., 2019). A related mutation at this amino acid position, I1011V, was reported in two studies (Table 1). Fourth, the G923V and L982W mutations were detected only in one study in 2003 (Bregues et al., 2003) and have not been reported since. Fifth, one mutation, F1534C, first documented in Vietnam in 2009 (Kawada et al., 2009), has been repeatedly detected in numerous *Ae. aegypti* populations in Asia, South and North Americas and Africa (Table 1). In India, F1534C was found to be linked to T1520I (R. B. Kushwah, Dykes, Kapoor, Adak, & Singh, 2015). More recently F1534C was found to co-occur with another mutation, A1007G, in Vietnam (Lien et al., 2018). F1534L, an alternative substitution at F1534, was also found in India (Kushwah et al., 2019).

In addition to IIS6- and IIS6-targeted sequencing, at least three studies (Chang et al., 2009; Haddi et al., 2017; Li et al., 2015) performed sequence analysis of the entire sodium channel coding region and uncovered two new mutations outside the IIS6 and IIS6 regions: D1763Y in the P-region connecting S5 and S6 in repeat IV (Chang et al., 2009) and V410L in IS6 (Haddi et al., 2017). While V410L was first documented in a Brazilian population in 2017 (Haddi et al., 2017), a paper published in 2018 reported identification of this mutation in populations collected as early as 2002 in Mexico (Saavedra-Rodriguez et al., 2018). Subsequent sequence analysis of IS6 uncovered the V410L mutation in other countries in Latin America. However, D1763Y was reported only in Taiwan and linked to V1016G (Chang et al., 2009; Chung et al., 2019).

Detection of species-specific *kdr* mutations is uniquely high in *Ae. aegypti*. To date, of 13 mutations, only four mutations have been reported in other insect pests. These are V1016G and F1534C/L in *Ae. albopictus* (Aguirre-Obando, Martins, & Navarro-Silva, 2017; Gao, Chen, Shi, Peng, & Ma, 2018; Kasai et al., 2019; Kasai et al., 2011; Pichler et al., 2019; Xu et al., 2016; Zhou et al., 2019) and V410L in *Cimex lectularis* (Dang, Toi, Lilly, Bu, & Doggett, 2015; Yoon et al., 2008). Furthermore, concurrence of two or more mutations was frequently documented in many of recent studies (Table 1), which is often associated with high levels of pyrethroid resistance. For example, concurrence of S989P and V1016G was detected in Asia populations that exhibit high resistance (Kasai et al., 2014; Srisawat et al., 2010; Srisawat et al., 2012). In another example, F1534C alone was found to be associated with a low level of resistance, but when linked with other mutations, such as V1016G or S989P + V1016G in Asian populations, it is associated with higher levels of resistance (Ishak, Jaal, Ranson, & Wondji, 2015; Leong et al., 2019; Li et al., 2015; Plernsub et al.,

2016; Wuliandari et al., 2015). Similarly, the F1534C + V1016I double mutation, which was repeatedly detected in many populations in Latin America, is often associated with higher levels of pyrethroid resistance (e.g., Vera-Maloof, Saavedra-Rodriguez, Elizondo-Quiroga, Lozano-Fuentes, & Black Iv, 2015). The frequency of individuals carrying only V1016I (lacking F1534) was extremely low (e.g., Alvarez, Ponce, Saavedra-Rodriguez, Lopez, & Flores, 2015; Vera-Maloof et al., 2015). The V410L + V1016I + F1534C triple mutation was detected in Mexican populations, and mosquitoes homozygous for the three mutations exhibited a greater level of knockdown resistance to both permethrin and deltamethrin (Saavedra-Rodriguez et al., 2018).

Development of the *Xenopus* oocyte expression system for *Ae. aegypti* sodium channels allowed direct functional studies of the impact of *Ae. aegypti* kdr mutations on sodium channel sensitivity to pyrethroids. In these studies, the experimental procedures involved introduction of single or multiple mutations into wild-type *Ae. aegypti* sodium channels, AaNa_v1-1 or AaNa_v2S, and sensitivity of mutant channels to pyrethroids was compared to wild-type channels in *Xenopus* oocytes using two-electrode voltage clamp techniques (Dong et al., 2014). Most of the mutations presented in Fig. 1, either alone or in combinations, have been functionally examined in the *Xenopus* oocyte expression system (Table 2). F1543C was found to confer a low level of AaNa_v1-1 or AaNa_vS2 channel resistance to type I pyrethroids (e.g., permethrin), but not to type II pyrethroids (e.g., deltamethrin) (Chen et al., 2019; Du et al., 2013; Haddi et al., 2017; Hirata et al., 2014). Similar results were obtained using the cockroach BgNa_v1-1a sodium channel (Hu, Du, Nomura, & Dong, 2011). V410L and V1016G, alone, conferred moderate levels of AaNa_v1-1 channel resistance to both type I (permethrin) and type II (deltamethrin) pyrethroids (Table 2). Interestingly, the V410L + F1543C double mutations conferred greater levels of AaNa_v1-1 channel resistance to permethrin, but not to deltamethrin (Haddi et al., 2017). V1016I alone did not alter the sensitivity of the AaNa_v1-1 channel to either permethrin or deltamethrin. However, V1016I enhanced F1543C-mediated AaNa_v1-1 channel resistance to both Type I and Type II pyrethroids even though F1543C alone conferred resistance to only Type I pyrethroids (Chen et al., 2019). Thus, V1016I can be considered as an enhancer of pyrethroid resistance (Chen et al., 2019). T1520I, like V1016I, often co-occurred with F1543C and it also enhanced F1543C-mediated AaNa_v1-1 channel resistance to permethrin, but not to deltamethrin (Chen et al., 2019). It would be interesting to determine in the future whether A1007G, a recently discovered mutation associated with pyrethroid resistance in Vietnam (Lie et al., 2018), also acts as an enhancer of F1543C-mediated resistance.

The unique effect of the F1534C mutation on type I pyrethroids, but not on type II pyrethroids, is consistent with bioassay results from various mosquito field populations (e.g., Yanola et al., 2011; Chang et al., 2009; Wuliandari et al., 2015; Harris et al., 2010). However, in some cases, mosquitoes carrying F1534C were resistant to both permethrin and deltamethrin (e.g., Kushwah et al., 2015; Kasai et al., 2019; Fan and Scott, 2020). In these cases, it is likely that additional mutations or background nucleotide sequence polymorphisms in the sodium channel gene could influence the interaction of type II pyrethroids with F1534C, resulting in resistance to type II pyrethroids. As described above, this appears to be the case for the V1016I mutation, which by itself does not confer resistance to either type of pyrethroids. However, when combined with F1534C, it causes

sodium channel resistance to both permethrin and deltamethrin in *Xenopus* oocytes (Chen et al., 2019). There might be other mechanisms of resistance in F1534C-carrying mosquitoes that could come into play to confer resistance to type II pyrethroids.

Functional analysis of S989P in different mosquito sodium channels led to somewhat different results. This mutation alone did not have any effect on the sensitivity of AaNa_v1-1 (Du et al., 2013) or AaNa_vS2 (Hirata et al., 2014) channels to permethrin or deltamethrin but it enhanced V1016G-mediated resistance of AaNa_vS2 channels to deltamethrin, but not to permethrin (Hirata et al., 2014). However, it did not enhance the V1016G-mediated resistance of AaNa_v1-1 channels (Du et al., 2013). These interesting differential effects of S989P on AaNa_v1-1 and AaNa_vS2 channel sensitivities to pyrethroids could be due to sequence differences between the two channels. In particular, AaNa_v1-1 and AaNa_vS2 contain different alternative spliced exons. *AaNa_v1-1* lacks optional exon a, whereas *AaNa_vS2* lacks optional exons b and f. In addition, *AaNa_v1-1* and *AaNa_vS2* use mutually exclusive exons c and d, respectively. Finally, there are three amino acid differences between the two clones. Further mutational analyses may lead to additional insights into differential sensitivities of AaNa_v1-1 and AaNa_vS2 to pyrethroids. The involvement of I1011V and D1763Y in pyrethroid resistance remains uncertain. I1011V did not alter the sensitivity of AaNa_v1-1 channels to either permethrin or deltamethrin, whereas I1011M, like F1534C, reduced the sensitivity to permethrin, but not to deltamethrin (Du et al., 2013). D1763Y alone or in combination with V1016G has no effect on AaNa_v1-1 channel sensitivity to pyrethroids (Du et al., 2013).

Based on chronological documentation of mutation detection, patterns of co-occurrence of mutations in field populations and results of functional analyses, a sequential selection model has been proposed for the V1016I and F1534C mutations: the V1016I mutation (alone confers no pyrethroid resistance) likely arose from the F1534C haplotype and the double mutations were subsequently selected because they confer a higher level of pyrethroid resistance (Chen et al., 2019; Vera-Maloof et al., 2015). Unlike V1016I, V410L alone confers resistance to both permethrin and deltamethrin (Table 2), V410L could be selected independent of F1534. Furthermore, in the case of mutations, such as F1534C, V410L, V1016G, which are present in multiple populations in various regions, these mutations may evolve independently from multiple origins and/or result from a single origin event that then spread widely (Chung et al., 2019; Kawada et al., 2016; Saavedra-Rodriguez et al., 2018). Mutations, such as V1016G/I and F1534C, were identified from mosquito populations that were also resistant to DDT (Aponte et al., 2019; Ishak et al., 2015; Kawada et al., 2016; Saha et al., 2019). It is possible that these mutations were selected initially due to extensive use of DDT for mosquito control prior to pyrethroid use. In support of this model, both F1534C and V1016G/I have been confirmed to reduce sodium channel sensitivity to DDT (Chen et al., 2019).

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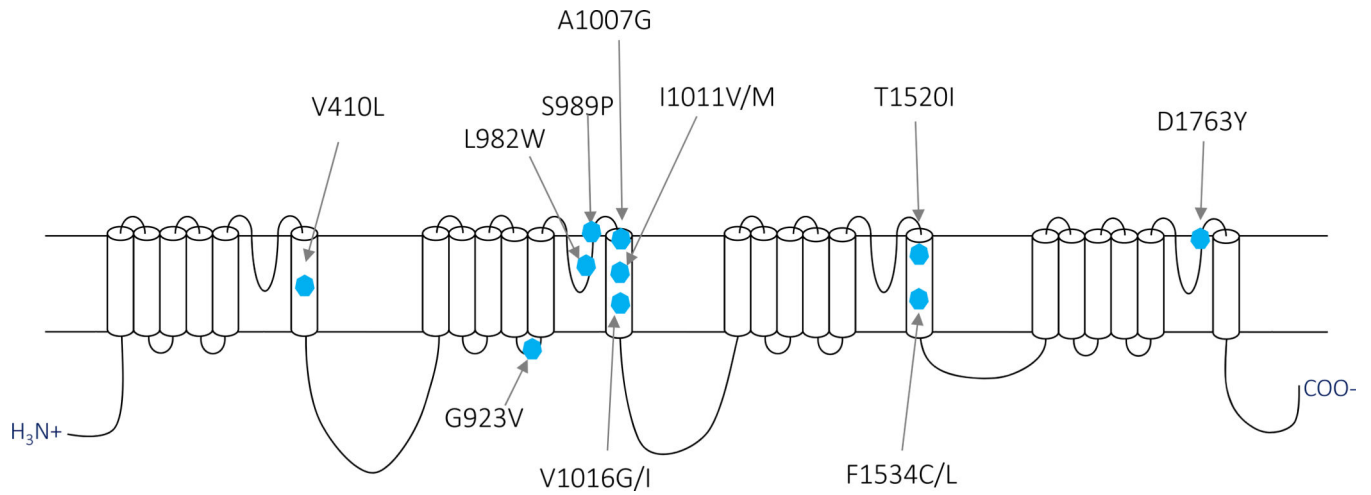


Figure 1. Sodium channel mutations that are associated with pyrethroid resistance in *Ae. aegypti*. Amino acid positions of mutations are numbered based on the house fly sodium channel protein, Vssc1 (Genbank accession number: [AAB47604](#)).

Table 1.Sodium channel mutations that are associated with pyrethroid resistance in *Aedes aegypti*.

Mutation ¹	Year ²	Country	Reference
V1016G ³	2002	Thailand	Prapanthadara et al., 2002; Brengues et al., 2003; Rajatileka et al., 2008; Stenhouse et al., 2013; Plernsub et al., 2016; Saingamsook et al., 2017
		Indonesia	Brengues et al., 2003; Wuliandari et al., 2015; Sayono et al., 2016; Hamid et al., 2017; Saingamsook et al., 2017; Hamid et al., 2018
		Vietnam	Kawada et al., 2009
		Myanmar	Kawada et al., 2014; Saingamsook et al., 2017
		Malaysia	Ishak et al., 2015; Rasli et al., 2018; Leong et al., 2019
		Singapore	Kasai et al., 2014; Pang et al., 2015
		Bhutan	Saingamsook et al., 2017
		Panama	Murcia et al., 2019
		Taiwan (China)	Chung et al., 2019
		Laos	Marcombe et al., 2019
I1011M + G923V	2003	Brazil	Brengues et al., 2003
		Guyana	Brengues et al., 2003
		Martinique	Brengues et al., 2003
		French overseas territories	Dusfour et al., 2015
L982W	2003	Vietnam	Brengues et al., 2003
I1011M	2007	Latin American	Saavedra-Rodriguez et al., 2007
		Brazil	Lima et al., 2011; Martins et al., 2013; Brito et al., 2018; Garcia et al., 2018; Araujo et al., 2019
I1011V	2007	Latin American	Saavedra-Rodriguez et al., 2007
		Thailand	Rajatileka et al., 2008
		Vietnam	Bingham et al., 2011
V1016I	2007	Latin American	Saavedra-Rodriguez et al., 2007
		Brazil	Martins et al., 2009; Lima et al., 2011; Martins et al., 2013; Aguirre-Obando et al., 2016; Collet et al., 2016; Dolabella et al., 2016
		Mexico	García et al., 2009; Siller et al., 2011; Garcia-Rejon et al., 2017; Lopez-Monroy et al., 2018
		Grand Cayman	Harris et al., 2010
		Vietnam	Bingham et al., 2011
		Martinique Island	Marcombe et al., 2012
		Venezuela	Alvarez et al., 2014
		Colombia	Aguirre-Obando et al., 2016
		United States	Cornel et al., 2016
		V1016G + D1763Y	2009
F1534C ³	2009	Vietnam	Kawada et al., 2009; Lien et al., 2018
		Thailand	Yanola et al., 2010; Yanola et al., 2011; Stenhouse et al., 2013; Plernsub et al., 2016; Saingamsook et al., 2017
		Grand Cayman	Harris et al., 2010

Mutation ¹	Year ²	Country	Reference
		Mexico	Aponte et al., 2013; Lopez-Monroy et al., 2018; Saavedra-Rodriguez et al., 2018; Kandel et al., 2019; Grissnan et al., 2019; Villanueva-Segura et al., 2019
		Myanmar	Kawada et al., 2014
		Singapore	Kasai et al., 2014; Pang et al., 2015
		Venezuela	Alvarez et al., 2014
		Brazil	Chapadense et al., 2015; Macoris et al., 2018; Araujo et al., 2019
		Indonesian	Wuliandari et al., 2015; Hamid et al., 2017; Saingamsook et al., 2017; Hamid et al., 2018; Mulyaningsih et al., 2018
		Southern China	Li et al., 2015
		Malaysia	Ishak et al., 2015; Leong et al., 2019
		India	Muthusamy et al., 2015; Kushwah et al., 2019
		Colombia	Atencia et al., 2016
		Cambodia	Saingamsook et al., 2017
		Pakistan	Saingamsook et al., 2017
		Myanmar	Saingamsook et al., 2017
		Portugal	Seixas et al., 2017
		Puerto Rico	Ponce-Garcia et al., 2017
		Saudi Arabia	Nazawi et al., 2017
		Jamaica	Francis et al., 2017; Aponte et al., 2018; Granada et al., 2018
		Sri Lanka	Fernando et al., 2018
		Burkina Faso	Badolo et al., 2019; Sombié et al., 2019
		Peru	Pinto et al., 2019
		Taiwan (China)	Chung et al., 2019
		Australia, New Zealand	Schmidt et al., 2019
		Laos	Marcombe et al., 2019
V1016G + F1534C	2009	Vietnam	Kawada et al., 2009
		Thailand	Stenhouse et al., 2013; Plernsub et al., 2016
		Myanmar	Kawada et al., 2014; Saingamsook et al., 2017
		Singapore	Pang et al., 2015
		Malaysia	Ishak et al., 2015
		Ghana	Kawada et al., 2016
		Sri Lankan	Fernando et al., 2018
		Saudi Arabia	Dafalla et al., 2019
		West Bengal	Saha et al., 2019
		Laos	Marcombe et al., 2019
V1016G + S989P	2010	Thailand	Srisawat et al., 2010; Yanola et al., 2011; Srisawat et al., 2012; Son-un et al., 2018;
		Myanmar	Kawada et al., 2014
		Singapore	Kasai et al., 2014
		Indonesia	Wuliandari et al., 2015; Sayono et al., 2016; Hamid et al., 2017b; Islami et al., 2018
		Southern China	Li et al., 2015
		Saudi Arabia	Nazawi et al., 2017

Mutation ¹	Year ²	Country	Reference
V1016G + F1534C + S989P	2011	Malaysia	Rasli et al., 2018; Leong et al., 2019
		Taiwan (China)	Chung et al., 2019
		India	Kushwah et al., 2019
		Australia, New Zealand	Schmidt et al., 2019
		Thailand	Yanola et al., 2011
		Myanmar	Kawada et al., 2014
		Indonesia	Wuliandari et al., 2015; Hamid et al., 2017b; Amelia-Yap ZH et al., 2019
		Southern China	Li et al., 2015
		Indonesian	Sayono et al., 2016
		Saudi Arabia	Nazawi et al., 2017; Dafalla et al., 2019
V1016I + F1534C	2013	Sri Lankan	Femando et al., 2018
		Malaysia	Leong et al., 2019
		Taiwan (China)	Chung et al., 2019
		Mexico	Aponte et al., 2013; Vera-Maloof et al., 2015; Deming et al., 2016; Lopez-Monroy et al., 2018; Saavedra-Rodriguez et al., 2018; Grissnan et al., 2019; Liebman et al., 2019; Villanueva-Segura et al., 2019
		Venezuela	Alvarez et al., 2014
		Brazil	Linss et al., 2014; Chapadense et al., 2015; Bona et al., 2016; Brito et al., 2018; Garcia et al., 2018; Macoris et al., 2018
		French overseas territories	Dusfour et al., 2015; Goindin et al., 2017
		Ghana	Kawada et al., 2016
		Portugal	Seixas et al., 2017
		Jamaica	Francis et al., 2017
S989P	2014	Puerto Rico	Ponce-Garcia et al., 2017
		Colombian Caribbean region	Granada et al., 2018; Aponte et al., 2019; Maestre-Serrano et al., 2019
		Southern Ecuador	Ryan et al., 2018
		Peru	Pinto et al., 2019
		Costa Rica	Zardkoohi et al., 2019
		Burkina Faso	Sombié et al., 2019; Badolo et al., 2019
		Myanmar	Kawada et al., 2014
		Indonesian	Wuliandari et al., 2015; Sayono et al., 2016
		Malaysia	Leong et al., 2019
		Indonesian	Wuliandari et al., 2015; Sayono et al., 2016
S989P + F1534C	2015	Indonesian	Wuliandari et al., 2015; Sayono et al., 2016
		Indonesian	Wuliandari et al., 2015; Sayono et al., 2016
F1534C + T1520I	2015	India	Kushwah et al., 2015
V410L	2017	Brazil	Haddi et al., 2017
		Colombia	Granada et al., 2018
		Mexico	Villanueva-Segura et al., 2020
V410L + F1534C	2017	Brazil	Haddi et al., 2017
		Mexico	Saavedra-Rodriguez et al., 2018; Villanueva-Segura et al., 2019

Mutation ¹	Year ²	Country	Reference
		Colombia	Granada et al., 2018
V410L + V1016I + F1534C	2018	Colombia	Granada et al., 2018
		Mexico	Saavedra-Rodriguez et al., 2018; Villanueva-Segura et al., 2019
A1007G + F1534C	2018	Vietnam	Lien et al., 2018
F1534L ³	2019	India	Kushwah et al., 2019
V1016G + F1534C + D1763Y	2019	Taiwan (China)	Chung et al., 2019; Biduda S et al., 2019
I1011M + V1016G	2019	Panama	Murcia et al., 2019

¹ Mutations are numbered according to the amino acid sequence of Vssc1 deposited in GenBank (Accession no: [AAB47604](#))

² Refers to the year that the mutation was first reported.

³ These mutations were also detected in *Ae. albopictus* (Aguirre-Obando et al., 2017; Gao et al., 2018; Kasai et al., 2019; Kasai et al., 2011; Li et al., 2018; Marcombe et al., 2014; Pichler et al., 2019; Rath et al., 2018; Xu et al., 2016; Zhou et al., 2019).

Table 2.Characterization of pyrethroid-resistance associated sodium channel mutations in *Xenopus oocytes*

Mutation ^a	Permethrin	Deltamethrin	DDT	Refs
F1534C	+	-	+	Hu et al., 2011; Du et al., 2013; Hirata et al., 2014; Haddi et al., 2017; Du et al., 2016; Chen et al., 2019
V1016I	-	-	+	Du et al., 2013; Chen et al., 2019
V1016I + F1534C	++	++	++	Chen et al., 2019
T1520I	-	-	-	Chen et al., 2019
T1520I + F1534C	++	-	+	Chen et al., 2019
V1016G	++	++	++	Du et al., 2013; Chen et al., 2019
S989P	-	-	nd	Du et al., 2013
D1794Y	-	-	nd	Du et al., 2013
V1016G+S989P	++	++	nd	Du et al., 2013
V1016G+D1794Y	++	++	nd	Du et al., 2013
S989P	-	+	nd	Hirata et al., 2014
V1016G	+	+	nd	Hirata et al., 2014
V1016G+S989P	+	+	nd	Hirata et al., 2014
V1016G+S989P+F1534C	++	++	nd	Hirata et al., 2014
V410L	++	++	nd	Haddi et al., 2017
V410L + F1534C	+++	++	nd	Haddi et al., 2017
I1011V	-	-	nd	Du et al., 2013
I1011M	+	-	+	Du et al., 2013; Du et al., 2016

^aMutations were introduced into the mosquito sodium channel, AaNav1-1, except for Hirata et al. (2014) where a different mosquito sodium channel, AaNav_{S2}, was used and Hu et al. (2011) in which the cockroach BgNav1-1a channel was used. "+" denotes a low level of resistance; "-" no resistance; "++" and "+++" higher levels of resistance and "nd" not determined.