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Research Paper

Multiple Sodium Channel Variants in the Mosquito *Culex quinquefasciatus*

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Abstract

Voltage-gated sodium channels are the target sites of both DDT and pyrethroid insecticides. The importance of alternative splicing as a key mechanism governing the structural and functional diversity of sodium channels and the resulting development of insecticide and acaricide resistance is widely recognized, as shown by the extensive research on characterizing alternative splicing and variants of sodium channels in medically and agriculturally important insect species. Here we present the first comparative study of multiple variants of the sodium channel transcripts in the mosquito *Culex quinquefasciatus*. The variants were classified into two categories, CxNa-L and CxNa-S based on their distinguishing sequence sizes of ~6.5 kb and ~4.0 kb, respectively, and generated via major extensive alternative splicing with minor small deletions/ insertions in susceptible S-Lab, low resistant HAmCq^{G0}, and highly resistant HAmCq^{G8} *Culex* strains. Four alternative Cx-Na-L splice variants were identified, including three full length variants with three optional exons (2, 5, and 21i) and one with in-frame-stop codons. Large, multi-exon-alternative splices were identified in the CxNa-S category. All CxNa-S splicing variants in the S-Lab and HAmCq^{G0} strains contained in-frame stop codons, suggesting that any resulting proteins would be truncated. The ~1000 to ~3000-fold lower expression of these splice variants with stop codons compared with the CxNa-L splicing variants may support the lower importance of these variants in S-Lab and HAmCq^{G0}. Interestingly, two alternative splicing variants of CxNa-S in HAmCq^{G8} included entire ORFs but lacked exons 5 to18 and these two variants had much higher expression levels in HAmCq^{G8} than in S-Lab and HAmCq^{G0}. These results provide a functional basis for further characterizing how alternative splicing of a voltage-gated sodium channel contributes to diversity in neuronal signaling in mosquitoes in response to pyrethroids, and possibly indicates the role of these variants in the development of pyrethroid resistance.

Key words: Sodium channel, transcript variants, alternative splicing, insecticide resistance, *Culex quinquefasciatus.*

INTRODUCTION

The insects' voltage-gated sodium channels are the targets for insecticides, such as DDT and pyrethroids [1-4], which are responsible for the rising phase of action potentials in the membranes of neurons and most electrically excitable cells [5]. Pyrethroids and DDT deliver their toxic, insecticidal effects primarily by binding to the sodium channel, thus altering its gating properties and keeping the sodium channel open for unusual long time, thereby causing a prolonged flow of sodium current that initiates repetitive discharges and prevents the repolarization phase of action potentials [5-7]. The common feature found in sodium channels is that relatively small changes, such as point mutations or substitutions [3, 8-14], short sequence insertions or deletions, or alternative splicing [15-23] in the structure of these channels significantly affect their behavior and are sufficient to change neuronal firing, resulting in different phenotypes. Modifications of the insect sodium channel structure can cause insensitivity of the channels to DDT and pyrethroids via a reduction in or an elimination of the binding affinity of the insecticides to proteins [6-7], and hence result in the development of insecticide resistance.

In mammalian systems, molecular characterization of voltage-gated sodium channel genes has revealed the existence of multiple genes [5, 24-26]: ten paralogous voltage-gated sodium channel genes have been identified in humans [25]; 8 in zebra fish [27]; and 6 in electric fish [28]. Several invertebrate species have also been found to include multiple sodium channel genes in their genome; for example, 4 sodium channel genes have been characterized in *Hirudo medicinalis* (leech) [29] and 2 in *Halocynthia roretzi* (ascidia) [30-31]. Compared to the fairly well defined multiple vertebrate sodium channel genes, it appears that a single sodium channel gene that has been well characterized in many insect species, homologous to *para* (currently *DmNa$_V$*) of *Drosophila melanogaster* [32-33] encodes the equivalent of the *α*-subunit of the mammalian sodium channels. While mammals rely on the selective expression of at least ten different sodium channel genes in various tissues to achieve sodium channel diversity [25], insects may produce a range of diverse sodium channels with different functional and pharmacological properties from a single sodium channel by extensive alternative splicing [16-23, 34].

Because of the importance of alternative splicing as a key mechanism for generating structural and functional diversity in sodium channels [19], following the first discovery of the existence of alternative splicing of the *para* sodium channel gene from *Drosophila melanogaster* [32], alternative splicing events were subsequently characterized in many medically or agricultural important insect and arachnid pest species [17, 19-23, 34-36]. As yet, however, there have been no reports of alternative splicing in *Culex quinquefasciatus*, an important mosquito vector of human pathogens such as St. Louis encephalitis virus (SLEV), West Nile virus (WNV), and the parasitic *Wuchereria bancrofti* nematode in many urban settings throughout the tropical and temperate regions of the world [37-39]. Here we present the first comparative study of full length sequences of the para-orthologue sodium channel transcripts from the *Culex quinquefasciatus* mosquito and examine multiple variants obtained through the mechanism of alternative splicing.

MATERIALS AND METHODS

Mosquito strains

Three strains of mosquito Cx. quinquefasciatus were studied. S-Lab is an insecticide susceptible strain provided by Dr. Laura Harrington (Cornell University). HAmCq^{G0} is a low insecticide resistant strain with a 10-fold level of resistance to permethrin compared with the laboratory susceptible S-Lab strain [40]. It was originally collected from Huntsville, Alabama, in 2002 and established in our laboratory without further exposure to insecticides [*41*]. The HAmCq^{G8} strain is the 8th generation of permethrin-selected HAmCq^{G0} offspring and has a 2,700-fold level of resistance [40]. All mosquitoes were reared at 25±2^{o}C under a photoperiod of 12:12 (L:D) h.

Amplification of the full length of sodium channel transcripts in *Cx. quinquefasciatus*

For each of the three mosquito populations, total RNA was extracted from the 4th instar larvae, and different tissues (head + thorax, and abdomen) from 2-3 day-old adult females before blood feeding using the acidic guanidine thiocyanate-phenol-chloroform method [42]. Messenger RNAs (mRNAs) were isolated using Oligotex-dT suspension (QIAGEN). The full length cDNA of the *Cx. quinquefasciatus* sodium channel gene was subsequently isolated from each of the mosquitoes populations by RT-PCR using the Expand Long Range, dNTPack kit (Roche) with a specific primer pair, KDR S16 (TGTTGGCCATATAGACAATGACCGA) /KDR AS09 (GCTTCTGAATCTGAATCAGAGGGAG), synthesized based on the respective 5′ and 3′ end sequences of the putative sodium channel cDNAs [43], accession numbers: JN695777, JN695778, and JN695779]. The PCR reaction was conducted following a PCR cycle of 92°C for 2 min, 10 cycles of 92°C for 10 s, 55°C for 15 s, and 68°C for 6 min, and 35 cycles of 92°C for 10 s, 55°C for 15 s, and 68°C for 8 min, with a final extension of 68°C for 10 min. All PCR products were cloned into PCR™ 2.1 Original TA cloning vector (Invitrogen) and sequenced. Cloning and sequence analyses of sodium channel cDNA fragments were repeated at least two times for each mosquito strain with different preparations of RNAs and mRNAs. The inserts of *Culex* sodium channel clones were sequenced and analyzed.

Quantitative real-time PCR (qRT-PCR)

The total RNA (0.5 µg/sample) from each mosquito sample was reverse-transcribed using SuperScript II reverse transcriptase (Stratagene) in a total volume of 20 µl. The quantity of cDNAs was measured using a spectrophotometer prior to qRT-PCR, which was performed with the SYBR Green master mix Kit and ABI 7500 Real Time PCR system (Applied Biosystems). Each qRT-PCR reaction (25 µl final volume) contained 1x SYBR Green master mix, 1 µl of cDNA, and a sodium channel transcript specific primer pair designed according to each of the sodium transcript or allele sequences (Table 1 shows the accession number for each of the sodium channel transcripts or alleles) at a final concentration of 3-5 µM. All samples, including the A 'no-template' negative control, were performed in triplicate. The reaction cycle consisted of a melting step of 50ºC for 2 min then 95°C for 10 min, followed by 40 cycles of 95ºC for 15 sec and 60ºC for 1 min. Specificity of the PCR reactions was assessed via a melting curve analysis for each PCR reaction using Dissociation Curves software [44]. Relative expression levels for the sodium channel transcripts were calculated by the $2^{-\Delta\Delta CT}$ method using SDS RQ software [45]. The 18S ribosome RNA, an endogenous control, was used to normalize the expression of targets [46-49]. Preliminary qRT-PCR experiments with a primer pair (Table 1) designed according to the sequences of the 18S ribosome RNA had revealed that the expression of this gene remained constant among all 3 mosquito strains, so the 18S ribosome RNA was used for internal normalization in the qRT-PCR assays. Each experiment was repeated three to four times with different preparations of RNA samples. The statistical significance of the gene expressions was calculated using a Student's *t*-test for all 2-sample comparisons and a one-way analysis of variance (ANOVA) for multiple sample comparisons (SAS v9.1 software); a value of $P \leq 0.05$ was considered statistically significant. Significant overexpression was determined using a cut-off value of a ≥2-fold change in expression [50].

Table 1. Oligonucleotide primers used in qRT-PCR reactions for amplifying sodium channel variants.

Mosquito population	Variants	Forward Primer	Reverse Primer
S-Lab	$CxNa_v$-Lv1	5′ AATCAGCTGTAAAAGTGATGGCGC 3′	5′ AGCTCGTAGTACCCTGAATGTTCT 3′
	$CxNa_v$-Lv2	5′ CACTGCAAAAGCCCGTAAAGTGAG 3′	5′ ATAGTATACTGGAACGATGATTGCA 3′
	$CxNa_v$-Lv3	5′ ACAAGGGCAAGAAGAACAAGCAGC 3′	5′ CTTTATACTGGCAGTGTCATCGTC 3′
	$CxNa_v$-Sv1	5′ ATCGATATCTGAGAGAACGTAGTT 3′	5′ TTCATCCTCGTCCTCATCGTCGTA 3′
$HAmCq^{G0}$	$CxNa_v$-Lv4	5′ GGTCGGAAGAAAAAGAAAAGAGA 3′	5′ TATCCTTTCCTTTACTAACTACTA 3′
	$CxNa_v$-Lv5	5′ GCCAAAAAAAGTACTACAACGCAA 3′	5′ TCCCGTCTGCTTGTAGTGAT 3′
	$CxNa_v$-Lv6	5′ AGCACAACCATCTCAGTTGGATAT 3′	5′ TCGTCGTCGAGTTCTTCGTCAATT 3′
	$CxNa_v$-Sv2	5′ CAAAAGTTCGACATGATCATCATG 3′	5′ TGAAGAACGACATCCCGAAGATG 3′
	$CxNa_v$-Sv3	5′ TACTACATGGACAGGATATTCAC 3′	5′ CAGGTTTATGAGCGAGAGCATCA 3′
$HAmCq^{G8}$	$CxNa_v$-Lv7	5′ TCGAGTGTTCAAGCTAGCAAA 3′	5′ AATGCAGAGCACAAACGTCAG 3′
	$CxNa_v$-Lv8	5′ TTCCAGTATACTATGCTAATTTAG 3′	5′ TTGGTGTCGACGTAGGACATGTT 3′
	$CxNa_v$-Sv4	5′ TCCAAGGTGATAGGCAATTCTATT 3′	5′ TCAATTCCTAGGTCCTCCTTGCT 3′
	$CxNa_v$-Sv5	5′ ACTACTACGAATGTCTTAATGTTT 3′	5′ TGTACTAAAATATAAATAGCTACG 3′

Results

Generation of sodium channel transcripts in *Cx. quinquefasciatus*

To examine the number of transcripts of the para-type sodium channel gene in the genome of *Culex* mosquitoes, RNAs isolated from S-Lab, $HAmCq^{G0}$ and $HAmCq^{G8}$ were subjected to PCR amplification using a primer pair: KDR S16 (TGTTGGCCATA TAGACAATGACCGA)/KDR AS09 (GCTTCTGAAT CTGAATCAGAGGGAG), synthesized based on the respective 5′ and 3′ end sequences of the putative sodium channel cDNAs (Table 1, 43). Two distinct molecular sizes of sodium channel cDNAs with ~6.5 and ~4 kb were generated by PCR amplification from each of the three mosquito strains, namely susceptible (S-Lab), intermediate ($HAmCq^{G0}$), and highly resistant ($HAmCq^{G8}$), when only a single primer pair, KDR S16/ KDR AS09 was used (Fig. 1). The PCR products of both the ~6.5 and ~4.0 kb fragments from each strain were then cloned and sequenced. Sequence analysis of insertions of clones (3, 3, and 2 clones for S-lab, $HAmCq^{G0}$, and $HAmCq^{G8}$, respectively) with ~6.5 kb and clones (2, 7, and 2 clones for S-lab, HAmCq^{G0}, and HAmCq^{G8}, respectively) with~4.0 kb PCR amplified products from each strain indicated that all the cDNA clones were indeed the sodium channel transcripts. Interestingly, nucleotide sequence analysis of these sodium channel transcripts revealed the existence of multiple variants in each of ~6.5 and ~4.0 kb PCR amplification products in each mosquito strains. These variants were then assigned to two categories, CxNa-L and CxNa-S, based on their sizes of ~6.5 and ~4.0 kb, respectively.

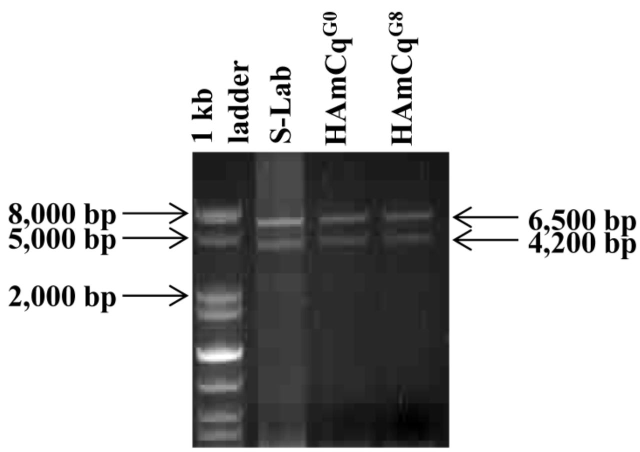

Figure 1. Polymerase chain reaction (PCR) amplification of *para*-type sodium channel transcripts from genomic RNAs of *Culex* mosquitoes. Sodium channel cDNA transcripts amplified from RNAs isolated from S-Lab, HAmCq^{G0} and HAmCq^{G8} mosquito strains were subjected to PCR amplification using a primer pair: KDR S16 (TGTTGGCCATATAGACAATGACCGA)/KDR AS09 (GCTTCTGAATCTGAATCAGAGGGAG), synthesized based on the respective 5' and 3' end sequences of the putative sodium channel genes [43].

The CxNa-L PCR products included three cDNA sequences of the sodium channel, CxNa-L$_v$1, CxNa-L$_v$2, and CxNa-L$_v$3, in the S-Lab strain, with molecular sequence sizes of 6246, 6273, and 6234 bps, respectively (Fig. 2); three sodium channel cDNA sequences, CxNa-L$_v$4, and CxNa-L$_v$5 and CxNa-L$_v$6, in the HAmCq^{G0} strain, with 6276, 6285, and 6063 bps, respectively (Fig. 3); and two cDNA sequences, CxNa-L$_v$7 and CxNa-L$_v$8, in the HAmCq^{G8} strain, with 6267 and 6273 bps, respectively (Fig. 4). In contrast, the CxNa-S PCR products contained only one cDNA sequence of the sodium channel in S-Lab, CxNa-S$_v$1, with a molecular size of 3891 bps (Fig. 2); two cDNA sequences in HAmCq^{G0}, CxNaS$_v$2 and CxNa-S$_v$3, with molecular sizes of 3615 and 3417 bps, respectively (Fig. 3); and two sodium channel cDNA sequences in HAmCq^{G8}, CxNa-S$_v$4 and CxNa-S$_v$5, with 4068 and 3987 bps, respectively, (Fig. 4). This discovery provides strong evidence supporting the existence of multiple transcripts of the sodium channel gene in the mosquito *Cx. quinquefasciatus,* as we previously suggested [51].

Structural analysis of deduced sodium channel protein sequences within each strain and/or among different strains of *Culex* mosquitoes

The putative amino acid sequences for the CxNa-L and the CxNa-S transcript sequences were compared for each of the three mosquito strains studied. In the S-Lab strain, of the three transcripts identified in the CxNa-L category, two, CxNa-L$_v$1 and CxNa-L$_v$2 (Accession numbers: JN695777 and JX424546), consisted of full length sodium channel sequences encoding the entire ORFs of the sodium channel proteins with 2082 and 2091 amino acid residues, respectively. These exons were numbered 1 through 33 (Fig. 5), based on the silkworm *Bombyx mori* sodium channel *BmNav* [35] and house fly sodium channel sequences [11, 52-53]. However, the *Culex* mosquito sodium channel lacked the exon 12 present in both the *BmNav* and *DmNa$_V$* sodium channel sequences. CxNa-L$_v$1 and CxNa-L$_v$2 shared very high sequence similarity (96%), except for a missing exon 5 as a result of the alternative splicing (Figs. 2 and 5) and several short insertions identified in the CxNa-L$_v$2 sequence (Figs. 2 and 5). The remaining transcript, CxNa-L$_v$3, incorporated several in-frame premature stop codons, with the first occurring at domain I segment 2 (IS2) (Figs. 2 and 5). Short deletions and insertions were also identified in CxNa-L$_v$3 compared with CxNa-L$_v$1 and CxNa-L$_v$2.

Similar length transcripts/variants were identified in the CxNa-L transcripts of the HAmCq^{G0} mosquitoes (Figs. 3 and 5), in which two of the three transcripts identified, CxNa-L$_v$4 and CxNa-L$_v$5 (Accession numbers: JN695778 and JX424547), were entire sodium channel ORFs, encoding 2092 and 2095 amino acid residues, respectively, and sharing 99% sequence similarity. Comparing the transcript sequences of CxNa-L$_v$4 and CxNa-L$_v$5 revealed that an alternative splicing exon 2, a short in-frame insertion in exon 12, and a short in-frame deletion in exon 21ii were present in CxNa-L$_v$5 (Figs. 3 and 5). The third CxNa-L transcript, CxNa-L$_v$6, in the HAmCq^{G0} mosquitoes was again the exception, incorporating several in-frame premature stop codons, with the first occurred in the linker between IIS6 and IIIS1, in addition to short insertions and deletions (Figs. 4 and 5). CxNa-L$_v$6 also exhibited an alternative splicing of exon 2 compared to that identified in CxNa-L$_v$5. The two CxNa-L transcripts, CxNa-L$_v$7 and CxNa-L$_v$8

(Accession numbers: JN695779 and JX424548), identified in HAmCq^{G8} were both full length sodium channel transcripts encoding entire ORFs of sodium channels proteins (Figs. 4 and 5). CxNa-L$_v$7 and CxNa-L$_v$8 shared very high sequence similarity (99%), except for an alternative splicing of exon 21i present in CxNa-L$_v$8 (Figs. 4 and 5). The above results indicate that multiple ~full length transcripts presented in the mosquitoes, with at least two transcripts in each mosquito strain, had entire ORFs. The other transcripts found in both S-Lab and HAmCq^{G0} incorporated in-frame premature stop codons and, as such, any resulting proteins would be truncated from those regions onward and thus less likely to be functional transcripts.

The sequences of the CxNa-S transcripts with the ~4.0 kb sized sodium channels in each of the mosquito strains were found to be similar to those of the full length CxNa-L sequences; i.e., one or more transcripts were present in each of the mosquito strains. The main difference in the CxNa-S transcripts compared with those of the full length sequences were the internal exons missing through the alternative splicing, along with some minor short deletions or insertions. In the S-Lab strain, only one transcript was observed, CxNa-S$_v$1, containing a single in-frame stop codon at the IIS6 region in the sequence (Fig. 2). However, the CxNa-S$_v$1 transcript also lacked exons 2, 5 to18, 21i, and 22 as a result of the alternative splicing, and thus had a short sodium channel sequence. Two transcripts, CxNa-S$_v$2 and CxNa-S$_v$3, were identified in the HAmCq^{G0} strain (Fig. 3). Both of these sequences exhibited alternative splicing of exons, in-frame stop codons, and short deletions and insertions. The CxNa-S$_v$2 sequences were found to have alternative splicing of exons 2, 12-26, whereas the CxNa-S$_v$3 sequences lacked exons 2-15, and parts of exons 21 and 22 (Fig. 3) due to the alternative splicing, once again resulting in a short sodium channel sequence. The HAmCq^{G8} strain contained 2 transcripts, CxNa-S$_v$4 and CxNa-S$_v$5, with entire ORFs, encoding 1356 and 1329 amino acid residues (Accession numbers: JX424549 and JX424550), respectively, and sharing 98% sequence similarity (Fig. 4). Compared with the PRFs of the CxNa-L transcripts, these two CxNa-S transcripts lacked exons 5-18 as a result of the exon alternative splicing (Figs. 4 and 5). Thus, among all the CxNa-S transcripts identified in the tested mosquitoes, only CxNa-S$_v$4 and CxNa-S$_v$5 in the highly resistant HAmCq^{G8} mosquitoes contained the entire ORFs of the sodium channels.

Expression analysis of sodium channel transcripts in *Culex* mosquitoes

The extent of the variation in alternative transcript expression was also addressed by determining the levels of expression of individual sodium channel transcripts in the 4th instar larvae and different tissues from the adult mosquitoes in each strain using qRT-PCR. Characterizing the developmental and regional expression of the sodium channel transcripts in mosquitoes is critical to our understanding of their relative biological importance. We therefore determined the relative expression levels of sodium channel RNAs for all the transcripts identified in all three mosquito strains, S-Lab, HAmCq^{G0} and HAmCq^{G8}. Total RNAs were extracted from whole bodies of 4th instar larvae, as well as the head+thorax, and abdomen tissues of 2-3 day old adults. The expression levels were determined using qRT–PCR and the expression ratios for the head + thorax and larval samples were then calculated relative to the quantity of the transcript expression in the corresponding abdomen samples for each strain (Fig. 6). The results show that the sodium channel expression in all three strains shared a number of common features. The expression levels were relatively high in the head + thorax tissues compared to the abdomen tissues; the full length sodium channel transcripts of CxNa-L with an ORF of ~6.5 kb had abundant expression compared with those of CxNa-S ~4.0 kb transcripts, the transcripts with in-frame-stop codons, and CxNa-L$_v$5 with in-frame-stop codons (Fig. 6). Comparing the transcripts with the full length ORFs in each of the three strains, even though the transcripts had undergone alternative splicing events the expression levels were similar, suggesting that the variants may have equivalent functional importance in the tissues and the mosquitoes. Indeed, the transcripts with in-frame stop codons were detected in both the S-Lab and HAmCq^{G0} mosquitoes, but at extremely low levels. The difference in the sodium channel expression between the CxNa-L and CxNa-S transcripts was particularly pronounced for S-Lab and HAmCq^{G0}, where the CxNa-S transcripts were expressed at levels more than 1000-fold lower than the CxNa-L sodium channel transcripts (Fig. 6a, b). In contrast, only about a 10-fold difference in expression between the CxNa-L and CxNa-S transcripts was identified in HAmCq^{G8} (Fig. 6c). This feature, plus the markedly higher expression in HAmCq^{G8}, might reflect their function in HAmCq^{G8}.

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CxNa-Lv1    MTEDLDSISEEERSLFRPFTRESLLVIEERIANEQAKQRELEKKRAEGETGFGRKKKKKE  60
CxNa-Lv2    MTEDLDSISEEERSLFRPFTRESLLVIEERIANEQAKQRELEKKRAEGETVPGRKKKKKE  60
CxNa-Lv3    MTDDLDSISEEERSLFRPFTRESLLVIEERIANEQAKQRELEKKRAEGETGFGRKKKKKE  60
CxNa-Sv1    MTDDLDRYLRE-RSLFRPFTRESLLVIEERIANEQAKQRELEKKRAEGE-----------  48

                                        exon3
CxNa-Lv1    IRYDDEDEDEGPQPDSTLEQGVPIPVRMQGSFPPELASTPLEDIDAFYANIKTFVVVSKG 120
CxNa-Lv2    IRYDDEDEDEGPQPDSTLEQGVPIPVRMQGSFPPELASTPLEDIDAFYANIKTFVVVSKG 120
CxNa-Lv3    IRYDDEDEDEGPQPDSTLEQGVPIPVRMQGSFPPELASTPLEDIDAFYANIKTFVVVSKG 120
CxNa-Sv1    IRYDDEDEDEGPQPDSTLEQGVPIPVRMQGSFPPELASTPLEDIDAFYANIKTFVVVSKG 108

                                      exon4       IS1
CxNa-Lv1    KDIFRFSATNALYVLDPFNPIRRVAIYILVHPLFSFFIITTILGNCILMIMPSTPTVEST 180
CxNa-Lv2    KDIFRFSATNALYVLDPFNPIRRVAIYILVHPLFSFFIITTILGNCILMIMPSTPTVEST 180
CxNa-Lv3    KDIFRFSATNASYVLDPFNPIRRVAIYILVHPLFSFFIITTILGNCILMIMPSTPTVEST 180
CxNa-Sv1    KDIFRFSATNALYVLDPFNPIRRVAIYILVHPLFSFFIITTILGNCILMIIPSTPTVEST 168

                  IS2                exon5           IS3
CxNa-Lv1    EVIFTGIYTFESAVKVMARGFILQPFTYLRDAWNWLDFVVIALAYVTMGIDLGNLAALRT 240
CxNa-Lv2    E-------------------------------------------YVTMGIDLGNLAALRT 197
CxNa-Lv3    EVIFTGIYTFDQL*SDGARFHITTVYLS*-RCMELVGLRSNSISICNYGYRFG*SRCIEN 239
CxNa-Sv1    E----------------------------------------------------------- 169

             exon6    IS4                               IS5
CxNa-Lv1    FRVLRAPKTVAIVPGLKTIVGAVIESVKNLRDVIILTMFSLSVFALMGLQIYMGVLTQKC 300
CxNa-Lv2    FRVLRALKTVAIVPGLKTIVGAVIESVKNLRDVIILTMFSLSAFALMGLQIYMGVLTQKC 257
CxNa-Lv3    IQGTTSSQNSGHRSRSQDHRRRCHRVRKESQRCDNFNNVFVVGVCFNGAADLHGRADAKV 299
CxNa-Sv1    ------------------------------------------------------------

                  exon7                            exon8
CxNa-Lv1    IKEFPTDGSWGNLTHENWERHHSNDSNWYFSETGDTPLCGNSSGAGQCEEGYVCLQGFGD 360
CxNa-Lv2    IKEFPTDGSWGNLTHENWERHHSNDSNWYFSETGDTPVCGNSSGAGQCEEGYVCLQGFGD 317
CxNa-Lv3    HQGVPDGRLVGQDPRELGAAPFERFQLVLFRNRGHAPLR-QFVGCWPM*GRICMFTRFWR 359
                         Δ1
CxNa-Sv1    ------------------------------------------------------------

                          exon9        IP                        IS6
CxNa-Lv1    NPNYGYTSFDTFGWAFLSAFRLMTQDYWENLYQLVLRSAGPWHMLFFIVIIFLGSFYLVN 420
CxNa-Lv2    NPNYGYTSFDTFGWAFLSAFRLMTQDYWENLYQLVLRSAGPWHMLFFIVIIFLGSFYLVN 377
CxNa-Lv3    *SKLRVYKF*YFRMGILICLSSHDPGLLGDLYQLVLRSAGPWHMLFFIVIIFLGSFYLVN 419
CxNa-Sv1    ------------------------------------------------------------

                        exon10
CxNa-Lv1    LILAIVAMSYDELQKRAEEEEAAEEEALREAEEAAAAKQAKLEAHAAAAAAAANPEIAKS 480
CxNa-Lv2    LILAIVAMSYDELQKRAEEEEAAEEEALREAEEAAAAKQAKLEAHAAAAAAAANPEIAKS 437
CxNa-Lv3    LILAIVAMSYDELQKRAEEEEAAEEEALREAEEAAAAKQAKLEAHAAAAAAAANPEIAKS 479
CxNa-Sv1    ------------------------------------------------------------ 178

              exon11
CxNa-Lv1    PSDFSCHSCELFVGQEKGNDDNNKEKMSIRSEGLESASLSLPGSPFNLRRGSRGSHQFTI 540
CxNa-Lv2    PSDFSCHSYELFVGQEKGNDDNNKEKMSIRSEGLESASLSLPGSPFNLRRGSRGSHQFTI 522
                                                 Δ2
CxNa-Lv3    PSDFSCHSYELFVGQEKGNDDNNKEKMSIRSEGLESASLSLPGSPLNLRRGSRGSHQFTI 539
CxNa-Sv1    ------------------------------------------------------------

                        exon12
CxNa-Lv1    RNGRGRFVGVPGSDRKPLVLSTYLDAQEHLPYADDSNAVTPMSEENGSRHSSYTSHQSRI 600
CxNa-Lv2    RNGRGRFVGVPGSDRKPLVLSTYLDAQEHLPYADDSNAVTPMSEENGSRHSSYTSHQSRI 594
                                                           Δ3
CxNa-Lv3    RNGRGRFVGVPGSDRKPLVLSTYLDAQEHLPYADDSNAVTPMSEENGSRHSSYTSHQSRI 599
CxNa-Sv1    ------------------------------------------------------------

                                   exon13
CxNa-Lv1    SYTSHGDLLGGMTKESRLRSRTQRNTNHSIVPPANMAASAASVTGAGSGAPNMPYVDTNP 660
CxNa-Lv2    SYTSHGDLLGGMTKESRLRSRTQRNTDHSIVPPANMAASAASVTGAGSGAPNMSYVDTNH 654
CxNa-Lv3    SYTSHGDLLGGMTKESRLRSRTQRNTNHSIVPPARTWRPRRRR*RVPARAR-PTCPTSTP 658
CxNa-Sv1    ------------------------------------------------------------

                                exon14                       exon15
CxNa-Lv1    KGQQRDFDQSQDYTDDAGKIKHNDNPFIEPSQTQTVVDMKDVMVLNDIIEQAAGRHSRAS 720
CxNa-Lv2    KGQQRDFDQSQDYTDHAGTIKHNDNPFIEPSQTQTVVDMKDVMVLNDIIEQAAGRHSRAS 714
CxNa-Lv3    TTRASSATLISPKTTQIMLVK*NTTTILSSSPLKPKP**KT*WC*NDIIEQAAGRHSRAS 720
                                                  Δ4
CxNa-Sv1    ------------------------------------------------------------

                                                 exon16           IIS1
CxNa-Lv1    DHGEDDDEDGPTFKHKAAEFGMRMIDIFCVWDCCWVWLKFQEWVSFIVFDPFVELFITPC 780
CxNa-Lv2    DHGEDDDEDGPTFKDKAVEFGMRMIDIFCVWDCCWVWLKFQEWVSFIVFDPFVELFITLC 782
                Δ5
CxNa-Lv3    DHGEDDDEDGPTFKDKAVEFGMRMIDIFCVWDCCWVWLKFQEWVSFIVFDPFVELFITLC 780
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CxNa-Sv1  ------------------------------------------------------------
                                      IIS2                exon17
CxNa-Lv1  IVVNTLFMALDHHDMNPDMERALKSGNYFFTATFAIEATMKLIAMSPKWYFQEGWNIFDF 840
CxNa-Lv2  IVVNTLFMALDHHDMNPDMERALKSGNYFFTATFAIEATMKLIAMKPQSDVLCQETVREH 842
CxNa-Lv3  IVVNTLFMALDHHDMNPDMERALKSGNYFFTATFAIEATMKLIAMSPKWYFQEGWNIFDF 840
CxNa-Sv1  ------------------------------------------------------------
             IIS3                    IIS4               exon18   IIS5
CxNa-Lv1  IIVALSLLELGLEGVQGLSVLRSFRLLRVFKLAKSWPTLNLLISIMGRTMGALGNLTFVL 900
CxNa-Lv2  FRFHHRSLELRVWRAFRRSRLRSFRLLRVFKLAKSWPTLNLLISIMGRTMGALGNLTFVL 907
                Δ6         Δ7
CxNa-Lv3  IIVALSLLELGLEGVQGLSVLRSFRLLRVFKLAKSWPTLNLLISIMGRTVGALGNLTFVL 900
CxNa-Sv1  ------------------------------------------------------------
                                        Exon1                 IIP
CxNa-Lv1  CIIIFIFAVMGMQLFGKNYIDNVDRFPDKDLPRWNFTDFMHSFMIVFRVLCGEWIESMWD 960
CxNa-Lv2  CIIIFIFAVMGMQLFGKNYIDNVDRFPDKDLPRWNFTDFMHSFMIVFRVLCGEWIESMWD 967
CxNa-Lv3  CIIIFIFAVMGMQLFGKNYTDNVDRFPDKDLPRWNFTDFMHSFMIVFRVLCGEWIESMWD 960
CxNa-Sv1  --------------------QRGPLPGQGPATVELHRLHALIHDRVPGAVRRVDRIHVGL 209
                       IIS6
CxNa-Lv1  CMLVGDVSCIPFFLATVVIGNLVVLNLFLALLLSNFGSSSLSAPTADNETNKIAEAFNWI 1020
CxNa-Lv2  CMLVGDVSCIPFFLATVVIGNLVVLNLFLALLLSNFGSSSLSAPTADNETNKIAEAFNRI 1027
CxNa-Lv3  CMLVGDVSCIPFFLATVVIGNLVVLNLFLALLCPTVGSSSLSAPTADNETNKIAEAFNRI 1020
CxNa-Sv1  HAGGRRVLHSLSSGHRSDRKFSRS*PFPSLAFVQLWFLEFVGAHSRQRNAQDRRGVQPDI 269
                     exon20           exon21i
CxNa-Lv1  SRFSNWIKANIAAALKFVKNKLTSQIASVQPAGKGVCPCISAEHGENELELTPDDILADG 1080
CxNa-Lv2  SRFSNWIKANIAAALKFVKNKLTSQIASVQPAGKGVCPCISAEHGENELELTPDDILADG 1087
CxNa-Lv3  SRFSNWIKANIAAALKFVKNKLTRQIASVQPAGEQDNHLSWIWKRRAWK*AGINSR*HPG 1081
                                                          Δ8
CxNa-Sv1  ALLQLDQGEHRGRAQVREKQVNKPDCVRAARR--------AAEHGENELELTPDDILADG 321
              exon21ii                              exon22
CxNa-Lv1  LLKKGVKEHNQLEVAIGDGMEFTIHGDLKNKGKKNKQLMNNSKVIGNSISNHQDNKLEHE 1140
CxNa-Lv2  LLKKGVKEHNQLEVAIGDGMEFTIHGDLKNKGKKNKQLMNNSKVIGNSISNHQDNKLEHE 1147
CxNa-Lv3  RRAAEKGRQGAQPAGGGDRRRDGVYDTRRPQEQGQEEQAADEQFQGDRQFYARTES*GHV 1150
                                                             Δ9
CxNa-Sv1  LLKKGVKEHNQLEVAIGDGMEFTIHGDLKHKGKKNKQLMNNSK----------------- 364
                                      exon23
CxNa-Lv1  LNHRGMSLQDDDTASIKSYGSHKNRPFKDESHKGSAETLEGEEKRDASKEDLGIDEELDD 1200
CxNa-Lv2  LNHRGMSLQDDDTASIKSYGSHKNRPFKDESHKGSAETLEGEEKRDASKEDLGIDEELDD 1207
CxNa-Lv3  LTGRKVPMAGR*HCQYKVLWQSQEPPLQGRKPQGQCRNAGGRRKARRQQGGPRN*RRTRR 1215
              Δ10
CxNa-Sv1  ---------DDDTASIKSYGSHKNRPFKDESHKGSAETLEGEEKRDASKEDLGIDEDLDD 415
                                       exon24
CxNa-Lv1  ECEGEEGPLDGEMIIHAEEDEVIEDAPADCFPDNCYKRFPALAGDDDAPFWQGWGNLRLK 1260
CxNa-Lv2  ECEGEEGPLDGEMIIHAEEDEVIEDAPADCFPDNCYKRFPALAGDDDAPFWQGRGNLRLK 1267
CxNa-Lv3  RVRG*-GGSSGRGNDHPRGRGRGDRGRAGRLLPGLLQAVPGTGRRRRRAVLAGLGQPAAQ 1275
                                         Δ11
CxNa-Sv1  ECEGEEGPLDGEMIIHAEEDEVIEDAPADSFPDNCYKRFPALAGDDDAPFWQGWGNLRLK 475
                    IIIS1                 exon25        IIIS2
CxNa-Lv1  TFQLIENKYFETAVITMILLSSLALALEDVHLPHRPILQDVLYYMDRIFTVIFFLEMLIK 1320
CxNa-Lv2  TFQLIENKYFETAVITMILLSSLALALEDVHLPHRPILQDVLYYMDRIFTVIFFLEMLIK 1327
CxNa-Lv3  DVPADREQVLETAVITMILLSSLALALEDVHLPHRPILQDVPYYMDRIFTVIFFLEMLIK 1336
                     Δ12
CxNa-Sv1  TFQLIENKYFETAVITMILLSSLALALEDVHLPHRPILQDVLYYMDRIFTVIFFLEMLIK 535
                            IIIS3          exon26       IIIS4
CxNa-Lv1  WLALGFRVYFTNAWCWLDFIIVMVSLINFVASLCGAGGIQAFKTMRTLRALRPLRAMSRM 1380
CxNa-Lv2  WLALGFRVYFTNAWCWLDFIIVMVSLINFVASLCGAGGIQAFKTMRTLRALRPLRAMSRM 1387
CxNa-Lv3  WLALGFRVYFTNAWCWLDFIIVMVSLINFVASLCGAGGIQAFKTMRTLRALRPLRAMSRM 1396
CxNa-Sv1  WLALGFRVYFTDAWCWLDFIIVMVSLINFVASLCGAGGIQAFKTMRTLRALRPLRAMSRM 595
                  exon27              IIIS5
CxNa-Lv1  QGMRVVVNALVQAIPSIFNVLLVCLIFWLIFAMMGVQLFAGKYFKCVDTNKTTLSHEIIP 1440
CxNa-Lv2  QGMRVVVNALVQAIPSTFNVLLVCLIFWLIFAIMGVQLFAGKYFKCVDTNKTTLSHEILP 1447
CxNa-Lv3  QGMRVVVNALVQAIPSIFNVLLVCLIFWLIFAIMGVQLFAGKYFKCVDTNKTTLSHEIIP 1456
CxNa-Sv1  QGMRVVVNALVQAIPSIFNVLLVCLIFWLIFAIMGVQLFAGKYFKCVDTNKTTLSHEIIP 655
                       exon28                  IIIP
CxNa-Lv1  DVNACIAENYTWENSPMNFDHVGKAYLCLFQVATFRGWIQIMNDAIDSRDIGKQPIRETN 1500
CxNa-Lv2  DVNACIAENYTWENSPMNFDHVGKAYLCLFQVATFKGWIQIMNDAIDSRDIGKQPIRETN 1507
CxNa-Lv3  DVNACIAENYTWENSPMNFDHVGKAYLCVFQVATFKGWIQIMNDAIDSRDIGKQPIRETN 1516
CxNa-Sv1  DVNACIAENYTWENSPMNFDHVGKAYLCLFQVAPFKGWIQIMNDAIDSRDIGKQPIRETN 715
                 IIIS6                                Exon29
CxNa-Lv1  IYMYLYFVFFIIFGSFFTLNLFIGVIIDNFNEQKKKAGGSLEMFMTEDQKKYYNAMKEMG 1560
CxNa-Lv2  IYMYLYFVFFIIFGSFFTLNLFIGVIIDNFNEQKKKAGGSLEMFMTEDQKKYYNAMKKMG 1567
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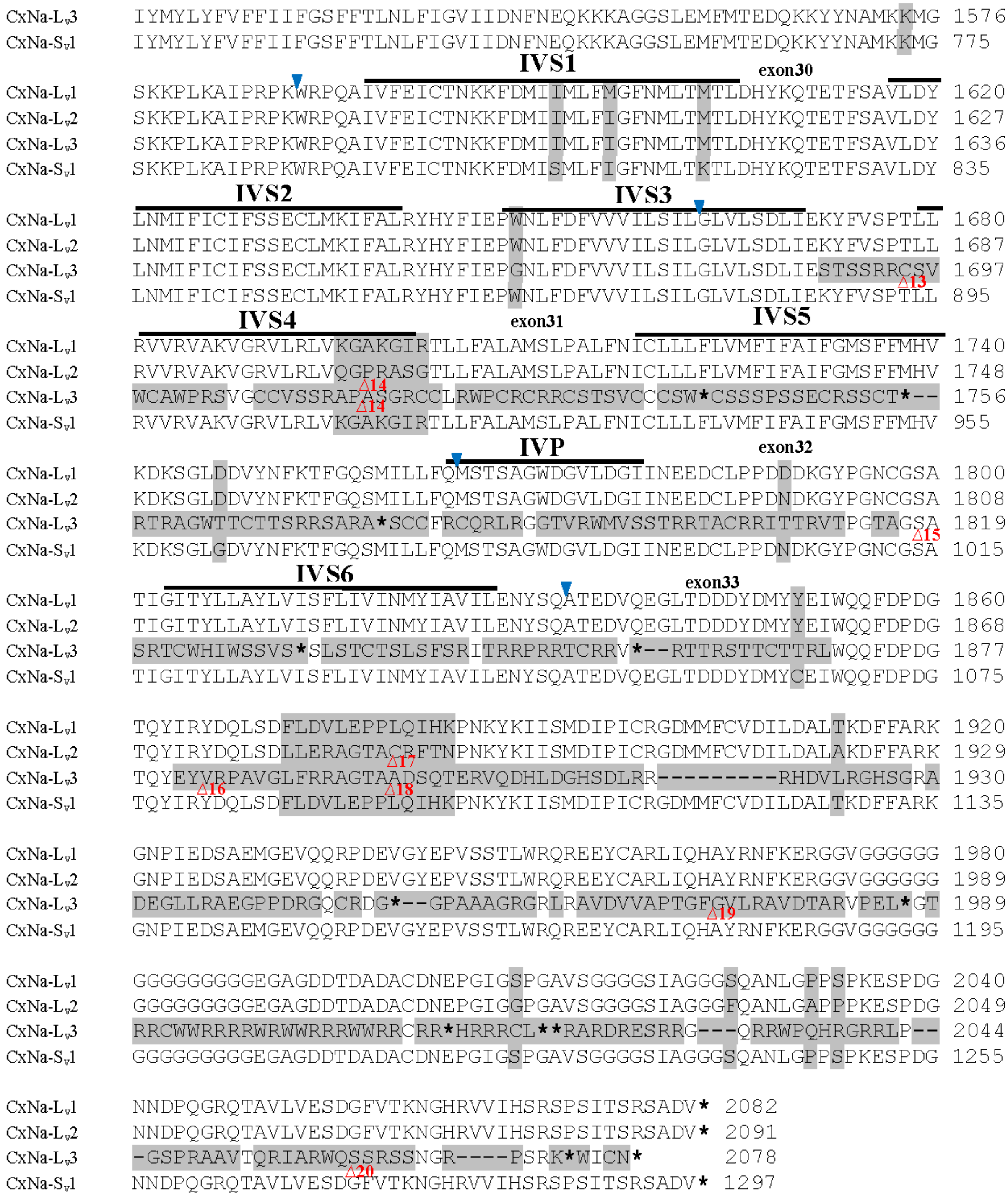
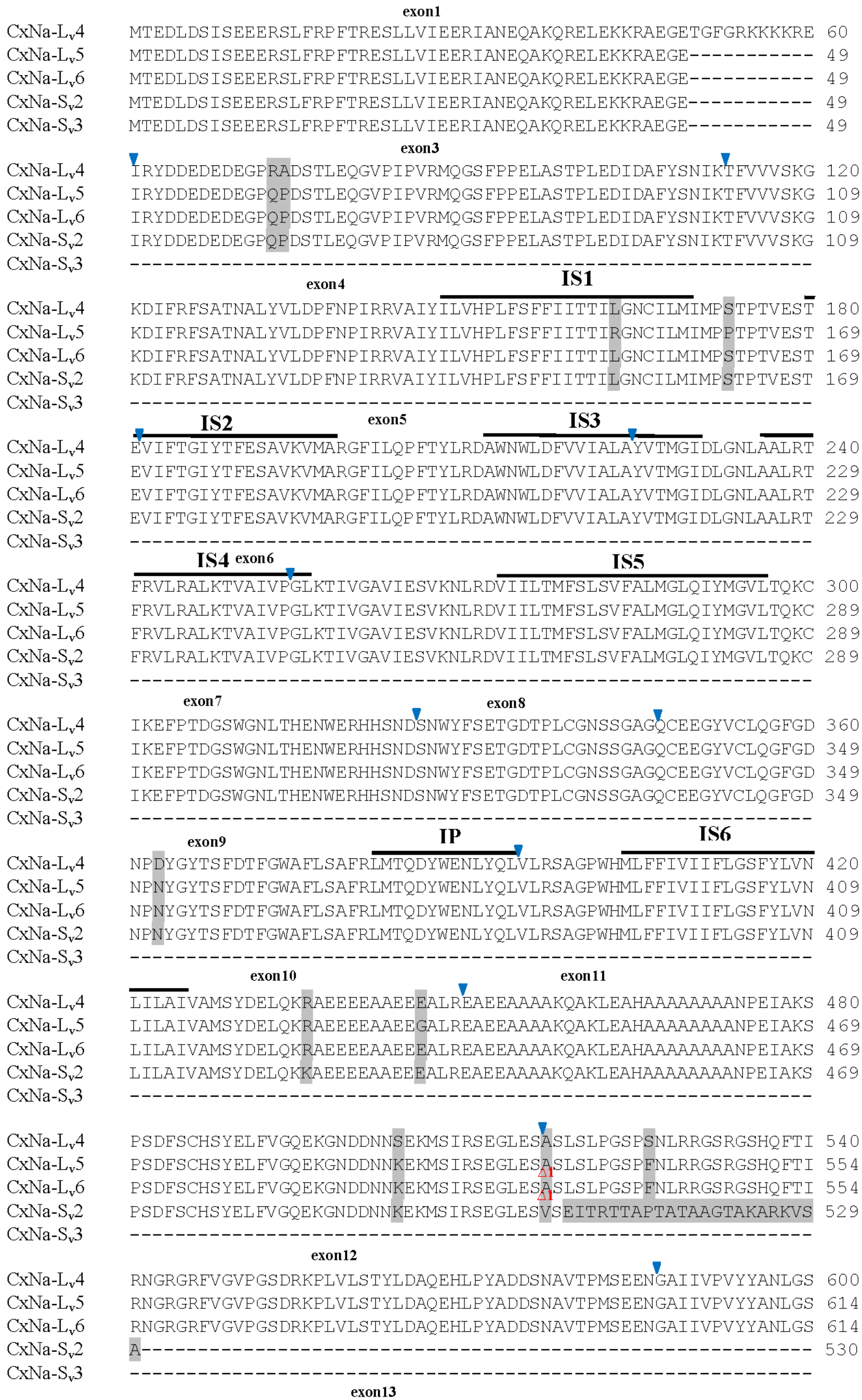


Figure 2. Alignment of deduced amino acid transcript sequences of the *para*-type sodium channel transcripts (Cx-Na) in S-Lab *Culex* mosquitoes. Transmembrane segments are indicated on the line over the sequence. Exons are indicated above the sequence with solid triangle symbols to indicate the boundaries between exons. The differences in the aa sequences are indicated by shading. A stop codon is marked by an asterisk (*). – indicates deletions. Δ indicates insertions with the sequences of Δ1: P; Δ2: VSEITRTTAPTATAAGTAKARKVSA; Δ3: GAIIVPVYYANL; Δ4:*I; Δ5: VSVYYFPT; Δ6: GPFR; Δ7: E; Δ8:*; Δ9: **SSR**VR; Δ10: *HCQY; Δ11:*; Δ12: G; Δ13: R; Δ14: R; Δ15: RRR; Δ16: T; Δ17: R; Δ18: A; Δ19: G; Δ20:**


exon1
CxNa-Lv4 MTEDLDSISEEERSLFRPFTRESLLVIEERIANEQAKQRELEKKRAEGETGFGRKKKKRE 60
CxNa-Lv5 MTEDLDSISEEERSLFRPFTRESLLVIEERIANEQAKQRELEKKRAEGE----------- 49
CxNa-Lv6 MTEDLDSISEEERSLFRPFTRESLLVIEERIANEQAKQRELEKKRAEGE----------- 49
CxNa-Sv2 MTEDLDSISEEERSLFRPFTRESLLVIEERIANEQAKQRELEKKRAEGE----------- 49
CxNa-Sv3 MTEDLDSISEEERSLFRPFTRESLLVIEERIANEQAKQRELEKKRAEGE----------- 49
exon3
CxNa-Lv4 IRYDDEDEDEGPRADSTLEQGVPIPVRMQGSFPPELASTPLEDIDAFYSNIKTFVVVSKG 120
CxNa-Lv5 IRYDDEDEDEGPQPDSTLEQGVPIPVRMQGSFPPELASTPLEDIDAFYSNIKTFVVVSKG 109
CxNa-Lv6 IRYDDEDEDEGPQPDSTLEQGVPIPVRMQGSFPPELASTPLEDIDAFYSNIKTFVVVSKG 109
CxNa-Sv2 IRYDDEDEDEGPQPDSTLEQGVPIPVRMQGSFPPELASTPLEDIDAFYSNIKTFVVVSKG 109
CxNa-Sv3 --
exon4
IS1
CxNa-Lv4 KDIFRFSATNALYVLDPFNPIRRVAIYILVHPLFSFFIITTILGNCILMIMPSTPTVEST 180
CxNa-Lv5 KDIFRFSATNALYVLDPFNPIRRVAIYILVHPLFSFFIITTIRGNCILMIMPPTPTVEST 169
CxNa-Lv6 KDIFRFSATNALYVLDPFNPIRRVAIYILVHPLFSFFIITTILGNCILMIMPSTPTVEST 169
CxNa-Sv2 KDIFRFSATNALYVLDPFNPIRRVAIYILVHPLFSFFIITTILGNCILMIMPSTPTVEST 169
CxNa-Sv3 --
IS2
exon5
IS3
CxNa-Lv4 EVIFTGIYTFESAVKVMARGFILQPFTYLRDAWNWLDFVVIALAYVTMGIDLGNLAALRT 240
CxNa-Lv5 EVIFTGIYTFESAVKVMARGFILQPFTYLRDAWNWLDFVVIALAYVTMGIDLGNLAALRT 229
CxNa-Lv6 EVIFTGIYTFESAVKVMARGFILQPFTYLRDAWNWLDFVVIALAYVTMGIDLGNLAALRT 229
CxNa-Sv2 EVIFTGIYTFESAVKVMARGFILQPFTYLRDAWNWLDFVVIALAYVTMGIDLGNLAALRT 229
CxNa-Sv3 --
IS4 exon6
IS5
CxNa-Lv4 FRVLRALKTVAIVPGLKTIVGAVIESVKNLRDVIILTMFSLSVFALMGLQIYMGVLTQKC 300
CxNa-Lv5 FRVLRALKTVAIVPGLKTIVGAVIESVKNLRDVIILTMFSLSVFALMGLQIYMGVLTQKC 289
CxNa-Lv6 FRVLRALKTVAIVPGLKTIVGAVIESVKNLRDVIILTMFSLSVFALMGLQIYMGVLTQKC 289
CxNa-Sv2 FRVLRALKTVAIVPGLKTIVGAVIESVKNLRDVIILTMFSLSVFALMGLQIYMGVLTQKC 289
CxNa-Sv3 --
exon7
exon8
CxNa-Lv4 IKEFPTDGSWGNLTHENWERHHSNDSNWYFSETGDTPLCGNSSGAGQCEEGYVCLQGFGD 360
CxNa-Lv5 IKEFPTDGSWGNLTHENWERHHSNDSNWYFSETGDTPLCGNSSGAGQCEEGYVCLQGFGD 349
CxNa-Lv6 IKEFPTDGSWGNLTHENWERHHSNDSNWYFSETGDTPLCGNSSGAGQCEEGYVCLQGFGD 349
CxNa-Sv2 IKEFPTDGSWGNLTHENWERHHSNDSNWYFSETGDTPLCGNSSGAGQCEEGYVCLQGFGD 349
CxNa-Sv3 --
exon9
IP
IS6
CxNa-Lv4 NPDYGYTSFDTFGWAFLSAFRLMTQDYWENLYQLVLRSAGPWHMLFFIVIIFLGSFYLVN 420
CxNa-Lv5 NPNYGYTSFDTFGWAFLSAFRLMTQDYWENLYQLVLRSAGPWHMLFFIVIIFLGSFYLVN 409
CxNa-Lv6 NPNYGYTSFDTFGWAFLSAFRLMTQDYWENLYQLVLRSAGPWHMLFFIVIIFLGSFYLVN 409
CxNa-Sv2 NPNYGYTSFDTFGWAFLSAFRLMTQDYWENLYQLVLRSAGPWHMLFFIVIIFLGSFYLVN 409
CxNa-Sv3 --
exon10
exon11
CxNa-Lv4 LILAIVAMSYDELQKRAEEEEAAEEEALREAEEAAAAKQAKLEAHAAAAAAAANPEIAKS 480
CxNa-Lv5 LILAIVAMSYDELQKRAEEEEAAEEGALREAEEAAAAKQAKLEAHAAAAAAAANPEIAKS 469
CxNa-Lv6 LILAIVAMSYDELQKRAEEEEAAEEEALREAEEAAAAKQAKLEAHAAAAAAAANPEIAKS 469
CxNa-Sv2 LILAIVAMSYDELQKKAEEEEAAEEEALREAEEAAAAKQAKLEAHAAAAAAAANPEIAKS 469
CxNa-Sv3 --
CxNa-Lv4 PSDFSCHSYELFVGQEKGNDDNNSEKMSIRSEGLESASLSLPGSPSNLRRGSRGSHQFTI 540
CxNa-Lv5 PSDFSCHSYELFVGQEKGNDDNNKEKMSIRSEGLESASLSLPGSPFNLRRGSRGSHQFTI 554
Δ1
CxNa-Lv6 PSDFSCHSYELFVGQEKGNDDNNKEKMSIRSEGLESASLSLPGSPFNLRRGSRGSHQFTI 554
Δ1
CxNa-Sv2 PSDFSCHSYELFVGQEKGNDDNNKEKMSIRSEGLESVSEITRTTAPTATAAGTAKARKVS 529
CxNa-Sv3 --
exon12
CxNa-Lv4 RNGRGRFVGVPGSDRKPLVLSTYLDAQEHLPYADDSNAVTPMSEENGAIIVPVYYANLGS 600
CxNa-Lv5 RNGRGRFVGVPGSDRKPLVLSTYLDAQEHLPYADDSNAVTPMSEENGAIIVPVYYANLGS 614
CxNa-Lv6 RNGRGRFVGVPGSDRKPLVLSTYLDAQEHLPYADDSNAVTPMSEENGAIIVPVYYANLGS 614
CxNa-Sv2 A--- 530
CxNa-Sv3 --
exon13

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CxNa-Lv4   RHSSYTSHQSRISYTSHGDLLGGMTKESRLRSRTQRNTNHSIVPPANMAASAASVTGAGS  660
CxNa-Lv5   RHSSYTSHQSRISYTSHGDLLGGMTKESRLRSRTQRNTNHSIVPPANMAASAASVTGAGS  674
CxNa-Lv6   RHSSYTSHQSRISYASHGDLLGGMTKESRLRSRTQRNTNHSIVPPANVAASAASVTGAGS  674
CxNa-Sv2   ------------------------------------------------------------
CxNa-Sv3   ------------------------------------------------------------

                                         exon14
CxNa-Lv4   GAPNMSYVDTNHKGQQRDFDQSQDYTDDAGKIKHNDNPFIEPSQTQTVVDMKDVMVLNDI  720
CxNa-Lv5   GAPNMSYVDTNHKGQQRDFDQSQDYTDDAGKIKHNDNPFIEPSQTQTVVDMKDVMVLNDI  734
CxNa-Lv6   GAPNMSYVDTNHKGQQRDFDQSQDYTDDAGKIKHNDNPFIEPSQTQTVVDMKDVMVLNDI  734
CxNa-Sv2   ------------------------------------------------------------
CxNa-Sv3   ------------------------------------------------------------

                   exon15                                exon16
CxNa-Lv4   IEQAAGRHSRASDHGVSVYYFPTEDDDEDGPTFKDKAVEFGMRMIDIFCVWDCCWVWLKF  780
CxNa-Lv5   IEQAAGRHSRASDHGVSVYYFPTEDDDEDGPTFKDKAVEFGMRMIGIFCVWDCCWVWLKF  794
CxNa-Lv6   TEPAAGRHSRASDHGVSVYYFPTEDDDEDGPTFKDKAVEFGMRMIDIFCVWDCCWVWLKF  794
CxNa-Sv2   ------------------------------------------------------------
CxNa-Sv3   -----------------------RTTTRTVRRSRTRRSSSGCG*STSSACGTAAGCGSKS  86
                          IIS1                              IIS2
CxNa-Lv4   QEWGSFIVFDPFVELFITLCIVVNTLFMALDHHDMNPDMERALKSGNYFFTATFAIEATM  840
CxNa-Lv5   QEWVSFIVFDPFVELFITLCIVVNTLFMALDHHDMNPDMERALKSGNYFFTATFAIEATM  854
CxNa-Lv6   QEWVSFIVFDPFVELFITLCIVVNTLLMALDHHDMNPDMERALKSGNYFFTATFAIEATM  854
CxNa-Sv2   ------------------------------------------------------------
CxNa-Sv3   SSRSGCPLSCSTRSSSCSSRSASWSTRCSWRSTTTT*TRTWSGRSRAVTTSSRRRSRGQR  146

                exon17      IIS3                                IIS4
CxNa-Lv4   KLIAMSPKWYFQEGWNIFDFIIVALSLLELGLEGVQGLSVLRSFRLLRVFKLAKSWPTLN  900
CxNa-Lv5   KLIAMSPKWYFQEGWNIFDFIIVALSLLELGLEGVQGLSVLRSFRLLRVFKLAKSWPTLN  914
CxNa-Lv6   KLIAMSPKWYFQEGWNIFDFIIVALSLLELGLEGVQGLSVLRSFRLLRVFKLAKSWPTLN  914
CxNa-Sv2   ------------------------------------------------------------
CxNa-Sv3   R*S*SR*APSGTSRKVGTFSISSSWPFRCSSSVXRAFRDCQYYVHSVCFECSS*QSRSPR  206
              exon18            IIS5
CxNa-Lv4   LLISIMGRTVGALGNLTFVLCIIIFIFAVMGMQLFGKNYTDNVDRFPDKDLPRWNFTDFM  960
CxNa-Lv5   LPISIMGRTVGALGNLTSVLCIIIFIFAVMGMQLFGKNYTDNVDRFPDKDLPRWNFTDFM  974
CxNa-Lv6   LLISIMGRTMGALGNLTFVLCIIIFIFAVMGMQLFGGNYIDNVDRFPDKDLPRWNFADFM  974
CxNa-Sv2   ------------------------------------------------------------
CxNa-Sv3   *TYSFPSWAERWAR*VI*RLCSALSSSSLP*WGCSCSARTTSTTWTASRTRTCHGGTLVT  266
                  IIP          Exon1          IIS6
CxNa-Lv4   HSFMIVFRVLCGEWIESMWDCMLVGDVSCIPFFLATVVIGNFVVLNLFLALLLSNFGSSS  1020
CxNa-Lv5   HSFMIVFRVLCGEWIESMWDCMLVGDVSCIPFFLATVVIGNFVVLNLFLALPLSIFGSSS  1034
CxNa-Lv6   HSFMIVFRVLCGEWIESMWDCMLVGDVSCIPFFLATVVIGNVVVLNLFLALLLSNFGSSS  1034
CxNa-Sv2   ------------------------------------------------------------
CxNa-Sv3   SCTHS*SCSGCCAASGSNPCGTACWWATCPAFRSSWPP***EI*SFLTFS*PCFCPTPRV  326
                exon20
CxNa-Lv4   LSAPTADNETNKIAEAFNRISRFSNWIKANIAAALKFVKNKLTSQIASVQPAEHGENERE  1080
CxNa-Lv5   LSAPTADNETNKIAEAFNRISRFSNWIKANIAAALKFVKNKLTSQIASVQPAD-------  1087
CxNa-Lv6   LSAPTADNETNKIAEAFNRDIALLQLDQG-EHRGRAQVREKQVNKPDCVRAARR*-----  1088
CxNa-Sv2   ------------------------------------------------------------
CxNa-Sv3   CRRPQPTTKRTRSPRRSTGYRASPTGSRRTSRPRSSS*KTS*QARLRPCSP---------  377

                 exon21ii                                 exon22
CxNa-Lv4   LTPDDILADGLLKKGVKEHNQLEVAIGDGMEFTIHGDLKNKGKKNKQLMNNSKVIGNSIS  1140
CxNa-Lv5   ----DILADGLLKKGVKEHNQLEVAIGDGMEFTIHGDLKNKGKKNKQLMNNSKVIGNSIS  1143
CxNa-Lv6   AAQPSQLDMERRQRGMSMYICRAW*K*AGINSR*HPGRREKGRQGAQPAGGGDRRRDGVY  1150
                                                  Δ2
CxNa-Sv2   ------------------------------------------------------------
CxNa-Sv3   --PDDILADGLLKKGVKEHNQLEVAIGDGMEFTIHGDPKNKGKKNKQLMNNSK-------  428
                                           exon23
CxNa-Lv4   NHQDNKLEHELNHRGMSLQDDDTASIKSYGSHKNRPFKDESHKGSAETLEGEEKRDASKE  1200
CxNa-Lv5   NHQDNKLEHELNHRGMSLQDDDTASIKSYGSHKNRPFKDESHKGSAETLEGEEKRDASKE  1203
CxNa-Lv6   DTRPQEQGQEEQAADEQFQGR*YHAYKVLWQSQESPLQGRKPQGQCRNAGGRRKARRQQG  1212
              Δ3
CxNa-Sv2   ------------------------------------------------------------
CxNa-Sv3   ------------------DDDTASIKSYGSHKNRPFKDESHKGSAETLEGEEKRDASKE  469
                                             exon24
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CxNa-Lv4   DLGIDEELDDECEGEEGPLDGEMIIHAEEDEVIEDAPADCFPDNCYKRFPALAGDDDAPF 1260
CxNa-Lv5   DLGIDEELDDECEGEEGPLDGEMIIHAEEDEVIEDAPADCFPDNCYKRFPALAGDDDAPF 1263
CxNa-Lv6   GPRN*RRTRRRVRG*GGSAGRGNDHPRGRGRSDRGRTGRLLPGQLLQAVPGAGRRRRRAV 1273
CxNa-Sv2   ------------------------------------------------------------
CxNa-Sv3   DLGIDEELDDECEGEEGPLDGEMIIHAEEDEVIEDAPADCFPDNCYKRFPALAGDDDAPF 529
                         IIIS1                         exon25   IIIS2
CxNa-Lv4   WQGWGNLRLKTFQLIENKYFETAVITMILLSSLALALEDVHLPHRPILQDVLYYMDRIFT 1320
CxNa-Lv5   WQGWGNLRLKTFQLIENKYFETAVITMILLSSLALALEDVHLPHRPILQDVLYYMDRIFT 1323
CxNa-Lv6   LAGLGQPAAQDVPADREQVLRDGRHHDDPAEGPGPRGCAPATPTNPAGRPVLHGQD-IHG 1333
                                          Δ4
CxNa-Sv2   ------------------------------------------------------------
CxNa-Sv3   WQGWGNLRLKTFQLIENKYFETAVITMILLSSLALALEDVHLPHRPILQDVLYYMDRIFT 589
                                    IIIS3                exon26     IIIS4
CxNa-Lv4   VIFFLEMLIKWLALGFRVYFTDAWCWLDFIIVMVSLINFVASLCGAGGIQAFKTMRTLRA 1380
CxNa-Lv5   VIFFLEMLIKWLALGFRVYFTNAWCWLDFIIVMVSLINFVASLCGAGGIQAFKTMRTLRA 1376
CxNa-Lv6   DLFFRDVDQVVGAR-LPGVLYERLVLARFHHCDGVLNQLRGFTLWSGWYSSIQNYANS*G 1392
CxNa-Sv2   ------------------------------------------------------------
CxNa-Sv3   VIFFLEMLIKWLALGFRVYFTNAWCWLDFIIVMLSLINFAIWVGAAD-IPAFRSMRTLRA 649
                                                Δ5
                                    exon27          IIIS5
CxNa-Lv4   LRPLRAMSRMQGMRVVVNALVQAIPSIFNVLLVCLIFWLIFAIMGVQLFAGKYFKCVDTN 1440
CxNa-Lv5   LRPLRAMSRMQGMRVVVNALVQAIPSIFNVLLVCLIFWLIFAIMGVQLFAGKYFKCVDTN 1436
CxNa-Lv6   TASATCHVPYAGYEGCRQCIGTGYTVHLQRVIGVFDLLVDFRHHGRPAVCRKVLQVRRHE 1451
CxNa-Sv2   --------------VVVNALVQAIPSIFNVLLVCLIFWLIFAIMGVQLFAGKYFKCVDTN 576
CxNa-Sv3   LRPLRACLSLGGHESCRQCIGTGYTVHLQRVIGVFDLLVDFRHHGRPAVCRKVLQVRRHE 709
                   exon28                                     IIP
CxNa-Lv4   KATLSHEIIPDVNACIAENYTWENSPMNFDHVGKAYLCLFQVATFKGWIQIMNDAIDSRD 1500
CxNa-Lv5   KTTLSHEIIPDVNACIAENYTWENSPMNFDHVGKAYLCLFQVATFKGWIQIMNDAIDSRD 1496
CxNa-Lv6   QDDTVARDHPGRERVHRGELHLGELPDEL*PRGEGLPVFVPG----GHVQGMDPDRERRD 1511
CxNa-Sv2   KTTLSHEIIPDVNACIAENYTWENSPMNFDHVGKAYLCLFQVATFKGWIQIMNDAIDSRD 636
CxNa-Sv3   QDDTVARDHPGRERVHRGELHLGELPDEL*PRGEGLPVFVPGGHVQGMDPDHERRDRLAG 769
                           IIIS6                          Exon29
CxNa-Lv4   IGKQPIRETNIYMYLYFVFFIIFGSFFTLNLFIGVIIDNFNEQKKRAGGSLEMFMTEDQK 1560
CxNa-Lv5   IGKQPIRETNIYMYLYFVFFIIFGSFFTLSLFIGVIIDNFNEQKKKAGGSLEMFMTEGQK 1556
CxNa-Lv6   RLARHWKAAHPRNQHLHVLVLCVLHHLRIVLHAEPLHRCHY*QL*---RTEEEGWGIARD 1567
CxNa-Sv2   IGKQPIRETNIYMYLYFVFFIIFGSFFTLNLFIGVIIDNFNEQKKKAGGSLEMFMTEDQK 696
CxNa-Sv3   HRKAAHPRNQHLHVLVLCVLHHLRIVLHAEPLHRCHY*QL*RTEEEGWGIARDVYDGGLK 829
                                             exon30     IVS1
CxNa-Lv4   KYYNAMKKMGSKKPLKAIPRPKWRPQAIVFEICTNKKFDMIIMLFIGFNMLTMTLDHYKQ 1620
CxNa-Lv5   KYYNAMKKMGSKKPLKAIPRPKRRPQAIVFEICTNKKFDMIIMLFIGFNMLTMTLDHYKQ 1616
CxNa-Lv6   VYDGGPKKVLQRNEEDGLEEATEGHSAAQVATTSNSVRNLHKQKVRHDHHVVHRLQHVDD 1624
CxNa-Sv2   KYYNAMKKMGSKKPLKAIPRPKWRPQAIVFEICTNKSST*SSCCSSASTC*R*RWITTSR 756
CxNa-Sv3   KVLQRNEEDGLEEATEGHSAAQVATTSNSVRNLHKQKVRHDHHVVHRLQHVDDDAGSLQA 889
                      IVS2                                 IVS3
CxNa-Lv4   TETFSAVLDYLNMIFICIFSSECLMKIFALRYHYFIEPWNLFDFVVVILSILGLVLSDLI 1680
CxNa-Lv5   TGTFSAVLDYLNMIFICIFSSECLMKIFALRYHYFIEPWNLFDFVVVILSILGLVLRDLI 1676
CxNa-Lv6   G---AGSLQAVGHVQRGAGLPEHDLHLYLQ*---RVSDEDLRAALPLLYRTVEPVRFRRR 1684
CxNa-Sv2   RKRSARCWTT*T*SSSVSSVASV**RSSRCATTTLSNRGTCSISSSSSCPFWAWC*AT*S 816
CxNa-Sv3   DGNVQRGAGLPEHDLHLYLQ*RVSDEDLRAALPLLYRTVEPVRFRRRHPVHFGPGAERPD 949

           exon31          IVS4                                 IVS5
CxNa-Lv4   EKYFVSPTLLRVVRVAKVGRVLRLVKGAKGIRTLLFALAMSLPALFNICLLLFLVMFIFA 1740
CxNa-Lv5   EKYFVSPTLLRVVRVAKVGRVLRLVKGAKGIRTLLFALAMSLPALFNICLLLFLVMFIFA 1735
CxNa-Lv6   HPVHFGPGAERPDR-----EVLRLADAAPCARGQGRSGAASRQGRQGHPDVAVCAGHVAA 1738
                                          Δ6
CxNa-Sv2   KSTSSRRRCSVWCAWPRSVGCCVSSRAPRASGRCCLRWPCRCRRCSTSVCCCSW*CPSSP 876
CxNa-Sv3   RKVLRLADAAPCGARGQGRSGAASRQGRQGHPDVAVCAGHVAAGAVQHLSAAVPGDVHLR 1009
                                                 IVP        exon32
CxNa-Lv4   IFGMSFFMHVKDKSGLDDVYNFKTFGQSMILLFQMSTSAGWDGVLDGIINEEDCLPPDND 1800
CxNa-Lv5   IFGMSFFMHMKDKSGLDGVYNFKTFGQSMILLFQMSTSAGWDGVLDGIINEEDCLPPDND 1795
CxNa-Lv6   GAVQHLSAAVPGDVHLRHLWDVVLHAR------EGQERAGRRVQLQDVRPEHDPAVSDVN 1794
CxNa-Sv2   SSGCRSSCT*RTRAGWTTCTTSRRSARA*SCCFRCQRLRGGTVCWMVSSTRRTACRRITT 936
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CxNa-Sv3   HLRNVVLHAREGQERAGRRVQLQDVRPEHDPAVSDVNVCGVGRCAGWYHQRGGLPAAGYR 1069
                           IVS6                         exon33
CxNa-Lv4   KGYPGNCGSATIGITYLLAYLVISFLIVINMYIAVILENYSQATEDVQEGLMDDDYDMYY 1860
CxNa-Lv5   KGYPGNCGSATIGITYLLAYLVISFLIVINMYIAVILENYSQATEDVQEGLTDDDYDMYY 1855
CxNa-Lv6   VCWVGRCAGWYHQRGGLLAAG*-------------------RQGLPRELRVGDDRHHVPA 1848
CxNa-Sv2   RVTRGTAGRRRSASRTCWRIWSSVS*SLSTCTSLSFSRITRRPRRTCRRV*RTTTTTCTT 996
CxNa-Sv3   QGLPRELRVGDDRHHAPAGISGHQFPDRYQHVHRCHSRELLAGHGGRAGGSDGRRLRHVL 1129

CxNa-Lv4   EIWQQFDPDGTQYIRYDQLSDFLDVLEPPLQIHKPNKYKIISMDIPICRGDMMFCVDILD 1920
CxNa-Lv5   EIWQQFDPDGTQYIRYDQLSDFLDVLEPPLQIHKPNKYKIISMDIPICRGDMMFCVDILD 1915
CxNa-Lv6   GISGHQFPDRYQHVHRCHPRELLAGHGGRAGGSDGRRLRHVLRDLAAVRSGRYAVHPVRP 1889
CxNa-Sv2   RSGSSSIRTVRSTSGTTSCRTFWTCRNRRCRFTNRTSTRSSRWTFRSVAAT*CSAWTFWT 1056
CxNa-Sv3   RDLAAVRSGRYAVHPVRPAVGLFGRAGTAAADSQTEQVQDHLDGHSDLSRRHDVLRGHSG 1189

CxNa-Lv4   ALTKDFFARKGNPIEDSAEMGEVQQRPDEVGYEPVSSTLWRQREEYCARLIQHAYRNFKE 1980
CxNa-Lv5   ALTKDFFARKGNPIEDSAEMGEVQQRPDEVGYEPVSSTLWRQREEYCARLIQHAYRNFKE 1975
CxNa-Lv6   AVG--LFGRAGTAAADS-QTEQVQDHLD--------------------------GHSDLSR 1921
CxNa-Sv2   R*RRTSSRGRSTRSRTVPRWVRSSSGRTRSVTSRFRRRCGANGRSTARG*YSTRTGTLRN 1116
CxNa-Sv3   RADEGLLRAEGQPDRGQCRDG*GPAAAGRGRLRAGFVDVVAPTGGVLRAVDTARVPEL*G 1249

CxNa-Lv4   RGGVGGGGGGGGGGGGGGEGAGDDTDADACDNEPGIGSPGAVSGGGGSIAGGGSQANLG 2040
CxNa-Lv5   RGGVGGGGGGGGGGGGGGEGAGDDTDADACDNEPGIGSPGAVSGGDSSIAGGGSQANLG 2035
CxNa-Lv6   RHDVLRGHSGRADEGLLRAEGQ---PDRGQCRDG*-----GPAAAGRGRLRAGFVDVVAP 1973
CxNa-Sv2   EAVLVAAAAVEVVEEEVVAKVPEMTPTPMPVITSPGSGVPARSAAVAAASPAEAPRLT*A 1176
CxNa-Sv3   TRRCWWRRRRWRWWRRRWWRRCRR*HRRRCL**RARDRESRRGQRRWRQHRRRRLPG*PR 1309

CxNa-Lv4   PPSPKESPDGNNDPQGRQTAVLVESDGFVTKNGHRVVIHSRSPSITSRSADV*        2092
CxNa-Lv5   PPSPKESPDGNNDPQGRQTAVLVESDGFVTKNGHRVVIHSRSPSITSRSADV*        2095
CxNa-Lv6   TGGVLRAVDTTRVPEL*----GTRRCWWRRRWRRRWRWWRRCRR*HRRRCL**        2021
CxNa-Sv2   RLTQRIVRWQ**SSRSSNGRPSRK*WICN*                               1205
CxNa-Sv3   AAVTQRIARWQ**SSRSSNGRPSRK*WICN*                              1339
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Figure 3. Alignment of deduced amino acid transcript sequences of the *para*-type sodium channel reanscripts (Cx-Na) in HAmCq^{G0} *Culex* mosquitoes. Transmembrane segments are indicated on the line over the sequence. Exons are indicated above the sequence with solid triangle symbols to indicate the bounderies between exons. The differences in the aa sequences are indicated by shading. A stop codon is marked by an asterisk (*). – indicates deletions. Δ indicates insertions with the sequences of Δ1: VSEITRTTAPTATAAGTAKARKVSA; Δ2: AA; Δ3: R; Δ4: *F; Δ5: L; Δ6: G.

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                       exon1                                          exon2
CxNa-Lv7    MTEDLDSISEEERSLFRPFTRESLLVIEERIANEQAKQRELEKKRAEGETGFGRKKKKKE  60
CxNa-Lv8    MTDDLDSISEEERSLFRPFTRESLLAIEERIANEQAKQRELEKKRAEGETGFGRKKKKKE  60
CxNa-Sv4    MTDDLDSISEEERSLFRPFARESLLVIEERIANEQAKQRELEKKRAEGETGFGRKKKKKE  60
CxNa-Sv5    MTEDLDSISEEERSLFRPFTRESLLVIEERIANEQAKQRELEKKRAEGETGFGRKEKKKE  60
                           exon3
CxNa-Lv7    IRYDDEDEDEGPQPDSTLEQGVPIPVRMQGSFPPELASTPLEDIDAFYSNIKTFVVVSKG  120
CxNa-Lv8    IRYDDEDEDEGPQPDSTLEQGVPIPVRMQGSFPPELASTPLEDIDAFYSNIKTFVVVSKG  120
CxNa-Sv4    IRYDDEDEDEGPQPDSTLEQGVPIPVRMQGSFPPELASTPHEDIDAFYSNIKTFVVVSKG  120
CxNa-Sv5    IRYDDEDEDEGPQPDSTLEQGVPIPVRMQGSFPPELASTPLEDIDAFYSNIKTFVVVSKG  120
                   exon4                         IS1
CxNa-Lv7    KDIFRFSATNALYVLDPFNPIRRVAIYILVHPLFSFFIITTILGNCILMIMPSTPTVEST  180
CxNa-Lv8    KDIFRFSATNALYVLDPFNPIRRVAIYILVHPLFSFFIITTILGNCILMIMPSTPTVEST  180
CxNa-Sv4    KDIFRFSATNALYVLDPFNPIRRVAIYILVHPLFSFFIITTILGNCILMIMPSTPTVEST  180
CxNa-Sv5    KDIFRFSATNALYVLDPFNPIRRVAIYILVHPLFSFFIITTILGNCILMIMPSTPTVEST  180
                IS2              exon5                IS3
CxNa-Lv7    EVIFTGIYTFESAVKVMARGFILQPFTYLRDAWNWLDFVVIALAYVTMGIDLGNLAALRT  240
CxNa-Lv8    EVIFTGIYTFESAVKVMARGFILQPFTYLRDAWNWLDFVVIALAYVTMGIDLGNLAALRT  240
CxNa-Sv4    E-----------------------------------------------------------  181
CxNa-Sv5    E-----------------------------------------------------------  181
            exon6  IS4                                  IS5
CxNa-Lv7    FRVLRALKTVAIVPGLKTIVGAVTESVKNLRDVIILTMFSLSVFALMGLQIYMGVLTQKC  300
CxNa-Lv8    FRVLRALKTVATVPGLKTIVGAVIESVKNLRDVIILTMFSLSVFALMGLQIYMGVLTQKC  300
CxNa-Sv4    ------------------------------------------------------------
CxNa-Sv5    ------------------------------------------------------------
                exon7                           exon8
CxNa-Lv7    IKEFPTDGSWGNLTHENWERHHSNDSNWYFSETGDTPLCGNSSGAGQCEEGYVCLQGFGD  360
CxNa-Lv8    IKEFPTDGSWGNLTHENWERHHSNDSNWYFSETGDTPLCGNSSGAGQCEEGYVCLQGFGD  360
CxNa-Sv4    ------------------------------------------------------------
CxNa-Sv5    ------------------------------------------------------------
                exon9                     IP                    IS6
CxNa-Lv7    NPNYGYTSFDTFGWAFLSAFRLMTQDYWENLYQLVLRSAGPWHMLFFIVIIFSGSFYLVD  420
CxNa-Lv8    NPNYGYTSFDTFGWAFLSAFRLMTQDYWENLYQLVLRSAGPWHMLVFIVIIFLGSFYLVN  420
CxNa-Sv4    ------------------------------------------------------------
CxNa-Sv5    ------------------------------------------------------------
                      exon10                              exon11
CxNa-Lv7    LILAIVAMSYDELQKRAEEEEAAEEEALREAEEAAAAKQARLEAHAAAAAAAANPEIAKS  480
CxNa-Lv8    LILAIVAMSYDELQKRAEEEEAAEEEALREAEEAAAAKQAELEAHAAAAAAAANPEIAKN  480
CxNa-Sv4    ------------------------------------------------------------
CxNa-Sv5    ------------------------------------------------------------

CxNa-Lv7    PSDFSCHSYELFVGQEKGNDDNNKEKMSIRSEGLESVSEITRTTAPTATAAGTAKARKVS  540
CxNa-Lv8    PSDFSCHSYELFVGQEKGNDDNNKEKMSIRSEGLESVSEITRTTAPTATAAGTAKARKVS  540
CxNa-Sv4    ------------------------------------------------------------
CxNa-Sv5    ------------------------------------------------------------
                         exon12
CxNa-Lv7    AASLSLPGSPFNLRRGSRGSHQFTIRNGRGRFVGVPGSDRKPLVLSTYLDAQEHLPYADD  600
CxNa-Lv8    AASLSLPGSPFNLRRGSRGSHQFTIRNGRGRFVGVPGSDRKPLVLSTYLDAQEHLPYADD  600
CxNa-Sv4    ------------------------------------------------------------
CxNa-Sv5    ------------------------------------------------------------

CxNa-Lv7    SNAVTPMSEENGSRHSSYTSHQSRISYTSHGDLLGGMTKESRLRSRTQRNTNHSIVPPAN  660
CxNa-Lv8    SNAVTPMSEENGSRHSSYTSHQSRISYTSHGDLLGGMTKESRLRSRTQRNTNHSIVPPAN  672
CxNa-Sv4    ------------------------------------------------------------
CxNa-Sv5    ------------------------------------------------------------
                          exon13                     exon14
CxNa-Lv7    MAASAASVTGAGSGAPNMSYVDTNHKGQQRDFDQSQDYTDDAGKIKHNDNPFIEPSQTQT  720
CxNa-Lv8    MAASAASVTGAGSGAPNMSYVDTNHKGQQRDFDQSQDYTDDAGKIKHNDNPFIEPSQTQT  732
CxNa-Sv4    ------------------------------------------------------------
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CxNa-Sv5   ------------------------------------------------------------

                                     exon15                    exon16
CxNa-Lv7   VVDMKDVMVLNDIIEQAAGRHSRASDHGVSVYYFPTEDDDEDGPTLKDKAVEFGMRMIDI 780
CxNa-Lv8   VVDMKDVMVLNDIIEQAAGRHSRASDHGVSVYYFPTEDDDEDGPTFKDKAVEFGMRMIDI 792
CxNa-Sv4   ------------------------------------------------------------
CxNa-Sv5   ------------------------------------------------------------
                                      IIS1
CxNa-Lv7   FCVWDCCWVWLKFQEWVSFIVFDPFVELFITLCIVVNTLFMALDHHDMNPDMERALKSGN 840
CxNa-Lv8   FCVWDCCWVWLKFQEWVSFIVFDPFVELFITLCIVVNTLFMALDHHDMNPDMERALKSGN 852
CxNa-Sv4   ------------------------------------------------------------
CxNa-Sv5   ------------------------------------------------------------

                  IIS2         exon17        IIS3               IIS4
CxNa-Lv7   YFFTATFAIEATMKLIAMSPKWYFQEGWNIFDFIIVALSLLELGLEGVQGLSVLRSFRLL 900
CxNa-Lv8   YFFTATFAIEATMKLIAMSPKWYFQEGWNIFDFIIVALSLLELGLEGVQGLSVLRSFRLL 912
CxNa-Sv4   ------------------------------------------------------------
CxNa-Sv5   ------------------------------------------------------------
                                exon18           IIS5
CxNa-Lv7   RVFKLAKSWPTLNLLISIMGRTMGALGNLTFVLCIIIFIFAVMGMQLFGKNYIDNVDRFP 960
CxNa-Lv8   RVFKLAKSWPTLNLLISIMGRTVGALGNLTFVLCIIIFIFAVMGMQLFGKNYTDNVDRFP 972
CxNa-Sv4   -----------------------------------------------------QRGPLPG 188
CxNa-Sv5   -----------------------------------------------------QRGPLPG 188
                   exon19          IIP                    IIS6
CxNa-Lv7   DKDLPRWNFTDFMHSFMIVFRVLCGEWIESMWDCMLVGDVSCIPFFLATVVIGNFVVLNL 1020
CxNa-Lv8   DKDLPRWNFTDFMHSFMIVFRVLCGEWIESMWDCMLVGDVSCIPFFLATVVIGNFVVLNL 1032
CxNa-Sv4   QGPATVELHRLHALIHDRVPGAVRRVDRIHVGLHAGGRRVLHSVLLGHRSDRKFCRSYTL 248
CxNa-Sv5   QGPATVVNPRLHALIHDRVPGAVRRV-IESMWDCMLVGDVSCIPFFLATVVIGNFVVLNL 247
                   exon20
CxNa-Lv7   FLALLLSNFGSSSLSAPTADNETNKIAEAFNRISRFSNWIKANIAAALKFVKNKLTSQIA 1080
CxNa-Lv8   FLALLLSNFGSSSLSASTADNETNKIAEAFNRISRFSNWIKANIAAALKFVKNKLTSQIA 1092
CxNa-Sv4   FLALLLSNFGSSSLSAPTAGNETNKIAEAFNRISRFSNWIKANIAAALKFVKNKLTSQIA 308
CxNa-Sv5   FLALLLSNFGSSSLSAPTADNETNKIAEAFNRISRFSNWIKANIAAALKFVKNKLTSQIA 307

                   exon21i                     exon21ii
CxNa-Lv7   SVQPAGKGVCPCISAEHGENELELTPDDILADGLLKKGVKEHNQLEVAIGDGMEFTIHGD 1140
CxNa-Lv8   SVQPA----------EHGENELELTPDDILADGLLKKGVKEHNQLEVAIGDGMEFTIHGD 1142
CxNa-Sv4   SVQPAGKGVCPCISAEHGENELELTPDDILADGLLKKGVKEHNQLEVAIGDGMEFTIHGD 381
                 Δ2
CxNa-Sv5   SVQPAGKGVCPCISAEHGENELELTPDDILADGLLKKGVKEHNQLEVAIGDGMEFTIHGD 380
                 Δ3
                 exon22                      exon23
CxNa-Lv7   LKNKGKKNKQLMNNSKDDDTASIKSYGSHKNRPFKDESHKGSAETLEGEEKRDASREDLG 1200
CxNa-Lv8   LKNKGKKNKQLMNNSKDDDTASIKSYGSHKNRPFKDESHKGSAETLEGEEKRDASKEDLG 1202
CxNa-Sv4   LKNKGKKNKQLMNNSQDDDTASIKSYGSHKNRPFKDESHKGSAETLEGEEKRGASKEDLG 467
                          Δ4
CxNa-Sv5   LKNKGKKNKQLMNNSKDDDTASIKSYGSHKNRPFKDESHKGSAETLEGEEKRDASKEDLG 440

                                 exon24
CxNa-Lv7   IDEELDDECEGEEGPLDGEMIIHAEEDEVIEDAPADCFPDNCYKRFPALAGDDDAPFWQG 1260
CxNa-Lv8   IDEELDDECEGEEGPLDGEMIIHAEEDEVIEDAPADCFPDNCYKRFPALAGDDDAPFWQG 1262
CxNa-Sv4   IDEELDDECEGEEGPLDGEMIIHAEEDEVIEDAPADCFPDNCYKRFPALAGDDDAPFWQG 527
CxNa-Sv5   IDEELDDECEGEEGPLDGEMIIHAEEDEVIEDAPADCFPDNCYKRFPALAGDDDAPFWQG 500
                              IIIS1                      exon25   IIIS2
CxNa-Lv7   WGNLRLKTFQLIENKYFETAVITMILLSSLALALEDVHLPHRPILQDVLYYMDRIFTVIF 1320
CxNa-Lv8   WGNLRLKTFQLIENKYFETAVITMILLSSLALALEDVHLPHRPILQDVLYYMDRIFTVIF 1322
CxNa-Sv4   WGNLRLKTFQLIENKYFETAVITMILLSSLALALEDVHLPHRPILQDVLYYMDRIFTVIF 587
CxNa-Sv5   WGSLRLKTLQLIENKYFETAVITMILLSSLALALEDVHLPHRPILQDVLYYMDRIFTVIF 560
                                       IIIS3               exon26  IIIS4
CxNa-Lv7   FLEMLIKWLALGFRVYFTNAWCWLDFIIVMVSLINFVASLCGAGGIQAFKTMRTLRALRP 1380
CxNa-Lv8   FLEMLIKWLALGFRVYFTNAWCWLDFIIVMVSLINFVASLCGAGGIQAFKTMRTLRALRP 1382
CxNa-Sv4   FLEMLIKWLALGFRVYFTNAWCWLDFIIVMVSLINFVASLCGAGGIQAFKTMRTLRALRP 647
CxNa-Sv5   FLEMLIKWLALGFRVYFTNAWCWLDFIIVMVSLINFVASLCGAGGIQAFKTMRTLRALRP 620
                                exon27             IIIS5
CxNa-Lv7   LRAMSRMQGMRVVVNALVQAIPSIFNVLLVCLIFWLIFAIMGVQLFAGKYFKCVDTNKTT 1440
CxNa-Lv8   LRAMSRMQGMRVVVNALVQAIPSIFNVLLVCLIFWLIFAIMGVQLFAGKYFKCVDTNKTT 1442
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CxNa-Sv4    LRAMSRMQGMRVVVNALVQAIPSIFNVLLVCLIFWLIFAIMGVQLFAGKYFKCVDTNKTT 707
CxNa-Sv5    LRAVSRMQGMRVVVNALVQAIPSIFNVLLVCLISWLIFAIMGVQLFAGKYFKCVDTNKTT 680
                         exon28                  IIP
CxNa-Lv7    LSHEIIPDVNACIAENYTWENSPMNFDHVGKAYLCLFQVATFKGWIQIMNDAIDSRDIGK 1500
CxNa-Lv8    LSHEIIPDVNACIAENYTWENSPMNFDHVGKAYLCLFQVATFKGWIQIMNDAIDSRDIGK 1502
CxNa-Sv4    LSHEIIPDVNACIAENYTWENSPMNFDHVGKAYLCLFQVATFKGWIQIMNDAIDSRDIGK 767
CxNa-Sv5    LSHEIIPDVNACIAENYTWENSPMNFDHVGKAYLCLFQVATFKGWIQIMNDAIDSRDIGK 740
                         IIIS6                      Exon29
CxNa-Lv7    QPIRETNIYMYLYFVFFIIFGSFFTLNLFIGVIIDNFNEQKKKAGGSLEMFMTEDQKKYY 1560
CxNa-Lv8    KPIRETNIYMYLYFVFFIIFGSFFTLNLFIGVIIDNFNEQKKKAGGSLEMFMTEDQKKYY 1562
CxNa-Sv4    QPIRETNIYMYLYFVFFIIFGSFFTLNLFIGVIIDNFNEQKKKAGGSLEMFMTEDQKKYY 827
CxNa-Sv5    QPIRETNIYMYLYFVFFIIFGSFFTLNLFIGVIIDNFNEQKKKAGGSLEMFMTEDQKKYY 800
                                          exon30        IVS1
CxNa-Lv7    NAMKKMGSKKPLKAIPRPKRRPQAIVFEICTNKKFDMIIMLFIGFNMLTMTLDHYKQTET 1620
CxNa-Lv8    NAMKKMGSKKPLKAIPRPKRRPQAIVFEICTNKKFDMIIMLFIGFNMLTMTLDHYKQTET 1622
CxNa-Sv4    NAMKKMGSKKPLKAIPRPKRRPQAIVFEICTNKKFDMIIMLFIGFNMLTMTLDHYKQTET 887
CxNa-Sv5    NAMKKMGSKKPLKAIPRPKRRPQAIVFEICTNKKFDMIIMLFIGFNMLTMTLDHYKQTET 860
                      IVS2                              IVS3
CxNa-Lv7    FSAVLDYLNMIFICIFSSECLMKIFALRYHYFIEPWNLFDFVVVILSILGLVLSDLIEKY 1680
CxNa-Lv8    FSAVLGYLNMIFICIFSSECLMKIFALRYHYFIEPWNLFDFVVVILSILGLVLSDLIEKY 1682
CxNa-Sv4    FSAVLDYLNMIFICIFSSECLMKIFALRYHYFIEPWNLFDFVVAILSILGLVLSDLIEKY 947
CxNa-Sv5    FSAVLDYLNMIFICIFSSECLMKIFALRYHYFIEPWNLFDFVVVILSILGLVLSDLIEKY 920

            exon31          IVS4                                IVS5
CxNa-Lv7    FVSPTLLRVVRVAKVGRVLRLVKGAKGIRTLLFALAMSLPALFNICLLLFLVMFIFAIFG 1740
CxNa-Lv8    FVSPTLLRVVRVAKVGRVLRLVKGAKGIRTLLFALAMSLPALFNICLLLFLVMFIFAIFG 1742
CxNa-Sv4    FVSPTLLRVVRVAKVGRVLRLVKGAKGIRTLLFALAMSLPALFNICLLLFLVMFIFAIFG 1007
CxNa-Sv5    FVSPTLLRVVRVAKVGRVLRLVKGAKGIRTLLFALAMSLPALFNICLLLFLVMFIFAIFG 980
                                                IVP        exon32
CxNa-Lv7    MSFFMHVKDKSGLDDVYNFKTFGQSMILLFQMSTSAGWDGVLDGIINEEDCLPPDNDKGY 1800
CxNa-Lv8    MSFFMHVKDKSGLDDVYNFKTFGQSMILLFQMSTSAGWDGVLDGIINEEDCLPPDNDKGY 1802
CxNa-Sv4    MSFFMHVKDKSGLDDVYNFKTFGQSMILLFQMSTSAGWDGVLDGIINEEDCLPPDNDKGY 1067
CxNa-Sv5    MSFFMHVKDKSGLDDVYNFKTFGQSMILLFQMSTSAGWDGVLDGIINEEDCLPPDNDKGY 1040
                         IVS6                               exon33
CxNa-Lv7    PGNCGSATIGITYLLAYLVISFLIVINMYIAVILENYSQATEDVQEGLTDDDYDMYYEIW 1860
CxNa-Lv8    PGNCGSATIGITYLLAYLAISFLIVINMYIAVILENYSQATEDVQEGLTDDDYDMYYEIW 1862
CxNa-Sv4    PGNCGSATVGITYLLAYLVISFLIVINMYIAVILENYSQATEDVQEGLTDDDYDMYYEIW 1127
CxNa-Sv5    PGNCGSATIGITYLLAYLVISFLIVINMYIAVILENYSQATEDVQEGLTDDDYDMYYEIW 1100

CxNa-Lv7    QQFDPDGTQYIRYDQLSDFLDVLEPPLQIHKPNKYKIISMDIPICRGDMMFCVDILDALT 1920
CxNa-Lv8    QQFDPDGTQYIRYDQLSDFLDVLEPPLQIHKPNKYKIISMDIPICRGDMMFCVDILDALT 1922
CxNa-Sv4    QQFDPDGTQYIRYDQLSDFLDVLEPPLQIHKPNKYKIISMDIPICRGDMMFCVDTLDALT 1187
CxNa-Sv5    QQFDPDGTQYIRYDQLSGFLDVLEPPLQIHKPNSNKIISMDIPICRGDMVFCVDILDALT 1160

CxNa-Lv7    KDFFARKGNPIEDSAEMGGVQQRPDEVGYEPVSSTLWRQREEYCARLIQHAYRNFKERGG 1980
CxNa-Lv8    KDFFARKGNPIEDSAEMGEVQQRPDEVGYEPVSSTLWRQREEYCARLIQHAYRNFKERGG 1982
CxNa-Sv4    KDFFARKGNPIEDSAEMGEVQQRPDEVGYEPVSSTLWRQREEYCARLIQHAYRNFKERGG 1247
CxNa-Sv5    KDFFARKGNPIEDSAEMGEVQQRPDEVGYEPVSSTLWRQREEYCARLIQHAYRNFKERGG 1220

CxNa-Lv7    VGGGGGGGGGGGGGGGEGAGDDTDADACDNEPGIGSPGAVSGGGGSIAGGGSQANLGPPS 2040
CxNa-Lv8    VGGGGGGGGGGGGGGGEGAGDDTDADACDNEPGIGSPGAVSGGGGSIAGGGSQANLGPPS 2042
CxNa-Sv4    VGGGGGGGGGGGGGGGEGAGDDTDADACDNEPGIGSPGAVSGGGGSIAGGGSQANLGPPS 1307
CxNa-Sv5    VGGGGGGGGGGGGGGGEGAGDDTDADACDNEPGIGSPGAVSGGGGSIAGGGSQANLGPPS 1280

CxNa-Lv7    PKESPDGNNDPQGRQTAVLVESDGFVTKNGHRVVIHSRSPSITSRSADV* 2089
CxNa-Lv8    PKESPDGNNDPQGRQTAVLVESDGFVTKNGHRVVIHSRSPSITSRSADV* 2091
CxNa-Sv4    PKESPDGNNDPQGRQTAVLVESDGFVTKNGHRVVIHSRSPSITSRSADV* 1356
CxNa-Sv5    PKESPDGNNDPQGRQTAVLVESDGFVTKNGHRVVIHSRSPSITSRSADV* 1329
```

Figure 4. Alignment of deduced amino acid transcript sequences of the *para*-type sodium channel transcripts (Cx-Na) in HAmCq^{G8} *Culex* mosquitoes. Transmembrane segments are indicated on the line over the sequence. Exons are indicated above the sequence with solid triangle symbols to indicate the bounderies between exons. The differences in the aa sequences are indicated by shading. A stop codon is marked by an asterisk (*). – indicates deletions. Δ indicates insertions with the sequences of Δ1: GAIIVPVYYANL Δ2: GEQHSHLSWIWSE; Δ3: GEQHNHLSWIWSE; Δ4: VIGNSISNHQDNKLEHELNHRGMSLQ.

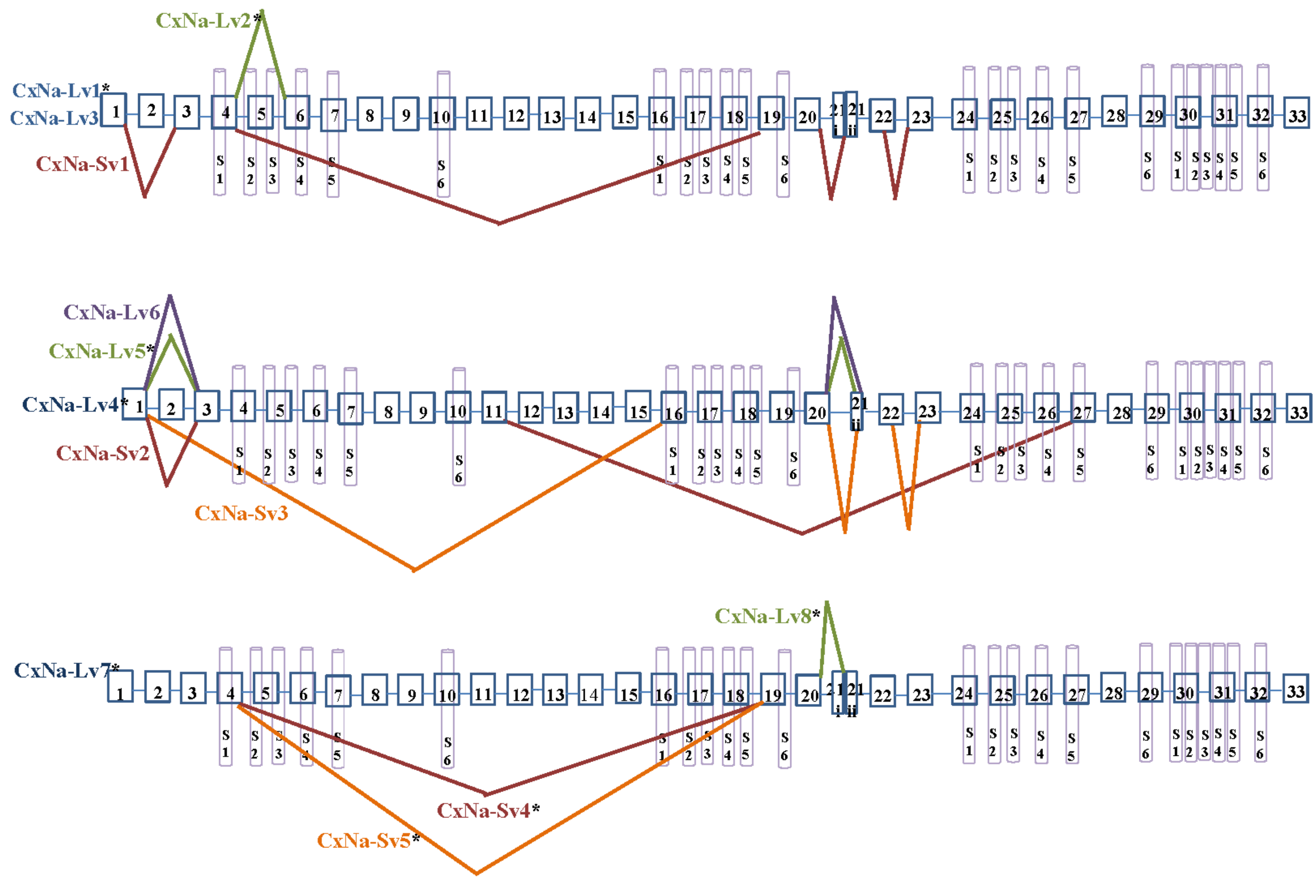

Figure 5. Alternative splicing of Cx-Nav from mosquitoes *Culex quinquefasciatus*. Boxes represent exons. The junctions of exons are indicated with straight lines or bridge lines. The schematic of the predicted 6 segments (S1 to S6) in each of the 4 domains (I, II, III, and IV) in the structure of Cx-Nav protein are shown. *The transcript had an entire ORF.

Discussion

Voltage-gated sodium channels are essential for the action potential generation of the neuron membrane and play a critical role in membrane excitability [6-7]. Over the last few years, a great deal of evidence has accumulated that supports the expression of diverse distinct sodium channel variants in insects through extensive alternative splicing of a single gene [16-23, 34]. The growing interest in alternative splicing of the sodium channels is propelled by its prominent contribution as a key mechanism generating the structural and functional diversity of sodium channels [15, 19]. Following the first reported cloning, sequencing and characterization of multiple variant transcripts from *Drosophila melanogaster* [32], the alternative splicing of sodium channels has now been characterized in many medically or agriculturally important insect and arachnid pest species, including *Drosophila melanogaster* [17, 20], the house fly *Musca domestica* [16], German cockroach *Blattella germanica* [19], the mosquito *Anopheles gambiae* [34], diamond-back moth *Plutella xylostella* [22], silkworm *Bombyx mori* [35], and varroa mite *Varroa destructor* [36]. The current study represents the first investigation of the transcripts of sodium channels in *Cx. quinquefasciatus* and has revealed multiple variants of sodium channels generated from extensive alternative splicing and small deletions/ insertions, which is consistent with the results of the previous studies of the sodium channels of other insect species.

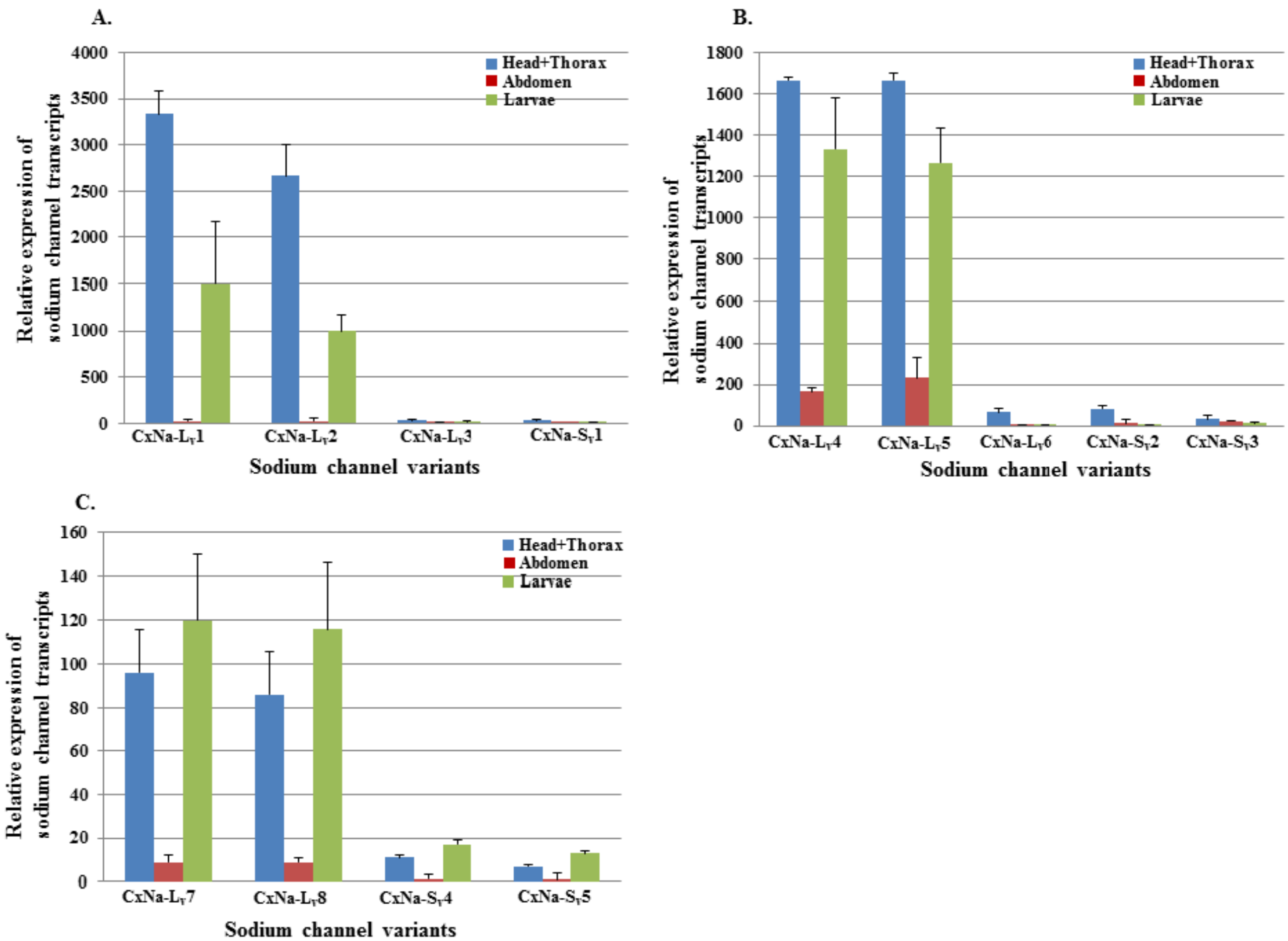

Figure 6. Expression of *Cx-Nav* in larvae and head+ thorax and abdomen tissues of 2-3 day-old female adult *Culex* mosquitoes. The relative level of gene expression shown along the Y axis represents the ratio of the gene expression in each tissue from the adults or larvae compared to that measured in the abdomen tissue of the same strain (ratio=1 indicates equal amounts). The experiments were performed three times. The results are shown as the mean ± S.E. No significant difference ($P \le 0.05$) in the levels of sodium channel transcript expression was found in samples labeled with the same alphabetic letter (i.e., a, b, or c).

These multiple variants of *para*-type sodium channel transcripts presented in the mosquito *Cx. quinquefasciatus* can be classified in terms of two categories, CxNa-L and CxNa-S, based on their distinguishing sizes of ~6.5 kb and ~4.0 kb, which were present in all three mosquito strains tested - the susceptible S-Lab strain, the low resistant HAmCq^{G0} strain, and the highly resistant HAmCq^{G8} strain. The main difference in the sequences obtained for these two subcategories is the presence of multiple internal exons obtained through alternative splicing. In all, nine alternatively splice variants were identified in *Culex* mosquitoes. In the CxNa-L sodium channel category, four splice variants were identified, of which three were full length variants with three optional exons (2, 5, and 21i) and one incorporated in-frame-stop codons. Exon 2 is located in the N-terminus, which is an optional exon corresponding to optional exon 2 of the sodium channel in the silkworm and optional exon j of the *para* in *Drosophila* and which is also conserved in other insect sodium channel genes [35]. Exon 5 is located between IS2 and IS3. Interestingly, skipping of exon 5 also occurs in the silkworm [35], German cockroaches [54] and the mosquito *Anopheles gambiae* [34], suggesting that exon 2 and exon 5 may be a conserved optional exon in insects. Exon 21 is located in the intracellular linker connecting domains II and III of the *Culex* mosquito sodium channels. The 5′ portion of exon 21, named 21i, is optional in *Culex* mosquitoes. Exon 21i corresponds to optional exon f in the *para* gene of *Drosophilar* and exon 22i in the silkworm [35]. These variants with optional exons 2, 5, and/or 21i are all entire ORFs of sodium channels, which may suggest the functional importance of these transcripts in mosquitoes. It has been reported that, when expressed in

Xenopus oocytes, the alternative splicing variants could exhibit different gating properties and generate sodium channel proteins with differing sensitivities to pyrethroids [18-19]. Whether these variants identified in the *Culex* mosquitoes also have different protein properties and different responses to pyrethroids remains to be seen.

Investigation of the putative amino acid sequences of alternative splicing variants in the CxNa-S sodium channel category, i.e. the ~4.5 kb transcripts, revealed that in contrast to the findings of CxNa-L, the alternative splicing identified in the sodium channel of *Culex* mosquitoes has resulted in large size or multi-exon-splicing. All the CxNa-S splicing variants in both the susceptible S-Lab and low resistance parental HAmCq^{G0} strains had in-frame stop codons, suggesting that these splicing variants and any resulting proteins would be truncated from those regions onward. As it has been reported that a truncated channel does not produce any sodium current when it expressed in *Xenopus* oocytes [19], the transcripts identified in our study that contain in-frame stop codons may not be functional transcripts. Furthermore, the ~1000 to ~3000-fold lower expression of the splice variants with stop codons compared to the CxNa-L splicing variances may further support the conclusion that these variances in mosquitoes are relatively unimportant. Nevertheless, two alternative splicing variants of CxNa-S splicing in HAmCq^{G8} had no in-frame stop codons but still had ORFs encoding sodium channel transcripts lacking exons 5 to18. In addition, these two variants in HAmCq^{G8} had relatively high expression levels, with only ~10-fold lower expression levels compared with the CxNa-L variants. Nevertheless, these variants both lacked IS4 and IIS4 as a result of the alternative splicing. Since the S4 segments act as voltage sensors that initiate voltage-dependent activation [34-35], the issue of whether these two alternative splicing variants identified in the highly resistant HAmCq^{G8} strain perform some function in the sodium channels of mosquitoes requires further investigation.

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Competing Interests

The authors have declared that no competing interest exists.

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