Supplementary Information

Target-Driven Evolution of Scorpion Toxins

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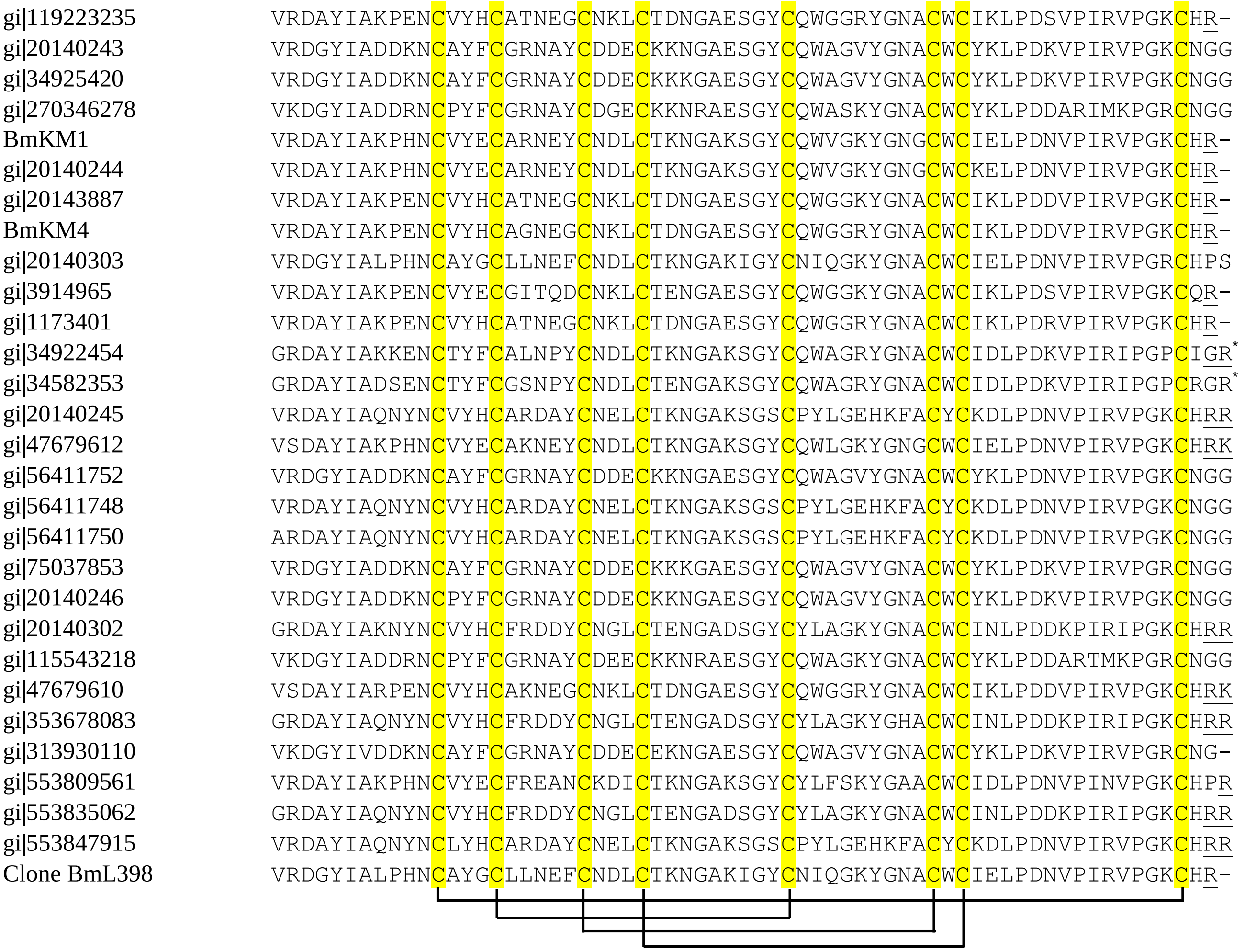

gi|119223235	VRDAYIAKPENCVYHCATNEGCNKLCTDNGAESGYCQWGGRYGNACWCIKLPDSVPIRVPGKCHR-
gi|20140243	VRDGYIADDKNCAYFCGRNAYCDDECKKNGAESGYCQWAGVYGNACWCYKLPDKVPIRVPGKCNGG
gi|34925420	VRDGYIADDKNCAYFCGRNAYCDDECKKKGAESGYCQWAGVYGNACWCYKLPDKVPIRVPGKCNGG
gi|270346278	VKDGYIADDRNCPYFCGRNAYCDGECKKNRAESGYCQWASKYGNACWCYKLPDDARIMKPGRCNGG
BmKM1	VRDAYIAKPHNCVYECARNEYCNDLCTKNGAKSGYCQWVGKYGNGCWCIELPDNVPIRVPGKCHR-
gi|20140244	VRDAYIAKPHNCVYECARNEYCNDLCTKNGAKSGYCQWVGKYGNGCWCKELPDNVPIRVPGKCHR-
gi|20143887	VRDAYIAKPENCVYHCATNEGCNKLCTDNGAESGYCQWGGKYGNACWCIKLPDDVPIRVPGKCHR-
BmKM4	VRDAYIAKPENCVYHCAGNEGCNKLCTDNGAESGYCQWGGRYGNACWCIKLPDDVPIRVPGKCHR-
gi|20140303	VRDGYIALPHNCAYGCLLNEFCNDLCTKNGAKIGYCNIQGKYGNACWCIELPDNVPIRVPGRCHPS
gi|3914965	VRDAYIAKPENCVYECGITQDCNKLCTENGAESGYCQWGGKYGNACWCIKLPDSVPIRVPGKCQR-
gi|1173401	VRDAYIAKPENCVYHCATNEGCNKLCTDNGAESGYCQWGGRYGNACWCIKLPDRVPIRVPGKCHR-
gi|34922454	GRDAYIAKKENCTYFCALNPYCNDLCTKNGAKSGYCQWAGRYGNACWCIDLPDKVPIRIPGPCIGR*
gi|34582353	GRDAYIADSENCTYFCGSNPYCNDLCTENGAKSGYCQWAGRYGNACWCIDLPDKVPIRIPGPCRGR*
gi|20140245	VRDAYIAQNYNCVYHCARDAYCNELCTKNGAKSGSCPYLGEHKFACYCKDLPDNVPIRVPGKCHRR
gi|47679612	VSDAYIAKPHNCVYECAKNEYCNDLCTKNGAKSGYCQWLGKYGNGCWCIELPDNVPIRVPGKCHRK
gi|56411752	VRDGYIADDKNCAYFCGRNAYCDDECKKNGAESGYCQWAGVYGNACWCYKLPDNVPIRVPGKCNGG
gi|56411748	VRDAYIAQNYNCVYHCARDAYCNELCTKNGAKSGSCPYLGEHKFACYCKDLPDNVPIRVPGKCNGG
gi|56411750	ARDAYIAQNYNCVYHCARDAYCNELCTKNGAKSGSCPYLGEHKFACYCKDLPDNVPIRVPGKCNGG
gi|75037853	VRDGYIADDKNCAYFCGRNAYCDDECKKKGAESGYCQWAGVYGNACWCYKLPDKVPIRVPGRCNGG
gi|20140246	VRDGYIADDKNCPYFCGRNAYCDDECKKNGAESGYCQWAGVYGNACWCYKLPDKVPIRVPGKCNGG
gi|20140302	GRDAYIAKNYNCVYHCFRDDYCNGLCTENGADSGYCYLAGKYGNACWCINLPDDKPIRIPGKCHRR
gi|115543218	VKDGYIADDRNCPYFCGRNAYCDEECKKNRAESGYCQWAGKYGNACWCYKLPDDARTMKPGRCNGG
gi|47679610	VSDAYIARPENCVYHCAKNEGCNKLCTDNGAESGYCQWGGRYGNACWCIKLPDDVPIRVPGKCHRK
gi|353678083	GRDAYIAQNYNCVYHCFRDDYCNGLCTENGADSGYCYLAGKYGHACWCINLPDDKPIRIPGKCHRR
gi|313930110	VKDGYIVDDKNCAYFCGRNAYCDDECEKNGAESGYCQWAGVYGNACWCYKLPDKVPIRVPGRCNG-
gi|553809561	VRDAYIAKPHNCVYECFREANCKDICTKNGAKSGYCYLFSKYGAACWCIDLPDNVPINVPGKCHPR
gi|553835062	GRDAYIAQNYNCVYHCFRDDYCNGLCTENGADSGYCYLAGKYGNACWCINLPDDKPIRIPGKCHRR
gi|553847915	VRDAYIAQNYNCLYHCARDAYCNELCTKNGAKSGSCPYLGEHKFACYCKDLPDNVPIRVPGKCHRR
Clone BmL398	VRDGYIALPHNCAYGCLLNEFCNDLCTKNGAKIGYCNIQGKYGNACWCIELPDNVPIRVPGKCHR-

Figure S1. Multiple sequence alignment (MSA) of the *Mesobuthus martensii* α-toxins. Apart from BmKM1 and BmKM4, two extensively studied toxins (1,2), all the other sequences are provided in their GenBank ID numbers (http://www.ncbi.nlm.nih.gov/) for avoiding confusion of their names. Clone BmL398 was isolated from a venom gland cDNA library of this scorpion species by our lab. Cysteines are shadowed in yellow and four disulfide bridges indicated by lines. Extra amino acids that will be removed after post-translational processing are underlined once and C-terminal amidation is indicated by an asterisk.

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gi|699624821|P.pubescens        KQAFDISIMILICLNMVTMMVETDDQSEDMENILYWINLVFIVLFTGECVLKLISLRHYYFTIGWNIFDFVVVILSIVGMFLA--EMIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|699624906|P.pubescens        KQAFDIVIMVLICLNMVTMMIETDDQSKLMQDILYWINLVFVVLFTGECVFKLFSLRYYYFTIGWNIFDFVVVILSIIGMFLA--EMIEKYFVSPTLFRVIRLARIGRILRLVKGAKG
gi|699624877|P.pubescens        QQVFDISIMILICLNMVTMMVETDDQSEEMENILYWINLVFIVLFTGECVLKLISLRHYYFTIGWNIFDFVVVILSIVGMFLA--EMIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|699689265|P.pubescens        QQAFDIVIMMLICLNMVTMMVETDTQSKQMEDILYWINLVFVIFFTCECVLKMFALRHYYFTIGWNIFDFVVVILSIVGMFLA--EIIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|699649878 P.pubescens        KQAFDVSIMILICLNMITMMVETDDQSQEKVNILHKINMLFVAIFTGECIFKMLALRHYYFTNGWNIFDFVVVILSIVGTVLS--DIIQKYFFSPTLFRVIRLARIGRILRLIRGAKG
gi|823463918|T.guttata          KQAFDISIMILICLNMVTMMVETDDQSEDMENILYWINLVFIVLFTGEFVLKLISLRHYYFTIGWNIFDFVVVILSIVGMFLA--EMIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|823463780|T.guttata          QQLFDITVMVLICLNMVTMMIETDDQTELKQNILYWINFVFVVLFTGECVFKIFSLRYYYFTIGWNIFDFVVVILSIIGMFLA--EVIEKYFVSPTLFRVVRLARIGRILRLIKGAKG
gi|823473529|T.guttata          QQAFDIVIMMLICLNMVTMMVETDTQSKQMEDILYWINFVFVIFFTCECVLKMFALRHYYFTIGWNIFDFVVVILSIVGMFLA--EIIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|449491396|T.guttata          KQVFDITIMILICLNMVTMMVETDDQSELKTSVLYKINLVFIVIFTGECVLKMFALRHYFFTVGWNIFDFVVVILSILGIVLS--DIIEKYFVSPTLFRVIRLARIGRVLRLIRGAKG
gi|449492116|T.guttata          KQAFDVSIMILICLNMVTMMVETDDQSQEKVNILHKINMLFVAIFTGECIFKMLALRHYYFTNGWNIFDFVVVILSIVGTVLS--DIIQKYFFSPTLFRVIRLARIGRILRLIRGAKG
gi|699632948|C.vociferus        KQAFDITIMVLICLNMVTMMIETDDQSELMQNILYWINLVFVVLFTGECVFKMFSLRYYYFTIGWNIFDFVVVILSIVGMFLA--KVIEKYFVSPTLFRVVRLARIGRILRLIKGAKG
gi|699632975|C.vociferus        QQVFDISIMILICLNMVTMMVETDDQSKEMENILYWINLVFIVLFTGECVLKLISLRHYYFTIGWNIFDFVVVILSIVGMFLA--EMIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|699677371|C.vociferus        KQAFDVSIMILICLNMVTMMVETDDQSQEKVNILHKINMLFVAIFTGECIIKMLALRHYYFTNGWNIFDFVVVILSIVGTVLS--DIIQKYFFSPTLFRVIRLARIGRILRLIRGAKG
gi|699659219|C.vociferus        KQVFDITIMILICLNMVTMMVETDDQSELKTSVLYKINLVFIVIFTGECVLKMFALRYYYFTIGWNIFDFVVVILSILGIVLS--DIIEKYFVSPTLFRVIRLARIGRVLRLIRGAKG
gi|543245193|G.fortis           KQAFDISIMILICLNMVTMMVETDDQSEEMENILYWINLVFIVLFTGEFVLKLISLRHYYFTIGWNIFDFVVVILSIVGMFLA--EMIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|543298808|G.fortis           KQVFDITIMILICLNMVTMMVETDDQSELKTSVLYKINLVFIVIFTGECVLKMFALRHYFFTVGWNIFDFVVVILSILGIVLS--DIIEKYFVSPTLFRVIRLARIRRLRRLGRGACT
gi|647570007|G.gallus           KQAFDIGIMVLICLNMVTMMIETDDQSELMQNILYWINFVFVVLFTGECVLKLFSLRYYYFTVGWNIFDFVVVILSIVGMFLA--KVIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|342837673|G.gallus           KQVFDITIMILICLNMVTMMVETDDQSETKTDILYKINLIFIVIFTGECVLKMFALRYYYFTIGWNIFDFVVVILSIAGIVLS--DIIEKYFVSPTLFRVIRLARIGRVLRLIRGAKG
gi|543348889|P.humilis          KQVFDISIMILICLNMVTMMVETDDQSKEMETILSRINLVFIVLFTGEFVLKLISLRHYYFTVGWNIFDFVVVILSIVGMFLA--EMIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|541981351|F.cherrug          KQAFDIAIMVLICLNMVTMMIETDDQSDLMQNILYWINLVFVVLFTGECVFKMFSLRYYYFTIGWNIFDFVVVILSIVGMFLA--KVIEKYFVSPTLFRVVRLARIGRILRLIKGAKG
gi|541971530|F.cherrug          KQVFDITIMILICLNMVTMMVETDDQSELKTSVLYMINLVFIVIFTGECVLKMLALRYYYFTIGWNIFDFVVVILSILGIVLS--DIIEKYFVSPTLFRVIRLARIGRVLRLIRGAKG
gi|696965978|C.canorus          KQAFDITIMVLICLNMVTMMIETDDQGQLMQDILYWINLVFVVLFTGECILKMFSLRYYYFTIGWNIFDFVVVILSIIGMFLA--DVIEKYFVSPTLFRVVRLARIGRILRLIKGAKG
gi|696965919|C.canorus          KQVFDISIMILICLNMVTMMVETDDQSKEMEAILSWINLVFIILFTGEFVLKLISLRHYYFTIGWNIFDFVVVILSIVGMFLA--EMIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|696965965|C.canorus          QQVFDISIMILICLNMVTMMVETDDQSKEMENILSWINLVFIVLFTGECVLKLISLRHYYFTIGWNIFDFVVVILSIVGMFLA--EMIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|696998543|C.canorus          KQVFDITIMILICLNMVTMMVETDDQSELKTSILYKINLVFIVIFTGECVLKMFALRYYYFTIGWNIFDFVVVILSILGIVLS--DIIEKYFVSPTLFRVIRLARIGRVLRLIRGAKG
gi|683906241|S.canaria          KQLFDIIIMVLICLNMVTMMIETDDQNELMQNILYWINLVFVVLFTGECVFKIFSLRYYYFTIGWNIFDFVVVILSIIGMFLA--EVIEKYFVSPTLFRVVRLARIGRILRLIKGAKG
gi|669281868|C.brachyrhynchos   KQVFDITIMILICLNMVTMMVETDDQSELKTSVLYKINLVFIVIFTGECVLKMFALRYYYFTVGWNIFDFVVVILSILGIVLS--DIIEKYFVSPTLFRVIRLARIGRVLRLIRGAKG
gi|669281061|C.brachyrhynchos   RQLFDITVMVLICLNMVTMMIETDDQTELMQNILYWINLVFVVLFTCECVFKIFSLRYYYFTIGWNIFDFVVVILSIIGMFLA--EVIEKYFVSPTLFRVVRLARIGRILRLIKGAKG
gi|726994909|C.cornix           KQVFDITIMILICLNMVTMMVETDDQSELKTSVLYKINLVFIVIFTGECVLKMFALRYYYFTVGWNIFDFVVVILSILGIVLS--DIIEKYFVSPTLFRVIRLARRRRGMRLIRGAKG
gi|719771801|T.guttatus         KQAFDISIMILICLNMVTMMVETDDQSKEMENILYWINLVFIVLFTGEFVLKLISLRHYYFTIGWNIFDFVVVILSIVGMFLA--EMIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
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gi|719776079|T.guttatus         QQAFDIVIMMLICLNMVTMMVETDTQSKQMENILYWINLVFVIFFTCECVLKMFALRHYYFTIGWNIFDFVVVILSIVGMFLA--EIIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|719730318|T.guttatus         KQVFDITIMILICLNMVTMMVETDDQSQLKTDVLYKVNLVFIVIFTGECVLKMFALRYYYFTIGWNIFDFVVVILSIVGIVLS--DIIEKYFVSPTLFRVIRLARIGRVLRLIRGAKG
gi|719791574|T.guttatus         KQAFDVSIMILICLNMVTMMVETDDQSQEKVNILHKINMLFVAIFTGECIIKMVALRHYYFTNGWNIFDFVVVILSIVGTVLS--DIIQKYFFSPTLFRVIRLARIGRILRLIRGAKG
gi|543743408|C.livia            KQIFDVSIMILICLNMVTMMVETDDQSQEKVNILHKINMLFVAIFTGECIIKMLALRHYYFTNGWNIFDFVVVILSIVGTVLS--DIIQKYFFSPTLFRVIRLARIGRILRLIRGAKG
gi|697486596|S.camelus          KQVFDISIMILICLNMVTMMVETDDQSKEMETILSRINLVFIILFTGECVLKLISLRHYYFTIGWNIFDFVVVILSIVGMFLA--EMIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|697486691|S.camelus          KQAFDISIMVLICLNMVTMMIETDDQSELMQNILYWINLIFVVLFTGECVLKLFSLRYYYFTIGWNIFDFVVVILSIVGMFLA--KVIEKYFVSPTLFRVVRLARIGRILRLIKGAKG
gi|697513240|S.camelus          KQVFDITIMILICLNMVTMMVETDDQSQFKTDVLYKVNLVFIVIFTGECVLKMFALRYYYFTIGWNIFDFVVVILSIVGIVLS--DIIEKYFVSPTLFRVIRLARIGRVLRLIRGAKG
gi|690446304|P.adeliae          KQAFDITIMVLICLNMVTMMIETDDQGELMQNILYWINLVFVVLFAGECVFKLFSLRYYYFTIGWNIFDFVVVILSVVGMFLA--KVIEKYFVSPTLFRVVRLARIGRILRLIKGAKG
gi|690439493|P.adeliae          KQAFDVSIMILICLNMVTMMVETDDQSQEKVNILHKINMLFVAVFTGECIIKMLALRHYYFTNGWNIFDFVVVILSIVGTVLS--DIIQKYFFSPTLFRVIRLARIGRILRLIRGAKG
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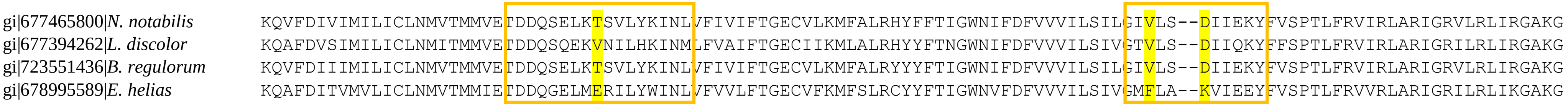
gi|690428680|*P.adeliae*	KQVFDITIMILICLNMVTMMVETDDQSELKTSVLYKINLVFIVIFTGECLLKMFALRYYYFTIGWNIFDFVVVILSILGIVLS--DIIEKYFVSPTLFRVIRLARIGRVLRLIRGAKG
gi|514743636|*A.platyrhynchos*	KQAFDISIMVLICLNMVTMMIETDDQGDLMQNILYWINLVFVVLFTGECVFKLFSLRYYYFTIGWNIFDFVVVILSIVGMFLA--KVIEKYFVSPTLFRVVRLARIGRILRLIKGAKG
gi|514743648|*A.platyrhynchos*	QQAFDISIMILICLNMVTMMVETDDQSKEMENILYWINLVFIVLFTGECVLKLISLRHYYFTIGWNIFDFVVVILSIVGMFLA--EMIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|514755303|*A. platyrhynchos*	KQVFDITIMILICLNMVTMMVETDDQSELKTDILYKINLVFIVIFTGECVLKMFALRYYYFTIGWNIFDFVVVILSIAGIVLS--DIIEKYFVSPTLFRVIRLARIGRVLRLIRGAKG
gi|729721552|*H. leucocephalus*	KQAFDITIMVLICLNMVTMMIETDGQGELMQNILYWINLVFVVLFTGECVLKLFSLRYYYFTVGWNIFDFVVVILSIVGMFLA--KVIEKYFVSPTLFRVVRLARIGRILRLIKGAKG
gi|701390492| *C. pelagica*	KQAFDITIMVLICLNMVTMMIETDDQGELMQNILYWINLVFVVLFTGECILKLFSLRYYYFTIGWNIFDFVVVILSIVGMFLA--EVIKKYFVSPTLFRVVRLARIGRILRLIKGAKG
gi|701375204|*C. pelagica*	QQAFDIVIMMLICLNMVTMMVETDTQSKKMEDILYWINFVFVIFFTCECVLKMFALRHYYFTIGWNIFDFVVVILSIVGMFLA--EIIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|701406438|*C. pelagica*	KQVFDITIMILICLNMVTMMVETDDQSELKTSVLYKINLVFIVIFTGECVLKMFALRHYYFTIGWNIFDFVVVILSILGIVLS--DIIEKYFVSPTLFRVIRLARIGRVLRLIRGAKG
gi|705688749|*C. macqueenii*	KQAFDITIMVLICLNMVTMMIETDDQGELMKSILYWINLVFVVLFTGECVLKLFSLRYYYFTIGWNIFDFVVVILSIVGMFLA--KVIEKYFVSPTLFRVVRLARIGRILRRIQGAKV
gi|705668534|*C. macqueenii*	QQVFDISIMILICLNMVTMMVETDDQSKEMETILYWINLVFIVLFTGECVLKLISLRHYYFTIGWNIFDFVVVILSIVGMFLA--EMIEKYFVSPTLFRVIRLDRIGRILRLIKGAKG
gi|705671331|*C. macqueenii*	KQVFDITIMILICLNMVTMMVETDDQSDLKTSVLYKINLVFIVIFTGECVLKMFALRYYYFTIGWNIFDFVVVILSILGIVLS--DIIEKYFVSPTLFRVIRLARIGRVLRLIRGAKG
gi|705695710|*C. macqueenii*	KQAFDVSIMILICLNMVTMMVETDDQSQEKVNILHKINMLFVAIFTGECIIKMLALRHYYFTNGWNIFDFVVVILSIVGTVLS--DIIQKYFFSPTLFRVIRLARIGRVLRLIRGAKG
gi|527269695|*M. undulatus*	KQAFDISIMILICLNMVTMMVETDDQSEDMENILYWINLVFIVLFTGEFVLKLISLRHYYFTVGWNIFDFVVVILSIVGMFLA--EMIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|527269392|*M. undulatus*	KQVFDISIMILICLNMVTMMVETDDQSKEMETILSRINLVFIILFTGECVLKLISLRHYYFTLGWNIFDFVVVILSIVGMFLA--EMIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|527269353|*M. undulatus*	KQEFDITIMVLICINMITMMIETDDQAELMQNILYWINLAFVVLFTGECVLKLFSLRYYYFTIGWNIFDFVVVILSIVGMFLA--KVIEKYFVSPTLFRVIRLARIGRILRLIRGAKG
gi|700400984|*O. hoazin*	KQAFDVSIMILICLNMITMMVETDDQSQEKVNILHKINMLFVAIFTGECIIKMLALRHYYFTNGWNIFDFVVVILSIVGTVLS--DIIQKYFFSPTLFRVIRLARIGRVLRLIRGAKG
gi|700375357|*O. hoazin*	KQVFDITIMILICLNMITMMVETDDQSELKTSVLYKINLVFIVIFTGECVLKMFALRYYYFTIGWNIFDFVVVILSILGIVLS--DIIEKYFVSPTLFRVIRLARIGRVLRLIRGAKG
gi|663239565|*C. anna*	KQVFDISIMILICLNMVTMMVETDDQSKEMETILSRINLVFIILFTGEFVLKLISLRHYYFTVGWNIFDFVVVILSIVGMFLA--EMIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|663288339|*C. anna*	KQVFDITIMILICLNMVTMMVETDDQSELKTAVLYKINLVFIVIFTGECVLKMFALRHYFFTIGWNIFDFVVVILSILGIVLS--DIIEKYFVSPTLFRVIRLARIGRVLRLIRGAKG
gi|675430434|*M. vitellinus*	KQVFDISIMILICLNMVTMMVETDDQSKEMETILSRINLVFIILFTGEFVLKLISLRHYYFTIGWNIFDFVVVILSIVGMFLA--EMIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|675407061|*M. vitellinus*	KQVFDITIMILICLNMVTMMVETDDQSELKTSVLYKINLVFIVIFTGECVLKMFALHYHFFTVGWNIFDFVVVILSILGIVLS--DIIEKYFVSPTLFRVIRLARIGRVLRLIRGAKG
gi|704177515|*B. rhinoceros*	KQVFDISIMILICLNMVTMMVETDDQSKEMEIILSRINLVFIILFTGEFVLKLISLRHYYFTIGWNIFDFVVVILSIVGMFLA--EMIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|704199054|*B. rhinoceros*	KQAFDITIMVLICLNMVTMMIETDDQGERMQDILYWINLVFVVLFTGECVFKMFSLRCYYFTVGWNIFDFVVVILSIIGTCLD--EMIQKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|704169897|*B. rhinoceros*	KQVFDITIMILICLNMVTMMVETDDQSEFKTSILNKINLVFIVIFTGECVLKMFALRYYYFTIGWNIFDFVVVIFSILGIVLS--DIIEKYFVSPTLFRVIRLARIGRVLRLIRGAKG
gi|542150274|*Z. albicollis*	KQVFDISIMILICLNMVTMMVETDDQSKEMETILSRINLVFIVLFTGEFVLKLISLRHYYFTIGWNIFDFVVVILSIVGMFLA--EMIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|733922787|*M. gallopavo*	KQVFDITIMILICLNMVTMMVETDDQSETKTDILYKINLVFIVIFTGECVLKMFALRYYFFTIGWNIFDFVVVILSIAGIVLS--DIIEKYFVSPTLFRVIRLARIGRVLRLIRGAKG
gi|733891677|*M. gallopavo*	NQAFDIAIMILICLNMITMMVETYEQSDTKTNVLNKINILFVAIFTTECILKLVALRQYYFSNAWNIFDIVVVIMSIVALLLSGIGKAFEHFLPPTLFRVIRLARIGRILRLIRAAKG
gi|686594368|*A. forsteri*	KQAFDISIMILICLNMVTMMVETDDQSEDMENILYWINLVFIVLFTGECVLKLISLRHYYFTIGWNIFDFVVVIISIVGMFLA--EMIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|686594360|*A. forsteri*	QQVFDISIMILICLNMVTMMVETDDQSKEMENILYWINLVFIVLFTGECVLKLISLRHYYFTLGWNIFDFVVVILSIVGMFLA--EMIEKYFVSPTLFRVSSDSRIGRILRLIKGAKG
gi|704329645|*C. carolinensis*	KQAFDITIMVLICLNMVTMMIETDDQSEMMQHILYWINLVFVVLFTGECVFKLFSLRYYYFTVGWNIFDFVVVILSIVGMFLA--KVIEKYFVSPTLFRVVRLARIGRILRLIKGAKG
gi|704298804|*C. carolinensis*	KQVFDITIMILICLNMVTMMVETDDQSEAKTSVLYKINLVFIVIFTGECVLKMFALRYYYFTIGWNIFDFVVVILSILGIVLS--DIIEKYFVSPTLFRVIRLARIGRVLRLIRGAKG
gi|683461587|*C. carolinensis*	RQAFDVVIMILICLNMISMMVETYEQSETKSNILNKINMLFVALFTTECVLKLVALRQYYFSNAWNIFDLVVVIMSLVALLLSGIGKAFEHFLPPTLFRVIRLARIGRILRLIRAARG
gi|699582281|*A. vittatum*	QQAFDIVIMMLICLNMVTMMVETDTQSEQMEDILYWINLVFVIFFTCECVLKMFALRHYYFTIGWNIFDFVVVILSIVGMFLA--EIIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|699600245|*A. vittatum*	KQVFDITIMILICLNMVTMMVETDDQSELKTSVLYKINLVFIVIFTGECVLKMFALRYYFFTIGWNIFDFVVVILSILGIVLS--DIIEKYFVSPTLFRVIRLARIGRVLRLIRGAKG
gi|723125282|*P. lepturus*	KQAFDITIMVLICLNMVTMMIETDDQGELMQNILYWINLVFVVLFTGECVLKLFSLRYYYFTIGWNIFDFVVVILSIVGMFLA--EVIEKYFVSPTLFRVVRLARIGRILRLIKGAKG
gi|678145895|*T. erythrolophus*	QQVFDISIMILICLNMVTMMVETDDQSKEMETILYWINLVFIVLFTGECVLKLISLRHYYFTIGWNIFDFVVVILSIVGMFLA--EMIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|698441979|*G. stellata*	KQAFDITIMVLICLNMVTMMIETDDQGELMQNILYWINLVFVVLFTGECVFKLFSLRYYYFTIGWNIFDFVVVILSIVGMFLA--KVIEKYFVSPTLFRVVRLARIGRILRLIKGAKG
gi|697017762|*F. glacialis*	KQAFDITIMVLICLNMVTMMIETDDQGELMQNILYWINLVFVVLFTGECVFKLFSLRYYYFTIGWNIFDFVVVILSIVGMFLA--EMIEKYFVSPMLFRVIRLARIGRILRLIKGAKG
gi|698407580|*C. cristata*	KQAFDITIMVLICLNMVTMMIETDDQGELMQNILYWINLVFVVLFTGECVFKLFSLRYYYFTIGWNIFDFVVVILSIIGMFLA--EVIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|677983296|*A. chloris*	KQVFDITIMILICLNMVTMMVETDDQSELKTSVLYQINLVFIVVFTGECVLKMFALRYYYFTVGWNIFDFVVVIFSILGIVLS--DIIEKYFVSPTLFRVIRLARIGRVLRLIRGAKG

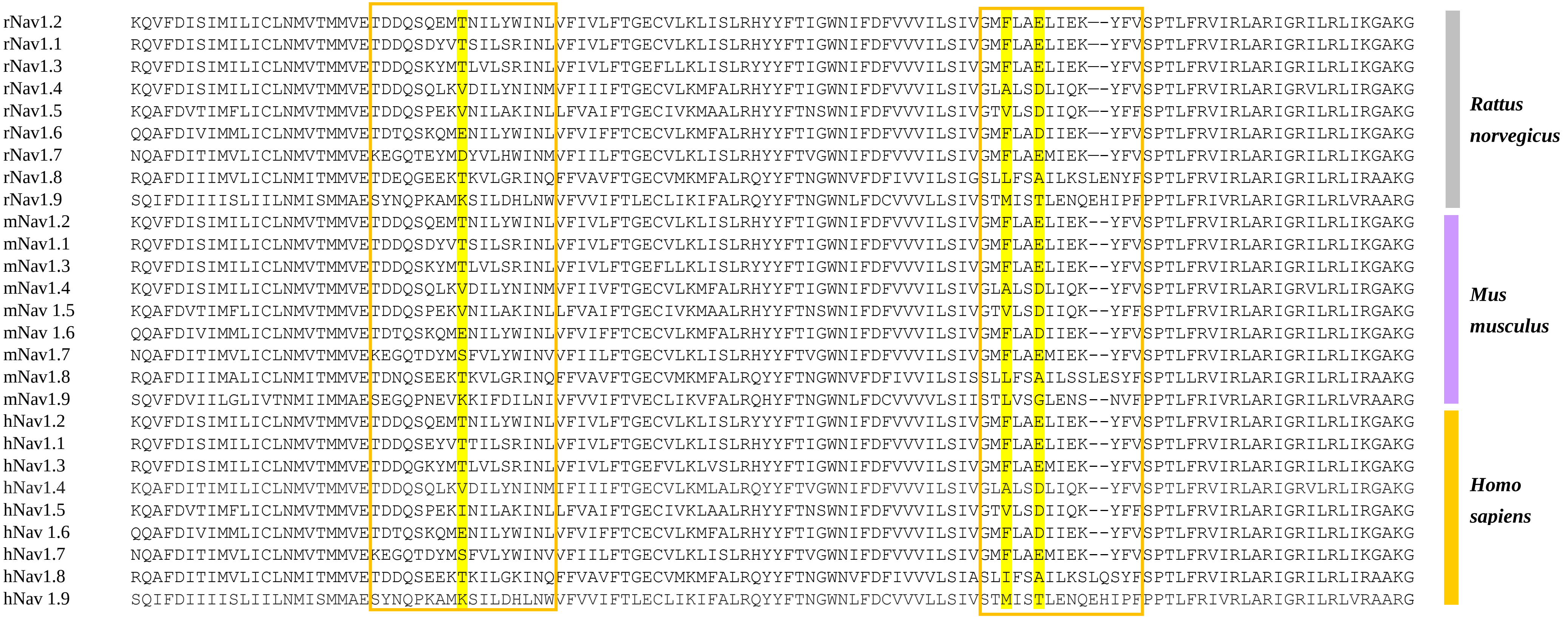


gi|677465800|*N. notabilis*	KQVFDIVIMILICLNMVTMMVETDDQSELKTSVLYKINLVFIVIFTGECVLKMFALRHYFFTIGWNIFDFVVVILSILGIVLS--DIIEKYFVSPTLFRVIRLARIGRVLRLIRGAKG
gi|677394262|*L. discolor*	KQAFDVSIMILICLNMITMMVETDDQSQEKVNILHKINMLFVAIFTGECIIKMLALRHYYFTNGWNIFDFVVVILSIVGTVLS--DIIQKYFFSPTLFRVIRLARIGRILRLIRGAKG
gi|723551436|*B. regulorum*	KQVFDIIIMILICLNMVTMMVETDDQSELKTSVLYKINLVFIVIFTGECVLKMFALRYYYFTIGWNIFDFVVVILSILGIVLS--DIIEKYFVSPTLFRVIRLARIGRVLRLIRGAKG
gi|678995589|*E. helias*	KQAFDITVMVLICLNMVTMMIETDDQGELMERILYWINLVFVVLFTGECVFKMFSLRCYYFTIGWNVFDFVVVILSIVGMFLA--KVIEEYFVSPTLFRVVRLARIGRILRLIKGAKG

Figure S2. MSA of voltage-sensor domain (VSD) (DIV) of bird Na$^+$ channels. Loops involved in interaction with α-toxins are boxed in orange where three sites responsible for binding to Lqh2, an α-toxin from *Leiurus quinquestriatus hebraeus* (3), are shadowed in yellow.

gi|343098400|*A. carolinensis*	KQAFDISIMILICLNMVTMMVETDDQTDAMETNLYRINLIFIVLFTGECVLKLISLRYYYFTIGWNIFDFVVVILSIVGMFLAEIIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|343098402|*A. carolinensis*	KQAFDISIMILICLNMVTMMVETDDQSKEMETILSRINLVFIILFTGECVLKLTSLRHYYFTIGWNIFDFVVVILSIVGMFLAEIIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|637324084|*A. carolinensi*	RQVFDISIMILICLNMITMMVETDDQSQQTEEILYRINLIFIVLFTGECVLKLISLRYYYFTIGWNIFDFVVVILSIVGMFLAEIIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|641451020|*A. carolinensis*	QQAFDILIMLLICLNMVTMMVETDTQSKQMEEILYWINLVFVIFFTCECVLKMFALRHYYFTIGWNIFDFVVVILSIVGMFLAEIIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|343098410|*A. carolinensis*	RQAFDIGIMSLICLNMVTMMVETDDEDESKKKILYWINVIFIILFTGEFLLKLIALRYYYFTIGWNIFDFVVVILSFIGMFLSELIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|343098406|*A. carolinensis*	NQAFDVCIMLLICLNMITMMVETDDQSPEKVNILYKINMVFVAIFTAECISKLVALRHYYFTNGWNIFDFVVVILSIVGSVLSDIIQKYFFSPTLFRVIRLARIGRILRLIRGAKG
gi|343098404|*A. carolinensis*	QQAFDIVIMILICLNMVTMMVETDDQSQTKIDILFQINLIFIVIFTTECFLKMIALRYYFFTVGWNIFDFVVVILSIAGLVLSDLIEKYFVSPTLFRVIRLARIGRVLRLIRGAKG
gi|675822220|*T. sirtalis*	KQAFDISIMILICLNMVTMMVETDDQTDAMETILYRINFIFIVLFTGECVLKLISLRYYYFTIGWNIFDFVVVILSIVGMFLAEIIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|675822222|*T. sirtalis*	KQAFDITIMILICLNMVTMMVETDDQSKEMEIILSRINLVFIILFTGECVLKLISLRHYYFTIGWNIFDFVVVILSIVGMFLAEIIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|675822218|*T. sirtalis*	QQVFDISIMILICLNMITMMVETDDQSKQTEDILYRINFIFIVLFTGECVLKLISLRYYYFTIGWNIFDFVVVILSIVGMFLAEIIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|675822226|*T. sirtalis*	QQAFDIVIMLLICLNMVTMMVETDTQSKQMEEILYWINFVFVIFFTCECVLKMFALRHYYFTIGWNIFDFVVVILSVVGMFLAEIIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|675822228|*T. sirtalis*	KQAFDIGIMILICLNMITMMVETADQDTSVEDILYWINLIFIVLFTAECLLKLIALRYYYFTIGWNIFDFVVVIFSIVGMCLSKIIEKFFVSPTLFRVVRLARIGRILRLIKGAKG
gi|60265593|*T. sirtalis*	QQAFDIIIMILICLNMVTMMVETDDQSQTKITILAQINLVFIIIFTSECFLKMIALRHYFFTNGWNIFDFVVVILSIVGLVLSDIIEKYFVSPTLFRVIRLARIGRVLRLIRGAKG
gi|60265591|*T. sirtalis*	QQAFDIIIMILICLNMVTMMVETDDQSQTKITILAQINLVFIIIFTSECLLKMIALRHYFFTNGWNIFDFVVVILSIVGLVLSDIIEKYFVSPTLFRVIRLARIGRALRLIRGAKG
gi|602644140|*P. bivittatus*	KQAFDISIMILICLNMVTMMVETDDQSKEMEIILSRINLVFIVLFTGECILKLISLRHYYFTIGWNIFDFVVVILSIVGMFLAEIIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|602651607|*P. bivittatus*	RQVFDISIMILICLNMITMMVETDDQSKQTEDILYRINLIFIVLFTGECVLKLISLRYYYFTIGWNIFDFVVVILSIVGMFLAEIIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|602629712|*P. bivittatus*	QQAFDIIIMLLICLNMVTMMVETDTQSKQMEEILYWINFVFVIFFTCECVLKMFALRHYYFTIGWNIFDFVVVILSIVGMFLAEIIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|602669993|*P. bivittatus*	HQLFDIAIMTLICLNMVTMMVEADDQSPEKTYVLNKINILFITLFTTECAMKLVALRHYYFINGWNIFDLVVVIMSIVGNVLSGIVVS-F-SPTLFRVIRLVRIGRILRLIRSARG
gi|602642869|*P. bivittatus*	NQAFDVCIMLLICLNMVTMMVETDDQSPEKVNILYRINMVFVAIFTAECIFKMTALRHYYFTNGWNIFDFVVVILSIVGSVLSDIIQKYFFSPTLFRVIRLARIGRILRLIRGAKG
gi|602636073|*P. bivittatus*	QQAFDIVIMILICLNMVTMMVETDDQSQTKINILAQINLIFIIIFTSECFLKMIALRYYFFTNGWNIFDFVVVILSIVGLVLADIIEKYFVSPTLFRVIRLARIGRVLRLIRGAKG
gi|602672446|*P. bivittatus*	RQAFDIGIMVLICLNMVTMMVETADQDSSVEEILYWINLIFVVIFTGECLLKLIALRYYYFTIGWNIFDFVVVIFSIVGMCLSQIIEKFFVSPTLFRVVRLARIGRVLRLIKGAKG
gi|565318632|*O. hannah*	KQAFDISIMILICLNMVTMMVETDDQSKEMEIILSRINLVFIILFTGECILKLISLRHYYFTIGWNIFDFVVVILSIVGMFLAEIIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|565312590|*O.* hannah	QQAFDILIMLLICLNMVTMMVETDTQSKQMEDILYWINFVFVIFFTCECVLKMFALRHYYFTIGWNIFDFVVVILSIVGMFLAEIIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|565307265|*O. hannah*	KQAFDIGIMILICLNMVTMMVETADQDSSVEEILYWINLFFIVIFTGECLLKLIALRYYYFTIGWNIFDFVVVIFSIVGMCLSQIIEKFFVSPTLFRVVRLARIGRVLRLIKGAKG
gi|565321537|*O. hannah*	QQVFDISIMILICLNMITMMVETDDQSKKTEDILYRINFIFIVLFTGECVLKLISLRYYYFTIGWNIFDFVVVILSIVGMFLAEIIEKYFVSPTLFRVIRLARIGRILRLIKGAKG
gi|565321905|*O. hannah*	KQAFELFIISLIFLNVVVMAVEYEGQDVAADQLLEKINCVFVALFTGECVMKLLALRLYFFKDSWNIFDLVVVILSIISLAATQLWQRLNFPPTILRVIRVIRISRLLRLIRGARG
gi|253910955|*T. couchii*	QQAFDIIIMILICLNMVTMMVETDDQSQTKINILAQINLVFIIIFTSECFLKMIALRHYFFTNGWNIFDFVVVILSIVGLVLSDIIEKYFVSPTLFRVIRLARIGRVLRLIRGAKG
gi|253910953|*T. elegans*	QQAFDIVIMILICLNMVTMMVETDDQSQTKINILAQINLVFIIIFTSECFLKMIALRHYFFTNGWNIFDFVVVILSIVGLVLSDIIEKYFVSPTLFRVIRLARIGRVLRLIRGAKG

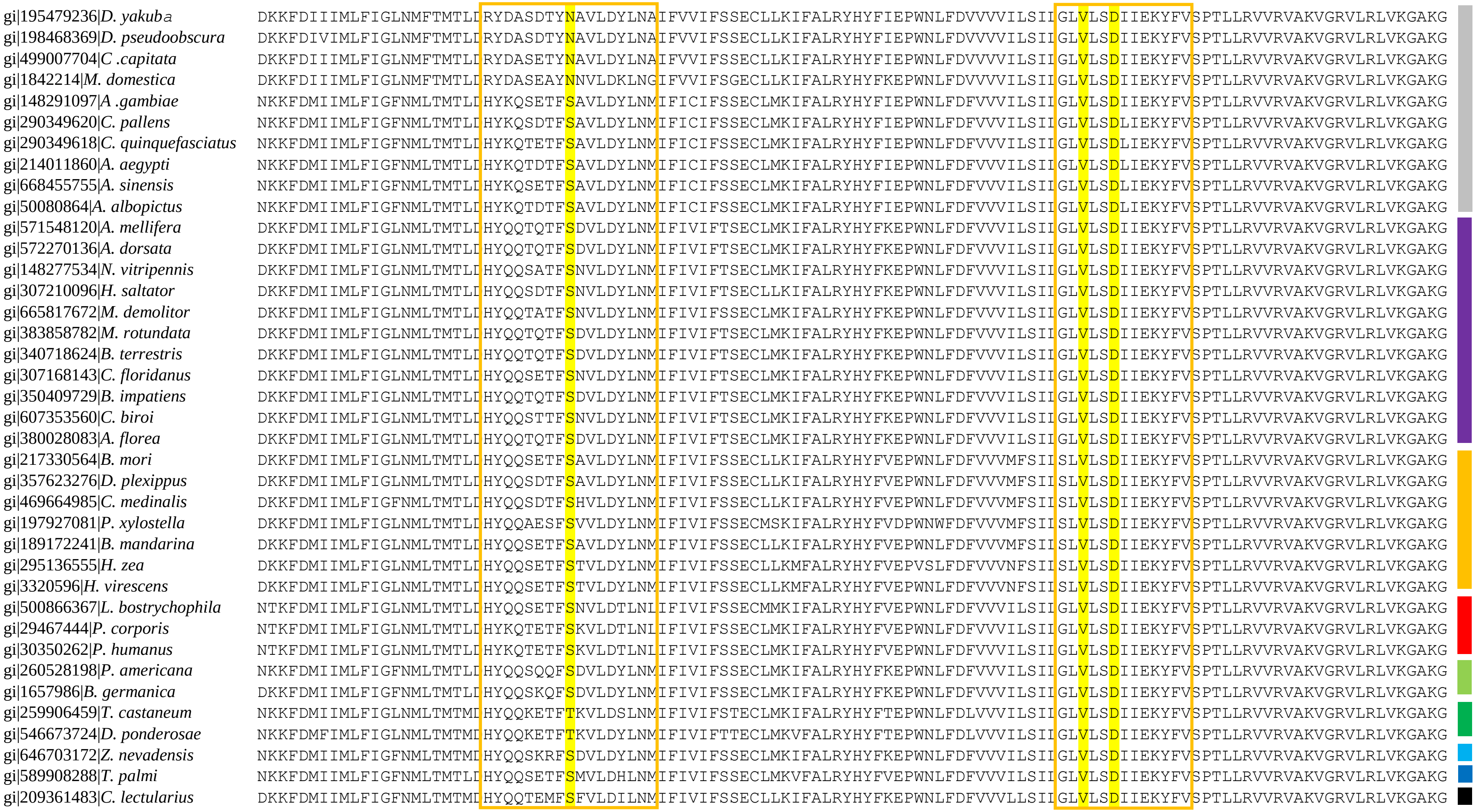
Figure S3. MSA of VSD (DIV) of lizard Na$^+$ channels. Sequence annotations are the same with those in Fig. S2 (the same below).



rNav1.2	KQVFDISIMILICLNMVTMMVETDDQSQEMTNILYWINLVFIVLFTGECVLKLISLRHYYFTIGWNIFDFVVVILSIVGMFLAELIEK--YFVSPTLFRVIRLARIGRILRLIKGAKG
rNav1.1	RQVFDISIMILICLNMVTMMVETDDQSDYVTSILSRINLVFIVLFTGECVLKLISLRHYYFTIGWNIFDFVVVILSIVGMFLAELIEK--YFVSPTLFRVIRLARIGRILRLIKGAKG
rNav1.3	RQVFDISIMILICLNMVTMMVETDDQSKYMTLVLSRINLVFIVLFTGEFLLKLISLRYYYFTIGWNIFDFVVVILSIVGMFLAELIEK--YFVSPTLFRVIRLARIGRILRLIKGAKG
rNav1.4	KQVFDISIMILICLNMVTMMVETDDQSQLKVDILYNINMVFIIIFTGECVLKMFALRHYYFTIGWNIFDFVVVILSIVGLALSDLIQK--YFVSPTLFRVIRLARIGRVLRLIRGAKG
rNav1.5	KQAFDVTIMFLICLNMVTMMVETDDQSPEKVNILAKINLLFVAIFTGECIVKMAALRHYYFTNSWNIFDFVVVILSIVGTVLSDIIQK--YFFSPTLFRVIRLARIGRILRLIRGAKG
rNav1.6	QQAFDIVIMMLICLNMVTMMVETDTQSKQMENILYWINLVFVIFFTCECVLKMFALRHYYFTIGWNIFDFVVVILSIVGMFLADIIEK--YFVSPTLFRVIRLARIGRILRLIKGAKG
rNav1.7	NQAFDITIMVLICLNMVTMMVEKEGQTEYMDYVLHWINMVFIILFTGECVLKLISLRHYYFTVGWNIFDFVVVILSIVGMFLAEMIEK--YFVSPTLFRVIRLARIGRILRLIKGAKG
rNav1.8	RQAFDIIIMVLICLNMITMMVETDEQGEEKTKVLGRINQFFVAVFTGECVMKMFALRQYYFTNGWNVFDFIVVILSIGSLLFSAILKSLENYFSPTLFRVIRLARIGRILRLIRAAKG
rNav1.9	SQIFDIIIISLIILNMISMMAESYNQPKAMKSILDHLNWVFVVIFTLECLIKIFALRQYYFTNGWNLFDCVVVLLSIVSTMISTLENQEHIPFPPTLFRIVRLARIGRILRLVRAARG
mNav1.2	KQVFDISIMILICLNMVTMMVETDDQSQEMTNILYWINLVFIVLFTGECVLKLISLRHYYFTIGWNIFDFVVVILSIVGMFLAELIEK--YFVSPTLFRVIRLARIGRILRLIKGAKG
mNav1.1	RQVFDISIMILICLNMVTMMVETDDQSDYVTSILSRINLVFIVLFTGECVLKLISLRHYYFTIGWNIFDFVVVILSIVGMFLAELIEK--YFVSPTLFRVIRLARIGRILRLIKGAKG
mNav1.3	RQVFDISIMILICLNMVTMMVETDDQSKYMTLVLSRINLVFIVLFTGEFLLKLISLRYYYFTIGWNIFDFVVVILSIVGMFLAELIEK--YFVSPTLFRVIRLARIGRILRLIKGAKG
mNav1.4	KQVFDISIMILICLNMVTMMVETDDQSQLKVDILYNINMVFIIVFTGECVLKMFALRHYYFTIGWNIFDFVVVILSIVGLALSDLIQK--YFVSPTLFRVIRLARIGRVLRLIRGAKG
mNav 1.5	KQAFDVTIMFLICLNMVTMMVETDDQSPEKVNILAKINLLFVAIFTGECIVKMAALRHYYFTNSWNIFDFVVVILSIVGTVLSDIIQK--YFFSPTLFRVIRLARIGRILRLIRGAKG
mNav 1.6	QQAFDIVIMMLICLNMVTMMVETDTQSKQMENILYWINLVFVIFFTCECVLKMFALRHYYFTIGWNIFDFVVVILSIVGMFLADIIEK--YFVSPTLFRVIRLARIGRILRLIKGAKG
mNav1.7	NQAFDITIMVLICLNMVTMMVEKEGQTDYMSFVLYWINVVFIILFTGECVLKLISLRHYYFTVGWNIFDFVVVILSIVGMFLAEMIEK--YFVSPTLFRVIRLARIGRILRLIKGAKG
mNav1.8	RQAFDIIIMALICLNMITMMVETDNQSEEKTKVLGRINQFFVAVFTGECVMKMFALRQYYFTNGWNVFDFIVVILSISSLLFSAILSSLESYFSPTLLRVIRLARIGRILRLIRAAKG
mNav1.9	SQVFDVIILGLIVTNMIIMMAESEGQPNEVKKIFDILNIVFVVIFTVECLIKVFALRQHYFTNGWNLFDCVVVVLSIISTLVSGLENS--NVFPPTLFRIVRLARIGRILRLVRAARG
hNav1.2	KQVFDISIMILICLNMVTMMVETDDQSQEMTNILYWINLVFIVLFTGECVLKLISLRYYYFTIGWNIFDFVVVILSIVGMFLAELIEK--YFVSPTLFRVIRLARIGRILRLIKGAKG
hNav1.1	RQVFDISIMILICLNMVTMMVETDDQSEYVTTILSRINLVFIVLFTGECVLKLISLRHYYFTIGWNIFDFVVVILSIVGMFLAELIEK--YFVSPTLFRVIRLARIGRILRLIKGAKG
hNav1.3	RQVFDISIMILICLNMVTMMVETDDQGKYMTLVLSRINLVFIVLFTGEFVLKLVSLRHYYFTIGWNIFDFVVVILSIVGMFLAEMIEK--YFVSPTLFRVIRLARIGRILRLIKGAKG
hNav1.4	KQAFDITIMILICLNMVTMMVETDDQSQLKVDILYNINMIFIIIFTGECVLKMLALRQYYFTVGWNIFDFVVVILSIVGLALSDLIQK--YFVSPTLFRVIRLARIGRVLRLIRGAKG
hNav1.5	KQAFDVTIMFLICLNMVTMMVETDDQSPEKINILAKINLLFVAIFTGECIVKLAALRHYYFTNSWNIFDFVVVILSIVGTVLSDIIQK--YFFSPTLFRVIRLARIGRILRLIRGAKG
hNav 1.6	QQAFDIVIMMLICLNMVTMMVETDTQSKQMENILYWINLVFVIFFTCECVLKMFALRHYYFTIGWNIFDFVVVILSIVGMFLADIIEK--YFVSPTLFRVIRLARIGRILRLIKGAKG
hNav1.7	NQAFDITIMVLICLNMVTMMVEKEGQTDYMSFVLYWINVVFIILFTGECVLKLISLRHYYFTVGWNIFDFVVVILSIVGMFLAEMIEK--YFVSPTLFRVIRLARIGRILRLIKGAKG
hNav1.8	RQAFDITIMVLICLNMITMMVETDDQSEEKTKILGKINQFFVAVFTGECVMKMFALRQYYFTNGWNVFDFIVVVLSIASLIFSAILKSLQSYFSPTLFRVIRLARIGRILRLIRAAKG
hNav 1.9	SQIFDIIIISLIILNMISMMAESYNQPKAMKSILDHLNWVFVVIFTLECLIKIFALRQYYFTNGWNLFDCVVVLLSIVSTMISTLENQEHIPFPPTLFRIVRLARIGRILRLVRAARG

Figure S4. MSA of VSD (DIV) of Na$^+$ channels from three mammalian species (*Rattus norvegicus*, *Mus musculus* and *Homo sapiens*).

gi|403447|*D. melanogaster*	DKKFDIIIMLFIGLNMFTMTLDRYDASDTYNAVLDYLNAIFVVIFSSECLLKIFALRYHYFIEPWNLFDVVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|194893768|*D. erecta*	DKKFDIIIMLFIGLNMFTMTLDRYDASETYNAVLDYLNAIFVVIFSSECLLKIFALRYHYFIEPWNLFDVVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|195132819|*D. mojavensis*	DKKFDIIIMLFIGLNMFTMTLDRYDASDTYNAVLDYLNAIFVVIFSSECLLKIFALRYHYFIEPWNLFDVVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|195048329|*D. grimshawi*	DKKFDIIIMLFIGLNMFTMTLDRYDASDTYNAVLDYLNAIFVVIFSSECLLKIFALRYHYFIEPWNLFDVVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|194768088|*D. ananassae*	DKKFDIIIMLFIGLNMFTMTLDRYDASDTYNAVLDYLNAIFVVIFSSECLLKIFALRYHYFIEPWNLFDVVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|195447454|*D. willistoni*	DKKFDIIIMLFIGLNMFTMTLDRYDASDTYNAVLDYLNAIFVVIFSSECLLKIFALRYHYFIEPWNLFDVVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|380690198|*B. dorsalis*	DKKFDIIIMLFIGLNMFTMTLDRYDASETYNAVLDYLNAIFVVIFSSECLLKIFALRYHYFIEPWNLFDVVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG



gi|195479236|*D. yakuba*	DKKFDIIIMLFIGLNMFTMTLDRYDASDTYNAVLDYLNAIFVVIFSSECLLKIFALRYHYFIEPWNLFDVVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|198468369|*D. pseudoobscura*	DKKFDIVIMLFIGLNMFTMTLDRYDASDTYNAVLDYLNAIFVVIFSSECLLKIFALRYHYFIEPWNLFDVVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|499007704|*C .capitata*	DKKFDIIIMLFIGLNMFTMTLDRYDASETYNAVLDYLNAIFVVIFSSECLLKIFALRYHYFIEPWNLFDVVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|1842214|*M. domestica*	DKKFDIIIMLFIGLNMFTMTLDRYDASEAYNNVLDKLNGIFVVIFSGECLLKIFALRYHYFKEPWNLFDVVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|148291097|*A .gambiae*	NKKFDMIIMLFIGFNMLTMTLDHYKQSETFSAVLDYLNMIFICIFSSECLMKIFALRYHYFIEPWNLFDFVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|290349620|*C. pallens*	NKKFDMIIMLFIGFNMLTMTLDHYKQSDTFSAVLDYLNMIFICIFSSECLMKIFALRYHYFIEPWNLFDFVVVILSIIGLVLSDLIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|290349618|*C. quinquefasciatus*	NKKFDMIIMLFIGFNMLTMTLDHYKQTETFSAVLDYLNMIFICIFSSECLMKIFALRYHYFIEPWNLFDFVVVILSIIGLVLSDLIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|214011860|*A. aegypti*	NKKFDMIIMLFIGFNMLTMTLDHYKQTDTFSAVLDYLNMIFICIFSSECLMKIFALRYHYFIEPWNLFDFVVVILSIIGLVLSDLIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|668455755|*A. sinensis*	NKKFDMIIMLFIGFNMLTMTLDHYKQSETFSAVLDYLNMIFICIFSSECLMKIFALRYHYFIEPWNLFDFVVVILSIIGLVLSDLIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|50080864|*A. albopictus*	NKKFDMIIMLFIGFNMLTMTLDHYKQTDTFSAVLDYLNMIFICIFSSECLMKIFALRYHYFIEPWNLFDFVVVILSIIGLVLSDLIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|571548120|*A. mellifera*	DKKFDMIIMLFIGLNMLTMTLDHYQQTQTFSDVLDYLNMIFIVIFTSECLMKIFALRYHYFKEPWNLFDFVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|572270136|*A. dorsata*	DKKFDMIIMLFIGLNMLTMTLDHYQQTQTFSDVLDYLNMIFIVIFTSECLMKIFALRYHYFKEPWNLFDFVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|148277534|*N. vitripennis*	DKKFDMIIMLFIGLNMLTMTLDHYQQSATFSNVLDYLNMIFIVIFTSECLMKIFALRYHYFKEPWNLFDFVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|307210096|*H. saltator*	DKKFDMIIMLFIGLNMLTMTLDHYQQSDTFSNVLDYLNMIFIVIFTSECLLKIFALRYHYFKEPWNLFDFVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|665817672|*M. demolitor*	DKKFDMIIMLFIGLNMLTMTLDHYQQTATFSNVLDYLNMIFIVIFSSECLMKIFALRYHYFKEPWNLFDFVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|383858782|*M. rotundata*	DKKFDMIIMLFIGLNMLTMTLDHYQQTQTFSDVLDYLNMIFIVIFTSECLMKIFALRYHYFKEPWNLFDFVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|340718624|*B. terrestris*	DKKFDMIIMLFIGLNMLTMTLDHYQQTQTFSDVLDYLNMIFIVIFTSECLMKIFALRYHYFKEPWNLFDFVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|307168143|*C. floridanus*	DKKFDMIIMLFIGLNMLTMTLDHYQQSETFSNVLDYLNMIFIVIFTSECLMKIFALRYHYFKEPWNLFDFVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|350409729|*B. impatiens*	DKKFDMIIMLFIGLNMLTMTLDHYQQTQTFSDVLDYLNMIFIVIFTSECLMKIFALRYHYFKEPWNLFDFVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|607353560|*C. biroi*	DKKFDMIIMLFIGLNMLTMTLDHYQQSTTFSNVLDYLNMIFIVIFTSECLMKIFALRYHYFKEPWNLFDFVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|380028083|*A. florea*	DKKFDMIIMLFIGLNMLTMTLDHYQQTQTFSDVLDYLNMIFIVIFTSECLMKIFALRYHYFKEPWNLFDFVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|217330564|*B. mori*	DKKFDMIIMLFIGLNMLTMTLDHYQQSETFSAVLDYLNMIFIVIFSSECLLKIFALRYHYFVEPWNLFDFVVVMFSIISLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|357623276|*D. plexippus*	DKKFDMIIMLFIGLNMLTMTLDHYQQSDTFSAVLDYLNMIFIVIFSSECLLKIFALRYHYFVEPWNLFDFVVVMFSIISLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|469664985|*C. medinalis*	DKKFDMIIMLFIGFNMLTMTLDHYQQSDTFSHVLDYLNMIFIVIFSSECLLKIFALRYHYFVEPWNLFDFVVVMFSIISLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|197927081|*P. xylostella*	DKKFDMIIMLFIGLNMLTMTLDHYQQAESFSVVLDYLNMIFIVIFSSECMSKIFALRYHYFVDPWNWFDFVVVMFSIISLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|189172241|*B. mandarina*	DKKFDMIIMLFIGLNMLTMTLDHYQQSETFSAVLDYLNMIFIVIFSSECLLKIFALRYHYFVEPWNLFDFVVVMFSIISLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|295136555|*H. zea*	DKKFDMIIMLFIGLNMLTMTLDHYQQSETFSTVLDYLNMIFIVIFSSECLLKMFALRYHYFVEPVSLFDFVVVNFSIISLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|3320596|*H. virescens*	DKKFDMIIMLFIGLNMLTMTLDHYQQSETFSTVLDYLNMIFIVIFSSECLLKMFALRYHYFVEPWNLFDFVVVNFSIISLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|500866367|*L. bostrychophila*	NTKFDMIIMLFIGLNMLTMTLDHYQQSETFSNVLDTLNLIFIVIFSSECMMKIFALRYHYFVEPWNLFDFVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|29467444|*P. corporis*	NTKFDMIIMLFIGLNMLTMTLDHYKQTETFSKVLDTLNLIFIVIFSSECLMKIFALRYHYFVEPWNLFDFVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|30350262|*P. humanus*	NTKFDMIIMLFIGLNMLTMTLDHYKQTETFSKVLDTLNLIFIVIFSSECLMKIFALRYHYFVEPWNLFDFVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|260528198|*P. americana*	NKKFDMIIMLFIGFNMLTMTLDHYQQSQQFSDVLDYLNMIFIVIFSSECLMKIFALRYHYFKEPWNLFDFVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|1657986|*B. germanica*	DKKFDMIIMLFIGFNMLTMTLDHYQQSKQFSDVLDYLNMIFIVIFSSECLMKIFALRYHYFKEPWNLFDFVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|259906459|*T. castaneum*	NKKFDMIIMLFIGLNMLTMTMDHYQQKETFTKVLDSLNMIFIVIFSTECLMKIFALRYHYFTEPWNLFDLVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|546673724|*D. ponderosae*	NKKFDMFIMLFIGLNMLTMTMDHYQQKETFTKVLDYLNMIFIVIFTTECLMKVFALRYHYFTEPWNLFDLVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|646703172|*Z. nevadensis*	NKKFDMIIMLFIGFNMLTMTMDHYQQSKRFSDVLDYLNMIFIVIFSSECLMKIFALRYHYFKEPWNLFDFVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|589908288|*T. palmi*	NKKFDMIIMLFIGFNMLTMTLDHYQQSETFSMVLDHLNMIFIVIFSSECLMKVFALRYHYFVEPWNLFDFVVVILSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG
gi|209361483|*C. lectularius*	DKKFDMIIMLFIGFNMLTMTMDHYQQTEMFSFVLDILNMIFIVIFSSECLLKIFALRYHYFKEPWNLFDFVVVLLSIIGLVLSDIIEKYFVSPTLLRVVRVAKVGRVLRLVKGAKG

Figure S5. MSA of VSD (DIV) of insect Na$^+$ channels. These sequences are derived from nine insect Orders (bar in grey: *Diptera*; purple: *Hymenoptera*; orange: *Lepidoptera*; red: *Phthiraptera*; pale green: *Blattodea*; green: *Coleoptera*; pale blue: *Thysanoptera*; blue: *Blattaria*; black: *Hemiptera*).

Supplementary Table 1 Parameter estimates and likelihood ratio statistics (2Δ*l*) for the scorpion α-toxin gene from *M. martensii*

Models	*p*	*l*	Parameter estimates	2Δ*l*	PSS
M0 (one-ratio)	1	-1497.0	$\omega = 0.85$		None
M1a (neutral)	2	-1448.0	$p_0 = 0.46$ ($p_1 = 0.54$)		Not allowed
			$\omega_0 = 0.08$ ($\omega_1 = 1$)	16.6	
M2a (selection)	4	-1439.7	$p_0 = 0.42$		2R*, 8K*, 9P*, 15E, 17A*,
			$p_1 = 0.30$ ($p_2 = 0.27$)		18R**, 37Q, 38W, 39V*, 54N
			$\omega_0 = 0.09$ ($\omega_1 = 1$)		
			ω_2 = **2.47**		
M7 (beta)	2	-1450.2	$p = 0.20$, $q = 0.15$	20.0	Not allowed
M8 (beta & ω)	4	-1440.2	$p_0 = 0.64$ ($p_1 = 0.38$)		2R*, 8K**, 9P**, 10H, 13V*, 15E*,
			$p = 0.32$, $q = 0.58$		17A*, 18R**, 37Q*, 38W*, 39V**,
			ω_s =**2.21**		54N*

Note: *p* is the number of parameters in the ω distribution; *l* is the log likelihood. Twice the log likelihood differences (2Δ*l*) between null models and their alternative models: M1a/M2a=16.6 and M7/M8=20.0, both having χ^2 significant values of $p < 0.001$. PSSs identified by the Naïve Empirical Bayes (NEB) methods under M2a and M8 with P (posterior probabilities) ≥ 0.9 are shown and those ≥ 0.99 are indicated by ** and ≥ 0.95 by *. Residues are numbered according to BmKM1. Two ω values in M2a and M8, as indicators of positive selection, are boldfaced. M8-specific PSSs are underlined once.

Supplementary Table 2 Log-likelihood values and parameter estimates under the fixed-sites models for Na$_v$ channel genes of birds

Model	*p*	*l*	r_2	κ	ω
A (homogeneous)	177	-6494.05	1	2.74	0.091
B (different *r*s)	178	-6468.83	1.62	2.68	0.090
C (different *r*s and πs)	187	-6454.74	1.41	2.57	0.090
D (different *r*s , κ and ω)	180	-6459.54	1.51	$\kappa_1 = 2.50$	$\omega_1 = 0.073$
				$\kappa_2 = 3.35$	$\omega_2 = 0.136$
E (different *r*s, κ, ω and πs)	189	-6447.52	1.33	$\kappa_1 = 2.55$	$\omega_1 = 0.074$
				$\kappa_2 = 2.87$	$\omega_2 = 0.131$
F (separate analysis)	354	-6340.11	1.52	$\kappa_1 = 2.53$	$\omega_1 = 0.077$
				$\kappa_2 = 2.92$	$\omega_2 = 0.120$

Note: *p* is the number of parameters including 166 branch lengths in the tree. The first partition refers to the two extracellular loops and the second the remaining portion of the VSD. r_2 is the rate of the second site partition relative to the rate of the first partition ($r_1 = 1$).

Supplementary Table 3 Log-likelihood values and parameter estimates under the fixed-sites models for Na_v channel genes of lizards

Model	p	l	r_2	κ	ω
A (homogeneous)	65	-3720.13	1	2.19	0.070
B (different rs)	66	-3689.90	2.20	2.13	0.064
C (different rs and πs)	75	-3683.19	1.76	2.12	0.064
D (different rs, κ and ω)	68	-3673.95	1.45	$\kappa_1 = 2.54$	$\omega_1 = 0.047$
				$\kappa_2 = 1.68$	$\omega_2 = 0.124$
E (different rs, κ, ω and πs)	77	-3666.68	1.18	$\kappa_1 = 2.60$	$\omega_1 = 0.047$
				$\kappa_2 = 1.58$	$\omega_2 = 0.119$
F (separate analysis)	130	-3615.89	1.77	$\kappa_1 = 2.53$	$\omega_1 = 0.049$
				$\kappa_2 = 1.72$	$\omega_2 = 0.102$

Note: p is the number of parameters including 54 branch lengths in the tree. Meanings for the two partitions of VSD and r_2 are the same with those in Supplementary Table 2 (the same below).

Supplementary Table 4 Log-likelihood values and parameter estimates under the fixed-sites models for Na_v channel genes of mammals

Model	p	l	r_2	κ	ω
A (homogeneous)	63	-3743.57	1	2.57	0.071
B (different rs)	64	-3675.42	3.00	2.59	0.067
C (different rs and πs)	73	-3663.73	2.58	2.54	0.065
D (different rs , κ and ω)	66	-3655.35	2.06	$\kappa_1 = 2.90$	$\omega_1 = 0.043$
				$\kappa_2 = 2.37$	$\omega_2 = 0.135$
E (different rs, κ and πs)	75	-3642.12	1.72	$\kappa_1 = 3.05$	$\omega_1 = 0.042$
				$\kappa_2 = 2.15$	$\omega_2 = 0.130$
F (separate analysis)	126	-3594.72	1.62	$\kappa_1 = 3.12$	$\omega_1 = 0.038$
				$\kappa_2 = 2.28$	$\omega_2 = 0.123$

Note: p is the number of parameters including 52 branch lengths in the tree.

Supplementary Table 5 Log-likelihood values and parameter estimates under the fixed-sites models for Na_v channel genes of insects

Model	p	l	r_2	κ	ω
A (homogeneous)	99	-4777.52	1	1.48	0.015
B (different rs)	100	-4749.93	2.11	1.42	0.013
C (different rs and πs)	109	-4747.02	1.71	1.43	0.013
D (different rs , κ and ω)	102	-4741.72	1.64	$\kappa_1 = 1.31$	$\omega_1 = 0.010$
				$\kappa_2 = 1.87$	$\omega_2 = 0.024$
E (different rs, κ ω and πs)	111	-4740.80	1.41	$\kappa_1 = 1.38$	$\omega_1 = 0.010$
				$\kappa_2 = 1.65$	$\omega_2 = 0.021$
F (separate analysis)	198	-4669.57	1.09	$\kappa_1 = 1.38$	$\omega_1 = 0.008$
				$\kappa_2 = 1.52$	$\omega_2 = 0.023$

Note: p is the number of parameters including 88 branch lengths in the tree.

Supplementary References

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