

**Supplementary data for:**

The neuropeptide SMYamide, a SIFamide paralog, is expressed by salivary gland innervating neurons in the American cockroach and likely functions as a hormone

Jan A. Veenstra

**Content**

Fig. S1. Deduced amino acid sequences of various Arthropod SIFamide precursors.	2
Fig. S2. Deduced amino acid sequences of various Polyneopteran SMYamide precursors.	3
Table S1. Analysis of SIFamide-related gene expression in <i>Periplaneta</i> transcriptome SRAs.	4
Fig. 3. Sequence alignment of various SIFamide GPCR homologs.	5
Fig. S4. Sanger sequence analysis of the RT-PCR product for <i>Periplaneta</i> SIFamide receptor 1.	12
Fig. S5. Sanger sequence analysis of the RT-PCR product for <i>Periplaneta</i> SIFamide receptor 2.	13

*Anisolabis maritima*

MQKSSSLLLLIVAVALLLILEDGALAYRKPPFNGSIFGKRGGSSAADFDAAVKALSSMCEIATEACSAWFPQNESNN\*

*Apis mellifera*

MVSTRVLAVVAALFVLAISVDAAYRKPPFNGSIFGKRNTITDYEITSRAMSSVCEVVSETCNAWLSRQDSN\*

*Blattella germanica*

MQNRVAATCLLLLAVLLFADLAAATYRKPPFNGSIFGKRGNVVEYDGTGKALSALCEIASEACSAWFPSADNN\*

*Bombus terrestris*

MSSRFVVAIVVALFILAIAVDAAYRKPPFNGSIFGKRSNAITDYELTSRAMSSVCETVSETCNAWLLARQDSN\*

*Bombyx mori*

MRADLIYFMFLVIILTTLATIEATYRKPPFNGSIFGKRNNVENDSSGRAIAALCEITTETCQAWYQALESQ\*

*Calopteryx splendens*

MNSKAIFIFAVVLVAFALLSGSADASSYRKPPFNGSIFGKRGGSGASADAESRGVAFSALCEVAIEACSSWMPASQDSK\*

*Drosophila melanogaster*

MALRFTLTLLLVTILVAAILLGSSEAAAYRKPPFNGSIFGKRNSLDYDSAKMSAVCEVAMEACPMWFPQNSDK\*

*Dryococelus australis*

MQRSGVLICAVMLAVLLLLSEPAATGKKPPFNGSIFGKRSNYVAEIRDYESTGRSLSAMCEIASEACASWFPQADKR\*

*Isoperla grammatica*

MQKSGVATCLLVLLVLLASDFAAANYRKPPFNGSIFGKRVGNSADYDGAALKSSMCEIAQEAACAAWFPQAEASK\*

*Ixodes scapularis*

MNSWKAFFMFGTLLVMVMMMACAAAYRKPPFNGSIFGKRSRADLNNADV KYAMCEAVWDTCTQWFPITQDGAQ\*

*Laupala kohalensis* (incomplete)

LRFAAVLCVALLVVAASAPSAASYRKPPFNGSIFGKRASAAGAAAAPTNAQEYEAAGKALSALCEVAAEAACA WFPQQNELN\*

*Lednia tumana*

MQKSALVKICIFLLALLAVCAEITTAAYRKPPFNGSIFGKRAGISIDYDNAGKALSSMCEIATEACAVWFPQRDESK\*

*Locusta migratoria*

MQTAACSRFLVVVLLALVMFTAASA AAATFRPPFNGSIFGKRNSIESAGSSTAVAAVCEIAAEAACA WLNNDK\*

*Medauroidea extradentata*

MQRSGVIVCVLVALLLLLSEPAVATGKKPPFNGSIFGKRSNYVAELRGCRGVVIADYESTGRSLSAMCEIASEACASWFPQADKR\*

*Nilaparvata lugens*

MVRICLAMCIVSIIILAFVDAAYKKPPFNGSIFGKRGSTIIIEYENAGKALSSMCEIASEACSAWFPPLPDN\*

*Periplaneta americana*

MQTRGVATCLLLLAVLLLAEFAAATYRKPPFNGSIFGKRGNAAADYD GAGKALSAMCEIASEACSAWFPQADNN\*

*Rhodnius prolixus*

MSRTL FVCCFTLVVALIFLDAAMATYKKPPFNGSIFGKRAGPSSDYETAGKALSTMCEIAAEAACA WFPVQDNN\*

*Teleogryllus comodus*

MAGKSFAASRRSARPLAVLLRAAAVLCVALLVVAASAPSAASYRKPPFNGSIFGKRAPAAAA PAPAASAQDYEAAGKALSALCEVA  
AEAACA WFPQQNELN\*

*Timema cristinae*

MNKYSIARCMLLLTLFMLTELTLANVKKPPFNGSIFGKRGNVGGELRDYDSAGRTL SVMCEIASEACSGWFPQPDKR\*

*Tribolium castaneum*

MQLALAKVFSVCIVVIILTSWIEMTEATYRKPPFNGSIFGKR GATIEYDSASKALSAMCEIASEACQWTFPSQEK\*

*Zootermopsis nevadensis*

MQNRVVATCVLLLAVLLLAEFATAAFRKPPFNGSIFGKRGSPTDYDGASKALSAMCEIASEACSAWFPQMD\*

Fig. S1. Deduced amino acid sequences of various Arthropod SIFamide precursors. Coding sequences are present in the supplementary spreadsheet of this paper.

***Blattella germanica***

MQFSQSVIFFLAILLLLTLSTTCNPGVPFRRLPFNGSMYGKRASSALPMDYDNKAFSSLCELAAEVCESTWYPQQVENN\*

***Dryococelus australis***

MLHKFALAIMIFACVLCVALAYNLRVPINGGMYGKRDGPSEYDARSKAVSTMCEMATEVCSAWLAQADPN\*

***Laupala kohalensis* (incomplete)**

-----LAIGFALHPVLAASGPFKKIPFNGSMYGKRASPSEYEAAAAGKALSALCEVAAEACSAWFPQQNELN\*

***Locusta migratoria***

MNRCSLVAMLVWAILLQTCLTEGIAFQKLPFNGAMYGKR TTSDVDFDSSNRAISSLCEETASEVCSASWYGQPD TN\*

***Medauroidea extradentata***

MIHISIARSLLVLVCTLLVTLGYNLRVPMNGGMYGKRDGPSEYDARGKAVSTMCEMATEVCSAWLAQADPN\*

***Periplaneta americana***

MKFACTVLSLVALLLAVLVACNPGPPFRRLPFNGSMYGKRTGNALPMDYDSNSKALSSLCEMAVEVCPWFPPQQENN\*

***Teleogryllus comodus***

MTEASTPAATAWCVRLLCLLLLVAALLEAAAARGSFQKIPFNGSMFYGKRAAAATEYELSHALSAMCEAAAAACTAWTQPDK\*

***Timema cristinae***

MILTAVLIAALSQNAMAANYRKLPFNGGMYGKRDTNLGELLGPQILT VRAKLCVRSPLKHAQCGFHSRRSTRATSDDLPR\*

***Zootermopsis nevadensis***

MKLSCSMIFLLALLLALLVDCNTGPPHRRVPFNGSMYGKRTANSLSDYDSNAKSLSSLCEVATEVCSAWFPQQTENN\*

**Fig. S2.** Deduced amino acid sequences of various Polyneopteran SMYamide precursors. Coding sequences are present in the supplementary spreadsheet of this paper.

SRA	Spots	SIFamide	SMYamide	SIFaR1	SIFaR2	
DRR014884	9576533	47	4.9	4	.4	0 .0 2 .2 Egg cases
DRR014885	9442758	27	2.9	1	.1	0 .0 4 .4 young larvae
DRR014886	9060819	5	.6	2	.2	4 .4 7 .8 An old-instar female larva
DRR014887	9962367	1	.1	4	.4	2 .2 2 .2 An old-instar male larva
DRR014888	8067940	5	.6	0	.0	0 .0 2 .2 Adult female
DRR014889	9773451	5	.5	0	.0	0 .0 0 .0 Adult male
SRR921630	10494946	0	.0	1	.1	2 .2 0 .0 Generic sample
SRR1184457	27531950	0	.0	0	.0	0 .0 0 .0 no specific information provided
SRR1184458	27531950	0	.0	0	.0	0 .0 0 .0 no specific information provided
SRR1322009	38573631	3	.1	0	.0	64 1.7 0 .0 testis
SRR2994649	26044067	3	.1	4	.2	0 .0 7 .3 control whole body
SRR2994650	26565524	16	.6	6	.2	5 .2 41 1.5 E.coli treated whole body pooled male and female
SRR3056857	25780592	3	.1	4	.2	0 .0 7 .3 Whole body pooled male and female
SRR3056858	26236239	16	.6	6	.2	5 .2 40 1.5 E.coli treated whole body pooled male and female
SRR3089536	22954896	1	.0	0	.0	1 .0 0 .0 chemosensory organs
SRR3089537	21727373	4	.2	0	.0	0 .0 2 .1 chemosensory organs
SRR3089538	21585983	52	2.4	107	5.0	2 .1 0 .0 chemosensory organs
SRR3289663	27096583	0	.0	0	.0	4 .1 12 .4 Foregut
SRR3289684	33591931	0	.0	0	.0	10 .3 0 .0 Midgut
SRR3289687	32043575	0	.0	0	.0	2 .1 7 .2 Foregut withTreatment by Cyclozaprid
SRR5097509	33490493	4088	122.1	2	.1	16 .5 28 .8 Brain stung
SRR5097510	100881736	7369	73.0	32	.3	42 .4 2 .0 Brain
SRR5097511	23403587	701	30.0	0	.0	12 .5 0 .0 Brain
SRR5097512	41305288	96	2.3	4569	110.6	4 .1 0 .0 SEG
SRR5097513	46825733	212	4.5	6279	134.1	0 .0 0 .0 SEG
SRR5097514	132099525	556	4.2	22945	173.7	0 .0 0 .0 SEG stung
SRR5097515	40310873	258	6.4	10940	271.4	0 .0 0 .0 SEG stung
SRR5097516	24170647	1694	70.1	0	.0	0 .0 10 .4 Brain stung
SRR5286150	43339233	12	.3	10	.2	2 .0 2 .0 male adult whole body
SRR5286151	34455335	61	1.8	7	.2	14 .4 28 .8 female adult whole body
SRR5286152	38897702	17	.4	0	.0	7 .2 14 .4 newly molted whole body
SRR5286153	64343011	51	.8	9	.1	2 .0 44 .7 8th larva whole body
SRR5286154	33964933	20	.6	11	.3	2 .1 12 .4 3rd larva whole body

**Table S1.** Analysis of SIFamide-related gene expression in *Periplaneta* transcriptome SRAs. Number of half-reads in the various transcriptome SRAs available at NCBI. SRA indicates the SRA identifier and Spots the total number sequences present in each SRA. The number of reads for the four genes are indicated in light blue, their relative number, i.e. their number per million reads are indicated in bold black. The final column gives a short description of the tissues analyzed. Note that the SIFamide gene is strongly expressed in the brain, but also in the subesophageal ganglion and chemosensory organs. The SMYamide gene is strongly expressed in the subesophageal ganglion, very little in the brain, but also in the chemosensory organs. The number of reads for the receptors are much smaller. Apart from the brain, there is a significant number of reads for SIFaR1 in the testes. Note however that the whole female body also has a significant number of reads for both SIFamide receptors and these reads are unlikely to be derived from the brain, as there are relatively few SIFamide reads in that particular SRA (SRR5286151).

```

Nilaparvata-2 -----
Zootermopsis-2 -----
Blattella-2 -----
Periplaneta-2 -----
Ixodes-1 -----
Ixodes-2 -----
Drosophila -----MAVNGRMRKRKRHSHTAGDMP TTTAPT TTT-----
Locusta -----RRRSGGSARCA-CAGAAAAPGGG-GAA
Nilaparvata-1 -----MMAPLRLP-----
Bombyx -----MKMAPLRLPVDYYTDDFLNFSTQN-----PNNERHHTRHN
Apis -----MLQPPSP LLEMASLRLPDSEEFVDVDP RRRSNNGNGPATTVSAVPPATVSTLL
Lednia -----
Rhodnius -----MVSLRLPDSEEYIEARGRALETGLAPTRA-----
Calopteryx -----
Isoperla -----
Blattella-1 MADLVESPAYHASSP LLEMASLRLPDSEEYEFENRRRADSLFTSSSTDSSSNTLHLPTRS
Periplaneta-1 MADPNIPPGYHASSP LLEMASLRLPDSEEYEFENRRRADSLFTSSSTDSSSNTLHLSTRS
Zootermopsis-1 -----
Anisolabis -----
Medauroidea -----
Dryococelus -----
Timema -----
Tribolium -----MGVLDLAAALRIDDEEEFYDERRRRA-----
Laupala -----
Teleogryllus ---MAEAGGLPASSP LLEMASLRLPDSEELDLDARRRGAAG---VLTSSSTPLHLAARA

```

```

Nilaparvata-2 -----
Zootermopsis-2 -----
Blattella-2 -----MTTSI-----
Periplaneta-2 -----
Ixodes-1 -----MRRAN-LTFNCYGLYHWT LSTL-----
Ixodes-2 -----MMRTRPPAMLGSSLLNADNVT-----
Drosophila -----TAGNGSDSGSFSSTSSAIKLSNGAITDTLLAAVLTTATATVAPAASSLISS
Locusta EERRQCALRV RGGGGGSGRWRSG-----GG---AAAVPGAPPLAMDA-----
Nilaparvata-1 -----
Bombyx -----HSHLRES-----HKNHVADMLSNSII--DA-----
Apis -----Q-----MRNEVG DYLN SLIV--EA-----
Lednia -----
Rhodnius -----SN--TSSRRLFV-----NSLLVDFVMDTLV--TN-----
Calopteryx -----
Isoperla -----
Blattella-1 QPYRQLPWNIS-----NRRILSE-----SGTKLVDFFGNTVL--EL-----
Periplaneta-1 QAYRQLPWNVS-----NRRI-SE-----GGNKLVDFIGNTYL--DL-----
Zootermopsis-1 -----M-S-----ETFENATCV-----
Anisolabis -----
Medauroidea -----M-----
Dryococelus -----M-----
Timema -----
Tribolium HLLAAVPMPEPNE-TM-----LTAEYLG NLF D--VV-----
Laupala -----
Teleogryllus HAYRPAPAPHPLS-NFSRSIVAE-----SSGYLTDFVGN TIK--VA-----

```

Fig. 3. Sequence alignment of various SIFamide GPCR homologs.

```

Nilaparvata-2 -----MFLPLSV---PRTQVI----
Zootermopsis-2 -----MDTSNECHPNRSFCDWWPNMT---SAEV-----
Blattella-2 -----INMDITDECRPNRSCPSWWTNAT---VADNDS----
Periplaneta-2 -----CRPNQSRCNWWPNMT---SEYHEL----
Ixodes-1 -----VPALTGTFTSGAGKSSDS--GNGSAFPMLATA----AVPLSVT
Ixodes-2 -----LRWGDSTQDDT--SQSET-----ANAVQ
Drosophila MSAAAATTTATSSQLAVVSTMQA-VALPGVSIPDA--TSSTYYANLLSMSPATTSLISVA
Locusta -SGSGSGPSAGGAVAVAVRHN-ASAGGATAAAG--GATGA-----VA--A
Nilaparvata-1 -----LAEEG--SAAAF-----GL--N
Bombyx -FNTRFVEN---SVL---PDM-----EP--LSSHM-----DL--E
Apis -IATSPG----SR-----G-PGM----DEHE--GASSA-----LL--N
Lednia -----
Rhodnius -TTPSASSS-----PSSP-----
Calopteryx -----
Isoperla -----
Blattella-1 -LTMNNHHHNGSAT---PSLHE-NGMDSIILEDSD--GNSS-----SI--N
Periplaneta-1 -VTMANNNNNNISF---NAFHD-GVMEASLQDES--AS-----GF--N
Zootermopsis-1 -LFSAMTNSKNGSL---NAFHG-SGIDGSVHDEP--GS-----GL--N
Anisolabis -----
Medauroidea -ALAGFN-----N-LTVDLLNHSRA--DNGT-----AW--L
Dryococelus -AVAALNASA-----D-FFLGVGNLGRP--GNGT-----PW--G
Timema -----M-----N-NGSGGGLVNEDE--AGGF-----LW--E
Tribolium -AHTESTK-----
Laupala -----KG--DGAD-----GI--F
Teleogryllus -TNHSAAG-----HDA--ADKG--EQGD-----AL--Y

```

```

Nilaparvata-2 ----Y-----TAS--TGATGEPsrNELRYSVVLTVVFCVAYLLVFCVGVVGNFAVVAVVC
Zootermopsis-2 ----Y-----NVSRIgPTTVTYVVKFRHPLSVTVVFCVAYALVFAVGVVGNCFVVGvvy
Blattella-2 ----F-----NVS---RNHTFIVYEFRHSLTvtISFCIAYALVFIgVVGNCfVVGvvy
Periplaneta-2 ----L-----NGS-LNGSTPTFAVfELRHSLSVTISFCIAYAVFVVGvVGNCFVVGvvy
Ixodes-1 Q-----EPPDTLVSEdWVDACFLRHtAPWAVAYCVAYSVFVVGIVGNCFVlKvvs
Ixodes-2 DNGSW-----NASDYDLYSIPDLWmRYSpgIVAVFCLAYSVVFVMGLLGNSFVvAVVA
Drosophila ATKSYNDsvLRWEQLDGNdFGFDPLyRHSLAMsIAFCVAYILVFLVGLIGNSFVIavvL
Locusta SA-NASSA-VMAAAAGPWHLlPDDLVyRHSAAMtAVYCAAYSLVFLlGLVGNCCVIAVvW
Nilaparvata-1 VT-RRLR--MDPAASNMSEPFwHDALyRHSLGMSVVFCAAYLlVfVVGlvGNVfVIAVvY
Bombyx ER-HYPS--RMNGTLNRSDfGGDEfMYRHSGAMtAVYCAAYLlVfVVGlvGNCFVIAVvY
Apis AS-KTAA--AANLtagDgQIPAVDRlyRHSMAMsAVYCVAYVlVfVVGlvGNsFVIAVvY
Lednia -----SNLNNVtSDIFPEYfYRHSPVlTVtFCIAYLlVfVVGmCGNSMvINvVf
Rhodnius ----AAGA-VADLPDSTSNQTYQHfYRHsIAMtIVFCVAYLlVfVVGlvGNCFVIMvvy
Calopteryx ----ALANS-SNATASLLAERlPTEfYRHSLAMtVvYCVAYLlVfAVGLVGNCFVIAVvY
Isoperla -----NNSTNSTYMPDLfYRHSLAMtVvYClAYLlVfAVGLVGNCFVIAVvY
Blattella-1 S--SLVN--G-TLATGMNFsMDPIPFYRHSLPMTAVYCFAYIlVfAVGLVGNCFVIAVvY
Periplaneta-1 A--SNVN--A-TL----GNATEPIPFYRHtFAMtAVYCFAYIlVfAVGLVGNCFVIAVvY
Zootermopsis-1 E--SILN--A-TA----ANGTDPiPFYRHsFAMtAMyCFAYIlVfAVGLVGNyFVIAVvY
Anisolabis -----N-V-T--GDSLnatVvTEfYRHsvAMtAlYCVAYLlVfAVGLVGNClVIAVvY
Medauroidea DD-GYGG-----LPTEmLYRHGFAMtVvYCVAYLlVfAVGLVGNsFVvAVvY
Dryococelus DD-----AGNATFAfDELvYRHsFAMtAVYCVAYLlVfAVGLVGNCFVIAVvY
Timema DY-GGYNL-T-YGNGTNGSfSYDEmVYRHtFAMtAlYCVAYLlVfAVGLVGNCFVIAVvY
Tribolium ----WAN----ATLNATETAfVPElFyRHsMAMtAVYCVAYLlVfAVGLVGNfFVIAVvF
Laupala NG-TFGI----NATNGTMTlSGDLfYRHtLVMTAVYCVAYVlVfAVGLlGNCFVIAVvF
Teleogryllus NG-SLAL----NASGNVtAALGADlFyRHSLAMtAVYCVAYVlVfAVGLlGNCFVIAVvF

```

Fig. 3. Sequence alignment of various SIFamide GPCR homologs.

Nilaparvata-2 RSPRM RTP - T N L F I A N L A C A D L L V N I I C L P F T L I S N I M T A W T M G W L V C K T I P Y M Q G V S V N  
 Zootermopsis-2 RSPRM RSP - T N L F I A N L A C A D L L V N V L C L P F T L V G N I M S A W I M G W V I C K T V P Y L Q G V S V S  
 Blattella-2 RSPRM RSP - T N L F I A N L A C A D L L V N V L C L P F T L V G N I M S A W I M G W V I C K T V P Y L Q G V S V S  
 Periplaneta-2 RSPRM RSP - T N L F I A N L A C A D L L V N V L C L P F T L V G N I M S A W I M G W V I C K T V P Y L Q G V S V S  
 Ixodes-1 R N T S M K N S A F H M L L V N L A V A D L M V I V F C L P V T L V G H L F G P W I L G L F V C K G V S Y L Q G V S V C  
 Ixodes-2 RSPRM RTV - T N Y F I V N L A M A D I L V V V F C I P A T L V S N I F V P W V L G W F M C K T M S Y L Q G V A V S  
 Drosophila R A P R M R T V - T N Y F I V N L A I A D I L V I V F C L P A T L I G N I F V P W M L G W L M C K F V P Y I Q G V S V A  
 Locusta RSPRM RTV - T N L F I A N L A A A D L L V V V V C L P A T L V S N I F V P W L L G W W M C K T V P Y V Q G V S V A  
 Nilaparvata-1 RSPRM RTV - T N F F I V N L A I A D I L V V V F C L P A T L L S N I F V P W I L G W W M C K T V P Y V Q G V S V A  
 Bombyx RSPRM RTV - T N F F I V N L A F A D I L V I V F C L P A T L M S N I F V P W L L G W L M C K T V P Y V Q G L S V A  
 Apis RSPRM RTV - T N F F I V N L A V A D I L V I V F C L P A T L L S N I F V P W L L G W F M C K A V A Y I Q G V S V A  
 Lednia RSPRM RTV - T N Y F I V N L A F A D I L V I V F C V P A T L M S N I F V P W I L G W W M C K M V P Y V Q G V S V A  
 Rhodnius RSPRM RNV - T N F F I V N L A V A D I L V I V F C L P A T L M S N I F V P W V L G W W M C K T V P Y V Q G V S V A  
 Calopteryx RSPRM RTV - T N Y F I V N L A A A D I L V I V F C L P A T L M S N I F V P W V L G W W M C K T V P Y V Q G V S V A  
 Isoperla RSPRM RTV - T N F F I V N L A I A D I L V V A F C I P A T L T A N I F V P W V L G W F M C K T V P Y I Q G V S V A  
 Blattella-1 RSPRM RTV - T N F F I V N L A V A D I L V I V F C L P A T L L S N I F V P W L L G W W M C K T V P Y V Q G V S V A  
 Periplaneta-1 RSPRM RTV - T N F F I V N L A V A D I L V I V F C L P A T L L S N I F V P W L L G W W M C K T V P Y V Q G V S V A  
 Zootermopsis-1 RSPRM RTV - T N F F I V N L A V A D I L V I I F C L P A T L L S N I F V P W L L G W W M C K T V P Y V Q G V S V A  
 Anisolabis RSPRM RTV - T N F F I V N L A V A D I L V I V F - L P A T L V A N I F V P W I L G W W L C K T I P Y V Q G V S V G  
 Medauroidea RSPRM RTV - T N Y F I V N L A V A D I L V I V F C L P A T L M A N I F V P W V L G K W M C K T V P Y V Q G V S V A  
 Dryococelus RSPRM RTV - T N Y F I V N L A V A D I L V I V F C L P A T L M A N I F V P W V L G W W M C K T V P Y V Q G V S V A  
 Timema RSPRM RTV - T N F F I V N L A V A D I L V I V F C L P A T L M A N I F V P W V L G W W M C K T V P Y V Q G V S V A  
 Tribolium RSPRM RTV - T N F F I V N L A V A D I L V I V F C L P A T L M S N I F V P W V L G W W M C K T V P Y I Q G V S V A  
 Laupala R T P R M R T V - T N F F I V N L A V A D I L V I V F C L P A T L M S N I F V P W V L G W W M C K T V P Y V Q G V S V A  
 Teleogryllus R T P R M R T V - T N F F I V N L A V A D I L V I V F C L P A T L M S N I F V P W V L G W W M C K T V P Y V Q G V S V A

Nilaparvata-2 A S I N T L V A I S F E R W L A I C Y P M R W O M T S R V C K L V I L L I W L F S L T I T L P W A L F F O L R P M G D -  
 Zootermopsis-2 A S I N T L V A I S V E R C L A I C Y P L K W O M T S R A C R V V V I I I W T F S L T I T L P W A I F F G L K P L E E -  
 Blattella-2 A S I N T L V A I S V E R C L A I C Y P L K W O M T S R A C R V V V V I I W T F S L I I T L P W A I F F G L R P L E E -  
 Periplaneta-2 A S I N T L V A I S V E R C L A I C Y P L K W O M T S R A C R V V V V I I W T F S L I I T L P W A I F F G L H P L E D -  
 Ixodes-1 A S V N T L V A I S I D R F L A I C H P M K R Q M T S R C R V I I A V I W S F S L T I T L P W T E F F R L M P M L S E  
 Ixodes-2 A S I N T L V A I S M D R C L A I C Y P L K C O L S T R S V R K I L V I I W T F S I A I T F P W A L F F T L Q P L H P S  
 Drosophila A S V S L I A V S L D R F L A I W W P L K - O M T K R R A R I M T I G I W V I A L V T T I P W L L F F D L V P A E E V  
 Locusta A S V S L I A V S L D R F L A I W W P L K F O I T K R R A R L I I A A I W L L A L T T T L P W A V F F D L V S V L D -  
 Nilaparvata-1 A S V S L I A V S I D R F L A I W P L K C O I T R R A R T I I A V I W L V A A T T T L P W A L F F D V V - I F S E  
 Bombyx A S V S L V A V S L D R F L A I W W P L K C O I T K R R S R M M I V F I W I F A I L V T T P W V E F F D L V V V F E E  
 Apis A S V S L I A V S L D R F L A I W W P L K C O I T K R R A R F I I V V I W E I A L T T T S P W L L F F D L V A I Y K D  
 Lednia A S V S L I C V S L D R F L A I W W P M K C O I T K R R A R F I I F L I W V I A F A T T I P W A I Y F D L V M - - E  
 Rhodnius A S V S L I A V T L D R F L A I W W P L K C O I T R R A R L M I L V I W V A L T T T I P W A L F F D L V V I F T D  
 Calopteryx A S V S L I A V T L D R F L A I W P L K C O I T K R R A R L M I F I I W V A L T T T I P W A L F F D L V I F R E  
 Isoperla A S V S L I A V S L D R L L A I W W P M K C O I T K R R A R I M I F V I W V I A L S T T I P W A I Y F D L V - I F H E  
 Blattella-1 A S V S L I A V S L D R F L A I W W P L K C O I T K R R A R F M I F V I W V I A L T T T I P W A L F F D L V I I F N D  
 Periplaneta-1 A S V S L I A V S L D R F L A I W W P L K C O I T K R R A R F M I F V I W V I A L T T T I P W A L F F D L V M V F N D  
 Zootermopsis-1 A S V S L I A V S L D R F L A I W W P L K C O I T K R R A R F M I F F I W V I A L T T T I P W A L F F D L V I I F N D  
 Anisolabis A S V S L I A V S V D R F L A I W W P L K C O I T K R R A R V M I V I I W V I A L T T T I P W A L F F - - - - V F R D  
 Medauroidea A S V S L I A V S A D R F L A I W W P L K C O I T K R R A R F V I V L I W V I A L S T T I P W A L F F E L V S V F N D  
 Dryococelus A S V S L I A V S L D R F L A I W W P L K C O I T K R R A R F M I V L I W V I A L T T T I P W A V F F D L V S I F R D  
 Timema A S V S L I A V S L D R F L A I W W P L K C O I T K R R A R I M I V F I W V I A L T T T I P W A V F F D L V S I F R D  
 Tribolium A S V S L I A V S L D R F L A I W W P L K C O I T K R R A R L M I V I I W E I A L T T T I P W A L F F D L V A V F N D  
 Laupala A S V S L I A V S L D R F L A I W W P L K C O I T K R R A R F I I L V I W V I A L T T T I P W A L F F D L V Q I Y A D  
 Teleogryllus A S V S L I A V S L D R F L A I W W P L K C O I T K R R A R F I I F V I W V I A L T T T I P W A L F F D L V V I Y S E

Fig. 3. Sequence alignment of various SIFamide GPCR homologs.

Nilaparvata-2 -----GSSMOTCLEETWPTPYSE-NVYFVLANLVMCYLLPLALISICYFRIWRRVCC  
 Zootermopsis-2 -----GSDVLICTESWSPSPHSG-NIYFVVAHLVMCYLFPLTLISLCYLLIWRVCR  
 Blattella-2 -----GSELQICTESWSPSPDSG-NVYFVVAHLVMCYLFPLTLISVCYLLIWRVCR  
 Periplaneta-2 -----GSDLQICTESWSPSQDSG-NIYFVVAHLVMCYLFPLTLISVCYLLIWRVCR  
 Ixodes-1 -----SNSLQVCREDWPTERMG-MLYFIVANLILCYLLPLCVITLCYIFIWLKVR  
 Ixodes-2 -----IPGISLCEVQWPDSTSS-TLYFILAHLVLCYLFPLLLIIVCYSCIWVKVR  
 Drosophila FSDALVSTYTOPQYLCQEVWPPGTDG-NLYFLLANLVAICYLLPMSLITLCYVLIWIKVST  
 Locusta -----DVRVCVEVWPDPTDG-ALYFLFENLLCFYVVPFALISLCYVLIWIKVCK  
 Nilaparvata-1 -----EPDVPMCEVWPDYLNGLYFLFANLIMCYIVPMILISLCYILIWIKVCK  
 Bombyx -----NPNVHLCEVWPNPLSE-VLYFVGNLIFCYILPMVMITMICYILIWIKVCR  
 Apis -----DEDLRLCEVWPRPKDE-TLFFLIENLTLCYVLPITILISLCYILIWIKVWR  
 Lednia -----EIDVSLCEVLPDPITG-ALNFLIANVLLCYILPTILISMICYVLIWIKVSK  
 Rhodnius -----NPEVKVCEVWPEYLNGLYFLIANLFCYILPMILISMICYVLIWIKVCK  
 Calopteryx -----MPETRLCEVWPPSLNG-DLYFLLANLVFCYVLPITILICLCYILIWIKVAR  
 Isoperla -----VPDVKLCMEVWPDSTNGGVLYFLIVNLLFCYILPMILISMICYVLIWIKVWK  
 Blattella-1 -----APDIQLCEVWPDSDLG-ALYFLIANLFCYILPMILISLCYILIWIKVWK  
 Periplaneta-1 -----APDVLLCEVWPDALDG-ALYFLIANLFCYILPMVILISLCYILIWIKVWK  
 Zootermopsis-1 -----APDVQLCEVWPDALDG-TLYFLIANLFCYILPMILISLCYILIWIKVWK  
 Anisolabis -----LPDNQLCEVWPDPMDG-ALYFLIANLMFFYILPMILISLCYILIWIKVWR  
 Medauroidea -----APDVQLCEVWPDMDG-NLYFLIDNLIICYILPLIVISLCYILIWIKVWR  
 Dryococelus -----APEIQLCEVWPDSDMG-DLYFLIANLVFCYILPMILISLCYILIWIKVWR  
 Timema -----APEIQLCEVWPEALDG-DLYFLIANLVFCYILPMILISMICYVLIWIKVWR  
 Tribolium -----APDVQLCEVWPDANMDG-ALYFLIANMVFICYILPMILITMICYVLIWIKVWR  
 Laupala -----APEVRLCEVWPDSDMG-ALYFLIANLMFCYLLPMVILISLCYILIWIKVWR  
 Teleogryllus -----APDVLLCEVWPDSDMG-ALYFLIANLMFCYLLPMVILISLCYILIWIKVWK

Nilaparvata-2 RKMPGGEVQM---YQELI IHRSKVKVIKMLFIVIVLFCASWLPLYVIFTRIKLGGDIQOP--  
 Zootermopsis-2 RTLPGEPHSHGGVVDLMIHRSKVKVIKMLLVVVVIFALS WLPLYVLFTRVKFGGPFSS-E  
 Blattella-2 RTLPGEPHPPQGGVMDMMIQRSKVKVIKMLLVVVVIFALS WLPLYVLFTRVKFGGPFSE--  
 Periplaneta-2 RTLPGEPHPPGGVMDMMIHRSKVKVIKMLLVVVVIFALS WLPLYVLFTRVKFGGPFSS-E  
 Ixodes-1 RRPGE-AH-DLGVENMIQRSKVKVIKMLLVVVVIFALS WLPLYVIFTRVKFGGPFSDGS  
 Ixodes-2 RSIPGE---SKHTEIMVQKSKIKVVKMLV VVVVIFVLSWLPLYVIFTRIKLDSPEEGS  
 Drosophila RSIPGEMSK-DAQMDRMQOKSKVKVIKMLVAVVILFVLSWLPLYVIFARIKFGSDISQ--  
 Locusta RDIPGD-ST-DAQLERMQHKS KIKVVKMLV VVVVIFL ASWLPLYVIFARIKLGGE LAA--  
 Nilaparvata-1 RHIPSD-TK-DAQERMQOKSKVKVVKMLVAVVILFVLSWLPLYVIFTRIKLGGDIDV--  
 Bombyx RSIPTD-TQ-DAQERMQOKSKVKVVKMLVAVVILFVLSWFLPLYVIFARIKLGGP IKK--  
 Apis RHIPSD-TK-DAQERMQOKSKVKVVKMLVAVVILFVLSWLPLYVIFTRIKLGD EQ----  
 Lednia RDIPTD-TK-DAQIERMQOKSKVKVVKMLV VVVVILFVLSWLPLYVIFASIKLGWDLSR--  
 Rhodnius RHIPSD-SK-DAQERMQOKSKVKVVKMLV VVVVILFVLSWLPLYVIFARIKLGGEISG--  
 Calopteryx RHIPTD-SK-DAIAERMQQQSKVKVIKMLVAVVIFVLSWLPLYVIFARIKLGGEVED--  
 Isoperla RHIPSD-TK-DAQIERMQOKSKVKVVKMLV VVVVILFVLSWLPLYVIFARIKLGGDIER--  
 Blattella-1 RTIPTD-TK-DAQERMQOKSKVKVVKMLVAVVILFVLSWLPLYVIFARIKLGGEVEI--  
 Periplaneta-1 RTIPTD-TK-DAQERMQOKSKVKVVKMLVAVVILFVLSWLPLYVIFARIKLGGEVEI--  
 Zootermopsis-1 RTIPTD-TK-DAQERMQOKSKVKVVKMLVAVVILFVLSWLPLYVIFARIKLGGEVEM--  
 Anisolabis RDIPTD-TK-DAQERMQOKSKIKVVKMLVIVVILFVLSWLPLYVICARIKLGGEREL--  
 Medauroidea RDIPTD-TK-DAQERMQOKSKVKVVKMLVAVVILFVLSWLPLYVIFTRIKLGERISR--  
 Dryococelus RDIPTD-TK-DAQERMQOKSKVKVVKMLVAVVILFVLSWLPLYVIFARIKLGGRTSR--  
 Timema RDIPTD-TK-DAQERMQOKSKVKVVKMLVAVVILFVLSWLPLYVIFARIKLGGEKTA--  
 Tribolium RHIPTD-TK-DAQERMQOKSKVKVVKMLVAVVILFVLSWLPLYVIFARIKLGGEHIEA--  
 Laupala RDIPTD-TK-DAQERMQOKSKVKVVKMLVAVVILFVLSWLPLYVIFARIKLGGETEL--  
 Teleogryllus RDIPTD-TK-DAQERMQOKSKVKVVKMLVAVVILFVLSWLPLYVIFARIKLGGETEL--

Fig. 3. Sequence alignment of various SIFamide GPCR homologs.



Nilaparvata-2 WE E P L V Y N L L P L A Q W L G A S N S C I N P V L Y A F F N K K F R A G F K A I L S S K S C F T T L R Y D T Y S F D  
 Zootermopsis-2 T E E A T I H S L L P V A Q W L G A S N S C V N P I L Y A F F N R K F R I G E K E I I T S R S C C S T L R Y N N E F R -  
 Blattella-2 S E E A A V H A I L P V A Q W L G A S N S C V N P I L Y A F F N K K F R A G F K A I V T S K S C C T P I R Y N N E Y S  
 Periplaneta-2 T E E A A I H A L L P I A Q W L G A S N S C V N P L Y A F F N R K F R I G F K A I L T S R S C C K P L R Y N N D F S -  
 Ixodes-1 A E Q A V I E V A A P V A Q W L G A S N S C I N P I L Y A F F N S K F R M G F K A I L L K C F C P K F S S R Q E R S C  
 Ixodes-2 V E W N L M L I L T P V A Q W L G A S N S C I N P V L Y A Y F N Q K F R K G F L A I I K S R S C C G T L R E P S Y - - S  
 Drosophila E E F E I L K K V M P V A Q W L G S S N S C I N P I L Y S - V N K K Y R R G F A A I I K S R S C C G R L R Y Y D N - - V  
 Locusta W E D E A L P V A T P V A Q W L G A S N S C I N P L L Y A F F N K K F R R G F A A I L R S R R C C G R L R Y Y E T - - V  
 Nilaparvata-1 W E T K L F V V L T P V A Q W L G S S N S C I N P I L Y A F F N N K Y R R G F S A I L R S R Q C C G T L R Y Y D T - - V  
 Bombyx W E E E M L P I V T P L A Q W L G A S N S C I N P I L Y A F F N K K Y R K G F V A I I K S R K C C G R L R Y Y E T - - I  
 Apis R E D E I V P I A T P I A Q W L G A S N S C I N P I L Y A F F N K K Y R R G F V A I L K S G R C C G K I R Y Y E T - - V  
 Lednia G E E D F L O Y A T P I A Q W L G S S N S C I N P I L Y A F F N K K F R R G F V A I W K S K R L C G R L R Y Y E T - - V  
 Rhodnius W E E D M L P M A T P V A Q W L G A S N S C I N P I L Y A F F N N K Y R R G F A A I L K S R K C C G T L R Y Y D T - - V  
 Calopteryx W E D K I L P V A T P L A Q W L G A S N S C I N P V L Y A F F N K K Y R R G F V A I V K S R K C C G R L R Y E T A - - I  
 Isoperla W E E E I L P I A T P I A Q W L G A S N S C I N P I L Y A F F N K K Y R R G F V A I L K S R R C C G - L R Y Y E T - - V  
 Blattella-1 W E D D I L L V A T P I A Q W L G A S N S C I N P I L Y A F F N K K Y R K G F I A I L K S R R C C G R L R Y Y E S - - V  
 Periplaneta-1 W E D D I L L V A T P I A Q W L G A S N S C I N P I L Y A F F N K K Y R K G F I A I L K S R R C C G R L R Y Y E S - - V  
 Zootermopsis-1 W E D D I L L V A T P I A Q W L G A S N S C I N P I L Y A F F N K K Y R K G F I A I L K S R R C C G H L R Y Y E S - - V  
 Anisolabis W E E D I L T F V F P I A Q W L G A S N S C I N P I L Y A F F N K K Y R R G F I A I L K S R K C C G R L H Y Y E S - - V  
 Medauroidea S E E E F I Q V V T P I A Q W L G S S N S C I N P I L Y A F F N K K Y R R G F V A I L K S R R C C G R L R Y Y E S - - V  
 Dryococelus W E E E I I Q V V T P I A Q W L G S S N S C I N P I L Y A F F N K K Y R R G F V A I L K S R R C C G R L R Y Y E S - - V  
 Timema W E E D V I Q V V T P I A Q W L G S S N S C I N P I L Y A F F N K K Y R R G F V A I F K S R R C C G R L R Y Y E S - - V  
 Tribolium W E E E I L P I A T P I A Q W L G A S N S C I N P I L Y A F F N K K F R R G F V A I I K S R R C C G R L R Y Y E T - - V  
 Laupala W E E D I L P I A T P I A Q W L G A S N S C I N P I L Y A F F N K K Y R R G F I A I L K S R K C C G R L R Y Y E T - - I  
 Teleogryllus W E E D V L P I A T P I A Q W L G A S N S C I N P I L Y A F F N K K Y R R G F I A I L K S R K C C G R L R Y Y E T - - I

Nilaparvata-2 GR-----NL-SSNY-GNRNDTVKR--PLTRMTISSAALRTGRIG--GSEGSLVTRTR--  
 Zootermopsis-2 -A-----SV-NSSF-SCRNTKNGSVLMTRMTITKSKGGKS-VRR--TRAGSLHRHN--  
 Blattella-2 -T-----SV-KSTF-SCRMNTKNGSILMSKKALNPSSTKT-RVP--TTGSNLQRHH--  
 Periplaneta-2 -----SSF-SCRMNGS-----VM--APASNLQRHN--  
 Ixodes-1 KMLSNTRTSM-----TAGRRKDSRMSDVF-----  
 Ixodes-2 VRG-----TTLRS-----GNT-RG-LSR-SENLEYVEHRAHSAK-AGLLINGGPDAQA--  
 Drosophila AIASS-TTSTRKSSHYPSSSRK-----SPSSPGLRKTNAVSYIYEHNSLRRHNLNLM  
 Locusta ALSGS--SVRKSE--NNN--NSS-----TRRPDTSVSYIFNNTGV-----  
 Nilaparvata-1 AMANSSSASVRKSSCYVNSNNPSVRRQ-----IMHQASQDSAVSYISNNTGV-----  
 Bombyx ALOSS-STSTRKSSWHYNNNNP--SIT-----RRSPPVDKNAVSYIFSHHTGV-----  
 Apis AMSS-STSMRKSSYYVNNNNNNNS-STRRTFHGPPVHQSNSVSYIFNHTGV-----  
 Lednia AMSSSASSTRKSSYYVNNNN--SSTR-----RSPEVAQDSAVSYISNNTGI-----  
 Rhodnius VRANSSTSLRKSSYYVTNNNNNNNS--TRRQLSQDTNVSYISNNTGV-----  
 Calopteryx -----TSSLRKSSYYMNS-----SMRQDTSVAYIYNNTGV-----  
 Isoperla ALMSSSGSSMRKSSYYVNN--NSST-----RRPEAGQDTSVSYICNNTGI-----  
 Blattella-1 -AMSS-SASLRKSSYYVNNN--NSST-----RRPPPQDTSVSYIFNNTGV-----  
 Periplaneta-1 -AMSS-SASMRKSSYYVNNN--NSST-----RR-PPGQDTSVSYIFNNTGV-----  
 Zootermopsis-1 -AMSS-TTSMRKSSYYVNNN--NSST-----RR-PPGQDTSVSYIFNNTGV-----  
 Anisolabis ALMSS-STSLRKSSYYVTNNN--NNST-----RRPPPQDTSVSYIFNNTGV-----  
 Medauroidea -MMSS-SASLRKSSYYVNN--NNST-----RRPPPQDTSVSYIYNNTGV-----  
 Dryococelus -MMSS-SASLRKSSYYVNN--NNST-----RRPPPQDTSVSYIFNNTGV-----  
 Timema -MMSS-SASLRKSSYYVNN--NSST-----RRPAPGQDTSVSYIFNNTGV-----  
 Tribolium AMSS-STSMRKSSHYVNN--NSS-----TRKLPQDNVSYIYNNTGV-----  
 Laupala AMSS-STSMRKSSYYTNN--NSS-----TRRPPGQDTSVSYIFNNTGV-----  
 Teleogryllus AMSS-STSMRKSSYYTNNN--NSS-----TRRPPGQDTSVSYIFNNTGV-----

Fig. 3. Sequence alignment of various SIFamide GPCR homologs.

```

Nilaparvata-2 -----TLSEFKAMNQVN-NNSSE-GPFYTEKV-----NGLTILSDMTL
Zootermopsis-2 -----SASAAAVQRIN-KNNEQFRKSYSRTECRNSISEAGENHRII--KSSSGS---HL--
Blattella-2 -----SASTAAVYKIN-KVNENYNNNYTNACKNPTTETG--HHII--KSSSVSALSHL--
Periplaneta-2 -----SASAAAVLKIN-KNN-----NY-TRCKNP--AS--HHIM--KSSSASALSQ---
Ixodes-1 -----
Ixodes-2 -----TNVKAPLVA-----
Drosophila MKQDSNLSQQMLLKQDSHGSRQFLIKQESSCSD---ASGTRRLLCQQDSNGSK---VSL
Locusta -----
Nilaparvata-1 -----
Bombyx -----
Apis -----
Lednia -----
Rhodnius -----
Calopteryx -----
Isoperla -----
Blattella-1 -----
Periplaneta-1 -----
Zootermopsis-1 -----
Anisolabis -----
Medauroidea -----
Dryococelus -----
Timema -----
Tribolium -----
Laupala -----
Teleogryllus -----

```

```

Nilaparvata-2 SANATFV-----
Zootermopsis-2 --NATSV-----
Blattella-2 --NATAV-----
Periplaneta-2 --NATAV-----
Ixodes-1 -----
Ixodes-2 -----
Drosophila SKQDSIVSYMEARRVAALSAQDRSVDSTATQQDTISIESRRPGALPAAATPPSLVDKRQK
Locusta -----
Nilaparvata-1 -----
Bombyx -----
Apis -----
Lednia -----
Rhodnius -----
Calopteryx -----
Isoperla -----
Blattella-1 -----
Periplaneta-1 -----
Zootermopsis-1 -----
Anisolabis -----
Medauroidea -----
Dryococelus -----
Timema -----
Tribolium -----
Laupala -----
Teleogryllus -----

```

Fig. 3. Sequence alignment of various SIFamide GPCR homologs.

```

Nilaparvata-2 -----
Zootermopsis-2 -----
Blattella-2 -----
Periplaneta-2 -----
Ixodes-1 -----
Ixodes-2 -----
Drosophila FVKQDSVISFVDQRPEPRRHQLVKQDSVISFADQRRGLLHKQDSLKTNRNSNDAPTHHVS I
Locusta -----
Nilaparvata-1 -----
Bombyx -----
Apis -----
Lednia -----
Rhodnius -----
Calopteryx -----
Isoperla -----
Blattella-1 -----
Periplaneta-1 -----
Zootermopsis-1 -----
Anisolabis -----
Medauroidea -----
Dryococelus -----
Timema -----
Tribolium -----
Laupala -----
Teleogryllus -----

```

```

Nilaparvata-2 -----
Zootermopsis-2 -----
Blattella-2 -----
Periplaneta-2 -----
Ixodes-1 -----
Ixodes-2 -----
Drosophila LKKTDSQLSYGTSSPRRNVELYE
Locusta -----
Nilaparvata-1 -----
Bombyx -----
Apis -----
Lednia -----
Rhodnius -----
Calopteryx -----
Isoperla -----
Blattella-1 -----
Periplaneta-1 -----
Zootermopsis-1 -----
Anisolabis -----
Medauroidea -----
Dryococelus -----
Timema -----
Tribolium -----
Laupala -----
Teleogryllus -----

```

Fig. 3. Sequence alignment of various SIFamide GPCR homologs.



Fig. S5. Sanger sequence analysis of the RT-PCR product for *Periplaneta* SIFamide receptor 2.

