

Electronic Supplementary Material

An ancient FMRFamide-related peptide-receptor pair induces defense behavior in a brachiopod larva

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Table S1: Peptides that were tested in *Terebratalia transversa* larvae and the necessary concentrations to induce defense stance.

peptide	batch 1	batch 2
DFLRFamide*	500 nM for full contraction	750 nM for full contraction
AFLRFamide*	1 µM for full contraction	2 µM for full contraction
FLRFamide**	3 µM for full contraction	3 µM for full contraction
LRFamide**	no contraction at 50 µM	no contraction at 50 µM
DFLRWamide	7.5 µM for full contraction	10 µM for full contraction
AFLRWamide	20 µM for full contraction	20 µM for full contraction
DFLRYamide	50 µM for full contraction	50 µM for full contraction
AFLRYamide	weak contraction in about 50% of the larvae at 50 µM	no contraction at 50 µM
DFLRLamide	weak contraction in about 50% of the larvae at 50 µM	no contraction at 50 µM
AFLRLamide	no contraction at 50 µM	no contraction at 50 µM
YMRFamide***	10 µM for full contraction	15 µM for full contraction
NSDGLamide*	no contraction at 50 µM	no contraction at 50 µM
TDKCPVYamide*		
AAKAPSSamide*		
CYLYDCINamide*		
MDPSQFGYGIamide*		
YSLDGIGSGLIamide*		
LSDYYAWAAQTRLamide*		
GWamide*		
RGWamide(*)		
LGWamide**		
Flamide**		
KPIIYEamide**		
WQGMKMWamide***		

* peptides predicted from *T. transversa* prepropeptide sequences

** shortened versions of peptides predicted from *T. transversa* prepropeptide sequences

*** peptides predicted from *Novocrania anomala* prepropeptide sequences

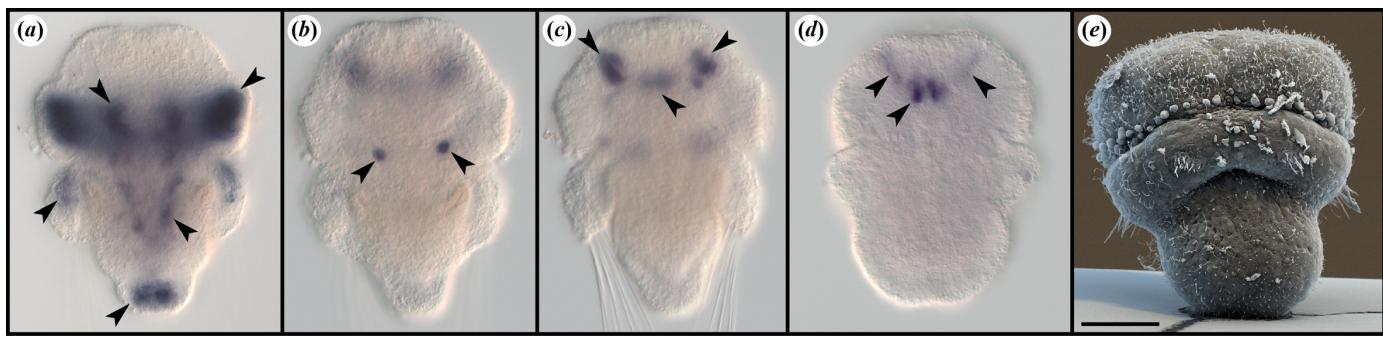


Figure S1: FLRFamide peptide and receptor expression in *T.transversa* larvae. **A-C** late larvae, *in situ* hybridization. Arrows indicate expression domains. **A** FLRFamide receptor expression. **B** ventral FLRFamide expression between apical and mantle lobe. (Same specimen as in C, with a focus on the ventral side.) **C** FLRFamide expression in apical lobe. **D** early larva with FLRFamide expression in apical lobe. **E** SEM picture of early larva. Scale bar = 30 μ m.

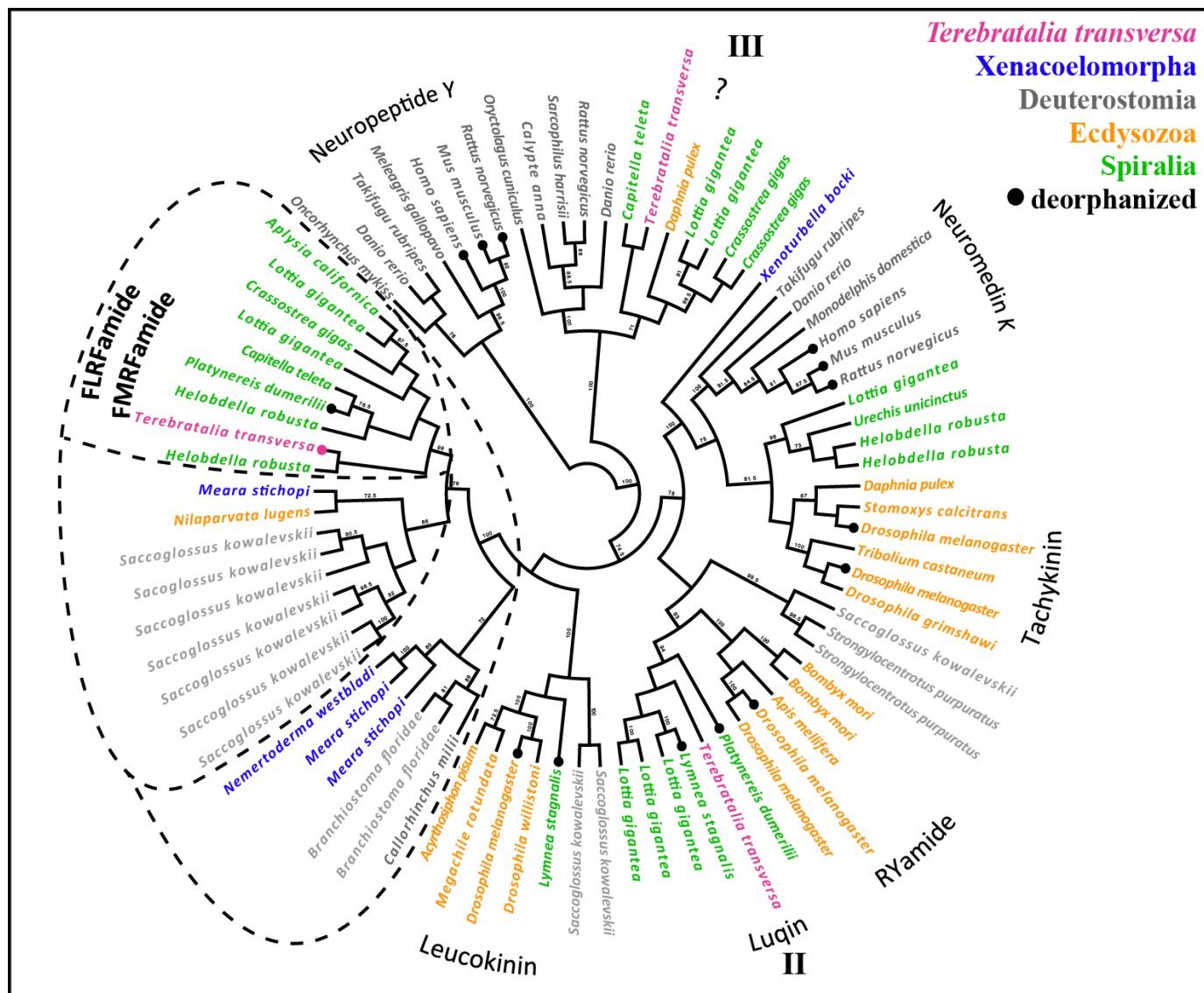


Figure S2: Phylogeny of the *T. transversa*-FLRFamide receptor. Cladogram of neuropeptide GPCRs that showed connections in the clustermap to the *T. transversa* FLRFamide receptor (Figure 3). The dashed lines indicate receptor groups related to the *T. transversa* FLRFamide receptor. Branches with filled circle at the end indicate a receptor that was deorphanized in a previous study.

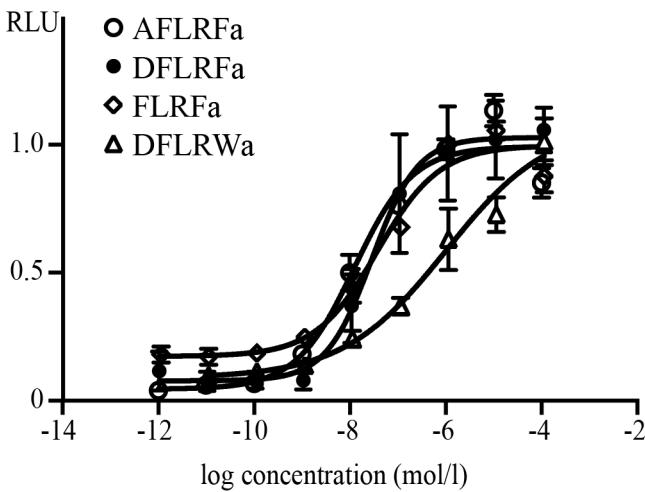


Figure S3: Combined dose-response curves of the *T. transversa* FLRFamide receptor to AFLRFamide, DFLRFamide, FLRFamide and DFLRWamide. RLU relative luminescence.

Terebratalia transversa FLRFamide prepropeptide sequence

MNRSVLLVAVVASFLLDHSGVGIYTSRYINPCSPWLKSTHFRKLCDESFWPSYGSQEKKDMIPYKRGFFGGGEYGEPEG
LSSFVDEPYRTGIGKRDFLRFGKRRYDDNERSDQLEALNRVTRDFLRFGKRDFLRFGRSYNLRGYDSDNKRKHMRKRR
SIKNESDKPSSIESNDVYNKGALLRSDKKMSAAKARDIKRAFLRFGRRLQKEYQQQDRKRAFLRFGKNTGINLDNRPT
SDTKTETNSETDNGSAKTVTNDVIDNNVDSKRAFLRFGKKCASKRAFLRFGKSEKNLISSSNKSMSSPLFGKEHT
KRAFLRFGKSVNRE*

Novocrania anomala YMRFamide prepropeptide sequence

MAKEAEMALLAAVLVIQLMVIPSGASQEKEAAKNMEFNQLRDDLSQLQQQLDDIKVLTAEVPEDKRFDTPTYLT
YLRFGRGIVDPYPYEHPSDAVDGFFDKPHYGFNKRHYLRFGRGIIDSNPHGYPNSALEGAMADGFEKKNKRYLRFGRS
VDRYVETPETHHLSKRSVKQKTPENGKTEISPPKKETIKDKRYMRFGKKSIEDEKRYMRFGKSDEDAQKRYMRFGK
KSDNNEEKRYMRFGKKSDDEKEKRYMRFGKKSDEPDE

signal peptide - cleavage site - C-terminal amidation site – predicted FLRFamide like neuropeptides

Terebratalia GPCR sequences:

Terebratalia transversa FLRFamide receptor (deorphanized) (R1)

MAAAKEFGIIRKRESYFKPVYIVNDKLLNTTTIRATTGLPLGHDTTMVTSMELNCTLPNCTNTTNDTTNGGSPPIPLADSAQA
LIITFYTLAIILAAFGNILAIIFSTGRRSRGDLRTYLLNLALADLAMSVCFCIPFTFPPTIYHYWQFGSAMCTIVLFLQTATVVVS
VSTNMAIGIDRFLAVTFPLRSRASRKQKVVKRIVVVIWCLSFLASPNSVVAQTVDLNGYYQCCTEKWPGGKQPKLIFGIFILIF
TYIIPLVILLTTYGIAIAMKLWRRQAPGEANRARDEAQQLQSKRKVKMLFTIVLLFGVCWLPLHTFINVLDNPPELAQNADQRILEI
IYICFHWLAMSNSFQNPIIYGFNDNFRADFWDLIFVCLPCCSKAKYFKNRRMSYTRSPANRWQQSSFHLLERRASARPLRNGST
SSSSDTKHNKFNSFARSKTDTALLVHEPSKGRTFIKRNGAKRSKQPMALVVPTVTYNSRDNDVTSREMEKLLEGIEPDTEKTN
SIK*

Terebratalia transversa orphan luquin GPCR homolog (R2)

FISMFSAITVLSITGNVLVCFAVLRNQTMRSSYFFILNLAVSDILMATMCIPFTFVANVLLDWWPGHVMCPLVNFLQAMAVFLSAFTLIVISL
DRYVAIIFPLKARLTTKQIKVVGIVWCCAVIPPIPIAVYGRVIRYVGRAYCQEWTDDNNNRFVYSLCILGLQFFVPLMVLYTYIRIGIVIWGK
KFPGEAEYNRDQRMATSKRKMVKMCCCCVVIMYIICWLPHYHCITIHGSIDDTFYDEEHAPTLWFAYWLAMSNSCCNPLIYFYMNSKFRRSFKKTF
CLLCCCKRISGRTCQESVKIKRTNTYATNTTKESSGSKNGTTVKTSCSSRQRTSGDELAMIDILPV*

Terebratalia transversa orphan neuropeptide GPCR (R3)

LRHLTPLLTLSLVGRAHFFILKSIIYYWSCRIMSKELLSYLERFNGDKNLTPMGNAADRTYSNIGIIIAYTVIIVISLFGNVLVCQVYRNKRL
QTVTNIFIVNLAIADILMSSLNIPFTIRTLTDEWVLGSFVCHVFYFIPMVSVYVSTFTLTLIAIDRHQVIVYPLRPKITKRYGVIVLGVITLA
ITLAAFAILAREADIHIVSREVWKCKMDYPHPSVLFHDSITLITISIQCYCLPFVIISIMYGRIAKRLWSRGPLGHHLTRAQELHHSKTKKKSRM
LVIVCIFGLCWPLNLHYILTDFTDKLTFRHNSKAFIACHWLAFSSVGYNPFPVYCWLNDAFRKEVKHILQCSRKDDSKIHPRGADKKQQPSL
TTRRTSSIRSTYISSLKVKKDIDIQKISPDYQGDIDMQDKISPDYQQLTAMLQQDVPMRDINQLTNANQYSERAYPKAMQRDSESISPDERSLI
EALRGAPHASEEDLDDIL*

Terebratalia transversa orphan neuropeptide GPCR, related to P. dumerilii NpY-4 and insect FMRFa receptor (R4)

MTCLRTMLENTSPGKLSMSREITNMTAGLTGVNQTYGNMSVCGHIPDPSTDIIIMFQFIAWGIIGSILVLGGCVGNILAIIVLNRHSMGTFSTYLSALAIFDTIILLCFLFSFSLPTIWNITWDSTYIDIIYPKMHLVIYPLTLISQQCTIYVTAFTIQRYCAINWPLKRNKCLSSRTQALIVITILILGSVIYNSPRMIEFTFYQCYSLQTNQVLQKIVPSEFGSDPTFRKVYHIYLFIISVIFMVPFLVLVIFNTLLWLAVRRSKKLQIQA
STVKENNITIMLIAIVVVFLICQILPIADNIFMVTSTATLNNNKYIKFTTISNLMA
LNSSINFILYCMFGQRFRQIFLNLFCCKELNINFEGRSRSIRWTRLSSFRSTLRDNKDGGNQPLRDNKDGGNQ

Terebratalia GPCR cloning primer:

[Non-gene specific adapters with restriction enzyme sites are given in brackets]

R1:

Forward: [ACAATAGAATTCCGCCACC] ATGGCAGCTGCAAAAGAGTTTC

Reverse: [ACAATAGCGGCCGC] TAATCCCAGACAGAATGCTACCC (includes partial 3' UTR)

R2:

Forward: [ACAATAGGATCCGCCCA] CCATGTTTCTGCAATCAC

Reverse: [ACAATAGCGGCCGC] GGCCTTGTTGAATTCTTGT (includes partial 3' UTR)

R3:

Forward: [ACAATAGAATTCCGCCACC] ATGTCAAAGGAACTACTGAGTTACTT

Reverse: [ACAATAGCGGCCGC] CCTGCACATCAGATAGATGA (includes partial 3' UTR)

R4:

Forward: [ACAATAGGATCCGCCACC] ATGATAGAGAATACATCACCTACGG

Reverse: [ACAATAGCGGCCGC] CTACACAAGTAATTACCTTGG

Accession numbers of neuropeptide receptor reference sequences

[Xbc.rna.tri.15475.1, *X.bocki*, in transcriptome SRX1343818] [Locus_45236.0_Transcript_1/0, *N.westbladi*, in transcriptome SRX1343819]
[Msti.rna.tri.15359.1, *M.stichopi*, in transcriptome SRX1343814] [Msti.rna.tri.31113.1, *M.stichopi*, in transcriptome SRX1343814]
[Msti.rna.tri.31092.1, *M.stichopi*, in transcriptome SRX1343814] [Locus_51813.1_Transcript_3/0, *H.spinulosa*, in transcriptome SRX1343820] [AKQ63075.1, *P. dumerilii*, Luqin receptor, deorphanized] [AKQ63063.1, *P.dumerilii*, FMRFamide receptor, deorphanized]
[O44426, *L. stagnalis*, Luqin receptor, deorphanized] [P92045, *L.stagnalis*, Lymnokinin receptor, deorphanized] [P49146, *H.sapiens*, Neuropeptide Y receptor type 2, deorphanized] [P97295, *M.musculus*, Neuropeptide Y receptor type 2, deorphanized] [Q9ERC0, *R.norvegicus*, Neuropeptide Y/peptide YY-Y2 receptor, deorphanized] [P29371, *H.sapiens*, Neuromedin-K receptor, deorphanized] [P47937, *M.musculus*, Neuromedin-K receptor, deorphanized] [P16177, *R.norvegicus*, Neuromedin-K receptor, deorphanized] [FBpp0076853, *D.melanogaster*, Leucokinin receptor, deorphanized] [FBpp0084470, *D.melanogaster*, RYamide receptor, deorphanized] [FBpp0081791, *D.melanogaster*, Tachykinin receptor 1, deorphanized] [FBpp0084873, *D.melanogaster*, Tachykinin receptor 2, deorphanized] [ELT88896, *C.teleta*] [XP_009016737, *H.robusta*] [XP_009054576, *L.gigantea*] [XP_009060043, *L.gigantea*] [XP_005090267, *A.californica*] [EKC27293, *C.gigas*] [XP_009027087, *H.robusta*] [XP_007899584, *C.mili*] [XP_009054574, *L.gigantea*] [BAO01094, *N.lugens*] [XP_002730513, *S.kowalevskii*] [XP_002596257, *B.floridae*] [XP_002734699, *S.kowalevskii*] [XP_002738788, *S.kowalevskii*] [XP_002731479, *S.kowalevskii*] [XP_002742045, *S.kowalevskii*] [XP_002596255, *B.floridae*] [NP_001161681, *S.kowalevskii*] [XP_002732003, *S.kowalevskii*] [XP_006812800, *S.kowalevskii*] [XP_009060304, *L.gigantea*] [XP_003700723, *M.rotundata*] [XP_002732001, *S.kowalevskii*] [NP_001161604, *S.kowalevskii*] [NP_001098693.1, *T.rubripes*] [XP_001342488.2, *D.rerio*] [XP_009064591.1, *L.gigantea*] [XP_009067028.1, *L.gigantea*] [XP_009050865.1, *L.gigantea*] [XP_009064514.1, *L.gigantea*] [ELT99672.1, *C.teleta*] [XP_009017792.1, *H.robusta*] [XP_009017796.1, *H.robusta*] [XP_009062052.1, *L.gigantea*] [XP_008498708, *C.anna*] [Q1ACB1, *O.mykiss*] [F1R5V3_DANRE, *D.rerio*] [E9HAW0_DAPPU, *D.pulex*] [G3X054_SARHA, *S.harrisii*] [G1NS97_MELGA, *M.gallopavo*] [J9JKR1_ACYPI, *A.pisum*] [B3XXN5_BOMMO, *B.mori*] [B4MM03_DROWI, *D.willistoni*] [K1PQW2_CRAGI, *C.gigas*] [B3XXN2_BOMMO, *B.mori*] [H3ILX9_STRPU, *S.purpuratus*] [H3ILY0_STRPU, *S.purpuratus*] [H9K8U7_APIME, *A.mellifera*] [Q8VHD7_RAT, *R.norvegicus*] [G1TPU6_RABIT, *O.cuniculus*] [K1Q8V2_CRAGI, *C.gigas*] [D6WD17_TRICA, *T.castaneum*] [Q6AWE5_DROME, *D.melanogaster*] [I4IY86_TAKRU, *T.rubripes*] [F1R3V0_DANRE, *D.rerio*] [F7E6B1_MONDO, *M.domestica*] [B4JUW2_DROGR, *D.grimshawi*] [Q94736_STOCA, *S.calcitrans*] [E9FUQ7_DAPPU, *D.pulex*] [Q8T8D1_UREUN, *U.unicinctus*]