

Supplementary Figure 1. Synteny oxford dot plots between *T. maipoensis* and 9 other taxa.

Supplementary Figure 2. Pairwise comparison of macrosynteny mixing rate between 9 cnidarian genomes and 1 ctenophore genome.

Supplementary Figure 3. Maximum-likelihood tree of UDP-glucuronosyltransferase (Pfam00201).

Supplementary Figure 4. Maximum-likelihood tree of cnidopsins and other opsin genes.

Supplementary Figure 5. Sequence percentage similarity matrix of cnidopsins.

Supplementary Figure 6. Sequence alignment of cnidopsin group 1a.

Supplementary Figure 7. Sequence alignment of cnidopsin group 1b.

Supplementary Figure 8. Sequence alignment of cnidopsin group 2 and 3.

Supplementary Figure 9. Maximum-likelihood tree of jellyfish toxins (JFT).

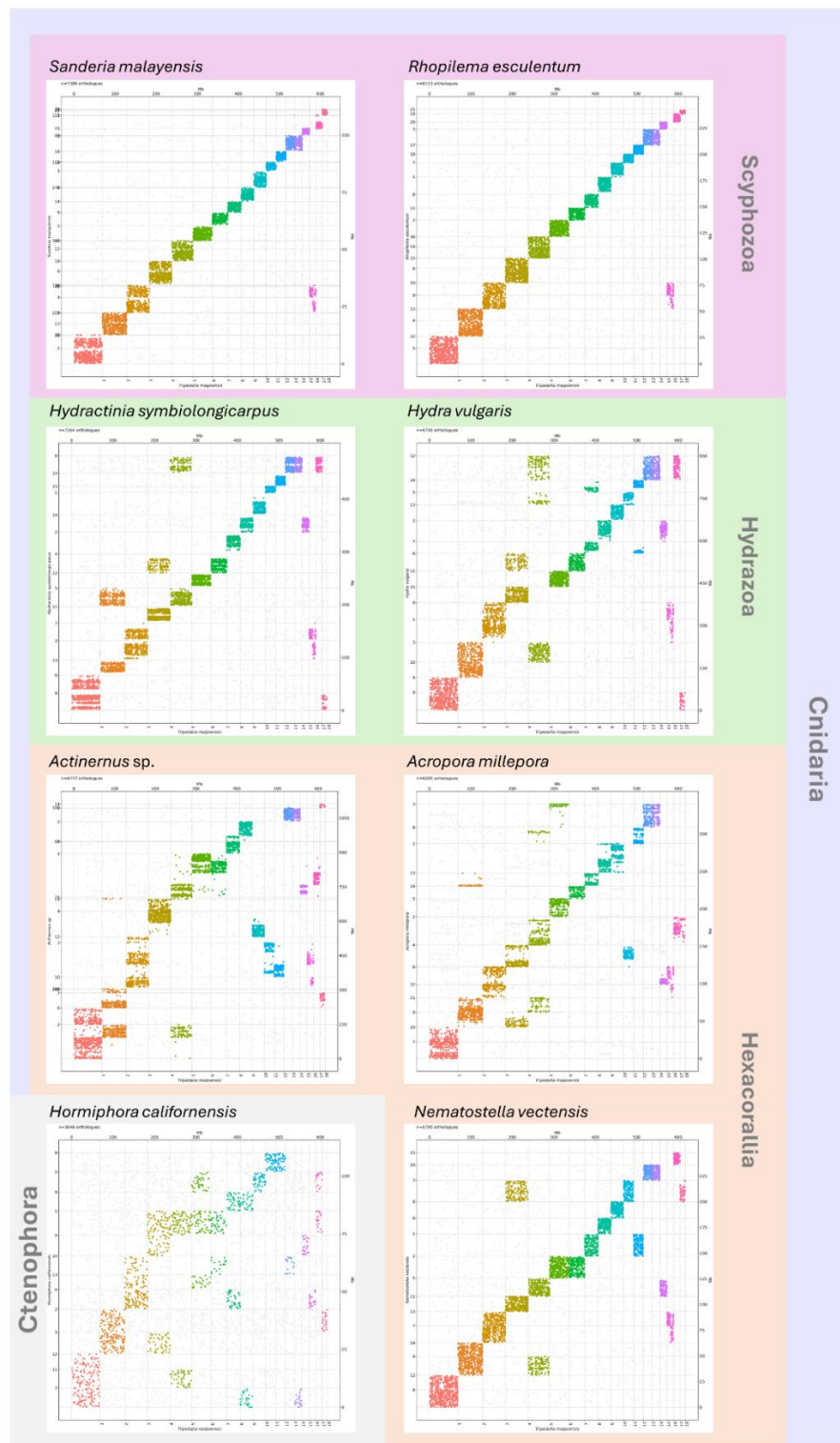
Supplementary Figure 10. Neuropeptides identified in *T. maipoensis*.

Supplementary Figure 11. Sequence alignments of miR-100, miR-2022, miR-2030 and miR-2036.

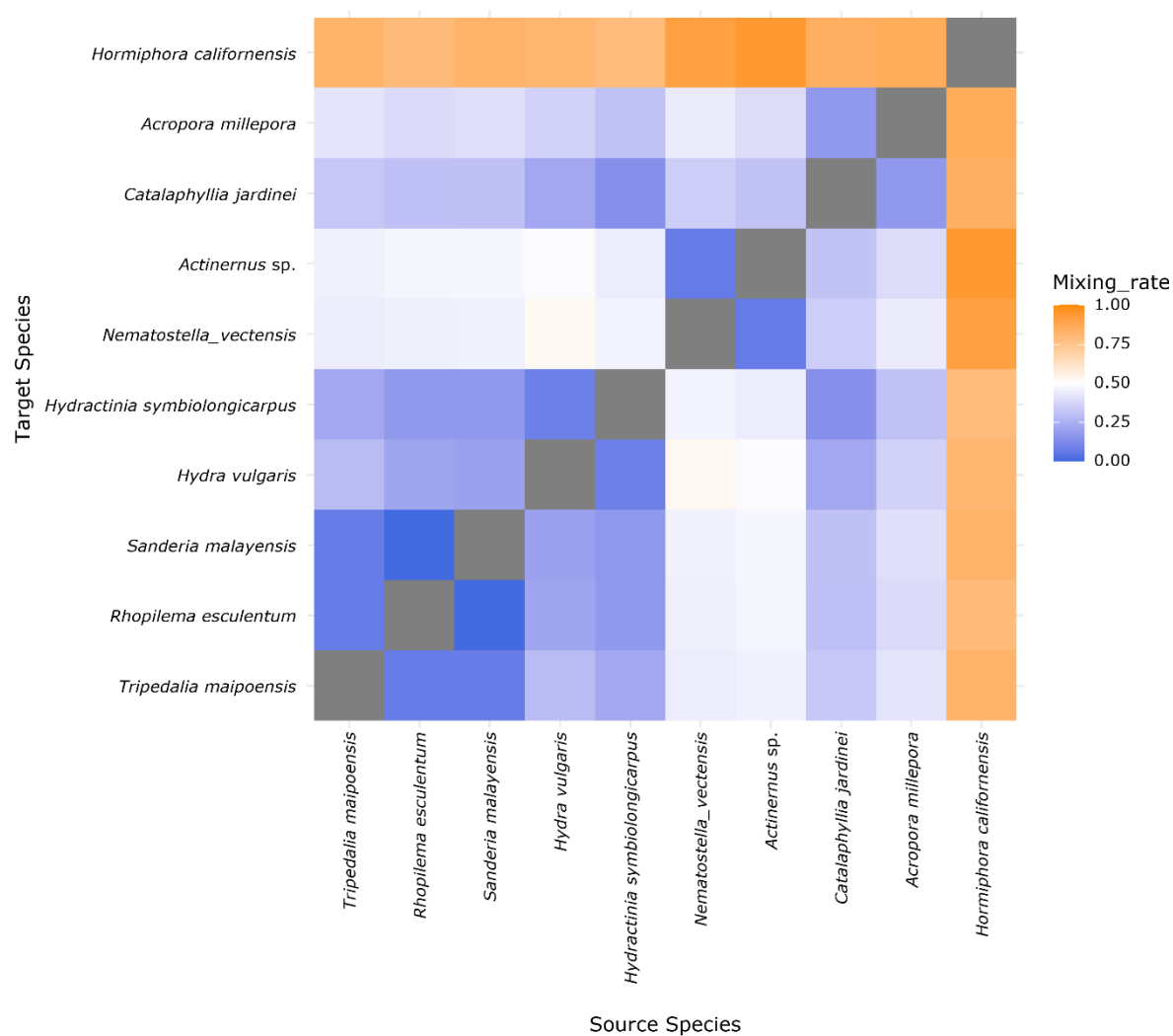
Supplementary Figure 12. Sequence alignments of conserved novel microRNAs in cubozoan, scyphozoans and anthozoans (mir-*CCI* to mir-*CC9*).

Supplementary Figure 13. Sequence alignments of conserved novel microRNAs in scyphozoans and cubozoan (mir-*MCI* to mir-*MC7*).

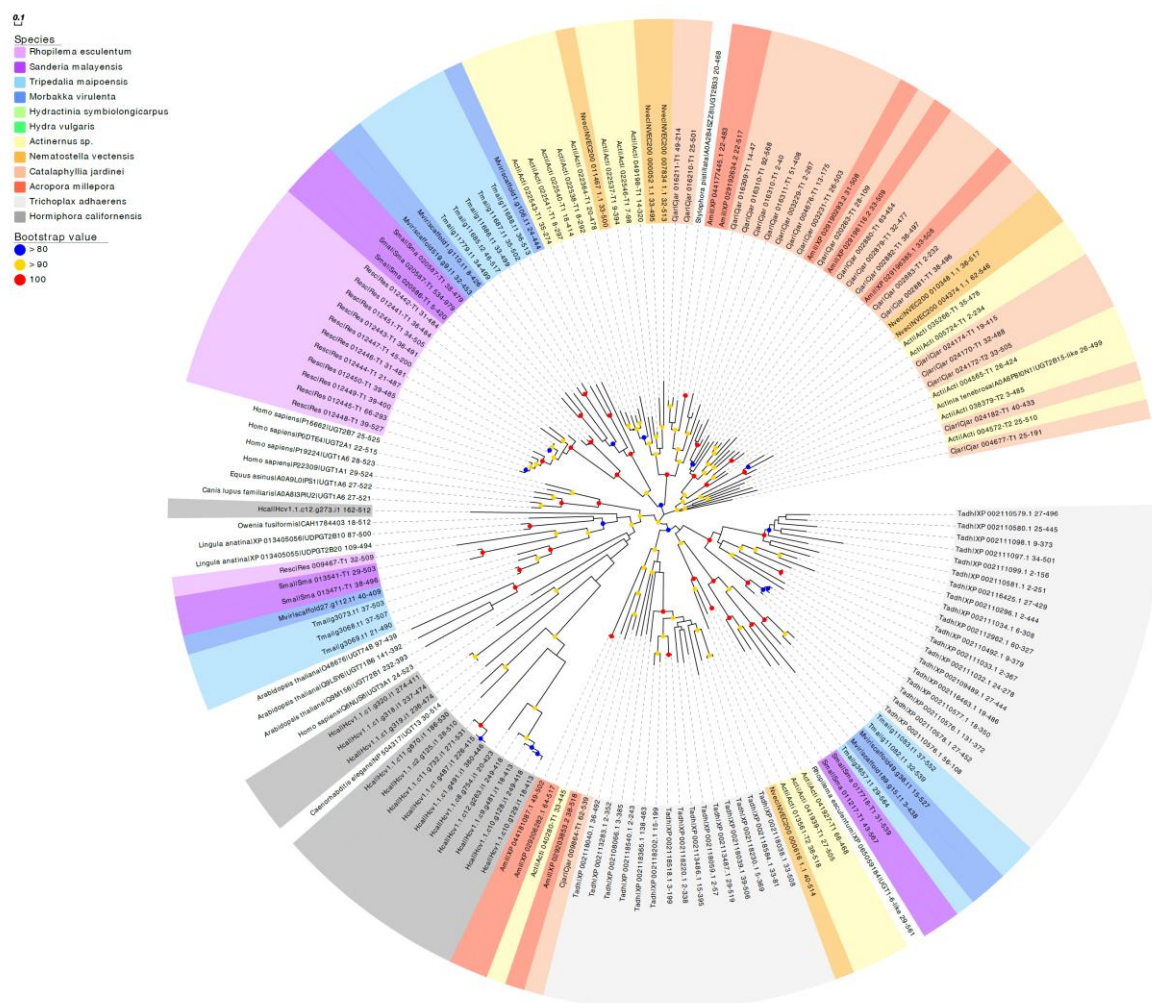
Supplementary Figure 14. Sequence alignments of conserved novel microRNAs in scyphozoans (mir-*SCI* to mir-*SC9*).



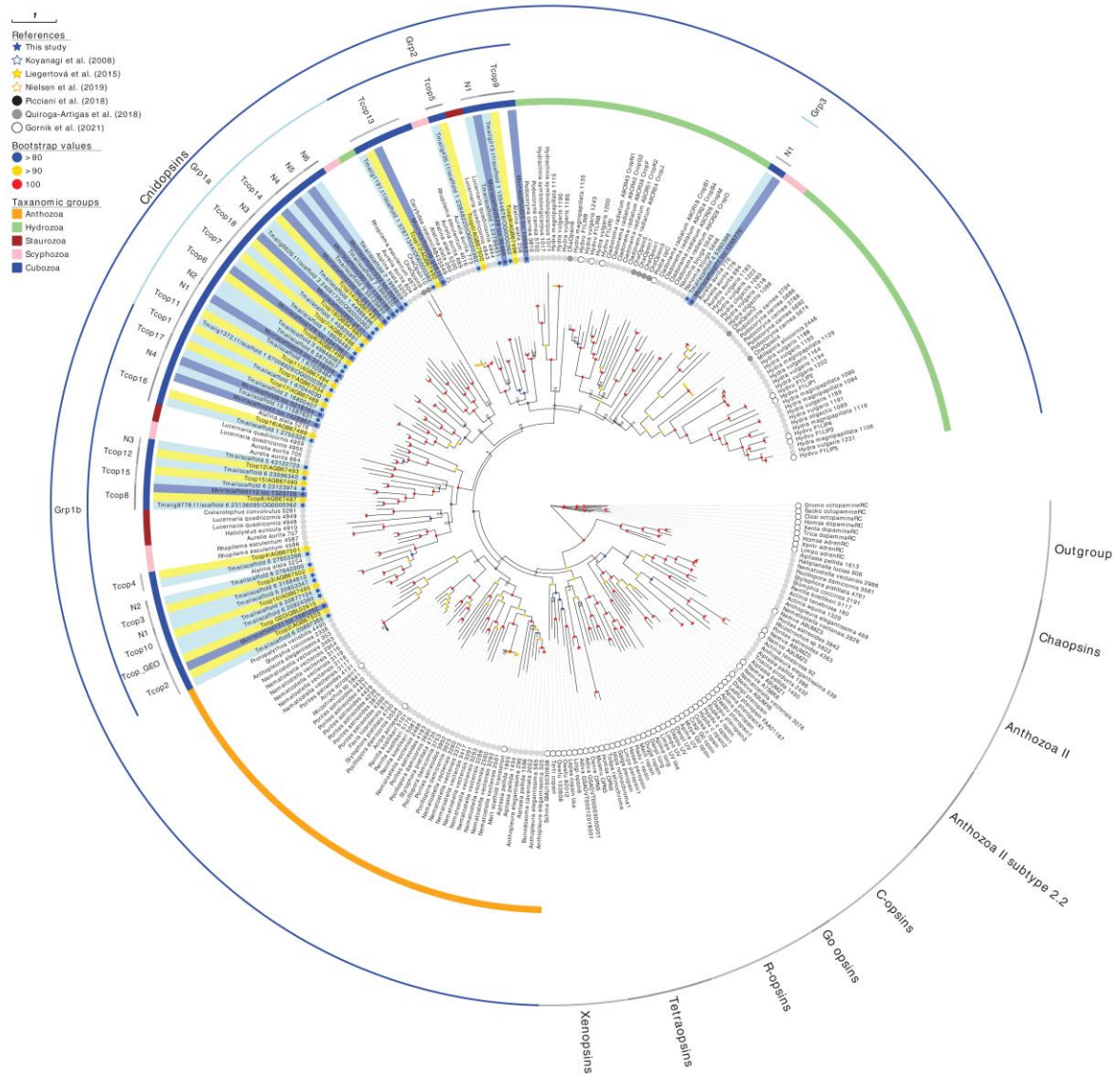
Supplementary Figure 1. Synteny Oxford dot plots between *T. maipoensis* (x-axis) and 7 other taxa (y-axis), namely *Sanderia malayensis*, *Rhopilema esculentum*, *Hydractinia symbiolongicarpus*, *Hydra vulgaris*, *Nematostella vectensis*, *Actinernus sp.*, *Acropora millepora*, *Nematostella vectensis* and *Hormiphora californensis*.



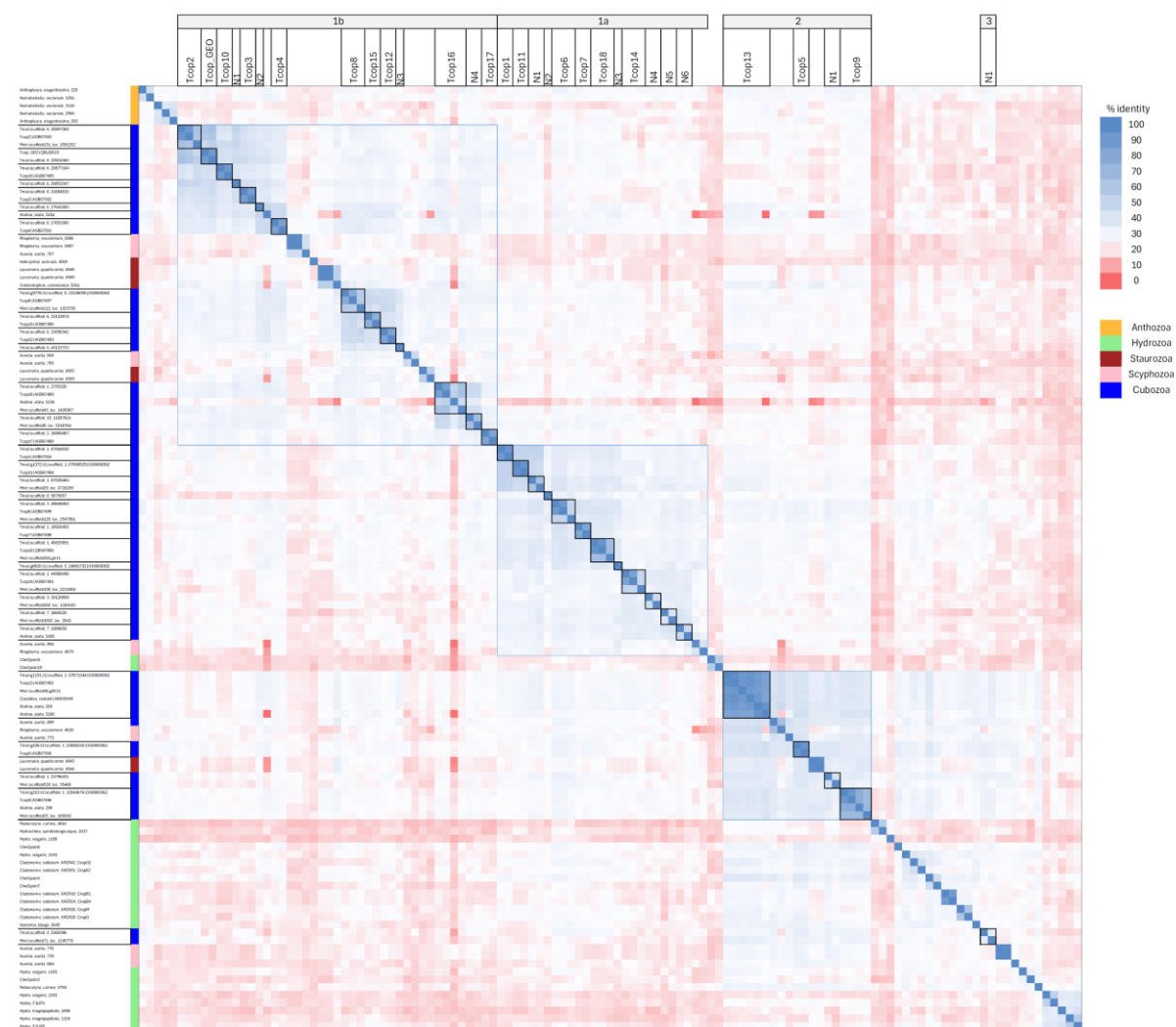
Supplementary Figure 2. Pairwise comparison of macrosynteny mixing rates between 9 cnidarian genomes and 1 ctenophore genome.



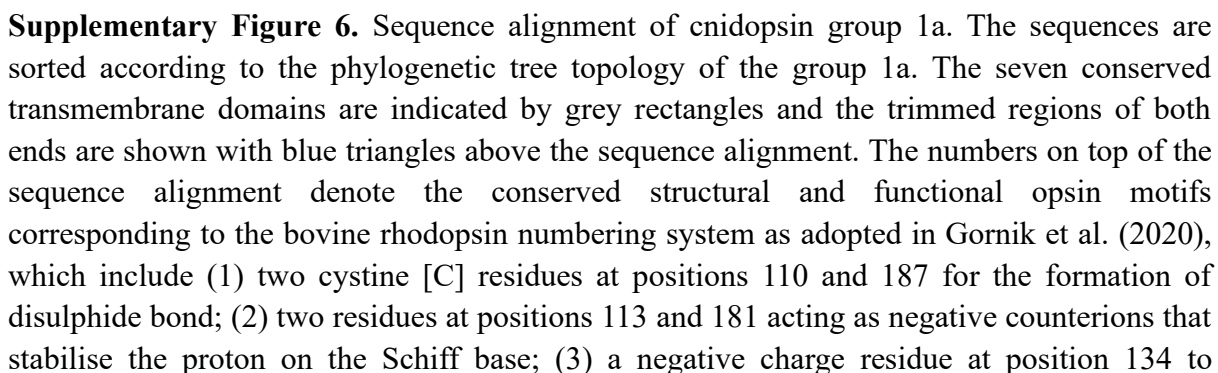
Supplementary Figure 3. Maximum-likelihood tree of UDP-glucuronosyltransferase (Pfam00201) constructed from domain sequences with 1,000 bootstraps.



Supplementary Figure 4. Maximum-likelihood tree of cnidopsins and other opsin genes constructed with 1,000 bootstraps. Cnidopsins identified from *Tripedalia maipoensis* and *Morbakka virulenta* are highlighted in lightblue and blue, respectively, while the reference sequences from *Tripedalia cystophora* are highlighted in yellow. The outermost annotated ring describes the grouping of cnidopsins and other G protein-coupled receptors, followed by an inner and innermost annotated ring of the grouping and gene orthology of *Tripedalia cystophora* cnidopsins as described in Liegerová et al. (2015). For outgroup, octopamine receptors, dopamine receptors and adrenergic receptors were used as described in Gornik et al. (2021).



Supplementary Figure 5. Sequence percentage similarity matrix of cnidopsins. The sequences are sorted according to the phylogenetic tree topology in Supplementary Figure 4. The sequence names are displayed along the y-axis whereas the cnidopsin orthology and grouping are indicated along the x-axis.



stabilise the inactive opsin molecule, as part of the conserved motif of “E/DRY” (positions 134-136) in rhodopsin; (4) a conserved lysine [K] residue at position 296 for a covalent linkage with the 11-cis retinal chromophore via a Schiff base; (5) a conserved NPxxY motif from position 302 to 306 and an NKQ motif (310–312) in rhodopsin that involve in maintaining the structural integrity upon photopigment activation. Cubozoan cnidopsins that are orthologous to the 18 cnidopsins in *Tripedalia cystophora* (Tcop1-18) described by Liegertová et al. (2015) are labelled on the right panel.

[illegible]

Supplementary Figure 7. Sequence alignment of cnidopsin group 1b. The sequences are sorted according to the phylogenetic tree topology of the group 1b. The seven conserved transmembrane domains are indicated by grey rectangles and the trimmed regions of both ends are shown with blue triangles above the sequence alignment. The numbers on top of the sequence alignment denote the conserved structural and functional opsin motifs corresponding to the bovine rhodopsin numbering system as adopted in Gornik et al. (2020), which include (1) two cystine [C] residues at positions 110 and 187 for the formation of disulphide bond; (2) two residues at positions 113 and 181 acting as negative counterions that stabilise the proton on the Schiff base; (3) a negative charge residue at position 134 to stabilise the inactive opsin molecule, as part of the conserved motif of “E/DRY” (positions 134-136) in rhodopsin; (4) a conserved lysine [K] residue at position 296 for a covalent linkage with the 11-cis retinal chromophore via a Schiff base; (5) a conserved NPxxY motif from position 302 to 306 and an NKQ motif (310–312) in rhodopsin that involve in maintaining the structural integrity upon photopigment activation. Cubozoan cnidopsins that are orthologous to the 18 cnidopsins in *Tripedalia cystophora* (Tcop1-18) described by Liegertová et al. (2015) are labelled on the right panel.



Supplementary Figure 8. Sequence alignment of cnidopsin group 2 and 3. The sequences are sorted according to the phylogenetic tree topology of the group 2 and 3. The seven conserved transmembrane domains are indicated by grey rectangles and the trimmed regions of both ends are shown with blue triangles above the sequence alignment. The numbers on top of the sequence alignment denote the conserved structural and functional opsin motifs corresponding to the bovine rhodopsin numbering system as adopted in Gornik et al. (2020), which include (1) two cystine [C] residues at positions 110 and 187 for the formation of disulphide bond; (2) two residues at positions 113 and 181 acting as negative counterions that stabilise the proton on the Schiff base; (3) a negative charge residue at position 134 to stabilise the inactive opsin molecule, as part of the conserved motif of “E/DRY” (positions 134-136) in rhodopsin; (4) a conserved lysine [K] residue at position 296 for a covalent linkage with the 11-cis retinal chromophore via a Schiff base; (5) a conserved NPxxY motif from position 302 to 306 and an NKQ motif (310–312) in rhodopsin that involve in maintaining the structural integrity upon photopigment activation. Cubozoan cnidopsins that are orthologous to the 18 cnidopsins in *Tripedalia cystophora* (Tcop1-18) described by Liegertová et al. (2015) are labelled on the right panel.

Predicted signal sequence and cleavage sites

>g9373.t1_FRamide

MNKPSMVLRALWFTFFAVTLTCKVATA DAEESTKSETYENWTTDEEIENQVPVSQNTPCQGEVCLYGNRQLARRK
LLKRKTVCQGCGCLLNPEAKSIDHELDAAEMDTYGIQYNQLPRDSSCQGQMCWFRGKREKFTASNIQERLLAQLSF
LRELKKEMNVNNGKRAQGELGCTGQMCWFRGKKEYDESQDQSSCVGQMCWFRGKRTLNTKGSKSTGQMC
WFRGKRNMILTGDGALKKELNEQKRCTGQMCWFRGGSLDEKGVGDGAARQKGKR SKKEDMCTGQMCWFRG
GRSLDEKGVNGVAANQNGKRTQKETMCTGQMCWFRGRKELQAHQMGCKGQMCWFRGKR NAKHEIKN

>g5906.t1_VWamide

MMKAPSYKIGLVVLMQLIVSLHVT CATDMNEESATSPVKRARSEEEVQEDAVVKALERGIFAR R DVISQPPGVWG
RSSERSFRARAFNRDRHLFRPQSPAERRALQDSIEHLLQAKEALDVLNKRASQPPGVWGKRVAAPPGVWGKRTV
RTQPPGVWGKR TVRTQPPGVWGKR RALEFMEREYALGQVESKDEDTIAARSASVSQPPGVWGRRSISERSHSEEQ

>g1334.t1_RFamide

MELNTKALVLAFLAIAIVCATA ADTKLAQQSRRQEEEDSQLFLSEEDDLVDERSVNAKRIIEQWLRGRFGKR DLRGR
FGREADSGDTVTENKARETLSQWLRGRFGR REASDQWLRGRFGR REATAQWLRGRFGR REAMDQWLRGRFGR REVA
DQWLRGRFGR REVNSQWLRGRFGR REVATQWLNGRFGR REVADQWLRGRFGRQVEEDIEDELFDRELDQWLRGRF
GRGTEQWLNGRFGR RESNEQWLRGRFGR REMDQWLRGRFGR REAEQWLRGRFGRREMEQWLRGRFGR REVEQWL
RGRFGR REAEQWLRGRFGRRESNESEDKVEQWLRGRFGRNLEQGH LGVENSQGNMGEPNVARAVKTEDRSATVGV
KDQRQDSSQVEAELSGQGISDKRNL

>g9177.t1_LWamide

MWASYAAPFLGLIFMLAIASA APREDTEDQSASKRDASDKEQMGYKANTVRETLIKGYPNRPSVLWGRDVAKD
NLKNTEISEGLQPGMWGKR RLSQPRLNMLWGR RAVVENPNMGLWGREGKEMFER RPKVGLWGR RSSSPGKLGLWGR
QLQESGYQPRD RPLGGLWGKEIRARDVENNQSSDELERELENKIISLFSQLKSAKER REATEKRKPGVVGIWGK

>g9270.t1_RAamide

MSGIYFFLLLGIGTYFLCQSFA KGECNTDSTEYAYKRSDCLEDLLGLEPTEFADFLEEQPRSGKRELSVQDYRPRAGREG
DQDYRPRAGRQTLTRPRGGREYSARPRAGREYTIKISSEMQGFRPRAGRESAARPRAGRENIQRPRAGRESLNR
PRAGRENLERPRAGRENLERPRAGREYGRPRAGREYGRPRAGREYGRPRAGREYGRPRAGRQMLGRPRAG
REFLERPRAGRNVLVLNDGDNEGSNKRFSYIRSKVHAESQQDVSRTQEEKGGELLDERESDHVVDAMDEEAAN

FMNENQPRSGKRELSGKAALALRLKREGAIRSAMGKISGSTFAASSALEMNNQPRSGKRSILLGKHGIGSLQAG
KKDSTDMKRGLNFPVEEDGQQLVFLMANKRENKKNDISKELEKLAGSLSKRRREV

>g10227.t1_RY-precursor

MAKRLIFSAFLAIFICLVEAKAWKRESNHHFAKGSKDPQWPRYKSESNHDPQWPRYKSELEHKDQWPRYKKSQ
ERNSPQWPRYKSEALQWPRYKKEIFASEQWPRYKKEVKKHSPQWPRYKSELKSPQWPRYKKEASKSAHWPR
YKSEAIRPAQWPRYKKEASKSAHWPRYKSEASRPAQWPRYKKEAESEAQWPRYK

>g1341.t1_RYamide

MVKIYIFALFCVAYVQSAVISTYIVRDVNPRDLHNHDMIFTDDSSSQGVKKREATLMKANTNTWNRMAFKHQER
RDTTPWVKGRYRGVVFHQNLRLQREGAVKVPREDYEKKVMAFLNNRLAREADKFHGQLYARQMWHHQRYGR
EGKNGRNQGAPISPFVDEEKRTDEEKNILKGSFDKRRRAAPGWHHGGRYGRVYGYQQGSMKRETPVWAKGRYG
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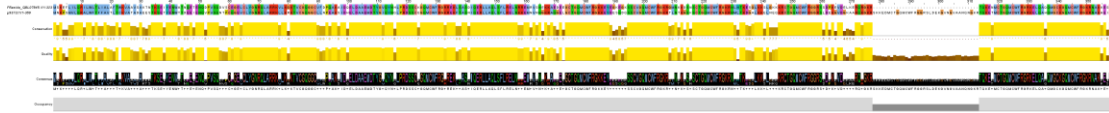
>g3266.t1_RRFamide

MSAIKLYQSQDLFQSIRCLHLKMNQQTQAFLLVTLQIVFQNGCSLENSQLEEVSYRVSTDANADFLLCRDMFITS
VKRLAHSRMRDEWTVENSQYQKKRRGRFGKRTLGNNGNWQESQHFHDNSGKLEKIARQIIAFCQFKILDSDERE
LDAFKRDRHDFRHDEAEKKSSLSQSSRSSEKFLEGDDPIWLPLATAMMTENDRELGAYNIQESKEEDDAYDMID
QHAAFDMAKKETAGMKPNRRRFGKKEVISAGSNTKPTRRRFGKEVSRGSTNLSQRSTNEEKSTREAVIGETSLPQE
KPRKRISKASKTDYQRRRFGKREKNESYRMFTNVPHNRRFGKREDSENYLMVADLPQNRRFGKREDGDEYLGNE
YYGKILGDNRKEQD

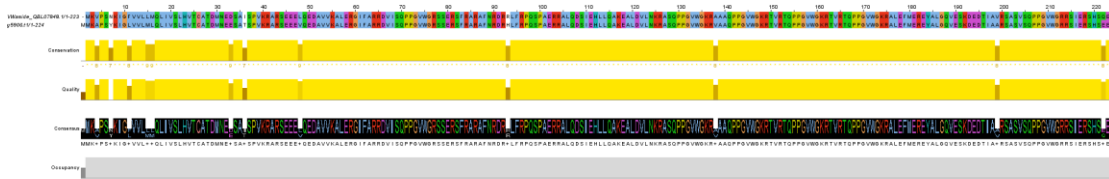
Annotations: Signal sequence Cleavage site

Sequence alignments of identified neuropeptide genes

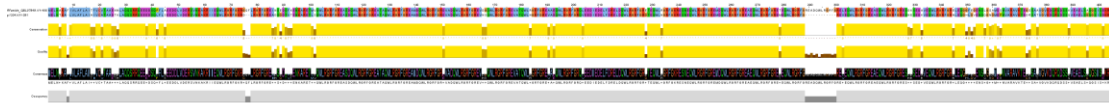
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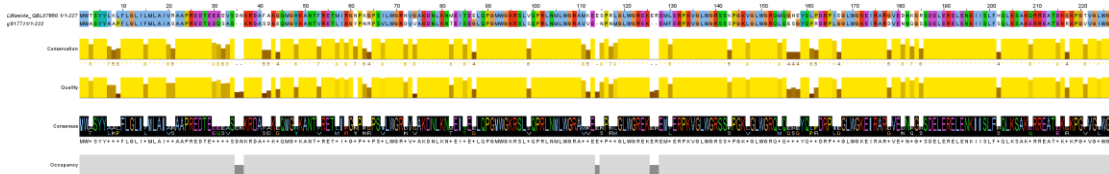
VWamide_g5906.t1



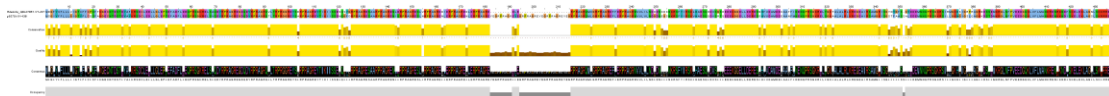
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LWamide_g9177.t1



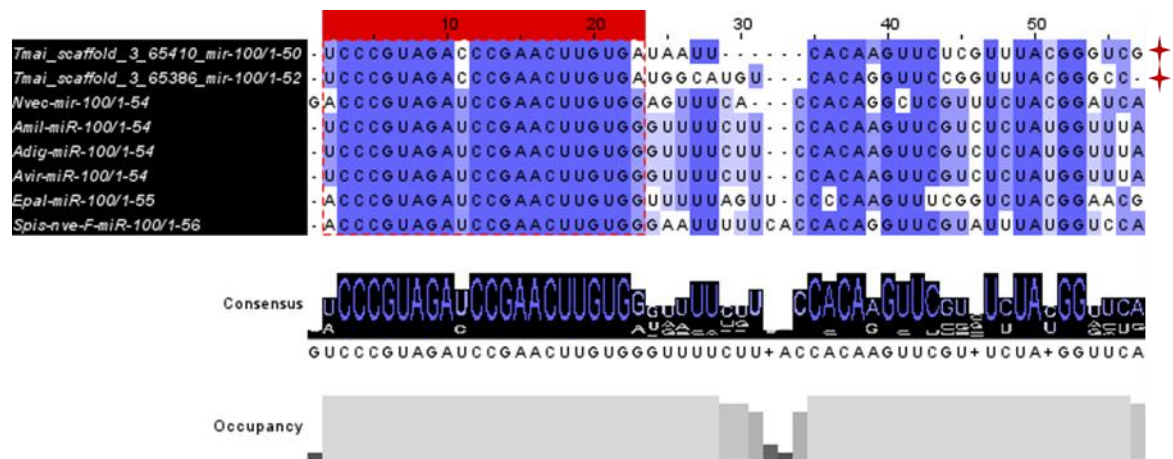
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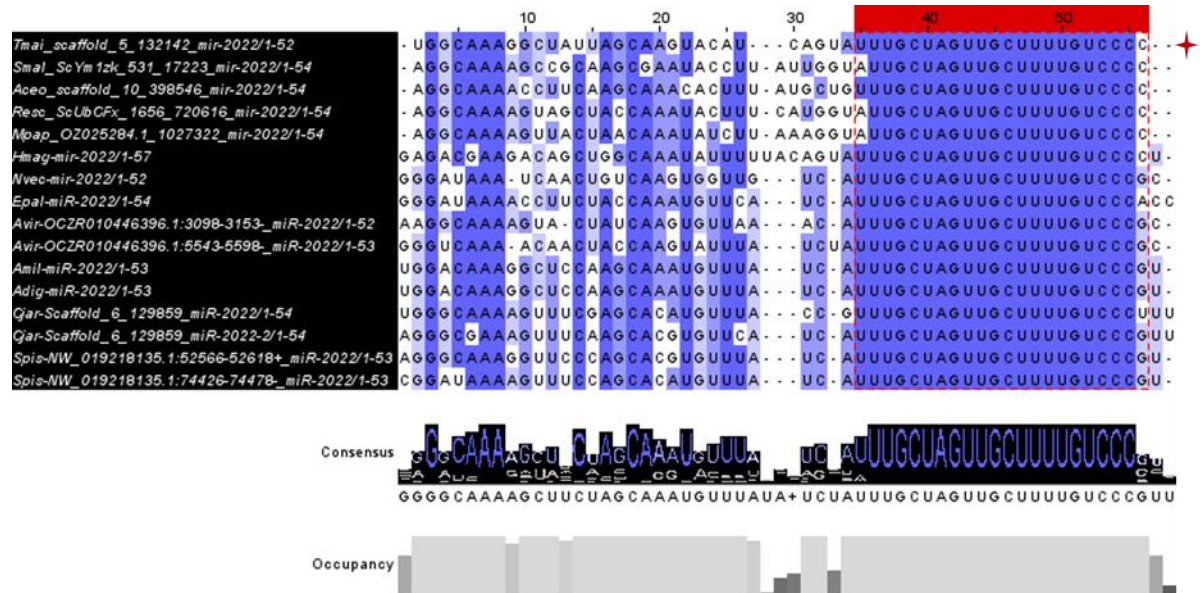
[illegible]

Genomic tracks for chromosome 10p11.21. The top track shows the gene structure of PLEKHA7 with exons numbered 1 to 12. Below are tracks for Coverage, RefSeq, and ClinVar. The bottom track shows the genomic context with a scale from 10,000,000 to 10,000,000 bp.

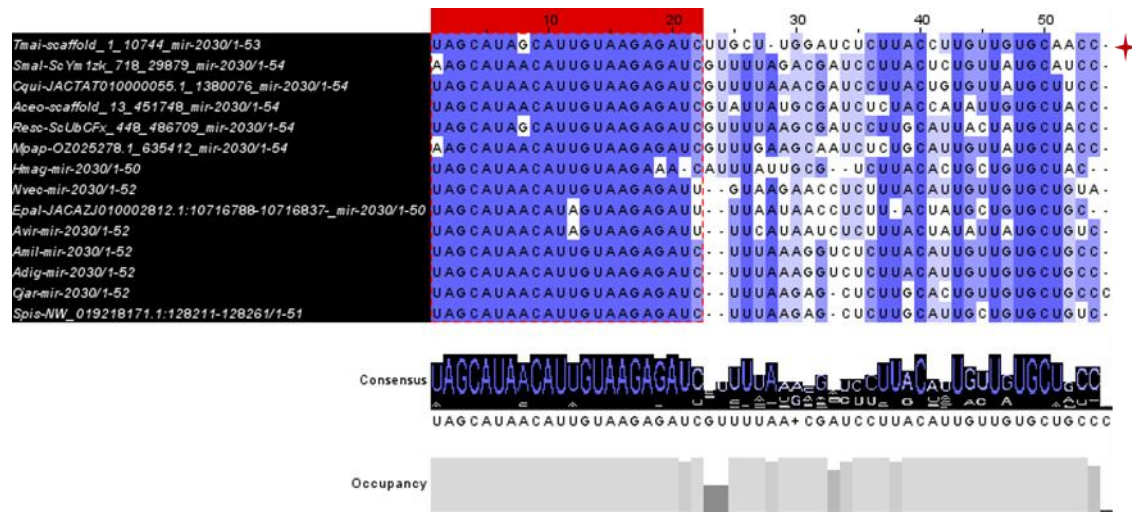
miR-100:



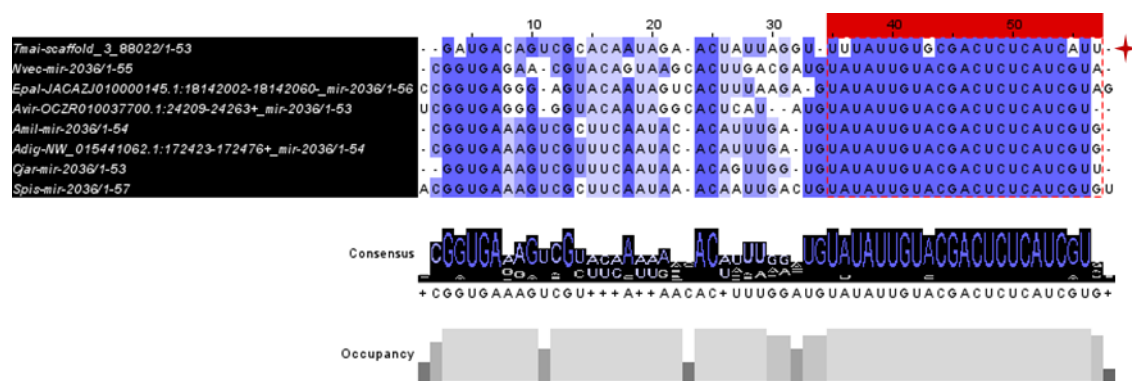
miR-2022:



miR-2030:



miR-2036:

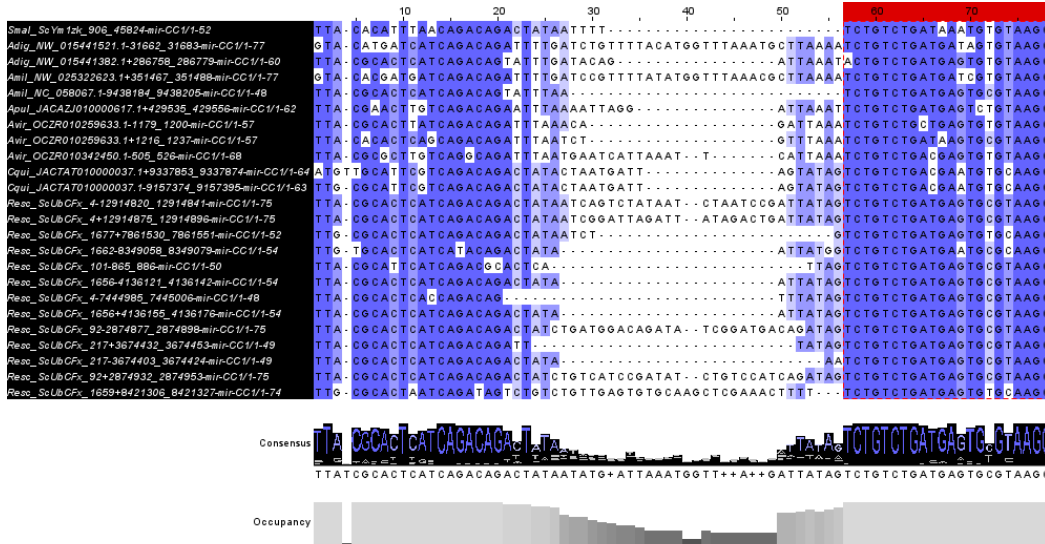


Supplementary Figure 11. Sequence alignment of miR-100, miR-2022, miR-2030 and miR-2036.

mir-CC1:



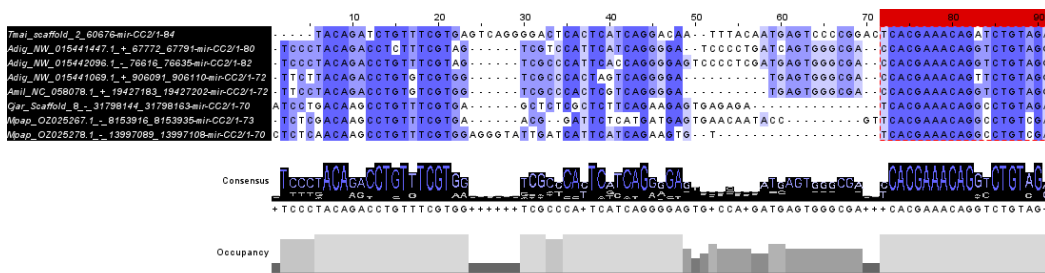
Ref: *Smal_ScYm1zk_906_45824*



mir-CC2:



Ref: *Tmai_scaffold_2_60676*

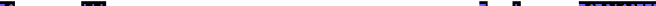


[illegible]

Consensus TGGCCAAACGATAAAATGTTGGTTACCCCAACATGTTGGGTCGTCCAACAATTATCGTTGGCCGGG

Occupancy

Position	10	20	30	40	50	60	70	80	90	
<i>Cytl_JACTA7010000224_1_436035min-CC4/1-88</i>	---	T	C	A	G	T	-	-	-	-
<i>Cytl_JACTA7010000224_1_436035min-CC4/1-88</i>	---	T	C	A	G	T	-	-	-	-
<i>Adig_NW_01541256_1_301713_301733min-CC4/1-82</i>	---	T	C	A	G	T	-	-	-	-
<i>Anti_NC_058089_1_140495B3_1404963min-CC4/1-82</i>	---	T	C	A	G	T	-	-	-	-
<i>Resn_SaLbGFX_4_3792342_3792362min-CC4/1-54</i>	---	T	C	A	G	T	-	-	-	-
<i>Resn_SaLbGFX_795_7612466_7612466min-CC4/1-60</i>	---	T	C	A	G	T	-	-	-	-

Consensus 
C+ C+ TCAGAG CAAAAACG TCAGAA+ AAA+ C9AAACAAT CCACTAGACTTA GTCATT TGTTCGGAATCTTTT TCAGAG TTTT GCTG+ TGAAG

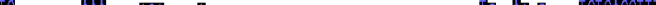
Occupancy 



Figure 2 displays the consensus sequence and occupancy of the 60 nt seed region. The top panel shows sequence logos for 10 nt windows (10-19, 20-29, 30-39, 40-49, 50-59, 60-69) for various miRNAs. The bottom panel shows the consensus sequence (TCCGACGGTAAATTTTCGGACACTACTTTTGTTCGAAATTTAAATCGTTCGAA) and the occupancy of each nucleotide position (0.0 to 1.0).



Map: 02025277_1_822295min-CCG1-462
 Aliq_WW_01544134_1_218767_218807min-CCG1-187
 Acco_paf006_50_+122415_122435min-CCG1-463

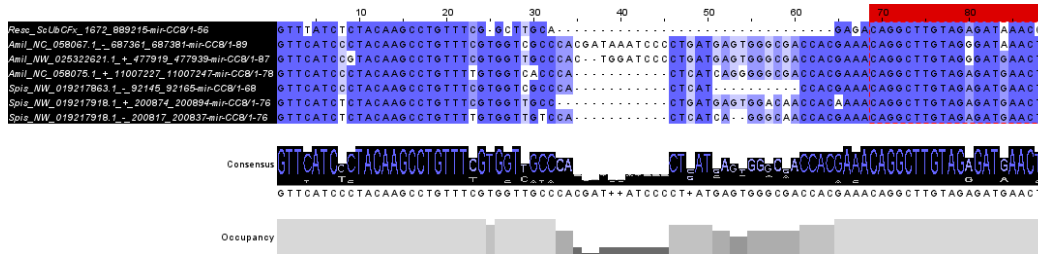
Consensus

Occupancy

mir-CC8:



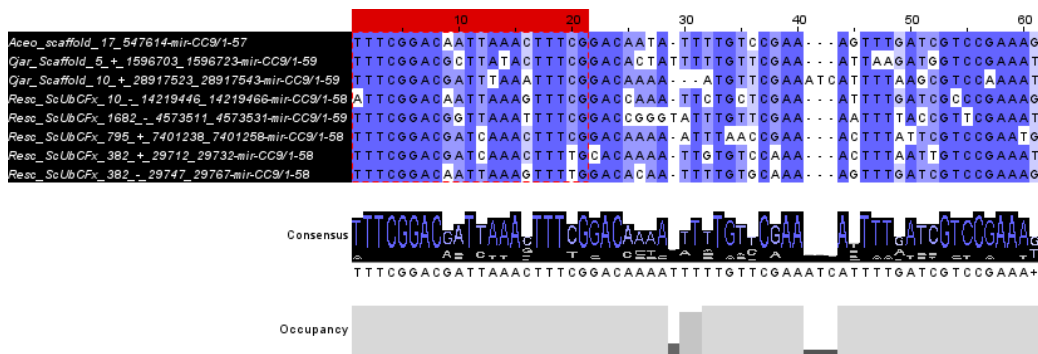
Ref: *Resc_ScUbCFx_1672_889215*



mir-CC9:



Ref: *Acoe_scaffold_17_547614*



Supplementary Figure 12. Sequence alignments of conserved novel microRNAs in cubozoan, scyphozoans and anthozoans (mir-CC1 to mir-CC9).



Figure 2 displays the phylogenetic conservation of the 5' region of the 155 nt miR-1-55 mature sequence. The figure is organized into three main horizontal sections: sequence alignment, consensus, and occupancy.

Sequence Alignment: The top section shows the 5' region of the mature miR-1-55 sequence for five species, aligned to a reference sequence. The reference sequence is shown in black text on a black background. The species and their corresponding sequences are:

- Tma_jacfold_9_212333-mir-MC/1-1-51*: TGCAC TGATCTCTAAATAAAGACTGAGATCTTTATTTAGAAATCAGTGAAT
- Cqui_JACFAT010000032.1-55255355525556-mir-MC/1-1-54*: AGCACTGATCTCTAAAAAAGAACTTTTTCGCAAAAACTTTTATTTAGAGTTCAAGTGATCG
- Cqui_JACFAT010000036.1-28662692866290-mir-MC/1-1-58*: TGCAC TGATCTCTGAAAAAAGAACTTTCTTTAGAGATCTTTTATTTAGAGATCAGTGGATCG
- Mhap_OZ025267.1+44631394463160-mir-MC/1-1-55*: AGCACTGATCTCTAAAAAAGAACTTTCTTTAG...CTTTTATTTAGAGATCAGTGAAGC
- Sma1_ScYm12k_24-45906604590681-mir-MC/1-1-59*: TGCAC TGATCTCTAAATAAAGACTGAGATTGAGCTTCTATTATTTGAGATCAGTCTGTA
- Sma2_ScYm12k_435+1082310844-mir-MC/1-1-56*: TGCAC TGATCTCTAAATAAAGACTGAGATTGAGCTTCTATTATTTGAGATCAGTCTG...

Consensus: The middle section shows the consensus sequence, which is a representation of the most common nucleotide at each position across the aligned sequences. The consensus sequence is shown in black text on a black background, with some positions highlighted in blue and red. The consensus sequence is: TGCAC TGATCTCTAAATAAAGACTGAGATTGAGCTTCTATTATTTGAGATCAGTCTG...

Occupancy: The bottom section shows the occupancy of each nucleotide across the species. The occupancy is represented by a bar chart where the height of each bar indicates the number of species that have a specific nucleotide at that position. The occupancy is shown in black text on a black background.

Figure 1 displays the consensus sequence and occupancy of miR-124-3p binding sites. The top panel shows the consensus sequence: CAAA-C-CT-GA-C-T-T-C-C-GA-CA-C-G-C-A-A-T-T-G-T-A-G-T-G-A-G-T-G-A-A-A-A-G-T-C-G-A-G-C-A-T-T. The bottom panel shows the occupancy of the binding sites, with a scale from 0 to 100. The occupancy is high for the first 10 positions and decreases for the remaining positions.

seqFold_6_151844mir-NC31-187
 RefSeq_CPX_212_556488.656488mir-NC31-187
 seqFold_7_2151802_2151831mir-NC31-55

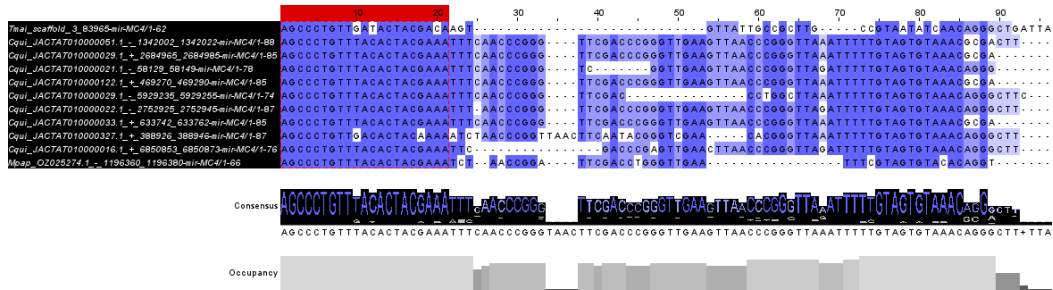
Consensus
 TGAACAC...GCTCTTCAGATTCATGAAAG...TGTGCGCTATTTTCATTCTGATGACTGCAAGGACTGTTTCAGT

Occupancy

mir-MC4:



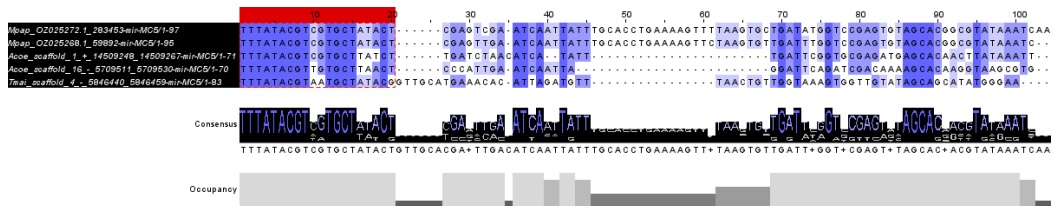
Ref: *Tmai_scaffold_3_83965*



mir-MC5:



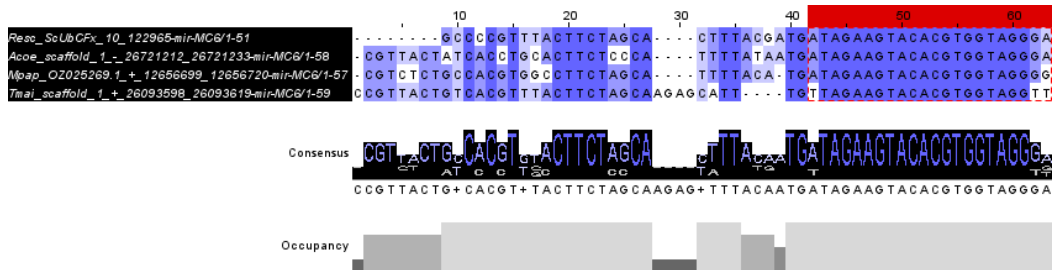
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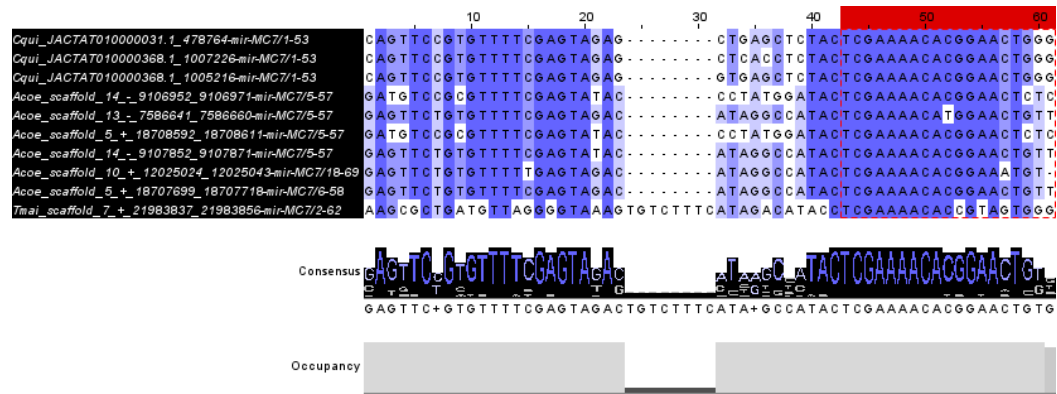
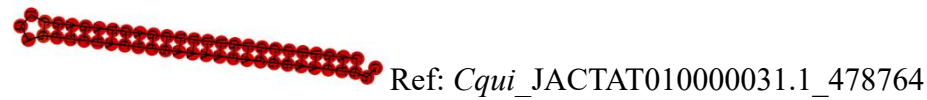
mir-MC6:



Ref: *Resc_ScUbCFx_10_122965*



mir-*MC7*:



Supplementary Figure 13. Sequence alignments of conserved novel microRNAs in scyphozoans and cubozoan (mir-*MC1* to mir-*MC7*).

Acco_gearfold_9_351554-mir-SC2/1-52
 Cgqi_fasta_1_JAGTA7010000007.1-1901011.1901032-mir-SC2/1-52
 Hkap_fasta_O2025280.1+1232754_12327775-mir-SC2/1-52
 Hkap_fasta_O2025280.1-1764919_1764940-mir-SC2/1-51
 Hkap_fasta_O2025280.1+6942029_6942050-mir-SC2/1-85

10 20 30 40 50 60 70 80
 TGGACAATCAAACCTTCGGACA...TTTGTGCGAAAGTTTAATGTGCGAA
 CGGACAATCAAACCTTCGGACA...GAAATGTGCGAAAGTTTAATCAACATCGGT
 CGGACAATCAAACCTTCGGACA...CGAATTTGCGAAAGTTTAATGTGCGAA
 CGGACAATCAAACCTTCGGACA...ATTATGTGCGAAAGTTTAATGTGCGAA
 CGGACAATAAACCTTCGGACA...CAAGTTATTTCTGACCAATAAACATTGAAACAGATTGTGCGCAAGTTTGAATGTCGAA

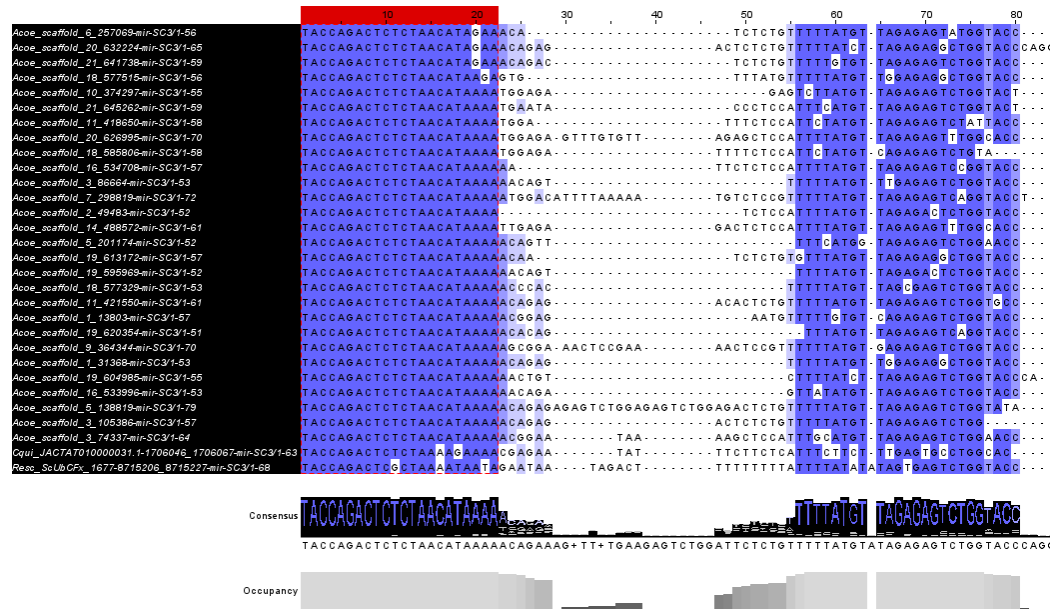
Consensus
 CGGACAATCAAACCTTCGGACA...TTTGTGCGAAAGTTTAATGTGCGAA

Occupancy

mir-SC3:



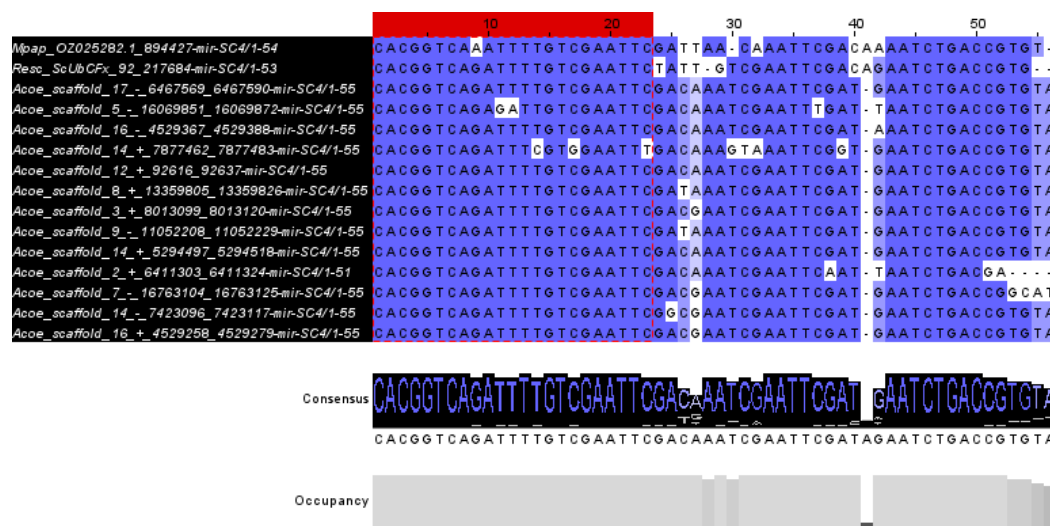
Ref: *Acoe_scaffold_6_257069*



mir-SC4:



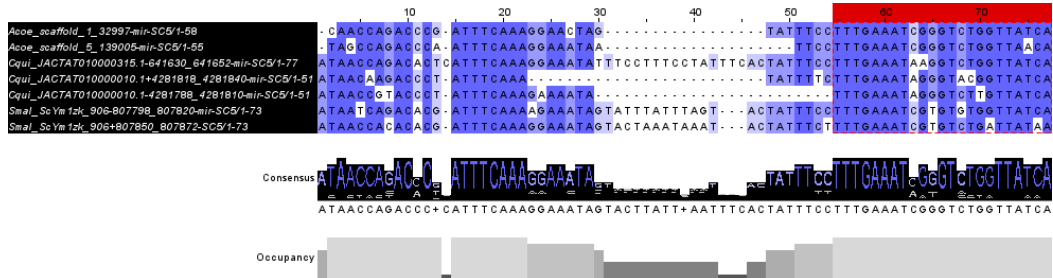
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mir-SC5:



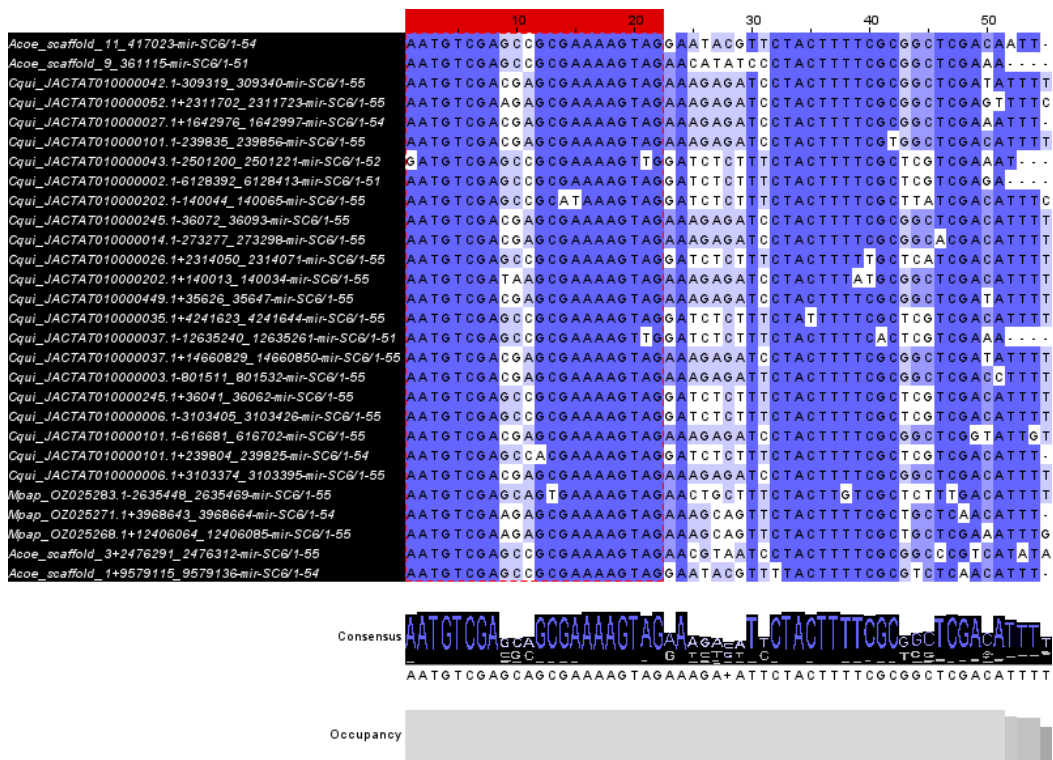
Ref: *Acoe_scaffold_5_139005*



mir-SC6:



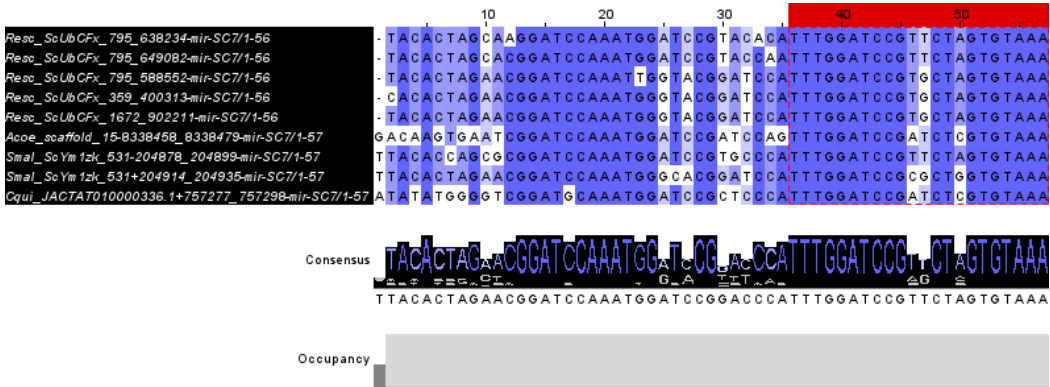
Ref: *Acoe_scaffold_11_417023*



mir-SC7:



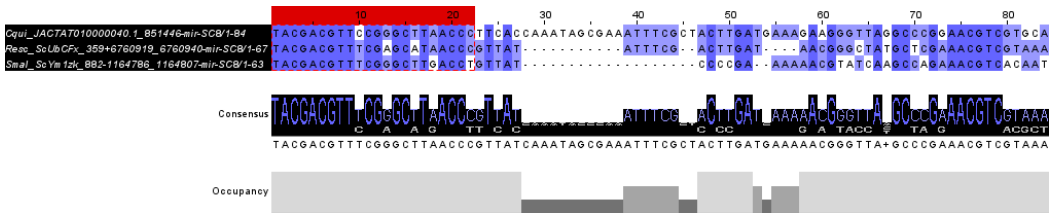
Ref: *Resc_ScUbCFx_795_638234*



mir-SC8:



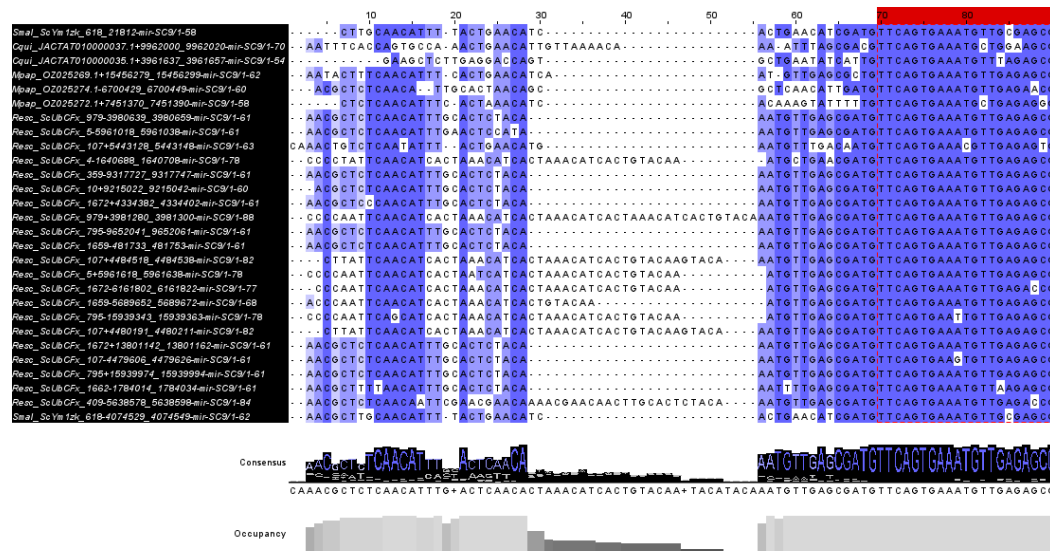
Ref: *Cqui_JACTAT010000040.1_851446*



mir-SC9:



Ref: *Smal*_ScYm1zk_618_21812



Supplementary Figure 14. Sequence alignments of conserved novel microRNAs in scyphozoans (mir-SC1 to mir-SC9).