

Supplementary Data for:

Coleoptera Genome and Transcriptome Sequences reveal numerous Differences in Neuropeptide Signaling between Species

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Note: In the supplementary figures depicting neuropeptide precursor alignments, putative neuropeptides in those precursors have been underlined in red.

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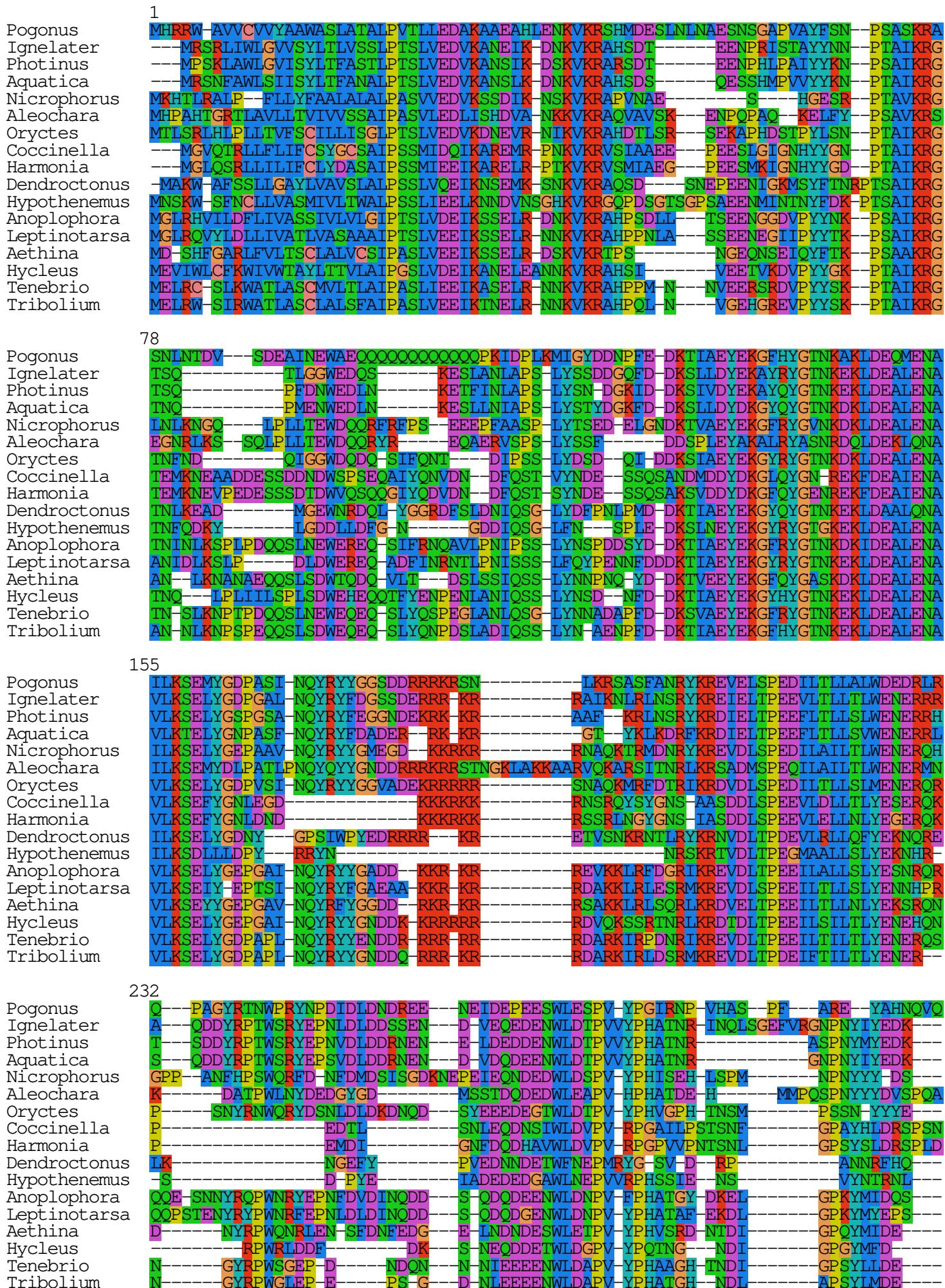
Hypothenemus hampei

Tribolium Natalisin-R	MSTTESWKEEEGNGS --- FQGFDYGLLVISSQTPVVVDSENFMPPIWVKVLWT VIFMVM	57
Sequence-1	----- M	1
Sequence-3	----- ILYAGM	6
Tribolium Tachykinin-R	MNFTQEFLYTTGYSIMDNSSEYDTTNIS --- YNDTEEGGNQFILPVWRQVLWSILYAGM	57
Tribolium Natalisin-R	IL IATGGNCIVIWIVTAHRRMRTVTNYFLVNLSADLLTFNCIFNFSYMIQRDWPFGS	117
Sequence-1	VIVATGGNLIVIYIVLAHKRMRTVTNYFL-----	30
Sequence-2	---ATGGNLIVIYIVLAHKRMRTVTNYFL RELKIA -----	32
Sequence-3	VIVATGGNLIVIYIVLAHKRMRTVTNY-----	33
Tribolium Tachykinin-R	VIVATGGNLIVI WIV FSHKRMRTVTNYFLNLSDVADTMVSTLNVTNFVYMLNSHWPFGE	117
Tribolium Natalisin-R	LYCIISNFIANATVAASVFTLTGISCDRYLAIHVPLQPRMSKRASLITITFIWLASMTVA	177
Tribolium Tachykinin-R	LYCKISQFIAVLSVCASVFLSMSISIDRYMAIMTPLRPRMGRVTVLLAVTTWLLGVIIG	177
Tribolium Natalisin-R	FPCLLYSTTITNKYK-GVERTGCILIWPDGKVVGSHDFAYQMFFLIITYVIPVTL MSFS	236
Sequence-5	----- MTYT	4
Tribolium Tachykinin-R	SPSLMFFRTYTMPYKDGEERVICYPEWPDGTTNESMMYEAYNVGFLFVTVVPIGS MTYT	237
Tribolium Natalisin-R	Y TING KELWGSRSIGEMTQRQIDSIRS KRKVVKM F IFVV F IFACWL PYHGYFL YVYYDT	296
Sequence-5	YARIGIEL WGSQSIGECTQRQMDNIKS KR-----	33
Tribolium Tachykinin-R	YARIGIEL WGSQSIGECTQRQMENIRSKRRVV KMMMV VII FAVCWL PYHLYFIV ISYFP	297
Tribolium Natalisin-R	DIIFS KYQHVYLAFYWFAMSNA MVNPLIY WMNARYVKT -----	336
Sequence-4	---SAYIQETFLAIYWLAMSNMSMYNP <small>II</small> YCWMNARY-----	33
Tribolium Tachykinin-R	EITN STYIQETYLAIYWLAMSNMSMYNP<small>II</small>YCWMNAR FRRGFKQFFSCLPFIHVSPGALTR	357
Tribolium Natalisin-R	----- 336	
Tribolium Tachykinin-R	REVLTSRRRSYSGSPDHNRIRRNG 381	

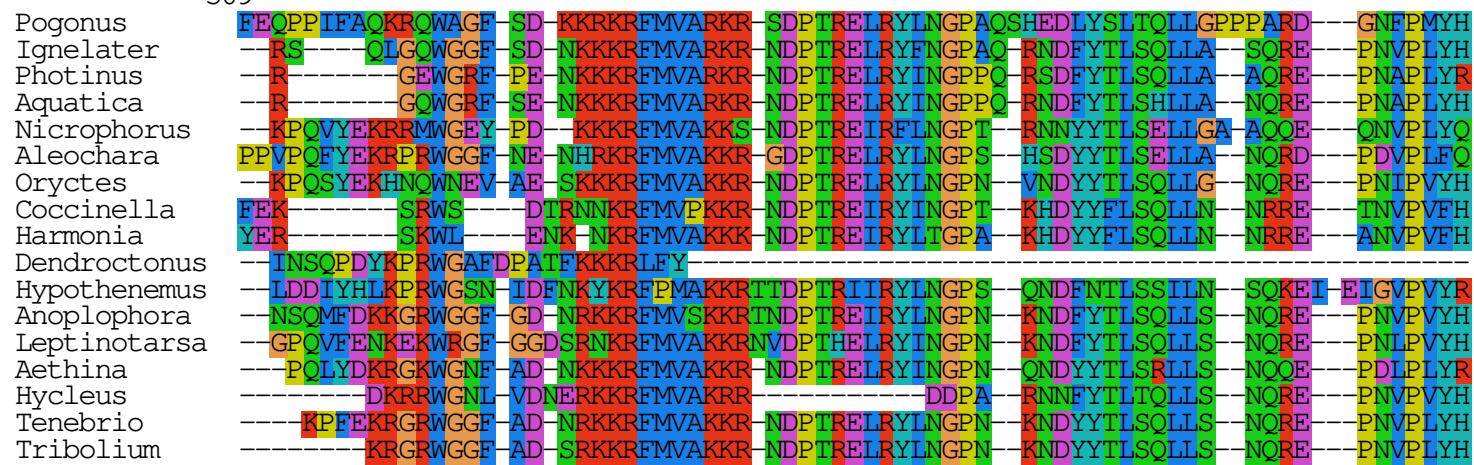
Hycleus phaleratus

Tribolium_Natalisin-R	MSTTESWKEEEGNGS --- FQGFDYGLLVISSQTPVVVD SGENFMPPIWVKVLWT VIFMVM	57
Sequence-1	----- GNQFILP WQALWSILYAGM	21
Tribolium_Tachykinin-R	MNFTQEFLYTTGYSIMDNSSEYDTTNIS --- YNDTEEG GNQFILP VWRQVLWSILYAGM	57
Tribolium_Natalisin-R	IL IATGGNCIVIWIVTAHRRMRTVTNYFLVNLSADLLTFNCIFNFSYMIQRDWPFGS	117
Sequence-1	VIVATGGNLIVI WIV LAHKRMRTVTNYFL-----	50
Sequence-4	----- NLSIADTMVSTLNVTNFVYMLNSHWPFGE	30
Tribolium_Tachykinin-R	VIVATGGNLIVI WIV FSHKRMRTVTNYFLNLSDVADTMVSTLNVTNFVYMLNSHWPFGE	117
Tribolium_Natalisin-R	LYC IISNFIA NATVAASVFTLTGISCDRYLAIHVPLQPRMSKRASLITITFIWLASMTVA	177
Sequence-4	LYCKITQFIAVLSVCASVFS-----	50
Tribolium_Tachykinin-R	LYCKISQFIAVLSVCASVFLSMSISIDRYMAIMTPLRPRMGRVTVLLAVTTWLLGVIIG	177
Tribolium_Natalisin-R	FPCLLYSTTITNKYK-GVERTGCILIWPDGKVVGSHDFAY QMFFLIITYVIPVTL MSFS	236
Sequence-2	----- YNVAFLFVTVVPIGSMTYT	20
Tribolium_Tachykinin-R	SPSLMFFRTYTMPYKDGEERVICYPEWPDGTTNESMMYEAY YNVGFLFVTVVPIGSMTYT	237
Tribolium_Natalisin-R	Y TING KELWGSRSIGEMTQRQIDSIRS KRKVVKM F IFVV F IFACWL PYHGYFL YVYYDT	296
Sequence-2	YARIGIEL WGSQSIGECTQRQ ENIKSKR-----	49
Sequence-3	----- WL PYHLYFIVTSYFP	15
Tribolium_Tachykinin-R	YARIGIEL WGSQSIGECTQRQMENIRSKRRVV KMMMV VII FAVCWL PYHLYFIV ISYFP	297
Tribolium_Natalisin-R	DIIFS KYQHVYLAFYWFAMSNA MVNPLIY WMNARYVKT -----	336
Sequence-3	EITNSP YIQETYLAIYWLAMSNMSMYNP<small>II</small>YCWMNA -----	50
Tribolium_Tachykinin-R	EITNST YIQETYLAIYWLAMSNMSMYNP<small>II</small>YCWMNA FRRGFKQFFSCLPFIHVSPGALTR	357

Figure S1. When the *Tribolium* natalisin receptor is used as query in a tblastn search of genomic short reads from either *Hypothenemus* or *Hycleus*, the predicted protein sequences after translatability of the reads with the smallest e-values are more similar to the *Tribolium* tachykinin receptor. Yellow highlighting indicates identical amino acid residues between the short sequences and the two *Tribolium* receptors, red highlighting indicates different residues. Black highlighting indicates conceptual translation of residues that are part of an intron. Note that in all cases the short sequences are more similar to the tachykinin receptor. Hence, one can conclude that neither of these species has a natalisin receptor.



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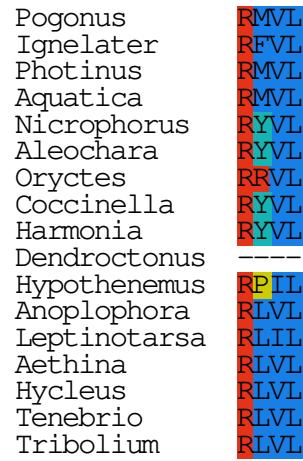
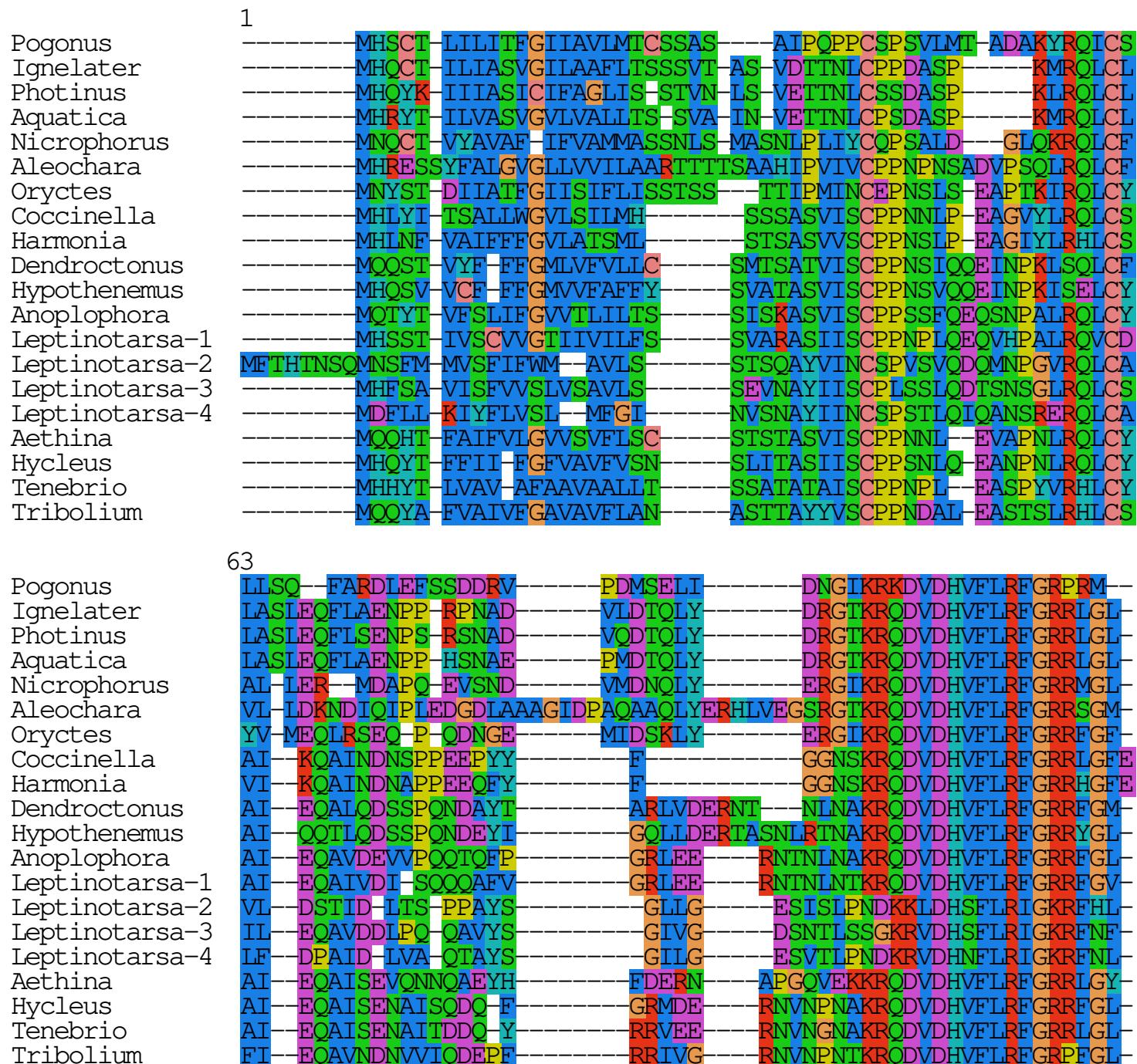


Figure S2. Sequence comparison of Coleoptera baratin precursors.

**Figure S3** Sequence comparison of Coleoptera myosuppressin precursors.

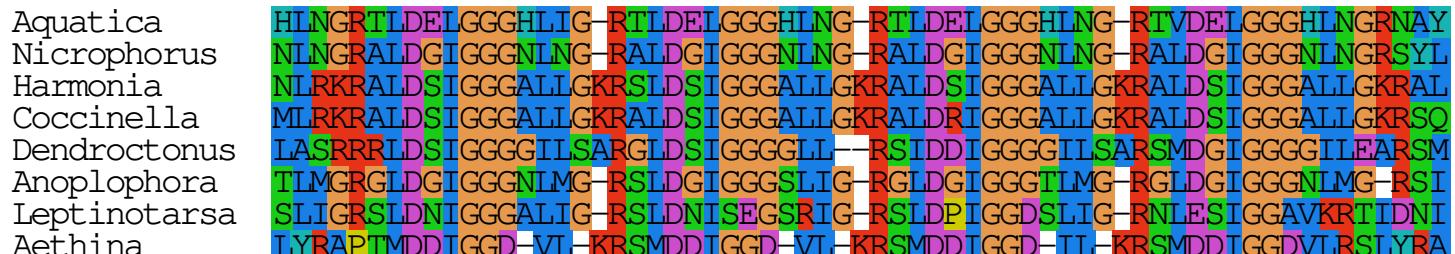
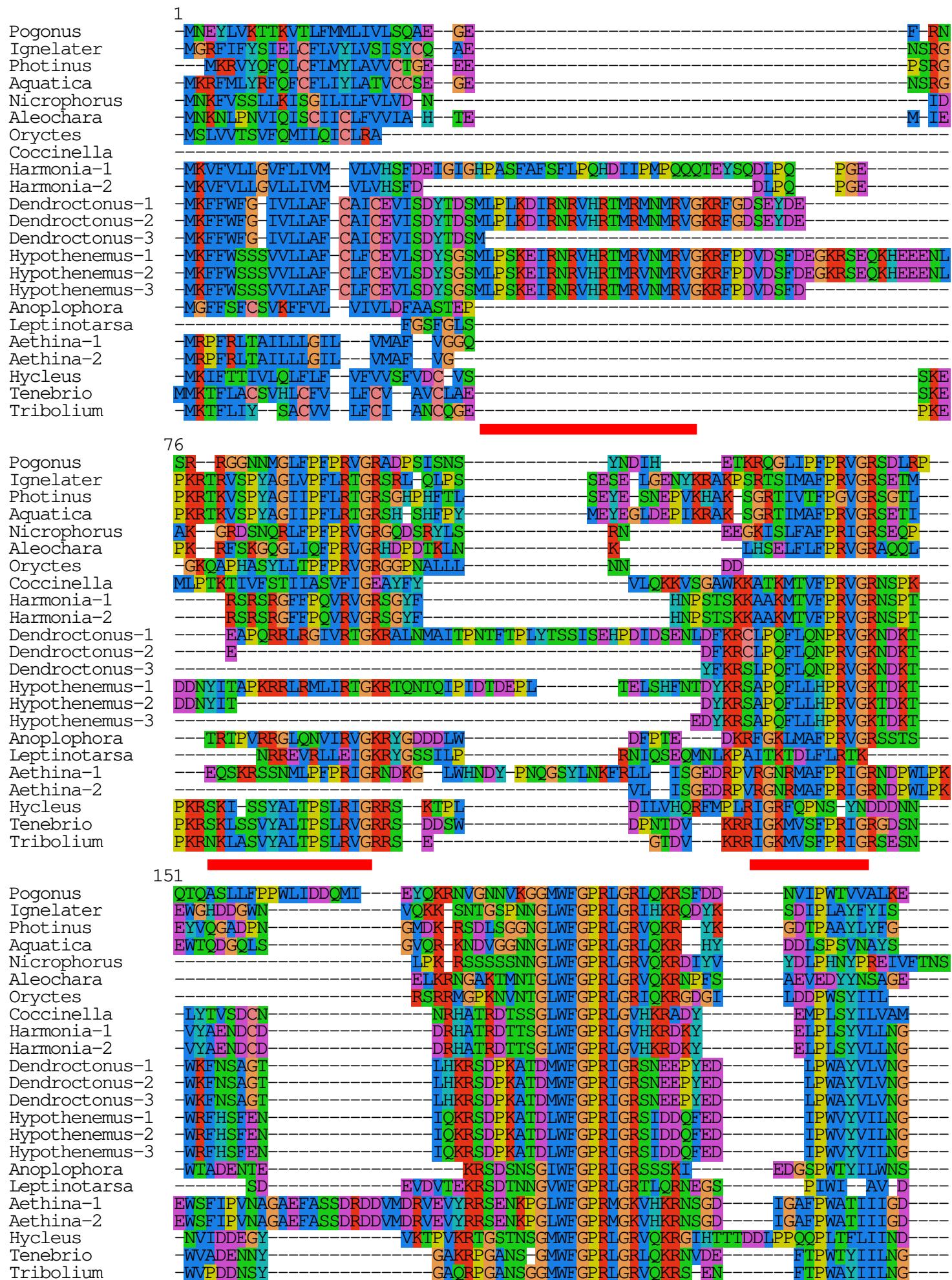
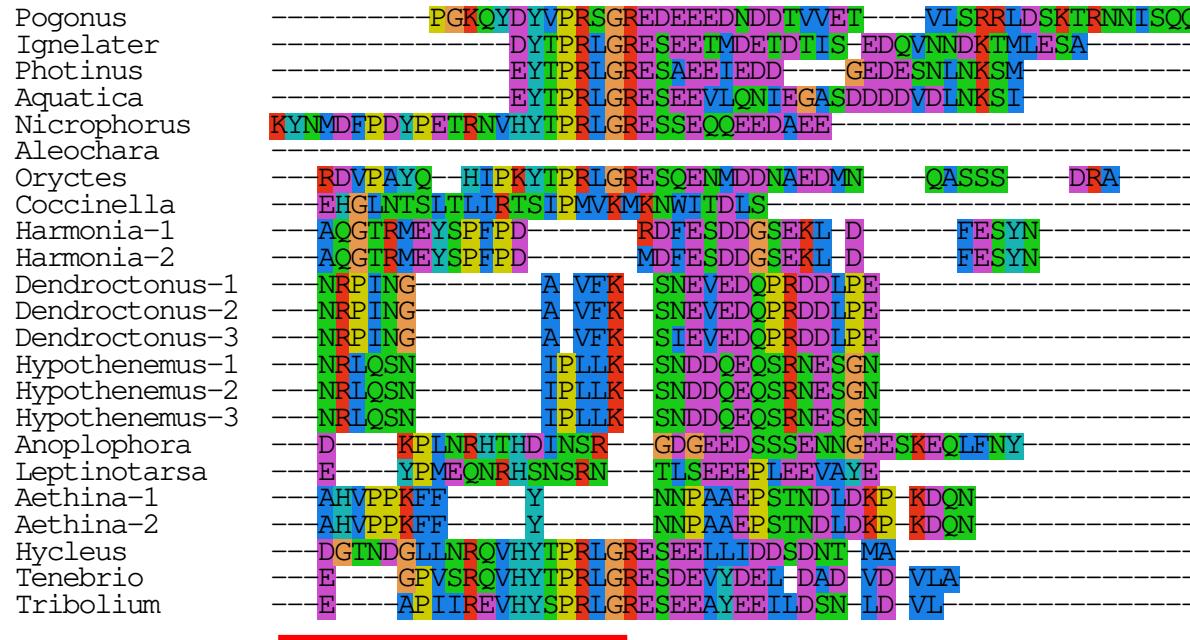


Figure S4. Sequence alignment of parts of the orcokinin B precursors from the species indicated. Note that the putative convertase cleavage sites consists of a single Arg residue in most species, but that in the two Coccinellids, *Harmonia* and *Coccinella*, all have been replaced by a Lys-Arg pair, while in *Aethina* several single Arg convertase cleavage sites have similarly been mutated into Lys-Arg doublets.



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**Figure S5.** Alignment of periviscerokinin precursors.

ARCHOSTEMATA

Micromalthus debilis (GDOQ01020265.1)

MALLWALVLFPAAILASGTEQEPEGIYPQYYSSRYPDREEPPFAFPEEFQSDQRKTVQKSYEETIAEDLSKLSNNLQ
 QKLRFASALLKQENPYETDPADRIEISNLQNSLCILNAEIDNYFRQKAGVFSKGNTQAKRSVWDSKKSTFDAG
 RTSPIELANKSPNIYKSNSNLANAYAQPLFTMKFDATPTDGRTPVQRKRREITKAQLENLLQDEIEKQEEEWE
 RIEGEDDFKKRSQNPPTVVLKSGEV DIPKRVPFNPWGGKRLEQFPVN RDLLGVWESKRDRMPFDPWGGKRARVP
 FDSWGGKRDRTAFDSWGGKRDRLPFDSWGGKRSSRVKLSPWAEGKRPKQRKSQFYSWGGKRSKYDATLV*

Priacma serrata (GACO01006445.1)

-----GGDDTDQKRTKFFSWAGKRTNDGTEPSFEFLPSFKRSHSSPNIVVKPREMALPKWTPFHSGGKRAVQV
 ESERLPISRVPFNAWGGKRSSHDNEFLPLHQEMTSLPHNPEGEFFKSVQTIEVEVSQLGWKEKSVLTLRN
 IKMLPL*

ADEPHAGA

Pogonus chalceus (JU438197.1)

MYYKNSVIQTSKILLQIILYIQLQFANTNCMTIPNDNSNMLQTKSIRNRLNLLEDDNIFGTETPTDTKYNSGN
 DMENNNNGDIFTKETDNENIIFTEDSDIDDKKLNVNKIVSLLDPNEVTPKMIEKLSQFKTFYLSNSNDKTLINL
 CTLSRNYINDVCLLIQSSNRSNGLYNPYIYQRCVKLPLLDFYHCVNEKQNYDETAKENEFKRVPFHAWGGKRF
 RQVATEKVQSSKVPFNAWGGKRQMNYDDGDEYCKRSKSFHSWGGKRTVRETANVL*

Gyrinus marinus (GAUY02012896.1)

-----QMLMDASKQVDDEAKHVKFLGWIGKRFSNANGNNIPKRIPFHAWGGKRSNQVGTEKIQASRVPFNAWG
GKRTYNMNSDESTPDNMYEINTPSSVLPYLVYKRSRTKFHSWGGKRNY*

Figure S6. Predicted partial or complete Coleoptera leucokinin precursors as obtained from conceptual translation of TSA transcripts found at NCBI. The nucleotide identifiers are indicated in parentheses. Lys-Arg and Gly-Lys-Arg processing sites are indicated in purple and the amino acid sequences of the mature peptides are in green.

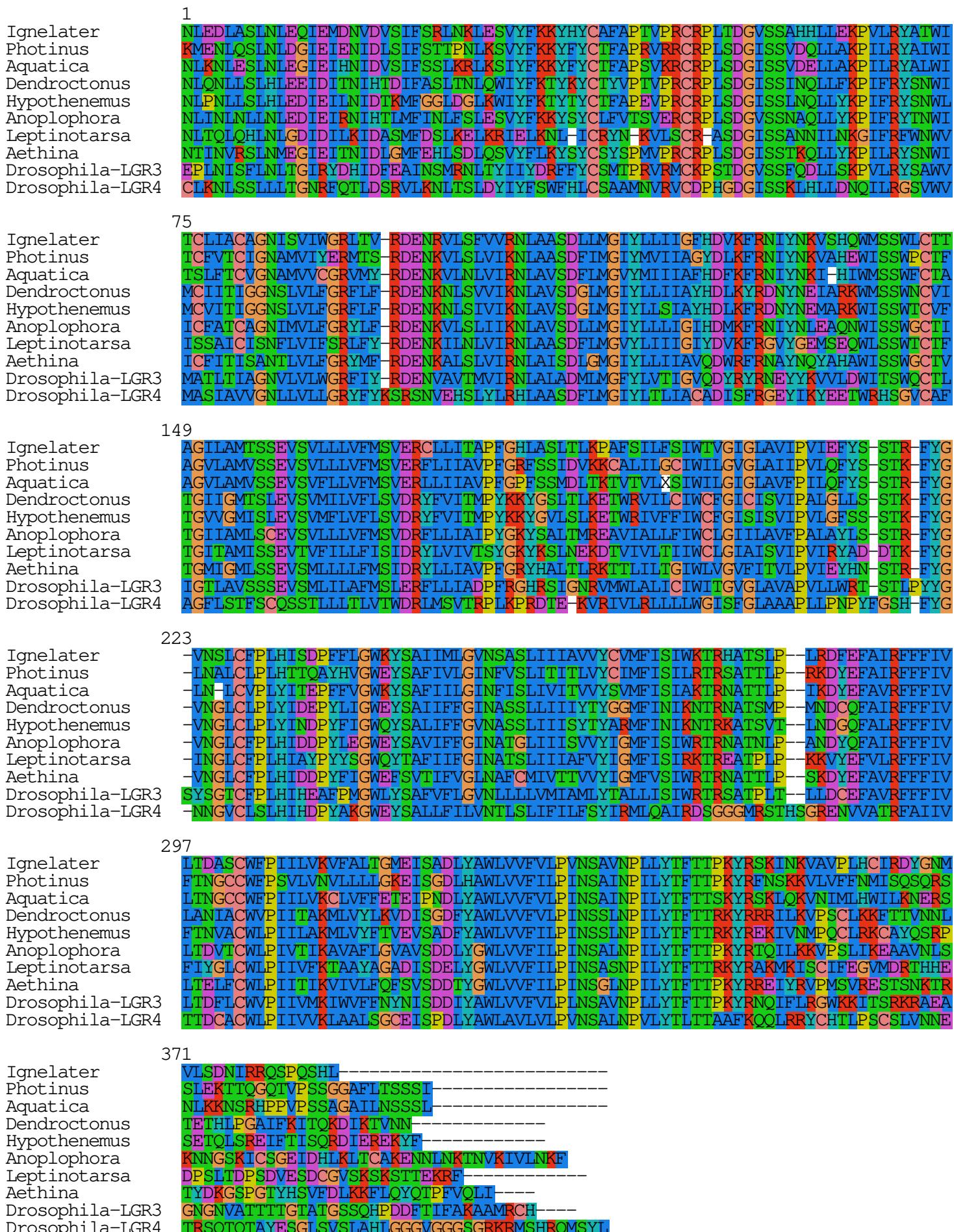


Figure S7. Alignment of N-terminal parts of Coleoptera LGR3's; for comparison the LGR3 and LGR4 from *Drosophila* have been added.

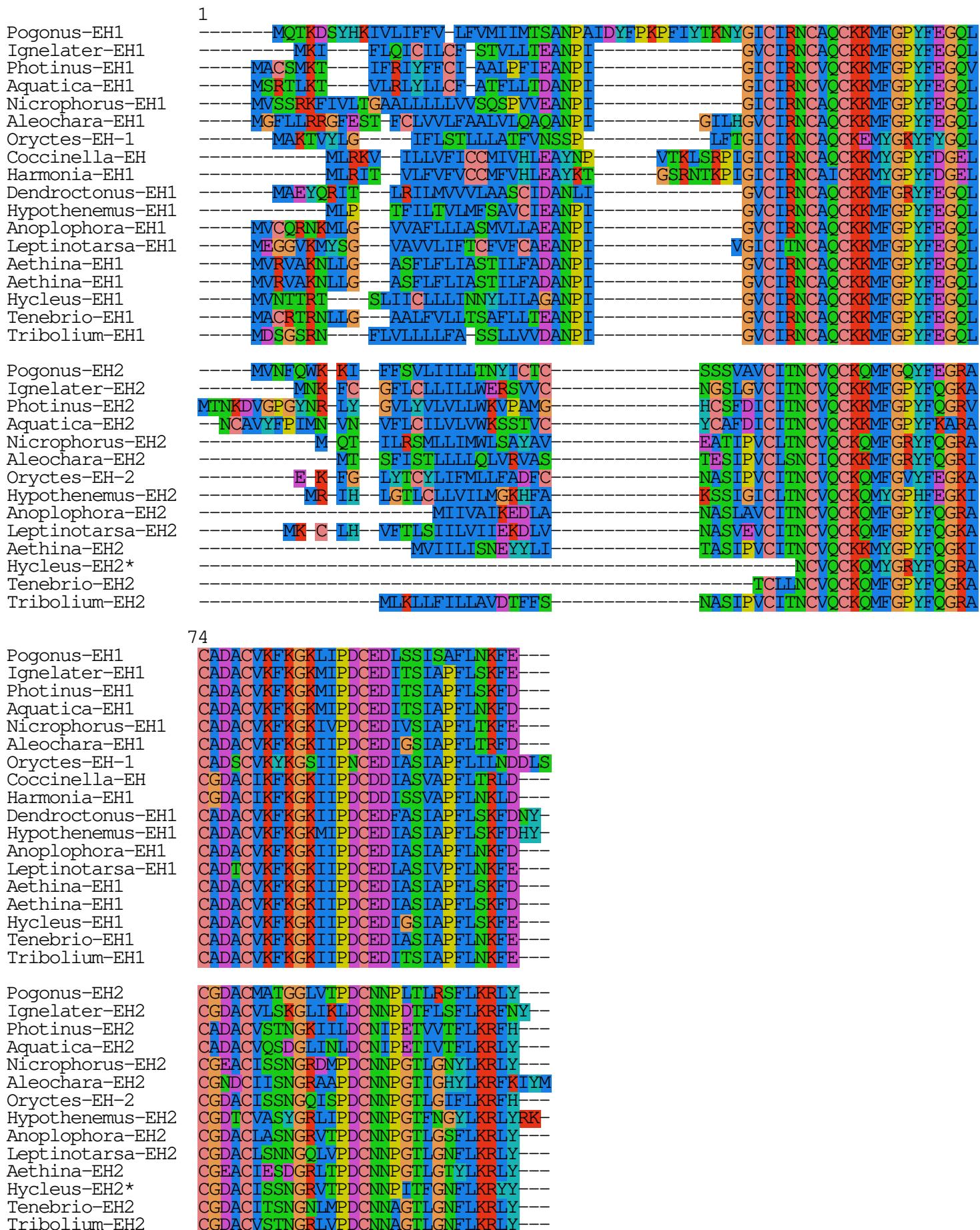


Figure S8. Alignment of Coleoptera eclosion hormone sequences. The asterisk for the second *Hycleus* eclosion hormone indicates the conceptual translation of a pseudogene.

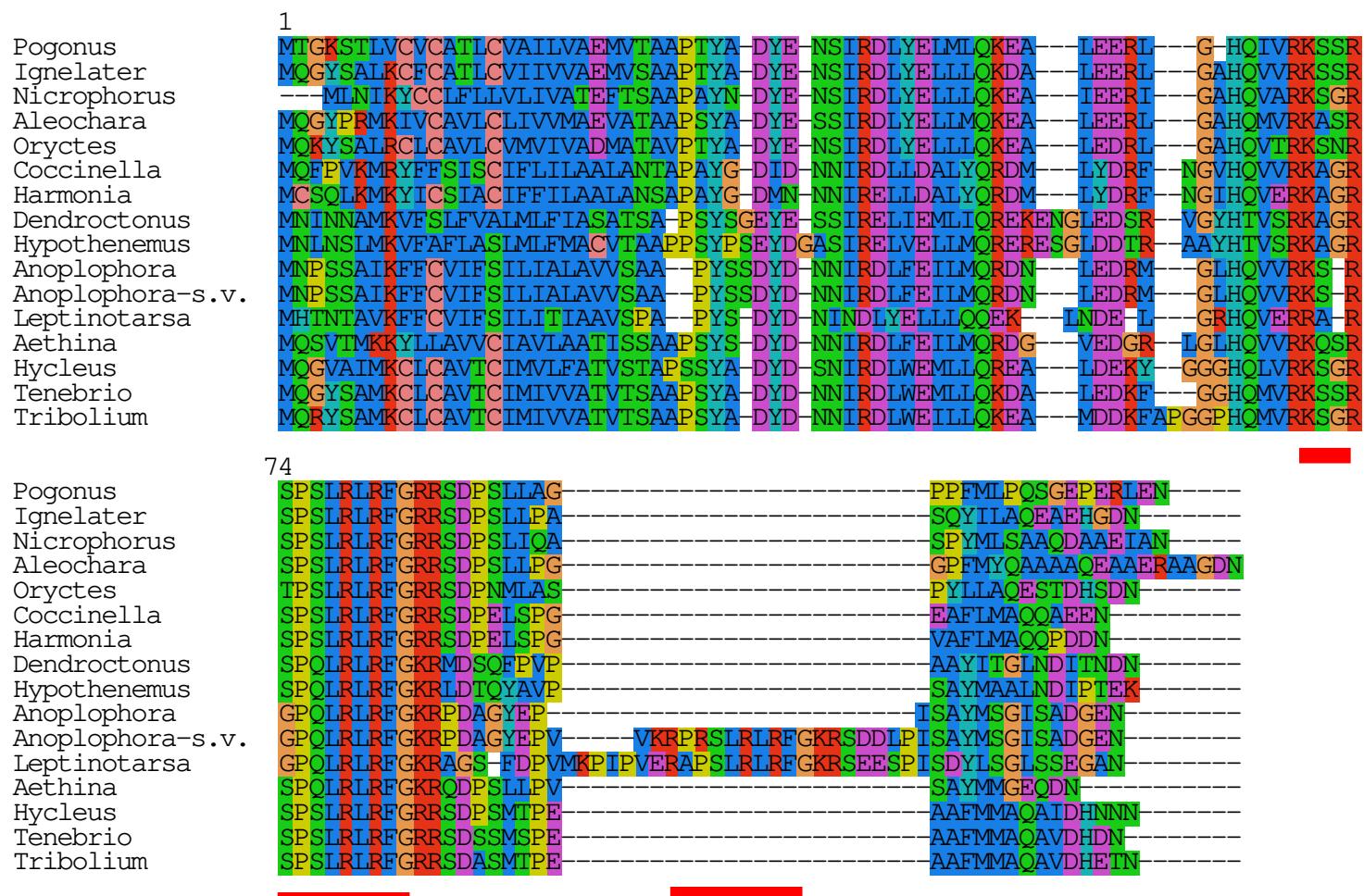
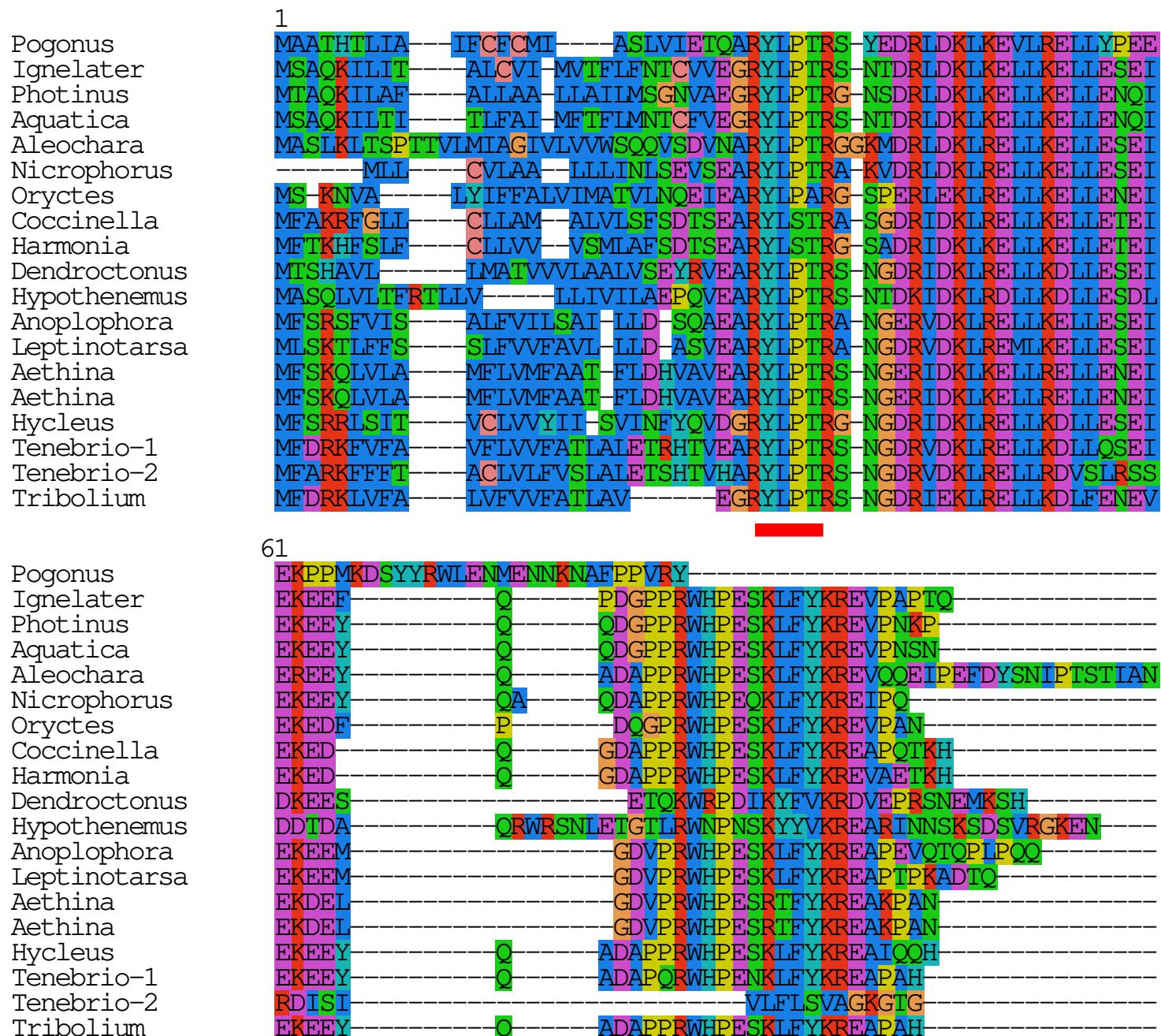
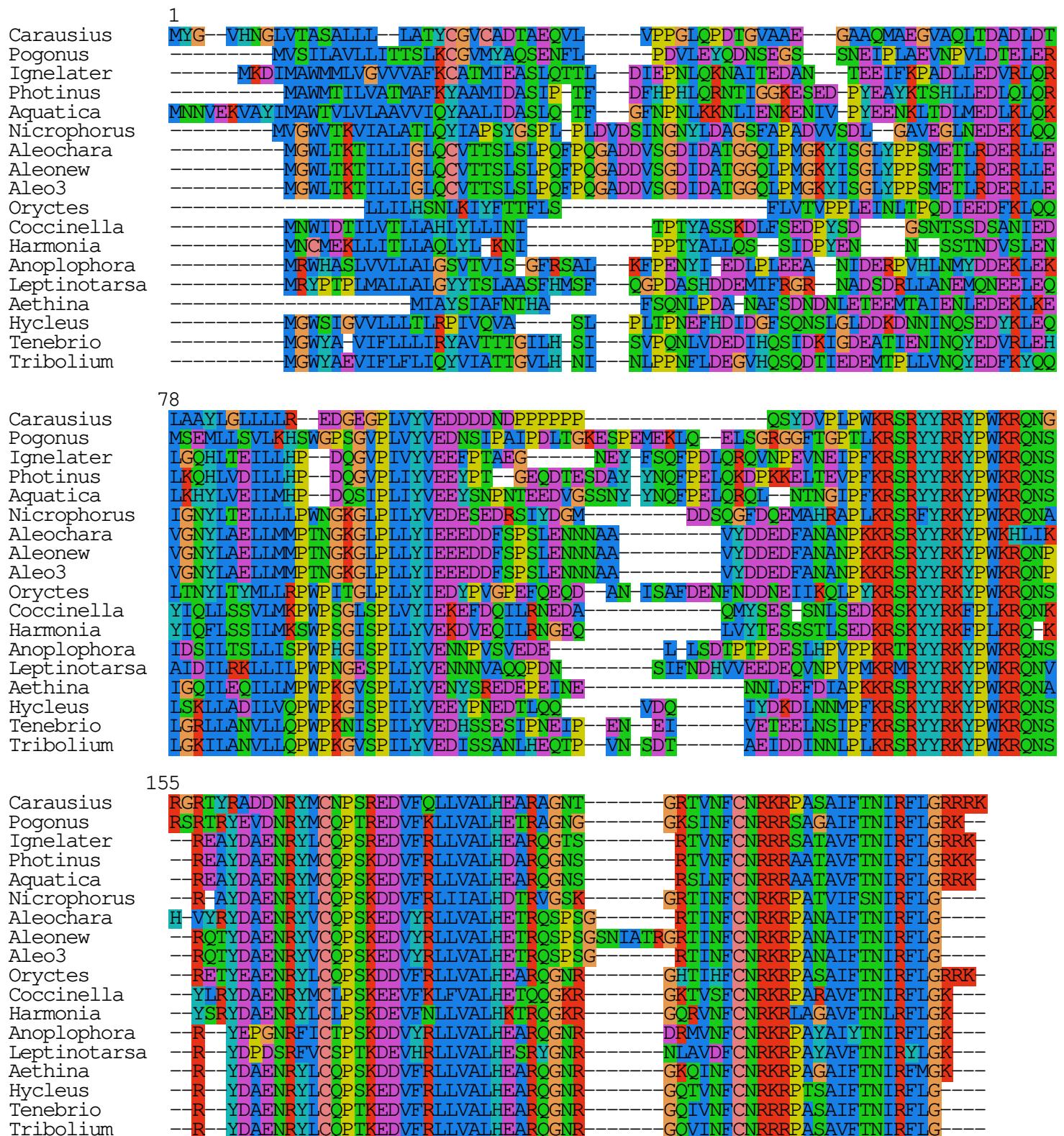


Figure S9. Alignment of Coleoptera sNPF precursors. Note that these sequences are well conserved. The apparent exceptions of *Leptinotarsa* and *Anoplophora* are due to the appearance of an extra exon. *Anoplophora* s.v., *Anoplophora* splice variant.

**Figure S10.** Alignment of proctolin precursors.

**Figure S11.** Alignment of RFLamide precursors.

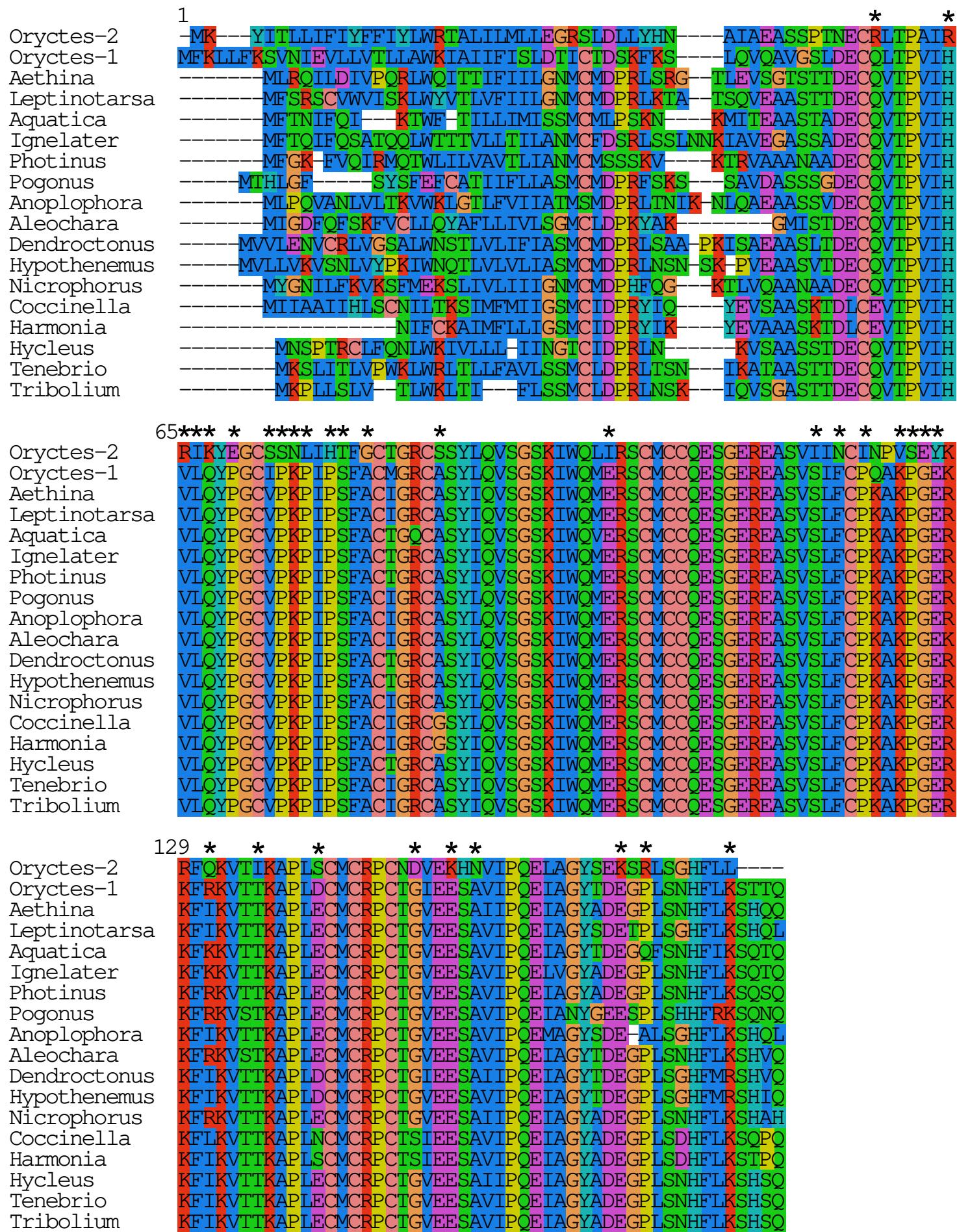
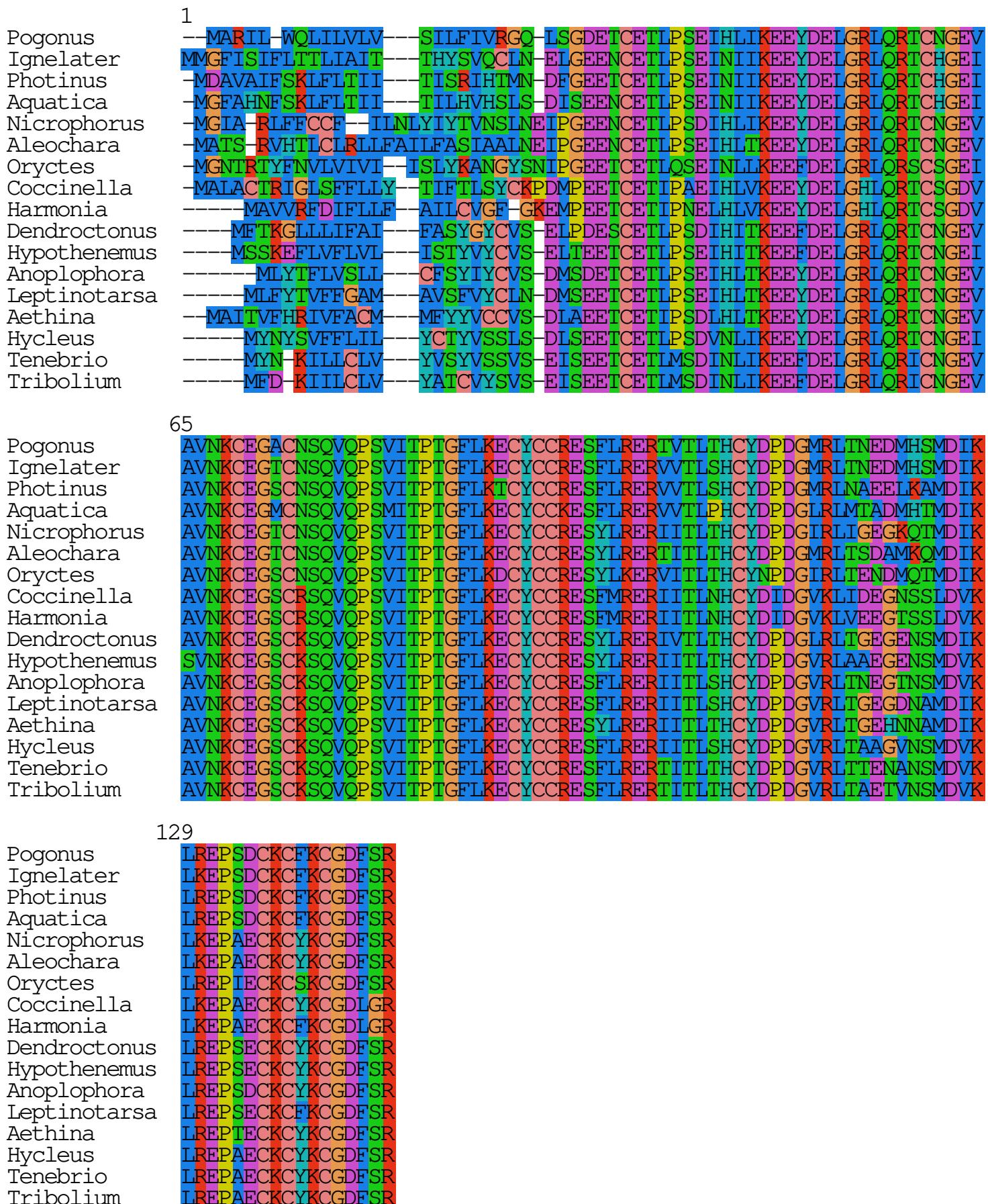
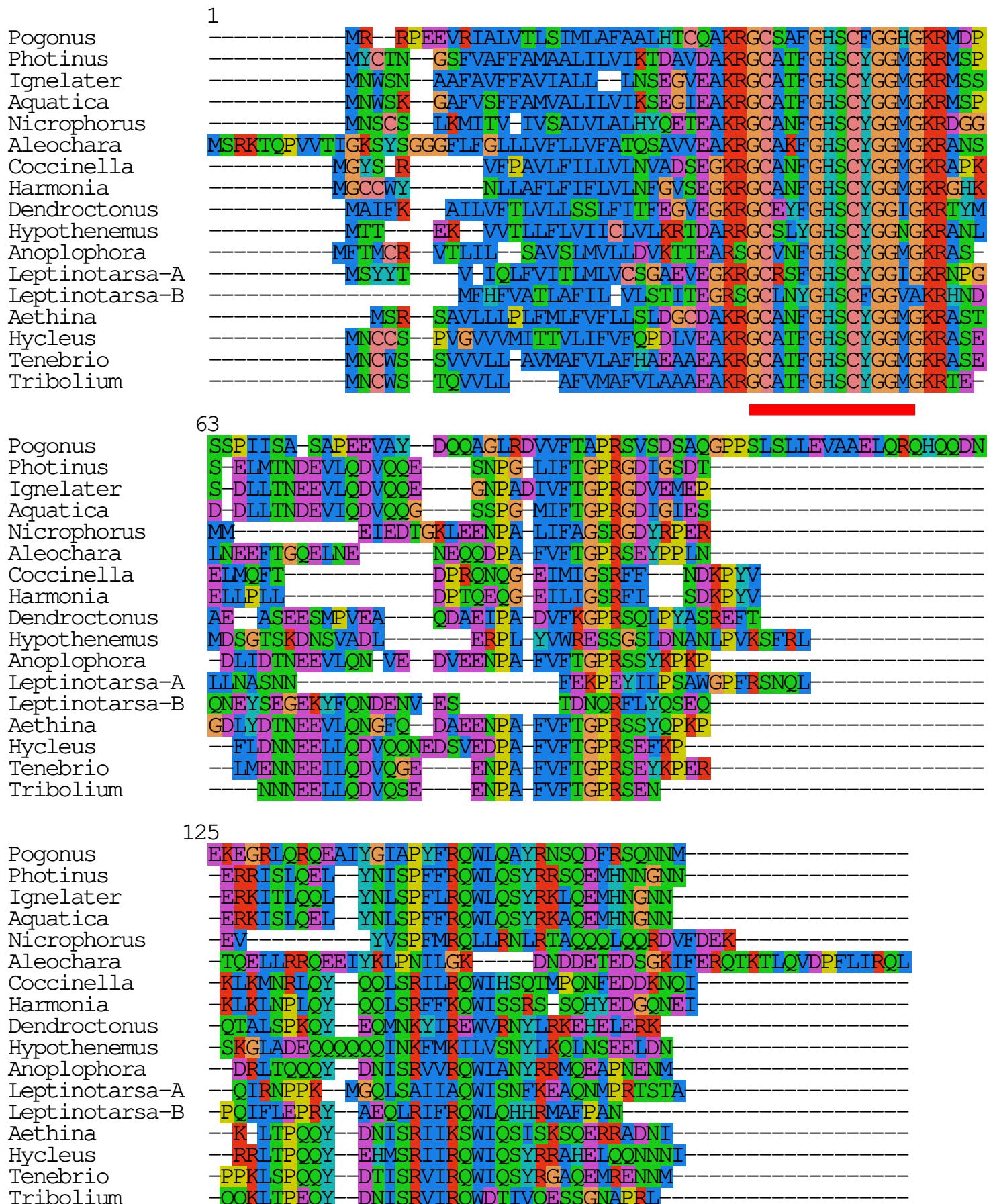
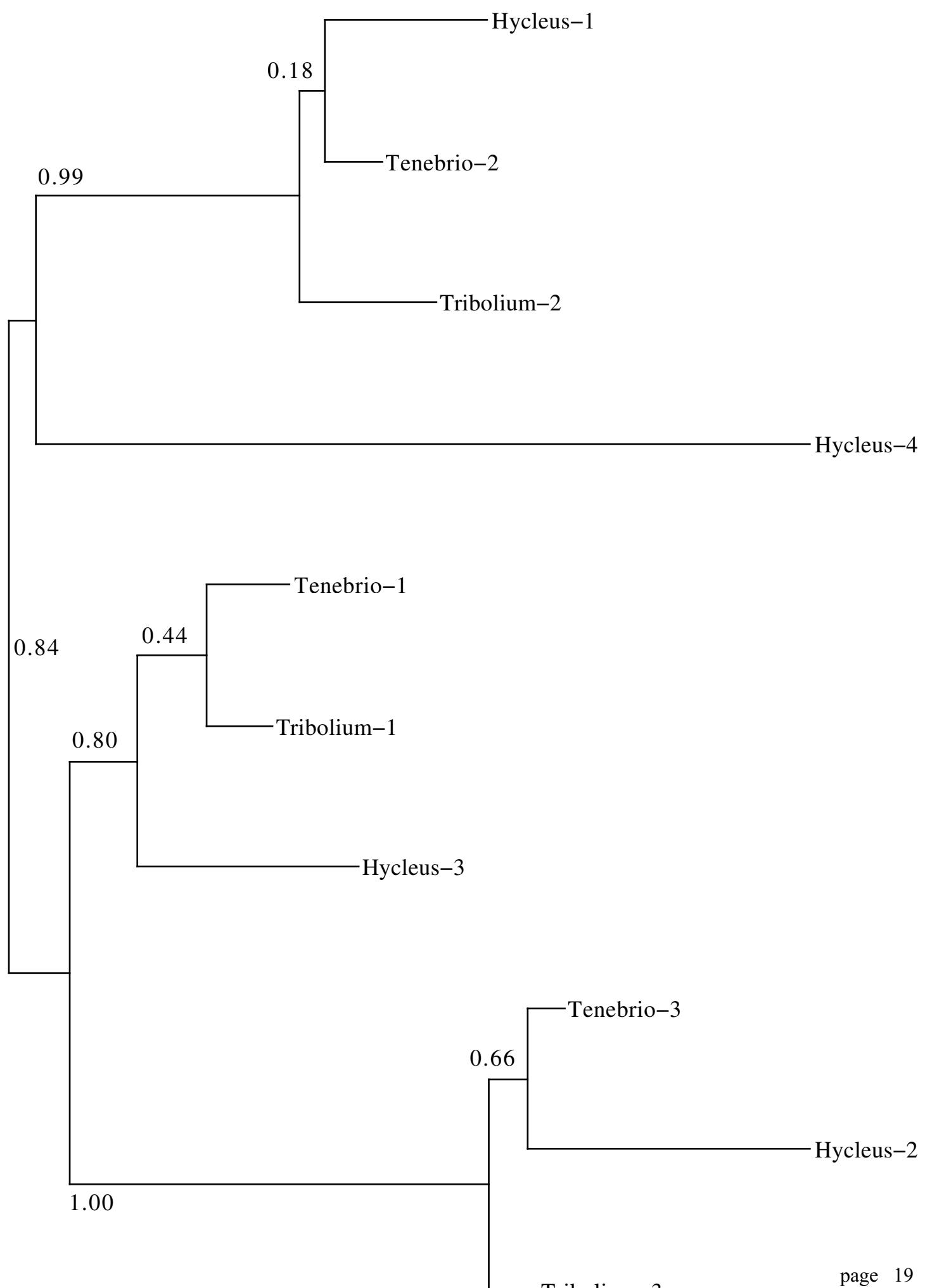


Figure S12. Sequence comparison of Coleoptera bursicon A sequences. Note that the first sequence, that of the second Oryctes bursicon A has a large number of amino acid residues that are different from the consensus sequence; those are indicated by the asterisks on top of the sequences.

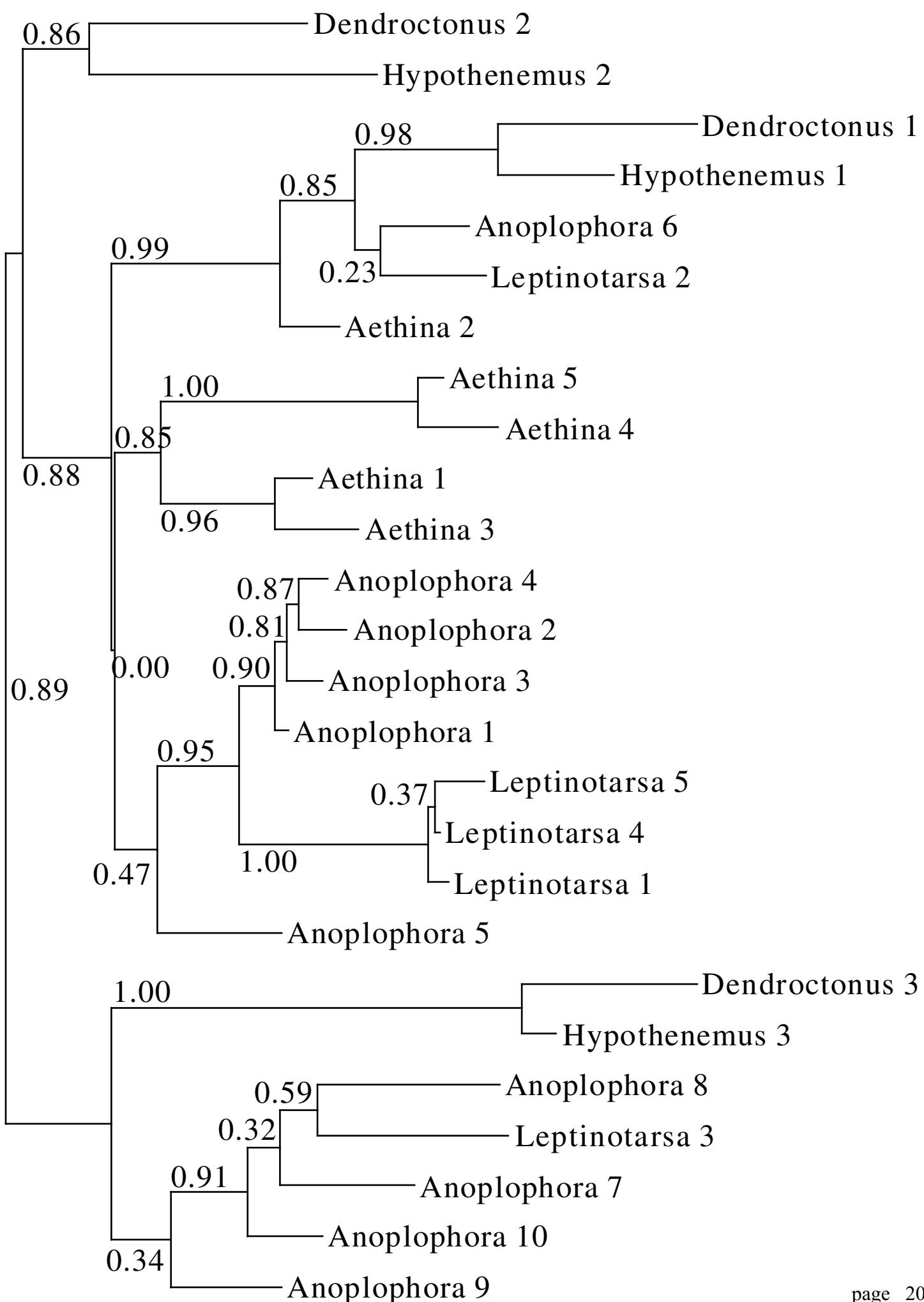
**Figure S13.** Sequence comparison of Coleoptera bursicon B sequences.

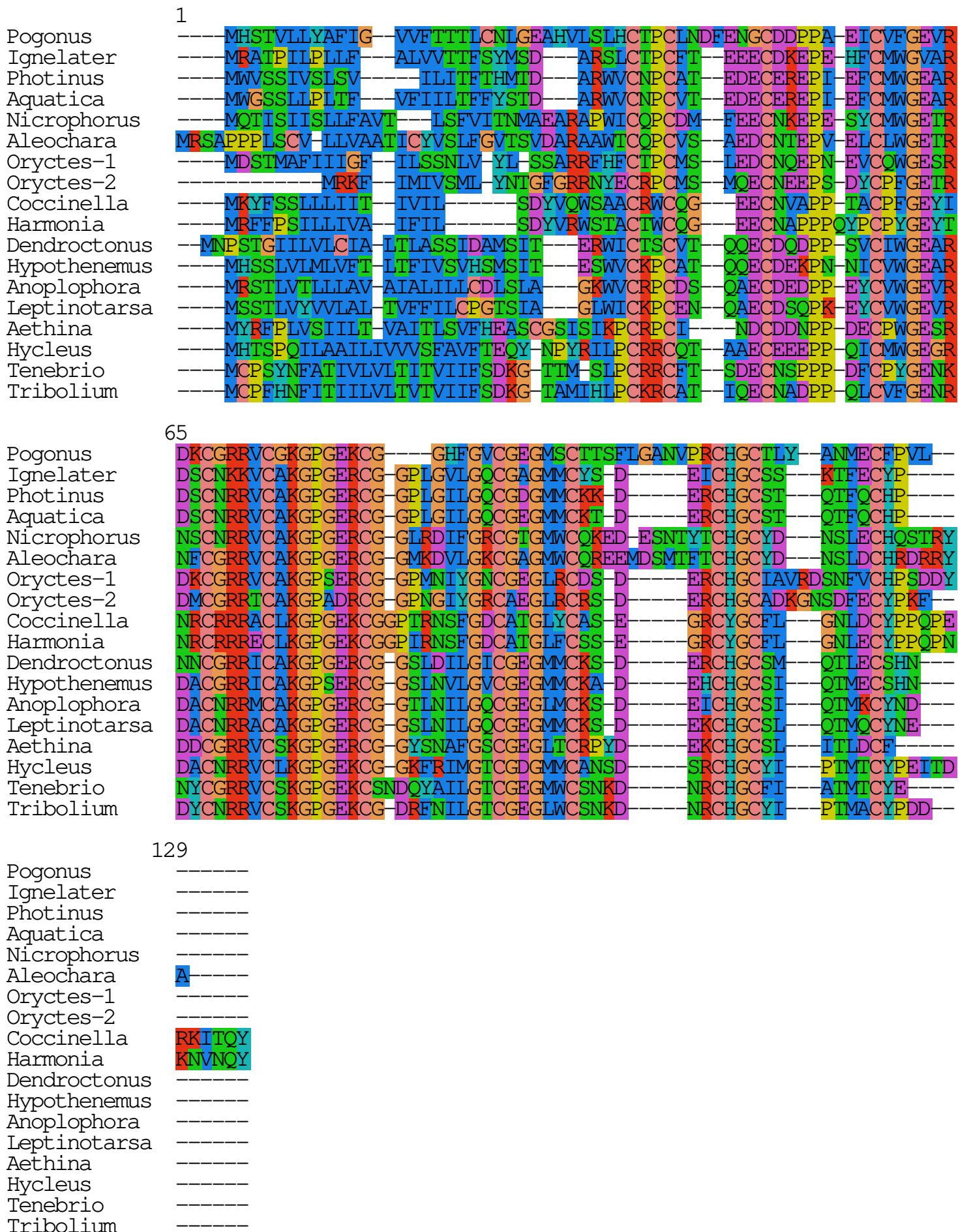
**Figure S14.** Alignment of CCHamide-2 precursors.

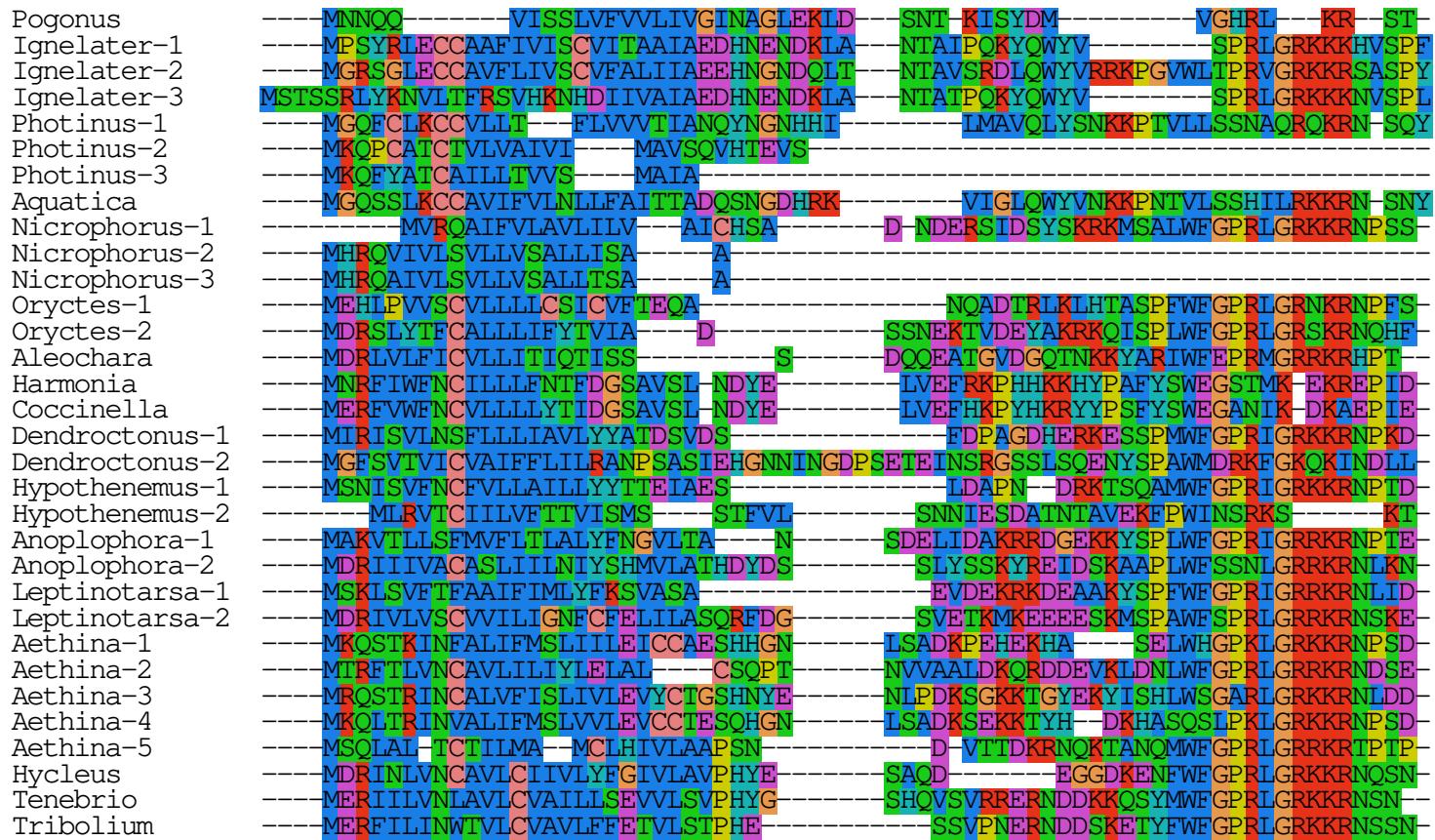
0.2

**Figure S15.** Phylogenetic tree of Tenebrionidea insulins.

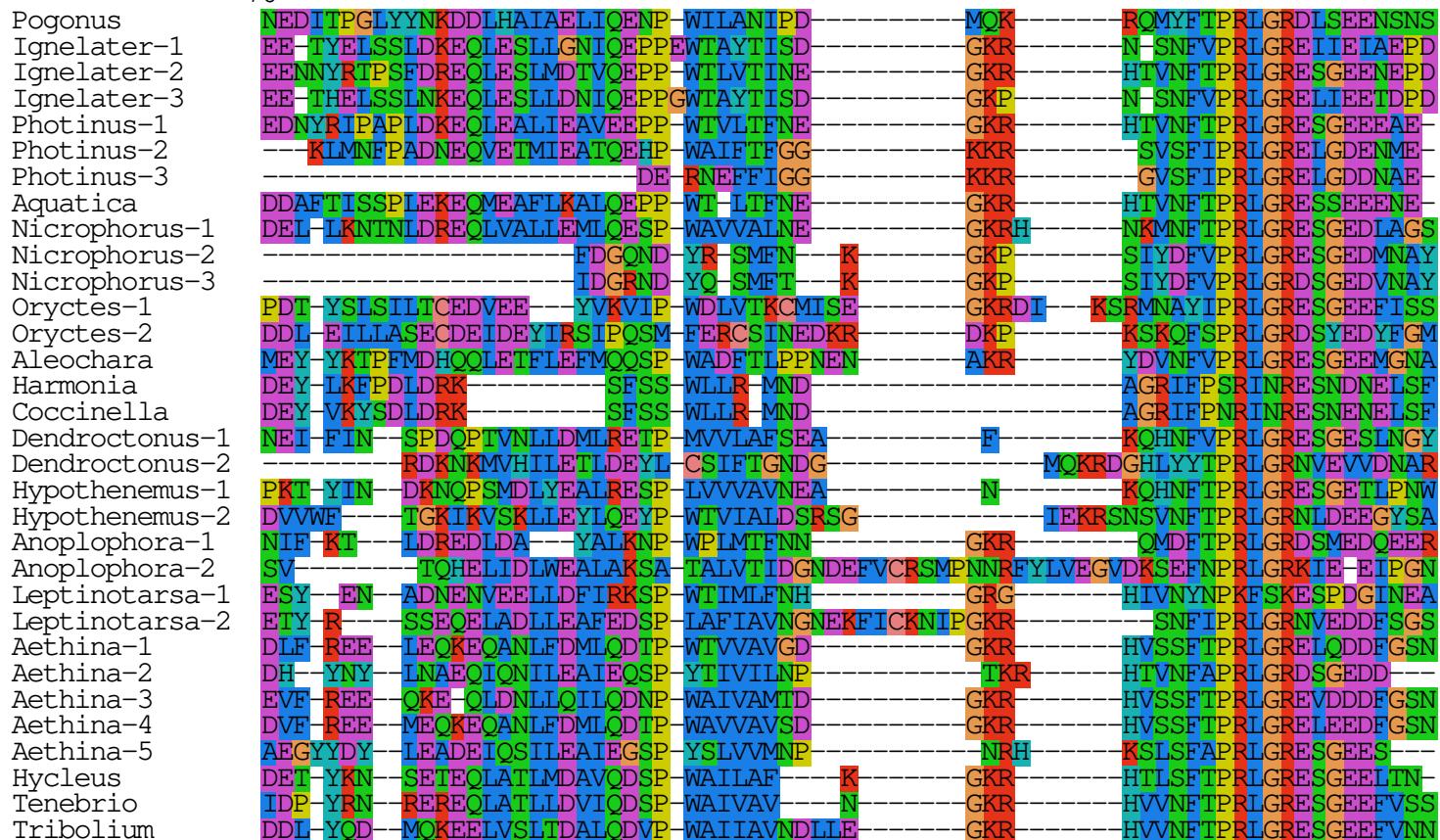
0.5

**Figure S16.** Phylogenetic tree of insulins from *Dendroctonus*, *Hypothenemus*, *Anoplophora*, *Leptinotarsa*, *Aethina*

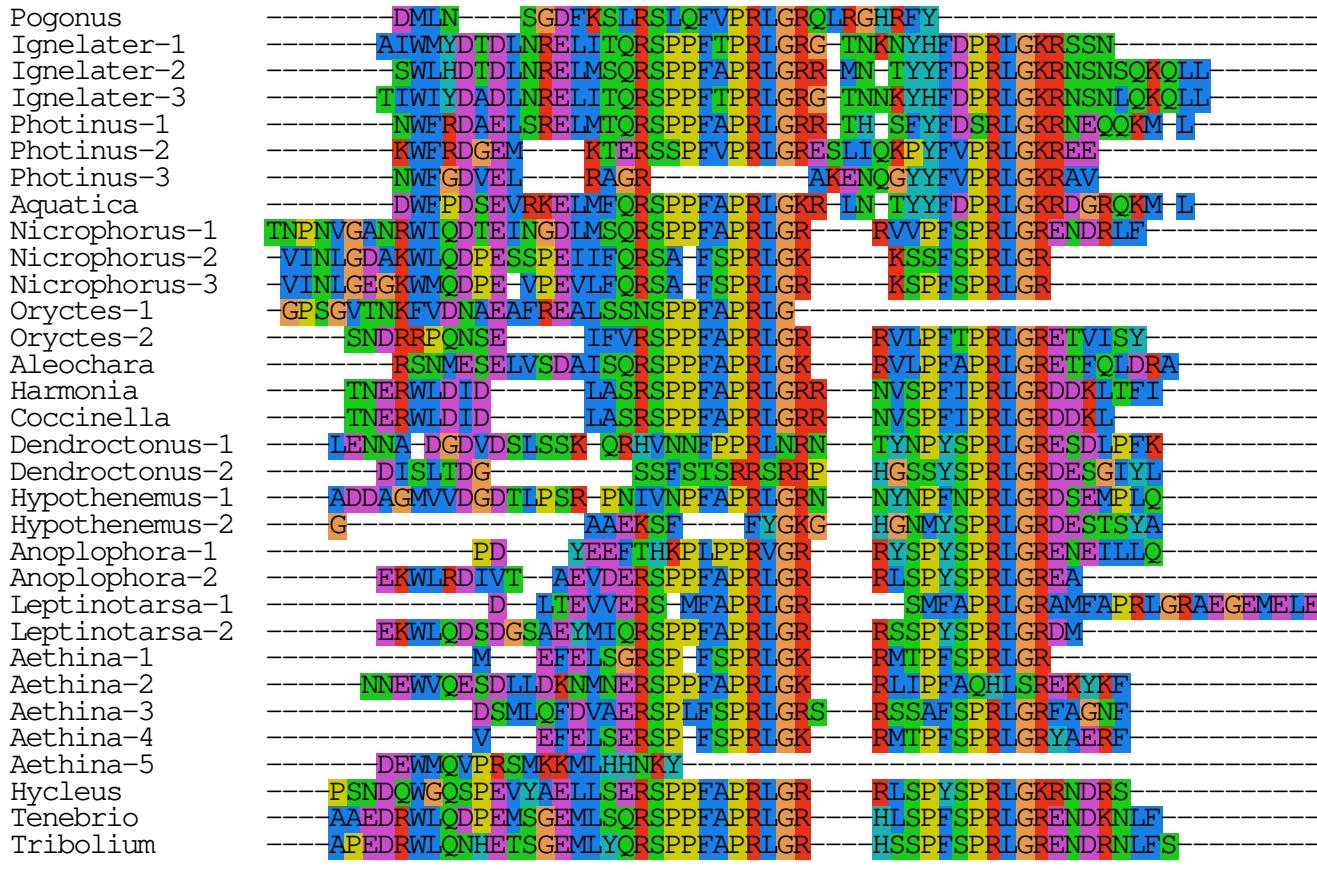
**Figure S17.** Alignment of Neuroparsin precursors.



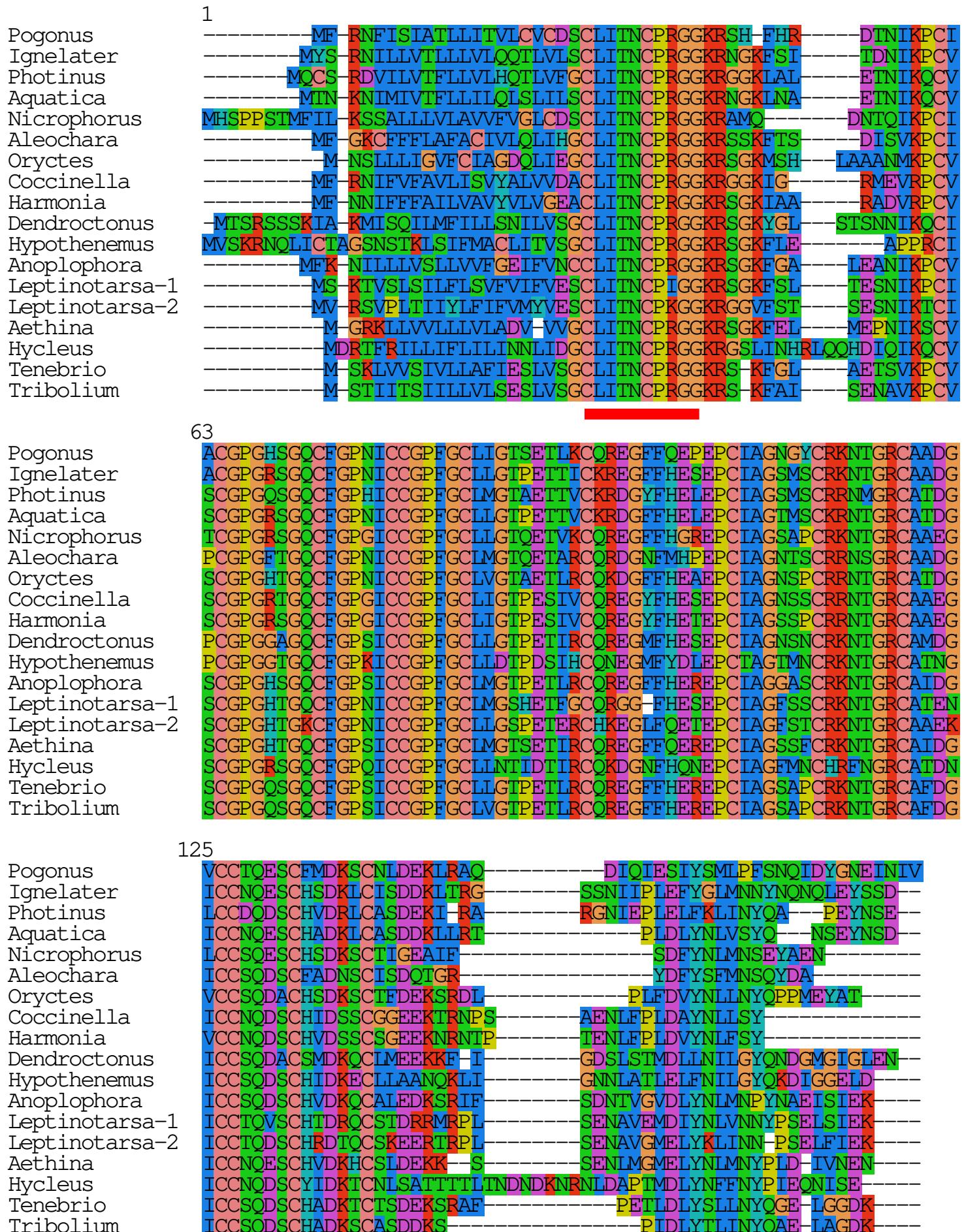
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**Figure S18.** Alignment of pyrokinin precursors.

**Figure S19.** Alignment of relaxin precursors.

**Figure S20.** Alignment of vasopressin precursors.

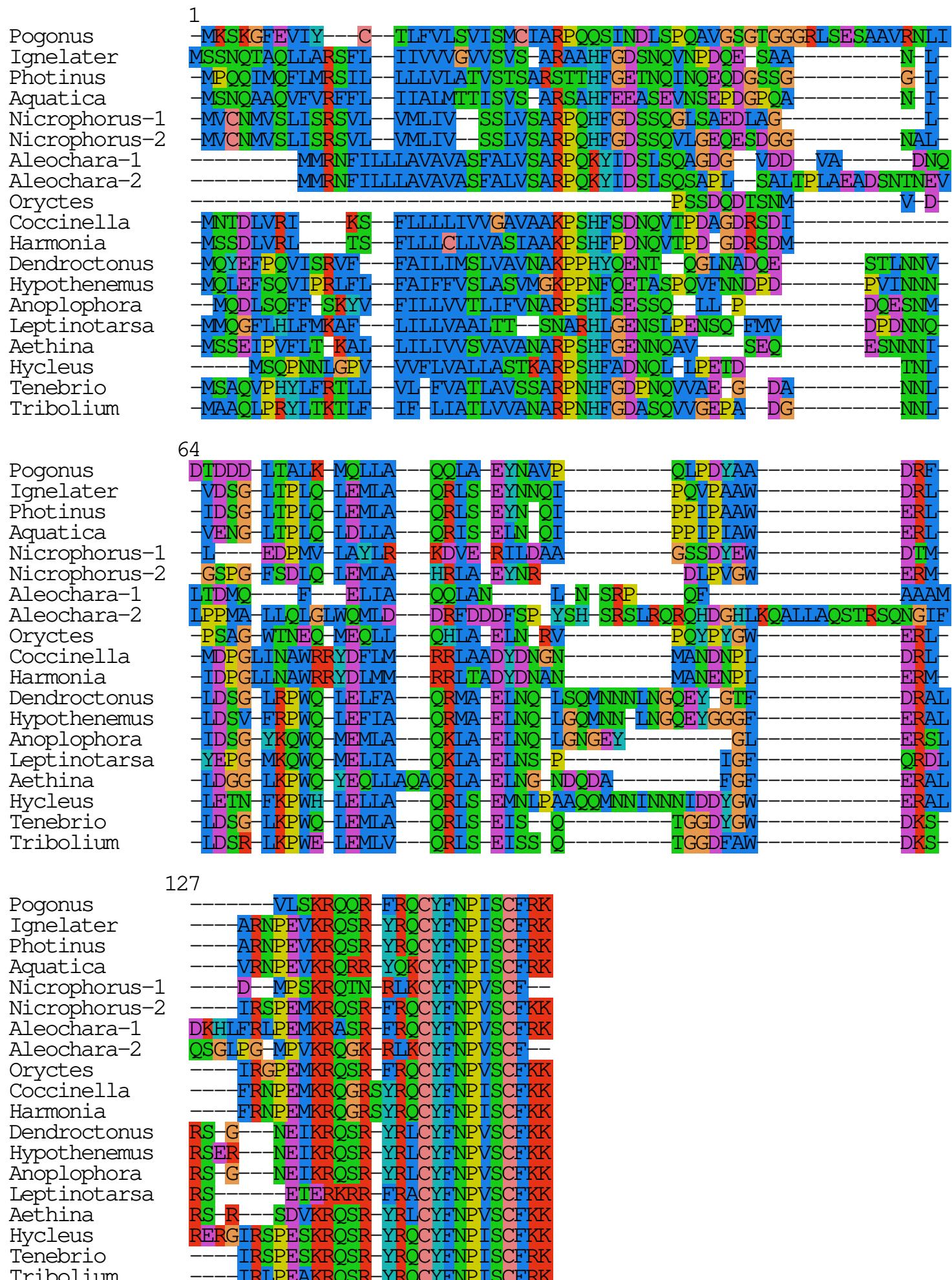
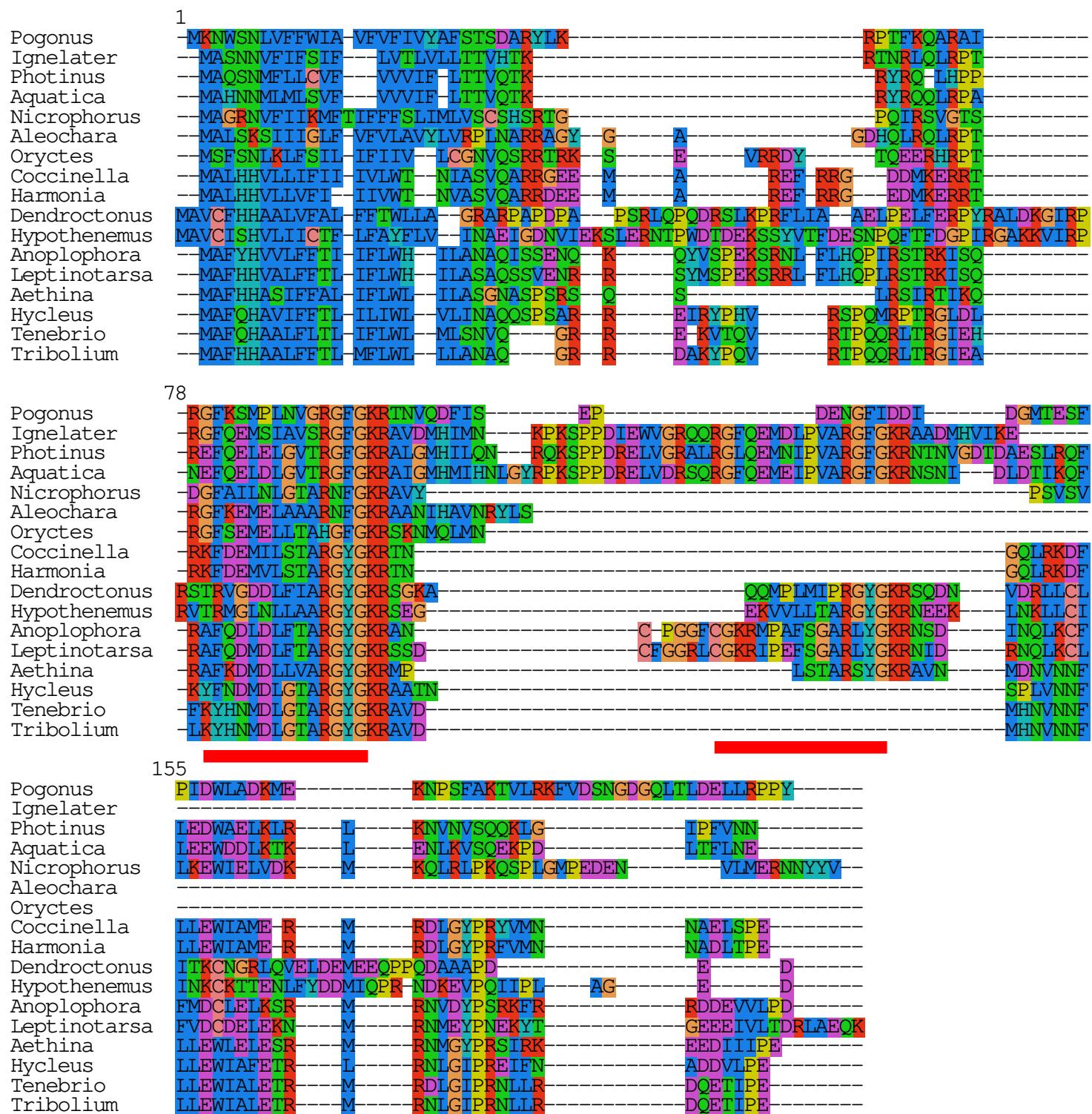


Figure S21. Alignment of allatostatin CCC precursors.

**Figure S22.** Alignment of allatotropin precursors.

Pogonus

MKTLIILMVISFAFATPYQYIRNRQYQIKHFKNSNQIARTHELPNSLQTLFKSFLKRCSNLGNDDCSNGYLPE
IGNDDYLGSGNTPGKRCSNLGNDDC

Ignelater

MNGIILLFGIVLSLDYTWGSPTKRCANMFDEGCINGGVGGAGRDEEWIKDPGNTPGKRCANMFDEGCINGGIGG
SGRDEEWIKDPGNTPGKRCANMFDEGCINEGIGGAGSDEEWIKNPGPKRNLLFKNIPRLYSRSVHKNHQGS
TRRFG

Photinus, pseudogene: has no signal peptide and is not expressed

QIRPKCANLFDESCLKNLIDSALEQWLSNGHVPGKRNLNDEGKYTFL

Aquatica

MVKYLVSFLIIISISLGSKYVNSARIKRCANLFGEDCINGGTIGGGPDQLWITGNGKQKFLHNLPKVNPQKCVNA
FDTNCINNRRLQEQSNQAWTYPESTYRKENIQDKKVLMLYLILKGHSIPTKRCANLMDESCCINGGIVGSGDD
EEWLTGDGSPGRR

Nicrophorus

MIRFMVLFILVLGTDAKPKPCHHRDQACLENQVAIHRPPGIFKFKSLSDFFNRVDIVTSKRASCSNLYDEGCING
GLPGAGDDEDWLNGNGLGKRASCSNLYDEGCINGGLPGAGDDEDWLNGNGLGKRAGHKDWMNKSGIGKRASCSN
LYDEGCINGGLPGAGDDEDWLNGNGLGKRASCNIYDEGCINGGLPGAGNDEDWLNGNGLGKRASCNIYDEGCINGGLPGAGNDEDWLNGNGLGKRASCSNLYDEGCINGGLP
GASEDQAWLTGNGLGKRVNIPQPGYNKNNFKGNKNSKTPIMY

Aleochara

MIRIITLPVVFSAVFLISDGAPCANLFDEGCTYRLLKESAMDQGWLRKNHLGKRTFQEHTQIGIDFFFHRLHNI
LNDRCNNFFDINCIEPNIENHQRVDVNPDAIRAFALQLHEDILNRCAQNAMEDKNCNFIQNFTPKDDDKWIA

Oryctes

MKLLTLILLSVGTINGGYIGKKYCPGLFGDSCASGGLDGSADDEDWLNKPNPGKRCIANVFDGSCSYGATLPG
AAADEYWKQHGSPGKRCSNVFDESCNGAVVNGGSDEDFLNGNGPGKRCSNLFDESCSNAGLIGAGDDESWLN
GGGSPGKRCSNLFDESCSNGLIGAGDDENWLNGGGSPGKRYLPKQWYKAGPGVKTAKRHVSDKVHESLLRK
MAKIRHM

Coccinella

MRKQQFFMLVLTIHSQLIECRSYSAAILEEVLRNSHQNLSPEDVHLFNELYNRRLPVRKRCANMFDEGSC
NIGIHGSDEDWLKDNSPGKR

Harmonia

MKFLFALIMTTIYLFLLVESRSFSSLSFEEAVGRNSHPKLSREDIELFNEYRRRSLPVRKRCANMFDEGCSN
GIHGSDEDWLKDNSPGKR

Dendroctonus

MNRIITLLAISVALSAAMSVPNVYHKTSNSLKQEQLAALINQLREKQVYQEVKRECLRMGPLGCLNDPRKHLL
TMYNPGKRCANFGDDGCLSGGVQGAGSDSDWLASGFTPGKRCANFGDEGCVTGGVGAASDSDWIGGGFPGKR
CANLGDEGCANGGVGPAAADTDWIDGGYPGKRCANLGDEGCVTGGVSGAGSDSDWISGGFSPGKRCANLGDEG
CANAGVGGAGGSDADWLSGDFNPGR

Hypothenemus

MKCIFIVLAVSVCLSFVRSFPNNPDTYRQSTQLKQKLSEIIIEDLMRDKERYRGSIREYDGCGSAGVPGAGS
DWIGGGFTPGKRCANFGDDGCANGGIPGANADQDWLDGGFSPGKRCANLGDEGCANGGVEAGGSDADWLNGNFNPGKR

Anoplophora-1

MNFLLAITAVLGTSQGMYLQRNPHQAVARQSRNEPTVLKSLGDFFHRMHAAATKRCAYLLDESCNNGIPGSGSDSDWL

DGGFN**PGKR**SLNLFEEGIVNKGVSGAAADNDWLNGGFN**PGKR**CANTMDES**C**GNGGIPGSGEDRDWLDDGSAN**PG**
KRRLNGIEGVPGSGNDNDWLHGDNTP**PGKR**

Anoplophora-2

MKCLLAITVLLGSSQG**FYVERNPHQLAIARQSRNQPTIFQSLGDLFHRMHATAT****KR**CAYLLDEA**C**NNGGIPG
 AGSDSDWLTQGFN**PGKR**SSKVLEEEPNDLNPEPDDDRPNGDFNLGKH**C**VNAMDESCDNEEIPGAGPERDWLH**R**
 GFHPGKPLHPFEEDNDVPVAADGGWLSSFSIV**C**PNFMDV**C**GNVGGKRVPGSGADRDLWLEGGGN**PGKR**RLNG
 IPGSGKDNDWLHGDNTP**PGKR**

Leptinotarsa

MNTSTLFLIVLLGFTTAVPLQQWHANKYSSEYHKPNQRGLFSSLGKLFPSSN**KR**ILNSRGGFSSGERSLNLF
 DSVANSKISGSGSDSDWINGGFSP**PGKR**CANLMGES**C**NNGGVPGSGSDDDWIHGGFS**PGKR**SLNLFFDDGAANSKI
 SGSGSDTEWIDGGFSP**PGKR**CANLMDESCSNGGVPGSGSDDDWIHGGAT**PGKR**SLNLFFDDGAANSKISGSGSDSE
 WIHGGFS**PGKR**SLNLLNYASNSKIPGSGSDWLNLDFNS**GRR**GIKSSEKIGHVFGG

Aethina

MRGIIIVVLAMVLAVTLARYVRRHPADVAMTRAKIHL**KR**NINDPELFKQLGDFFHRMHSASIRC**C**SPGLLDEG**C**SN
 GGIPGSGTADWINNYG**PGKR**CVNILSES**C**DNGGIPGAGSDDDWIHDNS**PGKR**CANILDEGC**C**NNGGVPGSGSDA
 DWINNNSP**PGK**

Hycleus-1

MKAIFIIVLVFLSLANCYYLAPHANRPVKVVPLRNLNDLLYRLNGLAL**RR**CANLFDES**C**ANGMSGAGADDDWL
 NGNTN**PGKKR**CANLFDESCSNGDINGAGSDDDWLNGDTN**PGRR**

Hycleus-2

MKFVKILLLLLLSTYAIDIHQSNRSLKLLKKLINSENYGFLGRL**KR**RHCANLYDES**C**INVHLPLAKIDEDWL
 RKGFN**PGK**

Tribolium-1

MKTAFLLLFVLMPAVLCYYVHPNLHYFPARVNYRSAPAKSWGALFHRLQLAS**KR**CANTFDESC**C**INDVINGAGSD
 EAFLNGGDN**PGKR**CVNTFDESCSNGDINGAGSDDDWLHGDDT**PGRR**

Tribolium-2

MRPVVLVLMIFSVSYARYLEPYNYGYPRPLVDFLNRLSLSDKI**KR**CGNTFDESCANLPIIGASSDESWLAHSSP
GKRCANVWGESCCINGGIIGGGSDQSWLQGDDN**PGRR**

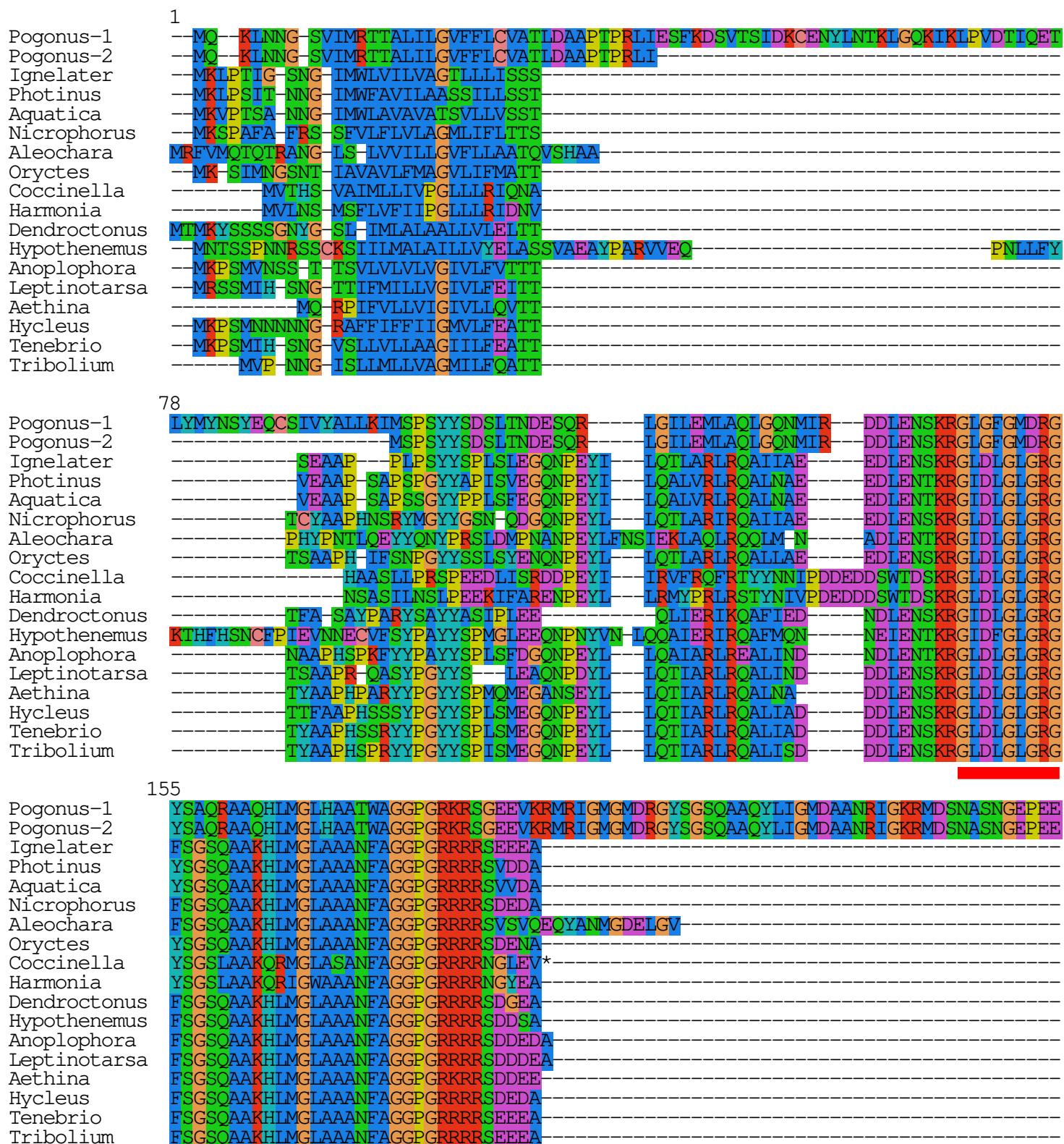
Tenebrio-1

MKAASFVLLAIALPAAYCFYLQPNEYHVPARLGTRNAPVYKSLGDLFHRLHLAS**KR**CVNTVDESC**C**INGGGNDAGND
 EDFLNGGDT**PGKR**CANLYDES**C**SNGGINGAGADDWLHGGNN**PGRR**

Tenebrio-2

MKLSLLIFAVAVALSTACGLYMPKHRPRDEEVFQLLASFFNQLGRRANM**KR**CANTFDESC**C**LNGPIGGATSDENWL
 SNGSP**PGKR**CSNIFGSS**C**VDGGTAGAGADEDFLGGGGG**PGRR**

Figure S23. Sequences of the various Calcitonin B genes from Coleoptera. Mature peptide sequences are in light blue the conserved C-terminal proline in dark blue, Gly residues that expected to be transformed into C-terminal amide in green and convertase cleavage sites and dibasic amino acid residues anticipated to be removed by carboxypeptidases in red. Signal peptides are highlighted in yellow.

**Figure S24.** Alignment of DH31 precursors.

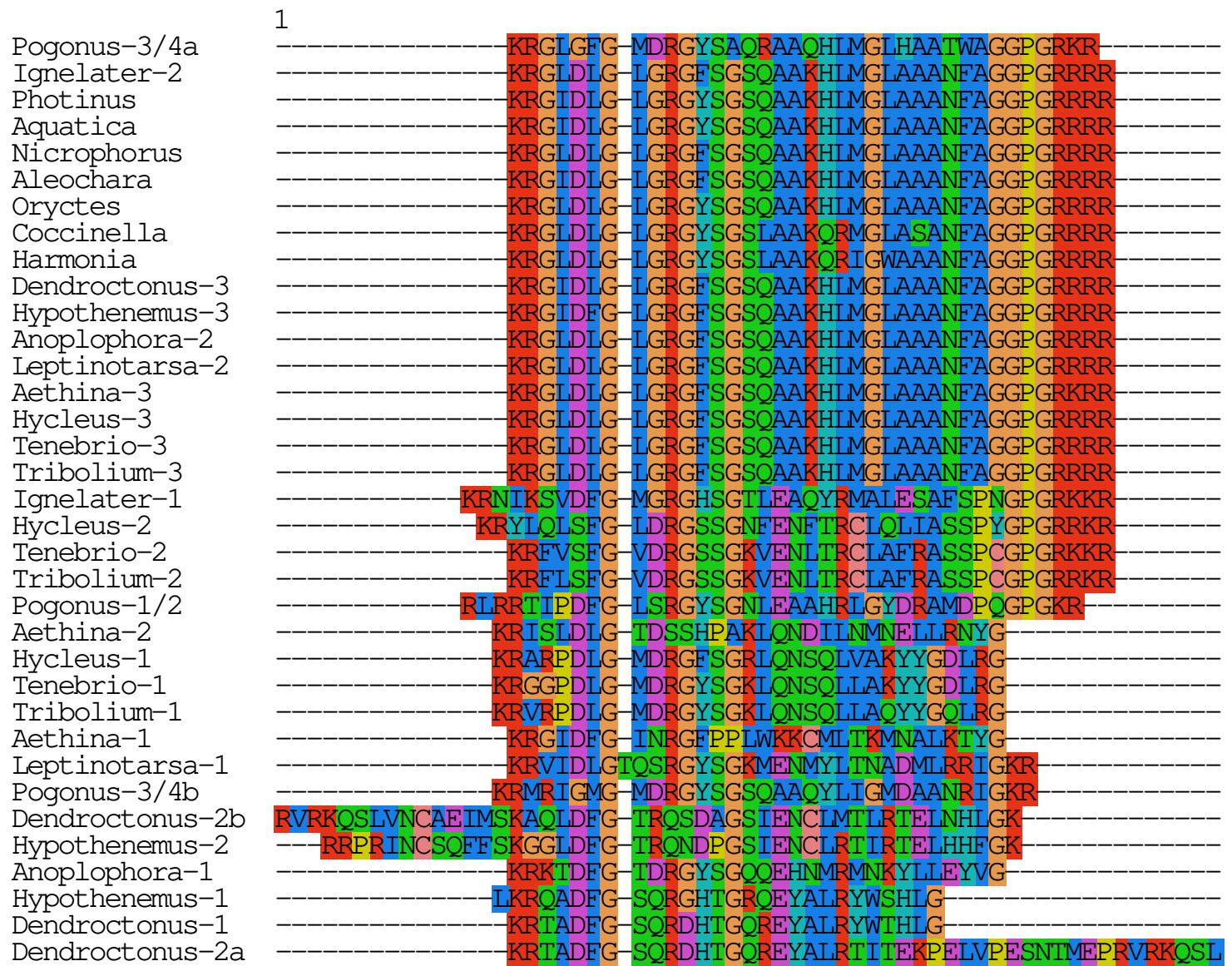
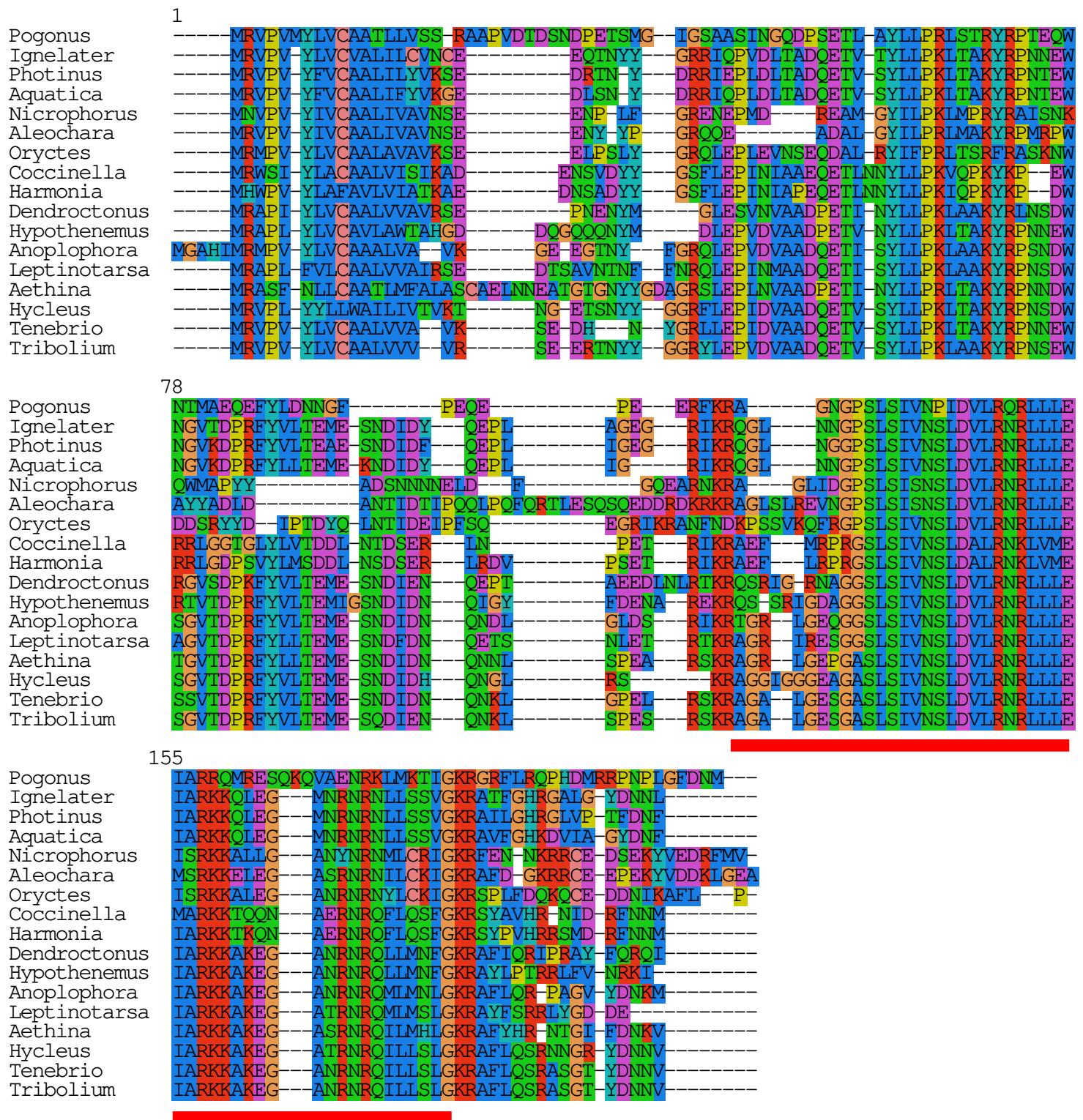


Figure S25. Alignment of DH31 and other putative neuropeptides encoded by transcripts from Coleoptera DH31 genes. Numbers behind the names indicates the transcript number (some species seem to have only one transcript). When this number is followed by a letter it indicates the putative peptide on that particular transcript. For example Pogonus-3/4a indicates DH31 itself that is coded by two transcripts in Pogonus and a refers to the first putative neuropeptide on this transcript. Pogonus-3/4b refers to the second putative neuropeptide on this transcript. Note that sequence conservation is very strong for DH31, but much less for the other putative neuropeptides encoded by these genes.

**Figure S26.** Alignment of DH47 precursors.

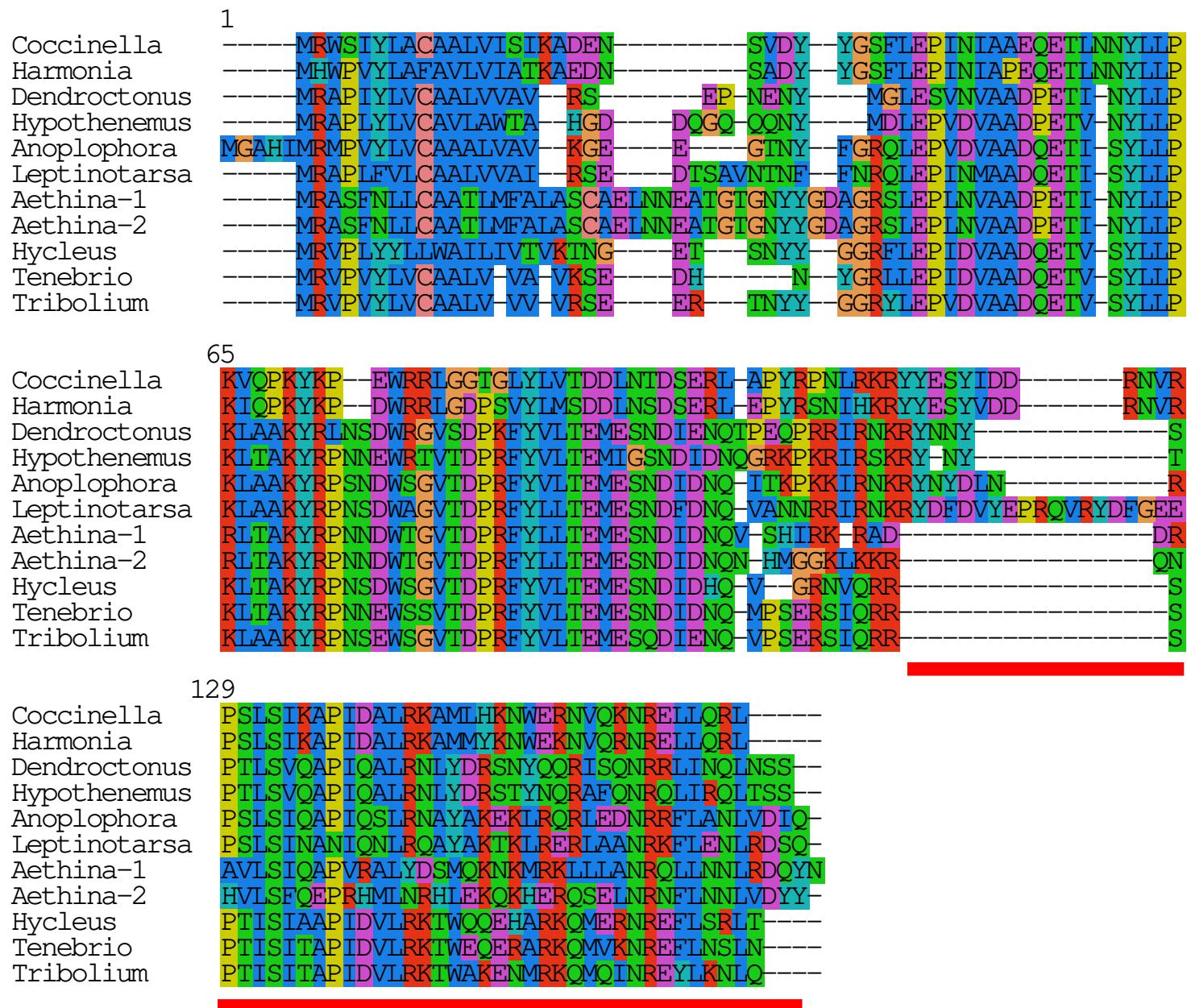
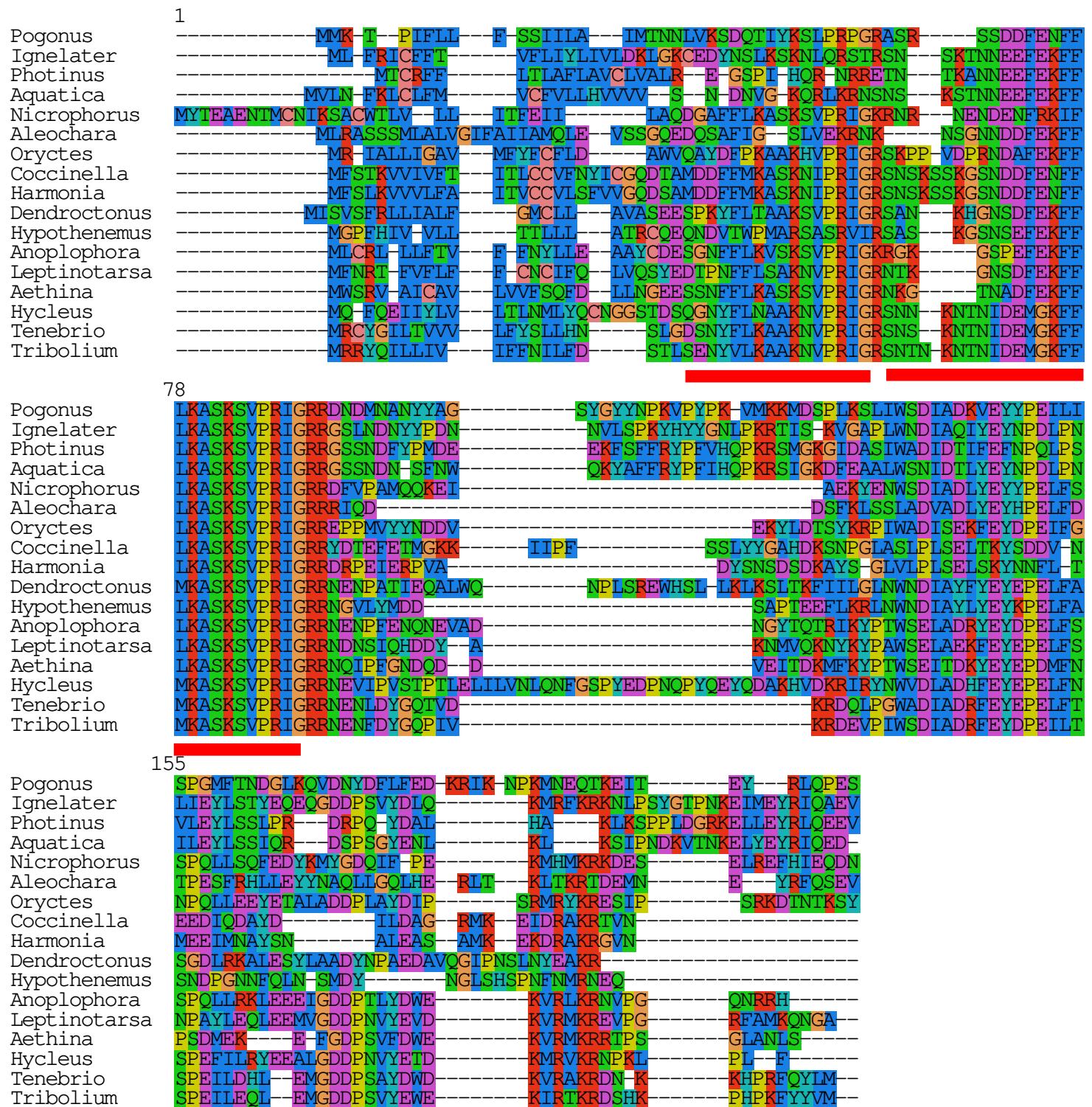


Figure S27. Alignment of DH37 precursors.

**Figure S28.** Alignment of ETH precursors.

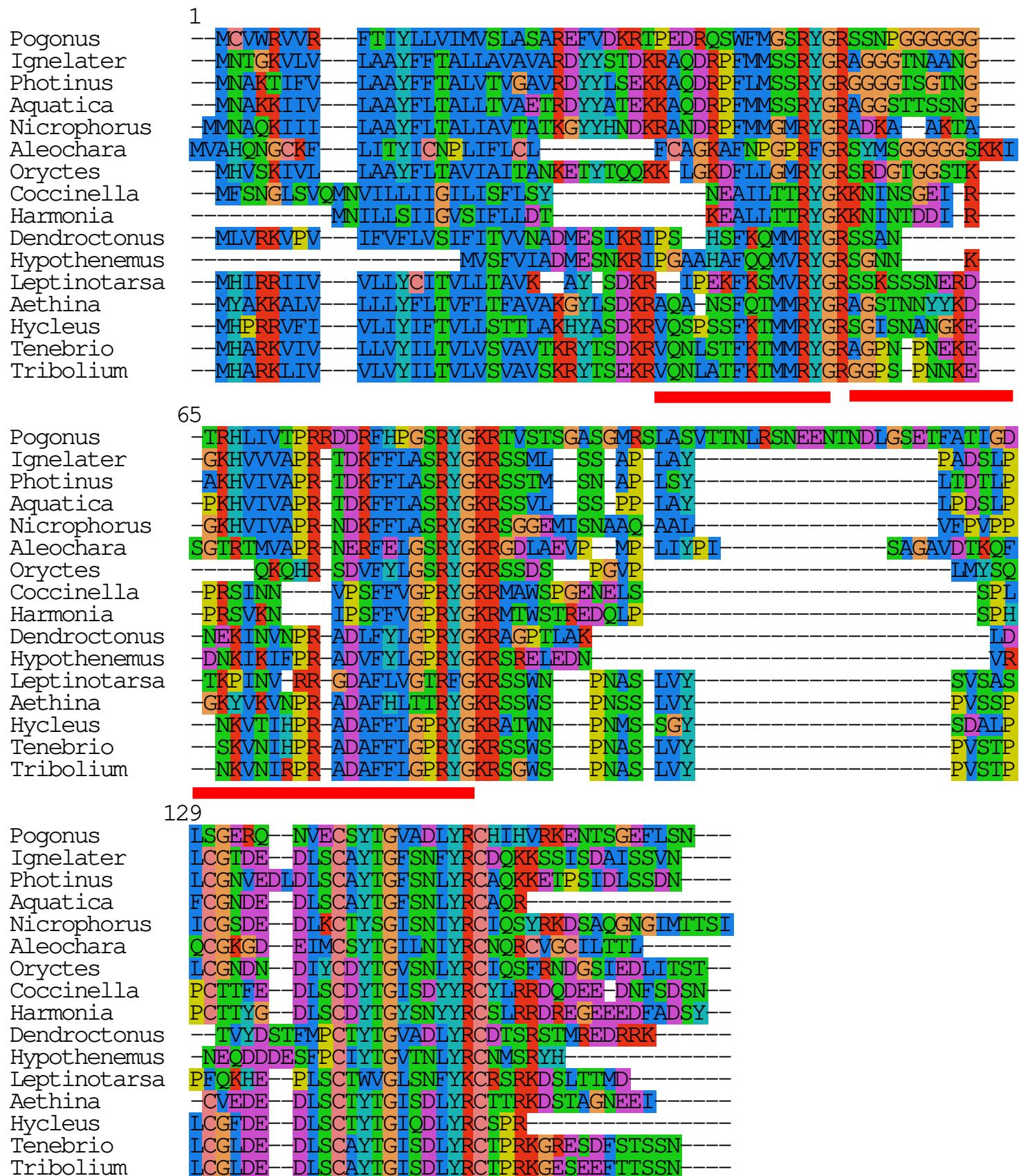
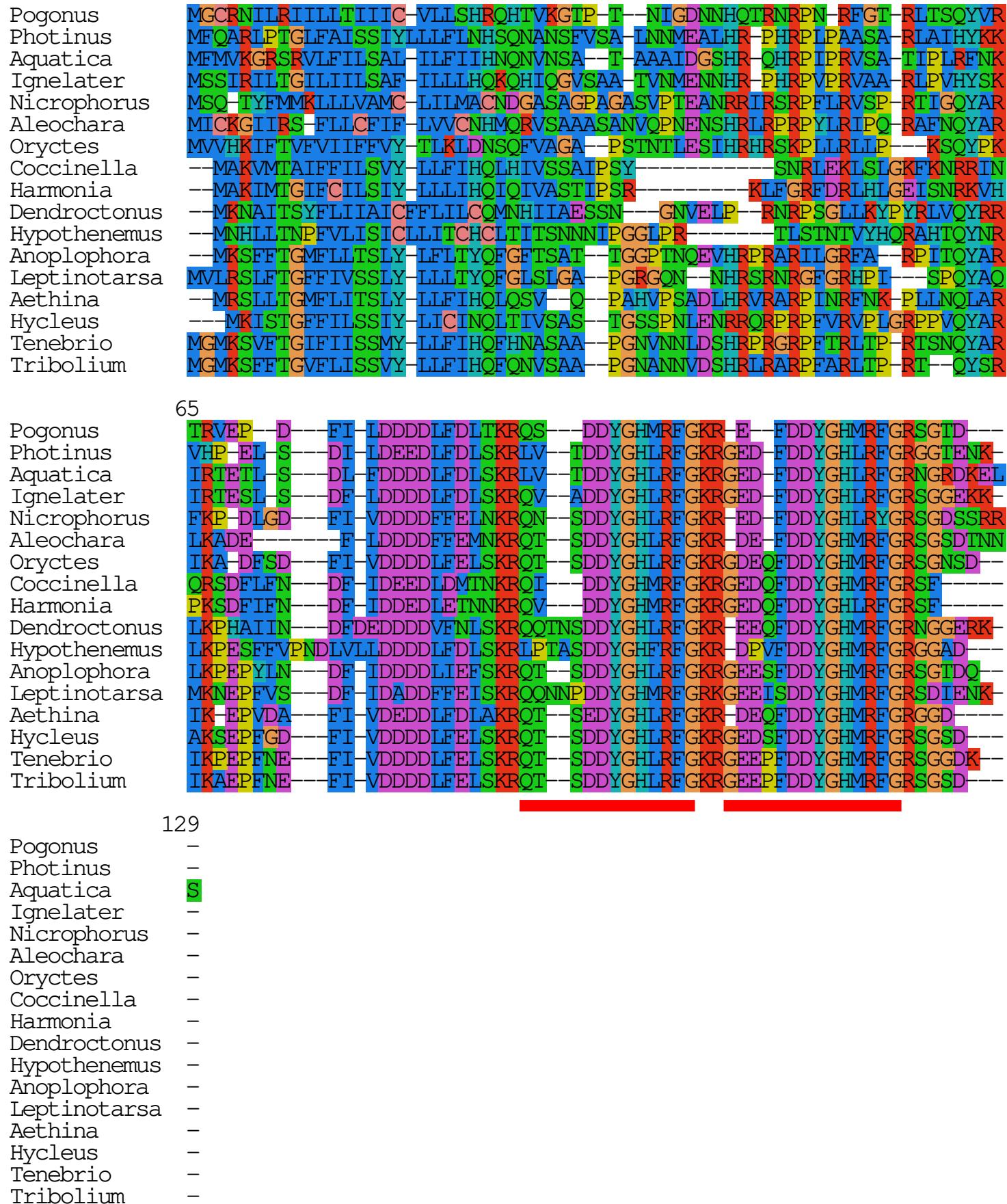
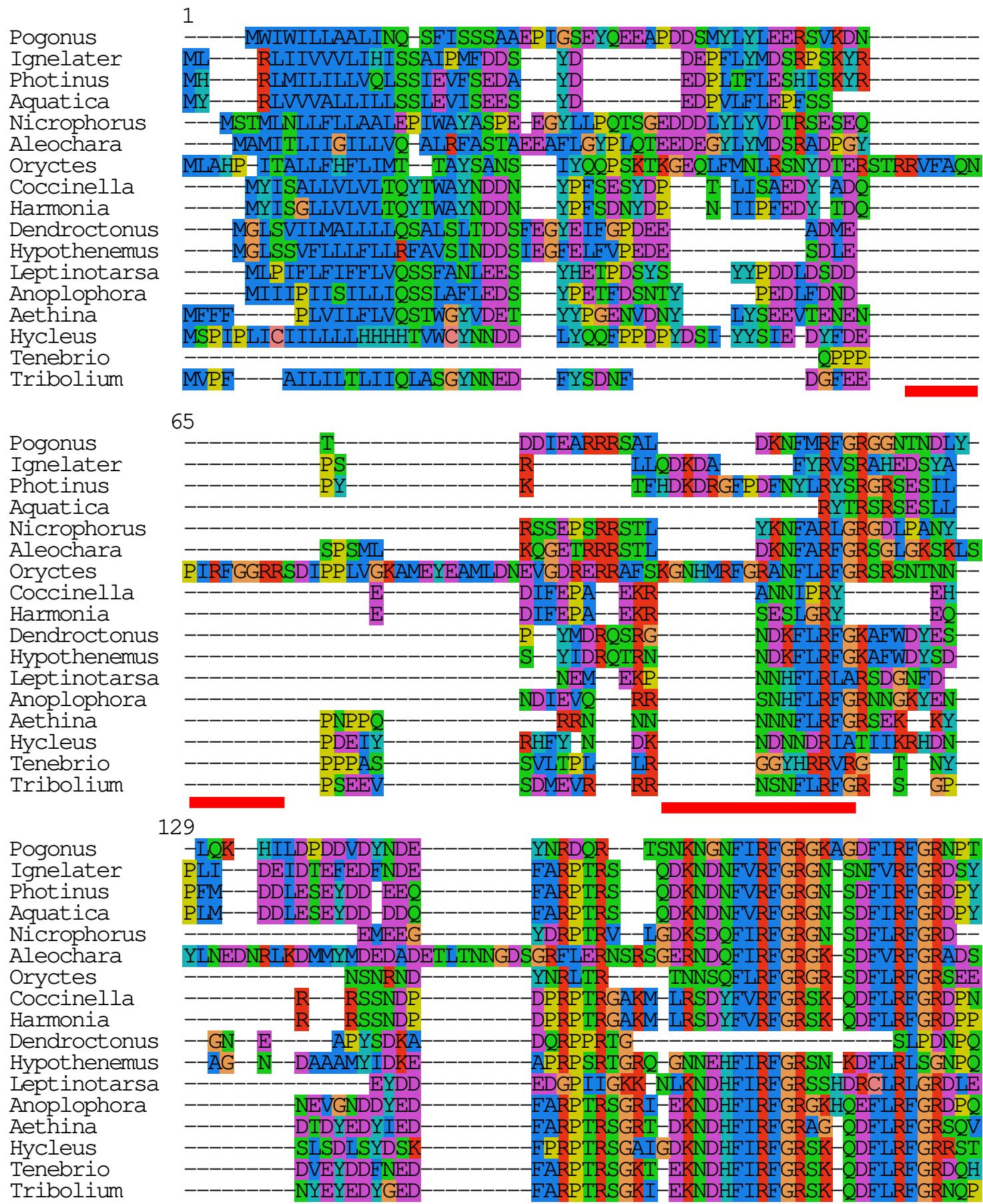


Figure S29. Alignment of RYamide precursors.

**Figure S30.** Alignment of Sulfakinin precursors.



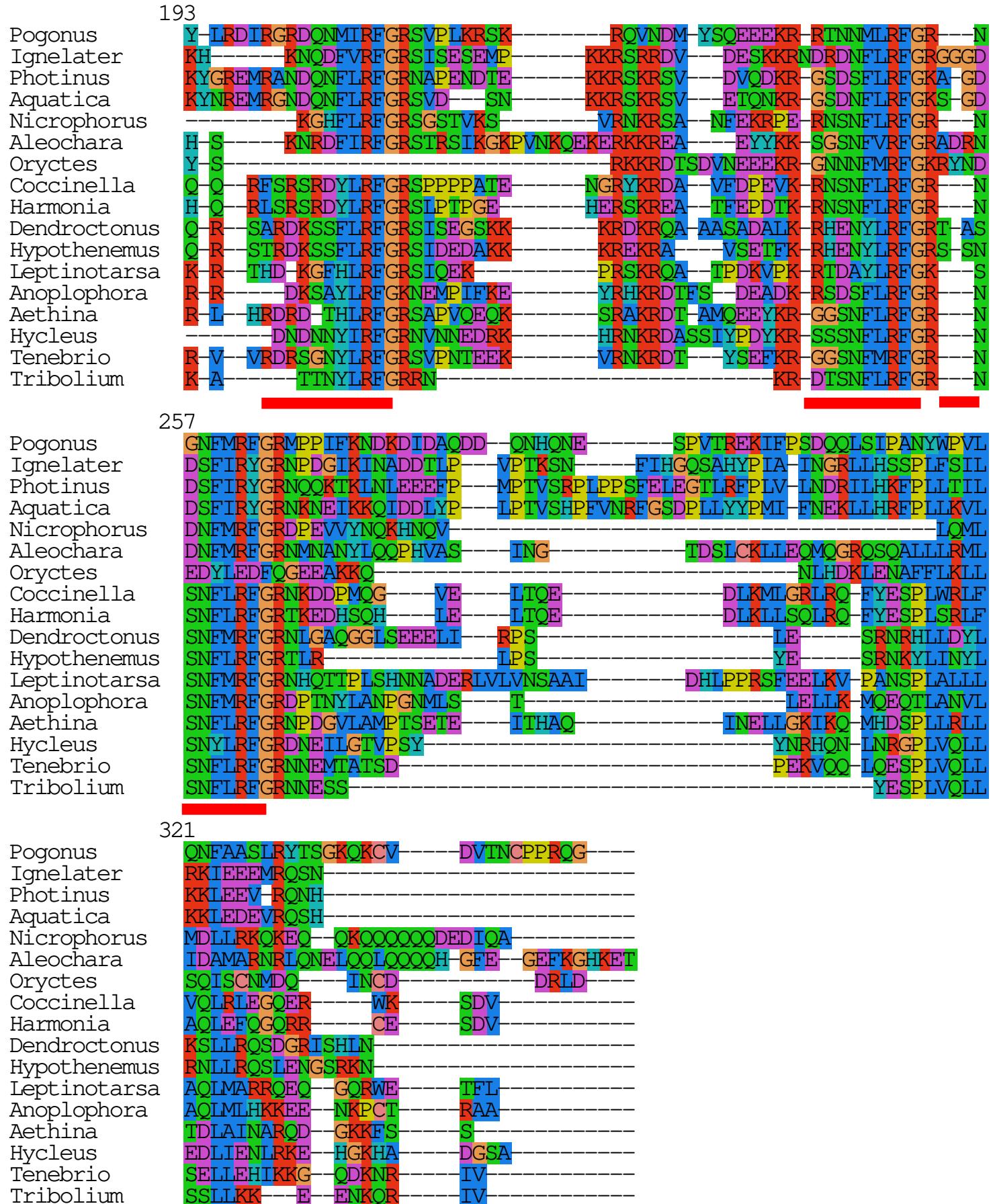
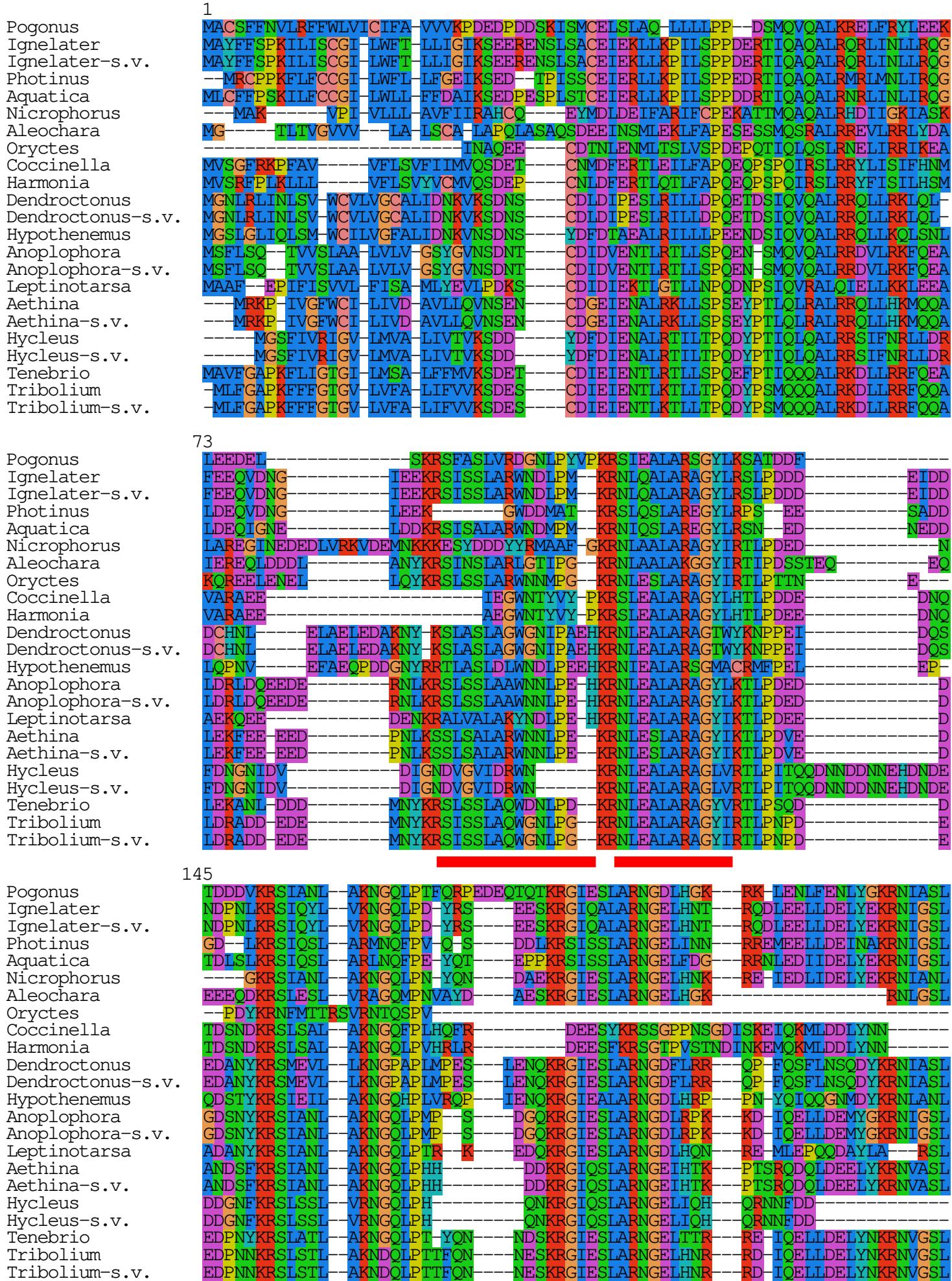
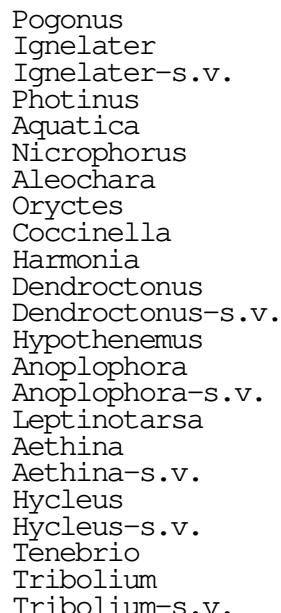
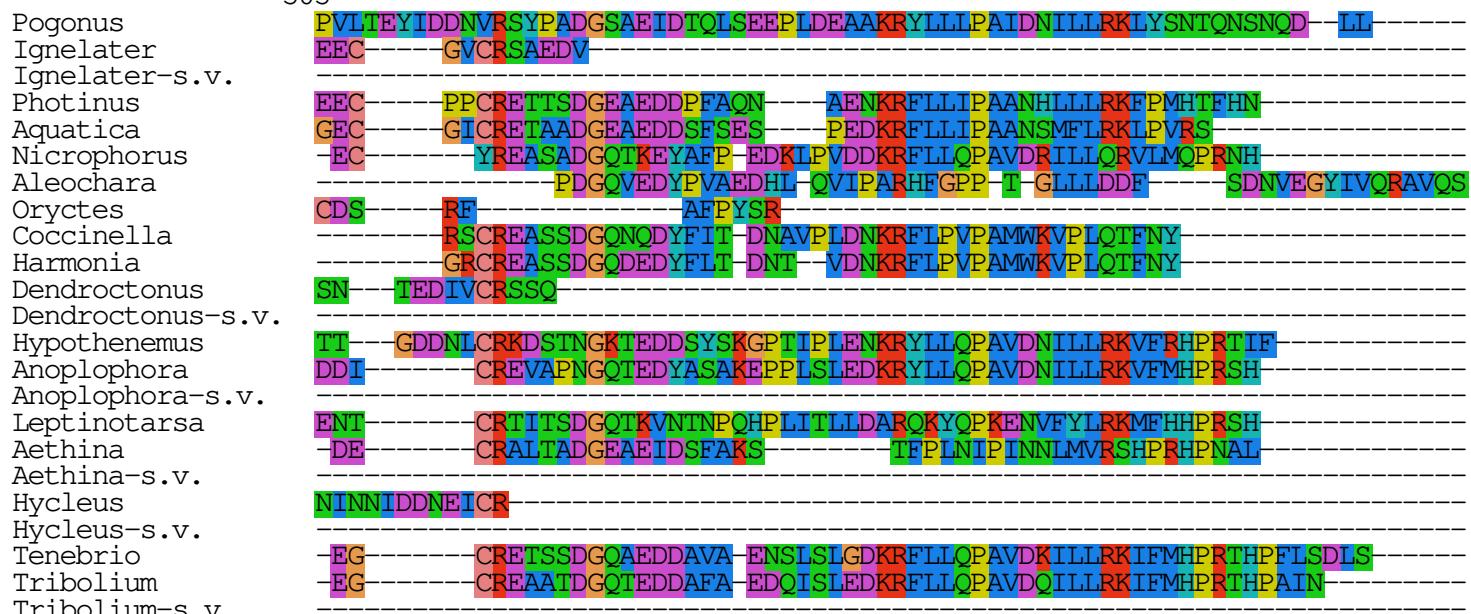
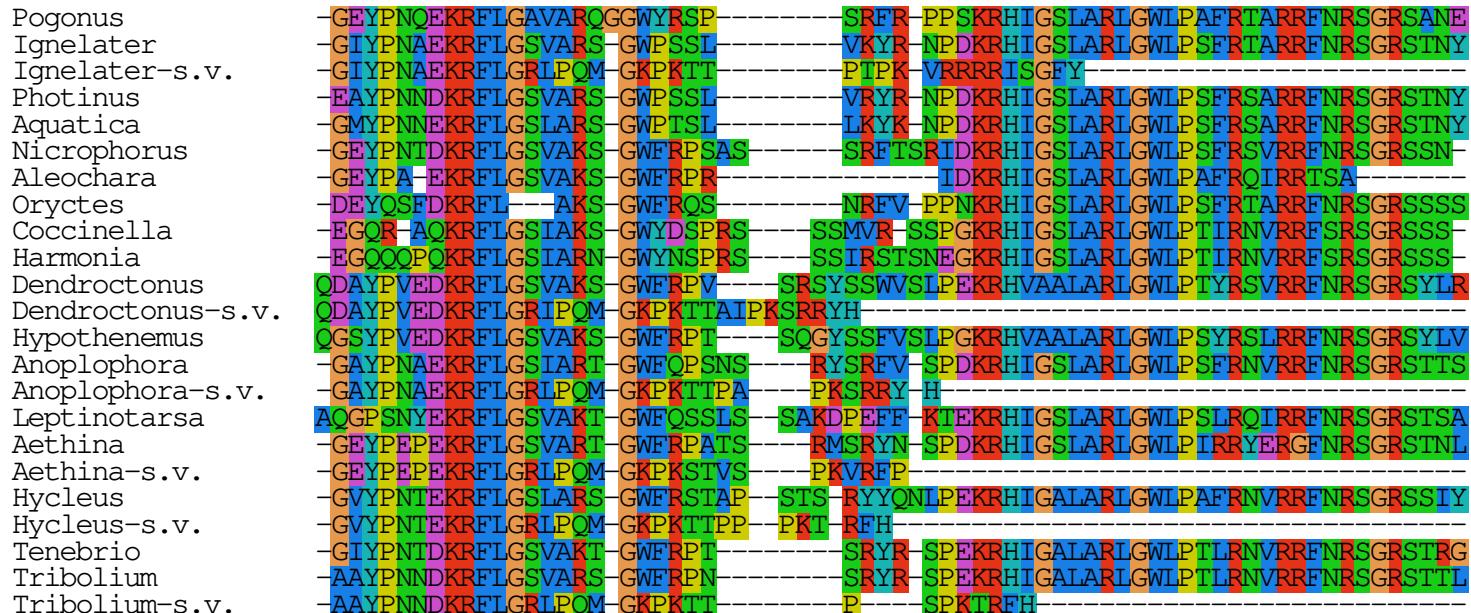


Figure S30. Alignment of FMRFamide precursors.





**Figure S32.** Alignment of NPLP1 precursors; s.v., splice variant.

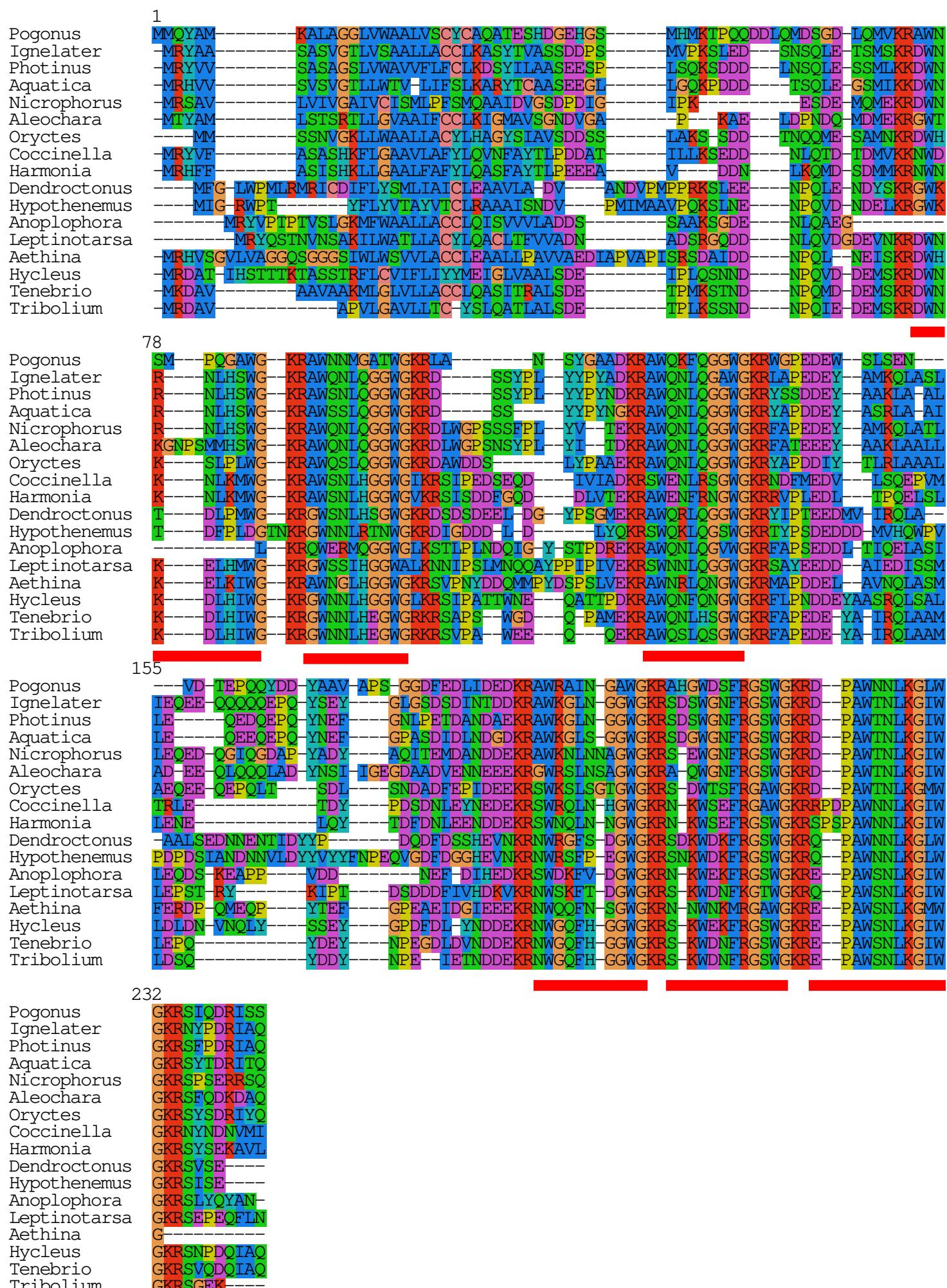
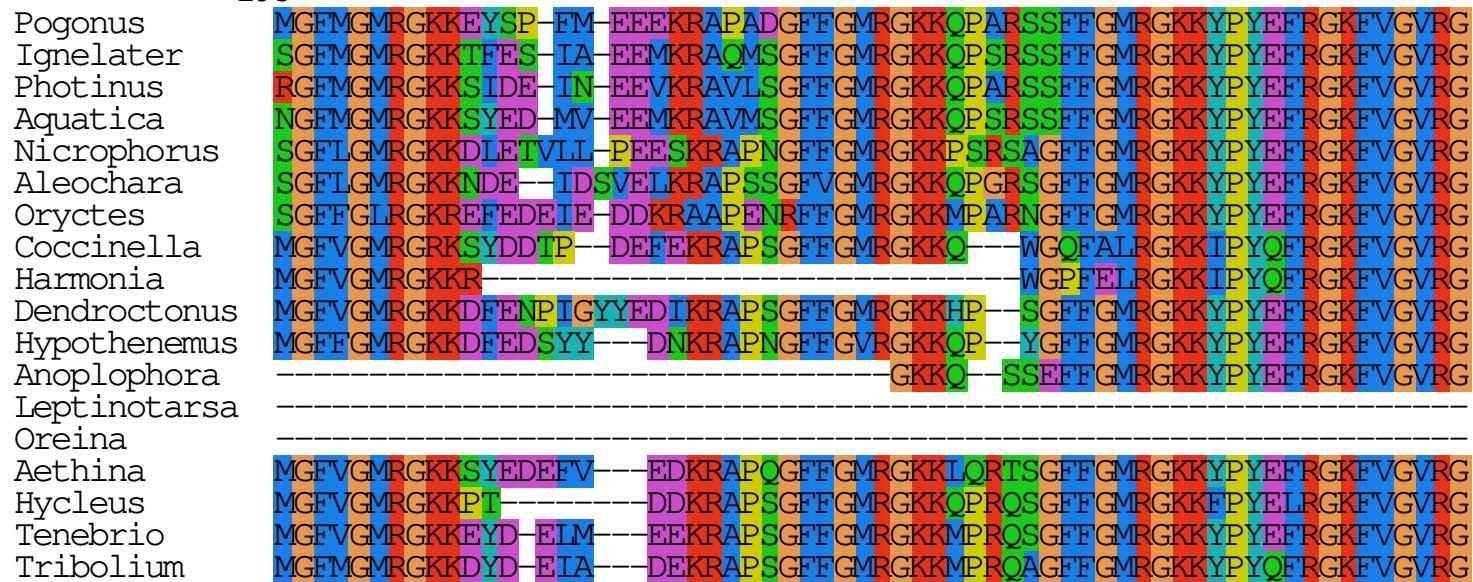


Figure S33. Alignment of allatostatin B precursors.



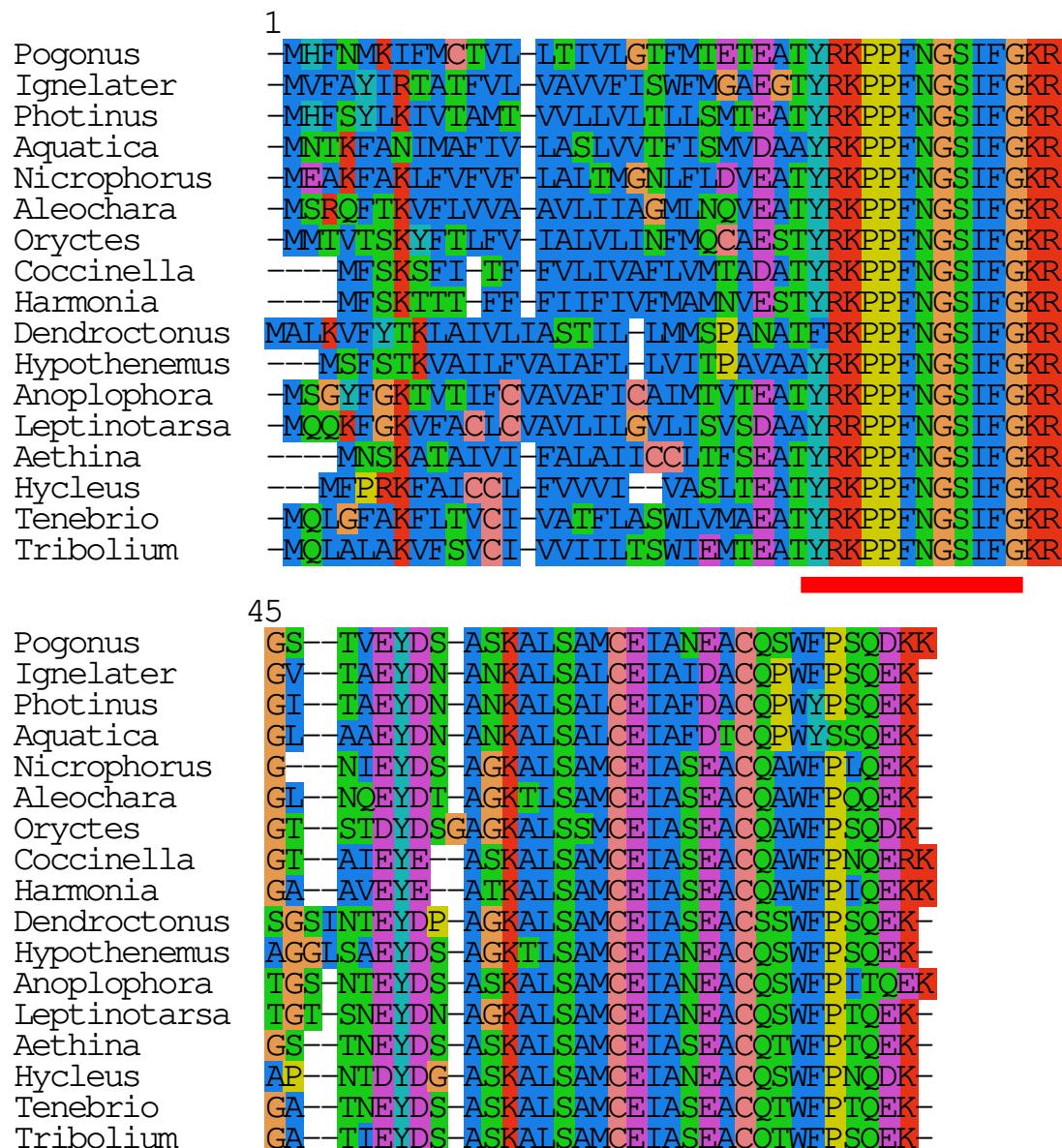
193

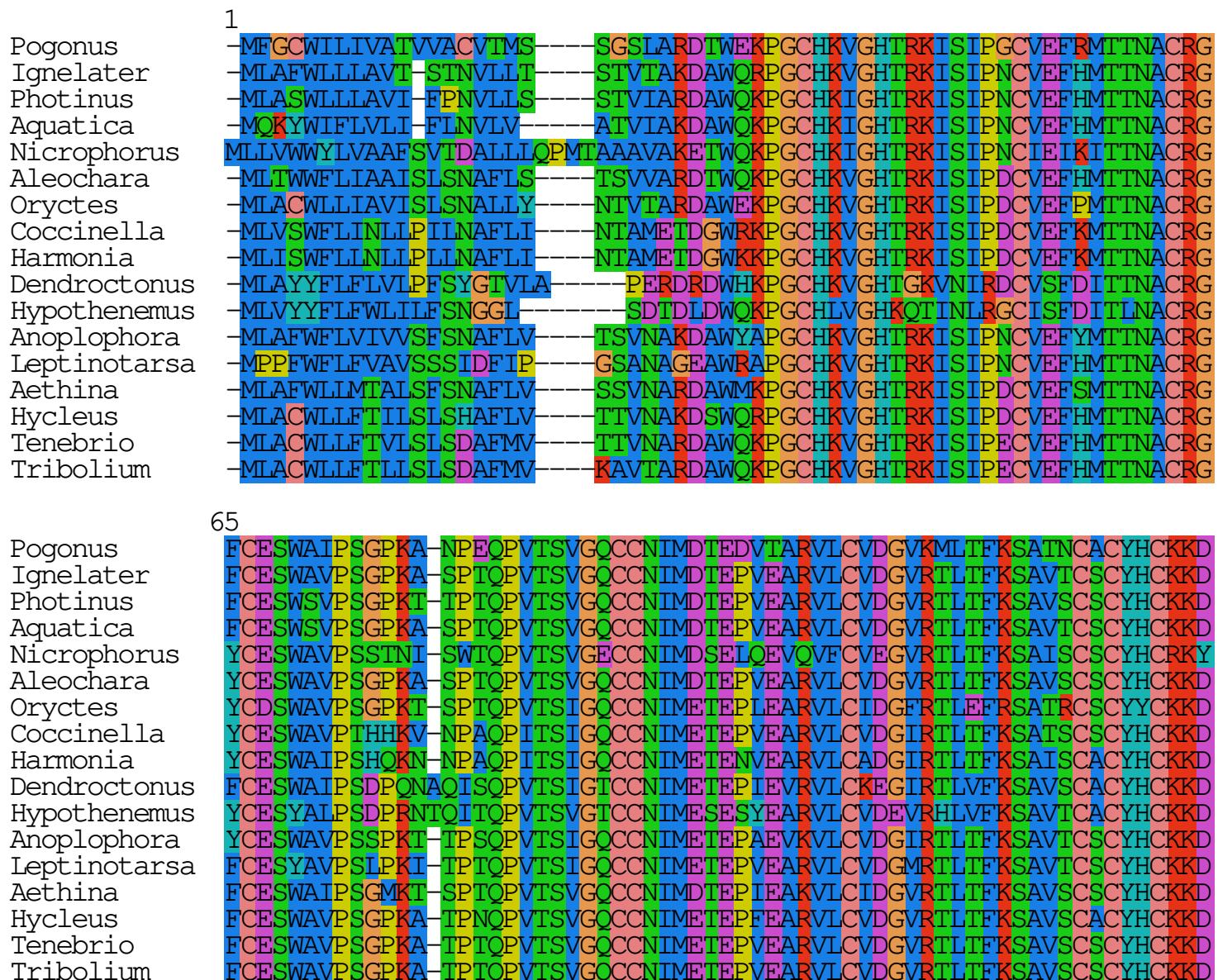


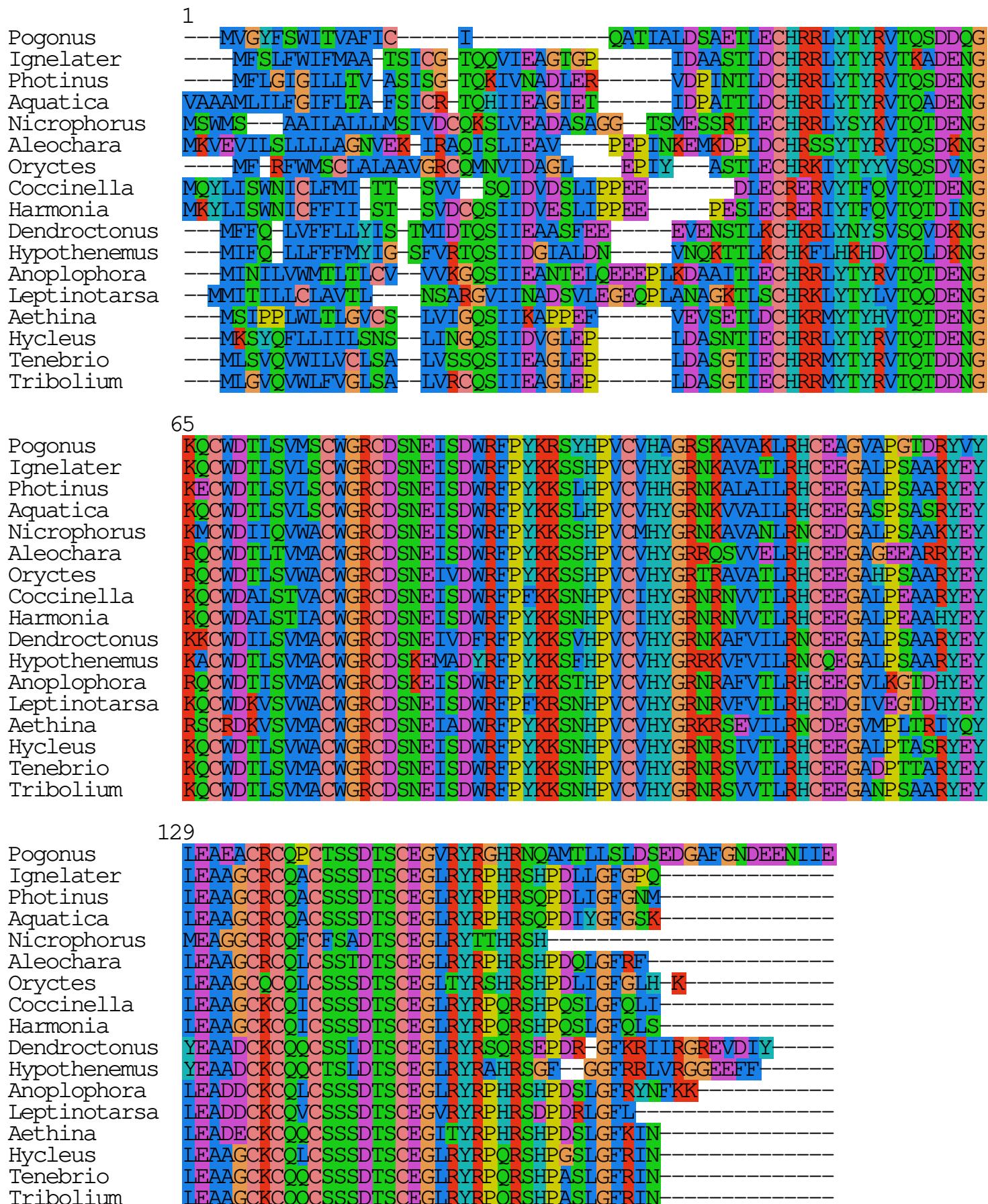
257

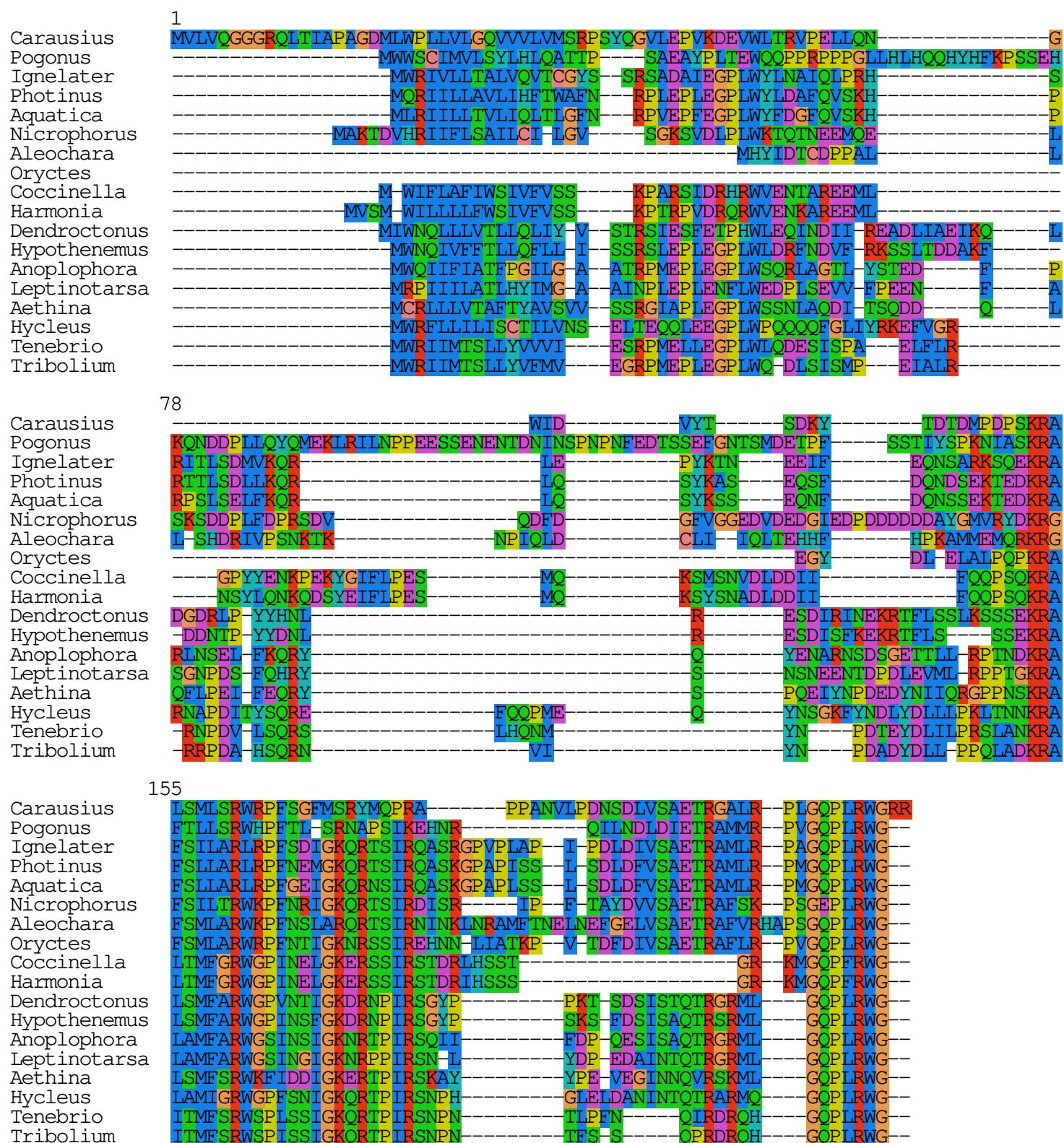
**Figure S34.** Alignment of Tachykinin precursors.

**Figure S35.** Alignment of CCAP precursors.

**Figure S36.** Alignment of SIFamide precursors.

**Figure S37.** Alignment of GPA2 precursors.

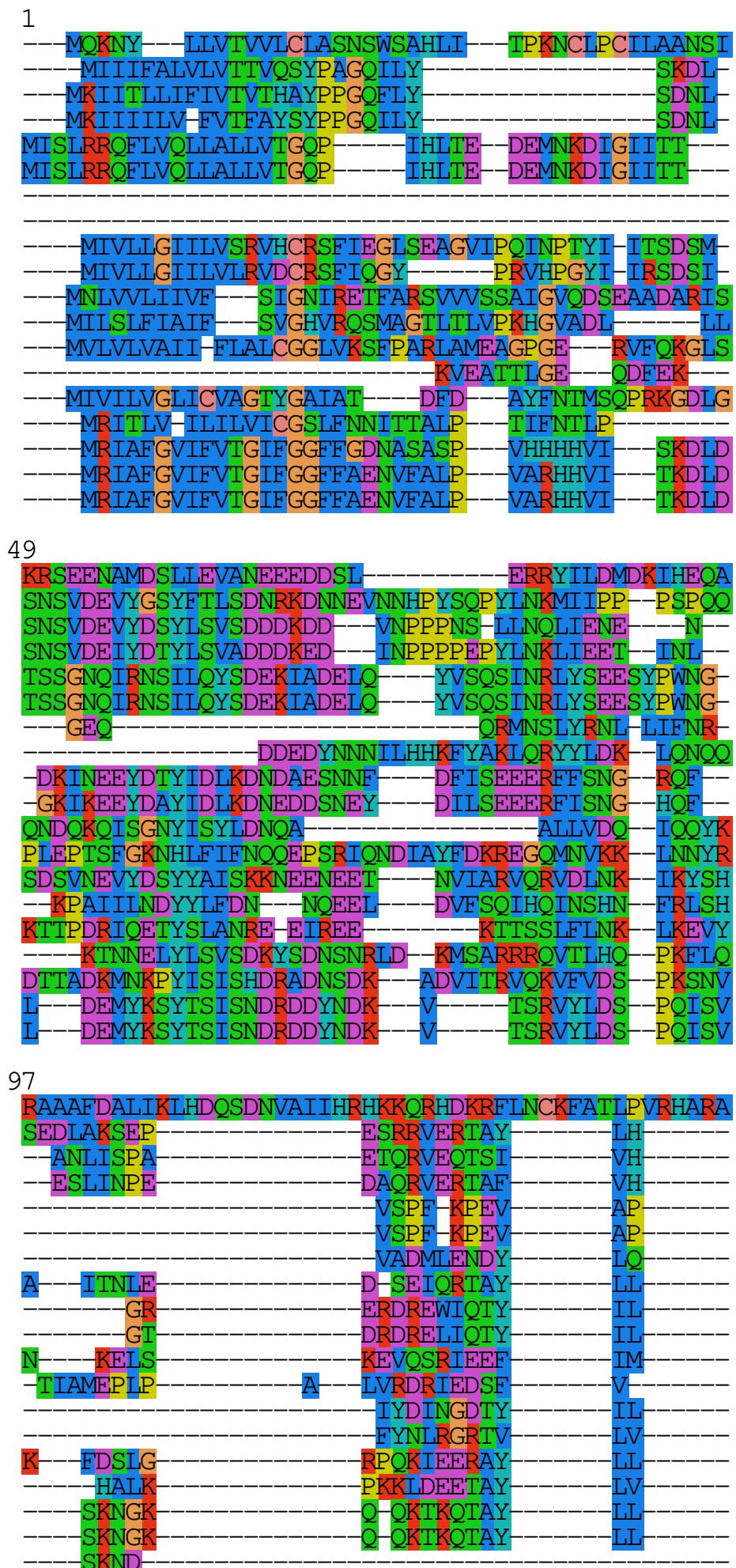
**Figure S38.** Alignment of GPB5 precursors.

**Figure S39.** Alignment of Hansolin precursors.

Pogonus
Ignelater
Photinus
Aquatica
Nicrophorus-splice-variant-1
Nicrophorus-splice-variant-2
Aleochara
Oryctes
Coccinella
Harmonia
Dendroctonus
Hypothenemus
Anoplophora
Leptinotarsa
Aethina
Hycleus
Tenebrio
Tribolium
Tribolium-splice-variant

Pogonus
Ignelater
Photinus
Aquatica
Nicrophorus-splice-variant-1
Nicrophorus-splice-variant-2
Aleochara
Oryctes
Coccinella
Harmonia
Dendroctonus
Hypothenemus
Anoplophora
Leptinotarsa
Aethina
Hycleus
Tenebrio
Tribolium
Tribolium-splice-variant

Pogonus
Ignelater
Photinus
Aquatica
Nicrophorus-splice-variant-1
Nicrophorus-splice-variant-2
Aleochara
Oryctes
Coccinella
Harmonia
Dendroctonus
Hypothenemus
Anoplophora
Leptinotarsa
Aethina
Hycleus
Tenebrio
Tribolium
Tribolium-splice-variant



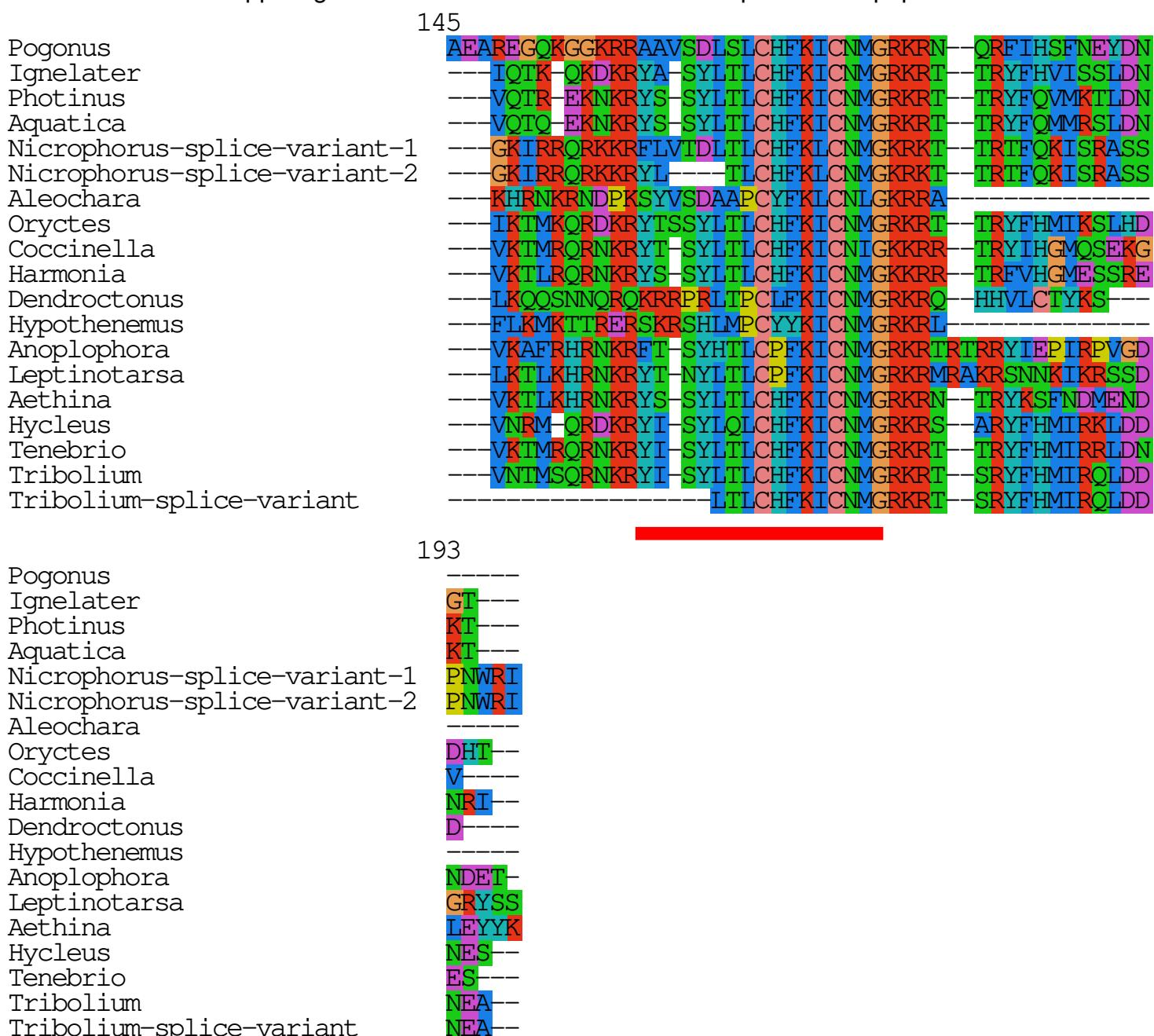


Figure S40. Alignment of CNMamide precursors.

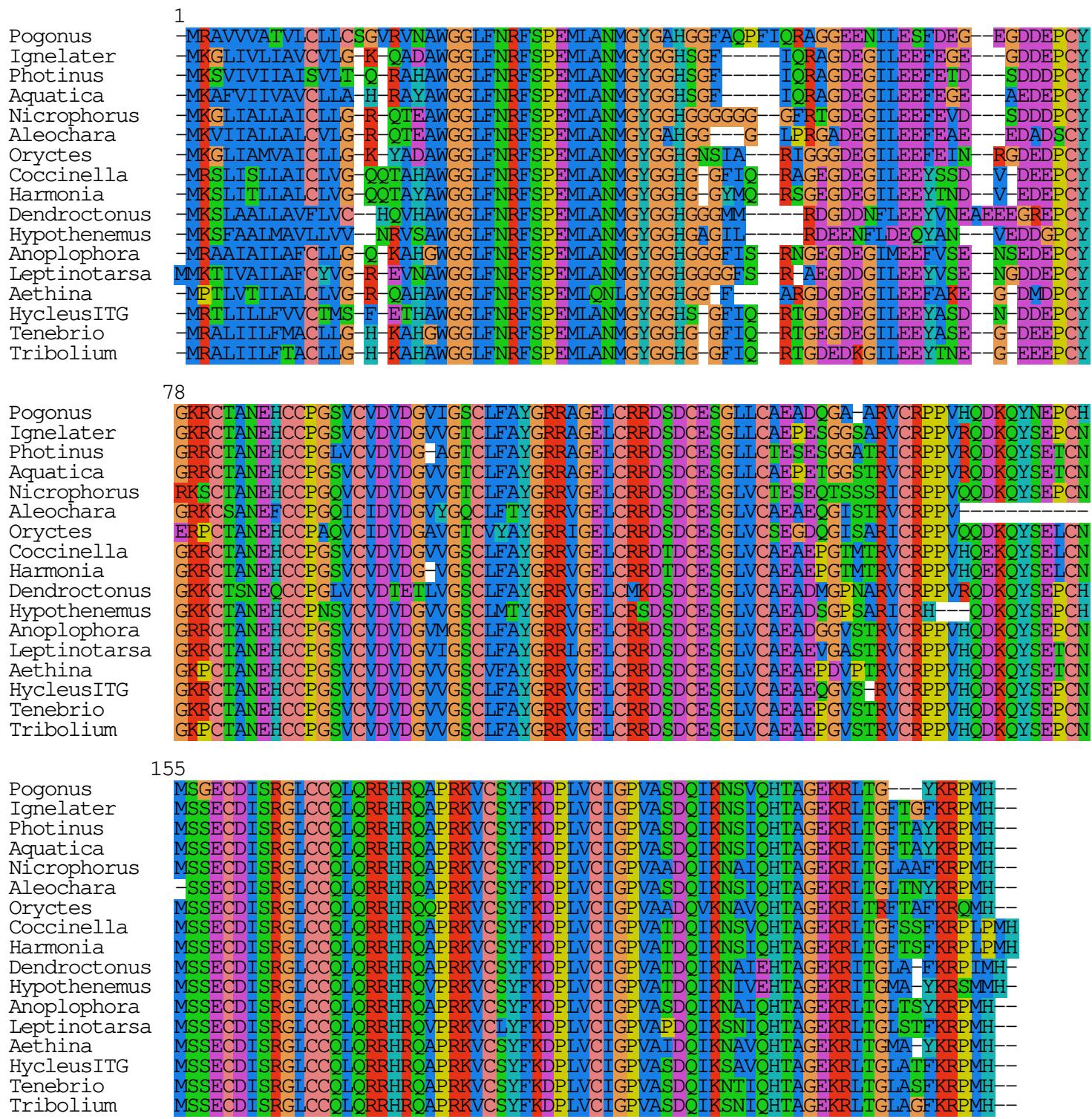


Figure S41. Alignment of ITG-like precursors.

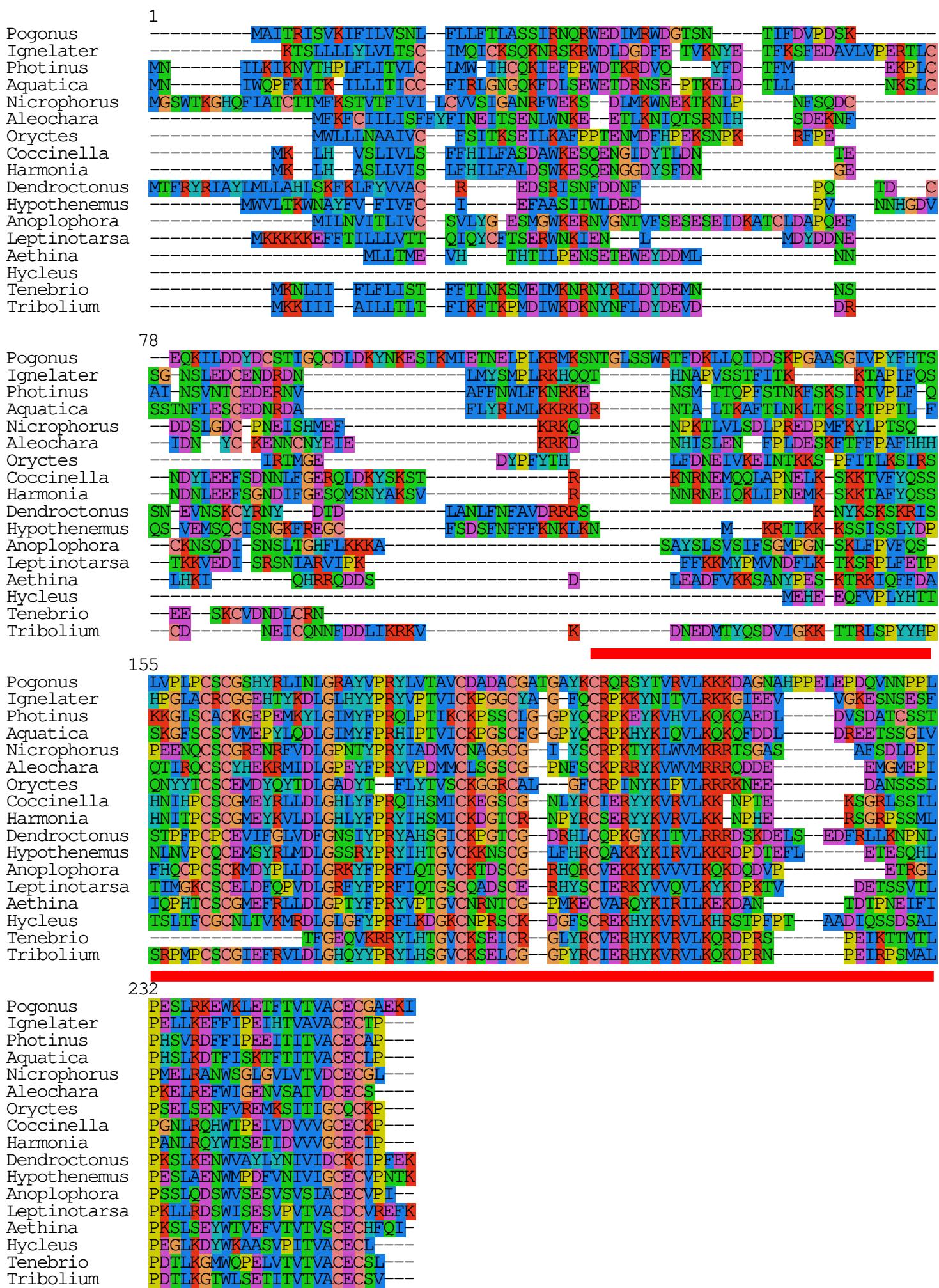


Figure S42. Alignment of PTTH precursors.

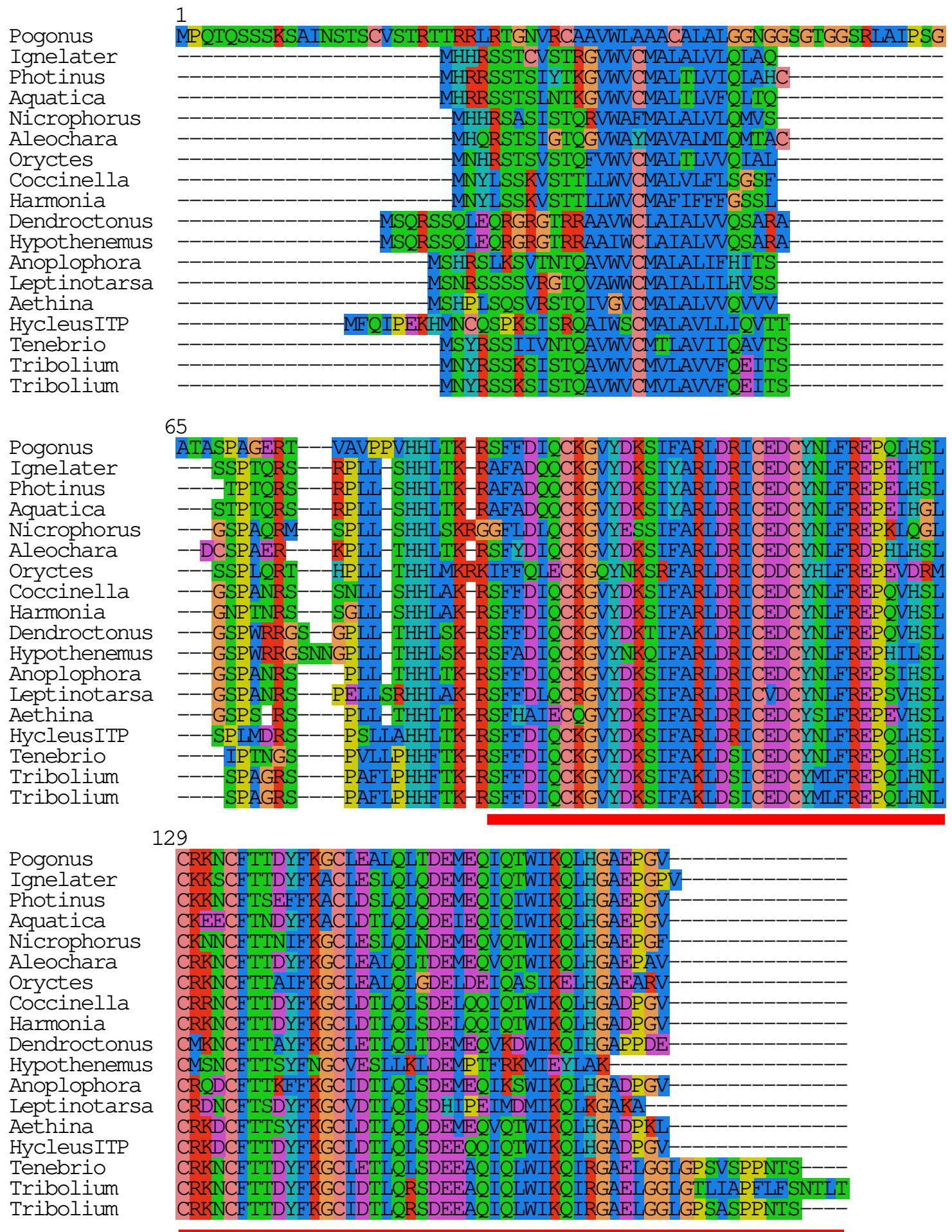
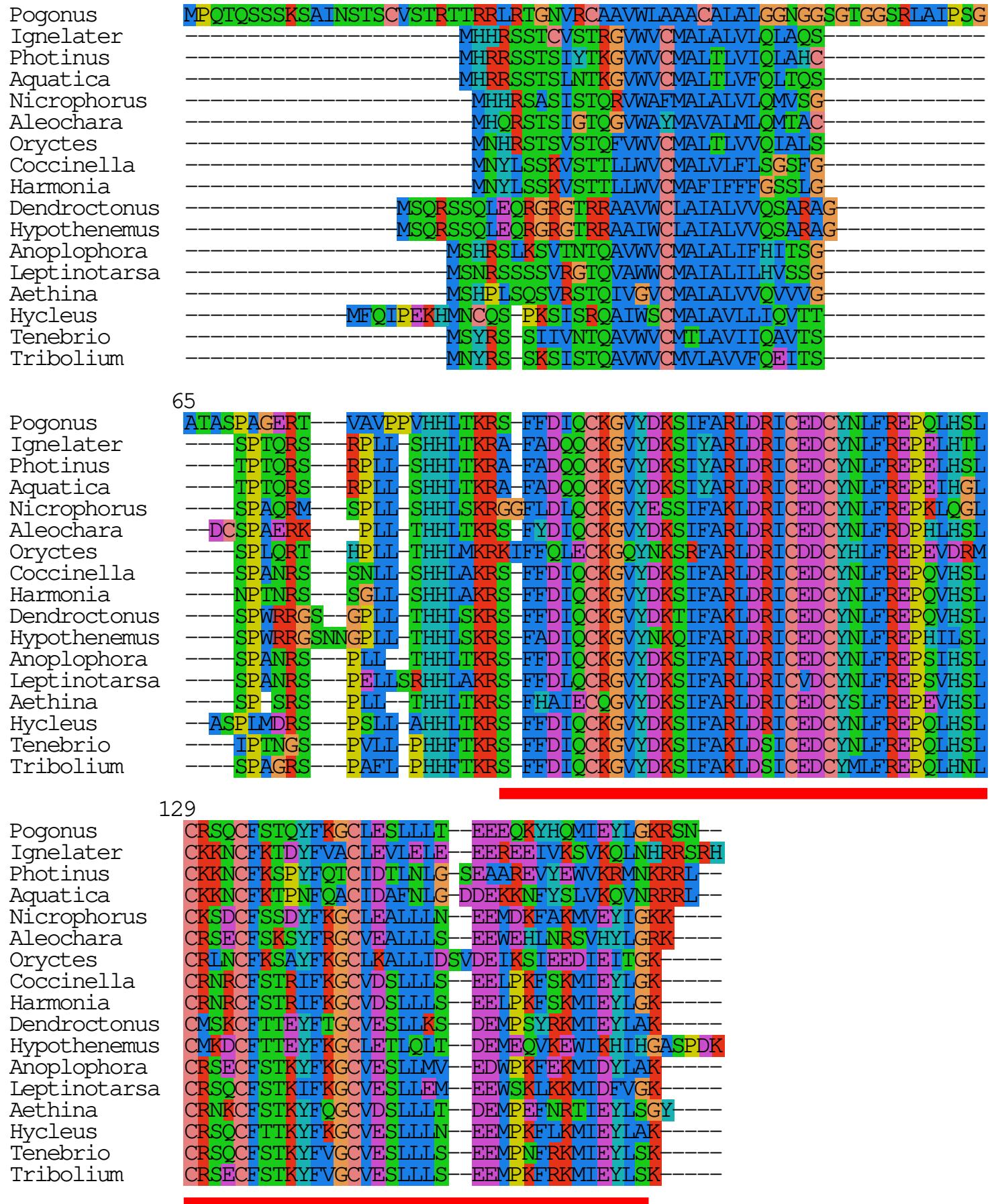


Figure S43. Alignment of ITP-A precursors.

**Figure S44.** Alignment of ITP-B precursors.