

Supplementary material for:

**Transcriptome profiling of the Pacific oyster *Crassostrea gigas* visceral ganglia over a reproduction cycle identifies novel regulatory peptides.**

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**Supplementary figure 1:**

Novel (neuro)peptide precursors characterized in *C. gigas*: Expression during development, in adult tissues and in the visceral ganglia over a cycle of reproduction.

A: Alignment of *C. gigas* NPP sequences with the sequence of related precursors from other species. Amino acids with similar physicochemical properties are in the same color. Signature sequence features are indicated in red above the sequences.

B Expression pattern of NPP transcripts during development (data retrieved from GigaTON, Rivière et al. 2015); Oo: Oocytes; Mor: Morula; Bla: Blastula; Gas: Gastrula; Tro: Trochophore larvae; D-sha: D-shaped larvae; Umb: Umbo larvae; Ped: Pediveliger larvae; Juv: Juvenile.

C: Expression pattern of NPP transcripts in adult tissues (data retrieved from GigaTON, Rivière et al. 2015). MU: Adductor muscle; FGO: Female gonad (mix stages); MGO: Male gonad (mix stages); Gills; Hem: Hemolymph; LPA: Labial palps; Man: mantle; Dgl: Digestive gland.

D Expression pattern of NPP transcripts in the visceral ganglia during the cycle of reproduction..

Bilaterian family

## A



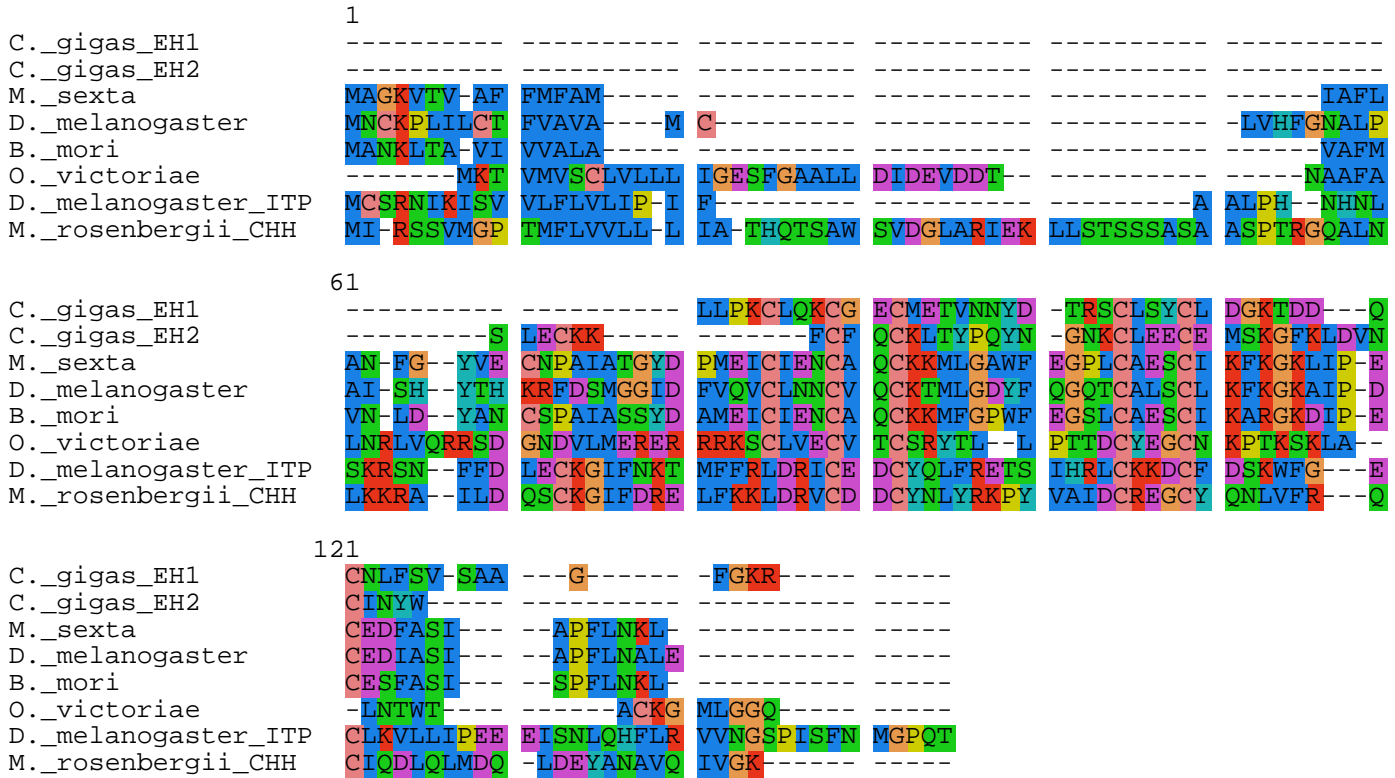
| Condition | Mean Correct Responses |
|-----------|------------------------|
| IO        | ~2.7                   |
| StF1      | ~2.1                   |
| StM1      | ~2.6                   |
| StF2      | ~1.6                   |
| StM2      | ~2.5                   |
| StF3      | ~2.6                   |
| StM3      | ~2.9                   |

A: Alignment of *Cragi-IL17/CFSH1*(CGG\_contig\_09983 genome scaffold 678 232727 to 233272 (+)), *Cragi-IL17/CFSH2* (CGG\_contig\_07061 genome scaffold160 103046 to 103573 (+), *P. yessoensis* (XP\_021370734.1), *L. gigantea* (XP\_009051410.1), *A. californica*(XP\_005097238.1), *S. paramamosain\_1b* (Veenstra, 2016), *S. paramamosain\_2a* (Veenstra, 2016) , *C. maenas\_1* (Veenstra, 2016) , *L. vannamei\_1a* (Veenstra, 2016) *CFSH*, *D. rerio* (NP\_001018623.1), *H. sapiens* (AAH69152.1) IL17 sequences.

D: Expression pattern of *Cragi-IL17/CFSH1*(CGG\_contig\_09983) and *Cragi-IL17/CFSH2* (CGG\_contig\_07061) encoding genes

## Cragi-EH (Eclosion Hormone)

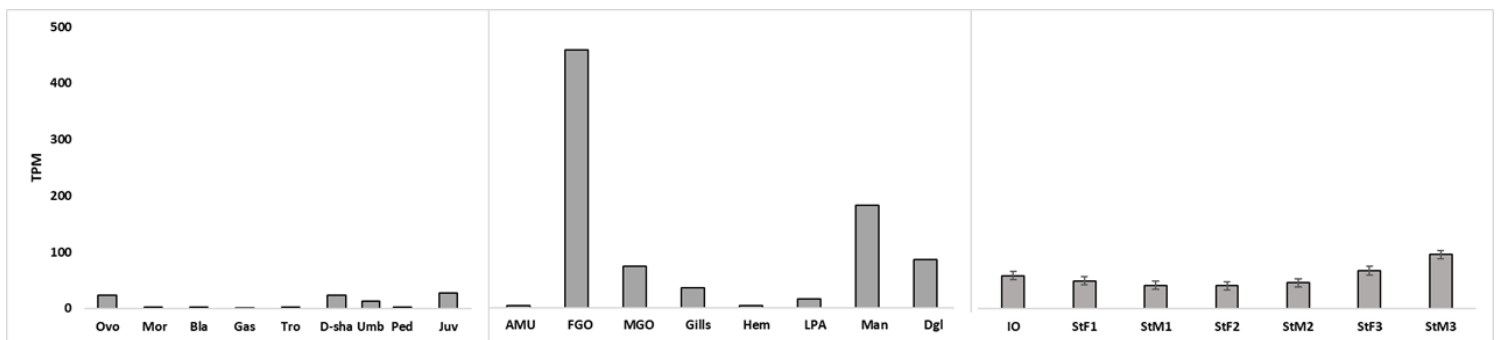
A



B

C

D



A : Alignment of *C. gigas* (CGG\_contig\_11351), *M.sexta*(AAA29310.1), *D.melanogaster* (AAF55423.1), *B.mori* (NP\_001037307.1), *O.victoriae*(ASK86246.1) EH, *D. melanogaster* ITP (NP\_611931.3) and *M. rosenbergii* (AAF29534.1) CHH sequences.

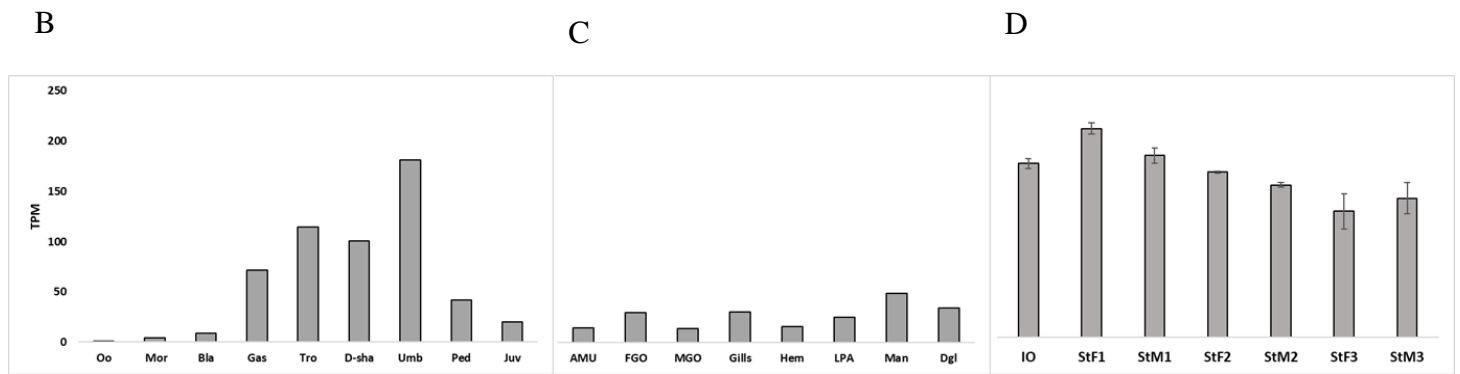
B : Expression pattern of *Cragi-EH* (CHOYP\_contig\_053689) encoding gene during development

C : Expression pattern in adult tissues.

D: Expression pattern of *Cragi-EH* (CGG\_contig\_11351) encoding gene in the visceral ganglia during the cycle of

## Cragi-Nesfatin

[illegible]



A : Alignment of *C. gigas* (CGG\_contig\_14701), *A. californica* (XP\_012940282.1), *C. teleta* (ELT97554.1) , *D. melanogaster* (AF044203). ; *O. victoriae* (ASK86275.1); *D. rerio* (NP\_958901.1) and *H. sapiens sp* (P80303.3) nesfatin sequences.

B : Expression pattern of Cragi-Nesfatin encoding gene (CHOYP-NUCB1.1.1) during development.

C : Expression pattern in adult tissues.

D : Expression pattern of Cragi-Nesfatin (CGG-contig-14701) encoding gene in the visceral ganglia during the cycle of reproduction.

## A



D

D : Expression pattern of Cragi-Neuroparsin (CGG-contig-00821) encoding gene in the visceral ganglia during the cycle of reproduction.

## Cragi-Urotensin

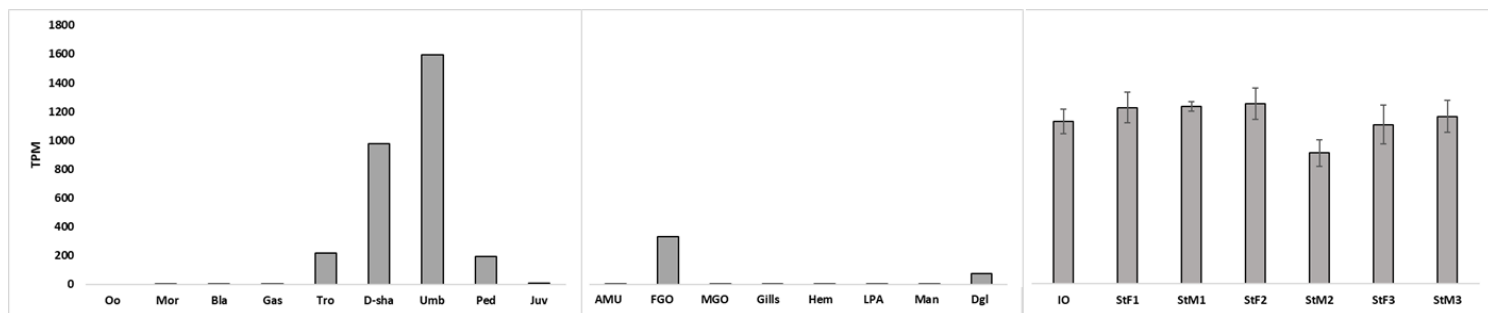
**A**

|                |   |             |            |             |             |             |             |
|----------------|---|-------------|------------|-------------|-------------|-------------|-------------|
| C._gigas       | 1 | -----       | MNSLSLLCLV | VLSLALLIGNY | VCSSEPPQFF  | ADEDEISE-P  | TEEQR--MM-  |
| A._californica |   | ---MISKSL   | MVMSVYVLV  | ALLAAMHGST  | -----PASAM  | PLEDDNTD-I  | --DDKR--SM- |
| C._teleta      |   | MRSTLTNTGL  | VALATLLLLL | ETSAALSSSS  | LLREEPEKITP | QESVER--    | ---SKR--S-- |
| D._rerio       |   | ---MI---    | -CKLFVFC-- | --SVLLLLSCS | LLSAHPVTDT  | AEMTNI GPDS | VEEAGGVSM D |
| 61             |   |             |            |             |             |             |             |
| C._gigas       |   | --INNKYQTH  | LM--KNAFRD | -----       | SGNLKR---   | --L-----    | -----LAQKR  |
| A._californica |   | --LLSKYMAL  | MNRLQEQRRQ | -----       | EGDLSD---   | --LVGSPYVQ  | Q---RSQKR   |
| C._teleta      |   | ---AHDLLKR  | LNQLDNTFSS | YYDYLLSSSE  | ASDNYD---   | --VQESKFLV  | EESPSSIQKI  |
| D._rerio       |   | DFSVDLTDV   | LQRAAAAAHS | PLLNRDSIKM  | SGQISKDALK  | EFLLKPYRL   | VPPSGLL---  |
| 121            |   |             |            |             |             |             |             |
| C._gigas       |   | FSSD--RQTRG | GVALCLWKVC | PAGPWLVOK   | ----        |             |             |
| A._californica |   | FKSPMQSRSG  | GMSLCLWKVC | PAAPWLISK   | ----        |             |             |
| C._teleta      |   | APAFKRQASS  | GAQICIWKVC | PPTPWRRARK  | SRAN        |             |             |
| D._rerio       |   | GSRRQFRKRG  | GGADCFWKYC | V-----      |             |             |             |

B

C

D



A : Alignment of *C. gigas* (CGG\_contig\_30764, CGG\_contig\_09857, CGG\_contig\_22724, CGG\_LOC105343318 ), *A. californica* (XP\_012945633.1), *C. teleta* (ELU12290.1) and *D. rerio* (XP\_005157985.1) urotensin II sequences.

B : Expression pattern of Cragi-Urotensin encoding gene (CHOYP-contig-004921) during development.

C : Expression pattern in adult tissues.

D : Expression pattern of Cragi-Urotensin (CGG-contig-30764) encoding gene in the visceral ganglia during the cycle of reproduction.

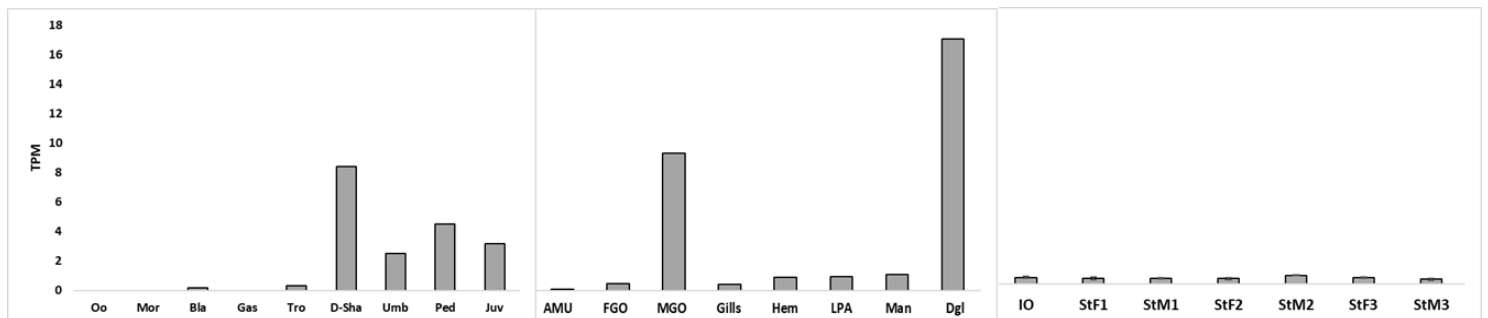


## Cragi-Prokineticin

[illegible]

B

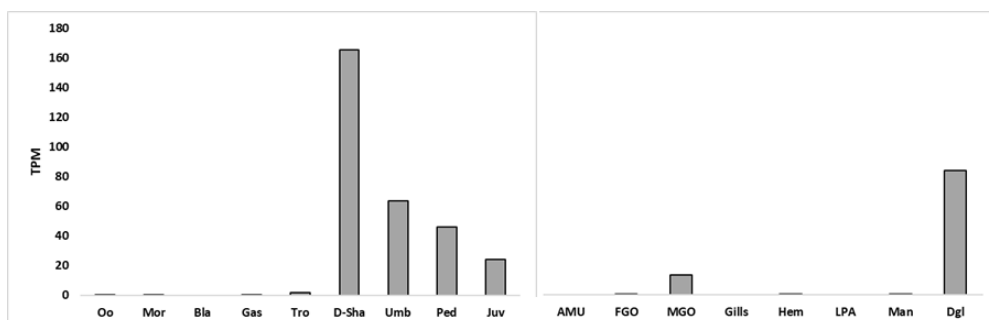
## Cragi-Prokineticin 1



C

D

## Cragi-Prokineticin 2

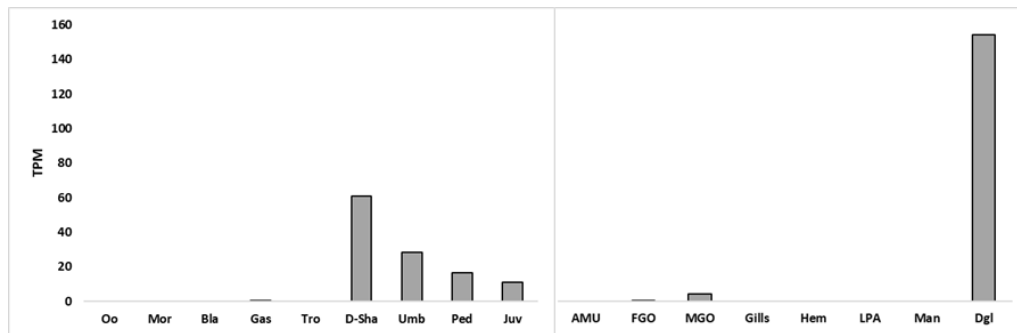


B

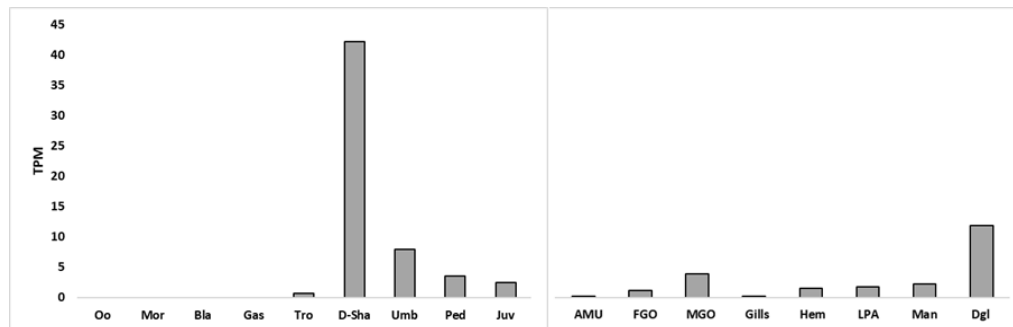
C

D

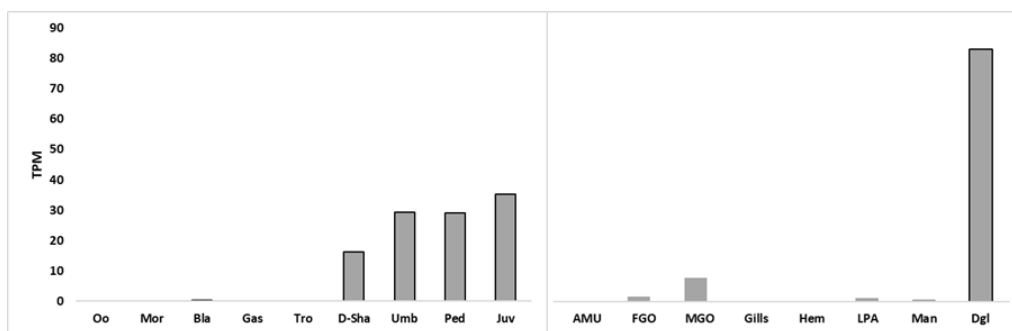
## Cragi-Prokineticin 3



## Cragi-Prokineticin 4



## Cragi-Prokineticin 5



A : Alignment of *C. gigas* 1 (CGG\_contig\_16708), *C. gigas* 2 (CHOYP\_contig\_004850\*), *C. gigas* 3 (CHOYP\_contig\_003956\*), *C. gigas* 4 (CHOYP\_contig\_004051\*), *C. gigas* 5 (CHOYP\_BM8F.2.2, EKC19072.1 *P. yessoensis* (XP\_021370383.1), *P. dumerilii* (Conzelmann et al. 2013), *A. mellifera* (XP\_003250271.1), *P. vannamei* (ADM53424.1) astakine and *D. rerio* (ACV04795.1) Prokineticin sequences. \* Data retrieved from GigaTON (Rivière et al, 2015)

B : Expression pattern of Cragi-Prokineticin 1 (CHOYP\_contig\_002587); Cragi-Prokineticin 2 (CHOYP\_contig\_014127); Cragi-Prokineticin 3 (CHOYP\_contig\_003956); Cragi-Prokineticin 4 (CHOYP\_contig\_004051) and Cragi-Prokineticin 5 (CHOYP\_BM8F.2.2) encoding genes during development.

C : Expression pattern in adult tissues.

D : Expression pattern of Cragi-Prokineticin1 (CGG\_contig\_16708) encoding gene in the visceral ganglia during the cycle of



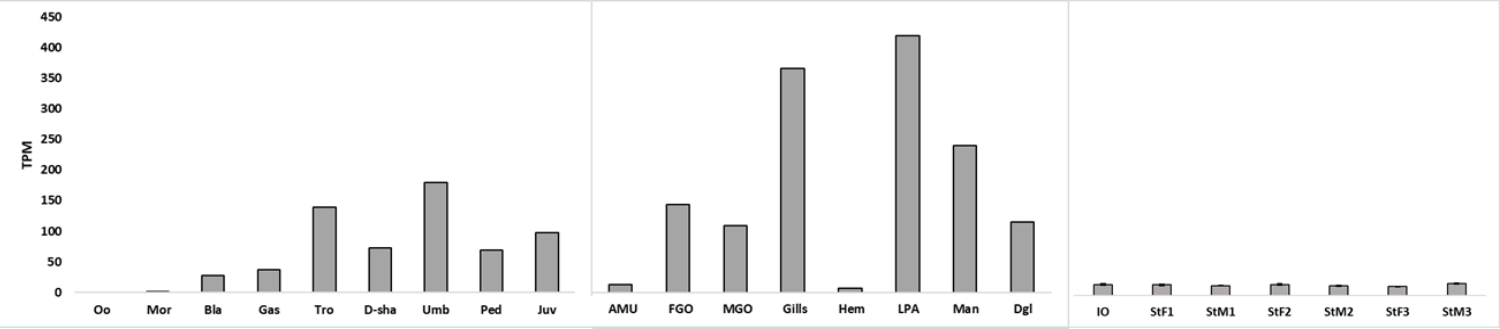
B

C

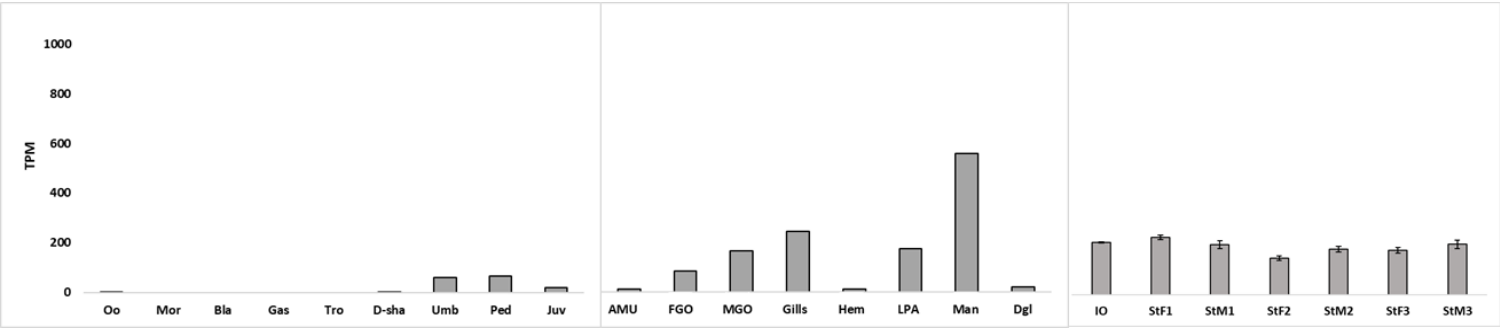
D

Cragi-PTTH 2

Cragi-PTTH 3



Cragi-PTTH 4



A : Alignment of *C. gigas* PTTH1 (CGG\_contig\_11546, genome Scaffold257 [810982 to 811595 + 810776 to 810900 + 810895 to 810992 + 814723 to 814836), *C. gigas* PTTH2 (CGG\_contig\_28778; EKC39796; XP\_011450369.1 ), *C. gigas* PTTH3 (CGG\_contig\_24835, genome Scaffold43954 [239163 to 239624 + 238782 to 239042] ), *C. gigas* PTTH4 (CGG\_contig\_10820 EKC21705, XP\_011448209.1), *P. yessoensis* (XP\_021368706.1), *A. californica* (XP\_012942268.1), *C. teleta* (ELU11356.1), *D. mel* (NP\_001285550.1), *M. sexta* ( AAG14368.1 ), *T. castraneum* (XP\_971946.1) PTTH sequences, *D. mel* (AAF52896.1) , *B. floridae* (XP\_002586716.1) and *S. pistillata* (PFX31008.1) trunk sequences .

B : Expression pattern of Cragi-PTTH 3(CHOYP\_TRK.1.1), PTTH 4 (CHOYP\_BRAFLDRAFT\_113863.1.1) encoding genes during development.

C : Expression pattern in adult tissues.

D : Expression pattern of Cragi-PTTH 1(CGG\_contig\_11546), PTTH 2 (CGG\_contig\_28778), PTTH 3 (CGG\_contig\_24835), PTTH 4 (CGG\_contig\_10820) encoding genes in the visceral ganglia during the cycle of reproduction.

## Protostome families

QNNQN

1

C. gigas  
C. virginica  
M. yessoensis  
A. californica  
P. dumerilii  
A. mellifera

91

C. gigas  
C. virginica  
M. yessoensis  
A. californica  
P. dumerilii  
A. mellifera

181

C. gigas  
C. virginica  
M. yessoensis  
A. californica  
P. dumerilii  
A. mellifera

271

C. gigas  
C. virginica  
M. yessoensis  
A. californica  
P. dumerilii  
A. mellifera

361

C. gigas  
C. virginica  
M. yessoensis  
A. californica  
P. dumerilii  
A. mellifera

451

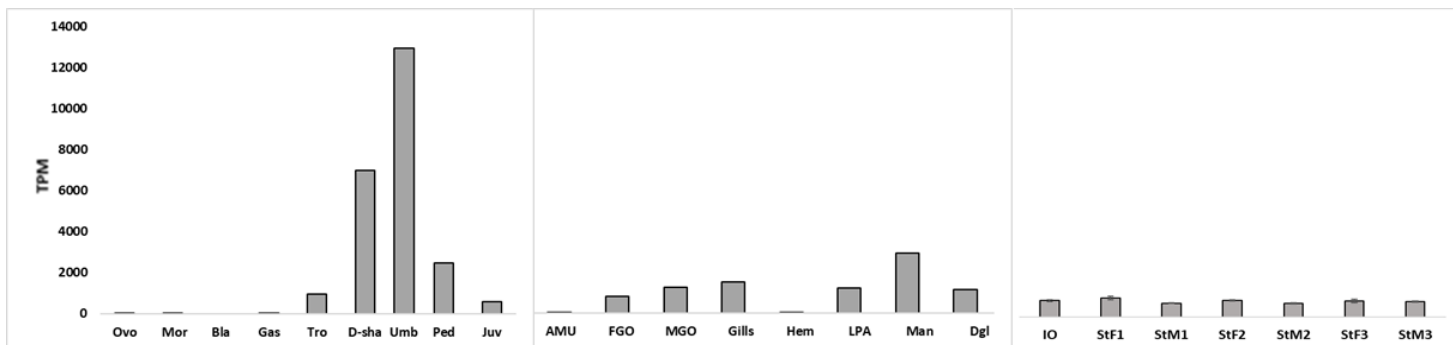
C. gigas  
C. virginica  
M. yessoensis  
A. californica  
P. dumerilii  
A. mellifera

541

C. gigas  
C. virginica  
M. yessoensis  
A. californica  
P. dumerilii  
A. mellifera

C

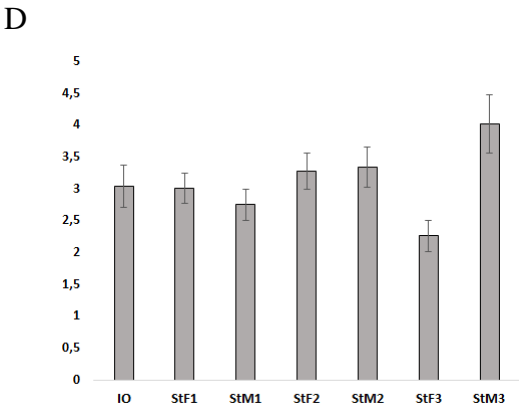
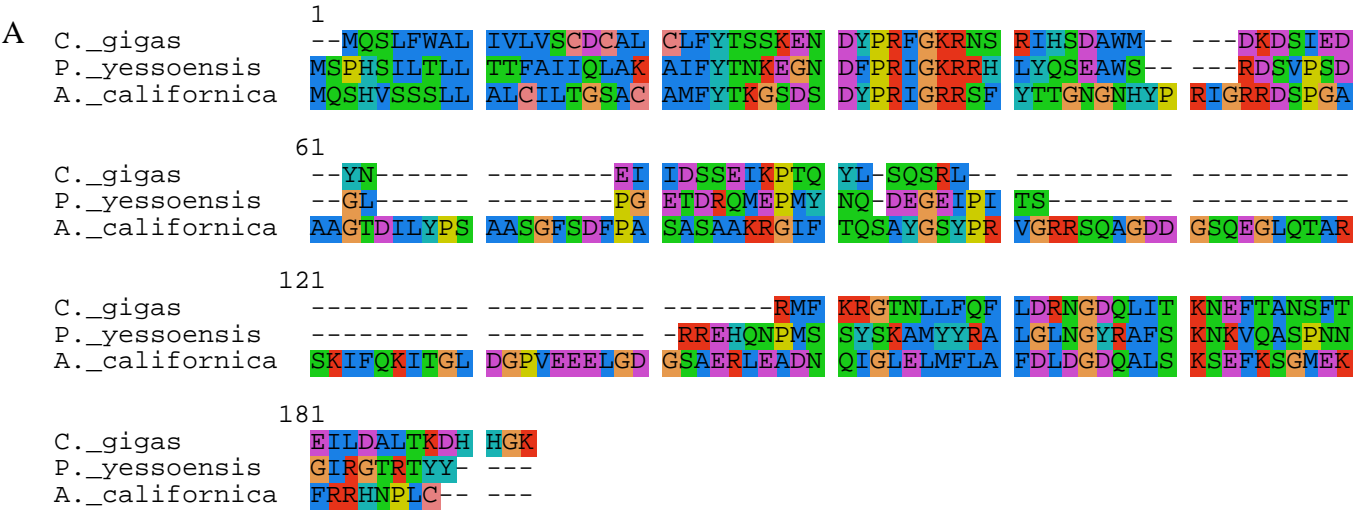
D



**B** : Expression pattern of Cragi-GNQQN encoding genes (CHOYP-contig-035767) during development.

D : Expression pattern of Cragi-GNQQN encoding gene (CGG\_contig\_21126) in the visceral ganglia during the cycle of reproduction.

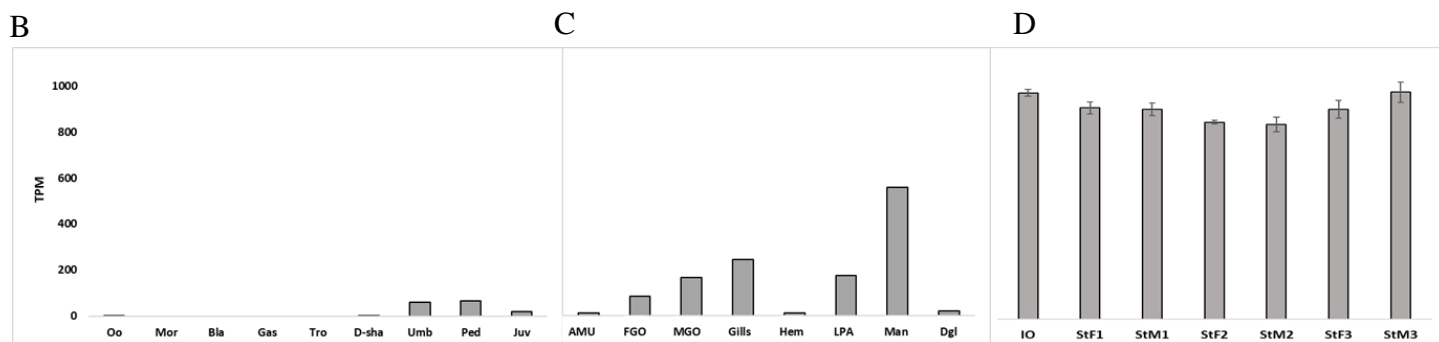
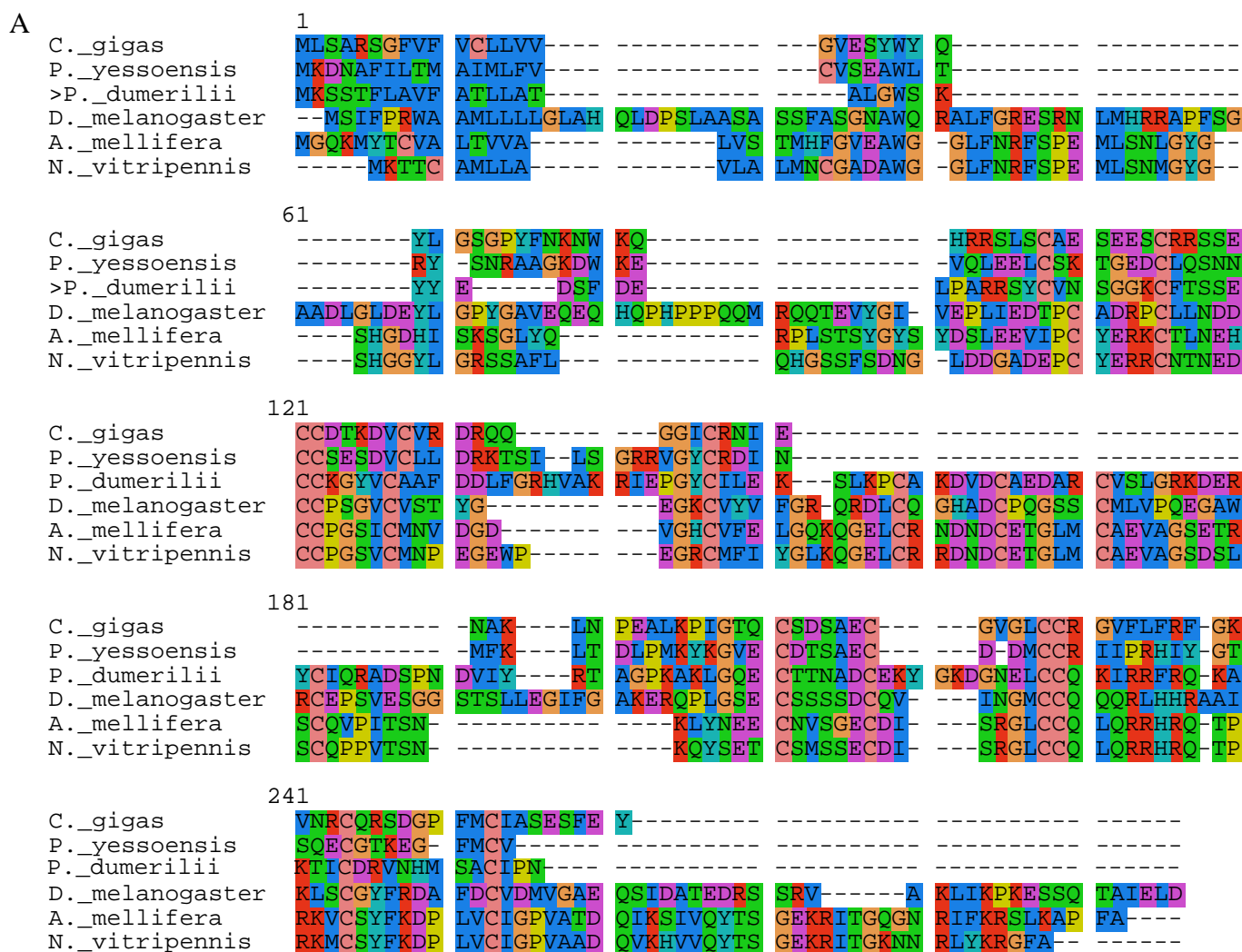
Cragi-Pleurin



A : Alignment of *C. gigas* (CGG\_contig\_22966 ), *P. yessoensis* (AXN93514.1) and *A. californica* (NP\_001191504.1) pleurin sequences.

D: Expression pattern of Cragi-Pleurin encoding gene (CGG\_contig\_22966) encoding gene in the visceral ganglia during the cycle of reproduction.

## Cragi-Prohormone 3



**A** : Alignment of *C. gigas* (CGG\_contig\_31012, XP\_011427435.1) and *P. yessoensis* (XP\_021368853.1), *P. dumerilii* (AHB62385.1), *D. melanogaster* (ABO52824.1), *A. mellifera* (XP\_001122204.2) and *N. vitripennis* (XP\_001604833.1) prohormone 3 sequences.

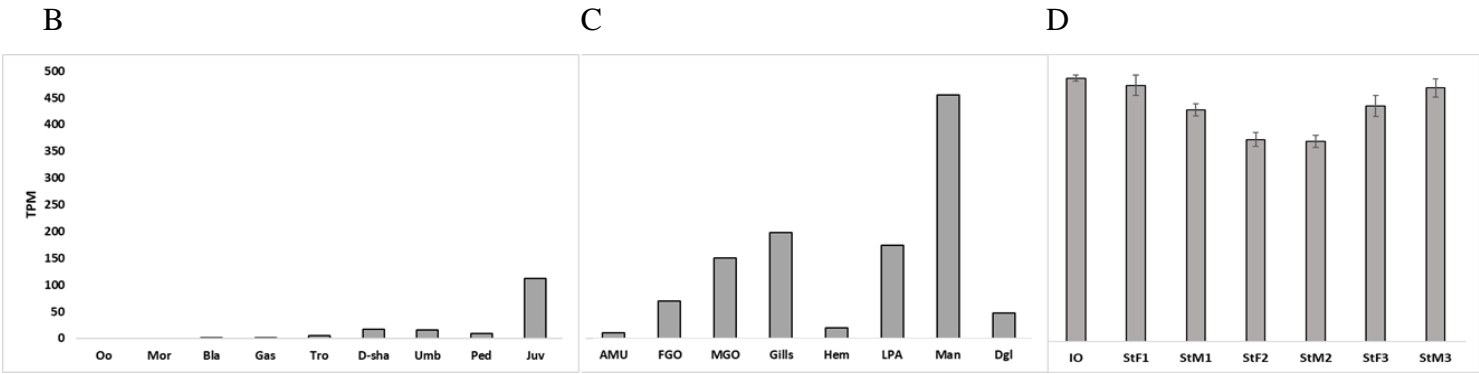
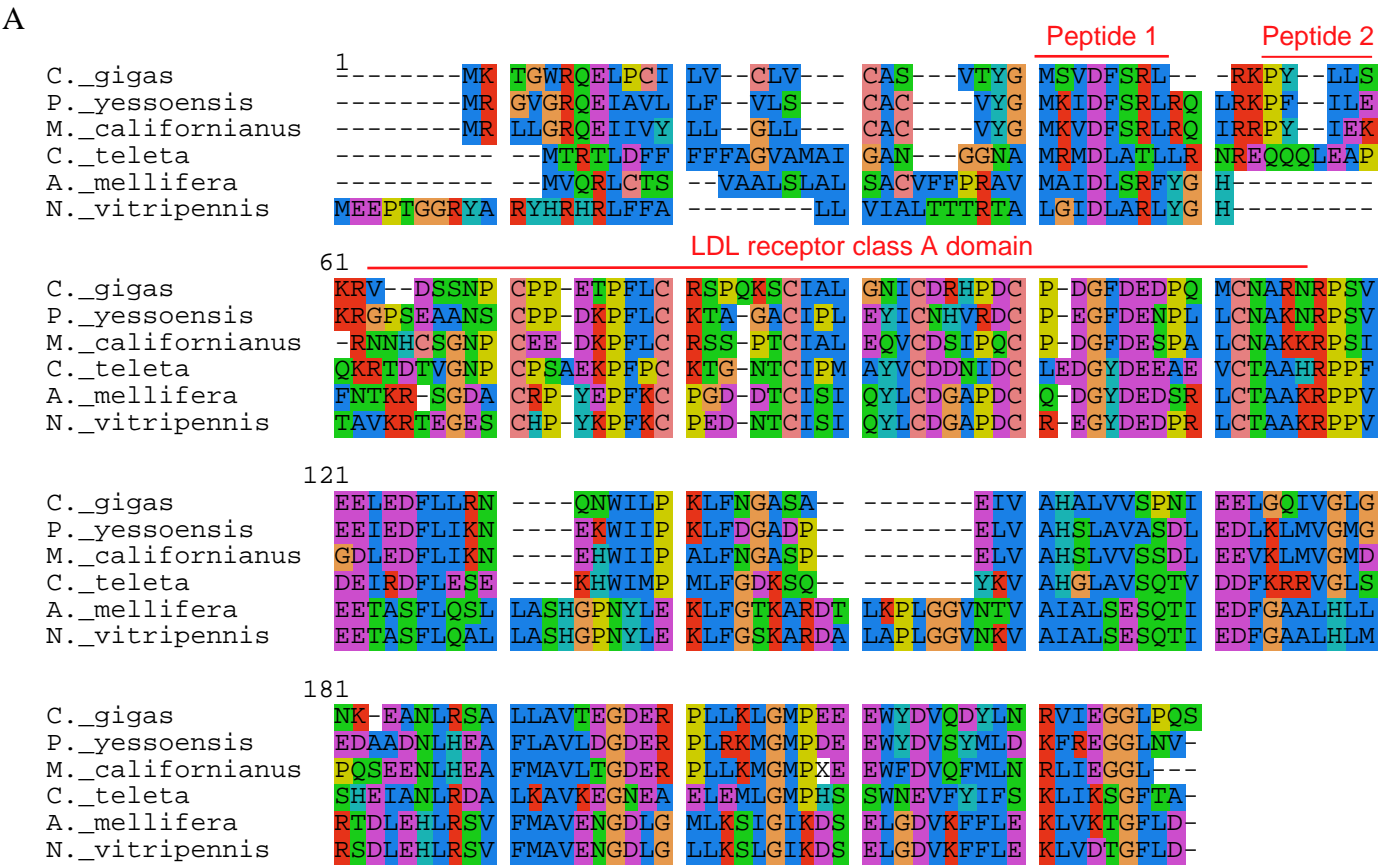
**B** : Expression pattern of Cragi-Prohormone 3 encoding gene (CHOYP-contig-038859) during development.

**C** : Expression pattern in adult tissues.

**D** : Expression pattern of Cragi-Prohormone 3 encoding gene (CGG\_contig\_31012) in the visceral ganglia during the cycle of reproduction.



Cragi-Prohormone 4



A : Alignment of *C. gigas* (CGG\_contig\_28466, CGG\_contig\_14367, CU997943.1), *P. yessoensis* (XP\_021370383.1), *M. californianus* (GE760547), *C. teleta* (EY614440), *A. mellifera* (XP\_392764.1) and *N. vitripennis* (XP\_001603909) prohormone 4 sequences.

B : Expression pattern of Cragi-Prohormone 4 encoding gene (CHOYP-LOC658640.1.2) during development.

C : Expression pattern in adult tissues.

D : Expression pattern of Cragi-Prohormone 4 encoding gene (CGG\_contig\_28466, CGG\_contig\_14367) in the visceral ganglia during the cycle of reproduction.

# Cragi-QSamide/PXXXamide precursors

A

|                           | QSamide |            |            |       |            |             |            |         |        |            | PXXXamide  |            |            |            |            |     |     |     |     |     |
|---------------------------|---------|------------|------------|-------|------------|-------------|------------|---------|--------|------------|------------|------------|------------|------------|------------|-----|-----|-----|-----|-----|
| Cragi-QSamide1            | ---     | MHYLNTV    | FKAI       | FLCVI | FVCA       | TNGRN       | L-ES       | RRVR    | RQNAV  | KATE       | YFARLAL    | ---        | E          | RMPTDCLQIG | CGLVLDLKE  | SG  |     |     |     |     |
| Cragi-QSamide2            | ---     | MHCLNTV    | LIAS       | VLGGM | LNGI       | TYGKY       | M-DK       | HGVH    | RRNADV | KSME       | YFIRMAM    | ---        | N          | PDPDCLHDP  | CGLIDLEANG |     |     |     |     |     |
| Cragi-QSamide3            | ---     | MLQMLRTOIL | ASVCCLLIA  | VSSL  | VDSRF      | LDQDEARFK   | ROTADMRLSE | LHAI    | REILNR | ---        | LPTGCSQYA  | CGLVDIFRS  | ---        |            |            |     |     |     |     |     |
| Patye-QSamide             | ---     | MICTKVV    | AKVL       | LVC   | AV         | FGCI        | ACGRS      | V-EGK   | LKRLR  | ROTGDVKTAE | YFARLAL    | ---        | E          | RRPTGCDLIG | CGLIDIVASG |     |     |     |     |     |
| Lotgi-QSamide             | ---     | MNNLQVC    | ILTV       | IFSLC | MALF       | TDGAS       | ---        | LSHLRNK | ROTADV | RTTE       | YLARLGLGRG | LNSY       | GCRDIA     | CGVVDIYRSG |            |     |     |     |     |     |
| Aplca-QSamide             | ---     | MNKAQLS    | VLVA       | ILSFF | LLTL       | THSAA       | VPDAVSSRSK | ROSADV  | RTAE   | YLAWIALGGK | VPSR       | GCDRVA     | CGVVDVYSSG |            |            |     |     |     |     |     |
| Pladu-QSamide             | ---     | MPNSQVL    | IATV       | IFCVA | VSCVCSKSIO | VFNRRHNERLK | RQIGDLQIAH | HQAQ    | ---    | IGLK       | HWP        | KGCVEIG    | CGLFDIAASG |            |            |     |     |     |     |     |
| Cragi-PYKIamide           | ---     | MLRTOIL    | ASVCCLLIA  | VSSL  | VDSRF      | LDQDEEA     | ---        | ---     | ---    | ---        | ---        | ---        | ---        | ---        | ---        | --- | --- | --- | --- | --- |
| Cragi-PYKI-like/PALIAMide | ---     | MLRTOIL    | ASVCCLLIA  | VSSL  | VDSRF      | LDQDEEA     | ---        | ---     | ---    | ---        | ---        | ---        | ---        | ---        | ---        | --- | --- | --- | --- | --- |
| Cragi-QS3-like/PYKIamide  | ---     | MLRTOIL    | ASVCCLLIA  | VSSL  | VDSRF      | LDQDEARFK   | ROTADMRLSE | LHAI    | REILNR | ---        | LPTGCSQYA  | CGLVDIFRRY | ---        |            |            |     |     |     |     |     |
| Lotgi-PXXXamide           | ---     | MPRFGVA    | LCSIVLFVVL | VSTL  | ---        | TESKSLSRQK  | ROLADLKTSE | LSALIS  | SLGGR  | YAP        | R          | GCKDVA     | CGLVDIFKRR |            |            |     |     |     |     |     |
| Capte-PXXXamide           |         |            |            |       |            |             |            |         |        |            |            |            |            |            |            |     |     |     |     |     |
| Dappu-PXXXa               |         |            |            |       |            |             |            |         |        |            |            |            |            |            |            |     |     |     |     |     |

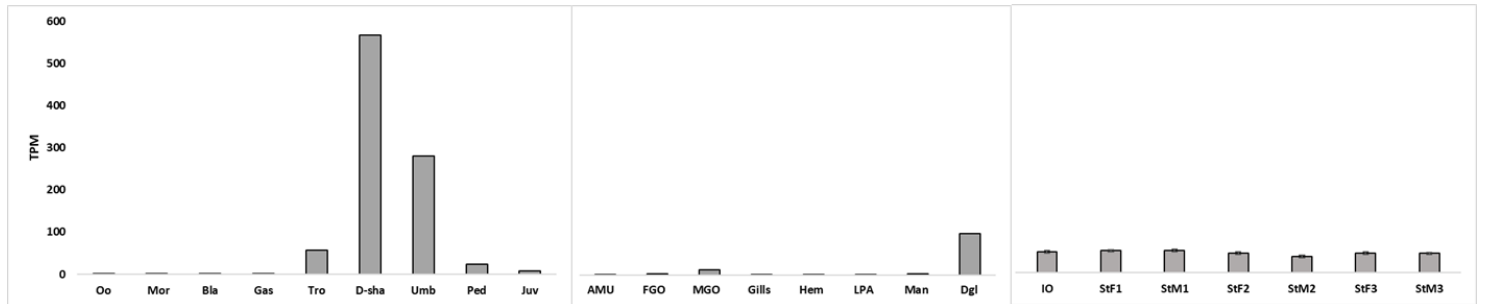
|                           | PXXXamide |             |            |            |     |     |            |           |     |     | PXXXamide |     |     |     |     |     |     |     |     |     |
|---------------------------|-----------|-------------|------------|------------|-----|-----|------------|-----------|-----|-----|-----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cragi-QSamide1            | ---       | KKKRTD      | TQSS       | PYHKQ      | --- | R   | LLKIVQLLIP | KEQ       | --- | --- | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cragi-QSamide2            | ---       | RKKRLNTQPN  | LHIN       | ---        | --- | --- | TATLLDP    | NEO       | --- | --- | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cragi-QSamide3            | ---       | ---         | ---        | ---        | --- | --- | ---        | ---       | --- | --- | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Patye-QSamide             | ---       | KKRDGRTAS   | AYQGQIDDES | RAKLIAMLLT | GS  | AE  | TRRK       | ---       | --- | --- | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Lotgi-QSamide             | ---       | KKKRGDTGKL  | GD         | ---        | --- | --- | SSVEVG     | ---       | --- | --- | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Aplca-QSamide             | ---       | KKKRTTPQLAE | V          | ---        | --- | --- | SEAE       | ---       | --- | --- | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Pladu-QSamide             | ---       | KKRKSYSNNV  | EDE        | ---        | --- | FLN | NENELRRESN | RLVLLORIA | --- | --- | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cragi-PYKIamide           | ---       | DKRSWSDORM  | AEI        | ---        | --- | --- | ---        | ---       | --- | --- | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cragi-PYKI-like/PALIAMide | ---       | DKRSWSDORM  | AEI        | ---        | --- | --- | ---        | ---       | --- | --- | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cragi-QS3-like/PYKIamide  | ---       | DKRSWSDORM  | AEI        | ---        | --- | --- | ---        | ---       | --- | --- | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Lotgi-PXXXamide           | ---       | KRQADSGLMQ  | LI         | ---        | --- | --- | ---        | ---       | --- | --- | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Capte-PXXXamide           |           |             |            |            |     |     |            |           |     |     |           |     |     |     |     |     |     |     |     |     |
| Dappu-PXXXa               |           |             |            |            |     |     |            |           |     |     |           |     |     |     |     |     |     |     |     |     |

|                           | PXXXamide |     |     |     |     |     |     |     |     |     | PXXXamide |     |     |     |     |     |     |     |     |     |
|---------------------------|-----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Cragi-QSamide1            | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cragi-QSamide2            | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cragi-QSamide3            | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Patye-QSamide             | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Lotgi-QSamide             | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Aplca-QSamide             | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Pladu-QSamide             | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cragi-PYKIamide           | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cragi-PYKI-like/PALIAMide | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Cragi-QS3-like/PYKIamide  | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Lotgi-PXXXamide           | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Capte-PXXXamide           | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Dappu-PXXXa               | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- | ---       | --- | --- | --- | --- | --- | --- | --- | --- | --- |

B

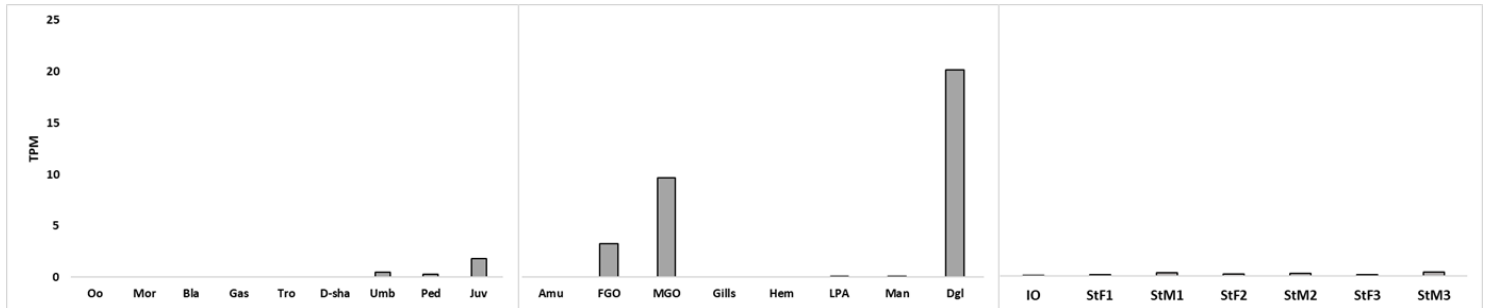
Cragi-QS1



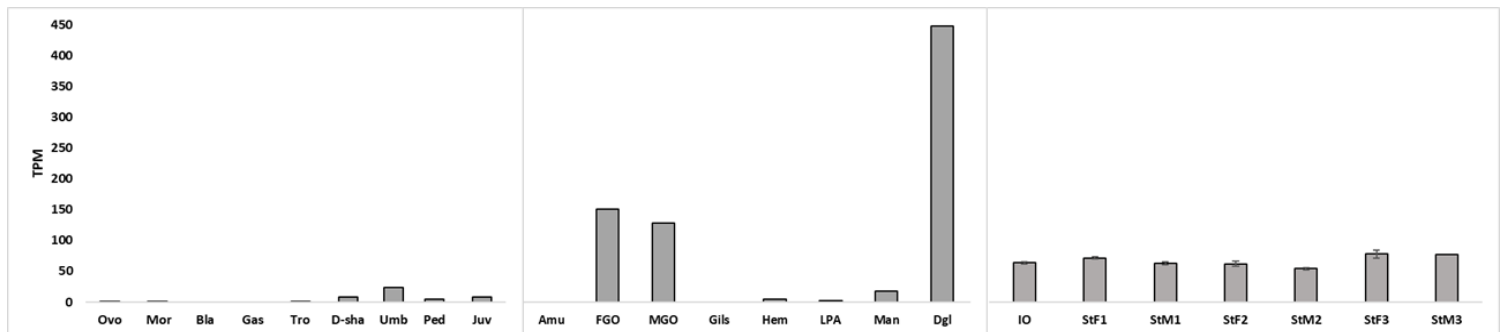
C

D

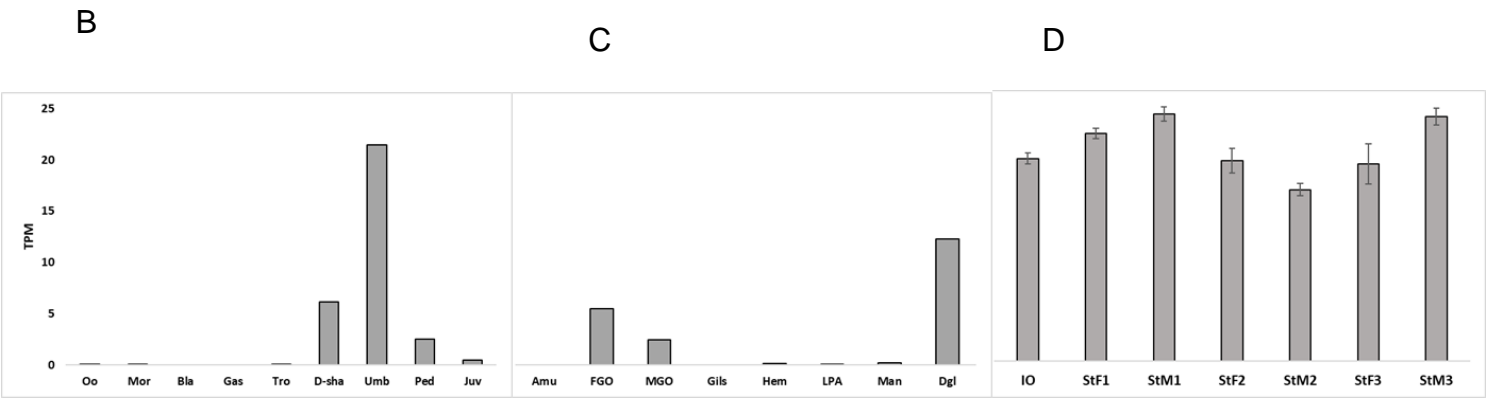
Cragi-QS2



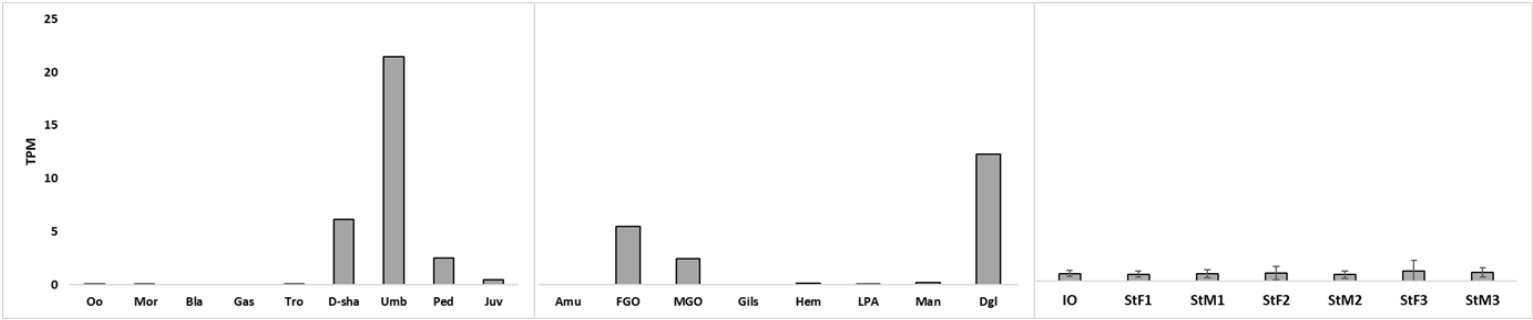
Cragi-QS3



Cragi-PYKI amide



Cragi- QS3-like/PYKIamide



A : Alignment of *C. gigas* Cragi-QSamide1 (CGG\_contig\_22528, CHOYP\_contig\_003053, ERK20562, CGI\_10005831), Cragi-QSamide2 (CGG\_contig\_22774, CHOYP\_contig\_042522) , Cragi-QS3 (CGG\_contig\_22898, CHOYP\_contig\_043914), *P. yessoensis* (XP\_021353029.1), *L. gigantea* (XP\_009051664.1 ) , *A. californica* (XP\_012937765.1) and *P. dumerilii* (AHB62379.1) QSamide sequences and Cragi-PYKI amide (CGG\_contig\_15711, CHOYP-contig-025841) Cragi-PIKI-like/PALIAMide (XR-01142063.4), Cragi-QS3-like/PYKIamide (CGG\_contig\_23609, CHOYP\_contig\_004027), *L. gigantea* (ESO97822.1), *C. teleta* (ELT91968.1 ) and *D. pulex* (EFX77601.1) PXXXamide sequences.

B : Expression pattern of Cragi-QSamide1 (CHOYP\_contig\_003053), Cragi-QSamide2 (CHOYP\_contig\_042522), Cragi-QSamide3 (CHOYP\_contig\_043914), Cragi-PYKIamide ( CHOYP-contig-025841) and Cragi-QS3-like/PYKIamide (CHOYP-contig-004207) encoding genes during development.

C : Expression pattern in adult tissues.

D : Expression pattern of Cragi-QSamide1(CGG\_contig\_22528), Cragi-QSamide2 (CGG\_contig\_22774), Cragi-QSamide3 (CGG\_contig\_22898), Cragi-PYKIamide (CGG\_contig\_15711) and Cragi-QS3-like/PYKIamide (CGG\_contig\_23609) encoding transcripts in the visceral ganglia during the cycle of reproduction.

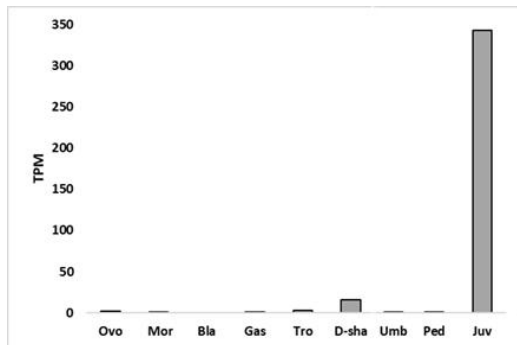
## Lophotrochozoan-specific neuropeptides

## Cragi-CCWamide

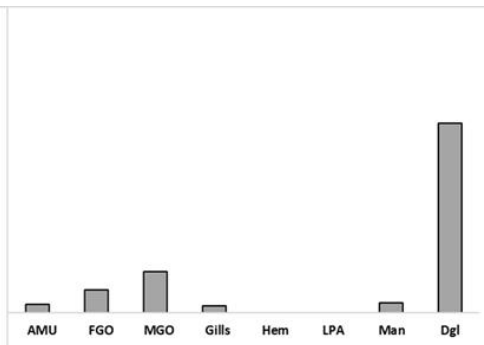
A



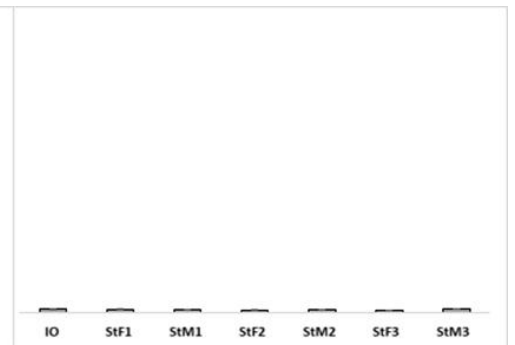
B



C



D



A : Alignment of *C. gigas* ( CGG\_contig\_20696), *L. gigantea* (XP\_009055999.1), *A. californica* (XP\_005090647.1 ) and *P. dumerilii* (FP002705) CCW sequences.

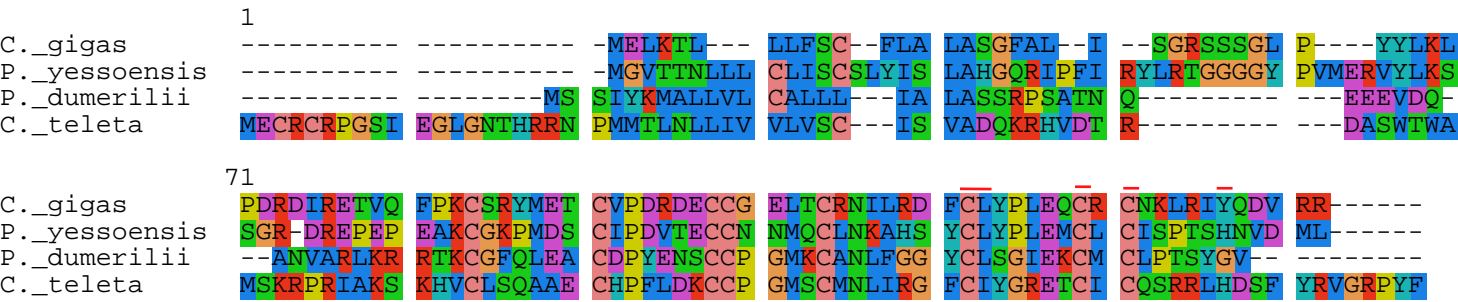
B : Expression pattern of Cragi-CCWamide encoding gene (CHOYP-contig-025497) during development.

C : Expression pattern in adult tissues.

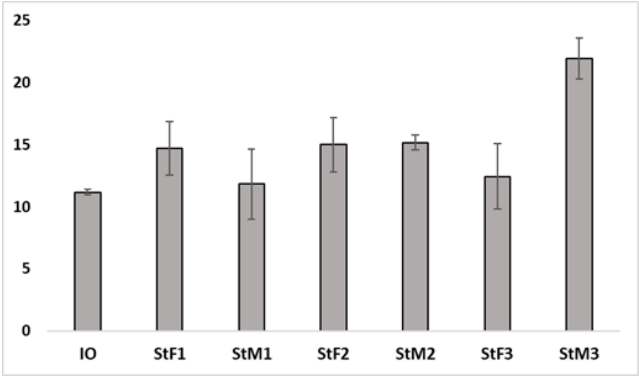
D : Expression pattern of Cragi-CCWamide (CGG-contig-20696) encoding gene in the visceral ganglia during the cycle of reproduction.

Cragi-CLCCY

A



D

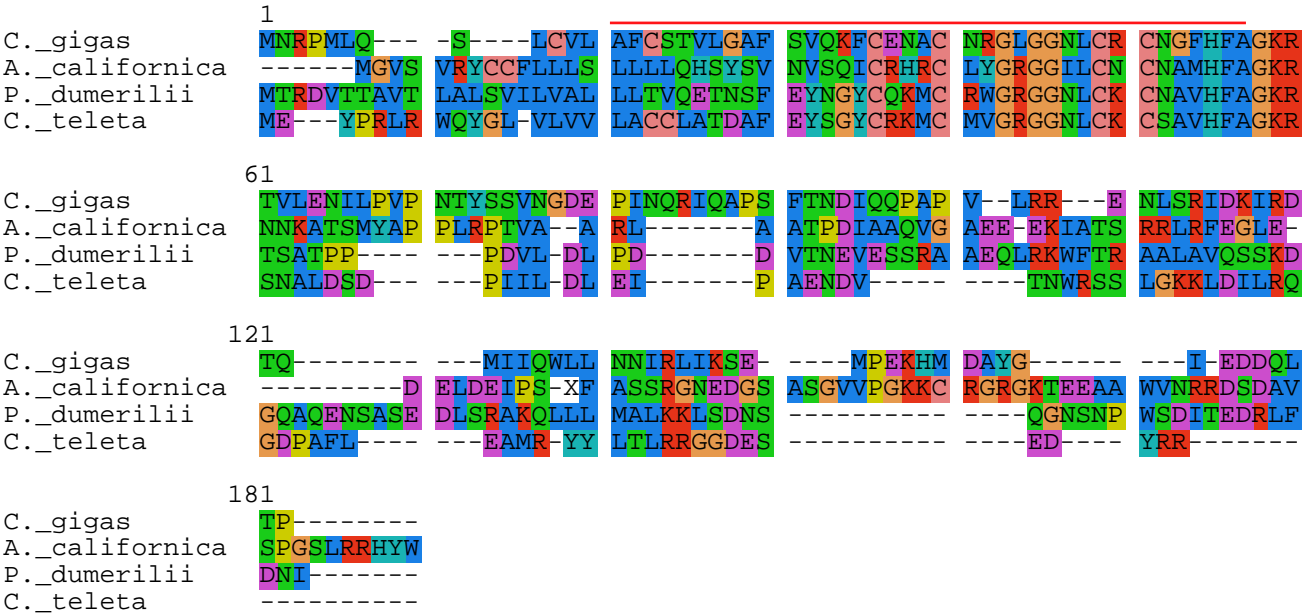


A : Alignment of *C.gigas* (CGG\_contig\_18747),*P. yessoensis* (XP\_021368903.1 ), *P. dumerilii* (AHB62377.1 ) and *C. teleta* (ELU16055.1) CLCCY sequences.

D : Expression pattern of Cragi-CLCCY encoding gene (CGG\_contig\_18747) in the visceral ganglia during the cycle of reproduction.

Cragi-HFAamide

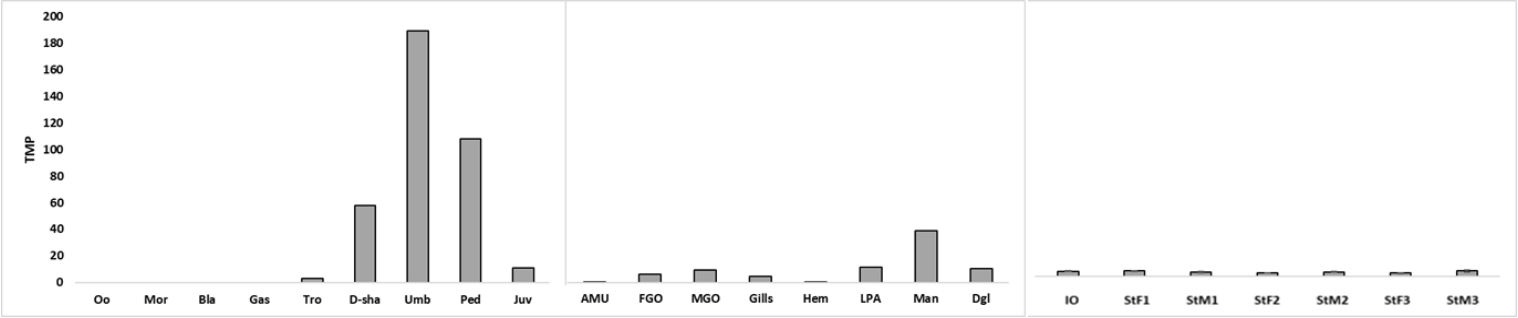
A



B

C

D



A : Alignment of *C. gigas* (CGG\_contig\_26304), *A. californica* (EB352987.1), *P. dumerilii* (PMID:24359412) and *C. teleta* (ELU01627.1) sequences.

B : Expression pattern of Cragi-HFAamide encoding gene (CHOYP-contig-035470) during development.

C : Expression pattern in adult tissues.

D : Expression pattern of Cragi-HFAamide encoding gene (CGG\_contig\_26304) in the visceral ganglia during the cycle of reproduction.

# Cragi-LXRX

A

1

|                  |            |            |            |             |            |
|------------------|------------|------------|------------|-------------|------------|
| C._gigas         | -----M     | ERCGSVFLIL | TVMCVSSWSL | QTSEIRSLQK  | RSLEVADQLR |
| M._californianus | -----      | --MVVPVFLV | LVFCFSTFTE | -TVRSHTITK  | REAEKPSELQ |
| H._asinina       | CCQWRQSDLI | TPNTQAMYAF | KMLLLPVFGV | LLTISIAG--- | -LSQGSISGK |
| P._dumerilii     | -----      | -----MKEL  | T-CLLVVVAT | IQLALSAAAP  | -SQNKVVRMK |

61

|                  |            |            |            |            |             |            |
|------------------|------------|------------|------------|------------|-------------|------------|
| C._gigas         | LENE---LK  | RVKRLQE--- | -----      | ---DEELSR  | VKRFKEH---  | ELSRQKRTN- |
| M._californianus | LENE---LR  | RVKRLKDTFA | VLPRVKRMKR | DEHSERELSR | IKRLKRSCLKP | GLSRVKRTKR |
| H._asinina       | LESQ---LS  | RVKRLD---P | GVPELSRTKR | RDVENDGLSR | MKRYNTDEMP  | SLS-----   |
| P._dumerilii     | FHQPDFTALV | RIKRQR-RSA | MEPTLVRVKR | D---EQELKR | YKREKRDVEE  | ELQRVKRQKR |

121

|                  |            |            |           |            |             |            |
|------------------|------------|------------|-----------|------------|-------------|------------|
| C._gigas         | -----      | -----      | -----EN   | GENDALKRVK | RLKRAVL---  | -----      |
| M._californianus | ASELVLPELS | RAKRFKDLFV | DS-----   | ---FMLSRVK | RLKRSPLYKTL | TVDHYEPLSL |
| H._asinina       | -----      | -----      | ---REKRGE | IENAALSRMK | RGDVD-----  | ---DNPVLS  |
| P._dumerilii     | DTEELHRFK  | RQKRDTEAEL | EYKKRHSGE | VEDPELARTK | RFKRE-----  | ---AEPPLQ  |

181

|                  |            |            |            |            |            |            |
|------------------|------------|------------|------------|------------|------------|------------|
| C._gigas         | ---EKKLEKE | IALHRMKRGE | VSHASKHASN | K-----     | LSNSENTKQK | HKSVKQDFKK |
| M._californianus | RMKRSQRSVE | FSLHE----- | ---KDLRV   | KRFHDPNTIE | LSLVKRLKRS | E-RVKR-IP- |
| H._asinina       | REKRQDLSEN | IGLSRMKRGD | E-SHAELSRE | K-RHDADSLA | LSRTKREGKN | LLRVKR-VP- |
| P._dumerilii     | RYKREKRDAE | AEIHRFKRQK | RDTEQELERY | KRDRDLEEPE | LSRTKREKRD | ---VES-SP- |

241

|                  |            |            |            |            |             |            |
|------------------|------------|------------|------------|------------|-------------|------------|
| C._gigas         | KRMEMAMKRH | GNLGKHRVMK | KGDP--RFHH | NPTRNGHLR  | HRMMTRGRFH  | GNDRKHHMRN |
| M._californianus | ---S---    | -----      | ---NVHK    | IKNRNRKNLN | ---YLKGT--- | ---QK---   |
| H._asinina       | DSVELDRAKR | G-----     | ---EVIL    | IKRRNTDD   | -----       | -----      |
| P._dumerilii     | ---TLAKEKK | GL-KEKKSRA | AGDRRRNQKA | LKREKKTNMN | HRWEKHQKRN  | G--NKHHISP |

301

|                  |            |            |            |            |            |            |
|------------------|------------|------------|------------|------------|------------|------------|
| C._gigas         | ILKRHHVQ-- | -----      | ---HHR     | SDV--KKPKR | HIKRS----- | -----      |
| M._californianus | -----      | RHFKALEHL  | DRNIDLKTAK | QELSRQSG-- | -----      | -ALXRKREKN |
| H._asinina       | -----      | -----      | -----      | -----      | -----      | -----      |
| P._dumerilii     | RMKKNVLRKE | RRSDLLANHA | LRNVHLVSQR | NKLGSKLAKH | RVKRTQKIHQ | GHHYNKLEAD |

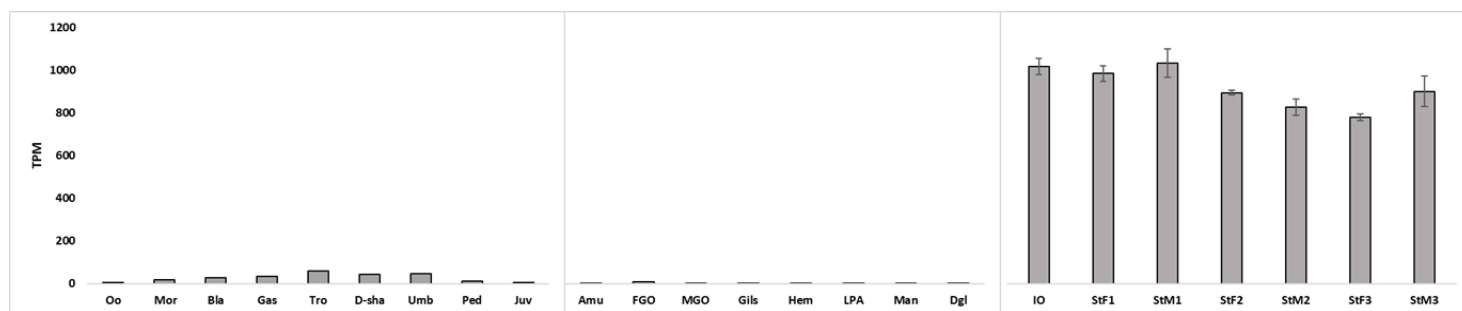
361

|                  |            |            |            |            |           |       |
|------------------|------------|------------|------------|------------|-----------|-------|
| C._gigas         | -----      | -----      | -----      | -----      | -----     | ----- |
| M._californianus | RAQ-----   | -----      | -----      | -----      | -----     | ----- |
| H._asinina       | -----      | -----      | -----      | -----      | -----     | ----- |
| P._dumerilii     | VAKLGLRLRD | IALNLHKANL | KNAAKRRVKR | THAKKNKKIA | AKKH----- | ----- |

B

C

D



A : Alignment of *C. gigas* ( CGG\_contig\_08893 ), *M. californianus* (ES394341.1), *H. asinina* (GT276618) and *P. dumerilii* (Conzelmann et al. 2013) LXRX sequences.

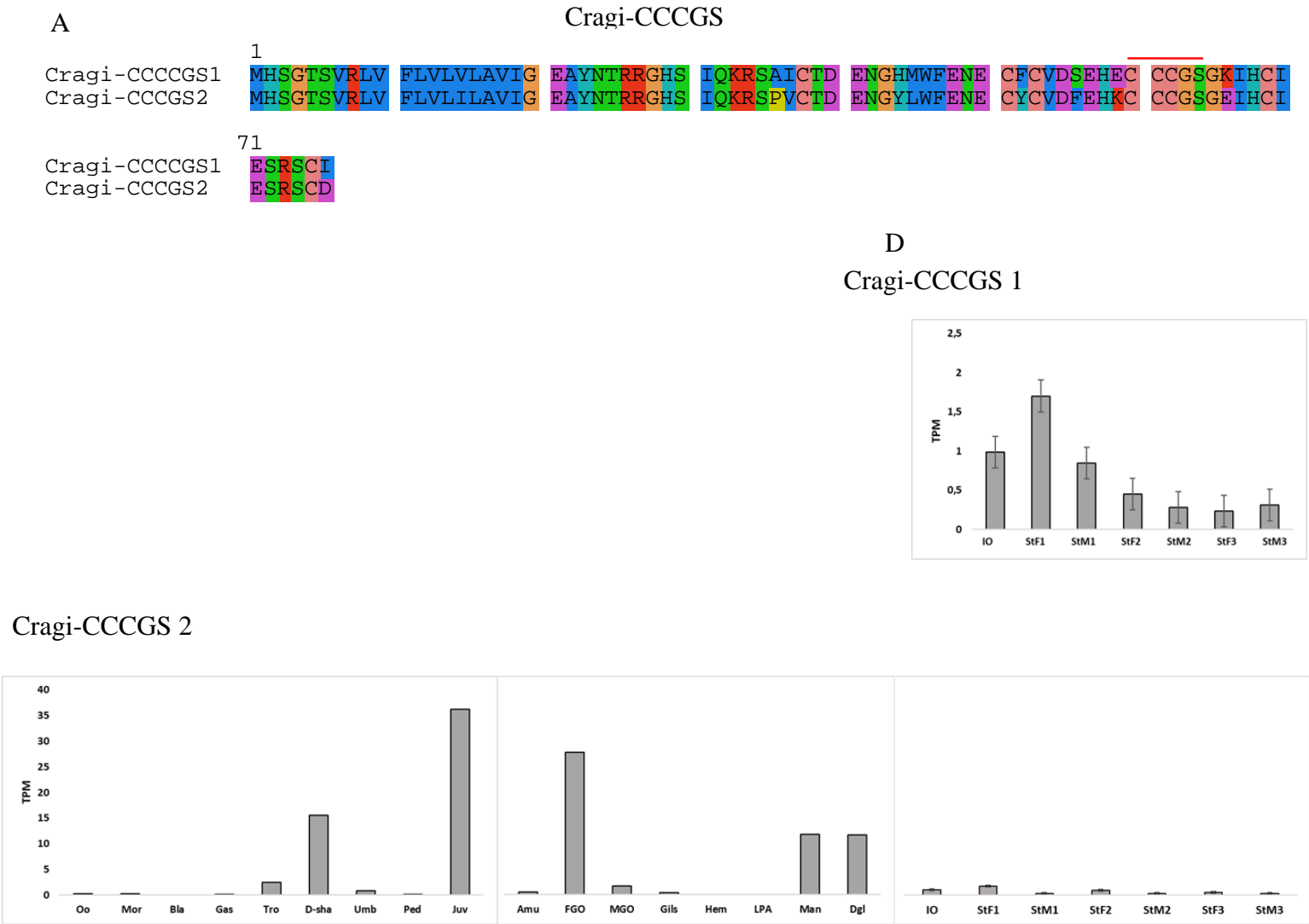
B : Expression pattern of Cragi-LXR encoding gene (CHOYP-SS1G-08041.1.1) during development.

C : Expression pattern in adult tissues.

D : Expression pattern of Cragi-HFAamide encoding gene (CGG\_contig\_08893) in the visceral ganglia during the cycle of reproduction.



## Mollusk-specific neuropeptides



A : Alignment of *C. gigas* *Cragi-CCCCGS1* (CGG\_contig\_25260, CGG\_contig\_16024 ), and *Cragi-CCCCGS2* (CGG\_contig\_26223, CHOYP\_contig\_014739).

B : Expression pattern of *Cragi-CCCCGS2* encoding gene (CHOYP-contig-014739) during development.

C : Expression pattern in adult tissues.

D : Expression pattern of *Cragi-CCCCGS 1* (CGG\_contig\_25260) and *Cragi-CCCCGS 2* (CGG\_contig\_26223) encoding genes in the visceral ganglia during the cycle of reproduction.

## Cragi-Clionin

A

1

|                |             |            |            |            |           |            |
|----------------|-------------|------------|------------|------------|-----------|------------|
| C._gigas       | --MNTCLIAV  | I---LALTIS | TSMVKSSPIF | ----PVVELS | SMHPRDACL | ICSICFHS-- |
| A._californica | --MTSSMILAV | L-AI-SLSCL | LTAVTSSPLG | P-VQPAISLS | KMEPDHACL | MCNICFPDLE |
| S._officinalis | MVKPTAFFTV  | -----VLVIA | AVFMKPANLY | --PTPSVPLE | EMDPRDACL | QCSNCFGD-- |
| T._diomedea    | -MSPFVCFAS  | LATIAMFAIL | LPLTSATPLE | PVIDPVIALE | EMEAYHACL | ICHNCFVE-- |

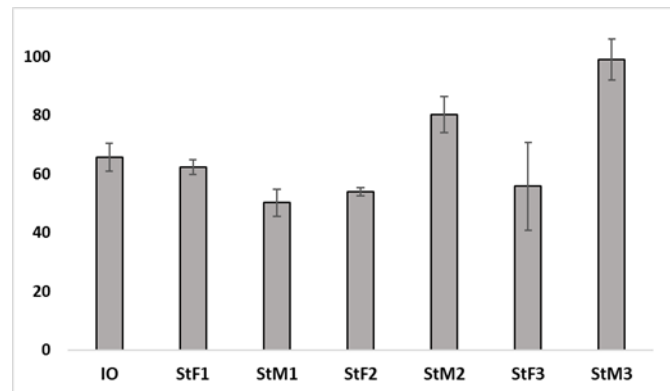
61

|                |             |            |            |            |            |            |
|----------------|-------------|------------|------------|------------|------------|------------|
| C._gigas       | EEENSMLKCAN | NFCLSDYGFN | --GLGYLWIG | KEC---AHWP | SLEKFVAADN | VLKN-----  |
| A._californica | DTGLLLDCSN  | KVCGPIMAGM | CAMEKLVWL  | HNCRO---YD | MVQKMWAPHG | AL-----    |
| S._officinalis | QYQPLLDGAN  | DVCPKVKSF  | -----EVG   | YECSTILRHP | NLGRSSLFKF | -----      |
| T._diomedea    | DKEKMMKCTN  | QVCGTLYEDK | CALDQFIWL  | HHCRH---YN | MIESMWSSKK | RTHDVKDQFN |

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|                |    |
|----------------|----|
| C._gigas       | -- |
| A._californica | -- |
| S._officinalis | -- |
| T._diomedea    | LE |

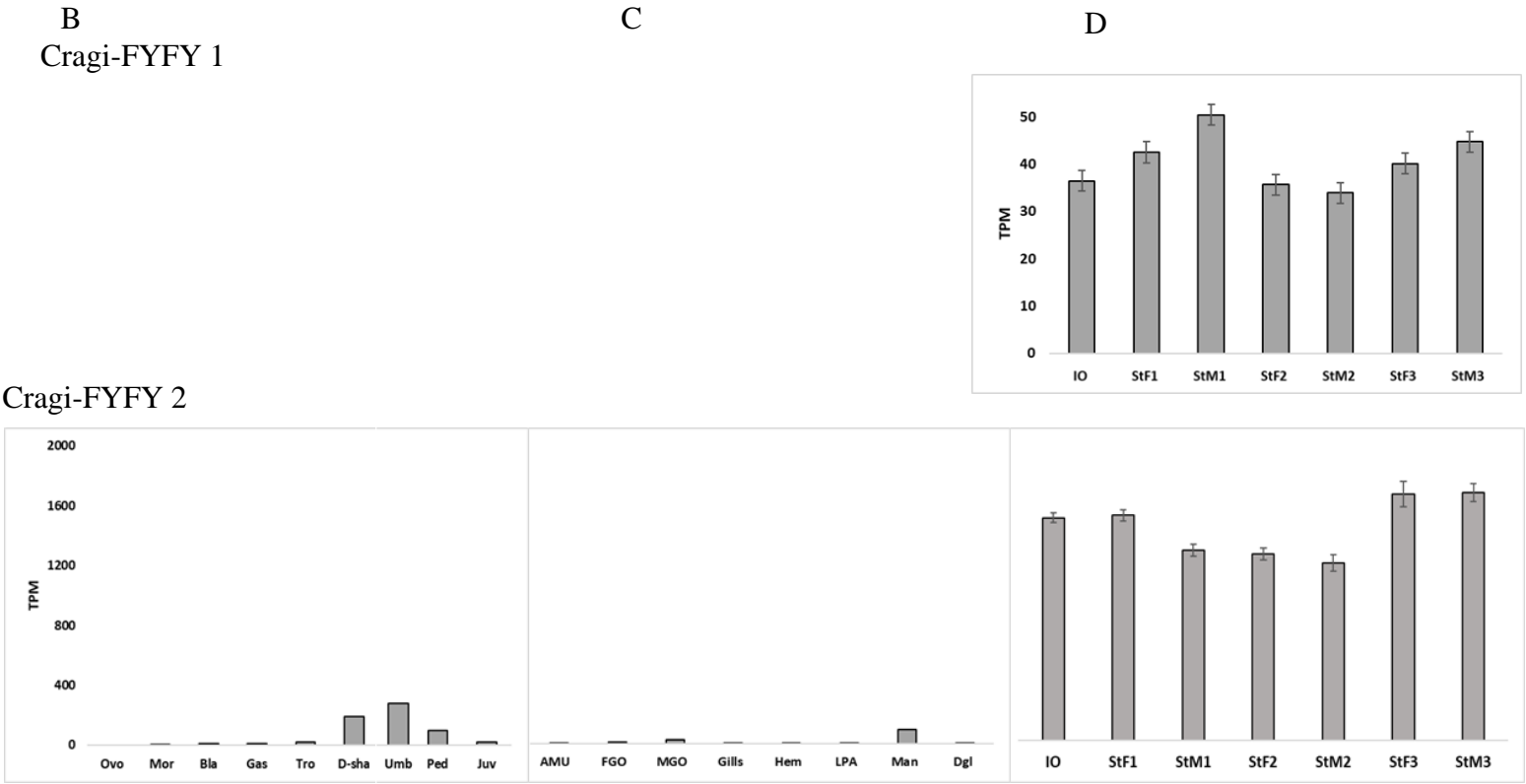
D



A : Alignment of *C. gigas* (CGG\_contig\_22908, XP\_011412322.1), *A. californica* (XP\_005099829.1), *S. officinalis* (Zatylny-Gaudin et al., 2016) and *T. diomedea* (I1SKI5) Clionin sequences.  
D: Expression pattern of Cragi-Clionin encoding gene (CGG\_contig\_22908) in the visceral ganglia during the cycle of reproduction.

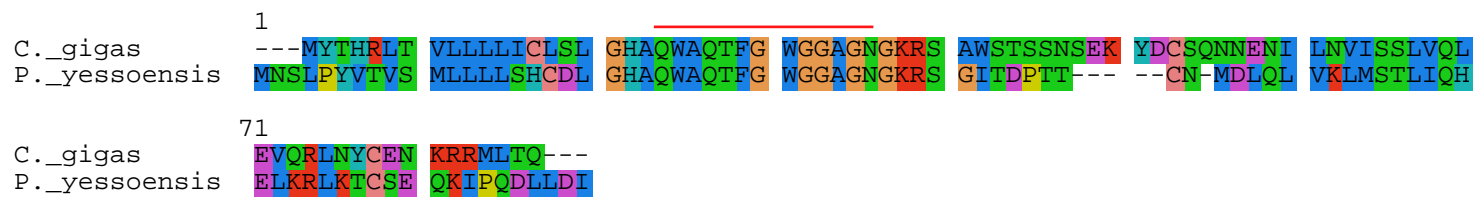
## A

|               | 1          | 71          | 141        | 211        | 281         | 351        | 421         | 491 | 561 | 631 |
|---------------|------------|-------------|------------|------------|-------------|------------|-------------|-----|-----|-----|
| C._gigas1     | MCAMKVLGVC | FIVIFALQQR  | LFVRAETIVR | YQEQLETA   | CE-ENAVCTE  | LMALP----  | -----DNYFSL |     |     |     |
| C._gigas2     | MYSLV-LTTT | LIMVLTIAD   | --GKDVFI-- | ---TRNSPI  | CP-PLAVCGN  | VFEFDRMMDD | GSVEPERRHI  |     |     |     |
| P._yessoensis | MCS-----   | WIAVLATF-V  | ACTRGTSITR | YQH--FPLPM | CS-EDTVCSE  | ISELPSIKEE | -----NNYFSW |     |     |     |
| L._gigantea   | -----      | -MATLTLSE-  | ----PIFLHR | ---PNEPRPG | CPSANTICST  | IYQDS----P | ENIGDEYLVN  |     |     |     |
| C._gigas1     | KSCSCPNGYT | CPERPGR-TST | LPFGRGRWYG | LCRPVSDIPE | CSPGGEVARKQ | YNGVRDIGYL | KYTQIHCLCP  |     |     |     |
| C._gigas2     | TNCFCNNSRV | CPFNRE-N-M  | IYQSRTQQEV | LCEPVRDLPR | CRPGMVARRM  | YVDSMDFNDK | SYIAIRCICP  |     |     |     |
| P._yessoensis | MMCQCPGESV | CPKSPG-KQT  | IEVTGSKWYG | MCRPKSNIRS | CSPGGEVGEEY | YMGVREIPFG | TFTRVHCLCP  |     |     |     |
| L._gigantea   | SICSCQNGQS | CPVDIDHNNT  | LQVDDGVWYG | LCQPTKDFSE | CKVNDVATRS  | FENSDFQGH  | GYSELNCICT  |     |     |     |
| C._gigas1     | GDGQIGMVPS | TVWTKTR-RD  | DPRADSVFEF | ECADQTH--- | -----       | -----SGSP  | S-----RKRS  |     |     |     |
| C._gigas2     | LNLPSSRPR  | VKATVYRNLQ  | FEGFDRIHNY | KCNEEDV--- | -----       | -----EEYK  | -----       |     |     |     |
| P._yessoensis | LLEYEEGQP- | -----E-MD   | ASNPKSIYQL | ICSGTGR--- | -----       | -----LQAA  | MKRGRGGSRY  |     |     |     |
| L._gigantea   | SENKVLENK  | IRSEDS--EY  | GPGVDK---- | ICGEEVEKRG | IDQGMSSFYK  | RSQDNLSSF  | YKRGSGLSAF  |     |     |     |
| C._gigas1     | RGRGGF-RKF | Y-----F     | YK-----    | -----      | -----       | -----      | -----       |     |     |     |
| C._gigas2     | -----      | -----       | -----K     | -----      | -----       | -----      | -----       |     |     |     |
| P._yessoensis | GGGKNG-RRF | Y-----F     | YRK-----   | -----      | -----       | -----      | -----       |     |     |     |
| L._gigantea   | YKRGNGLSSF | YKRGHDLSAF  | YKRSRGLNSF | YKRRSPFQHV | SSFYKRD LGH | VSSFYKRAPE | NSLSSFYKRS  |     |     |     |
| C._gigas1     | -----      | -----       | -----      | -----      | -----       | -----      | -----       |     |     |     |
| C._gigas2     | -----      | -----       | -----      | -----      | -----       | -----      | -----       |     |     |     |
| P._yessoensis | -----      | -----       | -----      | -----      | -----       | -----      | -----       |     |     |     |
| L._gigantea   | HDIGMNSFYK | RSRDNGMSSF  | YKRSPNDLSN | FYKRVPVGLR | NFYKRSVGND  | MLGFYKRTPS | SYDLSSFYKR  |     |     |     |
| C._gigas1     | -----      | -----       | -----      | -----      | -----       | -----      | -----       |     |     |     |
| C._gigas2     | -----      | -----       | -----      | -----      | -----       | -----      | -----       |     |     |     |
| P._yessoensis | -----      | -----       | -----      | -----      | -----       | -----      | -----       |     |     |     |
| L._gigantea   | ASGNNALSSF | YKRARENDQL  | SGFYKRAAGN | DDLSSFYKRS | SGNDDLASFY  | KRAAGNDDL  | SFYKRAAGND  |     |     |     |
| C._gigas1     | -----      | -----       | -----      | -----      | -----       | -----      | -----       |     |     |     |
| C._gigas2     | -----      | -----       | -----      | -----      | -----       | -----      | -----       |     |     |     |
| P._yessoensis | -----      | -----       | -----      | -----      | -----       | -----      | -----       |     |     |     |
| L._gigantea   | NLSSFYKRAA | GNDGLSNFYK  | RAAGNDGLSS | FYKRAAGNDN | LSNFYKRAAG  | NDDLSSFYKR | AAGNNDLSSF  |     |     |     |
| C._gigas1     | -----      | -----       | -----      | -----      | -----       | -----      | -----       |     |     |     |
| C._gigas2     | -----      | -----       | -----      | -----      | -----       | -----      | -----       |     |     |     |
| P._yessoensis | -----      | -----       | -----      | -----      | -----       | -----      | -----       |     |     |     |
| L._gigantea   | YKRAAGNDDL | SGFYKRAAGN  | DNLYNFYKRA | PVASQLSNFY | KRGLSSNDLV  | AFYKRALNG  | VSSFYKRANS  |     |     |     |
| C._gigas1     | -----      | -----       | -----      | -----      | -----       | -----      | -----       |     |     |     |
| C._gigas2     | -----      | -----       | -----      | -----      | -----       | -----      | -----       |     |     |     |
| P._yessoensis | -----      | -----       | -----      | -----      | -----       | -----      | -----       |     |     |     |
| L._gigantea   | DLSSFYKRAV | DDVSNFYKRS  | GEDDQTNDNS | LSSFYKRSLN | GGQNSFYKRS  | PLDHDVSHFY | KRGLDDVSSF  |     |     |     |
| C._gigas1     | -----      | -----       | -----      | -----      | -----       | -----      | -----       |     |     |     |
| C._gigas2     | -----      | -----       | -----      | -----      | -----       | -----      | -----       |     |     |     |
| P._yessoensis | -----      | -----       | -----      | -----      | -----       | -----      | -----       |     |     |     |
| L._gigantea   | YKRANANLQS | FYKRARDNTS  | NFYKRSPQQ  | SSFYKRAPKD | NVSSFYKRDS  | SDNKDVAFYK | RSV         |     |     |     |

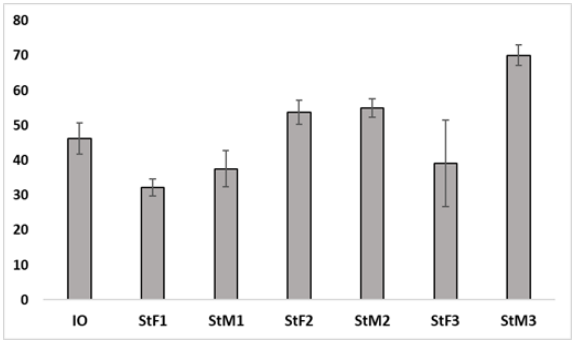


Cragi-GNamide

A



D



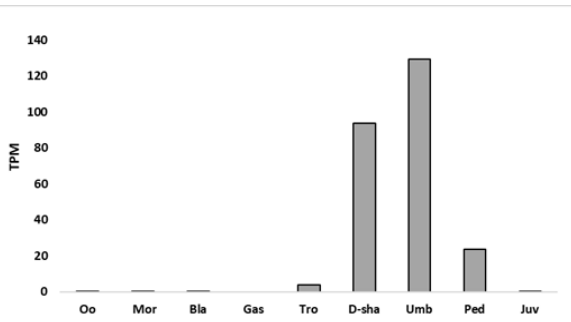
A : Alignment of *C. gigas* (CGG\_contig\_01838) and *P. yessoensis* (MH045254) sequences.  
D : Expression pattern of Cragi-GNamide encoding gene (CGG\_contig\_01838) in the visceral ganglia dduring the cycle of reproduction.

## Cragi-GRWRN

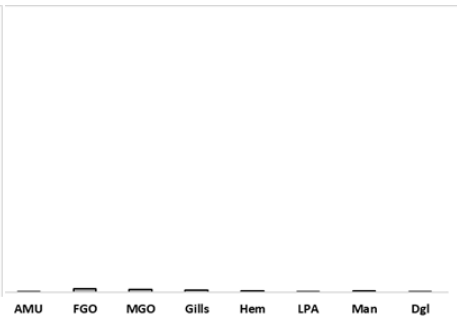
A



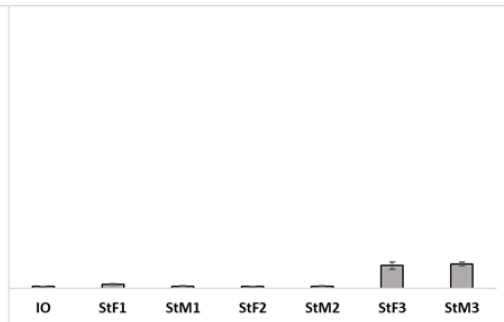
B



C



D



A : Alignment of *C. gigas* (CGG\_contig\_15181), *C. virginica* (XP\_022337980) and *P. yessoensis* (XP\_021372506) GRWRN sequences.

B : Expression pattern of Cragi-GRWRN encoding gene (CHOYP-contig-008923) during development.

C : Expression pattern in adult tissues.

D : Expression pattern of Cragi-GRWRN encoding gene (CGG\_contig\_15181) in the visceral ganglia during the cycle of reproduction.

## Cragi-GSWN

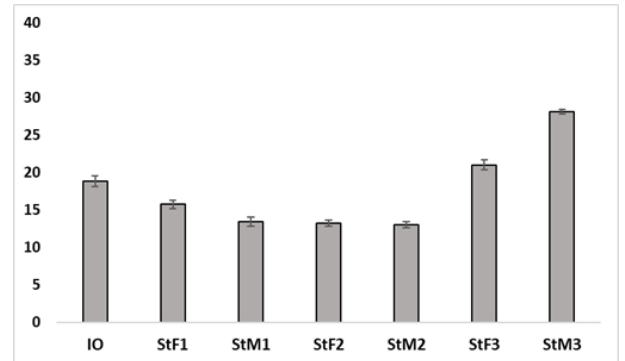
A

C.*\_gigas* MPRQLYLTALF L---VLVALL LSSTPEVSAK SSSCSYACMKT LFSCKRRS---EGDCC  
 C.*\_virginica* MSRLYTFILA M---TLIAS- IFTTTEVGAK SSSCSYDCMK S LFACKRRS---EGDCC  
 L.*\_gigantea* --MKRFTLLA L---LLVAIL YISQS--ESK CQGVTKCMVN LFTCKREQIS VN--IAADCC  
 A.*\_californica* --MELTSPLL LVISSFLAVY VINFTDAGPL DPCVFQCMRG VLKCKREQSV VGLSQKVDCC  
 P.*\_yessoensis* --MKAFCIL- ----LALAV FMASMTTTS G RSCYFMCLRD LILCKRTT---GTGCC

C.*\_gigas* NKFTTECFHTC DLAA---PPC ---AGKRGS WNKRYQVVSQG YED-RE-LYP YY-----  
 C.*\_virginica* NKFTDCFLAC GLSA---PPC ---AGKRGS WNKRYEVPGR YED-GE-LYP YY-----  
 L.*\_gigantea* KEYFECFNEC KPGSR--PPC KNTAPGKRGS WNKRYSSRYG SDILGD-IIS GY-----  
 A.*\_californica* QKYKMCYLVC RPDAESVPPC -GGAKGKRGS WNKRTQAANT CNNGHGHVP DFDDVVDLGG  
 P.*\_yessoensis* DKFLG---SC APGCG--VKN AKCKGTKRGS WNKRADYEE- -NPLEE-YYP MI-----

C.*\_gigas* ---  
 C.*\_virginica* ---  
 L.*\_gigantea* ---  
 A.*\_californica* RFY  
 P.*\_yessoensis* ---

D

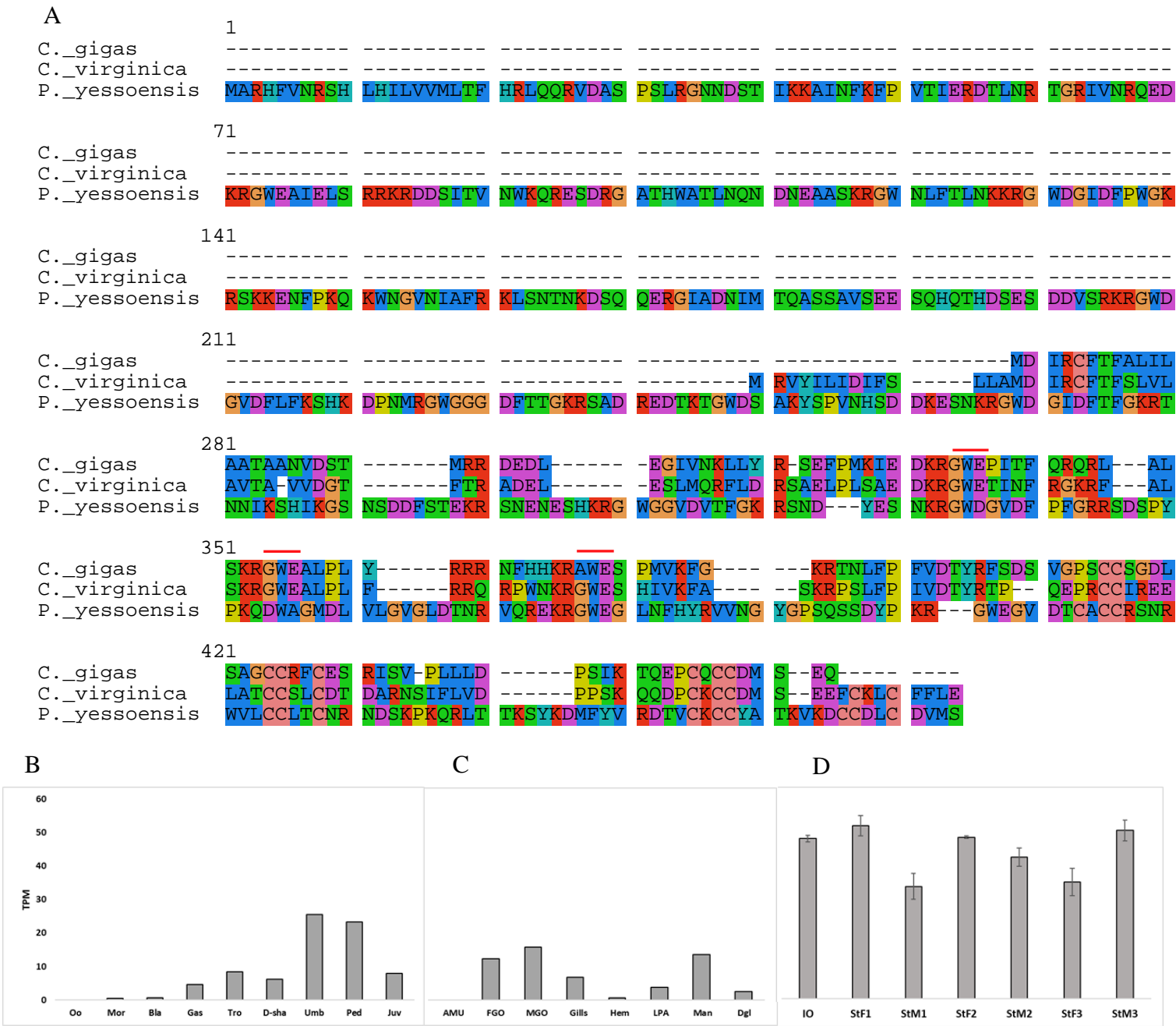


A : Alignment of *C. gigas* (CGG\_contig\_26157 ), *C. virginica* (XR\_002636430.1), *L. gigantea* (XM\_009047650.1), *A. californica* (XM\_013087810.1) and *P. yessoensis* (XR\_002464777.1) GSWN sequences.

D : Expression pattern of Cragi-GSWN encoding gene (CGG\_contig\_26157) in the visceral ganglia during the cycle of reproduction.



Cragi-GWE



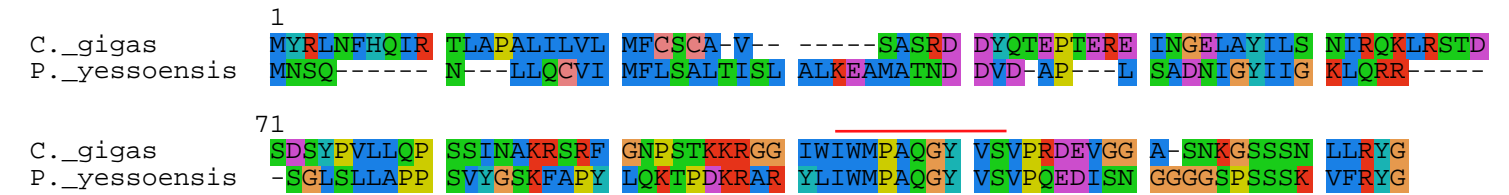
A : Alignment of *C. gigas* (CGG\_contig\_24907), *C. virginica* (XP\_022318468.1) and *P. yessoensis* (MH045253) GWE sequences.

B : Expression pattern of Cragi-GWE encoding gene (CHOYP-contig-049345) during development.

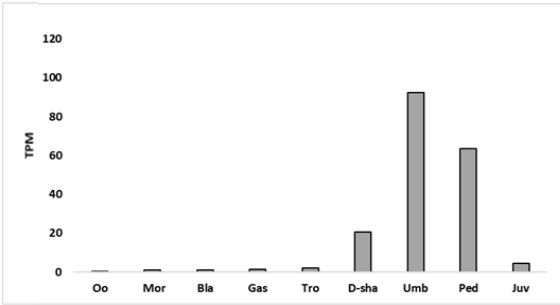
C : Expression pattern in adult tissues.

Cragi-IWMPxxGYxx

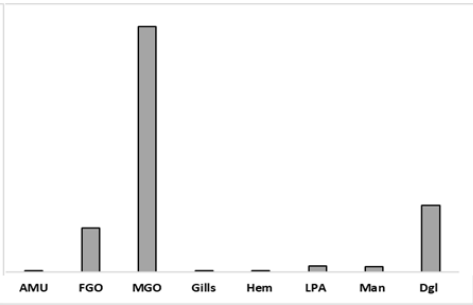
A



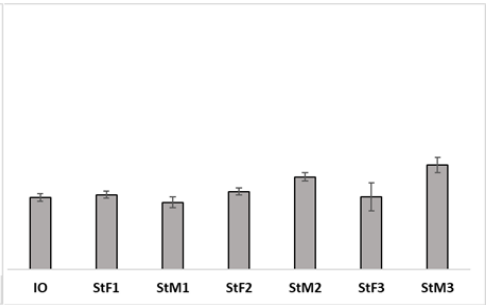
B



C



D



A : Alignment of *C. gigas* (CGG\_contig\_09620) and *P. yessoensis* (MH045262) IWMPxxGYxx sequences.

B : Expression pattern of Cragi-IWMPxxGYxx encoding gene (CHOYP-contig-025394) during development.

C : Expression pattern in adult tissues.

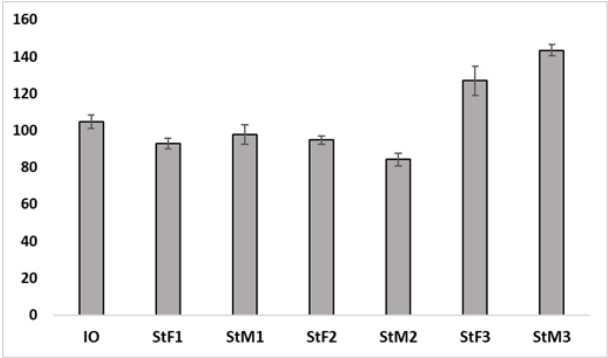
D : Expression pattern of Cragi-IWMPxxGYxx encoding gene (CGG\_contig\_09620)in the visceral ganglia during the cycle of reproduction.

Cragi-LXRYamide

A



D



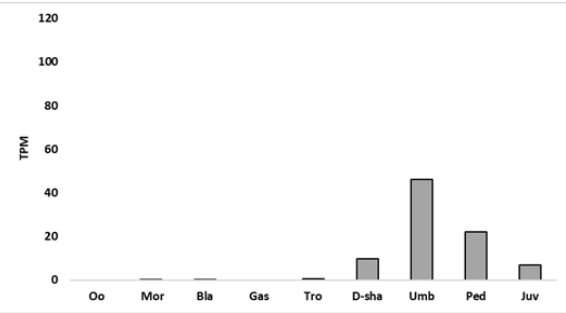
A : Alignment of *C. gigas* (CGG\_contig\_20802) and *P.yessoensis* (AXN93520.1) LXRYamide sequences.  
D : Expression pattern of Cragi-LXRYamide encoding gene (CGG\_contig\_20802) in the visceral ganglia during the cycle of reproduction.

Cragi-RTLFamide

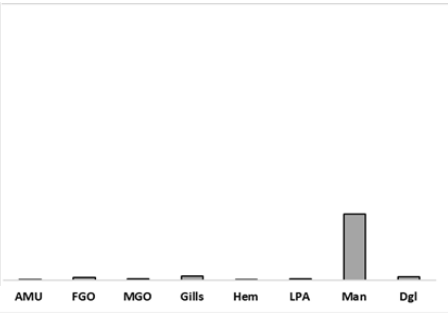
A



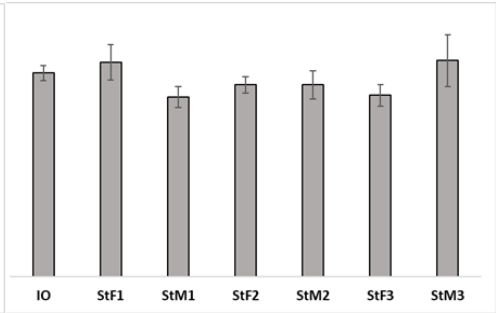
B



C



D



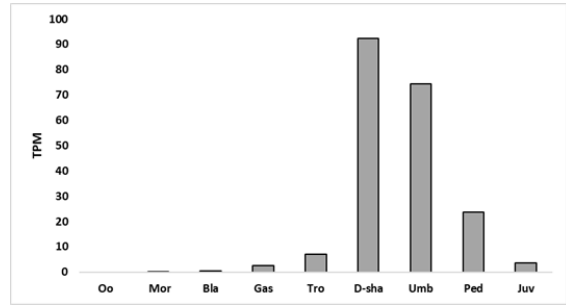
A : Alignment of *C. gigas* (CGG\_contig\_04103) and *L. gigantea* (XM\_009063916.1) RTLFamide sequences.  
B : Expression pattern of Cragi-RTLFamide encoding gene (CHOYP\_contig\_039290) during development.  
C : Expression pattern in adult tissues.  
D : Expression pattern of Cragi-RTLFamide encoding gene (CGG\_contig\_04103) in the visceral ganglia during the cycle of reproduction.

Cragi-SLRFamide

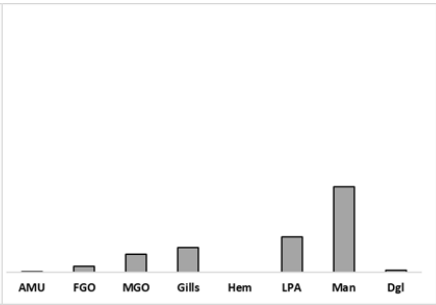
A



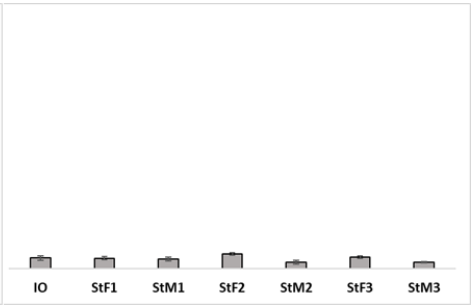
B



C



D



A : Alignment of *C. gigas* (CGG\_contig\_15407), *C. virginica* (XP\_022327616.1 ) and *P. yessoensis* (XP\_021354453.1) SLRFamide sequences.

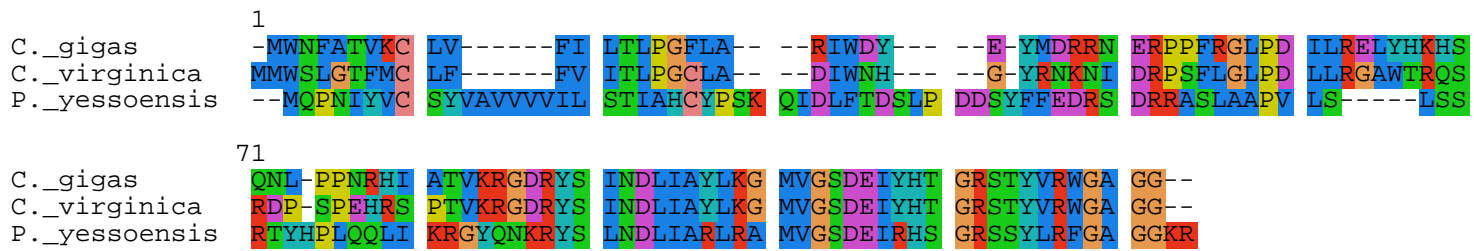
B : Expression pattern of Cragi-SLRFamide encoding gene (CHOYP-contig-005956) during development.

C : Expression pattern in adult tissues.

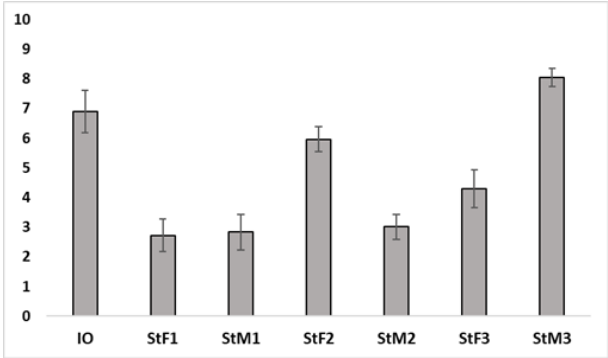
D : Expression pattern of Cragi-SLRFamide encoding gene (CGG\_contig\_15407) in the visceral ganglia during the cycle of reproduction.

Cragi-WGAGamide

A



D



A : Alignment of *C. gigas* (CGG\_contig\_15208), *C. virginica* (XP\_022335259.1) and *P. yessoensis* (A0A210QE07) WGAGamide sequences.

D : Expression pattern of Cragi-WGAGamide encoding gene (CGG\_contig\_15208) in the visceral ganglia during the cycle of reproduction.