1 **Prediction of the neuropeptidomes of members of the Astacidea**
2 **(Crustacea, Decapoda) using publicly accessible transcriptome**
3 **shotgun assembly (TSA) sequence data**
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Abstract

The decapod infraorder Astacidea is comprised of clawed lobsters and freshwater crayfish. Due to their economic importance and their use as models for investigating neurochemical signaling, much work has focused on elucidating their neurochemistry, particularly their peptidergic systems. Interestingly, no astacidean has been the subject of large-scale peptidomic analysis via *in silico* transcriptome mining, this despite growing transcriptomic resources for this taxon. Here, the publicly accessible astacidean transcriptome shotgun assembly data were mined for putative peptide-encoding transcripts; these sequences were used to predict the structures of mature neuropeptides. One hundred seventy-six distinct peptides were predicted for *Procambarus clarkii*, including isoforms of adipokinetic hormone-corazonin-like peptide (ACP), allatostatin A (AST-A), allatostatin B, allatostatin C (AST-C) bursicon α , bursicon β , CCHamide, crustacean hyperglycemic hormone (CHH)/ion transport peptide (ITP), diuretic hormone 31 (DH31), eclosion hormone (EH), FMRFamide-like peptide, GSEFLamide, intocin, leucokinin, neuroparsin, neuropeptide F, pigment dispersing hormone, pyrokinin, RYamide, short neuropeptide F (sNPF), SIFamide, sulfakinin and tachykinin-related peptide (TRP). Forty-six distinct peptides, including isoforms of AST-A, AST-C, bursicon α , CCHamide, CHH/ITP, DH31, EH, intocin, myosuppressin, neuroparsin, red pigment concentrating hormone, sNPF and TRP, were predicted for *Pontastacus leptodactylus*, with a bursicon β and a neuroparsin predicted for *Cherax quadricarinatus*. The identification of ACP is the first from a decapod, while the predictions of CCHamide, EH, GSEFLamide, intocin, neuroparsin and RYamide are firsts for the Astacidea. Collectively, these data greatly expand the catalog of known astacidean neuropeptides and provide a foundation for functional studies of peptidergic signaling in members of this decapod infraorder.

1. Introduction

Among the most iconic infraorders of decapod crustaceans is the Astacidea, a taxon that is comprised of clawed marine lobsters and freshwater crayfish. Five superfamilies are generally recognized within the Astacidea (*e.g.* Bracken-Grissom et al., 2014), including four currently extant ones: the Nephropoidea (homarid lobsters), the Enoplometopoidea (reef lobsters), the Astacoidea (Northern Hemisphere freshwater crayfish) and the Parastacoidea (Southern Hemisphere freshwater crayfish). At present approximately 800 living species are recognized within the Astacidea, with the majority contained within the Astacoidea and Parastacoidea (*e.g.* Crandall and Buhay, 2008). In fact, one family within the Astacoidea, *i.e.* the Cambaridae, accounts for approximately half of all living astacideans (*e.g.* Crandall and Buhay, 2008).

The iconic nature of the Astacidea is due largely to the fact that many members of this infraorder are highly prized and commercially important food species. For example, the American lobster *Homarus americanus* represents a multimillion-dollar per year fishery in New England and Atlantic Canada (the value of the 2013 landing for Maine alone was estimated at \$364.5 million; <http://bangordailynews.com/2014/02/28/news/state/maine-lobster-landings-value-jumped-23-million-in-2013/>), while extensive aquaculture initiatives are ongoing for many freshwater crayfish species (*e.g.* Hunter, 1994). Members of the Astacidea are also of global significance as invasive species (*e.g.* the environmental adaptability and fecundity of the freshwater crayfish *Procambarus clarkii* has allowed it to colonize large portions of North America, South America, Europe, Asia, and Africa, often at the expense of native aquatic species [*e.g.* Barbaresi et al., 2004; Klose and Cooper, 2013; Matsuzaki et al., 2009; Yue et al., 2010]). Many astacideans are also commonly used laboratory animals, both for educational exercises

(*e.g.* Baierlein et al., 2011; Cooper et al., 2011; Leksrisawat et al., 2010; Robinson et al., 2011) and for biomedical studies of neural network organization and neuromodulation (*e.g.* the stomatogastric and cardiac neuromuscular systems of lobsters and crayfish are among the premiere models for studying the basic principles that underlie the generation, maintenance and modulation of rhythmically active motor behaviors [*e.g.* Fénelon et al., 2003; Hooper and DiCaprio, 2004; Marder and Bucher, 2007; Selverston, 2005; Skiebe, 2001]).

Given their economic and biomedical importance, particularly their importance as models for understanding neuromodulatory control, members of the Astacidea have been the subjects of significant peptidomic analyses, particularly via mass spectral means (*e.g.* Bulau et al., 2004; Cape et al., 2008; Chen et al., 2010; Christie et al., 2006, 2008a; Dickinson et al., 2007; 2008, 2009a, 2009b; Fu et al., 2005a; Jia et al., 2014; Jiang et al., 2012; Li et al., 2002; Ma et al., 2008, 2009a; Skiebe et al., 2002, 2003; Stemmler et al., 2005, 2006, 2007a, 2010; Yasuda et al., 2004; Yasuda-Kamatani and Yasuda, 2004). Interestingly, and despite growing transcriptomic resources for members of this decapod group (*e.g.* Glazer et al., 2013; Manfrin et al., 2013, 2015; Stepanyan et al., 2006; Tom et al., 2013, 2014; Towle and Smith, 2006), only small-scale *in silico* transcriptome mining for peptide precursors has been conducted for members of the Astacidea (*e.g.* Christie et al., 2008b, 2010; Dickinson et al., 2007, 2008, 2009; Stevens et al., 2009). Here, we have conducted an infraorder-wide, large-scale peptidomic investigation of the Astacidea, mining the publicly accessible transcriptome shotgun assembly (TSA) data for putative peptide-encoding transcripts and predicting peptidomes for three astacidean species (*P. clarkii*, *Pontastacus leptodactylus*, *Cherax quadricarinatus*) using this sequence information. The structures of 176 distinct peptides were predicted for *P. clarkii*, including members of the adipokinetic hormone-corazonin-like peptide (ACP), allatostatin A (AST-A), allatostatin B

(AST-B), allatostatin C (AST-C) bursicon α , bursicon β , CCHamide, crustacean hyperglycemic hormone (CHH)/ion transport peptide (ITP), diuretic hormone 31 (DH31), eclosion hormone (EH), FMRFamide-like peptide (FLP), GSEFLamide, intocin, leucokinin, neuroparsin, neuropeptide F (NPF), pigment dispersing hormone (PDH), pyrokinin, RYamide, short neuropeptide F (sNPF), SIFamide, sulfakinin and tachykinin-related peptide (TRP) families, while 46 distinct, mature peptides, including isoforms of AST-A, AST-C, bursicon α , CCHamide, CHH/ITP, DH31, EH, intocin, myosuppressin, neuroparsin, red pigment concentrating hormone (RPCH), sNPF and TRP, were predicted for *P. leptodactylus*. An isoform of bursicon β and an isoform of neuroparsin were predicted for *C. quadricarinatus*. The identification of ACP from *P. clarkii* is the first detection of a member of this peptide group in any Decapoda, while the identifications of CCHamide, EH, GSEFLamide, intocin, neuroparsin and RYamide are the firsts for the Astacidea. Taken collectively, the data presented here both greatly expand the number of known astacidean peptides and provide a foundation from future *in silico* and functional studies of peptidergic signaling in members of this important crustacean taxon.

2. Materials and methods

2.1. Database searches

Database searches were conducted on or before January 10, 2015, using methods modified from a well-vetted protocol (*e.g.* Christie, 2008a, 2008b, 2014a, 2014b, 2014c, 2014d, 2014e, 2014f, 2015a, 2015b; Christie and Chi, 2015; Christie et al., 2008b, 2010a, 2011a, 2011b; Gard et al., 2009; Ma et al., 2009b, 2010). Specifically, the database of the online program tblastn (National Center for Biotechnology Information, Bethesda, MD;

<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) was set to “Transcriptome Shotgun Assembly (TSA)” and restricted to data from the Astacidea “(taxid:6712)”. Known crustacean peptide precursors were input into tblastn as the query sequences, and all hits returned by a given search were fully translated using the “Translate” tool of ExPASy (<http://web.expasy.org/translate/>) and then checked manually for homology to the target query. The complete list of peptide families searched for in this study, as well as the specific queries used, is provided in Table 1; this table also provides the BLAST-generated maximum score and E-value for each of the transcripts identified as encoding a putative neuropeptide precursor.

2.2. Peptide prediction

The structures of mature peptides were predicted using a well-established workflow (*e.g.* Christie, 2008a, 2008b, 2014a, 2014b, 2014c, 2014d, 2014e, 2014f, 2015a, 2015b; Christie and Chi, 2015; Christie et al., 2008b, 2010a, 2011a, 2011b, 2011c, 2013; Gard et al., 2009; Ma et al., 2009b, 2010). Specifically, each of the deduced precursor proteins was assessed for the presence of a signal peptide using the online program SignalP 4.1 (<http://www.cbs.dtu.dk/services/SignalP/>; Petersen et al., 2011); the D-cutoff values for the program were set to “Sensitive”. Prohormone cleavage sites were identified based on the information presented in Veenstra (2000) and/or by homology to known arthropod pre/preprohormone processing schemes. When present, prediction of the sulfation state of tyrosine residues was conducted using the online program “Sulfinator” (<http://www.expasy.org/tools/sulfinator/>; Monigatti et al., 2002). Disulfide bonding between cysteine residues was predicted by homology to known peptide isoforms and/or by using the online program “DiANNA” (<http://clavius.bc.edu/~clotelab/DiANNA/>; Ferrè and Clote, 2005).

Other post-translational modifications, *e.g.* cyclization of amino (N)-terminal glutamine/glutamic acid residues and carboxyl (C)-terminal amidation at glycine residues, were predicted by homology to known arthropod peptide isoforms. Figure 1 shows three examples of mature peptide structural prediction using the workflow just described; the mature structures of all peptides predicted in this study are provided in Tables 2-4. All protein/peptide alignments were done using the online program MAFFT version 7 (<http://mafft.cbrc.jp/alignment/software/>; Katoh and Standley, 2013).

3. Results

In this study, 36 distinct peptide families/subfamilies were searched for within the extant astacidean TSA sequence database (Table 1). In the interest of space, only those searches that resulted in the identification of putative precursor-encoding transcripts are described here (Table 1), with the data presented in alphabetical order based on family name. All precursor proteins listed as “full-length” exhibit a functional signal sequence (including a “start” methionine) and are flanked on their C-terminus end by a stop codon. Proteins described here as “partial” lacked a start methionine (referred to as C-terminal partial proteins), a stop codon (referred to as N-terminal partial proteins), or both of these features (referred to as internal protein fragments).

3.1. Adipokinetic hormone-corazonin-like peptide

A single *P. clarkii* transcript was identified as encoding a putative ACP precursor via a tblastn search of the extant astacidean TSA database using a partial ACP preprohormone from the remipede *Speleonectes cf. tulumensis* (Christie, 2014a) as the query sequence (Table 1). Translation of this transcript revealed a 100 amino acid full-length precursor protein (Procl-

prepro-ACP; Fig. 1A). Four peptides were predicted from Procl-prepro-ACP (Table 2 and Fig. 1A), one of which, pQITFSRSWVPQamide, possesses structural similarity to previously described members of the ACP family (*e.g.* Hansen et al., 2010).

3.2. Allatostatin A

Two transcripts, one from *P. clarkii* and the other from *P. leptodactylus*, were identified as encoding putative AST-A precursors using the sequence of a known *P. clarkii* prepro-AST-A (Accession No. **BAE45266**; Yasuda-Kamatani and Yasuda, 2006) as the input query (Table 1). Translation of the *P. clarkii* transcript revealed a 523 amino acid full-length prepro-hormone (Procl-prepro-AST-A; Fig. 2A). Alignment of Procl-prepro-AST-A and **BAE45266** is shown in Figure 3A. As can be seen from this figure, with the exception of a 23 amino acid insertion in **BAE45266**, the two proteins are essentially identical in sequence (one conservative amino acid substitution), and thus may represent splice variants of the same gene. Thirty-six peptides were predicted from Procl-prepro-AST-A (Table 2 and Fig 2A), 27 of which possess the C-terminal motif –YXFGLamide, where *X* represents a variable residue, which is the hallmark of the AST-A family (Christie et al., 2010c). Of the AST-As, 24 are present as single copies within Procl-prepro-AST-A, with three copies of one isoform, SGPYAFGLamide, present in the precursor protein (Table 2 and Fig 2A). The nine linker/precursor-related peptides predicted from Procl-prepro-AST-A are all distinct in structure (Table 2 and Fig 2A).

Translation of the *P. leptodactylus* transcript revealed it to encode a 93 amino acid internal fragment of a precursor protein (Ponle-prepro-AST-A; Fig. 4A). Five peptides, three distinct isoforms of AST-A (two full-length and one partial) and two distinct linker/precursor-related peptides, were predicted from Ponle-prepro-AST-A (Table 3 and Fig. 4A).

3.3. Allatostatin B

A single *P. clarkii* transcript was identified as encoding a putative AST-B precursor using the sequence of *Litopenaeus vannamei* prepro-AST-B (Christie, 2014f) as an input query (Table 1). Translation of this transcript revealed a 92 amino acid N-terminal partial protein (Procl-prepro-AST-B; Fig. 2B). Five distinct peptides were predicted from the extant portion of Procl-prepro-AST-B (Table 2 and Fig. 2B), two of which, VGWSSMHGTWamide and TNWNKFHGSWamide, possess the $-WX_6$ Wamide C-terminal motif that is the hallmark of the AST-B family (Christie et al., 2010c).

3.4. Allatostatin C

Two transcripts, one from *P. clarkii* and the other from *P. leptodactylus*, were identified as encoding putative AST-C precursors using the sequence of a prepro-AST-C from the lobster *H. americanus* (Dickinson et al, 2009b) as the input query (Table 1). Translation of the *P. clarkii* transcript revealed a 164 amino acid full-length prohormone (Procl-prepro-AST-C; Fig. 2C). Four distinct peptides were predicted from Procl-prepro-AST-C (Table 2 and Fig. 2C), one of which, GNGDGRLYWRCYFNAVSCF (disulfide bridging predicted between the two cysteine residues), possesses structural features consistent with its inclusion in the AST-C family, *i.e.* an unamidated C-terminus similar to the standard $-PISCF$ motif and a disulfide bridge between the cysteine residues located at positions 2 and 9 from the C-terminus (Christie et al., 2010c).

Translation of the *P. leptodactylus* transcript revealed a 72 amino acid C-terminal partial protein (Ponle-prepro-AST-C; Fig. 4B). Two peptides, SYWKQCAFNAVSCFamide (disulfide

bridging between the two cysteine residues) and a partial linker/precursor-related sequence, were predicted from the extant portion of Ponle-prepro-AST-C (Table 3 and Fig. 4C); SYWKQCAFNAVSCFamide is the same AST-C variant derived from the *H. americanus* prepro-AST-C used as the initial input query (Dickinson et al, 2009b).

3.5. Bursicon α

Two transcripts, one from *P. clarkii* and the other from *P. leptodactylus*, were identified as encoding putative bursicon α precursors using the sequence of *Homarus gammarus* pre-bursicon α (**Accession No. ADI86242**; Sharp et al., 2010) as the input query (Table 1). Translation of this transcript revealed a 142 amino acid full-length prehormone (Procl-pre-bursicon α ; Fig. 2D). The bursicon α derived from Procl-pre-bursicon α is 121 amino acids in length and is predicted by DiANNA analysis to possess disulfide bridges between its first and third, second and sixth, fourth and ninth, seventh and tenth, and eighth and eleventh cysteine residues (Table 2 and Fig. 2D); this *P. clarkii* peptide differs from that predicted from the *H. gammarus* query at just eight residues, all but one of which is a conservative amino acid substitution (alignment not shown).

Translation of the *P. leptodactylus* transcript revealed a 41 amino acid N-terminal partial protein (Ponle-pre-bursicon α ; Fig. 4C). While very little of this putative precursor protein is extant, the sequence that can be predicted differs at just three residues from the corresponding portion of the *P. clarkii* protein described above, all in the putative signal peptide (alignment not shown); the predicted partial *P. leptodactylus* bursicon α (Table 3 and Fig. 4C) is identical in amino acid sequence to its *P. clarkii* counterpart (alignment not shown).

3.6. Bursicon β

Two transcripts, one from *P. clarkii* and the other from *C. quadricarinatus*, were identified as encoding putative bursicon β precursors using the sequence of a pre-bursicon β from *H. americanus* (Christie et al., 2010a) as the input query (Table 1). Translation of the *P. clarkii* transcript revealed an 89 amino acid C-terminal partial protein (Procl-pre-bursicon β ; Fig. 2E). This partial peptide, likely the C-terminal portion of an isoform of bursicon β , differs at just seven positions from the corresponding portion of the bursicon β predicted from the *H. americanus* query protein (alignment not shown); four of the seven variable residues are conservative substitutions (alignment not shown).

Translation of the *C. quadricarinatus* transcript revealed a 48 amino acid C-terminal partial protein (Chequ-pre-bursicon β ; Fig. 5A). This partial peptide (Table 4 and Fig. 5A), the C-terminal portion of a putative bursicon β peptide, differs at 8 positions (all conservative amino acid substitutions) from the corresponding portion of its *H. americanus* counterpart (alignment not shown).

3.7. CCHamide

Three transcripts, two from *P. clarkii* and one from *P. leptodactylus*, were identified as encoding putative CCHamide precursors using the sequence of *L. vannamei* prepro-CCHamide (Christie, 2014f) as the input query (Table 1). Translation of one of *P. clarkii* transcripts revealed a 118 amino acid full-length preprohormone (Procl-prepro-CCHamide I; Fig. 1B), while translation of the other sequence revealed a 204 amino acid C-terminal partial protein (Procl-prepro-CCHamide II; Fig. 2F). Four distinct peptides were predicted from Procl-prepro-CCHamide I (Table 2 and Fig. 1B), one of which, SCSQFGHSCFGAHamide (a disulfide bridge

between the position 2 and 9 cysteines), possesses the structural motif XCXXW/Y/FGXXCXGXHamide (disulfide bridging between the position 2 and 9 cysteine residues), which is the hallmark of members of the CCHamide family (Hansen et al., 2011). Five distinct peptides, all different from those derived from Procl-prepro-CCHamide I, were predicted from Procl-prepro-CCHamide II (Table 2 and Fig. 2F), including the partial peptide +GGCLNYGHSLGAHamide, which appears to be the C-terminus of an N-terminally extended CCHamide (see below).

Translation of the *P. leptodactylus* transcript revealed a 246 amino acid full-length preprohormone (Ponle-prepro-CCHamide; Fig. 4D). This precursor protein is very similar to the extant portion of Procl-prepro-CCHamide II (*i.e.* 70.1% identical/87.7% similar in amino acid composition over the extant portion of the *P. clarkii* protein; alignment not shown), and it is likely to be the *P. leptodactylus* homolog of this precursor. Seven peptides were predicted from Ponle-prepro-CCHamide (Table 3 and Fig. 4D), including HRVMKGGCLNYGHSLGAHamide (disulfide bridging between the position 8 and 15 cysteines), which appears to be an N-terminally extended isoform of CCHamide; the partial CCHamide predicted from Procl-prepro-CCHamide II is identical in sequence to the sixth through nineteenth residues of HRVMKGGCLNYGHSLGAHamide, and may well be identical to this CCHamide isoform.

3.8. Crustacean hyperglycemic hormone (CHH)/ion transport peptide

Six transcripts, three from *P. clarkii* and three from *P. leptodactylus*, were identified as encoding putative CHH/ITP precursors using the sequence of *H. americanus* CHH A (**Accession No. P19806**; de Kleijn et al. 1995) as the input query. Translation of the three *P. clarkii*

transcripts revealed one sequence to encode an 85 amino acid internal protein fragment (Procl-prepro-CHH I; Fig. 2G1), and the other two to encode 139 (Procl-prepro-CHH II; Fig. 2G2) and 120 (Procl-prepro-CHH III; Fig. 2G3) amino acid full-length proteins. Each of the three *P. clarkii* CHH precursors is predicted to give rise to one isoform of CHH (a partial peptide from Procl-prepro-CHH I) and one linker/precursor-related peptide (Table 2 and Fig. 2G). Analysis of the CHH predicted from Procl-prepro-CHH II by DiANNA suggests disulfide bridging between the first and fifth, second and fourth, and third and sixth cysteine residues in this peptide (Table 2), a pattern of disulfide bonding stereotypical of members of the CHH superfamily (Böcking et al., 2002). Analysis of the CHH predicted from Procl-prepro-CHH III by DiANNA, which possesses only five cysteines, suggests disulfide bridges between the first and third and second and fifth cysteine residues in the peptide (Table 2).

Translation of the three *P. leptodactylus* transcripts revealed two to encode full-length precursor proteins of 137 (Ponle-prepro-CHH I; Fig. 4E1) and 140 (Ponle-prepro-CHH III; Fig. 4E3) amino acids, respectively, and the third, a 76 amino acid internal protein fragment (Ponle-prepro-CHH II; Fig. 4E2). Each of the full-length proteins contains a single isoform of CHH and one (Ponle-prepro-CHH I) or two (Ponle-prepro-CHH III) linker/precursor-related peptides; the extant portion of Ponle-prepro-CHH II contains just a CHH (Table 3 and Fig. 4E). All of the *P. leptodactylus* CHH and linker/precursor related peptides possess distinct structures (Table 3 and Fig. 4E). Analysis by DiANNA of the three predicted *P. leptodactylus* CHHs suggests each peptide possesses the stereotypical first and fifth, second and fourth, and third and sixth pattern of disulfide bonding (Table 3).

3.9. Diuretic hormone 31

Two transcripts, one from *P. clarkii* and the other from *P. leptodactylus*, were identified as encoding putative DH31 precursors using the sequence of *H. americanus* prepro-calcitonin-like diuretic hormone (**Accession No. ACX46386**; Christie et al. 2010b) as the input query (Table 1); calcitonin-like diuretic hormone is a synonym for DH31. Translation of the *P. clarkii* transcript revealed a 136 amino acid full-length prohormone (Procl-prepro-DH31; Fig. 2H). Five distinct peptides were predicted from Procl-prepro-DH31, including GLDLGLGRGYSGSQAAKHLMGLAAANFAGGPamide, a peptide possessing the structural hallmarks of the DH31 family (*i.e.* an overall length of 31 amino acids, an amidated C-terminus, and 13 highly conserved residues, XXDXGLXRGXSGXXXAKXXXXXXXXANXXXGPamide [Christie et al., 2010c]).

Translation of the *P. leptodactylus* transcript revealed a 66 amino acid C-terminal partial protein (Ponle-prepro-DH31; Fig. 4F). Two peptides were predicted from the extant portion of Ponle-prepro-DH31 (Table 3 and Fig. 4F), a linker/precursor-related peptide and the DH31 isoform GLDLGLGRGFSGSQAAKHLMGLAAANYAGGPamide.

3.10. Eclosion hormone

Two transcripts, one from *P. clarkii* and the other from *P. leptodactylus*, were identified as encoding putative EH precursors using the sequence of *Daphnia pulex* pre-EH (Dircksen et al. 2011) as the input query (Table 1). Translation of the *P. clarkii* transcript revealed an 85 amino acid full-length prehormone (Procl-pre-EH; Fig. 2I). A single 58 amino acid isoform of EH was predicted from Procl-pre-EH (Table 2 and Fig. 2I). Analysis of this EH isoform by DiANNA suggests disulfide bridging between the first and fourth, second and third and fifth and sixth cysteines of the peptide (Table 2).

Translation of the *P. leptodactylus* transcript revealed an 88 amino acid full-length protein (Ponle-pre-EH; Fig. 4G). A single 56 amino acid isoform of EH was predicted from this prehormone (Table 3 and Fig. 4G), with disulfide bridging likely between its first and fourth, second and third and fifth and sixth cysteines (Table 3).

3.11. FMRFamide-like peptide

Four transcripts, all from *P. clarkii*, were identified as encoding FLP precursors using the sequence of *P. clarkii* prepro-FLRFamide A (**Accession No. BAE06262**; Yasuda-Kamatani and Yasuda, 2006) as the input query (Table 1). Translation of one of these sequences revealed a 384 amino acid full-length prehormone (Procl-prepro-FLP I; Fig. 2J1), while translation of the others revealed C-terminal partial proteins of 29, 166 and 158 amino acids, respectively. The shortest of the partial proteins is identical in sequence to the C-terminus of Procl-prepro-FLP I; however, the two other partial proteins are distinct from the C-terminus of this full-length precursor, as well as from one another. Thus, both the 166 and 158 amino acid partial proteins are considered here to be parts of two distinct prehormones and have been named Procl-prepro-FLP II (Fig. 2J2) and Procl-prepro-FLP III (Fig. 2J2), respectively. Eighteen peptides were predicted from Procl-prepro-FLP I (Table 2 and Fig. 2J1), including nine possessing – FMRFamide-like C-termini, *i.e.* –FLRFamide (seven peptides), –YLRamide (one peptide) or –FPRamide (one peptide). Of these FLPs, all but one are encoded as single copies within the prehormone; the exception, SRNFLRFamide, is present twice in Procl-prepro-FLP I (Table 2 and Fig. 2J1). The nine linker/precursor-related peptide predicted from Procl-prepro-FLP I possess distinct structures (Table 2 and Fig. 2J1). Eight peptides were predicted from the extant portion of Procl-prepro-FLP II (Table 2 and Fig. 2J2), including four FLPs and four distinct

linker/precursor-related peptides. Of the FLPs, two are encoded as single copies (both distinct from those predicted from Procl-prepro-FLP I), with two copies of SRNFLRFamide (a peptide also predicted Procl-prepro-FLP I) present in the extant portion of Procl-prepro-FLP II (Table 2 and Fig. 2J2). Five distinct peptide were predicted from the extant portion of Procl-prepro-FLP III (Table 2 and Fig. 2J3), including two members of the FLP family: GFSDKNFMRFamide and GGHNVLRFamide. While the sequence of Procl-prepro-FLP I is similar to that of the two known *P. clarkii* prepro-FLPs (*i.e.* **Accession Nos. BAE06262** and **BAE06263**; Yasuda-Kamatani and Yasuda, 2006), it is distinct enough from both to likely represent the product of a different gene (Fig. 3B). Similarly, the differences in the sequence of Procl-prepro-FLP II and Procl-prepro-FLP III from each other and the other known *P. clarkii* prepro-FLPs suggest that they too are likely to be encoded by distinct genes (alignments not shown).

3.12. GSEFLamide

A single *P. clarkii* transcript was identified as encoding a putative GSEFLamide precursor using the sequence of *Tigriopus californicus* prepro-GSEFLamide Ia (Christie, 2014b) as the input query (Table 1). Translation of this transcript revealed a 136 amino acid C-terminal partial protein (Procl-prepro-GSEFLamide; Fig. 2K). Twelve peptides were predicted from the extant portion of Procl-prepro-GSEFLamide (Table 2 and Fig. 2K), including seven possessing the C-terminal motif –GSEFLamide (two copies of AVGSEFLamide and five copies of AMGSEFLamide), the hallmark of the GSEFLamide family, as well as three variant GSEFLamides (AMGAEFLamide, ATGLEFLNERVMGSELLamide and YMGSEFSamide) and two distinct linker/precursor-related peptides.

3.13. Intocin

Two transcripts, one from *P. clarkii* and the other from *P. leptodactylus*, were identified as encoding putative intocin precursors using the sequence of *L. vannamei* prepro-intocin (Christie, 2014f) as the input query (Table 1). Translation of the *P. clarkii* transcript revealed a 77 amino acid N-terminal partial preprohormone (Procl-prepro-intocin; Fig. 2L). Two distinct peptides were predicted from the extant portion of Procl-prepro-intocin (Table 2 and Fig. 2L), one of which, CFITNCPPGamide (a disulfide bridge between the position 1 and 6 cysteine residues), possesses the structural hallmarks of the intocin family, *i.e.* an overall length of nine amino acids, the structure CXXXNCPXGamide, and a disulfide bridge present between the position 1 and 6 cysteine residues (Stafflinger et al., 2008). DiANNA analysis of the linker/precursor-related protein derived from the extant portion of Procl-prepro-intocin, SGASTQIARSRTCSSCGPGLRGRCLGPEICCGEEVGCFLATREA, suggests disulfide bonding between the first and second, third and fifth, and fourth and sixth cysteines in this peptide (Table 2).

Translation of the *P. leptodactylus* transcript revealed a 153 amino acid full-length precursor protein (Ponle-prepro-intocin; Fig. 4H). Four distinct peptides were predicted from Ponle-prepro-intocin (Table 3 and Fig. 4H), including CFITNCPPGamide (disulfide bonding between the position 1 and 6 cysteines), which is the same isoform of intocin predicted from the *P. clarkii* partial protein identified above. Each of the linker/precursor-related peptides derived from Ponle-prepro-intocin is also predicted to possess disulfide bonding (Table 3), *i.e.* bridges between the first and sixth, second and fourth and third and fifth cysteines in SGASTQIARSRTCSSCGPGLRGRCLGPEICCGEGVGCFLATREA, between the first and second cysteines in MCRAENLVPLTCTNTDLKTCGRLREamide and between the second and

fifth and third and fourth cysteines in

CAASGLCCTEMKCEFDGSCVESREASVEDQQVERQRFAFLPNLSDDQWSL.

3.14. Leucokinin

Three transcripts, all from *P. clarkii*, were identified as encoding leucokinin precursors using the sequence of *Eucyclops serrulatus* prepro-leucokinin (Christie, 2015b) as the input query (Table 1). Translation of this set of transcripts revealed one to encode a 356 amino acid N-terminal partial protein, and the remaining two internal protein fragments of 155 and 154 amino acids, respectively. The two internal fragments share a common eight amino acid region of overlap with the N-terminal partial protein, though both are distinct sequences. For convenience of discussion the longer of the two internal fragments has been combined with the N-terminal partial protein to produce a 503 amino acid N-terminal sequence, named here Procl-prepro-leucokinin I (Fig. 2M1), with the 154 amino acid fragment named Procl-prepro-leucokinin II (Fig. 2M2). Twenty-seven distinct peptides were predicted from the extant portion of Procl-prepro-leucokinin I (Table 2 and Fig. 2M1), ten of which possess the C-terminal motif – FXXWA/Gamide, which is the hallmark of the leucokinin family (Christie et al., 2010c). One of the linker/precursor-related peptides derived from Procl-prepro-leucokinin I, QGTDVSPLALPGGQPSPCLPYRLPTHPLIRCELS, is predicted by DiANNA to possess a disulfide bridge between its two cysteine residues. Thirteen peptides were predicted from the extant portion of Procl-prepro-leucokinin II (Table 2 and Fig. 2M1), six of which possess structures consistent with their inclusion as members of the leucokinin family. Of the peptides derived from Procl-prepro-leucokinin II, all but three, one isoform of leucokinin and two linker/precursor-related peptides, were also predicted from Procl-prepro-leucokinin I (Fig. 2M).

3.15. Myosuppressin

A single *P. leptodactylus* transcript was identified as encoding a putative myosuppressin precursor using the sequence of *H. americanus* prepro-myosuppressin (**Accession No. ACX46385**; Stevens et al., 2009) as the input query (Table 1). Translation of this transcript revealed a 99 amino acid full-length prehormone (Ponle-prepro-myosuppressin; Fig. 4I). Four peptides were predicted from Ponle-prepro-myosuppressin (Table 3 and Fig. 4I), including pQDLDHVFLRFamide, a known and broadly conserved crustacean isoform of myosuppressin (Christie et al., 2010c).

3.16. Neuroparsin

Six transcripts, two from *P. clarkii*, three from *P. leptodactylus* and one from *C. quadricarinatus*, were identified as encoding putative neuroparsin precursors using the sequence of the lobster *Jasus lalandii* pre-neuroparsin (**Accession No. AHG98659**; Marco et al., 2014) as the input query (Table 1). Translation of the two *P. clarkii* sequences revealed one to encode a 95 amino acid C-terminal partial protein (Procl-pre-neuroparsin I; Fig. 2N1), and the other a 101 amino acid full-length prehormone (Procl-pre-neuroparsin II; Fig. 2N2). A single peptide was predicted from each of the *P. clarkii* pre-neuroparsins:

SPLCPERNEITPEDLNTCKYGVVLGWCGNPACGKGPGDPCGGRWEENGICGEGMYCVC
GYCAGCTTTLECALGRFC (disulfide bridges predicted between the first and twelfth, second and tenth, third and sixth, fourth and ninth, fifth and seventh and eighth and eleventh cysteines)
from Procl-pre-neuroparsin I (Table 2 and Fig. 2N1) and
APRCEHHDRPPPNCKYGTVRDWCRNGVCAKGPGESCGGNWFQKGKCGMGTFQCQCG

VCTGCSTVDGSCANGHLMC (disulfide bridges predicted between the first and eighth, second and fourth, third and seventh, fifth and sixth, ninth and eleventh, and tenth and twelfth cysteines) from Procl-pre-neuroparsin II (Table 2 and Fig. 2N2).

Translation of the three *P. leptodactylus* transcripts revealed one to encode a 107 amino acid full-length prehormone (Ponle-pre-neuroparsin I; Fig. 4J1), and the other two, identical 101 amino acid precursor proteins (Ponle-pre-neuroparsin II; Fig. 4J2). Like the *P. clarkii* pre-neuroparsins, each of the *P. leptodactylus* proteins is predicted to give rise to a single isoform of neuroparsin:

SPLCPERNEITPEDLNTCKYGVVLGWCGNAACGKGPGDSCGGRWEENGICGEGMYCVC
GYCAGCTTTLECALGRFC (bridging predicted between the first and twelfth, second and tenth, third and sixth, fourth and ninth, fifth and seventh and eighth and eleventh cysteines) from Ponle-pre-neuroparsin I (Table 2 and Fig. 4J1) and

APRCDHHDHRRPPNNCKYGTVRNWCNNGVCAKGPGESCGGHWYEQGKCGIGTFCLCGI
CIGCSTIDGSCADGPLIC (bridging predicted between the first and tenth, second and fourth, third and seventh, fifth and sixth, eighth and eleventh, and ninth and twelfth cysteines) from Ponle-pre-neuroparsin II (Table 2 and Fig. 4J2); these proteins are nearly identical in sequence to their *P. clarkii* counterparts (*P. leptodactylus* neuroparsin I differs from *P. clarkii* neuroparsin I at just two residues [both conservative amino acid substitutions], while *P. leptodactylus* neuroparsin II differs from *P. clarkii* neuroparsin II at 12 positions [nine of 12 being conservative substitutions]).

Translation of the *C. quadricarinatus* transcript revealed a 101 amino acid full-length prehormone (Chequ-pre-neuroparsin; Fig. 5B). As with the other pre-neuroparsin discussed earlier, a single neuroparsin isoform was predicted from Chequ-pre-neuroparsin (Table 4 and

Fig. 5B):

APRCNTHDRPPPSNCKYGTVRNWCRNGVCAKAPGESCGGHWYEHGKCGIGTFCLCGV
CIGCSTIDGRCADGPLMC (disulfide bridging predicted between the first and eighth, second
and fourth, third and seventh, fifth and sixth, ninth and twelfth, and tenth and eleventh cysteines).
This peptide appears to be the counterpart of *P. clarkii* and *P. leptodactylus* neuroparsin II,
differing from the former protein at 15 residues (ten of which are conservative substitutions) and
from the latter sequence at eight positions (six being conservative substitutions).

3.17. Neuropeptide F

Two transcripts, both from *P. clarkii*, were identified as encoding putative NPF
precursors using the sequence of *L. vannamei* prepro-NPF I (**Accession No. AEC12204**; Christie
et al., 2011d) as the input query (Table 1). Translation of one transcript revealed a 104 amino
acid full-length precursor (Procl-prepro-NPF I; Fig. 2O1), while translation of the other revealed
a 56 amino acid N-terminal partial protein (Procl-prepro-NPF II; Fig. 2O2). Two distinct
peptides were predicted from Procl-prepro-NPF I (Table 2 and Fig. 2O1), including
VASGGRHDSSAADALQAMHEAAIAGLLGPTEIQYPNRPNMFKSPVELRQYLDALNAYY
AIAGRPRFamide, a peptide possessing structural homology to member of the NPF family, *i.e.*
an overall length of 30 or more amino acids, the C-terminal motif –GRPRFamide, and tyrosine
residues at positions 10 and 17 from the C-terminus (Christie et al., 2010c). A single partial
peptide was predicted from Procl-prepro-NPF II (Table 2 and Fig. 2O2). This partial sequence,
KPDPTELAAMADAIAIKYLQELTKYYSQVSRP+, is highly similar to that of the N-terminus of
NPF isoform predicted from the *L. vannamei* query (differing at just six residues, four of which
are conservative amino acid substitutions), and thus likely represents a portion of an NPF

isoform distinct from that derived from Procl-prepro-NPF I (Table 2 and Fig. 2O). Given the conserved structure of NPF isoform, it is likely that the full-length peptide derived from Procl-prepro-NPF II will ultimately be determined to be KPDPTELAAMADAIAIKYLQELTKYYSQVSRPRFamide.

3.18. Pigment dispersing hormone

A single *P. clarkii* transcript was identified as encoding a putative PDH precursor using the sequence of a PDH preprohormone from the crayfish *Orconectes limosus* (**Accession No. AAB26385**; de Kleijn et al., 1993) as the input query (Table 1). Translation of this transcript revealed a 51 amino acid C-terminal partial protein (Procl-prepro-PDH; Fig. 2P). Two distinct peptides were predicted from Procl-prepro-PDH (Table 2 and Fig. 2P), one of which, NSELLNTLLGSQGLSNMRSAamide, possesses structural similarity to members of the PDH family (Christie et al., 2010c).

3.19. Pyrokinin

A single *P. clarkii* transcript was identified as encoding a pyrokinin precursor using the partial sequence of *L. vannamei* prepro-CAPA (Christie, 2014f) as the query sequence (Table 1). Translation of this transcript revealed a 75 amino acid C-terminal partial protein (Procl-prepro-pyrokinin; Fig. 2Q). Six peptides (five full-length and one partial) were predicted from Procl-prepro-pyrokinin (Table 2 and Fig. 2Q), all of which possess C-terminal motifs similar to – LXXPRXamide, the hallmark of the CAPA/periviscerokinin/pyrokinin superfamily (Christie et al., 2010c).

3.20. Red pigment concentrating hormone

A single *P. leptodactylus* transcript was identified as encoding a RPCH precursor using the sequence of *C. quadricarinatus* prepro-RPCH (**Accession No. AAV80404**; Martinez-Perez et al., 2007) as the input query (Table 1). Translation of this transcript revealed a 95 amino acid C-terminal partial protein (Ponle-prepro-RPCH; Fig. 4K). Three distinct peptides were predicted from Ponle-prepro-RPCH (Table 3 and Fig. 4K), including pQLNFSPGWamide, a peptide whose structure is identical to authentic RPCH (Christie et al., 2010c).

3.21. RYamide

A single *P. clarkii* transcript was identified as encoding a putative RYamide precursor using the partial sequence of *L. vannamei* prepro-RYamide (Christie, 2014f) as the input query (Table 1). Translation of this transcript revealed a 134 amino acid full-length preprohormone (Procl-prepro-RYamide; Fig. 2R). Six distinct peptides were predicted from Procl-prepro-RYamide (Table 2 and Fig. 2R), two of which, pQGFYSQRYamide and FIGGSRYamide, possess the –RYamide C-terminus that is the hallmark of members of the RYamide family (Christie et al., 2010c). Analysis of one of the linker/precursor-related peptide predicted from Procl-prepro-RYamide, SGSPPESEFSSAITPEADDTDMTATLLLGDVLCFLVDVPDIYRCL, suggests the presence of a disulfide bond between its two cysteine residues (Table 2).

3.22. Short neuropeptide F

Two transcripts, one from *P. clarkii* and the other from *P. leptodactylus*, were identified as encoding sNPF precursors using the sequence of *D. pulex* prepro-sNPF (**Accession No.**

EFX90018; Colbourne et al., 2011) as the input query (Table 1). Translation of the *P. clarkii* transcript revealed a 129 amino acid full-length preprohormone (Procl-prepro-sNPF; Fig. 2S). Six distinct peptides were predicted from Procl-prepro-sNPF (Table 2 and Fig. 2S), including three, APPSMRLRFamide, DMGWQVAQRSMPSLRLRFamide and DSRTPALRLRFamide, that possess the structural hallmarks of members of the sNPF family, *i.e.* the C-terminal motif – RXRFamide and an overall length of ten or so amino acids (Christie et al., 2010c).

Translation of the *P. leptodactylus* transcript revealed a 128 amino acid full-length protein (Ponle-prepro-sNPF; Fig. 4L). Like the *P. clarkii* precursor, six distinct peptides were predicted from Ponle-prepro-sNPF (Table 3 and Fig. 4L), including the sNPFs APPSMRLRFamide, DMGWQVAQRSMPSLRLRFamide and DSRMPALRLRFamide, the former two of which were also predicted from Procl-prepro-sNPF.

3.23. SIFamide

A single *P. clarkii* transcript was identified as encoding a putative SIFamide precursor using the sequence of *H. americanus* prepro-Val¹-SIFamide (**Accession No. ABV21807**; Dickinson et al., 2008) as the input query (Table 1). Translation of this transcript revealed a 76 amino acid full-length preprohormone (Procl-prepro-SIFamide; Fig. 2T), which is identical in sequence to a known *P. clarkii* SIFamide precursor (*i.e.* **Accession No. BAC55940**; Yasuda et al., 2004 [Fig. 3C]). Two peptides were predicted from Procl-prepro-SIFamide (Table 2 and Fig. 2T), one of which, GYRKPPFNGSIFamide, is a known and broadly conserved crustacean SIFamide isoform (Christie et al., 2010c).

3.24. Sulfakinin

A single *P. clarkii* transcript was identified as encoding a putative sulfakinin precursor using the sequence of *H. americanus* prepro-sulfakinin (**Accession No. ABQ95346**; Dickinson et al., 2007) as the input query (Table 1). Translation of this transcript revealed a 103 amino acid C-terminal partial protein (Procl-prepro-sulfakinin; Fig. 1C). Six distinct peptides were predicted from the extant portion of Procl-prepro-sulfakinin, including two, pEFDEY_(SO₃H)GHMRFamide and GGDY_(SO₃H)DDY_(SO₃H)GHLRFamide, that possess the structural hallmark of the sulfakinin family, *i.e.* the C-terminal motif –Y_(SO₃H)GHM/LRFamide (Christie et al., 2010c).

3.25. Tachykinin-related peptide

Two transcripts, one from *P. clarkii* and the other from *P. leptodactylus*, were identified as encoding putative TRP precursors using the sequence of *H. americanus* prepro-TRP (**Accession No. ACB41786**; Christie et al., 2008a) as the input query (Table 1). Translation of the *P. clarkii* transcript revealed a 225 amino acid full-length preprohormone (Procl-prepro-TRP; Fig. 2U), which is identical in sequence to a known *P. clarkii* TRP precursor (*i.e.* **Accession No. BAC82426**; Yasuda-Kamatani and Yasuda, 2004 [Fig. 3D]). Twelve peptides were predicted from Procl-prepro-TRP (Table 2 and Fig. 2U), including seven copies of APSGFLGMRamide, a known and broadly conserved crustacean TRP (Christie et al., 2010c). The five linker/precursor-related peptide derived from Procl-prepro-TRP possess distinct structures (Table 2 and Fig. 2U).

Translation of the *P. leptodactylus* transcript revealed a 73 amino acid internal protein fragment (Ponle-prepro-TRP; Fig. 4M). Five peptides were predicted from Ponle-prepro-TRP (Table 3 and Fig. 4M), including three copies of APSGFLGMRamide and two distinct linker/precursor-related peptides (one full-length and one partial).

4. Discussion

4.1. Prediction of crayfish peptidomes using transcriptome shotgun assembly data

In silico genome/transcriptome mining has been, and continues to be, a powerful method for neuropeptide discovery in crustaceans (*e.g.* Christie, 2014a, 2014b, 2014c, 2014d, 2014e, 2014f, 2015b; Christie et al., 2008b, 2010a, 2011c, 2013; Dirksen et al., 2011; Gard et al., 2009; Ma et al., 2009b, 2010; Toullec et al., 2013; Ventura et al., 2014; Yan et al., 2012). When a large collection of assembled nucleotide sequences is available for a species, an extensive peptidome can rapidly be deduced using a simple bioinformatics workflow, *e.g.* the 158 distinct mature peptides recently identified from the penaeid shrimp *L. vannamei* (Christie, 2014f). In the study presented here, the publicly accessible TSA sequences for members of the Astacidea were mined for putative neuropeptide-encoding transcripts using the BLAST algorithm tblastn and known crustacean pre/preprohormones as input queries. Peptide-encoding sequences from three crayfish were identified via this strategy: *P. clarkii* (33 transcripts), *P. leptodactylus* (17 transcripts) and *C. quadricarinatus* (two transcripts); these three species encompass the two extant superfamilies of the freshwater crayfish, namely the Astacoidea (*P. clarkii* and *P. leptodactylus*) and the Parastacoidea (*C. quadricarinatus*), as well as the three extant families of these astacidean subgroups, *i.e.* the Astacidae (*P. leptodactylus*), the Cambaridae (*P. clarkii*), and the Parastacidae (*C. quadricarinatus*).

Translation of the 33 *P. clarkii* transcripts allowed for the structural prediction of 176 distinct peptides, including isoforms of ACP, AST-A, AST-B, AST-C, bursicon α , bursicon β , CCHamide, CHH/ITP, DH31, EH, FLP, GSEFLamide, intocin, leucokinin, neuroparsin, NPF, PDH, pyrokinin, RYamide, sNPF, SIFamide, sulfakinin and TRP. This collection of sequences

is one of, if not the, largest peptidome thus far deduced via *in silico* transcriptome mining for any single crustacean species, significantly expanding the catalog of peptidergic signaling agents for *P. clarkii*, as well as for the Astacidea, and even the Decapoda, generally. Likewise, the 46 distinct *P. leptodactylus* peptides predicted here, which include members of AST-A, AST-C, bursicon α , CCHamide, CHH/ITP, DH31, EH, intocin, myosuppressin, neuroparsin, RPCH, sNPF and TRP families, are of significance as they are the first peptides predicted for this species, and in conjunction with the *P. clarkii* sequences, include isoforms of peptide families previously unknown from the Astacidea (see below). While just two peptides were predicted for *C. quadricarinatus*, the identification of a neuroparsin from this species is the first from any member of the Astacidea (isoforms of this peptide family were also predicted from *P. clarkii* and *P. leptodactylus*). Taken collectively, the structural data presented here not only greatly expand the catalog of neuropeptides known for the Astacidea, but also provide platforms for future anatomical studies of neuropeptide distribution in crayfish and for assessment of the functional roles played by peptidergic signaling systems in astacideans.

4.2. Identification of the first members of the CCHamide, eclosion hormone, GSEFLamide, intocin, neuroparsin and RYamide families from the Astacidea

A wide array of peptides was identified in this study, particularly from *P. clarkii*. While most of the peptides predicted here are novel, at least for the species from which they were identified, isoforms of many of them, including some identical sequences, have been described from other members of the Astacidea, particularly from *P. clarkii* itself (e.g. Yasuda et al., 2004; Yasuda-Kamatani and Yasuda, 2004, 2006) and/or from the lobster *H. americanus* (e.g. Cape et al., 2008; Chen et al., 2010; Fu et al., 2005a; Jia et al., 2014; Jiang et al., 2012; Ma et al., 2008).

Specifically, members of the AST-A, AST-B, AST-C, bursicon α , bursicon β , CHH/ITP, DH31, FLP, leucokinin, myosuppressin, NPF, PDH, pyrokinin, RPCH, sNPF, SIFamide, sulfakinin and TRP families have all been identified from at least one astacidean prior to this study. In contrast, the isoforms of CCHamide, EH, GSEFLamide, intocin, neuroparsin and RYamide predicted here, while known from other decapods, are the firsts for the Astacidea. Specifically, CCHamide (predicted in this study from both *P. clarkii* and *P. leptodactylus*) was identified previously from the penaeid shrimp *L. vannamei* and *Penaeus monodon* (Christie, 2014f), while isoforms of EH (predicted here from both *P. clarkii* and *P. leptodactylus*) are known from both of these shrimp (Christie, 2014f; Christie et al., 2010a), as well as from the penaeid *Marsupenaeus japonicus* (Christie et al., 2010a) and the brachyuran crab *Callinectes sapidus* (Christie et al., 2010a). Members of the GSEFLamide family (predicted here from *P. clarkii*) were previously described from the penaeids *L. vannamei* and *M. japonicus* (Christie, 2014b, 2014f) and the brachyuran *Eriocheir sinensis* (Christie, 2014b), while intocin (predicted here from both *P. clarkii* and *P. leptodactylus*) was known from *L. vannamei* (Christie, 2014f). Neuroparsins (predicted in this study from *P. clarkii*, *P. leptodactylus* and *C. quadricarinatus*) have been identified from a number of decapods, including the penaeid shrimp *L. vannamei*, *M. japonicus* and *P. monodon* (Christie, 2014f; Christie et al., 2010a; Ma et al., 2010), the brachyuran *C. sapidus* (Christie et al., 2010a), the anomuran *Petrolisthes cinctipes* (Christie et al., 2010a), and the palinuran *J. lalandii* (Marco et al., 2014). Similarly, members of the RYamide family (predicted here from *P. clarkii*) have been identified from a variety of decapods (e.g. Christie, 2014f; Fu et al., 2005b; Hui et al., 2013; Li et al., 2003; Ma et al., 2009b, 2010; Stemmler et al., 2007b). At present, how members of these peptides families are distributed in the nervous systems of astacideans, or for that matter any decapod, remains unknown, as are functional roles they may play in these

animals. While nothing is currently known about the functional roles played by members of the GSEFLamide or RYamide families in any arthropod, work in insects suggest that the crustacean CCHamides may be involved in the regulation of feeding-related behaviors (Ida et al., 2012; Li et al., 2013) and/or the modulation of olfaction (Farhan et al., 2013), while the EHs may play a role in the control of molting (*e.g.* Mesce and Fahrbach, 2002; Truman, 2005; Zitnan et al., 2007) and intocin in the regulation of diuresis (Aikins et al., 2008). In insects, the neuroparsins have been shown to play roles in reproductive control (*e.g.* Badisco et al., 2007), and they may well serve similar function in the Crustacea (Yang et al., 2014).

4.3. Identification of the first adipokinetic hormone-corazonin-like peptide from a member of the Decapoda

One of the highlights of this study was the prediction of an isoform of ACP, pQITFSRSWVPQamide, from the crayfish *P. clarkii*. This discovery is not only the first detection of an isoform of this peptide family in a member of the Astacidea, but is also the first description of an ACP from any member of the Decapoda. This peptide family is proposed as a structural intermediate between the adipokinetic hormone and corazonin families (Hansen et al., 2010 [Fig. 6A]), and thus the origin of it being named adipokinetic hormone-corazonin-like peptide or ACP. At the time of its discovery, the ACP family was proposed as hexapod-specific (Hansen et al., 2010), as isoforms of it, as well as its receptor, appeared broadly conserved in insects, but were absent in the genome of the cladoceran crustacean *D. pulex*, the only crustacean for which a fully sequenced genome was available (Colbourne et al., 2011), and one of just a few crustaceans for which significant transcriptomic resources were publicly extant. With the recent public deposition of a number of large TSA datasets for members of the Crustacea, the first

isoforms of ACP from this subphylum have recently been discovered (Fig. 6B), the first from the remipede *S. cf. tulumensis* (Christie, 2014a) and subsequently from the copepods *T. californicus* (Christie, 2014b) and *E. serrulatus* (2015b). Interestingly, the copepod and *P. clarkii* isoforms of ACP are longer than the known insect/remipede variants (Fig. 6B), being 11-13 amino acids in length rather than the stereotypical 10 residues (Christie, 2014a; Hansen et al., 2010). Also of note, two isoforms of ACP are present in each of the known copepod preprohormone (Fig. 6C), whereas just a single isoform is present in the insects, remipede and crayfish precursors (Christie, 2014a; Hansen et al., 2010 [Fig. 6C]). While nothing is currently known about the distribution or function of ACPs in crustaceans, in insects, members of this peptide family are present in neuronal somata in the brain and in axons and neuropil in the ventral nerve chord (Hansen et al., 2010; Patel et al., 2014). The organization of the neuronal ACP release sites in insects suggests a local endocrine (paracrine) role for this peptide, most likely functioning as a locally released neuromodulator (Hansen et al., 2010; Patel et al., 2014).

4.4. Summary

The Astacidea is a decapod crustacean infraorder of both commercial and biomedical importance. While members of this taxon have been the subjects of numerous peptide discovery studies, none has involved large-scale transcriptome mining. Here, such an investigation was undertaken, with peptide-encoding transcripts identified for three crayfish species: *P. clarkii*, *P. leptodactylus* and *C. quadricarinatus*. The extant TSA data for *P. clarkii* alone allowed for the prediction of 176 distinct peptides, which represent one of, if not the largest peptidome thus far predicted via *in silico* transcriptome mining for any single crustacean species. The peptides predicted here encompass 25 distinct peptide families, and include the first astacidean isoforms

of CCHamide, EH, GSEFLamide, intocin, neuroparsin and RYamide, as well as the first member of the ACP family predicted from any decapod. These data more than double the catalog of known astacidean peptides, and serve as a resource for future functional studies of peptidergic signaling in members of the Astacidea and other crustacean taxa.

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1128 **Figure legends**

1129 Figure 1. Three examples of the *in silico* workflow used for the prediction of putative mature
 1130 peptide structures. (A) Predicted processing scheme for *Procambarus clarkii* (Procl)-prepro-
 1131 adipokinetic hormone-corazonin-like peptide (ACP). The structure of the putative mature ACP
 1132 isoform is shown in red, with those of four putative mature linker/precursor-related peptides

1133 shown in blue. In this panel, the pyroglutamic acid residue present in the putative mature ACP is
 1134 indicated by “pQ”. **(B)** Predicted processing scheme for Procl-prepro-CCHamide I. The
 1135 structure of the putative mature CCHamide isoform is shown in red, with those of three putative
 1136 mature linker/precursor-related peptides shown in blue. In this panel, the inverted red bracket
 1137 indicates a disulfide bond between the two cysteine residues in the putative mature CCHamide.
 1138 **(C)** Predicted processing scheme for the extant portion of Procl-prepro-sulfakinin. The
 1139 structures of two putative mature sulfakinin isoforms are shown in red, with those of four
 1140 putative mature linker/precursor-related peptides (one partial) shown in blue. In this panel, the
 1141 presence of sulfated tyrosine residues in the two putative mature sulfakinins is indicated by
 1142 “Y_(SO₃H)”, while the presence of pyroglutamic acid in one sulfakinin is indicated by “pE”.

1143

1144 Figure 2. Putative *Procambarus clarkii* (Procl) pre/preprohormones deduced from transcriptome
 1145 shotgun assembly sequence data. **(A)** Procl-prepro-allatostatin A. **(B)** The amino (N)-terminal
 1146 portion of Procl-prepro-allatostatin B. **(C)** Procl-prepro-allatostatin C. **(D)** Procl-pre-bursicon
 1147 α . **(E)** The carboxyl (C)-terminal portion of Procl-pre-bursicon β . **(F)** The C-terminal portion of
 1148 Procl-prepro-CCHamide II. **(G1)** An internal protein fragment of Procl-prepro-crustacean
 1149 hyperglycemic hormone I. **(G2)** Procl-prepro-crustacean hyperglycemic hormone II. **(G3)**
 1150 Procl-prepro-crustacean hyperglycemic hormone III. **(H)** Procl-prepro-diuretic hormone 31. **(I)**
 1151 Procl-pre-eclosion hormone. **(J1)** Procl-prepro-FMRFamide-like peptide I. **(J2)** The C-terminal
 1152 portion of Procl-prepro-FMRFamide-like peptide II. **(J3)** The C-terminal portion of Procl-
 1153 prepro-FMRFamide-like peptide III. **(K)** The C-terminal portion of Procl-prepro-GSEFLamide.
 1154 **(L)** The N-terminal portion of Procl-prepro-intocin. **(M1)** The N-terminal portion of Procl-
 1155 prepro-leucokinin I. **(M2)** An internal protein fragment of Procl-prepro-leucokinin II. **(N1)** The

1156 C-terminal portion of Procl-pre-neuroparsin I. (**N2**) Procl-pre-neuroparsin II. (**O1**) Procl-prepro-
 1157 neuropeptide F I. (**O2**) The N-terminal portion of Procl-prepro-neuropeptide F II. (**P**) The C-
 1158 terminal portion of Procl-prepro-pigment dispersing hormone. (**Q**) The C-terminal portion of
 1159 Procl-prepro-pyrokinin. (**R**) Procl-prepro-RYamide. (**S**) Procl-prepro-short neuropeptide F. (**T**)
 1160 Procl-prepro-SIFamide. (**U**) Procl-prepro-tachykinin-related peptide. In this figure, signal
 1161 peptides are shown in gray, while all mono/dibasic cleavage loci are shown in black. For each
 1162 sequence, the isoform(s) of the peptide for which the precursor is named is/are shown in red,
 1163 with all linker/precursor related peptides shown in blue. The “+” symbol indicate the presence of
 1164 additional, unknown, amino acid residues at the N- and/or C-terminus.

1165

1166 Figure 3. Alignment of selected *Procambarus clarkii* (Procl) precursors deduced in this study
 1167 with previously known preprohormones. (**A**) Alignment of Procl-prepro-allatostatin A and
 1168 **BAE45266** (Yasuda-Kamatani and Yasuda, 2006). (**B1**) Alignment of Procl-prepro-
 1169 FMRFamide-like peptide I and **BAE06262** (Yasuda-Kamatani and Yasuda, 2006). (**B2**)
 1170 Alignment of Procl-prepro-FMRFamide-like peptide I and **BAE06263** (Yasuda-Kamatani and
 1171 Yasuda, 2006). (**C**) Alignment of Procl-prepro-SIFamide and **BAC55940** (Yasuda et al., 2004).
 1172 (**D**) Alignment of Procl-prepro-tachykinin-related peptide and **BAC82426** (Yasuda-Kamatani
 1173 and Yasuda, 2004). In the line immediately below each sequence grouping, “*” indicates amino
 1174 acids that are identically conserved between the two proteins, while “.” and “:” denote amino
 1175 acids that are similar in structure between the two sequences (*i.e.* conservative amino acid
 1176 substitutions).

1177

1178 Figure 4. Putative *Pontastacus leptodactylus* (Ponle) pre/preprohormones deduced from
 1179 transcriptome shotgun assembly sequence data. **(A)** An internal protein fragment of Ponle-
 1180 prepro-allatostatin A. **(B)** The carboxyl (C)-terminal portion of Ponle-prepro-allatostatin C. **(C)**
 1181 The amino (N)-terminal portion of Ponle-pre-bursicon α . **(D)** Ponle-prepro-CCHamide. **(E1)**
 1182 Ponle-prepro-crustacean hyperglycemic hormone I. **(E2)** An internal protein fragment of Ponle-
 1183 prepro-crustacean hyperglycemic hormone II. **(E3)** Ponle-prepro-crustacean hyperglycemic
 1184 hormone III. **(F)** The C-terminal portion of Ponle-prepro-DH31. **(G)** Ponle-pre-eclosion
 1185 hormone. **(H)** Ponle-prepro-intocin. **(I)** Ponle-prepro-myosuppressin. **(J1)** Ponle-pre-
 1186 neuroparsin I. **(J2)** Ponle-pre-neuroparsin II. **(J3)** The C-terminal portion of Procl-prepro-
 1187 FMRFamide-like peptide III. **(K)** The C-terminal portion of Ponle-prepro-red pigment
 1188 concentrating hormone. **(L)** Procl-prepro-short neuropeptide F. **(M)** An internal protein
 1189 fragment of Ponle-prepro-tachykinin-related peptide. In this figure, signal peptides are shown in
 1190 gray, while all mono/dibasic cleavage loci are shown in black. For each sequence, the isoform(s)
 1191 of the peptide for which the precursor is named is/are shown in red, with all linker/precursor
 1192 related peptides shown in blue. The “+” symbol indicate the presence of additional, unknown,
 1193 amino acid residues at the N- and/or C-terminus.

1194

1195 Figure 5. Putative *Cherax quadricarinatus* (Chequ) prehormones deduced from transcriptome
 1196 shotgun assembly sequence data. **(A)** The carboxyl (C)-terminal portion of Chequ-pre-bursicon
 1197 β . **(B)** Chequ-pre-neuroparsin. In this figure, signal peptides are shown in gray, with the
 1198 isoform of the peptide for which the precursor is named shown in red. In panel **A**, the “+”
 1199 symbol indicate the presence of additional, unknown, amino acid residues at the amino-terminus
 1200 of the bursicon β precursor.

1201

1202 Figure 6. Comparisons of crustacean adipokinetic hormone-corazonin-like peptide isoforms and

1203 precursor proteins. (A) Structural comparison between known astacidean isoforms of red

1204 pigment concentrating hormone (RPCH), adipokinetic hormone-corazonin-like peptide (ACP)

1205 and corazonin. Structural features (amino acid residues and carboxyl (C)-terminal amidation)

1206 that are identically conserved between all three peptides are colored red. Amino acids that are

1207 shared by ACP and corazonin, but not RPCH, are colored green. A pair of conserved, but not

1208 identical, amino acids shared by RPCH and ACP, but not corazonin, is colored blue. (B)

1209 Alignment of known crustacean ACP isoforms. Structural features (amino acid residues and C-

1210 terminal amidation) that are identically conserved between all isoforms are colored red, with

1211 conserved, but not identical, amino acids shared by all isoforms highlighted in yellow. (C).

1212 Alignment of a representative insect (*Tribolium castaneum* [Trica]; **Accession No. ADF28807**

1213 [Hansen et al., 2010]) and all known crustacean ACP preprohormones. In all protein sequences,

1214 the isoform(s) of ACP (including the C-terminal glycine residue that serves as an amidation

1215 signal) is colored red. In the line immediately below each sequence grouping, “*” indicates

1216 amino acids that are identically conserved between the two proteins, while “.” and “:” denote

1217 amino acids that are similar in structure between the two sequences (*i.e.* conservative amino acid

1218 substitutions). Other abbreviations: Procl, *Procambarus clarkii* (Astacidea); Spetu,

1219 *Speleonectes* cf. *tulumensis* (Remipedia); Tigca, *Tigriopus californicus* (Copepoda); Eucse,

1220 *Eucyclops serrulatus* (Copepoda). Other references to sequence data: *P. clarkii* (this study), *S.*

1221 cf. *tulumensis* (Christie, 2014a), *T. californicus* (Christie, 2014b), *E. serrulatus* (Christie, 2015b).

Table 1

Table 1. Putative peptide-encoding transcripts identified via <i>in silico</i> mining of transcriptome shotgun assembly (TSA) sequence data for the Astacidea							
Peptide Family	TSA transcript/protein identifications					tblasn search statistics	
	Species	Transcript accession number	Transcript length*	Deduced protein length [†]	Deduced protein type [‡]	BLAST score	E-value
ACP	<i>P. clarkii</i>	GBEV01011002	607	100	F	28.5	2.9
AST-A	<i>P. clarkii</i>	GBEV01001712	3193	523	F	770	0.0
	<i>P. leptodactylus</i>	GAFS01069035	282	93	I	73.6	2e-14
AST-B	<i>P. clarkii</i>	GBEV01040422	504	92	N	67.8	2e-12
AST-C	<i>P. clarkii</i>	GBEV01001327	1778	164	F	30.4	1.1
	<i>P. leptodactylus</i>	GAFY01028258	272	72	C	115	8e-33
Allatotropin							
Bursicon α	<i>P. clarkii</i>	GBEV01070554	1792	142	F	258	4e-82
	<i>P. leptodactylus</i>	GAFS01049726	235	41	N	57.0	2e-10
Bursicon β	<i>P. clarkii</i>	GBEV01054566	310	89	C	171	8e-54
	<i>C. quadricarinatus</i>	GADE01008158	478	48	C	87.8	5e-21
CCHamide	<i>P. clarkii</i>	GBEV01004199	2529	118	F	83.6	3e-18
		GBEV01015793	5007	204	C	31.6	0.82
	<i>P. leptodactylus</i>	GAFS01018472	1270	246	F	32.3	0.41
Corazonin							
CCAP							
CHH/ITP	<i>P. clarkii</i>	GBEV01000359	257	85	I	115	1e-32
		GBEV01014441	2949	139	F	83.2	8e-18
		GBEV01008025	2300	120	F	63.9	2e-11
	<i>P. leptodactylus</i>	GAFS01024727	802	137	F	173	6e-53
		GAFS01049968	228	76	I	140	1e-42
		GAFS01042936	913	140	F	81.6	2e-18
DENamide							
DH31	<i>P. clarkii</i>	GBEV01003269	1863	136	F	169	5e-49
	<i>P. leptodactylus</i>	GAFS01038688	268	66	C	79.3	1e-18
DH44							
DXXRamide							

ETH							
EH	<i>P. clarkii</i>	GBEV01101142	527	85	F	54.3	2e-09
	<i>P. leptodactylus</i>	GAFS01039705	1028	88	F	57.0	1e-09
FLP	<i>P. clarkii</i>	GBEV01020536	2003	384	F	311	4e-99
		GBEV01106719	381	29	C	38.1	0.012
		GBEV01105142	548	166	C	117	7e-38
		GBEV01189645	1045	158	C	132	4e-34
FXGGXamide							
GSEFLamide	<i>P. clarkii</i>	GBEV01013249	5907	136	C	143	2e-37
ILP							
Intocin	<i>P. clarkii</i>	GBEV01109169	281	77	N	89.7	2e-22
	<i>P. leptodactylus</i>	GAFS01000331	737	153	F	172	1e-52
Leucokinin	<i>P. clarkii</i>	GBEV01013648	1138	356	N	34.3	0.37
		GBEV01006537	466	155	I	32.7	0.75
		GBEV01006538	463	154	I	33.9	0.30
Myosuppressin	<i>P. leptodactylus</i>	GAFS01019764	732	99	F	127	6e-36
Neuroparsin	<i>P. clarkii</i>	GBEV01003881	1062	95	C	132	3e-37
		GBEV01000414	5114	101	F	69.3	2e-13
	<i>P. leptodactylus</i>	GAFS01000216	1154	107	F	137	2e-38
		GAFY01019447	2524	101	F	70.9	4e-14
		GAFS01005142	2584	101	F	70.9	4e-14
	<i>C. quadricarinatus</i>	GADE01014957	484	101	F	64.7	4e-13
NPF	<i>P. clarkii</i>	GBEV01005906	1178	104	F	34.3	0.036
		GBEV01020719	425	56	N	55.8	3e-10
Orcokinin							
PDH	<i>P. clarkii</i>	GBEV01236728	230	51	C	26.6	4.8
Proctolin							
Pyrokinin	<i>P. clarkii</i>	GBEV01049409	253	75	C	63.2	9e-13
RPCH	<i>P. leptodactylus</i>	GAFS01040704	563	95	C	95.1	2e-24
RYamide	<i>P. clarkii</i>	GBEV01010112	2521	134	F	95.5	1e-22
sNPF	<i>P. clarkii</i>	GBEV01004780	4804	129	F	32.0	0.53
	<i>P. leptodactylus</i>	GAFS01014236	1058	128	F	32.3	0.34
SIFamide	<i>P. clarkii</i>	GBEV01000221	2358	76	F	92.4	6e-22
Sulfakinin	<i>P. clarkii</i>	GBEV01027368	475	103	C	148	6e-45

TRP	<i>P. clarkii</i>	GBEV01001769	1980	225	F	303	1e-99
	<i>P. leptodactylus</i>	GAFS01072672	219	73	I	128	4e-39

Peptide family abbreviations: ACP, adipokinetic hormone-corazonin-like peptide; AST-A, allatostatin A; AST-B, allatostatin B; AST-C, allatostatin C; CCAP, crustacean cardioactive peptide; CHH/ITP, crustacean hyperglycemic hormone/ion transport peptide; DH31, diuretic hormone 31; DH44, diuretic hormone 44; ETH, ecdysis-triggering hormone; EH, eclosion hormone; FLP, FMRFamide-like peptide; ILP, insulin-like peptide; NPF, neuropeptide F; PDH, pigment dispersing hormone; RPCH, red pigment concentrating hormone; sNPF, short neuropeptide F; TRP, tachykinin-related peptide.

Species abbreviations: *P. clarkii*, *Procambarus clarkii*; *P. leptodactylus*, *Pontastacus leptodactylus*; *C. quadricarinatus*, *Cherax quadricarinatus*.

*Length in nucleotides
†Length in amino acids
‡Deduced protein type: F, full-length; N, amino-terminal partial; I, internal protein fragment; C, carboxyl-terminal partial.

Query proteins: ACP, *Speleonectes* cf. *tulumensis* prepro-ACP (Christie, 2014a); AST-A, *P. clarkii* prepro-AST-A (**Accession No. BAE45266**; Yasuda-Kamatani and Yasuda, 2006); *Litopenaeus vannamei* prepro-allatostatin B (Christie, 2014f); AST-C, *Homarus americanus* prepro-AST-C-like peptide (Dickinson et al., 2009b); allatotropin, *Tigriopus californicus* prepro-allatotropin (Christie, 2014b); bursicon α , *Homarus gammarus* pre-bursicon α (**Accession No. ADI86242**; Sharp et al., 2010); bursicon β , *H. americanus* pre-bursicon β (Christie et al., 2010a); CCHamide, *L. vannamei* prepro-CCHamide (Christie, 2014f); corazonin, *Daphnia pulex* prepro-corazonin (**Accession No. EFX86608**; Colbourne et al., 2011); CCAP, *H. gammarus* prepro-CCAP (**Accession No. ABB46292**; Chung et al., 2006); CHH/ITP, *H. americanus* prepro-CHH A (**Accession No. P19806**; de Kleijn et al., 1995); DENamide, *D. pulex* prepro-DENamide (Dirksen et al., 2011); DH31, *H. americanus* calcitonin-like diuretic hormone (**Accession No. ACX46386**; Christie et al. 2010b); DH44, *Argulus siamensis* prepro-DH44 (Christie, 2014c); DXXRLamide, *T. californicus* prepro-DXXRLamide Ia (Christie, 2014b); ETH, *D. pulex* prepro-ETH (Dirksen et al., 2011); EH, *D. pulex* prepro-EH (Dirksen et al., 2011); FLP, *P. clarkii* prepro-FLRFamide A (**Accession No. BAE06262**; Yasuda-Kamatani and Yasuda, 2006); FXGGXamide, *T. californicus* prepro-FXGGXamide Ia (Christie, 2014b); GSEFLamide, *T. californicus* prepro-GSEFLamide Ia (Christie, 2014b); ILP, *D. pulex* prepro-insulin/IGF/relaxin-like peptide 2 (**Accession No. EFX70023**; Colbourne et al., 2011); intocin, *L. vannamei* prepro-intocin (Christie, 2014f); leucokinin, *Eucyclops serrulatus* prepro-leucokinin (Christie, 2015b); myosuppressin, *H. americanus* prepro-myosuppressin (**Accession No. ACX46385**; Stevens et al., 2009); neuroparsin, *Jasus lalandii* pre-neuroparsin (**Accession No. AHG98659**; Marco et al., 2014); NPF, *L. vannamei* prepro-NPF I (**Accession No. AEC12204**; Christie et al., 2011d); orcokinin, *H. americanus* prepro-orcokinin I (**Accession No. ACB41787**; Dickinson et al., 2009a); PDH, *Orconectes limosus* prepro-PDH (**Accession No. AAB26385**; de Kleijn et al., 1993); proctolin, *Penaeus monodon* prepro-proctolin (Christie, 2014f); pyrokinin, *L. vannamei* prepro-CAPA (Christie, 2014f); RPCH, *C. quadricarinatus* prepro-RPCH (**Accession No. AAV80404**; Martinez-Perez et al., 2007); RYamide, *L. vannamei* prepro-RYamide (Christie, 2014f); sNPF, *D. pulex* prepro-sNPF (**Accession No. EFX90018**; Colbourne et al., 2011); SIFamide, *H. americanus* prepro-Val¹-SIFamide (**Accession No. ABV21807**; Dickinson et al., 2008); sulfakinin, *H. americanus* prepro-sulfakinin (**Accession No. ABQ95346**; Dickinson et al., 2007); TRP, *H. americanus* prepro-TRP (**Accession No. ACB41786**; Christie et al., 2008a)

Table 2

Table 2. Predicted peptidome of <i>Procambarus clarkii</i>	
Peptide group	Predicted peptide structure
Adipokinetic hormone-corazonin-like peptide	pQITFSRSWVPQamide
Adipokinetic hormone-corazonin-like peptide linker/precursor-related peptide	SGPLVSPAGAPSLDVDPCRDT LSTLTDVASHLVELMDDVSDLTQDDAALALRLKHALLARQ LT
Allatostatin A	YDY _(SO3H) Y _(SO3H) LDDQDDASRLQLVLQAAATDPSYVDDYGNHQNNYGFGLa TPNYAFGLa pQGMYSFGLa PDMYSFGLa PDLYSFGLa ADMYSFGLa ADLYSFGLa SGNYNFGLa SRQYSFGLa SGQYSFGLa PRNYAFGLa TSDEEDDEDQYYPYGLa PRVYGFGLa SYDFGLa TAGPYAFGLa AGPYAFGLa SGPYAFGLa TGPYAFGLa ADPYAFGLa PNPYAFGLa DGMYSFGLa AGQYSFGLa SGPYSFGLa SGAYSFGLa AGPYSFGLa
Allatostatin A linker/precursor-related peptide	SVSEALSSQDVSKADDLSPTI DTSPSEDIQDDKTSKHYGVGVE EIDED EDDDAE EPDMDLY SDDGLDDY _(SO3H) DDLDEEVEGLSELEQY _(SO3H) GEDL

	ADSYGLAFNGGDALEMGLa EDY _(S03H) DSSDQYSLa ESGDESHQDEIQGSVEETSS
Allatostatin B	VGWSSMHGTWa TNWNKFHGSWa
Allatostatin B linker/precursor-related peptide	QDQQASTPGQSDA PDLDDTQLEALED GEEIQAAED
Allatostatin C	GNGDGRLYWRCYFNAVSCF
Allatostatin C linker/precursor-related peptide	RLPQALP PQYMEVVRPVLPNVALEQQGTLQGAPLQISETVTTP AAIVLDKLMFALQKALDDTPASPSSGAPASYPRTRTYAAGPMDLQ
Bursicon α	DECSLT ^P VIHILSYQGCTSKPIPSFACQGRCTSYVQVSGSKLWQTERSCMCCQESGEREASVILNCPNARKGEPKQKILTRAPIDCMCRPCTDVEEGTVLAEIANFIHDS ^P MGNV ^P FLK
Bursicon β	+VVRTCEEDLAVNKCEGECISKVQPSVNTPSGFLKDCRCCRETHLRARDVTLTHCYDADGNRLTGERGSLSIKLREPADCQCFKCGDSTR
CCHamide	SCSQFGHSCFGAHa +GGCLNYGHSC ^L LGAHa
CCHamide linker/precursor-related peptide	DGGEQYPRQOEPSPLYPEVNPLAEFETRQDLPIDEVPAVTNREIVANV NWLAVLS LRQKTSSQASPSAQSIGYF ASMPALPRPLLDIIMDALNTPEHINMYPYAVINSLS ^D Q ^R SGVPA FLSPAGNQLGDEGVDLRLGGVVGGDDLEGVATRVKGD ^S L ^G STGSQDQDAMLYYGSIDDDY _(S03H) SDARY QVFSYKSPVVATSAASRVFHSTDSQDW IHLQKEQKQ ^Q EEQ ^Q Q ^Q ENQ ^H EEQ ^Q GTREYVPQRTLASQANWW
Crustacean hyperglycemic hormone/ion transport peptide	pQVFDQACKGIYDRAIFRKLELVCDDCYNLYRKPKVATT ^C R+ AVFDPECKGY ^Y DRKIWAKLNLACEDC ^N LFRKPGVETEC ^R KGC ^F KSSMFTSCVSD ^L FLPVDKYQALAA ^F VRGT LYNELNRICEDC ^N IYRRDSQLAMCKDNC ^F KNSWFEEC ^V NSLLLNDQ ^M ENYNKKISYINGQDL
Crustacean hyperglycemic hormone/ ion transport peptide linker/precursor-related peptide	RSVEGSSRLEQLLSSGSSSSSEPLSFLSQDH ^S VN RSLSSEDLQVLPQTTSPALSHASDLPEDHG ^L PLSTPLYHSIR SFYKIRPGTLKEFEY _(S03H) VNCQGT ^F D
Diuretic hormone 31	

Diuretic hormone 31 linker/precursor-related peptide	GLDLGLGRGYSGSQAAKHLMGLAAANFAGGPa ASLNREA AVVEIDDPDY _(SO3H) VLELLTRLGHSII ANELEKFVRSSGSA SSDDALDHDDNLYAHDQVAELAESS
Eclosion hormone	AANKVSVCIKNCAQCKVMYHDHFKGGLCADFCVQSEGRFIPDCARPQTLIPFFLQRL
FMRFamide-like peptide	AYSDRNYLRFa SRNFLRFa DHSRNFLRFa SPHKSYRHLVKGNRNFPRFa GDRNFLRFa AHDNRNFLRFa DRNFLRFa APQRNFLRFa AYSDRNFLRFa NRNFLRFa GFSDKNFMRFa GGHNVLRFa
FMRFamide-like peptide linker/precursor-related peptide	APVPPVAAALSSPSQGFSGSNNSEDDSVAQPE LLKYFLPGSQAWMDDGGDLYPISQEGS NDDD SDLGDY _(SO3H) DNRDEGGLSDSL SDMEDLSLAGGPMDFPAGLLDEVQEDLPFQE SVDSQLNALSCDGC DGPKTSVSSTPSPTPVQPTST DISTHSIEASNPSGSTSQRM AVDPTFY _(SO3H) DYGSMLSY _(SO3H) SPSSWARGFQPEEEIITVSSDPQDVN DRSNEY _(SO3H) PPSSPSESSESLVAVSPAEYSRNV SDVE SDLGDY _(SO3H) HNSDDETLDNSLV PDMEDMFVAGGPMEFLNSLEGEVVKDDLPLEKQAAYRH SVTRQLNAQSCDGC DGPKTQETTPHPPLPLRSSQRPLPSMTSLTRSSPQNPGRALLIA +AALSPPSHDFSFINNREESEGLPEKGVLYFNPGRQGWVDGAEDYHPFAEDVS SLDTRSNTLCLVGCDEEPKTQDHTCLPTS FHPPTTRVERDIQSPEPGQOTSQ AASKTFYDFRKTPSYSPSSWARGFQP
GSEFLamide	AVGSEFLa AMGSEFLa

GSEFLamide linker/precursor-related peptide	AMGAEFLa
	ATGLEFLNERVMGSELLa
	YMGSEFSa
Intocin	AKASEFE
	HYEPAFMHALIYDP
	CFITNCPPGa
Intocin linker/precursor-related peptide	SGASTQIARSRTCSSCGPGLRGRCLGPEICCGEEVGCFLATREA
Leucokinin	pQPFYHWGa
	ATFNPWGGKQSDPIHTASLTSDAFNDSPSKGNTFTPWGa
	GFSAWAa
Leucokinin linker/precursor-related peptide	AFSAWAa
	pQAFNAWAa
	pQAFSAWAa
	HSFSAWAa
	HTFSSWAa
	pQAFSPWAa
	pQAFSSWAa
	HAFSSWAa
	QGTDVSPALALPGGQSPSPCLPYRLPTHPLIRCELS
	SPHKFGLGLPINLREVYLALFQNARSQPL
	LQATEGEL
	SAEY _(SO3H) YLEDESPLAVKEDIIPYVTTFSDEGQTTEEIKEGSTATWGGNRVPSVSDGKNDILTEEEPSDFFLLNENLPFPDVGNLRY
	EVESYVKLENIKSSLEE
	AESGKSTQKDDQSQESNTSEVKRARYSAWVa
	PDLQAIQDAV
	LKY _(SO3H) QEPQMAE
	SLSTVFSNIMS
	SNYDFEQNIL
	TKVSPWAEKLLDKADSTTNVATVESSPFNDQERASFNEWIGNAE
	SDTEE
	NDNDE
	EDDE
	SNNDE
	SDNEE
	SENDE+
	+DFEQNILKTKVSPWAEKLSKADSTTNVATVESSPFNDQERASFNEWIGNA

	SDNDE
Neuroparsin	<u>S</u> <u>P</u> <u>L</u> <u>C</u> <u>P</u> <u>E</u> <u>R</u> <u>N</u> <u>E</u> <u>I</u> <u>T</u> <u>P</u> <u>E</u> <u>D</u> <u>L</u> <u>N</u> <u>T</u> <u>C</u> <u>K</u> <u>Y</u> <u>G</u> <u>V</u> <u>V</u> <u>L</u> <u>G</u> <u>W</u> <u>C</u> <u>G</u> <u>N</u> <u>P</u> <u>A</u> <u>C</u> <u>G</u> <u>K</u> <u>G</u> <u>P</u> <u>G</u> <u>D</u> <u>P</u> <u>C</u> <u>G</u> <u>G</u> <u>R</u> <u>W</u> <u>E</u> <u>E</u> <u>N</u> <u>G</u> <u>I</u> <u>C</u> <u>G</u> <u>E</u> <u>G</u> <u>M</u> <u>Y</u> <u>C</u> <u>V</u> <u>C</u> <u>G</u> <u>Y</u> <u>C</u> <u>A</u> <u>G</u> <u>C</u> <u>T</u> <u>T</u> <u>T</u> <u>L</u> <u>E</u> <u>C</u> <u>A</u> <u>L</u> <u>G</u> <u>R</u> <u>F</u> <u>C</u> <u>A</u> <u>P</u> <u>R</u> <u>C</u> <u>E</u> <u>H</u> <u>H</u> <u>D</u> <u>R</u> <u>P</u> <u>P</u> <u>P</u> <u>N</u> <u>N</u> <u>C</u> <u>K</u> <u>Y</u> <u>G</u> <u>T</u> <u>V</u> <u>R</u> <u>D</u> <u>W</u> <u>C</u> <u>R</u> <u>N</u> <u>G</u> <u>V</u> <u>C</u> <u>A</u> <u>K</u> <u>G</u> <u>P</u> <u>G</u> <u>E</u> <u>S</u> <u>C</u> <u>G</u> <u>G</u> <u>N</u> <u>W</u> <u>F</u> <u>E</u> <u>Q</u> <u>G</u> <u>K</u> <u>C</u> <u>G</u> <u>M</u> <u>G</u> <u>T</u> <u>F</u> <u>C</u> <u>Q</u> <u>C</u> <u>G</u> <u>V</u> <u>C</u> <u>T</u> <u>G</u> <u>C</u> <u>S</u> <u>T</u> <u>V</u> <u>D</u> <u>G</u> <u>S</u> <u>C</u> <u>A</u> <u>N</u> <u>G</u> <u>H</u> <u>L</u> <u>M</u> <u>C</u>
Neuropeptide F	VASGGRHDSSAADALQAMHEAAIAGLLGPTEIQYPNRPNMFKSPVELRQYLDALNAYYAIAGRPRFa KPDPTELAAMADAIAIKYLQELTKYYSQVSRP+
Neuropeptide F linker/precursor-related peptide	GSHGPQORTEEVYDY
Pigment dispersing hormone	NSELLNTLLGSQGLSNMRSAa
Pigment dispersing hormone linker/precursor-related peptide	+PEEGSVSLVLLPLPSNYHQTGTGTAQ
Pyrokinin	+FSPRLa SDFQFSPRLa ADFQFSPRLa ADFAFSPRLa DGEETSDDARGSIPTMYVPRPa PYFSPRLa
RYamide	pQGfYSQRYa FIGGSRYa
RYamide linker/precursor-related peptide	SDTREAVAV SGLYVNRNGRSTLPQGLPEIKIRSS SGSPPESEFSSAITPEADDTDMTATLLLGD ^{SVLC} FLVDVPDIYR ^{CL} PASEEANN
Short neuropeptide F	APPSMRLRFa DMGWQVAQRSMPSLRLRFa DSRTPALRLRFa
Short neuropeptide F linker/precursor-related peptide	GPAPSDY _(SO3H) DTVNEVY _(SO3H) SWLSEHGLD TVDQEEPLYDHDLV DSSY _(SO3H) GQEEQDVASQEQ
SIFamide	GYRKPPFNGSIFa
SIFamide linker/precursor-related peptide	

	AGGDSLYEPGKALASACQVAVEACAAWFPGP
Sulfakinin	
Sulfakinin linker/precursor-related peptide	<p>pEFDEY_(SO3H) GHMRFa</p> <p>GGDY_(SO3H) DDY_(SO3H) GHLRFa</p> <p>+MAAILLTRAASAPA</p> <p>PSSLARVLAPVV</p> <p>HRLEEGRLTPALVEELVADFEDPELMDFDAAa</p> <p>SLRGGQHDP SILH</p>
Tachykinin-related peptide	
Tachykinin-related peptide linker/precursor-related peptide	<p>APSGFLGMRa</p> <p>EEQDTADRE</p> <p>DVSTPLENIVPAVNDYTLQDSFPASLYGLRDDNGPVVLAVPWRV</p> <p>SGEEAFGEAGMDSELETLL</p> <p>HFDDSEIDAYIQALTAMVDGQQE</p> <p>AFFGDSSDEEMSVAGVD</p>
<p>Abbreviations: a, amidated C-terminus; +, likely partial peptide with additional unknown amino acids; pQ/pE, pyroglutamic acid; <u>C</u>, disulfide bonded cysteine (see text for descriptions of bonding partners); Y_(SO3H), sulfated tyrosine.</p> <p>Prediction of sulfation state of tyrosine residues and disulfide bridging between cysteines was done only for full-length peptides.</p> <p>A detailed description of the workflow used for peptide prediction is provided in Section 2.2.</p>	

Table 3

Table 3. Predicted peptidome of <i>Pontastacus leptodactylus</i>	
Peptide group	Predicted peptide structure
Allatostatin A	+SDEEDNEDEQYYAYGLa PRVYSFGLa SYGFGLa
Allatostatin A linker/precursor-related peptide	SFGSLDDY _(SO3H) DDLDDDEVGLSELGQYTDDL AATYGFDLGNDDALESGLa
Allatostatin C	SYWKQCAFNAVSCFa
Allatostatin C linker/precursor-related peptide	+PDQSPQYPQPQRMLDLVDDDGSLDAALINYLFAQMVERLRNNADIKDLQ
Bursicon α	DECSLTPVIHILSYQGCTSK+
CCHamide	HRVMKGGCLNYGHSC_LGAHa
CCHamide linker/precursor-related peptide	ASVPVVRPLLDIIMDALNTPEHTNNQYPFAVVSSI SEPRSGVPA FLTPDEGVDLRMa VVGVDLETLRTRVKGD LGSMGQDQDAMVYYGSLDDY _(SO3H) SDDRY HVVPY PPVATSAASRVAQAAGSQTWGSSRLQNELKQAEELQEEEEQKEEQEEQEEQHEEQEEQOQGARDHVHQRSLASQDSWW
Crustacean hyperglycemic hormone/ion transport peptide	pQVFDQACKGIYDRALFKKLDRCDDCYNLYRKPKVATTCRENCYANSVFRQCLDDLLLIDVVDEYISGVQIVa pQVFDQACKGIYDRAIFKKLDRCEDCYNLYRKPYVATTCRQNCYANSVFRQCLDDLLLIDVVDEYISGVQTVa AVFDTACKGYDRKIWATLNRACEDCQNLFRKPGVEAGCRKGC_FDTSMFTACVANLMLAVDKYEAMAGFVRGT
Crustacean hyperglycemic hormone/ ion transport peptide linker/precursor-related peptide	RSVDGSSRLERLLSSGSSSEPLSFLSEDHSVN EDVLVLPQATSAALSDTNVLPQNHS DMEPQYHSIT
Diuretic hormone 31	GLDLGLGRGFSGSQAAKHLMGLAAANYAGGPa
Diuretic hormone 31 linker/precursor-related peptide	SSDDTLDLPHDDNLYAHDQAAELAESS
Eclosion hormone	NKVSVCIKNCAQCKVMYHDHFKGGLCADFCVQSEGRFIPDCARPQTLPFFLQRL
Intocin	CFITNCPPGa

Intocin linker/precursor-related peptide	<p>SGASTQIARSRTCSSCGPGLRGRCLGPEICCGEGVGCFLATREA MCRAENLVPLTCTNTDLKTCGRLEa CAASGLCCTEMKCEFDGSCVESREASVEDQQVERQRFAPLNLSDDQWSL</p>
Myosuppressin	pQDLDHVFLRFa
Myosuppressin linker/precursor-related peptide	<p>IPPPICLNQQLPLSPYA LCAALSNISEFSRAMEEY_(SO3H) LDAQAIKSIIPVNEPEV SQ</p>
Neuroparsin	<p>SPLCPERNEITPEDLNTCKYGVVLGWCNAACGKGPGDSCGGRWEENGICGEGMYCVCGYCAGCTTTLECALGRFC APRCDHHDPPNNCKYGTVRNWCNRNGVCAKGPGESCGGHWYEQGKCGIGTFCLCGICIGCSTIDGSCADGPLIC</p>
Red pigment concentrating hormone	pQLNFSPGWa
Red pigment concentrating hormone linker/precursor-related peptide	<p>AGPVVVAGGVGSEPAGLHSASQAGLAAVSSNAAGDNCGAIPVSAAMHIYRLI TEAARLIQCQEEYMa</p>
Short neuropeptide F	<p>APPSMRLRFa DMGWQVAQRSMPSLRLRFa DSRMPALRLRFa</p>
Short neuropeptide F linker/precursor-related peptide	<p>GPAPPDY_(SO3H) DTVNEVYSWLSEHGLD TVDQEEPLYDHELV DSPYQGEEDIGSHEQ</p>
Tachykinin-related peptide	APSGFLGMRa
Tachykinin-related peptide linker/precursor-related peptide	<p>HDDDDSEIDAYIQALTAMVDGQQQ AYYGESADE+</p>
<p>Abbreviations: a, amidated C-terminus; +, likely partial peptide with additional unknown amino acids; pQ, pyroglutamic acid; C, disulfide bonded cysteine (see text for descriptions of bonding partners); Y_(SO3H), sulfated tyrosine. Prediction of sulfation state of tyrosine residues and disulfide bridging between cysteines was done only for full-length peptides. A detailed description of the workflow used for peptide prediction is provided in Section 2.2.</p>	

Table 4

Table 4. Predicted peptidome of <i>Cherax quadricarinatus</i>	
Peptide group	Predicted peptide structure
Bursicon β	+THLRAREVKLTHCYDADGNRLAGERGALVITLREPSECQCLKCGDSTR
Neuroparsin	APRCNTHDRPPPSNCKYGTVRNWCNRNGVCAKAPGESCGGHWYEHGKCGIGTFCLCGVCIGCSTIDGRCADGPLMC
Abbreviations: +, likely partial peptide with additional unknown amino acids; <u>C</u> , disulfide bonded cysteine (see text for descriptions of bonding partners). Prediction of disulfide bridging between cysteines was done only for full-length peptides. A detailed description of the workflow used for peptide prediction is provided in Section 2.2.	

A. Procl-prepro-adipokinetic hormone-corazonin-like peptide (from GBEV01011002)

MVAWQ**MM**LALVCLAIVPTMA**Q**ITFSRSWVPQ**GK**RS**G**PLVSPAGAPSLD**V**DPCRD**T**RLSTLTDVASHLVELMDDVSDLTQDDAALALRLKHALLAR**Q**R**L**T

↓ Signal peptidase (cleavage locus highlighted above)

QITFSRSWVPQ**GK**RS**G**PLVSPAGAPSLD**V**DPCRD**T**RLSTLTDVASHLVELMDDVSDLTQDDAALALRLKHALLAR**Q**R**L**T

↓ Prohormone convertase (cleavage loci highlighted above)

QITFSRSWVPQ**GK**R**G**PLVSPAGAPSLD**V**DPCRD**T**R**L**STLTDVASHLVELMDDVSDLTQDDAALALRLKHALLAR**Q**R**R****L**T

↓ Carboxypeptidase (cleavage loci highlighted above)

QITFSRSWVPQ**G****S**G**P**LVSPAGAPSLD**V**DPCRD**T****L**STLTDVASHLVELMDDVSDLTQDDAALALRLKHALLAR**Q**

↓ Peptidylglycine- α -amidating monooxygenase (amidation locus highlighted above)

QITFSRSWVP**Q**amide

↓ Glutaminyl cyclase (cyclization locus highlighted above)

pQITFSRSWVP**Q**amide

B. Procl-prepro-CCHamide I (from GBEV01004199)

MVRGVPSVVIVVVGLALLTSQAW**G**SCSQFGHSCFGAHGKRDGGEQYPRQ**Q**EPSPLYPEVNPLAEFETRQDLPIDEVPAVTNREIVANVRNWLAVLSRRLRQKTSSQASPSAQSIGYFK

↓ Signal peptidase (cleavage locus highlighted above)

SCSQFGHSCFGAHGK**R**DGGEQYPRQ**Q**EPSPLYPEVNPLAEFETRQDLPIDEVPAVTNREIVANVR**N**NWLAVLSR**R**LRQKTSSQASPSAQSIGYFK

↓ Prohormone convertase (cleavage loci highlighted above)

SCSQFGHSCFGAHGK**R**DGGEQYPRQ**Q**EPSPLYPEVNPLAEFETRQDLPIDEVPAVTNREIVANVR**N**NWLAVLS**R**RRLRQKTSSQASPSAQSIGYFK

↓ Carboxypeptidase (cleavage loci highlighted above)

SCSQFGHSCFGAH**G**DGGEQYPRQ**Q**EPSPLYPEVNPLAEFETRQDLPIDEVPAVTNREIVAN**V**NWLAVLS**L**RLRQKTSSQASPSAQSIGY**F**

↓ Peptidylglycine- α -amidating monooxygenase (amidation locus highlighted above)

SCSQFGHS**C**FGAHamide

↓ Enzymatic disulfide bond formation (bridged cysteines highlighted above)

SCSCQFGHSCFGAHamide

C. Procl-prepro-sulfakinin (C-terminal partial protein from GBEV01027368)

+MAA**I**LLTRAASAPAR**P**SSSLARVLAPV**V**RRHLEEGRLTPALVEELVADFEDPELMDFHDAAG**K**REFDEYGHMRFG**K**RGDYDDYGHLLRF**G**SLRGGQHDPSILH

↓ Prohormone convertase (cleavage loci highlighted above)

+MAA**I**LLTRAASAPAR**P**SSSLARVLAPV**V**RRHLEEGRLTPALVEELVADFEDPELMDFHDAAG**K**REFDEYGHMRFG**K**RGDYDDYGHLLRF**G**SLRGGQHDPSILH

↓ Carboxypeptidase (cleavage loci highlighted above)

+MAA**I**LLTRAASAP**A**PSSSLARVLAPV**V**RRHLEEGRLTPALVEELVADFEDPELMDFHDAAG**K**REFDEYGHMRFG**K**RGDYDDYGHLLRF**G**

↓ Peptidylglycine- α -amidating monooxygenase (amidation loci highlighted above)

HRLEEGRLTPALVEELVADFEDPELMDFHDA**A**amide**E**FDE**Y**GHMR**F**amideGGD**Y**DD**Y**GHLL**R**amide

↓ Tyrosylprotein sulfotransferase (tyrosine targets highlighted above)

EFDE**Y**_(SO3H)GHMR**F**amideGGD**Y**_(SO3H)DD**Y**_(SO3H)GHLL**R**amide

↓ Glutaminyl cyclase (cyclization locus highlighted above)

pEFDE**Y**_(SO3H)GHMR**F**amide

A. Procl-prepro-allatostatin A (from GBEV01001712)

MLGRHGAGAASLLLLSVTATVAA^{YDYLDDQDDASRLQLVLQAAATDPSYVDYGNHQNNYGFGLGKRTPNYAFGLGKRQGMYSFGLGKR}
^{GLGKR}^{PDLYSFGLGKKADMYSFGLGKRADLYSFGLGKSGNYNFGLGKR}^{SVSEALSSQDVSKADDLSPTIRKKRDTSPSEDIQDDKTSKHYGVGVEK}
^{REIDEDKR}^{SRQYSFGLGKREDDDAEKRSQQYSFGLGKR}^{PEMDLYKRPRNYAFGLGKR}^{TSEDEDDDDQYYPYGLGKR}^{PRVYGFGLGKR}^{SDDGLDDY}
^{DLDDDEEVEGLSELEQYGEDLKR}^{ADSYGLAFNGGDALEMGLRRSYDFGLGKR}^{TAGPYAFGLGKRAGPYAFGLGKR}^{SGPYAFGLGKR}^{SGPYAFGLG}
^{KRSGPYAFGLGKR}^{TGPYAFGLGKKADPYAFGLGKR}^{PNPYAFGLGKR}^{DMYSFGLGKKAGQYSFGLGKR}^{SGPYSFGLGKR}^{EDYSSDQYSLGRRSGAY}
^{SFGLGKR}^{AGPYSFGLGKR}^{ESGDESHQDEIQGSVEETSS}

B. Procl-prepro-allatostatin B (N-terminal partial protein from GBEV01040422)

MQHLRAAWTLVALMVTVVQLAAS^{QDQQASTPGQSDAKRVGWSMHWGTWKR}^{PDLDDTQLEALEDKR}^{TNWNKFHGSWGKR}^{GEEIQAEDKRT+}

C. Procl-prepro-allatostatin C (from GBEV01001327)

MMACRSVDCHSRHQAIMLFRGTPVTATAAPSHATAAALPLLVSMLVASAAA^{RLPQALPR}^{QYMEVVRPVL}^{PNVALEQQGT}^{LQGA}^{PLQISETVT}^{TPRK}
^{RAAIVL}^{DKLMFALQKALDDTPAS}^{PSSGAPASYPRT}^{RTYAAGPMDLQRR}^{GNGDGR}^{LYWRCYFNAVSCF}

D. Procl-pre-bursicon α (from GBEV01070554)

MGRISWVLVVMGMASMGVVS^{GDECSLTPVIHILSYQGCTSKPIPSFACQGRCTSYVQVSGSKLWQTERSCMCQESGEREASVILNCPNARKGEPKQ}
^{KKILTRAPIDCMCRPCTDVEEGTVLAQEI}^{ANFIHDS}^{PMGNVPFLK}

E. Procl-pre-bursicon β (C-terminal partial protein from GBEV01054566)

^{+VVRTCEEDLAVNKCEGECISKVQPSVNTPSGFLKDCRCCRETHLRARDVTLTHCYDADGNRLTGERGSLSIKLREPADCQCFCGSDTR}

F. Procl-prepro-CCHamide II (C-terminal partial protein from GBEV01015793)

^{+GGCLNYGHSCLAGH}^{KRASMPALPRPLLDI}^{IMDALNTP}^{EHINNMYPYAVINSLSDQRSGVPA}^{RRFLSPAGNQLGDEGV}^{DLRLGGVVGDDDL}^{EGVAT}
^{RVKGD}^{SLGSTGSDQD}^{AMLYYGSIDDDYSDARY}^{KRQVFSYKSPVVATSAASRVF}^{HSTDSQDWKRIHLQKEQKQEEQ}^{QQQENQHEEQ}^{QGTREYVPQR}
^{TLASQANWRR}

G1. Procl-prepro-crustacean hyperglycemic hormone I (internal protein fragment from GAZR01016298)

^{+ASLGSSGVHG}^{RSVEGSSRLQLSSGSSSSEPLS}^{SFLSQDHSVNR}^{QVFDQACKGIYDRAIFRKLELV}^{CDDCYNLYRKPKVATTCR+}

G2. Procl-prepro-crustacean hyperglycemic hormone II (from GAZR01016298)

MSQSMVWSSVMVVVVVVISSCY^{RLSSEDLQVLPQTTSPALSHASDL}^{PEDHGFLSTPLYHSIR}^{KRAVFD}^{PECKGYDRKIWAKLNLACEDC}^{QNLF}
^{KPGVETECRKGCFCSSMFTSCVSDLFLPV}^{DKYQALAA}^{FVRGT}

G3. Procl-prepro-crustacean hyperglycemic hormone III (from GAZR01016298)

MLLLQASTARSSCVWFLLILGLLSQSQNTSG^{SFYKIRPGTLKEFEYVNCQGT}^{FDKLLYNELN}^{RICEDCQNIYRRDSQLAMKCKDNC}^{FKNSWFEECVN}
^{SLLLNDQ}^{MENYNKKISYINGQDL}

H. Procl-prepro-diuretic hormone 31 (from GBEV01003269)

MSLMNSTGAVLVCLVSVLVLLSAVNS^{ASLNREARAVVEIDDPDYVLELLTRLGHSII}^{RANELEKFVRSSGSA}^{KRGDLGLGRGYSGSQA}^{AHKLMGLA}
^{AANFAGGPGRRRR}^{SSDDALDHDDNLYAHDQVAELAESSR}

I. Procl-pre-eclosion hormone (from GBEV01101142)

MIGSRKVVSALVLVLSVTLEALPLAAA^{AANKVSVC}^{IKNCAQCKVMYHDFKGG}^{LCA}^{DFCVQSEGRFI}^{PD}^{CARPQT}^{LI}^{PF}^{FLQRLE}

J1. Procl-prepro-FMRFamide-like peptide I (from GBEV01020536 and GBEV01106719)

MPLAAWVLLATFTWCSYAHAAHA^{APVPPVAAALSSPSQGFGSNNSEDD}^{SVAQPEKRL}^{LLKYFLPGSQAWMDG}^{GDLYPISQEGS}^{KRAYSDRNYLRFGR}
^{NDDDKSR}^{SNFLRFGR}^{SDLG}^{YDNREGG}^{LSDSLDKSR}^{SNFLRFGR}^{RDHSR}^{NFLRFGR}^{SDMED}^{LSL}^{AGGPMDFPAG}^{LLDEVDQEDLPFQE}^{KRSPHKSYR}
^{HLVKGNRNFPRFGR}^{GDRNFLRFGR}^{SVDSQLNALSCD}^{CGDGPKTSVSSTPSPTPVQ}^{PVTSTKKDISTHSIEASNP}^{SGSTSQRM}^{KRAVDPTFYDYG}^{SML}
^{SYSPSSWARGFQPEEEIITV}^{SS}^{EDPQDVNR}^{AHDRNFLRFGR}^{DRNFLRFGR}^{KDRSNEYPPSSP}^{SESSESLVAVSPA}^{EYSRNV}^{RAPQRNFLRFGR}

J2. Procl-prepro-FMRFamide-like peptide II (C-terminal partial protein from GBEV01105142)

^{+KRAYSDRNF}^{FLRFGR}^{SDVEKRN}^{NFLRFGR}^{SDLG}^{YHNSDDETLN}^{SLVKS}^{RN}^{FLRFGR}^{RPDMED}^{MFVAGGPME}^{FLNSLEGEV}^{VKDDLPLEKQAA}
^{YR}^{HRR}^{SRN}^{FLRFGR}^{SVTRQ}^{LNAQSCD}^{CGDGPKTQETT}^{PHPLPLRSSQR}^{PLPSMTSLTRSSPQ}^{NPG}^{RALLIA}

J3. Procl-prepro-FMRFamide-like peptide III (C-terminal partial protein from GBEV01189645)

^{+AALSPPSHDFS}^{INNREESEGLPEKGV}^{LYKFN}^{PGRQGW}^{DGAEDYH}^{PF}^{AEDVSR}^{RGFSDKN}^{FMRFGR}^{GGHN}^{VLR}^{FRGR}^{SLD}^{TR}^{NTL}^{CLVGCDEEPK}
^{TQDHTCL}^{PTS}^{FHPTTR}^{VERDIQ}^{SFEPGQ}^{QTSQR}^{KKRAASKTFYD}^{FRKTPSYSPSSW}^{ARGFQ}

K. Procl-prepro-GSEFLamide (from C-terminal partial protein from GBEV01013249)

^{+KRAVGSEFLG}^{KRAKASEFER}^{KRAMGSEFLG}^{KRAMGSEFLG}^{KRAMGSEFLG}^{KRAMGSEFLG}^{KRAMGSEFLG}^{KRAMGSEFLG}^{KRAMGSEFLG}^{KRAMGSEFLG}
^{EFLG}^{KRYMGSEFSGKR}^{HYEPAFM}^{HALIYDP}^{KRAVGSEFLG}

L. Procl-prepro-intocin (N-terminal partial protein from GBEV01109169)

MQVGVMIVA^{AAVVVGNA}^{ACFITNCPGGK}^{RS}^{GASTQIARSRTCSSCGP}^{LGRCLGPEICCGEEVGC}^{FLATREARM+}

M1. Procl-prepro-leucokinin I (N-terminal partial protein from GBEV01013648 and GBEV01006537)

MVVGGRWVVVWAWLVVASVDVPTFPQGTDVSPALPPGGQSPPCLPYRLPHTPLIRCELSKRQPFYHWGGKRSPHKFGLGLPINLREVYLALFQNARS
QPLRLQATEGELKRATFNFWGGKQSDPIHTASLTSDAFNDSPSKGNTFTPWGGKRSAEYYLEDESPLAVKEDIIPYVTTFSDEGQTEEIKEGSTATW
GGNRVPSVSDGKNDILTEEEPSPDFFLLNENLPFPDVGNLRYKREVESYVKSLNIKSSLEEKKAESGKSTQKDDQSQESNTSEVKRARYSAWVGKRP
DLQAIQDAVRRLKYQEPQMAERRGFSAWAGKRSLSTVFSNIMSKKAFSAWAGKRSNYDFEQNILKKTKVSPWAEKLLDKADSTTNVATVLESSPFNDQ
ERASFNEWIGNAEKRQAFNAWAGKRSDTEEKRQAFSAWAGKRNDNDEKRHSFSAWAGKRREDDEKRHTFSSWAGKRNNDEKRQAFSPWAGKRSDNE
EKRQAFSSWAGKRSENDE+

M2. Procl-prepro-leucokinin II (internal protein fragment from GBEV01006538)

+DFEQNILKTKVSPWAEKLSKADSTTNVATVLESSPFNDQERASFNEWIGNAKKRQAFNAWAGKRSDTEEKRQAFSAWAGKRSDNDEKRHSFSAWAG
KRREDDEKRHAFSSWAGKRNNDEKRQAFSPWAGKRSDNEEKRQAFSSWAGKRSENDE+

N1. Procl-pre-neuroparsin I (C-terminal partial protein from GBEV01003881)

+SCLLTLCISILLILQNAASPLCPERNEITPEDLNTCKYGVVLGWCNPACGKGPGDPCGGRWEENGICGEGMYCVCYGCAGCTTTLECALGRFC

N2. Procl-pre-neuroparsin II (from GBEV01000414)

MRTLTSMTTFVVAFYCLVLLFQEAASAPRCEHHDPRPPNNCKYGTVRWCRNGVCAKGPGESCGGNWFEQGKCGMGTFCQCGVCTGCSTVDGSCANG
HLMC

O1. Procl-prepro-neuropeptide F I (from GBEV01005906)

MQGMVMVGTVVAAVVATLVSGVASGGRHDSSAADALQAMHEAAIAGLLGPTEIQYPNRPNMFKSPVELRQYLDALNAYYAIAGRPRFGKRGSHPQR
TEEVYDY

O2. Procl-prepro-neuropeptide F II (N-terminal partial protein from GBEV01020719)

MYRHIVSAMVVAAVVGVLEMGAVEGKPDPTELAAMADAIKYIQELTKYYSQVSRP+

P. Procl-prepro-pigment dispersing hormone (C-terminal partial protein from GBEV01236728)

+PEEGSVSLVLLPLPSNYHQTTTGTAQKRNSELLNTLGSQGLSNMRSAGRR

Q. Procl-prepro-pyrokinnin (C-terminal partial protein from GBEV01049409)

+FSPRLGKKSDQFSPRLGKKADFQFSPRLGKKADFAFSPRLGKRDEETSDDARGSIPTMYVFRPGRPYFSPRLG

R. Procl-prepro-RYamide (from GBEV01010112)

MTRGACPTLVLLAALLTLAASQGFYSQRYGKRSDTREVAVRSGLYVNRNGRSTLPQGLPEIKIRSSRFIGGSRYGKRSGSPPESEFSSAITPEADD
TDMTATLLLGDSVLCFLVDVPDIYRCLKKPASEEANN

S. Procl-prepro-short neuropeptide F (from GBEV01004780)

MGVSAVKCWVTLCCCVLLSQTLSAGPAPSDYDVTNEVYSWLSEHGLDRAPPSMRLRFGKRDMGWQVAQRSMPSLRLRFGKRTRVDQEEPLYDHDLV
RKDSRTPALRLRFGKRDSYSGQEEQDVASQEQ

T. Procl-prepro-SIFamide (from GBEV01000221)

MCVQTRMLVAVAVVLVLAVLSDPVSAGYRKPPFNGSIFGKRAGGDSLYEPGKALASACQVAVEACAAWFGPEKK

U. Procl-prepro-tachykinin-related peptide (from GBEV01001769)

MVRGAAGLMVMVVTGALAEEQDTADRERRAPSGFLGMRGKKDVSTPLENIVPAVNDYTLQDSFPASLYGLRDDNGFPVVLAVFWRVKKAPSGFLGM
RGKKSGEAFGEAGMDSELETLKKRAPSGFLGMRGKKAPSGFLGMRGKKAPSGFLGMRGKKHFDDESEIDAYIQALTAMVDGQGEKRAPSGFLGMRG
KKAFFGDSSDEEMSVAGVDKRAPSGFLGMRG

A. Alignment of BAE45266 and Procl-prepro-allatostatin A

```

BAE45266      MLGRHGAGAASLLLLSVTATVAAYDYLLDDQDDASRLQLVLAQAATDPSYVDDYGNHQ
Procl-prepro-AST-A MLGRHGAGAASLLLLSVTATVAAYDYLLDDQDDASRLQLVLAQAATDPSYVDDYGNHQ
*****

BAE45266      NYGFGLGKRTPNYAFGLGKRQGMYSFGLGKRPMYSFGLGKRPDLYSFGLGKKADMYSFG
Procl-prepro-AST-A NYGFGLGKRTPNYAFGLGKRQGMYSFGLGKRPMYSFGLGKRPDLYSFGLGKKADMYSFG
*****

BAE45266      L GKRADLYSFGLGKSGNYNFG LGKRSVSEALSSQDVSKADDLSPTIRKKRDTSPSEDIQ
Procl-prepro-AST-A L GKRADLYSFGLGKSGNYNFG LGKRSVSEALSSQDVSKADDLSPTIRKKRDTSPSEDIQ
*****

BAE45266      DDKTSKHYGVGVEKREIDEDKRSRQYSFGLGKREDDAEKRSQYSFGLGKREPMDLYK
Procl-prepro-AST-A DDKTSKHYGVGVEKREIDEDKRSRQYSFGLGKREDDAEKRSQYSFGLGKREPMDLYK
*****

BAE45266      RPRNYAFGLGKRTSDEEDDEDQYYPYGLGKRPRVYGFGLGKRSDDGLDDYDDLDEEVE
Procl-prepro-AST-A RPRNYAFGLGKRTSDEEDDEDQYYPYGLGKRPRVYGFGLGKRSDDGLDDYDDLDEEVE
*****

BAE45266      GLSELEYGEDLKRADSYGLAFNGGDALEMLGRRSYDFGLGKRTAGPYAFGLGKRAGP
Procl-prepro-AST-A GLSELEYGEDLKRADSYGLAFNGGDALEMLGRRSYDFGLGKRT-----
*****

BAE45266      YAFGLGKRAGPYAFGLGKRAGPYAFGLGKRAGPYAFGLGKRSGPYAFGLGKRSGPYAFGL
Procl-prepro-AST-A -----AGPYAFGLGKRAGPYAFGLGKRSGPYAFGLGKRSGPYAFGL
*****

BAE45266      GKRSGPYAFGLGKRTGPYAFGLGKKADPYAFGLGKRPNPYAFGLGKRDMYSFGLGKKAG
Procl-prepro-AST-A GKRSGPYAFGLGKRTGPYAFGLGKKADPYAFGLGKRPNPYAFGLGKRDMYSFGLGKKAG
*****

BAE45266      QYSFGLGKRSGPYSFGLGKREDYDSSDQYSLGRRSGAYSFGLGKRAGPYSFGLGKRESGD
Procl-prepro-AST-A QYSFGLGKRSGPYSFGLGKREDYDSSDQYSLGRRSGAYSFGLGKRAGPYSFGLGKRESGD
*****

BAE45266      ESHQDEIQGSVEETSS
Procl-prepro-AST-A ESHQDEIQGSVEETSS
*****

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B1. Alignment of BAE06262 and Procl-prepro-FMRFamide-like peptide I

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BAE06262      MTKSCVMPLAAWVLLATFTWC SYAHAAHAASVPPVVAALSSPSQGFSGNNSEDDSVAQPE
Procl-prepro-FLP-I -----MPLAAWVLLATFTWC SYAHAAHAAPVPPVVAALSSPSQGFSGNNSEDDSVAQPE
*****

BAE06262      KRLKLYFHPGSQAWMDGDDLPIPSQEGSKRAYSDRNFLRFRGRSDVDKRNRLRFRGRSD
Procl-prepro-FLP-I KRLKLYFHPGSQAWMDGDDLPIPSQEGSKRAYSDRNFLRFRGRNDDDKRSRNLFRFRGRSD
*****

BAE06262      LGDYDNSDDETLDNSHVKRS-----
Procl-prepro-FLP-I LGDYDNRDEGGLSDSLDKRSRNLFRFRGRDHSRNLFRFRGRSDMEDLSLAGGPMDFPAGLLD
*****

BAE06262      -----RNLFRFRGRSVNRQLNARSCDGCDE
Procl-prepro-FLP-I EVDQEDLPFQEKRSPhKSYRHLVKGNRNFPRFGRGDRNFRFRGRSVDSQLNALSCDGCDE
*****

BAE06262      HNYPGTNPIPPPIPVQSMVPAKQDIA-HTIESPELDDSTSHRMKRAAIPTANDYGSMSY
Procl-prepro-FLP-I PK-TSVSSTPSPTPVQPVSTKKDISTHSIEASNPSGSTSQRMKRAVDPTFYDYGMSLSY
: . . . . * . * * * . : . : * : * : * : . . * : * : * : . * * * * *

BAE06262      SPIAWARGFQPEEQIITVSSDPQDVNKRALDRNFRFRGRDRNFRFRGRNRNFRFRGKRD
Procl-prepro-FLP-I SPSSWARGFQPEEQIITVSSDPQDVNKRADRNFRFRGRD-----RNFRFRGKRD
* : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : * : *

BAE06262      RSNEYRPSSPSESSESIVAVSPA EYSRNVRA PQRNFLRFG
Procl-prepro-FLP-I RSNEYPPSSPSESSESIVAVSPA EYSRNVRA PQRNFLRFG
*****

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B2. Alignment of BAE06263 and Procl-prepro-FMRFamide-like peptide I

```

BAE06263      MTRSCVMPLAAWVLLATFTWC SYAHAAHAAPVPPVVAALSSPSQGFSGNNSEDDSVAQPE
Procl-prepro-FLP-I -----MPLAAWVLLATFTWC SYAHAAHAAPVPPVVAALSSPSQGFSGNNSEDDSVAQPE
*****

BAE06263      KRLKLYFHPESQAWMDGDDLPIPSQEGSKRAYSDRNFLRFRGRSDVDKRNRLRFRGRSD
Procl-prepro-FLP-I KRLKLYFHPESQAWMDGDDLPIPSQEGSKRAYSDRNFLRFRGRNDDDKRSRNLFRFRGRSD
*****

BAE06263      LGDYDNSDDETLDNSLVKRS-----

```

```

BAC82426          MVRGAAIGLMVMVVVTGALAEEQDADRERRAPSGFLGMRGKDVSTPLENIVPAVNDYT
Procl-prepro-TRP  MVRGAAIGLMVMVVVTGALAEEQDADRERRAPSGFLGMRGKDVSTPLENIVPAVNDYT
                  *****

BAC82426          LQDSFPASLYGLRDDNGPVVLAVPWVKKAPSGFLGMRGKKSGEAAFGEAGMDSELETL
Procl-prepro-TRP  LQDSFPASLYGLRDDNGPVVLAVPWVKKAPSGFLGMRGKKSGEAAFGEAGMDSELETL
                  *****

BAC82426          KRAPSGFLGMRGKKAPSGFLGMRGKKAPSGFLGMRGKKHFDDESEIDAYIQALTAMVDGQ
Procl-prepro-TRP  KRAPSGFLGMRGKKAPSGFLGMRGKKAPSGFLGMRGKKHFDDESEIDAYIQALTAMVDGQ
                  *****

BAC82426          QEKRAPSGFLGMRGKKAFFGDSSDEEMSVAGVDKRAPSGFLGMRG
Procl-prepro-TRP  QEKRAPSGFLGMRGKKAFFGDSSDEEMSVAGVDKRAPSGFLGMRG
                  *****

```

A. Ponle-prepro-allatostatin A (internal protein fragment from GAFS01069035)

+SDEEDNEDEQYYAYGLGKRPRVYSFGLGKR SFGSLDDYDDLDDDEVEGLSELGQYTDLLKRAATYGFDLGNDDALESGLGRRSYGFGLGKRS+

B. Ponle-prepro-allatostatin C (C-terminal partial protein from GAFY01028258)

+PDQSPQPYQPQRMLDLVDDGSLDAALINYLFAKQMVRLRNADIKDLQKR RSYWKQCAFNAVSCFGKRK

C. Ponle-pre-bursicon α (N-terminal partial protein from GAFY01028258)

MGRISWVLVMMGMASVGVLGDECSLTPVIHILSYQGCTSK+

D. Ponle-prepro-CCHamide (from GAFS01018472)

MPVPRSSAILLLAFPLVLLWAPPITGHRVMKGGCLNYGHSCLGAHGKRASVPVVPRLLDIIMDALNTPHTNNQYPFAVVSSI SEPRSGVPAARRFL
TPDEGVDLRMGRVVGVDLLETLRTRVKGDRLGSMGQDQDAMVYGSLLDDYSDDRYKRHVVPYKRPPVATSAASRVQAAGSQTWGSSRLQNELKQ
AEELQEEEQKEEQEEQEEQHEEQEEQEQQGARDHVVHQRSLASQDSWRR

E1. Ponle-prepro-crustacean hyperglycemic hormone I (from GAFS01024727)

MVACRRMWSLVVVVVVVVSLGPSGVRGRSVDGSSRLERLLSSGSSEPLSFLSEDHSVNRQVFDQACKGIYDRALFKKLDRVCCDCYNLYRKPK
VATTCRENCYANSVFRQCLDDLLIDVVDEYISGVQIVGK

E2. Ponle-prepro-crustacean hyperglycemic hormone II (internal protein fragment from GAFS01049968)

+NKRQVFDQACKGIYDRAIFKKLDRVCEDCYNLYRKPYVATTCRQNCYANSVFRQCLDDLLIDVVDEYISGVQTVG+

E3. Ponle-prepro-crustacean hyperglycemic hormone III (from GAFS01042936)

MSQSMVWSSVMVVVLTSSCTGRSLSEDLVLVLQATSAALSNTNVLQNHSPHRRDMEFPQYHSITKR AVFDTACKGYDRKIWATLNRACEDCQNLF
RKPGVEAGCRKGCFTDSMFTACVANLMLAVDKYEAMAGFVRGT

F. Ponle-prepro-diuretic hormone 31 (C-terminal partial protein from GAFS01038688)

+KRGLDLGLGRGFSGSQAAKHLMGLAAANYAGGPGRRRRSSDDTLDLPHDDNLYAHDQAAELAESSR

G. Ponle-pre-eclosion hormone (from GAFS01039705)

MIGSRKVVSALLVLSVMLAALLVLP LSAAAA NKVSVCIKNCAQCKVMYHDFKGGLCADFCVQSEGRFIPDCARPQTLIPFFLQRL

H. Ponle-prepro-intocin (from GAFS01000331)

MQVGVMIVAAVVVGNAACFITNCPGGKRSGASTQIARSRTCSSCGPGLRGRCLGPEICCGEGVGCFLATREARM CRAENLVPLTCTNTDLKTCG
RLREGRC AASGLCCTEMKCEFDGSCVESREASVEDQQVERQRFAPL PNLSDDQWSL

I. Ponle-prepro-mysuppressin (from GAFS01019764)

MMYGSRSWCSLLLVSVVVALGVCVGEAIPPPICLNQQLPLSPYAKKLCAALSNISEFSRAMEEYLDQAISII PVNEPEVKRQDLDDHVFLRFGR
SQ

J1. Ponle-pre-neuroparsin I (from GAFS01000216)

MRTTISCTSGVSCLLAFCSILLILLILQ NATASPLCPERNEITPEDLNTCKYGVVLGWCGNAACGKGPGDSCGGRWEENGICGEGMYCVCGYCAGCTT
TLECALGRFC

J2. Ponle-pre-neuroparsin II (from GAFY01019447 and GAFS01005142)

MRTLTSFITFVVAFYCLVLLFQEA AAAAPRCDHHD RPPNNCKYGTVRNWC RNVCAKGPGESCGGHWEYEQGKCGIGTFCLCGICIGCSTIDGSCADG
PLIC

K. Ponle-prepro-red pigment concentrating hormone (C-terminal partial protein from GAFS01040704)

+LLMVAVVVLASCVSGLNFSPGWGKRAGPVVAVGGVSEPA GLHSAQAGLA AVSSNAAGDNCGAIPVSAAMHIYRLIRTEAARLIQCQEEYMG

L. Ponle-prepro-short neuropeptide F (from GAFS01014236)

MGVSVVKCWVALVCCGVLLSQTSA GPAPPDYDTVNEVYSWLSEHGLDRAPPSMRLRFGRDMGWQVAQRSMPSLRLRFGRKRTVDQEEPLYDHEL
V
RKDSRMPALRLRFGRDSPYQG EEDIGSHEQ

M. Ponle-prepro-tachykinin-related peptide (internal protein fragment from GAFS01072672)

+KKAPSGFLGMRGKKAPSGFLGMRGKKHDDDDSEIDAYIQALTAMVDGQQQKRAPSGFLGMRGKKAYYGESADE+

Figure 5

A. Chequ-pre-bursicon β (C-terminal partial protein from GADE01008158)

+THLRAREVKLTHCYDADGNRLAGERGALVITLREPSECQCLKCGDSTR

B. Chequ-pre-neuroparsin (from GADE01014957)

MRTLTFEISFVVTFYCLVLLFQEAAAAPRCNTHDRPPPSNCKYGTVRNWCNNGVCAKAPGESCGGHWYEHGKCGIGTFCLCGVCIGCSTIDGRCADG
PLMC

Figure 6

A. Structural comparison of known astacidean red pigment concentrating hormone (RPCH), adipokinetic hormone-corazonin-like peptide (ACP) and corazonin isoforms

RPCH	pQ L NF--SPG W ---amide
ACP	pQ I T F --S R S W VPQamide
Corazonin	pQ- T FQ Y S R G W TN-amide

B. Alignment of known crustacean ACP isoforms

Procl-ACP	pQ I T F S R S W VPQ-amide
Spetu-ACP	pQ V T F S R D W N A --amide
Tigca-ACP-I	pQ I T F S R D W L A G S amide
Tigca-ACP-II	SP V T F D R D W L A G S amide
Eucse-ACP-I	pQ I T F S R D W L A G S amide
Eucse-ACP-II	AS P I S F S R D W L A S -amide
Eucse-ACP-III	TF V T F S R D W L A S-amide
Eucse-ACP-IV	AK P I S F S R E W L A S -amide

C. Alignment of known crustacean ACP isoforms

Trica	MALKF-R-----VFALVAVLVLMAWMFTGTQA Q V T F S R D W N P-- G K R T-----
Spetu†	MNQRLDRVYPVMGSFLISLVLLSAFTQMTVA Q V T F S R D W N A-- G K R S-----
Procl	-----MVAWQMMLALVCLAIVPTMA Q I T F S R S W V P- Q G K R S G P L V S P -----
Tigca	MSKRS-TLFDVIFVCVMSAIVLA---STVD Q I T F S R D W L A G S G K R S- P V T F D R D W L A G
Eucse-I	MKNSF-----PSNLCLSLALLFFIASMADA Q I T F S R D W L A G S G K R A S P I S F S R D W L A-
Eucse-II	MRTSI-----ALCIVVVVAYLACMPEQTQA Q I T F S R D W L A G S G K R A K P I S F S R E W L A-
	: . . *:****.* . ***;
Trica	-----ENTDLHNTLKTA-----SAVCHLLMNQVRQLASCDNNNELEP
Spetu†	-----GAAALYPDCAMP-ERALLSEVSKLIHNEAQRMVSCQ-----
Procl	AGAPSLD-----VDPCRDTRLSTLTDVA-----SHLVEL-MDDVSDLTQDDAALALRL
Tigca	S G K R S V-----GSYPGKVMLPPREAIEELANYLLLQLS-----DSPMA---
Eucse-I	S G K R T P V T F S R D W L A S G K R S G D N T D S W I C V P K F A V A P L V H F M V E H E Q K E S Y D F Q P H A ---
Eucse-II	S G K R S V-----DQDAWICVPKITLAPLNFNVLVDHEQKDTYNGHPVN---
	: . .
Trica	GATIFSGRR-----
Spetu†	AWTVLRG-----
Procl	KHALLARQRR--LT
Tigca	KMSFMKHLQGHSEA
Eucse-I	STGTLHEDQ-----
Eucse-II	QEA-----