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Prediction of a neuropeptidome for the eyestalk ganglia of the lobster *Homarus americanus* using a tissue-specific *de novo* assembled transcriptome

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Abstract

In silico transcriptome mining is a powerful tool for crustacean peptidome prediction. Using homology-based BLAST searches and a simple bioinformatics workflow, large peptidomes have recently been predicted for a variety of crustaceans, including the lobster, *Homarus americanus*. Interestingly, no *in silico* studies have been conducted on the eyestalk ganglia (lamina ganglionaris, medulla externa, medulla interna and medulla terminalis) of the lobster, although the eyestalk is the location of a major neuroendocrine complex, *i.e.*, the X-organ-sinus gland system. Here, an *H. americanus* eyestalk ganglia-specific transcriptome was produced using the *de novo* assembler Trinity. This transcriptome was generated from 130,973,220 Illumina reads and consists of 147,542 unique contigs. Eighty-nine neuropeptide-encoding transcripts were identified from this dataset, allowing for the deduction of 62 distinct pre/preprohormones. Two hundred sixty-two neuropeptides were predicted from this set of precursors; the peptides include members of the adipokinetic hormone-corazonin-like peptide, allatostatin A, allatostatin B, allatostatin C, bursicon α, CCHamide, corazonin, crustacean cardioactive peptide, crustacean hyperglycemic hormone (CHH), CHH precursor-related peptide, diuretic hormone 31, diuretic hormone 44, eclosion hormone, elevenin, FMRFamide-like peptide, glycoprotein hormone α2, glycoprotein hormone β5, GSEFLamide, intocin, leucokinin, molt-inhibiting hormone, myosuppressin, neuroparsin, neuropeptide F, orcokinin, orcomyotropin, pigment dispersing hormone, proctolin, pyrokinin, red pigment concentrating hormone, RYamide, short neuropeptide F, SIFamide, sulfakinin, tachykinin-related peptide and trissin families. The predicted peptides expand the *H. americanus* eyestalk ganglia neuropeptidome approximately 7-fold, and include 78 peptides new to the lobster. The transcriptome and predicted neuropeptidome described here provide new resources for investigating peptidergic signaling within/from the lobster eyestalk ganglia.

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Keywords

Crustacea; Decapoda; neurohormone; neuropeptide; transcriptomics; X-organ-sinus gland system

1. Introduction

The American lobster, *Homarus americanus*, is an iconic and economically important species due to its popularity as a luxury food item; the value of the lobster fishery in 2015 for the state of Maine alone was valued at approximately half a billion dollars (State of Maine Department of Marine Resources; http://www.maine.gov/dmr/commercial-fishing/landings/documents/11-15LandingsBySpecieswithBonus.Table.pdf). In addition to its commercial importance, *H. americanus* is one of several decapod crustaceans that have long been used to elucidate the basic principles governing the generation, maintenance and neuromodulation of rhythmically active motor behaviors, and as such, serve as models for understanding the control of walking, breathing and chewing in vertebrates (for review see: Blitz and Nusbaum, 2011; Christie et al., 2010a; Cooke, 2002; Fénelon et al., 2003; Hooper and DiCaprio, Marder and Bucher, 2007; Marder et al., 1995; Nusbaum et al., 2001; Selverston, 2005; Selverston and Ayers, 2006; Selverston et al., 1998; Skiebe, 2001; Stein, 2009). A major contribution to our understanding of rhythmic motor behavior that has come from work conducted using the lobster and other decapods is that numerically simple, "hard-wired" neural circuits are capable of producing a wide array of distinct motor outputs. This functional flexibility is due largely to the actions of locally-released and circulating chemicals that modify the intrinsic properties of circuit elements at the molecular, cellular and system levels. While a number of classes of chemical are known to serve as locally-released and/or hormonally-delivered neuromodulators, the peptides are by far the largest and most diverse group of these compounds (*e.g.*, Christie et al., 2010a).

Much work has focused on identifying native neuropeptides in *H. americanus*. Early studies employing biochemical isolation and sequencing of peptides and/or the targeted molecular cloning of the genes/transcripts encoding them allowed for the identification of members of several peptide families from the lobster, *e.g.*, crustacean hyperglycemic hormone (CHH)/molt-inhibiting hormone (MIH), FMRFamide-like peptides (FLPs) and proctolin (*e.g.*, Chang et al., 1990; de Kleijn et al., 1994, 1995; Schwarz et al., 1984; Soyez et al., 1991; Tensen et al., 1991; Trimmer et al., 1987). However, large-scale peptide discovery in this species did not begin until the advent of biological mass spectrometry as a means for peptide identification. Via accurate mass matching and tandem mass spectrometric sequencing, a large peptidome, encompassing approximately 20 distinct families, was rapidly elucidated (*e.g.*, Cape et al., 2008; Chen et al., 2010; Christie et al., 2006, 2008a; Dickinson et al., 2009a, 2009b; Fu et al., 2005; Jiang et al., 2012; Li et al., 2002; Ma et al., 2008, 2009a; Stemmler et al., 2005, 2006, 2007, 2010). While clearly a powerful means for peptidome discovery, peptides that are present in low abundance, are large, possess extensive post-translational modifications, and/or possess sequences that do not ionize well can be very difficult to identify via mass spectrometry (*e.g.*, Christie et al., 2010a). In contrast, *in silico* genome/transcriptome mining with subsequent bioinformatics peptide prediction is not limited by these factors (*e.g.*, Christie et al., 2010a), and thus can be used to complement

and augment peptide discoveries made using mass spectral and other means (*e.g.*, Christie, 2014a, Christie et al., 2011a; Torfs et al., 2002). In fact, *in silico* mining of a mixed tissue neural transcriptome (**BioProject No. PRJNA300643**; D. Schulz and E. Marder, unpublished direct GenBank submission) was recently employed for expansion of the *H. americanus* neuropeptidome (Christie et al., 2015).

The eyestalk ganglia of decapod species, which consist of the lamina ganglionaris, medulla externa, medulla interna and medulla terminalis, have long been known to be rich sources of neuropeptides (*e.g.*, Christie, 2011). The sinus gland, a major neuroendocrine release site present in the eyestalk of decapods, is derived largely from neurons whose somata reside in the X-organ, a cluster of loosely associated cell bodies located within the medulla terminalis (e.g., Christie, 2011). Using mass spectrometry and other means, approximately 40 peptides encompassing about one dozen families (*i.e.*, allatostatin C [AST-C], corazonin, CHH/MIH, crustacean hyperglycemic hormone precursor-related peptide [CPRP], myosuppressin, orcokinin, orcomyotropin, pigment dispersing hormone [PDH], red pigment concentrating hormone [RPCH], short neuropeptide F [sNPF], SIFamide and tachykinin-related peptide [TRP]) have been identified from the eyestalk ganglia/sinus gland of *H. americanus* (*e.g.*, de Kleijn et al., 1994, 1995; Dickinson et al., 2009b; Fu et al., 2005; Ma et al., 2008; Stemmler et al., 2005, 2006, 2010). A number of these peptides have been shown (or implicated) to play critical roles in the control of key physiological processes in the lobster, including, but not limited to, molting, growth and reproduction (*e.g.*, Christie et al., 2010a). Interestingly, and despite its clear importance in understanding physiological control in the lobster, no significant transcriptomic resources have been developed for the eyestalk ganglia of *H. americanus*. In the study presented here, an eyestalk ganglia-specific transcriptome for the lobster was assembled *de novo* and used to predict a peptidome for this portion of the *H. americanus* nervous system. The transcriptome, which consists of 147,542 transcripts, has been publicly deposited (**BioProject No. PRJNA338672**) to provide a resource for future molecular studies of physiological control within and by the eyestalk ganglia. The peptidome predicted using this resource expands the peptidome known for this portion of the lobster nervous system approximately 7-fold, and includes a number of neuropeptides previously unknown from the lobster. These new peptide discoveries provide an expanded foundation from which to initiate anatomical and physiological studies of peptidergic signaling within and from the eyestalk ganglia.

2. Materials and methods

2.1. *De novo* transcriptome assembly

2.1.1. Animals and tissue dissection—American lobsters, *H. americanus*, (N=4) were purchased from local (Brunswick, ME, USA) seafood retailers. All animals were housed in recirculating natural seawater aquaria at 10–12°C and were fed approximately weekly on a diet of chopped shrimp. For the isolation of the eyestalk ganglia, animals were cold-anesthetized by packing in ice for approximately 20–30 min. After anesthetization, the eyestalks were removed and the eyestalk ganglia were dissected from the overlying carapace and surrounding musculature in chilled (approximately 4°C) physiological saline

(composition in mM/l: 479.12 NaCl, 12.74 KCl, 13.67 $CaCl_2$, 20.00 $MgSO_4$, 3.91 Na_2SO_4, 11.45 Trizma base, and 4.82 maleic acid [pH = 7.45]).

2.1.2. RNA isolation—Freshly dissected eyestalk ganglia pairs (N=4 pairs) were placed into sterile RNAase-free 1.5 ml microfuge tubes containing 300 μl of TRIzol Reagent (catalog no. 15596018; Thermo Fisher Scientific Inc., Waltham, MA, USA) and manually homogenized using a sterile RNAse-free disposable pestle (catalog no. 9950-901; Argos Technologies Inc., Elgin, IL, USA). RNA was isolated from the resulting homogenate using a Direct-zol RNA MiniPrep (catalog no. R2052; Zymo Research, Irvine, CA, USA) spin column system according to the manufacturer-supplied protocol. RNA quality was assessed using an Agilent 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA). All RNA samples were stored at −80°C until being shipped on dry ice to the Georgia Genomics Facility (University of Georgia, Athens, GA, USA) for library preparation and sequencing.

2.1.3. cDNA library production and Illumina sequencing—Double-stranded cDNA libraries were prepared from total RNA using a KAPA Stranded mRNA-seq kit (catalog No. KK8420; Kapa Biosystems, Wilmington, MA, USA) following the manufacturer's instructions; 3 μg of total RNA/sample was used for library generation. In brief, total RNA samples were purified with two oligo-dT selection (poly(A) enrichment using oligo-dT beads). Samples were then fragmented and reverse transcribed into double-stranded complementary cDNA using random primers, with second strand synthesis marked using dUPT. Each eyestalk ganglia library was tagged with a unique indexed adapter. A Qubit 2.0 Fluorometer (Invitrogen, Carlsbad, CA, USA), AATI Fragment Analyzer (Advanced Analytical Technologies, Ankeny, IA, USA), and Kapa qPCR assays were used to determine the quality and quantity of the final pool of libraries. Paired-end Illumina sequencing (150 base pairs [bp]) was performed on a NextSeq 500 system (Illumina, San Diego, CA, USA) using the high output kit v2 with 300 cycles.

2.1.4. Transcriptome assembly—Prior to transcriptome assembly, raw sequencing reads were assessed for quality using FASTQC (v1.0.0) software (Illumina Basespace Labs). Specifically, each RNA-Seq eyestalk library was quality filtered using FASTQ Toolkit (v. 2.0.0) by trimming the first 9 bp of each read, removing all Illumina adapters (TruSeqLT universal primer), culling all low quality reads (Phred cutoff score=30), and setting the minimum read length to 50 bp. This quality filtering resulted in the removal of <1% of the reads present in each library, leaving from ~27 to ~38 million filtered reads per eyestalk ganglion sample (Table 1).

Filtered reads were *de novo* assembled using Trinity (v2.0.6) software (https://github.com/trinityrnaseq/trinityrnaseq/wiki; Grabherr et al., 2011) on the National Center for Genome Analysis Support's (Indiana University, Bloomington, IN, USA) Mason Linux cluster. Each node of the computer system includes four Intel Xeon L7555 8-core processors running at 1.87 GHz with 512 GB of memory. For the assembly, reads from all eyestalk ganglia libraries were combined and the minimum sequence length in the assembly was set to 324 bp. For the *de novo* assembly, the initial parameters of Trinity were set as follows: maximum memory, 200GB; CPU, 32; normalize maximum read coverage, 50; minimum contig length, 324. A summary of the assembly statistics (Table 2) was obtained using the script

TrinityStat.pl (v2.0.6). Quality filtered raw reads were mapped against the *de novo* assembled transcriptome using Bowtie2 (v2.1.0; Langmead et al., 2009) software (Table 3).

2.2. Peptidome prediction

2.2.1. Transcriptome mining—Searches of the *H. americanus* eyestalk ganglia transcriptome were conducted using BLAST software installed on an Intel-processor-based BEOWULF computer cluster (Pacific Biosciences Research Center, University of Hawaii at Manoa, Honolulu, HI, USA) using a protocol that has proven highly effective for peptide-encoding transcript discovery in a wide array of arthropod species (*e.g.,* Christie, 2008a, 2008b, 2014a, 2014b, 2014c, 2014d, 2014e, 2014f, 2015a, 2015b, 2015c, 2015d, 2016a, 2016b; Christie and Chi, 2015a, 2015b, 2015c; Christie and Pascual, 2016; Christie et al., 2008b, 2010b, 2011b, 2013b, 2015; Gard et al., 2009; Ma et al., 2009b, 2010). Specifically, the *H. americanus* eyestalk transcriptome assembly was selected as the database to be searched using the tblastn algorithm, and a known neuropeptide precursor was input to the program as the protein query. The complete list of pre/preprohormones searched for, as well as the specific queries used, is provided in Table 4. All hits returned by a given search were translated using the "Translate" tool of ExPASy (http://web.expasy.org/translate/), and then checked manually for homology to the query sequence. The BLAST-generated maximum score and E-value for each of the transcripts identified as encoding a putative neuropeptide precursor are provided in Table 4.

2.2.2. Peptide prediction—The structures of mature peptides were predicted using a well-established workflow (*e.g.,* Christie, 2008a, 2008, 2014a, 2014b, 2014c, 2014d, 2014e, 2014f, 2015a, 2015b, 2015c, 2015d, 2016a, 2016b; Christie and Chi, 2015a, 2015b, 2015c; Christie and Pascual, 2016; Christie et al., 2008b, 2010b, 2011b, 2011c, 2011d, 2013, 2015; 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 of SignalP 4.1 were set to "Sensitive" to better match the sensitivity of version 3.0 of this freeware program. 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 tyrosine residues were present, prediction of their sulfation state 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, *i.e.*, 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 Table 5. All protein/peptide alignments were done using the online program MAFFT version 7 (http://mafft.cbrc.jp/alignment/software/; Katoh and Standley, 2013). To determine amino acid identity/similarity between peptides, the sequences in question were aligned using MAFFT version 7; amino acid identity/similarity was subsequently determined using the alignment output. Specifically, percent

identity was calculated as the number of identical amino acids divided by the total number of residues in the longest sequence (x100). Amino acid similarity was calculated as the number of identical and similar amino acids divided by the total number of residues in longest sequence (x100).

3. Results

3.1. *De novo* assembly of an eyestalk ganglia-specific transcriptome

Four samples, each consisting of the two eyestalk ganglia from a single lobster (three males and one female), were used as the source of RNA for transcriptome development (Table 1). RNASeq yielded approximately 33 million paired-end reads per library (Table 1), which were collectively assembled using Trinity; a total of 130,973,220 trimmed and quality filtered paired-end reads ranging in length from 50 to 150 bp were input into Trinity for *de novo* assembly (Table 2). The transcriptome assembled by Trinity consists of 147,542 transcripts and 110,841 "Trinity predicted genes" (Table 2). Of the "Trinity predicted genes", 96,484 are singletons (87%), with the remaining genes (14,357 in total) possessing from two to 55 "Trinity predicted isoforms". The average transcript length is 1,241 bp (Table 2); half of the contigs (N50 in Table 2) were at least 2,160 bp long, with the shortest and longest assembled sequences being 324 and 27,389 bp, respectively (Table 2). Mapping for the Illumina-generated reads against the complete 147,542-transcript assembly using Bowtie yielded an overall alignment rate of 91%, with the majority of reads (52%) mapping just once (Table 3). The transcriptome just described, as well as all associated data, have been deposited in GenBank under **BioProject No. PRJNA338672**.

3.2. Prediction of an eyestalk ganglia-specific neuropeptidome

Transcripts encoding precursors for 48 distinct peptide families/subfamilies were searched for within the *de novo* assembled *H. americanus* eyestalk ganglia-specific transcriptome described in the previous section (Table 4). In the interest of space, only those searches that resulted in the identification of protein-encoding transcripts (33 families/subfamilies) are described in the following subsections, with the data presented in alphabetical order based on peptide family name. All precursor proteins listed as "full-length" exhibit a functional signal sequence (including a "start" methionine) and are flanked on their C-terminal 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 fragment proteins).

3.2.1. Adipokinetic hormone-corazonin-like peptide (ACP)—Three transcripts were identified as encoding putative ACP precursors (Table 4). Translation of these sequences revealed each to encode an identical 104 amino acid protein (Homam-prepro-ACP; Table 4 and Fig. 2A); this protein is identical in sequence to the query protein used to identify the transcripts encoding it (Christie et al., 2015). Three distinct peptides were predicted from Homam-prepro-ACP (Table 5 and Fig. 2A): the ACP isoform pQITFSRSWVPQamide and two linker/precursor-related sequences. While all three of the peptides predicted from Homam-prepro-ACP are known *H. americanus* peptides (Christie et al., 2015), they are identified from the lobster eyestalk ganglia for the first time here.

3.2.2. Allatostatin A (AST-A)—Two transcripts were identified as encoding putative AST-A precursors (Table 4). Translation of these sequences revealed one to encode the N-terminus and the other the C-terminus of a 561 amino acid full-length preprohormone (Table 4 and Fig. 2B). This protein appears to be a splice variant (sv) of the *Homarus* AST-A precursor used for identifying the transcripts encoding it (*i.e.*, Homam-prepro-AST-A [termed here sv1] of Christie et al. [2015]; alignment not shown); Homam-prepro-AST-A sv2 (the protein identified here) possesses two copies of the AST-A isoform TGPYAFGLamide that are missing in the sv1 protein. Twenty-three distinct isoforms of AST-A and 14 distinct linker/precursor-related sequences were predicted from Homam-prepro-AST-A sv2 (Table 5 and Fig. 2B), all of which, while known *H. americanus* peptides (*e.g.*, Christie et al., 2015; Ma et al., 2008), are identified for the first time from the eyestalk ganglia in this study.

3.2.3. Allatostatin B (AST-B)—Three transcripts were identified as encoding putative AST-B precursors (Table 4). Translation of these transcripts revealed that one encodes the N-terminal partial protein, and the two others encode distinct C-terminal partial sequences. Given a common region of overlap between the N-terminal partial protein and two C-terminal partial precursors, two distinct full-length preprohormones can be generated by combining the partial sequences. One full-length precursor is 274 amino acids long, while the other preprohormone is 310 amino acids in length. As the longer protein differs from the shorter only in that it possesses a single 36 amino acid insertion (Fig. 3A), the two precursors appear to be splice variants of a common gene; these precursors are consequently named Homam-prepro-AST-B sv1 (the shorter protein; Table 4 and Fig. 3A) and Homam-prepro-AST-B sv2 (the longer protein; Table 4 and Fig. 3A). Fifteen distinct peptides were predicted from this pair of preprohormones collectively (Table 5 and Fig. 3A); seven of these possess the C-terminal motif $-WX_6$Wamide, the hallmark of the AST-B family (*e.g.*, Christie et al., 2010a). All of the peptides predicted from Homam-prepro-AST-B sv1/sv2 except TNWNKFQGSWamide, an AST-B isoform identified previously via mass spectrometry from the lobster brain, pericardial organ and stomatogastric ganglion (Ma et al., 2008), are novel discoveries for this species. All of these peptides are new discoveries for the *H. americanus* eyestalk ganglia.

3.2.4. Allatostatin C (AST-C)—Three transcripts were identified as encoding putative AST-C precursors (Table 4). Translation of one of these transcripts revealed a 65 amino acid C-terminal partial protein (Table 4 and Fig. 2C1) that is identical in sequence to a previously identified *H. americanus* partial precursor (*i.e.*, Homam-prepro-AST-C I of Christie et al. [2015]). Three distinct peptides (one partial and two full-length) were predicted from the extant portion of Homam-prepro-AST-C I (Table 5 and Fig. 2C1), one of which, pQIRYHQCYFNPISCF (disulfide bridging between the two cysteine residues), is a known lobster eyestalk AST-C isoform (Stemmler et al., 2010). The partial and full-length linker/precursor related peptides derived from Homam-prepro-AST-C I, while previously known *H. americanus* peptides (Christie et al., 2015), are new discoveries for the lobster eyestalk ganglia.

Translation of the second transcript revealed a 105 amino acid full-length preprohormone (Table 4 and Fig. 2C2) that is identical in sequence to a known *H. americanus* precursor protein, *i.e.*, Homam-prepro-AST-C II (Christie et al., 2015; Dickinson et al., 2009b). Two peptides were predicted from Homam-prepro-AST-C II (Table 5 and Fig. 2C2), one of which, SYWKQCAFNAVSCFamide (disulfide bridging between the two cysteine residues), is a known *Homarus* eyestalk AST-C isoform (Dickinson et al., 2009b); while previously identified from the lobster, this is the first report of the linker/precursor-related peptide derived from Homam-prepro-AST-C II in the *H. americanus* eyestalk ganglia.

Translation of the third transcript revealed a 148 amino acid full-length precursor protein that is distinct in sequence from Homam-prepro-AST-C I and II, and for convenience of future discussion has been named Homam-prepro-AST-C III (Table 4 and Fig. 1A). Four distinct peptides were predicted from Homam-prepro-AST-C III (Table 5 and Fig. 1A), one of which, GNGDGRLYWRCYFNAVSCF (disulfide bridging between the two cysteine residues), is a novel *H. americanus* isoform of AST-C; the three linker/precursor-related peptides derived from Homam-prepro-AST-C III are also new peptide discoveries for the lobster.

3.2.5. Bursicon α—A single transcript was identified as encoding a putative bursicon α precursor (Table 4). Translation of this sequence revealed a 100 amino acid N-terminal partial protein (Homam-pre-bursicon α v2; Table 4 and Fig. 2D). A single partial isoform of bursicon α (81 amino acids in length) was predicted from Homam-pre-bursicon α (Table 5 and Fig. 2D); except for its last three residues, this partial peptide is identical in amino acid sequence to an isoform of bursicon α predicted from a prehormone described in an earlier study (Christie et al., 2015; for convenience of later discussion this earlier identified variant is termed here Homam-pre-bursicon α v1).

3.2.6. CCHamide—Nine transcripts were identified as encoding putative CCHamide precursors (Table 4). Five of the transcripts encode an identical 116 amino acid preprohormone (Homam-prepro-CCHamide I; Table 4 and Fig. 2E); this precursor is identical in sequence to that of a CCHamide I preprohormone identified in a previous study (Christie et al., 2015). Three peptides, including the CCHamide isoform SCSQFGHSCFGAHamide (disulfide bridging between the two cysteine residues), were predicted from Homam-prepro-CCHamide I (Table 5 and Fig. 2E). Although all three peptides were previously identified from the lobster, this is their first identification from the eyestalk ganglia of *H. americanus*.

Translation of the remaining four transcripts revealed four distinct putative splice variants of a second CCHamide gene (Homam-prepro-CCHamide II sv1–sv4; Table 4 and Fig. 3B). The four CCHamide II precursors differ from one another in two alternatively spliced regions, one involving the presence/absence of a nine amino acid insertion within the preprohormone, and the other involving one of two alternative C-termini, one of which is five amino acids long (the short C-terminus) and the other of which is a 19 amino acid segment (the long C-terminus). Homam-prepro-CCHamide II sv1, which is identical in sequence to a previously identified *H. americanus* CCHamide II precursor (the tblastn query sequence used for the identification the CCHamide II transcripts identified here from the

eyestalk ganglia; Christie et al., 2015), lacks the insertion and possesses the short C-terminus (first row of sequence in Fig. 3B). Homam-prepro-CCHamide II sv2 possesses the nine amino acid insertion and the short C-terminus (second row of sequence in Fig. 3B). Homam-prepro-CCHamide II sv3 lacks the nine amino acid insertion and possesses the long C-terminus (third row of sequence in Fig. 3B). Homam-prepro-CCHamide II sv4 possesses both the nine amino acid insertion and the long C-terminus (fourth row of sequence in Fig. 3B). Eight distinct peptides were predicted from the collective set of Homam-CCHamide II variants (Table 5 and Fig. 3B), six of which, including the CCHamide isoform HRVLKGGCLNYGHSCLGAHamide (disulfide bridging between the two cysteine residues) are known *H. americanus* peptides (Christie et al., 2015), though identified here for the first time from the eyestalk ganglia. The remaining two peptides, both linker/precursor-related sequences, are new discoveries for *H. americanus*.

3.2.7. Corazonin—Five transcripts were identified as encoding putative corazonin precursors (Table 4). Translation of four of these revealed identical 109 amino acid full-length precursors (Homam-prepro-corazonin), with the fifth transcript encoding the C-terminus of the full-length protein (Table 4 and Fig. 2F). Homam-prepro-corazonin is identical in sequence to a previously identified *H. americanus* corazonin precursor (Christie et al., 2015). Three distinct peptides were predicted from Homam-prepro-corazonin (Table 5 and Fig. 2F): pQTFQYSRGWTNamide (authentic corazonin) and two linker/precursor-related sequences. Corazonin is a known *H. americanus* eyestalk ganglia peptide (Ma et al., 2008); the two linker/precursor-related peptides, while known from the lobster (Christie et al., 2015), are new discoveries for the eyestalk system.

3.2.8. Crustacean cardioactive peptide (CCAP)—Two transcripts were identified as encoding putative CCAP precursors (Table 4). Translation of these transcripts revealed that each encodes an identical 140 amino acid full-length protein (Homam-prepro-CCAP; Table 4 and Fig. 2G); Homam-prepro-CCAP possesses the same amino acid sequences as a known *H. americanus* CCAP precursor (Christie et al., 2015). Six peptides were predicted from Homam-prepro-CCAP (Table 5 and Fig. 2G), one being authentic CCAP, *i.e.*, PFCNAFTGCamide (disulfide bridging between the two cysteine residues). While all of the peptides derived from Homam-prepro-CCAP have been identified previously from the lobster (*e.g.*, Christie et al., 2015; Ma et al., 2008), this is their first identification from the *H. americanus* eyestalk ganglia.

3.2.9. Crustacean hyperglycemic hormone (CHH) superfamily—The CHH superfamily of peptides consists of two distinct subgroups, the CHH proper subfamily and the molt-inhibiting hormone (MIH) subfamily (*e.g.*, Böcking et al., 2002; Chan et al., 2003; Chung et al., 2010; Fanjul-Moles, 2006). Members of the CHH subgroup are typically 70 or so amino acids long and possess a stereotypical pattern of disulfide bridges between six identically conserved cysteine residues (namely bridging between the first and fifth, the second and fourth, and the third and sixth cysteines). The preprohormones that give rise to CHH subfamily members also contain a linker/precursor-related peptide between the signal sequence and the CHH, a peptide commonly referred to as crustacean hyperglycemic hormone precursor-related peptide or CPRP. In contrast, members of MIH subgroup are

typically longer than the CHHs, their arrangements of disulfide bridging between cysteine residues are more variable than that seen in the CHHs, and the precursors from which they are cleaved lack a CPRP.

3.2.9.1. CHH subgroup: Five transcripts were identified as encoding putative CHH precursors (Table 4). Four of the transcripts encode full-length preprohormones that possess distinct but highly similar amino acid sequences, and have been named here Type I CHH precursors (Table 4 and Fig. 4A). One of the deduced proteins is 134 amino acids in overall length and is identical in sequence to a known *H. americanus* X-organ-sinus gland system CHH precursor (**Accession No. P19806**; de Kleijn et al., 1995); for ease of discussion, this preprohormone has been named Homam-prepro-CHH Ia v1 (Table 4 and Fig. 4A). One isoform of CHH and one isoform of CPRP were predicted from Homam-prepro-CHH Ia v1 (Table 5 and Fig. 4A), both known eyestalk system peptides (*e.g.*, de Kleijn et al., 1995). Two 133 amino acid preprohormones are also members of the Type I grouping. The sequences of these two proteins are identical except for a single substituted residue (position 2) in their signal peptides, with that being a methionine in one protein (Homam-prepro-CHH Ia v2; Table 4 and Fig. 4A) and a phenylalanine in the other (Homam-prepro-CHH Ia v3; Table 4 and Fig. 4A); to the best of our knowledge, both of these precursors, while similar to known lobster CHH preprohormones (*e.g.*, **Accession No. 2105187B**; de Kleijn et al., 1995), are described here for the first time. The CHH and CPRP derived from Homam-prepro-CHH Ia v2 and v3 possess identical structures (Table 5 and Fig. 4A), with the CPRP being one amino acid shorter than that derived from Homam-prepro-CHH Ia v1 (*i.e.*, missing the position 17 asparagine) and the CHH having five substituted positions relative to its Homam-prepro-CHH Ia v1 counterpart. CHH derived from Homam-prepro-CHH Ia v2 and v3 is a known eyestalk system peptide (de Kleijn et al., 1995); the CPRP is a new lobster variant. The final Type I protein deduced here is 131 amino acids long and, while its N-terminus is similar to those of the previously described preprohormones, its C-terminus is quite distinct, and hence it has been named Homam-prepro-CHH Ib (Table 4 and Fig. 4A); with the exception of a 12 amino acid N-terminal extension and a single substituted residue (in the CPRP), this protein is identical to a previously known Homarus CHH precursor and hence has been named Homam-prepro-CHH Ib v2 (Table 4 and Fig. 4A). The CPRP derived from Homam-prepro-CHH Ib v2 is identical to that predicted from Homam-prepro-CHH Ia v2 and v3, while its CHH is distinct from all of those described earlier (Table 5 and Fig. 4A) and is described here from the lobster eyestalk ganglia for the first time here. Regardless of amino acid sequence, each of the CHHs identified in this study was predicted by DiANNA to possess a stereotypical pattern of disulfide bonding, namely bonds between the first and fifth, the second and fourth, and the third and sixth cysteines.

The protein deduced from the fifth and final CHH-encoding transcript identified here is 111 amino acids long and, like those described above, is a full-length precursor; this protein is identical in sequence to a recently predicted CHH preprohormone (Christie et al., 2015), with the exception that it is missing a 16 amino acid N-terminal extension. Given its sequence identity to the known precursor (both are likely splice variants of a common gene), but its variation from the other proteins in the Type I series, we have named this preprohormone Homam-prepro-CHH II sv2 (Table 4 and Fig. 4B). One isoform of CPRP

and one isoform of CHH were predicted from Homam-prepro-CHH II v2 (Table 5 and Fig. 4B), both distinct from those derived from the members of the Type I precursor series. While known lobster peptides, this is the first description of the CHH and CPRP derived from Homam-prepro-CHH II v2 from the *H. americanus* eyestalk system. Analysis of the CHH derived from Homam-prepro-CHH II by DiANNA suggest it possesses disulfide bridging between its first and fifth, the second and fourth, and the third and sixth cysteines.

3.2.9.2. Molt-inhibiting hormone (MIH) subgroup: Three transcripts were identified as encoding putative MIH precursors (Table 4). Translation of one of these sequences revealed a 112 amino acid full-length prehormone that is nearly identical in sequence to a known lobster MIH precursor (**Accession No. CAA60644**; de Kleijn et al., 1994); it differs by a single substituted residue, namely one in the signal peptide portion of the prehormone (an alanine for glycine substitution at position 6). The protein identified here has been named Homam-prepro-MIH I v2 (Table 4 and Fig. 4C). The MIH predicted from Homam-prepro-MIH I v2 (Table 5 and Fig. 4C) is a known *H. americanus* eyestalk system peptide (de Kleijn et al., 1994). The remaining two transcripts encode novel full-length MIH prehormones of 111 (Homam-pre-MIH II; Table 4 and Fig. 4C) and 119 (Homam-pre-MIH III; Table 4 and Fig. 4C) amino acids. A single isoform of MIH was predicted from Homam-pre-MIH II and Homam-pre-MIH III, each possessing a distinct sequence (Table 5 and Fig. 4C); these two MIHs are new discoveries for *H. americanus*. While DiANNA analyses suggest that the MIHs derived from Homam-pre-MIH I v2 and Homam-pre-MIH II possess disulfide bonding between their first and fifth, the second and fourth, and the third and sixth cysteines, a different bridging pattern was predicted for the MIH produced from Homam-pre-MIH III, *i.e.*, bridges between its first and third, second and fourth, and fifth and sixth cysteines.

3.2.10. Diuretic hormone 31 (DH31)—Two transcripts were identified as encoding putative DH31 precursors (Table 4). Translation of these transcripts revealed one to encode a 135 amino acid full-length preprohormone and the other a 128 amino acid full-length precursor (Table 4 and Fig. 3C). These two proteins appear to be splice variants of a common gene and hence have been named Homam-prepro-DH31 sv1 and Homam-prepro-DH31 sv2, respectively; the former precursor is identical in sequence to a previously identified *H. americanus* DH31 preprohormone (Christie et al., 2010c). Six distinct peptides were predicted from Homam-prepro-DH31 sv1/2 (Table 5 and Fig. 3C), including the DH31 isoform GLDLGLGRGFSGSQAAKHLMGLAAANFAGGPamide. This DH31 isoform, and four of the five linker/precursor-related sequences, are previously known lobster peptides (Christie et al., 2010c). However, they are identified here from the *H. americanus* eyestalk ganglia for the first time. The fifth linker/precursor-related peptide is a new discovery for *H. americanus*.

3.2.11. Diuretic hormone 44 (DH44)—Two transcripts were identified as encoding putative DH44 precursors (Table 4). Both transcripts encode the same 285 amino acid protein (Table 4 and Fig. 2H), which is identical in sequence to a previously described *H. americanus* DH44 precursor (Christie et al., 2015). Six distinct peptides were predicted from Homam-prepro-DH44 (Table 5 and Fig. 2H): the DH44 isoform ASGLSLSIDASMKVLREALYMEIIRKKQRQQMQRAQHNQKLLNSIamide and five

linker/precursor-related sequences. While all of the peptides predicted from Homam-prepro-DH44 are known lobster peptides (Christie et al., 2015), this is their first identification from the eyestalk ganglia.

3.2.12. Eclosion hormone (EH)—Two transcripts were identified as encoding putative EH precursors (Table 4). Translation of these transcripts revealed one to encode an 88 amino acid full-length protein (Homam-pre-EH I; Table 4 and Fig. I1), and the other, an 82 amino acid prehormone (Homam-pre-EH II; Table 4 and Fig. I2); these proteins are identical in sequence to a pair of EH precursors identified previously from *H. americanus* (Christie et al., 2015). A single EH isoform was predicted from each protein (Table 5 and Fig. 2I1–I2); these EHs possess distinct structures. Analysis of the EH isoforms derived from both Homam-pre-EH I and Homam-pre-EH II using DiANNA suggests disulfide bridges between the first and second, third and fourth, and fifth and sixth cysteines in it. While both of the predicted EH isoforms are known *H. americanus* peptides (Christie et al., 2015), this is the first identification of each EH from the eyestalk ganglia.

3.2.13. Elevenin—A single transcript was identified as encoding a putative elevenin precursor (Table 4). This transcript encodes a 127 amino acid full-length protein (Homam-prepro-elevenin; Table 4 and Fig. 5A). Three distinct peptides were predicted from Homam-prepro-elevenin (Table 5 and Fig. 5A). One of these peptides, VDCRKFVFAPVCRGIIA (disulfide bridging between the two cysteines), possesses structural homology to known arthropod elevenin isoforms; it is approximately 72% identical/89% similar in amino acid composition to the elevenin predicted from the *Nilaparvata lugens* precursor used to identify the transcript encoding Homam-prepro-elevenin. This is the first report of an elevenin from *H. americanus*.

3.2.14. FMRFamide-like peptide (FLP)—Two transcripts were identified as encoding putative FLP precursors (Table 4). Both transcripts encode identical 358 amino acid full-length proteins (Homam-prepro-FLP; Table 4 and Fig. 2J); this protein was identified in a previous study as an *H. americanus* FLP precursor (Christie et al., 2015). Eighteen distinct peptides were predicted from Homam-prepro-FLP (Table 5 and Fig. 2J), nine of which are FLP isoforms. While all 18 of the peptides predicted from Homam-prepro-FLP have been reported previously from the lobster, this is the first identification of them from the *H. americanus* eyestalk ganglia.

3.2.15. Glycoprotein hormone

3.2.15.1. α-subunit 2 (GPα2): One transcript was identified as encoding a putative GPα2 precursor (Table 4). Translation of this transcript revealed a 120 amino acid full-length prehormone (Homam-pre-GPα2; Table 4 and Fig. 5B). A single 104 amino acid isoform of GPα2 was predicted from Homam-pre-GPα2 (Table 5 and Fig. 5B); this peptide shares extensive amino acid conservation with other members of this peptide family, *e.g.*, it is 69% identical/94% similar in sequence to the GPα2 predicted from the *N. lugens* precursor (**Accession No. BAO00955**; Tanaka et al., 2014) used to identify the transcript encoding it. Analysis of the *H. americanus* GPα2 by DiANNA suggests disulfide bridging between the peptide's first and tenth, second and eighth, third and fifth, fourth and ninth, and sixth and

seventh cysteine residues; homology to known GPα2 isoforms (*e.g.*, Sudo et al., 2005) suggest that peptide is also glycosylated, potentially at its position 34 asparagine. This is the first identification of a GPα2 from *H. americanus*.

3.2.15.2. β-subunit 5 (GPβ5): Two transcripts were identified as encoding putative GPβ5 precursors (Table 4). Translation of these transcripts revealed each to encode an identical 163 amino acid full-length prehormone (Homam-pre-GPβ5; Table 4 and Fig. 5C). A single 125 amino acid isoform of GPβ5 was predicted from Homam-pre-GPβ5 (Table 5 and Fig. 5C), this GPβ5 is 54% identical/81% similar in sequence to the GPβ5 predicted from the *N. lugens* precursor (**Accession No. BAO00956**; Tanaka et al., 2014) used to identify the transcript encoding it. Analysis of this GPβ5 isoform by DiANNA suggests disulfide bridging between its first and tenth, second and fifth, third and fourth, sixth and eighth, and seventh and ninth cysteines. This is the first identification of a GPβ5 from *H. americanus*.

3.2.16. GSEFLamide—Three transcripts were identified as encoding putative GSEFLamide precursors (Table 4). Two of the transcripts encode N-terminal partial preprohormones of 268 and 278 amino acids, while the third transcript encodes a 35 amino acid C-terminal partial protein, which is identical to the query sequence (Christie et al., 2015) used to identify the transcripts encoding these partial proteins. Two full-length preprohormones can be generated by combining each of the N-terminal fragments with the C-terminal partial sequence. As these two proteins differ from one another only by the presence/absence of a 10 amino acid insertion, they are likely splice variants of a common gene. Given this hypothesis, they have been named here Homam-prepro-GSEFLamide sv1 (Fig. 3D; the shorter precursor), and Homam-prepro-GSEFLamide sv2 (Fig. 3D, the longer precursor). Twelve distinct peptides were predicted from these two proteins (Table 5 and Fig. 3D), six of which are isoforms of GSEFLamide. Two of the GSEFLamides, AMGSEFLamide AVGSEFLamide, and one of the linker/precursor-related peptides, QYEPEFAHTLDYDT, while known lobster peptides (Christie et al., 2015), are new discoveries for the eyestalk ganglia. The remaining nine peptides are described here for *H. americanus* for the first time.

3.2.17. Intocin—Three transcripts were identified as encoding putative intocin precursors (Table 4). All three transcripts encode a common 154 amino acid protein (Table 4 and Fig. 2K) that is identical in sequence to a previously described *H. americanus* intocin precursor (Christie et al., 2015). Six distinct peptides were predicted from Homam-intocin (Table 5 and Fig. 2K): CFITNCPPGamide (disulfide bridging between its two cysteine residues) and five linker/precursor-related sequences. All six of the peptides predicted from Homam-intocin are known *H. americanus* peptides (Christie et al., 2015). However, their descriptions here are the first identifications of them from the lobster eyestalk ganglia.

3.2.18. Leucokinin—Two transcripts were identified as encoding putative leucokinin precursors (Table 4). Translation of one transcript revealed a 606 amino acid full-length preprohormone (Table 4 and Fig. 2L1), while translation of the other revealed a 130 amino acid C-terminal partial protein (Table 4 and Fig. 2L2). The N-terminus of the full-length preprohormone (named here Homam-prepro-leucokinin v2) is identical in sequence, save a

single substituted residue, to that of a previously identified *H. americanus* N-terminal partial leucokinin precursor (Christie et al., 2015; referred to here prepro-leucokinin v1). The C-terminal partial protein discovered here differs from the C-terminus of Homam-prepro-leucokinin v2 at three positions, and has been named Homam-prepro-leucokinin v3. Thirty-two distinct peptides were predicted from the combination of Homam-prepro-leucokinin v2 and v3 (Table 5 and Fig. 2L1–2), 13 of which are isoforms of leucokinin. Of this collective set of peptides, all are new discoveries for the lobster eyestalk ganglia, with 10 being described here for *H. americanus* for the first time.

3.2.19. Myosuppressin—One transcript was identified as encoding putative myosuppressin precursors (Table 4). Translation of this transcript revealed a 100 amino acid preprohormone (Homam-prepro-myosuppressin; Table 4 and Fig. 2M); this protein is identical in sequence to a previously described *H. americanus* myosuppressin precursor (**Accession No. ACX46385**; Stevens et al., 2009), the query sequence used to identify the transcript encoding it. Four peptides were predicted from Homam-prepro-myosuppressin (Table 5 and Fig. 2M): the myosuppressin isoform pQDLDHVFLRFamide and three distinct linker/precursor-related sequences. While all four of the peptides predicted from Homam-prepro-myosuppressin are known lobster peptides (*e.g.*, Christie et al., 2015; Ma et al., 2008), and pQDLDHVFLRFamide was identified from the lobster eyestalk ganglia via mass spectrometry in a previous study (Ma et al., 2008), this is the first identification of the three linker/precursor-related sequences from the eyestalk ganglia.

3.2.20. Neuroparsin—Five transcripts were identified as encoding putative neuroparsin precursors (Table 4). Translation of one transcript revealed a 98 amino acid full-length prehormone (Homam-pre-neuroparsin I; Table 4 and Fig. 2N1); this protein is identical in sequence to a known *H. americanus* pre-neuroparsin (Christie et al., 2015), which was the query protein used for the identification of neuroparsin-encoding transcripts reported here. A single 74 amino acid neuroparsin isoform was predicted from Homam-pre-neuroparsin I (Table 5 and Fig. 2N1). Analysis of this neuroparsin isoform by DiANNA suggests that disulfide bridges are present between its first and seventh, second and third, fourth and tenth, fifth and eleventh, sixth and eighth, and ninth and twelfth cysteine residues (Table 5). While this is a previously predicted lobster peptide (Christie et al., 2015), this is the first description of the neuroparsin derived from Homam-pre-neuroparsin I from the *H. americanus* eyestalk ganglia.

Translation of another transcript also revealed a 98 amino acid full-length prehormone (Table 4 and Fig. 2N2); this protein is distinct in amino acid sequence from Homam-pre-neuroparsin I, and thus was named Homam-pre-neuroparsin II. A 76 amino acid isoform of neuroparsin was predicted from this prehormone (Table 5 and Fig. 2N2), with disulfide bridges predicted between its first and eleventh, second and seventh, third and fourth, fifth and twelfth, sixth and eighth, and ninth and tenth cysteines by DiANNA (Table 5); this is the first description of this peptide from *H. americanus*.

The third neuroparsin transcript encodes a 101 amino acid full-length prehormone (Homam-pre-neuroparsin III; Table 4 and Fig. 2N3). Like Homam-pre-neuroparsin II, a 76 amino acid isoform of neuroparsin was predicted from Homam-pre-neuroparsin III (Table 5 and Fig.

2N3); this peptide was predicted by DiANNA to possess disulfide bridges between its first and twelfth, second and tenth, third and sixth, fourth and fifth, seventh and ninth, and eighth and eleventh cysteines (Table 5). It is a novel *H. americanus* neuroparsin isoform.

The remaining two transcripts encode identical 103 amino acid full-length prehormones (Homam-pre-neuroparsin IV; Table 4 and Fig. 2N4). Like the neuroparsin precursors described earlier, a single neuroparsin isoform was predicted from Homam-pre-neuroparsin IV (Table 5 and Fig. 2N4). This peptide, which is 77 amino acids long, was predicted by DiANNA to possess disulfide bonding between its first and fourth, second and eighth, third and sixth, fifth and seventh, ninth and twelfth, and tenth and eleventh cysteines (Table 5). The neuroparsin predicted from Homam-pre-neuroparsin IV is a new discovery for *H. americanus*.

3.2.21. Neuropeptide F (NPF)—Three transcripts were identified as encoding putative NPF precursors (Table 4). Translation of one transcript revealed a 132 amino acid full-length preprohormone (Homam-prepro-NPF I sv2; Table 4 and Fig. 2O). With the exception of a 28 amino acid N-terminal extension, Homam-prepro-NPF I sv2 is identical in sequence to a known *H. americanus* NPF precursor (Christie et al., 2015), and the two are likely splice variants of a common gene (alignment not shown). For ease of discussion, the precursor described in Christie et al. (2015) has been renamed here Homam-prepro-NPF I sv1; Homam-prepro-NPF I sv1 was the protein query used for the discovery of the NPF-encoding transcripts discovered in this study. Two peptides were predicted from Homam-prepro-NPF I sv2 (Table 5 and Fig. 2O): the NPF isoform ARPDNSAADTLQAIHEAAMAGILGSAEVQYPNRPSMFKSPVELRQYLDALNAYYAI AGRPRFamide and a linker/precursor related sequence, both of which, while previously identified from *H. americanus* (Christie et al., 2015) are new discoveries for the lobster eyestalk ganglia.

Translation of the remaining two transcripts revealed full-length proteins of 79 and 116 amino acids, which appear to be splice variants of a second NPF gene, and have been named here Homam-prepro-NPF II sv1 (the shorter protein; Table 4 and Fig. 3E) and Homam-prepro-NPF II sv2 (the longer protein; Table 4 and Fig. 3E). Two peptides were predicted from each preprohormone (Table 5 and Fig. 3E): an isoform of NPF and a linker/precursor-related sequence. While the linker/precursor-related peptide is shared by the two splice variants, the NPF isoform derived from Homam-prepro-NPF II sv2 possesses a 37 amino acid insertion relative to the NPF predicted from Homam-prepro-NPF II sv1, *i.e.*, KPDPNQLAAMADALKYLQELDKYYSQVSRPSLRSSPGPASQIQALEKALKFLQLQE LGKMYSLRARPRFamide vs. KPDPNQLAAMADALKYLQELDKYYSQVSRPRFamide. All of the peptides predicted from Homam-prepro-NPF II sv1 and sv2 are new discoveries for *H. americanus*.

3.2.22. Orcokinin—Four transcripts were identified as encoding putative orcokinin precursors (Table 4). Translation of one transcript revealed a 156 amino acid N-terminal partial protein, with a second transcript encoding a portion (152 amino acids) of the same sequence. With the exception of a 30 amino acid N-terminal extension, this partial protein is identical in amino acid sequence to the N-terminus of a known *H. americanus* orcokinin

precursor, namely Homam-prepro-orcokinin II (**Accession No. ACD13197**; Dickinson et al., 2009a), and likely represents a splice variant of this gene; it was named here Homam-prepro-orcokinin II sv2 (Table 4 and Fig. 2P1). The remaining two transcripts encode C-terminal partial proteins of 118 and 96 amino acids, which also differ from one another at a single residue. The longer C-terminal partial preprohormone is identical in amino acid sequence to the C-termini of two known *H. americanus* orcokinin precursors, namely Homam-prepro-orcokinin I (**Accession No. ACB41787**; Dickinson et al., 2009a) and II (see above), and is missing the portion of the protein that allows for differentiating between the two sequences; this partial protein is referred to here as Homam-prepro-orcokinin I/II in Table 4 and Figure 2P2. The other C-terminal partial protein appears to represent the C-terminus of a novel orcokinin precursor and has been named Homam-prepro-orcokinin IV (Table 4 and Fig. 2P3). It should be noted that a protein named Homam-prepro-orcokinin III currently exists in GenBank (**Accession No. ACD13198**; Dickinson et al., 2009a); although the nucleotide sequence from which Homam-prepro-orcokinin III was deduced is distinct from that encoding Homam-prepro-orcokinin II, (**Accession No. ACD13197**; Dickinson et al., 2009a), the proteins encoded by these two nucleotide sequences are identical. Nine distinct peptides were predicted from the collective set of orcokinin precursors derived from the eyestalk ganglia (Table 5 and Fig. 2P1–3): three orcokinins, NFDEIDRSGFGFH, NFDEIDRSGFGFN and NFDEIDRSGFGFV, the orcomyotropin FDAFTTGFGHN, and five linker/precursor-related sequences, all of which have been previously described from *H. americanus* (*e.g.*, Dickinson et al., 2009a; Ma et al., 2008). With the exception of the linker/precursor-related peptides GPIKAAPARSSPQQDAAAGYTDGAPV and SAE, all have also been reported from the lobster eyestalk system (*e.g.*, Dickinson et al., 2009a; Ma et al., 2008).

3.2.23. Pigment dispersing hormone (PDH)—Five transcripts were identified as encoding putative PDH precursors (Table 4). Translation of one transcript revealed a 79 amino acid full-length preprohormone (Homam-prepro-PDH I; Table 4 and Fig. 2Q1). This protein is identical in sequence to a known *H. americanus* PDH precursor (Christie et al., 2015), which was the query sequence used for the discovery of the PDH-encoding transcripts reported here (alignment not shown). Two peptides were predicted from Homam-prepro-PDH I (Table 5 and Fig. 2Q1): a linker/precursor-related sequence and the PDH isoform NSELINSILGLPKVMNDAamide. Both of the peptides predicted from Homam-prepro-PDH I have been described from the lobster previously (*e.g.*, Christie et al., 2015; Ma et al., 2008), with NSELINSILGLPKVMNDAamide identified from the eyestalk system via mass spectrometry (*e.g.,* Ma et al., 2008).

The remaining four transcripts encode identical 79 amino acid full-length proteins (Homam-prepro-PDH II; Table 4 and Fig. 2Q2); Homam-prepro-PDH II, while similar to Homam-prepro-PDH I in amino acid sequence (alignment not shown), is distinct from it, and likely represents the product of a different gene. Two peptides were predicted from Homam-prepro-PDH II (Table 5 and Fig. 2Q2): a linker/precursor-related sequence and the PDH isoform NSELINSLLGIPKVMNDAamide. Both peptides are distinct in structure from their counterparts derived from Homam-prepro-PDH I, and are new discoveries for *H. americanus*.

3.2.24. Proctolin—Two transcripts were identified as encoding putative proctolin precursors (Table 4). Translation of these transcripts revealed that they encode identical 88 amino acid full-length preprohormones (Homam-prepro-proctolin; Table 4 and Fig. 2R), which are identical in sequence to the *H. americanus* prepro-proctolin that was used as the query sequence for the identification of the transcripts encoding them (Christie et al., 2015). Six distinct peptides, including RYLPT, *i.e.*, authentic proctolin, were predicted from Homam-prepro-proctolin (Table 5 and Fig. 2R). While all of the peptides derived Homam-prepro-proctolin were identified previously from *H. americanus* (*e.g.*, Christie et al., 2015; Ma et al., 2008), they are new discoveries from the lobster eyestalk ganglia.

3.2.25. Pyrokinin—One transcript was identified as encoding a putative pyrokinin precursor (Table 4). This transcript encodes a 385 amino acid full-length preprohormone (Homam-prepro-pyrokinin; Table 4 and Fig. 2S). The partial *H. americanus* pyrokinin precursor used as the query sequence in the search that identified the transcript encoding Homam-prepro-pyrokinin (Christie et al., 2015) is identical in sequence to the corresponding portion of the full-length protein (alignment not shown), and likely represents an internal fragment of it. Thirteen distinct peptides were predicted from Homam-prepro-pyrokinin, including 10 isoforms of pyrokinin and three linker/precursor-related sequences (Table 5 and Fig. 2S). Seven of the pyrokinins and all three linker/precursor-related peptides have been described previously from *H. americanus* (Christie et al., 2015), though all are new discoveries for the lobster eyestalk ganglia. The remaining three pyrokinins, ADFAFSPRLamide, DSEDSSVESRNTKTQASIPRPamide and AYFSPRLamide, are new discoveries for *H. americanus*.

3.2.26. Red pigment concentrating hormone—One transcript was identified as encoding a putative RPCH precursor (Table 4), translation of which revealed a 98 amino acid full-length preprohormone (Homam-prepro-RPCH; Table 4 and Fig. 2T). Three distinct peptides were predicted from Homam-prepro-RPCH (Table 5 and Fig. 2T): pQLNFSPGWamide, authentic RPCH, and two linker/precursor-related sequences. While RPCH has been identified previously from the lobster eyestalk system (*e.g.*, Ma et al., 2008), the two linker/precursor-related peptides predicted from Homam-prepro-RPCH are new discoveries for *H. americanus*.

3.2.27. RYamide—One transcript was identified as encoding a putative RYamide precursor (Table 4). Translation of this transcript revealed a 135 amino acid full-length preprohormone (Homam-prepro-RYamide; Table 4 and Fig. 2U). Six distinct peptides were predicted from Homam-prepro-RYamide (Table 5 and Fig. 2U), two of which, pQGFYTQRYamide and FIGGSRYamide, possess −RYamide C-termini, the hallmark of the RYamide family (Christie et al., 2010a). All of the peptides derived from Homam-prepro-RYamide are new discoveries for *H. americanus*.

3.2.28. Short neuropeptide F (sNPF)—Four transcripts were identified as encoding putative sNPF precursors (Table 4). Two of these transcripts encode identical 96 amino acid full-length preprohormones, with the remaining pair encoding identical 128 amino acid full-length precursor proteins. As the two proteins are identical in sequence except for a 32

amino acid insertion/deletion, they likely represent splice variants of a common gene, and hence have been named Homam-prepro-sNPF sv1 (the shorter variant; Table 4 and Fig. 3F) and Homam-prepro-sNPF sv2 (the longer precursor; Table 4 and Fig. 3F). Four distinct peptides were predicted from Homam-prepro-sNPF sv1 (Table 5 and Fig. 3F), two of which possess the structural hallmarks of the sNPF family, *i.e.*, the C-terminal motif −RLRFamide and an overall length of approximately 10 amino acids (Christie et al., 2010a). Six peptides were predicted from Homam-prepro-sNPF v2 (Table 5 and Fig. 3F), three of which, the sNPFs GPPSLRLRFamide and DMGWQVAQRSMPSLRLRFamide and the linker/precursor-related peptide VPAPQDY$_{(SO3H)}$DAVNEVYDWLVDHGLE, are shared with Homam-prepro-sNPF sv1. The three remaining peptides derived from Homam-prepro-sNPF v2 include the sNPF isoform DTSTPALRLRFamide and two distinct linker/precursor-related sequences. Two of the sNPFs, GPPSLRLRFamide and DTSTPALRLRFamide, while known lobster peptides (Ma et al., 2008), are new discoveries for the eyestalk ganglia. The remaining sNPF and all of the linker/precursor-related peptides are new discoveries for *H. americanus* in a general sense.

3.2.29. SIFamide—Two transcripts were identified as encoding putative SIFamide precursors (Table 4). Translation of one transcript revealed an 80 amino acid full-length preprohormone, while translation of the other sequence revealed a 76 amino acid full-length precursor protein (Table 4 and Fig. 3G). The longer preprohormone is identical in sequence to a known SIFamide precursor (**Accession No. ABV21807**; Dickinson et al., 2008), while the shorter protein differs from it by a four amino acid deletion and one substituted residue (Fig. 3G); both are considered here to be splice variants of a common gene. For ease of discussion, the longer protein has been named Homam-prepro-SIFamide sv1 and the shorter, Homam-prepro-SIFamide sv2. Two peptides were predicted from each of the two *Homarus* SIFamide precursors (Table 5 and Fig. 3G); both preprohormones share the SIFamide isoform VYRKPPFNGSIFamide, a known peptide from the lobster eyestalk ganglia (*e.g.*, Ma et al., 2008). However, they possess distinct linker/precursor-related peptides, *i.e.*, AGADPREYTVFEPGKGLASVCQVAVEACAAWFPVQE, a known *H. americanus* peptide (Dickinson et al., 2008), which is described here for the first time from the eyestalk ganglia, and AGADPLFEPGKGLASVCQVAVEACAAWFPVQE, which has not previously been identified in the lobster.

3.2.30. Sulfakinin—One transcript was identified as encoding a putative sulfakinin precursor (Table 4). Translation of this transcript revealed a 120 amino acid full-length precursor protein (Homam-prepro-sulfakinin; Table 4 and Fig. 1B); Homam-prepro-sulfakinin is identical in sequence to a known lobster sulfakinin precursor (**Accession No. ABQ95346**; Dickinson et al., 2007). Five distinct peptides were predicted from Homam-prepro-sulfakinin (Table 5 and Fig. 1B), two of which, pEFDEY$_{(SO3H)}$GHMRFamide and GGGEY$_{(SO3H)}$DDY$_{(SO3H)}$GHLRFamide, are isoforms of sulfakinin. While all of the peptides predicted from Homam-prepro-sulfakinin have been described previously from the lobster (Dickinson et al., 2007), they are new discoveries for the eyestalk ganglia.

3.2.31. Tachykinin-related peptide (TRP)—One transcript was identified as encoding a putative TRP precursor (Table 4). Translation of this transcript revealed a 229 amino acid

full-length preprohormone (Table 4). With the exception of a missing 20 amino acid N-terminal extension in its signal peptide, this protein is identical in amino acid sequence to a known lobster TRP precursor (**Accession No. ACB41786**; Christie et al., 2008a), and the two precursors are likely splice variants of a common gene. For ease of later discussion, the previously known preprohormone has been termed Homam-prepro-TRP sv1, while the precursor predicted here has been termed Homam-prepro-TRP sv2 (Fig. 2V). Seven distinct peptides were predicted from Homam-prepro-TRP sv2 (Table 5 and Fig. 2V), two of which, APSGFLGMRamide and TPSGFLGMRamide, are isoforms of TRP. The TRP isoform APSGFLGMRamide has been identified previously from the eyestalk ganglia of *H. americanus* (Ma et al., 2008); the remaining peptides derived from Homam-prepro-TRP sv2, while known from the lobster (Christie et al., 2008a), are new discoveries from the eyestalk system.

3.2.32. Trissin—Two transcripts were identified as encoding putative trissin precursors (Table 4). Translation of these transcripts revealed each to encode a full-length preprohormone, with one protein being 187 amino acids in length, and the other 186 amino acids long. The two precursors are identical in amino acid sequences except that the shorter protein is missing the position 61 serine of the longer sequence. The two proteins have been named Homam-prepro-trissin v1 (Table 4 and Fig. 5D; the longer sequence) and Homam-prepro-trissin v2 (Table 4 and Fig. 5D; the shorter sequence). Three peptides were predicted from each trissin precursor (Table 5 and Fig. 5D), two of which, the putative trissin isoform WSSSEVSCTSCGSECQSACGTRNFRACCFNFQ (disulfide bridges predicted by DiANNA between the first and sixth, second and fourth, and third and fifth cysteine residues) and the linker/precursor-related peptide PSPSLNQLQHQNLHQRYTPSPTSIKI, are present in both preprohormones. The third peptide derived from Homam-prepro-trissin v1 and v2 is a linker/precursor-related sequence; the peptides derived from Homam-prepro-trissin v1 and v2 differ from one another in the presence/absence of the serine residue discussed earlier. The *H. americanus* trissin isoform is approximately 50% identical/75% similar in amino acid composition to the trissin derived from the *Drosophila melanogaster* precursor (**Accession No. AAF55203**; Adams et al., 2000) used to identify the transcripts encoding the two lobster trissin preprohormones. To the best of our knowledge, this is the first report of trissin (and trissin linker/precursor-related peptides) from *H. americanus*.

4. Discussion

4.1. Development of new molecular resources for investigations of peptidergic signaling in *Homarus americanus*

In the study presented here, high-throughput nucleotide sequencing was conducted on the eyestalk ganglia of the lobster *H. americanus* using the Illumina NextSeq platform. This sequencing resulted in the generation of approximately 131,000,000 high-quality reads, which were *de novo* assembled into a transcriptome consisting of ~150,000 unique contigs. While other nucleotide data sets have been generated for *H. americanus* (*e.g.*, McGrath et al., 2016; Stepanyan et al., 2006; Towle and Smith, 2006), including neural-specific ones (*e.g.*, **BioProject No. PRJNA300643**; D. Schulz and E. Marder, unpublished direct GenBank submission), the transcriptome generated here is by far the largest currently extant

for this species, and is the only one that includes the eyestalk ganglia, the location of X organ-sinus gland complex, a major neuroendocrine center in this commercially and biomedically important decapod (*e.g.*, Christie, 2011). The public deposition of this eyestalk transcriptome, as well as the raw reads from which it was generated, should provide a powerful resource for investigations of neural control in the lobster, including, but certainly not limited to, the peptidergic modulation of physiology and behavior that was the impetus for this study.

4.2. The peptidome of the lobster eyestalk ganglia contains members of most known crustacean peptide families

Via the mining of the eyestalk ganglia-specific transcriptome developed here, ~90 neuropeptide encoding transcripts were identified. Analyses of the proteins deduced from these transcripts allowed for the prediction of 262 distinct neuropeptides. This eyestalk peptidome includes isoforms of ACP, AST-A, AST-B, AST-C, bursicon α, CCHamide, corazonin, CCAP, CHH, CPRP, DH31, DH44, EH, elevenin, FLP, GPα2, GPβ5, GSEFLamide, intocin, leucokinin, MIH, myosuppressin, neuroparsin, NPF, orcokinin, orcomyotropin, PDH, proctolin, pyrokinin, RPCH, RYamide, sNPF, SIFamide, sulfakinin, TRP and trissin, as well as a large number of linker/precursor-related peptides. Of the identified peptides, 19 are reidentifications of known *H. americanus* eyestalk peptides, 165 are ones previously described from the lobster, but new discoveries from the eyestalk ganglia, and 78 are new to the lobster in a general sense (see Table 5). All members of the elevenin, GPα2, GPβ5, RYamide and trissin families are included in the latter grouping, being the first isoforms of these peptide families identified from *H. americanus*.

Although they were searched for, no transcripts encoding members of 13 other peptide families were found in the eyestalk transcriptome (see Table 4). Of these 13 families, isoforms of only two, bursicon β and insulin-like peptide (ILP), have been identified in the lobster (e.g., Christie et al., 2015). It is possible that our lack of detection of bursicon β- and ILP-encoding transcripts (as well as those for members of the other 11 families), is due to the eyestalk system not possessing members of these peptide families. Alternatively, our lack of detection of transcripts for some or all of these peptide groups could be a result of incomplete coverage of the transcriptome that was mined. As additional molecular data are generated for the *H. americanus* eyestalk system, it will be interesting to see if members of these peptide families can be identified from this portion of the nervous system or if these groups are truly absent from the eyestalk ganglia.

4.3. Local modulatory vs. hormonal roles for *Homarus americanus* eyestalk peptides

The eyestalks of lobsters are complex structures; in addition to their functions in vision, they are involved in the control of reproduction and are the site of the X-organ-sinus gland complex, a major neuroendocrine organ (*e.g.*, Christie, 2011). Because the eyestalk transcriptomes used in this study included all parts of the eyestalk ganglia (lamina ganglionaris, medulla externa, medulla interna and medulla terminalis), the roles played by the peptides we have identified are likely diverse. First, they could exert their effects within the eyestalk ganglia themselves, modulating the neuronal output of these ganglia and the interactions that take place within them. Future research examining the peptide receptors

located within the eyestalk ganglia transcriptome could help shed light on which neuropeptides are likely to exert such local modulatory effects.

In addition to local regulation, the neuropeptides identified here could serve as neurohormones, exerting their effects on other parts of the nervous system or on other tissues. Peptides synthesized within the X-organ are released from the sinus gland into the hemolymph, allowing them to exert effects throughout the body. The present study does not distinguish those peptides that are synthesized in the X-organ-sinus gland complex from those that are present in other parts of the eyestalk ganglia, and so cannot determine which peptides might be released from the sinus gland. However, previous mass spectral studies (*e.g.*, Fu et al., 2005; Ma et al., 2008) have shown that a number of peptides identified here (see peptides highlighted in yellow in Table 5), for example, the orcokinins, orcomyotropin and many of the orcokinin linker/precursor-related peptides, are present in the lobster sinus gland. As additional studies using mass spectrometry are conducted, it will be interesting to see what additional peptides discovered here are also present in the sinus gland of *H. americanus* and which are absent from this neuroendocrine organ; such studies will help clarify which peptides are local modulators, which are neurohormones, and which are likely to serve both roles.

4.4. Conclusions and future directions

This study has significantly expanded both the number of individual peptides and the number of families of peptides known to be present in the *H. americanus* eyestalk ganglia. The peptides identified here provide a rich resource for examining local control within the eyestalk ganglia as well as hormonal control of other tissues, including the highly modulated neuronal networks that control rhythmic movements in the lobster. These include the stomatogastric nervous system, which controls the foregut, and the cardiac ganglion, which controls the rhythmic contractions of the heart. Both of these neuronal networks are bathed in hemolymph, the cardiac ganglion due to its location within the lumen of the heart and the stomatogastric system due to the location of the stomatogastric ganglion within the ophthalmic artery. A number of the peptides identified here from the eyestalk ganglion (*e.g.*, RPCH) are known to modulate one or both of these networks in members of the Decapoda (*e.g.*, Cruz-Bermúdez and Marder, 2007; Thirumalai et al., 2006); the present study identifies others whose modulatory effects are presently unknown, but could be examined to increase our understanding of neurohormonal modulatory systems.

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Figure 1.

Two examples of the *in silico* workflow used for the prediction of putative mature *Homarus americanus* eyestalk ganglia peptide structures. (**A**) The predicted processing scheme for prepro-allatostatin C (AST-C) III. The structure of the putative mature AST-C isoform is shown in red, with the structures of three putative mature linker/precursor-related peptides shown in blue. In the AST-C isoform, the presence of a disulfide bridge between the two cysteine residues is indicated by an inverted red bracket. (**B**) The predicted processing scheme for prepro-sulfakinin. The structures of two putative mature sulfakinin isoforms are shown in red, with those of three putative mature linker/precursor-related peptides shown in blue. "$Y_{(SO3H)}$" indicates the presence of sulfated tyrosine residues in the putative mature sulfakinins. In one of the two sulfakinins, the presence of pyroglutamic acid is indicated by "pE".

A. Prepro-adipokinetic hormone-corazonin-like peptide

MVGWQVMLAVMCLALAPTLAQITFSRSWVPQGKRSGGITGPLVTPGGGSDRGADPCKDVRLATLTQVASHLADLMDDTFDLPQDDAALALRLKHGLV
ARRRRMS

B. Prepro-allatostatin A

MVGEHGGLGTCVLVVVVLLLTTTTTTAHDYLEDLDDPDTSRLLDVLQYYDTEPSYLYDYGKRHSNYGFGLGKRTPGYAFGLGKREGLYSLGLDKRSD
LYSFGLGKKSGSYNFGLGKRSVGDLPEVSKVEDGASPRTKRDVSITEDTLEDKRAKYSFGIGKRESSKNKRSKLYGFGLGKRDSGEERREDDDMENR
TQQYSFGLGKQDPDMEIEKRPRNYAFGLGKRESDEDSDKRSQMYSFGLGKRDPDMDMDKRPRDYAFGLGKRASSDEDDEERYYAYEQGKRPTAYSFG
LGKRAFSETDDYDNVNDNDDGDDELELSDLEQYSDDLKRATSYGFGLGKRSDAPDSGFGRRSYDFGLGKRAGRYAFGLGKRTGPYAFGLGKRTGPYA
FGLGKRTGPYAFGLGKRTGPYAFGLGKRTGPYAFGLGKRVGPYAFGLGKKAGHYAFGLGKRAGPYAFGLGKRSGPYAFGLGKRADPYAFGLGKKAGQ
YSFGLGKRSGPYSFGLGKRSDSDSDQYTLGRRSGVYSFGLGKRAGPYSFGLGKREVSDDDHDEDEQDIGVEEEMSS

C1. Prepro-allatostatin C I (C-terminal partial protein)

+QQQQQQQQQQQQQQQQQQQQQGEEEVKRKRMFVPLSGLPGELPTIKRQIRYHQCYFNPISCFRRK

C2. Prepro-allatostatin C II

MLTRFVSVVAVVAVVALLGVSRVSAKALPDQDPQVYGQMPHMLDPAGNHLIDDDGSLDAVLINYLFAKQMVERLRNNADIKDLQRKRSYWKQCAFNA
VSCFGKRK

D. Pre-bursicon α v2 (N-terminal partial protein)

MGGLSWVLMVLGVATVVWSDECSLTPVIHILSYPGCVSKPIPSFACQGRCTSYVQVSGSKLWQTERSCMCCQESGEREASVVLNCPKVRKGEPTRRK
VSV+

E. Prepro-CCHamide I

MSRGMINVFLVVLGVVALSSQAWGSCSQFGHSCFGAHGKRDGDQYARQEPSPLYPEANQLPEFEQRQEDRLSVDEAVTDREIVANARNWLAVLSHRL
RQRTSPQSSPSAQSLGYFQ

F. Prepro-corazonin

MVKMYSQTLVMVVVVVVFAVTLAAAQTFQYSRGWTNGRKRSDPNVGVTELLADPPRRLSAHSHPHPPTHTLPKNIEERLRALEAGLNAVLKANSINF
SPGGDEEYYAEN

G. Prepro-crustacean cardioactive peptide

MTNMSWCGRVGILGVTTVLLLVLLAAHAQAGPVAKRDIGDLLEGKDKRPFCNAFTGCGKKRSDPSMEGLASSSELDALAKHVLAEAKLWEQLQSKME
MMRSYASRMENHPVYRRKRSTPHTQPRQHLTSTPQQKVETEKQ

H. Prepro-diuretic hormone 44

MVLFRAAMLGVMGLFPLAWCLSLGGGRADTSSLLSLPHPQELSQDDLQPFLSRQGNTDSAGAPSSVADYTGYDKSEVLRGLEDPTSSSAYRLQEALS
EAVAAAAAAAAEGAEGVRDGAAALSPTANEGVTLEDLVPYDPGYYLYPAFLNRGDEAMTGGSSGINSLRKVRNSNRSNRSNSSSGISGSNTSSNSNTN
NNSPDTISMAKRTWPNGFSRRRASGLSLSIDASMKVLREALYMEIIRKKQRQQMQRAQHNQKLLNSIGKRDVTRQLQQEGIQGVYQRGQRK

I1. Pre-eclosion hormone I

MVGSRKVVVSVLLVLSVMLMALLLLPSAAAAANKVSVCIKNCAQCKIMYHDHFKGGLCADLCVQSGGKFIPDCGRPQTLIPFFLQRLE

I2. Pre-eclosion hormone II

MSFKREVVVVVMTVVVLMTLATLSDAATFTSMCIRNCGQCKEMYGDYFHGQACAESCIMTQGISIPDCNNPATFNRFLKRFI

J. Prepro-FMRFamide-like peptide

MIVAAWVLLTTLTWCCQAHAAPVPPVVAALDPPTDALLPAQSQEDDLFALPEKRLLKYFLPASQAWGGDAYPIGQEGTKRGYSDRNYLRFGRSDDNS
KRSGRNFLRFGRSDTNDYEGEEMPESPEKRNRNFLRFGRDQNRNFLRFGRSGSPMEFATDLQEDVELPVEEKRGAHKNYLRFGRGNRNFLRFGRGDR
NFLRFGRSVDRQLSSLSCEDCDEEQKAREFTSTPSPTTIQPLARTKRDVSAVLSDDSIESSVLRQINAHRIKRAAAQNFYIPMAWASELQPEEDGID
VTSFEEPQVAKRFSHDRNFLRFGKRDGSDDYPSSSSSAESPAPVVVVRPVEYPRYVRAPSKNFLRFG

K. Prepro-intocin

MQLGVVVVVMTVVVGSTTACFITNCPPGGKRSGPTAQLGRTRTCTACGPGLQGRCLGPEICCVLGIGCFLGTREARMCHAENLVPVTCANRDLKSCG
RMQEGRCAAAGLCCTEMKCEFDSSCTVEGREERVGKQRAERQHLTFLSSLPEDQWNL

L1. Prepro-leucokinin v1

MVTVGRWVSLWVRLAVALSSGAASVSFVTSEVMDVSPLALPHGRHPNLCTPDHVPSHPIIRCEVGKRQAFHPWGGKRSSFKTAPGLPLSLREVYLAL
FQNARPPPPPSEGELKRASFNPWGGKRSDPLLPASQHEPNTKRNTFAPWGGKRAAGYFTHDTNPLIIEEDLIPYIGVLSDDGEAEDVVKRESFSAW
GGKRGSFPADDWEEEEPTDLFVLDGSLPYPPVDRLRYKREAETYTNTLVNSDDSGVKTEAENIKPDTKYDQTAEASSTTVAKRTRFSAWAGKRPDLQ
VIEDAVRKMAEHEPKTTERRAFSAWAGKRSSDVNLQDGEDDEPVSAWIGRRLQDANTDDKRTFSAWAGKRSPSMDLSGNQDKRTFRAWAGKRSSGDE
LDDHFLDKKTRFSPWAGKRAEGTLSRLSESTLKAALDENSPEDNVDNHKRPSFSAWAGKRSESNEKRPSFNAWAGKRSDSDEKRPSFSAWAGKRSDI
DEKRPSFNAWAGKRSDSDEKRPSFSAWAGKRSSETDKRQGFSAWAGKRNNGGSDDPTHSNNPQQISSILQQLQHQGLEFLHKRLPINDWGNKRVPFS
TWGGKRASPISEDSQLSDLYTSQL

L2. Prepro-leucokinin v2 (C-terminal partial protein)

+AWAGKRSESNEKRPSFNAWAGKRSDSDEKRPSFSAWAGKRSSETDKRQGFSAWAGKRNNGGSDDPTHSNNPQQISSILQQLQHQGLEFLHKRLPIN
DWGNKRVPFSTWGGKRASPISEDSQLSDLYTSQL

M. Prepro-myosuppressin

MVFRSCSWSCLLVVGVVVVMGVCVGVGETMPPPICLSQQVPLSPFAKKLCSALINISEFSRAMEEYLGAQAIERSMPVNEPEVKRQDLDHVFLRFGR
SQQ

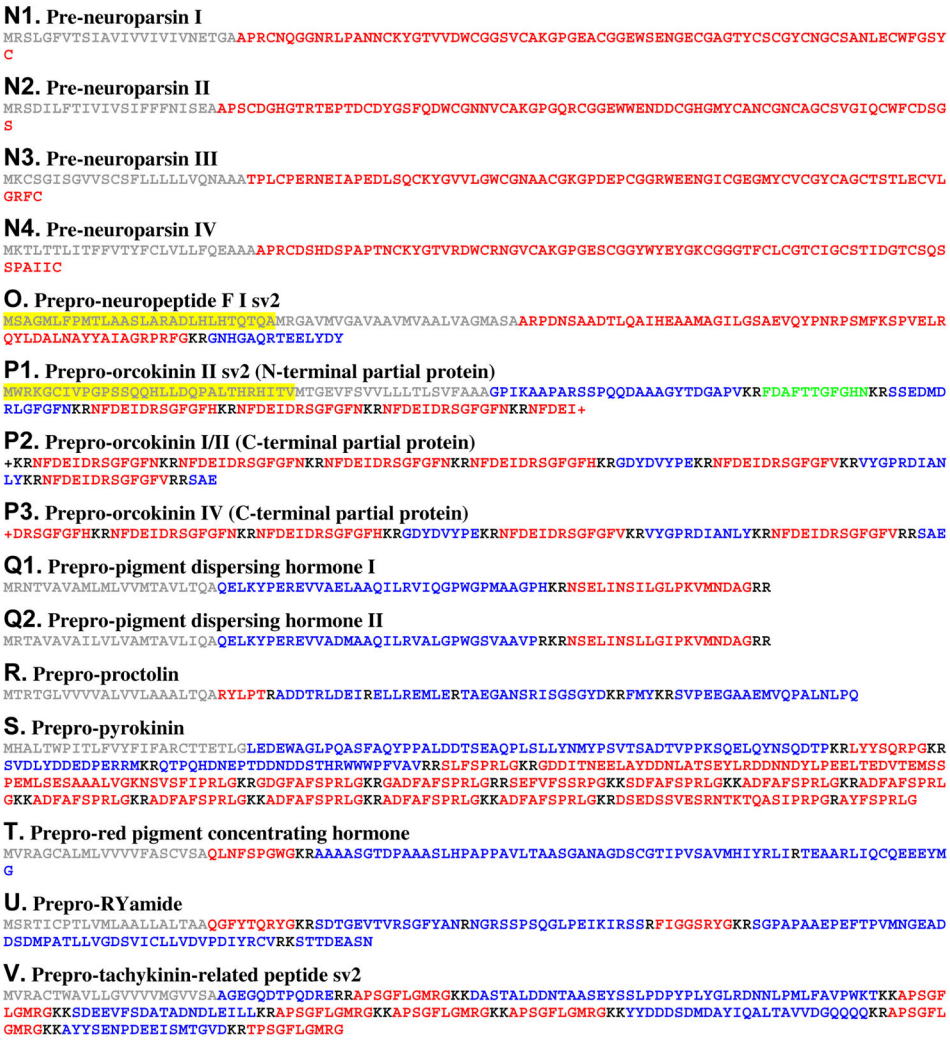
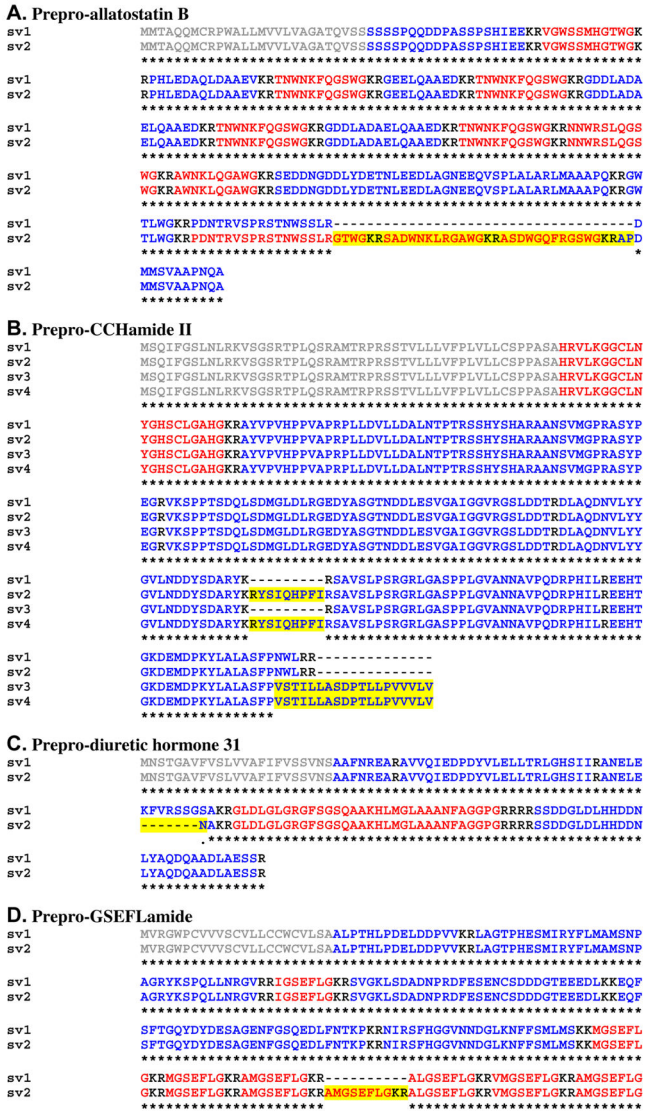


Figure 2.

Putative *Homarus americanus* pre/preprohormones deduced from transcriptome shotgun assembly sequence data. This figure does not include predicted preprohormones for which multiple putative splice variants were identified in the eyestalk ganglia transcriptome. (**A**) Prepro-adipokinetic hormone-corazonin-like peptide. (**B**) Prepro-allatostatin A. (**C1**) The carboxyl (C)-terminal portion of prepro-allatostatin C I. (**C2**) Prepro-allatostatin C II. (**D**) The amino (N)-terminal portion of pre-bursicon α variant (v) 2. (**E**). Prepro-CCHamide I. (**F**) Prepro-corazonin. (**G**) Prepro-crustacean cardioactive peptide. (**H**). Prepro-diuretic hormone 44. (**I1**) Pre-eclosion hormone I. (**I2**) Pre-eclosion hormone II. (**J**) Prepro-FMRFamide-like peptide. (**K**) Prepro-intocin. (**L1**) Prepro-leucokinin v1. (**L2**) The C-terminal portion of prepro-leucokinin v2. (**M**) Prepro-myosuppressin. (**N1**) Pre-neuroparsin I. (**N2**) Pre-neuroparsin II. (**N3**) Pre-neuroparsin III. (**N4**) Pre-neuroparsin IV. (**O**) Prepro-neuropeptide F splice variant (sv) 2. (**P1**) The N-terminal portion of prepro-orcokinin II sv2. (**P2**) The C-terminal portion of prepro-orcokinin I/II. (**P3**) The C-terminal portion of prepro-orcokinin IV. (**Q1**) Prepro-pigment dispersing hormone I. (**Q2**) Prepro-pigment dispersing hormone II. (**R**) Prepro-proctolin. (**S**) Prepro-pyrokinin. (**T**) Prepro-red pigment

concentrating hormone. (**U**) Prepro-RYamide. (**V**) Prepro-tachykinin-related peptide sv2. In this figure, signal peptides are shown in gray, while all mono/dibasic cleavage loci are shown in black. For each sequence, the isoform(s) of the peptide for which the precursor is named is/are shown in red, with all linker/precursor related peptides shown in blue. The "+" symbol indicate the presence of additional, unknown, amino acid residues at the N- and/or C-termini of the protein in question. It should be noted that there is an N-terminal extension prior to theorized start of the signal peptide in the neuropeptide F and orcokinin precursors shown in **O** and **P1** (highlighted in yellow in each panel). These extensions may be the result of an artifact in the process of assembling the transcript encoding each of these proteins, or may have true biological significance, *e.g.*, they may function as potential regulatory elements. In **P1**, an isoform of orcomyotropin has been colored green.



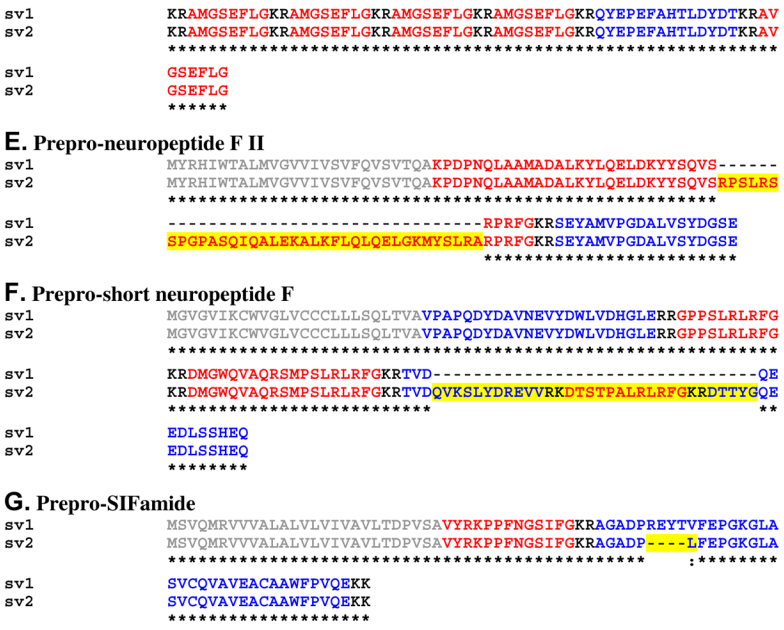


Figure 3.

Alignment of *Homarus americanus* preprohormones for which putative splice variants were identified from eyestalk ganglia transcriptome shotgun assembly data. (**A**) Prepro-allatostatin B. (**B**) Prepro-CCHamide II. (**C**) Prepro-diuretic hormone 31. (**D**) Prepro-GSEFLamide. (**E**) Prepro-neuropeptide F II. (**F**) Prepro-short neuropeptide F. (**G**) Prepro-SIFamide. In this figure, signal peptides are shown in gray, while all mono/dibasic cleavage loci are shown in black. For each sequence, the isoform(s) of the peptide for which the precursor is named is/are shown in red, with all linker/precursor related peptides shown in blue. Residues (or gaps) that vary from the first splice variant in each alignment are highlighted in yellow. In the line below each sequence grouping, amino acids that are identically conserved are indicated by “*”, while conservative amino acid substitutions are marked by “:” or “.”.

Figure 4.

Alignment of crustacean hyperglycemic hormone (CHH)/molt inhibiting hormone (MIH) precursors. (**A**) Alignment of prepro-CHH Type I variants. (**B**) Alignment of CHH Type I and Type II precursors. (**C**) Alignment of putative MIH prehormones. In this figure, signal peptides are shown in gray, while all mono/dibasic cleavage loci are shown in black. For each sequence, the isoform of CHH/MIH is shown in red, with isoforms of CHH precursor-related peptide shown in pink. Residues (or gaps) that vary between from the top protein in each alignment are highlighted in yellow. In the line below each sequence grouping, amino acids that are identically conserved are indicated by “*”, while conservative amino acid substitutions are marked by “:” or “.”.

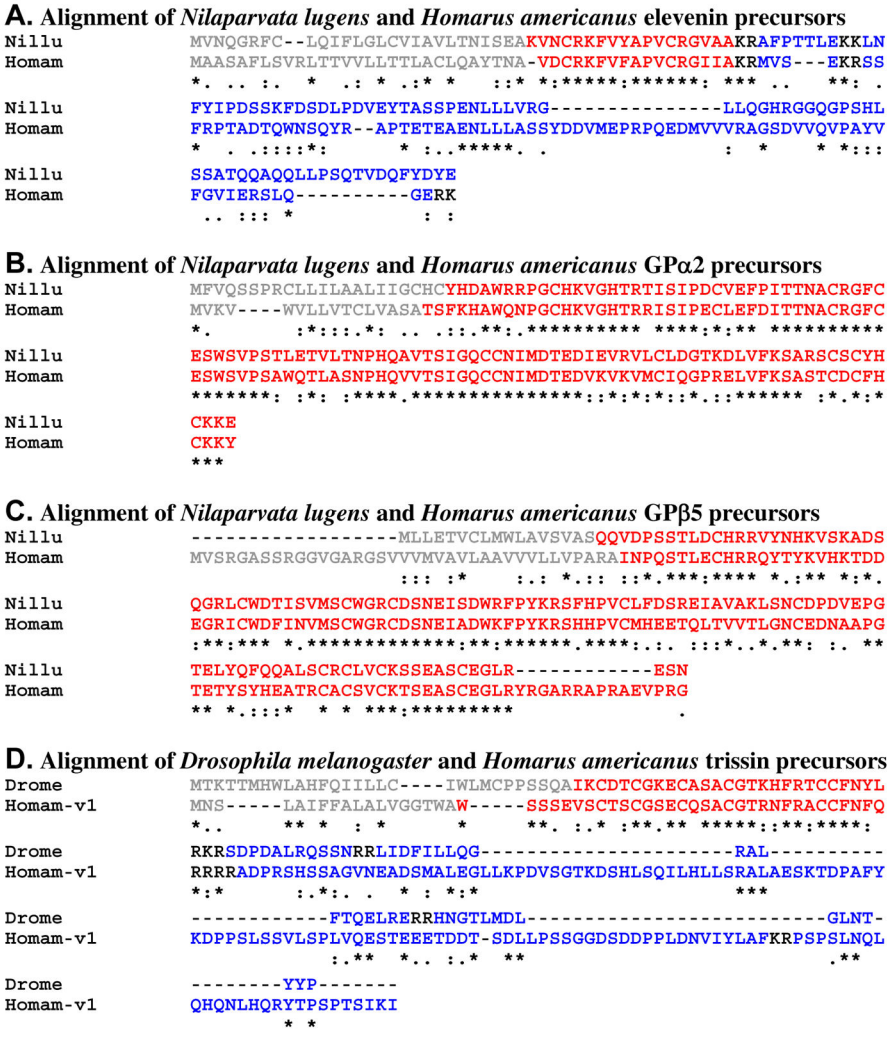


Figure 5.

Alignments of selected *Homarus americanus* precursor proteins and the insect queries used for their identifications. (**A**) Alignment of *Nilaparvata lugens* (Nillu) and *H. americanus* (Homam) prepro-elevenins. (**B**) Alignment of *N. lugens* and *H. americanus* glycoprotein hormone α2 (GPα2) precursors. (**C**) Alignment of *N. lugens* and *H. americanus* glycoprotein hormone β5 (GPβ5) precursors. (**D**) Alignment of *Drosophila melanogaster* (Drome) and *H. americanus* prepro-trissins (lobster variant 1 shown). In this figure, signal peptides are shown in gray, while all mono/dibasic cleavage loci are shown in black. For each sequence, the isoform(s) of the peptide for which the precursor is named is/are shown in red, with all linker/precursor related peptides shown in blue. In the line below each sequence grouping, amino acids that are identically conserved are indicated by "*", while conservative amino acid substitutions are marked by ":" or ".".

Table 1

Summary of *Homarus americanus* eyestalk ganglia samples and their Illumina sequencing

Sample	Sex	Total RNA concentration (ng/μl)	Raw reads
E1	M	223	26,829,856
E2	M	268	38,085,928
E3	F	316	32,879,458
E4	M	262	33,177,978

Table 2

Homarus americanus eyestalk ganglia transcriptome assembly statistics

Total number of bases assembled	183,106,109
Total number of reads assembled	130,973,220
Total number of assembled contigs	147,542
Total number of Trinity “genes”	110,841
Minimum contig length (bp)	324
Maximum contig length (bp)	27,389
Average contig length (bp)	1,241
Median contig length (bp)	637
N25 (bp)	4,193
N50 (bp)	2,160
N75 (bp)	869
Total GC count (bp)	74,888,256
GC content for the complete assembly (%)	40.90

Abbreviations: bp, base pairs; GC, guanine-cytosine.

Transcriptome assembly statistics were generated using Trinity software. Reads used for the de novo assembly were trimmed for Illumina adapters and quality filtered (Phred score=30)

Read length ranged from 324-27389 bp.

Table 3

Summary of the results of mapping RNA-Seq reads to the complete *Homarus americanus* eyestalk ganglia assembly

Total reads used for mapping	130,973,220
Total mapped reads *	119,120,860
Overall alignment (%)	91%
Reads mapped 1 time (#)	68,454,575
Reads mapped 1 time (%)	52%
Reads mapped >1 time (#)	50,666,285
Reads mapped >1 time (%)	39%

* 97% of the mapped reads aligned as clusters (read pairs)

Table 4

Putative *Homarus americanus* neuropeptide-encoding transcripts identified via *in silico* transcriptome mining of a *de novo* assembled transcriptome for the eyestalk ganglia (lamina ganglionaris, medulla externa, medulla interna and medulla terminalis) and the proteins deduced from them

Peptide family	Transcript/protein identifications					tblastn search statistics	
	Transcript		Deduced protein				
	Identification No.	Length*	Name	Length†	Type	Score	E-value
ACP	TR10790|c0_g1_i3	1033	Prepro-ACP	104	F	211	4e-55
	TR10790|c0_g1_i2	1077	Prepro-ACP	104	F	211	4e-55
	TR10790|c0_g1_i1	1685	Prepro-ACP	104	F	211	4e-55
AST-A	TR19564|c0_g1_i1	1395	Prepro-AST-A sv2	378	N	552	1e-156
	TR19564|c1_g1_i1	3284	Prepro-AST-A sv2	189	C	357	7e-98
AST-B	TR68637|c1_g2_i2	647	Prepro-AST-B	157	N	120	1e-27
	TR68637|c1_g1_i2	950	Prepro-AST-B sv1	153	C	69	3e-12
	TR68637|c1_g1_i1	1058	Prepro-AST-B sv2	189	C	69	3e-12
AST-C	TR39168|c1_g1_i1	1163	Prepro-AST-C I	65	C	97	1e-20
	TR48133|c0_g1_i1	1624	Prepro-AST-C II	105	F	174	4e-44
	TR20160|c0_g1_i1	1609	Prepro-AST-C III	148	F	29	3.4
Allatotropin							
Bursicon α	TR30087|c0_g1_i1	372	Pre-bursicon α v2	100	N	209	2e-54
Bursicon β							
CAP2b							
CCHamide	TR54993|c0_g1_i5	2717	Prepro-CCHamide I	116	F	237	6e-63
	TR54993|c0_g1_i4	3448	Prepro-CCHamide I	116	F	237	6e-63
	TR54993|c0_g1_i3	3452	Prepro-CCHamide I	116	F	237	6e-63
	TR54993|c0_g1_i2	3459	Prepro-CCHamide I	116	F	237	6e-63
	TR54993|c0_g1_i1	3476	Prepro-CCHamide I	116	F	237	6e-63
	TR75050|c0_g1_i1	3267	Prepro-CCHamide II sv1	252	F	470	1e-132
	TR75050|c0_g1_i3	3294	Prepro-CCHamide II sv2	261	F	462	1e-130
	TR75050|c0_g1_i2	1277	Prepro-CCHamide II sv3	266	F	458	1e-129

Peptide family	Transcript/protein identifications						tblastn search statistics	
	Transcript		Deduced protein					
	Identification No.	Length*	Name	Length†	Type	Score	E-value
	TR75050|c0_g1_i4	1304	Prepro-CCHamide II sv4	275	F	451	1e-126
Corazonin	TR17179|c1_g1_i6	1716	Prepro-corazonin	109	F	135	2e-32
	TR17179|c1_g1_i3	1716	Prepro-corazonin	109	F	135	2e-32
	TR17179|c1_g1_i2	1715	Prepro-corazonin	109	F	135	2e-32
	TR17179|c1_g1_i1	1715	Prepro-corazonin	109	F	135	2e-32
	TR17179|c1_g1_i4	631	Prepro-corazonin	22	C	49	3e-06
CCAP	TR69144|c0_g1_i2	1098	Prepro-CCAP	140	F	254	5e-68
	TR69144|c0_g1_i1	1113	Prepro-CCAP	140	F	254	5e-68
CHH (CHH subgroup)	TR1098|c3_g6_i2	649	Prepro-CHH Ia v1	134	F	260	6e-70
	TR1098|c3_g6_i3	497	Prepro-CHH Ia v2	133	F	243	7e-65
	TR1098|c3_g6_i4	589	Prepro-CHH Ia v3	133	F	241	3e-64
	TR1098|c3_g6_i1	747	Prepro-CHH Ib v2	131	F	217	7e-57
	TR25279|c0_g1_i1	673	Prepro-CHH II sv2	111	F	87	1e-17
CHH (MIH subgroup)	TR12133|c0_g1_i1	1451	Pre-MIH I v2	112	F	199	1e-51
	TR9706|c0_g1_i1	837	Pre-MIH II	111	F	87	1e-17
	TR58586|c0_g1_i1	1736	Pre-MIH III	119	F	48	7e-06
DENamide							
DH31	TR60801|c0_g1_i2	2393	Prepro-DH31 sv1	135	F	211	4e-55
	TR60801|c0_g1_i1	2371	Prepro-DH31 sv2	128	F	190	1e-48
DH44	TR48947|c0_g1_i2	1198	Prepro-DH44	285	F	416	1e-116
	TR48947|c0_g1_i1	1551	Prepro-DH44	285	F	416	1e-116
DXXRLamide							
ETH							
EH	TR8891|c0_g1_i1	555	Pre-EH I	88	F	120	1e-27
	TR33899|c2_g1_i1	2290	Pre-EH II	82	F	57	2e-08
Elevenin	TR73513|c0_g1_i1	884	Prepro-elevenin	127	F	39	0.003
FLP	TR10371|c0_g1_i2	3112	Prepro-FLP	358	F	562	1e-160

Peptide family	Transcript/protein identifications						tblastn search statistics	
	Transcript		Deduced protein					
	Identification No.	Length*	Name	Length†	Type	Score	E-value
	TR10371|c0_g1_i1	3101	Prepro-FLP	358	F	562	1e-160
FXGGXamide							
GPα2	TR7537|c0_g1_i1	2897	Pre-GPα2	120	F	175	2e-44
GPβ5	TR31071|c0_g1_i2	2321	Pre-GPβ5	163	F	170	8e-43
	TR31071|c0_g1_i1	2293	Pre-GPβ5	163	F	170	8e-43
GSEFLamide	TR71156|c0_g1_i2	966	Prepro-GSEFLamide sv1	268	N	39	0.003
	TR71156|c0_g1_i1	996	Prepro-GSEFLamide sv2	278	N	39	0.003
	TR33789|c3_g2_i1	1470	Prepro-GSEFLamide	35	C	75	3e-14
HIGSLYRamide							
ILP							
Intocin	TR17969|c0_g3_i1	1121	Prepro-intocin	154	F	309	2e-84
	TR17969|c0_g2_i1	1121	Prepro-intocin	154	F	309	2e-84
	TR17969|c0_g1_i1	1121	Prepro-intocin	154	F	309	2e-84
Leucokinin	TR63890|c0_g1_i1	2739	Prepro-leucokinin v1	606	F	920	0.0
	TR63890|c0_g1_i2	1230	Prepro-leucokinin v2	130	C	64	1e-09
Myosuppressin	TR50807|c0_g1_i1	1600	Prepro-myosuppressin	100	F	169	2e-42
Neuroparsin	TR33370|c0_g1_i1	726	Pre-neuroparsin I	98	F	207	5e-54
	TR49668|c0_g1_i1	1130	Pre-neuroparsin II	98	F	105	2e-23
	TR70851|c4_g1_i1	1490	Pre-neuroparsin III	101	F	101	5e-22
	TR43449|c0_g1_i2	1897	Pre-neuroparsin IV	103	F	100	2e-21
	TR43449|c0_g1_i1	878	Pre-neuroparsin IV	103	F	100	2e-21
NPF	TR52169|c0_g1_i1	1470	Prepro-NPF I sv2	132	F	161	4e-40
	TR47662|c0_g1_i1	1008	Prepro-NPF II sv1	79	F	34	0.081
	TR47662|c0_g1_i2	1119	Prepro-NPF II sv2	116	F	28	4.4
NPLP1							
NPLP2							
NPLP3							

Peptide family	Transcript/protein identifications					tblastn search statistics	
	Transcript		Deduced protein				
	Identification No.	Length*	Name	Length†	Type	Score	E-value
NPLP4							
Orcokinin	TR61279|c0_g2_i1	514	Prepro-orcokinin II sv2	156	N	225	8e-59
	TR61279|c0_g1_i1	504	Prepro-orcokinin II sv2	152	N	217	2e-56
	TR4139|c0_g1_i1	1307	Prepro-orcokinin I/II	118	C	251	1e-66
	TR4139|c0_g1_i2	1241	Prepro-orcokinin IV	96	C	203	3e-52
PDH	TR23970|c0_g1_i1	1058	Prepro-PDH I	79	F	122	2e-28
	TR9861|c0_g4_i1	481	Prepro-PDH II	79	F	101	4e-22
	TR9861|c0_g3_i1	481	Prepro-PDH II	79	F	101	4e-22
	TR9861|c0_g2_i1	481	Prepro-PDH II	79	F	101	4e-22
	TR9861|c0_g1_i1	714	Prepro-PDH II	79	F	101	4e-22
Proctolin	TR11540|c0_g1_i2	962	Prepro-proctolin	88	F	111	4e-25
	TR11540|c0_g1_i1	951	Prepro-proctolin	88	F	111	4e-25
PTTH							
Pyrokinin	TR21391|c0_g1_i1	1766	Prepro-pyrokinin	385	F	528	1e-150
RPCH	TR11499|c0_g1_i1	770	Prepro-RPCH	98	F	101	4e-22
RYamide	TR37059|c0_g1_i1	707	Prepro-RYamide	135	F	160	6e-40
sNPF	TR46457|c1_g1_i2	677	Prepro-sNPF sv1	96	F	137	9e-33
	TR46457|c1_g1_i1	663	Prepro-sNPF sv1	96	F	137	9e-33
	TR46457|c1_g1_i4	773	Prepro-sNPF sv2	128	F	182	1e-46
	TR46457|c1_g1_i3	759	Prepro-sNPF sv2	128	F	182	1e-46
SIFamide	TR65061|c0_g1_i1	1734	Prepro-SIFamide sv1	80	F	132	2e-31
	TR65061|c0_g1_i2	1302	Prepro-SIFamide sv2	76	F	117	7e-27
Sulfakinin	TR6787|c0_g1_i1	1023	Prepro-sulfakinin	120	F	196	1e-50
TRP	TR41108|c0_g1_i1	2211	Prepro-TRP	129	F	442	1e-124
Trissin	TR50290|c0_g2_i1	943	Prepro-trissin v1	187	F	58	7e-09
	TR50290|c0_g1_i1	940	Prepro-trissin v2	186	F	56	2e-08

* Length in nucleotides.

† Length in amino acids.

Peptide family abbreviations: ACP, adipokinetic-corazonin-like peptide; AST-A, allatostatin A; AST-B, allatostatin B; AST-C, allatostatin C; CAP2b, cardioacceleratory peptide 2b; CCAP, crustacean cardioactive peptide; CHH, crustacean hyperglycemic hormone; MIH, molt-inhibiting hormone; DH31, diuretic hormone 31; DH44, diuretic hormone 44; ETH, ecdysis-triggering hormone; EH, eclosion hormone; FLP, FMRFamide-like peptide; GPα2, glycoprotein hormone α2; GPβ5, glycoprotein hormone β5; ILP, insulin-like peptide; NPF, neuropeptide F; NPLP1, neuropeptide-like precursor 1; NPLP2, neuropeptide-like precursor 2; NPLP3, neuropeptide-like precursor 3; NPLP4, neuropeptide-like precursor 4; PDH, pigment dispersing hormone; PTTH, prothoracicotropic hormone; RPCH, red pigment concentrating hormone; sNPF, short neuropeptide F; TRP, tachykinin-related peptide.

Other abbreviations: C, carboxy-terminal partial protein; F, full-length protein; N, amino-terminal partial protein; sv, splice variant; v, variant.

Query proteins: ACP, *Homarus americanus* prepro-ACP (deduced from **Accession No. GEBG01018127**; Christie et al., 2015); AST-A, *H. americanus* prepro-AST-A (deduced from **Accession No. GEBG01007827**; Christie et al., 2015); AST-B, *Procambarus clarkii* prepro-allatostatin B (deduced from **Accession No. GBEV01040422**; Christie and Chi, 2015c); AST-C, *H. americanus* prepro-AST-C I (deduced from **Accession No. GEBG01004053**; Christie et al., 2015) and *H. americanus* prepro-AST-C II (deduced from **Accession No. EY291152**; Dickinson et al, 2009b); allatotropin, *Tigriopus californicus* prepro-allatotropin (deduced from **Accession No. JW513825**; Christie, 2014c); bursicon α, *H. americanus* pre-bursicon α (deduced from **Accession No. GEBG01013055**; Christie et al., 2015); bursicon β, *H. americanus* pre-bursicon β (deduced from **Accession No. CN854188**; Christie et al., 2010b); CAP2b, *Nilaparvata lugens* prepro-CAP2b splicing variant a (**Accession No. BAO00940**; Tanaka et al., 2014); CCHamide, *H. americanus* prepro-CCHamide I (deduced from **Accession No. GEBG01016648**; Christie et al., 2015) and *H. americanus* prepro-CCHamide II (deduced from **Accession No. GEBG01015625**; Christie et al., 2015); corazonin, *H. americanus* prepro-corazonin (deduced from **Accession No. GEBG01047508**; Christie et al., 2015); CCAP, *H. americanus* prepro-CCAP (deduced from **Accession No. GEBG01001997**; Christie et al., 2015); CHH, *H. americanus* prepro-CHH A (**Accession No. P19806**; de Kleijn et al., 1995); MIH, H. americanus pre-gonad-inhibiting hormone (**Accession No. CAA60644**; de Kleijn et al., 1994); DENamide, *Daphnia pulex* prepro-DENamide (Dircksen et al., 2011); DH31, *H. americanus* prepro-CLDH (**Accession No. ACX46386**; Christie et al., 2010c); DH44, *H. americanus* prepro-DH44 (deduced from **Accession No. GEBG01010013**; Christie et al., 2015); DXXRLamide, *T. californicus* prepro-DXXRLamide Ia (deduced from **Accession No. JW528324**; Christie, 2014c); ETH, *D. pulex* prepro-ETH (Dircksen et al., 2011); EH, pre-EH I (deduced from **Accession No. GEBG01042722**; Christie et al., 2015); elevenin, *N. lugens* prepro-elevenin (**Accession No. BAO00952**; Tanaka et al., 2014); FLP, *H. americanus* prepro-FLRFamide (deduced from **Accession No. GEBG01004307**; Christie et al., 2015); FXGGXamide, *T. californicus* prepro-FXGGXamide Ia (deduced from **Accession No. JV193177**; Christie, 2014c); GPα2, *N. lugens* pre-glycoprotein hormone α2 (**Accession No. BAO00955**; Tanaka et al., 2014); GPβ5, *N. lugens* pre-glycoprotein hormone β5 (**Accession No. BAO00956**; Tanaka et al., 2014); GSEFLamide, *H. americanus* prepro-GSEFLamide (deduced from **Accession No. GEBG01035690**; Christie et al., 2015); HIGSLYRamide, *Carcinus maenas* prepro-HIGSLYRamide (deduced from **Accession No. DV111329**; Christie et al., 2008b); ILP, *H. americanus* prepro-ILP (deduced from **Accession No. GEBG01059205**; Christie et al., 2015); intocin, *H. americanus* prepro-intocin (deduced from **Accession No. GEBG01052869**; Christie et al., 2015); leucokinin, H. americanus prepro-leucokinin (deduced from **Accession No. GEBG01042414**; Christie et al., 2015); myosuppressin, *H. americanus* prepro-myosuppressin (**Accession No. ACX46385**; Stevens et al., 2009); neuroparsin, *H. americanus* pre-neuroparsin (deduced from **Accession No. GEBG01017677**; Christie et al., 2015); NPF, *H. americanus* prepro-NPF (deduced from **Accession No. GEBG01010211**; Christie et al., 2015); NPLP1, *N. lugens* prepro-NPLP1 (**Accession No. BAO00966**; Tanaka et al., 2014); NPLP2, *Drosophila melanogaster* prepro-NPLP2, isoform A (**Accession No. AAF49832**; Adams et al., 2000); NPLP3, *N. lugens* prepro-NPLP3 (**Accession No. BAO00967**; Tanaka et al., 2014); NPLP4, *N. lugens* prepro-NPLP4 (**Accession No. BAO00968**; Tanaka et al., 2014); orcokinin, *H. americanus* prepro-orcokinin I (**Accession No. ACB41787**; Dickinson et al., 2009a); PDH, *H. americanus* prepro-PDH (deduced from **Accession No. GEBG01005888**; Christie et al., 2015); proctolin, *H. americanus* prepro-proctolin (deduced from **Accession No. GEBG01005712**; Christie et al., 2015); PTTH, *N. lugens* prepro-PTTH (**Accession No. BAO00973**; Tanaka et al., 2014); pyrokinin, *H. americanus* prepro-pyrokinin (deduced from **Accession No. GEBG01039267**; Christie et al., 2015); RPCH, *Cherax quadricarinatus* prepro-RPCH (**Accession No. AAV80404**; Martinez-Perez et al., 2007); RYamide, *P. clarkii* prepro-RYamide (deduced from **Accession No. GBEV01010112**; Christie and Chi, 2015c); sNPF, *P. clarkii* prepro-sNPF (deduced from **Accession No. GBEV01004780**; Christie and Chi, 2015c); SIFamide, *H. americanus* prepro-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); trissin, D. melanogaster prepro-trissin (**Accession No. AAF55203**; Adams et al., 2000).

Table 5

Predicted neuropeptidome of the *Homarus americanus* eyestalk ganglia

Peptide family	Predicted peptide structure
ACP	pQITFSRSWVPQa
ACP-PRP	SGGITGPLVTPGGGSDRGADPCKDVRLATLTQVASHLADLMDDTFDLPQDDAALALRLKHGLVA
	MS
AST-A	HSNYGFGLa
	TPGYAFGLa
	SDLYSFGLa
	SGSYNFGLa
	AKYSFGIa
	SKLYGFGLa
	PRNYAFGLa
	SQMYSFGLa
	PRDYAFGLa
	PTAYSFGLa
	ATSYGFGLa
	SYDFGLa
	AGRYAFGLa
	TGPYAFGLa
	VGPYAFGLa
	AGHYAFGLa
	AGPYAFGLa
	SGPYAFGLa
	ADPYAFGLa
	AGQYSFGLa
	SGPYSFGLa
	SGVYSFGLa
	AGPYSFGLa
AST-A-PRP	HDY$_{(SO3H)}$LEDLDDPDTSRLLDVLQYYDTEPSYLYDYa
	EGLYSLGLD
	SVGDLPEVSKVEDGASPRT
	DVSITEDTLED
	ESSKN
	DSGEE
	EDDDMENRTQQYSFGLGKQDPDMEIE
	ESDEDSD
	DPDMDMD
	ASSDEDDEERYYAYEQa

Peptide family	Predicted peptide structure
	AFSETDDY$_{(SO3H)}$DNVNDNDDGDDELELSDLEQY$_{(SO3H)}$SDDL
	SDAPDSGFa
	SDSDSDQYTLa
	EVSDDDHDEDEQDIGVEEEMSS
AST-B	**VGWSSMHGTWa**
	TNWNKFQGSWa
	NNWRSLQGSWa
	AWNKLQGAWa
	PDNTRVSPRSTNWSSLRGTWa
	SADWNKLRGAWa
	ASDWGQFRGSWa
AST-B-PRP	**SSSSPQQDDPASSPSHIEE**
	PHLEDAQLDAAEV
	GEELQAAED
	GDDLADAELQAAED
	SEDDNGDDLYDETNLEEDLAGNEEQVSPLALARLMAAAPQ
	GWTLWa
	PDNTRVSPRSTNWSSLRDMMSVAAPNQA
	APDMMSVAAPNQA
AST-C	pQIRYHQCYFNPISCF
	SYWKQCAFNAVSCFa
	GNGDGRLYWRCYFNAVSCF
AST-C-PRP	+QQQQQQQQQQQQQQQQQQQQQGEEEV
	MFVPLSGLPGELPTI
	KALPDQDPQVYGQMPHMLDPAGNHLIDDDGSLDAVLINYLFAKQMVERLRNNADIKDLQ
	ARVPQQAP
	PQYLEVVRPVLPNTALEPLGSLQDAPQQIAETVSTP
	AAIVLDKLMFALQKALDDSPAAPPGQPAPYSRPRTYAAGPMDLQ
Bursicon α	**1**
CCHamide	SCSQFGHSCFGAHa
	HRVLKGGCLNYGHSCLGAHa
CCHamide-PRP	DGDQYARQEPSPLYPEANQLPEFEQRQEDRLSVDEAVTDREIVANA
	NWLAVLSHRLRQRTSPQSSPSAQSLGYFQ
	AYVPVHPPVAPRPLLDVLLDALNTPTRSSHYSHARAANSVMGPRASYPEa
	VKSPPTSDQLSDMGLDLRGEDYASGTNDDLESVGAIGGVRGSLDDT
	DLAQDNVLYYGVLNDDY$_{(SO3H)}$SDARY
	SAVSLPSRGRLGASPPLGVANNAVPQDRPHIL
	EEHTGKDEMDPKYLALASFPNWL
	YSIQHPFIRSAVSLPSRGRLGASPPLGVANNAVPQDRPHIL

Peptide family	Predicted peptide structure
	EEHTGKDEMDPKYLALASFPVSTILLASDPTLLPVVVLV
Corazonin	pQTFQYSRGWTNa
Corazonin-PRP	SDPNVGVTELLADPP
	LSAHSHPHPPTHTLPKNIEERLRALEAGLNAVLKANSINFSPGGDEEY$_{(SO3H)}$YAEN
CCAP	PFCNAFTGCa
CCAP-PRP	GPVA
	DIGDLLEGKD
	SDPSMEGLASSSELDALAKHVLAEAKLWEQLQSKMEMMRSYAS
	MENHPVY
	STPHTQPRQHLTSTPQQKVETEKQ
CHH (CHH subgroup)	pQVFDQACKGVYDRNLFKKLDRVCEDCYNLYRKPFVATTCRENCYSNWVFRQCLDDLLLSDVIDEYVSNVQMVGK
	pQVFDQACKGVYDRNLFKKLNRVCEDCYNLYRKPFIVTTCRENCYSNRVFRQCLDDLLMIDVIDEYVSNVQMVGK
	pQVFDQACKGVYDRNLFKKLNRVCEDCYNLYRKPFIVTTCRQNCFEGDTFPRCVMDLGLDLELFLEFRDMIKa
	AVFDSACKGYYDREFWGKLSRVCWDCENLFRQPGYQDKCSEGCFVTTDFTQCVKALLLNVEEYNELAELVRa
CPRP	RSVEGASRMEKLLSSSNSPSSTPLGFLSQDHSVN
	RSVEGASRMEKLLSSISPSSTPLGFLSQDHSVN
	RSWLIDGDEDLQLSQYHSLN
CHH (MIH subgroup)	WFTNDECPGVMGNRDLYEKVAWVCNDCANIFRNNDVGVMCKKDCFHTMDFLWCVYATERHGEIDQFRKWVSILRAa
	IYIFNECPGRLGNGELHDKVDLVCDDCYNLFRDSALAVTCRGNCFTNHYFDMCIFATARRNQMQMYRRWVSILSAa
	2
DH31	GLDLGLGRGFSGSQAAKHLMGLAAANFAGGPa
DH31-PRP	AAFNREA
	AVVQIEDPDY$_{(SO3H)}$VLELLTRLGHSII
	ANELEKFVRSSGSA
	SSDDGLDLHHDDNLYAQDQAADLAESS
	ANELENA
DH44	ASGLSLSIDASMKVLREALYMEIIRKKQRQQMQRAQHNQKLLNSIa
DH44-PRP	3
	NSNRSN
	SNSSSGISGSNTSSNSNTNNNSPDTISMA
	TWPNGFS
	DVTRQLQQEGIQGVYQRGQ
EH	AANKVSVCIKNCAQCKIMYHDHFKGGLCADLCVQSGGKFIPDCGRPQTLIPFFLQRLE
	ATFTSMCIRNCGQCKEMYGDYFHGQACAESCIMTQGISIPDCNNPATFNRFLKRFI
Elevenin	**VDCRKFVFAPVCRGIIA**
Elevenin-PRP	**MVSE**
	SSFRPTADTQWNSQYRAPTETEAENLLLASSYDDVMEPRPQEDMVVVRAGSDVVQVPAYVFGVIERSLQGE
FLP	GYSDRNYLRFa
	SGRNFLRFa

Peptide family	Predicted peptide structure
	NRNFLRFa
	DQNRNFLRFa
	GAHKNYLRFa
	GNRNFLRFa
	GDRNFLRFa
	FSHDRNFLRFa
	APSKNFLRFa
FLP-PRP	APVPPVVAALDPPTDALLPAQSQEDDLFALPE
	LLKYFLPASQAWGGDAYPIGQEGT
	SDDNS
	SDTNDY$_{(SO3H)}$EGEEMPESPE
	SGSPMEFATDLQEDVELPVEE
	SVDRQLSSLSCEDCDEEQKAREFTSTPSPTTIQPLART
	DVSAVLSDDSIESSVLRQINAHRI
	AAAQNFYIPMAWASELQPEEDGIDVTSFEEPQVA
	DGSDDY$_{(SO3H)}$PSSSSSAESPAPVVVVRPVEYPRYV
GPα2	**4**
GPβ5	**5**
GSEFLamide	**IGSEFLa**
	MGSEFLa
	AMGSEFLa
	ALGSEFLa
	VMGSEFLa
	AVGSEFLa
GSEFLamide-PRP	**ALPTHLPDELDDPVV**
	LAGTPHESMIRYFLMAMSNPAGRYKSPQLLNRGV
	SVGKLSDADNPRDFESENCSDDDGTEEEDL
	EQFSFTGQY$_{(SO3H)}$DY$_{(SO3H)}$DESAGENFGSQEDLFNTKP
	NIRSFHGGVNNDGLKNFFSMLMS
	QYEPEFAHTLDYDT
Intocin	CFITNCPPGa
Intocin-PRP	SGPTAQLGRTRTCTACGPGLQGRCLGPEICCVLGIGCFLGTREA
	MCHAENLVPVTCANRDLKSCa
	MQEGRCAAAGLCCTEMKCEFDSSCTVEGREE
	VGKQRAE
	QHLTFLSSLPEDQWNL
Leucokinin	pQAFHPWGa
	ASFNPWGa
	NTFAPWGa

Peptide family	Predicted peptide structure
	pESFSAWGa
	TRFSAWAa
	AFSAWAa
	TFSAWAa
	TFRAWAa
	TRFSPWAa
	PSFSAWAa
	PSFNAWAa
	pQGFSAWAa
	VPFSTWGa
Leucokinin-PRP	LSSGAASVSFVTSEVMDVSPLALPHGRHPNLCTPDHVPSHPIIRCEVa
	SSFKTAPGLPLSLREVYLALFQNARPRPPPPSEGEL
	SDPLLPASQHEPNT
	AAGYFTHDTNPLIIEEDLIPYIGVLSDDGEAEDVV
	GSFPADDWEEEEPTDLFVLDGSLPYPPVDRLRY
	EAETYTNTLVNSDDSGVKTEAENIKPDTKY$_{(SO3H)}$DQTAEASSTTVA
	PDLQVIEDAVRKMAEHEPKTTE
	SSDVNLQDGEDDEPVSAWIa
	LQDANTDD
	SPSMDLSGNQD
	SSGDELDDHFLD
	AEGTLSRLSESTLKAALDENSPEDNVDNH
	SESNE
	SDSDE
	SDIDE
	SSETD
	NNGGSDDPTHSNNPQQISSILQQLQHQGLEFLH
	LPINDWGN
	ASPISEDSQLSDLYTSQL
Myosuppressin	pQDLDHVFLRFa
Myosuppressin-PRP	VCVGVGETMPPPICLSQQVPLSPFA
	LCSALINISEFSRAMEEY$_{(SO3H)}$LGAQAIERSMPVNEPEV
	SQQ
Neuroparsin	APRCNQGGNRLPANNCKYGTVVDWCGGSVCAKGPGEACGGEWSENGECGAGTYCSCGYCNGCSANLECWFGSYC
	APSCDGHGTRTEPTDCDYGSFQDWCGNNVCAKGPGQRCGGEWWENDDCGHGMYCANCGNCAGCSVGIQCWFCDSGS
	TPLCPERNEIAPEDLSQCKYGVVLGWCGNAACGKGPDEPCGGRWEENGICGEGMYCVCGYCAGCTSTLECVLGRFC
	APRCDSHDSPAPTNCKYGTVRDWCRNGVCAKGPGESCGGYWYEYGKCGGGTFCLCGTCIGCSTIDGTCSQSSPAIIC
NPF	ARPDNSAADTLQAIHEAAMAGILGSAEVQYPNRPSMFKSPVELRQYLDALNAYYAIAGRPRFa
	KPDPNQLAAMADALKYLQELDKYYSQVSRPRFa

Peptide family	Predicted peptide structure
	KPDPNQLAAMADALKYLQELDKYYSQVSRPSLRSSPGPASQIQALEKALKFLQLQELGKMYSLRARPRFa
NPF-PRP	GNHGAQRTEELYDY$_{(SO3H)}$
	SEYAMVPGDALVSYDGSE
Orcokinin	NFDEIDRSGFGFH
	NFDEIDRSGFGFN
	NFDEIDRSGFGFV
Orcomyotropin	FDAFTTGFGHN
Orcokinin-PRP	GPIKAAPARSSPQQDAAAGYTDGAPV
	SSEDMDRLGFGFN
	GDY$_{(SO3H)}$DVYPE
	VYGPRDIANLY
	SAE
PDH	NSELINSILGLPKVMNDAa
	NSELINSLLGIPKVMNDAa
PDH-PRP	QELKYPEREVVAELAAQILRVIQGPWGPMAAGPH
	QELKYPEREVVADMAAQILRVALGPWGSVAAVP
Proctolin	RYLPT
Proctolin-PRP	ADDTRLDEI
	ELLREMLE
	TAEGANSRISGSGYD
	FMY
	SVPEEGAAEMVQPALNLPQ
Pyrokinin	LYYSQRPa
	SLFSPRLa
	GDDITNEELAY$_{(SO3H)}$DDNLATSEYLRDDNNDYLPEELTEDVTEMSSPEMLSESAAALVGKNSVSFIPRLa
	GDGFAFSPRLa
	GADFAFSPRLa
	SEFVFSSRPa
	SDFAFSPRLa
	ADFAFSPRLa
	DSEDSSVESRNTKTQASIPRPa
	AYFSPRLa
Pyrokinin-PRP	LEDEWAGLPQASFAQYPPALDDTSEAQPLSLLYNMYPSVTSADTVPPKSQELQYNSQDTP
	SVDLY$_{(SO3H)}$DDEDPERRM
	QTPQHDNEPTDDNDDSTHRWWWPFVAV
RPCH	pQLNFSPGWa
RPCH-PRP	**AAAASGTDPAAASLHPAPPAVLTAASGANAGDSCGTIPVSAVMHIYRLI**
	TEAARLIQCQEEEYMa
RYamide	**pQGFYTQRYa**

Peptide family	Predicted peptide structure
	FIGGSRYa
RYamide-PRP	**SDTGEVTVRSGFYAN**
	NGRSSPSQGLPEIKIRSS
	SGPAPAAEPEFTPVMNGEADDSDMPATLLVGDSVICLLVDVPDIYRCV
	STTDEASN
sNPF	GPPSLRLRFa
	DMGWQVAQRSMPSLRLRFa
	DTSTPALRLRFa
sNPF-PRP	**VPAPQDY$_{(SO3H)}$DAVNEVYDWLVDHGLE**
	TVDQEEDLSSHEQ
	TVDQVKSLYDREVV
	DTTY$_{(SO3H)}$GQEEDLSSHEQ
SIFamide	VYRKPPFNGSIFa
SIFamide-PRP	AGADPREYTVFEPGKGLASVCQVAVEACAAWFPVQE
	AGADPLFEPGKGLASVCQVAVEACAAWFPVQE
Sulfakinin	pEFDEY$_{(SO3H)}$GHMRFa
	GGGEY$_{(SO3H)}$DDY$_{(SO3H)}$GHLRFa
Sulfakinin-PRP	VSAPARPSSLARVLAPVV
	QRLEESHLPPALVEELVQDFEDPELLDFHDAAa
	SLTHSDQHHHHDTTVN
TRP	APSGFLGMRa
	TPSGFLGMRa
TRP-PRP	AGEGQDTPQDRE
	DASTALDDNTAASEYSSLPDPYPLYGLRDNNLPMLFAVPWKT
	SDEEVFSDATADNDLEILL
	YYDDDSDMDAYIQALTAVVDGQQQQ
	AYYSENPDEEISMTGVD
Trissin	**WSSSEVSCTSCGSECQSACGTRNFRACCFNFQ**
Trissin-PRP	**6**
	PSPSLNQLQHQNLHQRYTPSPTSIKI
	7

Peptides shown in bold black font are new discoveries for *H. americanus*, while those shown in normal black font are known from the lobster, but new discoveries for the eyestalk ganglia. Peptides shown in gray font are known lobster eyestalk ganglia peptides. Peptides highlighted in yellow are present in sinus gland.

[1]**DECSLTPVIHILSYPGCVSKPIPSFACQGRCTSYVQVSGSKLWQTERSCMCCQESGEREASVVLNCPKVRKGEPTRRKVSV+**

[2]**YFYKIRSGTQKEFELINCKQFNKTYYTELSRVCDDCQNIYRKYYNVGVDCKKDCFDNEWFPKCVTYIEHDHLLEEYKKMKEYL NLRDL**

[3]LSLGGGRADTSSLLSLPHPQELSQDDLQPFLSRQGNTDSAGAPSSVADYTGYDKSEVLRGLEDPTSSSAYRLQEALSEAVAAAAAAAAEGA EGVRDGAAALSPTANEGVTLEDLVPYDPGYYLYPAFLNRGDEAMTGGSSGINSLRKV

4 **TSFKHAWQNPGCHKVGHTRRISIPECLEFDITTNACRGFCESWSVPSAWQTLASNPHQVVTSIGQCCNIMDTEDVKVKVMCIQGP RELVFKSASTCDCFHCKKY**

5 **INPQSTLECHRRQYTYKVHKTDDEGRICWDFINVMSCWGRCDSNEIADWKFPYKRSHHPVCMHEETQLTVVTLGNCEDNAAPG TETYSYHEATRCACSVCKTSEASCEGLRYRGARRAPRAEVPRa**

6 **ADPRSHSSAGVNEADSMALEGLLKPDVSGTKDSHLSQILHLLSRALAESKTDPAFYKDPPSLSSVLSPLVQESTEEETDDTSDLLPS SGGDSDDPPLDNVIYLAF**

7 **ADPRSHSAGVNEADSMALEGLLKPDVSGTKDSHLSQILHLLSRALAESKTDPAFYKDPPSLSSVLSPLVQESTEEETDDTSDLLPSS GGDSDDPPLDNVIYLAF**

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, crustacean hyperglycemic hormone; CPRP, crustacean hyperglycemic hormone precursor-related peptide; DH31, diuretic hormone 31; DH44, diuretic hormone 44; EH, eclosion hormone; FLP, FMRFamide-like peptide; NPF, neuropeptide F; PDH, pigment dispersing hormone; RPCH, red pigment concentrating hormone; sNPF, short neuropeptide F; TRP, tachykinin-related peptide; PRP, precursor-related peptide.

Abbreviations in peptide structures: a, carboxyl-terminal amide group; C, one of a pair of cysteines that are linked by a disulfide bridge; pE/pQ, pyroglutamic acid; $Y_{(SO3H)}$, sulfated tyrosine.

See text for descriptions of the disulfide bridging patterns for all peptides with more than one pair of bridged cysteine residues.