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SHORT COMMUNICATION

Identification of putative neuropeptidergic signaling systems in the spiny lobster, *Panulirus argus*

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Abstract

Members of the decapod infraorder Achelata, specifically species from the genus *Panulirus*, have storied histories as models for investigating the basic principles governing the generation, maintenance, and modulation of rhythmic motor behavior, including modulation by locally released and circulating peptides. Despite their contributions to our understanding of peptidergic neuromodulation, little is known about the identity of the native neuropeptides and neuronal peptide receptors present in these crustaceans. Here, a *Panulirus argus* nervous system-specific transcriptome was used to help fill this void, providing insight into the neuropeptidome and neuronal peptide receptome of this species. A neuropeptidome consisting of 266 distinct peptides was predicted using the *P. argus* assembly, 128 having structures placing them into a generally recognized arthropod peptide family: agatoxin-like peptide, allatostatin A (AST-A), allatostatin B, allatostatin C, bursicon, CCHamide, crustacean cardioactive peptide, crustacean hyperglycemic hormone/molt-inhibiting hormone, diuretic hormone 31 (DH31), ecdysis-triggering hormone (ETH), FMRFamide-like peptide (FLP), glycoprotein hormone (GPH), GSEFLamide, inotocin, leucokinin, myosuppressin, natalisin, neuroparsin, neuropeptide F, orcokinin, orcomyotropin, periviscerokinin, pigment-dispersing hormone, pyrokinin, red pigment-concentrating hormone, RYamide, short neuropeptide F (sNPF), SIFamide, sulfakinin, tachykinin-related peptide (TRP), and trissin. Twenty-five putative neuronal receptors, encompassing 15 peptide groups, were also identified from the *P. argus* transcriptome: AST-A, bursicon, CCHamide, DH31, diuretic hormone 44, ETH, FLP, GPH, inotocin, insulin-like peptide, myosuppressin, natalisin, periviscerokinin, sNPF, and TRP. Collectively, the reported data provide a powerful resource for expanding studies of neuropeptidergic control of physiology and behavior in members of the genus *Panulirus* specifically, and decapods generally.

Keywords *In silico* transcriptome mining · Neuropeptide · Neurohormone · Neuropeptide receptor · Crustacea · Malacostraca · Decapoda · Achelata

Introduction

The stomatogastric and cardiac neural networks of decapod crustaceans have served for over half a century as important models for understanding the basic principles governing the generation, maintenance, and modulation of rhythmic motor behaviors (e.g., Blitz and Nusbaum 2011; Christie et al. 2010a; Cooke 2002; Dickinson et al. 2016; Harris-Warrick et al. 1992; Hooper and DiCaprio 2004; Marder and Bucher 2007; Marder et al. 1995; Nusbaum et al. 2001; Selverston 2005; Selverston and Ayers 2006; Selverston and Moulins 1987; Selverston et al. 1998; Skiebe 2001; Stein 2009). A major insight obtained from work conducted on these decapod models is that numerically simple "hardwired" neural circuits are capable of producing an essentially infinite array of distinct motor outputs. This flexibility in motor behavior has been shown to be due, at least in large part, to the actions of locally released and circulating chemical compounds that act to change the properties of individual circuit elements, thereby allowing a single network to be reconfigured into a myriad of functionally distinct ones. While a number of chemical classes have been shown to function as

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neuromodulators in decapod species (e.g., Christie 2011), peptides are by far the largest and most diverse of these groups (e.g., Christie et al. 2010a).

Species from the brachyuran, astacidean, and achelatan genera *Cancer*, *Homarus*, and *Panulirus*, respectively, are among the decapods most commonly used for studying peptidergic neuromodulation. While much work has focused on identifying and characterizing the native neuropeptides and neuronal peptide receptor in *Cancer* and *Homarus* species (e.g., Cape et al. 2008; Chen et al. 2010; Christie and Pascual 2016; Christie et al. 2015, 2017; Dickinson et al. 2019a, b; Huybrechts et al. 2003; Jiang et al. 2012; Li et al. 2003; Ma et al. 2008, 2009), little is known about the native peptidergic signaling systems present in members of the genus *Panulirus*. In fact, there has been just one large-scale study focused on neuropeptide discovery in a member of this genus, *i.e.*, a mass spectral investigation directed at characterizing the neuropeptidome of *Panulirus interruptus* (Ye et al. 2015), and no data are extant on the identity of native neuronal peptide receptors in any *Panulirus* species. In the study presented here, a *Panulirus argus* nervous system (supraoesophageal ganglion [brain], suboesophageal ganglion, and thoracic ganglia)-specific transcriptome was used to help fill this void, providing insight into the native neuropeptides and neuronal peptide receptors of this species, and, by proxy, expanding our knowledge of peptidergic signaling systems in this genus, generally.

Materials and methods

Database searches

Searches of the *P. argus* neural-specific transcriptome for transcripts encoding putative neuropeptide precursors and neuronal peptide receptors were conducted on or before October 15, 2019, using a well-established protocol (e.g., Christie and Hull 2019; Christie and Pascual 2016; Christie and Yu 2019; Christie et al. 2013, 2015, 2017, 2018a, b; Dickinson et al. 2019a, b). In brief, the database of the online program tblastn (National Center for Biotechnology Information, Bethesda, MD; http://blast.ncbi.nlm.nih.gov/Blast.cgi) was set to Transcriptome Shotgun Assembly (TSA) and restricted to data from **BioProject No. PRJNA476144** (Ernst, Fitak, Schmidt, Derby, Johnsen, and Lohmann, unpublished direct GenBank submission); this assembly is the only publicly accessible neural-specific transcriptome currently available for any member of the genus *Panulirus*. Previously reported arthropod peptide precursor and receptor sequences, most from decapod species (Christie and Hull 2019; Christie and Pascual 2016; Christie and Yu 2019; Christie et al. 2008, 2010b, c, 2015, 2017, 2018a, c 2019; de Kleijn et al. 1994; Dickinson et al. 2007, 2008, 2009a, b, 2019a, b; Stevens et al. 2009; Tensen et al. 1991), were used as the query proteins for the BLAST searches. The complete list of peptide precursors and receptors searched for, as well as the specific queries used, is provided in Supplemental Table 1.

Prediction of mature peptide structures

The putative mature structures of *P. argus* neuropeptides were predicted using a well-established workflow (e.g., Christie and Hull 2019; Christie and Pascual 2016; Christie and Yu 2019; Christie et al. 2013, 2015, 2017, 2018a, b). Specifically, the hits returned by each BLAST search were translated using the Translate tool of ExPASy (http://web.expasy.org/translate/) and assessed for completeness. Precursor proteins listed as full length exhibit a functional signal sequence (including a start methionine) and are flanked on their carboxyl (C)-terminus by a stop codon. Proteins listed as partial lack a start methionine (referred to as C-terminal partial proteins), a stop codon (referred to as amino [N]-terminal partial proteins), or both of these features (referred to as internal fragment proteins). Each full-length or N-terminal partial precursor was subsequently assessed for the presence of a signal peptide using the online program SignalP 3.0 (http://www.cbs.dtu.dk/services/SignalP/; Bendtsen et al. 2004). Prohormone cleavage sites were identified based on information presented in Veenstra (2000) and/or by homology to known arthropod pre-/preprohormone processing schemes. When present, the sulfation state of tyrosine residues was predicted by homology to known peptide isoforms or by 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 or by using the online program DiANNA (http://clavius.bc.edu/~clotelab/DiANNA/; Ferrè and Clote 2005). Other posttranslational modifications, *i.e.*, cyclization of N-terminal glutamine/glutamic acid residues and C-terminal amidation at glycine residues, were predicted by homology to known arthropod peptides.

Provisional annotation of putative neuronal peptide receptors

A workflow developed to provide provisional annotation for a variety of proteins, including peptide receptors (e.g., Christie and Hull 2019; Christie and Yu 2019; Christie et al. 2013, 2015, 2017, 2018b; Dickinson et al. 2019b), was used to annotate the putative *P. argus* receptor proteins identified in this study. First, nucleotide sequences were translated and assessed for completeness as described above. Next, each *P. argus* receptor was used to query the *D. melanogaster* proteins in FlyBase (version FB2019_01; http://flybase.org/blast

/index.html; Thurmond et al. 2019) and the non-redundant arthropod proteins in NCBI for the most similar sequence in each dataset. Finally, protein structural motifs were predicted for each *P. argus* receptor using the online program Pfam (version 32.0; http://pfam.xfam.org/; El-Gebali et al. 2019). This workflow was conducted on or before October 29, 2019.

Amino acid alignments and calculations of amino acid identity/similarity

Amino acid alignments were carried out using the online program MAFFT version 7 (http://mafft.cbrc.jp/alignment/software/; Katoh and Standley 2013). Amino acid identity/similarity between selected peptide precursors, peptides, and peptide receptors was calculated using the MAFFT alignment outputs. Specifically, percent identity was calculated as the number of identical amino acids divided by the total number of residues in the longest sequence (x100), while amino acid similarity was calculated as the number of identical and similar amino acids divided by the total number of residues in the longest sequence (x100).

Results

Identification of putative Panulirus argus *neuropeptide-encoding transcripts and precursor proteins*

As the first step in the identification of a neuropeptidome for *P. argus*, a nervous system-specific transcriptome was searched for sequences encoding putative peptide precursor proteins. Forty-one generally recognized arthropod peptide families were targeted: adipokinetic hormone–corazonin-like peptide (ACP), agatoxin-like peptide (ALP), allatostatin A (AST-A), allatostatin B (AST-B), allatostatin C (AST-C), allatotropin, bursicon, CCHamide, CCRFamide, CNMamide, corazonin, crustacean cardioactive peptide (CCAP), crustacean hyperglycemic hormone (CHH), diuretic hormone 31 (DH31), diuretic hormone 44 (DH44), ecdysis-triggering hormone (ETH), eclosion hormone (EH), elevenin, FMRFamide-like peptide (FLP), glycoprotein hormone (GPH), GSEFLamide, HIGSLYRamide, inotocin, insulin-like peptide (ILP), leucokinin, myosuppressin, natalisin, neuroparsin, neuropeptide F (NPF), orcokinin/orcomyotropin, periviscerokinin, pigment-dispersing hormone (PDH), proctolin, pyrokinin, red pigment-concentrating hormone (RPCH), RYamide, short neuropeptide F (sNPF), SIFamide, sulfakinin, tachykinin-related peptide (TRP), and trissin (Supplemental Table 1). Transcripts encoding putative precursors for all families except ACP, allatotropin, CCRFamide, CNMamide, corazonin, DH44, EH, elevenin, HIGSLYRamide, ILP, and proctolin were found in the *P. argus* assembly (Supplemental Table 1). Translation of the identified transcripts revealed single full-length or partial precursor proteins for all groups except AST-A, AST-B, AST-C (Fig. 1a), CCHamide (Fig. 1b), CHH, ETH (Fig. 1c), leucokinin, natalisin, neuroparsin, NPF, orcokinin/orcomyotropin, PDH, and RYamide (Fig. 1d), where multiple full-length or partial pre-/preprohormones were identified (Supplemental Table 1 and Supplemental Fig 1). For AST-C (Fig. 1a), CCHamide (Fig. 1b), CHH, neuroparsin, NPF and PDH, multiple genes appear to contribute, at least in part, to precursor protein diversity (Supplemental Table 1 and Supplemental Fig. 1).

Prediction of a neuropeptidome for Panulirus argus

Two hundred and sixty-six distinct mature peptides were predicted from the *P. argus* precursor proteins deduced from the nervous system-specific assembly. The predicted neuropeptidome includes 128 peptides whose structures place them into a generally recognized arthropod peptide family (Table 1), as well as 138 linker/precursor-related sequences that may or may not function as neuropeptides in their own right (Supplemental Table 2). In *P. argus*, some families are represented by a single peptide, e.g., ETH, inotocin, periviscerokinin, SIFamide, and TRP, while others are represented by multiple, in some cases large numbers, of distinct isoforms, e.g., 20 AST-As, 15 natalisins, 12 leucokinins, and 12 pyrokinins.

Comparison of the complement of peptides predicted for *P. argus* with that identified previously via mass spectrometry/molecular cloning from *P. interruptus* neural tissues (Dickinson et al. 2009b; Jia et al. 2013; Stemmler et al. 2005, 2007; Yasuda-Kamatani and Yasuda 2004; Ye et al. 2015) suggests that many neuropeptide isoforms are shared by the two species (shown in bold font in Table 1). For example, the AST-B isoforms ANWNKFHGSWamide, ADWNKFHGSWamide, GNWNKFHGSWamide, and GDWNKFHGSWamide appear to be present in both *P. argus* and *P. interruptus*. While isoforms of AST-A, AST-B, AST-C, CHH, DH31, FLP, myosuppressin, orcokinin, orcomyotropin, sNPF, SIFamide, PDH, and TRP have been reported previously from *P. interruptus* (Dickinson et al. 2009b; Jia et al. 2013; Stemmler et al. 2005, 2007; Yasuda-Kamatani and Yasuda 2004; Ye et al. 2015), the isoforms of ALP, bursicon, CCHamide, CCAP, ETH, GPH, GSEFLamide, inotocin, leucokinin, natalisin, neuroparsin, NPF, periviscerokinin, pyrokinin, RPCH, RYamide, sulfakinin, and trissin predicted using the *P. argus* transcriptome are, to the best of the author's knowledge, the first reports of members of these families from any *Panulirus* species. Given the high levels of structural conservation seen for other families, it seems

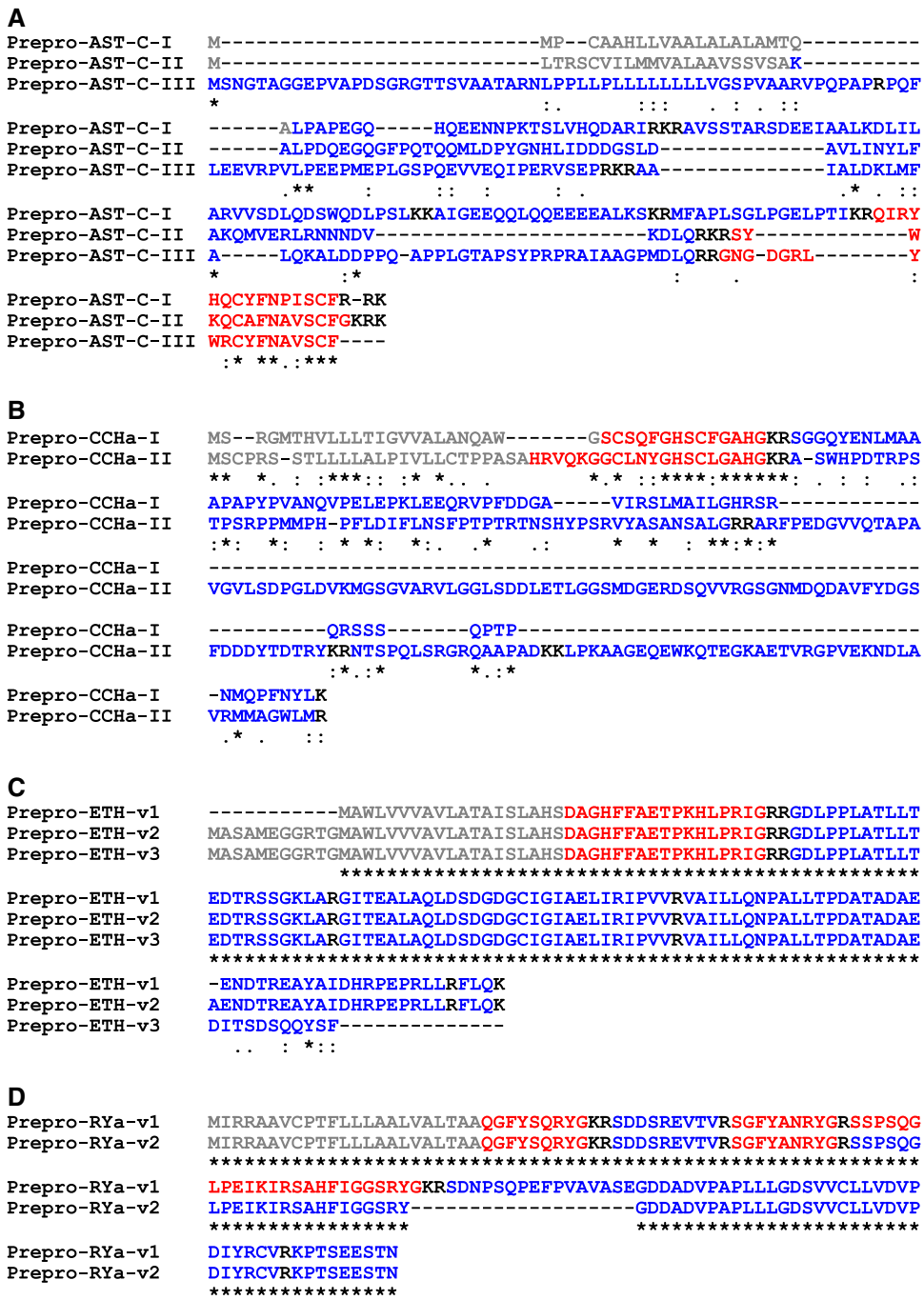

Fig. 1 MAFFT alignments of select *Panulirus argus* precursor proteins from four different peptide families. **a** Alignment of allatostatin C (AST-C) precursors. **b** Alignment of CCHamide (CCHa) precursors. **c** Alignment of ecdysis-triggering hormone (ETH) precursors. **d** Alignment of RYamide (RYa) precursors. 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 "." For both AST-C and CCHamide, precursor diversity appears to be due to the presence of multiple paralog genes, while for ETH and RYamide, precursor diversity appears to be due to alternative splicing of single genes

likely that many isoforms of these newly identified peptide groups will also ultimately be found in *P. interruptus* as well.

Identification of putative Panulirus argus *neuropeptide receptor-encoding transcripts and proteins*

In addition to searches for peptide precursors, the *P. argus* assembly was also queried for transcripts encoding putative neuronal peptide receptors, including those for ACP, AST-A, AST-B, AST-C, bursicon, CCHamide, CNMamide, corazonin, CCAP, CHH, DH31, DH44, ETH, FLP, GPH, inotocin, ILP, leucokinin, myosuppressin, natalisin, NPF, periviscerokinin, PDH, proctolin, pyrokinin, RPCH, RYamide, sNPF, SIFamide, sulfakinin, TRP, and trissin (Supplemental Table 1). Transcripts encoding 25 putative receptor proteins encompassing 15 peptide groups were found in the assembly (Supplemental Table 1). Translation of the identified sequences suggests the expression of at least one AST-A, three bursicon, one CCHamide, four DH31, one

Table 1 Putative mature structures of *Panulirus argus* isoforms from generally recognized arthropod peptide families

Family	Peptide structure
ALP	SCIRRGGPCDHRPNDCCYNSSCRCNLWGTNCRCQRMGLFQKWa
Allatostatin A	HNNYAFGLa
	$SPDY_{(SO3H)}SFGLa$
	pEGMYSFGLa
	ADLFSFGLa
	SGNYNFGLa
	TRQYSFGVa
	PNDYAFGLa
	SRQYAFGLa
	PRYYAFGLa
	PTTYSFGLa
	TASYGFGLa
	SYDFGLa
	SGPYAFGLa
	ADLYSFGLa
	ADPYAFGLa
	DGMYAFGLa
	AGQYSFGLa
	TDPYSFGLa
	SGSYSFGLa
	DGPYSFGLa
Allatostatin B	ADWSSMHGTWa
	GDWNKFHGSWa
	ADWNKFHGSWa
	ANWNKFHGSWa
	GNWNKFHGSWa
	ANWNKFQGSWa
	AYWNKFQGSWa
	SDWSSLQGTWa
	AWDNFHGSWa
	PDSTRVSPRSTNWSSLRGTWa
	SPDWNSLRGAWa
	TPDWAQFRGSWa
Allatostatin C	pQIRYHQCYFNPISCF
	SYWKQCAFNAVSCFa
	GNGDGRLYWRCYFNAVSCF
Bursicon	1/2
CCHamide	SCSQFGHSCFGAHa
	HRVQKGGCLNYGHSCLGAHa
CCAP	PFCNAFTGCa
CHH (CHH)	AVFDQSCKGVYDRSLFQKLDLVCDDCYNLYRKPYVATGCRENCFARDVFPMCVDSLGLDVDLYMAIRAMLQ
	AVFDQSCKGVYDRSLFQKLDLVCDDCYNLYRKPYVATGCRSGPTHLTASTVLFMISF
	AVFDTACKGLYDRKIWAKLNRACEDCQNVFREPALDMDCRKGCFATPVFFMCVRELLLPAKEYKALAHLLRa
CHH (MIH)	RFAFDECPGMIGNRDAYDKVEQVCDDCYNLYRDEELAVKCRKDCFFNNDFFVCLFAMERDNEFKDFIRRLSIINA-GRW
	RFIDHECVGALGNRDIYEKVVWVCDDCANIFRNNNVGESCKKNCFYNVDFQWCVYATERHGDMEHFKRWVSILSAa
DH31	**GLDLGLGRGFSGSQAAKHLMGLAAANYAGGPa**
ETH	DAGHFFAETPKHLPRIa
FLP	GYNSRNYLRFa
	GGRNFLRFa

Table 1 (continued)

Family	Peptide structure
	GRNFLRFa
	NPSRNFLRFa
	GAHKNYLRFa
	GNRNFLRFa
	GEDRNFLRFa
	SYNRSFLRFa
	NRNFLRFa
	APQRNFLRFa
GPH	3
GSEFLamide	IGSEFLa
	MGSEFLa
	AMGSEFLa
Inotocin	CFITNCPPGa
Leucokinin	ASFNPWGa
	VRFSAWAa
	AFNAWAa
	TFSTWAa
	LNDDGNQHSLTRFSAWAa
	TFNAWAa
	SNNDDKQQAFNPWAa
	pQSFSAWAa
	pQAFSAWAa
	pQAFNAWAa
	pQAFGAWAa
	PTFGAWVa
Myosuppressin	**pQDLDHVFLRFa**
Natalisin	SPPAPPLPPAQEGDASFWLARa
	PADPLDGHPSGEAAGPWDTEDDDDGSDEDDESVKLSGRSGSGTFWVARa
	DGGPFWIARa
	pQEGGGPFWISRa
	SEALPPLDEPLLWPIRGRKSADARTFWAARa
	pEVTARAPFWVARa
	GGEAGLTAPFWIARa
	DGEMSPIWVARGKEEGETHPFWVARa
	pEGETHPFWIARa
	pESERHPFWVARa
	pEEDNSSLWITRa
	pEETHPFWVARa
	pEGERDPYWIARa
	pEPENNAFWAARa
	HDDPDTQKHPFATSFVAQRa
Neuroparsin	APKCPIQISTATPENCTFGTTTDWCRNTICAKGPGEECKEEEWWANECTLGTYCACGFCSGCFVNVLCHTPSFC
	GPACPHRNEIVPEDLSKCKYGVVLGWCGNLACGKGPGDMCGGPWEEHGNCGKGMYCVCGHCAGCSSKLKCAL-GRFC
NPF	TRHDSSETADALQAIHEAVMAGVLGSAEVQYPNRPSIFKSPVELRKYLDALNAYYAIAGRPRFa
	KPDPSQLAAMADAIKYLQELDKLYSQVSRPSPRSAPGPASQIQALEKTLKFLQLQELGKMYSLRARPRFa
Orcokinin	NFDEIDRAGLAFA
	NFDEIDRAGLGFA
	NFDEIDRSGFGFT
	NFDEIDRAGFGFV

Table 1 (continued)

Family	Peptide structure
	NFDEIDRSGFGFV
	NFDEIDRAGLGFH
Orcomyotropin	**FDAFTTGFGHS**
Periviscerokinin	pQDLIPFPRVa
PDH	NAELINSILGLPKVMNDAa
	NAELLNTLLGSQDLGYMRNAa
Pyrokinin	FYYAPRPa
	SPFSPRLa
	SMAFSPRLa
	VSFVPRLa
	TPGFAFSPRLa
	AANFPFVPRPa
	pENFAFSPRLa
	SGFAFGPRLa
	ADFAFSPRLa
	GDFAFSPRLa
	ADFAFRPRLa
	ADFAFIPRLa
RPCH	pQLNFSPGWa
RYamide	pQGFYSQRYa
	SGFYANRYa
	SSPSQGLPEIKIRSAHFIGGSRYa
sNPF	APPSMRLRFa
	DMGWQVAHRSMPSLRLRFa
	DNRTPALRLRFa
SIFamide	**GYRKPPFNGSIFa**
Sulfakinin	pEFDEY$_{(SO3H)}$GHMRFa
	SGGEY$_{(SO3H)}$DDY$_{(SO3H)}$GHLRFa
TRP	**APSGFLGMRa**
Trissin	WSSSEVSCASCGSECQAACGTRNFRACCFNFQ

Only full-length peptide isoforms are included in this table

Peptides identically conserved in *P. argus* and *Panulirus interruptus* are shown in italic font [*P. interruptus* data from Dickinson et al. (2009b), Jia et al. (2013), Stemmler et al. (2007), Yasuda-Kamatani and Yasuda (2004) and Ye et al. (2015)]

Peptide family abbreviations: *ALP* agatoxin-like peptide, *CCAP* crustacean cardioactive peptide, *CHH* crustacean hyperglycemic hormone, *DH31* diuretic hormone 31, *ETH* ecdysis-triggering hormone, *FLP* FMRFamide-like peptide, *GPH* glycoprotein hormone, *MIH* molt-inhibiting hormone, *NPF* neuropeptide F, *PDH* pigment-dispersing hormone, *RPCH* red pigment-concentrating hormone, *sNPF* short neuropeptide F, *TRP* tachykinin-related peptide

Abbreviations in peptide structures: *pQ/pE* amino-terminal pyroglutamic acid, $Y_{(SO3H)}$ sulfated tyrosine, *C* cysteine residue participating in a disulfide bond, *a* carboxyl-terminal amide group

[1]Bursicon α-subunit peptide:

DECSLTPVIHILSYPGCTSKPIPSFACQGRCTSYVQVSGSKLWQTERSCMCCQESGEKEASITLNCPKARKGEPKRKKILTRAPIDCMCR-PCTDVEESTVLAQEIANFIQYSPMGNVPFL

[2]Bursicon β-subunit peptide:

ASSYGIECETLPSTIHVVKEEY$_{(SO3H)}$DEAGRVERTCEEDLAVNKCEGACLSKVQPSVNTPSGFLKDCRCCRETHLRSRDVTLTHCYDSDG NRLTGPKSSLEVKLREPADCQCFKCGDST

[3]Glycoprotein hormone β5 subunit peptide:

INPQSTLECHRREYSYKVHKTDDNGLVCWDFINVMSCWGRCDSNEIADWKFPYKRSHHPVCIHEETKLSEKILRHCDEGAAPGTE-TYKYYEATRCACSVCKSSEASCEGLRYRGARRAPRINVPRa

DH44, four ETH, two FLP, three GPH, one inotocin, one ILP, one myosuppressin, one periviscerokinin, one sNPF, and one natalisin/TRP receptor in the *P. argus* nervous system (Supplemental Table 1 and Supplemental Fig. 2). Protein diversity for the FLP receptor appears due to alternative splicing of a single gene, while multiple genes appear to contribute, at least in part, to the diversity seen for the other groups for which multiple putative receptors were identified.

To increase confidence in the annotations of the peptide receptors identified from the *P. argus* transcriptome, each protein was used as the query sequence in blastp searches of the annotated *D. melanogaster* and non-redundant arthropod protein datasets curated in FlyBase and NCBI, respectively. For the putative inotocin receptor a BLAST search of the beetle, *Tribolium castaneum*, dataset in NCBI was substituted for the FlyBase search, as *Drosophila* lacks an inotocin signaling system, whereas that of *Tribolium* is well documented (Stafflinger et al. 2008). The expectation for these searches was that they would return an isoform of the protein family for which the *P. argus* receptor was annotated. For all *P. argus* receptors, this expectation was found to be largely true (Supplemental Tables 3 and 4). For example, the BLAST search of FlyBase using the putative *P. argus* AST-A receptor (Panar-AST-AR; Fig. 2) as the input protein returned *D. melanogaster* allatostatin A receptor 1 isoform B (Drome-AST-AR1-B; **Accession No. AAF45884**; Adams

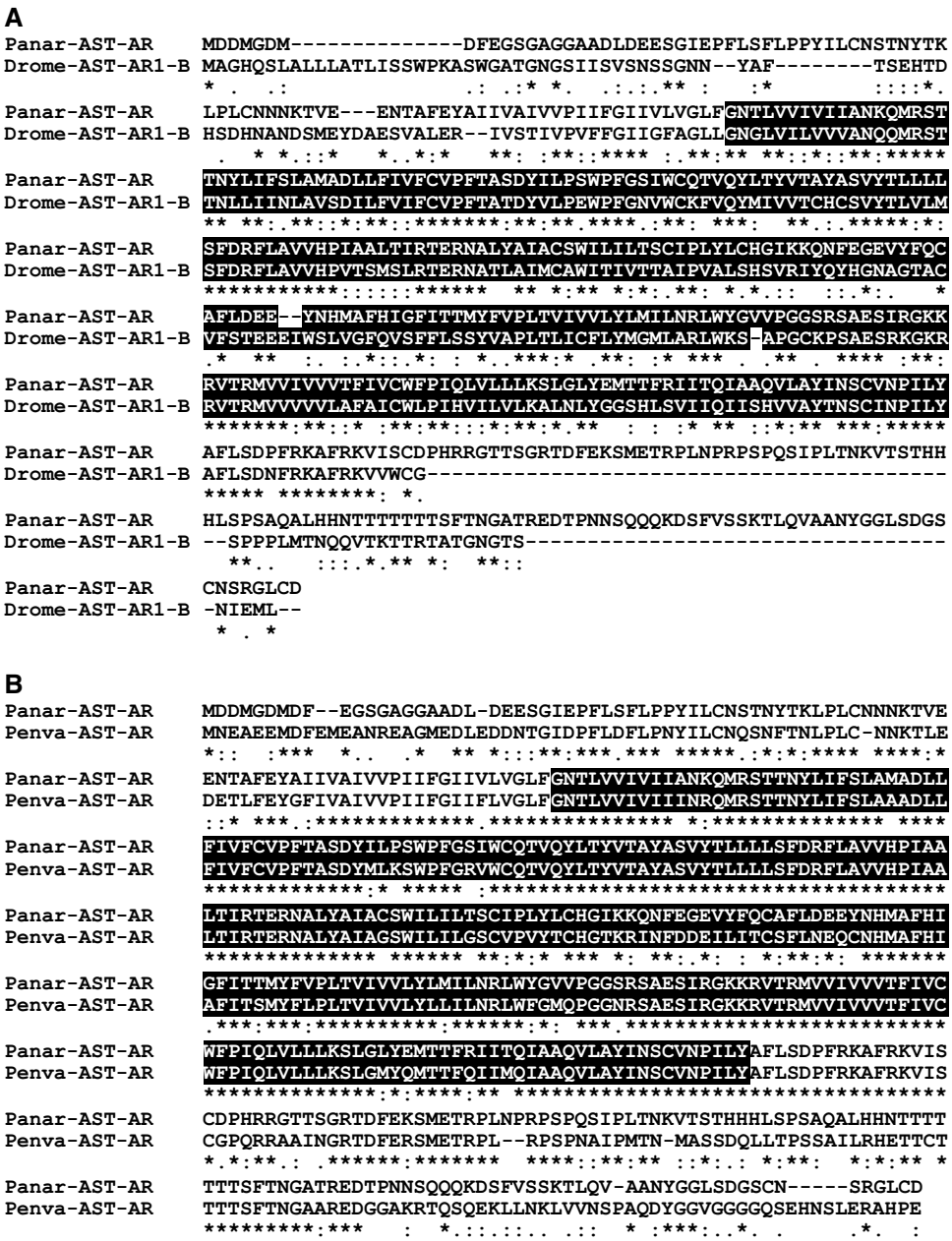

Fig. 2 Alignments of *Panulirus argus* and related allatostatin A receptors. **a** MAFFT alignment of the putative *P. argus* allatostatin A receptor (Panar-AST-AR; deduced from **Accession No. GHUJ01003623**) and *Drosophila melanogaster* allatostatin A receptor 1 isoform B (Drome-AST-AR1-B; **Accession No. AAF45884**). **b** MAFFT alignment of Panar-AST-AR and an allatostatin A receptor from the shrimp, *Penaeus vannamei* (Penva-AST-AR; **Accession No. XP_027222495**). In the line immediately below each sequence grouping, "*" indicates identical amino acid residues, while ":" and "." denote amino acids that are similar in structure between sequences. In this figure, rhodopsin family seven-transmembrane receptor domains identified by Pfam analyses are highlighted in black

et al. 2000) as the top hit (Supplemental Table 3 and Fig. 2a), while the search of the non-redundant arthropod protein dataset in NCBI revealed an allatostatin A receptor from the shrimp, *Penaeus vannamei* (Penva-AST-AR; **Accession No. XP_027222495**; unpublished direct GenBank submission), to be the top hit for Panar-AST-AR (Supplemental Table 4 and Fig. 2b). In addition to the BLAST analyses, each putative *P. argus* receptor was subjected to Pfam structural domain prediction (Supplemental Table 5), with the domain complement identified compared to those found by the program for the receptor's top FlyBase and NCBI non-redundant arthropod protein hits (data not shown). The expectation for these Pfam searches was that identical or highly similar domain complements would be returned for all three proteins in a given grouping, which was true for essentially all comparisons. For example, a single rhodopsin family seven-transmembrane receptor domain was identified by Pfam in Panar-AST-AR (Supplemental Table 5 and Fig. 2), a domain also predicted by the program for both Drome-AST-AR1-B (Fig. 2a) and Penva-AST-AR (Fig. 2b).

While the workflow used to provide provisional annotations to the putative *P. argus* peptide receptors identified here supports their being members of the protein families to which they are ascribed, it is important to note that none have actually been shown biochemically, or via other means, to bind any peptide specifically, which is true for the vast majority of the putative peptide receptors thus far identified from members of the Decapoda. This said, for the few decapod receptors that have been formally deorphanized, high levels of amino acid conservation are seen between them and their *P. argus* counterparts, e.g., 71%/88% amino acid identity/similarity between *P. argus* DH31 receptor I variant 1 and the *Carcinus maenas* DH31 receptor (**Accession No. AXF54360**), which is activated specifically by the native *C. maenas* DH31 peptide (Alexander et al. 2018).

Discussion

Despite their importance as models for investigating the basic principles underlying the generation, maintenance, and modulation of rhythmic motor behavior, including peptidergic control of neural network output [e.g., modulation of the motor output of the stomatogastric nervous system, which controls the rhythmic movements of the foregut, by cholecystokinin-like peptides (Turrigiano et al. 1994), which likely are identical or highly similar to the sulfakinins predicted here], the native neuropeptidergic signaling systems of members of the decapod genus *Panulirus* remain little studied. Using a *P. argus* nervous system-specific transcriptome, a neuropeptidome and a neuronal peptide receptome were predicted for this species. The *P. argus* neuropeptidome consists of 266 distinct peptides and includes members of 31 generally recognized families. The identified peptides include the first ALP, bursicon, CCHamide, CCAP, ETH, GPH, GSEFLamide, inotocin, leucokinin, natalisin, neuroparsin, NPF, periviscerokinin, pyrokinin, RPCH, RYamide, sulfakinin, and trissin isoforms described from any member of the genus *Panulirus*. In addition, putative neuronal receptors for 15 peptide groups were identified using the *P. argus* transcriptome, including two for which no peptide precursors were found, *i.e.*, DH44 and ILP.

While searched for, no transcripts encoding putative ACP, allatotropin, CCRFamide, CNMamide, corazonin, DH44, EH, elevenin, HIGSLYRamide, ILP, or proctolin precursors were identified from the *P. argus* transcriptome. Likewise, no putative *P. argus* ACP, AST-B, AST-C, allatotropin, CNMamide, corazonin, CCAP, CHH, leucokinin, natalisin, NPF, PDH, proctolin, pyrokinin, RPCH, RYamide, SIFamide, sulfakinin, or trissin receptor-encoding transcripts were found in the assembly. Given the identification of putative *P. argus* DH44 and ILP receptors, it is highly likely that precursors for both peptide families exist in this species. Similarly, the identification of putative AST-B, AST-C, CCAP, CHH, leucokinin, natalisin, NPF, PDH, pyrokinin, RPCH, RYamide, SIFamide, sulfakinin, and trissin precursors suggests that receptors for these groups are present in *P. argus* as well. The identification of ACP, corazonin, EH, and proctolin in other members of the Achelata, e.g., *Jasus edwardsii* (Christie and Yu 2019), suggests that these signaling systems are also likely present in *P. argus*. At present, there is too little information concerning the presence of CCRFamide, CNMamide, and elevenin signaling systems in members of the Decapoda to assess whether or not their lack of detection in *P. argus* is a reflection of their truly being absent in the nervous system of this species, or if they, like the above-mentioned groups, are present in *Panulirus*, but just that transcripts for them did not assemble or were missed during the mining of the assembly. The lack of identification of an allatotropin signaling system in *P. argus* adds to the bolus of data, suggesting that this peptide group has been lost in decapod crustaceans (e.g., Christie and Pascual 2016; Christie and Yu 2019; Christie et al. 2015, 2017; Fu et al. 2005). Similarly, the lack of detection of a *P. argus* HIGSLYRamide precursor-encoding transcript adds to a growing dataset, suggesting that this peptide family is brachyuran specific (e.g., Christie and Pascual 2016; Christie and Yu 2019; Christie et al. 2008, 2015, 2017; Fu et al. 2005).

While large-scale searches for both peptide precursors and receptors have been carried out for just a handful of decapod species, including the *P. argus* data reported here, one emerging finding is that there seems to be no hard and fast rule concerning peptide isoform diversity and the number of receptors for a given peptide family (e.g., Christie et al. 2015; Dickinson et al. 2019b). In some cases, there appear to be single receptors for groups with a large number

of distinct peptide isoforms, e.g., AST-A, while for others, the reverse appears true, e.g., ETH, where there appears to be a single peptide, but multiple putative receptor genes. For groups with multiple receptors, it seems likely that this is one mechanism by which functional flexibility can be expanded for a peptide family (e.g., Dickinson et al. 2019b). The data reported here provide a resource for beginning to test this hypothesis in members of the genus *Panulirus* specifically, and decapods generally.

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Compliance with ethical standards

Conflict of interest None.

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