Invertebrate Neuroscience (2020) 20:7
https://doi.org/10.1007/s10158-020-0239-5

ORIGINAL ARTICLE

Assessment and comparison of putative amine receptor complement/diversity in the brain and eyestalk ganglia of the lobster, *Homarus americanus*

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Received: 28 October 2019 / Accepted: 3 March 2020 / Published online: 26 March 2020

Abstract

In decapods, dopamine, octopamine, serotonin, and histamine function as locally released/hormonally delivered modulators of physiology/behavior. Although the functional roles played by amines in decapods have been examined extensively, little is known about the identity/diversity of their amine receptors. Recently, a *Homarus americanus* mixed nervous system transcriptome was used to identify putative neuronal amine receptors in this species. While many receptors were identified, some were fragmentary, and no evidence of splice/other variants was found. Here, the previously predicted proteins were used to search brain- and eyestalk ganglia-specific transcriptomes to assess/compare amine receptor complements in these portions of the lobster nervous system. All previously identified receptors were reidentified from the brain and/or eyestalk ganglia transcriptomes, i.e., dopamine alpha-1, beta-1, and alpha-2 (Homam-DAα2R) receptors, octopamine alpha (Homam-OctαR), beta-1, beta-2, beta-3, beta-4, and octopamine–tyramine (Homam-OTR-I) receptors, serotonin type-1A, type-1B (Homam-5HTR1B), type-2B, and type-7 receptors; and histamine type-1 (Homam-HA1R), type-2, type-3, and type-4 receptors. For many previously partial proteins, full-length receptors were deduced from brain and/or eyestalk ganglia transcripts, i.e., Homam-DAα2R, Homam-OctαR, Homam-OTR-I, and Homam-5HTR1B. In addition, novel dopamine/ecdysteroid, octopamine alpha-2, and OTR receptors were discovered, the latter, Homam-OTR-II, being a putative paralog of Homam-OTR-I. Finally, evidence for splice/other variants was found for many receptors, including evidence for some being assembly-specific, e.g., a brain-specific Homam-OTR-I variant and an eyestalk ganglia-specific Homam-HA1R variant. To increase confidence in the transcriptome-derived sequences, a subset of receptors was cloned using RT-PCR. These data complement/augment those reported previously, providing a more complete picture of amine receptor complement/diversity in the lobster nervous system.

Keywords Dopamine · Octopamine · Tyramine · Serotonin · Histamine · In silico transcriptome mining

Introduction

Decapod crustaceans have long served as important models for advancing our understanding of nervous system function. For example, the process of neurosecretion was first formally demonstrated using the neuroendocrine X-organ–sinus gland complex of a member of the Brachyura (Bliss 1951; Passano 1951), while the first data demonstrating that GABA is an inhibitory neurotransmitter were obtained from studies conducted on members of the Astacidea (Otsuka et al. 1966, 1967). Similarly, red pigment concentration hormone from the nervous system of a caridean shrimp was among the first neuropeptides to be isolated and fully characterized (Fernlund and Josefsson 1968, 1972), and much of what is known about the basic principles governing the generation,

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10158-020-0239-5) contains supplementary material, which is available to authorized users.

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maintenance, and modulation of rhythmic motor behavior comes from work conducted on the decapod stomatogastric and cardiac neuromuscular systems (e.g., Blitz and Nusbaum 2011; Christie et al. 2010; 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).

Interestingly, and somewhat surprisingly given their important contributions to the field of neuroscience, molecular resources for decapod nervous systems, e.g., deep transcriptomes, are quite limited (e.g., Bao et al. 2015; Christie et al. 2017 2018a, b; Northcutt et al. 2016). One species for which multiple neural transcriptomes have been generated and publicly deposited is the American lobster, *Homarus americanus*. A publicly accessible mixed nervous system (supraoesophageal ganglion [brain], abdominal nerve cord, cardiac ganglion, and stomatogastric nervous system) assembly exists for *H. americanus* (BioProject No. PRJNA300643; Northcutt et al. 2016), as do several nervous system region-specific ones, e.g., those for the brain (BioProject No. PRJNA379629; Christie et al. 2018a) and eyestalk ganglia (BioProject No. PRJNA338672; Christie et al. 2017). These and other *Homarus* assemblies have proven to be powerful resources from which to identify genes/proteins in specific areas of interest, e.g., those involved in neuropeptidergic signaling (e.g., Christie et al. 2015, 2017, 2018c) and those involved in the establishment of neuronal circadian signaling systems (Christie et al. 2018a, b), providing substrates for initiating molecular and physiological studies of neural control in *H. americanus*, and by proxy, other members of the Decapoda.

The *H. americanus* mixed nervous system transcriptome was recently used to identify putative neuronal amine receptors in the lobster (Northcutt et al. 2016), with the overarching goal of providing insight into the global complement of amine receptors in the *Homarus* nervous system (Northcutt et al. 2016). The data obtained from the mixed nervous system assembly suggest that the *H. americanus* neuronal amine receptor complement consists of at least three dopamine (dopamine alpha-1 [Homam-DAα1R], dopamine beta-1 [Homam-DAβ1R], and dopamine alpha-2 [Homam-DAα2R]), six octopamine (octopamine alpha [Homam-OctαR], octopamine beta-1 [Homam-Octβ1R], octopamine beta-2 [Homam-Octβ2R], octopamine beta-3 [Homam-Octβ3R], octopamine beta-4 [Homam-Octβ4R], and octopamine–tyramine [Homam-OTR]), four serotonin (serotonin type-1A [Homam-5HTR1A], serotonin type-1B [Homam-5HTR1B], serotonin type-2B [Homam-5HTR2B], and serotonin type-7 [Homam-5HTR7]), and four histamine (histamine type-1 [Homam-HA1R], histamine type-2 [Homam-HA2R], histamine type-3 [Homam-HA3R], and histamine type-4 [Homam-HA4R]) receptor genes (Northcutt et al. 2016). This dataset provides an important contribution to our understanding of aminergic signaling in the lobster nervous system. It is, however, limited by the facts that a number of the deduced receptors are fragmentary and that the assembler used to produce the mixed nervous system transcriptome, i.e., the CLC Genomics Workbench Server, does not preserve evidence of protein isoforms (e.g., Honaas et al. 2016). Thus, in an attempt to provide a broader and more complete picture of amine receptor complement/diversity in *H. americanus*, we have taken the proteins deduced from the mixed nervous system assembly [as well as members of several other arthropod amine receptor families not searched for/reported in Northcutt et al. (2016)] and used them to query the *Homarus* brain- and eyestalk ganglia-specific transcriptomes (Christie et al. 2017, 2018a), both of which were assembled using Trinity (Grabherr et al. 2011), an assembler that is well known for preserving evidence of protein isoforms (e.g., Honaas et al. 2016), for transcripts encoding putative homologs. As the data that follow will show, all of the *H. americanus* amine receptors identified previously from the mixed nervous system transcriptome, or variants of them, were reidentified from the brain- and/or eyestalk ganglia-specific assemblies, with full-length isoforms predicted for many receptors that were formerly fragmentary. In addition, evidence suggestive of alternative splicing was found for most receptors, including some variants that were assembly-specific, and thus potentially differentially distributed between the brain and eyestalk ganglia. Finally, three putative amine receptors not described in Northcutt et al. (2016) were identified from the brain and eyestalk ganglia transcriptomes. Taken collectively, the data presented in this report provide a significant expansion in our understanding of amine receptor complement and diversity in the *H. americanus* nervous system, and thus provide a broader foundation from which to initiate future molecular and physiological studies of aminergic signaling at the level of receptors in this and other members of the Decapoda.

Materials and methods

Transcriptome mining

Searches of the *H. americanus* brain and eyestalk ganglia transcriptomes were conducted using a protocol modified from one employed previously to identify G protein-coupled and other types of receptors in crustaceans (e.g., Christie et al. 2013, 2015, 2018a; Christie and Yu 2019; Dickinson et al. 2019; McCoole et al., 2011, 2012). In brief, the database of the online program tblastn (National Center for Biotechnology Information, Bethesda, MD; https://blast.ncbi.nlm.nih.gov/Blast.cgi) was set to Transcriptome

Shotgun Assembly (TSA) and restricted to data from the *H. americanus* brain- (BioProject No. PRJNA379629; Christie et al. 2018a) and eyestalk ganglia-specific (BioProject No. PRJNA338672; Christie et al. 2017) transcriptomes. Previously reported arthropod amine receptor proteins (i.e., Adams et al. 2000; Northcutt et al. 2016), primarily those from *H. americanus* itself, were used as the query sequences for the BLAST searches. All receptors searched for and the specific queries used are provided in Supplemental Table 1.

Transcripts identified as encoding putative amine receptors were translated using the Translate tool of ExPASy (https://web.expasy.org/translate/) and assessed for completeness. Proteins listed as full-length include a start methionine and are flanked on their carboxyl (C)-terminus by a stop codon (Supplemental Table 1). Proteins described here as partial lack either a start methionine, referred to as C-terminal partial proteins, or a stop codon, referred to as amino (N)-terminal partial proteins, or both of these features, referred to as internal fragment proteins (Supplemental Table 1). The amino acid sequences of all *H. americanus* amine receptors deduced from the brain and/or eyestalk ganglia are provided in Supplemental Figure 1.

Protein sequences were aligned using the online program MAFFT version 7 (https://mafft.cbrc.jp/alignment/software/; Katoh and Standley 2013). Amino acid identity/similarity between selected receptors was calculated using the MAFFT alignments. Specifically, percent identity was calculated as the number of identical amino acids in the two proteins divided by the total number of residues in the longest sequence (×100), while amino acid similarity was calculated as the number of identical and similar amino acids in the two sequences divided by the total number of residues in longest protein (×100).

Protein annotation

A well-established workflow was used to provide provisional annotation for three novel *H. americanus* amine receptors deduced from brain and/or eyestalk ganglia transcripts (e.g., Christie et al. 2013, 2015, 2018a; Christie and Yu 2019; Dickinson et al. 2019; McCoole et al. 2011, 2012). This workflow was also used to reconfirm the initial annotations of full-length receptors that were previously reported as partial proteins. Specifically, the longest full-length variant or partial protein of each *Homarus* receptor was used as the query sequence in blastp searches of the *Drosophila melanogaster* proteins curated in FlyBase (version FB2019_01; Thurmond et al. 2019) and the non-redundant arthropod proteins curated at NCBI (taxid:6656) to identify the most similar sequence in each dataset (Supplemental Tables 2 and 3, respectively). In addition, protein structural motifs were analyzed for each *H. americanus* sequence (Supplemental Table 4) using the online program Pfam version 32.0 (https://pfam.xfam.org/; El-Gebali et al. 2019); the identified domain complements were then compared to those predicted by the program for the receptor's top FlyBase and non-redundant arthropod protein hits.

Cloning of selected amine receptor sequences

Total RNAs were purified from freshly dissected individual *H. americanus* brains and eyestalk ganglia pairs using TRI Reagent (Life Technologies, Carlsbad, CA) and Direct-zol RNA MiniPrep spin columns (Zymo Research, Irvine, CA, USA) as described previously (Christie et al. 2017, 2018a). First-strand complementary DNAs (cDNAs) were generated from 500 ng of DNase I-treated total RNA using Superscript III Reverse Transcriptase (Life Technologies) and custom-made random pentadecamers (IDT, San Diego, CA, USA). Oligonucleotide primers (Supplemental Table 5) were designed using the Primer3 v2.3.7 (Rozen and Skaletsky 2000) module in Geneious v10.1.3 (Biomatters Ltd., Auckland, New Zealand; Kearse et al. 2012) to amplify a subset of the amine receptors. Transcripts were amplified from brain or eyestalk ganglia cDNAs using SapphireAmp Fast PCR Master Mix (Takara Bio USA, Inc., Mountain View, CA, USA) in a 20 µl reaction volume with 0.5 µl cDNA and 0.4 µl of the respective oligonucleotide primers (10 µM). PCR conditions consisted of 95 °C for 2:00 min, then 40 cycles of 95 °C for 0:20 s, 58 °C for 0:20 s, and 72 °C for 1:45 min, with a final extension at 72 °C for 5:00 min. The resulting PCR products were electrophoresed on 1.5% agarose gels stained with SYBR Safe (Life Technologies), cloned into pCR2.1TOPO TA vector (Life Technologies), and sequenced at the Arizona State University DNA Core laboratory (Tempe, AZ, USA).

Assessment of phylogenetic relationships among putative amine receptor proteins

The phylogenetic relationships among the predicted *H. americanus* amine receptors and those identified in other arthropods were inferred from a multiple sequence alignment constructed using default MUSCLE (Edgar 2004) settings in Geneious v10.1.3. Evolutionary analyses were conducted in MEGA X (Kumar et al. 2018) using the maximum likelihood method based on the Le and Gascuel model (Le and Gascuel 2008). The tree with the highest log likelihood (− 13,535.74) is shown. Initial trees for the heuristic search were obtained automatically by applying neighbor joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Jones–Taylor–Thornton model (Jones et al. 1992) and then selecting the topology with highest log likelihood value. A discrete gamma distribution was used to model evolutionary rate differences among sites with two categories (+G, parameter = 1.0687). The analysis

involved 58 amino acid sequences. All positions with less than 95% site coverage were eliminated such that fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position (partial deletion option), with 200 total positions retained in the final dataset. Additional phylogenetic trees (not shown) with similar topologies were also generated using UPGMA, neighbor joining, and minimum evolution algorithms in MEGA X. Accession numbers of sequences used to construct the phylogenetic trees are listed in Supplemental Table 6.

Results

Reidentification of previously described amine receptors in the brain and eyestalk ganglia

Recently, a transcriptome generated using multiple portions of the *H. americanus* nervous system (BioProject No. PRJNA300643) was used to identify transcripts encoding putative lobster neuronal amine receptors (Northcutt et al. 2016). Three dopamine (Homam-DAα1R, Homam-DAβ1R, and Homam-DAα2R), six octopamine (Homam-OctαR, Homam-Octβ1R, Homam-Octβ2R, Homam-Octβ3R, Homam-Octβ4R, and Homam-OTR), four serotonin (Homam-5HTR1A, Homam-5HTR1B, Homam-5HTR2B, and Homam-5HTR7), and four histamine (Homam-HA1R, Homam-HA2R, Homam-HA3R, and Homam-HA4R) receptors were predicted from the collective set of identified transcripts (Northcutt et al. 2016). All receptors but one, Homam-DAα1R, were reidentified from both assemblies (Table 1); Homam-DAα1R was reidentified from the brain-specific assembly, but not from the eyestalk ganglia-specific transcriptome (Table 1).

Many of the putative amine receptors deduced from brain and/or eyestalk ganglia transcripts are identical in amino acid sequence, or nearly so, to those identified previously from the mixed nervous system transcriptome (Table 1). For example, the sequence of the DAβ1R identified from the *H. americanus* mixed nervous system assembly is identical to that of a variant deduced from a brain transcript, while the sequence of the 5HTR7 identified from the mixed nervous system transcriptome differs from a variant deduced from both brain and eyestalk transcripts at two positions, i.e., a glutamic acid for aspartic acid substitution at position 49 and threonine for alanine substitution at position 488 (Fig. 1). Sequence information obtained from the proteins deduced from the brain and/or eyestalk ganglia assemblies also extended amino acid coverage for a number of partial proteins deduced from the mixed nervous system transcriptome, four of which are now putative full-length receptors, i.e., Homam-DAα2R, Homam-OctαR, Homam-OTR, and Homam-5HTR1B (Fig. 2).

Identification of new amine receptor gene products

In addition to reidentifying receptors previously described from the mixed nervous system assembly, evidence was found for the expression of three additional putative amine receptor genes in the *H. americanus* brain and eyestalk ganglia transcriptomes, receptors that were not searched for/reported by Northcutt and colleagues (Northcutt et al. 2016). Specifically, using the *D. melanogaster* dopamine/ecdysteroid receptor as the query protein, transcripts encoding a putative *Homarus* homolog (Homam-DopECR) were found in both the brain and eyestalk ganglia transcriptomes (Table 1). Identical putative DopECRs (399 amino acids in length) were deduced from brain and eyestalk ganglia transcripts (Fig. 3), with the lobster receptor being 43% identical/68% similar in amino acid sequence to its *Drosophila* counterpart (Fig. 3a). Similarly, using the sequence of an isoform of the *D. melanogaster* alpha-2-adrenergic-like octopamine receptor as the input query, evidence for the expression of a gene encoding a putative *H. americanus* homolog, Homam-Octα2R, was found in both the brain and eyestalk ganglia assemblies (Table 1). The longest variant of Homam-Octα2R is 44% identical/68% similar in amino acid sequence to the *Drosophila* protein used to identify the transcript encoding it (Fig. 4). In addition, the search for OTR-encoding transcripts provided evidence for the expression of a gene encoding a putative paralog, Homam-OTR-II (Table 1 and Fig. 5), of the partial protein reported previously from the mixed nervous system assembly, which was renamed here Homam-OTR-I (Table 1 and Fig. 4).

The *H. americanus* brain- and eyestalk ganglia-specific transcriptomes were also searched for putative homologs of the *D. melanogaster* tyramine (TyrR) and tyramine II (TyrR-II) receptors (Supplemental Table 1), neither of which were reported by Northcutt and colleagues from the mixed nervous system assembly (Northcutt et al. 2016). No putative TyrR- or TyrR-II-encoding transcripts were found in either the brain or eyestalk ganglia transcriptomes (Supplemental Table 1), suggesting that *H. americanus* may lack genes for these two receptors or that their expression is limited to portions of the lobster nervous system other than the brain and eyestalk ganglia.

Many amine receptors exhibit evidence for the existence of variants

A striking feature of the amine receptor sequences reported previously from the *H. americanus* mixed nervous system transcriptome was the lack of evidence for splice and/or other variants (Northcutt et al. 2016). We hypothesize that that this lack of isoform diversity is an artifact of the assembler used to produce the mixed nervous system transcriptome. This transcriptome was produced using the CLC

Table 1 Identification and transcriptome detection of putative *Homarus americanus* amine receptors

Amine	Receptor	Detection		
		Mx*	Br	EG
Dopamine	Dopamine alpha-1 receptor	+	+	–
	Dopamine beta-1 receptor variant 1	+	+	+[a]
	Dopamine beta-1 receptor variant 2	–	+	+[a]
	Dopamine beta-1 receptor variant 3	–	+	–
	Dopamine beta-1 receptor variant 4	–	+	–
	Dopamine alpha-2 receptor variant 1	+	+	+
	Dopamine alpha-2 receptor variant 2	–	+	–
	Dopamine alpha-2 receptor variant 3	–	+	–
	Dopamine alpha-2 receptor variant 4	–	+	+
	Dopamine/ecdysteroid receptor	NR	+	+
Octopamine–tyramine	Octopamine alpha receptor	+	+	+
	Octopamine alpha-2 receptor variant 1	NR	–	+
	Octopamine alpha-2 receptor variant 2	NR	+	–
	Octopamine beta-1 receptor variant 1a	+	–	+
	Octopamine beta-1 receptor variant 1b	–	+	–
	Octopamine beta-1 receptor variant 2	–	–	+
	Octopamine beta-2 receptor a	+	–	+
	Octopamine beta-2 receptor b	–	+	+
	Octopamine beta-3 receptor a	+	+	–
	Octopamine beta-3 receptor b	–	+	–
	Octopamine beta-3 receptor c	–	–	+
	Octopamine beta-4 receptor a	+	–	+
	Octopamine beta-4 receptor b	–	+	–
	Octopamine beta-4 receptor c	–	–	+
	Octopamine–tyramine receptor I variant 1	–	+	–
	Octopamine–tyramine receptor I variant 2a	–	+	–
	Octopamine–tyramine receptor I variant 2b	+	–	+
	Octopamine–tyramine receptor II variant 1	NR	+	–
	Octopamine–tyramine receptor II variant 2a	NR	+	–
	Octopamine–tyramine receptor II variant 2b	NR	–	+
Serotonin	Serotonin receptor 1A	+	+	+
	Serotonin receptor 1B variant 1	+	+	+
	Serotonin receptor 1B variant 2	–	+	–
	Serotonin receptor 2B variant 1	+	–	–
	Serotonin receptor 2B variant 2	–	+	+
	Serotonin receptor 7 variant 1a	+	–	–
	Serotonin receptor 7 variant 1b	–	+	+
	Serotonin receptor 7 variant 2	–	+	+
Histamine	Histamine type-1 receptor variant 1	+	–	–
	Histamine type-1 receptor variant 2a	–	+	–
	Histamine type-1 receptor variant 2b	–	+	–
	Histamine type-1 receptor variant 3	–	–	+
	Histamine type-2 receptor	+	+	+
	Histamine type-3 receptor a	+	–	–
	Histamine type-3 receptor b	–	+	+
	Histamine type-4 receptor	+	+	+

Mx, mixed nervous system transcriptome; Br, brain-specific transcriptome; EG, eyestalk ganglia-specific transcriptome

*Mixed nervous system transcriptome data taken from Northcutt et al. (2016); NR, not searched for or not reported in Northcutt et al.

[a]The eyestalk ganglia protein is a partial sequence that ends before the regions that differentiate Homam-DAβ1R-v1 from Homam-DAβ1R-v2, and thus it is not possible to determine which (if either) variant this partial protein represents

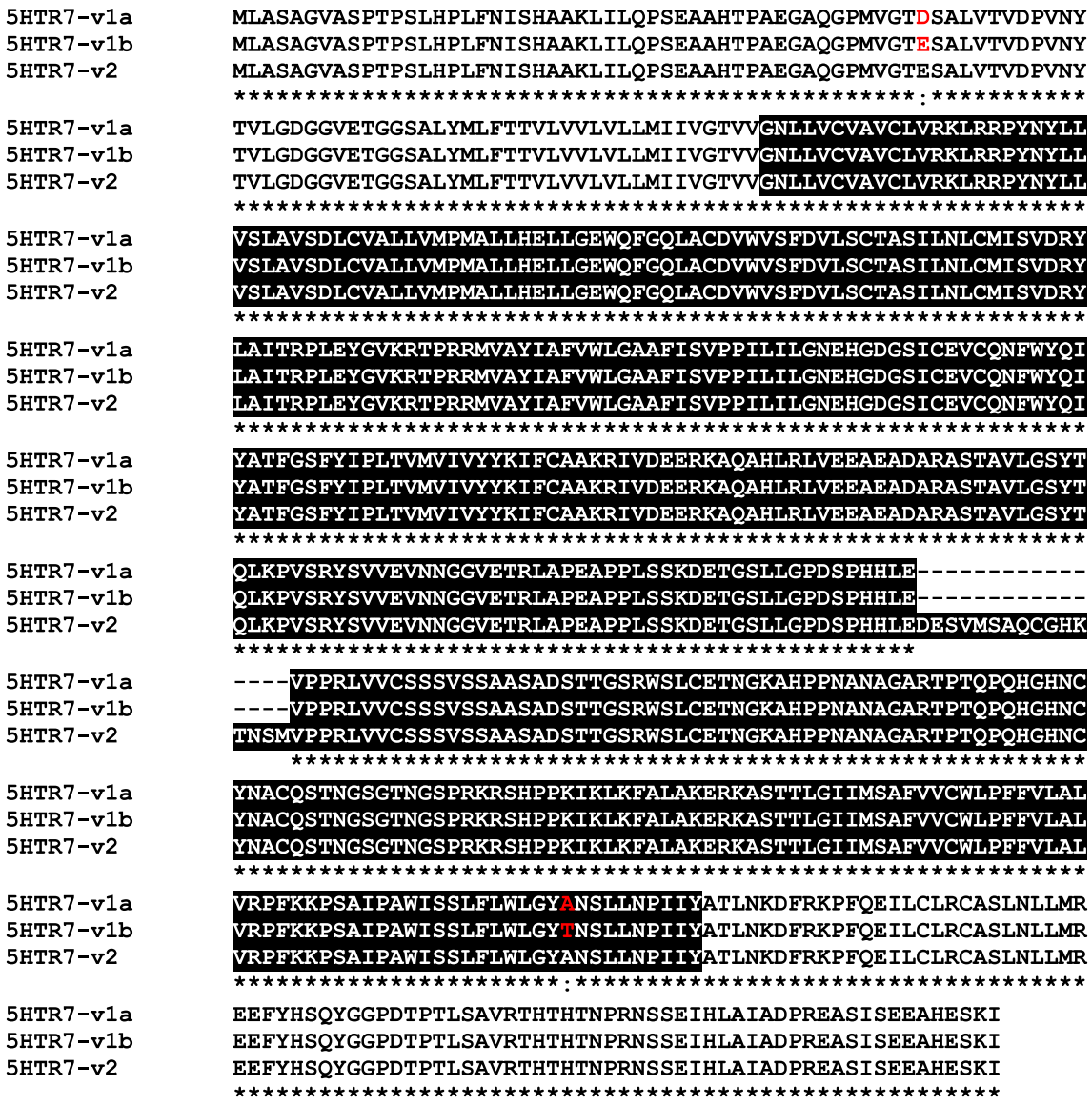

Fig. 1 MAFFT alignment of *Homarus americanus* serotonin type-7 receptor (Homam-5HTR7) variants. In the line immediately below each sequence grouping, "*" indicates identical amino acid residues, while ":" and "." denote amino acids that are similar in structure between sequences. In this figure, rhodopsin family seven-transmembrane receptor domains identified by Pfam analyses are highlighted in black. Amino acid residues that vary between Homam-5HTR7-v1a and Homam-5HTR7-v1b are shown in red (color figure online)

Genomics Workbench Server 5.0.1 (Northcutt et al. 2016), while both the brain and eyestalk ganglia transcriptomes were produced using Trinity (Christie et al. 2017, 2018a). A major difference that has been reported for Trinity vs. CLC assemblies is the former assembler's ability to preserve transcript isoform diversity, which is not a feature of the latter (e.g., Honaas et al. 2016). As expected, splice and/or other variants were found for many of the putative amine receptors identified from the *Homarus* brain- and/or eyestalk ganglia-specific transcriptomes (Table 1). A number of these variants are assembly-specific, suggesting the possibility of different amine receptor complements in different portions of the lobster nervous system. Specifically, putative splice variants were identified for Homam-DAβ1R, Homam-DAα2R, Homam-Octα2R, Homam-Octβ1R, Homam-OTR-I (Fig. 5), Homam-OTR-II (Fig. 5), Homam-5HTR1B (Fig. 2), Homam-5HTR2B, Homam-5HTR7 (Fig. 1), and Homam-HA1R. In addition, isoforms that vary by a single or a small number of amino acid substitutions or insertions/deletions were noted for many receptors, i.e., Homam-Octβ1R, Homam-Octβ2R, Homam-Octβ3R, Homam-Octβ4R, Homam-OTR-I (Fig. 5), Homam-OTR-II (Fig. 5), Homam-5HTR7 (Fig. 1), Homam-HA1R, and Homam-HA3R. Whether or not the presence of receptor variants

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5HTR1B-v1       MRVATAPAVTPPAGGSPHHHTTHMTHWSPTSSPPGVVVDVGGPASLPWWDDWTNTTDEEA
5HTR1B-v2       ------------------------------------------------------------

5HTR1B-v1       RALLDVDETVDADDGTLSGGNWTLEGGNWTMIDGNWTMMDGNWTLVGGNWTEEEGATLSM
5HTR1B-v2       ------------------------------------------------------------

5HTR1B-v1       QVMFTAITAVILGVMILATIIGNVFVIAAILLERNLQSVANYLILSLAVADLLVAALVMP
5HTR1B-v2       ------------------------------------------------------------

5HTR1B-v1       LGAVYEVSKEWTLGPELCDMWTASDVLCCTASILHLVAIALDRFWAVTKVDYIHQRSPKR
5HTR1B-v2       ------------------MWTASDVLCCTASILHLVAIALDRFWAVTKVDYIHQRSPKR
                                   ************************************

5HTR1B-v1       VGGMIAVIWSVSFLISAAPMMGWKDPEWHERLRNKMCIISQDVGYQIFATITSFYLPLAV
5HTR1B-v2       VGGMIAVIWSVSFLISAAPMMGWKDPEWHERLRNKMCIISQDVGYQIFATITSFYLPLAV
                ************************************************************

5HTR1B-v1       ILVLYWRVYQAARKRIRRKIGTNAHSALCRGPVTISETTTFTRMSSQPSPEKTSNGSTLN
5HTR1B-v2       ILVLYWRVYQAARKRIRRKIGTNAHSALCRGPVTISETTTFTRMSSQPSPEKTSNGSTLN
                ************************************************************

5HTR1B-v1       GHPVDGGREDTTTSAVVAAVTSAAAAQVVPRHLRRREVESRKERKAAKTLGIITGAFVMC
5HTR1B-v2       GHPVDGGREDTTTSAVVAAVTSAAAAQVVPRHLRRREVESRKERKAAKTLGIITGAFVMC
                ************************************************************

5HTR1B-v1       WLPFFILAVLMPLCTVSCHFNPYLIATFLWLGYFNSTLNPIIYTVFSPEFRNAFKRILCG
5HTR1B-v2       WLPFFILAVLMPLCTVSCHFNPYLIATFLWLGYFNSTLNPIIYTVFSPEFRNAFKRILCG
                ************************************************************

5HTR1B-v1       RKRPFQRQSSRFSSVRFTRS
5HTR1B-v2       RKRPFQRQSSRFSSVRFTRS
                ********************
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Fig. 2 MAFFT alignment of *Homarus americanus* serotonin type-1B receptor (Homam-5HTR1B) variants. 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. New sequence coverage for Homam-5HTR1B-v1 provided by full-length proteins deduced from the brain eyestalk ganglia is shown in red (color figure online)

manifests in multiple functional roles for the products of a given receptor gene remains to be determined. However, the presence of these variants, including possible tissue-specific ones, clearly raises this possibility.

Vetting receptor family annotations of new and previously identified partial proteins with significant new sequence coverage

To increase confidence in the protein family annotations ascribed to the three new receptor discoveries (i.e., Homam-DopECR, Homam-Octα2R, and Homam-OTR-II), as well as to those ascribed to the previously identified partial proteins that now have significant new sequence coverage (i.e., Homam-DAα2R, Homam-OctαR, Homam-OTR-I, Homam-5HTR1A, Homam-5HTR1B, and Homam-HA2R), the deduced *Homarus* proteins were subjected to the annotation/vetting workflow described in Sect. 2.2. Specifically, the longest variant of each *Homarus* receptor was used to query the annotated *D. melanogaster* proteins curated in FlyBase and the non-redundant arthropod proteins curated in NCBI to identify the most similar protein in each dataset (Supplemental Tables 2 and 3, respectively). The expectations for these analyses were that the top BLAST hits for each sequence would be an isoform of the protein family to which it had been ascribed. Additionally, each of the *Homarus* receptors was further vetted for annotation by subjecting it to structural/functional motif analysis using the online program Pfam and comparing the complement of structural/functional motifs identified (Supplemental Table 4) to those predicted by the program for the protein's top hits from the FlyBase and NCBI non-redundant arthropod protein searches. Here, the expectation was that the *Homarus* receptor and its top FlyBase *D. melanogaster* and NCBI non-redundant arthropod protein hits would contain the same, or very similar, complements of Pfam-predicted domains. As can be seen from the data presented in Supplemental Tables 2–4, this was indeed the case for all the receptors subjected to the annotation/vetting workflow. For example, the top FlyBase hit for Homam-DopECR was the *D. melanogaster* dopamine/ecdysteroid receptor (Accession No. AAF47893; Adams et al. 2000; Fig. 3a), while its top NCBI non-redundant arthropod protein hit was the G protein-coupled receptor 21 from the shrimp, *Penaeus vannamei* (Accession No. XP_027231351; unpublished direct GenBank submission; Fig. 3b), a receptor that also appears

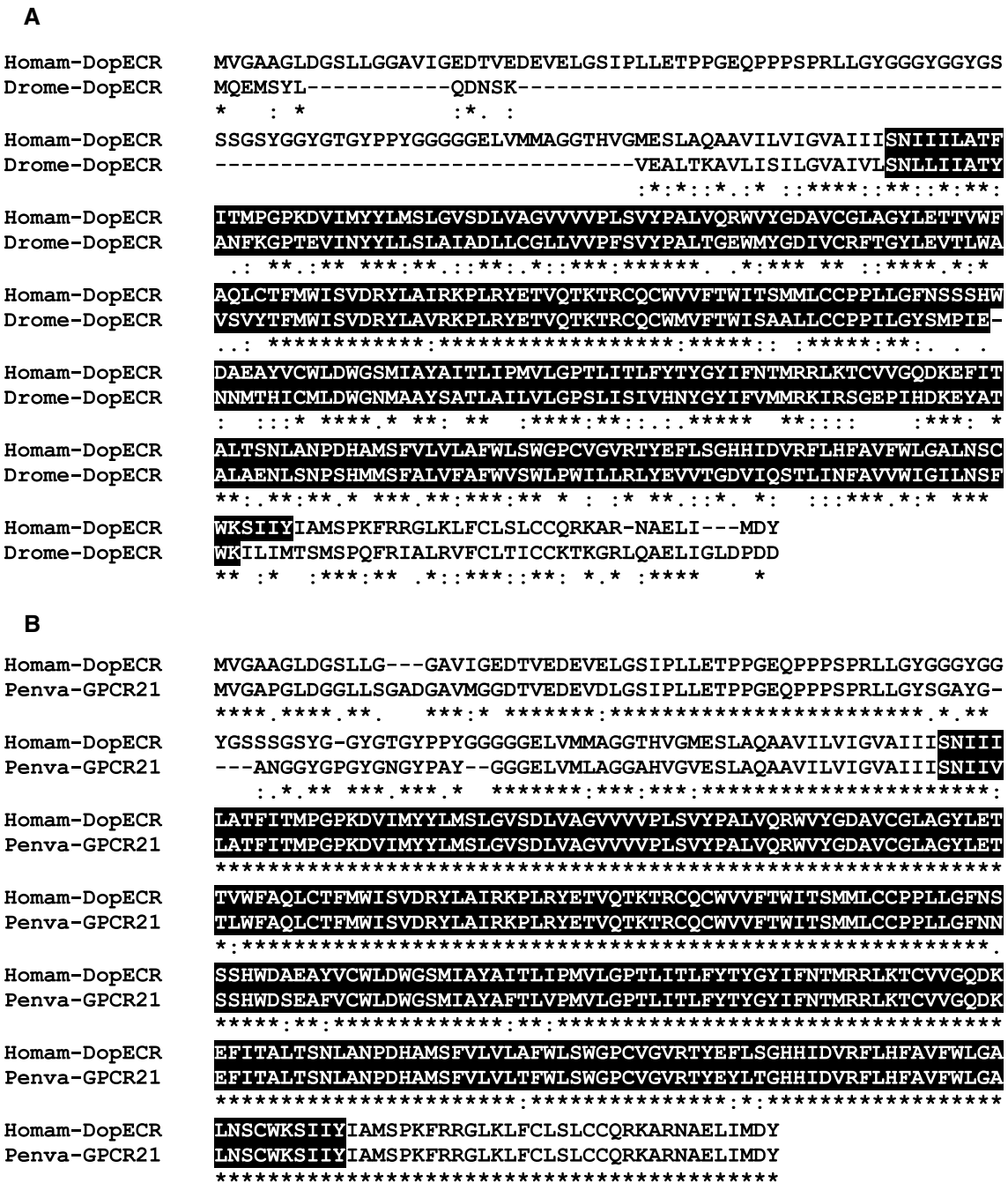

Fig. 3 MAFFT alignments of *Homarus americanus* and related dopamine/ecdysteroid receptor. (A) Alignment of Homam-DopECR and the *Drosophila melanogaster* dopamine/ecdysteroid receptor (Drome-DopECR; Accession No. AAF47893; Adams et al. 2000). (B) Alignment of Homam-DopECR and *Penaeus vannamei* G protein-coupled receptor 21 (Penva-GPCR21; Accession No. XP_027231351; unpublished direct GenBank submission). 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

to be a member of the DopECR family. Pfam analysis identified a single rhodopsin family seven-transmembrane receptor domain in Homam-DopECR (Fig. 3), which is the sole domain predicted by the program for the *Drosophila* and *Penaeus* proteins (Fig. 3a, b, respectively).

Full-length cloning of select Homarus americanus *amine receptors*

To further vet the authenticity of the sequences of the transcripts and, by proxy, proteins identified from the brain

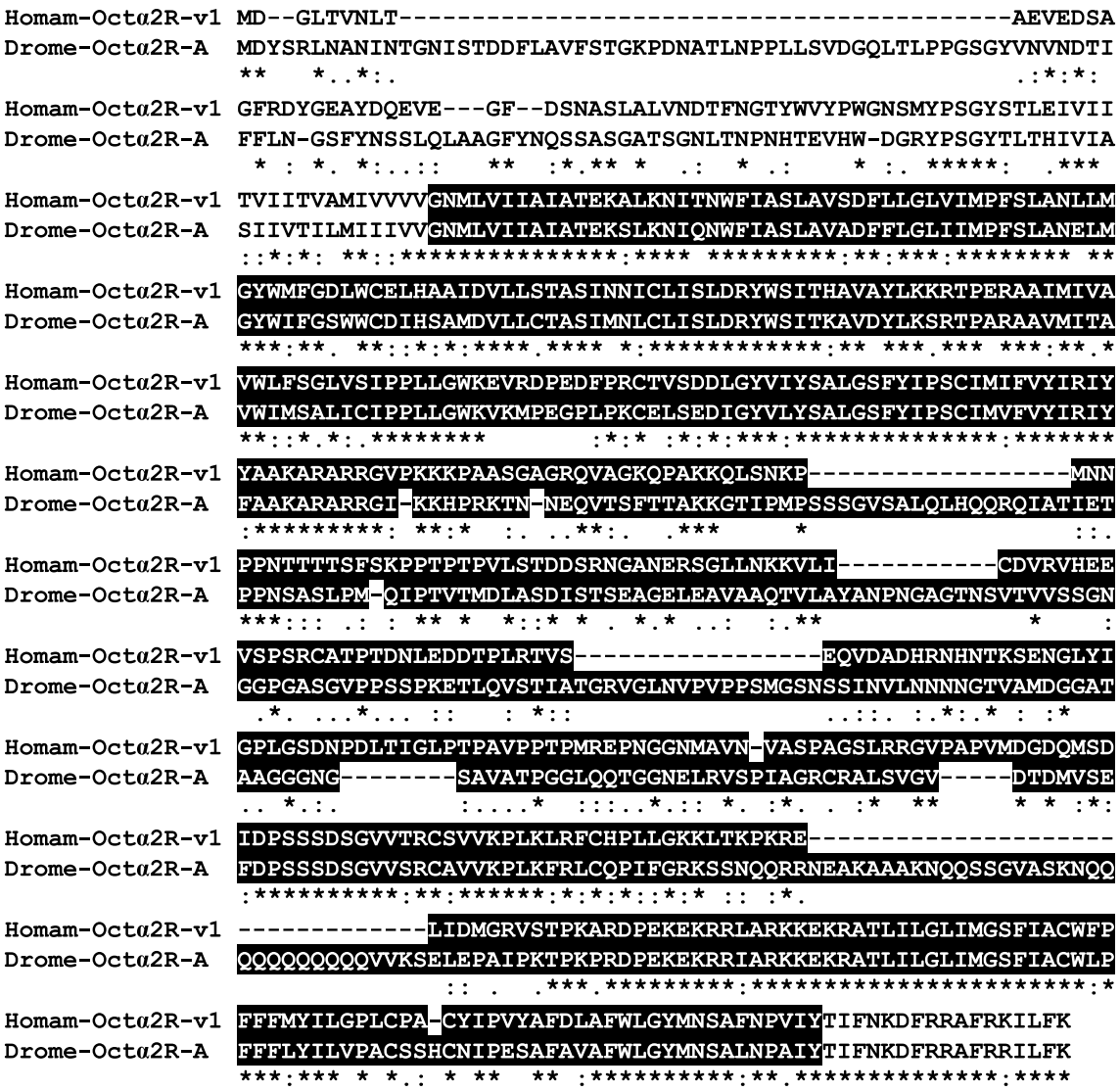

Fig. 4 MAFFT alignment of *Homarus americanus* octopamine alpha-2 receptor variant 1 (Homam-Octα2R-v1) and *Drosophila melanogaster* alpha-2-adrenergic-like octopamine receptor, isoform A (Drome-Octα2R-A; Accession No. AAF55598; Adams et al. 2000). In the line immediately below each sequence grouping, "*" 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

and eyestalk ganglia transcriptomes, full-length open reading frames for nine putative amine receptor-encoding transcripts were amplified from brain/eyestalk ganglia cDNAs. Among the sequences validated were the three newly identified transcripts (Homam-DopECR, Homam-Octα2R, and Homam-OTR-II), two transcripts with sequence extensions (Homam-OTR-I and Homam-5HTR1B), two receptor variants (Homam-DAβ1R variants 1 and 2), and two previously reported transcriptomic sequences (Homam-HA3R and Homam-5HTR7). For all of the targets, identity between the PCR-generated consensus and transcriptome-derived nucleotide sequences exceeded 99%. The cloned sequences have been submitted to GenBank: Homam-DopECR (Accession No. MN606036), Homam-DAβ1Rv1 (Accession No. MN606037), Homam-DAβ1Rv2 (Accession No. MN606038), Homam-Octα2R (Accession No. MN606039), Homam-OTR-I (Accession No. MN606040), Homam-OTR-II (Accession No. MN606041), Homam-HA3R (Accession No. MN606042), Homam-5HTR7 (Accession No. MN606043), and Homam-5HTR1B (Accession No. MN606044).

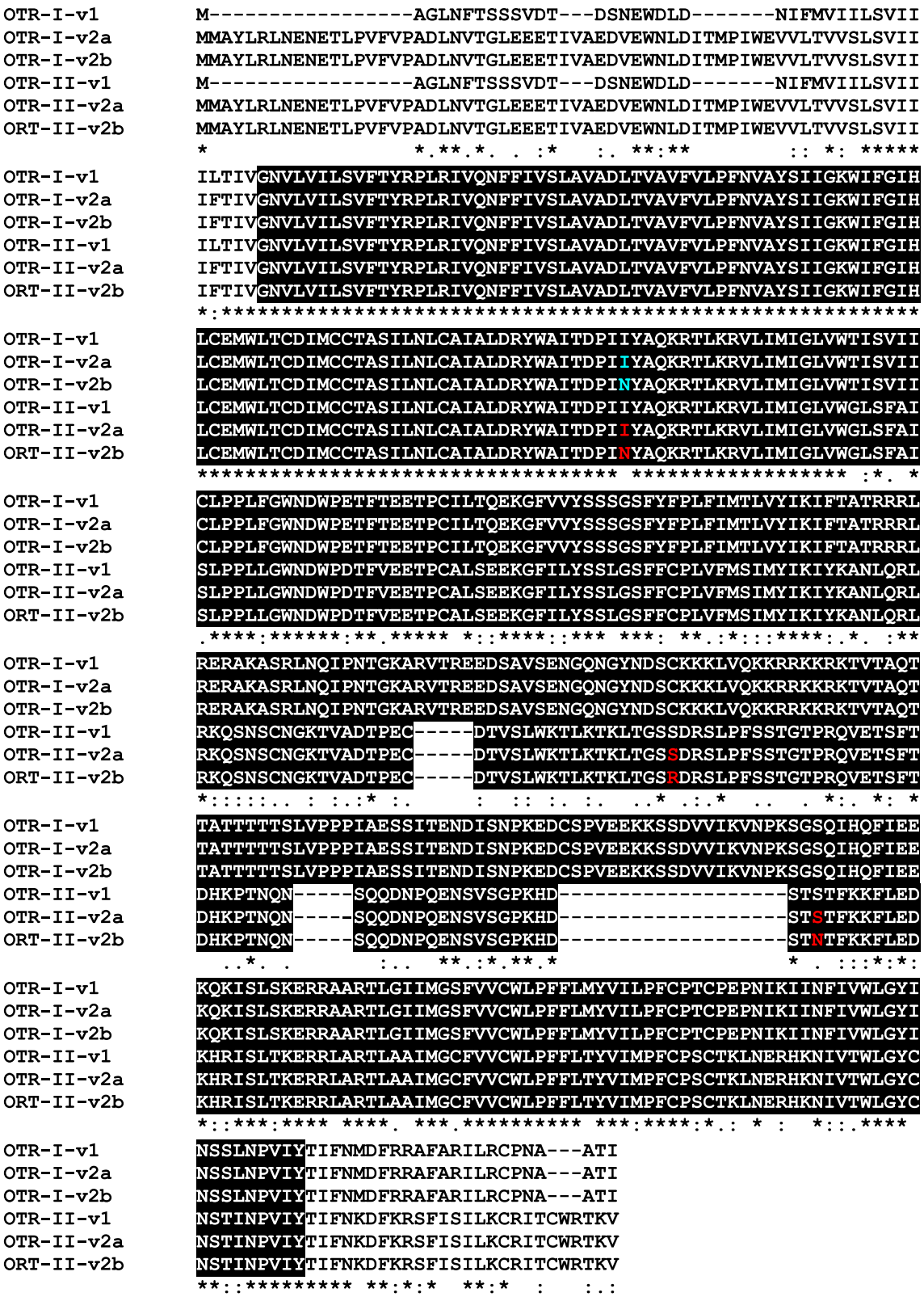

Fig. 5 MAFFT alignment of *Homarus americanus* octopamine–tyramine receptor I and II (Homam-OTR-I and II) proteins and their variants. In the line immediately below each sequence grouping, "*" indicates identical amino acid residues, while ":" and "." denote amino acids that are similar in structure between sequences. In this figure, rhodopsin family seven-transmembrane receptor domains identified by Pfam analyses are highlighted in black. Amino acid residues that vary between Homam-OTR-I-v2a and Homam-OTR-I-v2b are shown in blue, while those residues that vary between Homam-OTR-II-v2a and Homam-OTR-II-v2b are shown in red (color figure online)

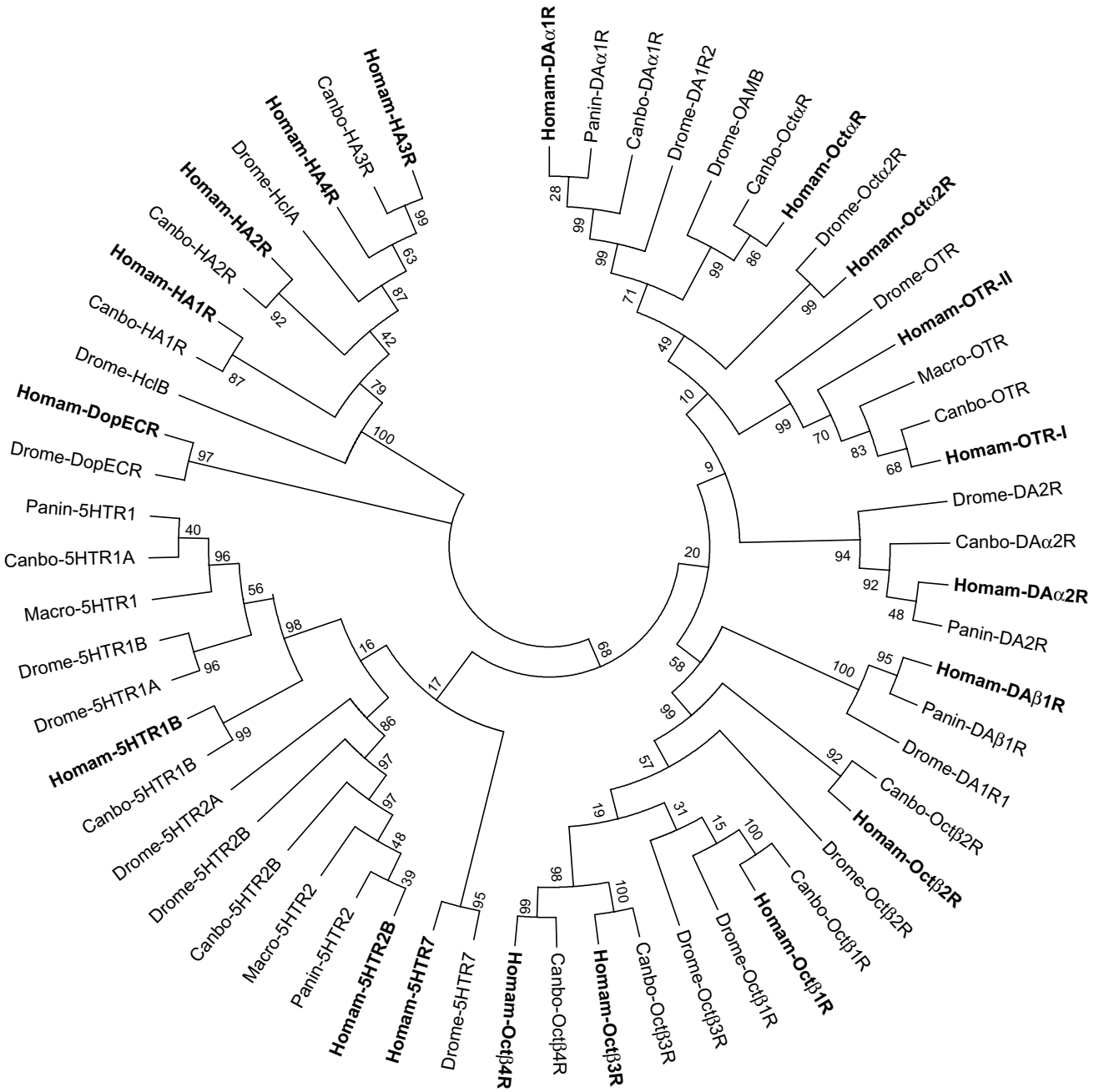

Fig. 6 Phylogenetic relationships among putative *Homarus americanus* amine receptors and select decapod and *Drosophila melanogaster* amine receptors. Maximum likelihood tree depicting relationships among putative amine receptor sequences. The percentage of trees in which the associated taxa clustered together (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Species abbreviations: Canbo, *Cancer borealis*; Drome, *Drosophila melanogaster*; Homam, *Homarus americanus*; Macro, *Macrobrachium rosenbergii*; Panin, *Panulirus interruptus*. Receptor subtypes abbreviations: 5HTR1A, serotonin receptor type 1A; 5HTR1B, serotonin receptor type 1B; 5HTR2, serotonin receptor type 2; 5HTR2A, serotonin receptor type 2A; 5HTR2B, serotonin receptor type 2B; 5HTR7, serotonin receptor type 7; DA1R1, dopamine 1-like receptor 1; DA1R2, dopamine 1-like receptor 2; DA2R, dopamine 2-like receptor; DAα1R, dopamine alpha-1 receptor; DAα2R, dopamine alpha-2 receptor; DAβ1R, dopamine beta-1 receptor; DopECR, dopamine/ecdysteroid receptor; HA1R, histamine 1 receptor; HA2R, histamine 2 receptor; HA3R, histamine 3 receptor; HA4R, histamine 4 receptor; Hcl, histamine-gated chloride channel; OAMB, octopamine receptor in mushroom bodies; OctαR, octopamine alpha receptor; Octα2R, octopamine alpha-2 receptor; Octβ1R, octopamine beta-1 receptor; Octβ2R, octopamine beta-2 receptor; Octβ3R, octopamine beta-3 receptor; Octβ4R, octopamine beta-4 receptor; OTR, octopamine–tyramine receptor; OTR-I, octopamine–tyramine receptor I; OTR-II, octopamine–tyramine receptor II. Accession numbers of sequences used in the phylogenetics analyses are listed in Supplementary Table 6

Putative Homarus americanus *amine receptors cluster in a clade-specific manner with other putative decapod and* Drosophila melanogaster *amine receptors*

As a final means of vetting the annotations assigned to the *H. americanus* receptors reported here, maximum likelihood-based phylogenetic relationships among other decapod and *D. melanogaster* amine receptors were examined (Fig. 6 and Supplemental Table 5). The *Homarus* receptors clustered with similarly annotated sequences, suggesting evolutionary conservation of the amine receptor system within the Decapoda.

Discussion

In the study presented here, amine receptor complement/diversity in the nervous system of *H. americanus* was re-evaluated using publicly accessible brain- and eyestalk ganglia-specific transcriptomes. These nervous system region-specific assemblies were produced using Trinity, which is well known for preserving evidence of splice/other variants. The data obtained from the mining of the brain and eyestalk ganglia transcriptomes complement and augment those reported previously from the mining of a mixed *H. americanus* nervous system transcriptome produced using the CLC Genomics Workbench Server, an assembler not known for preserving evidence of protein isoform diversity. All of the receptors identified from the mixed nervous system transcriptome were reidentified from brain and/or eyestalk ganglia transcripts. The proteins deduced from the brain and/or eyestalk ganglia transcripts provide full coverage for a number of receptors that were previously partial proteins. These findings also extend the complement of lobster nervous system amine receptors with the identification of transcripts encoding three new proteins. Evidence for putative splice and/or other variants was found for essentially all of the putative *Homarus* amine receptors. A number of these variants appear to be assembly-specific, suggesting the possibility of distinct brain and eyestalk ganglia amine receptor complements. If different receptors and/or their variants serve distinct functional roles, then the presence of region-specific receptor may provide a means for expanding the functional flexibility of aminergic signaling systems in different portions of the lobster nervous system.

Acknowledgements Lisa Baldwin and Dr. Colin Brent are thanked for reading and commenting on earlier versions of this article. This work was supported by funds from the National Science Foundation (IOS-1353023 [to AEC]; IOS-1856307 [to AEC]; IOS-1354567 [to PSD]; IOS-1856433 [to PSD]), the National Institutes of Health (INBRE Grant 8P20GM103423-12), the Cades Foundation (to AEC), and base CRIS funding from the US Department of Agriculture (Project No. 2020–22620-022-00D; to JJH). Mention of trade names or commercial products in this article is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the USDA; the USDA is an equal opportunity provider and employer.

Compliance with ethical standards

Conflict of interest None.

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