De novo transcriptome assembly of the cubomedusa *Tripedalia cystophora*, including the analysis of a set of genes involved in peptidergic neurotransmission

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**Abstract**

**Background:** The phyla Cnidaria, Placozoa, Ctenophora, and Porifera emerged before the split of proto- and deuterostome animals, about 600 million years ago. These early metazoans are interesting, because they can give us important information on the evolution of various tissues and organs, such as eyes and the nervous system. Generally, cnidarians have simple nervous systems, which use neuropeptides for their neurotransmission, but some cnidarian medusae belonging to the class Cubozoa (box jellyfishes) have advanced image-forming eyes, probably associated with a complex innervation. Here, we describe a new transcriptome database from the cubomedusa *Tripedalia cystophora*.

**Results:** Based on the combined use of the Illumina and PacBio sequencing technologies, we produced a highly contiguous transcriptome database from *T. cystophora*. We then developed a software program to discover neuropeptide preprohormones in this database. This script enabled us to annotate seven novel *T. cystophora* neuropeptide preprohormone cDNAs: One coding for 19 copies of a peptide with the structure pQWLRGRFamide; one coding for six copies of a different RFamide peptide; one coding for six copies of pQPQGVVamide; one coding for eight different neuropeptide copies with the C-terminal LWamide sequence; one coding for thirteen copies of a peptide with the C-terminal GRYamide sequence; and one coding for seven copies of a cyclic peptide, of which the most frequent one has the sequence CTGQMCWFRamide. We could also identify orthologs of these seven preprohormones in the cubozoans *Alatina alata*, *Carybdea xaymacana*, *Chironex fleckeri*, and *Chiropsalmus quadrumanus*. Furthermore, using TBLASTN screening, we could annotate four bursicon-like glycoprotein hormone subunits, five opsins, and 52 other family-A G protein-coupled receptors (GPCRs), which also included two leucine-rich repeats containing G protein-coupled receptors (LGRs) in *T. cystophora*. The two LGRs are potential receptors for the glycoprotein hormones, while the other GPCRs are candidate receptors for the above-mentioned neuropeptides.

**Conclusions:** By combining Illumina and PacBio sequencing technologies, we have produced a new high-quality de novo transcriptome assembly from *T. cystophora* that should be a valuable resource for identifying the neuronal components that are involved in vision and other behaviors in cubomedusae.

**Keywords:** Cnidaria, Cubozoa, Transcriptome, Vision, Opsin, Neuropeptide, Glycoprotein hormone, Biogenic amine, GPCR, LGR

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Background

Cnidarians are basal, multicellular animals such as Hydra, corals, and jellyfishes. They are interesting from an evolutionary point of view, because they belong to a small group of phyla (together with Placozoa, Ctenophora, and Porifera) that evolved before the split of deuterostomes (e.g. vertebrates) and protostomes (most invertebrates, such as insects), an event that occurred about 600 million years ago [1]. Cnidarians have an anatomically simple nervous system, which consists of a diffuse nerve net that sometimes is condensed (centralized) in the head or foot regions of polyps, or fused as a giant axon in polyp tentacles, or as a giant nerve ring in the bell margins of medusae [2–13].

The nervous systems from cnidarians are highly peptidergic: A large number of cnidarian neuropeptides have been chemically isolated and sequenced from cnidarians and their preprohormones have been cloned [14–33].

The cnidarian preprohormones often contain a high number of immature neuropeptide copies, ranging from 4 to 37 copies per preprohormone molecule [16–18, 20, 21, 23, 26, 27, 29, 33]. Each immature neuropeptide copy is flanked by processing signals: At the C-terminal sides of the immature neuropeptide sequences, these signals consist of the amino acid sequences GKR, GKK, or GR(R). The Arg (R) and Lys (K) residues are recognized by classical prohormone convertases (PC-1/3 or PC-2), which liberate the neuropeptide sequences, while the Gly (G) residues are converted into C-terminal amide groups by the enzyme peptidylglycine α-amidating monoxygenase [29, 34–36].

At the N-terminal sides of the immature cnidarian neuropeptide sequences, we very often find a Gln (Q) residue, which is cyclized into a pyroglutamate group (pQ) and which protects the N-terminus of the neuropeptide against enzymatic degradation [16–18, 20, 21, 29]. In contrast to higher metazoaans, however, the N-terminal processing sites preceding these Q residues are normally not dibasic residues, but often acidic (E or D) residues, or T, S, N, L, or V residues, suggesting the existence of novel endo- or aminopeptidases carrying out processing of cnidarian preprohormones [16–18, 20, 29]. These findings make it sometimes difficult to predict the N-terminus of a mature neuropeptide sequence from a cloned neuropeptide preprohormone. If a Q residue is found N-terminally of a PC 1/3 cleavage site preceded by acidic (E, D) or T, S, N, L or V residues, cleavage probably occurs N-terminally of this Q residue, yielding a protecting N-terminal pyroglutamate residue.

Cnidarian neuropeptides have a broad spectrum of biological activities, including stimulation of the maturation and release of oocytes (spawning) in hydrozoan medusa, stimulation or inhibition of metamorphosis in hydrozoan planula larvae, stimulation of nerve cell differentiation in hydrozoan polyps, and stimulation or inhibition of smooth muscle contractions in hydrozoans and sea anemones [28, 32, 33, 37–46].

In proto- and deuterostomes, neuropeptides normally act on G protein-coupled receptors (GPCRs), which are transmembrane proteins located in the cell membrane [47]. In cnidarians, one such GPCR has recently been identified (deorphanized) as the receptor for a hydromedusan neuropeptide that stimulates oocyte maturation [33]. GPCRs are metabotropic receptors that transmit their activation via second messengers and, because of the many steps involved, act relatively slowly. In cnidarians, however, some neuropeptides activate ionotropic receptors, such as the hydrozoan RFamide neuropeptides, which activate trimeric cell membrane channels belonging to the degenerin/epithelial Na⁺ channel (DEG/ENaC) family [48–52]. This peptidergic signal transmission via ligand-gated ion channels can be very fast.

Cnidarians probably also use protein hormones for their intercellular signaling. Already 25 years ago, we were able to clone a protein hormone receptor from sea anemones that was structurally closely related to mammalian glycoprotein receptors such as the ones that are activated by follicle stimulating hormone (FSH), luteinizing hormone (LH), or thyroid stimulating hormone (TSH) [53, 54]. Glycoprotein hormones are normally heterodimers. Such dimer subunits, however, have not been identified from cnidarians, so far.

Finally, cnidarians also use biogenic amines as neurotransmitters [55] and we have recently identified (deorphanized) a GPCR from Hydra magnipapilla that was a functional muscarinic acetylcholine receptor [56, 57]. The occurrence of this receptor gene, however, appears to be confined to hydrozoans and does not exist in other cnidarians [57].

The phyllum Cnidaria is generally subdivided into six classes: Hydrozoa (Hydra and colonial hydrozoans, such as Hydractinia), Anthozoa (such as sea anemones and corals), Scyphozoa (jellyfishes), Staurozoa (stalked jellyfishes), Cubomedusa (box jellyfishes), and Myxozoa (small obligate parasites). The nervous systems in animals belonging to these six classes all have the above-mentioned properties, for example they are all peptidergic, and their anatomy is diffuse with occasional centralizations [3–11]. However, many cubozoans, such as Tripedalia cystophora, have complex eyes, grouped together as six eyes on each of the four rhopalia, of which two eyes (the upper and lower lens eyes) are camera-type, image-forming eyes. These lower lense eyes are even able to adjust their pupils to light intensity [58–61]. One can expect that the innervation of these eyes and their signal processing must be unusually complex compared to the more basal signal transmission, occurring in other non-cubozoan cnidarians.
In our current paper, we are presenting a highly contiguous transcriptome database from *T. cystophora*, which was based on the combined use of Illumina and PacBio sequencing, that could help us to identify the neuronal components that are involved in the innervation and processing of vision in cubomedusae. We have also compared the quality of our transcriptome with that of other cubozoan transcriptomes, which showed that our transcriptome was of high quality. Finally, we have tested the transcriptome and identified a set of novel genes involved in peptidergic neurotransmission.

Results

De novo transcriptome by PacBio sequencing

We isolated RNA from 12 *T. cystophora* medusae, converted it into cDNA, and sequenced it, using the PacBio (Pacific Biosciences) sequencing technology (Additional file 1A-D). Comparison of this PacBio database with the Illumina reads (see below) gave us the information that some transcripts were missing in the PacBio database. We, therefore, carried out a second PacBio sequencing round of the same *T. cystophora* cDNA sample as mentioned above with the expectation that this would improve the completeness of the combined PacBio data set (Additional file 2A-C). All parameters in this second sequencing round were the same as in the first round. This second sequencing round improved our dataset considerably. In the following we give the combined data from the first and second sequencing rounds: Reads of interest (ROI; for definition see Additional file 1A), 645,865; containing 275,377 (42.64%) full length non-chimeric transcripts. After the Quiver polishing procedure (see Methods) we ended up with 88,588 high quality transcripts (mean quality index > 0.99) and 106,394 length non-chimeric transcripts. After the Quiver polishing procedure (see Methods) we ended up with 88,588 high quality transcripts (mean quality index > 0.99) and 106,394 length non-chimeric transcripts.

Error correction of the PacBio transcripts using Illumina reads

We also sequenced around 223 million paired-end reads from the Illumina X Ten platform, using *T. cystophora* cDNA derived for the same sample as the PacBio data. Around 204 million clean reads were generated, of which 99.3% had a base accuracy of 99 and 97.7% reads had a base accuracy of 99.9%. For an RNA-Seq pipeline outcome summary and quality assessment see Additional file 3. These short reads were subsequently used for correcting the PacBio consensus isoform sequences following two error correction pipelines, Proovread and LoRDEC (long read de Bruijn graph error correction) [62, 63] (see Additional file 4A and B).

Comparison of the *T. cystophora* transcripts with a set of eukaryotic universally conserved orthologues

In Additional file 5A-E we have compared the assembled transcripts of our *T. cystophora* transcriptome with those from other eukaryotes. From a Venn diagram (Additional file 5E), which can be regarded as an estimate of transcript assembly quality, one can conclude that from the 46,348 unigenes (transcripts) present in our database, 23,286 unigenes had universally conserved ortholog genes in common with the SwissProt, InterPro, Kyoto Encyclopedia of Genes and Genomes, and Eukaryotic Orthologue Group databases (=50%). These numbers compare well with other transcriptome databases.

Annotations of transcripts coding for neuropeptide preprohormones

Most cnidarian neuropeptide preprohormones have basic cleavage sites (KR, RR) at the C-terminal parts of their immature neuropeptide sequences, preceded by a glycine (G) residue, which, after cleavage of the preprohormone, is converted into a C-terminal amide group [21, 29]. Furthermore, cnidarian preprohormones very often have multiple copies of the immature neuropeptide sequences [21, 29]. Therefore, we wrote a software program in Python3 that was based on these preprohormone features and that only filtered protein-coding sequences from the transcriptome database that contained at least three similar amino acid sequences, each ending with the sequence GKR, GKK, or GR. The flow chart of our program is given in Additional file 6 and the software is given in Additional file 7. Furthermore, we have deposited our software at [64].

The application of our software program to the combined *T. cystophora* transcriptome databases (PacBio first and second round, and Illumina databases) detected seven putative neuropeptide preprohormones. Furthermore, many of these preprohormones could also be detected in transcriptomes from other cubozoan species:

(i) One complete preprohormone (having both a signal sequence and a stop codon in its cDNA) containing 19 copies of the neuropeptide sequence pQWLRGRFamide (named Tcy-RFamide-1) and one copy of pQFLGRFamide (named Tcy-RFamide-2) is present in the database from *T. cystophora* (Fig. 1, Table 1). It is interesting that, like in other cnidarian RFamide preprohormones [21, 29], these neuropeptide sequences are very often preceded by acidic (D or E) residues, suggesting that these
residues are processing sites and that the proposed neuropeptide sequences are correct. Similarly, we found a complete RFamide preprohormone in the transcriptome database from *A. alata* [65] that contained 18 copies of the neuropeptide pQWLRGRFamide, which is identical to Tcy-RFamide-1 (Fig. 1, Table 1). Most peptide sequences are preceded by acidic residues, while two peptide sequences are preceded by S residues (Fig. 1).

In the transcriptome database from the cubomedusa *Carybdea xaymacana*, we could identify an incomplete RFamide preprohormone lacking the signal sequence that contained 11 copies of a neuropeptide sequence that was identical to Tcy-RFamide-1 (Fig. 1, Table 1). This incompleteness of the preprohormone was likely due to multiple gaps present in the *C. xaymacana* Illumina transcriptome.

Similarly, the transcriptome assembly from the cubomedusa *Chiropsalmus quadrumanus* contained an incomplete preprohormone, having one copy of a neuropeptide identical to Tcy-RFamide-1 (Fig. 1, Table 1).

Finally, the transcriptome database from the cubomedusa *Chironex fleckeri* contained one incomplete preprohormone sequence coding for seven RFamide neuropeptides that were identical to Tcy-RFamide-1. Most peptides are copied by acidic residues, while one copy is preceded by a G and other copies by K residues.
| Species       | Preprohormone name | Peptide name   | Predicted peptide sequence | Copies |
|--------------|--------------------|----------------|----------------------------|--------|
| *T. cystophora* | Tcy-RFamide        | RFamide-1      | pQWLGRFamide               | 19     |
|              |                    | RFamide-2      | pQFLRGRFamide              | 1      |
| *A. alata*    | Aal-RFamide        | RFamide-1      | pQWLGRFamide               | 18     |
| *C. xaymacana*| Cxa-RFamide        | RFamide-1      | pQWLGRFamide               | 11     |
| *C. quadrumanus* | Cqu-RFamide     | RFamide-1      | pQWLGRFamide               | 1      |
| *C. fleckeri* | Cfl-RFamide        | RFamide-1      | pQWLGRFamide               | 7      |
| *T. cystophora* | Tcy-RFamide-II    | RFamide-II-1   | RFamide                    | 6      |
|              |                    | RFamide-II-1   | RFamide                    | 3      |
| *T. cystophora* | Tcy-VWamide        | VWamide-1      | pQPPGVWamide               | 6      |
|              |                    | VWamide-1      | pQPPGVWamide               | 6      |
| *A. alata*    | Aal-VWamide        | VWamide-1      | pQPPGVWamide               | 5      |
| *C. xaymacana*| Cxa-VWamide        | VWamide-1      | pQPPGVWamide               | 4      |
| *C. fleckeri* | Cfl-VWamide        | PAamide-1      | pQSPAamide                 | 1      |
|              |                    | NWamide-1      | pQGNWamide                 | 1      |
| *T. cystophora* | Tcy-LWamide        | Peptide-1      | GNPKGSILWamide             | 1      |
|              |                    | Peptide-2      | pQPGMWamide                | 1      |
|              |                    | Peptide-3      | SLVQPRNLNLWamide           | 1      |
| *A. alata*    | Aal-LWamide        | Peptide-1      | RAPKPFILWamide             | 1      |
|              |                    | Peptide-2      | pQPGMWamide                | 1      |
|              |                    | Peptide-3      | ALVKPRLDLLWamide           | 1      |
| *C. xaymacana*| Cxa-LWamide        | Peptide-2      | pQPGMWamide                | 1      |
| *C. fleckeri* | Cfl-LWamide        | Peptide-2      | pQPGMWamide                | 1      |
|              |                    | Peptide-3      | ALVKPRLDLLWamide           | 1      |
| *T. cystophora* | Tcy-RAamide        | RAamide-1      | RPRAamide                  | 13     |
|              |                    | RSamide-1      | pQPRSamide                 | 3      |
|              |                    | RGamide-1      | pQVLRPRGamide              | 1      |
| *A. alata*    | Aal-RAamide        | RAamide-1      | RPRAamide                  | 14     |
|              |                    | RGamide-2      | pQPRGamide                 | 3      |
| *C. xaymacana*| Cxa-RAamide        | RAamide-1      | RPRAamide                  | 2      |
Because PC 1/3-mediated processing could occur in between the RRR sequences (Additional file 8), the most likely products are six copies of RFamide. These RFamide sequences are very short compared to other known neuropeptides. For example, the shortest mammalian neuropeptide known is the tripeptide thyrotropin-releasing-hormone (TRH), pQHPamide [66], which, in contrast to the RFamide peptide, is N-terminally protected. We are, therefore, skeptical about the preprohormone status of Tcy-RFamide-II. A similar preprohormone as Tcy-RFamide-II can be identified in the A. alata database. Because this database only consists of Illumina reads, the complete preprohormone was difficult to assemble and the protein remained, therefore, incomplete (Additional file 8, Table 1).

No RFamide-II preprohormones could be identified in the transcriptome databases from the other cubomedusae.

(iii) In our T. cystophora transcriptome we could annotate a complete preprohormone that contained six copies of the proposed neuropeptide pQPPGVWamide (named Tcy-VWamide-1; Fig. 2, Table 1). Five of these neuropeptide sequences are preceded by either S or T residues, a phenomenon that we observed earlier [21, 29] suggesting, again, processing at unusual amino acid residues. A preprohormone that contained six copies of a neuropeptide that was identical to Tcy-VWamide-1 could also be annotated from the transcriptome of A. alata (Fig. 4, Table 1). Also here, most neuropeptide sequences are preceded by either S or T residues, suggesting unusual processing.

Also, in the transcriptome of C. xaymacana we could identify a complete preprohormone that contained five copies of a neuropeptide identical to Tcy-VWamide-1 (Fig. 2, Table 1). In addition, we could identify an incomplete preprohormone in the transcriptome from C. fleckeri that contained four neuropeptide copies identical to Tcy-VWamide-1. This precursor might also contain two other neuropeptide copies that were not annotated in the current study.
sequences that are different from Tcy-VWamide-1 (Fig. 2, Table 1).

We could not find a VWamide preprohormone in the transcriptome of C. quadrumanus, probably due to insufficient sequencing depth.

(iv) We could annotate a complete preprohormone in T. cystophora (named Tcy-LWamide) that contained seven neuropeptide copies with the C-terminal amino acid sequence LWamide and one copy of a peptide with the C-terminal MWamide sequence (Fig. 3, Table 1). For this preprohormone, it is difficult to predict the N-termini of each neuropeptide sequence, due to the uncertainties of N-terminal neuropeptide processing (Fig. 3, Table 1; see, however, below).

A similar complete preprohormone can be predicted from the transcriptome of A. alata (Fig. 3, Table 1), which has six copies of an LWamide, one copy of a MWamide, and one copy of an IWamide neuropeptide.

The transcriptomes from C. xaymacana, and C. fleckeri only contain incomplete fragments of an LWamide preprohormone, having one to three copies of the LWamide or MWamide neuropeptides (Fig. 3, Table 1).

We aligned the LWamide preprohormones from the four cubomedusa species, we could see that they contained discrete LWamide or MWamide peptide subsequences that were lying in a certain order from the N- to the C-termini. For example, peptide-2 (the second peptide from the N-terminus) in the preprohormones from T. cystophora, A. alata, C. xaymacana, and C. fleckeri always had the sequence ELQPGMWamide. When we would accept the existence of a hypothetical aminopeptidase processing C-terminally from the L residue [21], this subfamily would consist of four identical copies of pQPGMWamide (Table 2). Thus, each cubomedusan species would contain one copy of this predicted peptide situated at peptide position-2 of the LWamide preprohormone.

Peptide-3 (the third peptide from the N-terminus) always had the sequence A(or S)L(or M)VR(or K, or Q)PR(or K)LNL(or M)LWamide. This, then, is again a discrete peptide subfamily with a PRL or PKL core and an LWamide C-terminus (Table 2). Peptides-4 and -5, however (the fourth and fifth peptide from the N-terminus) have the C-terminus PR(or K)L(or M, V, or A)GLWamide and appear, therefore, to be related to each other (Table 2). Peptide-6 (the sixth peptide from the N-terminus) always has the C-terminal sequence PGKVGLWamide, which is different from the peptides located at the other positions (Table 2). In conclusion, discrete sequence signatures can be recognized in the peptide subfamilies positioned at peptide positions 1, 2, 3, 4, 5, and 6 (Table 2). We call the peptides belonging to these subfamilies peptide-1 to peptide-6 and not LWamide-1 to LWamide-6, because the peptides belonging to family-2 have the C-terminus MWamide.

![Fig. 2 Amino acid sequences of the complete VWamide preprohormone from T. cystophora, A. alata, C. xaymacana, and C. fleckeri. Residues and peptide sequences are highlighted as in Fig. 1. The VWamide preprohormone from T. cystophora (named Tcy-VWamide) contains six copies of Tcy-VWamide-1 (pQPPGVWamide), which are preceded by either S, T, or A residues. The VWamide preprohormone from A. alata contains six copies of a neuropeptide identical to Tcy-VWamide-1, which are preceded by either S, T, or R residues. The VWamide preprohormone from C. xaymacana contains five copies of Tcy-VWamide-1, each copy is preceded by either S, or T residues. The VWamide preprohormone from C. fleckeri contains four copies of Tcy-VWamide-1, one copy of a peptide with the PAamide C-terminal sequence (pQSPAamide), and one copy of a peptide with the NWamide C-terminal sequence (pQGNWamide) (Nielsen et al. BMC Genomics (2019) 20:175 Page 7 of 20)
Because of these discrete sequence signatures, the preprohormone fragments from *C. xaymacana* and *C. fleckeri* (Fig. 3) can easily be identified as a fragment containing (counted from the N- to the C-terminus) one copy of a peptide-2, −3, and −4 (*C. xaymacana*); and a fragment containing one copy of a peptide-2 and -3 sequence (*C. fleckeri*).

(v) In the transcriptome from *T. cystophora* we could annotate a complete preprohormone (name: Tcy-RAamide; Fig. 4) that contained thirteen copies of an RPRAamide neuropeptide, three copies of a PRSamide, and one copy of a PRGamide neuropeptide. In two cases (pQPRSamide, the first peptide, and pQVLTRPRGamide, the fourth peptide sequence counted from the N-terminus of the preprohormone, Fig. 4), the mature structures of the neuropeptide sequences can be readily predicted, because their Q residues are preceded by an acidic (E) or basic (R) residue, while for the other neuropeptide sequences the N-termini are uncertain (Fig. 4, Table 1). A similar complete RAamide preprohormone can be identified in the transcriptome from *A. alatina* (named Aal-RAamide, Fig. 4, Table 1). This preprohormone contains fourteen copies of a neuropeptide with the C-terminal sequence RPRAamide and three copies of a neuropeptide with a QPRGamide C-terminus. These last three peptides might have the mature structure pQPRGamide, while the N-termini of the other peptides are uncertain (Fig. 4).

In the transcriptome from *C. xaymacana*, *C. quadrumanus*, and *C. fleckeri*, we identified incomplete RAamide preprohormone fragments that contained between one and three copies of an RAamide or RSamide neuropeptide (Fig. 4, Table 1). One of the peptides located on the second preprohormone fragment from *C. quadrumanus* (Fig. 4) is preceded by an acidic residue and has the likely structure pQPRGamide (Table 1).

(vi) We identified a complete RYamide preprohormone (named Tcy-RYamide) in the transcriptome from *T. cystophora* that contained four copies of an RYa-mide neuropeptide. The first peptide located near the N-terminus of the preprohormone (Fig. 5) (named Tcy-RYamide-1) has the likely sequence TPPWVKGRYamide and is protected at its N-terminus by the two proline residues at positions 2 and 3 (protective imide bonds between residues 1 and 2, and 2 and 3) (Tables 1 and 3). The second peptide counted from the N-terminus of the preprohormone has the QMWHRQRYamide (named Tcy-RYamide-2) and is protected at its N-terminus by a pyroglutamate residue (Fig. 5, Tables 1 and 3). The
The third peptide counted from the N-terminus has the likely sequence APGWHHGRYamide (named Tcy-RYamide-3) and is N-terminally protected by its proline residue at amino acid position-2 (Fig. 5, Tables 1 and 3). The fourth peptide counted from the N-terminus has the probable sequence TPLWAKGRYamide and is protected at its N-terminus by the proline residue at amino acid position-2 (Fig. 5, Table 1 and 3).

In the transcriptome from *A. alatina* we could also annotate a complete preprohormone that was very similar to the RYamide preprohormone from *T. cystophora* (Fig. 5). When counted from the N-terminus by the C-terminus of this preprohormone, we could identify a peptide-1 that was nearly identical to Tcy-RYamide-1; a peptide-2 that was nearly identical to Tcy-RYamide-2; a peptide-3 that was completely identical to Tcy-RYamide-3; and a peptide-4 that was nearly identical to Tcy-RYamide-4 (Fig. 5, Tables 1 and 3).

In the transcriptome from *C. xaymacana* we could annotate an RY-amide preprohormone fragment that contained one copy of a peptide that was identical to Tcy-RYamide-3, and one that was very similar to Tcy-RYamide-4 (Fig. 5, Table 3).

| Species   | Peptide name          | Proposed mature peptide sequence | Position from the N-terminus of the preprohormone |
|-----------|-----------------------|----------------------------------|--------------------------------------------------|
| *T. cystophora* | Tcy-LWamide / peptide-1 | GNP KGSILWamide                  | 1                                                 |
| *T. cystophora* | Aal-LWamide / peptide-1 | RAP RKPFILWamide                 |                                                   |
| *T. cystophora* | Tcy-LWamide / peptide-2 | pQP GMWamide                     | 2                                                 |
| *T. cystophora* | Aal-LWamide / peptide-2 | pQP GMWamide                     |                                                   |
| *C. xaymacana*  | Cfl-LWamide / peptide-2 | pQP GMWamide                     |                                                   |
| *C. fleckeri*   | Cxa-LWamide / peptide-2 | pQP GMWamide                     |                                                   |
| *T. cystophora* | Tcy-LWamide / peptide-3 | SLYQPRLNLWamide                  | 3                                                 |
| *A. alatina*    | Aal-LWamide / peptide-3 | AMVRPAVLNLWamide                 |                                                   |
| *C. xaymacana*  | Cfl-LWamide / peptide-3 | ALVRPAVLNLWamide                 |                                                   |
| *C. fleckeri*   | Cxa-LWamide / peptide-3 | ALVKPRDLWamide                   |                                                   |
| *T. cystophora* | Tcy-LWamide / peptide-4 | AMKEESPRLGLWamide                | 4                                                 |
| *A. alatina*    | Aal-LWamide / peptide-4 | ALKS KP MGLWamide                |                                                   |
| *C. xaymacana*  | Cfl-LWamide / peptide-4 | ALKEN PGMLWamide                 |                                                   |
| *T. cystophora* | Tcy-LWamide / peptide-5 | REMLERPKVLWamide                 | 5                                                 |
| *A. alatina*    | Aal-LWamide / peptide-5 | GKM GC PQAGLWamide               |                                                   |
| *T. cystophora* | Tcy-LWamide / peptide-6 | SSKPGKVGLWamide                  | 6                                                 |
| *A. alatina*    | Aal-LWamide / peptide-6 | TSEP GK VGLWamide                |                                                   |
| *T. cystophora* | Tcy-LWamide / peptide-7 | PDRPTEGLWamide                   | 7                                                 |
| *A. alatina*    | Aal-LWamide / peptide-7 | DADA VD LWamide                  |                                                   |
| *T. cystophora* | Tcy-LWamide / peptide-8 | KGKG PTVGLWamide                 | 8                                                 |
| *A. alatina*    | Aal-LWamide / peptide-8 | KP KDAGI Wamide                  |                                                   |

Table 2 Distinct LWamide neuropeptide subfamilies located on the cubomedusan LWamide preprohormones. These neuropeptide subfamilies are located at a certain order (last column) counted from the N- to C-termini of the preprohormones. Identical amino acid residues are highlighted in yellow, non-identical residues in grey.
In the *C. fleckeri* transcriptome we could annotate an RYamide preprohormone fragment that contained one copy of a peptide that was very similar to Tcy-RYamide-2 (Fig. 5, Table 3).

(vii) We could annotate a complete preprohormone in the Illumina Sequence Read Archive, SRA (NCBI accession number SRR779134), but not in the PacBio or Transcriptome Shotgun Assembly (TSA) database of *T. cystophora* that contains seven similar copies of an FRamide peptide (Fig. 6, Table 1). In *A. alata*, the preprohormone (named Aal-RAamide) contains 14 peptide copies with the RPRAamide C-terminus, and three copies of pQPRamide (Table 1). In *C. xaymacana*, we identified a small preprohormone fragment (named Cxa-RAamide) that contained two peptide copies with the RPRAamide C-terminal sequence, and one copy with the VPRAamide C-terminal sequence (Table 1).

In the *C. quadrumanus* transcriptome we identified two small preprohormone fragments (probably part of one preprohormone named Cqu-RAamide) that contained two copies of a RPRAamide peptide and one peptide with the sequence pQPRamide (Table 1).

In *C. fleckeri* we identified a small preprohormone fragment (named Cfl-RAamide) that contained three copies of a peptide with the C-terminal sequence RPRAamide (Table 1).

Two of these copies were identical to Tcy-FRamide-1, two other copies were identical to Tcy-FRamide-3, one copy was identical to Tcy-FRamide-2, while one copy had a new sequence CEGQMCWFRamide (Fig. 6, Table 1).

We found an incomplete preprohormone fragment in the transcriptome from *C. fleckeri* that contained one copy of an FRamide peptide identical to Tcy-FAamide-1, and another one identical to Tcy-FAamide-2 (Fig. 6, Table 1). It is interesting that for all these proposed cubozoan FRamide peptides only the amino acid residues in position 2 were variable (being either T, K, V, or E), while the others were preserved.

**Presence of glycoprotein hormone transcripts**

TBLASTN screening using various mammalian and insect glycoprotein hormone sequences as a query identified four complete glycoprotein hormone subunits in our combined transcriptome database from *T. cystophora* (Fig. 7). When we applied the same procedure to the transcriptome database from *A. alata* we could identify four orthologues to the *T. cystophora* glycoprotein hormone subunits (Fig. 7).

Generally, glycoprotein hormone (GPH) subunits have eleven cysteine residues, of which 10 are used for intramolecular cystine bridges, while one (number 6 in Fig. 7) is...
used for connecting the two subunits to form a functional ligand. Figure 7 shows that the four cubomedusan GPH subunits probably form the same cysteine bridges as the other metazoan GPHs, for example the two subunits from *Drosophila bursicon*.

**Annotation of leucine-rich repeats-containing G protein-coupled receptors (LGRs)**

The presence of four glycoprotein hormone subunits (yielding at least two heterodimeric glycoprotein hormone ligands) in *T. cystophora* strongly suggests the presence of Leu-rich G protein-coupled receptors (LGRs), which in mammals, insects, and other invertebrates are the receptors for glycoprotein hormones [47, 67–70]. Furthermore, already in 1993, we cloned an LGR from sea anemones [53, 54], indicating that LGRs might be present in all cnidarians. Using the sea anemone LGR and several mammalian and insect LGRs as queries in a TBLASTN search, we were able to identify two LGR transcripts in the database from *T. cystophora* and one LGR in the transcriptome from *A. alata* (Table 4, Fig. 8, Additional file 9). Figure 8b explains that LGRs can be classified into three types, type-A, -B, and -C, depending on specific domains in their extracellular N-termini [70]. These criteria identify the two *T. cystophora* LGRs as being type-A and -B, respectively (Fig. 8a). The single LGR from *A. alata* is a type-B LGR and an orthologue of the type-B LGR from *T. cystophora*.

We assume that the absence of the A-type LGR family member in *A. alata* is due to insufficient coverage of the assembled transcriptome from this cubomedusa.

**Presence of opsins**

It is known that cubomedusae and other cnidarians produce opsins [13, 61, 71–75]. We used cnidarian, and other invertebrate and vertebrate opsins as queries in a TBLASTN search of our *T. cystophora* transcriptome and found five different opsins (Fig. 9 and Table 4). A similar screening of the *A. alata* transcriptome yielded two opsins, which were orthologues of two of the *T. cystophora* opsins (Fig. 9).
Presence of neuropeptide and biogenic amine GPCRs

We used all Drosophila neuropeptide and biogenic amine GPCRs [47] as queries in TBLASTN searches of our T. cystophora transcriptome. In this way, we identified 22 GPCRs, which the TBLASTN search software described as neuropeptide GPCR-like and 28 GPCRs which the search software described as biogenic amine GPCR-like (Table 4). A similar TBLASTN search of the A. alatina transcriptome identified 22 neuropeptide GPCR-like receptors and 18 biogenic amine GPCR-like receptors. When we carried out a phylogenetic tree analysis of these receptors together with the T. cystophora and A. alata opsins and LGRs (see above), we found that the opsins and LGRs were sorted as discrete, structurally related clusters (Fig. 10). For the receptors that the TBLASTN software identified as neuropeptide or biogenic amine GPCRs, however, no such homogeneous clustering could be observed and all receptors were distributed randomly (Fig. 10). These results make it difficult to predict whether a certain GPCR is a neuropeptide or biogenic amine receptor.

Fig. 6 Amino acid sequences of the FRamide preprohormones from T. cystophora, A. alata, and C. fleckeri. Residues and peptide sequences are highlighted as in Fig. 1. The T. cystophora preprohormone produces four copies of a neuropeptide with the sequence CTGQMCWFRamide (named Tcy-FRamide-1), two copies of CKGQMCWFamide (Tcy-FRamide-2), and one copy of CVGQMCWFamide (Tcy-FRamide-3). The preprohormone sequence of A. alata produces two copies of a peptide identical to Tcy-FRamide-1, two copies of a peptide identical to Tcy-FRamide-3, one copy of a peptide identical to Tcy-FRamide-2, and two copies of CEGQMCWFamide (Table 1). The preprohormone fragment from C. fleckeri contains one copy of a peptide identical to Tcy-FRamide-1 and one peptide copy identical to Tcy-FRamide-2 (Table 1). All peptides contained in the three preprohormones are nearly identical in structure and only vary in the second amino acid residue, being either T, K, V, or E.

Table 3 Distinct neuropeptide subfamilies located on the RYamide preprohormones from different cubomedusan species. These neuropeptide subfamilies are located at discrete positions and counted from the N- to C-termini of the preprohormones. The amino acid residues are marked as in Table 2.

| Species          | Peptide name          | Proposed mature peptide sequence | Position from the N-terminus of the preprohormone |
|------------------|-----------------------|----------------------------------|-----------------------------------------------|
| T. cystophora    | Tcy-RYamide-1 / Peptide-1 | TPPWVKGRYamide                  | 1                                             |
|                  | Aal-RYamide-1 / Peptide-1 | TPPWIKGRYamide                  |                                               |
| T. cystophora    | Tcy-RYamide-2 / Peptide-2 | PQMHRQRYamide                   | 2                                             |
|                  | Aal-RYamide-2 / Peptide-2 | PQLWFLQRYamide                  |                                               |
| C. fleckeri      | Cfl-RYamide-2 / Peptide-2 | PQLMYKGRYamide                  |                                               |
| T. cystophora    | Tcy-RYamide-3 / Peptide-3 | APGWHHGRYamide                  | 3                                             |
|                  | Aal-RYamide-3 / Peptide-3 | APGWHHGRYamide                  |                                               |
| C. xaymacana     | Cfl-RYamide-3 / Peptide-3 | APGWHHGRYamide                  |                                               |
| T. cystophora    | Tcy-RYamide-4 / Peptide-4 | TPLWAKGRYamide                  | 4                                             |
|                  | Aal-RYamide-4 / Peptide-4 | GPMWFGRYamide                   |                                               |
| C. xaymacana     | Cfl-RYamide-4 / Peptide-4 | NPWAKGRYamide                   |                                               |
In this article we described a high-quality transcriptome database from the cubomedusa *T. cystophora*, which was constructed by the combined use of Illumina and PacBio sequences, and which we made freely accessible to global researchers (NCBI accession numbers SRR7791343-SRR7791345 and GGWE0100000). The longer PacBio sequences were needed for the correct assembly of the shorter Illumina sequences, especially when these Illumina sequences coded for neuropeptide preprohormones, which often contained repetitive sequences (see, for example, Table 1). The PacBio sequences were also needed for the correct annotations of full length GPCRs (Figs. 8, 9, and 10). The Illumina sequences, on the other hand, were necessary to correct for point mutations in the PacBio sequences. In addition, we developed a bioinformatics tool to search the transcriptome database for the presence of neuropeptide preprohormones, which turned out to be a highly versatile script and superior to ordinary TBLASTN searches, using neuropeptide sequences from bilaterian metazoans as queries. Finally, we tested the transcriptome database for its quality and completeness by annotating several components of peptidergic signaling and by comparing these results from our transcriptome with those from other freely accessible transcriptomes from cubozoans [65, 76, 77].

We identified the same number of neuropeptide preprohormone genes (seven) in the transcriptomes from *T. cystophora*, and *A. alata*, while we found six neuropeptide genes in the *C. fleckeri* transcriptome, five neuropeptide genes in the *C. xaymacana* transcriptome, and two neuropeptide genes in the *C. quadrumanus* transcriptome (Table 1, Figs. 1, 2, 3, 4, 5 and 6). In most cases only incomplete preprohormone fragments could be identified in the transcriptomes from *C. fleckeri*, *C. xaymacana*, and *C. quadrumanus*, while always complete preprohormones (including a signal peptide and a stop codon) were identified in the transcriptome from *T. cystophora*, and with one exception in the transcriptome from *A. alata* (Figs. 1, 2, 3, 4, 5 and 6). These findings already suggest that the transcriptomes from *T. cystophora* and *A. alata* [65] are of much better quality (more complete) than the...
transcriptomes from \textit{C. fleckeri}, \textit{C. xaymacana}, and \textit{C. quadrumanus} \cite{76,77}.

When we annotated GPCRs, we discovered 50 neuropeptide and biogenic amine GPCRs in the transcriptome from \textit{T. cystophora} and 34 of these GPCRs in the transcriptome from \textit{A. alata} (Table 4). For the LGRs, these numbers were two in the \textit{T. cystophora} transcriptome and one in the \textit{A. alata} transcriptome. For the opsins, we found five in the \textit{T. cystophora} transcriptome and two in the \textit{A. alata} transcriptome. These somewhat lower numbers of annotated GPCRs in the \textit{A. alata} transcriptome \cite{65} might be due to the fact that this transcriptome is only assembled from short Illumina transcripts, while our \textit{T. cystophora} transcriptome contains a large number of long PacBio transcripts with a length of up to 5000 bp (Additional file 2A), which would favor the detection of longer proteins, such as GPCRs.

The number of opsins (five) that we found in our transcriptome is lower than the number of opsin genes (eighteen: Tcop1-Tcop18) claimed by Liegertova et al. \cite{74} to be present in the genome from \textit{T. cystophora}. However, this last claim cannot be checked, because the genomic sequences from \textit{T. cystophora} have not been made publicly available \cite{74}. Our identified opsin Tcy 38276 (Fig. 9) is identical to the \textit{Tripedalia} c-opsin cloned previously \cite{75} and opsin Tc-neo \cite{73}; it corresponds to the opsin gene Tcop18 \cite{74}. Our opsin Tcy 32089 (Fig. 9) was cloned previously as the lens eye opsin Tc-leo \cite{73} and corresponds to the opsin gene Tcop13 \cite{74}. Tcy 9518 and Tcy 4539 (Fig. 9) correspond to the opsin genes Tcop5 and Tcop9, respectively \cite{74}. The last of the five opsins that we identified, Tcy 37162 (Fig. 9), is new.

Some of the neuropeptides that we predicted from the seven \textit{T. cystophora} preprohormone cDNAs (Tables 1, 2...
and 3) are identical or very similar to earlier chemically isolated and sequenced cnidarian neuropeptides. The Tcy-RFamide preprohormone (Table 1), for example, contains 19 copies of the predicted neuropeptide sequence pQWLRGRFamide (Tcy-RFamide-1), which is identical to the isolated and sequenced scyphozoan neuropeptide Cyanea-RFamide-I and very similar to the hydrozoan neuropeptides Pol-RFamide-II and Hydra-RFamide-I (Table 5). The C-terminal GRFamide sequence has been found in isolated or cloned neuropeptides from every cnidarian species investigated so far and the GRFamide neuropeptide family, therefore, appears to be universal in Cnidaria. The Tcy-VWamide preprohormone (Table 1) produces six copies of the predicted neuropeptide pQPPGVWamide (Tcy-VWamide-1), which resembles a previously isolated sea anemone neuropeptide metamorphosin-A [19], and the Hydra neuropeptide Hym331/Hydra-LWamide-I [32] (Table 6). Also, peptides belonging to the Tcy-LWamide preprohormone, such as peptides −4, −5, and −6 (Tables 1 and 2) clearly resemble metamorphosin-A, with which they have the C-terminal sequence GLWamide in common (Table 6). Preprohormones containing GLWamide peptides have recently been identified in the transcriptomes from the hydrozoans Clythia hemispheria and Cladonema pacificum [33]. Thus, like the GRFamides, also GLWamide neuropeptides appear to be widespread in cnidarians.

The Tcy-RAamide preprohormone contains 13 copies of RPRAamide (Table 1). RPRAamide peptides and a corresponding preprohormone have recently been identified in the transcriptome from the hydrozoan C. pacificum [33], suggesting that also these neuropeptides might have a broad distribution.

The last two preprohormones presented in Table 1, Tcy-RYamide and Tcy-FRamide (see also Fig. 5, and Fig. 6), are completely novel sequences and also their neuropeptide constituents have not been published earlier. These results show that our T. cystophora transcriptome contains novel and highly useful data for understanding neurotransmission in cubozoans and possibly also in other cnidarians.

As a next practical step, we will raise specific antisera against the major neuropeptides produced by the seven preprohormones (Table 1) and clarify which neuronal subpopulations can be stained by them. These experiments will certainly give us important information on the neuroanatomy of T. cystophora and will also tell us which of these peptidergic nerve nets will innervate the eyes.

In conclusion, we are presenting a high-quality transcriptome from T. cystophora, which will be a useful resource for the scientific community to better understand the biology of early metazoans and the evolution of important tissues and organs, such as nervous systems and eyes.

Methods
T. cystophora culture and collection
T. cystophora (Conant 1897) were cultured in 250 l tanks with recycled sea water at 28 °C and fed with Artemia salina once a day. The light: dark cycle was 8:16 h. We sampled medusae of various stages with a bell diameter ranging between 3.5 and 9 mm. A total of 12 medusae were collected after 48 h of starvation.

RNA extraction
Total RNA was extracted using the NucleoSpin RNAII-kit (Macherey-Nagel, Düren, Germany) following the manufacturer’s instruction. Total RNA was dissolved in RNase-free water and RNA integrity was verified by gel electrophoresis. The RNA concentration and purity was...
measured with a Nanodrop ND-2000 spectrophotometer (NanoDrop products, Wilmington, DE, USA).

**cDNA library construction**

Total RNA samples were shipped on dry ice to an affiliation of the Beijing Genome Institute (BGI Tech Solutions, Hong Kong) for library preparation, sequencing, and bioinformatic analysis (coordinated by BGI Tech Solutions, Shenzhen, China). Sample quality and RNA concentrations were checked using the Agilent model 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) and approved for sequencing (RNA Integrity Number, RIN: 9.7, and 28S/18S: 1.7). 8.6 microgram RNA was used to construct two cDNA libraries.
separately. The PacBio Iso-Seq libraries with a size of 0-5 kb (Pacific Biosciences, Menlo Park, CA, USA) were generated for sequencing on two SMRT cells and one RNA-Seq library was prepared for sequencing with Illumina X Ten (Illumina, San Diego, CA, USA).

PacBio Iso-Seq de novo assembly and read correction
Additional file 4A and B give an overview of the PacBio sequencing procedures and data processing. The bioinformatic data processing and error corrections were conducted at Beijing Genome Institute (BGI Tech Solutions, Shenzhen, China) following the PacBio Iso-Seq De novo protocol. The raw reads generated from the SMRT (Single Molecule Real Time) pipeline were separated into FL (full length) non-chimeric, non-FL and chimeric ROI. The chimerae, which were artificial contatemers fusion genes, were removed by this step. The FL non-chimeric ROI’s were defined as having the 5′ prime, 3′ prime-adapters and a polyA tail. The FL non-chimeric ROI’s were assembled to generate transcripts of all FL non-chimeric and non-FL non-chimeric sequences. For each assembled transcript, the Quiver error self-correction (polishing) software was run [78]. These corrected transcripts were divided into a high quality (hq; expected accuracy ≥99%, or QV ≥ 30) and a low quality (lq; expected accuracy < 99%, due to insufficient coverage or rare transcripts) subset. Even though the error rate was reduced by this procedure, a further correction was performed using Illumina RNA-Seq reads from the same sample (see below) and two additional bioinformatic packages, proovread [62] and Long Read de Bruijn Graph Error Correction (LoRDEC) [63]. Default parameters applied in proovread were -t 5 -b 200 -e 0.4 -s 3 -T 4 and k-mers 21 and 25 were used in LoRDEC. For details on the bioinformatic pipeline see Additional file 4.

Illumina RNA-Seq data processing
Illumina sequencing was performed with the Illumina X Ten machine using standard procedures and FastQC tools [79, 80]. Raw reads were subjected to quality filtration [81]. The filtering procedure performed to obtain “clean reads” with a high quality score, included the following steps: 1) Reads with adaptor sequences were removed 2) Reads in which the percentage of unknown bases (N) > 10% were removed 3) Low quality reads consisting of more than 40% low quality bases (value ≤ 5) and having a Phred score less than 20, were also removed.

Identification of neuropeptides, protein hormones, and GPCRs
We developed a software program to identify putative neuropeptides (Additional files 6 and 7). This program was compiled using Python3 [82]. Our software was based on recognizing and counting prohormone convertase processing sites in the amino acid sequence. Because many mature neuropeptides are amidated, the preprohormone often contains a C-terminal glycine before the basic amino acid processing sites. This was accounted for in the

Table 5 Amino acid residue identities (highlighted in yellow) between Tcy-RFamide-1 and some chemically isolated and sequenced ("established") cnidarian neuropeptides

| Species            | Peptide name       | Peptide sequence | Reference |
|--------------------|--------------------|------------------|-----------|
| T. cystophora      | Tcy-RFamide-1      | pQWLGRGFamide    | This paper|
| C. lamarckii       | Cyanea-RFamide-1   | pQWLGRGFamide    | [24]      |
| P. penicillatus    | Pol-RFamide-II     | pQWLGRGFamide    | [15]      |
| H. magnipapillata  | Hydra-RFamide-I    | pQWLGRGFamide    | [22]      |

Table 6 Amino acid residue identities (highlighted in yellow) between the isolated and sequenced sea anemone neuropeptide metamorphosin-A, the Hydra peptide Hym331, and some of the T. cystophora peptides contained in the Tcy-VWamide and Tcy-LWamide preprohormones (see Table 1)

| Species                        | Peptide name             | Peptide sequence   | Reference |
|--------------------------------|--------------------------|--------------------|-----------|
| An. elegantissima              | Metamorphosin A          | pQPQGLWamide       | [19]      |
| T. cystophora                  | Tcy-VWamide-1           | pQPQGLWamide       | This paper|
| H. magnipapillata              | Hym331                   | GPPGVLamide        | [25, 32]  |
| T. cystophora                  | Tcy-LWamide-peptide-2    | pQPQGLWamide       | This paper|
| T. cystophora                  | Tcy-LWamide-peptide-4    | PRLGLamide         | This paper|
| T. cystophora                  | Tcy-LWamide-peptide-5    | PKVGLamide         | This paper|
| T. cystophora                  | Tcy-LWamide-peptide-6    | GKVGLamide         | This paper|
searches (e.g. searching for ‘GKR’, ‘GKK’ and ‘GR’ motifs). For each sequence from the TSA (transcriptome shotgun assembly), the sequence was translated into all 6 reading frames and split into possible open reading frames. In all of the open reading frames, the number of processing sites was counted. If there were at least 3 processing sites within an open reading frame, the putative neuropeptide sequences were aligned to assess the similarity of the mature peptides. The open reading frames with highly similar peptide sequences were then manually curated to reject further unlikely preprohormones that were not discarded during the automated screening. The flow chart of the program can be seen in Additional file 6. The code can be during the automated screening. The flow chart of the program can be seen in Additional file 6. The code can be found at [64] and in Additional file 7. The presence of signal peptides was determined by SignalP 4.1 [83, 84]. We identified glycoprotein hormones and GPCRs in the T. cystophora and A. alata transcriptomes by homology based searches. These searches were done with known cnidian and other invertebrate and vertebrate protein sequences as search queries using TBLASTN [85] with default settings.

Phylogenetic tree analyses and accession numbers
For phylogenetic tree analyses (Figs. 8, 9, 10), the protein sequences were aligned using t-coffee. The alignments were read and analyzed in PAUP* by neighbor joining using p-distance and bootstraps of 100 repeats. The majority rule consensus trees were visualized using iTOL. Only bootstrap values above 50 were given in the figures. For Fig. 7, the following accession numbers for the Drosophila sequences were used: Dmel-bursaβ, NP_650983; Dmel-bursaβ, NP_609712. For Fig. 8, the following accession numbers were used: Hma LGR1, XP_002155960; Hma LGR2, NP_001267732; Ael LGR, CAA82186; Nve LGR1, XP_001641580; Nve LGR2, XP_001635321; Nve LGR3, XP_001638153; Hsa FSHR, NP_000136; Hsa TSHR, NP_000360; Hsa LHR, NP_000224; Hsa LGR4, NP_060960; Hsa LGR5, NP_003658; Hsa LGR6, NP_001017403; Hsa LGR7, NP_006747; Hsa LGR8, AAL69324. For Fig. 9, Hsa ops-in-blue, NP_001699; Hsa ops-in-red, NP_064445; Hsa ops-in-green, NP_000504; Hsa-rhodopsin, NP_000530.

Additional files

- **Additional file 1:** A: Quality assessment of PacBio data. B: Read length distribution of ROIs from the first PacBio sequencing round. C: Read length classification summary of the first PacBio sequencing round. D: PacBio output summary from the first PacBio sequencing round. (DOCX 76 kb)
- **Additional file 2:** A: Read length distribution of all ROIs from combined data from the first and second PacBio sequencing rounds. B: Read length classification summary of the combined data from the first and second sequencing rounds. C: PacBio output summary of the combined PacBio data from the first and second sequencing rounds. (DOCX 61 kb)
- **Additional file 3:** Illumina HiSeq X Ten pipeline output summary. (DOCX 17 kb)

Abbreviations
Aal: Aiptasia alata; AeI: Anthopleura elegantissima; BGI: Beijing Genome Institute; Burs: Buriscorn; CFl: Chironex fleckeri; Cq: Chiropterus quadratus; Cxa: Carybdea xaymacana; DEG/ENaC: Degenin/epithelial Na + channel; Dme-burs: Drosophila melanogaster bursicon; DPAP: Dipeptidyl aminopeptidase II; FL: Full length; FSH: Follicle-stimulating-hormone; GPCR: G Protein-coupled receptor; GPH: Glycoprotein hormone; Hma: Hydra magnipapillata; hq: High quality; Hsa: Homo sapiens; ICE: Iterative clustering for error correction; LGR: Leucine-rich repeats containing G protein-coupled receptor; LH: Luteinizing hormone; LoRDEC: Long read de Bruijn graph error correction; Lq: Low quality; Nve: Nemastoma vectens; PacBio: Pacific Bioscences; PC: Phorhormone convertase; p2: Pyroglutamate residue (cycdized glutamine residue); RO: Reads of insert; SMRT: Single molecule real time; SRA: Sequence read archive; Tcy: Tripedalia cystophora; TRH: Tryptoisin-releasing-hormone; TSA: Transcriptome shotgun assembly; TSH: Thyroid-stimulating-hormone

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Availability of data and materials
The DNA sequences reported in this paper have been submitted to the GenBank Data Bank with accession numbers: MH423430-MH423435, MH464085, MH464087-MH464105, MH605357-MH705358, and MH835292-MH835333. Our Transcriptome Shotgun Assembly project has been deposited at DDBJ/EMBL/GenBank under the accession numbers SRX791343-SRX791345, and GWE00000000. The version described in this paper is the first version, GWE01000000.

Authors’ contributions
All authors contributed to the research project design and manuscript preparation. Conceived and designed the experiments SKDN, TLK, FH, AG, CIPG. Performed the experiments SKDN, TLK, FH. Analyzed the data SKDN, TLK, FH, CIPG. Wrote the paper: CIPG (with input from the other authors). All authors read and approved the final manuscript. SKDN and TLK contributed equally to the paper.

Ethics approval and consent to participate
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Consent for publication
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Competing interests
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