Review of: 105020-v0

The article titled "Neuropeptides from a Praying Mantis: What the Loss of Pyrokinins and Tryptopyrokinins Suggests About the Endocrine Functions of These Peptides" by Jan Veenstra uses publicly available data (mainly genomic data) and reports the loss of receptors for pyrokinins and tryptopyrokinins. The analyses consist of homology searches using the BLAST algorithm with known precursor and receptor sequences from phylogenetically related species. The method is well established and benefits from the growing number of publicly available transcriptomic and genomic datasets. However, this approach—using known sequences as queries—is limited by the degree of sequence conservation between the target and query species. If a sequence is highly derived in a particular species, BLAST searches may not identify the precursor and/or receptor. Most of the data used by the author were sequenced by large consortia (not peer-reviewed data), with limited information about the quality of the genome and transcriptome data. For this reason, I strongly recommend evaluating the quality of each dataset analyzed in the paper using BUSCO. The main reason is that in a genome with 75% BUSCO completeness, missing precursor or receptor sequences would not be surprising, but this does not necessarily mean that the precursors are truly absent in the investigated species. Similarly, another concern is that the author uses several raw reads (referred to as SRAs in the paper) that were likely not quality controlled. This could result in the identification of sequences with low-quality and/or unpaired reads. These reads are usually discarded and not included in the final assembly. Therefore, I suggest that for each SRA, the author carefully check the quality of the resulting assembly to ensure that the loss of a precursor or receptor is strongly supported by high-quality transcriptome or genome assemblies.

The introduction lacks a clear and up-to-date description of pyrokinin precursors and receptors in insects. This are the focus of the paper and should be well described in the introduction. The author suggests that these important neuropeptides are not essential for the physiology of the study species. In general, pyrokinins are a complex group of neuropeptides, with at least four commonly identify precursors —pyrokinins, capa (or periviscerokinin), tryptopyrokinins, and pyrokinin-like— and two to three receptors described in insects. Depending on the taxon, some of these precursors may be present or absent, but pyrokinins in the suboesophageal ganglion and ventral nerve cord are typically present in nearly all insects. Many papers address this topic, including works by the author (https://doi.org/10.3389/fphys.2014.00454) as well as by Reinhard Predel (https://doi.org/10.1186/s12862-016-0621-4; https://doi.org/10.1007/s00018-006-6187-3; https://doi.org/10.1002/cne.21183; https://doi.org/10.1016/j.bbrc.2017.02.135; https://doi.org/10.1016/j.jinsphys.2021.104326). In most of these papers, both neuropeptides and their localization in the insect CNS have been corroborated by mass spectrometry and immunohistochemistry. The receptors have also been investigated in several species with the most remarkable work in *Drosophila* (http://dx.doi.org/10.1016/j.bbrc.2005.07.038; http://dx.doi.org/10.1016/S0006-291X(02)02709-2) and in *Tribolium* (https://doi.org/10.1016/j.peptides.2014.11.004; DOI: 10.1038/srep06800). These are only few suggestions. I suggest that the author include a section describing the key aspects of these neuropeptides, the distribution of cells expressing these neuropeptides across the insect CNS, and the co-evolution between neuropeptides and their receptors in insects. As the author used also *Blattella*, a helpful reference would be https://doi.org/10.1002/cne.21183. I believe this will be useful for readers, especially those less familiar with this specific group of neuropeptides.

Moreover, the introduction lacks a clear description of the aims. While I agree that the results are relevant and interesting, the scope is not well introduced. What motivated the author's search for pyrokinin neuropeptides in this specific group? A clearer explanation would help readers understand why the absence of these neuropeptides is surprising and extremely interesting.

Based on the results presented in the paper, the precursor sequences of pyrokinin neuropeptides and their receptors appear to be absent from both the genome and transcriptome of the study species, as well as in several other mantises. The author suggests that the loss of pyrokinin receptors is because these neuropeptides are "irrelevant" for mantises. I find this to be a strong statement, which is not supported by further experimental evidence. The author also describes additional neuropeptide precursors that show considerable differences compared to other species, such as RYamide. In the discussion, the author extensively examines the known functions of these neuropeptides (pyrokinins) in insects and the involvement of this group of peptides in feeding and digestion. He hypothesizes that these neuropeptides are released into the hemolymph in anticipation of feeding in several insects. Thus, in a predator like mantises that cannot anticipate when they will start feeding, they do not need peptides that may be related to the anticipation of feeding and digestion. Based on this hypothesis, other predators should have also lost tryptopyrokinins and pyrokinins, which is not the case. The conclusions of the paper in its current form are speculative and not supported by the results.

During the last decade, thanks to advances in genome and proteome sequencing, it has been possible to confirm the loss of several peptidergic systems in different taxa and lineages. One interesting aspect to investigate for each of these losses is which neuropeptides may have compensated for the physiological functions. In mantises, for instance, was the loss of pyrokinin and tryptopyrokinin peptides, along with their associated physiological functions, compensated by other neuropeptides? Is it possible that periviscerokinin could compensate for the key physiological functions of other pyrokinins? Additionally, it would be interesting to investigate the genetic mechanisms that could have led to the loss of a specific neuropeptide and its receptors.

Specific comments:

Line 30: Is "speudogene" correct? Did the author mean "pseudogene"?

Lines 58–60: Is the author referring to pyrokinins, such as capa neuropeptides? A reference should be added to clarify this.

Line 89: The sentence "For most genes this yielded partial neuropeptide precursor sequences" suggests low quality or coverage. The presence of introns is only partially relevant since the BLAST search should identify each CDS. One possibility is that the assembly, before scaffolding with Hi-C, did not include contigs shorter than 2K nucleotides, so it is possible that several shorter sequences have been filtered out before the final assembly. Please remove the sentence.

Line 91–92: Can the author provide details about the tissues used for RNA-seq? Certain precursors, like tryptopyrokinin, may only be detected in specific tissues, and the sequence might contain several repetitions that are difficult to assemble and sequence, even with third-generation sequencing technology (https://doi.org/10.1016/j.jinsphys.2021.104326; https://doi.org/10.1016/j.bbrc.2017.02.135).

Line 92: Is the author confident that SRR25309874 refers to an RNA-seq dataset?

Line 103: The statement "When the core sequence of a neuropeptide is small, it is often difficult to detect in a genome assembly, particularly when genomes are as large as in Tenodera." is not supported by evidence, it is personal observation. Additionally, better tools exist for large-scale alignment of RNA-seq data to reference genomes. The genome assembly of *Tenodera* is at the chromosome level and phased, so blasting transcripts should return only the CDSs. As already mentioned, some assemblers have a default threshold that discards all contigs below 2 Kb. In the case of neuropeptide genes, this can be an issue.

Line 114–115: “To provide evidence that the absence of the pyrokinins is not limited to a single species, genomic SRAs from four additional Mantodea, i.e. Deroplatys truncata (SRR25068526), Hymenopus coronatus (SRR25046609), Mantis religiosa (SRR25010894) and Metallyticus violocea (SRR25078554) were analyzed in the same fashion.” Are these species chosen because they were the only ones available in public databases, or is there a rationale behind this choice? The author could provide better motivation here and describe the phylogenetic relationships more clearly.

Line 119–120: Did the author perform BLAST searches on raw reads? Please clarify.

Line 163-165: The author refers to abundantly expressed neuropeptide precursors described in Table 1, but it is not clear how the quantification was carried out. Did the author use Kallisto (https://doi.org/10.1038/nbt.3519)? Moreover, this information should also be taken with caution, as the quantifications are not based on biological replicates; therefore, they are only informative for the presence or absence of precursors in different tissues. Similarly, Table 2 do not contain biological replicates.

Lines 184-190: Since the precursors and their receptors are generally well conserved during evolution, if the receptor or precursor undergoes significant changes, it may be difficult to identify the sequence using homology searches. In the case of tryptopyrokinin, it is also possible that a precursor containing several paracopies is difficult to assemble. This has long been a problem in locusts, for example (https://doi.org/10.1016/j.bbrc.2017.02.135; https://doi.org/10.1016/j.jinsphys.2021.104326). Moreover, as the author comments, the tryptopyrokinin genes are still expressed in other mantises. Based on the available species and phylogeny, it would be helpful to determine in which clade the apparent loss of these two peptidergic systems occurred, possibly using a phylogenetic tree similar to the one described in https://doi.org/10.1111/syen.12596.

Lines 197-202: This paragraph is hard to understand. The pyrokinin precursor is usually expressed in the suboesophageal ganglion, which can be dissected together with the “head,” while periviscerokinin is normally found in the abdomen. In Deroplatys truncata, apparently only one read of PVK was sequenced in the thorax, while tryptoPK was extremely abundant in the thorax. This suggests issues with the samples, and therefore the results are not well supported by the current knowledge of the distribution of these neuropeptides. How can the author rule out any problems during sample preparation for these samples? I suggest removing Table 2 entirely and rewriting the paragraph to only mention that tryptoPK was identified in Deroplatys truncata.

Lines 207–212: The reason why “a tryptopyrokinin gene may have escaped detection” in Table S1 could be low quality? Can the author add BUSCO scores for each assembly produced with those SRAs in Table S1 and S2? This will help understanding if the results is based on high quality data or not.

Lines 235–239: It is hard to comment on the tree of Figure 6. There is no information on how the tree in Figure 6 was built and if the nodes have any type of statistical support. Were Bayesian or maximum likelihood methods used? Moreover, the alignment in fasta format should be added in the supplementary material for the readers. Please provide details.

Lines 278–287: This paragraph is speculative, and without biological replicates, the evidence is weak. I recommend removing the paragraph.

Lines 316-320: It is hard to understand if the loss of pyroknins can be beneficial due to the broad distribution of this peptides across insects, one question could be if the key physiological functiouns of pyrokins and tryptoPK have been compensated by other neuropeptides.

Lines 330-332: Similar results were also described in *Schistocerca gregaria*. The mass spectra of the lateral cardiac nerve which is connected by tryptoPK neurons of the gnathal ganglia showed several ion signals matching to tryptoPKs (https://doi.org/10.1016/j.jinsphys.2021.104326).

Lines 412-413. The conclusion is not supported by the results.