Hereafter, I will respond only to the major aspects of the paper. My answers are in italics.

General point: Regarding the BUSCO estimates for the complete data sets (not limited to Tenodera sinensis), after reading the author's response, I still recommend using BUSCO to thoroughly assess the quality of each dataset used in the study. In my opinion, it should be the responsibility of the person who conducted the genome sequencing and submitted the data to provide such information, as is typically done with data produced in scientific papers or by sequencing consortia like the Tree of Life Team at Sanger or ERGA, who routinely include this information in the data notes. Releasing data without details on sequencing coverage (which can vary between species also from the same taxonomic group) and quality control complicates the ability to properly assess the quality of publicly available data.

I don't understand why unpaired reads would be a problem while low-quality reads are neither problematic. The numbers of low quality reads in SRAs are generally very low and low quality reads typically have a few unreliable nucleotides, but generally maintain sufficient homology with query sequences and thus can still be used to determine whether or not a specific gene might be encoded.

Were the data submitted to NCBI trimmed using Trimmomatic? I suggest verifying the quality of the data, as supplementary materials from several high-quality publications typically include this information.

This is not a review on pyrokinins, which by itself would be interesting to write, but I did not try to do that here.

The introduction lacks a clear and up-to-date description of pyrokinin precursors and receptors in insects. I believe this is important for readers, and it doesn't need to be as comprehensive as a full review. A simple paragraph outlining the known information would be clear and helpful for less experienced readers. Several papers could be cited without the need to turn this into a detailed review (see my previous comments).

"Use it or loose it" that is how it goes in gene evolution. … ".

This explanation sounds overly simplified and is not convincing without more details. The author cannot completely prove that the pyrokinin gene is absent.

I understand the reviewer does not agree with my hypothesis that the function of these peptides has become irrelevant. I do think that is the case and consequently, there is in my opinion is no need to look for other peptides to compensate. Note though, that I do suggest that the neurons producing these cells may still be present and that other aspects of the functional relevance of these neurons may persist.

Regarding the hypothesis, I found that it focuses only on specific aspects of the study species (or group), and I am concerned that there may be other, more comprehensive hypotheses to consider. Additionally, I find the hypothesis difficult to test, unless there are other groups that may experience similar losses, which does not seem to be the case, as the author states for other insect species (dragon flies or ant lions). Many peptidergic systems are missing from molecular

data across different groups of insects, and there are unfortunately no clear explanations for why they are missing or not yet identified. Therefore, forcing the entire discussion (and conclusion) into a single explanation is not comprehensive without further evidence.

If you read the publication on the Tenodera genome, which is open access, you will see that is not the case.

According to the assembly program used in the paper (Ma et al., 2023), the default "read_cutoff = 1k" may have caused the loss of some neuropeptide exons. This likely occurred before scaffolding the genome.

The legend to table 1 states: “The number of spots coding dilp7, gonadulin, IGF and the ûve sirps in transcriptome SRAs prepared from diûerent body parts of T. sinensis.” In plain English, I counted the number of reads that contained coding sequence for each of these individual neuropeptides in each of the SRAs. No, there are no biological replicates. The interest of Table 1 is to show that despite the facts that (1) sirp3 is a very unusual peptide that (2) it was not found in other species, it nevertheless seems to be highly expressed. Based on (1) and (2) one might be inclined, at least I was, to think it is a pseudogene. It may well be, but it seems to be abundantly expressed.

Without using biological replicates, I find it difficult to rely on this approach to draw any solid conclusions. As I mentioned, there are several factors that could reduce the coverage of specific genomic regions, and this possibility cannot be ruled out. I don't mean to imply that the author is wrong, but I cannot base conclusions solely on his experience.

Sure, that is what I have done here, it is figure 6. In the text it is stated: In order to visualize the presence of tryptopyrokinin and its receptor in the Mantodea a phylogenetic tree was made. A recent revision of the Mantodea phylogeny has shown that several families in this group are polyphyletic (Ma et al., 2023). It was therefore difficult to assess the correct phylogenetic position of quite a few species and these could thus not be included in the tree (Fig. 6).

The author could also include information about node support and the phylogenetic inference methods used by Ma et al., 2023. I do not believe it is the reader’s responsibility to obtain this information.

That it is not consistent with our knowledge about the distribution of this peptide from other insect species is obvious and I mention that. In fact, that makes it so interesting. The point is, in this experiment the expression appears to be in the thorax (not only that, it seems to be massive), while none has been found in the head sample, that must contain the suboesophageal ganglion as that sample does reveal both SMYamide and inotocin, two neuropeptides that are typically expressed there. So it is not simply due to an error in which SRR25068532 is has been labeled has being constructed from the thorax does in fact originate from the head (incorrectly labeled SRAs are present in the databanks). If the reviewer wants to suggests that in fact SRR25068532 was produced from the abdomen and mislabeled, then that is a possibility, but that seems unlikely in light of the lack of any evidence for tryptopyrokinin in the abdomina of fourty species ((Koehler & Predel, 2009). I state in the text that it is unclear whether this is the normal site of expression of tryptopyrokinin in those mantises that still have a tryptopyrokinin gene.

I am not criticizing the author of the current paper. I am simply skeptical that the results can be consistently confirmed in the future. To support such a strong assumption, it would be necessary to include more biological replicates. Furthermore, I assume that the transcriptome data of Deroplatys truncata were not generated solely from CNS tissue but likely included the entire thorax, making it difficult to pinpoint the exact location of tryptoPK expression. Additionally, the BLAST results for SMYamide show only 3 reads, and inotocin shows 13 reads out of several thousand; these counts are very limited.

I will not do so. It is very clear from the data that (1) possibly all mantises have lost the pyrokinin-2 receptor gene and quite possibly the pyrokinin gene itself, (2) that several, if not many, mantises have lost the tryptopyrokinin gene, while almost all mantises have lost the pyrokinin-1 receptor and (3) that there are species that still have a tryptopyrokinin gene but no longer have a specific receptor for this gene (the pyrokinin-1 receptor); such species could be quite common. This latter point is very interesting and it is possible that the ligand survived because it codes for so many copies of the neuropeptide and that this allows it to activate still another receptor (if so, it might be the periviscerokinin receptor). Now there is one species, and only one, for which there is, admittedly preliminary, data that suggests that in those species where the ligand persists but the receptor gene has been lost, the ligand is expressed in a novel location. That is interesting, even if it is preliminary, so I am not going to remove that from the manuscript.

The data presented in Table 2 are difficult to interpret, and without any biological replicates, it is challenging to evaluate their reliability. Additionally, the time points selected for RNA extraction are not clearly explained. I would be surprised if other readers did not find the table confusing. It is not only surprising that tryptoPK is so abundant in the thorax, but also that some of the other neuropeptides were identified by just a single read out of millions. Another point I find unclear in the author's response is the claim regarding the loss of receptor genes and the tryptopyrokinin gene, which the author likely could not definitively prove, despite efforts to locate the pyrokinin genes. I believe the paper would benefit from removing Table 2, as the results are already sufficiently clear in the study species without the inclusion of Deroplatys truncata.

This boils down to what I consider to be the most urgent question to answer with regard to many insect neuropeptides, including the pyrokinins and the tryptopyrokinins. What is the essential function of each of these peptides? The problem with understanding the physiology of many insect neuropeptides is that most of them have been isolated by what one could call a pharmacological assay. It has an effect on some system, usually a muscle, so we know it is a biologically active peptide, but we don't really know what its primary function is. A good example in vertebrates is vasopressin. Its primary function is to maintain sufficient water in the extracellular space. So it acts as anti-diuretic hormone. Within this function is contracts smooth vascular muscle. But once you have lost a lot of blood, it will also stimulate the release of glucose (this will lead to intracellular water moving into the extracellular space). So it all depends on how you discover the peptide, if that is from stimulation of glucose production or smooth muscle contraction, it may be difficult to understand its true function. That is also the case here, and that is what I try to do here. I try to make a contribution to better understand the functions of these peptides. I understand you want testable hypotheses. So I included now a bit more clearly what "priming" of the salivary gland might entail. IF (still a big IF) it does indeed stimulate the production of salivary enzymes, it is easier to understand why loosing that function is beneficial.

I completely agree with this aspect and share the need for a better and more comprehensive understanding of the physiology of this group of neuropeptides (similar to many other neuropeptide families). However, to achieve a deeper understanding of their physiology, specific experiments are necessary to test different hypotheses. These should involve a combination of methods, including biological assays, expression experiments, and mass spectrometry. The main evidence presented in the current paper is the general absence (or extremely reduced expression) of this neuropeptides in this group of insects.

I changed the wording of the sentence.

I would suggest moving the concluding sentence to the end of the discussion and, if possible, expanding the conclusion. It could be rewritten in a more comprehensive manner, suggesting how the results presented in this study could be used to test the author's hypothesis in future research.