

Date: Dec 23, 2021
To: "Basanta Khakurel" basantakhakurel@gmail.com;basanta.khakurel@southeastern.edu
From: "Ichthyology & Herpetology" leo@ichsandherps.org
Subject: Decision on Ichthyology & Herpetology submission ASIH-D-21-00124

Ref.: Ms. No. ASIH-D-21-00124
Species Delimitation in Eastern Pine Snakes (*Pituophis melanoleucus*)
Ichthyology & Herpetology

Dear Dr. Khakurel:

Thank you very much for submitting your manuscript, Species Delimitation in Eastern Pine Snakes (*Pituophis melanoleucus*). The reviewers and Associate Editor appreciated the attention to this interesting topic, but they raised substantial concerns about the paper. Based on the reviews, we will not be able to accept this manuscript for publication in Ichthyology & Herpetology.

I realize you will be disappointed with this decision, but I hope the comments of the reviewers and editors will be of some assistance to you in preparing the manuscript for any future submission. Thank you again for thinking of Ichthyology & Herpetology. We look forward to further contributions from you and your colleagues.

Best regards,

Bryan L. Stuart
Associate Editor
Ichthyology & Herpetology

Reviewers' comments:

Editor's Responses to Questions and Comments to Author

Associate Editor Bryan Stuart: The data in this manuscript were recently published in Nikolakis et al. 2021 paper (cited as in press in BioRxiv in the manuscript but now published as Nikolakis et al. 2021 Fine-scale population structure within an Eastern Nearctic snake complex (*Pituophis melanoleucus*), *Zoologica Scripta* DOI: 10.1111/zsc.12522). The *Zoologica Scripta* paper asks essentially the same question, is there genetic evidence for three lineages (subspecies) across a landscape with biogeographic barriers, and using the same dataset comes to the same conclusion that there is evidence for widespread gene flow and little support for recognizing the three subspecies. The only novel addition in this manuscript that was apparent to me was running bpp (a species delimitation approach) on the same dataset. Although this pine snake genetic work is highly meritorious given the conservation plight of the taxa, I do not see enough of a new contribution in the present manuscript from what has just been published in Nikolakis et al. 2021 to warrant publishing this as a second paper in Ichthyology & Herpetology.

Reviewer #1: Species Delimitation in Eastern Pine Snakes (*Pituophis melanoleucus*)

Below is the review to where I made it to in the ms prior to looking at the figures and captions. Based on the figures and captions, I think this paper needs to be rejected outright---they don't make a lot of sense given what I know about the system. And the lack of monophyly mentioned in the ms for *Pituophis* tells me that there is likely a large error in how the authors did this. There are serious problems in the methods/results. It seems like the authors just took UCE data from another paper and use to make a publication with little understanding of these data or the system.

Initial review:

This is a great system to work on and detail. The general ideas would work well for the journal here and the system is appropriate. However, I found the writing to be a bit stilted with sentences throughout that don't really flow from one to the next. I think the writing could be improved to make this a better read, but the info is all good. It just, at times, reads more like an earlier draft vs a polished manuscript. I think the introduction in general needs a real re-organization and check for grammar/presentation in order to be best presented (i.e., introduce the system, the organism, the issues surrounding its taxonomy, and how you can tackle that with data/analyses, and the hypotheses/goals). The intro really jumps around too much in my opinion from organism to other aspects and then back again, I found this very hard to follow and quite disjointed. The last couple paragraphs of the intro also read like methods to me vs intro info.

The first paragraph of the intro felt quite stilted in presentation (almost reads like bullet points in a powerpoint slide), it's just a statement of facts with no flow really connecting or building them up. Given that this is an organismal journal, I don't think this general sort of approach is a necessary way to start the paper. I'd revise either the content/flow of paragraph 1 or the general organization of the intro to avoid this choppy feel. This isn't a fatal flow, just something I think would improve the reader experience overall. One option would be to just cut that paragraph (paragraph 2 is a nice way to start) or move it elsewhere (but still revise it a bit).

38/39---I'd say something more about the validity of subspecies here in general if you're going to bring this up as significant and also expand on it further as far as why its relevant, what it means etc. The following paragraph acts immediately like subspecies are "something" but I think you'll find a lot of herpetologists think they're useless, so if that's not the case, it

needs justification.

44---"Individuals of different subspecies who appear to show signs of introgression have been observed in the wild"---this makes a lot of assumption on subspecies representing real entities the way it is presented

This is not really a complete sentence:

"Some of

50 such molecular data are DNA barcode (Herbert et. a. 2003), UCEs (Bejerano et al. 2004; Winker et. al. 2018), ddRADseq (Peterson et al. 2012, Reitzel et. al. 2013), Sequence capture (Anderman et. al. 2020)."

The results could also be organized to mimic the order of the methods, it would read a lot more easily. I also think in general, the methods and results need more fleshing out and detailing as to what each method is aiming to accomplish/why it's being done. I found the details of the results somewhat lacking (line 137---detail this or why even mention it?).

Reviewer #2: The ms "Species Delimitation in Eastern Pine Snakes (*Pituophis melanoleucus*)" for review in Ichthyology and Herpetology uses UCE data and phylogenetic inference to determine if multiple species can be delimited in the eastern pine snakes. From their analyses, it certainly appears that this taxon cannot be delimited further. The paper is suitable for publication in Ichthyology and Herpetology, though I do have some suggestions for improvement.

Major problems

1. Some of the material refers to another paper, Nikolakis et. al. (2021), that was likely written by these authors. Therefore, it seems that population clusters within this taxon exist. However, it isn't clear just from this submitted paper what is actually happening. I would suggest they add a section to clarify this. Also, because of gene flow among subspecies or other geographic groups might exist they still might form groups that are not easily identifiable using typical phylogenetic approaches and looking for monophyly. Or, importantly, there is some other type of spatial/geographic structure. So, typically these kinds of phylogeographic studies start with some clustering approach to understand if spatial groups with some admixture can be discerned using Structure, DAPC, or SMNF (which was performed previously I believe). I would also try to see if there is there is any areas of lower migration over space and related to these subspecies using the EEMS program. This could also determine if this species does form clusters then there may be somewhat limited migration between them.
2. As a strong test of the existence of subspecies as lineages, I would group individuals into the three subspecies and determine if BPP supports any of these.
3. What about F_{st} s among populations and subspecies? This is a standard popgen metric to report. They could do this to show that there is no real structure (F_{st} with a statistical test). They can also show F_{st} by locus to help support their idea that UCEs are not very useful for shallow-time studies like this.
4. In the discussion section Gene Flow Across a Barrier, there is no actual estimation of gene flow in this study. There are several methods to infer gene flow as a rate using genomic data. I think that this is necessary if they keep a section with this title.
5. They should make a formal statement about the continued use of subspecies in *P. melanoleucus*. The results here look like this should be discontinued.

Minor problems:

1. I would rephrase line 11 and say: These results confirm prior findings that little or no divergence among the three putative subspecies, which suggests *P. melanoleucus* represents a single species.
2. On line 22, they suggest that "the multispecies coalescent (MSC) method uses both molecular phylogenetics and population genetics to counter problems, such as long-branch attraction." I have never heard of the MSC being phrased as using popgen and phylogenetics to counter LBA. That is not an accurate description of the method, and LBA is not something typically considered in the context of the MSC as far as I know.
3. Here, they address different markers but don't actually mention any of the properties of these markers: "Each type has different evolutionary properties that lead to the marker capturing different types of variation. Some such molecular data are DNA barcode (Herbert et. a. 2003), UCEs (Bejerano et al. 2004; Winker et. al. 2018), ddRADseq (Peterson et al. 2012, Reitzel et. al. 2013), Sequence capture (Anderman et. al. 2020)."
4. Designate the subspecies on network in Fig.4.
5. I think they should provide more details here rather than reference another paper: "The majority of the variation recovered was observed in the extreme regions of each UCE and there was little correlation between the variable sites and locus lengths (see Nikolakis et. al. 2021 for further details)"
6. On Line 149 I would rewrite this to say: The delimitation posterior probabilities were obtained using a guide tree which accounts for phylogenetic uncertainty." Also, how does the guide tree account for uncertainty?
7. I don't follow the statement here, were different loci used to estimate phylogeny? "The tree (Figure 4) indicates that there is little variation among the topologies estimated from different pine snakes over the eastern side of the United States."
8. I would revise the following on line to say: "If different loci had supported different phylogenetic trees, we would have expected the nodes of the trees to connect to the tips in a web-like structure..."
9. This reads strangely. Do they mean the eastern pinesnakes diverged from the other groups within *Pituophis* 3 to 6 mya?:

"The eastern pine snake diverged within the Pituophis about 6 to 3 million years ago (Pyron and Burbrink 2009)."

10. One line 176, I wouldn't say "we get" but rather "the same tree is inferred..."

11. On line 196, I would say "Previous studies examining movement showed that *P. melanoleucus* is very mobile with home ranges spanning from 35 hectares to over 105 hectares (Nikolakis et. al. 2021) which might contribute to gene flow among the different populations."

12. Do they mean "majority" in line 146 instead of "plurality"? "When we combined the probabilities obtained from all the datasets to a single file and made a histogram, the plurality of the datasets indicated that there is an ingroup of one species of *Pituophis melanoleucus* and the outgroup of *P. ruthveni*, *P. catenifer*, and *Pantherophis obsoletus* (Figure 2)."

13. In line 202, I would say "Phylogenetic inference of vertebrates using UCEs has increased (Gustafson et. al. 2019)."

Is the manuscript current with respect to the literature, methods, and concepts involved?

Reviewer 1: NO

Are the figures necessary and satisfactory or do they need improvement or additions?

Reviewer 1: ABSOLUTELY NOT

Are the interpretations and conclusions justified?

Reviewer 1: NO

Is the manuscript current with respect to the literature, methods, and concepts involved?

Reviewer 2: Fairly there are a few issues mentioned in my review.

Are the figures necessary and satisfactory or do they need improvement or additions?

Reviewer 2: Ok

Are the interpretations and conclusions justified?

Reviewer 2: Yes, though see my comments.

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