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Review of “Testing the Taxonomic Validity of Preble’s Meadow Jumping Mouse (*Zapus hudsonius preblei*)” Overall, I agree with the authors’ approach to investigating the taxonomic validity of Preble’s Meadow Jumping Mouse. Specifically, I believe it is a good idea to use multiple lines of evidence (not just genetic data) to clarify taxonomic borders. Typically these lines of evidence are genetics, morphology, and behavior (and sometimes geography). The authors do present genetic and morphological evidence and speak of ecological or perhaps behavioral evidence although this is not well defined in this report. My comments will focus more on the genetic aspects of this study than on the morphological aspects as is consistent with my experience and expertise.

From a genetic standpoint, this study uses an appropriate genetic marker (mtDNA sequence data) and does an excellent job analyzing the data from a phylogenetic standpoint. I see no problems with the sampling scheme, the technical aspects of the lab work, the appropriateness of the marker used, or the phylogenetic analysis. This study provides a great data set from which to **begin** to answer the question at hand. I do not feel, however, that this study by any means resolves the taxonomic question. Further, I feel that some of the conclusions made by the authors are debatable.

In the literature there exists a huge controversy about how to define a species that has resulted in a myriad of different species concepts (Biological Species Concept, Phylogenetic Species Concept, Evolutionary Species Concept, etc.). Trying to define a subspecies is even more nebulous but has resulted in a similar discussion of how to define a “unit” for conservation below the species level. Several authors (mainly Moritz) have described an Evolutionary Significant Unit (ESU) based purely on genetic data and indicate that a valid ESU must be reciprocally monophyletic. Others suggest different concepts including the one (Crandall et al. 2000) used in this report. My point here is that there is not one “accepted” definition in the literature of how to define a subspecies or even a species. How you delineate the boundaries of a subspecies depends upon which definition or groups of definitions you use. Different species or ESU concepts can be applied to the same data with widely different results. For example, in 2000, the AOU recognized the Gunnison Sage-Grouse as a new species based on the Biological Species Concept. Gunnison Sage-Grouse exhibit differences in behavior and morphology and are reproductively isolated from Greater Sage-Grouse. Genetic data from that study show a lack of gene flow between the two species and mtDNA haplotypes and nuclear microsatellite alleles that are unique to the Gunnison Sage-Grouse. However, if you apply Moritz’s criterion of reciprocal monophyly, the Gunnison Sage-Grouse do not even qualify as an ESU.

The authors of this study use three criteria to determine whether or not the Preble's subspecies is a valid one from the genetic standpoint: reciprocal monophyly, Ramey's AMOVA test, and the criterion of Crandall et al. 2000. The author's state in this report that they feel the reciprocal monophyly definition is too strict. I agree with this idea particularly in light of my experience with the genetics of Sage-Grouse. The second test is Ramey's assertion that the subspecies boundary exists when there is more variation among groups than within groups using AMOVA analysis. This measure, while discussed in 3 papers published by Ramey is not well tested in the literature or accepted as a standard measure. The authors make the statement that this measure is less restrictive than the reciprocal monophyly definition and I am not sure that in all cases it really is. I would like to see a critical review of this measure before I would use it as a standard. Finally, the authors use the criterion put forth by Crandall et al 2000. I agree that this criterion conceptually is a good one but may be more difficult to apply with empirical data.

In this study the authors find that *Z. h. campestris* is most closely related to *Z. h. preblei* and that *Z. h. luteus* is most closely related to *Z. h. pallidus*. Further, they show that all four haplotypes found in *Z. h. preblei* are shared with *Z. h. campestris*. This does seem to suggest that somehow *Z. h. preblei* are a subset of *Z. h. campestris*. At this point, I wished the authors had presented a map showing the range of each subspecies and where the corresponding haplotypes were found. I tried to do this using their data from Table 1. From what I could understand from this table, it appears as though all the *Z. h. campestris* samples from Custer, SD shared haplotypes with *Z. h. preblei*. Thus, there are no haplotypes in Custer, SD found so far that belong in the upper cluster of the *preblei/campestris* clade. Is Custer, SD the most southern portion of the sampled range of *Z. h. campestris*? Could it be that the samples from Custer, SD represent instead the northern most part of the range of *Z. h. preblei*? I have no idea (it would be great to have more samples sequenced from that area), but it would be nice to see the haplotype data superimposed on a map so that one could investigate those questions.

Certainly the fact that no unique haplotypes are found in *Z. h. preblei* and that all four *Z. h. preblei* haplotypes are shared with *Z. h. campestris* is compelling evidence suggesting that *Z. h. preblei* and *Z. h. campestris* may be one in the same. It is interesting that the four *Z. h. preblei* haplotypes all group together which does suggest a founder event from *Z. h. campestris* with restricted gene flow. The authors use this evidence (along with morphological evidence) to conclude that, in fact, *Z. h. preblei* and *Z. h. campestris* **are** synonymous and even go so far as to suggest that *Z. h. preblei* does not qualify for protection under ESA as a DPS. One major problem with this conclusion is that the genetic data that they gathered is from only one locus (or one window of evolution). Further, this locus represents only the matrilineal history, which could very well differ from the evolutionary history of that species or subspecies. It has been shown in other rodent species that mtDNA patterns can be widely different than patterns in the nuclear genome due to introgression and (Prager et al. 1993, Ruedi et al. 1997). In fact, Ruedi et al. (1997) found that despite distinctive nuclear differences between subspecies of pocket gophers, mtDNA haplotypes were found to be very similar due to introgression. Thus, I would be very skeptical to conclude **undeniably** that *Z. h. preblei* and *Z. h.*

campestris are synonymous without including nuclear data. I would also like to see more data from each “population” of *Z. h. campestris*, particularly in the Custer, SD area. A population level study of *Z. h. preblei* and *Z. h. campestris* using nuclear and mitochondrial markers would do a better job of providing a definitive answer.

The authors claim that using all three criteria, they reject the idea that *Z. h. preblei* is a valid subspecies. Certainly the data show that *Z. h. preblei* are not reciprocally monophyletic. This concept, however, can be overly restrictive (in my opinion) and only utilizes genetic data, which in my mind, is problematic. The second criterion based on AMOVA has not been well tested (at least that I know about, see below) and therefore I am not comfortable using it to define a subspecies. The third criterion is conceptually a good one. It is based on comparing recent and historic exchangeability and is set up in a hypothesis-testing framework. Crandall et al. 2000 suggest that “individuals from different populations are genetically exchangeable if there is ample gene flow between populations” and by ample gene flow they suggest “unique alleles, low gene flow estimates ($Nm < 1$) or phylogenetic divergence concordant with geographic barriers”. The authors state that the populations are genetically exchangeable because of shared haplotypes and no unique alleles. It would be interesting to estimate levels of gene flow. My biggest problem with this criterion is how the authors report their finding of ecological exchangeability. They state that they found ecological exchangeability based on a review of the literature. They give no explanations of what variables were compared or even any citations of any of the literature that was reviewed. This gives me no avenue to repeat the analysis that they did or even to judge whether or not I think it is valid. The authors emphasize how their study is based on testable hypotheses and the scientific method. Therefore, I find it troublesome that they included this assessment of the literature and made strong conclusions without reporting any of the data or the citations.

Specific comments:

Pg 3 – It is unclear from this report whether the Preble’s Meadow Jumping Mouse was listed as a DPS or subspecies.

Pg 3 – Include a range map. The description of the species range states that it extends from “the Pacific Coast of Alaska eastward to the Atlantic Coast; from the northern limit of tree growth south into central Colorado, Nebraska, eastern Kansas, Missouri, Tennessee, and northern Georgia” – What about the samples you obtained from New Mexico and Arizona?

Pg 4 – The authors state that they use population genetic methods that they only touch upon with AMOVA. I would like to see a real population level study comparing at least populations of *Z. h. preblei* and *Z. h. campestris*.

Pg 4 – I am skeptical of Ramey’s method of defining subspecies based on the relationship between variability among vs. within populations. How does this method work across different molecular markers? Is it robust when comparing populations of different sizes? Is it robust to differences in sample sizes among groups? Further, I am not convinced

that it is always less restrictive than reciprocal monophyly.

Pg 13 – I would not state based on this data set alone that *Z. h. preblei* are not markedly separated from other populations. This data suggest that they may not be separate, but without further analysis I don't believe the question can be answered undeniably.

Pg 14 – I take issue with the fact that the authors state that for a mere 57,000 dollars (50,000 of which went toward genetic work) they have been able to redefine the taxonomic classification of *Zapus hudsonius*. While I do agree that they have made a good start to answering the question, without the addition of nuclear markers their data is severely limited. I believe that the cost of adding nuclear markers and additional samples to address the *preblei/campestris* question at a population level will not be trivial and that is misleading to USFWS and other agency personnel to suggest otherwise.

Sara Oyler-McCance's answers to specific questions to consider for review of Dr. R.R. Ramey's report on genetic analysis of Preble's Meadow Jumping Mouse

Please analyze the techniques used in the population and phylogenetic evaluation of *Zapus hudsonius preblei* and other taxa. Were appropriate methodologies and markers used?

The use of mitochondrial control region data is an appropriate marker to use to begin to address the taxonomic question at hand. It is important, however, to include nuclear markers as well before definitive answers about taxonomic delineations are made. The authors used the proper methodology for the phylogenetic analysis. I am less comfortable with the "population analysis" mostly because it is based solely on only one test, AMOVA, and the conclusions drawn by the authors regarding the ratio of between vs. within group variation are based on a metric that is largely untested (see comments regarding this elsewhere).

Are the conclusions about the taxonomic validity of *Z.h. preblei* logical and defensible as presented in the manuscript?

I have no problems with the study itself except for some of the conclusions made by the authors. I feel that in some cases they have made recommendations based on an incomplete data set. Their data may suggest that *Z. h. preblei* and *Z. h. campestris* are synonymous yet without collecting data from nuclear loci, I would not say definitively that they are and I feel it is wrong to suggest reclassifying *Z. h. preblei* without collecting nuclear data and doing a more complete population level analysis first.

Are there possible alternative interpretations of the genetics data? Are there additional or divergent taxonomic conclusions that could be drawn from the genetics data?

Other studies have found a discordance between mitochondrial and nuclear data sets. It is possible that nuclear data might reveal a difference between the two subspecies that

was masked in the mtDNA through introgression. Further, I would be interested in seeing more data from the Custer, SD sampling site. It seems a little odd to me that 5 of the 7 *Z. h. campestris* samples that most closely resemble *Z. h. preblei* all are found in one location and that there are no other *Z. h. campestris*-like samples in that sampling locale.

Do you agree with the interpretation about possible mechanisms of reduced gene flow between *Z.h. preblei* and other subspecies of *Z. hudsonius*?

I do agree with the authors that the data seem more consistent with a southward colonization from *Z. h. campestris*. Again, it would be really nice to have a figure showing haplotype frequencies superimposed on a map. It appears as though the connection between the two subspecies is through Custer, SD (are these samples misidentified?). Is this the closest population to the *Z. h. preblei* group? It would be interesting to have estimates of gene flow using coalescent theory. Due to the low haplotype diversity within *Z. h. preblei*, it seems reasonable that there is reduced gene flow (compared to *Z. h. campestris*). A population level analysis including populations from both subspecies could better answer that question.

Do you agree with the concepts of Crandall et al. (2000)* for defining evolutionarily significant units?

Conceptually I think the concepts of Crandall et al are reasonable. The nice thing about this concept is that it focuses on the importance of adaptive distinctiveness in populations and because it combines genetic and ecological data. The hypothesis testing aspects of it are less appealing to me because of the inherent problems with applying hypothesis testing to observational data. Additionally, I don't feel that the concepts of Crandall et al (2000) are necessarily any better than some of the other concepts that are in the literature.

Are there clear ecological distinctions between *Z. h. preblei* and closely related taxa that would suggest a need for specific conservation actions for this taxon?

I know nothing about the ecological distinctions between the subspecies and am concerned that the authors used this as a criterion ala Crandall et al. 2000 yet failed to report what variables they used or even cite the literature that they examined to make this assessment.