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Dear Gary:

As I told you in my email, I am not trained in any way to evaluate specific DNA or genetic questions. My responses to your questions reflect this as do my general review comments. I first provide my review comments followed by answers to the sheet of questions you emailed.

**General Comments:**

Perhaps the larger issue of "What defines a species or sub-species?" is being missed in the quest for determining the legal status of the Preble's meadow jumping mouse. Ramey et al.'s report focuses on a primarily genetic, and secondarily morphometric, comparison of *Z. h. preblei* to other *Z. hudsonius* subspecies, which represents a typological view of species. Most definitions of a species include the term "does not interbreed with individuals of another species" (see Meffe and Carroll 1997 for a good discussion of the plethora of species concepts). The inability to interbreed can arise from ecological, physiological, behavioral, or physical/geographic barriers. The ability of *Z. h. preblei* to interbreed with *Z. h. campestris* needs to be addressed before the place of *Z. h. preblei* in the *Z. hudsonius* lineage can be evaluated. While Ramie et al. make a compelling argument for genetic and morphometric similarity between *Z. h. preblei* and *Z. h. campestris*, there was no evaluation of ecological, behavioral, physiological, or physical factors critical for determining taxonomic validity of *Z. h. preblei*.

The primary definition of taxonomy is "The classification of organisms in an ordered system that indicates natural relationships." Because natural relationships were not discussed, I think the title of the Ramey et al. report should be changed to "Testing Genetic and Morphometric Relationships of Preble's Meadow Jumping Mouse (*Z. h. preblei*) to Other Nearby *Z. hudsonius* Subspecies", or something similar. If the focus of the paper were clarified in the title and throughout the paper, then I would agree that Ramey et al. were justified in the conclusion that genetic and morphometric data for *Z. h. preblei* was indistinguishable from another *Z. hudsonius* subspecies, *Z. h. campestris*. However, the conclusion that *Z. h. preblei* should be lumped with *Z. h. campestris* is not warranted by Ramey's genetic and morphometric analyses. Changing the taxonomic identity of *Z. h. preblei* should not be done until determining whether ecological, behavioral, physiological, or physical barriers exist that may prevent *Z. h. preblei* from inbreeding with *Z. hudsonius*.

Finally, the "discrete" requirement that a DPS is "markedly separated from other populations of the same taxon by physical, physiological, ecological, or behavioral factors" is the key to the *Z. h. preblei* versus *Z. hudsonius* issue. If you believe that only genetic evidence should be used to define a DPS, then Ramey et al.'s assessment of *Z. h. preblei* as not worth protecting is logical. However, if you believe that more than genetics should be used to define a DPS, then Ramey et al's assessment is not logical or valid.

**Specific Details:**

1. I found many grammatical errors in this report; however I do not note or comment on these.
2. Throughout report – change "taxonomic differences" to "genetic and morphologic differences".
3. Page 3, paragraph 3 – The sentence "However, these authors did not gather data in such a manner as to be able to rigorously test whether *Z. h. preblei* formed a monophyletic group" needs to be changed to "However, these authors did not design their studies to answer the question of whether *Z. h. preblei* formed a monophyletic group".
4. Page 4 first paragraph (continued from previous page) and Page 10 first paragraph (continued from previous page) – Why is Crandall et al. (2000) the only criteria considered for defining a "single population"? (Note: In context, I am guessing that single population means same subspecies?) Was this agreed on before hand? Please explain the logic given that multiple definitions of species and subspecies exist (Meffe and Carroll 1997).
5. Page 5 first paragraph (continued from previous page) – The authors state that it is "critical to test whether hybridization occurs between *Z. h. preblei* and *Z. p. princeps*", but make no similar statement about the importance of evaluating whether *Z. h. preblei* can interbreed with other *Z. hudsonius* subspecies. The issue of interbreeding between *Z. h. preblei* and *Z. h. campestris* needs to be addressed.
6. Page 5 – In this methods paragraph, the authors state that they examined the literature for evidence of ecological differences, but it is not clear how or when they did this. In the last paragraph of results (page 9 first paragraph) Ramey et al. states "A review of the literature reveals that no quantitative evidence exits to reject the hypothesis of historic or recent ecological exchangeability… between *Z. h. preblei* and *Z. h. campestris*." First, what literature was reviewed? Second, and perhaps most importantly, what ecological characteristics were compared? It seems that genetic characteristics are being used as a proxy for ecological characteristics. Third, behavior, ecology, physiology, and physical/geographic factors need to be discussed as part of an taxonomic comparison. If the title and conclusions are to be left as written, then at the very least, this section needs to be vastly expanded and a table of results with literature cited produced.
7. Page 9-10 – What is a single population? Please define.
8. Page 9-10 – Basing subspecies rules on ones own work is poor scientific procedure. At the very least, provide other references for this rule or justification for this rule.
9. Page 10 – Discriminant results for a less conservative $P \geq 0.5$ rule should be included in this report for comparison to the conservative $P \geq 0.95$ rule.
10. Page 10 - A table of posterior probabilities for each specimen should be included in this report because it is a key line of evidence.
11. Page 11-12 – I agree that basing a subspecies classification on morphology of 3 specimens is not scientifically defensible. Is this really all that was done to justify *Z. h. preblei* as a subspecies?
12. Page 12 first paragraph of Conclusions section – I agree that Ramey et al. examined these 3 lines of evidence as presented. However, delete the statement in the following

paragraph (page 13) that they checked for ecological differences, which are not represented by the 3 criteria checked.

13. Page 13 second paragraph – "and the unsupported assumption that geographic isolation …" Please provide evidence that there is no geographic isolation.
14. Page 13 third paragraph – "The "discrete" requirement that a DPS is "markedly separated from other populations of the same taxon by physical, physiological, ecological, or behavioral factors" is the key to the *Z. h. preblei* versus *Z. hudsonius* issue. If you believe that only genetic evidence should be used to define a DPS then Ramey et al.'s assessment of *Z. h. preblei* as not worth protecting is logical. However, if you believe that more than genetics should be used to define a DPS, then Ramey et al's assessment is not logical or valid.
15. Figure 1 should either be in the back with other figures, or all figures should be within text.
16. Figure 2 should be reworked for a black and white printer.
17. Figure 3 is missing.
18. Table 3 – A 95% CI must be added to this table so that differences in mean measurements can be easily evaluated by the reader. Also, present SE rather than SD because what is statistically compared is the distribution of means, not the distribution of the sample.
19. Table 4 is difficult to follow visually – clean up columns and headings.
20. Page 27 – Delete hypotheses following Table 4 unless they are related to something in the text, in which case they need a to be tied via official table status.

**CDOW Questions:**

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

Morphology: The $P \geq 0.95$ rule is subjective. Ramey et al needs to present discriminant results for a $P \geq 0.5$ rule for comparison. The $P \geq 0.5$ rule is commonly used for discriminant classification (SAS 1990, Lance et al. 2000). Moreover, presenting results for both rules will provide the a full evaluation of the morphological discrimination between the 2 species. Also, it is critical that a table of posterior probabilities for each specimen be included in this report as it is a key line of evidence.

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

If you believe that genetic evidence should be used to define taxonomic validity, then Ramey et al.'s assessment of *Z. h. preblei* as not different that *Z. h. campestris* (i.e., not taxonomically valid) is logical. However, if you believe that ecological, physiological, behavioral, and geographic factors should be used to define taxonomic validity, then Ramey et al's assessment is not logical or valid.

3. Are there possible alternative interpretations of the genetics data?

Do not know.

4. Are there additional or divergent taxonomic conclusions that could be drawn from the genetics data?

Do not know.

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

Do not know.

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

I fully agree that evolutionarily significant units (ESU) should be defined based on both ecological data in conjunction with genetic data rather than on genetic data alone. However, the Ramey report argues for combining *Z. h. preblei* with *Z. h. campestris* based primarily on genetic data, and offers no ecological data. Citing this paper seems contradictory to the intent of the Crandall et al. (2000) paper.

7. 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?

There is no way to answer this question based on the Ramey et al. report as ecological distinctions were not discussed. To answer this, Ramey et al. should present an analysis of similarities and differences between *Z. h. preblei* and *Z. h. campestris* with respect to ecology, physiology, behavior, and geography.

**Literature Cited:**

Crandall, K. A., Bininda-Edmonds, O. R. P., Mace, G. M. and Wayne, R. K. 2000. Considering evolutionary processes in conservation biology: returning to the original meaning of "evolutionary significant units". Trends in Ecology and Evolution: 15(7):290-295.

Lance, R. F., M. L. Kennedy, and P. L. Leberg. 2000. Identification bias in discriminant function analyses used to evaluate putatively different taxa. Journal of Mammalogy 81:245-249.

Meffe, G. K., and C. R. Carroll. 1997. The species in conservation. Pages 57–86 *in* G. K. Meffe, and C. R. Carroll, editors. Principles of conservation biology. Sinauer Associates, Inc., Sunderland, Massachusetts, USA.

SAS Institute. 1990. SAS/STAT user's guide, Version 6. Forth edition. Volume 1. SAS Institute, Cary, North Carolina, USA.