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Mr. Peter Plage
U.S. Fish and Wildlife Service
Colorado Field Office
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17 May 2006

Subject: Second Peer Review of 12-Month Finding on a Petition to Delist the Preble's Meadow Jumping Mouse (*Zapus hudsonius preblei*) and Proposed Delisting of the Preble's Meadow Jumping Mouse (ES/CO: T&E: Preble's Mail Stop 65412)

Dear Mr. Plage:

On 16 June 2005, I submitted a letter at the request of the U.S. Fish and Wildlife Service, reviewing the Service's "Finding on Petition ...and Proposed Delisting of the Preble's Meadow Jumping Mouse, *Zapus hudsonius preblei*" (Federal Register 63(92):26517-26530) along with various scientific papers, reports, and letters cited in the petition and proposal. At that time I tentatively concluded that "the weight of scientific evidence I reviewed appears to support that Preble's meadow jumping mouse (*Z. h. preblei*) is not a distinct subspecies and appears to be synonymous with *Z. h. campestris*."

However, I also cautioned that the principal study used to support this conclusion (Ramey et al. 2004) had some methodological problems and over-extrapolated some of its findings. I further stated that I disagreed with Ramey et al. (2004) that combining *Z. h. preblei* with *Z. h. campestris* (or any other subspecies) necessarily means that former *Z. h. preblei* populations cannot comprise a Distinct Population Segment (DPS), and that a combined *preblei-campestris* subspecies would be of no conservation concern. Finally, I concluded that given these uncertainties, delisting had the potential to do irrevocable harm to biodiversity. I therefore recommended that before the delisting proposal was finalized, it would be prudent to perform "further genetic analysis using nuclear DNA coupled with research on morphological, physiological, behavioral, or ecological



similarities or differences between *Z. h. preblei* and other subspecies.” I strongly urged the Service to at least wait for results of additional genetics analyses that were then underway before finalizing the delisting proposal.

This second letter responds to a second request from the Service, to provide peer review of new information on *Z. hudsonius* pertinent to the delisting proposal -- especially the new genetics information referenced above, which I was pleased to learn the Service did wait for. The request for review from the Service was accompanied by two manuscripts: an updated and published version of the Ramey et al. study (Ramey et al. 2005), and a draft of the new and much more extensive genetics study performed by scientists at the U.S. Geological Survey (King et al. 2006). Based on this new information, I was asked by the Service to respond to the same two specific questions I was asked in the first review:

1. Do you support the conclusion that the best scientific and commercial information available indicates that Preble's is not a discrete taxonomic entity?
2. Could you support finalizing the proposal to delist Preble's based on the information currently available?

Based on the new information, I can now more confidently answer as follows:

1. **No:** The best scientific and commercial information strongly supports that *Z. h. preblei* is a discrete taxonomic entity, is a valid subspecies as currently classified, and should not be synonymized with *Z. h. campestris* or any other subspecies.
2. **No:** The currently available information strongly undermines the proposal to delist Preble's, which is a well-supported subspecies (or at the very least a DPS) that is in danger of extinction without protection under the Endangered Species Act.

Below I support these answers with my review of the two documents provided by the Service.

Ramey et al. (2005)

Ramey et al. (2005) is a slightly revised version of the previously reviewed Ramey et al. (2004) manuscript. The authors appear to have minimally responded to problems pointed out by me and other reviewers on the earlier, unpublished version. For example, the “hypothesis-testing approach” used by the authors in investigating the genetic, morphological, or ecological uniqueness of *Z. h. preblei* continues to treat “failure to detect” a difference as “proof positive” that there is no difference, despite problems of small genetic samples, overly conservative statistical tests, and failure to consider more revealing measures or stronger inferences. In general, it feels like the authors were attempting to “stack the deck” and build a case for delisting the subspecies, rather than objectively testing alternative scientific hypotheses.



The genetic samples used were small, including a single, short (346 bp) region of mtDNA and only 5 microsatellite fragments, and were potentially subject to molecular degradation or contamination (see King et al. 2006 for a full review of these and other methodological issues). Moreover, the tests of uniqueness the authors used (including greater between-subspecies variance than within-subspecies variance, and multiple private alleles at higher frequency than shared alleles at a majority of loci) are overly stringent and not generally accepted by phylogenists. As pointed out by King et al. (2006), these criteria for uniqueness are often not even met among accepted species, let alone subspecies.

The so-called morphometric analysis presented by Ramey et al. (2005) is misleading in that it doesn't actually test what it purports to test, ignores more meaningful measures in its focus on a limited suite of relatively uninformative skull measurements, and discounts without justification the statistically significant differences that it nevertheless revealed. The authors assert that their analysis of nine cranial measurements rejects the hypothesis that cranial shape differs between *Z. h. preblei* and *Z. h. campestris* and is counter to the findings of Krutzsch (1954). However, Ramey et al.'s analysis didn't evaluate cranial shape in any meaningful way, but rather recorded a handful of linear measurements, all of which are highly correlated with either length or width of skull. Ramey et al.'s claim that *Z. h. preblei* "failed the test of uniqueness *using the original criteria*" (emphasis added) is simply untrue, because they did not directly test Krutzsch's original diagnostic criteria, which included shape of skull structures and pelage characteristics as well as linear cranial measurements. In fact, the one cranial characteristic Ramey et al. (2005) measured that was claimed by Krutzsch (1954) to be diagnostic (interorbital breadth) was found by *both* studies to be significantly smaller in *Z. h. preblei* (more on this below).

Ramey et al. dismiss all of the other discriminating characteristics reported by Krutzsch (1954) (e.g., differences in pelage coloration and shape of auditory bullae and skull foramina) as "not readily quantifiable." This slight-of-hand attempt to discredit previous work by a trained morpho-taxonomist is both unfortunate and incorrect. In fact, it is quite easy to directly quantify pelage differences using a colorimeter, as I had recommended in my first review. Moreover, the relative size and shape of the auditory bullae could be objectively compared by taking multiple measurements of the bullae; and whether or not the incisive foramina are truncated, as described by Krutzsch (1954), is an objective, binary criterion.

Dismissing these criteria as "not readily quantifiable" appeared to make it easier for Ramey et al. (2005) to argue for no significant difference between subspecies, even though the few cranial measures they did record actually revealed statistically significant differences! In the first paragraph of their results, the authors acknowledge that *Z. h. preblei* was significantly smaller than *Z. h. campestris* in interorbital breadth, but significantly larger for both zygomatic and mastoid breadth¹. However, they go on to

¹ I also note that the original 2003 manuscript produced by Ramey et al. also reported the upper tooth row to be significantly larger in *Z. h. preblei* than in *Z. h. campestris*, with no mention of this difference in the 2004 and 2005 versions.



state that these differences were “very small and of questionable biological significance.” What is the basis for this speculative conclusion about biological significance (which belongs in Discussion, not Results)? I believe many a trained evolutionary or functional morphologist would come to a different conclusion when confronted with these data. A smaller interorbital breadth coupled with wider zygomatic and mastoid breadth hints at functional differences in skull shape that might reflect adaptations to different ecological niches for these two subspecies, especially in light of the importance of zygomatic and mastoid characteristics to masticatory function in mammals (Turnbull 1970, Herring 1993). That is an interesting hypothesis worthy of further scientific investigation, not something to be tossed off as “of questionable biological significance.”

There are other instances where Ramey et al. (2005) appear to emphasize certain results more than warranted or necessary to reinforce their conclusions. For example, the second paragraph of the Results section references a principle components (PC) score plot based on morphological measurements of three subspecies of *Z. hudsonius* (Figure 2) and states that “*Z. h. preblei* specimens fall entirely within *Z. h. campestris* along the PC1 axis.” Although this sentence may be technically true, parsing it in this way to focus attention on only the first of several PC axes seems a bit misleading, given that two of the *Z. h. preblei* points actually fall *outside* the convex polygon enclosing *Z. h. campestris* points in Figure 2.

Ramey et al. (2005) also again failed to support their conclusions regarding lack of “ecological exchangeability” between *Z. h. preblei* and other subspecies with any data or analyses. Although the Introduction section of the paper states that the authors “*tested* genetic and ecological exchangeability” (emphasis added) no “tests” of ecological exchangeability are described. What environmental variables were considered, how were they measured, and what quantitative or statistical tests were applied? Notably, there is no Methods subsection corresponding to the Results subsection entitled “Testing Ecological Exchangeability.” The “results” presented in this section are actually just conclusory statements that “there is no published evidence of adaptive differences... or ecological differences...” This entire subsection of the paper ought therefore to have been placed in the Discussion section rather than in Results. This may seem like a picky, academic point, but as convincingly argued by Conroy et al. (2006) and Beier et al. (2006), it is dangerous for scientific papers to mix discussion items within results sections, because it can mislead readers into believing the authors’ speculations are supported by scientific methods and data, thus risking premature acceptance and repeated mis-citation of untested hypotheses as scientific facts (Conroy et al. 2006). Finally, although I am not an expert on biogeography in the central U.S., even a cursory look at a few maps suggests that there are notable ecological differences between areas occupied by *Z. h. preblei* and *Z. h. campestris*, including differences in dominant vegetation communities (Küchler 1970).

I don’t believe further or more detailed review of Ramey et al. (2005) is necessary or fruitful, because their results are now essentially moot given the much more complete, comprehensive, and methodologically sound analysis provided by King et al. (2006).



King et al. (2006)

King et al. (2006) provided a much more robust and defensible analysis of genetic variation in *Z. hudsonius* than Ramey et al. (2005), and came to opposite conclusions. Their analysis of phylogeographic structure among five subspecies of *Z. hudsonius* used a significantly larger collection of samples (305) over a greater proportion of both mitochondrial and nuclear genomes than Ramey et al. (2005) (1380 bp over two regions of mtDNA and 21 microsatellite loci). It also used a more rigorous biogeographic sampling design for quantifying genetic variation within and between populations (much larger sample sizes of individuals from multiple locations). In contrast to Ramey et al. (2005), who extracted DNA from dried museum skins, King et al. (2006) consistently sampled fresh tissues for DNA extraction, which minimizes chances of molecular degradation or contamination. King et al. (2006) also used more appropriate statistical methods for portraying genealogical relationships. For example, King et al. (2006) argue convincingly that Ramey et al.'s (2005) use of F_{ST} to estimate variation between subspecies that share no haplotypes was biologically and statistically inappropriate, and that Φ_{ST} (which incorporates sequence divergence between haplotypes) is more biologically appropriate and more revealing of evolutionary patterns. I also agree with King et al.'s (2006) discussion that the test criteria used by Ramey et al. (2005) were statistically biased toward making type II errors (failure to reject a false null hypothesis). In other words, Ramey et al.'s (2005) test criteria appear stacked against chances of detecting real genetic differences between subspecies.

The analysis of King et al. (2006) demonstrates clear and significant differences between all five subspecies they examined, which are indicative of distinct evolutionary lineages. Moreover, they were able to demonstrate some genetic divergence *within* subspecies, as in the north-south differences within *Z. h. preblei*, suggesting that the subspecies may comprise at least two DPSs. The strong concurrence among patterns observed by King et al. (2006) using both mitochondrial DNA and nuclear DNA, and using a variety of robust analytical tests, is highly convincing.

Overall, it seems that King et al.'s (2006) conclusions are on very solid ground. Given that their analysis supports the current published taxonomy for these organisms, and that the burden of proof for taxonomic changes lies in falsifying accepted taxonomy, there is no other conclusion than that these five subspecies should retain their current designations as distinct subspecies, and that perhaps *Z. h. preblei* represents at least two DPSs (although this point requires further investigation).

I'll conclude my review of King et al. (2006) by commenting on one statement in their Methods section that I found somewhat troubling: "Attempts to obtain tissue or DNA samples for standardization with the Ramey et al. (2005) microsatellite DNA scoring were unsuccessful." Does this mean that Ramey et al. refused to provide requested samples for standardization? Cross-validation or calibration of samples between studies should be strongly encouraged in studies like these--particularly if funding was provided by federal agencies. Given the importance of the results to conservation and land-use



decisions, I can see no valid reason why the researchers would not share their samples to help ensure that the Service is basing their decisions on best available science. Note that King et al. (2006), with their larger and fresher samples of DNA and careful quality control, found no sharing of haplotypes between subspecies, in contrast to the extensive haplotype sharing reported by Ramey et al. (2005). This is puzzling, and raises concerns about potential sample identification errors or cross-contamination of samples in the Ramey et al. study.

Conclusions

Best available science indicates that *Z. h. preblei* is evolutionarily and taxonomically distinct from other subspecies of meadow jumping mouse, and provides no evidence that the Service was in error in originally listing *Z. h. preblei* as threatened. I applaud the Service for awaiting results of this significant new study by King et al. before finalizing the delisting proposal.

I will close by cautioning the Service to beware of studies wrapped in the lingo of "objective science," but structured in a way that appears to further an agenda rather than to honestly and objectively evaluate alternative hypotheses and let the chips fall where they may.

I hope you find these comments useful.

Sincerely,

A handwritten signature in black ink that reads "Wayne D. Spencer". The signature is written in a cursive, flowing style.

Dr. Wayne D. Spencer
Senior Conservation Biologist



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