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Mr. Seth Wiley
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RE: Notice of Rule 69 FR 16944 to remove Preble's meadow jumping mouse from protection as Threatened species under Endangered Species Act

The American Society of Mammalogists (ASM) is a non-profit, professional scientific society consisting of over 4,000 members from the United States and 60 other countries worldwide. It was founded in 1919 and is the world's oldest and largest organization devoted to the study of mammals. ASM is deeply concerned about the future of mammals worldwide in increasingly threatened habitats, and thus we strongly support conservation and responsible use of wild mammals based on sound and accurate scientific research.

We find that the decision by the USFWS to remove *Zapus hudsonius preblei* from the list of Threatened species under the Endangered Species Act (USFWS 2005b) at this time is unwarranted and unsubstantiated by the available scientific data. We have come to this conclusion after reviewing the delisting petitioners' briefs, two unpublished studies by staff at the Denver Museum of Natural History (DMNH; Ramey et al. 2004a; Ramey et al. 2004b), 14 independent scientific reviews of the first DMNH study by Ramey et al. (2004a), and opinions of additional experts we have contacted. Our primary arguments against delisting are three-fold: 1) most of the independent reviews of the Ramey et al. (2004a) study supported continued protection (i.e., retention of listed status); 2) the scientific evidence produced by Ramey et al. (2004a) was incomplete and misinterpreted; 3) the two studies on which USFWS's decision to delist *Zapus hudsonius preblei* are based (Ramey et al. 2004a; 2004b) have not been through sufficient scientific peer review, which can only be done under the supervision of a scientific journal editor. This latter point, as a general rule for government agencies making ESA decisions, has been well articulated by Ramey (2004) himself.

We find the report by Ramey et al. (2004a) to be inconclusive, at best, and methodologically flawed, at worst, with respect to resolving the question of the taxonomic validity of *Zapus hudsonius preblei*. Of the 14 independent reviews of the study by Ramey et al. (2004a) (USFWS 2005a website), only 5 state the unqualified opinion that the study was conclusive with respect to its demonstration of taxonomic synonymy between *Z. h. preblei* and *Z. h. campestris*. At least four (arguably six; USFWS 2005b) of the 14 reviewers felt strongly that this taxonomic conclusion was unwarranted and that there were methodological problems with the study. The remaining five reviewers expressed some reservations about the study's conclusion of synonymy. One reviewer notes that Ramey et al. (2004a) ignore the pattern in their own data of pronounced genetic differentiation between *Z. h. preblei* and *Z. h. campestris* based on frequencies of the four mtDNA haplotypes. Failure by Ramey et al. (2004a) to report F_{st} values, but interpret them as artificially inflated, raises questions about selective reporting of results in favor of their *a priori* hypothesis.

Synonymizing an endangered subspecies is not to be taken lightly, and the failure of a single study, employing a single mtDNA marker, to detect genetic differentiation according to the strict criterion of "reciprocal monophyly" (problematic due to the impact of ancestral polymorphism on the time required for groups to become reciprocally monophyletic after gene flow ends) (Ramey et al. 2004a) is not sufficient. Indeed, mitochondrial sequence data would not be expected to resolve a dichotomy with such a recent divergence time. As noted by one reviewer (M. Douglas, cited in USFWS 2005a), 355 base pairs is far short of the generally accepted *minimum* sequence data requirement of 1,000 base pairs for population-level analyses to be conclusive. Further, the practice of using a single-locus gene tree to define taxonomy has repeatedly been refuted in the literature (Brumfeld et al. 2003; Edwards and Beerli 2000) and is generally not accepted as sole evidence for the reclassification of any taxonomic groups. Failure to use additional nuclear loci to resolve the genetic divergence question or microsatellite DNA to investigate patterns of gene flow between populations reflects the incomplete and inadequate nature of the study and supports our conclusion that the evidence produced therein is not sufficient to refute previous studies of genetic and morphologic differentiation.

The morphologic analysis reported in Ramey et al. (2004a) was inadequate in that it ignored bacular data entirely and it failed to include skull-height measurements or analysis of the qualitative skull characters cited by Krutzsch (1954). Instead, they included many characters that likely are highly correlated, which limits the statistical power to discriminate. Finally, it would have been more appropriate to perform a Principal Components Analysis of the morphometric characters, rather than the Discriminant Analysis that was performed instead.

A possible methodological problem not raised by the reviewers is that Ramey et al. (2004a) relied exclusively on museum specimens and did not indicate how or if verification of the species and subspecies identity was made for their study. Misidentifications of specimens borrowed from numerous museums is a common problem hampering such studies, and is of particular concern with this study due to the apparent lack of prior experience by its investigators with this taxon. Having just a few misidentifications in the group of specimens included in the two studies could invalidate the genetic and morphologic results of Ramey et al. (2004a; 2004b).

We further note that four of the five reviewers who believed that Ramey et al. (2004a) made the case for synonymy, went further to emphasize that there was ample evidence to suggest that the loss of *Z. h. preblei* would constitute a critical loss of genetic and ecological diversity and/or that the newly combined *Z. h. campestris* itself is imperiled across its range due to fragmentation and loss and degradation of its habitat. Indeed, Hafner et al. (1998) consider *Z. h. campestris* to be a species of conservation concern: (Vulnerable (VU): B1; B2c; IUCN Red List Category; IUCN 1994).

In response to the concerns of the 14 original reviewers, Ramey et al. (2004b) produced a second unpublished study concluding that *Z. h. campestris* (into which they had already subsumed *Z. h.*

preblei) should be synonymized with the more widely distributed *Z. h. intermedius*. However, this study suffers from most of the same methodological and theoretical problems that plagued the original study.

A key component of the question of genetic distinctiveness between subspecies is how recently gene flow has occurred, in this case, between Front Range populations of *Z. h. preblei* and other *Z. hudsonius* populations. The second study by Ramey et al. (2004b) used the program MDIV to test for the presence of gene flow and concluded that there has been "very recent gene flow" between these two populations. We asked the author of the MDIV program, Dr. Rasmus Nielsen to review Ramey's study, and in his review he commented that: "The results obtained by Ramey *et al.* (2004a) could very well be consistent with the absence of gene-flow during the past, say, 10,000 years. The program MDIV is not designed to identify when the divergence of the populations gene-flow has taken place, only if it has taken place (Rasmus Nielsen, *in litt.*)." Therefore, the possibility of genetic isolation of *Z. h. preblei* for the past 10,000 years remains, even if we assume they used the technique correctly.

The petition for delisting *Z. h. preblei*, filed by Coloradans for Water Conservation and Development (CWCD), obviously is influenced heavily by the overstated conclusions of the Ramey, et al. (2004a) study. In their petition, CWCD presented unpublished data on numerous "new localities" for the species since the 1998 listing. New localities are not surprising, given the far more intensive trapping surveys that took place after listing, rather than before. However, the fact remains that the species is still absent from 3-4 counties in Colorado and Wyoming where it had been previously collected, and its range is now bisected by the urban area of Denver. Urban development subsequent to 1998, when threats to the survival of Preble's meadow jumping mouse were exhaustively detailed by USFWS, can have only further fragmented its distribution. It is possible to address questions concerning the impact of this fragmentation on current gene flow, information that is essential for predicting persistence of these populations; however no effort has been made in this regard. We see this as an additional shortcoming of the Ramey et al. (2004a; 2004b) studies and the proposal to delist in general.

CWCD makes a repeated point of the original intent of Congress that the Distinct Population Segment (DPS) option of the ESA be used "sparingly." Yet they present no data to indicate that use of that option to protect the population(s) currently classified as *Z. h. preblei* would violate that intent, if this population was indeed synonymized with *Z. h. campestris* (or even with *Z. intermedius*). In fact, the scientific consensus from the several reviewers mentioned above is that this disjunct population, regardless of its taxonomic status, is threatened by habitat loss and does warrant ESA protection either as a DPS or as part of a vulnerable combined taxon *Z. h. campestris*. We agree with this view and urge the USFWS to maintain ESA protection of the Front Range populations of *Z. hudsonius*, either as part of a distinct taxon or as a DPS of the newly combined taxon, pending completion of more conclusive genetic and morphologic analyses.

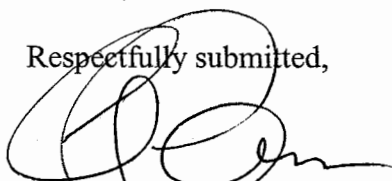
A final essential point and one that was not addressed at all by Ramey et al. (2004a; 2004b) or by the CWCD is the potential ecological distinctiveness of the populations in question. Ecological divergence can be indicative of separate and distinct evolutionary trajectories, and would further support protection of those populations under the Endangered Species Act. Due to the high potential for such divergence between these geographically distant groups, we urge that any decision regarding the protected status of meadow jumping mouse populations currently recognized as *Z. h. preblei*, *Z. h. campestris*, and *Z. h. intermedius* include field-collected data designed to test the ecological distinctiveness of these populations. Fieldwork should also be conducted to test the hypothesis that populations of meadow jumping mice in northeastern Wyoming are continuous with those in southeastern Wyoming and adjacent Colorado. If these populations are indeed disjunct, then gene flow between them will be impossible.

In summary, we have reviewed the available data and, in so doing, identified several specific misinterpretations and shortcomings of the evidence and conclusions presented by petitioners and one

unpublished study partially funded by a petitioner. Results from our review do not support the conclusion that *Zapus hudsonius preblei* should be delisted. The available evidence, primarily one study, which has not received the standard vetting of the scientific community, namely peer review as part of the publication process, is not sufficient to serve as the basis for a decision to delist this taxon. We are concerned that a delisting decision based on this evidence could be interpretable as an inappropriate application of the scientific process in agency decision-making (Union of Concerned Scientists 2005). Ironically, the senior author of the study upon which the USFWS has based its decision (Ramey et al. 2004a), criticized the ESA listing process in an appearance before Congress as too often being based on studies that were not properly peer-reviewed (Ramey 2004). We strongly agree with this aspect of his general advice to the Congressional committee. Ramey's failure to meet the criteria he himself advocated reduces the ability of USFWS to rely on that study as the basis for the decision to delist Preble's meadow jumping mouse.

In conclusion, the American Society of Mammalogists (ASM) recommends that the USFWS not remove *Zapus hudsonius preblei* from the list of Threatened species under the Endangered Species Act at this time. Further, ASM stands ready to make available our expertise on the technical questions raised by proposed rule 69 FR 16944. We greatly appreciate the opportunity to provide comments on this very important issue and will be glad to expand on or clarify these comments if needed.

Respectfully submitted,



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American Society of Mammalogists

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