

REVIEW: Report by R.R. Ramey et al. (2004b) on *Zapus* subspecies

Most of the problems noted in the Ramey et al. (2004a) review are not addressed in the current report. If anything, this report contains an even greater dearth of information than would be necessary to clarify points criticized in the first report. Rather than re-iterating those concerns, I simply refer to my previous review for details. Major issues pertain to molecular approaches used, analytical approaches employed, and ambiguous results that do not support the conclusions of the authors. A few major concerns are reiterated below (those with most serious consequences listed first):

Questions by FWS:

(1) a) Are methods appropriate? - No.

- The molecular data are quite limited (only 355 base pairs of sequence) and provide insufficient resolution. Selected marker (i.e., Control region of mtDNA) cannot resolve recent (<10'000 years) divergence. Given this, results remain inconclusive. Low nucleotide variation is reported and is suggestive of minimal divergence.
- A gene tree is equated with a species tree. More than one independent locus should be assessed to infer phylogenetic relationships among taxa.
- Insufficient detail is provided to ascertain findings and conclusions.
- Issues associated with museum-based molecular work are not addressed (low yield and quality of "ancient DNA"; potential contamination with other DNAs; genotyping errors)
- Details on analyses are missing (e.g., were all samples included in analyses? Previous report stated that certain individuals were excluded, but exclusion criteria were ill-defined).
- Details on collection data are still lacking. How reliable are the locality definitions? Was identification of specimens confirmed by reexamination? Inclusion of a few miss-identified specimens would weigh heavily in both molecular and morphological analyses.
- Why is a Neighbor joining phylogram presented, instead of an MP, ML or BA tree? Authors state that other analysis produced "similar" trees, but phylograms of these should be provided so that tree topology and nodal support can be examined.

b) Is the proposed approach to delineate subspecies based on within versus among population mtDNA variation appropriate? – No.

- The criterion of “greater genetic diversity among putative taxa than within” is still a flawed concept (and its iterative use does not make it valid). Genetic diversity depends on population size and population history. Paetkau (1999) emphasized that population demographics do influence retention of genetic diversity, including ancestral haplotypes and time to complete lineage sorting (or reciprocal monophyly).

- Conclusions based on AMOVA are not justified. High percentage of within vs among subspecies diversity is influenced by resolution of the marker and demographics of the population (e.g., bottlenecks, population fluctuations, effective population size, etc.).

(2) Are data presented in the report supporting author’s conclusion of lumping *Zapus hudsonius preblei*, *Z. h. campestris* and *Z. h. intermedius*? – No.

- I still cannot follow the logic. If *Z. h. preblei*, *Z. h. campestris* and *Z. h. intermedius* should be synonymized based on shared haplotypes, then the other two *Z. hudsonius* subspecies (*luteus* and *pallidus*) must be synonymized as well. Five *Z. h. intermedius* and four *Z. h. campestris* specimen fall within the *luteus/pallidus* clade, and two *Z. h. pallidus* fall within the *preblei/campestris/intermedius* clade (Fig. 2). The latter is not well supported (87% is not exactly “strong bootstrap support,” as stated on page 4).

- Shallow branches and low bootstrap support for most nodes suggest lack of resolution, again revealing limitations of molecular data (e.g., insufficient number of base pairs and only a single locus).

- No *Z. h. preblei* haplotypes (C/P) were found in any of the analyzed *Z. h. intermedius*. As previously pointed out, frequencies of C/P haplotypes are skewed, with low number of *Z. h. campestris* showing C/P haplotypes. Also, if this was due to recent gene flow (rather than insufficient resolution) then haplotypes that are numerous within geographically adjacent populations of *Z. h. campestris* would be expected, especially under the stepping-stone model of gene flow the authors evoke.

(3) Do results of MDIV analysis support conclusion that *Zapus hudsonius preblei*, *Z. h. campestris* are a single connected population? – No.

- MDIV analysis does not allow distinction between very recent versus more ancient gene flow.
- Further, lack of resolution is a more likely explanation than recent gene flow. See also comment above on haplotype frequencies.

(4) Are there possible alternative interpretations of the data? – Yes.

How likely are these possibilities? – More likely than author's interpretation.

- Alternative conclusions are most certainly possible. However, limitations of the genetic data hamper any conclusions and render as speculative any taxonomic interpretations.
- Identical haplotypes in *Z.h.preblei* and *Z.h.campestris* could be explained by:
 - Retention of ancestral polymorphism and incomplete lineage sorting.
 - Homoplasmy (similar character state but independent evolutionary origin).
 - Genotyping error

For details see my review of Ramey et al. (2004a).

(5) What additional analysis would be needed to verify the study's assertions and why?

- First and foremost, additional sequence data from fast evolving, independent markers (nuclear loci) would be needed to increase resolution (as recommended by Haig 1998). Data based on a different marker might still remain incongruent, but that in itself reveals important aspects of the phylogenetic history of a species (Hey et al. 2003).

For further suggestions see my review of Ramey et al. (2004a).

(6) Is new information presented in Ramey et al. (2004b) sufficient to address voiced concerns about findings and conclusions of Ramey et al. (2004a) report? – No.

- Dearth of information on methods and limitations of the data affect resolution of analyses and thus render results inconclusive. Relationships among haplotypes are not (or but poorly) resolved in the neighbor joining tree (Fig. 2).

References Cited:

Haig, S.M. 1998. Molecular contributions to conservation. *Ecology* 79(2):413—425.

Hey, J.M., C.H. Daugherty, A. Cree, and L.R. Maxson. 2003. Low genetic divergence obscures phylogeny among populations of *Sphenodon*, remnant of an ancient reptile lineage. *Molecular Phylogenetics and Evolution* 29:1-19.

Paetkau, D. 1999. Using genetics to identify intraspecific conservation units: a critiques of current methods. *Conservation Biology* 13(6):1507—1509.

Ramey, R.R., H-P. Liu, and L. Carpenter 2004a. Testing taxonomic validity of Preble's Meadow Jumping Mouse (*Zapus hudsonius preblei*). Report to the Governor of Wyoming and U.S. Fish and Wildlife Service.