

Review of Report, "Testing the Taxonomic Validity of Preble's Meadow Jumping Mouse (*Zapus hudsonius preblei*)," by R. R. Ramey II, H-P. Liu & L. Carpenter

Review Submitted by Mary V. Ashley, Ph.D., Dept. of Biological Science, University of Illinois at Chicago

Question 1: Do the morphological, ecology, and mtDNA data presented in the report support the authors' conclusions on synonymizing *Z. h. campestris* and *Z. h. preblei*?

Although I am not an expert on morphological data analysis, I found that the authors' strongest case for synonymizing the two subspecies comes from their thorough morphological study. Discriminant function analysis based on 33 *Z. h. preblei* and 39 *Z. h. campestris* specimens had poor discriminating ability, correctly assigning only 48% of specimens. It should be noted that the authors applied a fairly strict criterion of $p > 0.95$ posterior probability. However, it does not appear that the two subspecies are morphologically distinct. This is a fairly straightforward conclusion based on solid data set.

A cautionary note on morphology, however, comes from the work of Conner and Shenk (2003). They were able to develop a discriminant function based on repeated measures (single measurement sets were not sufficient) to distinguish between *Z. h. preblei* and another species, *Zapus princeps princeps*. They report a 70% error rate in species identification for specimens from southeastern Wyoming. The point is that *Zapus* species and subspecies are morphologically very similar.

With regards to ecological distinctiveness, the authors present no information either way. Although they use lack of evidence in the literature for ecological differences to argue for synonymizing, they present no references and it is difficult to judge how thoroughly they looked. Did they include the 'gray' literature and technical reports? At this point, lack of evidence represents lack of information, not evidence *for* synonymizing.

With regards to the mtDNA data, the results are interesting and can be interpreted differently depending on one's viewpoint. The author's position is that *Z. h. preblei* and *Z. h. campestris* are not reciprocally monophyletic for mtDNA haplotypes, and no unique mtDNA haplotypes were found in *Z. h. preblei*. This is the crux of their argument for synonymizing the subspecies based on genetic data, and based on very strict criterion, it is a valid argument. However, inspection of the results in Figure 2 and Table 1 indicate that there are four *Z. h. preblei* haplotypes and these are all at fairly high frequencies within *preblei*. While these haplotypes are also found in *Z. h. campestris*, the shared haplotypes are at very low frequency in *Z. h. campestris*. Clearly, based on haplotype frequencies, the two 'subspecies' are genetically quite distinct. Furthermore, the phylogram indicates a significant break between the 'mostly preblei' lineages and the 'mostly campestris' lineages, with 96% bootstrap support for the node. There is significant structure in the mtDNA data set, and it *nearly* corresponds to *preblei/campestris* subspecies.

Question 2: Could you support synonymizing *Z. h. campestris* and *Z. h. preblei* without additional genetics studies (i.e. microsatellite data)? If not, what additional analysis is needed?

No, I feel more data is needed, based on the patterns of the mtDNA data presented here. DNA microsatellite data would certainly help clarify the genetic picture, although in my opinion neutral genetic markers will never provide a definitive answer to whether ecological, behavioral or physiological differences exist. But given that there is evidence for population genetic structure that largely corresponds to the traditional taxonomy, I would recommend a microsatellite study, with data analysis including Bayesian approaches to identifying cryptic population structure (eg. *Structure* analysis of Pritchard et al.). Multi-locus assignment tests should be conducted. *Z. princeps* from sympatric and allopatric populations should also be included in the analysis, to address the possibility of gene flow between these taxa.

Question 3: What is the importance of potential ecological, behavioral, or physiological differences between *Z. h. campestris* and *Z. h. preblei* in substantiating or refuting synonymy?

Such differences might exist and have been shown for closely related species and subspecies of rodents. I am not an expert on *Zapus*, and most of my information comes from the materials I was sent, and references therein. I don't think there is a clear enough understanding of the ecology and biology of these subspecies to address this question.

Question 4: What is the likelihood that the *Z. h. preblei* is substantially reproductively isolated from other groups within the *Z. hudsonius* complex, especially from *Z. h. campestris*?

If you mean could they breed together in captivity, I doubt that there is reproductive isolation. In the wild, it seems to me that the critical issue here is the range of *Z. h. preblei*, which, according to the map provided in the materials, is geographically isolated from other *Z. hudsonius* populations. There would appear to be no opportunity for gene flow from the rest of the species complex, so the population from Colorado and southeastern Wyoming (whatever its taxonomic status), is reproductively isolated. Furthermore, if it is lost, it will not be naturally recolonized.

Question 5: Would the loss of what is now *Z. h. preblei* represent a substantial diminution of the *Z. h. campestris* taxon? Its Range? Biological characteristics? Evolutionary legacy? Other?

As stated in number 4 above, loss of *Z. h. preblei* would represent a loss from an important part of the species range. The rest is less clear and mostly subjective from my understanding and reading the material that was provided.

22 June 2004

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Dear Dr. Willey:

I have completed the review of the report "Testing the Taxonomic Validity of Preble's Meadow Jumping Mouse (*Zapus hudsonius* (*Z. h.*) *preblei*). Please find the review and my CV attached.

Thanks for allowing me to be part of the review process. Sorry I took a little longer than promised.

Sincerely,

Robert D. Bradley
Associate Professor

This is an excellent piece of work on a controversial issue. I like the way the authors set up the study by specifically testing a series of hypotheses related to the bigger picture. That way any rejecting leads to a valid conclusion. My assessment is based simply on the data provided – I have no prior experience with this taxon. My review is constructed along the lines of the questions provided in the cover letter.

1. The data presented do support the synonymizing *Z. h. campestris* and *Z. h. preblei*. Both the morphological and mtDNA analyses are convincing in that the two taxa actually represent a single taxon.
2. I could support the placement of the two taxa in synonymy without further data. Nuclear data would be nice – but is not necessary given the clear pattern in morphological and mitochondrial data.
3. Ecological, physiological, or behavioral differences are or would be extremely important in building a case that *Z. h. preblei* is significantly different from *Z. h. campestris*. For example, if one of the two taxa occurs only at high elevations and the other at low elevations; or if one occupies a forested area and the other occupies a grassland. In the absence of significant differences concerning ecological, physiological, or behavioral patterns between the two taxa; in my opinion, these parameters are of lesser importance.
4. Reproductive isolation is always a tough call. They may be physically isolated due to changing distribution patterns and by default reproductively isolated. However, if the two taxa were to become sympatric then they may or may not be reproductively isolated. My guess from the data and distribution is that the two are not reproductively isolated. The best example is with the mtDNA data.
5. It does not appear that that loss of *Z. h. preblei* would significantly impact *Z. h. campestris* in terms of genetics. However, it appears that the range or distribution might be impacted given the isolated nature of *Z. h. preblei*.

Review of:

Testing the taxonomic validity of Preble's meadow jumping mouse (Zapus hudsonicus preblei).

Reviewed by:

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I was asked to address five issues in this report. I will address each of them to various degrees based on my understanding of the concepts and analyses employed. I will then provide an overall opinion on the content and context of this study with regard to the relevant conservation issues.

1. Do the data support conclusions that *Z. h. campestris* and *Z. h. preblei* should be synonymized?

I believe that the data support a lack of substantive morphological, ecological, and molecular differentiation between these two subspecies. This is not surprising, and in fact is a very common outcome of molecular analyses of taxonomic subspecies that are in close geographic proximity, are ecologically similar, and appear to have no surmounting biogeographic obstacles to movements across an historical landscape (e.g., as it might have existed during a Late Pleistocene pluvial period). The mtDNA data, specifically, support the contention that populations within the ranges of these two subspecies do not form "reciprocally monophyletic" evolutionary lineages relative to one another, although further sampling of additional specimens and localities, and use of other phylogeographic statistical procedures, might reveal a significantly non-random geographic partitioning of genetic variation into subsets of populations that are largely congruent with a subspecies dichotomy. The latter comment comes from examination of the mtDNA gene phylogeny in the report, in which all four mtDNA haplotypes labeled as "C/P" form a loose, non-supported by bootstrap replication procedures, aggregate that might have geographic connections between Colorado and northern populations along major river drainages. If this were true, it might suggest a separate, albeit shallow, geographic history of range connection between certain subsets of populations relative to others within the overall two-subspecies aggregate.

2. Are additional genetics studies required?

Two potential problems are associated with the use of a single genetic marker: it might not reflect "overall" evolutionary affinities between lineages due to sorting or to introgression effects; and it might not be evolving rapidly enough to capture an evolutionary distinction between lineages. The first issue is not likely to be a serious problem in this case. The geographic structure of *campestris* and *preblei* (small, disjunct populations occurring at the edge of the species western distribution) suggests that, while

gene flow between populations may have been frequent under past climatic and habitat conditions, it is not likely to be occurring with high frequency at this time. Perhaps, an original and very distinct "*preblei*" lineage was swamped by a general dispersal of "*campestris*" genes into its range, but this seems unlikely to have happened simultaneously across the entire range of *preblei* given the generally fragmented nature of its populations. The second issue is also not likely to be an issue, because while microsatellites or SNPs might allow one to detect finer-scale population structure than mtDNA, they are not likely to provide a signature of substantial evolutionary subdivision at the level of taxonomic subspecies if the mtDNA did not do so.

3. What is the importance of ecological, behavioral, or physiological differences between subspecies in supporting or refuting synonymy?

I have little to say about this issue, in part because I doubt that one would find interesting biological differences between populations representing the different subspecies (they all should occupy a range of rather similar, discrete microhabitats that would select for quite similar phenotypic traits relative to, say *hudsonius* habitats embedded within in a far northern boreal forest), and in part because, without a genetic signature of historical evolutionary separation between lineages (e.g., reciprocal monophyly, or at least significant frequency differences), I would not support recognition of taxonomic distinctness based solely on ecological, behavioral, or physiological traits.

4. What is the likelihood that *preblei* is substantially reproductively isolated from *campestris*?

I see neither a genetic nor a biogeographic reason to predict that populations within these two subspecies are reproductively isolated from one another. Note that reproductive isolation has never been a criterion employed by mammalogists to recognize distinct taxa at the level of subspecies, so the question actually is not relevant to the issue of a substantial and recognizable history of isolation and divergence between populations.

5. Would loss of *preblei* represent a substantial diminution of *campestris* range, biology, or evolutionary legacy?

I suspect that populations of what is now considered *preblei* represent a substantial and important portion of the overall viability of what is now considered *campestris*. My opinion has to do with the fact that both subspecies represent a set of disjunct, peripheral populations at the western edge of the range of the species. Macroecologists identify core vs. peripheral ecological characteristics of species ranges, and these include, for example, a core to peripheral decrease in overall quality and continuity of habitat; and thus a core to peripheral decrease in overall population connectivity, population abundance, and population viability. I suspect that both subspecies represents, in large part, a set of populations that are disconnected from one another, restricted to small, discrete and isolated microhabitats surrounded by decidedly non-*hudsonius* habitats, and thus quite vulnerable to local extinctions without the possibility of rescue via dispersal from surrounding populations. Nevertheless, because of their peripheral isolation to,

perhaps, a subset of what might be considered as ecologically and physiologically extreme environments for the species, these populations could well contain a set of ecological traits that have selective advantage in extreme environments and therefore are unique and interesting (perhaps irreplaceable) within the context of the species as a whole. As such, even if the two subspecies are taxonomically synonymized, I would strongly urge agencies and recovery teams to regard all remaining populations as potentially valuable within a recovery plan.

Final comment

While I see no reason to support an opinion that *preblei* and *campestris* should be retained as separate taxonomic subspecies, I believe that there is still a case to be made for considering the collective set of populations originally represented as separate subspecies as an evolutionary lineage of conservation concern. That this is, perhaps, a highly distinct evolutionary lineage is supported by the very clear and deep “reciprocally monophyletic” separation between *luteus/pallidus* vs. a *campestris/preblei* mtDNA lineages (although, I would have liked to see sequence divergence data in the report, and don’t understand why the authors would not want to show results of all analyses rather than just saying (pg. 19) that “Other methods of phylogenetic analysis produced very similar trees.”

What this study has done, in my opinion, is not reduced the level of conservation concern for a set of vulnerable populations along the western periphery of the species’ distribution, but rather, established that the issue of evolutionary distinction needs to be addressed at a different geographic and sampling scale. If one looks at the proportion of the overall species distribution sampled in this study, it becomes very apparent to me that one cannot really say anything yet about context of the *campestris/preblei* within the phylogeographic structure of the species. Yes, they are quite distinct from *luteus/pallidus* populations as far south as New Mexico/Arizona and as far east as Kansas/Nebraska, but that still leaves us with at least two alternative hypotheses—either *campestris/preblei* represents its own unique evolutionary lineage (e.g., ESU) within *Z. hudsonius*; or it represents a western (and / or southern) extension of a lineage that is widespread throughout a larger portion of the species. If I had to make a prediction, after looking at distributional details in the map produced by Dr. Hafner within the context of the overall distribution of the species, I would guess that the mtDNA lineage represented in this study by populations assigned to *luteus* and *pallidus* is the one that represents a subset of a more widely-distributed lineage, and that the *campestris/preblei* lineage really is an evolutionarily distinct subset of the species. This possibility, however, cannot be evaluated without additional population and geographic sampling, although such could be accomplished efficiently by using the same mtDNA genetic marker as used in this report.

5 July 2004

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RE: Review of Ramey et al. "Testing the taxonomic validity of Preble's Meadow Jumping Mouse (*Zapus hudsonius* (*Z.h.*) *preblei*)"

Dear Seth;

I was recently contacted by your office (in a letter dated 3 June 2004) to review the above report by Dr. Rob Ramey II and co-authors, and herein include my review of that report (also enclosed). It might be helpful to you to know a bit about my background, so that my comments can be judged in the context of my strengths and limitations. As requested, I have also included an updated CV.

First, I am not a mammalogist, and I have not tracked down the taxon-oriented papers cited by Ramey et al. (such as papers or reports by Hafner, Riggs, etc.); I have accepted many things about the biology of these mice (such as the correct identification of taxa sampled for molecular data; see 'Results' section, bottom of p. 7) at face value. I am a herpetologist and my research group focuses almost exclusively on addressing evolutionary and conservation issues in amphibians and reptiles. I have had over 25 yrs of research experience in the use of genetic and molecular markers (allozymes, chromosomes, microsatellites, and sequences of mitochondrial and nuclear gene regions) for population genetic, phylogeographic, and phylogenetic studies of various groups. More to the point of this review, a lot of our work in the past 5-6 yrs has increasingly focused on conservation issues with species of special concern (frogs, salamanders, and turtles in particular), specifically on the issue of treating formally recognized taxonomic entities as hypotheses that should be tested by clearly defined criteria.

I did go through all of the background material sent to me ("Preble's Meadow Jumping Mouse Recovery Plan, Colorado", maps, figures, "Final Rule to List the Preble's Jumping Mouse", etc.) to try to become familiar with the history of the listing of *Z.h. preblei*, the conservation issues, and what has been invested to date in the recovery efforts. I will address each of the points below raised in the initial letter.

1 – Do the morphology, ecology, and mtDNA data presented in the report support the authors' conclusion to synonymize *Z.h. campestris* and *Z.h. preblei*?

The general answer to this question is “yes” but with qualifications. The authors were very clear on p. 4 about setting forth their hypotheses “*a priori*” for testing the taxonomic uniqueness of *Z.h. preblei*, and I am an advocate of this approach (Sites and Crandall, 1997). Ramey et al. adopt the mtDNA “reciprocal monophyly” test of Moritz (1994), and while this test specifies an unambiguous criterion for acceptance/rejection of a distinct ESU (evolutionary significant unit), it does assume that the mtDNA sequences used are evolving under a neutral model (Funk and Omland, 2003). Further, more recent theoretical work suggests that the coalescence of any single genetic locus may have a large variance, even in lineages of the same age, due to many stochastic processes (Irwin, 2002; Hudson and Turelli, 2003).

The mtDNA gene tree presented by Ramey et al. (Fig. 2) is consistent with an interpretation of the introgression *campestris* mtDNA into *Z.h. preblei*, but the topology is asymmetrical and provides no evidence for the reciprocal flow of *preblei* mtDNA back into *Z.h. campestris* – if mating is random in regions of overlap and hybrid and backcross genotypes have approximately the same fitness as parentals, then gene flow should be somewhat symmetrical then we should see at least some *preblei* mtDNA haplotypes introgressing into the Wyoming populations of *campestris*. This pattern would also be expected if hybrid and backcross genotypes showed some fitness reduction relative to parentals, so long as this was approximately the same level of reduction in both directions.

I point out the above alternatives because the gene tree pattern shown in Fig. 2 COULD also result either from: (1) incomplete sorting of mtDNA haplotypes (due to a very recent split between populations); or (2) a “selective sweep” favoring introgression (either ongoing or historically) of *campestris* mtDNA on the *preblei* nuclear background. If incomplete lineage sorting is an issue, then *a priori* one should also see some *preblei* haplotypes in Wyoming populations of *campestris*, because the process is normally stochastic. This appears to be the pattern, for example, between *campestris* and *pallidus* haplotypes: each of these entities segregates a few haplotypes in the “other’s clade” (Fig. 2). The issue of a selective sweep (or the alternative of selection acting against *preblei* mtDNA on the *campestris* nuclear background) is harder to get at, and although I think Ramey et al. make a good case for ongoing gene flow (or at least very recent gene flow) on the basis of absence of morphological differentiation between *campestris* and *preblei*, they did not implement any test of the selective neutrality on their mtDNA sequences (Funk and Omland, 2003; Ballard et al. 2004). They thus cannot unequivocally rule out the possible role of natural selection for the observed phylogeographic patterns of the mtDNA locus (Fig. 2) – if such as sweep has occurred then the “diversity test” proposed by the authors (bottom of p. 4) will fail, and the conclusions drawn at the bottom of p. 9 are compromised (see Rand, 1996, for specific reference of this issue to conservation biology). Fortunately, several statistical tests for neutrality of sequences can be implemented with widely available software (see Morando et al., 2004, for an example), and I would encourage Ramey et al. to do this before submitting their manuscript to a refereed journal.

The strongest case made by Ramey et al. for conspecificity of *Z.h. campestris* and *Z.h. preblei* is the morphological evidence. Here I think their methods are rigorous and unambiguous, the patterns of morphological variation are in general agreement with the recovered mtDNA genealogy, and I agree with their conclusions. One last point;

Ramey et al. implement the “crosshair classification” of Crandall et al. (2000) as a test for ecological distinctiveness (which may reflect adaptive change that would not be detected by molecular markers; p. 5), and then speak of “failure of evidence to reject hypotheses of genetic and ecological exchangeability between *Z.h. preblei* with *Z.h. campestris*, . . . “ (bottom of p. 9). They have not implemented a statistical test of genetic or ecological exchangeability, and so far as I am aware, the only way to do this currently is by implementing Templeton’s (2001) tests for “cohesion species”. Without a statistical test of ecological exchangeability, I don’t think the authors can say much here.

2 – Could you support synonymizing *Z.h. campestris* and *Z.h. preblei* without additional genetics study (i.e., microsatellite data)? If not, what additional analysis is needed and why?

Because so much rests on this decision, I would interpret the Ramey et al. report as tentative evidence for synonymizing *Z.h. campestris* and *Z.h. preblei*, and recommend two things. First, implement tests for neutrality of the mtDNA sequences, and if the null hypothesis (neutrality) cannot be rejected, then Ramey et al. have a stronger basis from which to infer ongoing or historically very recent gene flow between these entities. Second, Ramey et al. (or someone) should definitely conduct a follow-up study using nuclear markers, ideally using the same samples used by Ramey et al. Microsatellites could certainly be used for this, but unless a gene library has already been developed for a closely related species, and unless the microsatellite loci for the “target species” have been shown to amplify (by PCR) and to be polymorphic within *Z.h. campestris*, *Z.h. preblei*, and all other relevant taxa, this will be an expensive and time-consuming approach. I suggest a more feasible follow-up study could be based on “amplified fragment length polymorphisms” (AFLPs); this approach will provide many more loci (albeit they cannot be scored individually as co-dominant) for less cost, and they have been shown to be very informative when trying to assess relationships between closely related taxa (or populations) on the basis of incomplete information from morphological and mtDNA data (see Sullivan et al. 2004, for a recent example). If such a study revealed that the AFLP data recovered both *Z.h. campestris* and *Z.h. preblei* as a single strongly supported clade, the taxonomic conclusion of Ramey et al. would be confirmed. An alternative result might show that these two entities were recovered as separate clades, which would suggest that the mtDNA alone had introgressed asymmetrically from one distinct entity into another (not an uncommon pattern; Funk and Omland, 2003), and that morphological characters were conserved and did not “track” the splitting event.

3 – What is the importance of potential ecological, behavioral, or physiological differences between *Z.h. campestris* and *Z.h. preblei* in substantiating or refuting synonymy?

The ecological points raised by Crandall et al. (2000) are important and might reflect divergence between *Z.h. campestris* and *Z.h. preblei* in some adaptive characteristics driven by ecological differences, and as pointed out by Ramey et al., these

are not likely to be detected by molecular markers. However, without implementation of a rigorous statistical design, such as Templeton (2001), I don't think anything can be added to clarify the issue at present.

4 – What is the likelihood that *Z.h. preblei* is substantially reproductively isolated from other groups within the *Z. hudsonius* complex, especially from *Z.h. campestris*?

See my response to question 2 above – Ramey et al. have a good provisional case for **absence of isolation** between *Z.h. campestris* and *Z.h. preblei*, but follow-up work is needed.

5 – Would the loss of what is now *Z.h. preblei* represent a substantial diminution of the *Z.h. campestris* taxon? Its range, biological characteristics, evolutionary legacy, etc.??

Regardless of the taxonomic reality of *Z.h. preblei* (and I think FWS must delist *Z.h. preblei* if subsequent study validates Ramey et al.; at all costs we need to avoid investing time and resources in bogus taxa that were the result of faulty or incomplete early studies), the map in my supplemental material (Fig. 2, p. 12) shows that what is now *Z.h. preblei* represents a very large and disjunct portion of *Z.h. campestris*, and its loss would almost certainly represent a substantial loss of the evolutionary legacy of *campestris*. This may be especially true in this case because of the peripheral distribution of *Z.h. preblei* relative to *Z.h. campestris* – these kinds of populations are thought to represent a major component of future “speciation potential” of any taxon (Frey, 1993; Lesica and Allendorf, 1995). The application of high resolution molecular markers such as microsatellites might very well reveal that unique alleles are segregating in these peripheral demes, and while such a result would not support continued taxonomic recognition of this entity, it would highlight the evolutionary value of the populations in this part of the range.

In closing, I want to offer my support to the authors for their clear formulation of alternative hypotheses, and criteria by which these were tested in this study. This kind of approach is still frustratingly uncommon, and the authors have done a very good job. I hope my comments here are taken in the context of constructive criticism, and that they will be useful to the authors and to your office.

Sincerely,

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Peer Review of Preble's Jumping Mouse Report by Dr. Rob Ramey II et al.

Dr. Lisette Waits, Associate Professor, Department of Fish and Wildlife, Univ. of Idaho

I will start by addressing the following questions and then add two additional comments at the end of review.

- (1) Do the morphology, ecology, and mtDNA data presented in the report support the authors' conclusions on synonymizing *Z. h. campestris* and *Z. h. preblei*?
- (2) Could you support synonymizing *Z. h. campestris* and *Z. h. preblei* without additional genetics study (i.e., microsatellite data)? If not, what additional analysis is needed and why?
- (3) What is the importance of potential ecological, behavioral, or physiological differences between *Z. h. campestris* and *Z. h. preblei* in substantiating or refuting synonymy?
- (4) What is the likelihood that the *Z. h. preblei* is substantially reproductively isolated from other groups within the *Z. hudsonius* complex, especially from *Z. h. campestris*?
- (5) Would the loss of what is now *Z. h. preblei* represent a substantial diminution of the *Z. h. campestris* taxon? Its Range? Biological characteristics? Evolutionary legacy? Other?

Question 1:

Yes, I think that the authors provide convincing evidence for synonymizing these two subspecies since the hypothesis testing did not reject the hypothesis that the two are essentially the same for the morphological and genetic data. From the report and the draft recovery plan, it is not clear if we have enough data on the ecology and behavior of *preblei* to know if there are any significant differences.

Question 2:

I can support synonymizing subspecies without additional microsatellite data. I do think that microsatellite data are important if the USFWS would like to thoroughly address the question of whether these populations represent an ESU.

Question 3:

This question is really philosophical and depends on what species/subspecies definition one accepts. I think genetic data can provide important information for subspecies classifications but I do not think it is the only data that should be used. I feel that subspecies classifications can be justified based on substantial morphological, ecological, or behavioral differences even if mtDNA data do not demonstrate long-term separation.

Question 4:

I don't feel that I can adequately answer this question without additional data. The sharing of mtDNA haplotypes could represent historic not current gene flow. Detailed field studies or microsatellite analysis (preferred approach) will be necessary to address this question.

Question 5:

This is a difficult question. If we take only a mtDNA diversity perspective then the answer is no. I do not see any evidence of unique biological or ecological characteristics but I am not certain this has been thoroughly evaluated for *preblei*. Because of potential recent isolation (within the last 5,000 yrs) of this population, it may be on a unique evolutionary trajectory that might have future importance under Waples (1991, 1995) definition of evolutionary legacy.

Other General Comments:

- 1) When evaluating whether preble's would qualify as an ESU the authors do not apply Waples (1991, 1995) definition. Since this definition was cited by NMFS/USFWS in the 1996 joint policy that addresses ESUs, I think it would be important and useful to apply Waples' ESU definition.
- 2) On the bottom of page 9, the authors state that based on the Crandall approach the two species would be considered a single population for management purposes. I think it is a premature overstatement to conclude this without microsatellite data. The Crandall approach or others might support classification as separate populations maybe even different management units (under Moritz 1994) or ESUs (under Waples definition) depending on the results of microsatellite analysis.

Citations

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