Testing the Taxonomic Validity of Preble's Meadow Jumping Mouse (*Zapus hudsonius preblei*)

Report to the Governor of Wyoming and U.S. Fish & Wildlife Service (Revised)

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New in this report:
- Results of discriminant analysis using repeated skull measurements.
- Haplotype tables and phylogeny revised to be more informative

Abstract
We examined three lines of evidence to test the taxonomic validity of *Z.h. preblei*. These included: 1) phylogenetic and population genetic analysis of 176 mitochondrial DNA sequences, 2) morphometric analysis skull measurements of 80 individuals, and 3) a critical review of the basis of Krutzsch's qualitative description of *Z.h. preblei* as a subspecies. Phylogenetic analysis of mtDNA sequence data revealed that *Z.h. preblei* was not unique relative to *Z.h. campestris*, all *Z.h. preblei* mtDNA haplotypes were found within individuals of *Z.h. campestris*. *Z.h. luteus* is most closely related to *Z.h. pallidus*. Population genetic analysis revealed greater mtDNA variation within rather than among *Z.h. preblei* and *Z.h. campestris.* The lowest mtDNA variation was found within *Z.h. preblei*. Our morphometric analyses (analysis of variance and linear discriminant analysis of repeated skull measurements) refutes the quantitative morphological basis for Krutzsch's description of *Z.h. preblei* as a subspecies. Rather than being smaller in most skull dimensions than *Z.h. campestris*, *Z.h. preblei* was significantly larger for two measurements, smaller for one, and insignificant for 6 others. Discriminating ability with a jackknifed posterior probability of $\geq$0.95 was poor, with 48% (35 of 72) of the specimens correctly classified to each subspecies. The skull shape and pelage differences noted by Krutzsch have no quantitative basis and must be considered as "unsupported opinion". The lack of genetic, morphological, or published ecological evidence for distinctiveness of *Z.h. preblei* from *Z.h. campestris*, means that these subspecies should be synonymized (considered the same subspecies - *Z.h. campestris*). *Z.h. preblei* does not appear to be sufficiently unique to qualify as a Distinct Population Segment under the Endangered Species Act.

Introduction:
There is some controversy surrounding the taxonomic validity of Preble's meadow jumping mouse (Z*apus hudsonius preblei*) and conservation efforts under the Endangered Species Act (ESA) based on the presumed genetic uniqueness of this subspecies. This controversy is based upon the apparent weakness of the original taxonomic inference (Krutzsch 1954) which was an important component to the listing of *Z.h. preblei* under the ESA. The weakness of the original taxonomic designation includes: limited numbers of specimens used to describe the subspecies (3 adult skulls, 4 adult skins, 7 juvinile skins), qualitative descriptions that would not meet modern standards, and similarity in physical appearance of *Zapus* species and subspecies. The taxonomy of Krutzsch (1954) was not critically questioned by the scientific community until this study was proposed by the Denver Museum of Nature & Science in August 2002 and the results released in December 2003.

Z.h. preblei is one of 12 subspecies of the meadow jumping mouse (*Z. hudsonius*), a species whose range covers approximately half of North America. The range of *Z. hudsonius* extends from the Pacific Coast of Alaska eastward to the Atlantic Coast; from the northern limit of tree growth south into central Colorado, Nebraska, eastern Kansas, Missouri, Tennessee, and northern Georgia (Krutzsch 1954, Whitaker 1972). The range of *Z.h. preblei* is restricted to the base of the Front Range in Colorado and into southeastern Wyoming. The presumed cause of its uniqueness is the retreat of moist riparian habitat across the eastern plains of Colorado that occurred following the opening of the Holocene, approximately 10,000 years ago (Hafner 1981, 1987).

To date, most of the research has focused on distinguishing *Z. hudsonius preblei* from the western jumping mouse (*Z. princeps princeps*). Connor and Shenk (2003) used discriminant analysis of skull measurements to distinguish specimens of *Z. h. preblei* from *Z. princeps princeps*. An unpublished report by Riggs et al. (1997) claimed that based on mitochondrial control region sequences *Z. h. preblei* forms ``a homogenous group recognizably distinct from nearby populations and adjacent species of the genus." However, these authors did not gather data in such a manner as to be able to rigorously test whether *preblei* formed a monophyletic group. Furthermore Riggs et al. did not provide any statistical tests to support their conclusions. The data set used in the unpublished report by Riggs et al. (1997) is privately held by by Bioshpere Genetics Inc, Berkeley, CA..

If *Z. hudsonius preblei* is found to be indistinguishable from other subspecies of *Z. hudsonius,* then conservation efforts under the Endangered Species Act are being directed toward an organism that is more common and widespread then previously thought. If *Z. h. preblei* is found to be unique, relative to other subspecies of *Z. hudsonius,* then it may deserve conservation attention under the ESA, so long as it does not freely hybridize with *Z. princeps,* a common species whose distribution may overlap the western boundary of *Z. h. preblei*.

We tested the genetic distinctiveness and taxonomic validity of the Preble's meadow jumping mouse relative to other subspecies of the same species that are found in

bordering states. Our comparisons included samples of *Z. h. luteus* (from New Mexico and Arizona), *Z. h. campestris* (from Wyoming,, Montana, and South Dakota), and *Z. h. pallidus* (from Kansas and Nebraska). We used phylogenetic and population genetic methods to analyze DNA sequence data, as well as modern subspecies and distinct population concepts (Ball and Avise 1992, Crandall et al. 2000). We also retested Krutsch's original conclusions regarding cranial differences between *Z. h. preblei and Z. h. campestris*, using larger sample sizes. And finally, we examined Krutzsch's qualitative descriptions of skull shape and pelage differences between *Z. h. preblei and Z. h. campestris*.

1) Analysis of Mitochondrial DNA sequence variation

Methods:

Conceptual approach:

We used the scientific method to provide an objective test of the genetic distinctiveness of the Preble's meadow jumping mouse. Using hypotheses laid out in advance of data collection, we used the criteria of Ball and Avise (1992) and Moritz (1994) to test the taxonomic uniqueness of *Z. h. preblei* relative to other subspecies of *Z. hudsonius*. These authors were the first to provide a conceptual basis for recognizing subspecies (which are generally equated with evolutionary significant units or ESUs) that has both an evolutionary and quantitative basis. Ball and Avise (1992), and Moritz (1994) provided the following criteria for recognizing subspecies or ESU's: the subspecies or ESU must represent a major division in the diversity of the gene pool of a species based on concordant distributions of multiple genetically-based traits; it must have a plausible evolutionary mechanism for differentiation, and it must be on separate mitochondrial DNA lineages (reciprocal monophyly). The criteria of reciprocal monophyly for mitochondrial DNA requires that subspecies be separated long enough (e.g. generations since separation = 2 times the effective population size) for them to be on separate evolutionary pathways. While strict reciprocal monophyly is a clear-cut standard, it may be refuted if additional sampling reveals even one shared mitochondrial DNA type among subspecies. We prefer a less restrictive standard, specifically, there must be greater diversity among putative subspecies than within them. We previously used the approach outlined above in taxonomic revision of wild sheep (Ramey 1995, Wehausen and Ramey 2000, Tserenbatta et al. in press).

In our original research proposal "Testing the Taxonomic Validity of the Preble's Meadow Jumping Mouse", we asked the following question "Are Preble's meadow jumping mice a unique subspecies relative to other nearby *Z. hudsonius* subspecies?" We then laid out the following hypotheses and critical tests:

"Hypothesis 1A: Preble's is a unique taxon, distinguishable from other subspecies of *Z. hudsonius* using mitochondrial DNA sequence data. The alternative hypothesis (Hypothesis1B) is that Preble's will not be unique or distinguishable.

Critical test: Mitochondrial DNA sequence data for all samples show a pattern of reciprocal monophyly, or greater molecular variance among subspecies than within subspecies (in pairwise comparisons involving *Z. h. preblei*.) If we find that Preble's

cannot be distinguished on the basis of mitochondrial DNA sequences, it will be unlikely that it will be differentiated for nuclear microsatellite DNA. However, if Hypothesis 1A cannot be refuted, then screening all samples for microsatellite loci becomes crucial to test if hybridization occurs between *Z. h. preblei* and *Z.p. princeps.*"

Following our initial test using the criteria above, we also applied the conceptual approach of Crandall et al. (2000). These authors propose a hypothesis testing approach for recognizing distinct population segments using the criteria of genetic and ecological distinctiveness on recent and historic timescales. They advocate that ecological differences among populations can drive adaptive change that would not be detected by molecular markers alone. Therefore, we examined the literature for evidence of ecological differences between subspecies. We applied the conceptual approach using the crosshair classification of Table 1 in Crandall et al. (2000). We define "recent" as within the past 10,000 years (Holocene) and "very recent" as within the past several hundred years.

Acquisition of samples:
DNA samples were obtained from specimens in museum collections at the Denver Museum of Nature & Science, the University of Kansas, the Nebraska State Museum, and the University of New Mexico. We included only two ear punch tissue samples from live captured animals because they were needed to fill in a sampling area and photographs of these individuals were available . By relying on museum specimens, our results are repeatable. Additional questions may also be asked about each specimen at a later date, such as morphological distinctiveness. Museum research collections have the advantage of being open to public inspection and scientific research.

We sampled across the range of each putative subspecies, in order to sample the maximum extent of genetic variation across subspecies. This meant that we sampled more locations but fewer individuals per location. We included a limited sample from each of the subspecies of *Z. princeps* for use as an outgroup for phylogenetic analyses. Previous work by J. Cook (unpublished data) revealed a broad separation and reciprocal monophyly between *Z. princeps* and *Z. hudsonius* utilizing cytochrome *b* sequences, making *Z. princeps* an ideal outgroup for phylogenetic analyses.

Laboratory Methods:
Genomic DNA was extracted from frozen liver tissue and museum skin samples (5-10mg) using Qiagen DNeasy Tissue kit (Qiagen Inc.). Two specimens were from ear punch samples provided by Pioneer Environmental that had accompanying photographs (virtual vouchers). For frozen tissues, we followed the protocol provided in the Qiagen DNeasy Tissue kit. For skin samples, we modified the protocol slightly – samples were incubated at ATL buffer with proteinase K overnight at 56°C. 510bp of control region were amplified via the polymerase chain reaction (PCR) using primer L15320 and ZAP5P1r. The amplification conditions were as follows: in a 25 μl total volume, containing 5 μl of Invitrogen optimizer buffer D (17.5 mM $MgCl_2$, pH 8.5) (Invitrogen, Inc.), 2.5 μl of dNTPs (2.5 mM each), 1.25 μl of each primer (10 μM), 1 unit *Taq* polymerase, 1μl of template (200-300 ng), and 13.8 μl of sterile water. The temperature

profile for the PCR reaction consisted of an initial 2 min denaturation step at 94°C, followed by 30 cycles of 1 min at 94°C, 1 min at 58°C, 2 min at 72°C, and a final extension step at 72°C for 7 min. Amplified DNA was resolved by electrophoresis on 1.5% agarose gel that was stained with ethidium bromide to check for length, quality and quantity.

Some DNA extracts, most notably those of older museum specimens (prior 1980), did not amplify well or at all. We suspect that this occurred because the older museum specimens were treated with arsenic during skin preparation. We were able to amplify DNA from these older museum specimens using nested PCR. Two primers, L15398 and H16498 were designed to amplify ca. 430 bp control region fragment within the L15320/ZAP5P1r primer combination. The relative positions and priming directions of the control region primers are shown in Figure 1. Genomic DNA was first amplified using primer L15320 and ZAP5P1r. The PCR products were cleaned using the Exo/SAP method. The PCR products were incubated at 37°C for 30 min and then at 85°C for another 15 min with five units of Exonuclease I (ExoI, Amersham) and 0.5 unit Shrimp Alkaline Phosphatase (SAP, Amersham). Subsequently the cleaned PCR product was reamplified using primer L15398 and H16498.

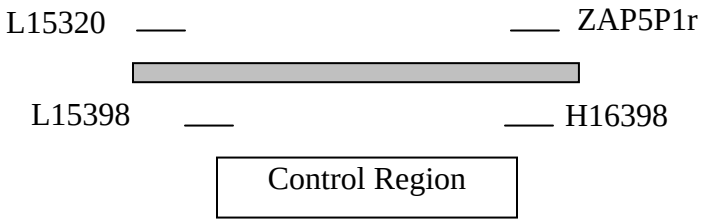

Figure 1. Location of primers used for PCR amplification of mitochondrial Control Region.

Automated Sequencing. The amplified PCR product was incubated at 37°C for 30 min and then at 85°C for another 15 min with five units of Exonuclease I (ExoI, Amersham) and 0.5 unit Shrimp Alkaline Phosphatase (SAP, Amersham) to cleave nucleotides one at a time from an end of excess primers and to inactivate single nucleotides. Approximately 10-30 ng of cleaned PCR product was used as a template in a cycle sequencing reaction using the CEQ DTCS Quick Start Kit (Beckman Coulter, Inc.). The following cycling conditions were used: 96°C for two min, then 30 cycles of 96°C for 20 s, 50°C for 20 s, and 60°C for four min. The cycle-sequenced product was cleaned using the Beckman Coulter protocol. Fluorescent dye-labeled DNA was combined with 4 µl stop solution (equal volume of 100 mM EDTA and 3 M NaOAc pH 5.2), 1 µl glycogen (20 mg/ml), and 10 µl milli-Q H_2O, mixed well, and precipitated with 60 µl cold 95% (v/v) ethanol/water. Fluorescent dye-labeled DNA was recovered by centrifuging at 13,000 rpm for 20 min at 4° C. Pellets were washed with 100 µl 70% (v/v) ethanol/water, air dried and resuspended in 40 µl of dimethylformamide. Resuspended samples were added

to the appropriate wells of the CEQ sample plate, overlayed with mineral oil, and run on the Beckman Coulter CEQ8000. Sequences were determined for both strands and were edited and aligned using Sequencher™. All DNA sequences were determined by sequencing in the forward and reverse directions, with additional runs used to eliminate ambiguous base calls. Aligned and edited sequences were checked back against raw chromatograms to insure base calling accuracy.

Data Analysis. Consensus sequences were aligned using Sequencher and verified manually. Phylogenetic hypotheses based on distance and parsimony methods were conducted using PAUP* 4.0b10 (Swofford, 2002). A Bayesian analysis using MrBayes 3.04 (Huelsenbeck and Ronquist, 2001) was conducted as another means of estimating phylogeny. The HKY model with variable sites assumed to follow a discrete gamma distribution (e.g., HKY + I+ Γ; Hasegawa et al., 1985) was selected as the best fit for the dataset (Modeltest 3.06; Posada and Crandall, 1998).

Maximum-parsimony (MP) analyses were conducted with equal weighting, using the heuristic search option with tree bisection reconnection branch-swapping and 10 random additions. Bootstrapping with 1000 replications (as implemented in PAUP*) was used to evaluate node support. HKY distances were used to generate a neighbor-joining (NJ) tree based on the clustering method of Saitou and Nei (1987). Node support was assessed by completion of 1000 bootstrap replications (Felsenstein, 1985) in PAUP*, using the fast-search option. Bayesian analyses were performed based on the HKY model with invariable and variable sites with a discrete gamma distribution (e.g., HKY + I+ Γ; Hasegawa et al., 1985) model of evolution. Several short runs were first conducted using the default random tree option to determine when the log likelihood sum reached a stable value (by plotting the log-likelihood scores of sample points against generation time). Then metropolis-coupled MCMC simulations were run with four chains using the default random tree option for 1,000,000 generations and Markov chains were sampled at intervals of 10 generations to obtain 100,000 sample points. The last 95,000 sampled trees with branch lengths (the first 5000 trees having been removed as "burn-in") were used to generate a 50% majority rule consensus tree. The percentage of samples that recovered specific clades on this topology represents that clade's posterior probability; these are the P values, and $P \geq 95\%$ was considered evidence of significant support for a clade (Huelsenback and Ronquiest, 2001).

ARLEQUIN 2.0 was used to perform an analysis of molecular variance (AMOVA) to partition the amount of genetic variation in a hierarchical fashion within and between the most closely related subspecies to *Z. h. preblei* (Excoffier et al. 1992). Statistical significance of differentiation at these levels was quantified and tested using ARLEQUIN 2.0 (Schneider et al. 2000). ARLEQUIN 2.0 was also used to estimate mtDNA nucleotide diversity.

Results:

We sequenced mitochondrial control region from 58 *Z. hudsonius preblei*, 33 *Z. h. campestris*, 32 *Z. h. luteus*, 35 *Z. h. pallidus*, 7 *Z. princeps princeps*, 3 *Z. p. idahoensis*,

and 7 *Z. p. utahensis*. The alignment of 151 sequences (Table 1), excluding four specimens from Wyoming, one from Kansas, one from Montana, and one from South Dakota (see explanation below), of the partial mitochondrial control region from four *Zapus hudsonius* subspecies yielded 355 bp. Overall nucleotide composition was biased towards thymine (T)(34.3%) and adenine (A)(29.8%), followed by cytosine (C)(26.0%) and guanine (G)(9.9%).

Three variable sites (all transitions) were observed among 54 specimens of *Z. h. preblei* resulting in four haplotypes. [Note: four specimens of *Z. h. preblei* from Albany Co., Wyoming had almost identical sequences to *Z. p. princeps.* These four specimens were presumed misidentified and thus not included.] Twenty-nine variable sites (19 transitions, 8 transversions, and 2 indels) were observed among 31 specimens of *Z. h. campestris* resulting in sixteen haplotypes. Four sequences (two haplotypes) of *Z. h. campestris*, three from Lawrence Co., South Dakota and one from Crook Co., Wyoming, are more similar to sequences of *Z. h. luteus* and *Z. h. pallidus* than to other sequences of *Z. h. campestris*. One specimen of *Z. h. campestris* from Carter Co., Montana and one specimen from Custer Co., South Dakota has similar sequences to *Z. p. utahensis*. We presume they were misidentified and thus not included (Table 2).

Thirty variable sites were observed among 34 specimens of *Z. hudsonius pallidus* resulting in twelve haplotypes. Two sequences of *Z. h. pallidus* from Clay Co., South Dakota are more similar to sequences of *Z. h. campestris* and *Z. h. preblei* than to other sequences of *Z. h. pallidus*. One specimen of Z. h. pallidus from Douglas Co., Kansas has similar sequences to *Z. p. utahensis*. They are presumed misidentified and thus not included. Six variable sites were observed among 32 specimens of *Z. h. luteus* resulting in eight haplotypes.

Phylogenetic analysis of mtDNA sequences based on maximum parsimony, distance and Bayesian methods yielded concordant results that differed only in the positioning of terminal taxa (Figure 2, Table 1). Phylogenetic analysis of mtDNA sequence data revealed that *Z. h. campestris* is most closely related to *Z. h. preblei* and that *Z. h. luteus* is most closely related to *Z. h. pallidus.* These two clades had strong bootstrap support (Figure 2). *Z. h. preblei* and *Z.h. campestris* were not reciprocally monophyletic. All four of the mtDNA haplotypes found in *Z. h. preblei* were also found in *Z. h. campestris*. No unique mtDNA haplotypes were found in *Z. h. preblei*.

Genetic variation within subspecies as indicated by mtDNA nucleotide diversity was lowest in *Z. h. preblei* (0.0027, SD=0.0020) and approximately nine times higher in *Z. h. campestris* (0.0243, SD=0.0129). Nucleotide diversity in *Z.h. luteus* (0.0041, SD=0.0029) was twice that of *Z.h. preblei* but three times lower than in *Z. h. pallidus* (0.0135, SD=0.0075).

In a pairwise comparison between *Z. h. preblei* and *Z.h. campestris*, analysis of molecular variance revealed that most of the genetic variation was within (64%) rather than among these subspecies (37%), thus refuting hypothesis 1A and failing our test of genetic uniqueness. We did not include the highly divergent sequences of the 4 Albany Co.

specimens in this analysis because it is likely that they are specimens of *Z.p. princeps* that were misidentified as *Z. h. preblei* .

Utilizing the criteria of genetic and ecological exchangeability as proposed by Crandall et al. (2000) for distinct populations, the mtDNA data does not refute the hypothesis of historic or recent genetic exchangeability (interbreeding) between *Z.h. preblei* with *Z.h. campestris*. This is because all four *Z.h. preblei* mtDNA haplotypes are found in *Z.h. campestris* from near the Black Hills of South Dakota. These mtDNA haplotypes that are shared between *Z.h. preblei* and *Z.h. campestris* span a range of up to 700km, from central Colorado to southeastern Montana. The fact all *Z.h. campestris* haplotypes are not found in the range of *Z.h. preblei* is consistent with founder effects and range expansion, not evidence of restricted genetic exchange. A review of the literature reveals that no quantitative evidence exists to reject the hypotheses of historic or recent ecological exchangeability (ecological similarity) between *Z.h. preblei* with *Z.h. campestris*. While it is possible that genetic exchange between these two putative subspecies is currently limited, this alone does not support them as being recognized as a distinct population segment (case 8, Crandall et al. 2000).

Discussion:
Our analysis of mtDNA sequence data refutes Hypothesis 1A, that *Z.h. preblei* is a unique taxon, distinguishable from other subspecies of *Z. hudsonius* (in this case *Z.h. campestris*) using mitochondrial DNA sequence data. The results of the mtDNA analysis reveal that *Z.h. preblei* is a less genetically variable population of *Z.h. campestris*.

The high level of mtDNA variation (nucleotide diversity) found in *Z.h. campestris* compared to *Z.h. preblei* does inflate the F_{ST} estimate, making these subspecies seem more diverged than the shared mtDNA haplotypes indicate.

While it is possible that the low level of mtDNA variation found in *Z.h. preblei* is the result of isolation and a northern migration into the range of *Z.h. campestris*, the pattern is more consistent with the hypothesis that the range of *Z.h. preblei* is the result of a recent southward colonization from the range of *Z.h. campestris*. Two observations support this later conclusion: first, no unique mtDNA haplotypes were found in *Z.h. preblei* and second, all of these haplotypes were closely related. The reduced mtDNA variation is consistent with a founder effect (e.g. population bottlenecks during a southern colonization). In contrast, if *Z.h. preblei* had been a long term resident along the Front Range and had evolved in isolation from *Z.h. campestris*, more unique mtDNA haplotypes would be expected – a situation found with *Z.h. luteus* compared to *Z.h. pallidus*. In either case, the shared mtDNA haplotypes indicate recent genetic exchange.

The failure of evidence to reject hypotheses of genetic and ecological exchangeability between *Z.h. preblei* with *Z.h. campestris*, using the approach of Crandall et al. (2000), means that *Z.h. preblei* with *Z.h. campestris* should be treated as a single population. If evidence from future trapping efforts supports a lack of current genetic exchangeability (e.g. genetic isolation) between *Z.h. preblei* and *Z.h. campestris*, these two subspecies

would still be considered a single population for management purposes, using the criteria proposed by Crandall et al. (2000).

2) Morphometric analyses: Retesting Krutzsch's conclusions with larger sample sizes, analysis of variance, and discriminant analysis.

Methods:

To test the hypothesis that size differences in skull measurements reported by Krutzsch (1954) are representative of differences among subspecies, we compared 39 adult *Z.h. preblei* and 41 adult *Z.h. campestris* specimens using analysis of variance (ANOVA). Specimens were measured at the zoology collections at the Denver Museum of Nature & Science, and the University of Kansas Museum of Natural History. We utilized the same 9 skull measurements of Krutzsch (1954): occipitonasal length (from anteriormost projection of nasal bones to posteriormost projection of supraoccipital bone), condylobasal length (posteriormost part of exoccipital condyles to anteriormost projections of premaxillary bones), palatal length (anterior border of incisors to anteriormost point of postpalatal notch), zygomatic length (anteriormost point of zygomatic process of maxillary to posteriormost point of zygomatic process of squamosal), zygomatic breadth (greatest distance across zygomatic arches of cranium at right angles to long axis of skull), mastoidal breadth (greatest distance across mastoid bones perpindicular to long axis of skull), braincase breadth (greatest distance across braincase perpindicular to long axis of skull), interorbital breadth (least distance across top of skull between orbits), and upper tooth row length (anterior border of P4 to posterior border of M3). Our palatal length is larger than what Conner and Shenk (2003) reported due to differences in where measurements were taken.

Four repeated measurements (Conner and Shenk 2003) were taken with digital calipers and recorded to the nearest hundredth of a millimeter. Only adult skulls were measured, as determined by tooth eruption and wear. In a several cases, fewer measurements were taken because of breakage or not taken because of previous breakage. Calipers were moved away from the skull and reset for each measurement. A single observer (L. Carpenter) measured all skulls in the study. We used the mean of the repeated measurements in both ANOVA and discriminant analysis (Connor and Shenk 2003).

We tested the cranial distinguishability of *Z.h. preblei* from *Z.h. campestris* from a multivariate perspective with linear discriminant analysis using SYSTAT 9.0. Forward, backward, and interactive stepwise procedures to develop the simplest discriminant models to eliminate statistically unimportant variables and to maximize the ratio of sample size to variables included in the model (Williams and Titua 1990). We used jackknifed estimates of posterior probabilities and classification ability for discriminant models (Afifi and Clark 1990). We used a previously published criterion for testing the hypothesis of distinguishability between subspecies: $\geq$90% of specimens correctly classified at jackknifed posterior probabilities of $p \geq 0.95$ (Wehausen and Ramey 2000). This criterion was more discriminating than just the percentage of specimens correctly classified at a posterior probability of $p > 0.5$. Males and females were pooled in the analyses because of a lack of cranial sexual dimorphism in *Z. princeps* and *Z. hudsonious*

(Connor and Shenk 2003). This apparent lack of sexual dimorphism was also tested using stepwise discriminant analysis.

Results:

Analysis of variance

Our analysis of skull measurement data refutes the hypothesis above and the claim made by Krutzsch (1954) that *Z.h. preblei* is *"averaging smaller in most skull measurements"* than *Z.h. campestris*. A total of 3 measurement variables were found to be significantly different at a level of $p<0.05$. Two of these measurements (zygomatic breadth and mastoid breadth, were significantly ***larger*** in *Z.h. preblei* than in *Z.h. campestris*, in the *opposite* direction to Krutzsch's claims that *Z.h. campestris* is larger. *Z.h. campestris* was only larger for one measurement (*interorbital breadth*) and it was only marginally significant (larger in *Z.h. campestris)* ($p=0.037$). All other measurements were not significantly different (Table 3).

Discriminant analysis

Four variables were determined to have the greatest discriminating power. These included: zygomatic breadth, mastoidal breadth, breadth of skull, and condylobasal length. A total of 33 *Z.h. preblei* and 39 *Z.h. campestris* were used in the discriminant analysis. The null of hypothesis of equal covariances among subspecies was not rejected ($p=0.147$). Discriminating ability with a jackknifed posterior probability of ≥ 0.95 was poor, with 48% (35 of 72) of the specimens correctly classified to each subspecies.

Discussion:

Our morphometric analysis refutes the quantitative morphological basis for Krutzsch's description of *Z.h. preblei* as a subspecies. Krutzsch (1954) described *Z.h. preblei* as *"averaging smaller in most skull measurements "* but using ANOVA, we found only one out of nine variables to be significantly smaller in *Z.h. preblei.* The three significant differences that we did find should be viewed within the context of variation typically found among populations.

Z.h. preblei failed the test of morphological distinguishability from *Z.h. campestris* using discriminant analysis of the same skull measurements as Krutzsch (1954) and a substantially larger sample size. The correct classification of specimens by the DFA was far less (48%) than the criterion that $\geq$90% of specimens be correctly classified at jackknifed posterior probabilities of $p\geq 0.95$ (Wehausen and Ramey 2000). This is a refutation of Krutzsch's (1954) only quantitative basis for concluding that *Z.h. preblei* are morphologically distinguishable and therefore a unique subspecies relative to *Z.h. campestris*.

As with other taxonomy papers of the period, Krutzsch's description in 1954 of *Z. h. preblei* as a newly recognized subspecies was based upon qualitative descriptions without statistical tests, and presumed geographic isolation. It represented the opinion of the author. The only quantitative comparison that Krutzsch (1954) used to support this "new" subspecies description, was based on measurements of only 3 adult specimens of

Preble's that he compared to 40 specimens of *Z.h. campenstris*. He examined the skin of a fourth adult specimen and the skins of 11 juveniles of *Z. h. preblei*. The three adult *Z. h. preblei* specimens were reported to be smaller in all skull dimensions.

3) A critical evaluation of Krutzsch's qualitative descriptions

We examined the basis of Krutzsch's qualitative differences in skull shape and pelage to determine the strength of the evidence that he used to infer that *Z.h. preblei* is a unique subspecies.

Three of the skull shape differences distinguishing *Z.h. preblei* and *Z.h. campestris* noted by Krutzsch (1954) had no reported measurements. Therefore the skull shape differences noted by Krutzsch have no quantitative basis and must be considered as "unsupported opinion". These shape descriptions include: "*incisive foramia not truncate posteriorly; auditory bullae smaller, less well inflated; and frontal region usually more inflated*". Additionally, one of the skull shape differences ("*frontal region usually more inflated"*) did not have an accompanying qualitative description for either subspecies individually (Table 4).

When Krutzsch's pelage descriptions of each subspecies are listed side by side (Table 2), and compared to what he stated were distinguishing pelage differences, it is clear that two of the three pelage differences were made without a description of one or both subspecies. For example, one pelage difference *("upper parts generally dull, averaging lighter"*) had no comparative description for *Z.h. campestris*. The second pelage difference *("sides duller"*) did not have an accompanying description for either subspecies. The only pelage difference where there was a description for both subspecies was "*less black tipped hair*" on the dorsal band. These three differences in pelage between *Z.h. preblei* and *Z.h. campestris* noted by Krutzsch (1954) are entirely qualitative and must also be considered as "unsupported opinion". The underpinnings of Krutsch's qualitative descriptions are without a quantitative basis, and fail the tests of falsifiability, comprehensiveness, repeatability, and sufficiency required by evidential reasoning (Lett 1990).

Conclusions:

Taxonomy

We examined three lines of evidence to test the taxonomic validity of *Z.h. preblei.* These included: 1) phylogenetic and population genetic analysis of mitochondrial DNA sequences, 2) morphometric analysis of skull measurements, and 3) a critical review of the logical basis of Krutzsch's description of *Z.h. preblei* as a subspecies. Our results failed to support the genetic distinctiveness of *Z.h. preblei* from *Z.h. campestris*. Our morphometric analysis refutes the quantitative morphological basis for Krutzsch's description of *Z.h. preblei* as a subspecies. The skull shape and pelage differences noted by Krutzsch have no quantitative basis and must be considered as "unsupported opinion".

The lack of genetic, morphological, or published ecological evidence for genetic distinctiveness (including adaptive divergence) of *Z.h. preblei* from *Z.h. campestris*, means that these subspecies should be synonymized (considered the same) and referred to as *Z.h. campestris*.

The lack of genetic, morphological, and ecological evidence supporting divergence of *Z.h. preblei* from *Z.h. campestris*, the weakness of the original taxonomic inference of *Z.h. preblei* being a subspecies (Krutzsch 1954), and the unsupported assumption that geographic isolation has driven genetic divergence between these putative subspecies, all point to *Z.h. preblei* being synonymous with *Z.h. campestris*. We therefore synonymize *Z.h. preblei* with *Z.h. campestris*

Does the evidence support consideration of Distinct Population Segment listing?

In a broader perspective, the range of *Z.h. preblei* represents less than 5% of the range of a species whose range is approximately *half* of North America (along streams and in meadows). This is not a compelling argument for *Z.h. preblei* to be a candidate for a distinct population segment designation (DPS). A DPS designation requires that a population be "discrete" and "of significance" (US Fish & Wildlife Service 1996). The "discrete" requirement, that a DPS is "markedly separated from other populations of the same taxon by physical, physiological, ecological, or behavioral factors" using evidence from "quantitative measures of genetic or morphological discontinuity" (US Fish & Wildlife Service 1996) is not supported by our genetic or morphological analyses.

The "significance" requirement that, "evidence that loss of the discrete population segment would result in a significant gap in the range of a taxon" is not supported because of the broad distribution of *Z. hudsonius* (Figure3). *Z.h. preblei* is a peripheral population of *Z. hudsonius*, that does not rank as distinct using the criteria (spatial distance, life history, time, and ecology) proposed by Lesica and Allendorf (1994).

Hypothesis testing and peer review

Krutzsch's (1954) unsupported opinions about shape differences in skulls and coloration of skins, as well as skull measurement comparison based on a sample size of 3 *Z.h. preblei*, have carried the weight as the "best available science" in the listing of *Z.h. preblei*. However, the logical basis of these opinions was not critically evaluated by the USFWS, or others, during the listing process, despite the weakness of Krutzsch's (1954) inference by modern standards. The identification of *Z.h. preblei* specimens by museum curators or consultants similarly relied on Krutzsch (1954). The description of *Z.h. preblei* as a new subspecies is typical of the taxonomic work that appeared in the literature in the early to mid twentieth century. During that time, species and subspecies descriptions had little or no quantitative basis, relied on small sample sizes, and were based largely on opinion (Ramey 1993, Wehausen and Ramey 1993, 2000). Essentially, a species or subspecies was "*what a good taxonomist said it was"*.

The original review of the *Z.h. preblei* listing would have benefited from a critical peer review by more broadly trained systematic biologists and molecular/morphometric analyses to specifically test the taxonomic validity of subspecies. The Federal peer review standards proposed by the Office of Management and Budget (2003) are a good example of how peer review can strengthen the scientific justification for proposed ESA listings, delistings, and Biological Opinions. Also, genetic analyses with the specific goal of treating taxonomic categories as testable hypotheses (Ramey 1993, 1995; Wehausen and Ramey 1993, 2000) would have been appropriate in this case and others. In the case of *Z.h. preblei*, a genetic analysis was performed by Riggs et al. (1997) but not with the subspecies validity question in mind or critical hypothesis testing. Similarly, the listing rule (USFWS 1998) appeared to have accepted the taxonomy of Krutzsch (1954) without question. Our review differs from those previously (Riggs et al. 1997; Hafner 1997; USFWS 1998) because it involves hypothesis testing, utilizes multiple lines of evidence, and incorporates modern concepts of subspecies and distinct population segments. Our analyses suggest that a large expenditure of conservation effort under the ESA is being directed towards populations of a subspecies (*Z. h. campestris*) that are more widespread than previously thought.

Scientific investigation involves critical thinking and evidential reasoning (Lipps 1999, Lett 1990, Platt 1964). Unsupported opinion and anecdotal observations are not scientific. In the case of endangered species management, facts (quantitative evidence) can be gathered in such a way as to answer specific questions, often at greater economy than courses of action whose basis is falsified later. Testing taxonomic classifications does not take as long, or cost as much, as one might initially think. The molecular data has taken approximately one year of part-time effort at a cost of approximately $50,000. Our morphometric measurements, analysis, and write up has taken only three weeks of effort, at a cost of approximately $7,000. Our analyses have benefited greatly from the availability of museum specimens in zoological research collections. Without these collections, this biodiversity research would not have been possible.

In the future, we strongly urge the USFWS to work with the scientific community in developing incentives to apply both critical peer review and molecular/morphometric analyses to test the quantitative basis of all proposed subspecies and distinct population segment listings. To not do so, invites a potential for misallocation of scarce conservation resources to populations that are not genetically or ecologically unique, and can erode public confidence in the implementation of the Endangered Species Act.

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Comments and constructive criticisms on this report are appreciated. Please direct these to rramey@dmns.org. Thank you.

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Appendix I:
Catalog of specimens examined for skull morphometry. Specimens are listed in the order they were examined.
Denver Museum of Nature & Science, *Z.h. preblei*: 9572, 9864, 10380, 9843, 9853, 9570, 9569, 9562, 9561, 9315, 9205, 9204, 9868, 9862, 10355, 10404, 10269, 10354, 10169, 10265, 10267, 2822, 10604, 9876, 10618, 10630, 10621, 9564, 9312, 10635, 9877, 10620, 10611, 9571, 10266, 10610, 9579, 10613, and10615. Denver Museum of Nature & Science, *Z.h.campestris*:8512. University of Kansas Natural History Museum, *Z.h.campestris*: 101551, 101552, 101554, 101555, 101558, 101560, 87040, 87041, 87042, 87034, 87035, 87036, 87037, 112664, 112657, 20835, 20836, 20837, 20838, 20839, 20840, 20842, 20843, 20844, 20845, 20846, 20847, 20848, 20849, 20851, 20850, 20852, 41450, 41451, 42467, 42468, 42469, 42471, 42517, and 42518.

Figure 2. Neighbor-joining phylogram based on partial control region sequences using a HKY substitution model, depicting phylogenetic relationships among subspecies of *Zapus hudsonius*. One hundred seventy six sequences were obtained for this study (Table 1 and 2). In order to provide a reasonable size tree, one sequence from each haplotype was used. Bootstrap percentages are given when >=50%. Other methods of phylogenetic analysis produced very similar trees.

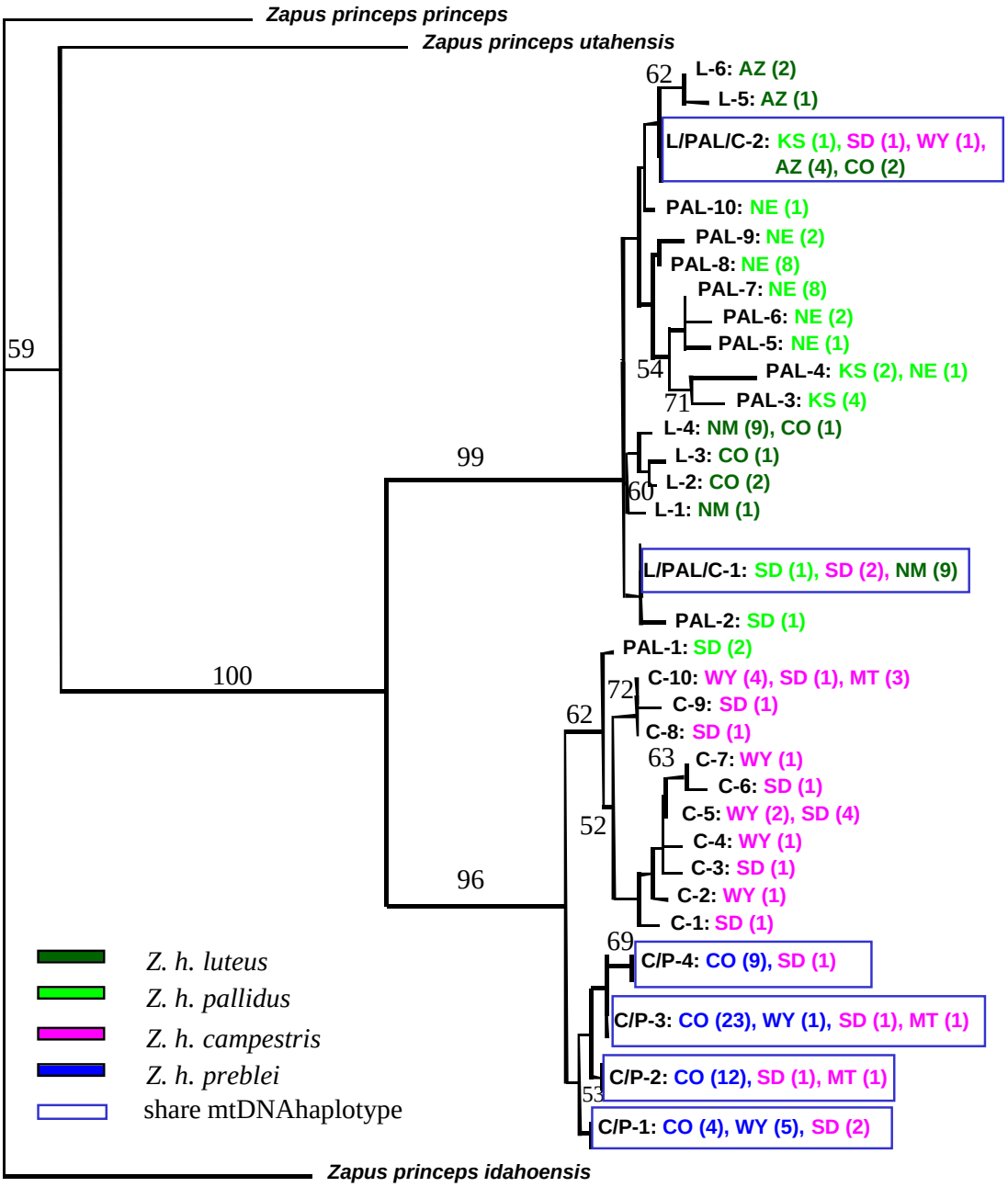

Table 1. Specimens of *Z. hudsonius* used in phylogenetic analysis, listed by museum and tissue archive catalog number (DMNH = Denver Museum of Nature & Science; TK = Texas Tech (tissue archive); KU = University of Kansas; UNSM = University of Nebraska State Museum; MSB and NK (Tissue archive) = Museum of Southwestern Biology; PIONEER = Pioneer Environmental Services.)

Representative individuals used in phylogenetic analysis	Additional specimens with identical mtDNA haplotype:.ID, state, and county	subspecies	haplotype
MSB40951, AZ:Apache		Z.h. luteus	L6
	MSB40994, AZ:Apache	Z.h. luteus	
MSB89194, AZ:Navajo		Z.h. luteus	L5
MSB86344, AZ:Apache		Z.h. luteus	L/PAL/C2
	MSB91627, AZ:Navajo	Z.h. luteus	
	MSB91675, AZ:Apache	Z.h. luteus	
	NK1584, AZ:Apache	Z.h. luteus	
	DMNH8635, CO:Las Animas	Z.h. luteus	
	DMNH8633, CO:Las Animas	Z.h. luteus	
	KU41451, WY:Crook	Z.h. campestris	
	KU153706, KS:Leavenworth	Z.h. pallidus	
	KU112661, SD: Lawrence	Z.h. campestris	
UNSM20596, NE:Buffalo		Z.h. pallidus	PAL10
UNSM26492, NE:Buffalo		Z.h. pallidus	PAL9
	UNSM20879, NE:Buffalo	Z.h. pallidus	
UNSM13217, NE:Cherry		Z.h. pallidus	PAL8
	UNSM12980, NE:Garden	Z.h. pallidus	
	UNSM12991, NE:Garden	Z.h. pallidus	
	UNSM26316, NE:Hall	Z.h. pallidus	
	UNSM20744, NE:Hall	Z.h. pallidus	
	UNSM20747, NE:Hall	Z.h. pallidus	
	UNSM26462, NE:Merrick	Z.h. pallidus	
	UNSM13067, NE:Thomas	Z.h. pallidus	
UNSM17482, NE:Antelope		Z.h. pallidus	PAL7
	UNSM17495, NE:Antelope	Z.h. pallidus	
	UNSM17498, NE:Antelope	Z.h. pallidus	
	UNSM17499, NE:Antelope	Z.h. pallidus	
	UNSM13084, NE:Dixon	Z.h. pallidus	
	UNSM14008, NE:Dodge	Z.h. pallidus	
	UNSM13118, NE:Holt	Z.h. pallidus	
	UNSM13343, NE:Lancaster	Z.h. pallidus	
UNSM13119, NE:Holt		Z.h. pallidus	PAL6
	UNSM13065, NE:Thomas	Z.h. pallidus	
UNSM17727, NE:Boyd		Z.h. pallidus	PAL5
UNSM20600, NE:Buffalo		Z.h. pallidus	PAL4
	KU109633, KS:Osage	Z.h. pallidus	
	KU109634, KS:Osage	Z.h. pallidus	

KU153597, KS:Macon	KU153598, KS:Macon KU153784, KS:Douglas KU153707, KS:Leavenworth	Z.h. pallidus Z.h. pallidus Z.h. pallidus Z.h. pallidus	PAL3
MSB37154, NM:Otero	MSB61696, NM:Otero MSB61684, NM:Otero MSB61690, NM:Otero MSB61693, NM:Otero MSB61712, NM:Otero MSB58369, NM:Rio Arriba NK871, NM:Otero NK884, NM: Socorro DMNH8630: CO:Las Animas	Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus	L4
DMNH8631, CO:Las Animas		Z.h. luteus	L3
DMNH8632, CO:Las Animas	DMNH8634, CO:Las Animas	Z.h. luteus Z.h. luteus	L2
NK9976, NM:Bernalillo		Z.h. luteus	L1
MSB58370, NM:Rio Arriba	MSB56980, NM:Sandoval MSB56986, NM:Sandoval MSB56987, NM:Sandoval MSB56991, NM:Sandoval MSB56993, NM:Sandoval MSB62096, NM:Sandoval MSB62103, NM:Valencia NK856, NM:Sandavol KU112665, SD:Lawrence KU109963, SD:Lawrence KU110033, SD:Bennett	Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. luteus Z.h. campestris Z.h. campestris Z.h. pallidus	L/PAL/C1
KU110022, SD:Bennett		Z.h. pallidus	PAL2
UNSM27388, SD:Clay	UNSM27389, SD:Clay	Z.h. pallidus Z.h. pallidus	PAL1
DMNH10638/TK86190, WY:Weston	DMNH10639/TK86191, WY:Weston KU101558, SD:Pennington KU123593, MT:Carter KU123598, MT:Carter KU123599, MT:Carter	Z.h. campestris Z.h. campestris Z.h. campestris Z.h. campestris Z.h. campestris	C10
KU112663, SD:Lawrence		Z.h. campestris	C9
KU101564, SD:Pennington		Z.h. campestris	C8
KU20839, WY:Crook		Z.h. campestris	C7
KU83559, SD:Harding		Z.h. campestris	C6
KU20844, WY:Crook		Z.h. campestris	C5

	KU42471, WY:Weston KU87040, SD:Harding KU83557, SD:Harding KU87042, SD:Harding KU112660, SD:Lawrence	Z.h. campestris Z.h. campestris Z.h. campestris Z.h. campestris Z.h. campestris	
KU20843, WY:Crook		Z.h. campestris	C4
KU109970, SD:Lawrence		Z.h. campestris	C3
KU42469, WY:Weston		Z.h. campestris	C2
KU101552, SD:Pennington		Z.h. campestris	C1
DMNH10614/TK86183, CO:El Paso	DMNH10331/TK86088, CO:Teller DMNH10606/TK86165, CO:El Paso DMNH10604/TK86169, CO:El Paso DMNH10612/TK86170, CO:El Paso DMNH10605/TK86173, CO:El Paso DMNH10618/TK86182, CO:El Paso DMNH10611/TK86185, CO:El Paso DMNH10635/TK86196, CO:Douglas KU109972, SD:Custer	Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. campestris	C/P4
DMNH9204/XM871, CO:Boulder	DMNH9205/XM872, CO:Boulder DMNH9312/XM874, CO:Gilpin DMNH9046/XM876, CO:Boulder DMNH9314/XM877, CO:Boulder DMNH9203/TK51406, CO:Jefferson DMNH9880/TK86021, CO:Boulder DMNH9854/TK86026, CO:Douglas DMNH9876/TK86029, CO:Douglas DMNH9857/TK86030, CO:Douglas DMNH9865/TK86031, CO:Douglas DMNH9868/TK86032, CO:Douglas DMNH9843/TK86034, CO:Boulder DMNH10169/TK86048, CO:Boulder DMNH10266/TK86080, CO:Douglas DMNH10269/TK86083, CO:Douglas DMNH10354/TK86090, CO:Boulder DMNH10408/TK86098, WY:Albany DMNH9564/TK86105, CO:Boulder DMNH9561/TK86109, CO:Larimer DMNH9576/TK86115, CO:Douglas DMNH9574/TK86116, CO:Douglas DMNH10520/TK86124, CO:Jefferson DMNH10602/TK86163, CO:Elbert KU110013, SD:Custer	Z.h. prebleii Z.h. campestris	C/P3

	KU123597, MT:Carter	Z.h. campestris	
DMNH9579/XM1166, CO:El Paso	DMNH9313/XM875, CO:El Paso DMNH9315/XM879, CO:El Paso DMNH10380/TK86093, CO:El Paso DMNH9565/TK86106, CO:El Paso DMNH9563/TK86107, CO:El Paso DMNH9566/TK86118, CO:El Paso DMNH9573/TK86120, CO:Douglas DMNH9572/TK86121, CO:Douglas DMNH9571/TK86122, CO:Douglas DMNH9574/TK86166, CO:El Paso DMNH10607/TK86167, CO:El Paso KU109978, SD:Custer KU123592, MT:Carter	Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. campestris Z.h. campestris	C/P2
DMNH10405/TK86095, WY:Albany	DMNH10258/TK86074, WY:Laramie DMNH10270/TK86081, CO:Larimer DMNH10404/TK86094, WY:Platte DMNH10406/TK86096, WY:Albany DMNH10407/TK86097, WY:Albany DMNH9568/TK86117, CO:Larimer PIONEER9A43, CO: Larimer PIONEER9B89, CO:Larimer KU109984, SD:Custer KU109985, SD:Custer	Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. prebleii Z.h. campestris Z.h. campestris	C/P1

Table 2. Specimens of *Z. princeps* used as outgroups in phylogenetic analysis and specimens that have an identical mtDNA haplotype or are on the same clade as the mtDNA haplotypes of representative individuals. Only the mtDNA haplotypes of the three represenative *Z. princeps* individuals were used in phylogenetic analysis. Note that some individuals previously identified as *Z. hudsonious* have mtDNA haplotypes identical to *Z. princeps*. These individuals were presumed to be misidentified and not included in phylogenetic or population genetic analyses.

Representative individuals of *Z. princeps* used in phylogenetic analysis	Additional specimens with identical mtDNA haplotype or mtDNA on the same clade with strong bootstrap support	Subspecies as per museum tag
DMNH9316, WY:Laramie		Z.p. princeps
	DMNH10327/TK86085,CO:Teller	Z.p. princeps
	DMNH10328/TK86086,CO:Douglas	Z.p. princeps
	DMNH10330/TK86089, CO:Douglas	Z.p. princeps
	DMNH10873/TK103545, CO:Conejos	Z.p. princeps
	DMNH10875/TK103589, CO:Las Animas	Z.p. princeps
	DMNH10874/TK103593, CO:Las Animas	Z.h. prebeli
	DMNH10257/TK86070,WY:Albany*	Z.h. prebeli
	DMNH9567/TK86123,WY:Albany*	Z.h. prebeli
	DMNH9569/TK86113,WY:Albany*	Z.h. prebeli
	DMNH10698/TK86202,WY:Albany*	
DMNH10274/TK86075, WY:Teton		Z.p. utahensis
	DMNH10559/TK86135, WY:Teton*	Z.p. utahensis
	DMNH10535/TK86155, WY:Teton	Z.p. utahensis
	DMNH10542/TK86175, WY:Teton	Z.p. utahensis
	DMNH9921/TK86039, WY:Park*	Z.p. idahoensis
	DMNH9923/TK86040, WY:Park *	Z.p. idahoensis
	DMNH9925/TK86041, WY:Park *	Z.p. idahoensis
	KU109994, SD:Custer*	Z.h. campestris
	KU123595, MT:Carter*	Z.h. campestris
	KU30814, KS:Douglas*	Z.h. pallidus
DMNH9595/TK86112, WY:Fremont		Z.p. idahoensis
	DMNH9837/TK86028,WY:Fremont	Z.p. idahoensis
	DMNH9839/TK86037,WY:Fremont	Z.p. idahoensis

*Sister taxa on the same clade as resprentative individual, with strong bootstrap support. For computation simplicity, these individuals were not used in phylogenetic analysis.

Table 3 Summary statistics for mean of repeated cranial measurements for *Z.h. campestris* and *Z.h. preblei*. Using ANOVA, 3 of the cranial measurements were significantly different ($p<0.05$) between subspecies: zygomatic breadth ($P=0.0071$), mastaoidal breadth ($P=0.012$), and interorbital breadth ($p=0.022$). *Z.h. preblei* was larger for both zygomatic breadth and mastaoidal breadth, while *Z.h. campestris* was larger for interorbital breadth. Using single measurements from three adult specimens of *Z.h. preblei*, Krutzsch (1954) stated that *Z.h. preblei* was "averaging smaller in most cranial measurements" compared to *Z.h. campestris.* Our results refute this claim.

Subspecies/ Measurement	**Number**	**Mean**	**S.D.**	**Min.**	**Max.**
Z.h. campestris					
Occipitonasal length	37	23.046	0.609	21.623	24.048
Condylobasal length	39	19.944	0.571	19.083	20.92
Palatal length	39	10.105	0.305	9.313	10.635
Zygomatic length	40	9.548	0.338	8.678	10.163
Zygomatic breadth	**39**	**10.972**	**0.377**	**10.055**	**11.728**
Mastoidal breadth	**39**	**10.261**	**0.292**	**9.53**	**10.82**
Braincase breadth	40	10.321	0.263	9.765	10.7
Interorbital breadth	**38**	**4.326**	**0.176**	**3.863**	**4.833**
Upper tooth row length	40	3.689	0.14	3.365	3.945
Z.h. preblei					
Occipitonasal length	37	22.941	0.445	22.065	23.933
Condylobasal length	35	19.858	0.457	18.55	20.823
Palatal length	40	10.057	0.272	9.323	10.645
Zygomatic length	40	9.454	0.254	8.82	9.993
Zygomatic breadth	**37**	**11.193**	**0.31**	**10.52**	**12.113**
Mastoidal breadth	**38**	**10.4282**	**0.28**	**9.62**	**10.855**
Braincase breadth	38	10.345	0.211	9.81	10.838
Interorbital breadth	**40**	**4.24**	**0.145**	**3.9**	**4.495**
Upper tooth row length	39	3.725	0.112	3.418	3.97

Table 4. Qualitative morphological comparisons made by Krutzsch (1954). The left column lists the descriptions for *Z.h. preblei* and the right column list descriptions for *Z.h. campestris*. The center column (bold italics) lists the differences Krutzsch (1954) used to distinguish *Z.h. preblei* from *Z.h. campestris.*

Z.h. preblei		*Z.h. campestris*
	From topotypes of Z.h. campestris, Z.h. preblei differs as follows:	
Size medium		Size large
Color dull		(no description)
	Upper parts generally dull, averaging lighter	
Back from near Clay color to near Tawny-olive with admixture of black hair forming poorly defined dorsal band		Back from near Ochaceous-Tawny to near Ochaceous-buff with admixture of black-tipped hair forming distinct dorsal band
	less black tipped hair	
Sides lighter than back from near Clay color to near cinnamon-buff		Sides lighter than back, from near Ochaceous-buff to near yellow Ocher with black hair interspersed
	Sides duller	
Lateral line distinct and clear Ochaceous-Buff		Lateral line usually distinct, of clear Ochaceous-buff
Belly white – sometimes with faint wash of clear Ochaceous-Buff above		Belly white, usually with moderate suffusion of near Ochaceous-buff
Tail bicolored, brownish to light brownish-black above, grayish-white to yellowish-white below		Tail bicolored, brownish to brownish-black above, grayish-white to yellowish-white below
Ears dark, narrowly edged with color of sides		Ears dark, edged with Ochaceous-buff
Feet grayish-white above		Feet grayish-white above
	Averaging smaller in most cranial measurements	
Incive foramia relatively narrow and elongate		Incive foramia long and usually truncate at posterior border
	Incisive foramia narrower, not truncate posteriorly	
Auditory bullae moderately inflated		Auditory bullae well inflated
	Auditory bullae smaller, less well inflated	
Pterygoid fossae relatively broad		Pterygoid fossae broad
Postpalatal notch broadly rounded		(no description)
Interorbital region relatively narrow		(no description)
	Least interorbital constriction narrower	
Zygomatic arch not widely bowed		Zygomata relatively wide-spread and long
Frontal region well inflated		(no description)
	Frontal region usually more inflated	
Distance from incisors to postpalatal notch relatively short		(no description)

(no description)	Large medial projection on inferior ramus of zygomatic process of maxillary
(no description)	Condylbasial length and occipitonasal length relatively great
(no description)	Mastoid region and palatal region relatively broad
(no description)	Interparietal bone usually broad

Hypotheses to explain the pattern of shared mtDNAs across the range of Z.h. preblei and Z.h. campestris

1) Range of Z.h. preblei is a recent colonization from Z.h. campestris (mtDNAs represent a northward range expansion and hybridization)

2) Z.h, preblei evolved in isolation and spread north colonizing the range of Z.h. campestris (mtDNAs represent a northward range expansion and hybridization)

Reduced gene flow has led to the pattern of reduced gene flow among the range of Z.h. preblei and Z.h. campestris.