REPORT **EXHIBIT A**

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GENOMA

For the State of Wyoming, Office of the Attorney General – Patrick J. Crank, Attorney General and Robert A. Nicholas, Senior Assistant Attorney General

An assessment of the threatened subspecific status of the Preble's meadow jumping mouse (*Zapus hudsonius preblei*) based on current molecular data sets

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I Introduction

Preble's meadow jumping mouse (*Zapus hudsonius preblei*) is one of twelve proposed subspecies within the *Zapus hudsonius* (meadow jumping mouse) species complex. *Zapus hudsonius* is found throughout North America ranging from West to East coast and as far north as Alaska and as far south as central New Mexico, Mississippi, and Alabama (see distribution map from Ramey et al. 2005, Figure 1). The distribution of the Preble's meadow jumping mouse (PMJM) does not overlap with other meadow jumping mouse subspecies and corresponds to the Front Range corridor running from Colorado Springs, Colorado to Cheyenne, Wyoming (Ramey et al. 2005, Figure 1).

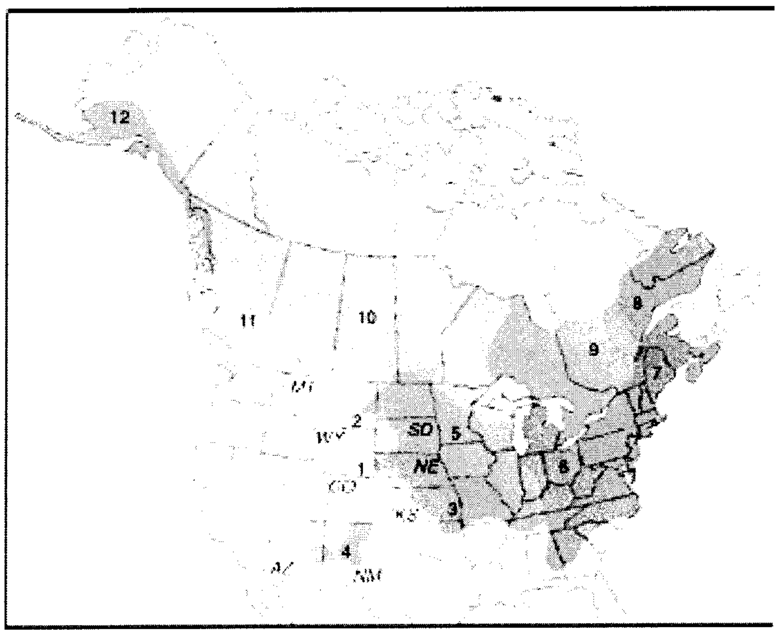


Fig. 1. Map of North America showing distribution and subspecies of *Zapus hudsonius* (Krutzsch 1954, Hafner et al. 1981): (1) *Z. h. preblei*, (2) *Z. h. campestris*, (3) *Z. h. pallidus*, (4) *Z. h. luteus*, (5) *Z. h. intermedius*, (6) *Z. h. americanus*, (7) *Z. h. acadicus*, (8) *Z. h. ladas*, (9) *Z. h. canadensis*, (10) *Z. h. hudsonius*, (11) *Z. h. tenellus*, and (12) *Z. h. alascensis*.

On May 13, 1998 the PMJM was designated as a threatened subspecies by the U.S. Fish and Wildlife service (http://mountain-prairie.fws.gov/preble/). However, a recent study has called into question the appropriateness of such a designation (Ramey et al. 2005). The Ramey et al. (2005) study reanalyzed and expanded a previous morphological study (Krutch 1954) used in the listing process but found no significant morphometric differences between the PMJM and other nearby *Z. hudsonius* subspecies. Additionally, Ramey et al. (2005) was unable to find significant underlying genetic differentiation between PMJM and the other subspecies using microsatellite and mitochondrial DNA sequence data. Due primarily to the findings of Ramey et al. in February 2005 the U.S.F.W. service issued a 12-Month Finding on a petition to delist the PMJM as a threatened subspecies under the U.S. Endangered Species Act. In early 2006 a new study was released (King et al. 2006) that questioned the conclusions of Ramey et al. 2005 and called for a continuation of the threatened subspecies status for the PMJM. In their study, King et al. also analyzed microsatellite and mitochondrial DNA sequences but approached their study from a drastically different sampling scheme as compared to the Ramey et al. 2005 study. The Ramey et al. study had widespread and fairly dense sampling but few individuals were taken from each locality, on the other end of the spectrum, the King et al. study sampled few localities but large numbers of individuals from each locality.

In this study, we investigate the seemingly different conclusions of these two studies and consider them in light of the sampling schemes employed. We also combine the data sets where possible and extend the analytical approaches used to determine, according to the current data, if the PMJM represents a distinct subspecies within the *Z. hudsonius* species.

II Ramey et al. 2005

A. Microsatellite Data

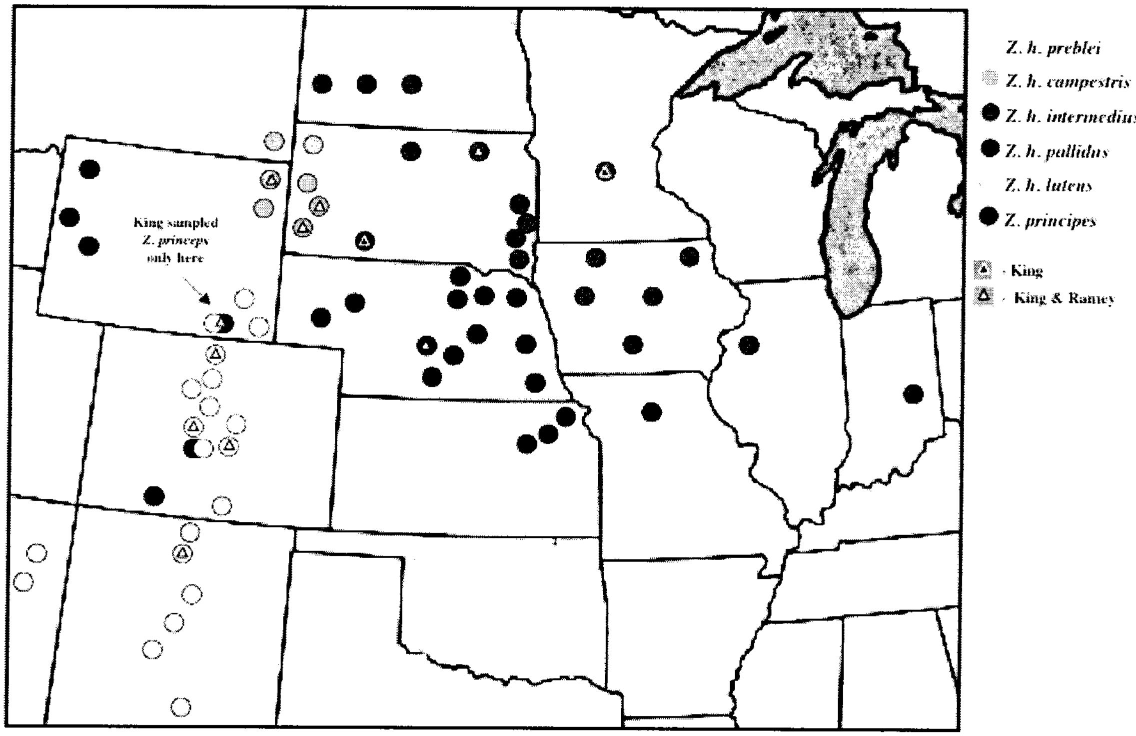
We reanalyzed the Ramey et al. 2005 microsatellite data using all six microsatellite loci from five *Zapus hudsonius* subspecies, *Zapus hudsonius preblei*, *Zapus hudsonius campestris*, *Zapus hudsonius intermedius*, *Zapus hudsonius pallidus*, and *Zapus hudsonius luteus*. Figure 2 shows the localities of the samples taken in both the Ramey et al. and King et al. studies. Table 1 also provides the state and county names for each locality, the numbers of samples taken, and GPS coordinates for a central locality within the county. We converted the Ramey et al. microsatellite data into a format (Appendix 1) appropriate to run on the computer program STRUCTURE (Pritchard et al. 2000). STRUCTURE organizes individuals into clusters or populations that minimize Hardy-

Weinberg and Linkage disequilibria. In this way researchers let the data determine the population boundaries rather than assigning individuals to populations based solely on geographic location. Our strategy was to allow for an ancestral admixture model in selecting the optimal number of population clusters (K) for the entire data set regardless of *a priori* subspecies determination. We selected an optimal K by adhering to the following suggestions from the STRUCTURE help files

"There are a couple of informal pointers which might be helpful in selecting K. The first is that it's often the situation that Pr(K) is very small for K less than the appropriate value (effectively zero), and then more-or-less plateaus for larger K … In this sort of situation where several values of K give similar estimates of log Pr(X|K), it seems that the smallest of these is often ``correct". It is a bit difficult to provide a firm rule for what we mean by a ``more-or-less plateaus". I think that a sensible way to think about this is in terms of model choice. That is, we may not always be able to know the TRUE value of K, but we should aim for the smallest value of K that captures the major structure in the data. … A corollary of this is that when there is no population structure, you will typically see that the proportion of the sample assigned to each population is roughly symmetric (~1/K in each population), and most individuals will be fairly admixed. If some individuals are strongly assigned to one population or another, and if the proportions assigned to each group are asymmetric, then this is a strong indication that you have real population structure. … In summary, you should be skeptical about population structure inferred on the basis of small differences in K if (1) there is no clear biological interpretation for the assignments, and (2) the assignments are roughly symmetric to all populations and no individuals are strongly assigned."

After an optimal K was selected, we then looked for evidence of admixture between clusters by identifying individuals that have similar assignment probabilities (inferred ancestry) to more than one cluster or no assignment probability greater than 0.80 to any cluster. We also looked for evidence of admixture between subspecies by identifying individuals from a subspecies that were assigned to clusters with individuals predominately from other subspecies. Like Ramey et al. 2005 and King et al. 2006, we used a burn-in of 15,000 followed by 100,000 replicates and tested K = 1 through K = 10. We performed these analyses 10 separate times in order to adequately search the likelihood space.

Figure 2. Distribution of all localities used in this study. Colored dots with no symbol indicate localities that were sampled only by Ramey et al. Color dots and symbols are explained in key below.



Extended results for all runs are given in support files (Supporting Documents/Data & Result Files/ Ramey MS Structure Results). Table 2 shows a summary of the likelihood scores for each of the 10 runs and the average likelihood scores for all runs at each K. A visual representation of average likelihood scores reveals a leveling-off of scores after K = 3 (Figure 3). We also noted possible leveling-off points at K = 5 and K = 9. In order to select a preferred K value we compared assignment probabilities for best score values at each alternative (see Supporting Documents/Data & Result Files/ Ramey MS Structure Results/Ramey Structure K = 3/5/9) and determined that K = 3 not only had the least admixed population assignments but also was the most concordant with "a clear biological interpretation for the assignments" or sets of subspecies designations. This result was also similar to the ΔK ad hoc statistic result of the King et al. 2006 study discussed below. Unfortunately a statistical test for selecting K is not available in STRUCTURE. Table 3 shows the inferred ancestry for each sample

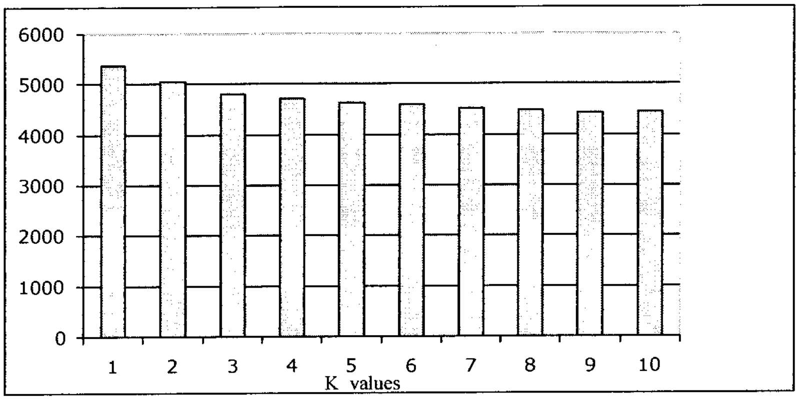


Figure 3. A visual summary average likelihoods scores for each K value estimated in STRUCTURE based on the Ramey et al. data, Purple bars represent absolute values of these scores, where the lower the bar the better the score. The optimal K value is the lowest value of K with a 'good' score or one that divides the individuals into populations that explain most of the variation.

for the best score at K=3. Population boundaries between clusters appeared to be semi-permeable as a number of individuals (13% of total) showed assignment probabilities < 0.80. Additionally a number of individuals (11% of total) had their highest probability of assignment to clusters of non-subspecific individuals (cluster 1 = *Z. h. preblei*, cluster 2 = *Z. h. pallidus* + *Z. h. luteus*, and cluster 3 = *Z. h. campestris* + *Z. h. intermedius*). These cases occurred most frequently in *Z. h. intermedius*, followed by *Z. h. campestris*, *Z. h. pallidus*, *Z. h. preblei,* and *Z. h. luteus*, in that order (Table 3). These results demonstrate that there is indeed limited gene flow among the three populations identified by the STRUCTURE analysis.

Our STRUCTURE results indicated that the five subspecies samples by the Ramey et al. study can be divided into three populations roughly equivalent to the three clusters identified above. To quantify the degree of admixture between *Z. h. preblei, Z. h. campestris* + *Z. h. intermedius*, and *Z. h. pallidus* + *Z. h. luteus*, we used coalescent-based methods to estimate relative measures of Θ ($4Ne\mu$, a measure of effective population size and mutation rate) and interpopulation migration rates (Nm) using the program MIGRATE (Beerli and Felsenstein 2001) based on the Brownian motion model. Appendix 2 shows the formatted infile. A summary of the results of this analysis is given in Table 4. Conditions of analysis and extended results for each run are given on accompanying disk (Supporting Documents/Data & Result Files/ Ramey MS Migrate Results). Table 4 (B) converts Migrate output into migration rates (Nm) that can be compared across studies. One advantage that these likelihood-based estimates have over traditional estimates of gene flow via Fst statistics is that asymmetrical migration rates can be estimated between populations. When considering gene flow into and out of *Z. h.*

preblei, we see that the most restricted migration is from the *Z. h. campestris* + *Z. h. intermedius* cluster into the *Z. h. preblei* cluster (0.46) and the highest migration from the *Z. h. preblei* cluster into the *Z. h. campestris* + *Z. h. intermedius* cluster (2.14). The immigration and emigration into and away from the all clusters ranged from 0.46 to 5.76; comparing this to other migration rates in rodents shows that comparatively high rates are found in these *Z. hudsonius* 'subspecies'. For instance in the African ground squirrel (*Xerus inauris*) migration rates (Nm) between populations within the species were estimated to be 0.64 to less than 0.001 (Herron et al. 2005), between population of common voles (*Microtus arvalis*) estimates ranged from 3.3 to 0.15 (Hamilton et al. 2006), between population of Tuco-tuco (*Ctenomys rionegrensis*) estimates ranged from 0.17 to less than 0.001 (Wlasiuk et al. 2003), between population of two deer mice species (*Peromyscus keeni* and *Peromyscus maniculatus*) estimates ranged from 1.00 to less than 0.001 and 4.74 to less than 0.001 respectively (Zheng et al. 2003). In all of these cases, we find migration rates lower than the lowest estimate between any of the *Z. hudsonius* populations and only a few higher, however, only in one of the above cases (*Peromyscus maniculatus*) have subspecies based on molecular data been described. This calls into question support of subspecific designation based on these microsatellite data.

B. Mitochondrial DNA Sequence Data

Ramey et al. 2005 sequenced a 346 bp piece of the mitochondrial control region (CR) gene to test for reciprocal monophyly between *Z. h. preblei* and its neighboring subspecies. King et al. 2006 also sequenced this same region of the mtDNA and we have combined these data sets and performed various analyses with them below. Here we will only mention briefly a couple of interesting points noted when comparing Ramey et al.'s CR data with their microsatellite data.

In their study, Ramey et al. found that *Z. h. preblei* contained few unique CR haplotypes and most haplotypes were also found in low frequencies within the range of *Z. campestris* (Table 5). The low frequencies of these shared haplotypes within *Z. h. campestris* caused King et al. to question the quality of these data. King et al. 2006 (p22, line 666) states:

"For example, Ramey et al. (2005) reported the presence of *Z. h. preblei* haplotypes in DNA extracted from five dried museum skins of *Z. h. campestris* collected from Custer County, SD. The authors suggested this finding indicated recent gene flow and alluded to the presence of these haplotypes as a critical element in the decision to recommend synonymy of these subspecies. In the present study, 31 *Z. h. campestris* sampled recently from the same site in Custer County, SD used by Ramey et al. (2005), along with 30 additional specimens from neighboring Crook County, WY were subjected to mtDNA CR and CytB sequence analysis. All 61 individuals were determined to posses *Z. h. campestris*-specific mtDNA haplotypes. Moreover, the same conclusion was reached with the microsatellite loci, as no *Z. h. campestris* individual from either of these collections was assigned to *Z. h. preblei*. Given the prominent role the haplotypes obtained for the five museum skins from Custer County, SD and two additional specimens from Carter County, MT have played in the conclusions drawn by Ramey et al. (2005), it is unsatisfactory that an *a posteriori* analysis was not considered as part of a routine quality assurance/quality control effort. Since no attempts were made to reproduce the previous CR results, to confirm the findings with another region of mtDNA, or to apply an additional finer resolution technique such as microsatellite DNA analysis, combined with our failure to detect *Z. h. preblei* haplotypes among 61 *Z. h. campestris* from the same and an adjacent location, the conclusions drawn by Ramey et al. (2005) should be considered questionable."

The point made above by King et al. is well taken and when much is dependent on these few results assurances should be taken that these samples were not misidentified or that the DNA isolated from these samples has not been cross contaminated. One way to control against this is to look at the microsatellite profiles for each of the *Z. h. campestris* individuals that have a '*Z. h. preblei*' CR haplotype. If these individuals were misidentified before DNA extraction or contaminated with *Z. h. preblei* DNA after extraction then their microsatellite genotypes should also show a '*Z. h. preblei*' profile and have a high probability assignment to the *Z. h. preblei* cluster. Table 5 shows the groupings of all identical CR haplotypes from both studies. From Table 5 we see that

ZhcaK110013, ZhcaK109984, ZhcaK109985, ZhcaK123592, ZhcaK109978, and ZhcaK109972, all have '*Z. h. preblei*' CR haplotypes. However, assignment probabilities from Table 3 show that ZhcaK110013 is assigned to the *Z. h. campestris/intermedius* cluster with a probability of 0.988. Also, all the others samples in question have their highest probability assignment to the *Z. h. campestris/intermedius* cluster, ZhcaK109985 (0.969), ZhcaK109978 (0.937), ZhcaK109972 (0.694), with the exception of ZhcaK109982 which shows similar assignment probabilities to all three clusters and ZhcaK123592 for which no microsatellite data are given. This indicates that these samples were neither misidentified, miscataloged, nor cross contaminated. Conflicting nuclear and mtDNA signals could be the products of different levels of resolution targeted by the different markers and types of analysis. The fast evolving microsatellite markers coupled with the **population-**level STRUCTURE analysis illustrate the current interactions of these populations whereas the slower evolving shared CR haplotypes may be indicative of historical interactions or mitochondrial introgression. If the subspecific category is to represent historical isolation in addition to current population structure, high levels of concordance between these analyses should be required. If simple allele frequency differences (highly dependent on sampling scheme) were allowed to fill this requirement most if not all colonization and bottleneck events would also instantaneously spawn new subspecies, something many scientists would find discomforting. An alternative explanation for different assignments based on mtDNA versus nuclear (microsatellite) markers is the potential for sex biased dispersal of mtDNA alleles (maternally inherited) given the different and asymmetric migration rates for the diagnosed populations.

A number of the CR mtDNA haplotypes from *Z. h. preblei*, *Z. h. campestris*, and *Z. h. pallidus*, individuals from the Ramey et al. study were identical or nearly identical to *Z. princeps* haplotypes (see Network 3 and 4 in Figure 10). Ramey et al. interpreted these results as cases of misidentification. Unfortunately, no microsatellite data were generated to test these in the same way the *Z. h. campestris* individuals with *Z. h. preblei* haplotypes were tested above. The four *Z. h. preblei* individuals that were 'misidentified' all came from Albany County, Wyoming. *Z. princeps* were also sampled from this county, which merits consideration of a different interpretation than 'misidentification'. It is possible that some gene flow is occurring at this much deeper interspecific level. If so, this may be indicative of a tradition of 'over-splitting' taxa by biologist within the *Zapus* genus. Results from previous studies indicate that gene flow between *Z. princeps* and *Z. h. preblei* and other subspecies is not only likely but probable. As summarized in Beauvais 2001:

"The relatively large zone of co-occurrence in southeast Wyoming raises the issue of potential hybridization between the 2 species [*Z. h. preblei* and *Z. princeps*]. Hybridization between related species in areas of co-occurrence is known to occur in several other free-ranging vertebrates (see examples in Pague and Grunau 2000). Hybridization between *Z. hudsonius* and *Z. princeps* in Wyoming is suggested by recent analyses of variation in mitochondrial DNA. Although these analyses can distinguish the 2 species in other parts of their ranges (e.g., the South Platte basin in Colorado), they are unable to reliably assign species identity to *Zapus* specimens from southeast Wyoming. **The general consensus among regional mammalogists is that *Z. hudsonius X Z. princeps* hybridization is the most parsimonious explanation for such results** (Riggs et al. 1997, Pague and Grunau 2000, Schorr 2001)."

It may be that the genus *Zapus* may be suffering not only from a tendency to split taxa but also from non-rigorous delimitation of species boundaries. This makes any discussion of subspecies dubious. Biologists may be better served by preceding debate on subspecific classification with substantial and meticulous examinations of species boundaries. In other words 'you can't have cupboards if you ain't got walls' *Neil Young-Old Laughing Lady*. A detailed analysis of this potential hybrid zone that incorporates both nuclear (microsatellite) and mitochondrial markers would contribute substantially to clarification of our current issue.

III King et al. 2006

A. Microsatellite Data

King et al. 2006 screened 320 samples for 21 microsatellite loci across the same five *Zapus* subspecies as above. We reanalyzed the King et al. microsatellite to verify their results and also to observe patterns in assignment probabilities for the optimal number of populations (K). Again, Figure 2 shows the localities of the samples taken in both the Ramey et al. and King et al. studies. Table 1 also provides the state and county names for each locality, the numbers of samples taken, and GPS coordinates. We converted the King et al. microsatellite data into a format (Appendix 3) appropriate to run on the computer program STRUCTURE (Pritchard et al. 2000). Conditions for the King et al. STRUCTURE analysis and method for selecting the optimal K were identical to the analysis of the Ramey et al. data and are given above. Resulting output files for all runs are given in support files (Supporting Documents/Data & Result Files/ King MS Structure Results).

We confirm the findings of King et al. 2006 and select an optimal K value of three (Table 6, Figure 4). The result is identical to the K value selected with the Ramey et al. data set only in the current analysis we see a more profound leveling of likelihood

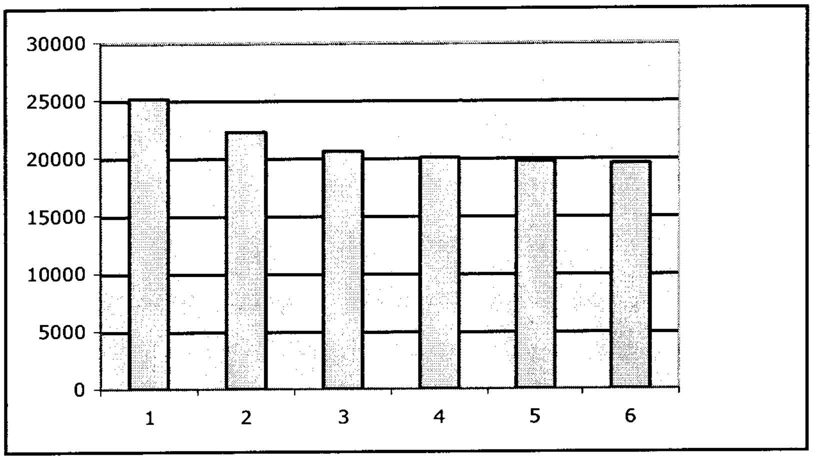


Figure 4. A visual representation of the absolute values of likelihood scores from ten separate STRUCTURE runs for King et al. data set. K values range from 1 to 6. Little improvement of likelihood scores is evident after K = 3.

scores at K = 3. The composition of resultant clusters was also very similar to our reanalysis of the Ramey et al. data (*Z. h. preblei*, *Z. h. pallidus* + *Z. h. luteus*, and *Z. h. campestris* + *Z. h. intermedius*). Table 7 shows the inferred ancestry for each sample. Population boundaries between clusters appeared to be much more distinct than in the STRUCTURE analysis of the Ramey et al. data. For instance, few individuals showed assignment probabilities < 0.80 and all of these occurred in the *Z. h campestris/intermedius* cluster. Additionally no individuals had their highest probability of assignment to clusters of non-subspecific individuals.

The differences in results and conclusion of these studies seem to be largely due to the sampling schemes employed by each study. King et al. rightly point out that sampling is critical in intraspecific studies and is distinct from systematic studies. King et al. argue for dense sampling at specific locations with sparse sampling across locations throughout the distribution of the subspecies. King et al. correctly point out that the basis of inference by Ramey et al. (frequency differences instead of evolutionary relationships) is highly dependent upon sampling individuals at a given location with the Ramey et al.

sampling design lacking in terms of individuals per site. Yet the conclusions reached by King et al. are also highly suspect in that leaving large geographic gaps between sampling sites when the taxon is known to range within those gaps leads to artificial inferences of population structure when, in fact, a gradient of variation may exist with

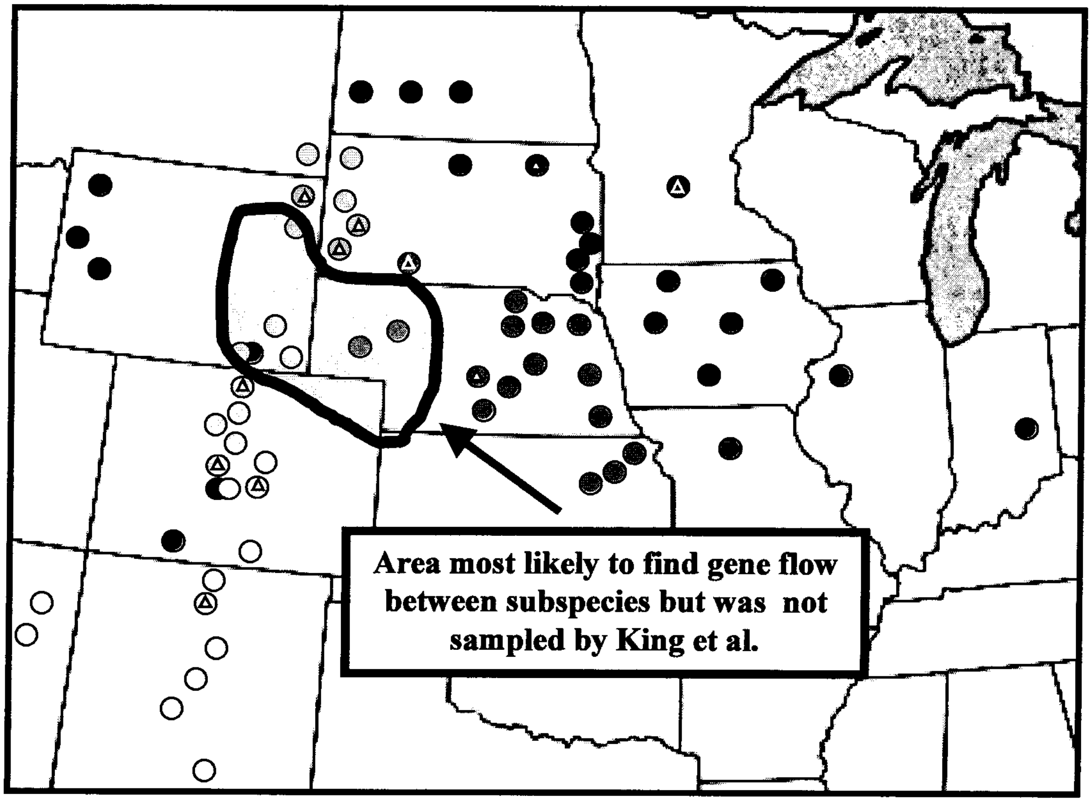


Figure 5. Sampling distribution showing critical region (shaded) not sampled by the King et al. study.

gene flow across the gradient. Thus the optimal sampling strategy for such studies is a combination of the two approaches. Below we attempt an approximation of this scheme by combining the CR data sets of Ramey et al. and King et al. Unfortunately, the scoring of microsatellite allele size on different machines can be tricky and the lack of generalized size standards run by these lab groups made it is impossible to combine the microsatellite data into a single analysis. Ideally some of the samples scored in the first study (Ramey et al al. 2005) should have been sent to the second (King et al. 2006) to be run and the results calibrated.

Both studies have limitations in their sampling strategies. The conclusions by King et al. of population structure are particularly suspect given the sampling design of their study. For example, King et al. fail to sample in areas most likely to show gene flow between subspecies (Figure 5). These areas include, *Z. h. preblei* from southern Wyoming, where you would find individuals most likely to show evidence of gene flow between *Z. h. campestris*, *Z. h. pallidus,* and even *Z. princeps* based on geographic proximity and previous studies (see above), and *Z. h. pallidus* from western Nebraska. King et al. have just a single locality sampled for *Z. h. luteus* and just two sites sampled for the critical *Z. h. campestris* and *Z. h. intermedius*. This is particularly problematic with the widespread distribution of *Z. h. intermedius* across 11 states with sampling in only the NE corner of South Dakota and an adjacent site in central Minnesota. The central problem here is a taxonomic issue relative to the entire species complex and possibly sister species within the genus, thus the entire species complex should be sampled to resolve the issue.

Like our STRUCTURE analysis of the Ramey et al. microsatellite data set our analysis of the King et al. data indicated that the five subspecies samples can be divided into three populations equivalent to the three clusters identified above. Again to quantify the degree of admixture between *Z. h. preblei, Z. h. campestris* + *Z. h. intermedius*, and *Z. h. pallidus* + *Z. h. luteus*, we used MIGRATE (Beerli and Felsenstein 2001) to estimate migration between these clusters. Conditions of analysis and extended results for each run are given in the supporting material (Supporting Documents/Data & Result Files/ King MS Migrate Results). Table 8 shows the estimated genetic diversity (Θs) for each cluster as well as the migration rates (Mxy) between all clusters (x and y). Table 8 (B) shows estimates of population migration rates (Nm) between clusters. The results of Table 8 (B) are similar to those estimated from the Ramey et al. microsatellite data (Table 4). We see nearly equal rates of migration out of *Z. h. preblei* but interestingly an increase of migration into *Z. h. preblei* from the other two clusters (1.21 and 2.45 as opposed to 0.46 and 0.47). Conversely lower migration rate estimates between the *Z. h. campestris* + *Z. h. intermedius*, and *Z. h. pallidus* + *Z. h. luteus* clusters resulted in the analysis of the King et al. data. As a whole, we draw similar conclusion here as with the analysis of the Ramey et al. data with migration rates again on par with and even in a little excess of other within species comparisons where subspecies are not recognized (see above).

B. Mitochondrial DNA Sequence Data

The King et al. studies generated sequence data from two mtDNA genes. Like Ramey et al. they sequenced a piece of the control region only slightly larger. We will discuss this below in the section on the combined analysis. King et al. also generated a ~1 Kb piece of the cytochrome b (CytB) mitochondrial gene for 292 individuals from 13 localities (Figure 2) representing the subspecies *Z. h. preblei*, *Z. h. campestris*, *Z. h. intermedius*, *Z. h. pallidus*, and *Z. h. luteus* as well as a single *Z. princeps* sample. In order to test the monophyly of the King et al. subspecies samples, we combined them with 27 outgroup plus one *Z. h. luteus* sequences provided by R. Ramey and J. Cook. The outgroup samples included 21 *Z. princeps* (ZP), two *Z. trinotatus* (ZT), three *Napaeozapus insignis* (Ntinsig), one *Ratus ratus* (Rratus), and one *Mus musculus* (Mmus). In order to combine these data, the total length of sequence had to be trimmed to 518 bp. The high throughput multiple sequence alignment program MUSCLE (Edgar 2004) was used to align sequences. From the 320 individual sequences 48 distinct haplotypes were found (Appendix 4). No haplotypes were shared between subspecies groups. Maximum parsimony (MP) trees were generated in PAUP* (Swofford 1999) by heuristic searches with 100 random additions and using the TBR branch swapping method (see Appendix 5 for PAUP* haplotype data file). Figure 6 shows the resulting 50% majority rule consensus tree for the 48 MP trees. We also generated Bayesian tree topologies with MRBAYES (Huelsenbeck and Ronquist 2001) using 1,000,000 iterations and a burn-in of 47,000. We used Modeltest (Posada and Crandall 1998) to select the GTR + G + I model as the optimal model of evolution. Because of the short length of the DNA fragment used, separate models for each codon position were not estimated. Tree topologies from the two methods were identical in all major divisions and differed only slightly by levels of resolution, for this reason, only the 50% consensus MP tree is shown and bootstrap and posterior probability values from both analyses combined and placed on the tree (Figure 6).

Figure 6 shows monophyletic groupings with good nodal support for *Z. princeps*, *Z. hudsonius*, and *Napaeozapus insignis*. This is not terribly surprising due to the low numbers of individuals and localities sampled from *N. insignis* and *Z. princeps*. Figure 6 also shows a monophyletic grouping for *Z. h. pallidus* and one for a combined *Z. h. luteus* + *Z. h. pallidus* group. However, given that only a single locality from *Z. h. luteus* was sampled, only limited conclusions can be drawn. All samples from *Z. h. preblei, Z. h. campestris*, and *Z. h. intermedius* combined to form a single monophyletic group.

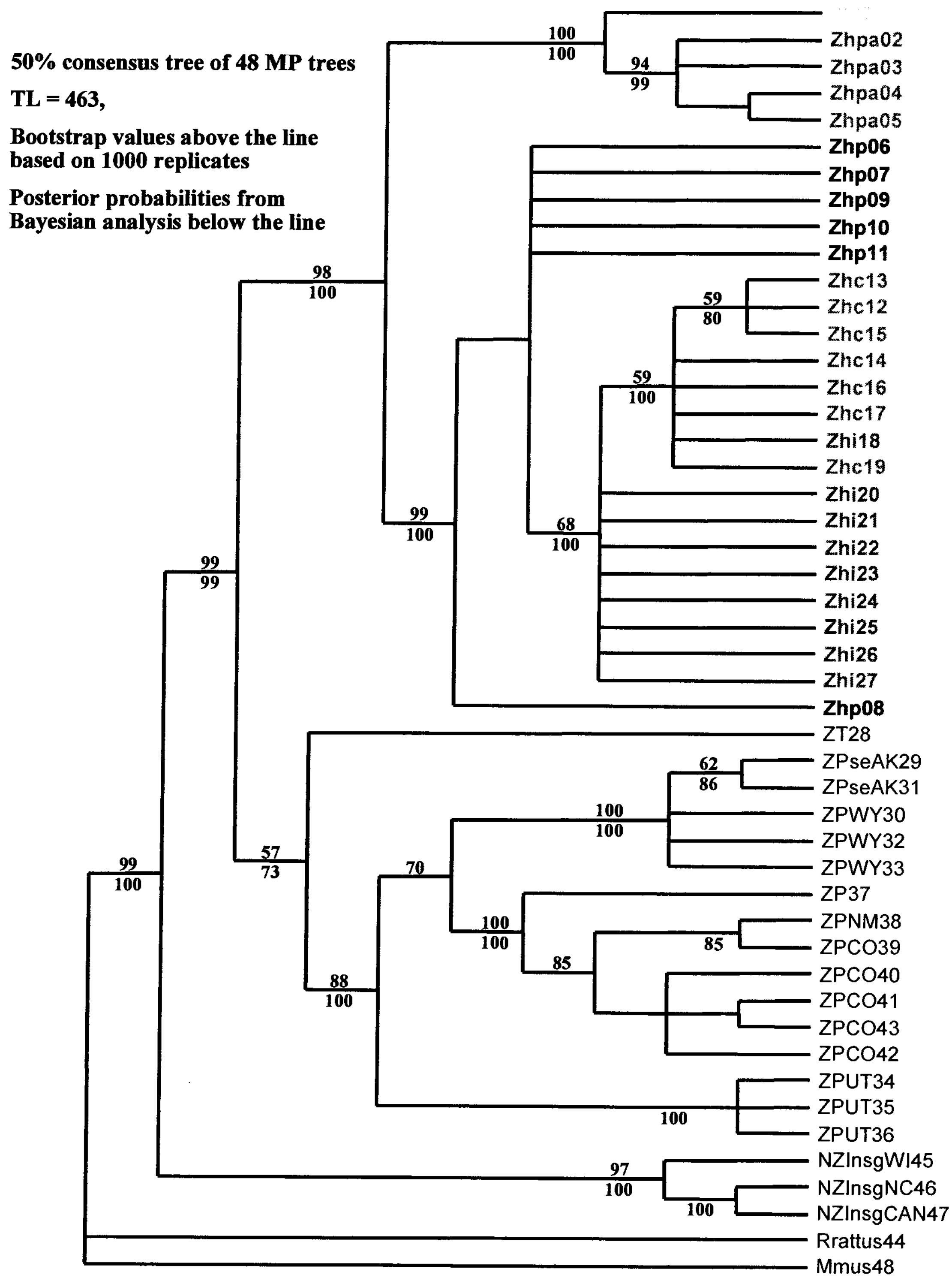


Figure 6. Phylogenetic tree based on part of the CytB mtDNA gene. Numbers at taxa represent haplotype numbers listed in Appendix 4.

However, none of these subspecies forms a monophyletic group by themselves and thus fail this particular subspecies test and indeed fail even an evolutionarily significant unit (ESU) test of Moritz (1994). *Z. h. campestris* comes closest to forming a monophyletic group with a single *Z. h. intermedius* haplotype nested within it. As a whole this analysis, although severely restricted by the sampling design as discussed above, provides some preliminary evidence for designation of a two subspecies within the *Z. hudsonius* samples investigated, one including the *Z. h. pallidus* + *Z. h. luteus* samples and another including the *Z. h. preblei* + *Z. h. campestris* + *Z. h. intermedius* samples.

IV Combined Data Analysis

A. Combined control region phylogenetics

As mentioned above the only data we were able to combine between the Ramey et al. 2005 and King et al 2006 studies were the sequences generated from the mitochondrial control region. Total sample size for the combined analysis was 520 individuals (including several *Z. princeps* samples) from 14 states. Individuals were pooled by their county and state or origin because exact GPS coordinates were not available for all samples (Table 1). GPS coordinates for all localities were taken from roughly the geographic center of the county. Sequences were aligned in MUSCLE (Edgar 2004) and then trimmed to a total base pair length of 347. Sequences collapsed into 63 distinct haplotypes. Nine of the haplotypes were shared either between our five focal *Z. hudsonius* subspecies and/or *Z. hudsonius* and *Z. princeps* (Table 5).

We estimated phylogenetic relationships based on Bayesian, maximum likelihood (ML), and maximum parsimony (MP) criteria. Topologies and well-supported nodes were similar for all three optimality criteria used. Figure 7 shows a 50% majority rule consensus tree of 23,329 most parsimonious trees with a tree length of 146. Trees were

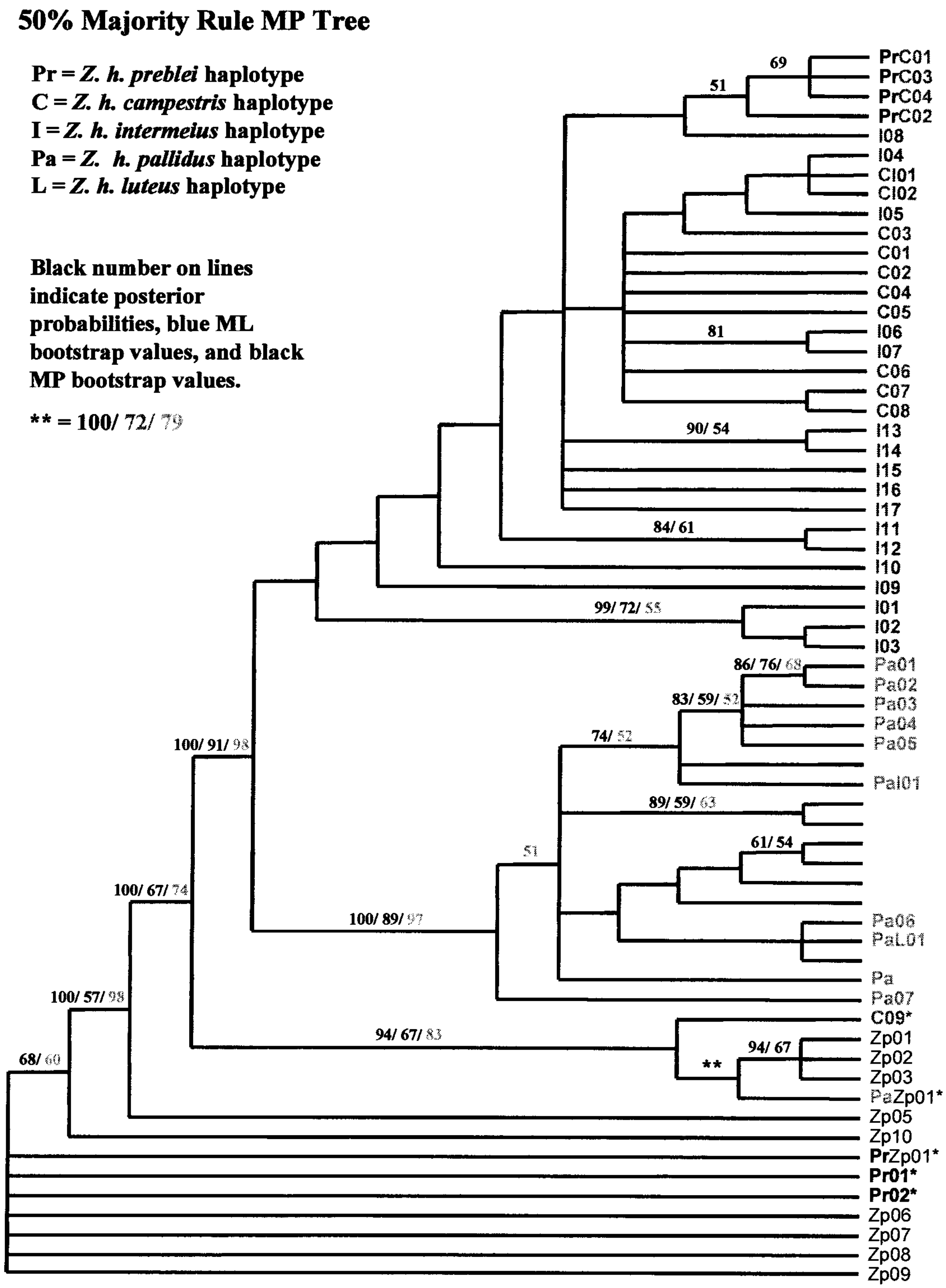


Figure 7. Phylogenetic tree based on the combined CR mtDNA data sets from Ramey et al. 2005 and King et al. 2006 studies. Lists of haplotypes represented by each haplotype label are found in Table 5. Colors correspond to different *Z. hudsonius* subspecies. * = *Z. hudsonius haplotypes* associated with *Z. princeps* haplotypes.

generated using 100 random additions and the TBR branch swapping method. Bootstrap values were based on 1000 replicates where a maximum of 2×10^7 rearrangements was set for each replicate. We generated our Bayesian topologies with MRBAYES (Huelsenbeck and Ronquist 2001) using 1,000,000 iterations and a burn-in of 46,000. We used Modeltest (Posada and Crandall 1998) to select the TVM + I + G model as the optimal model of molecular evolution. Maximum likelihood analysis was performed with GARLI v0.94 (Zwickl 2006, http://www.bio.utexas.edu/grad/zwickl/web/garli.html) under the TVM + I + G model and 100 replicates used for ML bootstrap values.

Results from the CR phylogenetic analysis are similar to the results from the CytB phylogenetic analysis except with less distinct clustering of subspecies and groups of subspecies. This is not surprising because the current analysis incorporated samples from regions avoided by the King et al. study that were located in area most likely to see gene flow between different subspecies/populations. Noticeably widespread across the tree topology are the *Z. h. preblei* samples. Our analysis included some samples of *Z. h. preblei* dropped from the Ramey et al. study on the basis of their similarity to *Z. princeps* sequences. As mentioned above, gene flow between these species is suspected in southeastern Wyoming and these samples seem equally likely to be a result of interspecific gene flow as a result of misidentification. Some haplotypes found both in *Z. h. pallidus* and *Z. h. campestris* also clustered with *Z. princeps* haplotypes (C09, PaZp01) and together formed a moderately supported (posterior probabilities and bootstrap values = 100/67/74, Figure 7) sister group to most *Z. hudsonius* populations. These results at a minimum merit further study on species boundaries between *Z. hudsonius* and *Z. princeps*. Quantification of levels gene flow between these species could then serve to add a base line level of gene flow between proposed *Z. hudsonius* subspecies and aid in the identification of proper subspecific boundaries if such boundaries exist.

Although many of the nodes in Figure 7 are either unresolved or poorly supported we can draw limited conclusions based on some of the moderately support ones. We see that most *Z. hudsonius* samples cluster into a well-supported (100/91/98) monophyletic group. Within this monophyletic group, we see most *Z. h. luteus* and *Z .h. pallidus* samples clustering into a well-supported (100/89/97) group nested within the larger *Z. hudsonius* clade. However, no support for exclusive clustering of any of the *Z. hudsonius* subspecies is evident anywhere in the tree. In fact, even combining most *Z. h. campestris*, *Z. h. preblei*, and *Z. h. intermedius* into a single group did not result in a well-supported clade (as opposed to the CytB result in Figure 6). These results could be indicative of different marker resolution, different sampling schemes, or a combination of both. If exclusivity or near-exclusivity of taxa based on mtDNA markers is to be taken as evidence of historical isolation between populations and thus incorporated into subspecific designation then the results in Figure 6 and Figure 7 question subspecific status for any of the individual *Z. hudsonius* subspecies. However the formation of two subspecies by combining *Z. h. pallidus* with *Z. h. luteus*, and *Z. h. campestris* with both *Z. h. preblei* and *Z. h. intermedius* would merit subspecific status under the near-exclusivity criterion.

B. Nested clade analysis (NCA) on control region

When assessing patterns of genetic variation at the intraspecific level, it is often difficult to distinguish current population structure from population history using traditional population genetic estimates such as Fst (Templeton et al. 1995). For instance, two population sharing similar alleles at similar frequencies could be the product of ongoing gene flow (current population structure) or a past range expansion of the organism (population history). NCA uses haplotype frequencies in conjunction with the genealogical relationships and geographic distribution of the haplotypes in a novel methodology that allows the researcher to distinguish between structure and different historical events (Templeton et al. 1995). Such an analysis was lacking from both the King et al. and the Ramey et al. studies. Thus their studies possibly confound population history and population structure.

To implement the NCA, a parsimony haplotype network was first constructed for the mitochondrial control region sequences using the program TCS (version 1.21, Clement et al. 2000). Haplotypes were connected using a 95% parsimony limit that imposed a maximum of seven mutational steps between connections. Four separate networks plus and unconnected single haplotype (Zp05) resulted (Figures 8-10).

The independent networks were then connected into a total network using TCS by relaxing the 95% parsimony criterion (Figure 10). A number of ambiguous connections or loops in the resulting haplotype networks were resolved using the criteria set forth in Crandall & Templeton (1993). The total network was then nested (Templeton 1998) and input into GEODIS (version 2.4, Posada et al. 2000) together with geographic sampling information (Appendix 7, input file). We then performed permutation tests (1000) to determine association between phylogeny and geographic distribution. Clade distances (Dc) and nested clade distances (Dn) were measured and interior and tip clade differences estimated. Templeton's revised (2004) inference key (Modified 11 November 2005) was then applied to the clades with significant results from GEODIS to determine the outcome of the NCA.

Appendix 8 provides the extended GEODIS results and Table 9 summarized the general conclusions from the NCA inference key. A total of 33 clades with both geographic and genetic variation from various nesting levels were input into GEODIS; of these clades only 17 resulted in significant results that lent themselves to interpretation. This hints at the necessity of the need for a sampling scheme that employs both large numbers of localities (as in Ramey et al. 2005) and large numbers of individuals per locality (as in King et al. 2006). However, even with our limited sampling scheme a number of important conclusions can be drawn. Of the 63 distinct haplotypes eleven were shared between species and subspecies. Noting the distribution of these haplotypes on our networks (Figs. 7-9), we see most of the shared haplotypes are interior clades indicating ancestral types.

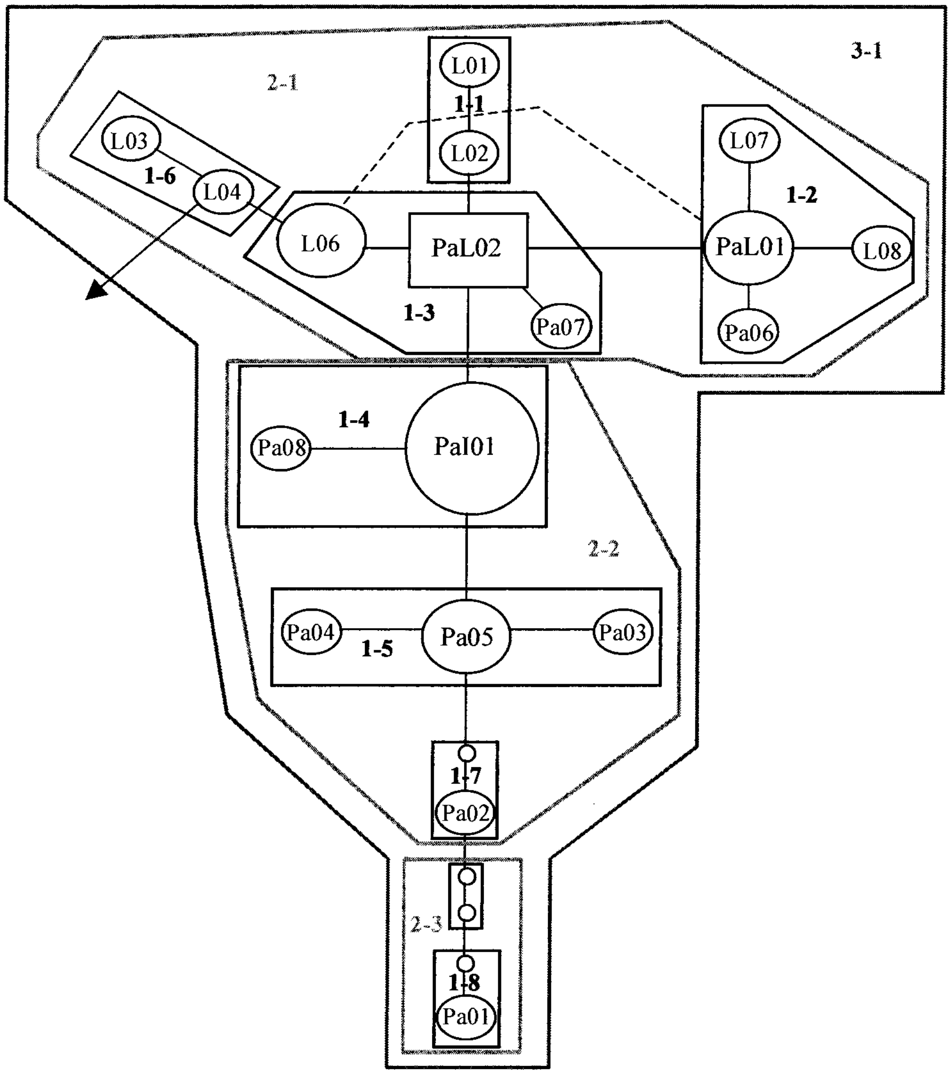


Figure 8. Haplotype network 1 estimated in TCS with 1, 2, and 3 step nesting groups shown. Ovals and squares represent haplotypes where labels correspond to labels in Table 5 and size roughly correlated with frequency of haplotype. Lines separating haplotypes and empty circles represent single mutational steps. Arrows indicate connections to other networks. Dashed lines represent broken loops. Colored boxes correspond to different nesting levels. Network consists of haplotypes mostly from *Z. h. pallidus* and *Z. h. luteus* individuals.

Network 1 (Figure 8) consisted of mostly haplotypes from *Z. h. pallidus* and *Z. h. luteus* individuals, with a single haplotype (PAI01) also being found in *Z. h. intermedius*. The distribution of these haplotypes between subspecies showed non-exclusive clustering within the network. Also clades 1-3 and 2-1 both spanned the geographic divide between *Z. h. luteus* and *Z. h. pallidus* populations (Figure 11), thus including non-subspecific populations. However, the NCA inference for clade 1-3 (Table 9) indicated possible allopatric fragmentation across this geographic divide. NCA inferences for clades 2-1

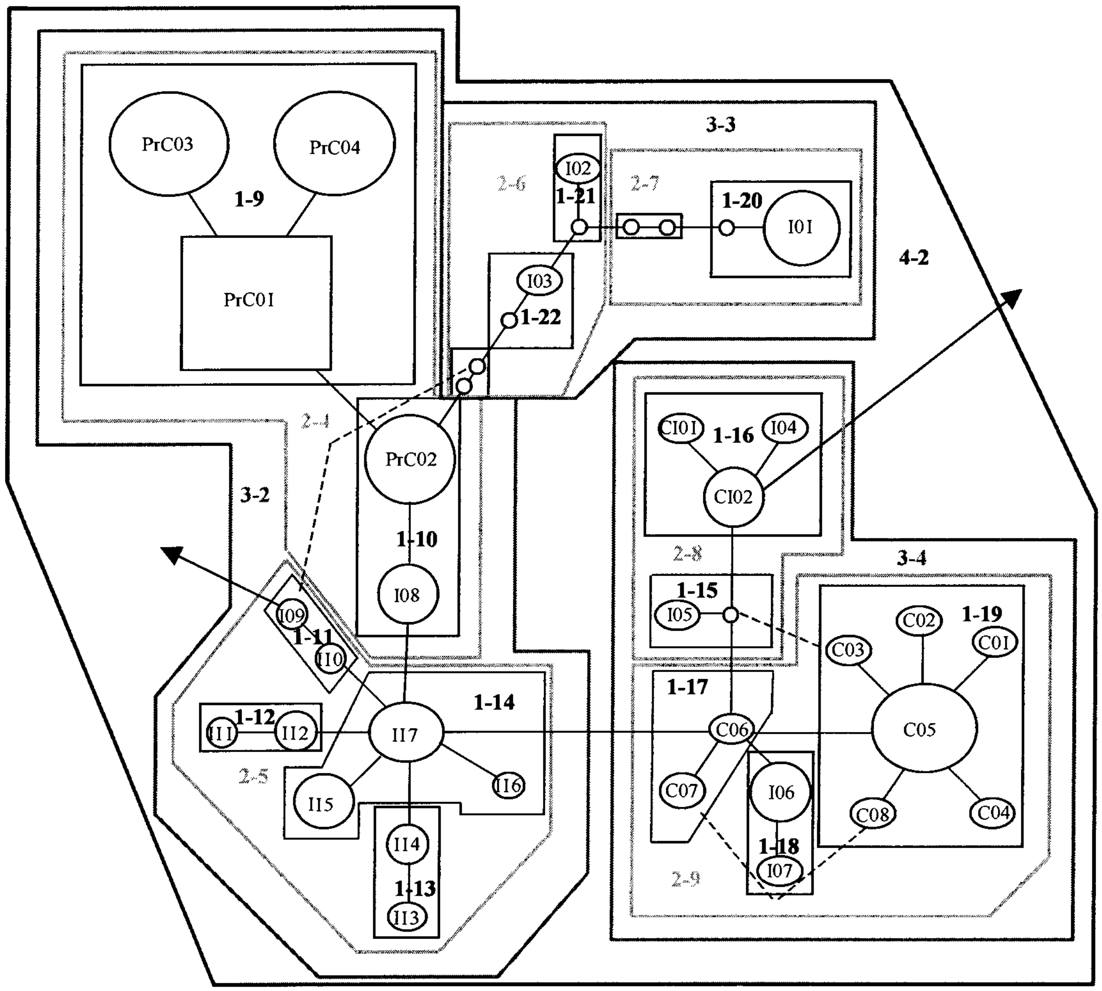


Figure 9. Haplotype network 2 estimated in TCS with 1, 2, 3, and 4 step nesting groups shown. Schematics are the same as in Figure 7. Network consists of haplotypes mostly from *Z. h. preblei*, *Z. h. campestris* and *Z. h. intermedius* individuals.

and 3-1 indicate restricted gene flow with isolation by distance and a contiguous range expansion but these results depend on adequate sampling for *Z. hudsonius* populations in eastern Colorado and Kansas. Although further investigation via more dense sampling in New Mexico, Kansas and eastern Colorado is merited to illuminate the extent of separation between the *Z. h. luteus* populations of New Mexico and various *Z. h. pallidus* populations of Kansas and Nebraska, taken as a single unit (*Z h. pallidus* + *Z. h. luteus*) evidence based on the CR sequence data seems to indicate some separation from the other *Z. hudsonius* populations sampled in these studies. Evidence for this includes, clustering into a single network separated from all other networks by a minimum of 16 mutational steps (Figure 10) and the inference of possible fragmentation within clade 5-1 (Figure 12, Table 5) between clades 4-1 (network 1) and 4-2 (network 2).

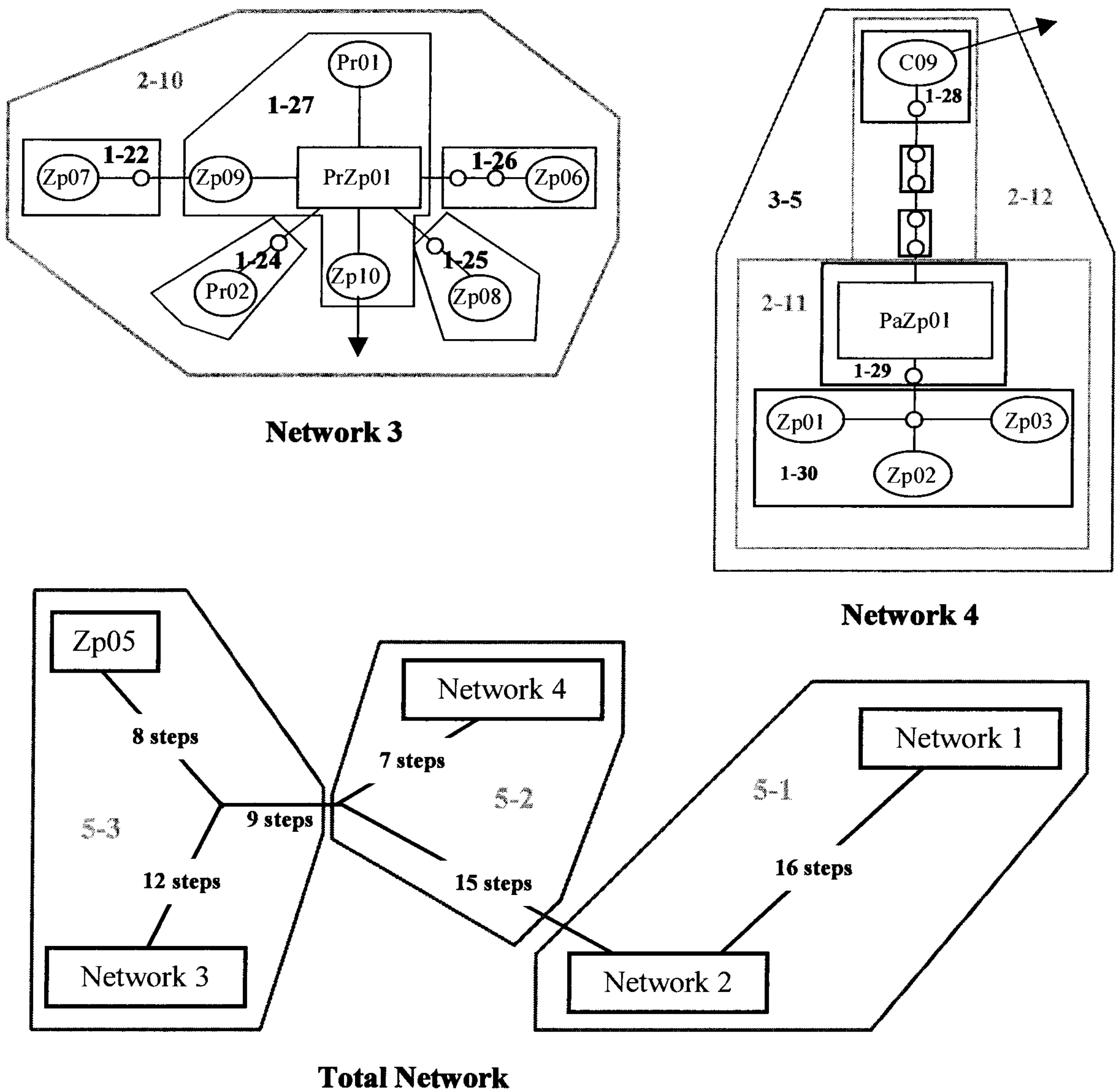


Figure 10. Haplotype networks 3, 4 and the total network. The total network represent connections above the 95% parsimony cut-off. Schematics are the same as in Figure 7. Networks 3 and 4 consist of haplotypes mostly from *Z. princeps* individuals but contain some individuals from *Z. hudsonius* subspecies indicating possibly low levels of gene flow.

Network 2 (Figure 9) consists of haplotypes primarily derived from *Z. h. preblei*, *Z. h. campestris*, and *Z. h. intermedius* individuals. Like network 1 haplotypes from different subspecies show some clustering but no subspecies form exclusive groups. Further, all the *Z. h. preblei* haplotypes found in this network are shared with *Z. h. campestris* haplotypes (although in every case the group is dominated by *Z. h. preblei* samples). Clades 1-9 (Figure 11) and 3-2 (figure 12) illustrate the geographical connection between these haplotypes. At no clade level do the *Z. h. preblei* haplotypes separate out. Taking frequency differences into account the NCA inferences for these clades indicate that contiguous range expansion is the best-supported conclusion in both cases (Table 9). At the deeper clade level (Clade 4-2) NCA indicates that restricted gene flow occurs within the *preblei/campestris/intermedius* cluster but is best explained by

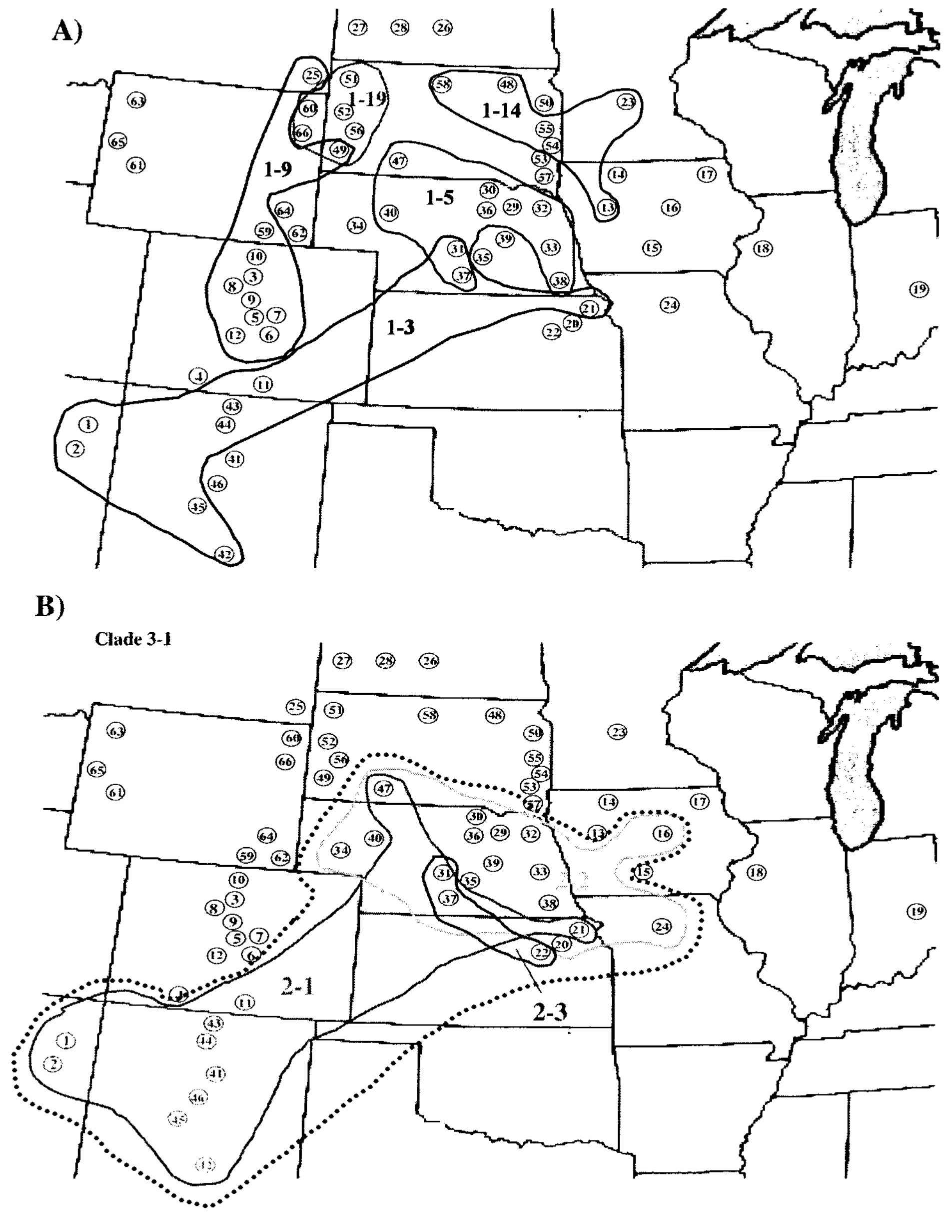


Figure 11. Geographic spread of selected clades used in NCA. Population numbers correspond to those in Table 1.

long distance dispersal for the populations 15, 18, and 19 (Table 1 and Figure 12) and isolation by distance for the vast majority of all other *preblei, campestris,* and *intermedius* populations.

Networks 3 and 4 and haplotype Zp05 were comprised primarily of *Z. princeps* individuals with the exception of a few *Z. hudsonius* individuals. These divergent *Z. hudsonius* samples were discarded by Ramey et al. 2005 under the assumption that they were misclassified. This may very well be the case but as mentioned above the possible introgression of *Z. princeps* haplotypes into *Z. hudsonius* populations should be

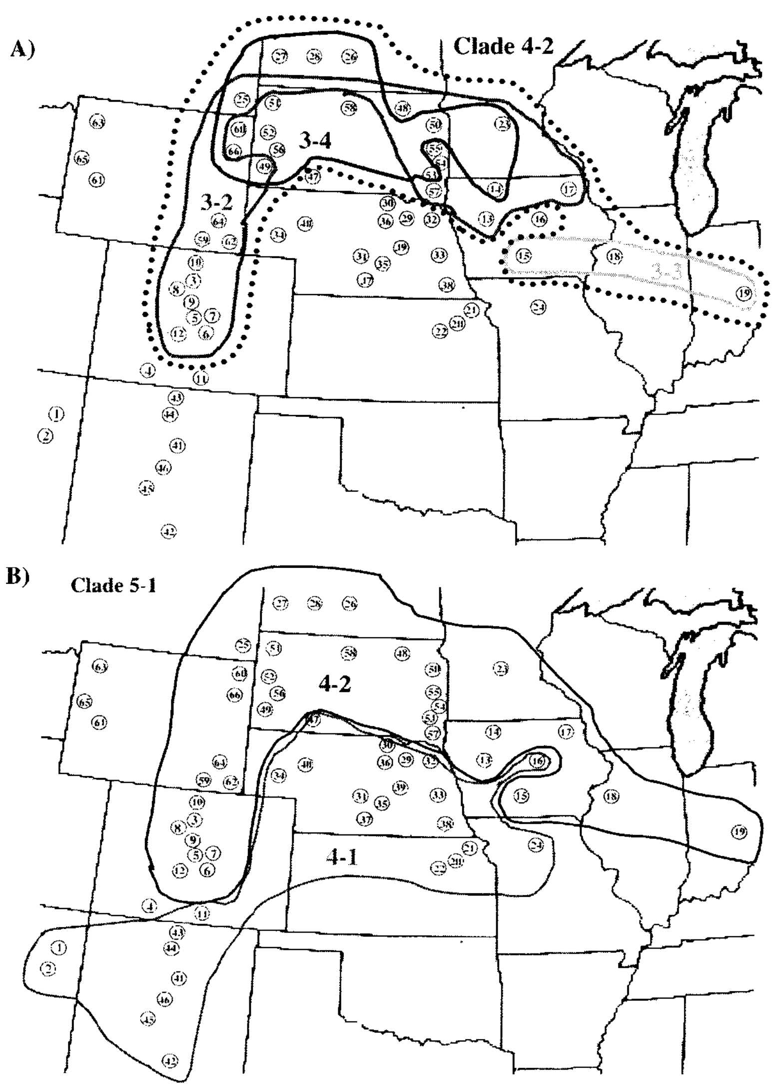


Figure 12. Geographic spread of selected clades used in NCA. Population numbers correspond to those in Table 1.

considered further. Few *Z. princeps* were sampled and thus few conclusions can be drawn from NCA aside from inadequate geographic sampling in Clade 5-1 (Table 9).

V. Subspecies designation

Much debate has centered around diagnosing units below the species level (Green 2005). At this time no established universally accepted criteria exist for diagnosing subspecies. Other units such as populations (Waples and Gaggiotti 2006) and evolutionary significant units have received more attention but still a number of competing criteria are used (Moritz 2002, Waples 2005). Crandall et al. 2000 established a methodology for rejecting or accepting evidence of distinctiveness based on genetic, ecological, recent, and historical categories. Crandall et al. established recommended management actions based on the relative strength of evidence for 8 separate cases. A criticism of the King et al. 2006 study is that no criteria are offered. Ramey et al. 2005 used criteria from Crandall et al. 2000 to diagnose Distinct Vertebrate Population Segments (DPS) and concluded that 'our results for *Z. h. preblei* and its neighboring populations [*Z. h. campestris* and *Z. h. intermedius*] do not appear to support the discrete requirement'. Cases such as that of the *Z. h. preblei* illustrate the need for explicit criteria to be established at the species, subspecies, and DPS level. The 'fuzziness' of boundaries at each of these levels makes this a challenge but eclectic approaches using ecologists, taxonomists, population geneticists, and phylogeneticists make it attainable.

VI. Conclusion

A number of general conclusions can be drawn from our reanalysis of the Ramey et al . 2005 and King et al. 2006 data sets. First, the highest resolution analysis performed in this study was the STRUCTURE analysis using microsatellite data. With both the King et al. and the Ramey et al. data sets, we saw clusters of subspecies into three groups consisting of *Z. h. pallidus* + *Z h. luteus, Z. h. intermedius* + *Z. h. campestris,* and *Z. h. preblei,* best accounted for the genetic variation. Although some admixture was evident in the STRUCTURE analysis and moderate levels of migration rates were estimated between these clusters, we still believe relatively good population boundaries exist between these three groups.

Analysis of the mtDNA data revealed two more inclusive groupings. Phylogenetic analysis of the CytB and CR data sets fairly consistently revealed two major clades. One consisted of a combined *Z. h. intermedius* + *Z. h. campestris* + *Z. h. preblei* group and the other a combined *Z. h. pallidus* + *Z h. luteus* group. In most analysis these groups were monophyletic or nearly so but almost without exception none of the subspecies ever formed monophyletic groups within these distinct clades. The same 'non-exclusive' pattern was evident in the haplotype networks. Add to this the NCA results where range expansion or restricted gene flow with isolation by distance were inferred for most clades at most level and preponderance of evidence seems to indicate some but negligible levels of divergence between subspecies within the groups. The most parsimonious conclusion based on the current available data suggests that *Z. h. pallidus* + *Z h. luteus* may represent a distinct 'subspecies' and *Z. h. intermedius* + *Z. h. campestris* + *Z. h. preblei* form another.

However, as mentioned several times in the body of this report, in this instance, solid conclusions can only be drawn from a study sampling many individuals from many localities. We believe the species, subspecies, and DPS boundaries within the *Zapus* genus will remain problematic until a study is conducted that samples extensively (20-30 individuals) from scores of localities within *Z. princeps*, *Z. trinotatus* and each of the 12 *Z. hudsonius* subspecies. Areas of overlap and where population from different species and subspecies are in close proximity should not be avoided, as in the King et al. 2006 study, but rather should be targeted. In this way science can best serve to direct the limited resources available for conservation.

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Table 1. Localities for all samples the King et al. & Ramey et al. studies. Abbr = abbreviations used in combined data set to indicate county and state of sample. Zhl = *Zapus hudsonius luteus*, Zhpr = *Zapus hudsonius preblei*, Zhi = *Zapus hudsonius intermedius*, Zhpa = *Zapus hudsonius pallidus*, Zhc = *Zapus hudsonius campestris*, ZPp = *Zapus princeps princeps*, ZPid = *Zapus princeps idahoensis*, ZPut = *Zapus princeps utahensis*. Samples were pooled by county and GPS coordinates taken from geographical center of county.

	State	County	Abbr	N	Species	GPS coordinates
1	Arizona	Apache	ApAZ	3	Zhl	N 35° 50’ W 109° 32’
2		Navajo	NCAZ	4	Zhl	N 35° 42’ W 110° 37’
3	Colorado	Boulder	BCCO	9	Zhpr	N 40° 07’ W 105° 29’
4		Conejo	CCCO	1	Zhpr	N 37° 15’ W 105° 58’
5		Douglas	DCCO	74	Zhpr	N 39° 18’ W 105° 12’
6		El Paso	ECCO	61	Zhpr	N 39° 04’ W 104° 15’
7		Elbert	EbCO	1	Zhpr	N 39° 16’ W 103° 34’
8		Gilpin	GCCO	1	Zhpr	N 39° 52’ W 105° 40’
9		Jefferson	JCCO	1	Zhpr	N 39° 30’ W 105° 18’
10		Larimer	LCCO	33	Zhpr	N 40° 40’ W 105° 38’
11		Las Animas	LACO	8	Zhl	N 37° 13’ W 103° 23’
12		Teller	TCCO	2	Zhpr/ZPp	N 39° 46’ W 105° 18’
13	Iowa	Buena Vista	BVIA	1	Zhi	N 42° 38’ W 95° 12’
14		Emmet	ECIA	3	Zhi	N 43° 24’ W 94° 50’
15		Marion	MCIA	1	Zhi	N 41° 19’ W 93° 06’
16		Tama	TCIA	1	Zhi	N 42° 04’ W 92° 24’
17		Winneshiek	WCIA	1	Zhi	N 43° 18’ W 91° 47’
18	Illinois	Henry	HCIL	1	Zhi	N 41° 06’ W 90° 12’
19	Indiana	Wayne	WCIN	1	Zhi	N 39° 36’ W 85° 02’
20	Kansas	Douglas	DCKS	2	Zhpa	N 38° 57’ W 95° 23’
21		Leavenworth	LCKS	2	Zhpa	N 39° 20’ W 94° 59’
22		Osage	OCKS	2	Zhpa	N 38° 38’ W 95° 48’
23	Minnesota	Morrison	MCMN	21	Zhi	N 46° 13’ W 94° 34’
24	Missouri	Macon	MAMO	2	Zhpa	N 39° 45’ W 92° 52’
25	Montana	Carter	CCMT	5	Zhc	N 45° 23’ W 104° 42’
26	North Dakota	Burleigh	BCND	6	Zhi	N 47° 14’ W 100° 12’
27		Dunn	DCND	5	Zhi	N 47° 23’ W 102° 52’
28		Mercer	MCND	1	Zhi	N 47° 20’ W 102° 01’
29	Nebraska	Antelope	ACNE	4	Zhpa	N 42° 04’ W 97° 58’
30		Boyd	BONE	1	Zhpa	N 42° 52’ W 98° 42’
31		Buffalo	BCNE	25	Zhpa	N 40° 47’ W 99° 09’
32		Dixon	DCNE	1	Zhpa	N 42° 41’ W 97° 02’
33		Dodge	DGNE	1	Zhpa	N 41° 42’ W 96° 50’
34		Garden	GCNE	2	Zhpa	N 41° 41’ W 102° 20’
35		Hall	HCNE	3	Zhpa	N 40° 55’ W 98° 22’
36		Holt	HONE	2	Zhpa	N 42° 27’ W 98° 39’
37		Kearney	KCNE	11	Zhpa	N 40° 30’ W 98° 57’
38		Lancaster	LCNE	1	Zhpa	N 40° 51’ W 96° 43’

39		**Merrick**	**MCNE**	**1**	**Zhpa**	**N 41^{O} 07' W 97^{O} 60'**
40		**Thomas**	**TCNE**	**1**	**Zhpa**	**N 41^{O} 58' W 100^{O} 33'**
41	**New Mexico**	**Bernalillo**	**BCNM**	**1**	**Zhl**	**N 35^{O} 30' W 106^{O} 46'**
42		**Otero**	**OCNM**	**7**	**Zhl**	**N 32^{O} 48' W 105^{O} 45'**
43		**Rio Arriba**	**RANM**	**2**	**Zhl**	**N 36^{O} 32' W 106^{O} 47'**
44		**Sandoval**	**SCNM**	**26**	**Zhl**	**N 35^{O} 52' W 106^{O} 48'**
45		**Socorro**	**SONM**	**1**	**Zhl**	**N 33^{O} 50' W 107^{O} 11'**
46		**Valencia**	**VCNM**	**1**	**Zhl**	**N 34^{O} 46' W 106^{O} 58'**
47	**South Dakota**	**Bennett**	**BeCSD**	**18**	**Zhpa**	**N 43^{O} 10' W 101^{O} 50'**
48		**Brown**	**BrCSD**	**33**	**Zhi**	**N 45^{O} 37' W 98^{O} 26'**
49		**Custer**	**CCSD**	**29**	**Zhc**	**N 43^{O} 43' W 103^{O} 01'**
50		**Deuel**	**DCSD**	**3**	**Zhi**	**N 44^{O} 40' W 96^{O} 49'**
51		**Harding**	**HCSD**	**3**	**Zhc**	**N 45^{O} 27' W 103^{O} 33'**
52		**Lawrence**	**LaSD**	**3**	**Zhc**	**N 44^{O} 29' W 103^{O} 44'**
53		**Lincoln**	**LCSD**	**2**	**Zhi**	**N 43^{O} 15' W 96^{O} 48'**
54		**Minnehaha**	**MCSD**	**3**	**Zhi**	**N 43^{O} 42' W 96^{O} 44'**
55		**Moody**	**MOSD**	**1**	**Zhi**	**N 43^{O} 50' W 96^{O} 40'**
56		**Pennington**	**PCSD**	**9**	**Zhc**	**N 44^{O} 13' W 102^{O} 30'**
57		**Union**	**UCSD**	**1**	**Zhi**	**N 42^{O} 59' W 96^{O} 42'**
58		**Walworth**	**WCSD**	**5**	**Zhi**	**N 45^{O} 22' W 100^{O} 03'**
59	**Wyoming**	**Albany**	**AbWY**	**16**	**Zhp/ZPp**	**N 41^{O} 18' W 105^{O} 32'**
60		**Crook**	**CCWY**	**33**	**Zhc**	**N 44^{O} 38' W 104^{O} 46'**
61		**Fremont**	**FCWY**	**3**	**ZPid**	**N 43^{O} 04' W 108^{O} 14'**
62		**Larimae**	**LCWY**	**2**	**Zhpr**	**N 41^{O} 09' W 104^{O} 33'**
63		**Park**	**PaWY**	**3**	**Zpid**	**N 44^{O} 31' W 109^{O} 25'**
64		**Platte**	**PCWY**	**1**	**Zhpr**	**N 41^{O} 58' W 104^{O} 46'**
65		**Teton**	**TCWY**	**4**	**ZPut**	**N 43^{O} 59' W 110^{O} 12'**
66		**Weston**	**WCWY**	**4**	**Zhc**	**N 43^{O} 52' W 104^{O} 35'**

Table 2- A summary of likelihoods scores for each STRUCTURE run for Ramey et al. data, average likelihood scores for each K for all runs, and a visual representation of the absolute values of these scores.

	K									
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Run 01	-5366.8	-5057.1	-4802.5	-4695.5	-4590.1	-4582	-4508.1	-4471.6	-4420.6	-4725.8
	-5364.7	-5049.4	-4799.6	-4694.9	-4585	-4530.1	-4517.5	-4507.9	-4463	-4402.4
	-5365.7	-5055.5	-4805.7	-4697.2	-4593.3	-4596.5	-4502.8	-4460.6	-4425	-4409.8
	-5365.9	-5065.1	-4794.3	-4726.5	-4815.9	-4533.3	-4505.8	-4520	-4421.9	-4411.1
	-5360.4	-5075.1	-4801.5	-4717.4	-4586.6	-4527.1	-4509.3	-4461.3	-4413.6	-4452.7
	-5361.2	-5077.3	-4803.9	-4814.8	-4672	-4603.4	-4504	-4445.3	-4421.6	-4434.7
	-5367.2	-5054.1	-4795.4	-4703	-4585	-4802.7	-4508	-4467.9	-4403.9	-4412.6
	-5364.6	-5085.8	-4801.4	-4759.2	-4582.6	-4571.2	-4516.7	-4468.3	-4417.4	-4415.1
	-5365.4	-5058.8	-4801	-4707	-4585.5	-4523.2	-4515.8	-4479.8	-4425.5	-4411
Run 10	-5359.7	-5084.7	-4805.5	-4702.4	-4582.5	-4576.3	-4504.3	-4449.3	-4409.2	-4428.9
Aver. -Ln	5364.16	-5066.29	-4801.08	-4721.79	-4617.85	-4584.58	-4509.23	-4473.2	-4422.17	-4450.41

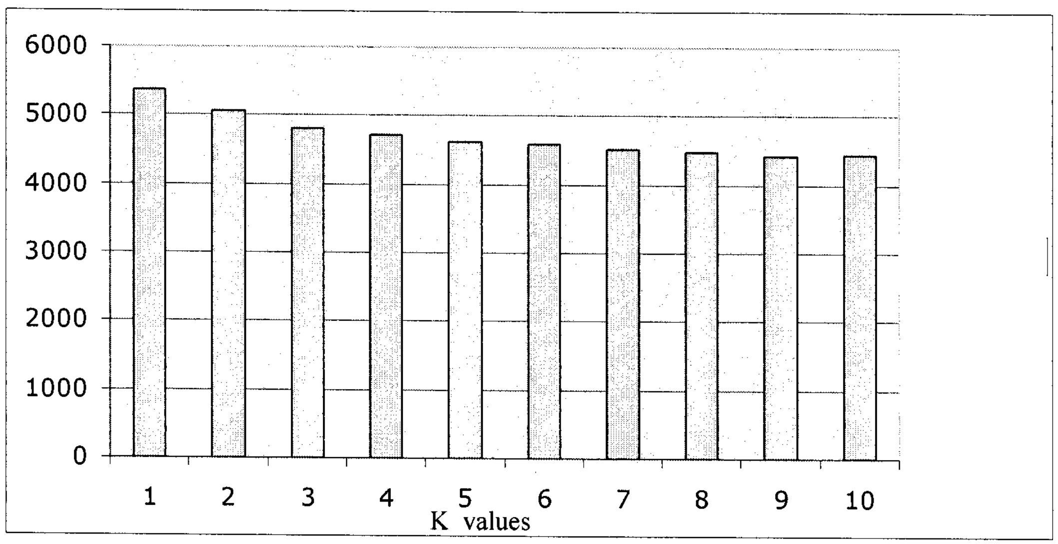

Table 3. Inferred ancestry of all individuals based on the run with the best likelihood score (-4794.3, run 4, Table 2) at K = 3. Probabilities in bold text indicate cluster with highest assignment. Individuals in bold red text indicate individuals with mixed ancestry (no probability > 0.80) and individuals that belong to one subspecies but have highest probability ancestry assigned to a cluster with predominately individuals from a different subspecies are indicated in green. Importantly, individuals indicated in blue text are ones that are *Z.h. campestris* who have a high probability assignment to cluster 3 but have *Z.h. preblei* control region mitochondrial DNA. Cluster 1 consists of predominately *Z.h. preblei* individuals, Cluster 2 consists of predominately *Z.h. luteus* and *Z.h. pallidus* individuals, and, Cluster 3 consists of predominately *Z.h. campestris* and *Z.h. intermedius* individuals.

Inferred ancestry of individuals:

Label	(%Miss)	: Inferred clusters		
		: Clstr 1	Clstr 2	Clstr 3
1 ZhprNM871	(0)	: **0.983**	0.010	0.008
2 M872Zhpr	(0)	: **0.860**	0.035	0.105
3 M876Zhpr	(0)	: **0.970**	0.013	0.018
4 **M877Zhpr**	(0)	: **0.763**	**0.107**	**0.130**
5 TK86021Zhpr	(0)	: **0.840**	0.039	0.121
6 TK86034Zhpr	(0)	: **0.899**	0.026	0.076
7 **TK86048Zhpr**	(0)	: **0.182**	**0.792**	0.027
8 TK86090Zhpr	(0)	: **0.873**	0.018	0.108
9 TK86105Zhpr	(0)	: **0.973**	0.013	0.014
10 **TK86074Zhpr**	(0)	: **0.477**	**0.476**	0.047
11 TK86094Zhpr	(0)	: **0.895**	0.092	0.013
12 9A34Zhpr	(0)	: **0.977**	0.011	0.012
13 9B89Zhpr	(0)	: **0.948**	0.024	0.028
14 M874Zhpr	(0)	: **0.976**	0.010	0.014
15 TK86081Zhpr	(0)	: **0.980**	0.010	0.011
16 TK86109Zhpr	(0)	: **0.962**	0.024	0.014
17 TK86117Zhpr	(0)	: **0.942**	0.023	0.036
18 TK86095Zhpr	(0)	: **0.952**	0.019	0.029
19 TK86096Zhpr	(0)	: **0.970**	0.014	0.016
20 TK86097Zhpr	(0)	: **0.965**	0.028	0.007
21 TK86098Zhpr	(0)	: **0.973**	0.010	0.017
22 TK86026Zhpr	(0)	: **0.986**	0.006	0.008
23 TK86029Zhpr	(0)	: **0.959**	0.030	0.011
24 TK86030Zhpr	(0)	: **0.918**	0.072	0.009
25 TK86031Zhpr	(0)	: **0.979**	0.009	0.012
26 TK86032Zhpr	(0)	: **0.958**	0.020	0.022
27 TK86080Zhpr	(0)	: **0.971**	0.007	0.022
28 TK86083Zhpr	(0)	: **0.978**	0.014	0.008
29 TK86115Zhpr	(0)	: **0.977**	0.010	0.013
30 TK86116Zhpr	(0)	: **0.977**	0.013	0.011
31 TK86120Zhpr	(0)	: **0.981**	0.008	0.011

32	TK86121Zhpr	(0)	:	**0.983**	0.008	0.009	
33	TK86122Zhpr	(0)	:	**0.986**	0.006	0.008	
34	TK86196Zhpr	(0)	:	**0.946**	0.038	0.016	
35	TK86163Zhpr	(0)	:	**0.977**	0.009	0.014	
36	M875Zhpr	(0)	:	**0.987**	0.007	0.006	
37	TK51406Zhpr	(0)	:	**0.893**	0.016	0.090	
38	TK86124Zhpr	(0)	:	**0.980**	0.012	0.008	
39	TK86088Zhpr	(0)	:	**0.944**	0.045	0.011	
40	M879Zhpr	(0)	:	**0.988**	0.006	0.006	
41	M1166Zhpr	(0)	:	**0.951**	0.008	0.041	
42	TK86093Zhpr	(0)	:	**0.978**	0.009	0.013	
43	TK86106Zhpr	(0)	:	**0.985**	0.009	0.006	
44	TK86107Zhpr	(0)	:	**0.974**	0.012	0.013	
45	TK86118Zhpr	(0)	:	**0.971**	0.009	0.019	
46	TK86165Zhpr	(0)	:	**0.982**	0.007	0.011	
47	TK86166Zhpr	(0)	:	**0.981**	0.011	0.008	
48	TK86167Zhpr	(0)	:	**0.954**	0.035	0.011	
49	TK86169Zhpr	(0)	:	**0.986**	0.006	0.008	
50	TK86170Zhpr	(0)	:	**0.983**	0.008	0.009	
51	TK86173Zhpr	(0)	:	**0.976**	0.010	0.014	
52	TK86182Zhpr	(0)	:	**0.988**	0.006	0.006	
53	TK86183Zhpr	(0)	:	**0.982**	0.008	0.010	
54	TK86185Zhpr	(0)	:	**0.983**	0.008	0.009	
55	**ZhcTK86190**	(0)	:	0.100	**0.229**	**0.671**	
56	**TK86191Zhc**	(0)	:	**0.755**	0.015	**0.230**	
57	KU123597Zhc	(0)	:	0.028	0.046	**0.926**	
58	KU123598Zhc	(0)	:	0.014	0.009	**0.977**	
59	**KU123599Zhc**	(0)	:	0.095	**0.535**	**0.370**	
60	KU101558Zhc	(0)	:	0.148	0.026	**0.827**	
61	KU109972Zhc	(0)	:	0.023	**0.283**	**0.694**	--- Zhp mtDNA
62	KU109978Zhc	(0)	:	0.053	0.010	**0.937**	--- Zhp mtDNA
63	**KU109984Zhc**	(0)	:	**0.382**	**0.411**	0.207	--- Zhp mtDNA
64	KU109985Zhc	(0)	:	0.014	0.017	**0.969**	--- Zhp mtDNA
65	KU110013Zhc	(0)	:	0.005	0.007	**0.988**	--- Zhp mtDNA
66	**KU7Zhc**	(0)	:	**0.466**	**0.054**	0.480	
67	KU8Zhc	(0)	:	0.026	0.015	**0.959**	
68	KU13Zhc	(0)	:	0.036	0.017	**0.947**	
69	KU14Zhc	(33)	:	0.016	0.011	**0.973**	
70	KU18Zhc	(0)	:	0.008	0.023	**0.969**	
71	KU19Zhc	(0)	:	0.007	0.012	**0.980**	
72	KU20Zhc	(0)	:	0.007	0.098	**0.895**	
73	KU23Zhc	(16)	:	0.013	0.023	**0.964**	
74	**KU24Zhc**	(0)	:	**0.718**	0.068	0.215	
75	**KU25Zhc**	(0)	:	**0.261**	0.028	**0.711**	
76	**KU26Zhc**	(0)	:	**0.225**	0.022	**0.753**	

77 KU1235Zhc	(0)	: 0.007	0.008	**0.985**
78 KU123593Zhc	(0)	: 0.006	0.008	**0.986**
79 KU27Zhc	(16)	: 0.012	0.015	**0.972**
80 KU28Zhc	(16)	: 0.007	0.010	**0.983**
81 KU29Zhc	(0)	: 0.067	0.008	**0.925**
82 **KU30Zhc**	(0)	: **0.228**	0.046	**0.726**
83 KU34Zhc	(0)	: 0.013	0.008	**0.979**
188 **K127252Zhia**	(0)	: 0.008	**0.953**	0.040
189 **K112830Zhi**	(0)	: 0.005	**0.980**	0.014
190 **K116263Zhi**	(0)	: **0.389**	**0.315**	**0.295**
191 K116264Zhi	(0)	: 0.027	0.090	**0.883**
192 **K116266Zhi**	(0)	: 0.034	**0.481**	**0.485**
193 **K116269Zhi**	(0)	: 0.006	**0.958**	0.035
194 **K104062Zhi**	(0)	: 0.165	**0.410**	**0.425**
195 **K108068Zhi**	(0)	: 0.025	**0.728**	**0.247**
84 DMNS7764Zhi	(0)	: 0.013	0.008	**0.978**
85 K115895Zhi	(0)	: 0.008	0.040	**0.952**
86 K115896Zhi	(0)	: 0.007	0.009	**0.984**
87 K115897Zhi	(0)	: 0.010	0.031	**0.960**
88 K123021Zhi	(0)	: 0.013	0.020	**0.967**
89 K123022Zhi	(0)	: 0.014	0.034	**0.952**
90 K123031Zhi	(0)	: 0.007	0.133	**0.860**
91 K123032Zhi	(0)	: 0.023	0.013	**0.964**
92 K123033Zhi	(0)	: 0.008	0.015	**0.976**
155 **K140721Zhi**	(0)	: **0.521**	0.132	**0.347**
156 **K140722Zhi**	(0)	: **0.199**	**0.385**	**0.416**
157 **K153176Zhi**	(0)	: 0.010	**0.202**	**0.788**
158 K153177Zhi	(0)	: 0.017	0.030	**0.953**
159 K153180Zhi	(0)	: 0.039	0.014	**0.947**
160 K153181Zhi	(0)	: 0.040	0.043	**0.917**
161 **K153190Zhi**	(0)	: **0.156**	0.059	**0.785**
162 K153196Zhi	(0)	: 0.013	0.028	**0.960**
163 K147018Zhi	(0)	: 0.017	0.011	**0.972**
164 K147020Zhi	(0)	: 0.019	0.017	**0.964**
165 K153201Zhi	(0)	: 0.119	0.017	**0.864**
166 K153203Zhi	(8)	: 0.091	0.013	**0.897**
167 **K153205Zhi**	(0)	: 0.044	**0.919**	0.038
168 K153212Zhi	(0)	: 0.021	0.062	**0.918**
169 K115700Zhi	(0)	: 0.013	0.026	**0.962**
170 K115702Zhi	(0)	: 0.010	0.040	**0.950**
171 **K115710Zhi**	(0)	: 0.026	**0.600**	**0.374**
172 **K120017Zhi**	(0)	: 0.042	**0.701**	**0.256**
173 K120018Zhi	(0)	: 0.026	0.012	**0.962**
174 K120019Zhi	(0)	: 0.018	0.050	**0.931**
175 **K153215Zhi**	(0)	: 0.027	**0.197**	**0.775**

176	**K153221Zhi**	(0)	:	0.088	**0.792**	**0.120**
177	K115730Zhi	(0)	:	0.008	0.012	**0.980**
178	K115731Zhi	(0)	:	0.006	0.052	**0.942**
179	K115732Zhi	(0)	:	0.023	0.018	**0.958**
180	K116265Zhi	(0)	:	0.025	0.012	**0.963**
181	K159190Zhi	(0)	:	0.094	0.053	**0.853**
182	K153230Zhi	(0)	:	0.099	0.035	**0.865**
183	K153229Zhi	(0)	:	0.016	0.007	**0.977**
93	**ZhpaUNL1UNS**	(0)	:	0.054	**0.655**	**0.292**
94	ZhpaUNL2UNS	(0)	:	0.015	**0.916**	0.069
95	ZhpaUNL3UNS	(0)	:	0.017	**0.957**	0.026
96	**ZhpaUNL4UNS**	(0)	:	0.010	**0.372**	**0.617**
97	**ZhpaUNL5UNS**	(0)	:	0.107	**0.652**	**0.241**
98	ZhpaUNL7UNS	(0)	:	0.016	**0.971**	0.013
99	ZhpaUNL8UNS	(0)	:	0.010	**0.946**	0.044
100	ZhpaUNL9UNS	(0)	:	0.031	**0.817**	0.152
101	ZhpaUNL12UN	(0)	:	0.068	**0.865**	0.067
102	ZhpaUNL16UN	(0)	:	0.015	**0.945**	0.040
103	**ZhpaUNL23UN**	(0)	:	0.015	**0.355**	**0.630**
104	ZhpaUNL26UN	(0)	:	0.019	**0.892**	0.090
105	ZhpaUNL27UN	(0)	:	0.077	**0.913**	0.010
106	ZhpaUNL28UN	(0)	:	0.022	**0.953**	0.025
107	ZhpaUNL35UN	(0)	:	0.006	**0.979**	0.015
108	ZhpaUNL36UN	(0)	:	0.030	**0.860**	0.111
109	**ZhpaUNL37UN**	(0)	:	0.009	**0.738**	**0.253**
110	ZhpaUNL41UN	(0)	:	0.013	**0.962**	0.025
111	ZhpaUNL42UN	(0)	:	0.023	**0.952**	0.025
112	ZhpaUNL46UN	(0)	:	0.023	**0.953**	0.024
113	ZhpaUNL51UN	(0)	:	0.010	**0.961**	0.029
114	ZhpaUNL55UN	(0)	:	0.035	**0.896**	0.070
115	ZhpaUNL56UN	(0)	:	0.023	**0.970**	0.007
116	KU40Zhpa	(0)	:	0.155	**0.801**	0.044
117	KU44Zhpa	(0)	:	0.028	**0.961**	0.011
118	KU45Zhpa	(0)	:	0.080	**0.867**	0.053
119	KU47Zhpa	(0)	:	0.008	**0.970**	0.023
120	**KU48Zhpa**	(0)	:	0.031	**0.716**	**0.252**
121	KU51Zhpa	(0)	:	0.022	**0.964**	0.014
122	KU52Zhpa	(0)	:	0.014	**0.976**	0.011
184	**UNL60Zhpa**	(0)	:	0.087	0.018	**0.896**
185	**UNL61Zhpa**	(0)	:	0.126	0.040	**0.834**
186	KU53Zhpa	(0)	:	0.018	**0.975**	0.007
187	KU54Zhpa	(0)	:	0.010	**0.982**	0.008
123	DMNH8630Zhl	(0)	:	0.009	**0.978**	0.013
124	DMNH8631Zhl	(0)	:	0.016	**0.964**	0.019

125	DMNH8632Zhl	(0)	:	0.010	**0.982**	0.008
126	DMNH8633Zhl	(0)	:	0.008	**0.978**	0.014
127	DMNH8634Zhl	(0)	:	0.009	**0.975**	0.016
128	DMNH8635Zhl	(0)	:	0.008	**0.985**	0.007
129	NK856Zhl	(0)	:	0.011	**0.979**	0.010
130	NK871Zhl	(0)	:	0.008	**0.986**	0.007
131	NK884Zhl	(0)	:	0.034	**0.942**	0.023
132	NK1584Zhl	(0)	:	0.019	**0.951**	0.031
133	NK9976Zhl	(0)	:	0.014	**0.977**	0.009
134	MSB2Zhl	(0)	:	0.021	**0.960**	0.019
135	MSB4Zhl	(0)	:	0.019	**0.964**	0.017
136	MSB5Zhl	(0)	:	0.056	**0.923**	0.021
137	MSB6Zhl	(0)	:	0.014	**0.954**	0.032
138	MSB7Zhl	(0)	:	0.091	**0.882**	0.026
139	MSB8Zhl	(0)	:	0.038	**0.947**	0.015
140	MSB9Zhl	(0)	:	0.022	**0.969**	0.009
141	MSB11Zhl	(0)	:	0.006	**0.985**	0.009
142	MSB12Zhl	(0)	:	0.028	**0.963**	0.009
143	MSB14Zhl	(0)	:	0.010	**0.984**	0.006
144	MSB16Zhl	(0)	:	0.009	**0.953**	0.038
145	MSB18Zhl	(0)	:	0.012	**0.974**	0.014
146	MSB19Zhl	(0)	:	0.022	**0.858**	0.121
147	MSB20Zhl	(0)	:	0.072	**0.914**	0.013
148	MSB21Zhl	(0)	:	0.008	**0.978**	0.014
149	MSB23Zhl	(0)	:	0.013	**0.977**	0.011
150	MSB24Zhl	(0)	:	0.014	**0.975**	0.011
151	MSB25Zhl	(0)	:	0.009	**0.854**	0.137
152	MSB26Zhl	(0)	:	0.011	**0.859**	0.130
153	MSB27Zhl	(0)	:	0.026	**0.959**	0.015
154	MSB30Zhl	(0)	:	0.008	**0.982**	0.009

Table 4. Summary of results for MIGRATE analysis of Ramey et al. microsatellite data between three hypothesized populations based on three separate runs. A) Theta (Θ) is equal to the estimated effective population size and Mxy is equal to the relative importance of migration from cluster on 'x' axis into cluster on 'y' axis relative to mutation rate in introducing new variants into the population. B) Nm estimates based on Migrate results run 2.

A)

Runs	**Clusters**	Θ		**Mxy(m/μ)**		**Chains**
			Zhpr	**Zhc/Zhi**	**Zhpa/Zhl**	
Run 1	**Zhpr**	1.49165	-------	1.4871	1.1340	**Short = 10**
	Zhc/Zhi	4.75791	1.9401	-------	4.1631	**Long = 3**
	Zhpa/Zhl	4.04230	1.3132	5.1773	-------	
Run 2	**Zhpr**	1.86589	-------	0.9990	1.0143	**Short = 10**
	Zhc/Zhi	3.73727	2.2950	-------	4.4632	**Long = 3**
	Zhpa/Zhl	4.60656	1.0230	4.9991	-------	
Run 3	**Zhpr**	1.14171	-------	1.5501	1.4162	**Short = 10**
	Zhc/Zhi	3.80171	1.7774	-------	5.3583	**Long = 3**
	Zhpa/Zhl	6.90139	0.6874	3.2829	-------	

B)

		Nm(xy)	
	Zhpr	**Zhc/Zhi**	**Zhpa/Zhl**
Zhpr	---	**0.46**	**0.47**
Zhc/Zhi	**2.14**	---	**4.17**
Zhpa/Zhl	**1.18**	**5.76**	---

Number of migrants from 'x' axis cluster into 'y' axis cluster

Table 5. A list of collapsed (identical) control region mitochondrial DNA haplotypes based on a combined data set from Ramey et al. 2005 and King et al. 2006. A total of 63 different haplotypes were found. Haplotypes are named to represent subspecies where haplotype is found. Pr= *Z. h. preblei*, C = *Z. h. campestris,* Pa = *Z. h. pallidus*, I = *Z. h. intermedius*, L = *Z. h. luteus*, and Zp = *Z. princeps*.

List of haplotype names:

1. PrC01
DCCOZhprTK86026 [60]
AbWYZhprTK86098
AbWYZhprTK86124
BCCOZhprTK86021
BCCOZhprTK86034
BCCOZhprTK86048
BCCOZhprTK86090
BCCOZhprTK86105
BCCOZhprXM871
BCCOZhprXM872
BCCOZhprXM876
BCCOZhprXM877
DCCOZhprTK86029
DCCOZhprTK86030
DCCOZhprTK86031
DCCOZhprTK86032
DCCOZhprTK86080
DCCOZhprTK86083
DCCOZhprTK86115
DCCOZhprTK86116
EbCOZhprTK86163
GCCOZhprXM874
JCCOZhprTK51406
LCCOZhprTK86109
ZHprDCCOMAY215
ZHprDCCOMAY229
ZHprDCCOMAY234
ZHprDCCOMAY268
ZHprDCCOMAY281
ZHprDCCOMAY374
ZHprDCCOMAY385
ZHprDCCOMAY408
ZHprDCCOMAY416
ZHprDCCOMAY452
ZHprDCCOMAY494
ZHprDCCOMAY497
ZHprDCCOMAY517
ZHprDCCOMAY532
ZHprDCCOMAY694

ZHprDCCOMAY714
ZHprDCCOMAY748
ZHprDCCOMAY798
ZHprDCCOMAY817
ZHprDCCOMAY822
ZHprDCCOMAY880
ZHprDCCOMAY946
ZHprDCCOMAY964
ZHprDCCOMAY9813
ZHprDCCOMAY9814
ZHprDCCOMAY940
ZHprDCCOWH98100
ZHprDCCOWH98107
ZHprDCCOWH98109
ZHprDCCOWH98110
ZHprDCCOWH98301
ZHprLCCOSP169
ZHprLCCOSP223
ZHprLCCOSP861
ZHprLCCOYG9803
CCSDZhcaK110013

2. PrC02
LCCOZhpr9A43 [35]
LCCOZhpr9B89
LCCOZhprTK86081
LCCOZhprTK86117
LCWYZhprTK86074
PCWYZhprTK86094
ZHprLCCOBG9801
ZHprLCCOBG9802
ZHprLCCOCER9801
ZHprLCCOCER9802
ZHprLCCOCER9803
ZHprLCCOCER9804
ZHprLCCOCER980
ZHprLCCOHRK981
ZHprLCCOHRK982
ZHprLCCOHRK984
ZHprLCCOMC9801
ZHprLCCOMC9803
ZHprLCCONFP9801
ZHprLCCONFP9802
ZHprLCCOPGC9801
ZHprLCCOSP125
ZHprLCCOSP170
ZHprLCCOSP243
ZHprLCCOSP336
ZHprLCCOSP367
ZHprLCCOSP375
ZHprLCCOSP674

ZHprLCCOSP746
ZHprLCCOYG9801
AbWYZhprTK86095
AbWYZhprTK86096
AbWYZhprTK86097
CCSDZhcaK109984
CCSDZhcaK109985

3. PrC03
DCCOZhprTK86120 [58]
DCCOZhprTK86121
DCCOZhprTK86122
ECCOZhprTK86093
ECCOZhprTK86106
ECCOZhprTK86107
ECCOZhprTK86118
ECCOZhprTK86166
ECCOZhprTK86167
ECCOZhprXM1166
ECCOZhprXM875
ECCOZhprXM879
ZHprDCCOMAY127
ZHprDCCOMAY254
ZHprDCCOMAY368
ZHprDCCOMAY429
ZHprDCCOMAY706
ZHprDCCOMAY785
ZHprDCCOWH9801
ZHprDCCOWH9802
ZHprDCCOWH9803
ZHprDCCOWH98102
ZHprDCCOWH98103
ZHprDCCOWH9810
ZHprDCCOWH98106
ZHprDCCOWH98108
ZHprDCCOWH98120
ZHprDCCOWH98300
ZHprDCCOWH98303
ZHprDCCOWH98304
ZHprDCCOWH98305
ZHprDCCOWH98306
ZHprDCCOWH98309
ZHprDCCOWH98311
ZHprDCCOWH98312
ZHprDCCOWH98313
ZHprECCO003
ZHprECCO004
ZHprECCO005
ZHprECCO011
ZHprECCO015
ZHprECCO016

ZHprECCO020
ZHprECCO021
ZHprECCO027
ZHprECCO080
ZHprECCO087
ZHprECCO088
ZHprECCO091
ZHprECCO092
ZHprECCO093
ZHprECCO095
ZHprECCO100
ZHprECCO102
ZHprECCO103
ZHprECCO104
CCMTZhcaK123592
CCSDZhcaK109978

4. PrC04
DCCOZhprTK86196 [39]
ECCOZhprTK86165
ECCOZhprTK86169
ECCOZhprTK8617
ECCOZhprTK8613
ECCOZhprTK86182
ECCOZhprTK86183
ECCOZhprTK86185
TCCOZhprTK86088
ZHprDCCOWH9805
ZHprDCCOWH9811
ZHprDCCOWH98104
ZHprDCCOWH98121
ZHprECCO00
ZHprECCO007
ZHprECCO008
ZHprECCO010
ZHprECCO013
ZHprECCO018
ZHprECCO019
ZHprECCO024
ZHprECCO025
ZHprECCO026
ZHprECCO079
ZHprECCO081
ZHprECCO082
ZHprECCO083
ZHprECCO084
ZHprECCO085
ZHprECCO086
ZHprECCO089
ZHprECCO090
ZHprECCO094

ZHprECCO096
ZHprECCO097
ZHprECCO098
ZHprECCO099
ZHprECCO101
CCSDZhcaK109972

5. PrZp01
AbWYZhprTK86070 [10]
AbWYZhprTK86123
DCCOZPPTK86086
DCCOZPPTK8608
TCCOZPPTK8605
ZpAbWY001
ZpAbWY004
ZpAbWY005
ZpAbWY006
ZpAbWY007

6. Pr01
AbWYZhprTK86202 [1]

7. Pr02
AbWYZhprTK86113 [1]

8. Pa01
BCNEZhpaUNL9 [10]
OCKSZhpaKU47
OCKSZhpaKU48
ZhpaBCNE030
ZhpaBCNE032
ZhpaBCNE040
ZhpaBCNE047
ZhpaKCNE021
ZhpaKCNE024
ZhpaKCNE025

9. Pa02
DCKSZhpaKU40 [4]
LCKSZhpaKU44
MAMOZhpaKU5
MAMOZhpaKU52

10. Pa03
BONEZhpaUNL7 [1]

11. Pa 04
HONEZhpaUNL42 [4]
TCNEZhpaUNL55
ZhpaBeCSD008
ZhpaBeCSD014

12. Pa05
ACNEZhpaUNL2 [18]
ACNEZhpaUNL3
ACNEZhpaUNL4
ACNEZhpaUNL5
DCNEZhpaUNL23
DGNEZhpaUNL26
HONEZhpaUNL41
LCNEZhpaUNL46
ZhpaBCNE031
ZhpaBCNE036
ZhpaBCNE039
ZhpaBCNE043
ZhpaBCNE046
ZhpaKCNE019
ZhpaKCNE026
ZhpaKCNE027
ZhpaKCNE028
ZhpaBCNE048

13. L01
NCAZZhluMSB6 [1]

14. L02
ApAZZhluMSB4 [2]
ApAZZhluMSB40951

15. L03
LACOZhluDMNH8631 [1]

16. L04
LACOZhluDMNH8632 [2]
LACOZhluDMNH8634

17. L05
BCNEZhpaUNL1 [2]
BCNEZhpaUNL12

18. PaI01
BCNEZhpaUNL16 [32]
GCNEZhpaUNL27
GCNEZhpaUNL28
HCNEZhpaUNL35
HCNEZhpaUNL36
HCNEZhpaUNL37
MCNEZhpaUNL51
TCIOZhinKU116269
ZhpaBCNE029
ZhpaBCNE033
ZhpaBCNE034
ZhpaBCNE038
ZhpaBCNE041
ZhpaBCNE042
ZhpaBeCSD004
ZhpaBeCSD005
ZhpaBeCSD006
ZhpaBeCSD007
ZhpaBeCSD009
ZhpaBeCSD010
ZhpaBeCSD011
ZhpaBeCSD012
ZhpaBeCSD013
ZhpaBeCSD015
ZhpaBeCSD016
ZhpaBeCSD017
ZhpaKCNE018
ZhpaKCNE020
ZhpaKCNE023
ZhpaBCNE049
ZhpaBeCSD002
ZhpaBeCSD003

19. L06
LACOZhluDMNH8630 [10]
OCNMMSB9
OCNMZhluMSB61684
OCNMZhluMSB61690
OCNMZhluMSB61693
OCNMZhluMSB61696
OCNMZhluMSB61712
OCNMZhluNK871
RANMZhluMSB58369
SONMZhluNK884

20. L07
BCNMZhluNK9976 [2]

VCNMZhluMSB30

21. Pa06
BeSDZhpaKU54 [1]

22. PaL01
BeCSDZhpaKU53 [26]
RANMZhluMSB58370
SCNMZhluMSB23
SCNMZhluMSB24
SCNMZhluMSB25
SCNMZhluMSB26
SCNMZhluMSB27
SCNMZhluMSB56980
SCNMZhluNK856
ZhlSCNMMSB3826
ZhlSCNMMSB3827
ZhlSCNMMSB3828
ZhlSCNMMSB382
ZhlSCNMMSB3831
ZhlSCNMMSB3832
ZhlSCNMMSB3833
ZhlSCNMMSB3834
ZhlSCNMMSB3835
ZhlSCNMMSB3836
ZhlSCNMMSB3838
ZhlSCNMMSB3840
ZhlSCNMMSB3841
ZhlSCNMMSB3842
ZhlSCNMMSB3843
ZhlSCNMMSB3844
ZhlSCNMMSB3845

23. L08
ZhlSCNMMSB3837 [2]
ZhlSCNMMSB3839

24. Pa07
BCNEZhpaUNL8 [5]
ZhpaBCNE035
ZhpaBCNE044
ZhpaBCNE045
ZhpaKCNE022

25. PaL02
ApAZZhluMSB5 [7]
ApAZZhluNK1584

LACOZhluDMNH8633
LACOZhluDMNH8635
LCKSZhpaKU45
NCAZZhluMSB7
NCAZZhluMSB8

26. I01
MCIAZhinKU108068 [1]

27. I02
HCILZhinKU127252 [1]

28. I03
WCINZhinKU112830 [1]

29. I04
DCNDZhinKU123033 [1]

30. CI01
LaSDZhcaKU112663 [2]
WCSDZhiKU115730

31. CI02
BCNDZhinKU115700 [28]
BCNDZhinKU115702
BCNDZhinKU115710
BCNDZhinKU120018
BCNDZhinKU120019
CCMTZhcaK123593
CCMTZhcaK123598
CCMTZhcaK123599
DCNDZhinKU123021
DCNDZhinKU123022
DCNDZhinKU123031
DCNDZhinKU123032
MCNDZhinDMNS7764
PCSDZhcaK101558
PCSDZhcaKU101564
WCSDZhiKU115731
WCSDZhiKU115732
WCSDZhinKU159190
WCWYZhcaTK86190
WCWYZhcaTK86191
ZhcCCSD061
ZhcCCSD066
ZhcCCSD070
ZhcPCSD079
ZhcPCSD080

ZhcPCSD081
ZhcPCSD082
ZhcPCSD083

32. I05
BCNDZhinKU120017 [1]

33. C01
CCWYZhcaKU20839 [1]

34. C02
CCWYZhcaKU20843 [2]
ZhcCCWY054

35. C03
WCWYZhcaKU42469 [1]

36. C04
ZhcCCWY034 [4]
ZhcCCWY037
ZhcCCWY053
ZhcCCWY088

37. C05
CCWYZhcaKU20844 [50]
HCSDZhcaKU83557
HCSDZhcaKU87040
HCSDZhcaKU87042
LaSDZhcaKU112660
WCWYZhcaKU42471
ZhcCCSD056
ZhcCCSD057
ZhcCCSD058
ZhcCCSD059
ZhcCCSD060
ZhcCCSD062
ZhcCCSD063
ZhcCCSD065
ZhcCCSD067
ZhcCCSD068
ZhcCCSD069
ZhcCCSD072
ZhcCCSD073
ZhcCCSD075
ZhcCCSD076
ZhcCCSD077
ZhcCCSD085
ZhcCCSD086
ZhcCCWY028

ZhcCCWY030
ZhcCCWY031
ZhcCCWY032
ZhcCCWY033
ZhcCCWY035
ZhcCCWY036
ZhcCCWY038
ZhcCCWY039
ZhcCCWY040
ZhcCCWY041
ZhcCCWY042
ZhcCCWY043
ZhcCCWY044
ZhcCCWY045
ZhcCCWY046
ZhcCCWY047
ZhcCCWY048
ZhcCCWY049
ZhcCCWY050
ZhcCCWY051
ZhcCCWY052
ZhcCCWY055
ZhcCCWY087
ZhcCCWY089
ZhcPCSD084

38. I06
DCSDZhinKU147018 [8]
DCSDZhinKU153196
ECIAZhinKU116263
ECIAZhinKU11626
LCSDZhinKU153203
ZhiMCMNMSB41532
ZhiMCMNMSB80783
ZhiMCMNMSB80784

39. I07
DCSDZhinKU153201 [1]

40. C06
ZhcCCSD071 [3]
ZhcCCSD074
ZhcCCSD078

41. C07
PCSDZhcaKU101552 [1]

42. C08
LaSDZhcaKU109970 [1]

43. I08
ECIAZhinKU116264 [4]
UCSDZhinKU153229
WCIAZhinKU104062
ZhiMCMNMSB80770

44. I09
BrCSDZhinKU140722 [1]

45. I10
LCSDZhinKU153205 [2]
MCSDZhinKU153215

46. I11
ZhiMCMNMSB41533 [2]
ZhiMCMNMSB80767

47. I12
ZhiBrCSD003 [5]
ZhiBrCSD010
ZhiBrCSD017
ZhiBrCSD018
ZhiBrCSD032

48. I13
ZhiMCMNMSB80780 [2]
ZhiMCMNMSB80786

49. I14
ZhiMCMNMSB41518 [8]
ZhiMCMNMSB80766
ZhiMCMNMSB80768
ZhiMCMNMSB80771
ZhiMCMNMSB80773
ZhiMCMNMSB80774
ZhiMCMNMSB80779
ZhiMCMNMSB80782

50. I15
BrCSDZhinKU147020 [18]
BrCSDZhinKU153176
BrCSDZhinKU153177
BrCSDZhinKU153180
BrCSDZhinKU153181
ZhiBrCSD005
ZhiBrCSD006
ZhiBrCSD007
ZhiBrCSD009
ZhiBrCSD011

ZhiBrCSD014
ZhiBrCSD016
ZhiBrCSD023
ZhiBrCSD026
ZhiBrCSD027
ZhiBrCSD028
ZhiBrCSD029
ZhiBrCSD030

51. I16
ZhiMCMNMSB80785 [1]

52. I17
BVIAZhinKU116266 [18]
BrCSDZhinKU140721
MCSDZhinKU153209
MCSDZhinKU153212
MOSDZhinKU153221
WCSDZhinKU15319
ZhiBrCSD004
ZhiBrCSD008
ZhiBrCSD012
ZhiBrCSD013
ZhiBrCSD015
ZhiBrCSD019
ZhiBrCSD024
ZhiBrCSD031
ZhiMCMNMSB80769
ZhiMCMNMSB80772
ZhiMCMNMSB80778
ZhiMCMNMSB80781

53.C09
CCMTZhcaK123595 [1]

54. Zp01
PaWYZPIdTK86039 [2]
PaWYZPIdTK86041

55. Zp02
TCWYZPUtTK86075 [3]
TCWYZPUtTK86155
TCWYZPUtTK86175

56. Zp03
TCWYZPUtTK86135 [1]

57. PaZp01

DCKSZhpaKU30814 [2]
PaWYZPIdTK86040

58. Zp05
FCWYZPIdTK86028 [3]
FCWYZPIdTK86037
FCWYZPIdTK86112

59. Zp06
CCCOZPPTK103545 [1]

60. Zp07
LACOZPPTK103593 [1]

61. Zp08
ZpAbWY002 [2]
ZpAbWY003

62. Zp09
LACOZPPTK103589 [1]

63. Zp10
LCWYZPPDMNH9316 [1]

Table 6. A summary of likelihoods scores for each STRUCTURE run for King et al. data, average likelihood scores for each K for all runs, and a visual representation of the absolute values of these scores.

Runs	K								
	1	2	3	4	5	6	7	8	9
1	-25,162.00	-22,257.80	-20,596.30	-20,067.20	-19,544.60	-20,006.30	-19,721.60	-20,104.20	-77,538.20
2	-25,165.80	-22,257.10	-20,593.60	-19,903.10	-19,378.00	-19,247.30	-19,745.60	-77,544.40	-80,319.90
3	-25,168.50	-22,257.70	-20,590.40	-20,067.00	-19,377.00	-19,994.60	-20,092.90	-19,976.70	-19,629.00
4	-25,157.90	-22,258.40	-20,589.90	-19,904.90	-19,715.10	-20,061.60	-19,708.60	-19,173.80	-19,113.90
5	-25,162.30	-22,266.00	-20,595.00	-20,067.50	-19,918.20	-19,243.90			
6	-25,156.70	-22,257.10	-20,593.00	-19,902.30	-19,542.30	-19,020.50			
7	-25,160.50	-22,263.30	-20,591.60	-20,069.80	-19,936.20	-19,025.60			
8	-25,155.90	-22,258.70	-20,591.50	-20,066.80	-19,717.40	-20,006.30			
9	-25,160.60	-22,259.00	-20,593.30	-20,071.40	-19,939.80	-19,020.50			
10	-25,157.50	-22,254.20	-20,597.30	-20,069.30	-19,931.20	-20,004.40			
Aver. -Ln	**-25,160**	**-22,258**	**-20,593**	**-20.018**	**-19,699**	**-19.563**	**-19,817**		

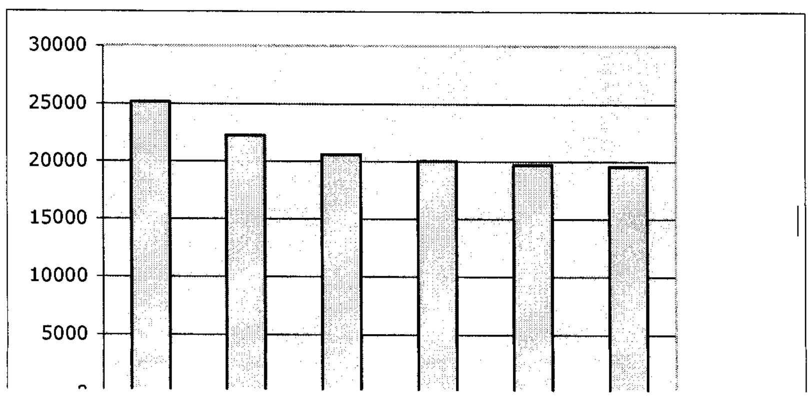

Table 7. Inferred ancestry of all individuals based on the run with the best likelihood score at K = 3 from the King et al. 2006 microsatellite data. Probabilities in bold text indicate cluster with highest assignment. Individuals in bold red text indicate individuals with mixed ancestry (no probability > 0.80) and individuals that belong to one subspecies but have highest probability ancestry assigned to a cluster with predominately individuals from a different subspecies are indicated in green. Individuals 1-94 are *Z. h. preblei*, species for all other samples are given in label. Abbreviations for sample sites are as in Table 1.

Inferred ancestry of individuals:

Label	(%Miss) :	Inferred clusters 1	2	3
1 LCC01_CER-9	(0) :	0.002	**0.997**	0.001
2 LCC01_CER-9	(0) :	0.002	**0.997**	0.001
3 LCC01_CER-9	(0) :	0.003	**0.995**	0.001
4 LCC01_CER-9	(0) :	0.002	**0.997**	0.001
5 LCC01_CER-9	(0) :	0.002	**0.996**	0.001
6 LCC01_CER-9	(9) :	0.002	**0.996**	0.001
7 LCC01_HRK-9	(0) :	0.003	**0.996**	0.001
8 LCC01_HRK-9	(0) :	0.002	**0.997**	0.001
9 LCC01_HRK-9	(0) :	0.003	**0.995**	0.002
10 LCC01_HRK-9	(0) :	0.002	**0.997**	0.001
11 LCC01_MC-98	(0) :	0.002	**0.997**	0.001
12 LCC01_MC-98	(0) :	0.002	**0.997**	0.001
13 LCC01_NFP-9	(0) :	0.002	**0.997**	0.001
14 LCC01_NFP-9	(0) :	0.003	**0.996**	0.001
15 LCC02_BG-98	(0) :	0.005	**0.993**	0.001
16 LCC02_BG-98	(0) :	0.017	**0.982**	0.001
17 LCC02_PGC-9	(0) :	0.002	**0.997**	0.001
18 LCC02_SP-12	(0) :	0.003	**0.995**	0.001
19 LCC02_SP-16	(0) :	0.004	**0.995**	0.001
20 LCC02_SP-17	(0) :	0.003	**0.996**	0.001
21 LCC02_SP-22	(0) :	0.003	**0.996**	0.001
22 LCC02_SP-24	(0) :	0.002	**0.996**	0.001
23 LCC02_SP-33	(0) :	0.003	**0.996**	0.001
24 LCC02_SP-36	(0) :	0.006	**0.993**	0.002
25 LCC02_SP-37	(0) :	0.012	**0.986**	0.002
26 LCC02_SP-67	(0) :	0.003	**0.995**	0.001
27 LCC02_SP-74	(0) :	0.002	**0.996**	0.001
28 LCC02_SP-86	(0) :	0.006	**0.993**	0.001
29 LCC02_YG-98	(0) :	0.003	**0.996**	0.001
30 LCC02_YG-98	(0) :	0.005	**0.994**	0.001
31 DCC01_MAY-1	(0) :	0.002	**0.997**	0.001
32 DCC01_MAY-1	(9) :	0.002	**0.997**	0.001
33 DCC01_MAY-2	(0) :	0.002	**0.997**	0.001

34	DCC01_MAY-2	(0)	:	0.004	**0.995**	0.001
35	DCC01_MAY-2	(0)	:	0.004	**0.995**	0.001
36	DCC01_MAY-2	(0)	:	0.002	**0.996**	0.002
37	DCC01_MAY-2	(0)	:	0.002	**0.997**	0.001
38	DCC01_MAY-2	(0)	:	0.002	**0.997**	0.001
39	DCC01_MAY-3	(0)	:	0.002	**0.996**	0.002
40	DCC01_MAY-3	(0)	:	0.002	**0.995**	0.003
41	DCC01_MAY-3	(0)	:	0.003	**0.996**	0.001
42	DCC01_MAY-4	(0)	:	0.002	**0.997**	0.001
43	DCC01_MAY-4	(0)	:	0.003	**0.996**	0.001
44	DCC01_MAY-4	(0)	:	0.002	**0.997**	0.001
45	DCC01_MAY-4	(0)	:	0.003	**0.996**	0.001
46	DCC01_MAY-4	(0)	:	0.005	**0.994**	0.001
47	DCC01_MAY-4	(0)	:	0.003	**0.996**	0.001
48	DCC01_MAY-5	(0)	:	0.008	**0.987**	0.005
49	DCC01_MAY-5	(0)	:	0.002	**0.997**	0.001
50	DCC01_MAY-6	(0)	:	0.002	**0.997**	0.001
51	DCC01_MAY-7	(0)	:	0.002	**0.996**	0.001
52	DCC01_MAY-7	(0)	:	0.002	**0.998**	0.001
53	DCC01_MAY-7	(0)	:	0.002	**0.996**	0.001
54	DCC01_MAY-7	(4)	:	0.002	**0.996**	0.001
55	DCC01_MAY-7	(0)	:	0.002	**0.997**	0.001
56	DCC01_MAY-8	(0)	:	0.002	**0.997**	0.001
57	DCC01_MAY-8	(0)	:	0.002	**0.997**	0.001
58	DCC01_MAY-8	(0)	:	0.002	**0.997**	0.001
59	DCC01_MAY-9	(4)	:	0.003	**0.995**	0.001
60	DCC01_MAY-9	(0)	:	0.002	**0.997**	0.001
61	DCC01_MAY-9	(0)	:	0.003	**0.996**	0.001
62	DCC01_MAY-9	(0)	:	0.002	**0.997**	0.001
63	DCC01_MAY-9	(0)	:	0.007	**0.990**	0.003
64	DCC01_MAY-9	(4)	:	0.006	**0.988**	0.006
65	DCC02_WH-98	(0)	:	0.002	**0.997**	0.001
66	DCC02_WH-98	(0)	:	0.005	**0.994**	0.002
67	DCC02_WH-98	(0)	:	0.002	**0.997**	0.001
68	DCC02_WH-98	(0)	:	0.002	**0.997**	0.001
69	DCC02_WH-98	(0)	:	0.002	**0.997**	0.001
70	DCC02_WH-98	(0)	:	0.002	**0.983**	0.015
71	DCC02_WH-98	(0)	:	0.002	**0.997**	0.001
72	DCC02_WH-98	(0)	:	0.001	**0.997**	0.001
73	DCC02_WH-98	(0)	:	0.001	**0.998**	0.001
74	DCC02_WH-98	(0)	:	0.002	**0.997**	0.001
75	DCC02_WH-98	(0)	:	0.002	**0.997**	0.001
76	DCC02_WH-98	(9)	:	0.002	**0.996**	0.001
77	DCC02_WH-98	(4)	:	0.003	**0.930**	0.067
78	DCC02_WH-98	(0)	:	0.002	**0.996**	0.002
79	DCC02_WH-98	(0)	:	0.002	**0.997**	0.001

80 DCC02_WH-98 (0) : 0.002 **0.996** 0.001
81 DCC02_WH-98 (4) : 0.002 **0.997** 0.001
82 DCC02_WH-98 (0) : 0.012 **0.986** 0.002
83 DCC02_WH-98 (0) : 0.001 **0.997** 0.001
84 DCC02_WH-98 (0) : 0.002 **0.997** 0.001
85 DCC02_WH-98 (0) : 0.002 **0.997** 0.001
86 DCC02_WH-98 (9) : 0.002 **0.997** 0.001
87 DCC02_WH-98 (0) : 0.002 **0.997** 0.001
88 DCC02_WH-98 (0) : 0.002 **0.996** 0.001
89 DCC02_WH-98 (0) : 0.039 **0.956** 0.005
90 DCC02_WH-98 (0) : 0.002 **0.997** 0.001
91 DCC02_WH-98 (4) : 0.002 **0.996** 0.001
92 DCC02_WH-98 (4) : 0.002 **0.997** 0.001
93 DCC02_WH-98 (4) : 0.002 **0.997** 0.001
94 DCC02_WH-98 (4) : 0.002 **0.997** 0.001
95 ECC01_Zhp-0 (0) : 0.002 **0.996** 0.001
96 ECC01_Zhp-0 (0) : 0.002 **0.997** 0.001
97 ECC01_Zhp-0 (0) : 0.004 **0.995** 0.002
98 ECC01_Zhp-0 (0) : 0.003 **0.995** 0.001
99 ECC01_Zhp-0 (0) : 0.002 **0.997** 0.002
100 ECC01_Zhp-0 (0) : 0.010 **0.988** 0.002
101 ECC01_Zhp-0 (0) : 0.002 **0.996** 0.002
102 ECC01_Zhp-0 (0) : 0.002 **0.997** 0.001
103 ECC01_Zhp-0 (0) : 0.003 **0.996** 0.001
104 ECC01_Zhp-0 (0) : 0.002 **0.997** 0.001
105 ECC01_Zhp-0 (0) : 0.010 **0.987** 0.003
106 ECC01_Zhp-0 (0) : 0.002 **0.997** 0.001
107 ECC01_Zhp-0 (0) : 0.002 **0.997** 0.001
108 ECC01_Zhp-0 (0) : 0.002 **0.997** 0.001
109 ECC01_Zhp-0 (0) : 0.002 **0.996** 0.002
110 ECC01_Zhp-0 (0) : 0.003 **0.995** 0.002
111 ECC01_Zhp-0 (0) : 0.002 **0.996** 0.001
112 ECC01_Zhp-0 (0) : 0.004 **0.995** 0.002
113 ECC01_Zhp-0 (0) : 0.006 **0.993** 0.002
114 ECC01_Zhp-0 (0) : 0.003 **0.995** 0.002
115 ECC01_Zhp-0 (0) : 0.003 **0.996** 0.001
116 ECC01_Zhp-0 (0) : 0.002 **0.997** 0.001
117 ECC02_Zhp-0 (0) : 0.003 **0.995** 0.002
118 ECC02_Zhp-0 (0) : 0.002 **0.997** 0.001
119 ECC02_Zhp-0 (0) : 0.006 **0.992** 0.002
120 ECC02_Zhp-0 (0) : 0.003 **0.996** 0.001
121 ECC02_Zhp-0 (0) : 0.003 **0.996** 0.002
122 ECC02_Zhp-0 (0) : 0.004 **0.993** 0.003
123 ECC02_Zhp-0 (0) : 0.003 **0.996** 0.001
124 ECC02_Zhp-0 (0) : 0.002 **0.997** 0.001
125 ECC02_Zhp-0 (0) : 0.002 **0.997** 0.001

126	ECC02_Zhp-0	(0)	:	0.002	**0.997**	0.001
127	ECC02_Zhp-0	(0)	:	0.004	**0.994**	0.001
128	ECC02_Zhp-0	(0)	:	0.002	**0.997**	0.001
129	ECC02_Zhp-0	(0)	:	0.003	**0.996**	0.001
130	ECC02_Zhp-0	(0)	:	0.002	**0.997**	0.001
131	ECC02_Zhp-0	(0)	:	0.002	**0.997**	0.001
132	ECC02_Zhp-0	(0)	:	0.003	**0.995**	0.001
133	ECC02_Zhp-0	(0)	:	0.004	**0.994**	0.002
134	ECC02_Zhp-0	(0)	:	0.002	**0.997**	0.001
135	ECC02_Zhp-0	(0)	:	0.002	**0.997**	0.001
136	ECC02_Zhp-0	(0)	:	0.002	**0.996**	0.002
137	ECC02_Zhp-0	(0)	:	0.002	**0.997**	0.001
138	ECC02_Zhp-1	(0)	:	0.002	**0.997**	0.001
139	ECC02_Zhp-1	(0)	:	0.002	**0.996**	0.001
140	ECC02_Zhp-1	(0)	:	0.014	**0.983**	0.003
141	ECC02_Zhp-1	(0)	:	0.072	**0.927**	0.002
142	ECC02_Zhp-1	(0)	:	0.019	**0.979**	0.003
143	CCWY_Zhc-02	(0)	:	**0.996**	0.003	0.001
144	CCWY_Zhc-03	(0)	:	**0.977**	0.022	0.001
145	CCWY_Zhc-03	(0)	:	**0.988**	0.011	0.002
146	**CCWY_Zhc-03**	(4)	:	**0.546**	**0.452**	0.002
147	CCWY_Zhc-03	(0)	:	**0.990**	0.008	0.001
148	CCWY_Zhc-03	(0)	:	**0.993**	0.006	0.001
149	CCWY_Zhc-03	(0)	:	**0.995**	0.003	0.002
150	CCWY_Zhc-03	(0)	:	**0.986**	0.004	0.010
151	CCWY_Zhc-03	(0)	:	**0.979**	0.019	0.002
152	CCWY_Zhc-03	(0)	:	**0.980**	0.019	0.001
153	CCWY_Zhc-03	(0)	:	**0.979**	0.017	0.004
154	CCWY_Zhc-04	(0)	:	**0.990**	0.007	0.004
155	**CCWY_Zhc-04**	(0)	:	**0.696**	**0.303**	0.002
156	CCWY_Zhc-04	(0)	:	**0.977**	0.021	0.002
157	CCWY_Zhc-04	(0)	:	**0.980**	0.018	0.001
158	**CCWY_Zhc-04**	(0)	:	**0.680**	**0.318**	0.002
159	CCWY_Zhc-04	(0)	:	**0.895**	0.103	0.002
160	CCWY_Zhc-04	(0)	:	**0.992**	0.006	0.001
161	CCWY_Zhc-04	(0)	:	**0.996**	0.002	0.001
162	CCWY_Zhc-04	(0)	:	**0.921**	0.077	0.002
163	CCWY_Zhc-04	(0)	:	**0.881**	0.118	0.001
164	CCWY_Zhc-05	(0)	:	**0.852**	0.146	0.001
165	CCWY_Zhc-05	(0)	:	**0.985**	0.012	0.002
166	CCWY_Zhc-05	(0)	:	**0.994**	0.005	0.002
167	CCWY_Zhc-05	(0)	:	**0.983**	0.016	0.001
168	CCWY_Zhc-05	(0)	:	**0.977**	0.021	0.002
169	CCWY_Zhc-05	(0)	:	**0.995**	0.003	0.002
170	CCWY_Zhc-08	(0)	:	**0.985**	0.012	0.003

171	CCWY_Zhc-08	(0)	:	**0.987**	0.011	0.002
172	**CCWY_Zhc-08**	(0)	:	**0.692**	**0.306**	0.001
173	CCSD_Zhc-05	(0)	:	**0.993**	0.005	0.002
174	CCSD_Zhc-05	(0)	:	**0.995**	0.003	0.001
175	CCSD_Zhc-05	(0)	:	**0.972**	0.026	0.001
176	CCSD_Zhc-05	(0)	:	**0.992**	0.007	0.002
177	CCSD_Zhc-06	(0)	:	**0.954**	0.043	0.004
178	CCSD_Zhc-06	(14)	:	**0.953**	0.045	0.002
179	CCSD_Zhc-06	(4)	:	**0.874**	0.122	0.004
180	CCSD_Zhc-06	(0)	:	**0.936**	0.063	0.002
181	CCSD_Zhc-06	(0)	:	**0.994**	0.005	0.001
182	CCSD_Zhc-06	(0)	:	**0.995**	0.003	0.001
183	CCSD_Zhc-06	(0)	:	**0.977**	0.019	0.004
184	CCSD_Zhc-06	(0)	:	**0.994**	0.004	0.002
185	CCSD_Zhc-06	(0)	:	**0.980**	0.019	0.001
186	CCSD_Zhc-06	(0)	:	**0.996**	0.002	0.002
187	CCSD_Zhc-07	(0)	:	**0.994**	0.004	0.001
188	CCSD_Zhc-07	(0)	:	**0.995**	0.003	0.002
189	CCSD_Zhc-07	(0)	:	**0.990**	0.008	0.002
190	CCSD_Zhc-07	(0)	:	**0.986**	0.012	0.002
191	CCSD_Zhc-07	(0)	:	**0.993**	0.006	0.002
192	CCSD_Zhc-07	(0)	:	**0.990**	0.009	0.001
193	CCSD_Zhc-07	(0)	:	**0.988**	0.008	0.004
194	CCSD_Zhc-07	(0)	:	**0.993**	0.006	0.001
195	CCSD_Zhc-07	(0)	:	**0.996**	0.003	0.001
196	CCSD_Zhc-07	(4)	:	**0.991**	0.007	0.002
197	CCSD_Zhc-08	(0)	:	**0.995**	0.003	0.001
198	CCSD_Zhc-08	(0)	:	**0.994**	0.004	0.001
199	CCSD_Zhc-08	(0)	:	**0.904**	0.093	0.003
200	CCSD_Zhc-08	(0)	:	**0.992**	0.006	0.002
201	CCSD_Zhc-08	(0)	:	**0.990**	0.008	0.002
202	CCSD_Zhc-08	(0)	:	**0.995**	0.004	0.001
203	CCSD_Zhc-08	(0)	:	**0.991**	0.007	0.002
204	BRCSD_Zhi-0	(0)	:	**0.991**	0.005	0.004
205	BRCSD_Zhi-0	(4)	:	**0.991**	0.006	0.002
206	BRCSD_Zhi-0	(0)	:	**0.995**	0.003	0.002
207	BRCSD_Zhi-0	(0)	:	**0.997**	0.001	0.002
208	BRCSD_Zhi-0	(0)	:	**0.995**	0.003	0.002
209	BRCSD_Zhi-0	(0)	:	**0.995**	0.002	0.003
210	BRCSD_Zhi-0	(0)	:	**0.996**	0.002	0.002
211	BRCSD_Zhi-0	(0)	:	**0.996**	0.001	0.002
212	BRCSD_Zhi-0	(0)	:	**0.995**	0.003	0.002
213	BRCSD_Zhi-0	(0)	:	**0.996**	0.002	0.002
214	BRCSD_Zhi-0	(0)	:	**0.928**	0.053	0.019
215	BRCSD_Zhi-0	(0)	:	**0.995**	0.003	0.003

216	BRCSD_Zhi-0	(0)	:	**0.991**	0.006	0.002
217	BRCSD_Zhi-0	(0)	:	**0.997**	0.002	0.002
218	BRCSD_Zhi-0	(0)	:	**0.993**	0.004	0.003
219	BRCSD_Zhi-0	(0)	:	**0.996**	0.002	0.002
220	BRCSD_Zhi-0	(0)	:	**0.996**	0.002	0.002
221	BRCSD_Zhi-0	(0)	:	**0.997**	0.001	0.001
222	BRCSD_Zhi-0	(0)	:	**0.996**	0.002	0.003
223	BRCSD_Zhi-0	(0)	:	**0.996**	0.002	0.002
224	BRCSD_Zhi-0	(0)	:	**0.988**	0.010	0.002
225	BRCSD_Zhi-0	(0)	:	**0.992**	0.003	0.006
226	BRCSD_Zhi-0	(0)	:	**0.993**	0.002	0.005
227	BRCSD_Zhi-0	(0)	:	**0.995**	0.003	0.002
228	BRCSD_Zhi-0	(0)	:	**0.990**	0.008	0.002
229	BRCSD_Zhi-0	(0)	:	**0.997**	0.002	0.001
230	BRCSD_Zhi-0	(0)	:	**0.997**	0.002	0.002
231	BRCSD_Zhi-0	(0)	:	**0.976**	0.003	0.021
232	MCMN_MSB-41	(0)	:	**0.993**	0.004	0.003
233	MCMN_MSB-41	(0)	:	**0.755**	0.005	**0.240**
234	MCMN_MSB-41	(0)	:	**0.961**	0.003	0.036
235	**MCMN_MSB-80**	(0)	:	**0.725**	0.003	**0.272**
236	MCMN_MSB-80	(0)	:	**0.994**	0.004	0.002
237	MCMN_MSB-80	(0)	:	**0.990**	0.003	0.007
238	MCMN_MSB-80	(0)	:	**0.979**	0.014	0.007
239	MCMN_MSB-80	(0)	:	**0.988**	0.009	0.003
240	MCMN_MSB-80	(0)	:	**0.977**	0.003	0.020
241	MCMN_MSB-80	(0)	:	**0.991**	0.004	0.004
242	MCMN_MSB-80	(0)	:	**0.976**	0.011	0.013
243	MCMN_MSB-80	(0)	:	**0.974**	0.002	0.024
244	MCMN_MSB-80	(0)	:	**0.919**	0.003	0.077
245	MCMN_MSB-80	(0)	:	**0.953**	0.004	0.043
246	MCMN_MSB-80	(0)	:	**0.981**	0.014	0.005
247	MCMN_MSB-80	(0)	:	**0.993**	0.004	0.003
248	MCMN_MSB-80	(0)	:	**0.990**	0.002	0.008
249	MCMN_MSB-80	(0)	:	**0.910**	0.004	0.086
250	MCMN_MSB-80	(0)	:	**0.991**	0.005	0.003
251	MCMN_MSB-80	(0)	:	**0.987**	0.010	0.003
252	**MCMN_MSB-80**	(0)	:	**0.736**	0.003	**0.262**
253	BCSD_Zhpa-0	(0)	:	0.003	0.001	**0.995**
254	BCSD_Zhpa-0	(0)	:	0.013	0.006	**0.981**
255	BCSD_Zhpa-0	(0)	:	0.002	0.031	**0.967**
256	BCSD_Zhpa-0	(0)	:	0.002	0.002	**0.995**
257	BCSD_Zhpa-0	(0)	:	0.014	0.002	**0.984**
258	BCSD_Zhpa-0	(0)	:	0.007	0.002	**0.990**
259	BCSD_Zhpa-0	(0)	:	0.002	0.002	**0.996**
260	BCSD_Zhpa-0	(0)	:	0.006	0.007	**0.987**

261	BCSD_Zhpa-0	(0)	:	0.005	0.002	**0.993**
262	BCSD_Zhpa-0	(0)	:	0.003	0.002	**0.995**
263	BCSD_Zhpa-0	(0)	:	0.003	0.005	**0.993**
264	BCSD_Zhpa-0	(0)	:	0.002	0.003	**0.995**
265	BCSD_Zhpa-0	(0)	:	0.009	0.014	**0.976**
266	BCSD_Zhpa-0	(0)	:	0.002	0.003	**0.995**
267	BCSD_Zhpa-0	(0)	:	0.002	0.007	**0.991**
268	BCSD_Zhpa-0	(0)	:	0.005	0.001	**0.994**
269	KBCNE_Zhpa-	(0)	:	0.005	0.001	**0.994**
270	KBCNE_Zhpa-	(0)	:	0.008	0.002	**0.990**
271	KBCNE_Zhpa-	(0)	:	0.008	0.002	**0.990**
272	KBCNE_Zhpa-	(0)	:	0.003	0.001	**0.996**
273	KBCNE_Zhpa-	(4)	:	0.002	0.001	**0.997**
274	KBCNE_Zhpa-	(0)	:	0.006	0.005	**0.990**
275	KBCNE_Zhpa-	(4)	:	0.003	0.004	**0.992**
276	KBCNE_Zhpa-	(0)	:	0.002	0.001	**0.997**
277	KBCNE_Zhpa-	(0)	:	0.019	0.032	**0.950**
278	KBCNE_Zhpa-	(9)	:	0.009	0.003	**0.988**
279	KBCNE_Zhpa-	(0)	:	0.005	0.003	**0.992**
280	KBCNE_Zhpa-	(0)	:	0.003	0.005	**0.992**
281	KBCNE_Zhpa-	(0)	:	0.021	0.009	**0.970**
282	KBCNE_Zhpa-	(0)	:	0.003	0.005	**0.992**
283	KBCNE_Zhpa-	(0)	:	0.007	0.007	**0.986**
284	KBCNE_Zhpa-	(0)	:	0.008	0.002	**0.991**
285	KBCNE_Zhpa-	(0)	:	0.003	0.003	**0.994**
286	KBCNE_Zhpa-	(0)	:	0.013	0.083	**0.904**
287	KBCNE_Zhpa-	(0)	:	0.004	0.007	**0.990**
288	KBCNE_Zhpa-	(0)	:	0.004	0.002	**0.995**
289	KBCNE_Zhpa-	(0)	:	0.004	0.004	**0.991**
290	KBCNE_Zhpa-	(0)	:	0.004	0.002	**0.995**
291	KBCNE_Zhpa-	(0)	:	0.003	0.002	**0.994**
292	KBCNE_Zhpa-	(0)	:	0.008	0.007	**0.986**
293	KBCNE_Zhpa-	(0)	:	0.018	0.004	**0.978**
294	KBCNE_Zhpa-	(0)	:	0.009	0.004	**0.987**
295	KBCNE_Zhpa-	(0)	:	0.008	0.024	**0.969**
296	KBCNE_Zhpa-	(0)	:	0.006	0.024	**0.970**
297	KBCNE_Zhpa-	(0)	:	0.005	0.004	**0.991**
298	KBCNE_Zhpa-	(0)	:	0.002	0.002	**0.997**
299	KBCNE_Zhpa-	(0)	:	0.019	0.009	**0.972**
300	KBCNE_Zhpa-	(0)	:	0.027	0.002	**0.971**
301	SCNM_MSB-38	(0)	:	0.001	0.001	**0.998**
302	SCNM_MSB-38	(0)	:	0.001	0.001	**0.998**
303	SCNM_MSB-38	(0)	:	0.001	0.001	**0.998**
304	SCNM_MSB-38	(0)	:	0.001	0.002	**0.997**
305	SCNM_MSB-38	(0)	:	0.002	0.002	**0.996**

306	SCNM_MSB-38	(0)	:	0.002	0.002	**0.996**
307	SCNM_MSB-38	(0)	:	0.001	0.001	**0.997**
308	SCNM_MSB-38	(0)	:	0.001	0.001	**0.997**
309	SCNM_MSB-38	(0)	:	0.002	0.002	**0.997**
310	SCNM_MSB-38	(0)	:	0.001	0.001	**0.998**
311	SCNM_MSB-38	(0)	:	0.002	0.003	**0.995**
312	SCNM_MSB-38	(0)	:	0.001	0.002	**0.997**
313	SCNM_MSB-38	(0)	:	0.001	0.001	**0.998**
314	SCNM_MSB-38	(0)	:	0.001	0.001	**0.998**
315	SCNM_MSB-38	(0)	:	0.001	0.001	**0.998**
316	SCNM_MSB-38	(0)	:	0.002	0.002	**0.997**
317	SCNM_MSB-38	(0)	:	0.001	0.001	**0.998**
318	SCNM_MSB-38	(0)	:	0.001	0.001	**0.998**
319	SCNM_MSB-38	(0)	:	0.001	0.001	**0.998**
320	SCNM_MSB-38	(0)	:	0.001	0.001	**0.998**

Table 8. Summary of results for MIGRATE analysis of King et al. microsatellite data between three hypothesized populations based on four separate runs. Because of slight insistencies in the first three runs a forth was attempted in which length of the run was doubled A) Theta is equal to the estimated effective population size and Mxy is equal to the relative importance of migration from cluster on 'x' axis into cluster on 'y' axis relative to mutation rate in introducing new variants into the population. B) Nm estimates based on the results of the Migrate run 4.

A)

Runs	**Clusters**	Θ	**Mxy(m/μ)**			**Chains**
			Zhpr	**Zhc/Zhi**	**Zhpa/Zhl**	
Run 1	**Zhpr**	**1.27503**	---	4.5161	1.9411	**Short = 10**
	Zhc/Zhi	**1.27120**	7.5431	---	3.5490	**Long = 3**
	Zhpa/Zhl	**1.39109**	2.6982	3.1101	---	
Run 2	**Zhpr**	**1.19892**	---	4.3438	1.9371	**Short = 10**
	Zhc/Zhi	**1.31505**	5.9209	---	3.2387	**Long = 3**
	Zhpa/Zhl	**1.47370**	3.1404	3.2982	---	
Run 3	**Zhpr**	**1.20181**	---	4.9202	1.8748	**Short = 10**
	Zhc/Zhi	**1.31586**	21.2357	---	7.3766	**Long = 3**
	Zhpa/Zhl	**1.41384**	2.9735	3.0185	---	
Run 4	**Zhpr**	**1.39925**	---	3.4569	1.7511	**Short = 20**
	Zhc/Zhi	**1.39302**	5.9200	---	3.1183	**Long = 6**
	Zhpa/Zhl	**2.98891**	2.1854	2.2228	---	

B)

		Nm(xy)	
	Zhpr	**Zhc/Zhi**	**Zhpa/Zhl**
Zhpr	---	**1.21**	**2.45**
Zhc/Zhi	**2.06**	---	**1.09**
Zhpa/Zhl	**1.63**	**3.32**	---

Number of migrants from 'x' axis cluster into 'y' axis cluster

Table 9. A summary of results for the nested clade analysis as per the Templeton (2004) inference key. Clades are labeled as in Figures 7-9. Geographical distribution of important clades (in bold) are shown in Figures 10-13.

Clades	NCA inferences
Clade 1-3	Allopatric fragmentation but the sampling scheme may be inadequate because only 6 samples were taken from Kansas and it is unclear if *Z. hudsonius* exists here.
Clade 1-5	Contiguous Range Expansion
Clade 1-9	Contiguous Range Expansion
Clade 1-10	Inconclusive
Clade 1-14	Restricted Gene Flow w/IBD
Clade 1-19	Restricted Gene Flow w/IBD
Clade 2-1	Restricted Gene Flow w/IBD
Clade 2-2	Restricted Gene Flow w/IBD
Clade 2-4	Restricted Gene Flow w/IBD
Clade 2-5	Insufficient Genetic Resolution to discriminate between range expansion/colonization and restricted dispersal / gene flow
Clade 2-9	Inconclusive
Clade 3-1	Contiguous range expansion but like with clade 1-3 this depends on adequate sampling in Eastern Colorado and Kansas
Clade 3-2	Contiguous Range Expansion
Clade 3-4	Restricted Gene Flow w/IBD
Clade 4-2	When you compare all nested clades 3-2, 3-3, and 3-4 you get restricted gene flow / dispersal with some long distance dispersal but it depends on the sampling design in the area between 3-2+3-4 and 3-3. If you just compare 3-2 and 3-4 ignoring 3-3 samples then you get Restricted Gene Flow with IBD.
Clade 5-1	Possible fragmentation but may need better sampling between clades 4-1 and 4-2.
Clade 5-3	Inadequate Geographical Sampling