

THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DEPARTMENT OF BIOLOGY

AN EXAMINATION OF POLAR BEAR AND BROWN BEAR PHYLOGENY USING
NUCLEAR GENES AS MOLECULAR MARKERS

LOUISE MONCLA
Spring 2012

A thesis
submitted in partial fulfillment
of the requirements
for baccalaureate degrees
in Biology and Musical Arts
with honors in Biology

Reviewed and approved* by the following:

Dr. Beth Shapiro
Assistant Professor of Biology
Thesis Supervisor

Dr. Jim Marden
Professor of Biology
Honors Adviser

* Signatures are on file in the Schreyer Honors College.

ABSTRACT

Recent discussion of global climate change has brought polar bears (*Ursus maritimus*) into the spotlight. Although their close relationship to the brown bear (*Ursus arctos*) has long been accepted, ambiguity still remains regarding their divergence time and the role of hybridization in polar bears' species history. Past phylogenies built on mitochondrial DNA have elucidated that polar bears and brown bears' mitochondrial lineages are reciprocally monophyletic, and that polar bear mitochondria are most closely related to a clade of extant brown bears inhabiting the Admiralty, Baranof, and Chichagof Islands (ABC Islands) Alaska. However, mitochondrial data only reflect the maternal lineage of a species. Here I use nuclear gene sequences obtained from multiple polar bear and brown bear individuals to construct species phylogenies with aims of determining both the phylogenetic relationship of polar bears and brown bears, and the role of hybridization in their evolutionary histories. I targeted four nuclear genes in 31 polar bear and brown bear tissue samples obtained from the Alaska area, and constructed both single gene and multiple gene trees. Different analyses of my data are consistent with two proposed hypotheses of polar bear evolution. My data also show possible evidence of recent polar bear-brown bear hybridization, and group ABC Island brown bears with brown bears rather than with polar bears.

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ACKNOWLEDGMENTS

I conducted the work for this thesis in the lab of Dr. Beth Shapiro at The Pennsylvania State University under the direction of Dr. Tara Fulton. I would like to extend my greatest thanks for the help from both Dr. Fulton and Dr. Shapiro for both implementing this study as well as reviewing this thesis. My sincere thanks also goes to Emily Pronchik, Lakshmi Kolandra, and Ben Rich for assisting me in DNA extractions that were necessary for the completion of this work, and to Dr. Mathias Stiller for advising me on several important lab protocols.

INTRODUCTION

Polar bears (*Ursus maritimus*) are one of the species for which projected climate change could be the most devastating. Acting as the top carnivores in their northern habitats, polar bears are an important part of the arctic ecosystem. In order to inform conservation efforts to keep this species active on the planet, an understanding of their evolutionary history is indispensable.

Polar bears are part of the Ursidae family, which contains a total of eight extant species: the giant panda (*Ailuropoda melanoleuca*), spectacled bear (*Tremarctos ornatus*), Asiatic black bear (*Ursus thibethanus*), sloth bear (*Melursus ursinus*), sun bear (*Helarctos malayanus*), American black bear (*Ursus americanus*), brown bear (*Ursus arctos*), and polar bear (*Ursus maritimus*) (Kurten et al. 1964). The Ursidae family is further divided into three subfamilies: Ailurpodinae, which includes the giant panda, Tremarctinae, which includes the spectacled bear, and Ursinae, which includes the remaining six ursids. The Ursinae group is comprised of the Asiatic black bear, sloth bear, sun bear, American black bear, brown bear, and polar bear. The Ursinae subfamily is thought to have undergone rapid species diversification about 5 million years ago (mya) (Kurten et al. 1964), leading to the extant species observed today. Despite years of research on bear phylogeny, the topologies elucidated by nuclear and mitochondrial sequence data are often conflicting (Pages 2008, Yu 2004, Krause 2008), and the Ursinae phylogeny is still not completely resolved. Within that phylogeny, one of the most perplexing dilemmas that still exists is the relationship between brown bears and polar bears.

Although interspecies mating between brown bears and polar bears sometimes produce viable offspring, these bears remain classified as distinct species because of their differences in morphology, metabolism, physiology, and social and feeding behaviors (Stirling and Guravich 1998). In terms of their evolutionary relationship, one hypothesis posits that polar bears evolved

from brown bears that became isolated on the Siberian coast between 800,000 and 150,000 years ago (Kurten et al. 1964). Several investigators have attempted to construct polar bear phylogeny using mitochondrial sequence data, and examinations of DNA from extant polar bears have revealed that all extant polar bear matriline fall within the brown bear lineage (Cronin et al. 1991, Shields et al. 1991, Talbot et al. 1996, Shields et al. 2000, Lindqvist et al. 2010). Of particular interest is a clade of brown bears inhabiting the Admiralty, Baranof, and Chichagof Islands (ABC Islands) located near southwest Alaska (Figure 1), whose mitochondrial genomes are more similar to extant polar bears than to other brown bears. In phylogenetic analyses of mitochondrial genes, polar bears cluster within the clade containing ABC Island brown bears (Cronin et al. 1991, Shields et al. 1991, Talbot et al. 1996, Shields et al. 2000, Lindqvist et al. 2010, Edwards et al 2011). This unusual pattern could be the result of two proposed scenarios. Hypothesis 1: Polar bears have only very recently diverged from within the brown bear diversity and their morphological differences are due to rapid adaptation to their specific, arctic climate (Lindqvist et al 2010, Davison et al. 2011). Hypothesis 2: The polar bear and brown bear lineages diverged much more distantly in the past than is proposed by the first hypothesis, but the existing genetic pattern is due to more recent hybridization between the two species. Recent analyses incorporating brown bears sampled from across their Holarctic distribution, including several populations that are now extinct, showed that the existing mitochondrial pattern may have evolved following hybridization between brown bears and polar bears, which resulted in the introgression of a brown bear matriline into the extant polar bear lineage (Edwards et al 2011).

The hypothesis of multiple hybridization events (hypothesis 2) is supported by recent evidence that polar and brown bears interbreed in the wild and produce fertile, hybrid offspring (Kelly et al. 2010). As sea ice dwindles in the polar bears' arctic home, they are forced to spend

more time hunting on land. As a result, they regularly come in contact with populations of terrestrial brown bears, facilitating interbreeding. Past climate changes could have had similar effects, resulting in hybridizations throughout polar and brown bear history.

Recent evidence supporting hypothesis 2 has also arisen from the inclusion of mitochondrial data from two newly discovered ancient polar bear bone samples in a phylogenetic analyses (Lindqvist et al. 2010, Davison et al. 2011, Edwards et al. 2011). The ancient bone samples (a jawbone and rib bone) are dated to 130-110 thousand years ago (kya) (Ingolfsson et al. 2009; analyzed in Lindqvist et al. 2010) and 115 kya (Davison et al. 2011), respectively. However, the coalescence time of all modern polar bear matriline is much more recent, estimated to be between 20-51 kya (Lindqvist et al. 2010, Ho et al. 2008, Edwards et al. 2011). Thus, recent speciation would separate the two ancient polar bear samples from the modern polar bear lineage. To explain this phenomenon, a recent study of brown bears and polar bears from across a large geographic distribution including the ancient and modern samples proposed two possible hybridization scenarios (Edwards et al. 2011). In the first, the most recent common ancestor (MRCA) of modern polar bears emerged from within the brown bear diversity between approximately 75-100 kya. Polar bears then hybridized multiple times with brown bear populations in Ireland and the ABC Islands, resulting in the shared mitochondrial lineage observed in extant bears. The second scenario suggests that the polar bear lineage diverged even earlier, between 400 kya and 2 mya, prior to the diversification of both brown and polar bears. Polar bears then hybridized multiple times with brown bears, resulting in at least two complete mitochondrial replacement events (Edwards et al. 2011).

While informative, mitochondrial data are limiting because the mitochondrion only represents the maternal lineage of a species' evolution. On the contrary, nuclear genes, although

more slowly evolving, show a record of both the maternal and paternal lineages. Past work done to estimate the ursid phylogeny (Pages et al. 2007) has identified several nuclear genes that can be amplified and sequenced. However, these studies sequenced only a single individual from each ursid species. Here, we expand on this analysis by including sequences from a larger number of polar and brown bear individuals. Unlike past research addressing the timing of divergence between brown bears and polar bears, our study targets nuclear genes in multiple brown bear and polar bear individuals. This approach allows us to test relative phylogenetic support for our two hypotheses regarding the timing of divergence between brown bears and polar bears. We aim to improve the current bear phylogeny and investigate the role of hybridization in the evolution of brown bears and polar bears.

MATERIALS AND METHODS

Sample Selection

We acquired modern tissue samples from 13 polar bears and 18 brown bears sampled from various regions of Alaska and Canada (Figure 1). Sample name, accession numbers, and location of origin are displayed in Table 1. Initially, we targeted seven genes for analysis: *Aminolevulinate, delta-, synthase 2 (ALAS)*, *Amyloid Beta Precursor Protein (APP)*, *Selenocysteine tRNA (TRSP)*, *Apolipoprotein B (APOB)*, *Proteolipid protein 1 (PLP)*, *Interphotoreceptor binding protein (IRBP)*, and *Feline sarcoma protooncogene (FES)*. After initial screening, we found that *PLP*, *IRBP*, and *FES* showed no variation among individuals or between species, so we excluded them from further analysis. We chose to continue work with *ALAS*, *APP*, *APOB*, and *TRSP*. For amplification of *APP*, *TRSP*, and *APOB*, we used previously published primers (Bardeleben et al. 2005, Jiang et al. 1998, Amrine-Madsen et al. 2003,

Murphy et al. 2001). Dr. Tara Fulton designed the forward and reverse primers for amplification of *ALAS*. Primer sequences, references, and corresponding gene name are listed in Table 2. The American black bear (*Ursus americanus*) was used as the outgroup for these analyses, and the corresponding gene sequences were acquired through GenBank; accession numbers are listed in Table 3. A published black bear sequence of *APP* was not available, so a black bear sequence was not included in the *APP* gene analyses.

DNA Extraction, and PCR Amplification and Clean-Up

To ensure that PCR products would not contaminate pre-PCR samples, DNA extraction and PCR amplification were performed in a separate room from all other procedures. We extracted DNA with the DNEasy Tissue Kit (Qiagen) by following manufacturer's protocol. We amplified the genes via PCR under the conditions specified in Table 4. For every sample, we added reaction components in the following concentrations. For amplification of *APP* and *TRSP*: 0.25U of Taq Promega, 0.29 mM dNTPs (0.25 μ l of 25 mM stocks), 0.23 μ M of Taq Promega 5X Buffer, 0.93 μ M each of the forward and reverse primers (2 μ l of 10 μ M stocks), and 9 μ l of water, for a total reaction volume of 21.5 μ l. For amplification of *ALAS* and *APOB*: 5U Taq Promega, 0.25 mM dNTPs (0.25 μ l of 25 mM stocks), 0.2 μ M of Taq Promega 5X buffer, 0.8 μ M each of the forward and reverse primers (2 μ l of 10 μ M stocks), and 12.5 μ l of water, for a total reaction volume of 25 μ l. Reaction mixtures for *APP* and *TRSP* amplification contained 0.5 μ l of sample, and reaction mixtures for *ALAS* and *APOB* amplification contained 1 μ l of sample. After amplification, we stored all PCR products in a freezer until they could be visualized. To visualize, we ran all PCR products on a 1% or 2% agarose gel and visualized the bands under UV light after staining with ethidium bromide.

We used one of two methods for cleaning all the PCR samples before sequencing in order to remove unincorporated dNTPs and PCR waste. The first method involved a Millipore plate. First, we added 80 μ l of nuclease-free water to each PCR tube. The entire contents of each tube were then transferred to a Millipore plate attached to a vacuum manifold. We used the vacuum to suck through all the liquid in each well of the plate until the membrane was completely dry (about 10 minutes). The vacuum was then removed, and the process was repeated with 50 μ l of nuclease-free water. We then removed the plate from the vacuum manifold, and the bottom was blotted with paper towels to remove excess moisture. Following that, we added 50 μ l of nuclease-free water to each Millipore well and the entire plate was then shaken at 600 1/min for 10-30 minutes to resuspend the PCR products. The liquid in each well was then transferred to a clean 96-well PCR plate via pipetting, sealed with a sticker and stored in the freezer until sequencing PCR was performed.

The second method of clean-up involved SPRI DNA-binding beads in 0.05% Tween-20. We transferred all PCR products to a clean, 96-well PCR plate via pipetting, and added water to each well so that the total volume in each well was 30 μ l. We added beads in a 1.2:1 bead:liquid ratio (36 μ l of beads to each 30 μ l well). The wells were then covered with dome caps and vortexed until the bead/sample mixture was homogenous. We centrifuged the plate at 2000 x g for 2-3 minutes to pellet the beads to the bottom of the plate. To draw the pelleted beads up to the sides of the wells, we slowly placed the plate in a magnetic tray. While avoiding touching the beads on the sides of the wells, we removed the liquid in each well by pipetting, and ejected it as waste. We then added 150 μ l of 70% ethanol to each well and allowed it to rest for 1 minute. Avoiding the beads, the liquid was again removed from each well by pipetting and ejected. This step was repeated once more. The plate was then allowed to dry completely. Once

completely dry, we added 20 μ l of TE with 0.05% Tween-20 to each well. We then vortexed the plate to mix completely, and then spun the plate down in the centrifuge up to 250 x g to re-pellet the beads. Again, we drew the beads up to the sides of the wells using a magnetic tray. Avoiding the beads, we transferred the liquid in each well to a clean 96-well PCR plate and stored in the freezer until sequencing.

Sequencing

We used a Nanodrop 2000 to determine the concentration of cleaned PCR products before sequencing. The sequencing PCR was set up as follows: 0.25 μ l of BigDye v.3.1, 1.75 μ l of BigDye Buffer, 1.6 μ l of 1 μ M primer, up to 100-200 ng of template/cleaned PCR product, and enough water to bring the total reaction volume to 10 μ l. Cycling conditions followed the manufacturer's recommended protocol.

To clean the sequencing PCR products, we followed the instructions for the ethanol/EDTA precipitation method, which was the recommended method described in the Big Dye v.3.1 manual.

Sequence Alignment

We viewed the sequencing chromatograms in Seqman Pro, a part of the Lasergene 9.1.0 software package, and manually trimmed the primer sequences. Sequences were aligned by eye in Se-Al v2.0 all Carbon (Rambaut, 1996). We then identified single nucleotide polymorphisms (SNPs) by eye, and identified potentially informative genes. We defined genes as potentially informative if they contained any sequence diversity among individuals of the same species, or any diversity between brown bears and polar bears. For some genes, we could not acquire bi-

directional reads; for these genes, we included single direction reads in the analysis. The individuals successfully sequenced for each gene are represented in Table 5. We trimmed the multiple alignment files so as to include all identified SNPs but to exclude as much missing data as possible, and exported the trimmed multiple alignment files as NEXUS files to be used for phylogenetic analysis.

Phylogenetic Analysis

We constructed individual gene trees for all four genes in PAUP v4.0b10* (Swofford, 2002) using neighbor joining and maximum parsimony methods (Appendices A and B). We then used maximum likelihood and Bayesian methods to construct trees for our concatenated data. Concatenated gene trees were formed using two different sets of data. In the first, only individuals for whom we could acquire sequence for all four genes were included in the analysis. We specified this data set “Complete Bears Only.” In the second data set, we included all individuals for whom we had data for any of the genes, where the missing data was specified. We designated this data set “All Bears.” These two data sets were used to construct trees via maximum likelihood and Bayesian inference.

To find the best model of evolution for each gene, we used jModelTest 0.1.1 (Posada, 2008) and computed the likelihood score with the maximum likelihood optimized calculation for *ALAS*, *APOB*, and *APP*. jModelTest 0.1.1 had difficulty applying the calculations to the *TRSP* data, so BIONJ was used instead of maximum likelihood. The selected models of evolution are displayed in Table 7.

We used RAxML 7.0.3 (Stamatakis, 2006) to construct Maximum Likelihood trees for each individual gene (Appendix C) and for the concatenated data, using 100 bootstrap replicates

for each tree. We used MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003) to construct Bayesian inference trees for the concatenated data by running the Markov chain Monte Carlo (MCMC) for 5,000,000 generations and sampling the parameter values from the posterior every 500 generations. Each gene was partitioned, designated as unlinked, and assigned its own model of evolution as determined by jModelTest 0.1.1 (Table 7). To determine run convergence, we examined the standard deviation of split frequencies. We determined that the runs had converged once the standard deviation of split frequencies approached zero and had values less than or equal to 0.01. To check that we had run the analysis for enough time, we examined the potential scale reduction factor (PSRF), a convergence diagnostic put out by MrBayes v3.1.2 after the analysis is stopped. If the analysis has accumulated a good sample from the posterior probability distribution, the PSRF values should all be very close to 1. All of our PSRF values were within less than 0.01 of 1 when we stopped our analyses, indicating that the runs had converged.

RESULTS

We used the single gene trees constructed using maximum parsimony, neighbor-joining, and maximum likelihood methods to ensure that individual gene phylogenies did not conflict with each other. When using concatenated gene sequences to infer species phylogeny, it is important to check that the phylogeny produced by the concatenated data sequence is not merely the result of bias from a single gene. Thus, we compared our twelve individual gene trees to ensure that they did not suggest strongly conflicting phylogenies.

While none of the single gene trees matched exactly, only the *APOB* maximum likelihood gene tree contained any nodes with bootstrap support >70%, so none of the trees are

in strong conflict. Most showed two large groups: one containing only brown bears, and a second containing polar bears with a few brown bear individuals. The only exceptions to this trend were the *APP* trees, which showed a single polytomy including all polar and brown bears (Appendices A-C). However, this is because there were zero parsimony-informative sites within the *APP* sequence (Table 6). With the exception of the *APOB* likelihood tree, most of the single gene trees had extremely low bootstrap support on all of their nodes, meaning that the actual branching order generated is not supported. The *APOB* likelihood tree had very strong support on two nodes, dividing the bears into two large clades (Appendix C). The first clade contained only brown bears (100% bootstrap support); the second clade included all the polar bear individuals as well as five brown bears, EP35, EP36, EP38, EP50, and EP55 (90% bootstrap support). This general topology was supported in the neighbor-joining and maximum parsimony *APOB* trees, although without bootstrap support.

In all of the *APOB* and *TRSP* trees, two brown bears from Little Diomed Island, Alaska (EP35 and EP36) were grouped with the polar bears. Additionally, the maximum parsimony and neighbor-joining *ALAS* trees grouped EP36 with polar bears as well. Another brown bear from Alaska (EP55) was grouped with the polar bears in all of the *APOB* trees and in the maximum parsimony and maximum likelihood *TRSP* trees. Two other brown bears from Mt. Michelson, Alaska and Admiralty Island, Alaska (EP38 and EP50, respectively) were also grouped with polar bears in the *APOB* neighbor-joining and parsimony trees, but not in any of the other single gene trees.

The “Complete Bears Only” Bayesian and maximum likelihood trees match almost exactly (Figures 4 and 5). Both show two clades diverging at the same time from a common ancestor. The first contains only brown bear individuals, and the second contains all of the polar

bear individuals as well as two brown bears (EP35 and EP36). In both the Bayesian and maximum likelihood trees, EP43 (from Fairbanks, AK) is shown as the earliest branching lineage of the brown bear lineage. Within the brown bear lineage, the Bayesian tree draws a highly supported clade of five brown bears (0.99 Bayesian maximum posterior clade probability, BPP) from throughout the Alaska area (EP39, EP40, EP46, EP49, EP53, and EP72). This clade is not highly supported in the maximum likelihood tree. The clade containing polar bears is shown on both trees as a single polytomy (91% bootstrap support and 0.87 BPP on maximum likelihood and Bayesian trees, respectively), containing all the polar bear individuals, as well as two brown bears, EP35 and EP36.

The trees generated using the “All Bears” dataset also matched each other, although the Bayesian and likelihood trees differ slightly in their topology of the brown bear clade. In the Bayesian tree (Figure 2), the brown bear clade is depicted as a single polytomy (1.0 BPP). The polar bear clade (0.9485 BPP) is also depicted as a polytomy that contains all polar bear individuals as well as three brown bears, EP35, EP36, and EP55. The maximum likelihood tree (Figure 3) depicts a slightly different topology; in this tree, the common ancestor to all extant brown bears split and diverged into two large brown bear clades; however, both of these clades are unsupported. Polar bears are then shown emerging out of the second brown bear clade, with EP55 as the sister taxon to the polar bear lineage (80% bootstrap support). Again, the polar bear clade contains all of the polar bear individuals as well as EP35 and EP36.

DISCUSSION

Our data recover support for both of our hypotheses. While the Bayesian analyses recovered consistent phylogenies with both the “Complete Bears Only” and “All Bears” data

sets, the maximum likelihood analyses produced differing tree topologies. The maximum likelihood analysis of the concatenated “All Bears” data set (Figure 3) shows polar bears evolving from within the brown bear diversity, making brown bears paraphyletic with respect to polar bears (supporting hypothesis 2). In contrast, likelihood analysis of the concatenated “Complete Bears Only” data set (Figure 5) recovers two lineages diverging at the same time, with one evolving into a clade containing only brown bears, and the other into a clade containing all polar bears and a few brown bears. There are several possible reasons why our two data sets support different phylogenies. The first is that the “All Bears” data set contained more individuals than the “Complete Bears Only” data set. The key difference between the two likelihood trees is that in the “All Bears” tree, polar bears are shown as evolving out of a clade containing six brown bears: EP50, EP45, EP38, EP41, EP43, and EP55. However, four of these six individuals (EP50, EP45, EP41, and EP55) were not included in the “Complete Bears Only” data set and do not appear in the “Complete Bears Only” likelihood tree. Thus, the “Complete Bears Only” tree most likely fails to show paraphyly merely because of the absence of these brown bear individuals.

A second possible reason is that our entire concatenated dataset contained only 16 variable sites; furthermore, only 8 of those sites were informative. Thus, our trees were produced from a very limited number of informative SNPs, and our data probably only reflect a very small proportion of the actual diversity between polar and brown bear gene sequences. As a result, although the “All Bears” data set contained only a few more individuals, that difference was enough to support different phylogenies under maximum likelihood. Additionally, many nodes on our trees are not supported, meaning that along these nodes, the branching order is essentially random.

Evidence for Polar Bear-Brown Bear Hybridization

Although our results do not conclusively support a single hypothesis, there are a few trends that appear consistently. The first is that the brown bears EP35, EP36, and EP55 group within the polar bear clade in all four of our concatenated data trees. Both EP35 and EP36 are from Little Diomed Island, Alaska, and EP55 is from the Healy, AK area (Figure 1). To determine which genes are responsible for this grouping, we also examined these individuals' placements on the single gene trees. EP35 fell within the polar bear clade in two of the single gene trees (*APOB* and *TRSP*) and EP36 fell within the polar bear clade in three of the single gene trees (*APOB*, *TRSP*, and *ALAS*). Of the three genes for which sequence was acquired for EP55 (*APOB*, *TRSP*, and *ALAS*), this individual only appeared with the polar bears in one tree (*APOB*). Although our concatenated trees do not show this difference, each of these three brown bears have a different ratio of brown bear to polar bear-type alleles.

This phenomenon could be due to a number of different possibilities. The first explanation is recent hybridization between these bears' ancestral lines and polar bears. Recent data that show that polar bears and brown bears can and do mate in the wild (Kelly et al. 2010) suggest that bear interbreeding could have occurred throughout both species' histories. These three Alaskan brown bears from our analysis (EP35, EP36, and EP55) may be evidence of recent breeding between bear species throughout Alaska. While polar bears tend to mate on sea ice, brown bears mate on land; as follows, the most likely location for a polar and brown bear to meet and mate is on islands and coastal areas that span the boundary of these two species' ranges. As global warming continues, northern sea ice melts earlier in the spring, and breeding polar bears are forced onto land to hunt (Kelly et al. 2010). This atypical behavior brings them within the same ecological range as brown bears, facilitating the possibility of interbreeding. Islands are

particularly advantageous locations for hybridization because they are accessible to both marine and terrestrial species. Both EP35 and EP36 are from Little Diomedede Island (Figure 1), whose location within both species' ranges could allow for frequent interbreeding in this area. Thus, EP35 and EP36 could be the resulting hybrids of such interspecies contact. In contrast, EP55 was found in central Alaska (Figure 1). Because polar bears rely on the marine environment for food and mating, it is less likely that frequent hybridization between polar bears and brown bears would occur in a non-coastal area like Healy, Alaska.

A second explanation for our results is incomplete lineage sorting (ILS). Like hybridization, ILS can result in gene phylogenies that are discordant with species phylogeny. Unlike hybridization however, ILS is a random process that affects all individuals with the same probability. If ILS has occurred with the genes examined here, then we would expect that for any given gene, all brown bear individuals would group within the polar bear clade with equal likelihood. In contrast, our data show the same three individuals (EP35, EP36, and EP55) grouping within the polar bear clade in all four concatenated trees and many single gene trees. Therefore, we hypothesize that the relationship between these bears and polar bears is more likely due to hybridization than to ILS.

Human error may also explain our results. Human error could have occurred at several levels, and involved mislabeling, extraction contamination, and misidentification of bear samples. One simple way to check that these bears are actually brown bears is by examining their mitochondrial DNA. Because the relationship of polar bear mitochondria as monophyletic to extant brown bears is well established, constructing the phylogenies of these three bears' mitochondrial DNA would quickly identify them as brown bears, mislabeled polar bears, or hybrids. To exclude contamination of a brown bear sample with polar bear DNA during

extraction, we could re-extract the DNA from our samples and repeat the analysis. Finally, acquiring a second sample of the same bears from the original museum and analyzing that would allow us to ensure that the original sample had not merely been mislabeled.

Phylogenetic Position of ABC Island Brown Bears

A second trend in our results is that ABC Island bears (EP49-EP52) consistently group with brown bears rather than polar bears. This differs from the known mitochondrial phylogeny, where the ABC Island bears cluster as the sister group to extant polar bears. For example, in the Bayesian “All Bears” tree (Figure 2), all four ABC Island bears fall into a clade with the other brown bears (1.0 BPP) rather than with the polar bears. Although the “Complete Bears Only” data set only included one ABC Island Bear (EP49), it also grouped with the other brown bears (Figures 4 and 5). If the established mitochondrial relationship between brown bears and polar bears reflects their true species phylogeny, then trees estimated using both mitochondrial and nuclear data should match. However, our nuclear data place these ABC Island bears with other brown bears. This placement could lend support for our second hypothesis and suggest that the close relationship between ABC Island brown bear mitochondria and polar bear mitochondria may reflect the history of these populations’ maternal lineages rather than their true species phylogeny. To prove this hypothesis, further analyses of the mitochondrial make-up of these particular bears is necessary. It is entirely possible that although these bears come from the ABC Islands, their mitochondrial sequences fall outside of the ABC Island brown bear clade. If this were the case, then the placement of their nuclear sequences with other brown bears would be expected. Thus, although these bears’ inclusion in the brown bear clade is interesting, we cannot definitively conclude support for either of our hypotheses without further research.

Further Studies

One important piece of information gained from our work has been the amount of information contained in seven nuclear genes. Through our analyses we have found that the genes *PLP*, *FES*, and *IRBP* are completely uninformative for inferring species phylogeny with respect to brown bears and polar bears. While *ALAS*, *APOB*, *APP*, and *TRSP* were somewhat informative, they also contained very few informative sites. In order to further define polar bear phylogeny, it will be vital to identify new nuclear markers. The challenge in this pursuit is finding markers that are evolving more slowly than mitochondrial genes, yet quickly enough that polar and brown bear sequences would be different. Thus, targeting new nuclear introns is a logical next step. Although it may seem attractive to examine microsatellites, past analyses of these sequences for studying species divergence has shown that they often evolve too quickly to be informative. Past work using microsatellites in black, brown, and polar bears (Paetkau et al. 1997) has shown that these regions are not informative for inferring phylogenies on large time scales. Microsatellites evolve by following the stepwise mutation model (Kimura and Crow 1964) whereby they gain or lose single repeats. Although in theory this allows for an infinite number of allele states, in reality, allele sizes are constrained. As a result, analyses based on microsatellite data tend to underestimate the genetic distance between species over long time scales.

Another important factor for future work is geographic range. Our data set primarily contained bears from throughout the Alaska area; however, polar and brown bears have a much larger geographic range. Expanding the geographic range of our sampled bears could allow for more diversity to be uncovered and better supported phylogenies to be constructed.

Conclusions

Polar bears are a vital part of the arctic ecosystem. Acting as the top predators in their habitat, polar bears play an indispensable role in maintaining the arctic food chain. Recent ecological research is beginning to make clear the importance of apex consumers for maintaining ecosystems (Estes et al. 2011). Removal or depletion of a single top feeder species can completely devastate an ecosystem by altering the populations of species lower in the food chain. Not only does this upset the balance of biodiversity within an ecosystem, but it can also lead to habitat destruction, collapse of valuable species, alterations of atmospheric makeup, and alteration of disease dynamics (Estes et al. 2011). Apex consumers like polar bears play a large part in controlling their ecosystems from the top down by maintaining healthy trophic cascades. Thus, conservation of polar bears could act as a way to conserve all of the smaller organisms living within the same ecosystem. The arctic ecosystem is finely balanced, with few organisms adapted to live in the hostile climate. With the current state of global climate change, polar bears' habitats are decreasing, placing extreme stress on their species. In order to conserve this valuable ecosystem, maintenance of its top predator should be a priority. In order to inform conservation efforts, it is necessary to understand both how polar bears have evolved, and the role of hybridization with brown bears in their long-term evolution. Understanding a species' phylogeny allows us to gain deeper knowledge of the existing biodiversity on the planet today, and how that biodiversity is divided amongst clades within a species. By mapping the biodiversity that exists among and within species, conservation efforts can be guided to maximize diversity preservation. Elucidating the role of hybridization in polar bears' species history is important because it provides insight into how the brown bear and polar bear species have remained distinct throughout past periods of interbreeding. Additionally, hybrids may play

an important role in species survival during periods of climate change by allowing essential genes to be maintained in bear populations. As such, conserving hybrid animals may be a crucial step in preserving both bear species.

REFERENCES

- Amrine-Madsen, H., Koepfi, K.P., Wayne, R.K., Springer, M.S. (2003). A new phylogenetic marker, apolipoprotein B, provides compelling evidence for eutherian relationships. *Mol. Phylogenet. Evol.* 2, 225-240.
- Bardeleben, C., Moore, R.L., Wayne, R.K. (2005). Isolation and molecular evolution of the selenocysteine tRNA (Cf TRSP) and RNase P RNA (Cf RPPH1) genes in the dog family, Canidae. *Mol. Phylogenet. Evolut.* 2, 347-359.
- Cronin, M.A., Armstrup, K.S., Garner, G.W., and Vyse, E.R. (1991). Interspecific and intraspecific mitochondrial DNA variation in North American bears (*Ursus*). *Can. J. Zool.* 69, 2985-2992.
- Davison, J., Ho, S.Y.W., Bary, S., Korsten, M., Vulla, E., Hindrikson, M., Ostbye, K., Ostbye, E., Lauritzen, S.-E., Austin, J., et al. (2011). Late-Quaternary biogeographic scenarios for a wild mammal model species, the brown bear (*Ursus arctos*). *Quat. Sci. Rev.* 30, 418-430.
- Edwards, C.J., Suchard, M.A., Lemey, P., Welch, J.J., Barnes, I., Fulton, T.L., Barnett, R., O'Connell, T.C., Coxon, P., Monaghan, N., Valdiorsera, C.E., Lorenzen, E.D., Willersleve, E., Baryshnikov, G.F., Rambaut, A., Mark, G.T., Bradley, D.G., Shapiro, B. (2011). Ancient hybridization and an irish origin for the modern polar bear matriline. *Current Biology* 21, 1-8.
- Estes, J.A., Terborgh, J., Brashares, J.S., Power, M.E., Berger, J., Bond, W.J., Carpenter, S.R., Essington, T.E, Holt, R.D., Jackson, J.B.C., Marquis, R.J., Oksanen, L., Orksanen, T., Paine, R.T., Pickett, E.K., Ripple, W.J., Sandin, S.A., Scheffer, M., Schoener, T.W., Shurin, J.B., Sinclair, A.R.E., Soule, M.E., Virtanen, R., Wardle, D.A. (2011). Trophic downgrading of planet Earth. *Science* 333, 301-306.
- Ho, S.Y., Saarma, U., Barnett, R., Haile, J., and Shapiro, B. (2008). The effect of inappropriate calibration: Three case studies in molecular ecology. *PloS ONE* 3, e1615.
- Ingolfsson, O., and Wiig, O. (2009). Late Pleistocene fossil find in Svalbard: The oldest remains of a polar bear (*Ursus maritimus* Phipps, 1744) ever discovered. *Polar Res.* 28, 455-462.
- Kelly, B.P., Whiteley, A., and Tallmon, D. (2010). The arctic melting pot. *Nature* 468, 891.
- Kimura, M. and Crow, J.F. (1964). The number of alleles that can be maintained in a finite population. *Genetics* 49, 725-738.

- Krause, J., Unger, T., Noçon, A., Malaspinas, A.S., Kolokotronis, S.O., Stiller, M., Soibelzon, L., Spriggs, H., Dear, P.H., Briggs, A.W., Bray, S.C., O'Brien, S.J., Rabeder, G., Matheus, P., Cooper, A., Slatkin, M., Pääbo, S., Hofreiter, M. (2008). Mitochondrial genomes reveal an explosive radiation of extinct and extant bears near the Miocene-Pliocene boundary. *BMC Evol Biol.* 8:220.
- Kurten, B. (1964). The evolution of the polar bear, *Ursus maritimus* (Phipps). *Acto Zool. Fenn.* 108, 1-30.
- Lindqvist, C., Schuster, S.C., Sun, Y., Talbot, S.L., Qi, J., Ratan, A., Tomsho, L.P., Kasson, L., Zeyl, E., Aars, J., et al. (2010). Complete mitochondrial genome of a Pleistocene jawbone unveils the origin of polar bear. *Proc. Natl. Acad. Sci. USA* 107, 5053-5057.
- Murphy, W.J., Eizirik, E., Johnson, W.E., Zhang, Y.P., Ryder, O.A., O'Brien, S.J. (2001). Molecular phylogenetics and the origins of placental mammals. *Nature* 409, 614-8.
- Paetkow, D., Waits, L.P., Clarkson, P.L., Craighead, L., Strobeck, C. (1997). An empirical evaluation of genetic distance statistic using microsatellite data from bear (Ursidae) populations. *Genetics* 4, 1943-1957.
- Pages, M., Calvignac, S., Klein, C., Paris, M., Hughes, S., and Hanni, C. (2008). Combined analysis of fourteen nuclear genes refines the Ursidae phylogeny. *Mol. Phylogenet. Evol.* 47, 73-83.
- Posada, D. (2008). JModeltest: Phylogenetic model averaging. *Molecular Biology and Evolution* 25, 1253-1256.
- Rambaut, A. (2009). FigTree v1.3.1: Tree figure drawing tool.
- Ronquist, H., Huelsenbeck, J.P. (2003). MrBayes v3.1.2: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19,1572-1574.
- Shields, G.F., Adams, D., Garner, G., Labelle, M., Pietsch, J., Ramsay, M., Schwartz, C., Titus, K., and Williamson, S. (2000). Phylogeography of mitochondrial DNA variation in brown bears and polar bears. *Mol. Phylogenet. Evol.* 15, 319-326.
- Shields, G.F., and Kocher, T.D. (1991). Phylogenetic Relationships of North American ursids based on analysis of mitochondrial DNA. *Evolution* 45, 218-221.
- Stamatakis, A. (2006). RAxML-vI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22, 2688-2690.
- Stirling I, Guravich D. (1998). *Polar Bears*. Ann Arbor: University of Michigan Press.
- Swofford, D.L. (2002). *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods)*. Sinauer Associates, Sunderland, MA.

Talbot, S.L., and Shields, G.F. (1996). Phylogeography of brown bears (*Ursus arctos*) of Alaska and paraphyly within the Ursidae. *Mol. Phylogenet. Evol.* 5, 477-494.

Yu, L., Li, A.Q., Ryder, O.A., and Zhang, Y.P. (2004). Phylogeny of the bears (Ursidae) based on nuclear and mitochondrial genes. *Mol. Phylogenet. Evol.* 32, 480-494.

TABLES

Table 1: Brown Bear and Polar Bear Sample Names, Accession Numbers, and Locations

Species	Sample Name	PSU Accession Number	Museum Accession Number	Country of Origin	State of Origin	Specific Location	Sample Type
<i>Ursus maritimus</i>	BR01	UP08.TF005	2372KF	USA	Alaska	Diomede	Tissue
<i>Ursus maritimus</i>	BR03	UP08.TF001	2367KB	USA	Alaska	Barrow	Tissue
<i>Ursus maritimus</i>	BR05	UP08.TF002	990083KD	USA	Alaska	Barrow	Tissue
<i>Ursus maritimus</i>	LM01	UP08.TF017	980369KB	USA	Alaska	Savoonga	Tissue
<i>Ursus maritimus</i>	LM03	UP08.TF014	970201KA	USA	Alaska	Nuiqsut	Tissue
<i>Ursus maritimus</i>	LM05	UP08.TF012	990658KB	USA	Alaska	Gambell	Tissue
<i>Ursus maritimus</i>	BR30	UP10.TF280	X3256705A	Canada	Northwest Territory	North Beaufort	Tissue
<i>Ursus maritimus</i>	BR70	UP10.TF282	X3254605A	Canada	Northwest Territory	North Beaufort	Tissue
<i>Ursus maritimus</i>	BR71	UP10.TF279	X1004400A	Canada	Northwest Territory	North Beaufort	Tissue
<i>Ursus maritimus</i>	BR79	UP10.TF331	X1003706A	Canada	Manitoba	West Hudson	Tissue
<i>Ursus maritimus</i>	BR80	UP10.TF363	X3248906A	Canada	Manitoba	West Hudson	Tissue
<i>Ursus maritimus</i>	BR83	UP10.TF368	X3311107A	Canada	Manitoba	West Hudson	Tissue
<i>Ursus maritimus</i>	BR84	UP10.TF373	X3313206A	Canada	Manitoba	West Hudson	Tissue
<i>Ursus arctos</i>	EP35	UP10.TF223	UAM101310	USA	Alaska	Little Diomede Island	Tissue
<i>Ursus arctos</i>	EP36	UP10.TF224	UAM101335	USA	Alaska	Little Diomede Island	Tissue
<i>Ursus arctos</i>	EP37	UP10.TF225	UAM19765	USA	Alaska	Anchorage	Tissue
<i>Ursus arctos</i>	EP38	UP10.TF226	UAM24112	USA	Alaska	Mt. Michelson	Tissue
<i>Ursus arctos</i>	EP39	UP10.TF227	UAM24962	USA	Alaska	Big Delta	Tissue
<i>Ursus arctos</i>	EP40	UP10.TF228	UAM33812	USA	Alaska	Fairbanks	Tissue
<i>Ursus arctos</i>	EP41	UP10.TF229	UAM33815	USA	Alaska	Talkeetna Mountains	Tissue
<i>Ursus arctos</i>	EP43	UP10.TF230	UAM33982	USA	Alaska	Fairbanks	Tissue
<i>Ursus arctos</i>	EP45	UP10.TF232	UAM49381	USA	Alaska	Gulf of Alaska	Tissue
<i>Ursus arctos</i>	EP46	UP10.TF233	UAM48385	USA	Alaska	Gulf of Alaska	Tissue
<i>Ursus arctos</i>	EP49	UP10.TF236	UAM63856	USA	Alaska	Chichagof Island	Tissue
<i>Ursus arctos</i>	EP50	UP10.TF237	UAM63857	USA	Alaska	Admiralty Island	Tissue
<i>Ursus arctos</i>	EP51	UP10.TF238	UAM63858	USA	Alaska	Chichagof Island	Tissue
<i>Ursus arctos</i>	EP52	UP10.TF239	UAM63869	USA	Alaska	Admiralty Island	Tissue
<i>Ursus arctos</i>	EP53	UP10.TF240	UAM70670	Canada	Yukon Territory	NA	Tissue
<i>Ursus arctos</i>	EP54	UP10.TF241	UAM70604	USA	Alaska	Talkeetna	Tissue
<i>Ursus arctos</i>	EP55	UP10.TF242	UAM70620	USA	Alaska	Healy	Tissue
<i>Ursus arctos</i>	EP72	UP10.TF257	UAM92550	USA	Alaska	Beechey Point	Tissue

Table 2: Primers and Gene Reference

Gene	Gene Name and Amplified Region	Primer Reference	Forward (F) and Reverse (R) Primer Sequences	Sequence Length (base pairs)
<i>TRSP</i>	<i>Selenocysteine tRNA</i>	Bardeleben et al. 2005	F: GGGCTTCTGAAAGCCGACTT R: CCGCCCCGAAAGGTGGAATTG	392
<i>APOB</i>	<i>Apolipoprotein B, partial exon 26</i>	Jiang et al. 1998 Amrine-Madsen et al. 2003	F: GTGCCAGGTTCAATCAGTATAAGT R: CCAGCAAAATTTTCTTTTACTTCAA	963
<i>ALAS</i>	<i>Aminolevulinate, delta-, synthase 2, intron 7</i>	Newly designed by Dr. Tara Fulton	F: CATCACCTTGTGGATTTATTA R: AGAGGTAGGAGATCACAACC	~ 900*
<i>APP</i>	<i>Amyloid Beta (A4) Precursor Protein, 3' UTR</i>	Murphy et al. 2001	F: TCCAAGATGCAGCAGAACG R: CTAATGTGTGCACATAAAACAGG	937
<i>PLP</i>	<i>Proteolipid protein, intron 3</i>	Newly designed by Dr. Tara Fulton	F: GGCCTCATCCCCACCCAA R: TGGTTAGAAATCAGCATCGGC	950
<i>IRBP</i>	<i>Interphotoreceptor binding protein, exon 1</i>	Fulton and Strobeck 2006	F: GGTAGAGTGCCGACAAAGATC R: GCTTTCTGGAGGTCCAGGGC	1184
<i>FES</i>	<i>Feline sarcoma protooncogene, exon 14</i>	Venta et al. 1996	F: GGGGAACTTTGGCGAAGTGTT R: TCCATGACGATGTAGATGGG	420

* *ALAS*'s sequence length is estimated to be approximately 900 base pairs. The presence of a microsatellite region in *ALAS* has made its exact length difficult to ascertain.

Table 3: GenBank Accession Numbers for American Black Bear Sequences

Gene	Species	Genbank Accession Number
<i>TRSP</i>	<i>Ursus americanus</i>	AY609123.1
<i>APOB</i>	<i>Ursus americanus</i>	GU930905.1
<i>ALAS2</i>	<i>Ursus americanus</i>	AB294723.1
<i>APP</i>	<i>Ursus americanus</i>	unavailable

Table 4: Amplification PCR Conditions

Gene Designation	Gene Name and Amplified Region	Amplification Conditions in Brown Bears	Amplification Conditions in Polar Bears
<i>TRSP</i>	<i>Selenocysteine tRNA</i>	Incubation: 95° C for 2 min. 40 cycles of: 95° C for 30 sec, 54° C for 30 sec, 72° C for 45 sec Final Elongation: 72° C for 5 min. Hold: 4° C forever	Incubation: 95° C for 2 min. 40 cycles of: 95° C for 30 sec, 54° C for 30 sec, 72° C for 45 sec Final Elongation: 72° C for 5 min. Hold: 4° C forever
<i>APOB</i>	<i>Apolipoprotein B, partial exon 26</i>	Incubation: 94° C for 3 min. 30 cycles of: 95° C for 45 sec, 55° C for 30 sec, 72° C for 1:30 min Final Elongation: 72° C for 10 min. Hold: 4° C forever	Incubation: 94° C for 3 min. 30 cycles of: 95° C for 45 sec, 55° C for 30 sec, 72° C for 1:30 min Final Elongation: 72° C for 10 min. Hold: 4° C forever
<i>ALAS</i>	<i>Aminolevulinate, delta-, synthase 2, intron 7</i>	Incubation: 95° C for 2 min. 40 cycles of: 95° C for 45 sec, 54° C for 45 sec, 72° C for 1:30 min Final Elongation: 72° C for 5 min. Hold: 4° C forever	Incubation: 95° C for 2 min. 40 cycles of: 95° C for 45 sec, 52° C for 45 sec, 72° C for 1:30 min Final Elongation: 72° C for 5 min. Hold: 4° C forever
<i>APP</i>	<i>Amyloid Beta Precursor Protein, 3' UTR</i>	Incubation: 95° C for 2 min. 45 cycles of: 95° C for 30 sec, 64° C for 30 sec, 72° C for 45 min Final Elongation: 72° C for 5 min. Hold: 4° C forever	Incubation: 95° C for 2 min. 45 cycles of: 95° C for 30 sec, 65° C for 30 sec, 72° C for 45 min Final Elongation: 72° C for 5 min. Hold: 4° C forever

Table 5: Gene Sequences Acquired for Each Individual

	<i>ALAS</i>	<i>APP</i>	<i>APOB</i>	<i>TRSP</i>
BR01	Bi-directional	Bi-directional	Bi-directional	Bi-directional
BR03	Bi-directional	Bi-directional	Bi-directional	Bi-directional
BR05	Bi-directional	Reverse Only	Bi-directional	Bi-directional
LM01	Bi-directional	Bi-directional	Bi-directional	Bi-directional
LM03	Bi-directional	Bi-directional	Bi-directional	Reverse Only
LM05	Bi-directional	Reverse Only	Bi-directional	Reverse Only
BR30	Bi-directional	Bi-directional	Bi-directional	Bi-directional
BR70	*	Bi-directional	Bi-directional	Bi-directional
BR71	Forward Only	*	Bi-directional	Bi-directional
BR79	Bi-directional	Bi-directional	Bi-directional	Bi-directional
BR80	Bi-directional	Bi-directional	Bi-directional	Forward Only
BR83	Bi-directional	Bi-directional	Bi-directional	Bi-directional
BR84	Bi-directional	Bi-directional	Bi-directional	Bi-directional
EP35	Bi-directional	Bi-directional	Bi-directional	Bi-directional
EP36	Forward Only	Reverse Only	Bi-directional	Bi-directional
EP37	Bi-directional	*	Bi-directional	Bi-directional
EP38	Bi-directional	Reverse Only	Bi-directional	Bi-directional
EP39	Bi-directional	Bi-directional	Bi-directional	Bi-directional
EP40	Bi-directional	Bi-directional	Bi-directional	Bi-directional
EP41	*	Reverse Only	Bi-directional	Bi-directional
EP43	Reverse Only	Bi-directional	Bi-directional	Bi-directional
EP45	Bi-directional	Bi-directional	*	Bi-directional
EP46	Reverse Only	Bi-directional	Bi-directional	Bi-directional
EP49	Reverse Only	Bi-directional	Bi-directional	Bi-directional
EP50	*	Reverse Only	Bi-directional	Bi-directional
EP51	*	Bi-directional	Bi-directional	Bi-directional
EP52	*	Reverse Only	*	Bi-directional
EP53	Forward Only	Reverse Only	Bi-directional	Bi-directional
EP54	*	Bi-directional	Bi-directional	Bi-directional
EP55	Forward Only	*	Bi-directional	Bi-directional
EP72	Bi-directional	Bi-directional	Bi-directional	Bi-directional

* = no sequence acquired

Table 6: Number of Characters, Variable Sites, and Parsimony Informative Sites for Single Gene and Concatenated Data Sets

Data Set	Total Number of Characters	Number of Variable Sites	Parsimony Informative Variable Sites
<i>TRSP</i>	392	2	2
<i>APOB</i>	849	8	4
<i>ALAS</i>	852	5	2
<i>APP</i>	533	1	0
Concatenated Data	2626	16	8

Table 7: Model of Evolution Determined by jModelTest 0.1.1

Gene	Model of Evolution Determined by jModelTest 0.1.1	Corresponding Number of Rates
<i>TRSP</i>	K80	2
<i>APOB</i>	TrN	6
<i>ALAS</i>	TIM+I	6
<i>APP</i>	HKY	2

FIGURES

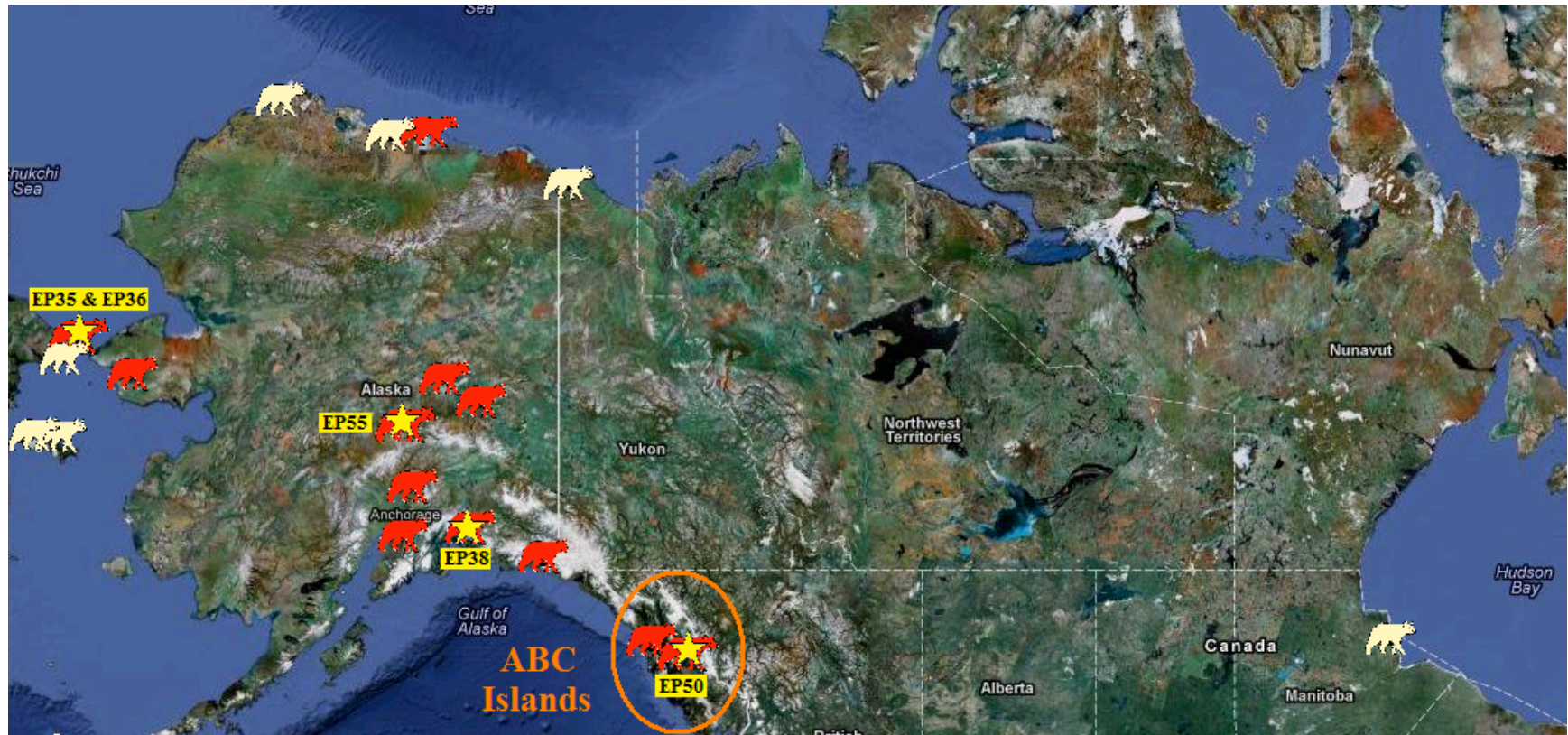


Figure 1: Geographic Range of Polar Bear and Brown Bear Samples

All samples were obtained from the Alaska area. Each bear on the map represents a single location of sample acquisition. Red bears represent brown bear sample locations and white bears represent polar bear sample locations. The starred bears indicate brown bears who grouped within the polar bear clade in some analyses as described in the text. The starred bears' sample names are indicated in yellow tags. The location of the ABC Islands is indicated in orange.

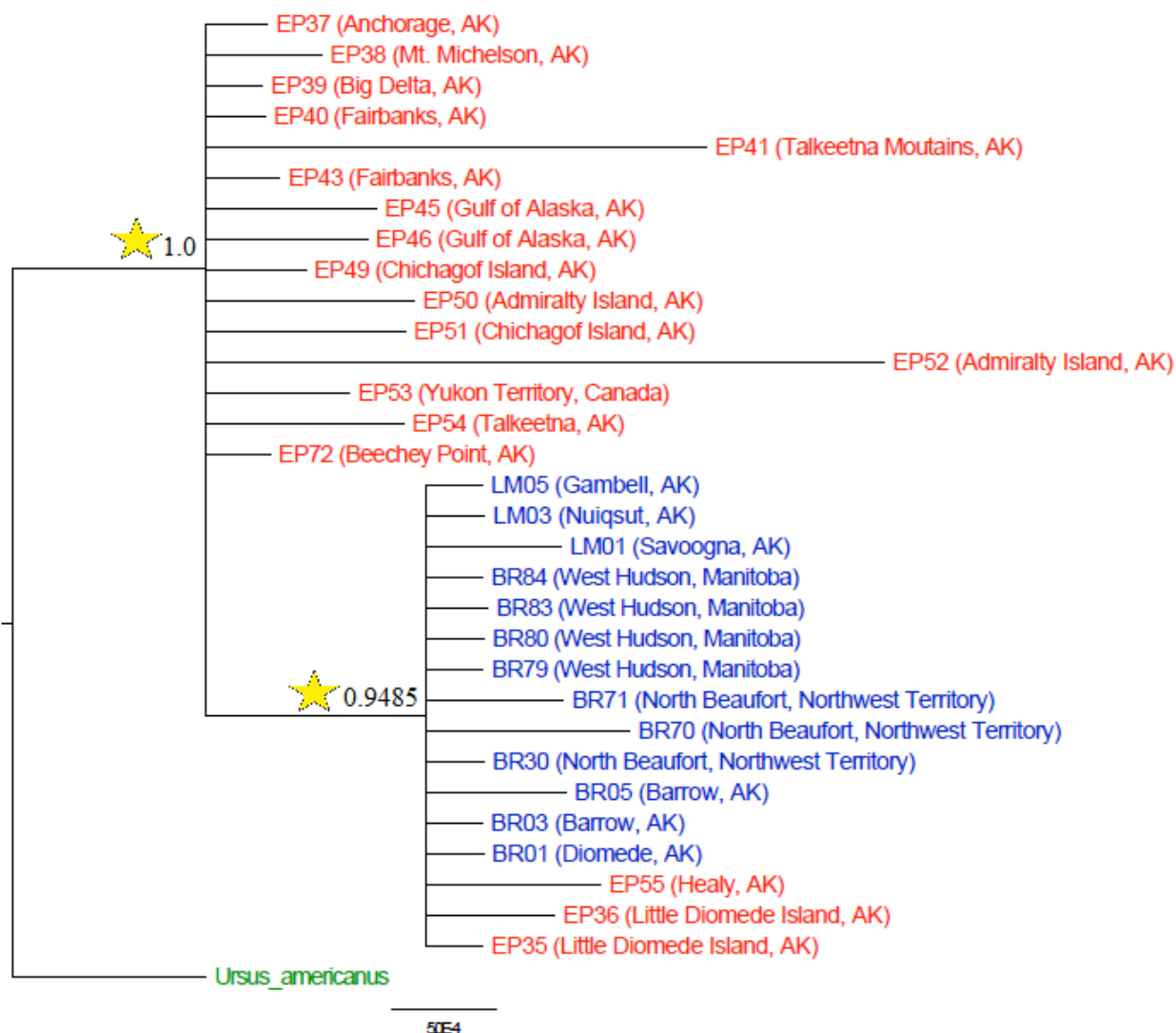


Figure 2: Bayesian Inference Tree Constructed Using “All Bears” Data Set

All brown bear individuals are listed in red and all polar bear individuals are listed in blue. Stars indicate branches with high support (>90% bootstrap or >0.9 BPP).

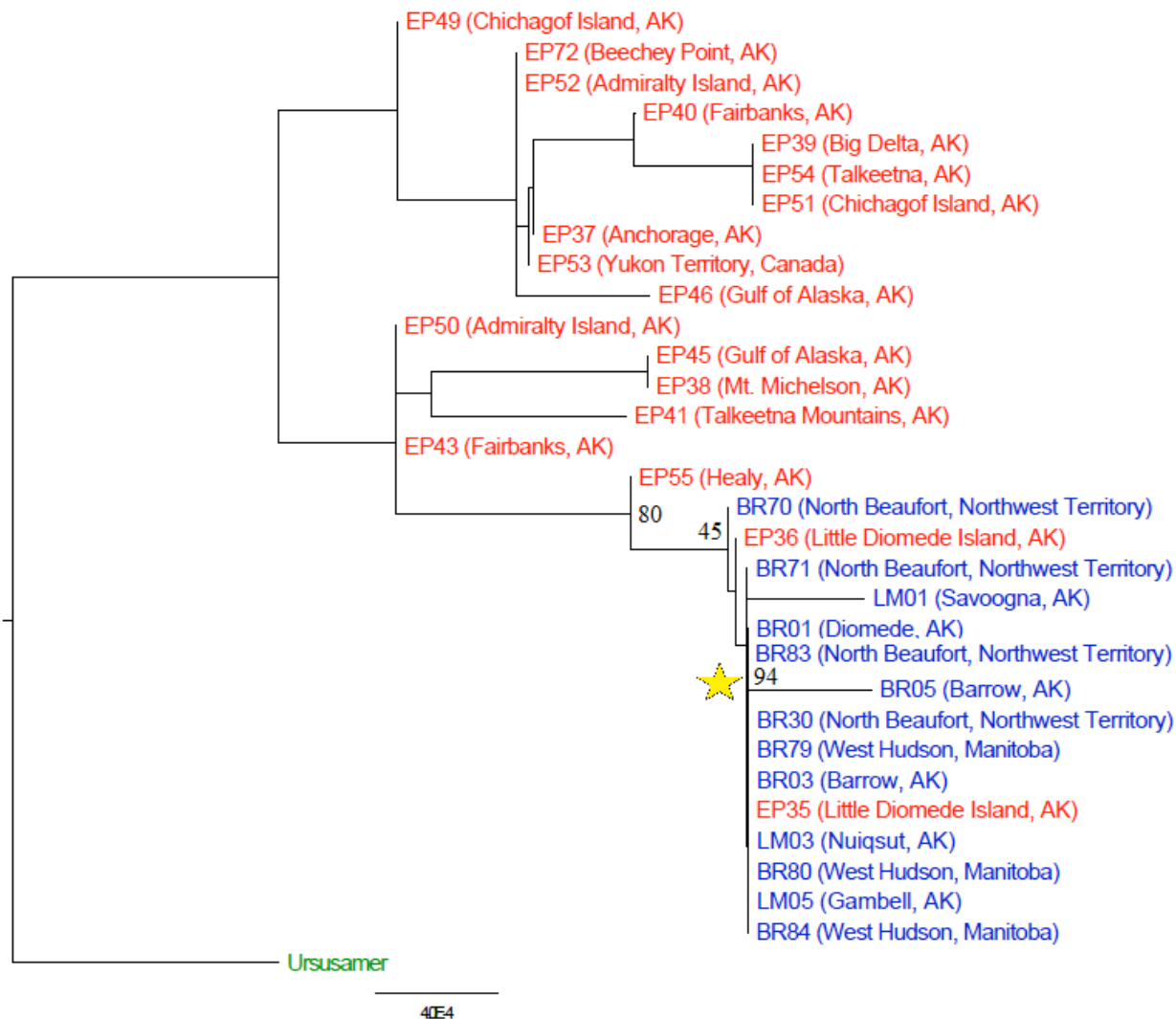


Figure 3: Maximum Likelihood Tree Constructed Using “All Bears” Data Set
 All brown bear individuals are listed in red and all polar bear individuals are listed in blue. Stars indicate branches with high support (>90% bootstrap or >0.9 BPP).

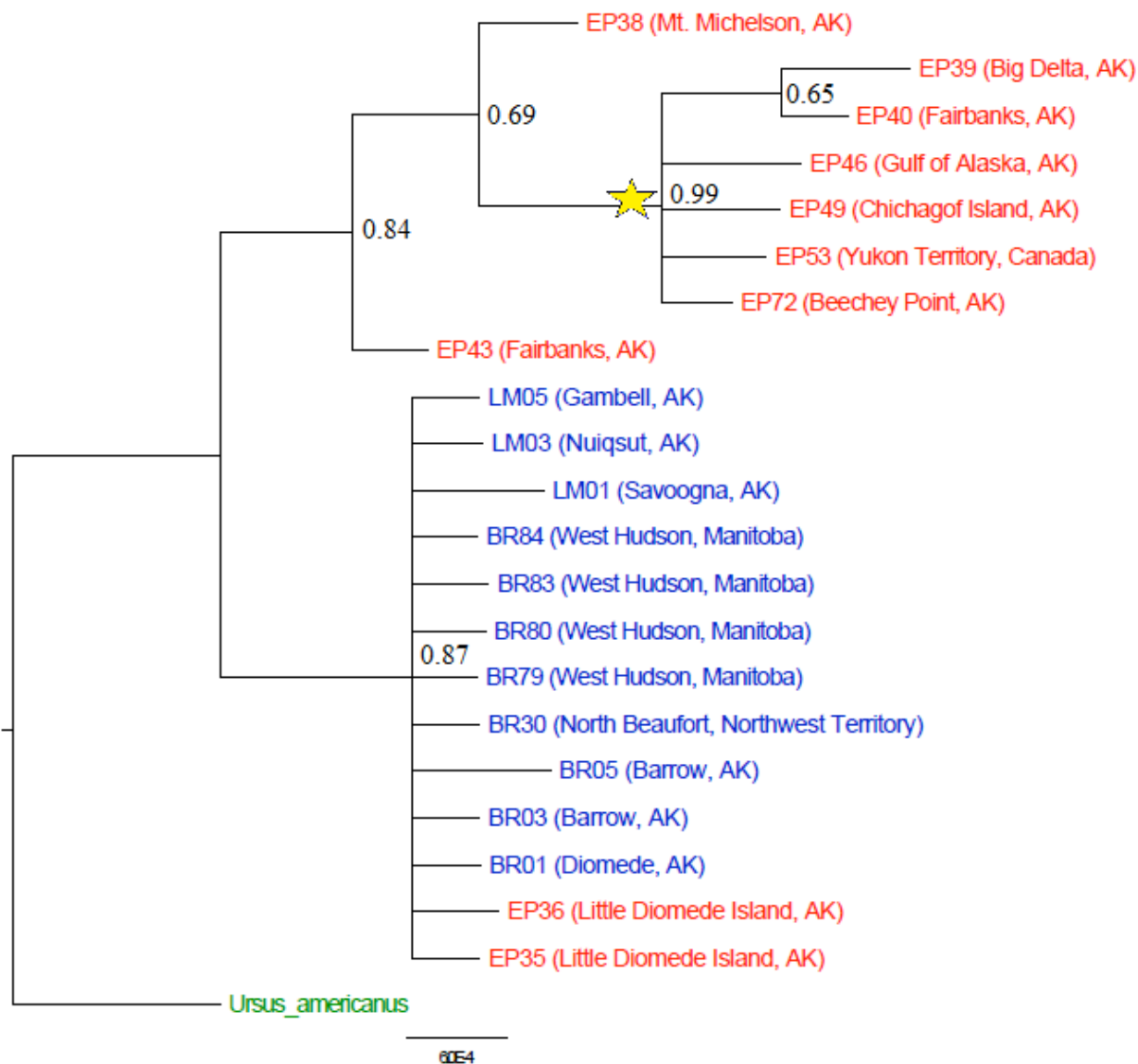


Figure 4: Bayesian Inference Tree Constructed Using “Complete Bears Only” Data Set
 All brown bear individuals are listed in red and all polar bear individuals are listed in blue. Stars indicate branches with high support (>90% bootstrap or >0.9 BPP).

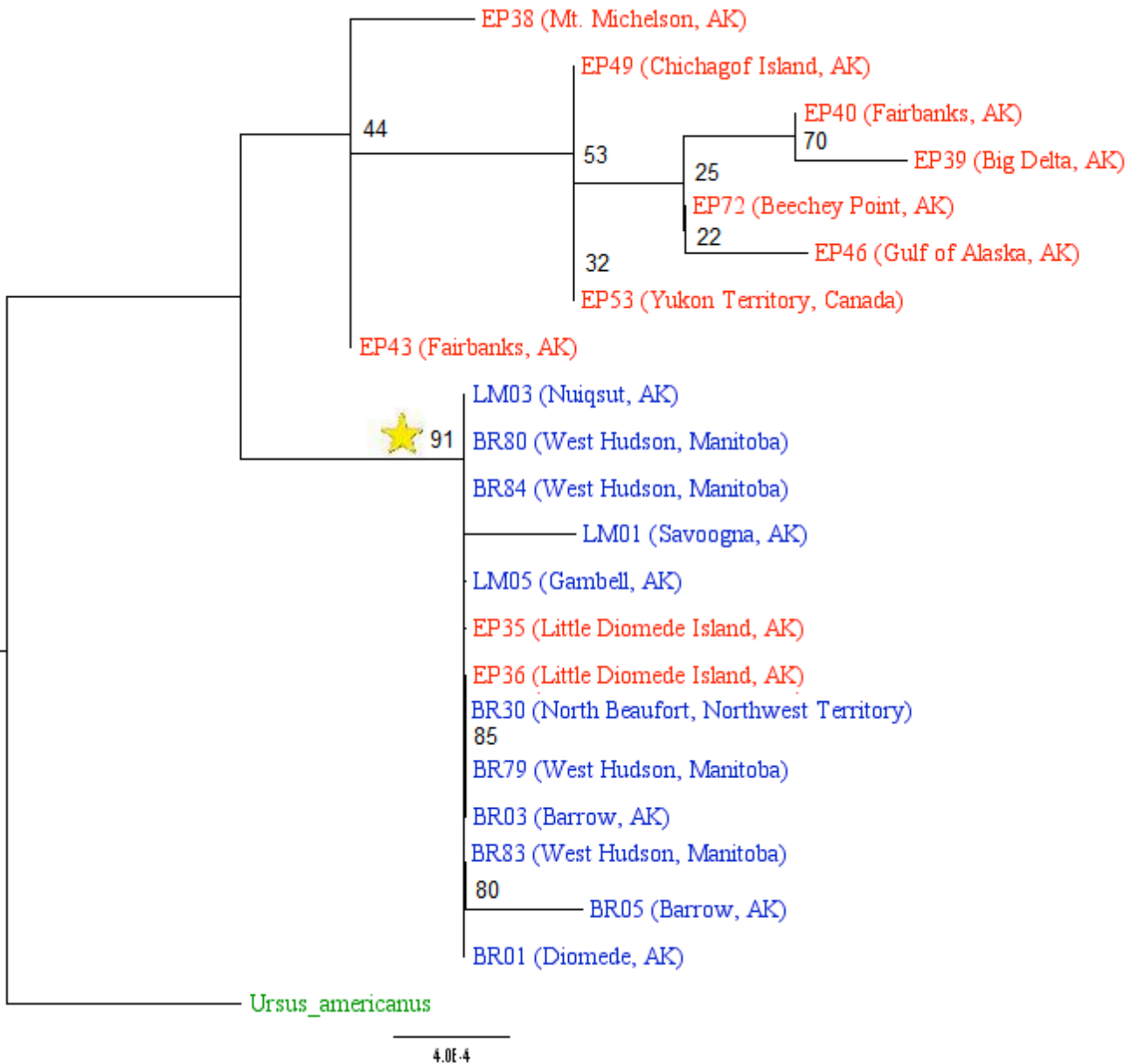
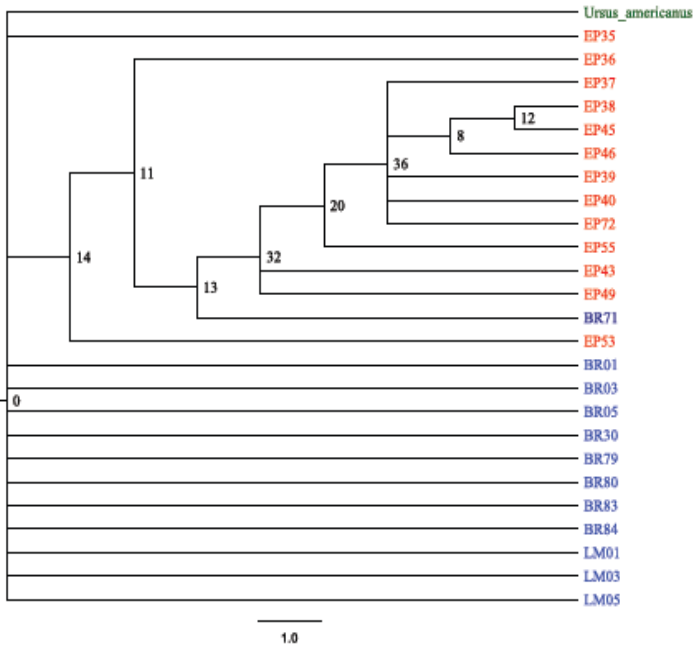


Figure 5: Maximum Likelihood Tree Constructed Using “Complete Bears Only” Data Set
 All brown bear individuals are listed in red and all polar bear individuals are listed in blue. Stars indicate branches with high support (>90% bootstrap or >0.9 BPP).

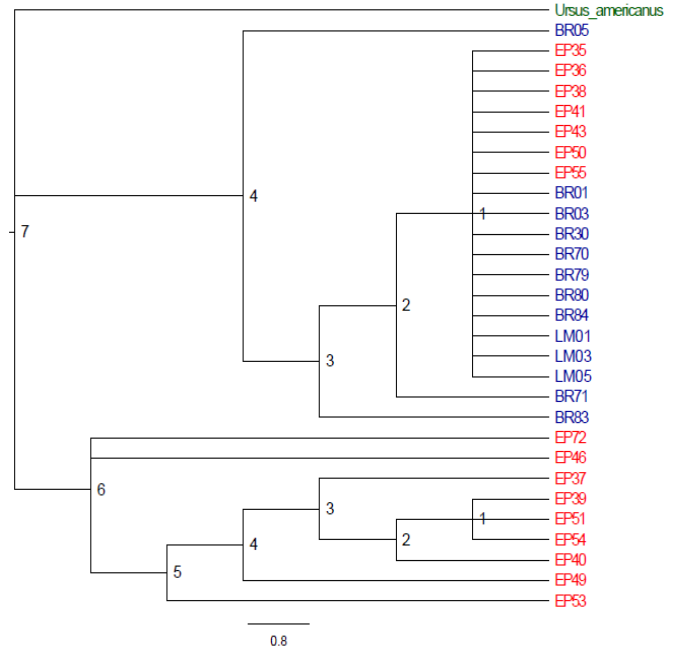
APPENDICES

Appendix A: Single Gene Trees Generated Using Maximum Parsimony Analysis

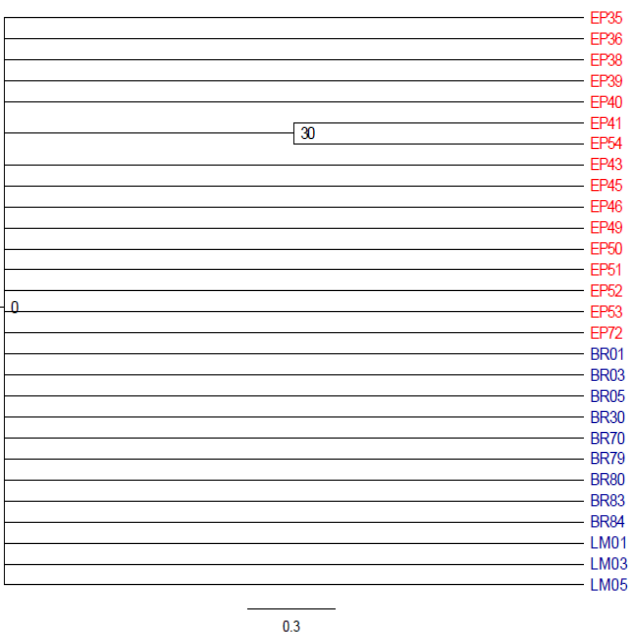
1. *ALAS*



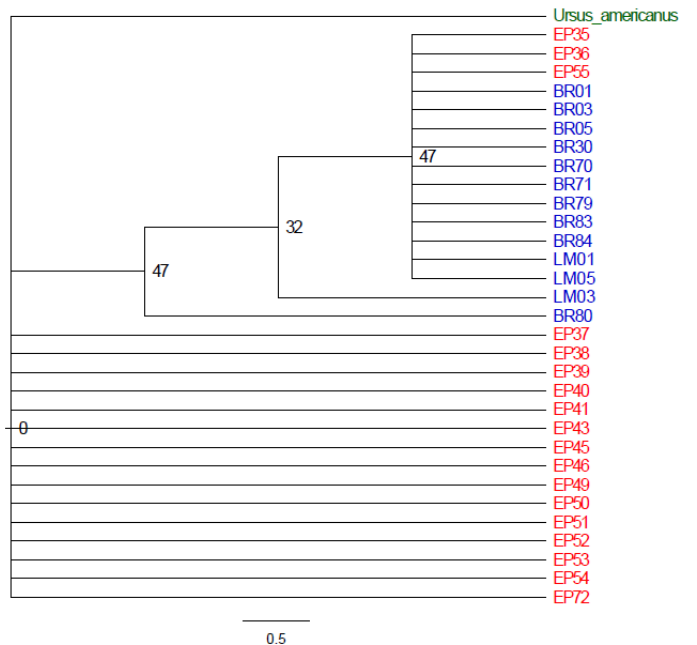
2. *APOB*



3. *APP*

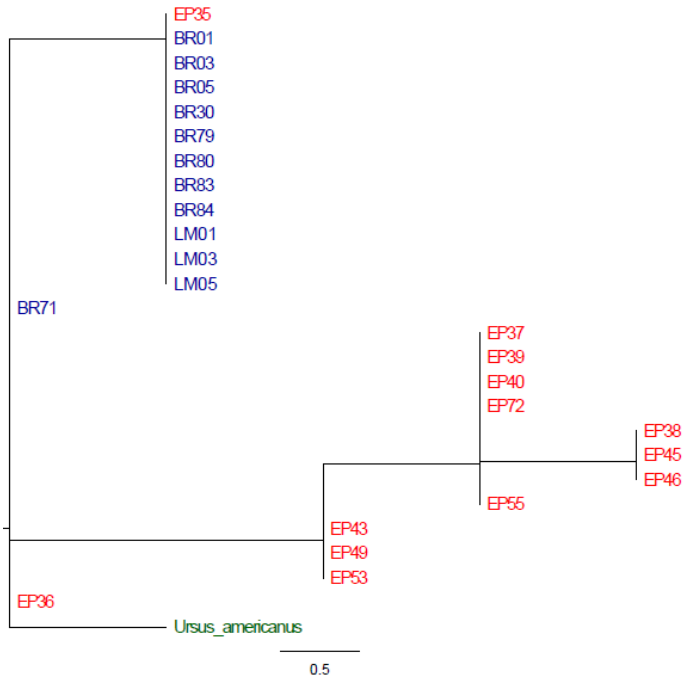


4. *TRSP*

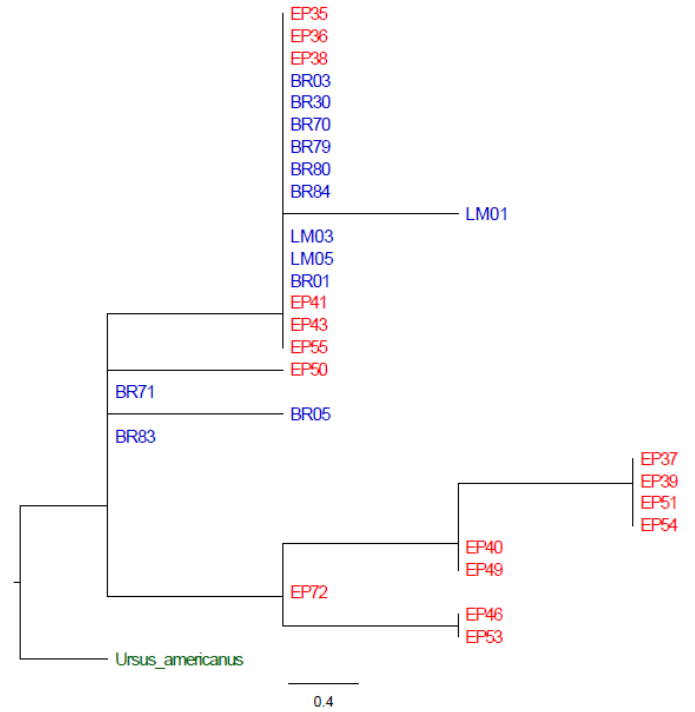


Appendix B: Single Gene Trees Generated Using Neighbor-Joining Analysis

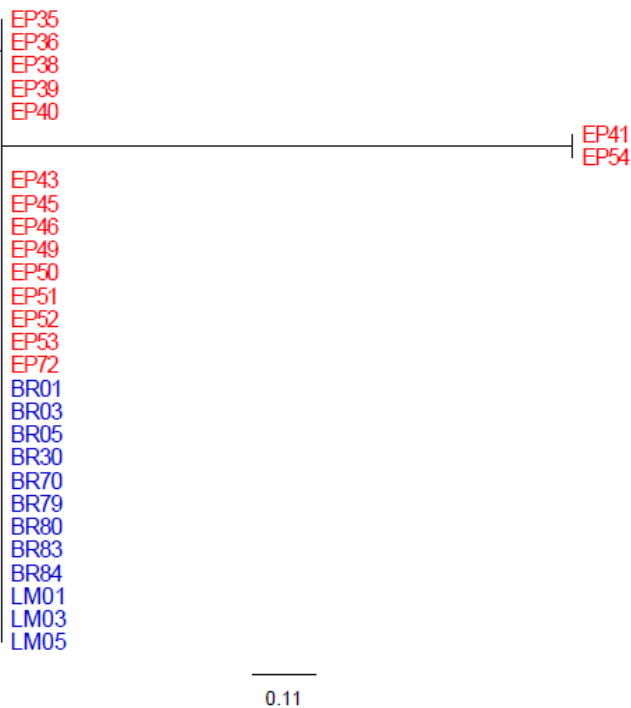
1. *ALAS*



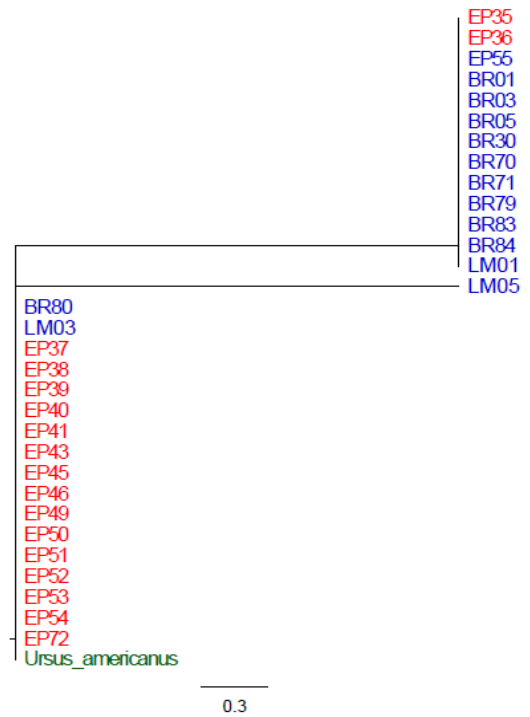
2. *APOB*



3. *APP*

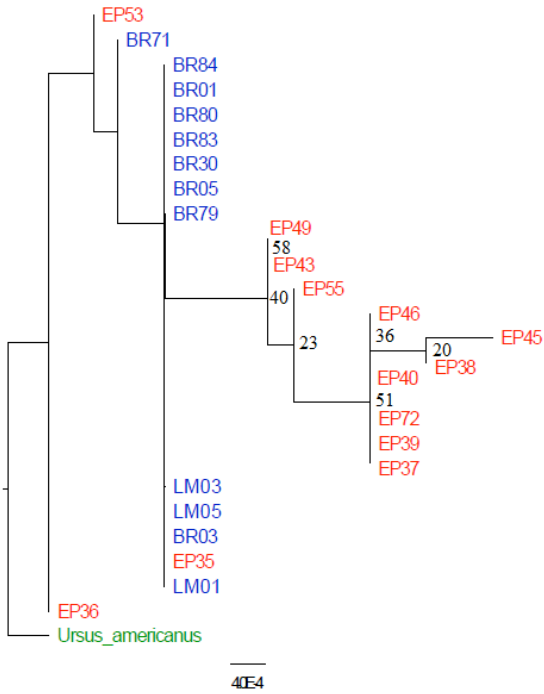


4. *TRSP*

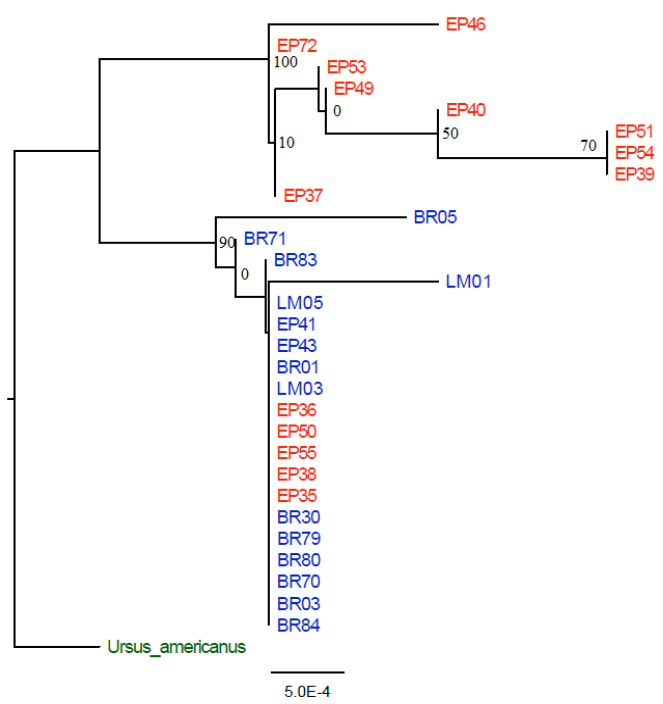


Appendix C: Single Gene Trees Generated Using Maximum Likelihood Analysis

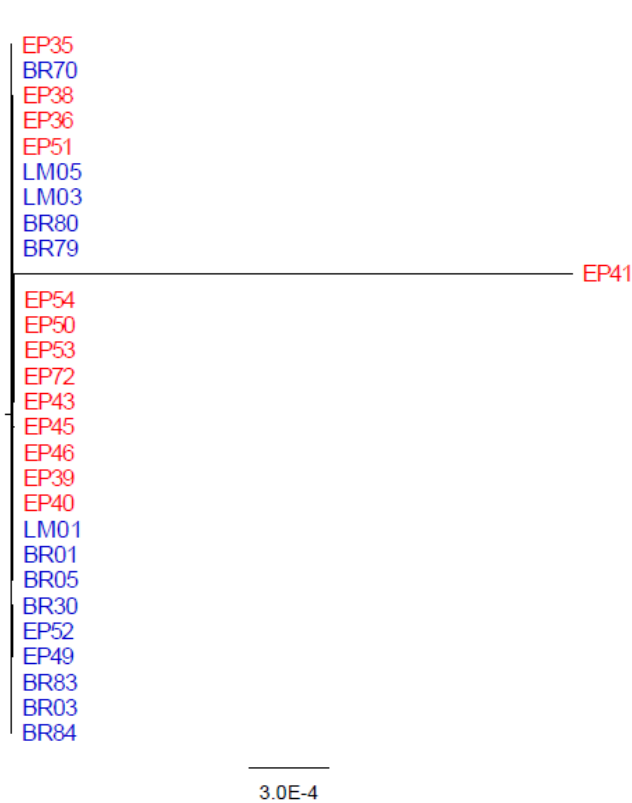
1. ALAS



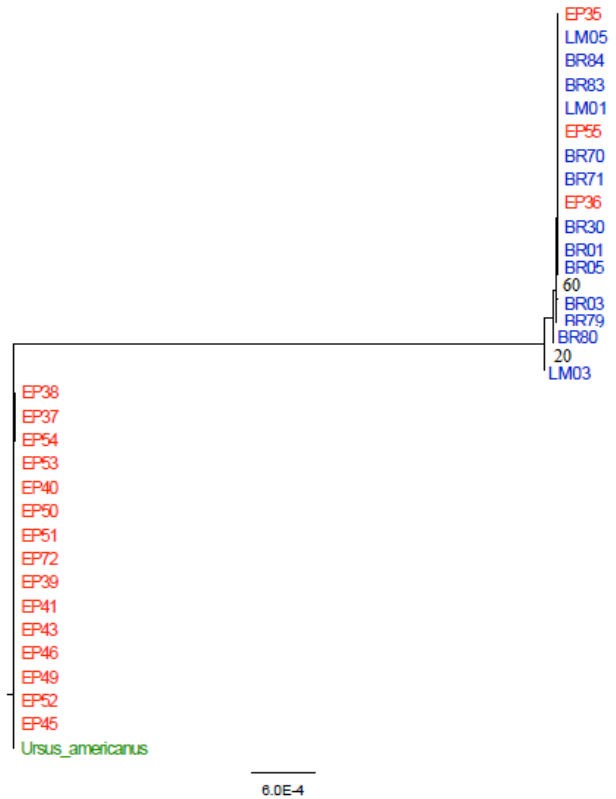
2. APOB



3. APP



4. TRSP



ACADEMIC VITA of Louise Hillier Moncla

Louise Hillier Moncla
945 Field Club Rd.
Pittsburgh, PA 15238
lhm5029@psu.edu

Education: Bachelor of Science Degree in Biology, Penn State University, Spring 2012
Bachelor of Musical Arts Degree, Penn State University, Spring 2012
Honors in Biology
Thesis Title: An Examination of Polar Bear and Brown Bear Phylogeny Using Nuclear Genes as Molecular Markers
Thesis Supervisor: Dr. Beth Shapiro

Related Experience:

- Research Experience examining polar bear and brown bear phylogeny
- involved DNA tissue extraction, gene amplification, sequencing, and phylogenetic analysis
 - Fall 2010 – Spring 2012
 - Advisor: Dr. Beth Shapiro
- Gene Annotation Project
- Collaborated with group members to annotate genes for GONUTS database
 - Successfully annotated the functions for several bacterial genes that are now displayed on the database
 - Spring 2011

Awards:

Dean's List every semester
Phi Beta Kappa

Work Experience:

- Freelance Trumpet Performer
- Hired to play for churches, schools, and small ensembles
 - Spring 2008 – present
- Private Trumpet Instructor
- Privately taught students ranging in age from 5th to 8th grade
 - Summer 2009 – Fall 2010