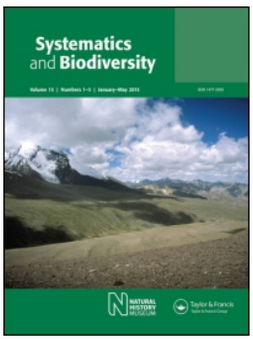

Systematics and Biodiversity

ISSN: 1477-2000 (Print) 1478-0933 (Online) Journal homepage: http://www.tandfonline.com/loi/tsab20

Uncovering discordance between taxonomy and evolutionary history in Florida raccoons

Alexa L. Trujillo & Eric A. Hoffman

To cite this article: Alexa L. Trujillo & Eric A. Hoffman (2016): Uncovering discordance between taxonomy and evolutionary history in Florida raccoons, Systematics and Biodiversity, DOI: 10.1080/14772000.2016.1214190

To link to this article: http://dx.doi.org/10.1080/14772000.2016.1214190

Systematics and Biodiversity (2016), 1–12

Taylor & Francis
Taylor & Francis Group

Research Paper

Uncovering discordance between taxonomy and evolutionary history in Florida raccoons

ALEXA L. TRUJILLO & ERIC A. HOFFMAN

University of Central Florida, 4000 Central Florida Blvd, Orlando, FL, 32816, USA

(Received 11 March 2016; accepted 21 June 2016)

Due to perceived isolation of near shore islands, there are currently five subspecies of raccoons (*Procyon lotor*) recognized in Florida, USA. In this study, we elucidated molecular genetic differences between subspecies using 173 samples from eight localities collected throughout Florida. We sequenced two mitochondrial genes and genotyped eight nuclear microsatellite loci to test two hypotheses: (1) the five morphologically and geographically defined subspecies of raccoons in Florida represent genetically distinct populations and (2) due to differing habitat availability between island and mainland subspecies, island populations would exhibit reduced levels of genetic diversity compared with mainland populations. Our mitochondrial results identified 37 unique haplotypes, many of which are shared between described subspecies. However, our analyses of genetic differentiation suggest a recent restriction of gene flow among three clusters of raccoons, which do not correlate to traditional geographies for subspecies identification. Finally, we provide evidence of reduced genetic diversity in island populations compared with their mainland counterparts using both mitochondrial and microsatellite data, which demonstrate that haplotype diversity, allelic richness, and heterozygosity are significantly reduced in island sites. These data stress the importance of using multiple lines of evidence when naming taxa to ensure concordance between evolutionary history and taxonomy.

Key words: Florida, microsatellites, mitochondria, molecular, phylogenetics, population structure, *Procyon lotor*, raccoon

Introduction

The occurrence of geographic barriers may restrict gene flow and cause once-contiguous populations to become separated. Once populations have been separated, the restricted gene flow, over time, causes populations to diverge owing to genetic drift and potential differential selection in the separate habitats (Templeton, 1981). These evolutionary processes often lead to physiological and morphological differences, and subsequent genetic differentiation, between the once panmictic populations. There are many different ways to classify these distinct populations (e.g., species, subspecies, evolutionary significant units, distinct population segments) and all are commonly used in biology to describe levels of distinction. However, distinctness is often assumed without rigorous analyses or data collection. When populations appear to be morphologically or geographically distinct, isolation is usually assumed and taxa are named based on that superficial distinction without verifying whether the nomenclature reflects the evolutionary history (Ryder, 1986; Zink, 2004). There is a trend towards using genetic data to determine existence of evolutionary history and genetic diversity to add support in determining taxon distinction. This is because problems arise when using only one line of evidence (e.g., morphology) to determine taxon differentiation (Wiens & Penkrot, 2002) and are exacerbated when researchers assume that historic taxonomy reflects separately evolving lineages. Indeed, it is well established across numerous taxa that morphological or geographic differentiation do not necessarily equate to patterns of genetic differentiation (e.g., Babik et al., 2005; Burbrink, Lawson, & Slowinski, 2000; Degner, Stout, Roth, & Parkinson, 2007; DiBattista, Waldrop, Bowen, & Rocha, 2012; Grady & Quattro, 1999; Triponez et al., 2011; Wiens & Penkrot, 2002; Zink, 2004).

Insular populations, in particular, are often assumed to represent distinct populations as a result of their apparent geographic isolation and, in many cases, morphological distinction. Supplementing geographic and morphological data with genetic data can aid in strengthening evidence

Correspondence to: Eric A. Hoffman. E-mail: eric.hoffman@ucf.edu

ISSN 1477-2000 print / 1478-0933 online

http://dx.doi.org/10.1080/14772000.2016.1214190

of isolation. Genetic identification of discrete evolutionary lineages among island populations is important for aiding conservation efforts to maintain the evolutionary trajectory of isolated populations. Moreover, genetic data provide evidence of whether insular populations tend to exhibit reduced levels of genetic diversity and are at greater risk of extinction when compared with related mainland taxa (Allendorf & Luikart, 2007). Other studies have found confounding lines of evidence between molecular data and taxonomy of both invertebrate and vertebrate species on islands (e.g., Eldridge, Meek, & Johnson, 2014; Furness et al., 2010; Juan, Ibrahim, Oromi, & Hewitt, 1996; Poulakakis et al., 2003; Robertson, Stephenson, & Goldstien, 2011). Along these lines, a growing number of studies on islands are showing the necessity to incorporate multiple lines of evidence to determine whether isolation exists before naming and describing new taxa.

Raccoons (*Procyon* spp.) provide an ideal study system in which to investigate questions related to taxonomic and phylogenetic congruence in accordance with island subspecies and their genetic diversity. There are more than 50 named types (i.e., species or subspecies) of raccoons ranging from Central Canada, across North and South America, into the southern Amazon; however, the current taxonomy is not well-supported (Helgen & Wilson, 2003). In particular, within the state of Florida, USA, there are currently five recognized raccoon subspecies: *P. l. elucus* (mainland Florida raccoon; Bangs, 1898), *P. l. inesperatus* (Matecumbe Bay raccoon; Nelson, 1930), *P. l. auspicatus* (Key Vaca raccoon; Nelson, 1930), *P. l. incautus* (Torch Key raccoon; Nelson, 1930), and *P. l. marinus* (Ten Thousand Islands raccoon; Nelson, 1930). Four of these subspecies reside exclusively on islands in south Florida. These island subspecies, described by Nelson (1930), were delimited based on geographic and morphological characters such as skull shape, size, and pelage colouration (Table S1, see online supplemental material, which is available from the article's Taylor & Francis Online page at http://dx.doi.org/10.1080/14772000.2016.1214190). Nelson (1930) also included average quantitative morphological measurements to help delineate subspecies. However, there are problems associated with Nelson's (1930) delimitation method: (1) there is large overlap in the morphological characters that demarcate subspecies; (2) although some quantitative measurements are used, the morphological characteristics are largely subjective and qualitative; (3) sample sizes used by Nelson (1930) to differentiate subspecies were small and inconsistent for both quantitative (e.g., four to eight specimens) and qualitative (e.g., 12−20) characteristics; and (4) geographic isolation is used to assume reproductive isolation. Lazell Jr. (1989) attempted to replicate Nelson's (1930) morphological measurements, but obtained contradictory results and suggested that there were only three subspecies in Florida: *P. l. elucus* (the mainland raccoon), *P. l. marinus* (the Ten Thousand Islands raccoon), and *P. l. auspicatus* (the Key raccoon).

The overlap in morphological characters between subspecies makes it challenging to differentiate between subspecies if geographic location of the sample is unknown (Lotze & Anderson, 1979), and the inconsistency of morphological studies illuminates the need to find alternative, independent lines of evidence in which to form the basis of differentiation. This is especially important given that studies have suggested eradication of Florida raccoons in specific areas where they may have negative impacts on endangered species (e.g., sea turtles and Lower Keys marsh rabbits) (Garmestani & Percival, 2005; Schmidt, McCleery, Lopez, Silvy, & Schmidt, 2010). Genetic data have been useful for identifying patterns of gene flow and differentiation in raccoons from other regions. Cullingham, Kyle, Pond, and White (2008) used mtDNA and tested for genetic evidence of four named subspecies, but the data only supported evidence for three subspecies and the authors suggested the use of only two names (*P. l. elucus* and *P. l. lotor*) to describe the subspecies they examined. Three more recent studies used microsatellite markers to assess structure and found evidence of two genetic clusters within different sampled regions located in the eastern USA (Cullingham, Kyle, Pond, Rees, & White, 2009; Kyle et al., 2014; Santonastaso, Dubach, Hauver, Graser, & Gehrt, 2012).

In this study, we used genetic data to elucidate if Nelson's (1930) nomenclature of Florida raccoons was congruent with their evolutionary history. We used the mtDNA control region (CR), cytochrome *b* (cyt *b*) gene, and variation present in eight nuclear microsatellite loci to test two hypotheses pertaining to raccoon evolutionary history. First, we tested the hypothesis that if Nelson's (1930) naming corresponds to evolutionary history, then we will find genetic differences between all five subspecies. However, we predicted that all Florida raccoons would be panmictic. Support for this prediction is based on the preponderance of evidence that suggests that raccoons exhibit both substantial natural and artificial gene flow (Helgen, Maldonado, Wilson, & Buckner, 2008), including the ability to swim across seawater (Lazell Jr., 1989). Additionally, artificial gene flow has been documented via human-aided translocations, especially for hunting (Kennedy & Lindsay, 1984; Lotze & Anderson, 1979). Second, we hypothesized that island populations would exhibit reduced levels of genetic diversity compared with mainland populations. We predicted that this trend would be demonstrated regardless of whether island sites comprise unique subspecies. This prediction is supported by studies of different taxa that have shown lower levels of genetic diversity in island populations versus their mainland counterparts (e.g., Boessenkool, Taylor, Tepolt, Komdeur, & Jamieson, 2007; Hay, Daugherty, Cree, & Maxson, 2003; White & Searle, 2007). In particular, other studies of Keys endemic taxa show reduced

diversity relative to mainland sister taxa (Ellsworth, Honeycutt, Silvy, Bickham, & Klimstra, 1994; Tursi, Hughes, & Hoffman, 2013). Finally, we discuss these results in light of general patterns of discordance between taxonomy and evolutionary history.

Materials and methods

Sampling

To test whether current raccoon nomenclature is congruent with evolutionary history, we obtained a total of 173 raccoon samples from eight localities throughout Florida to represent the five currently named subspecies of raccoons that occur in Florida, USA (Table 1; Fig. 1). Sample collection was completed with the help of pest control companies, parks, and taxidermists throughout Florida who collected samples via live-trapping or the collection of road-kill (by taking either hair samples or ear clips) and storing them in tubes filled with Drie-rite™ desiccant, as a preservative. In the Lower Keys (Big Pine Key to Key West), we collected a total of 23 samples representing the putative subspecies of *P. l. incautus*. Additionally, we acquired five samples from the Middle Keys (*P. l. auspicatus*), 24 from Key Biscayne (*P. l. inesperatus*), 18 from throughout the remainder of the Upper Keys (*P. l. inesperatus*; collected from Key Largo to Lower Matecumbe Key), and 13 samples from Ten Thousand Islands (*P. l. marinus*). The 84 samples of the putative mainland subspecies, *P. l. elucus*, were comprised of samples collected from three mainland sites (Miami = 35, Central Florida = 24, Tampa = 25) and six singleton samples from throughout mainland Florida (Table 1).

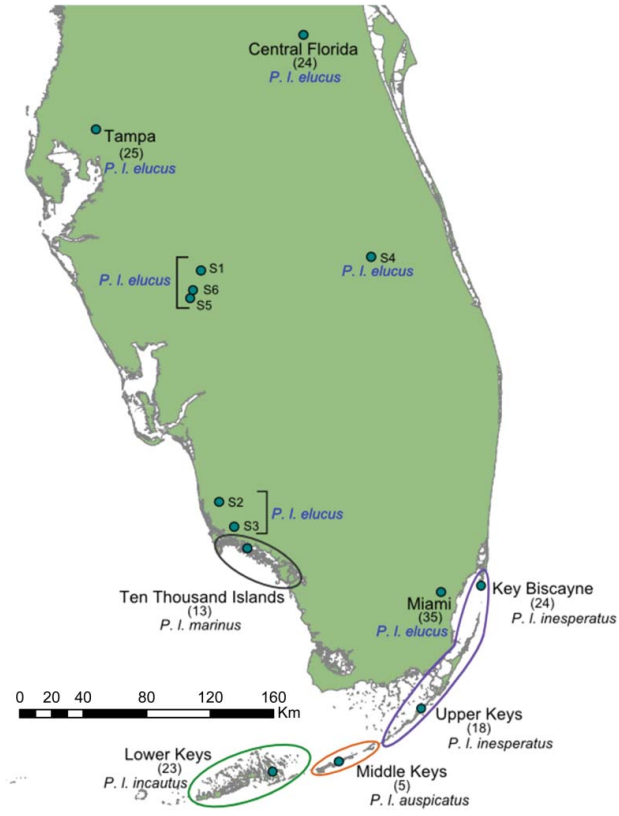

Fig. 1. Map of eight sample localities in Florida, including six scattered singleton samples (S1−S6), and the putative subspecies name for the samples at each location. Numbers in parentheses indicate number of samples per site.

Genetic data collection

All tissue samples were extracted using either the Qiagen DNeasy Blood and Tissue kit or a Serapure Bead (Rohland & Reich, 2012) extraction method. We extracted DNA from hair follicles using the Qiagen DNeasy Blood and Tissue kit following modifications suggested by Tursi et al. (2012).

To evaluate the evolutionary history of Florida raccoons, we amplified two mitochondrial genes, control

Table 1. Basic diversity statistics for mainland and island geographic sites of *Procyon lotor* in Florida. Basic diversity statistics show number of individuals used (n), number of haplotypes, number of segregating sites, nucleotide diversity (π), haplotype diversity (h), allelic richness (AR), heterozygosity (H_E), and standard deviation (SD) for each estimate.

	Mitochondrial Diversity					Microsatellite Diversity		
Location	n	No. of haplotypes	No. of segregating sites	π (SD)	h (SD)	n	AR (SD)	H_E (SD)
Mainland								
Tampa	13	6	13	0.00145 (0.00063)	0.821 (0.082)	25	8.9865 (0.08)	0.848 (0.08)
Central FL	20	14	49	0.00576 (0.00117)	0.958 (0.028)	24	8.86825 (3.72)	0.8495 (0.09)
Miami	21	10	32	0.00396 (0.00077)	0.810 (0.080)	31	8.450625 (3.95)	0.82225 (0.11)
Island								
Ten Th Islands	12	2	1	0.00008 (0.00007)	0.167 (0.134)	13	7.989625 (3.55)	0.81325 (0.13)
Key Biscayne	17	4	4	0.00024 (0.00012)	0.331 (0.143)	24	5.99025 (2.97)	0.698375 (0.19)
Up/Mid Keys	13	4	15	0.00379 (0.00050)	0.679 (0.089)	23	7.619125 (3.11)	0.798875 (0.12)
Lower Keys	16	4	10	0.00142 (0.00059)	0.442 (0.145)	22	7.59325 (3.12)	0.8095 (0.1)

region (CR) and cytochrome *b* (cyt *b*), by conducting polymerase chain reactions (PCRs) with DNA from each individual. For all individuals, we used the forward primer L15997 (Ward, Frazier, Dew-Jager, & Pääbo, 1991) and the reverse primer H00651 (Kocher et al., 1989) to achieve full coverage of the CR (~1100 bp) in a 20 μL reaction using 5−50 ng DNA, 2 μL 10× PCR buffer, 1.6 μL 25mM $MgCl_2$, 1.6 μL 10mM dNTPs, 1 μL 10 μM each primer, and 0.2 μL Taq DNA polymerase. Amplifications proceeded as follows: initial denaturation of 95°C for 5 min, 30 cycles of 94°C for 30 s, annealing at 60°C for 30 s, and 72°C for 30 s, followed by a final extension period at 72°C for 2 min (Cullingham et al., 2008). Likely the result of DNA degradation, some samples failed to amplify well with the L15997/H00651 primer pair. To compensate for degradation of certain samples, we replaced the original reverse primer with an internal reverse primer, PLO-CRL1 (Cullingham et al., 2008), using the same PCR protocol as above, to amplify a smaller CR fragment (~600 bp). To amplify the complete cyt *b* gene (1140 bp), we used the primers MTCB-F and MTCB-R, which were designed for mammals and previously tested on *P. lotor* (Naidu, Fitak, Munguia-Vega, & Culver, 2012). We performed cyt *b* PCR amplifications in a 20 μL reaction using 5-50ng DNA, 2 μL 10× PCR buffer, 2 μL 25mM $MgCl_2$, 1.6 μL 10mM dNTPs, 0.2 μL DMSO, 1 μL 10 μM each primer, and 0.2 μL Taq DNA polymerase with an initial denaturation step of 95°C for 10 min, 35 cycles of 95°C for 45 s, annealing between 57°C and 53°C for 1 min, and 72°C for 2 min, followed by a final extension period at 72°C for 10 min (Naidu et al., 2012). PCR products were cleaned either using shrimp alkaline phosphatase and exonuclease I or cleaned at the University of Arizona Genetics Core (UAGC) or at Eurofins Genomics when sent for sequencing. We edited CR and cyt *b* sequences in Sequencher v5.1 (Gene Codes Inc., Ann Arbor, MI, USA) and aligned them using the ClustalW method in MEGA6 (Tamura et al., 2011).

We also amplified the DNA from each individual at eight microsatellite loci (PLO-M15, PLO-M17, PLO-M2, PLO-M20, PLO2-117, PLO2-14, PLO-M3, PLO3-86) developed and optimized for *P. lotor* by Cullingham, Kyle, and White (2006). We performed microsatellite PCR amplifications in a 15 μL reaction using 5-50ng DNA, 1.5 μL 10× PCR buffer, 0.975 μL 25mM $MgCl_2$, 1.2 μL 10mM of dNTPs, 0.75 μL fluorescent dye, 0.375 μL 10μM each primer, and 0.15 μL Taq polymerase, edited from Cullingham et al. (2006) with an initial denaturation step of 95°C for 5 min, 30 cycles of 95°C for 30 s, annealing between 60°C and 55°C for 1 min, and 72°C for 1 min, followed by a final extension period at 72°C for 7 min. After amplification, we sent the PCR product to UAGC for genotyping. Genotypes were scored in GeneMarker v2.6.3 (SoftGenetics, LLC). In accordance with data archiving guidelines, we have deposited our sequences and multilocus genotypes in GenBank (accession numbers: KX357306−357379).

Statistical analyses

Phylogenetic reconstruction. Due to the hypervariable nature of the CR and the inability to estimate homology within this region, a 450 bp fragment (bp 523−972) was discarded from the phylogenetic analysis. Since cyt *b* is a coding gene, we confirmed that the sequences did not contain any stop codons by translating sequences to amino acids. After concatenating the trimmed CR and cyt *b* sequences, individuals that did not have sequence for both genes (65 individuals) were eliminated from downstream analyses of sequence data. We ran Partition Finder (Lanfear, Calcott, Ho, & Guindon, 2012) with unique haplotypes to determine which partitioning scheme and models of evolution fit the data. To estimate evolutionary relationships of Florida raccoons, we constructed a Bayesian phylogeny of Florida raccoon samples with MrBayes v3.2.2 (Ronquist et al., 2012), using each unique haplotype only once and including two GenBank *P. lotor* samples (accession numbers: AB291073 and AB297804) from outside of Florida, as outgroups. Conditions for MrBayes included two independent runs of 5×10^6 generations with the first 10,000 trees discarded as burn-in. We also analysed the MrBayes output data in Tracer v1.5 (Clement, Posada, & Crandall, 2000) to confirm stationarity and sufficient sampling of the posterior. Finally, we built a haplotype network using TCS v1.21 (Clement et al., 2000) to determine the relationships among similar haplotypes.

Genetic differentiation and gene flow. To determine whether the microsatellites conformed to the expectations of neutral markers, we calculated deviation from Hardy−Weinberg Equilibrium (HWE) using Fisher's Exact Tests in the program GenePop v4.2.1 (Rousset, 2008) with a sequential Bonferroni correction (Rice, 1989) to account for multiple comparisons. Given that the data indicated no consistent patterns deviating from HWE (see Results), we used all sample sites and all loci in downstream analyses.

In order to evaluate whether Nelson's (1930) subspecies represent distinct genetic clusters, we ran a Bayesian-based clustering method for multilocus data to determine the number of clusters (K) supported by the data. Here, we utilized STRUCTURE v2.3.4 (Pritchard, Stephens, & Donnelly, 2000), with 10 runs for each value of K ranging from 1 to 9 (10,000,000 iterations with 100,000 burn-in period). To determine the greatest probability of clusters across all STRUCTURE runs, we used the Evanno et al. (2005) criteria as implemented in Structure Harvester (Earl & vonHoldt, 2012). We also tested for substructure

within each population using STRUCTURE to identify if additional clusters could be identified within the clusters identified from the initial screen (Degner, Silva, Hether, Daza, & Hoffman, 2010). It is important to note that since we had a sample size of five for the Middle Keys, these individuals were grouped with Upper Keys (excluding Key Biscayne) based on results from STRUCTURE (see Results), for downstream microsatellite analyses. To further characterize differentiation among putative subspecies, we estimated global and pairwise F_{ST} among all sample sites using GenePop.

We tested for a pattern of isolation by distance (IBD) using the genetic distances/similarities function in the isolation by distance web service v3.23 (Jensen, Bohonak, & Kelley, 2005), which uses Mantel tests with 10,000 randomizations to determine if limited dispersal across space was detected. Due to the non-linear arrangement of sample sites in this study, we used log-transformed geographic distances for this analysis (Rousset, 2008). Additionally, to determine whether the regional groupings identified by STRUCTURE (see Results) better describe the genetic structure than groupings identified by Nelson (1930), we conducted an analysis of molecular variance (AMOVA; Excoffier, Smouse, & Quattro, 1992) in GenAlEx v6.5 (Peakall & Smouse, 2006, 2012) using two different groupings (i.e., *a priori* and *a posteriori*; see Results).

Genetic diversity. In order to evaluate whether levels of genetic diversity on island sample sites were lower than sites on the mainland, we estimated nucleotide diversity (π) and gene diversity (h) of mtDNA variation, and allelic richness (AR) and expected heterozygosity (H_E) of microsatellite variation. We determined π and h using the concatenated cyt *b* and CR dataset in DnaSP v5.10 (Librado & Rozas, 2009) and we calculated AR and H_E with FSTAT v1.2 (Goudet, 1995). To identify significant differences between mainland and island geographic sites of mtDNA diversity, we performed Welch's t-tests in R (R Core Team 2013). For differences in microsatellite genetic diversity between mainland and island sites, we ran a two-way analysis of variance (ANOVA) in R (R Core Team 2013), testing for the effects of locus and diversity.

Results

Phylogenetic reconstruction

In total, our phylogenetic analysis included 1991 base pairs (bp), consisting of 851 bp of trimmed CR and the complete cyt *b* gene (1140 bp) sequenced for 108 individuals throughout Florida (*P. l. elucus* = 52, *P. l. marinus* = 12, *P. l. inesperatus* = 24, *P. l. auspicatus* = 5, and *P. l. incautus* = 15). From these samples, we identified 37 unique haplotypes defined by 64 variable sites, 36 of which were parsimony informative (Table 1). We generated the final phylogenetic tree with four partitions (CR and each codon position of cyt *b*) and the best model of DNA evolution for each partition was: HKY+I+G, K80+I, F81, and HKY, respectively (Felsenstein, 1981; Hasegawa, Kishino, & Yano, 1985; Kimura, 1980). Well-supported clades (> 95% posterior probability) in the phylogenetic tree uncovered paraphyly among subspecies (Fig. 2), refuting the hypothesis that subspecies named by Nelson (1930) would represent monophyletic groups. All samples but one (H36) fell within Lineage I of Cullingham et al. (2008), which is the lineage that contains other *P. l. elucus* samples. H36 fell within Lineage II, which is predominantly composed of Midwestern United States samples and is devoid of *P. l. elucus* haplotypes.

In order to build the 95% statistical parsimony haplotype network, we removed a total of 15 bp from the ends of sequence fragments that were used in the phylogenetic analysis to create equal fragment sizes for all samples. In correspondence with the phylogenetic tree, the haplotype network (Fig. 3) did not reveal any distinct haplogroups differentiating the subspecies defined by Nelson (1930). One exception may be haplotypes 14 and 15, which are the only haplotypes found in Ten Thousand Islands (*P. l. marinus*) and these haplotypes are not shared among any other subspecies. In addition to a lack of monophyly among subspecies, many haplotypes are shared between pairs of described subspecies. H13 is a shared haplotype between *P. l. incautus* (Lower Keys) and *P. l. auspicatus* (Middle Keys). H1 is shared between *P. l. elucus* (Miami) and *P. l. incautus* (Lower Keys), and H6 is shared between *P. l. inesperatus* (Upper Keys) and *P. l. incautus* (Lower Keys). Due to H36 being too different (31 steps), it did not fall within the 95% probability limit achieved at a maximum of 19 steps. Therefore, haplotype H36 (putative *P. l. elucus* collected in Central Florida) was not included in network (Fig. 3).

Genetic differentiation and gene flow

We successfully genotyped 168/173 samples for all eight microsatellite loci. Fifty-five of 56 locus-sample site comparisons conformed to HWE expectations after a Bonferroni correction. The one comparison that was out of HWE equilibrium was Miami at PLO-M17 ($P = 0.0002$). However, with no overall pattern of locus by site out of HWE, all loci and sites were included in downstream analyses. With all sample sites included, we found most support for K = 3 clusters from the STRUCTURE analysis (Figure S1, see supplemental material online). The three clusters did not reveal a split between the mainland and island sample sites or the five putative subspecies. Instead, they supported a mainland Florida (including Ten

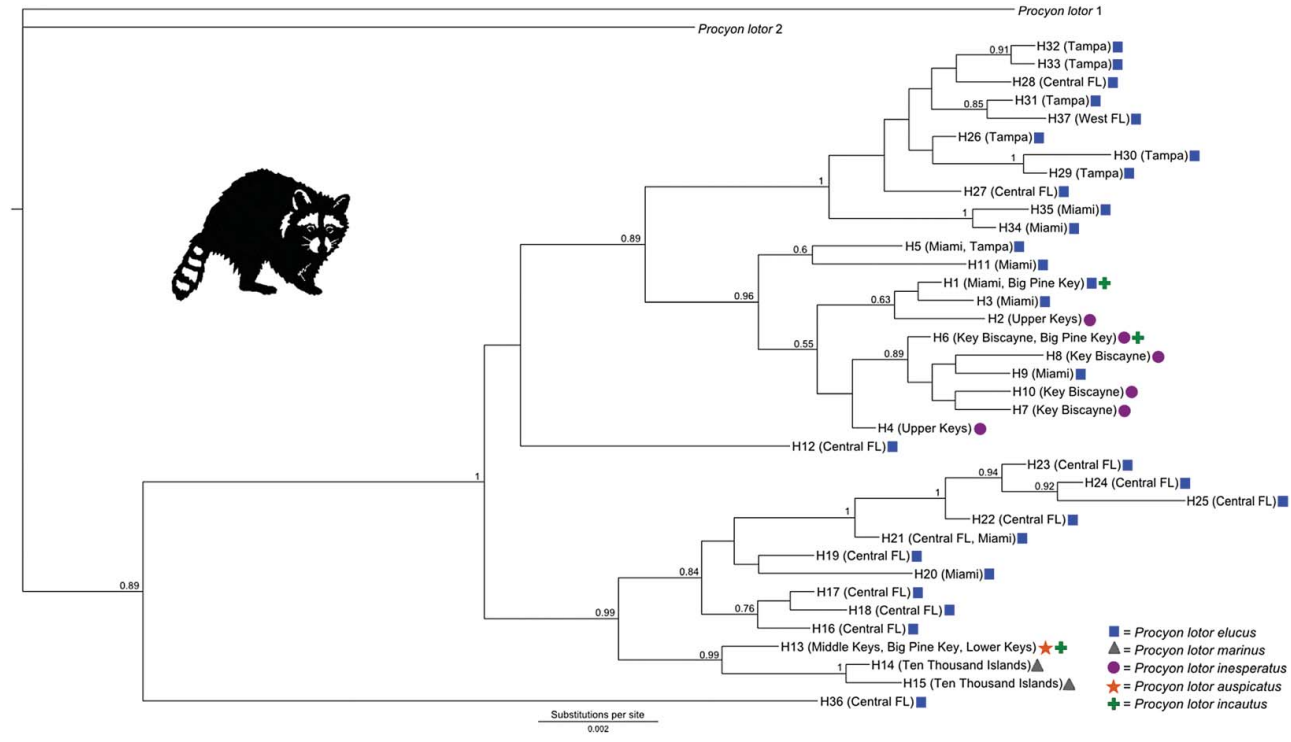

Fig. 2. Concatenated mitochondrial control region and cytochrome *b* haplotype phylogeny generated in MrBayes v3.2.2, with node posterior probabilities labelled when $P \geq 0.5$. Haplotype labels correspond to haplotypes in Fig. 3.

Thousand Islands) population, a Florida Keys (excluding Key Biscayne) population, and a Key Biscayne population (Fig. 4). Furthermore, testing for substructure within these clusters revealed no evidence of additional structuring. Global F_{ST} was moderate (0.066), whereas pairwise F_{ST} estimates (Table S2, see supplemental material online) were significant and ranged from 0.009 (between Central Florida and Miami) to 0.15 (between Key Biscayne and Middle/Upper Keys).

Additionally, we found no evidence of a relationship between genetic distance and geographic distances among all sample sites ($r = -0.1977$, $P = 0.7545$). However, we did find that the modified clusters defined by STRUCTURE better described the among-region pattern of genetic structuring found in Florida as compared with the current subspecies naming. Here, AMOVAs were run using *a priori* groups (defined by Nelson (1930)) and *a posteriori* groups (as defined by grouping identified by STRUCTURE in this study). For both analyses, the highest amount of genetic variation was found within sample sites (Table S2, see supplemental material online). However, variance among regions increased from 0% to 5% variance explained after differentiating the sites was altered to match the three clusters that STRUCTURE identified (i.e., *a posteriori* grouping), showing that the genetic regions identified by this study better explained patterns of isolation (Table S2, see supplemental material online).

Genetic diversity

Overall, average nucleotide diversity (π) was 0.00372 in the mainland geographic sites as compared with 0.00138 for the island sites (Table 1). The average estimates for gene diversity (h) between mainland and island sites were 0.863 and 0.405, respectively (Table 1). However, π was not significantly different between mainland and island sites ($t = -1.545$, $df = 3.765$, $P = 0.202$), whereas h was significantly greater in the mainland sites than the island sites ($t = -3.899$, $df = 4.059$, $P = 0.017$). Average allelic richness (AR) of microsatellites in mainland geographic sites was estimated to be 8.768 versus 7.298 for island sites (Table 1). Additionally, expected heterozygosity (H_E) among mainland sites averaged 0.84 versus 0.78 for island sites. The two-way ANOVA results indicated that mainland genetic diversity was significantly higher than island diversity for both AR and H_E (AR: $P < 0.001$, Figure S2, see supplemental material online; H_E: $P = 0.0059$, Figure S3, see supplemental material online).

Discussion

In this study, we employed genetic analyses to evaluate evolutionary histories, patterns of differentiation, and genetic diversity in the mainland Florida raccoon (*P. l. elucus*) and its four island sister subspecies (*P. l. marinus*,

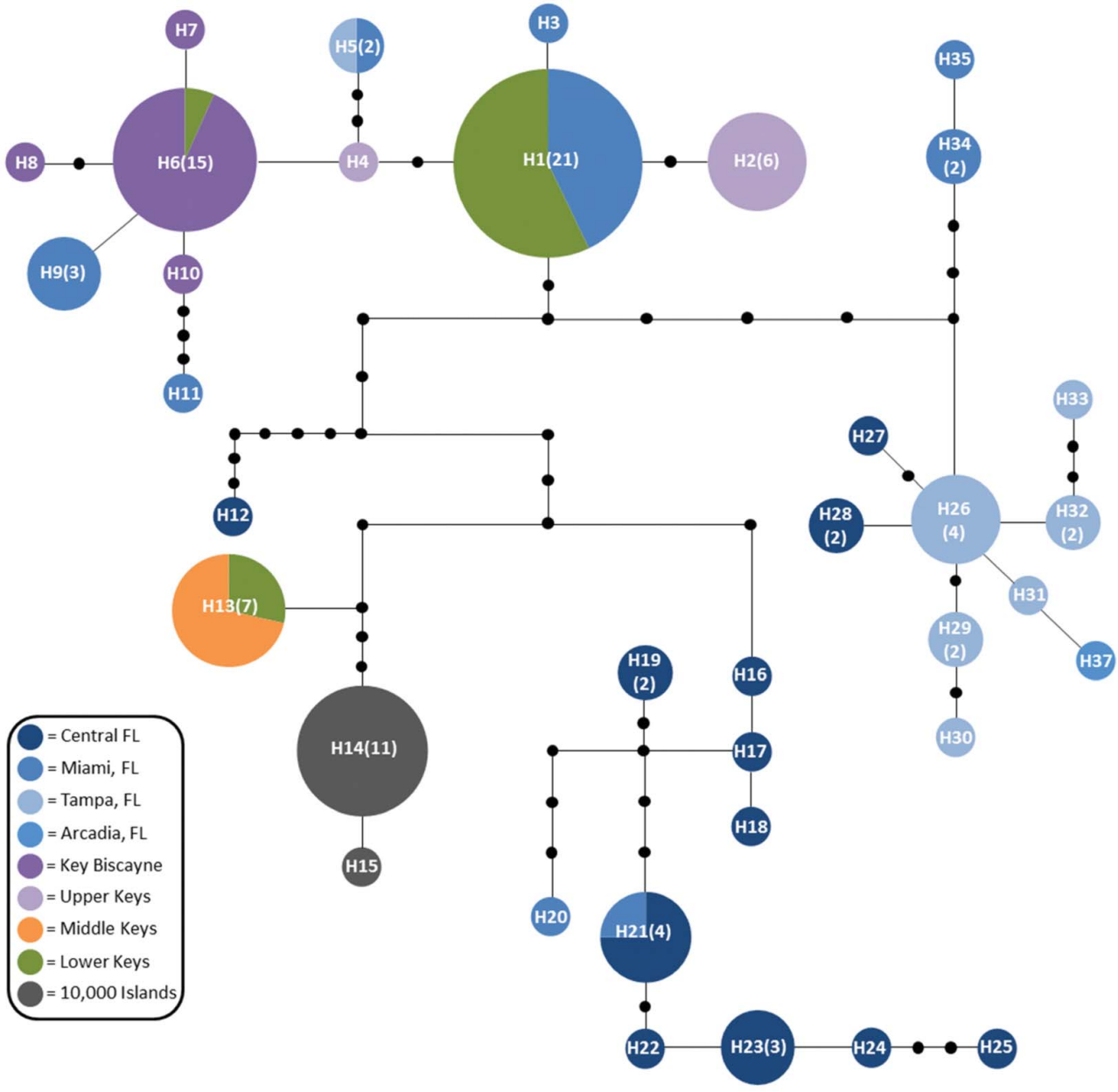

Fig. 3. 95% Parsimony network of concatenated mitochondrial control region and cytochrome *b* haplotypes generated in TCS v1.21. Circles represent distinct haplotypes; pie sizes and numbers in parentheses indicate the number of samples with that haplotype, no number indicates one individual; and colours represent subspecies: blue = *P. l. elucus*, purple = *P. l. inesperatus*, orange = *P. l. auspicatus*, green = *P. l. incautus*, and grey = *P. l. marinus*.

P. l. inesperatus, *P. l. auspicatus*, and *P. l. incautus*) to evaluate whether the current nomenclature (described in 1930) corresponds to the evolutionary history of these raccoons. This study provides evidence for the discordance between earlier subspecies designations based on morphology and geography and the evolutionary history elucidated here. In accordance with our predictions, the results do not demonstrate genetic support for Nelson's (1930) taxonomy, but do support the typical trend in which island sites display reduced levels of genetic diversity relative to mainland populations. Overall, these data shed new light on the evolutionary history of *P. lotor* subspecies, and the consequences of incongruences between taxonomy and phylogeny.

Genetic structure

This study illuminated four genetic patterns that would have been undetectable without the genetic analyses

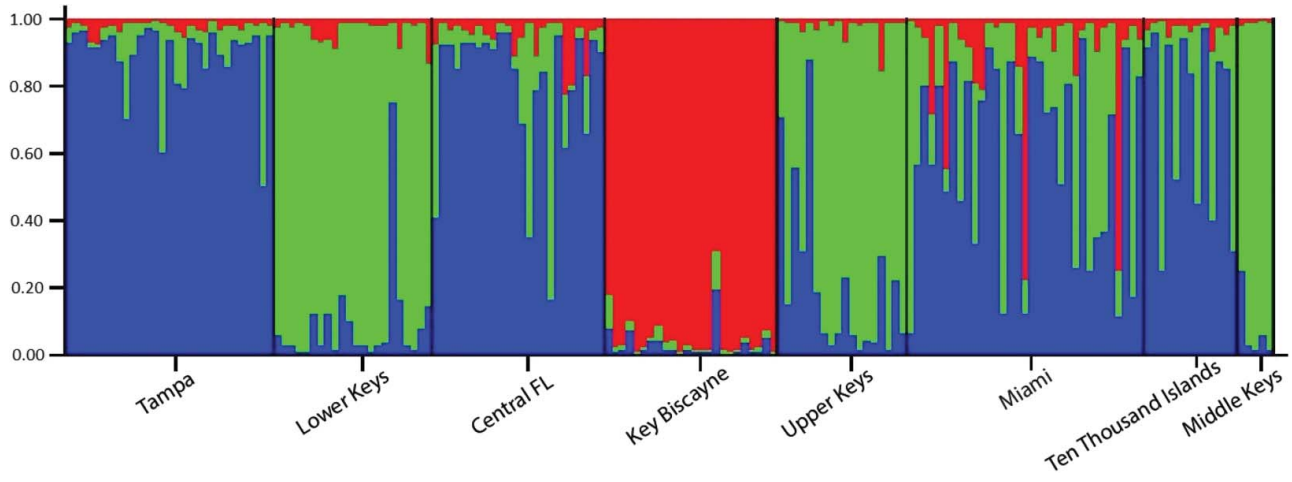

Fig. 4. Output of the STRUCTURE analysis with K = 3. The output shows structure between the Keys (green), Key Biscayne (red), and the mainland (blue), with no structure between Ten Thousand Islands and the mainland.

employed by this study: contemporary evidence of distinct groups, patterns of historic panmixia, the presence of recent gene flow between the mainland and island sites, and evidence of long-distance dispersal. First, we found molecular evidence for two genetically distinct island groups using microsatellite data: Key Biscayne and all other Florida Keys. Surprisingly, the Key Biscayne sample site is a single distinct population. This Key Biscayne population displayed the highest amount of pairwise differentiation compared with the Upper Keys sample site ($F_{ST} = 0.15$) of the same named subspecies (*P. l. inesperatus*). Additionally, the STRUCTURE analysis revealed that a few individuals from Key Biscayne may have migrated to Miami, but the significant genetic differentiation between sites suggests that this is not a frequent occurrence. In contrast, the remaining Keys all grouped together as a single cluster. These data suggest that high gene flow exists throughout the Keys (excluding Key Biscayne) and that there is restricted gene flow between all Keys and the mainland. Second, since the Florida Keys have been isolated from the mainland for about 6,000 to 10,000 years (Lazell Jr., 1989), we expected to uncover a pattern of historic differentiation among subspecies. Yet, all of the patterns of island isolation discussed above are based on contemporary estimates of gene flow (i.e., microsatellites). Estimates of evolutionary history utilizing mtDNA variation tend towards a lack of monophyly between clades with haplotypes shared among subspecies, suggesting historic panmixia. These differences between mtDNA and microsatellite patterns are useful in determining whether populations exhibit historic or contemporary genetic isolation, providing further information on the degree of population divergence (Crandall, Binindа-Emonds, Mace, & Wayne, 2000). Third, in contrast to the patterns described above, there appears to be contemporary gene flow between Ten Thousand Islands and all mainland sample sites. Why does Ten Thousand Islands exhibit a genetic pattern different than the other island sites? It is likely the natural formation of the islands that provides insight into the genetic patterns. The Ten Thousand Islands were formed by the build-up of peat and oyster beds over time (Hoffmeister, 1974), whereas the Keys were formed during glacial retreat coupled with rising sea level which isolated the islands from the mainland (Lazell Jr., 1989). These differences in origin cause alterations in how the islands are contemporaneously separated from the mainland. The Keys are disjointed, with about 3000 metres of seawater between mainland and Keys. In contrast, the Ten Thousand Islands are separated from the mainland by small waterways, which raccoons are probably able to cross (Lazell Jr., 1989). The fourth interesting genetic pattern that was uncovered by this study was the presence of an individual, collected from Central Florida, that exhibited a haplotype (i.e., H36) that was more than twice as divergent (at 1.5% uncorrected sequence divergence) as the next most divergent haplotype (H12). In comparison with the haplotype groups of Cullingham et al. (2008), this sample most closely grouped within Cullingham's lineage II, a lineage generally found in the Mid-Western United States. There are two likely explanations for the occurrence of this sample in central Florida: artificial translocation or natural long-distance dispersal. In finding a genetic outlier like H36, we need to consider that human interferences may obscure our interpretations of genetic data. Indeed, forced migrations have been documented, especially when raccoon hunting was a popular past-time (Kennedy & Lindsay, 1984; Lotze & Anderson, 1979) and even in recent years as raccoons are often seen as nuisance animals and are trapped and relocated. Alternatively, H36 could be a rare long-distance disperser. Natural long-distance dispersal has been documented previously in raccoons, with individuals recorded

as travelling over 200 km (Zeveloff, 2002). Although we cannot rule out natural dispersal, it seems that the distance travelled in this case (approximately 3000 km from the Midwestern United States) increases the likelihood that this individual was an artificial transplant.

Genetic diversity

Determining the levels of genetic diversity is important when comparing and contrasting island and mainland populations of a species because habitat fragmentation can lead to reduced genetic diversity and may restrict the ability of populations to adapt and persist (Bichet, Moodley, Penn, Sorci, & Garnier, 2015; Frankham, 1997). For example, Eldridge et al. (1999) found reduced fitness of island populations of the black-footed rock-wallaby, compared with mainland populations, due to low levels of genetic variation. Even in widespread, common species, fragmentation and isolation can lead to reduced genetic diversity of island populations (Bichet et al., 2015). Despite being widespread, this study sought to determine whether island populations of raccoon exhibit reduced genetic diversity. Other species living in sympatry to the Keys population of raccoons have been found to be genetically distinct and exhibit reduced genetic diversity compared with mainland counterparts. Genetic patterns of these species: Key deer (Villanova, 2015), Lower Keys marsh rabbit (Tursi et al., 2012), and silver rice rat (Indorf & Gaines, 2013), lead to our prediction that our data would elucidate this pattern even if levels of genetic divergence did not support Nelson's (1930) subspecies naming. For the raccoons studied here, island sites do indeed show a pattern of reduced genetic diversity, which may be a factor of founder effect or a population bottleneck (Maruyama & Fuerst, 1985; Mayr, 1970). Regardless of the mechanism, the patterns of reduced genetic diversity in the Keys raccoon support our data that these populations are isolated relative to the mainland raccoon.

Subspecific naming

Determining whether nomenclature and evolutionary history are concordant is important so that scientists do not split species unnecessarily, thus wasting effort and funds on widespread and abundant taxa (e.g., American puma: Culver, Johnson, Pecon-Slattery, & O'Brien, 2000; willow flycatcher: Zink, 2015) or lump species that are actually distinct and deny protection for taxa in need of support (e.g., Kemp's Ridley sea turtle: Bowen & Avise, 1996). Recently, studies have used genetic information to guide the accuracy of naming that was historically guided by morphology (e.g., Burbrink et al., 2000) or geography (Hay, Sarre, Lambert, Allendorf, & Daugherty, 2010). Overall, the results of our study do not support the current subspecies naming of Florida raccoons based on historic and contemporary patterns of genetic structure. These data provide adequate evidence to suggest two revisions in the current taxonomy. First, the use of the Ten Thousand Islands raccoon subspecies (*P. l. marinus*) should be discontinued and synonymized with *P. l. elucus*, as there is no evidence of differentiation from the mainland. Second, the Keys group (excluding Key Biscayne) should be synonymized to *P. l. auspicatus*, as suggested by Lazell Jr. (1989) in a study using blood protein analyses and supported by microsatellite data in this study. The Key Biscayne population must be further studied to determine whether it is distinct enough to warrant management and should keep its current name (*P. l. inesperatus*). This would leave Florida with three raccoon subspecies: the Florida raccoon (*P. l. elucus*), the Keys raccoon (*P. l. auspicatus*), and the Key Biscayne raccoon (*P. l. inesperatus*). This revised taxonomy would benefit from a thorough morphological assessment since Nelson's (1930) morphological characters overlap in their descriptions and have been met with difficulty when other researchers have tried to reanalyse them (Lazell Jr., 1989).

In summary, this study highlights how purely morphological and/or geographic-based species designations can be inaccurate, leading to a taxonomy that does not reflect the species' evolutionary history. Modern species definitions tend to agree that species are delimited by unique evolutionary lineages (de Queiroz, 1998), which should not be based solely on morphology or geographic location. As we strive to recover the most accurate evolutionary history, total information criteria, including morphology and genetic data should be utilized to estimate the phylogenies that should reflect these modern species definitions. Therefore, where taxonomy and phylogeny are discordant, nomenclature should be changed to reflect evolutionary history.

Acknowledgements

We thank Rob and Jennifer Hammer of Florida Wildlife Management, Mark Gurley, Mike Cove, Phillip Hughes, Greg Territo, Elizabeth Golden, Ellie Willingham, Nancy Stedman, Jeremy Dixon, Karen Bettmann, and Nancy Chatelaine for help with sample collections throughout Florida. We thank everyone in the Parkinson lab and the Hoffman lab for their assistance with editing and moral support. Raccoon samples were collected according to IACUC protocol 13-03W from the University of Central Florida.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This project was funded by the University of Central Florida.

Supplemental data

Supplemental data for this article can be accessed here: http://dx.doi.org/10.1080/14772000.2016.1214190.

References

Allendorf, F. W., & Luikart, G. (2007). *Conservation and the genetics of populations*. Maden, MA: Blackwell Publishing.

Babik, W., Branicki, W., Crnobrnja-Isailovic, J., Cogalniceanu, D., Sas, I., Olgun, K., … Arntzen, J. W. (2005). Phylogeography of two European newt species - Discordance between mtDNA and morphology. *Molecular Ecology*, *14*, 2475−2491.

Bangs, O. (1898). The land mammals of peninsular Florida and the coast region of Georgia. *Proceedings of the Boston Society of Natural History*, *28*, 157−235.

Bichet, C., Moodley, Y., Penn, D. J., Sorci, G., & Garnier, S. (2015). Genetic structure in insular and mainland populations of house sparrows (Passer domesticus) and their hemosporidian parasites. *Ecology and Evolution, 5*, 1639−1652.

Boessenkool, S., Taylor, S. S., Tepolt, C. K., Komdeur, J., & Jamieson, I. G. (2007). Large mainland populations of South Island robins retain greater genetic diversity than offshore island refuges. *Conservation Genetics*, *8*, 705−714.

Bowen, B. W., & Avise, J. C. (1996). Conservation genetics of Marine Turtles. In *Conservation genetics: Case histories from nature* (pp. 190−237). New York: Chapman & Hall.

Burbrink, F. T., Lawson, R., & Slowinski, J. B. (2000). Mitochondrial DNA phylogeography of the polytypic North American rat snake (Elaphe obsoleta): A critique of the subspecies concept. *Evolution; International Journal of Organic Evolution*, *54*, 2107−2118.

Clement, M., Posada, D., & Crandall, K. A. (2000). TCS: A computer program to estimate gene genealogies. *Molecular Ecology*, *9*, 1657−1659.

Crandall, K., Bininda-Emonds, O., Mace, G. M., & Wayne, R. K. (2000). Considering evolutionary processes in conservation biology. *Trends in Ecology and Evolution*, *15*, 290−295.

Cullingham, C. I., Kyle, C. J., Pond, B. A., Rees, E. E., & White, B. N. (2009). Differential permeability of rivers to raccoon gene flow corresponds to rabies incidence in Ontario, Canada. *Molecular Ecology*, *18*, 43−53.

Cullingham, C. I., Kyle, C. J., Pond, B. A., & White, B. N. (2008). Genetic structure of raccoons in eastern North America based on mtDNA: Implications for subspecies designation and rabies disease dynamics. *Canadian Journal of Zoology*, *86*, 947−958.

Cullingham, C. I., Kyle, C. J., & White, B. N. (2006). Isolation, characterization and multiplex genotyping of raccoon tetranucleotide microsatellite loci. *Molecular Ecology Notes*, *6*, 1030−1032.

Culver, M., Johnson, W. E., Pecon-Slattery, J., & O'Brien, S. J. (2000). Genomic ancestry of the American puma (Puma concolor). *The Journal of Heredity*, *91*, 186−197.

de Queiroz, K. (1998). The general lineage concept of species, species criteria, and the process of speciation: A conceptual unification and terminological recommendations. In D. J. Howard & S. H. Berlocher (Eds.), *Endless forms: Species and speciation* (pp. 57−75). Oxford, England: Oxford University Press.

Degner, J. F., Silva, D. M., Hether, T. D., Daza, J. M., & Hoffman, E. A. (2010). Fat frogs, mobile genes: Unexpected phylogeographic patterns for the ornate chorus frog (*Pseudacris ornata*). *Molecular Ecology*, *19*, 2501−2515.

Degner, J. F., Stout, I. J., Roth, J. D., & Parkinson, C. L. (2007). Population genetics and conservation of the threatened southeastern beach mouse (*Peromyscus polionotus niveiventris*): Subspecies and evolutionary units. *Conservation Genetics*, *8*, 1441−1452.

DiBattista, J. D., Waldrop, E., Bowen, B. W., & Rocha, L. A. (2012). Twisted sister species of pygmy angelfishes : Discordance between taxonomy, coloration, and phylogenetics. *Coral Reefs*, *31*, 839−851

Earl, D. A., & vonHoldt, B. M. (2012). STRUCTURE HARVESTER: A website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources*, *4*, 359−361

Eldridge, M. D. B., King, J. M., Loupis, A. K., Spencer, P. B. S., Taylor, A. C., Pope, L. C., & Hall, G. P. (1999). Unprecedented low levels of genetic variation and inbreeding depression in an Island population of the Black-Footed Rock-Wallaby. *Conservation Biology*, *13*, 531−541.

Eldridge, M. D. B., Meek, P. D., & Johnson, R. N. (2014). Taxonomic uncertainty and the loss of biodiversity on Christmas Island, Indian ocean. *Conservation Biology*, *28*, 572−579.

Ellsworth, D. L., Honeycutt, R. L., Silvy, N. J., Bickham, J. W., & Klimstra, W. D. (1994). Historical biogeography and contemporary patterns of mitochondrial DNA variation in white-tailed deer from the Southeastern United States. *Evolution*, *48*, 122−136.

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Molecular Ecology*, *14*, 2611−2620.

Excoffier, L., Smouse, P. E., & Quattro, J. M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics*, *131*, 479−491.

Felsenstein, J. (1981). Evolutionary trees from DNA sequences: A maximum likelihood approach. *Journal of Molecular Evolution*, *17*, 368−376.

Frankham, R. (1997). Do island populations have less genetic variation than mainland populations? *Heredity*, *78*, 311−327.

Furness, R. W., Mable, B., Savory, F., Griffiths, K., Baillie, S. R., & Heubeck, M. (2010). Subspecies status of Common Eiders Somateria mollissima in Shetland based on morphology and DNA. *Bird Study*, *57*, 330−335.

Garmestani, A. S., & Percival, H. F. (2005). Raccoon removal reduces sea turtle nest depredation in the ten thousand Islands of Florida. *Southeastern Naturalist*, *4*, 469−472.

Goudet, J. (1995). FSTAT (Version 1.2): A computer program to calculate f-statistics. *Journal of Heredity*, *86*, 485−486.

Grady, J. M., & Quattro, J. M. (1999). Using character concordance to define taxonomic and conservation units. *Conservation Biology*, *13*, 1004−1007.

Hasegawa, M., Kishino, H., & Yano, T. A. (1985). Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution*, *22*, 160−174.

Hay, J. M., Daugherty, C. H., Cree, A., & Maxson, L. R. (2003). Low genetic divergence obscures phylogeny among

populations of Sphenodon, remnant of an ancient reptile lineage. *Molecular Phylogenetics and Evolution*, *29*, 1−19.

Hay, J. M., Sarre, S. D., Lambert, D. M., Allendorf, F. W., & Daugherty, C. H. (2010). Genetic diversity and taxonomy: A reassessment of species designation in tuatara (Sphenodon: Reptilia). *Conservation Genetics*, *11*, 1063−1081.

Helgen, K. M., Maldonado, J. E., Wilson, D. E., & Buckner, S. D. (2008). Molecular confirmation of the origin and invasive status of West Indian raccoons. *Journal of Mammalogy*, *89*, 282−291.

Helgen, K. M., & Wilson, D. E. (2003). Taxonomic status and conservation relevance of the raccoons (*Procyon* spp.) of the West Indies. *Journal of Zoology*, *259*, 69−76.

Hoffmeister, J. E. (1974). *Land from the sea: The geologic story of South Florida*. Coral Gables, Florida: University of Miami Press.

Indorf, J. L., & Gaines, M. S. (2013). Genetic divergence of insular marsh rice rats in subtropical Florida. *Journal of Mammalogy*, *94*, 897−910.

Jensen, J. L., Bohonak, A. J., & Kelley, S. T. (2005). Isolation by distance, web service. *BioMed Central Genetics*, *6*, 13.

Juan, C., Ibrahim, K. M., Oromi, P., & Hewitt, G. M. (1996). Mitochondrial DNA sequence variation and phylogeography of Pimelia darkling beetles on the Island of Tenerife (Canary Islands). *Heredity*, *77*, 589−598.

Kennedy, M. L., & Lindsay, S. L. (1984). Morphologic variation in the raccoon, procyon lotor, and its relationship to genic and environmental variation. *Journal of Mammalogy*, *65*, 195−205.

Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, *16*, 111−120.

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Pääbo, S., Villablanca, F. X., & Wilson, A. C. (1989). Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proceedings of the National Academy of Sciences*, *86*, 6196−6200.

Kyle, C. J., Rico, Y., Castillo, S., Srithayakumar, V., Cullingham, C. I., White, B. N., & Pond, B. A. (2014). Spatial patterns of neutral and functional genetic variation reveal patterns of local adaptation in raccoon (*Procyon lotor*) populations exposed to raccoon rabies. *Molecular Ecology*, *23*, 2289−2298.

Lanfear, R., Calcott, B., Ho, S. Y. W., & Guindon, S. (2012). Partitionfinder: Combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution*, *29*, 1695−1701.

Lazell, Jr., J. D. (1989). *Wildlife of the Florida Keys*. Washington, D.C. and Covelo, California: Island Press.

Librado, P., & Rozas, J. (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, *25*, 1451−1452.

Lotze, J.-H., & Anderson, S. (1979). *Procyon lotor*. *Mammalian Species*, *119*, 1−8.

Maruyama, T., & Fuerst, P. A. (1985). Population bottlenecks and nonequilibrium models in population genetics. II. Number of alleles in a small population that was formed by a recent bottleneck. *Genetics*, *111*, 675−689.

Mayr, E. (1970). *Populations, species, and evolution*. Cambridge, MA: Belknap.

Naidu, A., Fitak, R. R., Munguia-Vega, A., & Culver, M. (2012). Novel primers for complete mitochondrial cytochrome b gene sequencing in mammals. *Molecular Ecology Resources*, *12*, 191−196.

Nelson, E. W. (1930). Four new raccoons from the keys of Southern Florida. *Smithsonian Miscellaneous Collections*, *82*, 1−17.

Peakall, R., & Smouse, P. E. (2006). Population genetic software for teaching and research. *Molecular Ecology Notes*, *6*, 288−295.

Peakall, R., & Smouse, P. E. (2012). GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research−an update. *Bioinformatics (Oxford, England)*, *28*, 2537−2539.

Poulakakis, N., Lymberakis, P., Antoniou, A., Chalkia, D., Zouros, E., Mylonas, M., & Valakos, E. (2003). Molecular phylogeny and biogeography of the wall-lizard Podarcis erhardii (Squamata: Lacertidae). *Molecular Phylogenetics and Evolution*, *28*, 38−46.

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*, 945−959.

R Development Core Team. (2013). *R: A language and environment for statistical computing*. Vienna, Australia: R Foundation for Statistical Computing.

Rice, W. R., (1989). Analyzing tables of statistical tests. *Evolution, 43*, 223−225.

Robertson, B. C., Stephenson, B. M., & Goldstien, S. J. (2011). When rediscovery is not enough: Taxonomic uncertainty hinders conservation of a critically endangered bird. *Molecular Phylogenetics and Evolution*, *61*, 949−952.

Rohland, N., & Reich, D. (2012). Cost-effective, high-throughput DNA sequencing libraries for multiplexed target capture. *Genome Research*, *22*, 939−946.

Ronquist, F., Teslenko, M., Van Der Mark, P., Ayres, D. L., Darling, A., Höhna, S., … Huelsenbeck, J. P. (2012). Mrbayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, *61*, 539−542.

Rousset, F. (2008). GENEPOP'007: A complete re-implementation of the genepop software for Windows and Linux. *Molecular Ecology Resources*, *8*, 103−106.

Ryder, O. A. (1986). Species conservation and systematics: The dilemma of subspecies. *TREE*, *1*, 9−10.

Santonastaso, T. T., Dubach, J., Hauver, S. A., Graser, W. H., & Gehrt, S. D. (2012). Microsatellite analysis of raccoon (Procyon lotor) population structure across an extensive metropolitan landscape. *Journal of Mammalogy*, *93*, 447−455.

Schmidt, P. M., McCleery, R. A., Lopez, R. R., Silvy, N. J., & Schmidt, J. A. (2010). Habitat succession, hardwood encroachment and raccoons as limiting factors for Lower Keys marsh rabbits. *Biological Conservation*, *143*, 2703−2710.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M., & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and parsimony methods. *Molecular Biology and Evolution*, *28*, 2731−2739.

Templeton, A. R. (1981). Mechanisms of speciation−A population genetic approach. *Annual Review of Ecology and Systematics*, *12*, 23−48.

Triponez, Y., Buerki, S., Borer, M., Naisbit, R. E., Rahier, M., & Alvarez, N. (2011). Discordances between phylogenetic and morphological patterns in alpine leaf beetles attest to an intricate biogeographic history of lineages in postglacial Europe. *Molecular Ecology*, *20*, 2442−2463.

Tursi, R. M., Hughes, P. T., & Hoffman, E. A. (2012). Taxonomy versus phylogeny: evolutionary history of marsh rabbits without hopping to conclusions. *Diversity and Distributions*, *19*, 1−14.

Villanova, V. L. (2015). *Genetic Structure and Demographic Analysis of Key deer (Odocoileus virginianus clavium)* (Unpublished master's thesis). University of Central Florida, Orlando.

Ward, R. H., Frazier, B. L., Dew-Jager, K., & Pääbo, S. (1991). Extensive mitochondrial diversity within a single Amerindian tribe. *Proceedings of the National Academy of Sciences of the United States of America*, *88*, 8720−8724.

White, T. A., & Searle, J. B. (2007). Genetic diversity and population size: Island populations of the common shrew, Sorex araneus. *Molecular Ecology*, *16*, 2005−2016.

Wiens, J. J., & Penkrot, T. A. (2002). Delimiting species using DNA and morphological variation and discordant species limits in spiny lizards (Sceloporus). *Systematic Biology*, *51*, 69−91.

Zeveloff, S. I. (2002). *Raccoons: A natural histroy*. Smithsonian Institution Press, Washington, DC.

Zink, R. M. (2004). The role of subspecies in obscuring avian biological diversity and misleading conservation policy. *Proceedings. Biological Sciences / The Royal Society*, *271*, 561−564.

Zink, R. M. (2015). Genetics, morphology, and ecological niche modeling do not support the subspecies status of the endangered Southwestern Willow Flycatcher (*Empidonax traillii extimus*). *The Condor*, *117*, 76−86.

Associate Editor: Christoph Bleidorn