A comparison of phylogenetic systematics among Middle American shrews of the genus *Cryptotis* (Mammalia: Soricidae) based on morphological versus molecular data

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Introduction

Members of the genus Cryptotis (Mammalia: Soricidae), the small-statured shrews, are found from eastern North America to Andean South America. The Cryptotis mexicanus group is a clade within the genus whose species are distinguished by enlargement of the forefoot and longer, broader foreclaws (Woodman and Morgan 2003). These modifications are believed to confer a distinct digging advantage to C. mexicanus group shrews. Forefoot morphology in C. mexicanus shrews is thought to be a shared characteristic that groups these organisms together. However, evolutionary relationships among these cryptic species are difficult to define using morphological variation. Variation in DNA sequences from the mitochondrial cytochrome b (cyt) gene and the nuclear apolipoprotein (ApoB) gene can be used to elucidate relationships between species and construct a molecular phylogeny (Ohdachi et al. 2006; Dubey et al. 2008). We sequenced 1140 bp of the cyt gene in 52 individuals and 517 bp of the ApoB gene in 33 individuals across seven Cryptotis species. From these data, we generated two maximum parsimony phylogram trees: one based on the cyt data alone, and the other based on 17 concatenated cyt and ApoB sequences. Phylogenies based on these molecular data will help to resolve the relationships of different Cryptotis species and confirm the morphological species delimitation.

Materials and Methods

Cryptotis tissue samples originating from localities in Guatemala, Costa Rica, and the United States were obtained from fresh tissue samples and from voucher specimens in the USNM collection (Table 1). Genomic DNA was extracted from the tissue samples and the resulting DNA concentration was measured using the QIAquick Gel Extraction Kit. DNA was PCR amplified for the cyt and ApoB nuclear intron regions using primers specific to soricid shrews (Woodman 2008). PCR products were gel-purified and directly sequenced using the ABI Prism 3130 Genetic Analyzer. Sequences 4.9 bp was used to align and edit sequences. Thirty-two sequences were successfully obtained for the cyt gene, and 33 sequences were successfully obtained for the ApoB gene. Twelve additional cyt sequences and four additional ApoB sequences from GenBank were used to supplement the analysis. PCR amplicons were purified using QIAquick PCR purification Kit. Pfu Turbo DNA Polymerase was used to perform the sequencing reaction. Sequence alignments were performed using ClustalW (http://www.ncbi.nlm.nih.gov/blast/Blast.cgi). Alignment was performed by visual inspection and partial sequence homology was used for scoring. Average genetic distances were estimated using the Kimura (1980) two-parameter model of nucleotide substitution.

Results

A comparison of the cyt and ApoB gene sequences were used to develop a molecular phylogeny for Cryptotis. Figure 2 present the maximum parsimony phylogram of 39 Cryptotis cyt sequences from five newly sequenced outgroup species. Bootstrap support values are indicated by the numbers near nodes. The cyt sequences from C. mexicanus, C. parvus, C. aurea, C. goodwini, and C. crocuta were not resolved. Further studies are necessary to resolve the relationships of these species.

Discussion

Phylogenetic analysis of the cyt gene sequences from the cryptic species within the C. mexicanus clade primarily confirm the morphological species delimitation. Relationships not resolved with mitochondrial DNA data have been resolved with nuclear DNA data. The cyt gene has been useful for resolving species relationships at higher taxonomic levels. Additional nuclear intron data are necessary to confirm the relationships of these groups. We sequenced cyt and ApoB sequences from five newly sequenced outgroup species. Bootstrap support values are indicated by the numbers near nodes. The cyt sequences from C. mexicanus, C. parvus, C. aurea, C. goodwini, and C. crocuta were not resolved. Further studies are necessary to resolve the relationships of these species.

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Our molecular analysis of shrew phylogenies clarified relationships within species, further resolved morphological species delimitation among the C. goodwini-like species (Woodman 2010). Other relationships were not resolved through mitochondrial and nuclear data and resulted in a polytomy. In addition, we found that several species shared identical haplotypes across regions. For these reasons, continued sampling of Cryptotis species in Central America and sequences from more nuclear markers are necessary to resolve the phylogenetics of Middle American Cryptotis.

References
