Molecules vs. Morphology: Systematics of Middle American shrews of the genus *Cryptotis* (Mammalia: Soricidae)

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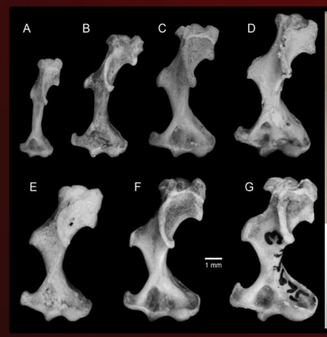
Introduction

Members of the genus *Cryptotis* (Mammalia: Soricidae), the small-eared shrews, range from eastern North America to Andean South America. The *Cryptotis mexicanus* group is a clade within the genus whose species are distinguished by modifications to the humerus and foreclaws, enlarging and broadening these structures (Woodman 2010; Woodman and Timm, 1999). These modifications suggest selected specialization and imply a distinct digging advantage to the *C. mexicanus* group. Humeri and forefoot morphology in *C. mexicanus* shrews are thought to be shared characteristics that can group these organisms systematically. However, evolutionary relationships amongst these diverse species are difficult to define based on morphological data alone. In order to expound evolutionary relationships among species, we sequenced four genes for 38 individuals across 12 species, using the short tailed shrew, *Blarina brevicauda,* as the closest outgroup species. Phylgenies based on this molecular data will help to resolve the relationships of different *Cryptotis* species and confirm the morphological phylogeny.



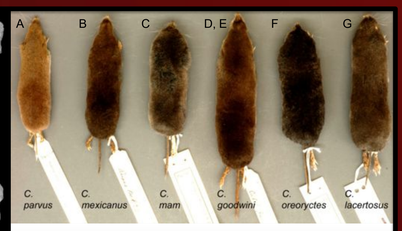


Figure 1A (above): Specimens of compared *Cryptotis* species.

Figure 1B (left): Left humeri of A, *Cryptotis parvus parvus*) B, *Cryptotis mexicanus*; C, *C. mam*); D, *C. goodwini*); E, *C. goodwini*; F, *C. oreoryctes*); G, *C. lacertosus*).

Materials and Methods

Cryptotis DNA samples were obtained from fresh tissue samples and from voucher specimens in the USNM collection. Samples originated from localities in Guatemala, Costa Rica, Columbia, Mexico, and the United States (Table 1). Genomic DNA was extracted from the tissue samples and the mitochondrial genes 16S and Cyt b and nuclear genes ApoB and BRCA1 were amplified using previously published (Dubey et al, 2008) and originally designed primers in a polymerase chain reaction (PCR). PCR products were tested for amplification through gel electrophoresis, purified and cycle-sequenced. We sequenced both forward and reverse strands of each sample using the ABI PRISM 3130 Genetic Analyzer, Sequencher 5.0 was used to align and edit the sequences. Additional sequences, of the four genes, were acquired from Genbank for analysis for five additional individuals.

We performed phylogenetic analyses using PAUP* 4.0. A preliminary neighbor-joining tree was generated based on all 16S sequences. A maximum parsimony analysis with 1000 bootstrap replicates was generated from the 16S data, with *Blarina brevicauda* included as the closest outgroup species (Ohdachi et al 2006; Dubey et al 2008). We concatenated the sequences of all four genes for 43 samples and constructed a maximum parsimony tree with 1000 bootstrap replicates.

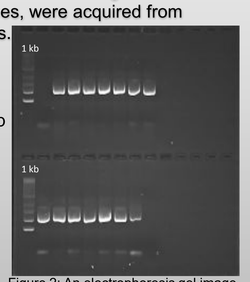


Figure 2: An electrophoresis gel image of the 16S mitochondrial gene.

Results

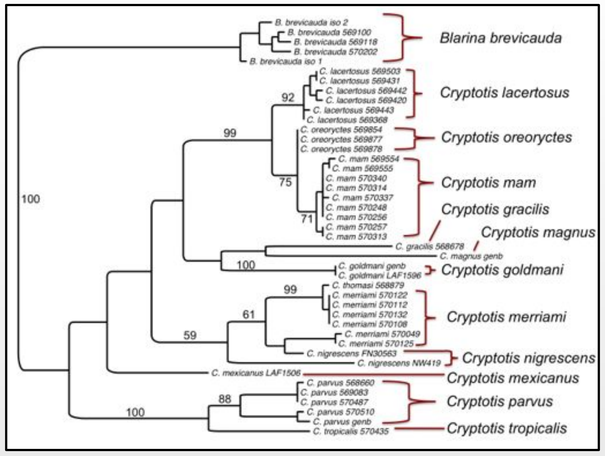


Figure 3: Maximum parsimony heuristic phylogram of 38 *Cryptotis* 16S sequences (998 bp), with five *Blarina* outgroup sequences. Bootstrap supporting values based on 1000 replicates are indicated near the nodes.

Table 1: List of compared species and localities of sample collection.

Species	Locality
C. mam	Huehuetenango, Guatemala
C. oreoryctes	Alta Verapaz, Guatemala
C. lacertosus	Huehuetenango, Guatemala
C. magnus	Oaxaca, Mexico
C. goldmani	Guerrero, Mexico
C. gracilis	Cartago, Costa Rica
C. mexicanus	Oaxaca, Mexico
C. thomasi	Valle del Cauca, Colombia
C. merriami	Alta Verapaz, Guatemala
	Baja Verapaz, Guatemala
	Zacapa, Guatemala
C. parvus	Virginia, USA
	Texas USA
	Kansas, USA
C. nigrescens	Monte Verde, Costa Rica
C. tropicalis	Huehuetenango, Guatemala
B. brevicauda	Michigan, USA
	Maine, USA
	Virginia, USA

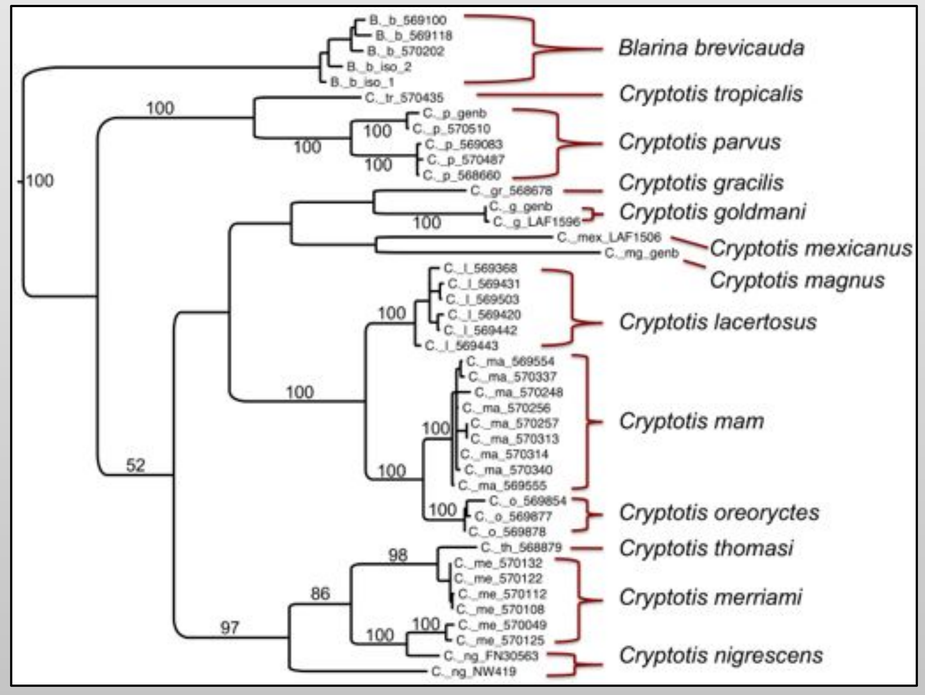


Figure 4: Maximum parsimony heuristic phylogram of 38 concatenated Cytb, ApoB, BRCA1, and 16S sequences (3225 bp), with five *B. brevicauda* as the outgroup. Numbers near nodes indicate support based on 1000 bootstrap replicates. We were unable to sequence BRCA1 for five of the *Cryptotis* samples.

Discussion

Heuristic maximum parsimony analysis of the 16S and concatenated sequence datasets revealed a highly supported monophyly of the three *C. goodwini*-like species from Huehuetenango and Alta Verapaz, GuatemalaÄ *C.lacertosus, C. mam,* and *C. oreoryctes*. The relative positions of these species in both the 16S and concatenated molecular phylogenies are concordant with a recently published morphological phylogeny, which places them as members of the *C.goldmani* subset of the *C. mexicanus* group (Woodman 2010). However, the basal nodes that group these clades lack strong bootstrap support.

The molecular position of *C. goldmani* proves intriguing. The enlarged forefeet and modified humerus suggest that *C. goldmani* is a highly derived member of the *C. mexicanus* group. A molecular analysis of cyt*b* in four species of *Cryptotis* supported this relationship (Ohdachi et al 2006). In this study, both phylogenies place *C. goldmani* in a clade with *C. gracilis* and *C. magnus* that is sister to the clade of the derived members of the *C. goodwini* subset. However, in both the 16S and the concatenated phylogenies, this node lacks strong bootstrap support.

The inconsistent placement of *C. mexicanus* based on 16S data was surprisingly different to the expected relationships based on morphological analyses. The highly derived members of the *C. mexicanus* group closely resemble *C. goldmani* and *C. goodwini* morphologically as shown in the concatenated phylogeny. However, the placement of *C. mexicanus* lacks sufficient bootstrap support in both phylogenies produced in this study.

Two distinct clades of *C. merriami* emerge from this analysisÄ one comprised of four specimens from Alta Verapaz and Baja Verapaz, and the other comprised of two specimens from Zacapa. Interestingly, *C. nigrescens* is closely nested with these two clades of *C. merriami*, with one *C. nigrescens* exhibiting a basal relationship and a second specimen falling between the two *C. merriami* clades. While these nodes have moderate support in the 16S phylogeny, they receive strong support in the concatenated phylogeny. Another intriguing placement that received strong support in both phylogenies is the nesting of *C. thomasi* with the *C. merriami* clades.

Also, concordant with the morphological data, our molecular phylogeny groups *C. parvus* and *C. tropicalis* together in a basal group with strong nodal support in both the 16S and concatenated phylogenies.

Our molecular analysis of systematics of middle American shrews of the genus *Cryptotis* clarified relationships within species and confirmed morphological groupings among the *C. goodwini*-like species, between *C. nigrescens* and *C. merriami*, and between *C. parvus* and *C. tropicalis* (Woodman 2010). Our results also suggest that *Cryptotis* diversified as a result of three separate invasion events into southern Mexico and Central America.

Acknowledgments

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