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Previous molecular allozymes, ITS2 sequences, mtDNA-RFLP, male genitalia and ultrastructure egg studies have shown varying degrees of divergence between populations of the neotropical malaria vector *Anopheles nuneztovari* from Brazil and Colombia/Venezuela. However, none of these studies strongly supports the hypothesis that *A. nuneztovari* is constituted of at least two cryptic species, based on a fixed inversion difference in polytene chromosomes. In this study, we sequenced 873 base pairs of DNA from the mitochondrial cytochrome oxidase subunit I (COI) of 16 populations of *A. nuneztovari*, throughout its geographic range in South America (Bolivia, Brazil, Colombia, Suriname, and Venezuela), and the outgroups *Anopheles dunhami* and *Anopheles darlingi*. The populations of *A. nuneztovari* sequenced yielded 22 distinct haplotypes. The uncorrected pairwise divergence ranged from 0.1% to 2.5%, with the highest values observed within Brazil. The divergence values between the populations from Brazil/Suriname and Bolivia/Colombia/Venezuela ranged from 0.9% to 2.2%, while the divergence values between the two sister-taxon *A. nuneztovari* and *A. dunhami* ranged from 1.6% to 3.2%, and between *A. nuneztovari* and *A. darlingi* ranged from 8.6% to 9.2%. The data matrix presented 42 (4.8%) variables sites, of which 32 (3.7%) are phylogenetically informative within *A. nuneztovari*. Including the outgroups *A. dunhami* and *A. darlingi*, the data matrix showed 53 (6.1%) and 108 (12.4%) variable sites, and 42 (4.8%) and 96 (11.0%) phylogenetically informative sites, respectively.

The nucleotides A and T made up 70% of all haplotypes, and C and G were observed at frequencies of 16% and 14%, respectively. Transitions were more common than transversions, and substitutions of T>C were the most frequent. Most of the transitions (87.2%) occurred at the third codon position. The strict consensus trees, using maximum parsimony, suggest that all populations of *A. nuneztovari* form a monophyletic group (94% bootstrap support) with respect to the selected outgroups. We suggest, based on uncorrected p-distance data, that populations from Brazil/Suriname and Bolivia/Colombia/Venezuela may represent at least two ancient evolutionary species within *A. nuneztovari*. However, if there are two or more cryptic species within *A. nuneztovari*, monophyly observed by the bootstrap analysis could be explained by the retention of ancestral mtDNA polymorphism after separation of these cryptic species. This research was supported by INPA/PPI-3190 and NIH.