Several species of highly toxic Centruroides inhabit Central Mexico. Relationships between common species and subspecies, C. infamatus infamatus, C. i. ornatus, C. limpidus limpidus, and C. i. tecomanus, are not resolved; existing taxonomy is based on a few morphological characters. Comparison of DNA sequences of ribosomal genes provides a new and powerful tool to examine such situations. Scorpions were collected from 12 different localities in Michoacán, Querétaro and Guerrero, México; collection sites varied in altitude (from 300 to 1940 m a.s.l.) and ecology. DNA sequences of the mitochondrial 16S rRNA gene reveal a complex taxonomic situation. At least three separate lineages are confirmed, with the 6-18 % sequence divergence. Comparison with other buthid species (Mesobuthus from West and Central Asia; our data) show that similar level of divergence is exhibited by congeneric morphospecies. It is suggested that C. i. ornatus may deserve a species status.