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DNA barcoding of new world cicada killers (Hymenoptera: Crabronidae)

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Abstract

We are engaged in a comprehensive study of cicada killers (*Sphecius* spp.), including their behavioral ecology. At one location, we observed interactions among three putative species of *Sphecius*, and used DNA barcoding to help clarify relationships among them. For this, we sequenced a fragment of the mitochondrial cytochrome *c* oxidase subunit I gene. During our study, a new taxonomic key of New World cicada killers, based on morphology, was published, and we expanded the barcoding project to test the congruence between barcodes and this key. In general, barcoding evidence supports morphological distinctions among species; sequence divergences between individuals of different species were within the range expected for congeneric Hymenoptera. However, two conflicts between barcoding and morphological evidence were noted. 1) Haplotypes of *Sphecius grandis* Say fall into two highly divergent clades, suggesting they are cryptic species. 2) Two clades of *S. convallis* Patton were found, and the sequences of one clade are virtually identical to those of *S. speciosus* Drury, suggesting that this clade of *S. convallis* is conspecific with *S. speciosus*. Alternative explanations are possible for this result, including hybridization and introgression between the two species. We conclude that our DNA barcoding evidence should be interpreted with caution, but that it has generated interesting questions we hope to resolve with field research coupled with analysis of suitable nuclear gene sequences.

Key words: cryptic species, introgression, Big Bend National Park, sympatric species, cytochrome *c* oxidase subunit I

Introduction

Cicada killers (*Sphecius* spp., Hymenoptera: Crabronidae) are ground-nesting wasps; nests may be solitary or in aggregations of up to several hundred (Evans & O'Neill 2007). These large provisioning wasps are named after the parental behavior of females, which capture and paralyze cicadas (Homoptera: Cicadidae) in nearby trees and carry them to their nests. There are currently 21 species in the genus worldwide (Pulawski 2006), five of which occur in the Western Hemisphere (Bohart 2000). Since 2004, we have collaborated on a comprehensive study of cicada killer biology, including field research of *Sphecius* physiological and behavioral ecology in Big Bend National Park (BBNP) in Texas, USA. At this location we have observed individuals of three putative species (*S. convallis* Patton, *S. grandis* Say, and *S. speciosus* Drury) interacting in the same trees and nesting areas, and females of the latter two preying on the same species of cicadas. This led us to question whether or not populations of these wasps were reproductively isolated from one another. The initial intent of this study was to use molecular evidence to help clarify the species boundaries among these sympatric wasps. We also wanted to determine the degree to which molecular evidence supported the morphological distinctions used in a recently published key to New World cicada killers (Holliday & Coelho 2006).

DNA barcoding uses relatively short, standard DNA sequences to identify species. A fragment from the 5' end of the mitochondrial cytochrome *c* oxidase subunit I gene (COX I) has shown much promise as a barcod-

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