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## Descriptions of two new, cryptic species of *Metasiro* (Arachnida: Opiliones: Cyphophthalmi: Neogoveidae) from South Carolina, USA, including a discussion of mitochondrial mutation rates

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### Abstract

Specimens of *Metasiro* from its three known disjunct population centers in the southeastern US were examined and had a 769 bp fragment of the mitochondrial gene cytochrome *c* oxidase subunit I (COI) sequenced. These populations are located in the western panhandle of Florida and nearby areas of Georgia, in the Savannah River delta of South Carolina, and on Sassafras Mt. in South Carolina. This range extends over as much as 500 km, which is very large for a species of cyphophthalmid harvestmen and presents a degree of physical separation among populations such that we would expect them to actually be distinguishable species. We examined the morphology, including the spermatopositors of males, and sequences from 221 specimens. We found no discernible differences in the morphologies of specimens from the different populations, but corrected pairwise distances of COI were about 15% among the three population centers. We also analyzed COI data using a General Mixed Yule Coalescent (GMYC) model implemented in the R package SPLITS; with a single threshold, the most likely model had four species within *Metasiro*. Given this level of molecular divergence, the monophyly of the population haplotypes, and the number of exclusive COI nucleotide and amino acid differences distinguishing the populations, we here raise the Savannah River and Sassafras Mt. populations to species status: *M. savannahensis* **sp. nov.**, and *M. sassafrasensis* **sp. nov.**, respectively. This restricts *M. americanus* (Davis, 1933) to just the Lower Chattahoochee Watershed, which in this study includes populations along the Apalachicola River and around Florida Caverns State Park. GMYC models reconstructed the two main haplotype clades within *M. americanus* as different species, but they are not exclusive to different areas. We estimate COI percent divergence rates in certain cyphophthalmid groups and discuss problems with historical measures of this rate. We hypothesize that *Metasiro* began diversifying over 20 million years ago.

**Key words:** Sassafras Mountain, Savannah River, harvestmen, DNA taxonomy, barcoding

### Introduction

Cyphophthalmid species typically have ranges of only a few kilometers and are distinguishable by subtle body proportions and genitalic differences, even when closely related (Giribet 2000; Giribet *et al.* 2012a). This feature appears to be a result of exceptionally poor dispersal abilities, and, when combined with their old age, allows them a unique role in testing hypotheses of historical landmass movements (see, for example, Boyer & Giribet 2007; Boyer *et al.* 2007b; Clouse & Giribet 2010; Giribet *et al.* 2012a; and Muriene *et al.* 2009). With an increase in cyphophthalmid sequence data, it has been possible to sort species with more confidence, as small differences in morphology and geographical location are usually accompanied by large numbers of molecular synapomorphies. In cases where specimens have been included in molecular phylogenies before a thorough morphological examination could be completed, sequence divergences have been used as a preliminary guide to species diversity (Clouse 2012).

In this context we investigated the strangely widespread US cyphophthalmid species *Metasiro americanus*, which lives in the western panhandle of Florida and nearby areas of Georgia, Sassafras Mountain in the Southern

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