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Phylogenetic relationships of Mesoamerican spider monkeys (*Ateles geoffroyi*): Molecular evidence suggests the need for a revised taxonomy

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ABSTRACT

Mesoamerican spider monkeys (*Ateles geoffroyi* sensu lato) are widely distributed from Mexico to northern Colombia. This group of primates includes many allopatric forms with morphologically distinct pelage color and patterning, but its taxonomy and phylogenetic history are poorly understood. We explored the genetic relationships among the different forms of Mesoamerican spider monkeys using mtDNA sequence data, and we offer a new hypothesis for the evolutionary history of the group. We collected up to ~800 bp of DNA sequence data from hypervariable region 1 (HV1) of the control region, or d-loop, of the mitochondrion for multiple putative subspecies of *Ateles geoffroyi* sensu lato. Both maximum likelihood and Bayesian reconstructions, using *Ateles paniscus* as an outgroup, showed that (1) *A. fusciceps* and *A. geoffroyi* form two different monophyletic groups and (2) currently recognized subspecies of *A. geoffroyi* are not monophyletic. Within *A. geoffroyi*, our phylogenetic analysis revealed no concordance between any of the classifications proposed for this taxon and their phylogenetic relationships, therefore a new classification is needed for this group. Several possible clades with recent divergence times (1.7–0.8 Ma) were identified within *Ateles geoffroyi* sensu lato. Some previously recognized taxa were not separated by our data (e.g., *A. g. vellerosus* and *A. g. yucatanensis*), while one distinct clade had never been described as different evolutionary unit based on pelage or geography (*Ateles geoffroyi* ssp. indet. from El Salvador). Based on well-supported phylogenetic relationships, our results challenge previous taxonomic arrangements for Mesoamerican spider monkeys. We suggest a revised arrangement based on our data and call for a thorough taxonomic revision of this group.

1. Introduction

Mesoamerican spider monkeys (*Ateles geoffroyi* sensu lato) have a widespread distribution throughout southern Mexico and Central America, and the taxon is suggested to contain up to nine geographically distinct forms or subspecies (Groves, 2001; Kellogg and Goldman, 1944; Rylands et al., 2006). The phylogenetic relationships among these forms and their taxonomic classification has proved contentious, as has the relationship of *A. geoffroyi* to other forms of spider monkeys. Previous studies, for example, have failed to establish whether Mesoamerican *A. geoffroyi* forms a monophyletic clade distinct from *Ateles fusciceps*, the only other currently recognized species of spider monkey found west of the Andes. *A. fusciceps* is distributed primarily along the Pacific coast of northern Ecuador and Colombia but extends into some parts of Panama, while forms of *A. geoffroyi* are found from Colombia to Mexico. Some authors have argued, based on pelage color, that *A. fusciceps* indeed represents a separate species from *A. geoffroyi* (Kellogg and Goldman, 1944), while others have suggested, based on either cranial measurements (Froehlich et al., 1991) or mtDNA sequence data (Collins and Dubach, 2000), that the former taxon is better recognized as a subspecies of the latter. For example, in a molecular phylogenetic study Collins and Dubach (2000) found that mtDNA samples assigned to *A. fusciceps* formed a monophyletic clade that was closely related to *A. geoffroyi*, but based on the genetic distance between the two clades for the COII gene (3.07%), they concluded that *A. fusciceps* and *A. geoffroyi* should

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be considered members of the same species (Collins and Dubach, 2000). However, their study only included samples from one of the currently recognized subspecies of *A. fusciceps* (*A. f. rufiventris* in the current taxonomy, but referred to as *A. f. robustus* in their study), collected from two sites, one in Colombia and one in Panama and representatives of only four of the currently recognized subspecies of *A. geoffroyi*. The authors did not analyze samples from the putative subspecies *A. f. fusciceps*, *A. g. azuerensis*, *A. g. grisescens*, or *A. g. geoffroyi*. Thus, the relationships among the various forms classified in these two morphologically distinct taxa remained unresolved. In our companion paper in this special issue, "Revisiting the phylogenetic relationships, biogeography, and taxonomy of spider monkeys (*Ateles* sp.) in light of new molecular data" (Morales-Jimenez et al., in revision), we use a robust phylogenetic analysis of close to 4 kb of mtDNA sequence data from three contiguous coding regions (*ND5*, *ND6* and cyt *b*) and demonstrate that *A. geoffroyi* and *A. fusciceps* (each represented by multiple samples from across their geographic range) indeed form two different monophyletic clades that diverged at approximately 2.2 Ma. Still, the intraspecific phylogeny of each of these two taxa, particularly that of the more widespread and variable Mesoamerican form (*A. geoffroyi* sensu lato), remains unresolved.

Traditionally, the different subspecies of *A. geoffroyi* have been recognized primarily on the basis of a combination of geography (e.g., country of origin) and pelage characteristics (Fig. 1), and, as noted above, some authors have recognized up to nine different subspecies of *A. geoffroyi* in Mesoamerica using these characteristics (Kellogg and Goldman, 1944) (Table 1). Other researchers, however, have questioned the use of pelage features for separating species and subspecies (Jacobs et al., 1995; Silva-Lopez et al., 1996) and have instead stressed the importance of evaluating the genetic variability that underlies pelage variation (Estrada et al., 2006). Mesoamerican spider monkeys are especially variable in pelage color (Fig. 1), and this trait seems to vary both among and within putative subspecies (Silva-Lopez et al., 1996), leading different authorities to propose dividing the species into different numbers of distinct subspecies (Table 1). To add to the confusion, a number of the putative subspecies of *A. geoffroyi* recognized by some researchers are considered questionable. For example, several authors question the validity of *A. g. pan* Schlegel, 1876, as the description of this subspecies was based on three individuals of unknown provenience, and the proposed distribution area lies within a region of coniferous forest that is unlikely to support spider monkeys (Konstant et al., 1985; Silva-Lopez et al., 1996). Similarly, although *A. g. grisescens* is included in the current IUCN Red List of Threatened Species, Red List assessors question the existence of this taxon, noting that "the two subspecies descriptions do not match, [and] it has never been observed in the wild" (Cuarón et al., 2008, accessed 03 January 2014). The taxonomic validity of *A. g. yucatanensis* has also been questioned, as pelage variation in this taxon is highly variable within populations and even within groups (Silva-Lopez et al., 1996). Finally, based on an assessment that the morphology of the type specimen for *A. g. panamensis* falls within the range of variation seen in *A. g. ornatus*, Napier (1976) has argued that *A. g. panamensis* should be considered a synonym of, and subsumed into, *A. g. ornatus* (see also Groves, 2001).

Thus far, Collins and Dubach's (2000) study has been the only one to apply genetic data to reconsidering the relationships among any of the Mesoamerican spider monkeys. Using mtDNA sequence data from both the control region, or d-loop, (~522 base pairs) and the COII gene (~711 base pairs) for four out of the nine subspecies of *A. geoffroyi* recognized by Kellogg and Goldman (1944), they found only limited concordance between the phylogenetic relationships inferred among these taxa using genetic data and the taxonomy proposed on the basis of pelage and geography. For example, in their study one sample from the Yucatan Peninsula in Mexico (putatively assigned to *A. g. yucatanensis*) was more closely related to a sample from Guatemala (tentatively assigned to *A. g. vellerosus*) than to other samples identified as *A. g. yucatanensis* (Collins and Dubach, 2000).

Based on their phylogenetic analysis, Collins and Dubach (2000) hypothesized the existence of two distinct clades of Mesoamerican spider monkeys: a "northern" clade containing one Honduran sample plus samples assigned to both *A. g. yucatanensis* (from Mexico, Belize and Guatemala) and *A. g. vellerosus* (from Mexico), and a "southern clade" containing samples from Panama. Within these clades, however, they were unable to detect distinct evolutionary lineages corresponding to particular proposed subspecies. Unfortunately, as Collins and Dubach (2001) noted, incomplete sampling may be responsible for the inability of some molecular data sets to resolve disputed relationships among spider monkeys and other closely related primates or to identify evolutionary distinct lineages within particular spider monkey taxa. Collins and Dubach (2001) also called attention to the importance of including in analyses multiple samples from each previously recognized subspecies of Central American spider monkeys in order to better understand the phylogenetic history of these animals.

Here, we reassess the evolutionary history of *A. geoffroyi* sensu lato. Using sequence data from the rapidly evolving mtDNA control region, we infer the phylogenetic relationships among a large number of individuals from samples collected across the geographic distribution of *A. geoffroyi*, and we evaluate whether different putative subspecies and sampled populations recognized by various authors and included in the 2013 IUCN Red List of Threatened Species form distinct monophyletic groups.

2. Methods

2.1. Samples

Blood, hair, and/or fecal samples of Mesoamerican spider monkeys from across the taxon's known geographic range were either collected in the field by the first author or were obtained by donation from in-country collaborators (Fig. 2). Following Rylands et al.'s (2006) classification of subspecies names (as it represents the taxonomy currently utilized by the IUCN: Cuarón et al., 2008), and based on the geographic provenance of samples, our dataset of 50 samples includes five of seven putative subspecies of *A. geoffroyi* as well as samples of the two putative subspecies of *A. fusciceps*, plus one sample of *A. paniscus* as an outgroup. Between 1 and 24 samples were available for each of the putative subspecies of *A. geoffroyi*. We also sampled animals from multiple geographically separated populations of two of the putative subspecies: *A. g. yucatanensis* and *A. g. vellerosus* (Table 2).

2.2. Molecular marker used

To examine the intraspecific phylogeny of *A. geoffroyi*, we sequenced portions of hypervariable region 1 (HV1) of the mitochondrial control region, which is a non-coding and highly polymorphic locus that has been widely employed in phylogenetic studies of various other primates as well as non-primate taxa (e.g., Bell et al., 2010; Charruau et al., 2011; Li et al., 2007; Nunez et al., 2011). Mitochondrial DNA is considered to be a very useful marker for intraspecific phylogenetic studies as it can be highly polymorphic even within a species, it tends to evolve faster than nuclear DNA, and it can be easily extracted and amplified from low quality or degraded samples because it is present in cells at much higher copy number than nuclear DNA (Avise, 2000, 2004). For intraspecific studies, HV1 of the control region is particularly

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