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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 little 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 a 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

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(3.07%), they concluded that *A. fusciceps* and *A. geoffroyi* should 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., 2015), we use a robust phylogenetic analysis of close to 4 kb of mtDNA sequence data from three contiguous coding regions (*ND5*, *ND6* and cytochrome *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, tends to evolve faster than nuclear DNA, and 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 useful as it

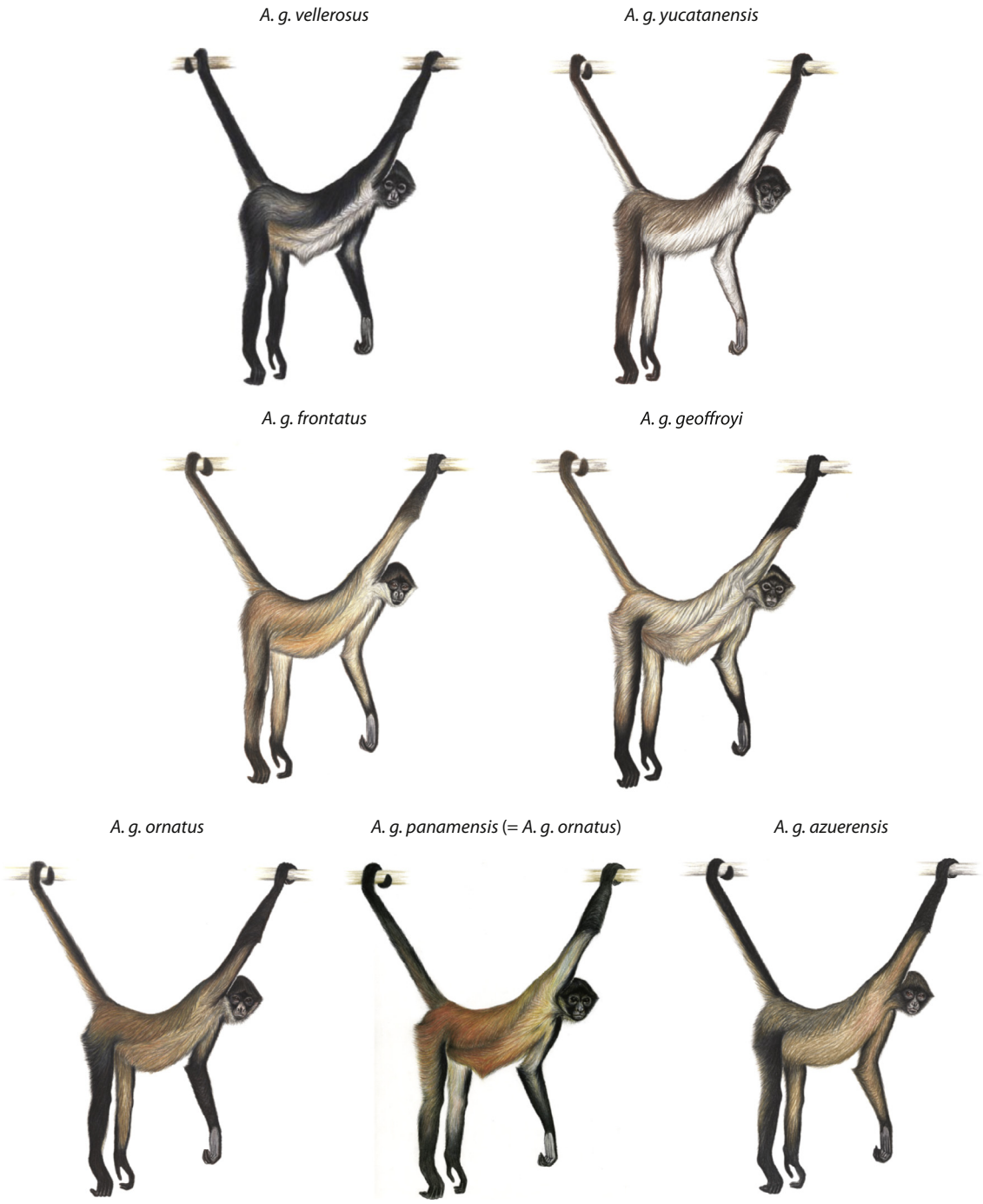

Fig. 1. Pelage characteristics of seven of the nine putative subspecies of *Ateles geoffroyi* recognized by Kellogg and Goldman (1944). Images provided by Stephen Nash.

Table 1
Alternative subspecies classifications for *Ateles geoffroyi*.

Kellogg and Goldman (1944)	Groves (2001)	Rylands et al. (2006) Cuarón et al. (2008)
A. g. geoffroyi	*A. g. geoffroyi*	*A. g. geoffroyi*
A. g. vellerosus	*A. g. vellerosus*	*A. g. vellerosus*
A. g. yucatanensis	*A. g. yucatanensis*	*A. g. yucatanensis*
A. g. pan		
A. g. frontatus		*A. g. frontatus*
A. g. ornatus	*A. g. ornatus*	*A. g. ornatus*
A. g. panamensis		
A. g. azuerensis		*A. g. azuerensis*
A. g. grisescens	*A. g. grisescens*	*A. g. grisescens*

evolves even more quickly than other mitochondrial regions and thus can be used to examine the phylogenetic relationships among taxa and individuals who shared a very recent last common ancestor (Avise, 2004).

2.3. DNA extraction, amplification, and sequencing

DNA was extracted from fecal, blood, and hair samples following protocols detailed in Morales-Jimenez et al. (2015). From each extraction, we sequenced a ~800 bp region of the mitochondrial control region encompassing HV1 by assembling a sequence contig spanning from positions 15,093 to 15,939 of the human reference sequence from shorter, overlapping fragments that were directly

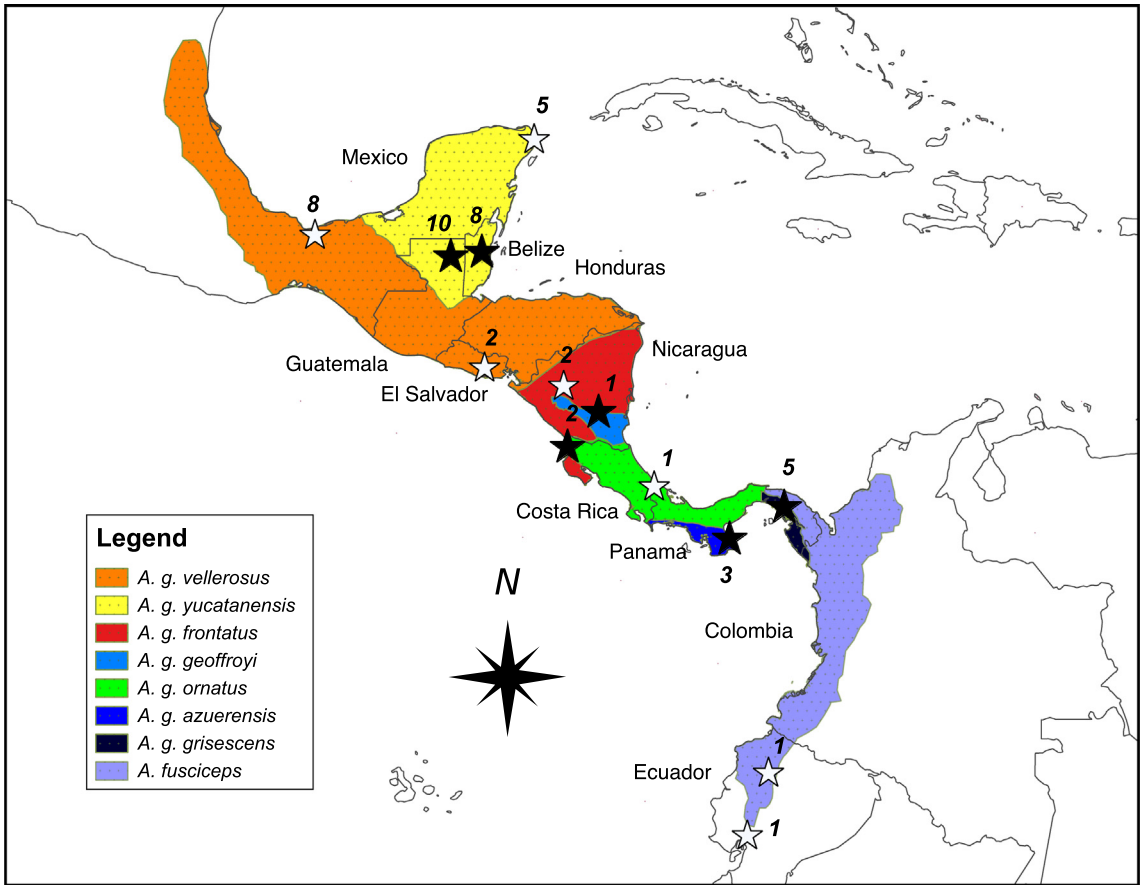

Fig. 2. Distribution map for putative subspecies of *Ateles geoffroyi* and for *A. fusciceps* (adapted from Rylands et al., 2006 and spatial data provided by the IUCN, http://www.iucnredlist.org/), with sample locations used in this study indicated by stars. The number of samples used from each site is indicated above the symbol. Black stars indicate samples with well-documented provenience, while white stars indicate that the sample came from a confiscated or captive animal with uncertain provenience that has been only assigned rough geographic coordinates. Where multiple samples are indicated at these rough coordinates (e.g., for *A. g. vellerosus* from Veracruz, México), they likely represent individuals collected from several locations around that general area. The samples of *A. f. fusciceps* were collected by the first author at the indicated coordinates in the Darien region of Panama. Although those coordinates fall within the distribution of *A. geoffroyi griscesens* included in IUCN maps, the animals clearly belong to the species *A. fusciceps*, both phenotypically and genetically.

amplified from our initial DNA extraction. These PCR amplifications were performed in 10 μl reactions using 5 μl of HotStart-IT® Taq Master Mix (2×), 0.2 μl each of the heavy and light strand primers (see Supplementary Data) at a concentration of 10 μM, 2 μl of unquantified DNA extraction, and 2.6 μl of HPLC water. Cycling conditions for all amplifications were as follows: initial denaturation 94 °C for 2 min; 35 cycles of denaturing at 94 °C for 30 s, annealing at 55 °C for 30 s and extension at 68 °C for 60 s; and final extension was at 68 °C for 5 min. PCR products were visualized on 1% agarose gels, purified using a standard ExoSAP treatment, and then used as a template for cycle sequencing reactions following protocols detailed in Morales-Jimenez et al. (2015). After ethanol precipitation and re-suspension in Hi-Di formamide, sequencing reaction products were separated and sequenced via capillary electrophoresis on an ABI 3730 DNA Analyzer (Applied Biosystems).

For a subset of nine fecal samples, we also independently sequenced the control region using as our cycle-sequencing template the product of an initial 'long-range' amplification of a roughly 5 kb mitochondrial region (Morales-Jimenez et al., 2015) in order to check for the possible amplification of nuclear copies of mtDNA pseudogenes or 'numts': (Zhang and Hewitt, 1996). Because it is much less common for very large mitochondrial fragments to be incorporated into the nuclear genome than smaller fragments, a lack of concordance between sequences generated from short- and long-range amplicons of the same sample would be cause for concern that our methods inadvertently yield 'numts' rather than true mitochondrial sequences. We also checked all amplification products for the following: (1) extra PCR bands in the initial visualization gel, (2) ambiguities (e.g., double bases) in the resulting sequence, and (3) unexpected phylogenetic placement for the sample. A complete list of amplification and sequencing primers are provided as Supplementary data.

Raw sequencing traces were reviewed using the software Sequencher 4.7 (Gene Codes Corporation) to check for miscalls due to either bad base spacing or over-fluorescence of particular dye nucleotides and to trim poor quality flanking regions from sequences. Sequencher 4.7 (Gene Codes Corporation) was also used to create contig assemblies of partial HV1 control region sequences for each sample.

2.4. Phylogenetic analyses

Sequences from our 50 samples were aligned with one another using the program Muscle v3.6 (Edgar, 2004) using the program default parameters: number of iterations = 16, maximum memory allocated = 30,000,000 mb. To infer the model of nucleotide substitution we used the softwares Modeltest 3.7 (Posada and Crandall,

Table 2
Samples of *Ateles geoffroyi*, *A. fusciceps*, and *A. paniscus* (outgroup) used in this study, along with the sample ID, putative taxonomic assignment based on pelage, and country of origin. Coordinates included in brackets [ ] reflect those samples for which we do not have precise locations. For these, we use the coordinates of the general area of origin, as indicated in Fig. 2.

Sample ID	Species	Country	Locality Name	Coordinates (decimal degrees)	Accession number
ALM126	*A. g. azuerensis*	Panama	La Miel, Las Tablas, Azuero	7.76 N, 80.28 W	KJ186889
ALM127	*A. g. azuerensis*	Panama	La Miel, Las Tablas, Azuero	7.76 N, 80.28 W	KJ186888
ALM120	*A. g. azuerensis*	Panama	La Miel, Las Tablas, Azuero	7.76 N, 80.28 W	KJ186890
AGNIC-1	*A. g. frontatus*	Nicaragua	El Espabel, St. Isabel Farm	12.01 N, 84.67 W	KJ186895
AGNIC-2	*A. g. frontatus*	Nicaragua	Guadalupe West	[12.88 N, 85.84 W]	KJ186893
AGNIC-3	*A. g. frontatus*	Nicaragua	Guadalupe West	[12.88 N, 85.84 W]	KJ186894
OCR1	*A. g. frontatus*	Costa Rica	Santa Rosa National Park	10.84 N, 85.71 W	KJ186896
24CR2	*A. g. frontatus*	Costa Rica	Santa Rosa National Park	10.84 N, 85.71 W	KJ186897
S063	*A. g. ornatus*	Panama	Summit Zoo	[9.51 N, 82.81 W]	KJ186887
AG73	*A. g. vellerosus*	El Salvador	Unknown	[13.49 N, 88.49 W]	KJ186892
AG76	*A. g. vellerosus*	El Salvador	Unknown	[13.49 N, 88.49 W]	KJ186891
S016	*A. g. vellerosus*	Mexico	Unknown, confiscated by Mexican authorities	[17.94 N, 94.17 W]	KJ186881
S017	*A. g. vellerosus*	Mexico	Unknown, confiscated by Mexican authorities	[17.94 N, 94.17 W]	KJ186885
S018	*A. g. vellerosus*	Mexico	Unknown, confiscated by Mexican authorities	[17.94 N, 94.17 W]	KJ186872
S019	*A. g. vellerosus*	Mexico	Unknown, confiscated by Mexican authorities	[17.94 N, 94.17 W]	KJ186882
S020	*A. g. vellerosus*	Mexico	Unknown, confiscated by Mexican authorities	[17.94 N, 94.17 W]	KJ186883
S024	*A. g. vellerosus*	Mexico	Unknown, confiscated by Mexican authorities	[17.94 N, 94.17 W]	KJ186868
S025	*A. g. vellerosus*	Mexico	Unknown, confiscated by Mexican authorities	[17.94 N, 94.17 W]	KJ186865
S026	*A. g. vellerosus*	Mexico	Unknown, confiscated by Mexican authorities	[17.94 N, 94.17 W]	KJ186867
103P	*A. g. yucatanensis*	Belize	Runaway Creek Nature Reserve	17.37 N, 88.58 W	KM347892
115P	*A. g. yucatanensis*	Belize	Runaway Creek Nature Reserve	17.37 N, 88.58 W	KJ186863
120P	*A. g. yucatanensis*	Belize	Runaway Creek Nature Reserve	17.37 N, 88.58 W	KJ186864
123P	*A. g. yucatanensis*	Belize	Runaway Creek Nature Reserve	17.37 N, 88.58 W	KJ186880
129P	*A. g. yucatanensis*	Belize	Runaway Creek Nature Reserve	17.37 N, 88.58 W	KJ399981
130P	*A. g. yucatanensis*	Belize	Runaway Creek Nature Reserve	17.37 N, 88.58 W	KJ399982
131P	*A. g. yucatanensis*	Belize	Runaway Creek Nature Reserve	17.37 N, 88.58 W	KJ399983
137P	*A. g. yucatanensis*	Belize	Runaway Creek Nature Reserve	17.37 N, 88.58 W	KJ399984
AA13	*A. g. yucatanensis*	Guatemala	Tikal National Park	17.22 N, 89.62 W	KJ186877
AA16	*A. g. yucatanensis*	Guatemala	Tikal National Park	17.22 N, 89.62 W	KJ186874
AA19	*A. g. yucatanensis*	Guatemala	Tikal National Park	17.22 N, 89.62 W	KJ186873
AA20	*A. g. yucatanensis*	Guatemala	Tikal National Park	17.22 N, 89.62 W	KJ186869
AA24	*A. g. yucatanensis*	Guatemala	Tikal National Park	17.22 N, 89.62 W	KJ186862
AA25	*A. g. yucatanensis*	Guatemala	Tikal National Park	17.22 N, 89.62 W	KJ186856
AA27	*A. g. yucatanensis*	Guatemala	Tikal National Park	17.22 N, 89.62 W	KJ186876
ALM30A	*A. g. yucatanensis*	Guatemala	Tikal National Park	17.22 N, 89.62 W	KJ186870
EMV16	*A. g. yucatanensis*	Guatemala	Tikal National Park	17.22 N, 89.62 W	KJ186875
EMV19	*A. g. yucatanensis*	Guatemala	Tikal National Park	17.22 N, 89.62 W	KJ186866
S081	*A. g. yucatanensis*	Mexico	Cancun	[21.14 N, 86.83 W]	KJ186878
S082	*A. g. yucatanensis*	Mexico	Cancun	[21.14 N, 86.83 W]	KJ186879
S083	*A. g. yucatanensis*	Mexico	Cancun	[21.14 N, 86.83 W]	KJ186884
S084	*A. g. yucatanensis*	Mexico	Cancun	[21.14 N, 86.83 W]	KJ186886
S085	*A. g. yucatanensis*	Mexico	Cancun	[21.14 N, 86.83 W]	KJ186871
ALM700	*A. f. fusciceps*	Ecuador	Reserva Ashiringa, Pichincha	[0.08 S, 78.98 W]	KJ452770
ALM703	*A. f. fusciceps*	Ecuador	Centro de Rescate Jambeli, Guayas	[2.61 S, 79.73 W]	KM347893
ALM51	*A. f. rufiventris*	Panama	Cerro Chucanti, Darien	8.82 N, 78.45 W	KJ186860
ALM52	*A. f. rufiventris*	Panama	Cerro Chucanti, Darien	8.82 N, 78.45 W	KJ186861
ALM53	*A. f. rufiventris*	Panama	Cerro Chucanti, Darien	8.82 N, 78.45 W	KJ186859
ALM56	*A. f. rufiventris*	Panama	Cerro Chucanti, Darien	8.82 N, 78.45 W	KJ186857
ALM63	*A. f. rufiventris*	Panama	Cerro Chucanti, Darien	8.82 N, 78.45 W	KJ186858
T4193	*A. paniscus*	Guyana	Elahe Via	[4.28 N, 58.41 W]	KM347891

1998) and MrModeltest 2.2 (Nylander, 2004) and chose the best model according to the Akaike Information Criterion (AIC). The model chosen by Modeltest 3.7 (TrN + I + G) was used for maximum likelihood analysis implemented in RAxML 7.2.8 (Stamatakis, 2006) using the default parameters, and we used 100,000 bootstrap replicates to assess the confidence of ML trees. The model chosen by MrModeltest 2.2 (GTR + I + G) was used for the Bayesian phylogenetic inference as implemented using MrBayes 3.1.2 (Ronquist and Huelsenbeck, 2003). For our Bayesian analysis, we ran 4 chains and 2 runs, which we allowed to run for 10,000,000 generations sampling trees and parameter values every 1000 generations. Both RAxML and MrBayes analyses were executed on the CIPRES Science Gateway v3.3 (Miller et al., 2010). For both of these analyses, we used *Ateles paniscus*, the Guianan spider monkey, as an outgroup based on our confirmation that *A. fusciceps* and *A. geoffroyi* form reciprocally monophyletic sister lineages, with *A. paniscus* as a more distantly related member of the spider monkey clade (Morales-Jimenez et al., 2015).

2.5. Divergence time estimation

To estimate divergence dates between lineages within the Mesoamerican spider monkeys based on HV1 sequences, we used the software BEAST v1.8.0 (Drummond and Rambaut, 2007). We constrained *A. fusciceps* and *A. geoffroyi* to form reciprocally monophyletic sister lineages, as we inferred in both this study (see results below) and in our broader analysis of spider monkey phylogenetics using sequence data from more slowly-evolving mtDNA regions (~3.5 kb of the *NAD5, NAD6,* and cytochrome *B* coding regions: Morales-Jimenez et al., 2015). To calibrate our divergence date estimates within *A. geoffroyi*, we set a normal prior for the split between *A. geoffroyi* and *A. fusciceps* at 2.20 ± 0.62 mya, which covers the 95% HPD range estimated for this divergence based on our broader analysis of *Ateles* phylogenetics (Morales-Jimenez et al., 2015). As for our phylogenetic analyses, we again specified *Ateles paniscus* as an outgroup for a clade of Mesoamerican spider monkeys (*A. geoffroyi* + *A. fusciceps*), and we assigned a normal

prior for the root of the tree – i.e., for the divergence of *A. paniscus* from *A. geoffroyi* + *A. fusciceps* – at 3.50 ± 0.86 mya, which again covers the 95% HPD estimated for this divergence in our broader study (Morales-Jimenez et al., 2015). Finally, we also constrained all of the clades within *A. geoffroyi* and *A. fusciceps* that were identified with > 80% bootstrap and posterior probability support in our phylogenetic analyses of HV1 sequence data (see below) to also be monophyletic.

We used the software PartitionFinder v1.1.0 (Lanfear et al., 2012) to select the best model of nucleotide substitution for our dating analysis under AIC, which was TrN + I + G, thus we used the following parameter settings for our BEAST analyses: Substitution Model = TN93; Base Frequencies = Estimated; Site Heterogeneity Model = Gamma (with 4 rate categories); Clock Model = Lognormal relaxed clock (uncorrelated). We assumed a Speciation: Yule Process model for the Tree Prior, with the starting tree generated randomly following the monophyly constraints noted above, and we specified a diffuse gamma prior distribution (with shape 0.001 and scale 1000) for the ucld.mean parameter. With these settings, we performed 10 separate MCMC runs, each with a chain length of 22 million generations with parameters values sampled every 1000 generations. The software Tracer v1.6 was used to visually inspect the BEAST log files and monitor convergence statistics for each run by effective samples sizes (ESS), after removing a burn-in period of 2 million generations. Log and tree files for the 10 runs were merged and resampled at a lower frequency (every 10,000 generations) using LogCombiner v1.6, and the software TreeAnnotator v1.6.1 was then used to derive a consensus tree with estimated divergence times and 95% confidence intervals based on the remaining 20,000 total trees sampled from the runs.

Once our phylogenetic and dating analyses were completed, we mapped the subspecies name assigned to each sample according to their geographic origin and based on Rylands et al.'s (2006) classification onto our resultant trees to explore whether samples assigned to the same subspecies clustered together in monophyletic clades.

3. Results

In total, we sequenced ~800 bp of the mtDNA control region for 49 samples putatively assigned to five of seven currently-recognized subspecies of *A. geoffroyi* and two subspecies of *A. fusciceps* (Table 2). For nine of these samples where we also sequenced the same region from an initial long-range amplicon, the sequences generated from shorter fragments amplified directly from our original extractions matched perfectly those generated from the long-range amplicon, suggesting that our method indeed yielded true mitochondrial sequences rather than 'numts.'

The results of both our maximum likelihood and Bayesian phylogenetic inferences of the relationships among HV1 sequences from different samples of *A. geoffroyi* sensu lato are shown in Fig. 3. Both of our analyses identified three main clades (**A**, **B**, and **C**) within *A. geoffroyi*. However, these clades do not reflect perfectly any of the subspecies classifications proposed to date. The **A** clade consists of samples putatively assigned to two subspecies, *A. g. vellerosus* and *A. g. yucatanensis* from Mexico, Belize and Guatemala. Control region sequences from these two putative subspecies are clearly intermixed and do not fall into distinct clades. The **B** clade consists mainly of a monophyletic set of samples from Nicaragua and Costa Rica, putatively assigned to *A. g. frontatus*, plus two samples from El Salvador putatively assigned to *A. g. vellerosus* as basal within the clade. Finally, the **C** clade comprised samples from Panama putatively assigned to two subspecies, *A. g. azuerensis* and *A. g. ornatus*.

Further examination of the tree shows additional examples of incongruence between inferred phylogenetic relationships and putative subspecies classifications. For example, as noted above, two samples from El Salvador described as *A. g. vellerosus* fall basally within the **B** clade and are more closely related to samples putatively assigned to *A. g. frontatus* than to other *A. g. vellerosus*. Similarly, one sample tentatively identified as *A. g. ornatus* (sample S063: Table 2) from Panama is closely related to *A. g. azuerensis*; given the variation in pelage coloration in *A. geoffroyi*, it is impossible to rule out the possibility that this specimen comes from the Azuero peninsula.

In spite of the lack of concordance between phylogenetic position and the subspecific assignments of some haplotypes, the phylogenetic relationships among different mtDNA haplotypes of *A. geoffroyi* in Mesoamerica are generally consistent with the country and broader geographic regions from which the samples originated (Fig. 2): the **A** clade comprised samples from Mexico, Belize and Guatemala; the **B** clade comprised samples from El Salvador, southwestern Nicaragua and northwestern Costa Rica; and the **C** clade included only samples from Panama, but no samples from that country or elsewhere assigned to *A. fusciceps* (Fig. 3). Based on our HV1 control region sequence data, the **A** clade has no further structure obvious within it, unlike the other two clades. Within the **B** clade, the two samples available from El Salvador (putatively of *A. g. vellerosus*) are positioned basally to a well-supported clade of Nicaraguan and Costa Rican samples. Finally, the **C** clade shows a further subdivision into two clades, one formed by samples from the Azuero Peninsula (Panama) and a closely related lineage formed by the sample S063 – putatively *A. g. ornatus* – from Panama.

Our BEAST dating analysis indicates that the first clade to diverge within *A. geoffroyi* sensu lato was the **C** clade at ~1.7 Ma (95% HPD confidence interval 0.8–2.7 Ma) (Fig. 4). The **B** clade diverged from the **A** clade soon thereafter, ~1.5 Ma (95% HPD confidence interval 0.6–2.4 Ma). Within the **C** clade, the Azuero haplotypes diverge from the S063 sample from Panama ~0.75 Ma (95% HPD confidence interval 0.16–1.4 Ma). Within the **B** clade, the Salvadorian haplotypes split from the Costa Rican plus Nicaraguan samples at ~0.83 Ma (95% confidence interval 0.24–1.5 Ma). Finally, all samples from the **A** clade shared a common ancestor ~0.85 Ma (95% confidence interval 0.30–1.5 Ma).

4. Discussion

4.1. Phylogenetic relationships among Central American spider monkeys

Prior to this research, the study by Collins and Dubach (2000) was the only one to have explored the phylogenetic relationships within the Mesoamerican spider monkeys, *A. geoffroyi* sensu lato, using molecular data. Based on a phylogenetic analysis of ~522 bp of sequence data from the mtDNA control region for seven samples (representing four putative subspecies) collected from different localities across Mesoamerica, they proposed the existence of at least two distinct *A. geoffroyi* clades: a northern clade composed of *A. g. yucatanensis* and *A. g. vellerosus* and a southern clade composed of *A. g. panamensis* (= *A. g. ornatus*, following Rylands et al., 2006 and Cuarón et al., 2008) and a sample whose subspecies designation was unassigned. In their study, the placement of an additional sample from Nicaragua (assigned to *A. g. frontatus*) was uncertain, but possibly basal within the northern clade. They thus concluded that no proposed subspecific taxonomy was consistent with the patterning of genetic variation among Central American *Ateles*. In this study, we also found evidence for "northern" and "southern" clades (our **A** and **C** clades), supporting

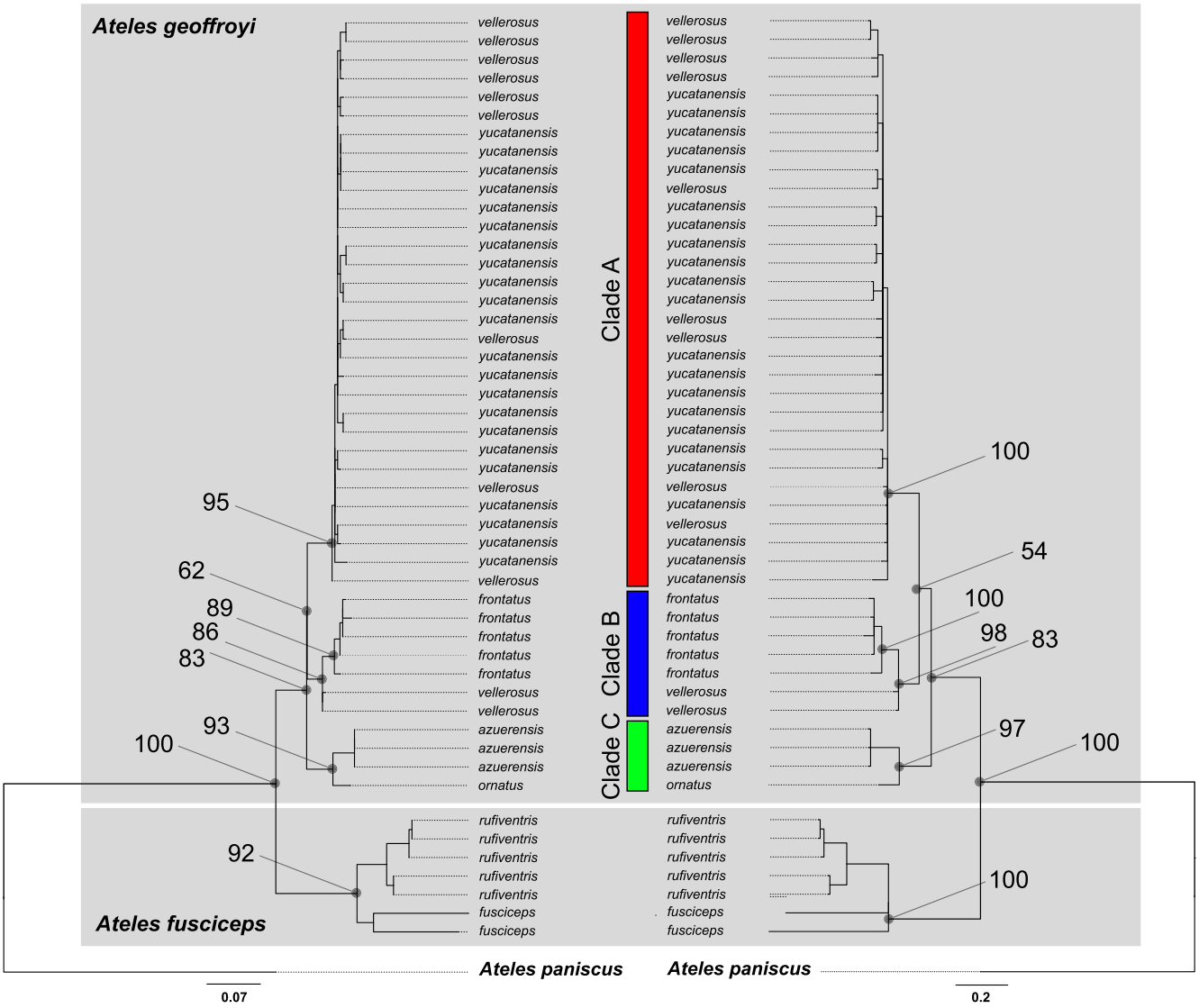

Fig. 3. Phylogenetic relationships for Mesoamerican spider monkeys *(Ateles geoffroyi* sensu lato and *Ateles fusciceps*) inferred in this study based on analysis of mtDNA sequence data for HV1 of the control region (Outgroup: *Ateles paniscus*) based on maximum likelihood analysis (left hand tree) and Bayesian inference (right hand tree). Both maximum likelihood and Bayesian analyses identified very similar tree topologies with three distinct, well-supported clades within the phylogeny. Support values (bootstrap support, in the case of the maximum likelihood tree, and posterior probabilities, in the case of the Bayesian tree) are indicated for key nodes. The three major clades (**A**, **B**, and **C**) recovered in both analyses are indicated by the colored boxes. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Collins and Dubach's (2000) conclusions. Our additional sampling in the central part of the *Ateles* distribution (i.e., southwestern Nicaragua, Costa Rica, and El Salvador) also identified a new (although less well-supported) third clade (our **B** clade) that seems restricted to this region, and Collins and Dubach's (2000) *A. g. frontatus* sample could very well represent this clade although their more limited sampling in this region did not allow them to identify it.

The phylogenetic tree inferred in this study is well resolved for most of the clades. The lowest support in our best phylogenetic reconstruction was for the node splitting the **A** and **B** clades (Bayesian posterior probability = 54%, likelihood bootstrap support = 62%), although each of the three clades individually had much higher support (89% or greater bootstrap support and 93% or greater posterior probability). The overall higher support values we found compared to Collins and Dubach's (2000) results may be due to the slightly longer fragment we sampled (~811 bp versus ~522 bp) as well as the fact that we used many additional samples from a wider geographic area. Our dating results suggest that the **C** clade – corresponding to Collins and Dubach's "southern" clade – split from other Mesoamerican spider monkeys ~1.7 Ma and that the **A** and **B** clades split soon thereafter, at ~1.5 Ma. Collins and Dubach (2000) estimated a date for the split between their "northern" and "southern" clades at ~1.3 Ma, which falls within the confidence interval found in this study. In general, our data suggest a possible 'Isolation by Distance' (Wright, 1943) pattern for *Ateles* in Mesoamerica; however adequate analysis to test for this pattern requires a more complete sampling of all major regions, and thus we hope to address this as we continue collecting and analyzing Mesoamerican samples of *Ateles* from regions still not represented in this study.

Our estimates of divergences among *A. geoffroyi* lineages in the Early Pleistocene fall within the time frame suggested by the biogeographic scenario proposed by Ford (2006) concerning the spread of spider monkeys into and across Mesoamerica. Ford (2006) analyzed the physiogeographic changes that occurred in South America from the late Miocene through the Pleistocene and how these changes may have affected the migration of primates into Central America. She suggested that at 8–10 Ma,

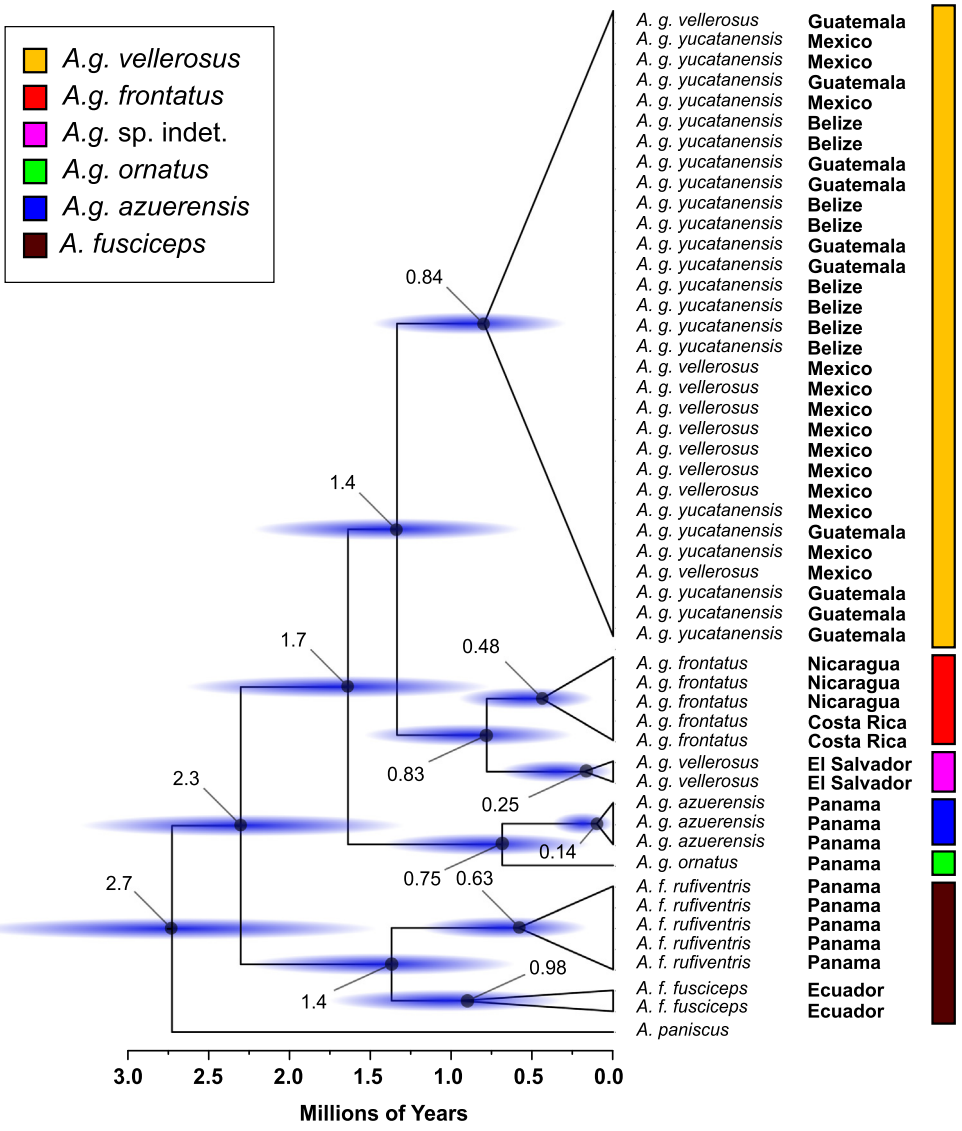

Fig. 4. Results of the Bayesian divergence time analysis showing the estimated age, in millions of years (plus 95% HPD confidence interval, in blue), for each major clade identified in our phylogenetic reconstruction. Also shown is the country of origin for each sample and the proposed subspecies names advocated here. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

the Cordillera Oriental in Colombia and Venezuela was a limiting barrier for the interchange of taxa between the Amazon and the northwestern regions of Colombia and Ecuador. When the connection between South and Central America was completed (around 3.0–3.5 Ma), the only source of lowland taxa for migration into Central America already would have been those living in the Cauca and Magdalena valleys in northern Colombia and the Chocó region. Regarding the colonization of Central America by spider monkeys, Ford (2006) suggests the migration of a source population around 2 Ma (which is entirely concordant with our results) from Panama up to the southern states of Mexico. Spider monkeys are the only non-human primates found on the Pacific coast of Mexico, and Ford (2006) suggests that a subsequent migration then occurred from this area south to El Salvador. Alternatively, the topology of our phylogenetic tree – including the presence a clade **B** comprising individuals from El Salvador, northwestern Costa Rica, and southwestern Nicaragua that are phylogenetically more proximate to the *vellerosus*/*yucatanensis* clade – is also consistent with an initial split within *A. geoffroyi* that divided the populations of Panama from those of Costa Rica to the north (similar to what was found by Cortés-Ortiz et al., 2003, for howler monkeys) and a subsequent split between populations in the central part of Central America from those of Mexico, Guatemala and Belize. Ford (2006) proposed a final later invasion of Central America by *A. fusciceps*, particularly into the northeastern region of Panama, a scenario which is also consistent with our data.

4.2. Implications for taxonomy and conservation

The classification and naming of organisms is important for policy makers as well as biologists. Conservation actions, including decisions about the management of captive and wild populations, are made based, in part, on how animals are named (Sinclair et al., 2005). *Ateles geoffroyi* has been classified into anywhere from five to nine subspecies based on morphological features such as pelage length and color (Groves, 2001; Kellogg and Goldman, 1944; Rylands et al., 2006). According to this study, however, none of the classifications proposed for *A. geoffroyi* are concordant with phylogenetic relationships inferred using mitochondrial control region sequence data. Taking into account the fact that dispersal

in spider monkeys is predominantly by females (Ahumada, 1989; Symington, 1988, 1990), the mtDNA tree is expected to be a very good predictor of the relationships between taxa and the observed structure is not an artifact of female philopatry.

We strongly agree that taxonomic classification should reflect phylogenetic relationships (Groves, 2001; Nelson, 1972; Sinclair et al., 2005), and therefore, based on the results presented here, the classification of *A. geoffroyi* needs to be reconsidered (although other loci should also be studied to further evaluate the hypothesis of phylogenetic relationships reconstructed here). The first important task is to determine whether or not the taxa currently subsumed within *A. geoffroyi* sensu lato should in fact be divided into more than one recognized species or subspecies. Groves (2001, 2004) suggests the use of the phylogenetic species concept (PSC) to delimit taxa, as this concept allows a falsifiable approach that could be tested. The PSC defines a species as the smallest cluster of individual organisms with a parental pattern of ancestry and descent and that is diagnosably separated from other clusters by a unique combination character states (Cracraft, 1983). Within primates, the PSC approach has recently been applied in reconsidering the evolutionary relationships among members of the *Saguinus nigricolis* species group (Matauschek et al., 2011), the leaf monkeys (Meyer et al., 2011), the genus *Lepilemur* (Craul et al., 2007), and the genus *Nomascus* (Thinh et al., 2010) among others. From a conservation perspective, the PSC carries benefits over other species concepts, as (1) it can be applied to allopatric populations (Agapow, 2005); (2) it can be more objective when compared with morphospecies, especially for cryptic species (Bruna et al., 1996); and (3) it is a better indicator of biodiversity (Meyer et al., 2011).

Applying the PSC to the results presented here, we find several well-supported monophyletic clades with recent divergence times (1.8 Ma–750 kyr) within *A. geoffroyi* sensu lato that might warrant consideration as distinct taxa. For comparability with the current classification of Mesoamerican *Ateles*, we tentatively distinguish these taxa at the subspecies rather than the species level. Given the recent crown age we reconstructed in this study for *A. geoffroyi* sensu lato (1.8 Ma), such an approach is also consistent with an age-based system for assigning taxonomic rank, where species level divergences usually predate the Pleistocene (Wildman and Goodman, 2004). Accordingly, populations from Mexico, Belize and Guatemala (the **A** clade) would be considered *A. g. vellerosus* (as the *vellerosus* name has priority over *yucatanensis*). Additionally, we find that there is not enough evidence to separate this taxon into two subspecies, a point also suggested by Silva-Lopez et al. (1996) who noted that pelage variation within and between populations is highly variable and not necessarily linked to particular populations. Although the inferred phylogenetic tree shows a few distinct lineages within the **A** clade, it is very unlikely the relationships among these lineages could be resolved with more data since the samples included in some of these seemingly distinct clades come from very distant localities. For example, in one lineage within the **A** clade a sample from the Yucatan Peninsula (Cancun, Mexico) groups with a sample from Tikal (Guatemala), ~700 km away.

Moving from north to south, the next possible taxon would be the **B** clade formed by spider monkeys from El Salvador, Nicaragua, and Costa Rica. The two Salvadoran samples are basal within this clade and virtually identical to one another (99.8% sequence identity); more samples from this country, as well as samples from individuals living in southern Guatemala, Honduras and northern Nicaragua, should be analyzed to determine the actual variation in and geographic range of the **B** clade before a final decision about the taxonomic status of the Salvadoran samples is made. Based on the data in hand, however, these samples do not belong in the *A. g. vellerosus* group and are more closely related to samples from Nicaragua and Costa Rica. At minimum, the subspecies name for samples from this region should change as they are clearly distinct from *A. g. vellerosus*.

Continuing southward, within the **B** clade the Nicaraguan and Costa Rican samples together form a monophyletic group (although it is important to mention these samples come from the southwest of Nicaragua and the northwest of Costa Rica, and no samples from the eastern parts of these countries were analyzed). Samples from these regions have previously been classified as *A. g. frontatus*, and their pelage coloration matches the description from Kellogg and Goldman (1944) as well as the distribution proposed by Rylands et al. (2006). Groves (2001), however, considers *A. g. frontatus* as a synonym of *A. g. geoffroyi*, from southeastern Nicaragua. Although the name *A. g. geoffroyi* would be senior to *A. g. frontatus*, in this study we were not able to include samples from individuals putatively belonging to *A. g. geoffroyi*, therefore to use that name, rather than *A. g. frontatus*, to refer to the samples from Costa Rica and Nicaragua would be premature. Additional taxonomic and genetic studies are needed to evaluate whether samples of eastern Nicaragua (*A. g. geoffroyi*) are distinct from those from southwestern Nicaragua and northwestern Costa Rica (*A. g. frontatus*) or if these two taxa are indeed synonymous.

The last two well-supported clades inferred from our dataset (the **C** clade and a subclade nested within it) are found in the samples collected from Panama. The Azuero Peninsula samples putatively classified as *A. g. azuerensis* form a monophyletic group. This clade diverged ~750 kyr from its closest relative, a sample putatively assigned to *A. g. ornatus* (sample S063) from a zoo in Panama. The precise collection locality of this last sample is unknown, but the pelage coloration matches the description by Kellogg and Goldman (1944) of *A. g. panamensis* (Fig. 1). However, the extent of variation in coloration patterns between *A. g. ornatus* and *A. g. azuerensis* has not been explored, and so we cannot exclude the possibility that the origin of this individual was the Azuero peninsula. Nonetheless, given the haplotype divergence of this sample, we continue considering S063 as *A. g. ornatus* pending analysis of additional samples from this area.

In summary, we propose that (1) specimens from Panama previously referred to as *A. g. panamensis* should be renamed as *A. g. ornatus* (as currently recognized by the IUCN: Cuarón et al., 2008), (2) specimens from the Azuero peninsula continue to be distinguished as *A. g. azuerensis* until further analysis of samples from other locations in Panama is possible, (3) specimens from El Salvador should be considered distinct from *A. g. vellerosus* and possibly as a distinct subspecies (*A. geoffroyi* ssp. indet.), (4) individuals from southwestern Nicaragua and northwestern Costa Rica should be considered as *A. g. frontatus*, and (5) individuals from Mexico, Guatemala and Belize, currently distinguished as *A. g. vellerosus* and *A. g. yucatanensis,* should be subsumed into a single subspecies, *A. g. vellerosus*. Whether or not individuals from Nicaragua currently considered as *A. g. geoffroyi* are distinct from those from southwestern Nicaragua and northwestern Costa Rica – as well as the phylogenetic identity of individuals from Honduras, central and western Panama, and eastern Costa Rica – remains to be determined.

For conservationists, it is critical to know whether a taxon is sufficiently differentiated genetically from others to warrant separate management and to maintain genetic diversity (Sinclair et al., 2005). In our study we detected several monophyletic clades, which could all be regarded as independent management units based on the mtDNA analysis presented here (although we agree that more loci will be needed to make adequate decisions on taxonomy). Support from nuclear markers is also required to confirm the validity of the distinct lineages we identified. This information will help researchers, managers and conservationists to assess the status of the different taxa and create programs to protect the most endangered forms. For example, *ex situ* collections could be

organized using this taxonomic framework, separating animals from different geographical regions or according to their haplotype, and breeding programs could be implemented for those taxa that are critically endangered. Furthermore, the establishment of a solid taxonomy based on phylogenetic relationships would allow researchers studying ecology and behavior to compare the results of their investigations considering similar evolutionary units. Finally, decision makers would be able to evaluate threats and risks affecting particular management units and direct efforts to those in more need.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ympev.2014.08.025.

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