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A phylogenomic perspective on the robust capuchin monkey (*Sapajus*) radiation: First evidence for extensive population admixture across South America

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ABSTRACT

Phylogenetic relationships amongst the robust capuchin monkeys (genus *Sapajus*) are poorly understood. Morphology-based taxonomies have recognized anywhere from one to twelve different species. The current IUCN (2017) classification lists eight robust capuchins: *S. xanthosternos*, *S. nigritus*, *S. robustus*, *S. flavius*, *S. libidinosus*, *S. cay*, *S. apella* and *S. macrocephalus*. Here, we assembled the first phylogenomic data set for *Sapajus* using ultra-conserved elements (UCEs) to reconstruct a capuchin phylogeny. All phylogenomic analyses strongly supported a deep divergence of *Sapajus* and *Cebus* clades within the capuchin monkeys, and provided support for *Sapajus nigritus*, *S. robustus* and *S. xanthosternos* as distinct species. However, the UCE phylogeny lumped the putative species *S. cay*, *S. libidinosus*, *S. apella*, *S. macrocephalus*, and S. *flavius* together as a single widespread lineage. A SNP phylogeny constructed from the UCE data was better resolved and recovered *S. flavius* and *S. libidinosus* as sister species; however, *S. apella*, *S. macrocephalus*, and *S. cay* individuals were recovered in two geographic clades, from northeastern and southwestern Amazon, rather than clustering by currently defined morphospecies. STRUCTURE analysis of population clustering revealed widespread admixture among *Sapajus* populations within the Amazon and even into the Cerrado and Atlantic Forest. Difficulty in assigning species by morphology may be a result of widespread population admixture facilitated through frequent movement across major rivers and even ecosystems by robust capuchin monkeys.

1. Introduction

Robust capuchin monkeys (*Sapajus*) comprise a widespread Neotropical primate genus found from the Colombian Llanos to the Guianas and throughout the Amazon basin, as well as in the Atlantic Forest, Cerrado, Caatinga, and Pantanal biomes of South America, to as far south as northern Argentina (Rylands et al., 2013). Robust capuchins are true habitat generalists, with an incredible diet breadth compared to other Neotropical primates. While fruit and insects form the bulk of their diets, their robust jaw morphology, coupled with behavioral adaptations for tool use and manipulative and extractive foraging, together allow them to exploit encased and hidden foods unavailable to most other non-human animals (Fragaszy et al., 2004; Lynch Alfaro et al., 2012b). This in turn allows them to occupy habitats usually inhospitable to primates.

Current primate taxonomy separates robust (*Sapajus*) and gracile (*Cebus*) capuchin monkeys in two genera, while earlier taxonomists lumped all capuchins into one genus, *Cebus*, despite recognizing morphological differences between the two types. For example, Elliot (1913) created a taxonomic key that divided the genus *Cebus* into

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Table 1
Taxonomies of robust capuchin monkeys.

Elliot (1913)	Hershkovitz (1949)	Cabrera (1957)	Hill (1960)	Groves (2001, 2005)	Silva-Júnior (2001, 2005)	Rylands et al. (2013)
Cebus apella	*Cebus apella*	*Cebus apella*	*Cebus apella*	*Cebus apella*	*Cebus (Sapajus) apella*	*Sapajus apella*
Cebus fatuellus		*C. a. apella*	*C. a. apella*	*C. a. apella*	*Cebus (Sapajus) macrocephalus*	*Sapajus macrocephalus*
C. f. fatuellus		*C. a. margaritae*	*C. a. margaritae*	*C. a. fatuellus*	*Cebus (Sapajus) libidinosus*	*Sapajus libidinosus*
C. f. peruanus		*C. a. macrocephalus*	*C. a. fatuellus*	*C. a. macrocephalus*	*Cebus (Sapajus) cay*	*Sapajus cay*
Cebus macrocephalus		*C. a. libidinosus*	*C. a. peruanus*	*C. a. peruanus*	*Cebus (Sapajus) nigritus*	*Sapajus nigritus*
Cebus libidinosus		*C. a. paraguayanus*	*C. a. tocantinus*	*C. a. tocantinus*	*Cebus (Sapajus) robustus*	*S. n. nigritus*
Cebus azarae		*C. a. pallidus*	*C. a. macrocephalus*	*C. a. margaritae*	*Cebus (Sapajus) xanthosternos*	*S. n. cucullatus*
C. a. azarae		*C. a. xanthosternos*	*C .a. libidinosus*	*Cebus libidinosus*		*Sapajus robustus*
C. a. pallidus		*C. a. versutus*	*C. a. cay*	*C. l. libidinosus*		*Sapajus xanthosternos*
Cebus frontatus		*C. a. nigritus*	*C. a. pallidus*	*C. l. pallidus*		*Sapajus flavius*
Cebus variegatus		*C. a. vellerosus*	*C. a. frontatus*	*C. l. paraguayanus*		
Cebus versuta		*C. a. robustus*	*C. a. xanthosternos*	*C. l. juruanus*		
Cebus cirrifer			*C. a. nigritus*	*Cebus nigritus*		
Cebus crassiceps			*C. a. robustus*	*C. n. nigritus*		
Cebus caliginosus			*C. a. magnus*	*C. n. robustus*		
Cebus vellerosus			*C. a. juruanus*	*C. n. cucullatus*		
			C. a. maranonis	*Cebus xanthosternos*		

'tufted' and 'non-tufted' groups based on whether hair tufts were present on the frontal region of the head. Hershkovitz (1949) cemented a general consensus about the validity of this division, with just one species (*Cebus apella* Linnaeus, 1758) recognized for the tufted group. Hill (1960) also considered all robust or tufted capuchins to be a single cosmopolitan species, *Cebus apella*. Groves (2001, 2005) divided capuchins in two species groups: (1) the *C. capucinus* group, comprising *C. capucinus*, *C. albifrons*, *C. olivaceus*, and *C. kaapori*; and (2) the *C. apella* group, with *C. apella*, *C. libidinosus*, *C. nigritus*, and *C. xanthosternos* (Table 1). Silva-Júnior (2001) separated the tufted or robust capuchins as a different subgenus (*Sapajus*) from the non-tufted or gracile capuchins (*Cebus*) based on distinct cranial, post-cranial, and pelage morphology; he emphasized that *Sapajus* skull and mandible are more robust than that of *Cebus*, because of differences in feeding ecology. Subsequently, genetic research validated the separation of robust and gracile capuchins as two distinct and diverse clades using mitochondrial (Lynch Alfaro et al., 2012a; Lima et al., 2017) and a combination of mtDNA and nuclear (Perelman et al., 2011) markers. Two *Alu* elements also provide strong evidence for the monophyly of robust versus gracile capuchins: *Alu* element S49P is present in *Sapajus* but not *Cebus* (Viana et al., 2015), and the AluSc8 insertion is found in *Cebus* but not *Sapajus* (Martins Jr. et al., 2015). A recent review justified the splitting capuchins into two genera (*Cebus* for gracile capuchins and *Sapajus* for robust capuchins) based on the distinctive morphology, biogeographic history, behavior, and ecology of each type (Lynch Alfaro et al., 2012b).

Taxonomists have also disagreed about the number of species of extant robust capuchins based on morphology (Table 1). Elliot (1913) recognized twelve species of robust capuchins, but Cabrera (1957) and Hill (1960), as noted above, placed all robust forms into one species, *Cebus apella*, while retaining 11 and 16 subspecies, respectively. For the four decades between 1960 and 2000, most researchers considered all robust capuchins to belong to a single species irrespective of place of origin and usually without regard for subspecies designations (e.g. Cole, 1992; Daegling, 1992; Ford and Hobbs, 1996; Masterson, 1997; Wright, 2005a; 2005b, 2007), leading to obfuscation of species or population differences within the robust capuchin literature (see Lynch Alfaro et al., 2014 for discussion). However, Torres de Assumpção (1983) pointed to distinct geographical variation in morphology among robust capuchin populations within Brazil, especially within the Atlantic Forest. More recent morphological analyses have provided evidence for multiple *Sapajus* species (Groves, 2001, 2005; Silva-Júnior, 2001, 2002, 2005; Rylands et al., 2005, 2012, 2013; Rylands and Mittermeier, 2009). The robust capuchin group is now considered by most taxonomists to comprise between four and eight species (Silva-Júnior, 2001; Groves, 2001; Rylands and Mittermeier, 2009; Rylands et al., 2005, 2012, 2013). The IUCN (2017) currently recognizes eight distinct species: *Sapajus flavius*, the blonde capuchin; *S. xanthosternos*, the yellow-breasted capuchin; *S. robustus*, the robust tufted capuchin; *S. nigritus*, the black-horned capuchin; *S. apella*, the brown capuchin; *S. macrocephalus*, the large-headed capuchin; *S. cay*, Azara's capuchin; and *S. libidinosus*, the bearded capuchin.

Recent biogeographic analyses based on mitochondrial DNA suggest that the age of the radiation of extant robust capuchins is about 2.5 My, with diversity accumulating first in the Atlantic Coastal Forest of Brazil and a recent expansion of robust capuchins throughout the Amazon Basin and Cerrado, Caatinga, and Central Grasslands in the last 500,000 years (Lynch Alfaro et al., 2012a; Lima et al., 2017). These analyses suggest that while the Atlantic Forest populations are relatively old and distinct and can be divided into up to four different species, the forms from the Amazon and savanna-like biomes are better considered to be members of a highly polymorphic single species or species complex (Lima et al., 2017).

Here, we use phylogenomic markers – ultraconserved elements (UCEs) (Faircloth et al., 2013) – to infer the phylogeny of robust capuchin monkeys, and to assess the evidence for congruence with species delineation based on morphology and mitochondrial markers. The UCE approach has been used successfully to answer historically contentious taxonomic questions (McCormack et al., 2012; Crawford et al., 2012), including Pleistocene radiations (McCormack et al., 2015; but see Giarla et al. 2015 for challenges in estimating a bifurcating tree even using UCEs when there is a rapid and recent radiation). Previous studies using nuclear markers for capuchin phylogeny have used a limited number of taxa and have also used captive individuals with unknown provenance as species exemplars (i.e. Perelman et al., 2011, Springer et al., 2012). This study marks the first test of robust capuchin phylogeny using phylogenomic markers to analyze genetic relationships across species-representative individuals from known provenance. We use SNP (Single Nucleotide Polymorphisms) data from the UCE results to refine our understanding of robust capuchin diversification in the Pleistocene, as this technique has been used successfully to elucidate phylogeny across a similar geologic time frame (McCormack et al., 2015).

2. Material and methods

2.1. Samples, DNA extraction and sequencing

We sampled 61 individuals from eight species of the genus *Sapajus* from 58 localities distributed throughout the Atlantic Forest, Amazon, Caatinga, Cerrado and Pantanal habitats in South America (Fig. 1, Table 2, Supplementary Table 1). Note that our study extends the *S. macrocephalus* morphotype to the east of the Madeira River, into the