

# Geographic origins and genetic diversity of Angolan colobus monkeys (*Colobus angolensis*) in North American zoos and its implications for *ex situ* population management

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## Background

Species Survival Plan (SSP) programs use a species' pedigree to try to maintain 90% of the founders' genetic diversity [1]. Knowing the geographic origin of the founder and how representative their genetic diversity is relative to that in the wild is also informative for *ex situ* genetic management. Unfortunately, this information is often vague or unknown.

As of 2020, 88 Angolan colobus monkeys (*Colobus angolensis*) were in the AZA population and the studbook indicates that all founders originated in Tanzania between 1986 and 1989 [2]. While this species is wide-ranging across central and eastern Africa, Tanzania contains three of the eight recognized subspecies (*C. a. palliatus*, *C. a. sharpei*, *C. a. ssp. nov.*).

Previously, an assessment of 13 individuals across three zoos suggested that their mitochondrial DNA haplotypes clustered with Kenyan, rather than Tanzanian, colobus haplotypes (McDonald pers comm.), however, limited genetic data from the Tanzanian *in situ* population was available at that time. Recently, more genetic data have become available, with haplotypes from an additional five populations of *C. a. palliatus* [3].

## Objective

This project characterizes the mitochondrial genetic diversity of *C. angolensis* individuals in North American zoos to:

1. understand how much of the diversity within wild populations is represented in the zoo population and
2. discover the geographic origin of the zoo's founding population.

## Sample Collection

We obtained 40 fecal samples from 26 individuals at five North American zoos. Zoos were chosen based upon:

- the number of individuals closely related to the founding population,
- the number of total individuals at their institution,
- their willingness to participate.

## Laboratory Work & Analysis

- Extracted DNA from fecal samples and amplified and sequenced a 1,795bp region of the mitochondrial genome (cytochrome *b* and a portion of the D-loop) using previously established methods [3-4].
- Integrated sequences into a 140 sequence dataset from nine wild Kenyan and Tanzanian populations (Figure 1), representing two subspecies (*C. a. palliatus* and *C. a. sharpei*).
- Characterized mitochondrial haplotype diversity, percent sequence difference, and phylogenetic relationships [3-4].
- Assigned zoo haplotypes to their founder and founder event using the AZA Angolan colobus studbook [2] and compared founder and haplotype representation of the current zoo population using the AZA Breeding and Transfer Plan [1].

## References

1. Gibson, D., Fogarty, D., & Lynch, C. (2020). *Angolan Colobus (Colobus angolensis) AZA species survival plan yellow program population analysis and breeding and transfer plan*. Chicago, IL: AZA Population Management Center.
2. Fogarty, D. (2018). *Angolan Colobus (Colobus angolensis) AZA Regional Studbook*, AZA Population Management Center: Chicago, IL: Springfield.
3. McDonald, M. M., Cunneyworth, P. M. K., Anderson, A. G., & Wroblewski, E. (*in revision*). Mitochondrial genetic diversity and divergence dating of Angolan colobus monkeys in the eastern forests of Kenya and Tanzania: implications for subspeciation and reconstructing historical biogeography. *American Journal of Primatology*.
4. McDonald, M. M., & Hamilton, H. (2010). Phylogeography of the Angolan black and white Colobus monkey, *Colobus angolensis palliatus*, in Kenya and Tanzania. *American Journal of Primatology*, 72(8), 715–724. <https://doi.org/10.1002/ajp.20828>

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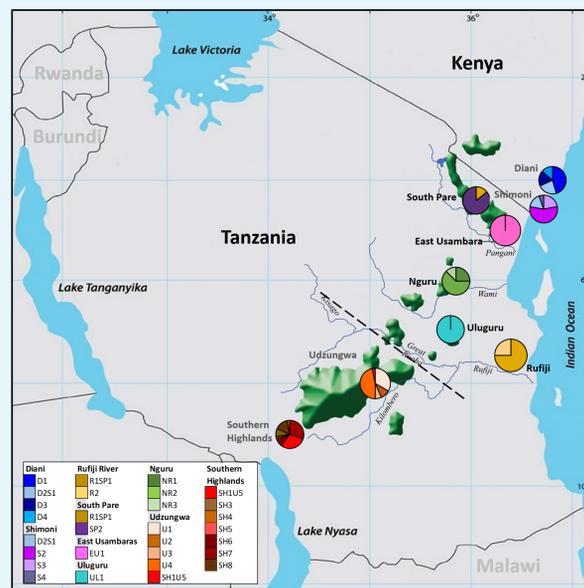


Figure 1: Map of nine wild study populations and the frequency of haplotypes in each [3].

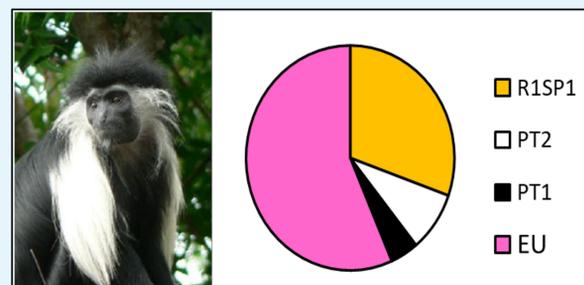


Figure 2: Picture of *Colobus angolensis* (McDonald) along with haplotype frequencies in the sampled zoo population (n=23); EU1 (n=13) = East Usambaras, R1SP1 (n=7) = Rufiji River/South Pare. PT1 (n=1) and PT2 (n=2) are new haplotypes identified in the zoo population that were not identified in the sampled wild population.

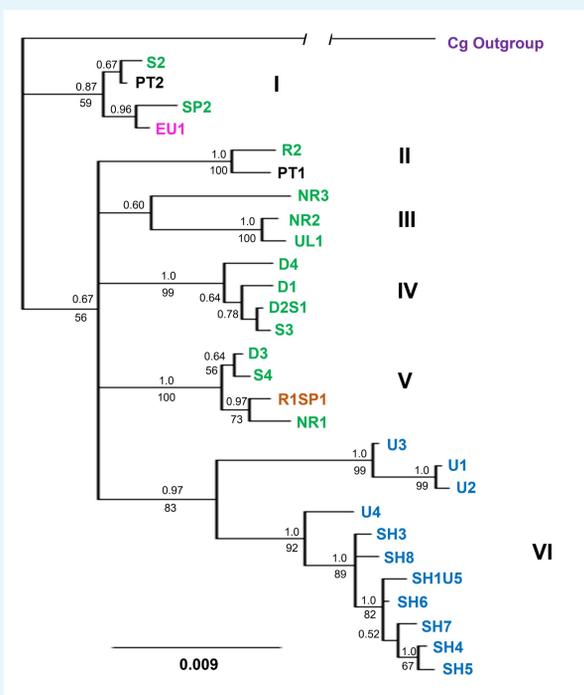


Figure 3: Bayesian tree with posterior probabilities (above branch) and bootstrap values from Maximum Likelihood (ML) analysis below branch. EU1 (pink), R1SP1 (orange) and new PT1 and PT2 (black) are the haplotypes found in the sampled North American zoo population. Roman numerals indicate haplogroups. All zoo haplotypes fall into the subspecies *C. a. palliatus* (*C. a. palliatus* = green; *C. a. sharpei* = blue). Outgroup is *Colobus guereza* (Raum et al., 2005). Figure adapted from [3].

## Results

- We sequenced samples from 23 zoo individuals, representing 9 out of 15 original founders, which resulted in 4 haplotypes.
- Most zoo individuals had an East Usambara mountains haplotype (EU1) (n = 13), or a haplotype shared between the South Pare mountains and Rufiji River (R1SP1) (n = 7) (Figures 1 & 2).
- Maximum Likelihood and Bayesian phylogenetic trees confirmed that all zoo founders were subspecies *C. a. palliatus* (Figure 3).
- Three individuals had two new haplotypes not found in the sampled wild population. PT1 (n = 1) is most closely related to the Rufiji River haplotype (R2) and PT2 (n = 2) is most closely related to a Shimoni, Kenya haplotype (S2).
- These two new haplotypes differed from their most similar haplotypes by 0-0.2% (cytochrome *b*) and 0.4-1.5% (D-loop) (Figure 3).

## Population Management Implications

- Founders 4, 6, 7 & 13 most likely came from the Rufiji River or the South Pare Mountains (R1SP1). Founders 16, 19 & 32 most likely originated from the East Usambara mountains (EU1). These two haplotypes are well represented in the current AZA population [1].
- Founders 9 (PT2) & 11 (PT1) possess the new haplotypes not detected in the wild and likely represent two different geographic origins. These two haplotypes are very underrepresented in the sampled AZA population.
- Founder 9's descendants should be considered for breeding, particularly if they are already deemed genetically valuable by established metrics.
- Founder 11's descendants have aged out of the population except for one individual. However, that individual belongs to a non-AZA accredited facility, limiting his contribution to the genetic diversity of the *ex-situ* population.

## Conclusions

- The North American zoo population of Angolan colobus originated from several distinct areas of Tanzania, and all belong to the subspecies *C. a. palliatus*.
- Mitochondrial genetic data from *ex situ* populations can help identify unique genetic diversity of difficult to obtain or recently extirpated wild populations, as well as inform population management decisions of existing zoo populations.
- Knowing the geographic origin of founding individuals can reveal unique haplotypes before their descendants age out of or leave the AZA SSP-managed population.
- When available, knowledge of unique haplotypes can be a beneficial part of the toolkit used when making breeding recommendations, as it can help further prioritize already genetically valuable individuals for breeding.