



Identification of African leopard (*Panthera pardus*) scat samples using DNA Barcoding

Joshua Ashley¹, Jaden Oh², and Danielle Lema³

¹All Hollows High School, ²Regis High School, ³American Museum of Natural History, Fordham University

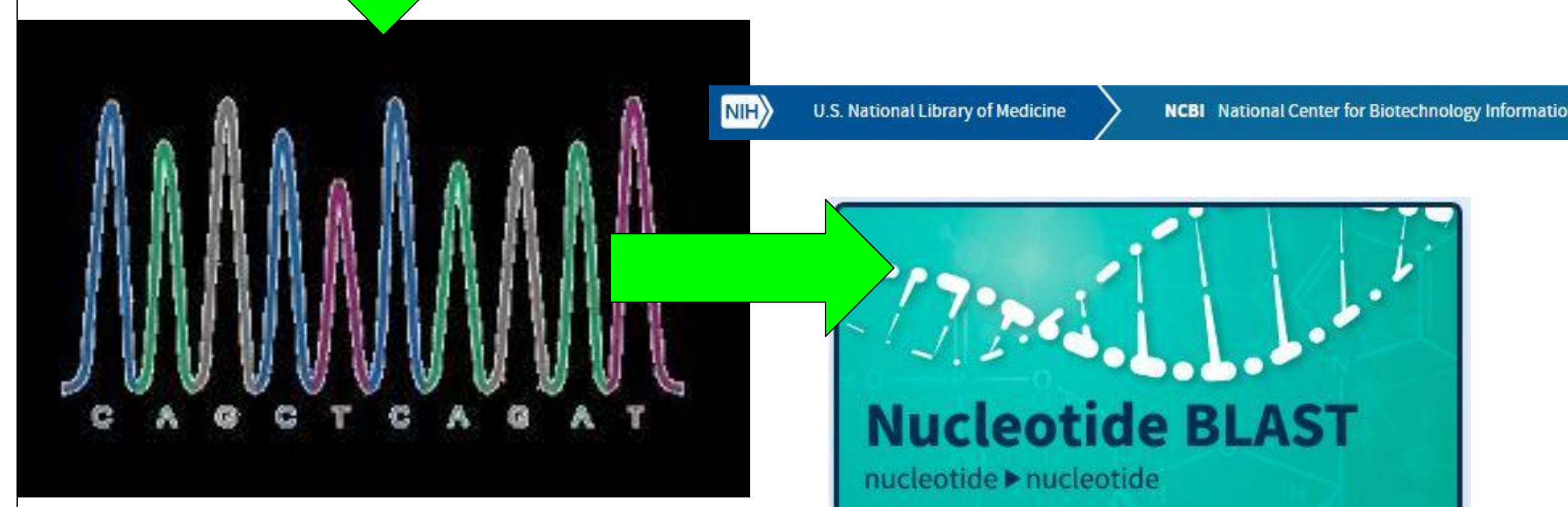
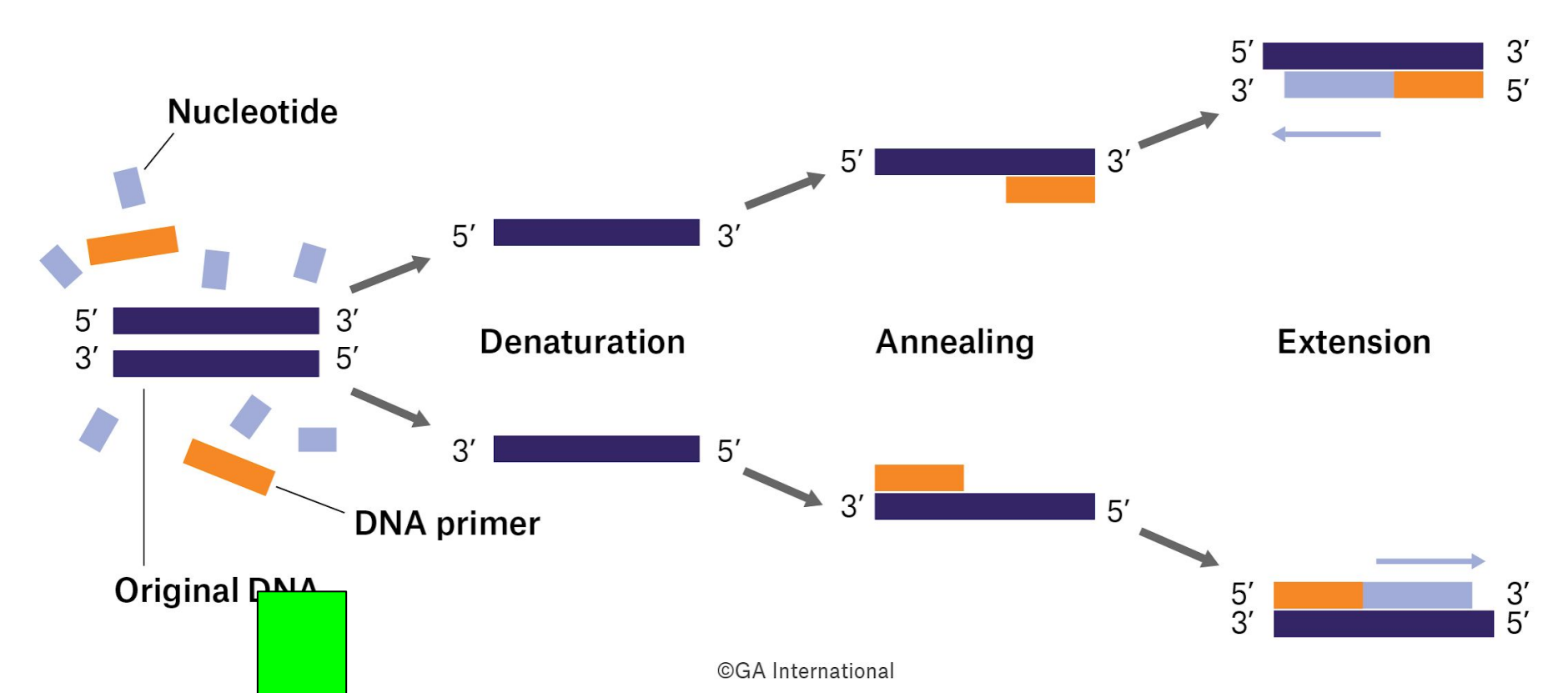
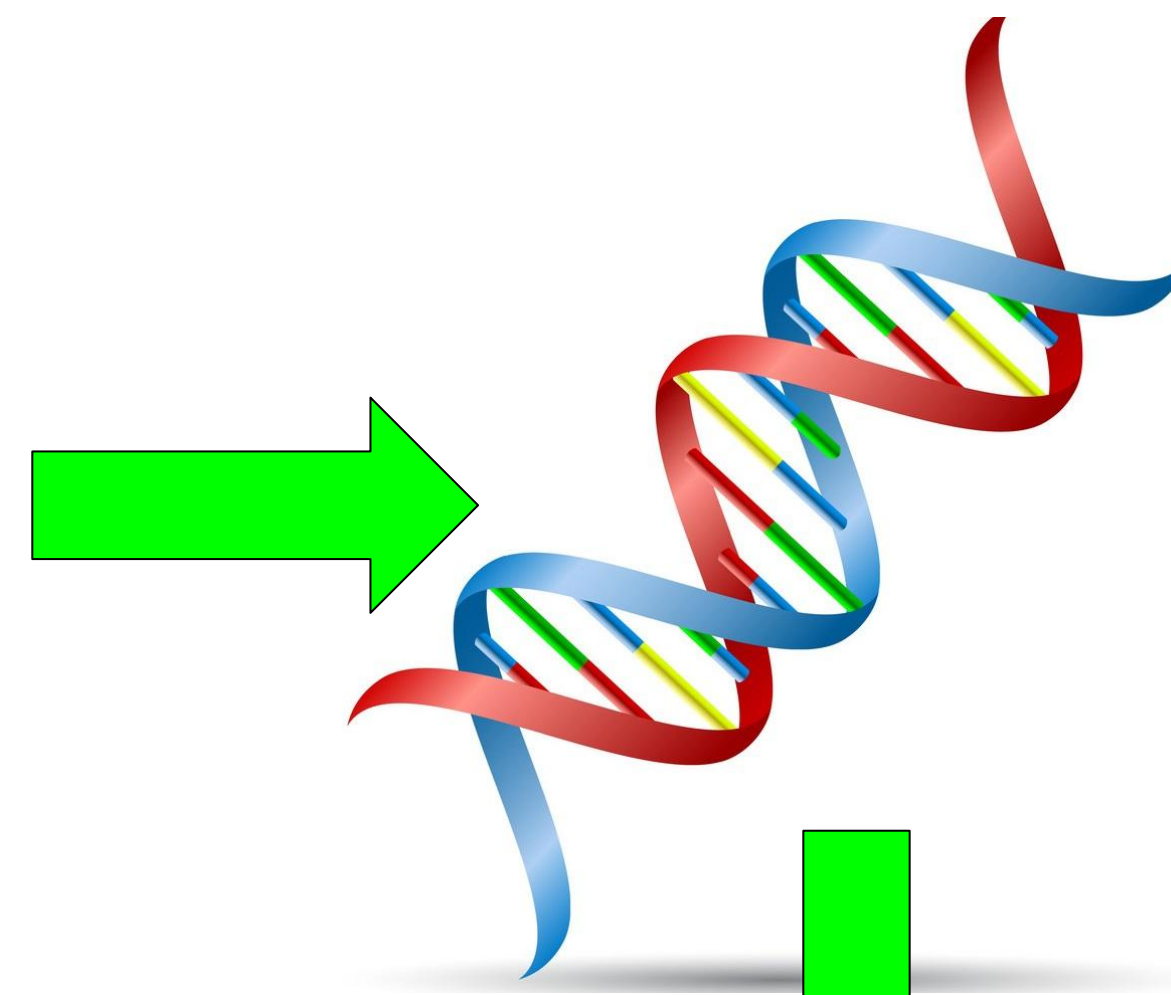


Abstract

The African leopard (*Panthera pardus*) is important for ecological balance. However, they are under large threat of population decline. The IUCN ranks them from Threatened to Critically Endangered. This project was intended to identify scat collected in West and Central Africa to its species of origin, using DNA barcoding. We hypothesized that the samples would be identified as African leopard.

Materials & Methods

- Dna extracted from scat samples and purified via the QIAGEN stool kit (QIAGEN, Inc.).
- PCR amplification of cytB, 12S and 16S mitochondrial loci
- Gel electrophoresis
- Nucleotide Sequencing by Genewiz, Inc.
- NCBI BLAST

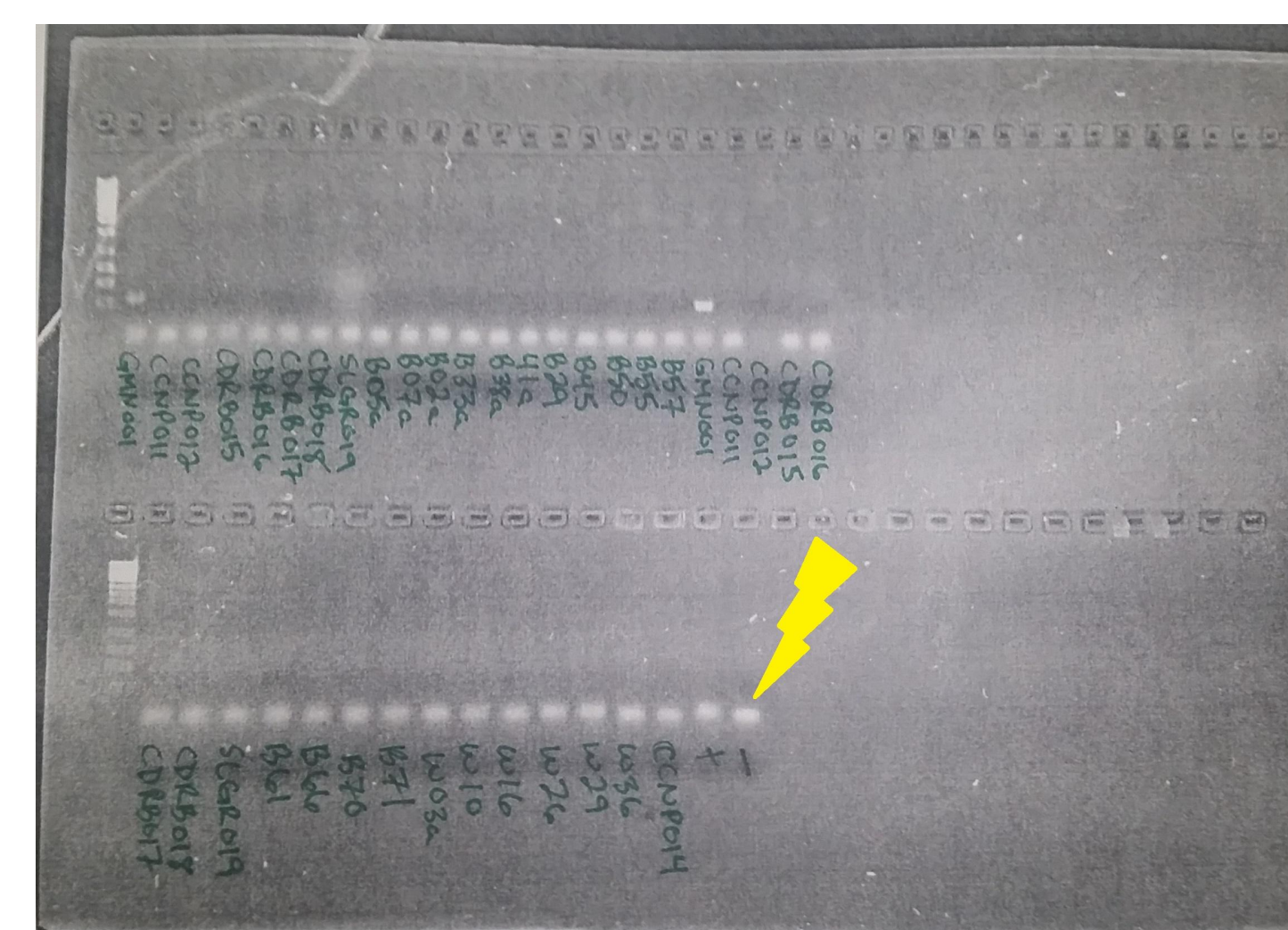


Results

- An average of 2.9665ng/mL of DNA were extracted from 30 scat samples
- Mitochondrial cytB, 12S, and 16S were PCR amplified and viewed in 1.5% agarose gels
- *Genewiz Sequencing and BLAST halted due to covid-19 closures*
- *Below are examples for other big cat species from previous research for what we would have expected to see (16S)*

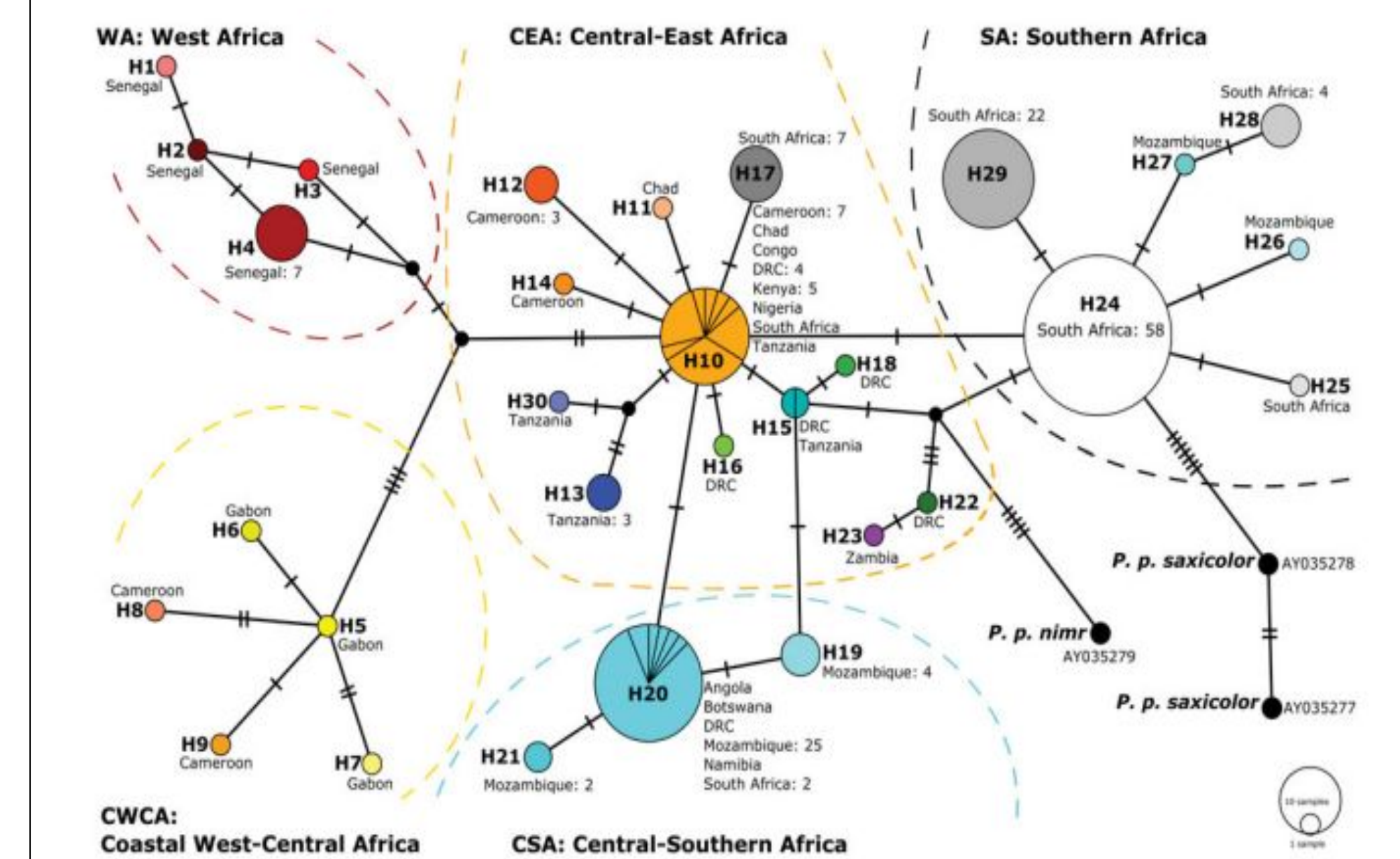
Forward Sequence				Reverse Sequence			
Puma_54	GGGCTGCGCTCGCGG	GTGACG	AGTT	Puma_54	GGCGGTTTAACTG	ATGCAC	
Puma_47	GGGCTGCGCTCGCGG	GTGACG	AGTT	Puma_47	GGCGGTTTAACTG	ATGCAC	
Puma_46	GGGCTGCGCTCGCGG	GTGACG	AGTT	Puma_46	GGCGGTTTAACTG	ATGCAC	
Puma_44	GGGCTGCGCTCGCGG	GTGACG	AGTT	Puma_44	GGCGGTTTAACTG	ATGCAC	
Puma_25	GGGCTGCGCTCGCGG	GTGACG	AGTT	Puma_25	GGCGGTTTAACTG	ATGCAC	
Puma_22	GGGCTGCGCTCGCGG	GTGACG	AGTT	Puma_22	GGCGGTTTAACTG	ATGCAC	
Oeolot_137	GGGCTGCGCTCGCGG	GTGACG	AGTT	Oeolot_137	GGCGGTTTAACTG	ATGCAC	
Oeolot_118	GGGCTGCGCTCGCGG	GTGACG	AGTT	Oeolot_118	GGCGGTTTAACTG	ATGCAC	
Oeolot_77	GGGCTGCGCTCGCGG	GTGACG	AGTT	Oeolot_77	GGCGGTTTAACTG	ATGCAC	
Oeolot_15	GGGCTGCGCTCGCGG	GTGACG	AGTT	Oeolot_15	GGCGGTTTAACTG	ATGCAC	
Jaeguar_14	GGGCTGCGCTCGCGG	GTGACG	AGTT	Jaeguar_14	GGCGGTTTAACTG	ATGCAC	
Jaeguar_9	GGGCTGCGCTCGCGG	GTGACG	AGTT	Jaeguar_9	GGCGGTTTAACTG	ATGCAC	
Jaeguar_8	GGGCTGCGCTCGCGG	GTGACG	AGTT	Jaeguar_8	GGCGGTTTAACTG	ATGCAC	
Jaeguar_5	GGGCTGCGCTCGCGG	GTGACG	AGTT	Jaeguar_5	GGCGGTTTAACTG	ATGCAC	

Tables & Figures



Discussion

- *Due to COVID-19 precautions we were unable to finish the research.*
- If we had identified the scat samples as African Leopard, we would have done another analysis comparing the nucleotide variation of our samples to other samples across Africa, from nucleotide sequences stored in Genbank.



References

- Anco, C., *et al.*, (2017) Mitochondrial DNA Part A, 29(3), 455–473.
- Chaves, P.B., *et al.*, (2012) Mol. Ecol. Res. 12: 18–35.
- Jacobson, A. P., *et al.*, (2016) *PeerJ*, 4, e1974.
- Nowell, K., Jackson, P. 1996. Wild cats. Status Survey and Conservation Action Plan. Gland, Switzerland: IUCN.
- Pauli, J. N., *et al.*, (2010) Cons. Bio., 24(1), 349–352.
- Stein AB, *et al.*, (2016). *Panthera pardus*. IUCN Red List Threat Species.

Acknowledgements

We would like to thank Evon Hekkala and Mark Siddall and the American Museum of Natural History's Sackler Institute of Comparative Genomics.

