

# DESIGNING A NILE CROCODILE EXPERIMENT

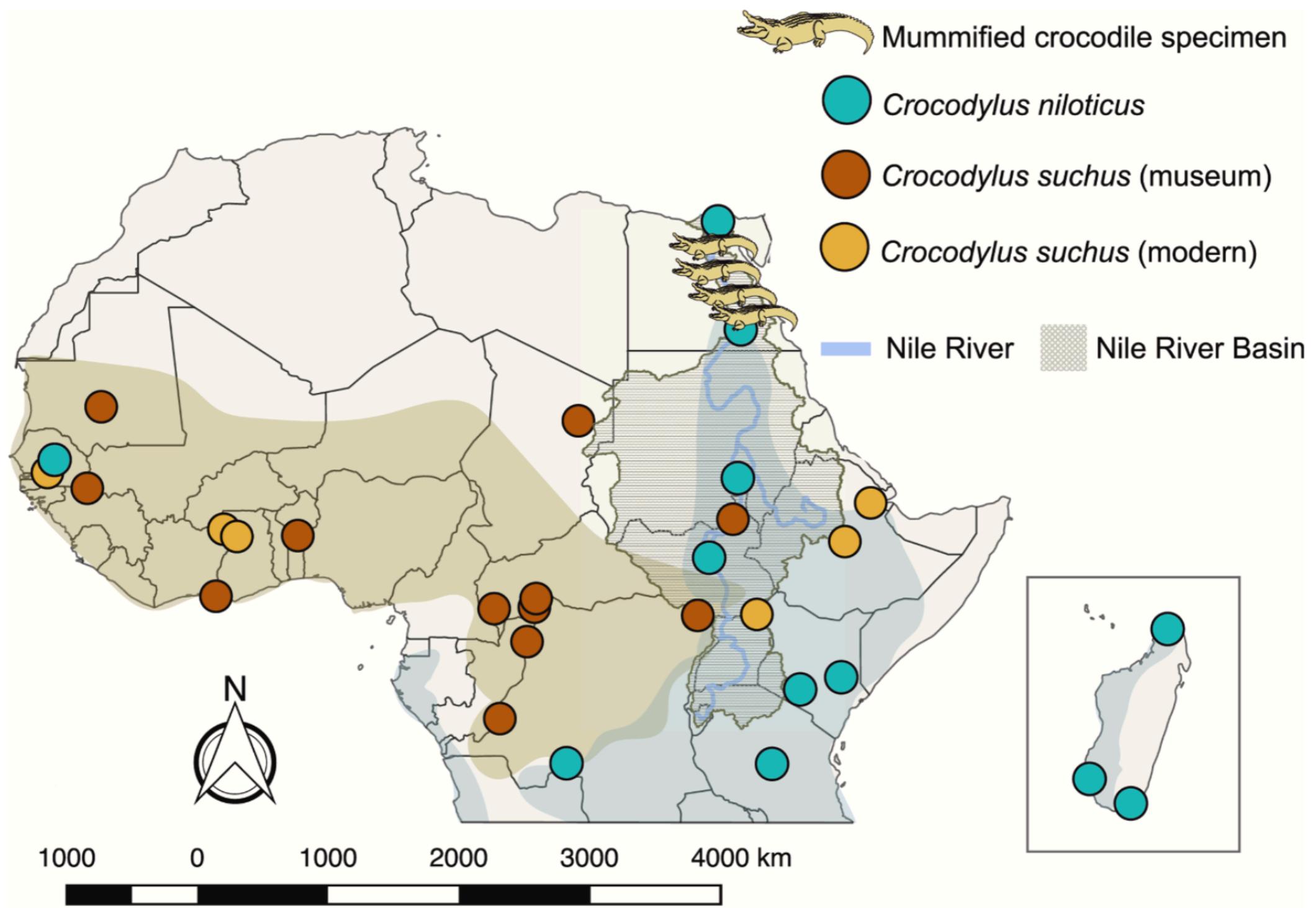


# Background

The taxonomic distinction between *Crocodylus niloticus* (Nile crocodile) and *Crocodylus suchus* (West African crocodile) is a subject of debate. Previously thought to be a single species, recent genetic studies have reclassified eastern and western populations of the African Nile crocodile into two deeply divergent species: *Crocodylus niloticus* ('Eastern clade') and *Crocodylus suchus* ('Western clade'). However identification based on morphology remains challenging, as *C. Suchus* and *C. niloticus* appear to be morphologically distinct. This has led to confusion in the identification of specimens and in our understanding of taxonomic relationships.

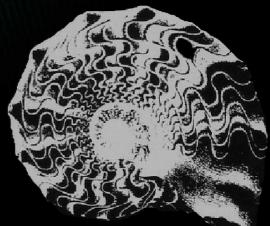
# Background

E.R. Hekkala, et al.



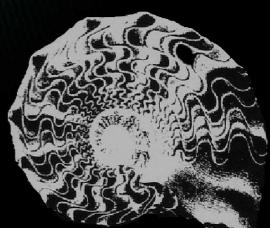
# Objective

An integrative taxonomic approach:  
Comparing morphology and phylogenetic  
diversity using the same specimens



# Study Design

- Sequence mitochondrial genomes from historical museum specimens (skulls)
- DNA degraded and low concentration.  
Therefore, enrichment protocol more likely to succeed: **Sequence Capture**



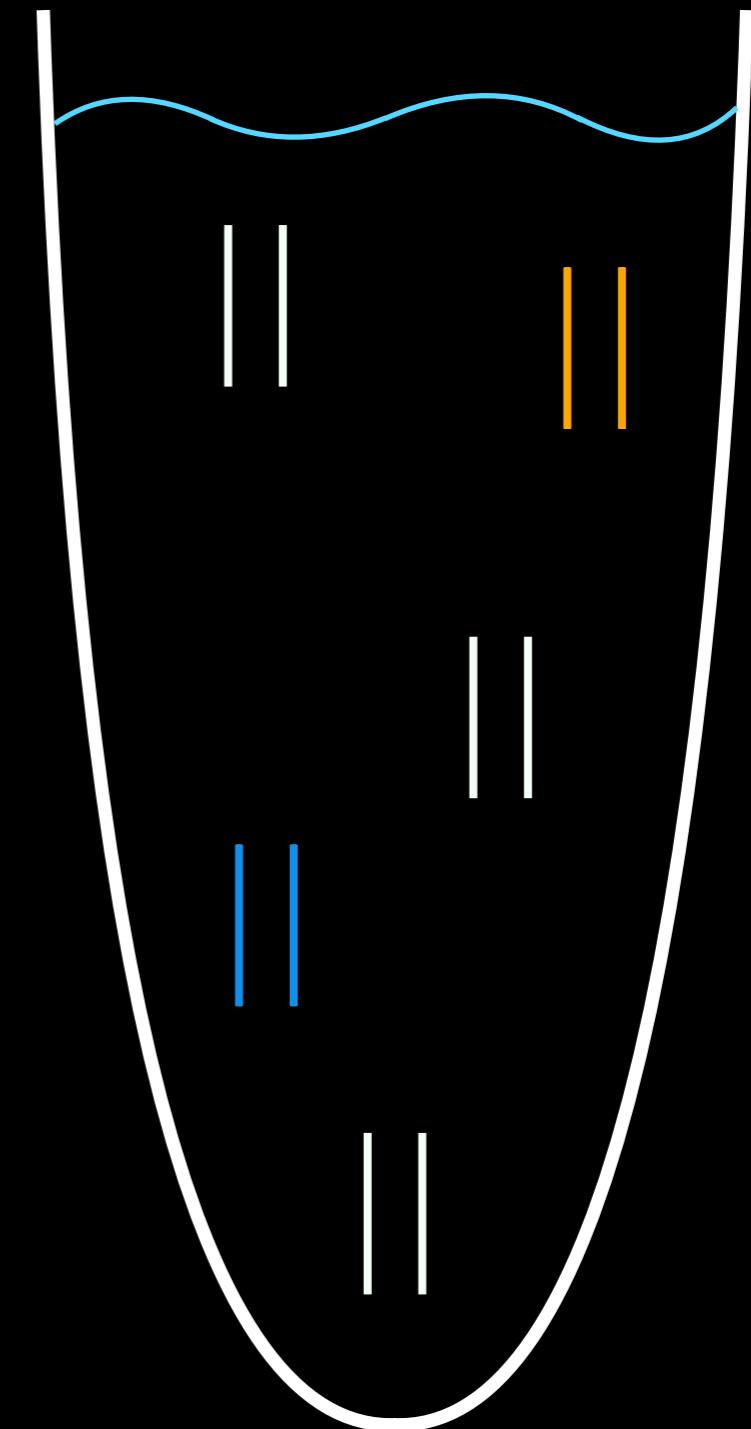
# Phylogenomic markers

Objective: How to  
sequence a large number  
of orthologous loci for a  
large number of  
individuals?

## Genome reduction methods

- Sequence capture

REMEMBER DAY 3?



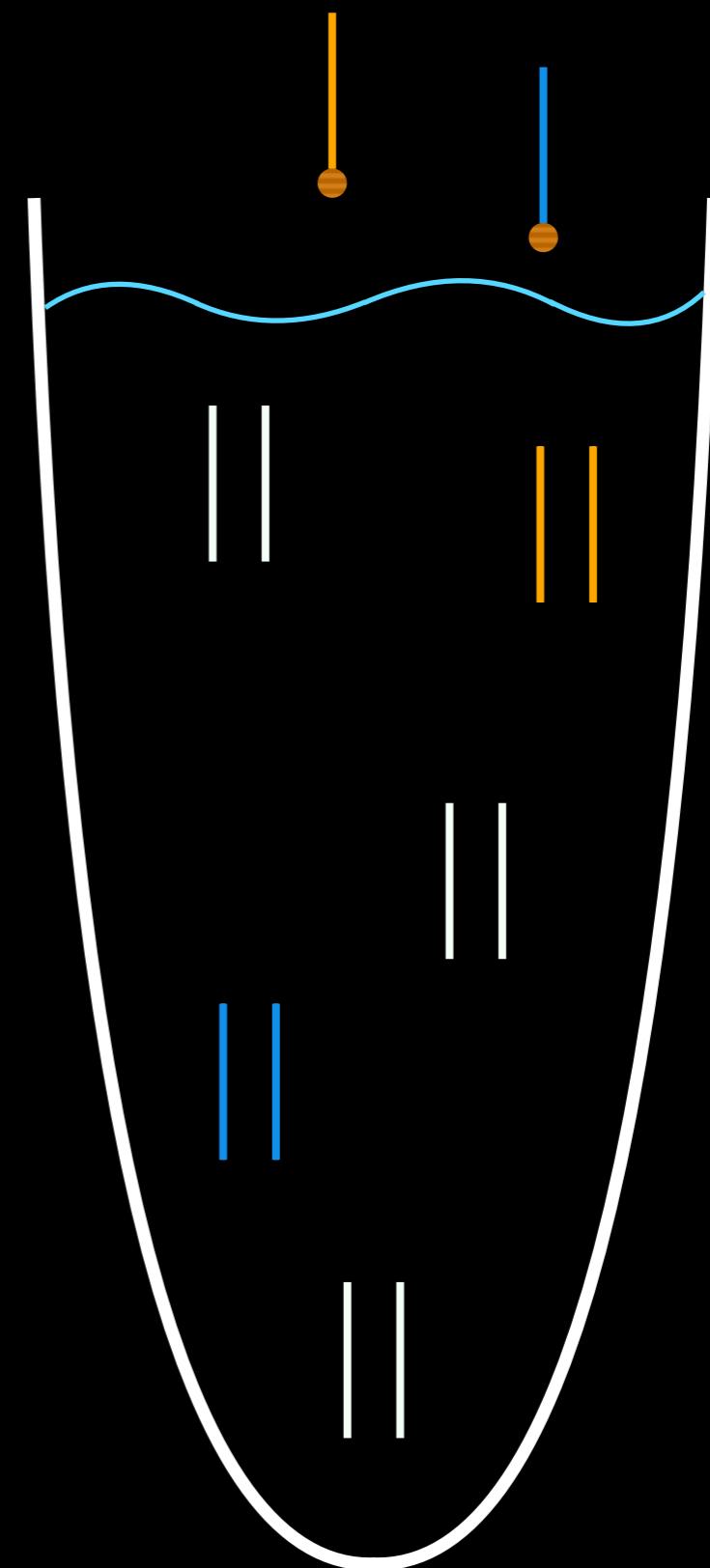
# Phylogenomic markers

Objective: How to  
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## Genome reduction methods

- Sequence capture

*Biotinylated probes*



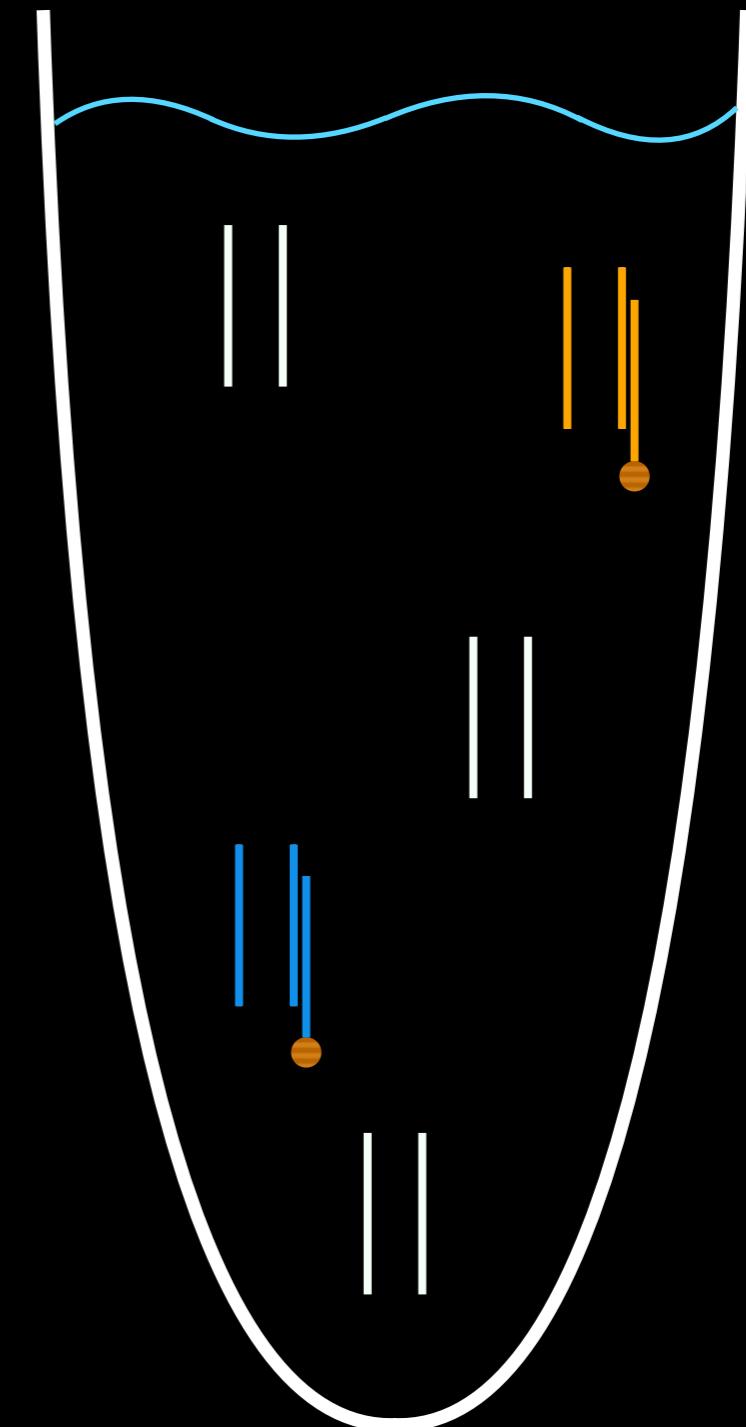
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## Genome reduction methods

- Sequence capture

*Probe hybridisation*



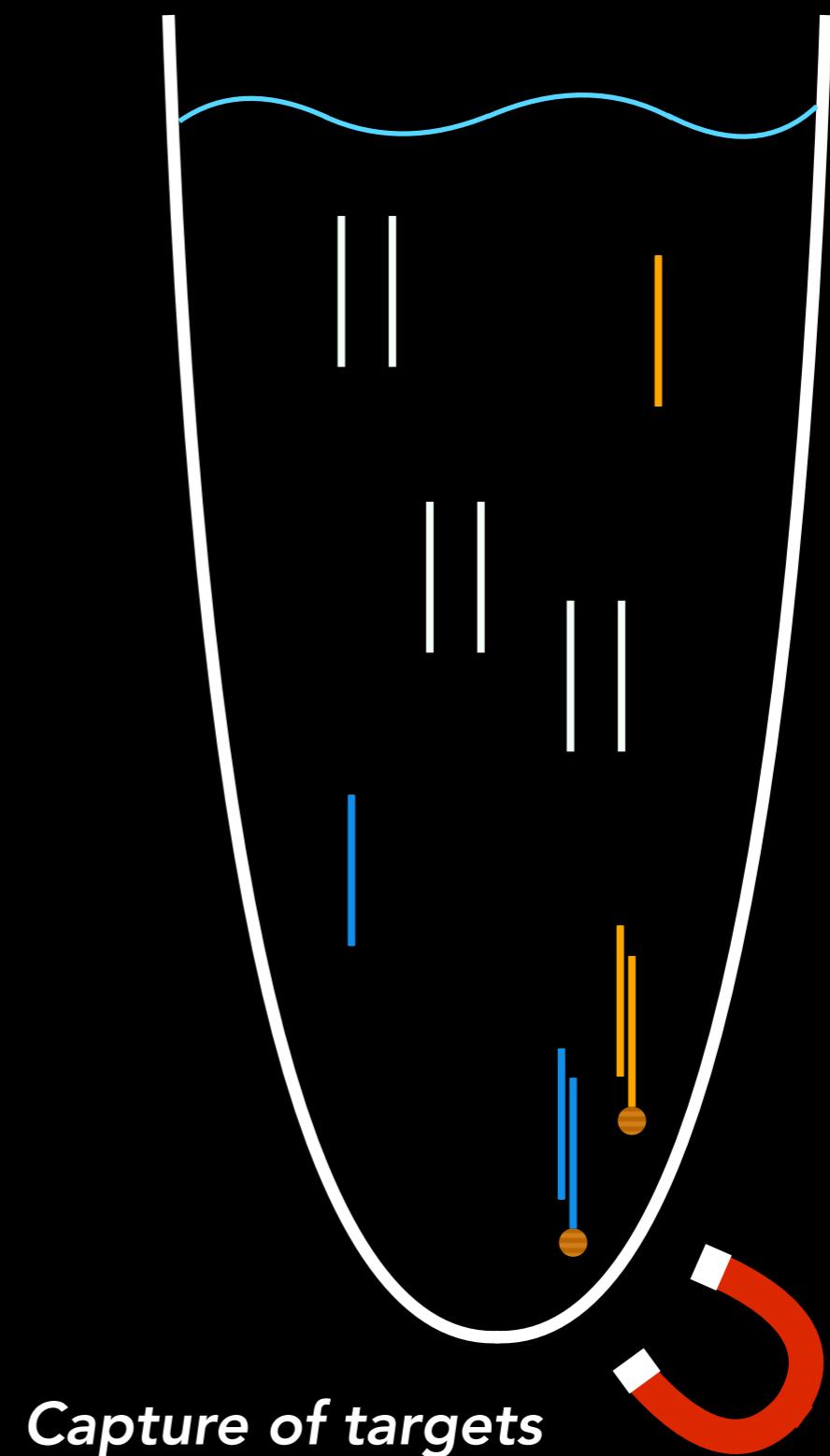
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- Sequence capture

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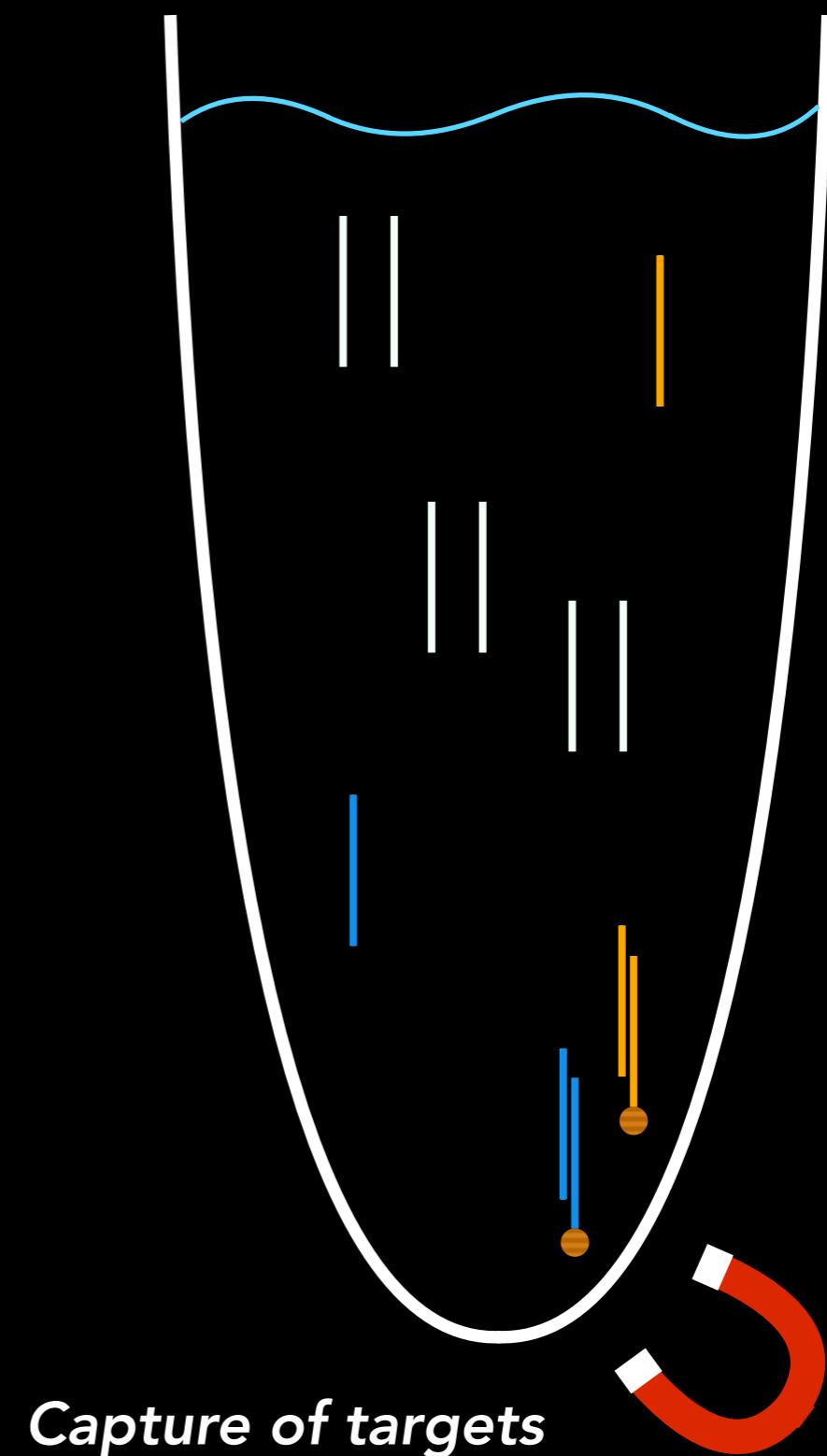
# Phylogenomic markers

Objective: How to  
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of orthologous loci for a  
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individuals?

## Genome reduction methods

- Sequence capture
  - Exons
  - Ultra-conserved elements
  - Mitogenomes etc.

REMEMBER DAY 3?



# Study Design

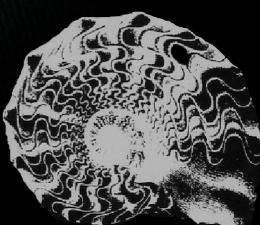
- Sequence mitochondrial genomes from historical museum specimens (skulls)
- DNA degraded and low concentration.  
Therefore, enrichment protocol more likely to succeed: Sequence Capture
- To design the probes for sequence capture, we will need a **reference mitochondrial genome!**



# Outstanding Questions

## Mitogenome Sequence Capture Design

- Are there existing mitochondrial genomes for both *C. niloticus* and *C. suchus*?
- Will we be able to map degraded DNA sequencing data to either or both reference genomes?



# Outstanding Questions

## Mitogenome Sequence Capture Design

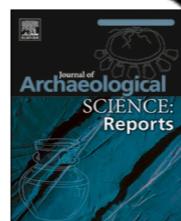
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The secrets of Sobek – A crocodile mummy mitogenome from ancient Egypt

Evon R. Hekkala<sup>a,c,\*</sup>, Matthew L. Aardema<sup>b,c</sup>, Apurva Narechania<sup>c</sup>, George Amato<sup>c</sup>, Salima Ikram<sup>d</sup>, Matthew H. Shirley<sup>e</sup>, Kent A. Vliet<sup>f</sup>, Seth W. Cunningham<sup>a,c</sup>, M. Thomas P. Gilbert<sup>g</sup>, Oliver Smith<sup>g,h</sup>



# Outstanding Questions

# Mitogenome Sequence Capture Design

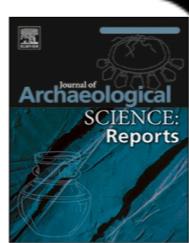


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## Methods

The adapters were initially removed using cutadapt v1.13 ([Martin, 2011](#)) and reads were aligned to mitochondrial reference sequences of *Crocodylus niloticus* (GB JF502243.1) and *Crocodylus suchus* (GB JF502244.1, an accession originally listed as *C. niloticus* in Genbank but currently recognized as *C. suchus*) using bowtie2 ([Langmead and Salzberg, 2012](#)) and then de-duplicated and filtered for minimum



# Outstanding Questions

# Mitogenome Sequence Capture Design

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**No difference in coverage between reference species suggests that we can use one reference mitogenome for probe design and doesn't matter which one.**

**Table 2**

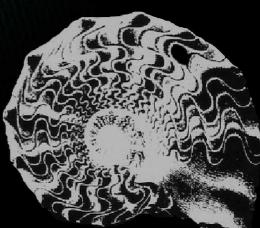
Next Generation Sequencing (NGS) read statistics for enriched libraries for each crocodile mummy sample (KomOmbo21 or KomOmbo22) and the extraction blanks.

Sample name	Sample source	Raw reads	Deduplicated raw reads	Mapped to JF502243.1 Crocodylus niloticus	Mapped to JF502244.1 Crocodylus suchus	Endogenous %	Coverage depth JF502243.1	Coverage depth JF502244.1
KomOmbo21 (Rep 1)	Scapular surface tissue	10,053,918	7,010,213	4585	4591	.065404573	12.3	12.3
KomOmbo22 (Rep 2)	Scapular bone	152,460,620	92,713,171	10,373	10,385	0.0118827	28.9	29
KomOmbo21 (Blank1)	NA	3101	2587	0	0	0	0	0
KomOmbo22 (Blank2)	NA	5035	4953	0	0	0	0	0

Coverage of sample equally high regardless of reference species used!

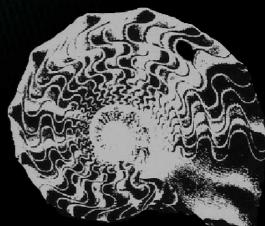
# YOUR TASK

- Find the two mitochondrial reference genomes mentioned on Genbank
- Complement this with additional *Crocodylus niloticus* and *Crocodylus suchus* mitochondrial genomes
- Download and align the sequences
- IF you are using Geneious Pro for the alignment, **see next page first!!!**
- Visually inspect the alignment
- Infer a mitochondrial phylogeny using ML or Bayesian Inference



# YOUR TASK

- Alignment in Geneious Pro can take a very long time, especially when using the standard alignment algorithm ("*Geneious alignment*")
- MAFFT is another alignment algorithm that is much faster and available as a plugin
- Download the plugin via the following link:  
<https://www.geneious.com/plugins/mafft>
- In Geneious: Tools -> Plugins -> Install plugin from a gplugin file
- Now use MAFFT as alignment algorithm



# QUESTIONS

- Are the two mitochondrial reference genomes used in the Hekkala *et al.* study good representatives for *C. niloticus* and *C. suchus*?
- If yes/no, can we proceed with designing our sequence capture probes based on either mitogenome? Based on the Hekkala *et al.* study, can we be confident that it will also work for our skulls with unknown identity?

