Phylogenetic and Phylogeographic meta-analysis of Cytochrome c Oxidase I barcode sequences of East African arthropods submitted into the Barcode of Life Database

Presented by:

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DRIP fellow

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  Dr Jandouwe Villinger
  Dr Steven Ger
- **Collaborators:**
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  Dr Jean-Baka Domelevo
  Dr Daniel Masiga

Background

The 658 base-pair 5’ region of mitochondrial cytochrome c oxidase I gene is used as the standard the barcode for most animal groups: A genetic key in identification of known species

The Consortium for the Barcode of Life (CBOL), launched in May 2004: To aid the rapid and inexpensive identification of millions of species using DNA barcodes

- International Nucleotide Sequence Database Collaborative (INSDC): GenBank, the European Molecular Biology Lab in Europe, and the DNA Data Bank of Japan
- Barcode of Life Database (BOLD): University of Guelph in Ontario

Background

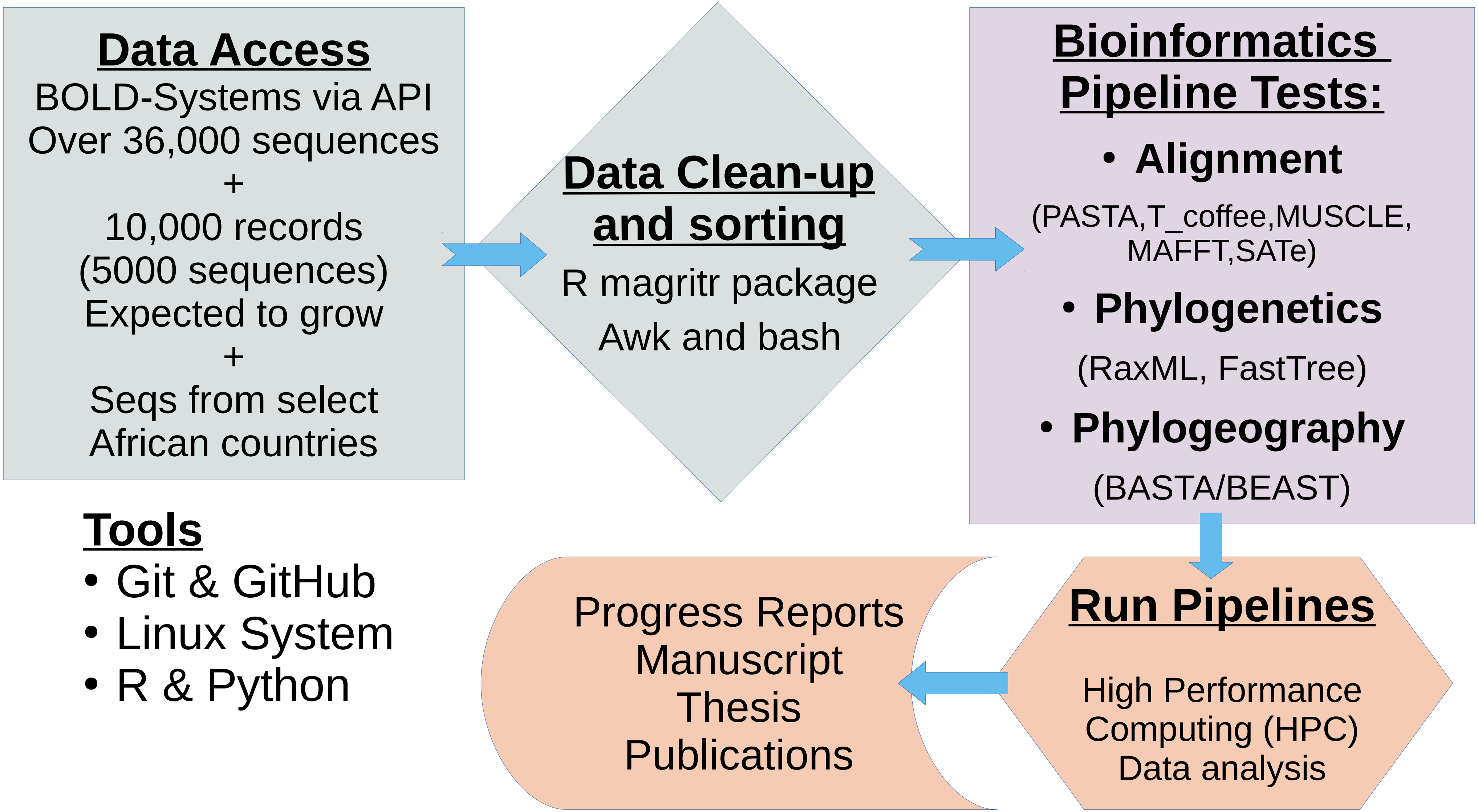
Problem statement:

Thousands of COI sequences from voucher arthropods submitted into the BOLD database and are yet to be analysed for phylogenetic diversity and phylogeographic distribution.

Objectives:

- Improve phylogeographic descriptions and phylogenetic diversity of arthropod in East Africa.
- Identify the cryptic species that may not yet be recognized and may be potential crop pests or vectors of human and animal diseases.

Workflow



Data Access

BOLD-Systems via API
Over 36,000 sequences
+
10,000 records
(5000 sequences)
Expected to grow
+
Seqs from select
African countries

Data Clean-up and sorting

R magritr package

Awk and bash

Bioinformatics Pipeline Tests:

- **Alignment**

(PASTA,T_coffee,MUSCLE,
MAFFT,SATe)

- **Phylogenetics**

(RaxML, FastTree)

- **Phylogeography**

(BASTA/BEAST)

Run Pipelines

High Performance
Computing (HPC)
Data analysis

Progress Reports
Manuscript
Thesis
Publications

Tools

- Git & GitHub
- Linux System
- R & Python

Data Mining and Clean up

Sequence retrival:

BOLD-Systems via API; using specific syntax:

“arthropoda Kenya Uganda Tanzania Rwanda Burundi”

Data clean up:

R, Awk and Bash

Metadata (80 columns)				
'COI-5P' = 35990 out of 37257 (1 sample)				
#nucleotides / unaligned seqs / #ns				
Over 700 (592)	Under 500 (705)	500 -700 (34693)	650 -660 (21886)	Over 500 (35285)
1 sample	1 sample	3 samples	2 samples	
Build.fasta: ProcessID | order | seq_len | seq				

Multiple Sequence Alignment

Large dataset:

- Accuracy
- Speed

Algorithms:

- Progressive (mafft/muscle)
- Progressive & transivity (pasta)
- Regressive (T_coffee)

MUSCLE: default	MAFFT: --large G-INS-1	T_coffee: -reg	PASTA: default	SATe
Speed	Speed	speed	speed	***
Low accuracy	High accuracy	High accuracy	High accuracy	***
• Refine • Align • Merge	• Align • Add sequence • Add_fragments • Merge	• Align • Evaluate: (CORE index TCS)	• Align • Add fragments	***

Multiple Sequence Alignment evaluation

Evaluation for Accuracy:

- T_coffee: **consistency based scoring**.

CORE index (html)

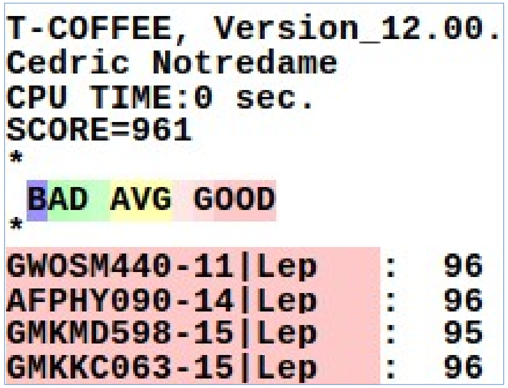
Transitive Consistency Scores (TSC) (html/ascii)

Purpose:

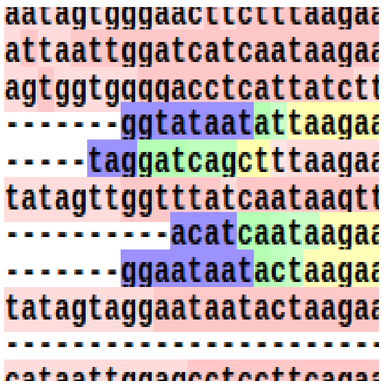
- Used to select the most suitable alignments.
- TCS ascii to used in applying different weights to columns in phylogenetic analysis

T_coffee consistency based Multiple Sequence Alignment evaluation

MAFFT

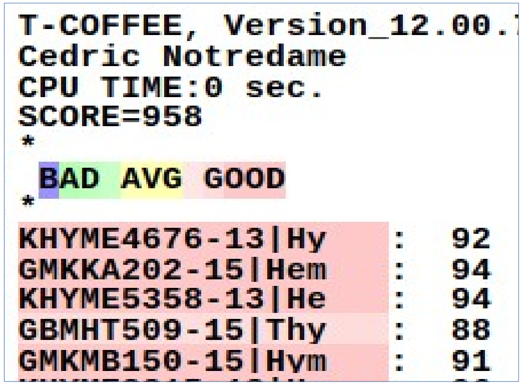


```
T-COFFEE, Version_12.00.
Cedric Notredame
CPU TIME:0 sec.
SCORE=961
*
 BAD AVG GOOD
*
GWOSM440-11|Lep     :  96
AFPHY090-14|Lep     :  96
GMKMD598-15|Lep     :  95
GMKKC063-15|Lep     :  96
```

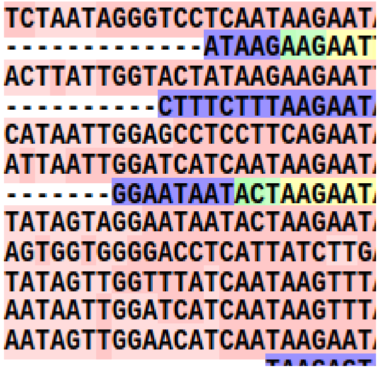


```
aatagtgggaacttctttaagaa
attaattggatcatcaataagaa
agtggtggggacctcattatctt
-------ggtataatattaagaa
-----taggatcagctttaagaa
tatagttggtttatcaataagtt
----------acatcaataagaa
-------ggaataatactaagaa
tatagtaggaataatactaagaa
-----------------------
```

MUSCLE

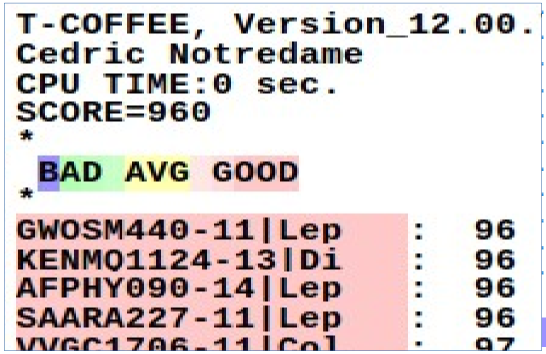


```
T-COFFEE, Version_12.00.
Cedric Notredame
CPU TIME:0 sec.
SCORE=958
*
 BAD AVG GOOD
*
KHYME4676-13|Hy     :  92
GMKKA202-15|Hem     :  94
KHYME5358-13|He     :  94
GBMHT509-15|Thy     :  88
GMKMB150-15|Hym     :  91
```

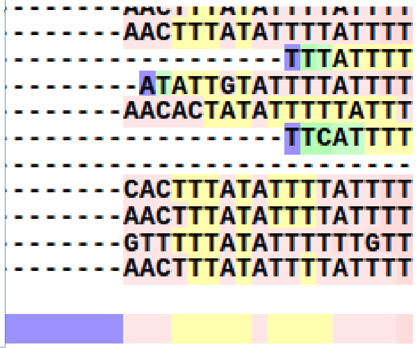


```
TCTAATAGGGTCCTCAATAAGAAT
-------------ATAAGAAGAAT
ACTTATTGGTACTATAAGAAGAAT
----------CTTTCTTTAAGAAT
CATAATTGGAGCCTCCTTCAGAAT
ATTAATTGGATCATCAATAAGAAT
-------GGAATAATACTAAGAAT
TATAGTAGGAATAATACTAAGAAT
AGTGGTGGGGACCTCATTATCTTG
TATAGTTGGTTTATCAATAAGTTT
AATAATTGGATCATCAATAAGTTT
AATAGTTGGAACATCAATAAGAAT
```

T_coffee

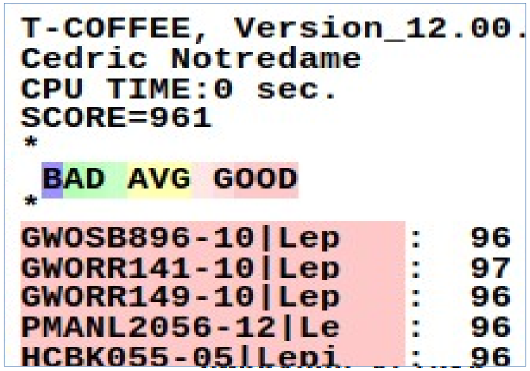


```
T-COFFEE, Version_12.00.
Cedric Notredame
CPU TIME:0 sec.
SCORE=960
*
 BAD AVG GOOD
*
GWOSM440-11|Lep     :  96
KENMQ1124-13|Di     :  96
AFPHY090-14|Lep     :  96
SAARA227-11|Lep     :  96
VYGC1706-11|Col     :  97
```

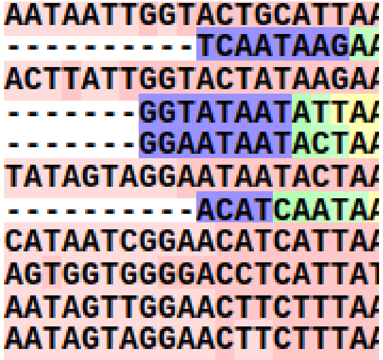


```
-------AACTTTATATTTTATTTT
-------AACTTTATATTTTATTTT
-----------------TTTATTTT
--------ATATTGTATTTTATTTT
-------AACACTATATTTTTATTT
-----------------TTCATTTT
-------------------------
-------CACTTTATATTTTATTTT
-------AACTTTATATTTTATTTT
-------GTTTTTATATTTTTTGTT
-------AACTTTATATTTTATTTT
```

PASTA



```
T-COFFEE, Version_12.00.
Cedric Notredame
CPU TIME:0 sec.
SCORE=961
*
 BAD AVG GOOD
*
GWOSB896-10|Lep     :  96
GWORR141-10|Lep     :  97
GWORR149-10|Lep     :  96
PMANL2056-12|Le     :  96
HCBK055-05|Lepi     :  96
```



```
AATAATTGGTACTGCATTAA
----------TCAATAAGAA
ACTTATTGGTACTATAAGAA
-------GGTATAATATTAA
-------GGAATAATACTAA
TATAGTAGGAATAATACTAA
----------ACATCAATAA
CATAATCGGAACATCATTAA
AGTGGTGGGGACCTCATTAT
AATAGTTGGAACTTCTTTAA
AATAGTAGGAACTTCTTTAA
```

Current progress...

- Build my Data set
- Setting up a RAxML8 and FastTree pipelines
- Improve on alignments: translation-alignment-threading of DNA
- High Performance Computing (HPC) analysis

Activity	Time in months (2018-2019)											
	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	March	April	May	June
Proposal writing and Literature Review	■	■	■									
Data Mining and Sorting			■									
Pipeline Development and Testing				■	■	■	■	■				
Data Analysis on HPC								■	■			
Manuscript Writing and submission										■	■	
Thesis writing and Defence								■	■	■	■	■

ACKNOWLEDGEMENT

Thank you for enabling a bioinformatics dream

BOLD SYSTEMS

icipe
African Insect Science for Food and Health

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Smithsonian
Institution

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NATIONAL MUSEUMS OF KENYA
WHERE HERITAGE LIVES ON