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:

Kibet Rono Gilbert
DRIP fellow

Supervisors:

Dr Scott Miller
Dr Jandouwe Villinger
Dr Steven Ger

Collaborators:

Dr Caleb Kipkurui
Dr Jean-Baka Domelevo
Dr Daniel Masiga

Background

Identification and classification of organisms:

Morphology-based identification systems – extensive information (ecology, anatomy, physiology); expensive, slow and needs expertise

Molecular-based system – efficient (fast and effective); dependent on reference libraries of DNA barcode -*short and standardized genes or regions thereof used in identification and discovery of species*

The Consortium for the Barcode of Life (CBOL), May 2004: To aid 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

A 658 base-pair 5' region of mitochondrial cytochrome c oxidase subunit I (COI/COXI) gene is the standard the barcode for most animal groups

Problem statement:

Thousands of East African COI sequences from voucher arthropods submitted into the BOLD database and are yet to be analysed comprehensively: ***phylogenetic*** **diversity** and **phylogeographic distribution**

Objectives:

- Improve phylogeographic and **phylogenetic** diversity descriptions 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 (tidyverse package)

Awk and bash

Bioinformatics Pipeline Tests:

- **Alignment**

(PASTA, T_coffee, MUSCLE, MAFFT, SATe)

- **Phylogenetics**

(RaxML, FastTree)

- **Phylogeography**

(BASTA/BEAST)

Tools

- Git & GitHub
- Linux System
- R & Python

Run Pipelines

High Performance Computing (HPC)
Data analysis

Progress Reports
Manuscript
Thesis
Publications

Data Mining and Clean up

Sequence retrival:

BOLD-Systems via API; using specific syntax:

“arthropoda Kenya Uganda Tanzania Rwanda Burundi”

Data clean up:

R (tidyverse package), 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
Fast speed	Fast speed	Fast speed	Fast speed	NA
Low accuracy	High accuracy	High accuracy	High accuracy	NA
• Refine • Align • Merge	• Align • Add sequence • Add_fragments • Merge	• Align • Evaluate: (CORE index TCS)	• Align • Add fragments	NA

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

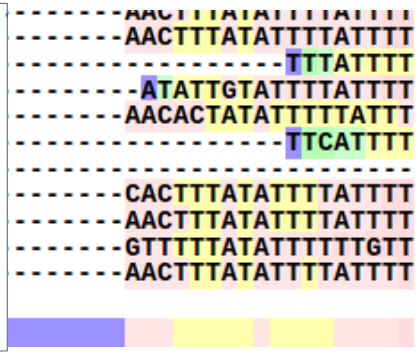
```
T-COFFEE, Version_12.00.
Cedric Notredame
CPU TIME:0 sec.
SCORE=961
*
 BAD AVG GOOD
*
GWOSM440-11|Lep    :   96
AFPHY090-14|Lep    :   96
GMKMD598-15|Lep    :   95
GMKKC063-15|Lep    :   96
```

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
```

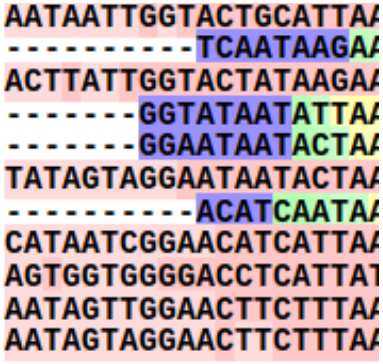
T_coffee

```
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
```



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
```



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
- Phylogeographic analysis

Timeline

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