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Genetic diversity of Mosquito Anopheles in Sudan

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Abstract: In this work we obtained a reasonably clean part of the DNA sequence of one of the Mosquito sample collected from Al-Fetyhab area, Sudan. We identified a clean sequence for about 120 base pairs of the CO1 gene region. The sequence of mosquito sample we analyzed was closely comparable to published sequences for various mosquito species, all published by researchers from Iran.

Keywords: Genetic diversity; Mosquito; Anopheles; DNA sequence

Introduction

Several insects are known to be vectors of human diseases. Mosquitoes are the first insects to be associated with the transmission of a disease effect human and animal health [Service, 2007; Yadav, 2009]. Malaria disease transmitted by mosquito is responsible for 500 million cases of illness and up to 2.7 million deaths annually, more than 90% of which occur in sub-Saharan Africa [Heyden, 2009]. Genetic diversity and gene flow between fragmented faunal populations is warranting an ever-increasing focus from a conservation perspective [Holt,2002; Bertola *et al*.,2011]. Ensuring phenotypic and genotypic similarities between populations of the same species relies upon essential connectivity, particularly across wide distribution ranges [Weiss and Fullerton, 2000]. Mosquitoes comprise two-winged flies and it belong to the family Culicidae in the order Diptera. There are approximately, 3,500 species of mosquitoes. The family Culicidae is divided into three subfamilies: *Toxorhynchitinae, Anophelinae,* and Culicinae Worldwide, there are 37 genera of mosquitoes [Service, 2007]. The subfamily *Anopheiinae* has three genera: the neotropical *Chagas'ia*, the Australasian

Bironella, and the nearly cosmopolitan *Anopheles*. The first two genera include 4 and 7 known species, respectively, while the genus *Anopheles* contains more than 350 species grouped in six subgenera: *Stethomyia* with 5 species, *Lophopodomyia* with 6 species, Kerteszia with 6 species, *Vyssorhynchus* with 25, *Anopheles* with about 150 species, and *Cellia* with about 160 species. The last four subgenera include all the known vectors of human malaria, as well as important vectors of Bancroftian filariasis and arbovirus [Coluzzi and Kitmiller, 1975].

Materials and methods:

The sample was collected from Al Fetyhab town, Omdurman city, Khartoum State. Al Fetyhab town lies on the western banks of the White River Nile. Extraction was performed by using a High Pure PCR Template Preparation Kit Dissection. The spin column protocol for tissue extraction was used for extraction and included the following chemicals that were obtained from the kit: Tissue Lyses Buffer, Protei“nase K, Binding Buffer, Inhibitor Removal, Wash Buffer and Elution Buffer. To start the extraction procedure 200pl Tissue Lyses Buffer as well as 40pl Protei”nase Kwere added to the six to seven millipede feet in the 1.5 ml Eppendorf tube and mixed using the Vortex-Genie 2. The samples were then placed inside an overnight heating block at 55’C. The Elution Buffer was warmed to 70 ℃ before usage and the samples were removed from the heating block. Binding Buffer (200dI) was then added to the Eppendorf Tubes and mixed. The samples were then reheated for ten minutes at 70 ℃. Afterwards 100pI Isopropanol were added and the samples mixed. The sample was then centrifuged, using a Spectrafuge 7M, at 8,000 x *g* for 30 seconds. A High Filter Tube was inserted into a Collection tube and the top part of the sample was pipetted and transferred to the Filter Tube. The top part of the sample contains the DNA. This was done carefully to make sure that none of the insoluble segments goes into the Filter Tube. The entire High Pure Filter assembly were inserted in the Spectrafuge and centrifuged at 8,000 x *g* for one minute

After centrifugation the Filter Tube were removed from the Collection Tube and placed in a new collection tube. The flow through liquid as well as the collection tube was discarded. Inside the upper reservoir of the filter tube, 500pl inhibiter removal was added and the mixture centrifuged 8,000 x *g* for one minute. The filter tube was removed and placed inside a new collection tube while the flow through liquid aswell as the collection tube was discarded. Wash buffer (500pI) was then added to thefilter tube and centrifuged at 8,000 x *g* for one minute. The previous step was repeated and the flow through liquid discarded. The filter top was then refitted inside the collection tube and centrifuged again at 8.000 x *g* for one minute. This was done to ensure that all the wash

buffer was removed

To elute the DNA the filter tube was inserted in a clean 1.5 ml Eppenforf tube and 200pI pre-warmed elution Buffer was added. The reaction was centrifuged at 8,000 x *g* for one minute. The Eppendorf Tube contained the eluted DNA for usage or storage The DNA was stored at -22'C when not used.

For Pre-PCR we used Universal Insect which can be used on a variety of insects and invertebrates. These primers are specially designed for the mitochondrial DNA gene Cytochrome Oxidase c subunit (CO1). The PCR procedure involved 2µl DNA, 2.5µl Buffer with $MgCl_2$, 5µl 2mM 2Dntp, 0.15µl Tag, 2,5µl 10 µM primer LCO1490, 2.5µl 10 µM primer HCO2198 and Distilled Water to form a 25µl reaction. The PCR program was as follows: Denaturation at 94 ℃ for eight minutes, annealing for 40 cycles at 94 ℃ for 30 seconds, 58 C for one minute 72 ℃ for three minutes and Elongation at 72 ℃ for 10 minutes. Agarose Gel Electrophoresis was done after pre-PCR to determine if the PCR worked and to test the quality of the DNA to determine whether the reagent was clean or not. Agarose gel-electrophoresis EPS-25 Series II machine was used to run the agarose gel and an Ultra Lum Electronic U.V. Transilluminator were used to view gel under UV light. The TAE buffer consisted of 16ml 50 × TAE and 784ml of distilled water. GelRed 2 × Bromophenol Blue dye was used to stain the DNA on gel and make it visible on Transilluminator. The 2 × loading buffer was made up by adding 200 µl × 10 loading buffer and 800µl Distilled water. To make the GelRed mixture, 750 µl 2×bromophenol Blue and 3.5 µl GelRed were mixed and covered with foil since the GelRed is very light sensitive. To run the gel electrophoresis, 1 µl GelRed 2 × bromophenol blue was mixed with 6µl of the Template DNA obtained from pre-PCR and loaded onto the gel. The gel was then run at 100V for 20 minutes. After agarose gel electrophoresis the Biospin PCR Kit was used to clean the PCR sample and remove any excess chemicals in order to get cleaner DNA. Post-PCR was done to prepare the final sample for sequencing. The AB13130 genetic analyzer was used to obtained sequencing from the DNA and the result were then interpreted through the software program Mega v.5.

Result and discussion:

In this work we managed to obtain a clean sequence for about 120 base pairs of the CO1 gene region and the result illustrated by the figures 1 and 2. The actual sequence of this region is:

TCATAAAGATATTGGTCAACAAATCATAAAGATATTGGTCAACAAATCAT
AAAGATATTGGTCAACAAATCATAAAGATATTGGTCAACAAATCATAAAG
ATATTGGTCAACAAATCATAAAGATATTGGTCAACAAATCA.

To see how similar the sequence is to sequences of other mosquitoes, we compared the sequence to other sequences available. Specifically, we used the BLAST function of the NCBI GENBANK database. (http://www.ncbi.nlm.nih.gov/genbank/). The sequence of mosquito sample we analyzed was closely comparable to published sequences for various mosquito species, all published by researchers from Iran. It is however not clear whether these mosquitoes actually come from Iran, or whether only the analyses were done in that country. The sequence from the mosquito from study area and the published ones of Anopheles by researchers from Iran showed 100% overlap over 83-84%% of its length. The mosquito species that show close identities to the mosquito from study area are *A. plumbeus, A. apoci; A. maculipennis; A. superpictus; A.culicifacies; A. claviger* (GenBank numbers JF966742 to JF966747). We constructed a tree to depict relationships among the species (Figure 2).

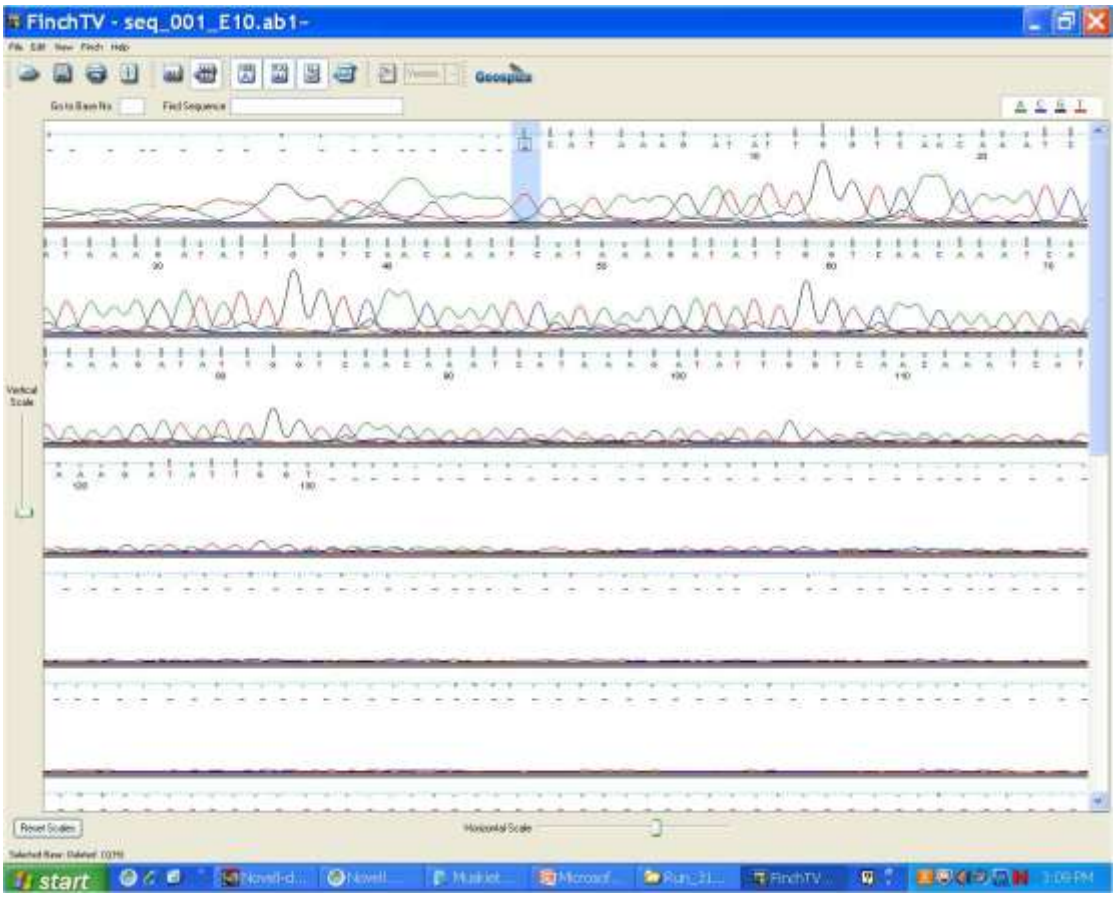

Figure 1: Seq001

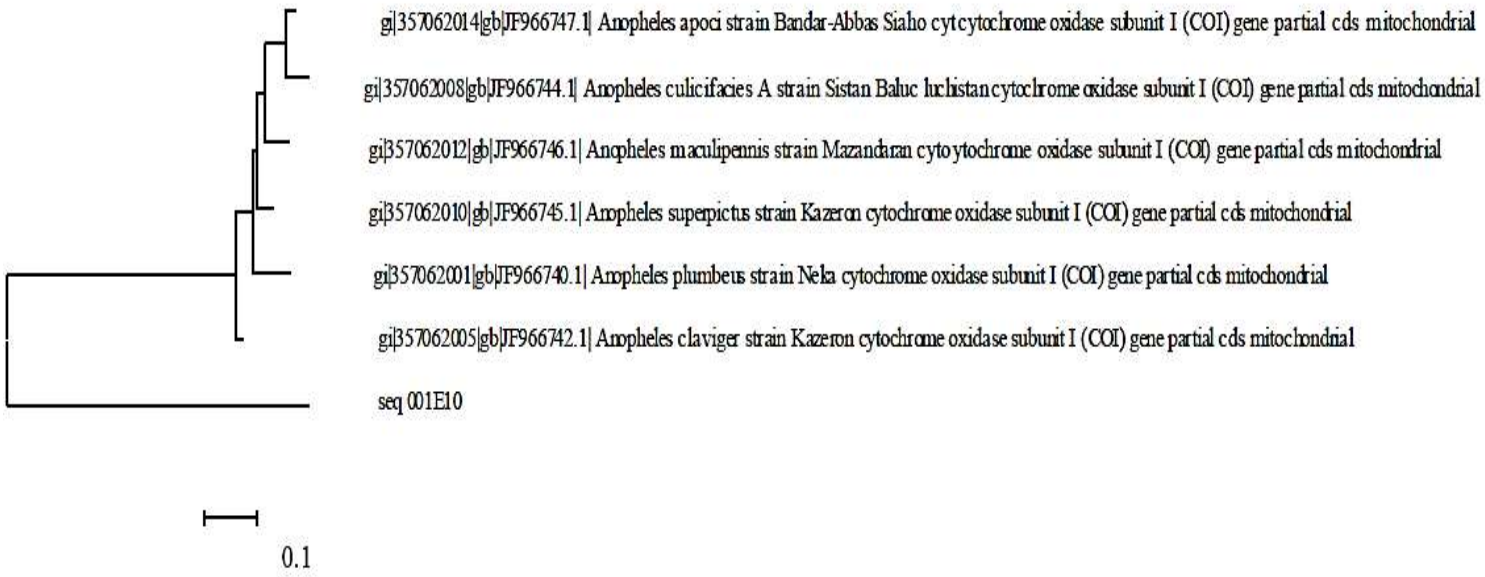

Figure 2: Tree depict relationships among the species

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