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Identification and confirmation of *Aedes albopictus* (Skuse, 1864) and *Aedes aegypti* (Linnaeus, 1762) using DNA Barcoding

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Abstract

Present study was performed to identify and confirm collected *Aedes albopictus* and *Aedes aegypti* mosquitoes up to species level. To evaluate molecular taxonomy and phylogeny, DNA barcoding was done using sanger did epoxy sequencing of mitochondrial cytochrome C oxidase subunit I (COI) gene of *Aedes* mosquitoes sample. Bioinformatics analysis was done by using NCBI'S BLAST software. By evidence of DNA barcoding, it was confirmed that present *Aedes* species were *Aedes albopictus* (*Skuse*) and *Aedes aegypti* (*Linnaeus*).

Keywords: *Aedes albopictus, Aedes aegypti*, cytochrome C, NCBI'S BLAST, DNA Barcoding

Introduction

Mosquitoes are most important groups of arthropods that live in aquatic habitats. They are likely antagonistic arthropod which transmits wide scope of pathogens that cause exceptional disease such has human malaria, dengue, filariasis and viral encephalitis (Rasool *et al.*, 2014) [1]. In this manner control of mosquitoes turns into that need of great importance to prevention wide pestilence diseases. Be that as it may, it is challenging to control and forestall serious species (Aneesh and Vijayan *et al.*, 2010) [2]. Studies demonstrates that 390 million individual on the world faces dengue infections each year with 96 million diseases affirmed clinically (Bhatt *et al.*, 2013) [3]. Moreover, the fast spread of mosquito borne load transportation and global travel (Straetemans M 2008; Medlock *et al.*, 2012) [4, 5]. Brief and wonderful species identification with massive precision can be accomplished through molecular methodology (Cywinska *et al.*, 2006) [6]. The main widespread epidemic of dengue happened in India during 1996 including regions in Dehli and Lucknow (Agarwal *et al.*, 1999) [7], which latter spread to all around (Shah *et al.*, 2004; Singh *et al.*, 2000) [8, 9]. Accurate recognition proof of the *Aedes* species engaged with arbovirus transmission is vital to plan methodologies for vector reconnaissance and control programme. Larva observation was directed in dengue flare-up regions in Malaysia from 2008 until 2009(Rohani *et al.*, 2014) [10]. Also many firmly related types of mosquitoes with shifting ecology and host inclinations are almost indivisible morphologically (Walton *et al.*, 2008) [11]. This present troubles is recognition of mosquitoes to a species types or even genus level (Reinert, 2000; Savag and Strickman, 2004; Reinert *et al.*, 2009) [12, 13, 14]. Concentrates on utilizing cytochrome C oxidase subunit I (Col) and NADH dehydrogenase subunit 5 (NDS) qualities showed a regulation degree of polymorphisms of the mitochondrial DNA (mt DNA), yet uncovered contrasts among tropical and subtropical populations (Mousson *et al.*, 2005; Patsoula *et al.*, 2006; Kamgang *et al.*, 2011; Zitko *et al.*, 2011) [15, 28, 17, 18]. The quantity of molecular strategies accessible for entomological work has developed tremendously since the origination of polymerase chain reaction or PCR. The presence of moderated areas in the DNA successions, for example, such as mitochondrial, ribosomal and molecular DNA markers, it conceivable to intensify part of creatures, whose genome is obscure (Kocher *et al.*, 1989; Fitzpatrick *et al.*, 2010; Albers *et al.*, 2013) [19, 20, 21]. We chose COI as the most enlightening quality for investigation of *Aedes albopictus* from various testing locales COI is likewise utilized for barcoding and distributed grouping information of various geological beginning are accessible for examination. To concentrate on nuclear quality variety inside and among of *Aedes albopictus* from various inspecting destinations from various inspecting destinations, we researched the second inward translated spacer (ITS2) of rRNA that is customarily used to distinguish interspecific contrasts. ITS 2 was effectively used to separate the *Aedes* species (Patsoula *et al.*, 2006) [22]. Hence, for phylogenetic investigation of firmly related species it is the most appropriate succession (Coleman and Vacquier, 2002) [23]. Gene sequence, for example, ITS-1 and ITS-2 of Rdna, COI and COII are useful in building phylogenetic relationship in the middle of species (Kaura *et al.*, 2010; Park *et al.*, 2008) [24, 25]. As a results molecular equipment that can also separate mosquito species from a small part of tissue in scientific identification of mosquitoes.

Materials and Methods

1. Mosquito collection

Aedes larva were collected from different regions of Udaipur district of Rajasthan in pre-monsoon, monsoon and post monsoon during the period April, 2016 to March, 2017. Sample were collected from different regions of Udaipur district (24°58’ N; 73°68’E) namely hill regions (Gogunda, Jhadole, Kotra and Girwa) and plain regions (Mavli, Udaipur city and Salumber). The collected larvae were reared to adult in the mosquito rearing laboratory at University College of Science, MLSU Udaipur Rajasthan.

2. Morphological identification

Aedes mosquitoes species were identified with the help of pictorial identification key (Rueda, 2004). Thorax of *Aedes aegypti* adult has white scales on the top and lyre shaped. *Aedes albopictus* has white silver line of the thorax. Each hind region has white bands and abdomen dark brown to back to back. This identification features were used and visualized using Stereoscopic microscope in the Laboratory. Molecular phylogeny of *Aedes* further confirms their identification.

3. Molecular Phylogeny of *AedeS*

Adult sample collections were send to Xcelris Genomis Lab, Ahmedabad for molecular phylogeny analysis. The following procedure was adopted:

1. DNA was isolated from the given sample through in house method.
2. Isolated DNA was amplified with HCO/LCO (mitochondrial cytochrome c oxidase subunit I (COI) genes specific primer using Veriti® 99 well thermal cycler (Model No.9902). A single discrete PCR amplicon band of 700 bp was observed.
3. The PCR amplicon was enzymatically (ExoSap) purified and further subjected to Sanger sequencing.

PCR Condition:

94°C---------------------------- 5 min
35 cycle of
94°C--------------------------- 40 Sec
47°C--------------------------- 45 Sec
72°C--------------------------- 45 Sec
Final extension:
72°C ------------------------- 20 min

4. Bi-directional DNA sequencing reaction of PCR amplicon was carried out with HCO and LCO primers using BDT v3.1 cycle sequencing kit on ABI 3730 xl Genetic Analyzer.

5. Sequencing PCR products

The PCR amplicon was purified enzymatically using Exo-SAP, as per the manufacturer instructions (Applied Bio system). After the purification the products were subjected to Sanger sequencing using ABI, 3730XL DNA analyzer using BdT v3.1 chemistry. Forward and reverse DNA sequencing reaction of PCR amplicons (Sample) of PCR product was carried out with 911 (5’-TTAACTTCAGGGTGACCAAAAAATCA-3’) and 912 (5’-TTACTACCAATCATAAAGATATTGG-3’) primers, separately. 911 and 912 are Cytochrome Oxidase specific primers.

Results and Discussion

Taxonomic species identification of larvae *Aedes* mosquitoes are more time consuming and very difficult due to morphological resemblance them. DNA barcoding utilizing COI quality is helpful for species conformity however because of their overflow and non-attendance of accessible Gene bank information base, it is likewise not achievable for all collected mosquitoes. Significant sequence similarity alignment obtained using BLAST is taken in figure 1 to 6. Phylogeny tree (at distance scale 0.0) obtained using BLAST is given figure 1 to 6, Percent identify in our case reported is 100% (figure 1 to 6), percent identify is a number that report how similar the query sequence is to the target sequence. The higher the percent identify is the move significant the mach. Query cover in our reported is 100 (figure 2). Query cover is a number that show how much of the query sequence is covered by the target sequence. This shows us how long time sequences are relative to each other. E value (excepted value in over case reported is 0.0 (Figure 1 to 6). E value is a number that show how many times we would expect as match by chance in database. The lower the E value is more significant the match.

Molecular Phylogeny of *Aedes*

In present study, to confirm species identification and assess molecular phylogeny of Aedes, Sanger sequencing of Mitochondrial Cytochrome Oxidase 1 (COI) gene was carried out by using Cytochrome Oxidase specific universal primer 911 (5`-TTTCTACAAATCATAAAGATATTGG-3`) and 912 (5`-TAAACTTCAGGGTGACCAAAAAATCA-3`).
Nucleotide sequence obtained for *Aedes albopicutus* using these primers are as follows:

Contig sequence got using primers-
GTGCCAGTTCCGTCCGGGCGGTGGTGCGGGGATTGT
AGTGTTAGACTTCCAGAACATGCTAGTAATATAGAC
ATACTTCCATCAAATGCAGGGAGGGAAAGAAGTAA
AAAACTGCCTTTAACTCCTTTCGATCCCAAAAATACC
GCTAAGTGATCTCCATCTGCCGAATTGAGCTGTTATT
TGTTGACCATGTAATAAAATTTACTGCTCCTAAAATA
GATGAGATTCCCGCTAAATGTAAAGAAAAAATTGCT
AAATCAACTGAAGCCCCAGCATGAGCTGTTCCAGAA
GAAAGGGGAGGATAAACCGTTCACCCTGTTCCAGCT
CCGTTTTCTACTATAGAACTAGAAAGCAGCAGTGTT
AAAGAGGGGGGTAATATTCAAAAACTTATATTATTT
ATTCGAGGAAAAGCTATATCAGGGGCTCCTAGTATT
AAGGGTACTAGTCAGTTTCCAAATCCTCCAATTATG
ATAGGTATTACTATAAAAAAAATTATAATAAAAGCA

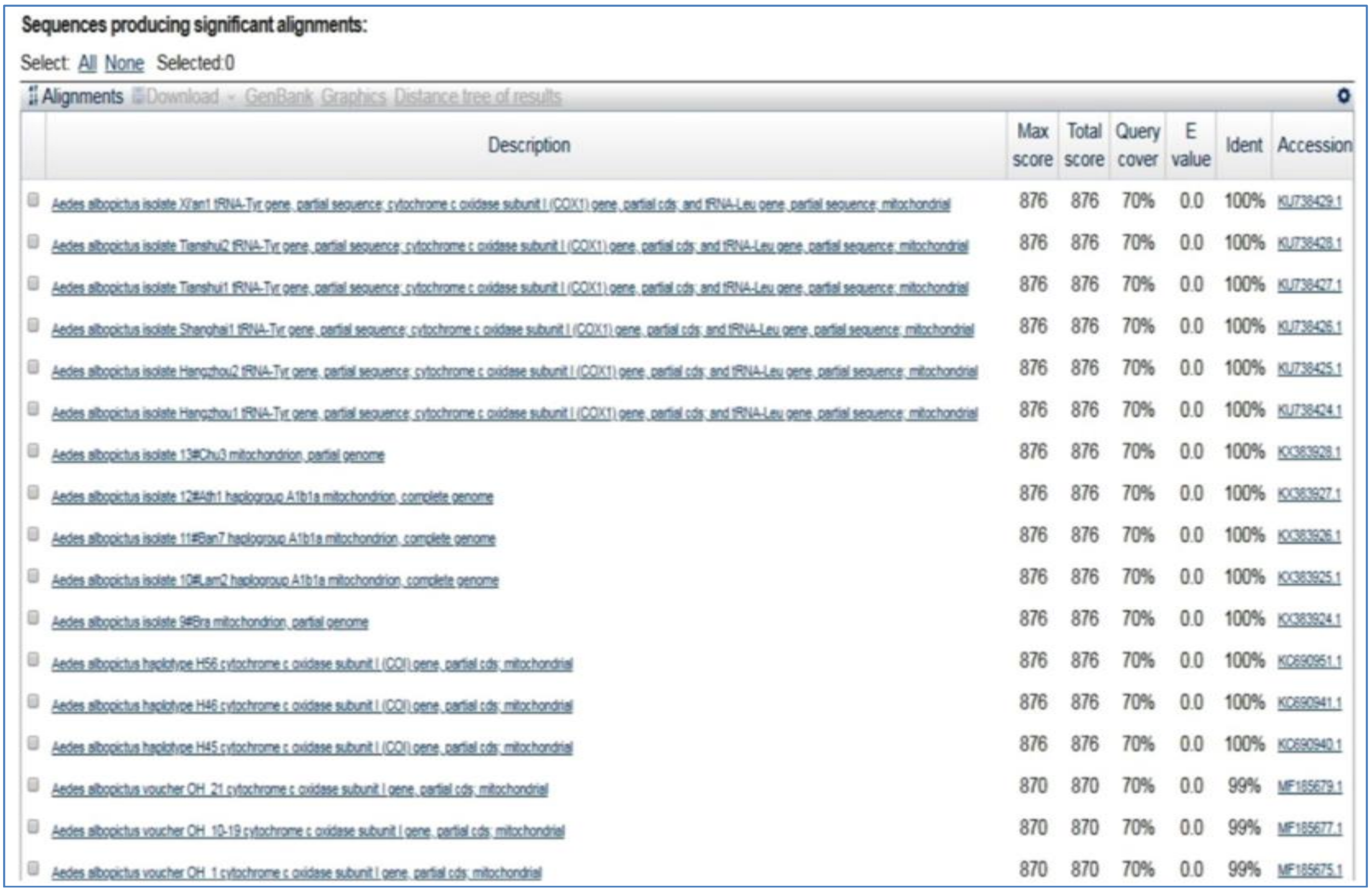
TGAGCAGTAACAATTACATTATAAATTTGATCATTTC
CAATAAATATACCAGGATGTCTAAGTTCAATACGAA
TTAAAACTCTTAGTGAAGTTCCGACTATTCCAGATCA
AATACCGAAAATAAAGTATAATGTTCCAATATCTTT
ATGATTTGTTGACCAAA.

Nucleotide sequence obtained for *Aedes aegypti* using these primers are as follows:

ATAAGTTTTTGAATACTACCTCCTTCATTGACTCTTC
TATTATCAAGCTCAATAGTAGAAAATGGGGCAGGAA
CTGGGTGAACAGTTTATCCTCCTCTCTCTTCAGGAAC
AGCTCATGCTGGAGCTTCTGTTGATTTAGCTATTTTT
TCTCTTCATTTAGCTGGAATTTCCTCAATTTTAGGGG
CAGTAAATTTTATTACAACTGTAATTAATATACGATC
GTCAGGAATTACTTTAGATCGACTACCTTTATTTGTT
TGATCTGTAGTTATTACAGCTATCTTATTACTTCTTTC
TCTTCCTGTTTTAGCTGGAGCTATTACTATGTTATTA
ACAGACCGAAACTTAAATACATCTTTCTTTGATCCAA
TCGGAGGAGGAGATCCTATTTTATACCAACACTTATT
CTGATTCTTTGGACAC.

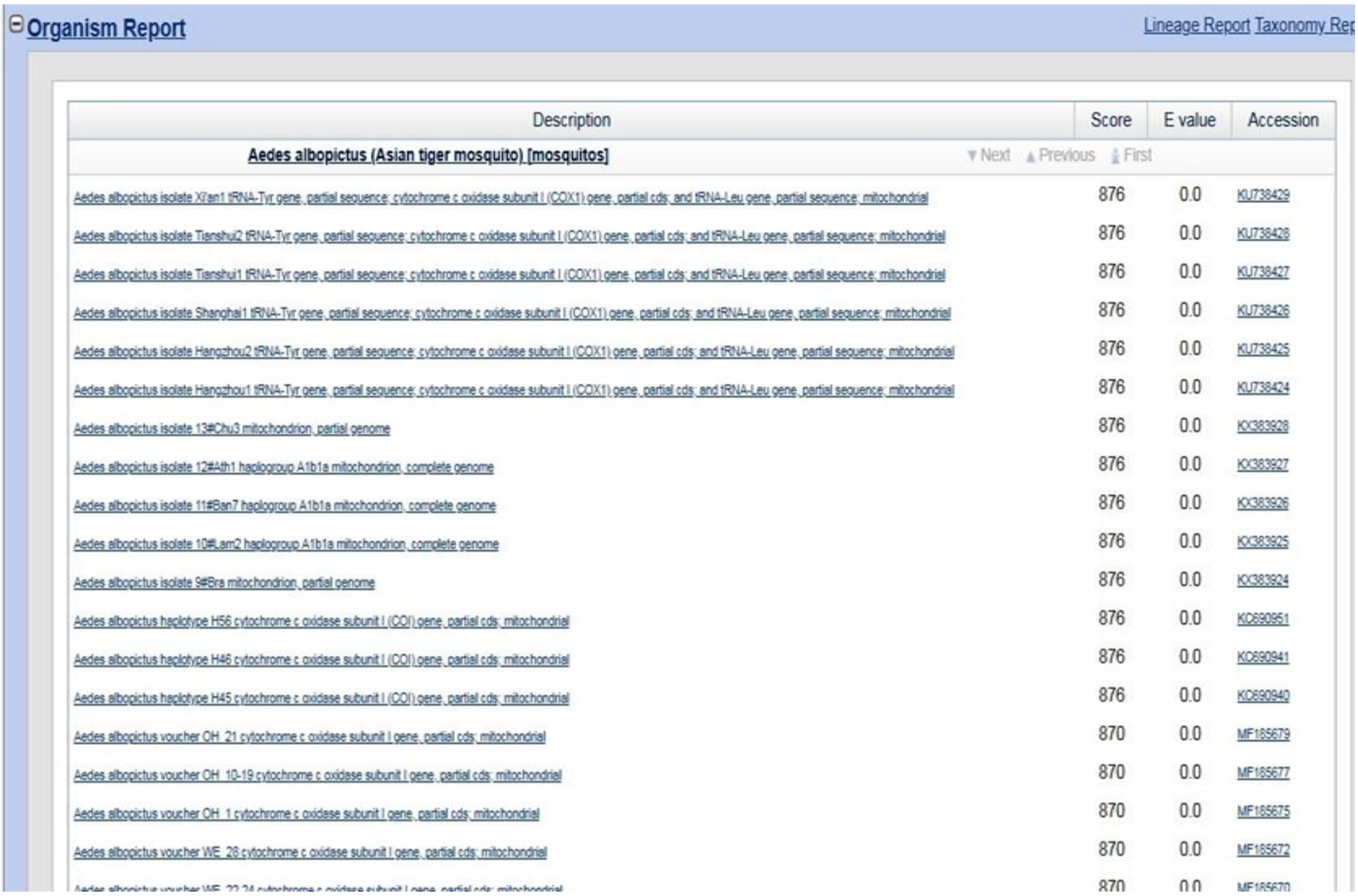
After getting contiguous sequence, this sequencing was analyzed on open access NCBI BLAST software and got NCBI hits, organism report and phylogeny tree (Figure 1 to 6).

Morphological identification methods are not appropriate for identification of closely related species, sibling species and sub species level. In this manner molecular phylogeny, r-DNA, mt gene COI methods helpful for identification of these species. In present study was carried out with the help of molecular phylogeny of *Aedes aegypti* and *Aedes albopictus;* Sanger sequencing of mitochondrial cytochrome oxidase 1 (COI) using cytochrome oxidase specific universal primer 911 (5`-TTTCTACAAATCATAAAGATATTGG-3`) and 912 (5`-TAAACTTCAGGGTGACCAAAAAATCA-3`). In this manner similar type study was also conducted by Das *et al.* (2016) [26] in Sonitpur district of Assam; they found *Aedes albopictus* species having highly conserved ribosomal (r) DNA and mitochondrial (mt) DNA gene sequences. Other study was also carried out by Anoopkumar *et al.* (2017) [27]; they used CO1-based DNA barcoding method for identification of *Aedes albopictus* and *Aedes aegypti* in Thrissur district of Kerala state.


Sequences producing significant alignments:

	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	Aedes albopictus isolate Xi'an1 tRNA-Tyr gene, partial sequence; cytochrome c oxidase subunit I (COX1) gene, partial cds; and tRNA-Leu gene, partial sequence; mitochondrial	876	876	70%	0.0	100%	KU738429.1
☐	Aedes albopictus isolate Tianshui2 tRNA-Tyr gene, partial sequence; cytochrome c oxidase subunit I (COX1) gene, partial cds; and tRNA-Leu gene, partial sequence; mitochondrial	876	876	70%	0.0	100%	KU738428.1
☐	Aedes albopictus isolate Tianshui1 tRNA-Tyr gene, partial sequence; cytochrome c oxidase subunit I (COX1) gene, partial cds; and tRNA-Leu gene, partial sequence; mitochondrial	876	876	70%	0.0	100%	KU738427.1
☐	Aedes albopictus isolate Shanghai1 tRNA-Tyr gene, partial sequence; cytochrome c oxidase subunit I (COX1) gene, partial cds; and tRNA-Leu gene, partial sequence; mitochondrial	876	876	70%	0.0	100%	KU738426.1
☐	Aedes albopictus isolate Hangzhou2 tRNA-Tyr gene, partial sequence; cytochrome c oxidase subunit I (COX1) gene, partial cds; and tRNA-Leu gene, partial sequence; mitochondrial	876	876	70%	0.0	100%	KU738425.1
☐	Aedes albopictus isolate Hangzhou1 tRNA-Tyr gene, partial sequence; cytochrome c oxidase subunit I (COX1) gene, partial cds; and tRNA-Leu gene, partial sequence; mitochondrial	876	876	70%	0.0	100%	KU738424.1
☐	Aedes albopictus isolate 13#Chu3 mitochondrion, partial genome	876	876	70%	0.0	100%	KX383928.1
☐	Aedes albopictus isolate 12#Ath1 haplogroup A1b1a mitochondrion, complete genome	876	876	70%	0.0	100%	KX383927.1
☐	Aedes albopictus isolate 11#Ban7 haplogroup A1b1a mitochondrion, complete genome	876	876	70%	0.0	100%	KX383926.1
☐	Aedes albopictus isolate 10#Lam2 haplogroup A1b1a mitochondrion, complete genome	876	876	70%	0.0	100%	KX383925.1
☐	Aedes albopictus isolate 9#Bra mitochondrion, partial genome	876	876	70%	0.0	100%	KX383924.1
☐	Aedes albopictus haplotype H56 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	876	876	70%	0.0	100%	KC690951.1
☐	Aedes albopictus haplotype H46 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	876	876	70%	0.0	100%	KC690941.1
☐	Aedes albopictus haplotype H45 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	876	876	70%	0.0	100%	KC690940.1
☐	Aedes albopictus voucher OH_21 cytochrome c oxidase subunit I gene, partial cds; mitochondrial	870	870	70%	0.0	99%	MF185679.1
☐	Aedes albopictus voucher OH_10-19 cytochrome c oxidase subunit I gene, partial cds; mitochondrial	870	870	70%	0.0	99%	MF185677.1
☐	Aedes albopictus voucher OH_1 cytochrome c oxidase subunit I gene, partial cds; mitochondrial	870	870	70%	0.0	99%	MF185675.1

Fig 1: NCBI hits obtained for *Aedes albopictus*



Description	Score	E value	Accession
Aedes albopictus (Asian tiger mosquito) [mosquitos]			
Aedes albopictus isolate Xi'an1 tRNA-Tyr gene, partial sequence; cytochrome c oxidase subunit I (COX1) gene, partial cds; and tRNA-Leu gene, partial sequence; mitochondrial	876	0.0	KU738429
Aedes albopictus isolate Tianshui2 tRNA-Tyr gene, partial sequence; cytochrome c oxidase subunit I (COX1) gene, partial cds; and tRNA-Leu gene, partial sequence; mitochondrial	876	0.0	KU738428
Aedes albopictus isolate Tianshui1 tRNA-Tyr gene, partial sequence; cytochrome c oxidase subunit I (COX1) gene, partial cds; and tRNA-Leu gene, partial sequence; mitochondrial	876	0.0	KU738427
Aedes albopictus isolate Shanghai1 tRNA-Tyr gene, partial sequence; cytochrome c oxidase subunit I (COX1) gene, partial cds; and tRNA-Leu gene, partial sequence; mitochondrial	876	0.0	KU738426
Aedes albopictus isolate Hangzhou2 tRNA-Tyr gene, partial sequence; cytochrome c oxidase subunit I (COX1) gene, partial cds; and tRNA-Leu gene, partial sequence; mitochondrial	876	0.0	KU738425
Aedes albopictus isolate Hangzhou1 tRNA-Tyr gene, partial sequence; cytochrome c oxidase subunit I (COX1) gene, partial cds; and tRNA-Leu gene, partial sequence; mitochondrial	876	0.0	KU738424
Aedes albopictus isolate 13#Chu3 mitochondrion, partial genome	876	0.0	KX383928
Aedes albopictus isolate 12#Ath1 haplogroup A1b1a mitochondrion, complete genome	876	0.0	KX383927
Aedes albopictus isolate 11#Ban7 haplogroup A1b1a mitochondrion, complete genome	876	0.0	KX383926
Aedes albopictus isolate 10#Lam2 haplogroup A1b1a mitochondrion, complete genome	876	0.0	KX383925
Aedes albopictus isolate 9#Bra mitochondrion, partial genome	876	0.0	KX383924
Aedes albopictus haplotype H56 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	876	0.0	KC690951
Aedes albopictus haplotype H46 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	876	0.0	KC690941
Aedes albopictus haplotype H45 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	876	0.0	KC690940
Aedes albopictus voucher OH 21 cytochrome c oxidase subunit I gene, partial cds; mitochondrial	870	0.0	MF185679
Aedes albopictus voucher OH 10-19 cytochrome c oxidase subunit I gene, partial cds; mitochondrial	870	0.0	MF185677
Aedes albopictus voucher OH 1 cytochrome c oxidase subunit I gene, partial cds; mitochondrial	870	0.0	MF185675
Aedes albopictus voucher WE 28 cytochrome c oxidase subunit I gene, partial cds; mitochondrial	870	0.0	MF185672
Aedes albopictus voucher WE 22-24 cytochrome c oxidase subunit I gene, partial cds; mitochondrial	870	0.0	MF185670

Fig 2: Organism report of *Aedes albopictus*

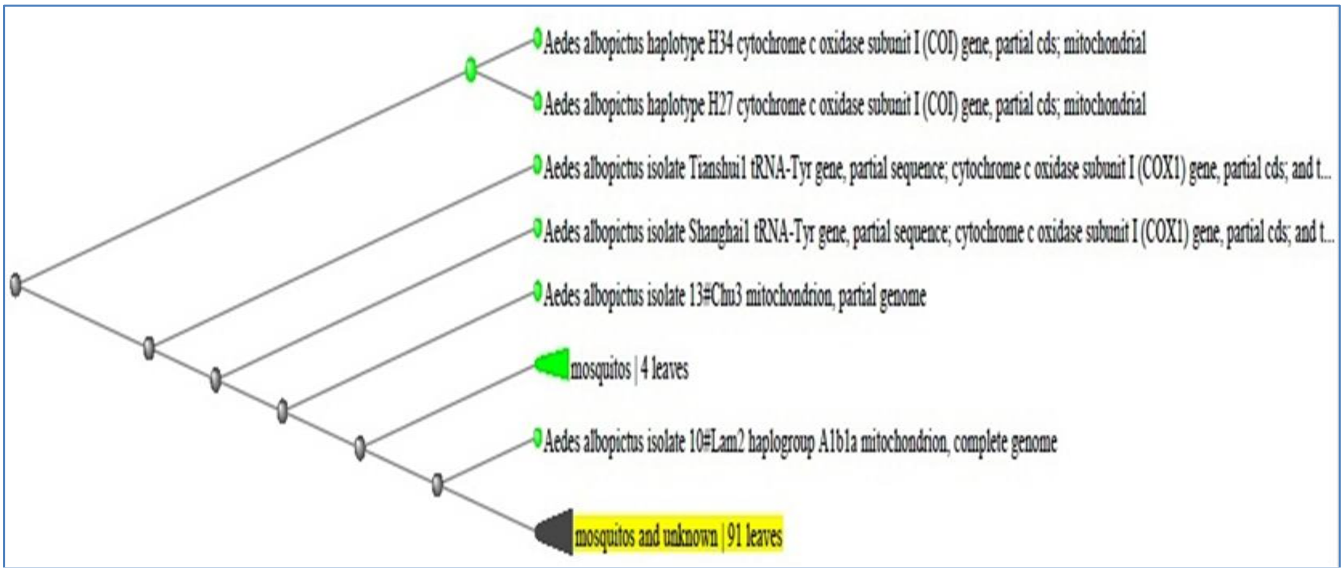
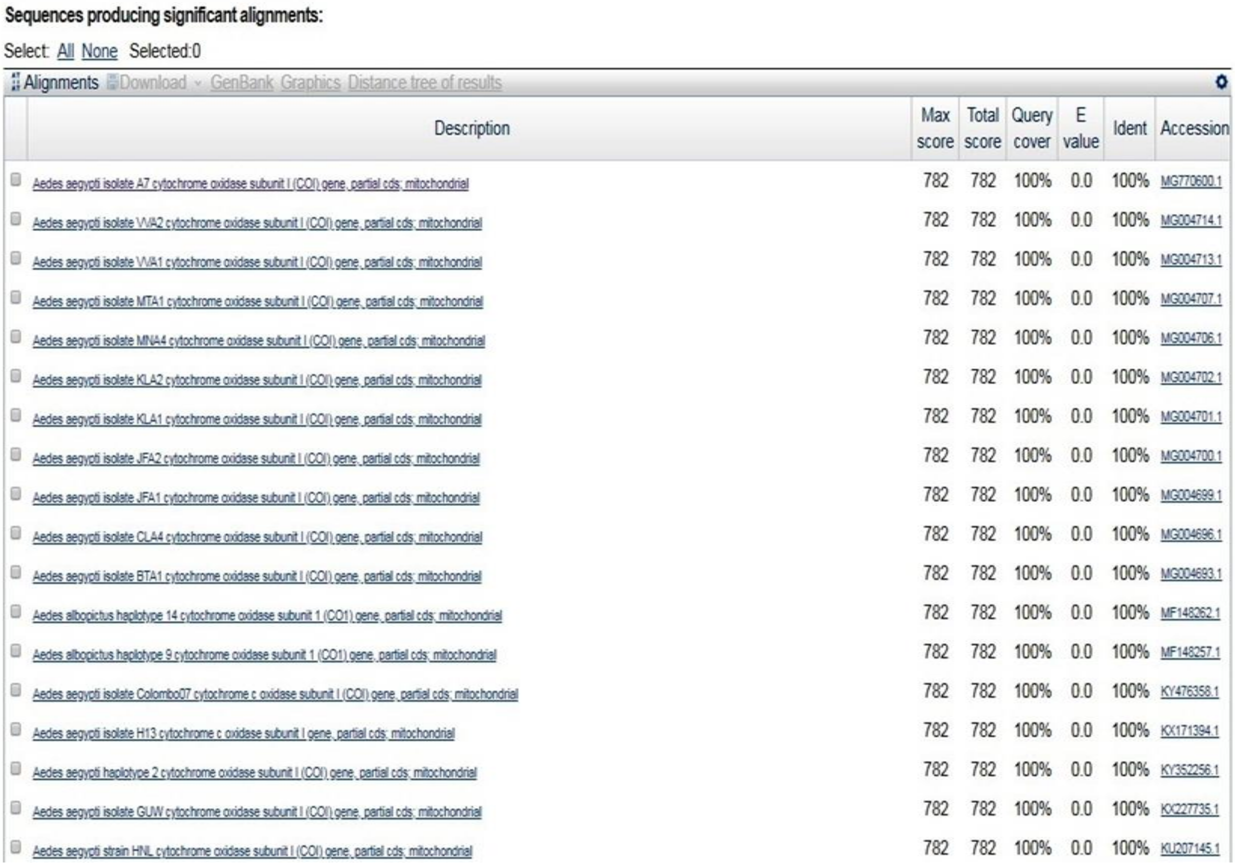
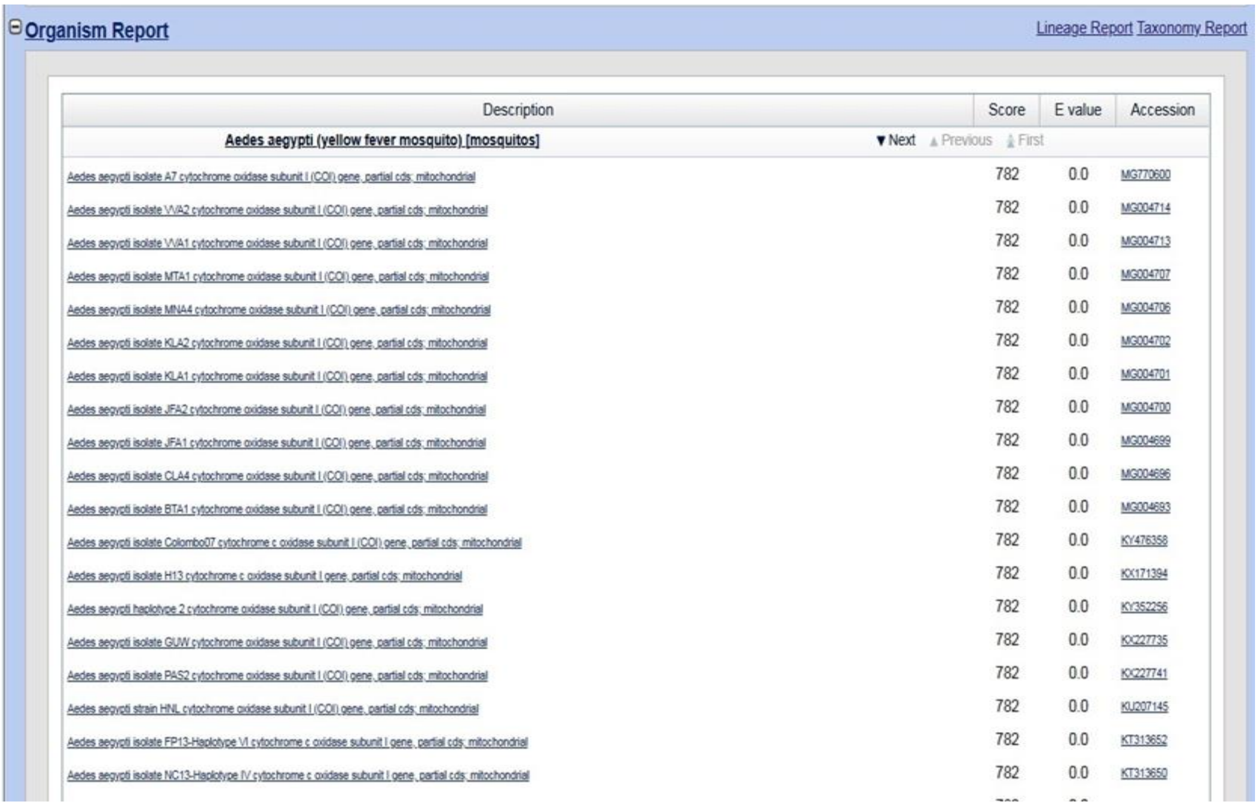


Fig 3: Phylogeny tree of *Aedes albopictus*



	Description	Max score	Total score	Query cover	E value	Ident	Accession
☐	Aedes aegypti isolate A7 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	782	100%	0.0	100%	MG770600.1
☐	Aedes aegypti isolate WA2 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	782	100%	0.0	100%	MG004714.1
☐	Aedes aegypti isolate WA1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	782	100%	0.0	100%	MG004713.1
☐	Aedes aegypti isolate MTA1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	782	100%	0.0	100%	MG004707.1
☐	Aedes aegypti isolate MNA4 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	782	100%	0.0	100%	MG004706.1
☐	Aedes aegypti isolate KLA2 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	782	100%	0.0	100%	MG004702.1
☐	Aedes aegypti isolate KLA1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	782	100%	0.0	100%	MG004701.1
☐	Aedes aegypti isolate JFA2 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	782	100%	0.0	100%	MG004700.1
☐	Aedes aegypti isolate JFA1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	782	100%	0.0	100%	MG004699.1
☐	Aedes aegypti isolate CLA4 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	782	100%	0.0	100%	MG004696.1
☐	Aedes aegypti isolate BTA1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	782	100%	0.0	100%	MG004693.1
☐	Aedes albopictus haplotype 14 cytochrome oxidase subunit 1 (CO1) gene, partial cds; mitochondrial	782	782	100%	0.0	100%	MF148262.1
☐	Aedes albopictus haplotype 9 cytochrome oxidase subunit 1 (CO1) gene, partial cds; mitochondrial	782	782	100%	0.0	100%	MF148257.1
☐	Aedes aegypti isolate Colombo07 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	782	782	100%	0.0	100%	KY476358.1
☐	Aedes aegypti isolate H13 cytochrome c oxidase subunit I gene, partial cds; mitochondrial	782	782	100%	0.0	100%	KX171394.1
☐	Aedes aegypti haplotype 2 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	782	100%	0.0	100%	KY352256.1
☐	Aedes aegypti isolate GUW cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	782	100%	0.0	100%	KX227735.1
☐	Aedes aegypti strain HNL cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	782	100%	0.0	100%	KU207145.1

Fig 4: NCBI hits obtained for *Aedes aegypti*



Description	Score	E value	Accession
Aedes aegypti (yellow fever mosquito) [mosquitos] ▼Next ▲Previous ▲First			
Aedes aegypti isolate A7 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	0.0	MG770600
Aedes aegypti isolate WA2 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	0.0	MG004714
Aedes aegypti isolate WA1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	0.0	MG004713
Aedes aegypti isolate MTA1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	0.0	MG004707
Aedes aegypti isolate MNA4 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	0.0	MG004706
Aedes aegypti isolate KLA2 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	0.0	MG004702
Aedes aegypti isolate KLA1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	0.0	MG004701
Aedes aegypti isolate JFA2 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	0.0	MG004700
Aedes aegypti isolate JFA1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	0.0	MG004699
Aedes aegypti isolate CLA4 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	0.0	MG004696
Aedes aegypti isolate BTA1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	0.0	MG004693
Aedes aegypti isolate Colombo07 cytochrome c oxidase subunit I (COI) gene, partial cds; mitochondrial	782	0.0	KY476358
Aedes aegypti isolate H13 cytochrome c oxidase subunit I gene, partial cds; mitochondrial	782	0.0	KX171394
Aedes aegypti haplotype 2 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	0.0	KY352256
Aedes aegypti isolate GUW cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	0.0	KX227735
Aedes aegypti isolate PAS2 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	0.0	KX227741
Aedes aegypti strain HNL cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial	782	0.0	KU207145
Aedes aegypti isolate FP13-Haplotype VI cytochrome c oxidase subunit I gene, partial cds; mitochondrial	782	0.0	KT313652
Aedes aegypti isolate NC13-Haplotype IV cytochrome c oxidase subunit I gene, partial cds; mitochondrial	782	0.0	KT313650

Fig 5: Organism report of *Aedes aegypti*

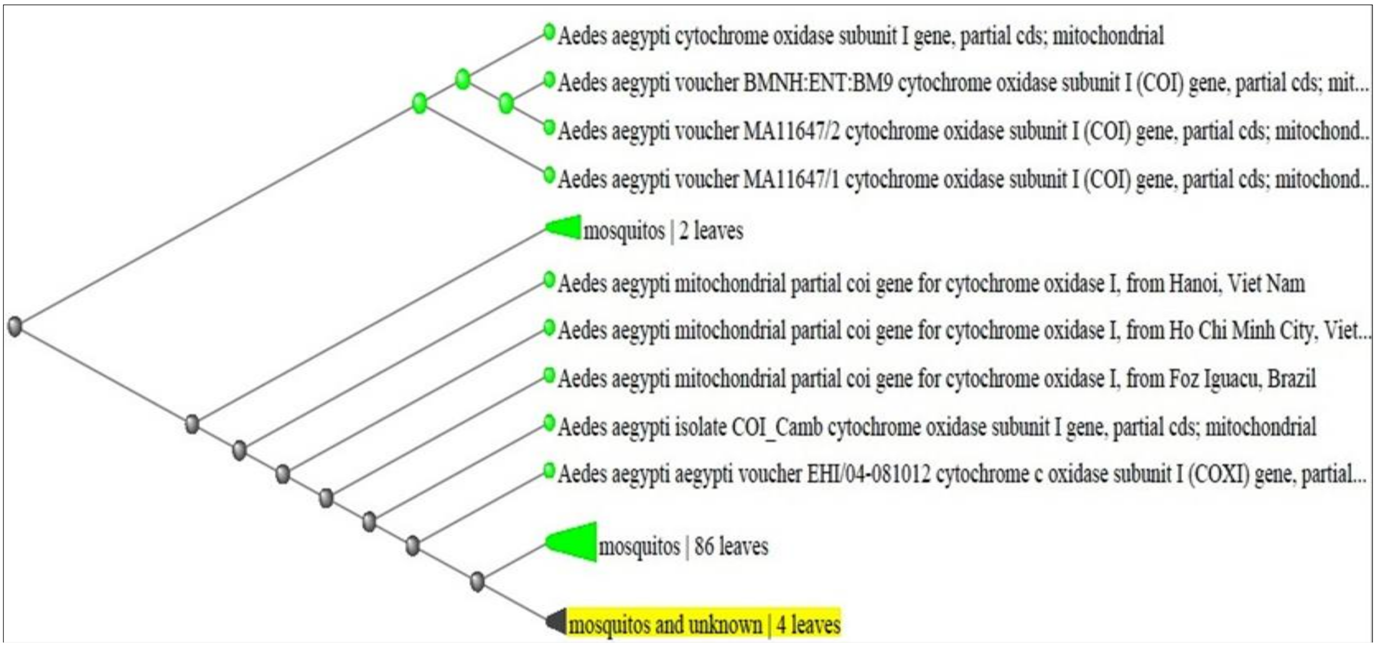


Fig 6: Phylogeny tree of *Aedes aegypti*

Conclusion

By evidence of molecular taxonomy and phylogeny concluded DNA barcoding, we are 100% sure that present samples were Aedes species namely *Aedes albopictus* and *Aedes aegypti* whose taxonomic classification and hierarchy is as follows:

Domain- Eukaryota/Eukarya; Kingdom- Animalia; Subkingdom- Bilateria; Infra kingdom- Protostomia; Super phylum – Ecdysozoa; Phylum- Arthropoda; Sub phylum-Hexapoda; Class- Insecta; Sub class- Pterygota; Infra class-Neoptera; Superorder- Holometabola; Order- Diptera; Suborder- Nematocera, Infra order- Culicomorpha, Family-Culicidae, Subfamily- Culicinae, Tribe- Aedini, Genus-*Aedes*, Subgenus- Aedes; Species- *Aedes aegypti* (Linneaus, 1762) and *Aedes albopictus* (Skuse, 1894).

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