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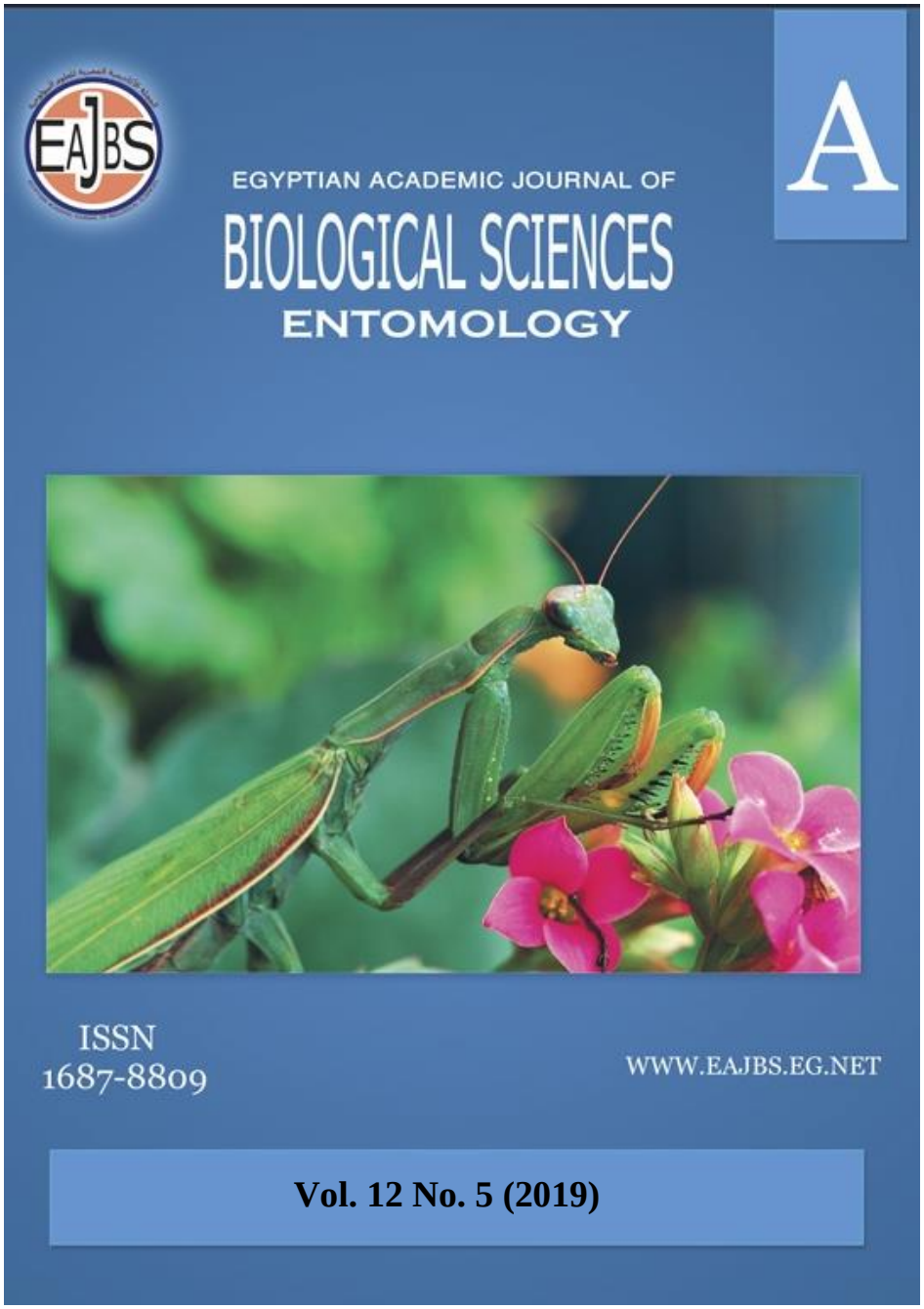

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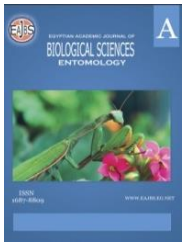

Molecular Barcoding of Green Bottle Fly, *Lucilia sericata* (Diptera: Calliphoridae) Using COI Gene Sequences In Upper Egypt.

Mohamed Elshehaby , Mahran Tony, and Abd elbaset M. A Abd elreheem
Zoology Department, Faculty of Science, Al Azhar University,Assiut, Egypt
Email: mohamedelshehaby44@yahoo.com

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ABSTRACT

The mitochondrial cytochrome oxidase I (*COI*) gene has been proposed as standard DNA barcoding marker for the identification of organisms. *COI* provides an ideal species identification marker in insects, the partial mitochondrial gene of (*COI*) for Egyptian specimens 6 area (*L. sericata*) of genus *Lucilia* were amplified and sequenced successfully from Egypt in addition to data collected from the Genbank from several representative taxa and neighboring countries. The obtained sequences were analyzed and edited using Bio Edit program and aligned using Basic Local Alignment Search Tool software (BLAST). Construction of phylogenetic trees was made using the Maximum Parsimony (MP) and Maximum Likelihood (ML). SDS-PAGE traditionally is a technique employed by biochemists to detect and characterize proteins in a mixture based on their molecular size. Protein was analyzed at 14% gel concentration for wide range detection of high and low molecular weight (MW) proteins. If analytical conditions are optimized and followed by sophisticated techniques, to detect any changes in protein patterns could be happened in six Egyptian specimens of *Lucilia sericata* according to different areas.

INTRODUCTION

Mitochondrial DNA (mtDNA) has been one of the most widely used molecular markers for phylogenetic studies in animals, because of its simple genomic structure (Avise 2004). Studying the mitochondrial DNA is very important to build the phylogenetic tree of living organisms as it is the most useful molecule to infer the phylogeography at the level of conspecific populations and closely related species (Walker and Avise1998) Among insects, the maximum number of mitochondrial genomes have been characterized in the order Diptera (Cameron *et al*. 2007; Shao and Barker 2007). Though mtDNA sequence data have proved valuable in determining phylogenetic relationships, the choice of the gene is also of great significance (Simon *et al*. 1994; Lunt *et al*. 1996). The size and structure of cytochrome oxidase subunit 1 (*COI*) gene have been well conserved in the animal groups analyzed so far, a feature that makes it especially suitable for evolutionary studies (Simon *et al*. 1994) . In recent years, comparison of mitochondrial DNA sequences has been used for population genetics and phylogenetic studies in genus *Lucilia* of medical, veterinary and economic importance (Hall *et al*. 2001; Zehner *et al*. 2004; Cummings and Krafsur 2005; Segura *et al*. 2006; Angella *et al*. 2007). The availability of an accurate postmortem interval (PMI) can influence the overall direction of an investigation and the interpretation of

entomological evidence may eventually be the deciding factor in the determination of guilt or innocence in a court of law. Identifying the insect species is an important first step in the investigation process, but morphological identification of immature stages can be difficult and sometimes impossible, due to the similarity between different species. In order to overcome the difficulties associated with the classical methods, others have been developed; one of them is the genetic identification. This method provides a rapid and accurate species determination, in any stage and even when specimens are damaged. Species from the Calliphoridae family give information relating to the accurate estimation of the PMI, as they are among the first insects to discover and colonize human remains. They are attracted to carrion and a large number of eggs are commonly placed in natural body openings and wounds that are exposed (Byrd and Castner **2001)**. This study describes DNA sequences data of the mitochondrial region of the subunit I of cytochrome oxidase (COI) gene. To date many geographical regions in Upper Egypt were studied, and SDS PAGE.

MATERIALS AND METHODS

Collection of Samples:

We started this study in May 2015. We collected Eighty samples of the adults *Lucilia sericata.* in May 2015 from six different Governorates in Egypt (Beni Suef, AL Menia, Assiut, Sohag, Qina and Aswan). And the sample put in sterile flacon tube then froze at -20°C. The collected samples are deposited in the Al-Azhar University, Department of Zoology, Assuit, Egypt

Table 1: Coordinates and altitudes for each locality of study area

No. Locality	Latitude	Longitude
Beni Suef	29.04'N	31.08'E
AL Menia	28.11'N	30.73'E
Assiut	27.10'N	31.18'E
Sohag	26.54'N	31.68'E
Qina	26.15'N	32.70'E
Aswan	24.08'N	32.88'E

DNA Extraction:

The systematic methods used to classify the living organisms depend on the morphological characters reference put that are green bottle fly species.it is 10-14 mm long and has brilliant metallic, blue-green or golden coloration with black markings .it has short, sparse black bristles (setae) and three cross-grooves on the thorax. The wings are clear with light brown veins basic sta bright yellow and the legs and antennae are black. The defining characteristic of *Lucilia sericata*, and most used when identifying the adult fly is the presence of three bristles on the dorsal mesothorax .This body region is located on middle of the back of the fly femoral of legs *Luclia sericata is blue –back. Also when looking at the occipital setae Luclia sericata* has 6-8 bristles on each side **(** Bishop1991). and recently on le of the molecular characters. This study depends on molecular characters to identify the Egyptian species of the genus *Lucilia*.

DNA Was Extracted Using the Following Method:

The application of molecular biology techniques to the analysis of complex genome depends on the ability to prepare pure, high molecular weight DNA. (Smith and Kelly 1994) .www.qiagen.com/handbooks

1. Procedure Using A Mortar And Pestle:

Grind up to 50 mg insects in liquid nitrogen using a mortar and pestle. Place the powder in 1.5 ml microcentrifuge tube. 2- Add 180 µl Buffer ATL (Animal Tissue Lyses). 3- Add 20

µl proteinase K. Mix thoroughly by vortexing, and incubate at 56°C until the insects are completely lysed. Vortex occasionally during incubation to disperse the sample, or place in a thermomixer, shaking water bath, or on a rocking platform.4- Vortex for 15 s. Add 200 µl Buffer AL (Lyses buffer) to the sample, and mix thoroughly by vortexing. Then add 200 µl ethanol (96–100%), and mix again thoroughly by vortexing. 5- Pipet the mixture from step 4 (including any precipitate) into the DNeasy Mini spin column placed in a 2 ml collection tube (provided). Centrifuge at ≥6000 x g (8000 rpm) for 1 min. 6- Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW1(Wash buffer 1), and centrifuge for 1 min at ≥6000 x g (8000 rpm). Discard flow-through and collection tube.7- Place the DNeasy Mini spin column in a new 2 ml collection tube (provided), add 500 µl Buffer AW2 (Wash buffer 2), and centrifuge for 3 min at 20,000 x g (14,000 rpm) to dry the DNeasy membrane. Discard flow-through and collection tube. 8- Place the DNeasy Mini spin column in a clean 1.5 ml or 2 ml microcentrifuge tube (not provided), and pipet 200 µl Buffer AE (Elution buffer) directly onto the DNeasy membrane. Incubate at room temperature for 1 min, and then centrifuge for 1 min at ≥6000 x g (8000 rpm) to elute. 9- Recommended: For maximum DNA yield, repeat elution once as described in step 8 and then NDNA samples were stored at -20°C until use.

2. Measurements of DNA Concentration And Purity (ng/µl):

Nanodrop Measures DNA conc. As Following:

1- Auto zero (blank) by Tris-EDTA (elution buffer).
2- One microliter of DNA sample in nanodrop machine, then result represented by graphic drawing on computer screen.3- DNA conc: measured by ng/microliter, always good conc. more than 20 ng/ µl. 4- DNA purity: calculated by ratio between 260 nm Absorbance and 280 nm Absorbance, always good purity between 1.7 and 1.9.

$$\text{DNA purity: } = \frac{\text{O.D 260}}{\text{O.D 280}}$$

Agarose Gel Electrophoresis Was Prepared Using The Following Protocol:

1- Reagents and Chemicals:

1-Agarose powder (Fisher Scientific, UK).Tris Borate EDTA buffer (TBE): for 1 liter of working 1X TBE, 10.8gm Tris base, 5.5gm boric acid (United Co., Egypt), and 0.74gm of EDTA (WINLAB, USA) were dissolved and the volume is brought to 1 liter by ddH2O.2- Ethidium Bromide (EthBr) stain: (Promega, USA) ready for use.

2- Electrophoretic Apparatus:

Minicell horizontal electrophoretic unit 8 wells (UNITEC, EC-370M, USA).

3- DNA Loading Buffer (6X) Preparation:

30% (v/v) glycerol was added to 0.25% (w/v) bromophenol blue and 0.25% (w/v) xylene cyanol FF and stored at 4°C.

4- Prestained 1% Agarose Gel Preparation:

0. 25g of agarose added to 25 ml TBE buffer into a 100 ml bottle, and dissolving in the microwave oven (Kelvinator®, USA) for about 50 seconds. Solution mixed twice during the microwaving, cooled down to ~ 50ºC, and then 1.0 µl of the Ethidium Bromide was added and mixed. The horizontal gel apparatus was sealed and the comb was inserted until its base is 2 mm from the base of the gel to make pores. Molten agarose was poured on to a gel tray while avoiding bubbles. Left in order to solidify and gel tray is then inserted into the proper position in the electrophoresis chamber, after that gel stand was filled with buffer TBE until it covers the gel completely. Comb removed and gel became ready to receive samples for electrophoresis.

5- Loading samples:

2 µl of 6X loading dye were added to 5 µl of samples (PCR product, or gDNA), Mixed well and then the mixture was injected into the gel pores (wells). The molecular weight (size) of each DNA fraction was determined by using 100 base pair DNA ladder marker (Solis BioDyne, Riia, Tartu, Estonia) from 100-3000 bp MW as a standard (3µl loaded), which is loaded and migrated the same gel with samples.

6- Running Agarose Gel:

Gel was run at a constant current 100V for ~1 hour until bromophenol blue passes ~two third the gel distance.

7 Gel Visualization:

The gel is then visualized using UV transilluminator (Upland CA.91786, M-20, USA) and was photographed using Digital Camera (Japan).

Amplification of Target Fragments:

Mitochondrial gene is sequenced throughout this study (cytochrome oxidase subunit I (*COI*) gene. Polymerase chain reaction (PCR) with specific primers situated in the flanking regions of the target fragments were performed to amplify the fragments of interest. The primers and the profiles used in this study are shown in table (2). PCR was performed in 25 µl volume of solution containing 0.1 µl of Taq polymerase, 1 µl of primer, 0.5 µl of DNTPs, 5.0 µl of 10x buffer, 3.0 µl of template DNA and the rest is deionized water until it reaches the requested volume. The PCR reaction was performed in a Standard (conventional) thermocycler with different temperature profiles. The PCR was checked on 1.5% agarose gel to test the existence of the DNA amplified and to know the size of the DNA fragments. In some cases, the PCR process was repeated and the PCR product was re-amplificated under the modified conditions.

Table 2: Primer used for amplification and sequencing of the mitochondrial gene.

Primer	Sequence
Forwad	5'-GGTCAACAAATCATAAAGATATTGG -3'
Reverse	5'TAAACTTCAGGGTGACCAAAAAATCA -3'.

The thermal profile used for gene**:-** conditions: initial denaturation at 95 ºC for 6 min; 37cycles at 94 ºC for 1 min, 36 ºC for 2 min, and 72 ºC for 3 min; final incubation at 72 ºC for 10 min; hold at 4 ºC for 24 hour(Kelly1995) .

Purification:

The PCR products were cleaned using QIAquick PCR Purification Kit Protocol; this protocol is designed to purify single- or double-stranded DNA fragments from PCR and other enzymatic reactions. For the cleanup of other enzymatic reactions, DNA Fragments purified from primer, nucleotides, polymerases, and salts using (QIAquick spin handbook 2008) columns in a microcentrifuge.www.qiagen.com/handbooks

1 Procedures:

1- 125 µl Buffer PB added to 25 µl of the PCR sample and mix.
2- Check that the color of the mixture is yellow.
3- QIAquick spin column placed in a provided 2 ml collection tube.
4- To bind DNA, the sample applied to the QIAquick column and centrifuge for 30–60s.
5- The tube containing the filtrate was discarded and the QIAquick column Placed back into the same tube. Collection tubes are re-used to reduce plastic waste.
6- 0.75 ml Buffer PE added to the QIAquick column and centrifuge for 30–60 s.
7- The tube containing the filtrate was discarded and the QIAquick column Placed back into the same tube. Centrifuge the column for an additional 1 min.
8- QIAquick column Placed in a clean 1.5 ml microcentrifuge tube.

9- 50 µl Buffer EB (10 mM Tris·HCl, pH 8.5), added to the center of the QIAquick membrane and centrifuge the column for 1 min.

Sequencing:

The PCR products were sequenced directly on automated sequencers with the primer listed in tables 3 and 4 with the primer listed in table 2.

Table 3: Cycle Sequencing Reaction Using

Component	Volume
BigDye Terminator	4 ul
5X Sequencing Buffer	4 ul
Primer (3.2 pmol)	1 ul
Template	20 ng
Nuclease Free Water	Up To 20 ul
Total	20 ul

Table 4: Thermal Profile of sequencing

Stage	Description	Temp.	Time
1	Denaturation	96	1 min
2	Amplification	96 Annealing 60	10 sec 5 sec 4 min
3	Hold	4	Pause

Bigdye Terminator V3.1 Cycle Sequencing Kit:

1 - Cycle Sequencing Purification Using Centri-Sep Kit:

1 - The upper-end cap removed and 0.8 ml of deionized water added.
2 - The upper-end cap replaced and vortex the column a few times to mix the water and gel material.
3 - The gel preserved at room temperature for at least 2 hours to hydrate.
4 - Invert the column to remove any air bubbles and allow the gel to settle.
5 - The upper-end cap removed, then the bottom cap removed, the column will drain completely by gravity.
6- The column inserted into the wash tube.
7 - The column centrifuged at 3000 rpm for 2 minutes to remove the interstitial fluid.
8- Purified PCR product loaded carefully onto the center of the gel material.
9-The column centrifuged at 3000 rpm for 2 minutes.
10 - The column discarded. The sample is in the sample collection tube.

Sequence Analysis:

Chromatograms and sequences were analyzed and edited using BioEdit program®, version 7.2.5 cited from (Thomas.Hall@abbott.com), according to the method of (Hall,1999). The obtained DNA sequences were aligned using Basic Local Alignment Search Tool software [BLAST, version 2.2.22 (http://blast.ncbi.nlm.nih.gov). cytochrome oxidase subunit I (*COI*) gene sequences reported in this study were deposited in GenBank sequence database provided by the National Center for Biotechnology Information. Multiple

alignment analysis was performed with CLUSTALW computer program (Thompson and Higgins,1994). in MEGA6 software (http://www.megasoftware.net).

Phylogenetic Construction:

The Maximum Likelihood (ML) and Maximum Parsimony (MP) trees were constructed from the distances using MEGA6 software (http://www.megasoftware.net). In order to improve the homology statements out group included by Basic Local Alignment Search Tool (nBLAST) (www.ncbi.nih.gov/BLAST/) in the NCBI database (National Center for Biotechnology Information, NIH, Bethesda, Maryland, USA (Tatusova and Madden1999). The bootstrap analysis employed 500 replicates. For the cytochrome oxidase subunit I (*COI*) gene, the K2 (Kimura 2-parameter)(Kimura,1980) + G (gamma) model of nucleotide substitution was determined as the most appropriate model according to Bayesian Information Criterion (BIC) and Akaike Information Criterion (AIC)(Nei and Kumar,2000) . Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. Genetic distances were creating in MEGA6 software (http://www.megasoftware.net).

Polyacrylamide Gel Electrophoresis:

The basic principle of protein electrophoresis is the movement of the charged protein molecules through a supporting medium towards an electrode with the opposite charge. After electrophoresis, the protein fractions were visualized by staining with COBB (Coomassie brilliant blue R-250 stain), which is a specific protein stain. The method was described by **(Laemmli1970)**.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE):

In a solution of SDS and β- mercaptoethaol. proteins dissociate into subunits (polypeptide chains) in which the diameter of the rods is thought to be constant while the long axis varies in proportion with the molecular weight (MW). The latter value can be determined by comparing the relative electrophoretic mobility of unknown proteins with the mobility of known protein markers.

RESULTS

Polymerase Chain-reacting of Cytochrome Oxidase Subunit I (COI) Gene Products:

Phylogenetic Relationships Based on Cytochrome Oxidase Subunit I (COI) Gene:

The (*COI*) molecule provides readily obtainable nucleotide sequences because of the high DNA transcript copy number in eukaryotes and ease of PCR primer design(Woese1987).

Polymerase Chain Reacting of Cytochrome Oxidase Subunit I (COI) Gene Products:

COI gene was amplified using a universal primer.The PCR products were approximately 680 bp for *COI* gene.

***COI* Sequences Data of Genus *Lucilia*:**

The data set of this study is comprised of sequences of *Lucilia.* Multiple sequences alignment was performed and trimmed from both ends, the total length of sequences after working multiple sequence alignment became ~643 bp

***COI* Sequences Gene of Collected Species:**

Figure (1) showed the obtained DNA sequences of *L. sericata* Egypt were aligned using Basic Local Alignment Search Tool software (BLAST) in GenBank is showing 99% similarity with other *L. sericata* that recorded in GenBank (Fig.2).

Phylogenetic Construction:

The analysis resulted in a topology with a mean log-likelihood values lnL= -565.088, BIC = 1216.614, AIC = 1152.280, nucleotide frequencies: f (A) = 0.345, f (C) = 0.154, f (G) = 0.154, f (T) = 0.345. The phylogenetic analysis using the Maximum likelihood method distinguishes clades comprises species of *Lucilia sericata*. In clade *Lucilia sericata* Qina and *Lucilia sericata* Aswan form a very well supported clade (high value of the bootstrap, closed related for them). While *Lucilia sericata* from each Sohag; Assiut; Al Menia and Beni Suef constitutes a high genetic similarity. The six Egyptian samples of species of genus *Lucilia* gathered in one cluster forming one clade from the calliphoridae. The Overall average of genetic distance between all six Egyptian *Lucilia sericata* samples was zero and showed very little genetic difference. In addition to mitochondrial *COI* gene sequences of *Lucilia sericata* species, *COI* sequences of the same region of *Apis mellifera* were used in the phylogenetic analysis as an outgroup is represented in figure (1).

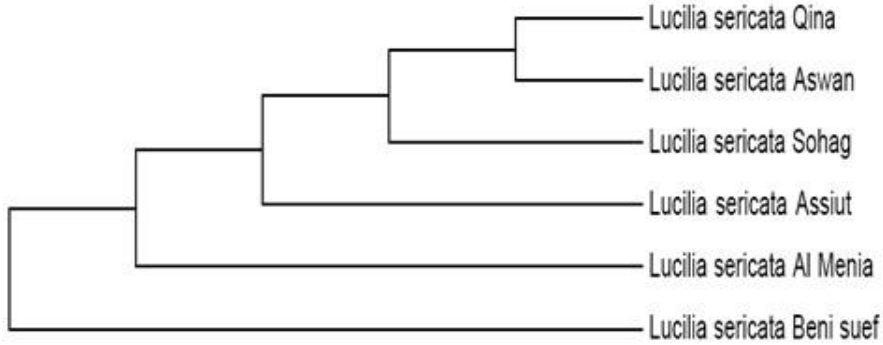

Fig.1. Rooted phylogenetic tree among Egyptian species of genus *Lucilia* based on partial sequence of *COI* gene using Maximum Likelihood method (Bootstrap value at node).

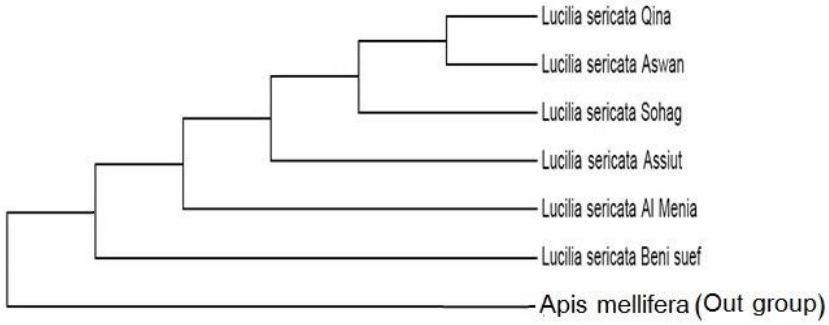

Fig..2: phylogenetic tree among Egyptian species of genus *Lucilia* and out group based on partial sequence of *COI* gene using Maximum Parsimony method (Bootstrap value at node 500 replications

***COI* Sequence Data of Local and Global *Lucilia sericata*:**

The data set of this study is comprised of 11 sequences includes sequences of *Lucilia sericata* and collected sequences of *Lucilia sericata* from Genbank. Multiple sequences alignment was performed and trimmed from both ends. Nucleotide composition: T (42.1%), C (14.5%), A (30.1%) and G (13.3%). Table (5) showed the overall average of genetic distance between *Lucilia sericata* of Egypt; China; Iran and Australia are 1.034, 0.99 and 0.99% respectively is very high range. Within *Lucilia sericata* of Egypt group, genetic distance range is 0.15 % is very low, within other sample groups.

Phylogenetic Construction:

Maximum Likelihood (ML) analysis resulted in a topology with Mean log-likelihood values lnL= -524.0561, BIC = 1205.565, AICc = 1090.630, nucleotide frequencies: f (A) = 0.36, f (C) = 0.13, f (G) = 0.13, f (T) = 0.36. The results of their study, analyses clade include specimens from the *Lucilia sericata* USA; Canada; China; Australia; South Africa; Germany; UK; Iran; Brazil; Spain and Egypt. Clade contains Genbank sequences (*COI*) of the species *Lucilia* from almost localities in its distribution. Range divided into tow inner groups: *Lucilia sericata* USA;

Canada; Germany; UK; Brazil; Spain and South Africa group represents samples and *Lucilia sericata* China; Iran and Australia group represent samples.

The results of this study indicated that sequences of *Lucilia sericata* clade, include specimens from China, Iran and Australia these populations constitute separate clade. Genbank sequences except China, Iran, and Australia of the species are closely related to those of Egypt which together form very well supported clade and is genetically high distinct and the level of genetic distance between the 0.15 %, is represented in Fig(3).

Table (5): Average genetic distances of *L. sericata* Egypt based on 680 bp fragment of *COI* with Genbank samples of *L. sericata*.

Sample	1	2	3	4	5	6	7	8	9	10	11
L. sericata USA											
L. sericata Canada	0.00										
L. sericata China	0.86	0.86									
L. sericata Australia	0.85	0.85	0.05								
L. sericata South Africa	0.00	0.00	0.86	0.85							
L. sericata Germany	0.00	0.00	0.86	0.85	0.00						
L.sericata UK	0.00	0.00	0.86	0.85	0.00	0.00					
L. sericata Iran	0.85	0.85	0.05	0.00	0.85	0.85	0.85				
L. sericata Brazil	0.00	0.00	0.86	0.85	0.00	0.00	0.00	0.85			
L. sericata Spain	0.00	0.00	0.86	0.85	0.00	0.00	0.00	0.85	0.00		
L. sericata Egypt	0.15	0.15	1.034	0.99	0.15	0.15	0.15	0.99	0.15	0.15	

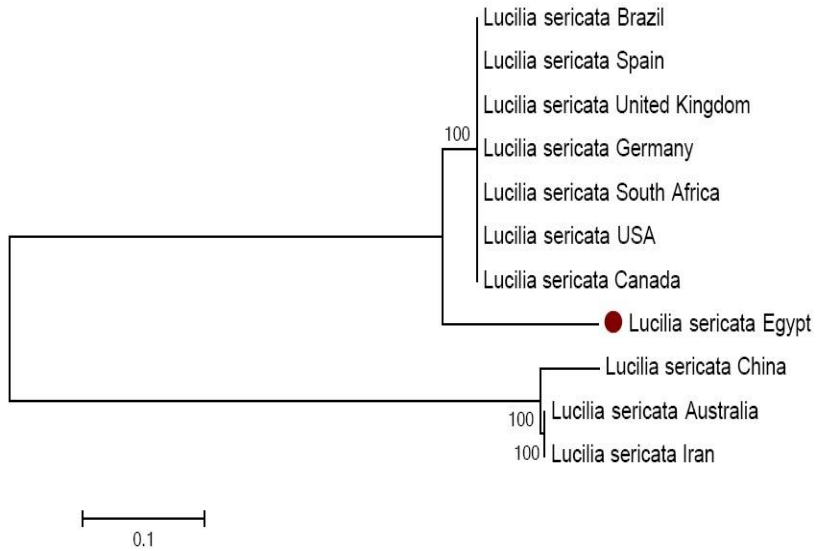

Fig (3). : Phylogenetic tree of 11 sequences of *Lucilia sericata* based on partial sequences of *COI* by Maximum Likelihood method (Bootstrap values at nodes).

SDS-PAGE .Protein Patterns of Samples Represented Six Governorates:

Table(6) and Fig(4) showed that an overall comparison of protein profiles of six governorates evidently showed the expression of 15 protein fractions ranging between 4.6-153.8 kDa.There were present all common bands except band No 1 in sample Assiut. Some bands were characteristics high concentration and some band low concentrations

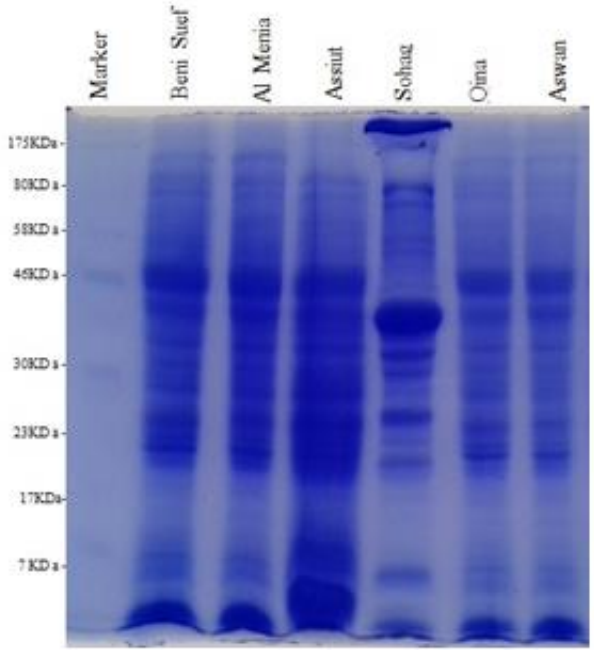

Fig.4: Polyacrylamid gel showing protein fractions stained with COBB separated at14%SDS PAGE in adults of *L.sericata* at six site represented six governorates of Egypt

Table 6 : Molecular weight and concentrations of SDS-PAGE Protein bands detected in adults of *L. sericata* at six sites represented six governorates of Egypt

No. band	Mol.Wt.	Beni Suef	Al Menia	Assiut	Sohag	Qina	Aswan
		amount	amount	amount	amount	amount	amount
1	153.8	5.8431	1.7677	-	8.2687	5.8821	5.8378
2	104.6	2.0202	2.2801	2.0923	1.7233	1.9231	3.3617
3	80	9.1256	3.5717	8.893	11.934	7.3428	15.163
4	58	2.7143	3.7388	10.616	6.6014	8.7023	3.4754
5	46	0.0318	0.3181	0.1809	9.2652	0.2072	0.26582
6	40.5	1.1424	1.3189	1.8985	31.866	2.4995	3.4244
7	34.06	3.302	2.9096	2.1766	2.5715	4.059	4.4144
8	30	5.4706	2.4222	2.8205	2.4059	6.1805	3.4523
9	27.5	12.472	2.9456	0.1157	7.368	9.7824	11.82
10	24.5	12.827	10.94	1.195	0.75906	10.799	16.647
11	23	14.953	2.4631	0.2889	6.9664	2.0579	4.7062
12	21.6	15.238	13.648	0.7062	8.0589	0.2542	0.55749
13	8.8	5.55	5.902	6.983	42.55	5.984	4.953
14	7	5.9355	5.106	3.6895	8.089	6.916	5.009
15	4.6	5.387	4.102	4.101	3.206	4.106	3.103

DISCUSSION

Phylogenetic Relationships Based On COI Gene:

Classical taxonomy relies primarily on morphological characteristics to elucidate the phylogenetic relations among organisms. Recently, the molecular approach based on comparing nucleotide sequences of RNA and DNA and sequences of amino acids of protein opens a new era in phylogenetic analysis. Both the classical morphology-based methods and molecular taxonomy are importance of all organisms are similar and morphology of an organism is actually the manifestations of its genome, proteome and transcriptome profiles. A combination of morphological based and molecular-based methods thus strengthens the exercise of the determination of phylogenetic relationships of organisms to a great extent (Patwardhan *et al.,* 2014).

In genus *Lucilia*, as in many animal groups, the molecular phylogenetic analysis offers a decisive method to distinguish species and their taxonomic relations. COI gene sequences of genus *Lucilia* are recorded on Genbank and considered universal gene for all eukaryotes. So, COI gene used for study of the phylogenetic relationship among species of genus *Lucilia* at species level, populations because higher variability of COI gene was universal for all eukaryotes. This results of this issue, based on COI data revealed that *Lucilia sericata* from Qina and Aswan are very well supported clade and high genetic similarity (high value of the bootstrap, closed related for them), while *Lucilia sericata* Sohag; Assiut;

Al Menia and Beni Suef constitute high genetic similarity between the populations of this fly. The six Egyptian samples of genus *Lucilia* gathered in one cluster forming one clade from the calliphoridae. The calculated genetic distance among the six studied samples provides molecular support to conventional phylogenetic relationships among species of genus *Lucilia.* Mitochondria play a central role in metabolism (Le and Blair, 2002).

They are the site of oxidative phosphorylation, essential for the production of ATP, as well as a variety of other biochemical functions, within these subcellular organelles is a genome, separate from the nuclear chromatin, referred to as mitochondrial DNA (mtDNA), very commonly used in studies of molecular phylogenetic (Avise, 2000).

At the same time, *COI* gene is considered a promising tool for tracing the history of more recent evolutionary events (Hillis and Dixon, 1991) and it has been widely used to study the phylogenetic relationships among different levels of taxa such as families (Alves-Gomes *et al.,* 1995), genera (Gatesy *et al.,* 1997), and species (Murphy and Collier, 1996).
The phylogenetic species concept defines a species as the smallest group of individuals that share a common ancestor, forming one branch on the tree of life (Campbell *et al.,* 2008). Using *COI* gene, provided assessment of the phylogenetic relationships between *Lucilia sericata* and the present work is in agreement with the results obtained by Harris and Arnold (2000) reported that species are considered near if have similar genetic. The phylogenetic relationship between six samples and phylogenetic results indicates to high same genetic between this species and the data revealed very few genetic differences among the species for the *COI* (Bajpai and Tewari, 2010). Also this results is in accordance with Baker *et al.,* (2006) who said that species are considered near species and closed from them if they have very little genetic difference. Similar results were found by Harris and Arnold (2000) they noticed the presence of the many similarities between the results of the molecular analyses. Microclimates and geographical variation play an important part in the diversity of this fly (Niemela, 2007 and Brundage *et al.*, 2011). No Significant differences were found in this study of fly in even this relatively small geographic area, indicating the importance of microclimates in the distribution of these flies, this indicates that there is no geographical variation between the species and all these species belongs to the same clade (Segura *et al*., 2006).

In this study, agree with Brundage *et al.*, (2011) showed that the actual diversity of calliphorids in the Santa Clara County region is higher than that demonstrated here. The present findings reaffirm a very close genetic similarity among caliphoradae as evidenced by earlier studies at molecular level (Kaul *et al*., 1981; Thakur, 1992 and Zehner *et al*., 2004). This study indicated that less of the variation within a species may be partly attributed to the high mobility of these flies. Such variability in larger geographical distributions may be attributed to diverse factors, such as age, gender, type of food, environmental conditions, genetic diversity, and geographic location (Estrada-Gomez *et al.*, 2014).

Lucilia sericata proteins are a promising source of bioactive peptides that potently and selectively modulate a wide variety of physiological targets. thus, the studies of the molecular diversity of *Lucilia sericata* proteins.

Can provide potential avenues for different discoveries (Harrison *et al*,2014).Consequently,the present study gives further insight and advance the knowledge into adult stages *Lu.cilia sericata* proteins diversity from both structural and functional of proteins viewpoints. Using SDS-PAGE, observed polypeptides bands with similar molecular masses as some of the previously reported most abundant secreted proteins from *Lucilia sericata* (Valenzuela *et al*., 2004).

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