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Molecular barcoding of green bottle fly, *Lucilia sericata* (Diptera: Calliphoridae) using COI gene sequences

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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, due to lack of introns, simple alignment, limited exposure to recombination and the availability of robust primer sites. Sequence variation in this region generally shows large interspecific, but small intraspecific divergence. *Lucilia sericata* (Diptera: Calliphoridae) is the common green bottle fly found in most areas of the world, and the most well-known of the numerous green bottle fly species. *L. sericata* has much importance in the field of forensic entomology. We have amplified and sequenced a 545 bp fragment of cytochrome oxidase subunit I (COI) gene of *L. sericata*. The consensus sequence was searched for its similarity using BLAST programme of NCBI. The phylogeny tree construction and sequence divergence study were done.

Keywords: cytochrome oxidase I, *Lucilia sericata*, phylogeny, divergence

1. Introduction
To analyze diversity among and within species, molecular systematics provides an overview of molecular methods currently used. Precise determination of closely related species, ecotypes or intraspecies variability can be done using DNA based taxonomy. This complements with traditional methods for species description and identification. Due to its simple genome structure, mitochondrial DNA is one of the most widely used molecular markers for phylogenetic studies in animals. Maximum number of mitochondrial genomes has been characterized in order Diptera among insects [1]. Relevance of barcoding in insect studies was investigated using sequence of *Diatraea saccharalis* [2]. This sequence has maximum homology (99%) with barcode sequence of *Crambidae* (Lepidoptera). Molecular barcoding of *A. cerana* demonstrates the efficiency of the barcoding gene in discriminating global phylogeographical variants among the *Apis* species complex [3].
Studies revealed that the blowflies, *Calliphora vomitoria* (Linnaeus), *C. vicina* (Rob-Desvoidy) and *Lucilia sericata* (Meigen) exhibited a limited ability to colonise pig liver baits buried in loose soil. *C. vomitoria* colonised baits buried at 5 cm but not deeper. Whereas *C. vicina* and *L. sericata* colonised remains at 10 cm but not at 20 cm. This provides the information to determine whether a body was stored above ground before being buried and/or the time since burial occurred [4].
Competitive interactions between insects and microbes were investigated using the blowfly *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) [5]. Studies showed that bacterial presence has no detrimental effect on survival of *L. sericata* from egg to adult, or on pupal size.
Arthropod studies help to gather evidence in Medico legal forensic entomology at events such as murder, suicide, rape, physical abuse and contraband trafficking. This can be helpful in determining a post mortem interval (PMI) and location of a death in question [6]. Many insects exhibit a degree of endemism, or have a well-defined phenology and hence their presence in association with other evidence can reveal potential links to time and locations where other events might have occurred. Forensic entomology is based on the analysis of those insects which successively colonize a corpse as decomposition progresses and on the rate at which the various stages of their progeny develop. To determine the time, mode and place of death, this information can be useful in criminal investigations.

The insects involved in this approach are mostly Dipterans, especially those belonging to the families Calliphoridae and Sarcophagidae. The first organisms to arrive are the blowflies (Family Calliphoridae). *C. vicina, L. sericata* or *Phormia terraenovae* which colonize the body even before the body starts any signs of bloating.

L. sericata has much more importance in the field of forensic entomology. Like most Calliphorids, the insect has been deeply studied and its life cycle and habits are well documented [7]. Due to this, the stage of the insect's development on a corpse is used to calculate a minimum period of colonization, so that it can be used as an aid in determining the time of death. Forensic entomology is an emerging field in forensic sciences and an important tool in criminal investigations. To determine time since death is easy in the early post mortem period, but in the late stages it becomes the major problem. But by studying insect evidence it is possible to determine post mortem interval in decomposed bodies. Blow flies in their different stages of development were found on fresh and decaying corpses as insect evidence [8].

In the present study, the sequencing of mitochondrial COI gene of *Lucilia sericata* has been done which can be used as its barcode for taxonomic identification. *L. sericata* is an important species to forensic entomology as it helps to calculate the post mortem index. Medically, research is ongoing centered on the secretions produced by *L. sericata* as an agent against MRSA and VRSA, and the larval applications for maggot therapy.

2. Materials and Methods

The experimental insect, *Lucilia sericata* (Diptera: Calliphoridae) is the common green bottle fly found in most areas of the world and the most well-known of the numerous green bottle fly species, were collected from Calicut University Botanical Garden (CUBG, India) during post monsoon period. The tissue from one of the thoracic legs was homogenized and the extracted genomic DNA was isolated using Genie Ultrapure Mammalian Genomic DNA Prep Kit (Genie, Bangalore).

2.1 Sequencing of genomic DNA

About 2ng of genomic DNA was PCR amplified for mitochondrial cytochrome oxidase subunit I (COI) gene using the forward primer with DNA sequence 5'-GGTCAACAAATCATAAAGATATTGG -3' and reverse primer with DNA sequence 5'-TAAACTTCAGGGTGACCAAAAAATCA -3'. The PCR products were resolved on a 2% TAE- agarose gel, for confirmation of the target gene amplification. After ascertaining the PCR amplification of the corresponding COI fragment, the remaining portion of the PCR product was column purified using Mo Bio UltraClean PCR Clean-up Kit (Mo Bio Laboratories, Inc. California). The purified PCR product was sequenced at SciGenome Labs Private Ltd., Cochin. The forward and reverse sequences obtained were trimmed for the primer sequences, assembled by using Clustal W and the consensus was taken for the analysis.

2.2 Phylogenetic analysis

The nucleotide sequence and peptide sequence were searched for its similarity using BLAST programme of NCBI [9]. The phylogenetic tree was plotted in neighbor joining method using MEGA 6 software [10].

3. Results and Discussion

The PCR amplification of the COI gene fragment of *L. sericata* yielded a single product of 545 bp. The BLAST search using sequence revealed that the sequence obtained in this study was novel and the sequence was deposited in NCBI GenBank (GenBank Accession Number: KM 096998). The results indicate that nucleotide sequence showed great similarity with sequence of same genus from Europe and American continents. The intraspecies divergence was less and interspecies divergence ranged from 0.7 to 3.8%. *L. sericata* was found to be 99.3% similar to *L. cuprina* (KJ 496771). The mitochondrial genome of closely allied species showed sequence diversity to enable their discrimination. The evolutionary divergence of *L. sericata* within the genus is given in the Table 1.

The COI gene in the mitochondrial genome has been proved to be an excellent source of information for the set of closely related families belonging to the order Diptera. Sequencing was done for COI gene for *Armigeres subalbatus*, to evaluate its relationship between the different species of mosquitoes and to generate a database for molecular barcoding [11]. The partial sequence of COI gene of *L. sericata* (GenBank Accession Number: KM 096998) is 100% identical with that of *L. sericata* isolated from USA, Spain, Belgium, Portugal and Brazil. This indicates that there is no geographical variation between the species and all these species belongs to the same clade. The COI sequence obtained in this study showed nucleotide variation of 0.7% to that of *L. cuprina* CO I gene (GenBank Accession Number: (KJ 496771), (KC 568275) and 3.4% to that of *Lucilia silvarum* (FR 719175), (JQ 801751) COI gene sequences. The N-J tree with nucleotide sequences revealed that it is closer to *L. cuprina, Hemipyrellia ligurriens, L. thatuna, L. silvarum, L. illustris* in their mitochondrial COI gene sequences. The phylogenetic tree of DNA plotted using neighbour joining method is attached as Figure 1.

Table 1: Evolutionary divergence between sequences of *Lucilia sericata* within the genus

Organism with Accession Number	% of divergence
L. sericata (KM 096998)	
L. sericata (KF 225236)	0.0%
L. sericata (KF 908125)	0.0%
L. sericata (JX 438041)	0.0%
L. sericata (KC 473902)	0.0%
L. sericata (JQ 246679)	0.0%
L. sericata (KF 225235)	0.0%
L. sericata (FJ 614824)	0.3%
L. cuprina (KJ 496771)	0.7%
L. cuprina (KC 568275)	0.7%
L. sericata (JN 257225)	0.9%
L. thatuna (DQ 453489)	1.7%
L. illustris (EU 880198)	3.1%
L. silvarum (JQ 801751)	3.4%
L. silvarum (FR 719175)	3.4%
L. illustris (JX 295709)	3.4%
L. bufonivora (KF 751384)	3.8%

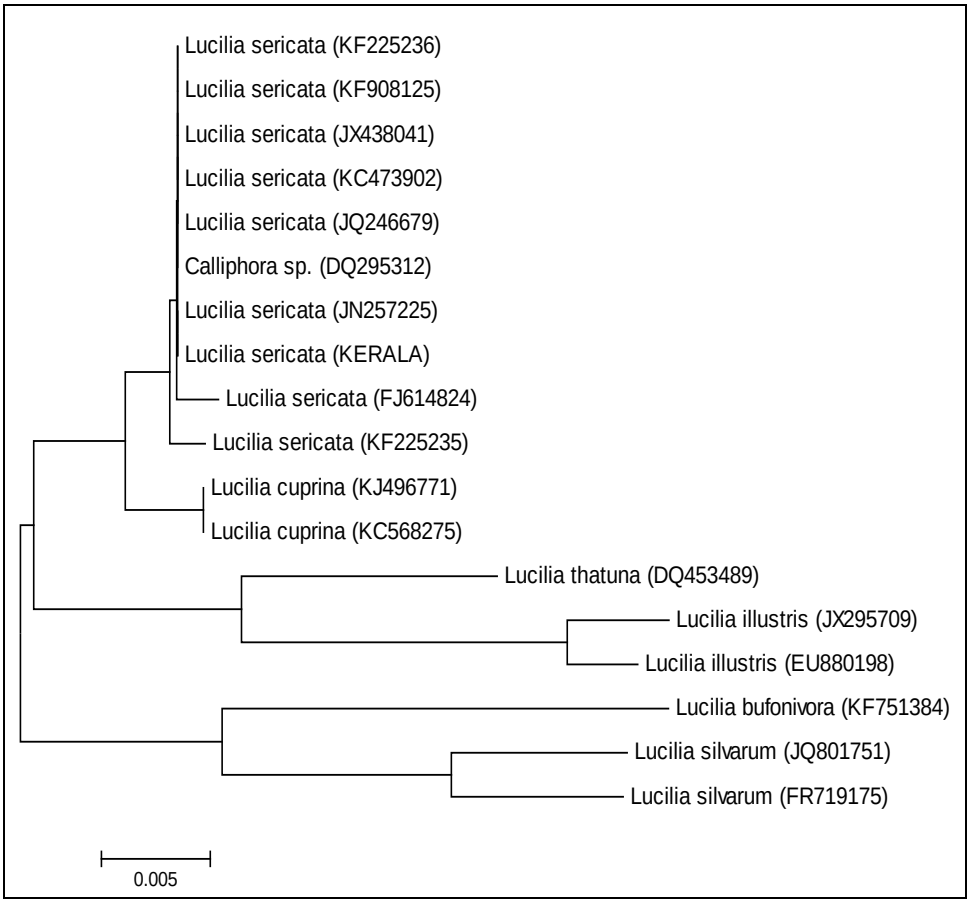

Fig 1: Phylogenetic tree of *Lucilia sericata* using neighbour joining method

4. Conclusion

DNA barcoding is an excellent source for genetic and phylogeographical variants. Variation in the nucleotide is fundamental property of all living organisms which can be used for their identification and phylogenetic status. Phylogenetically the nearest relatives of *L. sericata* is *L. cuprina*, showing 99.3% similarity and *H. ligurriens*, showing 99% similarity.

5. Acknowledgements

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