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Mitochondrial DNA studies: Revealing the first record of genus *Protophormia* from India

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Abstract

Calliphorids are forensically, ecologically and anthropologically very valuable, as they are astute forensic detectives; vectors of some human and veterinary diseases, scavenge on dead matter; responsible for myiasis in neglected and injured organisms, hold aesthetic value; used in maggot debridement therapy and are great pollinators. A new record of genus *Protophormia* from India based on *16SrRNA/NAD1/tRNA/Leu* gene fragment (earlier known from Pakistan and other regions of northern hemisphere) was found. This study clearly shows that molecular studies if used in the right direction can help in unveiling and estimating the actual biodiversity.

Keywords: mtDNA, *16SrRNA/NAD1/tRNA/Leu*, *Protophormia*, Calliphorids, Charakri

Introduction

Calliphorids are forensically very valuable insects. In present study first record of *Protophormia* from India was discovered and confirmed through molecular analysis. *Protophormia terraenovae* is the most cold tolerant calliphorid species known worldwide. It can withstand very low temperature conditions and thus are also known in the Arctic region and around the North Pole [4]. It is holarctic in distribution and is reasonably found in the whole northern hemisphere viz. North America, Texas, Florida, Pakistan, Turkey and north west Iran [2, 1]. The author thus appraises the application of molecular taxonomy in successfully ferreting out new genus and species.

Methodology:

Collection and Identification of *Protophormia*: These were collected with the help of fly sweep net from the sheep dung from Charakri, Himachal Pradesh in the month of May in the year 2012. The details of collection and the accession number obtained for this are mentioned in (Table 1). Some of the diagnostic features included: Blue coloured thorax and abdomen; approximately 10mm length, anterior spiracle brown, lower brown calyptra without hairs, both calyptras brown, mesonotum flattened on the centre, two post intra alar bristles, lateroposterior side of hind coxa had few hair, no post sutural acrostichial bristles. To further validate, this fly is common in Holarctic region (Nearctic and Palearctic), thus Indian Himalayas border Palearctic and oriental regions thus there is great possibility of this being present in India.

DNA analysis: Their identification was further confirmed through DNA analysis. After the successful extraction of DNA from these two samples, (using Qiagen Puregene Tissue DNA extraction kit, India) these were amplified with primer set 12585F/ 12866R for *16S rRNA/tRNA/Leu/ND1* gene. Then BLAST was performed for these sequences. When BLAST was performed for both the sequences, these showed 99% (sequence pt99) and 93% (sequence pt1) identity. This supports the new record. BLAST is only a preliminary test and it needs to be further validated through phylogenetic tree construction.

Phylogenetic Analysis: Phylogenetic trees were attempted among 4 calliphorid species namely *Calliphora vicina*, *Lucilia sericata*, *Lucilia cuprina* and *Lucilia porphyria* and these two query sequences for *16SrRNA* gene (12585F/ 12866R). Both UPGMA and Neighbour joining methods were applied for tree construction. The out group chosen was *Drosophila yakuba* (KF824873). The author could have chosen some other (more closer) relative to Calliphoridae as out group, but as the query sequence was doubtful, thus its identity could be most appropriately confirmed through *Drosophila* species which is a distant relative.

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As destructive method of DNA extraction was used thus these two samples could not be satisfactorily preserved as

type specimen for future appraisal.

Table 1: Details of species collection.

Species	Specimen voucher number	Location	Date of collection	Gender	Accession number
<i>Protophormia terraenovae</i> Robineau-Desvoidy	pt99	Charakri, Himachal Pradesh	23-05-2012	♀	KT375354

Results and Discussion

This fly is common in Holarctic region (Nearctic and Palearctic) and thus Indian Himalayas that border Palearctic and Oriental regions represent a great possibility of this being present in India. This has also been rightly identified through morphological traits. The further validation was done using molecular approach which showed 96-100% authenticity with both UPGMA and Neighbour Joining methods. Thus all these clearly stilt the discovery of new record of this species. However, a detailed analysis is accounted in the ensuing write up to elaborate our findings.

Phylogenetic analysis for confirmation of new record of *Protophormia terraenovae* through:

• UPGMA approach

As the name suggests, UPGMA method is unweighed cluster method which possesses a strict molecular clock constraint. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the number of differences method and are mentioned in the units of the number of base differences per sequence. The

analysis involved 11 nucleotide sequences. Bootstrapping was performed with 1000 replicates. The optimal tree finally generated had the sum of branch lengths equal to 53.82023810. There were a total of 333 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 [3]. As mentioned earlier, *Drosophila yakuba* was chosen as the out group. Posterior probabilities are the measure of percentage of replicate trees in which the associated taxa clustered together in the bootstrap test. These values are mentioned on the branches of the tree. A probability value above 70 is valid enough for 95% probability. In the present problem, the query sequence pt99 shows 96% posterior probability and is represented as a cluster or clade among the GenBank *Protophormia terraenovae* sequence (JX913743). This clearly indicates that the query sequence is that of *Protophormia terraenovae*. However, the other sequence has little to confirm for its similarity with *Protophormia terraenovae* as it gets separated out into a separate clade. Thus, the sequence pt99 can be to a good extent validated and confirmed as *Protophormia terraenovae*. (Figure 1) shows the phylogenetic tree constructed on the basis of UPGMA method.

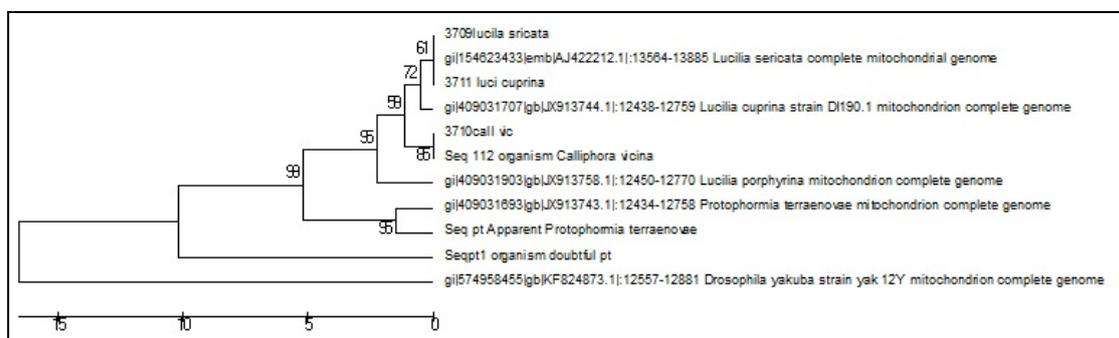


Fig 1: Evolutionary relationships of taxa inferred using the UPGMA method.

• Neighbour joining method

To further support our discussion, Neighbour joining approach was tried. The evolutionary distances were computed using the number of differences method and were in the units of the number of base differences per sequence. The analysis involved 11 nucleotide sequences. There were a total of 333 positions in the final dataset. Evolutionary

analyses were conducted in MEGA5. The optimal tree generated had the sum of branch length equal to 52.14843750 (Kumar and Nei, 1994). The out group chosen was again *Drosophila yakuba* (KF824873). This method, however, more strongly supported our apprehension about the query sequence by providing a posterior probability value of 100% and separating out as a clade (Figure 2).

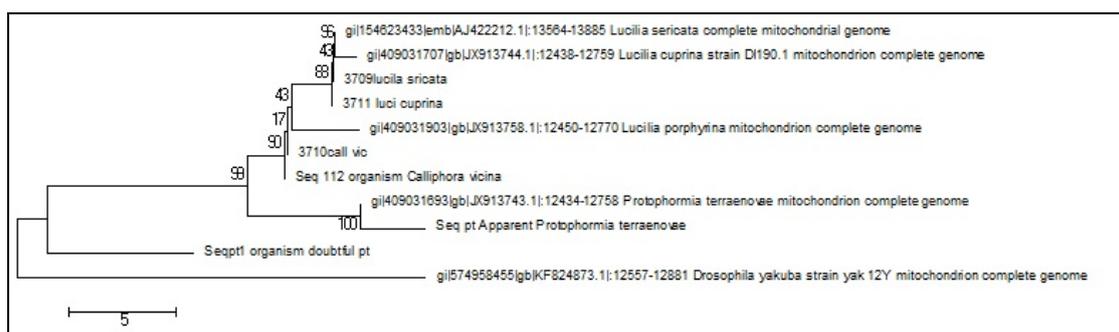


Fig 2: Evolutionary relationships of taxa inferred using the Neighbor-Joining method.

This discovery of first record of *Protophormia* in India is truly a new feather in the cap of molecular taxonomy and ascertained its hegemony as a midas tool in deciphering the colossal biodiversity in the easiest, fastest and most reliable way. DNA studies are nowadays predominant in phylogenetics. But morphological approach is still the most widely accepted approach among the taxonomists. This paper clearly describes the importance of mitochondrial DNA studies in the field of systematic. Till today, molecular taxonomy has not gained an authoritative status in taxonomy due to lack of sufficient data base. May be in the coming time this field of study also gains an equal status to morpho-taxonomy. Also, it is looked upon that non-destructive methods of DNA extraction must be used for DNA extraction so as to avoid loss of the type specimens.

Conclusion

This study clearly shows that molecular studies if used in the right direction can help in unveiling and estimating the actual biodiversity.

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Disclosure

The authors are not conversant of any memberships, financial holdings, affiliations that could raise a conflict of interest.

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