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High interspecific and intraspecific variations observed among members of family Calliphoridae

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Abstract
Phylogenetic studies were performed on family Calliphoridae to deduce interspecific and intraspecific variations. Alignment of a total of 53 sequences comprising of 10 Calliphorid species was carried out. Also for population studies, 23 sequences were picked from the Gen Bank. *Sarcophaga subvicina* was chosen as the out group which got clearly separated in the phylogenetic tree. Interspecific variation among different species showed a wide range of 1-15%. Considerably high intraspecific divergences were observed when these sequences were compared with the global Gen Bank submissions. The intraspecific divergence observed for *Chrysomya nigripes* was 4%; for *Lucilia sericata* 5%; for *Hemipyrellia ligurriens* 1.7%; for *Hemipyrellia pulchra* 1.5%; for *Chrysomya pinguis* 2% and for *Chrysomya albiceps* it was 3%. While, for other species divergence was found to be within limits. These observations were also in agreement with previous research.

Keywords: mtDNA, intraspecific, interspecific, blow flies

Introduction
Calliphorids are forensically important insects. In accordance with the updated checklist of Calliphoridae, 9 subfamilies, 30 genera and 119 species are known from India (Bharti, 2011) [1]. In the present study different populations of 10 calliphorid species were considered for phylogenetic studies. Also global Genbank sequences for the same species were included for intra specific studies.

Methodology
DNA was isolated from these insect samples. These were then subject to Polymerase Chain Reaction using specific *COI* primers. The amplicons were then sequenced, trimmed and submitted in GenBbank and accession numbers were obtained for all these sequences. Phylogenetic analysis was then performed using these sequences using MEGA5.

Observations and Results
Phylogenetic tree based on UPGMA approach using *COI* gene: The evolutionary history was inferred using the UPGMA method (Sneath and Sokal, 1973) [9]. Figure 1 represents the phylogenetic tree based on UPGMA method. Alignment of a total of 53 sequences comprising of 10 Calliphorid species was carried out. The aim was to choose conspecific specimens from different geographical localities to evaluate the extent of variations in various species and to evaluate the conservation of *COI* gene over geographical ranges. Calliphorid species were rightly allotted to the three subfamilies Chrysomyinae, Calliphorinae and Luciliina. Out of 210 variable locations 113 sites were considered parsimoniously informative. The optimal tree with the sum of branch length equal to 415.83 was observed. 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 were in the units of the number of base differences per sequence. The tree clearly represented Calliphorinae as the most ancient sub family among Lucilina and Chrysomyinae and Lucilina to be most recent subfamily that diverged lately in the geological time scale Pair wise alignment is a more reliable method than multiple alignments which is a complicated method. A single nucleotide variation is also very informative (Otranto and Stevens, 2002) [5]. In the present study, the interspecific difference between *Lucilia ampullacea* and *L. illustris* and *L. porphyrina* is 1% to10%. Similarly, *Chrysomya albiceps*, *C. pinguis* and *C. nigripes* vary between 2% and 12%. Interspecific difference between *Hemipyrellia ligurriens* and *H. pulchra* ranges from 1% to 3%. Intergeneric difference between *Hemipyrellia* and *Lucilia* was observed to be between 9 % and 10 %. While the intergeneric difference between *Calliphora* and other three genera namely *Lucilia*, *Hemipyrellia* and *Chrysomya* was found to lie between 12% and 15. Analyses were conducted using the Tamura-Nei model. There were a total of 15843 positions in the final dataset. Evolutionary analyses were conducted in MEGA5. The analysis involved 52 nucleotide sequences. Similar results were found by Wallman and Donnellan (2001) [11]. They found percentage sequence variation for three different species of *Calliphora* for COI gene to be between 1% to 11.1%. While, Sharma (2006) [7] found the percentage sequence divergence to be between 1 % to 13.7% in calliphorids. Thus the present study is in concurrent with the previous studies and explains for the reliability of this fragment of COI gene for interspecific variation studies.

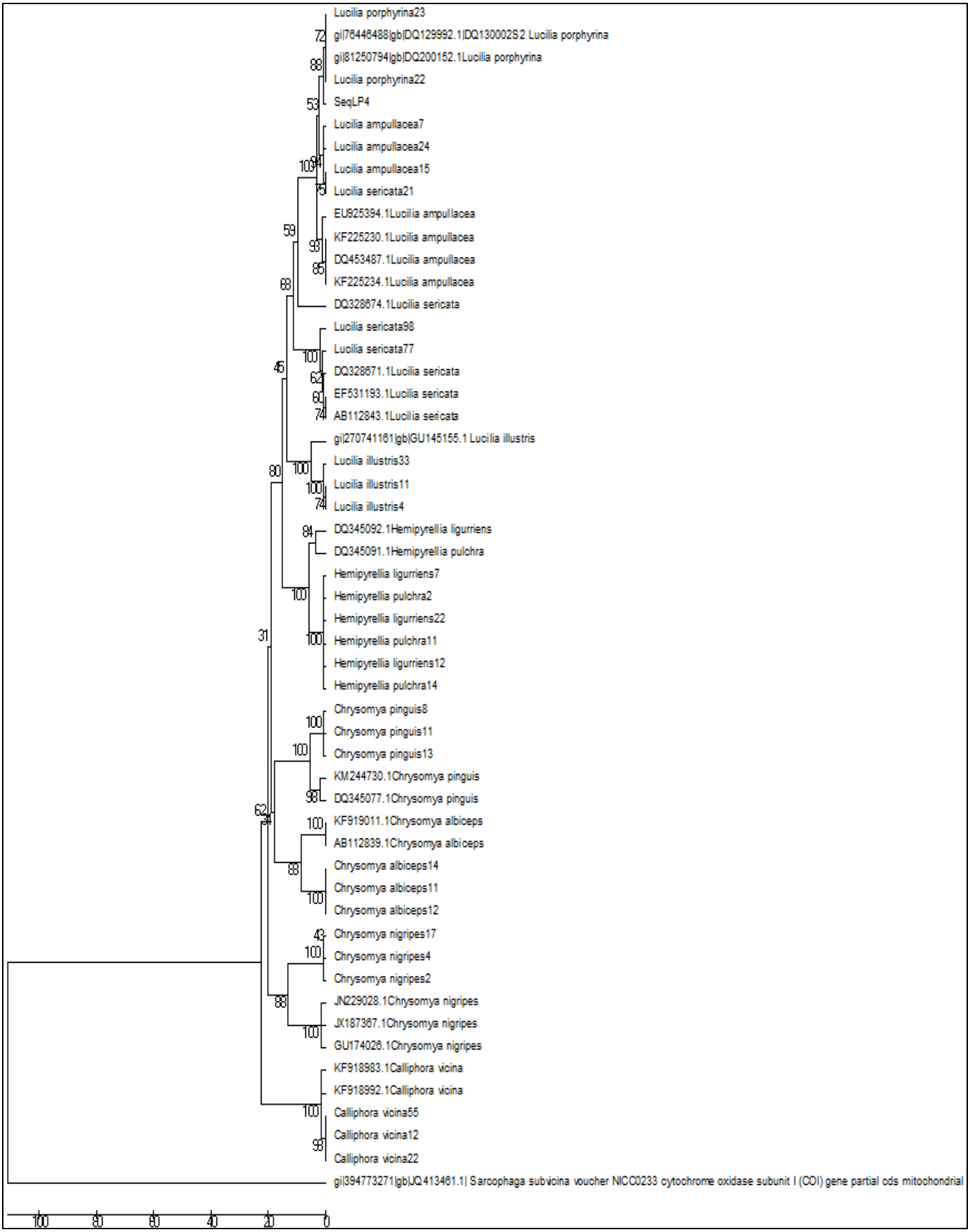

Fig 1: The evolutionary history was inferred using the UPGMA method. Evolutionary analyses were conducted in MEGA5.

Intraspecific variations

Among the various species sequenced for analysis for the present research intra specific sequence divergence was observed between the permissible limits ie. < 1% (Wells and Sperling, 1999 [13]; Wallman and Donnellan, 2001 [11]; Zehner *et al.,* 2004). This observation is in agreement with previous research. A maximum intraspecific variationof 0.4% was observed by Wagner and Wells (2000) [2] for the *Phormia regina* population in America. Similar results were seen by Harvey *et al.* (2003) [3] while analysing conspecific
populations of Calliphorid species. Similarly, Sapna (2006) observed 0.4% divergence for *Chrysomya rufifacies;* 0.6% for *Chrysomya megacephala*. In the present research, for *Lucilia ampulacea*, *Hemipyrellia ligurriens* and *Hemipyrellia pulchra* 0.1%; *Chrysomya pinguis* 0.2%; *Chrysomya albiceps*; 0.3% for *Chrysomya nigripes* 0.4%; *Lucilia sericata* 0.5%; while in case of *Calliphora vicina* hardly 0.05% and no intra specific variation was observed in case of *Lucilia illustris* and *Lucilia porphyrina*. The number of base substitutions per site from averaging over all sequence pairs within each group is shown. Analyses were conducted using the Tamura-Nei model. There were a total of 15843 positions in the final dataset. Evolutionary analyses were conducted in MEGA 5. However, considerably large intraspecific divergences were observed among the GenBank submissions from countries outside India. These results have also been reported earlier through various studies worldwide. Wallman *et al.* (2005) [12] found more than 4% intraspecific divergence between *Chrysomya rufifacies* from Australia. Sapna (2006) witnessed intraspecific divergence of 2.5% in case of one sample of *C. rufifacies;* 2% in *Lucilia illustris*. There is a wide array of GenBank submissions witnessing intraspecific variation greater than 1% viz. Saigusa *et al.* 2005 [5] reported these anomalies in *Chrysomya pinguis* samples; *Lucilia porphyrina* samples from Australia (GenBank accession numbers AY842611), Taiwan (GenBank accession numbers AY097336) showed divergence more than 7%; Hawaiian sample showed divergence of more than 6% (GenBank accession numbers AY074900). Similarly, intraspecific divergence of 1.2% for *Chrysomya nigripes* 4%; *Lucilia sericata* 5%; *Hemipyrellia ligurriens* 1.7%; *Hemipyrellia pulchra* 1.5% *Chrysomya pinguis* 2%; *Chrysomya albiceps* 3% were observed in present research. While other species reported divergence within limits. The GenBank sequences chosen for analysis that showed over estimated intraspecific divergence are:

- JN229028.1Chrysomya_nigripes
- DQ328671.1Lucilia_sericata
- DQ328674.1Lucilia_sericata
- EF531193.1Lucilia_sericata
- AB112843.1Lucilia_sericata
- DQ345092.1Hemipyrellia_ligurriens
- KM244730.1Chrysomya_pinguis
- DQ345077.1Chrysomya_pinguis
- DQ345091.1Hemipyrellia_pulchra
- KF919011.1Chrysomya_albiceps
- AB112839.1Chrysomya_albiceps
- GU174026.1Chrysomya_nigripes.

Conclusion

The reasons for these overestimations are explained by Simon *et al*. 1994 [8]; Hillis *et al*. 1996 [4]; Caterino *et al.* 2000 [2] and Harvey *et al.* 2003 [3]. They all believed that mtDNA haplotyping is based on intraspecific variations. The present findings are in agreement with previous studies.

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