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# DNA-Based Characterization of Flesh Flies (Diptera: Sarcophagidae)

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## Abstract

*The members belonging to the family Sarcophagidae includes species of medically, veterinary and forensic importance. Many studies have been carried out on the cytogenetics and biochemical genetics of these flies; however, very little work has been carried out using DNA based methods for their molecular characterization. Therefore, in the present study amplification of Cytochrome b gene was performed among five sarcophagids viz., Sarcophaga knabi, S. albiceps, S. dux, S. argyrostoma and S. ruficornis with a view to unravel the genetic relationship among these Indian flesh flies. DNA sequence was analysed using MEGA 4 software. Phylogenetic analysis was also performed which is in congruence with the results found earlier by using COI gene. The result shows the reliability of Cyt b gene as a diagnostic marker for the genetic study of these flesh flies.*

**Keywords**: Cyt b, Genetic relationship, Molecular marker, Flesh flies, *Sarcophaga.*

## Introduction

Flesh flies belonging to the family Sarcophagidae comprise 100 genera from all over the world with more than two thousand species, among which many species are being responsible for transmission of pathogenic bacteria and viruses and animal tissue myiasis[1-3]. These species are also forensically important[4-6]. However, the morphology of male genitalia, especially the development of ventral sclerotization has been the only character which offers good morphological evidence upon which grouping and phylogenetic conclusions can be drawn. A meaningful correlation between evolutionary change at morphological and molecular level could only be carried out by studying molecular data[7,8]. Although many studies have been carried out on the cytogenetics and biochemical genetics of Indian sarcophagid flies[9-12], while very little work has been carried out using DNA based methods for their genetic characterization and identification[13-17]. However, the studies using DNA based methods have been carried out in these flies from other areas of the world[18-26]. Studies with DNA based molecular markers in medically and veterinary important dipterans have revealed abundant genetic information that could be used successfully for identification of closely related species and phylogenetic relationships[27-32].

In the present study, sequencing of mitochondrial Cytochrome b (Cyt b) region have been carried out among five sarcophagids viz., *Sarcophaga knabi, S. albiceps, S. dux, S. argyrostoma and S. ruficornis* with a view to establish genetic relationship.

## Materials and Methods

For the extraction of genomic DNA, the process of Maniatis et al.[33] was followed with some modifications. The extracted DNA was precipitated by ethanol and 100 µl Tris:EDTA buffer (pH-8.0) has been used for the resuspension of extracted DNA.

**Amplification and sequencing of mitochondrial Cyt b region:** The primer pairs used for the amplification of Cyt b gene are CBJ 10933 5'TATGTTTTACCTTG AGGACAAATATC 3' and TSI-N-11683 5'AAATTCTATC TTATGTTTTCAAAAC 3'. For the amplification purpose,25 µl of reaction mixture was prepared containing genomic DNA (30ng), Taq polymerase (1.5U), each primer (10 picomole), 2 µl dNTP (2.5 mM each), 10 X buffer and the remaining volume was completed by milli Q water. The amplification profile of Cyt b region comprised of an initial denaturation of 5 min at 94°C (1 cycle), followed by 35 cycles of 1 min denaturation at 94°C, 1 min annealing at 55°C, 2 min extension at 72°C and final extension of 7 min at 72°C (1 cycle). Sequencing of amplified products was performed by Genei and sequenced genes were submitted to Gen Bank (Table-1).

**Table-1**
**Accession numbers submitted to Genbank**

| Species | Sequence | Accession numbers |
|---|---|---|
| *S. albiceps* | Cyt b, 774 | GQ390348 |
| *S. argyrostoma* | Cyt b, 774 | GQ390346 |
| *S. dux* | Cyt b, 774 | GQ390351 |
| *S. knabi* | Cyt b, 774 | GQ390347 |
| *S. ruficornis* | Cyt b, 774 | GQ390352 |

**DNA sequence data analysis:** For the purpose of alignment Clustal X software[34] was used. Different statistical data such as proportions of nucleotide, informative and polymorphic sites, transition bias and sequence divergence were calculated by Molecular Evolution Genetic Analysis 4 (MEGA 4) software[35]. From the same software Neighbor Joining (NJ) and Maximum Parsimony (MP) methods were used to resolve genetic relationships. For Bootstrap support calculation 1000 replicates was selected. Modeltest programme[36], run under the HyPhy[37] environment, in Phylemon determined that the K81uf+G[38] is best fitted to the data.

## Results and Discussion

This has been the first study to contribute a molecular database for Cyt b gene for Indian sarcophagids. Previous studies have presented the molecular data of COI and COI and II regions[13-17]. For Cyt b region, 774 bp long fragments were obtained and the alignment showed that 149 (19.25 %) sites were polymorphic and 71 (9.17%) sites were informative. Average proportions of T: C: A: G was 33: 13: 39: 15 and the value of transition bias were 0.94.

The sequence divergence among these species ranged from 3.2 % to 14.6 % with an average of 11.1 % (Table-2). Figures-1 and 2 represent the genetic relationship inferred with Neighbor Joining (NJ) and Maximum Parsimony (MP) methods. In both the methods, as an out group, *D. yakuba* species has been used. Higher than 50% bootstrap value are indicated in figures.

**Table-2**
**Sequence divergence or pairwise nucleotide distance for Cyt b gene sequence**

| | ***S. knabi*** | ***S. albiceps*** | ***S. dux*** | ***S. argyrostoma*** | ***S. ruficornis*** |
|---|---|---|---|---|---|
| *S. knabi* | - | | | | |
| *S. albiceps* | 0.032 | - | | | |
| *S. dux* | 0.101 | 0.094 | - | | |
| *S. argyrostoma* | 0.133 | 0.130 | 0.146 | - | |
| *S. ruficornis* | 0.136 | 0.134 | 0.124 | 0.084 | - |

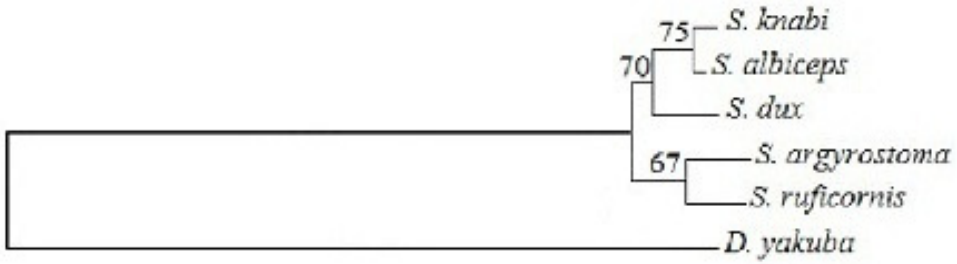


**Figure-1**
**Neighbor Joining dendogram for Cyt b region, Numbers above the branches indicate bootstrap support**

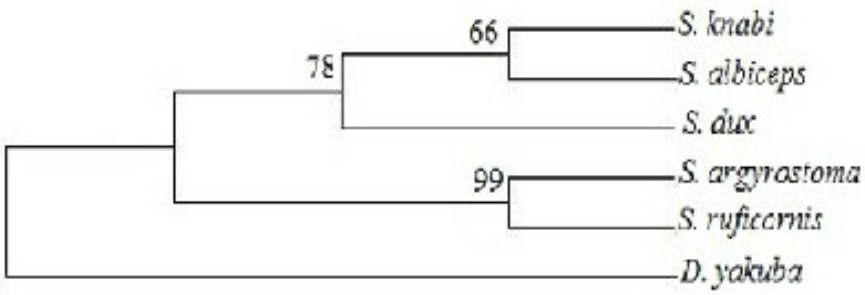


**Figure-2**
**Maximum Parsimony dendogram for Cyt b region, Numbers above the branches indicate bootstrap support**

The high frequency of transition bias in coding regions is typical of recently diverged species[39-41] supporting the close relationship among the species analyzed[42]. In coding regions the bias could be explained by selection on nonsynonymous transversions because the biochemical difference in the protein product tends to be greater for transversions[43,44]. The average proportions of T: C: A: G among the region analyzed shows AT bias. For mitochondrial regions, in many dipterans, it has been suggested that those enzymes which are needed for replication and transcription purposes shows optimum function at high AT proportion[45,46].

High sequence homology, as observed by sequence divergence values once again suggests that the species analyzed in the present study diverged very recently[21,47,48]. For genetic relationship analysis two different methods, i.e. NJ and MP, were used because single method cannot be reliable[49]. The tree produced by cyt b region showed two clusters- in one cluster *S. knabi, S. albiceps* and *S. dux* were grouped together while in another cluster *S. argyrostoma* and *S. ruficornis* were grouped. The close relationship among the species analyzed in the present study is also supported by other cytological, biochemical and molecular studies in these flies[11-14,50].

## Conclusion

The genetic relationship obtained by Cyt b sequencing data reveals close relationship among the five species which is in congruence with earlier studies. The present study, therefore, clearly indicates the importance of Cyt b region for obtaining the relationship among sarcophagid flies and the molecular data obtained from the present study constitute a database which can be used for the purpose of identification.

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