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GENETIC RELATIONSHIP OF FLESH FLIES OF THE GENUS *SARCOPHAGA* USING MITOCHONDRIAL CYTOCHROME OXIDASE SUBUNITS (SARCOPHAGIDAE: DIPTERA)

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ABSTRACT

Mitochondrial Cytochrome oxidase I and II (CO I+II) region was characterized among five sympatric species of the genus *Sarcophaga* with a view to unravel genetic relationship. Average nucleotide pair wise distance was 0.283, which indicates that these species are genetically very close. The phylogenetic tree obtained by distance and character based method groups *S. dux, S. albiceps* and *S. knabi* together in one cluster while *S. ruficornis* and *S. argyrostoma* were grouped together in another cluster.

KEY WORDS: Cytochrome oxidase subunits, flesh flies, genetic relationship, *Sarcophaga*

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INTRODUCTION

Mitochondrial genes are being extensively used as informative markers for resolving phylogenetic relationships in dipterous insects[1-3]. The presence of highly conserved and variable regions in Cytochrome Oxidase subunits enable one to use it for genetic characterization of different groups[4-6].

Only a few studies have utilized DNA based molecular markersin the subfamily Sarcophaginae from India, for genetic characterization, which comprises many synanthropic flies with considerable medical, veterinary and sanitary importance[7-10]In the present study, genetic characterization of five sympatric species of the genus *Sarcophaga* from India, namely, *Sarcophaga ruficornis*, *S. argyrostoma*, *S. dux*, *S. albiceps* and *S. knabi* has been carried out with Cytochrome oxidase subunit I and II (CO I+II) with a view to unravel genetic relationship.

MATERIALS AND METHODS

Genomic DNA was extracted from five species viz., *Sarcophaga ruficornis* (Fab.), *S. argyrostoma* (R. -D.), *S. dux* (Walker), *S. albiceps* (Meigen) and *S. knabi* (Parker) following the method of Maniatis et al[11] with minor modifications. The DNA was concentrated by ethanol precipitation and resuspended in 100 µl TE buffer (pH-8.0).

The primers used for the amplification of COI+II are S2792 5' ATACCTCGACGTTATTCAGA-3' and C2N3389 5'-TCATAAGTTCARTATCATTG-3'. The amplification reactions were performed in 25 µl reaction volume having 2.5 µl 10X buffer, 2 µl dNTP (2.5 mM each), 10 picomole of each primer, 1.5 U Taq polymerase, 30 ng of genomic DNA and the rest milli Q water. The amplification profile comprises initial denaturation at 94^{o}C for 3 min, 34 cycles of 94 ^{o}C for 1 min (denaturation), 45 ^{o}C for 1 min (annealing), 72 ^{o}C for 2 min (extension) and final extension of 7 min at 72 ^{o}C. The amplified products were sequenced at the laboratory of Bangalore Genei and the gene sequences were submitted to GenBank (Table 1).

Table 1
Species used in the present study and their GenBank accession numbers.

S. No.	Species	Sequence	Accession numbers
1	*Sarcophaga albiceps*	CO I +II	GQ390340
2	*S. argyrostoma*	CO I +II	GQ390341
3	*S. dux*	CO I +II	GQ390343
4	*S. knabi*	CO I +II	GQ390344
5	*S. ruficornis*	CO I +II	GQ390345

Sequences were aligned with Clustal X software[12]. Nucleotide ratio, variable and parsimony informative sites and nucleotide pair wise differences were calculated by MEGA 4[13]. Neighbor Joining (NJ) and Maximum Parsimony (MP) analyses were performed using MEGA 4[13]. Bootstrap support was calculated from 1000 replicates.

RESULTS AND DISCUSSION

The alignment of all the sequences together with that of *D. yakuba* shows 317 variable and 55 parsimony informative sites. However, the alignment is not straight forward due to the variation in size of amplicons. The T: C: A: G ratio was 40:13:34:13 among five species which show high AT content. In *D. yakuba* it has been suggested that the enzymes responsible for transcription and replication function optimally under high AT content[2,14]. The value of average pair wise nucleotide distance was 0.283, this low level of distance reveals close genetic similarity among them as evidenced by earlier studies at chromosomal and molecular level[7-10,15-16].

For phylogenetic tree analysis no single method can be reliable[17], therefore, two different methods were used. The phylogenetic tree obtained by distance and character based methods using *D. yakuba* as out group is represented in figs. 1-2.

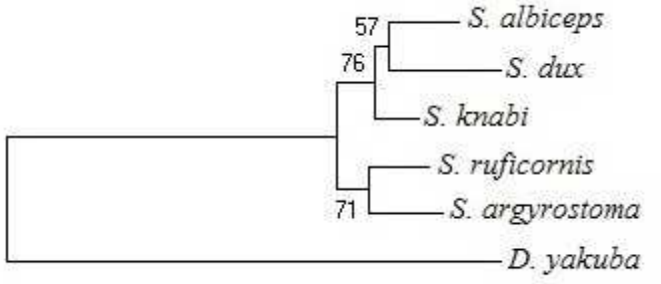

Figure.1
Neighbor joining tree obtained for COI+II region. Values above the branches indicate bootstrap support

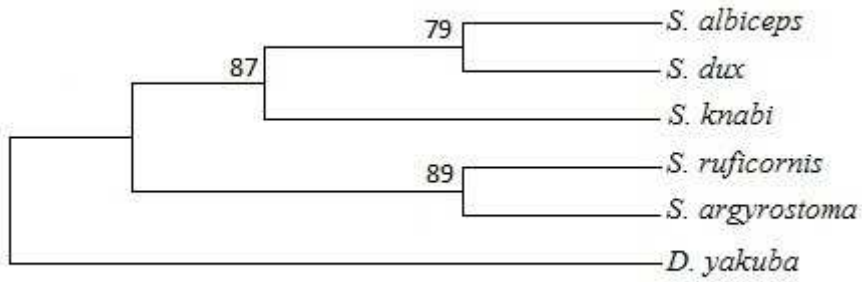

Figure.2
Maximum parsimony tree obtained for COI+II region. Values above the branches indicate bootstrap support

The phylogenetic tree, produced by two different methods, groups *S. dux, S. albiceps* and *S. knabi* together to form one cluster and *S. ruficornis* and *S. argyrostoma* are grouped together in another cluster.

CONCLUSION

The present study indicates the utility of COI+II region for the investigation of genetic relationship among sarcophagid flies, and the molecular data constitute a genetic database for their identification.

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REFERENCES

1. Angella A.F., Gil L.H.S., da Silva L.H.P. and Ribolla P.E.M., Population structure of the malaria vector *Anopheles darlingi* in Rondonia, Brazilian Amazon, based on mitochondrial DNA. Mem Ins Oswaldo Cruz, 102: 953-958, (2007).
2. Segura M.D., Callejas C., Fernandez M.P. and Ochando M.D., New contributions towards the understanding of phylogenetic relationships among economically important fruit flies (Diptera: Tephritidae). Bull Entomol Res, 96: 279-288, (2006).
3. Szalanski A.L., Owens C.B., Lewter J.A. and Broce A.B., Genetic structure of (Diptera: Culicidae) populations from Central United States based on mitochondrial ND5 sequences. Ann. Entomol Soc Am, 99: 157-163, (2006).
4. Desmyter S. and Gosselin M., CO I sequence variability between Chrysomyinae of forensic interest. Forensic Sci Int Genet, 3: 89-95 (2009).
5. Hemmerter S., Slapeta J. and Beebe N.W., Resolving genetic diversity in Australasian *Culex* mosquitoes: incongruence between the mitochondrial cytochrome c oxidase I and nuclear acetylcholine esterase 2.Mol Phylogenet Evol, 50: 317-325, (2009).
6. Kutty S.N., Pape T., Pont A., Weigmann B.M. and Meier R., The muscoidea (Diptera: Calyptratae) are paraphyletic: Evidence from four mitochondrial and four nuclear genes. Mol Phylogenet Evol, 49: 639-652, (2008).
7. Bajpai, N., Tewari, R. R. and Thakur, S., Genetic characterization of three *Sarcophaga* species with Allozymes and RAPD-PCR markers (Sarcophagidae: Diptera). National Academy Science Letter, 34: 69-73, (2011).
8. Bajpai N. and Tewari R. R., Mitochondrial DNA sequence based phylogenetic relationship among flesh flies of the genus *Sarcophaga* (Sarcophagidae: Diptera). Journal of Genetics, 89: 51-54, (2010).
9. Bajpai N. and Tewari R. R. Molecular characterization of the genus *Sarcophaga* using PCR-RFLP markers. National Academy Science Letters 33: 47-51, (2010 a).
10. Bajpai N. and Tewari R. R., Genetic characterization of sarcophagid flies by Random Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR). National Academy Science Letters 33: 103-106, (2010 b).
11. Maniatis T., Fritsch E.F. and Sambrook J., Molecular Cloning: a laboratory manual, Cold Spring Harbor Laboratory, New York, (1982).

12. Thompson J.D., Gibson T.J., Plewniak F., Jeanmougin F. and Higgins D.G., The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl Acids Res, 24: 4876-4882, (1997).
13. Tamura K., Dudley J., Nei M. and Kumar S., MEGA 4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol Biol Evol, 24: 1596-1599, (2007).
14. Wolstenholme D.R. and Clary D.O., Sequence evolution of *Drosophila* mitochondrial DNA. Genetics, 109: 725-744, (1985).
15. Kaul D., Tewari R.R. and Gaur P., The chromosomes of sarcophagid flies. La Kromosomo II, 24: 697-706, (1981).
16. Thakur S., Allozyme variation in flesh flies.D.Phil dissertation, University of Allahabad, India, (1992).
17. D. L. Swofford, G. J. Olsen, P. J. Waddell and D. M. Hillis. Phylogenetic inference. In: D. M. Hillis, and C. Moritz (eds.), *Molecular Systematics*, Sunderland, Massachusetts, Sinauer Associates, 1996, pp. 407-514.