Research Journal of Recent Sciences _____E-ISSN 2277-2502
Vol. **5(12),** 17-20, December **(2016)** *Res. J. Recent Sci.*

Review Paper

Mitochondrial DNA based studies in Sarcophagid flies from India

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Available online at: www.isca.in, www.isca.me
Received 10th October 2016, revised 20th November 2016, accepted 30th November 2016

Abstract

Now a days DNA based characterization has been extensively used as a tool in forensic studies because it accurately determines the post mortem interval. Initially the dead bodies are attracted by the flies belonging to the family Calliphoridae and Sarcophagidae. The flies of the Sarcophagidae family shows morphological similarity. In forensic studies, however, the exact identification of the fly is most significant step. Therefore, it is worthwhile to characterize the mitochondrial or nuclear region to resolve the problem related with similarity. In this review article an attempt has been made to summarize the mitochondrial DNA based studies pertaining to identification and phylogenetic relationship from India.

Keywords: *Sarcophaga*, COI, Cyt b, forensic, sequencing.

Introduction

In forensic entomology the PMI (Post Mortem Interval) is most crucial information that is needed in death investigation. For PMI identification in fresh bodies different body changes such as lowering of body temperature and stiffness are used while in putrefied bodies, such changes cannot be identified[1]. In these putrefied dead bodies different arthropods, especially flies of dipteran order are attracted among which flies of the family Sarcophagidae and Calliphoridae are common[2]. Since these flies represent the important insect order used for forensic entomology purpose.

Flies belonging to the Sarcophagidae family are widely distributed all over the world. Since the larvae of these species feeds on flesh or decaying organic matter of animal i.e. carrion or animal faeces. Therefore, many sarcophagid flies are important as they carry disease causing pathogen in human[3] and many species are important as forensic point of view for estimation of PMI[4].

However, the identification of the fly collected from carrion at crime spot is the most relevant step in forensic study as misidentification leads to ambiguous data[5]. Conventional study involves the identification based on morphology which is difficult because these flies are characterized by very similar appearance, the scutum with three black longitudinal stripes and the abdomen with black and white checkered pattern, the male genitalic character is the distinguishing feature among different species, however, there is a lack of agreement in taxonomic characters by systematists and their perception of what constitutes taxonomic categories[6]. Thus, delineation of the genera in the family Sarcophagidae has been a major problem. While proposing classification systematists have used a great variety of characters, external and genitalic, but no two authors have agreed completely on supergeneric, generic and subgeneric divisions. However, the differential evolutionary rates and subjective treatment of taxonomic categories by systematists are likely to contribute to the incongruities between different groups. To resolve the problem related with morphological similarity it has been suggested that molecular methods involving, mitochondrial and nuclear DNA regions have been characterized for the purpose of identification[7-12]. In India, however, very few workers were performing molecular based study in sarcophagid flies[13-19]. In this review paper, a summary of Indian work has been discussed pertaining to the molecular study involving mitochondrial gene for identification as well as phylogenetic relationship.

Phylogenetic studies in sarcophagid flies

The first mitochondrial DNA based analysis among sarcophagid flies from India has been performed by Bajpai and Tewari[13] by studying partial COI and ND5 mitochondrial regions. The COI region was 296 bp long while the ND5 region was 386 bp long. The genetic divergence value ranges from 0.03 to 0.10 for COI region and 0.04 to 0.20 for ND5 region. In COI region 71 variable sites were present while ND 5 region have 55 variable sites. The output of the study reveals that these species are genetically very similar. Further, partial mitochondrial CO I and II region was characterized in five sarcophagid species by Bajpai and Tewari[14] with a view to unravel genetic closeness among the species analysed. This region was 617bp long with 317 variable sites and the average of the genetic divergence value is 0.283. The result defines that these species are genetically very close. Sharma *et. al*[15] characterized mitochondrial COI region of three sarcophagid flies. The analysed region was 450 bp long having 50 variable sites. The

result shows that there exists very little interspecific variation ranging from 0.06 to 0.1. Sharma *et al*[16] studied COI mitochondrial DNA region among ten Indian flesh fly species which was 450 bp long with 161 variable sites. The genetic divergence values were found to be from 0.06 to 0.21. Bajpai[17] used Cyt b region for the phylogenetic analysis among five species of flesh flies. The region was 774 bp long among which 149 variable sites were present. The pairwise genetic distance value ranges from 0.03 to 0.14. The results grouped the species in two clusters: in one cluster *S. ruficornis* and *S. argyrostoma* were present while the other cluster consists of *S. dux*, *S. albiceps* and *S. knabi*. However, the genetic identity value reveals very less difference among these species.

Forensic studies in sarcophagid flies

Sharma *et al*[18] studied COI region among ten sarcophagid flies. The genetic divergence among the species analysed ranges from 0.04 to 0.14. Sharma *et al*[19] sequenced 465 bp long COI region of two flesh flies in which 99 variable sites were present where intraspecific and interspecific nucleotide pairwise distance has been calculated. The genetic divergence value ranges from 0.03 to 0.14. The result shows that there exists no significant intraspecific variation. Different statistical data obtained among sarcophagid flies are presented in Table-1.

The T:C:A:G nucleotide proportions of the analysed sarcophagid flies are AT rich. Because of high AT content the replication process function properly[20]. It seems possible that there may be some kind of selection favouring AT nucleotides because all the sarcophagids are interestingly endowed with regions of AT rich DNA as evidenced by bright fluorescence with AT base specific fluorochromes[21,22].

The genetic divergence value indicates sequence homologies which explain the closeness of the species[23-26]. A perusal of the genetic divergence values from the table indicates high sequence homology which establishes the fact that these species have diverged very recently and these species are very closely related.

Mitochondrial DNA has been preferably selected for molecular identification or for phylogenetic studies purpose at different taxonomic levels because of its maternal inheritance, higher evolutionary rate; more copies number and absence of inter molecular recombination[27].

Most of the studies are based on the mitochondrial DNA encoding COI region which showed that this region has the potential to be used as a molecular marker in different species of the genus *Sarcophaga*. Mitochondrial COI region has been considered to be ideal region as it bears variable as well as conserved sites for characterization, identification and forensic studies purpose[28,29]. However, it was suggested that if analysis has been performed by combination of different mitochondrial regions then we will get more accurate results as compared to single mitochondrial DNA fragment analysis[26].

The result of the present review clearly shows that the molecular approach is the direct approach which replaces the complex morphological based identification method. In summary, the mitochondrial gene fragments for different *Sarcophaga* species are now available that can be used as tool for identification purpose in case of forensic application from India.

Table-1
The regions analyzed, size, variable and informative sites, nucleotide composition and genetic divergence values

Region / locus analysed	Size in base pair (bp)	Variable sites	Informative sites	T: C: A: G ratio	Genetic divergence	References
CO I	296	71	26	40:15:31:14	0.03 to 0.10	Bajpai and Tewari[13]
ND5	386	55	26	47: 8: 31: 14	0.04 to 0.20	Bajpai and Tewari [13]
COI and II	617	317	55	40:13:34:13	0.283 (average)	Bajpai and Tewari [14]
COI	450	50	-	42:13:30:15	0.06 to 0.1	Sharma *et al*[15]
COI	450	161	65	41:16:30:13	0.06 to 0.21	Sharma *et al*[16]
COI	-	-	-	-	0.4-0.14	Sharma *et al* [18]
COI	465	99	65	-	0.15	Sharma *et al* [19]
Cyt b	774	149	71	33: 13: 39: 15	0.03 to 0.14	Bajpai [17]

Conclusion

Identification of sarcophagid flies by methods involving mitochondrial DNA has many advantages such as it is simple and fast. It is clear from the aforesaid discussion that only few studies are available involving sequencing of mitochondrial DNA. It is imperative, therefore, to focus in the field of molecular studies in these forensically important flies in future for unravelling identification of species.

References

1. Saukko P.J. and Knight B. (2004). Knights Forensic Pathology. Arnold, London, UK, 2004.

2. Kim Y.H., Shin S.E., Ham C.S., Kim S.Y., Ko K.S., Jo T.H. et al. (2014). Molecular identification of necrophagous Muscidae and Sarcophagidae fly species collected in Korea by mitochondrial cytochrome c oxidase subunit I nucleotide sequence. *The Scientific World Journal*, http://dx.doi.org/10.1155/2014/275085.

3. Byrd J.H. and Castner J.L. (2009). Forensic entomology: the utility of arthropods in legal investigations. CRC press, Boca Raton, Florida, USA.

4. Greenberg B. (1971). Flies and Disease: ecology, classification and biotic association. Volume1, Princeton University Press, Princeton, New jersey, USA.

5. Harvey M.L., Mansel M.W.L and Villet M.H. (2003). Molecular identification of some forensically important blow flies of Southern Africa and Australia. *Med. Vet. Entomol.,* 17, 363-369.

6. Aspoas B.R. (1991). Comparative micromorphology of third instar larvae and the breeding biology of some Afrotropical *Sarcophaga* (Diptera: Sarcophagidae). *Med. Vet. Entomol.,* 5, 437-445.

7. Zehner R., Amendt J., Schutt S., Sauer J., Krettek R. and Povolny D. (2004). Genetic identification of forensically important flesh flies (Diptera: Sarcophagidae). *Int. J. Legal Med.,* 118, 245-247.

8. Wells J.D., Pape T. and Sperling F.A.H. (2001). DNA based identification and molecular systematics of forensically important Sarcophagidae (Diptera). *J. Forensic Sci.,* 46, 1098-1102.

9. Meiklejohn K.A., Wallman J.F. and Dowton M. (2011). DNA-based identification of forensically important Australian Sarcophagidae (Diptera). *Int. J. Legal Med.*, DOI 10.1007/s00414-009-0395-y.

10. Tan S.H., Rizman-Idid M., Mohd-Aris E., Kurahashi H. and Mohamed Z. (2010). DNA-based characterization and classification of forensically important flesh flies (Diptera: Sarcophagidae) in Malaysia. *Forensic Sci. Int.,* 199, 43-49.

11. Guo Y.D., Cai J.F., Xiong F. and Wang H.J. et al. (2012). The utility of mitochondrial DNA fragments for genetic identification of forensically important sarcophagid flies (Diptera: Sarcophagidae) in China.*Trop. Biomed.,* 29, 51-60.

12. Napoleao K.S., Mello-Patiu C.A., Oliveira-Costa J., Takiya D.M., Silva R. and Moura-Neto R.S. (2015). DNA-based identification of forensically important species of Sarcophagidae(Insecta: Diptera) from Rio de Janeiro.*Brazil.Gen. Mol. Res.,* 15, 1-7.

13. Bajpai N. and Tewari R.R. (2010). Mitochondrial DNA sequence based phylogenetic relationship among flesh flies of the genus *Sarcophaga* (Sarcophagidae: Diptera). *J.Genet.,* 89, 51-54.

14. Bajpai N. and Tewari R.R. (2012). Genetic relationship of flesh flies of the genus Sarcophaga using mitochondrial cytochrome oxidase subunits (sarcophagidae: diptera). *Int. J. Pharm. Bio. Sci.,* 3, (B) 521 – 525.

15. Sharma M., Singh D. and Sharma A.K. (2014). Identification of three forensically important Indian species of flesh flies (Diptera: Sarcophagidae) based on cytochrome oxidase I gene. *Indian J. Forensic Med. and Toxicol.,* 2, 814-818.

16. Sharma M., Singh D. and Sharma A.K. (2015). Molecular identification of forensically important Indian species of flesh flies (Diptera:Sarcophagidae) by using COI gene of mitochondrial DNA. *J. Forensic Res.,* 6: http://dx.doi.org/10.4172/2157-7145.1000316

17. Bajpai N. (2016). DNA-based characterization of flesh flies (Diptera: Sarcophagidae). *Int. Res. J. Biol. Sci.,* 5,35-39.

18. Sharma M., Singh D. and Sharma A.K. (2015). Mitochondrial DNA based identification of forensically important Indian flesh flies (Diptera: Sarcophagidae). *Forensic Sci. Int.,* 247, 1-6.

19. Sharma M., Singh D. and Sharma A.K. (2015). Molecular identification of two forensically important Indian flesh flies (Diptera:Sarcophagidae). *Int. J. Advanced Res. in Sci., Eng. and Tech.,* 2, 814-818.

20. Segura M.D., Callejas C., Fernandez M.P. and Ochando M.D. (2006). New contributions towards the understanding of phylogenetic relationships among economically important fruit flies (Diptera: Tephritidae). *Bull. Entomol. Res.,* 96, 279-288.

21. Agrawal U.R., Bajpai N., Tewari R.R. and Kurahashi H. (2010). Cytogenetics of flesh-flies of the genus *Boettcherisca* (Sarcophagidae : Diptera). *Cytologia*, 75, 149-155.

22. Parise-Maltempi P.P. and Avancini R.M.P. (2000). Cytogenetics of the neotropical flesh fly *Pattonella intermutans* (Diptera : Sarcophagidae). *Genet. Mol. Biol.*, 23, 563-567.

23. Song Z.K., Wang X.Z. and Liang G.Q. (2008). Molecular evolution and phylogenetic utility of the Internal

Transcribed Spacer 2 (ITS 2) in Calyptratae (Diptera: Brachycera). *J. Mol. Evol.,* 67, 448-464.

24. Song Z.K., Wang X.Z. and Liang G.Q. (2008). Phylogenetic relationships among 15 sarcophagid fly species (Diptera: Sarcophagidae) based on partial sequences of mitochondrial cytochrome b and cytochrome oxidase subunit I genes. *Acta Entomologica Sinica,* 51, 298-306.

25. Hall M.J.R., Adams Z.J.O., Wyatt N.P., Testa J.M., Edge W., Nikolausz M., Farkas R. and Ready P.D. (2009). Morphological and mitochondrial DNA characters for identification and phylogenetic analysis of the myiasis-causing flesh fly *Wohlfahrtia magnifica* and its relatives, with a description of *Wohlfahrtia monegrosensis* sp. N. Wyatt & Hall. *Med. Vet. Entomol.,* 23, 59-71.

26. Wallman J.F. and Donnellan S.C. (2001). The utility of mitochondrial DNA sequences for the identification of forensically important blow flies (Diptera: Calliphoridae) in Southeastern Australia. *Forensic Sci. Int.*, 120, 60-67.

27. Machida R.J., Miya M.U., Nishida M. and Nishida S. (2004). Large scale gene rearrangements in the mitochondrial genomes of two calanoid copepods *Eucalanus bungii* and *Neocalanus cristatus* (Crustacea), with notes on new versatile primers fot the srRNA and COI genes. *Gene*, 332, 71-78.

28. Lunt D.H., Zhang D.X., Szymura J.M. and Hewitt G.M. (1996). The insect cytochrome oxidase I gene: evolutionary patterns and conserved primers for phylogenetic studies. *Insect Mol. Biol.,* 5, 153-165.

29. Stahls G. and Nyblom K. (2000). Phylogenetic analysis of the genus *Cheilosia* (Diptera, Syrphidae) using mitochondrial CO I sequence data. *Mol. Phylogenet. Evol.,* 15, 235-241.