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REVIEW ARTICLE

MITOCHONDRIAL COI GENE BASED IDENTIFICATION OF INDIAN SARCOPHAGID FLIES (DIPTERA: SARCOPHAGIDAE): A REVIEW

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ABSTRACT

Accurate identification of various species found in and around the corpse is very crucial for the determination of post mortem interval (PMI). In family Sarcophgidae, larvae and adults are very similar in external morphology, so accurate species identification is very difficult. To overcome this difficulty, cytochrome oxidase I (COI) gene of mitochondria is very good choice for species identification. In India, very few workers are doing research in this very promising field. In the present review paper, we are presenting the research of all the workers involved in the species identification of Indian flesh flies.

INTRODUCTION

HISTORICAL BACKGROUND

Forensic entomology is a science, which uses the knowledge of insects for the determination of to civil proceedings and criminal trials (Turchetto and Vanin, 2004). The first documented case of forensic entomology took place in 13th century China. In the book, "Hsi Yuan Chi Lu" (one possible translation is "the washing away of wrong") (McKnight, 1981), Chinese criminalist Sung Tz'u reported a case in which insects were used to identify a murderer. A murder was committed by slashing, and all villagers were ordered to bring their sickles to a single location.

The sickles were laid on the ground, and flies were attracted to a single sickle, presumably responding to traces of tissue and blood. On the basis, the owner of the sickle broke down and confessed. The applications of forensic entomology are numerous, encompassing any situation that may involve an interaction between insects and other arthropods, and the law. Therefore, the utility of the field is categorized under three separate headings: urban, stored product and medico legal forensic entomology [Hall, 1990; Harvey, 2006].

Urban forensic entomology generally deals with the interaction of insects with man-made structures and other aspects of human society and may include the infestation of buildings by termites, cockroaches etc. [Hall, 1990], and the breeding of flies in livestock and similar facilities [Hall, 2001].

The stored product aspect of forensic entomology involves the infestation of stored commodities by insects. Infestations may include the harvesting and storage of crops and subsequent invasion by an insect pest and domestic invasion of kitchen products. This aspect also encompasses the infestation of food sold by retailers to the public, which may result in prosecution and substantial fines [Hall, 2001].

Sarcophagidae is thought to have originated in the early Cretaceous (Pape *et al.*, 2006), however fossil evidence is scarce and little work has been done to estimate dates of divergence (Stevens *et al.*, 2006). Approximately 2600 Sarcophagidae species are currently recognized (Pape *et al.*, 2006), with molecular evidence supporting monophyly of the group (Wells *et al.*, 2001). Sarcophagidae are typically ovolarviparous (eggs hatching and larvae developing within the female), and exhibit a wide range of lifestyles, including saprophagy, inhabiting plants, coprophagy (feeding on faeces), inquilinism (living commensally in the nest or burrow of

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another organism) and parasitoidism (Pape *et al.*, 2006). In family Sarcophgidae, larvae and adults are very similar in external morphology, so accurate species identification is very difficult. Furthermore if the samples found in and around the corpse are broken than also it's very difficult to identify that particular sample. To solve this problem, COI gene of mitochondrial DNA is very much helpful in species identification, In India, this field is in its infancy stag still now after 29 years of its starting since 1987 (Kulshreshtha and Chandra, 1987). Nandi (2002) has done tremendous amount of work in the morphological identification of Indian species of family Sarcophagidae. But there are few workers in the field of molecular identification of various species under this family. In the present paper, we are presenting a brief review of all the Indian workers who are actively engaged in species identification of India sarcophagid flies using mitochondrial COI gene.

Molecular Identification of Sarcophagid Flies In India

Forensic entomology is in its early stage in India because very few workers are actively engaged in this field. Bajpai and Tewari (2010) studied the phylogenetic relationship between five species (*Sarcophaga ruficornis*, *Sarcophaga albiceps*, *Sarcophaga argyrostoma*, *Sarcophaga dux* and *Sarcophaga knabi*) of Sarcophagidae on the basis of COI and ND5. The COI gene revealed 71 variable sites in 296 bp long sequences and 26 sites were fund to be parsimony informative. The average nucleotide composition across all the species was T=40%, A=31%, C=15% and G=14%. The ts:tv ratio was 0.63. The ND5 amplicon which is 386 bp long shows 55 variables and 26 parsimony informative sites. Average composition of bases was T=47%, A=31%, C=8% and G=14%, with ts:tv ratio of 1.44 across the five species. The pairwise genetic distance between five species ranged from 0.037-0.106 and 0.049-0.207 for COI and ND5 genes respectively. So, they showed that analysis based on mitochondrial genes can be useful for unraveling phylogenetic relationships in the Sarcophagidae.

Bajpai and Tewari (2012) characterized five sympatric species of the genus *Sarcophaga* on the basis of Mitochondrial COI and II region 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.

Singh *et al.* (2014) sequenced 450 bp region of COI gene and phylogenetically analyzed three species of Sacophagidae. They showed that COI gene revealed 400 conserved, 50 variable and parsimony informative sites in the 450 bp long fragment. The average nucleotide composition across species was T=41.65%, A=29.56%, C=13.33% and G=15.48%. The ts:tv ratio was 1.24. The levels of interspecific variation varied from 6% to 10%. However, no significant intraspecific variation was observed within each of these three sarcophagid species. The genetic difference between *Musca autumnalis* and *P. misera* and *P. sericea* was found out to be 16% and for *P. albiceps*, it was 15%.

Sharma *et al.* (2015a) collected ten species of Sarcophagidae from four north Indian states and sequenced 465 bp fragment of COI gene. All the samples studied showed 0.0% intraspecific divergence. Interspecific genetic divergence was in the range of 4–14%. It is clear from the data that *P. misera* showed least genetic divergence of 4.0% with *P. sericea* and highest with *H. kempi* and *B. peregrina* (10.0%). *P. sericea* showed minimum percent genetic divergence with P. albiceps (5.0%) and maximum with *B. peregrina* and *H. kempi* (11.0%). *P. hirtipes* showed least genetic divergence with *P. ruficornis* (9.0%) and maximum with *P. macroauriculata* (14.0%). All the sequences of P. *albiceps* showed least genetic divergence with *P. sericea* (5.0%) and highest with *S. princeps* and *H. kempi* (11.0%). *P. macroauriculata* showed least genetic divergence with *P. ruficornis* (7.0%) and highest with *S. princeps*, *H. kempi* and *P. hirtipes* (13.0%); *S. princeps* showed maximum genetic divergence with *P. hirtipes* (14.0%) and minimum with *P. misera*, *P. ruficornis*, *B. peregrina* and *H. kempi* (10.0%). Sharma *et al.* (2015b) again sequenced COI gene from two forensically important flesh flies and demostrated that *S. prineps* showed interspecific variation of 15% with samples of *P. hirtipes*. *S. princeps* showed genetic divergence of 2% with two Malaysian samples (EF405949 and EF405948) and 3% with two Chinese samples (KM279655 and KM279654). 2% genetic divergence was seen between samples of Malysian (EF405949 and EF405948) and Chinese (KM279655 and KM279654) origin. However, no significant intraspecific variation was observed within each of these two sarcophagid species within their respective countries. The results of interspecific variation between other species were found to be higher, which showed the efficacy of COI to identify the species from different genus of Sarcophagidae family.

CONCLUSION

It is clear from the above review of literature that in India there are very few workers who are doing their research work in the field of forensic entomology. Forensic entomology is very good discipline and this review article will further encourage the future workers to choose this field in their research projects.

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