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Molecular Identification of Forensically Important Indian Species of Flesh Flies (Diptera: Sarcophagidae) by Using COI Gene of Mitochondrial DNA

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Abstract

Identification of the fauna collected from and around the corpse is an absolutely vital prerequisite for the estimation of post mortem interval (PMI) in the field of forensic entomology. Morphological identification of flesh flies (Diptera; Sarcophagidae) can be perplexing due similar attributes of the species. So, to simplify the identification method, amplification of cytochrome oxidase I (COI) gene is often tried. We illustrate the use of 450 bp fragment of COI gene for differentiation of ten forensically significant species of flesh flies in India. The outcome displays the robustness of COI gene as a diagnostic marker, since its nucleotide variability endows reliable distinction to be drawn between species. Four new COI gene sequences have been added to GenBank which may be of interest for correct species identification for future workers.

Keywords: COI; Forensic entomology; Mitochondrial DNA; PMI; Species identification

Introduction

Entomological evidence at a crime place can supply information on time, origin, kind, and location of death [1-4]. Postmortem interval (PMI), the time elapsed since death, is classically calculated by techniques such as measurement of *algor mortis*, *liver mortis*, and *rigor mortis* [3,4]. Necrophagous insects have proved to be helpful in forensic investigations [5,6]. Most forensic entomological clues are reliant on unquestionable species identification. This estimation is founded upon the time taken for insects evolving on a corpse to come to the stage present when the body is discovered [7,8]. Insect species have distinct developmental lifecycle timings and thus to utilize the correct developmental data, species need to be accurately recognized. This can be done on the basis of morphological differences [7,8].

The larval types and eggs of numerous forensically significant dipteran species are tough to distinguish as their morphological differences may be obscured or awfully maintained [8,9]. Definitive identification may be achieved by rearing larvae to adults but this can be time consuming and need the larvae to be assembled, reside and kept in situation optimal for proceeded development [8,9]. The members of family Sarcophagidae or flesh flies (Diptera) comprise over 2,500 species in over 100 genera globally, with numerous species being carrion breeders and initial corpse colonizers [10,11]. Such species have the potential to be utilized to estimate the PMI [1,12-14].

The use of DNA methods can not only supplement the traditional morphological methods but may furthermore give information on population substructures [8,15]. This identification can be carried out on any lifecycle stage without further rearing and on dead, maintained or reside trials. DNA techniques are also relatively insensitive to the methods of preservation or age of samples [16]. Latest use of molecular markers for forensic species has focused on the use of mitochondrial DNA [8,14]. Mitochondria are present in high numbers inside cells and, therefore, mitochondrial DNA is present in a much higher exact replicate number than nuclear DNA [17]. Hence it is the favored goal when considering incomplete or old tissue samples [17-21].

The mtDNA investigation of forensically relevant flies focuses mainly on the cytochrome oxidase subunits one (COI) [4,12,20-25]. Even partial sequences of this gene have been verified to have sufficient discrimination power, [18,19,25,26], which makes it specifically important for forensic applications. With this study we will be able to know the utility of COI for identification of ten forensically significant Indian sarcophagid fly species and build up genetic data for the database of sarcophagid flies from India.

Materials and Methods

Taxon sampling, identification and DNA extraction

30 specimens belonging to 10 species of Indian Sarcophagidae were collected from the four north Indian states. All the flies were collected from meat kept as bait by using sweep net and immediately killed by using potassium cyanide (KCN) killing jar. The identification was done by preparing the external genitalia slides followed by the keys given by Senior-White et al. [27], Pape [10] and Nandi [28]. After identification, the specimens were kept in insect boxes. DNA was extracted from the dried thorax of the fly specimens using the Coen method [29]. The extracted DNA was eluted in 200 µL elution buffer and kept at -20°C for long term storage. The fraction of extracted DNA was spectrophotometrically quantitated and diluted to 50 ng/µL prior to PCR amplification step.

Amplification and sequencing of COI gene

A partial fragment (450 bp region) of COI gene of the mitochondrial DNA was amplified using the primer pairs 5'-CAGCTACTTTATGAGCTTTAGG-3'(forward primer) and 5'-CATTTCAAGCTGTGTAAGCATC-3'(reverse primer) [22]. The

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PCR reaction volume was 25 µL, containing 2.5 µL of template DNA, 2.5 µL *Taq* polymerase, 2.5 µL dNTP, 2.5 µL 10 × buffer, 2.5 µL of each primer and Nuclease-free water was added to a total volume of 25 µL. The thermocycler (Bio-Rad, USA) was programmed with the following parameters; initial step at 94°C for 3 min then continued for 30 cycles each of 94°C for 30s; 48°C for 30s; and 72°C for 30s. An elongation of PCR products by 72°C for 5 min completed the reaction.

Sequence analysis

Sequencing of both sense and antisense strand was done using an ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit by ABI PRISM 3730 (Applied Biosystems, Foster City, U.S.A.). Big Dye terminator v3.1 was used as the sequencing agent. The sequences obtained have been deposited in GenBank by Sequin (http://www.ncbi.nlm.nih.gov/Sequin/index.html) and their accession numbers are listed in Table 1. For phylogenetic analysis and comparison, the available world sequences for seven species were downloaded from the NCBI website (Table 1). All the COI gene sequences were compared with Dipteral sequences on the NCBI website via the BLAST function for quick identification.

DNA sequence data analysis

Clustal X software [30] was used to align DNA sequences and after alignment homologous region was selected for phylogenetic investigations. Analysis of nucleotide composition, general transition;transversion ratio (ts;tv), variable and parsimony informative sites and pair wise nucleotide distances were calculated using MEGA5 [31]. The evolutionary history was estimated using the Neighbor-joining procedure and Maximum parsimony procedure [32] with 1000 replications in bootstrap test. There were a total of 450 positions in the last dataset. *Musca autumnalis* belonging to the family Muscidae was taken as an outgroup for phylogenetic analysis.

Results and Discussion

The aim of this research was to ascertain the reliability of COI gene to differentiate between some Indian flesh flies of forensic interest. So far, the publications on the molecular identification of forensically applicable flesh flies from India are constrained to one [33]. They researched five species of flesh flies on the basis of COI gene but that work was only constrained to phylogenetic investigation and not associated to forensic entomology. In our previous paper [25], we have sequenced ten species of Sarcophagidae in which the primer pair was different than the primer pair used for the sequencing of samples used in the present study.

It is clear from the nucleotide composition data that A+T composition dominated in all the species studied. The average nucleotide composition for the ten species was T=40.58.6%, A=30.10%, C=16.08%, G=13.24%. The data showed that maximum A+T bias was seen in *S. hirtipes* (72.2%) and lowest in *S. macroauriculata* (69.3%). The A+T content observed during the present study was found out to be in agreement to the characteristics of the base composition of the mitochondrial genome of other dipteran insects (ranging from 72.6% to 82.2%) [34]. Silvestre et al. [35] suggested that one hypothesis that attempts to explain A+T bias is that the DNA polymerase could use these bases in a more efficient way during mtDNA replication [36]. Sharma et al. [25] showed average nucleotide composition for ten species as T=37.20%, A=29.19%, C=16.61% and G=17.00%.

The 450 bp region has revealed 289 conserved and 161 variable and parsimony informative positions which showed that COI contains both conserved and highly variable regions across taxa which are very useful for the species identification. The present results are in complete agreement with Sharma et al. [25] who sequenced 465 bp region from the ten flesh fly Indian species and showed 342 conserved and 123 variable and parsimony informative positions. Bajpai and Tewari [33] also studied five sarcophagid species and showed that COI showed 71 variable sites in 296 bp long sequence and only 26 sites were found to be parsimony informative. Song et al. [37] noted 151 variable sites in the 552 bp long fragment of COI gene amplified from fifteen sarcophagid species of China origin. They also concluded that out of 151, 129 variable sites were in the third codon position.

All the species under investigation showed 0.0% intraspecific genetic divergence. While, interspecific genetic divergence was in the range of 6% to 21%. It is clear from the Table 2 that *P. misera* showed least genetic divergence of 6.0% with *P. albiceps* and highest with *S. orientaloides* (16.0%). *P. sericea* showed minimum percent genetic divergence with *P. albiceps* (9.0%) and maximum with *I. martellata* (19.0%). *P. hirtipes* showed least genetic divergence with *P. misera* (13.0%) and maximum with *S. orientaloides* (21.0%). All the sequences of *P. albiceps* showed least genetic divergence with *B. peregrina* (10.0%) and highest with *S. orientaloides* (16.0%). *P. macroauriculata* showed least genetic divergence with *P. misera* (13.0%) and highest with *S. orientaloides* (19.0%).

Sharma et al. [25] studied ten Indian flesh fly species and showed interspecific genetic divergence of 4-14%. Maximum genetic divergence of 14% was seen between samples of *S. princeps* and *S. hirtipes*. Minimum genetic divergence (4%) was seen between samples of *S. misera* and *S. serciea*. Guo et al. [38] investigated seven Chinese sarcophagid species from different populations on the basis of 272 bp region of COI gene. The mean levels of divergence between these seven sarcophagid species ranged from 4% to 8%. Subsequently, Guo et al. [39] showed interspecific variation varying from 7% to 10%. The maximum and minimum levels of divergence between the four sarcophagid species were similar and ranged from 7% to 11%. The minimum intraspecific values of the four species were all 0%. They further emphasized that the interspecific variation between species of different genera was higher than that from same genus, which indicated the ability of COI to identify the species from different genera of Sarcophagidae family.

The 450 bp COI fragment was successfully sequenced for all the specimens. All flies were rightly assigned into ten species with monophyletic separation in the NJ and MP trees (Figures 1 and 2). At the species level, the high bootstrap values (100%) provide percentage robust support for the monophyly of all the ten species under study. Phylogenetic trees constructed by both methods (NJ and MP) showed almost same topology. Within *H. kempi*, two specimens clustered together with three Malaysian samples (GenBank accession numbers GU174025, EF405946, EF405947) with a supporting bootstrap of 52-100%. Within *I. martellata*, two specimens (Accession numbers FJ440843 and FJ440844) clustered together with a supporting bootstrap of 38-100%. At the genus level, *P. albiceps* and *B. peregrina* cluster together, whereas *H. kempi*, *S. princeps* and *I. martellata* cluster together indicating the ability of this shorter COI fragment to identify the species from the same genus may not be as efficient as that of the longer fragments. More DNA fragments of different lengths and regions should be studied in the future.

All the samples belonging to species *P. albiceps* are clustered together with the sequences of the same species originating from France (JQ582062 and JQ582097) and Malaysia (EF405931 and EF405932) with bootstrap value ranging from 54-99%. With the high

S.No.	Species	Voucher ID	GenBank	Country	Collection locality	GPS (Longitude and Latitude)	Reference
1	*Sarcophaga misera* (Walker)	A136	JX507310	India	Harike, Punjab	31°09'56.58"N and 74°56'41.32"E	This study
		D90	JX507309	India	Dehradun, UK	30°19'32.37"N and 77°54'30.77"E	This study
		D218	JX507307	India	Andretta, HP	32°02'14.11"N and 76°34'12.59"E	This study
		JK6	JX507303	India	Kathua, J&K	32°23'52.48"N and 75°33'09.51"E	This study
		S107	EF405930	Malaysia	Pahang	03°20' 32"N and 101°53'01"E	Tan et al. (2010)
		S9	EF405930	Malaysia	Kuala Lumpur	03°07'51"N and 101°39'22"E	Tan et al. (2010)
2	*Sarcophaga sericea* (Walker)	A333	JX507337	India	Nawanshehr, Punjab	31°07'37.16"N and 76°07'14.40" E	This study
		D106	JX507339	India	Dehradun, UK	30°19'32.37"N and 77°54'30.77"E	This study
		D280	JX507341	India	Kangra, HP	32°05'55.45"N and 76°25'16.72"E	This study
		JK49	JX507343	India	Kathua, J&K	32°23'52.48"N and 75°33'09.51"E	This Study
3	*Sarcophaga hirtipes* (Wiedemann)	A103	JX507313	India	Patiala, Punjab	30°22'45.94"N and 76°17'22.89"E	This study
		D55	JX507318	India	Sahastradhara, UK	30°23'06.10"N and 78°07'42.81"E	This study
		D241	JX507319	India	Parour, HP	32°06'33.80" N and 76°07'42.81"E	This study
		JK1	JX507315	India	Kathua, J&K	32°23'52.48"N and 75°33'09.51"E	This Study
4	*Sarcophaga albiceps* (Meigen)	A348	JX507320	India	Roopnagar, Punjab	30°12'35.82" N and 73°59'33.19"E	This study
		D113	JX507322	India	Sahastradhara, UK	30°23'06.10"N and 78°07'42.81"E	This study
		D231	JX507324	India	Palampur, HP	32°06'33.80"N and 76°32'13.80"E	This study
		JK34	JX507326	India	Kathua, J&K	32°23'52.48" N and 75°33'09.51"E	This study
		PA1	EF405931	Malaysia	Kuala Lumpur	03°07'51"N and 101°39'22"E	Tan et al. (2010)
		S152	EF405932	Malaysia	Kuala Lumpur	03°07'51"N and 101°39'22"E	Tan et al. (2010)
		MIC/INCC 0212	JQ582062	France	Dompierre-sur-Busbre	46°31'16"N and 03°40'54"E	Jordaens et al. [42]
		MIC/INCC 0415	JG582097	France	Cameles	42°38'27"N and 02°41'48"E	Jordaens et al. [42]
5	*Sarcophaga macroauriculata* (Ho)	D116	JX507328	India	Dehradun, UK	30°19'32.37"N and 77°54'30.77"E	This study
		D143	JX507329	India	Dehradun, UK	30°19'32.37"N and 77°54'30.77"E	This Study
6	*Sarcophaga ruficornis* (Fabricius)	A365	JX507303	India	Patiala, Punjab	30°22'45.94"N and 76°17'22.89"E	This study
		A366	JX507302	India	Patiala, Punjab	30°22'45.94"N and 76°17'22.89"E	This study
		S21	EF405940	Malaysia	Kuala Lumpur	03°07'51"N and 101°39'22"E	Tan et al. (2010)
		SY5	Ef405941	Malaysia	Kelantan	05°58'21"N and 102°14'44"E	Tan et al. (2010)
7	*Sarcophaga kempi* (Senior-White)	D160	JX507328	India	Nahan, HP	30°33'35.76"N and 77°17'43.74"E	This study
		D161	JX507329	India	Nahan, HP	30°33'35.76"N and 77°17'43.74"E	This study
		S274	GU174025	Malaysia	Kuala Lumpur	03°07'51"N and 101°39'22"E	Tan et al. (2010)
		S102	EF405946	Malaysia	Kuala Lumpur	03°07'51"N and 101°39'22"E	Tan et al. (2010)
		S134	EF405947	Malaysia	Pahang	03°20'32"N and 101°53'01"E	Tan et al. (2010)
8	*Sarcophaga princeps* Wiedemann	A338	JX507351	India	Roopnagar, Punjab	30°12'35.82" N and 73°59'33.19"E	This study
		D18	JX507347	India	Mussoorie, UK	30°27'40.31"N and 78°03'09.51"E	This study
		D333	JX507350	India	Manali, HP	32°15'16.87 N and 77°09'47.11"E	This study
		JK30	JX507344	India	Kathua, J&K	32°23'52.48"N and 75°33'09.51"E	This study
		S71	EF405949	Malaysia	Kuala Lumpur	03°07'51"N and 101°39'22"E	Tan et al. (2010)
		S25	EF405948	Malaysia	Kuala Lumpur	03°07'51"N and 101°39'22"E	Tan et al. (2010)
9	*Sarcophaga peregrina* (Robineau-Desvoidy)	D407	JX507335	India	Bilaspur, HP	31°20'48.42"N and 76°44'36.70"E	This study
		D409	JX507334	India	Bilaspur, HP	31°20'48.42"N and 76°44'36.70"E	This study
		S-CH2	EF405927	Malaysia	Cameron Highlands	04°30'50"N and 101°2523E	Tan et al. (2010)
		S-CH9	EF405928	Malaysia	Cameron Highlands	04°30'50"N and 101°2523E	Tan et al. (2010)
10	*Sarcophaga martellata* Nandi	A304	JX507331	India	Patiala, Punjab	30°22'45.94"N and 76°17'22.89"E	This study
		A364	JX507330	India	Patiala, Punjab	30°22'45.94"N and 76°17'22.89"E	This study
		S153	FJ440843	Malaysia	Kuala Lumpur	03°07'51"N and 101°39'22"E	Tan et al. (2010)
		S155	FJ440844	Malaysia	Kuala Lumpur	03°07'51"N and 101°39'22"E	Tan et al. (2010)

Table 1: Showing voucher codes, collection data and accession numbers for the ten Indian (under investigation) and world flesh flies species.

bootstrap value ranging from 76%-100%, sequences obtained from the samples of *B. peregrina* grouped together with the same species originating from Malaysia (EF405927 and EF405928). Two samples of species *P. ruficornis* showed similarity with the Malaysian samples with high bootstrap support (39-100%). Two samples of *S. princeps* clustered together with two Malaysian samples (Bootstrap value 94-100%) with GenBank accession numbers EF405948 and EF405949. Similarly, DNA sequences for species *I. martellata* and *H. kempi* from India showed high bootstrap support with the Malaysian species.

Sharma et al. [25] phylogenetically analyzed ten Indian species of flesh flies and showed high bootstrap support for each species. All the ten species were separated with high bootstrap support (50-100%) which showed the robustness of this gene to distinguish between Indian flesh flies. Zehner et al. [40] investigated twelve species of Sarcophagidae and showed a bootstrap value of 52-90% in the NJ tree. Song et al. [36] noted ≥94% bootstrap support which strongly supported monophyly of the three clades including the *Parasarcophaga* clade, *Boettcherisca* clade and the *Liopygia* clade and suggested interior relationship as *S. albiceps*, *S. misera* and *S. sericea* for the *Parasarcophaga clade*. They also showed sister relationships between some of the species (with *S. scopariiformis* and *S. iwuensis; S. polystylata* and *S. hui; S. brevicornis* and *S. dux*). Our results are also in complete association with Guo et al. [21] who observed 100% bootstrap support for three species of flesh flies viz., *S. similis, S. albiceps* and *B. peregrina*. Qing et al. [41] showed 99% bootstrap support for 7 sarcophagous species of Chinese origin. Bajpai and Tewari [33] phylogenetically studied five species of Indian Sarcophagidae and demonstrated minimum evolution trees for COI and ND5 genes of mtDNA. They showed a bootstrap value of 59% to 94% which shows robustness of this gene to identify species within the genus *Sarcophaga*. Jordaens et al. [42] also showed a bootstrap value ranging from 99% to 100% while studying *S. misera*, *S. albiceps*, *S. peregrina*, *S. ruficornis*

[	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	]
[1]																															
[2]	0.0																														
[3]	0.0	0.0																													
[4]	0.0	0.0	0.0																												
[5]	6.0	6.0	6.0	6.0																											
[6]	6.0	6.0	6.0	6.0	6.0																										
[7]	6.0	6.0	6.0	6.0	6.0	0.0																									
[8]	6.0	6.0	6.0	6.0	0.0	0.0	0.0																								
[9]	9.0	9.0	9.0	9.0	10.0	10.0	10.0	10.0																							
[10]	9.0	9.0	9.0	9.0	10.0	10.0	10.0	10.0	0.0																						
[11]	9.0	9.0	9.0	9.0	10.0	10.0	10.0	10.0	0.0	0.0																					
[12]	9.0	9.0	9.0	9.0	10.0	10.0	10.0	10.0	10.0	0.0	0.0	0.0																			
[13]	10.0	10.0	10.0	10.0	10.0	9.0	9.0	9.0	9.0	13.0	13.0	13.0																			
[14]	10.0	10.0	10.0	10.0	10.0	9.0	9.0	9.0	9.0	13.0	13.0	13.0	0.0																		
[15]	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0	16.0	16.0	16.0	16.0	14.0	14.0																	
[16]	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0	16.0	16.0	16.0	16.0	14.0	0.0																	
[17]	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0	16.0	16.0	16.0	16.0	14.0	0.0	0.0																
[18]	13.0	13.0	13.0	13.0	13.0	13.0	13.0	13.0	16.0	16.0	16.0	16.0	14.0	0.0	0.0	0.0															
[19]	14.0	14.0	14.0	14.0	14.0	13.0	13.0	13.0	17.0	17.0	17.0	17.0	14.0	17.0	17.0	17.0	17.0														
[20]	14.0	14.0	14.0	14.0	14.0	13.0	13.0	13.0	17.0	17.0	17.0	17.0	14.0	17.0	17.0	17.0	17.0	0.0													
[21]	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	13.0	13.0	13.0	13.0	12.0	12.0	14.0	14.0	14.0	14.0	15.0	15.0											
[22]	11.0	11.0	11.0	11.0	11.0	11.0	11.0	11.0	13.0	13.0	13.0	13.0	12.0	14.0	14.0	14.0	14.0	15.0	15.0	0.0											
[23]	14.0	14.0	14.0	14.0	15.0	15.0	15.0	15.0	19.0	19.0	19.0	19.0	13.0	17.0	17.0	17.0	17.0	18.0	18.0	12.0											
[24]	14.0	14.0	14.0	14.0	15.0	15.0	15.0	19.0	19.0	19.0	19.0	19.0	13.0	17.0	17.0	17.0	17.0	18.0	18.0	12.0	0.0										
[25]	10.0	10.0	10.0	10.0	10.0	11.0	11.0	11.0	11.0	14.0	14.0	14.0	12.0	12.0	15.0	15.0	15.0	15.0	14.0	14.0	10.0	10.0	14.0	14.0							
[26]	10.0	10.0	10.0	10.0	10.0	11.0	11.0	11.0	11.0	14.0	14.0	14.0	12.0	12.0	15.0	15.0	15.0	15.0	14.0	14.0	10.0	10.0	14.0	14.0	0.0						
[27]	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	21.0	21.0	21.0	21.0	18.0	18.0	13.0	13.0	16.0	16.0	13.0	13.0							
[28]	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	21.0	21.0	21.0	21	0	18.0	18.0	13.0	13.0	16.0	16.0	13.0	13.0	0.0					
[29]	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	21.0	21.0	21.0	21.0	18.0	18.0	13.0	13.0	16.0	16.0	13.0	13.0	0.0	0.0					
[30]	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	16.0	21.0	21.0	21.0	21.0	18.0	18.0	13.0	13.0	16.0	16.0	13.0	13.0	0	0	0.0	0.0			

S.Nos: 1-4: *S. albiceps*; 5-8: *S. misera*; 9-12: *S. sericea*; 13-14: *S. peregrina*; 15-18: *S. hirtipes*; 19-20: *S. macroauriculata*; 21-22: *S. kempi*; 23-24: *S. martellata*; 25-26: *S. ruficornis*; 27-30: *S. princeps*

Table 2: Showing the pairwise sequence differences (%) for the 450 bp region of COI for ten flesh fly species.

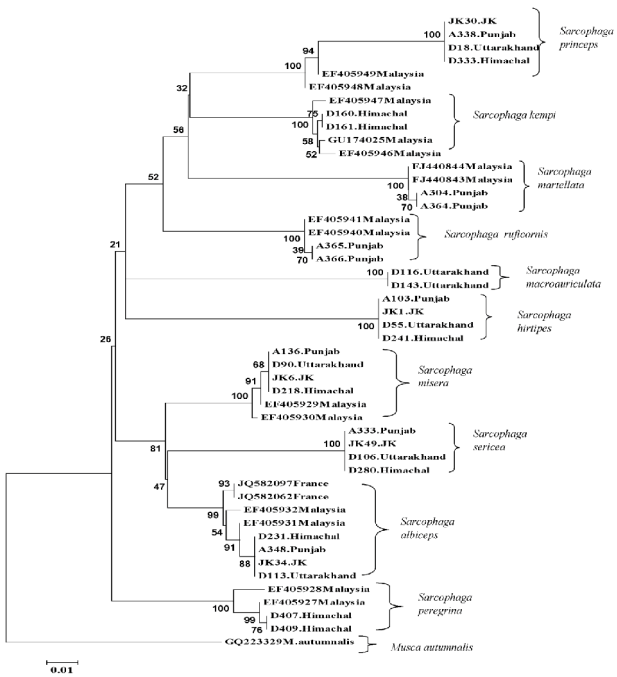

Figure 1: Neighbour-joining (NJ) tree of tamura-3-3parameter (T3P) distance for 30 COI gene sequences from ten species of Indian and world Sarcophagidae. *Musca autumnalis* of Muscidae is involved as an out group. Numbers on branches suggest the support value for that particular branch. Specimen voucher codes referred to in Tale 2 shown following collection. Evolutionary distance divergence is given by bar which indicates 0.01 substitutions per site.

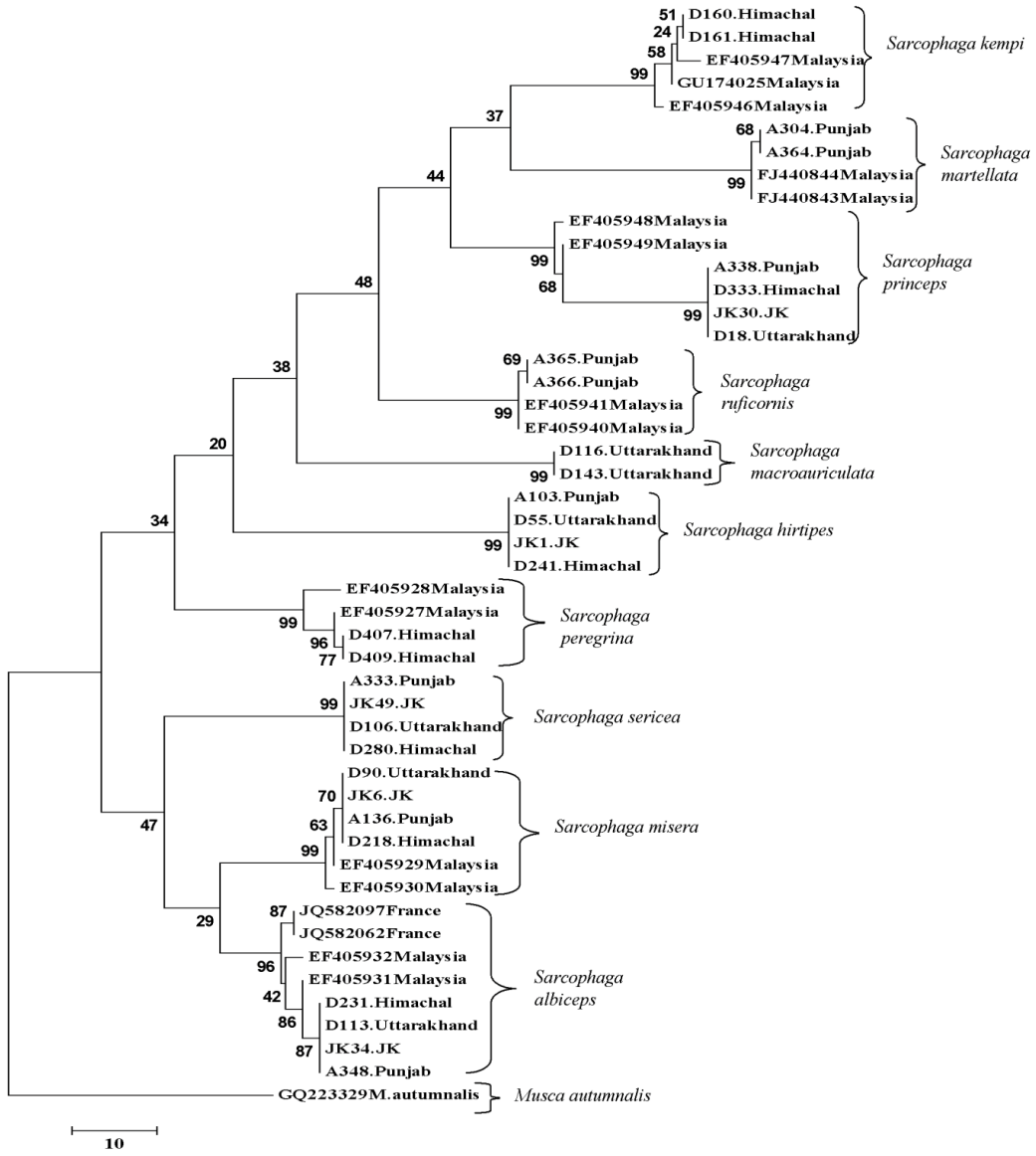

Figure 2: The evolutionary history was inferred using the Maximum Parsimony method. *Musca autumnalis* of Muscidae is taken as an out group. Numbers on branches suggest the support value for that particular branch. Specimen voucher codes given in the Table 1. Evolutionary distance divergene is given by bar which indicates 10 substitutions per site.

and *S. kempi* (a total 56 species were investigated in their study).

Conclusions

On the basis of this discussion, it can be concluded that COI gene of mtDNA is capable of identification and phylogenetic analysis of Indian sarcophagid flies. The sarcophagid fly fauna has remained neglected not only inside India but globally and, therefore, is unexploited as PMI indicators in forensic entomology. The primary step required to secure the use of sarcophagids in PMI estimator is the accurate species identification of a specimen from any life stage. The COI gene becomes an important marker for the fast species identification where the morphological based identification is not likely. This approach has proved thriving for the unquestionable identification of the Indian Sarcophagidae examined and is likely to be a perceptive typing scheme for sarcophagids associated with forensic casework. The present study is the first foremost try to recognize ten forensically important flesh fly species of Indian origin on the basis of COI gene of mtDNA. The resultant facts and figures will be rather helpful for the future workers in their investigations and for the advancement of specific markers for the identification purposes.

Conflict of Interest

The authors declare that they have no conflict of interest.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors. So, the ethical approval is not required.

References

1. Catts EP, Goff ML (1992) Forensic entomology in criminal investigations. Annu Rev Entomol 37: 253-272.
2. Byrne AL, Camann MA, Cyr TL, Catts EP, Espelie KE (1995) Forensic implications of biochemical differences among geographic populations of the black blow fly, Phormia regina (Meigen). J Forensic Sci 40: 372-377.
3. Campobasso CP, Introna F (2001) The forensic entomologist in the context of the forensic pathologist's role. Forensic Sci Int 120: 132-139.
4. Meiklejohn KA, Wallman JF, Dowton M (2011) DNA-based identification of forensically important Australian Sarcophagidae (Diptera). Int J Legal Med 125: 27-32.
5. Anderson GS (2001) Succession on carrion and its relationship to determining time of death. In; Forensic entomology; the utility of arthropods in legal investigations. Byrd, JH and Castner, JL (Eds.), Boca Raton, CRC.
6. Cainé LM, Real FC, Saloña-Bordas MI, de Pancorbo MM, Lima G, et al. (2009) DNA typing of Diptera collected from human corpses in Portugal. Forensic Sci Int 184: e21-23.
7. Smith KGV (1986) A manual of forensic entomology, The British Museum (Natural History), London and Cornell University Press.
8. Ames C, Turner B, Daniel B (2006) The use of mitochondrial cytochrome oxidase I gene (COI) to differentiate two UK blowfly species -- Calliphora vicina and Calliphora vomitoria. Forensic Sci Int 164: 179-182.
9. Otranto D, Stevens JR (2002) Molecular approaches to the study of myiasis-causing larvae. Int J Parasitol 32: 1345-1360.
10. Shewell GE (1987) Sarcophagidae. In: McAlpine JF, Peterson BV, Shewell, GE, Teskey HJ, Vockeroth JR, Wood DM (eds.) Manual of Nearctic Diptera. Agriculture Canada Monograph.
11. Pape T (1996) Catalogue of the Sarcophagidae of the world (Insecta; Diptera). Mem Entomol Int 8: 1-558.
12. Catts EP (1992) Problems in estimating the post-mortem interval in death investigations. J Agric Entomol 9: 245-255.
13. Wells JD, Pape T, Sperling FA (2001) DNA-based identification and molecular systematics of forensically important Sarcophagidae (Diptera). J Forensic Sci 46: 1098-1102.
14. Amendt J, Krettek R, Zehner R (2004) Forensic entomology. Naturwissenschaften 91: 51-65.
15. Hillis DM, Moritz C (1990) An overview of applications of molecular systematic. In: Hillis DM, Moritz C (eds.) Molecular Systematics. Sinauer Associates, Sunderland.
16. Pääbo S (1989) Ancient DNA: extraction, characterization, molecular cloning, and enzymatic amplification. Proc Natl Acad Sci U S A 86: 1939-1943.
17. Butler JM (2001) Sample collection and preparation (DNA extraction and quantification). In; Forensic DNA typing, Academic Press, San Diego.
18. Malgorn Y, Coquoz R (1999) DNA typing for identification of some species of Calliphoridae. An interest in forensic entomology. Forensic Sci Int 102: 111-119.
19. Vincent S, Vian JM, Carlotti MP (2000) Partial sequencing of the cytochrome oxydase b subunit gene I: a tool for the identification of European species of blow flies for postmortem interval estimation. J Forensic Sci 45: 820-823.
20. Guo Y, Jifeng C, Xinghua W, Lingmei L, Qinlai L, et al. (2010) Identification of forensically important sarcophagid flies (Diptera; Sarcophagidae) based on COI gene in China. Rom J Legal Med 18: 217-224.
21. Guo YD, Cai JF, Li X, Xiong F, Su RN, et al. (2010) Identification of the forensically important sarcophagid flies Boettcherisca peregrina, Parasarcophaga albiceps and Parasarcophaga dux (Diptera; Sarcophagidae) based on COII gene in China. Trop Biomed 27: 451-460.
22. Sperling FA, Anderson GS, Hickey DA (1994) A DNA-based approach to the identification of insect species used for postmortem interval estimation. J Forensic Sci 39: 418-427.
23. Wallman JF, Leys R, Hogendoorn K (2005) Molecular systematics of Australian carrion-breeding blowflies (Diptera; Calliphoridae) based on mitochondrial DNA. Invertebr Syst 19: 1-15.
24. Nelson LA, Wallman JF, Dowton M (2007) Using COI barcodes to identify forensically and medically important blowflies. Med Vet Entomol 21: 44-52.
25. Sharma M, Singh D, Sharma AK (2015) Mitochondrial DNA based identification of forensically important Indian flesh flies (Diptera: Sarcophagidae). Forensic Sci Int 247: 1-6.
26. Alessandrini F, Mazzanti M, Onofri V, Turchi C, Tagliabracci A (2008) MtDNA analysis for genetic identification of forensically important insects. Forensic Sci Int; Genet Suppl Ser 1: 584-585.
27. Senior-White RA, Aubertin D, Smart J (1940) Diptera. Family Calliphoridae. The Fauna of British India, including the remainder of the Oriental region. 6. xiii, Taylor & Francis, Ltd., London.
28. Nandi BC (2002) Fauna of India and the adjacent countries. Diptera, Sarcophagidae. Vol. 10 Director, Zoological Survey of India (Eds.), Kolkata, India.
29. Coen ES, Thoday JM, Dover G (1982) Rate of turnover of structural variants in the rDNA gene family of Drosophila melanogaster. Nature 295: 564-568.
30. Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higguns DG (1997) The CLUSTAL X Windows Interface; Flexible stretegies for Multiple Sequence Alignment aided by Quality analysis tools. Nucleic Acids Res 24: 4876-4882.
31. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, et al. (2011) MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol Biol Evol 28: 2731-2739.
32. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol 4: 406-425.
33. Bajpai N, Tewari RR (2010) Mitochondrial DNA sequence-based phylogenetic relationship among flesh flies of the genus Sarcophaga (Sarcophagidae: Diptera). J Genet 89: 51-54.
34. Junqueira AC, Lessinger AC, Torres TT, da Silva FR, Vettore AL, et al. (2004) The mitochondrial genome of the blowfly Chrysomya chloropyga (Diptera: Calliphoridae). Gene 339: 7-15.
35. Silvestre D, Dowton M, Arias MC (2008) The mitochondrial genome of the stingless bee Melipona bicolor (Hymenoptera, Apidae, Meliopini); sequence, gene organization and a unique tRNA translocation event conserved across the tribe Meliponini. Genet Mol Biol 31: 451-460.
36. Clary DO, Wolstenholme DR (1985) The mitochondrial DNA molecular of Drosophila yakuba: nucleotide sequence, gene organization, and genetic code. J Mol Evol 22: 252-271.
37. Song Z, Wang X, Liang G (2008) Phylogenetic relationship among 15 sarcophagid fly species (Diptera; Sarcophagidae) based on partial sequences of mitochondrial cytochrome b and cytochrome oxidase subunit I genes. Acta Entomologic Sin 51: 298-306.
38. Guo YD, Cai JF, Meng FM, Chang YF, Gu Y, et al. (2012) Identification of forensically important flesh flies based on a shorter fragment of the cytochrome oxidase subunit I gene in China. Med Vet Entomol 26: 307-313.
39. Guo Y, Cai J, Chang Y, Li X, Liu Q, et al. (2011) Identification of forensically important sarcophagid flies (Diptera: Sarcophagidae) in China, based on COI and 16S rDNA gene sequences. J Forensic Sci 56: 1534-1540.
40. Zehner R, Amendt J, Schütt S, Sauer J, Krettek R, et al. (2004) Genetic identification of forensically important flesh flies (Diptera: Sarcophagidae). Int J Legal Med 118: 245-247.
41. Qing C, Jie B, Li L, HongBin L, Hui T, et al. (2009) Sequential analysis of mitochondrial COI gene for seven common sarcosaphagous flies (Diptera) in Beijing and the establishment of their DNA barcodes. Acta Entomol Sin 52: 202-209.
42. Jordaens K, Sonet G, Richet R, Dupont E, Braet Y, et al. (2013) Identification of forensically important Sarcophaga species (Diptera: Sarcophagidae) using the mitochondrial COI gene. Int J Legal Med 127: 491-504.