ISSN: 2350-0328

International Journal of Advanced Research in Science, Engineering and Technology

Vol. 2, Issue 7 , July 2015

Molecular identification of two forensically important Indian flesh flies (Diptera: Sarcophagidae)

Manish Sharma[¥], Devinder Singh[¥], Ajay Kumar Sharma[§]

¥Department of Zoology & Environmental Sciences, Punjabi University, Patiala-147002, India

§Vector Management Division, Defense R&D Establishment, DRDO, Gwalior, Madhya Pradesh-474002, India

ABSTRACT: Insect collection from the crime scene and their identification is very important and crucial step in forensic entomology. *Sarcophaga princeps* (Walker) and *Parasarcopahga hirtipes* Wiedemann, are two impotent species belonging to Sarcophagidae family. Male and female specimens of both the species have been collected, examined and were identified based on DNA sequencing analysis. Male genitalia offer unambiguous species identification characteristics in the traditional taxonomy of flesh flies but the female flies are very similar to one another in general morphology. For molecular identification, mitochondrial COI was employed and a 465bp fragment was isolated. DNA sequence data was analyzed using MEGA 5 software. Sequence divergence between two species and nucleotide composition were calculated using maximum likelihood method. Phylogenetic analysis was done by constructing neighbor-joining and maximum parsimony trees by employing Tamura-3-parameter given in the same software. Results showed that molecular methods have the potential to be used as supplement to the traditional morphological identification process.

KEYWORDS: Cytochrome oxidase I, Flesh fly, Forensic entomology, PMI, Sarcophagidae

I. INTRODUCTION

Insect specimens collected at crime scenes can be used to estimate the minimum postmortem interval (PMI), season of death, presence of toxins, or corpse transportation [1,2]. For accuracy, forensic entomologists, where possible, utilize evidence from initial corpse colonizers, which include carrion-breeding species of blow flies (Diptera: Calliphoridae) and flesh flies (Diptera: Sarcophagidae) [2,3]. Flesh flies can provide precise PMI estimations as they are viviparous (lay live larvae), producing immatures that are ready to start feeding immediately on the corpse and contribute to its decomposition. Despite the considerable forensic potential of sarcophagids, their use to date in forensic investigations has been limited in comparison with calliphorids, as accurate species-level morphological identification at any life stage is very difficult [1–5]. Sarcophagids collected from crime scenes are generally therefore reared to adults to assist with taxonomic identifications; however, this is not always possible [2,3,6,7]. Many studies have demonstrated that the identification of adult flesh flies can done by using various molecular methods. Mitochondrial genes are proved to be important, including cytochrome oxidase subunit I (COI) and NADH dehydrogenase subunit 4 [2,8,9]. Moreover, the 658-bp barcoding region of COI has shown a lot of potential to effectively distinguish between Australian Sarcophagidae [10], along with the flesh fly *Sarcophaga* (Liopygia) *argyrostoma* [11], blow flies of eastern Australia [12], butterflies (Lepidoptera) [13], blackflies (Diptera: Simuliidae) [14], mayflies (Ephemeroptera) [15], and tachinids (Diptera: Tachinidae) [2,16]. So, the present investigation has been undertaken to identify two forensically important flesh fly species of Indian origin by using short fragment of mitochondrial COI gene.

II. MATERIALS AND METHODS

A. Insect Collection and preservation:

Eight specimens of insects belonging to two species of family Sarcophagidae (Table 1) were collected over the meat kept as bait with the help of entomological net. After collection, the flies were killed by keeping them in potassium cynanide killing jar. After that, flies were pinned and kept in the entomological boxes for further use.

ISSN: 2350-0328
International Journal of Advanced Research in Science, Engineering and Technology
Vol. 2, Issue 7 , July 2015

B. Identification, amplification and DNA sequencing

External genitalia of all the specimens was cut and immerged in 10% KOH solution overnight. Morphological identification of all the specimens was done by following the keys given by Senior White et al. [17], Nandi [18] and Pape [7]. The genitalia and 5th sternite were examined under microscope and illustrated. Finally, the genitalia was either kept with glycerol in glass vials with cork stopper or mounted

For molecular identification, DNA was extracted from the dried thorax of the fly specimens using the Coen method [19]. The extracted DNA was eluted in 200 µL elution buffer and kept at -20˚C for long term storage. After this, a partial fragment (450 bp region) of COI gene of the mitochondrial DNA was amplified using two primer sets as suggested by Sperling et al. [20].

Species	Voucher	Place of collection	GenBank
Sarcophaga princeps Senior-White	A342.Punjab	Roopnagar, Punjab	JX507345
	D26.UK	Mussoorie, Uttrakhand	JX507348
	D384.HP	Manali, Himachal Pradesh	JX507349
	JK31.JK	Kathua, Jammu & Kashmir	JX507346
	S71	Malaysia	EF405949
	S25	Malaysia	EF405948
	CSU140808CS74	Chengdu, China	KM279655
	CSU140808CS73	Chengdu, China	KM279654
Parasarcophaga hirtipes (Weidemann)	A269.Punjab	Hoshiarpur, Punjab	JX507312
	D58.UK	Sahastradhara, Uttarakhand	JX507317
	D274.HP	Parour, Himachal Pradesh	JX507316
Musca autumnalis DeGeer	JK3.JK	Kathua, Jammu & Kashmir	JX507314
	-	-	GQ223329

Table 1: Collection localities, voucher number, and GenBank record for present specimens and their world references.

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. The sequence data was analysed by using the MEGA 5 software.

III. RESULTS AND DISCUSSION

This study showed that mitochondrial COI gene fragment has a lot of potential for identification of Indian flesh flies. A 465-bp fragment of the COI gene was sequenced from eight specimens, representing two forensically important species of Indian Sarcophagidae. The 465 bp region under present study has revealed 366 conserved and 99 variable and 65 parsimony informative positions which indicated that mitochondrial COI gene have both conserved and highly variable regions across taxa which is very crucial for the species discrimination. Our results are in association with Song et al. [21] who noted 151 variable sites in the 552 bp long fragment of COI gene amplified from fifteen sarcophagid species belonging to China. They also emphasized that out of 151, 129 variable sites were in the third codon position. Bajpai and Tewari [22] also studied five sarcophagid species and observed 71 variable sites in 296 bp long sequences in which only 26 sites were parsimony informative.

Both the tree i.e., neighbor joining (Fig. 1) as well as on the maximum parsimony tree (Fig. 2) based on the sequence data showed several distinct congeneric clusters. The high bootstrap values (100%) provide an indication of good percentage support for the grouping nodes of all the species under present investigation. From the bootstrap support for each group and the level of nucleotide divergence between groups, it is thus evident that these gene sequences have potential for identification of sarcophagid flies. A high bootstrap support (69-93%) was also seen when we compare our samples with world samples .The monophyletic separation of all the samples of two species (Fig. 2) confirmed the sufficient resolution of the genetic marker. All the samples of both the species of Sarcophagidae were

ISSN: 2350-0328
International Journal of Advanced Research in Science, Engineering and Technology
Vol. 2, Issue 7 , July 2015

separated and the bootstrap values were all 100% and these results are in agreement with those of Zehner et al. [9], Bajpai and Tewari [22], Alessandrini et al. [23], Guo et al. [24], Guo et al. [25]. Tan et al. [26] and Stamper et al. [27].

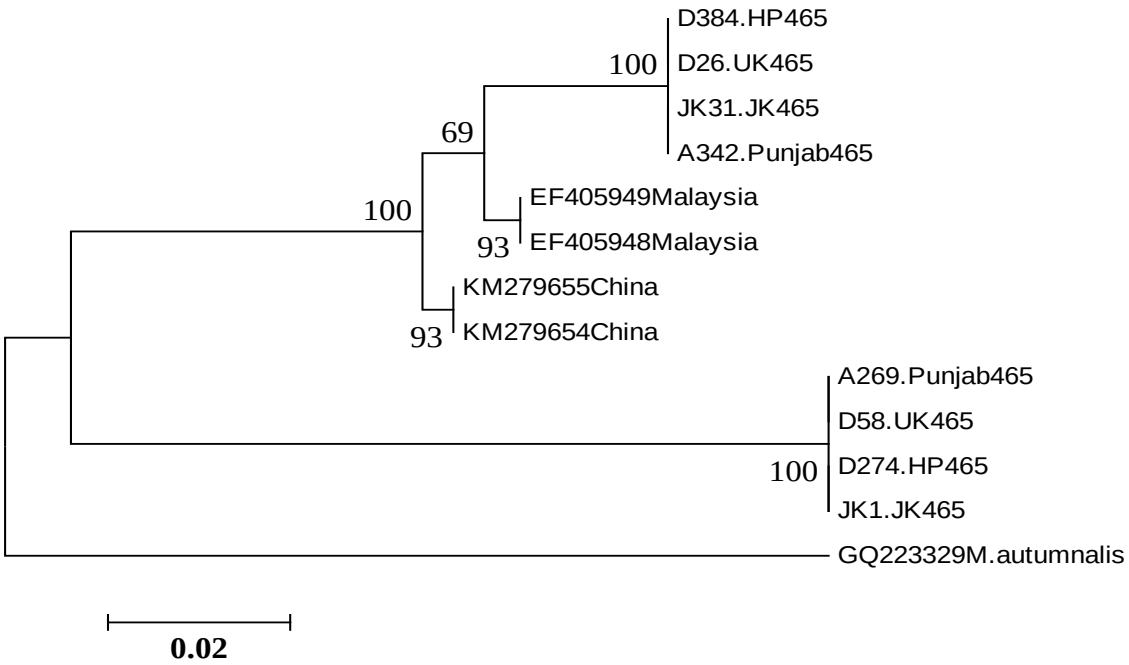

Fig. 1: Neighbour-joining (NJ) tree of Tamura-3-parameter (T3P) distances for 08 COI gene sequences from two species of Indian and world Sarcophagidae. Evolutionary distance divergence is given by bar which indicates 0.02 substitutions per site.

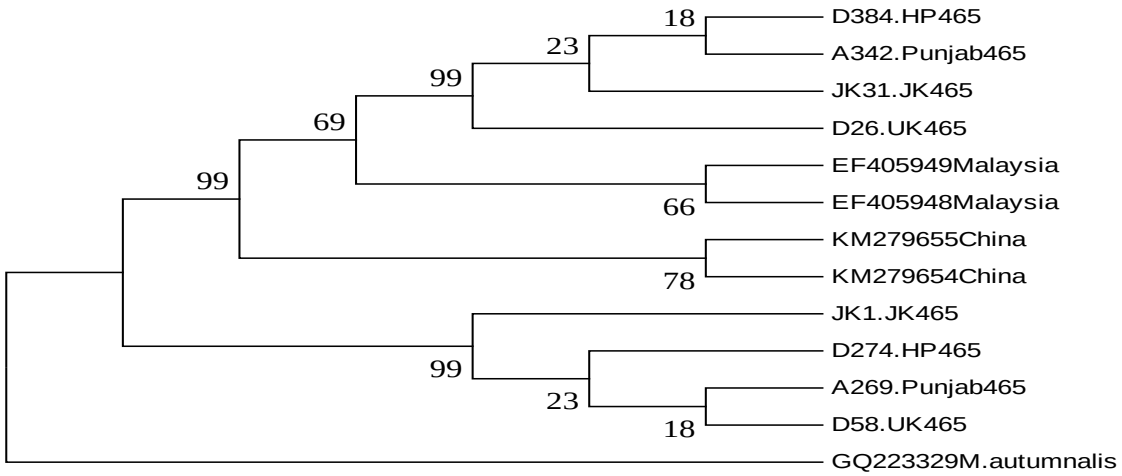

Fig.2: Most parsimonious tree using Tamura-3-parameter (T3P) distances for 08 COI gene sequences from two species of Indian and world Sarcophagidae.

Percent pairwise divergence between species was calculated and was presented in Table 2, and variation among all individuals of the species was calculated. The number of base substitutions per site from analysis between sequences was shown.

ISSN: 2350-0328

International Journal of Advanced Research in Science, Engineering and Technology

Vol. 2, Issue 7 , July 2015

[	1	2	3	4	5	6	7	8	9	10	11	12]
[1]												
[2]	0.0											
[3]	0.0	0.0										
[4]	0.0	0.0	0.0									
[5]	2.0	2.0	2.0	2.0								
[6]	2.0	2.0	2.0	2.0	0.0							
[7]	3.0	3.0	3.0	3.0	1.0	1.0						
[8]	3.0	3.0	3.0	3.0	1.0	1.0	0.0					
[9]	15.0	15.0	15.0	15.0	13.0	13.0	12.0	12.0				
[10]	15.0	15.0	15.0	15.0	13.0	13.0	12.0	12.0	0.0			
[11]	15.0	15.0	15.0	15.0	13.0	13.0	12.0	12.0	0.0	0.0		
[12]	15.0	15.0	15.0	15.0	13.0	13.0	12.0	12.0	0.0	0.0	0.0	0.0

S.Nos: 1-8:*S. princeps*; 9-12: *P.hirtipes*

Table 2: Showing the percent pairwise sequence differences for the 465 bp region of COI gene for two flesh fly species.

In Table 2, *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 and these results are in complete consonance with those of Harvey et al. [28].

IV. CONCLUSIONS

With the preceding discussion, it is clear that the investigated COI gene sequences have lot of potential for identification of Indian flesh flies. As no significant intraspecific variation was observed within each of these two sarcophagid species because this COI sequence is partial and short to infer required amount of information for species identification. Future workers are encouraged to do full length analysis to get best results for intraspecific variations.

REFERENCES

[1] E.P. Catts, "Problems in estimating the postmortem interval in death investigations", Journal of Agricultural Entomology, 9(4), pp. 245–255,1992.

[2] K.A. Meiklejohn, J.F. Wallman and M. Dowton, "DNA barcoding identifies all immature life stages of a forensically important flesh flies (Diptera: Sarcophagidae)", Journal of Forensic Sciences, 158(1), pp. 184-187, 2013.

[3] J.H. Byrd and J.L. Castner, "Insects of forensic importance" In: Byrd JH, Castner JL, editors. Forensic entomology: the utility of arthropods in legal investigations, 2nd edn. Boca Raton, FL: CRC Press, pp. 39–126, 2010.

[4] E.P. Catts and M.L. Goff, "Forensic entomology in criminal investigations", Annual Review of Entomology, 37, pp. 253–272, 1992.

[5] J. Amendt, R. Kretteka and R. Zehner, "Forensic entomology", Naturwissenschaften 91, pp. 51–65, 2004.

[6] G.E. Shewell, "Sarcophagidae", In: McAlpine JF, Peterson BV, Shewell GE, Teskey HJ, Vockeroth JR, Wood DM, editors. Manual of Nearctic Diptera, Vol. 2. Ottawa, Canada: Research Branch, Agriculture Canada, pp., 1159–1186, 1987.

[7] T. Pape, "Catalogue of the Sarcophagidae of the world (Insect: Diptera)", Gainesville, FL: Associated Publishers, 1996.

[8] J.D. Wells, T. Pape and F.A.H. Sperling, "DNA-based identification and molecular systematics of forensically important Sarcophagidae (Diptera)", Journal of Forensic Sciences, 46(5), pp. 1098–1102, 2001.

[9] R. Zehner, J. Amendt, S. Schutt, J. Sauer, R. Krettek and D. Povolny, "Genetic identification of forensically important flesh flies (Diptera: Sarcophagidae)", International Journal of Legal Medicine, 118, pp. 245–247, 2004.

[10] K.A. Meiklejohn, J.F. Wallman and M. Dowton, "DNA-based identification of forensically important Australian Sarcophagidae (Diptera)", International Journal Legal Medicine 125(1), pp. 27–32, 2011.

ISSN: 2350-0328

International Journal of Advanced Research in Science, Engineering and Technology

Vol. 2, Issue 7 , July 2015

[11] A. Draber-Monko, T. Malewski, J. Pomorski, M. Los and P. Slipinski, "On the morphology and mitochondrial barcoding of the flesh fly Sarcophaga (Liopygia) argyostoma (Robineau-Desvoidy, 1830) (Diptera: Sarcophagidae)—an important species in forensic entomology", Annals of Zoology, 59(4), pp. 465–493, 2009.

[12] L.A. Nelson, J.F. Wallman and M. Dowton, "Using COI barcodes to identify forensically and medically important blowflies", Medical and Veterinary Entomology, 21, pp. 44–52, 2007.

[13] M. Hajibabaei, D. Janzen, J. Burns, W. Hallwachs and P. Herbert, "DNA barcodes distinguish species of tropical Lepidoptera", Proceedings of National Academy of Science USA, 103, pp. 968–971, 2006.

[14] J. Rivera and D.C.Currie, "Identification of Nearctic black flies using DNA barcodes (Diptera: Simuliidae)", Molecular and Ecological Resources, 9, pp. 224–236, 2009.

[15] S.L. Ball, P.D.N. Hebert, S.K. Burian and J.M. Webb, "Biological identifications of mayflies (Ephemeroptera) using DNA barcodes", Journal of North American Benthological Society, 24, pp. 508–524, 2005.

[16] M.A. Smith, N.E. Woodley, D.H. Janzen, W. Hallwachs and P.D.N. Hebert, "DNA barcodes reveal cryptic host-specificity within presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae)", Proceedings of National Academy of Science USA, 103, pp. 3657–3362, 2006.

[17] R.A. Senior-White, D. Aubertin and J. Smart, "Diptera. Family Calliphoridae". The Fauna of British India, including the remainder of the Oriental region. **6**.xiii, Taylor & Francis, Ltd., London, pp. 288, 1940.

[18] B.C. Nandi, "Fauna of India and the adjacent countries. Diptera, Sarcophagidae. Director, Zoological Survey of India (Ed.), Kolkata, pp. Vol. 10**,** pp. 60, 2002.

[19] E.S. Coen, J.M. Thoday and G. Dover, "Rate of turnover of structural variants in the rDNA gene family of Drosophila melanogaster" Nature, 295, pp. 564-568, 1982.

[20] F.A.H. Sperling, G.S. Anderson and D.A. Hickey, "A DNA-based approach to the identification of insect species used for postmortem interval estimation. Journal of Forensic Sciences, 39(2), pp. 418-427, 1994.

[21] Z. Song, X. Wang and G. Liang, "Phylogenetic relationship 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(3), pp. 298-306, 2008.

[22] N. Bajpai and R.R. Tewari, "Mitochondrial DNA sequence-based phylogenetic relationship among flesh flies of the genus Sarcophaga (Sarcophagidae: Diptera)", Journal of Genetics, 89, pp. 51-54, 2010.

[23] F. Alessandrini, M. Mazzanti, V. Onofri, C. Turchi and A. Tagliabracci, "MtDNA analysis for genetic identification of forensically important insects", Forensic Science International: Genetics Supplement Series, 1, pp.584-585, 2008.

[24] Y. Guo, C. Jifeng, W. Xinghua, L. Lingmei, L. Qinlai, L. Xiang, C. Tunfeng, M. Zhong, w. Xiang and W. Jifang, "Identification of forensically important sarcophagid flies (Diptera: Sarcophagidae) based on COI gene in China", Romanian Journal of Legal Medicine, 18, pp. 217-224, 2010.

[25] Y.D. Guo, J.F. Cai, X. Li, F. Xiong, R.N. Su, F.L. Chen, Q.L. Liu, X.H. Wang, Y.F. Chang, M. Zhong, X. Wang and J.F. Wen, "Identification of the forensically important sarcophagid flies Boettcherisca peregrina, Parasarcophaga albiceps and Parasarcophaga dux (Diptera: Sarcophagidae) based on COII gene in China", Tropical Biomedicine, 27, pp. 451-460, 2010.

[26] S.W. Tan, M. Rizman-Idid, E. Mohd-Aris and H. Kurahashi, "DNA-based characterisation and classification of forensically important flesh flies (Diptera: Sarcophagidae) in Malaysia", Forensic Science International, 199, pp. 43-49, 2010.

[27] T. Stamper, G.A. Dahlem, C. Cookman and R.W. Debry, "Phylogenetic relationship of flesh flies in the subfamily Sarcophagidae based on three mtDNA fragments (Diptera: Sarcophagidae)", Systematics Entomology, 38(1), pp. 35-44, 2013.

[28] M.L. Harvey, I.R. Dadour and S. Gaudieri, "Mitochondrial DNA cytochrome oxidase I gene: potential for distinction between immature stages of some forensically important fly species (Diptera) in western Australia", Forensic Science International, 131, pp. 134-139, 2003.