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DNA based identification of scuttle flies (Diptera: Phoridae) & their forensic importance: A review

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Abstract
Scuttle flies (Diptera: Phoridae) comprises necrophagous flies commonly found in and around the place of crime and are vital indicators of indoor hygiene. Scuttle flies can be found in conditions such as enclosed rooms where necrophagous insects are difficult to found, thus are used as forensic evidence. This paper reviews the entire literature pertaining to DNA based identification of scuttle flies and their use in forensic entomology.

Keywords: COI gene, Diptera, forensic entomology, Phoridae, PMI estimation

Introduction

Scuttle flies are amongst the insects with varied ecological backgrounds and diverse mrophology [1, 2]. Family Phoridae consists of 4000 species under 230 genera [3,4]. These flies have low small heads, having dark eyes, bodies with black, brown, or yellowish colour, and humped backs. The presence of costal veins is an important feature in these flies which extends only half of along with the anterior margin of wing. Disney [1, 4], included the globose third antennal segment to the list of characters. These flies prefer indoor human waste as food so can be used as an major hygiene indicator [4].

Scuttle flies have important position in Diptera order of forensic entomology because these flies being small in size can penetrate small gaps and enter dead bodies faster as compared to forensically important calliphorid and sarcophagid flies [2, 5, 6]. Phorid flies represent themselves as potential indicator for minimum post-mortem interval (PMImin) estimation when in the absence of other forensically important flies [2, 7, 8]. Phoridae breed on dead decaying matter and some are parasites and still other develops in fungi. In larval stage, some species are predators and breed in dead snails (Genus *Spiniphora*) but *S. bergenstammi* (Mik) is frequently recorded from dirty milk bottles. Many species belonging to genera *Anevrina*, *Conicera*, *Diplonevra*, *Dohrniphora*, *Metopina*, *Tripleba* and *Megaselia* are regularly found in and around vertebrate corpse [9].

DNA based identification of scuttle flies

A possible new cryptic species complex in a population of scuttle flies attracted towards ant species was described using the 420 bp sequences of the COI gene. Mitochondrial DNA sequence comparisons of individuals from several locations and host ant species showed high and low sequence divergence in inter and Intra-population respectively [10].

In central western Argentina record of five parasitic fly species was food and a new host, *Solenopsis quinquecuspis* Forel was surveyed. A large form of *Pseudacteon obtusus* Borgmeier attacking *Solenopsis gayi* in Chile was found. *Solenopsis gayi* is said to be genetically closer to the Argentine fire ants (*saevissima*-group) than the North American *S. geminata*-group. Monophyly of *P. obtuse* was supported using mtDNA and showed the similarity of Chilean population with that of eastern Argentina population [11].

A preliminary study of 30 exemplars *Melaloncha* species was reported with the help of combination of nuclear (28S and CAD) and mitochondrial (12S, 16S, ND1, and CO1) genes. Phylogenetic results using 3,306 bp showed that monophyletic genus are *Melaloncha* and subgenus *M.* (*Udamochiras*). *M.* (*Udamochiras*) is a sister-group to *M.* (*Melaloncha*) and all multiple species groups are monophyletic [12].

Phylogenetic analysis of 70 species of *Melaloncha* Brues was carried out with the help of six genes (COI, 16S ribosomal DNA, 12S and 28S ribosomal DNA, NADH1 dehydrogenase, and CAD), moreover 47 morphological characters were also evaluated. A single most parsimonious tree was produced and similar results were also seen using Bayesian analysis of morphology + molecules characters with a few differences [13].

At the molecular level, a 658bp long fragment of COI gene was the basis of identification of larvae and pupae of six phorid species [14]. Another molecular phylogenetic study using nuclear and mitochondrial genes for a total of 2220bp was carried out in phorid genus *Anevrina*. *Anevrina* was recovered as a monophyletic lineage in both maximum parsimony and Bayesian analysis when compared with a sampling of 13 genera from 4 subfamilies [15].

Authors demonstrated relationships amongst 14 Chinese genera of Phoridae on the absis of sequences of the mitochondrial 12S and 16S rRNA genes morphologically and phyogenetically. A 77.7% A+T bias alignment of the concatenated sequences was observed The close relationship was found in *Diplonevra* and *Dohrniphora.* Similarly, *Anevrina*, *Conicera*, and *Spiniphora* showed close relationship. The *Metopina* genus and *Puliciphora* are found to be reciprocal sister groups and a close relationship was found in between *Gymnophora* and *Phalacrotophora* through cluster analysis [16].

Hash *et al*. [17] phylogenetically analyzed DNA sequences of nuclear CAD (rudimentary) and mitochondrial NDI, COI, and 2817 bp 16S genes recovered exemplars of the genus *Dohrniphora* as a monophyletic lineage.

55 Korean food-associated insect pests were identified using 658 bp region of COI gene. Mean genetic divergence among

individuals of a species between species belongs to one genus in *Blattaria* was 0.59%/13.18%, Coleoptera showed 0.84%/20.10%, in Hymenoptera it was 0.02%/22.61%, Lepidoptera observed 0.24%/3.84% and Diptera had 0.17%/15.90%. Phorid species studied were *Megaselia scalaris* (Loew), *Megaselia longicostalis* Wood, *Megaselia rufipes* (Meigen and *Megaselia subtumida* (Wood) [18].

In a first in-depth molecular phylogenetic study, *Dohrniphora* was dealt as monophyletic group based on 2,327 bp of nuclear CAD (rudimentary) and mitochondrial ND1, CO1, and 16S rDNA genes [19]. A complete mitochondrial genome (15,599 bp) of *Megaselia scalaris* was sequenced. It was observed that 37 genes showed identical arrangement with other insects and each of the base compositions on the heavy strands are like A: 38.87%, G: 13.74%, C: 9.46%, T: 37.93%, and the A + T content 76.80% [20].

Haggqvist *et al.* [21] supplemented morphological identification of Sweden phorid with molecular identification using COI and 28S markers and emphasized a separate status for *Megaselia subnitida* Lundbeck, 1920, which was previously taken as a synonym of *Megaselia lucifrons* Schmitz, 1918. The previously unknown third species *Megaselia albalucifrons* sp. n. in the group was also described.

Machkour-M'Rabet *et al.* [22] identified the parasitoid as *Megaselia scalaris* using COI and 16D rRNA genes. It is said to infest an endangered tarantula, *Brachypelma vagans* Ausserer 1875. The infestation was severe as more than 500 fly larvae were found within the host. A neighbor-joining phylogenetic tree using COI gene showed its relationship among other closely related dipteran flies [23].

A case 60 years old Iranian male patient suffering from urinary myiasis and urinary tract infection was reported by Solgi *et al.* [24]. Larvae recovered from urine of patient were identified morphologically and at the molecular level (using the COI gene) as *Megaselia scalaris* Loew [24].

To study phylogenetic relationships (using Bayesian and Maximum Likelihood methods) seven loci were used. These loci were 16S, COI, NDI, 28S, AK, CAD, and TPI. The subgenus *Mesophora* Borgmeier was kept deep inside the genus and synonymized with *Apocephalus* while pre-existing morphological taxonomic relationships were upheld. The species of *Mesophora* belongs to the *Apocephalus wheeleri* group [25]. Lancu *et al.* [26] confirmed the COI-based identification of adult female and III instar larvae of *Conicera similis* in accordance with GenBank reference sequence for *C. similis*.

20 species of *Pseudacteon* with already described species were analyzed using COI and nuclear sequences. However, due to the presence of presence of putative cryptic species the species richness has been underestimated [27].

Identification of 23 parasitic Diptera samples using partial 634bp fragment of COI gene and study of ecology of the parasitoids of termites of Thailand were carried out. *Macrotermes gilvus* (Hagen) soldier termites were found from 25 mounds. The BLAST results in *M. scalaris* observed 98.08–99.84% similarity to the NCBI database and 0.0–3.2% of intraspecific variation was observed. Samples showed 97.51–99.20% similarity to a lineage of *Megaselia scalaris* from China and 97.87–99.39% similarity was observed in a lineage of the same species from Cameroon [28].

The COI gene of 79 larvae of scuttle flies collected from 20 post mortems was sequenced and phylogenetically analyzed. Six mitochondrial haplogroups and two of them matched to foreign Phoridae fly species haplotypes, *Megaselia scalaris* (Loew, 1866) and *M. spiracularis* Schmitz 1938 were identified. However, the taxonomies of five other haplogroups, with nucleotide distances ranging from 1.68% to 2.26% from the *M. scalaris* group were not confirmed [4].

A largest molecular identification study comprising 175 Nordic samples belonging 145 species of *Megaselia* using 28S rDNA, ND1, COI and 16S genes was presented. Of the 22 well supported clades of informal *Megaselia* groups, 20 comes in a moderately well-supported monophyletic clade of 'core *Megaselia*' whereas 2 species groups comes outside of core *Megaselia* [29].

Characterization of 71 species of Phoridae which belongs to 9 genera was carried out with the help of molecular markers like COI, 28S rRNA, and *Arginine kinase*. Except four *Megaselia* Rondani, 1856 species, the results specified that morphologically delimited species were in agreement with the molecular analyses phylogenetic trees constructed using gene sequences. [30].

Pedraza-Lara *et al.* [31] sequenced COI gene from samples in the Valley of Mexico at a Nature Reserve after morphological identification. Authors have identified 42 species under nine families of Diptera from the 339 individuals analyzed. Out of identified families, 9 species were from Calliphoridae, 7 species from Sarcophagidae, and 6 species from Phoridae were the richest.

3 provinces and 1 municipality in China were the sites for the collection of Parasitic flies from *Spodoptera frugiperda.* These were identified as *Megaselia scalaris* using morphological characters and molecular markers (COI and 28S rRNA). The parasitic ability of *M. scalaris* is the first time reported against invasive pest *S. frugiperda.* A homologous similarity was showed with scuttle flies and clustered with *M. scalaris* [32].

Sequencing and annotation of complete mitochondrial genome of *Metopina sagittata* (Liu) and *Puliciphora borinquenensis* (Wheeler) species was evaluated. The complete mitochondrial genome is sequences of *M. sagittata* and *P. borinquenensis* were 15,640 bp having 75.97% of A+T content and 15,429 bp with an A+T content of 75.38%, respectively alongwith 13 protein-coding genes, 22 tRNA genes, 2 rRNA genes, and 1 control region located between rrnS and trnI which was 808 bp for *M. sagittata* and 746 bp for *P. borinquenensis*. The study also proved that Platypezidae and Phoridae are sister families and closely related phylogenetically. [33].

Conclusion

The present paper reviews the works on the mitochondrial COI gene-based identification of phorid species and their forensic importance. All the workers believed that this marker has a lot of potential to distinguish Indian as well as world species of the family Phoridae.

It can be supplemented with traditional morphological identification and therefore the workers are encouraged to pursue research in this area.

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