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Molecular Identification of Forensically Relevant Diptera: Missed Important Criteria

Forensic Sciences

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ABSTRACT

Molecular identification of forensically relevant Diptera is a valuable tool in forensic entomology. Many contemporary studies have been evaluated the discrimination between closely related species based on different cytochrome oxidase I gene (COI) fragments. One of those recently tested markers is 278-bp COI gene which has been proven its success in discrimination between closely related species belonging to one family. To make a thorough investigation to any molecular marker, we need to evaluate its discrimination power not only between closely related species but also between species from different families. Therefore, we herein assessed the utility of that marker in identification of 15 species of 3 families. The results of present investigation revealed odd relationship and distribution of some tested species in phylogenetic tree. These results confirmed that the inclusion of species from different families "ignored criteria' on the same level of importance as inclusion of closely related species.

Introduction:

Forensic entomology has a wealth of information to offer the criminal justice system. The study of entomological evidence is focused mainly on accurate calculation of variables [manner, place and time of death].[1] A dead body is an attractive site for insect colonization and development. Traditional (morphological-based) method is difficult and complicated. Thus, contemporary studies exhaustively evaluate gene sequences because these constitute the fastest and most accurate method of species identification.[2,3] Currently, COI gene has been suggested as a standard barcode gene for animal groups. However, scientists have found it less effective in some taxon groups.[4] The search for the most suitable gene fragment for species identification is not over, with several recent studies testing the efficiencies of different sequence of COI gene to look for the optimal DNA barcode gene.[5-7] Recently, different studies proved that 278-bp COI region has sufficient discrimination power.[8,9] Because these studies evaluate the discrimination power of that genetic marker only within closely related species; belong to one family. In tendency to make a thorough assessment to that marker, we need to compare between the distributions of most common forensically important species of different families in phylogenetic tree based on molecular marker and morphological method. This is of great importance because with database construction, unknown samples will be compared with all reference sequences.[6,7,10] Consequently, that will help in evaluating its power in identifying new cryptic species and validating the reliability of any independent marker.[11]

Materials and Methods:

In the present study, 278-bp COI sequences of forensically important flies; that have been deposited within GenBank, have been collected. The targeted marker was 278-bp fragment of the mitochondrial COI which corresponded to positions 2523-2798 on COI gene of Drosophila yakuba (GenBank accession number X03240). Fifty four sequences of 15 species and 3 families were aligned using ClustalW. Phylogenetic analyses were conducted in MEGA5. The 278-bp fragment of COI gene was assessed as a potential marker for the identification of common fly species *(Calliphoridae: Chrysomya megacephala, C. rufifacies, Lucilia sericata, L. bazini, L. porphyrina, L. caesar, Calliphora vicina, Protophormia terraenovae, Hemipyrellia ligurriens; Sarcophagidae: Sarcophaga peregrine, S. dux, S. albiceps, S. melanura; Muscidae: Musca domestica, Ophyra spinigera).*

Results and Discussion:

The monophyletic branches of the phylogenetic tree **(Figure 1.)** with high bootstrap values (≥94%); except for *S. peregrine* at 55%, initially revealed that this marker could be suitable for discrimination among these 15 species of three families, especially between some morphologically similar sister species. Within Muscidae and Sarcophagidae families, the tested species belonging to each family formed an isolated group. Within Calliphoridae family, the 9 tested species of 5 genuses (Chrysomya, Lucilia, Calliphora, Protophormia and Hemipyrellia) failed to group together. All Calliphorid species were not assigned appropriately. Two Chrysomyinae species (*C. megacephala, C. rufifacies*) are clustered together, whereas the only species belongs to Protophormia (*P. terraenovae*) joined with Muscidae group before joining with other Calliphoridae. All Lucilia species succeeded to join together while *H. ligurriens* embedded within this tribe. This relationship has been observed before by other studies analyzed longer COI fragment.[12] Unexpectedly, *C. vicina* embedded also within Lucilia tribe although it belongs to Calliphora. This odd relationship has not been observed before even with analysis based on using the same marker that could be attributed to inclusion of more species; belonging to different families, in the present investigation. That consequently confirms the importance of make thorough investigation to any marker before construction of local database and introduces this tool to real cases. The present investigation revealed that although success of 278-bp fragment to separate closely related species but it failed to distribute them in accordance with that based on morphological method. Therefore, its solo use may lead to misidentification. That is in agreement with other recent studies which revealed the danger of sole reliance on other short mitochondrial marker but these markers could have potential utility to use as supplementary means in identification. [6,7,13,14]

In conclusion, these results highlight an important concern for anyone proposing a forensic DNA-based identification marker. Many studies has been labelled different criteria for validate the reliability of any molecular marker such as monophyletic separation of species with high bootstrap support, absence of overlapping between calculated intra and inter-specific variations as well as distribution of different species belonging to one family. But the distribution of genus/subfamilies/families in agreement with anatomical taxonomy must also consider as an important criteria especially if we need to evaluate the reliability of any independent identification marker.

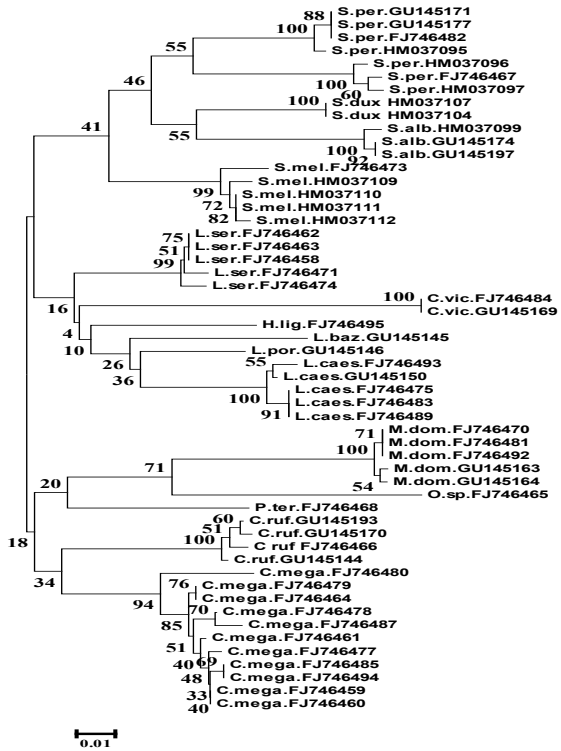

Figure 1. Neighbour-joining tree displaying relationships between sequences have been collected from GenBank database of 15 species belonging to different families based on 278-bp COI gene fragment. Bootstrap values indicate support for nodes and label for each sample include species name [*C. mega.: Chrysomya megacephala; C.ruf: C. rufifacies; C.vic: Calliphora vicina; P.ter.: Protophormia terraenovae; H.lig.: Hemipyrellia ligurriens; L.ser.: Lucilia sericata; L.baz.: L. bazini; L.por.: L. porphyrina; L.caes.: L. caesar; S.per.: Sarcophaga peregrine; S.dux: S. dux; S.alb.: S. albiceps; S.mel.: S. melanura; M.dom.: Musca domestica; O.sp.: Ophyra spinigera*] and accession number. The bar indicates 0.01substitutions per site.

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