

Molecular identification of *Stearibia nigriceps*: An example of the usefulness of Cytochrome b gene for the identification of entomofauna species

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Abstract. In Forensics, the study of entomological evidence is focused on the accurate calculation of the variables “manner and place of death” and “postmortem interval (PMI)”. Carrion-feeding fly species morphological differentiation is often a difficult task. DNA-related methodologies, those based on mitochondrial DNA (mtDNA) in particular, are a powerful alternative to achieve species identification. For this reason, we have used the Cytochrome b (Cyt-b) gene to identify the necrophagous fly species *Stearibia nigriceps* (Meigen, 1826) (Diptera; Piophilidae) involved in the postmortem process. © 2005 Elsevier B.V. All rights reserved.

Keywords: Mitochondrial Cytochrome b (Cyt-b); *Stearibia nigriceps*; Piophilidae; Species identification; Forensic entomology

1. Introduction

The evidence collected during a postmortem investigation, frequently carrion-feeding flies, must be properly identified to specific level. These flies, which may be in different life stages (larval or adult) are by far the most ordinary insect evidence connected with a death investigation. They have a great amount of information associated with time, manner and place of death [1], any of which, if ascertained appropriately is highly valuable in the

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course of a postmortem investigation [2]. However, species typifying is not always an easy job.

DNA-based methodologies are highly reliable since they possess the following advantages: a) they provide higher specificity, sensitivity and a more rapid identification [3] and b) the identification from any life stage of the insect and any preservation method is very unlikely to be hampered [4].

All in all, the major objective of this work is to improve the analysis of cadaveric entomofauna setting a standard through the Cytochrome b (Cyt-b) gene species identification involved in the postmortem process. The species selected for this study was the necrophagous fly *Steiribia nigriceps* (Meigen, 1826) (Diptera; Piophilidae).

2. Methodology

Seventeen larvae of *S. nigriceps* were genetically analysed. The overall DNA extraction process included a cell lysis stage with Proteinase K and SDS, followed by a standard phenol-chloroform method for DNA purification. A 358 bp long fragment of the Cyt-b was successfully amplified via the PCR technique under Parson et al. [5] modified conditions. All the samples were sequenced using the dRhodamine Terminator kit (Applied Biosystems) method in an automatic ABI Prism 310 DNA sequencer.

3. Results and discussion

Sequence data from the amplified Cyt-b region were obtained for all specimens.

All sequences were aligned using the computer programme ClustalX. This operation permitted to set a consensus sequence (Fig. 1) which contains an invariable span of 86 bp exclusive of *S. nigriceps*. Thus, this singular characteristic can be used for singling out this species from any entomological source whenever present. The consensus sequence was then submitted to the on-line search engine BLAST (<http://www.ncbi.nlm.nih.gov/BLAST>). As there was no information related to this species Cyt-b at the time of the study, the results from the aforementioned search did not yield a single direct match. Although the limited information harboured in the database pertaining to carrion-feeding

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1  TTG  GCT  CTT  TAC  TTG  GGT
19 TAT  GTT  TAA  TTA  TTC  AAA
37 TTT  TAA  CGG  GTT TAT TTT
55 TAG CTA TAC ATT ATA CAG
73 CAG ATA TTA ATT TAG CIT
91 TCA ATA GAG TTA ATC ATA
109 TTT GTC GTG ATG TAA ATT
127 ATG GTT GAT TAT TAC GAA
145 CAC  TAC  ATG  CTA  ATG  GRG
163 CRT  CAT  TCT  TCT  TTA  TTT
181 GTA  TTT  ATC  TTC  ATG  TAG
199 GAC  GAG  GAA  TTT  ATT  ATG
217 GGT  CAT  ATC  TTT  ATA  CCC
235 CTA  CCT  GAT  TAG  TTG  GAG
253 TAA  TTA  TTT  TAT  TCT  TAG
271 TAA  TAG  CAA  CAG  CCT  TTA
289 TAG  GAT  AYG  TAT  TRC  CT

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Fig. 1. Shown is the consensus sequence of *S. nigriceps* which starts at the 3' end of the forward primer. Underscored is the span of 86 bp. The variable bases have been shadowed in grey.

flies Cyt-b is far from abundant, it allowed to know that the closest species were of necrophagous/myiasis-causing nature.

Whereas the use of Cytochrome oxidase subunits I and II (COI and COII) (which also possess essential species-specific information) for insects is commonplace, the use of Cyt-b gene is fairly rare. This work, bearing in mind the meagre insectwise information gathered world-wide, shows that the Cyt-b genetic differences within the order Insecta, are high enough to help differentiate at the species level.

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