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Molecular identification of arthropods by cytochrome *b* analysis

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Abstract. This work has been developed to improve the analysis of cadaveric entomofauna. Taking into account that the cytochrome *b* is widely use to identify vertebrates, we have used it for the identification of some necrophagous species involved in the postmortem process. © 2004 Published by Elsevier B.V.

Keywords: Cytochrome *b*; *Lucilia sericata*; *Piophila casei*; *Dermestes frischii*

1. Introduction

Entomological knowledge can reveal the manner or location of death, although is most often used to estimate the time of death, or postmortem interval (PMI). Insects collected as evidence during a postmortem investigation should be preserved as eggs, larva or adult. Preimaginal stages can be difficult or impossible to identify to specific level, and an incorrect or uncertain identification can seriously harm or impede an investigation. This is because adult arrival times, egg phase duration and larval growth rates can vary between species. Proper species identification is usually an essential first step in the use of entomological evidence in a legal investigation [1]. An alternative source for species identification information is mitochondrial DNA. We selected the mtDNA region that codes for cytochrome *b*. This gene contains species-specific information and has been used in phylogenetic as well as in forensic investigations in a number of studies [2].

The main objective of this work is to improve the analysis of cadaveric entomofauna. Taking into account the difficulty to identify eggs, larva and maggots, we have used the

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Table 1
Clustal W(1.5) multiple sequence alignment

L. sericata GACTAAG-AATAAAATAATTACTCCT-ACTAATCAAGTTGTGTGTAAATAAGTATGAAC
P. casei GACTAAG-A**T**TAAAAT**C**ATTACTCCT**C**ACTAATCAAGTT**T**G**G**TGTAAATAA**A**TATGAAC
D. frischii GACTAAG**GC**AATAAAATAATTACTCCT**C**ACTAATCAAGTTG-GTGTAAATAAGTATGAAC
L. sericata CGTAATATATTCCTCG-TCCTACATGTAAGT-AAATACAAATAAAGAAAAATGATGC**T**-C
P. casei CGTAATATATTCCTCG**A**TCCTACATGTAAGT**G**AAATACAAATAAAGAAAATGATGCG-C
D. frischii CGTAATATATTCCTCG-TCCTACATGTAAGT-AAATACAAATAAAGAAAAATGATGCG**T**C
L. sericata C**C**TTAGCATGTATAGTTCGTAATAATCAACCAT**C**ATTTACGTCTCGGCAAAT**C**ATGATTT
P. casei CATTAGCATGTATAGTTCGTAATAATCAACCATAATTTACGTCTCGGCAAAT-ATGATTT
D. frischii CATTAGCATGTATAGTTCGTAATAATCAACCATAATTTACGTCTCGGCAAAT-ATGATTT
L. sericata ACTCT**A**TTAAAAGCTA-GTCTAATATC**C**TGCTGTGTAATGT**C**TAGCTAAAAATAA
P. casei ACTCTGTTAAAAGCTA**A**GTCTAATATC-TGCTGTGTAATGTATAGCTAAAAATAA
D. frischii ACTCTATTAAAAGCTA-GTCTAATATC-TGCTGTGTAATGTATAGCTAAAAATAA

cytochrome b to identify some important necrophagous species involved in the postmortem process.

2. Methodology

Lucilia sericata, a pioneer, *Piophila casei* arriving during fat saponification of corpses and *Dermestes frischii*, responsible of feeding on advanced decayed and mummified corpses, have been studied. They were properly identified and preserved in 96% EtOH for further studies. Three specimens of each species were selected for genetic analysis. In order to avoid human contamination, we have extracted DNA from muscle of one leg of each specimen. Cell lysis was performed using proteinase K and SDS and the DNA was purified by the phenol–chloroform method. Cytb was amplified under the conditions described by Parson et al. [2]. All the samples were sequenced using the dRhodamine Terminator kit (Applied Biosystems) method in an automatic ABI Prism 310 DNA sequencer. The sequences of insects of the same species were aligned using the Clustal V

Table 2
Cytb sequences producing significant alignments using the online BLAST search engine

	Score bits	*E*-value
L. sericata		
gi | 13384216 | gb | AF352790.1| *Chrysomya putoria* mitochondrion, cytb	341	3e – 91
gi | 1166529 | gb | U37541.1 | DMU37541 *Drosophila melanogaster*	283	6e – 74
gi | 7799401 | emb | AJ400907.1 | DME400907 *Drosophila melanogaster*	283	6e – 74
P. casei		
gi | 13384216 | gb | AF352790.1| *Chrysomya putoria* mitochondrion, cytb	230	1e – 57
gi | 12923 | emb | X03240.1 | MIDYRRN *Drosophila yakuba* complete mt	190	9e – 46
gi | 5101664 | emb | AJ242872.1 | CCA242872 *Ceratitis capitata* comp mt	174	5e – 41
D. frischii		
gi | 13384216 | gb | AF352790.1| *Chrysomya putoria* mitochondrion, cytb	418	e – 114
gi | 12923 | emb | X03240.1 | MIDYRRN *Drosophila yakuba* complete mt	337	7e – 90
gi | 1166529 | gb | U37541.1 | DMU37541 *Drosophila melanogaster* com mt	329	2e – 87

program. The obtained sequences were used to identify the species of the samples by aligning to the cytb gene sequence entries using the online BLAST search engine of NCBI.

3. Results and discussion

Sequence data from a 358-bp region cytb were obtained for all specimens. The results of alignment by Clustal V showed that the sequences of insects, which are closest relatives, are almost indistinguishable. On the contrary, insects that belong to different species showed a high degree of divergence (Table 1). The cytb sequences of species here studied are not included in the database of GenBank. Thus, no significant coincidences were observed. However, the closest BLAST alignments were found between our species and *Chrisomia* and *Drosophila* genera (Table 2).

For insects, it is very common to sequence some or all of cytochrome oxidase subunits I and II (COI and COII). For vertebrates species, it is most common the cytochrome b gene analysis. Other gene commonly used to identify species is 16S RNA.

Our work has demonstrated that there are several differences in the base sequences of the cytb gene between arthropod species. Thus, the cytb gene can be added to the genes above mentioned to improve our ability to identify the insect species.

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