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Genetic identification of forensically important Calliphoridae in Portugal

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Abstract. In Medico-legal Entomology identifying species is an important first step in the investigation. The present study has been developed to improve the genetic data of the cadaveric entomofauna in Portugal. © 2006 Published by Elsevier B.V.

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1. Introduction

Medico-legal entomology, one area in the broad field of entomology, is routinely applied in forensic applications.

The availability of an accurate postmortem interval (PMI) can influence the overall direction of an investigation and the interpretation of entomological evidence may eventually be the deciding factor in the determination of guilt or innocence in a court of law [1].

Identifying the insect species is an important first step in the investigation process, but morphological identification of immature stages can be difficult and sometimes impossible, due to the similarity between different species.

In order to overcome the difficulties associated with the classical methods, others have been developed; one of them is the genetic identification. This method provides a rapid and accurate species determination, in any stage and even when specimens are damaged.

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Species from the Calliphoridae family give information relating to the accurate estimation of the PMI, as they are among the first insects to discover and colonize human remains. They are attracted to carrion and a large number of eggs are commonly placed in natural body openings and wounds that are exposed.

This study describes DNA sequences data of the mitochondrial region of the subunit I of cytochrome oxidase (COI) gene. To date many geographical regions were studied, but Portugal presents a total lack of genetic data collected on the main species of forensic interest. The goal of this study is to improve the genetic data knowledge of cadaveric entomofauna in Portugal.

2. Material and methods

Maggots were collected from 24 human bodies during autopsy procedures in the National Institute of Legal Medicine, from June 2003 to August 2005. Maggots were killed in pure ethanol and preserved at − 80 °C until needed for DNA extraction. Maggots were placed in sterile Petri plates and washed in 20% of bleach solution to remove potential external contaminants, according to [2].

DNA was extracted using the two methods: DNeasy® Tissue Kit (Qiagen) following the manufacturer protocol for animal tissues and BioRobot® EZ1 workstation using EZ1 DNA Forensic Card (Qiagen), following the manufacturer protocol for purification of genomic DNA from tissue.

A region of COI gene was amplified using the PCR primers pairs C1-J-2495/C1-N-2800 and C1-J-2495/TL2-N-3014, the primer sequences were described in [3].The PCR was carried out in a thermocycler GeneAmp® PCR System 9700 (Applied Biosystems) and programmed according to [3]. PCR products were purified using MinElute™ PCR Purification Kit (Qiagen) according to the manufacturer's protocol.

PCR product was cycle sequenced using the PCR primers separately, with BygDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems), and removal of unincorporated dye terminators was accomplished by DyeEx™ 2.0 Spin Kit (Qiagen), all according to the manufacturer's recommendations. Each strand was sequenced using the same primers employed for PCR. Electrophoretic separation and detection of the sequencing reaction products were performed using an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems).

The obtained sequences were confirmed and aligned manually to identify species, using the online BLAST search engine of the National Center for Biotechnology Information (NCBI) [4]. Sequences where a similarity of the query is found are displayed according to the degree of sequence match.

3. Results and discussion

A fragment of COI was successfully amplified for the 141 maggots studied. The obtained sequences were compared to those in the database of GenBank and the maximum scoring segment pair (MSP) was found. The information content within the nucleotide sequence of the COI gene enabled the identification of 104 species (MSP = 100%). In 37 specimens, values from 97% to 99% were found (data not shown). In these maggots other primer pairs should be tried in order to obtain a 100% MSP values.

Table 1
Species found collected from 24 human bodies during autopsy

Case	Sex	Place	Season	Stage of decomposition	NSS	Species
1	Male	Field	Winter	Putrefied	3	*Calliphora vomitoria*
					1	*Calliphora vomitoria*
2	Male	House	Winter	Putrefied	1	*Calliphora vicina*
3	Male	House	Winter	Putrefied	5	*Calliphora vicina*
4	Male	House	Spring	Putrefied	1	*Calliphora vicina*
5	Male	Field	Spring	Putrefied	2	*Calliphora vomitoria*
6	Male	Field	Spring	Putrefied	2	*Calliphora vomitoria*
7	Male	House	Summer	Putrefied	7	*Chrysomya albiceps*
8	Male	Road	Summer	Putrefied	9	*Chrysomya albiceps*
					2	*Lucilia illustris*
9	Male	Field	Autumn	Putrefied	1	*Lucilia caesar*
10	Male	House	Winter	Putrefied	4	*Calliphora vicina*
11	Male	Field	Winter	Putrefied	5	*Calliphora vicina*
12	Male	?	Winter	Putrefied	3	*Calliphora vomitoria*
13	Male	?	Summer	Putrefied	2	*Chrysomya albiceps*
14	Male	?	Summer	Putrefied	7	*Chrysomya albiceps*
15	Male	House	Summer	Putrefied	5	*Lucilia sericata*
					3	*Lucilia sericata*
16	Male	House	Summer	Putrefied	2	*Calliphora vicina*
17	? (fetus)	Field	Summer	Putrefied	1	*Lucilia sericata*
					1	*Lucilia sericata*
18	Male	Field	Summer	Putrefied	3	*Chrysomya albiceps*
19	Male	?	Summer	Putrefied	7	*Chrysomya albiceps*
					1	*Calliphora vicina*
20	Male	Field	Summer	Putrefied	1	*Lucilia illustris*
21	Male	?	Summer	Putrefied	8	*Lucilia sericata*
22	Male	Field	Summer	Putrefied	6	*Chrysomya albiceps*
23	Female	House	Summer	Putrefied	5	*Lucilia sericata*
24	Female	?	Summer	Putrefied	6	*Chrysomya albiceps*

NSS—number of specimens studied.

This work does not include all insect species that an investigator might find during autopsy, but it represents their general appearance. Further collections should proceed to improve this study (Table 1).

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