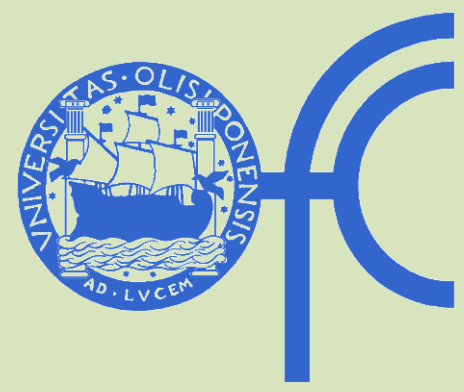


# FORENSIC ENTOMOLOGY: NUCLEAR AND MITOCHONDRIAL MARKERS FOR DIPTERA AND COLEOPTERA IDENTIFICATION

S. Ferreira <sup>(1,2)</sup>, A. R. Oliveira <sup>(1,2)</sup>, M. T. Rebelo <sup>(1,2)</sup>, D. Dias <sup>(1,2)</sup>

1) University of Lisbon, Faculty of Sciences, Department of Animal Biology, Lisbon, Portugal  
2) Center for Environmental and Marine Studies - CESAM, Lisbon, Portugal



## INTRODUCTION

The presence of entomological evidences can be of great importance to solve some forensic investigations. Hence, species identification of insect found in cadavers is extremely important. Molecular techniques are faster, easier and more reliable. In this direction becomes necessary the establishment of molecular markers suitability to differentiate species.

Thus, in this study it was intended to infer about COI, CytB and ITS2 suitability for insect species identification.



Cytochrome c oxidase subunit I (COI) is the most used *locus* for insects molecular identification, however there is no agreement about which *loci* should be used <sup>[1]</sup>. Also, in some studies, cytochrome B (CytB) has been used for this purpose, with successful results <sup>[2]</sup>. Furthermore, mitochondrial markers have been proven to have some limitations for insect species identification. For example, it was shown that there are high levels of overlap in inter and intraspecific distances to certain species <sup>[3]</sup>. Hence, it becomes necessary to complement insect specimens identification with a nuclear marker <sup>[4]</sup> such as second internal transcribed spacer (ITS2).

## RESULTS AND DISCUSSION

19 COI and 35 ITS2 samples were successfully identified (sequence identity  $\geq 98\%$ ). For other samples, identification values were below established boundary. No significant identification values were obtained for CytB sequences.



It was possible to identify more specimens crossing obtained results for all markers.

Phylogenetic analyses and nucleotide divergences were performed only for COI and CytB. ITS2 sequences length variation prevented an accurate alignment. Nevertheless, it was observed that there were almost no differences within species.



Maximum parsimony (MP) analyses for both, COI (Figure 1) and CytB, were in accordance.

COI and CytB intraspecific divergences showed a range of values above and interspecific divergences below 3% (threshold above what species can be distinguished).

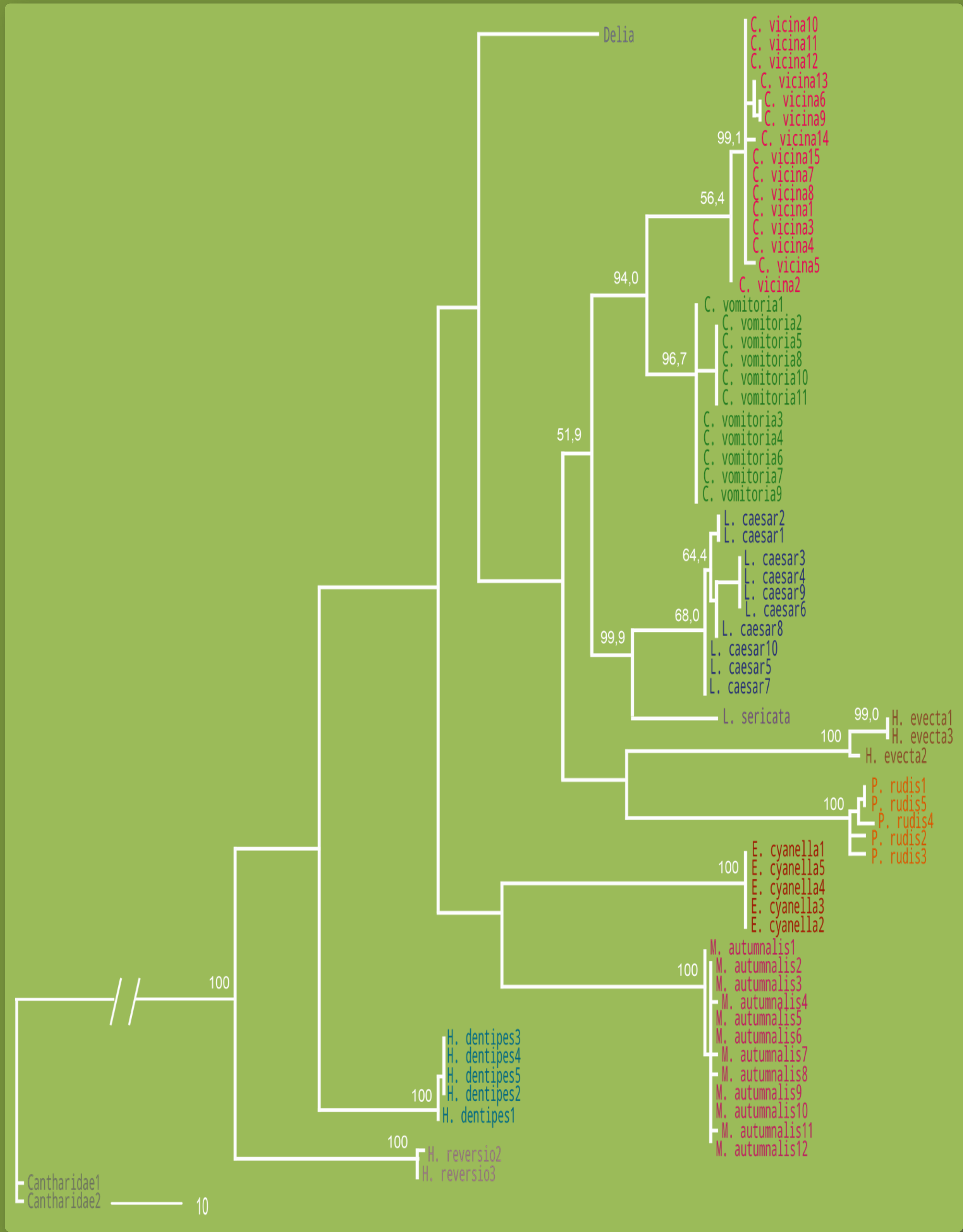


Figure 1 – Phylogram obtained through Maximum Parsimony method, for COI data of all studied specimens. Bootstrap values present on tree branches indicate support for nodes. C= *Calliphora*; L= *Lucilia*; H= *Helina*; P= *Pollenia*; E= *Eudasyphora*; M= *Musca*; H= *Hydrotaea*.

## MATERIAL AND METHODS

It was used DNA samples from Diptera species: *Calliphora vicina*, *Calliphora vomitoria*, *Musca autumnalis*, *Lucilia caesar*, *Lucilia sericata*, *Helina evecta*, *Helina reversio*, *Eudasyphora cyarella*, *Pollenia rudis* and *Hidrotea dentipe*, and Coleoptera from Cantharidae family. 52 COI sequences were already matched and identified; 21 COI, 14 CytB and 72 ITS2 amplicons were PCR amplified using specific primers (according to <sup>[5,6,7]</sup> respectively). Amplification products were purified and sequenced with the same primers pair. Sequences were matched in Blastn and BOLD-IDS. Filogenetic analysis (Maximum Parsimony) and nucleotide divergences (corrected - GTR model - and uncorrected – p-distance) were performed using PAUP\* v4.0b10 software.



## CONCLUSIONS

- ❖ The best results were obtained for COI, with good support.
- ❖ More accurate results can be obtained using more than one genetic marker.
- ❖ ITS2 has proven its suitability to differentiate insect species.
- ❖ More sequences of these markers, from necrophagous insects, are needed in databases, in order to improve molecular identification.



### REFERENCES

[1] J.D. Wells and J.R. Stevens, Application of DNA-based methods in forensic entomology, *Annu. Rev. Entomol.* 53 (2008) 103-120.  
[2] M. Sombela, The origin of groundnut infestation by the seed beetle *Caryedon serratus* (Olivier) (Coleoptera: Bruchidae). Results from cytochrome B and ITS1 gene sequences, *J. Stored Prod. Res.* 42 (2006) 97-111.  
[3] M. Wiemers and K. Fiedler, Does the DNA barcoding gap exist? - a case study in blue butterflies (Lepidoptera: Lycaenidae), *Front. Zool.* 4 (2007) 8.  
[4] M.J. Raupach, J.J. Astrin, K. Hannig, et al., Molecular species identification of Central European ground beetles (Coleoptera: Carabidae) using nuclear rDNA expansion segments and DNA barcodes, *Front. Zool.* 7 (2010) 26.  
[5] P.D. Hebert, A. Cywinski, S.L. Ball, et al., Biological identifications through DNA barcodes, *Proc. Biol. Sci.* 270(1512) (2003) 315-321.  
[6] W. Parson, K. Pagarano, H. Niederstatter, et al., Species identification by means of the cytochrome b gene, *Int. J. Legal Med.* 114(1-2) (2000) 23-28.  
[7] Z. Song, X. Wang, G. Liang, Species identification of some common necrophagous flies in Guangdong province, southern China based on the rDNA internal transcribed spacer 2 (ITS2), *Forensic Sci. Int.* 175(1) (2008) 17-22.

