Forensic Science International: Genetics Supplement Series 7 (2019) 132–134

Contents lists available at ScienceDirect

Forensic Science International: Genetics Supplement Series

journal homepage: www.elsevier.com/locate/fsigss

Application of HRM-PCR (high resolution melting PCR) for identification of forensically important Coleoptera species

Tadeusz Malewski[a], Marta Łoś[a], Ireneusz Sołtyszewski[b,*]

[a] Museum and Institute of Zoology, Polish Academy of Sciences, 64 Wilcza St., 00-679 Warsaw, Poland
[b] Department of Criminalistics and Forensic Medicine, University of Warmia and Mazury in Olsztyn, 11 Dybowskiego St., 10-719 Olsztyn, Poland

ARTICLE INFO

Keywords:
Coleoptera
HRM-PCR
Species identification
Forensic
Entomology

ABSTRACT

Precise estimation time of death is one of key task of forensic entomology. Especially interesting is Coleofauna present at all stages of cadaver decomposition. The morphological identification of Coleoptera species from varying life stages to species level is time-consuming and needs highly qualified entomologists. Among different molecular methods of species identification very promising is high-resolution melting PCR. It allows fast single-tube assignment of analyzed sample to species based on amplicon melting profile. The object of this study were different specimens of Coleoptera collected at pig cadavers in Łomna (central Poland) during 2012 - 2014. Specimes were identified to species by experts of corresponding Coleoptera families. From 120 collected specimens belonging to four families and twelve species HRM-PCR correctly identified specimens belonging to three families and eight species.

1. Introduction

Precise estimation time of death is one of key task of forensic entomology. Application of Calihoridae for post mortem interval estimation is well suited for initial period of cadaver decomposition but limited to of their larval stage. Further stages of cadaver decomposition are associated with other entomofauna. Especially interesting is Coleofauna present at all stages of cadaver decomposition. Identification of Coleoptera species is however more difficult that Calliphoridae. The morphological identification of Coleoptera species from varying life stages to species level can be challenging, time- consuming and costly. Moreover, the quality and level of taxonomic resolution achieved depends upon the individual knowledge and experience of the respective investigator, while professional taxonomists simultaneously are getting rare. In recent years, the developments in molecular biology opened the door for a variety of DNA sequence-based alternative approaches for determining organisms. Especially the idea of “DNA-barcoding” [1,2] has gained attention among biologists. This identification tool proved to be a powerful approach for discriminating a broad range of organisms, including forensically important [3,4]. However, using standard DNA-barcoding is expensive, time consuming and labor intensive. The high resolution melting (HRM) analysis is a highly sensitive closed-tube method for detecting smallest variations between DNA amplicons. Following a real- time PCR, the products are denatured by increased temperature and the resulting changes in fluorescence caused by the release of an intercalating DNA dye from the DNA duplex is precisely monitored in real time. By comparing the resulting melting curves of unknown samples, to melting curve profiles of known isolates, they can be assigned to known strains or species [5,6]. Following previous studies [3] we combined DNA-barcoding with the advantage of HRM analysis and tested its ability to identify specimens of 12 Carabidae species. Therefore, we designed a single pair of PCR primers for a short region of the mitochondrial standard DNA-barcode COI, presenting sufficient sequence diversity for the discrimination between target species.

2. Materials and methods

The object of this study were different specimens of Coleoptera collected at pig cadavers in Łomna (central Poland (52°21′ N; 20°47′ E) during 2012–2014. Ten specimens per species were selected to consider intraspecific variation. In total, 120 specimens belonging to 12 species were included in this study. Specimens were identified to species by experts of corresponding Coleoptera families. Genomic DNA was extracted from the thorax using a GenElute Mammalian Genomic DNA Purification Kit (Sigma-Aldrich, Milwaukee, WI), following the manufacturer’s instructions. DNA barcode sequences belonging to investigated Coleoptera families were retrieved from GenBank and

* Corresponding author.
E-mail address: ireneusz.soltyszewski@uwm.edu.pl (I. Sołtyszewski).

https://doi.org/10.1016/j.fsigss.2019.09.051
Received 5 September 2019; Accepted 23 September 2019
Available online 24 September 2019

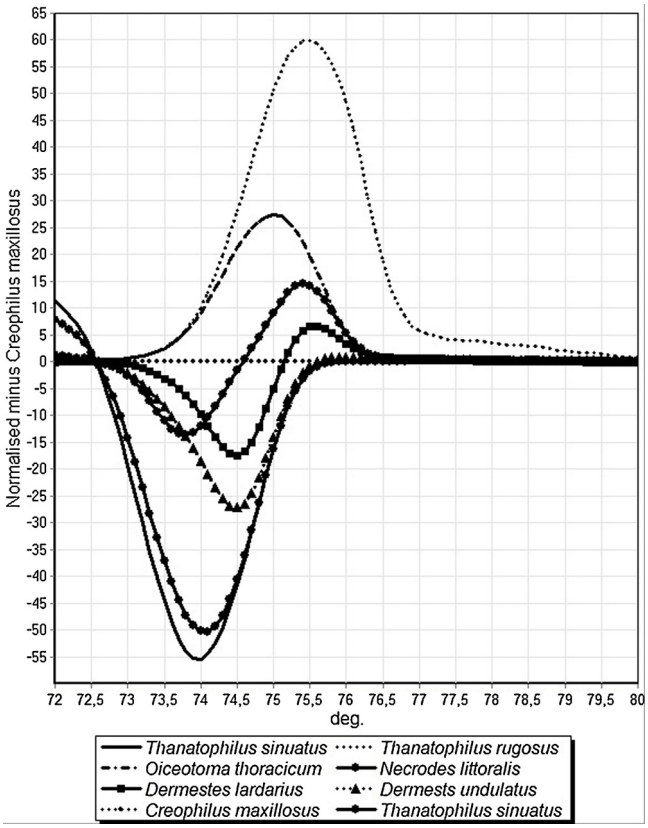

Fig. 1. An example of specimens' identification by HRM-PCR.

aligned with Clustal W [7]. Region with sufficient sequence diversity to differentiate analyzed species, flanked by conserved regions was selected manually as potential mini-barcode for the HRM analysis. Primers were designed using the Primer3 software [8]. The designed PCR primers were purchased from Sigma-Aldrich (Milwaukee, WI, USA).

Real time PCR reactions and the subsequent HRM analysis were performed in a Rotor-Gene 6500 thermal cycler (Corbett Research, Mortlake, NSW, Australia). Each PCR reaction mix was prepared using 10 µl Real Time 2 × PCR Master Mix EvaGreen (A&A Biotechnology, Gdynia, Poland), 2 µl of 5 µM each primer, 2 µl DNA extract, and 4 µl H_2O to total 20 µl volume. Amplification was performed under following thermal program: an initial denaturation step of 95 °C for 3 min followed by 40 cycles at 95 °C for 30 s, 61 °C for 30 s, and 72 °C for 30 s. Melting curve data were generated by monitoring the changes in fluorescence during the increase of temperature from 60 to 95 °C at 0.1 °C per second. The resulting fluorescence data were first visualized by difference plotting using the software Rotor-Gene 1.7.27. Therefore, the raw data were normalized in two regions (region 1: 71–72 °C; region 2: 88–89 °C) to allow the comparison of all melting curves from the same starting and ending signal level. The resulting normalized curves were subsequently used to construct difference graphs, depicting the variation in fluorescence of a sample to a selected reference.

3. Results

Based on multiple sequence alignment of COI sequences of Coleoptera species important for forensic entomology were designed pair of primer for HRM-PCR: forvard - silphfv GCHACWYTWCATGGAACWCAA and reverse silphrv TASAATTGCAAAHACSGCHCC. Amplicon 197 bp lenght spans from 257 - to 454 bp (numbering according to *Thanatophilus sinuatus* HQ165288.1). There were analyzed specimens belonging to 12 species from 4 families. Four carrion beetles (Silphidae) species: *Thanatophilus sinuatus* (Fabricius, 1775), *Thanatophilus rugosus* (Linnaeus, 1758), *Oiceotoma thoracicum* (Linnaeus, 1758), *Necrodes littoralis* (Linnaeus, 1758). Two of rove beetles (Staphylinidae) species: *Creophilus maxillosus* (Linnaeus, 1758), *Ontholestes murinus* (Linnaeus, 1758). Four of rove beetles (Histeridae) species: *Hister unicolor* (Linnaeus, 1758), *Margarinotus brunneus* (Fabricius, 1775), *Saprinus planiusculus* (Motschulsky, 1849), *Saprinus semistratus* (L.G. Scriba, 1790), and two of skin beetles (Dermestidae) species: *Dermestes lardarius* (Linnaeus, 1758), *Dermests undulatus* (Brahm, 1790).

HRM melting profiles were successfully obtained from specimens belonging to nine species. The crossing points (Cp) of real-time PCR, i.e. the cycle at which a statistically significant increase in fluorescence above background signal is first detected, ranged from 21.4 to 31.3 cycles. In general, the HRM-PCR analysis was able to correctly assign specimens to one of the 8 species. The obtained difference graphs of these eight species can clearly be distinguished by variation in peak position (positive peak, negative peak, or both), shape change and melting temperature, with *Creophilus maxillosus* selected as reference (Fig. 1). There were not obtained sufficient amplification and specimen assigning for four species of rove Histeridae family: *Hister unicolor*, *Margarinotus brunneus*, *Saprinus planiusculus and Saprinus semistratus.*

4. Discussion

This study was designed to evaluate the potential of high resolution melting in combination with HRM-PCR to discriminate between forensically important Coleopera species. By using HRM-PCR analysis we were able to successfully discriminate eight of the tested species. All species, except Histeridae family, can clearly be distinguished by significant shape variation in their melting curves. An additional benefit is that HRM-PCR allows to get answers in a much shorter time. It has also been successfully applied in the identification of microorganisms [9], oomycetes [10], plants [11], blowflies [3], and fishes [12]. Hence, this molecular technique may be a promising alternative identification tool without conventional Sanger-sequencing. The highly sensitive technique is applicable in any laboratory with access to an HRM-capable real-time PCR machine.

5. Conclusion

In the present study HRM-PCR analysis was found to be a suitable method to assign specimen to species in Coleoptera. This is a fast, powerful and simple alternative to identify species without the need of more costly DNA-sequencing.

Declaration of Competing Interest

All authors declare no conflict of interest.

References

[1] P.D. Hebert, A. Cywinska, S.L. Ball, et al., Biological identifications through DNA barcodes, Proc. Biol. Sci. 270 (2003) 313–332.
[2] S. Ratnasingham, P.D. Hebert, BOLD: The barcode of life data system, Mol. Ecol. Notes 7 (2007) 355–364.
[3] T. Malewski, A. Draber-Mońko, J. Pomorski, et al., Identification of forensically important blowfly species (Diptera: Calliphoridae) by high-resolution melting PCR analysis, Int. J. Legal Med. 124 (2010) 277–285.
[4] V.H. Peña, G.J. Fernández, A.M. Gómez-Palacio, et al., High-Resolution Melting (HRM) of the Cytochrome B Gene: a powerful approach to identify blood-meal sources in Chagas Disease vectors, PLoS Negl. Trop. Dis. 6 (2012) e1530.
[5] C.T. Wittwer, High-resolution DNA melting analysis: advancements and limitations, Hum. Mut. 30 (2009) 857–859.
[6] M. Li, L. Zhou, R.A. Palais, et al., Genotyping accuracy of high-resolution DNA melting instruments, Clin. Chem. 60 (2014) 864–872.
[7] M.A. Larkin, G. Blackshields, N.P. Brown, et al., Clustal W and Clustal X version 2.0, Bioinformatics 23 (2007) 2947–2948.
[8] A. Untergrasser, I. Cutcutache, T. Koressaar, et al., Primer3 - new capabilities and interfaces, Nucl. Acids Res. 40 (2012) e115.
[9] D. Jin, Y. Luo, Z. Zhang, et al., Rapid molecular identification of *Listeria* species by

use of real-time PCR and high-resolution melting analysis, FEMS Microbiol. Lett. 330 (2012) 72–80.
[10] J. Hu, R. Lyon, Y. Zhou, et al., PCR amplification of SNP loci from crude DNA for large-scale genotyping of oomycetes, Mycologia 106 (2014) 607–609.
[11] I. Ganopoulos, P. Madesis, N. Darzentas, et al., Barcode high resolution melting (Bar-HRM) analysis for detection and quantification of PDO fava santorinis (*Lathyrus clymenum*) adulterants, Food Chem. 133 (2012) 505–512.
[12] S. Behrens-Chapuis, T. Malewski, E. Suchecka, et al., Discriminating European cyprinid specimens by barcode high-resolution melting analysis (Bar-HRM)- a cost efficient and faster way for specimen assignment? Fish. Res. 204 (2018) 61–73.