Natural products application: Health, Cosmetic and Food

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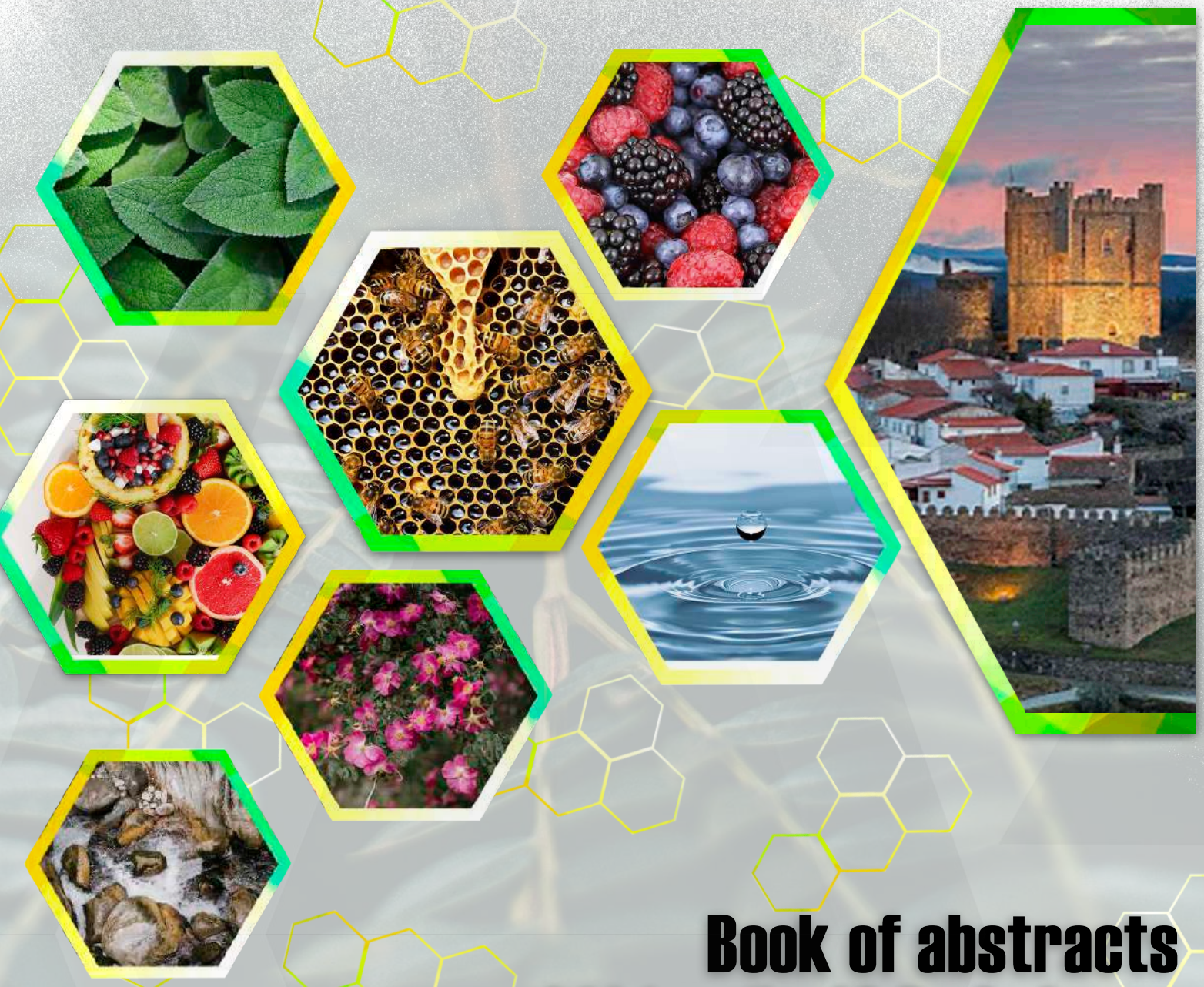

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Development of a new approach based on real-time PCR coupled with high resolution melting (HRM) analysis towards the entomological authentication of honey

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Honey is a natural product widely consumed around the globe, not only for its taste and nutritional value, but also for its health benefits. Being a product of high dietary relevance and increasing demand, it has also become a target of economically motivated adulteration. According to the 2014 European Parliament report on the food crisis, fraud in the food chain and the control thereof, honey is among the 10 food products most prone of being adulterated [1]. Up until now, honey authenticity was mainly focused on the issues of sugars addition and botanical and geographical origin. However, recently an increased attention has been paid to the entomological origin of honey. To this aim, different approaches have been proposed to differentiate honey produced by different *Apis mellifera* subspecies, including those from distinct mitochondrial (mt) DNA lineages [2]. This work aimed to develop a novel real-time PCR method coupled with HRM analysis that allows for the simultaneous differentiation of honeybee from maternal lineages A, M and C, for further application in honey authentication. In this sense, data previously obtained from the mitogenomes of a total of 112 honeybees of different lineages were considered for the development of new DNA markers. Considering the aim of further application in honey, new primer sets were designed to amplify short fragments that included different single nucleotide polymorphisms (SNPs) allowing for HRM application. Three primer sets were proposed, amsCOI-F/amsCOI-R targeting the Cytochrome oxidase I (COI) gene, amsND1-F-amsND1-R targeting the NADH-ubiquinone oxidoreductase chain 1 (ND1) gene and amsCox3-F/amsCox3-R targeting the Cytochrome oxidase subunit III (Cox3) gene. Each primer set was first tested using qualitative PCR using DNA extracted from honeybees of A, M and C mtDNA lineages. After optimizing the real-time PCR conditions, each primer set was tested using a series of mtDNA extracted from honeybees. While amsCOI-F/amsCOI-R allowed only for the separation of the honeybees in two clusters, with lineage C and M clustering together, both the amsCox3-F/amsCox3-R and amsND1-F/amsND1-R set of primers allowed to differentiate the three lineages in separate clusters, with high level of confidence. As future work, the methodology will be assayed in commercial honey samples.

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