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Identification of the entomological origin of European honey by high resolution melting analysis of a COI mini-barcode

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Abstract:

Honey is widely consumed worldwide and highly appreciated for its organoleptic, nutritional and health properties. Honey is also considered one of the foods most prone to be adulterated, either by admixing of honey with lower quality, by sugars' addition, or by origin mislabelling, among other possible frauds. Recently, great attention has been paid to the development of techniques for authenticating honey through its entomological origin, which is also related to its geographical origin, since bees carrying mitochondrial DNA (mtDNA) from distinct ancestries can be found throughout Europe. Moreover, consumers are increasingly concerned with ethical and environmental issues, paying attention to issues such as the protection of biodiversity and the mode of production. For these reasons, the development of methodologies to authenticate the entomological origin of honey contributes not only to assure consumers rights and avoid unfair competition by the identification of frauds, but also to promote and valorise autochthonous honeybee subspecies.

In this work, a one-step approach based on HRM analysis of a 150 bp fragment of the COI gene was developed to establish the entomological origin of honey by discriminating A, M and C mtDNA lineages and differentiating a SNP associated with a high frequency of C1 or C2 mitotypes in the Italian honey bee *A. m. ligustica* and the Carniolan honey bee *A. m. carnica*. The method showed high analytical performance and was able to successfully identify the entomological origin of honeys of known origin obtained from research apiaries/beekeepers. Therefore, it was applied to 44 commercial honeys from different countries. It confirmed the entomological authenticity of French PDO honeys that should be produced by the Corse ecotype *A. m. mellifera*. For the remaining honeys, the results were also in good agreement with the declared geographical origin. This method is also capable of indicating the mixture of honeys produced by honey bees of different lineages, although not allowing to identify the lineages or mitotypes in the mixture. This was the case of three honeys from Slovenia that did not cluster with C2 mitotype *A. m. carnica* as expected, suggesting the mixture of honeys produced by honeybees of different mitotypes.

Keywords: Honey, authenticity, HRM analysis, *Apis mellifera* subspecies.

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