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DEVELOPMENT OF MOLECULAR MARKERS FOR HONEY ENTOMOLOGICAL ORIGIN AUTHENTICATION

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Honey is the natural sweet substance produced by honey bees. According to the European Union legislation, it should be produced by the western honey bee, *Apis mellifera*. However, in Asia, honey is traditionally obtained from other bee species, mainly the eastern honey bee *Apis cerana*. So far, only a few protein-based methods have been proposed to assess honey entomological origin [1], which in fact is related to its geographical origin since bee species generally occupy different geographical ranges according to their evolutionary lineages [2].

In this work, DNA markers were developed for the specific identification of *A. mellifera* and *A. cerana* in honey. For this purpose, bees of *A. cerana* from Thailand, China and Vietnam and honey bees of 4 different subspecies of *A. mellifera* (*iberiensis*, *mellifera*, *ligustica*, *carnica*) from EU countries were used. Different sets of primers were designed targeting the 16S rRNA gene and the $tRNA^{leu}$ - COII intergenic region. The specificity and sensitivity of the designed primers were assayed by qualitative polymerase chain reaction (PCR). Primers targeting the intergenic region successfully differentiated *A. cerana* from *A. mellifera*. Positive amplifications were obtained for all the bees with 16S rRNA primers. However, the use of real-time PCR coupled with High Resolution Melting analysis allowed the separation of the two honey bee species in different clusters. The developed methodologies were applied to the analysis of authentic honey samples from Vietnam (produced from *A. cerana* and *A. mellifera* bees) and from Portugal allowing its successful entomological origin identification.

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[2] Pinto M.A.; Henriques D.; Neto M.; Guedes H.; Muñoz I.; Azevedo J.; Rúa P. Apidologie **2013**, *44*, 430-439.

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