



**Mitochondrial DNA-Based Identification, of
the Honeybee (*Apis mellifera* Linnaeus)
Subspecies in Egypt**

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Requirements for the Degree of
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(Entomology)**

BY

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ABSTRACT

MtDNA based RFLP-PCR technique was adopted, in conjunction with morphometric analysis to differentiate between three pure subspecies of *A. mellifera* collected from Egypt. A subset of 17 different measurements in the right fore- and hind- wings, were selected for comparison of the morphometrics of the studied honeybee subspecies, *A. m. lamarkii*, *A. m. ligustica*, and *A. m. carnica*. The statistical analysis revealed high weights for only 9 characters, grouping the three subspecies, which were the length of the right fore wing, widths of the right fore- and hind- wings, angles (B4, E9, J16, L13, & D7) and the cubital index of the right fore wing. Four mtDNA segments from Co I, Cyt b, lsrRNA genes & Co I-Co II intergenic region, were amplified and digested with 7 restriction enzymes, *Hinf* I, *EcoR* I, *EcoR* V, *Taq* I, *Bgl* II, *Dra* I and *Xba* I, for RFLPs. Co I/*Hinf* I restriction pattern is found to be specific for *A. m. lamarkii*, Cyt b/*Dra* I marked *A. m. carnica* and Co I-Co II/*Xba* I for *A. m. ligustica*. Co I-Co II/*Xba* I test is valuable, for the amplified Co I-Co II length polymorphism which discriminate Eastern European subspecies from Egyptian one and when digested with *Xba* I, it differentiated between the European, Italian and Carniolan, subspecies. Another discrimination test, which could be utilized, was the PCR amplification of the Cyt b gene fragment, followed by digestion with 2 restriction enzymes *Hinf* I and *Dra* I, as an independent test or to verify the previous test.

Key words: *Apis mellifera lamarkii* - *Apis mellifera ligustica* - *Apis mellifera carnica* - Morphometrics - ANOVA statistics - Stepwise statistics - MtDNA - cytochrome oxidase I - large subunit ribosomal RNA - Cytochrome b - Cytochrome oxidase I–Cytochrome oxidase II intergenic region - RFLP–PCR.

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LIST OF ABBREVIATIONS

A	:African honeybee type
<i>A. m.</i>	:<i>Apis mellifera</i>
ang.A4	:Angle A4
ang.B4	:Angle B4
ang.D7	:Angle D7
ang.E9	:Angle E9
ang.G18	:Angle G18
ang.J10	:Angle J10
ang.J16	:Angle J16
ang.K19	:Angle K19
ang.L13	:Angle L13
ang.N23	:Angle N23
ang.O26	:Angle O26
ANOVA	:Analysis Of Variance
ATPase	:Adenine Tri Phosphatase
bp	:base pair
C	:South Eastren European honeybee type
CO I	:Cytochrome Oxydase Subunit I Gene
CO II	:Cytochrome Oxydase Subunit II Gene
CO III	:Cytochrome Oxydase Subunit III Gene
COI-COII	:Intergenic Regin between Cytochrome Oxydase Subunit I and Cytochrome Oxydase Subunit II