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MOLECULAR IDENTIFICATION WITH MORPHOMETRIC AND ANATOMIC CHARACTERIZATION OF HONEY BEE (*APIS MELLIFERA LIGUSTICA, APIS MELLIFERA CARNICA AND APIS MELLIFERA MEDA)* IN NORTHERN IRAQ

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Abstract: Morphometric, anatomic and genetic diversity of Northern Iraq populations of honey bee (*Apis mellifera* L.) were investigated. Young adult worker bees were collected from 10 separate localities, from four provinces of Northern Iraq; (Duhok, Erbil, Sulaymaniyah and Halabcha) during June 2020, and five colonies were randomly selected from each locality. Thirty young workers were obtained from the brood nest of each colony. Together, morphological identification and molecular identification showed that *A. mellifera* isolates are *A. mellifera ligustica* (Genbank accession No. OL824844), *A. mellifera carnica* (Genbank accession No. OL824935) and *A. mellifera meda* (Genbank accession No. OP020430). The most obvious differences in the average values of morphological characteristics among the three genetically identified subspecies, were represented in body mass, body length, and the number of hamuli; *A. mellifera meda* was heavier than *A. mellifera ligustica* by 9.3 mg and heavier than *A. mellifera carnica* by 5.1 mg. The differences in the average values of anatomical characteristics among identified subspecies were detected in the length of hypopharyngeal glands acini and the main ducts of the same glands, salivary glands, number of ovarioles and the size of fat body cells. *A. mellifera ligustica* had bigger hypopharyngeal gland acini (195 ± 10.8 µm), and bigger salivary gland acini (209 ±18.2 µm), while *A. mellifera carnica* had longer main duct of hypopharyngeal gland (12.6 ± 0.949 mm) and more ovarioles *(*4.97 ± 0.54) in their ovaries. Significant differences were found in the length of fat body cells of the three subspecies and larger fat body cells were observed in *A. mellifera ligustica* (70 ±10 µm).

Keywords: anatomy; *Apis mellifera* subspecies; molecular identification; morphometric.

1. Introduction

Honey bees have fundamental role in plant pollination in natural ecosystems and agro-ecosystems. Western Asia, Europe, and Africa are the native distribution regions for the honey bee, *A. mellifera* L. (Miguel et al. 2011). Erik et al. (2020) determined that the origin of *A. mellifera* was either northern Africa or the Near East which consistent with morphology (Ruttner et al. 1978). They showed that phylogenetic mistake led to the previously proposed European and Afrotropical cradles of honey bees.

There are many transition zones between subspecies' distributions, which has caused progressive changes in neighboring subspecies' traits (Ilyasov et al. 2020). Beekeepers perform a variety of procedures on their honey bee hives. Requeening and migratory beekeeping are two examples of methods that over time cause variations in the traits of honey bee colonies. Honey bee

morphological traits can be assessed to identify honey bee populations and to serve as a productivity indicator for honey bee colonies (Abou-shaara et al. 2012).

Twenty-nine subspecies of *A. mellifera* are recently recognized using the conventional morphometric methods, based on the external characteristics (Sheppard & Meixner 2003; Miguel et al. 2011). Because of their distributions within certain geographic regions, these subspecies are frequently referred to as "geographic races." A honey bee subspecies can be identified using genetic markers in conjunction with morphometric traits (Ruttner 1992). Although morphological characteristics are fundamental methods of honey bee classification, but it is not well suited for characterization of honey bee subspecies and analyzing of phylogenetic relationships because they can be affected by environmental selection pressures (Franck et al. 2000). The genetic structure of honey bee populations may be influenced by the migration of colonies across border lines between neighboring countries. Ahmad (2018) found that the honey bee populations in Northern Iraq have a limited genetic diversity. With more than forty usable characteristics, morphological characterization is still regarded as a crucial tool in the classification of honey bees when combined with morphometric methods (Adl et al. 2007; Meixner et al. 2011). Among them, wing length, wing width, cubital veins, femur length, basitarsus and tibia lengths of workers are the easiest and the most quantitative parameters (Adl et al. 2007). However, this approach is not well suited to characterizing honey bees at a subspecies level and to studying phylogenetic relationships (Danforth et al. 2013; Miguel et al. 2011). The honey bee, *A. mellifera*, is widespread throughout the world and has a variety of subspecies that can be categorized using morphometric techniques (Ruttner et al. 1978). Numerous researches have been conducted on honey bees by using morphological characteristics (Abou-Shaara et al. 2012; Garnery et al. 2004). These characteristics were grouped into three main categories; which are length measurements, colors, and wing venation properties. There are many subspecies of western honeybee *A. mellifera* worldwide distributed, including *A. mellifera carnica*, the Carniolan honeybee, which is native to Slovenia (Ruttner 1988). Due to the specificity of population structure, biological traits, and resolutions of honey bee subspecies identification methods, the taxonomy of the honey bee *A. mellifera* has several problems. Transitional zones have influence on the distribution of subspecies which caused progressive changes in neighboring subspecies' traits. Currently, there is a lack of clarity regarding the number of honey bee subspecies and their division into evolutionary lineages. (Alburaki et al. 2011). *A. mellifera* subspecies split into four lineages (A, M, C, and O) by morphometry and molecular data (about 25 subspecies) subsequently after being first divided into three lineages (A, M, and C) by morphometry (about 20 subspecies) (Ruttner et al. 1978; Ruttner 1988; Palmer et al. 2000) and then divided into five lineages (A + sublineage Z), M, C, O, Y) by molecular data finally (about 30 subspecies) (Alburaki et al. 2011). The occurrence of transition zones between subspecies, which caused gradient changes in morphometric and molecular characteristics, posed the biggest obstacle to the differentiation of subspecies. Ruttner (1988) and Adl et al. (2007) confirmed that *A. mellifera meda* is native in Iraq and has similarities with both *A. mellifera ligustica* and *A. mellifera anatolica*. According to Fontana et al. (2018), there are five subspecies in the Middle East and Central Asia:

A. mellifera anatoliaca - Anatolia (Turkey and Iraq); *A. mellifera meda* Skorikov - Iran, northern Iraq and southwest Turkey; *A. mellifera pomonella* - Tien Shan mountains and Central Asia; *A. mellifera sinisxinyuan* - Xinyuan (Central Asia); *A. mellifera syriaca* - Israel, Jordan, Lebanon and Syria. They revealed that different subspecies will inevitably lose their distinctive genetic and phenotypic traits if they are forced to coexist in the same locality as a result of human activity.
The sequencing and characterization of the mt DNA genome have been considered useful methods for analyzing the phylogeny and genetic population structure of the *Apis* species and of *A. mellifera* subspecies. A number of molecular markers mainly mitochondrial DNA (mt DNA) such as COX1-COXII intragenic region and 16 rDNA were used to discriminate among five honey bee lineages and to discriminate among *A. mellifera* subspecies (Alajmi et al. 2019). The hypothesis is that the patterns of honeybee variations would reflect the geographic region on a morphometric, anatomic and molecular level. This study aimed to;

I. To confirm the existence of the mentioned subspecies by previous studies in this area, using recently molecular methods instead of traditional methods.
II. To study some morphometric characteristics of existed subspecies.
III. To study some anatomic characteristics of existed subspecies.
IV. To find out the relationships among studied parameters.

2. Materials and methods

This research was performed in the entomology laboratory of the College of Agricultural Engineering Sciences (60 km south east Turkish border)/ Department of Plant Protection at the University of Duhok with corporation of the Duhok Research Center at Duhok University.
In current study, the morphometric, anatomic and genetic diversity of honey bee populations in northern Iraq were investigated.

2.1. Sampling

Young adult worker bees were collected from 10 separate localities, from four provinces of Northern Iraq; (Duhok, Erbil, Sulaymaniyah and Halabcha) during June 2020, and five colonies were randomly selected from each locality. Thirty young workers were obtained from the brood nest of each colony. Radloff et al. (2003) proved that the samples of five colonies per locality with 10 bees per colony are adequate in the analysis of morphological data in studies of honey bee populations. Bees were taken inside wooden cages provided with food and passage way of air. All worker samples were transferred alive to the Entomology lab. and kept in deep freezer for 5 min to immobilize them (Alajmi et al. 2019) then subjected to morphometric, anatomic and molecular investigations.

2.2.Morphometric characteristics of the external parts of honey bee workers

The following external parts were measured;
Body mass, body length, forewing (length and width), hind wing (length and width), number of hamuli, cubital veins (length), femur length, tibia length, basitarsus (length and width), length of proboscis, and length of mandible (figure 1).

2.3. Anatomic characteristics of the internal parts of honey bee workers

The following internal parts of honey bee workers were measured;

I. Salivary glands; twenty acini from each worker were randomly selected, the diameters of the acini from both sides of the head were measured (ten acini from each side). The diameter of acini for each dissected worker was calculated.
II. Hypopharyngeal glands; the same as mentioned in salivary glands, as well as the length of the main duct of hypopharyngeal glands was measured.
III. Mandibular Gland; mandibular glands from both sides of the head were dissected; the length of each gland was calculated.
IV. Ovaries; the number of ovarioles of both sides of the worker ovary was counted (Ayoub 2011). In addition to the length of the ovary (the length of the longest ovariole was calculated).
V. Length of ten fat body cells inside the abdomen were also measured (figure 2 and 3). Anatomic study was performed according to the standard methods for *A. mellifera* anatomy and dissection (Carreck et al. 2013).

The measurements of each bee sample were subjected to analyzing to detect the relationships between some investigated parameters using simple linear regression.

2.4. Genomic DNA extraction, PCR and sequencing

The DNA was extracted from honey bee abdomen using an animal tissue DNA kit (Addbio-Korea Company). Mitochondrial 16S rDNA region of the extracted DNA was amplified using universal primers 16S rDNA (Macrogen, Korea) (Alajmi et al. 2019).

The PCR reactions were achieved in a final volume of $40\mu l$, which included 20µl 2× Taq PCR Master Mix (polymerase enzyme), 2µl of each reverse and forward primer (1pmol/ µl), 6 µl of genomic DNA (30-100 ng/ µl) and 10 µl of RNase - Free water. PCR amplification and subsequent DNA sequencing was carried out using the following mitochondrial *16S rDNA* primers: 16SF (5'-CAC CTG TTT ATC AAA AAC AT-3') and 16SR (5'-CGT CGA TTT GAA CTC AAA TC-3'), designed by Crozir and Crozier (1993) and used by Alajmi et al. (2019). The optimal performance of the PCR of the primers (16S rDNA) was obtained from an initial denaturation at $95°C$ for 15 sec. accompanied by 35 cycles of amplification (denaturation, $95°C$ for 15 sec, annealing, $62°C$ for 30 sec, and extension, 72 °C for 1 min.), with a last extension at 72°C for 5 min.

Amplified PCR products were visualized by 1% agarose gel electrophoresis stained with 3 µl of Eva Green® Fluorescent Gel Stain (Jena Bioscience, Germany). The electrophoresis was done at 100V/ cm gel a voltage source (80V) for 40 min., photography and visualization of bands were carried out using a transilluminator equipped with a digital camera.

2.5. Sequencing and phylogenetic analysis

All sequences obtained from this study had been submitted to Gen Bank. Bio Edit and align ITS sequences Version 7.0.0 were used from the program Molecular Evolutionary Gene Analysis software (Isis Pharmaceuticals, Inc., Carlsbad, CA, USA). The sequences were aligned and placed

in the Gen Bank database. The comparison between the similarities of groupings with a homologous grouping deposited in Gen Bank has been calculated by utilizing "Blast" instruments on the site of National Center for Biotechnology Information (NCBI). The Neighbor- joining method MEGA7 was exploited to construct the phylogenic tree. Branch support was estimated by bootstrap analysis with 1000 replicates (Felsenstein 1985); (Nei & Kumar 2000).

The statistical procedures were performed, the means and standard deviations of morphological and internal characters for each worker bee were calculated. Then data were analyzed by analysis of variance (ANOVA) to compare workers of the three molecularly identified subspecies for each character. Duncan's multiple range tests on the means of 14 morphological and 7 internal characters were used to detect significant differences among molecularly identified subspecies. Simple Liner regression was applied to detect relationships between some studied parameters, the data of each sample were used for these tests, body length and body mass were used as independent variables.

3. Results

3.1. Morphological observations and measurements

Morphologically the studded subspecies were completely differed, the first one (genetically identified as *A. mellifera liguistica*) had a light-yellow color. While the second (genetically identified as *A. mellifera carnica*) was dark in color with brown bands on their abdomen. The third subspecies; *A. mellifera meda*, was dark in color with two brown bands on their abdomen (figure 4). The results indicated that the most obvious differences in the average values of morphological characteristics among the previously mentioned subspecies were represented in body mass, body length, and the number of hamuli. More body mass (93 ± 7.94 milligrams) was noted in *A. mellifera meda* followed by *A. mellifera carnica* (88.67 ± 11.05 mg), while the weight of *A. mellifera ligustica* was 84 ± 4.98 mg. *Apis mellifera meda* was heavier than *A. mellifera lugistica* by 9.3 mg and heavier than *A. mellifera carnica* by 5.1 mg. Table (1) shows that longer bodies were found in both *A. mellifera meda* (11.6 ± 0.6 millimeters) and *A. mellifera carnica* (11.6 ± 0.01mm), while the length of *A. mellifera ligustica* was 10.5 ± 0.57 mm. The shorter forewing (9.18 ± 0.18 mm) was recorded in *A. mellifera ligustica* compared to other subspecies. More number of hamuli was observed in *A. mellifera carnica* (23 ± 1.25) followed by those in *A.mellifera meda* (22.5 ± 0.94) (table 1).

The differences in the average values of anatomical characteristics among the identified subspecies were appeared in the length of hypopharyngeal glands acini and the main ducts of the same glands, salivary glands, number of ovarioles and the size of fat body cells. *A. mellifera liguistica* had bigger hypopharyngeal gland acini (195 ± 10.8 microns), and bigger salivary gland acini (209 ±18.2 µm), while *A. mellifera carnica* had longer main duct of hypopharyngeal gland (12.6 ± 0.949 mm) and more ovarioles *(*4.97 ± 0.54) in their ovaries.

Significant differences were found in the length of fat body cells among three subspecies and larger fat body cells were observed in A. *mellifera liguistica* (70 ±10 µm) followed by *A. mellifera carnica* (60 ±7.6 µm) and (51 ± .75 µm) in *A. mellifera meda.*

3.2. Relationships between studied parameters

Results obtained from morphometric (table 1) and anatomic (table 2) studies of these sup-species; indicated to the presence of some positive relationships between studied parameters. Linear regression showed a significant positive relationship between body length (independent variable) and the length of forewing (r^2 = 56.299, p = = 0.000, n = 60). There was also a significant positive relationship between the body length and the length of hindwing (r^2 = 14.9107, p = 0.0023, n = 60). Workers with more body mass had more ovarioles (r^2 = 4.841, p = 0.091, n = 60). There was a significant positive relationship between length of worker ovary and the whole-body length of the worker (r^2 = 21.489, p = 0.0002, n = 60); longer ovaries were found in longer workers (figure 5).

3.3. Sequencing of 16S rDNA and phylogenetic analysis

The results of 16S rDNA sequencing of the *A.mellifera* isolates showed 629 and 341bp of special DNA fragments sequenced. Using BLAST search, to compare the resulting sequences with sequences of 16S rDNA accessed in Genbank, phylogenetic analysis showed that the obtained sequences shares 99% homology to *A. mellifera carnica*, strains: USA (MN250878), Serbia (JQ778289) and Eastern Serbia strains (JQ778288, JQ778286, JQ778285, JQ778284, JQ778283), and 100% homology to *A.mellifera liguistica* strains: Korea strains (MH341408, MH341407, KX908209), USA strains (NC_001566, MN714160, MN250878), China (MT859135), New Zealand (AP018435) and Eastern Serbia (JQ778289). Phylogenetic analysis showed that the obtained sequences share 100% homology to *A. mellifera meda* strain Germany (KY464957). Together, morphological identification and molecular identification showed that *A. mellifera* isolates are *A. mellifera ligustica* (Genbank accession No. OL824844) (figure 4 A), *A. mellifera carnica* (Genbank accession No. OL824935) (figure 4 B), and *A. mellifera meda* (Genbank accession No. OP020430) (figure 4 C).

4. Discussion

Honey bees have found opportunities to adapt to a diversity of ecological conditions. Interactions between bees and the local floral characteristics have led to morphological, biochemical, physiological, and behavioral adaptations to form several honey bee ecotypes. Because of its large honey storing ability, *A. mellifera ligustica* queens have been exported worldwide for more than 150 years (Woodward 1993).

In this study we investigated honey bee subspecies in Northern Iraq using universal primers 16S rDNA primers and confirmed the presence of subspecies; *A. m. ligustica*. *A. m. carnica* and the indigenous subspecies *Apis mellifera meda* (Ruttner 1988; Ahmad 2018). Ozdil et al. (2012) confirmed the existence of *A. mellifera meda* in the southeastern part of Turkey, while Ahmed

(2018) found only *A. mellifera meda* subspecies in the area. This discrepancy in the results can be attributed to sample size, sampling locations and target gene.

The significant variations of measurements in morphological and anatomical features resulted from the presence of more than one distinct honey bee subspecies (Ayoub 2017).

A. mellifera meda showed higher body mass (93 ±7.94 mg) than the other two genetically identified subspecies, probably caused by more adaptation to the environment of the area. The body mass of *A. m. carnica* (88.67 ±11.05 mg) was higher than that of *A. m. ligustica* (84 ±4.98 mg) which due to the longer body of *A. mellifera carnica* (11.6 ±0.01 mm) compared to body length of *A. mellifera ligustica* (10.5 ±0.57 mm). According to Ruttner (1988), *A. mellifera carnica* are large with dark body colour with short grey cover hair and high value of cubital index. The body mass of honey bee workers was ranged from 81 to 140 mg (Winston 1987). The workers are influenced by genetics and by environmental factors such as larval feeding (Daly & Morse 1991). More ovarioles (4 to 6) were counted in *A. mellifera carnica* than those in *A. mellifera ligustica* (2 to 4.5). Ovarioles are the functional unit of the female insect reproductive organs and the number of ovariole filaments per ovary is an important female reproductive character that affects fecundity across insect taxa (Makert et al. 2006). The highly eusocial species in the genus *Apis* have queens that can have more than 360 ovarioles in two ovaries, and workers that often have fewer than 10 ovarioles (Winston 1987; Michener 2003). Length of hypopharyngeal gland acini (184 ±13.39 µm) in *A. mellifera. carnica* was longer than those observed in young workers of *A. mellifera carnica* by Ayoub (2011), as well as lower body mass (88.67 ± 11.05) and similar number of ovarioles (4.97 ± 0.54) were found compared to those observed in young workers of *A. mellifera carnica* by the same author in south of Poland.

A. mellifera ligustica showed significantly bigger hypopharyngeal gland acini (195 ±10.8 µm) as well as bigger salivary glands of (209 ± 18.2 µm) than the other two genetically identified subspecies, these are desirable qualities characteristics because both glands have fundamental role in brood rearing in honey bee colonies. Deseyn & Billen (2005) concluded that there was positive relation between size of hypopharyngeal glands and the gland activity. They confirmed that these glands have essential role in larval rearing and feeding queens, they are synthesizing and secrete royal jelly.

Basitarsus length of *A. mellifera carnica* was 2.1 ± 0.03 mm, and similar to that found by Jevtić (2007) for the same subspecies in Serbia. Proboscis length of *A. mellifera carnica* (6.47 ± 0.06 mm) was slightly bigger than those found by Kakhramanov et al. (2020) in *A. mellifera carnica* (6.17 ± 1.5 mm) in Uzbekistan.

Environment factors have significant influence on morphological traits and the introduction of new honey bee subspecies into diverse regions may result in extensive population hybridization (Alqarni et al. 2011). Additionally, migratory beekeeping may have a significant impact on forming differences (Marghitas et al. 2008). The stability of morphological traits in unmanaged honey bee populations was low over time (Abou-Shaara et al. 2012).

It can be concluded that three subspecies were genetically identified in northern Iraq; *A. mellifera ligustica*, *A. mellifera carnica* and *A. mellifera meda* using 16S rDNA sequencing. The results of

current study indicated that the most obvious differences in the average values of morphological characteristics among the genetically identified subspecies; were represented in body mass, body length, and the number of hamuli. The differences in the average values of anatomical characteristics among the identified subspecies were appeared in the length of hypopharyngeal glands acini and the main ducts of the same glands, salivary glands, number of ovarioles and the size of fat body cells. Morphometric and anatomic studies of these sup-species; indicated to the presence of some positive relationships between studied parameters.

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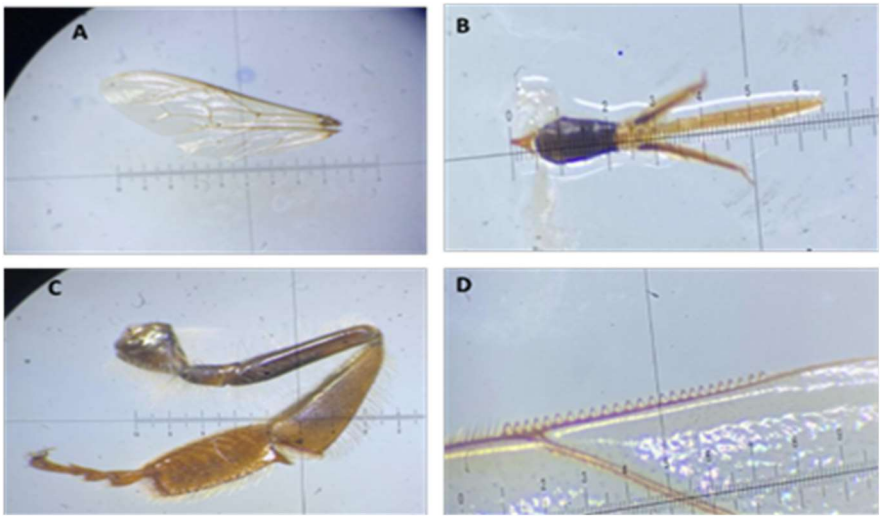

Figure 1. Some external studied parameters of honey bee workers; A. Fore wing, B; Proboscis, C; Hind leg, D; Hamuli.

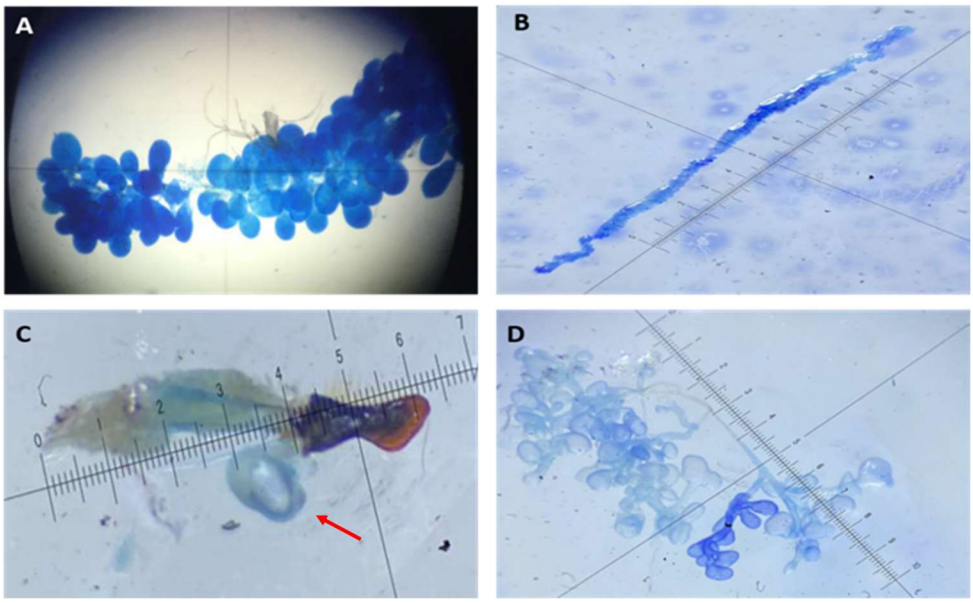

Figure 2. Studied glands of honey bee workers; A. hypopharyngeal gland, B; Main duct of hypopharyngeal gland, C; Mandibular gland, D; Salivary gland.

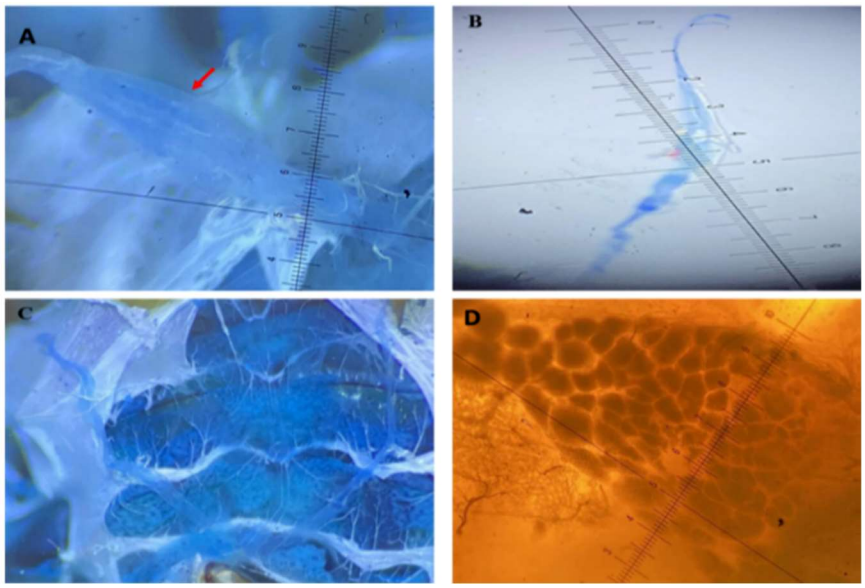

Figure 3. Some internal studied parameters of honey bee workers; A. worker overioles, B; Ovary with oviduct, C; worker abdominal cavity, D; fat body cells inside abdomen.

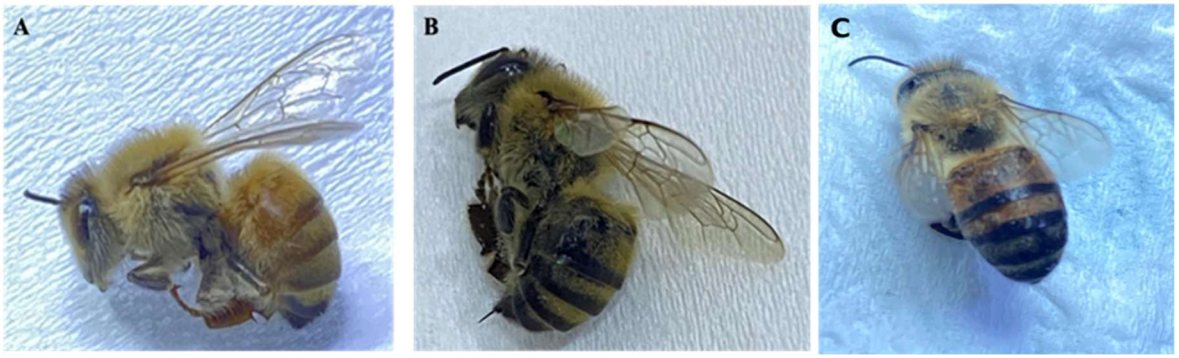

Figure 4. Molecularly identified subspecies, A; *Apis mellifera liguistica* B; *Apis mellifera carnica* C; *Apis mellifera meda.*

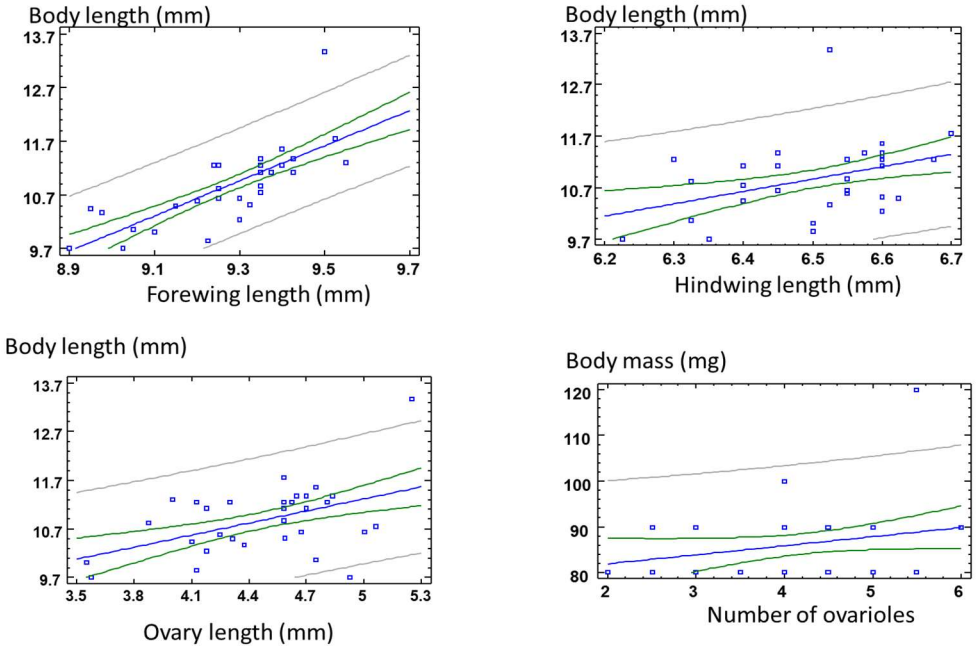

Figure 5. Relationships between studied parameters, A; Relationship between body length and forewing length, B; Relationship between body length and hindwing length, C; Relationship between body length and length of ovary, D; Relationship between body mass and number of ovarioles.

Table 1. External studied parameters of honey bee workers of the identified subspecies; *Apis mellifera ligustica, Apis mellifera carnica* and *Apis mellifera meda.*

External structures	*Apis mellifera ligustica*			*Apis mellifera carnica*			*Apis mellifera meda*		
	Min	Max	Average ±SD	Min	Max	Average ±SD	Min	Max	Average ±SD
Body mass (mg)	80	90	84 ±4.98 c	80	120	88.67 ±11.05 b	80	100	93±7.94 a
Body length (mm)	9.7	11.3	10.5 ±0.57 c	11.4	11.8	11.6 ±0.01 a	10.7	12.4	11.6±0.6 a
Forewing length (mm)	8.9	9.55	9.18 ±0.18 b	9.15	9.53	9.36 ±0.09 a	9.25	9.55	9.4±0.08 a
Forewing width (mm)	2.93	3.19	3.09 ±0.07 c	3.15	3.3	3.2 ±0.04 a	3.1	3.25	3.15±0.06 b
Hindwing length (mm)	6.23	6.68	6.47 ±0.13 b	6.4	6.7	6.54 ±0.088 a	6.4	6.7	6.57±0.11 a
Hindwing width (mm)	1.63	1.9	1.77 ±0.07 c	1.8	1.99	1.88 ± 0.059 a	1.8	1.85	1.81±0.02 b
Number of hamuli	18.5	23	21.2 ±1.17 b	20	24.5	23 ± 1.25 a	21.5	24.5	22.5±0.94 a
Cubital veins (Length) (mm)	2.48	3.45	2.81 ±0.26 b	2.6	3.59	3.1± 0.324 a	2	3.46	2.66±0.44 b
Femur length (mm)	2.48	2.69	2.61 ±0.05 b	2.6	2.73	2.65 ± 0.038 a	2.55	2.7	2.63±0.04 ab
Tibia length (mm)	2.9	3.25	3.11 ±0.1 b	3.05	3.28	3.16 ± 0.059 a	3.05	3.2	3.12±0.05 b
Basitarsus length (mm)	1.9	2.1	2 ±0.06 c	2.05	2.15	2.1 ± 0.03 a	2	2.1	2.04±0.04 b
Basitarsus width (mm)	1.14	1.23	1.19 ±0.02 a	1.15	1.53	1.21 ± 0.088 a	1.2	1.28	1.21±0.02 a
Proboscis (mm)	6.2	6.5	6.38 ±0.12 b	6.4	6.55	6.47 ± 0.061 a	6.2	6.45	6.3±0.09 c
Length of mandible (mm)	1.4	1.48	1.44 ±0.02 a	1.4	1.49	1.44 ± 0.028 a	1.35	1.5	1.4±0.04 b

Table 2. Internal parts of honey bee workers of the identified subspecies; *Apis mellifera ligustica* *Apis mellifera carnica* and *Apis mellifera meda***.**

Internal structures	*Apis mellifera ligustica*			*Apis mellifera carnica*			*Apis mellifera meda*		
	Min	Max	Average ±SD	Min	Max	Average ±SD	Min	Max	Average ±SD
Length of mandibular gland (Right) (mm)	0.95	1.45	1.19 ±0.17 a	0.7	1.3	1.1 ±0.158 a	0.9	1.45	1.19±0.15 a
Length of mandibular gland (Left) (mm)	0.85	1.5	1.19 ±0.18 a	0.85	1.5	1.1 ±0.192 b	0.9	1.45	1.19±0.15 a

Width of mandibular gland (mm)	0.4	0.9	0.61 ±0.15 a	0.53	0.9	0.63 ±0.113 a	0.5	0.8	0.67±0.11 a
Length of hypopharyngeal gland acini (µm)	178	213	195 ±10.8 a	160	212	184 ±13.39 b	176	200	187±6.95 b
Main duct of hypopharyngeal gland (mm)	9.68	13.5	11.8 ±0.95 b	10.3	14.2	12.6 ±0.949 a	7.7	15.1	11.5±2.16 b
salivary gland (µm)	185	248	209 ±18.2 a	172	218	199 ±12.9 b	173	224	190±15.1 c
Ovary Length (mm)	3.55	5	4.27 ±0.43 b	4.18	5.25	4.66 ±0.273 a	3	5.5	4.22±0.79 b
number of ovarioles	2	4.5	3.5 ±0.72 b	4	6	4.97 ±0.54 a	2	6	3.7±1.21 b
Length of fat body cells (µm)	60	80	70 ±10 a	50	80	60 ±7.6 b	42	61.5	51±5.75 c