# Identification of Honeybee Prevalent in Iraq According to the Geometric Morphometry Front Wing Using the Computer Program (identifly)

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**Abstract:**

This study included 13 regions: Basra, Babel and Wasit Province from Iraq. A total of 505 honeybee workers was used for this study. The geometric morphometry wing technique was used for identification Iraqi honeybee strains by applying computer program, the results of current study showed that the bee population distributed in two evolutionary lineages, the first one is evolutionary lineage C of the northern Mediterranean and southeastern Europe, and the evolutionary linage M of the western Mediterranean and northwestern Europe. Whereas the subspecies were diagnosed to the *Apis mellifera intermissa* that was included in the population group of the Grendland, Al-Baradiyah, Al-Harith, Hamdan, and Yusufan region, While the *Apis mellifera mellifera* was identified for each of the populations of Al-Gunaina, Shatt Al-Arab, Karmat Ali and Qurna region, Whereas the *Apis mellifera carnica* was identified for the population group of Al- Kharbtlia, Al-Tanuma, Babel and Wasit Province, This study is considered the first time of its type in Iraq.

**Keywords**: Diagnose, geometric wing, morphology, Iraq, honeybee races identification, Identifly.

## Introduction

The western honey bee or World bee *Apis mellifera* is one of the most important races of honey bees that spread widely all over the world, as it extending from Scandinavia to the Cape of Good Hope in the south, and from Dakar in the west to the Gulf of Oman in the East (Ruttner, 1987). His origin country Africa, Europe, and the Middle East and had introduced to America, Australia, and other countries. This caused the spread to the difference in the morphology characters, if bees are highly variable insects includes about 30 races recognized globally, including environmental patterns and lineage (Ruttner, 1988).

Many studies and researches had been done on honey bees because of their unique place among specialists in the field of insects, and they had received the largest share of multiple and disparate studies specializing in the most accurate morphological, genetic, anatomical, and biological. Among these studies are the morphology taxonomic that were used to distinguish

between the races in most citation. The scientist has developed many classification methods for bees and most of them are dependent on the shape of the wing, their length and width, the wing venation, the shape of the cells in the wings, No. hamuli (Ruttner, 1988) and the forewing coefficient (Cubital index), which are as considered good, accurate and approved methods (Tiago *et al*, 2008; Pilar *et al*, 2009), body size, coloration patterns (Abou-Shaara, 2009). These methods take a lot of time and effort, so there must be modern and new methods of automated measures and geometric morphometry had been used to distinguish Africanized honey bees from African and European subspecies, and to characterise the evolutionary lineages of *A. mellifera* (Tofilski, 2017). This method has also been used to analyse differences between three honey bee subspecies in Poland: *A. m. mellifera*, *A. m. carnica*, and *A. m. caucasica* (Tofilski, 2008).

As for the studies in Iraq unfortunately, they did not take sufficient attention and are almost very rare and old times, the first study was conducted in Iraq1977 and 1986 when many races were introduced to the country, including the Egyptian races hybridized with the Carniolan races, and then the Italian races were introduced (Muslim, 2013), In addition to the presence of several races in the Iraqi neighborhood that merged with the Iraqi bees. Therefore, this study aimed to determine the identity of Iraqi honey bees and their evolutionary lineage in Basra Province, southern Iraq, and Babil, Wasit Province, using the computer program identification.

## Materials & Methods

### Study areas

This study included 13 regions Al-Baradiyah, Al- Kharbtlia, Al-Gunaina, Karmat Ali, Al-Harith, Shatt Al-Arab, Hamdan, Yusufan, Tanuma, Grendland, Qurna (Basra Province), Al-Musayyib (Babel Province) and Al-Essowera (Wasit Province). The Global Positioning System (GPS) was used to determine the spatial and geographical boundaries within the study area which is located between two latitudes (29˚. 24˚ and 31˚. 29˚ north) and between two longitudes (46˚.52˚ and 48˚.71˚ East) (Yahya, 2013). Samples were collected from the area as follows: For each area, chosen randomly of 3-5 Hive/apiary and caught Foraging bees were in front of entrance to hive by forceps. at a rate of 10 bees/hive, which included the regions, Al-Baradiyah, Al- Kharbtlia, and Al-Geneina Total samples were 120 honeybee workers, 40 honeybee workers for to each e areas of Garmat Ali, Al-Harith, Hamdan, Yusufan, Tanuma, Grendland, 80 bees Al-Qurnah area in addition to Babel province, Al-Musayyib area 30 adult honeybee workers, and Wasit province, Al-Suwaira area 35 honeybee workers, the total samples for the study are 505 bees. They were placed in a plastic bottle perforated from the top, the samples were coded by writing the name of the area, the number of hives in the apiary, and the date of collecting the samples and placed in ice box until transported to the laboratory. Figure (1).

**Samples dissection**

The honeybee workers were dissected in an Hour bottle under the Dissecting Microscope of BEL type, and the wings were carefully separated using forceps and scalpels and placed in 70% ethanol alcohol. Then it was placed on a Temporary slide and examined and imaged with the Leica EZ4 Stereomicroscopes and the data for each site were recorded in a file until used in the software program identification.

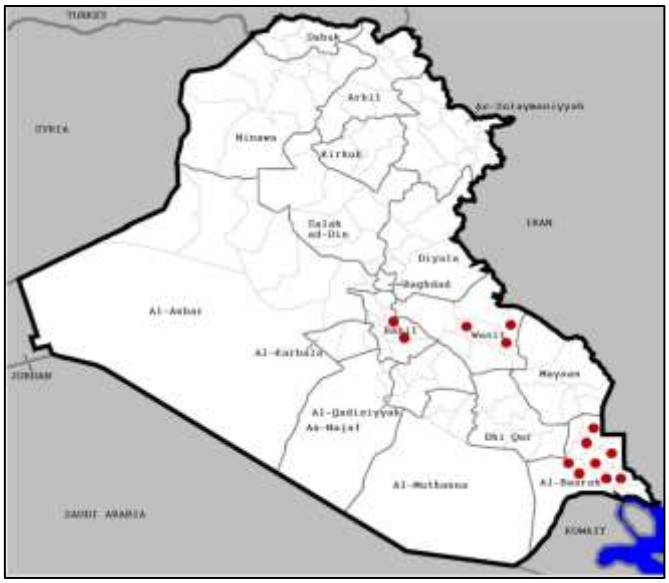


**Figure (1): Iraq map showing the regions for the *Apis mellifera* honey bee collection**.

**Samples Diagnose**

The wings samples were diagnosed of *A. mellifera* worker bees by applying the automated computer program. image is converted to gray, and then it is converted from the PNG format to the DW.PNG format, and all wing images are measured for each colony separately. (Anna *et al.*, 2018) Figure (2).

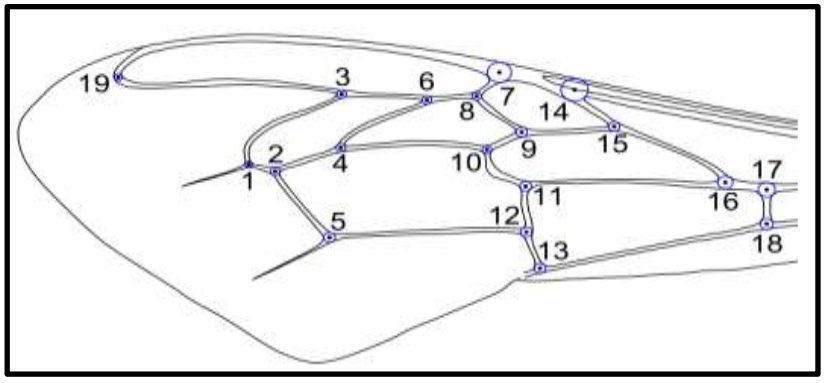


**Figure (2): Position of 19 landmarks on the forewing of honey bee workers. The landmarks should be placed in such a way that circles around them are tangent to the venation outline in at least three points.**

**Results & Discussion**

Diagnostic results showed that the honey bee *Apis mellifera* in the studied areas by using the forewing landmarks of the computer program (Identifly), the population honey bees back to the evolutionary lineage C of the northern Mediterranean, and the evolutionary line M of western

Mediterranean, while the subspecies or strains of the studied areas were diagnosed to the *intermissa* race included in the population group of the regions of Grendland, Al- Baradiyah, Al-Hartha, Hamdan and Yusufan, with a probability of 6.88553e-09, 4.90359e-05, 5.89726e-05, 3.4064e-06, and 7.1143e-08, respectively, Table (1), Figure. (3, 4, 5, 6 and 7).

**Table (1): Population group and evolutionary lineages of *A. mellifera intermissa* race, using the forewing landmarks of the Identify program.**

| Regions | Identification program | Probability | Evolutionary lineages | Probability |
|---|---|---|---|---|
| **Grendland** | *A. m. intermissa* | 6.88553e-09 | C | 0.000245554 |
| **Baradiyah** | *A.m. intermissa* | 4.90359e-05 | M | 0.000531144 |
| **AL-Harith** | *A. m. intermissa* | 5.89726e-05 | C | 7.85724e-08 |
| **Hamdan** | *A.m. intermissa* | 3.4064e-06 | M | 1.57481e-05 |
| **Yusufan** | *A. m. intermissa* | 7.1143e-08 | M | 0.00120157 |

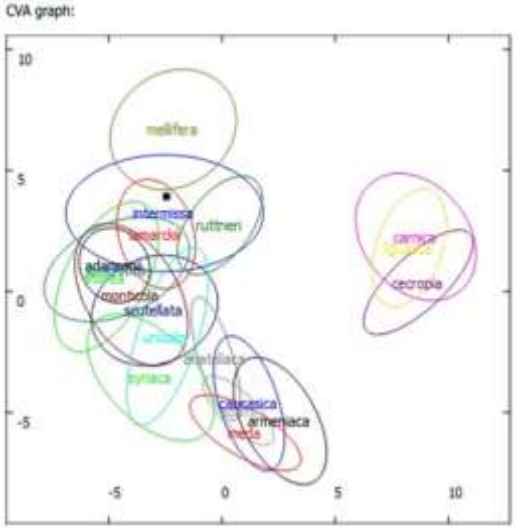


**Figure (3) diagram of the population group for intermissa race for Al- Grendland region.**

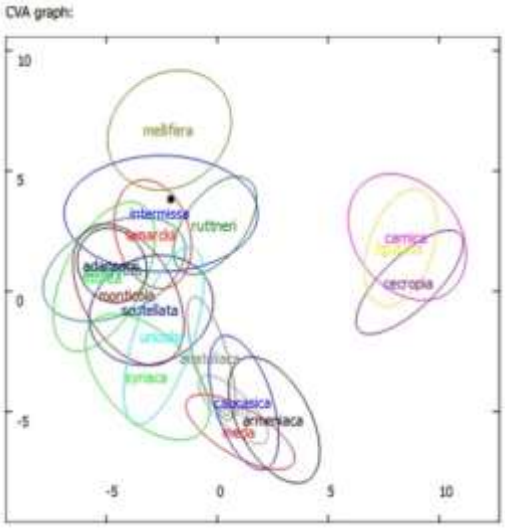


**Figure (4) diagram of the population group for intermissa race for Al- Baradiyah region.**

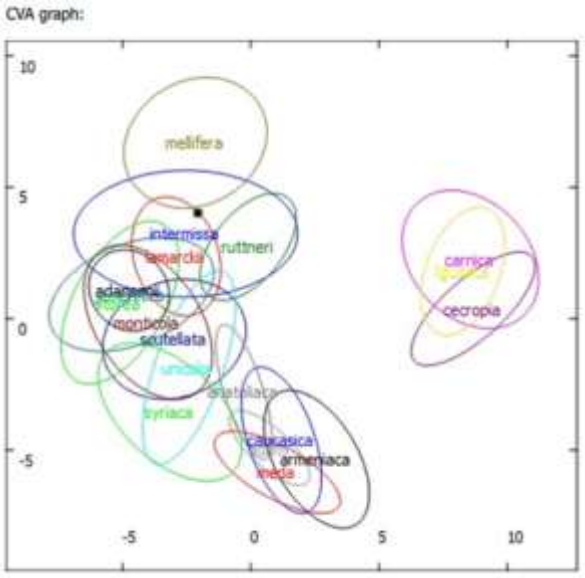


**Figure (5) diagram of the population group for intermissa race for Al- Harith region.**

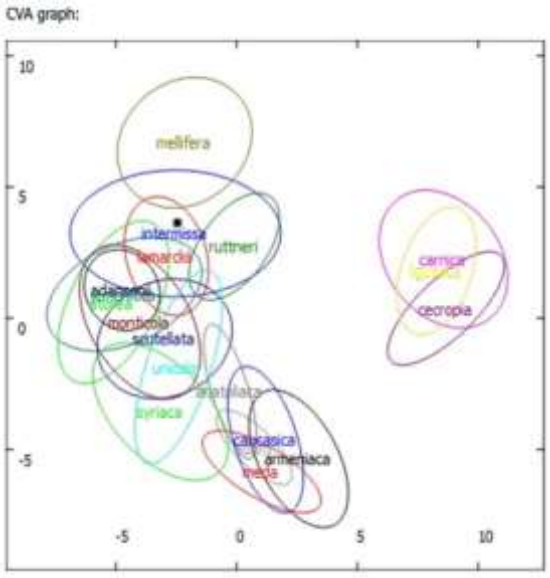


**Figure (6) diagram of the population group for intermissa race for Al- Hamdan region.**

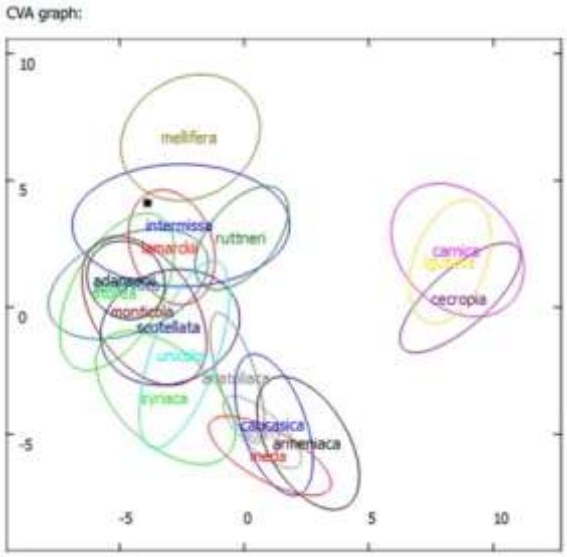


**Figure (7) diagram of the population group for race intermissa for Al- Yusufan region.**

While the *A. mellifera mellifera* race was diagnosed for each of the population groups in the regions of Al-Geneina, Shatt Al-Arab,
Karmat Ali, and Al-Qurna, with a probability of 0.0224152, 3.95266e-07, 0.0118798, and 1.86125 e-09, respectively, Table (2), Figure (8, 9, 10 and 11).

**Table (2): Population group and evolutionary lineages of *A. mellifera mellifera* race, using the forewing landmarks of the Identify program.**

| Regions | Identification program | Probability | Evolutionary lineages | Probability |
|---|---|---|---|---|
| **AL-Geneina** | *A. m. mellifera* | 0.0224152 | C | 1.30445e-06 |
| **Shatt Al-Arab** | *A.m. mellifera* | 3.95266e-07 | M | 7.77848e-05 |
| **Karmat Ali** | *A.m. mellifera* | 0.0118798 | M | 0.0433675 |
| **AL-Qurna** | *A. m. mellifera* | 1.86125e-09 | M | 8.43378e-10 |

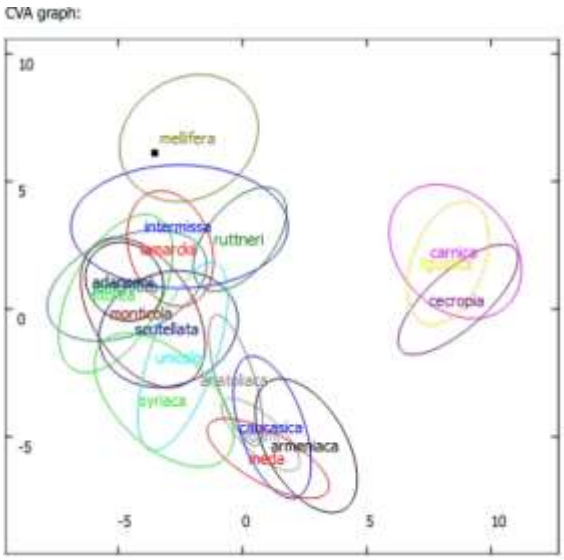


**Figure (8) diagram of the population group for mellifera race for Al- Geneina region.**

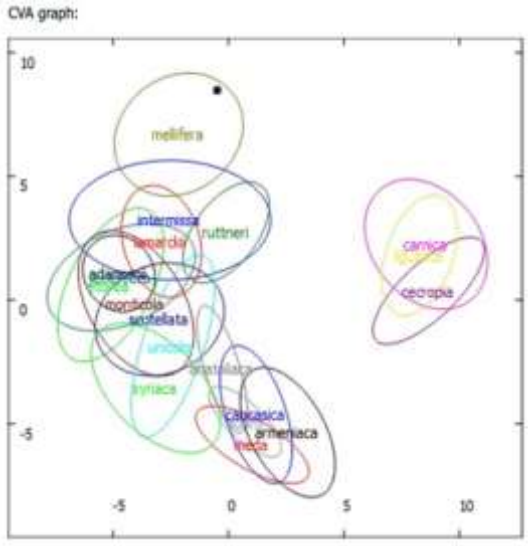


**Figure (9) diagram of the population group for mellifera race for Shatt Al-Arab region.**

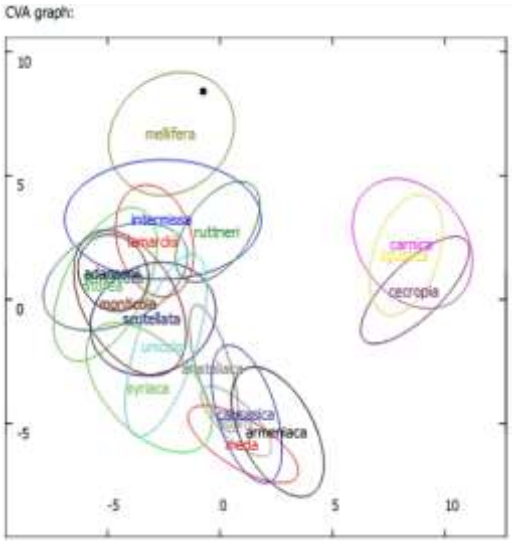


**Figure (10) diagram of the population group for mellifera race for Karmat Ali region.**

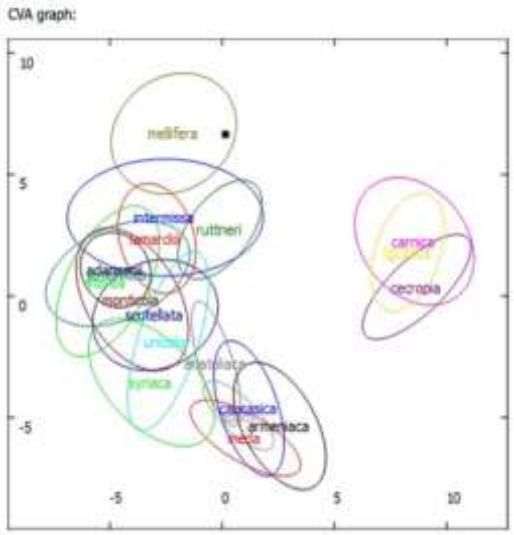


**Figure (11) diagram of the population group for mellifera race for AL-Qurna region.**

Whereas, the *A mellifera carnica* race was diagnosed for the population group of Al- Kharbtlia, Al-Tanuma, Babel, and Wasit Province, With a probability of 1.85477e-07, 1.52135 e-07, 2.27365 e-13, and 1.45931 e-07, respectively Table (3), Figure (12, 13, 14 and 15).

**Table (3): Population group and evolutionary lineages of *A. mellifera carnica* races, using the forewing landmarks of the Identify program.**

| Regions | Identification program | Probability | Evolutionary lineages | Probability |
|---|---|---|---|---|
| **Kharbtlia** | *A. m. carnica* | 1.85477e-07 | C | 0.000144007 |
| **Tanuma** | *A. m. carnica* | 1.52135e-07 | C | 1.26247e-05 |
| **Babel** | *A.m. carnica* | 2.27365e-13 | C | 1.61004e-17 |
| **Wasit** | *A. m. carnica* | 1.45931e-07 | C | 1.88832e-09 |

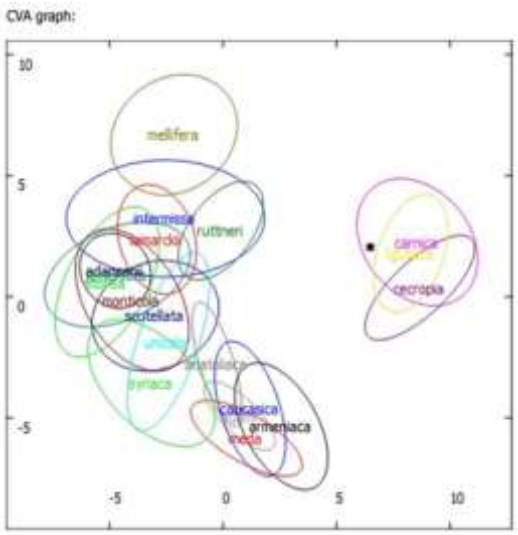


**Figure (12) diagram of the population group for carnica race for Kharbtlia region.**

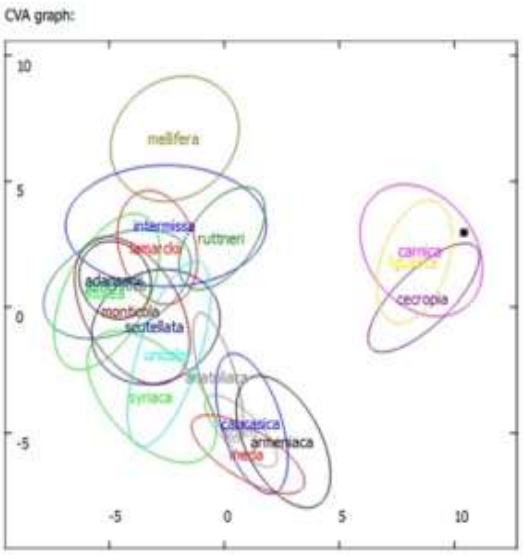


**Figure (13) diagram of the population group or carnica race for Tanuma region.**

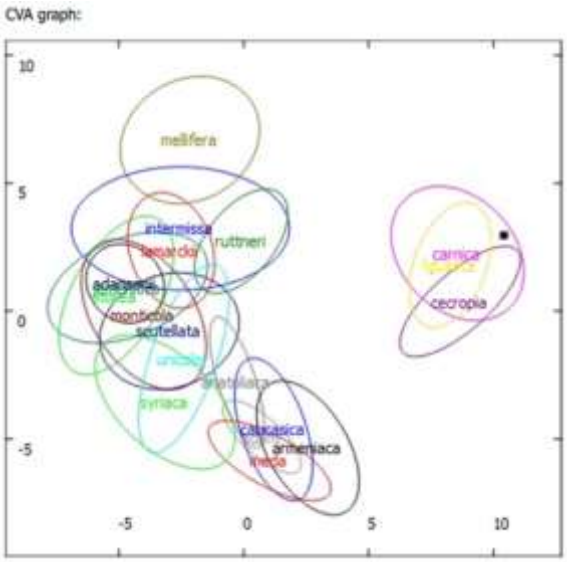


**Figure (14) diagram of the population group for carnica race for Babel Province.**

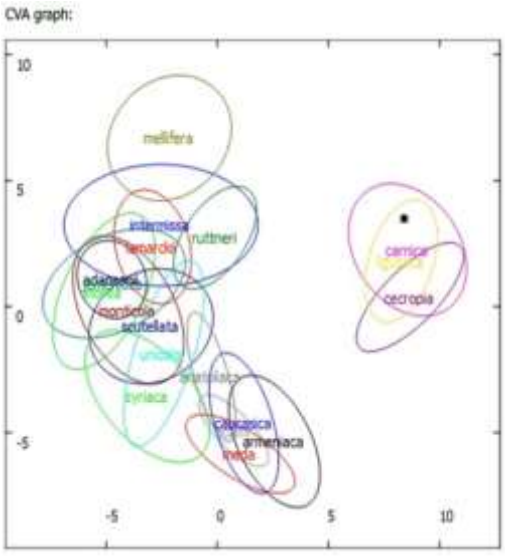


**Figure (15) diagram of the population group for carnica race for Wasit Province.**

The results show the study of the evolutionary lineages and subtypes of the population groups from the similarity of the evolutionary lineage C with the described race *carnica* in each of the regions of Al- Kharbtlia, Al-Tanuma, Babil, and Wasit This may suggest that this race keep its purity in these regions While, we note the asymmetry in the evolutionary lineage M with the described races *intermissa* in the regions of Grendland and Al-Hartha and the *mellifera* races in the Al-Geneina region that follows the evolutionary line C, which reflects the state of hybridization occurring between the population and whose causes include the trade of queens of honey bees and the breeding of migratory bees between regions (the researcher ). Ruttner and Kauhausen (1985) documented that honey bees in tropical Africa exhibit high variation in morphology characters (including wing venation) although the absence of natural barriers or artificial isolation zones that makes migration of bees a possibility that may cause bee hybridization. The multiplicity of honey bee races is the result of an adaptive response to different environmental conditions of climate, an abundance of food resources, and predation pressure (Ruttner, 1988) in a study by Marina *et al.* (2007) of the *A. m. mellifera* in Eastern

Europe (Poland, Belarus, and Ukraine), most samples of the northern part were classified as *A. m. mellifera*, but the crossbreeding rate increased towards the south, as it was classified as a hybrid between *A. m. mellifera* and subtypes of C and O lineages.

In a similar study by Barour *et al.* (2011) of the *A. m. intermissa* honey bee race in the regions of northern Algeria, using the landmark of the geometric characters of the forewing, he explained that the size of the wing differed greatly, as the largest was in the northwest and medium in size in the north of the center and the smallest in the northeast and this indicates The presence of more complex patterns within each region and apiarie. While Paweł *et al.* (2019) in the study of the effect of hybridization on the morphology features, including the geometry wing, three races of *A. mellifera*, its *mellifera, carnica,* and *caucasic*a, using artificial insemination, the study concluded that wing geometry in the first generation was similar to that of the mother colonies, except that it differed in reactionary hybridization from the mother's traits a lot.

All these gradual changes that occur in the morphology characters between the races in neighboring regions make the composition of honey bee population difficult to distinguish easily, so the need for a continuous review study of these patterns, The researcher stated Rustem, *et al.* (2020) in a review of the taxonomic structure of the subspecies of honey bee *A. mellifera*, and the results of this study distinguished 33 races, which were previously 26 races distributed as follows: 11 races throughout Africa, of which the *intermissa* races follows the developmental lineage A and 9 races in The Middle East and West Asia, which includes the *mellifera* races that follow the M evolutionary line, and 13 races in Europe, including the *carnica* races that follow the evolutionary lineage C, and these races were divided into 5 evolutionary lineages instead of 4 lineages which are the evolutionary lineage A and organized to it under the ratios Z and the evolutionary ratios M and the ratios C and the lineage O and the lineage Y, which includes one races, this leads us to say that honey bees in The studied areas were highly hybridized, and there is no 100% pure in the studied areas.

Also, these obtained results are inconsistent with what was mentioned by Muhammad & Ahmad (1977), taking into account the time dimension between the two studies, which is that the Iraqi bee race is very similar to its counterparts in the Syrian race, based on the report of the scientist Brother Adam, who The Iraqi race was considered to be a sub-variety of the Syrian race, as it was not among the studied population groups, including those related to the Middle East evolutionary line O, which includes the Syrian, Anatolian, Caucasian, and meda bees. This from one side and the other side, many races were Entered to Iraq, including the Egyptian race hybridized with the Carniolan race (Falih, 1977), which is considered the first attempt to crossbreeding Iraqi bees and the conclusions off Louay (1980) in which the Iraqi bees were classified within the group of yellow bees that include the Italian, Anatolian and Syrian bees.

This led to the spread of hybrid bees in central and southern Iraq, but the wars that Iraq went through led to a significant deterioration in the beekeeping of bees. As the queens of bees were Entered from standard races from Egypt, Iran, Syria, and America, the Italian races were Entered to the southern regions by Muslim (2013) and some African races were Entered to Babylon Province and the Carniolan race were Entered to Wasit and Erbil by some amateur beekeepers (personal contact with beekeepers). It led to the loss of the identity of the Iraqi hybrid race and the native Iraqi race.

The depends on the efficiency of discrimination the evolutionary lineage and subspecies in the program Identifly on the degree of purity of the races, it may reach 100% in pure races, and it may be different if hybrid races are belonging to different races resulting from a mating that give a low degree of probability and may be considered new hybrid patterns if it is not covered Induction program (Anna *et al*., 2018).

**Conclusions**

The method of diagnosing honey bee races using the geometry of the forewing in the Identify software computer program is easy, fast, and does not take a long time compared to other morphological methods, it is useful for knowing the races and degree of crossbreeding and Conservatism its good qualities, so it is considered better than other traditional methods.

**Acknowledgments**

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# تحديد هوية النحل المنتشر في العراق وفق الشكل الهندسي للجناح الامامي باستخدام البرامج الحاسوبي (identifly).

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**الملخص**

تضمنت هذه الدراسة 13 منطقة في محافظة البصرة وبابل وواسط، تم جمع505 *Apis mellifera*. هدفت الدراسة الى تعريف سلالات النحل العراقي بالاعتماد على الشكل الهندسي للجناح الامامي باستخدام البرنامج الحاسوبي identifly. وأوضحت نتائج الدراسة من ان المجتمعات السكانية المدروسة تعود الى نسبين تطوريين هما الخط التطوري C الخاص بشمال البحر الأبيض المتوسط وجنوب شرق اوربا، والخط التطوري M الخاص بغرب البحر الأبيض المتوسط وشمال غرب اوربا، في حين تم تشخيص النويعات او السلالات الى سلالة *Apis mellifera intermissa* التي تضمنتها المجموعة السكانية لمنطقة كرندلاند و البراضعية والهارثة وحمدان ويوسفان بينما تم تحديد السلالة *Apis mellifera mellifera* لكل من المجموعة السكانية لمنطقة الجنينة وشط العرب وكرمة علي والقرنة في حين تم تحديد سلالة*Apis mellifera carnica* للمجموعة السكانية لمنطقة الخربطلية والتنومة ومحافظة بابل ومحافظة واسط .

**الكلمات المفتاحية:** نحل العسل، سلالة نحل العسل الأوروبي، سلالة نحل العسل العراقي، التشخيص، الشكل الهندسي للجناح، المظهر الخارجي، برنامجIdentifly .