

First Record and Morphology Study of Red Dwarf Honey Bees *Apis florea* Fabricius (1787) in Basra Province

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Abstract. In this study, 25 Morphometric characters of dwarf honey bees *Apis florea*: 15 were for the body characters and 10 for wing venation, in four geographical areas in the province of Basra. a total Samples of 360 workers, Computer program ImageJ was used to measure Morphometric characters. The results of the statistical analysis of the averages showed a clear difference in the Basra area, as the least of them were in the characters of the Head length and width, femur length, width of the end of tibia, and 1st Wax mirror Length, followed by the Al-Siba area in the characters, tibia length, 3rd tergite length, and 3rd sternite length. As for the wing venation, which are forewing width, No. hamuli, A4 angle, B4 angle, G18 angle, and K19 angle. While the cluster analysis showed the hierarchical grouping method of Basra bees with some regional countries, the Basra bees group aligned with the Iranian bee group with a degree of lineage and genetic distance more than the rest of the groups, this study is considered a preliminary registration and diagnosis of *Apes florea* in Basra province.

Keywords. *Apis florea*, First Record, Morphometric, Characters, forewing, Dwarf honey bees, Basrah.

1. Introduction

The Red Dwarf Honeybee (RDH) *Apis florea* is classified superfamily Apoidea of the order Hymenoptera, the family Apidae, the subfamily Apinae, the Tribe Apini Latreille 1802, the Genus *Apis* Linnaeus 1787 and subgenus *Apis* (Micrapis) and the species *florea* Fabricius 1787, It was named so because of the reddish-brown color on the abdomen and its relatively small size compared to other honey bee species. Its origin is from Southeast Asia, Pakistan, India, Sri Lanka, Thailand, Southeast China and Malaysia, but in recent years its geographic range has been expanded to the west naturally and unintentionally via global transportation [7], [14]. This species was accidentally introduced in Saudi Arabia and Sudan [12], [14] and was discovered in Ethiopia [3] and was first recorded on the eastern border of the Red Sea around the port of Aqaba, Jordan [6] and extended to Iran, Oman and possibly Iraq and Abu Dhabi [24]. This expansion along the Arabian Peninsula and Africa proves that it is a successful colonialist, well adapted to the hot environmental conditions in both urban and rural areas and is a Strong Competitor Along with other species, especially *Apis mellifera* [4]. (RDH) are the Features construction of one small single comb that does not exceed the area of the hand and in the open nests [15]. is builds on the small trees, corners of hay stalks, generally preferring shaded sites. It also nests in corners of buildings, wells and on rock cliffs. The nest structure varies and depends on the nesting site. It is highly migratory preferring to migrate rather than defend whenever the colony is disturbed. [1], [18]. The many Morphometric studies on *A. florea* from India, Iran, Oman, Pakistan, Thailand and Sri-Lanka [7], [18] Cambodia, Myanmar, Nepal, and Vietnam [7] and Jordan [6]. There are many techniques to study the Morphometric characters of honey bees



Among these techniques is the use of body characters or standard Morphometric [17] to the use of coordinates of the wing venation pattern (landmarks coordinates) or geometric Morphometric [23], [18] used 20 standard Morphometric parameters and obtained three monoclusters. While [10] studied the characters of the hind leg, the basitarsus, and the color of the first and second tergum (Metasomal T1 and T2) and stinging.

The (RDH) It was not officially registered in Iraq Until he recorded it by [5] in the city of Mandali, 10 km west of the Iraqi-Iranian border, and then in the city of Khanaqin 60 km north of Mandali Its registration was confirmed by the Museum of Natural History in Iraq and the International Institute of Entomology in London. However, there is no study of its phenotypes in Iraq Therefore, the main objective of this research is a taxonomic study of the Morphometric characters of *A. florea* and comparing it with some geographical or environmental patterns in the regional countries through cluster analysis.

2. Materials and Methods

2.1. Study Areas and Sampling Collection

The study was conducted for the period from May 2017 to October 2019, (RDH) samples were collected from four geographical areas in Basra Province (which are 2.4 meters above sea level), which included AL-Siba ($30^{\circ} 19'39.9'' \text{N } 48^{\circ} 15'02.6'' \text{E}$), Abu Al-Khasib ($30^{\circ} 28'12.0'' \text{N } 47^{\circ} 53'55.9'' \text{E}$), Shatt al-Arab ($30^{\circ} 35'44.7'' \text{N } 47^{\circ} 46'42.9'' \text{E}$), and the Basrah Center ($30^{\circ} 32'13.8'' \text{N } 47^{\circ} 45'52.4'' \text{E}$) Fig. 1.

Two methods were used to collect samples. The first method was used: when full colonies were present and the second method: using Attractant bait traps (sugar solution) for bees in the areas where activity was monitored, which is usually Horticulture and nurseries using Aerial nets. 360 bees were collected from all the studied sites, including 35 bees for Al-Siba area, 70 bees for Abu Al-Khasib area, 75 bees for Shatt Al-Arab area and 180 bees for Basra Province Center. Samples were placed in bottles and icebox for a while Dissected in the laboratory.

2.2. Samples Dissection

After that, she was killed with hot water for 5 minutes For the proboscis studying, Were dissected samples into parts (head, thorax and abdomen) and placed in a dish consisting of 25 holes and learn to holes by the name of the sample and add the NaOH 10% at room temperature and leave for 1-2 days to get rid of tissues and Soluble fatty substances and pollen after the parts are washed with distilled water and to make permanent slides is done withdrawn water (Dehydration) from parts through a series of concentrations of alcohol 35%, 70%, 90% and 96% for 30 minutes for each concentration Then put into xylene 100 % for 1-2 minutes after which the prepared parts are fixed on a glass slide by a low viscosity DPX solution for wings sample, high viscosity for other parts and cover slide Then leave the slide on a hot plate with a temperature of less than 50°C and until completely dry [25], The prepared slides were examined and imaged by Leica Dissection microscope.

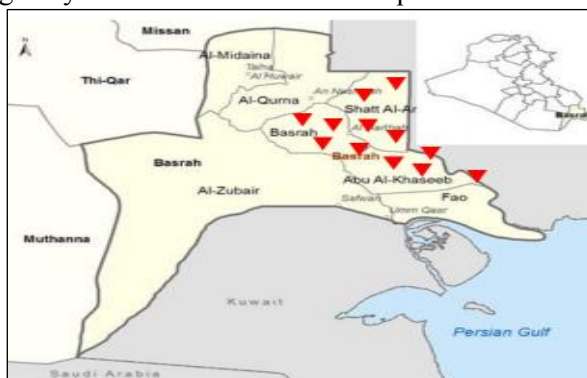


Figure 1. Basra map showing the areas where bee colonies were discovered *A. florea*.

2.3. Choice of Characters

The 25 Morphometric characters were selected, of which 15 were for the body and 10 for the wing: Body characters are Head length (HL), Head width (HW), proboscis length(PL), Antennal length (AL), hind femur length (HFL), Hind tibia length (HTL), the ratio of Length and Width of metatarsus (MLW- ratio), the width of the end of the tibia (HTW), ³rd tergite length (T3L), ³rd sternite length (S3L), ⁴th tergite length (T4L), ⁴th sternite length (S4L), ¹st Wax mirror Length (WM1L) Wax mirror1 width (WM1W), Wax between wax mirrors (WD). The characters of the wing are as follows Forewing Length (FWL), Forewing width (FWW), Hindwing Width(HWW), No. hamuli (NH), D7 angle, A4 angle, G18 angle, B4 angle, K19 angle, Cubital Index(CI) (Fig.2,3), use of a distance-measuring unit for image analysis in the digital image processor program ImageJ [11].

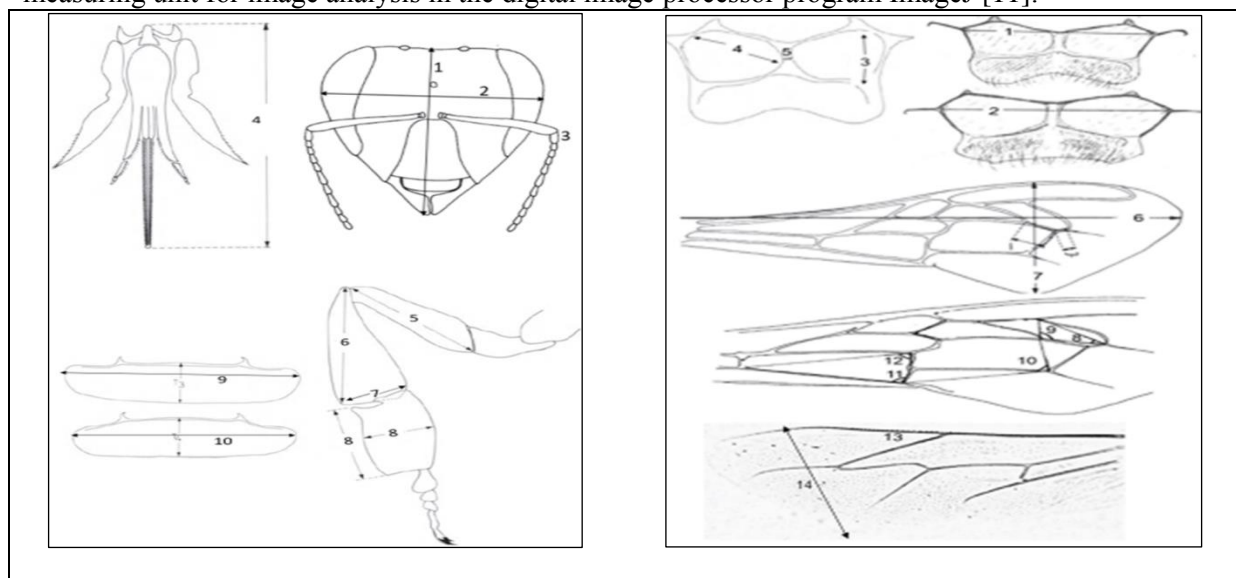


Figure 2. Morphometric characters of *A. florea* honey bees: 1 = head length, 2 = head width, 3 = Antennal length, 4 = proboscis length, 5 = Hind femur length, 6 = Hind tibia length, 7 = width of the end of tibia, 8= ratio of Length and Width of metatarsus, 9 = ³rd tergite length, 10 = ⁴th tergite length, for [18].

Figure 3. Morphometric characters of *A. florea* honey bees: 1 = ³rd sternite length, 2 = ⁴th sternite length, 3 = ¹st Wax mirror Length, 4 = Wax mirror1 width, 5 = distance between the Wax mirror1, 6 = forewing length, 7 = forewing width, A, B Cubital Index, 8 = A4 angle, 9 = B4 angle, 10 = D7 angle, 11 = G18 angle, 12 = E19 angle, 13 = No. hamuli, 14 = hindwing width, [18].

2.4. Samples Diagnose

Diagnosis of the insect as *A. florea* through taxonomic keys [9], [24]. Her diagnosis was confirmed by Prof. Dr. Kazem Saleh Al-Hadlak, Department of Biology, College of Sciences, Basra University.

3. Results and Discussion

3.1. The Body Morphology Characters

The results of the statistical analysis of 15 morphological characters of *A. florea* that were collected from four regions in Basra Province, Fig. 4 showed the superiority of the length of the head, in the Al-Siba, Shatt Al-Arab and Abu Al-Khasib regions, over the Basra region, as it reached 3.7737 mm, 3.722 mm and 3.6162 mm and 3.1808 mm respectively for the width of the head, Shatt Al-Arab, Al-Siba and Abu Al-Khasib surpassed Basra, which was the lowest, at 3.6423 mm, 3.6190 mm, 3.5827 mm and 3.1330 mm, respectively. As for the length of the proboscis, it was close to the values of the studied areas, as it reached 2.4838 mm, 2.4575 mm, 2.4238 mm and 2.4202 mm in each of Abu Al-Khasib, Basra, Shatt Al-Arab and Al-Siba, respectively. The length of the Antennal length was shorter

in the area of Abu Al-Khasib than in the areas of Al-Siba, Basra and Shatt Al-Arab, as it reached 2.3975 mm, 2.6168 mm, 2.5572 mm and 2.5543 mm, respectively, and there were no significant differences between them. Whereas, the femur length of the hind leg was the highest in the Siba region, followed by the Shatt Al-Arab and Abu Al-Khasib, while the lowest was in the Basra region, as it reached 1.6660 mm, 1.5800 mm, 1.4773 mm and 1.3858 mm, respectively. The leg length of the hind leg was the highest value in Basra region, reaching 2.0358 mm, and the lowest in Al-Siba, 1.8518 mm, while the two regions, Abu Al-Khasib and Shatt Al-Arab, did not differ from the two regions, reaching 1.9193 mm and 1.9192 mm respectively.

As for the characters of the ratio of Length and Width of the metatarsus, the Basra region is superior to all regions, the least of which was the Abu Al-Khasib area, and there was no significant difference between them, as it reached 2.8885 mm, 2.4475 mm, 2.2758 mm and 2.1775 mm, respectively, while the width The end of the tibia was in the Abi Al-Khasib area on all the areas that did not differ significantly, as it reached .72580 mm, while it was the lowest in the Basra region, as it increased .58020 mm, while the Siba and Shatt Al-Arab regions did not differ from their predicate as they reached .67230 mm and. Respectively.

As for the 3rd tergite length, the Basra region surpassed the rest of the areas that did not differ significantly from it, as it reached 5.6920 mm, while the least of it was the Al-Siba area, which reached 5.3848 mm, and the Shatt al-Arab and Abu Al-Khasib regions were equal, reaching 5.4673 mm and 5.4625 mm. Whereas, the 3rd sternite length was smaller than all in the Al-Siba area, which was significantly different, reaching 1.3673 mm, while there were no significant differences between the rest. The 4th tergite length was the highest value in the Abu Al-Khasib area, which was 5.0410 mm, and the lowest in the Al-Siba area was 4.4112 mm. Whereas, the values of all regions converged in the 4th sternite length characters, which did not differ significantly with Some of them. As for the 1st Wax mirror Length, the highest value was for the Al-Siba and Shatt Al-Arab regions, as it reached 1.2680 mm and 1.2053 mm, respectively The lowest of them were in the Basra and Abu Al-Khasib regions, and they reached 1.1897 mm and 1.1327 mm, respectively. The characters of the distance between the two mirrors was superior to the Basra region over all the regions, which did not differ significantly from it, as it reached .2867 mm, after which the Shatt al-Arab area reached .1578 mm. Abu Al-Khasib and Al-Siba converged with each other as they reached .1415 mm and .1432 mm respectively.

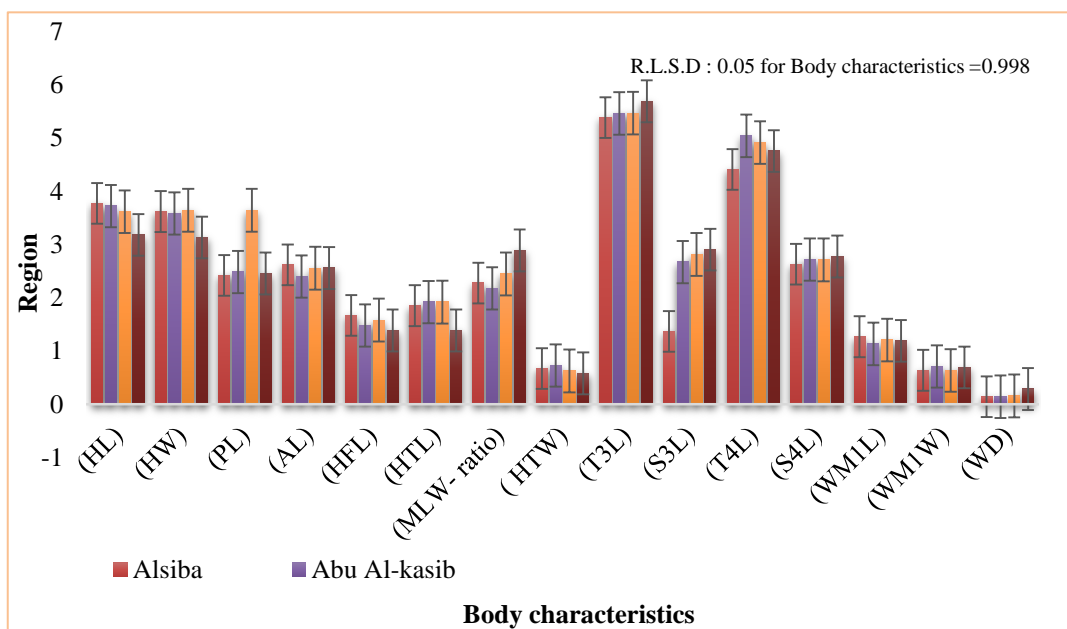


Figure 4. Body characters of workers *A. florea* collected from four geographical regions in Basra Province.

Through the results, it is evident that the *A. florea* in the Basra region were smaller in size than the rest of the other regions in the characters of head length, width, femur length and width, and the width of the end of tibia, the 1st Wax mirror Length, followed by the Al-Siba area in the characters, the length tibia, 3rd tergite length and 3rd sternite length. [19] stated that bees in low-altitude and hot, humid areas are of small size compared to high altitude areas. [21] explained that there are differences between the studied traits in regions of Thailand, where the average femur length was 1,721 mm, the tibia length was 2,183 mm, the length of the 3rd tergite length was 1,365 mm, the 3rd sternite length was 1.777 mm, and the antenna length was 2,819 mm. While [6] indicated that *A. florea* bees were distributed in two groups in Jordan using ten morphology characters, length tibia, femur, metatarsus, metatarsus width, 3rd 4th tergite length, forewing width, cubital a and cubital b. The first group was larger with length characters. length tibia, femur, metatarsus, 3rd 4th tergite length, length and width forewing the second group were with the characters cubital-a and cubital- b and metatarsus width. While [18] stated when comparing specimens of *A. florea* bees in my region in Sudan that they did not differ among themselves in the proboscis length, hind leg length and 3rd 4th tergite length as they reached 3.407 mm, 5.239 mm and 2.843 mm, respectively.

3.2. Wing Venation Pattern

10 morphology characters of the fore and hindwing were chosen to study the variance in the studied areas using the analysis of the averages and the standard deviation for each of the wing venation Fig. 5 What showed a clear difference in the studied characters was the superiority of the Shatt Al-Arab and Basra regions in the characteristic of the forewing length over the Al-Siba and Abu Al-Khasib regions, which did not differ significantly with it as it reached 6.1215 mm, 6.1173 mm, 5.7947 mm and 5.6990 mm, respectively, while the Shatt Al-Arab region surpassed In the characters of the forewing width on each of Basra, Abu Al-Khasib and Al-Siba, respectively, as it reached 2.0528 mm, 1.8600 mm, 1.6648 mm, and 1.6285 mm, respectively. As for the hindwing width, the Al-Siba exceeded all areas as it reached 1.5928 mm and Basra converged with Abu Al-Khasib As it reached 1.2980 mm and 1.2973 mm, while the Shatt Al-Arab was less superior, reaching 1.1710 mm, while the No. hamuli in the hindwing were superior to Basra and Abu Al-Khasib, respectively, without a significant difference between them over Shatt Al-Arab and Al-Siba , which did not differ significantly from Some of them, as they reached 13.1667, 12.5000, 11.1667 and 11.6667, respectively. As for the angle of the forewing, including the A4 angle, Basra and Shatt al-Arab were morally superior, as they reached 30.1747 and 30.0032, and the rest of the areas were not significantly differentiated While the B4 angle was superior in the Shatt Al-Arab region in a significant way over the rest of the areas that did not differ in a significant way between them, as it reached 104.5545, while all the regions differed significantly between them in the D7 angle and were arranged according to the following: Al-Siba , Abu Al-Khasib, then Shatt Al-Arab, then Basra 93.9432, 90.4937, 86.0167 and 83.8153, respectively, and Abu Al-Khasib and Basra were morally superior to the rest of the areas in G18 angle without significant differences between them, as they reached 95.3437 and 95.0880 respectively. Shatt Al-Arab also morally outperformed Al-Siba, which was the lowest rate, reaching 94.2275 and 92.5802, respectively, while Abu Al-Khasib and Basra outperformed the rest of the regions in a significant way without significant differences between them in the K19 angle, reaching 66.8278 and 66.2167 respectively, while it was followed by Al-Siba and Shatt Al-Arab, without significant differences between them, reaching 62.8828 and 62.0345, while the cubital index was is not significant in the studied areas, but it was highest in the Basra and Shatt Al-Arab regions, reaching 2.9950 and 2.9783, respectively, followed by Abu Al-Khasib and Al-Siba, which reached 2.5788 and 2.4785 respectively.

The results showed a clear difference in size upward, which appeared from Al-Siba to the Shatt Al-Arab, as the Al-Siba area was the least in measuring the characters, which is the width of the forewing, the No. hamuli, A4 angle, B4 angle, G18 angle and K19 angle, while the Shatt Al Arab area recorded the highest a value in the characters of forewing length, width, A4 angle, B4 angle and G18 angle, a fact indicated by [19] that bees increase in variation in size from east to west in different regions of

India while [18] indicated that bee samples *A.florea* in different regions not only differed in size but also in wing venation, as [2] mentioned that the forewing length and width were highest in Khuzestan, reaching 6.896 mm and 2.446 mm, respectively, and the lowest in Hormuzgan area reached 6.484 mm. And 2,188 mm, respectively, and through the analysis of the correlation coefficient and between the length of the forewing and its width, the relationship was positive high, reaching .599**, which was the highest in Basra and Shatt Al-Arab, and the lowest in Abu Al-Khasib and Al-Siba. The No. hamuli in the Basrah area was higher than in AL-Siba, [19] reported that the No. hamuli in Al-Ahsa were more than in Jubail, reaching 12.40 and 11.70 respectively.

[18] used the cubital index for the first time to distinguish subspecies. For the species of *Apis mellifera* and for its importance in distinguishing it, he used it in *A. florea*, through which he [2] explained that there was a difference in southern Iran, which reached 2.89, Hormuzgan reached 3.30 and Khuzestan reached 2.82, environmental conditions play an important role in influencing the morphology. For honey bees, [13] indicated that wing venation is affected by environmental factors, including temperature and season, while [8] indicated that wing venation is affected by the intensity of Selective pressure and the age of the adult bee.

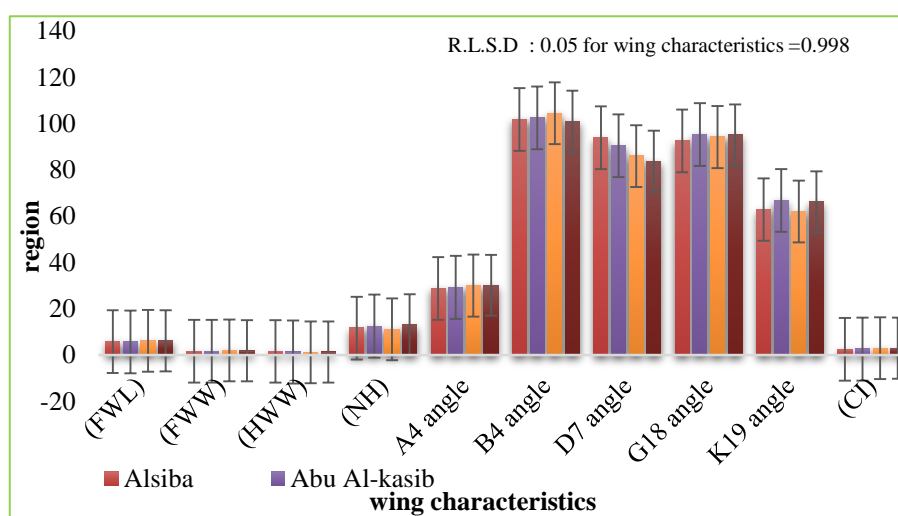


Figure 5. Wing venation pattern of workers *A. florea* collected from four geographical regions in Basra Province.

3.3. The Correlation Coefficient

The results of the correlation analysis for some of the studied characters showed that there is a high positive relationship at the probability level (1% **) between each of the head length and its width, which reached .832 ** and the forewing length and the width of It reached .599 **, while it was a high negative relationship between the width of the rear wing and the No. hamuli as it reached -.547 ** and a positive relationship (5% *) between the B4 angle and D7 angle amounted to .507 * and a positive relationship between the cubital index and A4 angle reached .447 * while the negative relationship between the cubital index and D7 angle was -.724 ** and the ratio of Length and Width of the metatarsus and width of the end of tibia if it reached -.524 * This reinforces the results of the study.

3.4. Cluster analysis of *A. Florea* Colonies with Regional Countries

From the results obtained for the hierarchical cluster analysis Fig. (6) to find out the degree of genetic relationship between *A. florea* in Basra with honey bees in regional countries, namely Iran, Pakistan, Oman, northern India, southern India (Sri Lanka), Vietnam, Myanmar, Thailand and Cambodia using morphology data These are the tibia length, the femur length, 3rd tergite length, 4th tergite length, the forewing length and the G18 angle from the data bank [16].

It was observed that the first clustering of geographical patterns between the group of Thailand and the group of Vietnam and the second cluster between the group of North India, Pakistan and Oman, and with a more genetic difference, the third cluster between the group of Pakistan and South India. From the group of Thailand, Vietnam, Myanmar, Cambodia, Nepal and the second main cluster included each of the group of North India, Pakistan, Oman, South India and Iran, as for the group of Basra bees, they were aligned with the second cluster, but to a more or less degree, and the genetic distance was closer to the Iranian group, mentioned [18], The characters of the forewing length, width, cubital index, and the No. hamuli in the hindwing of each of the southern Iranian bees are 6.706 mm, 2.313 mm, 2.89 and 12.89, respectively, Oman reached 6,516 mm, 2,248 mm, 3.08 and 11.37 respectively, and Pakistan 6,598 And 2,316 mm, 2.85 and 13.20 respectively, Sri Lanka 6.168 mm and 2,125 mm and 3.50 and 11.60 mm respectively, Southern India 6.252 mm and 2,140 mm and 3.32 and 10.50 mm respectively, Thailand 6,433 mm and 2.20 1 mm, 2.94 and 11.173, respectively, which are close to the values of wing venatin of *A.floea* in Basra, which were 6.121, 2.052, 2.995 and 12.50 respectively. This indicates that the species is *A. florea* and that the difference may be in genotype or ecotype. In the region.

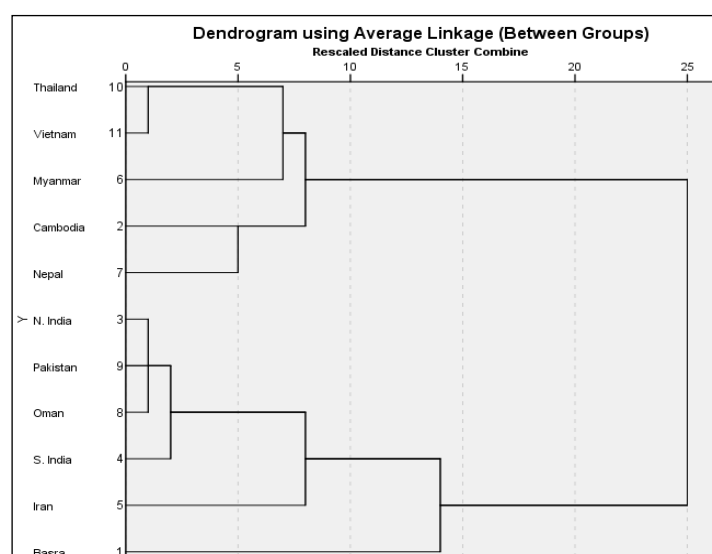


Figure 6. Hierarchical clustering dendrogram for *A. florea* Basra with averaged for countries.

Conclusions

- The *A. florea* in the Basra area was smaller than other areas in most of their body characters While the Al-Seiba area was the lowest in measuring the characters of wing venation, while the Shatt Al-Arab area recorded the highest value Therefore, we notice the alignment of Basra bees with the Iranian group, with a genetic distance less than the other groups Which suggest that the source of the dwarf honey bees *A. florea* in Basra is from the western side of Iran.

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