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SCREENING SUBSPECIES STATUS OF HONEY BEE WORKERS COLLECTED FROM SOME QUEEN REARING STATIONS UTILIZING GEOMETRIC MORPHOMETRICS

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ABSTRACT

The Egyptian honey bees, *Apis mellifera lamarckii*, in Egypt have been exposed to hybridization since the 1930s with other subspecies, especially *Apis mellifera carnica* (Carniolan bees). Current beekeeping practices are shaping the expectation that local honey bee stocks are distinct from the Egyptian subspecies, *A. m. lamarckii*. In this study, bee workers were collected from five queen-rearing stations located in four governorates: Gharbia, Alexandria, El-Behera, and Monufia. These samples underwent geometric morphometrics analysis to determine the subspecies they belong to with the highest probability. To achieve this, the forewings were separated and analyzed against a database that includes various honey bee subspecies found in Europe, Asia, and Africa. During sampling, workers that were accompanying bee queens were specifically selected. It is well-known that queen-rearing stations serve as sources for new queens for most apiaries, thereby aiding in the dissemination of a particular subspecies within local apiaries in Egypt. The majority of the samples exhibited a high probability of being Carniolan bees. Some samples showed relatedness to other bee subspecies in Africa and Europe, with only one sample displaying relatedness to *A. m. lamarckii*. This study supports the hybrid status of the bees collected from the queen-rearing

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stations and sheds light on the diminishing characteristics of indigenous bees in the studied samples due to hybridization with other subspecies over an extended period. Further studies are recommended to include other morphological characteristics and analysis approaches to delve deeper into the traits of local bee stocks.

Keywords: Honey bees; *Apis mellifera*; stock; queens; station; morphology.

INTRODUCTION

Honey bees, *Apis mellifera* L., colonies have been kept in Egypt for a long time. It has been documented that traditional beehives in Egypt were used in beekeeping during the Pharaonic periods, employing tools to manage bees and harvest honey (**Hammad, 2018; Sheppard *et al.,* 2001**). The indigenous honey bee subspecies in Egypt is *Apis mellifera lamarckii*, as documented in various studies (**Page *et al.,* 1981; Hussein, 2000; Abou-Shaara *et al.,* 2020)**. This subspecies underwent hybridization with Carniolan honey bees, *Apis mellifera carnica*, from Europe during the 1930s to enhance the characteristics of the native honey bees (**Hammad, 2018; Sheppard *et al.,* 2001; Abou-Shaara *et al.,* 2020**). It is worth mentioning that the *lamarckii* honey bees were not superior in terms of honey production and exhibited some undesirable behaviors such as swarming tendencies and aggressiveness (**Abou-Shaara *et al.,* 2020)**. The hybridization with Carniolan honey bees resulted in notable improvements in the characteristics of the bee stocks (**Abou-Shaara and Ahmed, 2015**). Over time, other honey bee subspecies have been introduced, either legally or illegally, leading to further hybridization in local bee stocks (**Page *et al.,* 1981**). Mostly Carniolan bees, along with Italian honey bees (*Apis mellifera ligustica*) and their developed stocks like the Buckfast honey bees, are commonly introduced as bee queens by beekeepers.

Modern beekeeping practices, based on using several techniques for queen rearing and hive management, have been employed by commercial beekeepers since the 1930s, while traditional

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beekeeping has declined except in a few locations in South Egypt (**Hussein, 2000; Al-Ghamdi *et al.,* 2016; Abou-Shaara *et al.,* 2020; Abou-Shaara, 2024a**). The presence of several queen-rearing stations has contributed to the dissemination of various bee queens in commercial apiaries. This underscores the significance of these stations in influencing the hybridization status of local bee stocks. Beekeepers typically replace their queens mostly during spring and again in autumn, further emphasizing the role of queen-rearing stations in impacting local bees (**Abou-Shaara, 2024b**). Various genetic and morphometric methods can be utilized to identify honey bee subspecies and their hybrids (**Meixner et al., 2013)**. Both methods are deemed adequate for accurately determining the subspecies of the test samples (**Aglagane *et al.,* 2022; Özdil *et al.,* 2022; Litvinoff *et al.,* 2023**). Considering the high costs associated with genetic analysis, morphometric analysis is regarded as a more cost-effective and rapid alternative.

The analysis of bee shape can be conducted using standard morphometrics analysis, which involves measuring various body parts (**Abou-Shaara *et al.,* 2013**), as well as geometric morphometrics (**Abou-Shaara, 2013**). The latter method is based on analyzing the forewing coordinates of bee workers. Typically, around 19 wing points (coordinates) are utilized in this analysis. Each subspecies possesses a unique wing shape and point coordinates, which aids in distinguishing subspecies when comparing wings from different samples (**Tofilski, 2008; Meixner *et al.,* 2013**). Therefore, this study involved utilizing worker samples collected from different queen-rearing stations. The forewings underwent geometric morphometrics analysis to differentiate the subspecies of the test wings. The results have been discussed in relation to the hybridization status of the tested samples, and further recommendations have been provided.

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MATERIALS AND METHODS

Sampling

In this study, five queen-rearing stations located in the northern Egyptian governorates - Gharbia (station 1), Alexandria (station 2), El-Behera (stations 3 and 5), and Monufia (station 4) - as depicted in **Figure 1**, were utilized. From each station, 10 worker bees were sampled. These worker bees are utilized as attendant workers for the queens in their shipping cages (10 cages per station, with one worker selected from each cage). Typically, the queens used within colonies from the queen stations are sourced from a select number of colonies, as they serve as the primary sources of larvae for rearing new queens. Consequently, a threshold of ten workers has been established as sufficient to achieve the objectives of this study. Therefore, cages containing virgin queens and workers were obtained from each station, and the workers were subsequently used in the study.

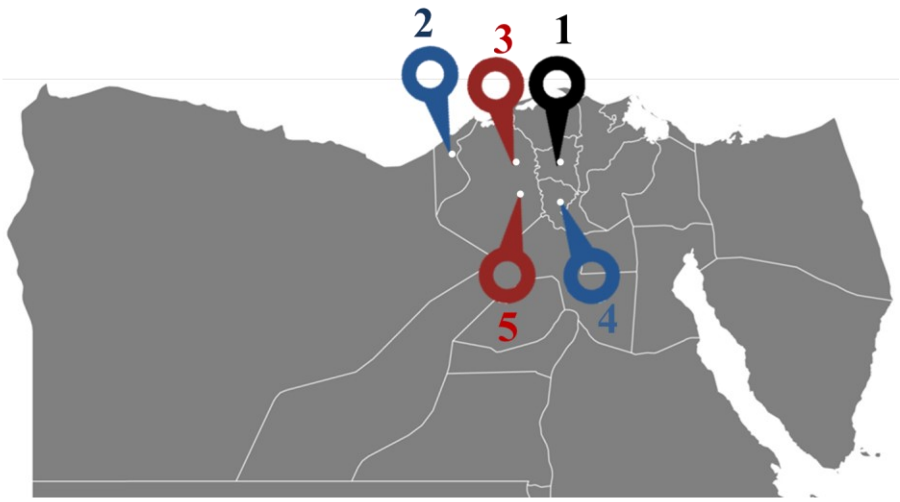

Figure 1: Map showing the approximate locations of the five queen stations used during the study in the northern Egyptian governorates: Gharbia (1), Alexandria (2), El-Behera (3, 5), and Monufia (4).

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Sample preparation

The workers were transported to the laboratory for subsequent analysis. They were placed in a freezer until analysis. Subsequently, the workers were dissected to separate their forewings, which were then scanned at a high resolution of 1200 dpi using a scanner (**Abou-Shaara, 2015**), sufficient for identifying the wing coordinates. The scanned wings were then used for geometric morphometrics analysis.

Wing analysis

The scanned wings were processed using an online platform, deepwings.ddns.net, by the Polytechnic Institute of Bragança, Portugal (**Rodrigues *et al.*, 2022**), which is capable of capturing the coordinates needed to locate the 19 wing points. This platform can compare the test wing point data with available datasets to identify subspecies and other characteristics, including hantel index, and discoidal shift, as well as determine the probability of the lineage to which the test sample belongs (**Figure 2**). The results of samples from each station have been presented and discussed.

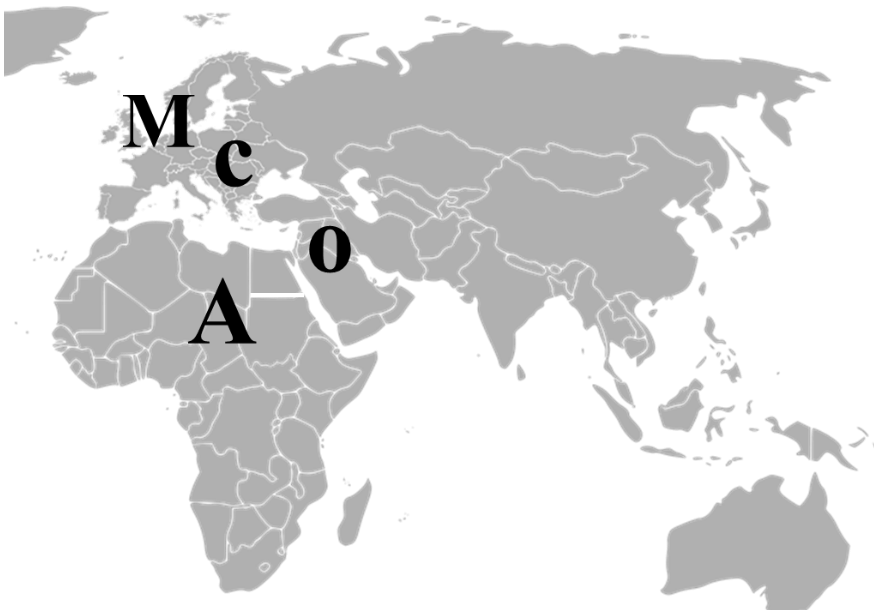

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Figure 2: Lineages of honey bee subspecies according to their geographical presence: Africa (A), Middle East and western Asia (O), central and southern Europe (C), and northern Europe and central Asia (M).

RESULTS AND DISCUSSIONS

Only three out of the ten samples were correctly identified for geometric morphometrics (**Figure 3**). These three samples differed from each other, with one belonging to *A. m. lamarckii*, while the other two belonged to *A. m. capensis* and *A. m. carnica*, respectively. The overall average indicates that the bees likely belong to *A. m. carnica* with a probability of 74.09% and to lineage C with a probability of 99.99% (**Table 1**). These results suggest a high level of hybridization among bees from Gharbia (Station 1), as there was no consistency found in the analyzed samples.

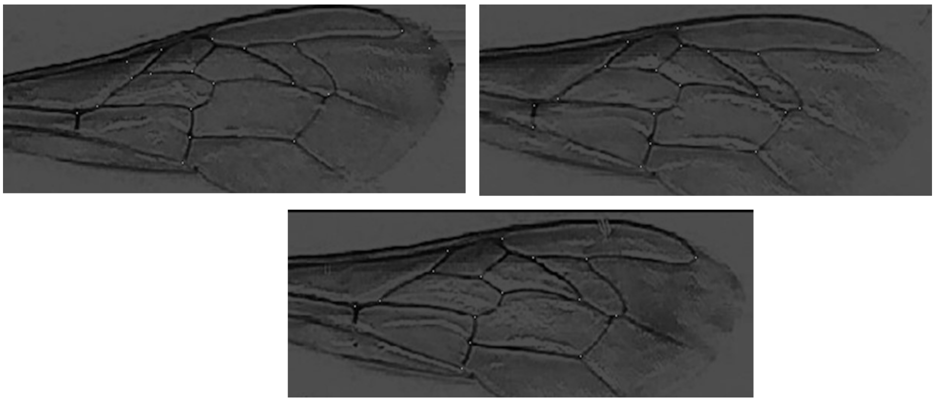

Figure 3: The identified wing landmarks for geometric morphometrics from samples collected at Gharbia (Station 1).

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Table 1: Wing characteristics and probabilities of lineage and subspecies for wing samples from Gharbia (Station 1)

Wing	Lineage	Lineage *P* (%)	Subspecies	Subspecies *P* (%)	Hantel index	Discoida shift
1	A	99.91	*A.m.capensis*	77.73	0.84	-0.63
2	C	96.17	*A.m.carnica*	71.34	0.87	3.60
3	A	99.57	*A.m.lamarckii*	72.55	0.84	1.35
Average	C	99.99	*A.m.carnica*	74.09	0.85	1.41

Four samples have demonstrated a good fit for identifying wing coordinates (**Figure 4**). All samples showed an association with *A. m. carnica* with probabilities ranging from 86.72% to 99.99%, whereas only one sample exhibited a link to *A. m. iberiensis* with a lower probability of 51.17%. The overall average indicates that the bees are likely of the *A. m. carnica* subspecies with a probability of 99.74% and belong to lineage C with a probability of 100% (**Table 2**). These outcomes suggest the prevalence of *A. m. carnica* characteristics in the analyzed samples from Alexandria (Station 2), as a consistent trend toward this subspecies was observed.

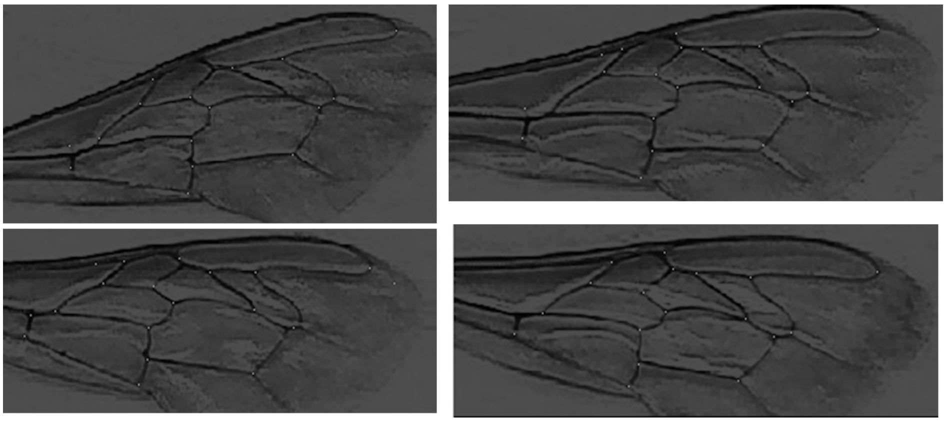

Figure 4: The identified wing landmarks for geometric morphometrics from samples collected at Alexandria (station 2)

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Table 2: Wing characteristics and probabilities of lineage and subspecies for wing samples from Alexandria (station 2)

Wing	Lineage	Lineage *P* (%)	Subspecies	Subspecies *P* (%)	Hantel index	Discoidal shift
1	C	100	*A.m.carnica*	99.45	3.13	23.58
2	C	100	*A.m.carnica*	99.99	2.77	-1.48
3	M	100	*A.m.iberiensis*	51.17	0.84	-0.13
4	C	100	*A.m.carnica*	86.72	0.87	-2.73
Average	C	100	*A.m.carnica*	99.74	1.42	4.95

Out of the 10 samples, seven were correctly identified for geometric morphometrics analysis (**Figure 5**). These samples displayed relatedness to three subspecies: *A. m. carnica* in four samples with probabilities ranging from 98.66% to 99.99%, *A. m. iberiensis* in two samples with probabilities between 61.89% and 93.07%, and *A. m. mellifera* in just one sample with a low probability of 47.68%. The overall average of the test samples indicates that the bees are likely of the *A. m. carnica* subspecies with a probability of 80.12% and belong to lineage C with a probability of 100% (**Table 3**). These findings suggest a hybridization status with a predominance of *A. m. carnica* characteristics in the analyzed samples from El-Behera (Station 3).

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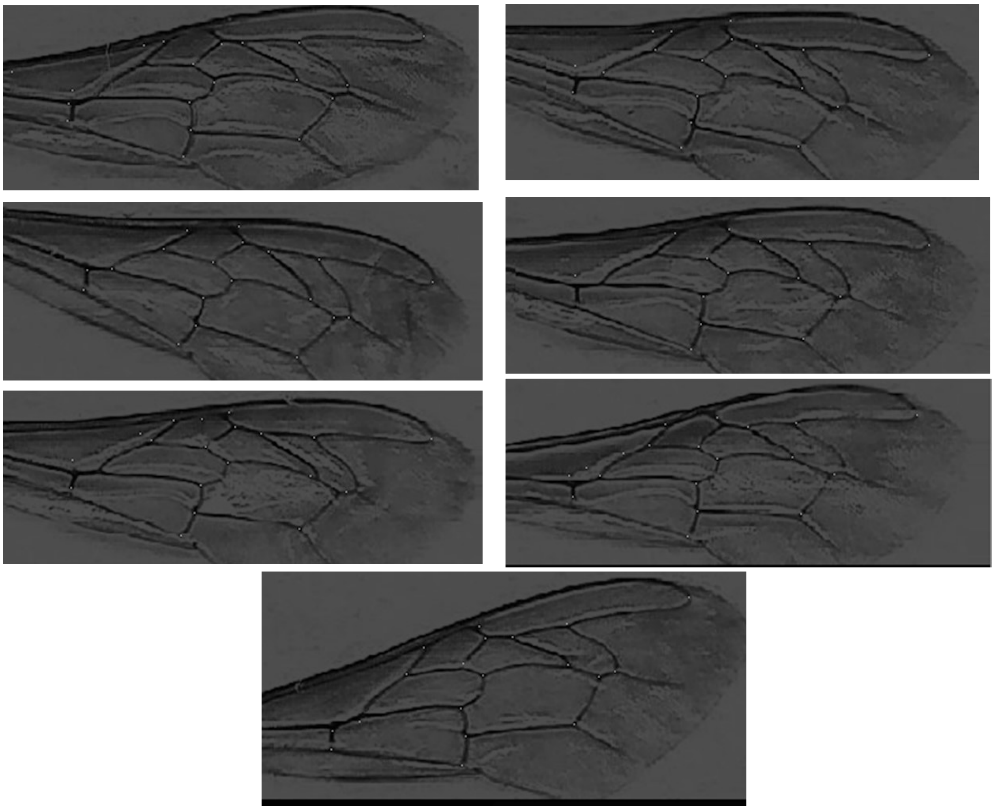

Figure 5: The identified wing landmarks for geometric morphometrics from samples collected at El-Behera (station 3)

Table 3: Wing characteristics and probabilities of lineage and subspecies for wing samples from El-Behera (station 3)

Wing	Lineage	Lineage P (%)	Subspecies	Subspecies P (%)	Hantel index	Discoidal shift
1	M	100	*A.m.iberiensis*	61.89	0.55	-111.83
2	C	100	*A.m.carnica*	99.99	0.90	-6.54
3	C	100	*A.m.carnica*	99.99	0.96	5.48
4	M	100	*A.m.mellifera*	47.68	0.84	1.30
5	C	100	*A.m.carnica*	98.66	1.03	1.48
6	C	100	*A.m.carnica*	99.16	1.20	-7.32
7	M	100	*A.m.iberiensis*	93.07	0.86	-2.69
Average	C	100	*A.m.carnica*	80.12	0.92	-4.16

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Samples from Monufia (**Station 4**) exhibited correct identifications for five out of the targeted 10 samples (**Figure 6**). These samples showed relatedness to *A. m. mellifera* in two samples with probabilities ranging from 91.01% to 99.99%, *A. m. carnica* in two samples with probabilities from 99.94% to 99.99%, and *A. m. iberiensis* in one sample with a probability of 76.28%. The overall average of the test samples from this station suggests that the bees are of the *A. m. carnica* subspecies with a probability of 99.99% and belong to lineage C with a probability of 100% (Table 4). These results indicate a hybridization status with a predominance of A. *m. carnica* characteristics in the analyzed samples.

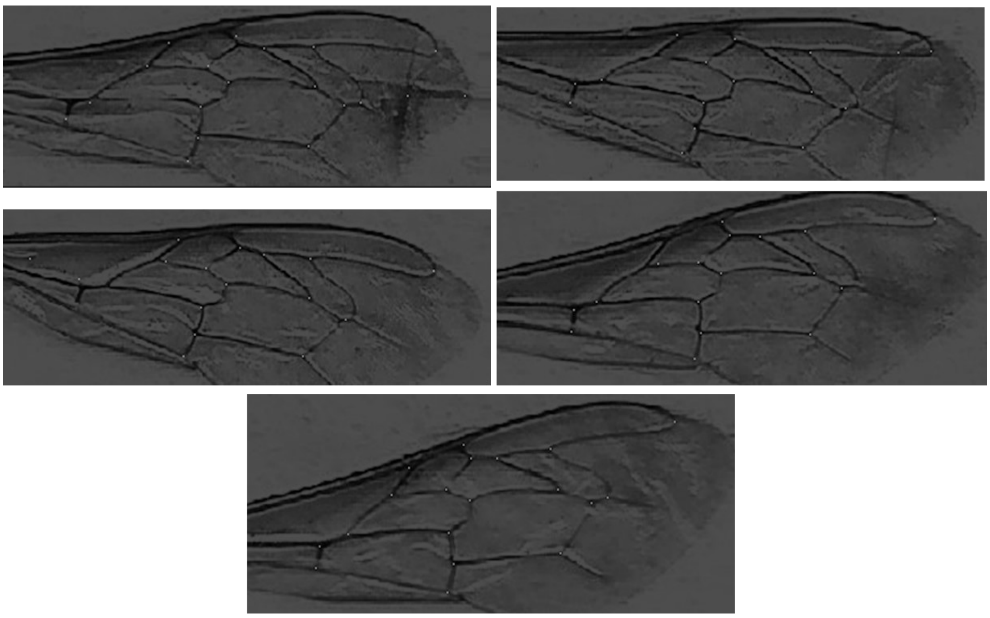

Figure 6: The identified wing landmarks for geometric morphometrics from samples collected at Monufia (station 4)

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Table 4: Wing characteristics and probabilities of lineage and subspecies for wing samples from Monufia (station 4)

Wing	Lineage	Lineage P (%)	Subspecies	Subspecies P (%)	Hantel index	Discoidal shift
1	M	100	*A.m.mellifera*	99.99	0.66	8.34
2	M	100	*A.m.iberiensis*	76.28	1.03	2.98
3	C	100	*A.m.carnica*	99.94	1.90	1.09
4	M	100	*A.m.mellifera*	91.01	0.93	0.68
5	C	100	*A.m.carnica*	99.99	2.27	94.15
Average	C	100	*A.m.carnica*	99.99	1.45	10.49

Only four samples were correctly identified for geometric morphometrics (**Figure 7**). All samples exhibited relatedness to *A. m. carnica* with probabilities ranging from 99.72% to 100%, while one sample showed relatedness to *A. m. caucasica* with a probability of 99.31%. The overall average suggests that the samples are of the *A. m. carnica* subspecies with a probability of 99.99% and belong to lineage C with a probability of 100% (**Table 5**). These results indicate the consistency of bees from El-Behera (Station 5) with *A. m. carnica*.

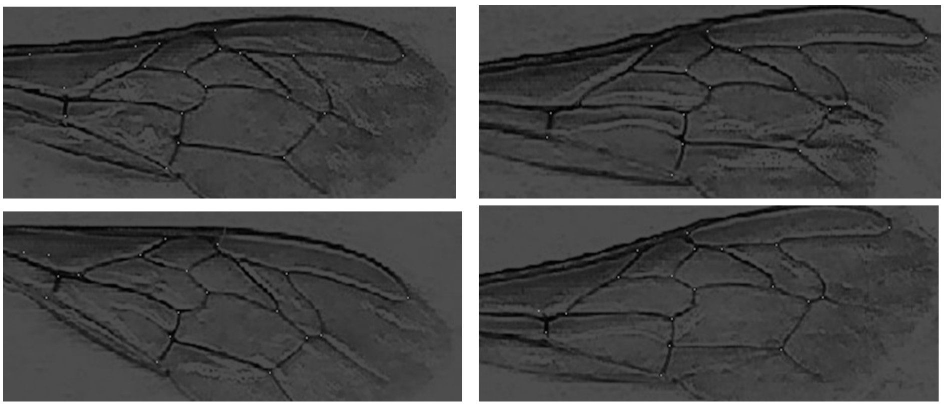

Figure 7: The identified wing landmarks for geometric morphometrics from samples collected at El-Behera (station 5)

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Table 5: Wing characteristics and probabilities of lineage and subspecies for wing samples from El-Behera (station 5).

Wing	Lineage	Lineage P(%)	Subspecies	Subspecies P(%)	Hantel index	Discoidal shift
1	C	100	*A.m.carnica*	99.72	0.90	1.049
2	C	100	*A.m.carnica*	100	2.56	60.24
3	C	100	*A.m.carnica*	99.99	1.15	71.73
4	O	100	*A.m.caucasica*	99.31	0.60	-4.60
Average	C	100	*A.m.carnica*	99.99	0.96	19.33

The online platform was not able to capture the coordinates required to locate the 19 wing points of the 10 scanned wings at each station, leading to discrepancies in the number of wings analyzed per station. This issue may stem from either the poor scanning quality of certain wings or technical glitches within the platform. Most of the samples showed a relationship to *A. m. carnica*, with probabilities ranging from 74.09% to 99.99% across the five stations. Other subspecies detected included *A. m. iberiensis* and *A. m. mellifera*, with only a single sample each for *A. m. capensis*, *A. m. lamarckii*, and *A. m. caucasica*. The distinction of the analyzed samples from the five stations from *A. m. lamarckii* (the indigenous bees of Egypt) was evident, as the Carniolan bees (*A. m. carnica*) were dominant. The hybridization between Carniolan bee stocks and the local bees significantly influenced the subspecies status of the analyzed samples. These samples showed a high probability of belonging to lineage C, ranging from 99.99% to 100%. In support of this study, the morphological characteristics measured in honey bee samples collected from southern Egypt (Assiut) argue against the purity of the bees (**Shaheen *et al.*, 2024**).

On the other hand, this study indicates inconsistencies in the bee stocks used at each queen station, although Carniolan bees dominate. The presence of other bee subspecies such as *A. m. mellifera* among the analyzed samples, albeit with low probabilities, suggests the use of queens from other countries in these stations. This study underscores the need for further research utilizing more samples for

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analysis and targeting additional morphological characteristics. Given the availability of various software tools for geometric morphometrics, such as MorphoJ (**Klingenberg, 2011**) and Geomorph (**Adams *et al.*, 2016**), and recognizing the significance of this analysis in identifying honey bee species/subspecies (**Santoso *et al.*, 2018; Henriques *et al.*, 2020; García *et al.*, 2022; Zaboli and Abbasi, 2023**), further studies in this area are recommended.

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الملخص العربى

إستكشاف حالة السلالة لشغالات نحل العسل المجموعة من بعض محطات تربية الملكات بإستخدام المورفولوجية الهندسية

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تعرض نحل العسل المصرى *Apis mellifera lamarckii* للتهجين منذ ثلاثينيات القرن العشرين مع سلالات أخرى، وخاصة *Apis mellifera carnica* (النحل الكرنيولى). وقد شكلت ممارسات تربية النحل الحالية التوقع بأن عشائر نحل العسل المحلية تختلف عن السلالة المصرية الأصلية، *A. m. lamarckii*. في هذه الدراسة، تم جمع شغالات النحل من خمس محطات لتربية الملكات تتواجد في أربع محافظات: الغربية والإسكندرية والبحيرة والمنوفية. خضعت هذه العينات لتحليل بإستخدام المورفولوجية الهندسية لتحديد السلالة الذي تنتمي إليه النحل بأعلى إحتمالية. لتحقيق ذلك، تم فصل الأجنحة الأمامية وتحليلها فى مقارنة مع قاعدة بيانات تتضمن سلالات مختلفة من نحل العسل الموجودة في أوروبا وآسيا وأفريقيا. أثناء أخذ العينات، تم إختيار الشغالات المرافقة لملكات النحل فى أقفاص السفرعلى وجه التحديد. ومن المعروف أن محطات تربية الملكات تعمل كمصدر للملكات الجديدة لمعظم المناحل، وبالتالي تساعد في نشر سلالة معينة داخل المناحل المحلية في مصر. وأظهرت غالبية العينات إحتمالية عالية لكونها من النحل الكرنيولى. وأظهرت بعض العينات إرتباطها بسلالات أخرى من النحل في أفريقيا وأوروبا، مع وجود عينة واحدة فقط أظهرت إرتباطها بـالنحل المصرى *A. m. lamarckii*. تدعم هذه الدراسة الحالة الهجينة للنحل الذي تم جمعه من محطات تربية الملكات وتلقي الضوء على تناقص خصائص النحل المحلي في العينات المدروسة بسبب التهجين مع السلالات الأخرى على مدى فترة طويلة. ويوصى بإجراء المزيد من الدراسات لتشمل خصائص مورفولوجية أخرى وأساليب تحليلية إضافية للتعمق في صفات عشائر النحل المحلية.