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Morphometric analysis of the Carniolan Honeybee (*Apis mellifera carnica*) in the Republic of Srpska

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Abstract

Beekeeping plays a significant role in animal husbandry, providing valuable products such as honey, beeswax, royal jelly, pollen, propolis, bee swarms, queens, and pollination services, essential for the survival of terrestrial ecosystems. The European honeybee (*Apis mellifera*) is classified into various subspecies, with the Carniolan bee (*Apis mellifera carnica*) being native to the Republic of Srpska. This research aimed to investigate the potential hybridization between native Carniolan bees and other subspecies, mainly the Greek (*Apis mellifera cecropia*) and Anatolian (*Apis mellifera anatoliaca*) bees, using geometric morphometry. Samples of adult bees were collected from 17 registered breeding centres across the Republic of Srpska and analyzed using the IdentiFly software, which relies on geometric morphometry for classification. The results have indicated some hybridization with the *A. m. cecropia* and *A. m. anatolica*, particularly in breeding centres located in warmer regions, such as Trebinje. However, no significant influence from the *A. m. ligustica* was observed. The northward spread of Greek and Anatolian bees, influencing the Carniolan bee population, is primarily due to the import of queens from these regions, with climate change acting as a significant contributing factor. The findings highlight the need for continued monitoring and selective breeding to preserve the native Carniolan bee and adapt it to the challenges posed by illegal queen import and climate change. Future research should focus on detailed analyses of bee

populations and the impact of climate change on the distribution of honeybee breeds.

Key words*:* Native bee preservation, Geometric morphometry, Crossbreeding, Breeding centres, Climate impact.

Introduction

Beekeeping is a specific branch of animal husbandry in which the beekeeper is not necessarily tied to their land. A person engaged in beekeeping is called a beekeeper. In beekeeping, we distinguish between migratory beekeeping (in which the beekeeper moves the hives to areas close to forage depending on the season) and stationary beekeeping, where the beekeeper places the hives mainly in one location (Crane, 1999). Besides honey, the main products of beekeeping include bee venom, royal jelly, beeswax, pollen, propolis, bee swarms, and queens. Beekeeping also produces other products that result from the processing of bee products, such as honeycomb, mead, honey brandy, honey liqueur, propolis cubes, gingerbread, and more (Nabhan & Buchmann, 1997). The importance of beekeeping, aside from the production of bee products, is also in pollination. Pollination is a vital process for the survival of terrestrial ecosystems and the human species (Diffenbaugh & Giorgi, 2012). More than 75% of major cultivated plants and 80% of flowering plants are pollinated by insects that carry pollen (Dedej et al., 1996). In total pollination activities, insects contribute about 80%, with bees contributing nearly 80%, making them the best pollinators (Ruttner, 1988). The bee is a social creature that can only survive as part of a group known as a bee colony (society). A bee colony consists of one queen (a reproductive female), drones (males), and worker bees (sexually undeveloped females) (Ruttner et al., 2000).

In 1758 Swedish scientist Linnaeus applied the binomial classification system for animals, where each animal was given two names. In this system, the European honeybee was named Apis mellifera. The first name (*Apis*) places the honeybee with a group of other bees, while the second name (*mellifera*) defines it as a distinct species (Linnaeus, 1758). The honeybee *Apis mellifera* is classified in the phylum *Arthropoda*, class *Insecta*. Since the bee's wings are membranous, it belongs to the order *Hymenoptera*. This order includes many species of social bees, both with and without stingers, as well as bumblebees. All these species are categorized under the *Apidae* family. This family is divided into two subfamilies: bumblebees (*Bombinae*) and bees (*Apinae*). The *Apinae* subfamily contains many genera, and for us, the *Apis* genus is of particular importance, as it includes four species of honeybees (Charles, 2000): European honey bee (*Apis mellifera*), Eastern honeybee (*Apis cerana*), Giant honeybee (*Apis indica*) and Dwarf honeybee (*Apis florea*). The European honeybee is particularly interesting to us because it is found in the European region. Across the broader European continent, the honeybee *Apis mellifera* L. is widespread with several varieties. However, four economically significant breeds of honeybees are widely accepted in scientific literature: *Apis mellifera mellifera* L. – the dark Dutch-German honeybee, *Apis mellifera caucasica* Gorb. – the Caucasian (dark and yellow) honeybee, *Apis mellifera ligustica* Spin. – the Italian (yellow) honeybee, and *Apis mellifera carnica* Polm. – the Carniolan (grey) honeybee. In the Republic of Srpska, the native bee is the grey bee *Apis mellifera carnica*, and one of the fundamental responsibilities is the preservation of this bee breed in our region.

To better understand the characteristics of bees in biosystematic research, morphological measurements were first applied in 1910 (Alpatov, 1929). A significant contribution to this research on the honeybee came from Russian scientist Aplatonov, who, studied the morphological characteristics of bees in relation to the areas they inhabit from 1924 to 1958 (Aplatonov, 1929). Initially, the differentiation of subspecies was based on descriptive methods, but these proved insufficient and were replaced by morphometric methods (Ruttner, 1988a). Morphometric methods are based on multiple measurements of many individuals (Alpatov, 1929). The subspecies of the honeybee (*Apis mellifera*) are typically distinguished using standard morphometric methods, which primarily rely on multivariate analysis of distances and angles. From a large number of characters used in earlier studies, Ruttner and colleagues (1978) selected 42 characters for the analysis of worker honeybees from a wide range of geographic locations. This set of characters, known as standard morphometry, has withstood the test of time and remains the most widely used method in a broad range of studies of geographic variation (Ruttner et al., 2000; Radloff et al., 2003; Diniz-Filho et al., 2000). Statistical shape analysis has made significant advances in recent years. A new method, often referred to as geometric morphometry (Bookstein, 1991), is based on the well-established shape theory (Kendall et al., 1999). Instead of using distances and angles, this method uses the coordinates of points called landmarks. These landmarks are positioned by shifting, scaling, and rotating. After positioning, the configurations of landmarks differ only in shape and can be analyzed using multivariate statistical methods (Zelditch et al., 2004).

This research aimed to investigate the potential hybridization between native Carniolan bees and other subspecies, mainly the Greek (*Apis mellifera cecropia*) and Anatolian (*Apis mellifera anatoliaca*) bees, using geometric morphometry.

Material and Methods

The bee samples analyzed were collected from 17 registered queen breeding and selection centres in the Republic of Srpska. The research was conducted at the Entomology Laboratory of the Institute for Genetic Resources at the University of Banja Luka. The bee samples were stored in 70% alcohol at a temperature of -5°C. For each location, 20 bees from several colonies per location were analyzed, and two front wings were removed from each bee (a total of 40 wings). The front wings were carefully separated, dried, placed between two microscope slides, and then photographed using a camera equipped with a 25 mm lens (RICOH FL-CC2514-2M). The wing photographs were saved in separate PNG files for each location. Each PNG file was processed separately, so the results for each breeding centre were obtained individually.

The analysis method is based on wing measurements. This method requires the identification of 19 points on the front wing photograph. It was implemented in the IdentiFly software (Tofilski, 2017), which is capable of identifying 19 subspecies and four evolutionary lines of honeybees. IdentiFly is semi-automatic classification software based on geometric morphometry. It was primarily developed for insect species identification but can also be used for classifying any objects that differ in shape. Furthermore, IdentiFly can be used as an alternative to tpsDig for digitizing landmarks in two dimensions. Currently, it is mainly used for identifying subspecies and lineages of honeybees (https://www.drawwing.org/identifly).

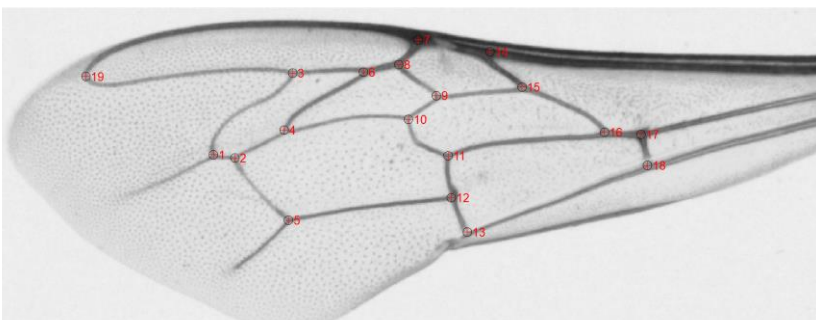

Fig. 1 Nineteen properly positioned points

Each of the 640 images was carefully reviewed, and point positions were corrected if necessary. After correction, data processing was carried out using Canonical Variate Analysis (CVA). This analysis is a multivariate statistical technique used to identify and analyze relationships between two sets of variables, particularly in the context of group comparisons (Johnson et al., 2007). When no hybridization is present, the population clusters distinctly within the space defined by CVA, meaning that individuals from that breeding centre are genetically similar to a known breed. When hybridization occurs, the analyzed bees do not form a distinct cluster but rather spread between two or more groups, showing genetic overlap.

Results and Discussion

Both breeding centres from Banja Luka were classified as the *A. m. carnica*, with the next highest probability being for *A. m. cecropia*, which is so small that we can consider there was no impact on the native breed. The results for the breeding centre in Bratunac, although with a higher probability for *A. m. carnica*, indicated that crossbreeding with the *A. m. cecropia* occurred, as there was no significant difference between the values. The results from Derventa show a minor influence of the *A. m. anatolica* while the results from Kozarska Dubica have shown no significant influence from other breeds, and the samples from these breeding centres were classified as *A. m. carnica*. For the breeding centre in Buljevići, there was a significant influence of the *A. m. cecropia*, while the other two breeding centres from Milići municipality have shown no influence from other breeds. Although the final result for the breeding centre in Petrovo has pointed to *A. m. carnica*, the next highest value was almost the same, and we can conclude that hybridization occurred between *A. m. carnica* and *A. m. cecropia*. According to the results, the breeding centres from Rudo and Sokolac, , belong to *A. m. carnica*, as the next highest values were significantly lower. In the breeding centre in Trebinje, there was a significant influence of *A. m. anatoliaca*, and *A. m. cecropia* also had a notable impact. Although the final result was *A. m. carnica*, it is evident that crossbreeding has occurred. For the breeding centres from the Zvornik municipality, there was no significant influence from other breeds, except for one in Kamenica, where there was an influence from *A. m. anatolica,* although it did not affect the final result.

A very significant observation is that the analysis has not identified the influence of the Italian bee on any breeding centre in the Republic of Srpska.

Values in Table 1 represent probabilities, where higher values indicate a stronger likelihood of belonging to a specific breed, which in this case is *A. m. carnica*, for all locations. Bolded numbers indicate values that show a significant mixture of the indicated breed with *A. m. carnica*.

Ruttner (1988) states that *A. m. carnica* is a morphologically quite uniform bee, essentially limited to the area between the Alps, the Carpathians, and the Dinaric Alps. The Mediterranean, including the Balkan region, is considered a global hotspot for climate change, with average warming and highly endangered populations (Diffenbaugh and Giorgi, 2012). Historical observations suggest that the Mediterranean has already experienced a particularly rapid increase in summer temperatures, a reduction in summer rainfall, and an increase in the number of consecutive dry days (Cramer et al., 2018; Lionello and Scarascia, 2018).

Tab. 1 The analysis results

Location of the breeding centre	*Apis mellifera carnica*	*Apis mellifera cecropia*	*Apis mellifera anatoliaca*
Banja Luka 1	0.50	0.000003	0.000002
Banja Luka 2	0.44	0.00008	0.000024
Bratunac	0.012	**0.0015**	0.000034
Derventa	0.27	0.000003	**0.00019**
Kozarska Dubica	0.43	0.00001	0.000035
Milići 1	0.11	**0.0011**	0.00002
Milići 2	0.0008	0.000001	0.000000
Milići 3	0.087	0.000001	0.000012
Petrovo	0.016	**0.015**	0.000003
Rudo	0.92	0.000009	0.000003
Sokolac	0.36	0.000002	0.000018
Trebinje	0.12	**0.0012**	**0.029**
Zvornik 1	0.89	0.000003	0.00001
Zvornik 2	0.32	0.000007	0.000068
Zvornik 3	0.36	0.000003	0.00001
Zvornik 4	0.073	0.000027	**0.00028**
Zvornik 5	0.081	0.0000002	0.000011

Climate change and the rise in average temperatures, along with the import of queens, are facilitating the spread of the *A. m. cecropia* and the *A. m. anatoliaca*) to the south, into the areas where the native *Apis mellifera carnica* breed is located. This claim is particularly evident when considering the results from the breeding centre in Trebinje. The value for the Carniolan breed was 0.1178, then 0.0291 for the Anatolian breed, and 0.0012 for the Greek breed, showing that significant mixing of breeds has occurred.

It is safe to say that the geographical location of Trebinje and higher average temperatures, which are higher compared to other localities in the Republic of Srpska, have contributed to a more significant spread of these two breeds. Additionally, the proximity to the border and the import of bees from neighbouring regions have further facilitated their spread. It is likely that their influence has been present in this locality for a longer period.

Conclusion

The main hypothesis in this research, that the method of geometric morphometrics will show that other bee breeds have not interbred with the native *Apis mellifera carnica* (Polman 1879) breed in the Republic of Srpska, has not been proved. Although the final results classify all the breeding populations as Carniolan, the analyzed samples indicate that there has been some interbreeding with the Greek bee (*Apis mellifera cecropia*, Kiesenwetter 1860) in four breeding centres and the Anatolian bee (*Apis mellifera anatoliaca*, Maa 1953) in three breeding centres. The result of the illegal import of queen bees, along with climate change, is the northward shift of the distribution ranges of these two bee breeds, which has enabled their influence on the Carniolan breed. It is very important to note that there has been no influence from the Italian bee (*Apis mellifera ligustica*, Spinola 1806).

Future research should focus on a more detailed analysis of breeding populations by applying both standard and geometric morphometrics to obtain more detailed data. Furthermore, a more detailed investigation of the impact of climate change on the distribution of bee breeds is necessary. Further selective breeding efforts should focus on preserving the native *Apis mellifera carnica* breed in our breeding populations through constant and continuous control of the queens selected for breeding. The selection process should prioritize developing strains that are more resistant to climate change, as the influence of *Apis mellifera anatoliaca* and *Apis mellifera cecropia* is expected to grow, leading to increased spontaneous hybridization. Additionally, more stringent import control measures, the implementation of stricter legal regulations, and educating beekeepers about the importance of preserving our native breed should be prioritized.

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Морфолошка анализа крањске медоносне пчеле (*Apis mellifera carnica*) у Републици Српској

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Сажетак

Пчеларство има значајну улогу у сточарству, пружајући вриједне производе као што су мед, пчелињи восак, матична мљеч и опрашивачке услуге, које су од есенцијалне важности за опстанак копнених екосистема. Европска медоносна пчела (Apis mellifera) класификована је у различите подврсте, а Крањска пчела (Apis mellifera carnica) је аутохтона у Републици Српској. Ова истраживања имала су за циљ да испита потенцијално укрштање између аутохтоне Крањске пчеле и других раса, посебно Грчке (Apis mellifera cecropia) и Анатолске (Apis mellifera anatoliaca) пчеле, користећи геометријску морфометрију. Узорци пчела прикупљени су из 17 регистрованих репроцентара широм Републике Српске и анализирани коришћењем софтвера IdentyFly, који се ослања на геометријску морфометрију за класификацију. Резултати су указали на укрштање са Грчком и Анатолском пчелом, посебно у пчеларским центрима смјештеним у топлијим регионима, попут Требиња. Међутим, није примећен значајан утицај Италијанске пчеле (Apis mellifera ligustica). Климатске промјене и пораст температура доприносе ширењу Грчке и Анатолске пчеле према сјеверу, што утиче на популацију Крањске пчеле. Налази указују на потребу за континуираним праћењем и селективним узгојем како би се сачувала аутохтона Крањска раса пчела и прилагодили изазовима које доносе климатске промене. Будућа истраживања требало би да се фокусирају на детаљне анализе пчелињих популација и утицај климатских промена на дистрибуцију раса медоносних пчела.

Кључне ријечи: очување аутохтоне пчеле, геометријска морфометрија, укрштање, репроцентри, утицај климе.

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