Genetic Local Differentiation of A.m.carnica Population as well as Subspecies A.m.macedonica, A.m.ligustica, A.m.mellifera, A.m.caucasica, in Germany, Alpine Region, Austria, Croatia, Serbia, Northern Kosovo, Albania and Macedonia

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Abstract
This Measurements of 11 traits of the right forewing of the Albanian native bee were made in this study. Using these traits, the relations between the Albanian bee population and populations belonging to subspecies A.m.carnica, A.m.ligustica, A.m.macedonica, A.m.mellifera and A.m.caucasica were evaluated. Based on these data, the analysis of the principal components and cluster analysis is made. According to them, it is found that the Albanian bee population can be classified in the group of the subspecies A.m.carnica. These analyzes highlight the existence of two distinct groups of subspecies A.m.carnica. The Albanian population is part of the group A.m.carnica breeding in Italy, Croatia and Serbia. During the last twenty years the Albanian bee population is differentiated more with A.m.carnica populations breeding in northern Italy and Croatia, and is much closer to the population of the subspecies A.m.carnica breeding in Serbia and A.m.macedonica.
Keywords: honey bee, morphometry, angle, cubital index

1. Introduction

Our native bee has black body, which is covered with silver-colored fluff circle from the second half circle of the back to the fifth circle of the back, this thing nears our bees with bees of carnica race (Nuri 1965). In native bees and carnica bees, worker bees which have half of the first circle of the back in yellow are found; their chest is covered with fluff gray. Foreign and domestic authors have given some considerations for the identity of our bee. The foreign scholars define our native bee as the southern border of the spread of carnica race (Ruttner 1963), or Infantidis (1979) defines Greek bees in its northern border, so at our southern border as "variety of carnica race". Others in the study of bees for the Kosovo and Metohi area (Vladkovic and Kulincevic 1958) claim for pure breeding of the carnica race. While in the studies for the Macedonian bee (J. Rihar 1958), are recorded that three morphological traits of these bees coincide with those of carnica race, while according to the authors group (Uzunov A., Kiprijanovska H., Andonov S., Naumovski M., Gregorc A. 2009) at the Macedonian territory, three bee races dominate; A.m.macedonica, A.m.carnica, A.m.ligustica and the sampling made in the area adjacent to Albania (Mavrovo) resulted in, the presence of the bee Apis mellifera carnica.

The first study was conducted by Nuri Qenan (1963). He realized measurement of the forewing length, forewing width, number of hooks, longitudinal diameter of tergite 4 and proboscis length. The study was

conducted in a limited number of families (15), which lie in the north-eastern area of the country. He concluded that our native bee is a variety of carnica race.

Dedej S (1996), realized morphometric measurements of forewing characters with computer method, as well as conducted the electrophoretic analysis of head and thorax of worker bees. 15 morphological traits that he analyzed were: distance a, distance b, distance c, distance d, angle A_4, angle B_4, angle D_7, angle E_9, angle G_{18}, angle J_{10}, angle J_{16}, angle K_{19}, angle L_{13}, angle N_{23}, angle O_{26}. He has defined our bee as a hybrid between A.m.carnica and A.m.macedonica and that Albanian territory can be considered as the southern border of distribution of A.m.carnica race.

Morphological analysis was extended further in 2002 by (K.Thomo., L.Shehu., Z.Bajrami., M.Koni), who conducted biometric research on bee population in Albania based on six morphometric traits. Measurement of traits is made with traditional methods, with microscope equipped with ocular micrometer. Traits that they analyzed were: forewing length, forewing width, proboscis length, cubital index, length of hairs on tergite 5, width of Tomentum in tergite 4. The study covered 20 districts of Albania, representing different geographical and climate areas. The result of the study was that our bee is a variety of carnica race and consists of homogeneous populations, without significant geographical differences.

Measurements of wing characters are very important for honey bee classification and in the discrimination between races. The simplest method in classification of bees is the measurement of fore wing characters, which are typical for each pure race (Kauhausen-Keller and Keller, 1994). Right wings are used by many authors during their morphometric analysis (Nazzi, 1992; Uzunov et al., 2009; Mladenović et al., 2011 and

Abou-Shaara et al., 2012). In this study, are made measurements of forewing angles, in 20 bee population from different districts of Albania and the values of these features are used to compared with the values of the same features in different subspecies. Traits that we analyzed were: angle A_4, angle B_4, angle D_7, angle E_9, angle G_{18}, angle J_{10}, angle J_{16}, angle K_{19}, angle L_{13}, angle N_{23}, angle O_{26} as shown in figure 1. Our aim in this study is evaluation of relations / differences between Albanian bee and other bees, with objective, definition of belonging, subspecies, in which it can be classified.
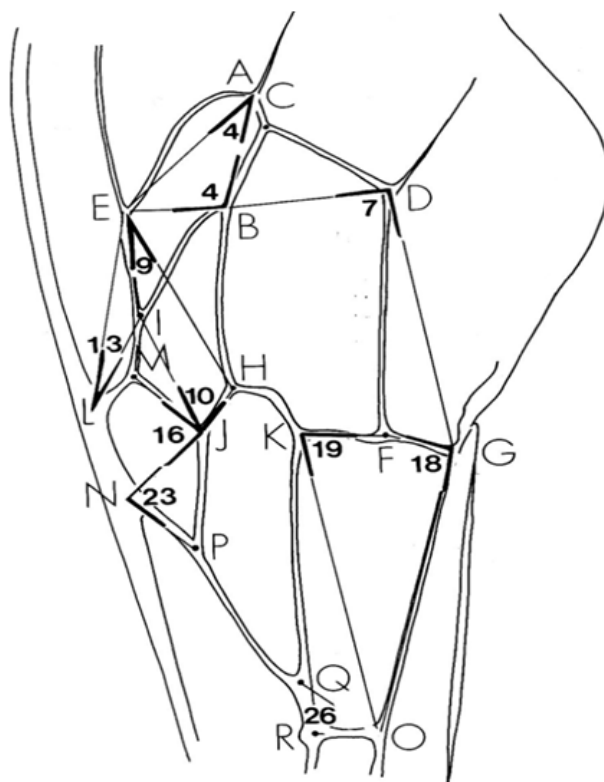

Figure 1. Forewing traits measure

2. Materials and methods

In period April-June 2012 and 2013, samples with 100 bees each, are collected from 60 different honeybee colonies. Districts where samples have been taken are: Tropoja, (2) Puka, (3) Kukës, (4) Shkodra, (5) Dibra, (6) Burrel, (7) Lezha, (8) Kruja, (9) Tirana, (10) Elbasan, (11) Gramsh, (12) Librazhd, (13) Korça, (14) Erseka, (15) Fier, (16) Lushnje, (17) Berat, (18) Përmet, (19) Vlora, and (20) Saranda, as shown in figure 2. In every district, 9 samples are collected from three different parks with satisfactory health status and who did not practice queen replacement or swarming from other countries, especially from areas that we have taken the samples. In total were collected 180 samples, from each sample 20 worker bee are randomly chosen and are dissected for biometric measurements.

Measurements of 11 angles, between the coordinates of 18 vein junctions, of the right forewing (Ruttner 1978), have been conducted in 3600 worker bees. To evaluate the changes that happened during the last 20 years in the local population of bees in Albania, the data published by Dedej, S. et al. (1996) for 11 traits was used. His study was conducted in 8 districts of Albania, which are: (1) Burreli, (2) Kavaja, (3) Kruja, (4) Kukes, (5) Pogradec, (6) Saranda, (7) Tepelena, (8) Tirana, as shown in figure 2.

Even neighboring countries conducted similar studies for the identification of bee races based on forewings traits. Average values of forewing angles of our bee are compared to the values of these features for subspecies A.m.carnica measured in some countries such as in: Germany (V.Maul, A.Hähnle. 1994), in Serbia (N. Nedić, G. Jevtić, G. Jež, B. Anđelković, S. Milosavljević, M. Kostić. 2011), in Northern Kosovo (M. Mladenović and V. D. Simeonova. 2014), in Croatia (S Dedej, A Biasiolo, R Piva. 1996 and Nazzi F. 1992), in Alpine region (V.Maul, A.Hähnle. 1994), in Austria (Morphometric bee data bank of the "Institut fur Bienenkunde" in Oberursel D), and in the North Eastern Italy (Nazzi F. 1992).
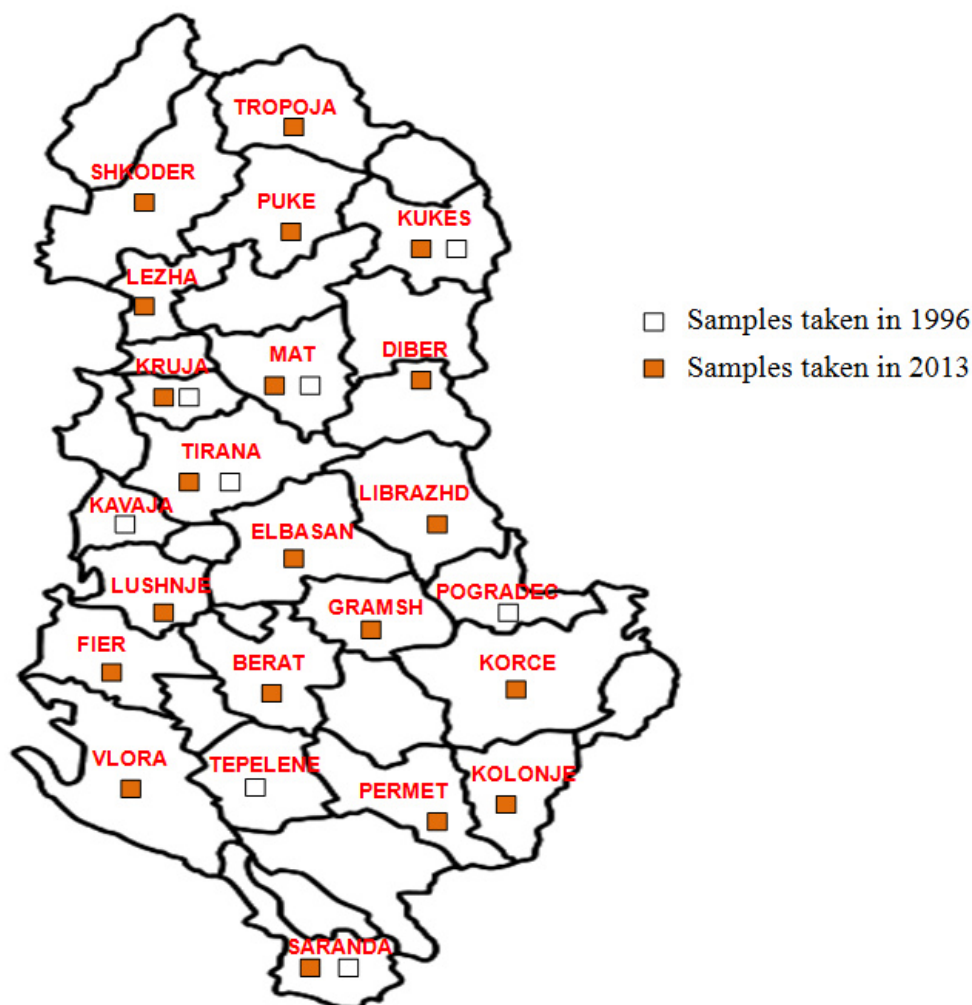
Figure 2. Districts where have been taken samples in studies conducted during 1996 and 2013

The method used for the measurement of morphological features is the scanning method (SPT). This is among the most advanced methods used in recent years for the realization of morphological analysis of the honey bee (Apis mellifera L). This method consists of a combination of a scanner with a Photoshop program (El-Aw, M.A.; Kh. A. Draz; Kh.S.Abd El-Hamid and H. Abo-Shara. 2012). This program enabled automatic measures and within a reduced time compared to the traditional method. Saving data is done automatically, and then these data are used for statistical analysis.

The basic information that was subjected to statistical treatment, represented by 180 samples from 20 different districts of the country. 11 morphological characters have been subjected to this analysis. To judge about the Albanian bee belonging, method of analysis of the principal components and cluster method were used. In the principal components analysis, subspecies and some populations of A.m.carnica are presented in the plan of first two main components. Cluster analysis was used to build dendrogram that show groupings of several honey bee subspecies and some populations of A.m.carnica, using for this purpose the Euclidean distances estimated using averages of features in the study, for each of the subspecies or populations of A.m.carnica.

3. Results and discussion

In table 1, morphological traits that are considered for taxonomic analysis, the average values of these traits for our bees measured in the periods 1996 and 2013, and estimated average values in different subspecies and populations of the bees are presented. Racial relations between the Albanian bee population and subspecies A.m ligustica, A.m.mellifica, A.m caucasica, and A.m.macedonica are studied using standard values of DAWINO protocol for the forewing traits of these subspecies.

Table 1. Average data for forewing angles, evaluated in subspecies and in different bee population

	A_4	B_4	D_7	E_9	G_{18}	K_{19}	J_{10}	J_{16}	N_{23}	L_{13}	O_{26}
A.m.carnica- Germany	29.2	110.6	97.3	23.1	90.4	78.2	53.5	91.9	91.7	13.1	36.5
A.m.carnica- Serbia	29.8	107.89	96.74	22.94	90.69	78.36	53.67	91.44	92.57	14.49	36.21
A.m.carnica- N. Kosovo	31.1	111	100.53	22.47	90.27	77.3	51.87	95.6	93.6	13.53	38.43
A.m.carnica- Croatia	29.89	110.59	98	23.22	89.87	78.26	53.03	92.08	92.3	12.86	36.44
A.m.carnica-Alp .Region	29.00	112.7	99.3	23.3	92.7	79.4	52.5	96.4	94.4	12.3	37.9
A.m.carnica- Austria	29.196	112.162	99.768	23.121	93.098	79.505	52.198	95.998	94.167	12.479	37.905
A.m.carnica- Italy	29.57	111.5	98.28	23.8	90.67	79.03	53.28	93.89	93.32	12.19	36.82
Albania 1996	29.78	110.95	99.22	23.44	90.20	76.51	55.82	90.6	93.13	13.54	35.65
Albania 2013	29.38	111.08	97.62	23.54	89.05	75.56	53.2	90.61	91.81	12.76	36.94
A.m.macedonica	31.1	107.5	98.4	22.4	91.2	78.6	54.8	88.1	--	14.5	36.6
A.m.ligustica	31.6	106.7	98.6	23.3	89.8	78.3	53	93.8	91	13.5	36.6
A.m.mellifera	33.9	100.9	103.6	18.7	93.8	80.8	50.4	91.6	95.81	15.3	37.5
A.m.caucasica	35.0	100.9	102.3	20.7	93.5	74.9	54.0	87.3	93.85	14.9	35.2

We haven't found a study of relations or distances between all these populations from the study of literature, one such study is done for the first time. Classification of different bee subspecies and populations, included in this study, using cluster analysis for this reason, appears as in Figure 3. Referring to the data of this analysis, we notice that five subspecies: A.m.ligustica, A.m.macedonica, A.m.carnica, A.m.caucasica and A.m.mellifera are separated and have no close relation between them. Such an outcome was expected, because we don't see that there are groupings by geographical affinities.

It is evident the A.m.carnica grouping of populations breeding in different countries from A.m.macedonica, A.m.ligustica, A.m.mellifera, A.m.caucasica subspecies. Such a grouping is expected, as these populations, despite the region where they are bred, are from the same origin of subspecies, which have expanded its natural extension through genes movement. Within subspecies A.m.carnica, two distinct groups are created according to geographic proximity: 1- A.m.carnica-Albania, A.m.carnica-Italy, A.m.carnica-Croacia, A.m.carnica-Austria, A.m.carnica-Alpine region, A.m.carnica-Germany. 2- separately grouped are A.m.carnica in Serbia and Northern Kosovo.
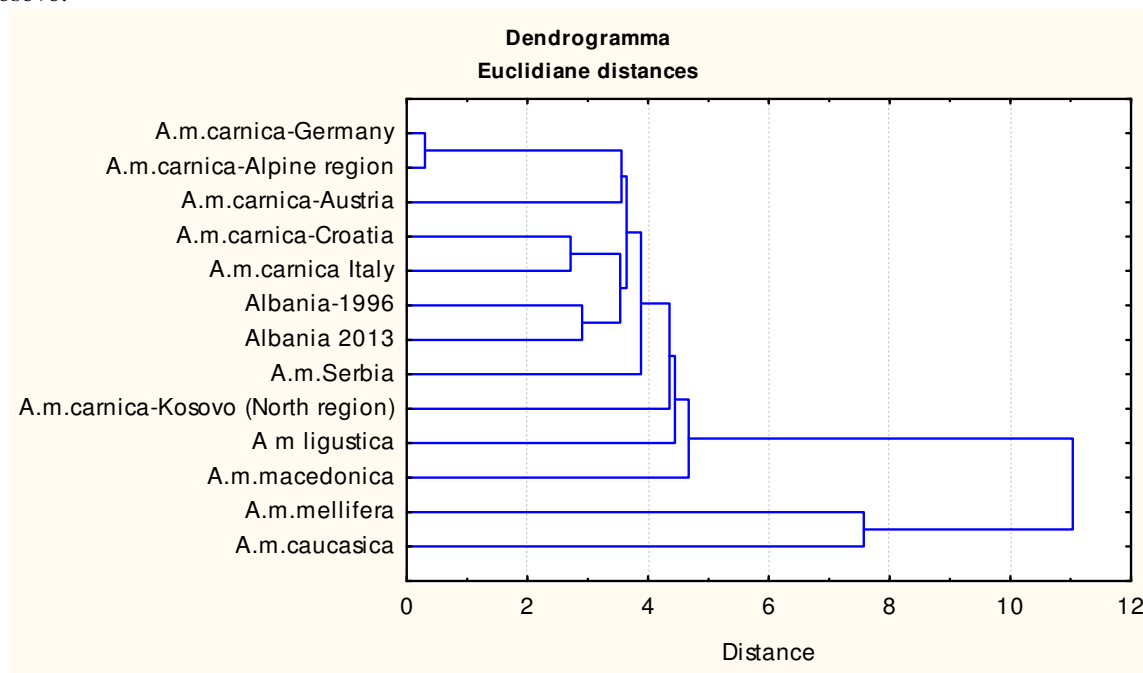

Figure 3. Groupings of subspecies / populations of bees, referring their differences in averages of forewing characters

Small variations are noted between A.m.carnica bee populations breeding in Germany and Alpine Region. Close relations are observed between populations breeding in Croatia and Italy, such a result is consistent with the phenomenon of isolation in distance. Similarly it is noticed that the Albanian bee population in different years is almost the same, although the method used in the measurement was different. Evolutionary processes occurring during the last 20 years, as a result of uncontrolled migration of genes from other populations, have not caused significant changes in the population, measurements in the bee population of the country during 1996 and 2013 do not identify essential changes. These measurements show that Albanian bee populations during all this period, despite changes incurred, continue to preserve traits with average values

without statistical significant differences between them for the studied traits.

During 1996 our population has had close relations with populations of northern Italy and Croatia. But in recent years there has been a differentiation from these two populations and closeness of the Albanian bee population with the population of A.m.carnica breeding in Serbia and A.m.macedonica, this can be explained by the fact that over the years there has been an uncontrolled movement of genes from these countries to Albania which could have influenced in the genetic makeup of the native bee population. Variability of traits occurred during this period as a consequence of different factors, among which we can separate the uncontrolled migration of genes as a principal one, leads to the growth of the closeness of this population with the A.m.macedonica bee population.

The problem of subspecies is discussed in Albania, still the genetic affiliation of our bees is not clear, so it is defined as a variety of A.m.carnica (Nuri Q. 1963); (Thomo K., Shehu L., Bajrami Z., Koni M. 2002) and as a hybrid between A.m. carnica and A. Macedonica (Dedej S. 1996). The data of our study are in favor of the hypothesis that the Albanian bee can be classified in the A.m.carnica subspecies, because it is seen that the Albanian bee population, is part of the group of populations belonging to subspecies A.m.carnica. Albanian population is part of the group of A.m.carnica that is bred in Italy, Croatia and Serbia. As it seems, it has no relation at all with subspecies A.m.caucasica, A.m.ligustica and A.m.mellifera. There is a closeness between A.m.macedonica and A.m.carnica that goes gradually, a study should be done in the population of bees in Serbia and northern Kosovo to determine whether they are A.m.carnica or A.m.macedonica race.

Referring to the results of principal components analysis (Figure 4), these results confirm the obtained results by cluster analysis. It is distinguished that the Albanian bee population studied in different years are very close between them, although their study method was different.
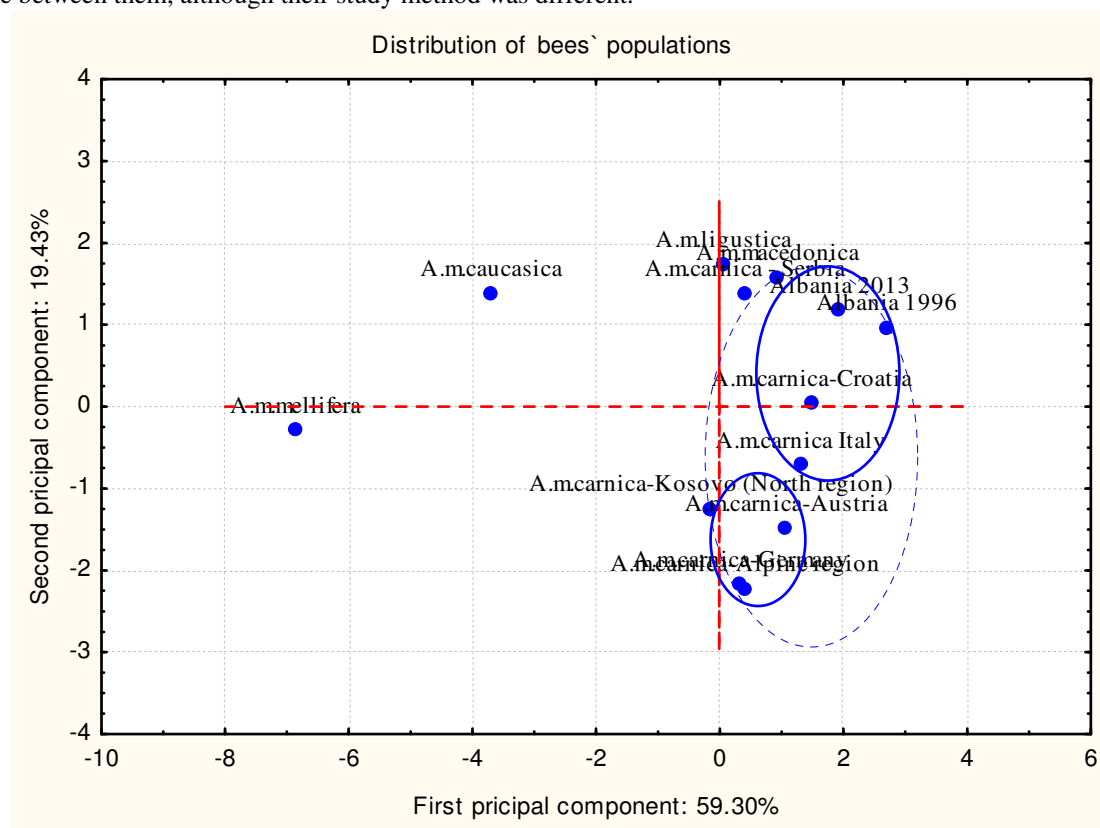

Figure 4. Grouping of bee populations of the country and subspecies A.m.macedonica, A.m.ligustica, A.m.mellifera, A.m.caucasica in the plan of two principal components

Separation in the first component explains about 60% of the changes. Under this division there is a differentiation of subspecies A.m.macedonica, A.m.ligustica, A.m.mellifera, A.m.caucasica and there is grouping of populations that belong to subspecies A.m.carnica. But A.m.carnica population is differentiated in the second component, which explains about 20% of the changes. It is seen that A.m.carnica populations found in Croatia, Albania, Serbia and Italy are united, while A.m.carnica populations in Austria, Germany and the Alpine region are isolated, this result is expected since we have differentiation by isolation in distance.

Albanian bee population has big differences from subspecies A.m.caucasica, A.m.ligustica and A.m.mellifera. It is noticed that the Albanian bee population has large proximity with the populations of bees

belonging to A.m.carnica subspecies. Ellipse of Albanian bee population by the second component division does not fall in contact with the ellipse of A.m.carnica populations breeding in northern Kosovo, Austria, Alpine region and Germany. During the last 20 years, this population has undergone changes in average indicators of analyzed traits. As a result of these changes, its distance from the bee population of Croatia and Italy has increased and the distance to the Serbian population is reduced.

Conclusions

Our analysis made for the first time, shows that from the five subspecies of honey bee (Apis mellifera L.), populations of Apis mellifera carnica are separated. On the other hand, it is indicated that it is possible that Albanian bee population can be classified in Apis mellifera carnica subspecies group than in A.m.macedonica subspecies group that is questioned by other researchers, for the reason that its group is located in the populations of this race from other countries.

Populations of subspecies A.m.carnica are divided in two distinct groups and Albanian bee population is part of the group A.m.carnica breeding in Italy, Croatia and Serbia. The results of the analysis of the principal components and cluster analysis show that this population has great difference from subspecies A.m.caucasia, A.m.ligustica and A.m.mellifera.

Import of Queens and uncontrolled gene migration from neighboring countries have caused genetic pressure on native bee populations, this has resulted in differentiation of this population with A.m.carnica populations breeding in northern Italy and Croatia, and as a consequence increasing of the proximity of this population with the population of the subspecies A.m.carnica breeding in Serbia and A.m.macedonica. Finally, we propose that a study about genetic differences in the populations of the Balkans can be made in relation to bees from Kosovo, Macedonia and Serbia.

Acknowledgement

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