GENETIC VARIATION OF *Apis mellifera* FROM SERBIA INFERRED FROM MITOCHONDRIAL ANALYSIS

Irene Muñoz[1], Jevrosima Stevanovic[2], Zoran Stanimirovic[2], Pilar De la Rúa[1]

[1]Dpto. de Zoología y Antropología Física, Facultad de Veterinaria, Universidad de Murcia, 30100 Murcia, Spain.
[2]Department of Biology, Faculty of Veterinary Medicine, University of Belgrade, Bul. oslobodjenja 18, 11000 Belgrade, Serbia.
e-mail: pdelarua@um.es

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S u m m a r y

Two honeybee subspecies inhabit Serbia; *Apis mellifera carnica* and *A. m. macedonica*. Both belong to eastern Mediterranean (C) evolutionary lineage. Furthermore three Serbian honeybee ecotypes restricted to particular regions, were defined through morphometry and cytogenetic analyses. In this study, mitochondrial data have been used to analyze the molecular diversity of the honeybee population from Serbia. Seven haplotypes of the C evolutionary lineage have been found, two of them are newly described (C2o and C2p) and restricted to two regions, which ultimately increased the number of haplotypes found in this lineage. Comparisons with surrounding honeybee populations suggest a hybrid situation between *A. m. carnica* and *A. m. macedonica* and also introgression from *A. m. ligustica*. The results should be considered when dealing with future conservation strategies, and for pathogen-parasite-tolerant breeding programs.

Keywords: *Apis mellifera carnica*, *Apis mellifera macedonica*, mitochondrial DNA, intergenic region, haplotypes, sequencing, population genetics.

INTRODUCTION

The honeybee *Apis mellifera* is endemic to Africa, Europe and Western Asia, but actually this species has spread worldwide due to multiple migrations and introductions (Moritz et al., 2005; Whitfield et al., 2006). Around 29 subspecies have been recognized based on morphological and ecological data (Engel, 1999; Sheppard and Meixner, 2003). These species have been grouped into four morphometric groups (Ruttner, 1988): the African group (A), the Northern and Western European group (M), the East Mediterranean group (C) and the Oriental group (O). The morphometric C-group included the subspecies of the Balkan region, plain of the Danube and diverse areas such as Sicily, the Apennine Peninsula and the Ukraine (Ruttner, 1988). The morphometric C-group consisted of five honeybee subspecies: *A. m. carnica, A. m. ligustica, A. m. macedonica, A. m. cecropia* and *A. m. siciliana* (lately included in the African group based on molecular analyses, Sinacori et al., 1998). Different studies have recently been done to depict the relationships among these subspecies, using molecular grounds (Bouga et al., 2005; Ivanova et al., 2010; Martimianakis et al., 2011).

Serbia is situated in the middle of the distribution area of the C-group. It is located in Southeastern Europe, covering two distinct geographic areas: the Pannonian Plain in the north between the Sava and Danube rivers and a southern mountainous part covering the central part of the Balkan Peninsula. The climate in Serbia varies from the continental type

Journal of Apicultural Science

in the north to moderate-continental in the south. In relation to beekeeping in the northern and central parts of Serbia, beekeepers practice extensive seasonal movements of their colonies. Besides intra-regional movements, they often migrate their colonies to Eastern Serbia. However, beekeepers in Eastern Serbia rarely move their colonies. When they do move them, the colonies are only moved short distances, whilst in Southern Serbia (including Syenichko-Peshterski Plateau) migratory beekeeping is not practiced.

Two subspecies of *A. mellifera* inhabit the territory of Serbia, *A. m. carnica* and *A. m. macedonica*, both belonging to the East Mediterranean C evolutionary lineage. Based on morphometric analyses (Ruttner, 1988), *A. m. carnica* is considered to be distributed across Central-eastern European countries such as Austria, Hungary, Romania, Bulgaria and former Yugoslavia (Slovenia, Croatia, Serbia, Bosnia and Herzegovina, Montenegro, the Republic of Macedonia), whilst *A. m. macedonica* was described as inhabiting parts of the Ukraine, Romania, Bulgaria, parts of former Yugoslavia and Northern Greece, giving no exact border between *A. m. carnica* and *A. m. macedonica*. Furthermore, cytogenetic and morpho - metric analyses (Stanimirovic et al., 1999a,b; Stevanovic, 2002; Stanimirovic et al., 2005a), as well as behavioural analyses (Pejovic, 2001; Cirkovic, 2002; Stanimirovic et al., 2002, 2005b; Stevanovic, 2007) described the presence of three honeybee ecotypes (defined in terms of locally adapted populations within a subspecies with a particular distribution): Banat (B), Timok (T) and Syenichko-Peshterski (S). Cytogenetic research revealed differences in the biometric and ultrastructural organization of chromosomes between the B and S honeybee ecotypes (Stanimirovic et al., 1999a,b). Chromosomal analyses comprising the three honeybee ecotypes from Serbia demonstrated the existence of a G-band polymorphism (Stanimirovic et al., 2005a). These observations were in accordance with previous results obtained by morphometric analysis of the same ecotypes using 30 morphometric characters (Stevanovic, 2002). This high morphometric variance suggests that the honeybee populations from Serbia may play a pivotal role in the understanding of the biogeographic transition of the subspecies *carnica* and *macedonica*.

Giving these preliminary results, the two aims of the present work were: 1) to test whether the Serbian honeybee ecotypes defined through cytogenetic and morphometric analyses are genetically differentiated, and 2) to depict the relationship of the honeybees from Serbia in relation to neighbouring *A. mellifera* subspecies.

MATERIAL AND METHODS

Sampling and DNA extraction

Adult honeybee workers were sampled from three Serbian regions where the ecotypes have been described: Banat (B), Timok (T) and Syenichko-Perhterski (S). Samples from the Southeastern (SE) region were also included (Tab. 1 and Fig. 1). A total of 37 colonies were sampled in Serbia and 24 reference honeybee colonies were additionally included from Italy (*A. m. ligustica*, N=5), Croatia (*A. m. carnica*, N=5), Bosnia and Herzegovina (*A. m. carnica*, N=5), Albania (hybrid population of *A. m. carnica* and *A. m. macedonica*, N=4, Dedej et al., 2000) and the Republic of Macedonia (*A. m. macedonica,* N=5) for comparison purposes (Tab. 1). Worker honeybee samples were preserved in absolute ethanol and kept at -20ºC. Total DNA was extracted from three right legs of each honeybee worker using a 5% Chelex solution (Walsh et al., 1991).

Mitochondrial DNA analysis

A single honeybee worker per colony was used for mtDNA analysis. The $tRNA^{leu}$-cox2 intergenic region were PCR amplified with the primers E2 (5'-GGCAGAATAAGTGCATTG -3') located at the 5' end of the gene $tRNA^{leu}$ and

Table 1.

Collection sites and mtDNA haplotype found in the sampled colonies of *A. mellifera*

Sample	Locality	Region/Country	Geographical Coord.	mtDNA
SER01	Belosavci	Banat/Serbia	44°19' N 20°41' E	C2e
SER02	Dumbovo	Banat/Serbia	45°34' N 19°39' E	C2e
SER03	Curug	Banat/Serbia	45°12' N 19°43' E	C2d
SER04	Dublje	Banat/Serbia	44°47' N 19°31' E	C2d
SER05	Ripanj	Banat/Serbia	44°38' N 20°32' E	C2d
SER06	Cerak	Banat/Serbia	44°44' N 20°24' E	C2i
SER07	Sevarice	Banat/Serbia	44°52' N 19°39' E	C2o
SER08	Veliko Gradiste	Banat/Serbia	44°42' N 21°38' E	C2d
SER10	Sedlari	Banat/Serbia	44°18' N 19°53' E	C2d
SER11	Valjevo	Banat/Serbia	44°16' N 19°53' E	C2d
SER19	Sabac	Banat/Serbia	44°52' N 19°42' E	C2e
SER20	Vrsac	Banat/Serbia	45°07' N 21°18' E	C2d
SER21	Pancevo	Banat/Serbia	44°51' N 20°39' E	C2d
SER09	Pester	Syenichko-Peshterski/Serbia	43°02' N 20°23' E	C2d
SER32	Kocarnik	Syenichko-Peshterski/Serbia	42°59' N 20°19' E	C2d
SER33	Borostica	Syenichko-Peshterski/Serbia	43°02' N 20°06' E	C2e
SER34	Dubovo	Syenichko-Peshterski/Serbia	43°06' N 21°40' E	C2d
SER35	Cvijetlje	Syenichko-Peshterski/Serbia	43°08' N 20°31' E	C2e
SER36	Livadak	Syenichko-Peshterski/Serbia	42°59' N 20°19' E	C2e
SER37	Grabovica	Syenichko-Peshterski/Serbia	42°18' N 18°53' E	C2e
SER38	Sare	Syenichko-Peshterski/Serbia	43°16' N 20°13' E	C2d
SER39	Pavlje	Syenichko-Peshterski/Serbia	43°17' N 20°36' E	C2p
SER40	Gonje	Syenichko-Peshterski/Serbia	43°17' N 19°54' E	C2p
SER12	Ilino	Timok/Serbia	43°50' N 21°59' E	C2d
SER13	Krivi Vir	Timok/Serbia	43°49' N 21°44' E	C1a
SER14	Lukovo	Timok/Serbia	43°48' N 21°49' E	C2c
SER15	Stupanj	Timok/Serbia	43°56' N 21°57' E	C2d
SER16	Metris	Timok/Serbia	43°54' N 22°11' E	C2i
SER17	Lubnica	Timok/Serbia	43°52' N 22°11' E	C2d
SER22	Vladicin Han	Southeast/Serbia	42°42' N 22°03' E	C2d
SER23	Presevo	Southeast/Serbia	42°18' N 21°39' E	C2d
SER24	Pirot	Southeast/Serbia	43°09' N 22°36' E	C2d
SER26	Vranjska banja	Southeast/Serbia	42°33' N 22°01' E	C2d

Journal of Apicultural Science

Table 1. Continued

SER27	Leskovac	Southeast/Serbia	42°59' N 21°57' E	C2d
SER28	Nis	Southeast/Serbia	43°19' N 21°53' E	C2e
SER30	Doljevac	Southeast/Serbia	43°12' N 21°48' E	C2d
SER31	Sinkovce	Southeast/Serbia	42°55' N 21°43' E	C2e
ARU03	Perugia	Italy	43°06' N 12°23' E	C1a
CAM01	Camugnano	Italy	44°10' N 11°05' E	C1a
CAM02	Camugnano	Italy	44°10' N 11°05' E	C1a
CAM03	Camugnano	Italy	44°10' N 11°05' E	C1a
CAM04	Camugnano	Italy	44°10' N 11°05' E	C1a
BRA04	Brac	Croatia	43°19' N 16°46' E	C2c
KRC02	Korcula	Croatia	42°56' N 17°07' E	C2e
KRC03	Korcula	Croatia	42°56' N 17°07' E	C2e
MLS04	Mali Losini	Croatia	44°31' N 14°27' E	C2e
STV04	Sutivanac	Croatia	45°06' N 13°57' E	C1a
BOS01	Ilova	Bosnia and Herzegovina	44°45' N 17°37' E	C2d
BOS02	Usce	Bosnia and Herzegovina	43°50' N 19°18' E	C2d
BOS03	Arandelovo	Bosnia and Herzegovina	44°53' N 17°30' E	C2d
BOS04	Trebinje	Bosnia and Herzegovina	42°42' N 18°20' E	C2d
BOS05	Gacko	Bosnia and Herzegovina	43°07' N 18°29' E	C2d
SAR01	Sarande	Albania	39°52' N 20°00' E	C2d
SAR02	Sarande	Albania	39°52' N 20°00' E	C2d
SAR03	Sarande	Albania	39°52' N 20°00' E	C2i
SAR04	Sarande	Albania	39°52' N 20°00' E	C2i
MAC02	Probistip	Macedonia	41°57' N 22°10' E	C2d
MAC03	Kriva Palanka	Macedonia	42°10' N 22°19' E	C2d
MAC04	Strumica	Macedonia	41°24' N 22°37' E	C2d
MAC05	Demir Hisar	Macedonia	41°11' N 21°12' E	C2d
MAC09	Kumanovo	Macedonia	42°06' N 21°42' E	C2d

H2 (5'-CAATATCATTGATGACC-3') located close to the 5' end of the gene cox2 (Garnery et al., 1991) in a total volume of 12.5 µL, containing 2 µL DNA, 10 mM Tris-HCl, 50 mM KCl, 1.5 mM MgCl2, 0.2 mM of each dNTP, 0.5 µM of each primer and 1.25 U of puReTaq DNA polymerase (GE Healthcare). The program cycle was as follows: denaturation for 5 min at 95ºC, 36 cycles of 45 s at 95ºC, 60 s annealing at 47ºC, and extension for 1 min 30 s at 72ºC followed by a final elongation step of 10 min at 72ºC. Amplicons of each sample were purified with isopropanol and ammonium acetate and submitted to sequencing (Secugen S.L., Madrid, Spain).

Multiple alignments were done by using the online version of the multiple alignment program for amino acid or nucleotide sequences (MAFFT version 6,

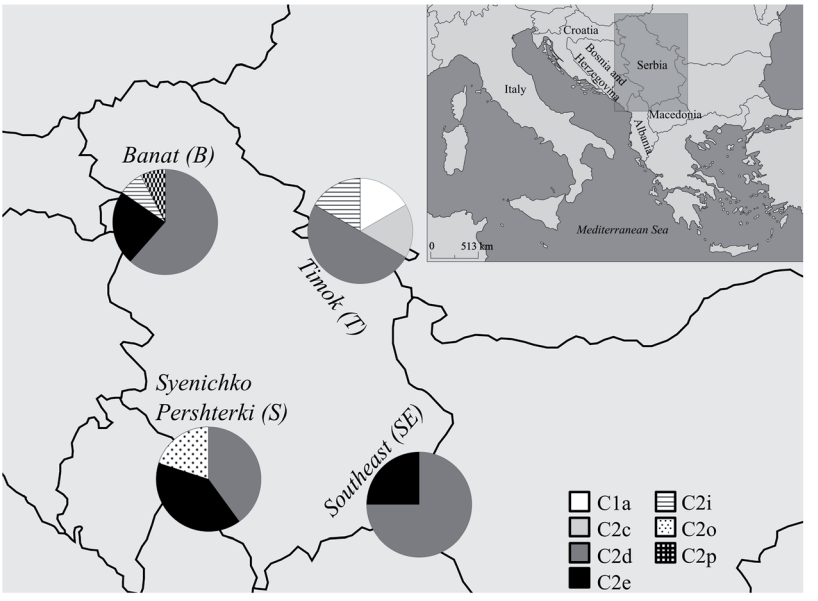

Fig. 1. Haplotype frequency pie charts in the different sampled regions from Serbia: Banat (N=13), Timok (N=6), Syenichko-Persterki (N=10) and Southeast (N=8).

KaToh and Toh, 2008) including published sequences for comparison (Franck et al., 2000; Sušnik et al., 2004; Muñoz et al., 2009). Haplotype networks were constructed using the median joining algorithm (Bandelt et al., 1999) with the software NETWORK 4.1.0.7 (fluxus-engineering.com).

RESULTS

The identified haplotypes with sequence data are showed in Table 1. All samples bear only one Q sequence in the analyzed mtDNA fragment corresponding to the predicted composition of this region. Seven polymorphic sites were detected (Tab. 2) within the sequenced 571-573 bp mtDNA fragment (sequences are deposited in the GeneBank database with accession numbers JQ977699-JQ977705, see Fig. 2). These seven polymorphic sites defined the seven haplotypes found in Serbia, two of them newly described and named C2o and C2p (after Franck et al., 2000). The new haplotypes share with the previously described C2e haplotype (Muñoz et al., 2009), a single nucleotide deletion in position 3475 bp (Crozier and Crozier, 1993), but were distinguishable from C2e for a T→A transversion at the position 3515 bp, and a T→C transition at the position 3450 bp present in C2o and C2p haplotypes, respectively (Tab. 2).

The C2d haplotype was spread all over Serbia. This haplotype was most frequent in samples from the B (0.615), T (0.500) and SE (0.750) regions. C2e haplotype was recorded in S, SE and B region with a frequency of 0.400, 0.250 and 0.231, respectively. Particular haplotypes were found in T (C1a and C2c), B and T (C2i), S (C2p) and B (C2o) regions. Serbia haplotype diversity ranged from 0.375 (SE) to 0.667 (T) (Tab. 3).

In the reference populations, C2d was detected in *A. m. carnica* and *A. m. macedonica* samples from Bosnia and Herzegovina and the Republic of Macedonia, respectively, whereas C1a was uniquely observed in *A. m. ligustica* from Italy. C1a was also observed in *A. m. carnica* from Croatia together with haplotypes C2c and C2e. Two haplotypes, C2d and C2i were present in

Journal of Apicultural Science

Fig. 2. Alignment of the sequences of the mitochondrial intergenic region found in the Serbian honey bees. The mtDNA fragment corresponds to positions 3363-3935 bp published by Crozier and Crozier (1993).

the hybrid population of *A. m. carnica* and *A. m. macedonica* from Albania (Tab. 3).

The data set included 12 haplotypes, seven from this work and five (C2a, C2b, C2f, C2g and C2h) which were previously published (Franck et al., 2000; Özdil et al., 2009) and included for comparison. In total, 573 base pairs (562 invariable sites, 8 variable sites and 3 gaps) were analyzed to identify haplotype relationships within the C evolutionary lineage.

Table 2.

Polymorphic sites (position in nucleotide changes and bp) of the intergenic tRNAleu-cox2 region of *A. mellifera* C-lineage. The mtDNA fragment corresponds to the positions 3363-3935 bp published by Crozier and Crozier (1993)

	Genbank accession number	69 (3432 bp)	87 (3450 bp)	112 (3475 bp)	152 (3515 bp)	225 (3488 bp)	270 (3633 bp)	405 (3768 bp)
C1a	JQ977699	C	T	T	T	G	T	C
C2c	JQ977700	-	T	T	T	G	T	C
C2d	JQ977701	-	T	T	T	G	C	T
C2e	JQ977702	-	T	-	T	G	C	T
C2i	JQ977703	-	T	T	T	A	C	T
C2o	JQ977704	-	T	-	A	G	C	T
C2p	JQ977705	-	C	-	T	G	C	T

Table 3.

Number of analyzed colonies (n), haplotype frequency and diversity (D) in the honey bee colonies from Serbia and the reference populations

Population	n	C1a	C2c	C2d	C2e	C2i	C2o	C2p	D
Banat (Serbia)	13			0.615	0.231	0.077	0.077		0.556
Syenichko-Peshterski (Serbia)	10			0.400	0.400			0.200	0.640
Timok (Serbia)	6	0.167	0.167	0.500		0.167			0.667
Southeast (Serbia)	8			0.750	0.250				0.375
A. m. ligustica (reference)	5	1.000							0.000
A. m. carnica (reference)	10	0.100	0.100	0.500	0.300				0.640
Hybrid population (reference)	4			0.500		0.500			0.500
A. m. macedonica (reference)	5			1.000					0.000

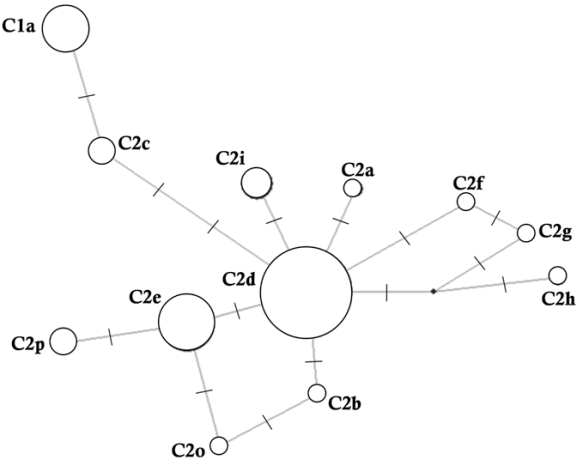

Fig. 3. Median-joining network connecting 12 haplotypes based on the sequence of the tRNA^{leu}-cox2 intergenic region. Each circle represents a distinct haplotype. Tick marks along branches indicate the number of mutational changes between nodes. Black dots correspond to unsampled haplotypes. The size of the circles is proportional to the observed frequency of each haplotype. Haplotypes C2a and C2b were described by Franck et al. (2000) while C2f, C2g and C2h by Özdil et al. (2009) in Turkey.

Journal of Apicultural Science

Parsimony analyses resulted in a network with four unresolved connections (Fig. 3). The C2d haplotype occupied a central position in the network, whereas the most distant haplotype C1a which was present only in *A. m. ligustica*, showed five mutations with respect to C2d. Newly described haplotypes C2o and C2p, were separated from C2e by one single mutation each. One haplogroup was differentiated and separated, including three haplotypes (C2f, C2g and C2h) present in *A. m. meda* and *A. m. anatoliaca* from Turkey.

DISCUSSION

The analysis of Serbian honey bees with molecular markers provides additional evidence of the underlying genetic diversity in the sequence of the intergenic $tRNA^{leu}$-cox2 region. A total of seven honey bee mitochondrial haplotypes were detected in Serbia. Two newly described haplotypes, C2o and C2p, defined after two newly detected polymorphic sites (one transversion and one transition), were found in the B and S regions, respectively. These findings indicate greater honey bee molecular diversity in Serbia compared to the other assessed Balkan countries, where present investigations and those of Muñoz et al. (2009) and Stevanovic et al. (2010) revealed only one (Republic of Macedonia), two (Albania) or four (Croatia) haplotypes. This is not surprising, as the territory of Serbia is a significant biodiversity center on the Balkan Peninsula. Serbia's status as a centre of biodiversity in Europe is to a high degree determined by its geological age, geomorphology, and climatic conditions and, in particular, by the role that the territory of Serbia played as an important refuge for a number of species during the Pleistocene glaciations (Hewitt et al., 1999). However, taking into consideration possible human-mediated queen importation, the high diversity of honey bees in Serbia could also be interpreted as the result of anthropogenic influences.

Honeybee colonies from the four Serbian regions present different haplotype frequency, C2d being more frequent in the southeast. Among the seven haplotypes found in Serbian honey bees, only C2d haplotype was detected in colonies from each analysed region, with frequencies of 0.750, 0.615, 0.500 and 0.400 in bees from the SE, B, T and S regions, respectively. These results may be interpreted in many ways. Due to high frequency and widespread distribution in all Serbian regions, the C2d is perhaps the ancestral haplotype in Serbia. However, the C2d haplotype was previously found in Greece, with a frequency of 0.8 (Muñoz et al., 2009), and also in Albania, Bosnia and Herzegovina and the Republic of Macedonia (Stevanovic et al., 2010; present study). These findings may suggest gene flow and the natural northward migration of *A. m. macedonica* along the river basins of Vardar, Struma, Pcinja, south and great Morava up to Sava. The same could be inferred from the findings of C2i in Serbian honey bees (with the frequencies of 0.167 and 0.077 in T and B regions, respectively), as this haplotype was detected only in *A. m. macedonica* from Albania (present study) and Greece (Muñoz et al., 2009). Beside the natural northward invasion of bees, introgression from *A. m. macedonica* into the Serbian populations could be due to human migrations that took place after the Ottoman invasions and after the First World War.

The haplotype C2e was detected in honey bee populations from the S, SE and B regions, with frequencies of 0.400, 0.250 and 0.231, respectively. Such a detection could be interpreted as the consequence of the introduction of *A. m. carnica* honey bees from Croatia to Serbia most probably through human-mediated queen trade. This haplotype was first described in Croatian coastal honey bees with a high overall frequency (0.45) (Muñoz et al., 2009).

Other haplotypes detected in Serbian honey bees (C1a, C2c and C2i) possibly came later than C2d and C2e as they are less frequent and their geographical distribution is not so widespread. C1a, C2c found only in bees from T region and C2i

found in T and B regions have also been detected in Italy, Slovenia and Greece, respectively. Such findings confirm the close relationship among the subspecies forming the C evolutionary group, as well as the intense commercial exchanges between beekeepers of this area.

The finding of C1a haplotype in honey bees from the Timok region suggests that introgression from *A. m. ligustica* might have occurred in these colonies. This is not surprising since *A. m. ligustica* is certainly the honey bee subspecies that is most implicated in human introductions because of its favorable characteristics (Franck et al., 2000). Two new haplotypes were observed solely in colonies from two regions: C2o in B and C2p in S, although at different frequencies, 0.077 the first and 0.200 the second. These two haplotypes could be putative discriminant markers for the ecotypes dispersed in these regions, which should be contrasted with a wider sampling.

In conclusion, the honeybee colonies located in Serbia present a hybrid situation between *A. m. carnica* and *A. m. macedonica,* although introgression from *A. m. ligustica* has been also observed. On the other hand, two of the cytogenetic-described honeybee ecotypes (B and S) showed particular haplotypes, whose presence and frequency should be determined in posterior studies. The results found in this region confirm a preliminary analysis and should be considered in future conservation strategies and for pathogen-parasites-tolerant breeding programs (Stevanovic et al., 2011).

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REFERENCES

Bandelt H. J., Forster P., Rōhl A. (1999) - Median-joining networks for inferring intraspecific phylogenies. *Mol. Biol. Evol*., 16: 37-48.

Bouga M., Harizanis P. B., Kilias G., Alahiotis S. (2005) - Genetic divergence and phylogenetic relationships of honey bee *Apis mellifera* (Hymenoptera: Apidae) populations from Greece and Cyprus using PCR - RFLP analysis of three mtDNA segments. *Apidologie,* 36: 335-344.

Cirkovic D. (2002) - Reproductive-productive and hygienic-grooming characterization of Syenichko-Peshterski honey bee ecotype, MSc thesis, Belgrade University.

Crozier R. H., Crozier Y. H. (1993) - The mitochondrial genome of the honey bee *Apis mellifera*: complete sequence and genome organisation. *Genetics,* 133: 97-117.

Dedej S., Delaplane K. S., Gocaj E. (2000) - A technical and economic evaluation of beekeeping in Albania. *Bee World*, 81: 87-97.

Engel M. S. (1999) - The taxonomy of recent and fossil honey bees (Hymenoptera: Apidae, *Apis*). *J. Hymenop. Res.,* 8: 165-196.

Franck P., Garnery L., Celebrano G., Solignac M., Cornuet J. M. (2000) - Hybrid origins of honey bees from Italy (*Apis mellifera ligustica*) and Sicily (*A. m. sicula*). *Mol. Ecol*., 9: 907-921.

Garnery L., Vautrin D., Cornuet J. M., Solignac M. (1991) - Phylogenetic relationships in the genus *Apis* inferred from mitochondrial DNA sequence data. *Apidologie,* 22: 87-92.

Hewitt G. M. (1999) - Post-glacial re-colonization of European biota. *Biol. J. Linn. Soc.,* 68: 87-112.

Ivanova E., Petrov P., Bouga M., Emmanouel N., Ivgin-Tukka R., Kence M. (2010) - Genetic variation in honey bee (*Apis mellifera* L.) populations from Bulgaria. *J. Apic. Sci.*, 54: 49-59.

KaToh K., Toh H. (2008) - Recent developments in the MAFFT multiple sequence alignment program. *Briefings in Bioinformatics,* 9: 286-298.

Martimianakis S., Klossa-Kilia E., Bouga M., Kilias G. (2011) - Phylogenetic relationships of Greek *Apis mellifera* subspecies based on sequencing of mtDNA segments (COI and ND5). *J. Apic. Res.*, 50: 42-50.

Moritz R. F. A., Härtel S., Neumann P. (2005) - Global invasions of the western honey bee (*Apis mellifera*) and the consequences for biodiversity. *Ecoscience,* 12: 289-301.

Muñoz I., Dall'Olio R., Lodesani M., De la Rúa P. (2009) - Population genetic structure of coastal Croatian honeybees (*Apis mellifera carnica*). *Apidologie,* 40: 617-626.

Özdil F., Yildiz M. A., Hall H. G. (2009) - Molecular characterization of Turkish honeybee populations (*Apis mellifera* L.) inferred from mitochondrial DNA RFLP and sequence results. *Apidologie,* 40: 570-576.

Pejovic D. (2001) - Investigations of hygienic and grooming behaviour of honey bee subspecies *Apis mellifera carnica* in disease resistance, MSc thesis, Belgrade University.

Ruttner F. (1988) - Biogeography and Taxonomy of Honeybees, Springer-Verlag; Berlin, Germany, 284 pp.

Sheppard W. S., Meixner M. D. (2003) - *Apis mellifera pomonella*, a new honey bee subspecies from Central Asia. *Apidologie,* 34: 367-375.

Sinacori A., Rinderer T. E., Lancaster V., Sheppard W. S. (1998) - A morphological assessment of *Apis mellifera* from Palermo, Italy. *Apidologie,* 29: 481-490.

Stanimirovic Z., Popeskovic D., Pejovic D. (1999a) - Biodiversity of the honeybee *Apis mellifera*, Linne (1758), from the Yugoslav regions I - the biometric variability of the Banate and Sjenicko - Peshtersky ecotypes chromosomes. *Acta Vet. Beograd,* 49: 199-206.

Stanimirovic Z., Vucinic M., Stevanovic J. (1999b) - Biodiversity of the honeybee *Apis mellifera*, Linne (1758), from some Yugoslav regions: II - Ultrastructural chromosomal differences between Banat and Syenichko-Peshterski honeybee ecotype. *Acta Vet. Beograd,* 49: 207-214.

Stanimirovic Z., Pejovic D., Stevanovic J., Vucinic M., Mirilovic M. (2002) - Investigations of hygienic behaviour and disease resistance in organic beekeeping of two honeybee ecogeographic varieties from Serbia. *Acta Vet. Beograd,* 52: 169-180.

Stanimirovic Z., Stevanovic J., Andjelkovic M. (2005a) - Chromosomal diversity in *Apis mellifera carnica* from Serbia. *Apidologie,* 36: 31-42.

Stanimirovic Z., Stevanovic J., Cirkovic D. (2005b) - Behavioural defenses of the honey bee ecotype from Sjenica - Pester against *Varroa destructor. Acta Vet. Beograd,* 55: 69-82.

Stevanovic J. (2002) - Investigations of morphometric and chromosomal variability in diversity preserving of Carniolan honey bee (*Apis mellifera carnica* Pollmann, 1879) in Serbia, MSc thesis, Belgrade University.

Stevanovic J. (2007) - Ecological-ethological defence mechanisms of *Apis mellifera carnica* against ectoparasite *Varroa destructor* on the territory of Serbia. PhD dissertation, PhD thesis, Belgrade University.

Stevanovic J., Stanimirovic Z., Radakovic M., Kovacevic R. S. (2010) - Biogeographic study of the honey bee (*Apis mellifera* L.) from Serbia, Bosnia and Herzegovina and Republic of Macedonia based on mitochondrial DNA analyses. *Rus. J. Genet*., 46: 603-609.

Stevanovic J., Stanimirovic Z., Genersch E., Kovacevic S. R., Ljubenkovic J., Radakovic M., Aleksic N. (2011) - Dominance of *Nosema ceranae* in honey bees in the Balkan countries in the absence of symptoms of colony collapse disorder. *Apidologie,* 42: 49-58.

Sušnik S., Kozmus P., Poklukar J., Meglič V. (2004) - Molecular characterisation of indigenous *Apis mellifera carnica* in Slovenia. *Apidologie,* 35: 623-636.

Walsh P. S., Metzqer D. A., Higuchi R. (1991) - Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques,* 10: 506-512.

Whitfield C. W., Behura S. K., Berlocher S. H., Clark A. G., Johnston J. S., Sheppard W. S., Smith D .R., Suarez A. V., Weaver D., Tsutsui N. D. (2006) - Thrice out of Africa: Ancient and recent expansions of the honey bee, *Apis mellifera*. *Science,* 314: 642-645.

RÓŻNORODNOŚĆ GENETYCZNA *APIS MELLIFERA* W SERBII NA PODSTAWIE BADAŃ MITOCHONDRIALNYCH

Muñoz I., Stevanovic J.,

Stanimirovic Z., De la Rúa P.

S t r e s z c z e n i e

Na terenie Serbii występują dwa podgatunki pszczoły miodenj: *Apis mellifera carnica* i *A. m. macedonica.* Oba te podgatunki należą do wschodnio-śródziemnomorskiej linii ewolucyjnej (C). Na podstawie analiz morfometrycznych oraz cytogenetycznych zidentyfikowano trzy ekotypy pszczoły miodnej występujące w określonych rejonach Serbii. W przedstawionych badaniach, do określenia różnorodności molekularnej serbskiej populacji pszczół wykorzystano wyniki analiz mitochondrialnych. Stwierdzono siedem haplotypów linii ewolucyjnej C, z których dwa to nieznane wcześniej haplotypy C2o i C2p o występowaniu ograniczonym do dwóch regionów, co w efekcie zwiększa liczbę haplotypów tej linii. Porównania z okolicznymi populacjami pszczół sugerują mieszanie się pszczół *A. m. carnica* i *A. m. macedonica* oraz introgresję *A. m. ligustica*. Uzyskane wyniki należy uwzględniać podczas ustalania programów hodowli zachowawczych oraz w programach hodowlanych zorientowanych na selekcję pszczół o zwiększonej tolerancji na patogeny i pasożyty.

Słowa kluczowe: *Apis mellifera carnica, Apis mellifera macedonica*, DNA mitochondrialne, region międzygenowy, haplotypy, sekwencjonowanie, genetyka populacyjna.