HONEY BEES IN SLOVAKIA

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Beekeeping - an ancient tradition, honey bees have been kept in Europe for several millennia

Bees - critically important for the environment
- provide essential pollination for a wide range of crops and wild plants

Apiculture sector - an important part of the EU agriculture

Insect pollination - currently under threat in Europe
- its economic value is estimated to
€ 22 billion
per year in the EU countries

Honey bees and human benefits - production of honey, pollen, wax, propolis and royal jelly

EU countries (2015) - around 630,000 beekeepers (most of them are hobby beekeepers), 16 millions of hives, producing 268,000 tons of honey

CCD, bees mortality – since 2005, unusual weakening of bee numbers and colony losses (**C**olony **C**ollapse **D**isorder), particularly in the USA and Western European countries

Possible factors - intensive agriculture and pesticide use, starvation and poor bee nutrition, viruses, environmental changes and attacks by pathogens (*Varoa destructor*) or invasive species

EU activities - SMARTBEES, SWARMONITOR, SUPER-B, BEE DOC, COLOSS, EPILOBEE....

Rational beekeping in the territory of **Slovakia** started in the late 18th century

First Union of Slovak beekeepers - founded in 1869, but regional organizations were active much earlier

Current structure of apiculture in Slovakia- formed 40 years ago by establishing **6** breeding stations and around **60** reproduction stations (apiaries)

Carniolan bee   (*Apis mellifera carnica*) - the most common and the only legal honey bee breed in Slovakia originating from the north-western region of Slovenia (Kranj)

Official lineages - Košičanka, Carnica Sokol, Vojničanka, Tatranka, Sitnianka and Mošovčanka

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Carniolan bee – autochthonous subspecies, well adapted to the geographic and climatic conditions in the Central Europe, shows high endurance, resistance to brood diseases and high honey yield

Carnica's nearest relatives:

Italian bee (*A. m. ligustica*)
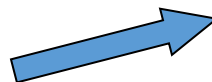
Macedonian bee (*A. m. macedonica*)

All 3 subspecies belong to **haplogroup C**

Research Institute for Animal Production Nitra:

is authorized as bee breeding station (Inst. of Apiculture)

has been approved as an artificial insemination service for all six breeding stations

offers morphometric control of breed purity and breed assignment

manages the Central Registry of Beehives in Slovakia, **Data from 2016:** registered more than 15,500 beekeepers and around 278,000 colonies

Slovak Carniolan honeybee - considered an endangered in
      Slovakia due to the crossbreeding

Morphometric method
frequently used to determine the origin of bees based
on morphological parameters (cubital index, forewing
length and width, femur length, tibia length...)

Cubital Index = Blue/Red

Pollen
basket

Coxa
Trochanter Femur
           Tibia

Basitarsus

Tarsi
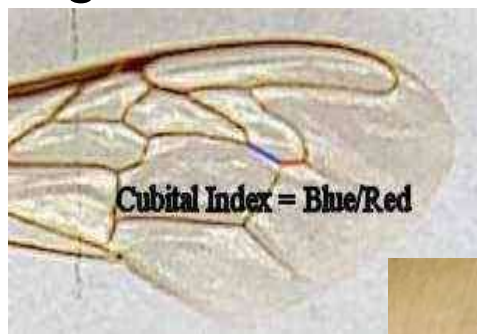
This method has no power to
provide detailed information
about the genetic structure of
the population

Microsatellites - useful for testing genetic variability on the level of subspecies and populations

Carniolan honey bee population in Slovakia – more than 300 bees analyzed using 16 microsatellite markers (Gasper *et al.*, 2013; Paál *et al.*, 2013; Šťastný *et al.*, 2014).

Population statistics: GenAlex, GeneClass, STRUCTURE

- 79 Slovak Carniolan workers from 19 regions of Slovakia, mostly collected at breeding and reproduction farms
- 85 reference samples of A.m.mellifera,A.m.macedonica, A.m. ligustica and Buckfast
- 10 highly polymorphic (PIC>0.5) microsatellite markers running in 2 multiplex PCR

Sample collection sites
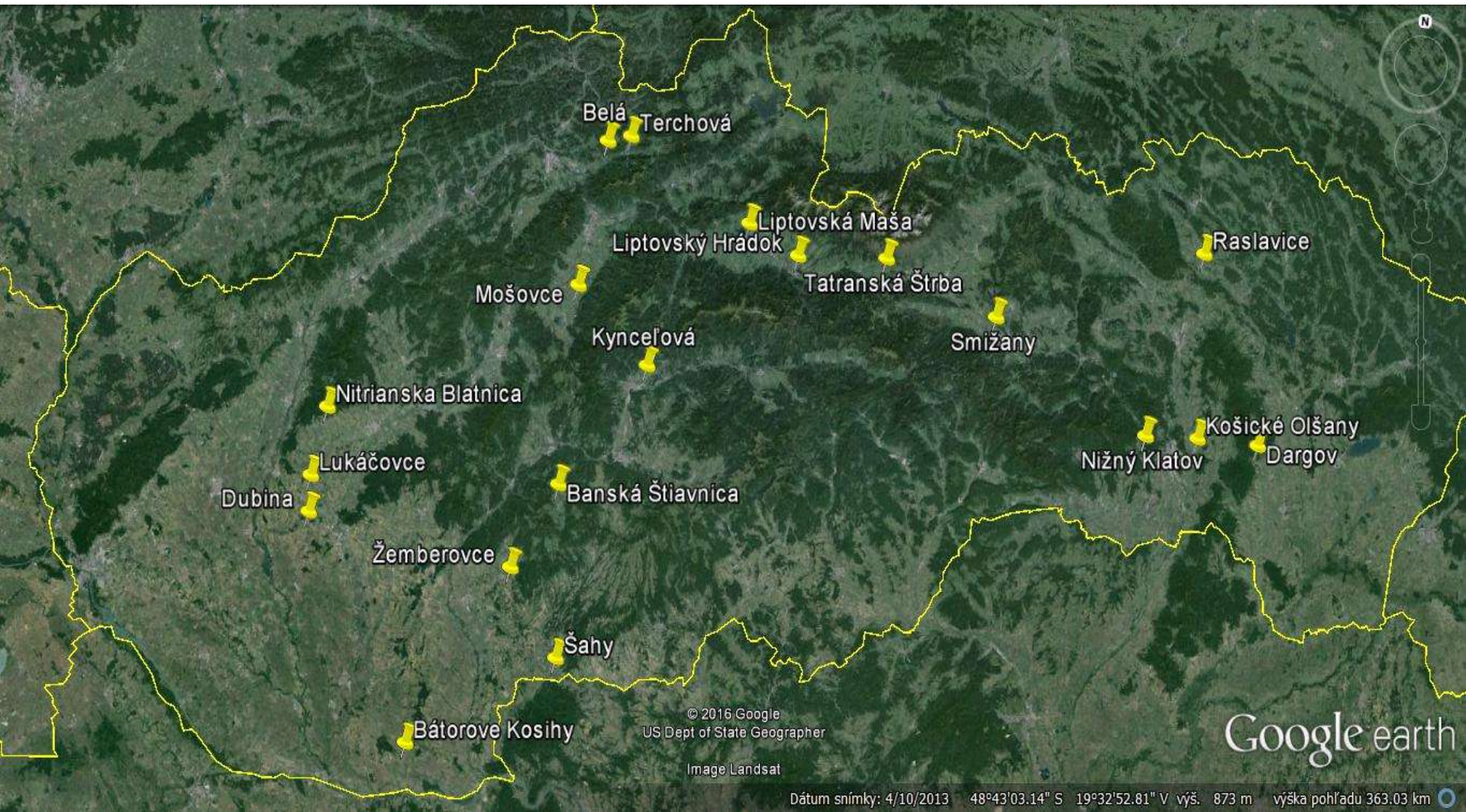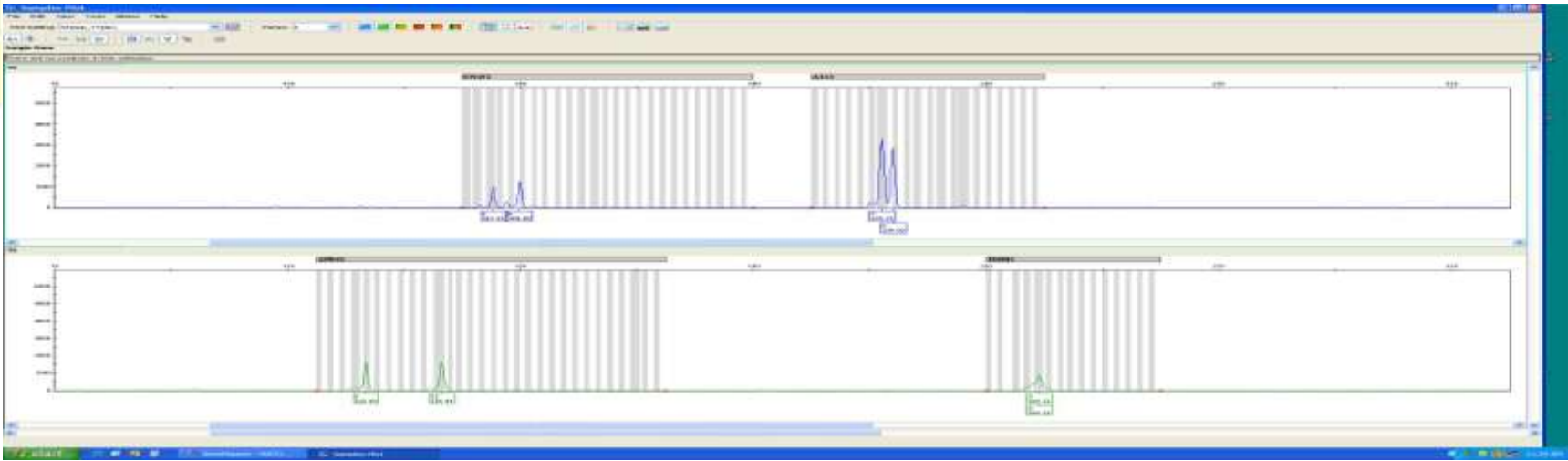
Marker	Ch	Size	Dye	MP	Marker	Ch	Size	Dye	MP
A024	7	91 - 115	NED	1	**A079**	1	94 - 120	PET	2
A007	8	100 - 140	FAM	1	**A113**	6	199 - 227	FAM	2
THE01	13	274 - 302	VIC	1	**A8**	2	100-132	NED	2
THE03	13	180 - 202	NED	1	**Ac11**	9	163-183	NED	2
C1602	16	235 - 290	FAM	1	**Ap224**	9	277-287	FAM	2

Self-assignment tests (GeneClass, 2.0)

a) Actual sample size:

Bayesian method – successfully assigned **95.1%**

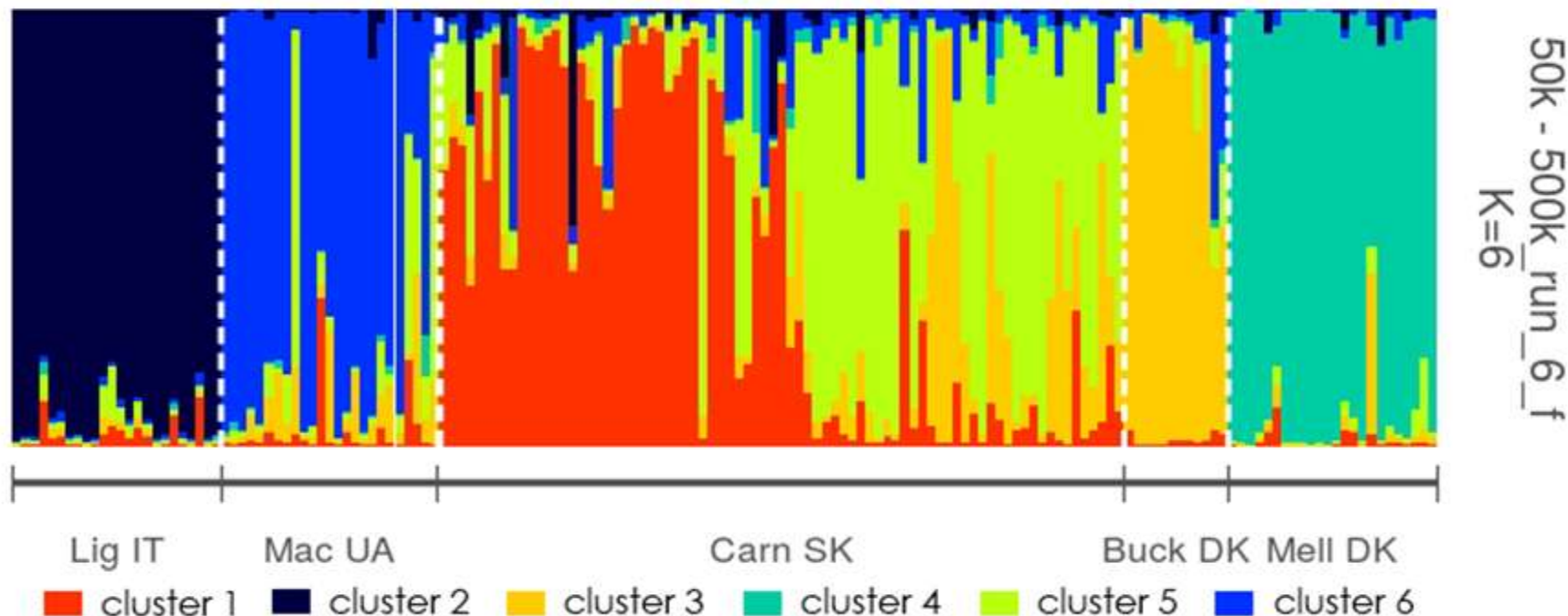Frequency-based method – successfully assigned **93.3%**

b) Simulated sample size using Monte Carlo Markov

   Chain resampling of 100 000 individuals

Bayesian method – assigned **87.8%**

Frequency-based method – assigned **92.1%**

Population structure: Bayesian assignment barplot (STRUCTURE)

The Bayesian assignment barplot for K= 6, length of the burn-in period 50,000, number of 500,000 MCMC iterations showing the genetic structure of individual bees in examined honeybee subspecies.

Analysis of mtDNA

mtDNA - about 16 kbp circular molecule, maternally inherited

PCR-RFLP method based on amplification and Dra*I digestion* of variable tRNAleu-cox2 region (**Dra*I* test**) has been widely used in honey bee maternal identification, phylo-geographical studies, introgression of foreign queens, ...

Dra*I* test - is unable to identify honey bees at the subspecies level

**Sequencing** - the ultimate method for assessing mtDNA variation
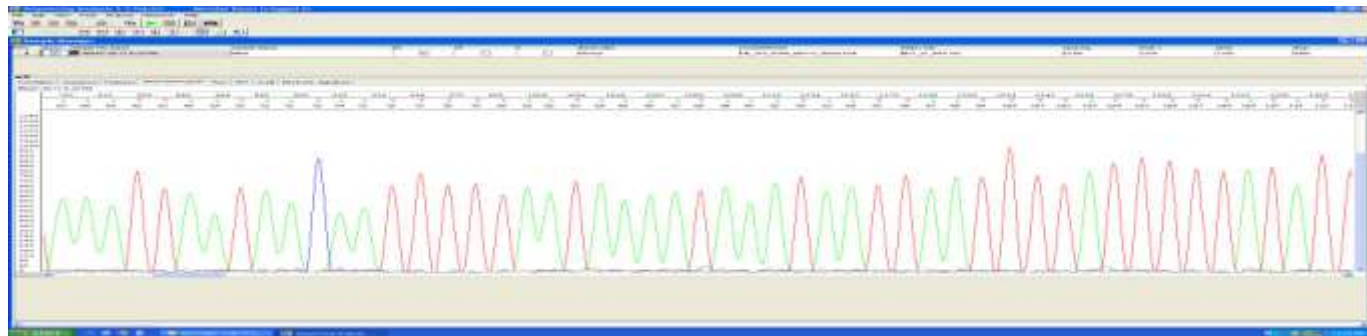
In our study:

- sequenced cca 570 bp PCR fragment of tRNAleu-cox2 intergenic region
- analysed 66 *A. m. carnica* workers, collected from 6 breeding and 21 reproduction farms
- **71.2%** of samples correspond to **5 known** haplotypes, belonging to C lineage (C2c, C1a, C2d, C2e and C2z)
- **28.8%** of samples have novel mutations

- **the most frequent haplotype C2c (36.4%)** is typical and dominant for Slovenian *A. m. carnica* population

- haplotype C2d (6.1%) has been observed in Croatia, Canary Islands, detected also in *A. m. macedonica and* Buckfast

- haplotypes C2e (4.5 %) and C2z (1.5 %) were detected in bees from Romania

- **interestingly, haplotype C1a, typical for *A.m. ligustica* was observed in 22.7% of bees from Slovakia**

- novel haplotypes (1- 6) are derived from C2c (16.6%), C2v (6.1%) and C2y (6.1%)
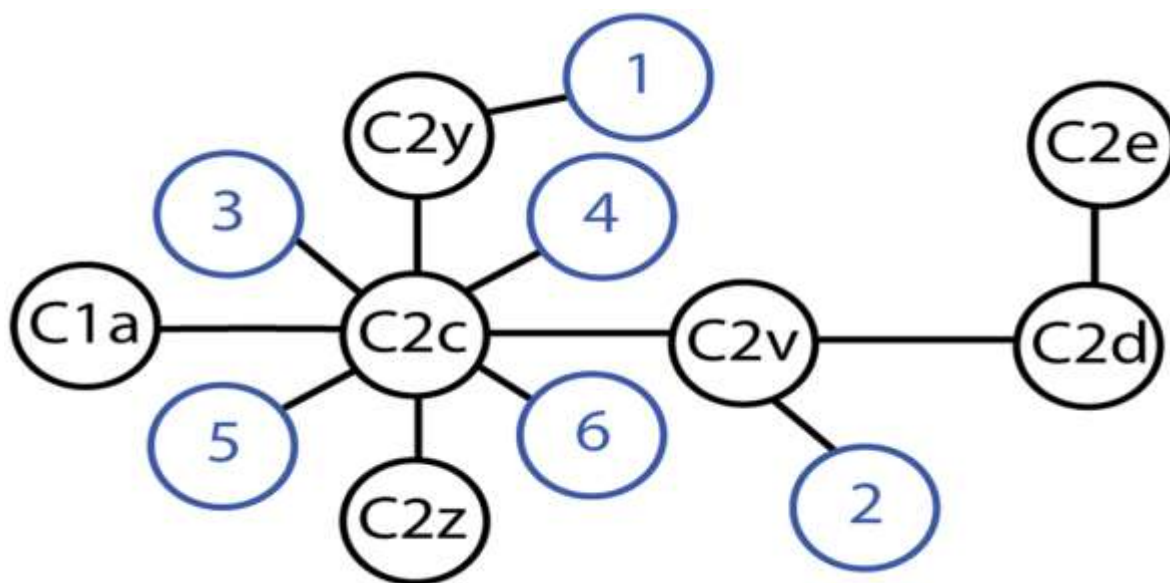
CONCLUSIONS

➤ honey bee population in Slovakia comprises predominantly Carniolan race (*Apis mellifera carnica*)

➤ apparent admixture of *A. m. ligustica (mtDNA analysis)*, *A. m. macedonica* and *Buckfast* bees (microsatellites) has been observed

➤ to efficiently preserve Carniolan honey bee as a genetic resource in Slovakia it is necessary to combine molecular methods with standard morphometric tools to perform reliable race assignment, especially at breeding stations

ACKNOWLEDGEMENTS

Thanks for your attention