New member in SWFS from Belarus: Scientific and Practical Center of the State Committee of forensic examinations Republic of Belarus

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The Scientific and Practical Center was established on 16th August, 1929. This is a scientific organization, accredited by the National Academy of Sciences of Belarus. As of 2013, forensic expertise is concentrated within the State Committee of Forensic Examination of the Republic of Belarus, to which the Scientific and Practical Center belongs.

The Centre's activities are both scientific and practical, carrying out examinations in the field of forensic science, forensic expertise, and scientific research. The Centre also engages in the development of modern methods, techniques and technical means for conducting expert studies.

The Molecular Biological Research laboratory (MBRlab) conducts DNA analysis of wild animals on investigative materials submitted for cases dealing with allegations of illegal hunting. Forests cover 40 percent of the territory of Belarus. Previously, molecular genetic studies of wild flora and fauna of Belarus were not conducted. To solve this issue, the MBRlab has developed scientific pilot studies of DNA-marker polymorphism and the genetic structure of wild species, in order to develop methodological approaches for expert application.

Wild boar, moose, roe deer and red deer are the main native species hunted in our country. The introduced fallow deer is also hunted. Mouflons and spotted deer live in open-air cages. The Republic of Belarus has one species that is listed in the Red Book of Belarus (a listing of protected species), the European bison. Populations live in designated national parks. However, illegal hunting outside the reserves is known to occur. The bison belongs to the order of even-toed ungulates, which also include domestic pig, sheep, goat, domestic bull; meat products of these species are a common household item.

Thus, any expert examination of biological samples should first determine the species of a sample, in order to identify whether a wild or domestic animal is being examined. Only after this is it necessary to identify to individual.

MBRlab has a strategy, which dictates that the traditional STR-technologies of the investigating laboratory are applied to expert studies of animal DNA. So, the laboratory has worked to establish the limits of marker cross-amplification for even-toed ungulate species. MBRlab studied STR locus cross-amplification on specimens of elk, roe deer, red deer and wild boar. We used four groups of STR-markers to study cross-amplification in species wild to Belarus: twelve bovine loci, six pig loci, 13 deer markers (species origin – American deer) and five caribou markers. Some results are shown in tables 1 and 2.

Table 1 – Cross-applicability of microsatellite markers in closely related species. The following parameters included analysis of PCR products for each target species: The first parameter is absence of amplification of the target product. There are the group of unamplifiable loci (letter "N") and the presence of the expected target product. 2 groups were isolated in the presence of the desired product. There are a group of monomorphic amplified STR loci (letter "M") and a group of polymorphic loci (letter "P").

Source species of marker	Name of locus	Target species				
		Bull *B.taurus*	Red deer *(C.elaphus)*	Roe deer (*C.capreolus)*	Moose (*A.alces)*	Wild boar *(S.scrofa)*
Bull - *Bos taurus*	ETH225	P	P	P	P	N
	TGLA122	P	P	P	M	N
	BM2113	P	N	N	N	N
	ETH10	P	N	N	N	N
	ETH3	P	N	N	N	N
	TGLA227	P	N	N	N	N
	INRA023	P	M	N	P	N
	TGLA53	P	P	N	P	N
	HEL1	P	N	N	N	N
	TGLA126	P	M	M	P	N
	CSSM036	P	M	P	M	N

Quantitative differences were seen in the spectrum of the detected alleles and differences in the molecular size ranges of alleles in the group of amplified loci.

Table 2– Allelic polymorphism of bull markers in representatives of the suborder Ruminants

Source species of marker	Name of locus	Ruminant target species							
		Family Bovid (*Bovidae*)		Family Deer (*Cervidae*)					
		Bull *B.taurus* *n=41*		Red deer *(C.elaphus)* *n=46*		Roe deer (*C.capreolus)* *n=38*		Moose (*A. alces)* *n=42*	
		Allele Size (bp)	N	Allele Size (bp)	N	Allele Size (bp)	N	Allele Size (bp)	N
Bull- *Bos taurus*	ETH225	140-152	6	145-164	3	145-155	4	143-155	6
	TGLA122	144-183	14	149-151	2	126-128	2	134	1
	BM2113	125-139	6	NA	-	NA	-	NA	-
	ETH10	209-225	7	NA	-	NA	-	NA	-
	ETH3	117-129	6	NA	-	NA	-	NA	-
	TGLA227	81-103	9	NA	-	NA	-	NA	-
	INRA023	200-216	8	198	1	NA	-	221-223	2
	TGLA53	152-188	16	173-183	3	NA	-	145/149	-
	HEL1	104-112	3	NA	-	NA	-	NA	-
	TGLA126	111-123	7	110	1	114	1	112-122	2
	CSSM036	163-185	5	158	1	160-162	2	172	1

N – number of alleles
Allele sizes are indicated for the MegaBACE 750 genetic analyzer.

The success of cross-amplification depends on the level of kinship. This allowed us to create a DNA-panel that successfully differentiates wild and domestic animals. A patent was filed for consideration in the Eurasian patent office in the city of Moscow, based on this work.

Pork is the national cuisine in Belarus, so a second scientific study examined genetic polymorphism of wild boar. Samples of wild boar (719 samples) and domestic pigs (304 samples including 6 breeds) were studied in parallel using 18 STR loci and SNP polymorphisms of the melanocortin 1 receptor gene (MCR1, 2 sites of single nucleotide substitutions) and the nuclear receptor NR6A1 (1 site).

The SNP study showed that there is an introgression of the genes of domestic pigs in wild boar animals, which is 6.8 ± 0.9% for the MCR1 gene and 1.5 ± 0.5% for the NR6A1 gene. It also showed that the frequencies of alleles of STR loci don’t differ in the group of hybrid wild boars from those of non-hybrid wild boars. This suggests that the processes of hybridization in wild boars took place relatively long ago in Belarus. Analysis of STR polymorphism in population of wild boar revealed significant regional differentiation of wild populations. The MDS results are shown on the figure 1. The analysis of allele frequencies (with the help of the Harlequin program and statistical data) shows statically valid differences between groups among the groups formed according to the administrative criterion.

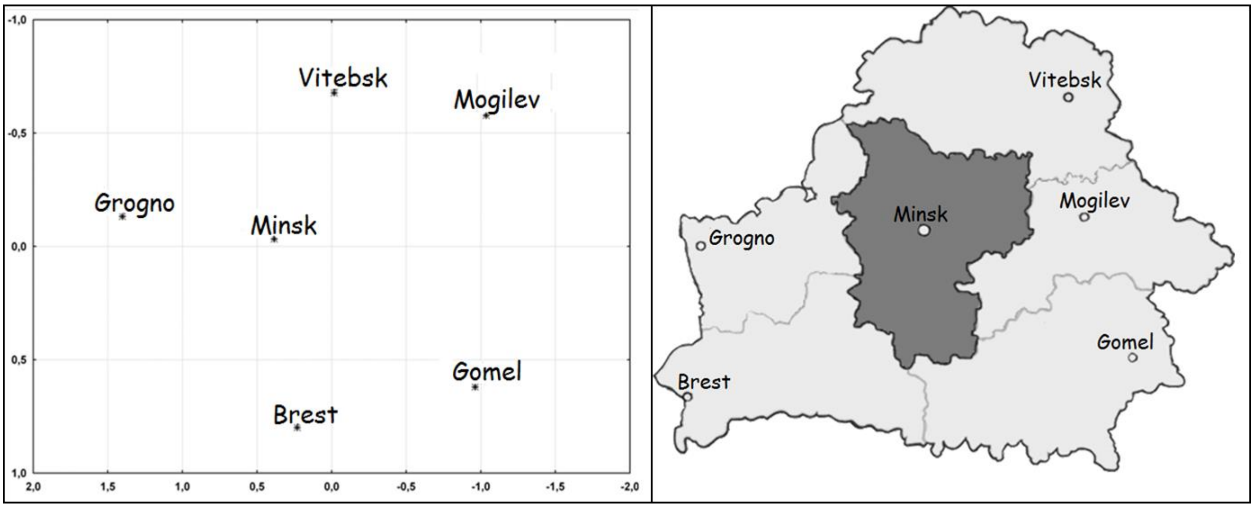

Figure 1 – The graph of MDS genetic distances Fst between populations of wild boar (the left figure shows the genetic distances between populations the right one is a map of the geographical position of these populations)

MBRlab also found that the STR polymorphisms of wild boar have significant differences with the STR polymorphisms of domestic pigs. This means that the calculation of the reliability of the inference should be based only on the frequencies of the alleles of the corresponding groups in expert studies. The study also showed that the frequency of alleles is statistically significantly different between individual breeds within a group of domestic pigs. This can potentially be used to identify breed of domestic pigs.

The study evaluates the differentiating power of the STR loci in solving the problem of differentiating wild and domestic pigs, based on the genetic profile of the sample. The results indicate that a genotype requires a minimum of 12 loci to enable inclusion of the sample to the group of wild or domestic pigs.

Table 3 – Power of the 20 tested STR markers to discrimination between wild boar and domestic pigs, sorted in an ascending order of F_{ST} values

Marker	F_{ST}*	P*	Average log-LR for assignment to the correct population**	Minimal log-LR for assignment to the correct population**	Percentage of incorrect assignments for log-LR cut-off point equal to 0**
FH3637	0.01305	0.00010	49.7	8.5	0.0%
FH1701	0.02358	0.00000	47.8	8.1	0.0%
FH1589	0.03593	0.00000	46.5	7.2	0.0%
FH2478	0.03760	0.00000	45.2	5.4	0.0%
FH1696	0.04362	0.00000	44.4	4.7	0.0%
FH1733	0.06186	0.00000	42.4	6.2	0.0%
FH2148	0.07601	0.00000	41.2	6.4	0.0%
FH1727	0.07624	0.00000	39.3	4.4	0.0%
S0005	0.07724	0.00000	36.9	4.1	0.0%
SW240	0.08640	0.00000	31.6	-1.1	0.3%
FH2709	0.09409	0.00000	29.0	-2.0	0.3%
SW857	0.09599	0.00000	23.9	-3.6	0.8%
S0766	0.09963	0.00000	21.1	-5.6	1.0%
FH1900	0.10234	0.00000	18.4	-6.1	0.7%
NLRIP	0.10516	0.00000	17.0	-4.9	1.1%
S0101	0.11486	0.00000	12.7	-5.9	1.6%
S0710	0.18639	0.00000	9.3	-6.6	3.4%
S0355	0.23236	0.00000	6.7	-7.5	5.2%
SW951	0.27174	0.00000	4.9	-2.9	7.3%
FH4219	0.38490	0.00000	3.2	-3.2	16.0%

* locus-by-locus AMOVA between wild boar and domestic pig populations from Belarus

** before sequential elimination of the marker from the first column

MBRlab also investigates the genetic polymorphism of red deer, roe deer and moose as shown in the following study. Red deer is a reintroduced species, and therefore its distribution isn’t uniform in the territory of the Republic of Belarus. 143 biological samples of deer were investigated by 16 tetra-nucleotide STR loci.

Red deer have statistically significant regional differentiation, like wild boar. The results are at the analysis stage. Sampling of regional groups was conducted and frequency of alleles established for all populations. A high number of suitable loci for forensic tasks were confirmed. Our laboratory completed the genotyping of samples of European moose (about 400 samples) and Roe deer (380 samples) at present. We investigated 18 STR dinucleotide and tetranucleotide loci on DNA of moose samples and 13 STR loci for roe deer. These loci are selected on the basis of cross-amplification. The research showed that the sets of target STR loci are a sufficient tool for solving various kinds of expert problems.

The legalization of methodological tools is a separate area in forensic examination. Officially approved methods are added to the Register of forensic methods and other methodological materials of the State Committee of Forensic Examinations of the Republic of Belarus. The Interdepartmental Scientific and Methodological Council in the field of forensic expert activity decide on the approval of methods, and their inclusion in the Register. The Council accepts them on the basis of reviewing the methodology and testing the methods in expert departments.

The MBRlab of the Scientific and Practical Center have developed methodological materials, three of which have already been included in the Register of Forensic Expert Techniques at present:

1. Method of specific PCR identification of wild animals of the *Cervidae* family and their differentiation from Bovid and Suids families // Kotova S.A., Spivak E.A., Rybakova V.I., Ryabtseva A.O., Tsybovsky I.S.// Scientific and Practical Center of the State Committee of Forensic Examinations of the Republic of Belarus/ Minsk: Law and Economics. - 2016. - 32 p.

2. Technique of DNA-identification of biological specimens of animals of the European boar (wild and domestic) // Kotova S.A., Ryabtseva A.O., Spivak E.A., Tsybovsky // Scientific and Practical Center of the State Committee of Forensic Examinations of the Republic of Belarus/ Minsk: Law and Economics - 2017. - 27 p.

3. Method of establishing the belonging of biological specimens to wild and domestic representatives of the species European boar by the method PCR-RFLP // Kotova S.A., Kipen V.N., Ryabtseva A.O., Tsybovsky I.S // Scientific and Practical Center of the State Committee of Forensic Examinations of the Republic of Belarus / Minsk: Law and Economics, - 2017. - 23 p.

In 2018, further methods will be developed to identify red deer, moose and roe deer. The laboratory will apply these techniques to expert examinations involving illegal hunting of these species. The number of these examinations exceeds 200 per year.

MBRlab track other recent socially significant events involving animals and objects of animal origin. The animal abuse and theft of dogs is of interest, although it is still quite small compared to Europe. In 2018, four examinations of animal abuse were conducted, and five in 2017. There is a completely unexpected problem caused by global warming. Reports are made on the appearance of new species in the territory of the Republic of Belarus – for example, the appearance of the golden jackal. Golden jackals were registered in Poland, Lithuania and Ukraine in recent years. The first case of finding a golden jackal in the Republic of Belarus occurred on December 31st in 2011. Residents of one of the regions of

Belarus reported on his appearance two years ago, but it wasn't possible to conduct all the necessary studies and register a jackal in the republic.

In late 2017, MBRlab received an appeal from the Department of Ecology of the Biological Faculty of the Belarusian State University. They asked to conduct a genetic study of the animal; they assume that it's a jackal. The mitochondrial DNA nucleotide sequence of an unknown sample was aligned to the reference sequences of representatives of the *Canidae* family that inhabit the territory of the Republic of Belarus. Results showed that the unknown sample coincides most with the mitochondrial DNA of the golden jackal from the population of Israel (figure 2).

№ п/п	Name of animal	Latin name	Accession No.	Identity
1	Wolf	*Canis lupus lupus*	AM711902.1	91
2	Dog	*Canis lupus familiaris*	NC_002008.4	90
3	Fox	*Vulpes vulpes*	NC_008434.1	75
4	Arctic fox	*Vulpes lagopus*	NC_026529.1	75
5	Raccoon dog	*Nyctereutes pronoides*	NC_013700.1	78
6	Golden jackal	*Canis aureus*	KT448274.1 KT448273.1	98
7	Coyote	*Canis latrans*	NC_008093.1	88

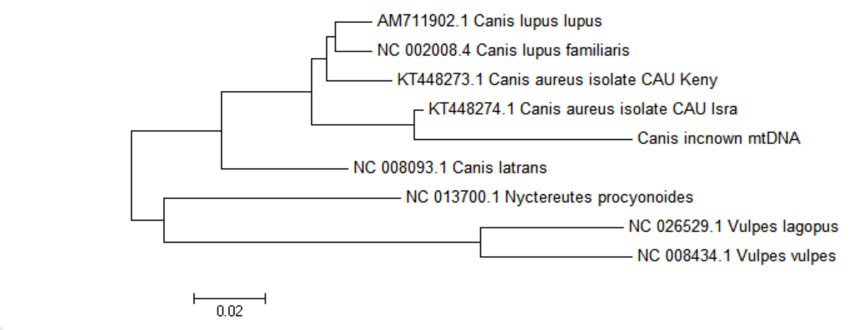

Figure 2 – Identity of an unknown sample with a reference sample (from above)
A phylogenetic tree - was built with MEGA 4.1 program (from below)

In 2017, MBRlab signed an agreement of scientific co-operation with the State Organization Minsk Zoo. Most of the employees of Minsk zoo are interested in the sex of birds that don't show sexual dimorphism. There is also interest in the establishment of paternity testing of lion-tailed macaque. In addition, an examination was conducted to determine maternity in the African wild dog, as well as the relationship of sibs from different litters, one of whom was abducted from the zoo.

In addition, small numbers of expert studies have been required for fox, bear, beaver, badger and bison.

Due to the number of investigations in which dogs appear, it is most likely that the laboratory will be engaged in a more detailed study of genetic polymorphism in *Canidae* family including are wolf, fox, raccoon dog and domestic dog

In the nearest future, a separate area will be assigned to the laboratory that will deal with problems of forensic identification of plant objects. First this sector will be engaged in research of molecular biological polymorphism of plants involved in logging (pine and spruce) and illegal circulation (hemp).

We thank you for your attention.