RESEARCH OF VARIABILITY IN PRESTICE BLACK PIED PIG USING DINUCLEOTIDE AND TETRANUCLEOTIDE MICROSATELLITE MARKERS

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Abstract

The aim of this study was to use 10 dinucleotide and 12 tetranucleotide microsatellite markers (MS) to characterize the variability in 89 breeding boards of Prestice Black Pied Pig (PC). Mean number of alleles at dinucleotide versus tetranukleodtide MS was 7.40 - 7.55, observed heterozygosity 0.740 - 0.696 and PIC 0.691 - 0.682.

We also evaluated diversity of PC and all other breeds kept in the Czech Republic (Large White – father line (LWF) and Large White – mother line (LWM), Landrace (L), Pietrain (P), Duroc (D), and Czech Meat (CM) using 10 dinucleotide MS. Total of 658 breeding boars were analyzed. Mean number of alleles per locus in all breeds was 12.9, ranging between 9 to 18 alleles. The greatest PIC was 0.691 in PC and lowest 0.506 in D. The expected and observed heterozygosity was 0.760, 0.635 resp. across all loci. Only Pietrain breed showed a significant deficit in heterozygosity (F_{IS}; $P < 0.05$), which was 0.068. The analysis of population structure with using algorithm based on the Markov chain Monte Carlo method in Structure software confirms that there was little admixture between seven studied breeds, except Large White – father line and Large White – mother line.

Key Words: Prestice-Black-Pied pig, dinucleotide and tetranucleotide microsatellites, diversity

Changes in pig breeding, substitution of traditional pig breeding and intense production have been leading to a gradual abandoning of local breeds and being replaced by commercial high productive breeds, and thus, they have practically extincted. Recent studies have been warning about the status of endangered animal genetic resources (Taberlet et al. 2008, 2011).

However, consciousness of a value and an importance of local breeds is increasing lately. Also, claims of consumers on a high quality of meat products and regional products are turning attention to native breeds and there is a will in order that defunct genetical resources can be kept and re-establish. This fact shows many studies investigating genetic diversity and variability of commercial and local pig breeds, which are describing genetic relationships and assess of their admixture (Wang et al. 2011, Berthouly-Salazer et al. 2012, Chen et al. 2012).

The goal of this study was to compare the panel of dinucleotide and tetranucleotide microsatellite markers to determine the genetic variability in genetic resource Prestice Black Pied Pig. Secondly, evaluation of diversity PC in relation to other breeds of pigs kept in the Czech Republic, due to the fact, that up until the PC has been declared as a genetic resource (1992) and kept as a separate population (1996), these breeds were using to his improvement. About the variability of breeds of pigs in the Czech Republic reported Vrtková et al. 2012, Matoušek et al. 2011.

Material and Methods

Animals

89 of Prestice Black Pied Pig – PC breeding boars registred in a herd book of genetic resource from 2002 to 2011 were analyzed by typing 10 dinucleotide and 12 tetranucleotide microsatellites.

Total of 658 breeding boars from six commercial breeds (D, P, LWF, LWM, CM, LA) and genetic resource PC collected from 2002 to 2011 were sampled i.e. 100 of following breeds, except CM and analyzed for 10 dinucleotide markers.

Microsatellites

Dinucleotide MS: S0107, S0386, SW353, S070, TNFB, S0355, S0068, SW24, SW936, SW72 (Putnová et al., 2003)

Tetranucleotide MS: SBH2, SBH18, SBH4, S0655, SBH23, SBH20, SBH1, SBH10, SBH13, 387A12F, SBH22, SBH19, SBH23 (Kourková et al., 2008).

The microsatellite markers were analyzed using an ABI PRISM® 310 Genetic Analyzer. Fragment analysis was carried out using GeneScan 3.7 and Genotyper 3.7 software.

Statistical analysis

The mean number of alleles per locus (MNA), polymorphism information content (PIC), observed heterozygosity (H_O) and expected heterozygosity (H_E) were obtained across different loci and populations using the Excel Microsatellite Toolkit v. 3.1.1. add-in utility for Microsoft Excel (Park, 2001). Genepop v. 4.2 (Rousset, 2007) was used to perform deviations form Hardy-Weinberg equilibrium (HWE) per locus using Markov chain algorithm implemented according to authors recommendation with 10,000 dememorizations, 200 batches and 5,000 interactions per batch. The software package Fstat v. 2.9.3.1 (Goudet, 2001) was used to calculate Wright's F_{IS} index according to Weir and Cockerham (1984). Genetic differentiation and population subdivision were tested by algorithm of Pritchard et al. (2000) implemented in software Structure v. 2.2. Individual animals were assigned to two or more subpopulations on their multilocus 10 microsatellite genotypes. The Structure is able to determine for each pig the proportion of genes originating from the "K" potential clusters. The Structure

algorithm based on the Markov chain Monte Carlo method (MCMC) was use to define the natural algorithm of the probability that a given genotype belongs to the assumed K clusters. The run length was set to burn-in period of 10^5 interactions followed by 10^5interactions suggesting by authors. The program was tested the range of possible clusters (K) from 2 to 9, and was run 10 times for each K.

Results

Comparison of dinucleotide and tetranucleotide MS panels to assess variability of PC pig

For evaluating of variability of PC breed, there were chosen two MS panels used for parentage control. Panel of 10 dinucleotide MS and panel of 12 tetranucleotide MS.

The table 1 is showing individual MS positions on chromosomes. On 3, 6, 7, 13 and 17 chromosome, there are both of dinucleotide nad tetranucleotide MS.

Comparison of suitability of dinucleotide and tetranucleotide MS panel for assessing variability PC is shown in table 2. 10 MS panel versus 12 MS panel: MNA was 7.40 – 7.55, observed heterozygosity 0.714 – 0.696, expected heterozygosity 0.733 – 0.724, PIC 0.691 – 0.682.

Biotype Diagnostic GmbH (Animaltype PCR Pig Amplification Kit) worked out population study of variability of tetranucleotide MS in LW, L a P breed kept in Germany. In our study in MS loci 387A12F, SBH4 a SBH10, we found alleles specific for PC (alleles not found in population study of commercial breeds).

In the table 3, there are given loci in which less than a half of all alleles known at commercial breeds appears at PC.

Table 1. Location of dinucleotide and tetranucleotide microsatellites in chromosomes

chromosome	dinucleotide MS	tetranucleotide MS
1		SBH1
3	SW72	SBH2
4	S0107	
6	SW353	SBH4
7	TNFB	S0655
9		SBH10
10	S0070	
11	S0386	
12		387A12F
13	S0068	SBH13
15	S0355, SW936	
16		SBH18
17	SW24	SBH19
18		SBH20
X		SBH22, SBH23
Y		SBH23

Table 2. Comparison of variability of dinucleotide and tetranucleotide MS panel in PC breed

panel 10 dinucleotide MS				panel 12 tetranucleotide MS			
MNA	H_O	H_E	PIC	MNA	H_O	H_E	PIC
7.4	0.714	0.733	0.691	7.55	0.696	0.724	0.682

Observed heterozygosity (H_O), expected heterozygosity (H_E),mean number of alleles (MNA), polymorphism information content (PIC)

Table 3. Chosen loci with low number of alleles in PC breed

MS locus	NA - comercial breeds	NA - PC breed	NA - PC (%)
SW353	12	4	30
TNFB	15	6	40
387A12F	18	8	44
S0655	14	6	42
SBH10	20	9	45
SBH20	24	8	33
SBH22	12	5	42

NA -Number of alleles

Assessing of diversity in PC breed comparing with other commercial breeds kept in the Czech Republic.

The total number of alleles found for dinucleotide microsatellite markers was 129 with mean number of alleles per locus 12.9, ranging between nine (*S0386* and *SW72*) to 18 alleles (*S0068* and *S0070*) (Table 4). All loci in this study were observed polymorphic for each breed. On the other hand, MNA ranged between 5.0 in Duroc and 8.3 in Pietrain boars (Table 5).

The PIC values per locus were greatest (0.867) in *S0070* and least (0.615) in *S0355* (Table 4). By focusing on different populations of boars, the greatest PIC was 0.691 in PC and lowest 0.506 in D (Table 5). The expected heterozygosity was 0.760 ± 0.027 across all loci (Table 4). Six out of 10 microsatellite markers exceeded PIC value 0.7 in *S0068, S0107, SW24, SW936, S0070*, and *TNFB*. Four markers *S0355, S0386, SW72* and *SW353* were close to 0.7. For all loci expected heterozygosity exceeded observed heterozygostiy.

Negative Wright's coefficient indicates a significant difference of F_{IS} smaller than zero, suggesting an excess of heterozygotes due to non-random mating. Negative F_{IS} coefficient was estimated only in CM breed. However, relatively high diversity values (MNA, H_E) were observed in all breeds. Especially, genetic resource PC has the highest H_E, which illustrating no risk status of this breed. The F_{IT} parameter reflected divergence between H_E and H_O had multilocus mean of 0.184, and ranged from 0.103 (*SW936*) to 0.281 (*S0355*). Individual F_{ST} values ranged between 0.076 (*SW936*) and 0.225 (*S0355*) and indicated genetic differentiation among breeds, since multilocus variability corresponded to 0.158.

The Structure algorithm was applied to measure the population structure and degree of admixture. All runs from K = 2 to K = 9 showed a pattern allowing a meaningful interpretation (Figure 1). The Ln Pr (X | K) increased sharply between K = 2 and K = 5, and stabilized between K = 6 and K = 8, dropping afterward. These results would indicate that the appropriate value of K would be between 6 and 8. The contribution of the assumed ancestral populations to the 7 breeds under study are graphically presented in Figure 1, for values of K ranging between 2 and 9. When K = 2, D was separated from the other. When K = 3, with LWF and LWM separating from the other breeds. Progressively, as K increased, the contributions of the assumed populations resulted in still incomplete separation of the 7 breeds. Genetic differences between mother and father lines of Large White were not significant, even if the K = 9. Assuming K = 7, the proportional contribution of the assumed ancestral populations to each one of the current breeds was computed. Each one of the breeds except LWF and LWM. On the other hand, genetic resource of PC breed was identified when K = 5.

Table 4. The overall results of genetic diversity as one population

Locus	NA	PIC	H_O[1]	H_E[1]	F_{IT}	F_{IS}	F_{ST}
S0068	18	0.712	0.640	0.735	0.1455	0.0134	0.1339
S0107	14	0.810	0.699	0.831	0.1779	0.0214	0.1599
SW24	12	0.830	0.697	0.847	0.1975	0.0349	0.1685
S0355	10	0.615	0.488	0.656	0.2809	0.0721*	0.2250
S0386	9	0.617	0.546	0.670	0.2090	0.0143	0.1976
SW353	12	0.624	0.507	0.663	0.2508	0.1264*	0.1425
SW936	12	0.712	0.680	0.749	0.1026	0.0292	0.0757
S0070	18	0.867	0.727	0.879	0.1979	-0.0093	0.2053
SW72	9	0.678	0.590	0.715	0.1949	0.0411	0.1604
TNFB	15	0.835	0.775	0.853	0.1061	-0.0057	0.1112
Multilocus	12.900	0.730	0.635 (0.006)	0.760 (0.027)	0.1835	0.0308	0.1576

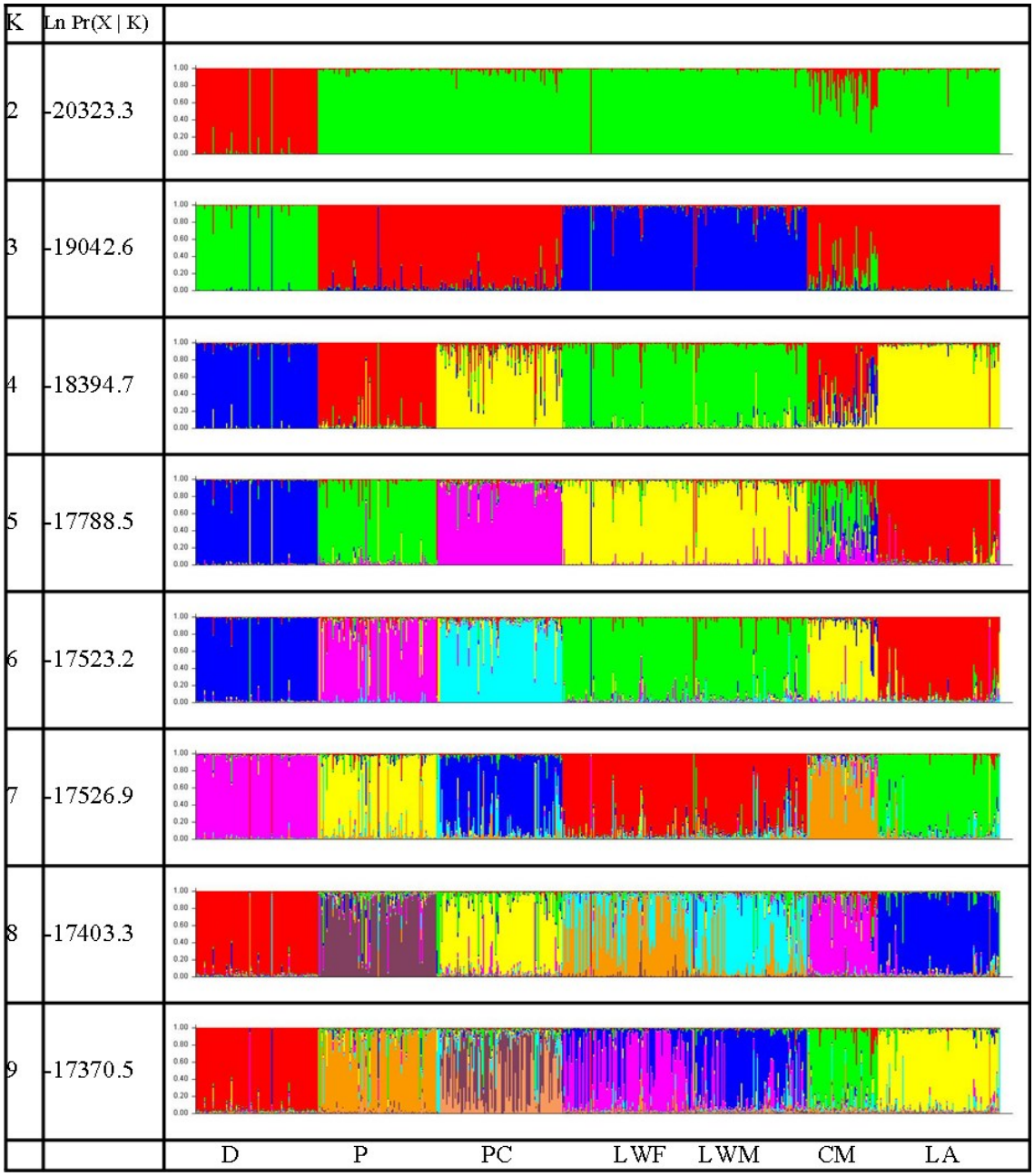
Observed heterozygosity (H_O), expected heterozygosity (H_E), and Wright's statistics (F_{IT}, F_{IS}, F_{ST}) for each locus and for all loci combined. Deviations from Hardy – Weinberg equilibrium and significance of the F_{IS} values are indicated by asterisks (*$p < 0.01$). [1]Standard errors in parentheses

Table 5. Genetic diversity within 7 pigs populations

Population	N	H_O[1]	H_E[1]	MNA[1]	PIC	BDHW	F_{IS}
D	100	0.561 (0.016)	0.565 (0.065)	5.0 (1.49)	0.506	0.0000	0.040
P	100	0.594 (0.016)	0.637 (0.059)	8.3 (2.41)	0.595	0.0000	0.068*
PC	100	0.714 (0.015)	0.733 (0.025)	7.4 (2.59)	0.691	0.0125	0.023
LWF	100	0.579 (0.016)	0.611 (0.050)	7.1 (2.08)	0.565	0.0000	0.052
LWM	100	0.677 (0.015)	0.685 (0.038)	7.5 (2.46)	0.641	0.3163	0.012
CM	58	0.692 (0.019)	0.688 (0.057)	6.70 (2.11)	0.646	0.2199	-0.004
LA	100	0.650 (0.016)	0.682 (0.031)	7.90 (1.97)	0.639	0.0000	0.048
Total	**658**	0.635 (0.006)	0.760 (0.027)	12.90 (3.31)	0.730	0.0784	

Observed heterozygosity (H_O), expected heterozygosity (H_E),mean number of alleles per breed (MNA), polymorphism information content (PIC), proportion of breed not complying with the Hardy-Weinberg equilibrium at $P < 0.05$ (BDHW), and F_{IS} per locus. Significance of the F_{IS} values are indicated by asterisks (*$p < 0.01$). [1-Standard] errors in parentheses

Figure 1. Graphical representation of the estimated membership fractions of individuals of the breed analyzed in each of the K inferred clusters, for K = 2 to K = 9



Discussion

Table 1 shows that on chromosomes 3, 6, 7, 13 and 17 dinucleotide and tetranucleotide MS appears. This fact provides a possibility of creation of haplotypes as another tentative parameter of genetic variability. In case of showing that they are somehow interesting, we will test both panels for using haplotypes for next characteristic of genetic resource PC.

For suitability of using panel 10 dinucleotide MS comparing with panel 12 tetranucleotide MS according to values observed heterozygosity Ho (0,714 x 0,696) and PIC (0,691 x 0,682) , both of panels does seem to be on the same level.

Stimulating fact for further study is that in loci with low number of tetranucleotide MS alleles (387A12F, SBH4 a SBH10) at PC we identified new alleles. If those alleles became specific for PC breed in the further study, it would be important benefit for breeding work with genetic resource PC.

The knowledge about the genetic diversity within the different commercial breeds (D P, LWF, LWM, CA, LA) and genetic resource (PC) is important to know, in terms of local genetic resources. Since the conventional breeds play a key role in global meat production (Druml et al. 2012), in recent years, increased consumer demand for high-quality local meat products has been noticed. It proves necessity to pay attention back to traditional breeds such as PC in our study. Several studies have reported observed heterozygosity values for different populations of the commercial breeds such as D, P, LA, LW (which is breed in two lines, father and mother, in Czech Republic) (Boitard et al., 2010, Druml et al., 2012, Fabuel et al., 2004, Laval et al., 2000, Megens et al., 2008, SanCristobal et al., 2006). The values ranging from 0.670 to 0.500 are comparable to our measured values ranging from 0.692 to 0.561. In comparing to PC breed, the observed heterozygosity was the highest 0.714.

In terms of genetic variability expressed by mean number of alleles per breed (MNA), P (8.3) has the highest value within our studied populations, also within other examined population reported in (Boitard et al., 2010, Druml et al., 2012, Fabuel et al., 2004, , Guastella et al., 2010, Laval et al., 2000, Megens et al., 2008, SanCristobal et al., 2006, Vicente et al., 2008).

The F_{IS} values were not significantly different from zero in all other populations except P (0.068) which suggesting a limited exchange of animals among farms and herds. The comparable results have been reported in Vicente et al., 2008. However, Druml et al., 2012 noticed no significant value of F_{IS} in Pietrain (0.01). Vicente et al., 2008 also showed significantly F_{IS} values for all examined commercial breeds Duroc (0.051), Landrace (0.038), Large White (0.065), and Pietrain (0.061).

The results obtained from STRUCTURE confirms that there was little admixture between 7 studied breeds, except LWF and LWM. Since these two breeds are kept separately from each other as a single line, we might assumed to differentiate from each other. Assuming K = 8, indication of differentiation of LWF and LWM is obvious. In addition to native breed, when K = 5 the clear differentiation of PC together with commercial breed (D, P, LWF, LWM, CM, LA) has been detected. The comparable results have reported in Druml et al., 2012 and Vicente et al., 2008).

Overall, the genetic diversity within the seven pig breeds kept in Czech Republic and analyzed in our study is relatively high. The analyzed breeds had a high level of differentiation to ancestral population, except of father and mother line of Large White. Our results showed that Czech genetic resource, Prestice Black Pied pig has the highest genetic variability and diversity compared to commercial breeds. As it is apparent from the part above, there exist exceptions in some loci.

In conclusion, there was proved the hypothesis of high variability in MS at genetic resource PC according to influence of commercial breeds which had been used for improving this breed during its breeding until 1992 when it was recognized as a genetic resource. This fact could be helpful for further breeding work with this breed.

References

BERTHOULY-SALAZAR C, THÉVON S, NHU VAN T, NGUYEN BT, PHAM LD, VU CHU C, and MAILLARD JCH (2012) Uncontrolled admixture and loss of genetic diversity in a local Vietnamese pig breed. Ecol Evol 2(5): 962–975

BOITARD S, CHEVALET C, MERCAT MJ, MERIAUX JC, SANCHEZ A, TIBAU J, and SANCRISTOBAL M (2010) Genetic variability, structure and assignment of Spanish and French pig populations based on a large sampling. Anim Genet 41:608-618.

DRUML T, SALAJPAL K, UROSEVIC M, GRILZ-SEGER G, and BAUMUNG R (2012) Genetic diversity, population structure and subdivision of local Balkan pig breeds in Austria, Croatia, Serbia and Bosnia-Herzegovina and its practical value in conservation programs. Genet Sel Evol 44(1): 5

FABUEL E, BARRAGAN C, SILIO L, RODRIGUEZ MC, and TORO MA (2004) Analysis of genetic diversity and conservation priorities in Iberian pigs based on microsatellite markers. Heredity 93:104-113.

GOUDET J (2001) FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www2.unil.ch/popgen/softwares/fstat.htm. Updated from Goudet (1995)

GUASTELLA AM, CRISCIONE A, MARLETTA D, ZUCCARO A, CHIES L, and BORDANO S (2010) Molecular characterization and genetic structure of the Nero Siciliano pig breed. Genet Mol Biol 33(4): 650–656

CHEN YC, HSU JT, CHEN CC, LEU YC, CHYR CYL, LIN DY, LIN. EC, CHEN CH, and WANG PH (2012) Investigation of Genetic Relationships Among Taiwan Black Pigs and Other Pig Breeds in Taiwan Based on Microsatellite Markers. Anim Biotechnol 23: 278–290

KOURKOVÁ L., VRTKOVÁ I., DVOŘÁK J., MATOUŠEK V., KERNEROVÁ N. (2008) Spolehlivější genetická metodika určování parentity prasat v ČR s využitím tetranukleotidových mikrosatelitových sekvencí DNA, Certifikovaná metodika,www.mze-vyzkum-infobanka.cz

LAVAL G, IANNUCCELLI N, LEGAULT C, MILAN D, GROENEN MAM, GIUFFRA E, ANDERSSON L, NISSEN PH, JORGENSEN CB, BEECKMANN P, GELDERMANN H, FOULLEY JL, CHEVALET C, and OLLIVIER L(2000) Genetic diversity of eleven pig breeds. Genet Sel Evol 32:187-203.

MATOUŠEK V., KERNEROVÁ N., VRTKOVÁ I. (2011): The variability of chosen genes and their associations with performance traits in sows of Přeštice Black-Pied breed. Research in Pig Breeding, 5:13-20.

MEGENS HJ, CROOIJMANS RPMA, SAN CRISTOBAL M, HUI X, LI N, and GROENEN MA (2008) Biodiversity of pig breeds from China and Europe estimated from pooled DNA samples: differences in microsatellite variation between two areas of domestication. Genet Sel Evol 40:103-128.

PARK, S. D. E. (2001) Trypanotolerance in West African Cattle and the Population Genetic Effects of Selection. [Ph.D. thesis] University of Dublin

PRITCHARD JK, STEPHENS M, and DONNELLY P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945-959.

PUTNOVÁ,L.,KNOLL, A., DVOŘÁK, V., DVOŘÁK, J. (2003) A novel porcine microsatellite panel for the identification of individuals and parentage control in the Czech Republic. Czech J. Anim. Sci. 48 (8):307-314.

ROUSSET F (2007) Genepop'007:Acomplete re-implementation of the GENEPOP software for Windows and Linux. Mol Ecol Notes 8:103-106.

SANCRISTOBAL M, CHEVALET C, HALEY CS, JOOSTEN R, RATTINK AP, HARLIZIUS B, GROENEN MAM, AMIGUES Y, BOSCHER MY, RUSSELL G, LAW A, DAVOLI R, RUSSO V, DÉSAUTÉS C, ALDERSON L, FIMLAND E, BAGGA M, DELGADO JV, VEGA-PLA JL, MARTINEZ AM, RAMOS M, GLODEK P, MEYER NJ, GANDINI GC, MATASSINO D, PLASTOW GS, SIGGENS KW, LAVAL G, ARCHIBALD AL, MILAN D, HAMMOND K, and CARDELLINO R (2006) Genetic diversity within and between European pig breeds using microsatellite markers. Anim Genet 37:189-198.

TABERLET P, COISSAC E, PANSU J, and POMPANON F (2011) Conservation genetics of cattle, sheep, and goats. CR Biol 334:247–254

TABERLET P, VALENTINI A, REZAEI HR, NADERI S, POMPANON F, NEGRINI R, and AJMONE-MARSAN P (2008) Are cattle, sheep, and goat endangered species? Mol Ecol 17:275–284

VICENTE AA, CAROLINO MI, SOUSA MC, GINJA C, SILVA FS, MARTINEZ AM, VEGA-PLA JL, CAROLINO N, GAMA LT.VICENTE AA, CAROLINO MI, SOUSA MC, GINJA C, SILVA FS, MARTINEZ AM, VEGA-PLA JL, CAROLINO N, and GAMA LT (2008) Genetic diversity in native and commercial breeds of pigs in Portugal assessed by microsatellites. J Anim Sci 86(10):2496-507

VRTKOVÁ I., STEHLÍK L., PUTNOVÁ L., KRATOCHVÍLOVÁ L., FALKOVÁ L. (2012) Genetic structure in three breeds of pigs populations using microsatellite markers in the Czech Republic. Research in Pig Breeding, 6(2):83-87

WANG J Y, GUO JF, ZHANG Q, HU HM, LIN HC, WANG CH, ZHANG Y, and WU Y (2011) Genetic Diversity of Chinese Indigenous Pig Breeds in Shandong Province Using Microsatellite Markers. Asian-Aust. J Anim Sci **24(1)**:28–36

WEIR BS, and COCKERHAM CC (1984) Estimating F-Statistics for the analysis of population structure. Evolution **38**:1358-1370.

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