

## GENETIC CONSERVATION OF MICROSATELLITE SEQUENCES IN *SUIDAE*\*

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### Abstract

The aim of the study was to identify genetic conservation between several species of *Suidae* (*Sus scrofa domestica*, *Sus scrofa scrofa*, *Sus vittatus*, *Phacochoerus aethiopicus*) and *Tayassuidae* (*Tayassu tajacu*) families, using microsatellite sequences (SW983, SWC27, SW902, SW1430, SW2411, SW2623, SW2160) characteristic of the domestic pig (*Sus scrofa domestica*) genome. The results obtained in the present study made it possible to consider the analysed markers as conservative in the *Suidae* species (*Sus scrofa domestica*, *Sus scrofa scrofa*, *Phacochoerus aethiopicus*, *Sus vittatus*). Clear differences in relation to the *Suidae* species compared were shown by *Tayassu tajacu* (*Tayassuidae* family), in which no genetic conservation was found in any of the microsatellite sequences analysed.

**Key words:** *Suidae*, microsatellite DNA markers, comparative study of genomes

Interspecific comparative analyses of the genomes are based on the phenomenon of genetic conservation. This concerns groups of linked or syntenic genes that often have the same relationships even in taxonomically distant species (Botstein et al., 1980; Babicz et al., 2008), nucleotide sequences of genes coding for the same products in different animal species (Kozubska-Sobocińska et al., 2006), microsatellite sequences (Kacirek et al., 2001; Behl et al., 2002; Rejduch et al., 2003 a; Kozubska-Sobocińska et al., 2008 b) and chromosome banding patterns (Rejduch et al., 2003 b; Kozubska-Sobocińska et al., 2008 a).

Microsatellite DNA markers, identified in different animal species using comparative analysis, provide modern and precise tools to study genetic structure and variation in both domesticated and feral animals (Ellegren et al., 1994; Laval et al., 2000; Kacirek et al., 2001; Behl et al., 2002; Knoll and Putnova, 2002).

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## Material and methods

### Experimental material

For comparative analyses the following species of the *Suidae* were used: domestic pig (*Sus scrofa domestica*) of the Polish Large White breed (five boars and five sows), wild boar (*Sus scrofa scrofa*) (six animals), Vietnamese pot-bellied pig derived from the Asian wild pig (*Sus vittatus*) (two animals), wart hog (*Phacochoerus aethiopicus*) (two animals) and collared peccary (*Tayassu tajacu*) (one sow).

The blood samples were obtained from animals from the Experimental Stations of the National Research Institute of Animal Production, zoological gardens in Wrocław and Warszawa, farm of the University of Life Sciences in Lublin and forensic studies.

### Analytical methods

#### Isolation of DNA

DNA isolation from peripheral blood leukocytes was carried out according to Kawasaki (1990) as modified by Coppieters et al. (1992).

#### Amplification

Amplifications of the DNA isolated from individual animals were performed through PCR using fluorescently labelled primers for 7 microsatellite markers: *SW983*, *SWC27*, *SW902*, *SW1430*, *SW2411*, *SW2623*, *SW2160* selected from the <http://www.projects.roslin.ac.uk/pigmap> database (Table 1 and Figure 1), enabling simultaneous amplification of all the markers tested in a single reaction sample (multiplex-type PCR).

Table 1. Starter sequences for 7 microsatellite sequences: *SWC27*, *SW983*, *SW902*, *SW2160*, *SW2623*, *SW2411* and *SW1430*

Locus	Primers	
	Primer A (5'-3')	Primer B (5'-3')
<i>SWC27</i>	CCATTTTCCAAAAACATGGG	FAM-CTGAGACTGTGCTGCTCACTG
<i>SW983</i>	ATAATGCTGCTATGAACACTGTAGTG	FAM- GCAGTCCCCTCTTAGG-TATATATCC
<i>SW902</i>	CTTGCCCTCAAAGAGTTGTAAGG	FAM- ATCAGTTGGAAATGATGGCC
<i>SW2160</i>	TTTCTCAGCAATCTGATTGGG	TAMRA-TCTGCTTTTTTCCCCCTG
<i>SW2623</i>	GATTCCTCTGCTCGAGATG	TAMRA-TCGGAGAATGAGGTAGCTG
<i>SW2411</i>	TTCCTATTCTGTCTGCCTTG	JOE-CCTGGACTCATTCTTGCTTTG
<i>SW1430</i>	AGGACTCAGAGAACAGAGGTGG	JOE- TGTTACACCTTGGCAGATTCC

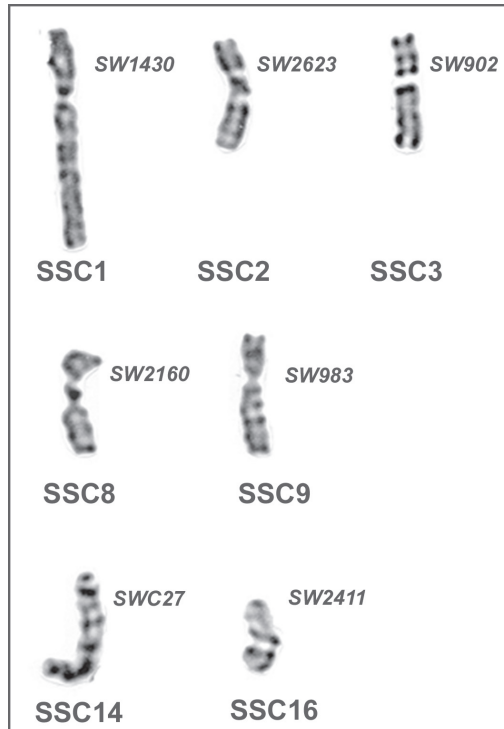


Figure 1. Chromosomal location of microsatellite markers: *SW1430*, *SW2623*, *SW902*, *SW2160*, *SW983*, *SWC27* and *SW2411* in *Sus scrofa domestica*

PCR reaction was performed according to the PCR-Protocol under the following conditions: starting denaturation at 93°C for 10 minutes, 35 cycles of denaturation at 93°C for 30 seconds, annealing at 60°C for 30 seconds, extension at 72°C for 1 minute, final extension at 72°C for 60 minutes. In the PCR reaction we used the following components: 20 ng double-stranded DNA, buffer for PCR reaction, mixture of dNTP deoxynucleotides (1.25 mM for each nucleotide), DNA Ampli Taq Gold polymerase (5 units/ $\mu$ l), deionized water and 2.5 pmol of each starter sequence. The PCR reaction products were subjected to vertical electrophoresis in 4% denaturing polyacrylamide gel in an ABI PRISM 377 DNA sequencer. The size of the analysed DNA fragments was determined in base pairs using Genotyper 2.1 software (Applied Biosystems).

## Results

The results of cross-species amplification in 7 microsatellite loci (*SW983*, *SWC27*, *SW902*, *SW1430*, *SW2411*, *SW2623*, *SW2160*) of *Suidae* (domestic pig, wild boar, Vietnamese pot-bellied pig, wart hog) and *Tayassuidae* (collared peccary) families are presented in Table 2.

Table 2. Comparison of microsatellite DNA sequences in species of *Suidae* and *Tayassuidae* families

Marker	Range of alleles (bp) / No. of alleles				Collared peccary ( <i>Tayassu tajacu</i> )
	Domestic pig ( <i>Sus scrofa domestica</i> )	Wild boar ( <i>Sus scrofa scrofa</i> )	Vietnamese pot-bellied pig ( <i>Sus vittatus</i> )	Wart hog ( <i>Phacochoerus aethiopicus</i> )	
SW983	109-123 / 4	109-123 / 4	117 / 1	109-123 / 3	-
SWC27	149-165 / 5	149-165 / 3	161 / 1	149-165 / 2	-
SW902	189-203 / 5	189-203 / 4	190-198 / 2	189-204 / 2	-
SW1430	152-178 / 5	152-178 / 4	178 / 1	164-168 / 2	-
SW2411	188-206 / 4	188-206 / 3	211 / 1	211-213 / 2	-
SW2623	132-142 / 5	132-142 / 5	129 / 1	132-142 / 2	-
SW2160	175-187 / 5	175-187 / 4	-	175-187 / 2	-

The results obtained showed a conservative nature of all 7 microsatellite DNA sequences analysed in 3 *Suidae* species: *Sus scrofa domestica*, *Sus scrofa scrofa*, *Phacochoerus aethiopicus*. Six of these markers (SW983, SWC27, SW902, SW1430, SW2411, SW2623) were also classified as conservative in *Sus vittatus* of the *Suidae* family. Clear differences in relation to the species compared were shown by *Tayassu tajacu* (*Tayassuidae* family), in which no genetic conservation was found in any of the 7 analysed microsatellite DNA sequences, characteristic of the domestic pig genome.

## Discussion

*Suidae* family includes three subfamilies: *Suinae*, *Phacocherinae* and *Babyroussinae*, of which *Suinae* (4 species compared: *Sus scrofa domestica*, *Sus scrofa scrofa*, *Phacochoerus aethiopicus*, *Sus vittatus*) was most strongly represented in our study. For comparative analyses we also used *Tayassu tajacu*, a species once classified as *Suidae* (*Tayassuinae* subfamily) and now belonging to the *Tayassuidae* family (Grubb, 1993).

The analysis of genetic conservation based on comparative tests of G-bands on chromosomes, performed in four *Suidae* species (domestic pig, wild boar, Vietnamese pot-bellied pig, wart hog) showed homologies and homeologies for whole chromosomes or their arms. The genetic conservation shown at the banding pattern level, as well as differences in chromosome number between the species compared, with the same number of autosome arms (NF) indicate that the interspecific differences are due to Robertsonian translocations. This high similarity of karyotypes confirmed the close phylogenetic relationship between *Sus* and *Phacochoerus* species (Rejduch et al., 2003 b; Kozubska-Sobocińska et al., 2008 a).

Genetic conservation of heterosomes in *Suidae* enabled interspecific *in situ* hybridization (FISH), in which porcine molecular probes were used to identify sex chromosomes in metaphase preparations of the wild boar, peccary and wart hog (Babicz et al., 2008).

Genetic characterization of *Suidae* based on microsatellite DNA sequences is conducted by many research centres in the world (Ellegren et al., 1994; Korwin-Kossakowska et al., 1998; Behl et al., 2002). These studies are performed mainly with the *Sus scrofa domestica* population and rarely concern wild species such as *Sus scrofa strofa*, *Phacochoerus aethiopicus*, *Sus vittatus* or *Tayassu tajacu* (Rejduch et al., 2003 a; Rejduch et al., 2003 b).

The results obtained in the present study make it possible to consider the analysed markers, characteristic of the domestic pig genome, as conservative in the analysed species (*Sus scrofa domestica*, *Sus scrofa scrofa*, *Phacochoerus aethiopicus*, *Sus vittatus*) of *Suidae*. Clear differences in relation to the *Suidae* species compared were shown by *Tayassu tajacu*, (*Tayassuidae* family), in which no genetic conservation was found in any of the 7 microsatellite DNA sequences analysed (*SW983*, *SWC27*, *SW902*, *SW1430*, *SW2411*, *SW2623*, *SW216*).

Considering that the species compared were represented by small groups of animals (*Phacochoerus aethiopicus*, *Sus vittatus*) or even single animal (*Tayassu tajacu*), the results obtained offer a basis for determining genetic conservation at the level of microsatellite sequences in the *Suidae* family, but cannot be used to characterize genetic variation of populations, for which the polymorphism of genetic markers has to be determined (Rejduch et al., 2003 a).

The microsatellite DNA markers identified in *Suidae* could be used as a precise tool to study the genome of both domesticated and wild animals belonging to this family (Laval et al., 2000; Kacirek et al., 2001; Behl et al., 2002; Babicz et al., 2008).

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### **Konserwatyzm genetyczny sekwencji mikrosatelitarnych u *Suidae***

#### STRESZCZENIE

Celem badań była identyfikacja konserwatyizmu genetycznego u kilku gatunków z rodziny *Suidae* (*Sus scrofa domestica*, *Sus scrofa scrofa*, *Sus vittatus*, *Phacochoerus aethiopicus*) i *Tayassuidae* (*Tayassu tajacu*), przy wykorzystaniu sekwencji mikrosatelitarnych (*SW983*, *SWC27*, *SW902*, *SW1430*, *SW2411*, *SW2623*, *SW2160*) charakterystycznych dla genomu świni domowej (*Sus scrofa domestica*).

Uzyskane wyniki pozwoliły na uznanie analizowanych markerów za konserwatywne u gatunków należących do *Suidae* (*Sus scrofa domestica*, *Sus scrofa scrofa*, *Phacochoerus aethiopicus*, *Sus vittatus*). Od porównywanych gatunków z rodziny *Suidae* wyraźnie różnił się *Tayassu tajacu*, należący do rodziny *Tayassuidae*, u którego nie stwierdzono konserwatyizmu genetycznego żadnej z analizowanych sekwencji mikrosatelitarnych.