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Cross Species Amplification of Coat Colour Genes in Nutria (*Myocastor coypus* Mol)*

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In this study, nutria-specific DNA fragments were amplified with cross-species primers for parts of coat colour genes (*MC1R*, *ASIP*, *TYRP-1*, *c-KIT*) conserved across a number of species. Twenty-three nutria-specific DNA fragments were generated using cross-species PCR (26.7% amplification success rate). Certain limitations of this approach were encountered, including strong mismatching in the primer binding sites. Sequences of 12 nutria-specific PCR products aligned in the BLASTx option showed homology with known protein sequences. Obtained sequences were submitted to GenBank with accession number KC758969 for *MC1R*, KF511655 for *ASIP*, KF511656 for *TYPR-1* and KU831489 for *c-KIT*. The highest homology for all translated queries was obtained with protein sequences belonging to species of the rodent family. Sequences of proteins encoded by genes involved in environmental adaptation (coat colour genes in rodents) were found to be strongly conserved among species.

Key words: nutria, cross-species, amplification, coat colour.

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Cross species amplification may be used to find sequences of interesting genes in a species for which no DNA sequence information is available. Efficient cross species primers can be used to develop genetic marker maps of closely related species for which the genome sequences have not yet been determined. Cross species amplification is also a routine approach in molecular phylogenetic studies in which sequence comparison of multiple taxa is applied to determine relationships and even to describe new taxonomical groups (NESI *et al.* 2015; WANG *et al.* 2015; MAROJU *et al.* 2016). A common approach for cross species amplification is to use PCR primers designed for conserved parts of a gene (mostly exonic sequences) in a number of more or less divergent species (PARKER *et al.* 2001; AITKEN *et al.* 2004; HOUSLEY *et al.* 2004). A limitation of this method is the unpredictable likelihood that the designed primers will amplify a homologous target in another species. The efficiency of the cross species primers depends mostly on the evolutionary distance, meaning that closer phylogenetic relationships improve the rate of cross species amplification. For example, PRIMMER *et al.* (2005) estimated that 50% of the primers designed for avian species worked for species diverged by 11 million years in passerines, and by 23 million years in non-passerines. Because of the greater chance of nucleotide mismatches at primer bind-

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ing sites between different species, the easiest way to design an efficient primer is to use parts of the genome conserved across a number of species (HOUSLEY *et al.* 2006). A range of available bioinformatics tools can help optimise the design of such primers. The most common method of searching for homological DNA and protein sequences is the BLAST tool option (Basic Local Alignment Search Tool) available on the website of NCBI GenBank. Comparative analysis of nucleotide sequences (BLASTn) can help identify the origin of an amplified fragment based on its homology with known DNA sequences in GenBank (ALTSCHUL *et al.* 1990). A search using translated DNA queries (BLASTx) can help to determine the nucleotide positions of putative coding regions in the amplified DNA sequence and the type of putative encoded protein (GISH & STATES 1993). Translated queries can then be compared with known amino acid sequences annotated in protein databases (BLASTp or PSI-BLAST) (ALTSCHUL *et al.* 1997) in order to find homology with known protein families and to describe their potential function (MARCHLER-BAUER *et al.* 2002).

In our study we used the cross species approach to amplify genes playing key roles in coat colour determination in nutria. A genomic reference sequence for this species is unavailable. Therefore our results are interesting in relation to the development of polymorphic DNA markers of coat colour traits used in nutria breeding.

Material and Methods

Animals and DNA extraction

Blood was collected from animals representing all nutria coat colour variants: standard, dominant black, ambergold, sable, pearl, greenland, pastel, white mark and snow white reared on farms in Poland under the control of the National Animal Husbandry Center. Blood samples were taken from the *vena saphena* into tubes containing K2EDTA as coagulant. Samples were stored at -20^{o}C for further analyses. DNA was extracted from blood using the Wizard Genomic DNA Purification kit (Promega) according to the manufacturer's protocol.

Primer design

Primers were designed using Primer3 (ROZEN & SKALETSKY 2000). Sequences of *ASIP*, *c-KIT*, *MC1R*, *TYR* and *TYRP-1* genes for a group of mammalian species were used in homology search through sequence alignments. Comparative analysis employed the MEGABLAST option and primers were designed in regions showing the highest homology. During cross species primer design, we prioritized sequencing records available for rodents (*Mus musculus*, *Rattus norvegicus*, *Cavia porcellus*) and lagomorphs (*Oryctolagus cuniculus*). If sequence alignment failed in rodents, we then retrieved sequences of other mammals (*Homo sapiens, Bos taurus, Equus caballus, Sus scrofa, Felis catus, Canis familiaris, Vicugna pacos*). The regions of interest included exonic sequences and 3' downstream and 5' upstream sequences of genes. Primer pairs were designed based on homology between multiple sequences or a single sequence of the given species. We designed and tested 86 primer pairs. Based on the alignment results we designed 11 primer pairs for *ASIP*, 46 for *c-KIT*, 10 for *MC1R*, 1 for *TYR*, and 18 for *TYRP-1*. Five of the above primer pairs were modified by adding forward and reverse M13 universal primer sequences to the 5' ends of original primers in order to improve the cycle sequencing results. Primers were designed for amplification of products no longer than 800 bp in length, with GC content in primer sequences at or above 50%, maximum melting temperature (Tm) 60^{o}C, and the incorporation of G or C clamp at the 3' end of the primer sequence.

PCR conditions

DNA was amplified in 20 µl reaction mixtures containing 0.2 µl 1 U HotStar Taq Polymerase (Quiagen), 2 µl 10x PCR Buffer (Quiagen), 2.4 µl 0.3 nM dNTP (Life Technologies), 2 µl 1 uMol primers (FOR + REV mixture), and 1 µl DNA (20-100 ng) using touchdown PCR thermal profile (DON *et al.* 1991) with annealing temperature (Ta) of 66^{o}C down to 55^{o}C or Ta of 55^{o}C down to 43^{o}C. Amplification products were electrophoresed on 2% agarose gel (LONZA). Gels were visualised using the Photo Doc-ItTM Imaging System (UVP).

Sequencing

PCR products were purified using Exonuclease I and Shrimp Alkaline Phosphatase (Fermentas). Sanger sequencing was performed using forward and reverse primers and BigDye Terminator chemistry (Applied Biosystems) according to the manufacturer's protocol. Sequencing products were electrophoresed on the ABI3130xl automated capillary sequencer (Applied Biosystems). Five animals representing each coat colour variants were sequenced.

Bioinformatic analysis

Raw sequencing data were evaluated in FinchTV (www.geospiza.com/finchtv). Forward

and reverse sequences were evaluated and arranged using GeneDoc (NICHOLAS *et al.* 1997). BLAST (ALTSCHUL *et al.* 1990) was used to determine sequence identity through sequence alignment of the investigated parts of selected genes across species. BLASTx was used to identify the coding parts of the obtained sequences based on homology with other amino acid sequences available in GenBank. A similar analysis was performed using BLASTn for comparison of nucleotide sequences. PsiBLAST was used to predict protein function.

Results

Table 1 describes primer sequences that amplified *Myocastor coypus* coat colour gene fragments. The curated sequences were aligned in BLASTx for prediction of homology with known genes. PsiBLAST was used for prediction of protein function based on affiliation to a particular protein family (Table 2).

The homology search against nucleotide and protein databases using curated sequences of nutria-specific PCR products allowed the identification of homology to whole exonic sequences or parts of coding regions of known coat colour genes. These included fragments of the 1st and 3rd exon of *ASIP*, the whole coding region of *MC1R*, the 4th exon of *TYRP-1*, and five exons of *c-KIT*. The use of cross species primers designed to amplify part of the *TYR* gene in nutria resulted in homology with Dtyrosyl-tRNA(TYR) deacylase 2-like protein. The sequences of nutria-specific DNA fragments were submitted to the GenBank database – *MC1R* (accession number KC758969), *ASIP* (accession number KF511655), *TYRP-1* (accession number KF511656) and *c-KIT* (KU831489).

Discussion

Available reference genomic sequences from species are important sources of information for extracting conserved parts of important genes in unknown mammalian genomes (MATHÉ *et al.* 2002). Regions of high homology can be identified by aligning nucleotide sequences between multiple mammalian species. Then primers can be designed to amplify the DNA of a species for which there is no prior knowledge of the sequence of the gene under investigation. The sequencing results of the amplified products are then aligned to all sequence records in public nucleotide databases such as GenBank. The exons and all other features of a gene sequence for a given species were determined by bioinformatics analysis (BLAST option). A limitation of the use of cross species primers is the possibility of amplifying pseudogenes, random fragments of the genome or other genes unlike those of the desired ones in the targeted species. In our study the primers designed using a conservative region of the *Cavia porcellus TYR* gene gave unintended amplification of a part of the Dtyrosyl-tRNA(TYR) deacylase 2-like gene, and 11 PCR products amplified by cross species designed primer pairs resulted in amplification of random DNA fragments.

The presence of polymorphisms at primer-binding sites, and template-primer mismatches (HOUSLEY *et al.* 2006), can hinder amplification of target-species DNA. Making certain modifications of the PCR protocol (e.g. changing the melting temperature of nucleic acids, or using Touchdown PCR proposed by DON *et al.* 1991) can increase the amplification yield of DNA from cross species primers.

Using cross species PCR primers, we were able to amplify nutria DNA fragments of putative *ASIP*, *TYRP-1* and *c-KIT* genes, and also the full coding region of *MC1R*, *ASIP*, *TYR*, *TYRP-1* and *c-KIT*, being well conserved among different species. These genes are known for their role in melanogenesis which is a common process among different mammalian species. Because of this, the well conserved structure of our target loci could be substantial for the great homology of encoded proteins influencing the similarities of melanogenesis in mammals (TAKEUCHI *et al.* 1996; MARKLUND *et al.* 1997; SCHMIDT-KÜNTZEL *et al.* 2005; ŚWITOŃSKI *et al.* 2013). An exception in our study was the unexpected amplification of a part of the Dtyrosyl-tRNA(TYR) deacylase 2-like gene using cross species primers primarily designed for the *TYR* coat colour gene.

Of the 86 primer pairs designed, 23 produced PCR products that were sequenced. The choice of the most conserved parts of the target region is an important factor in improving the efficiency of cross species primer design. LYONS *et al.* (1997) designed primers using only conserved sequences and obtained 35-52% successful amplifications. Another important factor is phylogenetic proximity between the species whose DNA is to be amplified and the species whose sequence is used for primer design. Using phylogenetic distance between species as the main criterion for primer design, JIANG *et al.* (1998) achieved 36.4-80.9% successful PCR amplifications. HOUSLEY *et al.* (2006) designed primers based on human sequences and successfully amplified DNA from 11 animals: 46.9-64.3% of the PCR reactions were positive, and varied depending on the phylogenetic relationships, as in JIANG *et al.* (1998). ZĄBEK (2010) amplified coding regions of a group of *E. caballus* genes using human genomic se-

Table 1

Myocastor coypus specific PCR products aligned with the BLASTx option

Gene	Exon	Species used in alignment	Starters sequence	Ta	Predicted length (bp)
ASIP	1	*Felis catus, Canis familiaris, Vicugna pacos, Bos taurus, Equus cabalus, Cavia porcellus, Rattus norvegicus, Oryctolagus cuniculus, Sus scrofa*	F: CCTTCTCTGTCCCATTCAGG R: CCACAATAGAGACAGAAGGGAAA	46°C	179
	3	*Felis catus, Vicugna pacos, Bos taurus, Equus cabalus, Cavia porcellus*	F: GCAGAAAAAGGCTTCGATGA R: TCAGCAGGTGAGGTTGAGC	50°C	124
c-KIT	2	*Felis catus, Canis familiaris, Vicugna pacos, Bos taurus, Equus cabalus, Cavia porcellus, Rattus norvegicus, Oryctolagus cuniculus*	F: TTTTCTTGGCAGGCACTTCT R: TTTACCTCTAACAAACACATAAATGGA	61°C	287
	7	*Felis catus, Canis familiaris, Vicugna pacos, Bos taurus, Equus cabalus, Cavia porcellus, Rattus norvegicus, Oryctolagus cuniculus, Sus scrofa*	F: TCCTTTGTAGATATGTGAACGAACTT R: CGTAAACTTAAATGTCACGGAAG	50°C	120
	8	*Felis catus, Canis familiaris, Vicugna pacos, Bos taurus, Equus cabalus, Cavia porcellus, Rattus norvegicus, Oryctolagus cuniculus, Sus scrofa*	F: CAGCAAAACCAGAAATCCTGA R: AAAATAATCCTCTCACCTCTGCTC	54°C	124
	14	*Felis catus, Canis familiaris, Vicugna pacos, Bos taurus, Equus cabalus, Cavia porcellus, Rattus norvegicus, Oryctolagus cuniculus,*	F:GGCCCACCCTGGTCATTA R: CCAGGAAGACTCCTTTGAATG	62°C	152
	17	*Felis catus, Canis familiaris, Vicugna pacos, Bos taurus, Equus cabalus, Cavia porcellus, Rattus norvegicus, Oryctolagus cuniculus, Sus scrofa*	F: AGACTTGGCAGCCAGGAATA R: CTCACATTTCCTTTGACCACA	53°C	117
	18-19	*Felis catus, Canis familiaris, Vicugna pacos, Bos taurus, Equus cabalus, Cavia porcellus, Oryctolagus cuniculus, Sus scrofa*	F: GGCTCCCTGTGAAGTGGAT R: CTTACATTTCAGCAGGTGCAT	55°C	245
MC1R	1	*Sus scrofa*	F: ATGTCATGGACGTGCTCATCT R: CCAGAAAGAGGTTGACGTTCT	47°C	408
	1	*Ctenomys sp, Myocastor coypus*	F:GAGGCTTCTGGGTTCCCTC R:AGCGCAGTGTATAGAAGATGGA*	53°C	270
	1	*Ctenomys sp, Myocastor coypus*	F: TCCATCTTCTATACACTGCGCT* R: TCACCAGGAGCACAGCAG	62°C	332
TYR	3	*Cavia porcellus*	F: TGTTAAATGTGAAATTAAGTGAAACAG R: ATTAAGTGTGTGTAGGGTCCATTG	55°C	307
TYRP-l	4	*Felis catus, Canis familiaris, Vicugna pacos, Bos taurus, Equus cabalus, Cavia porcellus, Rattus norvegicus, Oryctolagus cuniculus, Sus scrofa*	F:CTCCCACGGGAAAATATGAC R: CGGCGTTATACCTCCTTAGC	48°C	186

* – starters designed on *Myocastor coypus MC1R* sequence obtained from amplified DNA using *Sus scrofa* primer pair. F – forward starter sequence; R – reverse starter sequence; Ta – annealing temperature.

quences and achieved 48% successfully amplified horse-specific DNA fragments. According to ZĄBEK (2010) and HOUSLEY *et al.* (2006), a primer-template mismatch higher than 3 nucleotides results in failure to produce a PCR product. We found that the nucleotide sequences of the investigated genes differed between species more than the alignment of the encoded proteins did. In our study, 23 sequences amplified by cross species primers were sequenced and aligned, resulting in a 26.7 % success rate. The sequences of 12 nutria-specific PCR products aligned (Table 1) with the BLASTx option showed homology with known protein sequences (Table 2). Among them, 6 DNA fragments were amplified using primers designed in conserved regions of coat colour genes. Their alignments were confirmed using the PSI-BLAST option (Table 2).

Table 2

Myocastor coypus specific PCR product homology with known protein sequences

Gene (Accession number)	Exon	Coding sequence region (bp)	Protein length (aa)	Protein family
ASIP (KF511655.1)	1	<83..>178	32	Agouti Superfamily
	3	<361..465	34	Agouti Superfamily
c-KIT (KU831489)	2	<57.. 233>	59	–
	7	<337..405	23	–
	8	<514..>540	9	–
	14	<705..>806	34	Protein kinase superfamily
	17	<909..968	20	Catalytic domain of the protein tyrosine kinase
	18,19	1100..1154, <1213..>1350	46	Catalytic domain of the protein tyrosine kinase
MC1R (KC758969.2)	1	<15..962>	315	7 transmembrane receptors (G-proteins family)
TYRP-1 (KF511656.1)	4	<26..>162	45	–

aa – Amino acid;
- Lack of domain representing specific protein family.

In general the sequence data of single species was not enough to achieve an acceptable rate of cross species amplification. Even when a nutria-specific DNA fragment was amplified, its sequence was not homologous to the desired coding region. An exception was the effective amplification of the nutria *MC1R* fragment using primers designed based on the sequences of only two species, *Sus scrofa* and *Ctenomys sp*. The use of sequences from 5-8 species during primer design gave a higher probability of successful amplification. Some nutria-specific DNA fragments were obtained with primers designed based on sequences of highly divergent species. This was the case for *MC1R* gene amplification using primers designed based on the *Sus scrofa* sequence. *MC1R*s belong to the 7tm receptor family (7 transmembrane family receptors), showing high homology between species (ŚWITOŃSKI *et al.* 2013) and plays a role in adaptive quantitative variation in the wild (HOEKSTRA *et al.* 2006) as well as influences the dramatic variety of coat colour (GONÇALVES *et al.* 2012).

To increase the rate of cross species amplification, sequences encoding a highly conserved region of a protein can be used for primer design. The conserved subdomains play an important role in the catalytic function, either directly as components of the active site or indirectly by contributing to the formation of the active site through constraints imposed on secondary structure (HANKS *et al.* 1988). Because of this, these regions are characterized by structure highly conserved between species.

A conserved region is present in the *c-KIT* sequence. Exon 17 covers a highly conserved region of tyrosine kinases comprising the catalytic loop and parts of the activation loop (HUBBARD *et al.* 1994). Exons 17, 18 and 19 of this gene were found to be highly conserved (MARKLUND *et al.* 1998). Similarly, two exons obtained for *ASIP* showed high homology. These findings were confirmed using the PSI-BLAST option for *MC1R*, *ASIP* and *c-KIT* translated sequences (Table 2).

In this study we used homology with known nucleotide and protein sequences to identify DNA sequences specific to nutria. BLASTn was used for coding region determination. Analysis of the translated nucleotide queries helped determine the putative functions of the investigated sequences through a search against the protein family database (Table 2). The highest homology for all of the translated queries was obtained with *Oryctolagus cuniculus, Heterocephales glauber, Peromyscus maniculatus, Cavia porcellus, Mus musculus, Rattus norvegicus* and *Ctenomys sp*. All these species except *Oryctolagus cuniculus* belong to the rodent family, which includes nutria. Our results support the findings of JIANG *et al.* (1998) and HOUSLEY *et al.* (2006) showing that phylogenetic distance is

one of the most important factors in successful design of primers suitable for cross species amplification.

Despite the uniqueness of some species' DNA sequences in the investigated coding regions (expressed as different rates of cross species amplification), the sequences of the encoded proteins are highly conserved, playing a crucial role in environmental adaptation (coat colour genes in rodents). Therefore the occurrence of potential SNP variants of the investigated coding sequences can be used in phylogenetic analyses (HALL 2005; SHIMANDA *et al.* 2009, BEDFORD & HOEKSTRA 2015) to describe putative allelic variants of particular coat colour traits of rodents. HOEKSTRA *et al.* (2006) described polymorphism within beach mice *MC1R* which did not contribute to different colour in beach mice living in different environment. In nutria *MC1R* we identified polymorphism (MIGDAŁ *et al.* 2014) which was present in a population of dominant black, pastel, sable. Moreover analysis of the obtained sequences of the *ASIP* gene revealed a SNP linked with pearl colour (unpublished data). Our findings are consistent with the results obtained by LINNEN *et al.* (2013) who linked multiple colour variation with multiple mutation within the *ASIP* gene in *Peromyscus maniculatus*.

Our results demonstrate the ease and reliability of cross species amplification for annotation of genes from unsequenced genomes, nutria in our case: the use of primers designed in conserved parts of genes can increase the rate of cross species amplification.

References

AITKEN N., SMITH S., SCHWARZ C., MORIN P.A. 2004. Single nucleotide polymorphism (SNP) discovery in mammals: a targeted-gene approach. Mol. Ecol. **13**: 1423-1431.

ALTSCHUL S.F., GISH W., MILLER W., MYERS E.W., LIPMAN D.J. 1990. Basic local aligment search tool. J. Mol. Biol. **215**: 403-410.

ALTSCHUL S.F., MADDEN T.L., SCHÄFFER A.A., ZHANG J., ZHANG Z., MILLER W., LIPMAN D.J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucl. Acids Res. **25**: 3389-402.

BEDFORD N.L., HOEKSTRA H.E. 2015. Peromyscus mice as a model for studying natural variation. eLIFE **4**: eO6813.

DON R.H., COX P.T., WAINWRIGHT B.J., BAKER K., MATTICK J.S. 1991. 'Touchdown' PCR to circumvent spurious priming during gene amplification. Nucl. Acids Res. 25: 4008.

GONÇALVES G.L., HOEKSTRA H.E., FREITAS T.R.O. 2012. Striking coat colour variation in tuco-tucos (Rodentia: Ctenomyidae): a role for the melanocortin-1 receptor? Biol. J. Linnean Soc. **105**: 665-680.

GISH W., STATES D.J. 1993. Identification of protein coding regions by database similarity search. Nat. Genet. **3**: 266-272.

HALL B. 2005. Comparison of the accuracies of several phylogenetic methods using protein and DNA sequences. Mol. Biol. Evol. **22**: 792-802.

HANKS S.K, QUINN A.M., HUNTER T. 1988. The Protein Kinase Family: Conserved Features and Deduced Phylogeny of the Catalytic Domains. Science **241**: 42-52.

HOEKSTRA H.E., HIRSCHMANN R.J., BUNDEY R.A., INSEL P.A.,CROSSLAND J.P. 2006. A single amino acid mutation contributes to adaptive beach mouse color pattern. Science **313**: 101-104.

HOUSLEY D.J., RITZERT E., VENTA P.J. 2004. Comparative radiation hybrid map of canine chromosome 1 incorporating SNP and indel polymorphisms. Genomics **84**: 248-264.

HOUSLEY D.J., ZALEWSKI Z.A., BECKETT S.E., VENTA P.J. 2006. Design factors that influence PCR amplification success of cross-species primers among 1147 mammalian primer pairs. BMC Genomics **9**: 253.

HUBBARD S.R., WEI L., ELLIS L., HENDRICKSON W.A. 1994. Crystal structure of the tyrosine kinase domain of the human insulin receptor. Nature **372**: 746-754.

JIANG Z., PRIAT C., GALIBERT F. 1998. Traced orthologous amplified sequence tags (TOASTs) and mammalian comparative maps. Mamm. Genome **9**: 577-587.

LINNEN C.R., POH Y.P., PETERSON B.K., BARRETT R.D., LARSON J.G., JENSEN J.D., HOEKSTRA H.E. 2013. Adaptive evolution of multiple traits through multiple mutations at a single gene. Science **339**: 1312-1316.

LYONS L.A., LAUGHLIN T.F., COPELAND N.G., JENKINS N.A., WOMACK J.E., O'BRIEN S.J. 1997. Comparative anchor tagged sequences (CATS) for integrative mapping of mammalian genomes. Nat. Genet. **15**: 47-56.

MARCHLER-BAUER A., PANCHENKO A.R., SHOEMAKER B.A., THIESSEN P.A., GEER L.Y., BRYANT S.H. 2002. CDD: a database of conserved domain alignments with links to domain three-dimensional structure. Nucl. Acids Res. **30**: 281-283.

MATHÉ C., SAGOT M.F., SCHIEX T., ROUZÉ P. 2002. Current methods of gene prediction, their strengths and weaknesses. Nucl. Acids Res. **30**: 4103-4117.

MARKLUND L., MOLLER M.J., SANDBERG K., ANDERSSON L. 1997. A missense mutation in the gene for melanocyte-stimulating hormone receptor (*MC1R*) is associated with the chestnut coat color in horses. Mamm. Genome **7**: 895-899.

MARKLUND S., KIJAS J., RODRIGUEZ-MARTINEZ H., RÖNNSTRAND L., FUNA K., MOLLER M., LANGE D., EDFORS-LILJA I., ANDERSSON L. 1998. Molecular Basis for the Dominant White Phenotype in the Domestic Pig. Genome Res. **8**: 826-833.

MAROJU P.A., YADAV S., KOLIPAKAM V., SINGH S., QURESHI Q., JHALA Y. 2016. Schrodinger's scat: a critical review of the currently available tiger (*Panthera Tigris*) and leopard (*Panthera pardus*) specific primers in India, and a novel leopard specific primer. BMC Genet. **9**: 37.

MIGDAŁ Ł., ZĄBEK T., MIGDAŁ A., ŁAPIŃSKI S., MAJ D., BIENIEK J., FORNAL A., BUGNO-PONIEWIERSKA M. 2014. Effect of melanocortin 1 receptor (*MC1R*) polymorphism on coat colour variation in nutrias (*Myocastor coypus Mol.*). Anim. Genet. **45**: 909-911.

NESI N., JACOBS D.S., FELDHEIM K., BISHOP J.M.. 2015. Development and characterization of 10 microsatellite markers in the Cape horseshoe bat, Rhinolophus capensis (*Chiroptera, Rhinolophidae*) and cross-amplification in southern African Rhinolophus species. BMC Res. Notes **8**: 477.

NICHOLAS K.B., NICHOLAS H.B. Jr, DEERFIELD D.W. II 1997. GeneDoc: Analysis and Visualization of Genetic Variation. Embnew. News: **4**: 14.

PARKER H.G., YUHUA X., MELLERSH C.S., KHAN S., SHIBUYA H., JOHNSON G.S., OSTRANDER E.A. 2001. Meiotic linkage mapping of 52 genes onto the canine map does not identify significant levels of microrearrangement. Mamm. Genome **12**: 713-718.

PRIMMER C.R., PAINTER J.N., KOSKINEN M.T., PALO J.U., MERILA J. 2005. Factors affecting avian cross-species microsatellite amplication. J. Avian Biol. **36**: 348-360.

ROZEN S., SKALETSKY H.J. 2000. Primer3 on the WWW for general users and for biologist programmers. (In: Bioinformatics Methods and Protocols: Methods in Molecular Biology. Krawetz S., Misener S. Eds, Humana Press, Totowa, NJ): 365-386.

SCHMIDT-KÜNTZEL A., EIZIRIK E., O'BRIEN S.J., MENOTTI-RAYMOND M. 2005.Tyrosinase and Tyrosinase Related Protein 1 Alleles Specify Domestic Cat Coat Color Phenotypes of the albino and brown Loci. J. Hered. **96**: 289-301.

SHIMADA T., SATO J.J., APLIN K.P., SUZUKI H. 2009. Comparative analysis of evolutionary modes in *MC1R* color gene in wild mice and mustelids. Genes Genet. Syst. **84**: 225-231.

ŚWITOŃSKI M., MANKOWSKA M., SALAMON S. 2013. Family of melanocortin receptor (*MCR*) genes in mammals-mutations, polymorphisms and phenotypic effects. J. Appl. Genet. **54**: 461-72.

TAKEUCHI S., SUZUKI H., HIROSE S., YABUUCHI M., SATO C., YAMAMOTO H., TAKAHASHI S. 1996. Molecular cloning and sequence analysis of the chick melanocortin 1-receptor gene. Biochim. Biophys. Acta **1306**: 122-126.

WANG C., ZHANG S.J., DU X.Y., XU Y.M., HUO X.Y., LIAO L.F., CHEN Z.W. 2015. Development of novel DNA markers for genetic analysis of grey hamsters by cross-species amplification of microsatellites. Genet. Mol. Res. **13**: 14339-47.

ZĄBEK T. 2010. Amplification and analysis of *Equus caballus* DNA sequences, the homologues of other mammalian species. Roczniki Naukowe Zootechniki: Monografie i Rozprawy. (In Polish with English Summary).