HABILITATION THESIS SUMMARY

Part I. Academic achievements and scientific research activity

Professional and academic activity

My academic studies started with the graduation in 1999 of License in Biochemistry at the University of Bucharest, Faculty of Biology, followed by Master of Biochemistry and Molecular Biology within the same institution, in 2004. Since 2003, I have been following doctoral studies at the Faculty of Biology, University of Bucharest for a period of four years. The public defence for my PhD thesis took place in 2007, and since March 2008 I received the title of Doctor of Sciences, in Biology domain.

My professional debut took place in year 2000 when I was employed as teacher for two years. Since 2005, I have been working in the Department of Biochemistry and Molecular Biology, Faculty of Biology, University of Bucharest, where I occupied the positions of assistant professor, lecturer and associate professor. The didactic activity was complex, including courses, practical activities, guiding bachelor and dissertation theses, or supervision of PhD students from the Doctoral School of Biology and Ecology at the University of Bucharest.

Scientific research activity

My scientific activity started at the Department of Biochemistry and Molecular Biology, Faculty of Biology, University of Bucharest were I was hired in 2003 as a research assistant. In the following years I was promoted to scientific researcher, scientific researcher III and scientific researcher II (my current position).

As a result of my work, I managed to win three national research projects as Coordinator and three national research projects as Responsible partner in the competitive system. At the same time, I participated as a member of the research team at another 20 research projects, from which five are international and 15 national. The constant scientific work led to the publication of 5 books, 6 book chapters, 25 articles in ISI journals, 73 articles in BDI indexed journals, 6 articles in conference volumes, 16 abstracts at conferences outside the country, and 89 abstracts at conferences organized in Romania. All of these publications add up to 361 citations and the Hirsch Index is 9 (according to Google Academic). The scientific value of the elaborated works

is highlighted by their acceptance for publication in specialized journals from the main scientific flux, as well as their presentation at scientific symposiums or congresses and conferences (national or international).

Contributions to the development of scientific research directions

The main research areas that I have contributed so far are:

1. Molecular methods useful in selection assisted by markers in local breeds and fish species of economic interest. i. Developing molecular methods for detecting genetic diseases; ii. Identification and study of DNA markers correlated with different morpho-productive traits; iii. Determination the expression of genes correlated with increased productivity and efficiency of livestock and aquaculture activities.

2. Identification of pure species and hybrids using DNA markers. i. Identification of interspecific hybrids in fish; ii. Species identification using molecular techniques.

3. Molecular phylogeny and phylogenetic studies in endangered local breeds and species using nuclear and mitochondrial DNA markers. i. Studies of biodiversity and molecular phylogeny in local breeds; ii. Studies of biodiversity, phylogeography and molecular phylogeny of endangered species from Romanian fauna.

1. Molecular methods useful in marker assisted selection for local breeds and fish species of economic interest

Marker Assisted Selection (MAS) is a more recent concept used in all areas of agriculture, which refers to an indirect, non-traditional selection process based on molecular markers (DNA, RNA, proteins) associated directly or indirectly with certain traits of interest such as productivity, resistance to disease, or various stress factors. Currently, information about molecular markers is essential in the improvement process, along with traditional data from phenotypes analysis. The advantage of using genetic data is that they are very precise and their use is not limited by factors such as gender, age or number of individuals.

i. Developing molecular methods for detecting genetic diseases

Detecting DNA markers associated with various genetic diseases is extremely important from an economic point of view, allowing breeders to remove the affected animals from livestock and thus improve existing stocks.

a. Diagnosis of genetic diseases in horses. In the case of horse breeds, there are several genetic diseases for which we developed molecular screening methods to detect the affected animals. For SCID (Severe Combined Immunodeficiency) and JEB (Junctional Epidermolysis Bullosa) we developed two similar methods based on fragment analysis technique. Thus, the genome regions involved in the onset of the two diseases were amplified by PCR technique using fluorescent-labeled forward primers. Subsequently, the resulting amplification products were subjected to capillary electrophoresis with fluorescence detection in the presence of a molecular weight standard. Depending on the size of the amplified fragments, it can be established with certainty whether the analyzed individuals are healthy, carriers or affected. For the diagnosis of HYPP (Hyperkalemic Periodic Paralysis) we combined PCR-RFLP technique and analysis of fluorescently labeled fragments. The resulting products were analyzed by capillary electrophoresis with fluorescence detection, and depending on the size of the restriction fragments obtained, it can be established with certainty whether the individual under consideration is healthy, carrier or ill.

b. Diagnosis of genetic diseases in swine. In the case of swine breeds, Malignant Hyperthermia is a disease characterized by accelerating of skeletal muscle metabolism, muscle rigidity and rapid increase of body temperature, and its diagnosis was performed by the PCR-RFLP technique.

c. Diagnosis of genetic diseases in sheep. Regarding sheep, one of the main hereditary diseases is the Spider Lamb syndrome. Identification of carrier animals was performed by PCR-RFLP technique. Another extremely widespread disease in domestic sheep breeds is Scrapie. This is a transmissible endemic neurodegenerative disease produced by a transmissible agent from the prion group. Over time, various polymorphisms in the coding sequence of the sheep *PrP* gene have been identified, but only three of them have been shown to have a significant effect on the disease due to the amino acid changes generated in the protein sequence: in codon 136 a substitution that replace alanine with valine; in codon 154 arginine is replaced with histidine and in codon 117 glutamine in replaced with arginine or histidine.

Determination of genotypes susceptible to scrapie was performed by the Real-Time PCR technique followed by analysis of the melting curves generated after obtaining the amplicons. For the identification of mononucleotide polymorphisms, we used probes with sequences complementary to the polymorphic regions of the *PrP* gene.

d. Diagnosis of genetic diseases in cattle. Another category of domestic animals for which we developed molecular diagnostic methods were cattle. In this case, the investigations focused on two diseases with major economic impact: BLAD (Bovine Leukocyte Adhesion Deficiency) and DUMPS (Deficiency in Uridine Monophosphate Synthase). For both of them, the diagnosis of the specimens was performed using the PCR-RFLP technique. In the case of BLAD we amplified the genomic region involved in the disease using PCR technique and the resulting amplicons were digested with the *Taq I* restriction enzyme. In a similar way for DUMPS, a fragment containing the mutation site was amplified by PCR and the product obtained was digest with endonuclease *Ava I.*

All the diseases described so far support the usefulness of the implementation of the molecular methods in the veterinary diagnosis and the possibility of their use by the breeders for the purpose of controlling the health of the flocks. All these methods allow a rapid identification of the carriers which will lead to the elimination of the affected individuals from the stocks and ultimately the eradication of these diseases.

ii. Identification and study of DNA markers correlated with different morpho-productive traits.

Currently there are already identified hundreds of DNA polymorphisms correlated with different morpho-productive traits in all domestic animal species. For this reason, there is a gradual shift from phenotype-based selection to predominantly genotype-based selection. The most commonly used approaches for identifying and characterizing genes involved in the complex character determinism are the QTL (Quantitative Trait Loci) and the candidate gene method.

a. Analysis of genes associated with prolificacy in swine and sheep. Prolificacy is an essential feature in animal husbandry, with a major role in making production more efficient. In the case of swine and sheep, a whole range of genes associated with the increased number of products at each birth were identified. The development of molecular methods for their analysis will allow breeders to select genetically appropriate specimens.

In the case of swine, a first association between a candidate gene and the number of products was achieved in the case of α-estrogen receptor placed on chromosome 1. The presence of a mononucleotide polymorphism (T1665C) causes the appearance of two distinct alleles, A and B, and studies have shown a favorable association of allele B with prolificacy. Identification of the presence of allele B could be done using the PCR-RFLP technique.

Until now, more variations in productivity have been described in domestic sheep breeds, including ovulation and, implicitly, prolificacy. Several studies have shown that the ovulation rate and the number of products per birth can be adjusted by the interaction of several genes with major effects generic called fecundity genes (*Fec*). Thus, based on the identification of the genotypes present at these loci, selection programs can be conducted to increase the prolific level of sheep breeds. For screening the distribution of the *FecB* and $FecX^I$ gene alleles in sheep we used the PCR-RFLP technique.

b. Analysis of the loci involved in the determinism of the coat color in horses. In the case of horses, in special situations (e.g. the Lipizzaner breed), the selection of the specimens is done taking into consideration mainly the coat color. Thus, the black or chestnut individuals are removed from the selection process, while the grey ones are preserved. Therefore, for the determination of genotypes at the *Agouti* locus, we developed a technique that combines PCR amplification with capillary electrophoresis and fluorescent fragments detection. For determining the genotypes, the profile of the amplification products is analyzed. For the determination of genotypes at the *Extension* locus, the PCR-RFLP technique can be used. The primers flank the polymorphic region of the *MC1R* gene and the amplicons are digested with *Taq I* endonuclease.

c. Analysis of genetic markers associated with the quantity and quality of milk in sheep and cattle. The identification of genetic markers correlated with characteristics such as milk quality and quantity in cattle and sheep breeds is of major interest to the livestock sector. In order to easily differentiate between the genetic variants of the three major milk proteins types, we proposed an identification method based on the PCR-RFLP technique.

Among the genes associated with the different productive traits in sheep, β-lactoglobulin locus is the most study one. Using the same PCR-RFLP technique we were able to establish a method of differentiation between genotypes of β-lactoglobulin and αs1-casein encoding genes in sheep

iii. Determination of gene expression correlated with increased productivity and efficiency of animal breeding and aquaculture

A newer alternative to identifying the value of genes involved in the determinism of productive characters is represented by the analysis of gene expression. There is currently a relatively wide range of research techniques such as Real-Time PCR or Microarray.

a. Analysis of gene expression of leptin encoding genes and leptin receptor in swine. In swine, leptin is an important regulator of appetite, energetic metabolism and reproduction. The leptin

receptor gene is considered a genetic marker associated with body composition, growth rate, or obesity in domesticated swine breeds. Starting from the hypothesis that leptin and its receptor are molecules involved in obesity, the idea of conducting a study on the effects of expression levels of their coding genes in the adipogenesis process in swine has emerged. The comparative study of leptin (*lep*) and leptin receptor (*lepr*) genes expression levels was performed in several swine breeds specialized in meat production (Duroc, Belgian Landrace, Large White, Synthetic LS-345 and LSP-2000) and the Mangalitsa breed, specialized in fat production. The purpose of the researches was to correlate the levels of gene expression with the morpho-productive traits, as well as to compare the level of expression of the leptin receptor encoding gene in different tissues in the case of Mangalitsa breed.

Relative quantification of the *lep* gene expression was achieved by Real-Time PCR in the adipose tissue. The *gapdh* and *rpl32* genes were used as references. The expression was quantified for the adipose tissue comparatively for Mangalitsa, Duroc, Large White, Belgian Landrace, LS-345 and LSP-2000 breeds. In the case of the Mangalitsa, the expression was also analyzed comparatively between different tissues: liver, lung, spleen, kidney, heart, brain and skeletal muscle. The most important differences in relative expression were observed between Mangalitsa and Large White (about 6 times more expressed in the case of Mangalitsa breed), LSP-2000 (about 6.2 times higher) and LS-345 (about 6.5 times higher).

In the case of the leptin receptor gene expression, the lowest level was reported in the Belgian Landrace, while in the Mangalitsa and Large White were also observed low levels of expression compared to Duroc or LSP-2000 and LS-345. The highest level of expression appears on the Synthetic Line LS-345. After statistically comparation of the levels of relative leptin gene expression between Mangalitsa and other breeds, significant differences were found between Mangalitsa and Belgian Landrace/Synthetic Line LS-345; in the case of the other breeds the differences the expression were insignificant. Compared with Belgian Landrace the relative *lepr* gene expression level for the Mangalitsa is 3.7 times higher, and compared with LS-345, 2.6 times smaller.

In the case of relative expression of *lepr* gene in Mangalitsa at the level of different tissues, it was observed that the gene is expressed in all the analyzed tissues; the lowest level of relative expression was evidenced in the brain and the skeletal muscle, and the highest level in the lung. The results obtained suggested a leptin resistance in the case of the Mangalitsa breed. Thus,

although the level of relative gene expression is much higher, the specimens are leptin resistant, exhibiting increased accumulation of adipose tissue and a reduction in reproductive functions.

The results obtained in this study highlight the increased expression of the leptin gene in the Mangalitsa compared to other swine breeds specialized for meat production. Also, after analyzing the comparative results obtained for different tissues in the Mangalitsa, we can suggest the existence of a leptin resistance, which resolves with a very pronounced tendency for the accumulation of adipose tissue and with a prolificacy below average.

b. Identification of molecular markers useful for early sex determination in sturgeons. Early sex determination is a challenge of great importance in sturgeons' aquaculture, requiring the identification of effective solutions due to its major economic implications. Reducing age at which sex identification is possible would allow breeders to eliminate males, which would cause a significant decrease of food costs and maximize the space for female breeding, making aquaculture more efficient. So far, several approaches have been proposed to identify sex in sturgeons using genetic markers. The latest studies have been intent upon identifying candidate genes whose expression can be correlated with sex determinism and gonad development. Starting from the presented data and aiming to identifying a molecular method for early sex determination in sturgeons, a method with potential applications in aquaculture, two separate experiments were carried out.

The first study was conducted on *Acipenser stellatus* (stellate sturgeon) and was based on Real-Time PCR technique for comparative analysis of expression levels of candidate genes (*ar, dmrt1, sox9, wt1, foxl2, cyp17a, star, lh, igf1*) for males and females, in different organs. Analyzing the experimental data, it is noted that for gonads the *foxL2* does not show significant differences between females and males while the remaining genes have statistically significant differences. In males the expression is higher for all of the six genes analyzed. In the case of fin tissue, no statistically significant differences were observed between males and females. This analysis was conducted with purpose to possible discrimination between females and males without sacrificing specimens. When analyzing the kidney samples, the expression of the *foxL2, ar, dmrt1, sox9* and *star* genes was observed but no expression was detected for the *cyp17A1*. No statistically significant differences between males and females were observed in this case. In the liver, the *cyp17A1* gene expression was not detected, and no statistically significant differences between males and females were observed. Finally, in the case of white muscle, it is observed

the lack of expression of *cyp17A1* and *star*, while the expression of f*oxL2, ar, dmrt1* and *sox9* genes is present. Also here, differences in gene expression between males and females are insignificant. In conclusion, in the study of *Acipenser stellatus*, we observed different levels of *cyp17a1, ar, dmrt1,* and *sox9* gene expression in gonads for males and females suggesting that they are likely involved in testicular development and are potential markers useful for early sex determination in sturgeons. However, we did not report expression differences between males and females for any genes in fin or white muscle, tissues that can be harvested by non-invasive techniques, and could therefore be used to develop a molecular method for sex determination.

The second experiment analyzed an interspecific sturgeon hybrid, Best beluga. This is obtained by crossing Bester females with *Huso huso* (beluga sturgeon) males. The Bester is also an interspecific hybrid obtained by crossing female of *Huso huso* with males of *Acipenser ruthenus* (sterlet sturgeon). In the case of gonads, the expression of all six analyzed genes (*ar, dmrt1, sox9, wt1, foxl2, cyp17a, star, lh, igf1*) in males and females was observed. In the particular case of the *dmrt1* gene, we observed measurable expression only in the case of gonads, with no significant difference between the expression levels in males and females. The *cyp17a1* gene showed overexpression in the testes, compared to the ovaries, in the analyzed individuals; the same thing is observed for *star* gene. In the analysis of *sox9* gene expression it was observed that it does not differ between males and females. Likewise is the expression of *ar* gene for which we could not reveal significant differences between males and females. However, it is interesting that the gene showed, for both sexes, a significantly higher expression in gonads compared with kidney and white muscle. Our analyzes, together with similar studies from the literature, show that the genes investigated have a species-specific expression profile and also show differences from one stage of development to another.

2. Identification of pure species and hybrids using DNA markers

Correct identification of endangered fish species is very important for many reasons. A first goal of identification is to ensure the protection of these species, which is an important step in the implementation of conservation programs. A second reason is of economic nature. In the context of diminishing the effects of the natural environment, the food market was occupied by products from fish farms, many of them deliberately labeled wrongly.

i. Identification of interspecific hybrids in fish.

Until recently, identification of the hybrids was done solely on the basis of morphometric characters. However, identification of an individual as a hybrid based on morphology is not sufficient, so we have proposed to develop a method based on microsatellite markers for sturgeons, in order than to be extended to other species where the process of hybridization occurs frequently *in vivo*. Within the proposed method, we selected eight loci that were amplified by multiplex PCR, and the identification of alleles present at each locus was accomplished by the fluorescent-labeled fragments analysis. The eight microsatellites are into the category of disomic loci for *Huso huso*, *Acipenser stellatus* and *Acipenser ruthenus*. However, some of them have a polysomic profile in *Acipenser gueldenstaedtii* (Russian sturgeon), which greatly impedes the correct estimation of genotypes. As a result of analyzes carried out on a significant number of individuals of each species, the particular alleles with diagnostic value, namely alleles that appear only within one of the analyzed species, have been identified. Statistical analysis of genotypic data was performed by several methods:

1. *Factorial Correspondence Analysis (FCA).* Initially, FCA was performed only on the basis of genotypic data from individuals considered to belong to pure species. Some of the analyzed individuals, classified as pure species on the basis of morphological data, were found out of clusters corresponding to their species. This is an indication that they have been misidentified and are hybrids of unknown origin. In the second step of the analysis, the genotypic data for individuals identified based on morphometric data as hybrids were included in FCA as additional individuals. After the analysis, a fifth cluster, with the hybrids plus the taxonomically wrong identified specimens, was revealed.

2. Assignment test with STRUCTURE. The STRUCTURE software can perform a test to assign individuals belonging to a particular category based on their genotypes. The identification of the clusters corresponding to the specific categories is based on a Bayesian algorithm implemented in the program. In our study, the assignment test demonstrated the existence of five distinct clusters: one for the four pure species (sterlet, stellate, beluga and Russian sturgeon) and one for the hybrids. At the same time, some individuals classified morphologically as hybrids appear as a pure species as a result of this test.

3. Determination of hybrid categories. The hybrids confirmed by the STRUCTURE and FCA analysis were analyzed along with genitors' species using the NewHybrids software.

Our results, provided by DNA markers through the three successive statistical methods, showed that in most cases, with some exceptions, the molecular marker classification was similar to that based on morphological criteria. The new method based on DNA marker analysis is very efficient and can be improved by including in the analysis a larger number of microsatellites and several individuals belonging to pure species.

ii. Identification of pure species by molecular techniques.

Another common molecular method is based on the sequencing and analysis of a fragment of the mitochondrial gene encoding for the cytochrome oxidase subunit I. This nucleotide region is considered a "barcode" for the identification of vertebrate species, and its analysis is the base of "DNA barcoding" technique. Other mitochondrial markers successfully used to identify fish species are: D-loop control region, mitochondrial genes encoding for cytochrome b or for ribosomal RNA (rRNA) and transfer RNA (tRNA). The PCR-RFLP analysis of these sequences was proposed as a method for identifying sturgeon and salmonid species. Identification of species by PCR-RFLP or DNA barcoding, although extremely useful for identifying pure species, proved to be ineffective in hybrids.

a. Species identification by PCR-RFLP technique. In practice, an extended region of the mitochondrial genome of salmonids, including the D-loop region, as well as coding genes for rRNA and tRNA, was amplified and then the PCR amplification were digest with *Hinf I* restriction enzyme. After analysis of the restriction profiles, the differences between different salmonid species were clearly observed. The same method can also be used to identify different species of sturgeons. In this case, three restriction enzymes, *Rsa I*, *Ssp I* and *Tru9 I*, which act on a mitochondrial fragment containing the genes encoding for $tRNA^{Glu}$ and cytochrome b, respectively, were selected for correct identification.

b. Species identification by DNA barcoding technique. In order to test the usefulness of the molecular barcode represented by COI gene, the DNA barcoding technique was applied to identify valuable fish species of economically and biologically importance from Romania's wildlife and aquaculture, but also samples from the fish market. In our study, 64 samples of salmonids and 58 samples of acipenserids were collected, processed and initially identified from a taxonomic point of view based on morphology. The samples were subjected to DNA extraction and then to sequencing. Sequences were edited using the BioEdit Sequence Alignment Editor.

In the case of salmonids, while the majority of the analyzed samples were correctly identified, there were also many exceptions to classifications made using classical methods. Thus, all the individuals sampled from the Porumbacu, Avrig and Topolog rivers, initially identified morphologically as *Salmo trutta*, were classified as *Salmo farioides* following DNA barcoding analysis. Also, an individual who came from a fish farm from Caraș-Severin County, originally identified as *Oncorhynchus mykiss*, was classified as *Salmo farioides*. Another case is that of an individual morphologically classified as *Hucho hucho* (huchen) that has been identified as *Salmo sp*. based on the barcode region, and also for a sample morphological identified as *Salmo trutta*, where the analyzes showed that it would actually be *Salmo labrax*. Another nonconformity was represented by a sample came from aquaculture, where an individual classified as *Thymallus thymallus* was identified as *Thymallus aeliani*. Regarding biological samples from the fish market, for the vast majority, the results confirmed the data from the labels. However, a specimen classified as *Salvelinus fontinalis*, along with another misidentified, was marketed under the wrong name of *Salmo trutta*, brown trout.

In the case of acipenserids, the morphological identification was in accordance with the data resulting from the DNA barcoding analysis. In the case of hybrids, as expected, molecular identification was performed after the genitors' species, analyzing mtDNA. Regarding fish market samples, for two of them we found ambiguity; these have been identified as *Huso huso*, respectively hybrid between *Acipenser ruthenus* and *Huso dauricus*.

In parallel, the genetic distances between the COI sequences obtained by us were determined, and these were represented as histograms. In this case, a huchen sample from a fish farm was classified with maximum identity score as *Leucaspius delineatus* in the GenBank Database. *Leucaspius delineatus* is a member of the *Cyprinidae* family, with a small size, opposite to the huchen, a very large predator salmon. In this case, we considered GenBank entrance to be incorrect. Another biological sample morphologically identified as *Salmo trutta*, received maximum scores of identity and similarity with *Salmo labrax*, and the phylogenetic trees obtained support this result. This biological sample comes from an individual caught in the Cerna River, a tributary of the Danube from the south-western region of Romania. Although a clear conclusion cannot be drawn about the identity of this sample, especially since only two *Salmo labrax* entries are available in GenBank. In the case of sturgeons, all specimens of hybrids received maximum scores of identity and similarity with native genitors' species.

Therefore, we can state that the study presents the limitations of DNA barcoding technique in in terms of its usefulness in distinguishing between pure sturgeon species. In order to increase the accuracy of species identification, it is possible to couple the technique with genetic distance-based methods and with DNA-barcoding Character-Based Approach Algorithms. However, combining the barcoding DNA method with nuclear marker analyzes, the rate of correct identification could increase. Recently, Bayesian Fingerprinting has been proposed as an alternative to the barcoding DNA technique because could be applied to several difficult types of tissues.

3. Molecular phylogeny and phylogeography studies in endangered local breeds and species using nuclear and mitochondrial DNA markers

i. Studies of biodiversity and molecular phylogeny in local breeds. In our country there are some local breeds of domesticated animals threatened with extinction on which all efforts for salvation and conservation must be directed. Among these, we can list the following: Romanian Grey Steppe (cattle), Hucul (horse), Mangalitsa and Bazna (swine), Ratska, Tsurcana and Tsigai (sheep), as well as the whole buffalo population (*Bubalus bubalis*). For all these breeds, conservation efforts can be successful only if they are based on detailed studies of their genetic structure, as well as on stable conservation measures of the remaining nuclei of pure, genetically well-defined individuals.

a. Analysis of biodiversity and phylogeny of Hucul breed from Romania. The Hucul is the only local horse breed in our country, probably descended from wild horse populations from western Eurasia. The phylogenetic analyzes of Hucul were performed on both microsatellite nuclear markers and mitochondrial markers (D-loop control region). In case of phylogenetic analysis based on microsatellites, there is a clear divergence of the Hucul from the other analyzed breeds, which is to be expected if we consider that those are relatively new breeds. The analyses suggest the probability of a distant origin of Hucul, from Tarpan horse (*Equus ferus ferus*) and possibly even from Mongolian horses (*Equus ferus prezewalskii*) brought to the Carpathian region following Tartar invasions. Another study carried out a comparative analysis of the Hucul breed and other primitive horse breeds based on the sequence of a fragment from the D-loop mitochondrial region. Our sequences were aligned and compared to another 52 sequences from GenBank database belonging to 14 primitive breeds spread across the globe. There have been

found common haplotypes shared between Hucul and the Akhal Teke, Shetland, or Konik breeds. The results show a high level of genetic variability in all 15 primitive breeds analyzed. Hucul completely segregates from the primitive pony breeds, suggesting a separate position within the horses group, even if its structure is similar to the ponies.

The results of both studies, based on nuclear and mitochondrial markers, demonstrate a distinct origin of the Hucul compared to other primitive or modern horse breeds. Also, the results remove the hypothesis that the Hucul breed is part of the pony group and is related to the primitive Konik breed from Poland.

b. Analysis of biodiversity and phylogeny of local sheep breeds from Romania. Between the local breeds, the most important from economic and cultural point of view are Tsurcana, Ratska, Carabaşa (Teleorman Black Head) and Tsigai. Molecular phylogenetic analyzes for sheep were based on nuclear and mitochondrial DNA markers (D-loop and *cytb* gene fragments). A first study based on microsatellites was carried out analyzing the genetic diversity and phylogenetic relations for four sheep breeds from Romania: Karakul de Botosani, Carabaşă, Palas Milk Line and Palas Meat Line, the last two being specialized breeds. The study included analysis of the degree of heterozygosity, inbreeding, relationship between breeds and phylogeny. The following study was based on the sequences of the mitochondrial fragments of the D-loop region and of the cytochrome b encoding gene and had in the center Ratska breed. 12 distinct haplotypes with a haplotype diversity of 0.897 and a nucleotide diversity of 0.00437 were identified for the Ratska. Subsequently, two phylogenetic networks, one for the haplotypes identified for Ratska, and the other for all the breeds analyzed, were made. The haplotypes of the Ratska are distributed alongside with sheep's maternal lines A and B in a proportion of 35% and 65%, respectively. No haplotype from the Ratska is associated with maternal lines C, D or E. However, the distribution of haplotypes in the phylogenetic network does not indicate a clearly defined geographical position. Other three local breeds from Romania included in this study (Tsurcana, Tsigai and Carabaşa) are grouped at the level of haplogroup B.

The study based on mitochondrial markers was informative for the genetic diversity of the Ratska breed. It has been shown that this breed still has an increased level of diversity, demonstrating that the current population is healthy and has good potential for the formation of a purebred nucleus.

c. Analyzes of biodiversity, phylogeny and phylogeography in the Mangalitsa breed from Romania. In the case of the swine, one of the last primitive local breed in our country is Mangalitsa. As in the case of other local breeds, biodiversity and phylogeny analyzes were performed on both microsatellite markers and mitochondrial DNA. In the case of the microsatellite study, samples from several breeds were taken as follows: Synthetic Line 345 (LS-345), Synthetic Line LSP-2000 (LSP-2000), Pietrain, Large White, Landrace, Mangalitsa and wild boar. The study included analysis of the degree of heterozygosity, inbreeding, the relationship between breeds and phylogeny. It can be seen that the Mangalitsa is the closest to the wild boar, while synthetic lines and the Pietrain breed are grouped in the same cluster. In the case of the mitochondrial marker study, two fragments of mitochondrial DNA were amplified, one from the D-loop control region and the other from the encoding gene of cytochrome b. For the interpretation of phylogenetic relationships, the sequences obtained by us were compared with several GenBank sequences belonging to 24 swine breeds and four wild boar populations. The data revealed the presence of 86 variable sites (75 transitions, 10 transversions and one deletion), representing 4.9% of the total nucleotides analyzed, the sites being equally divided between the D-loop region and the *cytb* gene. Also, 32 different haplotypes were identified, five of which were identified in the Mangalitsa breed and one in the wild boar. In the phylogenetic tree, the haplotypes of the Mangalitsa are placed in the European clade, and two of them are grouped together with the haplotypes from the Romania and Spain wild boars.

The results obtained by the analysis of the mitochondrial and nuclear markers show us the existence of an increased level of genetic diversity within the Mangalitsa breed from Romania and highlight the character of its primitivity and its proximity to the wild boar, basically delimiting the Mangalitsa from other European breeds.

d. Analysis of biodiversity and phylogeny in local breeds of cattle and buffaloes in Romania. Among the local, primitive, cattle breeds, the only remaining one is the Romanian Grey Steppe. At the same time, the buffalo (*Bubalus bubalis*) represented in the past an important economic and social resource for our country. A first study on the genetic diversity and phylogenetic relationships of local cattle breeds from Romania was carried out using microsatellite. The study analyzed five cattle populations: Romanian Grey Steppe, Romanian Spotted, Romanian Black Spotted, Brown and Montbeliarde, and for the genetic characterization 11 microsatellites were used. As a result of the analyses, it was observed that the Romanian Grey Steppe totally

segregate of the other four analyzed breeds. However, regarding the genetic variability of the living Romanian Grey Steppe nucleus, the data are worrying; the lack of urgent conservation measures may lead to the extinction of the breed. Afterward, two other different studies on the genetic variability of bovine breeds from Romania were performed using the mitochondrial markers D-loop and *cytb* gene. They analyzed only three local cattle breeds: Romanian Grey Steppe, Romanian Black Spotted and Brown. For the D-loop region, a 661 bp sequence was aligned and compared with similar sequences from other Asian and European bovine species and breeds. In the case of the *cytb* gene we proceed in the similar way, but the sequence analyzed was 610 bp in length. From the phylogenetic tree constructed using the fragment of the D-loop region, can notice the placement of the three local breeds from Romania in very close positions at the level of the *Bos taurus* cluster. At the same time, the phylogenetic tree constructed using a sequence from the *cytb* gene places the Romanian Grey Steppe and Romanian Black Spotted in the same cluster, and the Brown at the level of a distinct cluster.

In the case of the Romanian buffalo, the only diversity study based on nuclear markers included the analysis of 22 specimens from the Research and Development Station for Buffalo, Şercaia, Braşov County. Genetic diversity was analyzed based on six microsatellites initially isolated in cattle. The analyses show us a healthy buffalo population with a high degree of genetic variability without a solid inbreeding phenomenon. However, this is only a preliminary study, based on only on six microsatellites, which must be extended to more individuals and more loci.

ii. Studies of biodiversity, phylogeography and molecular phylogeny of endangered species from Romanian fauna.

Phylogeography is the study of biological processes and historical events that have led to the current geographical distribution of animal and plant species. An extremely important study has been carried out on the existing sturgeons' populations from the Ponto-Caspian region, because phylogenetic relationships, especially the relations under the Acipenseriformes order, have remained controversial even in present time. In our study, 78 individuals were captured in the Lower Danube, from which 27 of *Acipenser stellatus*, 28 of *Acipenser gueldenstaedtii* and 23 of *Huso huso*. All of these individuals were included in the Black Sea group. The mitochondrial sequences for the D-loop region of individuals captured in the Danube were compared to sequences downloaded from GenBank belonging to individuals from the Caspian Sea, Azov Sea and Black Sea. In the case of individuals from Danube, we identified the presence of four distinct

haplotypes for the Russian sturgeon and seven different haplotypes for the stellate sturgeon, respectively beluga sturgeon. All sequences were compared to those from the Caspian, Black and Azov Seas, in order to highlight the existence of possible different subspecies. Finally, 23 distinct haplotypes for Russian sturgeon, 65 for the stellate sturgeon and 30 for the beluga sturgeon were identified. Based on the identified haplotypes for the three analyzed species, phylogenetic trees were construct using the Maximum Likelihood and Neighbor-Joining methods. It has been observed that haplotypes are randomly distributed to the resulting trees, not associated with a particular geographical origin. Therefore, the phylogenetic analysis did not confirm the classification in the different subspecies of the three analyzed species for the Ponto-Caspian region. It also did not identify the existence of separate populations or reproductive isolated units. Our results showed a reduced genetic differentiation and a substantial gene flow between individuals from different geographic origin. These are probably the consequence of exchanges of genetic information between populations, and are also due to anthropogenic influence, manifested mainly by uncontrolled repopulation.

Other studies have been focused on the analysis of salmonid populations from Romania, with particular emphasis on brown trout (*Salmo trutta*). Estimation of phylogenetic relationships was performed using the sequences of the 16SrRNA, 12SrRNA and also the concatenated data sets using three methods: Maximum Parsimony, Maximum Likelihood, and Neighbor-Joining. Phylogenetic analysis has highlighted that primitive salmonid species such as *Coregonus lavaraetus* or representatives of the *Thymallus* genus occupy a basal position in the tree topology. Also, the *Coregoninae* and *Thymallinae* subfamilies have been considered the oldest branches of the *Salmonidae* family. The data reveals a close relationship between *Salmo trutta*, *Salmon trutta trutta* (sea trout) and *Salmo labrax* (Black Sea trout). Another study was aimed to evaluating the anthropic impact on brown trout populations from the Făgăraș Mountains area. In this study, 118 individuals of brown trout belonging to four populations from the Făgăraș Nordic slope area were analyzed using nine microsatellite markers. Based on the genetic diversity assessment, it was observed that there is a high degree of genetic differentiation among the analyzed populations, especially when comparing the Ucea river population with the other three. In addition, two populations of inbreeding effect were identified, to be exact the populations of the Cârţişoara and Porumbacu rivers. Increasing fragmentation of habitats may reduce the

number of brown trout populations in the future, but also their genetic variability, contributing to their isolation.

Part II. Professional and academic career development plan.

The general objectives for the future development of academic career, including both the didactic and the research parts, presents two key directions:

A. Developing and deepening the areas of expertise already in place: i. Developing and extending expertise and competencies in the disciplines of biochemistry and molecular biology. ii. Acquiring high knowledge in specialized topics by constantly consulting scientific publications from the international flow and participating on national and international scientific events. iii. Disseminating the results of the scientific activity through the continuous publication in scientific papers from the specialized national and international journals. iv. Improving the teaching methods for courses, seminars or practical laboratory, in order to meet the general and specific objectives of the disciplines. v. Technological transfer of the results of scientific research (services, innovative products, technologies) through national and international research projects and collaborations with partners from other universities and research institutes, as well as from the private industry.

B. Expanding areas of expertise and developing new skills and competences: i. Addressing new research topics of international interest, within the specialized domains, with increased potential for development of knowledge, services, technologies, and innovative products. ii. Propose and develop new courses for students, master and PhD students, but also for other target groups. iii. Develop new approaches to scientific research in the field.

1. Development of teaching activity

To summarize my future objectives regarding the development of teaching activity, I propose the following: i. Improving the teaching strategies in order to develop a more student-centered learning method. ii. Continuous improvement of the scientific content of the courses and practical laboratory. iii. Organizing new courses and laboratories according with new international trends and objectives about scientific research. iv. Attracting students to practical research activities and their guidance to masteral and doctoral studies. v. Proposing new

scientific themes for the accomplishment of bachelor and master studies. vi. Organizing student meetings to discuss scientific themes of interest, impacting on research and everyday life. vii. Developing the ability of communication and transfer knowledge.

2. Development of scientific research directions

The main research directions that I want to develop within the Doctoral School of Biology from the University of Bucharest will be oriented towards:

i. Molecular methods applied in marker assisted selection. In this direction, I intend to conduct studies to identify and analyze DNA markers correlated with different morpho-productive traits for fish species of economic interest. Identification and investigation of such markers, which then can be correlated with meat production and quality, with disease resistance or rapid growth rates, can help establish the basics of marker assisted selection in aquaculture.

ii. Analysis of molecular mechanisms involved in stress resistance and early sex determination of fish species of economic interest. Studies evaluating the response to different stress factors for aquaculture species could provide fish farms the possibility of developing long-term strategies to make fisheries activities more efficient. It would also help to understand the cellular mechanisms involved in counteracting the effects of stressors on these species in captivity.

iii. Studies of population genetics, biodiversity, phylogenetic and molecular phylogeny. Population genetics studies are of great importance in the improvement of domestic animal species, and also contribute to highlighting factors that change the frequency of genes in panmictic populations, the role of reproductive isolation mechanisms of the species and also the importance of population size in the process of genetic drift of small populations.