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Autoreferat w języku angielskim

Załącznik 3 do wniosku
o przeprowadzenie postępowania habilitacyjnego

Hybridization and introgression impact on the mussel *Mytilus trossulus* genetic structure

Sopot, 2015

Appendix 3

Autoreferat

1. First and Last Names.
Małgorzata Karolina Zbawicka

2. Obtained diplomas, scientific/artistic titles and degrees – with their name, place and year of their receiving and the title of the PhD thesis.

Master of science in biologia – University of Gdański, Faculty of Biology, Geography and Oceanology, 1992. Master thesis title - „Study the role of proteins λO and λP in the plasmid λdv replication *Escherichia coli* dnaA46 mutant".

Ph.D. in biology - University of Gdański, Faculty of Biology, Geography and Oceanology, 2000. Title of PhD thesis – „ Polymorphism and transmission of mitochondrial DNA in population of the mussel *Mytilus trossulus* from the polish coast"

3. Information about employment at research/art centers.

1993 – 2000: assistant, Marine Biology Center, Polish Academy of Science, Laboratory of Genetics.

2001 - 2002: postdoctoral position, Marine Biology Center, Polish Academy of Science, Laboratory of Genetics
.
2002 - 2011: postdoctoral position, Institute of Oceanology, Polish Academy of Sciences Department of Genetics and Marine Biotechnology.
2011 – till now: assistant, Institute of Oceanology, Polish Academy of Sciences Department of Genetics and Marine Biotechnology.

4. Description of the scientific achievements*, as described in art. 16, Section 2 of the bill published on March 14, 2003, regarding scientific degrees and titles and regarding degrees and titles in the arts (Dz. U. nr 65, poz. 595 ze zm.):

a) title of the scientific/artistic achievement,

Hybridization and introgression impact on the mussel *Mytilus trossulus* genetic structure

b) (author/authors, title/titles of publications, date of publication, name of publisher),

1. Zbawicka M., Burzyński A., Wenne R. 2007. Complete sequences of mitochondrial genomes from the Baltic mussel *Mytilus trossulus*. Gene 406: 191-198

Number of citations by WoS: 24 (MNiSW – 20pkt, IF 2007 = 2.871)

2. Zbawicka M., Burzyński A., Skibinski D., Wenne R. 2010. Scottish *Mytilus trossulus* mussels retain ancestral mitochondrial DNA: Complete sequences of male and female mtDNA genomes. Gene, 456: 45-53

Number of citations by WoS: 19 (MNiSW – 20pkt, IF 2010 = 2.266)

3. Zbawicka M., Wenne R. Burzyński A. 2014. Mitogenomics of recombinant mitochondrial genomes of Baltic Sea Mytilus mussels. Molecular Genetics and Genomics, 289: 1275-1287 DOI 10.1007/s00438-014-0888-3

(MNiSW – 25pkt, IF 2014 = 2.831)

4. Zbawicka M., Drywa A., Śmietanka B., Wenne R. 2012. Identification and validation of novel SNP markers in European populations of marine Mytilus mussels. Marine Biology, 159:1347-1362

Number of citations by WoS: 5 (MNiSW – 35pkt, IF 2012 = 2.468)

5. Zbawicka M., Sańko T., Strand J., Wenne R. 2014. New SNP markers reveal largely concordant clinal variation across the hybrid zone between Mytilus spp. in the Baltic Sea. Aquatic Biology 21:25-36

Number of citations by WoS: 1 (MNiSW – 25pkt, IF 2014 = 1.118)

c) Aim of above publications and description of obtained results, including description of their possible utilization.

The aim of above publications was to expand our knowledge of impact of hybridization and introgression on the genetic structure of *Mytilus trossulus*. Hybridization is phenomenon which consists in breaking the reproductive barriers and mating animals of various species, allows flow from one gene to another gene pool by introgression.

Mussels of genus *Mytilus* are widespread in northern and southern hemispheres (Borsa et al. 2007; Gerard et al. 2008). There are commonly-known, benthic, filter-feeding inhabitants of marine waters. They are important components of marine coastal ecosystems. European populations of *Mytilus* are represented by *M. galloprovincialis*, *M. edulis* and *M. trossulus*. *M. trossulus* originated in the Pacific and colonized the northern Atlantic after opening the Bering Strait 3.5 M years ago. It gave rise to *M. edulis* and *M. galloprovincialis* (Riginos and Cunningham 2005). *M. edulis* is located in the northern part of the Atlantic and European seas, whereas *M. galloprovincialis* is distributed mainly in the Mediterranean Sea, Black Sea and along the Atlantic coastline of Western Europe. All three *Mytilus* taxa hybridize in areas where they are in contact (Gosling, 1992; Bierne et al., 2003a). Hybrid zones of *M. trossulus* and *M.*

edulis are localized in the Danish straits separating the Baltic and North Seas (Väinölä and Hvilsom, 1991; Riginos and Cunningham, 2005), and in North America.

Marine mussels *Mytilus* have an unusual system of mitochondrial DNA inheritance, referred to as doubly uniparental inheritance (DUI). Female type (F), presented in females and males, is transmitted to all offspring and male type (M), presented almost exclusively in heteroplasmic males is transmitted only to the sons (Zouros et al. 1994;. Skibiński et al. 1994). These two genomes are usually highly diverged at the sequence level. Occasionally the M genome can be replaced by the F in process called masculinization, in consequence the divergence between F and M genomes can be reduced. Study of issues related to DUI and masculinization, was one of the main topics of research in our laboratory in which I participated.

My research interest in the genetic structure of the mussel *M. trossulus* date back to my PhD thesis, which concerned the variation and transmission of mitochondrial DNA in population of the mussel *M. trossulus* from the Polish coast of Baltic. Result of my research pointed to the existence of two phylogenetically close female and male genomes and length variants identified in major noncoding region (Zbawicka et al. 2003a,b) In Baltic mussels we are dealing with masculinization (Wenne and Skibinski, 1995). Both original F and M mitochondrial genomes of *M. trossulus* were replaced by *M. edulis* mtDNA in the Baltic populations, and were similar to the F genome of *M. edulis*.not to the native *M. trossulus*. Highly diverged M genome occurs rarely in the Baltic mussels. However, recently recombination between those variants has been reported (Burzyński et al. 2003, 2006).

Continuing my work on the Baltic mussels I collected the sample from Gulf of Gdańsk, determined sex and izolated DNA. I used three nuclear DNA markers to identify the *Mytilus* species. The nuclear adhesive protein marker (Me 15/16) was diagnostic for all three taxa *M. edulis*, *M. trossulus*, and *M. galloprovincialis* (Inoue et al., 1995). Second marker was the internal transcribed spacer (ITS) regions between the 18S and 28S rRNA genes and detected with the restriction enzyme HhaI digestion, which differentiated *M. trossulus* from other *Mytilus* taxa (Heath et al., 1995). The last used marker was Efbis, intron in the elongation factor 1a, which was diagnostic for all three taxa (Bierne et al., 2003b; Kijewski et al., 2006). All study samples showed a thoroughly mixed genetic composition of *M. edulis* and *M. trossulus* markers.

The main theme of my first work composed for scientific achievement was to explore the full mtDNA sequences from two Baltic mussels. I selected two mtDNA haplotypes with

nonrecombined noncoding region for sequencing of whole molecule, based on the results from sequencing of the major noncoding region obtained from this sample in our laboratory (Burzyński et al. 2006). One originated from eggs (F - haplotype 3a) and had a small duplication of the central part of variable domain 1 (VD1) in noncoding region, the other came from sperm (M – 1b) and it was found that a normal M genome characteristic of *M. edulis*. I found that both genomes contained the same set of metazoan genes, had similar nucleotide composition but were diverged by 24% in nucleotide sequences. I noticed that the most diverged was the VD1 region. The F genome which I sequenced was longer than M by 147 bp. and the main difference was localized in the VD1 region. In relation to the earlier work carried out in our laboratory on the recombination, I checked genomes in this regard. I demonstrated lack of recombination in whole mtDNA of both studied variants. I found high similarity of the M genome of Baltic *M. trossulus* to the M genome of *M. edulis*. My conclusion was that the M genome was introgressed to the southern Baltic from the *M. edulis* populations in the North Sea, similarly to the F genome. I observed surprisingly low (K=0.005–0.007) divergence between the Baltic *M. trossulus*, *M. edulis* and *M. galloprovincialis* F genomes. This small difference show that all these F genomes were closely related to *M. edulis* haplotype. I tested also the occurrence of nuclear mitochondrial pseudogenes (numts), that was confirmed in many eucaryotes. I wanted to verify that the observed heteroplasmy of mtDNA variants does not come from nuclear DNA. I conducted hybridisation experiments involving probes complementary to selected fragments of the Baltic *M. trossulus* mtDNA. Numts have not been found. The results of the whole genome sequencing of two Baltic mtDNA genomes were presented in publication [**1**].

It was previously thought that after a recent invasion of Pacific mussels into the Atlantic, *M. trossulus* colonized only the Atlantic Canadian coasts and the Baltic Sea in Europe. Pure ancestral *M. trossulus* mussels have been found only in American populations (Riginos and Cunningham, 2005). Beaumont et al. 2008 the occurrence of *M. trossulus* mussels on the coasts of Western Scotland. North American F genome of *M. trossulus*. which has both M-like and F-like sequences in the control region (CR) has been completely sequenced by Breton et al., (2006). For the ancestral M genome of *M. trossulus* only sequence of three fragments were known: one covering a fragment of *lrrna*, the entire CR and a *cob* fragment (Cao et al., 2009), to further covering part of *cox1* and *cox3* (Riginos et al., 2004). The complete mtDNA sequences of the F and M genomes from Baltic *M. trossulus* presented in my earlier work [**1**]

have low genetic distance from the F and M genomes of *M. edulis* suggesting that they derive through recent introgression from *M. edulis* (Kijewski et al., 2006).

Continuing my work on the mtDNA of *M. trossulus* I wanted to answer the question of whether *M. trossulus* identified at Loch Etive in Scotland (Beaumont et al., 2008) derives from North American populations or from the Baltic. I collected the *Mytilus* sp sample consisting of the reproductively mature mussels from Loch Etive, determined sex and isolated DNA from gametes. Then I have shown the mixed ancestry for this population with three previously used nuclear markers [**1**]. I observed a high frequency of mussels with *M. trossulus* ancestry, many heterozygotes or mussels having intermediate hybrid index values. Based on population genetic structure I concluded that in Loch Etive mussels hybridisation was at an intermediate stage compared with North American populations, where there is little hybridisation, and Baltic populations where there is extensive introgression. My results pointed separate *M. trossulus* invasion to Scotland. I obtained the complete sequences of the three *M. trossulus* mtDNA genomes (one F and two M), and I have shown that they were very similar to the corresponding genomes from ancestral *M. trossulus* in America and divergent from the genomes of the Baltic *M. trossulus*. My work was the first report of ancestral *M. trossulus* mtDNA genomes in Europe and additionally I reported the first complete sequence of ancestral *M. trossulus* M genome. I confirmed the presence of DUI in Scotland population. I found that F and M genomes were diverged by 26% in nucleotide sequence, similar to other *Mytilus* F and M genomes. The gene arrangement in the sequenced genomes was also similar to that in other sequenced *Mytilus* mtDNA genomes. However the two sequenced M genomes differ by 960 bp which was caused by a duplication in the main noncoding region (CR). This duplication has not been so far observed in North American populations of *M. trossulus* (Cao et al. 2009). The results of study of Loche Etive mussels which have indicated the presence of ancestral *M. trossulus* mtDNA genomes in Europe were presented in publication [**2**].

I continued my work on Baltic *Mytilus*. In further research on the hybridization and introgression [**3**] I have presented the complete sequences of a representative set consisting of 11 mitochondrial genomes from Baltic *Mytilus*. Among haplotypes sequenced by myself, there was the haplotype close to the native *M. trossulus* mitochondrial genome, which was thought to have been entirely eliminated from this population. I found that it was F genome having recombination structure and numerous and long repeats in noncoding region, 3 x longer than in corresponding genomes from Scotland and Canada [**2**], (Breton et al., 2006). After phylogenetic and comparative analysis it turned out that the recombination was limited to the vicinity of the

CR in all sequenced genomes. Coding sequence comparison indicated that all paternally inherited genomes showed increased accumulation of nonsynonymous substitutions, including the genomes which switched their transmission route very recently. Additionally comparing the whole sequences of *Mytilus* mtDNA, it can be concluded that recombination events predate masculinization and the genomes with M-like CRs are preferentially masculinized. But I observed also masculinized genomes without M-like sequences, which suggested the existence of other factors than the recognition of particular sequences within the CR.

Interspecies hybridization in the Baltic Sea caused cytonuclear incompatibility - almost exclusive presence of *M. edulis* mtDNA in Baltic Sea *Mytilus* mussels and the nuclear background dominated by *M. trossulus* [**5**]. This phenomenon consequently created conditions for both structural and evolutionary mitochondrial instability of mtDNA. The result of this situation observed in my work were unique features of Baltic Sea hybrid populations of *Mytilus* mussels, such as high level of CR recombination, masculinization, heteroplasmy, length variation and structural rearrangements of mtDNA

Mytilus taxa are morphologically indistinguishable in European water because of adaptation of the shell to different environmental condition, in macrogeographic scale, the location has a greater impact than the genotype (Gardner and Thompson, 2009). Initially, allozyme data and a few diagnostic nuclear markers were used to definition the taxa, but their number was limited and they often have given inconsistent results, particularly in the areas of known or potential hybridization and/or introgression (i.e. Heath et al. 1995; Inoue et al. 1995; Bierne et al. 2003b; Kijewski et al. 2006). F genome of mtDNA was also used as a marker for defining genetic structure, it is single-locus and is known to undergo introgression across hybrid zones (Śmietanka et al. 2004). Even the studies of the distribution of *Mytilus* taxa at large European scale were limited to only one or three nuclear markers (Śmietanka et al. 2004; Kijewski et al. 2011).

Since the markers previously used were insufficient for identifying either the geographical origin of mussels and the precise identification of taxa, due to hybridization and morphological similarity, I have developed new reliable markers for the discrimination of *Mytilus* taxa and their hybrids. I have discovered new molecular markers based on single nucleotide polymorphisms (SNPs). SNPs are point mutations occurring at the nucleotide level and producing single nucleotide differences among or within individuals of a species. SNPs located in coding regions can be used to differentiate loci under selective pressure from neutral loci (Morin et al. 2004). I selected ten nuclear DNA fragments based on DNA, mRNA and EST

sequences available in GenBank, and designed specific primers to amplified selected fragments from all three taxa, 15 mussels. Based on the obtained by me genomic DNA sequences of the three taxa, I identified 37 candidate SNPs. SNP genotyping of 499 sampled individuals of mussels *Mytilus* from 24 populations from Europe was performed using the MassARRAY iPLEX Gold technology, following the protocol provided by Sequenom (Gabriel et al. 2009). Twenty one SNPs proved to be polymorphic in most of the sampled populations. They were localized in coding and non-coding sequences of some functionally important genes. I discovered eight novel SNPs localized in genes of the histone family, *hsp 70* and *p53* which may be applied as novel markers for *Mytilus* taxa on a large European scale. Five of them differentiated the *M. trossulus* genome, two *M. galloprovincialis*, and one *M. edulis*. Other SNPs differentiated populations within taxa.

Using Structure (Falush et al. 2007) and Correspondence analysis (Belkhir et al. 2003) SNP data very clearly demonstrated population genetic structure and separation between three *Mytilus* taxa *M. edulis*, *M. trossulus*, and *M. galloprovincialis*, as well as diversity among and within the studied populations and level of admixture. Additionally I showed the existence of a few regions where hybridization of the three *Mytilus* taxa was observed: the Baltic Sea, Scotland, and Norway, in particular hybridization between *M. edulis* and *M. trossulus*. Populations from these regions had almost all loci polymorphic. Method developed myself is a valuable tool for population studies of European *Mytilus* mussels. The results of this research accounted for the publication [**4**] describing identification and validation of novel SNP markers in *Mytilus* mussels.

Continuing studies on the SNP markers, I have been involved in their quest on a larger scale. My research was mainly focused on the hybrid zone between Baltic Sea and the Danish Straits. The Baltic Sea was colonized by *Mytilus* spp. mussels about 7000 years ago, after the most recent freshwater period and the emergence of this area as a marine (brackish) ecosystem (Zillén et al. 2008). In order to increase the number of available sequences needed for finding new SNPs, new cDNA library made from mantle tissue of a single male collected from the Gulf of Gdańsk (Baltic *M. trossulus* x *M. edulis* hybrid) were constructed in our laboratory. I aligned obtained expressed sequence tag (EST sequences) with appropriate GenBank EST sequences (mainly for *M. galloprovincialis* and *M. edulis*) using the ClustalX program (Thompson i wsp., 1997), then I used multiple sequence alignments for SNP discovery using the Staden computer programs (Staden et al. 2001). I found 340 putative SNPs, of which, for genotyping 642 individual mussels from Baltic, Danish Straits and from Pacific (reference population of *M.*

trossulus), I chose 60 polymorphic SNPs. I characterized 49 novel SNP markers that differentiate the populations of the North and Baltic Sea areas. I observed that for most of the examined SNPs, allele frequencies were changing abruptly, creating concordant narrow clines at the Baltic Sea entrance (Kattegat and the Sound). Based on this study, I concluded that the boundary separating populations with a predominance of *M. edulis* genes from those with a predominance of *M. trossulus* genes was located around the eastern islands of Falster and Moen in South Danish Straits. Additional conclusion was, that the majority of new SNPs markers showed a larger representation of *M. trossulus* than *M. edulis* genes in the nuclear DNA of Baltic *Mytilus* species. Using more nuclear markers it becomes apparent that the Baltic Sea mussels are much closer to *M. trossulus* than to *M. edulis*. I confirmed the existence of strong reproductive isolation, probably caused by a combination of exogenous (e.g. adaptation to brackish waters) and endogenous pre- and post-zygotic factors (e.g. selection against hybrids). I identified also a few markers with an elevated level of introgression of *M. edulis* alleles in the Baltic Sea *M. trossulus* populations in comparison to the reference *M. trossulus* population of the Pacific. My study revealed the complexity of the Baltic hybrid zone and clearly showed how populations of the Danish Straits and Øresund were more an admixture of the 2 taxa than a unimodal hybrid swarm in the inner Baltic. The results of this research are presented in publication [**5**] and indicate the high potential of the new SNPs in the study of hybridization zones.

In summary, the most important discoveries presented in publications comprising my scientific achievements are:

- Complete sequences of a representative set consisting of 16 mitochondrial genomes from mussels that had mixed genetic composition of *M. edulis* and *M. trossulus* markers and derived from the populations that are affected by hybridization and introgression.

- The first report of the presence of ancestral *M. trossulus* mtDNA genomes in Europe.

- The confirmation that interspecies hybridization in the Baltic Sea created conditions for both structural and evolutionary mitochondrial instability of mtDNA (structural rearrangements, multiplication, deletion and recombination).

- Finding that hybridization could have caused abrupt and rapid increase in the M genome incompatibility, favoring masculinization.

- Finding that in Baltic *Mytilus* mussels the nuclear background is dominated by *M. trossulus*

- Discovery the 80 new SNP markers which show the high potential in the study of hybridization zones of *Mytilus* taxa mussels.

The results, described above, show changes in the genetic structure and sequence of the genomes of mussel *Mytilus trossulus* in areas covered by the hybridization and introgression. As well as complementary the knowledge about the mechanism of the origin and evolution of recombined mitochondrial genomes in mussels with doubly uniparental inheritance (DUI).

Cited literature (except publications forming scientific achievement)

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5. Discussion of other scientific-research (artistic) achievements.

In 1992, I graduated with honors from the University of Gdańsk, Faculty of Biology, Geography and Oceanology. Master's Thesis "Study the role of proteins λO and λP in replication of plasmids λdv in the mutant *Escherichia coli* dnaA46" I performed at the Department of Molecular Biology under Prof. Karol Taylor supervision. In February 1993, I was employed as assistant in Marine Biology Center, Polish Academy of Science, in Laboratory of Genetics led by Prof. Roman Wenne. In the first years, my work concerned the studying the mitochondrial DNA of mussel (*Mytilus trossulus*) from the Gulf of Gdansk. The study was focused on issues related to mtDNA length heteroplasmy and has just discovered the phenomenon of DUI

occurring in these mussels. In the context of collecting samples for testing, I participated in multiple research cruises in the Gulf of Gdansk on k/h Oceanograf 2 as a member of the research team.

In 1996 I received French government scholarship and for 6 months I worked in Centre de CNRS Genetique Moleculaire in Gif-sur-Yvette under the supervision of Prof. Jean Claude Mounolou. I participated in the study of mitochondrial DNA of shrimp. My work concerned performing experiments involving the cloning and sequencing of the gene part. I conducted also phylogenetic comparative analysis. The results of these studies have been published (Garcia-Machado et al. 1999)

In 1996-1997 I was the coordinator of the grant on the polymorphism of mitochondrial DNA mussel from the Polish coast of Baltic Sea. Additionally in 1996-1999 I participated in the research projects realized in our laboratory of Genetics as an investigator (Appendix 5,II- I 2) I studied the diversity of mussels populations from Polish coast of Baltic. The highly diverged M genome has not been detected at high frequency in Baltic *M. trossulus*, thus the initially my study focuses on the F genome. I used PCR and RFLP analysis to characterize haplotypes in the coding region ND2- COIII. In the further study somatic tissues and gametes were surveyed for the presence of different types of mtDNA. My research results indicated the existence of two phylogenetically close female and male genomes. I used PCR to identify length variants in a major noncoding region. I found seventeen length variants presented in homo- and heteroplasmic individuals. Two length variants of the major noncoding region (CR) were the most frequent. Length heteroplasmy was found in 46.8% of males, and was mostly caused by the presence of the short length variant of the major noncoding region. This study provides evidence, that the short length variant was always transmitted to sperm and has taken over the role of the M genome. I have shown that the remaining length variants were presented in both males and females. The present study also pointed towards the potential utility of mtDNA length variation in studies of population differentiation of *Mytilus*.

The results of these studies have been served as a basic for my Ph.D. dissertation concerning the study of polymorphism and transmission of mitochondrial DNA in mussels *M. trossulus* which I performed under Prof. Roman Wenne supervision and defended in November, 2000. Additionally the results were published (Zbawicka et al. 2003a,b) Also with this issue was maintain my participation in the international join research project and 1 month stay at the University of Helsinki (Appendix 5,III- L 2).

Besides research and publications described as a scientific achievement, I also participated in other project associated with issues related to the recombination of mtDNA in mussel *Mytilus trossulus* from Baltic (Appendix 5,II- I 3, 6). This phenomenon was discovered in our laboratory of Genetics. I was responsible for the majority of work associated with sex determination, PCR amplification, sequencing. We provided evidence of the generality of mtDNA recombination in *Mytilus*. The subject of our research was the mtDNA region beginning in the 16S rRNA gene and terminating in the cytochrome b gene and includes a major noncoding region. Initially we found two recombinant variants that appear to be mosaic for F-like and M-like sequences. Both variants have the noncoding region from the M genome, and both are transmitted to sperm like the M genome. We have discovered that reported here recombinant genomes have the noncoding region derived from the *M. edulis* M genome. Continuing these studies we analyzed the mtDNA control region structure in a larger group of male and female Baltic *M. trossulus* mussels. Sample were analyzed by Southern blotting and PCR and after selected PCR products were sequenced. We showed that a great diversity of structural rearrangements was presented in both sexes. Sperm samples were dominated by recombinant haplotypes with M. edulis M-like control region segments, some having large duplications. By contrast, the rearranged haplotypes that dominate in eggs lack segments from this M genome. We have found that the rearrangements can be explained by a combination of tandem duplication, deletion, and intermolecular recombination. The results of these researches were presented in two publications (Burzyński et al. 2003, 2006).

In the next years I was included in the study of nuclear markers (Appendix 5,II- I 2). Initially, my work concerned the identification of *Mytilus* species in coastal areas, on a large geographic scale in Europe using the nuclear adhesive protein marker (Me15/16). I identified three expected DNA fragments diagnostic for each *Mytilus* taxon. Their sizes were 180 bp for *M. edulis*, 168 bp for *M. trossulus* and 126 bp for *M. galloprovincialis* and corresponded to three alleles (Inoue et al. 1995). I identified pure population and two types of hybrid specimens in this study *M. edulis*/*M. galloprovincialis* (EG) and *M. edulis*/*M. trossulus* (ET). The results of these researches were presented in publication on mtDNA lineages in the European populations of *Mytilus* spp. (Śmietanka et al. 2004). Subsequently, I continued my work focusing on Baltic populations of mussels *Mytilus*. I used three nuclear DNA markers to identify the *Mytilus* species: Me15/16, ITS, and Efbis.

I developed a new fully diagnostic method of the EFbis analysis by RFLP. The PCR products were doubly digested with Hin6I and RsaI endonucleases to enhance the exposure of

the diagnostic length variability. I tested this methods using some specimens of *M. trossulus* from Canada, *M. galloprovincialis* from north Spain and *M. edulis* from Holland. All samples from the Danish Straits and the inner Baltic Sea showed a thoroughly mixed genetic composition of *M. edulis* and *M. trossulus* markers. We observed extensive introgression of *M. edulis* alleles from the North Sea into populations throughout the Baltic for mtDNA and two nuclear markers (ME15/16 and ITS). Whereas introgression of *M. trossulus* alleles into the *M. edulis* background was observed in the opposite direction at the EFbis nuclear marker in populations from Kattegat (Danish Straits). The results described above have been published (Kijewski et al. 2006). I used the same set of three nuclear DNA markers in the next project (Appendix 5, III-A 1, 2) that was focused on distribution of *Mytilus* taxa in European coastal areas (Kijewski et al. 2011). In addition to a sharp cline between Atlantic and Mediterranean *M. galloprovincialis,* we observed a clear genetic distinction between the Black Sea and Mediterranean populations and a higher incidence of *M. trossulus* than reported so far in northern European populations. We reported for the first time occurrence of *M. trossulus* alleles in cold waters of Iceland, Barents Sea and White Sea.

The next research, in which I participated, was focused on the question of the origin of the *M. trossulus* mussels in European waters, which have retained their native mtDNA. We wanted to find out if they were part of a population present, but previously undetected, or is this a potentially human mediated, ongoing spread of an invasive species? We amplified with species-specific primers and sequenced an approximately 1,200-bp-long fragment spanning COIII and ND2 genes from both mitochondrial genomes of mussels *M. trossulus* on the global scale, with populations from both oceans sampled. We investigated populations from the west coast of the Japan Sea, Pacific coast of Canada, Atlantic coast of Canada, Loch Etive at Scotland and the Aleutian Islands. Phylogeographical analyses applied by us pointed at the West Atlantic as the source of the European *M. trossulus* mussels, at least the ones who retained their native mtDNA. We conducted the comparison with the well-known case: the introduction of congeneric mussel, *M. galloprovincialis,* from Mediterranean Sea to Asia to know the timing of the events. Our results most likely indicated that, this invasion was not a human-mediated process. Above researches were implemented within of a research project (Appendix 5, II-I 9) and were presented in publication on mitochondrial genomes of subarctic *M. trossulus*. (Śmietanka et al. 2013)

I also participated in European program BONUS plus implementing the project on Baltic Sea Genetic Biodiversity (Appendix 5,III-A 2, II-I 8). The result of this collaboration was the publication of seven ecologically important species of Baltic ecosystem (Wennerstrom

et al. 2013). In the above project I was responsible for *Mytilus* species and used SNP loci as a nuclear genetic data. SNP markers were developed by me in earlier study. Our main conclusion was, that in the Baltic Sea ecosystem where environmental gradients occured and where separate species have different origins (freshwater or marine), genetic patterns of variation and divergence were not shared among species. Each species displayed a unique genetic pattern of diversity and divergence and location the barriers to gene flow.

Currently I continue research of molecular biogeography of marine mussels. Information on genetic biodiversity is important for successful management and conservation of species. Recently, changes have been observed in the distribution of marine mussels. In the implementation of grant (Appendix 5, II-I 10) I developed a SNP method to identify the origin of populations of different taxa of mussels on a global scale and determine the presence of non-native mussels. Therefore we collected the samples belonging to five *Mytilus* taxa from six continents from the port, aquaculture and regions well removed from the putative influence of shipping, and remote islands. I examined also the taxonomic and geographical origin of food products from mussels available in Polish stores. I detected and documented for the first time the presence of *M. trossulus* on the coasts of Greenland using SNP markers. I discovered an unknown form of hybrid of three *Mytilus* taxa (*M. galloprovincialis*, *M. platensis* and *M. chilensis*) on the Atlantic coast of Argentina. Hybrids could arised as a result of the introduction of imported samples of farmed mussels. I discovered, that *M. galloprovincialis* populations in South Africa were resulting from the introduction of the Atlantic form of *M. galloprovincialis* not the Mediterranean as previously thought. SNP data appears to be a new tool, which could be used to illustrate the altering the distribution of native and introduced populations of mussels especially in the area influenced by aquacultures. All of the above results are preparing for publication.

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