Szent István University

The Analysis of Bullhead Catfishes (*Ameiurus spp.*) in Hungary with Species-specific Markers and Molecular Genetic Methods

Theses of the Ph.D. Dissertation

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2. BACKGROUD AND OBJECTIVES

2.1 Background

Bullhead catfishes (*Ameiurus spp.*) are non-indigenous but widely spread species in Hungary. Due to their conscious but poorly planned introduction, they were distributed from their native habitat in North America to a number of other countries over the last century. As a species suitable for aquaculture, Bullhead catfishes were introduced not only in the West, in the states of Idaho and California, but also in South America, Asia and Europe. Their introduction was so successful that within a few decades they spread to most European countries except for the British Isles (PINTÉR 1976). In their native habitat, catfishes were regarded as promising and economically important species because of their high growth potential but in Europe they did not meet the expectations of fish farmers. Their strong but delicate body, however, contributes to a beautiful aquarium display, which promoted their spead even in freshwater fishkeeping.

Signs of natural hybridization and introgression between species can be detected in many taxonomic groups (RIESEBERG 1997, ARGUE and DUNHAM 1999, HARDMAN and PAGE 2003, PAYSEUR et al. 2004, NURIA et al. 2009, SANZ et al. 2009). This process may have several evolutionary and ecological consequences such as the merging of taxonomical groups (RHYMER and SIMBERLOFF 1996), which may lead to the appearance of new, reproductively isolated hybrid species, or to the transmission of characteristics important for adaptation which happen through introgression (ARNOLD and MARTIN 2009).

In some cases, this phenomenon may be linked to the invasion of species or to their human-mediated introduction (JOSE MADEIRA et al. 2003, BUCCIARELLI et al. 2002). The latter influences populations of freshwater fish on a considerable scale (FERGUSON 1990, CROSS 2000). Introgression changes the genetic background of the invasive species involved in hybridization, which may

influence the adaptive spreading and the ecological and biological distribution of species. In the case of bullhead catfish species, this phenomenon has been observed even in Hungary.

Applying morphological and molecular genetic methods, the present dissertation examines the distribution, phenotypic traits and hybridization of bullhead catfishes introduced in Hungary. As they are non-indigenous, invasive, economically and ecologically harmful species, their academic study is of utmost importance for aquaculture in natural waters in Hungary.

2.2 Objectives

The main research objectives of the dissertation are the following:

1. to analyse the phenotypic traits of *Ameiurus* species based on a representative number of samples collected at different habitats in Hungary;

2. to develop a research method based on nuclear genetic markers in order to distinguish the three catfish species introduced in Europe (*A. melas*, *A. nebulosus*, and *A. natalis*) as well as their hybrids;

3. to perform mitochondrial sequence analyses to identify the Ameiurus species introduced in Hungary;

4. to prove the distribution frequency of pure species and hybrids based on genomic markers, mitochondrial sequences and phenotypic analysis.

3. MATERIALS AND METHODS

3.1 Sampling Locations

Populations of bullhead catfishes used in experiments were sampled from their natural habitat in Hungarian drainage basins with different menthods. Most of them were caught with fishing rods or electrofishing but some specimens were collected with fyke net trapping.

All research samples come from the following rivers and lakes:

the Dráva at Majláthpuszta, Pécs (52 specimens), the Hármas-Körös at Gyomaendrőd (8 specimens), Lake Vaja (75 specimens), Lake Pilisvörösvár (13 specimens), Lake Adács (56 specimens), the Körös at Dénesmajor (114 specimens), the Jászság Canal connected to the Tisza (6 specimens), the Kettős-Körös at Békéscsaba (11 specimens), Lake Lőrinci at Hatvan connected to the Zagyva (51 specimens), Lake Külső-Béda at Mohács connected to the Danube (30 specimens), and the Szikra Backwater at Tőserdő (50 specimens).

The fish samples collected in 1990 and identified as the hybrid subspecies *Ictalurus nebulosus pannonicus* were obtained from the Fish Collection at the Hungarian Natural History Museum. They comprised one holotype and 13 paratype samples. Small muscle biopsies were taken from these samples under the supervision of the museum personnel.

As a genetic control of the collected samples, 20 genetic samples from reference specimens for each of the three bullhead species introduced in Europe were used. These caudal fin clips collected from fishes in their native habitat in the USA were provided by Prof. Hanping Wang, Ohio State University South Centers.

Morphological examinations were performed at the Department of Aquaculture, Institute of Aquaculture and Environmental Safety,

Faculty of Agricultural and Environmental Sciences, Szent István University, Gödöllő, Hungary. DNA experiments were done in the research laboratories of KÖRET, the Regional University Center of Excellence in Environmental Industry Based on Natural Resources at Szent István University, Gödöllő, Hungary.

3.2 Morpholgical Identification

Morphological analysis was performed on the fish samples collected in Hungary. All parameters that previous were decribed in literatures as distinctive phenotypes were examined. Both subjectively evaluable and objectively measurable parameters were used. Based on the body coloring of the fish, two categories were distinguished: the olive greenbody with yellow belly, and the grey body with white belly. The morphological characters examined include the color of mandibular barbels, the presence of dark circumscribed spots appearing on the sides of the body, the occurrence of marble mottling or an irregular lateral line, as well as the number of rays in the caudal, anal and dorsal fins. In addition, serrations on the pectoral spine as distinctive phenotypes were also examined.

All morphological examinations were conducted after over-anesthetization of fish by clove oil (*Syzygium aromaticum*). Caudal fin clips collected from each specimen for the DNA analyses were stored in absolute ethanol at -20 C until further processing.

3.3 DNA Preparation

Genetic examination began with the maternal inheritance pattern of mtDNA. The methods used for DNA isolation depended on the origin and size of samples but in most cases, especially in Hungarian samples, the so-called “salt precipitation” procedure was used (MILLER et al. 1988). In case of small tissue samples phenol chloroform extraction provided the required quantity of pure DNA. From the formalin-fixed museum samples, DNA was isolated with a kit developed for this particular purpose.

3.4 Identification of Species-specific Genetic Markers

During the adaptation of the mitochondrial PCRs under low stringency, besides the expected DNA fragment, an additional PCR product was also amplified from *A. nebulosus* and *A. melas* samples (*Figure 1*) by cytochrome b-specific primers (CytB_L_14724F and Cytbasa_R). As the size of these fragments was different between the two species, they were used to develop species-specific genetic markers.

The amplified extra fragments were excised from agarose gel and isolated by QIA Quick Gel Extraction Kit (Qiagen), following the manufacturer's recommendations.

Each fragment was ligated into plasmid vector using pGEM-T Easy Vector System (Promega) and transformed into XL1 Blue Escherichia coli cells (Stratagene).

Vector specific T7 and SP6 primers were used to sequence 16 clones containing an insert of the proper size from both *A. nebulosus* and *A. melas* samples.

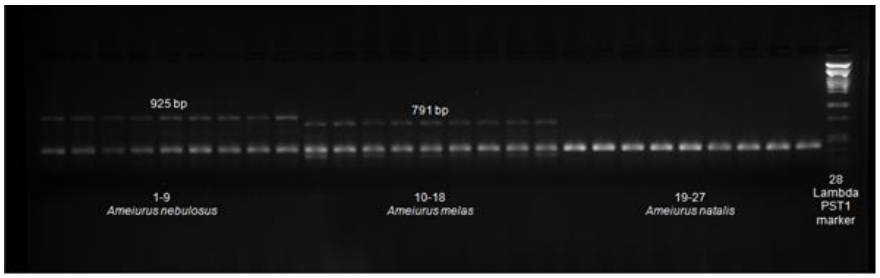

Figure 1. The species-specific fragment pattern amplified by the CytB_L_14724F and Cytbasa_R primers. Lanes from left: (1–9) *A. nebulosos*; (10–18) *A. melas*; (19–27) *A. natalis*; (28) molecular weight marker

3.5 Multiplex PCR Reaction for Species Identification

Specific multiplex and triplex PCR reactions were set up and optimized as recommended by HENEGARIU et al. (1997) for the identification of *A. nebulosus*, *A. melas* and their hybrids.

Based on the previously identified species-specific sequences, a specific primer pair (Neb_F, Neb_R) was designed with Primer Express 3.0.1 software (ABI) that amplified different size of genomic fragments from the two species. In addition, a control (P5 and 16sarL 59) primer pair, which amplified the partial sequence of the mitochondrial 16S rDNA gene, was also used in the multiplex reaction.

The annealing temperature, the duration of elongation as well as the concentration of additives and the two primer pairs were varied until all three products were amplified to similar band intensity from *A. nebulosus*, *A. melas* and their mixed DNA.

3.6 Mitochondrial DNA Analysis

The universal FF2d and FR1d primers were used for the identification of the species with mitochondrial sequence analysis (IVANOVA et al. 2007). These primers amplified a 708 bp fragment of the cytochrome oxidase I (COI) gene.

3.7 Sequencing of PCR Products

The PCR-amplified mitochondrial COI fragments and species-specific fragment containing plasmids were cleaned with NucleoSpin Gel and PCR Clean-up (MachereyNagel) and High speed plasmid mini (Geneaid) kits before sequencing. Then the BigDye Terminator Sequencing Kit (ABI) was used for sequencing.

The sequences were determined on ABI Prism 3130 (ABI) sequencer with POP7 polymer using a 50 cm capillary array**.** Sequences were analyzed using FinchTV 1.4 and Mega5 (TAMURA

et al. 2011) software, and were compared with the genetic sequences found in the GenBank database of NCBI using the BLAST search tool.

3.8 Statistical Analysis of Morphological Results

For the statistical evaluation of the morphological findings, a one way ANOVA test was performed followed by post-hoc Tukey HSD (HUZSVAI et al. 2012, EVANS et al. 1983).

The aim of the PCA is to generate a reduced number of data and produce aggregate results. When the analysis began, there was a large set of strongly correlated variables but their number was reduced with this method. Possibly correlated variables were transformed into a smaller number of uncorrelated variables. Then correlated variable groups were obtained to derive principal components.

PCAGEN 1.2.1 software was used for the calculation of correlation and the p value of correlation between the morphological parameters and genotype (WESSA 2015).

4. RESULTS

4.1 Identification and Characterization of New Nuclear Genomic DNA Markers from *A. melas* and *A. nebulosus*

Under the specific conditions of optimalization, one of the primer pairs (CytB_L_14724F and Cytbasa_R) used for the amplification of the B gene sequence on the mitochondrial cytochrome oxidase amplified one extra species-specific fragment besides the expected bands from both *A. nebulosus* and *A. melas* samples, while only Cytocrom-b specific bands were amplified from *A. natalis*.

These "extra fragments" could be amplified from all of the 20 *A. nebulosus* and 20 *A. melas* control samples from the native habitat in North America, and did not show any contradiction in any samples of the two species.

The full-length nucleotide sequence of the two species-specific fragments was determined and deposited to GenBank (species specific sequence accession numbers: *A. nebulusus* KX943306, *A. melas* KX943305).

The exact length of the sequence derived from *A. nebulosus* and *A. melas* was 925 bp (KX943306) and 791 bp (KX943305) respectively. The two sequences derived from the same locus but the *A. melas* sequence contained four deletions (133 bp), 14 single base pair substitutions and a 1 bp long insertion compared to the *A. nebulosus* sequence.

4.2 Development of a PCR-based Marker System for the Identification of *A. melas, A. nebulosus* and their Hybrids

For a more precise identification of the two species and their hybrids, a new primer pair (Neb_F és Neb_R) was developed that annealed to the consensus region of the two species-specific sequences.

This primer pair amplified a fragment of 591 bp and 475 bp from *A. nebulosus* and *A. melas*, respectively. In addition, it also amplified both fragments from hybrid genomes (*Figure 2*).

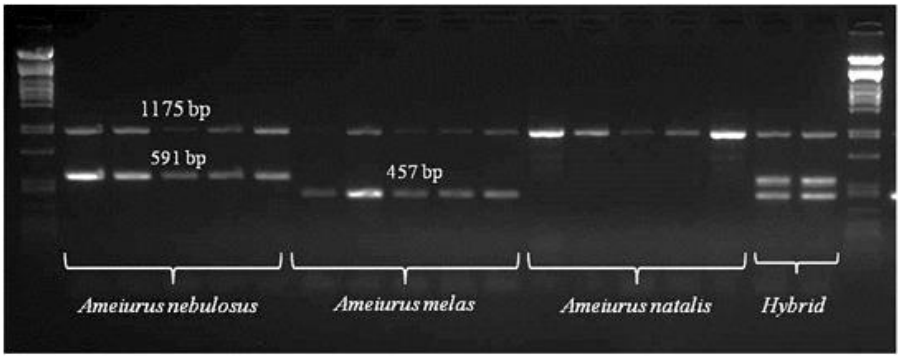

Figure 2 Multiplex PCR reaction developed for bullhead species that facilitates a more efficient identification than the original PCR reaction. The first and the last lanes contain Lambda PstI molecular weight marker.

Besides the species-specific markers, several mitochondrial primers were tested as possible controls for a multiplex PCR. Finally the P5 and 16sarL 59 primers were selected, which amplified a 1.175 bp long fragment of the 16S rDNA gene.

The optimized multiplex PCR amplified two fragments from the original *A. melas* and *A. nebulosus* samples and three from their F1 hybrids, while only the control fragment was amplified from *A. natalis*. However, amoung the Hungarian samples, *A. natalis* hadn't been identified.

All of the available 466 samples were examined with the multiplex PCR test. The results proved that 426 specimens were *A. melas*, and 37 specimens were *A. nebulosus*, whereas only two were hybrids specimens. *A. melas* specimens were present in all habitats, but the *A. nebulosus*-specific fragment pattern was found only in samples from Lake Vaja (19 specimens) and Tőserdő (19 specimens). In addition, the hybrid genotype (three fragments) was present only in two specimens sampled at Tőserdő.

4.3 Mitochondrial COI Gene Sequence Analysis

Mitochondrial sequence analyses were performed for species or maternal lineage identification. First the segment of the Cytochrome Oxidase I (COI) gene sequence used for DNA barcoding was determined (KOCHZIUS et al. 2010, WARD et al. 2005).
The universal oligos (FF2d, FR1d) worked well in the examined *Ameiurus* species and specific fragments with the expected size (708 bp) were amplified.

At least 25% of the available samples were sequenced from each habitat. They were complemented with the samples from those specimens that showed the presence of the *A. nebulosus* specific fragment at nuclear genomic DNA testing or whose coloring identified them unequivocally as *A. nebulosus*.

Altogether 176 mitochondrial COI gene sequences and three specimens of every species from the American control samples were analysed. The sequences of control samples from North America were 100% identical with the *A. melas*, *A. nebulosus* and *A. natalis* sequences in GenBank according to BLAST results.

There were only two of the 11 habitats (Lake Vaja and the Szikra Backwater at Tőserdő) where *A. melas* and *A. nebulosus* coexisted.

When the results of mitochondrial sequence analyses and the multiplex PCR test were contrasted, two specimens (one from the Szikra Backwater at Tőserdő and the other from the Jászság Canal) were identified as post F1 generation hybrids.

Altogether 138 of the Hungarian samples carried an *A. melas* mitochondrial sequence (GenBank species specific sequence accession numbers: KX909375–KX909513) while 37 mitochondrial sequences proved to originate from *A. nebulosus* (GenBank species specific sequence accession numbers: KX909514 - KX909551).
The maternal inheritance pattern of mtDNA and phenotypic sex data of hybrid specimens (3 from the Szikra Backwater at Tőserdő, 1 from the Jászság Canal) provide evidence that hybridization may happen in both direction of sexes.

4.4 Genetic Analysis of Samples from the Museum

DNA isolation from formalin fixed samples is a difficult process and the DNA finally isolated was crashed, poor in quality and small in amount. But the multiplex PCR test worked and gave satisfactory results.
In seven specimens the *A. melas* specific marker was found and five specimens were hybrid according to the genomic DNA tests. Unfortunately, the samples were not suitable for sequencing the mitochondrial COI gene.

4.5 Principal Component Analysis

Although only one sampling location, the Szikra Backwater at Tőserdő provided specimens with all three genotypes (*A melas, A. nebulosus* and their hybrids) identified with the multiplex PCR test, their morphological characters were contrasted with the genetic results. The analysis found that the main morphological differences between the species were the body coloring and the presence of marble mottling. The occurrence of these two characters was 100% identical with the results of the multiplex PCR test and it clearly distinguishes the two species. Hybrids showed maternal inheritance of these morphological characters.

5. CONCLUSIONS AND RECOMMENDATIONS

The use of phenotypic analysis as the primary method of identification of species and hybrid specimens (SPECZIÁR and BERCSÉNYI 2009, RUTKAYOVA et al. 2013) leads to a rather high chance of error and uncertainty, and sometimes it is difficult to evaluate individual parameters objectively.

A more reliable identification is possible with species and origin identification using genetic methods. The new PCR-based marker system is primarily suitable for the identification of F1 hybrids and provides limited information for post F1 generation hybrids. But it proved to be reliable for the identification of *A. melas* and *A. nebulosus* species in samples both from North America and Europe. Presumably F1 hybrid samples were also identified with this method.

Based on the results of genetic species identification, the applicability of morphological parameters often used as identification phenotype was evaluated. The evaluation clarified that neither of the two distinctive phenotypes most widely used for species identification (the number of rays in the anal fin and the serrations of the pectoral spine) is suitable to distinguish the two species in the case of Hungarian and most probably other European samples (HARKA and PINTÉR 1990, RUTKAYOVA et al. 2013).

Among the morphological parameters examined, the marble mottling of the body and grey/oil green body color or the light/yellow belly showed the strongest correlation with the genetically identified species. It was also confirmed by the principal component analyses of the samples from the Szikra Backwater at Tőserdő. The two species and their hybrids coexisted at this sampling location, and the presence of phenotypes cannot have been influenced by the natural conditions of the habitat.

The contradictory findings in case of phenotype parameters are supposedly the result of mixed hybrid genomes in post F1 generations. It confirms the assumption that in the species complexes present at the same habitat, the separation of morphological and genetic characters are decreasing in every generation due to the level of continuous hybridization, backcross, random segregations, and possible recombination of the chromosomes. The mixing of divergent genomes from different parent taxa may generate new genetic combinations leading to new transgressive phenotypes upon which natural selection can act (RIESEBERG et al. 1999, FISS et al. 1997, BELL and TRAVIS 2005, MÜLLER et al. 2010).

Consequently, further research is needed to examine morphological and genetic characteristics of Fx hybrids and also to modelling the introgession. The PCR-based marker system developed for bullhead species needs further nuclear genomic markers to reliably identify post F1 generation hybrids. Mathematical modeling of hybrid identification in post F1 generations need the mimimum of four nuclear genetic markers depending on the generation number, but upward of 70 markers are required for reliable discrimination between pure species and backcrossed hybrids (BOECKLEN and HOWARD 1997). It would be recommended to supplement the marker system with further nuclear DNA markers and test their applicability, sensibility and efficiency on artificially produced F1, F2 or even later generation hybrids.

When surveying the distribution of bullhead catfish species in Hungary, only *A. melas* was found at most sampling locations. *A. nebulosus* was only found at two of the 11 habitats. The presence of *A. natalis* cannot be proved.

Among the 466 specimens examined only two hybrids were found with the genomic DNA tests, while in two cases there was a discrepancy between the results of the mitochondrial genome and genomic DNA tests, which also points to a hybrid character in later generations. However, this is less than 1% of the specimens and this level of the hybridization is similar to that observed in other

intogressive species (MALLET 2005). Introgression changes the genetic background of the invasive species involved in hybridization, which may influence the adaptive spreading and the ecological and biological distribution of species. This phenomenon has been observed in the *Ameiurus* species in Hungary and monitoring these introgression changes in the future may provide models for important ecological processes.

Furthermore, based on mitochondrial genetic results, hybridization may have happened in both direction of sexes, because hybrid specimens were identified both with *A melas* and *A. nebulosus* mitochondrial DNA. This phenomenon is present only in one third of hybridized species and in most species natural hybridization is limited to only one combination (WIRTZ 1999). In the samples examined, all hybrids were fertile and had gonads with gametes.

The research findings confirm the hypotheses that the invasion of *A. melas* started and has not finished yet, and this species invading new habitats gradually replaces *A. nebulosus* not only in the natural waters in Hungary but even all over Europe (HARKA 1997, GARCIA-DE-LOMAS et al. 2009, WILHELM 1998, GANTE and SANTOS 2002, LUSK et al. 2010, POPA et al. 2006, NOWAK et al. 2010, KAPUSTA et al. 2010, MOVCHAN et al. 2014, WILHELM et al. 1999). Monitoring and studying its spread is mostly based on phenotypic analysis but genetic examinations are also recommended to prove previous research findings.

6. NEW SCIENTIFIC RESULTS

1. New nuclear genomic sequences for the identification of *A. melas* and *A. nebulosus* species were isolated and described, which makes the identification of *Ameiurus* species in both European and Hungarian populations possible.

2. A new, simple, quick, cost efficient and repeatable multiplex PCR-based marker system was developed for the identification of *A. melas* and *A. nebulosus* species and their hybrids and also for the discrimination of *A. natalis* species.

3. Based on mitochondrial sequence analyses and nuclear genomic DNA test, was proves that the two most reliable morphological parameters are the (grey/oil green) body or the (light/yellow) belly color and the marble mottling of the body the most usable phenotypes for the species identification of the original introduced *Ameiurus* species in Hungary.

4. The results of mitochondrial sequence analyses and nuclear genomic DNA test based on samples from 11 habitats in Hungary prove that the natural hybridization of *A. melas* and *A. nebulosus* species occurs with very low, less than 1% frequency.

5. Mitochondrial sequence analyses and nuclear genomic DNA test also provide evidence that natural hybridization of *A. melas* and *A. nebulosus* species happened in both direction of sexes and resulted in significant introgression over generations.

6. Morphological analyses combined with genetic examination and statistical evaluation of a representative number of samples confirm for the first time the observation that *A. melas* replaced or replace *A. nebulosus* that was more widely spead in natural waters in Hungary.

7. LIST OF PUBLICATIONS

7.1 Publications related to the topic of the dissertation

Beatrix Béres, Dóra Kánainé Sipos, Tamás Müller, Ádám Staszny, Milán Farkas, Katalin Bakos, László Orbán, Béla Urbányi, Balázs Kovács (2017): Species specific markers provide molecular genetic evidence for natural introgression of bullhead catfishes in Hungary; Peer J., 17 page.

Szabóné Béres et al. / AWETH Vol 5. 4. (2009) Szabóné **Béres Beatrix**, Müller Tamás, Bakos Katalin, Kovács Balázs, Urbányi Béla (2009)*:* Morfológiai és genetikai vizsgálatok magyarországi törpeharcsákon; "II. Gödöllői Állattenyésztési Tudományos Napok" elektronikus újságcikk, Animal welfare, ethology and housing systems; V. évfolyam, Különszám; p: 459-464; Gödöllő, 2009**.**október 16-17.

Béres Beatrix, Bakos Katalin, Müller Tamás, Kovács Balázs, Urbányi Béla (2010): A törpeharcsa fajok genetikai azonosítása, XVI. Ifjúsági Tudományos Fórum kézirat, Pannon Egyetem, Georgikon Kar, Keszthely, 2010. március 25. ISBN:978-963-9639-36-2; Állattan-Állatélettan-Takarmányozástan; Szekció; p:1-5

7.2 Conference presentations related to the topic of the dissertation

Beatrix Beres, Balazs Kovacs, Tamas Muller, Bela Urbanyi (2009): "Morfological determination and genetic identification of Hungarian bullhead catfish population" International

Conference, Trondheim, European Aquaculture Society, Trondheim, Norway 2009.08.14-17; p:104-105

Szabóné Béres Beatrix, Müller Tamás, Bakos Katalin, Kovács Balázs, Urbányi Béla (2009)*:* A magyarországi törpeharcsa állományok morfológiai és genetikai vizsgálata, "II. Gödöllői Állattenyésztési Tudományos Napok", Gödöllő, 2009. október 16-17. p:103

Béres Beatrix, Müller Tamás, Bakos Katalin, Kovács Balázs, Urbányi Béla (2010): A törpeharcsa fajok genetikai azonosítása, XVI. Ifjúsági Tudományos Fórum, Pannon Egyetem, Georgikon Kar, Keszthely, 2010. március 25. ISBN:978-963-9639-36-2 Állattan-Állatélettan-Takarmányozástan Szekció; p:1-5

Béres Beatrix (2010): A magyarországi törpeharcsák vizsgálata morfológiai bélyegek valamint molekuláris genetikai módszerek segítségével; Állattenyésztés-tudományi Doktori Iskola Fórum (ÁTDI), Gödöllő, 2010. április.30. kivonat: 11-15

Béres Beatrix, Csenki Zsolt, Váradi László: Újabb adalékok a törpeharcsa specialitásaihoz; Szent István Egyetem, Halgazdálkodási Tanszék, Gödöllő; XXVIII. Halászati Tudományos Tanácskozás; Szarvas, 2004. május 12-13

Béres Beatrix, Bakos Katalin, Müller Tamás, Urbányi Béla, Kovács Balázs (2010): Molekuláris genetikai vizsgálatok törpeharcsa populációkon; XXXIV. Halászati Tudományos Tanácskozás, Szarvas május 12-13.

Béres Beatrix: Az érdekes törpeharcsa; Halászati Napok, XXVII. Halászati Tudományos Tanácskozás; Szarvas, 2003. május 7-8.

7.3 Posters and abstracts related to the topic of the dissertation

Béres Beatrix, Csenki Zsolt, Váradi László: Újabb adalékok a törpeharcsa specialitásaihoz, XXVIII. Halászati Tudományos Tanácskozás kivonat: 60. p.2004

Béres Beatrix, Staszny Ádám, Kánainé Sipos Dóra, Urbányi Béla, KovácsBalázs: "A magyarországi törpeharcsa állományok morfológiai és genetikai vizsgálata" absztrakt és poszter, XXXIII. Halászati Tudományos Tanácskozás, Szarvas, 2009. július 02.

Beatrix Beres, Balazs Kovacs,Tamas Muller, Bela Urbanyi: "Morfological determination and genetic identification of Hungarian bullhead catfish population", Trondheim, European Aquaculture Society, Trondheim, Norway 2009.08.14-17

Béres Beatrix, Staszny Ádám, Kánainé Sipos Dóra, Urbányi Béla, KovácsBalázs (2009): "A magyarországi törpeharcsa állományok morfológiai és genetikai vizsgálata" absztrakt és poszter, XXXIII. Halászati Tudományos Tanácskozás, Szarvas; 2009. május 27-28.,p:46

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Szabóné Béres Beatrix, Kovács Balázs, Müller Tamás, Urbányi Béla (2009*):* A magyarországi törpeharcsa állományok morfológiai és genetikai vizsgálata**,** "II. Gödöllői Állattenyésztési Tudományos Napok" konferencia kiadvány; Gödöllő, 2009. október 16-17.p:103

Béres Beatrix, Bakos Katalin, Müller Tamás, Kovács Balázs, Urbányi Béla (2010): A törpeharcsa fajok genetikai azonosítása,

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Béres Beatrix, Bakos Katalin, Müller Tamás, Urbányi Béla, Kovács Balázs (2010): Molekuláris genetikai vizsgálatok törpeharcsa populációkon, kivonat; XXXIV. Halászati Tudományos Tanácskozás, Szarvas május 12-13. p:19

Béres Beatrix (2010): Fajspecifikus genetikai markerek törpeharcsa fajok vizsgálatához TUDOC nemzetközi konferencia poszter szekció p:18

7.4 Book chapters

Dr. Horváth László, **Béres Beatrix,** Dr. Urbányi Béla (2011): Ökológiai szemléletű tógazdálkodás - Haltenyésztés hidrobiológiai alapokon; ISBN 978-963-269-218-0; p:1-167

Dr. Horváth László, Dr. Tamás Gizella, Szerkesztette: **Béres Beatrix** (2011 második átdolgozott kiadás): Halivadék nevelés

7.5 Other publications unrelated to the topic of the dissertation

Han-PingWang, ZexiaGao, **Beatrix Beres**, Joseph Ottobre, Geoff Wallat, Laura Tiu, Dean Rapp, Paul O'Bryant, Hong Yao (2008): Effects of estradiol-17β on survival, growth performance, sex reversal and gonadal structure of bluegill sunfish Lepomis macrochirus; Aquaculture 285 (2008) 216–223

Dr. Horváth László**, Béres Beatrix,** Csorbai Balázs (2010): Hozamcsökkenő energiatorlódások a halastavi ökoszisztémákban, kézirat; Halászatfejlesztés 33 –

Fisheries&AquacultureDevelopment 33; ISBN:978-963-7120-31-2; p:5-18.

Dr. Horváth László, **Béres Beatrix,** Csorbai Balázs (2010): Szervesanyag-termelés és hasznosítás a halastavakban I. rész A természetes hozam biológiai alapjai**;** Halászat 103.évfolyam 1. szám, 2010 tavasz. p: 3-7

Dr. Horváth László, **Béres Beatrix,** Csorbai Balázs (2010): Szervesanyag-termelés és -hasznosítás a halastavakban II. rész; Halászat 103.évfolyam 3. szám, 2010 tavasz. p: 86-97

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Urbányi Béla, Müller Tamás, Bokor Zoltán, Szabó Tamás, **Béres Beatrix**, Horváth László (2011): Gazdasági haszonhalak és őshonos halfajok szaporítási és ivadéknevelési kutatásainak eredményei, Állattenyésztés és Takarmányozás folyóirat, 2011. 60. 3. p: 247–262

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