MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM

Investigation of sex-determining factors in African catfish (*Clarias gariepinus*)

Thesis of doctoral (PhD) dissertation

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Gödöllő

2024

The doctoral school's

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1. PRELIMINARY INFORMATION AND OBJECTIVES

African catfish (*Clarias gariepinus* BURCHELL, 1822) belongs to the *Siluriformes* order. It is an extremely suitable species for aquaculture production. Males exhibit higher growth rate, better feed utilization (HENKEN ET AL., 1987), and higher fillet yield (FAO, 2010), therefore monosex production could be advantageous. To create a monosex population with sex manipulation techniques, basic knowledge of the sex determination (SD) mechanism and the sexual development of the species of interest is essential. Despite the importance of African catfish, there was a debate earlier about its sex determination mechanism. Some authors suggested female heterogametic (ZZ/ZW) (OZOUF-COSTAZ ET AL., 1990; VÁRADI ET AL., 1999), whereas others proposed male heterogametic (XX/XY) (GALBUSERA ET AL., 2000; LIU ET AL., 1996) SD system. Recently, it was also suggested based on 'moderately sex-associated' markers that both XX/XY and ZZ/ZW systems coexist in Asian populations of African catfish (NGUYEN ET AL., 2021). Moreover, in addition to genetic sex determination, temperature-induced sex reversal was suggested in Dutch populations with masculinizing effect of higher temperature (SANTI ET AL., 2016; SANTI ET AL., 2017). Santi and colleagues found skewed sex-ratios (90-100% of males) after exposing larvae to 36 °C water temperature and proposed sex-reversal based on this data, though the hypothesis has not been confirmed with molecular techniques.

Sex-specific differences in the expression of sex related genes have not been investigated yet in African catfish in early developmental stages, only following gonad development. Our research group previously isolated a male-specific DNA marker (CgaY1, NCBI genebank ID: AF332597) suitable for molecular sexing from as early as zygote stage, which enables us to study the early sex-specific gene expression. Furthermore, the temperature-induced sex reversal can be tested with the help of the CgaY1 marker, and its presence

supports the idea of XX/XY SD mechanism. Besides, a sex-associated marker can be useful to determine the genomic region responsible for sexual development.

Recently, the whole genome sequence of an African catfish male was published without proofreading on an online server (NGUINKAL ET AL., 2023). This sequence offers numerous possibilities for future research. They successfully assembled a chromosome-level (28 chromosomes) genome and created two haplotypes (Hap1 and Hap2) in addition to the primary (Prim) assembly. Nguinkal and colleagues aligned the CgaY1 sequence to the Hap1 and Prim assemblies, but could not align it to Hap2, therefore they proposed that Hap2 represents the male phenotype. Based on the size of the assemblies, they recommended that African catfish possess ZZ/ZW SD mechanism, however, they did not characterize the sex chromosomes.

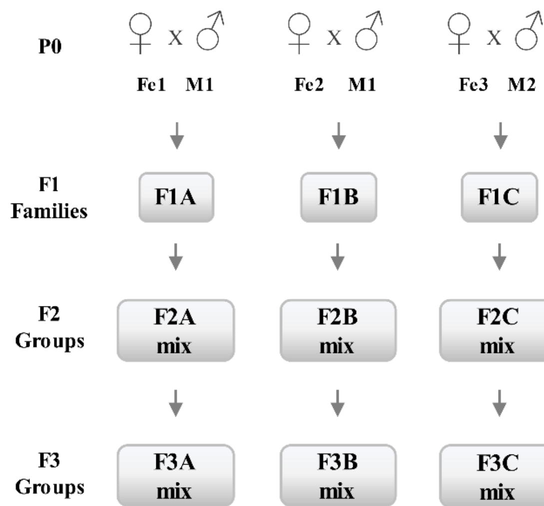
The objectives of my research were the following:

1. Multi-generation validation of the previously isolated CgaY1 marker on Hungarian African catfish stocks and testing it on Vietnamese stocks.
2. Testing 'moderately sex-associated' markers published by others.
3. Investigate the temperature-induced sex reversal suggested by others on Hungarian African catfish stocks.
4. Isolation of novel, sex-associated SNP markers from African catfish with high-throughput sequencing technique.
5. Study the sex-specific differences of sex-related genes in early developmental stages with molecular sexing in heads and bodies of African catfish larvae.

2. MATERIALS AND METHODS

2.1. African catfish genetic crosses and stocks

Five brooders (P0) with matching phenotypic and genotypic sex tested with the CgaY1 marker were crossed pairwise (one brooder was used twice) to create the genetic cross (**Figure 1.**) reared in a recirculating aquaculture system (RAS).

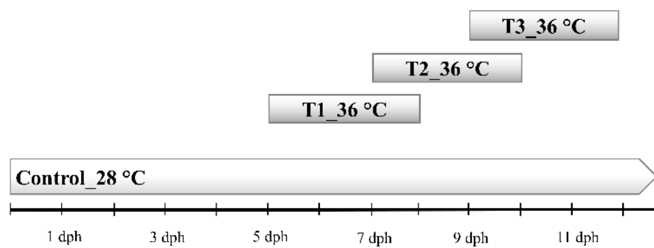


1. Figure: Genetic cross in RAS system. Females- Fe1, Fe2, Fe3, males- M1, M2

The resulting three F1 families (Family F1A, F1B and F1C) were grown in different tanks to maturation without subjecting them to molecular sexing. The F2 generation was produced by ten pairwise full-sib crosses from each F1 family and reared in three different tanks (Groups F2Amix, F2Bmix and F2Cmix) using equal amounts of offspring from each pairwise cross. F2 generation was grown to maturation in three different tanks when molecular and phenotypical (by visual observation of dissected gonadal morphology) sexing was performed. F3 generation was produced by ten pairwise crosses from each F2 groups using brooders with matching genotypic and phenotypic sex. Offspring from the ten crosses were mixed in equal amount in each group (Groups F3Amix, F3Bmix and

F3Cmix) and raised until three months post-hatching in different tanks when they were sexed molecularly and phenotypically (by aceto-carmine squash analysis of dissected gonadal tissue).

To test the temperature-induced sex reversal, I established four groups from each mix of the F3 generation (F3A Mix, F3B Mix, and F3C Mix), each group consisting of 1000 larvae. Then, each group was reared at 36±1°C for 72 hours at three developmental stages: 5-8 (T1), 7-10 (T2), and 9-12 (T3) days post-hatching. The fourth group, the control group, was reared at 28±1°C (**Figure 2.**)



2. Figure: Investigation of the temperature-induced sex reversal. Three groups were reared at 36°C in three different developmental stages (T1 – 5-8, T2 – 7-10, T3 - 9-12 days post-hatching), while the fourth, control group was reared at 28°C.

The P0 generation (5 individuals), three F2 groups (85, 86, 89 individuals), and three F3 groups (50, 48, 93 individuals) were used for validation of the sex-specific CgaY1 marker. To test some moderately sex-linked markers isolated by others, individuals with the CgaY1 marker corresponding to the phenotypic sex were used from the F2 groups (n=16). The temperature-induced sex reversal was investigated on F3 groups (heat-treated and control, n=444). The early sex-specific gene expression of sex related genes was studied on the F3 control groups (n=168).

The applicability of the CgaY1 marker was also tested on a Vietnamese stock of African catfish (n=61). Novel sex-linked SNP markers were isolated from P0 and F2 generations of a stock reared in the flow-through system of Bajcshal Ltd. (n=192).

2.2. Investigation of the sex determination system of African catfish

2.2.1. Phenotypic sex determination

To check the phenotypic sex, the gonads were removed *post-mortem* and placed on slides, then stained with a modified aceto-carmine staining method (GUERRERO & SHELTON, 1974) and investigated under a stereomicroscope.

2.2.2. Molecular sex determination

Following DNA isolation, a duplex PCR reaction was performed amplifying the CgaY1 male-specific marker (1,1 kb) and a control (K1) fragment (486 bp) from males and only the K1 fragment from females. Three primer sequences (Y1-1R, K1-1F, K1-1R) originated from a previous research (KOVÁCS, 2004) and I designed a new, forward primer (Y1-6F). The reaction was optimized, and the size of the PCR product was checked using agarose gel electrophoresis.

2.2.3. Investigation of the temperature effect

The phenotypic sex of all individuals in both the heat-treated and control groups (n=444) was checked (see 2.2.1). In addition, molecular sex determination was performed using the CgaY1 marker (see 2.2.2) by randomly selecting 123 phenotypic males (24, 19, 80 fish from the three crosses) from the heat-treated groups to check their genotypic sex.

2.2.4. Data analysis

For each individual, phenotypic sex was compared with the results of molecular sex determination. From the frequency of recombination, the relative genetic distance (centiMorgan) between the male-specific CgaY1 marker and the presumed sex-determining region was calculated. The deviation from the 1:1 sex ratio was tested in each group using a one-sample Z-test.

2.2.6. Investigation of putative sex-associated markers

Three 'moderately male-associated' (*dtna*, *add3*, *gucd1*) and two 'moderately female-associated' (*dctn4*, *pcdh2ab3*) loci were examined. The marker sequences were aligned to the African catfish genome previously sequenced by our research group (unpublished data) using BLAST software. Primers were designed for the flanking region of four markers, and after DNA isolation a PCR reaction was performed and the length of the products calculated *in silico* were checked using agarose gel electrophoresis.

2.2.7. Isolation of sex-linked SNP markers using ddRAD sequencing

The high-purity DNA samples were digested with PstI and MspI restriction enzymes and then size selection was performed using KAPA PureBeads to obtain fragments between 150-300 bp. Using T4 DNA ligase, double-stranded adapters were ligated to the fragments, then a unique identifier (barcode) was added to each sample with PCR reaction, using the NEBNext Multiplex Oligos for Illumina kit. The samples were 'pooled' in equal concentrations, quality and quantity control was performed on the 'pooled' library and the final, amplified library was diluted to a concentration of 10 pM. Sequencing was performed by Xenovea Ltd. on Illumina NextSeq 500 genome sequencing platform with a read length of 2x150 bp. The data was evaluated in collaboration with Julian Catchen (Department of Evolution, Ecology, and Behavior, University of Illinois, Urbana-Champaign, Urbana, IL). The association between markers and sex was tested using a non-parametric Fisher exact test and Analysis of Molecular Variance (AMOVA). The results were filtered for the highest fixation index (F_{ST}) values (>0.07).

2.3. Study of the sex-specific gene expression

The animals were over-euthanized and then cut into three parts under a stereomicroscope (Leica M205 FA). DNA was isolated from caudal fin tissues for molecular sex determination (see 2.2.2). RNA was isolated from heads and

trunks separately using TRI Reagent. Following DNase I treatment, cDNA was prepared from RNA using random hexamer primers and reverse transcriptase enzyme, then qPCR tests were performed with Step One Plus device, using Hot FIREPol EvaGreen qPCR Mix Plus.

The early expression profile of *vasa*, *foxl2*, *sox3*, *sox9*, *amhr2*, *pten*, and *mark2* genes was investigated. The reactions were optimised using standard curve analysis to an efficiency of 95-105%. From five potential reference genes (*ef1a*, *gapdh*, *rpl13*, *actb*, *18S*), I determined the most suitable reference gene for my studies, actb.

I studied four groups (male, female, head, trunk) at 10, 15, 20, 25, 30, and 40 days post-hatching (dph). Six pools of 8 dph fish were used as control groups. For each sample, I determined the average threshold cycle number (Ct) from the average of three technical replicates. The results were verified using gel electrophoresis and melt curve analysis. I calculated the delta Ct (ΔCt), delta-delta Ct (ΔΔCt), and RQ (relative quantification; RQ = 2^(-ΔΔCt)) values. The normal distribution of the groups was checked using Kolmogorov-Smirnov test. Depending on normality, I examined the deviation from the control group using ANOVA or Kruskal-Wallis test, and differences between sexes using t-test or Mann-Whitney test.

3. RESULTS AND DISCUSSION

3.1. Sex determination mechanism of African catfish

3.1.1. Results of the validation of the CgaY1 marker

During the multi-generational validation of the CgaY1 marker, molecular sexing predicted the phenotype with 96.4% fidelity. Reliability in the F2 generation was 97.3% (95.3-98.8%), while in the F3 generation groups, it was 95.3% (94.6-96%). Among the 451 individuals examined, I found differences between genotypic and phenotypic sex in only 16 cases (3.5%): CgaY1 could not be amplified in five males, whereas in eleven phenotypic females, it was present. These cases were confirmed by a second PCR reaction. The likely cause of these discrepancies is a recombination event between the sex-specific marker and the sex-determining region. In this case, the average frequency of recombination in the F2 and F3 generations was 2.69% and 4.51%, respectively. Thus, the measure of genetic distance is estimated to be between 2.69 cM (centiMorgan) and 4.51 cM per generation, with an average of 3.6 cM. No significant differences ($p > 0.05$) were found in either phenotypic or genotypic sex compared to the expected 1:1 sex ratio in any of the examined groups.

I also assessed the applicability of the CgaY1 marker in 61 individuals from Vietnam. In the Vietnamese population, a higher proportion (16.39%) of difference between phenotype and genotype were found: in six phenotypic males the CgaY1 marker could not be amplified, while in four females it was present. The higher rate of discrepancies can be attributed to the fact that phenotypic sex determination in the Vietnamese population was based on the shape of the genital papilla, since dissection of the gonads was not possible, which is less reliable.

3.1.2. Results of testing the temperature-effect

Out of 123 males randomly selected from the heat-treated groups based on phenotype, only four individuals (3.25%) failed to amplify the CgaY1 male-

specific fragment. This proportion did not differ significantly from the control group, where discrepancies between phenotype and genotype were found in 9 out of 191 examined individuals (4.71%). I could not find statistically significant differences from the 1:1 phenotypic sex ratio (the proportion of males was 44%, 53%, and 44% in the F3A, B, and C Mix treated groups, respectively) in any of the heat-treated groups, and comparing the sex ratios of the heat-treated groups to those of the control groups (the proportion of males was 58%, 58%, and 50% in the F3A, B, and C Mix control groups, respectively), I did not observe significantly higher male sex ratios. Additionally, I observed a high mortality rate in the heat-treated groups: 82±3% after 90 days post-hatching in the treated groups, compared to 51±5% in the control groups, and in many cases, I observed morphological abnormalities.

3.1.3. Results of testing putative sex-linked markers published by others

Among the 'moderately sex-linked' markers (*gucd1*, *add3*, *dtna* for male-specific and *dctn4*, *pcdh2ab3* for female-specific) published by other authors (NGUYEN ET AL., 2021), I was unable to detect the *gucd1* marker in the genome of African catfish sequenced by our research group (unpublished data) using the BLAST software. For the other four markers, I found only one significant match in the genome. I examined the applicability of these four potential markers in 8 male and 8 female individuals from the F2 generation reared in the RAS system. In all four PCR reactions, we observed the same product size, varying by marker, in both sexes, which fell within the size range predicted by *in silico* calculations (194 bp for *dtna*, 204 bp for *add3*, 226 bp for *dctn4*, 195 bp for *pcdh2ab3*).

3.1.4. Isolating sex-specific SNP markers

High-throughput sequencing resulted in the identification of 69,344 ddRAD loci from the genomes of 95 female and 97 male individuals. For further analyses, only those loci were used that were present in at least 50% of the

individuals from both sexes, leaving a total of 20,841 loci containing a total of 24,743 single nucleotide polymorphisms (SNPs) in females and 24,020 in males. The number of private alleles was 1,811 in females and 1,835 in males.

The mean value of the fixation index (F_{ST}) was 0.0036 for males and females. The twelve SNP markers with the highest (> 0.07) F_{ST} values were located at ten loci, with one locus containing three markers. The sixth (0.2592) and eleventh (0.1953) SNP markers had the highest F_{ST} values. The ten loci were aligned to the African catfish genome using the BLASTn algorithm (megablast) recently published (NGUINKAL ET AL., 2023) and seven of them, including the two with the highest F_{ST} and LOD values and the one containing three SNP markers, were located on chromosome 24. Five of the seven loci were located in close proximity to each other, in a region of approximately 1.2 Mb in length, containing more than forty protein coding sequences, including the gene encoding the androgen receptor (*ar*).

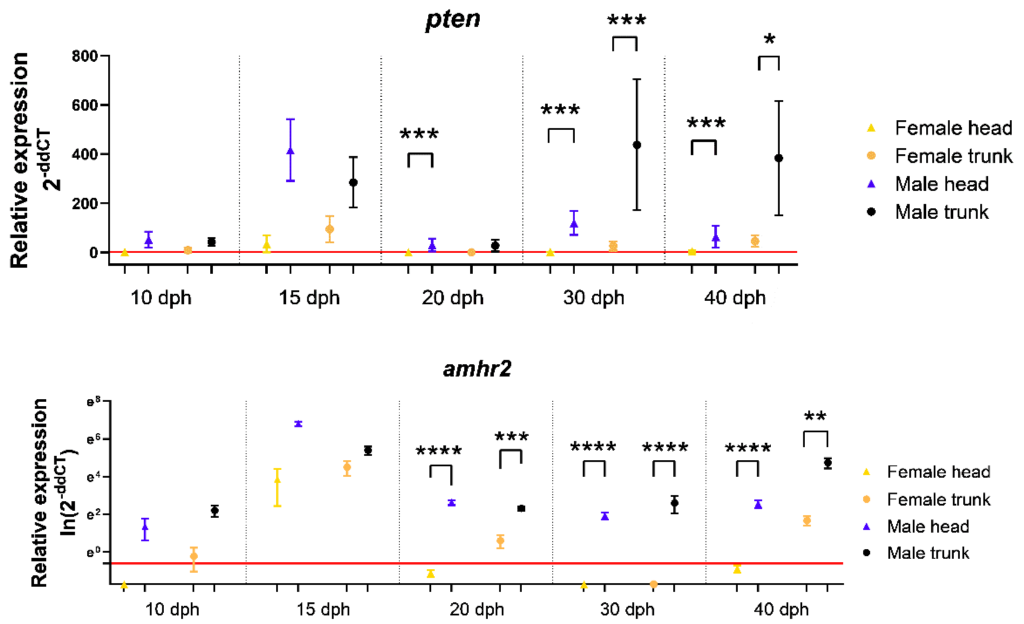
One of the loci was located on the sequence of a transcriptional regulator ATRX-like (Gene ID: 128512310) gene annotated by Nguinkal and colleagues (2023) and the SNP marker was located on an exon of this gene. In mammals, the gene encodes an X chromosome-linked zinc-finger helicase (STAYTON ET AL., 1994; VILLARD ET AL., 1997), mutation of which causes sex reversal in XY individuals (REARDON ET AL., 1995). One of the most strongly linked SNP markers wass located on an intron of a relaxin family peptide receptor 2-like (Gene ID: 128511954) gene, which in mammals encodes a G-protein coupled transmembrane receptor (HSU ET AL., 2002). The ligand of this receptor is the insulin-like factor 3 (INSL3), a relaxin-type peptide expressed in Leydig cells of the testis, which regulates Wolffian tube maintenance, maintenance of reproductive organs and testis descent (KUMAGAI ET AL., 2002) in mammals.

Interestingly, when I used BLAST analysis to align the 2,582 bp long male-specific CgaY1 marker (AF332597.1) to the Prim assembly of the African catfish genome (NC_071100.1) published in the NCBI database, I found it on the

unplaced genomic sequence (scaffold) number 36 (NW_026521003.1) with 99% coverage and 97.2% identity. I also successfully aligned the CgaY1 marker to the whole genome sequence sequenced by our research team and found it on a scaffold (scf484). The scf484 (89.533 bp) scaffold was also aligned to scaffold 36 of the Prim assembly published by Nguinkal et al. This region contains 21 annotated protein coding genes, including genes encoding histone proteins and ribose phosphate phosphokinase, as well as the *mark2* and *pten* genes, and the *amhr2* gene, which functions as a master sex determiner gene in other species and is important in vertebrate sexual differentiation.

3.2. A korai ivaronkénti génkifejeződés eredményei

I have examined the expression of *foxl2* and *sox9b* genes involved in ovarian differentiation, *sox3*, *amhr2*, *pten* and *mark2* genes involved in testis formation, and the *vasa* gene, which is typical for primordial germ cells (PGCs), earlier than 40 days after hatching, using molecular sexing. Higher mRNA levels were observed for *sox9b*, *foxl2*, *sox3* and *vasa* genes at 15 dph compared to the 8 dph mixed sex control group. By comparing the differences between sexes, among the genes involved in ovarian differentiation, *sox9b* was higher in female heads at 20 dph. Among the genes involved in testis differentiation, two genes located near the male-specific CgaY1 marker, *amhr2* and *pten*, showed significantly higher expression in both male heads and trunks in several developmental stages from 20 dph on (see **Figure 3**.). Based on the information available in literature, both genes regulate the proliferation of PGCs in mammals, and *pten* inhibits their proliferation in mammals (CHU & TARNAWSKI, 2004; KIMURA ET AL., 2003; YIN AND SHEN, 2008). In several species of bony fishes, the first sexual difference is the higher amount of PGCs in females, which in some cases directly decides the fate of the gonad (SIEGFRIED, 2010; TZUNG ET AL., 2015; YE ET AL., 2019).



3. Figure: *Pten* and *amhr2* genes have higher expression levels in males. Results of the mRNA level in female and male heads and trunks, at 10, 15, 20, 25, 30 and 40 dph, significant differences marked with asterisks (p).

4. CONCLUSIONS

The male-specific CgaY1 marker predicted the phenotype correctly in 96% of the individuals in a three-generation cross. I also found 84% reliability in a Vietnamese stock, which is genetically distant from the Hungarian stocks. In addition, female specific markers (*dctn4* and *pcdh2ab3*) published by others (NGUYEN ET AL., 2021) supporting the idea of a ZZ/ZW sex determination mechanism did not show differences between sexes in my experiments. Based on my results, I suggest that the African catfish stocks I studied possess a male heterogametic (XX/XY) sex determination mechanism only. Based on the recombination frequency, the average genetic distance between the sex determining locus and the male-specific CgaY1 marker is 3.6 cM, which suggests a close linkage.

Based on the results of high-throughput sequencing, I suggest that the genomic region involved in sexual development is located on chromosome 24 of the recently published (NGUINKAL ET AL., 2023) African catfish genome. However, the male-specific CgaY1 marker was aligned to unplaced genomic scaffold 36, which also contained the sequence of the *pten*, *mark2*, and *amhr2* genes. Based on these results, I suggest that the genomic region involved in sex determination might be located on chromosome 24, but the unplaced scaffold 36 also plays an important role in the process.

I could not detect sex reversal in the Hungarian stocks following heat treatment: I could not prove it either by molecular sexing or by observing skewed sex ratios. In addition, I observed high mortality in the treated groups, suggesting that the high-temperature treatment is not suitable for the establishment of monosex populations in Hungarian stocks.

The early expression of *vasa*, *foxl2*, *sox3*, *sox9*, *amhr2*, *pten* and *mark2* genes suggests that the first signs of sexual differentiation are detectable as early as 15 dph and that signals from the head (presumably the brain) are involved. I found a difference between sexes in the amount of *sox9* mRNA, which was higher

in female heads at 20 dph. In addition, the amount of *pten* and *amhr2* mRNAs, was higher in males in both heads and trunks at several developmental stages. *Pten* and *amhr2* genes regulate the proliferation of PGCs in mammals and the first sign of sexual dimorphism in several species of bony fish is the higher number of PGCs in females. Based on these data, I suggest that in African catfish these genes might be involved in the development of male phenotype by inhibiting PGC proliferation in males.

Suggestions

I suggest testing of the male-specific CgaY1 marker in African catfish stocks from Thailand. I also suggest to cross individuals with not matching phenotypic and genotypic sex tested with the CgaY1 marker, then to examine the sex ratio of the resulting progeny. I recommend a more detailed analysis of chromosome 24 and scaffold 36 of the genome published by Nguinkal et al. I suggest lowering the temperature of the heat treatment to sustain a lower mortality rate and investigating temperature induced sex-reversal in other lines, selected stocks or individual crosses (families).

I recommend a high-throughput transcriptome study at 15 dph, preferably in brains and gonads separately, and in the brains of sex-reversed individuals. I suggest to perform an *in situ* hybridization at this developmental stage to test if there is a difference in the number of PGCs between sexes and, if so, to investigate the role of the *pten* and *amhr2* genes in this process by gene knockout or gene silencing. I also suggest to further investigate *pten* and *amhr2* genes and to analyse the relationship between these genes.

5. NEW SCIENTIFIC RESULTS

1. I have determined that the Hungarian and Vietnamese populations of African catfish (*Clarias gariepinus*) examined exhibit exclusively male heterogametic (XX/XY) sex determination system.
2. Through genotyping of 451 individuals across three generations, I validated the previously isolated CgaY1 male sex-specific DNA marker. I confirmed that the CgaY1 male-specific marker is inherited in linkage with the sex-determining region, situated at a genetic distance of 3.4-3.6 cM from it.
3. By subjecting African catfish populations to heat treatments at 36°C conducted at 5-8, 7-10, and 9-12 days post-hatching, I refuted the hypothesis that temperature-induced sex reversal could occur in Hungarian populations.
4. I isolated more than 24,000 polymorphic SNP markers from African catfish females and a similar number of markers from males, of which twelve are presumed to be strongly sex-linked. I determined that these markers are located on chromosome 24 of the African catfish. This confirmed previous indications suggesting the likely location of the sex-determining region on this chromosome.
5. In African catfish, I examined early sex-specific gene expression differences for the first time using molecular sex determination, before the appearance of the first phenotypic signs of sex, in the head and trunk. I found that sexual differentiation likely begins around 15 days post-hatching.
6. Furthermore, I observed significant differences in the quantities of *sox9*, *amhr2*, and *pten* mRNAs between sexes before the differentiation of gonads. Consequently, I suggest that the products of these genes play a role in the process of sexual differentiation in African catfish.

6. PUBLICATIONS RELATED TO THE TOPIC OF THE DISSERTATION

6.1. Publications in scientific journals

Balogh, R. E., Csorbai, B., Guti, C., Keszte, S., Urbányi, B., Orbán, L. & Kovács, B. (2023): Validation of a male-specific DNA marker confirms XX/XY-type sex determination in several Hungarian strains of African catfish (*Clarias gariepinus*). *Theriogenology* 205, 106–113p.
https://doi.org/10.1016/j.theriogenology.2023.04.017

6.2. Oral presentations

Balogh, R. E., Csorbai, B., Guti, C., Urbányi, B., Catchen, J., Orbán, L. & Kovács, B. (2024): Revisiting the sex determination and sexual differentiation of African catfish (*Clarias gariepinus*). 59th Croatian & 19th International Symposium on Agriculture, Dubrovnik, Croatia, 12-15 February 2024

Balogh, R. E., Csorbai, B., Guti, C., Keszte, S., Bíró, A., Péter, D., Urbányi, B., Kovács, B. (2021): Early expression profile of sex-related genes in African catfish (*Clarias gariepinus*). 56th. Croatian & 16th International Symposium on Agriculture, Vodice, Croatia, 5-10 September 2021

6.3. Poster presentations

Balogh, R. E., Csorbai, B., Guti, C., Keszte, S., Urbányi, B., Orbán, L., Kovács, B. (2019): Applicability of an African catfish (*Clarias gariepinus*) sex specific DNA marker. 54 Croatian & 14 International Symposium on Agriculture, Vodice, Croatia, 17-22 February 2019

Guti, C., Kovács, B., Barta, E., Keszte, S., Kánai, S. D., **Balogh, R. E.**, Uri, C., Patócs, A., Pongor, S. L., Müller, T., Orbán, L., Urbányi, B. (2017): Whole transcriptome sequencing and sex-specific expression of the African catfish (*Clarias gariepinus*). Aquaculture Europe 17, Dubrovnik, Croatia, 17-20 October 2017

Kovács, B., Barta, E., Guti, C., Keszte, S., Kánai, S. D., **Balogh, R. E.**, Uri, C., Patócs, A., Pongor, S. L., Müller, T., Orbán, L., Urbányi, B. (2017): Preliminary results of de novo whole genome sequencing of the African catfish, *Clarias gariepinus* (Burchell, 1822). Aquaculture Europe 17, Dubrovnik, Croatia, 17-20 October 2017

7. PUBLICATIONS NOT RELATED DIRECTLY TO THE TOPIC OF THE DISSERTATION

7.1. Publications in scientific journals

Bláha, M., Weiperth, A., Patoka, J., Szajbert, B., **Balogh, R. E.**, Staszny, Á., Ferincz, Á., Lente, V., Maciaszek, R., Kouba, A. (2022): The pet trade as a source of non-native decapods: the case of crayfish and shrimps in a thermal waterbody in Hungary, *Environmental Monitoring and Assessment* 194(10), e795, https://doi.org/10.1007/S10661-022-10361-9

Piria, M., Jelkic, D., Gavrilovic, A., Horváth, Á., Kovács, B., **Balogh, R. E.**, Spelic, I., Radocaj, T., Vilizzi, L., Ozimec, S., Opacak, A. (2022): Finding a hybrid African catfish (*Clariobranchus*) in the Danube river, *Journal of Vertebrate Bilogy* 71(22008), 1-8p., https://doi.org/10.25225/JVB.22008

Keszte, S., Ferincz Á., Tóth-Ihász, K., **Balogh, R. E.**, Staszny, Á., Hegyi, Á, Takács, P., Urbányi, B., Kovács, B., (2021): Mitochondrial sequence diversity reveals the hybrid origin of invasive gibel carp (*Carassius gibelio*) populations in Hungary, *PeerJ*, 9, e12441 https://doi.org/10.7717/peerj.12441

7.2. Book chapters

Kovács B., Lehoczky, I., **Balogh, R. E**., Molnár T. (2022): A sügér biológiája és tenyésztése - A sügér genetikai háttere és genetikai erőforrásai, szerk.: Bokor, Z., Csorbai, B., Urbányi, B., Magyar Agrár- és Élettudományi Egyetem, Akvakultúra és Környezetbiztonsági Intézet, Gödöllő, Magyarország, 166 p. pp. 41-61.

Kovács B., **Balogh, R. E**., Péter, D. (2021): Horgászati szempontból jelentős pontyfélék biológiája és tenyésztése - Egyes horgászati szempontból jelentős pontyfélék genetikája, szerk.: Urbányi, B., Szabó, T., Horváth, Á., Magyar Agrár- és Élettudományi Egyetem, Akvakultúra és Környezetbiztonsági Intézet, Gödöllő, Magyarország, 262 p. pp. 53-71.

7.3. Oral presentations

Balogh R. E., Péter, D., Bíró, A., Kobolák, J., Varju-Katona, M., Szilágyi, G., Bokor, Z., Urbányi, B., Kovács, B. (2023): Selection programme for better performance of African catfish (*Clarias gariepinus*). 58th Croatian & 18th

International Symposium on Agriculture, Dubrovnik, Croatia, 12-16 February 2023

7.4. Poster presentation

Balogh R. E., Kovács, B., Urbányi, B., Marinović, Z., Kitanović, N., Gavrilović, A., Jug-Dujaković, J., I., Radočaj, T., Barić, O., Allison, J., Horváth, Á. (2023): Molecular species identification of Adriatic oysters. Aquaculture Europe 2023, Vienna, Austria, 18-21 September 2023

Balogh R. E., Péter, D., Bíró, A., Kobolák, J., Bokor, Z., Urbányi, B., Kovács, B. (2022): Multi generation selection and performance test of an African catfish (*Clarias gariepinus*, Burchell, 1822) line selected for better utilization of low fish-meal feed. Aquaculture Europe 2022, Rimini, Italy, 27-30 September 2022

Kitanović N., **Balogh R. E.**, Marinović Z., Kovács B., Csenki Zs., Urbányi B., Horváth Á. (2022): Steps towards establishing a primary culture of zebrafish previtellogenic ovarian follicles. 8th International Workshop on the Biology of Fish Gametes, Gdansk, Poland, 20-23 September 2022

Balogh R. E., Péter, D., Bíró, A., Keszte Sz., Urbányi, B., Lehoczky, I., Edviné M., E., Kovács, B. (2021): Genetic analysis of nine Hungarian common carp (*Cyprinus carpio*) strains with a novel multiplex PCR set for tetranucleotide microsatellites. Aquaculture Europe 2021, Funchal, Madeira, 4-7 October 2021

Balogh R. E., Varju-Katona, M., Péter, D., Bíró, A., Keszte Sz., Kobolák, J., Pataki, B., Kitanovic, N., Szilágyi, G., Urbányi, B., Kovács, B. (2021): Preliminary results of an African catfish (*Clarias gariepinus*) selection programme for better utilization of low fish-meal feed. Aquaculture Europe 2021, Funchal, Madeira, 4-7 October 2021

Balogh R. E., Varju-Katona, M., Ihász, K., Keszte Sz., Lujic, J., Šćekić, I., Kitanović, N., Urbányi, B., Kovács, B. (2020): Growth performance of African catfish (*Clarias gariepinus*) fed with low fish-meal feed. 55th Croatian & 15th International Symposium on Agriculture, Vodice, Croatia, 16-21 February 2020

Balogh R. E., Guti, Cs., Ihász, K., Keszte, Sz., Lehocky, I., Urbányi, B., Kovács, B. (2019): Population genetic analysis of wild common carp (*Cyprinus carpio*) populations from Hungary with a newly designed multiplex microsatellite set. Aquaculture Europe, Berlin, Germany, 7-10 October 2019

Kovács, B., Kánainé, S. D., **Balogh R. E.**, Guti, Cs., Ihász, K., Keszte, Sz., Urbányi, B. (2019): Microsatellite set for parentage analysis of African catfish, *Clarias gariepinus* (Burchell, 1822). Aquaculture Europe, Berlin, Germany, 7-10 October 2019

Balogh R. E., Keszte, Sz., Guti, Cs., Kánainé, S. D., Lehocky, I., Várkonyi, L., Bokor, Z., Bernáth, G., Urbányi, B., Kovács, B. (2019): A novel multiplex microsatellite set and its application on common carp (*Cyprinus carpio*). 54th Croatian & 14th International Symposium on Agriculture, Vodice, Croatia, 17-22 February 2019

Reining, M., **Balogh R. E.**, Balogh, E., Butz, H., Németh, K., Bakos, K., Gazsi, Gy., Kövesi, J., Csepeli, A., Kovács, R., Csenki, Zs., Urbányi, B., Patócs, A. (2016): In situ hybridization of DLK1 and DIO3 tumor suppressor genes in zebrafish, 8th International Symposium on Fish Endocrinology, Gothenburg, Sweden, 28 June -2 July 2016

BIBLIOGRAPHY

CHU, E. C. AND TARNAWSKI, A. S. (2004). PTEN regulatory functions in tumor suppression and cell biology. *Med. Sci. Monit.* **10**, 235–242.

GALBUSERA, P., VOLCKAERT, F. A. . AND OLLEVIER, F. (2000). Gynogenesis in the African catfish Clarias gariepinus (Burchell, 1822). *Aquaculture* **185**, 25–42.

GUERRERO, R. D. AND SHELTON, W. L. (1974). An aceto-carmine squash method for sexing juvenile fishes. *Progress. Fish-Cultist* **36**, 56–56.

HENKEN, A. M., BOON, J. B., CATTELL, B. C. AND LOBEE, H. W. J. (1987). Differences in growth rate and feed utilization between male and female African catfish , Clarias gariepinus (Burchell 1822). *Aquaculture* **63**, 221–232.

HSU, S. Y., NAKABAYASHI, K., NISHI, S., KUMAGAI, J., KUDO, M., SHERWOOD, O. D. AND HSUEH, A. J. W. (2002). Activation of Orphan Receptors by the Hormone Relaxin. *Science (80-.).* **295**, 671–674.

KIMURA, T., SUZUKI, A., FUJITA, Y., YOMOGIDA, K., LOMELI, H., ASADA, N., IKEUCHI, M., NAGY, A., MAK, T. W. AND NAKANO, T. (2003). Conditional loss of PTEN leads to testicular teratoma and enhances embryonic germ cell production. *Development* **130**, 1691–1700.

KOVÁCS, B. (2004). Investigation of the sex of African catfish with molecular genetic methods (in Hungarian). *PhD thesis*.

KUMAGAI, J., HSU, S. Y., MATSUMI, H., ROH, J., FU, P., WADE, J. D., BATHGATE, R. A. D. AND HSUEH, A. J. W. (2002). INSL3/Leydig insulin-like peptide activates the LGR8 receptor important in testis descent. *J. Biol. Chem.* **277**, 31283–31286.

LIU, S., YAO, Z. AND WANG, Y. (1996). Sex hormone induction of sex reversal in the teleost Clarias lazera and evidence for female homogamety and male heterogamety. *J. Exp. Zool.* **276**, 432–438.

NGUINKAL, J. A., ZOCLANCLOUNON, Y. A. B. AND BRUNNER, R. M. (2023). Haplotype-resolved assembly of the African catfish (Clarias gariepinus) provides insights for semi-terrestrial adaptation of airbreathing catfishes. *bioRxiv* 1–27.

NGUYEN, D. H. M., PANTHUM, T., PONJARAT, J., LAOPICHIENPONG, N., KRAICHAK, E., SINGCHAT, W., AHMAD, S. F., MUANGMAI, N., PEXACHOKNAGUL, S., NA-NAKORN, U., ET AL. (2021). An investigation of ZZ/ZW and XX/XY sex determination systems in North African catfish (Clarias gariepinus , Burchell , 1822). *Front. Genet.* **11**, 1–15.

Ozouf-Costaz, C., Teugels, G. G. and Legendre, M. (1990). Karyological analysis of three strains of the African catfish, Clarias gariepinus (Clariidae), used in aquaculture. *Aquaculture* **87**, 271–277.

Reardon, W., GIBBONS, R. J., WINTER, R. M. and BARAITSER, M. (1995). Male pseudohermaphroditism in sibs with the α-thalassemiamental retardation (ATR-X) syndrome. *Am. J. Med. Genet.* **55**, 285–287.

Santi, S., Gennotte, V., Toguyeni, A., Mélard, C., Antoine, N. and Rougeot, C. (2016). Thermosensitivity of the sex differentiation process in the African catfish , Clarias gariepinus: Determination of the thermosensitive period. *Aquaculture* **455**, 73–80.

Santi, S., Rougeot, C., Toguyeni, A., Gennotte, V. and Kebe, I. (2017). Temperature preference and sex differentiation in African catfish , Clarias gariepinus. *J. Exp. Zool.* **00**, 28–37.

Siegfried, K. R. (2010). In search of determinants: Gene expression during gonadal sex differentiation. *J. Fish Biol.* **76**, 1879–1902.

Stayton, C. L., Dabovic, B., Gulisano, M., Gecz, J., Broccoll, V., Glovanazzl, S., Bossolasco, M., Monaco, L., Rastan, S., Boncinelli, E., et al. (1994). Cloning and characterization of a new human Xq13 gene, encoding a putative helicase. *Hum. Mol. Genet.* **3**, 1957–1964.

Tzung, K. W., Goto, R., Saju, J. M., Sreenivasan, R., Saito, T., Arai, K., Yamaha, E., Hossain, M. S., Calvert, M. E. K. and Orbán, L. (2015). Early depletion of primordial germ cells in zebrafish promotes testis formation. *Stem Cell Reports* **4**, 61–73.

Váradi, L., Benkó, I., Varga, J. and Horváth, L. (1999). Induction of diploid gynogenesis using interspecific sperm and production of tetraploids in African catfish, Clarias gariepinus Burchell (1822). *Aquaculture* **173**, 401–411.

Villard, L., Lossi, A. M., Cardoso, C., Proud, V., Chiaroni, P., Colleaux, L., Schwartz, C. and Fontés, M. (1997). Determination of the genomic structure of the XNP/ATRX gene encoding a potential zinc finger helicase. *Genomics* **43**, 149–155.

Ye, D., Zhu, L., Zhang, Q., Xiong, F., Wang, H., Wang, X. and He, M. (2019). Abundance of early embryonic primordial germ cells promotes zebrafish female differentiation as revealed by lifetime labeling of germline. *Mar. Biotechnol.* **21**, 217–228.

Yin, Y. and Shen, W. H. (2008). PTEN: A new guardian of the genome. *Oncogene* **27**, 5443–5453.