Summary of results

The primary focus of this project was to investigate the evolutionary dynamics of chromosomal rearrangements within the family Pipidae. To achieve this, we analyzed members from the genera *Xenopus*, *Silurana*, and *Hymenochirus*.

Initially, we examined the dynamics of rearrangements and chromosomal morphology in the allotetraploid frog, *X. calcaratus* and how they affected the evolution of these frogs. Our findings revealed distinct characteristics in the subgenomes of *X. calcaratus*. The a-subgenome is more conserved and resembles the genome of the diploid species *X. tropicalis*, whereas the b-subgenome has differentiated more from the original progenitor and went through more modifications, showing significant differences in the structure of the chromosomes, including different repetitive sequences and heterochromatic blocks. We also discussed a specific chromosomal translocation observed in *X. mellotropicalis* but not found in *X. calcaratus*. This translocation is significant because it highlights the differences in chromosomal rearrangements between these two species. Our study proposed that a single allotetraploidization event led to the emergence of *X. mellotropicalis*, *X. epitropicalis*, and *X. calcaratus*. The translocation happened after the divergence of *X. calcaratus* but before the speciation of *X. mellotropicalis* and *X. epitropicalis*. Alternatively, if two independent allotetraploidization events occurred, probably the translocation occurred either diploid ancestor of the common one of *X. mellotropicalis* and *X. epitropicalis*, the most recent probably. This finding underscores the slow nature of genome evolution in these species and the role of chromosomal rearrangements in their divergence.

We investigated how polyploidization and divergence have influenced the evolution of repetitive elements in six species of African clawed frogs. Combining FISH and genomic data, we were able to map U1 and U2 small nuclear RNAs and histone H3 in both diploid and allotetraploid species. The results showed that the number and position of these repetitive elements were conserved in the diploid and tetraploid species *X. tropicalis* and *X. calcaratus*, both from subgenus *Silurana*, while variation was observed among the allotetraploid species from the subgenus *Xenopus*. We realized that allotetraploid species could have originated from two different independent polyploidization events that exhibited different patterns of repetitive element distribution. Younger allotetraploids, like *X. calcaratus*, have twice as many signals as their diploid relatives, whereas older allotetraploids showed more variation. These findings suggest that polyploidization initially duplicates tandem repeats, but their copy number can vary over time due to reduction and expansion, highlighting the complex evolutionary dynamics of repetitive elements in these frogs.

We began an in-depth analysis of the genome of *X. epitropicalis*. However, due to logistical constraints (waiting for the delivery of the frogs, breeding, sequencing, and analysis time), we were only able to compare our existing *X. borealis* sequence to analyze the factual expression of both subgenomes and to compare the homology between the two subgenomes, which revealed a similarity of around 93-94%. Although this preliminary data does not allow us to make definitive statements, it provides a good initial insight for the continuation of these experiments beyond this thesis frame.

Finally, we turned our attention to another frog, *H. boettgeri*. We discovered that the *Boettgeri* dwarf clawed frog from the Congo is tetraploid, possessing four sets of chromosomes (2n = 36). This finding suggests that the wild population is not conspecific with the captive populations, which are diploid (2n = 20A + 1B chromosomes). We indicated that the karyotype of tetraploid frogs could have been evolved through the fusion of two chromosomes followed by allotetraploidization, making it functionally diploid, as seen in the polyploid frogs of *Xenopus* subgenome. These findings highlight significant differences between wild and captive populations, suggesting the need for further research to clarify

the taxonomy and evolutionary history of the genus *Hymenochirus*. Furthermore, we proposed separating the captive population, previously known as *H. boettgeri*, from the wild population, which retained the name *H. boettgeri*. We decided to refer to the captive population as *Hymenochirus* sp.

Accompanying sections

Comments on contributions to co-authored publications

Knytl M.; **Fornaini N.R**. Measurement of Chromosomal Arms and FISH Reveal Complex Genome Architecture
and Standardized Karyotype of Model Fish, Genus Carassius. Cells, 10, 2343, (2021).
https://doi.org/10.3390/cells10092343. IF_{2021}=6.60. Q2 category.

In this publication I measured the length of chromosomes and cytogenetic analysis on chromosomal spreads as well as probes preparations. I also analyzed the pictures.

Knytl M, **Fornaini N.R.** , Bergelová B, Gvoždík V, Černohorská H, Kubíčková S, Fokam EB, Evans BJ, Krylov V.
Divergent subgenome evolution in the allotetraploid frog *Xenopus calcaratus*, Gene, 2023, Volume 851, 146974.
https://doi.org/10.1016/j.gene.2022.146974. IF_{2023}=3.68. Q2 category.

In this publication I prepared the probes for every single chromosome and for the whole genome. I conducted the painting FISH experiments as well as banding experiments. I prepared the ribosomal probes and used them for FISHexperiments. I also analyzed FISH experiments and I helped in manuscript preparation.

Fornaini N.R., Bergelová B, Gvoždík V., Černohorská H., Krylov V., Kubíčková S., Evans B.J., Knyt M.
Cytogenetic mapping of a repetitive DNA in selected African clawed frogs of the genus *Xenopus* (Pipidae). Eur J
Wildl Res 69, 81 (2023). https://doi.org/10.1007/s10344-023-01709-8. IF_{2023}=2.24. Q2 category

I conducted the cytogenetic experiments and analysis and I designed and prepared the probes. I also analyzed FISH results and prepared the publication pictures and I participated in manuscript preparation.

Gvoždík V, Knyt Ml, Zassi-Boulou A.G., **Fornaini N.R.**, Bergelová B. Tetraploidy in the Boettger's dwarf clawed frog (Pipidae: Hymenochirus boettgeri) from the Congo indicates non-conspecificity with the captive population, Zoological Journal of the Linnean Society, zlad119, (2023) https://doi.org/10.1093/zoolinnean/zlad119 IF_{2023}=3.83. Q1 category.

I prepared ribosomal and small nuclear probes and conducted subsequent FISH experiments. I also analyzed FISH results.

Fornaini N.R., Cernohorska H., do Vale Martins L., Knyt M. Cytogenetic analysis of the fish genus *Carassius* Indicates divergence, fission and segmental duplication as drivers of tandem repeat and microchromosome evolution, Genome Biology and Evolution, Volume 16, Issue 3 ,evae028, (2024).
https://doi.org/10.1093/gbe/evae028 . IF_{2023}=3.2. Q2 category.

I prepared the probes for repetitive DNA FISH and I conducted the experiments. I also prepared probes for microchromosome painting FISH and I conducted the experiments. I analyzed the data and edited the pictures, I prepared the phylogenetic tree and I participated in manuscript preparation.

List of abbreviations

BLAST	Basic local alignment search tool
cept1	Choline/ethanolamine phosphotransferase 1
CMA3	Chromomycin A3
CRISPR	Clustered regularly interspaced short palindromic repeats
DAPI	4′,6-diamidino-2-phenylindole
DMRT1	Doublesex and mab-3 related transcription factor 1
DNA	Deoxyribonucleic acid
F1	First filial generation
FISH	Fluorescence in situ hybridization
FISH-TSA	FISH with tyramide signal amplification
fn1	Fibronectin 1
G-banding	Giemsa banding
GISH	Genomic in situ hybridization
GO	Gene Ontology
gyg2	Glycogenin 2
H.	*Hymenochirus*
H3	Histone H3
mRNA	Messenger ribonucleic acid
Mya	Millions year ago
n	Number of chromosomes in a haploid cell
ndufs1	NADH:ubiquinone oxidoreductase core subunit S1
NOR	Nucleolus organizer region
p arm	Short chromosomal arm
q arm	Long chromosomal arm
RAG1	Recombination activating gene 1
RNA	Ribonucleic acid
RNA-seq	RNA sequencing
rRNA	Ribosomal ribonucleic acid
sf3b1	Splicing factor 3b subunit 1
snRNA	Small nuclear ribonucleic acid
Te	Transposable element
U1	Small nuclear 1
U2	Small nuclear 2
WCP	Whole chromosome painting probe
WGS	Whole genome sequence
X.	Xenopus
XCA	*Xenopus calcaratus*
XME	*Xenopus mellotropicalis*
XTR	*Xenopus tropicalis*
ZOO-FISH	Cross-species chromosome painting

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