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1 Introduction

1.1Polyploidization and its role in evolution

Polyploidization is the process of multiplying a complete chromosome set within a species, potentially leading to the creation of a new species. This occurs due to rare mitotic or meiotic events, such as nondisjunction, which result in gametes with a full set of duplicate chromosomes. When a diploid gamete fuses with a haploid gamete, it leads to a formation of a triploid zygote, but these triploids are typically unstable and can cause sterility. Conversely, the fusion of two diploid gametes produces a tetraploid zygote, which is generally stable. In nature, polyploidy can occur at various levels, including tetraploids (four chromosome sets), hexaploids (six chromosome sets), and other multiples of chromosome complements.
These events allow multiple sets of chromosomes to coexist within a single nucleus, and these sets can be stably inherited by offspring (Comai, 2005). Widespread and multiple polyploidization events produce polyploid species with higher resistance to diseases and environmental stress (Zhang et al., 2019). Polyploidization has a very important role in diversification and evolution, predominantly in plants (Falistocco et al., 2024). However, it has an important role also in some animal species, like some amphibians, fishes, insects and even in some mammals (Acharya and Ghosh, 2016; Li et al., 2018; Schmid et al., 2015; Zhou and Gui, 2017). The level of susceptibility to polyploidy varies according to the species. Polyploids are relatively frequent in flowering plants and it can be also found in some species of frogs, insects and fishes. However, mammals don’t seem to have a high tolerance to polyploidy, but some organs like the brain, muscles, bone marrow, and heart appear to be polyploid (Zhang et al, 2019). It is estimated that around 10% of spontaneous abortions in humans are attributed to the presence of polyploid zygotes (Yildirim et al., 2023).
Polyploids can be categorized into two groups: paleo-polyploids and neo-polyploids, based on the status of the parental chromosomes after polyploidization. Paleo-polyploid species evolved from ancestors that experienced polyploidization events in the distant past (Zhang et al, 2019). Over time, these paleo-polyploid species reverted to having two sets of chromosomes (diploid state) through a process known as diploidization. This involves reshuffling and rearranging the multiple sets of chromosomes inherited from their polyploid ancestors. In this process, polyploid genomes are converted into diploid-like state, reducing redundancy, coordinating subgenomic functions and undergoing chromosomal rearrangements (Feng et al., 2024). Rediploidization is crucial in evolution, since it stabilizes polyploid genomes, promoting genetic diversity and involves adaptations that lead to speciation and ecological success (Feng et al., 2024; Gundappa et al., 2021).
Contrary, neo-polyploids are species that have multiple sets of chromosomes right from the time of polyploidization. These sets of chromosomes come from merging the chromosomes of their parent species, but they remain independent of each other (Zhang et al., 2019). The recent improvement of sequencing technologies has made more complete genomes available, making comparative polyploid analysis easier (Kyriakidou et al., 2018). Polyploidization events are used also to date the speciation of various species.
Polyploidy can develop through two distinct mechanisms. One is autopolyploidy, that occurs within a single ancestor due to meiotic incompatibilities triggered by environmental factors.

The other mechanism is allopolyploidization, which begins with the hybridization of two or more divergent ancestors, leading to meiotic incompatibilities and subsequently preventing cytokinesis (Chen and Ni, 2007).
Autopolyploids are organisms that have more than two sets of chromosomes, all derived from a single ancestral species. They possess the capacity to generate various configurations of homologous chromosomes during meiotic metaphase I, leading to irregular segregation patterns, such as a 3:1 or 2:1 ratio with an additional laggard (laggard chromosomes fail to properly attach to the spindle apparatus, resulting in their random distribution to daughter cells) (Bretagnolle and Thompson, 1995). The resolution of these irregular segregation patterns into balanced products is unattainable, leading to the production of predominantly aneuploid gametes through the random segregation of multiple chromosome types. Allopolyploids exhibit a higher level of constraint in chromosome pairing during meiosis I, compared to autopolyploids, yet the stable preservation of both parental chromosomal complements necessitates the production of balanced gametes (Adams et al., 2005).
The divergence among homoeologous chromosomes accelerates the rediploidization process in a polyploid genome. Thus, at such a stage of the diploidization process, the chromosomes become disomically inherited, pairing with only one homolog during meiosis. The presence of such compartments in the genome has been referred to as 'subgenomes', whereby in allopolyploid genomes these subgenomes are primarily or entirely inherited from different ancestral species (Schiavinato et al., 2021). An important mechanism that follows the re-diploidization and plays a crucial role in the genome stabilization is the genome downsizing (Wang et al., 2021). This mechanism is the process of reduction in the size of genomes and is frequently observed in the reduction of the genome following polyploidy events. Despite undergoing multiple whole-genome duplications, some organisms have smaller genomes than expected, suggesting that there had been a massive loss of DNA over evolutionary time; this was particularly noted in angiosperm (Wang et al., 2021). Hybridization and allopolyploidization often create "genomic shock," that involves rapid genetic and epigenetic changes, due to conflicts between the parental genomes. This results in the dominance of one subgenome over others, bringing distortions in gene content and expression between subgenomes (Bird et al., 2018). The dominant subgenome usually presents some characteristics as a higher gene expression, a bigger retention of functional genes over evolutionary time and a lower accumulation of deleterious mutations or silencing compared to the subordinate subgenome (Bird et al., 2018). In polyploids, the dominance of a subgenome has important implications in the evolution, since it can influence the adaptive potential of polyploids, promoting their survivability in particular environments and influencing the speciation (Bird et al., 2018). The dominant subgenome is usually more stable and conserved, compared to the subordinate genome, which undergoes more mutations and chromosomal rearrangements (Bird et al., 2018; Session et al., 2016).

Polyploidy is common in plants, fish, and frogs, thus it’s still unclear if this mechanism indicates a fitness advantage (Barker et al, 2016; Laurent et al. 2017) In plants, hybrid vigor, or heterosis, is observed, where the polyploid offspring of two diploid progenitors are more vigorous and more resistant to diseases and environmental stress than either diploid parent (Comai, 2005). There are several possible explanations for this phenomenon. One theory suggests that the enforced pairing of homologous chromosomes in an allotetraploid prevents recombination between the genomes of the original progenitors, thereby preserving heterozygosity across generations. This heterozygosity, characterized by multiple gene copies, leads to a reduced accumulation of recessive mutations in the genomes of future generations.

Problems in meiosis can lead to unreduced gametes. These gametes, instead of having half of the chromosomal set, possess the full set, this condition is heritable, having a crucial role in evolution for polyploid species (Mason and Pires; 2015).
As mentioned before, unreduced gametes are a product of failures in meiosis, such as defects in chromosome segregation or spindle formation. Usually this would have been seen as an obstacle for reproduction, but under certain conditions, like very stressful environments, this condition can be triggered, an advantage for evolution and speciation. When unreduced gametes fuse with normal reduced gametes, they can generate offspring with an increased chromosome number, leading to a polyploid condition. Unreduced gametes are often registered hybridization between different species, and they are also produced by interspecies, promoting allopolyploidization (Mason and Pires; 2015).
Gene redundancy plays a crucial role in the advantages of polyploidization. Offsprings are protected against side effects of recessive mutations, because they multiple copies, according to the ploidy level, of any gene as in diploids, as mentioned before. This protective aftermath of polyploidy could have a crucial role in small, isolated populations that have as their only mating option inbreeding. Evolution might have selected polyploid individuals, because of their higher resistance to mutations. Gene redundancy offers the potential to diversify gene function over time. Extra copies of non-functional genes can be repurposed in entirely new ways, creating new opportunities for evolutionary selection (Adams and Wendel, 2005). Polyploidy can also influence sexuality by providing selective advantages. For instance, it can break certain self-incompatibility systems, promoting self-fertilization. This could come from interactions between parental genomes in allopolyploids (Comai et al., 2000), asexual reproduction can be promoted as well, which in both plants and animal, is often associated with polyploidy (Heslop-Harrison et al., 2023; Knytl et al., 2022; Lamatsch and Stöck, 2009; Stöck et al., 2021).
As well with already known advantages, polyploidism comes also with some disadvantages, some of them are confirmed while others are just hypothesized (Comai, 2005). Since cell volume is proportional to the DNA amount in the nucleus, doubling the genome should double the volume occupied by chromosomes. However, only a 1.6-fold increase in the surface area of the nuclear envelope is observed (Melaragno et al., 1993). This can potentially obstruct the normal balance of elements that are part of the mediation between chromosomes and nuclear components, that includes proteins bound to envelope. The reduced space on the nuclear envelope can also affect the peripheral positioning of centromeric and telomeric heterochromatin (Fransz et al., 2002). Polyploidy can increase the rate of spindle irregularities, leading to disordered segregation of chromatids and the formation of aneuploid cells.
These aneuploid cells, characterized by an irregular number of chromosomes, are more likely to be produced in meiosis involving multiple sets of chromosomes compared to diploid cells. Consequently, polyploidy can also be an obstacle for the normal cycle of mitosis and meiosis (Potapova and Gorbsky, 2017).

Another drawback of polyploidy involves potential variations in gene expression. The increase in chromosome copy number should affect all genes uniformly, resulting in a consistent increase in gene expression. However, this might change for genes involved in regulatory pathways that don’t scale proportionally with ploidy (Comai et al., 2005). Experimental evidence for such exceptions exists in several models. For instance, researchers compared mRNA levels per genome for 18 genes across different polyploid levels. As expected, most gene expression patterns increased with ploidy, but some genes exhibited a different expression pattern, not proportional to the level of ploidy. Approximately 10% of these genes were shown to be related to ploidy levels (Guo et al., 1996).

1.3 African clawed frogs (*Xenopus*) as model organism

African clawed frogs, genus *Xenopus*, (family Pipidae), are a population of frogs living in the area of central Africa. They are divided into subgenera *Xenopus* and *Silurana*, *Silurana* includes diploid and tetraploid species, while *Xenopus* includes tetraploid, octoploid, and dodecaploid species in (Tymowska, 1991; Evans et al., 2015; Furman et al., 2018).
Xenopus tropicalis (from subgenus *Silurana*) and *Xenopus laevis* (from subgenus *Xenopus*) are model organisms widely used in medicine and biology, for different purposes. *Xenopus laevis* and *X. tropicalis* are both aquatic animals, and it's easy to store them in an aquarium or in a tank, since they don't need any complicated care or particular environments. The size of the eggs (~1.2mm diameter) makes them easy to manipulate, helped by the large quantities they are laid. Usually *Xenopus* frogs deposit multiple eggs at once, *X. laevis* can produce around 1000 eggs with every single brood, while *X. tropicalis* can lay up to 3000 eggs. Under the right conditions, *Xenopus* females can be mated every two months, while males only once a month. For this reason they have always been considered a valuable tool for studying early embryonic development. *Xenopus* is widely used for studying embryonic development (Figure 3), the changes during the ages and stress response as well as diseases and malformations.

Xenopus models are very useful, besides the already mentioned oocyte and embryo characteristics also for the high reproductive capacity, swift external development, and straightforward genomic modification. *Xenopus* are used also as models for human diseases, since they share around 79% genes associated with a disease, in humans (Hellsten et al., 2010; Khokha, 2012; Tandon et al., 2017).
Moreover, they are rapid to breed, cheap, easy to manipulate with and have a high successful ratio of gene mutation using CRISPR/Cas, compared to mammalian models. It has been reported in many studies that CRISPR/Cas modifications can be used for phenotype analysis in the F1 generations of both animals, *X. laevis* and *X. tropicalis* (Bhattacharya et al., 2015; Blitz et al., 2013; Wang et al., 2015).

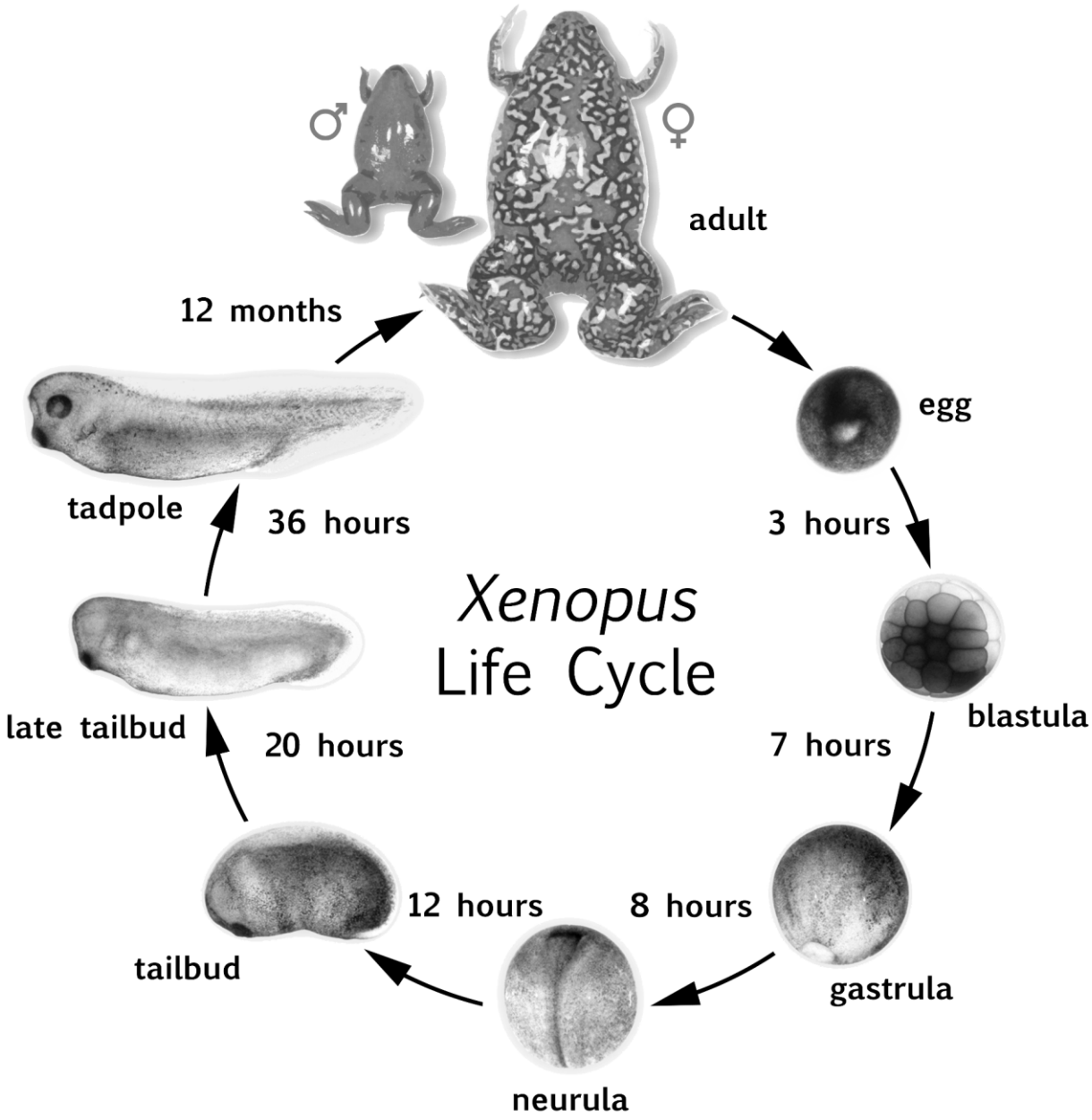

Figure 3. Life cycle of Xenopus laevis (Gerhart and Kirschner, 2020).

Lately *Xenopus* frogs were successfully used as a model Investigating spinal cord morphogenesis, functionality, and recovery (Borodinsky, 2017), as demonstration of their great adaptability and wide use for various biological fields. *Xenopus laevis* is also an excellent model for analysis on the development of the heart (Warkman and Krieg, 2007). The large size of the embryo allows the use of microinjections for gene manipulation, an approach used to study the gene function overexpressing or silencing a specific gene of interest.

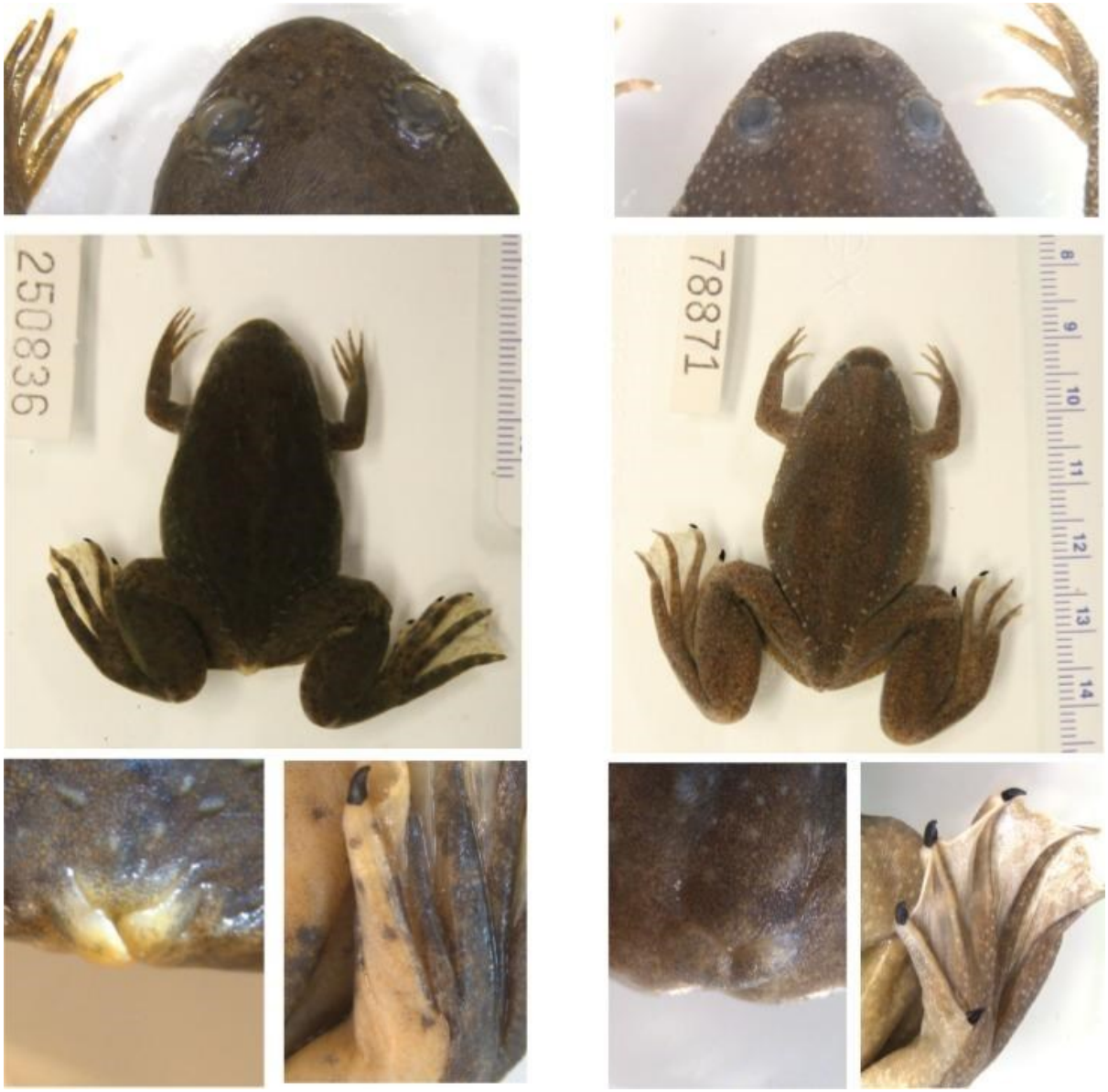

Figure 4. The examination of external features between the subgenera Xenopus (right, specimen of X. victorianus CAS 250836 [DCB-202]) and Silurana (left, X. calcaratus CAS 207759) highlights several distinctions. In Silurana, the skin texture is coarser, the eyes are comparatively smaller, and the subocular tentacle is shorter relative to coexisting Xenopus species (Evans et al., 2015).

Xenopus tropicalis genome is estimated to be approximately 1.7 billion base pairs, with ten chromosome pairs and is being sequenced to approximately 8X depth A range of tools, incorporating cDNA and protein homology, were employed to predict or to map gene models and their corresponding transcripts and proteins. In the last version of the assembly, around 99% of the genome is mapped to chromosomes (Fisher et al., 2023; Hellsten et al., 2010; Roe et al., 1985; Session et al., 2016). Researchers discovered the genome of *X. tropicalis* contains more than 20,000 protein-coding genes, which include orthologs of at least 1,700 genes associated with human diseases. This makes *X. tropicalis* a viable human diseases model. The size of the *X. laevis* genome is 2.7 billion base pairs spread across 18 chromosomes (Session et al., 2016). The genome of X. laevis is fully annotated as well. The annotation process revealed 36,175 genes, resulting in a proteome with 61,616 entries (UP000186698). Gene functions were determined on the homology to known genes, and the curation is still ongoing (Fisher et al., 2023). *Xenopus laevis* has a longer maturation time,

compared to *X. tropicalis*, taking 1-2 years to reach the adult form, able to lay eggs. *Xenopus tropicalis* is a simpler alternative (Tymowska, 1991).
The availability of the complete genome of *X. tropicalis* and *X. laevis* make them valuable tools for studying human diseases (Nenni et al., 2019) as well as their relatively phylogenetic close distance to humans (Figure 3). The fact that 99% of chromosomes are mapped on the genome, is an essential tool for cytogenetic studies. Knowing the position of a gene on a chromosome, in crucial in order to understand the chromosomal rearrangements in sister species, also it makes easier to find candidate genes for a particular rearrangement, like an inversion or a translocation (Knytl et al, 2018). The advantages of having diploid and tetraploid species with fully annotated genomes, allowed us to identify the position genes in both subgenomes of X. laevis and having good insights on possible chromosomal rearrangements or genome expansions and reductions in sister species.

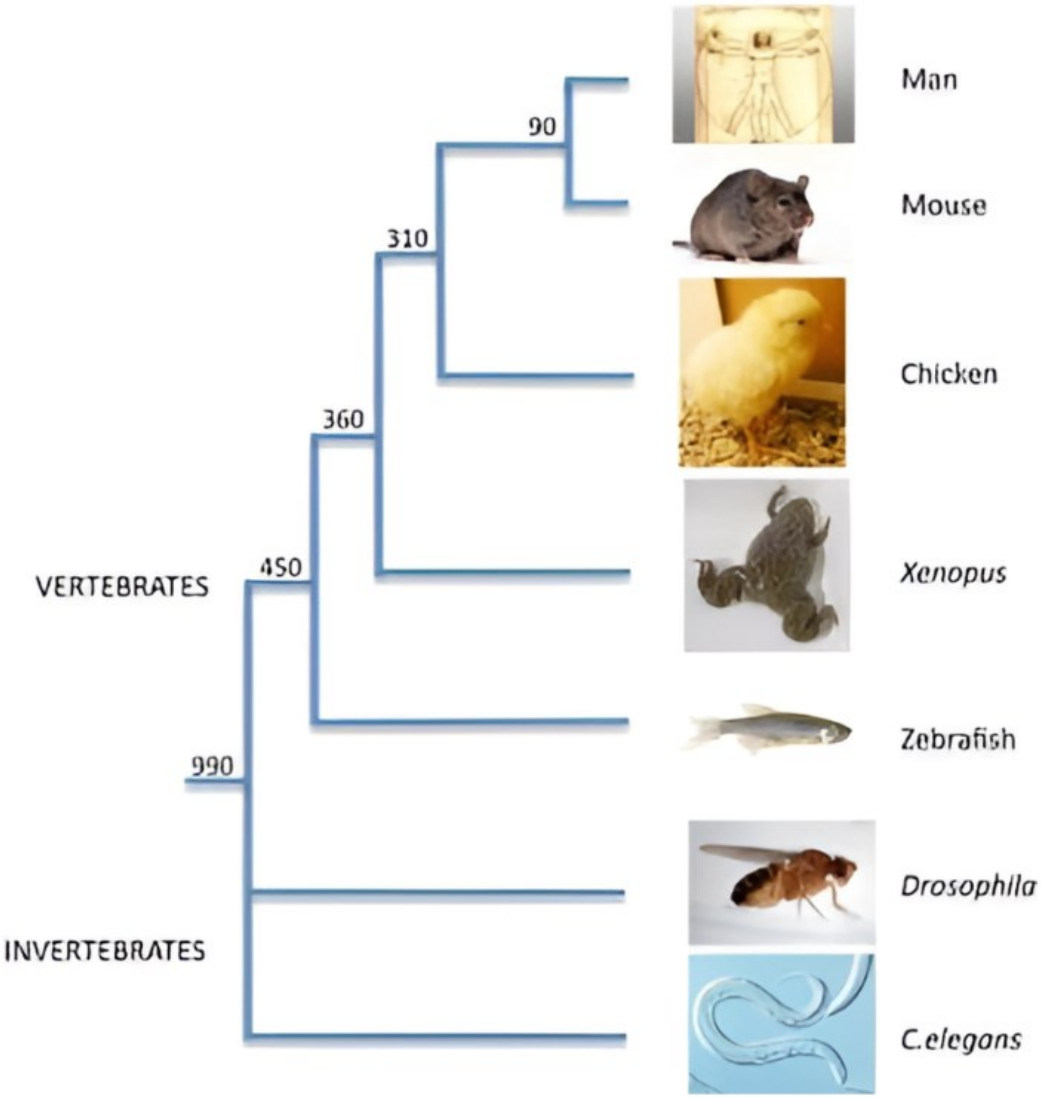

Figure 5. Phylogenetic tree illustrates the evolutionary relationships among the primary animal models frequently utilized in biomedical research. Periods of separation, expressed millions of years ago (Mya), are taken from studies using multi proteins and genes (Hedges, 2002; Hedges and Kumar, 2002; Wheeler and Brändli, 2009). Note that the branch lengths are not proportional to time (Wheeler and Brändli, 2009).

1.4 *Hymenochirus boettgeri* relevance in research

An interesting pipid species for our research is the dwarf clawed frog (Hymenochirus boettgeri).
Dwarf clawed frogs, found in captivity, are not commonly used as animals for the research but they are widely used as pets, kept in aquariums. However, the name '*H. boettgeri*' was usually used in laboratory studies and in publications, even when the frogs were taken from captive populations (Cauret et al., 2020; Mezzasalma et al., 2015; Miller et al., 2019; Höbel and Fellows). Nowadays this dwarf clawed frog is considered a valuable model species (Bredeson et al., 2024).
Xenopus and *Hymenochirus* are phylogenetic close, as can be seen in Figure 6.

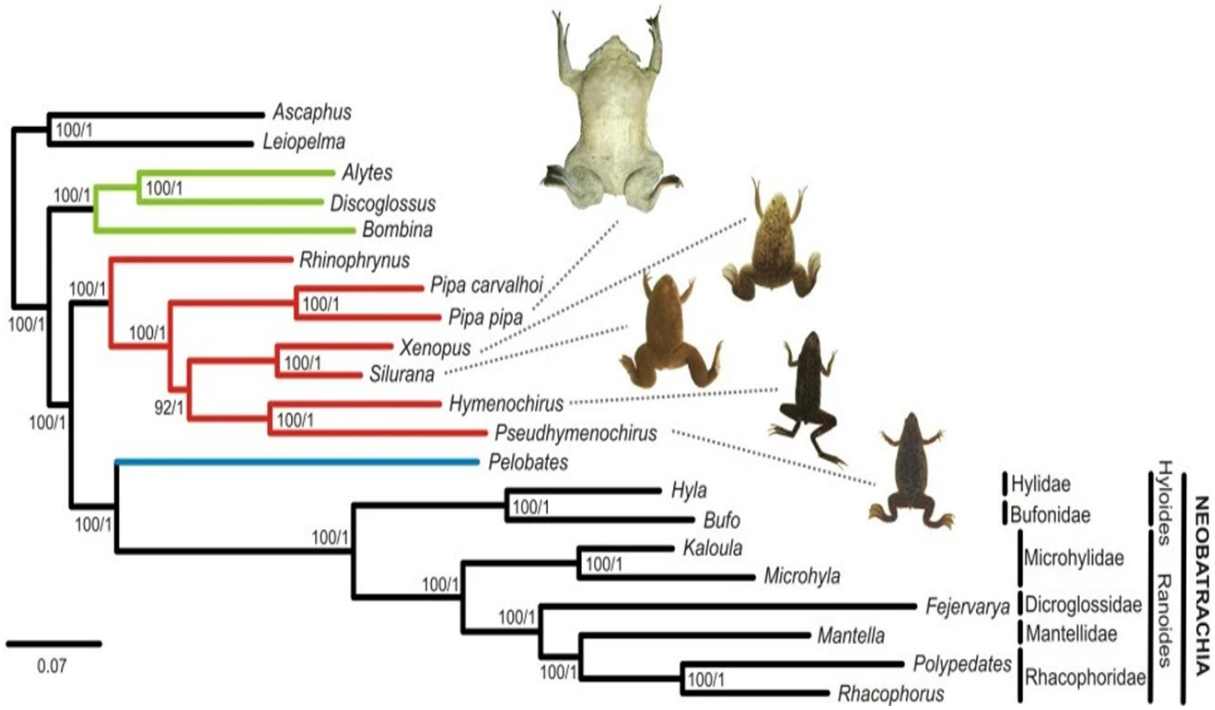

Figure 6. Phylogenetic tree illustrating the various frog species divergence. Adapted from (Irisarri et al., 2011).

Dwarf clawed frogs (*Hymenochirus* sp.) that we have nowadays in aquariums, kept as domestic pets, likely began in the 1950s, when pet fish companies imported these frogs from the wild to Europe and the USA (Olsson and Österdahl, 1960; Sokol, 1962). We don't have precise geographic information about the origin of this speciesIn addition to documents stating that dwarf clawed frogs were shipped from Leopoldville, which is now known as Kinshasa in the Democratic Republic of the Congo (Rabb and Rabb, 1963) or 'from Stanley Pool' (Sokol, 1962), an area north-east of Kinshasa (Sokol, 1962). However, Leopoldville might have just been the shipping point, and Stanley Pool the arrival point (since collectors could have landed there due to its ports), meaning they may not be the actual areas of origin.There was two species, at least for what it's known, traditionally known with the names *H. boettgeri* and *Hymenochirus curtipes*, that were likely imported in the 1950s and even several years after (Sokol, 1962, 1959), through time *H. boettgeri* became more popular than the frog *H. curtipes* (Rabb and Rabb, 1963; Sokol, 1969). *Hymenochirus curtipes* is known to thrive in open areas and requires higher temperatures in captivity, especially for breeding. Additionally, its tadpoles require big tanks and much more space (Sokol; 1962,1969b). For that reason, *H. curtipes* might have gradually disappeared from captivity due to its higher breeding specialization and

its demanding taking care (Kunz, 2002). Despite both species being mentioned in aquarium and herpetoculture literature, only one species seems to have been observed in aquaria in recent years (Kunz, 2003, 2002). It is usually referred to as *H. boettgeri*, even though it has been registered that the genus needs a taxonomic revision (Kunz, 2004).

The question if the two species analyzed were belonging to the same one of the captive populations with *H. boettgeri*. There has been some discussion about certain morphological differences, leading to the hypothesis that the captive population might be a 'domesticated' hybrid between *H. boettgeri* and *H. curtipes* (Cecere, 1998). For example, typical morphological features of *H. boettgeri* include an oval body, broad head, monochromatic dorsum, and sides covered with enlarged tubercles (de Witte; 1930, Arnoult and Lamotte, 1968; Perret, 1966). Aquarium population, showed different morphology, the body sides have homogeneous tubercles without differentiated verrucous, the body is oval to pear-shaped, the head is not distinctly broad, and the dorsum is often mottled (Kunz, 2004) (Figure 7).

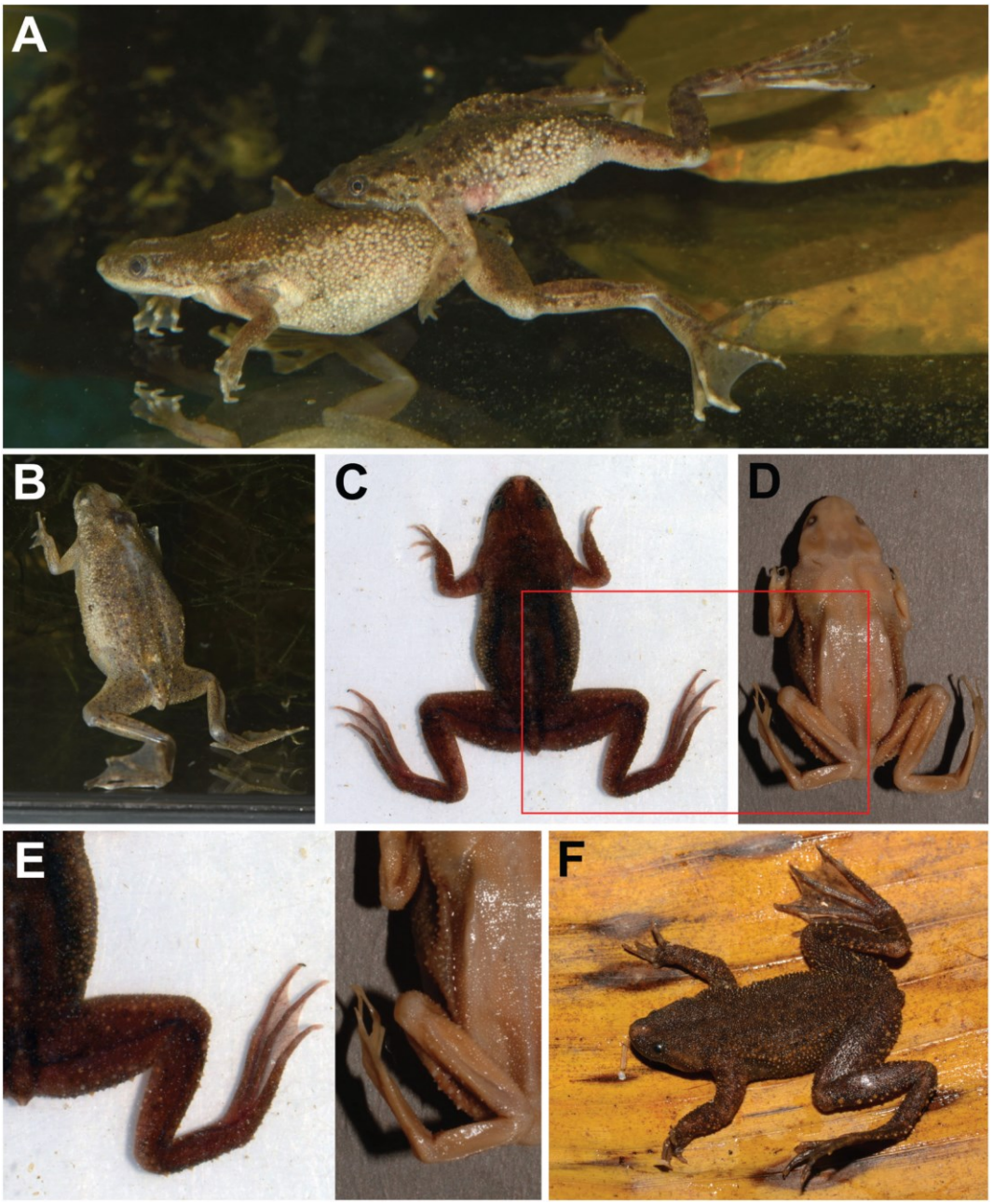

Figure 7. Dwarf clawed frogs, Hymenochirus sp. (captive population) and H. boettgeri, are shown in various views. (Gvoždík et al., 2024).

1.2 Polyploidization in *Xenopus* frogs

Amphibians is a vertebrate group composed of over 8,700 species (Frost, 1999), in which around 100 species across 19 families have been found to be polyploid (Mezzasalma et al., 2023).Amphibians stand out among vertebrate lineages because they can form populations and even entire species with both diploid and polyploid individuals capable of sexual reproduction (Bogart, 1980; Mezzasalma et al., 2015).

A notable hallmark setting African clawed frogs different from many other amphibian species is their remarkably elevated frequency of polyploid species (Evans, 2008; Evans et al., 2015; Tymowska, 1991).

So, *Xenopus* diploids can be used as system models for evolutionary and cytogenetic research related to polyploidy (Bogart and Bi, 2013; Fornaini et al., 2023; Knytl et al., 2018).

Pipid frogs (family Pipidae) represent an ancient evolutionary line of fully aquatic frogs, unlike those that need both aquatic and terrestrial environments to survive. They diverged from their sister lineage, Rhinophrynidae, over 150 Mya. The currently living pipid frogs are divided into two subfamilies, the American Pipinae (*Pipa Laurenti*) and the African Dactylethrinae (split ~110 Mya) containing two deeply divergent tribes (split ~100 Mya), *Dactylethrini* (*Xenopus* Wagler) and *Hymenochirini* (*Hymenochirus boulengeri* and *Pseudhymenochirus merlini*) (Dubois et al., 2021; Feng et al., 2017; Hime et al., 2021).

For almost thirty years, *Xenopus* frogs have been classified into two genera, *Xenopus* and *Silurana* (Cannatella and De Sa, 1993). These two distinct clades are defined by variations in morphology and the chromosome number of their diploid ancestors (20 for *Silurana* and 18 for *Xenopus*) (Cannatella and Trueb, 1988; Tinsley et al., 1996). However, the previously proposed paraphyletic relationship between *Silurana* and *Xenopus*, in relation to other pipid genera, was defined on morphological evidence (Cannatella and Trueb, 1988), and found no evidence nor confirmation by lately molecular phylogenetic analyses that confronted the predominance of Xenopodinae in relation to other pipid genera (Bewick et al., 2013; Hedtke et al., 2013; Irisarri et al., 2011).

The Pipidae family exhibits a high degree of variability in karyotypes, especially in chromosome numbers and sizes, largely due to the influence of polyploidization throughout their evolutionary history (Schmid et al., 2015; Tymowska, 1991). It has been hypothesized that the ancestral karyotype of *Pipidae* was 2n = 20. (Bredeson et al., 2024; Mezzasalma et al., 2015), as is seen in *Xenopus* (subgenus *Silurana*), *Hymenochirus*, *Pseudhymenochirus*, and *Pipa* (*Pipa carvalhoi* (Miranda-Ribeiro, 1937). Mezzasalma et al. (2015) also suggested three mechanisms for the chromosome number increasing in pipid frogs: fission, allopolyploidy and the addition of a B chromosome. In pipids, polyploidy, especially allopolyploidy, was thought to have only been found in *Xenopus* species, including *Silurana* subfamily (Schmid et al., 2015) however our recent studies found that other species outside *Xenopus* are polyploid. I will discuss this topic in the discussion.

In *Silurana* the haploid number is n = 10, while in the subgenus *Xenopus*, where the fusion of two chromosomes, 9 and 10, was registered (Session et al., 2016), the haploid number is n = 9. Recently, in *Hymenochirus*, was discovered a fusion of chromosomes 8 and 10 (Bredeson et al., 2024). It has been hypothesized that in Pipa genus a chromosomal fission occurred during the evolution line (Mezzasalma et al., 2015).

Usually, the scientific community that uses African clawed frogs as model in their studies, refers to all the species simply as "*Xenopus"* (Hellsten et al., 2010), but a previous study on *Xenopus* systematics refer them as two different subgenera, *Silurana* and *Xenopus* (Tinsley et al., 1996)*, Silurana* is considered a subgenus within the genus *Xenopus* (Evans et al., 2015). The subgenus *Silurana* encompasses the diploid *X. tropicalis* and the tetraploids *Xenopus*

epitropicalis, *Xenopus mellotropicalis* and *Xenopus calcaratus* (Evans, 2008; Evans et al., 2015). Based on a recent taxonomic reassessment of *X. laevis*, the subgenus *Xenopus* comprises 25 described species, including 14 tetraploids (X*enopus borealis*, X*enopus clivii*, X*enopus fraseri*, X*enopus gilli*, *Xenopus laevis*, *Xenopus largeni*, *Xenopus muelleri*, X*enopus petersii*, X*enopus poweri*, X*enopus pygmaeus*, *Xenopus victorianus, Xenopus allofraseri, Xenopus parafraseri and Xenopus fischbergi*), seven octoploids (X*enopus amieti*, *Xenopus andrei*, X*enopus boumbaensis*, *Xenopus itombwensis*, *Xenopus lenduensis*, *Xenopus wittei*, and *Xenopus vestitus*), and four dodecaploids (*Xenopus longipes, Xenopus eysoole, Xenopus kobeli* and *Xenopus ruwenzoriensis*) (Evans et al., 2015).

The molecular evolutionary history of *Xenopus* frogs is typically analyzed using segments of mitochondrial DNA. This analysis includes most of the mitochondrial 12S and 16S rDNA genes, the intervening tRNA val (tRNA which binds L-valine), cloned homeologs of the autosomal genes RAG1 and DMRT1, and a portion of the mitochondrial cytochrome oxidase I gene (Evans, 2007; Evans et al., 2004).

Thanks to this approach, a research that also used genetic data from four of the new species shown in Evans et al. (2015), it is suggested that tetraploidization occurred at least once in both *Silurana* and *Xenopus*. In contrast, octoploidization and dodecaploidization are believed to have occurred at least three times within the subgenus *Xenopu*s (Figure 1) (Evans et al; 2015). Phylogenetic analyses prove that these genome duplication events were driven by allopolyploidization rather than autopolyploidization. There is/are an ancestral (2n = 18) diploid(s) that is/are not available for studying, that is/are probably extinct(s) (Figure 1).

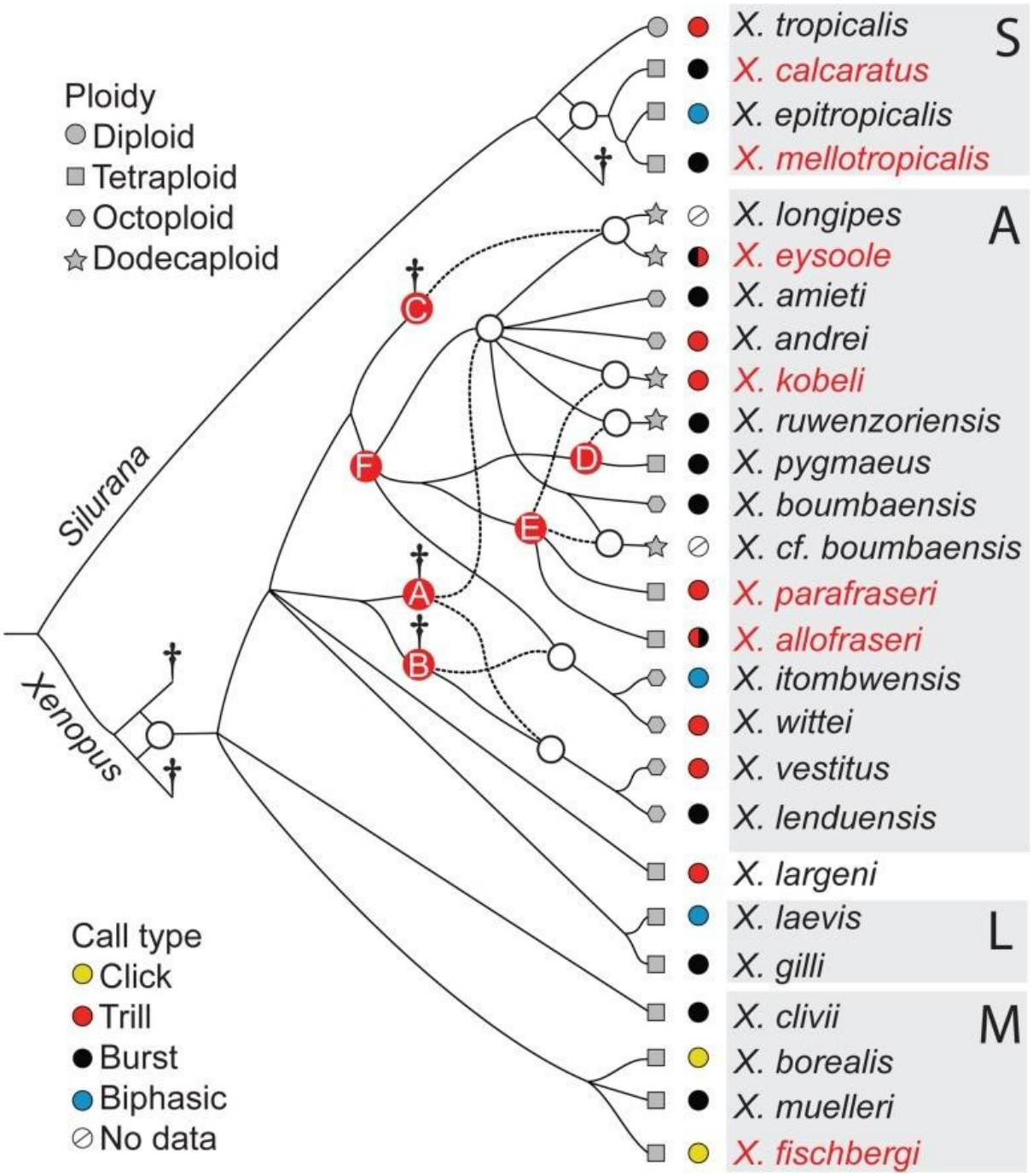

Figure 1. A summary phylogeny was inferred through a comparative analysis of mitochondrial and autosomal gene trees. Newly identified and resurrected species are highlighted in red. The letters S, A, L, and M represent the subgenus Silurana and the amieti, laevis, and muelleri species groups within the subgenus Xenopus, respectively. Dotted lines indicate paternal ancestral lineages, while circles at internal nodes denote allopolyploidization events. Shapes at branch tips indicate the ploidy of existing species, with colors next to these shapes reflecting vocalization. Daggers mark lost ancestors, including up to three diploid species (assuming allotetraploidization in the subgenus Xenopus) and at least three tetraploid ancestors (A, B, and C). Taken from (Evans et al., 2015).

GROUP	HAPLOID CHR NUMBER	TETRAPLOID CHR NUMBER	OCTOPLOID CHR NUMBER	DODECAPLOID CHR NUMBER
Silurana	10	40	/	/
Amlieti	9	36	72	108
Laevis	9	36	/	/
Muelleri	9	36	/	/

Table 1. The known chromosomal characteristic per group, in Xenopus genus.

Hybridization in African clawed frogs do not always lead to genome duplication. Some hybrids retain the same ploidy level as their parental species, such as those between *X. laevis* and *X. muelleri*, *X. laevis* and *X. gilli*, and *X. victorianus* and *X. borealis* (Evans et al., 1998; Yager, 1996).

The high diversity of *Xenopus* species (in total 29 species), makes them a perfect group for studying chromosomal rearrangements and polyploidization. The research on *Xenopus* taxa has also the potential to give some clues if chromosomal rearrangement and polyploidization are linked to speciation (Knytl et al., 2017).

Researchers have investigated the subgenus *Xenopus* and identified significant large-scale rearrangements. Notably, one of the most important findings is the fusion of chromosomes 9 and 10 in a diploid ancestor of allopolyploids, now extinct.

In the subgenus *Xenopus*, a comparison with the well-assembled genome of the diploid species *X. tropicalis* reveals many additional rearrangements in the *X. laevis* S-subgenome. This comparison underscores the relative genomic conservation of the *X. laevis* L-subgenome (Session et al., 2016). Regarding *Silurana* tetraploids, the difference between the a- and b-subgenomes is greater than the divergence between the more conserved a- subgenome and the diploid *X. tropicalis* genome (Evans, 2008; Evans et al., 2015). Also, in *Silurana* it has been identified a large-scale rearrangement: in the tetraploid species *X. mellotropicalis*, a nonreciprocal translocation has occurred between the pericentromeric regions of chromosomes 9b and 2b (Knytl et al., 2018, 2017). The rearrangement was identified using fluorescence in situ hybridization (FISH) hybridizing the whole chromosome painting (WCP) probes from *X. tropicalis* to *X. mellotropicali*s chromosomes (Zoo-FISH). It was unknown if the rearrangement occurred also in the progenitor of the three allotetraploid species in *Silurana* or after the divergence, from the other species, of the ancestor of *X. mellotropicalis*, this topic will be explained in section 3.2.

This is due to the unresolved evolutionary relationships among species in the subgenus *Silurana*, mainly because of the limited genomic data available from *X. calcaratus* and *X. epitropicalis* when the study was published. Such data could significantly aid in phylogenetic estimation when combined with genomic data from other *Silurana* species (Cauret et al., 2020; Hellsten et al., 2010).

Regarding polyploidy in the *Hymenochirus genus*, in a striking discovery, B chromosomes have been identified in the captive population of "*Hymenochirus boettgeri"*. (2n = 20A + 1B). Astonishingly, these B chromosomes appeared in nearly half of the karyotypes analyzed from two male specimens (30 out of 66 metaphase spreads from the intestine, spleen, gonads, and lung tips), while the rest exhibited the standard 2n = 20 configuration (Mezzasalma et al., 2015). However, older studies of "*H. boettgeri*" registered the chromosomal number being 2n

= 24 (Morescalchi, 1981, 1968; Tymowska, 1991), also the karyotype 2n = 22 was stated (Scheel, 1973). Unfortunately, this study only reported the chromosome number and no more information was given. Finally, a recent study recorded 2n = 18 chromosomes in *H. boettgeri*, with no B chromosomes detected (Bredeson et al., 2024). It remains uncertain whether the variations in chromosome number are due to differences in B chromosome count (except for the 2n = 18 case, where chromosome fusion occurred), as proposed by Mezzasalma et al. (2015), alternatively, it is possible that earlier researchers examined one or more different species where chromosome fissions had taken place (Morescalchi, 1981, 1968; Scheel, 1973).

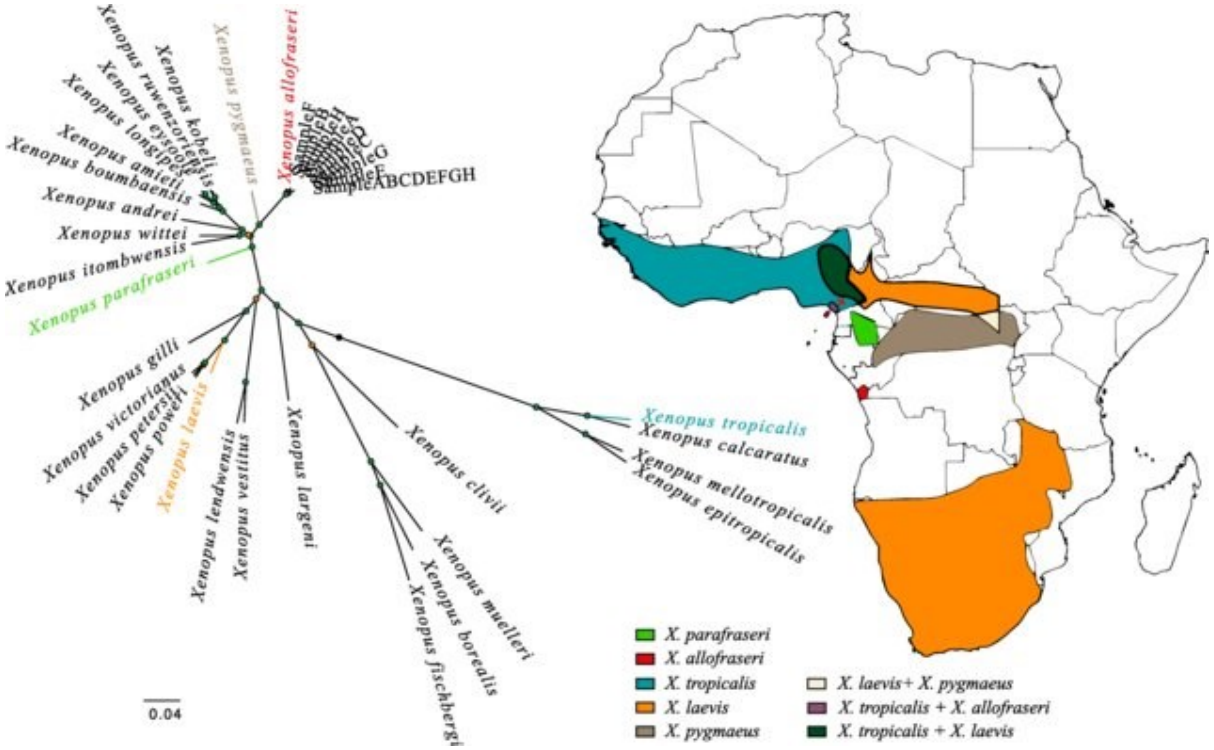

Figure 2. Geographic range of some Xenopus species in Africa and maximum-likelihood phylogenetic tree (Ducret et al, 2021).

2 AIMS

The purpose of this work is to investigate the subgenome evolution of another *Silurana* species, *Xenopus calcaratus*, in order to understand if the evolution of its two subgenomes is asymmetric, as seen in *X. laevis* and if the subgenome, supposed to be more conserved, is similar to the diploid ancestor, *X. tropicalis*.

In order to understand if the chromosomal rearrangements are shared among all the species of the same subgenus, I also wanted to confirm if the translocation between chromosome 2 and 9, found in *Xenopus mellotropicalis* (Knytl et al., 2018) was registered also in *X. calcaratus*.
To explore the chromosomal rearrangements after the allotetraploidization events, I mapped repetitive DNA elements on chromosomes of six *Xenopus* species, at least one species per group.

Finally, we confronted the karyotypes of one captive population of *Hymenochirus* with a wild population, in order to determine if they were the same species. We also performed the same cytogenetic analysis, we already performed in the *Xenopus* genus, on these two species to prove our theory that they were divergent species.

REVISIONS

Reviewer 1

-The Introduction (1.1 – 1.2) is according to me too much focused-on model systems and their role in developmental biology studies and human medicine, which is not a main topic of this thesis. On the other hand, some important issues of the thesis, such as subgenome evolution in polyploids, are not very well addressed in the Introduction. **DONE**

-I also missed a chapter describing the aims of the thesis after the Introduction. Without this, it was quite difficult to understand the following chapters. **DONE**

-The figures also need a little more care: Some figures (e.g. Figure 5) are entirely taken from the accompanying paper, including the text of the legend. Although the source paper is cited at the end of the legend, it is not common practice to take over the entire figure, including the legend. For other figures (e.g. Figures 6, 9), the legend is inadequate, and the figure is difficult to understand. Some figures (e.g. Figures 3, 4) have very poor resolution. **DONE**

Reviewer 2

-Mistakes in grammar: The level of susceptibility for polyploidy it's different according to the species (page 12). …but a previous study (page 15). **DONE**
-Examples of unintelligible sentence:
Page 15: Pipa has been hypothesized to occur chromosome fission (Mezzasalma et al., 2015). **DONE**
-I appreciate the phylogeny of genus Xenopus in Fig. 7, however, it would be useful to improve the scheme by adding the column with known chromosome characteristics (at least 2n) to make the figure more informative for the context of the thesis. **DONE**
-I, on the other hand, find unhappy the placement and content of Figure 6. Not only it is unnecessary to narrow the processes of polyploidization and re-diploidization to plants, but also the content of both pictures A+B is not explained either in the figure legend, nor it adheres to the main text. **DONE**
-The candidate chose to first introduce the model frog species, with many details of their applications which is interesting but so far, we do not know what will be important for the thesis objectives. Then the author jumps to the concept of polyploidy. Later he describes the Pipidae family and returns back to detailed description of frog species. I find this structure unhappy as the more general evolutionary concepts and questions, along with the identified knowledge gaps, should be introduced first and then the text should focus to the model organism and why it is suitable for study of the chosen scientific questions. **DONE**
-The information flow in certain sections also deserves caution. In chapter 1.3 about polyploidization (starts on page 12) only very later we repeatedly receive the information what is auto- and allopolyploidy (e.g. end of pages 13 and 14). This is something that should be

delineated in the first paragraph. Then it would make more sense to explain mechanisms that facilitate polyploidy emergence and subsequent polyploid genome evolution. **DONE**

-The problematics of unreduced gametes along with various mechanisms leading to them (not properly delineated by the candidate) are, for example, neatly summarized in Mason and Pires 2015 (doi: 10.1016/j.tig.2014.09.011). **DONE**

-Several key concepts and mechanisms following the re-diploidization process are not adequately introduced such as genome downsizing (e.g. Wang et al. 2021; doi:10.1111/tpj.15363) and subgenome dominance (Bird et al. 2018; doi: 10.1111/nph.15256). To my opinion, these concepts are important for the interpretation of the achieved (as well as future) results. **DONE**

-The polyploidization section also sometimes contains huge segments of text with a dense information content but without any (or just one/few) supporting reference(s), which applies especially to last two paragraphs of this section (page 14). **DONE**

-Page 8: Unclear statement: I do not understand in the context of X. tropicalis and X. laevis genomes what the author means by „fully sequenced ". What level of genome assembly has been achieved? **DONE**

-Page 12: I do not agree with this (also not very happily formulated) statement: „Polyploids have a relatively high frequency in flowering plants, this can be also found in some species of frogs and fishes. However, in higher vertebrates, as amphibians, reptiles, birds, and mammals, animals that are characterized by ….. a more complex structure compared to lower vertebrates like fish, they don't seem to have a high tolerance to polyploidy very well. "There are many examples of natural polyploidy in amphibians (both sexual and asexual) and in parthenogenetic reptiles. Moreover, I do not understand, upon which reasoning/based on which resource the author considers amphibians and reptiles as higher vertebrates. I find it misleading and incorrect. Also, my opinion is that it is better to categorize vertebrates to cold-blooded and warm-blooded. **DONE**

-Page 13: This statement not only contradicts the one I mention above but also it is problematic in other way: "Polyploidy is common in plants, fish, and frogs, indicating clear fitness advantages. "Tolerance to polyploidy does not necessarily mean ultimate fitness advantage. There is still ongoing debate on this matter (Barker et al. 2016; https://www.jstor.org/stable/newphytologist.210.2.391; Laurent et al. 2017; doi: 10.1371/journal.pone.0176384; Barker et al. 2024; doi: 10.1002/ajb2.16395). **DONE**

-Page 13: For polyploidy linked with asexual reproduction, I miss very important relevant sources such as e.g. Lamatsch and Stöck 2009 (doi:10.1007/978-90-481-2770-2_19), Stöck et al. 2021 (doi:10.1098/rstb.2020.0103). **DONE**

-Page 14: „Unlike, for example, teleost fishes where whole genome duplication became an established evolutionary process for the entire group, polyploidy in amphibians has arisen independently on multiple occasions within different families (Schmid et al., 2015). "

This is incorrect interpretation. In teleost and also non-teleost fishes, there were multiple additional polyploidization events at various taxonomic levels. Not only teleost-specific whole-genome duplication at the base of teleost lineage. **DONE**