

Univerzita Karlova

Přírodovědecká fakulta

Studijní program: Ekologie



Mgr. Barbora Augstenová

Evoluce určení pohlaví u plazů skupiny Toxicofera

Evolution of sex determination systems in toxicoferan reptiles

Disertační práce

Vedoucí práce: Michail Rovatsos, Ph.D

Konzultant: prof. Mgr. Lukáš Kratochvíl, Ph.D.

Praha, 2021

Acknowledgement

In the first place, I would like to thank my supervisors Michial Rovatsos and Lukáš Kratochvíl mainly for their support, guidance and patience during my studies and writing. I would like to thank also to prof. Petr Ráb from the Laboratory of Fish Genetic of the Institute of Animal Physiology and Genetics in Liběchov for allowing us to use their laboratory facilities and all the people from there. I also thank all the people from our cellar lab S28 as well as the people from the Ph.D. room 128 who were always there to help, give advice or listen to all my complaints (for this special thanks to Tomáš). Our work would not be so easy without the support of our (lab) technicians, so special thanks belong to Nuria Viñuela Rodríguez, Jana Thomayerová, Jana Vokurková and Jan Červenka as well as Šárka Pelikánová. I would like to express my gratitude to Münster Zoo, Prague Zoo, Plzeň Zoo, Zoopark Zájezd and all private breeders for samples from their animals. And lastly, I would like to thank my family for their support during all the years of my studies.

This thesis was partially supported by Grant Agency of Charles University (GA UK no.1073416).

Declaration of originality / Prohlášení o originalitě

I declare that this thesis has not been submitted for the purpose of obtaining the same or another academic degree earlier or at another institution. My involvement in the research presented in this thesis is expressed through the authorship order of the included publications and explained in detail in the “Outline of the publications” section of the dissertation. All literature sources I used when writing this thesis have been properly cited.

Prohlašuji, že tato práce nebyla předložena za účelem získání stejného nebo jiného akademického titulu dříve na této, ani na jiné instituci. Moje zapojení do výzkumu obsaženého v této práci je vyjádřeno pořadím autorů zahrnutých prací a podrobně vysvětleno v části „Přehled publikací“ disertační práce. Všechny literární zdroje, které jsem použila při psaní této práce, byly řádně citovány.

Prague, June 2021 / Praha, červen 2021,

Barbora Augstenová

Statements of contribution / Prohlášení o příspěvku

I declare that my research for this dissertation was conducted in collaboration with scientists from several countries. My personal contribution to the experimental design, data collection, analyses and preparation of manuscripts for each chapter is explained in detail below.

Prohlašuji, že moje výzkumné úsilí ústící v tuto disertační práci bylo vedeno v mezinárodní spolupráci. Můj osobní příspěvek ke koncepci, sběru dat, analýze a rukopisné přípravě pro každou z kapitol byl přesně vysvětlen níže.

Prague, June 2021 / Praha, červen 2021,

Barbora Augstenová

As supervisor of the PhD thesis and to the best of my knowledge, I confirm that the contribution of Mgr. Barbora Augstenová to the chapters and appendices of this thesis is accurately explained below.

Jako vedoucí disertační práce a podle mého nejlepšího vědomí potvrzuji, že příspěvek Mgr. Barbory Augstenové ke kapitolám a dodatkům v této práci byl přesně vysvětlen níže.

Prague, June 2021 / Praha, červen 2021,

Michail Rovatsos

Content

Abstrakt (Česky)	6
Abstract (English)	7
Introduction	9
Evolution of sex determination in amniotes	9
Sex chromosomes evolution	11
Phylogenetic overview of Toxicofera reptiles	13
Evolution of sex determination in Toxicofera	16
Aims of the thesis	22
Publications overview	23
Outline of the publications	24
Conclusions and future perspectives	27
References	29
Chapter 1	40
Chapter 2	53
Chapter 3	59
Chapter 4	69
Chapter 5	89
Chapter 6	106

Abstrakt (Česky)

Determinace pohlaví hraje důležitou roli v životaschopnosti populací a evoluci druhů, a i proto je determinace pohlaví důležitým předmětem studia již více než sto let. Tato práce se zaměřuje na evoluci určení pohlaví u plazů skupiny Toxicofera. Toxicofera jsou skupinou šupinatých plazů čítající více než 6000 druhů. Druhová bohatost této skupiny se odráží i v různorodosti způsobů určení pohlaví napříč fylogenetickým spektrem. Byly popsány jak druhy s environmentálně (ESD), tak s genotypově určeným pohlavím (GSD) s XX/XY nebo ZZ/ZW pohlavními chromozomy. Nicméně, současné znalosti týkající se determinace pohlaví Toxicofer nejsou mezi jednotlivými liniemi rovnoměrně rozložené. Hlavním cílem této práce je rozšíření současných poznatků zabývajících se evolucí pohlavních chromozomů pomocí cytogenetických metod u hadů, chameleonů a plazů skupiny Anguimorpha. První část práce se zabývá evolucí pohlavních chromozomů u hadů skupin Caenophidia a Henophidia. V případě hadů skupiny Caenophidia je práce zaměřena zejména na variabilitu v rozmístění repetitivních sekvencí a heterochromatinu na chromozomu W. Zatímco pohlavní chromozomy hadů skupiny Caenophidia jsou cytogeneticky poměrně dobře prostudované a snadno rozpoznatelné, pohlavní chromozomy zbývajících skupin hadů jsou ve většině případů homomorfní, málo diferenciovány a při použití obvyklých cytogenetických metod nejsou rozlišitelné. V naší práci jsme studovali deset druhů hadů skupiny Henophidia. Morfologicky rozlišené pohlavní chromozomy se nám podařilo detekovat pouze u jediného druhu madagaskarského hroznýše *Acrantophis cf. dumerili*. Druhá část práce se zabývá pohlavními chromozomy u chameleonů rodu *Furcifer*. U studovaných druhů se nám podařilo popsat přítomnost pohlavních chromozomů ZZ/ZW a mnohočetných pohlavních chromozomů Z₁Z₁Z₂Z₂/Z₁Z₂W. Třetí část práce je zaměřena na pohlavní chromozomy skupiny Anguimorpha. Současné znalosti týkající se určení pohlaví jsou omezené zejména na varany (Varanidae) a korovce (Helodermatidae). V naší práci se nám navíc podařilo detekovat putativní pohlavní chromozomy u tří druhů čeledi Anguinae, jmenovitě u druhů *Abronia lythrochila*, *Celestus warreni* a *Gerrhonotus liocephalus*.

Abstract (English)

Sex determination plays an important role in the viability of populations and species evolvability. This is one of the reasons why sex determination has become an important subject of many studies during more than the last 100 years. The thesis focuses on the evolution of sex determination systems in toxicoferan reptiles. Toxicofera is a group of squamate reptiles containing more than 6000 species. Their species richness is also reflected in the diversity of their sex determination systems. The presence of environmental sex determination (ESD) as well as genotypic sex determination (GSD) with either XX/XZ or ZZ/ZW sex chromosomes, was reported among the toxicoferan species; however, the current knowledge on sex determination in toxicoferan reptiles is not equally distributed across their lineages. The main aim of the theses is to expand our knowledge on sex chromosome evolution using cytogenetic methods in snakes, chameleons and anguimorphan lizards. The first part of the thesis deals with the sex chromosome evolution in caenophidian and henophidian snakes. It is focused mainly on the variability in the distribution of repetitive content as well as heterochromatinization of the W chromosome of caenophidian snakes. While the sex chromosomes of Caenophidia are cytogenetically quite well studied and easily recognizable, the homomorphic and poorly differentiated sex chromosomes of other snakes are mostly not detectable by commonly used cytogenetic methods. In our work, we examined ten species of non-caenophidian snakes. The differentiated sex chromosomes were detected only in one member, namely in the Madagascan boa *Acrantophis cf. dumerili*. The second part of the thesis focuses on the cytogenetic description of sex chromosomes in chameleons of the genus *Furcifer*: ZZ/ZW and Z₁Z₁Z₂Z₂/Z₁Z₂W multiple sex chromosomes were detected in studied species. The third part of the thesis is devoted to sex chromosomes in anguimorphan lizards. The knowledge on the sex determination mode in Anguimorpha was limited mainly to monitors (Varanidae) and beaded lizards (Helodermatidae). In our work, apart from these two families, we detected putative sex chromosomes also in *Abronia lythrochila*, *Celestus warreni* and *Gerrhonotus liocephalus* (Anguinae).

Introduction

Evolution of sex determination in amniotes

In amniotes, we can distinguish two main types of sex determination: **environmental sex determination** (ESD) and **genotypic sex determination** (GSD). Under **ESD**, the sex of the individual is determined post-zygotically based on environmental conditions (in amniotes the most commonly involved environmental factor is temperature and ESD is then assigned as temperature-dependent sex determination - TSD) (Charnov and Bull 1977, Head *et al.* 1987, Mrosovsky and Pieau 1991). Under **GSD**, sex is determined by a sex-specific genome, most commonly by the combination of sex chromosomes at the moment of egg fertilization (Leonard 2018). In amniotes, there are two basic forms of GSD: male heterogamety with XX/XY sex chromosomes (typical e.g. for mammals) and female heterogamety with ZZ/ZW sex chromosomes (typical e.g. for birds) (e.g. Ohno 1967, Beukeboom and Perrin 2014, Vicoso 2019). Two different mechanisms of genotypic sex determination were proposed: (1) sex determination can be linked to **the dominant locus on Y/W** and then the sex of the individual is determined by the presence or absence of this locus; (2) the sex of the individual is affected by the dosage of copies of the sex-determining locus linked to the X/Z chromosomes, resulting in **dosage-dependent mechanism** (Figure 1, Smith 2010, Kuroiwa 2018).

Whether a species does or does not possess sex chromosomes can play important role in diversification and speciation (Bull *et al.* 1983, O'Neill and O'Neill 2018). The presence or absence of sex chromosomes can also affect offspring sex ratio, which can even influence the survival or extinction of the whole population/species (Korpelainen *et al.* 1990, Mitchell and Janzen 2010). Compared to species with GSD, the species with ESD are more prone to biased sex ratios (Bókony *et al.* 2019). Recently there are several studies showing that climate change can lead to skewed sex ratios. Global warming can affect sex ratios e.g. in the clutches of sea turtles where mainly females hatch at higher temperatures (Hawkes *et al.* 2007, Tomillo and Spotila 2020). An equal or at least less biased offspring sex ratio can be expected for species with sex chromosomes. However, even in this case, sex ratios can depart from 1:1 due to e.g. sex-specific mortality of embryos (e.g. Goth and Booth 2005, Li *et al.* 2008), resorption of fetuses of a particular sex (Krackow 1992), meiotic drive (e.g. Rutkowska and Badyaev 2008) or occurrence of sex reversals (Quinn *et al.* 2007, Radder 2008, Holleley *et al.* 2015, Ehl *et al.* 2017).

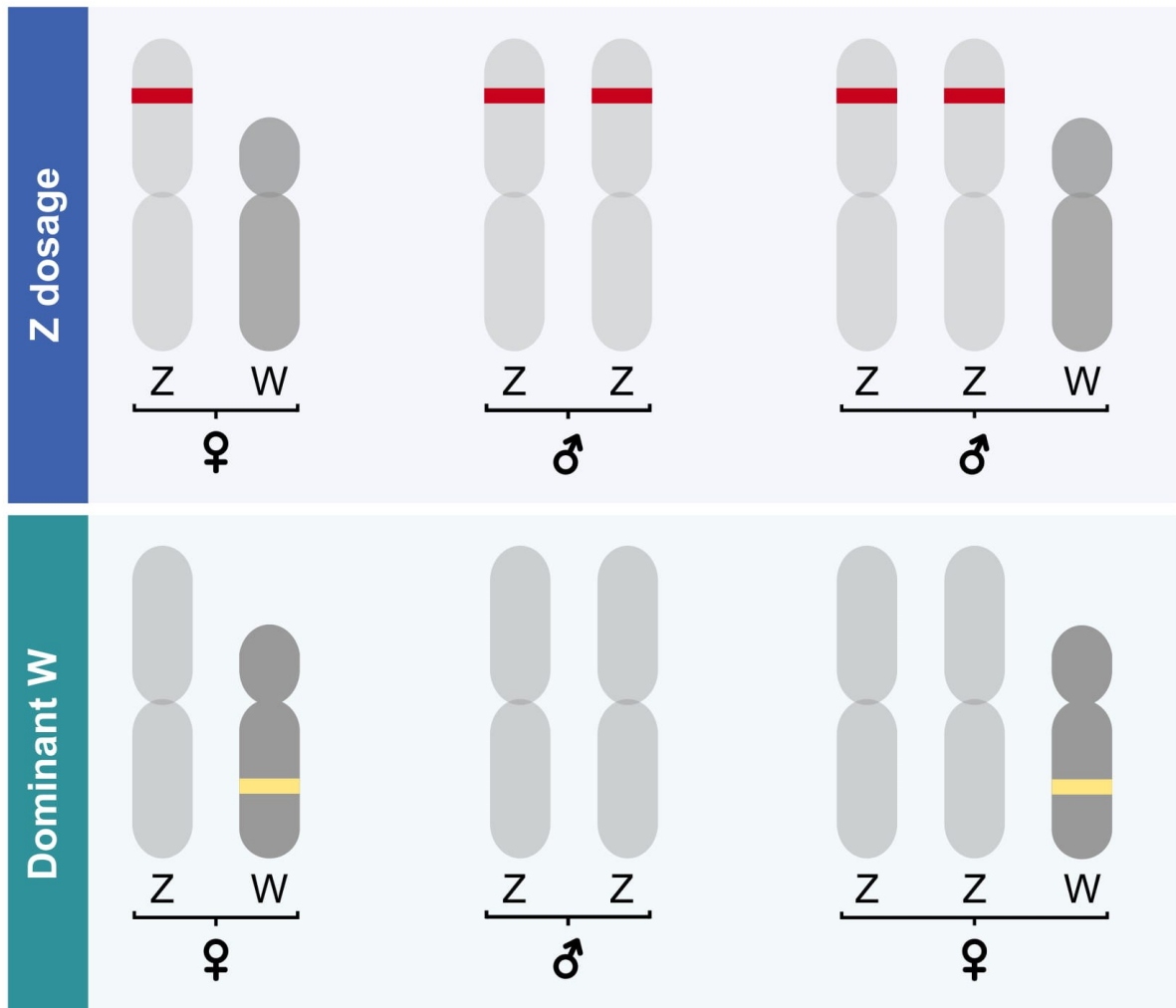


Figure 1: Mechanism of sex determination. Graphical overview of sex determination mechanisms in case of ZZ/ZW sex chromosomes. Figure constructed according to Smith 2010.

With the expanding knowledge on sex-determination systems there raises the question: which sex-determination system was ancestral for the amniotes? Environmental sex determination or genotypic sex determination? In several studies, **GSD** was postulated as the **ancestral** sex determination system in amniotes. The observation that sex chromosomes of monotreme mammals, birds, and the gecko *Gekko hokouensis* partially share homologous genomic regions (Grützner *et al.* 2004, Rens *et al.* 2007, Kawai *et al.* 2009) led to the speculation that the common ancestor of amniotes probably had “bird-like proto-sex chromosomes” (Marshall 2009, Zechner and Hameister 2011, O’Meally *et al.* 2012). This hypothesis was recently further expanded, indicating that the common ancestor of amniotes had a “super-sex chromosome”, which subsequently underwent multiple rearrangements (mainly fissions) involving chromosomal regions that formed the extant sex chromosomes in various

amniote lineages, including birds, gekkotan lineages, lacertid lizards, monotremes and viviparous mammals (Ezaz *et al.* 2017, Singchat *et al.* 2018, Singchat *et al.* 2020, Laopichienpong *et al.* 2021). Although it appears that only some parts of the genome are typically participating in the evolution of the sex chromosomes in amniotes, which can support the hypothesis of super-sex chromosome, there are alternative explanations such as independent **co-option of genomic regions** for the role of sex chromosomes (Marshall Graves and Peichel 2010, O'Meally *et al.* 2012, Ezaz *et al.* 2017, Kratochvíl *et al.* 2021).

On the other hand, the hypotheses of **ancestral ESD** assumes that the common ancestor of amniotes had environmental sex determination and during the time, sex chromosomes evolved independently in different groups of amniotes. This hypothesis gain support from the interpretation of the distribution of sex determination systems in a phylogenetic context (Pokorná and Kratochvíl 2009, Johnson Pokorná and Kratochvíl 2016).

Sex chromosomes evolution

According to the classical model of the evolution of sex chromosomes, the sex chromosomes originated from a pair of autosomes (Figure 2) when one of the chromosomes attained a locus responsible for sex determination (Ohno 1967, Charlesworth 1991, Charlesworth *et al.* 2005). This event is usually followed by suppression of recombination, which - according to the classical model - can be induced by the occurrence/accumulation of sexually antagonistic alleles linked to the sex-determining locus. Those alleles are advantageous to one sex while disadvantageous to the other (Rice 1987, Charlesworth 1996), however, other factors can play a role as well (Ponnikas *et al.* 2018). Suppression of recombination is typically caused by structural chromosomal rearrangements such as translocations, deletions, centric fusions or pericentromeric and paracentric inversions (e.g. Ayling and Griffin 2002, Charlesworth *et al.* 2005, Lemaitre *et al.* 2009). The suppression of recombination often leads to degeneration of the Y or W chromosome by the accumulation of repetitive sequences, usually connected with heterochromatinization and eventually further structural changes and gene loss (Charlesworth and Charlesworth 2000, Charlesworth *et al.* 2005, Steinemann and Steinemann 2005, Bachtrog 2013). Such structural changes can be connected with changes in chromosome morphology, leading to heteromorphic sex chromosomes (Charlesworth 1991, Charlesworth *et al.* 2005). Notably, changes in sex

chromosome morphology (homomorphic/heteromorphic) are not always correlated with changes in gene content, i.e. degree of sex chromosome differentiation.

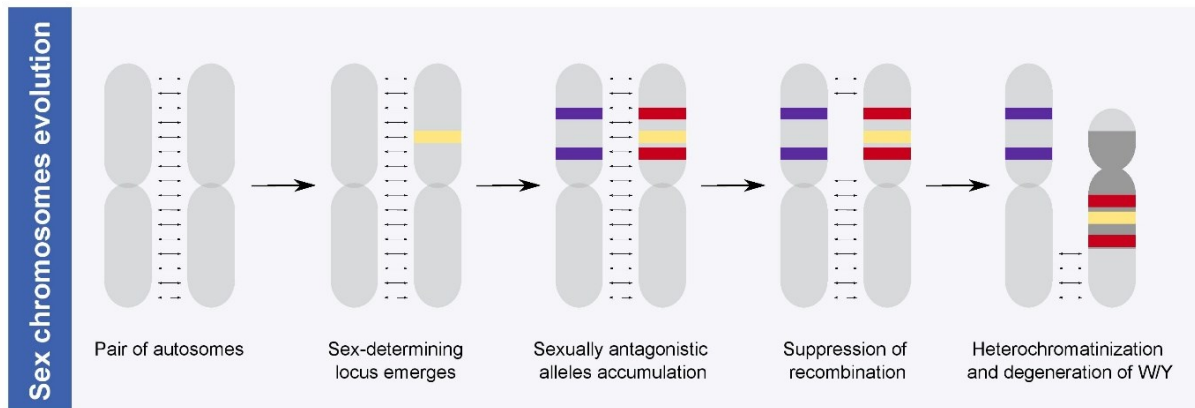


Figure 2: Classical model of sex chromosomes evolution. According to Charlesworth (1991).

Once evolved, sex chromosomes can be evolutionary stable in some lineages, while in other lineages we detect frequent switches between sex chromosomes and/or determination systems. The event that leads to the transition between two GSD systems is known as the **turnover of sex chromosomes** (Kitano and Peichel 2012, Palmer *et al.* 2019). The sex chromosome turnover can occur: (1) by the rise of a new sex-determining gene, which overwrites the effect of the previous one (Kondo *et al.* 2006, Tanaka *et al.* 2007), or (2) by transposition of the already existing sex-determining gene to a different chromosome, which leads to the emergence of new sex chromosomes with the same sex-determining gene, but with different gene content (Woram *et al.* 2003).

In some cases, the **high turnover rate** of sex chromosomes (when switches between the sex chromosomes are so common, that there is no evolutionary time for the degeneration of the unpaired chromosomes) can be one of the explanations for the presence of poorly differentiated sex chromosomes in some lineages (Schartl 2004). Alternatively, the model of “**Fountain of youth**” suggests that rare recombination of X and Y chromosomes (Z and W sex chromosomes, respectively) occurring in sex-reverted individuals can maintain sex chromosomes in a poorly differentiated state for a long evolutionary time (Perrin 2009).

The sex chromosome differentiation and degeneration cause problems with unequal gene copy number, which might affect gene expression (Ohno 1967, Charlesworth 1996, Gartler 2014, Brockdorff and Turner 2015, Gu and Walters 2017). In order to prevent the problems connected to unbalanced gene expression between sexes, different gene **dose regulatory mechanisms** evolved (Charlesworth 1996, Mank 2009, Mank *et al.* 2011,

Brockdorff and Turner 2015, Gu and Walters 2017, Rovatsos *et al.* 2021). Nowadays we usually distinguish between **dosage compensation** and **dosage balance** (Brockdorff and Turner 2015, Gu and Walters 2017). The dosage compensation is a mechanism that restates the ancestral state of gene expression in the heterogametic sex and achieves equal expression levels between sexes for the genes linked to the non-recombining part of the X/Z chromosomes (Mank 2009, Brockdorff and Turner 2015, Gu and Walters 2017). The dosage compensation can be either complete (“global”) and then all X/Z- specific genes are compensated in the heterogametic sex (Mank 2009, Mank *et al.* 2011, Gu and Walters 2017) or it can be incomplete (“partial”), where X/Z- specific genes in the heterogametic sex do not achieve the ancestral level (Mank 2009, Mank and Ellegren 2009, Mank *et al.* 2011, Vicoso *et al.* 2013, Gu and Walters 2017). Incomplete dosage compensation can be either **chromosome-wide**, where all genes in an extensive chromosomal region (or even full chromosome) are regulated (Mank 2009, Mank *et al.* 2011, Wheeler *et al.* 2016), or **gene-by-gene**, where the expression of only a limited number of genes is regulated (Melamed and Arnold 2007, Mank and Ellegren 2009). Dosage balance is a regulatory mechanism that equalizes the gene expression between the sexes, however, the gene expression is not necessarily adjusted to the ancestral state (Gu and Walters 2017). Notably, many species seem to tolerate the gene dose imbalance, caused by the sex chromosome differentiation process and did not evolve any gene dose regulatory mechanism (Rovatsos *et al.* 2021).

Phylogenetic overview of Toxicofera reptiles

This thesis is focused on Toxicofera, the group of squamate reptiles distributed in all continents except Antarctica (Vitt and Caldwell 2009). With more than 6000 species divided into around 50 families (Figure 3, Pyron *et al.* 2013, Uetz *et al.* 2021), they contain more than half species of the extant diversity of squamate reptiles (Uetz *et al.* 2021). The clade Toxicofera can be divided into three subclades: (1) Anguimorpha (e.g. anguids, beaded lizards and monitors), (2) Iguania (chameleons, dragon lizards, iguanas) and (3) Serpentes (snakes) (Figure 3, Pyron *et al.* 2013).

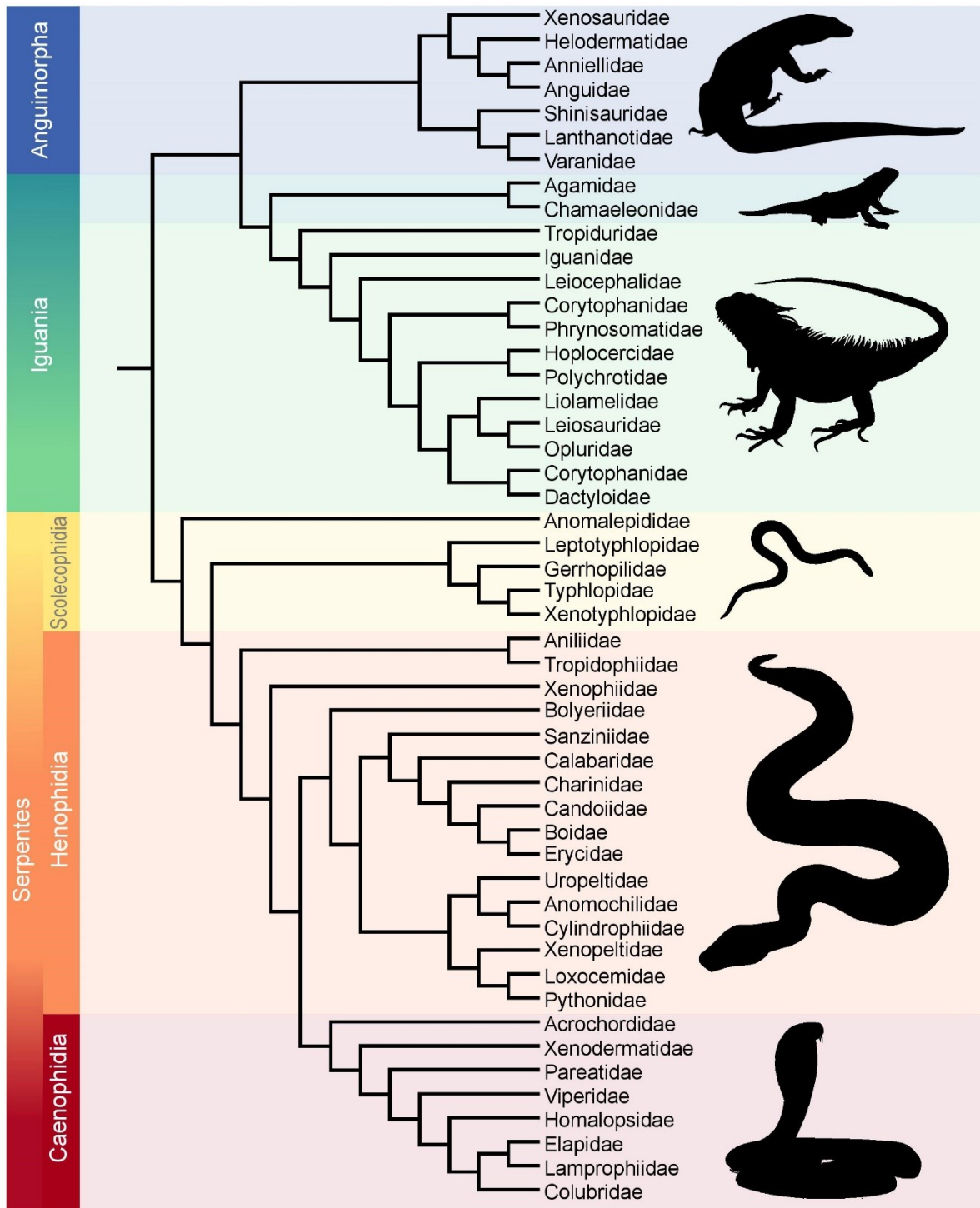


Figure 3: Phylogeny of Toxicofera. Phylogenetic overview of the toxicoferan families. Constructed according to Pyron *et al.* (2013, 2014a,b). The groups Scolecophidia and Henophidia are not monophyletic.

The name of the clade - Toxicofera - comes from the Greek word for toxin (*τοξίνη*) and Latin word *ferre*, meaning carry. According to the name, Toxicofera is a group of reptiles carrying toxins. The group includes reptile lineages with species that can produce toxins, however, not all toxicoferan species can produce toxins, nor all that produce toxins present a threat for humans (Fry *et al.* 2006, 2009, 2012, Hargreaves *et al.* 2014, 2017). It is assumed that the ability to produce toxins evolved in the common ancestor of Toxicofera approximately 170 MYA (Million Years Ago), indicating that many of the genes for toxin production are homologous (Fry *et al.* 2006). The hypothesis assumes that the toxins and venom glands were diversified later in evolution, reduced, or even lost in some lineages (reviewed in Fry *et al.* 2009). On the contrary, more recent work of Hargreaves *et al.* (2014, 2017) suggests that venom evolved at least twice among toxicoferan reptiles.

(1) Anguimorpha

The infraorder Anguimorpha with approximately only 240 species (Uetz *et al.* 2021) is divided into seven families: Anguidae, Anniellidae, Helodermatidae, Lanthanotidae, Shinisauridae, Varanidae and Xenosauridae (Figure 3, Pyron *et al.* 2013). Although this group contains only a small number of species, they are distributed worldwide (Vitt and Caldwell 2009) and quite diverse: from legless fossorial forms such as Anniellidae (Jennings and Hayes 1994, Kuhn *et al.* 2005) through semi-aquatic species (Harrisson and Haile 1961, Mendyk *et al.* 2015 Reinhardt *et al.* 2019) to large terrestrial monitors (Pianka 1995, Ciofi and De Boer 2004).

(2) Iguania

The suborder Iguania with almost 2000 species (Uetz *et al.* 2021) can be divided into three monophyletic groups: dragon lizards (Agamidae), chameleons (Chamaeleonidae) and 12 families of iguanas: Corytophanidae, Crotaphytidae, Dactyloidae, Hoplocercidae, Iguanidae, Leiocephalidae, Leiosauridae, Liolaemidae, Opluridae, Phrynosomatidae, Polychrotidae and Tropiduridae (Figure 3, Pyron *et al.* 2013). While dragon lizards and chameleons are strictly distributed in the “Old World” (Africa and Eurasia) and Australia (Vitt and Caldwell 2009), Iguanas are distributed in the “New World” (Southern and Northern America) (Vitt and Caldwell 2009), with the exception of the family Opluridae distributed in Madagascar and Comoro Islands (Vences *et al.* 2008, Vitt and Caldwell 2009). Interestingly, a single genus (*Brachylophus*) from the family Iguanidae is distributed in the South Pacific islands of Fiji and Tonga (Avery and Tanner 1970, Keogh *et al.* 2008).

(3) Snakes

The suborder Serpentes (snakes) with over 3800 species contains more than one-third of the extant species of squamate reptiles (Uetz *et al.* 2021). Despite their more or less uniform legless body shape, snakes were able to colonize different environments from subterranean habitats through deserts and from rain forests up to the open oceans (Heatwole 1999). Snakes are distributed in all continents with the exception of Antarctica (Vitt and Caldwell 2009). For simplicity, the snakes used to be still divided into three main groups: Scolecophidia, Henophidia and Caenophidia (Figure 3, Parris and Holman 1978, Heise *et al.* 1995, Lee and Scanlon 2002). The paraphyletic group Scolecophidia (Figure 3, Pyron *et al.* 2013) includes blind snakes and other fossorial forms (O’Shea 2018). The second, also paraphyletic group Henophidia includes mainly boas and pythons (Figure 3, Pyron *et al.* 2013, Uetz *et al.* 2021), often referred to in the literature as “basal snakes”. The third group, Caenophidia, is a monophyletic group of snakes, often called “advanced snakes” (Figure 3, Pyron *et al.* 2013, 2014a).

Evolution of sex determination in Toxicofera

The sex determination systems are more evolutionary stable in some lineages than in others. Compared to mammals (Cortez *et al.* 2014), reptiles with the exception of birds (Zhou *et al.* 2014, Mazzoleni *et al.* 2021), are in general quite variable in sex determination (e.g. Sarre *et al.* 2004, Ezaz *et al.* 2009a, Gamble 2010, Rovatsos *et al.* 2015b, Nielsen *et al.* 2019). This applies also to toxicoferan reptiles, where we can find lineages with environmental as well as genotypic sex determination, GSD under either female or male heterogamety (e. g. Ezaz *et al.* 2013, Rovatsos *et al.* 2015a,b, 2019a,b, Gamble *et al.* 2017, Altmanová *et al.* 2018, Augstenová *et al.* 2018a, Nielsen *et al.* 2018, 2019). While the presence of ESD was so far confirmed only in several species of dragon lizards (Harlow 2004), the GSD was confirmed in several lineages (Figure 4, Ezaz *et al.* 2013, Rovatsos *et al.* 2014b, 2015a,b, 2019a,b, Gamble *et al.* 2017, Altmanová *et al.* 2018, Augstenová *et al.* 2018a, Nielsen *et al.* 2018, 2019, Matsubara *et al.* 2019b, Alam *et al.* 2020, Sidhom *et al.* 2020, Kratochvíl *et al.* 2021) by using different methods from conventional cytogenetic methods to whole-genome sequencing. The more advanced molecular methods can allow us to reveal their homology, particular syntenic blocks are commonly referred to with the respect to homology to chicken chromosomes (GGA). The

partial gene content is so far known in seven lineages of Toxicofera (Figure 4), however, even with our constantly expanding knowledge many lineages are still not well studied.

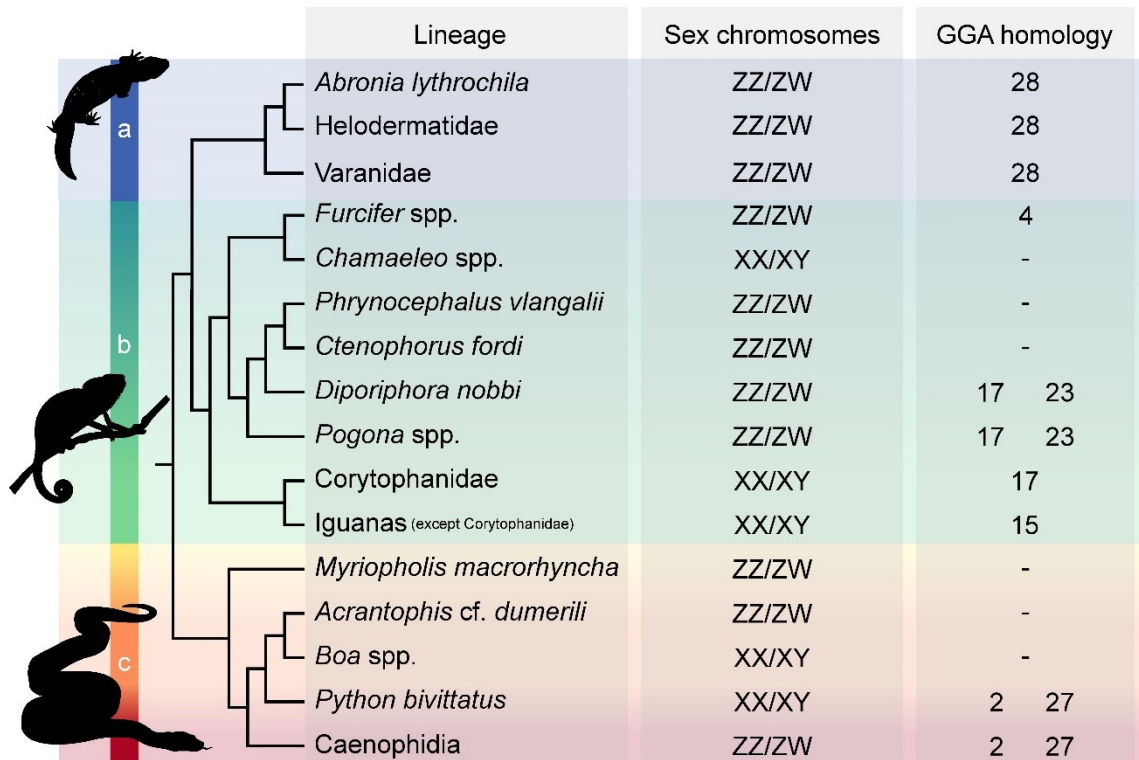


Figure 4: Phylogenetic distribution of sex chromosomes and sex chromosome homology in Toxicofera. Current knowledge on sex chromosome evolution and homology of the sex chromosomes to the chicken chromosomes (GGA) in Toxicofera. Unknown data on GGA homology are marked with dash (-). The figure was constructed based of the data from Ezaz *et al.* (2013), Rovatsos *et al.* (2014b, 2015a,b, 2019a,b), Gamble *et al.* (2017), Altmanová *et al.* (2018), Augstenová *et al.* (2018a), Nielsen *et al.* (2018, 2019), Matsubara *et al.* (2019b), Alam *et al.* (2020), Sidhom *et al.* (2020), Kratochvíl *et al.* (2021). Phylogenetic relations according to Pyron *et al.* (2013).

(1) Anguimorpha

In Anguimorpha, sex chromosomes were studied mainly in monitors (Varanidae) and bearded lizards (Helodermatidae) (Olmo and Signorino Rovatsos *et al.* 2019a, Augstenová *et al.* 2021). All studied species of monitors possess female heterogamety (Olmo and Signorino, Iannucci *et al.* 2019, Rovatsos *et al.* 2019a). The ZZ/ZW sex chromosomes were cytogenetically described as a pair of microchromosomes with heterochromatic W in several species of monitors (Iannucci *et al.* 2019). The presence of TSD was reported in *Varanus salvator* (Hairston and Burchfield 1992, Harlow 2004), but this report is considered dubious (Iannucci *et al.* 2019, Rovatsos *et al.* 2019a). Apart from the family Varanidae, the ZW sex

chromosomes were cytogenetically described also in three *Heloderma* species (Johnson Pokorná *et al.* 2014, Augstenová *et al.* 2021)

Recently, two papers support the homology of the Z and the W chromosome across the phylogeny of monitors (Iannucci *et al.* 2019, Rovatsos *et al.* 2019a). The Z chromosome of monitors are homologous to chicken chromosome 28 (GGA28) (Rovatsos *et al.* 2019a, Lind *et al.* 2019) and appeared to be homologous also to the Z chromosome of helodermatids and the anguid *Abronia lythrochila* (Anguidae). This finding suggested that the ZZ/ZW sex chromosomes of Anguimorpha evolved approximately 115-180 MYA (Rovatsos *et al.* 2019a).

Although around 30 species of the families Anguidae, Annielidae, Shinisauridae and Xenosauridae were cytogenetically studied (reviewed in Augstenová *et al.* 2021), the putative ZZ/ZW sex chromosomes were described only in three species from the family Anguidae, namely in *Abronia lythrochila*, *Celestus warreni* and *Gerrhonotus liocephalus* (Augstenová *et al.* 2021). The sex chromosomes of the remaining anguimorph species are unknown and likely homomorphic and poorly differentiated.

(2) Iguania

Dragon lizards

Species of the family Agamidae possess either environmental or genotypic sex determination with female heterogamety, which makes them the only toxicoferan reptiles with confirmed ESD (Harlow 2004). Multiple transitions between different sex determination systems were suggested for agamids (Ezaz *et al.* 2009b). However, sex determination is unstudied in most of the species. Although the presence of GSD was suggested in several agamid species (Harlow 2004, Ezaz *et al.* 2009b), the ZZ/ZW sex chromosomes were uncovered only in five species. In *Phrynocephalus vlangalii* the sex chromosomes were detected as a pair of macrochromosomes (Zeng 1997, Alam *et al.* 2020), while in the other four species (*Ctenophorus fordi*, *Diporiphora nobbi*, *Pogona barbata* and *Pogona vitticeps*), the sex chromosomes are a pair of microchromosomes (Ezaz *et al.* 2005, 2009b, Matsubara *et al.* 2019a, Alam *et al.* 2020).

The bearded dragon (*Pogona vitticeps*) is the only agamid species where sex determination was studied in more details. Sex chromosomes are a pair of microchromosomes with highly heterochromatic W, sharing partial homology with the chicken chromosomes GGA17 and GGA23 (Ezaz *et al.* 2013). Furthermore, the ZZ/ZW sex chromosomes of *Pogona vitticeps* are homologous with ZZ/ZW sex chromosomes of *Pogona barbata*, *Pogona minor*,

Pogona henrylawsoni and *Diporiphora nobbi* (Ezaz *et al.* 2013, Ehl *et al.* 2021), however, their sex determination appeared not to be homologous with ZZ/ZW sex chromosomes of *Ctenophorus fordi* (Ezaz *et al.* 2013).

Although the sex chromosomes of *Pogona vitticeps* seem to be well-differentiated, several cases of sex-reverted individuals were described (Quinn *et al.* 2007, Holleley *et al.* 2015, Ehl *et al.* 2017). The sex-reverted hatchlings were induced by high incubation temperature (above 32 °C) which led to fully fertile ZZ females (Quinn *et al.* 2007, Holleley *et al.* 2015, Ehl *et al.* 2017). The possible presence of sex reversals was described also in the species *Amphibolurus muricatus* (Quinn *et al.* 2011, Whiteley *et al.* 2021). Despite several studies describing the presence of TSD in this species (Warner and Shine 2005, 2007, 2008 Warner *et al.* 2013, Esquerré *et al.* 2014, Schwanz 2016), the recent work of Whiteley *et al.* (2021) suggests the presence of cryptic sex chromosomes in *Amphibolurus muricatus* due to occurrence of female-biased offspring sex ratio at extreme incubation temperatures and the detection of ovotestes in approximately 50% females hatched at these extreme temperatures. These similarities with *Pogona vitticeps* led the authors to conclude that *A. muricatus* could also possess GSD, however, the sex chromosomes have not been detected yet (Whiteley *et al.* 2021).

Chameleons

The sex determination of chameleons was described only in the genera *Furcifer* (ZZ/ZW) and *Chamaeleo* (XX/XY) (Rovatsos *et al.* 2015a, 2019b, Nielsen *et al.* 2018, Sidhom *et al.* 2020). The possible presence of female heterogamety was reported in *Bradypodion ventrale* (reported in Olmo and Signorino 2005), however, the data have not been published. The ZZ/ZW sex chromosomes were described in six species of the genus *Furcifer* (Rovatsos *et al.* 2015, 2019). In four species (*F. bifidus*, *F. pardalis*, *F. verrucosus*, *F. willsii*), multiple sex chromosomes ($Z_1Z_1Z_2Z_2/Z_1Z_2W$) were detected (Rovatsos *et al.* 2015a, 2019b). Furthermore, it is possible that multiple sex chromosomes evolved at least twice in the genus *Furcifer* (Rovatsos *et al.* 2019b).

Despite previous reports on the presence of ESD in *Chamaeleo calytratus* (Andrews 2005, Ballen *et al.* 2016), XX/XY sex chromosomes were recently detected by restriction site associated DNA (RAD) sequencing method (Nielsen *et al.* 2018). The presence of homologous XX/XY sex chromosomes was found also in the closely related species *Chamaeleo chamaeleon* (Sidhom *et al.* 2020).

Iguanas

All studied iguanas possess GSD with XX/XY sex chromosomes varying from poorly differentiated to well-differentiated heteromorphic sex chromosomes (Rovatsos *et al.* 2014b, Altmanová *et al.* 2018). Thanks to the whole-genome sequencing of the green anole *Anolis carolinensis*, we know the partial gene content of the X chromosome, which is homologous to the chicken chromosome 15 (GGA15) (Alföldi *et al.* 2011). The homology of sex determination was tested in other species of *Anolis* (Gamble *et al.* 2014, Rovatsos *et al.* 2014a) and later also across representatives from other iguanid families (Rovatsos *et al.* 2014b, Altmanová *et al.* 2018). It appeared that despite numerous cytogenetic differences and the presence of multiple sex chromosomes in several species, all families of iguanas possess homologous XX/XY sex chromosomes with the only exception of basilisks (Corytophanidae) (Altmanová *et al.* 2018, Nielson *et al.* 2019). The XX/XY sex chromosomes of the family Corytophanidae are homologous to the GGA17 genomic region (Nielson *et al.* 2019).

(3) Snakes

In the case of snakes, there is no evidence of temperature-dependent sex determination and therefore it is expected that all snakes possess GSD (Valenzuela and Lance 2004). For decades, ZZ/ZW sex determination system was assumed in all snakes (Beçak *et al.* 1964, Matsubara *et al.* 2006, 2016, Oguiura *et al.* 2009, Vicoso *et al.* 2013), however, the sex chromosomes were studied by cytogenetic methods mainly in caenophidian snakes. In the case of Caenophidia, the sex chromosomes are usually the 4th or 5th largest chromosome pair of their karyotype with a variable degree of differentiation among species (Beçak *et al.* 1964, Oguiura *et al.* 2009, Augstenová *et al.* 2018b). The W chromosome of Caenophidia is typically heterochromatic and enriched in repetitive content (Augstenová *et al.* 2018b), such as the GATA, GACA or AC satellite motifs, except for *Acrochordus javanicus* (Rovatsos *et al.* 2017, Augstenová *et al.* 2018b). Caenophidian snakes share similar diploid chromosome number, the morphology of sex chromosomes and other karyotypic traits (Olmo and Signorino, 2005, Oguiura *et al.* 2009). However, there are few exceptions, such as several elapid species with multiple sex chromosomes: ZZ/ZW₁W₂ in sea snake *Enhydrina schistosa* (Singh 1972) and Z₁Z₂/Z₁Z₂W in *Bungarus caeruleus* (Singh *et al.* 1970).

With the development of molecular methods, we are getting more information about the snake sex chromosomes and a few years ago the gene content of the snake Z chromosome was

partially uncovered. The Z chromosome of all studied caenophidian snakes is homologous to GGA2, GGA4 and GG27 (Vicoso *et al.* 2013; Rovatsos *et al.* 2015b). The Z-specific gene content is shared across all caenophidian families, but not with the henophidian snakes (Rovatsos *et al.* 2015b).

This finding was one of the indications that henophidian snakes might possess different sex chromosomes than caenophidian snakes. Several studies were indicating the presence of male heterogamety in “basal” snakes. One of such indications was the facultative parthenogenetic reproduction with only female offspring described in several species of boas and pythons, e.g. in *Boa constrictor*, *Epicrates maurus* and *Python bivittatus* (Booth *et al.* 2011a,b, 2014). The second indication was a sex-linked colour mutation of the ball python *Python regius* inherited from fathers to sons, which indicated a Y-specific inheritance (Mallery *et al.* 2016). On the contrary, ZZ/ZW sex chromosomes were previously reported in the Madagascar boa *Acrantophis dumerili* (Mengden and Stock 1980). Finally, in 2017 the group of Tony Gamble described the presence of XX/XY sex chromosomes in the python *Python bivittatus* and the boas *Boa constrictor* and *Boa imperator*. Furthermore, it appeared that those two systems are not homologous. While the Y chromosome of *Python bivittatus* shares at least partially gene content with the Z chromosome of caenophidian snakes, the gene content of the Y chromosome of the boas is different (Gamble *et al.* 2017). These authors also questioned the presence of ZZ/ZW in *A. dumerili* and attributed it to misidentification of the sex in the old study. However, the presence of ZZ/ZW sex chromosomes was later cytogenetically confirmed in *A. cf. dumerili* by Augstenová *et al.* (2018a). The rest of cytogenetically studied henophidian snakes possess homomorphic, poorly differentiated sex chromosomes (Matsubara *et al.* 2006, Vianna *et al.* 2016, Augstenová *et al.* 2019).

The least studied group of snakes are scolecophidian snakes. There is almost nothing known about their sex chromosomes. The putative ZZ/ZW sex chromosomes were recently described in one species, *Myriopholis macrorhyncha* (Leptotyphlopidae) (Matsubara *et al.* 2019b). However, whether these chromosomes are homologous to the caenophidian ZW sex chromosomes remains unknown.

Aims of the thesis

The main aim of the thesis was the exploration of sex chromosomes across a wide phylogenetic spectrum of toxicoferan reptiles, with the major focus on groups with not well-studied sex determination systems and the expansion of our knowledge on the sex chromosome evolution in the lineages with previously reported sex chromosomes. In the thesis, I aimed to:

- 1) Explore the evolutionary dynamics of repetitive elements in the W chromosomes of caenophidian (“advanced“) snakes across a wide phylogenetic spectrum **(Chapter 1)**.
- 2) Uncover sex chromosomes in non-caenophidian snakes by conventional and molecular cytogenetic methods **(Chapter 3, 4)**.
- 3) Cytogenetically examine and identify sex chromosomes in unstudied species of chameleons from the genus *Furcifer* **(Chapter 5)**.
- 4) Uncover sex chromosomes in unstudied anguimorphan lizards by cytogenetic methods **(Chapter 6)**.

Publications overview

Part I – Sex determination and sex chromosome evolution in snakes

- **Chapter 1:** Augstenová B., Mazzoleni S., Kratochvíl L., Rovatsos M. (2018): Evolutionary dynamics of the W chromosome in caenophidian snakes. *Genes*, 9: 5.
- **Chapter 2:** Rovatsos M., Augstenová B., Altmanová M., Sloboda M., Kodým P., Kratochvíl L. (2018): Triploid colubrid snake provides insight into the mechanism of sex determination in advanced snakes. *Sexual Development*, 12: 251-255.
- **Chapter 3:** Augstenová B., Johnson Pokorná M., Altmanová M., Frynta D., Rovatsos M., Kratochvíl L. (2018): ZW, XY, and yet ZW: Sex chromosome evolution in snakes even more complicated. *Evolution*, 72: 1701-1707.
- **Chapter 4:** Augstenová B., Mazzoleni S., Kostmann A., Altmanová M., Frynta D., Kratochvíl L., Rovatsos M. (2019): Cytogenetic analysis did not reveal differentiated sex chromosomes in ten species of boas and pythons (Reptilia: Serpentes). *Genes*, 10: 934.

Part II – Sex determination and sex chromosome evolution in chameleons

- **Chapter 5:** Rovatsos M., Altmanová M., Augstenová B., Mazzoleni S., Velenský P., Kratochvíl L. (2019): ZZ/ZW sex determination with multiple neo-sex chromosomes is common in Madagascan chameleons of the genus *Furcifer* (Reptilia: Chamaeleonidae). *Genes*, 10: 1020.

Part III – Sex determination and sex chromosome evolution in anguimorph lizards

- **Chapter 6:** Augstenová B., Pensabene E., Kratochvíl L., Rovatsos M. (2021): Cytogenetic evidence for sex chromosomes and karyotype evolution in anguimorph lizards. *Cells*, 10: 1612.

Outline of the publications

The thesis contains six published original investigations divided into three thematic parts. The first part is focused on the evolution of sex chromosomes in snakes (**Chapters 1-4**). The second part is focused on the detection of sex determination system in chameleons of the genus *Furcifer* (**Chapters 5**). The third part is focused on the sex chromosome evolution of anguimorph lizards (**Chapter 6**). My contribution to each chapter is summarized in Table 1.

Table 1: My contribution to the the Chapters 1-6 of the theses. Detailed overview of contribution of all authors for Chapter 1 and Chapters 3-6 is described in the end of each publication.

Chapetr	Original investigation	Journal	Contribution				
			Experimental design	Experimental procesures	Figure/table preparation	Data analyses	Manuscript preparation
1	Augstenová <i>et al.</i> 2018	<i>Genes</i>	x	x	x	x	x
2	Rovatsos <i>et al.</i> 2018	<i>Sex. Dev.</i>		x	x	x	x
3	Augstenová <i>et al.</i> 2018	<i>Evolution</i>	x	x	x	x	x
4	Augstenová <i>et al.</i> 2019	<i>Genes</i>	x	x	x	x	x
5	Rovatsos <i>et al.</i> 2019	<i>Genes</i>		x	x	x	x
6	Augstenová <i>et al.</i> 2021	<i>Cells</i>	x	x	x	x	x

Chapter 1 is focused mainly on the examination of the evolutionary dynamics of the repetitive content of the W chromosome of caenophidian snakes using cytogenetic methods. We studied 13 species of snakes from six families (Acrochordidae, Colubridae, Homalopsidae, Lamprophiidae, Viperidae, Xenodermatidae) out of eight caenophidian families. In order to cover most of the phylogeny, we combined our results with already existing data from the literature, which allowed us to add and compare also the species from the family Elapidae. In the study, we performed fluorescence *in situ* hybridization (FISH) with four microsatellite motifs typically accumulated on the sex chromosomes: GATA, GACA, AG and TTAGGG (the telomeric-like sequence). Apart from the distribution of the repetitive elements, we also compared the distribution of the heterochromatin on the W chromosome by C-banding. The W chromosomes of all studied species were heterochromatinic, while the microsatellite motifs were accumulated in all chromosomes with the exception of the W chromosome of the species *Acrochordus javanicus* (Acrochordidae), which is placed at the base of the caenophidian phylogeny (Pyron *et al.* 2014a). The presence of heterochromatic W in *A. javanicus* along with the lack of accumulation of the tested repetitive sequences questions the role of microsatellite accumulation in the heterochromatinization of the W chromosomes of caenophidian snakes as was previously suggested (Subramanian *et al.* 2003, O'Meally *et al.*

2010). The W chromosomes of caenophidian snakes show in general differences in the degree of heterochromatinization as well as in the distribution of the microsatellite motifs even in closely related species. This finding might suggest that the repetitive content of the W chromosomes show higher evolutionary variability compared to the evolutionary stable Z chromosome. In addition, we described the karyotypes of six species of caenophidian snakes for the first time.

During the study of the repetitive content of the W chromosomes in snakes, a triploid individual of the species *Elaphe bimaculata* was discovered (**Chapter 2**). The animal was a male with only a single testis. The triploidy was probably a result of a spontaneous event. The cytogenetic analyses detected the presence of ZZW sex chromosomes. This is an important finding which can help us in understanding the sex determination mechanism of caenophidian snakes. The presence of ZZW sex chromosomes in the male individual suggests the possibility of the “Z dosage” sex-determining mechanism; however, the testis of the animal was malformed and it was most likely sterile. More investigations will be needed to confirm the sex determination mechanisms in snakes.

The main aim of **Chapters 3 and 4** was to detect the sex chromosomes in previously unstudied henophidian snakes by conventional and molecular cytogenetic methods. **Chapter 3** is focused on the species from the Madagascan family Sanziniidae, which is the only family of basal snakes with previously detected ZZ/ZW sex chromosomes. In our study we examined four species: *Acrantophis dumerili*, *Acrantophis cf. dumerili*, *Acrantophis madagascariensis* and *Sanzinia madagascariensis*. The heteromorphic ZZ/ZW sex chromosomes were described only in *Acrantophis cf. dumerili*. The sex chromosomes correspond to the 4th chromosome pair of the karyotype, confirming a previous report, published 40 years ago by Mengden and Stock (1980). The heteromorphic sex chromosomes most likely occurred as a result of pericentric inversion. Neither C-banding nor the comparative genome hybridization detected sex specific differences in any of the studied species.

Chapter 4 is focused on the sex chromosomes of pythons and boas. We studied 10 species from 6 families of pythons and boas (Boidae, Calabariidae, Candoiidae, Charinidae, Pythonidae, Sanziniidae). The used cytogenetic methods did not lead to detection of sex chromosomes in any studied species. The sex chromosomes of these species are homomorphic and poorly differentiated. In this study, we described karyotypes of five species for the first time.

In **Chapter 5** we cytogenetically studied chameleons of the genus *Furcifer*. We examined six species: *F. antimena*, *F. bifidus*, *F. lateralis*, *F. minor*, *F. verrucosus* and *F. willsii*. In the case of *F. antimena* and *F. minor* only males were examined and sex chromosomes could not be identified. We detected ZZ/ZW sex determination system in *F. lateralis* and Z₁Z₁Z₂Z₂/Z₁Z₂W multiple sex chromosomes in *F. bifidus*, *F. verrucosus* and *F. willsii*. The phylogenetic position of these species suggests that multiple sex chromosomes probably evolved independently multiple times. Four of the studied species were cytogenetically studied for the first time. Interestingly, the diploid chromosome number in chameleons is in general quite variable (Rovatsos *et al.* 2017) and within six studied species of *Furcifer* chameleons, the diploid number varies from 22 to 28 chromosomes in male karyotypes.

Chapter 6 is focused on the identification of sex chromosomes in anguimorphan lizards by conventional and molecular cytogenetic methods, including comparative genome hybridization. We cytogenetically examined 23 species from five families (Anguidae, Helodermatidae, Shinisauridae, Varanidae and Xenosauridae). Among them, 22 species were cytogenetically examined for the first time. The ZZ/ZW sex chromosomes were successfully detected in all studied species of the families Helodermatidae and Varanidae. The sex chromosomes were detected as a pair of microchromosomes. The putative ZZ/ZW sex chromosomes were detected in three other species: *Abronia lythrochila*, *Celestus warreni* and *Gerrhonotus liocephalus*, however, due to the limited sample size of these species, additional individuals are required to confirm the presence of the ZZ/ZW sex chromosomes. Interestingly among the studied individuals, we detected a spontaneous triploid specimen of *Varanus primordius*.

Conclusions and future perspectives

This thesis brings new insights into the evolution of sex chromosomes in toxiciferan reptiles, namely in the caenophidian and henophidian snakes, the anguimorphan lizards and the chameleons of the genus *Furcifer*. Alongside our results on sex chromosomes, we described karyotypes of 15 species of snakes (**Chapters 1-4**), three species of chameleons (**Chapter 5**) and 22 species of anguimorphan lizards (**Chapter 6**).

We investigated the evolutionary dynamics of repetitive elements in the W chromosomes of caenophidian snakes across a wide phylogenetic spectrum and questioned the traditionally viewed role of the microsatellite accumulation on the sex chromosomes during their differentiation process (**Chapter 1**). The discovery of the triploid snake *Elaphe bimaculata* brought new insights into the sex determination mechanisms in caenophidian snakes (**Chapter 2**). The presence of ZZW sex chromosomes in the male individual would suggest Z dosage mechanism, however, we still need additional studies for a better understanding of the mechanisms behind the sex determination in Caenophidia.

From the previously published cytogenetic studies, it seems that the vast majority of the henophidian snakes possess poorly differentiated and homomorphic sex chromosomes which are not detectable by conventional or molecular cytogenetic methods (Beçak *et al.* 1964, Matsubara 2006, Vianna *et al.* 2016, **Chapter 3, 4**). The only known exception is *Acrantophis* cf. *dumerili* with heteromorphic ZZ/ZW sex chromosomes (Mengden and Stock 1980, **Chapter 3**). This finding together with the detection of two independent origins of XX/XY sex chromosomes in pythons and boas (Gamble *et al.* 2017) and the presence of ZZ/ZW sex chromosomes in *Myriopholis macrorhyncha* (Scolecophidia) (Matsubara *et al.* 2019) show us that the sex determination in snakes is much more complicated than was assumed for decades.

The investigation of sex chromosomes in chameleons of the genus *Furcifer* revealed the presence of ZZ/ZW sex chromosomes in the species *F. lateralis* and $Z_1Z_1Z_2Z_2/Z_1Z_2W$ sex chromosomes in *F. bifidus*, *F. verrucosus* and *F. willsii*. The phylogenetic distribution of the ZZ/ZW sex chromosomes and $Z_1Z_1Z_2Z_2/Z_1Z_2W$ multiple sex chromosomes, suggests that the multiple sex chromosomes evolved in the genus *Furcifer* at least twice (**Chapter 5**).

Our study on the evolution of sex chromosomes in anguimorphan lizards revealed ZZ/ZW sex determination systems in beaded lizards (Helodermatidae) and monitors (Varanidae). Furthermore, we detected putative sex chromosomes also in *Abronia lythrochila*, *Celestus warreni* and *Gerrhonotus liocephalus* (Anguidae) (**Chapter 6**).

Future studies should focus on the identification of sex chromosome gene content in henophidian snakes and anguimorphan lizards by next generation sequencing methods, such as whole-genome sequencing and RAD sequencing. The identification of sex chromosome gene content will allow further testing of the homology of sex chromosomes across a wider phylogenetic spectrum.

References

- Alam S. M. I., Altmanová M., Prasongmaneerut T., Georges A., Sarre S. D., Nielsen, S. V. et al. (2020):** Cross-species BAC mapping highlights conservation of chromosome synteny across dragon lizards (Squamata: Agamidae). *Genes*, 11: 698.
- Alföldi J., Di Palma F., Grabherr M., Williams C., Kong L., Mauceli E. et al. (2011):** The genome of the green anole lizard and a comparative analysis with birds and mammals. *Nature*, 477: 587–591.
- Altmanová M., Rovatsos M., Johnson Pokorná M., Veselý M., Wagner F., Kratochvíl L. (2018):** All iguana families with the exception of basilisks share sex chromosomes. *Zoology*, 126: 98–102.
- Andrews R. M. (2005):** Incubation temperature and sex ratio of the veiled chameleon (*Chamaeleo calyptratus*). *J. Herpetol.*, 39: 515–518.
- Augstenová B., Johnson Pokorná M., Altmanová M., Frynta D., Rovatsos M., Kratochvíl L. (2018a):** ZW, XY, and yet ZW: Sex chromosome evolution in snakes even more complicated. *Evolution*, 72: 1701–1707.
- Augstenová B., Mazzoleni S., Kratochvíl L., Rovatsos M. (2018b):** Evolutionary dynamics of the W chromosome in caenophidian snakes. *Genes*, 9: 5.
- Augstenová B., Mazzoleni S., Kostmann A., Altmanová M., Frynta D., Kratochvíl L., Rovatsos M. (2019):** Cytogenetic analysis did not reveal differentiated sex chromosomes in ten species of boas and pythons (Reptilia: Serpentes). *Genes*, 10: 934.
- Augstenová B., Pensabene E., Kratochvíl L., Rovatsos M. (2021):** Cytogenetic evidence for sex chromosomes and karyotype evolution in anguimorph lizard. *Cells*, 10: 1612.
- Avery D. F., Tanner, W. W. (1970):** Speciation in the Fijian and Tongan iguana *Brachylophus* (Sauria, Iguanidae) with the description of a new species. *Great Basin Nat.*, 30: 166–172.
- Ayling L. J., Griffin D. K. (2002):** The evolution of sex chromosomes. *Cytogenet. Genome Res.*, 99: 125–140.
- Bachtrog D. (2013):** Y-chromosome evolution: emerging insights into processes of Y-chromosome degeneration. *Nat. Rev. Genet.*, 14: 113–124.
- Ballen C. J., Shine R., Andrews R. M. Olsson M. (2016):** Multifactorial sex determination in chameleons. *J. Herpetol.*, 50: 548–551.
- Beçak W., Beçak M. L., Nazareth H. R. S., Ohno S. (1964):** Close karyological kinship between the reptilian suborder Serpentes and the class Aves. *Chromosoma*, 15: 606–17.
- Beukeboom L. W., Perrin N. (2014):** *The evolution of sex determination*. Oxford University Press, New York, USA
- Bókony V., Milne G., Pipoly I., Székely T., Liker, A. (2019):** Sex ratios and bimaturism differ between temperature-dependent and genetic sex-determination systems in reptiles. *BMC Evol. Biol.*, 19: 1–7.

- Booth W., Million L., Reynolds R.G., Burghardt G.M., Vargo E.L., Schal C., Tzika A.C., Schuett G.W. (2011a):** Consecutive virgin births in the new world boid snake, the Colombian rainbow boa, *Epicrates maurus*. *J. Hered.*, 102: 759–763.
- Booth W., Johnson D. H., Moore S., Schal C., Vargo E. L. (2011b):** Evidence for viable, non-clonal but fatherless *Boa constrictors*. *Biol. Lett.*, 7: 253–256.
- Booth W., Schuett G. W., Ridgway A., Buxton D. W., Castoe T. A., Bastone G., Bennett C., McMahan W. (2014):** New insights on facultative parthenogenesis in pythons. *Biol. J. Linn. Soc.*, 112: 461–468.
- Brockdorff T., Turner B. M. (2015):** Dosage compensation in mammals. *Cold Spring Harb. Perspect. Biol.*, 7: a019406.
- Bull J. J. (1983):** *Evolution of sex determining mechanisms*. The Benjamin/Cummings Publishing Company, Inc., San Francisco, USA.
- Charlesworth B. (1991):** The evolution of sex chromosomes. *Science*, 251:1030–1033.
- Charlesworth B. (1996):** The evolution of chromosomal sex determination and dosage compensation. *Curr. Biol.*, 6: 149–162.
- Charlesworth B., Charlesworth D. (2000):** The degeneration of Y chromosomes. *Philos. Trans. R. Soc. Lond. B*, 355: 1563–1572.
- Charlesworth D., Charlesworth B., Marais G. (2005):** Steps in the evolution of heteromorphic sex chromosomes. *Heredity*, 95: 118–128.
- Charnov E. L., Bull J. J. (1977):** When is sex environmentally determined? *Nature*, 267: 673.
- Ciofi C., De Boer M. E. (2004):** Distribution and conservation of the Komodo monitor (*Varanus komodoensis*). *Herpetol. J.*, 14: 99–107.
- Cortez D., Marin R., Toledo-Flores D., Froidevaux L., Liechti A., Waters P. D. et al. (2014):** Origins and functional evolution of Y chromosomes across mammals. *Nature*, 508: 488–493.
- Ehl J., Vukić J., Kratochvíl L. (2017):** Hormonal and thermal induction of sex reversal in the bearded dragon (*Pogona vitticeps*, Agamidae). *Zool. Anz.*, 271: 1–5.
- Ehl J., Altmanová M., Kratochvíl L. (2021):** With or without W? Molecular and cytogenetic markers are not sufficient for identification of environmentally-induced sex reversal in the bearded dragon. *Sex. Dev.*, 1–10.
- Esquerré D., Keogh J. S., Schwanz L. E. (2014):** Direct effects of incubation temperature on morphology, thermoregulatory behaviour and locomotor performance in jacks dragons (*Amphibolurus muricatus*). *J. Therm. Biol.*, 43: 33–39.
- Ezaz T., Quinn A.E., Miura I., Sarre S. D., Georges A., Graves J. A. M. (2005):** The dragon lizard *Pogona vitticeps* has ZZ/ZW micro-sex chromosomes. *Chromosome Res.*, 13: 763–776.
- Ezaz T., Sarre S. D., O’Meally D., Graves J. M., Georges A. (2009a):** Sex chromosome evolution in lizards: independent origins and rapid transitions. *Cytogen. Genome Res.*, 127: 249–260
- Ezaz T., Quinn**

- A.E., Sarre S. D., O’Meally D., Georges A., Graves J. A. M. (2009b):** Molecular marker suggests rapid changes of sex-determining mechanisms in Australian dragon lizards. *Chromosome Res.*, 17: 91–98.
- Ezaz T., Azad B., O’Meally D., Young M. J., Matsubara K., Edwards M. J. et al. (2013):** Sequence and gene content of a large fragment of a lizard sex chromosome and evaluation of candidate sex differentiating gene R-spondin 1. *BMC Genomics*, 14: 1–13.
- Ezaz T., Srikulnath K., Marshall Graves J. A. (2017):** Origin of amniote sex chromosomes: an ancestral super-sex chromosome, or common requirements? *J. Hered.*, 108: 94–105.
- Fry B. G., Vidal N., Norman J.A., Vonk F.J., Scheib H., Ramjan S.F.R. et al. (2006):** Early evolution of the venom system in lizards and snakes. *Nature*, 439: 584–8.
- Fry B. G., Vidal N., Van der Weerd L., Kochva E., Renjifo C. (2009):** Evolution and diversification of the Toxicofera reptile venom system. *J. Proteom.*, 72: 127–136.
- Fry B. G., Casewell N. R., Wüster W., Vidal N., Young B., Jackson T. N. (2012):** The structural and functional diversification of the Toxicofera reptile venom system. *Toxicon*, 60: 434–448.
- Gamble T. (2010):** A review of sex determining mechanisms in geckos (Gekkota: Squamata). *Sex. Dev.* 4: 88–103.
- Gamble T., Geneva A. J., Glor R. E., Zarkower D. (2014):** *Anolis* sex chromosomes are derived from a single ancestral pair. *Evolution*, 68: 1027–1041.
- Gamble T., Castoe T. A., Nielsen S. V., Banks J. L., Card D. C., Schield D. R. et al. (2017):** The discovery of XY sex chromosomes in a boa and python. *Curr. Biol.*, 27: 2148–2153.
- Gartler S. M. (2014):** A brief history of dosage compensation. *J. Genet.* 93: 591–595.
- Göth A., Booth D. T. (2005):** Temperature-dependent sex ratio in a bird. *Biol. Lett.*, 1: 31–33.
- Grützner F., Rens W., Tsend-Ayush E., El-Mogharbel N., O’Brien P. C., Jones R. C., Ferguson-Smith M. A., Marshall Graves J. A. (2004):** In the platypus a meiotic chain of ten sex chromosomes shares genes with the bird Z and mammal X chromosomes. *Nature*, 432: 913–917
- Gu L., Walters J. R. (2017):** Evolution of sex chromosome dosage compensation in animals: a beautiful theory, undermined by facts and bedeviled by details. *Genome Biol. Evol.*, 9: 2461–2476.
- Hairston C. S., Burchfield P. M. (1992):** The reproduction and husbandry of the water monitor *Varanus salvator* at the Gladys Porter Zoo, Brownsville. *Int. Zoo Yearb.*, 31:124–130
- Hargreaves A. D., Swain M. T., Logan D. W., Mulley J. F. (2014):** Testing the Toxicofera: comparative transcriptomics casts doubt on the single, early evolution of the reptile venom system. *Toxicon*, 92,: 140–156.
- Hargreaves A. D., Tucker A. S., Mulley J. F. (2017):** A critique of the toxicofera hypothesis. In: Gopalakrishnakone P., Malhotra A. (eds) *Evolution of Venomous Animals and Their Toxins*. Toxinology. Springer, Dordrecht. Netherlands; p. 69–86.

- Harlow P. S. (2004):** Temperature-dependent sex determination in lizards. In: Valenzuela N., Lance V. A., (eds) *Temperature-dependent sex determination in vertebrates*. Smithsonian Books. Washington, USA; p. 42–52.
- Harrisson T., Haile N. S. (1961):** Notes on a living specimen of the earless monitor lizard, *Lanthonotus borneensis*. *J. Ohio Herpetol. Soc.*, 3: 13–16.
- Hawkes L. A., Broderick A. C., Godfrey M. H., Godley B. J. (2007):** Investigating the potential impacts of climate change on a marine turtle population. *Glob. Change Biol.*, 13: 923–932.
- Head G., May R. M., Pendleton L. (1987):** Environmental determination of sex in reptiles. *Nature*, 329: 219–222.
- Heatwole H. (1999):** *Sea snakes*. Krieger Publishing Company. Malabar, Florida, USA.
- Heise P. J., Maxson L. R., Dowling H. G., Hedges S. B. (1995):** Higher-level snake phylogeny inferred from mitochondrial DNA sequences of 12S rRNA and 16S rRNA genes. *Mol. Biol. Evol.*, 12: 259–265.
- Holleley C. E., O'Meally D., Sarre S. D., Graves J. A. M., Ezaz T., Matsubara, K *et al.* (2015):** Sex reversal triggers the rapid transition from genetic to temperature-dependent sex. *Nature*, 523: 79–82.
- Iannucci A., Altmanová M., Ciofi C., Ferguson-Smith M., Milan M., Pereira J. C. *et al.* (2019):** Conserved sex chromosomes and karyotype evolution in monitor lizards (Varanidae). *Heredity*, 123: 215–227.
- Jennings M. R., Hayes M. P. (1994):** *Amphibian and reptile species of special concern in California*. California Department of Fish and Game, Inland Fisheries Division, Sacramento, California, USA.
- Johnson Pokorná M., Kratochvíl L. (2016):** What was the ancestral sex-determining mechanism in amniote vertebrates? *Biol. Rev.*, 91: 1–12.
- Johnson Pokorná M., Rovatsos M., Kratochvíl L. (2014):** Sex chromosomes and karyotype of the (nearly) mythical creature, the Gila monster, *Heloderma suspectum* (Squamata: Helodermatidae). *PLoS One*, 9: e104716.
- Kawai A., Ishijima J., Nishida C., Kosaka A., Ota H., Kohno S., Matsuda Y. (2009):** The ZW sex chromosomes of *Gekko hokouensis* (Gekkonidae, Squamata) represent highly conserved homology with those of avian species. *Chromosoma*, 118: 43–51.
- Keogh J. S., D. L. Edwards Fisher, R. N. Harlow P. S. (2008):** Molecular and morphological analysis of the critically endangered Fijian iguanas reveals cryptic diversity and a complex biogeographic history. *Philos. Trans. R. Soc. B*, 363: 3413–3426.
- Kitano J., Peichel, C. L. (2012):** Turnover of sex chromosomes and speciation in fishes. *Environ. Biol. Fish*, 94: 549–558.
- Kondo M., Hornung U., Nanda I., Imai S., Sasaki T., Shimizu A. *et al* (2006):** Genomic organization of the sex-determining and adjacent regions of the sex chromosomes of medaka. *Genome Res.*, 16: 815–826
- Korpelainen H. (1990):** Sex ratios and conditions required for environmental sex determination in animals. *Biol. Rev.*, 65: 147–184.

- Krackow S. (1992):** Sex ratio manipulation in wild house mice: the effect of fetal resorption in relation to the mode of reproduction. *Biol. Reprod.*, 47: 541–548.
- Kratochvíl L, Gamble T, Rovatsos M. (2021):** Sex chromosome evolution among amniotes: Is the origin of sex chromosomes non-random? *Philos. Trans. R. Soc. B*, in press.
- Kuhnz L. A., Burton R. K., Slattery P. N., Oakden J. M. (2005):** Microhabitats and population densities of California legless lizards, with comments on effectiveness of various techniques for estimating numbers of fossorial reptiles. *J. Herpetol.*, 39: 395–402.
- Kuroiwa A. (2018):** Sex determination and differentiation in birds. In: *Reproductive and Developmental Strategies*. Springer, Tokyo, Japan; p. 391–405.
- Laopichienpong N., Kraichak E., Singchat W., Sillapaprayoon S., Muangmai N., Suntrarachun S. et al. (2021):** Genome-wide SNP analysis of Siamese cobra (*Naja kaouthia*) reveals the molecular basis of transitions between Z and W sex chromosomes and supports the presence of an ancestral super-sex chromosome in amniotes. *Genomics*, 113: 624–636.
- Lee M. S., Scanlon J. D. (2002):** Snake phylogeny based on osteology, soft anatomy and ecology. *Biol. J. Linn. Soc.*, 77: 333–401.
- Lemaitre C., Braga M. D., Gautier C., Sagot M. F., Tannier E., Marais G. A. (2009):** Footprints of inversions at present and past pseudoautosomal boundaries in human sex chromosomes. *Genome Biol. Evol.*, 1: 56–66.
- Leonard J. L. (2018):** The evolution of sexual systems in animals. In: *Transitions between sexual systems* Springer, Cham; p. 1–58.
- Li W. M., Feng Y. P., Zhao R. X., Fan Y. Z., Affara N. A., Wu J.J. et al. (2008):** Sex ratio bias in early-dead embryos of chickens collected during the first week of incubation. *Poult. Sci. J.*, 87: 2231–2233
- Lind A. L., Lai Y. Y., Mostovoy Y., Holloway A. K., Iannucci A., Mak A. C. et al. (2019):** Genome of the Komodo dragon reveals adaptations in the cardiovascular and chemosensory systems of monitor lizards. *Nat. Ecol. Evol.*, 3: 1241–1252.
- Mank J. E. (2009):** The W, X, Y and Z of sex-chromosome dosage compensation. *Trends. Genet.* 25: 226–233
- Mank J. E., Ellegren H. (2009):** All dosage compensation is local: gene-by-gene regulation of sex-biased expression on the chicken Z chromosome. *Heredity*, 102: 312–320.
- Mank J. E., Hosken D. J., Wedell N. (2011):** Some inconvenient truths about sex chromosome dosage compensation and the potential role of sexual conflict. *Evolution* 65: 2133–2144.
- Marshall Graves J. A. (2009):** Weird animal genomes and the evolution of vertebrate sex and sex chromosomes. *Ann. Rev. Genet.*, 42: 565–586.
- Marshall Graves J. A., Peichel C. L. (2010):** Are homologies in vertebrate sex determination due to shared ancestry or to limited options? *Genome Biol.*, 11: 205.
- Matsubara K., Tarui H., Toriba M., Yamada K., Nishida-Umehara C., Agata K., Matsuda Y. (2006):** Evidence for different origin of sex chromosomes in snakes, birds, and mammals and step-wise differentiation of snake sex chromosomes. *Proc. Natl. Acad. Sci. U. S. A.*, 103: 18190–5.

- Matsubara K., Nishida C., Matsuda Y., Kumazawa Y. (2016):** Sex chromosome evolution in snakes inferred from divergence patterns of two gametologous genes and chromosome distribution of sex chromosomelinked repetitive sequences. *Zool. Lett.* 2:19.
- Matsubara K., O'Meally D., Sarre S. D., Georges A., Srikulnath K., Ezaz T. (2019a):** ZW sex chromosomes in Australian dragon lizards (Agamidae) originated from a combination of duplication and translocation in the nucleolar organising region. *Genes*, 10: 861.
- Matsubara K., Kumazawa Y., Ota H., Nishida C., Matsuda Y. (2019b):** Karyotype analysis of four blind snake species (Reptilia: Squamata: Scolecophidia) and karyotypic changes in Serpentes. *Cytogen. Genome Res.*, 157: 98–106.
- Mazzoleni S., Němec P., Albrecht T., Lymberakis P., Kratochvíl L., Rovatsos M. (2021):** Long-term stability of sex chromosome gene content allows accurate qPCR-based molecular sexing across birds. *Mol. Ecol. Resour.*, 00: 1–9.
- Melamed E., Arnold, A. P. (2007):** Regional differences in dosage compensation on the chicken Z chromosome. *Genome Biol.*, 8: 1–10.
- Mendyk R. W., Shuter A. Kathriner A. (2015):** Historical notes on a living specimen of *Lanthanotus borneensis* (Squamata: Sauria: Lanthanotidae) maintained at the Bronx Zoo from 1968 to 1976. *Biawak*, 9: 44–49.
- Mengden G. A., Stock A. D. (1980):** Chromosomal evolution in serpentes; a comparison of G and C chromosome banding patterns of some colubrid and boid genera. *Chromosoma*, 79: 53–64.
- Mitchell N. J., Janzen F. J. (2010):** Temperature-dependent sex determination and contemporary climate change. *Sex. Dev.*, 4: 129–140.
- Mrosovsky N., Pieau C. (1991):** Transitional range of temperature, pivotal temperatures and thermosensitive stages for sex determination in reptiles. *Amphib.-Reptil.*, 12: 169–179.
- Nielsen S. V., Banks J. L., Diaz Jr. R. E., Trainor P. A., Gamble T. (2018):** Dynamic sex chromosomes in Old world chameleons (Squamata: Chamaeleonidae). *J. Evol. Biol.*, 31: 484–490.
- Nielsen S. V., Guzmán-Méndez I. A., Gamble T., Blumer M., Pinto B. J., Kratochvíl L., Rovatsos, M. (2019):** Escaping the evolutionary trap? Sex chromosome turnover in basilisks and related lizards (Corytophanidae: Squamata). *Biol. Lett.*, 15: 20190498.
- O'Meally D., Patel H. R., Stiglec R., Sarre S. D., Georges A., Marshall Graves J. A., Ezaz T. (2010):** Non-homologous sex chromosomes of birds and snakes share repetitive sequences. *Chromosome Res.*, 18: 787–800.
- O'Shea M. (2018):** "Scolecophidia". *The Book of Snakes*. University of Chicago Press, Chicago, Illinois, USA; p. 39-64.
- Oguiura N., Ferrarezzi H., Batistic R. F. (2009):** Cytogenetics and molecular data in snakes: A phylogenetic approach. *Cytogenet. Genome Res.*, 127: 128–142.
- Ohno S. (1967):** *Sex Chromosomes and Sex-linked Genes*. Springer-Verlag Berlin/Heidelberg, Germany.

- Olmo E., Signorino G. G. (2005):** Chromorep: A Reptile Chromosomes Database. Available online: <http://chromorep.univpm.it>; Accessed 17. 4. 2021.
- O'Meally D., Ezaz T., Georges A., Sarre S. D., Graves J. A. (2012):** Are some chromosomes particularly good at sex? Insights from amniotes. *Chromosome Res.*, 20: 7–19.
- O'Neill M. J., O'Neill, R. J. (2018):** Sex chromosome repeats tip the balance towards speciation. *Mol. Ecol.*, 27: 3783–3798.
- Palmer D. H., Rogers T. F., Dean R., Wright A. E. (2019):** How to identify sex chromosomes and their turnover. *Mol. Ecol.*, 28: 4709–4724.
- Parris D. C., Holman, J. A. (1978):** An Oligocene snake from a coprolite. *Herpetologica*, 34: 8–264.
- Perrin N. (2009):** Sex reversal: A fountain of youth for sex chromosomes? *Evolution* 63: 3043–3049
- Pianka E. R. (1995):** Evolution of body size: varanid lizards as a model system. *Am. Nat.*, 146: 398–414.
- Pokorná M., Kratochvíl L. (2009):** Phylogeny of sex-determining mechanisms in squamate reptiles: Are sex chromosomes an evolutionary trap? *Zool. J. Linn. Soc.*, 156: 168–183.
- Ponnikas S., Sigeman H., Abbott J. K., Hansson B. (2018):** Why do sex chromosomes stop recombining? *Trends Genet.* 34: 492–503.
- Pyron R. A., Burbrink F. T., Wiens J. J. (2013):** A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. *BMC Evol. Biol.*, 13: 93.
- Pyron R. A., Hendry C. R., Chou V. M., Lemmon E. M., Lemmon A. R., Burbrink F. T. (2014a):** Effectiveness of phylogenomic data and coalescent species-tree methods for resolving difficult nodes in the phylogeny of advanced snakes (Serpentes: Caenophidia). *Mol. Phylogenet. Evol.*, 81: 221–231.
- Pyron R. A., Reynolds R. G., Burbrink F. T. (2014b):** A taxonomic revision of boas (Serpentes: Boidae). *Zootaxa*, 3846: 249–260.
- Quinn A. E., Georges A., Sarre S. D., Guarino F., Ezaz T., Graves J. A. (2007):** Temperature sex reversal implies sex gene dosage in a reptile. *Science*, 316: 411.
- Quinn A. E., Sarre S. D., Ezaz T., Marshall Graves J. A., Georges A. (2011):** Evolutionary transitions between mechanisms of sex determination in vertebrates. *Biol. Lett.*, 7: 443–448.
- Radder R. S., Quinn A. E., Georges A., Sarre S. D., Shine R. (2008):** Genetic evidence for co-occurrence of chromosomal and thermal sex-determining systems in a lizard. *Biol. Lett.*, 4: 176–178.
- Reinhardt T., van Schingen M., Windisch H. S., Nguyen T. Q., Ziegler T., Fink, P. (2019):** Monitoring a loss: Detection of the semi-aquatic crocodile lizard (*Shinisaurus crocodilurus*) in inaccessible habitats via environmental DNA. *Aquat. Conserv.*, 29: 353–360.
- Rens W., O'Brien P. C., Grützner F., Clarke O., Graphodatskaya D., Tsend-Ayush E. et al. (2007):** The multiple sex chromosomes of platypus and echidna are not completely identical and several share homology with the avian Z. *Genome Biol.* 8: R243.

- Rice W. R. (1987):** The accumulation of sexually antagonistic genes as a selective agent promoting the evolution of reduced recombination between primitive sex chromosomes. *Evolution* 41: 911–914.
- Rovatsos M., Altmanová M., Pokorná M., Kratochvíl L. (2014a):** Conserved sex chromosomes across adaptively radiated *Anolis* lizards. *Evolution*, 68: 2079–2085.
- Rovatsos M, Pokorná M, Altmanová M, Kratochvíl L. (2014b):** Cretaceous park of sex determination: sex chromosomes are conserved across iguanas. *Biol. Lett.*, 10: 20131093.
- Rovatsos M., Johnson Pokorná M., Altmanová M., Kratochvíl L. (2015a):** Female heterogamety in Madagascar chameleons (Squamata: Chamaeleonidae: *Furcifer*): differentiation of sex and neo-sex chromosomes. *Sci. Rep.*, 5: 1–9.
- Rovatsos M., Vukić J., Lymberakis P., Kratochvíl L. (2015b):** Evolutionary stability of sex chromosomes in snakes. *Proc. R. Soc. B*, 282: 20151992.
- Rovatsos M., Altmanová M., Johnson Pokorná M., Velenský P., Sanchez Baca A., Kratochvíl L. (2017):** Evolution of karyotypes in chameleons. *Genes*, 8: 382.
- Rovatsos M., Altmanová M., Johnson Pokorná M., Augstenová B., Kratochvíl L. (2018):** Cytogenetics of the Javan file snake (*Acrochordus javanicus*) and the evolution of snake sex chromosomes. *J. Zoolog. Syst. Evol. Res.*, 56: 117–125.
- Rovatsos M., Reháč I., Velenský P., Kratochvíl L. (2019a):** Shared ancient sex chromosomes in varanids, beaded lizards, and alligator lizards. *Mol. Biol. Evol.*, 36: 1113–1120.
- Rovatsos M., Altmanová M., Augstenová B., Mazzoleni, S., Velenský P., Kratochvíl L. (2019b):** ZZ/ZW sex determination with multiple neo-sex chromosomes is common in Madagascan chameleons of the genus *Furcifer* (Reptilia: Chamaeleonidae). *Genes* 10: 1020.
- Rovatsos M., Gamble T., Nielsen S., Georges A., Ezaz T., Kratochvil L. (2021):** Do male and female heterogamety really differ in expression regulation? Lack of global dosage balance in pygopodid geckos. *Proc. R. Soc. B.*, in press.
- Rutkowska J., Badyaev A. V. (2008):** Meiotic drive and sex determination: molecular and cytological mechanisms of sex ratio adjustment in birds. *Proc. R. Soc. B*, 363: 1675–86
- Santidrián Tomillo P., Spotila J. R. (2020):** Temperature-dependent sex determination in sea turtles in the context of climate change: uncovering the adaptive significance. *BioEssays*, 42: 2000146.
- Sarre S. D., Georges A., Quinn A. (2004):** The ends of a continuum: Genetic and temperature-dependent sex determination in reptiles. *BioEssays* 26: 639–645.
- Schartl M. (2004):** Sex chromosome evolution in non-mammalian vertebrates. *Curr. Opin. Genet. Dev.*, 14: 634–641.
- Schwanz L. E. (2016):** Parental thermal environment alters offspring sex ratio and fitness in an oviparous lizard. *J. Exp. Biol.*, 219: 2349.
- Sidhom M., Said K., Chatti N., Guarino F. M., Odierna G., Petraccioli A. et al. (2020):** Karyological characterization of the common chameleon (*Chamaeleo chamaeleon*) provides insights on the evolution and diversification of sex chromosomes in Chamaeleonidae. *Zoology*, 141: 125738.

- Singchat W., Ahmad S. F., Laopichienpong N., Suntronpong A., Panthum T., Griffin D. K., Srikulnath K. (2020):** Snake W sex chromosome: the shadow of ancestral amniote super-sex chromosome. *Cells*, 9: 2386.
- Singchat W., O'Connor R. E., Tawichasri P., Suntronpong A., Sillapaprayoon S., Suntrarachun S et al. (2018):** Chromosome map of the Siamese cobra: did partial synteny of sex chromosomes in the amniote represent "a hypothetical ancestral super-sex chromosome" or random distribution? *BMC Genomics*, 19: 939.
- Singh L. (1972):** Multiple W chromosome in a sea snake, *Enhydrina schistosa* Daudin. *Experientia*, 28: 95–9
- Singh L., Sharma T., Ray-Chaudhuri S. P. (1970):** Multiple sex-chromosomes in the common Indian Krait, *Bungarus caeruleus* Schneider. *Chromosoma*, 31: 386–391.
- Smith C. A. (2010):** Sex determination in birds: A review. *Emu*, 110: 364–377.
- Steinemann S., Steinemann M. (2005):** Retroelements: tools for sex chromosome evolution. *Cytogenet. Genome Res.*, 110: 134–143.
- Subramanian S., Mishra R. K., Singh L. (2003):** Genome-wide analysis of *Bkm* sequences (GATA repeats): Predominant association with sex chromosomes and potential role in higher order chromatin organization and function. *Bioinformatics*, 19: 681–685.
- Tanaka K., Takehana Y., Naruse K., Hamaguchi S., Sakaizumi M. (2007):** Evidence for different origins of sex chromosomes in closely related *Oryzias* fishes: substitution of the master sex-determining gene. *Genetics* 177: 2075–2081.
- Uetz P., Freed P, Aguilar R. Hošek J. (eds) (2021):** The Reptile Database, <http://www.reptile-database.org>; Accessed 10. 5. 2021.
- Valenzuela N., Lance, V. (eds) (2004):** *Temperature-dependent sex determination in vertebrates* Smithsonian Books Washington, DC, Columbia, USA p. 1–194.
- Vences M., Glaw F., Wollenberg K., Münchenberg T. (2008):** Molecular phylogeny and geographic variation of Malagasy iguanas (*Oplurus* and *Chalarodon*). *Amphib.-Reptil.*, 29: 319–327.
- Viana P. F., Ribeiro L. B., Souza G. M., Chalkidis H. D. M., Gross M. C., Feldberg E. (2016):** Is the karyotype of neotropical boid snakes really conserved? Cytotaxonomy, chromosomal rearrangements and karyotype organization in the Boidae family. *PLoS ONE*, 11: 1–16.
- Vicoso B. (2019):** Molecular and evolutionary dynamics of animal sex-chromosome turnover. *Nat. Ecol. Evol.*, 3: 1632–1641.
- Vicoso B., Emerson J. J., Zektser Y., Mahajan S., Bachtrog D. (2013):** Comparative sex chromosome genomics in snakes: differentiation, evolutionary strata, and lack of global dosage compensation. *PLoS Biol.*, 11: e1001643.
- Vitt L. J., Caldwell J. P. (2009):** *Herpetology: an introductory biology of amphibians and reptiles*. Academic press. London, UK.

- Warner D. A., Shine R. (2008):** The adaptive significance of temperature-dependent sex determination in a reptile. *Nature*, 451: 566–569.
- Warner D. A., Shine R. (2005):** The adaptive significance of temperature-dependent sex determination: experimental tests with a short-lived lizard. *Evolution*, 59: 2209–2221.
- Warner D. A., Shine R. (2007):** Fitness of juvenile lizards depends on seasonal timing of hatching, not offspring body size. *Oecologia*, 154: 65–73.
- Warner D. A., Uller T., Shine R. (2013):** Transgenerational sex determination: the embryonic environment experienced by a male affects offspring sex ratio. *Sci. Rep.*, 3: 2709.
- Wheeler B. S., Anderson E., Frøkjær-Jensen C., Bian Q., Jorgensen E., Meyer B. J. (2016):** Chromosome-wide mechanisms to decouple gene expression from gene dose during sex-chromosome evolution. *eLife*5, e17365.
- Whiteley S. L., Georges A., Weisbecker V., Schwanz L. E., Holleley C. E. (2021):** Ovotestes suggest cryptic genetic influence in a reptile model for temperature-dependent sex determination. *Proc. R. Soc. B*, 288: 20202819.
- Woram R. A., Gharbi K., Sakamoto T., Hoyheim B., Holm L. E., Naish K. et al. (2003):** Comparative genome analysis of the primary sex-determining locus in salmonid fishes. *Genome Res.*, 13: 272–280.
- Zechner U., Hameister H. (2011):** Sex chromosomes in vertebrates: XX/XY against ZZ/ZW. *Sex. Dev.*, 5: 266–271.
- Zeng X. M., Wang Y. Z., Liu Z. J., Fang Z. L., Wu G. F. (1997):** Karyotypes on nine species in the genus *Phrynocephalus*, with discussion of karyotypic evolution of Chinese *Phrynocephalus*. *Acta. Zool. Sin.*, 43: 399–410.
- Zhou Q., Zhang J., Bachtrog D., An N., Huang Q., Jarvis E. D. et al. (2014):** Complex evolutionary trajectories of sex chromosomes across bird taxa. *Science*, 346: 1246338.