First Report of Sex Chromosomes in Plated Lizards (Squamata: Gerrhosauridae)

Alexander Kostmann Lukáš Kratochvíl Michail Rovatsos

Department of Ecology, Faculty of Science, Charles University, Prague, Czech Republic

Keywords

CGH · FISH · rDNA · Sex chromosomes · Telomeres

Abstract

Squamate reptiles show high diversity in sex determination ranging from environmental sex determination to genotypic sex determination with varying degrees of differentiation of sex chromosomes. Unfortunately, we lack even basic information on sex determination mode in several lineages of squamates, which prevents full understanding of their diversity and evolution of sex determination. One of the reptilian lineages with missing information on sex determination is the family Gerrhosauridae, commonly known as the plated lizards. Several species of gerrhosaurids have been studied in the past by conventional cytogenetic methods, but sex-specific differences were not identified. In this study, we applied both conventional and molecular cytogenetic methods to metaphases from both sexes of the Peters' keeled plated lizard (*Tracheloptychus petersi*). We identified accumulations of rDNA loci in a pair of microchromosomes in metaphases from males, but only in a single microchromosome in females. The restriction of the observed heterozygosity to females suggests a putative ZZ/ZW system of sex chromosomes, which represents the first report of sex chro-

mosomes in a gerrhosaurid lizard. The lack of sex-specific signals in all other cytogenetic methods implies that the sex chromosomes of *T. petersi* are poorly differentiated in sequence content.

© 2021 The Author(s)

Published by S. Karger AG, Basel

Introduction

Amniotes possess 2 major systems of sex determination: genotypic sex determination, where the sex of an individual is set by its sex-specific genotype, and environmental sex determination, where the sex is set by environmental factors, predominantly temperature [Bull, 1983]. Most of the variability in sex determination systems among amniotes is concentrated to squamate reptiles, but even within this lineage the variability is heavily unequally distributed. It is estimated that roughly 60% out of approximately 11,000 recent squamate species belong to 5 clades with highly evolutionary stable XX/XY (iguanas, skinks) and ZZ/ZW (caenophidian snakes, lacertids, monitors) sex chromosomes [Rovatsos et al., 2014, 2015a, 2016, 2019; Kostmann et al., 2021]. The majority of the variability is thus concentrated to the other squamate lin-

eages, but their sex determination mode is up to now poorly studied.

In this context, sex determination is poorly studied in the lizards of the superfamily Scincoidea, which consists of 4 families: Scincidae, Xantusiidae, Cordylidae, and Gerrhosauridae [Pyron et al., 2013]. In skinks (family Scincidae), the far most diversified scincoidean lineage, XX/XY sex chromosomes were identified cytogenetically in few species [Hardy, 1979; Donnellan, 1985; Caputo et al., 1994], while ZZ/ZW sex chromosomes were reported in a single species [Patawang et al., 2017]. In 2 recent studies using whole-genome sequencing and exploring the differences in read depth coverage between sexes, XX/XY sex chromosomes were revealed in 2 skink species: *Scincus scincus* [Kostmann et al., 2021] and *Eulamprus heatwolei* [Cornejo-Páramo et al., 2020]. In addition, our recent study using a qPCR-based method revealed that homologous XX/XY sex chromosomes are present in 13 species of skinks, covering most of the phylogenetic diversity of the family [Kostmann et al., 2021]. Furthermore, although sex chromosomes in skinks are poorly differentiated, they are phylogenetically stable for at least 85 million years [Kostmann et al., 2021].

Female heterogamety was identified by analysis of RADseq data in the xantusiid *Xantusia henshawi* [Nielsen et al., 2020]. In addition, female heterogamety was predicted for the xantusiid *Lepidophyma smithii* from the type of facultative parthenogenesis [Kratochvíl et al., 2020]. It was observed that females of this species can parthenogenetically produce offspring of both sexes, which is not compatible with male heterogamety [Kratochvíl et al., 2020]. Cytogenetic studies of species from the families Cordylidae and Gerrhosauridae were mostly performed by conventional cytogenetic methods, and sex-specific differences were not observed in their karyotypes [Odierna and Olmo, 1980; Odierna et al., 2002].

In this study, we applied conventional and molecular cytogenetic methods to metaphases from both sexes of the Malagasy Peters' keeled plated lizard *Tracheloptychus petersi*, with the major aim to identify their sex chromosomes to fill the gap in our knowledge on the evolution of the sex determination in amniotes.

Material and Methods

Samples and Species Verification

Blood samples were collected from 2 males and 3 females of *T. petersi* originated from a legal import from Madagascar. All individuals were adults, and the sex was identified by external morphology (head shape, thickness of tail base) and everted hemi-

penes in males by palpation. Total DNA was isolated using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA). The standard DNA barcoding region of the mitochondrial gene cytochrome c oxidase I (*COI*) gene was amplified by PCR from DNA extracted from all 5 lizards, with primers optimized for reptiles [Nagy et al., 2012] and following a standard PCR protocol [Koubová et al., 2014]. The PCR products were purified and sequenced bidirectionally by Macrogen (Seoul, Korea). The *COI* sequences were subsequently trimmed in FinchTV, aligned in BioEdit v5.0.9 [Hall, 1999], analyzed in MEGA v10 [Kumar et al., 2018], and compared to sequences deposited in public databases by BLASTn [Altschul et al., 1990] to verify the taxon assessment. All sequences were deposited in GenBank under the accession numbers MW052709, MW052710 and MW326658.

Chromosome Preparation and Staining

Whole-blood cell cultures were set up to obtain mitotic chromosome suspensions as described in Mazzoleni et al. [2019]. Karyograms were constructed from Giemsa-stained metaphase chromosomes. Heterochromatic regions were visualized by C-banding according to Sumner [1972] with slight modifications.

Fluorescence in situ Hybridization with Probes for Repetitive Elements

We used fluorescence in situ hybridization (FISH) to visualize the topology of ribosomal DNA loci and telomeric sequences (TTAGGG)_n. The rDNA probe was prepared from a plasmid (pDmr.a 51#1) with an 11.5-kb insert encoding the 18S and 28S ribosomal units of *Drosophila melanogaster* [Endow, 1982] and labeled by dUTP-biotin using nick translation. The telomeric probe was prepared according to Ijdo et al. [1991] and Rovatsos et al. [2011]. The probes were hybridized to chromosome preparations following the protocol of Rovatsos et al. [2011].

Comparative Genome Hybridization

Comparative genome hybridization (CGH) was applied to both male and female metaphase chromosomes to reveal sex-specific genomic regions. The detailed protocol can be found in Rovatsos et al. [2015b].

Microscopy and Image Analyses

Images from at least 10 metaphases per method and sex were captured using either a Zeiss Axio Imager Z2 equipped with automatic Metafer-MSearch scanning platform (MetaSystems, Alt-lussheim, Germany) and a CoolCube 1 b/w digital camera (MetaSystems) or a Provis AX70 fluorescence microscope (Olympus, Tokyo, Japan) equipped with a DP30BW digital camera (Olympus).

Results and Discussion

The haplotype analysis revealed 3 haplotypes of *COI* gene. The BLASTn revealed 98.19, 98.79 and 98.95% similarity of sequences from our sequenced individuals to those from the specimens of *T. petersi* previously studied by [Nagy et al., 2012] across an alignment of 664 bp confirming the expected taxon assessment of our material.

Fig. 1. Giemsa-stained karyograms (**A, B**), distribution of C-banding (**C, D**), rDNA loci (**E, F**) and telomeric motifs (**G, H**), and comparative genome hybridization (**I, J**) in metaphases from both sexes of the Peters' keeled plated lizard (*T. petersi*). Z chromosomes are indicated when recognizable. ITRs are indicated by yellow arrows.

Two different haplotypes were found in each sex pointing that individuals within a given sex are not siblings, and the variability in cytogenetic markers between sexes thus cannot be attributed to kinship.

The examination of metaphases from 5 individuals revealed a diploid number of $2n = 34$, with 12 macrochromosomes and 22 microchromosomes (Fig. 1A, B). The macrochromosomes are bi-armed, either metacentric or submetacentric, but the morphology of microchromosomes is not possible to identify. Prominent accumulations of heterochromatin were identified in the pericen-

tromeric regions of the 4 larger chromosome pairs, but no sex-specific accumulation was observed (Fig. 1C, D). The karyotype of *T. petersi* is similar to its closest relative *Tracheloptychus madagascariensis* previously reported by Odierna et al. [2002]. A diploid chromosome number of $2n = 34$ was observed in the majority of gerrhosaurids ($2n = 34-36$) and cordylids ($2n = 32-46$) [Odierna and Olmo, 1980; Odierna et al., 2002; Olmo and Signorino, 2016], suggesting that the karyotype consisting of 12 macrochromosomes and 22 microchromosomes was probably present already in the common ancestor of

these families. On the contrary, diploid chromosome number is quite variable in skinks ($2n = 22\text{--}36$) and xantusiids ($2n = 24\text{--}40$) [Hass and Hedges, 1992; Olmo and Signorino, 2016].

FISH with the rDNA probe showed strong signals in the centromeric region of the fourth largest chromosome pair in both sexes of *T. petersi* (Fig. 1E, F). An additional signal was found on 1 microchromosome in females, but on 2 microchromosomes in males. The occurrence of the heterozygous state only in females indicates that this species possesses female heterogamety (ZZ/ZW sex chromosomes) with the Z chromosome having rDNA accumulation missing on the W. Sex-specific differences in accumulations of rDNA loci has been previously reported in many phylogenetically distant taxa. Within Scincoidea, the rDNA loci are accumulated in the X chromosome, but not in the Y in the common sandfish (*S. scincus*) [Caputo et al., 1994; Kostmann et al., 2021]. In an analogous case to *T. petersi*, rDNA loci are accumulated in the Z chromosome of the Kajika frog (*Buergeria buergeri*), but are missing in its W chromosome [Schmid et al., 1993]. In other cases, both sex chromosomes possess accumulations of rDNA loci, but they differ at their extent as in softshell turtles, where rDNA accumulations are usually much more expanded on the W [Badenhorst et al., 2013; Litterman et al., 2014; Rovatsos et al., 2017].

Odierna et al. [2002] examined the position of active rDNA loci by silver-staining across different species of cordylids and gerrhosaurids including *T. madagascariensis*, but did not find sex-specific differences in any of them. In *T. madagascariensis*, the accumulations of rDNA loci are localized in 2 microchromosomes, which could be homologous to the sex chromosomes of *T. petersi*. It seems that the complete or partial loss of rDNA accumulation from the W chromosome is an apomorphy of *T. petersi*. The signal intensity of the rDNA loci varies significantly even in *T. petersi* males between the 2 putative Z chromosomes (Fig. 1E). Notably, rDNA loci seem to be variable in number of repeats and chromosome positions at both inter- and intraspecific levels [Porter et al., 1991; Stults et al., 2008; Altmanová et al., 2016; reviewed by Sochorová et al., 2017; Mazzoleni et al., 2018; Degrandi et al., 2020]. The high mobility of rDNA clusters among genome regions could be caused by insertion of rDNA genes into chromosomes via translocation followed by amplification in the new sites and elimination in the old location [Dubcovsky and Dvořák, 1995]. The functional and evolutionary significance of the variability in the amount and position of rDNA loci still remains unclear. In humans, rDNA loci seem to act as “recombination

hotspots” resulting in extensive variability in rDNA quantity and genomic organization [Stults et al., 2008]. In some cases, like in fruit flies from the genus *Drosophila*, rDNA loci on the X and Y chromosomes seem to have a vital role in meiosis I [McKee and Karpen, 1990]. This variability in the amount and chromosomal topology of rDNA loci can be to a large extent neutral, and it can be partially tolerated by the cellular mechanism. For example, it was demonstrated that only 25–50% of the diploid number of rDNA loci are necessary for normal development in wildtype and mutant individuals of *Xenopus laevis* [Knowland and Miller, 1970]. Nevertheless, the different numbers of repeats of rDNA loci might be viable, but still could be connected with negative consequences, such as the development of cancer [Gibbons et al., 2015; Xu et al., 2017] and genome instability [Kobayashi, 2011].

In *T. petersi*, FISH with the telomeric probe revealed the expected terminal signals in all chromosomes and additional interstitial telomeric sequences (ITRs) in the centromeric and/or pericentromeric regions of all 12 macrochromosomes (Fig. 1G, H). ITRs are generally common in squamate reptiles with centromeric ITRs being the most common [Rovatsos et al., 2015c]. ITRs are often remnants of past chromosomal rearrangements, such as chromosome fusions and inversions [Rovatsos et al., 2015c]. Accumulations of telomeric sequences in reptiles also often occur on differentiated sex chromosomes [Matsubara et al., 2015], e.g., in caenophidian snakes [Augstenová et al., 2017] and a carphodactylid gecko [Pokorná et al., 2014]. Nevertheless, no sex-specific accumulations were detected in *T. petersi* (Fig. 1G, H).

CGH did not show accumulations of female-specific sequences on the W chromosome, indicating that the sex chromosomes of *T. petersi* are poorly differentiated in sequences (Fig. 1I, J). The results of CGH suggests that most of the W chromosome is pseudoautosomal and the W-specific region is small, below the detection limit of this method, which is estimated to approximately 2–3 megabases [Schoumans et al., 2004]. Nevertheless, previous studies revealed that CGH shows strong signals when the sex-specific chromosomal region is heterochromatic or enriched in repetitive elements, otherwise even bigger sex-specific regions might not be visualized by this method [Altmanová et al., 2016; Kostmann et al., 2021].

In summary, our study provides the first karyotype description of *T. petersi* and the first report of sex chromosomes in the gerrhosaurid lizards, expanding our knowledge on the evolution of sex determination systems in vertebrates. Future studies should focus on the identification of sex chromosome gene content, for example, by

next generation sequencing methodologies, and to explore the homology of sex chromosomes across scincoidean lizards. Next to skinks and xanthusiids, gerrhosaurids are another scincoidean lineage with poorly differentiated sex chromosomes, which indicates that recombination suppression on sex chromosomes might be slower in this group than in other reptiles, which often have highly differentiated sex chromosomes [Rovatsos et al., 2015a, 2016, 2019]. Comparison of the rates of recombination between scincoidean lizards and other lineages will allow us to explore the effect of recombination on the emergence and stability of sex chromosomes and sex determination systems.

Acknowledgement

We would like to express our gratitude to Petr Ráb and members of his laboratory for their support and to Jana Thomayerová, Anna Bauerová, and Nuria Viñuela Rodríguez for technical assistance.

References

- Altmanová M, Rovatsos M, Kratochvíl L, Johnson Pokorná M. Minute Y chromosomes and karyotype evolution in Madagascan iguanas (Squamata: Iguania: Opluridae). *Biol. J. Linn. Soc.* 2016;118(3):618–33.
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol.* 1990;215(3):403–10.
- Augstenová B, Mazzoleni S, Kratochvíl L, Rovatsos M. Evolutionary dynamics of the W chromosome in caenophidian snakes. *Genes.* 2017;9(1):5.
- Badenhorst D, Stanyon R, Engstrom T, Valenzuela N. A ZZ/ZW microchromosome system in the spiny softshell turtle, *Apalone spinifer*, reveals an intriguing sex chromosome conservation in Trionychidae. *Chromosome Res.* 2013;21(2):137–47.
- Bull JJ. Evolution of sex determining mechanisms (Menlo Park: Benjamin/Cummings, 1983.
- Caputo V, Odierna G, Aprea G. A chromosomal study of Eumeces and Scincus, primitive members of the Scincidae (Reptilia, Squamata). *Boll Zool.* 1994;61:155–62.
- Cornejo-Páramo P, Dissanayake DSB, Lira-Noriega A, Martínez-Pacheco ML, Acosta A, Ramírez-Suástegui C, et al. Viviparous reptile regarded to have temperature-dependent sex determination has old XY chromosomes. *Genome Biol Evol.* 2020;12(6):924–30.
- Degrandi TM, Gunsli RJ, Garner ADV, Oliveira EHC, Kretschmer R, Souza MS, et al. The distribution of 45S rDNA sites in bird chromosomes suggests multiple evolutionary histories. *Genet Mol Biol.* 2020;43(2):e20180331.
- Donnellan SC. *The evolution of sex chromosomes in scincid lizards [dissertation]*. Sydney: Macquarie University; 1985.
- Dubcovsky J, Dvorák J. Ribosomal RNA multi-gene loci: Nomads of the Triticeae genomes. *Genetics.* 1995;140(4):1367–77.
- Endow SA. Polytenization of the ribosomal genes on the X and Y chromosomes of *Drosophila melanogaster*. *Genetics.* 1982;100(3):375–85.
- Gibbons JG, Branco AT, Godinho SA, Yu S, Lemos B. Concerted copy number variation balances ribosomal DNA dosage in human and mouse genomes. *Proc Natl Acad Sci USA.* 2015;112(8):2485–90.
- Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl Acids Symp Ser.* 1999;41:95–8.
- Hardy GS. The karyotypes of two scincid lizards, and their bearing on relationships in genus *Leiopisma* and its relatives (Scincidae: Lygosominae). *New Zeal J Zool.* 1979;6:609–12.
- Hass CA, Hedges SB. Karyotype of the Cuban Lizard *Cricosaura typica* and its implications for xanthusiid phylogeny. *Copeia.* 1992;1992(2):563–5.
- Ijdo JW, Baldini A, Ward DC, Reeders ST, Wells RA. Origin of human chromosome 2: an ancestral telomere-telomere fusion. *Proc Natl Acad Sci USA.* 1991;88(20):9051–5.
- Knowland J, Miller L. Reduction of ribosomal RNA synthesis and ribosomal RNA genes in a mutant of *Xenopus laevis* which organizes only a partial nucleolus. I. Ribosomal RNA synthesis in embryos of different nucleolar types. *J Mol Biol.* 1970;53(3):321–8.
- Kobayashi T. Regulation of ribosomal RNA gene copy number and its role in modulating genome integrity and evolutionary adaptability in yeast. *Cell Mol Life Sci.* 2011;68(8):1395–403.
- Kostmann A, Kratochvíl L, Rovatsos M. Poorly differentiated XX/XY sex chromosomes are widely shared across skink radiation. *Proc R Soc B.* 2021;288:20202139.
- Koubová M, Johnson Pokorná M, Rovatsos M, Farkačová K, Altmanová M, Kratochvíl L. Sex determination in Madagascar geckos of the genus *Paroedura* (Squamata: Gekkonidae): are differentiated sex chromosomes indeed so evolutionary stable?. *Chromosome Res.* 2014;22(4):441–52.
- Kratochvíl L, Vukić J, Červenka J, Kubička L, Johnson Pokorná M, et al. Mixed sex offspring produced via cryptic parthenogenesis in a lizard. *Mol Ecol.* 2020;29(21):4118–27.
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K, MEGA X. MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol.* 2018;35(6):1547–9.

Statement of Ethics

All experimental procedures were carried out under the supervision and with the approval of the Ethics Committee of the Faculty of Science, Charles University, followed by the Committee for Animal Welfare of the Ministry of Agriculture of the Czech Republic (permissions No. 35484/2015-16 and 8604/2019-7).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

Funding Sources

This study was funded by the Czech Science Foundation project No. 20-27236J, the Charles University Grant Agency project 1518119, Charles University projects PRIMUS/SCI/46 and the Research Centre program 204069.

Author Contributions

A.K. performed the cytogenetic analyses; L.K. and M.R. contributed to the experimental part. All authors drafted and approved the final manuscript.

- Literman R, Badenhorst D, Valenzuela N. qPCR-based molecular sexing by copy number variation in rRNA genes and its utility for sex identification in soft-shell turtles. *Methods Ecol Evol*. 2014;5(9):872–80.
- Mazzoleni S, Rovatsos M, Schillaci O, Dumas F. Evolutionary insight on localization of 18S, 28S rDNA genes on homologous chromosomes in Primates genomes. *Comp Cytogenet*. 2018;12(1):27–40.
- Matsubara K, Uno Y, Srikulnath K, Matsuda Y, Miller E, Olsson M. No interstitial telomeres on autosomes but remarkable amplification of telomeric repeats on the W sex chromosome in the sand lizard (*Lacerta agilis*). *J Hered*. 2015;106(6):753–7.
- Mazzoleni S, Augstenová B, Clemente L, Auer M, Fritz U, Praschag P, et al. Turtles of the genera *Geoemyda* and *Pangshura* (Testudines: Geoemydidae) lack differentiated sex chromosomes: the end of a 40-year error cascade for *Pangshura*. *PeerJ*. 2019;7:e6241.
- McKee BD, Karpen GH. Drosophila ribosomal RNA genes function as an X-Y pairing site during male meiosis. *Cell*. 1990;61(1):61–72.
- Nagy ZT, Sonet G, Glaw F, Vences M. First large-scale DNA barcoding assessment of reptiles in the biodiversity hotspot of Madagascar, based on newly designed COI primers. *PLoS One*. 2012;7(3):e34506.
- Nielsen SV, Pinto BJ, Guzmán-Méndez IA, Gamble T. First report of sex chromosomes in Night lizards (Scincoidea: Xantusiidae). *J Hered*. 2020;111(3):307–17.
- Odierna G, Olmo E. Chromosomal evolution and DNA of cordylid lizards. *Herpetologica*. 1980;36:311–6.
- Odierna G, Canapa A, Andreone F, Aprea G, Barucca M, Capriglione T, et al. A phylogenetic analysis of Cordyliformes (Reptilia: Squamata): Comparison of molecular and karyological data. *Mol Phylogenet Evol*. 2002;23(1):37–42.
- Olmo E, Signorino GG. Chromorep: a reptiles chromosomes database. 2016. <http://chromorep.univpm.it>
- Patawang I, Chuaynkern Y, Supanuam P, Maneechot N, Pinthong K, Tanomtong A. Cytogenetics of the skinks (Reptilia, Scincidae) from Thailand; IV: Newly investigated karyotypic features of *Lygosoma quadrupes* and *Scincella melanosticta*. *Caryologia*. 2017;71:29–34.
- Pokorná M, Rens W, Rovatsos M, Kratochvíl L. A ZZ/ZW sex chromosome system in the thick-tailed gecko (*Underwoodisaurus milii*; Squamata: Gekkotia: Carphodactylidae), a member of the ancient gecko lineage. *Cytogenet Genome Res*. 2014;142:190–6.
- Porter C, Hamilton M, JW Jr S, Baker R. Location of ribosomal DNA in chromosomes of squamate reptiles: Systematic and evolutionary implications. *Herpetologica*. 1991;47:271–80.
- Pyron RA, Burbrink FT, Wiens JJ. A phylogeny and revised classification of Squamata, including 4161 species of lizards and snakes. *BMC Evol Biol*. 2013;13:93.
- Rovatsos MT, Marchal JA, Romero-Fernández I, Fernández FJ, Giagia-Athanosopoulou EB, Sánchez A. Rapid, independent, and extensive amplification of telomeric repeats in pericentromeric regions in karyotypes of arvicoline rodents. *Chromosome Res*. 2011;19(7):869–82.
- Rovatsos M, Pokorná M, Altmanová M, Kratochvíl L. Cretaceous park of sex determination: sex chromosomes are conserved across iguanas. *Biol Lett*. 2014;10(3):20131093.
- Rovatsos M, Vukić J, Lymberakis P, Kratochvíl L. Evolutionary stability of sex chromosomes in snakes. *Proc Biol Sci*. 2015a;282(1821):20151992.
- Rovatsos M, Johnson Pokorná M, Altmanová M, Kratochvíl L. Female heterogamety in Madagascar chameleons (Squamata: Chamaeleonidae: Furcifer): differentiation of sex and neo-sex chromosomes. *Sci Rep*. 2015b;5:13196.
- Rovatsos M, Kratochvíl L, Altmanová M, Johnson Pokorná M. Interstitial telomeric motifs in squamate reptiles: When the exceptions outnumber the rule. *PLoS One*. 2015c;10(8):e0134985.
- Rovatsos M, Vukić J, Altmanová M, Johnson Pokorná M, Moravec J, Kratochvíl L. Conservation of sex chromosomes in lacertid lizards. *Mol Ecol*. 2016;25(13):3120–6.
- Rovatsos M, Praschag P, Fritz U, Kratochvíl L. Stable Cretaceous sex chromosomes enable molecular sexing in softshell turtles (Testudines: Trionychidae). *Sci Rep*. 2017;7:42150.
- Rovatsos M, Reháč I, Velenský P, Kratochvíl L. Shared ancient sex chromosomes in varanids, beaded lizards, and alligator lizards. *Mol Biol Evol*. 2019;36(6):1113–20.
- Schmid M, Ohta S, Steinlein C, Guttenbach M. Chromosome banding in Amphibia. XIX. Primitive ZW/ZZ sex chromosomes in *Buergeria buergeri* (Anura, Rhacophoridae). *Cytogenet Cell Genet*. 1993;62(4):238–46.
- Schoumans J, Nielsen K, Jeppesen I, Anderlid BM, Blennow E, Brøndum-Nielsen K, et al. A comparison of different metaphase CGH methods for the detection of cryptic chromosome aberrations of defined size. *Eur J Hum Genet*. 2004;12(6):447–54.
- Stults DM, Killen MW, Pierce HH, Pierce AJ. Genomic architecture and inheritance of human ribosomal RNA gene clusters. *Genome Res*. 2008;18(1):13–8.
- Sochorová J, García S, Gálvez F, Symonová R, Kovařík A. Evolutionary trends in animal ribosomal DNA loci: introduction to a new online database. *Chromosoma*. 2017;127(1):141–50.
- Sumner AT. A simple technique for demonstrating centromeric heterochromatin. *Exp Cell Res*. 1972;75(1):304–6.
- Xu B, Li H, Perry JM, Singh VP, Unruh J, Yu Z, et al. Ribosomal DNA copy number loss and sequence variation in cancer. *PLoS Genet*. 2017;13(6):e1006771.