

Phylogenetic analysis of the genomes of several sexual and parthenogenetic lizards of the genus *Darevskia*

Urin A.^{1,2*}, Sukhanova X.¹, Korchagin V.², Komissarov A.^{1,2}, Ryskov A.²

¹ ITMO University, St. Petersburg, Russia

² Institute of Gene Biology, Moscow, Russia

* avel55793@gmail.com

Key words: partenogenesis, comparative genomic, genome assembling

Motivation and Aim: Parthenogenesis, a form of asexual reproduction where an organism develops from an unfertilized egg, is a rare phenomenon in vertebrates. The genus *Darevskia*, particularly renowned for its parthenogenetic species, presents a fascinating case study in evolutionary biology due to its complex and reticulated evolutionary history. Recent genomic studies, such as those detailed in the article by Freitas and colleagues [1], highlight the intricate evolutionary pathways that have given rise to these unique reproductive strategies within *Darevskia* species.

Comparative genomic analyses across various samples and species within this genus can provide significant insights into the mechanisms underlying parthenogenesis and the broader patterns of Squamata evolution. These investigations are crucial not only for understanding the genomic architecture and evolutionary dynamics of *Darevskia* but also for elucidating the genetic and molecular bases of parthenogenesis. By leveraging high-quality genomic assemblies, researchers can uncover new details about chromosomal evolution, sex determination systems, and reproductive barriers, advancing our knowledge of these rare yet captivating evolutionary phenomena.

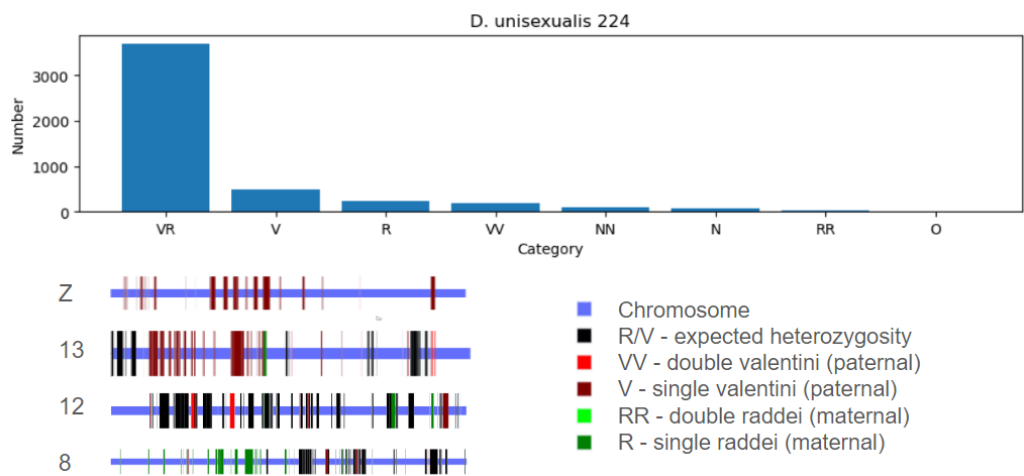
Methods and Algorithms: Sequencing results were obtained for the species *Darevskia unisexualis*, *D. raddei nairensis*, *D. armeniaca*, *D. mixta*, and *D. valentini*. Various samples were sequenced at different times using diverse methods, including Illumina paired-end sequencing, 10x Genomics, and PacBio HiFi sequencing. These methods enabled the assembly of the most complete possible genomes, with PacBio sequencing generating phased haplotypes. Assemblers such as Canu, Flye, and Hifiasm were utilized for the genome assemblies [2].

To assess sample heterozygosity, k-mer analysis was performed. For comparing genome and sex chromosome evolution, conserved genomic loci from the odb_10 [3] dataset were selected and compared within and between genomes. This was accomplished through alignment of the obtained sequences using ClustalW and evaluating the distance matrices using the k-nearest neighbors algorithm (KNN) method. The closely related genome of *Lacerta agilis* was used for visualizing gene locations

Results: Genome assemblies were obtained for three samples of *Darevskia unisexualis*, three of *D. raddei nairensis*, two of *D. valentini*, one of *D. mixta*, and one of *D. armeniaca*. The assemblies demonstrated good results in key quality metrics such as N50, L50, and BUSCO scores. K-mer analysis revealed that the genomes of *D. armeniaca* and *D. unisexualis* are predominantly heterozygous, while the other species exhibited significantly lower heterozygosity. An exception was one sample of *D. raddei nairensis*, which also showed a high level of heterozygosity. Bioproject access numbers PRJNA1029803, PRJNA1024511.

Comparative analysis of conserved genes indicated that one sample of *D. raddei* exhibited hybrid origins across much of its genome, deriving from different phylogenetic branches within the genus. Additionally, several loci, expected to be autosomal and present in two copies in the diploid genome based on the outgroup, were found to be in hemizygous state in all *D. raddei* and *D. unisexualis* samples, but not in *D. valentini*. The figure illustrates gene categories for the entire genome and highlights several specific loci. On chromosome Z, hemizygosity was expected, while chromosome 12 showed predominantly heterozygous loci. In contrast, chromosomes 13 and 8 presented unexpected hemizygosity, demonstrating intriguing variations in gene copy number and suggesting complex genomic evolution in these species.

Gene visualizing on the *L. agilis* chromosomes



Conclusion: High-quality draft genomes have been obtained, which can serve as valuable resources for various studies. These reliable results can be utilized by molecular biologists and geneticists for research into chromosomal evolution, sex determination, and reproductive mechanisms.

The heterozygosity observed in one sample of *D. raddei nairensis* is particularly intriguing, suggesting that this population might be closer to *D. unisexualis* than to other *D. raddei* samples. However, to confirm the hypothesis of increased heterozygosity and to better understand the genomic composition, further analysis of additional samples from this population is necessary.

Additionally, the varying ploidy observed in certain loci suggests that these regions may have been translocated from autosomes to sex chromosomes. This discovery highlights the dynamic nature of genome evolution in *Darevskia* species and underscores the need for ongoing genomic studies to fully elucidate these complex processes.

Funding: This research was funded by the Russian Science Foundation (RSF) Research Project No. 19-14-00083.

References

1. Freitas S., Westram A.M., Schwander T. et al. Parthenogenesis in Darevskia lizards: A rare outcome of common hybridization, not a common outcome of rare hybridization. *Evolution*. 2022;76(5):899-914. doi 10.1111/evo.14462
2. Ochkalova S., Korchagin V., Vergun A., Urin A., Zilov D., Ryakhovsky S., Girnyk A., Martirosyan I., Zhernakova D.V., Arakelyan M., Danielyan F. First genome of rock lizard Darevskia valentini involved in formation of several parthenogenetic species. *Genes*. 2022;13:1569. <https://doi.org/10.3390/genes13091569>
3. Kuznetsov D., Tegenfeldt F., Manni M., Seppey M., Berkeley M., Kriventseva E.V., Zdobnov E.M. OrthoDB v11: annotation of orthologs in the widest sampling of organismal diversity. *Nucleic Acids Res*. 2023;51(D1):D445-D451. doi 10.1093/nar/gkac998