

The Riverine Rabbit (*Bunolagus monticularis*) Genome: A Genomic Foundation to Assist the Conservation of a Critically Endangered Indigenous Ecosystem Health Indicator Species

The riverine rabbit (*Bunolagus monticularis*) is a critically endangered, endemic South African leporid. It is the only member of the genus *Bunolagus* and found in a limited distribution area. Also known as the bushman rabbit or bushman hare, the riverine rabbit is facing extinction due to agricultural expansion, climate change and a limited geographical distribution. The proposed eleven subpopulations are estimated to have less than 50 individuals each with the subpopulations geographically isolated (Collins and Du Toit 2016). This monotypic species diverged an estimate 10 mya (Matthee et al. 2004) and has a slow production rate producing litters of only one or two offspring (Duthie et al. 1989). Early studies concluded *Bunolagus* to be a habitat specialist found in the central Karoo of South Africa (Nama and Succulent Karoo biomes) (Mucina and Rutherford). Recent investigations found the species to be present in other areas, including the Fynbos biome (Collins and Du Toit 2016). These groups were thereafter defined as the Northern and Southern populations. The Northern population comprised of nine subpopulations and occurred at higher altitudes with the Southern population consisting of two subpopulations in a mid-altitude range. In the last 5 years additional mid-altitudinal subpopulations have been discovered. The discovery of these new populations outside the traditionally defined ranges raises the possibility that the population numbers may not be as low and perhaps the critically endangered status may be overstated. Information on the evolutionary relatedness between the geographically isolated populations is further limited. The most recent study by Matthee et al. (2022) used a single maternally inherited DNA marker (mtDNA) and conclude that more rigorous genomic investigations are needed to develop a comprehensive conservation program for this indicator species of ecosystem health. The study found some subpopulations more genetically isolated than others. They further state that “if the genetic differentiation between the Northern and Southern populations is confirmed by nuclear DNA analyses this will pose an interesting dilemma for targeting future conservation efforts in this phylogenetically unique leporid lineage”. There is further evidence for different intrapopulation evolutionary histories within the two populations and it is suggested that the Northern and Southern populations should be treated as two distinct conservation units. It is therefore clear that detailed genome information is required for this understudied yet critically important species. This information will greatly assist conservation efforts of this critically endangered indigenous ecosystem health indicator species.

This study would require 1 MSc student (Zoology) and 1 PhD student (Bioinformatics). This project entails firstly the correct identification and sample collection of *Bunolagus monticularis* for accurate and reliable results and thereafter appropriate bioinformatic and computational biology methodologies to produce a comprehensive genome. Current information on the different subpopulations and geographic distribution of these subpopulations are extrapolated and based on single locations with small sample sizes. The MSc (Zoology) student will be expected to expand on the current knowledge regarding the distribution and population numbers of *Bunolagus monticularis*. This will include the identification of current and possibly other subpopulations and the collection of samples. The detailed collection and cataloguing of samples are critical to the success of this project. This is a vastly understudied indigenous species and requires added information based on populations, distribution and geographic isolation to adequately assess its endangered status. For this, a large and encompassing sampling strategy is required. This will include currently know population locations and expanding into other locations. The collection of all this information will further assist the project in choosing a suitable reference individual for genome assembly and annotation or advice on rather using a museum reference. The envisioned addition to current literature and knowledge obtained from this will be suitable for a successful MSc degree. Furthermore, the samples collected by the MSc for their portion of the project will be used by the PhD student to construct a chromosome-level assembly of the *Bunolagus monticularis* genome. The PhD student will be expected to incorporate and condense all the genomic data, produced by various technologies, into a chromosome-level assembly and thereafter annotate. This will include data derived from Illumina, PacBio and Bionano technologies. The PhD workflow will follow de novo assembly of contigs, data obtained from PacBio technologies, and supplemented with Illumina data and scaffolded with Bionano technology. After scaffolding, gap filling and polishing will follow. The final assembly will be evaluated and proceed to genome annotation. The final assembly

and annotation will then be evaluated against the European rabbit, *Oryctolagus cuniculus*, reference genome OryCun2.0 (released August 2009). Low coverage sequencing of population sub-samples will further be incorporated to analyse the population and subpopulation genetic structures based on variants. The PhD thesis will greatly contribute to the currently limited genetic information with regards to the *Bunolagus monticularis* genome and genetic variation within the population and subpopulations. The work accomplished by both students will allow the project to come full circle and detail the population structure of *Bunolagus monticularis* as found genetically and in the field. Both the students would have greatly contributed to the current knowledge regarding the Riverine Rabbit and the population structure thereof.

The publication arising from the work completed by both the students will provide a detailed analyses of the distribution and population structure of the Riverine Rabbit (*Bunolagus monticularis*), including the first genome assembly and annotation of this monotypic species. The publication will be structured in three sections. The first will detail the distribution, populations, subpopulations and geographic locations of the Riverine Rabbit as determined by an expansive sampling process. This will shed more light on the current population structure and classification of the Riverine Rabbit as critically endangered. The subsequent section will focus on the first genome of *Bunolagus monticularis* and the contribution such a resource will have on conservation efforts. Lastly, population analyses will be demonstrated by low coverage sequencing data and provide a holistic picture of the Riverine Rabbit population and subpopulation structure. This publication will greatly assist in formulating conservation strategies for this ecosystem health indicator species and greatly assist in elucidating population genetic trends and ecological interactions.

Supervision of these students require a supervisor to adequately cater to their academic needs to ensure an environment free from obstacles and unnecessary hinderances. This includes provision of funding, consumables, computational resources and any additional requirements for the project. Regular informal and structured formal meetings will be held to ensure that the students have the necessary support to ensure continued and satisfactory progress. It will be well articulated that the students are expected to complete the work themselves and as such expand their research and academic capacity. It is critical that students acquire the necessary skills and experience during their projects to be employable in the sector of their choosing and further contribute to science. Guidance and advice on all aspects of the project will be provided to ensure the students feel supported and included. In my personal experience I have found that by demonstrating a passion for science and research, students are encouraged to explore and investigate on their own. By doing this they feel empowered and slowly but surely momentum is gained. It is of the utmost importance that a passion and thirst for science and research is promoted in students and that degrees are not merely conferred.

References:

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