

**Genetic structure of the endangered Fiji Banded Iguana, *Brachylophus bulabula*, in Viti Levu, Fiji**

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*Acknowledgements*

The author would like to thank the BIOL3110 Evolutionary and Conservation Genetics teaching staff for providing the data and guidance for this study.

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## ***Abstract***

The Fiji Banded Iguana, *Brachylophus bulabula*, are endangered as per listed on the International Union for Conservation of Nature and Natural Resources Red List of Threatened Species. The landscape genetics of this species is not well understood. Landscape genetics focuses on the flow of genetic information of a population across different habitats. Genetic structure is an essential element of landscape genetics. *B. bulabula* is under threat of habitat fragmentation but the genetic structure of this species is unknown. A study of 19 populations of *B. bulabula* in Viti Levu is conducted to investigate the genetic structure of these populations. This study shows that there is a very strong genetic structure of *B. bulabula*, as three different genetic groups were found in Viti Levu. The mountain range and river constructed barriers to the gene flow of this species hence, resulting in small isolated populations. Effective population size was low in two out of three population groups. The populations studied are also under the impact of isolation by distance. Inbreeding and genetic drift were also detected, which could potentially cause genetic divergence. Genetic monitoring and conservation actions should be implemented to maintain the genetic diversity of this endangered species.

**Keywords:** landscape genetics, genetic structure, isolation by distance, inbreeding, genetic drift

## Introduction

*Brachylophus bulabula*, commonly known as Fiji Banded Iguana, was previously grouped with *Brachylophus fasciatus* as one species. Molecular and morphological analyses by Keogh et al. in 2008 showed that *B. bulabula* is a separate species from *B. fasciatus*. *B. bulabula* is categorised as “Endangered” by the International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened Species (Fisher et al. 2012). According to the same IUCN Red List assessment report, *B. bulabula* are under threat of predation by mongoose, black rats, and feral cats, as well as habitat fragmentation. Keogh et al. (2008) suggested that the distribution of *B. bulabula* is limited to the larger northwestern islands of the Fijian Islands, the Viti group. Due to its recent discovery, not much is known about *B. bulabula*.

The landscape genetics of *B. bulabula* is focused on in this study for the conservation of this endangered iguana. Landscape genetics is fundamental in conservation management, especially for fragmented populations such as *B. bulabula* (Balkenhol et al. 2015). Genetic structure is a large area of focus within landscape genetics; it provides information about the genetic variation within and among populations as the result of ecological and genetic processes. Previous studies of the genetic structure of populations in the same order, Squamata, (Malone et al. 2003; Runemark et al. 2010; Felappi et al. 2015) showed that small isolated populations experienced random genetic drift and inbreeding depression leading to strong genetic structure, low genetic variation, and genetic divergence.

This study aims to investigate the genetic structure of *B. bulabula* on the Viti Levu, the largest island in Fiji. The question of the main focus is whether there is a genetic structure of *B. bulabula* populations found on the Viti Levu. To answer this question, genetic data from three populations of *B. bulabula* that were distributed on different sides of a mountain range and a river

were analysed. The hypothesis of isolation by distance was also tested to provide further information for the conservation management of this species. It is predicted that *B. bulabula* will show the pattern of strong genetic structure and isolation by distance.

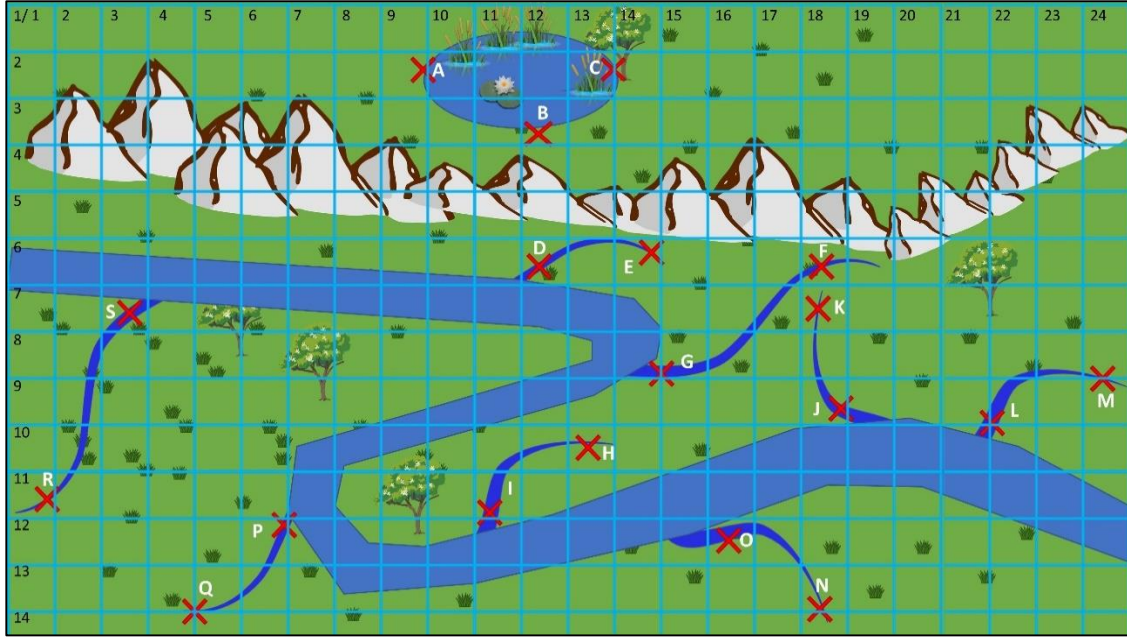
## Material and Methods

### *Sampling size and location*

A total of 19 populations of *B.bulabula*, represented by A to S, were sampled from Viti Levu. Each population represents a sampling site. In each population, seven individuals were sampled. In total, 133 individuals were sampled.

Samples were collected from both the northern and southern sides of a mountain. The mountain has an average elevation of 1300 metres. The southern side of the mountain is further divided by a river. The river has an average width of 80 kilometres. Samples were collected from the northern and southern sides of the river.

Population A to C, referred to as the “mountain” populations, were sampled from the northern side of the mountain. Population D to M referred to as the “north” populations, were sampled from the northern side of the river. Population N to S, referred to as the “south” populations, were sampled from the southern side of the river (see Figure 1).



*Figure 1.* Sampling site of each population of *B.bulabula* in Viti Levu, Fiji. Each red cross, X, represents a sampling site. Each alphabetical letter represents a sampled population. Populations A to C were sampled at the northern side of the mountain. Populations D to M were sampled at the northern side of the river. Populations N to S were sampled at the southern side of the river.

#### *DNA extraction and sequencing*

Blood samples were carefully collected from each individual by ensuring that no individuals are killed during the process. DNA was extracted using the GeneCatch™ Blood and Tissue Genomic Mini Prep Kit (Epoch Life Science, Inc) following the manufacturer's guidelines. The DNA were then sent to an external company, Diversity Arrays Technology Pty. Ltd. (DArT; Canberra, Australia - <http://www.diversityarrays.com>), for DNA sequencing. SNP discovery was performed for each sample using the standard DArTseq protocol (DArT 2018).

#### *Single nucleotide polymorphism dataset generation*

Single nucleotide polymorphisms (SNPs) were identified and called using in DArT proprietary pipeline DArTSoft14™ (Diversity Arrays Technology) standard procedures. Only homozygous and heterozygous forms were retained by removing monomorphic clusters. The

generated SNPs were filtered to only obtain SNPs with the average ratio of reading depths between alleles of 0.75, reproducibility average of >90%, and a minimum read depth of 5.

### *Data analysis*

The Excel add-in package GenAlEx 6.503 (Smouse and Peakall, 2012) was used for most of the analyses in this study.

Summary statistics of the data were generated using the Frequency tab of GenAlEx 6.503 to obtain genetic data of *B. bulabula* in this study. The summary statistics include the mean and standard error (SE) of the following: number of samples with data (N), number of different alleles ( $N_a$ ), number of effective alleles ( $n_e$ ), Shannon's information index (I), observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ), unbiased expected heterozygosity ( $uH_e$ ), and fixation index (F). A pairwise  $F_{ST}$  matrix ( $F_{STL}$ ) was also generated in the same process. This  $F_{STL}$  provided information about the genetic structure of *B. bulabula*.

Genetic distance data were generated through the Distance tab of GenAlEx. The genetic distance data were then used for a Principle Coordinates Analysis (PCoA) that was generated by the PCoA function of GenAlEx. The PCoA provides further information about the genetic structure. An ancestry matrix or admixture model was produced for all populations. The effective population size ( $N_e$ ) of the “mountain”, “north”, and “south” population groups were calculated using linkage disequilibrium.

To investigate isolation by distance, the SNPs data from “north” and “south” populations were separated into two different datasets. The “mountain” populations were excluded because not sufficient data were available. Genetic distances were generated for each data set by the Distance function of GenAlEx. Through the Spatial function of GenAlEx, a correlogram between genetic

similarity and geography distance was generated for each dataset along with the results of spatial structure analyses.



## Results

### *Genetic data*

Summary statistics of the genetic data are presented in Table 1. The data are consistent throughout all populations in all measurements, except F. Population A, B, and C had higher F than the rest of the populations.

Overall, the grand mean of N,  $N_a$ ,  $n_e$ , I,  $H_o$ ,  $H_e$ ,  $uH_e$  and F are 6.912, 1.767, 1.422, 0.385, 0.255, 0.253, 0.273, and -0.012, respectively, and the standard errors are 0.002, 0.002, 0.002, 0.001, 0.001, 0.001, 0.001, and 0.002, respectively.

Table 1. Summary statistics of the genetic data of *B. bulabula* sampled on Viti Levu. This table shows the number of samples with data (N), number of different alleles ( $N_a$ ), number of effective alleles ( $n_e$ ), Shannon's information index (I), observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ), unbiased expected heterozygosity ( $uH_e$ ) and fixation index (F).

Population		N	$N_a$	$n_e$	I	$H_o$	$H_e$	$uH_e$	F
Site A	Mean	6.876	1.732	1.389	0.360	0.213	0.236	0.254	0.072
	SE	0.009	0.010	0.008	0.006	0.005	0.004	0.005	0.010
Site B	Mean	6.878	1.733	1.400	0.368	0.214	0.242	0.261	0.088
	SE	0.010	0.010	0.008	0.006	0.005	0.004	0.005	0.010
Site C	Mean	6.912	1.745	1.401	0.370	0.215	0.242	0.261	0.081
	SE	0.008	0.010	0.008	0.006	0.005	0.004	0.005	0.010
Site D	Mean	6.944	1.719	1.401	0.364	0.265	0.240	0.259	-0.090
	SE	0.006	0.011	0.008	0.006	0.006	0.004	0.005	0.010
Site E	Mean	6.958	1.686	1.413	0.361	0.287	0.241	0.260	-0.150
	SE	0.005	0.011	0.009	0.006	0.007	0.005	0.005	0.010
Site F	Mean	6.901	1.780	1.440	0.397	0.266	0.262	0.283	-0.019
	SE	0.008	0.010	0.008	0.006	0.005	0.004	0.005	0.009
Site G	Mean	6.923	1.842	1.446	0.413	0.268	0.270	0.291	-0.003
	SE	0.007	0.009	0.008	0.005	0.005	0.004	0.004	0.009
Site H	Mean	6.808	1.790	1.436	0.398	0.260	0.262	0.283	0.001
	SE	0.012	0.010	0.008	0.006	0.005	0.004	0.004	0.010
Site I	Mean	6.920	1.787	1.431	0.396	0.256	0.261	0.281	0.006
	SE	0.007	0.010	0.008	0.006	0.005	0.004	0.004	0.009
Site J	Mean	6.924	1.834	1.445	0.412	0.269	0.270	0.291	-0.013
	SE	0.007	0.009	0.008	0.005	0.005	0.004	0.004	0.009
Site K	Mean	6.876	1.813	1.441	0.406	0.257	0.266	0.287	0.017
	SE	0.009	0.009	0.008	0.006	0.005	0.004	0.004	0.009
Site L	Mean	6.929	1.809	1.439	0.404	0.262	0.265	0.286	-0.003
	SE	0.007	0.009	0.008	0.006	0.005	0.004	0.004	0.009
Site M	Mean	6.932	1.743	1.412	0.374	0.248	0.247	0.266	-0.012
	SE	0.007	0.010	0.008	0.006	0.005	0.004	0.005	0.009
Site N	Mean	6.934	1.794	1.433	0.397	0.259	0.261	0.281	-0.003
	SE	0.007	0.010	0.008	0.006	0.005	0.004	0.004	0.009
Site O	Mean	6.923	1.752	1.411	0.375	0.259	0.247	0.266	-0.047
	SE	0.007	0.010	0.008	0.006	0.006	0.004	0.005	0.009
Site P	Mean	6.938	1.816	1.444	0.407	0.268	0.267	0.288	-0.013
	SE	0.006	0.009	0.008	0.006	0.005	0.004	0.004	0.009
Site Q	Mean	6.921	1.784	1.413	0.384	0.261	0.251	0.270	-0.044
	SE	0.007	0.010	0.008	0.006	0.005	0.004	0.004	0.009
Site R	Mean	6.901	1.673	1.390	0.349	0.253	0.232	0.250	-0.081
	SE	0.009	0.011	0.009	0.006	0.006	0.005	0.005	0.010
Site S	Mean	6.936	1.741	1.425	0.382	0.263	0.253	0.272	-0.037
	SE	0.006	0.010	0.008	0.006	0.006	0.004	0.005	0.009

### *Genetic structure*

Pairwise  $F_{ST}$  matrix (Table 2) has shown that Population A, B, and C had low  $F_{ST}$  within themselves but high  $F_{ST}$  compared to other populations.

The principal coordinates analysis (PCoA), visualised in Figure 2, showed patterns of genetic clusters, with “mountain”, “north”, and “south” populations each forming their own genetic cluster. Each population was also showing a genetic cluster to an extent.

Three different genetic groups were apparent in the ancestry matrix, or admixture model (Figure 3). Those three different genetic groups correspond to “mountain”, “north”, and “south” populations. There was also a small amount of mixing of genetic groups in each group but overall, there were obvious patterns of distinct genetic groups.

As shown in Table 3, the  $N_e$  is the smallest in the “mountain” populations and the largest in the “south” populations.

Table 2. Pairwise  $F_{ST}$  matrix of all the sampled populations (referred by site) of *B. bulabula*.

	SiteA	SiteB	SiteC	SiteD	SiteE	SiteF	SiteG	SiteH	SiteI	SiteJ	SiteK	SiteL	SiteM	SiteN	SiteO	SiteP	SiteQ	SiteR	SiteS
SiteA	0.000																		
SiteB	0.050	0.000																	
SiteC	0.047	0.048	0.000																
SiteD	0.135	0.132	0.130	0.000															
SiteE	0.135	0.133	0.132	0.053	0.000														
SiteF	0.111	0.108	0.105	0.043	0.058	0.000													
SiteG	0.101	0.100	0.098	0.050	0.067	0.039	0.000												
SiteH	0.110	0.109	0.106	0.044	0.046	0.041	0.039	0.000											
SiteI	0.107	0.105	0.102	0.056	0.046	0.048	0.044	0.034	0.000										
SiteJ	0.097	0.095	0.092	0.074	0.077	0.051	0.041	0.054	0.053	0.000									
SiteK	0.103	0.103	0.102	0.064	0.063	0.048	0.042	0.045	0.045	0.038	0.000								
SiteL	0.102	0.099	0.096	0.068	0.067	0.052	0.043	0.047	0.042	0.039	0.043	0.000							
SiteM	0.105	0.104	0.102	0.090	0.080	0.070	0.063	0.059	0.047	0.057	0.060	0.048	0.000						
SiteN	0.104	0.104	0.100	0.088	0.098	0.070	0.061	0.069	0.075	0.061	0.067	0.067	0.080	0.000					
SiteO	0.115	0.112	0.108	0.100	0.113	0.084	0.072	0.086	0.085	0.073	0.078	0.075	0.090	0.052	0.000				
SiteP	0.103	0.100	0.098	0.082	0.097	0.067	0.053	0.070	0.072	0.056	0.066	0.062	0.079	0.055	0.053	0.000			
SiteQ	0.113	0.110	0.108	0.093	0.111	0.079	0.064	0.083	0.083	0.067	0.075	0.073	0.090	0.061	0.046	0.040	0.000		
SiteR	0.136	0.135	0.132	0.101	0.118	0.091	0.076	0.095	0.095	0.083	0.093	0.086	0.109	0.094	0.091	0.057	0.065	0.000	
SiteS	0.115	0.114	0.109	0.091	0.108	0.076	0.062	0.081	0.083	0.069	0.077	0.072	0.091	0.070	0.066	0.043	0.048	0.046	0.000

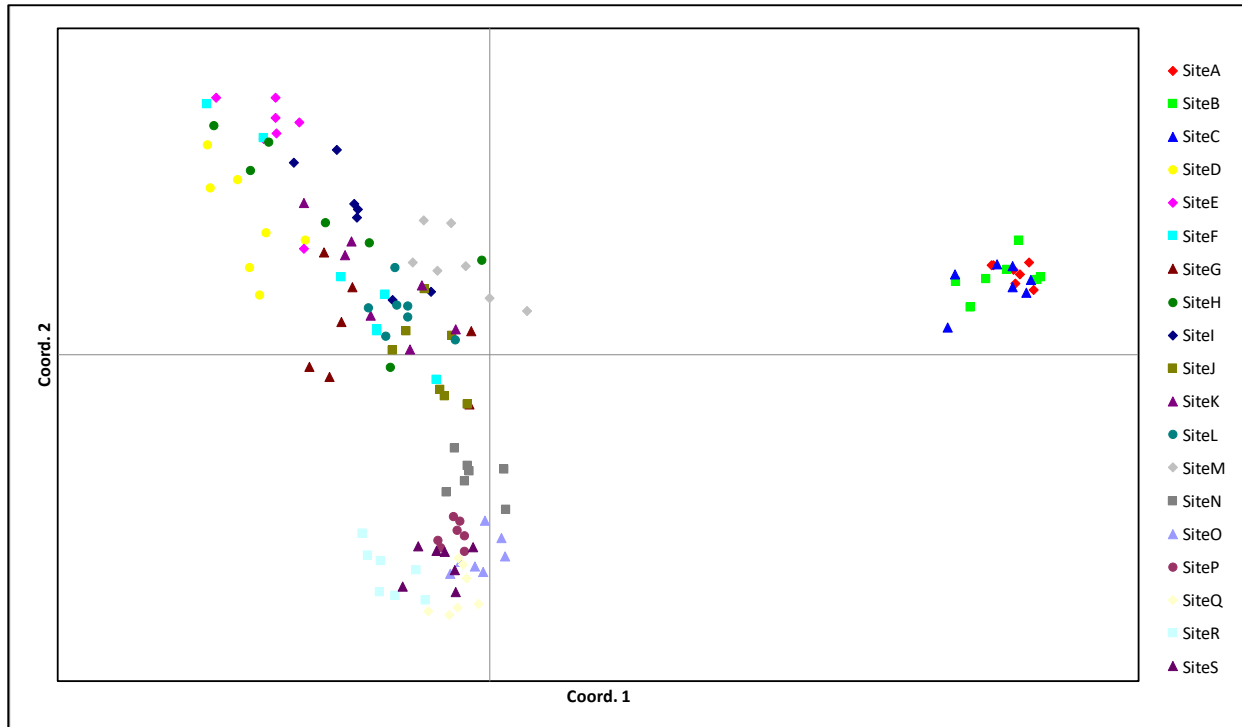


Figure 2. Principal Coordinates (PCoA) plot. Each symbol represents one individual of *B. bulabula*. Each population has a unique symbol as shown in the legends. The distance between pairs of symbols reflects the genetic distance of the individual pair.

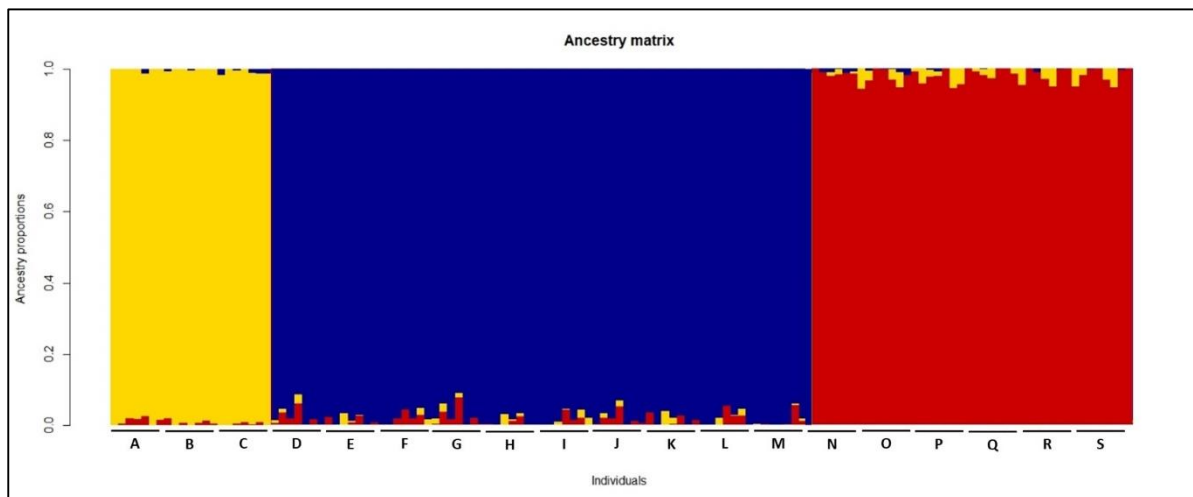


Figure 3. Ancestry matrix, or admixture model, of the genetic group of *B. bulabula* sampled. Individuals are grouped genetically based on Hardy-Weinberg equilibrium. The three different colours represent three different genetic groups. Blue, yellow, and red colours are in the order of top to bottom, respectively.

*Table 3.* The estimated effective population size ( $N_e$ ) and its 95% confidence interval of the “mountain”, “north”, and “south” populations of *B. bulabula*.

<b>Population</b>	<b>Estimated <math>N_e</math></b>	<b>95% Confidence interval</b>
“Mountain”	37	36.12 – 38.01
“North”	386	378.91 – 392.75
“South”	1844	1832.71 – 1861.39

#### *Isolation by distance*

The correlogram of the “north” population had an Omega value of 78.467 and a P-value of 0.001, suggesting that the correlogram is significant. The average genetic similarity ( $r$ ) from distance class 0 to 2 is higher than the upper 95% confidence interval limit of the null hypothesis of no spatial structure (U). However, from distance class 4 to 12,  $r$  is lower than the lower 95% confidence interval limit of the null hypothesis (L). The  $r$  of the distance class 2 to 4 is within the range of U and L.

The “south” population correlogram was also significant with an Omega value of 93.803 and a P-value of 0.001. The  $r$  in distance class 1 to 5 is higher than U, while the  $r$  from distance class 5 to 11 is within the U and L range. However, the  $r$  from distance class 13 to 17 dropped below L.

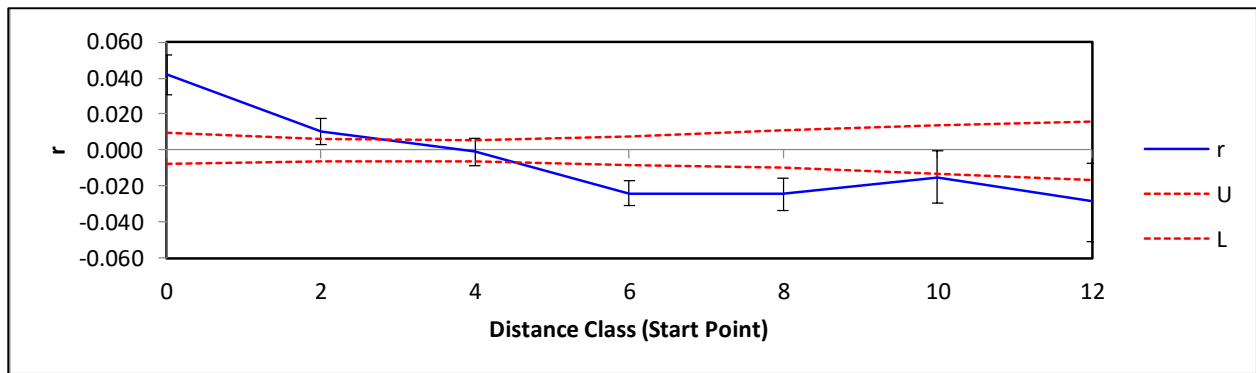


Figure 4a. Correlogram of average genetic similarity,  $r$  (y-axis), versus distance class (x-axis) of the “north” *B. bulabula* populations. The bar at each distance class shows the 95% confidence interval of  $r$ . The blue line,  $r$ , shows the average genetic similarity of the sampled populations. Red dashed lines,  $U$  and  $L$ , show the upper and lower limits of the 95% confidence interval of the null hypothesis of no spatial structure as determined by permutation.

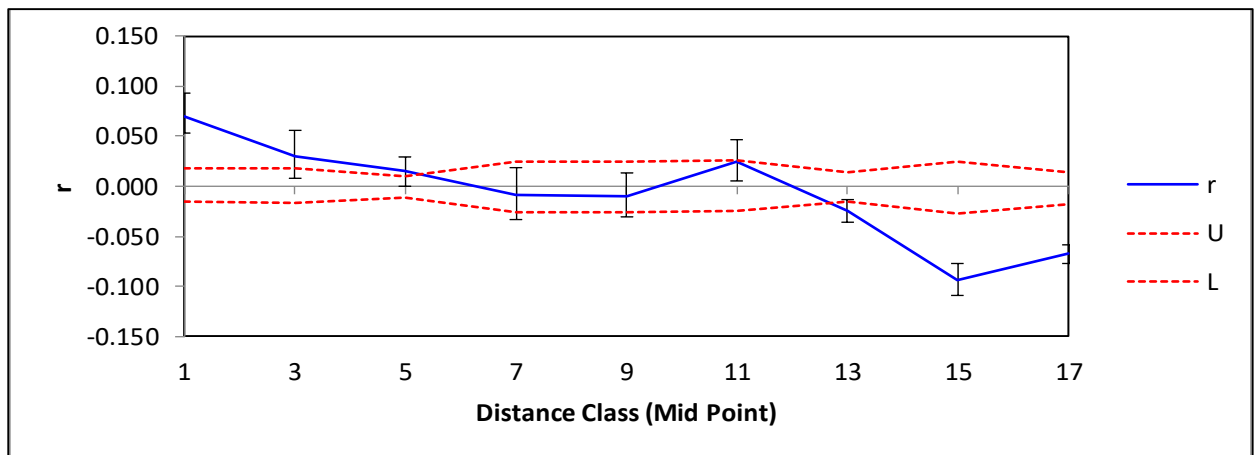


Figure 4b. Correlogram of average genetic similarity,  $r$  (y-axis), versus distance class (x-axis) of the “north” *B. bulabula* populations. The bar at each distance class shows the 95% confidence interval of  $r$ . The blue line,  $r$ , shows the average genetic similarity of the sampled populations. Red dashed lines,  $U$  and  $L$ , show the upper and lower limits of the 95% confidence interval of the null hypothesis of no spatial structure as determined by permutation.

## Discussion

### *Genetic data and genetic structure*

The results obtained suggested that the “mountain” *B. bulabula* populations formed their own genetic groups that are distinct from the “north” and “south” populations. The three populations of the “mountain” group showed considerably low  $n_e$  and high  $F$  compared to other populations (Table 1). The low  $n_e$  is a sign that the “mountain” populations have high homozygosity. Furthermore, the “mountain” populations had low pairwise  $F_{ST}$  within themselves but high  $F_{ST}$  relative to the “north” and “south” populations (Table 2). PCoA result also showed a genetic cluster of the “mountain” populations that were distanced from the other populations (Figure 2). The ancestry matrix further supports that the “mountain” populations form their own genetic cluster. These populations have lower genetic variation than the rest of the populations.

Unlike the “mountain” populations, the “north” and “south” populations did not show high  $F$  in Table 1. The pairwise  $F_{ST}$  within each group and among the group of the “north” and “south” populations were not high as when compared with the “mountain” populations (Table 2). Despite that, the PCoA (Figure 2) and the ancestry matrix (Figure 3) revealed the genetic structure of the “north” and “south” populations as they form their own genetic groups. Frankham et al. 2002 suggested  $F_{ST} \geq 0.15$  is significant for genetic divergence. Although the highest  $F_{ST}$  obtained (Table 2) was 0.136 (Site A versus Site R), continuous monitoring should be conducted to prevent further divergence.

The  $N_e$  of 37 in the “mountain” population raises concern as evidence since 1980 revealed that  $N_e$  of 50 is insufficient to prevent inbreeding depression in the wild for over five generations (Frankham et al. 2014). The same study cited also found that  $N_e \geq 100$  is required to maintain the



loss in total fitness to  $\leq 10\%$ . The low  $N_e$ , low  $n_e$ , and positive  $F$  (Table 1) show signs of inbreeding in the “mountain” populations. The “North” population has a  $N_e$  of 386 which is adequate for preventing inbreeding depression however it is inadequate to maintain evolutionary potential. Frankham et al. (2014) recommended that  $N_e \geq 1000$  is required to maintain evolutionary potential as  $N_e = 500$  is inadequate. The “south” population has a  $N_e$  of 1844 which is sufficient for preventing inbreeding depression and maintaining evolutionary potential.

The IUCN Red List assessment (Fisher and Harlow 2012) has reported that *B. bulabula*’s distribution has an upper elevation limit of 500 metres so, that is a plausible reason that *B. bulabula* are not able to disperse to the opposite side of the mountain as it was 1300 metres in average elevation.

#### *Isolation by distance*

Both correlograms of the “north” and “south” populations displayed a significant decline in genetic similarity as geographic distance increased (Figures 4a and 4b). The correlogram of the “north” population has a sharper decline than the correlogram in the “south” population. Despite having  $r$  falling within the U and L range in most distance classes, the “south” population still had a significant negative trend as supported by the P-value of 0.001. Isolation by distance is observed in the “north” and “south” populations of *B. bulabula*.

The significant P-values resulting from the spatial structure analyses of both “north” and “south” populations provided evidence of significant heterogeneity within each population (Banks and Peakall 2012). This suggests that there is a constraint to the distance that *B. bulabula* can disperse to, from their place of birth (Wagner and Fortin 2005), which could cause high relatedness

among the individuals of each population leading to the rapid decline of genetic similarity when moving across spatial distance.

Even though *B. bulabula* were reported to be good swimmers, they prefer to be arboreal (Sacramento Zoological Society 2016). This could explain the restricted dispersal and limited gene flow across the opposite of the water. Their ability to swim could also explain the higher relatedness among the “north” and “south” populations compared to the “mountain” populations.

### *Conclusion*

As predicted, the Viti Levu’s *B. bulabula* showed a strong genetic structure. The hypothesis was also supported as the results showed evidence of isolation by distance. It is apparent that the “mountain”, “north”, and “south” populations form their own genetic groups. These populations are also under isolation by distance. The mountain range and the river form barriers to gene flow in the *B. bulabula* populations in Viti Levu thus, leading to the three small isolated populations. The “mountain” populations are at risk of inbreeding depression due to their low  $N_e$ . Although the “north” and “south” populations have higher  $N_e$  than the “mountain” populations, all of these populations are nonetheless susceptible to genetic drift.

With the “mountain” populations at risk of inbreeding depression and the “north” populations at risk of losing evolutionary potential, conservation actions must be undertaken for *B. bulabula*. A regular genetic monitoring program should be introduced to conserve the endangered *B. bulabula* population in Viti Levu. Further studies should also be conducted to find viable solutions to the genetic restoration of this iguana. Translocation of the *B. bulabula* between the northern side of the mountain range and the northern and southern sides of the river should also be considered with prior planning and risk assessment.

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