



Microsatellites Loci Reveal Heterozygosity and Population Structure in Critically Endangered Southern River Terrapin (*Batagur affinis*) of Peninsular Malaysia

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Abstract

These freshwater turtles are found across Indochina, mostly in big rivers. Lack of genetic research has concentrated on Malaysia's Southern river terrapin (*Batagur affinis*) population. We used minimally intrusive ways to collect blood samples from a total of 80 individuals in four different sites in peninsular Malaysia. The genetic difference within and between locations was examined using five microsatellite loci. Our findings indicated that each locus was polymorphic. High amounts of heterozygotes were observed when the percentage of alleles in each locus was compared. Pairwise F_{ST} and Nei matrixes revealed considerable genetic differences across individuals from distinct geographical locations. Our population structure analysis shows a significant proportion of assigned individuals linked to certain collection locations.

Keywords: Turtles; Indochina; genetic; blood sample; polymorphic; population structure.

Objectives

Our purpose was to demonstrate that Southern river terrapin populations' genetic diversity and structure are related to the collection location and restricted migrations, with little effect at broader scales due to the Southern river terrapin's lack of broad migratory movements. Following that, we analysed and assessed the genetic diversity of Southern river terrapin populations. Finally, we hypothesised a genetic population structure characterised by a large proportion of individuals allocated to certain places. We demonstrate that a human disturbance affects the Southern river terrapin's genetic diversity by presenting our findings in various quantitative methods.

Materials and Methods

This study comprised 80 *Batagur affinis* individuals from four population regions crossing the East and West Malaysia peninsula;

1. Bukit Pinang (BP), Kedah (4.2221° N, 100.4370° E).
2. Bota Kanan (BK), Perak (4.3489° N, 100.8802° E).
3. Bukit Paloh, Kuala Berang (KB), Terengganu (5.0939° N, 102.7821° E).
4. Pasir Gajah, Kemaman (KE), Terengganu (4.2524° N, 103.2957° E).

A total of 20 individuals from the *B. affinis* population were sampled at each location in 2020. Blood was drawn using two venepuncture techniques:

- i. the subcarapacial venous plexus (SVP).
- ii. the jugular vein.

Statistical Analysis;

- CONVERT 1.31 (Glaubitz, 2004).
- FSTAT version 2.9.3.2 (Goudet, 1995).
- ARLEQUIN version 3.0 (Excoffier et. al., 2005).
- GenAlEx 6.5 (Peakall and Smouse, 2012).

Results

Table 1. Descriptive statistics for each population including a range of alleles, expected hetero-zygosity (H_e), and observed heterozygosity (H_o), allele richness (R_s), gene diversity (H_s), sample size (N)

Locality	Statistical						N
	H_o	H_e	Allele Range	p value	R_s	H_s	
KE	0.66	0.85	7-17	0.02	11.4	0.86	20
KB	0.49	0.87	9-16	0	13.6	0.88	20
BP	0.51	0.8	8-11	0	9	0.81	20
BK	0.37	0.81	7-13	0.01	10	0.81	20

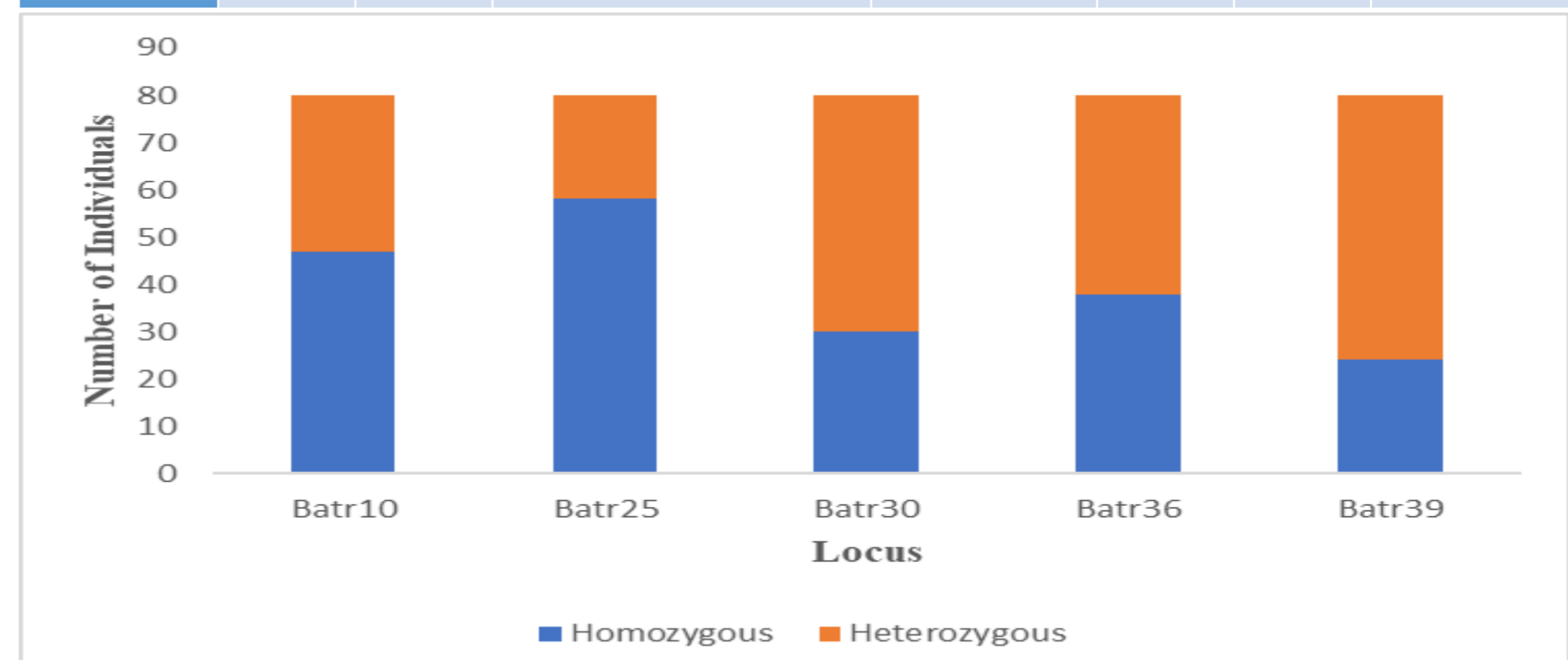


Figure 2. Frequencies of homozygous/heterozygous individual alleles represented for the five loci.

Table 2. Pairwise genetic distance based on the F_{ST} matrix (below diagonal) and Nei Matrix (upper diagonal), a measure of divergence among the *B. affinis* species populations. * $p < 0.05$

Region	Kemaman	Kuala Berang	Bukit Pinang	Bota Kanan
Kemaman	-	0.962	0.894	0.823
Kuala Berang	0.080*	-	0.749	0.574
Bukit Pinang	0.096*	0.083*	-	1.011
Bota Kanan	0.088*	0.064*	0.122*	-

Conclusions

Our results showed consistency with expected genetic diversity and population differentiation for a species affected by human activities. A novelty is the first study on two subspecies of *B. affinis*. Four populations spanning the East, and West coasts of peninsular Malaysia was disclosed using microsatellites. The study showed *B. affinis edwardmulli* more genetic diversity than *B. affinis affinis*, making this a beneficial research that extends beyond Malaysia to the Indochina region. This will serve as a key source for future genetic association and functional analysis to enhance breeding programmes for long-term sustainability.

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