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A study on the molecular characterization of *Mabuya multifasciata* at Darrang District, Assam.

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Abstract

Skinks are very widely distributed throughout the world. For their many unique characters, this species draws eyes of many scientific work. They are very colourful in their breeding season and are viviparous. For this special character there are many research work are going to know the true nature of the involved genes related to their reproductive behaviour.

Being naturally blessed, Assam is a home of many reptiles. A study was planned during 2011-2017 on *Mabuya multifasciata* in Darrang district of Assam to study the reproductive behaviour and the molecular characterization of genes being involved in it.

Key words : ***Mabuya multifasciata*, molecular charcaterization, Darrang, Assam.**

1.Introduction :

Skinks are very widely distributed throughout the world. For their many unique characters, this species draws eyes of many scientific work. They are very colourful in their breeding season and are viviparous. For this special character there are many research work are going to know the true nature of the involved genes related to their reproductive behaviour.

Being naturally blessed, Assam is a home of many reptiles. A study was planned during 2011-2017 on *Mabuya multifasciata* in Darrang district of Assam to study the reproductive behaviour and the molecular characterization of genes being involved in it.

2. Materials and methods :

2.1 Study area :

Darrang of Assam situated in India, was choosen as study area.

2.2 Molecular characterization of *Mabuya multifasciata*

Genomic DNA extraction, PCR amplification and sequencing method was followed those outlined in Sambrk and Russl *et.al* (2015).

2.2 (a) Sample collection

A total of three samples of *Mabuya multifasciata* across three sample sites of Darranf District were collected for analysis. The technique used for molecular study was non-invasive such as collecting samples from faeces, claws and scales. The individuals were marked as QQ1, MM1 and SS1.

2.2 (b) DNA extraction

DNA isolation was carried out using modified Phenol-Chloroform method :

Preparation of reagents :

SNET Reagent :

Tris-Cl (p H 8.0) = 20 m M (final concentration)

EDTA (p H 8.0) = 5 m M(final concentration)

NaCl = 400 m M (final concentration)

SDS = 1% (w/v)

T.E Buffer = 10 m M Tris+ 1 m M EDTA)

The solution was sterilized by filtration through a 0.45 μm nitrocellulose filter. The sterile solution was then stored in 50 ml aliquots at room temperature.

1. 6-10 mm of tails were removed under anesthesia.
2. Then it was transferred to 17 x 100 mm Falcon polypropylene tube.
3. (Lysis buffer + Proteinase K $\rightarrow$ Final concentration 400 μg /ml in SNET) was added to the mouse tail.
4. Transfer it to the 17 x 100 mm P.F.T.
5. Overnight incubation was done at 55 ^{o} C (till the Buffer was milky gray).
6. Equal volume of Phe : Chl : IAA was added , then the top of the tube was sealded, it was placed in rocking platform for 30 minutes at room temperature. (Higher molecular weight DNA was required to take care of minimize shearing forces).
7. The organic and aqeuous phases were seaparated by centrifugation. (Centrifuge was done in 17 x 100 mm P.Tube of 660 g for 5 min at room temperature).

8. Then the upper aquous phase was transferred to fresh Falcon/miicrocentrifuge.
9. The DNA was precipitated to the double volume of Isopropanol.
10. Then the ppt DNA was collected (centrifuge at 18000 g for 15 min at 4 ^{o} C.
11. Then the isopropanol was removed.
12. The pellate of DNA was rinsed with 1 mL of 70 % ethanol. (pellet was with ethanol was done twice with 0.5 % chilled 70 % ethanol)
13. Centrifuge was done again for 5 min for loose pellets.
14. Centrifuge (same) was done to remove 70 % ethanol, the the pellet was allowed to dry in air at room temperature for 15-20 minutes.(care was taken not to dry completely)
15. It was dissolved in 0.5 m L T.E (p H 8 at 4^{0} C). Overnight by rocking.
16. Then it was transferred to micro-centrifuge and stored at room temperature.

2.2 (c) Primer Sets

The present study used Four nuclear exons for genotyping of various population of *Mabuya multifas*ciata. They were 2MHCF, 2MHCR, MHCIF and MHCIR (all are 140 bp proteins).

Table 1 : PCR primer sequences used for amplification and for sequencing of DNA

Gene name	Primer name	Sequence (5″-3‟)	Position	T_m (^{0}C)
MHC CLASS	2MHCF	CAGCAGATGTATGGCTGTGA	410 [a]	54
	2MHCR	GCAGATCTCCTCCAGGTAG	578 [a]	54
1	MHCIF	GCCGAGTTCACGGCTTTCTACCCC	736 [a]	58
	MHCIR	CATGCTCCACGTGGCACTGGTA	873 [a]	58

PCR Amplification :

PCR Mix :

Forward primer = 0.2 µ L

Reverse primer = 0.2 µ L

10X HF Buffer = 1µ L

Taq DNA Polymerase Enzyme = 0.2 µ L

dNTPs = 0.2 µ L

Distilled water = Added to make

Table 2 : PCR conditions for amplification of MHC class1 gene

Step	Temperature (°C)	Time (minute)	Cycles
Initial denaturation	94	5	40
Denaturation	94	1	
Annealing	68	1	
Extension	72	2	
Final hold	72	10	

2.2 (d) DNA sequencin

Electrophoresis of the PCR products were done through agarose gels, visualized with ethidium bromide staining, removed from the gel and then purified using a QIAamp gel extraction kit (Qiagen). The DNA obtained as such were sequenced using DNA sequencer (at Bio Axis DNA Research Centre, Hyderabad).

2.2 (e) In Sillico Analysis of the sequences : The sequences does obtained were thereby subjected to BLAST to acquire alignment of the sequences. To acquire either their specificity or their corresponding relatedness the comparative analysis of sequences with the concerned species was done.

For alignment of the sequences CLUSTAL2 and MEGA7 were used. This was done at the BIF centre, Gauhati University, Guwahati, India . This allowed proper

characterization and comparative analysis of the sequences.

2.2 (f) Base composition and mutation : Using MEGA 7 software the characterization of the sequences was done by obtaining the base composition and their respective properties.

2.2 (g) Restriction Mapping : In the individuals the occurrence of restriction sites within a genome is a characteristic of every individuals. Considering this the restriction mapping for the gene sequence of MHC Class1 was done using SEQUENCE MANIPULATION SUITE2 available at www.bioinformatics.org

Then protein modelling was done from the proten sequences obtained from above were put forth for prediction of protein structures using online applications like www.swissmodel.expasy.org.


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LEGEND
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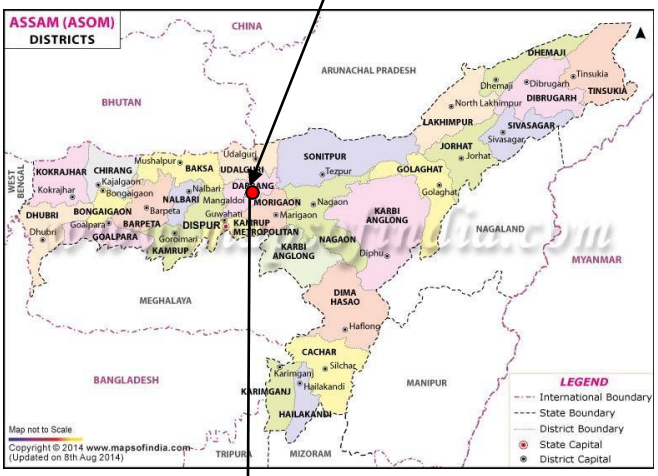
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DIMA HASAO
Haflong
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Silchar
Karimganj
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Hailakandi
HAILAKANDI
LEGEND
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State Boundary
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State Capital
District Capital
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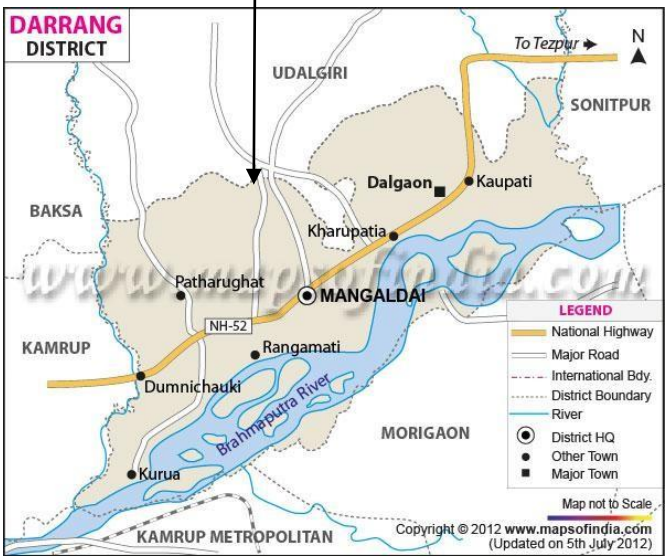
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River
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3 . Result :

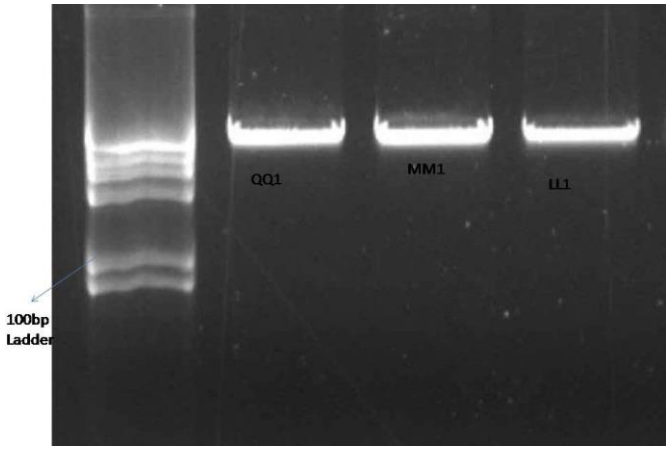

Fig 1: Amplified PCR

product for the gene MHC

Class 1 (720 bp)

QQ1= sample from male

MM1= sa,mple from female

LL1= sample from juvenile

3.1 Sequencing and submission of sequences to database :

The purified PCR products were sequenced and the sequences thus obtained were submitted to Genebank available and maintained by National Centre for Biotechnology Information (NCBI). The Accession numbers of the PopSets are listed in Table.

Table 5 : Accession number of the sequences (Genebank, NCBI)

PopSet	MHC Class1
QQ1	KY814743
MM1	KY814744
LL1	KY814745

3.2 Sequence analysis and characterization :

3.2.A Assessment of sequences for analysis :

The respective sequences thus obtained were then forwarded for analysis with intent to characterize the sequences and avail,sufficient information of the species *Mabuya multifasciata* with respect to the gene MHC Class1.

3.2.B . Base composition :

The sequences were firstly characterized by studying its base composition. Mega 7 software was used to study the nucleotide composition and the number of mutations recoded along with their respective positions where they occur. Using online vailable server site Sequence Multiplication Suit (SMS) , the restriction mapping for each individual sequences of the genes were obtained.

Table 6: Percentage base composition of sequences QQ1,MM1,LL1 for the gene MHC Class1

	T(U) %	C%	A%	G%	Total
QQ1	27.4	31.1	28.9	12.6	585
MM1	27.7	30.9	28.2	13.2	585
LL1	26.7	32.5	28.5	12.3	585

3.3.A. Restriction site mapping :

Considering that restriction sites that occur in a genome can be assessed as important molecular characteristic of gene sequences, the occurrence of various restriction sites along with their position and number of cuts induced by them were worked out using Sequence Manipulation Suite 2 available online. This assessment was done for each individual sequences for MHC Class1 gene.

The pattern of occurrence of restriction sites are represented for the gene MHC Class1 in the Table. Among the individuals the variation is obvious. Owing to the incidences of base mutation , the altered pattern of restriction site occurrences in MHC Class1 gene is well anticipated.

There has been alterations in the positions of the restriction sites , as well as in the type of restriction enzymes.lAbove all Acc III,c has evolved a new recognisation site in QQ1,MM1 and LL1 in QQ1,MM1 and LL1 at 217 and 113 instead of the usual site at 301. There has been also an instances of emergence of newr site. As an example of such site is occurrence of new site for the enzyme Afa I gt at position 477 of MHC Class 1 sequence of MM1 . On the other hand there is loss of restriction sites as in MM1 and LL1 where the site for Alu ag is lost.

Table 7: Comparative occurrence of Restriction Sites in the sequence MHC Class1

Site	QQ1	MM1	LL1
AccII cg|cg	217	113	None
Acc III cg|cg	301	301	301
AfaI gt|ac	477	218,477	477
AflII c|ttaag	28	28	28
AluI ag|ct	211	None	None
AseI at|taat	89	89	89
BfrI c|ttaag	28	28	28
BstUI cg|cg	217	None	None
HinfI g|antc	312	312	312
HpaII c|cgg	194	194	None
NlaIII catg|	559	559	559
PsiI tta|taa	98,176	98,176	98,176
RsaI gt|ac	477	218,477	477
SspI aat|att	87	87	87
VspI at|taat	89	89	89

4. Discussion :

The molecular characterization was carried to assess the property of the gene MHC1 which is involved in colouration. The gene has been involved is MHC1 (720bp) for three different population groups from three different areas have been identified and sequenced.

The genetic variability which is the effect of genetic variation on population have received extensive theoretical treatment and experimental variation. The molecular characterization revealed the variation in the base composition in all three populations (Table: 6) and also predicted variation in their restriction map for the sequences of MHC1 (Table : 7). The proteins sequenced from the MHC1 exhibits variation among the population groups. The MHC1 exhibited differences in the sequences of proteins obtained and presented varied structures . This suggests that the colouration involves in breeding (sexual trait) may be depend upon the expression level of the MHC1 gene in different period. Further study is needed here. The protein structure predicted for the male, female and juvenile was found to be different. Base composition variation in

composition of C and G percentage. The restriction map of each gene from the different population group obtained varied from each other (Table :7).

In this study introduction of restriction map analysis using sequencr manipulation Suite-2, it could well be predicted the genetic variability of MHC1 gene among the populations. After extensive study on brown anole lizard (*Anolis*) Gase *et al*.,2010 suggested that using microsatellite marker, that there has been possible loss of genetic diversity. The sequence obtained in this study will form the basis of future research on skink MHC sequence of *Mabuya multifasciata*, they contribute to current research on the effect of MHC on animal behaviour and mat choice. Reusch et al.,2001 suggested the heterozygosity at MHC affects mate choice in fish, mice (Penn and Potts 1999) and human (Wedekind *et al*,1995).

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