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Molecular Characterization and Genetic Diversity Assessment of *Lissemys punctata andersoni* from Dhubri district of Assam, India

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Abstract

The present study investigates the molecular characteristics and genetic diversity of *Lissemys punctata andersoni* across six populations in the Dhubri district of Assam using cytochrome b gene sequences. Base composition analysis revealed consistently AT-rich gene regions dominated by adenine, a common characteristic of mitochondrial DNA. Sequence alignment indicated both conserved and variable nucleotide regions across populations. In silico restriction site analysis using multiple enzymes demonstrated significant polymorphism, confirming genetic divergence among populations. Genetic clustering showed three distinct clades, suggesting population structuring and evolutionary divergence. The findings highlight the utility of cytochrome b as a molecular marker for genetic variability assessment and emphasize the importance of such studies for conservation and species management.

Key words: Genetic diversity, Molecular characterization, cytochrome-b, mitochondrial DNA, Conservation

Introduction

Mitochondrial genes, particularly the cytochrome b gene, are extensively used for phylogenetic analysis, population genetics, and evolutionary studies in reptiles, including freshwater turtles. The mitochondrial genome evolves relatively rapidly and exhibits maternal inheritance, making it a powerful genetic marker for distinguishing populations and understanding evolutionary relationships (Parham et al., 2001; Rohila & Tiwari, 2008b). Among turtles, mtDNA regions such as COI, cytochrome b, and ND4 have proven useful for elucidating phylogenetic connections and assessing intraspecific variation (Stuart & Parham, 2004). Cytochrome b, in particular, provides valuable information due to its functional role in the electron transport chain and its moderate mutation rate, enabling both species-level and population-level analysis (Iverson et al., 2007). *Lissemys punctata andersoni*, a subspecies of Indian softshell turtles, holds ecological and conservation significance in Assam. However, molecular studies assessing its genetic diversity remain scarce. This study aims to fill this gap by analyzing cytochrome b sequences from six populations in Dhubri district, providing insights into genetic variation, population structure, and molecular features.

Materials and Methods:

1. **Sample Collection and DNA Sequencing**

Tissue samples of *L. p. andersoni* were collected from six distinct populations within Dhubri district. Genomic DNA extraction, PCR amplification was done using standard protocols (Sambrook and Russel, 2001). Cytochrome b gene fragments were amplified using specific primers and sequenced. Generated sequences were submitted to GenBank.

2. Sequence Alignment and Base Composition

Sequences were aligned using BioEdit version 7.1.1 and MEGA-7 to identify conserved and variable regions. Base composition percentages for adenine (A), thymine (T), guanine (G), and cytosine (C) were calculated to evaluate nucleotide distribution patterns.

3. Restriction Site Analysis

To evaluate polymorphism, sequences were digested in silico using with 15 restriction enzymes: AluI, AvaI, HinfI, Hinf1, MboI, NdeI, NdeI–NheI, RsaI, TaqI, VspI, EcoRV, HincII, HpaI, RsaI, and ScaI. Differences in enzyme recognition sites among populations were used as indicators of genetic variability.

4. Genetic Diversity and Clustering

Multiple sequence alignments were used to identify nucleotide variations. Populations were grouped into clades based on similarity indices, highlighting genetic relationships and divergence patterns.

Results

Base Composition of Cytochrome b Gene

All six populations (Table 1 and 2) describe the collection site along with their spectrophotometer quantification of DNA sample and GenBank accession No. showed an AT-rich sequence pattern, with adenine being the most abundant nucleotide. This reflects typical mitochondrial genome characteristics where AT-rich regions dominate due to replication and transcriptional preferences.

Table-1. Spectrophotometer Quantification of DNA Sample

SL No.	GPS Location	Sample ID	OD260/280	Conc mg/µl
1	Chandakhola 26°1'30.58"N 89°53'329.40"E	LPA7	1.62	169
2	Maragaddahar 26°1'27.18"N 89°58'37.90"E	LPA8	1.55	308.2
3	Upartary 26°14'52.69"N 90°15'4.29"E	LPA9	1.87	151.51
4	Dighalgaon 26°14'55.86"N 90°15'16.00"E	LPA10	1.84	399.1
5	Kushum Beel 26°23'45.46"N 90°6'23.92"E	LPA11	1.86	284.2
6	Chakrashila Pt-I 26°17'32.55"N 90°22'30.75"E	LPA12	2.53	644.1

Table-2 GenBank Accession Number of the Cytochrome-b Gene Sequenced from *Lissmys punctata andersoni*

Sl No.	Gene sequenced	Sample ID	GenBank accession No.
1	Cytochrome-b	LPA7	KY489852
2	Cytochrome-b	LPA8	KY489853
3	Cytochrome-b	LPA9	KY489854
4	Cytochrome-b	LPA10	KY489855
5	Cytochrome-b	LPA11	KY489856
6	Cytochrome-b	LPA12	KY489857

Sequence alignment revealed:

1. Multiple conserved domains shared across all populations
2. Distinct variable regions demonstrating population-specific differences

These variations contribute to genetic differentiation and are key to understanding population structure.

Restriction Site Polymorphism

The in silico digestion analysis (Table-3) revealed several conserved restriction sites shared across all populations, along with distinct population-specific digestion profiles arising from mutational differences. The observed variation in restriction patterns highlights substantial polymorphism and indicates the existence of multiple mitochondrial haplotypes within the studied groups.

Table-3 Restriction Mapping of *L. punctata andersoni* (Cytochrome-b Gene)

Restriction Site	LPA7 (1149)	LPA8 (864)	LPA9 (1153)	LPA10 (891)	LPA11 (1039)	LPA12 (1045)
AluI (agct)	141, 1138 (2)	14, 186 (2)	143, 1120, 1145 (3)	36, 209 (2)	161, 355, 1028 (3)	141
Aval	196	241	198	264	-	196
HincII (gty|rac)	1007	-	-	-	1012	1007
HinfI (glantc)	517, 679, 1077 (3)	562, 724 (2)	521, 683 (2)	585, 747 (2)	294, 521, 683 (3)	517, 679 (2)
MboI (lgatc)	44, 87, 597 (3)	89, 132, 642 (3)	46, 89, 439, 601 (4)	112, 155, 665 (3)	46, 89, 237, 439, 601 (5)	44, 87, 597 (3)
NdeI (catatg)	449	494	453	517	453	449
NdeII (lgatc)	-	-	-	112, 155, 665 (3)	46, 89, 237, 439, 601 (5)	44, 87, 597 (3)
NheI (glctagc)	-	731	690	754	-	686
RsaI (gtlac)	-	250, 427, 514, 605, 708 (5)	-	273, 450, 537, 628, 731 (5)	690	-
TaqI (tcga)	-	269, 669 (2)	226, 628, 1106 (3)	95, 292, 692 (3)	220, 318, 628 (3)	224, 624 (2)
VspI (att|taat)	-	264	221	287	-	219
EcoRV (gat|atc)	-	-	362	-	-	-
HincII (gtyl|rac)	-	-	1012	-	-	-

Formation of Genetic Clades

Sequence similarity analysis grouped the populations into three distinct clades, each representing a genetically cohesive cluster. This pattern reflects notable evolutionary divergence within the species, likely driven by factors such as geographic separation and ecological isolation.

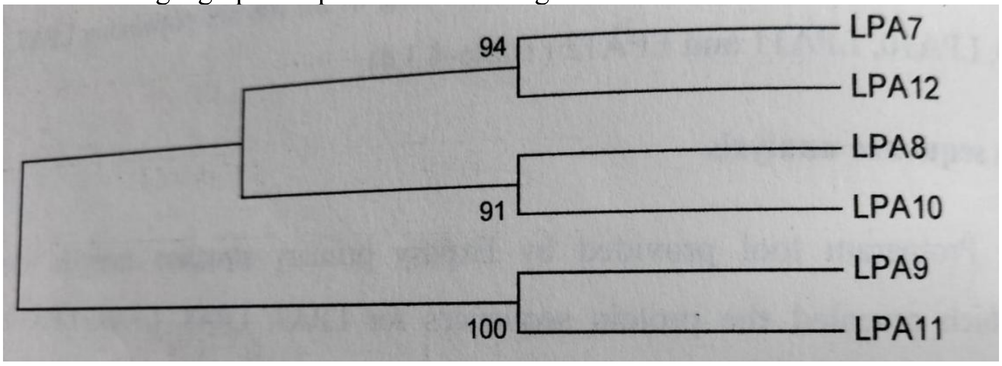

Fig: 1. Evolutionary relationship of *L. p. andersoni from Dhubri district*

Discussion

The molecular characterization of *L. p. andersoni* based on cytochrome b sequences provides a detailed understanding of genetic variability across populations in the Dhubri district. The AT-rich nucleotide bias observed in all samples is consistent with known mitochondrial genome structure, where AT-rich control regions facilitate replication and transcription. The strong dominance of adenine further reflects the evolutionary conservation of mitochondrial genes (Bell & Dutta, 2002; Rajeswska et al., 2012).

Despite this overall conservation, the presence of variable nucleotide regions indicates notable genetic divergence among populations. Such variations may arise from ecological differences, geographic separation, or reduced gene flow, all of which are common in semi-aquatic species with patchy habitats.

Restriction site analysis strengthens evidence of genetic variability. Changes in enzyme recognition patterns reflect underlying mutations, demonstrating polymorphism within the species. This molecular diversity is crucial for understanding population connectivity and can aid in identifying unique genetic stocks. Comparable studies on freshwater turtles (Osentoski & Lamb, 1995; Walker et al., 1997) also report similar patterns of mtDNA restriction site variation, underscoring its value in population genetics.

The emergence of three genetic clades signifies evolutionary structuring within the species. These clades likely represent populations that have experienced historical or ecological separation. Although genetically distinct, the populations retain core conserved regions, suggesting stability in essential mitochondrial functions such as oxidative phosphorylation.

From the conservation perspective, the detected genetic diversity highlights the importance of protecting all identified clades. Populations with distinct genetic signatures may harbor unique evolutionary potential, making them critical for the species' long-term survival. Habitat degradation, wetland loss, and human interference could disrupt gene flow and further fragment populations, making genetic monitoring essential.

Overall, the cytochrome b gene proves to be a powerful molecular marker for assessing genetic diversity, identifying evolutionary relationships, and guiding conservation strategies for *L. p. andersoni*.

Conclusion

This study provides a comprehensive molecular assessment of *Lissemys punctata andersoni* populations in Dhubri district. The key findings include: AT-rich cytochrome b gene sequences with high adenine content, Conserved and variable regions indicating balanced evolutionary stability and divergence, Significant restriction site polymorphism revealing genetic diversity, Formation of three distinct genetic clades among populations. These results demonstrate that cytochrome b is highly effective for studying genetic variation and population structure in freshwater turtles. The molecular insights generated here can guide conservation planning and species management.

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