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Sequence Divergence and Phylogenetic Status of Four Species of Testudinidae Family

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Abstract

Phylogenetic study is most important approach to understand the evolutionary history of the species and Mitochondrial DNA (mtDNA) specially ribosomal RNA have been robustly used for this application due to its slow evolutionary rate in mitochondrial genome. Sequence divergence and phylogenetic status of four species of Testudinidae family was estimated and constructed using 12S rRNA mitochondrial gene to develop the accurate methodology species identification and phylogenetic study which will helps in the conservation, forensic and as well as in management plans of endangered species. Sequence divergence between the four species of Testudinidae family were ranges from 0.021 to 0.064 and within the species was 0.0003 in T. horsfieldii, where as in rest of three species sequence divergence was 0.000. Species specific nucleotides were also reported in the four species of tortoise for species identification.

Keywords: Tortoise; mtDNA; PCR; Sequence divergence; Species specific nucleotide; Phylogeny

Introduction

Molecular phylogenetic analysis is more reliable method for build a phylogenetic trees than the particular other methods; these methods have been used by many authors [1-3]. Genetic comparisons are helpful to understand ancestry of related species at higher taxonomic levels. Consequential morphological characteristics can be difficult to determine ancient divergences of the species, [4-6]. Phylogenetic suggestion must consider the population processes that produced the gene tree by three methods, first methods applied by mathematical population genetic methods, by calculating the conditional probabilities of genealogies given different species trees or population histories [7-9] second, by estimating population genetic parameters using methods that incorporate into the analysis both the large variance inherent in the coalescent process as well as improbability about the genealogical reconstruction [10,11] and third, by calculating outline statistics that do not use the gene tree for parameter estimation [12,13]. For instance, pairwise divergence time has been used to infer sister relationships among a group of taxa. 12S rRNA is highly conserved and applied to illustrate the phylogeny of higher categorical levels such as phyla or subphyla [14]. Species identification is one of the most important goal in the molecular biology, forensic and conservation biology and various mtDNA have used for this purpose [15,16]. The aim of the present analysis is to provide a preliminary insight into the sequence divergence and phylogenetic status of four species of Testudinidae family using 12S rRNA. This study is also helpful into find out the unique SNPs (Single Nucleotide Polymorphism) for species identification.

Material and Methods

All sequence data were obtained from GenBank on the NCBI website (http://www.ncbi.nlm.nih.gov/) for the comparisons of 12S rRNA gene, of four species of Testudinidae family. Sequences were aligned using BioEdit software with Clustal W program. Sequence divergence and phylogenetic trees were constructed for 12S rRNA sequence alignments using the Maximum Parsimony in the Molecular Evolutionary Genetics Analysis (MEGA) software package version 5.0. [17]. Nucleotide diversity were calculated using DnaSP v5 [18].

Result and Discussion

Based on the 12S rRNA analysis, sequence divergence in the all four tortoise species viz., *T. kleinmanni*, *T. marginata T. graeca graeca* and *T. horsfieldii* were ranged from 0.021 to 0.064, whereas lower sequence divergence were between the *T. kleinmanni* and *T. kleinmanni* (0.021) and maximum divergence were between the *T. graeca graeca* and *T. horsfieldii* (0.064). Sequence divergence estimated within the four tortoise species was (0.000) except T.horsfieldii (0.003) (Table 1). In this study nucleotide diversity in *T. kleinmanni* and *T. graeca graeca* were 0.00036 and 0.01020 respectively. In the *T. graeca graeca* nucleotide diversity estimated high rather then the *T. kleinmanni*. Nucleotide diversity was found less due to 12S rRNA is much conserved region and hence not suitable for to analyze genetic diversity within the species but can be used for species identification and to understand the genetic diversity across the different taxa.

Based on phylogenetic tree analysis, *T. kleinmanni* is very closer to the *T.marginata* with higher bootstrap value of 89 then the *T. graeca graeca* and *T. horsfieldii* having bootstrap value of 67 (Figure 1). Unique SNPs for species identification were observed at specific nucleotide positions in the four tortoise species (Table 2). But in the

	Sequence divergence between the group				Within group
	T. kleinmanni	*T. marginata*	*T. graeca graeca*	*T. horsfieldii*	
T. kleinmanni					0.000
T. marginata	0.021				0.000
T. graeca graeca	0.060	0.060			0.000
T. horsfieldii	0.051	0.061	0.064	0.000	0.003

Table 1: Sequence divergence of mitochondrial 12S rRNA between the turtle species of Testudinidae family.

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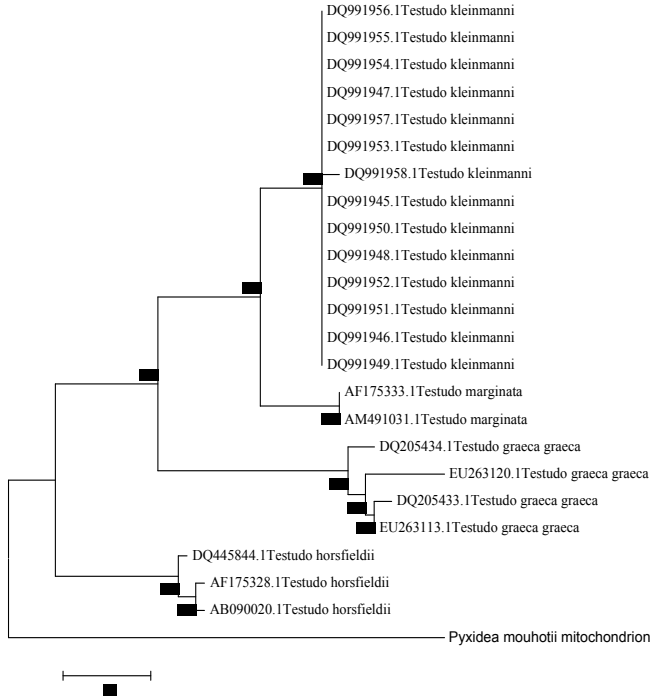

Figure 1: Maximum parsimony (M-P) tree constructed of 12S rRNA with four turtle species.

5	5	D	6	7	7	7	7	7	7	7	7	7	7	
3	5		7	0	2	2	3	3	4	4	5	6	6	
1	3		8	8	1	9	4	9	8	9	6	0	1	
T.kleinmanni	T	A		T	G	C	C	T	A	G	C	G	A	C
T.kleinmanni	T	A		T	G	C	C	T	A	G	C	G	A	C
T.kleinmanni	T	A		T	G	C	C	T	A	G	C	G	A	C
T.kleinmanni	T	A		T	G	C	C	T	A	G	C	G	A	C
T.marginata	T	A		T	G	C	C	T	A	G	C	G	A	C
T.marginata	T	A		T	G	C	C	T	A	A	C	G	G	C
T. graeca graeca	T	A		T	G	C	C	T	A	A	C	G	G	C
T. graeca graeca	T	T		T	C	G	T	C	A	G	T	A	A	T
T. graeca graeca	T	T		T	C	G	T	C	A	G	T	A	A	T
T.horsfieldii	C	A	C	C	A	A	C	T	G	G	C	G	A	C
T.horsfieldii	C	A	C	C	A	A	C	T	G	G	C	G	A	C

Table 2: Species specific nucleotide position in 12S rRNA gene. Nucleotide positions are as the sequence of *T. kleinmanni* (Accession no. NC007699.1).

species *T. kleinmanni* and *T. marginata* no species specific nucleotide were observed due to very closely related species or 12S r RNA have very conserved sequence in species. This gene can be used for species identification through localizing species specific nucleotide or SNPs.

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