INTRODUCTION

Squamate reptiles number approximately 8000 living species and are a major component of the world’s terrestrial vertebrate diversity. However, the established relationships of higher-level groups have been questioned in recent molecular analysis. Expanded molecular data include DNA sequences, totaling 6192 base pairs from nine nuclear protein coding genes viz. c-mos, RAG1, RAG2, R35, HOXA13, JUN, α-enolase, amelogenin and MAFB, and for 19 taxa representing all major lineages (Vidal and Hedges 2005).

In the early part of 2004, the first molecular study was done by Vidal and Hedges to sample major squamates lineage, including all lizards, amphisbaenian families and most snake families, where they discovered some unconventional relationship. With sequences of two nuclear genes [c-mos and RAG1], snakes were not found to be nested with Anguimorpha. In addition, a close relationship was established between lacertid lizard and amphisbaenians. The classical association of snakes with either the varanid lizards or amphisbaenians was therefore, rejected. Moreover, the classical squamates dichotomy between Iguania and Scleroglossa was also not supported.

In the same year 2004, a second molecular study carried out by Townsend et al., reached similar conclusions with additional sequence data and support.

Evolutionary rates in DNA sequence exhibit heterogeneities of several types. Variable substitution rates are apparent as follows: - Across nucleotide positions within a codon, among non-homologous genes with a lineage, among classes of DNA within a genome, among genome within an organismal lineage.

Under the neutral mutation theory, the heterogeneities across nucleotide sites, genes, and genomes within a phylogenetic lineage are interpreted to reflect differential levels of functional constraint across DNA sequences, possibly in conjunction with variation in the underlying rate of mutation.
The classical phylogeny of living reptile pairs, crocodilians with birds, tuitas with squamates, and places turtles at the base of the tree. However, new evidence from two nuclear genes and analyses of mt. DNA and 22 additional nuclear genes, join crocodilians with turtles and places the latter at the base of the tree. Morphological and paleontological evidence for this molecular phylogeny is unclear. Molecular time estimate support a Triassic origin for the major group of living reptile.

The phylogenetic analyses of the present study will be discussed and compared with the previous morphological analysis.
**HISTORICAL BACKGROUND**

Markert and Moller (1959) discuss the multiple forms of enzymes: tissue, ontogenetic and species-specific patterns. Shaklee et al., (1973) - Specialized lactate dehydrogenises isozymes-the molecular and genetic basis for the unique eye and liver LDHs of teleost fish.


mitochondrial genomic sequence was done by Macey et al., (2004). Lee (2005) observed squamate phylogeny, taxon sampling, and data congruence. Vidal and Hedges (2005) gave the molecular phylogeny of squamates reptiles inferred from nine nuclear protein-coding genes.


OBJECTIVES

However, there have been various efforts to study relationship among reptiles elaborate in-depth studies about phylogenetic relationship among reptiles in the semi-arid of Agra region have been hitherto neglected. Therefore, still no general statement on phylogenetic relationship among reptiles could be standardized. This inadequacy necessitates a detailed study on these reptiles. The approach to the problem will be made in the following manner:

(1) To study the phylogeny through Molecular marker.

(2) To study phylogeny through isozymal analysis.
MATERIALS AND METHODS

MATERIALS-

Lizards, Snakes, Turtle would be procured from the different parts of Agra region. The species, which are selected, lizard - *Calotes versicolor*; Snake – *Typlops & Ptyas mucosus* and Turtle - *Kachuga tentoria*.

METHODS-

A) Molecular marker analysis

B) Isozymal analysis

(A) Molecular marker analysis-

In molecular analysis we will use the mitochondrial DNA because it is composed of highly conservative gene as, they do not undergo recombination process. In mitochondrial DNA, we have selected the 16S rRNA gene of Lizard, Snake, and Turtle. Total genomic DNA extracted from muscle, liver and blood sample from the standard phenol/chloroform protocol (Sambrook *et al.*).

16SrRNA gene analysis-

The primers 16S F (light chain 5’ CGC CTG TTT ATCAA AAC AT-3’) 16S R (Heavy chain 5’ CCG GTC TGA ACT CAG ATC ACGT-3’) of Palumbi *et al.*, (1991) will be used to amplify a section of mitochondrial 16S ribosomal RNA gene.

Amplification reaction performed by PCR condition, [ initial denaturation at 94°C and denaturation at 94°C, annealing at 55°C with extention at 72°C], (Vences *et al.*, 2000). To detect PCR product, reaction mixture will be subjected to of 1% low melting agarose gel. The gel will be visualized under UV.

Sequencing of 16S rRNA gene-
The purpose of sequencing will be to determine the accuracy of PCR and consequently analyzing the sequences. PCR amplification and sequence reaction will be carried out for all three animals. Sequenced product will be analyzed by restriction analyses.

**Phlogenetic analysis-**

Sequence will be read out by autoradiogram. Sequences will be obtained analyzed for closest homology database search by standard BLAST search provided by NCBI. The sequence will be aligned with default parameters using Bioedit, MEGA-4, Treev32, chromaslite201, Network softwares.

Phylogenetic tree will be constructed based on Kimura (1980). After the tree construction, analysis will be done by Vector NTI, Advance II, Invitrogen.

**(B) Isozymal analysis-**

Isozymes have proven to be exceptionally valuable in comparison and characterization of multilocus protein system in species. Studies of multi-molecular form of proteins are also important not only for determining interspecific relationships but also in relating genetically controlled variant among population of the same species.

In the present study, the Isozymal analysis of four soluble proteins viz. Lactate dehydrogenase (LDH), Malate dehydrogenase (MDH), Alcohol dehydrogenase (ADH) and Glucose 6phosphate dehydrogenase (G6PDH) while a non-soluble protein esterase will be carried out.

The techniques of separating isozyme and iso-allele forms of protein by electrophoresis in starch and acrylamide gels will purify four vital dehydrogenase and non-specific estrase of all the three animals. After electrophoresis, the gel will be stained with coomassie brilliant blue. The above mentioned isozyme are used because the regulation of these enzymes expression, including tissue specificity and intensity of expression (i.e. qualitative and quantitative) will notify us the main characters of the target species.