SUMMARY

The present study was carried out on 8 species of termites of three genera namely *Heterotermes*, *Microtermes* and *Odontotermes* collected from different locations of Haryana region. Phylogenetic relatedness was calculated amongst these by analysis of two mitochondrial ribosomal genes i.e. 12S and 16S rRNA.

**12S rRNA**

- Partial fragment of 12S rRNA was amplified using universal primers which yielded approx 410bp fragment.
- Sequences data was submitted to NCBI and accession numbers were obtained.
- All the species showed a higher AT content (Average 65.35%) as compared to GC content (Average 34.64%).
- Multiple sequence alignment using Clustal omega showed Transition/Transversion Ratio (R/ratio of the number of transitions to the number of transversions for a pair of sequences) of 2.6, which indicated presence of more transitions over transversions.
- Phylogenetic analysis was done using MEGA 6 software.
- Pairwise genetic distance values (Kimura 2 parameter) were calculated which varied from 0.004 to 1.152 among various sequences under study.
- Phylogenetic tree was drawn on the basis of multiple sequence alignment for twenty one species of termites. Two major clusters were formed. Various species of *Odontotermes* genus i.e. *O. assmuthi*, *O. bhagwati* (two populations), *O. bruneus*, *O. ceylonicus*, *O. escherichi*, *O. gurdaspurensis*, *O. hainanensis*, *O. mathuri*, *O. obesus* (two populations), *O. parvidens* and *O. redemanii* formed a single cluster, along with two species of genus *Microtermes* i.e. *M. mycophagus* and *M. unicolor*. *Coptotermes*, *Heterotermes* and *Reticulotermes* genera formed another cluster.
16S rRNA

- Genomic DNA using universal insect primers for partial fragment of mitochondrial 16S ribosomal RNA. Sequencing yielded approximately 430 bp fragment in termite species under study.
- The nucleotide data was biased as A+T content varied from 61.91 % to 63.69%, whereas G+C content varied from 36.31% to 38.09%.
- Multiple sequence alignment using Clustal Omega showed that more transitions were found in the present data as compared to transversions.
- Transition/Transversion Ratio (R/ratio of the number of transitions to the number of transversions for a pair of sequences) was 3.6.
- Pairwise genetic distance values using Kimura 2 parameter. The K2P distance varied from 2.209 to 0.003 among various sequences under study.
- Maximum likelihood and Neighbor joining trees drawn using MEGA 6 software revealed that individuals belonging to same genus group close, showed minimum divergence between them as all the twelve species of Odontotermes were present in a single cluster. The precise grouping of the different Isoptera species in the present study is in accordance with the geographical location and it clearly depicts the utility of such genetic tool in establishing the overall picture of relationship and taxonomic positioning of the lesser known species.

Significance

- The sequencing obtained may be used as a template to design specific primers or to construction molecular markers to differentiate between members of a family.
- Molecular data in association with morphological data can be used to solve the taxonomical problems and study the extent of genetic diversity.
- The data can be used to know degree of genetic relatedness among various species all around the world and to produce a census of all biological diversity and modernize taxonomy.
• The sequencing obtained may be used as reference data for further studies in Global Bioidentification System.
• The information obtained from the study can be used for molecular characterization and comparative studies between sequences of various termite species.
• The data obtained can be used for further evaluation of mitochondrial ribosomal genes of termites.
• Sequencing data obtained can be used for construction of phylogenetic trees and hence, to know the evolutionary history of termites and whether the species are native or exotic.
• The information generated in the present study can be used high resolution of ambiguous species in combination with other genes such as COI, COII, NDI, cytb etc.