Diversity of Subterranean Termites in South India Based on COI Gene

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Abstract

The diversity of subterranean termites collected from various locations in South India were characterised based on the COI gene using specific primers. Sequence analysis and divergence among the species was assessed. Genbank accession numbers were obtained for the different species. Phylogenetic tree based on neighbour-joining method was drawn on the basis of multiple sequence alignment, which revealed clustering of individuals according to the genera. Among the species, Odontotermes longignathus was more prevalent than others. The utility of COI gene to study the systematics of termites, their evolution and relatedness that would have implication on their management is discussed.

Keywords: Subterranean termites; COI gene; Genbank; Phylogenetic tree; Odontotermes longignathus

Introduction

Termites (Isoptera) represent up to 95% of soil insect biomass [1,2] show an elaborated morphology and complex behaviour. The family Termitidae is considered to be the most evolved group with about 85% of all known genera and nearly 70% known species Ohkuma et al. Termites are considered as the most abundant invertebrates that are classified in about 280 genera, and over 2800 species within 14 subfamilies [1,3-5]. In India about 300 species within seven families have been reported [6]. The subterranean termites which are of economic importance Wang et al. [7] to agriculture are mostly found in temperate climates Groves et al. [8]. Termite on systematics was exclusively based on taxonomical identification based morphological characters of individuals belonging to various castes (e.g., soldiers or workers) Kambhampati and Eggleton [9,10] which is of industrial value.

Termites are the serious pests of agricultural and horticultural crops that mainly destroys the roots and above ground parts and feed on paper, wood and timber Murthy et al. [10]. They are found to considerably damage the artificial structures and commodities due to their large colony size, varying feeding preferences and nesting behaviour Wang et al. [7]. It has been estimated that worldwide the overall cost annually for the control of damage caused by termites is more than $20 billion NY [13].

The understanding of termites in various biological processes is extremely limited regardless of their importance in agriculture and hence it is very important to understand their biology and ecology, which greatly relies on accurate species identification Singla et al. [14]. Termite on systematics was exclusively based on taxonomical identification based morphological characters of individuals belonging to various castes (e.g., soldiers or workers) Kambhampati and Eggleton [3]. Nevertheless, the caste differentiation, eusocial behaviour, varying physiological functions and crypto-biotic structure had contributed to ambiguities in their morphological identification [7,10] and species diagnosis has become a challenging task Kirtin et al. [15].

The use of molecular methods which are fast and reliable, complementary to the morphological identification [2,16], are helpful in estimating evolutionary relatedness between the species Singla et al. [14]. Studies on mitochondrial genome sequences such as the AT-rich region, 16S rDNA and cytochrome oxidase genes have shown an efficient alternative for species identification and phylogenetic studies [2,17,16,19-23,35]. The mitochondrial DNA is more abundant as the mitochondrial genes evolve more rapidly, than the nuclear genome Wang et al. [7], therefore at species level mitochondrial DNA is more suitable Masters et al. [24] and various other regions also can be sequenced [8,25,26].

Cytochrome c oxidase subunit I gene (COI) is one of the three mitochondrial DNA (mtDNA) encoded subunits of respiratory complex IV. It is a key enzyme in aerobic metabolism. COI gene is the most conservative protein-coding gene in the mitochondrial genome. Since, the mutation rate in COI gene is fast enough it can differentiate precisely the closely related termite species and assess their phylogeny, in understanding the evolutionary relationships, the gene has been extensively used to understand the diversity and genetic relatedness.

In the present study, we characterized twelve species of termites of family termitidae obtained from various locations in South India based on the mitochondrial COI gene and studied their phylogenetic relation.

Materials and Methods

Collection and identification of termite samples

Termite specimens collected from different locations in South India were preserved in absolute alcohol and stored at -80°C at the Division of Molecular Entomology, NBAIR-ICAR, Bangalore, India. Taxonomical identification of these specimens was done at the Division of Entomology Indian Agricultural Research Institute, New Delhi, Institute of Wood Science Technology Bangalore, and Centre for Insect taxonomy, University of Agricultural Sciences, Bangalore.

Isolation of genomic DNA

Isolation of genomic DNA was carried out using Qiagen DNeasy blood tissue kit following the established protocols. Termite samples were stored in absolute alcohol, were first washed well with distilled

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water followed by dissection of the head region. The dissected head was dried and collected in 1.5ml eppendorf tube to which 180 µl of ATL buffer was added and homogenised using micro pestle. 20 µl of proteinase K was added to it and mixed methodically by vortexing and then incubated in water bath at 56ºC overnight. 100 µl of AL buffer was added and kept further for incubation at 56ºC for 10 minutes, after incubation 100 µl of 100% ethanol was added to the eppendorf tube and vortexed. The solution was then transferred into the mini spin columns with silica member that binds the genomic DNA. The columns were then centrifuged at 8000 rpm for 5 minutes and the flow-through collected in a tube was discarded. The column was then further given two washes with two different buffers present in kit i.e., AW1 and AW2 and centrifuged at 8000 rpm for 5 minutes simultaneously. These columns were then transferred to a new fresh 1.5 µl eppendorf tubes in order to elute out the DNA bound to the Silica member in the column. The elution was carried out by pipetting 100 µl of sterile water to the columns, centrifuged at 8000 rpm for 5 minutes. The DNA was then checked at 1% agarose gel and stored in 4ºC until PCR was done.

Quantification of DNA by nanodrop spectrophotometer

The extracted DNA was quantified using nanodrop spectrophotometer and the amount of DNA present in the samples was identified. Since, all the organic compounds show a characteristic absorption, the nitrogenous bases in DNA show a strong absorption at a wavelength of 260 nm.

PCR amplification of CO1 gene

The DNA obtained was then amplified for a portion of mitochondrial CO1 gene fragment, using the universal primers CO-1 F 5’ GGTCAAAAATCATAAAGATATTGG 3’ and CO-1 R 5’ TAACTTCAGGCTGACCAAAAAATCA 3’. The amplified CO1 product was sequenced at M/S. Eurofins Pvt Ltd, Bangalore. The sequence data was submitted to NCBI genbank and the accession numbers were obtained for the populations of termites (Table 1).  

Phylogenetic analysis

Phylogenetic tree was constructed using neighbour-joining method and the evolutionary distances were computed using p-distance method with a boot strap consensus of 1000 replicates Tamura and Nie [28]. Constructed phylogenetic tree was visualised using tree viewer program.

Results and Discussion

The genomic DNA was collected from 12 populations of termites and the CO1 gene was characterized, using the universal primers CO-1 F 5’ GGTCAAAAATCATAAAGATATTGG 3’ and CO-1 R 5’ TAACTTCAGGCTGACCAAAAAATCA 3’. The amplified CO1 product was sequenced at M/S. Eurofins Pvt Ltd, Bangalore. The sequence data was submitted to NCBI genbank and the accession numbers were obtained for the populations of termites (Table 1).

Nucleotide analysis

The complete gene analysis of nucleotide sequence for each of the collected termite species showed a considerably high percentage of A+T base composition content, with an average composition of A+T=54.88% and G+C=45.11% of various species (Table 2).

The variation in A+T (%) among the different populations was 4.63% and 5.68% with respect to G+C (%). The populations from Attur, Sivaganaga, Thrissur and Marthalli had the least variation in the base composition of A+T (%) (54.17 to 54.89) and (45.28 to 45.83), respectively. The estimated Transition/Transversion bias (R) is 1.65. Substitution pattern and rates were estimated under the Tamura-Nei [28] model. The nucleotide frequencies were A=32.20%, T/U=24.96%, C=27.27%, and G=15.57%. A tree topology was automatically computed for estimating the ML values W et al. [29]. The maximum Log likelihood for this computation was -1053.298, which involved analysis of 12 nucleotide sequences.

Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 206 positions in the final dataset. Evolutionary analyses were conducted in MEGA6 Szalanski et al. [30] software.

Divergence and percent identity

The divergence and percent identity was calculated using MEGA 6 software based on the sequence alignment. The overall average was found to be 0.06 for the total 48 nucleotide sequences. The number of base differences per site from between sequences is shown. Standard error estimate(s) are shown above the diagonal Tamura et al. [31]. There were a total of 190 positions in the final dataset.

Sequence analysis and data interpretation

Chromatograms were edited in order to remove the ambiguous bases. These edited sequences were then aligned using Basic Local Alignment Search Tool (BLAST), with the sequences of same or related genera retrieved from the nucleotide database (PUBMED) of National Centre for Biological Information (NCBI). The CO1 nucleotide sequences of the termite species included in our present study were aligned and compared with the species obtained from PUBMED, using CLUSTAL W alignment Thompson et al. [27].
Odontotermes longignathus population from Attur, Dasarahalli and Marthahalli had negligible divergence with the habitat. Population of termite populations of species similarity across the geographical barriers did not vary, notwithstanding the habitat. Therefore the occurrence of termite populations of similar trend (Figure 2). Our observations are in broad conformity among the collections could be due to the reports by earlier workers [6,10]. The greater occurrence of different species indicated from Mysore (Hypotermes xenotermitis) and Marthahalli indicated low divergence of 0.03% Tamura et al. [31]. Therefore the occurrence of termite populations of species similarity across the geographical barriers did not vary, notwithstanding the habitat. Population from Mysore (Hypotermes xenotermitis) and Marthahalli indicated similar trend (Figure 2). Our observations are in broad conformity with the reports by earlier workers [6,10]. The greater occurrence of Odontotermes longignathus among the collections could be due to the widest niche breadth [6,31,33].

**Phylogenetic analysis**

A total of 48 strains were used for the phylogenetic analysis. The phylogenetic tree built using Hypotermes xenotermitis, Hypotermes makhamensis, Odontotermes longignathus, Nasutitermes octopilis, Odontotermes escherichi, Hypoteres sp were divided into two major clusters and three smaller clusters. The phylogenetic tree displayed in (Figure 3), shows that all the species belonging to Hypotermes xenotermitis were clubbed together with bootstrap score of 97, 53, 1 and 68. All the Hypotermes xenotermitis collected from Ratnagiri, India were grouped together with the bootstrap score of 97. The first major cluster was further divided into 6 sub-clusters. In all the 6 sub-clusters only the strains collected from Ratnagiri, India were clubbed together.

The Hypotermes xenotermitis strains collected from Pune, India formed two clusters. In one cluster, most of the Hypotermes xenotermitis species collected from Pune, India were grouped together with bootstrap score of 53. In another cluster, only four sequences with the ID of KT879848, KT879846, KT879847 and KT879845 were grouped together with the bootstrap score of 63. These four gene sequences of Hypotermes xenotermitis species collected from Pune showed divergent from the other gene sequences collected from the same place. Apart from Hypotermes xenotermitis strains, four Hypotermes makhamensis strains were also collected from Pune and Sivaganga. All the four sequences along with the Hypotermes xenotermitis strain collected from Ratnagiri, India were grouped together and formed a cluster. The two strains (KT879848 and KT879846) collected from Pune were closely related and grouped together with bootstrap score of 87, whereas the two strains collected from Sivaganga were also closely related and grouped together to form a sub-cluster with bootstrap score of 98. The Hypotermes xenotermitis strain collected from Pune, India formed two clusters. In one cluster, most of the Hypotermes xenotermitis species collected from Pune, India were grouped together with bootstrap score of 53. In another cluster, only four sequences with the ID of KT879848, KT879846, KT879847 and KT879845 were grouped together with the bootstrap score of 63.

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**Sl.no** | Place of collection | Code | Latitude | Longitude | Molecular identity | GenBank Accession no.
---|---|---|---|---|---|---
1 | Mysore | Mys TE-2 | 12.296° | 76.639° | Hypotermes xenotermitis | KT224387
2 | Attur | Attur TE-7 | 13.099° | 77.568° | Odontotermes longignathus | KT224388
3 | PKP | PKP TE-1 | 12.38° | 75.05° | Odontotermes escherichi | KT224389
4 | Sivaganga | Sivaganga TE-1 | 9.843° | 78.481° | Odontotermes longignathus | KT224390
5 | Marat | Marat TE-2 | 12.95° | 77.70° | Hypotermes sp. | KT224391
6 | Attur | Attur TE-8 | 13.099° | 77.568° | Odontotermes longignathus | KT254244
7 | Sivaganga | Sivaganga TE-2 | 9.843° | 78.481° | Hypotermes makhamensis | KT274763
8 | Sivaganga | Sivaganga TE-3 | 9.843° | 78.481° | Hypotermes makhamensis | KT274765
9 | Marat | Marat TE-1 | 12.95° | 77.70° | Hypotermes xenotermitis | KT274764
10 | KAU, Thrissur | BCCP59/2015 | 10.528° | 76.214° | Odontotermes longignathus | KT719274
11 | Dasarahalli | Dast-1 | 13.096° | 77.835° | Odontotermes longignathus | KM015486
12 | Ooty | Ooty-TE-2 | 11.00° | 78.00° | Nasutitermes octopilis | KM857478

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**Table 1:** Place of collection of Termite species and their GenBank accession numbers.

**Table 2:** A+T and G+C content (%) for partial COI of the different termite species.
Figure 2: Matrix showing the divergence and percent identity among the 12 species under study and 36 species of termites retrieved from the database (out-group taxa).

| Percent identity | divergence |
|------------------|------------|
| longignathus     | collected from DAST were formed a single clade which was appeared to be very divergent from other strains used to construct the phylogenetic tree. Overall, the phylogenetic tree analysis suggested that the Hypotermes xenotermitis strains collected from Ratnagiri were appeared to be closely related with the Hypotermes xenotermitis strains collected from Pune, India. The occurrence of different species of termites could be attributed |
Figure 3: Phylogeny of some South Indian termites
to varied ecology, food availability, geography and the dispersed pattern [20]. Knowledge of their diversity, distribution and understanding evolutionary relationships would provide insights to tailor effective management strategies (Table 3) [10,14,32,34-37].

| Sl.no | Organism name |
|-------|---------------|
| 1     | KM015486_Odontotermes_longignathus_strain_DAST-1 |
| 2     | KM657478_Nasutitermes_octotipis_strain_Ooty-TE-2 |
| 3     | KT224387_Hypotermes_xenotermitis_strain_Mys_TE-2 |
| 4     | KT7724955_Hypotermes_xenotermitis_strain_PPPU-5** |
| 5     | KT879849_Hypotermes_xenotermitis_strain_PPPU-20** |
| 6     | KT879849_Hypotermes_xenotermitis_strain_PPPU-15** |
| 7     | KT879849_Hypotermes_xenotermitis_strain_PPPU-13** |
| 8     | KT879849_Hypotermes_xenotermitis_strain_PPPU-12** |
| 9     | KT879849_Hypotermes_xenotermitis_strain_PPPU-11** |
| 10    | KT879849_Hypotermes_xenotermitis_strain_PPPU-10** |
| 11    | KT879849_Hypotermes_xenotermitis_strain_PPPU-9** |
| 12    | KT879849_Hypotermes_xenotermitis_strain_PPPU-6** |
| 13    | KT879849_Hypotermes_xenotermitis_strain_PPPU-5** |
| 14    | KT879849_Hypotermes_xenotermitis_strain_PPPU-19** |
| 15    | KT879849_Hypotermes_xenotermitis_strain_PPPU-18** |
| 16    | KT879849_Hypotermes_xenotermitis_strain_PPPU-17** |
| 17    | KT879849_Hypotermes_xenotermitis_strain_PPPU-16** |
| 18    | KT224388_Odontotermes_longignathus_strain_Attur_TE-7 |
| 19    | KT224389_Odontotermes_escherichii_strain_PKP_TE-1 |
| 20    | KT224391_Hypotermes_sp._Marat_TE-2 |
| 21    | KT254244_Odontotermes_longignathus_strain_Attur_TE-6 |
| 22    | KT274763_Hypotermes_makhamensis_strain_Sivanganga_TE-2 |
| 23    | KT887717_Hypotermes_xenotermitis_strain_PPPU-20** |
| 24    | KT887717_Hypotermes_xenotermitis_strain_PPPU-19** |
| 25    | KT887717_Hypotermes_xenotermitis_strain_PPPU-18** |
| 26    | KT887717_Hypotermes_xenotermitis_strain_PPPU-17** |
| 27    | KT887717_Hypotermes_xenotermitis_strain_PPPU-16** |
| 28    | KT887717_Hypotermes_xenotermitis_strain_PPPU-15** |
| 29    | KT887717_Hypotermes_xenotermitis_strain_PPPU-14** |
| 30    | KT887717_Hypotermes_xenotermitis_strain_PPPU-13** |
| 31    | KT887717_Hypotermes_xenotermitis_strain_PPPU-12** |
| 32    | KT887717_Hypotermes_xenotermitis_strain_PPPU-11** |
| 33    | KT274764_Hypotermes_xenotermitis_strain_Marat_TE-1 |
| 34    | KT879843_Hypotermes_xenotermitis_strain_PPPU-14** |
| 35    | KT879843_Hypotermes_xenotermitis_strain_PPPU-3** |
| 36    | KT879843_Hypotermes_xenotermitis_strain_PPPU-2** |
| 37    | KT887707_Hypotermes_xenotermitis_strain_PPPU-10** |
| 38    | KT887707_Hypotermes_xenotermitis_strain_PPPU-9** |
| 39    | KT887707_Hypotermes_xenotermitis_strain_PPPU-8** |
| 40    | KT887707_Hypotermes_xenotermitis_strain_PPPU-7** |
| 41    | KT887707_Hypotermes_xenotermitis_strain_PPPU-6** |
| 42    | KT887707_Hypotermes_xenotermitis_strain_PPPU-5** |
| 43    | KT887707_Hypotermes_xenotermitis_strain_PPPU-4** |
| 44    | KT887707_Hypotermes_xenotermitis_strain_PPPU-3** |
| 45    | KT887707_Hypotermes_xenotermitis_strain_PPPU-2** |
| 46    | KT887707_Hypotermes_xenotermitis_strain_PPPU-1** |
| 47    | KT274765_Hypotermes_makhamensis_strain_Sivanganga_TE-3 |
| 48    | KT719274_Odontotermes_longignathus_strain_BCCP_59_Thrissur |

Note: where ** marked indicate sequences from the NCBI

Table 3: Chronological order of termite species as indicated in the similar/fly matrix.
References

1. Ohkuma M, Yuzawa H, Amornsak W, Somnunwat Y, Takematsu Y, et al. (2004) Molecular Phylogeny of Asian termites (isoptera) of the families Termitidae and Rhinotermitidae based on mitochondrial COII sequences Mol Phylogenet Evol 31: 751-710.

2. Roy V, Demanche C, Livet A, Harry M (2006) Genetic differentiation in the soil-feeding termite Cubitermes sp. affinis subarquatus: occurrence of cryptic species revealed by nuclear and mitochondrial markers. BMC Evolutionary Biology 6:102.

3. Kambhampati S, Eggleton P (2000) Phylogenetics and taxonomy. In: Abe T, Bignell DE, Higashi M (eds). Kluwer Academic Publishing, Netherlands pp: 1-23.

4. Daegan JG, Alfried PV, Eggleton P (2007) A comprehensive phylogenetic analysis of termites (isoptera) illuminates key aspects of their evolutionary biology. Mol Phylogenet Evol 44: 953-967.

5. Eggleton P, Tayasu I (2001) Feeding groups, lifetypes and the global ecology of termites. Ecol Res 16: 941-967.

6. Kumar D, M Pardeshi (2011) Biodiversity of Termites in Agro-ecosystem and Relation between their Niche Breadth and Pest Status. Journal of Entomology and Biology 6:102.

7. Murthy S, Lakshmi Sharma V, Lakshmi R, Kumari M (2013) Novel technologies for subterranean termite control. Sociobiology 70: 467-513.

8. Wang C, Zhou X, Shujun Li, Schwinghammer M, Scharf ME, et al. (2009) Survey and Identification of Termites (Isoptera: Rhinotermitidae) in Indiana Ann Entomol Soc Am 102: 1029-1036.

9. Chen JH, Pan D, Groves C (2006) Molecular phylogeny of Nycticus eburneus inferred from mitochondrial genomes. Int J Primatol 27: 1187-1200.

10. Mufty S, Rajeshwari K, Ramya R, Venkatakeas SL, Jalali T, et al. (2015) Genetic diversity among Indian termites based on mitochondrial 12S rRNA gene. Euro J Zool Res 4: 1-6.

11. Pearce MJ, Waite BS (1994) A list of termite genera (Isoptera) with comments on taxonomic changes and regional distribution. Sociobiology 23: 247-259.

12. Manjula A, Satyavathi S, Gunasekaran P, Rajendran J (2011) Comparison of seven methods of DNA extraction from termitearium for functional metagenomic DNA library construction. J Sci Ind Res 70: 945-951.

13. Su NY (2002) Novel technologies for subterranean termite control. Sociobiology 40: 95-101.

14. Singla M, Lakshmi Sharma V, Chander Sobti R, Sodhi M, Kumari M (2013) Genetic Relationship Among Indian Termites Based on DNA Sequence of Mitochondrial 12S Ribosomal RNA. Gene. Int J J 2: 1-5.

15. Kirton GL (2005) The importance of accurate termite taxonomy in the broader perspective of termite management. Proceedings of the Fifth International Conference on Urban Pests. P&Y Design Network, Malaysia.

16. Virgilio M, Delatte H, Backeljau T, De Meyer M (2010) Macrogeographic population structuring in the cosmopolitan agricultural pest Bactrocera cucurbitae (Diptera: Tephritidae Mol Ecol 19: 2713-2724.

17. Jenkins TM, Dean RE, Verkerk R, Forschler BT (2001) Phylogenetic analyses of two mitochondrial genes and one nuclear intron region illuminate European subterranean termite (Isopreta: Rhinotermitidae) gene flow, taxonomy, and introduction dynamics. Mol Phylogenet Evol 20: 286-293.

18. Misra TR, Matsumoto YT (2000) Molecular phylogeny and biogeography of the nasute termite genus Nasutitermes (Isopreta: Termitidae) in the pacific tropics. Mol Phylogenet Evol 17: 1-10.

19. Austin JW, Szalanski AL, Uva P, Bagneres AG, Kence A (2002) A comparative genetic analysis of the subterranean termite genus Reticulitermes (Isopreta: Rhinotermitidae). Ann Entomol Soc Am 95: 753-760.

20. Marini M, Mantovani B (2002) Molecular Relationships among European Samples of Reticulitermes (Isoptera, Rhinotermitidae). Mol Phylogenet Evol 22: 454-459.

21. Foster BT, Anthony I, Cognato I, Roger EG (2004) DNA-Based Identification of the Eastern Subterranean Termite, Reticulitermes flavipes (Isopreta: Rhinotermitidae). J Econ Entomol 97: 95-101.

22. Kirsten AG, Lori JN, Edward LV, Michael IH (2005) Phylogenetic analyses of mtDNA sequences corroborate taxonomic designations based on cuticular hydrocarbons in subterranean termites. Mol Phylogenet Evol 35: 689-700.

23. Austin JW, Szalanski AL, Cabrera, BJ (2004) Phylogenetic Analysis of the Subterranean Termite Family Rhinotermitidae (Isoptera) by Using the Mitochondrial Cytochrome Oxidase II Gene. Annals of Entomological Society of America 97: 548-555.

24. Masters JC, Bioniotto M, Crovella S, Roos C, Pozzi L, et al. (2007) Phylogenetic Relationships Among the Lorisosidea As Indicated by Craniodental Morphology and Mitochondrial Sequence Data. Am J Primatol 69: 6-15.

25. Pan D, J, Chen, Groves C (2007) Mitochondrial control region and population genetic patterns of Nycticius bengalensis and N. pygmaeus. Int J Primatol 28 : 791-799.

26. Somura H, Hori H, Manome Y (2012) Structure Analysis of Mitochondrial DNAs of 12S rRNA, 16S rRNA, and Cytochrome Oxidase Subunit 1(COI) Regions in Slow Lorises (Genus Nycticebus) May Contribute to Improved Identification of Confiscated Specimens. ISRN 498731.

27. Thompson JD, Higgins DG , Gibbons TJ, CLUSTAL (1994) improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position specific gap penalties and weight matrix nucleic Acids Res 22: 4673.

28. Tamura KS, Nie M (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Mol Biol Evol 10: 512-526.

29. Ye W, Lee CY, Scheffrahn RH, Aleong JM, Su NY (2004) Phylogenetic relationships of nearctic Reticulitermes species (Isoperta: Rhinotermitidae) with particular reference to Reticulitermes arenicola Goellner. Mol Phylogenet Evol 30: 815-822.

30. Szalanski AL, Austin JW, Owens CB (2003) Identification of Reticulitermes spp. (Isoperta: Rhinotermitidae) by polymerase chain reaction – restriction fragment length polymorphism. J Econ Entomol 95: 514-1519.

31. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6 Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol 30: 2725-2729.

32. Deivendran (2013) Genetic Diversity in the soldiers of the Subterranean Termite Macrotermes convulsionarius (Konig, 1779) Termitidae, Isoperta. Int J Adv Lif Sci. 6: 318-324.

33. Anantharaju, T, Gurjeet Kaur, Lakshmi G (2014) Sampling and identification of termites in Northeastern Puducherry. JEZS 2: 225-230.

34. Donovan SE, Jones DT, Sands WA, Eggleton P (2000) Morphological phylogenetics of termites (Isoperta). Biol J Linn Soc 70: 467-513.

35. Thompson GJ, Miller LR, Lenz M, Crozier RH (2000) Phylogenetic analysis and trait evolution in Australian lineages of drywood termites (Isoperta, Kalotermitidae). Mol Phylogenet Evol 17: 419-429.

36. Gentz M, Daniel Rubinoff C, Kenneth Grace J (2008) Phylogenetic Analysis of Subterranean Termites (Coptotermes spp., Isoperta: Rhinotermitidae) Indicates the Origins of Hawaiian and North American Invasions: Potential Implications for Invasion Biology. Proc Hawaiian Entomol Soc 40: 1-9.

37. Abe T, Bignell DE, Higashi M (eds.) (2000) Termites Evolution, Sociality, Symbioses and Ecology. Springer Publishers, USA pp: 1-23.