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GENETIC DIVERSITY AND PHYLOGENY OF SUBTERRANEAN TERMITES FROM MAHARASHTRA USING MITOCHONDRIAL COI GENE

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ABSTRACT: Termites are eusocial insects that live in colonies with a division of labor and overlapping generations of older individuals that help raise younger individuals. Subterranean termites are associated with the ground which excavate and make passages through the soil and construct shelter tubes to cross inhospitable territory (such as concrete), to reach above ground food sources. This study investigated the genetic diversity among the 100 termite samples collected from five different regions of Maharashtra, India using Cytochrome Oxidase subunit I (COI) DNA barcoding. PCR was performed to amplify mitochondrial COI fragment which was then sequenced and analyzed. All 100 nucleotide sequences of COI gene were deposited into the GenBank database and obtained the Accession number. Neighbour Joining (NJ) and Maximum Likelihood (ML) method on the basis of multiple sequence alignment were used in Molecular Evolutionary Genetic Analysis (MEGA 7) software to define the molecular phylogeny. The results of the both phylogenetic analyses revealed almost identical topology among the species and clustering of individuals according to their genera. Based on the gene sequencing, termites were found to be *Hypotermes xenotermitis* and phylogenetic analysis showed the diversity and clustering of termites based on the geographical location as well as environmental factors.

KEYWORDS: Subterranean termites, Maharashtra, mtCOI gene, Genbank, phylogenetic tree, *Hypotermes xenotermitis*

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1. INTRODUCTION

Like ants, termites are entirely eusocial and have profound ecological significance in the tropics.

Termite species differ in their basic biology and ecology, including colony size, nesting, feeding, swarming, and reproductive behavior. Most species inhabit tropical and subtropical ecosystems, where they are particularly ample in terms of biomass and are the principal decomposers of organic matter. Termites are considered as the most abundant invertebrates that are classified in about 280 genera, and over 2800 species within 14 subfamilies [1,2,3]. In India, seven families from about 300 species have been reported [4]. The evolution of termites is of great interest, due to their diversity of diet, social structures, and phenotypes. Environmental factors such as temperature and rainfall can act as filters that limit, for instance, the distribution of species and define part of a species niche, especially over larger geographic scales [5,6]. The diversity of species is also correlated with the environmental factors and the geographical locations. The decline of taxonomic richness with increasing latitude is a generally accepted diversity trend [7,8]. Termite diversity generally declines with increased elevation. This is usually explained by the reduction in temperature, linked to metabolic rate [9]. Nucleic acid sequence information, primarily from mitochondrial DNA (mtDNA) has allowed us to make enormous strides towards resolving phylogenies during the last twenty years [10]. Mitochondrial genes evolve comparatively faster than nuclear genes making them suitable markers for analyzing relatively close relationship [11]. These genes have been widely used for phylogenetic reconstructions. Mitochondrial DNA is easy to isolate and amplify [12]. Morphological identification of termite species can be difficult as diagnostic morphological markers can be rare and are often restricted to soldiers or alates. For such taxa, sequencing of gene fragments (DNA barcoding) is now an important molecular tool widely used to elucidate phylogenetic relationships between taxa and to identify species [13]. Termites are believed to be the earliest-evolving social insects [14]. The use of molecular methods which are fast and reliable, complementary to the morphological identification [15], are helpful in estimating evolutionary relatedness between the species [16] Termites genome is a typical metazoan mitochondrial genome of around 16 kb which encodes for 37 genes, including 22 transfer RNAs (tRNAs), 13 protein-coding genes, and 2 ribosomal RNA genes [17]. Phylogenetic information for resolving deeper relationships can be provided by mitochondrial DNA gene rearrangements. 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 [18,19]. The mitochondrial DNA is more abundant as the mitochondrial genes evolve more rapidly, than the nuclear genome [20]. Cytochrome c oxidase subunit I gene (COI) is key enzyme in aerobic metabolism and one of the three mitochondrial DNA (mtDNA) encoded subunits of respiratory complex IV. 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 relationship, the gene has been extensively used to understand the diversity and genetic relatedness [21]. In the present study, we have sequenced mtCOI gene of

hundred termite samples collected from different regions of Maharashtra and studied phylogenetic relationship.

2. MATERIALS AND METHODS

2.1 Sampling and morphological identification

100 termite samples from five regions of Maharashtra viz. Aurangabad, Nashik, Ratnagiri, Kashid and Pune were collected (Figure 1). The collected specimens were identified as per Chottani, (1997), preserved in absolute ethanol and maintained at 4-8°C storage until extraction of genomic DNA.

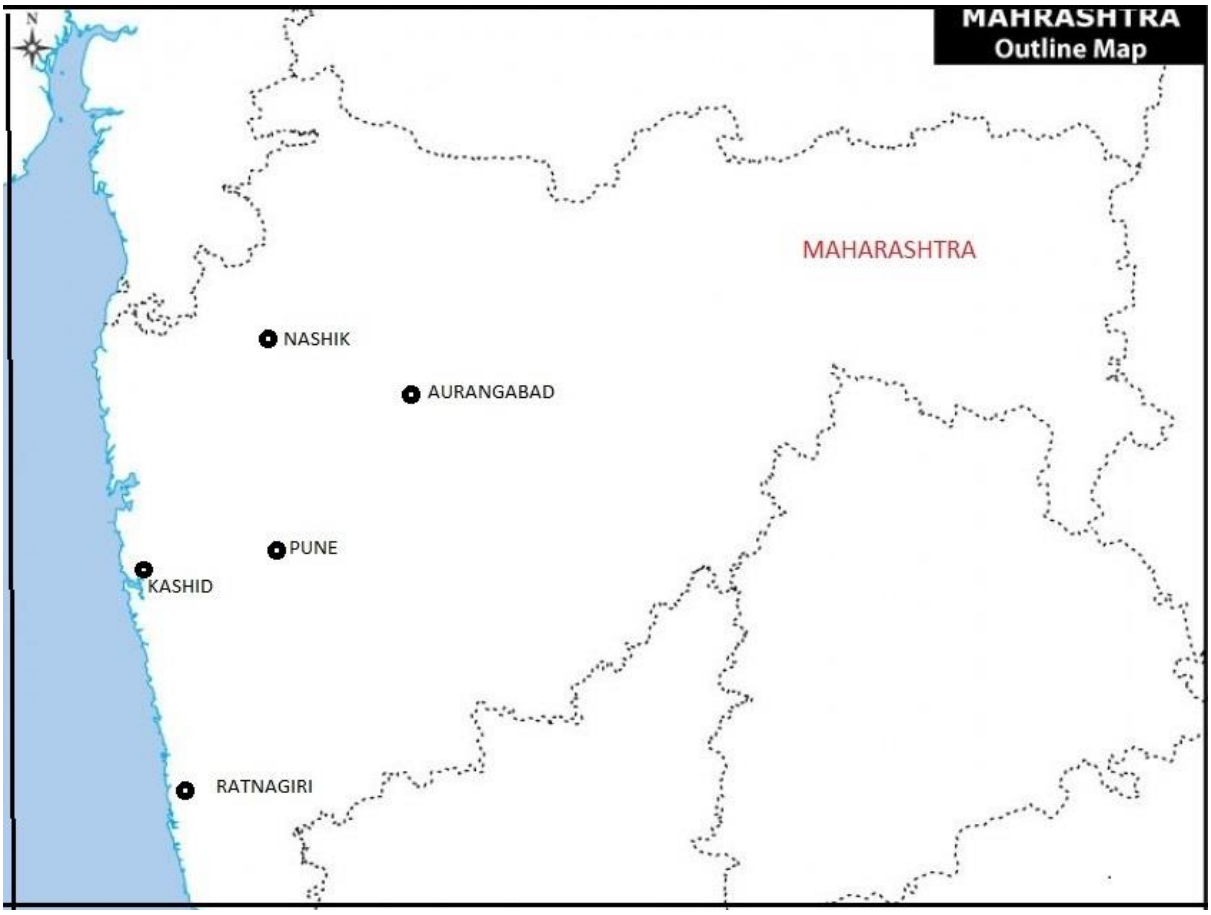

Figure 1: Map of termite collections sites in Maharashtra, India

2.2 Genomic DNA isolation

The total genomic DNA was extracted from 25 mg leg tissue using Dneasy Blood & tissue DNA kit (Qiagen, germany) following standard protocol. The tissue was dried and collected in 1.5ml eppendorf tube to which 180 µl ATL buffer was added and homogenised using micro pestle. 20 µl proteinase K was added to it and mixed thoroughly by vortexing and incubate at 56°C until the tissue was completely lysed. 200 µl AL buffer was added to the sample with the final mixing. Then 200 µl of 100% ethanol was added to the eppendorf tube, mixed again by vortexing. The solution was then transferred into the mini spin columns with silica member that binds the genomic DNA and then centrifuged at 8000 rpm for 1 min. The flowthrough collected in a tube was discarded. The column was then further given two washes with two different buffers present in kit i.e., AW1 centrifuged for 1 min at 8000rpm and AW2 and centrifuged at 14000 rpm for 3 min. Flow-through and collection tube

was discarded. These mini spin columns were then transferred to a new clean 2 ml microcentrifuge tubes and 200 µl Buffer AE was directly pipetted onto the DNeasy membrane. Incubated at room temperature for 1 min then centrifuged for 1 min at 8000rpm to elute. DNA was stored at -20°C, after checking on 1.0% agarose gel (Figure 2).

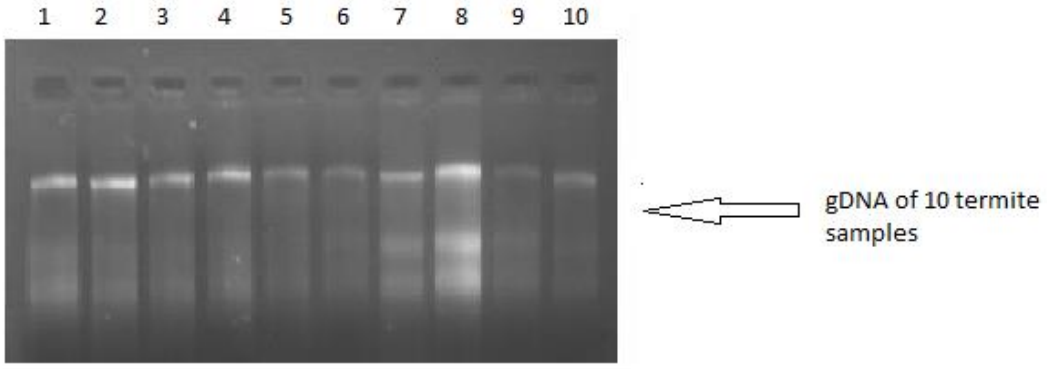

Figure 2: The Agarose (1.0%) gel electrophoresis of mitochondrial COI genePCR amplified products of termites of Maharashtra. 1-10 is the genomic DNA extracted from 10 samples. Similarly agarose gel electrophoresis of remaining 90 samples was also done.

2.3 PCR amplification of COI gene and sequencing

The extracted DNA was checked by 1% agarose gel electrophoresis using standard protocol. For amplification of mtCOI segment, the published primer pair LCO1490 and HCO2198 was used (Folmer *et. al*., 1994). The 25 µl PCR reaction mixture contains 10 pmol of each primer, 100-200 ngDNA template, 1X PCR buffer, 2.5 mM$MgCl_2$, 0.1 mM each dNTP and 1 U high fidelity Taq DNA Polymerase. The thermal profile for PCR was set as initial denaturation at 94°C for 10 min, followed by 40 cycles at 94°C for 30 sec, 46°C for 30s & 72°C for 1 min and subsequent storage at 4°C & amplification was performed using 2720 thermal cycler (Applied Biosystems). The PCR product was purified using Invitrogen PCR product purification kit. Amplified DNA was then checked on 2% agarose gel using a DNA ladder of 100 bp and the gel was visualized in BIO-RAD gel Doc XR gel documentation system. Sequencing was done bi-directionally in 48 capillary array ABI3130 (Applied Biosystems) automated sequencer following Sanger sequencing method.

2.4 Sequence analysis & Dataset preparation

The sequence data was retrieved in the form of chromatograms and submitted to genbank for obtaining the accession numbers. Chromatograms were edited in order to remove the ambiguous bases and 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 COI 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.

2.5 Phylogenetic analysis

We screened the Genbank database to acquire the publicly available COI sequence of related termites species in which sequence of *H. xenotermitis, H. makhamensis, Odontotermes escherichi, O. billitoni, O. formosanus, O. longignathus, O. hainanensis, O. oblongatus and O. minutes* was taken as out-group in the dataset. The screened sequences were aligned using ClustalW software & alignment was converted to MEGA format using MEGA 7 software. Phylogenetic analysis was carried out for a dataset consisting of mtCO1 sequences from 100 termite samples including the out-group. The phylogenetic analysis was performed under the optimality criteria of Neighbour-joining (NJ) and Maximum Likelihood Method (ML). Bootstrap values were calculated with 1000 replicates.

3. RESULTS AND DISCUSSION

The genomic DNA was collected from 100 populations of termites and the COI gene was characterized, using the universal primers. The amplified COI product was sequenced. The sequence was submitted to NCBI genbank and the accession numbers were obtained for the termite populations (Table 1). PCR amplification of the genomic DNA of the yielded partial mtCO1 sequences of varied lengths ranging between 421-600 bp.

Table 1: Details and corresponding accession numbers of de novo sequences of termites collected from regions of Pune, Ratnagiri, Kashid, Aurangabad and Nashik.

Sr. No.	Region	Sample name	Size of sequence	Query Coverage	Max identity	NCBI BLAST result	Accession number
1	Pune	PPPU-01	421	100%	100%	*Hypotermes xenotermitis*	KT879830
2	Pune	PPPU-02	464	100%	95%	*Hypotermes makhamensis*	KT879831
3	Pune	PPPU-03	464	100%	95%	*Hypotermes makhamensis*	KT879832
4	Pune	PPPU-04	440	100%	95%	*Hypotermes makhamensis*	KT879833
5	Pune	PPPU-05	504	100%	99%	*Hypotermes xenotermitis*	KT879834
6	Pune	PPPU-06	504	100%	99%	*Hypotermes xenotermitis*	KT879835
7	Pune	PPPU-07	466	100%	99%	*Hypotermes xenotermitis*	KT879836
8	Pune	PPPU-08	466	100%	99%	*Hypotermes xenotermitis*	KT879837
9	Pune	PPPU-09	504	100%	99%	*Hypotermes xenotermitis*	KT879838
10	Pune	PPPU-10	504	100%	99%	*Hypotermes xenotermitis*	KT879839
11	Pune	PPPU-11	504	100%	99%	*Hypotermes xenotermitis*	KT879840
12	Pune	PPPU-12	504	100%	99%	*Hypotermes xenotermitis*	KT879841
13	Pune	PPPU-13	504	100%	99%	*Hypotermes xenotermitis*	KT879842
14	Pune	PPPU-14	504	100%	99%	*Hypotermes xenotermitis*	KT879843
15	Pune	PPPU-15	504	100%	99%	*Hypotermes xenotermitis*	KT879844
16	Pune	PPPU-16	504	100%	99%	*Hypotermes xenotermitis*	KT879845

17	Pune	PPPU-17	504	100%	99%	*Hypotermes xenotermitis*	KT879846
18	Pune	PPPU-18	504	100%	99%	*Hypotermes xenotermitis*	KT879847
19	Pune	PPPU-19	504	100%	99%	*Hypotermes xenotermitis*	KT879848
20	Pune	PPPU-20	504	100%	99%	*Hypotermes xenotermitis*	KT879849
21	Ratnagiri	PPRA-01	483	100%	98%	*Hypotermes xenotermitis*	KT887698
22	Ratnagiri	PPRA-02	483	100%	98%	*Hypotermes xenotermitis*	KT887699
23	Ratnagiri	PPRA-03	483	100%	98%	*Hypotermes xenotermitis*	KT887700
24	Ratnagiri	PPRA-04	483	100%	98%	*Hypotermes xenotermitis*	KT887701
25	Ratnagiri	PPRA-05	483	100%	98%	*Hypotermes xenotermitis*	KT887702
26	Ratnagiri	PPRA-06	483	100%	98%	*Hypotermes xenotermitis*	KT887703
27	Ratnagiri	PPRA-07	483	100%	98%	*Hypotermes xenotermitis*	KT887704
28	Ratnagiri	PPRA-08	483	100%	98%	*Hypotermes xenotermitis*	KT887705
29	Ratnagiri	PPRA-09	483	100%	98%	*Hypotermes xenotermitis*	KT887706
30	Ratnagiri	PPRA-10	483	100%	98%	*Hypotermes xenotermitis*	KT887707
31	Ratnagiri	PPRA-11	483	100%	98%	*Hypotermes xenotermitis*	KT887708
32	Ratnagiri	PPRA-12	483	100%	98%	*Hypotermes xenotermitis*	KT887709
33	Ratnagiri	PPRA-13	483	100%	98%	*Hypotermes xenotermitis*	KT887710
34	Ratnagiri	PPRA-14	483	100%	98%	*Hypotermes xenotermitis*	KT887711
35	Ratnagiri	PPRA-15	483	100%	98%	*Hypotermes xenotermitis*	KT887712
36	Ratnagiri	PPRA-16	483	100%	98%	*Hypotermes xenotermitis*	KT887713
37	Ratnagiri	PPRA-17	483	100%	98%	*Hypotermes xenotermitis*	KT887714
38	Ratnagiri	PPRA-18	483	100%	98%	*Hypotermes xenotermitis*	KT887715
39	Ratnagiri	PPRA-19	483	100%	98%	*Hypotermes xenotermitis*	KT887716
40	Ratnagiri	PPRA-20	483	100%	98%	*Hypotermes xenotermitis*	KT887717
41	Kashid	PPKA-01	600	99%	97%	*Hypotermes xenotermitis*	KT898507
42	Kashid	PPKA-02	600	99%	97%	*Hypotermes xenotermitis*	KT898508
43	Kashid	PPKA-03	600	99%	97%	*Hypotermes xenotermitis*	KT898509
44	Kashid	PPKA-04	600	99%	97%	*Hypotermes xenotermitis*	KT898510
45	Kashid	PPKA-05	600	99%	97%	*Hypotermes xenotermitis*	KT898511
46	Kashid	PPKA-06	600	99%	97%	*Hypotermes xenotermitis*	KT898512
47	Kashid	PPKA-07	600	99%	97%	*Hypotermes xenotermitis*	KT898513
48	Kashid	PPKA-08	600	99%	97%	*Hypotermes xenotermitis*	KT898514
49	Kashid	PPKA-09	600	99%	97%	*Hypotermes xenotermitis*	KT898515
50	Kashid	PPKA-10	576	99%	97%	*Hypotermes xenotermitis*	KT898516
51	Kashid	PPKA-11	600	99%	97%	*Hypotermes xenotermitis*	KT898517
52	Kashid	PPKA-12	600	99%	97%	*Hypotermes xenotermitis*	KT898518

53	Kashid	PPKA-13	603	99%	97%	*Hypotermes xenotermitis*	KT898519
54	Kashid	PPKA-14	600	99%	97%	*Hypotermes xenotermitis*	KT898520
55	Kashid	PPKA-15	576	99%	97%	*Hypotermes xenotermitis*	KT898521
56	Kashid	PPKA-16	600	99%	97%	*Hypotermes xenotermitis*	KT898522
57	Kashid	PPKA-17	525	99%	98%	*Hypotermes xenotermitis*	KT898523
58	Kashid	PPKA-18	525	99%	98%	*Hypotermes xenotermitis*	KT898524
59	Kashid	PPKA-19	600	99%	97%	*Hypotermes xenotermitis*	KT898525
60	Kashid	PPKA-20	525	99%	98%	*Hypotermes makhamensis*	KT898526
61	Aurangabad	PPAU-01	522	100%	99%	*Hypotermes xenotermitis*	KT898527
62	Aurangabad	PPAU-02	522	100%	99%	*Hypotermes xenotermitis*	KT898528
63	Aurangabad	PPAU-03	522	100%	99%	*Hypotermes xenotermitis*	KT898529
64	Aurangabad	PPAU-04	522	100%	95%	*Hypotermes makhamensis*	KT898530
65	Aurangabad	PPAU-05	522	100%	99%	*Hypotermes xenotermitis*	KT898531
66	Aurangabad	PPAU-06	522	100%	95%	*Hypotermes makhamensis*	KT898532
67	Aurangabad	PPAU-07	522	100%	99%	*Hypotermes xenotermitis*	KT898533
68	Aurangabad	PPAU-08	522	100%	99%	*Hypotermes xenotermitis*	KT898534
69	Aurangabad	PPAU-09	522	100%	99%	*Hypotermes xenotermitis*	KT898535
70	Aurangabad	PPAU-10	550	100%	99%	*Hypotermes makhamensis*	KT898536
71	Aurangabad	PPAU-11	522	100%	99%	*Hypotermes xenotermitis*	KT898537
72	Aurangabad	PPAU-12	522	100%	99%	*Hypotermes xenotermitis*	KT898538
73	Aurangabad	PPAU-13	522	100%	99%	*Hypotermes xenotermitis*	KT898539
74	Aurangabad	PPAU-14	522	100%	99%	*Hypotermes xenotermitis*	KT898540
75	Aurangabad	PPAU-15	522	100%	99%	*Hypotermes xenotermitis*	KT898541
76	Aurangabad	PPAU-16	522	100%	99%	*Hypotermes xenotermitis*	KT898542
77	Aurangabad	PPAU-17	522	100%	99%	*Hypotermes xenotermitis*	KT898543
78	Aurangabad	PPAU-18	522	100%	99%	*Hypotermes xenotermitis*	KT898544
79	Aurangabad	PPAU-19	522	100%	99%	*Hypotermes xenotermitis*	KT898545
80	Aurangabad	PPAU-20	522	100%	99%	*Hypotermes xenotermitis*	KT898546
81	Nashik	PPNA-01	591	98%	98%	*Hypotermes xenotermitis*	KT898547
82	Nashik	PPNA-02	591	98%	98%	*Hypotermes xenotermitis*	KT898548
83	Nashik	PPNA-03	591	98%	98%	*Hypotermes xenotermitis*	KT898549
84	Nashik	PPNA-04	591	98%	98%	*Hypotermes xenotermitis*	KT898550
85	Nashik	PPNA-05	591	98%	98%	*Hypotermes xenotermitis*	KT898551
86	Nashik	PPNA-06	591	98%	98%	*Hypotermes makhamensis*	KT898552
87	Nashik	PPNA-07	591	98%	98%	*Hypotermes xenotermitis*	KT898553
88	Nashik	PPNA-08	591	98%	98%	*Hypotermes xenotermitis*	KT898554

89	Nashik	PPNA-09	591	98%	98%	*Hypotermes xenotermitis*	KT898555
90	Nashik	PPNA-10	591	98%	98%	*Hypotermes xenotermitis*	KT898556
91	Nashik	PPNA-11	591	98%	98%	*Hypotermes xenotermitis*	KT898557
92	Nashik	PPNA-12	591	98%	98%	*Hypotermes xenotermitis*	KT898558
93	Nashik	PPNA-13	591	98%	98%	*Hypotermes xenotermitis*	KT898559
94	Nashik	PPNA-14	591	98%	98%	*Hypotermes xenotermitis*	KT898560
95	Nashik	PPNA-15	591	98%	98%	*Hypotermes xenotermitis*	KT898561
96	Nashik	PPNA-16	591	98%	98%	*Hypotermes xenotermitis*	KT898562
97	Nashik	PPNA-17	591	98%	98%	*Hypotermes xenotermitis*	KT898563
98	Nashik	PPNA-18	591	98%	98%	*Hypotermes xenotermitis*	KT898564
99	Nashik	PPNA-19	591	98%	98%	*Hypotermes xenotermitis*	KT898565
100	Nashik	PPNA-20	591	98%	98%	*Hypotermes xenotermitis*	KT898566

PPPU- Pune, PPRA- Ratnagiri, PPAU- Aurangabad, PPRA- Ratnagiri, PPNA- Nashik

3.1 Nucleotide analysis

Evolutionary analysis was conducted in MEGA7 software (22). 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=55.37% and G+C= 44.73 % of various regions (Table 2). There was high A+T content in the studied sequences consistent with what is expected in insect mitochondrial DNA [23]. The populations from Aurangabad had the least variation in the base composition of A+T (54.93%) and most in Ratnagiri (56.10%), respectively. The Transition/Transversion rate ratios are k1 = 6.297 (purines) and k 2 = 7.338 (pyrimidines). The overall Transition/Transversion ratio Bias R is 3.347, where R = [A*G*k1 + T*C*k2]/[(A+G)*(T+C)]. The nucleotide frequencies were A=29.97%, T/U=24.97%, C=26.91% and G=18.16%. For estimating the ML value, a tree topology was automatically computed [24]. The maximum log likelihood for this computation was -1211.575, which involved 107 nucleotide sequences. Codon positions included were 1^{st}+2^{nd}+3^{rd}+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 411 positions in the final dataset. The overall result of nucleotide analysis showed AT-rich gene which is common in insects. This pattern suggests that the sequence divergence is often accompanied by high AT mutation pressure or less probably resulting from the selection for the incorporation of amino acids encoded by AT rich codon families. Further, the transversion is higher than transition indicating less variation in the termite population which is confirmed by the number of conserved sites/sequences (in multiple sequence alignment) obtained from MEGA. In termites AT-bias is particularly evident at 3^{rd} codon position [25, 26]. The gene sequence data of mtCOI gene showed a predominance of transversions over transitions.

Table 2: A+T and G+C content (%) for partial COI of termite species from different regions

Sr.no	Region	Average A+T %	Average G+C %
1	Pune	55.82	44.25
2	Ratnagiri	56.10	43.9
3	Kashid	55.02	44.98
4	Aurangabad	54.93	45.53
5	Nashik	54.99	45.00

3.2 Phylogenetic analysis

A Phylogenetic tree of the species using Maximum-Likelihood method was drawn on the basis of multiple sequence alignment of mtCOI gene. A similarity index, generated from the sequence identity matrix, revealed that maximum similarity (and thus a close proximity) exists among members of *H. Xenotermitis* and *H. makhamensis* .The phylogenetic tree consists of the outgroup *Odontotermes* spp. and *Hypotermes* spp. for the analysis of phylogenetic tree by Neighbour joining method (Figure 3) and Maximum Likelihood method (Figure 4). The Neighbour joining tree consists of two major clusters. The first major cluster was further divided into two subclusters consisting of population from Kashid and Ratnagiri with a bootstrap score of 63 and 99. The second major cluster consists of three subclusters. The first subcluster was further divided into two clusters consisting of Pune and Nashik populations with a bootstrap value 64 and 99 respectively. The second subcluster represents termites from Aurangabad having 67 bootstrap value. The third cluster was made up of three termite samples from Aurangabad and two termite samples from Pune having bootstrap of 98 and 89. In all the subclusters only the strains collected from Ratnagiri were clubbed together and was *Hypotermes xenotermitis*. In one subluster, there were three sequences from Aurangabad and two sequences from Pune region together with a bootstrap value of 98 and 89. These sequences showed divergent from the other sequences collected from the same place. The two strains collected from Pune were closely related and grouped together with bootstrap score of 89, whereas the three stains collected from Aurangabad was also closely related and clubbed with bootstrap of 98. Almost similar topology is shown in the Maximum Likelihood tree.

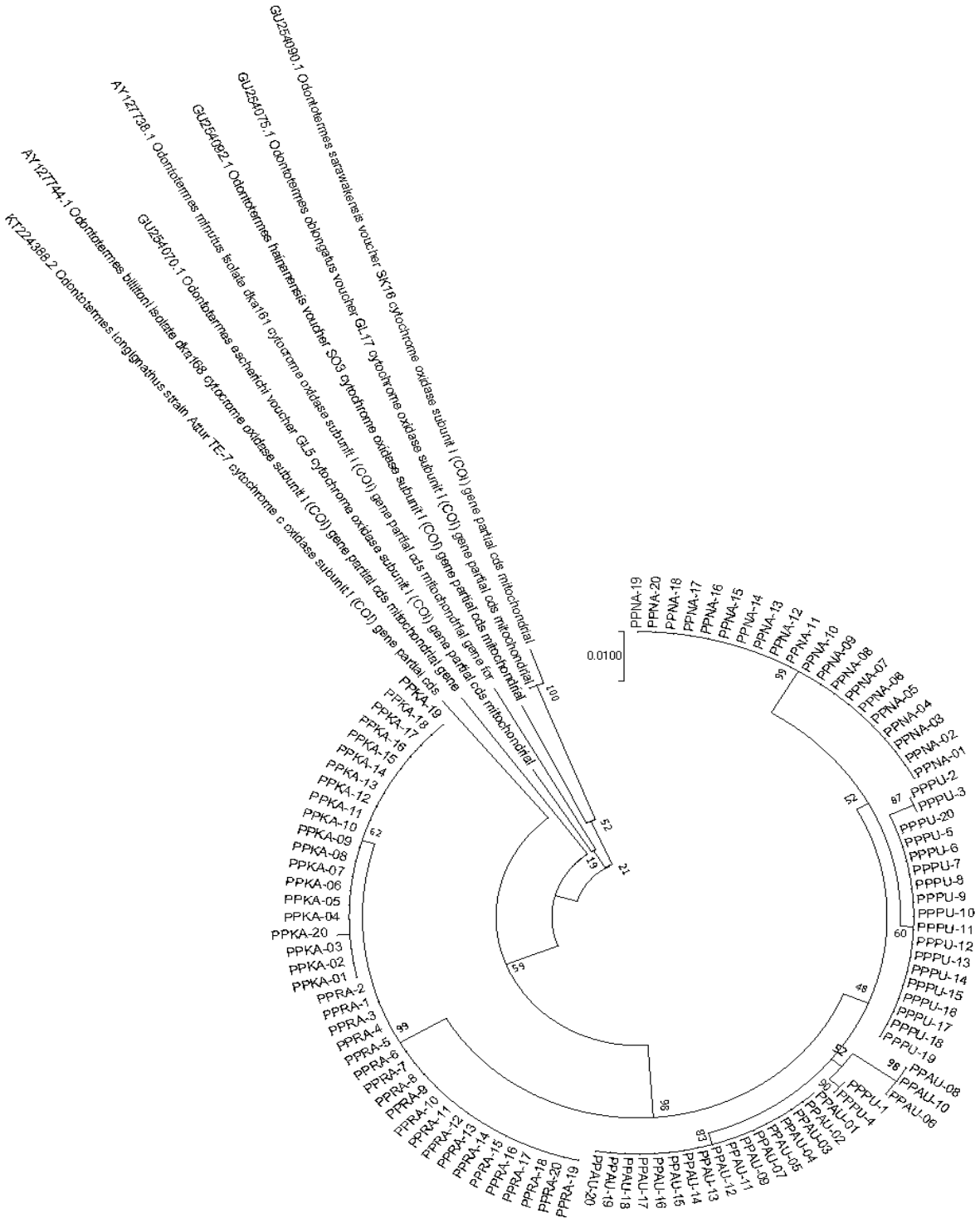

Figure 3: The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 0. 0.27082207 is shown. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. The analysis involved 107 nucleotide sequences. There were total of 411 positions in the final dataset. The three shows two major clusters. The first major cluster was further divided into two subclusters consisting of population from Kashid and Ratnagiri. The second major cluster consists of three subclusters. The first subcluster was further divided into two clusters consisting of Pune and Nashik populations. The second subcluster represents termites from Aurangabad. The third cluster was made up of three termite samples from Aurangabad and two termite samples from Pune. Evolutionary analyses were conducted in MEGA7.

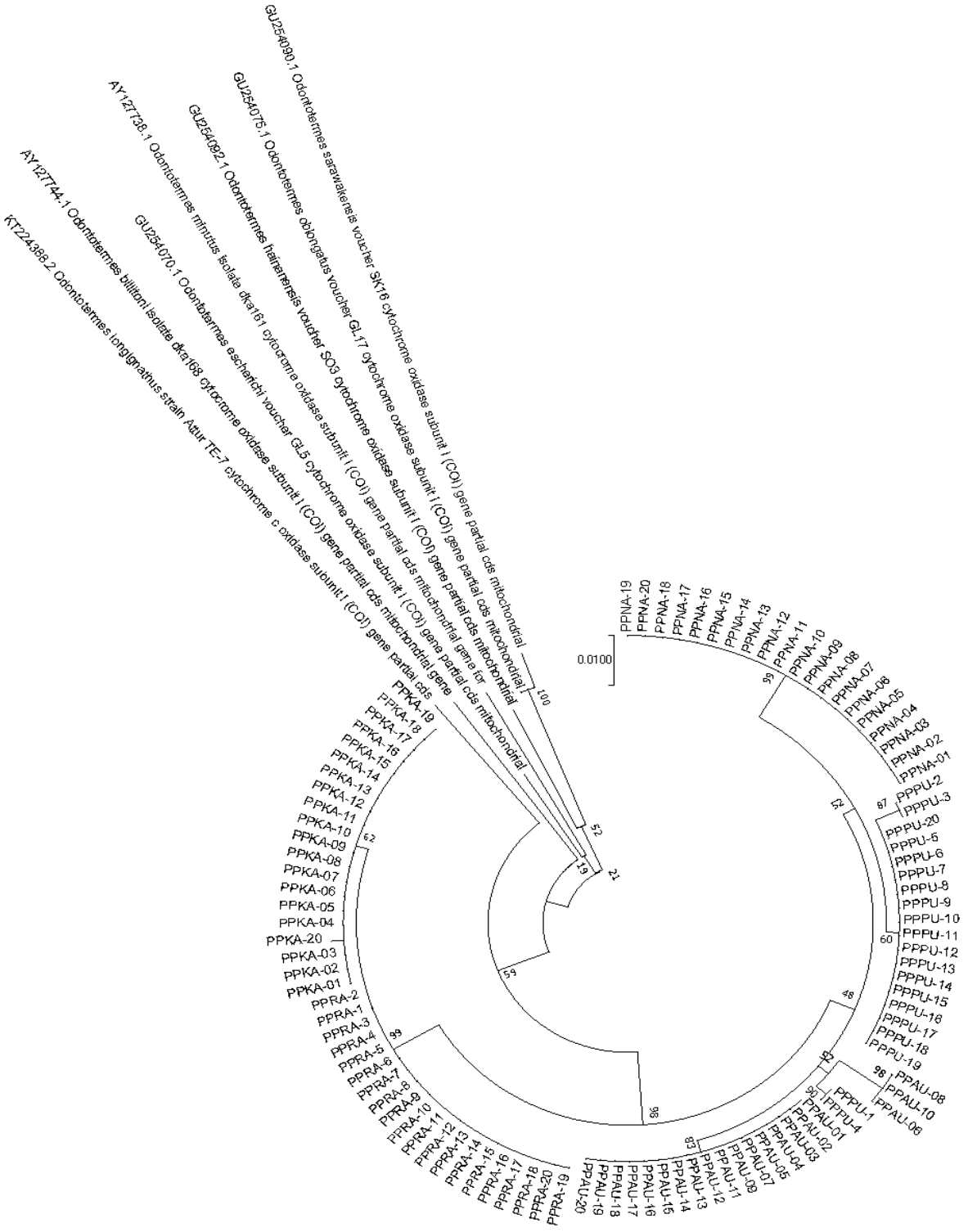

Figure 4: The evolutionary history was inferred using the Maximum Likelihood method based on the Tamura-Nei model. The tree with the highest log likelihood (-1211.58) is shown. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. The analysis involved 107 nucleotide sequences. There were total of 411 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

Our study has shown the distribution of termites and their diversity within different regions of Maharashtra. Effects of different environmental factors i.e., temperature, relative humidity and precipitation on population dynamics, density and foraging activities of termites [27]. Both the Neighbour joining and maximum likelihood-based trees offer same topology phylogenetic tree. The data set had low sequence variability for the termites populations of Ratnagiri, Kashid and Nashik regions as the termites of the area were clustered together in their respective clade, but the models were consistent in estimating the relationships within the genus; though divergence was low, as might be expected due to less mutation in the gene level or less invasion of termites from other regions. Though the samples from Pune and Aurangabad showed the separate clades but the clades further showed subclades of some termite indicating the divergence from their respective group. The Blast analysis showed that the majority of termites sequenced belonged to the *Hypotermes xenotermitis* with 95-99% similarity, but there were some species of *Hypotermes makhamensis* also present which may be due to the invasion or mutation in their gene level. It is possible that the shared mitochondrial DNA sequence is the result of long-term lineage sorting, or recent introgression or hybridization in congruent with the studies of Gentz [28] which revealed a complexity in mitochondrial DNA relationships between Coptotermes taxa in the same geographic region. The overall study has shown the genetic diversity of termites depending upon their geographic location and environmental factor as the termites from Kashid and Ratnagiri region are closely related (from the phylogenetic tree) because of their location near to sea and relatively high humidity than the termites of other regions located in the interior of Maharashtra having less humidity. This can be correlated with the study of Liu [29,30,31] suggesting environmental factors influence both abundance and genetic diversity in the species.

4. CONCLUSION

Our study is the first investigation to compute the phylogeny of termites from Maharashtra region indicating the genetic diversity is based on the environmental factor and the geographical location. This kind of information is valuable in studies related to characterization and molecular comparisons between the sequences of different species of Indian termites. The information generated on the basis of this data can be used for the molecular identification of Indian termite species, their prevalence and classification, which are difficult to differentiate on the basis of morphological parameters.

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CONFLICT OF INTEREST

Authors have no any conflict of interest.

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