

Molecular characterization of *Ceylonitermellus sahyadriensis* Emerson, 1960 (Termitidae, Nasutitermitinae) - a rare species from Kerala part of Western Ghats, India

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ABSTRACT: Molecular characterization of *Ceylonitermellus sahyadriensis* (Termitidae, Nasutitermitinae), using the cytochrome oxidase I (COI) gene was studied, along with its morphological characteristics. The combined molecular analysis with its morphological figures provided a comprehensive species description, the first genetic profile of this species and giving a baseline data for the genus *Ceylonitermellus*. The phylogenetic analysis validated the species' taxonomic position and revealed its evolutionary relationships within Nasutitermitinae. This integrated approach is particularly significant given the genus's rarity and limited distribution, contributing to our understanding of termite diversity in the Oriental region. The molecular data generated will serve as a crucial reference for future systematic studies and species identification of this endemic genus. © 2025 Association for Advancement of Entomology

KEY WORDS: Termite, endemism, cytochrome oxidase, integrated validation, phylogenetic analysis

INTRODUCTION

Ceylonitermellus Emerson, 1960 is a rare genus, which is endemic to oriental region erected by Emerson 1960 (Termitidae, Nasutitermitinae), was initially understood as an endemic to Sri Lanka (Hemachandra *et al.*, 2010). Amina and Rajmohana's later description of *C. periyarensis* from Kerala, India shed light into the distribution of the species (Amina and Rajmohana, 2013). The new species *C. sahyadriensis* was recently described by Renjith *et al.* (2022). Nasutitermitinae is a widely distributed termite subfamily (Sen-sarma and Verma, 1983). The genus is very rare and presumed to be endemic to Sri Lanka till the description of *C. periyarensis* from Kerala, India

(Amina and Rajmonha, 2013; Renjith *et al.*, 2022). In this study, molecular characterization of *C. sahyadriensis* was carried out using COI gene and morphological description was also made. The resulting molecular data were combined with detailed morphological observations, for genetic profile of this. Phylogenetic analysis was studied for its evolutionary relationships within *Nasutitermitinae*.

MATERIALS AND METHODS

Specimens were collected from a tree bark of *bombax ceiba* in Mamalakandam, Idukki District, Kerala State, Southern India. The samples were collected using an aspirator and kept in 80% alcohol.

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Measurements and dissections (80% ethanol) of the specimens were carried out using stereo zoom microscopes, a Labomed Luxeo 4D binocular microscope with an attached camera, and pixelpro software. The magnification range was 8X to 35X. According to Roonwal and Chhotani (1989), Sands (1998) and Chhotani (1997), soldiers and workers were described using morphological terms, measurements, and indices.

DNA extraction, amplification and sequencing:

Soldier termite heads were used to harvest the DNA. Distilled water was used to clean the specimen, then the specimen was then spread out on filter paper to dry completely. Using the Machery-Nagel NucleoSpin® Tissue Kit and according to the manufacturer's instructions, genomic DNA was extracted from the tissues. Agarose gel electrophoresis was used to evaluate the quality of the extracted DNA. The amount of template given to the polymerase chain reaction was calculated based on the concentration of DNA in each sample using a DNA spectrophotometer (Thermo Fisher).

For the polymerase chain reaction amplification, cytochrome oxidase c subunit I (COI) primers are utilized (Sequences, 5' to 3': Forward LCO: GGTCACAAATCATAAAGATATTGG, Reverse HCO: TAAACTTCAGGGTGACCAAAAAA TCA). In a 10 µL reaction volume, individual PCR reactions were conducted using a mixture of 5µL of 2X Sapphire Master Mix, 4µL of D/W, 0.25µL of forward primer chloride, 0.25 µL of reverse primer chloride, and 1µL of DNA template [2-8 ng/l]. PCR reactions were run at 98.0°C for 30 seconds, followed by 10 cycles at 98.0°C for 5 seconds, 45°C for 10 seconds, and 72.0°C for 15 seconds, followed by 30 cycles at 98.0°C for 5 seconds, 45°C for 10 seconds, 72.0°C for 15 seconds, and 72.0°C for 60 seconds (Folmer *et al.*, 1994). The GenElute PCR Clean-Up Kit (Sigma-Aldrich) was used to purify the PCR products, which were then submitted to sequencing using the BigDye Terminator Cycle Sequencing Kit version 3.1. (Applied Biosystems).

Phylogenetic analysis: The chromatograms were edited using the BioEdit software. To collect meaningful data, unclear and skipped bases were

removed. The sequences were then converted to FASTA format for subsequent processing. The generated sequence was compared to similar sequences from the NCBI nucleotide database using the Basic Local Alignment Search Tool (BLAST N) tool (Altschul *et al.*, 1997). Before tree construction, the sequences were aligned using Clustal Omega's multiple alignment technique with the default values. The tree was created using the Maximum Likelihood (ML) technique with the MEGA X software. This technique is based on the uniform rate substitution among sequences approach and the Kimura 2-parameter distance. To ensure the accuracy of the constructed tree, 1000 bootstrap replication phylogenies were examined (Felsen and Stein, 1985). The necessary termite sequences were obtained from the NCBI GenBank database

RESULTS AND DISCUSSION

Family: Termitidae Latreille (1802);

Genus: *Ceylonitermellus* Emerson (1960); *Ceylonitermellus* Emerson. In: Emerson, American Museum; Novitates.

Type species: *Ceylonitermellus hantanae* (Holmgren)

Ceylonitermellus sahyadriensis Emerson

Materials examined: Specimen: 12 soldiers and 10 workers, India, Kerala, Idukki (Mamalakandam), 11/05/22, Edwin Joseph. Colony Code : CMSZ126

Description:

Imago. **Unknown**

Soldier (Fig. 1, Table 1): The species exhibits monomorphic characteristics with a pear-shaped (pyriform) head capsule displaying a golden yellow coloration. The head features sparse pubescence, comprising two prominent hairs and numerous shorter ones, with a slight hump when viewed laterally. The reddish-brown rostrum is slender and cylindrical, matching the head length, and darkens towards the apex where multiple hairs are present. Dorsally, the cephalic gland duct is partially

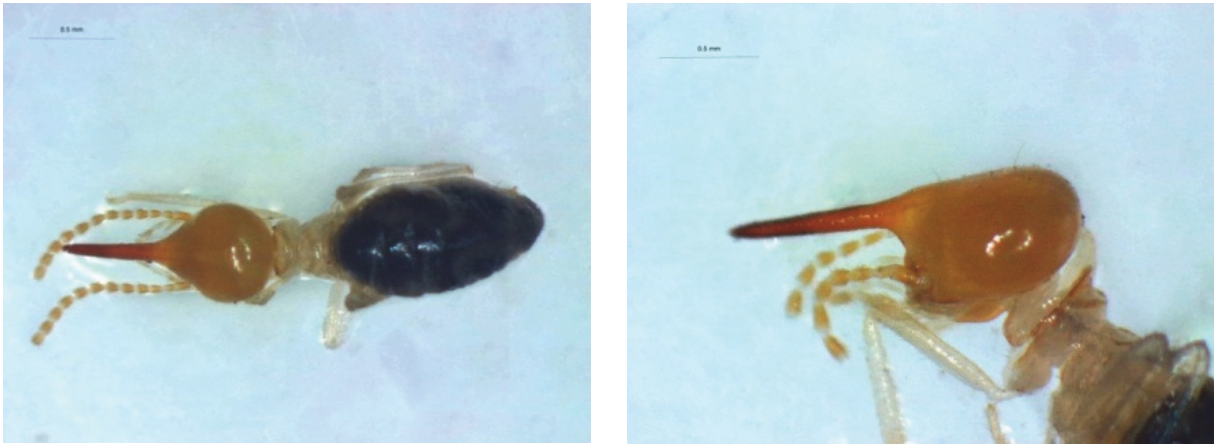


Fig. 1 *Ceylonitermellus sahyadriensis* soldier. (a) Dorsal view (b) lateral view of the head.

observable, extending from the rostrum to the gland reservoir. The 12-segmented antennae show a yellowish-brown coloration, with the second segment being the shortest, while segments three through five are approximately equal in length. The mandibles are reduced (vestigial) and lack spine-like projections. The pronotum exhibits a saddle-shaped structure, appearing lighter yellow dorsally and narrower than the head width. The abdomen demonstrates slight transparency, revealing the internal gut, and is adorned with both short and long bristles. The species possesses small, bi-segmented cerci and legs with a 2:2:2 arrangements of apical tibial spurs, complemented by four-segmented tarsi.

Worker (Fig. 2): The species displays monomorphic characteristics with an unsclerotized, oblong body structure. The sub-circular head capsule reaches its maximum width at the antennal base, exhibiting a pale yellow coloration and dense setation. In lateral view, the postclypeus shows slight swelling with notable flattening, while the clypeus presents a transparent, pale yellowish appearance. Both the epicranial suture and fontanelle are poorly defined, and the labrum maintains a pale yellow coloration. The antennae comprise 13 segments, with the third segment being the shortest. The pronotum demonstrates a saddle-shaped configuration and is significantly narrower than the head width. The oblong abdomen exhibits transparency, revealing visible gut coiling, and features dense pubescence. The species possesses small, two-segmented cerci and legs with four-segmented tarsi, accompanied by apical tibial spurs arranged in a 2:2:2 pattern.

The mandibular structure is asymmetrical: the left mandible features an apical tooth exceeding the first marginal tooth in size, with a 45-degree angle between them. A pronounced posterior margin separates the first and second marginal teeth. The third marginal tooth, intermediate in size between the apical and second marginal, is separated by a distinct notch with prominent molar development. The right mandible is characterized by an elongated apical tooth, with the first and second marginal teeth positioned at a narrow angle, lacking the posterior margin separation observed in the left mandible. The molar ridges are prominently developed.

Among Indian *Nasutitermitinae*, three genera are distinguished by their reduced head capsule sclerotization. *Ceylonitermellus* differs from *Aciculitermes* by its longer third antennal segment relative to the second, while both these genera

Table 1. Morphometry of *Ceylonitermellus sahyadriensis* soldier (n = 10)

Characters	Range	SE
Total body Length	3.11-3.64	0.056949
Total Head Length	1.56-1.66	0.010349
Head Length Without rostrum	0.72-0.77	0.008921
Head Width	0.68-0.72	0.005212
Rostrum Length	0.82-0.90	0.007118
Pronotum Length	0.15-0.18	0.002906
Pronotum Width	0.34-0.37	0.003333

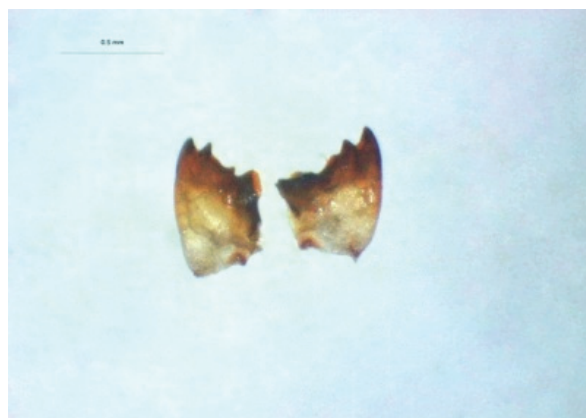


Fig. 2 *Ceylonitermellus sahyadriensis* worker. (a) Dorsal view (b) mandible worker

possess 14-segmented antennae, contrasting with *Emersonitermes*' 12-segmented antennae. For comparison image of the *Ampoulitermes zacharia*, Joseph and Mathew (2024) soldier is provided (Fig. 3), which shows how distinguished the *Ceylonitermellus* from other species in morphology.

Distribution: India: Kerala, Wayanad, Meppadi; Kerala, Idukki, Mamalakkandam as per present study

Molecular analysis: *C. sahyadriensis* sample had a mitochondrial COI gene. DNA fragment of 580 base pair was deposited in NCBI database with accession number OQ471904. The base composition of COI gene fragment presented 33% (A), 30 (T), 15 (G), and 22 (C) with total A+T content 63 per cent. G+C content 37 Per cent. The characteristic property of insect sequence with rich

in A+T content is evident in the current sequence (Navajas *et al.*, 1996). Related termite sequences such as *Bulbitermes laticephalus* (NC_034059.1), 1 *Nasutitermes longirostris* (KY224677.1), *Trinervitermes geminatus* (JF923343.1) and *Cryptotermes dudleyi* (OM415296.1) were accessed from the NCBI data base to construct phylogenetic tree (Fig. 4) using *Cryptotermes dudleyi* as an outgroup. Based on the sequences Evolutionary Divergence between Sequences was calculated (Table 2).

Table 2. Estimates of evolutionary divergence between sequences

Species	1	2	3	4	5
1. <i>Ceylonitermellus sahyadriensis</i>					
2. <i>Bulbitermes laticephalus</i>	0.1009				
3. <i>Nasutitermes longirostris</i>	0.0983	0.1027			
4. <i>Cryptotermes dudleya</i>	0.2462	0.2371	0.2577		
5. <i>Trinervitermes geminatus</i>	0.1059	0.1069	0.1086	0.2398	



Fig. 3 Soldier of *Ampoulitermes zacharia*

The evolutionary history of *C. sahyadriensis* was deduced through the application of the Maximum Likelihood approach and the Kimura 2-parameter model (Kimura, 1980). The illustrated tree is the one with the highest log probability (-1773.92). Next to the branches is the percentage of trees where the related taxa were clustered together. By employing the Neighbor-Join and BioNJ algorithms on a matrix of pairwise distances calculated using

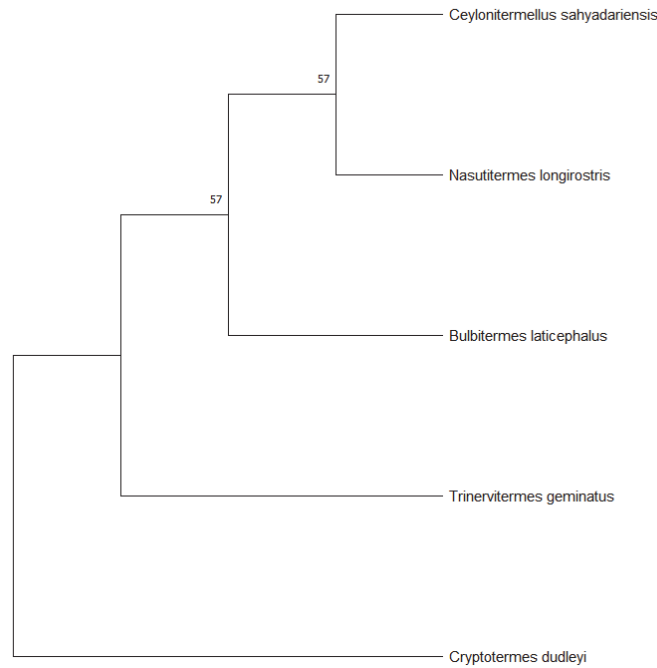


Fig. 4 Phylogenetic tree of *Ceylonitermellus sahyadriensis*.

the Maximum Composite Likelihood (MCL) technique, the initial tree or trees for the heuristic search were automatically generated. The topology with the highest log likelihood value was then chosen. There were five nucleotide sequences in this investigation. Codon positions included were 1st+2nd+3rd+Noncoding. The final dataset contained 580 locations in total. (Kumar et al., 2018). The tree shows *C. sahyadriensis* makes a clade with *Nasutitermes longirostris* showing that they are the most related. The equality of evolutionary rate between sequences A (*Ceylonitermellus sahyadriensis*) and B (*Nasutitermes longirostris*), with sequence C (*Bulbitermes laticephalus*) used as an outgroup in Tajima's relative rate test (Table 3) (Tajima, 1993). The $\div 2$ test statistic was 0.02 ($P = 0.88640$ with 1 degree[s] of freedom) P -value less than 0.05 is often used to reject the null hypothesis of equal rates between lineages. This analysis involved 3 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated (complete deletion option). There were a total of 580 positions in the final dataset.

Presence of *C. sahyadriensis* in an area other than the type locality first reported here. The presence of the new species and report of the rare species *C. sahyadriensis* highlights the remarkable diversity of the area.

Morphologically the soldiers of the genus *Ceylonitermellus* show similarity with genus *Aciculitermes* and both of these genus show morphological similarity with genus *Emersonitermes* (Renjith et al., 2022). *C. sahyadriensis*, the species under discussion show affinity to *C. kotuae* (Amina and Rajmohana, 2013). Lack of data availability has been a major challenge in molecular studies in India. On the basis of the similar sequences obtained from the data base the phylogeny of the species was studied. *C. sahyadriensis* formed a monophyletic clade with *Nasutitermes longirostris* which thus show highest phylogenetic relation among the species. This clade form a paraphyletic clade with *Bulbitermes laticephalus*. *C. sahyadriensi* has a poly phyletic relation with *Trinervitermes geminatus*. The present study reiterates the diversity of India and also sheds light to the distribution pattern of the

Table 3. Results from the Tajima's test for 3 Sequences

Configuration	Count
Identical sites in all three sequences	501
Divergent sites in all three sequences	4
Unique differences in Sequence A	24
Unique differences in Sequence B	25
Unique differences in Sequence C	26

Sequences A-*Ceylonitermellus sahyadariensis*; Sequences B - *Nasutitermes longirostris*; Sequences C- *Bulbitermes laticephalus*.

rare genus *Ceylonitermellus* and the species *C. sahyadriensis*. This comprehensive study, combining distributional data, morphological analysis, and molecular characterization, significantly advances our understanding of *C. sahyadriensis* and the genus *Ceylonitermellus*. The findings not only expand the known geographic range of this rare species but also provide valuable molecular markers for future taxonomic investigations. This research highlights the substantial knowledge gaps in Indian termite taxonomy and emphasizes the need for continued systematic studies in this biodiversity-rich region. The integration of molecular tools with traditional taxonomy, as demonstrated in this study, offers a robust framework for future investigations into termite diversity, evolution and conservation.

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