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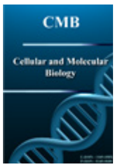

Original Article

Mitochondrial DNA sequence-based identification of two subterranean termite species, from Riyadh Province, Kingdom of Saudi Arabia

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Abstract

Termites are economically important wood-destroying and agricultural pests. The termite fauna almost consists of 2900 described species in 286 genera worldwide. In the present study, hundreds of termite samples from 42 different locations in the Riyadh province were collected. These samples were previously used for morphometric identification and reported two subterranean termite species, *Coptotermes heimi* and *Psammotermes hypostoma*, in the family Rhinotermitidae. In the present study, these samples were analysed using DNA barcoding with the mitochondrial cytochrome c oxidase subunit 1 gene to confirm the conventional taxonomical identification on a molecular basis. The obtained COI gene sequences of all 42 termite specimens were submitted to GenBank (accession numbers: ON529959-ON529969, OP825131-OP825132, and OP890882-OP890910). Eleven of the 42 samples were thus identified as *C. heimi* and the remaining 31 samples as *P. hypostoma*, which were phylogenetically analysed. All the 11 *C. heimi* sequences were grouped in a single clade, indicating close relatedness. While 31 sequences of *P. hypostoma* constituted two clades in the phylogenetic tree. Pairwise nucleotide sequence identity and divergence analysis showed that *C. heimi* sequences showed high nucleotide identities of 87.6-99.5% and less divergence ranging from 0.5% to 13.6%. Similarly, sequences of *P. hypostoma* also showed a high nucleotide identity of 78.6-100% and low divergence among them ranging from 0-10.7%. A further application, significance, and shortcomings of COI-based DNA barcoding have been discussed. DNA barcoding using the COI gene is a reliable tool to distinguish *C. heimi* and *P. hypostoma* genotypes.

Keywords: Termites, Rhinotermitidae, DNA barcoding, Saudi Arabia

1. Introduction

Termites are eusocial insects inhabiting tropical, subtropical, and temperate regions of the world. Termites are generally recognized as harmful insects and might almost destroy several cellulose-based materials [1]. They destroy household articles, barriers, wooden poles, fruits, and ornamental/shade trees in addition to agricultural crops like sugarcane [2], wheat, peas, and chilies. Termites contribute to ecosystem processes and carbon and nitrogen cycles. Termites live in huge colonies, mostly in underground nests, and are familiar because of their depredations. In Saudi Arabia, reports on termite fauna are very rare except for few studies [3].

Coptotermes heimi (Wasmann 1902) and *Psammotermes hypostoma* Desneux, 1902 are subterranean termites acting as voracious pests to dwellings, furniture, silos, grain, and crops, or any material containing cellulose [3]. Subterranean termites species are widely distributed throughout the world's arid and semi-arid areas [4]. Control methods to manage these termites, including entomopathogenic fungi, nematodes, and chemicals [2, 5, 6, 7].

Traditional termite identification relies on morphological characteristics, which can be difficult due to species similarities and the presence of cryptic species. Molecular identification techniques offer a more precise and reliable approach to identifying termite species. By analyzing specific regions of the termite's DNA, genetic sequences can be compared and the species or genetic lineage of a particular termite specimen can be determined. However, in the Kingdom of Saudi Arabia (KSA), molecular identification of termite fauna is currently limited. Correct identification of termite species is necessary for successful integrated pest management programs and conservation efforts. Theerefore, the present study aimed at the identification of the termite fauna of the Riyadh Province using molecular

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techniques (DNA barcoding).

DNA barcoding enables the researcher to identify the organism species in a faster and economical manner, by using a unique gene region for specimen identification [8]. DNA barcoding is elusive to animal taxonomy. The mitochondrial gene 'Cytochrome Oxidase I' [9] is an efficient DNA barcoding tool [10].

The present research analysed the mitochondrial cytochrome oxidase subunit I, (COI-based DNA) barcodes of two phenotypically identified termite species, *C. heimi* and *P. hypostoma* collected from different localities in the Riyadh region.

2. Materials and Methods

2.1. Sample Collection and Genomic DNA Extraction

Termite specimens were either hand-picked or collected using an aspirator from diverse habitats, preserved immediately in 96% ethanol, placed in an ice box, and brought into the laboratory where they were stored at 4°C in a refrigerator. The sample collection sites and their detailed information are given in Table 1. The genomic DNA of the specimens was isolated using a modified protocol of the cetyltrimethylammonium bromide (CTAB) method [11]. The detailed steps for the protocol were strictly followed [12].

2.2. Amplification of Cytochrome Oxidase I gene

The isolated genomic DNA from all the specimens was used as a template for COI gene amplification by using universal primers (forwards primer: LCO 1490: 5'-GGTCAACAAATCATAAAGATATTGG-3' and reverse primer; HCO 2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3) [13]. PCR conditions were set as described previously [12]. After the PCR, COI gene amplification was confirmed through 1% agarose gel electrophoresis. The positive samples were used for further downstream sequencing.

2.3. Sequencing and Phylogenetic Analysis

The desired amplicon band size (680-700 bp) of the PCR products was purified using Illustra GFX PCR DNA and Gel Band Purification Kit (Healthcare Life Sciences, USA) and sent for DNA sequencing to Macrogen Inc. Seoul, South Korea. The obtained sequences were analysed using Lasergene package (DNASTAR, Madison, Wisconsin). BLASTn (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to search out closely related sequences from databases. Closely related sequences showing 80% or more sequence identity were selected and retrieved from databases in FASTA format. These sequences were further used in pairwise multiple sequence alignment (global alignment). The MEGA7.0 software was then used for multiple sequence alignment using the Clustal W algorithm and phylogenetic analysis using the maximum likelihood algorithm, while tree display and manipulation were performed through Tree View Software. MegAlign application was used to pairwise distance analysis for evolutionary divergence between sequences [14].

3. Results

The samples of *C. heimi* and *P. hypostoma* were collected from sites in the desert ecosystems of the central regions of the Kingdom of Saudi Arabia and covered different types of habitats, including agricultural and native undisturbed, disturbed, and terrestrial ecosystems in the Riyadh province (Table 1) in the year 2020. The samples were previously morphologically identified as *C. heimi* and *P. hypostoma* [15]. The amplified PCR products analysed in 1% agarose gel electrophoresis, showed bright bands of approximately 700 bp size.

3.1. Nucleotide sequence identity/divergence and phylogenetic analysis of *Coptotermes heimi*

COI gene sequences of 11 specimens of the termite species *Coptotermes heimi* were analyzed, and barcode sequences were submitted to GenBank with accession numbers ON529959-ON529969. Basic Local Alignment Search Tool (BLAST) analysis inferred their allocation to Rhinotermitidae. No available COI sequences for *C. heimi* were found in GenBank. Closely related sequences were members of genera *Microcerotermes*, *Neocapritermes*, *Pericapritermes*, and *Amitermes*, which belong to the family Termiditae and subfamily Termitinae. The COI gene sequence of a fruit fly *Bactrocera zonata* (MG770092) was used as an outgroup sequence. The sequences of closely related termite species were retrieved from GenBank database in FASTA format. Phylogenetic analysis showed that all the specimens grouped together into a single clade and were confirmed to be highly closely related to one another and being members of the same species (Figure 1).

A second closely related clade was made by members of genera *Microcerotermes*, *Neocapritermes*, *Pericapritermes*, and *Amitermes*, which belong to the family Termitidae, and subfamily Termitinae while the members of Hodotermes and Microhodotermes made a distant clade that belongs to the Hodotermitidae family. A Pairwise sequence distance of COI gene sequences of *C. heimi* with sequences of other termite species has been shown in (Figure 2). Pairwise sequence identity percentage analysis of the termite *C heimi* samples has shown a high homology

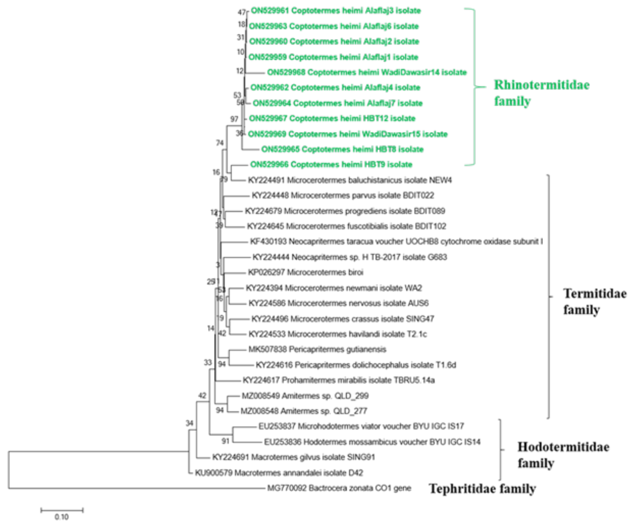


Fig. 1. Phylogenetic tree constructed using MEGA 7.0 software and the maximum-likelihood algorithm. The phylogram depicts the relationship of 11 *Coptotermes heimi* COI gene sequences (indicated with green font color) with those of other closely related members of the same family and with those of other families of termites. The sequences used were labeled with species names along with accession numbers. Bootstrap values (1000 replications) are indicated at each node.

Table 1. Detailed information of *Coptotermes heimi* and *Psammotermes hypostoma* specimens collected from different places in Riyadh Region, the Kingdom of Saudi Arabia.

Sample No	Species name	Accession Number	Locality/Area	Coordinates
1	*Coptotermes heimi*	ON529959	Al Aflg	22°01.933’N, 46°33.794’E
2		ON529960	Al Aflg	22°00.395’N, 46°31.972’E
3		ON529961	Al Aflg	22°00.399’N, 46°31.983’E
4		ON529962	Al Aflg	22°00.398’N, 46°31.974’E
5		ON529963	Al Aflg	22°00.388’N, 46°31.989’E
6		ON529964	Al Aflg	22°00.390’N, 46°31.985’E
7		ON529965	Hawtet bani Tamim	23°37.267’N, 46°38.028’E
8		ON529966	Hawtet bani Tamim	23°37.807’N, 46°31.786’E
9		ON529967	Hawtet bani Tamim	23°36.759’N, 46°39.260’E
10		ON529968	Wadi Ad Dawasir	20°27.345’N, 44°43.213’E
11		ON529969	Wadi Ad Dawasir	20°27.608’N, 44°43.583’E
1	*Psammotermes hypostoma*	OP825131	Rawdhet Khoraim	25°23.176’N, 47°16.270’E
2		OP825132	Rawdhet Khoraim	25°23.228’N, 47°16.665’E
3		OP890882	Rawdhet Khoraim	25°23.006’N, 47°16.313’E
4		OP890883	Rawdhet Khoraim	25°24.145’N, 47°12.625’E
5		OP890884	Rawdhet Khoraim	25°26.069’N, 47°12.846’E
6		OP890885	Rawdhet Khoraim	25°26.833’N, 47°12.389’E
7		OP890886	Rawdhet Khoraim	25°34.367’N, 47°08.566’E
8		OP890887	Rawdhet Khoraim	25°34.356’N, 47°08.560’E
9		OP890888	Al-Muzahmiya	24°29.490’N, 46°22.115’E
10		OP890889	Al Hair	24°26.291’N, 46°50.108’E
11		OP890890	Al Hair	24°26.315’N, 46°50.110’E
12		OP890891	Al Hair	24°26.289’N, 46°50.123’E
13		OP890892	Al Hair	24°32.678’N, 46°14.555’E
14		OP890893	Al Dwadmi	24°28.772’N, 44°20.898’E
15		OP890894	Al Dwadmi	24°28.884’N, 44°21.538’E
16		OP890895	Al Dwadmi	24°28.667’N, 44°21.106’E
17		OP890896	Al Dwadmi	24°31.613’N, 44°21.294’E
18		OP890897	Al Dwadmi	24°34.095’N, 44°29.272’E
19		OP890898	Afif	23°50.494’N, 42°53.029’E
20		OP890999	Afif	23°55.567’N, 42°56.978’E
21		OP890900	Afif	24°10.895’N, 43°13.505’E
22		OP890901	Afif	23°56.425’N, 42°57.487’E
23		OP890902	Afif	23°56.420’N, 42°57.435’E
24		OP890903	Afif	23°56.405’N, 42°57.433’E
25		OP890904	Al Bijadyah	24°17.704’N, 43°40.620’E
26		OP890905	Al Bijadyah	24°18.227’N, 43°44.254’E
27		OP890906	Al Bijadyah	24°17.912’N, 43°44.195’E
28		OP890907	Sajir	25°12.646’N, 44°36.105’E
29		OP890908	Sajir	25°13.391’N, 44°35.957’E
30		OP890909	Sajir	24°09.077’N, 44°38.767’E
31		OP890910	Sajir	24°09.076’N, 44°38.788’E

(87.6-99.5%). In the sequences retrieved from GenBank, *Microcerotermes baluchistanicus* (KY224491) had the highest nucleotide sequence identity of 92.4% with one of the *C. heimi* samples, showing that *M. baluchistanicus*, being a member of the Termitidae, appears to be close enough to *C. heimi*. Whereas, nucleotide sequence identity with Hodotermitidae family members ranged from 75–78.6% (Table 2).

The sequence divergence for all 11 sequences of *C. heimi* at intraspecific and interspecific levels was analyzed using the MegAlign software application (DNA Star Inc., Madison, WI, USA). The 11 studied specimens showed an intraspecific sequence divergence (0.5% to 13.6%), demonstrating a low level of divergence and a highly conserved barcode sequence. This suggests that all specimens are likely to belong to the same species (Table 2). Interspecific sequence divergence between studies specimens and other species sequences belonging to Hodotermitidae retrieved

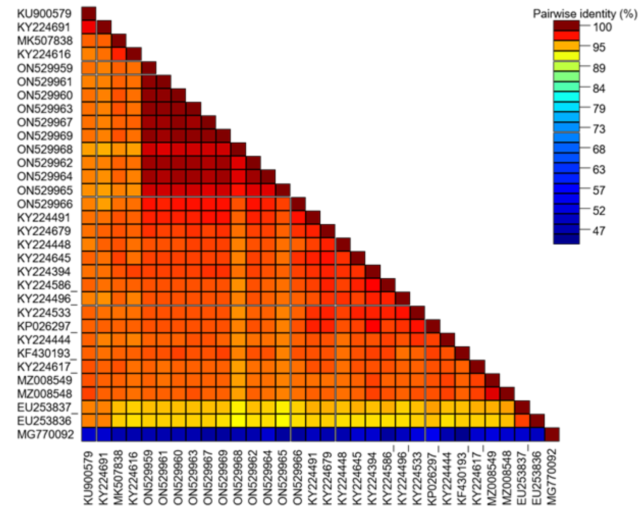


Fig. 2. Pairwise sequence distance of COI gene sequences of *Coptotermes heimi* with sequences of other termite species aligned by CLUSTAL W using sequence demarcation tool (SDT).

from databases showed a high degree of divergence with a minimum interspecific value of 20.1% with *Microhodotermes viator* (EU253837).

1.2. Nucleotide sequence identity/divergence and phylogenetic analysis of *Psammotermes hypostoma*

The obtained 31 COI gene sequences of *Psammotermes hypostoma* species specimens were analyzed and submitted to the GenBank and following accession numbers OP825131- OP825132 and OP890882- OP890910; were obtained. The BLAST analysis showed that all the sequences belong to the same family Rhinotermitidae. Moreover, no COI gene sequence representing *Psammotermes hypostoma* was found in the databases. However, closely related sequences found were members of genus *Coptotermes* and *Amitermes* which belong to family Termitidae and subfamily Termitinae.

Phylogenetic analysis revealed that all sequences were grouped into two clades, indicating that they evolved from two ancestors. These two clades were distinct from all other species' sequences and constituted distinct clades. This confirmed that they were extremely connected, being members of the same species but perhaps evolved from two separate ancestors and demonstrating distance from members of other termite groups (Figure 3). Pairwise sequence identity percentage analysis revealed that analyzed samples grouped in two distinct clades in the phylogenetic tree, one clade of 10 samples (Table 3; shaded with yellow color) showed nucleotide sequence identity of 87-100%,

Table 2. Matrix of pairwise nucleotide sequence identity as well as divergence percentage of 11 *Coptotermes heimi* COI gene sequences with those of other related sequences retrieved from NCBI database. Part of matrix is shaded with yellow background showing intraspecific nucleotide identity of *C. heimi* and unshaded part of matrix is exhibiting interspecific nucleotide sequence identity between *C. heimi* and other termite species. The green shaded part of the matrix shows intraspecific sequence divergence of *C. heimi*.

	ON529959	ON529960	ON529961	ON529962	ON529963	ON529964	ON529965	ON529966	ON529967	ON529968	ON529969
ON529959	***	0.5	0.8	1.7	0.5	1.9	4.6	8.5	1.1	4.9	0.9
ON529960	99.5	***	0.9	1.2	0.6	2	5.1	8.3	0.9	5.1	0.8
ON529961	99.2	99.1	***	2.2	0.9	2.3	4.7	9.2	1.5	5.4	1.4
ON529962	98.3	98.8	97.9	***	1.9	1.7	5.9	7.8	2	6	1.5
ON529963	99.5	99.4	99.1	98.2	***	2	5.1	9	1.2	5.1	1.1
ON529964	98.2	98	97.7	98.3	98	***	6	9.3	2.5	5.7	2
ON529965	95.6	95.1	95.4	94.4	95.1	94.2	***	11.9	5.4	9.4	5.2
ON529966	92.1	92.2	91.5	92.7	91.6	91.3	89	***	8.5	13.6	8.3
ON529967	98.9	99.1	98.5	98	98.8	97.6	94.8	92.1	***	5.1	0.8
ON529968	95.3	95.1	94.8	94.2	95.1	94.5	91.1	87.6	95.1	***	5.2
ON529969	99.1	99.2	98.6	98.5	98.9	98	95	92.2	99.2	95	***
EU253837	78.6	78.3	77.9	78.2	78.5	77.7	75.9	78.2	78.6	75.7	78.5
EU253836	77.7	77.4	77.1	76.8	77.6	76.2	75	77.4	77.6	75	76.9
KF430193	85.8	86	85.6	85.6	85.8	85.2	82.7	85.8	86	82.6	86.3
KP026297	88.7	88.5	88.1	88.1	88.9	87.5	86.1	88.9	88.9	85.3	89
KU900579	82.1	81.8	81.5	81.8	82	81.1	80.2	82	82.1	78.9	82
KY224394	89.8	89.6	89	89.2	89.6	88.5	87.6	89.9	90.5	86.3	90.4
KY224444	87.8	87.6	87.3	86.9	88.2	86.7	85.2	87.5	88.1	84.6	87.8
KY224448	88.7	88.9	88.2	88.7	89.2	87.9	86.7	88.1	89.3	85.6	89.3
KY224491	91.5	91.3	90.7	91.1	91.6	90.7	88.1	**92.4**	91.9	87.3	91.8
KY224496	89.3	88.9	88.5	88.5	89.5	88.2	87.3	87.9	89	85.6	89
KY224533	88.4	87.9	87.6	87.5	88.2	87.2	85.8	87.8	88.1	84.7	88.4
KY224586	88.5	88.4	87.8	88.4	88.7	87.6	86.3	89.3	88.9	84.7	88.9
KY224616	86.9	86.7	86.3	86.1	86.7	85.3	84.6	86.7	87.2	83.4	86.9
KY224617	87.9	87.5	87.2	87.2	87.8	86.9	85.8	87.3	88.1	84	87.9
KY224645	89.9	89.8	89.2	89.5	90.1	88.9	86.9	90.4	89.6	85.6	89.9
KY224679	90.2	89.8	89.5	89.8	90.4	89.3	87.3	90.7	89.9	86.3	90.2
KY224691	81.7	81.4	81.1	81.4	81.5	80.3	79.5	80.3	81.7	78.5	81.7
MK507838	88.1	87.9	87.3	87.8	88.2	87.2	85.6	87.2	87.9	84.6	88.1
MZ008548	88.2	87.8	87.5	87.3	88.4	86.9	86.1	87	88.1	84.1	87.6
MZ008549	87.6	87.2	87	86.7	87.5	86.6	85.3	87	87.9	84	87.6
MG770092	5.5	5.5	5.5	4.6	5.5	4.6	4.6	4	4.6	5.5	5.5
	ON529959	ON529960	ON529961	ON529962	ON529963	ON529964	ON529965	ON529966	ON529967	ON529968	ON529969

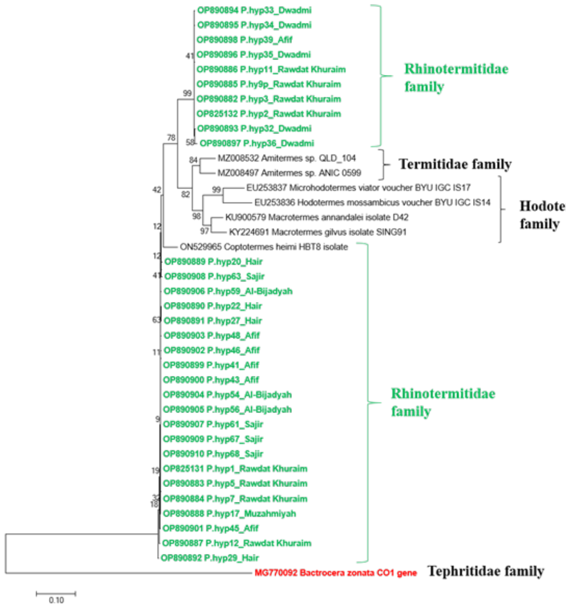


Fig. 3. Phylogenetic tree created with MEGA 7.0 software using maximum-likelihood algorithm. The phylogenetic tree depicts the relationship of 31 *Psammotermes hypostoma* COI gene sequences (indicated with green font color) with those of other closely related members of the same family and with termite families. The sequences used are labeled with the species name and accession number with a bootstrap value (1000) shown at the base of each node.

while the second clade of 21 samples (Table 3; shaded with orange color) showed identity of 78.6-100%. The nucleotide sequence identity of most samples between two clades was as low as 74.6%; however, one of the samples (accession number ON529965) showed a greater identity of 94.9% with *C. heimi*. Nucleotide sequence identity with members of other termite families varied from 67.2 to 87.7%.

Intraspecific sequence divergence varied from 0 to 10.7% among the 31 specimens tested, demonstrating low divergence and indicating all samples belong to the same species (Table 3; shaded with green color). Interspecific sequence divergence of *Psammotermes hypostoma* sequences with those from other Hodotermitidae species retrieved from databases revealed a high degree of divergence, with a minimum interspecific value of 13.1% with *Amitermes sp*.

(MZ008532). Pairwise sequence distance of COI gene sequences of *Psammotermes hypostoma*

along with sequences of other termite species has been shown in (Figure 4).

3. Discussion

The use of universal primers LCO 1490 and HCO 2198 to amplify and sequence COI gene sequences from two subterranean termite species, *Coptotermes heimi,* and *Psammotermes hypostoma*, provides vital information for the study of termite evolution and taxonomy. The DNA barcoding technique has effectively defined the boundaries among species, demonstrating its usefulness for accurately identifying specimens and uncovering new species [16, 17]. Because of the high amount of conservation of mitochondrial genes across taxa, sequences from different termite species can be compared, revealing insights into their evolutionary relationships [18, 19]. Based on the findings, the current study provides a significant resource for future research into the evolutionary links of subterranean termite species.

Several scientists have employed COI gene sequencing to investigate termite evolution and taxonomy. In ormosan subterranean termite, COI gene sequences were used to evaluate the genetic diversity and breeding organization [20]. Whereas, mitochondrial DNA sequences of termites from Peninsular Malaysia has reported 11 species in odontotermes geneus when they used the cytochrome oxidase subunit I genes and concluded that species identification through DNA method has yielded more species of termites [21]. The disparity in the number of sequences retrieved from each species could be attributed to variances in the quantity or accessibility of these species in the research area. According to the results, the nucleotide sequences being compared are substantially similar, with a range of 87.6-100% identity. Furthermore, the divergence between the sequences is relatively low, ranging from 0-0.7%. This implies that the sequences under consideration are most likely from closely related organisms or genetic variants within the same organism. The high percentage of sequence identity shows that there are few differences between the nucleotide sequences, but the low divergence indicates that these differences are insignificant. In line with the present findings, genetic diversity has been reported in samples of Odontotermes formosanus collected from different areas in the Anhui province of China [22]. Several other studies reported the varied genetic differentiation and concluded that it might be helpful to understand the insight of termite dispersal and may elevate the termite colony resistance against pathogens [23, 24]. Overall, these findings indicate that DNA barcoding may reliably differentiate between closely related termite species while also revealing previously unknown genetic variation within populations.

Similarly, DNA-based identification of Genus *Coptotermes* spp. soldier caste has been reported from Indonesia to examine phylogenetic relationships and genetic divergence among *Coptotermes* species [25]. According to this information, the present sequence analysis of both *C. heimi* and *P. hypostoma* species showed highly conserved single clade for phylogenetic study (Figures 1 and 3) in-

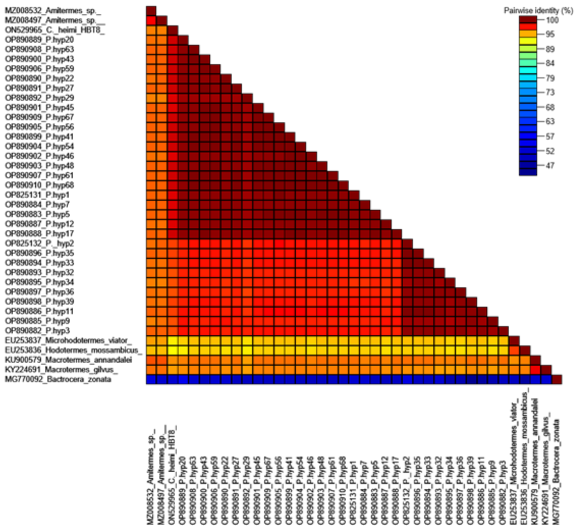
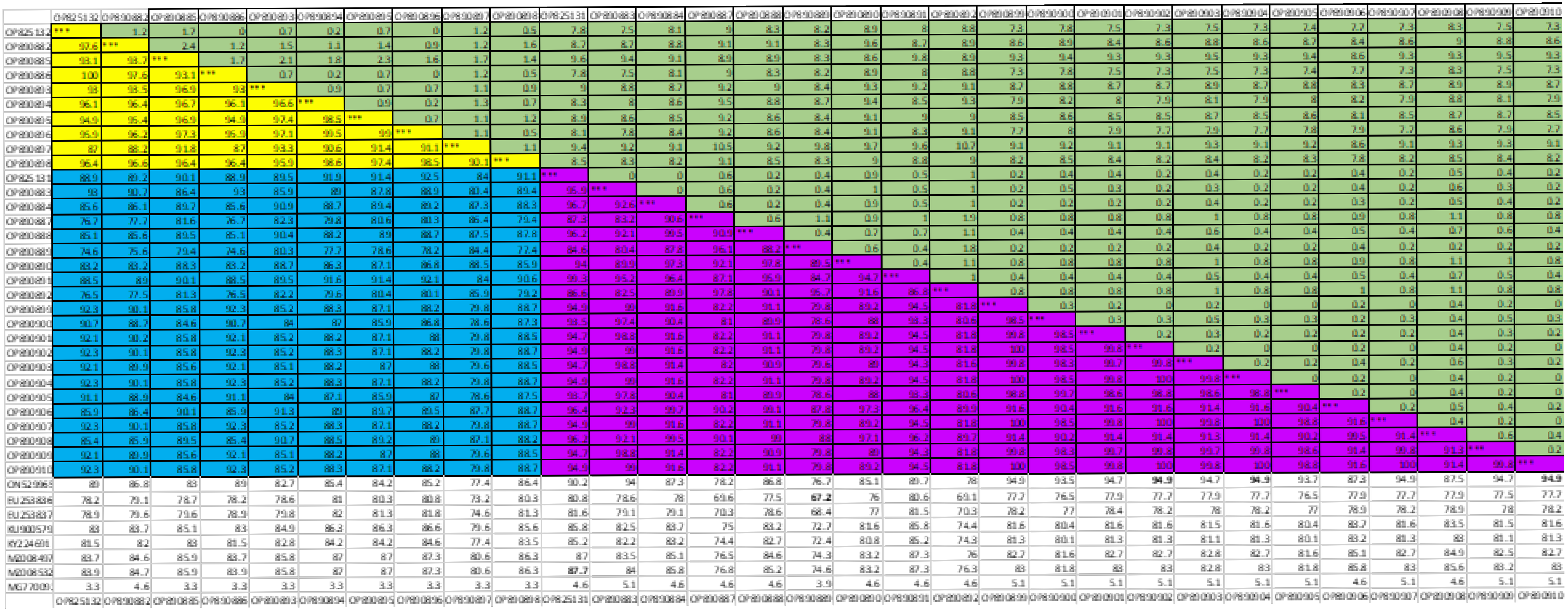


Fig. 4. Pairwise sequence distance of COI gene sequences of *Psammotermes hypostoma* along with sequences of other termite species aligned by CLUSTAL W using sequence demarcation tool (SDT).

Table 3. Matrix of pairwise nucleotide sequence identity as well as divergence (shaded with green background) percentage of 31 *Psammotermes hypostoma* COI gene sequences with those of other related sequences retrieved from NCBI database. *Psammotermes hypostoma* samples (shaded with yellow background) are samples of Clade I showing nucleotide sequence identity with one another and samples of Clade II (shaded with purple background) showing nucleotide sequence identity with one another. The matrix shaded with blue background shows nucleotide sequence identity of *P. hypostoma* samples between two clades.

	OP825132	OP810882	OP810885	OP810886	OP810893	OP810894	OP810895	OP810896	OP810897	OP810898	OP825131	OP810883	OP810884	OP810887	OP810888	OP810889	OP810890	OP810891	OP810892	OP810899	OP810900	OP810901	OP810902	OP810903	OP810904	OP810905	OP810906	OP810907	OP810908	OP810909	OP810910
OP825132	***	1.2	1.7	0	0.7	0.2	0.7	0	1.2	0.5	7.8	7.5	8.1	9	8.3	8.2	8.9	8	8.8	7.3	7.8	7.5	7.3	7.5	7.3	7.4	7.7	7.3	8.3	7.5	7.3
OP810882	97.6	***	2.4	1.2	1.5	1.1	1.4	0.9	1.2	1.6	8.7	8.7	8.8	9.1	9.1	8.3	9.6	8.7	8.9	8.6	8.9	8.4	8.6	8.8	8.6	8.7	8.4	8.6	9	8.8	8.6
OP810885	98.1	98.7	***	1.7	2.1	1.8	2.3	1.6	1.7	1.4	9.6	9.4	9.1	8.9	8.9	8.3	8.6	9.8	8.9	9.3	9.4	9.3	9.3	9.5	9.3	9.4	8.6	9.3	9.3	9.5	9.3
OP810886	100	97.6	98.1	***	0.7	0.2	0.7	0	1.2	0.5	7.8	7.5	8.1	9	8.3	8.2	8.9	8	8.8	7.3	7.8	7.5	7.3	7.5	7.3	7.4	7.7	7.3	8.3	7.5	7.3
OP810893	93	98.5	96.9	98	***	0.9	0.7	0.7	1.1	0.9	9	8.8	8.7	9.2	9	8.4	9.3	9.2	9.1	8.7	8.8	8.7	8.7	8.9	8.7	8.8	8.3	8.7	8.9	8.9	8.7
OP810894	96.1	96.4	96.7	96.1	96.6	***	0.9	0.2	1.3	0.7	8.3	8	8.6	9.5	8.8	8.7	9.4	8.5	9.3	7.9	8.2	8	7.9	8.1	7.9	8	8.2	7.9	8.8	8.1	7.9
OP810895	94.9	95.4	96.9	94.9	97.4	98.5	***	0.7	1.1	1.2	8.9	8.6	8.5	9.2	8.6	8.4	9.1	9	9	8.5	8.6	8.5	8.5	8.7	8.5	8.6	8.1	8.5	8.7	8.7	8.5
OP810896	95.9	96.2	97.3	95.9	97.1	98.5	99	***	1.1	0.5	8.1	7.8	8.4	9.2	8.6	8.4	9.1	8.3	9.1	7.7	8	7.9	7.7	7.9	7.7	7.8	7.9	7.7	8.6	7.9	7.7
OP810897	87	88.2	91.8	87	93.3	90.6	91.4	91.1	***	1.1	9.4	9.2	9.1	10.5	9.2	9.8	9.7	9.6	10.7	9.1	9.2	9.1	9.1	9.3	9.1	9.2	8.6	9.1	9.3	9.3	9.1
OP810898	96.4	96.6	96.4	96.4	95.9	98.6	97.4	98.5	90.1	***	8.5	8.3	8.2	9.1	8.5	8.3	9	8.8	9	8.2	8.5	8.4	8.2	8.4	8.2	8.3	7.8	8.2	8.5	8.4	8.2
OP825131	88.9	89.2	90.1	88.9	89.5	91.9	91.4	92.5	84	91.1	***	0	0	0.6	0.2	0.4	0.9	0.5	1	0.2	0.4	0.4	0.2	0.4	0.2	0.2	0.4	0.2	0.5	0.4	0.2
OP810883	93	90.7	86.4	93	85.9	89	87.8	88.9	80.4	89.4	95.9	***	0	0.6	0.2	0.4	1	0.5	1	0.2	0.5	0.3	0.2	0.3	0.2	0.2	0.4	0.2	0.6	0.3	0.2
OP810884	85.6	86.1	89.7	85.6	90.9	88.7	89.4	89.2	87.3	88.3	96.7	92.6	***	0.6	0.2	0.4	0.9	0.5	1	0.2	0.2	0.2	0.2	0.4	0.2	0.2	0.3	0.2	0.5	0.4	0.2
OP810887	76.7	77.7	81.6	76.7	82.3	79.8	80.6	80.3	86.4	79.4	87.3	83.2	90.6	***	0.6	1.1	0.9	1	1.9	0.8	0.8	0.8	0.8	1	0.8	0.8	0.9	0.8	1.1	0.8	0.8
OP810888	85.1	85.6	89.5	85.1	90.4	88.2	89	88.7	87.5	87.8	96.2	92.1	99.5	90.9	***	0.4	0.7	0.7	1.1	0.4	0.4	0.4	0.4	0.6	0.4	0.4	0.5	0.4	0.7	0.6	0.4
OP810889	74.6	75.6	79.4	74.6	80.3	77.7	78.6	78.2	84.4	77.4	84.6	80.4	87.8	96.1	88.2	***	0.6	0.4	1.8	0.2	0.2	0.2	0.2	0.4	0.2	0.2	0.4	0.2	0.2	0.2	0.2
OP810890	83.2	83.2	88.3	83.2	88.7	86.3	87.1	86.8	88.5	85.9	94	89.9	97.3	92.1	97.8	89.5	***	0.4	1.1	0.8	0.8	0.8	0.8	1	0.8	0.8	0.9	0.8	1.1	1	0.8
OP810891	88.5	89	90.1	88.5	89.5	91.6	91.4	92.1	84	90.6	99.3	95.2	96.4	87.1	95.9	84.7	94.7	***	1	0.4	0.4	0.4	0.4	0.5	0.4	0.4	0.5	0.4	0.7	0.5	0.4
OP810892	76.5	77.5	81.3	76.5	82.2	79.6	80.4	80.1	85.9	79.2	86.6	82.5	89.9	97.8	90.1	95.7	91.6	86.8	***	0.8	0.8	0.8	0.8	1	0.8	0.8	1	0.8	1.1	0.8	0.8
OP810899	92.3	90.1	85.8	92.3	85.2	88.3	87.1	88.2	79.8	88.7	94.9	99	91.6	82.2	91.1	79.8	89.2	94.5	81.8	***	0.3	0.2	0	0.2	0	0	0.2	0	0.4	0.2	0
OP810900	90.7	88.7	84.6	90.7	84	87	85.9	86.8	78.6	87.3	93.5	97.4	90.4	81	89.9	78.6	88	93.3	80.6	98.5	***	0.3	0.3	0.5	0.3	0.3	0.2	0.3	0.4	0.5	0.3
OP810901	92.1	90.2	85.8	92.1	85.2	88.2	87.1	88	79.8	88.5	94.7	98.8	91.6	82.2	91.1	79.8	89.2	94.5	81.8	99.8	98.5	***	0.2	0.3	0.2	0.2	0.2	0.2	0.4	0.3	0.2
OP810902	92.3	90.1	85.8	92.3	85.2	88.3	87.1	88.2	79.8	88.7	94.9	99	91.6	82.2	91.1	79.8	89.2	94.5	81.8	100	98.5	99.8	***	0.2	0	0	0.2	0	0.4	0.2	0
OP810903	92.1	89.9	85.6	92.1	85.1	88.2	87	88	79.6	88.5	94.7	98.8	91.4	82	90.9	79.6	89	94.3	81.6	99.8	98.3	99.7	99.8	***	0.2	0.2	0.4	0.2	0.6	0.3	0.2
OP810904	92.3	90.1	85.8	92.3	85.2	88.3	87.1	88.2	79.8	88.7	94.9	99	91.6	82.2	91.1	79.8	89.2	94.5	81.8	100	98.5	99.8	100	99.8	***	0	0.2	0	0.4	0.2	0
OP810905	91.1	88.9	84.6	91.1	84	87.1	85.9	87	78.6	87.5	93.7	97.8	90.4	81	89.9	78.6	88	93.3	80.6	98.8	99.7	98.6	98.8	98.6	98.8	***	0.2	0	0.4	0.2	0
OP810906	85.9	86.4	90.1	85.9	91.3	89	89.7	89.5	87.7	88.7	96.4	92.3	99.7	90.2	99.1	87.8	97.3	96.4	89.9	91.6	90.4	91.6	91.6	91.4	91.6	90.4	***	0.2	0.5	0.4	0.2
OP810907	92.3	90.1	85.8	92.3	85.2	88.3	87.1	88.2	79.8	88.7	94.9	99	91.6	82.2	91.1	79.8	89.2	94.5	81.8	100	98.5	99.8	100	99.8	100	98.8	91.6	***	0.4	0.2	0
OP810908	85.4	85.9	89.5	85.4	90.7	88.5	89.2	89	87.1	88.2	96.2	92.1	99.5	90.1	99	88	97.1	96.2	89.7	91.4	90.2	91.4	91.4	91.3	91.4	90.2	99.5	91.4	***	0.6	0.4
OP810909	92.1	89.9	85.6	92.1	85.1	88.2	87	88	79.6	88.5	94.7	98.8	91.4	82.2	90.9	79.8	89	94.3	81.8	99.8	98.3	99.7	99.8	99.7	99.8	98.6	91.4	99.8	91.3	***	0.2
OP810910	92.3	90.1	85.8	92.3	85.2	88.3	87.1	88.2	79.8	88.7	94.9	99	91.6	82.2	91.1	79.8	89.2	94.5	81.8	100	98.5	99.8	100	99.8	100	98.8	91.6	100	91.4	99.8	***
ON529966	89	86.8	88	89	82.7	85.4	84.2	85.2	77.4	86.4	90.2	94	87.3	78.2	86.8	76.7	85.1	89.7	78	94.9	93.5	94.7	94.9	94.7	94.9	93.7	87.3	94.9	87.5	94.7	94.9
EU253886	78.2	79.1	78.7	78.2	78.6	81	80.3	80.8	73.2	80.3	80.8	78.6	78	69.6	77.5	67.2	76	80.6	69.1	77.7	76.5	77.9	77.7	77.9	77.7	76.5	77.9	77.7	77.9	77.5	77.7
EU253887	78.9	79.6	79.6	78.9	79.8	82	81.3	81.8	74.6	81.3	81.6	79.1	79.1	70.3	78.6	68.4	77	81.5	70.3	78.2	77	78.4	78.2	78	78.2	77	78.9	78.2	78.9	78	78.2
KU900579	83	83.7	85.1	83	84.9	86.3	86.3	86.6	79.6	85.6	85.8	82.5	83.7	75	83.2	72.7	81.6	85.8	74.4	81.6	80.4	81.6	81.6	81.5	81.6	80.4	83.7	81.6	83.5	81.5	81.6
KY224691	81.5	82	83	81.5	82.8	84.2	84.2	84.6	77.4	83.5	85.2	82.2	83.2	74.4	82.7	72.4	80.8	85.2	74.3	81.3	80.1	81.3	81.3	81.1	81.3	80.1	83.2	81.3	83	81.1	81.3
MZ008497	83.7	84.6	85.9	83.7	85.8	87	87	87.3	80.6	86.3	87	83.5	85.1	76.5	84.6	74.3	83.2	87.3	76	82.7	81.6	82.7	82.7	82.8	82.7	81.6	85.1	82.7	84.9	82.5	82.7
MZ008532	83.9	84.7	85.9	83.9	85.8	87	87	87.3	80.6	86.3	87.7	84	85.8	76.8	85.2	74.6	83.2	87.3	76.3	83	81.8	83	83	82.8	83	81.8	85.8	83	85.6	83.2	83
MG770092	3.3	4.6	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	4.6	5.1	4.6	4.6	4.6	3.9	4.6	4.6	4.6	5.1	5.1	5.1	5.1	5.1	5.1	5.1	4.6	5.1	4.6	5.1	5.1
	OP825132	OP810882	OP810885	OP810886	OP810893	OP810894	OP810895	OP810896	OP810897	OP810898	OP825131	OP810883	OP810884	OP810887	OP810888	OP810889	OP810890	OP810891	OP810892	OP810899	OP810900	OP810901	OP810902	OP810903	OP810904	OP810905	OP810906	OP810907	OP810908	OP810909	OP810910



	OP825132	OP890882	OP890885	OP890886	OP890893	OP890894	OP890895	OP890896	OP890897	OP890898	OP825131	OP890883	OP890884	OP890887	OP890888	OP890889	OP890890	OP890891	OP890892	OP890899	OP890900	OP890901	OP890902	OP890903	OP890904	OP890905	OP890906	OP890907	OP890908	OP890909	OP890910
OP825132	***	1.2	1.7	0	0.7	0.2	0.7	0	1.2	0.5	7.8	7.5	8.1	9	8.3	8.2	8.9	8	8.8	7.3	7.8	7.5	7.3	7.5	7.3	7.4	7.7	7.3	8.3	7.5	7.3
OP890882	97.6	***	2.4	1.2	1.5	1.1	1.4	0.9	1.2	1.6	8.7	8.7	8.8	9.1	9.1	8.3	9.6	8.7	8.9	8.6	8.9	8.4	8.6	8.8	8.6	8.7	8.4	8.6	9	8.8	8.6
OP890885	93.1	93.7	***	1.7	2.1	1.8	2.3	1.6	1.7	1.4	9.6	9.4	9.1	8.9	8.9	8.3	8.6	9.8	8.9	9.3	9.4	9.3	9.3	9.5	9.3	9.4	8.6	9.3	9.3	9.5	9.3
OP890886	100	97.6	93.1	***	0.7	0.2	0.7	0	1.2	0.5	7.8	7.5	8.1	9	8.3	8.2	8.9	8	8.8	7.3	7.8	7.5	7.3	7.5	7.3	7.4	7.7	7.3	8.3	7.5	7.3
OP890893	98	98.5	96.9	93	***	0.9	0.7	0.7	1.1	0.9	9	8.8	8.7	9.2	9	8.4	9.3	9.2	9.1	8.7	8.8	8.7	8.7	8.9	8.7	8.8	8.3	8.7	8.9	8.9	8.7
OP890894	96.1	96.4	96.7	96.1	96.6	***	0.9	0.2	1.3	0.7	8.3	8	8.6	9.5	8.8	8.7	9.4	8.5	9.3	7.9	8.2	8	7.9	8.1	7.9	8	8.2	7.9	8.8	8.1	7.9
OP890895	94.9	95.4	96.9	94.9	97.4	98.5	***	0.7	1.1	1.2	8.9	8.6	8.5	9.2	8.6	8.4	9.1	9	9	8.5	8.6	8.5	8.5	8.7	8.5	8.6	8.1	8.5	8.7	8.7	8.5
OP890896	95.9	96.2	97.3	95.9	97.1	99.5	99	***	1.1	0.5	8.1	7.8	8.4	9.2	8.6	8.4	9.1	8.3	9.1	7.7	8	7.9	7.7	7.9	7.7	7.8	7.9	7.7	8.6	7.9	7.7
OP890897	87	88.2	91.8	87	93.3	90.6	91.4	91.1	***	1.1	9.4	9.2	9.1	10.5	9.2	9.8	9.7	9.6	10.7	9.1	9.2	9.1	9.1	9.3	9.1	9.2	8.6	9.1	9.3	9.3	9.1
OP890898	96.4	96.6	96.4	96.4	95.9	98.6	97.4	98.5	90.1	***	8.5	8.3	8.2	9.1	8.5	8.3	9	8.8	9	8.2	8.5	8.4	8.2	8.4	8.2	8.3	7.8	8.2	8.5	8.4	8.2
OP825131	88.9	89.2	90.1	88.9	89.5	91.9	91.4	92.5	84	91.1	***	0	0	0.6	0.2	0.4	0.9	0.5	1	0.2	0.4	0.4	0.2	0.4	0.2	0.2	0.4	0.2	0.5	0.4	0.2
OP890883	93	90.7	86.4	93	85.9	89	87.8	88.9	80.4	89.4	95.9	***	0	0.6	0.2	0.4	1	0.5	1	0.2	0.5	0.3	0.2	0.3	0.2	0.2	0.4	0.2	0.6	0.3	0.2
OP890884	85.6	86.1	89.7	85.6	90.9	88.7	89.4	89.2	87.3	88.3	96.7	92.6	***	0.6	0.2	0.4	0.9	0.5	1	0.2	0.2	0.2	0.2	0.4	0.2	0.2	0.3	0.2	0.5	0.4	0.2
OP890887	76.7	77.7	81.6	76.7	82.3	79.8	80.6	80.3	86.4	79.4	87.3	83.2	90.6	***	0.6	1.1	0.9	1	1.9	0.8	0.8	0.8	0.8	1	0.8	0.8	0.9	0.8	1.1	0.8	0.8
OP890888	85.1	85.6	89.5	85.1	90.4	88.2	89	88.7	87.5	87.8	96.2	92.1	99.5	90.9	***	0.4	0.7	0.7	1.1	0.4	0.4	0.4	0.4	0.6	0.4	0.4	0.5	0.4	0.7	0.6	0.4
OP890889	74.6	75.6	79.4	74.6	80.3	77.7	78.6	78.2	84.4	77.4	84.6	80.4	87.8	96.1	88.2	***	0.6	0.4	1.8	0.2	0.2	0.2	0.2	0.4	0.2	0.2	0.4	0.2	0.2	0.2	0.2
OP890890	83.2	83.2	88.3	83.2	88.7	86.3	87.1	86.8	88.5	85.9	94	89.9	97.3	92.1	97.8	89.5	***	0.4	1.1	0.8	0.8	0.8	0.8	1	0.8	0.8	0.9	0.8	1.1	1	0.8
OP890891	88.5	89	90.1	88.5	89.5	91.6	91.4	92.1	84	90.6	99.3	95.2	96.4	87.1	95.9	84.7	94.7	***	1	0.4	0.4	0.4	0.4	0.5	0.4	0.4	0.5	0.4	0.7	0.5	0.4
OP890892	76.5	77.5	81.3	76.5	82.2	79.6	80.4	80.1	85.9	79.2	86.6	82.5	89.9	97.8	90.1	95.7	91.6	86.8	***	0.8	0.8	0.8	0.8	1	0.8	0.8	1	0.8	1.1	0.8	0.8
OP890899	92.3	90.1	85.8	92.3	85.2	88.3	87.1	88.2	79.8	88.7	94.9	99	91.6	82.2	91.1	79.8	89.2	94.5	81.8	***	0.3	0.2	0	0.2	0	0	0.2	0	0.4	0.2	0
OP890900	90.7	88.7	84.6	90.7	84	87	85.9	86.8	78.6	87.3	98.5	97.4	90.4	81	89.9	78.6	88	98.3	80.6	98.5	***	0.3	0.3	0.5	0.3	0.3	0.2	0.3	0.4	0.5	0.3
OP890901	92.1	90.2	85.8	92.1	85.2	88.2	87.1	88	79.8	88.5	94.7	98.8	91.6	82.2	91.1	79.8	89.2	94.5	81.8	99.8	98.5	***	0.2	0.3	0.2	0.2	0.2	0.2	0.4	0.3	0.2
OP890902	92.3	90.1	85.8	92.3	85.2	88.3	87.1	88.2	79.8	88.7	94.9	99	91.6	82.2	91.1	79.8	89.2	94.5	81.8	100	98.5	99.8	***	0.2	0	0	0.2	0	0.4	0.2	0
OP890903	92.1	89.9	85.6	92.1	85.1	88.2	87	88	79.6	88.5	94.7	98.8	91.4	82	90.9	79.6	89	94.3	81.6	99.8	98.3	99.7	99.8	***	0.2	0.2	0.4	0.2	0.6	0.3	0.2
OP890904	92.3	90.1	85.8	92.3	85.2	88.3	87.1	88.2	79.8	88.7	94.9	99	91.6	82.2	91.1	79.8	89.2	94.5	81.8	100	98.5	99.8	100	99.8	***	0	0.2	0	0.4	0.2	0
OP890905	91.1	88.9	84.6	91.1	84	87.1	85.9	87	78.6	87.5	98.7	97.8	90.4	81	89.9	78.6	88	98.3	80.6	98.8	99.7	98.6	98.8	98.6	98.8	***	0.2	0	0.4	0.2	0
OP890906	85.9	86.4	90.1	85.9	91.3	89	89.7	89.5	87.7	88.7	96.4	92.3	99.7	90.2	99.1	87.8	97.3	96.4	89.9	91.6	90.4	91.6	91.6	91.4	91.6	90.4	***	0.2	0.5	0.4	0.2
OP890907	92.3	90.1	85.8	92.3	85.2	88.3	87.1	88.2	79.8	88.7	94.9	99	91.6	82.2	91.1	79.8	89.2	94.5	81.8	100	98.5	99.8	100	99.8	100	98.8	91.6	***	0.4	0.2	0
OP890908	85.4	85.9	89.5	85.4	90.7	88.5	89.2	89	87.1	88.2	96.2	92.1	99.5	90.1	99	88	97.1	96.2	89.7	91.4	90.2	91.4	91.4	91.3	91.4	90.2	99.5	91.4	***	0.6	0.4
OP890909	92.1	89.9	85.6	92.1	85.1	88.2	87	88	79.6	88.5	94.7	98.8	91.4	82.2	90.9	79.8	89	94.3	81.8	99.8	98.3	99.7	99.8	99.7	99.8	98.6	91.4	99.8	91.3	***	0.2
OP890910	92.3	90.1	85.8	92.3	85.2	88.3	87.1	88.2	79.8	88.7	94.9	99	91.6	82.2	91.1	79.8	89.2	94.5	81.8	100	98.5	99.8	100	99.8	100	98.8	91.6	100	91.4	99.8	***
ON52996	89	86.8	83	89	82.7	85.4	84.2	85.2	77.4	86.4	90.2	94	87.3	78.2	86.8	76.7	85.1	89.7	78	94.9	93.5	94.7	94.9	94.7	94.9	93.7	87.3	94.9	87.5	94.7	94.9
EU253836	78.2	79.1	78.7	78.2	78.6	81	80.3	80.8	73.2	80.3	80.8	78.6	78	69.6	77.5	67.2	76	80.6	69.1	77.2	76.5	77.9	77.7	77.9	77.7	76.5	77.9	77.7	77.9	77.5	77.7
EU253837	78.9	79.6	79.6	78.9	79.8	82	81.3	81.8	74.6	81.3	81.6	79.1	79.1	70.3	78.6	68.4	77	81.5	70.3	78.2	77	78.4	78.2	78	78.2	77	78.9	78.2	78.9	78	78.2
KU900579	83	83.7	85.1	83	84.9	86.3	86.3	86.6	79.6	85.6	85.8	82.5	83.7	75	83.2	72.7	81.6	85.8	74.4	81.6	80.4	81.6	81.6	81.5	81.6	80.4	83.7	81.6	83.5	81.5	81.6
KY224601	81.5	82	83	81.5	82.8	84.2	84.2	84.6	77.4	83.5	85.2	82.2	83.2	74.4	82.7	72.4	80.8	85.2	74.3	81.3	80.1	81.3	81.3	81.1	81.3	80.1	83.2	81.3	83	81.1	81.3
MZ008497	83.7	84.6	85.9	83.7	85.8	87	87	87.3	80.6	86.3	87	83.5	85.1	76.5	84.6	74.3	83.2	87.3	76	82.7	81.6	82.7	82.7	82.8	82.7	81.6	85.1	82.7	84.9	82.5	82.7
MZ008532	83.9	84.7	85.9	83.9	85.8	87	87	87.3	80.6	86.3	87.7	84	85.8	76.8	85.2	74.6	83.2	87.3	76.3	83	81.8	83	83	82.8	83	81.8	85.8	83	85.6	83.2	83
MG77009	3.3	4.6	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	4.6	5.1	4.6	4.6	4.6	3.9	4.6	4.6	4.6	5.1	5.1	5.1	5.1	5.1	5.1	5.1	4.6	5.1	4.6	5.1	5.1
	OP825132	OP890882	OP890885	OP890886	OP890893	OP890894	OP890895	OP890896	OP890897	OP890898	OP825131	OP890883	OP890884	OP890887	OP890888	OP890889	OP890890	OP890891	OP890892	OP890899	OP890900	OP890901	OP890902	OP890903	OP890904	OP890905	OP890906	OP890907	OP890908	OP890909	OP890910

dicating all 42 specimens belong only to two species previously identified based on phenotypic traits [15]. Maximum likelihood algorithm had been previously used for phylogenetic relationships of termites by other studies [26, 27]. In several morphological and molecular studies of termites, molecular data confirmed the morphological data [28]. The identified termite species are most commonly distributed in the dry habitat area of the Kingdom of Saudi Arabia. Due to the availability of suitable habitats, in dry areas, these termite species appear to have a greater geographical spread. As these species prefer arid environments, a larger amount of precipitation, moist soils, and cooler air temperatures in the Kingdom of Saudi Arabia and other parts of the world make it rare for the species to be found there [3, 15, 29]. It is predicted that additional termite records for the Kingdom of Saudi Arabia may be added through future collecting.

5. Conclusions

Finally, the use of universal primers LCO 1490 and HCO 2198 to amplify and sequence COI gene sequences from *Coptotermes heimi* and *Psammotermes hypostoma* gives essential information for the study of termite evolution and taxonomy. The current study added to our understanding of genetic diversity and evolutionary links of subterranean termite species and served as a foundation for future research into these insects' evolutionary history.

Conflict of Interests

The author has no conflicts with any step of the article preparation.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

No human or animals were used in the present research.

Informed Consent

The authors declare not used any patients in this research.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

Conceptualization, A.S.A. and M.R.S.; specimen collection, K.D.S., M.H., and K.G.R.; Methodology, M.H., M.R.S and K.D.S; Software, M.T. A.S.A., and W.S.A.; writing-original draft preparation, M.H; and K.G.R.; review and editing, K.D.S., W.S.A., M.T., and A.S.A., and M.R.S.

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