International Journal of Tropical Insect Science
https://doi.org/10.1007/s42690-020-00287-5

ORIGINAL RESEARCH ARTICLE

First interception of two wood feeding potential invasive *Coptotermes* termite species in India

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Received: 23 December 2019 / Accepted: 25 September 2020

Abstract

Several termite species are significant economic pests, causing damage to wood in both man-made structures and also in forest ecosystem. The species belongs to genus *Coptotermes* (Rhinotermitidae: Isoptera: Blattodea) are widespread and few of them are among the world's most dangerous wood pests. Two species of *Coptotermes* were collected, identified and described from yemene, mora and tali logs imported from Columbia, Suriname and Equitorial Guinea. These two intercepted species are non-native to India which are *Coptotermes testaceus* (Linnaeus) and *Coptotermes sjöstedti* Holmgren. Identification was initially made with morphological characters and then with molecular DNA barcoding for a 348 bp fragment of 16S rRNA gene. Maximum likelihood Phylogenetic analysis results showed that data recovered as monophyletic which confirms the species identity. *Coptotermes testaceus* and *C. sjöstedti* are in single clade. *Coptotermes testaceus* is neotropical in origin and *C. sjöstedti* is native to Africa. Both the species are known to be serious pests in human habitats and forest ecosystem in the regions of their distributions. This first interception suggests possible chances of entry and establishment in India and provides an important alert for authorities concerned to take the necessary steps in monitoring and preventing its possible introduction. The importance of quarantine and potential infliction of damage they may cause if unnoticed and future measures to be taken are discussed.

Keywords *Coptotermes testaceus* · *C. sjöstedti* · DNA barcoding · 16S rRNA gene · Invasive · Maximum likelihood · Monophyletic

Introduction

Through globalization and trade, huge numbers of exotic species of insects move from continent to continent, resulting in significant economic loss. Many termite species are major economic pests, causing wood damage both in man-made buildings and in the forest environment. Around 28 termite species are considered invasive throughout the world and have spread outside their native ranges, often with significant economic implications (Buczkowski and Bertelsmeier 2017). The most important subterranean genera are the genus *Coptotermes* Wasmann (Rhinotermitidae) in which few species are known to be invasive. For example, a subterranean Asian termite, *Coptotermes gestroi* (Wasmann) has been introduced by trade into the new world and now causing significant economic loss (Gay 1967; Ghesini et al. 2011; Li et al. 2013).

Coptotermes is one of the most common subterranean termite genera of economic importance and some species are effective invaders. Nevertheless, the taxonomic validity of many species of *Coptotermes* remains unclear, given their significant pest status. The *Coptotermes* termite genus currently consists of 69 existing species distributed in Asia, Australia, Africa, and the New World (Krishna et al. 2013). Because this genus is specious, widespread, pestiferous and lacks robust interspecific diagnostic morphology, *Coptotermes* has more synonymous species than any other genus of termite (Krishna et al. 2013). The challenge of morphologically sorting *Coptotermes* is exacerbated by

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Published online: 31 October 2020

intraspecific character variation (Emerson 1971) and increased body mass with colony age (Grace 2014).

Of the 21 *Coptotermes* species that we considered valid, 16 species currently have major pest status (Krishna et al. 2013). This confirms the ecological success of this genera and their ability to establish themselves in disturbed environments. It also confirms the worldwide economic effect of the genus (Rust and Su 2012). However, due to extensive research on some of these species in non-native areas, the general perception that the genus is a major invader may have been distorted. While it is widely accepted that when associated with human activity *Coptotermes* is a 'great invader' (Evans 2010; Evans et al. 2013; Chouvenc et al. 2016). India is importing timber from different countries where many wood feeding termites can sneak in which may leads to economic and ecological impact. The problem may be accentuated when pest species introduce establishes in Indian condition. Herewith we report the interception of two non-native species of *Coptotermes*: *Coptotermes testaceus* (Linnaeus) and *Coptotermes sjöstedti* Holmgren from a quarantine station of India. The importance of quarantine and potential infliction of damage they may cause if not monitored and future measures to be taken are discussed.

Material and methods

Collection of termites

Termite specimens (soldiers and workers) were collected by Plant Quarantine Station, Tuticorin from imported mora logs (*Maclora tinctoria*), tali wood logs (*Erythrophleum* sp.) and yemene wood (*Gmelina* sp.). The collected specimens were stored in 70% ethyl alcohol and held as a permanent collection. The voucher specimens (UAHS/ RFID/2019001, UAHS/RFID/2019002, UAHS/RFID/2019003 and UAHS/ RFID/2019004) were deposited at Department of Entomology, College of Agriculture, UAHS, Shivamogga, Karnataka.

Termite identification

Morphological identification

For morphological identification, the soldier caste was used. The specimens collected were identified to species level using the keys provided by Maiti (2006). Important taxonomic characters studied were photographed using Leica M205C (Wetzlar and Mannheim, Germany) with auto montage. Description of both the species identified was provided.

Molecular identification

Genomic DNA extraction

Total genomic DNA from a single worker termite from *Coptotermes* species was extracted. The species *Coptotermes* was washed with distilled water and air dried on a piece of sterilized filter paper. The sample was frozen in liquid nitrogen and was ground to fine a powder. The extract was taken in 1.5 ml micro centrifuge tubes. Added 800 μl of sterile STE buffer (50 mM Sucrose, 25 mM Tris-HCl pH 7.0, 10 mM EDTA) and grinded using pestle. It was also treated with Proteinase K at 55 °C for 30 min, followed by addition of 10% SDS and incubated for 3 h. The gDNA was purified by adding an equal volume (approximately 500 μl) of phenol:chloroform mixture (25:24) to the extraction buffer mixture. The DNA was precipitated and resuspended with ethanol in 25 μl TE buffer (10 mM Tris, 1 mM EDTA, pH 8) (Yeap et al., 2009) and stored at −20 °C until further use.

Quantification of *Coptotermes* gDNA

The concentration by *Coptotermes* gDNA samples was determined by nanodrop analysis (qualitative and quantitative) and electrophoresing DNA samples on 0.8% agarose gel. By comparing the intensity of ethidium bromide fluorescence with that of the known DNA concentration norms, the concentration of the interest fragment was predicted. Two μl CWSB DNA sample was loaded with an agarose gel of 0.8%. The gel was run for 30 min at 90 V. The DNA bands were then visualized and photographed under ultraviolet light. Two μl of genomic DNA were qualified and quantified using a spectrophotometer (NanoDrop, Thermo Scientific) at 260 nm (OD260) and 280 nm (OD280). The quantity of DNA was directly recorded from spectrophotometer. DNA quality was indicated by the ratio of OD260/OD280, which should be in the range of 1.8 to 2.0.

Molecular identification of *Coptotermes* species using 16S rRNA gene

From the gDNA, a fragment of ~348 base pairs of the 16S rRNA gene was amplified. The forward primer Chiar16SF (5'-TARTYCAACACGRCGTC) and reverse primer Chiar16SR (5'-CYGTRCDAAGGTAGCATA) were used for PCR amplification purpose. The PCR amplification reactions were conducted using a thermal cycler (Eppendorf, CA) in 25 μl volume containing 3 ul genomic DNA, 1 ul of 10 mM dNTP, 0.7 ul of 10pM primer, 2.5 ul of 10× reaction buffer, 0.7 ul of 25 mM $MgCl_2$, 0.3 ul of DMSO, 1 ul of 2 unit of Taq DNA polymerase (Genie) with the following conditions: initial denaturation at 94 °C for 5 min, 35 cycles of (94 °C-40s;

47 °C-45 s; 72 °C-60s) and a final extension of 72 °C for 10 min. The PCR negative control contained the identical amount of PCR mixture with 5 μl distilled water instead of DNA template. A PCR positive control was also included, containing the PCR mixture plus DNA that had been successfully put through the PCR reaction on previous studies. PCR amplification products were separated by electrophoresis in a 1.5% agarose gel in TAE buffer (40 mM Tris-acetate [pH 8.0], 1 mM EDTA) at 100 V for 1 h. The gel was then visualized under UV light.

Cloning, sequencing and sequence analysis of the *Coptotermes* 16S rRNA gene

Using a NucleoSpin® Gel and PCR clean up kit (Macherey-Nagel) as directed by the manufacturer, *Coptotermes* 16S gene PCR products noted as single sharp and transparent bands on agarose gel were eluted and purified. The standard recombinant DNA techniques used in cloning were, according to the Sambrook> and Russell (2001). The *E. coli* DH5α cells were transformed with PCR-amplified ~348 bp *Coptotermes* 16S gene ligated in pTZ57R (T/A cloning vector) using Fermentas DNA ligation kit (#K1214). The transformed cells (20 μl) were spread on LB agar plates containing X-gal (270 μg/ml), IPTG (120 μg/ml) and ampicillin (100 μg/ml). The plates were then incubated at 37 °C for 24 h to screen blue and white colonies. Cloning was confirmed by colony PCR, plasmid mobility check and restriction analysis of recombinant plasmid DNA containing *Coptotermes* 16S gene. The *Coptotermes* 16S gene was sequenced, to determine the homology with the known sequences in the NCBI GenBank database. Sequencing was performed in triplicates of the above clones in an automated sequencer (ABI prism® 3730 XL DNA Analyzer; Medauxin, Bengaluru) using M13 universal primers, both in the forward and reverse directions.

Coptotermes 16S rRNA gene bioinformatics and phylogenetic analysis

Homology search was carried out using NCBI-Basic Local Alignment Search Tool (BLAST, http://www.ncbi.nlm.nih.gov) identification database. With the sequence alignment editor, BioEdit version 7.0.5.3, the differences in nucleotide sequences of all specimens were determined. Using the BioEdit Clustal W program, *Coptotermes* 16S rRNA sequences were aligned. The sequences were further analyzed using MEGAX (Kumar et al. 2018) to obtain conspecific and congeneric distances, whilst Maximum Likelihood (ML Heuristic Method: Nearest-Neighbor-Interchange) trees were constructed using the Kimura-2-parameter (K2P) distance model (Kimura 1980) employing MEGAX (Kumar et al. 2018). All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). The sequences were deposited to NCBI GenBank database.

Results

Coptotermes testaceus (Linnaeus, 1758)

Morphological characters of collected specimens were studied and described (Fig. 1)

Materials examined: 1. INDIA: Tamil Nadu, Tuticorin, Reg No. IR21Tut2018010712, Dt. 26.ix.2018, Coll. N. Kasturi and D.K. Nagarju, Intercepted in imported yemene wood from Columbia. 2. INDIA: Tamil Nadu, PQS, Tuticorin, Reg. No. IR 9888, Dt. 06.vii.2018, Coll. Maharaj Singh, Intercepted in imported mora logs from Suriname. 3. INDIA: Tamil Nadu, PQS, Tuticorin, Dt. 06.vii.2018, Coll. R.K. Jain, Intercepted in imported mora logs from Suriname.

Description

General appearance:

Soldier: Head-capsule straw-coloured to yellow, fontanelle region paler; mandibles dark brown, rest of body pale. Head moderately hairy and with one bristle on either side of fontanelle; pronotum with a small hairs on anterior margin; rest of the body densely hairy. Eyes and ocelli absent; Legs with femora long; tibiae long and slender; apical tibial spurs 3:2:2; tarsi four jointed and hairy. Abdomen elongate, and densely hairy; cerci 2-jointed.

Head: Head capsule pyriform, longer than broad (length to mandible base 1.25 mm, width 1.08 mm); converging anteriorly; posterior margin rounded. Fontanelle glandular line visible from head-surface; the opening is round at posterior margin of clypeus with a thick brown chitinous rim. Antennae with 14 segments, segment three longer than two or four. Labrum subtriangular; with a pointed hyaline apex; a little longer than broad (length 0.29 mm, width 0.24 mm); broadest near base; with a pair of long bristles just below hyaline tip. Mandibles thin, sabre-shaped, with strongly inwardly pointed apices. Left mandible with fairly large basal projection and four small well marked crenulations. Right mandible without any crenulations. Postmentum long, club-shaped; broadest at anterior one-third and from there narrowing down to thin waist just below the middle and again widening out at base (length 0.62 mm, maximum width 0.32 mm, minimum width 0.21 mm). Pronotum flat, subreniform, much broader than long, anterior and posterior margin with a weak emargination, antero lateral corners strongly convex.

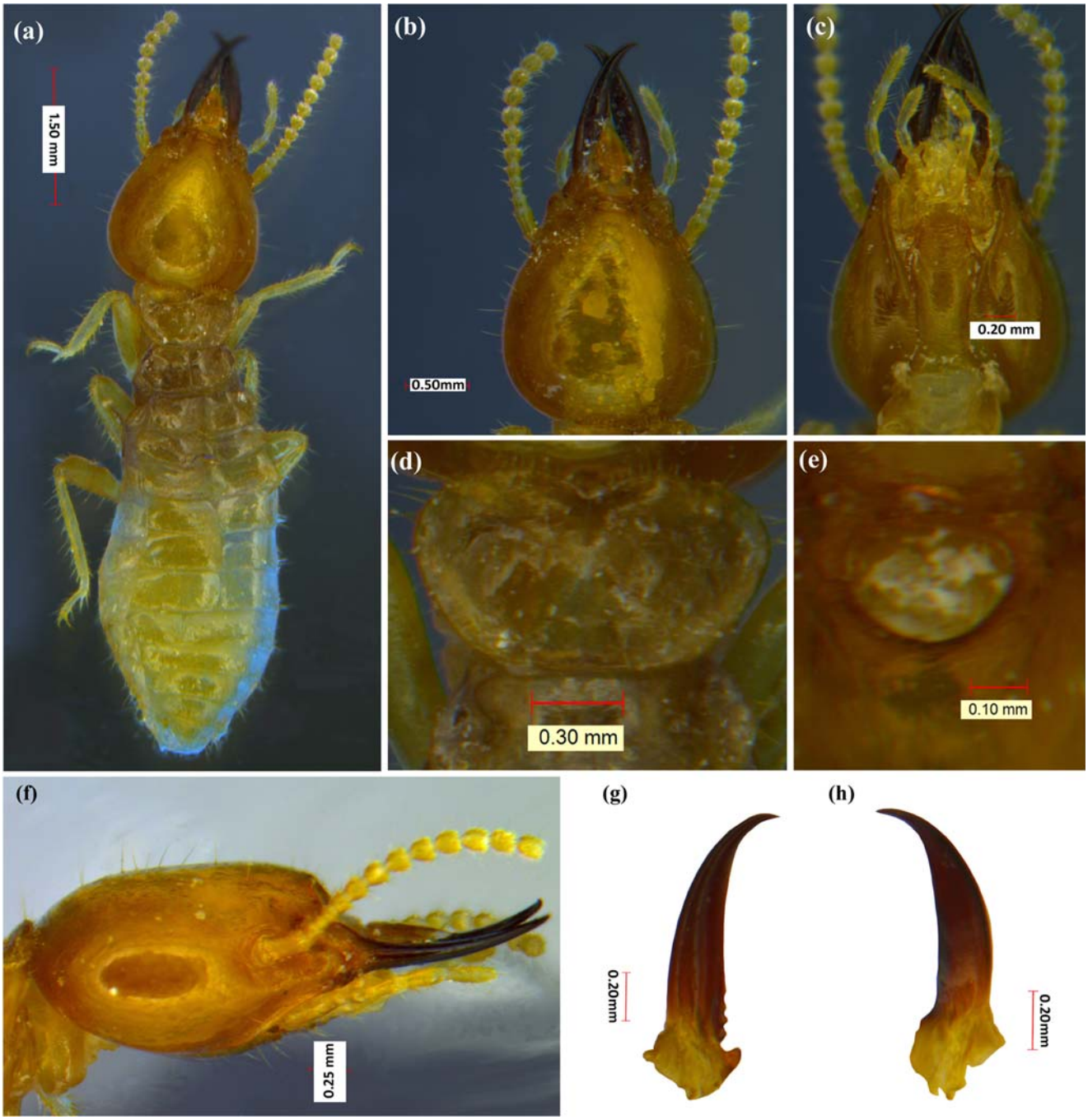

Fig. 1 **a** *Coptotermes testaceus* soldier, **b** Dorsal view of head, **c** Ventral view of head, **d** Pronotum dorsal view, **e** Frontal gland opening, **f** Lateral view of head, **g** Left mandible of soldier, **h** Right mandible of soldier

Coptotermes sjöstedti Holmgren, 1911

Figure 2 materials examined: INDIA: Tamil Nadu, Tuticorin, Reg No., IR21Tut2019000898; Dt. 06.ii.2019, Coll. N. Kasturi; Intercepted in imported Tali logs of Equitorial Guinea.

Description

General appearance

Soldier: Head-capsule straw-coloured to yellow, fontanelle region paler; antennae straw-coloured; mandibles reddish

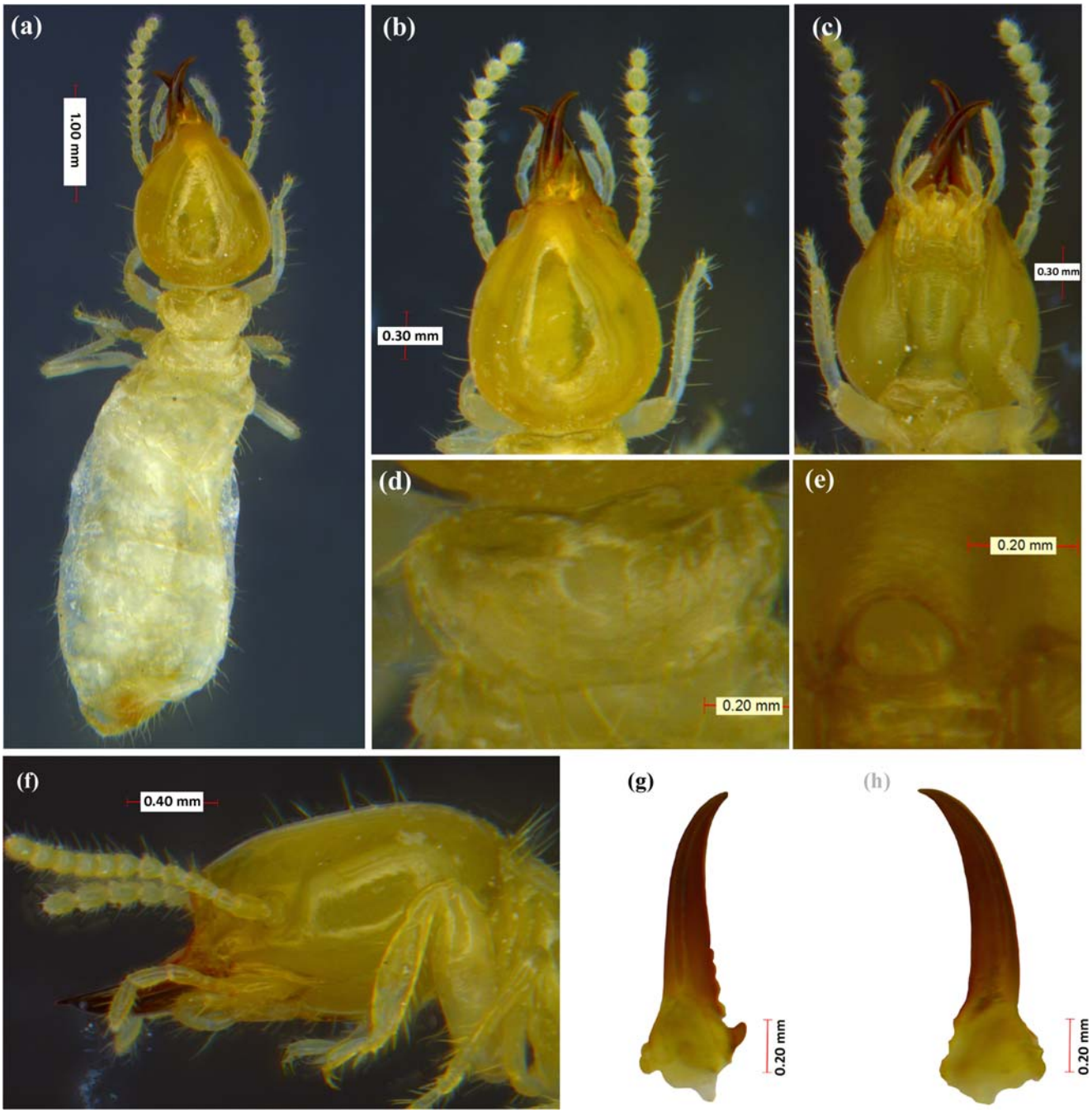

Fig. 2 **a** *Coptotermes sjöestedti* soldier, **b** Dorsal view of head, **c** Ventral view of head, **d** Pronotum dorsal view, **e** Frontal gland opening, **f** Lateral view of head, **g** Left mandible of soldier, **h** Right mandible of soldier

to dark brown, rest of body pale. Head with a few erect bristles and with one bristle on either side of fontanelle; pronotum with a long hairs along the margin and longer ones on body. Eyes and ocelli absent; Legs with femora long; tibiae long and slender; apical tibial spurs 3:2:2; tarsi four jointed. Abdomen elongate, with a row of hairs on posterior margins of terga and some scattered ones on body; cerci 2-jointed.

Head :Head capsule pyriform, slightly longer than broad (length to mandible base 1.07 mm, width 0.94 mm); strongly converging anteriorly; posterior margin rounded. Fontanelle glandular line visible from head-surface; the opening is round with a thin brown chitinous rim connecting the posterior aspect of postclypeus. Antennae with 12 segments, segment three variable, subequal to four and smaller than two. Labrum subtriangular, with a pointed hyaline apex; a little longer than broad (length 0.21, width

0.18 mm); broadest near base; with a pair of long bristles just below hyaline tip. Mandibles thin, sabre-shaped, with inwardly pointed apices. Left mandible with fairly large basal projection and four small well marked crenulations at the basal half. Right mandible without any crenulations. Postmentum long, club-shaped; broadest at anterior one-third and from there narrowing down to thin waist just below the middle and again widening out at base (length 0.50 mm, maximum width 0.31 mm, minimum width 0.21 mm). Pronotum flat, subreniform, much broader than long, anterior margin with a week emargination, antero lateral corners slightly rounded, posterior margin substraight with a small depression in middle.

The imported wood materials were fumigated with Methyl Bromide @48 g/CuM for 24 h to destroy all the termites to avoid their entry further.

Coptotermes molecular identification and phylogenetic analysis

The genomic *Coptotermes* gDNA obtained was of high quality using the presented methodology. The concentration of *Coptotermes* species gDNA varied between 42.5 and 110.0 ng/μl. The 260:280 absorbance ratios met pure DNA demands and ranged from 1.76 to 1.82 in most instances. The gDNA extraction method's effectiveness has been shown by amplifying and sequencing the 16S rRNA gene. PCR for *Coptotermes* gDNA samples had produced the expected PCR amplicon size (~ 348 bp), which was verified with an agarose gel of 1.5%.

The expected *Coptotermes* 16S rRNA gene PCR amplicon (~348 bp) was eluted from the agarose gel using Nucleospin Extract II as per manufacture's protocol (Machery Nagal, Germany) subsequently cloned and sequenced. The ligated PCR products were incubated along with competent DH5α cells. Transformed and non-transformed cells were selected by Blue-white screening. It is a rapid and efficient technique for the identification of recombinant bacteria. For a better selection of transformed cells substreaking was followed on LB agar with ampicillin and smeared with Xgal and IPTG. Later a colony, which contained the PCR insert, was referred back to the replica plate. The colony was isolated by a sterile culture loop and streaked onto a LB agar plate with 100 μg/ml ampicillin. The plate was incubated at 37 °C for 16–24 h or until colonies appeared on the medium subjected to colony PCR for reconfirmation of the correct transformants.

Three single colonies from each transformation reaction, that contained a fragment *Coptotermes* 16S rRNA gene of the expected size, were cultured and purified for plasmid DNA extraction using GeneJET Plasmid Midiprep Kit (Thermo Scientific) following the manufacturer's instructions.

The colony PCR confirmed transformants from the substreaked plates were inoculated in LB broth (containing ampicillin) for multiplication. Plasmids were isolated from the multiplied bacterial cells and thus isolated plasmids were visualized on 1% agarose gel electrophoresis and compared with control plasmids (without insert) that were provided with ligation kit (Thermo Scientific, Fermentas, Lithuania). These purified recombinant plasmids with the *Coptotermes* 16S rRNA gene were resuspended in 30 μl of elution buffer. 10 μl plasmid samples from each resuspended replications were sent for Sanger's sequencing. Sequencing reactions were performed in both M13 forward and reverse direction. The rest of the plasmids were preserved in −80 °C. The sequence obtained from automated sequencer from ABI prism. The sequences were compared using NCBI BLAST with the sequences already available in NCBI GenBank database. The results revealed that the sequences submitted were 97.20 to 99.16% identical with the previously published *C. testaceus*. Whereas *C. sjöestedti* sequence showed 96.62% to 97.91% homology with 100% query coverage. These results confirm the *Coptotermes* species identity. *Coptotermes testaceus* 16S rRNA gene sequences were deposited with NCBI GenBank accession number MK559590, MK559591, MK559592 and MK559593. Whereas *C. sjöestedti_IR21Tut2019000898* 16S rRNA gene got NCBI GenBank accession number MN540914.

The detailed analysis of the gene nucleotide sequence showed the significantly high percentage of content of A + T base composition in each of the two species being examined. The sequence base composition of various species of termites depicted average composition of 22.33% (A), 44.38% (T), 8.66% (C), and 24.62% (G) (Table 1). The overall transition/transversion bias is R = 1.64. The comparison of *C. testaceus* with KM588291_*C. testaceus*_Trinidad and Tobago sequence did not show any nucleotide variation except at 307 and 389 position (C-T). Whereas *C. sjöestedti* showed 9 nucleotide

Table 1 Maximum composite likelihood estimate of the pattern of nucleotide substitution

	A	T	C	G
A	–	*4.06*	*0.79*	19.74
T	*2.05*	–	7.19	*2.25*
C	*2.05*	36.85	–	*2.25*
G	17.91	*4.06*	*0.79*	–

Note: Each entry shows the probability of substitution (r) from one base (row) to another base (column) [1]. For simplicity, the sum of r values is made equal to 100. Rates of different transitional substitutions are shown in bold, and those of transversionsal substitutions are shown in *italics*. The nucleotide frequencies are 22.33% (A), 44.38% (T/U), 8.66% (C), and 24.62% (G). This analysis involved 26 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 254 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [2]

variations with KT215899_*Coptotermes sjöestedti* sequences. Using MEGA-X (Molecular Evolutionary Genetics Analysis Version X), phylogenetic analysis was carried out. The aligned sequences, accessible *Coptotermes* species sequences in NCBI GenBank have been used to build phylogenetic tree (Fig. 3). Whereas the gene sequence of *Cubitermes fungifaber* 16S rRNA gene served as an out-group. The Maximum Likelihood phylogenetic tree showed that the individuals of the same species clustered together based on the 16S rRNA gene sequence similarity, regardless of their collection site and host. The classification of the *C. testaceus* and *C. sjöestedti* species is in accordance with the geographic locations and their taxonomic positions.

Discussion

Despite ecological benefits of termites, they are also major pests that cause damage to human-built structures (Su and Scheffrahn 1998; Scheffrahn et al. 2015) and tropical

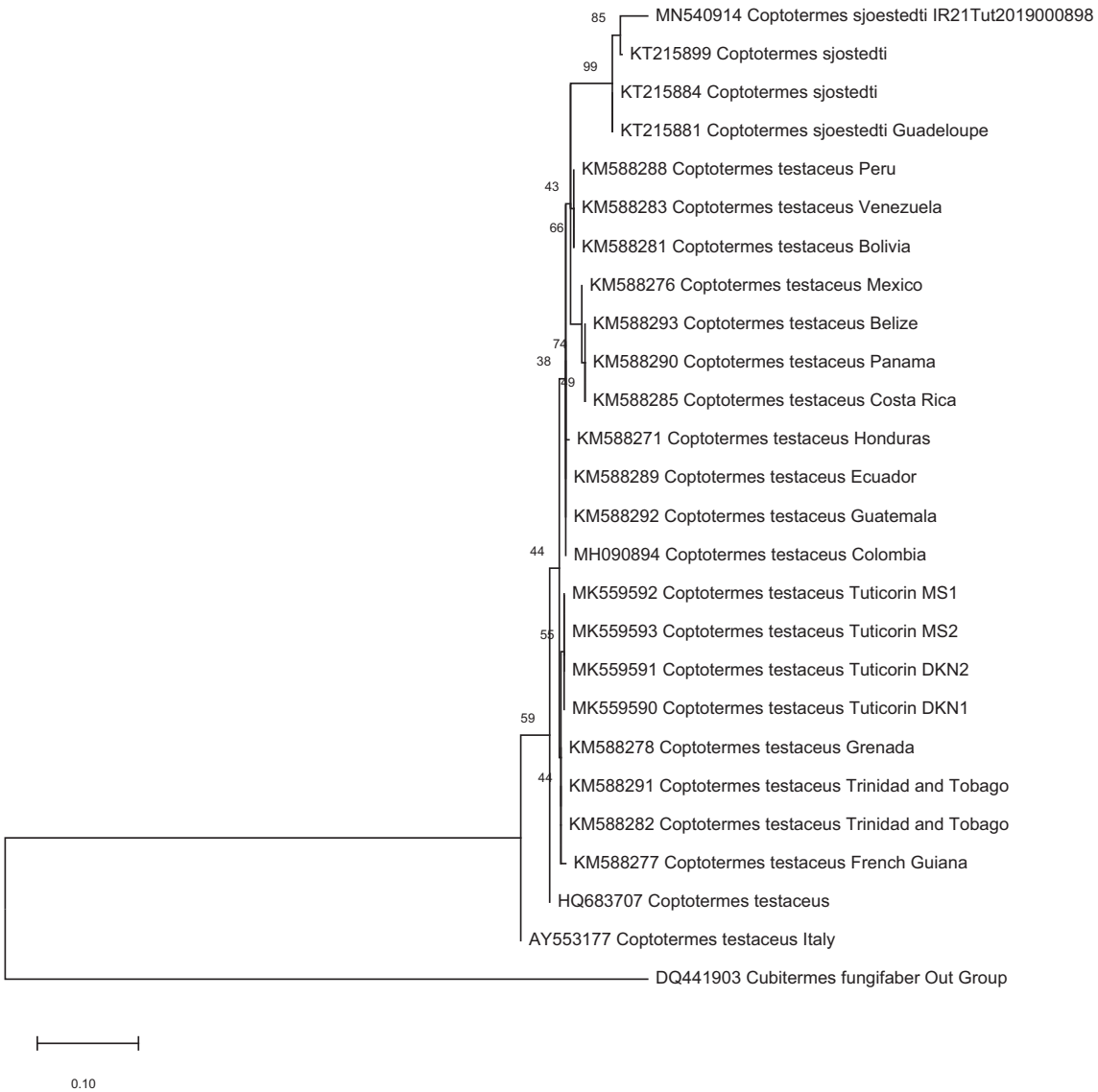

Fig. 3 The phylogenetic relationships of *Coptotermes* species samples, inferred from the 348 bp 16S rRNA gene sequence using the Maximum likelihood method based on the Kimura 2-parameter model. Bootstrap values (2,000 replications) reproduced in <60% replicates were collapsed and the bootstrap values are shown at the branch points. The GenBank accession numbers of the corresponding reference sequence are indicated. The analysis involved 26 nucleotide sequences. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. Codon positions included were 1st+2nd +3rd+ Noncoding. All positions containing gaps and missing data were eliminated. The nucleotide sequences were aligned using the MUSCLE and the evolutionary analyses were conducted in MEGA-X. There were a total of 983 positions in the final dataset

agriculture (Rouland-Lefèvre 2011). Of the 300 termites so far known from India, approximately 35 species have been identified as harmful to agricultural crops and timbers (Rajgopal 2002). He classified *C. ceylonicus* and *C. heimi* within the genus *Coptotermes* as structural pests. The dominant species tends to be *C. heimi*, with a wide distribution from the Pakistan Indus River Valley to Nepal and most of India. Mahapatro and Chatterje (2018) studied the existence of thirteen termites species belonging to three structural damage-related families in India. Although there is no systematic work on the structure loss caused by termites, both *Heterotermes* and *Coptotermes* (Rhinotermitidae) in India are serious pests. *Coptotermes* are the main cause of damage to wood-based construction materials in Malaysia in urban areas and rural settlements (Tho and Kirton 1992). Although mostly trees are targeted by *Coptotermes*, they also sometimes damage crops. *Coptotermes formosanus*, for example, was recorded in China and Japan as harmful to groundnuts and other food crops (Sands 1973). The amount of damage that happens can be affected by seasonal changes in foraging groups. *Coptotermes curvignathus* may attack healthy young rubber trees in Malaysia within 3–4 weeks. Adding any species to the current one has a lot of economic and ecological effects on India where climate suitability can aid in developing and spreading further. Species movement around the world and new interactions between allopatric organisms can also have unforeseen consequences.

Species of *Reticulitermes*, *Heterotermes* and *Coptotermes* are some of the worst attackers (Bourguignon et al. 2016). The *Coptotermes* genus is considered to be largely invasive and mainly distributed in tropics (Scheffrahn et al. 2015). In the new world they recorded one native species and three exotic species of *Coptotermes*, despite the worldwide classification of 69 *Coptotermes* species (Krishna et al. 2013). There are currently only 21 species that are considered valid (Chouvenc et al. 2016). There is confusion about the validity of species due to the widespread existence of *Coptotermes* and the authors' non-comparison of type specimens. There are cases that have taken hundreds of years to overcome *Coptoterms* taxonomic confusion (Li et al. 2010). Termite taxonomy and species classification have long been problematic and only recently molecular diagnostic methods have been used to answer questions regarding invasive termites origins and sinks (Evans et al. 2013). Of the six species described in Fauna of India and adjacent countries by Roonwal and Chhotani (1989), only four species are known to occur in India.

There is taxonomic confusion for correct species identity of the Indian *Coptotermes*. The reports of occurrence of *C. gestroi* and *C. travians* are uncertain (Chouvenc et al. 2016). Among the remaining four species validity of *C. beckeri*, *C. ceylonicus* and *C. kishori* is uncertain. Chouvenc et al. (2016) suggests junior synonomy for these three species under *C. amanii* (Sjöstedt), *C. brunneus* Gay and *C. kalshoveni* Kemmer. Among these three, *C. amanii* is African origin, *C. brunneus* is Australian orgin and *C. kalshoveni* is appears to be serious pests of wood in South East Asia (Indonesia and Malyasia).

The worldwide economic influence of the *Coptotermes* genus is considered high (Rust and Su 2012). *Coptotermes* are known to be 'a great invader' and 'as urban pests' once combined with human activity. Since the *Coptotermes* may feed on heartwood and softwood, the weather available may be ideal for setting up and spreading. In the United States, where efforts were made to understand the pest risk assessment of importation of unprocessed eucalyptus logs, economically, environmentally and socially, moderate to heavy damage was reported (Kliejunas et al. 2001). Damage can, however, rely on the species aggressiveness and climatic suitability. *Coptotermes testaceus* and *C. sjöstedti* are in single clade. Two species of termites, *C. testaceus* and *C. sjöstedti* have been identified in this study based on both morphological and molecular characteristics. *Coptotermes testaceus* is native to the neotropical region, but the species is also recorded in Trinidad and Tobago. *Coptotermes sjöstedti* is native to Senegal from Ethiopia and was not reported outside the Africa. *Coptotermes sjöstedti* mainly occurs in West Africa's lowland Equatorial rainforest belt. It also occurs in woodland remnants and thickets in the moister savannas (Harris 1966a, b) at the extreme eastern limits of its distribution. It has the potential to nest inside living trees in the tree trunks, there is every opportunity to spread to new geographical areas. Soil-covered runways may suggest *C. sjöstedti*'s existence on tree trunks or logs. Logs may also have internal galleries and a honeycomb-like structure similar to a soil or carton nest (Becker 1975). At the time of shipment, log fumigation must reduce the risk of introductions. It is possible to use a less environmentally harmful fumigant like sulfuryl fluoride (Cabi 2016). A strict policy recommendation for the importing country and strict monitoring of imported material could minimize the intrusion of termites into a new geographical region, reducing the risk both economically and ecologically. The report may helps all the quarantine stations of India to be alert and for the wood exporting countries.

Although the competitive displacement of native species by an invasive species is not reported in case of termites, the ecological and economic importance of invasive termites is extremely large. The most significant positive environmental impacts can be attributed to the activities of termites as soil ecosystem engineers. The most important negative impacts are the activities of termites as structural and agricultural pests. In recent decades the tendency of expansion of the range of harmful species of termites has led to an increase in economic damage. Invasive termites have been shown to adapt their reproductive phenology in response to climate change (Chouvenc et al. 2016). In parts of Florida, the dispersal flight season of *C. formosanus* and *C. gestroi* has begun to overlap

due to changes in local climate, and lead to to hybridization between the two species and the potential evolution of highly destructive "super-termites" due to hybrid vigor. Such a possibility should not be eliminated and need to be monitored over the years in India.

Conclusions

Molecular and phylogenetic analysis demonstrated the precise technique of species-level *Coptotermes* detection. *Coptotermes* 16S rRNA gene was cloned and sequenced. Analysed nucleotide sequences were found without pseudogenes and indels; they match with high similarity in nucleotide Basic Local Alignment Search Tool search identification. DNA barcoding is not replacing traditional morphological species identification; the combined use of morphological and molecular analysis can provide a very powerful tool for identifying pest at the species level. Maximum likelihood (ML) phylogenetic tree analysis showed that the individuals of the same species clustered together based on the 16S rRNA gene sequence similarity, regardless of their collection site and host. These results suggest that the use of molecular approach is crucial in ensuring accurate species identification. In conclusion, no previous attempts have been made to provide a molecular identification and DNA barcode for the *Coptotermes* species from India, therefore the data represented herein are new. Nevertheless, the robust quarantine system and measures introduced in India can intercept and stop any termites incursions. There are no records of invasive termites being introduced into India so far, but robust quarantine measures are required for unexpected entry. Further, interception of quarantine pests triggers the revision of Pest Risk Analysis and updation of import regulations.

Acknowledgments We thank the Plant Protection Advisor, Directorate of Plant Protection, Quarantine and Storage, Faridabad, India for encouragement and facility. CMK thanks Ministry of Environment, Forest and Climate change, GoI for funding under AICOPTAX (F.No. 22018-28/2019-CS(Tax).

Compliance with ethical standards

Conflict of interest The authors declare that no conflict of interest exists.

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