Intrusion pathway of invasive Asian subterranean termite, *Coptotermes gestroi* (Wasmann) from the Neotropics into the Indian mainland

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***Coptotermes* is one of the most widespread subterranean termite genus of economic significance with few species considered as truly invasive. *Coptotermes gestroi* (Wasmann) is also known to be invasive and has taxonomic confusion on its correct identity. Originally described from Myanmar, it is considered to occur in North East India and Thailand. However, it is found to cause serious economic losses in some Asian countries, Brazil, the Caribbean islands and peninsular Florida, USA. In the present study, the insect specimens which were recovered from imported wooden packaging material of a consignment at Goa, India, received from Harrisonburg, Virginia, USA have been reported. The termite species received was identified as *C. gestroi* through morphological characters and DNA barcoding. The possible infliction caused if invasive populations of *C. gestroi* enters India, in particular southern India, is discussed.**

Keywords: *Coptotermes gestroi*, intrusion pathway, invasive species, morphological and molecular identification, subterranean termites.

COPTOTERMES Wasmann (Blattodea: Isoptera: Rhinotermitidae) is widespread and few species are among the world's highly threatening wood pests. The Asian subterranean termite, *Coptotermes gestroi* (Wasmann) and the Formosan subterranean termite, *Coptotermes fomosanus* Shiraki are the two most devastating termite species in the world which cause an annual loss of US $40 billion[1]. Buczkowski and Bertelsmeier[2] through predictive climate modelling provided risk assessment for 13 of the world's most invasive termites, which include *C. gestroi* at a global scale. Among several species of *Coptotermes*, *C. gestroi* is infamously known to be invasive and has taxonomic confusion on its correct identity. The species was first described from Myanmar (formerly Burma; Wasmann 1902) and was believed to occur only in this region, including Thailand and North East India[3]. Due to morphological variation in a few characters, new species names were given, but eventually synonyms were given and adopted in India, the Pacific Islands, Pakistan and Southeast Asia. In the Neotropical region, *C. gestroi* was first described as *C. vastator* and then under the name *C. havilandi* Holmgren it spread from Asia to Brazil in 1936, into the Caribbean[4], and into peninsular Florida, USA[5].

Along with the junior synonyms for *C. gestroi*, such as the destructive *C. heimi* (Wasmann)[6], confusion and misidentification with other valid species of the genus have also commonly occurred. In Southeast Asia, *C. gestroi* was sometimes wrongly identified as *C. travians* (Haviland), whereas the true *C. travians* was also misidentified as *C. havilandi* in peninsular Malaysia[7]. In the Pacific Islands, *C. gestroi* was mistakenly identified as *C. formosanus* in Guam[8,9]. As described by Li *et al.*[10], the following are all now considered as junior synonyms of *C. gestroi*: *C. havilandi* Holmgren, *C. heimi* (Wasmann), *C. javanicus* Kemner, *C. obliqus* Xia and He, *C. pacificus* Light, *C. parvulus* Holmgren, *C. vastator* Light, and *C. yaxianensis* Li. In addition, although it is yet to be formally synonymized, the Sri Lankan species, *C. ceylonicus* Holmgren was also considered as a part of the *C. gestroi* complex[11], and similar enough in morphology to *C. gestroi* to possibly be the same species. Due to such confusions, the actual occurrence of *C. gestroi* in India is in doubt, which needs thorough sampling and taxonomic studies. After a report by Roonwal and Chhotani[3], there is no clear evidence of its presence in India. Maiti[12] clearly mentions that the holotype was not designated by Wasmann and syntype was not traceable in Wassman's collections in the Natural History Museum, Maastricht, The Netherlands. In the fauna of Indian Isoptera (termites) and the adjacent countries[3] and in the taxonomic monograph published by Maiti[12], there is no description of imago stages. Hence the report of swarming of *C. gestroi* in north-eastern Puducherry by Harit *et al.*[13] is doubtful, wherein they have identified the species based on the imago caste which the previous taxonomists have not described from India so far. Harit *et al.*[13] also cited the publication by Rathore and Bhattacharyya[14] which documents the fauna of termites in Gujarat, where the species does not exist, nor does the publication contain the species description of *C. gestroi*. Our strong emphasis here is on the fact that if the species is present in India, it has not been collected and described after 1961. Krishna *et al.*[15] considered only 21 *Coptotermes* species as valid among 69 species described worldwide. Out of the 69 valid species listed by them[15], 50% is described from limited material (e.g. one caste described, single colony of origin, or a single alate, etc.)[11]. Another lacuna in these studies was that there was no comparison with previously described specimens. Hence thorough scrutiny of the validity of Indian *Coptotermes* is warranted. Further, there is a need of describing imago caste to avoid confusion.

Among 21 valid *Coptotermes* species, 16 are considered as largely invasive. In this study, the presence of *C. gestroi* collected from imported wooden packaging

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Table 1. Measurements of *Coptotermes gestroi* imagoes received in comparison to previous studies[7,24]

	Characters	Present study material	Kirton and Brown[7]; Li *et al.*[24]
1	Head length to tip of labrum	1.45–1.52	1.34–1.66
2	Head length to lateral base of mandible	1.08–1.12	0.85–1.15
3	Maximum head width	1.38–1.50	1.34–1.53
4	Maximum diameter of eyes	0.38–0.42	0.39–0.46
5	Minimum diameter of eyes	0.35–0.40	0.34–0.40
6	Maximum diameter of ocellus	0.15–0.17	0.13–0.18
7	Minimum diameter of ocellus	0.10–0.12	0.10–0.13
8	Maximum width of pronotum	1.25–1.30	1.14–1.44
9	Minimum width of pronotum	0.75–0.80	0.76–0.94

$N = 6$.

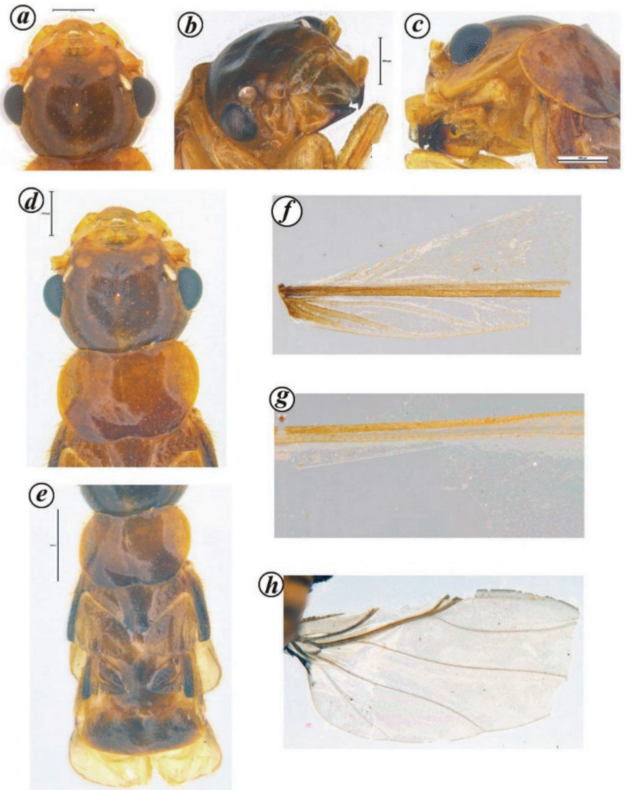

Figure 1. ***a***, Head (dorsal view); ***b***, head (mandibles in view); ***c***, head (lateral view); ***d***, head with pronotum; ***e***, thorax in part; ***f–h***, wing remnants.

from Harrisonburg, Virginia, USA, has been reported. The possible infliction caused, if entered, is discussed.

Dead insect specimens (approximately 50 nos) were found in the packaging material. The imago specimens were used for morphological identification. The morphological characters examined were photographed using Leica M205C (Wetzlar and Mannheim, Germany) which has auto montage facility. The samples of *C. gestroi* received were imagoes and majority were damaged. Morphological comparison was made with previously published materials and measurements of *C. gestroi* imagoes were found to agree with those given by Kirton and Brown[7] (Table 1). Head capsule colour light to moderate dark brown. Head subcircular with length almost similar to width (head length 1.45 mm; maximum head width 1.38 mm) and covered with several setae. Antenna with 19–21 segments; first and second sparsely hairy, remaining hairy; first cylindrical, longest; second cylindrical, shorter and narrower than the first; third shortest; third to twentieth moniliform, last antennomere elongate elliptic. Labrum with anterior margin broadly rounded, broadest at middle, sides slightly converging posteriorly; central area convex; anterior border transparent; anterior with six long setae, central area with several long setae. Eyes subcircular (maximum diameter 0.38 mm; minimum diameter 0.35 mm). Ocellus elongated (maximum diameter 0.15 mm; minimum diameter 0.10 mm). Pronotum flat, brownish, narrower than the head, wider anteriorly, posterior margin with median notch and densely hairy. Abdomen oblong, densely hairy. Wings membrane hairy with brownish veins (Table 1 and Figure 1).

The specimens were further processed for molecular identification and voucher specimens were deposited in the National Insect Museum of National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru, India. Further, the identity of the specimen was confirmed through DNA barcoding by amplifying the CO1 gene. A small tissue of the specimen was used for DNA extraction employing the Qiagen DNeasy® kit and the remaining parts of the specimen were stored as voucher at 70°C at NBAIR. DNA was used for PCR amplification of the 5′-terminus of CO1 gene (658 bp)[16]. The following primers, viz. forward: LCO 1490 5′-GGTCAACAAATCATAAAGATATTGG-3′ and reverse: HCO 2198 5′-TAAACTTCAGGGTGACCAAAAAATCA-3′ were used for amplification of the CO1 gene. PCR reaction was carried out in 200 μl volume, flat-capped tubes which had 50 μl reaction volume containing: 5 μl GeNeiTM *Taq* buffer, 1 μl GeNeiTM, 10 mM dNTP mix, 1 μl (20 pmol/μl) forward primer 1 μl (20 pmol/μl) reverse primer 1 μl, GeNeiTM *Taq* DNA polymerase (1 U/μl), 5 μl DNA (50 ng/μl) and 36 μl sterile water. Standard PCR conditions were followed using a BioRad C1000™ Thermal Cycler. The amplified products were viewed on 1.5% agarose gel electrophoresis[17] and sequenced using the ABI3130 (M/S Chromous, Bengaluru, India) platform. The samples were

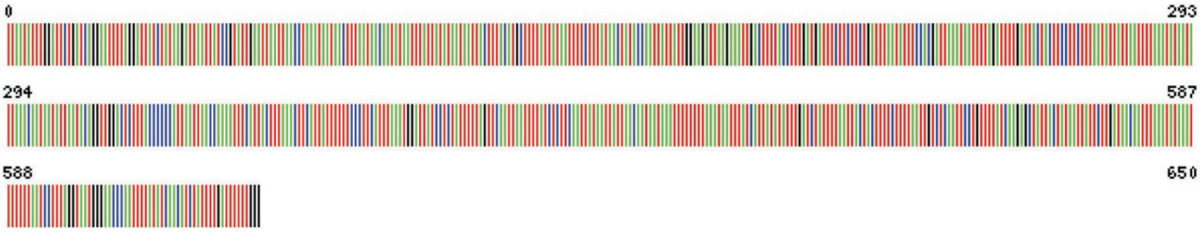

Figure 2. DNA barcode of *Coptotermes gestroi* with accession number MW575256.

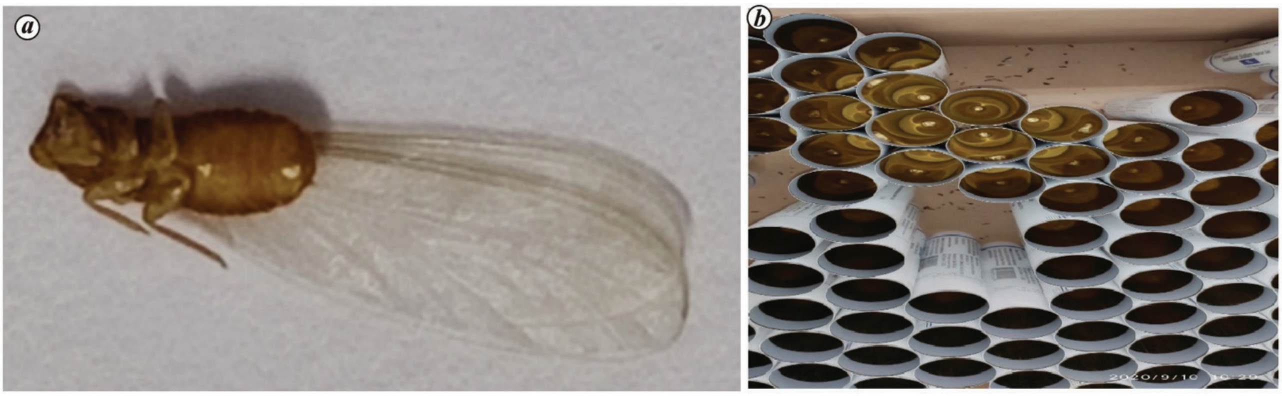

Figure 3. Dead insects and their parts observed in the packing material corrugated box after deboxing. ***a***, Collected termite species. ***b***, Photograph of the corrugated box in which termites were observed.

checked for homology, insertions and deletions, stop codons and frame shifts using NCBI-BLAST and ORF finder. Blast analysis revealed that the sequence obtained showed 99% match to KC887198 (China), KC887197 (China) and KJ934505 (Malaysia). Further the specimen was confirmed as *Coptotermes gestroi* and GenBank accession number (MW575256) was received. DNA barcode was obtained from BOLD systems (Figure 2).

So far, around 28 termite species are considered as invasive and among them genus *Coptotermes* is known to cause significant economic loss around the world[2,10,18]. Recently, two species of *Coptotermes*, viz., *C. testaceus* and *C. sjöstedti* have been intercepted in India[19] and thus it is important to have continuous monitoring of any invasive species entering the country. The negative impact of *C. gestroi* is huge involving environmental, economic[7] and social (where historic structures are at risk) aspects[5,20]. According to the global projection of termite invasion risks under scenarios of climate change (with predictions for 2050 and 2070), the spread of *C. gestroi* is well anticipated[2,10]. With these prediction models, we hypothesize, that if native populations of *C. gestroi* are present in India, they would have been reported often as the country possesses both geographic and climatic suitability for their distribution. After six decades of its first report in India[3], the first confirmative transportation pathway of *C. gestroi* straight from the neotropics to the Indian mainland, raises serious concerns on the quarantine measures taken at the place of origin for checking the spread of serious invasive pest species. Furthermore, from visual inspection, this pest species most probably has entered through the wooden structures/packaging material used for shipping a consignment from Harrisonburg, Virginia, USA, as the consignment was intact during transit and on receipt until the box was opened. During the course of transit, the termite images emerged and subsequent mortal remains (in the absence of any possible escape route) were observed and later recovered after opening the box (Figure 3). It is well proven that boat infestations[21] have accelerated the spread of *C. gestroi* along the coasts of tropical Florida[22] and numerous West Indian islands[23].

The discovery of this unwelcome and serious invasive species in India can be considered as a fortunately failed reintroduction owing to anthropogenic dispersal. As the adults were recorded, there will be high chances of colonization in the area of invasion. Also, it raises serious concerns about the execution of standard protocols for quarantine measures to be followed during export at the place of packaging (Harrisonburg, Virginia, USA). Finally a word of caution to all the quarantine authorities, exporters and importers involved. In this era of biosecurity threat and biological invasion, we need to be careful and proactive and while handling international trade.

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Laevicaulis haroldi (Veronicellidae: Gastropoda), a potential future invader to India

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Invasive alien species are considered one of the greatest threats to biodiversity, ecosystem services, economy and human health. Global climate change will only exacerbate the impact of several invasive species in the introduced range. Hence the control and management of invasive species is crucial. Spatial tools such as GIS/RS and ecological niche models can help understand the potential region where the species might invade and predict invasive spread under different climate change scenarios. This study explores if the newly introduced slug from South Africa, *Laevicaulis haroldi* (Purcell's hunter slug or caterpillar slug) will become invasive in India under current as well as future climate scenarios. Our result suggests that most parts of western and Peninsular India are vulnerable to the invasion, and suitable regions will only increase

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