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First record of the invasive Asian subterranean termite, *Coptotermes gestroi*, from Egypt

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Abstract

The Asian subterranean termite, *Coptotermes gestroi* (Wasmann 1896) (Isoptera Rhinotermitidae), is a structural pest endemic to Southeast Asia that has been spread anthropogenically to much of the tropics worldwide. We report a land-based establishment of *C. gestroi* in Alexandria, Egypt, the northernmost locality of this species and over 4000 km from its nearest previous locality in northern India. An earlier study suggested that *C. gestroi*, misidentified as *Reticulitermes lucifugus* (Rossi 1792), was in Alexandria in 2010. We confirm *C. gestroi* in Alexandria based on soldier morphology and COII sequence data. We also report that *Coptotermes alexandrinus* Mohammad et Ramadan 2023, is a junior synonym of *C. gestroi*. The world distribution of *C. gestroi* is presented.

Key words: *Coptotermes*, establishment, invasive species, world distribution, genetic sequencing, Palearctic Region.

Introduction

The termite genus *Coptotermes* Wasmann 1896 occurs in all tropical and subtropical zoogeographic regions of the world (Chouvenc *et al*., 2016). Two *Coptotermes* species, *Coptotermes formosanus* Shiraki 1909 and *Coptotermes gestroi* (Wasmann 1896), both major pests, have been spread by maritime transport locally (Hochmair and Scheffrahn, 2010; Hochmair *et al*., 2023), regionally (Scheffrahn and Su, 2005), globally (Scheffrahn and Crowe, 2011). *C. gestroi* exhibit significant morphological similarities to *Coptotermes heimi* (Wasmann 1902) (Roonwal and Chhotani, 1962), according to Procheş and Ramdhani (2012)'s definition of the western Palearctic Region, *C. heimi* spread from Pakistan and India to Oman (Chhotani, 1988), the United Arab Emirates (Chouvenc *et al*., 2016), and Saudi Arabia (Sharaf *et al*., 2021).

Although *Coptotermes* sp. was unknowingly first reported in Alexandria, Egypt as *Reticulitermes lucifugus* (Rossi 1792) (El-Sebay *et al*., 2010), *Coptotermes* sp. was not confirmed in Alexandria until 2022 by Eldakak *et al*. (2022). Subsequently, this unidentified species was described as *Coptotermes alexandrinus* Mohammad et Ramadan 2023 (Mohammad *et al*., 2023) based on non-diagnostic morphological characters. Herein, we report that the Alexandria *Coptotermes* is actually *C. gestroi* and we synonymize *C. alexandrinus* into *C. gestroi* based on morphological and molecular characters. We provide the current worldwide distribution of *C. gestroi* and compare the soldier of *C. gestroi* with that of its closest sibling, *C. heimi*.

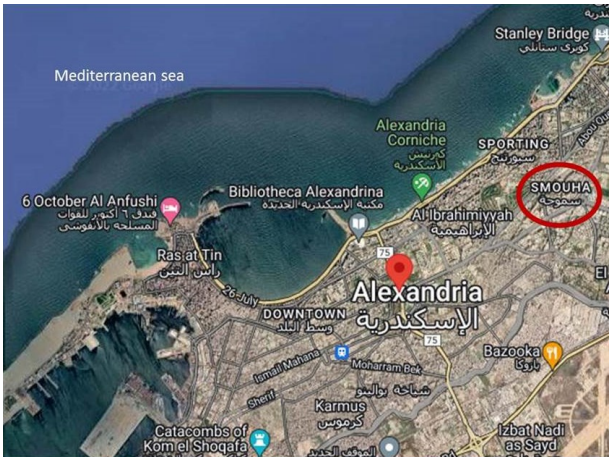

Figure 1. Areal street view of *C. gestroi* localities in Alexandria, Egypt. Source: Google Maps®.

Materials and methods

Collection and identification of termites

Soldiers and workers of *Coptotermes* were collected from an infested building at Smouha, Alexandria, Egypt (31.2020N 29.9164E) in November 2020 and September 2022 (figure 1). One soldier and some workers are deposited at the Ain Shams University Collection (ASUC), Department of Entomology, Ain Shams University, Egypt, other soldiers and workers are deposited in the University of Florida Termite Collection (UFTC), Davie, Florida, as AFR3834 (Scheffrahn, 2019). Some specimens were stored in 95% ethanol and other was preserved in −20 °C for molecular studies. For photography (figures 2 and 3), soldiers were suspended in hand sanitizer pooled in a plastic Petri dish and photographed using a Leica M205C stereomicroscope controlled by Leica Application Suite ver. 3.0 montage software. A distribution map of *C. gestroi* (figure 4) was produced using ArcGIS Pro Intelligence 3.0 software from localities in supplemental material table S1 and S2.

DNA sequencing

Total genomic DNA was extracted only from the heads and thoraces of both termite soldiers and workers to avoid contamination with gut fauna. The specimens were washed with distilled water and left to dry on a piece of

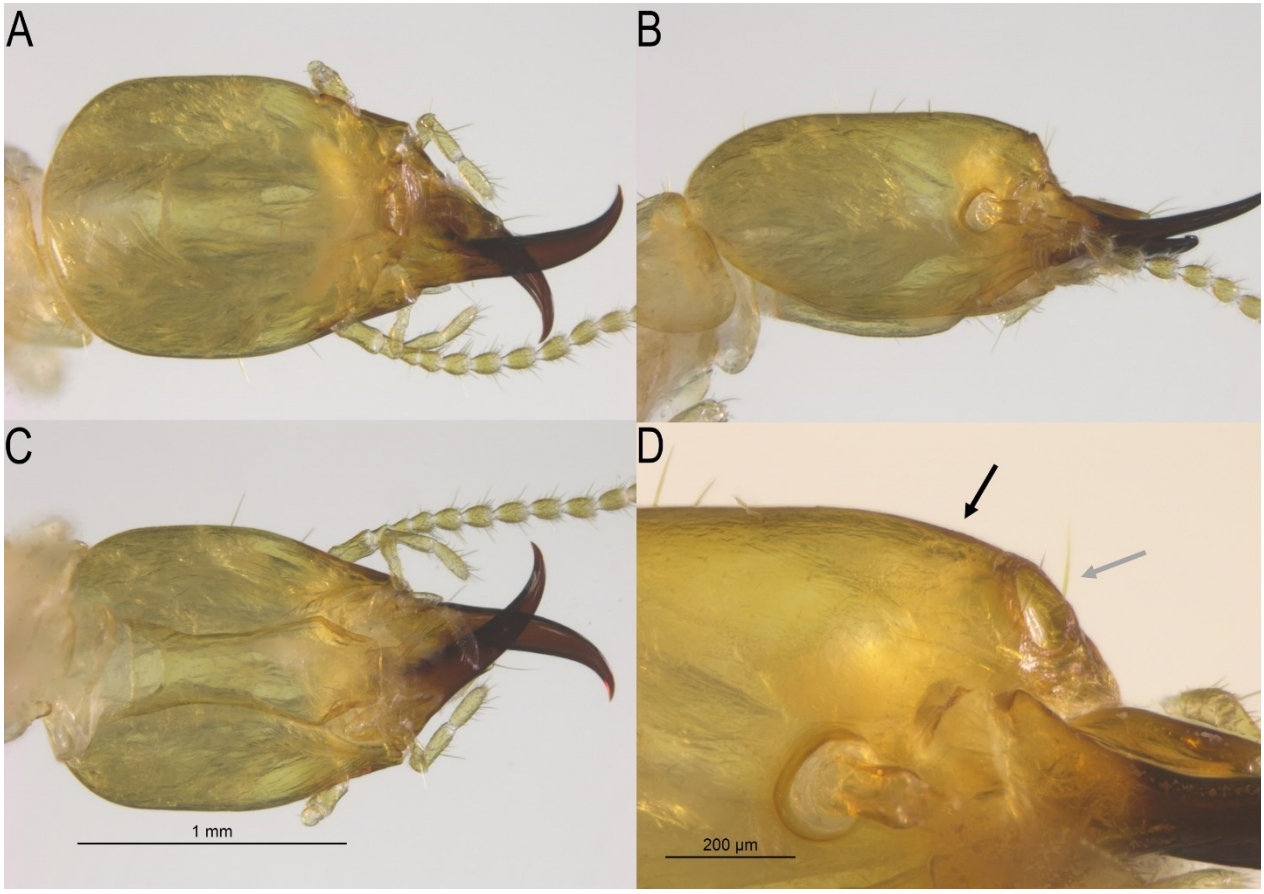

Figure 2. *C. gestroi* soldier head capsule from Alexandria, Egypt: (**A**) dorsal, (**B**) lateral, (**C**) ventral, and (**D**) frons; frontal slope (black arrow) and fontanellar setae (gray arrow).

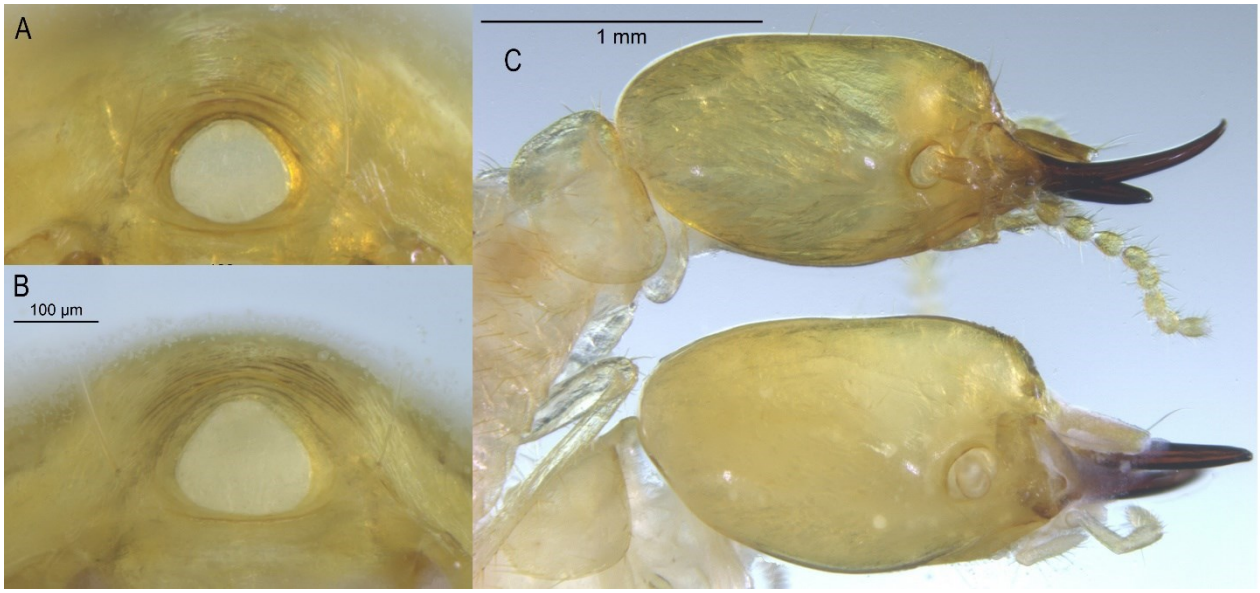

Figure 3. *Coptotermes* soldier fontanelle (**A**) *C. gestroi* from Egypt and (**B**) *C. heimi* from Pakistan (UFTC no. ASA164); (**C**) lateral head capsules of soldiers (**A**) and (**B**).

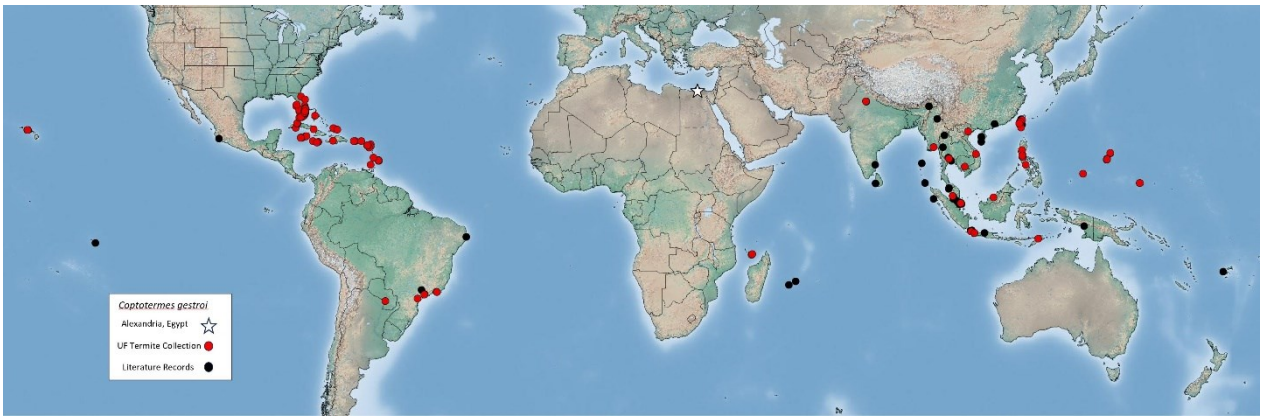

Figure 4. World distribution map of *C. gestroi* based on published literature (supplemental material table S1 and S2).

filter paper. Specimens were ground in liquid nitrogen using a pestle in a 1.5 ml microcentrifuge tube. Subsequently, 180 µl ATL buffer and 20 µl of proteinase K were added, and the mixture was incubated for 2 hours at 56 °C, followed by overnight incubation at 37 °C to enhance DNA yield. Extraction carried out using the DNeasy® Blood and Tissue Kit (Qiagen, Inc., Hilden, Germany). Total DNA was eluted into 100 µl of AE-buffer and subsequently stored at −20 °C until further use.

From the total extracted DNA, a 622 bp and 512 bp portion of 18S and 28S, respectively, were amplified, using the primers (5'-GGAGAGGGAGCCTGAGAA AC-3') and (5'-CTTTCGCTTCTGTCCGTCTT-3') for 18S according to Ghesini *et al*. (2014) and F (5'-CCCGTCTT-GAAACACGGGACCA A-3'), R (5'-CCACAGCGCCA-GTTCTGCTTAC-3') for 28S according to Muraji and Tachikawa (2000). PCR was performed under the following reaction conditions: initial denaturation at 95 °C for 3 minutes; denaturation at 94 °C for 40 seconds, annealing at 50 °C for 45 seconds, extension at 72 °C for 1 minute; final extension at 72 °C for 7 minutes for 35 cycles. Purification and sequencing were performed by Macrogen (Seoul, South Korea) and the sequences were submitted to GenBank by Bankit tool in the NCBI GenBank database. For the region of COII analysed, a 794 bp product was generated using the primers A-tLeu (5'-ATGGCAGATTAGTGCAATGG-3') and B-tLys (5'-GTTTAAGAGACCAGTACTTG-3') (Liu and Beckenbach, 1992). Thermal cycling conditions were as follows: initial denaturation at 95 °C for 90 seconds, followed by 35 cycles of denaturation for 60 seconds at 95° C, annealing for 60 seconds at 55 °C, extension for 2 minutes at 72 °C and a final extension for 10 minutes at 72 °C. The total reaction volume was 25 µl and was comprised of 2 µl of DNA template, 5x GoTaq Flexi Buffer, 25 mM $MgCl_2$, 10 mM dNTP's, 10 mM of each primer, 10% PVP-40, and 2.5U GoTaq Flexi DNA Polymerase with remaining volume made up with nuclease free water. According to the manufacturer's instructions, PCR products of the right size were purified using the ExoSAP-ITTM Express PCR Product Cleanup Reagent (ThermoFisher Scientific, Waltham, Massachusetts, USA). The purified PCR product was quantified using a NanoDrop Lite Spectrophotometer (ThermoFisher Scientific, Waltham, Massachusetts, USA) and sequenced using the SeqStudio Genetic Analyzer (Applied Biosystems). Contiguous files were assembled using DNA Baser (Version 4.36) (Heracle BioSoft SRL, Pitesti, Romania), and aligned using ClustalW as part of the package MEGA7 (Kumar *et al*., 2016). The homology search made use of the NCBI-Basic Local Alignment Search Tool (BLAST) identification database. The Tamura-Nei model was used to build a molecular phylogeny for the COII locus using the Maximum Likelihood technique with 1,000 repetitions.

Results

Infestation conditions

In November 2020, a resident of Alexandria was preparing to move her furniture to a new house when she observed decay in the hardwood drawers of cabinets. She discovered further damage in the parquet flooring and damage around the doors (figure 5). She shared her observations and requested help from a pest control group on the social media Facebook website. We noted in her inquiry and determined that she had a termite infestation. We requested that she send samples to us before the infestation was treated and the damage repaired. In September 2022, we obtained a second sample of termites collected by the same resident but this time on a different floor within the same building.

Morphological identification

Of the tree major invasive *Coptotermes* species, the soldiers of *C. formosanus* are easily separated from *C. gestroi* and *C. heimi* by the former having two pairs of frontal setae lateral to the fontanelle versus a single pair of frontal setae in the latter two species (Scheffrahn *et al*., 2015). Seventeen morphological measurements of *C. gestroi* and *C. heimi* soldiers did not discriminate between the two species based upon Principal Components Analysis (Yeap *et al*., 2010). Discriminant Function Analysis of eight measurements also yielded measurement overlap (Yeap *et al*., 2009) - our sample measurements at mm (figure 2): head length to base of mandibles (1.28 to 1.42), head length to fontanelle (1.20 to 1.32), maximum head width (0.96 to 1.12), head width at base of mandibles (0.68 to 0.7), maximum width of postmentum (0.32 to 0.4), minimum width of postmentum (0.24), postmentum length

Figure 5. *C. gestroi* damage in infested residence in Alexandria, Egypt: (**a**) parquet flooring, (**b**) hardwood cabinet drawers, and (**c**) door frame.

(0.80 to 1.1), pronotum length (0.34 to o.44), and pronotum width (0.76 to 0.84). We, however, found qualitative differences between the two species as originally reported by Roonwal and Chhotani (1962) in which the vertex of *C. heimi* has a more convex lobe directly posterior to the fontanelle; while the middle of the vertex is slightly concave (figure 3). In *C. gestroi*, the anterior frons slopes gradually toward the fontanelle without the middle having any concavity. Additionally, *C. heimi* has more narrowly spaced undulations in a belt lying dorsal to the fontanellar opening and in *C. gestroi*, there are fewer, less fine undulations in a narrower belt. There are fewer, shorter, and thinner setae on the vertex of *C. heimi* compared to *C. gestroi* (figure 3).

Genetic identification

In the validation process of the identification, the 18S, 28S and COII genes were utilized and submitted to the GenBank database with the following accession numbers: ON775495 for 18S, OP162718 for 28S and OQ929505 for COII. *Coptotermes* have low molecular variation among populations locally and globally (Li *et al*., 2009; Blumenfeld *et al*., 2021). Yeap *et al.* (2009) showed that *C. gestroi* and *C. heimi* form separate groups within a common clade using the COII gene. They found a genetic divergence between *C. gestroi* and *C. heimi* of 2.63% based on the Kimura 2-parameter yet Yeap *et al*. (2009) concluded that *C. heimi* should be a junior synonym of *C. gestroi* although our morphological results show otherwise.

Furthermore, based on the phylogeny generated utilizing the COII locus the level of variance observed between *C. gestroi* and *C. heimi* (approximately 6%) along with moderate to strong bootstrap support (82) supports that these are two distinct species (figure 6). This level of variance is observed among other species in the phylogeny and in some cases, is higher than among other species presented in this analysis and falls well within the range of acceptable interspecific variation for COII.

Synonymy

We argue that *C. alexandrinus* is a junior synonym of *C. gestroi*. Firstly, the verbal descriptions of Eldakak *et al.* (2022) and Mohammad *et al.* (2023), being almost identical, strongly point to a singular species. Both of these descriptions are rather non-diagnostic however, their accompanying figures match the revised description of *C. gestroi* (Scheffrahn *et al*., 2015). Secondly, the sampling locality of our study is very close proximity to the same two localities reported by in El-Dakkak *et al.* (2022) and Mohammad *et al.* (2023): 31°12'18.62"N 29°55'8.86"E and 31°17'83.10"N 29°93'20.35"E. Thirdly, the 18S and 28S accession sequences as given by Eldakak *et al.* (2022) and Mohammad *et al.* (2023) are 100% identical to those generated in this study for *C. gestroi* and are short sequences (only 300 bp long; 50%), suggesting poor quality reads. Eldakak *et al.* (2022) and Mohammad *et al.* (2023) do not present COI data in their papers (no accession number). The COII accession number they present (MZ935741) is less than

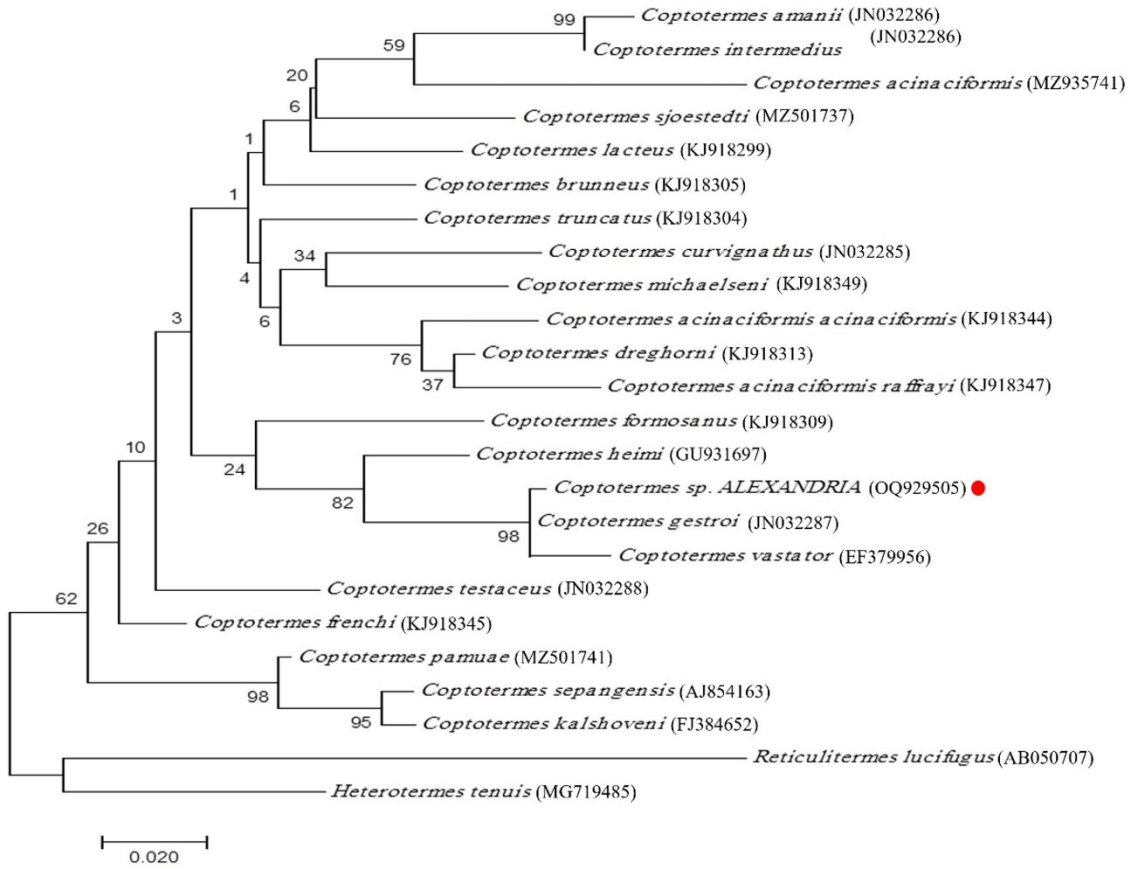

Figure 6. Maximum Likelihood phylogenetic tree at 1,000 replicates based on the Tamura-Nei model for confirming the identity of the *Coptotermes* specimen collected in Alexandria, denoted with red dot; scale bar = percent nucleotide difference. GenBank accession numbers included. *C. vastator* is a junior synonym of *C. gestroi*.

50% of the region needed (again, approximately a 300 bp product for what should be an approximately 700 bp product) for discrimination, so the 8% difference score with *Coptotermes frenchi* Hill 1932, a non-invasive Australian species, is inaccurate. The phylogenetic analysis given by Eldakak *et al.* (2022) showed that their "*Coptotermes* sp." grouped with the African *Coptotermes* clade and thus they proposed that it might be a new species, but poor sequence quality suggest otherwise.

Discussion

Because of the widespread distribution of *C. gestroi*, it has at least six junior synonyms, the most prominent in the literature has been *Coptotermes vastator* Light 1929 (Kirton and Brown, 2003; Yeap *et al*., 2007; Krishna *et al*., 2013). The presence of numerous junior synonyms has produced confusion and contradictions in the scientific literature, making correct identification and classification of the genus difficult (Chouvenc *et al*., 2016). Furthermore, due to the exceptional adaptability of *C. gestroi* to its habitat, there are significant differences between different populations (Huang *et al*., 2000; Li *et al*., 2011; Grace, 2014).

In this study, the two collected populations had different head capsule shapes at first sight. The characteristics of the head capsule alone could be problematic in identification (Wikantyoso *et al*., 2021). In addition, the first population collected contained only a few soldiers and workers. *Coptotermes* studies had not begun in Egypt until 2020, and the fontanellar setae were not studied in the first population. Because 18S and 28S genes are ultraconserved elements (Hellemans *et al*., 2022), the morphological traits of the sample, combined with the 18S and 28S sequences of this population, revealed that it belonged to *Coptotermes* but could not be identified to the species level. One pair of fontanellar setae was observed in the second population, and the COII obtained confirmed that the sample was indeed *C. gestroi.*

Shipboard infestations have the greatest potential to transport *C. gestroi* to new localities (Scheffrahn and Su, 2005; Hochmair and Scheffrahn, 2010; Scheffrahn and Crowe, 2011; Hochmair *et al*., 2023). *Coptotermes* has also been dispersed globally by ancient overwater dispersals (Bourguignon *et al*., 2016). Although Ghesini *et al*. (2011) documented a *C. gestroi* infestation on large yacht in Sicily, no land-based establishment has yet been found in Europe. Alexandria is the largest seaport on the Mediterranean coast (Aref, 2023) and could host future *Coptotermes* establishments. Quarantine protocols must be improved to stop further introductions along the Nile River to Cairo and other cities in Egypt and beyond.

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