

Morphological and molecular analysis of *Coptotermes* sp. an invasive subterranean termite newly recorded in Egypt

Moustafa ELDAKAK¹, Rehab R. E. MOHAMMAD², Hedaya H. KARAM², Hanan M. RAMADAN², Abdelazizi M. EL-MINSHAWY²

¹Department of Genetics, Faculty of Agriculture, Alexandria University, Egypt

²Department of Applied Entomology and Zoology, Faculty of Agriculture, Alexandria University, Egypt

Abstract

During the last ten years, the problem of subterranean termites in Alexandria, Egypt has worsened, causing great economic losses. Throughout our study to survey the termite species in Alexandria, a species was found that differed from any of the previously recorded ones in Egypt. The aim of this study was to identify this invasive subterranean species in the region. Samples were collected from infested buildings and trees, and their morphological characteristics along with their DNA barcoding loci (COII, 18S and 28S) were determined. Moreover, phylogenetic comparison with other related species were also considered for the most accurate identification. The analysis of all the previously mentioned parameters proved that it is *Coptotermes* sp. This genus is identified and recorded for the first time in Egypt and North Africa. This study demonstrates that the combination of molecular and morphological methods is essential for accurate species identification, which aids in the correct control of destructive pests.

Key words: taxonomy, *Coptotermes*, Rhinotermitidae, barcoding, subterranean termite, Egypt.

Introduction

There are about 3,106 living and fossil termite species all over the world are recognized, classified in 12 families. More than 1000 species are found in the African continent. Termite diversity in North Africa is low, with about 11 species (El-Sebay *et al.*, 2010).

Few taxonomic studies have been conducted on the termites of Egypt. Kemner (1932) described *Kalotermeis sinaicus* Kemner based on five soldiers and some workers collected in Sinai, Ghesini *et al.* (2014) excluded *Kalotermeis sinaicus* from genus *Kalotermeis* and redescribed it as a new genus: *Longicaputermeis*. El-Sherif and El-Kaschef (1973) described the morphological and taxonomic characteristics of *Anacanthotermeis ochraceus* (Burmeister), *Psammotermeis hybostoma* Desneux, and *Amitermeis desertorum* Desneux. Moein (1997) recorded the mound building termite *Microcerotermeis eugnathus* Silvestri from the Northern Western coast of Egypt. El-Sebay *et al.* (2010) surveyed and illustrated 7 species of termites in Egypt, five of them are subterranean species: *A. ochraceus*, *P. hybostoma*, *A. desertorum*, *M. eugnathus* and *Reticulitermeis lucifugus* (Rossi); and two species are drywood species: *Cryptotermeis brevis* (Walker) and *Kalotermeis flavicollis* (F.); Ghesini and Marini (2017) recorded *Amitermeis vilis* (Hagen) from the Greek Orthodox Monastery of Saint Catherine in Sinai for the first time.

Coptotermes Wasmann (Isoptera Rhinotermitidae) is one of the most economically important subterranean termite genera and some species are successful invaders (Chouvenec *et al.*, 2016). It is widely distributed in Asia, Africa, Central South America and Australia with greatest diversity in Asia (Lee *et al.*, 2015). It is the most destructive genus targeting timber and buildings but, it lacks clear and distinct morphological and diagnostic characteristics to differentiate the different species. This has resulted in several taxonomic synonyms that lead to the current assemblage of 67 extant species (Emerson, 1971). Variation

of non-specific characters and increased body mass with colony age compound the difficulty of morphologically sorting species of termites (Grace *et al.*, 1995). So, at the beginning of the twenty-first century, molecular genetics helped to study and identify species and their distribution (Wang and Grace, 2000a; 2000b; Szalanski *et al.*, 2003, Vargo, 2004). Genetic markers are now used routinely to identify individual termite colonies in field studies of termite bait efficacy (Vargo, 2003; 2004; Messenger *et al.*, 2005). *Coptotermes* species, especially in the soldier caste are morphologically similar. Therefore, the combination of molecular and morphological approaches is necessary for accurate species differentiation.

The aim of this study is to report the existence of *Coptotermes* sp. as a new pest in the Egyptian fauna based on the combined morphological and molecular genetic studies.

Materials and methods

Morphological data

Samples were collected from two localities in Alexandria, first one including (Ramlh station, El Azarita, El Shatby (31°12'18.62"N 29°55'8.86"E), second one including Moharam Bik, El Ibrahimia, Sidi Gaber and Smouha (31°17'83.10"N 29°93'20.35"E). Part of the sample was preserved in -20 °C for molecular genetic studies while the other part was stored in ethanol (85%) and used for morphological studies.

Samples were prepared on slides for microscopic examination. Specimens were boiled in 10% solution of caustic soda (NaOH) in water path for half an hour then washed by distilled water several times. The specimens were passed through a series of alcohol gradient concentrations from 75% to 95% and then transferred to clove oil for 15 minutes. The head, thorax and abdomen were separated under stereoscopic binocular microscope

(Zeiss, 475052-9901) and mounted in Euparal under separated covers on the same slide. Mounted slides were dried at 45 °C for 2 weeks.

The morphological characteristics of the selected soldiers, alates, and workers were microscopically examined and identified. The photographs were taken by stereo microscope and the details were studied. All the illustrated diagnostic characteristics were measured by using a micrometric lens attached to the stereoscopic microscope Model B-150 OPTIKA.

The following morphometric characteristics were measured: (8 soldiers), length of head capsule; height of head without postmentum; maximum width of head; maximum length of left mandible; length of pronotum; width of pronotum and length of hind tibia. (4 alates), length of anterior wing with scale; maximum width of anterior wing; length of pronotum; width of pronotum; width of head with eyes; length of head capsule; diameter of eye; maximum diameter of ocellus and length of hind tibia.

For scanning electron microscope examination (SEM), specimens were dried in 95% ethanol for 24 hours. The next procedures were carried out at the SEM unit of Faculty of Science, Alexandria University. The dehydrated specimens were fixed on a stub by using double face sticky tape, then placed in sputter chamber scanning electron microscope (JOELFC-1100E) to coat with a thin layer of gold. A computer-controlled SEM (JOEL model JSM- IT200) was used to scan the specimens from different angles to capture the best micrographs.

Terminology used in this study follows Krishna *et al.* (2013) also, identification of species was confirmed by using taxonomical key of Kambhampati and Eggleton (2000) for families and subfamilies, Roonwal and Chhotani (1989), Scheffrahn and Su (1994) for genus, and Maiti (2006) for species.

The specimens were kept in Department of Applied Entomology and Zoology, Faculty of Agriculture, Alexandria University, Egypt.

DNA extraction, PCR and sequencing

DNA was extracted only from the head and thorax of soldiers and workers (abdominal segments were excluded due to the presence of protozoa in the hindgut) by using iNtRon, biotechnology DNA extraction kit, South

Korea according to protocol B for tissue and Rodent tail in the manufacturer manual, (about 25 individuals for each sample).

Four genes were chosen: two mitochondrial genes, protein-coding cytochrome oxidase subunits (COII) and (COI), and two nuclear ribosomal 28S and 18S. These markers were amplified in one fragment according to the length of the sequences targeted. The PCR primers and amplification protocols are listed in table 1. PCR amplification was performed on thermal cycler (Piq Primus 25). PCR amplicons were bidirectionally sequenced using sequencing primers same as primers used in PCR protocol which are shown in table 1 and the BigDye® Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Inc.) on an ABI 3730 capillary sequencer following manufacturer's instructions. PCR products were fractionated on an agarose gel to check for specificity and to monitor for contamination using a negative control. Each sequence was edited using Sequencer 4.0 (Gene Codes 1999). The edited sequences were compared with related sequences from the nucleotide database by using Basic Local Alignment Search Tool (BLAST) of the National Centre for Biotechnology Information (NCBI) <http://www.ncbi.nlm.nih.gov>.

Drywood termite *C. brevis* used as a positive indicator (control) to verify the results of the sequencing and alignment data with the GenBank database. It is widely spread in Egypt and easy to distinguish by the robustly sclerotized head of soldier.

DNA retrieved sequences for the different examined loci COII, 28S and 18S were submitted to the GenBank (website) using BANKIT tool and given the accession number (MZ935741), (MZ852495) and (MZ855471), respectively.

Phylogenetic analysis

The phylogenetic analysis and the evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura and Kumar, 2004) and are in the units of the number of base substitutions per site. The proportion of sites where at least 1 unambiguous base is present in at least 1 sequence for each descendent clade is shown next to each internal node in the tree. This analysis involved 11 nucleotide sequences. All ambigu-

Table 1. Sequences of primers and PCR conditions used.

| Gene | Primer name | Sequence (5'-3') | Sources | | | |
|------|-------------------|------------------------------------|------------------------------|--------------------|-------------------|--------|
| COII | TL2J3037 | ATGGCAGATTAGTGCAATGG | Liu and Beckenbach, 1992 | | | |
| | TKN3785 | TTTAA GAGACCAGTACTTG | Simon <i>et al.</i> , 1994 | | | |
| COI | LCO | GGT CAA CAA ATC ATA AAG ATA TTG G | Folmer <i>et al.</i> , 1994 | | | |
| | HCO | TAA ACT TCA GGG TGA CCA AAA AAT CA | Folmer <i>et al.</i> , 1994 | | | |
| 18S | 1F | TAC CTG GTT GAT CCT GCC AGT AG | Giribet <i>et al.</i> , 1996 | | | |
| | 1.2F | TGC TTG TCT CAA AGA TTA AGC | Whiting, 2002 | | | |
| 28S | 28SA | GAC CCG TCT TGA AGC ACG | Whiting <i>et al.</i> , 1997 | | | |
| | 28SB | TCG GAA GGA ACC AGC TAC | Whiting <i>et al.</i> , 1997 | | | |
| Gene | Heat | Denaturation | Annealing | Extension | Final extension | Cycles |
| COII | 94 °C (2 minutes) | 94 °C (1 minute) | 50 °C (1 minute) | 72 °C (90 seconds) | 72 °C (7 minutes) | 40 |
| COI | 94 °C (2 minutes) | 94 °C (1 minute) | 50 °C (1 minute) | 72 °C (90 seconds) | 72 °C (7 minutes) | 40 |
| 18S | 94 °C (2 minutes) | 94 °C (1 minute) | 50 °C (1 minute) | 72 °C (90 seconds) | 72 °C (7 minutes) | 40 |
| 28S | 94 °C (2 minutes) | 94 °C (1 minute) | 50 °C (1 minute) | 72 °C (90 seconds) | 72 °C (7 minutes) | 40 |

ous positions were removed for each sequence pair (pair-wise deletion option). There was a total of 769 positions in the final dataset. Evolutionary analyses were conducted in MEGA X (Kumar *et al.*, 2018).

Results and discussion

The termite species under study was identified as *Coptotermes* sp., the most destructive species of subterranean termites recently dwellings in Alexandria Governorate. El-Sebay *et al.* (2010) misidentified this species as *R. lucifugus*, by revisiting his description and illustration, it was found that they are exactly the characters of *Coptotermes*.

Coptotermes sp. feeds voraciously on any material that contains cellulose such as living trees by consuming the heartwood, leading to several degrees of trees mortality. It can eat structural timbers from the inside out, leaving a thin film of blistered surface wood, which can be found in timber framing in homes and buildings, large colonies in the walls and mudding bivouacs were seen in the infested buildings (figure 1C-D). We have noticed that the release

of winged reproductives occurs annually in summer. They leave the nest in great numbers, hundreds of them were observed flying around streetlights in April, May and June every year, especially on hot days where weather conditions (25 ± 5 °C, $65 \pm 5\%$ RH and less than 15 km/h wind speed) considered favourable for alate flight.

The genus *Coptotermes* is characterized by the presence of pear shaped to sub rectangular head, with a pointed labrum in the soldier caste (Pearce *et al.*, 1993). Mandibles are slender, sharply pointed and slightly incurved without or with very small marginal teeth at the base. Most distinctive in the soldier caste is the large fontanelle (opening) at the front of the head which exudes a white defence secretion when the termite is disturbed. *Coptotermes* species have been shown to possess, as for other members of the Rhinotermitidae, sunken pores on their legs which may produce a defensive secretion against predators (Bacchus, 1979). We tried to identify the species of samples under study using the Maiti (2006) key, but it did not correspond to any of the species described therein, therefore the identification of species needs more study, it could be a new species.

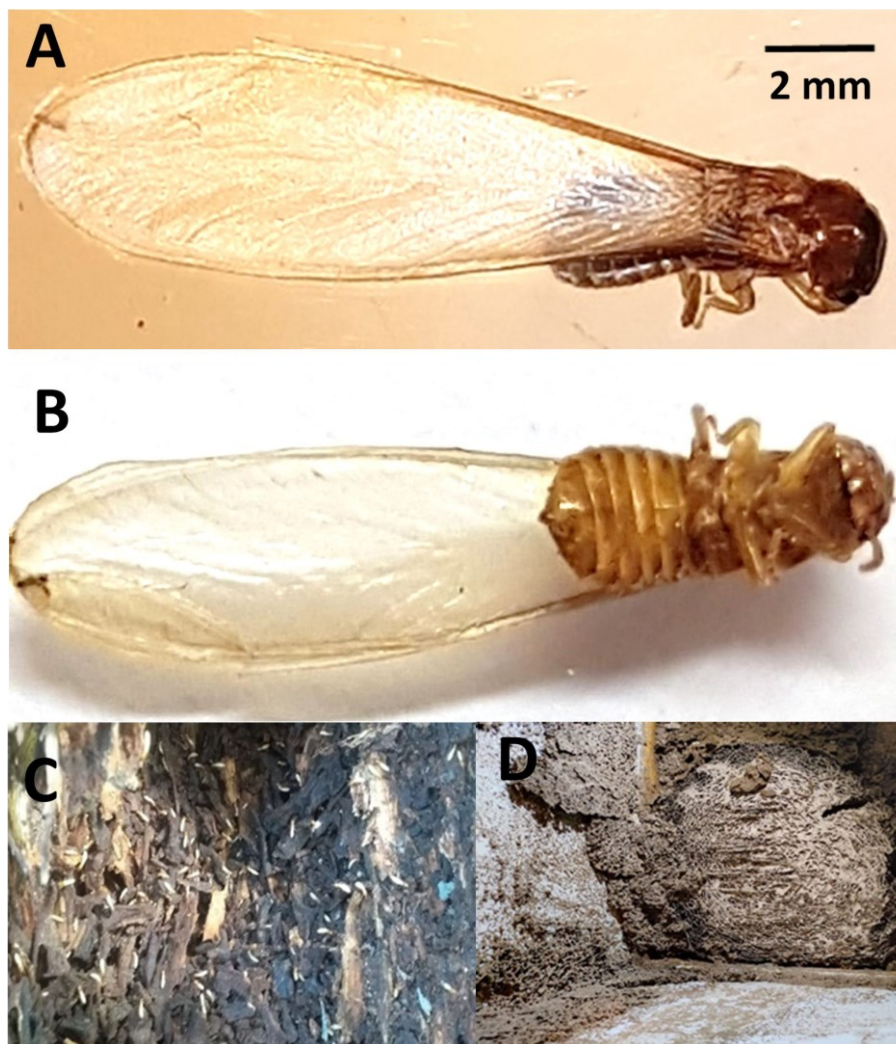


Figure 1. Habitus images of dorsal and ventral view of *Coptotermes* sp. (A, B). Infestation symptoms photos taken from the colonies of the examined samples: soldiers and workers inside an infested tree (C), mudding bivouac inside an infested apartment (D).

Characters of *Coptotermes* sp. under study

Like other species of termite, a *Coptotermes* sp. colony contains three primary castes: the workers, soldiers, and reproductives.

Soldier: body length is about 6.3 to 7 mm, with light brown head capsule and whitish body (figure 2A). Head slightly rectangular, 1.39-1.72 mm in length, distinctly longer than broad; widest in middle 1.11-1.33 mm, with a few scattered hairs (figure 2C); labrum light brown, narrowly pointed longer than broad with a hyaline tip that has a pair of hairs on it (figure 2E Lb). Mandibles reddish brown in colour, 0.83-0.94 mm in length, curved apically (figure 2E Lm). Antennae light brown consist of 14 to 15 segments, the 2nd segment longer than the 3rd, while 3rd and 4th being subequal (figure 2E-F An). Fontanelle somewhat circular in shape. A pair of setae occur at its rim (figure 2B, 2E Fo). The postmentum is about more than two times as long as broad at the widest point, with the waist midway between the posterior margin and the widest point (figure 2D, 2F Pm). The pronotum light brown in colour, about twice as broad 1.11 mm as long 0.56 mm, anterior and posterior margins slightly curved

in the middle (figure 2A Pr), but the posterior depression is more clear than the anterior one. The legs and abdomen are pale with 4 segmented tarsus ends with 2 claws and with a pair of tibial spurs (figure 2A).

Workers: are soft bodied, pale yellow, reach a length of 5 mm. and are blind. The head is round when seen from above and somewhat flattened. The black jaws slightly protrude, and the antennae have 15 or 16 segments. It has no special characteristics and resembles the alates (figure 3A).

Imago (alate): head capsule almost rounded in shape (figure 3B) and directed downwards (figure 3D), slightly depressed on the vertex; fontanelle indistinct; eyes moderately large, 0.28-0.33 mm in diameter (figure 3D); ocelli small and elongate, 0.17 mm in diameter (figure 3D oc); antennae with 17-20 segments, 3rd and 4th almost subequal, sometimes the third is the smallest (figure 3B). Left mandible, apical tooth clearly longer than 1st marginal tooth; 2nd marginal tooth fully developed and distinct from 1st and 3rd, both edges longer than those of 1st; 3rd marginal hind edges separated from molar prominence by a distinct gap; molar prominence broadly rounded in outline, proximal marginal weakly indented (figure 3B Lm). Right

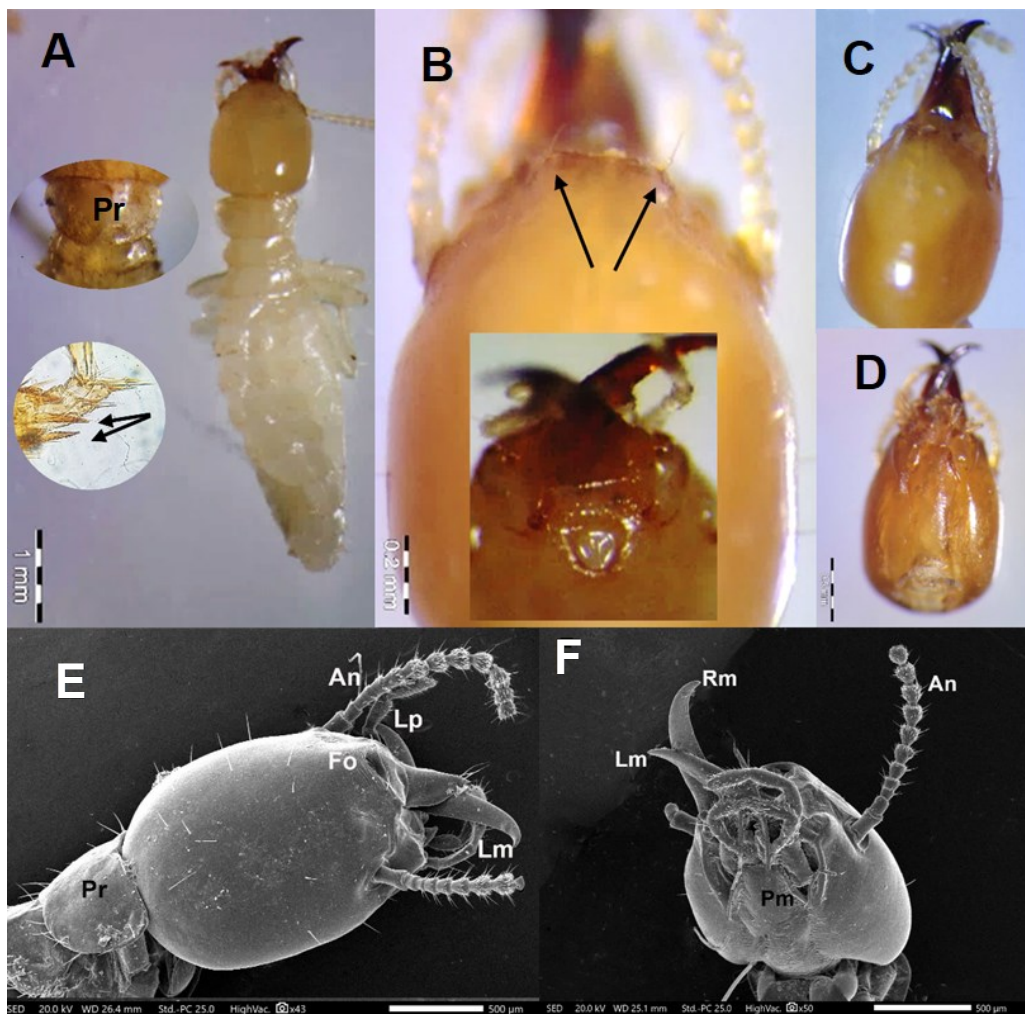


Figure 2. *Coptotermes* sp. soldier. **A)** dorsal view, arrows showing two hind tibial spurs, Pr. magnified pronotum; **B)** dorsal view of head, arrows showing two fontanelle setae and fontanelle opening anterior view; **C)** dorsal view of head; **D)** ventral view of head showing postmentum; **E)** SEM of head and pronotum, An. antennae, Lb. labrum, Fo. fontanelle, Pr. pronotum; **F)** SEM of head capsule ventral view, An. Antennae, Pm. Postmentum, Lm. left mandible, Rm. right mandible.

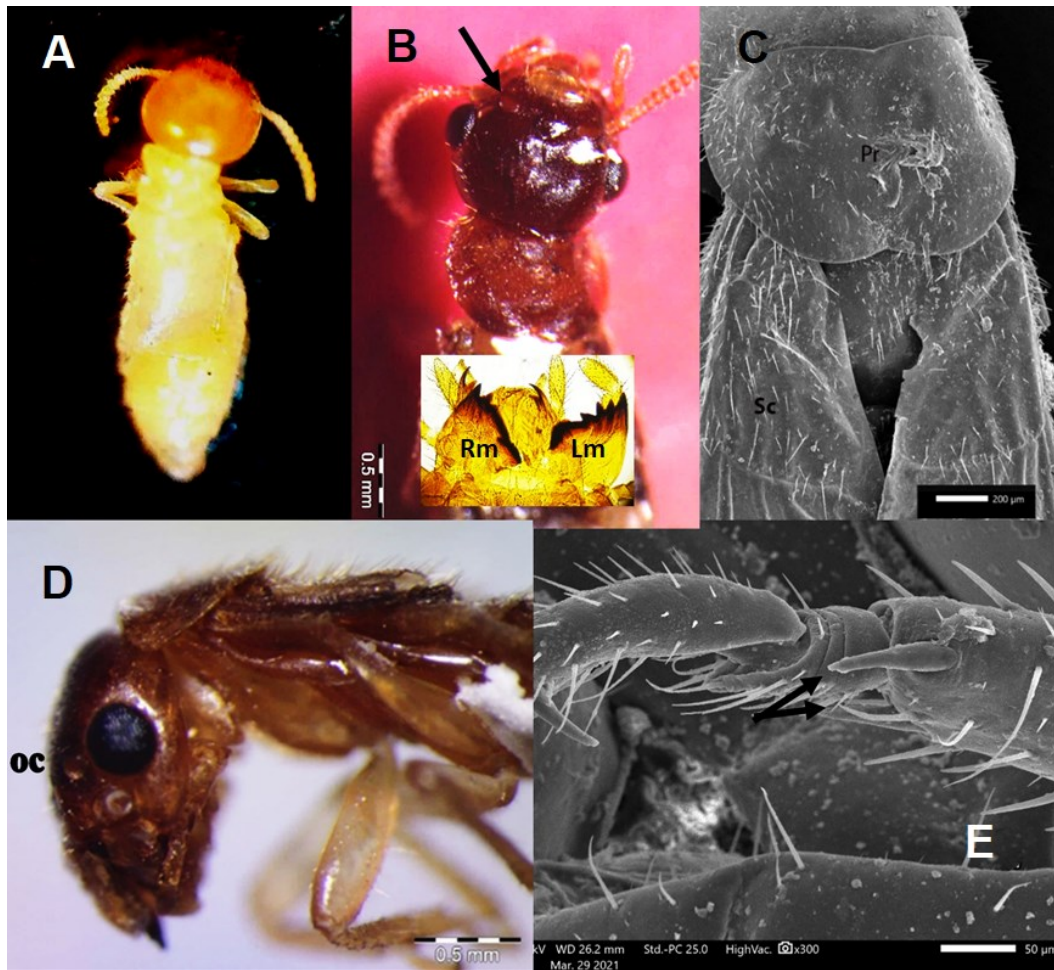


Figure 3. *Coptotermes* sp. worker and alate. **A)** dorsal view of a worker; **B)** dorsal view of head, prothorax, and microscopic photograph showing mandibles of alate, arrow showing antennal spot; **C)** SEM of alate thorax, Pr. pronotum, Sc. forewing scale; **D)** lateral view of head and thorax of alate, oc. ocellus; **E)** SEM of alate hind tibia and tarsus, arrows showing tibial spurs.

mandible, 1st marginal tooth with anterior edges bearing a small subsidiary tooth at base; 2nd marginal tooth fully developed and separate from 1st, exposed posterior edge longer than that of 1st, more or less straight; notch at proximal end of right molar plate absent, molar ridges prominent (figure 3B Rm). Pronotum almost as wide as head, about 1.44 mm, anterior margin weakly incurved with a weak median notch, antero-lateral corners narrowly rounded, posterior margin obviously emarginated (figure 3C Pr). Wing membranous, about two times as long as body, 10.82-11.11 mm in length (figure 1A-B), anterior wing-scale very large, almost twice as long as the hind wing-scale (figure 3C Sc). Hind tibial length 1.11-1.28 mm with a pair of tibial spurs (figure 3E), tarsus 4 segmented ends with 2 claws, arolium absent. Head and pronotum moderately hairy, abdomen densely hairy.

Sequencing and DNA barcoding

Specimens were easily identified as the genus *Coptotermes* but the recognition of the species morphologically was very difficult. So, to confirm the identification, we carried out DNA barcoding using four different DNA loci (18S, 28S, COI and COII genes). The results of the genetic sequencing of the four mentioned loci showed

that 18S, 28S and the most precise COII were the best and most accurate in identifying the examined samples to be *Coptotermes* sp. after performing BLAST of these sequences with the NCBI database.

COI did not show any results after agarose gel electrophoresis for its PCR product, so it was completely excluded at this stage, while 18S, 28S and COII showed sharp bands of their PCR products on the agarose gel (figure 4).

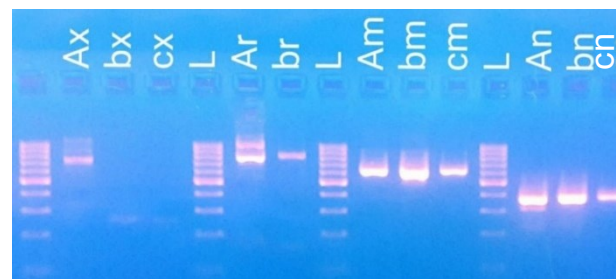


Fig 4. Gel electrophoresis for PCR products of DNA barcoding of the three different loci; x: COI, r: COII, m: 18S, n: 28S; A and c: *Coptotermes* samples, b: *Cryptotermes brevis* sample.

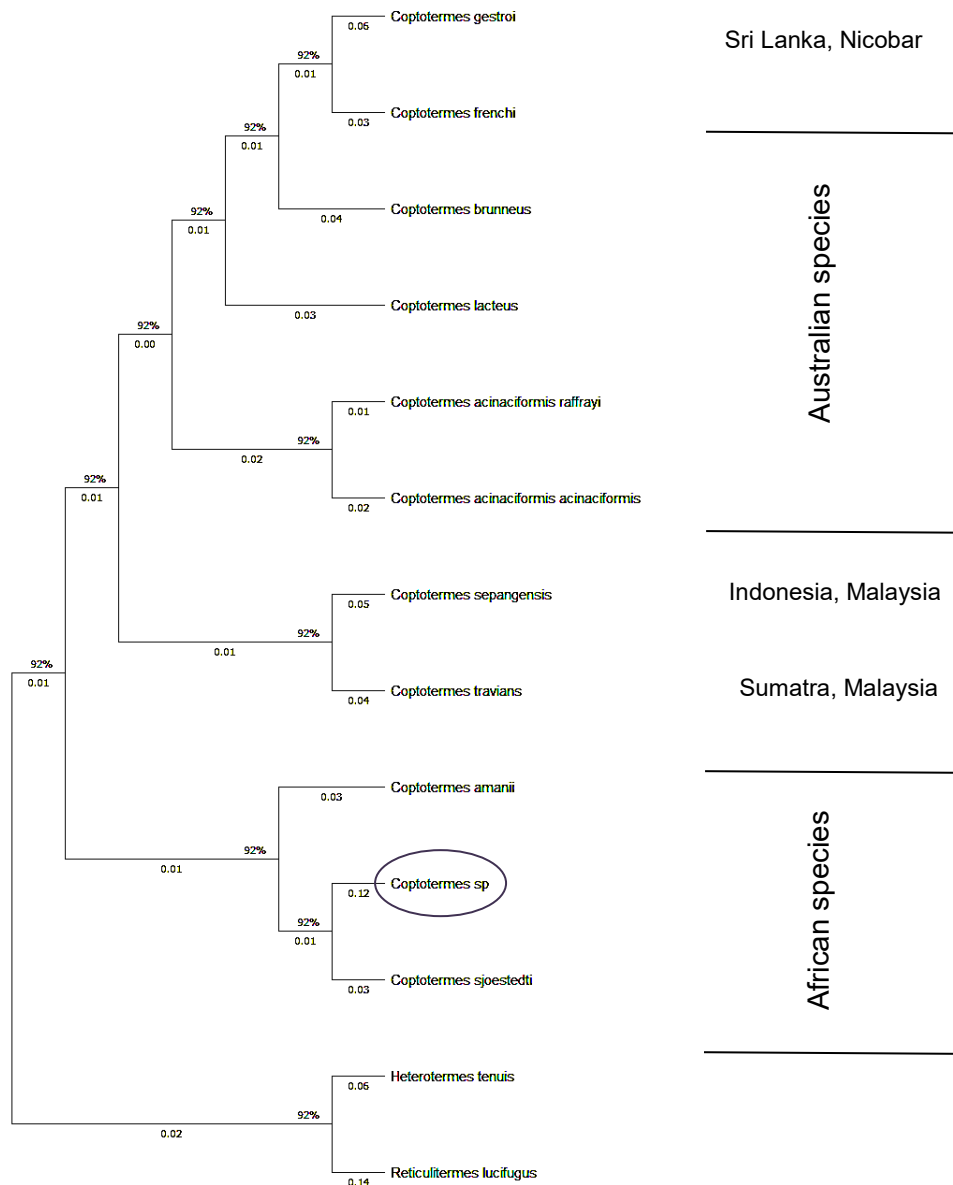


Figure 5. The phylogenetic tree of the related *Coptotermes* species in GenBank database against the studied *Coptotermes* sp. sample.

Phylogenetic analysis

After confirming that the collected samples to be *Coptotermes* morphologically and also on the molecular level, phylogenetic analysis was performed to analyse the genetic relationship between our sample and the sequences of related samples in the GenBank database. The phylogenetic tree shown in (figure 5) was made through the alignment of the sequence data of the gene Cytochrome oxidase II of our suggested *Coptotermes* species against the Cytochrome oxidase 2 sequences of the related *Coptotermes* species, and we added an outgroup of the cytochrome oxidase 2 sequences of *Heterotermes tenuis* (Hagen) and *R. lucifugus* species.

As shown in the phylogenetic tree our examined species under the genus *Coptotermes* was shown to be grouped in the African *Coptotermes* species cluster. Thus, we propose that the examined sample might be a newly species under the genus *Coptotermes* of a most

probable African genetic origin and this will be confirmed with further studies.

Conclusion

The prevalence of subterranean termites has augmented in Alexandria recently, and this study confirmed that this invasive species is *Coptotermes* sp. This is the first report for genus *Coptotermes* to be established in North Africa.

This conclusion was achieved by combining the morphological and DNA barcoding methods for identifying species. Specimens were collected from several districts in Alexandria Governorate. It appears that more efforts should be intensified to reduce the risk of spread of this invasive structural pest into other areas of Egypt and the humid subtropical Mediterranean Basin.

Acknowledgements

The authors present deep thanks to Mohsen M. Ramadan (State of Hawaii Department of Agriculture, Division of Plant Industry, Plant Pest Control Branch), for reviewing the manuscript.

References

- BACCHUS S., 1979.- New exocrine gland on the legs of some Rhinotermitidae (Isoptera).- *International Journal of Insect Morphology and Embryology*, 8 (2): 135-142.
- CHOUVENC T., LI H.-F., AUSTIN J., BORDEREAU C., BOURGUIGNON T., CAMERON S. L., CANCELLO E. M., CONSTANTINO R., COSTA-LEONARDO A. M., EGGLETON P., EVANS T. A., FORSCHLER B., GRACE J. K., HUSSENER C., KRECEK J., LEE C.-Y., LEE T., LO N., MESSENGER M., MULLINS A., ROBERT A., ROISIN Y., SCHEFFRAHN R. H., SILLAM-DUSSÈS D., ŠOBOTNÍK J., SZALANSKI A., TAKEMATSU Y., VARGO E. L., YAMADA A., YOSHIMURA T., SU N.-Y., 2016.- Revisiting *Coptotermes* (Isoptera: Rhinotermitidae): a global taxonomic road map for species validity and distribution of an economically important subterranean termite genus.- *Systematic Entomology*, 41: 299-306.
- EL-SEBAY Y., EL-AKKAD M. K., ABBASS M. K., EL-BASSIOUNY A. R., 2010.- A revision and illustrated identification keys to soldiers and adults of termite, order: Isoptera, in Egypt.- *Egyptian Journal of Agricultural Research*, 88 (2): 381-400.
- EL-SHERIF L. S., EL-KASCHEF A. H., 1973.- Survey and taxonomy of the termites of Egypt.- *Bulletin of the Entomological Society of Egypt*, 57: 283-297.
- EMERSON A. E., 1971.- Tertiary fossil species of the Rhinotermitidae (Isoptera), phylogeny of genera, and reciprocal phylogeny of associated Flagellata (Protozoa) and the Staphylinidae (Coleoptera).- *Bulletin of the American Museum of Natural History*, 146: 243-304.
- FOLMER O., BLACK M., HOEH W., LUTZ R., VRIJENHOEK R., 1994.- DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates.- *Molecular Marine Biology and Biotechnology*, 3: 294-299.
- GHESSINI S., MARINI M., 2017.- Termites of the Monastery of Saint Catherine (Sinai, Egypt).- *Bulletin of Insectology*, 70 (1): 1-8.
- GHESSINI S., SIMON D., MARINI M., 2014.- *Kaloterms sinaicus* Kemner (Isoptera, Kalotermitidae): new morphological and genetic evidence, and assignment to the new genus *Longicaputermes* gen. nov.- *Insectes Sociaux*, 61 (2): 123-131.
- GIRIBET G., CARRANZA S., BAGUÑA J., RIUTORT M., RIBERA C., 1996.- First molecular evidence for the existence of a Tardigrada + Arthropoda clade.- *Molecular Biology and Evolution*, 13: 76-84.
- GRACE J. K., YAMAMOTO R. T., TAMASHIRO M., 1995.- Relationship of individual worker mass and population decline in a Formosan subterranean termite colony (Isoptera: Rhinotermitidae).- *Environmental Entomology*, 24: 1258-1262.
- KAMBHAMPATI S., EGGLETON P., 2000.- Taxonomy and phylogeny of termites, pp. 1-23. In: *Termites: evolution, sociality, symbiosis, ecology* (BIGNELL D. E., HIGASHI M., ABE T., Eds).- Kluwer Academic, Dordrecht, The Netherlands.
- KEMNER N. A., 1932.- Zwei Termiten aus Sinai, mit Beschreibung der neuen Art *Kaloterms sinaicus*.- *Entomologisk Tidskrift*, 53: 87-91.
- KRISHNA K., GRIMALDI D. A., KRISHNA V., ENGEL M. S., 2013.- Treatise on the Isoptera of the world.- *Bulletin of the American Museum of Natural History*, 377: 1-2704.
- KUMAR S., STECHER G., LI M., KNYAZ C., TAMURA K., 2018.- MEGA X: molecular evolutionary genetics analysis across computing platforms.- *Molecular Biology and Evolution*, 35: 1547-1549.
- LEE T. R. C., CAMERON S. L., EVANS T. A., HO S. Y. W., LO N., 2015.- The origins and radiation of Australian *Coptotermes* termites: from rainforest to desert dwellers.- *Molecular Phylogenetics and Evolution*, 82: 234-244.
- LIU H., BECKENBACH A. T., 1992.- Evolution of the mitochondrial cytochrome oxidase II gene among 10 orders of insects.- *Molecular Phylogenetics and Evolution*, 1 (1): 41-52.
- MAITI P. K., 2006.- A taxonomic monograph on the world species of termites of the family Rhinotermitidae (Isoptera: Insecta).- *Memories of the Zoological Survey of India*, 20 (4): 1-281.
- MESSENGER M. T., SU N., HUSSENER C., GRACE J. K., 2005.- Elimination and reinvasion studies with *Coptotermes formosanus* (Isoptera: Rhinotermitidae) in Louisiana.- *Journal of Economic Entomology*, 98: 916-929.
- MOEIN S. I., 1997.- Record of the mound building termite *Microcerotermes eugnathus* Silvestri (Isoptera: Termitidae, Termitinae) in the Northern Western coast of Egypt.- *Alexandria Science Exchange Journal*, 18: 393-403.
- PEARCE M. J., BACCHUS S., LOGAN J. W. M., 1993.- *What termite? - A guide to identification of termite pest genera in Africa*.- Technical leaflet 4. Natural Resources Institute, Chatham, UK.
- ROONWAL M. L., CHHOTANI O. B., 1989.- *The fauna of India and the adjacent countries Isoptera, (Termites) Vol. 1, zoological survey of India*.- National Government Publication, Calcutta, India.
- SCHEFFRAHN R. H., SU N.-Y., 1994.- Keys to soldier and winged adult termites (Isoptera) of Florida.- *The Florida Entomologist*, 77 (4): 460-474.
- SIMON C., FRATI F., BECKENBACH A., CRESPI B., LIU H., FLOOK P., 1994.- Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers.- *Annals of the Entomological Society of America*, 87: 651-701.
- SZALANSKI A. L., AUSTIN J. W., OWENS C. B., 2003.- Identification of *Reticulitermes* sp. (Isoptera: Reticulitermatidae) from South Central United States by PCR-RFLP.- *Journal of Economic Entomology*, 96: 1514-1519.
- TAMURA K., NEI M., KUMAR S., 2004.- Prospects for inferring very large phylogenies by using the neighbor-joining method.- *Proceedings of the National Academy of Sciences of the United States of America*, 101: 11030-11035.
- VARGO E. L., 2003.- Genetic structure of *Reticulitermes flavipes* and *R. virginicus* (Isoptera: Rhinotermitidae) colonies in an urban habitat and tracking of colonies following treatment with hexaflumuron bait.- *Environmental Entomology*, 32: 1271-1282.
- VARGO E. L., 2004.- Windows on the underworld: DNA markers present new vistas for termite biology and management, pp. 55-57. In: *Proceedings of the 2004 national conference on urban entomology*, 20-22 May 2004, Phoenix, Arizona, USA.
- WANG J., GRACE J. K., 2000a.- Esterase differences among *Coptotermes formosanus* (Isoptera: Rhinotermitidae) populations.- *Sociobiology*, 36: 1-6.
- WANG J., GRACE J. K., 2000b.- Genetic relationship of *Coptotermes formosanus* (Isoptera: Rhinotermitidae) populations from the United States and China.- *Sociobiology*, 36: 7-19.
- WHITING M. F., 2002.- Mecoptera is paraphyletic: multiple genes and phylogeny of Mecoptera and Siphonaptera.- *Zoologica Scripta*, 31: 93-104.
- WHITING M. F., CARPENTER J. M., WHEELER Q. D., WHEELER W. C., 1997.- The Strepsiptera problem: phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology.- *Systematic Biology*, 46: 1-68.

Authors' addresses: Hanan M. RAMADAN (corresponding author: dhanmr@yahoo.com), Rehab R. E. MOHAMMAD (rehab.elsayed2013@alexu.edu.eg), Hedaya H. KARAM (Hedayak@yahoo.com), Abdelazizi M. EL-MINSHAWY (abdelaziz.elminshawy@alexu.edu.eg), Department of Applied Entomology and Zoology, Faculty of Agriculture, Alexandria University, Aflaton street, El-Shatby, Alexandria, Egypt; Moustafa ELDAKAK (moustafa.eldakak@gmail.com), Department of Genetics, Faculty of Agriculture, Alexandria University, Aflaton street, El-Shatby, Alexandria, Egypt.

Received November 6, 2021. Accepted March 21, 2022.