



Research Article

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Coptotermes alexandrinus sp. nov. (Rhinotermitidae) and *Incisitermes snyderi* (Kalotermitidae) A Recent Addition to the Termite Fauna of Alexandria, Egypt

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Abstract

Termites cause great economic losses in Alexandria, Egypt. During a survey of the termite species in Alexandria, *Incisitermes snyderi* (Light) was recorded for the first time in Egypt and North Africa as a genus and species. A second new species was collected and identified as *Coptotermes* sp. It did not match the characters of any previously described *Coptotermes* species. Therefore, the aim of this study was to describe this subterranean species using morphological characteristics and DNA barcoding loci (COII, 18S and 28S). Moreover, phylogenetic comparison with other related *Coptotermes* species were also considered for the most accurate identification. Analysis of all the previously mentioned parameters proved that *C. alexandrinus* sp. nov. Mohammad and Ramadan is a new species named after Alexandria, the city in which it is widely distributed. Genus *Coptotermes* is 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.

Abbreviations: *Coptotermes*; *Rhinotermitidae*; Barcoding; Egypt

Introduction

Termites are eusocial insects, comprise about 3176 known species (2976 living and 200 fossils) were recognized all over the world [1]. More than 1000 species of termites are found in the African continent but the diversity in North Africa is low, with about 11 species seven of which are recorded from Egypt [2]. Few morphological and taxonomical studies on termites were conducted the termite fauna of Egypt [2-5]. During a survey and taxonomic study on termites in Alexandria, Egypt two genera were discovered to be new to the Egyptian fauna i.e. *Coptotermes* (Rhinotermitidae) and *Incisitermes*. (Kalotermitidae) [6].

Genus *Incisitermes* was established by Krishna [7]. He included in this new genus 23 described and 2 undescribed species from the following geographic regions: Papuan, Indo-Malayan, Nearctic and Neotropical. The known distribution of *Incisitermes* is extended to

both the Nearctic and Neotropical Americans [8-10]. This genus is considered to be a dry wood termite.

Coptotermes is one of the most economically important subterranean termite genera and some species are successful invaders [11]. It is widely distributed in Asia, Africa, Central South America and Australia with greatest diversity in Asia [12]. It is the most destructive genus targeting timber and buildings. Variation of non-specific characters and increased body mass with colony age caused the difficulty of morphologically sorting species of termites [13]. So, at the beginning of the twenty-first century, molecular genetics helped to advance study and identify species and their distribution [14-17]. Genetic markers are now used routinely to identify individual termite colonies in field studies of termite bait efficacy [17-19]. *Coptotermes* species, especially in the soldier caste are morphologically similar. The aim of this study

is to report the existence of *Incisitermes snyderi* (Light, 1933) as a new pest in the Egyptian fauna and *Coptotermes alexandrinus* sp. nov. as a new species to science based on the combination of morphological and molecular genetic studies.

Materials and Methods

The current study has been carried out in Faculty of Agriculture, Alexandria University, Department of Applied Entomology & Zoology and Department of Genetics through three years (2019, 2020 and 2021). The studied species were collected from different localities in Alexandria Governorate. Voucher specimens were deposited in the insect collection at Department of Applied Entomology and Zoology, Faculty of Agriculture, Alexandria University, Egypt.

Collecting samples: Samples of *C. alexandrinus* sp. nov. were collected from two localities in Alexandria, while six alate samples of *I. snyderi* collected from Raas El-teen (31.20179, 29.87641) during a swarming season in August 2020. Part of *Coptotermes* samples was preserved in -20 °C for molecular genetic studies while the other part was stored in ethanol (85%) and used for morphological studies. The description of the species under study based on the available collected caste, either soldiers, workers or alates.

Preparation of the permanent slides: Samples were prepared on slides for microscopic examination according to Walker & Crosby [20]. Mounted slides were dried at 45°C for 2 weeks. Mounted slides were microscopically examined and photographed. All the illustrated diagnostic characteristics were measured using a micrometric lens attached to the stereoscopic microscope (Model B-150 OPTIKA).

Preparation for Scanning Electron Microscope: Specimens were dried in 95% ethanol for 24 hours. The next procedures were carried out at the SEM Unit, Faculty of Science, Alexandria University. The dehydrated specimens were fixed on a stub using double face sticky tape then placed in sputter chamber Scanning Electron Microscope (JOELFC-1100E) to be coated 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 and specimens' identification: Terminology and Identification of specimens was carried out using taxonomical keys of Gay [21] and Kambhampati & Eggleton [22] for families and subfamilies, Krishna et al. [23] for genera, Maiti [24] for *Coptotermes* species and Scheffrahn et al. [25] for *Incisitermes* species.

Phylogenetic analysis: After confirming that the collected samples to be *Coptotermes* morphologically and also on the molecular level, phylogenetic analysis was performed to analyze the genetic relationship between the sample under study and the sequences of the related samples in the GenBank database, previously published in [26].

The phylogenetic tree was made through the alignment of the sequence data of the gene COII of our suggested *Coptotermes* species against the COII sequences of the related *Coptotermes* species present in the GenBank. DNA retrieved sequences of *C. alexandrinus* for the different examined loci COII, 28S and 18S were submitted to the GenBank (website) using BANKIT tool and given the accession numbers (MZ935741), (MZ852495) and (MZ855471), respectively.

Results and Discussion

Taxonomic and morphological studies of termites are very rare in Egypt. Therefore, the current work aimed to study termites in Alexandria, Egypt from these points of view which revealed the presences of two newly recorded genera of termites i.e., *Coptotermes* (Rhinotermitidae) and *Incisitermes*. (Kalotermitidae). In addition to using DNA barcoding and molecular analysis in termite identification for the first time in Egypt.

Incisitermes snyderi (Light, 1933)

This species belongs to the drywood termite (Family: Kalotermitidae).

a. Synonyms: *Kalotermes snyderi*, 1933

Description

Diagnostic characters of Imago (alate): Orange to medium or ferruginous brown in color, moderately hairy (Figure 2A & C). Head: (Figures 1A & 2D) slightly oblong, eyes sub oval, with 1.2 mm diameter, ocelli rounded, very close to eyes, antennae with 18 - 20 segments. Left mandible about 1.2 mm or less; with anterior margin of 2nd marginal tooth slightly longer than posterior margin of 1st one, right mandible molar area subequal to posterior margin of 2nd marginal tooth (Figures 1B & 2B). Thorax: Pronotum as wide as head or wider (Figure 1C) with anterior notch. Forewing (Figure 1D), length from suture 8mm, sclerotized wing venation distinctly more pigmented proximally versus distally, median vein unsclerotized, unpigmented, terminating near tip of wing and running remotely to radial sector with two cross veins between radial sector and media. Tibial spurs 3:3:3; tarsi 4 segments; arolium present (Figure 1E & 2E).

Distribution: United States; Mexico; Panama; Bermuda; Bahamas; Cuba; Turks and Virgin Islands.

Examined material: Six alate from Raas El-teen (31.20179, 29.87641) during their swarming in August 2020.

Remarks: This termite was not previously record in Egypt as a genus or species; thus, it is a new record in this work.

Coptotermes alexandrines Mohammad and Ramadan sp nov. (Family: Rhinotermitidae)

By using the key of Maiti [24] to identify the species of samples under study, it did not match with any of the species described therein. Due to the lack of distinct morphological and diagnostic characters to distinguish the different species resulted in several

taxonomic synonyms that lead to the current assemblage of 67 extant species [27]. Therefore, more genetic studies were carried out which prove that, it could be a new species. The present

authors published research dealing with the morphological and molecular analysis of the genus *Coptotermes* as an invasive subterranean termite newly recorded in Egypt [26].

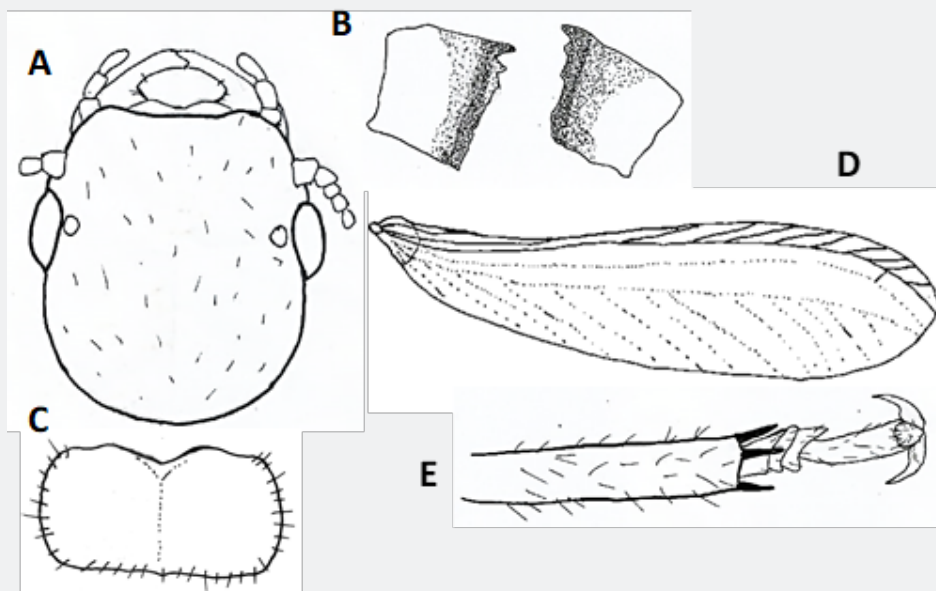


Figure 1: Drawing of *Incisitermes snyderi* alate A: head (dorsal view), B: left and right mandibles, C: pronotum (dorsal view), D: fore wing, E. hind tibia and tarsus.

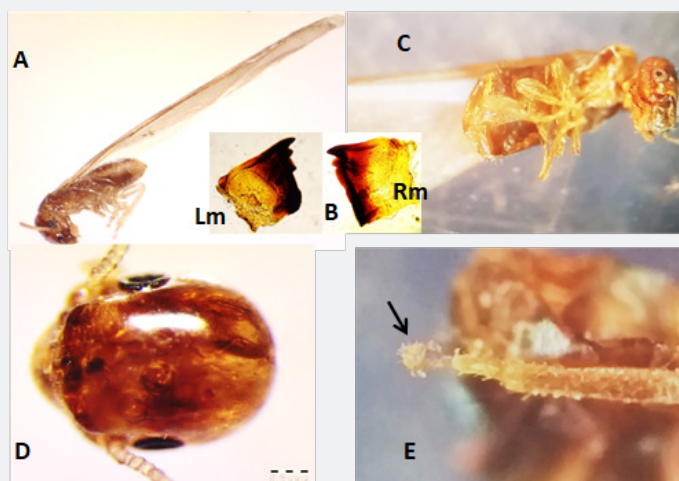


Figure 2: *Incisitermes snyderi*. alate A: lateral view, B: right and left mandibles, C: ventral view D: head capsule. E: hind leg, arrow showing arolium. Lm: Left mandible, Rm: right mandible.

Diagnostic characters of Imago (alate): General coloration moderate to dark brown about 12mm in length. Head: rounded in shape and directed downwards; fontanelle indistinct; eyes moderately large, 0.28-0.33 mm in diameter (Figure 3A); ocelli small and elongate; antennae with 19-21 segments, 3rd and 4th almost subequal. Left mandible, apical tooth clearly longer than 1st

marginal tooth; 2nd marginal tooth fully developed and distinct; molar prominence broadly rounded in outline, proximal marginal weakly indented (Figure 3C Lm). Right 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 3C Rm). Thorax: 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 (Figures 3B & 4G Pr). Wing membranous, about two times as long as body, 10.82-11.11

mm in length (Figures 4B & C), anterior wing-scale very large, almost twice as long as the hind wing-scale (Figure 4G Sc). Hind tibial length 1.11-1.28 mm with a pair of tibial spurs (Figure 4H), tarsus 4 segmented ends with 2 claws, arolium absent. Head and pronotum moderately hairy, abdomen densely hairy.

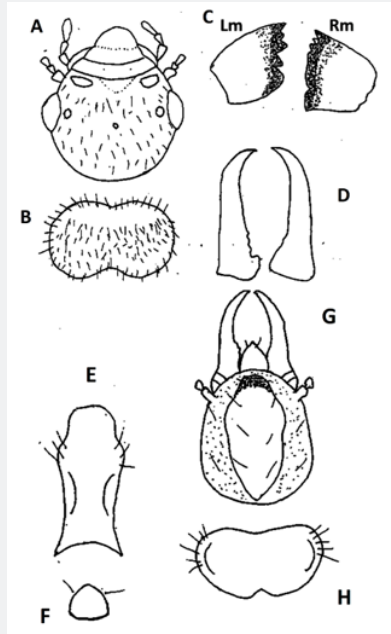


Figure 3: Drawing of *Coptotermes alexandrinus* : A-C imago, A: head, B: pronotum, C: left & right mandibles; D-H soldier, D: left & right mandibles E: postmentum, F: fontanelle, G: dorsal view of head H: pronotum.

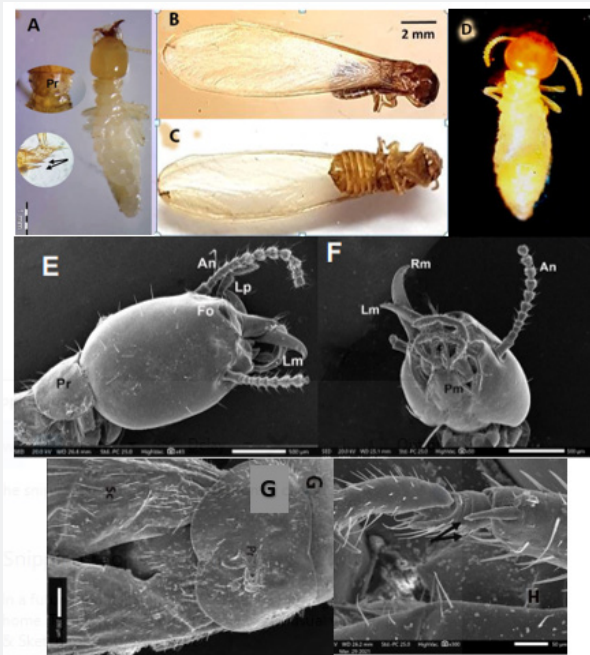


Figure 4: *Coptotermes alexandrinus* sp.nov. A: soldier; B: imago dorsal view C: Imago ventral view D: worker E: SEM photo dorsal view of head and pronotum; F: SEM photo of head capsule ventral view, G: SEM of alate thorax, H: SEM of alate hind tibia and tarsus, arrows showing tibial spurs. An. Antennae, Fo. Fontanelle, Lp. labium, Lm. left mandible; Pm. Postmentum; Pr. Pronotum; Rm. right mandible; Sc. forewing sc

Diagnostic characters of Soldier: body length is about 6.3 to 7 mm, and whitish in color (Figure 4A). Head: light brown. Labrum narrowly pointed longer than broad with a hyaline tip that has a pair of hairs on tip (Figure 4E Lb). Mandibles reddish brown in color, 0.83-0.94 mm in length, curved apically (Figure 3D). Antennae light brown consist of 14 to 15 segments, the 2nd segment longer than the 3rd, while 3rd and 4th being subequal (Figures. 4E & F An). Fontanelle somewhat circular in shape. A pair of setae occur at its rim (Figures 3F & 4E Fo). The postmentum is about more than two times as long as broad at the widest point. **Thorax:** pronotum light brown in color, about twice as broad 1.11 mm as long 0.56 mm, anterior and posterior margins slightly curved in the middle (Figure 3H). The legs and abdomen are pale, legs with 4 segmented tarsus ends with 2 claws and with a pair of tibial spurs (Figure 4A). 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 4D).

Holotype: collected from infested trees, Lagunaria patersonii (Andrews) G.Don (Family: Malvaceae), from Antoniadis Park, Smouha (31°17'83.10"N 29°93'20.35"E).

Paratype: collected from Faculty of Agriculture, Al-Shatby district (31°12'18.62"N 29°55'8.86"E).

Remarks: El-Sebay et al. [2] misidentified this species as *Reticulitermes lucifugus*, by revising their description and illustration, it was found that the illustrated characters exactly similar to genus *Coptotermes*.

Moreover, by comparing the morphological characters of African *Coptotermes* species described in Harris [28] with the collected *Coptotermes* specimens under study, we found that the imago cast are differ from *Coptotermes amanii* (Sjöstedt, 1911) and *C. sjostedti* Holmgren, 1911 in the color of wings which is brown in both of them while our samples with transparent wings. On the other side, inspected samples were very close in many characters to *C. intermedius* Silvestri, 1912 but differ in body color, width of labrum in addition, the yellow color surrounding R, Rs and Cu veins in *C. intermedius*.

Etymology: This species was named *alexandrinus* after Alexandria city, Egypt in which it is widely distributed..

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