Ann. For. Res. 65(1): 4808-4818, 2022
ISSN: 18448135, 20652445

ANNALS OF FOREST RESEARCH
https://www.e-afr.org/

MOLECULAR IDENTIFICATION AND MORPHOLOGICAL STUDY OF GROUND BEETLE, *CYMINIDIS ANDREA,* MENETRIES, 1832(COLEOPTERA: CARABIDAE) FROM ERBIL GOVERNORATE KURDISTAN REGION- IRAQ

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ABSTRACT

The present study includes a details description of the ground beetle, *Cyminidis andrea* Menetries, 1832 (Coleoptera: Carabidae) and its molecular identification. The specimens were collected from the underground and stones in different localities of Erbil governorate Kurdistan Region- Iraq during, March tile July 2022. According to molecular analysis, *Cymindis andreae* was used as a source of samples for PCR amplification of the fragments (550 bp) of the mtCOI gene for phylogenetic analysis. To match our sequence of nucleotides with sequences of other insect species that have been recorded, we exploited a section of the mtCOI gene from the collected insect which aliment the NCBI GenBank by the BLAST tool. The outcomes have gotten from the BLAST indicated that the sequences obtained newly identified species based on the mitochondrial COI gene and recorded as a new species in GenBank. The identified *Cymindis andreae* in the Kurdistan region clustered in a polyphyletic clade available from other countries. The COI sequence of *Cymindis andreae* was submitted to GenBank with accession OP361098. The base pairing of the mitochondrial COI gene sequence was highly adenine & thymine bps.mt COI gene can be practiced as a marker for the detection of related species, as detected from the likenesses between the phylogenetic association constructed by COI and morphological facts. The morphological study showed that the diagnosis characteristics of the species are, mandibles triangular, apically with a single tooth. Third segment of labial palps securiform, 1.4 times as long as 2nd segment. Antenna filiform, 1st antennomere cylindrical, 2 times as long as 2nd. Elytra dark yellow, surface with seven striae. Protibial tubular shaped, inner margin with cleaning organ. Right paramere nearly rounded, apical, and inner margin without setae. The habitus and diagnostic parts of the species were photographed. We achieved that the

Keywords: Molecular identification; morphological study; mt COI gene; *Cyminidis andrea*; sequencing.

INTRODUCTION

Carabidae, which includes ground beetles, is a sizable international family of beetles (Gonçalves and Paulo, 2017). The family with over 40,000 species, about 2,000 of whom are located in North America and 2,700 in Europe (Kromp, 1999). The species typically lives by the edges of ponds and rivers, under the branches of trees, beneath wood, even amongst pebbles or

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soil. According to Gonzales and Paulo (2017), the majorities of members in the family are carnivorous and continue pursuing whichever invertebrate feed they can outnumber. Carabid beetles contribute significantly to the mortality of weed seeds, insects, and slugs in agroecosystems. Aphids, moth larvae (including armyworm, cutworm, and gypsy moth larvae), beetle larvae (including the maize rootworm, Colorado potato beetle, and this significant cucumber beetle), mites, and springtails are just a few of the nuisance creatures they consume (Kromp, 1999). In Northern Hemisphere agricultural fields, carabids are widespread (Daly *et al*., 1998). An essential ground beetle genus is *Cymindis* Latreille. Despite the exception of Australia, all zoogeographic regions of the majority of species' ranges are covered (Ball and Hilchie 1983). These are indigenous to North Africa, the Near East, and the Palearctic region (that incorporates Europe). And over 75% of the species are located in the Palaearctic, and the rest are located in the Holarctic, Oriental, New World, and tundra to Costa Rica (Arnett and Thomas, 2000; Bousquet, 2012). There are several species known to prey on weed seeds (Holland, 2002). As nighttime insects, these species like to attack during darkness, and the location in which they reside is ideal for them since there is a plentiful supply of food. They can be distributed mainly in woodlands and fields as well as in gardens in which they are most frequently observed hunting down dangerous pests. Derwesh (1965) listed 61 species as existing in Iraq. In 1966, Shalaby *et al*. counted 32 species. Six species are mentioned by El-Haidari et al. in 1972. In 1976, Abdul-Rassoul added 10 new species. The precise detection of species is essential to biodiversity preservation and research management in an era when climate change and widespread environmental degradation. However, regular species diversity can be challenging and takes a time complex as well, frequently requiring highly specialized expertise, and consequently, it is a restrictive issue in ecological research and evaluations of biodiversity (Monaghan *et al*., 2005). Molecular approaches that depend on PCR have been crucial in the advancement of biological sciences since they have allowed for the development of inexpensive, quick, and reliable approaches and equipment for detecting species in recent years. Molecular methods have been practiced effectively for distinguishing insects (Jalalizand *et al*.,2012). There have previously been numerous attempts to apply molecular approaches in entomology. Rather than employing morphological techniques, DNA sequencing and molecular analysis are the best tools for understanding insect epidemiology (Brauner *et al*., 2002). Species identification of Coleoptera, however, requires a sophisticated technique (Greenberg 2002). Given these facts, DNA testing looks to hold promise for resolving the identification of species conundrum because DNA is durable and stable (Wallman and Donnellan 2001). For this purpose, partially genomic conservative areas, such as the mitochondrial COI gene, can really be sequenced (Foottit *et al*., 2008; Hebert et al., 2003; Jalalizand *et al*.,2012). Various creatures, along with some species of insects, can be accurately identified via DNA sequencing, particularly those who have identical morphologies. Because of its exceptionally high-resolution phylogenetic signal, the mitochondrial cytochrome-c oxidase subunit 1 gene (CO1) is frequently utilized for species separation and investigating the hidden variation in similar species (Hebert *et al*., 2003, Hebert *et al*., 2010). The identification by molecular analysis of Central European ground beetles is the subject which has been focused by Raupach *et al* (2010)'s research. At the

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family level, a lot of carabids may be quickly identified. Adult beetles have noticeable mandibles and palps, long, thin legs, striate elytra, and sets of punctures with tactile setae. They seem to be well insects. Several have mostly pubescent antennae and an organ for cleaning them. Many of them are drab, glossy, or dark in hue. A few are pubescent, many have vibrant or sparkly colours (Triplehorn and Johnson, 2005; Riddick and Capinera, 2008). The authors of the study objectives include collecting samples from a wide range of locations in the Erbil Governorate, Kurdistan Region of Iraq, and molecularly identifying the species. A comprehensive explanation of the species. taking pictures of the habitus and some significant components, particularly the male genitalia, and noting environmental details like the time and place of collection and dispersion locations.

MATERIALS AND METHODS

First: Molecular study

Five adult dried samples of ground beetles were used in the study:

1. **DNA Extraction:**

The whole genomic included mtDNA extraction was done in adults, the DNA was taken out using ZYMO Quick-DNA Tissue/Insects Microprepa Kit (USA-D6015) according to the instructions of the manufacturer. Then the DNA was kept at -20°C for the next steps applications. The quantification and qualification of DNA was checked by a Nanodrop (Thermo scientific-UK).

2. **Polymerase Chain Reaction (PCR) Amplification**:

Mitochondrial DNA with primers of a specific gene were manufactured for using the sequences of cytochrome c oxidase subunit I (Table 1), produced by Company of Micro-gene in South Korea, and then the primers were amplified using normal PCR for every species. The band size of amplicons was 550bps. The amplification was prepared in 50µl of ultimate mixtures consisted of; 2X Master Mix (AMPLIQON A/S Stenhuggervej, 22), 10 Pico-mol of primer pairs, DNase free water and DNA (Table 2), using Bioresearch PTC-200 Gradient thermocycler. The PCR program outline comprised primary denaturation at 95 C^o, 5 min, tracked down by 35 cycles (denaturation at 95C^o, 40 sec., annealing stage at 60C^o, 40 sec., and extension at 72C^o, 1 min followed by last step final extension at 72C^o,10 min. after that stored at -20 °C to the next steps application. Furthermore1.5% of agarose gel used to run the amplicons in (1X TAE, 30 minutes 70Volts, colored by ethidium bromide), visualized by UV trans-illuminator.

Table (1): Mitochondrial COI primers sequences.

Gene name	Nucleotide Sequences	Product size	Reference
Cytochrome Oxidase C- I (COI)	F: C1-J-1718 (5'- 3') GGAGGATTTGGAAATTGATTAGTTCC	550bp	(Simon *et al..,* 1994)
	R: C1-J-1718 (5'- 3') ACTGTAAATATATGATGAGCTCA		

Table (2): Reaction mixture for the PCR amplification of mtCOI gene.

No.	PCR requirements	Concentration	Volume (µl)
1	Taq DNA, Master-Mix	2x	25
2	Forward Primer	10 Pico-mol	3
3	Reverse Primer	10 Pico-mol	3
4	DNase free Water	-	15
5	Template DNA	50ng/µl	4
Total			50

3. Mt-COI Sequencing

At the Micro-gene Center in Korea, specimens of the mtCOI incomplete gene amplicons were sequenced utilizing the Applied Biosystems ABI Prism Terminator Sequencing Kit. By using Finch TV program, chromatograms of the COI gene were modified, and base calls were verified.

4. Sequence alignment and submission.

The partial of mt-COI gene sequences were implemented to the exploration program alignments of the DNA sequence Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi), which is accessible at the NCBI (National Center for Biotechnology Information) website as well to compare and laboratory alignments or query sequence with previous added biological sequences to realize more similarity with other targets.

Second: Morphological study

The current research is conducted on 20 individuals that were directly aspirated and hand-collected in ground and beneath stones in three distinct areas (Grdarashra, Ainkawa, and Shaqlawa) of the Kurdistan region of Iraq between April and June 2022. To dissolve the samples' pieces, they were submerged for 10 to 15 minutes in water bath. The mouthpart and abdomen were immersed in a heated 10% KOH solution for twenty-four hours in order to dissolve the body's fatty components and damage the muscles. Then positioned in distilled water for a further two-to-three-minute period to balance the alkali. The components were submerged in ethanol 25%, by using dissect microscope the samples were dissected, next moved to ethanol 50%, 75%, and 100% for two min. through each concentration to dehydrate the water, and then submerged in xylol for 2 minutes to check for translucency. Lastly, for preparing slides for investigation, the portions are put on slides with such a droplet of DPX reagent and capped by cover slides (Lane and Grosskey, 1993; Mawlood et al., 2016; Abdulla et al., 2020). Through the use of a digital camera, pictures of the physiques and significant pieces were taken (Ucmas series microscope camera). In a binocular microscope, the reported sizes of body parts are expressed in points on an eyepiece linear micrometer. Body width was evaluated based on the pronotum, and body length was recorded as from apex of the clypeus to the apex of the elytra (dorsal view). The species is identified according the key of (Rasool et al., 2017). The samples were placed for display in the insect museum at Plant Protection department, College of Agricultural Engineering Sciences- Salahaddin University-Erbil, Kurdistan region, Iraq.

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RESULTS AND DISCUSSION

First: Molecular study

PCR amplification of partial COI gene

The primers pairs of the specific mitochondrial gene were manufactured for utilizing the COI gene sequences which produced by the company of micro-gene made in South Korea. The primers got a clear band of 550bp. The amplicons were run and pictured via 1.5% Agarose gel.

(Figure1).

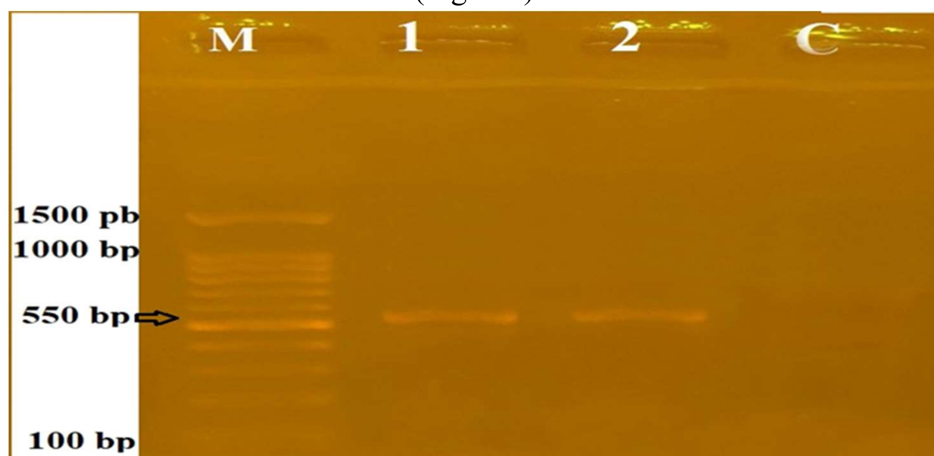

Figure 1 PCR amplification of partial cytochrome C oxidase I gene from insects Cymindis andreae. M; indicate: ladder 100 bp, lane 1and 2: 550 bp of PCR products of from species of insects and C is negative control.

Partial Sequence of COI gene

The sequence of the DNA, a forward primer C1-J-1718 was achieved in each sample through ABI 3130X genetic analyzer (Applied Biosystem). The amplicons of the specimen were used as a source of DNA template for sequence specific PCR amplification.

Molecular Identification of genus and species

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The insect samples of partial COI gene are alimented inside of NCBI GenBank by BLAST program (http://blast.ncbi.nlm.nih.gov/) then were utilized to liken our sequences of nucleotide with other saved insect sequences species. The outcomes have reached by the BLAST tool showed that the maximum query sequences of identity number were 100% species recognized (genus bases only, since *Cymindis andreae* showed a novelty in Genbank), these alignments demonstration to submit this new query sequences to Genbank-NCBI then received accession numbers OP361098 (Table 2). According to the analysis results of molecular data, the above test samples can be identified as a new record based on the COI gene.

Phylogenetic inferences

MEGA 11 program of phylogenetic study upon COI partial gene sequences of nucleotides exposed alliance studied insect species on predictable line. Beginning to the sequence divergence likeness data and created of phylogenesis, it was showed that the species belons to particular genera were adjacent to each other. The species grouped in one cluster with high similarity of GenBank insect species. *Cymindis andreae* showed new species and were recorded in GenBank

Table 2 Indicate BLAST analysis to identify of observed sample

for the first time that has not been recorded before in GenBank (Figure 2).

sample	Insect Identified	Accession Numbers	**Query Cover %**	**Identic Number %**	**BLAST with Similar Genus**	**Accession Number of Similar Genus**
1	*Cymindis andreae*	OP361098 (New Record)	**100**	**92.31**	*Cymindis vaporariorum*	KU911755
			100	**91.99**	*Cymindis interior*	JX260688
			100	**91.35**	*Cymindis axillaris*	KU908678
			100	**91.35**	*Cymindis humeralis*	KM444727
			100	**91.03**	*Cymindis cribricollis*	JF887588
			100	90.71	*Cymindis macularis*	KJ965656
			67	93.40	*Cymindis planipennis*	KX751749
			100	83.78	*Cymindis arizonensis*	MW340157
			98	84.42	*Cymindis platicollis*	MN345026

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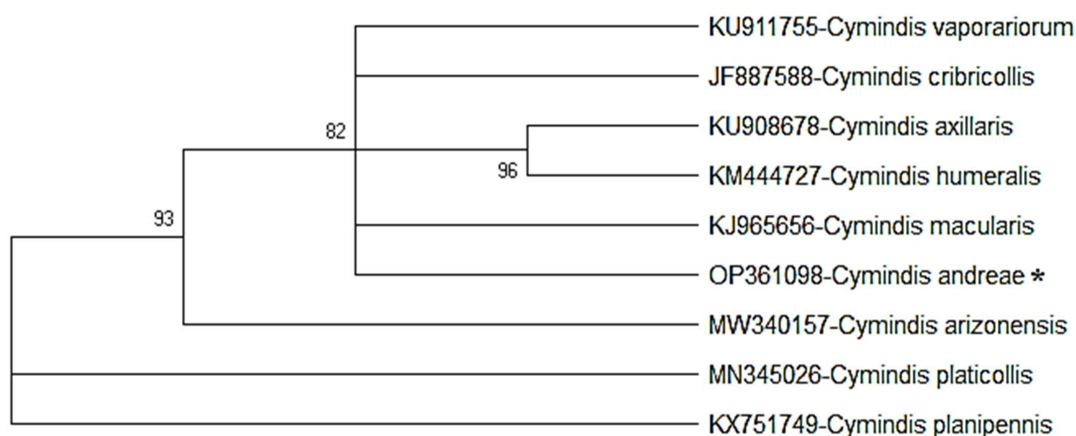

Figure 2. Employing Maximum Likelihood with boost strap of Mega 11 program, show phylogenetic positioning of each insect species with similar GenBank sequences of COI partial gene that available in GenBank. The stars (*) is query sample.

Second: Morphological study

Description

***Cyminidis andreae* Menetries, 1832**

Body (Fig. 3a): Elongated oval, flat, yellow – pale brown. Medium sized. Length 7.8-9.6mm.

Head: The pronotum (with eyes) somehow narrower than the pale brown-brown, oval-shaped and as wide as the pronotum. Eyes dark brown and oval-shaped, slightly prominent. Vertex brown finely punctuates without setae. Frons brown finely punctuates, each side with two long brown setae. Clypeus pale brown finely punctuate. Gena pale brown finely punctuates without setae. Labrum (Fig.3b) dark brown, triangular shaped, larger than clypeus anterior margin moderately emarginated six long setae. Mandibles (Fig.3c) dark brown, triangular, apical part with a single tooth, scrobe without setae. ventral surface without setae, brush absent. Maxillae (Fig.3d) Yellow-brown, stipes nearly oval-shaped, lacinia tubular shaped, apical part hook like outer margin densely short, yellow setose, maxillary palps four segmented, fourth section tubular shaped, 1.25 times long such as the second. Mentum (Fig.3e) dark brown, labial palps brown, three-segmented, 3rd segment securiform, 2times long such as 2nd. Antenna brown, filiform, length 3.2 – 3.8 mm, 1st antennomere cylindrical, 2 times as long as 3rd once, 3rd antennomere 1.3 times as long as 4th , 5th -10th antennomeres nearly same long, 11th antennomere oval shaped, 1.2 times as long as 10th .

Thorax: Pronotum brown, cordiform shaped, finely punctuate, anterior margin slightly concave, posterior margin straight, anterior and posterior angles rounded, laterally convex with two brown setae, the surface without setae. Prosternum brown, nearly trapezoid-shaped, surface finely punctate sparsely pale brown short setose. Prosternal process nearly rounded, finely punctate. Scutellum pale brown, triangular shaped, surface finely punctate. Elytra (Fig. 3g) yellow-brown, slightly convex, with the brown crisscross group at post-medium, somewhat extended to apex, not

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reaching lateral border and apex, the base of elytra with square spot, suture brown during its length; surface bare with seven bands, bands 1-2 merged near the base, bands 3-6 joined near the apex. Hind wing, pale yellow, distal part with three short curved branches of radius sector (Rs1, Rs2, and Rs3). Stigma is present at 1/3 of the apical part below the costa vein. Medium cells and cuneus cells are nearly rectangular shaped. Wedge cell oval shaped. For legs (Fig.3h) dark brown, fore coxa circular shaped, trochanter small and triangular, femur cylindrical shaped, fore tibia tubular shaped, inner margin with cleaning organ, apical part with two short spurs. Protarsus five segmented, 1st segment 1.3 long like second, fifth section three times as long 1st segment., claw small and pectinate. The middle legs resemble the first one and differed by the tibia is slender and without cleaning organs. Hind legs (Fig.3i) resemble the first one and differed by, the hind coxa bot shaped, trochanter large, elongated oval, extended to the posted margin of 1st abdomen sternum, inner margin without cleaning organ, metatarsus four segmented.

Abdomen: Brown consists of six visible sternites, finely punctate, sparsely pale brown setose with a pair of brown setae on each 3rd – 6th sternites, posterior margin of last abdominal sternite (6th) slightly rounded. Ventrally with six visible, smooth tergites, finely pubescent laterally.

Male genitalia: The 9th band of abdominal is oval, the apical surface lacking setae. The median lobe (Fig.3k) is nearly cylindrical, apex acute slightly bent to the right, 1\3 of apical part weakly sclerotized, slightly curved, length1.5 - 1.8 mm. The right paramere is nearly rounded. The left paramere is nearly oval, twice longer than the right paramere, apex rounded, apical and inner margin without setae.

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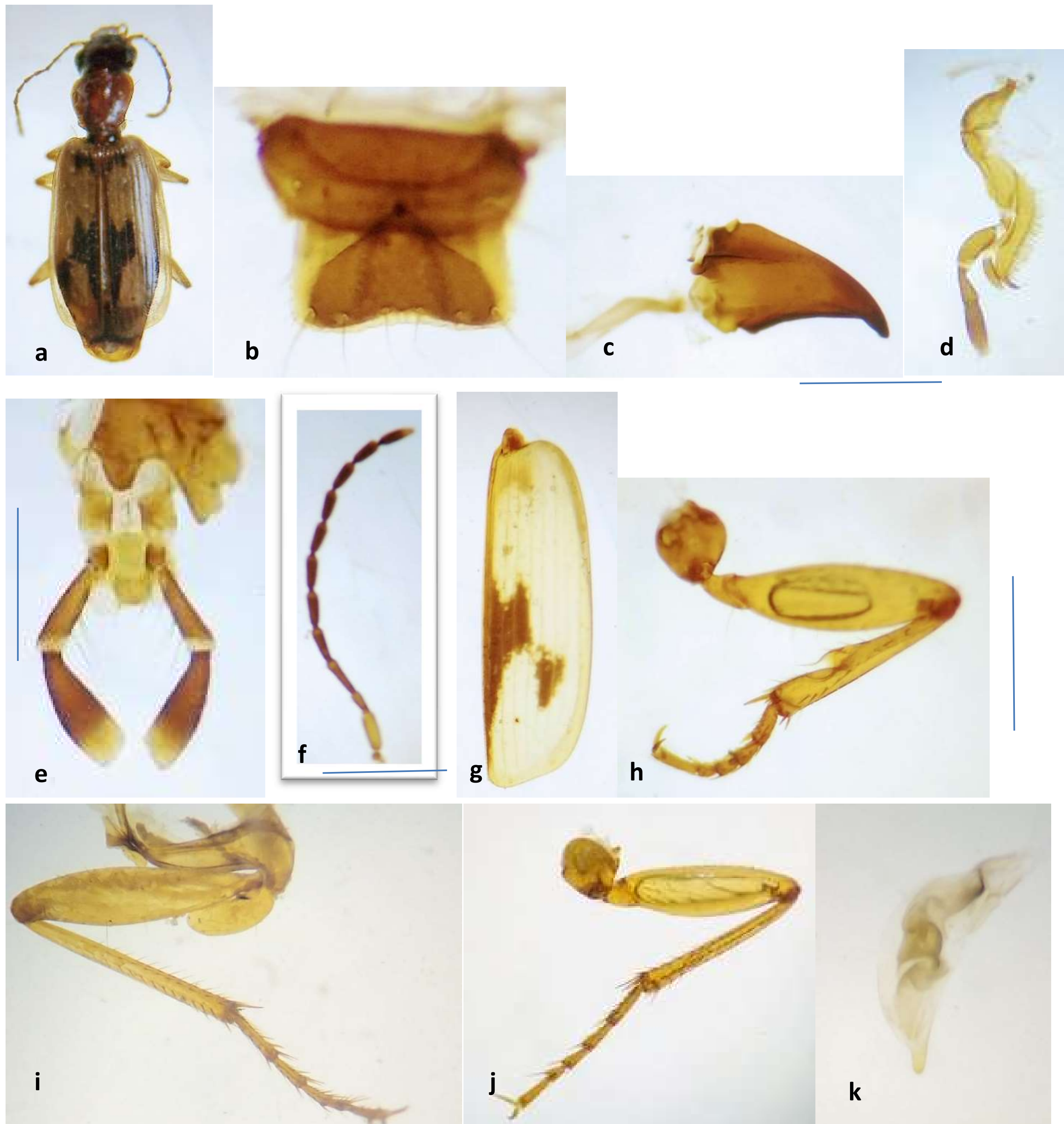

Fig. 3 *Cyminidis andreae*

a. Habitus (6X) b. Labrum b. Right mandible c. Maxilla d. Labium f. Antenna g. Elytra h. Fore leg i. Middle leg j . Hind leg k. Aedeagus (Lateral view).
Scale bare: a, b, c, d and k= 0.2mm; g, h, I, and g = 0.5mm

Conclusions

Based on the analyses of our various datasets, we have come to the conclusion that the bands of (550 bp) of the gene of mt CO1 was amplified with PCR from the *Cymindis andreae* for distinguishing and phylogenetic studies. The part of the COI gene of the insect samples are

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alimented inside of NCBI GenBank by BLAST program, were used to compare our nucleotide have sequenced with other stored species of insect sequences. Our study confirms the high potential of DNA sequence data for successful species identification of even closely related ground beetle species. The morphological study includes; detailed description the species with many new characteristics not previously mentioned by other researchers; especially in mouthparts and male genitalia. Photographing the habitus and important parts which used in identification of the species particularly the mouth parts, elytra and male genitalia. The species is one of important predators and most widespread in Kurdistan Region- Iraq. Therefore, it is necessary to comprehensive survey of different regions of Iraq, to collect the samples and update the database of the family Carabidae.

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