

Original Research Paper

# Barcode of Life Data Systems (BOLD) Versus GenBank Molecular Identification of a Dragonfly from the UAE in Comparison to the Morphological Identification

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**Abstract:** Dragonflies are insects in the order Odonata. They inhabit freshwater ecosystems and are found in the UAE. To date, few checklists have been published for the local dragonflies and the used identification keys are not comprehensive of Arabia. The aim of this study was to provide a molecular identification of a dragonfly based on the mitochondrial Cytochrome c Oxidase subunit I (COI) gene using the National Center for Biotechnology Information (NCBI) database and the Barcode of Life Data Systems (BOLD) in comparison with the morphology. The insect's DNA was extracted and the PCR was performed on the target gene. The insect was identified initially as *Anax imperator* based on the NCBI database and as *Anax parthenope* based on the BOLD. However, the morphological identification was in agreement with the one produced by the BOLD. The results of this study is a demonstration of how, in some cases, the DNA-based identification does not provide a conclusive species designation and that a morphology-based identification is needed.

**Keywords:** Dragonfly, *Anax imperator*, *Anax Parthenope*, DNA Barcoding, COI Gene

## Introduction

Dragonflies constitute the suborder (Anisoptera). They belong to the order Odonata, which includes other two suborders: Zygoptera (damselflies) and Anisozygoptera (Dijkstra *et al.*, 2013). The Odonates were amongst the pioneers of flight in the animal kingdom; they showcase a unique flying mechanism and wing venation. They are equipped with unique copulatory structures and have complex and elaborate mating system, thus making them a heavily investigated subject of behavioral and ecological studies (Carle *et al.*, 2015). In general, Odonates are characterized by having a large prognathic heads with large compound eyes, chewing mouthparts and setaceous antennae. Their prothorax (wingless thoracic segment) is small and functions as a neck, while the mesothorax – and metathorax are fused into a large pterothoracic segment equipped with two pair of elongate wings (Abbott, 2009). Each segment produces a pair of legs that are pushed to the front, so it can form a basket used for hunting preys or perching (Pessacq, 2008). Anisoptera and Zygoptera have more morphological distinct characters beside the visible sturdiness of the

prior's body. Zygopterous forewing and hindwing are about the same size, while the Anisopterous hindwings are basally broader than the forewings and have different venation patterns near the base, which are very helpful in identification. Their eyes usually touch on the top of the head (with the exception of the Petaluridae and Gomphidae) (Abbott, 2009). The abdomen (10 segments) includes the digestive and reproductive systems. Its length helps put more weight behind the wings for added aerodynamic swiftness. In all Anisoptera, sexual dimorphism is apparent in body weight; like all insects, the females are bigger and heavier than the males (Woodward, 2001). The Males have cleft on the ventral side of the second segment, which includes the copulatory structures. The spermaries are in the ninth segment. Whereas females have larger abdomens. Their reproductive tract is on the underside of the eighth segment and is covered by an ovipositor (Paulson, 2011). Dragonflies are considered excellent flyers exhibiting impressive flight skills. Thanks to their densely-veined hemolymph containing wings, they can make sufficient aerodynamic forces by periodically flapping the wings (Hou *et al.*, 2017).

**Table 1:** Compiled checklist of recorded Anisoptera species. three families exist; Gomphidae, Aeshnidae and Libellulidae. (\*) are from (Feulner and Reimer, 2007), (\*\*) from (Feulner and Judas, 2013) and the rest are from the first checklist (Giles, 1998)

Scientific name	Common name
<b>GOMPHIDAE</b>	
<i>Lindenia tetraphylla</i>	Arabian Lobetail
<i>Paragomphus sinaiticus</i>	
<i>Paragomphus genei</i>	
<b>AESHNIDAE</b>	
<i>Anax imperator</i>	Emperor
<i>Anax parthenope</i>	Lesser Emperor
<i>Hemianax ephippiger</i>	Vagrant Emperor
<b>LIBELLULIDAE</b>	
<i>Pantala flavescens</i>	Globe Skimmer
<i>Orthetrum sabina</i>	Oasis Skimmer
<i>Orthetrum chrysostigma</i>	Girdled Skimmer
<i>Orthetrum taeniolatum</i>	Azure (Blue) Skimmer
<i>Orthetrum ransonneti</i> *	
<i>Diplacodes lefebvrei</i>	Purple Darter
<i>Trithemis annulata</i>	Purple Blushed Darter
<i>Trithemis arteriosa</i>	Gulley Darter
<i>Trithemis kirbyi</i>	Orange Darter
<i>Crocothemis erythraea</i>	Carmine Darter
<i>Crocothemis sanguinolenta</i> *	Bloody Scarlet
<i>Selysiothemis nigra</i>	Desert Darter
<i>Sympetrum fonscolombii</i> *	Red-veined Darter
<i>Urothemis thomasi</i> **	
<i>Zygonyx torridus</i>	Ringed Cascader

Identifying by DNA barcode is superior to the conventional method in many aspects; some animals, like insects, go through morphologically distinct developmental stages making identification a bit tricky. In addition, numerous animals have accumulated genetic diversity without displaying it morphologically and molecular identification is not affected by these cryptic species or phenotypic plasticity. In some occasions, the species specimens might be damaged rendering them unrecognizable, so a small tissue sample for DNA barcoding can provide resolution. The theory of DNA barcodes is to find a single, universal segment of the DNA sequence for identification of all taxa. Despite the extensive research in this area, the perfect single and universal DNA barcoding marker has not been and most unlikely to be discovered. In 2013, the Barcode of Life Database (BOLD) included at least 2.7 million records of biological species. In the context of Anisoptera, Carle *et al.* (2015) published a phylogeny containing 510 species of 184 genera in 11 families by analyzing over 10,000 nucleotides from nuclear and mitochondrial genome (using COI).

The identification of dragonflies is typically done based on characteristics that are not readily seen in the field (Feulner and Reimer, 2007). Nevertheless, many species are clearly morphologically distinctive while others are ambiguous requiring a competent field

observer. Morphological identification based on the coloration is sometimes complicated with Odonata. Colors are unreliable characteristic because some adults continue to lose their vibrant colors as they grow. Not to mention the newly emerged individuals, which may have different colors from that of an adult conspecific. Unfortunately, the only two resources for identification of the UAE's dragonflies are not comprehensive of Arabia and they exclude many dragonflies of Asian origins, which are usually locally found (Table 1). The aim of this study was to provide a molecular identification of a dragonfly based on the mitochondrial cytochrome c oxidase subunit I (COI) gene using the NCBI and BOLD databases in comparison with the morphology.

## Materials and Methods

### Insect Collection

The dragonfly specimen was captured manually from the vicinity of the Maqam Campus of the United Arab Emirates University, Al-Ain, United Arab Emirates (Lat.: 24.196221, Lng.: 55.679975) on the 19<sup>th</sup> of March 2017.

### DNA Extraction and PCR

Genomic DNA was extracted from the insect's leg muscle tissue using an automated DNA extraction machine Maxwell 16 (Promega, Madison, USA) according to the manufacturer's protocol. DNA was stored at -20°C. PCR reactions were conducted using the following oligonucleotide primer pair, which amplify a segment of the COI gene: LCO1490: 5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198: 5'-TAACTTCAGGGTGACCAAAAAATCA-3' (Folmer *et al.*, 1994). The template DNA was amplified in a 25 µL reaction mixture containing 50 ng of DNA, 10 pmol of each primer pair and 12.5 µL 2x PCR Master Mix (Qiagen, Hilden, Germany). Reaction mixtures were preheated at 95°C for 5 min. Amplifications were carried out for 35 cycles (95°C for 30 s, 45.5°C for 30 s and 72°C for 1 min) and a final extension cycle at 72°C for 5 min in a Swift MaxPro thermocycler (ESCO, Singapore). Every PCR included a negative control (no-template DNA) to detect any contamination. Gel electrophoresis was performed using 1.5% agarose gel, which was stained by ethidium bromide. The bands on the gel were visualized on ultraviolet light transilluminator and the photograph was taken using a gel documentation system (Major Science, Taiwan).

### DNA Sequence Analysis

PCR products were cleaned and sequenced (Sanger sequencing) by the Macrogen Company (Seoul, South

Korea). Sequences were analyzed by Mega 6 software (Tamura *et al.*, 2013), NCBI BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) and BOLD (<http://www.boldsystems.org/>).

## Results and Discussion

The primer pair used in this study amplified the target region of the COI gene and produced the expected single band ( $\approx 700$  bp) on the agarose gel (Fig. 1). The BLAST search of the DNA sequence revealed a 99% similarity with *A. imperator* at a sequence coverage 100% and an  $E$ -value = 0 (Table 2). The sequence appeared in a big cluster of *A. imperator* on the neighbor-joining tree (Fig. 2). However, the BOLD database showed 99.84% similarity with *A. parthenope* (Table 3) and the sequence appeared in a cluster of *A. parthenope* on the phylogenetic tree (Fig. 3). Based on the morphology, the insect was identified as *A. parthenope*. The DNA sequence was submitted in the GenBank with an accession number MH669065.

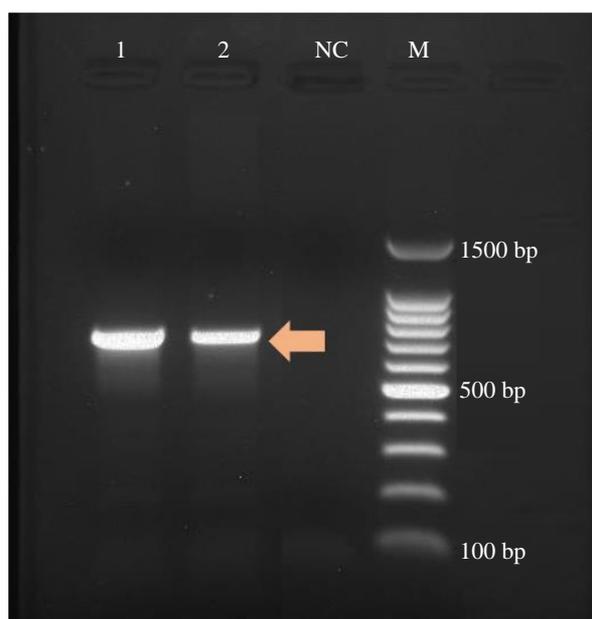
The traditional taxonomic identification is based on morphological characters and often is a challenging and somewhat a daunting chore, which demands experienced taxonomists in order to be done accurately. About two decades ago, an innovative molecular tool has been developed for determining species and their phylogenies. It was based on DNA sequences of short standardized gene segments and was named DNA barcodes (Ajmal *et al.*, 2014). In the UAE, DNA barcoding has been used in the identification of some arthropods (Al-Deeb and Enan, 2018; Al-Deeb *et al.*, 2015).

In order to identify the dragonfly of this study using a molecular tool, the DNA sequence of a fragment of the COI gene was compared to other DNA sequences in the NCBI and BOLD databases. The BLAST search in the NCBI database showed 99% and 98% similarity with *A. imperator* and *A. parthenope*, respectively. Therefore, applying the 2% sequence similarity rule to this case will identify the sample as *A. imperator* based on the 99% DNA similarity and as *A. parthenope* based on the 98% DNA similarity. In addition, after conducting the multiple alignment the sequence appeared in a cluster of *A. imperator* on the neighbor-joining tree. However, according to the BOLD database the sequence appeared in a big cluster of *A. parthenope* on the phylogenetic tree. This demonstrated that in some cases identifying an organism based on DNA alone could show some identification differences between databases. However, in this study the final species designation was made based on morphology, which came in agreement with the DNA sequence similarity produced by the BOLD database. Thus, the insect sample was identified as a female *A. parthenope*. It had a yellow and green face and the S1 abdominal segment was yellowish green (Fig. 3).

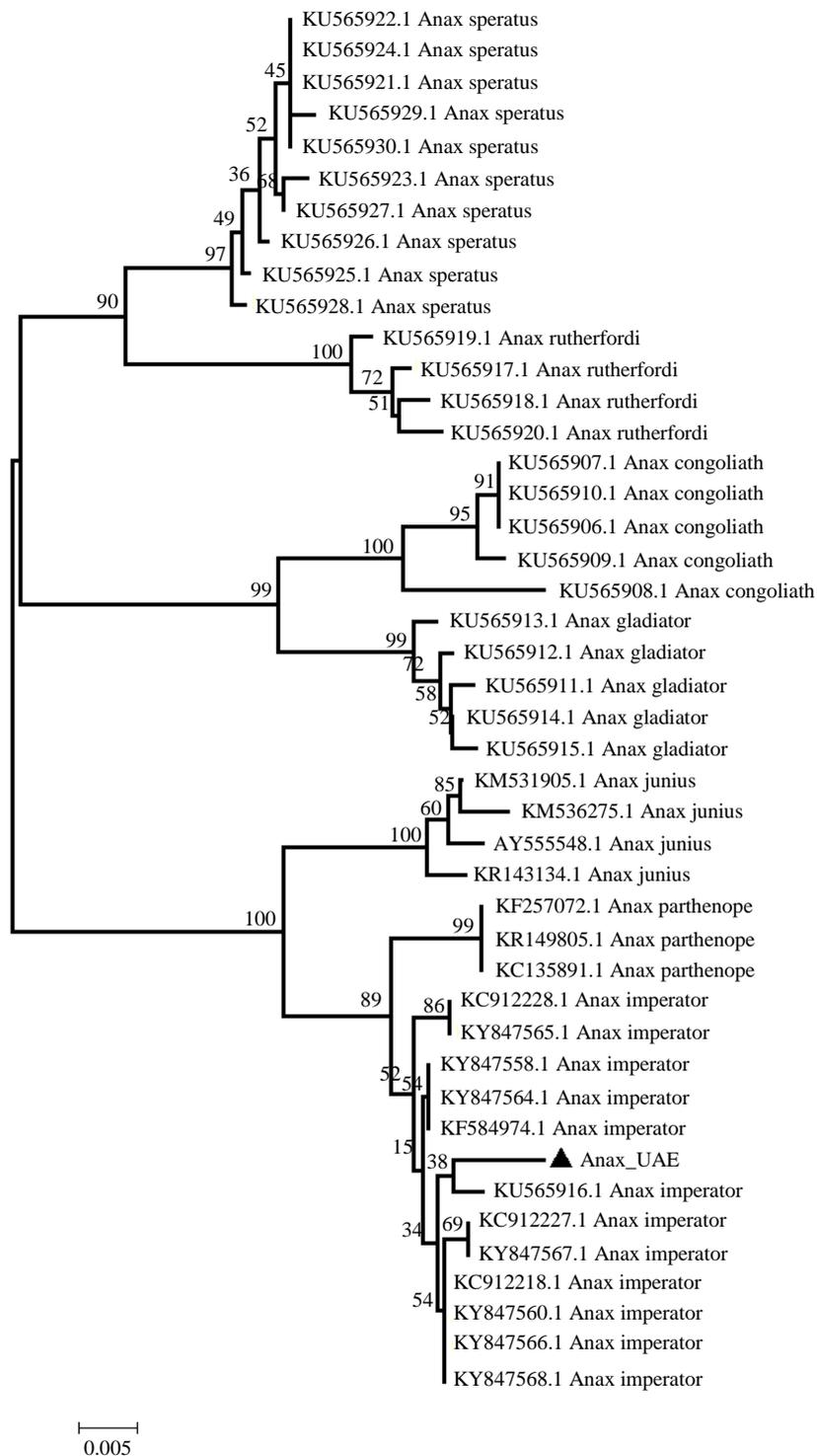
On the forewing there were strong costa and nodus. The pterostigmas were present, thin and long and reddish brown in color (Fig. 4). In addition, the R3 vein was sharply curved directly under the pterostigma (Fig. 5). Moreover, there were two foliated anal appendages on the last abdominal segment. Furthermore, on the head, the occipital margin was slightly protruding and squarish with a tubercle at each side.

Although DNA-based species identification looks very appealing to non-experts in morphology-based taxonomy, it is not always successful. In such cases, its limitations can be overcome by morphological identification. Some studies highlighted and discussed the problems with the use of DNA barcodes for species delimitation (Brower, 2006; Will and Rubinoff, 2004; DeSalle *et al.*, 2005). However, a group of taxonomists suggested the use of integrative taxonomy, which uses large number of characters including DNA (Will *et al.*, 2005). We are in favor of this approach because it capitalizes on the power of the traditional taxonomy as well as the power of the DNA barcoding.

From a different perspective, this study shows that adults of *A. parthenope* are active in March, which is the time of the year in which temperatures are around mid to high thirties in the UAE, which is much milder compared to the ones in the summer. As predators, adults of *A. parthenope* feed on other insects, which are active during this time period because the spring season is a very biologically active time in the UAE.



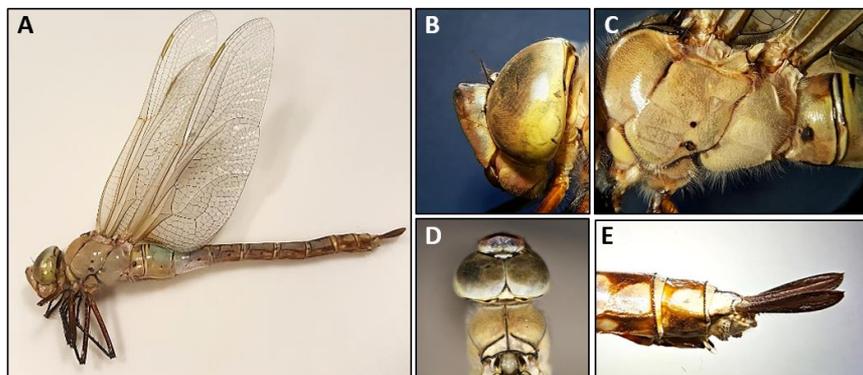
**Fig. 1:** Agarose gel (1.5%) stained with ethidium bromide showing two bands of COI gene produced by the LC01490 and HCO2198 primers and amplified by PCR. Lane M: 100-bp DNA ladder (Promega, Madison, USA); lane NC is negative control



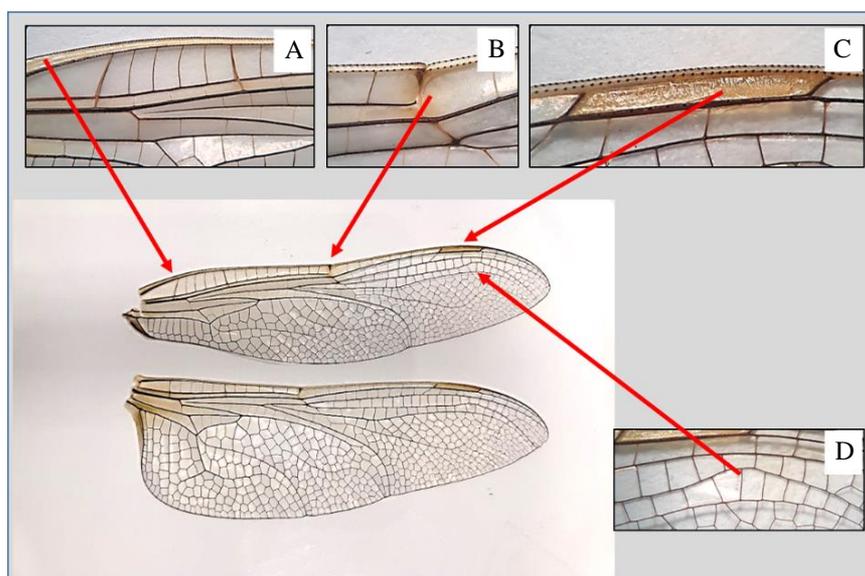
**Fig. 2:** Neighbor-Joining (Saitou and Nei, 1987) unrooted tree showing genetic similarity between the UAE dragonfly (MH669065) Cytochrome Oxidase subunit 1 (COI) gene and GenBank records. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches (Felsenstein, 1985). Genetic distances were computed using the Kimura 2-parameter method (Kimura, 1980) and are in the units of the number of base substitutions per site. Sequence alignments and tree generation were conducted in MEGA6 (Tamura *et al.*, 2013). The dragonfly from the UAE is placed in a cluster of *A. imperator*



**Fig. 3:** Unrooted tree using Kimura 2 Parameter distance model produced by Barcode of Life Data System (BOLD). The dragonfly from the UAE is placed in a cluster of *A. parthenope*



**Fig. 4:** Adult female of *A. parthenope*: (A) whole body (lateral), (B) head (lateral), (C) thorax (lateral), (D) head and prothorax (dorsal) showing occipital margin that is slightly protruding and squarish with a tubercle at each side and (E) the end of abdomen segments S8, S9 and S10 with two foliated anal appendages and the ovipositor underneath them



**Fig. 5:** Wings of female *A. parthenope*: (A) strong costa, (B) strong nodus, (C) prostigmas are thin and long and reddish brown in color and (D) the R3 vein is sharply curved directly under the pterostigma

**Table 2:** Molecular identification of a dragonfly from UAE as *Anax imperator* (MH669065) based on DNA similarity between cytochrome oxidase subunit 1 (COI) gene and GenBank species using NCBI BLAST

Best match species	Accession number	Sequence identity	Sequence coverage	E-value
<i>Anax imperator</i> mitochondrion, complete genome	KX161841.1	99%	100%	0
<i>Anax imperator</i> voucher RMNH.INS.502987	KU565916.1	99%	99%	0
<i>Anax parthenope</i> voucher P002	KC135891.1	98%	99%	0
<i>Anax parthenope</i> voucher CUAP 01-A1	KR149805.1	98%	96%	0
<i>Anax junius</i> voucher ODON 0057.02	KR143134.1	96%	98%	0
<i>Anax imperator</i> voucher DF_300	KF584974.1	99%	90%	0
<i>Aeshnidae</i> sp. CC14A-07	KX781748.1	96%	98%	0
<i>Anax junius</i>	AY555548.1	96%	98%	0
<i>Anax tristis</i> voucher RMNH.INS.502406	KU565931.1	96%	99%	0
<i>Anax imperator</i> voucher Ai16D	KY847568.1	99%	86%	0
<i>Anax imperator</i> voucher Ai21A	KY847566.1	99%	86%	0
<i>Anax imperator</i> voucher Ai61A	KY847563.1	99%	86%	0
<i>Anax imperator</i> voucher Ai98A	KY847562.1	99%	86%	0

Unless otherwise mentioned all the above sequences are cytochrome oxidase subunit 1 (COI) partial gene sequences. <sup>a</sup> The typical threshold for a good E-value from a BLAST search is  $10^{-5}$  or lower.

**Table 3:** Molecular identification of a dragonfly from UAE as *Anax parthenope* based on DNA similarity between cytochrome oxidase subunit I (COI) gene and Barcode of Life Data System (BOLD)

Phylum	Class	Order	Family	Genus	Species	Similarity (%)
Arthropoda	Insecta	Odonata	Aeshnidae	<i>Anax</i>	<i>parthenope</i>	99.84
=	=	=	=	<i>Anax</i>	<i>parthenope</i>	99.84
=	=	=	=	<i>Anax</i>	<i>sp. 1</i>	99.68
=	=	=	=	<i>Anax</i>	<i>parthenope</i>	99.68
=	=	=	=	<i>Anax</i>	<i>sp. 1</i>	99.52
=	=	=	=	<i>Anax</i>	<i>parthenope</i>	99.52
=	=	=	=	<i>Anax</i>	<i>parthenope</i>	99.52
=	=	=	=	<i>Anax</i>	<i>parthenope</i>	99.35
=	=	=	=	<i>Anax</i>	<i>parthenope</i>	99.35
=	=	=	=	<i>Anax</i>	<i>parthenope</i>	99.35
=	=	=	=	<i>Anax</i>	<i>imperator</i>	99.19
=	=	=	=	<i>Anax</i>	<i>imperator</i>	99.07
=	=	=	=	<i>Anax</i>	<i>imperator</i>	99.07

It is rich in growing plants and their associated herbivorous insects and thus many organisms, including *A. parthenope*, try to utilize this growth conducive period before the arrival of the scorching summer. To our knowledge, this paper is the first molecular record of a dragonfly in the UAE. We hope it will encourage taxonomists to sequence the DNA barcodes of all the known dragonfly species in the country.

## Conclusion

Although DNA barcoding has enough power to differentiate between intraspecific and interspecific variation, the current study is an example on how the DNA-based identification, in some cases, does not provide the accurate species identification and could assign the insect to the wrong species. In addition, it shows that the morphological identification can resolve problems arising from DNA-based identification. In short, integrative taxonomy could be the right middle ground.

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## Author's Contributions

**Noora Almansoori:** Performed the laboratory work, provided an insect image (Fig. 4A) and participated in writing the manuscript.

**Mohamed Rizk Enan:** Participated in data analysis and in writing the manuscript.

**Mohammad Ali Al-Deeb:** Conceived and designed the study, collected insects, contributed reagents and tools, provided insect images, participated in the laboratory work, analyzed the data and wrote the manuscript.

## Ethics

All the authors read and approved the manuscript. The authors declare no conflict of interest.

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