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Morphological study and Molecular identification of *Maiestas knighti* (Webb and Viraktamath, 2009) (Hemiptera: Cicadellidae) in Erbil Province, Kurdistan Region –Iraq

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ABSTRACT

A new record, *Maiestas knighti* (Hemiptera: Cicadellidae) is described and a molecular identified from Iraq. Samples were collected by Arial insect net from various Cucurbitaceae plants (melon, cucurbit, pumpkin, cucumbers and snake cucumber) between 25th/May till 2nd/August/2021 and 5th/June tile 2nd/September/ 2022. The important characteristic of species is indicated that, the mandibles and maxillae are needle shaped. The antennae are setaceous, pale brown, consist of 38 antennomers, 1st antenomer cup shaped, 2nd antenomer nearly rectangular, 2.6 times as long as the 1st antennomer, 3rd antenomer elongated oval, 38th antenomer oval, nearly equal in length with the 37th antennomer. Pygophore slightly sclerotized, posterior part narrower than anterior, apically covered with dense and long brown setae. The subgenitalia plate pale brown, long, a slightly convex caudal margin, apically rounded. Genital style is brown, hook shaped apically, basal part broader than apical part. The aedeagus dark brown, low sclerotized and dorsoventrally flattened. Photographs of the importance parts were provided. Localities, plant hosts and date of collected are indicated. The molecular identification determines a 550 bp bands of mitochondrial gene COI, which was amplified with PCR from the *Maiestas knighti* for constructing and distinguish a phylogenetic tree. The COI gene sequences of insect species were aliment insides NCBI GenBank using BLAST programs and utilized to compare the sequenced nucleotides others *Maiestas* species sequence. The outcome illustration that obtained sequences was *M. knighti* identified species based on the mitochondria COI gene. The sequence COI of *M. knighti* was submitted to GenBank with accession OQ709767 Kafroshi17.

Keywords: Morphological, Molecular, *Maiestas knighti*, Iraq.
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INTRODUCTION

Hemiptera is the fifth largest order of insects after Coleoptera, Diptera, Hymenoptera, and Lepidoptera, with approximately 82.000 described species [1, 2]. Cicadellidae Latreille, 1802 (leafhoppers) is important family belonging to the superfamily Cicadoidea, recently consist of 35,000-45,000 species and over 2600 described genera for the world [3]. *Maiestas knighti* (Webb and Viraktamath, 2009) It's among the most significant species belongs to the families, which are serious pest in both Nymphal and adult stages, they caused suck plant sap and transmit various pathogens [4], The genus *Maiestas* Distant, 1917 distribution in Palearctic region, there are three species in Iran, *M. trifasciata* (Lindberg, 1954), *M. schmidtgeni* (Wagner, 1939) and *M. horvathi* (Then, 1896) [5]. [6] The *Maiestas* species, which pose challenges or uncertainties in identification, are the subject of DNA barcode development experiments. Mitochondrial genes (Mt COI) were used in the molecular characterization process, which involved DNA extraction, PCR amplification, and sequencing for *M. dorsalis* and *M. krameri.* The study's goals include a detailed description and a molecular identification of, *Maiestas knighti* Webb and Viraktamath,2009 as new species from Iraq.

MATERIALS AND METHOD

Insect Samples were obtained from a variety of Cucurbitaceae plants, including (melon, cucumbers, pumpkins, snake cucumbers and cucurbits) in different locations near the city of Erbil, Kurdistan region during, 25th/May/ 2021 tile 2nd /August/2021 and 5th/June/2021 tile 2nd/September/ 2022.

Morphological identification

The present paper is based on 20 specimens (15 Male and 5 Female) which collected during 25th/May/ 2021 tile 2nd /August/2021 and 5th/June/2021 tile 2nd/September/ 2022. by Arial insect net through various Cucurbitaceae plants (cucurbit, melon, snake cucumber pumpkin, and cucumbers) from some locality of Erbil provinces. The sample were placed in warm waters for 15-20 minute to softens their parts. After that separated parts by 2 micro pin and put in KOH 10%, thein placed on (heater source) with shaking for 15-20 minute for dissolvent the lipid. After being placed in distilled water for 3-4 minutes. in order to destroy the alkali. The parts are placed in 25% ethyl alcohol and dissected under binocular microscope, then transferred to 50%, 75% and 100% ethyl alcohol successively for 2 minutes each concentration to dehydration of waters. The clearing parts are then placed in dishes with xylene for 5 minute after that were fixed on slide with DPX solution and covered by cover slide for subsequent examination [7, 8, 9]. The digital computerized microscope and compound microscope using for describing the insect body parts, after that the important parts and the habitus were photographers by using a digital camera (Ucmas series microscope camera), then length of the parts is measures using a linear micrometer. Species identifies based on the taxonomic key available in the previously published literature [10, 11]. Furthermore, Dr. Hanna Hani Al-safar of the Iraqi Natural History Research Centre and Museum at the University of Baghdad, Baghdad, Iraq, has confirmed this species. The specimens are preserved in Museum of Plant Protection Department in College of Agricultural Engineering Sciences in Salahaddin University, Erbil- Iraq.

Molecular identification

For the procedural studies, the molecular studies by [12] was following.

1. Extraction of DNA:

DNA genome was obtained by extracting it from ten adult's subjects using the ZYMO Quick-DNA Tissue/Insect Micro-Prep Kit (No. D6015) following the instruction construction. The genomic DNA was isolated and kept at -20°C for future use in applicate downstream. The quality of DNA was assessed utilize a Nano Drop spectrophotometers from Thermo Scientific UK.

2. Amplification by Polymerase Chain Reaction (PCR):

Cytochrome Oxidase c subunit I (COI):

Mitochondrial specific-gene primers were designed utilizing cytochrome c oxidase I subunit sequences synthesized by Company Micro-gene (South Korea) (Table 1) and then amplified by PCR for each specimen. The Primers generated a 550 bp band, amplification PCR for COI was performs using 50 µL of partial gene extracted partial gene, resulting in a final reaction mixture containing; 2x Taq DNA polymerase master mix (AMPLIQONA/S Stenhuggervej22) was utilize for partial gene amplification of 10 picomoles (pmol) of primer pair, template DNA and DNase-free water (Table 2) using Bioresearch PTC-200 Gradient thermos-cycling process.

The PCR programs consisted of three steps the first steps: included denaturation at 5 min at 95 °C, the second step 35 cycle of denaturation at 40 min for 95 °C, annealing of primers at 40 min for 60 °C and extension for 1 min at 72 °C. The final step included further extension at 10 min for 72 °C after which the samples were stored at -20 °C for subsequent use.

Table (1): COI a Pair of oligo nucleotide

Gene names	Sequences Nucleotide	Products size	Reference
Cytochrome Oxidase c subunit I (COI)	Forward C1-J-1718 3' (GGAGGA TTTGGAAATTGATTAGTTCC) 5' Reverse HCO2198 5'(TAAACTTCAGGGTGACCAAAAAAT) 3'	550bp	[13]

Table (2): PCR reaction mix for amplifying the gene COI

No.	PCR component	Concentrations	Volumes (µl)
1	Master Mix	2x	25
2	Forward Primers	10 Picomol	3
3	Reverse Primers	10 Picomol	3
4	DNases free Water	-	15
5	DNA Template	50ng/µl	4
	Totals		50

3. Visualizations of DNA fragment

After 30 minutes in the electrophoretic electrics field, ethidium bromide dye is added to a 1.5% agaroses gel in 1X TAE buffer, and the positions of the bands are determined by examining the gels under UV-trans illumination.

4. Sequencing DNA Analysis

PCR produced partial gene COI samples were sequenced using the ABI Prism Terminators Sequence Kit (Appling Bio-system) at the Micro-Gene Centers in Korea utilize Finch TV programming soft wares, gene COI chromatogram were check and base calls were verified.

5. Sequence submission and Alignment

The COI gene sequences were utilized in Basic Local Alignment Search Tool (BLAST), which a searches tool that applies sequences alignment(https://blastncbinlmnihgov/Blastcgi), and is obtainable at the (Nationals Biotechnology Information Centers) NCBI websites to compared and alignments lab or query sequences with others biology sequences to identify high similar with the another targets.

RESULTS AND DISCUSSION

First: Morphological Identification

Description of *Maiestas knighti* Webb and Viraktamath, 2009

Insect body: Cylindrical, yellow -pale brown, length 2.9-3.5 mm and width 0.8-0.9 mm. The head region: Nearly triangular, pale yellow -pale brown, length 0.6-0.8 mm. Vertex brown, slightly convex. Frons yellow -pale brown, slightly convex, with low dense with fine punctures. Clypeus pale brown- brown slightly convex. Eye oval elongate, prominent brown, length 0.3-0.4 mm, the distance between eyes 0.5- 0.6 mm. Two ocelli present. Antenna (Fig.1d) pale brown, length 0.9-1.2 mm, consist of 38 antennomers, 1st antenomer cup shaped, 2nd antenomer nearly rectangular, 2 times as long as the 1st antenomer. 3rd antenomer elongated oval, 38th antenomer oval, nearly equal in size with 37th antenomer. Mouthparts piercing sucking type, pale brown- brown, soft, length 0.7- 0.8 mm. Labrum (Fig.1e) stylet-like, 0.2-0.3 mm. Mandible and Maxilla (Fig.1f, g) needle-like, length 0.4-0.5 mm. Labium three segmented, 3rd palpimer 1.2 time as long as the 2nd palpimer.

Thorax: Pale yellow -pale browns, prothorax smaller than the mesothorax and metathorax, surface with elongate brown spots and a low dense of fine punctures. Procoxal cavity closed, prosternal process rounded and brown. Scutellum triangular, surface with five spots. Fore wing (Fig.1h) membranous, yellow -pale brown, length 2.6-2.9 mm, with irregular spots on the surface, R vein branched to R1, R2+3 and R4+5; M vein consist of M1+2 and M3+4 at apically, have a single Cu and A1, A2, also have four apical cells. Hind wing brightly pale yellow. Fore legs (Fig.1i) yellow -pale brown, coxa nearly bulb shaped, trochanter triangular, femur elongated oval, tibia tubular, latterly with one row of spines, length 0.4-0.7 mm, apical part bears two brown spurs. Tarsus consist of three tarsomers, 2nd tarsomer 1.4 times as long as the 1st tarsomere. Claws simple, stylet shaped. Middle legs resemble foreleg except, the length of mesotibia 0.8-1.0 mm. Hind legs resemble forelegs except length of the metatibia 1.7-1.9 mm.

Abdomen: Elongate oval, soft, brown- dark brown. Abdominal dorsal view consists of eight visible tergites, anterior margin of 7th tergite straight, posterior margin low convex. Abdominal ventral view consists of seven visible sternites, anterior margin of 6th sternite convex, posterior margin straight.

External male genitalia: (Fig.1j) Brown, length 0.7-1.2 mm. Pygophore pale brown-brown, slightly sclerotized, posterior part narrower than anterior, apically with dense covering of long brown setae. Valve subgenitalia plate pale brown, long, caudal margin slightly convex, apically rounded. Styles brown, apical slightly hook shaped, basal part broader than apical. Aedeagus (Fig.1 k,l) dark brown, low sclerotized, dorso-ventrally flat or connected, length 0.7-0.8 mm. [14] described the male genitalia and indicated that the lateral margin of subgenitalia plate is slightly convex.

The female is similar to the male except, slightly longer, the length 3.8-4.1 mm.

Fig.1 a, b &c Habitat (Dorsal view; Ventral view and Lateral view) d. Antenna e. Labrum f. Mandible g. Maxilla h. Forewing i. Foreleg J. pygofore k. Aedeagus (dorsal view); l. Aedeagus (lateral view) scale bare (a,c= 13 X b=14 X d, f,g,I,j,k,l= 0.25 mm e= 0.10 mm h= 0.5mm)

Second: Molecular Identifications

The PCR products were electrophorese and visualized on a 1.5% agarose gel to verify the convenient part of the cytochromes oxidase subunits 1gene. A 3000 bp ladder was used as a reference. The documentary image from the BioDoc Analyzer gel indicates that all selected samples of up to 550 bp in length (Figure2). Mitochondrial gene-specific primers were designed (universal primers) for use with cytochromes c oxidases I subunits sequences synthesize by Micro-Genes Company (South

Korea). The primer can generate a bands of ~550 bp. PCR products was electrophorese and visualization an agarose gel 1.5% (Figure 2)

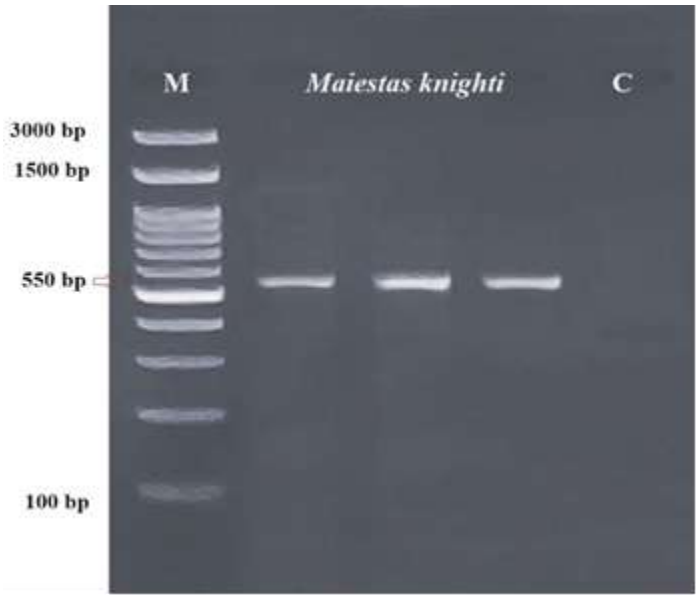

Fig2. Amplification PCR of partial cytochromes C Oxidase I gene regions of *M. knighti* Lane M; indicate: ladder lanes number 1 – lanes number 4: 550 bp of PCR product of insect and C is negative controls

Partial cytochrome c oxidase I Sequence genes

DNA Sequencing was performed using forward primer C1-J-1718, separate by ABI 3130X genetic analyzer (Apply Bio-systems). PCR products from the ten specimens were used as a sources of template DNA for specifics PCR amplification.

Molecular Identification of genus *Maiestas knighti*

The species *Maiestas knighti* BLAST programs from Gen banks (http://blastncbinlmnihgov/) is feds 550-bp sequence COI sample. Applies for comperes our amplify sequence with sequence of another species *Maiestas* [12] The BLAST results confirmed that the query sequences showed high similarity to insects identification records in the NCBI gene banks. This alignment observed to submitted our concern sequence to the NCBI Gen bank, and the accessions number are lists in the tables bellows.

Table (3) sequences gene COI partial in NCBI and aliment with same sequence after submissions

Insect Identify	Accessions Number	Query Covers %	Identic Numbers %	Accession Numbers of BLAST Identification
Maiestas knighti	OQ709767	100	98.6	KF227139
		100	99.08	KF227130
		100	98.18	KF2271132

Phylogenetic tree

The nucleotide sequence of COXI was used to construct a phylogenetic tree, which confirmed the expected grouping of the two investigated insect specimens. The results of the phylogenetic tree analysis among species in the family Cicadellidae displayed in (Fig. 3) showed that the species *Maiestas knighti* is similar to previously described species, with a 94% difference from the other family. The specimen groups in one clusters with highest similar off into GenBank insect's species.

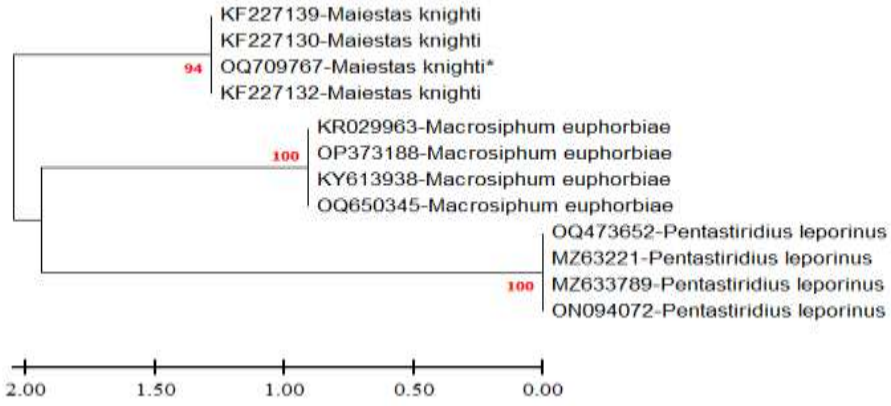

Fig. 3 Phylogeny trees of *M. knighti* species from Iraq the phylogeny tree was prod utilized MLm with the Tamura-Nei model in MEGA11 software's and bootstrap analysis with 94 replicate. As inputted data, partial DNA sequence of concatenated partial COXI mitochondrial gene were used.

The use of molecular primers that can detected a changes in the base sequence of specimen-specific DNA, such as the mitochondrial gene, enable the successful detection of genetic variations in certain species. Moreover, the mitochondrial cytochrome CO1 gene is highly conserved and lack introns in animals. This allows the construction of primers that can work with a wide ranges of species and the alignment of generated DNA sequences for population genetics and phylogenetic studies [15]. [16] conducted a study on the phylogenetic relationships between genera and species within the leafhopper tribe Deltocephalini's with a specific focus on the large genus *Maiestas*

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دراسة مورفولوجية وتحديد جزيئي لحشرة Maiestas Knighti(Hemiptera: Cicadellidae) (Webb and Viraktamath, 2009) في محافظة أربيل، إقليم كردستان – العراق

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• تاريخ استلام البحث 2024/2/14 وتاريخ قبوله 2024/3/31 .

الخلاصة

تم تسجيل جديد ووصف مع الدراسة الجزيئية للنوع ***Maiestas knighti*** **(Hemiptera: Cicadellidae)** . جمعت العينات باستعمال الشبكة الهوائية من بعض النباتات (البطيخ ، الخيار ، القرع ، اليقطين ، القثاء والبطيخ) وللفترة مابين ٢٥ ايار الى 2 آب 2021 و5 حزيران الى 2 ايلول 2022. الصفات المهمة المميزة للنوع هو ان الفكوك العليا والمساعدة أبرية الشكل . اللآمس بني اللون ، شعري الشكل يتكون من 38 عقلة ، العقلة الأولى فنجانية الشكل والثانية مستطيلة تقريبا طولها بقدر 2.6 طول العقلة الأولى ، العقلة الثالثة بيضوية الشكل والعقلة 38 بيضوية ومساوية لطول العقلة 37. المحفظة التناسلية في الذكر قليل التصلب ، جزءها الخلفي أضيق من الأمامي ، يحتوي قمتها شعيرات كثيفة ، طويلة بنية اللون. القلم التناسلي بني اللون ، جزءه القمي كلابي الشكل. الصفيحة تحت التناسلية باهتة اللون بنية، طويلة ، حوافها الخلفية قليلة التحدب وقمتها كروية الشكل. القضيب ذو لون بني داكن ، قليل التصلب ومسطح من الجهتين الظهرية والبطنية . تم تصوير بعض الأجزاء المهمة . ذكرت العوائل النباتية ، المناطق و تاريخ جمع الحشرة.

وقد استخدمت تقنية تفاعل البوليمراز المتسلسل (PCR) تم تحديد الجزيئية ل(550 قاعدة نيوكليوتيدية) من جين COI الخاص بالميتوكندريا باستخدام تفاعل البلمرة المتسلسل من *Maiestas knighti*لبناء وتمييز شجرة التطور. تمتضخيم جزء من جين COI لأنواع الحشرات المدروسة واستخم امقارنة النوكليوتيد المتتابع مع سلاسل الأخرى للحشرات باستخدام برامج BLAST داخل NCBI GenBank تبين أن التسلسلات المحصل عليها تمثل نوع *Maiestas knighti* بناء على جين COI الميتوكندريا تم تقديم COIل *Maiestas knighti* وتم تسجله في بنك الجينات برقم Kafroshi17 OQ709767.

الكلمات المفتاحية: مظهرية، الجزيئي، *Maiestas knighti* في العراق.