

Morphological and molecular identification of the scorpion *Androctonus crassicauda* (Olivier, 1807), using COI and 12S rRNA genes from the Provinces of Dhi Qar and Wasit, Iraq

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Abstract

The scorpion fauna of Iraq remains comparatively less studied than that of several neighboring Middle Eastern countries. This study aimed to identify and characterize one scorpion species found in northern Dhi Qar Province and southern Wasit Province, both morphologically and molecularly. Samples were collected in the field. Morphological identification relied on external features and morphometric measurements, while COI gene expression, 12S rRNA, and polymerase chain reaction (PCR) were used for molecular identification. The results identified a scorpion species belonging to the Buthidae family, *A. crassicauda*, which is abundant and widespread. Morphological and molecular findings confirmed the identification. BLAST analysis showed a 99% similarity with reference sequences, and phylogenetic trees indicated high genetic convergence with limited variation within the species.

Keywords: Survey, (Arachnida, Scorpiones), Morphological identification, Molecular identification. *A. crassicauda*

التشخيص المظهري والجزيئي للعقرب *Androctonus crassicauda*
بأستخدام جيني COI و 12S rRNA من محافظتي ذي قار وواسط/العراق

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الخلاصة

لا تزال أنواع العقارب في العراق أقل دراسة نسبياً من تلك الموجودة في العديد من دول الشرق الأوسط المجاورة. هدفت هذه الدراسة إلى تحديد وتوصيف نوع واحد من العقارب الموجودة في شمال محافظة ذي قار وجنوب محافظة واسط، وذلك مورفولوجياً وجزيئياً. جُمعت العينات ميدانياً. اعتمد التحديد المورفولوجي على السمات الخارجية والقياسات المورفومترية، بينما استُخدم التعبير الجيني لـ COI و 12S rRNA، وتفاعل البوليميراز المتسلسل (PCR) للتحديد الجزيئي. كشفت النتائج عن نوع من العقارب ينتمي إلى عائلة Buthidae، وهو *A. crassicauda*، الذي كان الأكثر وفرة وانتشاراً. أكدت النتائج المورفولوجية والجزيئية هذا التحديد. أظهر تحليل BLAST تشابهاً بنسبة 99% مع التسلسلات المرجعية، وأشارت الأشجار التطورية إلى تقارب جيني عالٍ مع تباين محدود داخل النوع.

الكلمات المفتاحية: مسح، (Arachnida)، (Scorpiones)، التشخيص المظهري، التشخيص الجزيئي، *A. crassicauda*.

1.Introduction

The scorpion fauna of Iraq remains comparatively less studied than that of several neighboring Middle Eastern countries. The most comprehensive modern review of Iraqi scorpions recognized 19 species belonging to 13 genera and five families, namely Buthidae, Euscorpiidae, Hemiscurpiidae, Iuridae, and Scorpionidae. Among these, Buthidae is the most

diverse family, comprising 15 species in nine genera, whereas each of the remaining families is represented by a single species. The same review emphasized that historical records from Iraq include a number of doubtful identifications and locality records, and 18 previously reported species were excluded from the accepted Iraqi fauna after taxonomic reassessment [1].

The history of scorpion research in Iraq extends back to the nineteenth century. Peters (1861) described *Hemiscorpius lepturus* from Mendeli near Baghdad, while Simon (1880) described *Buthus saulcyi* from Mosul [1]. With respect to the species investigated in the present study, *Androctonus crassicauda* [2] was already known from the region by the end of the nineteenth century. The earliest literature record explicitly cited for Iraq in the modern checklist is Kraepelin (1899) [3], who listed the species under the name *Buthus crassicauda* from Mesopotamia [1], [3]. Subsequent Iraqi records appeared under different combinations, including *Prionurus crassicauda* in Penther (1912), *Buthus crassicauda* in Kennedy (1937), and *Buthus* (*Prionurus*) *crassicauda* in Whittick (1955). Later authors used the currently accepted name, *Androctonus crassicauda* [1].

It is therefore important to distinguish the earliest Iraqi record from the first dedicated taxonomic study of the species in Iraq. The latter was published by Al-Azawi and Bassat (2016) [4], who described and illustrated the principal taxonomic characters of *A. crassicauda* based on specimens collected from Baghdad, Diyala, Salah ad-Din, Al-Qadisiyah, Wasit, and Dhi Qar provinces [4]. Their study was presented as the first taxonomic study of *A. crassicauda* in Iraq; however, it was not the first historical record of the species from the country, as earlier records had already been reported in the literature [1], [3].

Androctonus crassicauda is currently regarded as one of the most widely distributed scorpion species in Iraq. The 2021 national checklist documented it from 15 Iraqi provinces: Basra, Babil, Dhi Qar, Maysan, Al-Qadisiyah, Wasit, Najaf, Salah ad-Din, Karbala, Al-Anbar, Baghdad, Diyala, Nineveh (Mosul), Erbil, and Duhok [1]. These records indicate that the species occurs across the major geographical regions of Iraq, from the northern and northeastern parts of the country to the central, western, and southern regions [1].

Taxonomically, *A. crassicauda* belongs to the family Buthidae and was originally described by Olivier in 1807 from Kashan, Esfahan Province, Iran [1], [2]. Its broader geographical range extends across much of the Middle East and adjacent areas, including Iraq, Iran, Turkey, Syria, Jordan, Israel, Kuwait, Saudi Arabia, Oman, Bahrain, the United Arab Emirates, Yemen, Armenia, Azerbaijan, and the Sinai region of Egypt [1]. Its broad occurrence within Iraq, together with its medical importance, makes accurate taxonomic identification and documentation of its geographical distribution particularly relevant when interpreting biological, ecological, or toxicological studies of Iraqi populations.

The order Scorpions are classified within the Arachnida class that also includes, which belongs to the Arthropoda phylum [5]. Members of this order are found in other continents except Antarctica, scorpions, inhabiting deserts, mountains, rainforests, and coastal environments [4]. Recently, there are at least 2,500 species of scorpion on the Earth planet, inhabiting most ecosystems worldwide [8]. In There are about 117 scorpion species in the Middle East region, 22 of them are highly venomous [9]. Scorpions have a distinctive body structure consisting of a cephalothorax (prosoma) and an opisthosoma (abdomen), which is further divided into a broad anterior part called the mesosoma and a narrow posterior part

called the metasoma. The metasoma is composed of five segments ended with Nelson's stapes, which contains the venom glands [10].

The eight legs and segmented tail are the key characteristic of all scorpions. The appendages known as pedipalps, along with two prominent pincers. The tail is long and slender curves over the posterior part of the abdomen. This tail contains a swollen venom gland and a stinger. Scorpions are characterized by their small eyes and remarkably poor vision. As predatory arthropods, scorpions rely primarily on their strong senses of touch and sound to detect and hunt their prey. Scorpions have very hard bodies, their length ranging in from 3 to 18 cm, scorpions are night creatures found in most environments such as deserts, caves, mountains, and even under rocks. When they threatened or disturbed, they are act to

The aim of study was to identify morphologically and molecularly of a scorpion species *Androctonus crassicauda* in northern Dhi Qar Province and Southern Wasit, Iraq.

2. Materials and Methods

Table 1- Specimens collection Sites for the Current Study in North Dhi Qar and South Wasit and their Environmental Classification.

No.	District	Location	Type of area	Collection areas	Coordinates
1	Rifai	North Dhi Qar	Agricultural	Nazim area No. 4 - Al-Musayfi area - As'idan area - Al-Hassan neighborhood area	N"00'36"31 E "60 '16"45
2	Qalat Sukkar	North Dhi Qar	Agricultural	Al-Batrounat area - Sayyid Hamid village - Al Abu Hassan village -	N "36'24"31 E "00 '18"45
3	Fajr	North Dhi Qar	Desert-agricultural	Tal Al-Aqareb area - Hassan Ibn Thabit area - Al-Malih area - Al-Badir area	N"00'30"31 E"00'27"45
4	Wasit	Southern Wasit	Desert-agricultural	Al-Hay district (brick factory area) - Al-Bashair - Badra and Jassan	N 46""36°32 E" 06' 49° 45

2.1 Specimens Collection

120 specimens were collected from twelve locations across 4 stations in north part of Dhi Qar Province and south part of Wasit Province during the duration between August 2025 and July 2026. Samples were collected at night using ultraviolet light, which induces fluorescence in scorpions. The samples were collected using forceps and stored in sterile plastic bottles containing 70% ethanol until laboratory analysis was completed.

2.2 DNA Extraction

Genomic DNA was extracted from scorpion tissues using a commercial insect extraction kit, according to the manufacturer's instructions. The extraction steps included tissue homogenization, cell lysis with lysate and proteinase K, separation of the aqueous phase with chloroform, and transfer of the filtrate to extraction columns to obtain purified DNA. Electrophoresis of the PCR products was performed on a 2% agarose gel that was placed under to a light with a UV source, then; the results were captured with a mobile phone camera.

3. Results

3.1 Morphological identify

Morphological identification was done for. The diagnosis relied on external feature morphological identification, such as body color, shape, combs, mesosomes, metasomas, and telsons. The results represent a presence of one scorpion species belonging to the Buthidae family, *A. crassicauda*, a species previously recorded in Dhi Qar Province.

3.2 Family Buthidae

Buthidae family's members have medium to large body size, long, slender pedipalps with serrated toes, and long metasomas ending in telsons that containing the venom gland with the stinger. They also possess a pair of median eyes and several lateral eyes, a triangular or pentagonal sternum, and variations in the number of teeth on the combs. Their coloration varies among yellow, brown, and black depending on the environment, and they are widely distributed across hot, semi-arid, and temperate regions.

3.3 General Classification

Species *A. crassicauda* (Olivier, 1807)

3.4 Morphological Characteristics

The body is characterized by a black or dark brown color tending towards red. The average total length is 68.61 mm in males and 71.39 mm in females. The prosoma and mesosome regions are dark in color, with slender footpads and pedipalps with long toes.

The mandibular claws consist of a fixed toe and a movable toe, each bearing distinctive diagnostic teeth. They measure 36.92 mm in length in males and 39.81 mm in females.

The pedipalps are characterized by long, toothed toes bearing rows of granules, with three granules near the terminal tooth. This is an important feature in species identification (Figure 3).

The carapace is trapezoidal and rough-surfaced due to chitinous granules. It bears a pair of central eyes and several small lateral eyes. It measures 6.74 mm in length in males and 8.11 mm in females (Figure 4).

The pectinae are long and slender, located behind the sternum. They have an average of 28 teeth in males and 20 in females.

Meosoma consists of seven rough, dark brown to black segments, characterized by distinct crests and granules (Figure 1).

As about legs, they consisting of four pairs of medium length, yellowish-brown to dark brown, ending in fine claws, and covered with chitinous hairs and granules.

Metasoma consists of five strong, thick segments terminating in a venom vesicle, characterized by distinct crests and dense granules. It measures 30.74 mm in males (Figure 5 A) and 32.16 mm in females (Figure 5 B).

Telson consists of a venom vesicle and a downward-curving needle. It measures 9.87 mm in males (Figure 2 A) and 9.69 mm in females (Figure 2 B) and is an important diagnostic feature of the species.

The bristles of Trichobothria are distributed on the phalanges of the foot appendages, particularly the femur, patella, and chela. They are arranged symmetrically on the inner and outer surfaces of the chela, while on the femur and patella they are arranged in longitudinal lines. This is an important taxonomic characteristic in identifying the species.



Figure -1 Mesosome in the scorpion *A. crassicauda* from the dorsal view, A. Female mesosome showing the dorsal body segments (tergets) and the distribution of surface granules, B. Male mesosome showing the dorsal body segments (tergets) and the distribution of surface granules.

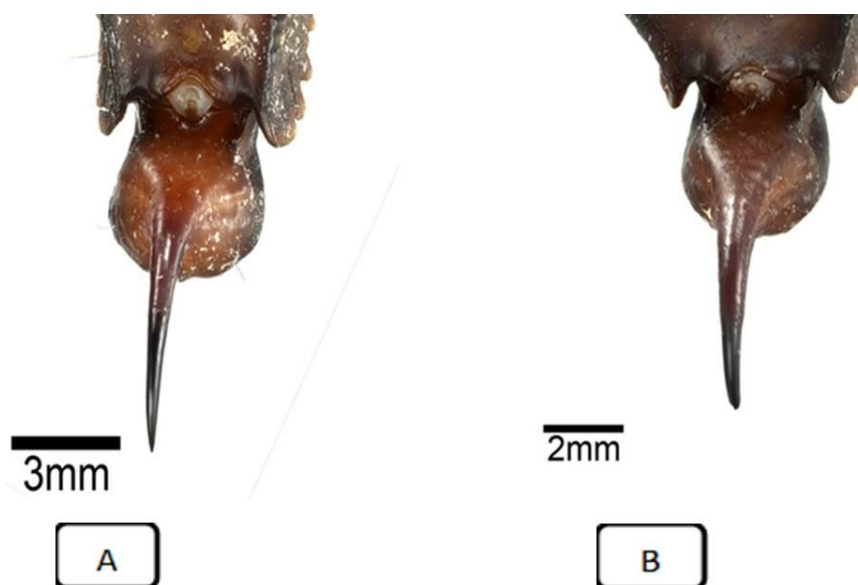


Figure -2 The telson and venom stinger in the female and male, A. The telson and venom stinger in the female, showing the shape of the venom vesicle and the elongation of the venom stinger, B. The telson and venom stinger in the male, showing the shape of the venom vesicle and the elongation of the venom stinger



Figure -3 Structures of the pedipalp chela in the crassicauda species, A. Side view of the movable finger showing elongation and curvature towards the apex with rows of teeth visible on the incisal edge, B. Side view of the fixed finger showing widening of the base and gradual curvature towards the tip, with regular teeth on the inner margin.

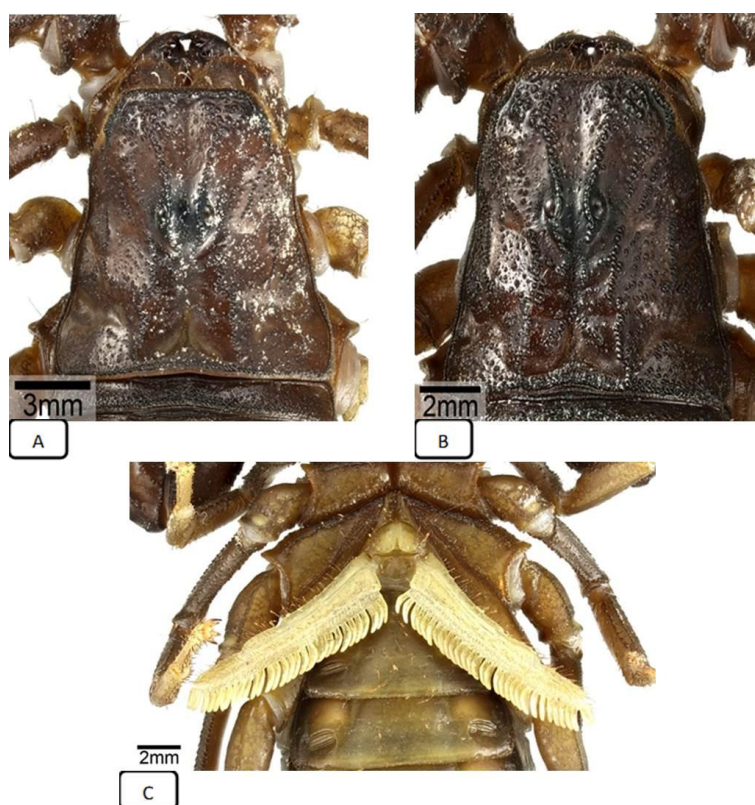


Figure -4 The carapace of the scorpion *A. crassicauda*, A. Dorsal view of the carapace in the male, B. Dorsal view of the carapace in the female, C. Stemopectinal area in the male.



Figure -5 Metasoma and telson in the scorpion *A. crassicauda*, A. Dorsal view of the metasoma and telson in the male, B. Dorsal view of the metasoma and telson in the female.

3.5 Molecular Analysis

Electrophoresis on agarose gel showed successful amplification of the COI and 12S rRNA genes using PCR. Clear bands were observed at approximately 710 base pairs for the COI gene and 360 base pairs for the 12S rRNA gene, confirming the quality of the amplification process and the suitability of the products for molecular analysis.

BLAST analysis showed a high degree of concordance with the reference sequences in the GenBank database, with a similarity of 99% in the *A. crassicauda* samples. The phylogenetic trees also showed that all local isolates clustered with reference sequences within a single biological branch with limited genetic variation within the species, confirming the accuracy of molecular diagnosis using the 12SrRNA and COI genes.

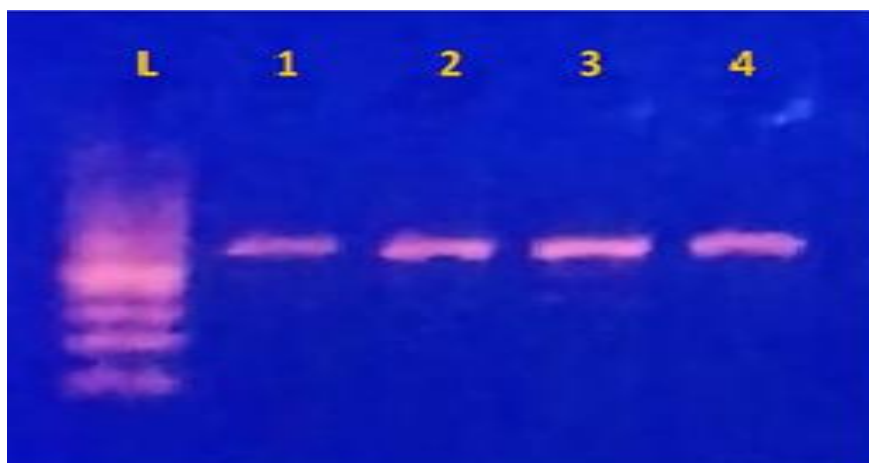


Figure -6 Electrophoresis on agarose gel of polymerase chain reaction (PCR) products of S rRNA 12 and COI genes.

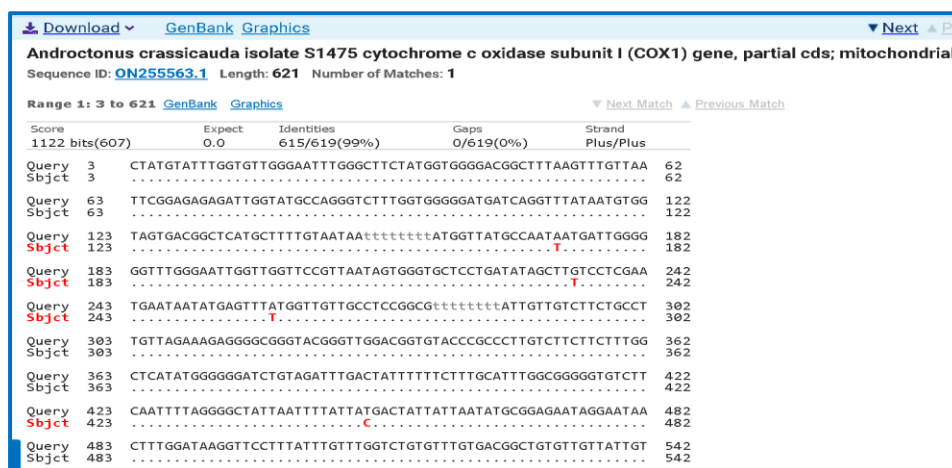


Figure -7 The BLAST alignment pattern of the *A. crassicauda* COI gene sequence shows high similarity to the GenBank reference sequence (ON255563.1).

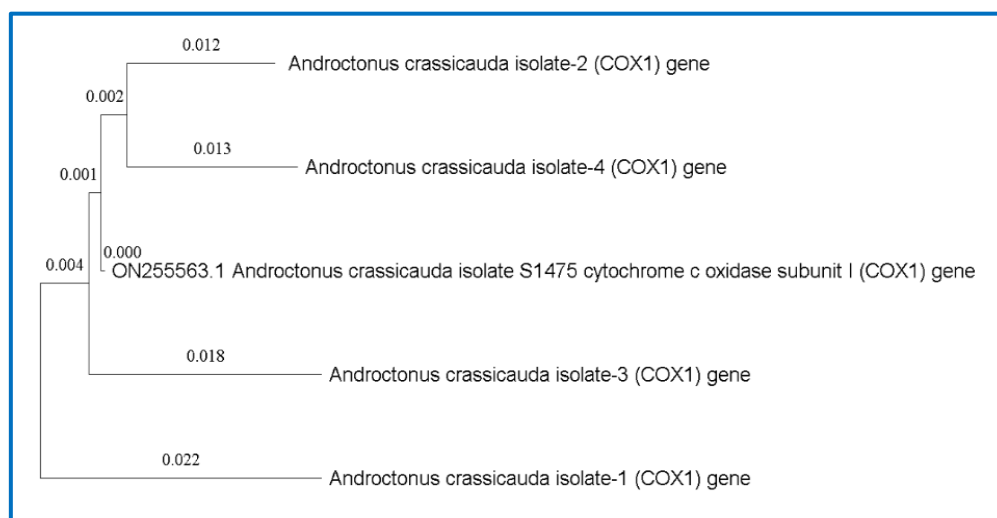


Figure -8 Maximum probability phylogenetic tree based on COI gene sequences of *A. crassicauda*

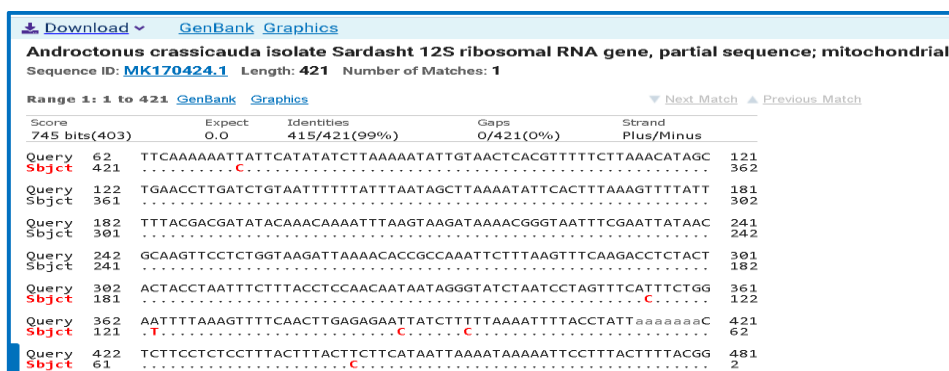


Figure -9 BLAST alignment of the 12S rRNA gene sequence of *A. crassicauda* shows high similarity to the GenBank reference sequence (MK170424.1).

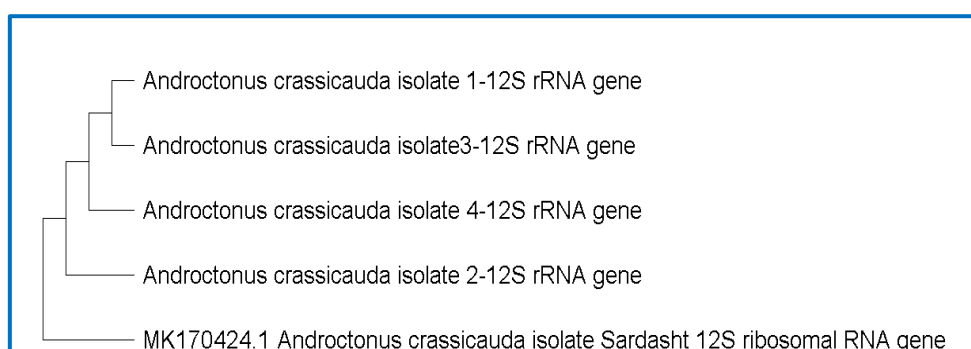


Figure -10 Maximum Probability Phylogenetic Tree Based on 12S rRNA Gene Sequencing of *A. crassicauda*.

4. Discussion

The current study showed that all identified specimens belonged to the Buthidae family, including one species *A. crassicauda*. This result confirms the dominance of this family in the northern Dhi Qar and southern Wasit regions, which is consistent with previous studies indicating that Buthidae is the most widespread family in arid and semi-arid environments in Iraq and the Middle East [18]; [19]. This is attributed to the morphological and functional characteristics that help it adapt to harsh environmental conditions, such as high temperatures and low humidity.

The study showed that *A. crassicauda* was the abundant species, with 75 out of 120 specimens, indicating its dominance in the study area. Its widespread distribution is attributed to its high adaptability to agricultural, desert, and semi-desert environments, as well as its tolerance to high temperatures and drought. Its morphological characteristics showed clear consistent with the previous established taxonomic descriptions. Specimens were recognized by the black or dark brown body color, strong metasomas, and long footpads, along with clearly defined granules and crests on the shield and metasomas, these characteristics consistent with what reported in the international taxonomic references [6]; [20]; [21]. The footpads also exhibited a unique arrangement of granules and teeth, underscoring the importance of these characteristics in the diagnosis and differentiation of Buthidae species.

Morphometric measurements revealed clear sexual dimorphism; females scorpions were larger than males. The average length was 71.39 mm and 68.61 mm for females and males respectively. Females also have higher values in most anthropometric measurements, which

is attributed to the relation between body size and reproductive capacity. In contrast, some parts of the canines showed relative development in males, which may be related to reproductive behavior and sexual selection. These results underscore the importance of integrating between the phenotypic traits and morphometric measurements to ensure accurate diagnosis, especially among morphologically similar species. The study also showed the successful extraction and amplification of DNA using PCR for the COI gene and 12S rRNA. Clear bands appeared at the expected sizes, indicating the quality of the genetic matter and the efficiency of the amplification process. BLAST analyses also showed high similarity to reference sequences in the GenBank database, with *A. crassicauda* showing a high match to the reference sequence ON255563.1.

BLAST analyses of the COI and 12S rRNA genes also showed high similarity to reference sequences in the GenBank database. Additionally, phylogenetic trees showed that local isolates clustered with reference sequences within the same biological branches, indicating high genetic convergence and limited variation within the species. *A. crassicauda* scorpion showed high similarity to the reference sequences ON255563.1 and MK170424.1, confirmed the molecular diagnosis. Overall, the results confirm that the COI and 12S rRNA genes are highly efficient molecular markers to identify the scorpion species. The consistency between the BLAST results and the phylogenetic trees supports the identification of HGK,UA. The study highlights and confirmed the importance of molecular analysis in taxonomic studies, particularly for phenotypically similar species.

5. Conclusions

The study identified *A. crassicauda* scorpion species that is belonging to the Buthidae family, *A. crassicauda* was the most abundant and widespread species in the study area (75 specimens). Phenotypic identification, supported by molecular analysis using COI and 12S rRNA gene sequencing confirmed the species' identity. BLAST analyses and phylogenetic trees confirmed a high degree of genetic similarity with reference sequences.

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