Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(4): 1397 – 1412 (2025)
www.ejabf.journals.ekb.eg

Morphological and Molecular Identification of Four Species of Aquatic Beetles (Coleoptera: Hydrophilidae, Dytiscidae) in Thi Qar Governorate, Southern Iraq

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ARTICLE INFO

Article History:
Received: May 28, 2025
Accepted: July 12, 2025
Online: July 22, 2025

Keywords:
Aquatic beetles,
COI gene,
Species identification,
Thi Qar

ABSTRACT

Aquatic insects are a group of arthropods belonging to the class *Insecta* that live in or spend part of their life cycle in water. They form a dominant and functionally important component of freshwater ecosystems, occupying diverse ecological niches as herbivores, detritivores, predators, and prey. This study investigated the morphological and molecular identification of four aquatic beetle species—two from the family *Dytiscidae* and two from *Hydrophilidae*. Specimens were collected from various freshwater habitats in Thi Qar Governorate, southern Iraq. Morphological identification was carried out using standard taxonomic keys. Molecular analysis involved DNA extraction and amplification of the mitochondrial cytochrome oxidase subunit 1 (COI) gene. The resulting sequences were compared with reference data from GenBank to confirm species identity. Morphological characteristics were consistent with established species descriptions, and high genetic similarity to GenBank reference sequences confirmed the identifications. This is the first taxonomic study of aquatic beetles in this region and provides new distributional records for Iraq's aquatic fauna. The findings highlight the value of integrating morphological and molecular approaches in freshwater biodiversity assessment. The identification of four aquatic beetle species was confirmed through molecular analysis. The combination of traditional morphological methods and DNA barcoding proved effective for accurate species diagnosis. The mtCOX1 gene is a reliable marker for determining the molecular identity of aquatic beetles, supporting species differentiation and evolutionary studies.

INTRODUCTION

Aquatic insects represent a group of arthropods (Phylum: Arthropoda) belonging to the class Insecta that live in or spend part of their life cycle in water **(Arimoro & Ikomi, 2008)**. This group includes approximately 45,000 species that inhabit diverse freshwater environments and are found in ponds, marshes, lakes, and rivers worldwide, constituting an important component of aquatic biodiversity **(Balaram, 2005)**.

Aquatic insects are a dominant and functionally significant part of freshwater ecosystems. They occupy a wide range of ecological roles—as herbivores, detritivores,

predators, and prey—forming vital links in aquatic food chains and facilitating energy transfer to terrestrial ecosystems **(Wallace & Webster, 1996)**. Diving beetles of the family *Dytiscidae* are among the most common aquatic beetles. They are voracious predators typically found in stagnant bodies of water such as ponds and swamps **(Miller & Bergsten, 2016)**. Water-scavenger beetles of the family *Hydrophilidae* have adapted to feed on decaying organic matter and inhabit various freshwater environments, including stagnant ponds, slow-moving streams, and wetlands; some species can even survive in polluted water **(Debnath, *et al.*, 2013)**. Beetles from both families, *Dytiscidae* and *Hydrophilidae*, are valuable bioindicators due to their diverse sensitivity to environmental disturbances such as pollution and habitat degradation **(Ribera *et al.*, 2001)**.

Variations in the structure of beetle communities appear closely linked to pollution levels, with reduced species richness, abundance, and diversity observed in degraded sites. These biological changes often reflect water quality deterioration before it is detected through conventional physical and chemical analyses **(Benetti & Garrido, 2010)**. The presence of certain species of narrow-range diving beetles may indicate clean, unpolluted waters, whereas the presence of more tolerant scavenger beetles may suggest increased organic enrichment **(Fairchild *et al.*, 2000)**.

Taxonomic studies of beetles from the families *Dytiscidae* and *Hydrophilidae* remain incomplete in many regions of the world, despite their ecological significance and potential as bioindicators—particularly in biodiversity-rich yet underexplored areas **(Bilton, *et al.*, 2019)**. Although traditional taxonomy based on morphological traits remains fundamental for species identification, it presents challenges when dealing with cryptic species groups or morphologically similar developmental stages **(Alarie & Michat, 2013)**. Integrating traditional morphological methods with modern molecular diagnostics offers a more robust and comprehensive approach to accurately assess beetle diversity. The advent of molecular tools—particularly DNA barcoding—has revolutionized taxonomy by providing an integrated and reliable system for species identification and delineation **(Hebert *et al.*, 2003)**.

Despite the broad geographical distribution of aquatic beetles in Iraq, studies involving their morphological and molecular identification—particularly in Thi Qar Governorate—are almost nonexistent. Therefore, this study aims to perform a diagnostic analysis of four aquatic beetle species using morphological traits alongside molecular sequencing of the COX1 gene to confirm species identification. This research contributes to expanding the national taxonomic database and emphasizes the importance of incorporating molecular diagnostics to complement traditional taxonomy, especially in regions with insufficiently studied biodiversity.

MATERIALS AND METHODS

Study area

Field sampling was conducted in Thi Qar Governorate, one of the southern provinces of Iraq. Six sites were selected to represent the governorate's main districts: Nasiriyah, Jubaish, Souq Al-Shuyukh, Shatrah, Rifai, and Qalat Sukkar. In each district, a permanent station was established for the monthly collection of adult-stage specimens of the target beetle species. These stations encompassed diverse freshwater habitats, including ponds, swamps, sections of marshes, rivers, small streams, and irrigation canals.

Sample collection and morphological identification

Samples were collected monthly from December 2022 to November 2023 using locally made sieves and dipping nets. Collected specimens were preserved in 70% ethanol and transported to the laboratory for analysis. Morphological identification was performed using a stereomicroscope and based on regional and international taxonomic keys **(Abdul-Karim, 1978; Hansen, 1991; Foster & Friday, 2011)**. Diagnostic features and specimens were photographed using a digital camera with a display attachment for documentation.

Molecular identification

DNA was extracted from beetle tissues following the protocol of **(Sambrook *et al.*, 1989)**. Soft tissues, such as legs or thoracic muscles, were selected for DNA extraction. The mitochondrial cytochrome oxidase subunit 1 (mtCOX1) gene was amplified using polymerase chain reaction (PCR) technology. Specific primers targeting the gene—LCO1490 and HCO2198, also known as Folmer primers—were used for amplification **(Folmer *et al.*, 1994)**, as detailed in Table (1).

Table 1. Primer and base pair sequence of mtCOX1 gene

Primer	Primer sequence	gene	Source
LCO1490	5'- GGTCAACAAATCATAAAGATATTGG -'3	*mtCOX1*(700bp)	(Folmer *et al*., 1994)
HCO2198	5'-TAAACTTCAGGGTGACCAAAAAATCA-'3		

Polymerase chain reaction (PCR) products were verified by 1.5% agarose gel electrophoresis, and samples were sent to Macrogen Company for sequence analysis using a Genetic Analyzer with a minimum of 25µL of PCR product. Sequences were

modified and formatted using MEGA-X and compared to those in GenBank using BLAST to confirm species identity. Species names were recorded directly with the subject sequence. Only a similarity score of 97% or greater was considered for identification in the current study.

RESULTS

1. Morphological identification

Four beetle species were morphologically identified: two species belonging to the family Dytiscidae (*Rhantus suturalis* and *Eretes sticticus*) and two species belonging to the family Hydrophilidae (*Berosus spinosus* and *Hydrobius fuscipes*) based on key diagnostic characteristics such as body shape, wing punctuation, and antennal morphology. The observed features were consistent with previous descriptions in regional taxonomic references. The following are descriptions of the recorded species:

1.1 Rhantus suturalis (W.S. MacLeay, 1825)

Number of specimens examined: 488 (273 ♀, 215 ♂)

Description:

The body is elongated, oval, and streamlined, measuring approximately 10.5–12 mm in length. This species displays a distinctive coloration pattern: the pronotum is pale with a central dark spot; the sternites are entirely black; and the elytra are dark brown with lighter, often reddish-brown, threadlike margins.

The head is oval, smooth, and black, featuring a pale patch between the eyes that typically overlaps with the pale anterior margin. The mouthparts are of the gnawing type, equipped with prominent mandibles, a pair of four-segmented maxillary palps, and a pair of three-segmented pale-yellow labial palps. The antennae are filiform, pale yellow, and composed of 11 segments—seven of equal size followed by four smaller segments. The eyes are convex and slightly project laterally.

The pronotum is slightly convex and broad, pale brown to yellowish in color, and typically bears a well-defined central dark mark. The posterior margin is arched with a small central notch, while the anterior margin, adjacent to the head, is nearly straight with sharply angular, forward-projecting corners. A row of fine pits runs along the length of the anterior margin.

The ventral plate of the anterior thorax is dark brown and raised, with a prominent sternal process ending in a sharp triangular point. The medial thoracic ventral plate is black and elevated centrally, flanked by a pair of suprasternal plates. The posterior thoracic ventral plate, known as the posterior sternum, is large, black, solid, and broad, centrally located and divided into two large triangular lobes. Above these lobes are suprasternal plates; below are large, heart-shaped posterior coxal plates.

The elytra are light brown with a slight metallic sheen and measure approximately 9 mm in length. They are usually densely covered with dark spots, appearing darker than the anterior dorsal plate. The elytral margins are light-colored except near the apex. These markings and engravings on a golden-yellow base give an overall dark brown appearance. A distinct dark patch is observed in the final third of each elytron. Each wing also contains two longitudinal rows of fine punctures and a microscopic pattern of irregular, net-like reliefs that taper near the basal margin.

The hindwing is thin, elongated, and membranous, measuring about 13 mm in length. It is triangular when extended for flight and exhibits a relatively simple venation pattern characteristic of the *Dytiscidae* family. The primary veins and those along the wing's outer edge are darkened, while adjacent membranous areas are opaque.

The abdomen is entirely black in both sexes. Dorsally, eight membranous tergites (dorsal plates) are visible. The first four are nearly equal in size, while the remaining decrease progressively toward the posterior. The first segment has a wavy posterior edge, and the eighth is small with a rounded rear margin. Ventrally, six abdominal sternites (plates) can be distinguished. The first is divided by the posterior coxa; the second and third are the largest. A narrow groove separates these two segments, making them appear fused. The terminal segment has a rounded outer edge (Fig. 1).

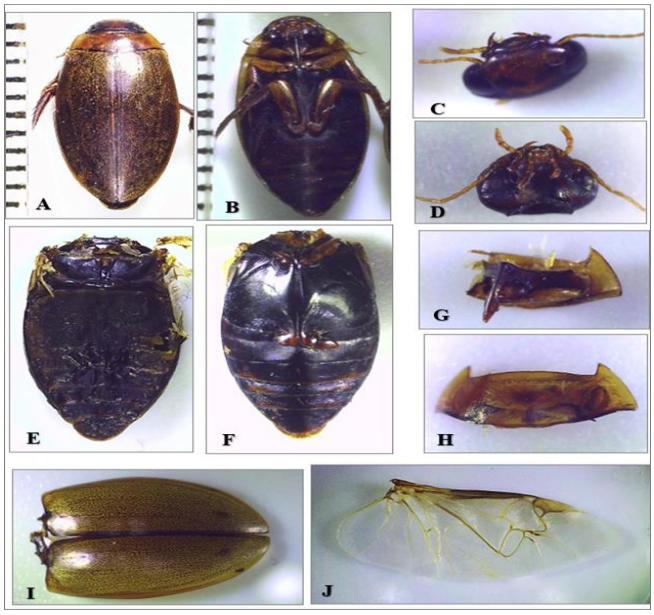

Fig. 1. *Rhantus suturalis*. A- Dorsal view of female. B- Ventral view of the male. C- Dorsal view of the head. D- Ventral view of the head. E- Dorsal plates (targa). F- Abdominal plates (sternites). G- sternal process of the first thoracic segment. H- Pronotum. I- Forewing (Elytra). J- Hindwing (Membranous)

1.2 Eretes sticticus (Linnaeus,1767)

Number of specimens examined: 17 (7 ♀, 10 ♂)

Description:

The adult body length ranges from 14–15 mm, with a width of 6–7 mm. The beetle is characterized by an elongated oval shape and a light brown coloration with golden-yellow margins. Distinctive black spots and patterns are present on the head and pronotum.

The head is flat, disc-shaped, dorsally compressed, and brown with well-defined black markings. A black spot is located on the forehead between the eyes, and a black band marks the posterior margin. The compound eyes are large, globular, and prominently positioned laterally. The antennae are yellow, filiform, and consist of 11 segments. The mandibles are strong and adapted for gnawing. The maxillary palps are bright yellow, four-segmented, with the terminal segment slightly swollen and the largest in size. The labial palps are also bright yellow and composed of three segments.

The pronotum is broad and yellowish brown, with short, blunt anterior corners. The anterior margin is arched and features a single row of black-pigmented pits along its length. The posterior margin is also arched and includes a central black band that divides into two lobes. Ventrally, the anterior thorax displays a convex brown plate with a downward-projecting sternal prominence.

The dorsal plate of the mesothorax is small, while the dorsal plate of the posterior thorax is large, segmented, and completely concealed by the elytra when at rest. The medial thoracic ventral plate is narrow, with a shallow depression between the coxae of the middle legs. The ventral plate of the posterior thorax is large and light brown, with two lateral projections extending downward. These projections form thin extensions with arched ends, connecting to the posterior coxal plate below and the suprasternal plate above.

The elytra measure 11–13 mm in length and 6–8 mm in width. They are light brown with shiny golden margins. The posterior half of each elytron has serrated lateral edges lined with fine yellow hairs. These serrations extend nearly to the apex, where the margin becomes smooth. The elytra exhibit a unique pattern of black spots, with denser concentrations forming large dark patches in certain areas. Three marginal spot clusters are present on each elytron, beginning near the middle and extending toward the apex. Two longitudinal rows of dimpling extend from the base toward the apex, marked at intervals with black spots. A zigzag band of black pigmentation is visible in the final third of the elytra. Notably, the posterior apex of each elytron is sharply raised, forming a pointed tip and creating a triangular gap between the tips when the elytra are closed.

The hindwings are transparent, membranous, and triangular, measuring approximately 15–16 mm in length when extended in flight. The venation follows the typical pattern observed in other species of the *Dytiscidae* family.

The dorsal surface of the abdomen consists of eight brown tergites (dorsal plates). The lateral edges of the last five segments extend outward, giving a stepped appearance. Long, scattered setae are visible on tergites 1–6, while segments 7 and 8 are densely covered with fine setae. The eighth tergite is triangular and the smallest in size.

Ventrally, the abdomen includes six brown, sclerotized sternites (plates). The first is completely divided by the posterior coxae. The second is narrowed at the midpoint of its anterior margin, and the third is the largest. The remaining three decrease progressively in size, with the sixth being the smallest (Fig. 2).

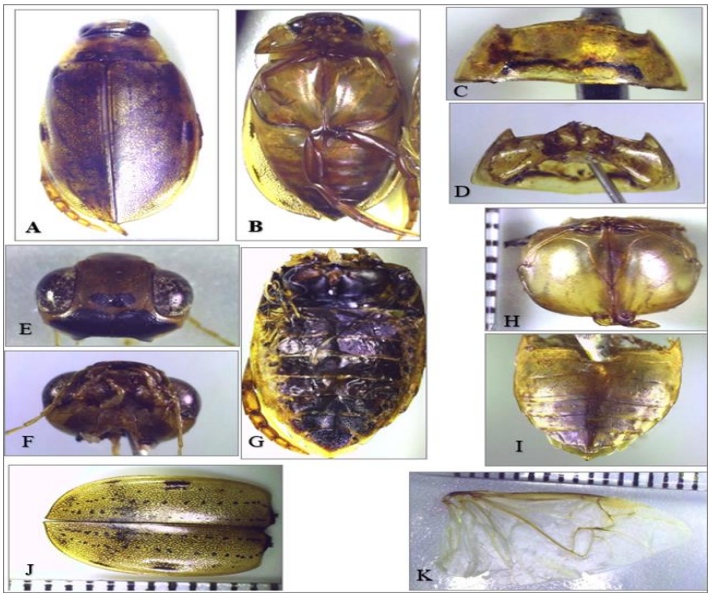

Fig. 2. *Eretes sticticus*. A- Dorsal view of female. B- Ventral view of the male. C- Pronotum. D- ventral plate of the anterior thorax. E- Dorsal view of the head. F- Ventral view of the head. G- Dorsal plates (targa). H- Ventral plates of the mesothorax and metathorax I- Sternites of abdominal segments. J- Forewing (Elytra). K- Hindwing (Membranous)

1.3 Berosus spinosus (Steven,1808)

Number of specimens examined: 376 (219♀, 157 ♂)

Description:

The body is oval, measuring 4.5–5.8 mm in length, and is yellowish-brown in color. The dorsal surface of the anterior thoracic plate (pronotum) features pits of varying sizes, with dark spots that are more pronounced along the lateral margins. The elytra exhibit ten longitudinal rows of fine pits, interspersed with dark spots and pigmentation.

The head is slightly elongated and brown, with shallow depressions of various sizes scattered across its dorsal surface. A distinct Y-shaped groove is present, with the lateral arms flattened. Beneath these arms, on either side of the head, are two large, laterally projecting compound eyes. The ventral surface of the head, particularly the posterior half, is covered with fine hairs.

The antennae are composed of nine segments and are yellowish-brown. The final three segments are enlarged and densely setose, forming a club-like structure. The maxillary palps are yellow and consist of four segments. The labial palps are shorter, with three segments.

The pronotum is convex, with an arched anterior margin and a sinuous posterior margin. Its surface is dotted with pits of varying sizes, most prominent in the center, along with dark pigmentation concentrated in the posterior half. The lateral edges of the pronotum curve downward to articulate with the pleuron, which in turn connects to the ventral plate of the anterior thorax. This ventral plate is small, brown, and features a minor sternal protuberance.

The elytra are yellowish-brown and approximately 4 mm long. Their outer surface bears ten longitudinal rows of pits and grooves, with dark pigmentation and spotting most apparent along the suture (the junction between the two elytral halves). A distinguishing feature of this species is the presence of a small, clear spine near the apical tip of each elytron.

The hindwing is membranous and approximately 6 mm long. The main veins are dark and well-defined, whereas the secondary veins are thinner and less distinct. A membranous fold is also present near the terminal wing cell, located along the middle of the outer wing margin.

The abdomen consists of eight dorsal plates (tergites), ranging from dark brown to black. These tergites are smooth and hairless, except for the eighth, which is densely covered with black hairs and has an arched posterior edge. On the ventral side, five black sclerotized plates (sternites) are visible. The first sternite features a depression along its anterior margin, which accommodates the coxa of the hind leg. The fifth sternite has an arched posterior margin. All ventral plates are clothed with short, yellow setae (Fig. 3).

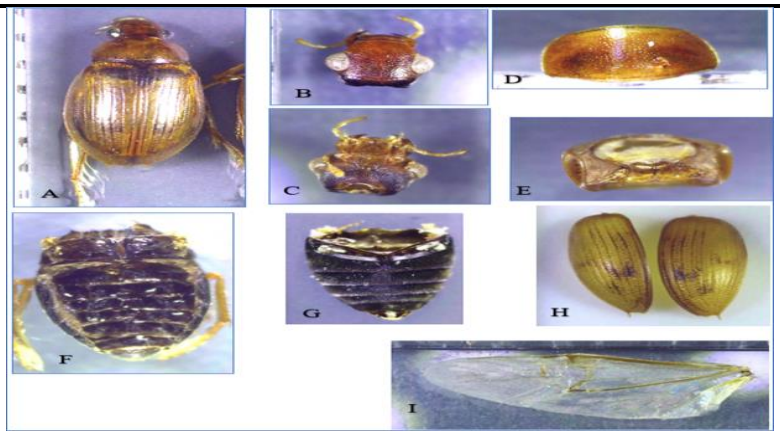

Fig. 3. *Berosus spinosus* A- Dorsal view of female. B- Dorsal view of the head. C- Ventral view of the head D- Pronotum. E- sternum of the first thoracic segment. F- Dorsal plates (targa). G- Abdominal plates (sternites). H- Forewing (Elytra). I- Hindwing (Membranous)

1.4 Hydrobius fuscipes (Linnaeus,1758).

Number of specimens examined: 287 (136 ♀, 151 ♂)

Description:

The body is oval, convex dorsally and flat ventrally, measuring 7–8 mm in length and 3.5–4 mm in width. It is uniformly dark brown to black with a metallic sheen, while the lateral margins and appendages are lighter in color. The solid forewings (elytra) are marked by ten longitudinal rows of fine pits.

Head:

The head is small, rounded, and convex, with a smooth dorsal surface. The ventral surface is often covered with short, dense hairs. A Y-shaped notch is visible on the forehead, with its lateral arms extending from the lateral edges of the head—tangent to the compound eyes—toward the center of the head. The compound eyes are weakly convex and slightly protrude laterally. Rows of small hairs are present behind each eye.

The antennae are yellow, composed of nine segments. The last three segments are enlarged, club-shaped, and dark brown. The mouthparts are prognathous and gnawing. The maxillary palps are nearly as long as the antennae and consist of four segments, with the terminal segment darkened at the tip. The labial palps are reddish-yellow, tipped with dark coloration, and consist of three segments of approximately equal length.

Pronotum:

The pronotum is convex and broader at the base. It is dark brown, with a flat anterior margin and an undulated posterior margin. The lateral areas of the dorsal surface bear a group of large pits, while the inner surface is covered in fine hairs, which are more

concentrated at the sides. The ventral surface is densely pitted and grooved, with a covering of fine hairs.

The ventral plate of the anterior thorax is raised medially and undivided. The mesothoracic ventral plate is posteriorly elevated and often bears a sharp sternal process. The ventral plate of the posterior thorax is characterized by a thin sternal process that does not extend posteriorly.

Elytra:

The elytra are triangular with gently curved edges, dark brown in color, and measure up to 6 mm in length and 3 mm in width. Each elytron has ten longitudinal rows of fine punctures extending from base to apex. The underside of the elytra is distinctly concave, patterned with fine sculpturing and covered in fine hairs.

Hindwings:

The hindwings are membranous and triangular, up to 9 mm long and 4.5 mm wide. When at rest, they fold beneath the elytra and assume a lanceolate shape during flight.

Abdomen:

Five black ventral plates (sternites) are visible, each bearing a dense and uniform covering of bristles. The posterior margins of the plates are brown to reddish-brown. The posterior margin of the fifth ventral plate is slightly rounded and bears several coarse bristles at its apex (Fig. 4).

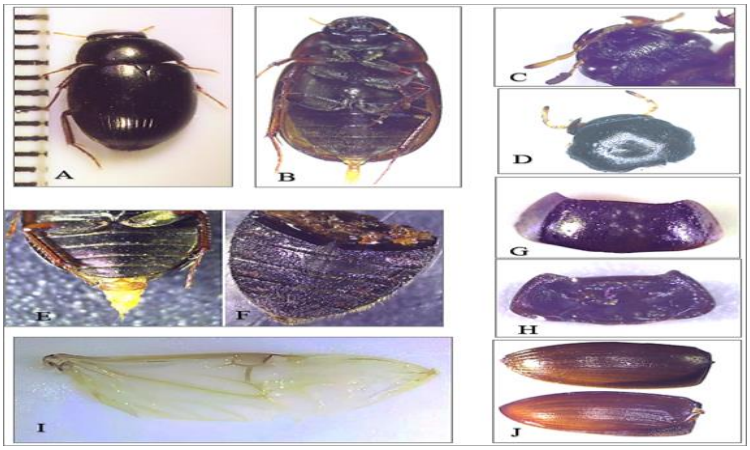

Fig. 4. *Hydrobius fuscipes* A- Dorsal view of female. B- Ventral view of the male. C- Ventral view of the head D- Dorsal view of the head. E- Abdominal sternites in male. F- Abdominal sternites in female. G- Pronotum. H-sternum of the first thoracic segment. I- Hindwing (Membranous). J- Forewing (Elytra)

2. Molecular identification

The results of the polymerase chain reaction (PCR) technique for all extracted DNA samples showed clear bands of 700 base pairs when compared to the DNA ladder indicator carried over with the samples using a 1.5% agarose gel (Fig. 5).

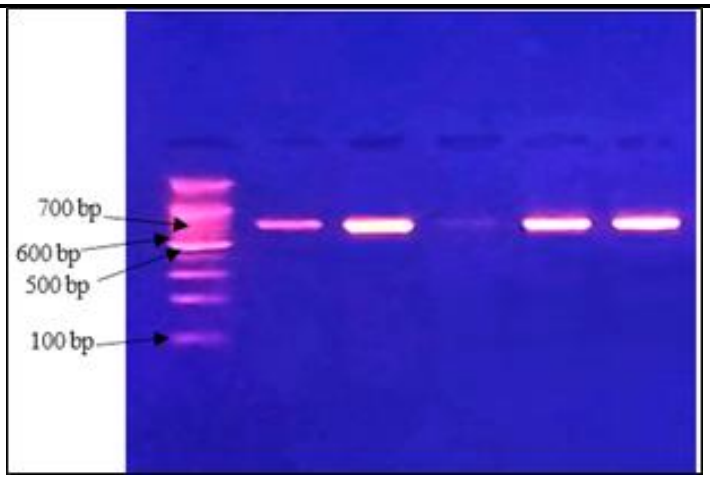

Fig. 5. Electrophoresis results of PCR products for the COX1 gene of the beetle species examined in the current study

Table (2) shows the matching percentages of the target gene segments mtCOX1 for the samples examined in the current study, whose sequences were compared with the reference sequences recorded in the GenBank (NCBI) database using the BLAST (Basic Local Alignment Search Tool). The extracted nucleotide sequences were entered into the BLAS interface for comparison with the Nucleotide Collection database to determine the extent of their matching with previously recorded sequences. The results were analyzed based on the matching percentage and sequence length (Query coverage) to determine the closest sequences to the target gene. The sequencing results of the mtCOX1 gene for the examined species were documented in GenBank as sources for the first time in a study on aquatic beetle species in Thi Qar Governorate, southern Iraq, and were recorded with a new serial number (Accession Number) for each segment.

Table 2. Matching ratios for the target gene segments mtCOX1 and accession number of the registered species

Species	Accession number of the registered species	Accession Number of reference sample	Matching percentage
Rhantus suturalis	LC872750	FN263063.1	98%
Eretes sticticus	LC872751	JX461227.1	97%
Berosus spinosus	LC872754	MZ607671.1	99%
Hydrobius fuscipes	LC872753	MZ660195.1	98%

DISCUSSION

Rhantus suturalis has one of the widest geographic distributions among diving beetles, occurring on nearly every continent except Antarctica **(Nilsson & Hájek, 2019)**. As noted by **Balk *et al.* (2004)**, its range extends across North and South America,

Europe, Asia, Africa, and Australia, inhabiting diverse climatic zones from temperate to tropical. This widespread distribution reflects the species' strong dispersal capacity and ecological plasticity, supported by its ability to fly long distances and rapidly colonize new habitats **(Miller & Bergsten, 2016)**.

The present study's findings are consistent with the morphological description of *R. suturalis* provided by **Nilsson and Holmen (1995)**. The species displays an elongated, oval body with a smooth, glossy dorsal surface. Its coloration is striking—dark brown with lighter, filamentous margins on the hindwings. Variations in size and coloration were observed among collected specimens, likely reflecting adaptations to local environmental conditions. **Miller and Bergsten (2016)** reported that populations from temperate regions tend to be larger and darker, whereas those from tropical regions are typically smaller with lighter-colored elytra.

Eretes sticticus has been recorded across North Africa, the Middle East, Central Asia, and parts of southern Europe. Its range extends from Morocco across the Arabian Peninsula to Iran, Pakistan, India, and western China **(Nilsson & Hájek, 2023)**. The limited number of specimens collected in this study may be attributed to the species' preference for temporary aquatic habitats and the influence of environmental variability on habitat availability. Additionally, its nocturnal habits hinder daytime detection, as this species is primarily active at night and is often attracted to artificial light sources **(Nitzu, 2022)**.

E. sticticus is characterized by an elongated, oval body, typically yellowish- to reddish-brown in color, with distinctive black spots on the elytra and anterior dorsal plate. The body surface is smooth and moderately glossy, consistent with descriptions by **Miller and Bergsten (2016)**. **Miller (2022)** also reported overlapping black spots of varying size on the elytra, as well as large, laterally protruding, rounded eyes and long, dorsally projecting antennae.

Berosus spinosus is widespread across the Palearctic region and has been documented in countries including France, Russia, Mongolia, and Finland **(Litovkin a&nd Efimov, 2020)**. Notably, the species was first recorded in Japan in Fukuoka Prefecture, expanding its known distribution to East Asia **(Watanabe & Nakajima, 2021)**. Morphologically, *B. spinosus* is relatively large compared to similar species and can be identified by distinctive dark markings on the anterior dorsal plate **(Rodionova *et al.*, 2021)**.

In terms of physiological adaptation, *B. spinosus* demonstrates a strong tolerance to desiccation. Research from southern France showed that during drought periods, individuals burrow into moist substrates of dried ponds and enter a state of torpor until water returns. This behavior allows survival in fluctuating aquatic environments **(Christian, 1975)**.

Hydrobius fuscipes is considered one of the most widely distributed species within the genus *Hydrobius*. Based on morphological and molecular analyses, it is common throughout Europe and is also found in large areas of Canada and the United

States. Its range extends into Siberia, parts of northern Asia including the Himalayas, and into North Africa and the temperate Middle East **(Fossen, 2016; UK Beetles, 2024)**.

The morphological traits of *H. fuscipes* observed in this study align with those described by **Queney and Prévost (2021)**. Adults measure up to 8 mm in length, with an oval, dorsally convex, and ventrally flattened body. The coloration is generally dark brown to black with a slight metallic sheen, while the lateral margins and appendages are paler. The head is convex with slightly protruding eyes, and the hindwings display ten longitudinal rows of fine pits.

Molecular confirmation and taxonomic significance

The molecular identification of the four aquatic beetle species was conducted using polymerase chain reaction (PCR) and amplification of the mitochondrial cytochrome c oxidase subunit 1 (mtCOX1) gene, employing a universal invertebrate primer. The resulting sequences confirmed the morphological identifications, and a genetic database was established in GenBank, with each specimen assigned a unique accession number. This is the first study to molecularly document aquatic beetle species in Thi Qar Province.

Species identification was verified by comparing the obtained sequences with reference entries in GenBank using a minimum similarity threshold of 97%. The studied species showed high similarity to globally distributed reference sequences:

- *Rhantus suturalis*: 98% similarity with a specimen from Iran
- *Eretes sticticus*: 97% similarity with a specimen from India
- *Hydrobius fuscipes*: 98% similarity with a Finnish specimen
- *Berosus spinosus*: 99% similarity with a Finnish specimen

These results underscore the value of integrating molecular and morphological approaches in biodiversity research. Accurate species identification is critical for taxonomic studies, ecological monitoring, and conservation planning. While traditional morphological methods remain fundamental, they can be limited by phenotypic plasticity and the presence of cryptic species.

DNA barcoding, particularly using the mtCOX1 gene, has proven to be a reliable and effective tool for species identification and delineation **(Chac & Thinh, 2023)**. The COX1 gene contains highly conserved regions suitable for primer binding, along with sufficient variability for distinguishing closely related species. This makes it a powerful marker for large-scale biodiversity assessments and evolutionary studies **(Kachhawa, 2023)**.

CONCLUSION

The current study reached several conclusions, including that some phenotypic diagnostic characteristics can be relied upon to differentiate between species, such as the insect's general shape, size, color, and patterns on the elytra, head appendages, and wing

venation. Furthermore, the identification of four species was confirmed through molecular analysis, which enhanced the accuracy of the classification. The combination of traditional morphological identification and DNA barcoding proved effective in confirming the diagnosis. The study also concluded that the mtCOX1 gene is an effective tool for determining the molecular identity of aquatic beetles, facilitating species discrimination and conducting evolutionary studies.

CONFLICT OF INTEREST

Both authors declare that the article has no conflict of interest.

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