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Morphological and Molecular study of Seven New Recorded of Ostracod in - Kurdistan Region, Iraq

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Abstract: The current study conducted the first characterization of morphological and molecular of seven Ostracoda species new to Iraqi fauna which are; *Heterocypris salina, H. spadix, Dolerocypris sinensis*, *Cyprinotus unoi*, *Eucypris virens*, *Sclerocypris exserta* and *Notodromas monacha* that belongs to two families (Cyprididae and Notodromatidadae) collected from 17 different sites which are stream, lakes and rivers in the boundary of Province, from September 2021 to October 2022. For biological purposes, samples of aquatic shore plants (*Cynodon dactylon, Polygonum sp., Nerium oleander* and *Nasturtium officinale*), algal municipal (*Anabaena* and *Chlorella vulgaris*), were also collected zooplanktonic net was used in the sampling mesh-size (55 µm pore size).Samples placed in an oxygen instrument for about one week after being transformed into the laboratory to allow the ostracod species to grow. After the maturation period, adult species were fixed (were preserved) in 70% and 100% ethanol for morphological and molecular analysis respectively. PCR product of COI was sequenced using forward primer COI F 5'(ACCCGCTGAATTTAAGCAT)3' and reverse primer COIR 5'(CTCTTCAGATACTTTTCAAC) 3' then registered in the GenBank database with their accession numbers. The phylogenetic tree was constructed; the studied species were recognized (as new records to the Iraqi fauna of ostracods) and described from Iraq for the first time. The goal of present study is the molecular study the species besides the phenotypic identification for more accurate taxonomic results.

Keywords: Molecular study, Morphology identifications, Ostracod, PCR, Phylogenetic analysis.

Introduction

Ostracodes are species of crustaceans that range in size from 0.3 to 5 mm. Their calcitic bivalved carapace is hinged upon the dorsal margin, which can completely cover and defend the non-mineralized body parts and projection. Its carapace varied in shape and ornaments. Ostracodes have eight pairs of appendages, which serve a variety of function, such as swimming, feeling, crawling, feeding, and copulating. They can reproduce in both sexual and non-sexual ways according to (Zwair, 2023; Brandão *et al.,* 2024). Ostracodes can be found in both fresh and saltwater environments with pH levels ranging from naturally alkaline to neutral, they can be affected by the environmental parameters (Bellin *et al.*, 2021; Czajkowska, 2022).

Investigations from 218 sites collected samples of inland aquatic habitats of Sicily (Southern Italy) and neighboring islands, in result 46 species were noticed, among them

(*Candonopsis novaezelandiae, Physocypria kerkyrensis, Cypria subsalsa, Eucypris mareotica, Vestalenula boteai,* ect.) the two former species were new for Sicily (Pieri *et al.*, 2020). Also, in the province of Kütahya in Turkey's Aegean area exerts influence on the association between the ecological characteristics of freshwater ostracods with habitat preferences. The outcome twenty-three species were listed, 16 of which were new to the area, among them, *Limnocythere inopinata, Heterocypris incongruens, Ilyocypris bradyi, and Cypridopsis vidua***,** etc.(Külköylüoğlu *et al.*, 2018). Although, a study utilized ostracod as environmental indicators in the springs of a tiny lowland River Krpiel valley in Poland, Results *Candona sp. Cypria ophtalmica, Psychrodromus sp.,* and *Cypridopsis vidua* were four most prevalent species among thirteen recorded species (Szlauer-Lukaszewska *et al.*, 2021). Technology became more developed in the last invention for the first sexual (mixed) population of the *Cypridopsis vidua* from the northeast of the USA (Martens *et al.*, 2023). Furthermore, in Iraq samples were taken in the Holy Karbala province's Aldewehiyah district from the Karbala Governorate to detect the ostracods (Zwair, 2021) These study described the genus (Latef & Ali, 2023) *Pseudocandona* sp., which was revealed for the first time in Iraq. Generally, in Kurdistan region only article about *Cyprinotus incongreuns* has been found in the Greater Zab River (Ali, 2007). Latef & Ali (2023) worked for the first time in Iraq on molecular ostracod in a different water bodies from Erbil province. As a result, *Heterocypris incongreuns* was recorded as a novel species in Iraq. In recent years, molecular technology was widely used to prove the taxonomy analysis. Morphological identification had some restrictions, due to the phenotypically similarities between some of the ostracod species However, the utilization of molecular identification markers has proven instrumental in overcoming this complexity and accurately assigning species to their respective taxonomic classifications (Taher & Alyousuf, 2023). The limited availability of DNA sequence data for numerous species, typically restricted to a single sequence per species, poses significant challenges in accurately aligning sequencing data and pointed to substantial divergence among taxa (Koyee & Abdullah, 2023). In that manner, ostracod samples were collected in some ponds and streams of the eastern Iberian Peninsula. In the consequence, *Cypris pretusi* recorded as a novel species according to the molecular study based on the *Cox1* gene was amplified by using COX1-OligoF and COX1-OligoR primers (Mesquita-Joanes *et al.*, 2020). Also, a molecular study was applied on 12 ostracoda species in the perennial freshwater lake of Singanallur, India, they discovered that *Cypretta campechensis* was a new species through examination of the mitochondrial cytochrome oxidase subunit (Kalpana *et al.*, 2021).

Currently, there is a lack of published evidence regarding the distribution and molecular study of ostracods in Iraq generally. This study aimed to achieve a more robust and comprehensive understanding of species identification, by combining two approaches of morphological confirmations and DNA fingerprint.

Materials & Methods

Sample collection

The ostracod samples were gathered from seventeen various sites, including (Khabat, Khalan, Khalifan, Bekhal, Gali Ali bag, Zargali, Hafiz bridge, Khanaqa, Wasanan, Qalat, Choman, Smaquli dam outlet, Smaquli Dam, Smaquli girtik, Altun kupri, Degala and Taq taq) situated in boundaries of Erbil Province (Fig. 1) from September 2021to October 2022. For biological purposes numeral plants, algae, and zooplankton net of mesh-sized (55 µm pore size) were used. Samples were put under an oxygen instrument for about one week after being transformed into the laboratory and then ostracod species initiated growth. After the maturation period Samples were pulled out from the bottle and fixed in 70% and 100% ethanol for morphological and molecular analyses.

Morphological identification

The traditional morphological recognition of ostracods were based on their size, shape and their appendages was performed using a Wild Herrbrugg microscope camera lucida zeichentubus and the classification was subjected to identification keys (Karanovic, 2012).

Molecular identification: The entire genomic DNA was extracted from the specimens by using the Qiagen kit (Hilden, Germany); then the extracted genomes were preserved at -20°C depending on the manufacturer's instructions. Then Nanodrop device (American company Thermo-Scientific) was used at wave lengths 260-280 nm to evaluate the quantity and quality of the extracted DNA. Later, it was conducting a polymerase chain reaction (PCR) test using COIF 5′-ACCCGCTGAATTTAAGCAT -3′ and COIR 5′-CTCTTCAGATACTTTTCAAC -3′ to detect the sequences of samples. The PCR reaction mixture was run in a total volume of 50 µL consisted of 25 µl master mix, 1.5 µl of each primer (forward and reverse), 2 µl DNA template, and 20 µl PCR grade water. The PCR conditions was pragmatic as follow (5 minutes at 94°C, 35 cycles of 45 seconds step: 94°C, 50°C, 72°C and a final extension step 7 minutes at 72°C). The PCR products were confirmed by agarose gel electrophoresis. The PCR products, with an expected size of 700bp, were sent to Macrogene Company in Korea for sequencing by consuming the forward primers of COI. The achieved sequences were matched and aligned with formerly registered sequences in the National Center for Biotechnology Information (NCBI) GenBank. The sequences of all noticed species in the current study were submitted in NCBI-Gen Bank. To gain further understanding of the genetic variation and relationships among the species under study, a Maximum Likelihood analysis was accomplished using the MEGA X (Kumar *et al.* (2018).

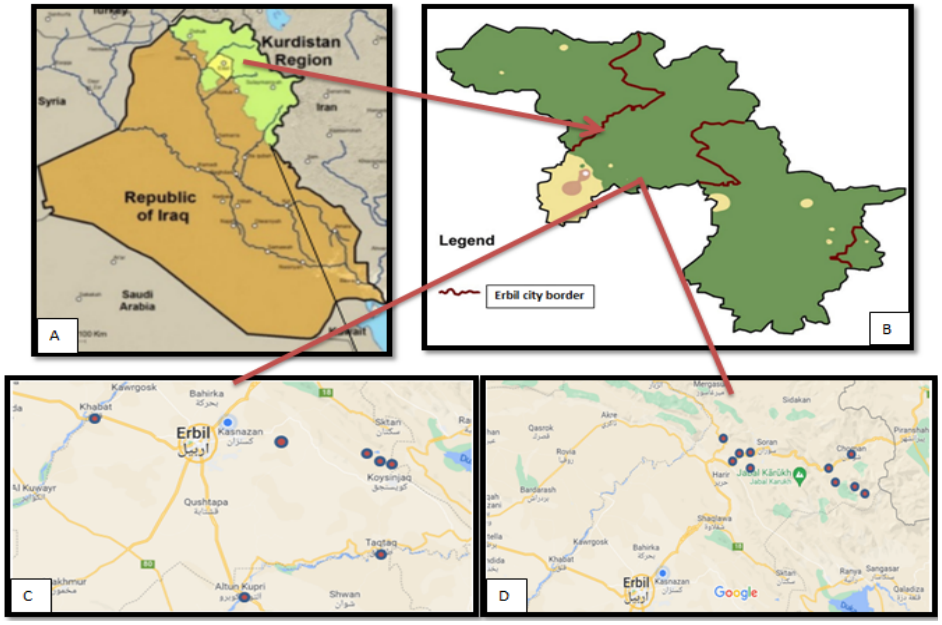

Fig (1): A- Map of Iraq B- Erbil province C&D- Map of studied area and the sampling sites.

Results

The current study has documented seven new records in northern Iraq, which are *Heterocypris salina*, *H. spadix*, *Dolerocypris sinensis*, *Cyprinotus unoi*, *Eucypris virens* and *Sclerocypris exserta* were belong to Cyprididae and *Notodromas monacha belongs to* Family Notodromatidadae. In spite of morphological evidence, DNA barcoding was applied to approve the morphotype documentation.

Morphological description

The following only distinguish character of each species were mentioned here:

1-*Heterocypris salina* (Brady, 1868) [**Fig. 2]**

The carapace (Ca) dark-brown in colour with a group of eight adductor muscle scars look as in five. Its dimensions is 1.04 mm. First antenna Antenna A1 comprises of eight segments, 7th segment is longest, with four strong, extensive apical setae and a short α seta. Second antenna A2 included five segments, the exopodite with three unequal dorso-distal smooth setae. The first endopodal segment with well-developed natatory (5+1) setae, which is used for swimming, ventrally bears short aestethasc Y, with short plumose seta. The coxal-endite of Mandible (Md) has six minute teeth inserted with tinny setae and two ventro-distal pilose, short setae. Also the endopodite of Md is broad, with eight unequal slight setae and short pilose β seta. Maxilla (Mx) consist of maxillular palp and

endites. First maxillular palp bears six smooth setae. Two lateral setae exist on 3rd endite. Branchial plate bears twenty plumose setae. The Protopodite of first thoracic leg (L5) has 12 plumose setae, also exist b and d pilose setae. The first protopodite of second thoracic leg (L6) has d1 and d2 seta, with minute setae on both sides. The first segment of the third thoracic leg (L7) is lengthy and has three smooth setae (d1, d2, dp). Likewise the second and third segments with e and f pilose seta. The furca bears tiny setae at the ventral margin, Sa and Sp setae are identical in length, encompassing the mid-length of the claws.

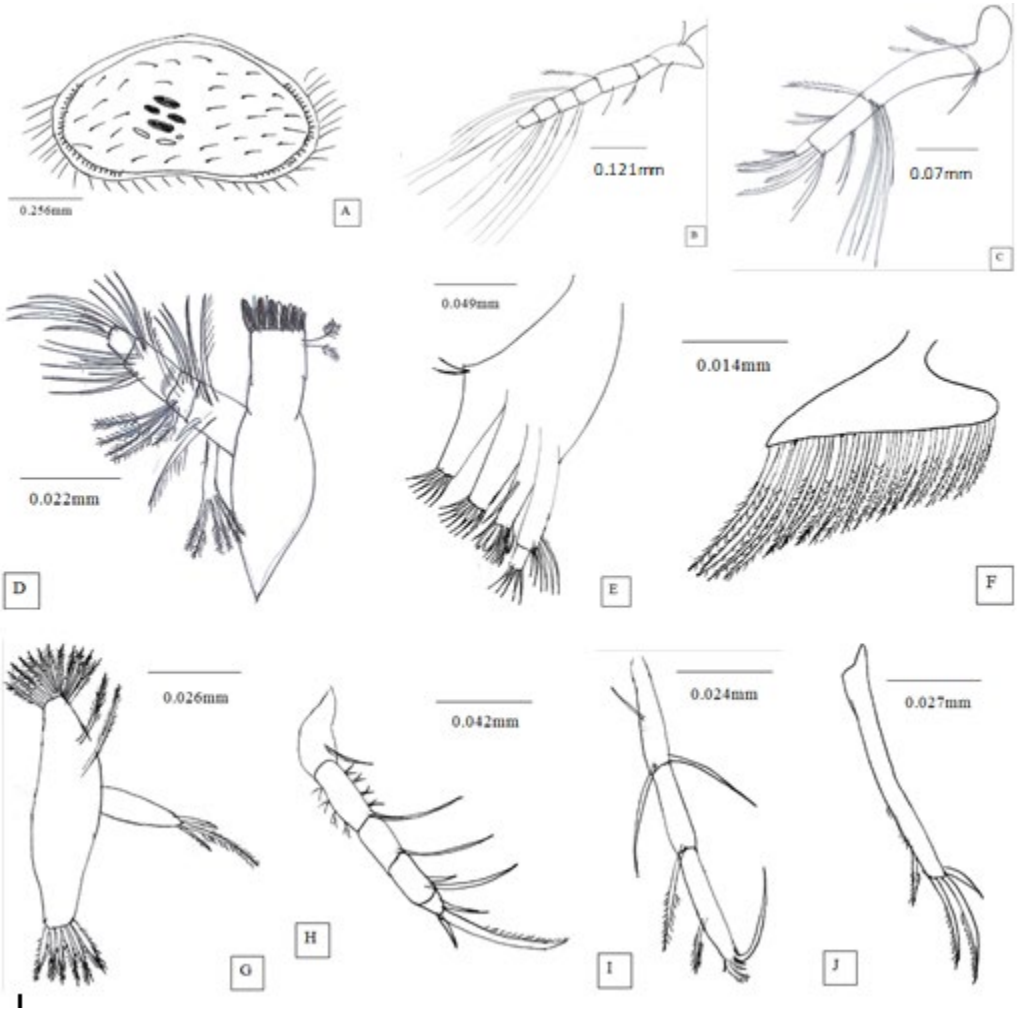

Fig. (2): Lucida drawing of *Heterocypris salina* A-Whole amount, B-Antennula, C-Antenna, D- Mandible, E- Maxilluie, F- Branchial plate, G- First limb, H- second limb, I-Third limb, J- Furca.

2-*Heterocypris spadix* Munakata, Tanaka et Kakui, 2001 (Fig. 3)

Carapace length 0.84mm, carapace pale yellowish in colour, blotchy with dark brown. The fourth podomere of A1 had two long plumose dorso distal setae. While, Fifth podomere with two short plumose ventrodistal setae. Seventh podomere with

three distal setae and aesthetics ya. The A2 is similar to A2 of *H. salina*. The fifth podomere of it with plumed seta g reaching 75% of claw GM, and Gm. The vibratory plate of Md noted with four rays, and next podomere present distal Y seta. The first palpal podomere of ML with seven dorsodistal setae of uneven length (two of them plumed). Vibratory plate muscle bears 15 plumed rays. The protopodite of L5 has two plumed setae "b, and d" with 13 distal plumed setae in unequal length. L6 consist of six podomeres, the last one present longer plumed h3 seta than h1. L7 included four podomeres, existing pincer organ formed by 3rd and 4th podomeres. The furca is slender without a cluster of tiny setae at the ventral margin.

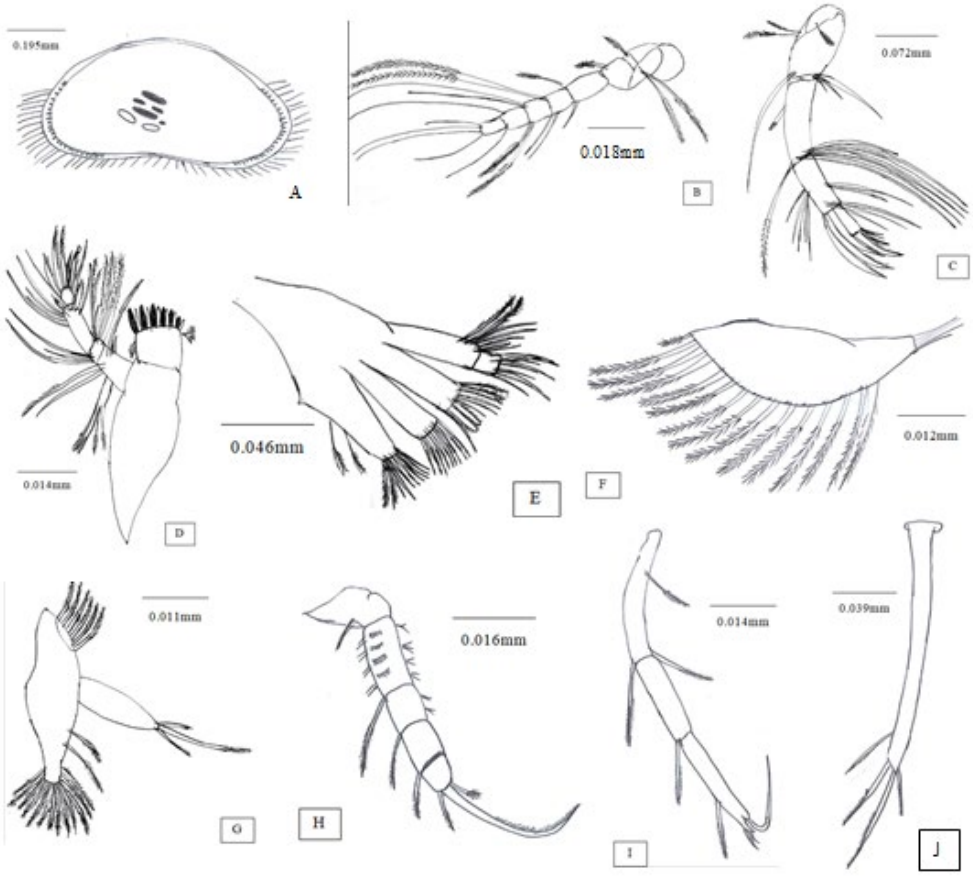

Fig. (3): Lucida drawing of *Heterocypris spadix* A-Whole amount, B- Antennula, C- Antenna, D- Mandible, E- Maxilluie, F- Branchial plate, G- First limb, H- second limb, I- Third limb, J- Furca.

3-*Dolerocypris sinensis* G. O. Sars, 1903 (Fig. 4)

The Ca length is 1.48mm, generally is reaching 2 mm in length. The shell is elongated and laterally compressed, slightly convex dorsally, the ventral margin is straight or somewhat concave. The frontal edge is more rounded than the posterior one,

with long setae at the posterior end. The A1 is seven segmented. The base of the protopodite present V-shaped articulation. The terminal segment has 3 unequal long, smooth setae and 1 midsized aesthetasc ya. The first endopodite of A2 with six short, natatory setae on the inner edge; and aesthetasc Y short, which is 2- segmented. Also, present Z1-3 seta on the second endopodite, Z1 well developed, longer than the claws. The Coxal part of Md with six tough teeth and two median-sized setae internally between the three largest teeth. Terminal endopodite segment with dissimilar long, 3-claw-like setae and 3 short setae. The first palp segment of Mx with 4 long setae (the apical setae is bristle).The second segment has 7 smooth, unequally setae. The Vibratory plate with 23 plumose setae. The protopodite of L5 with two two proximal medium-sized d and b plumose setae. While, the exopodite plate has six (5 long, one short) pilose setae. The L6 has medium-sized plumose d2 setae on the 1st segment, and h2 claw on the terminal segment (longer than the last 3 segments) and weakly serrated. The L7 bears long, smooth d1, d2 and dp setae on the 1st segment, and h1 is very short terminal seta. The furca is ending with 2 claws and 2 setae. The Sp seta is very small and smooth

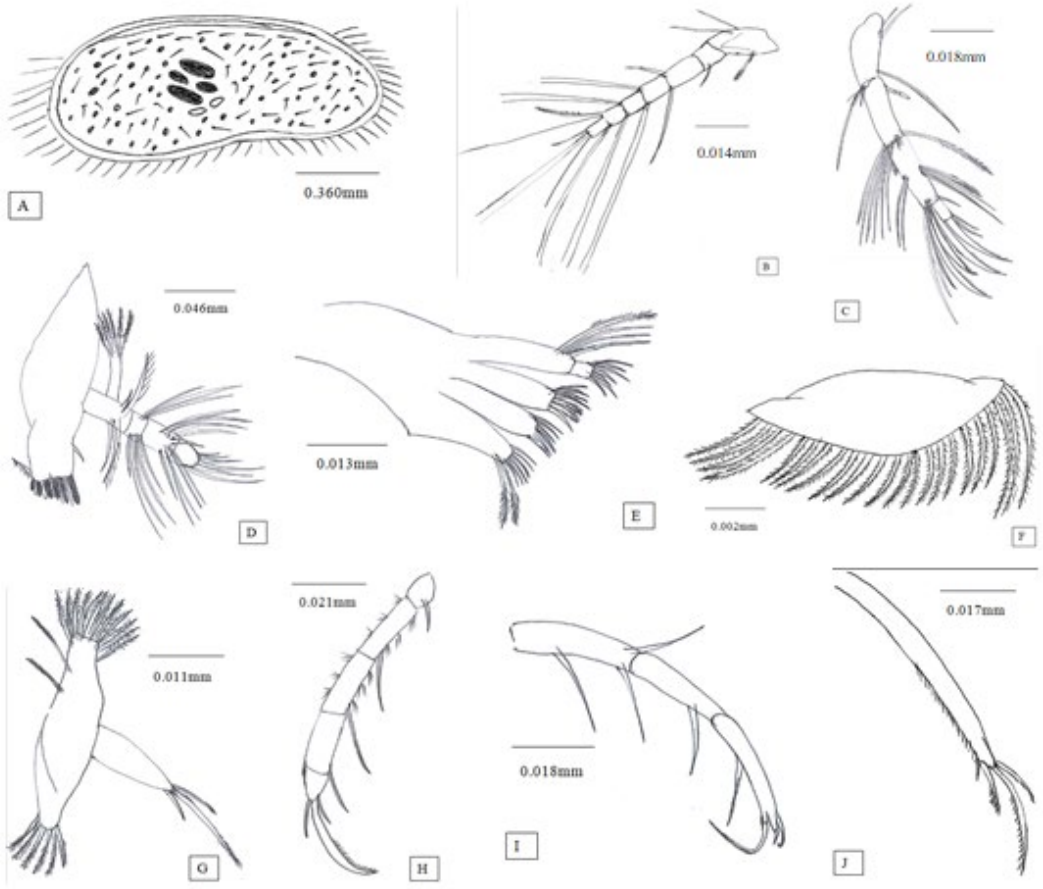

Fig. (4): Lucida drawing of *Dolerocypris sinensis* A-Whole amount, B- Antennula, C- Antenna, D- Mandible, E- Maxilluie, F- Branchial plate, G- First limb, H- second limb, I- Third limb, J- Fur.

4-*Cyprinotus uenoi* Brehm, 1933 (Fig. 5)

The right valve lapping the anterior point of maximum highs of the left valve. RV is higher than LV. The valves' surface is smooth centrally, while small shallow pits are near the valve borders, in particular towards anterior and posterior margins. The adductor muscle scars arranged in a circular shape on the carapace. The first segment of A1 bears minor seta dorsally and two lengthy sub-apical setae ventrally. The sixth segment has four long, pilose setae. The swimming setae of A2 are long, getting behind the ends of the claws; one of them is short and reaches the middle of the swimming setae. The Mandibular-coxa of Md is stout, with 7 teeth of different sizes and three small sub-distal setae, with bristled ventral setae. The last segment of Md with 3 stout claw-like setae and 2 solvent setae. The first maxillary segment of ML has a set of 6 unequal setae on the outer apical edge and two unequal long sub-apical setae. The Branchial plate bears 18 pilose setae.

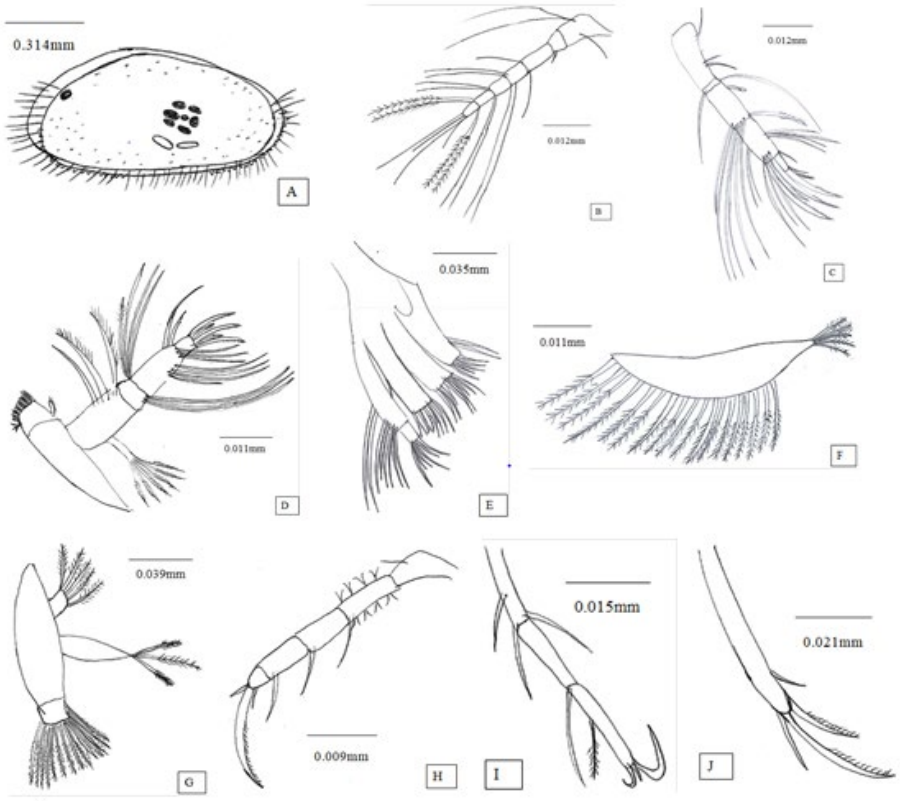

Fig. (5): Lucida drawing of *Cyprinotus uenoi,* A-Whole amount, B- Antennula, C- Antenna, D- Mandible, E- Maxilluie, F- Branchial plate, G- First limb, H- second limb, I- Third limb, J- Fur.

The protopodite of L5 without (d, d2, and a) setae, while the exopodite with 5 rays. The L6 is robust with five articulated segments. The basis segment with small d2 seta. The L7 present seta d1, d2 and dp on first segment. The furca is straighter in ♀ than in males, seta Sa is moderately long, absent 40% distance from G.

5- *Eucypris virens* (Jurine, 1820) (Fig. 6)

The carapace is sub-rectangular in side view. The left valve is larger than the right and covers nearly the total margin. The greatest height located at the anterior-media axis. The central muscle scars comprise "2 elongated mandibular scars, 5 large and 1 minor adductor scars". The 4th endopodite of A1 bearing 4 natatory with 1 median seta on the distal end. The major endopodite of A2 with six natatory setae on the inner side, the frontal seta is shortest, followed by one lengthiest than the four other natatory setae, while one pilose seta is noticed at ventro distal end. The basis protopodite of Md bears 6 chitinised teeth, the exopodite bears four thin setae distally, and the last endopodite bear 4 stout, smooth claws with a thin seta. The proximal maxillary palp of Mx carried 1 long, thin, and 5 mid-long ending setae, the next podomeres of palp with 5 medians and one very small seta. The protopodite of L5 bearing b and d seta, also 2 (a) setae ascending from a proximal end of protopodite. The exopodite of the same leg signified by 6 pilose long setae. The protopodite of L6 had tiny ventro-distal seta, while their endopodites 1, 2 and 3 terminating by e, f and g seta respectively. Terminal segment of L7 has 1 hook like and 1 small straight seta. The furca is well-developed, Sa seta is approximately the same length as the Gp claw.

6- *Sclerocypris exserta* Sars, 1924 (Fig. 7)

The carapace is large and sub-quadrate in shape. The left valve is larger than the right. The greatest height is located in the anterior part, and the frontal margin is more rounded. The ventral margin is straight. The second endopodite of A1 bearing 2 long (the longest is plumose) seta on the distal dorsal border and 2 (short and median) setae on the distal ventral rim. The basal endopodite of A2 with 6 short natatory setae on the inner face. The first protopodite of Md bears on inferior end 7 chitinised teeth. The outer edge of the 2nd protopodite bears a branchial plate that has six (4 long, 1medi-um, and 1 short) setae distally.

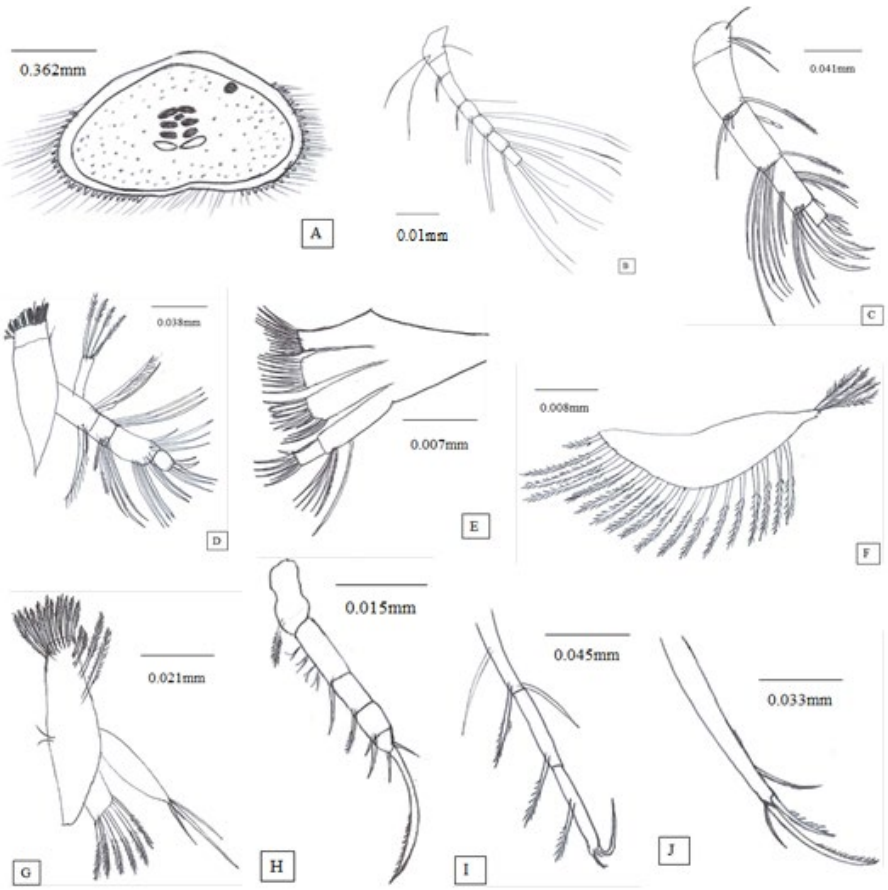

Fig. (6): Lucida drawing of *Eucypris virens* A-Whole amount, B- Antennula, C- Antenna, D- Mandible, E- Maxilluie, F- Branchial plate, G- First limb, H- second limb, I- Third limb, J- Furca.

A robust and wide, pilose seta present at distal end of 2nd endopodite. The first podomere of maxillary palp of Mx bear -ing 1 +5 long setae. The inner masticatory bearing 8-9 identical setae distally, with two sub-distal long and pilose setae, and two apical smooth setae. The branchial plate bears 16 plumose setae. The protopodite of L5 bearing plumose b, d, and smooth a seta, dorsal border bears about 12 unequal setae, one of them is the longest. The terminal podomere of L6 bears small h1, and h3 setae, h3 is about 6 times larger than its podomere. The third endopodite of L7 with one short straight and 1 hook-shaped with two tooth-like small setae, in spite of reflecting seta. The furca is muscular, long and straight, the Sa seta is approximately has similar length of Sp seta.

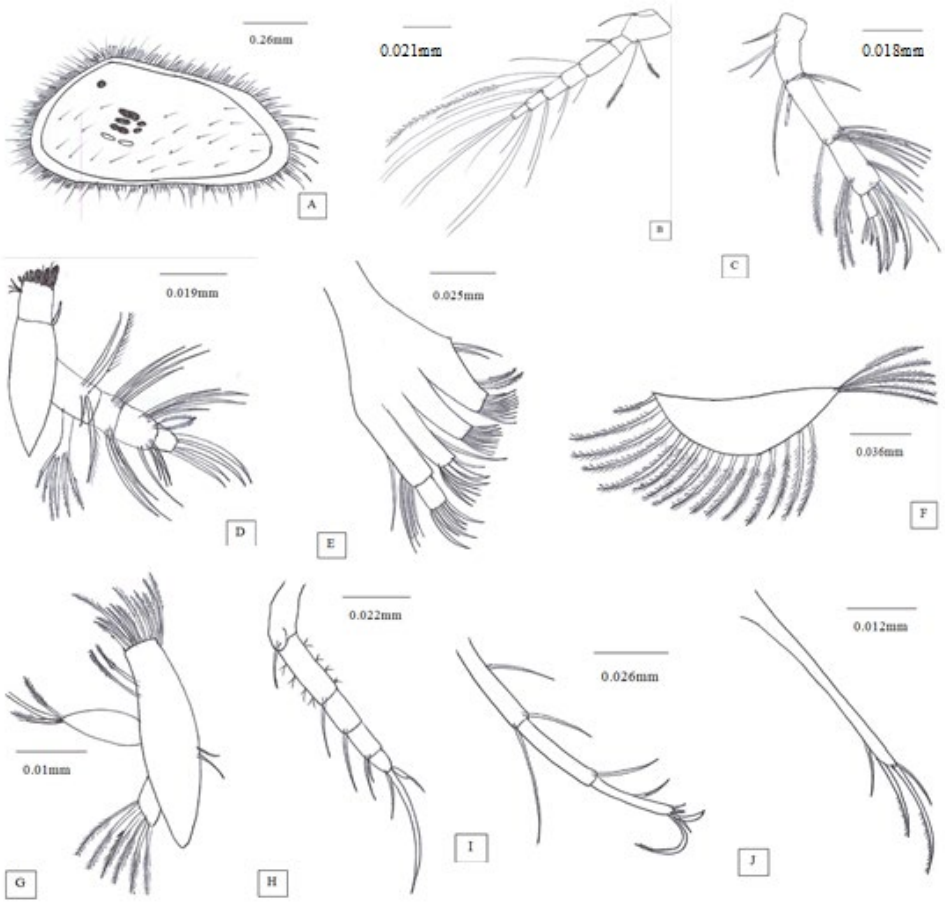

Fig. (7): Lucida drawing of *Sclerocypris exserta*, A-Whole amount, B- Antennula, C- Antenna, D- Mandible, E- Maxilluie, F- Branchial plate, G- First limb, H- second limb, I- Third limb, J- Furca.

7- *Notodromas monacha* (O.F. Müller, 1776) (Fig. 8)

The present sample is male, its carapace is 1.05-1.25 mm in length, and the shell is compact and nearly sub-pentagonal in shape. The male shell lacks a spine-like protrusion at the border between the ventral and posterior margins, which is present in the female. The posterior edge is narrower than the anterior one. The sixth segment of A1is elongate, with four long and pilose setae. The seventh section gets longer and narrower, bears one short and two long setae also exist aesthetasc ya. The first endopodite of A2 had very long and strong Y aesthetasc ventrally, also long natatory setae at the inner edge reaching the end of the last endopodite. The final endopodite has two claws (GM and Gm) with one thin seta, and aesthetasc y3. The branchial plate on the outer edge of Md bears (1+5) unequal pilose rays. A long robust pilose seta occurred centrally in 2nd endopodite despite of other seta. The principal segment of maxillary palp has one sub-apical robust with four (1 pilose and 3 sooth) apical setae. Two long thread-like seta where present between the

2nd and 3rd endites. The vibratory plate muscle with 16 plumose setae. The endopodites of L5 in male are asymmetrical. The left palp was noticeably shorter than the right, and it is pointed considerably distally to the large clasper. The end of palp bearing three unequal setae h1-3; the central one is long. The L6 is actual small, five-segmented, 1st segment lacking setae. The first segment of L7 bears d1, d2 and dp setae. The Furca is curved. Claw Gp with seta Sp of about the same length.

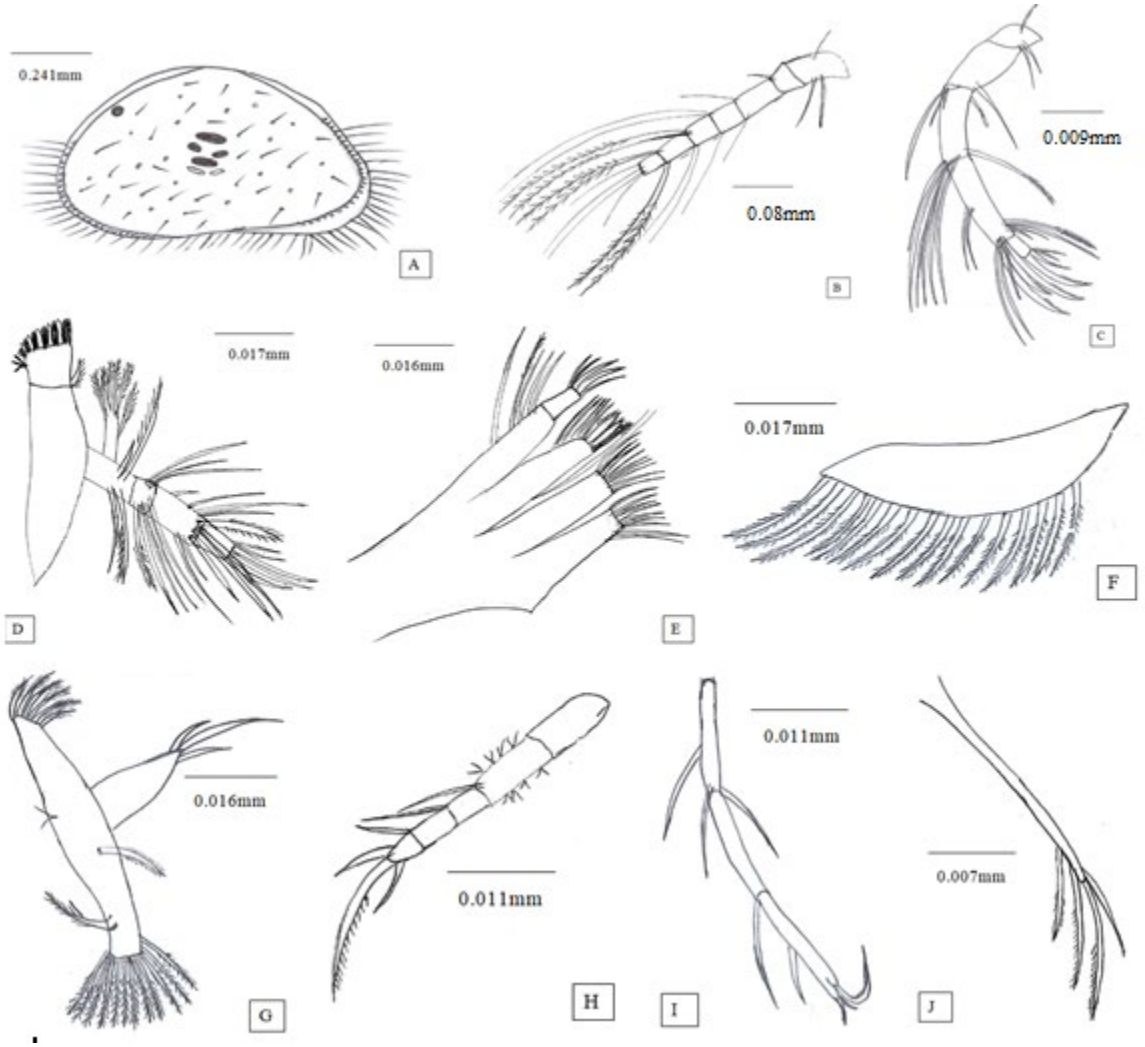

Fig. (8): Lucida drawing of *Notodromas monacha* A-Whole amount, B- Antennula, C- Antenna, D- Mandible, E- Maxilluie, F- Branchial plate, G- First limb, H- second limb, I- Third limb, J- Furca.

Molecular analysis

In the current study, integrating morphological confirmations with DNA fingerprint, have done for more precision and dependability of species identification. The results of the alignments indicated a high level of resemblance between the COI sequences of the studied species and the reference sequences in the GenBank, which accession numbers of (OR563931, OR563932, OR563952, OR763237,

OR759088, PP133793 and OR563933) for (*Heterocypris spadix*, *Heterocypris salina*, *Dolerocypris sinensis*, *Cyprinotus uenoi*, *Eucypris virens*, *Sclerocypris exserta* and *Notodromas monacha*) respectively (see table 1).

Discussion

The study of genetic characteristics of ostracod species in Iraq, specifically in Erbil city, had not been documented before the study of Latef & Ali (2023). Previous studies on ostracods in the region Iraq primarily focused on classical taxonomical characteristics such as size, carapace shape, and appendages. This approach can be challenging due to the existence of similar-looking species variations within the same species. However, the accurate identification criteria for these species required the application of molecular analyses.

The maximum likelihood sequence was conducted on data from Cytochrome Oxidase subunit I (partial COI). Molecular analyses of COI confirm the presence of 7 species belonging to two families (Fig. 9).

In fig. (9) clade (A) *H. spadix* relation revealed that 92% similarity with (LC626010- LC557032) recorded by (Kakui *et al.*, 2021; Munakata *et al.*, 2021) in Japan respectively. This species is very similar with *H. salina* but it differs in “the marginal infolds on valves are less developed, the ventral margins of the valves bend inwardly”. In clade (B) our result showed that *H. salina* has 96% similarity with (LC324691, LC324690, LC319787) recorded in Egypt by Ali *et al.* (2018). Also, our specimens match in the morphological study with Egyptian species, in spite of few differences. The observed differences can be attributed to the phenomenon of morphological plasticity, which is known to be influenced by environmental factors (Ali *et al.* (2018). Regarding *Dolerocypris sinensis*, *Cyprinotus uenoi*, and *Sclerocypris exserta* in clade (C&D) deposited in GenBank for the first time, they do not have any preceding data for matching. This important step aims to establish it as a valuable marker for future identification of these species. On the other hand, in clade (E) *Eucypris virens* presented 100% matches with (GQ914385, GQ914387 and GQ914372) which were recorded in Estonia and Latvia respectively (Bode *et al.*, 2010). *E. virens* one of the common species found in Studied sites, this might be due to the ability to tolerate and adapt to different ecological variables (Külköylüoğlu *et al.*, 2022). About *N. monacha* in clade (F) showed 100% parallel with (DQ889116) recorded by Costa *et al.* (2007) in the United Kingdom, and OA887660 by Tran Van *et al.* (2021) In Switzerland. *N. monacha* is worldwide species and their carapace is very similar to *N. trulla*, except variation in the posterior ventral flange of the females. In *N. monacha* the females' flange on the left valve overlaps the right one while in *N. trulla* the situation vice versa (Smith & Kamiya, 2014). Finally, *Bennelongia strellyensis* was used as out group that recorded by Martens *et al.* (2012) in Western Australia. In total, to enhance the taxonomic validity and improve our

understanding of phylogenetic relationships among various ostracod species, molecular data has been incorporated.

Generally, in Iraq there is no documented previous molecular data on ostracod in NCBI to match the similarities (likelihood available) and dissimilarities of sequences with our recorded species. This study became reasonable marker for future research in Iraq.

The global biodiversity plays a crucial role in influencing the global climate, which in turn have emotional impact on all facets of life (Sulieman *et al.*, 2022). In the present study maximum numbers of Ostracod were noticed in slow water flow regions, this approved by (Quante *et al.*, 2022). They suggested that highest density and diversity of ostracoda are found in area that is lentic or slowly running waters. The result comes in accordance with Khalifa & Ajeel (2022), they worked on the East of Al-Hammar Marshes in Iraq. Otherwise, the reason for the presence of variability in the richness species in the study sited is prospective to be made happen by environmental traits such as DO, pH, base geography, alkalinity and saltiness. High disintegration of organic matter could increase DO depletion in water and affect aquatic life (Dhahir & Ali, 2017). However, in Lap An Lagoon, central Vietnam (Tan *et al.*, 2021), worked on basic area sediment fraction, grain size, organic matter, and total organic carbon, which have a direct effect on the ostracod composition and distribution. The greatest invertebrate taxa exhibited a confident correlation with particulate organic matter (Alrubayi *et al.*, 2011). It is difficult to recognize ostracods based on their morphology because their carapace shape that can vary due to the environmental effects. It might be difficult and hence unreliable to identify ostracods species merely based on morphology, in addition to morphology, so molecular taxonomic techniques have been widely applied to identify species and establish phylogenetic relationships (Echeverría-Galindo *et al.*, 2021). Environmental situations are responsible for morphotype variation, which can further confuse identification (Dung *et al.*, 2013).

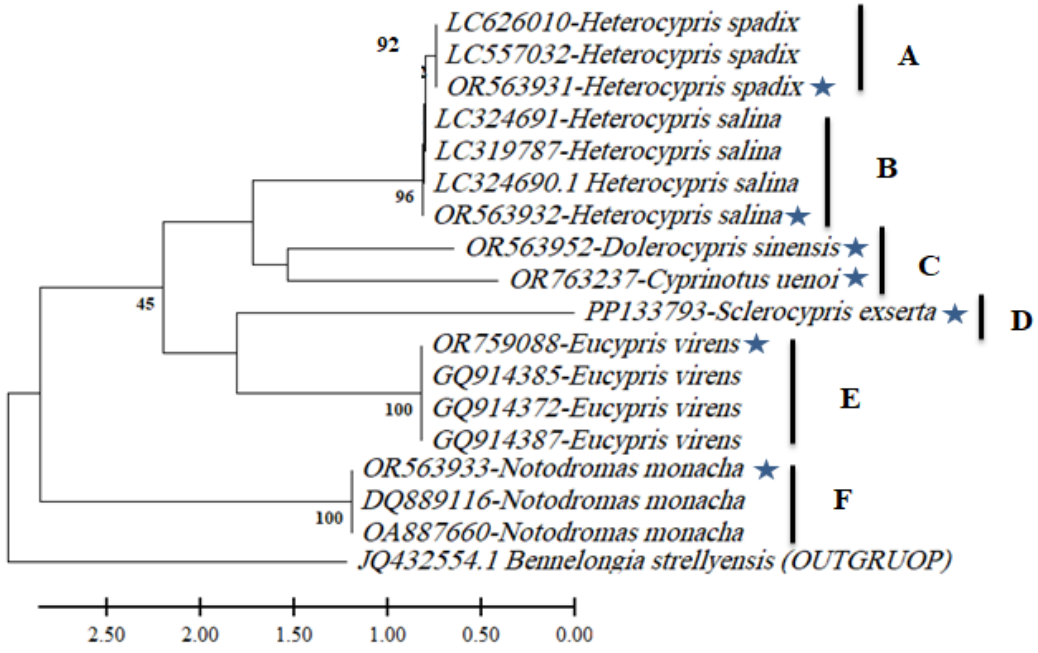

Fig.(9):Phylogenetic tree of samples from Iraq: Kurdistan region(*) the phylogenic was conducted using the Maximum Likelihood method based on the Tamura- Nei model in MEGA11 software and bootstrap analysis with 100 re-sampling. Partial DNA sequences of concentrated partial COI gene were used as input data.

Table (1): Percentage distribution of ostracoda based on gene according to blast in Genbank od NCBI.

Samples	Identified	Sample Accession Number	Query Cover %	Identic Number %	Genbank Accession Number	Country Identification
1	*Heterocypris salina*	OR563932	100	100	LC324690	Egypt
			100	100	LC319787	Egypt
			100	99.53	LC324691	Egypt
2	*Heterocypris spadix*	OR563931	100	100	LC626010	Japan
			100	100	LC557032	Japan
3	*Dolerocypris sinensis*	OR563952	New Record			Iraq: Erbil
4	Cyprinotus uenoi	OR763237	New Record			Iraq: Erbil
5	*Eucypris virens*	OR759088	100	100	GQ914385	Estonia
			100	99.84	GQ914372	Latvia
			100	99.53	GQ914387	Estonia
6	*Sclerocypris exserta*	PP133793	New Record			Iraq: Erbil
7	*Notodromas monacha*	OR563933	100	100	DQ889116	United Kingdom
			100	100	OA887660	Switzerland

Conclusion

A combination of two techniques were employed to verify the identification of an ostracod species in the freshwater environment of Erbil city-Iraq, which marks its first recorded occurrence in the country. The study utilized both a phenotype-based analysis method, involving microscopic examination, and a DNA-based method to ensure accurate identification.

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Contributions of authors

B. A. L., collected the samples and wrote the manuscript.

L.A.A., proposed the research project, supervised the study, and revised the manuscript.

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Conflict of interests

The authors declared no conflict of interest.

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الوصف المظهري والجزيئي لسبعة أنواع من الدرعيات تسجل لاول مرة في إقليم كردستان-العراق

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المستخلص: الدراسة الحالية هي أول دراسة مظهرية وجزيئية لسبعة أنواع جديدة من الدرعيات في العراق وهي (*Heterocypris salina*، *H. spadix*، *Dolerocypris sinensis*، *Cyprinotus unoi*، *Eucypris virens*، *Sclerocypris exserta* و*Notodromas monacha*) التي تنتمي إلى العائلتين (Cyprididae وCypridididae وNotodromatidadae. تم جمعها من 17 محطه من الجداول والبحيرات والأنهار في حدود محافظة أربيل، خلال الفترة من سبتمبر 2021 إلى أكتوبر 2022. وللاختبارات البايولوجية تم جمع عينات نباتية من(النباتات فى) المياه الضحلة (*Cynodon dactylon*، *Polygonum sp.*، *Nerium oleander* و *Nasturtium officinale*)، اضافة الى الطحالب (*Anabaena* و*Chlorella vulgaris*)، استخدمت شبكة جمع الهائمات النباتية والحيوانية لهذا الغرض. كما تم تربيه العينات في احواض التربيه مزوده بجهاز الأكسجين لمدة أسبوع تقريبًا بعد الرجوع إلى المختبر بعد بدء أنواع الصدفيات في الظهور و النمو(الدرعيات بالتكاثر) ووصولها الى مرحله النضج، تم تثبيت وحفظ العينات البالغة في 70% و100% من الإيثانول للأجراء الأختبارات المورفولوجية والجزيئية. تم دراسه التسلسل الجزيئي للجين COI بواسطة البادئ الأمامي COI F '5'(ACCCGCTGAATTTAAGCAT)3 والبادئ العكسي 3 '5'(CTCTTCAGATACTTTTTCAAC) 'COIR وسجلت في قاعدة البيانات الجينيه GenBank و منحت ا ارقام التسجيل الخاصة بها. ثم تم رسم شجرة النشوء والتطور للأنواع المدرسه. تم التعرف عليها وصفها لأول مرة في العراق. استهدفت الدراسه تحديد انواع الدرعيات جزيئيًا إلى جانب الوصف المظهري للأنواع (الحصول) وتم الحصول على نتائج تصنيفية أكثر دقة.

الكلمات المفتاحية: الدراسة الجزيئية، الدراسة المظهرية، الدرعياتش، اختبار ال PCR، اختبار تتطور السلالات.