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Short Communication

Morphological and Molecular Characterization of *Heterocypris incongruens* (Ramdohr 1808), A New Species Ostracod in Kurdistan Region-Iraq

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Abstract

The present paper is intensive on screening new Ostracod's species obtained from four selected sites in different areas, Erbil Governorate, Kurdistan Region, Iraq. Samples have been collected during October 2021, March and June 2022. *Heterocypris incongruens* (Ramdohr 1808) was observed in water after taking some algae (*Chlorella vulgaris, Anabaena*, etc.., and aquatic plants (*Polygonum* sp., *Nasturtium officinale* and *Cynodon dactylon*) with its residue near-shore water. Later separated the sample and fixed in 70% ethanol. After the morphological study, the PCR product of 28s rRNA was sequenced and deposited in the GenBank database with accession numbers OP600471-OP600474. According to phylogenetic analysis, the examined *Heterocypris incongruens* were identified and are being reported from Iraq.

Keywords: Ostracod, Molecular analysis, Phylogenetic tree

Introduction

Global climate is significantly affected by global biodiversity, which in turn affects all aspects of life (Sulieman *et al.,* 2022). The species *Heterocypris incongruens* (Ramdohr 1808) is a member of the phylum Arthropod's Cyprididae family. It's a tiny aquatic crustacean that ranges in size from 0.3 to 5 mm. Small calcite and tough carapace component of mineralized skeletal tissue with two dorsally linked valves. The hinged shell that can entirely cover and defend the non-mineralized body parts and appendages, makes them most recognizable. Mostly have rounded shells that are

embellished with pits, and ridges, with other features. It has a complex body inside its carapace that normally has eight pairs of appendages. These appendages serve a variety of purposes while the shell is open such as swimming, sensing, crawling, feeding, and mating.

It can be found in most aquatic ecosystems, including marine and freshwater (lake, river and pond). It is also occasionally found as a parasite species in fishes' digestive systems. This community's species vary according to location because they are impacted by several water properties, including temperature, base geography, saltiness, pH, alkalinity, and DO (Bellin et al., 2021; Rossetti et al., 2020). Furthermore, it has been vital to decades of academic and applied palaeontology research in a variety of areas, including evolutionary and environmental studies (Czajkowska, 2022). Additionally, it provides precise details on the dynamics of permafrost and the climate throughout the late Quaternary (Machado et al., 2020; Tan et al., 2021). The first discovered *Chlamydotheca unispinosa* (Baird, 1862) was on the Caribbean island of Montserrat by (Schmidt et al., 2018).

Karanovic et al., (2020) conducted research for molecular identification of every specimenwas was analyzed. To harvest DNA, genetic markers were used are (ITS, 28S, 18S, COI). They report two new species, *Ishizakiella occidentalis* and *I. miurensis*, whose shell shapes match the molecular phylogeny, according to the findings.

In an investigation in the perennial freshwater lake of Singanallur, India. The *Cypretta campechensis* was the first record of the ostracod species, based on the molecular examination of the mitochondrial cytochrome oxidase subunit (mt-COI). LCO1490 and HCO2198 universal primers were used to amplify the gene, which was subsequently sequenced and verified using NCBI GenBank (MN641913). (Kalpana et al., 2021).

Through the use of a molecular approach, Huyen and Karanovic (2022) discovered four new species of Parasterope, including *P. busanensis, P. single, P. sohi,* and *P. sagami*. Based on the partial 16S rRNA. The following primers were used: forward and reverse, 16S MYOF1, 16S MYOF2, 16S MYOR1 and 16S MYOR2.

On the other hand, two new species were listed. *Vestalenula gravata* of the Darwinulidae family and *Microloxoconcha Semicircularis* in the Cytheroidea family. (Smith & Chang, 2022).

Geometric morphometric techniques are effective tools for distinguishing between closely related Ostracod species and for investigating the relationship among their morphological variability, taxonomy, and paleoecology. In this work, valve outline analysis enables differentiation between

Riocypris whatleyi and Cypridopsis silvestrii juvenile non-marine ostracods by highlighting variations in the posterior valve area and surface ornamentation (Ramos et al., 2021).

Arthropods are ecologically important because of their sheer numbers and extreme diversity. They play extremely important roles in maintaining the ecosystem and can also be beneficial for humans (Schapheer et al., 2021). Ostracods were found in small numbers in Iraq (Hassan, 2018) and collected specimens from ten locations in the Baghdad and wasit governorates. He revealed thirteen species of ostracods among them *Ilyocypris forester*, *Stenocypris hislopi*, *Cyclocypris* sp, *Eucypri* sp, etc. which indicate that the population of *Stenocypris hislopi* reached up to 3000. Also, (Zwair, 2021) introduced *Pseudocandona* sp for the first time in Iraq. Generally, the study of the Ostracodes fauna poorly documented in the Kurdistan region. Five ostracode species have been described from Baluti Formation (Upper Triassic) near Sarki Village situated about (11 km) Southeastern Amadiya City, Dohuk province, Northern Iraq, there are *Fabanella* sp., *Hungarella moorei*, *Ogmoconcha* sp., *Ogmoconcha bristolensis* and Cytherella acuta (Al-Khashab & Al-Halawachi, 2018). Prior to this study, Al-Meshleb (2012) was the only researcher who discovered a species of *Heterocypris giesbrechtii* in Iraq. No study has yet been conducted on the prevalence of *Heterocypris* in Iraq. The aim of this study was to investigate the ostracods species living in the Kurdistan region. We did this in the hope of obtaining different types of ostracods in a watery environment.

Materials and methods

Sample collection

The Ostracod samples were collected from 4 different sites which include Altun kupri about 55km, Xalan site 100km, Bridge Hafiz 94 km and Xanaqa 119 km distance from Erbil city respectively (Figure 1). Specimens were gathered by taking algae which include *Chlorella vulgaris, Anabaena, cylindrica* and *Oscillatori* and plants belong to *Polygonum* sp., *Nasturtium* sp. and *Cynodon dactylon* with its sediment near shore water, during October 2021, March and June 2022. The samples were transferred to the laboratory and placed under an oxygen instrument for about one week, then ostracod species began to grow and it was removed from the bottle and then fixed in 70% ethanol. Identification of ostracods sample was performed in the laboratory of Education College, Salahaddin University- Erbil, by using a compound microscope (OPTIKA B-350) and a small amount of glycerin. Characteristics were examined using identification keys (Fernando & Biology, 1981; Karanovic, 2012).

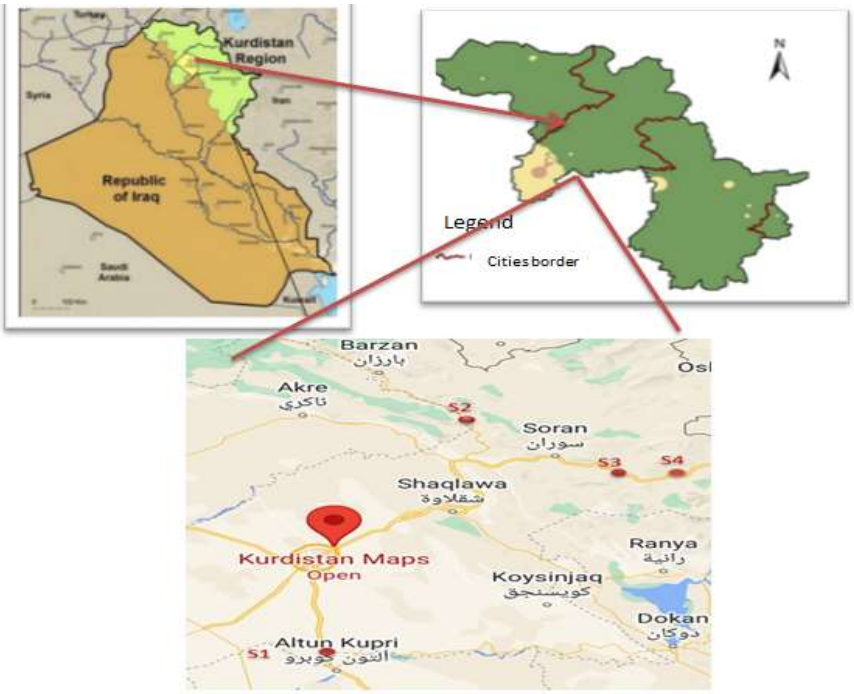

Figure 1. Map of the Kurdistan region shows the study sites

PCR Technique and genomic extraction

Heterocypris incongruens was discovered and sequenced using PCR technology. By utilizing the GeneAll® ExgeneTM for Clinic Cell SV small kit, which was acquired from GeneAll® (Songpa-gu, Seoul, KOREA), by the manufacturer's instructions, and 50 mL of elution buffer, total genomic DNA was extracted from the specimens. Using a nanodrop spectrophotometer to calculate concentrations of isolated DNA. Before starting a PCR, the extracted genomes were kept at -20°C.

PCR amplification

A region of the 28SrRNA gene was amplified by PCR. The universal primers included forward primer C1 (5ACCCGCTGAATTTAAGCAT) at 5'-3' position and reverse primer C3 (CTCTTCAGATACTTTTCAAC) at 5'-3' position .PCR cocktail was run in a total volume 50 µL reaction mixture containing 25 µL of 2× PCR master mix (AMPLIQON, Denmark), 1.0 µL of each primer (10 pmol), 1.5 µL of genomic DNA template. The volume was completed to 50 µL with free nuclease water. Each gene was independently amplified. The subsequent program was used to continue the PCR after that: the thermal cycling conditions comprised of initial denaturation at 94c for 5 min, 35 cycles of each denaturation at 94c for 45 sec, annealing

temperatures at 51 sec for 45 sec and extention at 72c for 45 sec, and final extention at 72c for 5 min. the PCR product was confirmed by using agaros gel electrophoresis. The samples prepared and run in 2% gel of agaros verify the size of the fragment after which the DNA is dyed with ethedium bromide to make it visible under ultraviolet light. Having a PCR product predicted size of 365bp.

DNA sequencing analysis

PCR product of DNA samples were organized for sequencing by using forward primers of 28s rRNA in Zheen International Hospital, Erbil, Kurdistan region, Iraq. The National Center for Biotechnology Information (NCBI) Gen Bank was used to compare the alignment findings of the sequences with previously deposited sequences. The sequences of *Heterocypris incongruens* in this study were submitted in NCBI-Gen Bank.

Results

There does not appear to be any preceding literature on this species in Iraq. Specimens of four revealed sites that under the microscopic inspection and ordering keys with molecular sequences discovery have been diagnosed as *Heterocypris incongruens* as a new species in Iraq (Fig. 2 and 3).

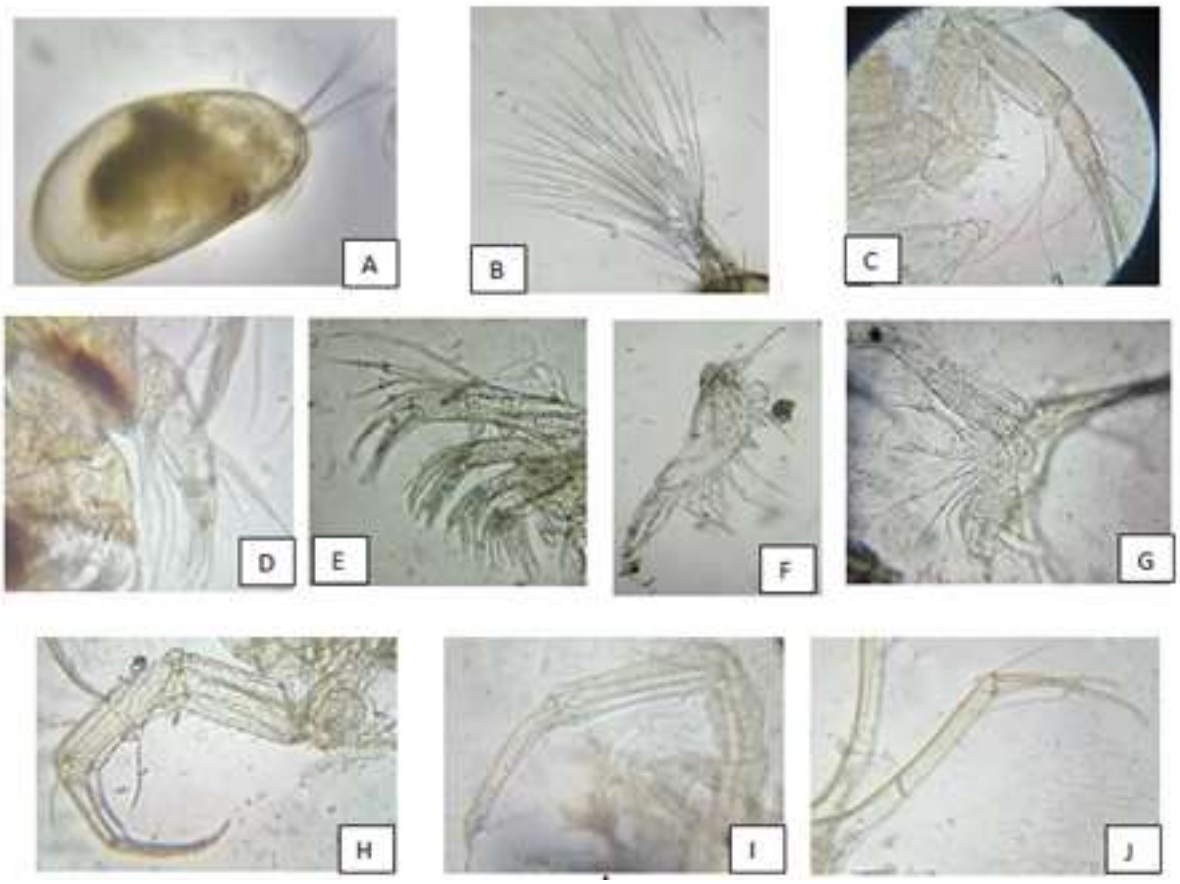

Figure 2. APhotomicrograph of *Heterocypris incongruense* A-Whole amount, B- Antennula, C- Antenna, D- Mandibula, E- Maxillula, F- Branchial plate, G- First limb, H- second limb, I- Third limb, J- Furca

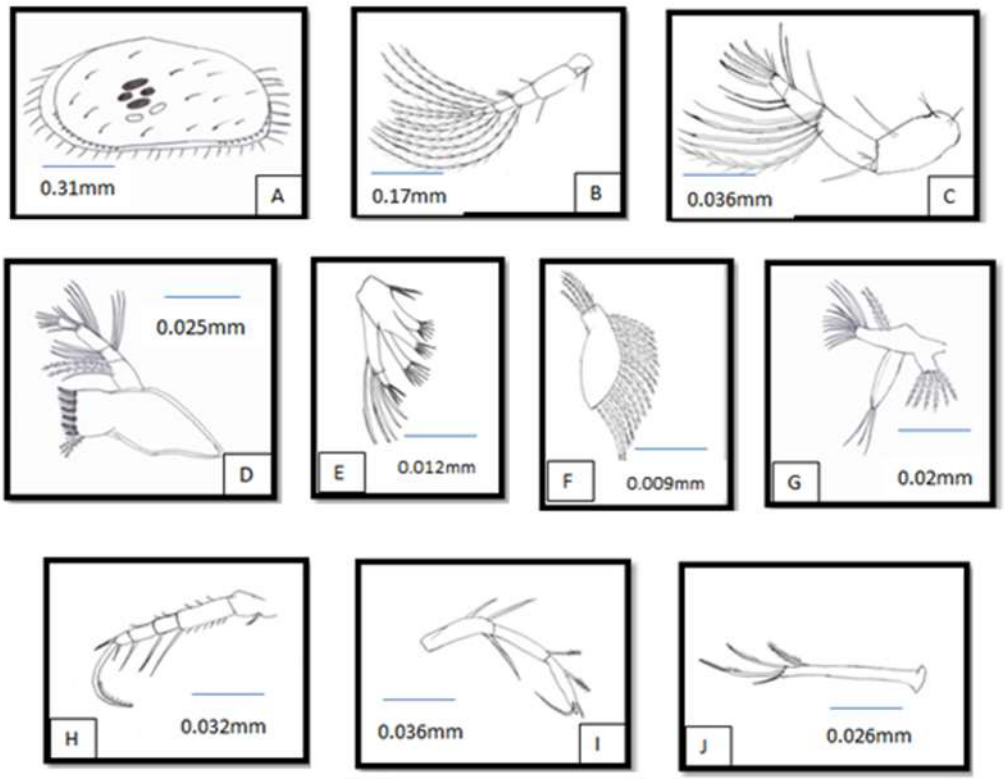

Figure 3. Lucida drawing of *Heterocypris incongruence* A-Whole amount, B- Antennula, C- Antenna, D- Mandibula, E- Maxillula, F- Branchial plate, G- First limb, H- second limb, I- Third limb, J- Furca

Heterocypris incongruens

The anterior region of the shell of this species is repeatedly covered in tiny tubercles that are arranged along the margin in a regular row of twenty to thirty, only on the right or smaller valve. Also along the margin of the valves, a series of regularly spaced denticles can be seen. The carapace, which encases the entire body, is made up of two valves joined dorsally. It passes through moulting, until it reaches adulthood. The carapace's two valves close with extreme precision, with one valve being slightly larger than the other and therefore covering it. By letting go of the adductor muscles that protrude from the shell and using the appendages to force the valves apart, the carapace can be opened.

The outer lamella and the inner lamella are the two primary components of the valve. The first one creates the outermost surface and makes up the majority of the valves. Numerous tiny pores, which are openings in the carapace wall through which sensory setae emerge, are present in this layer. Has a radial (marginal) pore canals on the borders of the valves as well. The valve layer inside the carapace is the second one. They both cross the area known as the fused zone.

Their length varies from 0.8 to 1.1 mm, and the upper portions of the shell are orange-yellow, fading to brown. It has a complicated soft body that has been shrunk such that the carapace may completely encapsulate the body's non-calcified regions. This makes it difficult to distinguish between the head, thorax, and belly. The mandibles, maxilla, antennae, and antennules are the four pairs of appendages that the head supports. Because there isn't a distinct line separating the head and thorax, it has been argued on occasion that the first pair of thoracic appendages should be considered the fifth appendage. Typically have eight pairs of appendages that serve a variety of purposes, including sensing, movement, cleaning, mating, and eating.

The first appendage, known as the antennule or first antenna, is situated close to the hinge's anterior end. It has six segments and is uniramous. Are long and slender, and together with the antennae, they are flexible enough to be employed for swimming. The lengthy setae are spread out when the ostracods is at rest in order to detect movement in the water. The antennae, which are biramous and have two branches, are the second set of limbs (the endopod and exopod). Utilized to swimming, crawling and drag food to the mouth region. The third appendages at either side of the mouth are the mandibles. Consist of two primary components: the coxa, which bears teeth on the inside to masticate food, and the palp, which sweeps food into the mouth. The fourth limb is the maxillula. There are two primary sections. The endopod and endites, which are utilized for eating, are in the anterior parts. The huge branchial plate in the posterior portion of it aids in respiration by circulating water through the carapace cavity.

The first pair of thoracic legs altered to a masticatory appendage. , it is usually the smallest of the walking legs. These appendages consist of protopod which is the proximal portion, and endopodite with exopodiet at the inner and outer side of protopodite respectively. The second pair of thoracic legs is interconnected. The first piece of this limb is the protopod, while the other segments are the endopod. The limb is positioned in the posterior section of the carapace. It is a walking limb and is sometimes used as a clasping appendage. Usually ends with a claw. The third thoracic legs Five jointed. Located in the posterior region of the carapace. It is either a cleaning limb bent upwards into the carapace, or a walking leg, analogous in shape to the second limb, the first segment of its limb is the protopod, while the other segments are the endopod.

The final appendage, a rod-like structure located at the back of the body, is known as the caudal ramus (also known as the furca or uropodal ramus). Typically, it has a rigid ramus or base with several setae and claws sticking out of it. Both the extremely long terminal claw and the sub-

terminal claw have hair at their ventral ends. Additionally, there are two setae: one on the proximal ventral side of the sub terminal claw and another one at the distal end of the terminal claw. The caudal ramus and furcal attachment are joined internally. This attachment has two branches that are positioned at an acute angle at the proximal end. Long, wide, and straight define the furcas attach's median region. Arched and tapering at its terminal end, the articulation extremity is rounded. In some populations, the caudal ramus is employed to move the male sexual organs during copulation, also used for movement. Regarding the molecular investigation, the blast result in the DNA (28srRNA) sequence of *Heterocypris incongruence* in GenBank indicated 99% similarity to this species with accession No. (op600471-op600474) (Table.1).

Table 1: Pairwise alignment of 28srRNA sequence of *Heterocypris incongruens*. Query is the study or sample sequence and Subject is the GenBank sequence

samples	Identified	Accession Numbers	Query Cover %	Identic Number %	Accession Number of BLAST Identification	Country
1	Heterocypris incongruens	OP600471	100	100	AB675003	Japan
2	Heterocypris incongruens	OP600472				
3	Heterocypris incongruens	OP600473	90	97.8	EU370438	Germany
4	Heterocypris incongruens	OP600474				
5		Out group			LC557033	Japan

Phylogenetic inferences

According to phylogenetic analysis based on the 28S rRNA nucleotide sequence, the examined *Heterocypris incongruens* were grouped along predicted lines. Sequence divergence similarity data and a phylogeny were used to determine how closely related species within different genera to one another (Fig. 4).

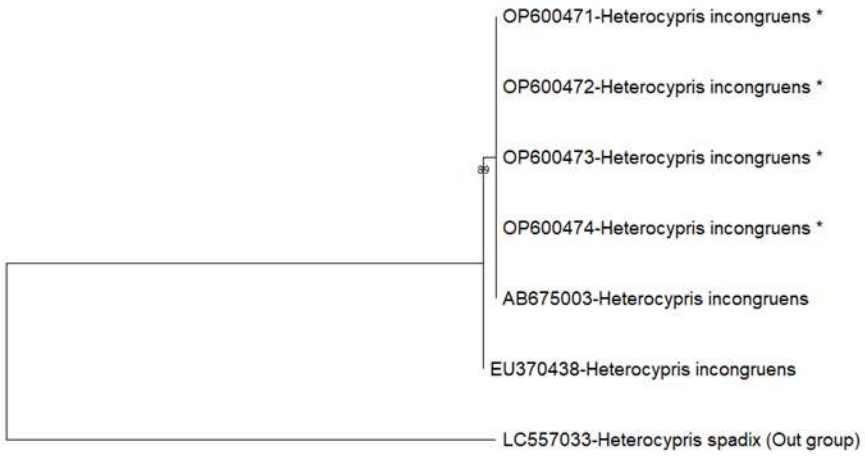

Figure 4. Phylogenic tree of Heterocypris incongruens samples from Iraq: Kurdistan region-Iraq . The phylogenic tree was constructed using the Maximum Likelihood method based on the Tamura-Nei model in MEGA11 software and bootstrap analysis with 100 re-samplings. Partial DNA sequences of concatenated partial 28S rRNA gene were used as input data.

Discussion

For the first time, *Heterocypris incongruens* was recorded by (Ramdor 1808). Then, additional researchers throughout the globe work on it in diverse contexts. Considering that Iraq had no prior knowledge of it. The current record is the first to be set in this nation. The morphological analysis of the species in the illustrations is consistent with (Karanovic, 2012; Purper & Würdig-Maciel, 1974; Rossetti et al., 2020). Otherwise, the reason of presence one species in an area and its absent in another are likely to be caused by ecological traits such as base geography, saltiness, pH, alkalinity and DO. Some studies, (Parra & Espinoza-Villalobos, 2020; Szlauer-ŁUkaszewska & Pešić, 2020) indicated that various ecological conditions and calcification mechanisms are believed to be the root of the different outcomes for different species. According to certain experts who studied 'aquatic creatures' calcification or development was accelerated by low pH levels. Also, in an investigation done by (Hassan, 2018) with the drop in water temperatures and the decline in the abundance of other crustacean groups came the appearance of an expanding ostracods colony. The highest number was recorded in January, March and April.

Concerning the molecular study, The BLAST tool from (http://blast.ncbi.nlm.nih.gov/) is used to analyze gene sequence samples with a size of 700–800 bp to compare our amplified sequences to other *Heterocypris incongruent* sequences that had been saved. The BLAST results showed that 4 *Heterocypris incongruens* had the highest identity number query sequence with 100% identity.

This aliment suggests that we submit our query sequences to NCBI. According to the alignment of 28srRNA sequence of *Heterocypris incongruens* that shown in Table (1) and Figure 4 Suggested that this species was recorded in Gen Bank three times only. The first time was in Japan (AB675003), then in Germany (EU370438) and the last one current record in Iraq (op600471-op600474). There are other Heterocypris species described in Japan and recorded in Gen Bank such as *H. spadix* (LC557033). However, further research is needed to investigate the other species of Ostracoda in our freshwater environments.

Conclusion

The present paper is intensive on screened new Ostracods species morphologically and molecular analysis. Otherwise, the result indicated that various ecological situations and calcification mechanisms are believed to be the origin of the different outcomes for different species.

References

Al-Khashab, S. A., Al-Halawachi, J. M. (2018). Ostracoda Species from Upper Triassic of Baluti Formation (Amadiya Section) in Northern Iraq.

Al-Meshleb, N. F. (2012). Systematic and ecological study of recent Ostracoda from Al-Faw town , south of Iraq. Mesopotamian Journal of Marine Sciences, 27(2), 88–103.

Bellin, N., Spezzano, R., Rossi, V. (2021). Assessing the extinction risk of heterocypris incongruens (Crustacea: Ostracoda) in climate change with sensitivity and uncertainty analysis. Water, 13(13), 1828. https://doi.org/10.3390/w13131828

Czajkowska, M. (2022). High-resolution environmental changes recorded in ostracod and mollusc fauna from the Holsteinian palaeolake at Ortel Królewski II, eastern Poland. Boreas, 51(4), 793–809. https://doi.org/10.1111/bor.12588

Hassan, M. (2018). Habitat requremient of ostracoda from water bodies in middle of iraq. Biochem. Cell Archive, 18(2), 2197–2202.

Huyen, Ph. T. and Karanovic, I. (2022) Four new Parasterope (Ostracoda, Myodocopina) from the Northwest Pacific and their phylogeny based on 16S rRNA. ZooKeys, 1095: 13–42. https://doi.org/10.3897/zookeys.1095.77996

Kalpana, R., Saravana Bhavan, P., Udayasuriyan, R., Sheu, J-R., Jayakumar, T., Fong, TH. (2021). First record of a seed shrimp (Ostracoda: Podocopida) Cypretta campechensis (Cyprididae) in a perennial lake (Coimbatore, India): Its molecular identification. International Journal of Fauna and Biological Studies , 8(2)

Karanovic, I. (2012). Recent freshwater ostracods of the world: Crustacea, Ostracoda, Podocopida. In Recent Freshwater Ostracods of the World: Crustacea, Ostracoda, Podocopida. Book, Springer publisher. https://doi.org/10.1007/978-3-642-21810-1

Karanovic, I., Huyen, Ph. T., Yoo, H., Yuriko Nakao, Y. and Akira Tsukagoshi, A. (2020). Shell and appendages variability in two allopatric ostracod species seen through the light of molecular data. Contributions to Zoology, (89) 247-269

Machado, C. P., Coimbra, J. C., & Bergue, C. T. (2020). Provinciality of ostracoda (Crustacea) in the northeastern and eastern brazilian shelves based on neontological and paleontological analyses. Revista Brasileira de Paleontologia, 23(1), 3–31. https://doi.org/10.4072/rbp.2020.1.01

Parra, G., & Espinoza-Villalobos, D. (2020). Effects of CO2-driven acidification on the ostracod Cypridopsis vidua: what are its likely environmental consequences? Toxicological & Environmental Chemistry, 102(5–6), 284–301. https://doi.org/10.1080/02772248.2020.1779723

Purper, I., & Würdig-Maciel, N. L. (1974). Occurrence of Heterocypris incongruens (Ramdohr), 1808 - Ostracoda - in Rio Grande do Sul, Brazil. Discussion on the allied genera: Cyprinotus, Hemicypris, Homocypris and Eucypris. Pesquisas Em Geociências, 3(3), 69. https://doi.org/10.22456/1807-9806.21855

Ramos, L. Y., Pérez, A. P., Coviaga, C. A., Schwalb, A., Ariztegui, D., Alperin, M., & Cusminsky, G. C. (2021). Morphological diversity and discrimination tools of the non-marine ostracod cypridopsis silvestrii across temporal and spatial scales from patagonia. Anais Da Academia Brasileira de Ciencias, 93. https://doi.org/10.1590/0001-3765202120200635

Rossetti, G., Pieri, V., Bolpagni, R., Viaroli, P., & Nizzoli, D. (2020). Variability in environmental conditions strongly impacts ostracod assemblages of lowland springs in a heavily anthropized area. Water (Switzerland), 12(11), 3276. https://doi.org/10.3390/w12113276

Schapheer, C., Pellens, R. & Scherson, R. (2021). Arthropod-Microbiota Integration: Its Importance for Ecosystem Conservation. Fronteir Microbiology, **12**:702763. https://doi.org/10.3389/fmicb.2021.702763

Schmidt, R. E., Shoobs, N. F., McMullin, E. R. (2018). Occurrence of the large ostracod, Chlamydotheca unispinosa (Baird, 1862), in temporary waters of Montserrat, Lesser Antilles. ZooKeys, 2018(748), 89–95. https://doi.org/10.3897/zookeys.748.22323

Smith, R. J., Chang, C. Y. (2022). Two new species of non-marine Ostracoda (Crustacea) of the genera Vestalenula Rossetti & Martens, 1998 (Darwinuloidea) and Microloxoconcha Hartmann, 1954 (Cytheroidea) from Tsushima, Japan, and a summary of the non-marine ostracod fauna of the island. Zootaxa, 5150(4), 529–555. https://doi.org/10.11646/zootaxa.5150.4.4

Sulieman, A. M., Idris, A. I., Alshammari, N., Alanazi, N., Al-Azmi, M., Hamadou, W., Albadri, G., Khamisabadi, H.(2022). The "Molecular Biodiversity of Bacteria Isolated from Medicago sativa Rhizosphere in Hail District, Saudi Arabia: Molecular Biodiversity of Medicago sativa associated Bacteria. Cellular and Molecular Biology, 68(2):1-7. https://doi.org/10.14715/cmb/2022.68.2.1

Szlauer-ŁUkaszewska, A., Pešić, V. (2020). Habitat factors differentiating the occurrence of Ostracoda (Crustacea) in the floodplain of a small lowland River Kra piel (N-W Poland). Knowledge and Management of Aquatic Ecosystems, 2020-January(421), 23. https://doi.org/10.1051/kmae/2020012

Tan, C. W. J., Gouramanis, C., Pham, T. D., Hoang, D. Q., & Switzer, A. D. (2021). Ostracods as pollution indicators in Lap An Lagoon, central Vietnam. Environmental Pollution, 278, 116762. https://doi.org/10.1016/j.envpol.2021.116762

Victor, R., & Fernando, C. H. (1981). An illustrated key to the freshwater ostracod genera of the Oriental region. In University of Waterloo Biological Series No (Vol. 23). Department of Biology, University of Waterloo.

Zwair, H. (2021). New Record Species Of Freshwater Ostracoda From Middle Of Iraq. Nveo-Natural Volatiles & Essential Oils Journal| Nveo, 12787–12794.