ZANCO Journal of Pure and Applied Sciences

TYPE Original article
DOI http://dx.doi.org/10.21271/ZJPAS.37.2.4

OPEN ACCESS

*Corresponding author

Berivan Asaad Latef:
berivan.latef@su.edu.krd

RECEIVED :16 /05 /2024
ACCEPTED :21/02/ 2025
PUBLISHED :30/ 04/ 2025

KEYWORDS:
Ostracod, Morphology, Molecular analysis, Phylogenetic tree.

Morphological and Molecular Characterization of Three New Records of Ostracod Species in Kurdistan Region

Berivan A. Latef * ,Luay A. Ali
Department of Biology, Education College, Salahaddin University- Erbil, Erbil, Kurdistan Region-Iraq

ABSTRACT

Research on Ostracod identification in Iraq is currently limited, specifically in Erbil Province with relatively few studies conducted on this topic. As a result, there is a significant knowledge gap regarding the diversity and distribution of ostracod species within the country. This study fills significant gaps by study the morphology and molecular analysis of three novel ostracoda species which are (*Stenocypris hislopi, Cypridopsis vidua* and *Candona neglecta*), that belong to two families (Cyprididae and Candonidae), collected from 17 different sites in boundary Erbil Province, during September 2021 to October 2022. For biological analysis, a number of aquatic plants, green algae, and zooplanktonic net were used. Meanwhile, some physical and chemical parameters were taken. Following a comprehensive morphological study, the PCR product of the COI gene was subjected to sequencing, and the resulting sequences were deposited in the GenBank database under unique accession numbers. Subsequently, phylogenetic analysis was conducted, leading to the identification of the examined species, which are now being reported from the Kurdistan region. This study focused on molecular inquiry besides morphology identification. This finding implies that molecular methods ought to be used in subsequent biodiversity studies in order to assess species diversity accurately.

1.Introduction

Ostracods are tiny crustaceans, less than 5 mm in length, with two-valve calcified carapace casing the whole body. They are lives in aquatic habitats, including marine and non-marine. The occurrence of it is related to water nature, air temperature and even chemical properties. The taxonomy of freshwater Ostracods is based mainly on the size, shape, and ornament of the left and right carapaces. (Wang et al., 2022, Zwair, 2023).

In an investigation conducted at 47 sites in the 13 provinces of Turkey (Rasouli et al., 2014), results show that three species (*Eucypris kerkyrensis, Cypridopsis elongata,* and *Bradleystrandesia parva*) were new records for the Ostracoda fauna of Turkey. On the other hand, forty-two marl peat sediment samples from eight stratigraphic stages in the Qa'aJahran-Dhamar region of Yemen yielded eight global ostracod species, which are *Sarscypridopsis aculeata, Pseudocandona albicans Fabaeformiscandona breuili,* with described physico-chemical parameters(Mohammed et al., 2018). Although, (Mesquita-Joanes et al., 2020) present a novel species called *Cypris pretusi* that was discovered in tiny transient streams and ponds around the Eastern Iberian Peninsula and Minorca (Balearic Islands). Otherwise, (Tran Van et al., 2021) study on the molecular aspect of *Cyprideis torosa*, *Darwinula stevensoni* and *Notodromas monacha* in Belgian lakes, due to the small bodies of individual, the whole genome amplification method also used due to this factor use of Illumina-based sequencing technology. Through the use of a molecular approach, (Pham and Karanovic, 2022) revealed four new species of *Parasterope busanensis, P. single, P. sohi,* and *P. sagami*, based on the partial 16S rRNA sequences. Additionally, in Iraq, samples were taken in the Al-Hindiyah from the Karbala Governorate (Zwair, 2023). The outcome shows that *Pseudocypretta maculata* was identified for the first time in Iraq. Generally, in the Kurdistan region only (Ali, 2007) collected specimens from 3 sites of the Greater Zab River (Qandil, Efraz, and Khabat area), in which *Cyprinotuis incongruens* represented as new records . Also, (Latef and Ali, 2023) were the first researchers in Iraq to study molecular analysis of ostracod in seventeen water bodies from the Erbil Governorate boundary. In consequence, *Heterocypris incongreuns* were documented as new species in Iraq. However, the existing study was conducting COI gene analysis to confirm the morphological study.

To date, there is a dearth of published information regarding the distribution and molecular study of ostracod in Erbil Province-Kurdistan Region/Iraq. However, this study aims to bridge this gap by documenting the first recorded instance of ostracod in this region of Iraq, along with molecular evidence to verify its presence.

2. Materials and methods

A comprehensive survey of Ostracod species was conducted from September 2021 to October 2022 in 17 different sites (Taq taq, Degala, Altun kupri, Smaquli girtik, Smaquli Dam, Smaquli dam outlet, Choman, Qalat, Wasanan, Khanaqa, Hafiz bridge, Khalifan, Zargali, Gali Ali bag, Bekhal, Khalan, and Khabat) within Erbil city, Iraq. (Fig.1). In the present investigation for the biological aspects, the number of plants and algal community were recorded, along with the collection of zooplanktonic nets. The taxonomic identification was confirmed based on classification key (Karanovic, 2012). For molecular analysis, genomic DNA was extracted from the specimens using the Qiagen kit (Hilden, Germany) following the manufacturer's instructions. The extracted genomes were stored at -20°C. A region of the COI gene was amplified using PCR, with the forward primer COI F5' (ACCCGCTGAATTTAAGCAT) 3' and reverse primer COIR 5' (CTCTTCAGATACTTTTCAAC) 3'. The PCR reaction mixture was run in a total volume 50 µL consisted of 25 µl master mix, 1.5 µl of each primer, 2 µl DNA template, and 20 µl PCR grade water. The PCR conditions was applied 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 for

sequencing using the forward primers of COI in Korea. The obtained sequences were compared and aligned with previously deposited sequences in the National Center for Biotechnology Information (NCBI) GenBank. All the sequences of the reported species in this study were submitted to NCBI-GenBank. To obtain additional insights into the genetic variation and relationships among the studied species, utilizing the Maximum Likelihood was conducted using the MEGA X (Kumar et al., 2018).

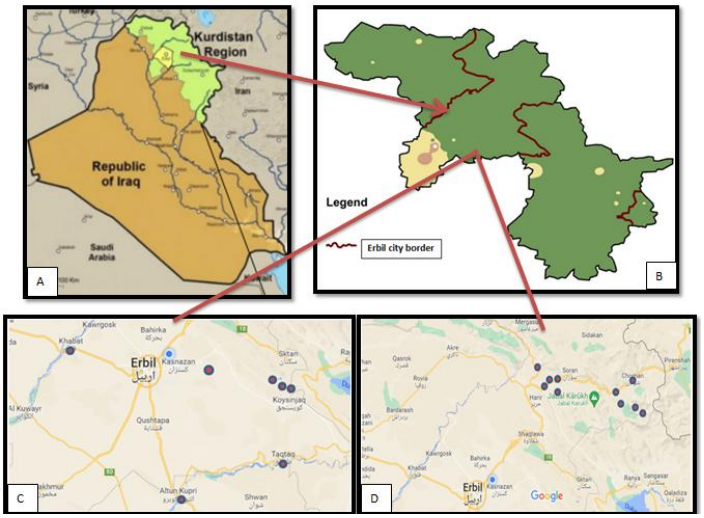

Figure 1: A- Map Of Iraq and Erbil Province B- Erbil province. C&D- studied area and the sampling sites.

3.Results

3.1 Morphological analysis (Note: in each species only point out discriminate character)

The present study has reported 3 novel species in the Kurdistan region which are (*Stenocypris hislopi, Cypridopsis vidua* that belong to family Cyprididae and *Candona neglecta* regarded to Candonidae family). Their species previously documented in Iraq, but the morphological features were described only. This study applied molecular technique for the first time in Iraq and Erbil city

3.1.1- Stenocypris hislopi

Carapace (Ca) is light brown in color, elongated, 1.46 mm in length. The anterior and posterior margins are rounded and ventral straight but slightly concave in the middle. The septa are present in the anterior, posterior and ventral margins. Eight adductors with two mandibular muscle scars are located at the center of the carapace, also six long hairs appear at the posterior edge. On the first antenna (A1) the Rome organ is present at the apical ventral side of the second protopodite with a small seta attached to the dorso-distal end. Second antenna (A2) is five jointed, the group of long swimming setae of the second podomere exceeds the tip of the distal claws at the dorsal edges. The coxal plate of Mandible (Md) bears a series of seven teeth, and the exopodite plate of the basis protopodite bears six plumose setae. The proximal maxillary segment of maxilla (ML) with a group of four setae on the outer apical edge (two of them are plumose). Also, the first endite of MLwith one sub apical pilose and eight apical setae. The vibratory plate bears seventeen pilose setae. The protopodite of first thoracic leg (L5) bears a smooth b seta near the junction with the endopodite. The masticatory process is armed with ten apical and four sub-apical plumose setae. The second thoracic leg (L6) is five segmented, the two fused podomeres of protopodite bear small pilose d2 setae. The first endopodite have dorso distal e and very small seta. The third thoracic leg (L7) its basal segment with (d1, d2 and dp) seta. The third podomere bears one long backward-pointed spinose bristle and one denticulated vital claw. The furca is noticeably asymmetrical. The right one is convex and comprehensive dorsally, with a denticulated dorsal margin. While the left one is slender and almost straight, the dorsal margin is smooth. It have very small smooth sp seta (Fig. 2).

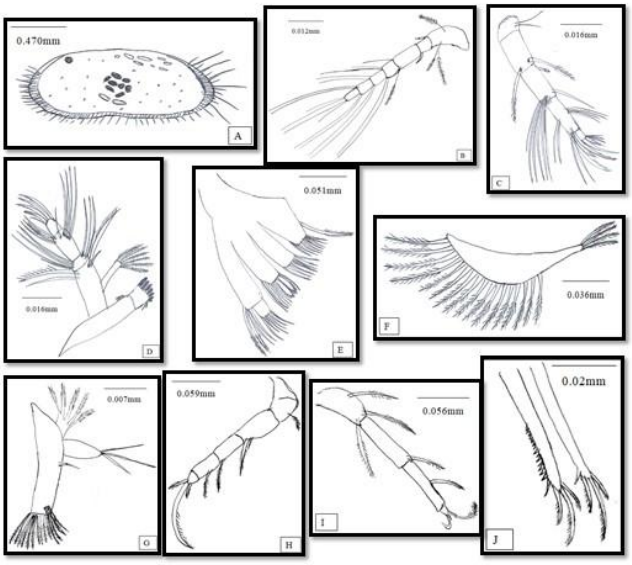

Figure 2: Lucida drawing of *Stenocypris hislopi* A-Whole amount, B- Antennula, C- Antenna, D- Mandibula, E- Maxillula, F- Branchial plate, G- First limb, H- second limb, I- Third limb, J- Furca

3.1.2- Cypridopsis vidua

The length of carapace reaches 0.52 mm (usually 0.5–0.6 mm), its Ovate in both lateral and dorsal views, usually with four lights to dark green transverse bands on a yellowish carapace. The anterior end is bluntly pointed, and the posterior is more rounded. The An1 comprise seven podomeres. Well-developed natatory seta. The exopodite of A2 is reduced to a minor plate with one long and two small setae. The second endopodal segment with two dorsal and two ventral bristle setae. The Protopodite of MD bears a vibratory plate noted with four smooth rays. The third endopodite with distal Y plumose seta and four distal smooth claws. The first maxillary palp of ML with six (5+1) setae. The 2nd palp has four setae similar to claw. The first endite present three sub-distal setae and three distal smooth claws. The vibratory plate muscle exist 14 rays. The protopodite of L5 present (a) seta, which is small in size, while b and d setae are absent. The exopodite have four plumose rays. On the L6 seta dl missing, while d2 well-developed. The fourth podomere with terminal claw heavily built h2, dorsodistal seta h1, while seta h3 is tiny or absent. The protopodite of L7 with smooth setae d2 and dp, while seta d1 is absent. The Furca is reduced to a short triangular base, which noticed long terminal flagellum, and a short sub-apical seta in females, while absent in males (Fig.3).

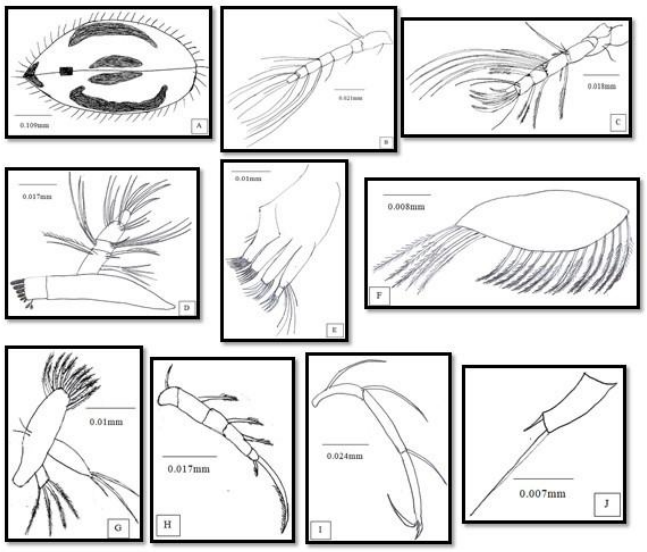

Figure 3: Lucida drawing of *Cypridopsis vidua* A-Whole amount, B- Antennula, C- Antenna, D- Mandibula, E- Maxillula, F- Branchial plate, G- First limb, H- second limb, I- Third limb, J- Furca

3.1.3- Candona neglecta

The Ca about 0.8 to 1.1 mm in length. The valves usually elongate, with a straight dorsal margin. The posterior part is more rounded than the anterior and bears a number of long cilia. Also, a series of septa appear at the ventral edge of the shell. The A1 is seven jointed. The second protopodite has a very small seta on the dorso-distal end, and terminal podomere bears three very long natatory and two shorter setae distally. The first endopodite of A2 bears a hairy seta at the ventral side, while at the dorsal end, there are five natatory setae, with a small setae centrally. About the Md, at the free end of coxa a series of six teeth are noted; below the last tooth there are bundles of little setae. The last endopodite bears three setae and three claws, two claws are long, and the other is smaller. The proximal maxillary segment of ML present six unfit setae on the outer apical edge. The third endite has four sub-apical, four smooth apical setae, and two strongly serrated Zahnborsten. The vibratory plate existing sixteen pilose setae. The protopodite of L5 bears two long smooth b and d setae. The exopodite plate is formed by a small process bearing six long plumose setae. The L6 is five segmented, the protopodite with small smooth d2 setae. The L7 Five jointed, the basal part with two setae (d1 and dp). The last

segment with two long and one small setae. The furca is rod like, long and narrow. The (right) ventral furca is denticulated at the ventral side (Fig. 4).

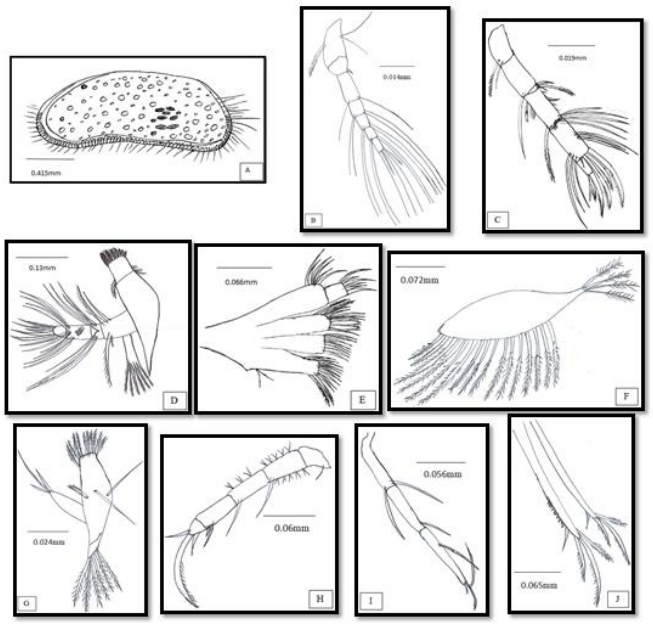

Figure 3: Lucida drawing of *Candona neglecta* A-Whole amount, B- Antennula, C- Antenna, D- Mandibula, E- Maxillula, F- Branchial plate, G- First limb, H- second limb, I- Third limb, J- Furca

3.2 Molecular analysis

Despite morphological confirmations, DNA barcoding was used to approve morphological identification. the blast result in the cytochrome oxidase subunit (COI) sequence of all species in GenBank designated 99% parallel to this species with accession numbers (PP140919, OR563930 and OR759087) for *Stenocypris hislopi, Cypridopsis vidua* and *Candona neglecta*) that illustrated in table (1).

Table (1): Percentage distribution of ostracod based on gene according to blast in GenBank of NCBI

Sample Accession Number	Identified	Query Cover %	Identic Number %	Genbank Accession Number	Country Identification
PP140919	Stenocypris hislopi	100	100	MH937424	India
OR563930	Cypridopsis vidua	100	100	OP912986	Portugal
OR759087	Candona neglecta	100	100	MN013113	Poland

4. Discussion

Generally, molecular studies of ostracoda species in Iraq, particularly in Erbil city has never been documented till we begin with first research in 2023. Despite of detect many taxonomical characteristics of ostracoda species, which include size, shape and appendages, while the accurate identification criteria was molecular analysis, that applied for each species. The utilization of sequence data obtained from the COI gene has become a widely recognized and extensively used method in various fields such as phylogeny, systematics, and species identification (Bashê and Al-Qassab, 2024)

The maximum likelihood sequence method was conducted on data from the Cytochrome Oxidase subunit I (partial COI) for species phylogenetic analyses. Molecular analyses of COI prove the existence of 3 species belonging to two families (Fig.5). in clade (A) *Stenocypris hislopi* has 100% resemblance with (MH937424) that recorded by (Laskar, 2018) in India. *S. hislopi* initially recorded as *Chrissia hislopi*. But, the study done by (Karuthapandi and Rao, 2016) clearly confirms that *S. hislopi* does exist and can be identified by the presence of septa around the carapace margin. *Cypridopsis vidua* in clade (B) has 100% similarity with (OP912986) recorded by (Gomes et al., 2023) in Portugal. In Iraq, *c. vidua* was documented for the first time by (Khalifa and Ajeel, 2022) in the East Al-Hammar marshes. They revealed that this species exhibits the ability to modest rise in salinity up to 8 ppt, and notably low tolerance for insufficient oxygen levels. Concerning *Candona neglecta* has a strong relation with (MN013113), which was recorded by (Wysocka et al., 2019) in Poland. This species mostly found in stations that have shallow water with muddy substrates, this result parallel with preceding study recorded by (Tunoğlu et al., 2012) from the Afşin-Elbistan Coal Basin of Turkey. As a final point, *bennelongia strellyensis* was utilized as an outgroup in Western Australia by (Martens et al., 2012). The aim of this research was to strengthen the taxonomic accuracy and enhance our comprehension of the phylogenetic connections between different ostracod species. To achieve this, molecular data was incorporated into the analysis.

Generally, there is no recorded molecular

data in NCBI on ostracoda in Iraq to compare the similarities and differences of sequences of our recorded samples. The current documented result became basis for future study in the country.

In this study, there is a presence of variability in the richness of species in the study sites, which are likely influenced by ecological factors such as geographical location, water temperature, pH, alkalinity, and dissolved oxygen levels. This finding aligns with previous studies conducted by (Parra and Espinoza-Villalobos, 2020, Szlauer-Łukaszewska and Pešić, 2020, Tan et al., 2021), who proposed that diverse ecological conditions and variations in calcification mechanisms contribute to the differential abundance of species. Furthermore, the study by Al-Seria (2018) corroborated these findings by demonstrating that the decrease in water temperatures and the concurrent decline in the abundance of other crustacean groups coincided with the expansion of an ostracod colony.

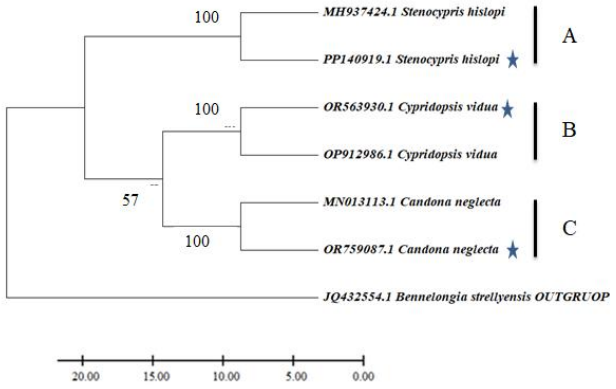

Figure 5: Maximum Likelihood (Kimura 2-parameter model) was applied to infer the evolutionary history, all nodes supported by high bootstrap

Conclusions

The morphological and molecular analysis of three newly discovered Ostracod species which are (*Stenocypris hislopi, Cypridopsis vidua* and *Candona neglecta*) from 17 different sites in boundary Erbil Province Kurdistan were studied. Applying phylogenetic relationships for all recorded species with other relatively closed species.

Acknowledgment: Molecular technique laboratory in Biology department, College of education-Salahaddin University in Erbil, Iraq, delivered support for the present study.

Financial support: No financial support.

Potential conflicts of interest. The authors declared no conflict of interest, it's part of the PhD thesis.

References

ALI, L. A. 2007. *A study of macroinvertebrates community in the Middle Sector of Greater Zab River/Iraq.* Ph.D. Thesis, , Univ. of Baghdad. Iraq.

BASHÊ, S. K. & AL-QASSAB, S. E. 2024. Morphological and Molecular Identification of Some Terrestrial Snails: First Report. *Zanco Journal of Pure and Applied Sciences* 36 (1) 94-104.

GOMES, N., DE ARAÚJO COSTA, D., MARTINS, D., ROCHA, S., SOUSA-PINTO, I. & ANTUNES, C. 2023. Insights into the fauna associated with Egeria densa at the upper section of the international Minho River estuary (NW Iberian Peninsula) 3 decades after its establishment. *Journal of Coastal Conservation,* 27**,** (36) 1-20.

KARANOVIC, I. 2012. Recent freshwater ostracods of the world: Crustacea, Ostracoda, Podocopida. In 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

KARUTHAPANDI, M. & RAO, D. 2016. FRESHWATER OSTRACODS (CRUSTACEA: OSTRACODA) OF TELANGANA, INDIA. *Records of the Zoological Survey of India,* 116 (3) 241-277.

KHALIFA, M. G. & AJEEL, S. G. 2022. New record of Cypridopsis vidua (Muller, 1776) (Ostracoda, Podocopida: Cyprididae) from East Al-Hammar Marshes, Iraq. *International Journal of Aquatic Biology,* 10(4) 299-302.

KUMAR, S., STECHER, G., LI, M., KNYAZ, C. & TAMURA, K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular biology and evolution,* 35(6) 1547-1549.

LASKAR, B. A., ADIMALLA, H., KARUTHAPANDI, M., JAISWAL, D. AND CHANDRA, K., 2018. Freshwater Ostracods of Eastern Ghats India. Freshwater Biology Regional Centre, Zoological Survey of India. *Plot 366/1 Hyderguda, Attapur, Hyderabad, Telangana 500048, India.*

LATEF, B. A. & ALI, L. A.-Q. 2023. Morphological and Molecular Characterization of Heterocypris incongruens (Ramdohr 1808), A New Species Ostracod in Kurdistan Region-Iraq. *Journal of Wildlife and Biodiversity, 7*(4), 36-48

MARTENS, K., HALSE, S. & SCHON, I. 2012. Nine new species of Bennelongia De Deckker & McKenzie, 1981 (Crustacea, Ostracoda) from Western Australia,

with the description of a new subfamily. *European Journal of Taxonomy,* 0 (8) 1-56.

MESQUITA-JOANES, F., AGUILAR-ALBEROLA, J., PALERO, F. & RUEDA, J. 2020. A new species of Cypris (Crustacea: Ostracoda) from the Iberian Peninsula and the Balearic Islands, with comments on the first ostracod named using the Linnean system. *Zootaxa,* 4759 (1) 113–131.

MOHAMMED, M., FRENZEL, P., KEYSER, D., HUSSAIN, F., ABOOD, A., SHA'AF, A., ALZARA'E, S. & ALAMMARI, S. 2018. A humid early Holocene in Yemen interpreted from palaeoecology and taxonomy of freshwater ostracods. *Journal of Micropaleontology*, 37(1) 167–180.

PHAM, H. T. M. & KARANOVIC, I. 2022. Four new Parasterope (Ostracoda, Myodocopina) from the Northwest Pacific and their phylogeny based on 16S rRNA. *ZooKeys,* 1095**,** 13-42.

RASOULI, H., AYGEN, C. & KÜLKÖYLÜOĞLU, O. 2014. Contribution to the Freshwater Ostracoda (Crustacea) Fauna of Turkey: Distribution and Ecological Notes. *Turkish Journal of Fisheries and Aquatic Sciences,* 14 (2) 11-20.

TRAN VAN, P., ANSELMETTI, Y., BAST, J., DUMAS, Z., GALTIER, N., JARON, K. S., MARTENS, K., PARKER, D. J., ROBINSON-RECHAVI, M., SCHWANDER, T., SIMION, P. & SCHÖN, I. 2021. First annotated draft genomes of nonmarine ostracods (Ostracoda, Crustacea) with different reproductive modes. *Journal of G3*, 11(4), jkab043.

TUNOĞLU, C., BESBELLI, B. & ERTEKIN, İ. 2012. Ostracoda (Crustacea) association and a new species (Dolerocypris anatolia nov. sp.) from the Pliocene-Pleistocene Afşin-Elbistan (Kahraman Maraş) Coal Basin of Turkey. *Geologica Carpathica,* 63 (2) 165-174.

WANG, Q., HORNE, D. J., FAN, J., WEN, R., SMITH, R. J., WANG, M. & ZHAI, D. 2022. Ilyocypris leptolinea Wang & Zhai, sp. nov., an ostracod (Ostracoda, Crustacea) from the late Quaternary of Inner Mongolia, northern China. *ZooKeys,* 1137**,** 109-132.

WYSOCKA, A., KILIKOWSKA, A., MORI, N., IEPURE, S., KIJOWSKA, M. & NAMIOTKO, T. 2019. Monophyletic status of European morphogenera of the subfamily Candoninae Kaufmann, 1900 (Ostracoda: Candonidae) in relation to their mtDNA phylogenies. *Journal of Crustacean Biology,* 39 (5) 567-573.

ZWAIR, H. 2023. Perspective Chapter: OstracodaFormation and Evolution of Earth's Crust . IntechOpen; 2023. Available from: http://dx.doi.org/10.5772/intechopen.112211.