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Integrating Molecular and Chemical Analyses for Assessing Blue Swimmer Crab Portunus Health and Genetic Diversity in Pakistani Coastal Waters

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SM designed and conducted experiments, and collected data. SJ conceived and designed the study. NHM analysed and interpreted the data. TSR contributed to the methodology and validation. FAA performed data analysis and interpretation. MI and AI contributed to the methodology and validation.

Key words
DNA barcoding, Haplotype, Nucleotide, Evolutionary, Cryptic varieties, Maritime

ABSTRACT

The blue swimming crab species *Portunus pelagicus* and *Portunus segnis* represent crucial components of commercial fisheries in the Northern Arabian Sea (NAS). This study employs partial coding regions of the cytochrome oxidase subunit 1 (*COI*) gene for DNA barcoding, with accession numbers OL840323 and OL840324 assigned *to Portunus pelagicus* and *Portunus segnis*, respectively, and deposited in the GenBank database. Analysis revealed high haplotype diversity and low nucleotide diversity within the populations of Portunus spp. Neutrality tests, specifically Tajima's D and Fu's F, yielded non-significant results. However, mismatch analysis indicated a potential population expansion event in the Arabian Sea. Evolutionary analyses were conducted comparing 21 sequences of *Portunus* spp. from GenBank, including 2 from Pakistan and 19 from various other regions, based on COI variation. Results from the analysis of molecular variance (AMOVA) suggested significant phylogeographic structuring ($P < 0.05$). The study highlights the efficiency of DNA barcoding in species identification, particularly in delineating cryptic varieties. Additionally, seasonal variations in the concentrations of ten trace elements, in carapace meat samples from 210 blue swimming crabs (*Portunus pelagicus* and *Portunus segnis*) collected from two locations in Karachi, Pakistan: West Wharf Fish Harbor (n = 100) and Korangi Creek (n = 110). Data collected during monsoon including zinc (Zn), iron (Fe), copper (Cu), cobalt (Co), chromium (Cr) as essential trace elements revealed the following order of essential trace elements: Fe > Zn > Cu > Co > Cr. In contrast, toxic trace elements such as aluminum (Al), lead (Pb), mercury (Hg), arsenic (As), and cadmium (Cd) were found in the order: Pb > Cd > Al > As > Hg and in non-monsoon period essential and toxic elements were found in the order: Fe>Zn>Cu>Cr>Co and As>Pb>Cd>Al>Hg. Metal concentrations were assessed using Atomic Absorption Spectroscopy (AAS), which was chosen for its sensitivity and accuracy in quantifying trace elements. The presence of elevated levels of essential trace elements (Fe, Zn, and Cu) in the aquatic environment is attributed to industrial and maritime activities in the Arabian Sea. Understanding the dynamics of these populations and their trace element uptake is significant for conservation, fishery management, and public health.

INTRODUCTION

The blue swimmer crab, *Portunus pelagicus* (Linnaeus, 1758), also known as the flower crab, is a significant species in the commercial fisheries sector, valued for its economic and ecological contributions. Found predominantly in the coastal regions of the Indo-Pacific, *P. pelagicus, and P. segnis* both as a predator and as a prey, are vital to the benthic environment (Maryani *et al*., 2023). This species is widely distributed along the coastal waters

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of Pakistan, particularly in the Karachi region, where it supports local fisheries and contributes to the livelihoods of coastal communities (Siddiqui *et al*., 2008).

Global production exceeded 200,000 tons in 2013 (Noori *et al*., 2015). These crabs hold both commercial and ecological importance across various regions, including the Northwestern Indian Ocean (NWIO) (FAO, 2016). The blue swimming crab, comprising species such as *Portunus pelagicus* (Linnaeus, 1758) and *Portunus sanguinolentus* (Herbst, 1783), among others, is particularly noteworthy. More than 200 crab species, including commercially significant ones like *Portunus pelagicus*, *Charybdis feriata*, *Portunus sanguinolentus*, and *Scylla serrata*, have been reported in Pakistani waters (Kazmi, 2003), making them suitable for both international trade and local consumption (FAO, 2016).

Traditional taxonomic identification of the blue swimmer crab relies on morphological characteristics such as carapace shape, size, and coloration patterns, which can be variable and subject to misinterpretation, especially among juvenile or geographically distinct populations. This variability underscores the need for robust molecular tools to complement traditional taxonomy and accurately delineate species boundaries. The advent of DNA barcoding, a molecular technique based on sequencing short, standardized gene regions, has revolutionized species identification by providing a reliable method for discriminating between closely related taxa (Saher *et al*., 2019). By targeting highly conserved regions of the genome, such as mitochondrial DNA, DNA barcoding offers a rapid and cost-effective means of species identification that transcends morphological variation (Joesidawati *et al*., 2023).

In the case of the blue swimmer crab, DNA barcoding presents an opportunity to overcome taxonomic challenges associated with morphological variability and cryptic speciation. By targeting specific regions of the genome unique to *P. pelagicus*, such as the cytochrome c oxidase subunit I (*COI*) gene, researchers can accurately distinguish it from closely related congeners and facilitate the identification of mislabeled or adulterated seafood products in the market. In recent years, several studies have highlighted the efficacy of DNA barcoding in species identification and biodiversity assessment (Meier *et al*., 2006). These studies have demonstrated the utility of DNA barcoding across diverse taxa, including crustaceans, and underscore its potential for enhancing our understanding of species diversity and distribution patterns.

In addition to genetic factors, heavy metal contamination in marine crabs is an emerging concern with significant implications for public health. Heavy metals, such as mercury, lead, cadmium, and arsenic, can accumulate in marine organisms, entering the food chain and posing serious health risks to humans who consume contaminated seafood. Long-term exposure to these metals has been linked to various health issues, including neurological disorders, kidney damage, and increased risk of cancers (Doi and Minegishi, 2020; Huo *et al*., 2016). The concentrations of these contaminants in edible species like *P. pelagicus* and *P. segnis* can vary significantly based on environmental factors and geographical location, rendering regular monitoring and risk assessments crucial (Zhou *et al*., 2022). By integrating genetic and environmental assessments, this study aims to provide comprehensive insights into the health and sustainability of *Portunus pelagicus* and *P. segnis* populations, contributing to better fisheries management practices while safeguarding consumer health. Anthropogenic activities such as industrial discharge, mining operations, and agricultural runoff contribute to the release of these metals into marine ecosystems, where they can accumulate in aquatic organisms, posing risks to human health and marine biodiversity. The assessment of heavy metal concentrations in marine organisms is therefore crucial for understanding environmental contamination levels and evaluating potential risks to human consumers (Shi *et al*., 2019). Additionally, molecular approaches, such as DNA barcoding using partial mitochondrial cytochrome c oxidase subunit I (*COI*) gene sequences, offer complementary insights into species identification, genetic diversity, and population structure of *Portunus* spp. (Lai *et al*., 2010). This study presents a comprehensive assessment of heavy metal concentrations and partial *COI* gene sequences of *Portunus* spp., focusing on the blue swimmer crab (*P. pelagicus*) and closely related congeners. By combining chemical analyses of heavy metal levels with molecular data, we aim to elucidate patterns of metal accumulation, assess potential health risks associated with crab consumption, and enhance our understanding of evolutionary linkages and genetic diversity within the genus *Portunus*. Through the integration of chemical and molecular approaches, this research aims to provide insightful information about the ecological health of coastal ecosystems, the sustainability of seafood resources, and the genetic diversity of swimming crabs. Such insights are essential for informing management and conservation strategies aimed at preserving marine biodiversity and safeguarding human health. This study represents a significant contribution to the fields of environmental toxicology, molecular ecology, and seafood safety, with implications for both scientific research and policymaking in the realm of marine conservation and public health.

MATERIALS AND METHODS

Collection of samples and morphological analysis

Samples comprising 210 fresh crabs and tin-packed crab meat were collected from the local fisheries market at West Wharf Fish Harbor (24.8536° N, 66.9836° E) and a seafood processing plant at Korangi Fish Harbor (24.8844° N, 67.1443° E). Upon collection, specimens underwent morphological assessment and were promptly stored in ice before transfer to the Aquatic Diagnostics and Research Centre in Karachi, Pakistan. Species identification was conducted up to the possible species level using morphological traits (Jirapunpipat *et al*., 2008). The first walking leg was carefully removed, sliced, and immediately preserved in 99% ethanol for subsequent DNA analysis. Species were identified based on external morphology, followed by DNA extraction for analyzing the *COI* gene barcode region in each specimen (Lu *et al*., 2022).

Extraction of DNA

The genomic DNA extracted from crab muscles was isolated using the Bio-Basic DNA isolation kit BS-88504. The extracted DNA was resuspended in 80 µL of T.E buffer, and its concentration was spectrophotometrically estimated before being stored in the freezer until further use. To eliminate protein contaminants, 2 µL of RNase with 50 µL of TE buffer was added to the sample, followed by incubation in a water bath for 2 h at 37°C. DNA quantification was performed using both gel electrophoresis and the spectrophotometric method (Sambrook and Russell, 2001).

PCR amplification

Dilutions were adjusted to a standard 1:20 ratio to achieve a working concentration of 15 ng DNA/µL. PCR amplification of the partial coding regions of the cytochrome oxidase subunit 1 (*COI*) gene was conducted using the primer set LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'), as described by Folmer *et al*. (1994). The PCR reaction mixture (total volume 25 µL) consisted of 0.5 units of GoTaq DNA polymerase (Promega), 1.2 mM $MgCl_2$, 0.3 µM of each primer, 200 µM of each dNTP, and 15 ng of genomic DNA. PCR amplification was carried out using a temperature gradient thermocycler. The PCR conditions comprised an initial denaturation step at 95°C for 2 min, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 42°C for 1 min, and extension at 72°C for 2 min. A final elongation step at 72°C for 7 min concluded the PCR program. The amplified products were visualized by electrophoresis in 1x TBE buffer stained with SYBR green on a 1.5% agarose gel at 60 mA. Negative controls were included in each amplification assay, and a 1 kb molecular weight marker (Nippon Genetics) served as a reference for band size.

DNA sequencing and analysis

PCR products from each sample were purified using the FastGene Gel/PCR extraction kit (Biobasic). The purified DNA fragments were then submitted for sequencing to Macrogen Company (Seoul, Korea). DNA sequence data were analyzed using applied biosystems sequence scanner v1.0 software (SPSS, Chicago, IL). Sequence results were utilized for species identification, initially searching for sequence similarity via the NCBI BLAST (basic local alignment search tool) website (www.ncbi.nlm.nih.gov/BLAST). Sequences were aligned using the Clustal W tool in MEGA X (MEGA Inc., Ocheyedan, IA) and submitted to GenBank for accession numbers.

Heavy metal analysis

The carapace meat samples from tin-packed blue swimming crabs were transported in dry ice to the Centralized Science Laboratories at the University of Karachi (UoK). Eight grams (dry weight) of meat samples were precisely weighed and transferred to digestion tubes. To aid the digestion, 5 mL of concentrated nitric acid and 5 mL of concentrated sulfuric acid were applied to the sample. The mixture was heated on a hot plate at 60°C for 30 min or until it approached near dryness. After cooling, additional nitric acid was added up to 10 mL to oxidize any remaining organic matter. The gradual addition of nitric acid continued until effervescence ceased, and the digestion was returned to the hot plate for an additional hour.

Following cooling, a small quantity of deionized water was added to increase the volume to approximately 20 mL. The digests were then filtered using Whatman 41 filter paper into 30 mL volumetric flasks and made up to volume with deionized water.

Metal estimation of aluminum (Al), chromium (Cr), zinc (Zn), copper (Cu), lead (Pb), cadmium (Cd), arsenic (As), mercury (Hg), cobalt (Co), and iron (Fe) in the digests were performed using an Atomic Absorption Spectrometer (AAS) 3100 by Perkin Elmer (USA), model Analyst 700. The analyses were conducted at least in triplicate, and all concentrations are expressed in milligrams of element per hundred grams of fresh mass (mg/100g).

RESULTS AND DISCUSSION

The findings of this study underscore the complex interplay between genetic diversity and environmental factors influencing the health of blue swimmer crab populations. The significant genetic variability found

among *P. pelagicus* populations in China (Jiang *et al*., 2021) reinforces the notion that maintaining genetic diversity is critical for the resilience of species in the face of environmental changes. Lai *et al*. (2023) further elucidated how environmental conditions affect genetic structures, suggesting that fisheries management should consider local environmental dynamics to enhance the sustainability of crab populations.

Additionally, the rising concern over heavy metal contamination in marine crabs, as discussed by Doi and Minegishi (2020) and highlighted by Zhou *et al*. (2022) presents a significant challenge for both ecological health and food safety. The accumulation of metals such as mercury and cadmium in *P. pelagicus* and *P. segnis* raises alarms regarding the potential health risks for consumers, necessitating stricter regulatory frameworks and monitoring programs to mitigate these risks and protect public health.

Furthermore, the interplay between genetic health and environmental stressors, including pollution, indicates that effective management strategies must be multi-faceted. By drawing on recent findings regarding genetic assessments and contamination levels, stakeholders can develop targeted conservation efforts that not only preserve genetic diversity but also safeguard the health of marine ecosystems and human populations.

Genetic diversity and haplotype networks

COI mtDNA sequences were aligned using Clustal-X, and their alignment quality was verified through correct amino acid translation. Nucleotide diversity (π) and haplotype diversity (h) were calculated for the entire population and individual sampling locations. The analysis involved 21 nucleotide sequences, resulting in a final dataset of 687 positions. For *Portunus segnis*, the sequence length was 621 bp with a nucleotide composition of A=25.1%, C=21.3%, G=18.2%, and T=35.4%. For *Portunus. pelagicus*, the sequence length was 676 bp with a nucleotide composition of A=26.5%, C=20.6%, G=17.5%, and T=35.5%. Pairwise genetic distances among Egypt's five crab species sampled showed the highest value (0.038) between *L. corrugatus* and *P. pelagicus*, and the lowest (0.148) between *C. hellerii* and *C. natator* (Table I).

A median-joining haplotypic network was created to illustrate the genetic relationships among the sequenced crab samples and those retrieved from GenBank. The disparity index per site for each sequence pair showed an overall average of 0.022, indicating significant differences in base composition biases, with P-values smaller than 0.05 considered significant. The nucleotide diversity (π) was 0.151. All analyses were conducted using MEGA11.

Table I. Nucleotide frequencies of both crab species in (%).

***Portunus* spp.**	**T**	**C**	**A**	**G**	**Total**
Portunus segnis	35.4	21.3	25.1	18.2	621
Portunus pelagicus	35.5	20.6	26.5	17.5	676

Base substitution analysis

The number of base substitutions per site between sequences is presented, with standard error estimates shown above the diagonal, obtained through a bootstrap procedure with 300 replicates. Analyses were conducted using the Tajima-Nei model, with the rate variation among sites modeled with a gamma distribution (shape parameter=1). The analysis included 21 nucleotide sequences, and all ambiguous positions were removed for each sequence pair using the pairwise deletion option. The final dataset comprised 687 positions. Results from Tajima's Neutrality Test are detailed in (Table II). The number of base substitutions per site between sequences is shown. Standard error estimates are shown above the diagonal and were obtained through a bootstrap procedure (300 replicates). Analyses were conducted using the Tajima-Nei model (Tajima and Nei, 1984). The rate variation among sites was modeled with a gamma distribution (shape parameter =1). This analysis involved 21 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 687 positions in the final dataset Evolutionary analyses were conducted in MEGA11 (Tamura *et al*., 2021) (Supplemenaty Table I).

Table II. Results from Tajima's neutrality test.

Number of sequences (m)	21
Number of segregating sites (S)	255
Ps (S/n)	0.371179
Θ (Ps/a1)	0.103170
Nucleotide diversity (π)	0.151237
Tajima test statistic *(D)*	1.921004

m, number of sequences; *n,* total number of sites; *S,* Number of segregating sites; p_s, *S/n*; Θ, p_s/a_1; π, nucleotide diversity, and *D* is the Tajima test statistic.

Phylogenetic analysis

A phylogenetic tree was constructed using maximum parsimony for 21 species of Portunus, with sequences from GenBank serving as references. The phylogenetic tree, supported by 250 bootstrap replicates, forms a well-supported clade (Fig. 1). The analysis included *P. segnis* and *P. pelagicus* (OL840323 and OL840324) along with sequences

Table III. Essential and toxic trace elements content (mg/100g) in the meat of *Portunus pelagicus* and *Portunus segnis* crabs (mg/100g) in non-monsoon and in monsoon BDL Below the Detection Limit < 0.00000001 mg/100g.

Species and crabs samples mean values	Essential trace elements					Toxic trace elements				
	Iron	Chromium	Zinc	Copper	Cobalt	Cadmium	Mercury	Arsenic	Lead	Aluminum
Non-monsoon										
Portunus pelagicus	446.7	0.057	3.63	1.21	0.612	0.103	0.0000000255	0.011	0.013	0.222
Portunus segnis	573.2	0.015	7.61	1.16	0.904	0.024	0.0000000314	0.065	0.139	0.0834
Mean	509.5	0.036	5.62	1.185	0.758	0.0635	0.0000000284	0.038	0.076	0.0528
Monsoon										
Portunus pelagicus	328.7	0.957	2.63	1.62	0.612	0.245	BDL	0.589	0.071	0.101
Portunus segnis	127.8	0.715	9.61	1.87	0.304	0.098	BDL	2.647	0.436	0.0971
Mean	456.5	0.836	6.12	1.745	0.458	0.171	BDL	1.618	0.253	0.086

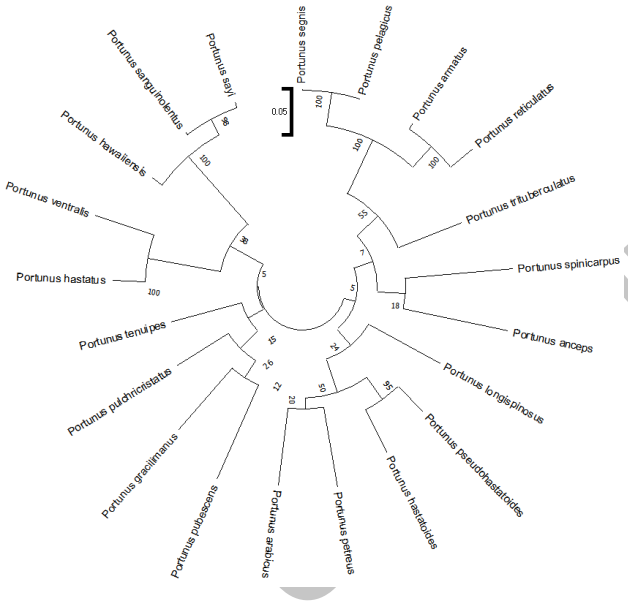

Fig. 1. Bootstrapping for phylogenetic tree construction, phylogenetic relationship among species of *Portunus* from the different parts of the world with *Portunus segnis* and *Portunus pelagicus* exported from Pakistan based on the sequence of the COI region by maximum likelihood (ML) method.

GU321237, MZ393893, MZ393892, MZ393886, OL588010, MW264449, MW277922, KT365746, KT365745, KT365747, KT365742, KT365743, KT365737, KT365736, KT365738, JX398098, JX398099, and JX398092 from GenBank, based on the COI region. Using the maximum likelihood method and the general time reversible model, the tree with the highest log likelihood (-4948.76) was selected. The tree's branches indicate the percentage of trees in which the associated taxa clustered together. Initial trees for the heuristic search were obtained using Neighbor-Join and BioNJ algorithms applied to a pairwise distance matrix estimated with the maximum composite likelihood (MCL) approach, selecting the topology with the superior log likelihood value. Branch lengths are measured in substitutions per site. The analysis, involving 21 nucleotide sequences and a total of 687 positions, showed two major clades. One clade grouped *P. pelagicus*, *P. segnis*, *P. armatus* and *P. reticulatus* with all representatives of the family Portunidae. Evolutionary analyses were conducted in MEGA11. The evolutionary history among OL840324, OL840323, KY587391, KY587767, KY695086, KY587390, KY695087, KY587389, KY587772, KY587387, MF002106, and KY587386 was concluded by using the Maximum Likelihood method and the Kimura 2-parameter model. The bootstrap consensus tree, constructed from 100 replicates, represents the evolutionary history of the taxa analyzed, with branches having less than 50% of bootstrap support being collapsed. The final dataset comprised 768 positions, and evolutionary analyses were conducted in MEGA11 (Fig. 2).

Trace elements

The study found that various factors, including season, length, weight, and the physical and chemical status of water, influence metal accumulation in tissues. (Zaynab *et al*., 2022). The mean carapace width (CW), mean carapace length (CL), and weights of the species examined were similar for *P. segnis* and *P. pelagicus* ($p > 0.05$). During the monsoon season, higher concentrations of copper, lead, cadmium, and aluminum were detected, followed by iron, zinc, copper, cobalt, chromium, and mercury. The essential trace elements were ordered as Fe > Zn > Cu > Co > Cr, while the toxic trace elements were ordered as Pb>Cd>Al>As > Hg (Table III).

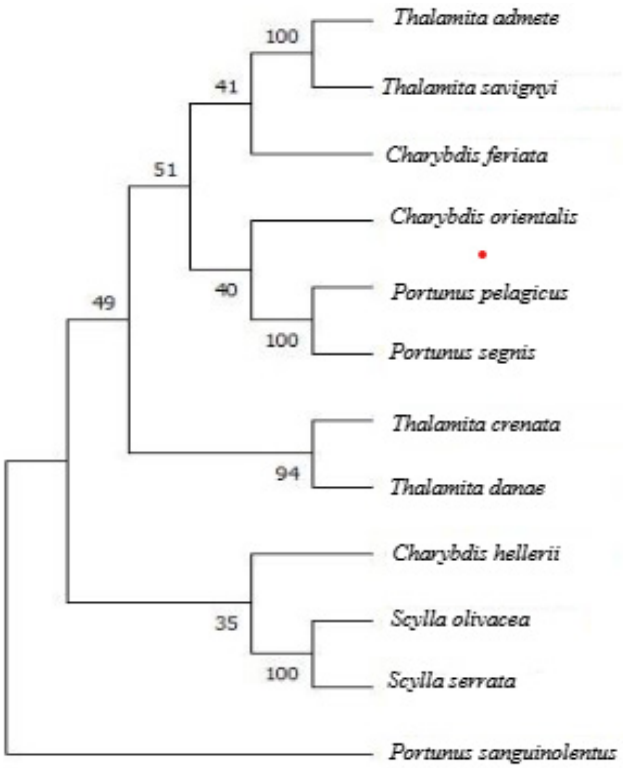

Fig. 2. Bootstrapping for phylogenetic tree construction, Phylogenetic relationship among species of *Portunus*, *Charybdis*, *thalamita*, and *scylla* exported from Pakistan based on the sequence of the COI region by maximum likelihood (ML) method.

In the non-monsoon season, higher amounts of arsenic, lead, cadmium, and chromium were detected compared to the monsoon season. The essential trace elements were ordered as Fe>Zn>Cu>Cr>Co and the toxic trace elements were ordered as As>Pb>Cd>Al>Hg (Table III). The blue swimming crab accumulates heavy metals such as iron, zinc, lead, and cadmium throughout the year, with significant increases in arsenic during the non-monsoon season and lead during the monsoon season. These concentrations were higher than the permissible limits recommended by FAO (1998), posing health risks.

The accumulation of trace elements is influenced by factors such as salinity, pH, metal bioavailability, tissue composition, pollution load, and environmental hydrodynamics. Essential trace elements like iron, copper, zinc, cobalt, and chromium are vital for human physiological and biochemical processes, and crab meat is a good source of these minerals. However, values varied significantly among crab species due to factors like seasonal variation, species, age, sex, environmental conditions, and feeding patterns. Previous studies found that copper concentrations were higher than lead, cadmium, and chromium in *P. pelagicus* and *P. segnis* muscle tissues during the monsoon season. The study concluded that both *P. pelagicus* and *Portunus segnis* tend to accumulate heavy metals, with increased industrialization leading to marine pollution, disrupting the food chain, and adversely affecting human health.

The genetic diversity and evolutionary relationships of the blue swimming crabs, *Portunus segnis* and *Portunus pelagicus*, were analyzed using COI mtDNA sequences. *P. segnis* exhibited a sequence length of 621 bp with nucleotide frequencies of A=25.1%, C=21.3%, G=18.2%, and T=35.4%, while P. *pelagicus* had a sequence length of 676 bp with nucleotide frequencies of A=26.5%, C=20.6%, G=17.5%, and T=35.5%. The genetic distances between the crab species sampled in Egypt showed the highest value (0.038) between *L. corrugatus* and *P. pelagicus,* and the lowest (0.148) between *C. hellerii* and *C. natator*. Median-joining haplotype networks (MJN) visualized the evolutionary connections among the crab samples, revealing moderate genetic variation within the populations ($\pi = 0.151$) (Tamura *et al*., 2021). Disparity Index analysis indicated significant base composition biases among the sequences ($P < 0.05$). The significant genetic variability found among *Portunus* populations aligns with the work of Akin and Ozturk (2023) utilized *COI* gene sequences for molecular identification and phylogenetic characterization of this species. Their research underscores the utility of molecular markers in delineating species and understanding evolutionary relationships within the *Portunidae* family, which is essential for informed conservation strategies.

Phylogenetic relationships among 21 *Portunus* species were inferred using maximum likelihood and maximum parsimony methods. The phylogenetic tree constructed from COI sequences revealed two major clades within the Portunidae family, with strong bootstrap support (250 replicates). This analysis incorporated both newly obtained sequences and reference sequences from GenBank, demonstrating clear genetic distinctions among *P. pelagicus*, *P. segnis*, *P. armatus* and *P. reticulatus* (Tamura *et al*., 2021; Kimura, 1980). Trace element analysis of *P. segnis* and *P. pelagicus* showed that these species accumulate various metals in their tissues. Higher concentrations of iron, zinc, copper, chromium, and cobalt as essential and toxic trace elements arsenic, lead, cadmium, and aluminum were detected during the non-monsoon season.

While Fe>Zn>Cu>Co>Cr are essential trace elements and lead, cadmium, aluminum, and arsenic were more prevalent during the monsoon season as toxic elements. Seasonal variations significantly affected arsenic, lead, cadmium, aluminum, and cobalt concentrations.

However, the levels of these elements exceeded FAO (1998) permissible limits, posing potential health risks. Environmental factors such as salinity, pH, metal bioavailability, and pollution influence trace element accumulation in crabs. Industrialization and marine pollution contribute to heavy metal bioaccumulation in marine organisms, disrupting the food chain and impacting human health (Blewett and Wood, 2015). *P. pelagicus* and *P. segnis* demonstrated a considerable capacity to accumulate heavy metals, indicating the need for regular monitoring to mitigate health risks associated with their consumption (Monastero *et al*., 2017). These findings underscore the importance of understanding genetic diversity, evolutionary relationships, and environmental impacts on marine species to ensure sustainable management and public health safety.

CONCLUSIONS

Our study investigates the genetic diversity, phylogeography, and trace metal accumulation in *Portunus pelagicus* and *Portunus segnis* crabs in the Northern Arabian Sea. Using the cytochrome oxidase subunit 1 (*COI*) gene for DNA barcoding, we found significant haplotype diversity and low nucleotide diversity, suggesting potential cryptic diversity. Population expansion within the Arabian Sea was indicated by neutrality tests and mismatch analysis, showing dynamic evolutionary processes. Phylogenetic analyses revealed significant phylogeographic structuring among *Portunus* spp., highlighting the importance of considering spatial genetic variation in conservation and management. Combining genetic and morphological data allowed for accurate species identification despite morphological variability. We also found seasonal fluctuations in heavy metal concentrations in crab tissues, with levels exceeding FAO (1998) limits, posing health risks. Our research emphasizes the need for environmental monitoring and regulatory measures to mitigate pollution. This study enhances our understanding of crab populations' ecological dynamics and genetic diversity in the Northern Arabian Sea, supporting integrated approaches in molecular genetics, environmental science, and fisheries management to preserve marine biodiversity and protect human health.

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Supplementary material

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Statement of conflict of interest

The authors have declared no conflict of interest.

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Supplementary Material

Integrating Molecular and Chemical Analyses for Assessing Blue Swimmer Crab Portunus Health and Genetic Diversity in Pakistani Coastal Waters

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Supplementary Table I. Estimates of evolutionary divergence between sequences.

Portunus trituberculatus	***Portunus trituber-culatus***	***Por-tunus segnis***	***Portunus pelagi-cus***	***Por-tunus pulchric-ristatus***	***Portunus pseudo-hasta-toides***	***Por-tunus pubes-cens***	***Portunus sanguino-lentus***	***Por-tunus hastatus***	***Portunus ha-waiiensis***	***Por-tunus armatus***	***Por-tunus sayi***	***Por-tunus ventralis***	***Portunus longispi-nosus***	***Por-tunus petreus***	***Por-tunus arabicus***	***Por-tunus anceps***	***Por-tunus reticula-tus***	***Por-tunus hasta-toides***	***Por-tunus tenuipes***	***Por-tunus gracili-manus***
Portunus segnis	0.18577																			
Portunus pelagicus	0.18309	0.00161																		
Portunus pulchricristatus	0.20952	0.22544	0.22561																	
Portunus pseudohastatoides	0.18979	0.22827	0.22827	0.18984																
Portunus pubescens	0.21162	0.25033	0.24372	0.19674	0.19434															
Portunus sanguinolentus	0.24312	0.22837	0.22838	0.24612	0.23723	0.23677														
Portunus hastatus	0.25379	0.25616	0.26080	0.21927	0.23763	0.23978	0.22240													
Portunus hawaiiensis	0.22208	0.22528	0.22279	0.22997	0.22475	0.23210	0.04066	0.21654												
Portunus armatus	0.17536	0.07062	0.07201	0.21193	0.22480	0.24010	0.23419	0.28197	0.20673											
Portunus spinicarpus	0.22002	0.25659	0.25281	0.23053	0.22011	0.25982	0.29286	0.25700	0.27730	0.24915										
Portunus sayi	0.23278	0.22236	0.22043	0.23283	0.23549	0.23500	0.01019	0.21934	0.04014	0.22481	0.27701									
Portunus ventralis	0.26273	0.27108	0.27790	0.23545	0.25172	0.28808	0.27640	0.11026	0.25976	0.28557	0.25432	0.25123								
Portunus longispinosus	0.23097	0.22029	0.21857	0.20026	0.19537	0.26324	0.28709	0.25755	0.25517	0.23081	0.21529	0.26327	0.21749							
Portunus petreus	0.23030	0.24485	0.24716	0.23036	0.15732	0.24051	0.26094	0.27088	0.25438	0.24349	0.22240	0.24594	0.27383	0.21004						
Portunus arabicus	0.21739	0.24500	0.24183	0.22784	0.14625	0.22997	0.26734	0.25972	0.25733	0.23028	0.25747	0.24881	0.23529	0.17596	0.18265					
Portunus anceps	0.21189	0.27298	0.26603	0.23681	0.27552	0.26269	0.31715	0.24492	0.29063	0.26599	0.25117	0.29991	0.25078	0.23158	0.23663	0.28142				
Portunus reticulatus	0.20570	0.09936	0.09702	0.23983	0.22094	0.27956	0.26929	0.31921	0.25284	0.03835	0.28342	0.27275	0.31922	0.25006	0.25261	0.24623	0.32659			
Portunus hastatoides	0.23280	0.24260	0.23927	0.18267	0.09430	0.20679	0.23896	0.21926	0.24592	0.22608	0.25628	0.23566	0.25931	0.19803	0.19158	0.19159	0.29148	0.23122		
Portunus tenuipes	0.23844	0.25181	0.24839	0.15533	0.21228	0.21510	0.23128	0.24800	0.24830	0.25877	0.23154	0.22800	0.26901	0.17566	0.19034	0.22826	0.22788	0.28642	0.21197	
Portunus gracilimanus	0.19987	0.23853	0.24192	0.15828	0.20618	0.21533	0.26945	0.26218	0.27337	0.24534	0.21237	0.26591	0.26583	0.22242	0.19666	0.21552	0.28745	0.27222	0.21861	0.19841