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Molecular barcoding of the Persian Gulf mangrove associated brachyuran crabs

Sana Sharifian[1], Ehsan Kamrani[2], Maria A. Nilsson[3,4], Hanieh Saeedi[5]

1 *Persian Gulf and Oman Sea Ecological Research Center, Iranian Fisheries Sciences Research Institute, Agricultural Research Education and Extension Organization (AREEO), Bandar Abbas, Hormozgan, Iran*

2 *Fishery Department, University of Hormozgan, Bandar Abbas, Iran*

3 *Senckenberg Research Institute and Natural History Museum, LOEWE-Centre for Translational Biodiversity Genomics, 60325 Frankfurt am Main, Germany*

4 *Senckenberg Research Institute and Natural History Museum, Senckenberg Biodiversity and Climate Research Centre (BikF), 60325 Frankfurt am Main, Germany*

5 *Senckenberg Research Institute and Natural History Museum, Department of Marine Zoology, 60325 Frankfurt am Main, Germany*

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Corresponding author: Sana Sharifian (sharifian_sana@yahoo.com)

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Abstract

Brachyuran crabs constitute the dominant fauna in intertidal and supratidal coasts of mangrove forests. We sampled the most commonly occurring crab species from the biodiversity rich Persian Gulf mangrove forest. We identified crabs from Camptandriidae, Dotillidae, Ocypodidae, Macrophthalmidae, and Sesarmidae as the most common species in the sampled regions. Molecular barcoding was applied to determine 11 species (29 specimens) of the mangrove crabs. Two mitochondrial genes were used to barcode the specimens and these were included in a larger phylogenetic data set. Many of the analyzed species showed a close phylogenetic relationship with species from the Northern Arabian Sea. The results provide the first steps to study the genetic diversity of the mangrove crab community along the Iranian coasts to support protection and management of mangrove ecosystems and its associated taxa.

Key words

Crustacea, Camptandriidae, Dotillidae, Ocypodidae, Macrophthalmidae, Sesarmidae, 16S, COI, DNA barcoding, Persian Gulf.

1. Introduction

Mangroves are inter-tidal ecosystems unique to the tropics and subtropics, which are habitat to many brackish organisms and have a high genetic diversity (Nagelkerken et al. 2008; Saeedi et al. 2018; Sharifian et al. 2021c, 2020, 2017; Walters et al. 2008). The mangrove ecosystem is one of the most important areas in terms of ecological and economic importance in protected areas, rich in swamps which control the tidal hydrodynamics, and in the production of fishery resources (Kathiresan and Bingham 2001, Nagelkerken et al. 2008, Zahed et al. 2010). Brachyuran crabs are one of the most dominant fauna in many mangrove forests in the Indo-Pacific

area, making up about 80% of the macrofaunal biomass and can reach densities of 80-90 individuals m^2 (Nagelkerken et al. 2008, Soundarapandian et al. 2008, Davie et al. 2015b, 2015a, Ngo-Massou et al. 2018). In the Persian Gulf, the burrowing crabs *Ocypode* Weber, 1795 and *Uca* Leach, 1814 were reported as the most abundant crabs in the mangrove coastal zone. The macrofaunal community of Persian Gulf mangroves showed temporal and spatial heterogeneity in species diversity and composition with higher taxonomic diversity at summer in the deltaic zone and vegetated habitats than the coastal zone and mudflats (Hajializadeh et al. 2020, 2022, Vahidi et al. 2020, Nozarpour et al. 2023), as well as differences in the distribution of abundant species and biological traits across all habitats of the Persian Gulf (Hajializadeh et al. 2020). In the Persian Gulf mangroves, macrofaunal communities were characterized by low diversity and low functional redundancy indicating low trait modalities such as morphological, physiological and behavioral characteristics (Delfan et al. 2021). Most of the mangrove crabs are directly dependent on mangrove ecosystems for their survival, shelter, and nutritional support (Sharifian et al. 2017, Ngo-Massou et al. 2018, Saeedi et al. 2018, Sharifian et al. 2021c). They play a significant role in formation of detritus, nutrient restoration, and dynamism of the mangrove ecosystem along with other organisms (Nagelkerken et al. 2008, Soundarapandian et al. 2008, Ngo-Massou et al. 2018).

Establishing a comprehensive list of the aquatic species present in mangroves and estuaries is a critical step for the development of marine conservation programs. Unfortunately, data on the distribution and diversity of aquatic crab communities in mangrove forests are too scarce to provide basic knowledge for fisheries and conservation plans of the Persian Gulf's mangrove ecosystems (Naderloo 2017). The Persian Gulf has diverse and heterogeneous marine habitats with remarkably high biodiversity, especially in terms of brachyuran crabs (Naderloo, 2017; Naderloo et al., 2011). During our sampling in the northern Persian Gulf, we found mostly grapsoid and ocypodoid crabs as the most abundant. Hajializadeh et al. (2022) found *Opusia indica* Alcock, 1900, *Manningis arabicum* Jones & Clayton, 1983, and *Nasima dotilliformis* Alcock, 1900 as the dominant species in un-vegetated habitats and *O. indica*, *M. arabicum*, and *Parasesarma persicum* Naderloo & Schubart, 2010 as the dominant species in vegetated habitats of Persian Gulf mangroves. The highest species richness of grapsoid and ocypodoid crabs is along the Indo-West Pacific (Sharifian et al. 2020). Approximately 13.5% of the species richness in the Indian and western Pacific Ocean is contained in The Persian Gulf (Sharifian et al. 2020). Our previous studies indicated that most of the dominant crab species are endemic to the Persian Gulf and the Sea of Oman, and some of these mangrove crabs are vulnerable to habitat loss in future (Sharifian et al. 2021b). Grapsoid and ocypodoid crabs of the Persian Gulf have many commonalities in terms of environment and morphology (Sharifian et al. 2020, 2021b). This emphasizes the importance to systematically classify the Persian Gulf's grapsoid and ocypodoid crabs using molecular methods.

Mangrove forests are a fragmented habitat, where species occur as discrete local populations connected by the passive and active migration of individuals (Dibacco et al. 2006, Ragionieri et al. 2010). The Iranian mangrove forests cover an estimated 93.37 km^2 of shorelines (Zahed, 2010), despite this only two species of mangrove *Avicennia marina* Forssk. Vierh and *Rhizophora mucronata* Poir. are found in the Persian Gulf mangroves. These mangroves are known as one the most biodiverse marine ecosystems (Zahed et al. 2010). The mangrove forests of the Persian Gulf and Oman Sea have exhibited an increase in surface area from 64.12 to 197.46 km^2 during 1977 to 2017 (Salehipour Milani 2018). Along the Iranian coasts, changes of mangrove area from 47.35 km^2 in 1977 to 94.03 km^2 in 2017 were reported (Salehipour Milani 2018). Naderloo (2017) reported a total of 37 families, 150 genera and almost 256 species of brachyuran crabs from the Persian Gulf of which about 30 species inhabit mangrove forest of this region. Mangrove crabs are known to have high larval dispersal potential and gene flow among geographically separated populations (Laurenzano et al. 2013, Fratini et al. 2016, Sharifian et al. 2020, 2021a). Molecular barcoding data, in particular, 16S and COI mitochondrial DNA gene regions (mtDNA) have been found to be useful to address the unresolved issues related to the population genetics and ecology of mangrove crabs (Ragionieri et al. 2010, Laurenzano et al. 2013, Shih et al. 2015, Fratini et al. 2016, Buranelli et al. 2019). In this study, we used DNA barcode sequences of the most abundant mangrove crabs in the Persian Gulf to identify and characterize the unique fauna of the mangrove forests. The sequences were also used to reconstruct phylogenetic trees to compare identities of species from Pakistan.

2. Material and Methods

2.1. Study area and sampling

Specimens were collected between 2018 to 2019 from seven sites. These sites were selected where high densities of crabs were expected based on previous studies of mangrove crabs in the Persian Gulf (Fig. 1) (Naderloo 2017; Naderloo et al. 2011). The sampling areas include various localities of mangrove forests along the tidal mud zones located in A) Qeshm Island: 1) Mangroves of Laft (26.88°N, 55.76°E), 2) Soheili (26.78°N, 55.76°E), 3) Fardis Park (26.71°N, 55.90°E), 4) Kuweii (26.94°N, 55.98°E), 5) Dargahan (26.96°N, 56.05°E), and 6) Peiposht (26.90°N, 55.88°E); B) Bandar Khamir: 7) Mangrove of Bandar e Pol (26.98°N 55.64°E) and ; C) Coasts of Bandar Abbas: 8) Suru Beach (27.15°N, 56.23°E), and 9) Nayband mangrove (27.18°N, 56.39°E) (Fig 1).

Sampling was performed in three inter-tidal regions: high tidal, inter-tidal, and low tidal (Naderloo 2017; Naderloo et al. 2011; Sharifian et al. 2020). Specimens were

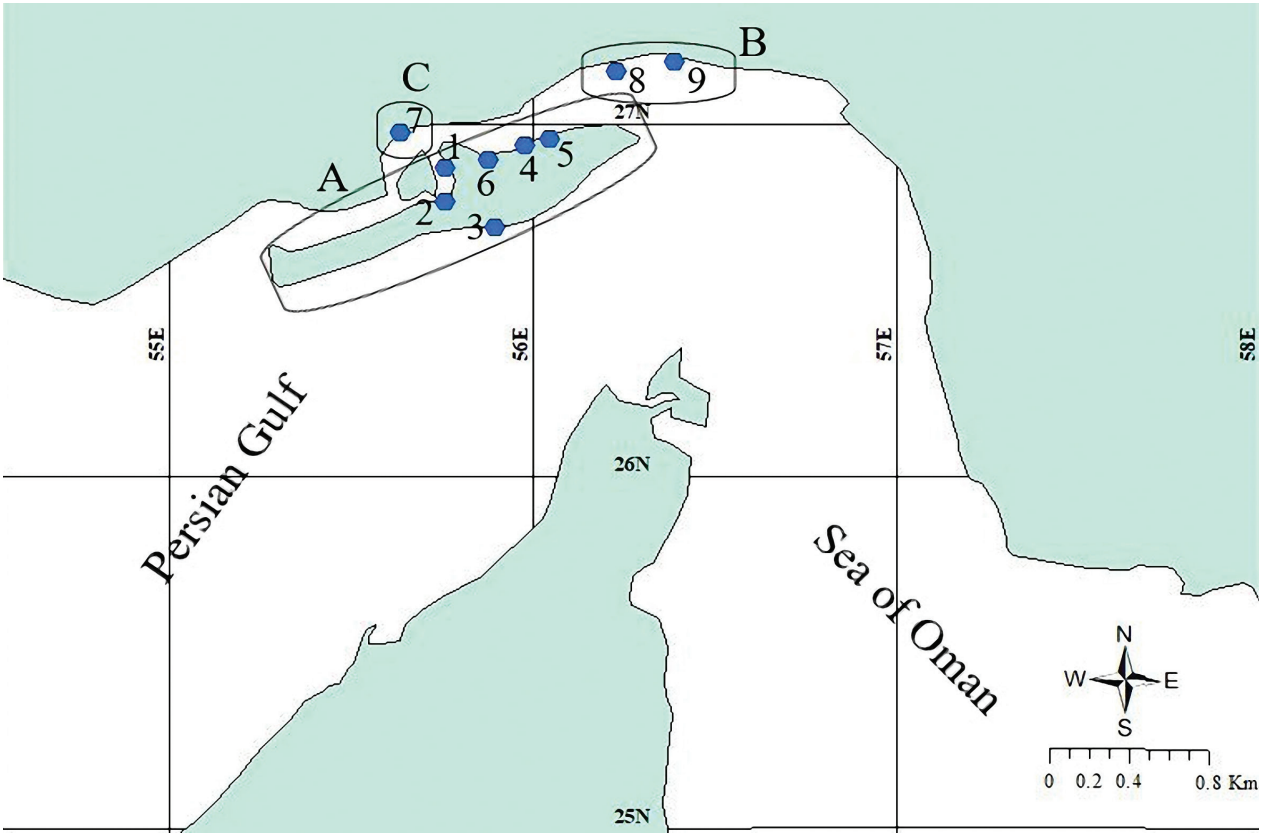

Figure 1. Study area located in the Persian Gulf. **A** Qeshm Island: 1) Mangroves of Laft 2) Soheili, 3) Fardis Park, 4) Kuweii, 5) Dargahan, and 6) Peiposht. **B** Bandar Khamir: 7) Mangrove of Bandar e Pol. **C** Coasts of Bandar Abbas: 8) Suru Beach, and 9) Nayband mangrove.

collected mainly by hand and by sieving and digging in mud beds. All specimens were kept in 96% ethanol, and transferred to the Senckenberg Research Institute and Natural History Museum in Frankfurt, Germany, for molecular analysis. The specimens were morphologically identified (Barnes, 2010; Naderloo, 2017; Naderloo et al., 2011) and were examined and verified by Dr. Jose Christopher Mendoza (National University of Singapore). From the 70 specimens, 11 species from five families Camptandriidae Stimpson, 1858, Dotillidae Stimpson, 1858, Macrophthalmidae Dana, 1851, Ocypodidae Rafinesque, 1815, and Sesarmidae Dana, 1851 were morphologically identified (Figs S1–S6). Photos of specimens and their diagnostic characters were taken using a camera attached to a stereo microscope at the Senckenberg Research Institute and Natural History Museum in Frankfurt**.**

2.2. DNA extraction

The pereiopods from 29 of the specimens from Camptandriidae (13 individuals), Dotillidae (8 individuals), Macrophthalmidae (2 individuals), Ocypodidae (4 individuals), and Sesarmidae (2 individuals) (Table S1) were stored in separate containers for further analysis. In the laboratory, purification of total DNA from muscle tissue was performed using the Spin-Column protocol or the QIAGEN DNA extraction kit (DNeasy Blood & Tissue Kit) according to the manufacturer's specification. The quantity of the extracted DNA was measured using a DS-11 (DeNovix) spectrophotometry.

2.3. PCR

Published primers for the genes 16S (16Sar, 16Sbr) and COI (LCO1490, HCO2198 and COL14, COH6) were used for PCR (Folmer et al. 1994; Palumbi et al. 1991; Schubart and Huber, 2006) (Table 1). For COI, both primer pairs were used. The primers from Folmer et al (1994) were used for the initial PCRs, if the amplification failed the modified primers COL14/COH6 were used.

The PCR was run under the following conditions: 16S: Initial denaturation: three minutes at 94°C, 40 cycles with 45 seconds at 94°C for denaturing, one minute at 48°C for annealing, and one minute at 72°C for elongation, followed by a final elongation of 10 minutes at 72°C. COI: initial denaturation: 5 minutes at 94°C, 40 cycles with 50 seconds at 94°C for denaturing, 70 seconds at 47–45°C for annealing, and one minute at 72°C for elongation and a final elongation at 10 minutes at 72°C.

2.4. Sequencing and data analysis

The PCR products were visually evaluated on a 1% agarose gel. Successfully amplified PCR products were

Table 1. The primers used for amplification of 16S and COI.

Primer name	Primer sequence	Reference
16Sar	C G C C T G T T T A T C A A A A A C A T	(Palumbi et al., 1991)
16Sbr	C C G G T C T G A A C T C A G A T C A C G T	(Palumbi et al., 1991)
LCO1490	G G T C A A C A A A T C A T A A A G A T A T T G G	(Folmer et al., 1994)
HCO2198	T A A A C T T C A G G G T G A C C A A A A A A T C A	(Folmer et al., 1994)
COL14	G C T T G A G C T G G C A T A G T A G G	Schubart and Huber, 2006
COH6	T A D A C T T C D G G R T G D C C A A A R A A Y C A	Schubart and Huber, 2006

sequenced from both strands using the PCR primers on an ABI sequencer 3730 DNA analyzer (Applied Biosystems, Foster City, USA) by the laboratory center of the Senckenberg Biodiversity and Climate Research Centre Frankfurt (SBiK-F). The resulting sequences were analyzed with Geneious (1.1.0) (Kearse et al. 2012). Quality control and trimming resulted in 49 sequences (28 from 16S/21 from COI) (Table S2). An additional 74 sequences of 16S, and 69 of COI sequences, from the five families, as well as Varunidae Milne Edwards, 1853 were selected from the NCBI database (see Tables S3 and S4 for details). The additional sequences were selected with the aim to analyse the relationship between the Persian Gulf species with species and individuals from other geographical regions. Three data sets were generated, one for COI, one for 16S, and a concatenated alignment for 16S and COI. *Pseudocarcinus gigas* Lamarck, 1818 (16S:HM637969/COI: HM638058) and *Charybdis helleri* Milne-Edwards, 1867 (16S:KX060527/COI: KX060332) were used as outgroups in the respective data sets. The data sets were aligned with MUSCLE (Edgar, 2004) in AliView (Larsson, 2014) and manually inspected. The molecular identification and barcoding of crabs were performed for all three data sets with Maximum Likelihood (ML) using IQ-TREE (http://iqtree.cibiv.univie.ac.at) (Nguyen et al. 2015, Trifinopoulos et al. 2016). ModelFinder, as implemented in IQ-TREE, identified the suitable evolutionary model for each data set (Kalyaanamoorthy et al. 2017). Branch support was calculated by 1000 bootstrap replicates of SH-aLRT as well as 1000 replicates of ultrafast bootstrap support (Hoang et al. 2018). Branches with SH-aLRT $\geq$ 80% and UFboot $\geq$ 95% were considered significant. FigTree 1.4.4 (available at http://tree.bio.ed.ac.uk/software/figtree) was used to visualize the resulting phylogenetic trees.

3. Results

Molecular analysis of the Persian Gulf crabs resulted in 49 new sequences (16S: 28 sequences / COI: 21 sequences) that have been deposited in GenBank (Table S2). Molecular analysis infers that these correspond to 11 species from five families. One species in Macrophthalmidae (Fig. 2) could not be determined to the species level. The species tentatively named *Ilyograpsus* sp. may represent a so far undescribed species that may be endemic to the Persian Gulf. The phylogenetic results showed most of the identified sequences grouped with published sequences from same species from Pakistan.

3.1. Camptandriidae

From the family Camptandriidae, we identified three species, *Manningis arabicum*, *Nasima dotilliformis* and *Opusia indica*, in the mangrove forests. We molecularly identified three of the 11 described Camptandriidae genera using the data sets. *Nasima* Manning, 1991 and *Manningis* Al-Khayat & Jones, 1996 are reconstructed as sister genera, while *Opusia* Ng, Rahayu & Naser, 2009 in our analysis is the sister genus to the other genera in Dotillidae; although, with low support values (64%/62%). The six individuals of *Manningis arabicum* from the different locations in the Persian Gulf (Qeshm Island (Soheili, Koweii, Peiposht and Fardis Park), Bandar Abbas (Suru coast), Bandar Khamir (Bandare e Pol)) grouped with two published individuals from Pakistan (Fig. 2; Figs S7, S8). *Nasima dotilliformis* from Qeshm Island (locations: Dargahan, Soheili, Laft), Bandar Abbas (Nayband), and Bandar Khamir (Bandar e Pol) formed a monophyletic clade with published individuals from Pakistan (support values 100%/100%) (Fig. 2; Figs S7, S8). *Opusia indica* from Bandar Abbas (Suru coasts) and Bandar Khamir (Bandare e Pol) grouped with published sequences from same species from Pakistan (100%/100%) (Fig. 2, Figs S7, S8).

3.2. Dotillidae

From Dotillidae we identified four species from three different genera (*Ilyoplax* Stimpson, 1858, *Scopimera* De Haan, 1833, and *Dotilla* Stimpson, 1858) (Fig. 2, Fig. S8). The two species from *Ilyoplax*, *I. frater* Kemp, 1919 and *I. stevensi* Kemp, 1919 were reconstructed as sister species (Fig. 2). *Scopimera crabricauda* Alcock, 1900 from Bandar Abbas (Suru coasts) clustered (97%/100%) with *Scopimera* sp. from Pakistan (Fig. 2; Figs S7, S8), and all analysed species/individuals from *Scopimera* form a monophyletic group. *Dotilla blanfordi* Alcock, 1900 from Bandar Abbas (Suru coasts) clusters with other species from Taiwan, Singapore, and Egypt (Fig. 2; Figs S7, S8).

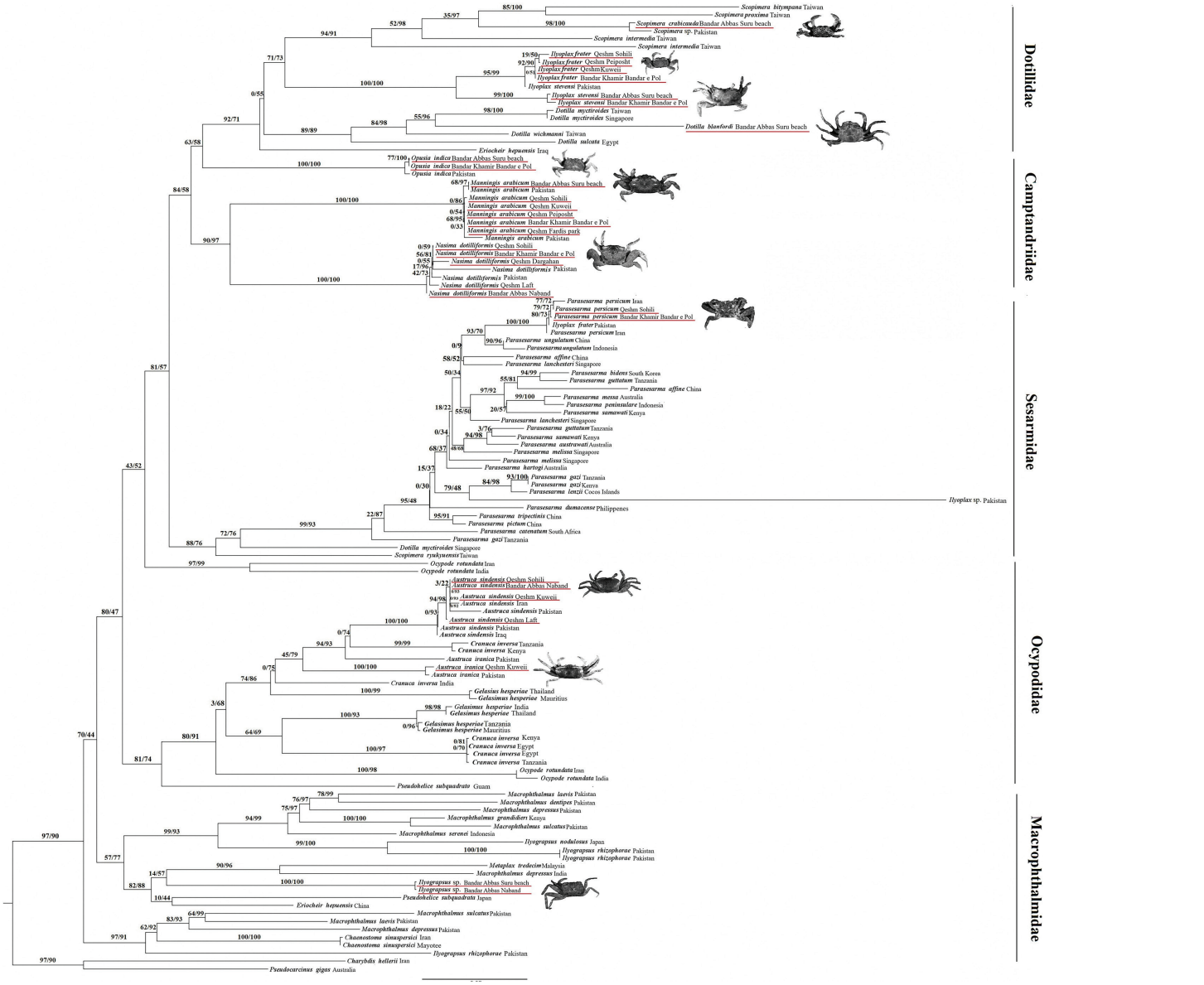

Figure 2. A phylogenetic reconstruction of grapsoid and ocypodoid crabs based on a concatenated alignment of CO1 and 16S sequences. Support values are listed at nodes (SH-aLRT support (%)/ultrafast bootstrap support (%)). Scale bar show substitutions per site. Red lines show specimens sampled from the Persian Gulf for this study. Crab images by Sharifian, 2019.

3.3. Macrophthalmidae

From Macrophthalmidae only one genus was identified, *Ilyograpsus* Barnard, 1955. The species could not be determined to the species level using sequence and morphological data from two individuals. The species did not group with the congeneric *I. rhizophorae* Barnard, 1955 and *I. nodulosus* Sakai, 1983 (Fig. 2, Fig. S5) in the phylogeny. This potentially new species forms a long, genetically distinct lineage inside Macrophthalmidae. The two individuals of *Ilyograpsus* sp. form the sister group to *Metaplax* H. Milne Edwards, 1852 /*Macrophthalmus* Desmarest, 1823 (Fig. 2). The new species is different in the shape of carapace, second gonopod, and third maxilliped (Fig. S5) compared to the other two Persian Gulf species, *I. rhizophorae* Barnard, 1955 and *I. nodulosus* Sakai, 1983.

3.4. Ocypodidae

Two species from Ocypodidae were collected, *Austruca sindensis* Alcock, 1900 and *A. iranica* Pretzmann, 1971. *A. sindensis* Alcock, 1900 from Qeshm (Soheili, Laft and Kuweii) and Bandar Abbas (Nayband) were clustered with sequences from the same species from Pakistan and Iraq (100%/100%) (Fig. 2). Similarly, *A. iranica* from Qeshm (Kuweii) clustered with individuals of the same species from Pakistan (Fig. 2). Based on 16S sequences, the two *A. iranica* individuals (Persian Gulf) and the Pakistan individual (MG024475) had a pairwise sequence distance of 12%. For COI the sequence distance between the individuals from Pakistan and the Persian Gulf was less than 1% (MF614779) and may be a result of misidentified individuals.

3.5. Sesarmidae

Only one species from Sesarmidae was collected, *Parasesarma persicum*. *P. persicum* collected from the Qeshm stations (Soheili) and Bandar Khamir (Bandar e Pol) in the Persian Gulf clustered (96%/100%) with the sequences of the same species from other areas (Nayband Bay, Bushehr) in the Persian Gulf (Fig. 2; Figs S7, S8). The sequences from two species, *Ilyoplax* sp. and *Ilyoplax frater*, clustered inside the Sesarmidae instead of the Dotillidae in the COI phylogeny (Fig. S8). Most likely these COI sequences stem from misidentified species, likely *Parasesarma persicum* (*Ilyoplax frater* MK431533), or an unknown *Parasesarma* De Man, 1895 (*Ilyoplax* sp. MK503851).

4. Discussion

Most mangrove forests around the world are currently being degraded for land-use types, threatening the biodiversity of the area, especially the endemic species (Fratini et al. 2016, Richards and Friess 2016, Thomas et al. 2017). Future destruction may lead to the local extinction of species. As crabs are the dominant taxon of the mangrove community their habitat degradation may lead to disturbances of their ecological functions leading to negative effects on bioturbating, nutrient recycling, and microbial decomposition, as well as adverse lateral influence on the distribution, and abundance of tree species of mangrove forests (Alongi 2009, Buranelli et al. 2019, Sharifian et al. 2020, 2021b, 2021a).

The mangrove forests of the Iranian coasts of the Persian Gulf has a higher species richness of mangrove crabs, as well as a higher number of geographic distribution records, compared to its Southern coasts (Naderloo et al. 2013, Sharifian et al. 2021b). Despite the high diversity of mangrove crabs few studies have described and analyzed morphometric characteristics and sequence data from crabs in this region (Naderloo, 2017, 2011; Naderloo et al., 2011; Naderloo and Türkay, 2011), (Naderloo and Schubart, 2010; Naderloo and Schubart, 2009).

To assess the species diversity, mangrove crabs were sampled in the Persian Gulf from seven sites covering three inter-tidal regions. Molecular analysis of the collected individuals identified 11 species. The Persian Gulf harbors about 30 crab species that inhabit mangrove forests (Naderloo et al., 2011), thus our sampling covers about one third of the crab fauna in this habitat.

Our results from molecular barcoding analysis of 29 mangrove crab individuals highlight a phylogenetic relationship between Persian Gulf crabs and species from the waters of Pakistan. Previous molecular studies have shown that mangrove crabs have a planktonic larval stage that results in genetically homogeneous populations covering large regions (Ragionieri et al. 2010, Shih et al. 2015, Buranelli et al. 2019). However, the barcoding genes COI/16S are conserved and faster evolving markers are needed in order to analyze population structures between the Persian Gulf and Pakistan, if these exist.

Due to their underrepresentation in molecular studies the Persian Gulf mangrove crab community may harbour both cryptic as well as new species. We may have discovered a new species, *Ilyograpsus* sp. (Macrophthalmidae Dana, 1851). *Ilyograpsus* sp. does not cluster with the two other included species (*I. rhizophorae* and *I. nodulosus*) that occur in the Persian Gulf. However, the other three described *Ilyograpsus* species were not included in our molecular analysis. *Ilyograpsus* sp. form an isolated lineage in the phylogeny, suggesting that it is genetically distinct from the species in our taxon sampling. Further morphological and molecular analysis is needed to understand the taxonomic position of *Ilyograpsus* sp.

5. Conclusions

Our study provides for the first time molecular data of the most abundant mangrove crabs of the Persian Gulf, barcoding one third of the species occurring in this habitat. In general, mangrove crabs of the Persian Gulf were closely related to species and individuals from the waters of Pakistan. Future research is necessary to assess the population ecology of the mangrove crab fauna across the Indo-Pacific Ocean to determine the influence of biotic and abiotic factors on their genetic variation and distribution ranges.

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Supplementary Material 1

Figures S1–S8, Tables S1–S4

Authors: Sharifian S, Kamrani E., Nilsson M, Saeedi H (2023)
Data type: .pdf
Explanation note: Figure S1. Morphological features of Camptandriidae (*Manningis arabicum* and *Nasima dotilliformis*). — **Figure S2.** Morphological features of Camptandriidae (*Opusia indica*) and Sesarmidae (*Parasesarma persicum*). — **Figure S3.** Morphological features of Dotillidae (*Ilyoplax frater* and *I. stevensi*). — **Figure S4.** Morphological features of Dotillidae (*Dotilla blanfordi* and *Scopimera bracricauda*). — **Figure S5.** Morphological features of Macrophthalmidae (*Ilyoplax* sp.). — **Figure S6.** Morphological features of Ocypodidae (*Austruca iranica* and *A. sindensis*). — **Figure S7.** 16S phylogeny of Grapsoidae and Ocypodoidae. — **Figure S8.** COI phylogeny of Grapsoidae and Ocypodoidae.

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