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Identification of Mitochondrial species-Chiromantes boulengeri in Arvand River, Persian Gulf

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Abstract

In this study, Identification of *Chiromantes boulengeri* was carried out in 2014 in two parts of the Arvand River in Minoo Island (Minoo City Bridge and Omm ol Ejaj region) based on the characteristics of its mtDNA gene. In order to investigate the molecular analyzes of this species with the species close to the NCBI gene bank, DNA was extracted from the phenol-chloroform method and the 16S rRNA mitochondrial genes were amplified by PCR and finally sequenced. Based on the polymorphism analysis, two mutation regions were observed in *Chiromantes boulengeri* species, which indicated its evolution and adaptation for better survival in different ecological and atmospheric conditions in the study area.

Keywords: *Chiromantes boulengeri*; Arvand River; mt DNA; Phylogeny.

1. Introduction

There are many marine habitats in the Persian Gulf that have high biodiversity (Jones *et al.,* 1994). One of the important water sources that feed the northern part of the Persian Gulf is the Arvand River (Figure 1). There are many marine habitats in the Persian Gulf with high biodiversity (Jones *et al.,* 1994). One of the important water sources feeding the northern part of the Persian Gulf is the Arvand River (Figure 1), which has significant impact on biological and physical factors of tidal habitats, considering prevailing ecological conditions of the river due to changes in salinity, high turbidity and tidal current. *Chiromantes boulengeri* species (Calman, 1920) are common crabs in the river that often live in offshore. According to the distribution of *Chiromantes boulengeri* in Arvand River and the conditions governing the region, to study of the *Chiromantes boulengeri* species (Calman, 1920), two different regions

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of Arvand River were selected, Minoo City Bridge and Omm ol Ejaj region.

Naderloo and Schubart (2009) re-described *Chiromantes boulengeri* (Calman, 1920) from Bahmanshir River and proposed a closer sister species relationship of the two East Asian species, *C. haematocheir* by De Haan, 1833, and *C. dehaani*.

Shahdadi and Schubart (2017) reconstructed phylogenetic relationships of *Perisesarma* and related genera to evaluate the phylogenetic importance and taxonomic usefulness of the epibranchial tooth. Their molecular analysis proposed most species of *Perisesarma* cluster solidly together with species of *Parasesarma*, but without being reciprocally monophyletic.

2. Materials and methods

2.1. Methods

Samples were collected in October 2014 from the crustaceans in tidal zones along the Arvand River in Abadan, Minoo Island. The crab species were collected by hand and kept in 70% alcohol for molecular analyzes and transferred to the lab for morphological study (Nozarpour, 2015) and gene sequencing for accurate identification of the species. The DNA was separated from the legs' muscle of seven samples (six males and one female) by phenol-chloroform method (Michele, 2002).

2.2. Data

In this study, mitochondrial 16S rRNA (16S) gene amplification using 16S reverse primer and 16S forward primer took place. Sequence data were recorded in the NCBI Gene Bank. Phylogenic sequencing analyzes were compared with the species that exist in different parts of the world in gene bank to verify the relationship between the genotypes of the species C. *boulengeri* (Calman, 1920) based on 5000 repetitions in Maximum Composite Likelihood and Neighbor-Joining in Mega6

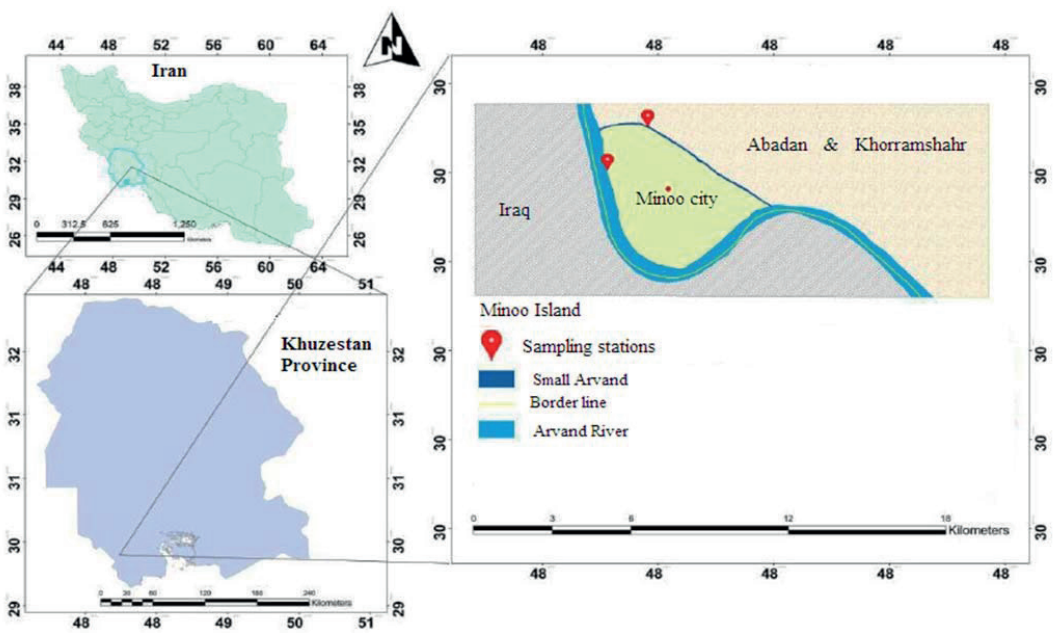

Figure 1. Geographical location of the study area

software (Tamura *et al.,* 2013). The gene in the world gene bank included of C. *dehaani* (H. Milne Edwards, 1853) FN296221، *Sesarmops intermedium* by De Haan in 1835, FN296222، *Bresedium brevipes* (De Man, 1889) AM1800685، *Chiromantes haematocheir* (De Haan, 1833) DQ131499، *Chiromantes ortmani* AJ784016، *Chiromantes eulimene* (De Man, 1897) AJ784017، *Armases cinereum* (Bosc, 1802) AJ784010، *Parasesarma liho* FN659068، *Sarmatium striaticarpus* AM180680، *Neosarmatium smithi* AJ784014، *Armases elegans* AJ784011، *Neosarmatium meinerti* FN392171، *Armases recordi* (Milne Edwards, 1853) AJ250637، *Aratus sp.* HG939511، *Neosarmatium fourmanoiri* FN392195، *Parasesarma leptosoma* AJ784024, and Sesarmops sinensis AY497290 were selected as the outsider. DNA Polymorphism analyzes were calculated between seven sequences identified from Arvand River and sequences of close species by DnaSP5 software (Librado and Rozas, 2009).

3. Results and Discussion

Seven sequences from *Chiromantes boulengeri* were recognized by molecular identification that resembled 99% of the S. boulengeri species identified by Calman (1920) from Basra (Iraq) (access numbers: FN296219- FN296220). The species C. *boulengeri* (Figure 2) is from the Sesarmidae Dana family 1851, and the Chiromantes Gistel genus 1848.

According to phylogeny trees (Figure 3), the species were divided into two groups along with smaller groups in their interstices, and molecular analyzes indicated a close relationship between the Chiromantes genus and the Neosarmatium genus.

DNA Polymorphism Analysis on seven sequences of C. *boulengeri* species from two regions in Arvand River included of 10 polymorphs and 11 mutations, and the nucleotide diversity was estimated to be Pi=0.11957.

According to phylogeny trees (Figure 3), the C. *boulengeri* species are located in the exogenous position relative to the branches of C. *haematocheir, C. dehaani, C. ortmanni* and *C. eulimene*, and also the sister relationship between *C. boulengeri* species and there are no other species. Therefore, there is no evidence about the existence of a close genetic relationship between the *C. boulengeri* and *C. dehaani* species, which was morphologically compared to the Calman (1920) study. According to the phylogeny tree, the C. *boulengeri* species has a longer evolutionary time much longer than C. *dehaani* species, and there is a significant genetic

Figure 2. A male *Chiromantes boulengeri* (Calman, 1920) in Arvand River from Left) front, and Right) back

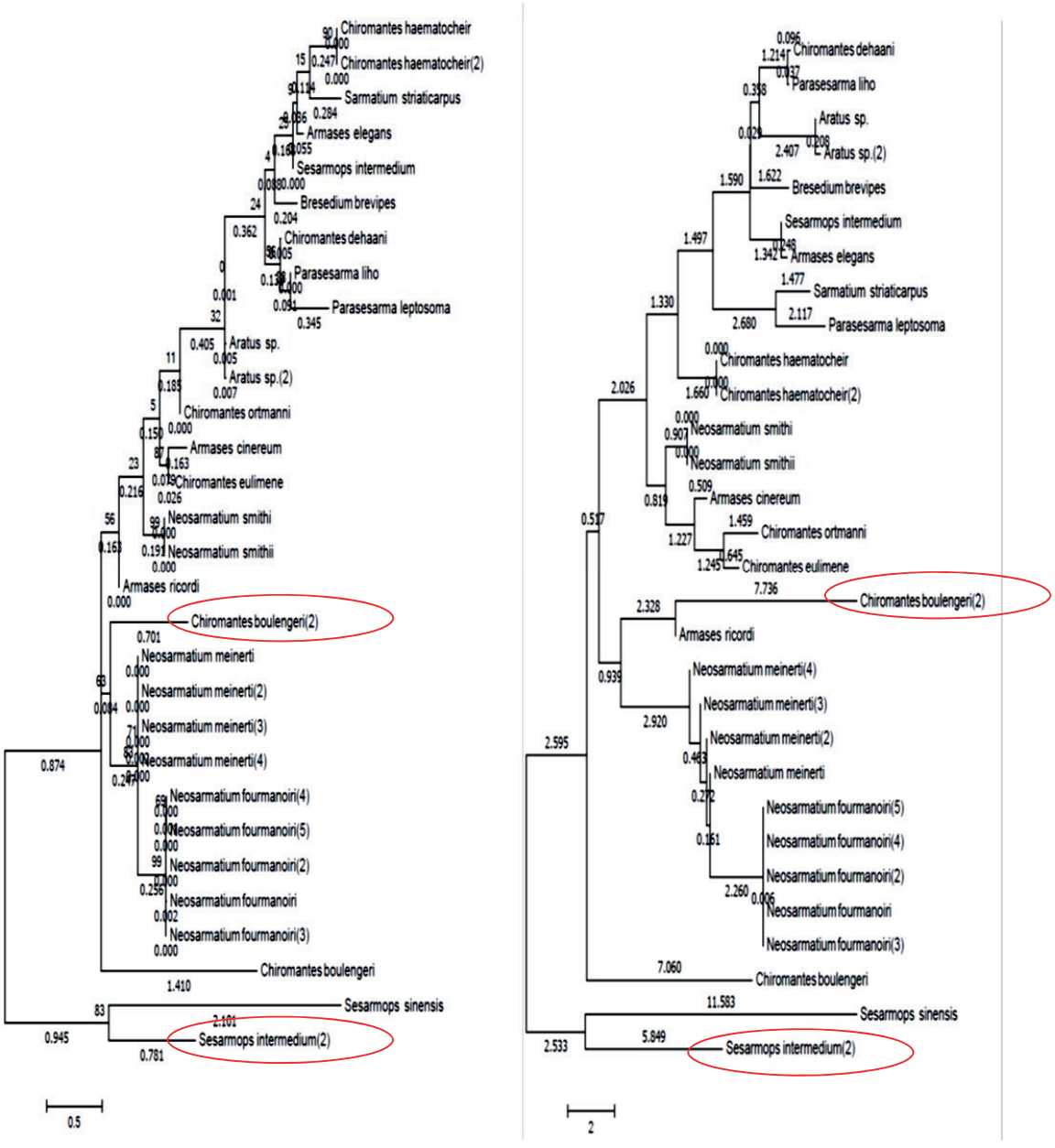

Figure 3. Phylogenic relationships of the selected species of Chiromantes genus and other Sesarmidae based on Left) Maximum composite Likelihood with 5000 replications, and Right) based on Neighbor-Joining method

differentiation between them. Also, the results of DNA Polymorphism analysis between seven sequences identified in two areas of the Arvand River, showed genetic mutations, probably due to the distribution of larvae in the river and the effects of environmental conditions governing the region. Compared with morphological characteristics *C. boulengeri* from Arvand River (Nozarpour, 2015) and *C. boulengeri* from Bahmanshir (Naderloo and Schubart, 2009), propose evolutionary changes in *C. boulengeri* from Arvand River.

Conclusion

The present study provided an understanding of the characteristics of the mtDNA gene of Chiromantes boulengeri (Calman, 1920) in the tidal waters of Arvand River in the Minoo Island (Minoo City Bridge and Omm ol Ejaj region). Environmental conditions affect on the morphology and polymorphism of the C. boulengeri species.

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