Taxonomic and phylogenetic relationships of freshwater crabs of the genus *Potamon* (Crustacea: Brachyura: Potamidae) from Iran

Universität Regensburg

DISSERTATION ZUR ERLANGUNG DES DOKTORGRADES DER
NATURWISSENSCHAFTEN (DR. RER. NAT.) DER FAKULTÄT FÜR
BIOLOGIE UND VORKLINISCHE MEDIZIN DER UNIVERSITÄT
REGENSBURG

vorgelegt von
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aus
Sabzevar, Iran

im Jahr
2013

Das Promotionsgesuch wurde eingereicht am:
30.10.2013

Die Arbeit wurde angeleitet von:
PD Dr. Christoph Schubart

Unterschrift:

Dedication

This work is dedicated to:

My wife, Maryam for encouragement, patience and continuous support

My little, lovely daughter, Ariana

My parents, who their love and sacrifices made possible for me to accomplish the education

My advisor, PD Dr. Christoph Schubart for his fatherly support

Abstract of the Dissertation

Taxonomic and phylogenetic relationships of freshwater crabs of the genus *Potamon* (Crustacea: Brachyura: Potamidae) from Iran

by

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In an era of climate change and habitat destruction, assessing current biodiversity as soon as possible is of crucial importance. To uncover the real biodiversity, taxonomic problems in species complexes and phylogenetic delimitations of species need to be clarified. *Potamon* is a well-known freshwater crab genus with a distribution from the Middle East through southern Europe and the far north of Africa. Phylogeny of *Potamon* has been a source of disagreement among specialists over the last few decades. In order to understand the evolution of the group it is crucial to resolve the phylogenetic relationships among the species of *Potamon* and to discover intraspecific endemic units using different tools. The purposes of the current study were: 1) to reconstruct the phylogenetic history of different species of the genus *Potamon* in Iran, 2) to find out, if there is consistency of morphological with molecular characters, 3) to determine intraspecific molecular variability among populations of *Potamon elbursi* in order to understand initial steps of regional differentiation as well as importance of endemic forms for conservation issues. In the course of the study two mitochondrial (16S rRNA and Cox1) and one nuclear (28S rRNA) genes were partially amplified and sequenced. A significant divergence was shown among three distinct lineages previously considered to belong to *Potamon persicum* complex and associated with their geographical distributions. One lineage corresponds to a formerly synonymised species, *P. elbursi*, which is here revalidated and redescribed. Another lineage (western Iran) still must be considered a cryptic lineage, since it is genetically distinct, but the current morphological evidence cannot separate it from *P. persicum*. Consistent molecular and morphological divergence was also recorded from the westernmost slopes of the Zagros Mountains where *Potamon ilam*, a sister species to *Potamon mesopotamicum*, is described as a new species to science. Overall, our results show that *Potamon* radiated at different time intervals in the Middle East, leaving a genetic footprint of historical differentiation processes and following successive isolation within different watersheds. The current locally confined and unique biological diversity of Iran can be explained mainly by different topography and climate regimes resulting from mountains uplifting. Genetic assessment of populations of *P. elbursi* elucidates a significant genetic differentiation among populations without any linkage with geographical dispersal. Some populations show high endemicity, resulting from strong genetic drift or geographic barriers. Endangered aquatic ecosystems and occurrence of genetically distinct endemic and isolated populations of *P. elbursi*, call for considering the Alborz Mountains for possible future conservation plans.

Acknowledgments

I express my sincere appreciation to my advisor, PD Dr. Christoph Schubart, for his endless patience, and fatherly support and guidance thorough my four and half years of graduate studies.

Further thanks are due to my PhD mentors, Prof. Dr Christoph Oberprieler and Dr. Sara Fratini, for their comments and beneficial advice in course of my PhD.

My thank also goes to Prof. Jürgen Heinze (University of Regensburg) and his staff for the use of the necessary facilities and continuing support.

My visit to Natural History Museum, Wien (NHMW); Natural History Museum, London (NHM) was funded by the “Synthesis” Project of the European Union, which is greatly appreciated. I am grateful to Dr. Jørgen Olesen (ZMUC), Dr. Paul Clark (NHM), Prof. Michael Türkay (SMF), Prof. Peter K. L. Ng (ZRC), Dr. Peter Dworschak and Dr. Alireza Sari (ZUTC) for loaning me material from their collections in course of my PhD.

My thanks also go to my uncle Mr. Esmaeil Keikhosravi for his great help in collecting specimens. It also goes to other people who accompanied me with sampling: Fatollah Houshmand, Behzad Fathinia, Morteza Zarei and Madjid Moradmand.

Dr. Mehdi Abedi deserves special praise for hosting me several months and providing much needed support, help and advice.

Dr. Reza Naderloo, Dr. Catia Bartilotti, Dr. Ivana Silva, Nicolas Thiercelin, Jürgen Trettin for all sort of support, including troubleshooting, brainstorming and friendship. Warm thanks also go to Peter Koller, Nicole Rivera, Adnan Shahdadi, Claudia Laurenzano, Masaki Suefuji, Marion Füßl and Manuel Stemmer.

I express my gratitude to Mrs. Christiane Wanke and Doris Rothgaenger, for helping to make a comfortable situation for my family while they lived here.

I am deeply indebted to my parents-in-law, Mr. Yadollah Houshmand and Mrs. Kobra Keikhosravi for supporting my family.

I also wish to thank my brother and sisters for their support, their understanding, their power to arm me with new resources at each of my visits.

I appreciate the Akademisches Auslandsamt of the University of Regensburg for financial support.

Table of Contents

Lists of figures

Lists of tables

Chapter 1

General Introduction

What are freshwater crabs?

True freshwater crabs are those brachyuran crustaceans that spend their entire life cycle in freshwater habitats (Fig. 1.1). As opposed to these true freshwater crabs, other species (e.g. from the families Sesarmidae, Varunidae, Hymenosomatidae) may live as adults in freshwater, but in most cases larval development takes place in the marine environment. Freshwater crabs are distributed in the tropical and warm-temperate to temperate zones of all continents except Antarctica (Shih *et al.*, 2006; Yeo *et al.*, 2008). Independence of freshwater crabs from the marine realm and living in freshwater ecosystems is the consequence of evolution of some characteristics to overcome the unfavorable abiotic conditions. They lack of planktonic larval stages, but emerge from the egg with the regular shape of a small crab which is a strategy to reduce the risk arising from the environment, e.g., predation and desiccation (Diesel *et al.*, 2000). They also produce a small number of yolky eggs different from marine crabs and use a parental care strategy to diminish the loss of their limited offspring due to dehydration and washing off by currents (Shine, 1978; Micheli *et al.*, 1990). These characteristics evolved from ecological demands and are considered as tendency to follow a K-strategy to overcome the unsuitable condition of freshwater ecosystems. Absorbing salt from urine and food, and restriction of water loss are other evolved characteristics in freshwater crabs. These physiological adaptations are used to compensate for diffusive losses and pre-adaptation to terrestrial life (Wolcott, 1992; Morris & Van Aardt, 1998).

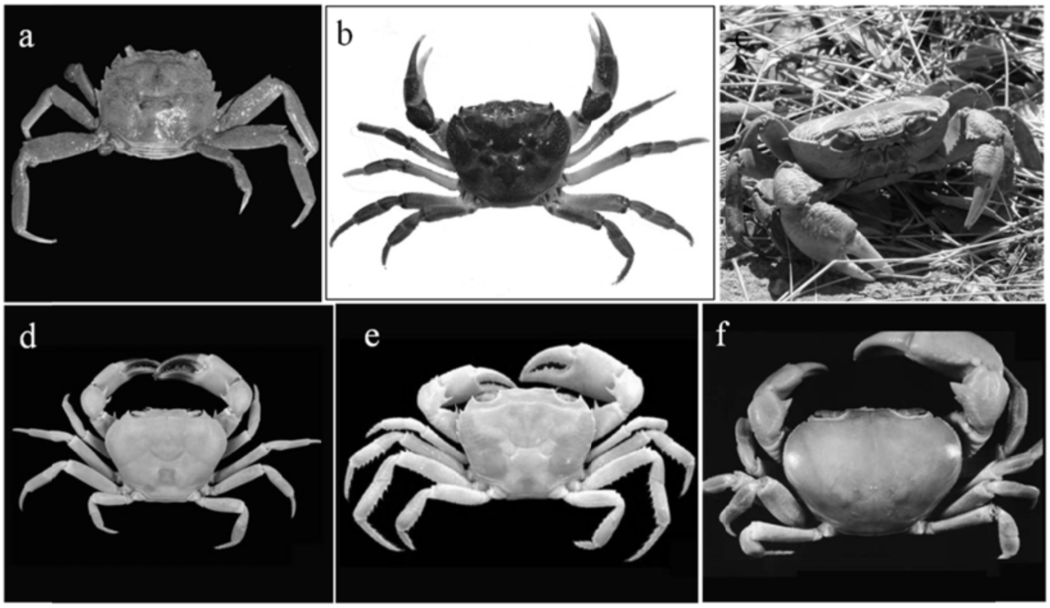


Figure 1.1: Morphological diversity of different freshwater crabs belonging to different families. a: *Trichodactylus panoplus* (Cláudio Timm - Flickr, CC BY-NC-SA 2.0); b: *Potamon fluviatile* (own work); c: *Potamon potamios* (Frente – Wikimedia Commons, CC BY-SA 3.0); d: *Potamonautes lirrangensis*; e: *Platythelphusa echinata* (d-e from Reed S.K & Cumberlidge N., 2006); f: *Hainanpotamon orientale* (from Yeo, D.C.J & Naruse T., 2007).

Behavioral Ecology of freshwater crabs

Freshwater crabs play a key role in the ecology of tropical inland waters worldwide (Rodríguez & Magalhães, 2005; Dobson *et al.*, 2007a, b; Yeo *et al.*, 2008). They are found in almost all clean freshwater bodies in the tropics and subtropics from moist lowland forests to rugged mountains. There are also many representatives with semi-terrestrial and terrestrial modes of life. They are adapted to live in a wide range of habitats including fast flowing or sluggish lowland rivers, streams, lakes, freshwater swamps, waterfalls, wetlands, karsts, caves, forest floor, rice fields and so forth (Fig. 1.2) (Yeo *et al.*, 2008). They use gills for breathing, but some species possess a pseudo-lung to breathe also in terrestrial habitat. The ability of air breathing has been evolved several times in freshwater crabs since it is present in three families (Gecarcinucidae, Potamidae and Pseudothelphusidae) (Bliss, 1968; Greenaway *et al.*, 1983; McMahon & Wilkens, 1983; Cumberlidge, 1986, 1991).

Freshwater crabs are sexually dimorphic. Females are often smaller than males (occasionally reach larger size) because of lengthening of exuviate intervals, and higher mortality rate due to carrying eggs, and intense vagility during the pre-ovulatory period (Gherardi *et al.*, 1988a; Micheli *et al.*, 1990). The first and second pair of males' pleopods of crabs (G1 and G2, respectively) are specialized for delivering sperms to females' gonopores (Ng *et al.*, 2008). Sexual dimorphism of their morphological traits, a shared with their marine cousins, is the form of the abdomen. In male abdomen is slender and triangular, resembling an inverted "T", with locking mechanism for abdomen, but in female is broader and rounded that is due to carrying fertilized eggs on the abdomen (Fig. 1.3).

These mainly stream dwelling crabs are primarily nocturnal animal, hiding during the day in sheltered places, and their locomotion activity is often confined to the dark time of the

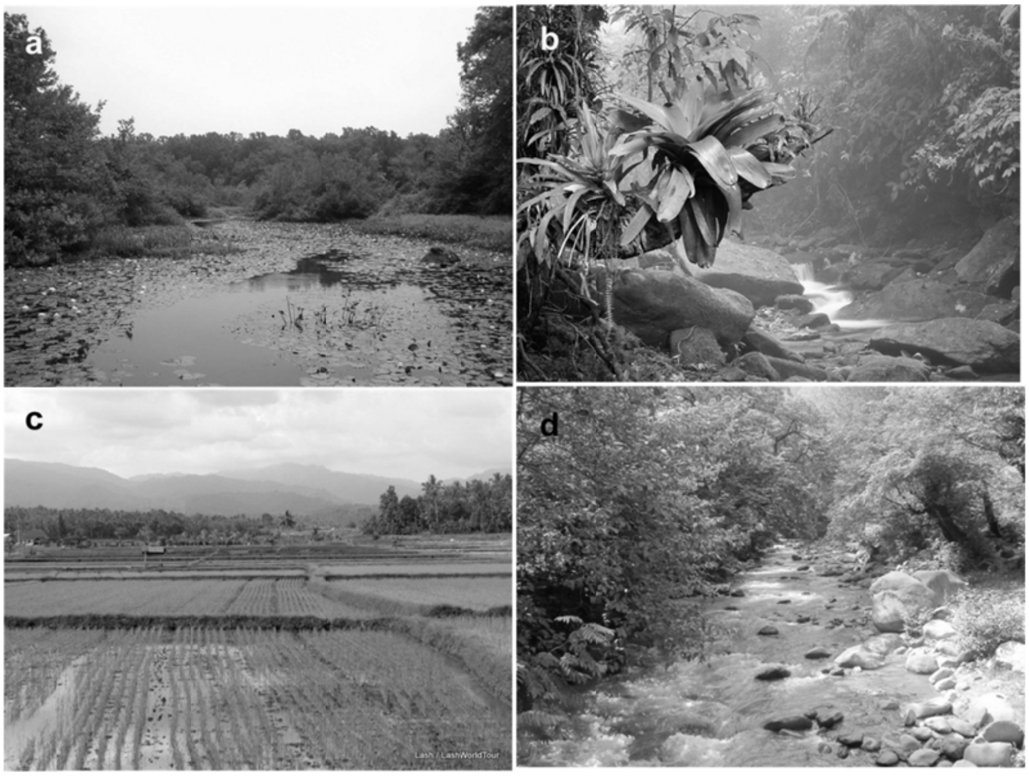


Figure 1.2. Habitat diversity in freshwater crabs. a: wetlands; b: forest; c: rice fields; d: rivers.

day. They are opportunistic animal and eat whatever is available. They generally feed on fallen leaves, insects (adults or larvae), worms and snails, and even frogs and fish. (Kabish, 1968; Schneider, 1971; Gherardi *et al.*, 1987; Yeo *et al.*, 2008). The cannibalistic behavior also has been documented in freshwater crabs either in captivity or in the wild (Williams, 1961; Hill & O'Keeffe, 1992; Somers & Nel, 1998).

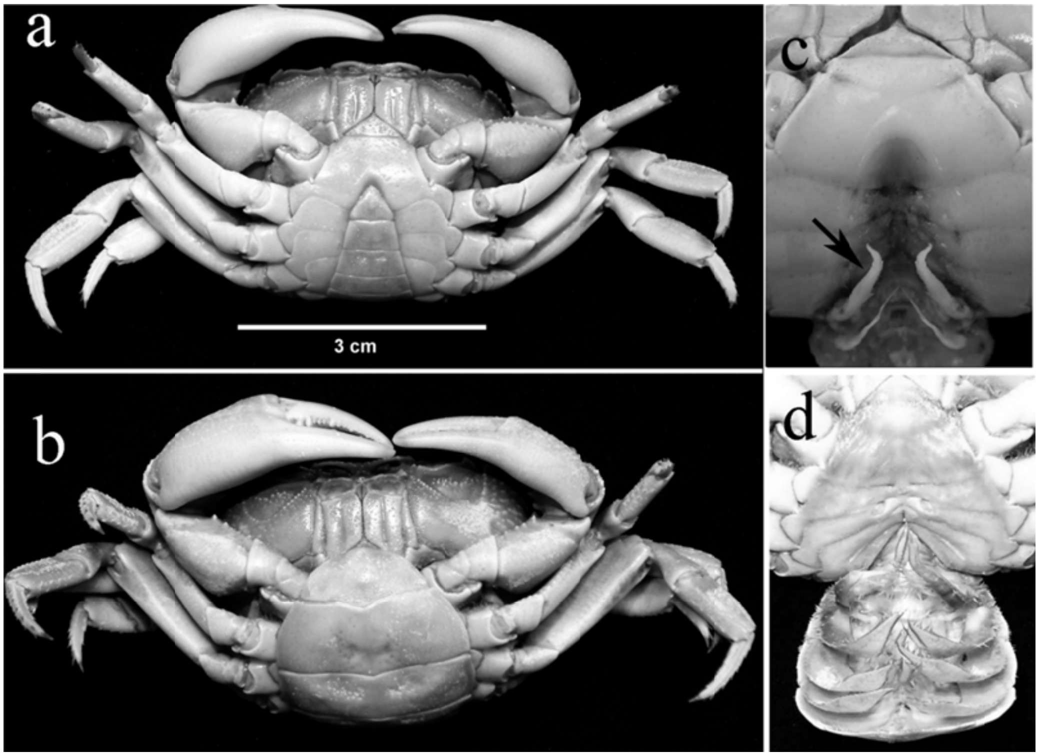


Figure 1.3. Morphological dimorphism in freshwater crabs. a: male abdomen; b: female abdomen; c: G1 (arrow) and G2 in male (pleopods 3-5 absent); d: Ovigerous pleopods in female.

Freshwater crabs experience a direct development, in which the eggs hatch as juveniles, with the embryonic stages passing within the egg (Yeo *et al.*, 2008). The broods comprise only a few hundred relatively large eggs (compared to hundreds of thousands small eggs for marine crabs) at a diameter of around 1 mm (0.04 in). The juveniles keep growing via several molts to become adult (Diesel *et al.*, 2000; Dobson, 2004).

Freshwater crabs and human life

The history of many freshwater crabs is tightly linked to human demography, especially when they supply needs for humans. *Potamon fluviatile* was considered as food source in ancient and modern Greek culture (Brandis, 1997). The former species' name was *Potamon edule*, which is a derived word from the Latin word "edulis" meaning edible (Pretzmann, 1987b; Jesse *et al.*, 2009). The occurrence of this species in the two relatively small islands of Malta and Gozo (off southern Italy) is attributed to colonisation mediated by anthropogenic transport (Jesse *et al.*, 2009). Presence of *Potamon ibericum* in southern France is also one of the examples of human introduction of freshwater crabs. This species was co-transported with the Turkish crayfish *Astacus leptodactylus* (for aquaculture purpose) (Charmantier, 1992; Noël & Guinot, 2007).

Freshwater crabs are also medically important as intermediate hosts of paragonimiasis in Asia, Africa, and the Neotropics. Paragonimiasis, as a food-borne zoonosis, has infected more than 20 million people worldwide by one of the 15 species of lung flukes (i.e. genus *Paragonimus*) that possibly indicates a wide consumption of freshwater crabs by humans (Toscano *et al.*, 1994; WHO, 1995; Maleewong, 2003; Adams, 2006). Ingesting inadequately cooked or raw crabs, carrier of the parasite, transmits the *Paragonimus* (Platyhelminthes) to humans (Nwokolo, 1974; Blair *et al.*, 1999; Rodríguez & Magalhães, 2005). Freshwater crabs are also host to the developing larvae of biting black flies (*Simulium* spp.), carrier (vectors) of the parasite *Onchocerca volvulus*. The transmission of such parasite causes hundreds of thousands human onchocerciasis (river blindness) in Africa (Williams, 1964, 1991; Crosskey, 1990).

Conservation perspective of freshwater crabs

As a whole, protection of freshwater ecosystems is of crucial importance due to the following three reasons: First, it includes approximately 12% of all species, despite covering only 0.8% of Earth's surface (Stiassny, 1996; McAllister *et* al., 1997). Second, such diversified ecosystem is extremely threatened by human activities such as dam construction and river regulation. Third, human mediated isolation of freshwater habitats provides a limited opportunity for inhabitants to disperse across. Therefore, freshwater habitats and their belonging biodiversity are among the most threatened ecosystems worldwide. Freshwater crabs, as ecologically highly important invertebrates in such enriched ecosystem, are mainly threatened by anthropogenic activity (Revenga *et al.*, 2005; Silk & Ciruna, 2005; Dudgeon *et al.*, 2006; Strayer, 2006). Despite, many species of freshwater crabs are in risk of extinction, conservation assessment of them has not received enough attention since the beginning of the 21st century. A fine scale conservation assessment of freshwater crabs in Malaysia evidenced that several species are differently threatened (Ng & Yeo, 2007). A similar study on Sri Lankan freshwater crabs showed that 37 species (72%) are threatened with global extinction as a result of water quality deterioration (e.g. unregulated use of pesticides), habitat fragmentation and undistributed habitat (Bahir *et al.*, 2005). This severe threatened status is mainly because of their biology and restriction to freshwater systems. Such restriction is more noticeable when is accompanied with vast destruction of tropical forest and aquatic ecosystems, and habitat defragmentation (Bahir *et al.*, 2005; Ng & Yeo, 2007; Cumberlidge *et al.*, 2009). In addition, freshwater crabs are also extirpating from desiccation and water exploitation in temperate regions. There are unfortunately insufficient data for over 600 out of 1280 species to complete thoroughly Red List assessment of freshwater crabs. Of the remaining, 209 species are listed in three threatened categories. Of this threatened category, 16.3% are sitting in critically endangered level and they may be on the brink of extinction. As a conclusion, all known species in Americas, Africa, Asia, Australia and Europe are highly threatened and there is an emphasis on significant extinction risk of many species (Sodhi *et al.*, 2004; Dudgeon *et al.*, 2006; Strayer, 2006; Cumberlidge & Daniels, 2008; Cumberlidge *et al.*, 2009). Hence, it is here assumed that the lack of conservation plans for most threatened species of freshwater crabs is exposing them to a high risk of extinction. Therefore, all facts put together justify an urgent assessing of current diversity for conservation purposes in the era of fast habitat devastation.

Biogeographic importance of freshwater crabs

Most genera of freshwater crabs are endemic to their respective zoogeographical regions and are considered conservative in their distribution potential (Cox, 2001). (1) They have generally low fecundity. (2) They are poor dispersers because they need regular access to water for oxygen absorption and periodic excretion of nitrogen. They are only able to cross short land bridges during rainy seasons or high humidity conditions (Gherardi et al., 1988a, b; Morris & Van Aardt, 1998; Daniels, 2003, 2006a). (3) They are highly philopatric; means individuals have tendency to stay in their point of origin or birthplace. This is mainly because of brood care and lack of planktonic larval (Daniels *et al.*, 2006a). (4) Nevertheless, freshwater crabs may tolerate some degree of salinity but they have a low capacity for transoceanic dispersal (Morris & Van Aardt, 1998). The occurrence of the Gecarcinucidae all over the Sunda Shelf to the Philippines, Sulawesi and Halmahera, and further to New Guinea can be explained by rafting dispersal. Therefore, they incidentally surpass the short marine passages by rafting (Klaus *et al.*, 2009). As a result, above mentioned features justify the importance of freshwater crabs as good indicators for zoogeographic patterns, palaeogeographical relationships, and history of river basins (Ng & Rodriguez, 1995; Klaus *et al.*, 2009, 2010).

Taxonomy and diversity of freshwater crabs

Freshwater crabs are a diverse group of eubrachyurans and strikingly represent one-fifth of all brachyurans with more than 1280 species (involved in 220 genera) (Cumberlidge & Ng, 2009). Nevertheless, the real global diversity of freshwater crabs is estimated to be higher about 1430 species (table 1.1). As an example, using molecular techniques suggests a huge potential for cryptic diversity (up to 35 different OTU's) exist within the *Paratelphusa* lineage in Sulawesi, whereas currently only 13 species are considered valid. Hence, freshwater crab's diversity must be regarded as still being in its "discovery" phase (Yeo *et al.*, 2008; Poettinger & Schubart, 2013). Diversity and species richness of freshwater crabs is shown with details in figure 1.4.

The first description of freshwater crab species goes back to 18th century when *Potamon fluviatile* (as *Cancer fluviatile*) (Herbst, 1785) and *Spiralothelphusa hydrodroma* (Herbst, 1794) were described from Italy and South India, respectively. This level of taxonomy was continued in the 19th century by placing Old World freshwater crabs in one genus, *Potamon* Savigny, 1816. After about twenty years, the first African freshwater crabs' representative, *Potamonautes niloticus* (H. Milne-Edwards, 1837) and *Potamonautes obesus* (H. Milne-Edwards, 1868) were described. The number of genera continuously increased to eleven by the end of 19th century. At the beginning of 20th century a new method for classification of the potamoid freshwater crabs was described by Rathbun (1904-1906). Her classification was based on external morphology i.e. overall shape, carapace structure and setation. This method was widely used until the mid-20th century. Rathbun's classification system became less common because of high variability of selected characters. Finally, Bott (1955, 1967, 1970) introduced morphology of the first pleopods (adult males bear two pairs of pleopods that are modified into copulatory structures known as first and second gonopods) for the classification of freshwater crabs, a character still used in today's studies. Pretzmann (1962) was the first who applied this system to classify freshwater crabs from the Middle East and later developed the method for other Asian and American freshwater crabs (Pretzmann, 1966a, b, 1972). Therefore, the second half of 20th century is assigned to an era of dramatic increasing description of potamoids. Later studies at the beginning of 21th century involved molecular genetic analyses in taxonomy. These data confirmed the high accuracy and liability of using the gonopods in taxonomy of freshwater crabs, in particular for the genus *Potamon*. It also stressed that morphometric analysis is valuable method in taxonomy when there is no difference in gonopod morphology (Jesse *et al.*, 2011). Currently, true freshwater crabs are assigned to five families: Trichodactylidae is a family of crabs, in its own superfamily, Trichodactyloidea. They distribute over Central and South America including some offshore islands, such as Ilhabela, São Paulo. Pseudothelphusidae, in superfamily Pseudothelphusoidea, occur in the Americas (from northern Mexico) and the entire South American temperate zone (and also from some of the Caribbean islands). The two families, Potamonautidae and Potamidae, both belong to the superfamily Potamoidea (Cumberlidge & Ng, 2009). The former exist in the tropical part of Africa and Madagascar (and some other adjacent islands), but the latter lives in North Africa, southern Europe and Asia. Potamidae is classified into two subfamilies with relatively distinct distribution. The subfamily Potaminae is confined to southern Europe, North Africa, Socotra Island, Middle East, northeastern India and Myanmar. Otherwise, the Potamiscinae is an Oriental group with the main diversity in East and Southeast Asia. And the latter group, Gecarcinucidae, is present in Australia and Oriental region (Yeo & Ng, 2004; Cumberlidge & Ng, 2009; Klaus *et al.*, 2009) (table 1.1).

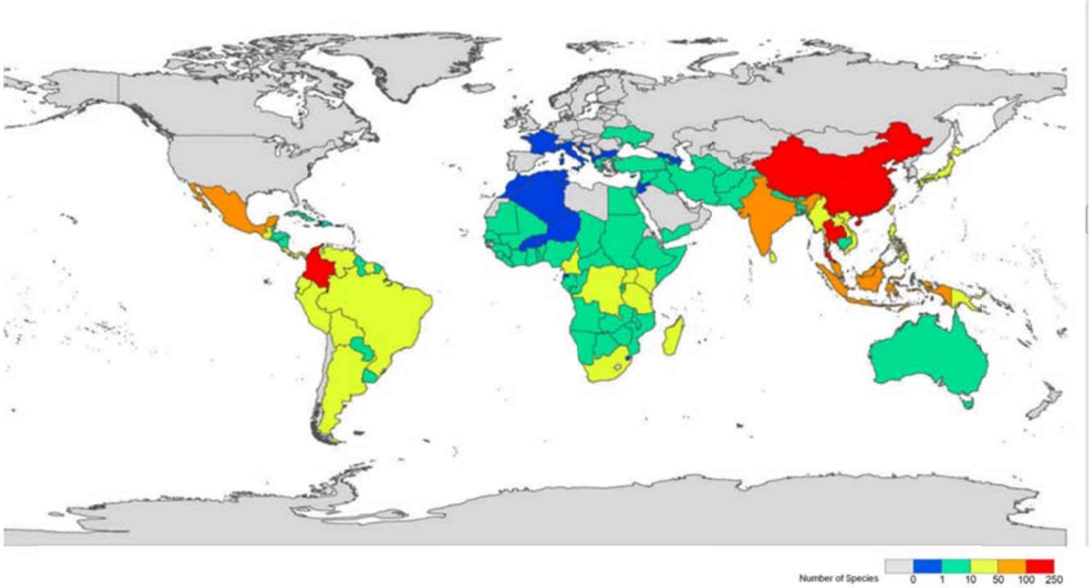
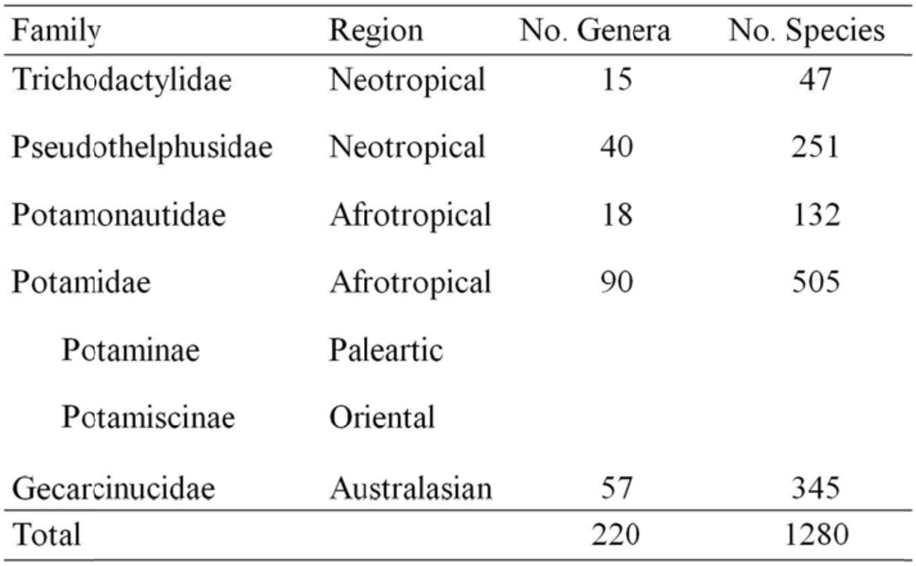


Figure 1.4. Diversity of freshwater crabs in different countries where they are present. The countries where freshwater crabs are absent are shown in grey. Based on data by Cumberlidge *et al*., 2009.

Table 1.1. Freshwater crab diversity by zoogeographical region and family. Based on data by Cumberlidge &Ng, 2009.



Family	Region	No. Genera	No. Species
Trichodactylidae	Neotropical	15	47
Pseudothelphusidae	Neotropical	40	251
Potamonautidae	Afrotropical	18	132
Potamidae	Afrotropical	90	505
Potaminae	Paleartic		
Potamiscinae	Oriental		
Gecarcinucidae	Australasian	57	345
Total		220	1280

Origin of freshwater crabs

There are some hypotheses about the origin of freshwater crabs that are still under debate and neither of them has an unequivocal explanation. A multiple invasion or paraphyletic hypothesis was the first expressed on favouring of the idea that limnic habitat were separately occupied by a marine stem-group of freshwater crabs (Bott, 1969, 1970, 1972). Ng and Rodríguez (1995) hypothesized a monophyly of freshwater crabs. They favoured a Gondwanan origin for primary freshwater crabs paralleling that of freshwater parastacid crayfish. It was argued that most of the freshwater crab families are present on large landmass fragments of the former Gondwanan continent and they are sharing the bilobed terminal segment of the mandibular palp (Rodríguez, 1986; Ng *et al.*, 1995). Later studies questioned the validity of this hypothesis. Firstly, the inconsistency in the shape of the mandibular palp characters in the Potamonautidae makes it inappropriate for higher phylogenetic relationships (these characters may be homoplastic) (Cumberlidge & Ng, 2009); secondly, due to disagreement of taxonomical and mainly molecular data with area relationship predicted by consecutive splitting up of Gondwana (Daniels *et al.*, 2006b; Klaus *et al.*, 2006, 2009, 2011; Cumberlidge *et al.*, 2008). Thirdly, because of morphological discontinuities that revealed monophyly of the Pseudothelphusidae and the Paleotropical freshwater crabs, and believing separate phylogenetic origin for Trichodactylidae (von Sternberg *et al.*, 1999; von Sternberg & Cumberlidge, 2001). Different scenarios have been recently discussed in the review of Klaus *et al*. (2011) to understand how well each hypothesis fits to the current state of knowledge. At the current point, distribution of the three Old World freshwater crabs (Gecarcinucidae, Potamonautidae and Pseudothelphusidae) is attributed to dispersal rather than splitting up of Gondwana. The Gecarcinucidae independently evolved on the Indian subcontinent before collision with the Eurasian plate and dispersed secondarily into Southeast Asia. Therefore, the African origin of Gecarcinucidae may not be validated (Klaus *et al.*, 2010; Klaus & Streit, 2011). The monophyly of Pseudothelphusidae and Old World freshwater crabs can be explained either by rafting between Neotropics and Afrotropics or a common marine stem group (Cumberlidge & Ng, 2009). Limited distribution of potamids evidences the likelihood of secondarily dispersion into Eurasia from a Gondwanan fragment, because potamids have not actually crossed Wallace’s Line and their territory is not expanded into the main Indian subcontinent (Yeo *et al.*, 2008; Klaus *et al.*, 2011). The Trichodactylidae is placed closely to the marine portunid subfamily Carcininae within the superfamily Portunoidea (Sternberg *et al*., 1999; Sternberg and Cumberlidge, 2001).

Genus *Potamon*

Potamon is a well-known freshwater crab genus with a distribution expanding from the western periphery of the distribution area of the family Potamidae; ranging from the Middle East through southern Europe and the far north of Africa. The genus *Potamon* is the earliest described and one of the best known freshwater crab genera in the world (Yeo & Ng, 2004; Ng *et al.*, 2008). Pretzmann is the pioneer, who entirely studied the genus and for the first time introduced subgenera for *Potamon*. He recognized five subgenera within *Potamon* based on the morphology of the first gonopods (G1): *P. (Potamon)* Savigny, 1816; *P.* (*Euthelphusa*) Pretzmann, 1962; *P.* (*Pontipotamon*) Pretzmann, 1962; *P.* (*Orientopotamon*) Pretzmann, 1962; *P.* (*Centropotamon*) Pretzmann, 1962 (Fig. 1.5). He also introduced a remarkable number of new species and subspecies of *Potamon* (Pretzmann, 1962, 1965, 1966a, 1971, 1976a, b). Brandis *et al*. (1998, 2000) studied the genus *Potamon* and examined material deposited in different museums mainly collected by Pretzmann from Europe, North Africa and the Middle East. His essay resulted in the description of two new species and elevation of three subspecies to species level, while many of Pretzmann's subspecies and other infraspecific taxa were synonymised. He reduced the number of subgenera to four by synonymising the subgenus *Centropotamon* under *Orientopotamon* giving priority to the latter (see Brandis *et al*., 2000). Later, Ng *et al*. (2008) omitted all the subgenera, but a phylogenetic study on the evolution of *Potamon* in the Aegean region reclaimed the validity of subgenera, as they turned out to be congruent with geographic distribution and geological events. Their study also revealed some unknown species to science and the necessity of revalidation for some synonymised species introduced by Pretzmann (Jesse *et al.*, 2011). A total of twenty three nominate species are included in the genus *Potamon* of which eight species, i.e., *P. bilobatum* Brandis *et al*. 2000; *P. persicum* Pretzmann, 1962; *P. ruttneri* Pretzmann, 1962; *P. strouhali* Pretzmann, 1962; *P. transcaspicum* Pretzmann, 1962, *P. elbursi* Pretzmann, 1962 (see Keikhosravi and Schubart, 2013), *P. ilam* (Keikhosravi and Schubart, in press; see Chapter 2) and *P. ibericum* (Bieberstein, 1808) can be found in Iran.

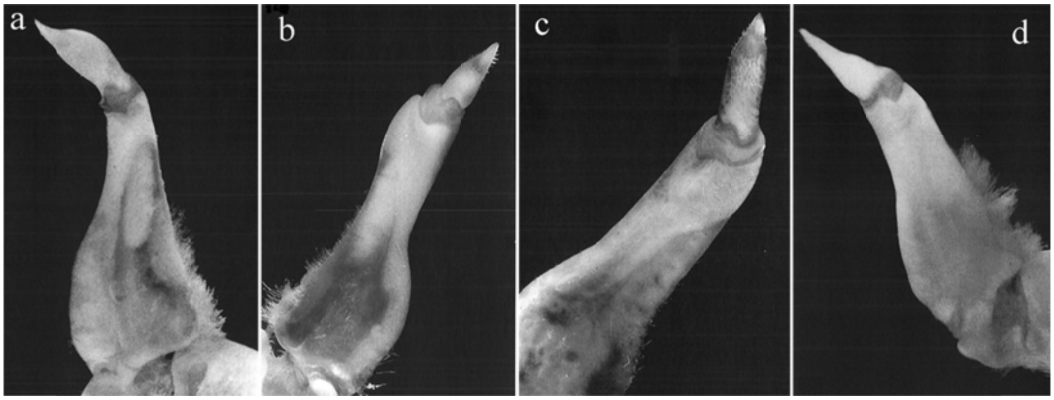


Figure 1.5. First gonopod in four subgenera of *Potamon*. a: *P.* (*Euthelphusa*) *fluviatile*; b: *P.* (*Orientopotamon*) *mesopotamicum*; c: *P.* (*Potamon*) *potamios*; d: *P.* (*Pontipotamon*) *ibericum.* Pictures from Brandis *et al*., 2000.

The aim of the present work

Phylogenetic relationships among freshwater crabs of the genus *Potamon* in the Middle East in particular have been historically neglected and, as a consequence, their phylogeny has been a source of disagreement among specialists (e.g. Pretzmann, 1962, 1963, 1965, 1966a, 1976b; Bott, 1967, 1970; Brandis *et al.*, 1998, 2000). Therefore, it seems crucial to resolve the phylogenetic relationships among the species of *Potamon* and to discover evolutionary significant units of species using different tools to provide an insight into the real biogeography and diversity of this genus. In this regard, this study aims to address the following goals:

- Identification and differentiation between species of the genus *Potamon* from Iran - in particular *P. persicum* complex- using morphological and molecular data in order to answer the following questions: 1) Is morphology-based (and to some degree, geography-based) classification of *Potamon* congruent with molecular similarities and thus the underlying phylogeny? 2) How reliable are morphological characters in separating these species compared to DNA sequences?

- Reconstruction of phylogenetic relationships among different species of *Potamon* in Iran, using different molecular markers to construct a robust framework for understanding speciation history.

- Intraspecific variability among populations of *Potamon elbursi* using molecular methods to understand initial steps of regional differentiation, reconstruct past and current genetic structure and differentiation patterns, and to infer if populations have any conservation value. Additional goal is tracking the likely impact of the Pleistocene glaciations on the population structure.

Chapter 2

Description of a New Freshwater Crab Species of the Genus *Potamon* (Decapoda, Brachyura, Potamidae) from Iran, Based on Morphological and Genetic Characters

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In press: *Crustaceana Monographs*

A new freshwater crab species of the genus *Potamon* Savigny, 1816, from Iran is described. *Potamon ilam* sp. nov. differs from the closely related species *Potamon persicum* Pretzmann, 1962 and *P. mesopotamicum* Brandis *et al*., 1998, by the shape of the first gonopod and other carapace characters. Other differences between these taxa are evident from nuclear 28S rRNA and mitochondrial 16S rRNA gene sequences. The phylogenetic data also show noticeable geographic variation within *P. persicum* from different parts of its range. The present work increases the number of species in the genus *Potamon* to twenty-two.

Introduction

Potamon Savigny, 1816, is a well-known freshwater crab genus with a distribution ranging from the Middle East through to southern Europe and North Africa. Pretzmann (1962, 1965, 1966a, 1971, 1976a, b) revised the taxonomy of the freshwater crabs of the Middle East and described a number of new species and subspecies of *Potamon*. However, Pretzmann's collections did not cover the entire range of *Potamon* in Iran and left large areas unexplored. Brandis *et al*. (2000) revised the entire genus *Potamon* and described *Potamon bilobatum* Brandis, Storch & Türkay, 2000, from Iran, and synonymized a number of Pretzmann's subspecies and infraspecific taxa. A total of twenty-one species are now included in the genus *Potamon* (Brandis *et al*., 2000; Ng *et al*., 2008; Jesse *et al*., 2010, 2011) of which six species, i.e., *P. bilobatum* Brandis *et al*., 2000; *P. persicum* Pretzmann, 1962; *P. ruttneri* Pretzmann, 1962; *P. strouhali* Pretzmann, 1962; *P. transcaspicum* Pretzmann, 1962, and *P. ibericum* (Bieberstein, 1808) are found in Iran.

Taxonomic revisions of *Potamon* focused on the Middle East are important for two reasons. First, it has been suggested that the European freshwater crab fauna originated in the Middle East (Pretzmann, 1987a, b; Klaus & Gross, 2010; Jesse *et al*., 2011) and so a better resolution of the taxonomy and phylogeny of *Potamon* in this area promises to provide important insights into the biogeography and diversity within this genus (Brandis *et al*., 2000). Second, the Middle East was recently a center of orogenic activities (viz., the current structure of Zagros Mountains) that may have resulted in the isolation of river systems in the past (Kinzelbach, 1980; Banarescu, 1991; Rangzan & Iqbaluddin, 1995).

The present describes a new species from the Zagros Mountains of Iran based on morphological and molecular evidence. This is the seventh species of *Potamon* from Iran.

Material and Methods

Specimens were collected during three field trips in 2009 and 2010 (Fig. 2.1, table 2.1). Specimens were preserved in 70 % ethanol for morphological studies. One walking leg from each individual was removed and preserved in 100% ethanol for molecular studies. Two specimens of *P. mesopotamicum* (SMF 23315) from Syria and *P. transcaspicum* (ZUTC Pot.1088) from Iran (see table 2.1) were used for tissue and DNA extraction for phylogenetic analyses. The studied specimens are deposited in the following collections: Zoology Museum, University of Tehran (ZUTC); Senckenberg Research Institute and Natural History Museum, Frankfurt am Main (SMF); Natural History Museum, London (NHM); Naturhistorisches Museum, Wien (NHMW); Zoological Reference Collection of the Raffles Museum of Biodiversity Research, National University of Singapore (ZRC); and Netherlands Centre for Biodiversity Naturalis, Leiden (RMNH).

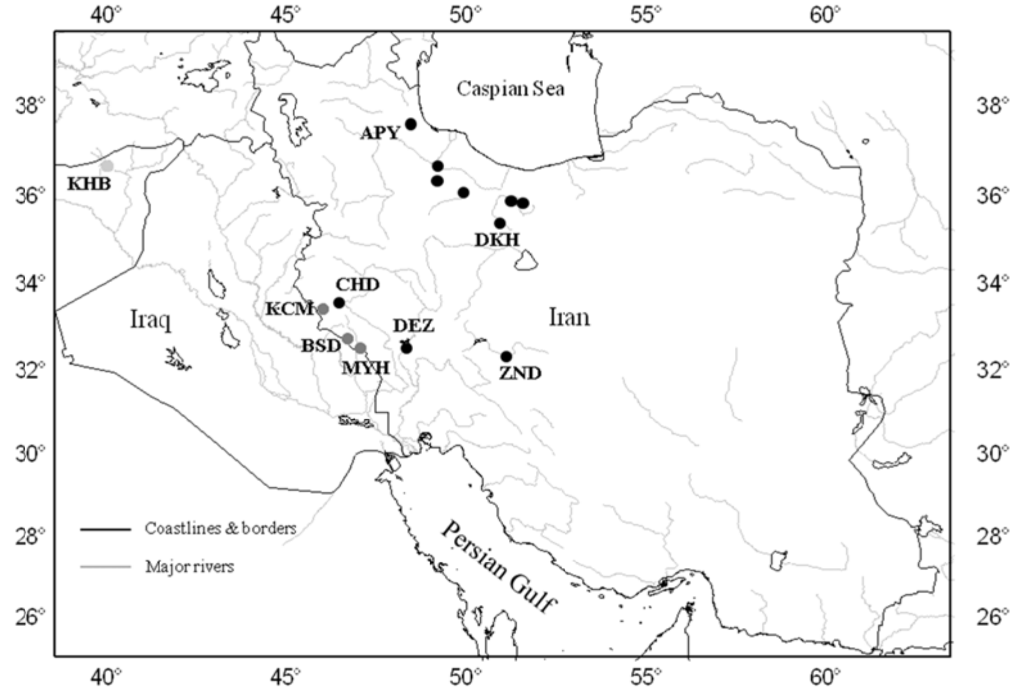

Figure 2.1. Map of Iran showing the principal rivers. Black circles represent the sampling sites for *Potamon persicum* Pretzmann, 1962, dark gray circles represent sampling sites for *P. ilam* sp. nov., and light gray circles represent sampling sites for *P. mesopotamicum* Brandis, Storch &Türkay, 1998. For abbreviations see table 2.1.

DNA was isolated from the muscle tissue extracted from walking legs using a modified Puregene method (Gentra Systems). One mitochondrial (16S rRNA) and one nuclear (28S rRNA) ribosomal gene were partially amplified using the following primer combinations: 16L29 (5'-CATATTATCTGCCAAAATAG-3') and 16HLeu (5'-YGCCTGTTTATCAAAAACAT-3') (Schubart, 2009) for the 16S rRNA, 28L9 (5'-GACCCGTCTTGAAACACGG-3') (newly designed) and 28Sb (5'-TCGGAAGGAACCAGCTACTA-3') (Whiting *et al.*, 1997) for the 28S rRNA. Polymerase chain reaction (PCR) was carried out under the following conditions: 94°C 45s / 48°C 1min / 72°C 1min (40 cycles) for 16S rRNA (16S) and 97°C 45s / 58°C 1min / 72°C 1min (40 cycles) for 28S rRNA (28S) (denaturing / annealing / extension, respectively). The sequences were obtained with an ABI Prism 310 Genetic Analyser (Applied Biosystem, Foster City, USA) or by outsourcing to LGC Genomics. The sequences were corrected manually with BioEdit (version 5.09; Hall, 1999), aligned with Mafft (version 6; Katoh *et al*., 2002), and deposited at the European Molecular Database EMBL (accession numbers in table 1.1). In addition, a sequence of *P. persicum* from GenBank (FM180116) (Klaus *et al*., 2009) was included in the analyses.

A phylogeny based on a 644 basepair alignment of the 16S mitochondrial DNA (mtDNA) was inferred by Bayesian Inference (BI) and performed with MrBayes (version. 3.1.2; Huelsenbeck & Ronquist, 2001). The appropriate substitution model was previously evaluated using MrModeltest (version. 2.3; Nylander, 2004). The best model was selected by the Akaike information criterion (AIC). Four Montecarlo Markov chains (MCMC) were run for 2,000,000 generations, saving trees every 500 generations. The –ln L converged on a stable value between 5,000 and 10,000 generations (burn-in phase). Consequently, the first 20,000 generations were excluded from the analysis. The topology and posterior probabilities of the phylogeny were determined by constructing a 50% majority-rule consensus tree using the sumpt option in MrBayes. *Potamon ibericum* (ZUTC Pot.1089) from Iran served as outgroup for phylogenetic reconstructions because it belongs to a distinct morphological group (a former subgenus).

A statistical parsimony network analysis was constructed using the program TCS version 1.21 (Clement *et al.*, 2000) for twelve nuclear 28S rRNA sequences (accession numbers in table 2.1) with an alignment length of 646 bases, in order to represent the relationships among the closely related sequences (table 2.1, Fig. 2.6).

Characters of the first gonopod, median tooth of gastric mill, cheliped, walking legs, carapace and abdomen were observed under light microscopy. Figures were prepared using a microscope fitted with a camera lucida. The gastric mill terminology follows that used by Naderloo *et al*. (2010).

Taxonomy

Family Potamidae Ortmann, 1896

Genus *Potamon* Savigny, 1816

Potamon ilam sp. nov. (Fig. 2.2a-c)

Material examined — Holotype: 1 ♂ (38.27 x 31.01 carapace width x carapace length in mm) (SMF 39025), Iran: Ilam Province, 82 km southeast of Mehran, road to Dehloran, Bishehderaz River, 361 m asl, 32º49.412´N 46º58.346´E, leg. A. Keikhosravi, B. Fathinia & M. Moradmand, 17.04.2009. Paratypes: 1 ♂, 1 ♀, 1 j (j) (44.64 x 35.20, 46.10 x 37.26, 22.69 x 18.15) (SMF 39026), 2 ♂♂, 1 ♀, 1 j (35.13 x 28.02, 35.90 x 28.34, 46 x 36.17, 26.50 x 21.69) (NHMW 25425), 1 ♂, 1 ♀, 1 j (42.80 x 33.81, 38.05 x 30.46, 21.46 x 17.18) (RMNH-D 54892), 1 ♂, 1 ♀, 2 j (31.25 x 24.24, 37.74 x 30.36, 18.50 x 14.73, 16.85 x 13.63) (ZUTC Pot.1077), same collection data as holotype; 1 ♂, 4 ♀♀, 6 j (34.19 x 27.30, 40.98 x 32.77, 33.40 x 26.73, 35.42 x 28.20, 34.24 x 27.31, 18.86 x 15.38, 28.80 x 22.78, 19.35 x 15.41, 28.90 x 23.10, 23.90 x 18.65, 27.80 x 21.53) (SMF 39027), Ilam Province, 43 km southwest of Ilam, road to Mehran, Konjacham River, 33°17.350'N 46°15.130'E, leg. A. Keikhosravi, B. Fathinia & M. Moradmand, 16.04.2009; 1 ♂, 1 ♀, 5 j (41.74 x 32.76, 46.30 x 36.36, 27.08 x 21.91, 25.40 x 20.21, 26.74 x 21.74, 17.61 x 14.06, 15.68 x 12.66) (SMF 39028), 2 ♂♂, 1 ♀, 4 j (37.60 x 29.48, 29.10 x 23.74, 34.50 x 27.93, 26.31 x 21.47, 19.80 x 16.13, 16.75 x 13.44, 17.32 x 14.03) (ZRC 2012.0162), 1 ♂, 3 j (37.89 x 30.80, 24.65 x 19.83, 26.76 x 21.45, 16.40 x 13.11) (ZUTC Pot.1078), Ilam Province, 12 km northwest of Dehloran, near to Meymeh, Meymeh River, 32°44.6'N 47°9.366'E, leg. A. Keikhosravi, B. Fathinia & M. Moradmand, 17.04.2009.

Diagnosis — Posterolateral region of the carapace gently flexed downward. Terminal segment of the first gonopod (G1) conical in shape, mesial part straight, slight distal depression, lateral part slightly convex (Fig. 2.3a-c).

Description — Carapace distinctly broader than long ; upper surface of carapace convex, glabrous, smooth; anterolateral region granular; posterolateral region with curved finely granulated ridges (carinae), nearly parallel, variably sized, last one longest, nearly parallel with posterior margin; posterolateral margins strongly converging posteriorly.

Cervical groove distinct, deeper anteriorly; H-shaped depression shallow, distinct. Cephalothoracic region slightly convex; mesogastric region distinct, almost smooth; mesogastric grooves well defined; epigastric region visible, separated from postorbital crest by shallow groove. Frontal ridge low, granulated; frontal region flexed downward, strongly granulated, depressed medially, almost bilobed, with field of short, faint setae; frontal margin gently sinuous, decorated by blunt, hardly visible granules; postfrontal crest sharp, very prominent and detached from anterolateral region by deep cervical groove. Inner supraorbital margin smooth, outer margin finely tuberculated under magnification; infraorbital margin finely tuberculated. Exorbital tooth triangular, distinct, blunt, lateral side slightly serrate. Anterolateral margin arched, bending inward posteriorly; first epibranchial tooth short, blunt; epibranchial denticles (31- 43) short, slightly serrate. Posterolateral region gently flexed downward, with short and hardly visible setae sparsely covering it. Epistome smooth laterally with some short setae; slightly granulated medially.

Third maxilliped ischium with deep median sulcus; exopod with flagellum nearly as long as merus. Anterior part of suborbital lobe smooth, glabrous; posterior region with curved lines of tubercles.

Thoracic sternites smooth, glabrous, pilose only at margins of segments. ♂ pleon long, triangular, smooth; lateral margins with short, dense setae; abdominal segments 2-6 progressively longer; telson longer than abdominal segment 6, tip rounded.

Male chelipeds medium-sized, unequal. Merus with upper margin serrate; inner-ventral margin granular, granules with wide base, progressively larger from proximal to distal; outer-ventral margin granular, progressively larger from proximal to distal; outer surface smooth, becoming serrated near upper margin; inner surface smooth; ventral surface with distinct spine-shaped tooth on distal part near inner-ventral margin. Carpus with large acute medial spine on upper-inner margin, 1-2 smaller spines at base of large spine; outer surface with transverse granulated rows, slightly depressed distally. Palm with outer surface slightly swollen, nearly smooth, transverse rows of small granules on median portion, becoming larger dorsally; ventral margin completely smooth; dorsal margin denticulate; inner surface of palm smooth. Movable dactyl as long as propodal pollex, dorsal longitudinal row of small granules, disappearing at distal half; cutting edges with distinct row of conical teeth.

Pereiopods 2-5 (P2 – P5) relatively long, P3 longest (about 1.4 times as long as CW), P5 shortest; dorsal margin of merus, carpus and propodus of P2-P5 slightly serrate, dorsal margin of merus with subdistal notch; carpus with longitudinal carinae, medially on posterior surface, carpus of P5 without carinae; propodus with two rows of spines along ventral margin, spines larger distally; propodus of P2 with three rows of spines; dorsal margin of dactylus with two rows of 4-8 sharp spines, proximal to distal progressively larger, ventral margin with two rows of 2-4 spines, proximal to distal progressively larger; spines of dorsal margin slightly larger than those of ventral margin; three rows of short setae along dorsal and ventral margins.

Male first gonopod (G1) sinuous, gently curving outward; terminal segment conical in shape, mesial margin nearly straight, slightly concave distally, lateral part slightly convex. Flexible zone well developed. Subterminal segment slender; mesial edge subapically swollen, laterally bent, proximally covered by setae, disappearing distally; lateral margin strongly curved (sinus shape), with scattered setae near base (Fig. 2.3a-c).

Gastric mill plate elongated, goblet shaped, slender at base; median tooth with three teeth; first one largest with bilobed appearance; second tooth slightly bilobed, laterally extended; third one very small, hardly discernible (Fig. 2.4).

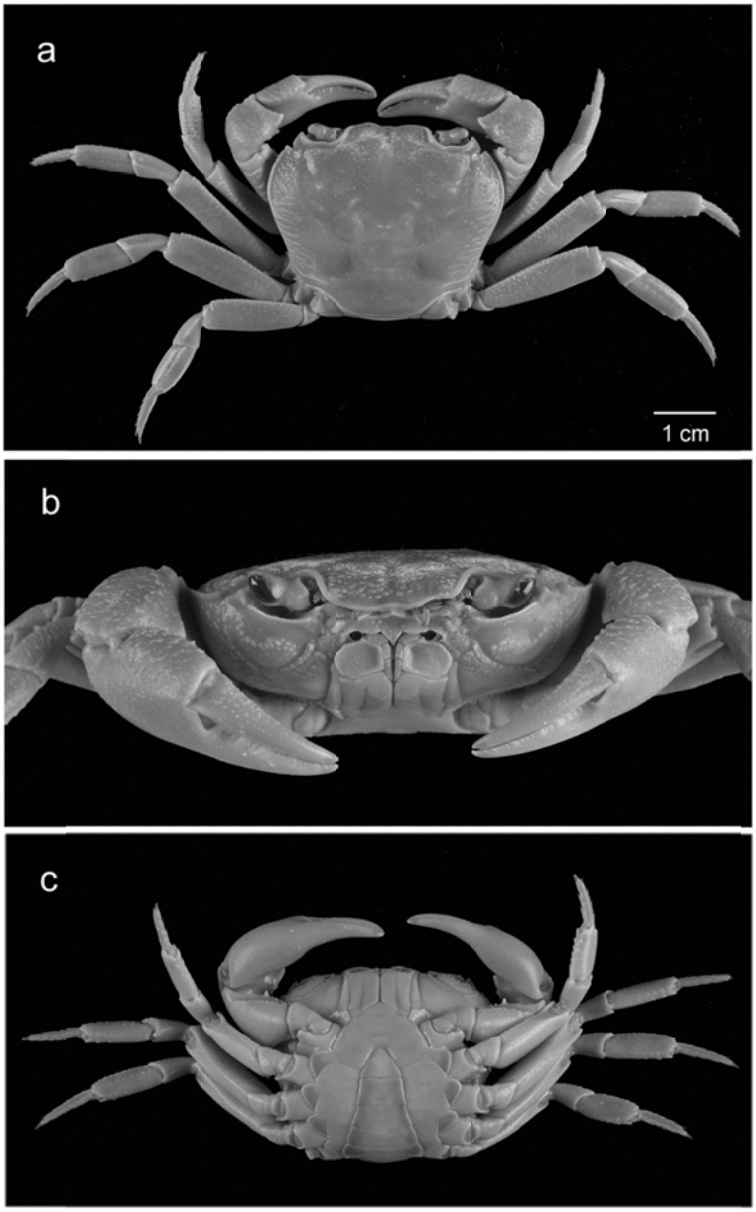

Figure 2.2. ***Potamon ilam*** **sp. nov., holotype (SMF 39025). a, dorsal view; b, frontal view; c, ventral view.**

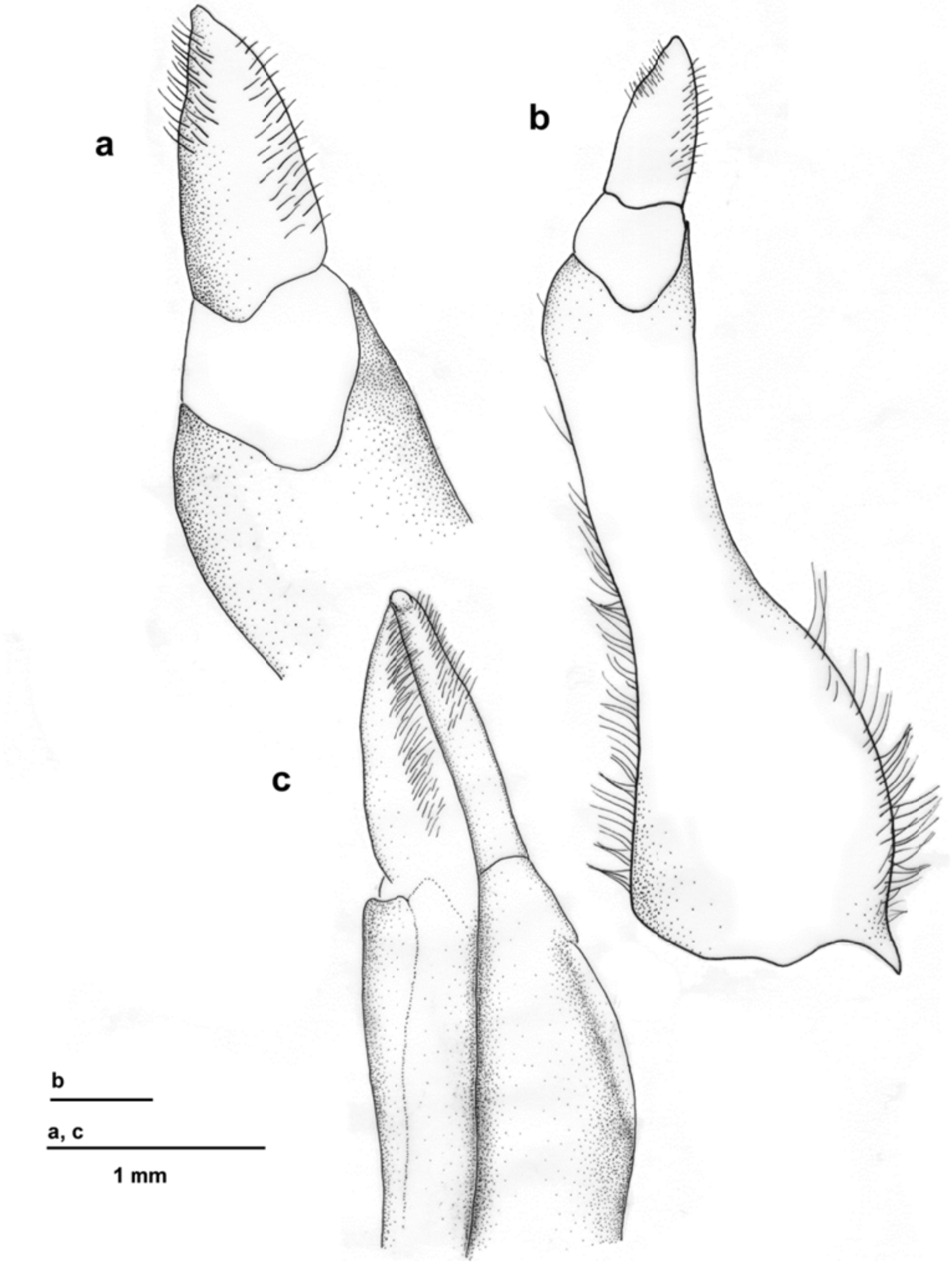

Figure 2.3. ***Potamon ilam*** **sp. nov., holotype (SMF 39025). a-b, right G1 (dorsal view); c, ventral aspect of terminal article.**

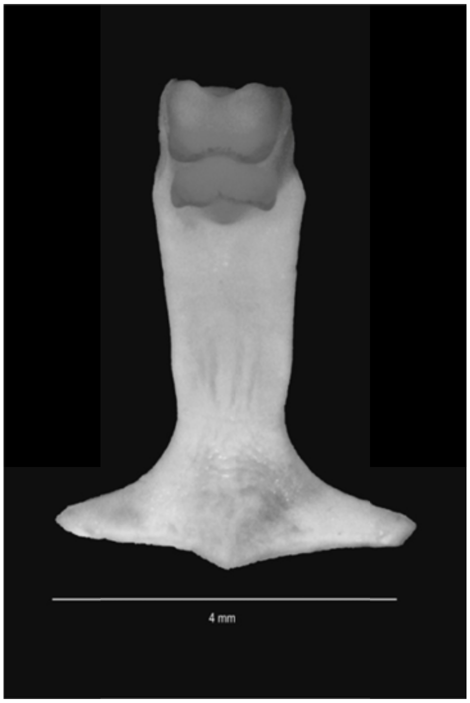

Figure 2.4. *Potamon ilam* sp. nov., paratype (SMF 39028). Ventral surface of median tooth plate of gastric mill.

Remarks — *Potamon ilam* sp. nov. is most similar to *P. persicum* and *P. mesopotamicum* in terms of G1 morphology . The new species is easily distinguished from these and other species of *Potamon* by characters of the carapace and G1. The terminal article of the G1 in *P. ilam* is conical, with the mesial margin slightly concave distally (Fig. 2.3a-c) whereas that of *P. persicum* and *P. mesopotamicum* is nearly triangular, with straight mesial margins in the proximal half, becoming sharply convergent distally (Brandis *et al*., 2000: figs. 11c-d, 12 c-d). The posterolateral carapace region in *P. ilam* is gently flexed downward, with relatively long curving parallel tuberculated ridges (Fig. 2.2a), whereas in *P. persicum* and *P. mesopotamicum* this margin is arc-shaped, with only short tuberculations (Brandis *et al*., 2000: figs. 11a-b, 12a-b). The anterolateral margin of the carapace of *P. persicum* and *P. ilam* is slightly cristate, whereas that of *P. mesopotamicum* is distinctly cristate. These characters, together with others that distinguish between *P. persicum*, *P. mesopotamicum* and *P. ilam* are summarized in table 2.2.

Size — *Potamon ilam* sp. nov. is a medium-sized species, the largest ♂ encountered measured 35.2 mm in carapace length (CL) and 44.64 mm in carapace width (CW), and the largest ♀ 36.4 mm CL and 46.3 mm CW.

Etymology — The species name is derived from the type locality in Ilam Province in the west of Iran whose capital city is Ilam.

Colour — Carapace and walking legs dark grey/blue. The outer surfaces of the fingers of the chelipeds (dactylus, propodus), and of the carpus and merus are all bright orange.

Distribution — *Potamon ilam* n. sp. is found in most of the rivers in Ilam Province that drain into the Tigris River (except for some rivers in the eastern part), but it is not found in the Tigris River itself (where *P. mesopotamicum*, *P. persicum,* and *P. magnum* occur) (Pretzmann, 1962).

Molecular results

The tree based on mtDNA (16S rRNA gene) had a total alignment length of 644 basepairs, and GTR +I (with proportion of invariable sites = 0.8358) was chosen as the best evolutionary model of substitution by MrModeltest. The model was used to construct a phylogenetic tree with ten specimens with MrBayes.

Two main clades can be distinguished (Fig. 2.5), one that unites *P. ilam* sp. nov. and *P. mesopotamicum*, and a second one that unites different populations of *P. persicum*. These clades are well supported by posterior probability values and group taxa that are found in the eastern and western slopes of the Zagros Mountains. The clade that includes *P. persicum* is subdivided into two groups (that each has weak support) that correspond well with the geographical distribution of the taxa. The remarkably low genetic distances (based on 16S rRNA) between the two clades of *P. persicum* (0.008 to 0.01, mean = 0.009) are unexpected and warrant further investigations of the genetic and morphometric structure of the populations over the entire range of this species (Keikhosravi & Schubart, in prep).

A 646 basepair DNA fragment of the nuclear 28S rRNA was obtained for a subset of specimens (see table 2.1). The conservative nature of the 28S rRNA locus means that it has only limited resolution power in this context and only four genotypes were identified by this marker.

The haplotypes were separated by less than six substitutions from neighboring haplotypes, and the most frequent haplotype (six individuals from five populations) was found in *P. persicum*. Two haplotypes were found among three populations of *P. ilam* separated by 1-3 mutation steps from *P. persicum* and 4-6 mutation steps from *P. mesopotamicum* (see Fig. 2.6).

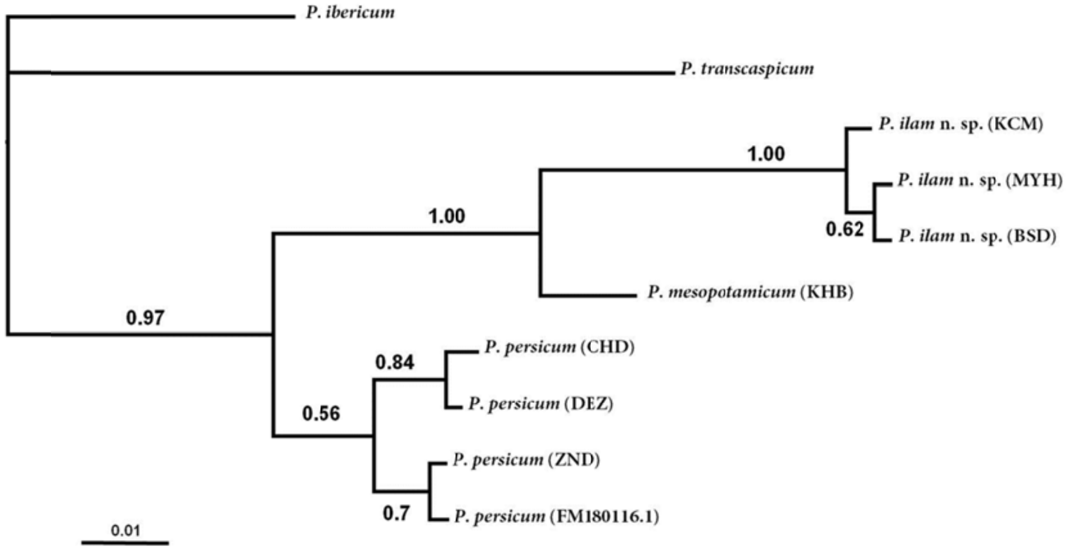

Figure 2.5. Phylogenetic relationships of *Potamon ilam* sp. nov. and related species according to a Bayesian analysis (GTR+I) of a 644 basepair alignment of 16S ribosomal mtDNA. Posterior probability values show support for the corresponding clades. Terminal taxa labels are provided in Fig. 2.1 and table 2.1.

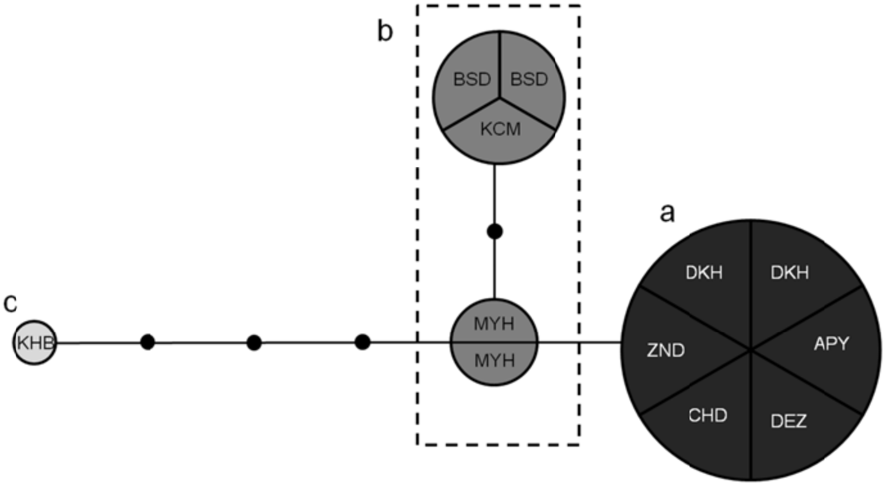

Figure 2.6. Maximum parsimony spanning network constructed with TCS of *Potamon ilam* sp. nov. and related species, based on a 646 basepair alignment of 28S ribosomal nuclear DNA. The size of the circle is proportional to the frequency of the haplotypes; each line represents one substitution and dots on lines indicate additional substitutions. a, *P. persicum* Pretzmann, 1962, from five different rivers in Iran; b, *P. ilam* sp. nov. collected from three adjacent rivers in Iran; c, *P. mesopotamicum* Pretzmann, 1962 from Syria. See Fig. 2.1 and table 2.1 for haplotype labels.

Table 2.1. Specimens used for DNA sequencing with collection number, locality, coordinate and GenBank accession numbers.

Species	Voucher number	Locality	Coordinate	Label	Accession no. of 16S	Accession no. of 28S
P. ibericum	ZUTC Pot.1089	Iran, Gorgan, Naharkhoran.	36°45.68'N 54°28.74'E	------	HE963848	----------
P. ilam n. sp.	SMF-39028	Iran, Ilam Prv., Meymeh R.	32°44.60'N 47°9.366'E	MYH	HE963847	HE970761
P. ilam n. sp.	ZUTC Pot.1078	Iran, Ilam Prv., Meymeh R.	32°44.60'N 47°9.366'E	MYH	-----------	HE970762
P. ilam n. sp.	SMF-39026	Iran, Ilam Prv., Bishehderaz R.	32º49.41´N 46º58.34´E	BSD	HE963846	HE970759
P. ilam n. sp.	SMF-39025	Iran, Ilam Prv., Bishehderaz R.	32º49.41´N 46º58.34´E	BSD	-----------	HE970760
P. ilam n. sp.	SMF-39027	Iran, Ilam Prv., Konjacham R.	33°17.35'N 46°15.13'E	KCM	HE963845	-----------
P. ilam n. sp.	SMF-39027	Iran, Ilam Prv., Konjacham R.	33°17.35'N 46°15.13'E	KCM	-----------	HE970758
P. persicum	SMF-39030	Iran, Khuzestan Prv., Dezful, Dez R.	32°23.28'N 48°23.67'E	DEZ	HE963843	HE970753
P. persicum	SMF-39029	Iran, Ilam Prv., Chardavol R.	33°40.17'N 46°41.48'E	CHD	HE963842	HE970752
P. persicum	SMF-39031	Iran, Isfahan, Zayandehrood R.	32°38.32'N 51°35.97'E	ZND	HE963844	HE970754
P. persicum	SMF-39038	Iran, Khalkhal, Arpachay R.	37°40.92'N 48°30.23'E	APY	-----------	HE970757
P. persicum	SMF-39032	Iran, Tehran, Darakeh R.	35°49.09'N 51°22.85'E	DKH	-----------	HE970755
P. persicum	ZUTC Pot.1082	Iran, Tehran, Darakeh R.	35°49.09'N 51°22.85'E	DKH	-----------	HE970756
P. mesopotamicum	SMF-23315	Syria, Al-Hassakah Prv., Al-Khabur R.	36°42'N 40°11'E	KHB	HE963849	HE970763
P. transcaspicum	ZUTC Pot.1088	Iran, Sabzevar, Zardkoohi R.	36°17.29' N 57°23.34'E	------	HE963841	-----------

Table 2.2. Distinct morphological differences between *Potamon ilam* n. sp., *P. persicum* Pretzmann, 1962 and *P. mesopotamicum* Brandis, Storch &Türkay, 1998.

Character	***Potamon ilam* n. sp.**	***Potamon mesopotamicum***	***Potamon persicum***
G1 (terminal segment)	Conical	Triangular or nearly triangular	Nearly triangular
	Mesial part nearly straight only slightly concave distally	Proximal half of mesial part straight, getting sharply convergent in distal half or distinctly triangular	Proximal half of mesial part straight, getting sharply convergent in distal half
	Lateral part slightly convex	Lateral part almost straight	Lateral part Gently straight
Cervical Groove	Distinct and deep	Shallow	Very shallow
Anterolateral surface	Long rows of small granules(>12), transversely arranged	Short or long rows of small granules, transversely arranged	Many short rows of larger granules (>6), irregularly arranged
Anterolateral teeth	Short and blunt	Slightly long and sharp, slightly upward	Short and blunt
Anterolateral margin	Slightly cristate, lined with small granules	Distinctly cristate, lined with small granules	Slightly cristate, lined with small granules
Posterolateral surface ridge	Curved tuberculation, relatively long, nearly parallel	No tuberculation	Short tuberculation
Posterolateral region	Gently flexed downward	Arc shaped	Arc shaped
Mesogastric sulcus	Shallow and short mesogastric	Deep and more elongated	Deep and more elongated
Notch between exorbital and first anterolateral teeth	Small, almost contiguous view	Large U-shaped notch	Small, almost contiguous view
Colour	Dark grey bluish	Greyish-brown or grey bluish	Dark green
Largest male body size (carapace width x carapace length in mm)	44.64 x 35.2 mm	55.2 x 45.2 mm	79.05 x 64.64 mm

Comparative material — *Potamon persicum*, Iran: 3 ♂♂, 1 j (SMF 39029), 3 ♂♂, 1 ♀ (NHMW 25426), 2 ♂♂, 1 j (ZUTC Pot.1079), Ilam Province, 25 km West of Ilam, Chardavol River, Shemsheh Strait, 33°40.177'N 46°41.489'E, leg. A. Keikhosravi, B. Fathinia & M. Moradmand, 15.04.2009; 2 ♂♂, 1 j (NHMW 25427), 2 ♂♂, 1 j (SMF 39030), 2 ♂♂ (ZUTC Pot.1080), Khuzestan Province, Dezful, Dez River, 32°23.282'N 48°23.679'E, leg. A. Keikhosravi & M. Moradmand, 18.04.2009; 4 ♂♂, 1 ♀ (SMF 39031), 3 ♂♂ (NHMW 25428), 3 ♂♂, 1 j (ZUTC Pot.1081), Isfahan Province, Isfahan, Zayandehrood River, 32°38.322'N 51°35.977'E, leg. A. Keikhosravi & M. Moradmand, 19.04.2009; 2 ♂♂ (SMF 39032), 2 ♂♂ (RMNH-D 54893), 3 ♂♂ (NHMW 25429), 2 ♂♂, 1 j (ZUTC Pot.1082), Tehran Province, Tehran, Darakeh, Darakeh River, 35°49.092'N 51°22.855'E, leg. A. Keikhosravi, 28.04.2009; 2 ♂♂, 1 ♀ (RMNH-D 54894), 2 ♂♂ (NHMW 25430), 3 ♂♂ (SMF 39033), 2 ♂♂, 1 ♀ (ZUTC Pot.1087), Guilan Province, Roodbar, Sepidrood River, 36°49.060'N 49°25.256'E, leg. A. Keikhosravi & M. Houshmand, 23.12.2009; 2 ♂♂, 2 j (SMF 39034), 1 ♂, 1 j (RMNH-D 54895), 2 ♂♂, 2 j (ZUTC Pot.1083), Alborz Province, Taleghan, Thaleghan River, 36°10'8.22"N 50°45'45.45"E, leg. F. A. Houshmand, 18.08.2009; 3 ♂♂, 2 ♀♀ (SMF 39035), 4 ♂♂, 1 ♀ (NHMW 25431), 3 ♂♂, 2 j (RMNH-D 54896), 2 ♂♂, 1 ♀, 1 j (ZUTC Pot.1084), northwest of Qazvin, 55km to Qazvin, Molaali River, Trib to Shahrood R., 36°27.742'N 49°30.868' E, leg. Y. A. Houshmand & A. Keikhosravi, 03.09.2010; 1 ♀, 1 ♂, 1 j (SMF 39036), 1 ♂, 2 j (ZUTC Pot.1085), Tehran, Lavasan, 3km west of Latyan Dam 35°48'46.44"N 51°36'03.54"E, leg. Y. Houshmand & A. Keikhosravi, 13.11.2009; 2 ♂♂, 3 ♀♀ (SMF 39037), 3 ♂♂, 3 ♀♀, 1 j (NHMW 25432), 30 km northeast of Tehran, Kamard, Trib to Jajrood River, 35°46'20.98"N 51°45'15.80"E, leg. A. Keikhosravi, Y. A. Houshmand & Heydari, H., 01.10.2009; 1 ♂, 2 ♀♀ (SMF 39038), 2 ♀♀ (ZUTC Pot.1086), 5 km north of Khalkhal, Arpachay River, 37°40.926'N 48°30.237'E, leg. Y. Houshmand & A. Keikhosravi, 02.09.2010; 1 ♂ (NHM 1920.2.5.3-4), northwest of Qazvin, leg. P. A. Buxton, R. Gurney, 1920; 3 ♂♂, 2 ♀♀, 13 j (NHM 1899.10.6.1-5), Azarbayejan-e Gharbi Province, near Orumiyeh, Northwest of Iran, leg. R. J. Gunther, 1899; 1 ♂ (ZMUC CRU-9522), Kermanshah, no further locality information, leg. S. W. Kaiser, 1937; 1 ♂ (SMF 2640), Tehran Province, Tehran, leg. F. Bruhns; 1 ♂, 1 ♀ (SMF 4157), road of Abu-Ali, 30 km off Tehran, leg. J. Theodorides, 1959. Iraq: 1 ♂ (NHM 1934.8.29.1-6), stream on Kirkuk road, 16 km west of Sulaymaniyah, 760 m, 35°33'N 45°16'E, leg. MacFadyen, 1933. Turkey: 2 ♂♂, 1 ♀ (SMF 5881), 2 ♂♂, 2 ♀♀ (SMF 5882), Van, Mengene Daglari, SE of Van Gölu, Baskale, leg. Lampe, 1972.

Potamon mesopotamicum, Syria: Holotype: 1 ♂ (SMF 23311), Source of Al-Khabur River, Ras Al-Ain, 36°51'N 40°04'E, leg. F. Krupp, D. Kock & H. Martens, 1989; paratypes: 13 ♂♂, 13 ♀♀, 14 j (SMF 23312), same data as holotype; 1 ♂ (SMF 23315), Al-Hassakah Province, Al-Khabur River, Tell Atash, 36°42'N 40°11'E, leg. F. Krupp, D. Kock & G. Eppler, 1988; 2 ♂♂ (SMF 23318), Al-Khabur River, Ras Al-Ain, 36°51'N 40°04'E, leg. F. Krupp, D. Kock & G. Eppler, 1988; 1 ♂ (SMF 23320), Al-Khabur River, Tell Shaikh Hamed, 35°37'N 40°45'E, leg. F. Krupp & H. Martens, 1989; 1 ♂ (SMF 23326), Al-Khabur River, Bahrat Khatuniya, 36°24'N 41°13'E, leg. F. Krupp & W. Schneider, 1986; 3 ♂♂ (SMF 23331), Euphrates at Halabiyeh, leg. R. Kinzelbach, 1978; 1 ♂ (SMF 23332), Euphrates tributary N of Maadan, leg. R. Kinzelbach, 1978. Iraq: 1 ♂, 1 ♀ (ZRC 2009.0693), Al-Huwaizah Marshes.

Discussion

The phylogeny and taxonomy of freshwater crabs in general, and particularly within the genus *Potamon* in the Middle East, has only recently been studied in detail by specialists. Formerly, five subgenera were recognized within *Potamon* based on G1 morphology: *P. (Potamon)* Savigny, 1816; *P. (Euthelphusa)* Pretzmann, 1962; *P. (Pontipotamon)* Pretzmann, 1962; *P. (Orientopotamon)* Pretzmann, 1962; *P. (Centropotamon)* Pretzmann, 1962. Brandis *et al*. (2000) synonymised the subgenus *Centropotamon* under *Orientopotamon* giving priority to the latter. Later, Ng *et al*. (2008) omitted all the subgenera, but a phylogenetic study on evolution of *Potamon* in the Aegean region supported the recognition of these subgenera that were also congruent with geographic distributions and past geological events (Jesse *et al*., 2011).

All rivers populated by *P. ilam* rise on the westernmost slopes of the Zagros Mountains in western Iran. These rivers belong to the Euphrates-Tigris system and drain westward into the Tigris River in eastern Iraq. *Potamon ilam* and *P. mesopotamicum* are phylogenetically closer to each other than both are to *P. persicum* in terms of mtDNA, and both are distributed beyond the Zagros Mountains. These taxa share a large drainage system: *P. mesopotamicum* occurs in the Khabur River, the Euphrates River (in the region from the mouth of the Khabur River upstream) and in the Al-Huwaizah marshes, Iraq (downstream of Tigris River) (Brandis *et al.*, 1998; Naser, 2009). The presence of *P. magnum* and *P. persicum* in the headwaters of the Tigris River suggests that *P. ilam* may have limits to its dispersal in this direction. The fact that three different species of freshwater crabs have evolved to co-exist in a single drainage system emphasizes their abilities to adapt to different local conditions.

The average 16S rRNA nucleotide divergence between the clades for *P. persicum* and *P. ilam* (*P. ilam* and *P. mesopotamicum*) is 2.3%. This represents a separation of about 3.6 my based on the substitution rate proposed by Schubart *et al*. (1998) (0.65% per million years for non-endemic Jamaican freshwater crab species). The separation time between these taxa also corresponds to isolation events associated with the orogenic events occurring in the Zagros Mountains at that time. The Zagros Mountains formed when the Arabian and Eurasian plates collided during the Miocene (Takin, 1972; Agard *et al.*, 2011), but the current uplift (Zagros Simply Folded Belt) of the Zagros Mountains took place 4-5 mya, concomitant with the opening of the Red Sea (Rangzan & Iqbaluddin, 1995; Agard *et al*., 2011). The generally poor dispersal abilities of freshwater crabs mean that vicariance events such as mountain formation could have resulted in the reproductive isolation of crab populations (Banarescu, 1991; von Sternberg *et al.*, 1999; Shih *et al.*, 2006, 2009). The formation of the Zagros Mountains in the recent past may have blocked gene flow between populations of *Potamon* that then speciated, producing *P. ilam* and *P. mesopotamicum*.

The present study brings the number of species in the genus *Potamon* to 22. This number may increase further when the results of studies on the *P. persicum* complex have been completed, because this taxon may comprise one or more cryptic species (Keikhosravi & Schubart, unpublished). The vast range of this species in Iran (about 518,000 km^2), which includes a diverse topography and climate zones, requires characterization of the evolutionary diversity within the *P. persicum* complex. Future morphological and molecular studies based on larger sampling are needed to resolve the phylogenetic relationships within the *P. persicum* and *P. mesopotamicum* complexes, and to reveal the true diversity of Iran’s freshwater crabs.

Acknowledgements

The first author would like to express his special gratitude to the people who helped to collect specimens: Mr. Yadollah Houshmand, Fatollah Houshmand, Behzad Fathinia and Madjid Moradmand. We are grateful to Dr. Jørgen Olesen (ZMUC), Dr. Paul Clark (NHM), Prof. Michael Türkay (SMF), and Prof. Peter K. L. Ng (ZRC) for loaning us material from their collections. We extend our gratitude to Dr. Reza Naderloo for his useful comments and excellent illustrations, and to Nicole Rivera and Richard Landstorfer for their help with TCS and MrBayes. Prof. Jürgen Heinze (University of Regensburg) and his staff are also thanked for the use of their facilities and for their continuing support throughout this project.

Chapter 3

Revalidation and redescription of *Potamon elbursi* Pretzmann, 1976 (Brachyura, Potamidae) from Iran, based on morphology and genetics

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Published in: *Central European Journal of* Biology, 8(12), 2013

In the current study, we redescribe and revalidate a freshwater crab species of the genus *Potamon* Savigny, 1816, from northern Iran. *Potamon elbursi* Pretzmann, 1962, differs from the species it has been synonymised with, *Potamon persicum* Pretzmann, 1962, mainly by the shape of the male first gonopods. Consistent and marked genetic divergence was also recognised in the mitochondrial 16S rRNA and cytochrome oxidase subunit I genes. This study elevates *P. elbursi* to species rank and therefore increases the number of valid species of the genus *Potamon* to a total of twenty-two.

Introduction

Potamon Savigny, 1816, is a well known freshwater crab genus with a distribution ranging from the Middle East through southern Europe and the far north of Africa (Savigny, 1816). Currently, there are a total of twenty-one recognized species in the genus *Potamon* (Jesse *et al*., 2011). The phylogeny and taxonomy of the genus in the Middle East has only recently been studied and dealt with in detail by few specialists. Pretzmann (Pretzmann, 1962, 1965, 1966a, 1971, 1973,1976a, b) was the first to extensively survey freshwater crabs in the Middle East, introducing a remarkable number of new species and subspecies (as well as other infraspecific categories) of *Potamon*. Brandis *et al*. (1998, 2000) studied the first male gonopods (G1) of *Potamon* from different museum collections, mainly gathered by Pretzmann. This survey resulted in the description of two new species and elevation of three subspecies to species level, while many of Pretzmann's subspecies and other infraspecific taxa were synonymised. In the process, Brandis *et al*. (Brandis *et al*., 2000) synonymised four subspecies/species with *Potamon persicum* Pretzmann, 1962: *Potamon* (*persicum*) *elbursi* Pretzmann, 1976, *Potamon* (*persicum*) *kermanshahi* Pretzmann, 1962, *Potamon* (*magnum*) *vangölium* Pretzmann, 1976, and *Potamon armenicum* Pretzmann, 1962. More recently, A. Keikhosravi and C.D. Schubart (in press) documented noticeable diversity within *P. persicum* based on molecular and morphological evidences, that is probably related to the diverse topography and climate within the species' range. We thus consider *P. persicum* to represent a species complex, to which we will refer to as the *Potamon persicum* complex and the constituent units / species need to be redescribed.

With the present study, we provide sufficient genetic and morphological evidence that freshwater crab populations of *P. persicum* inhabiting streams in the Alborz Mountain Range are distinct and the corresponding taxon described by Pretzmann (1962) requires to be revalidated and elevated to species rank, *i.e. Potamon elbursi* Pretzmann, 1962. Therefore, these specimens are here redescribed and the species revalidated as eighth species of *Potamon* from Iran.

Experimental Procedures

A total number of seventy two individuals from seven populations were collected in 2011. Specimens were preserved in 70% ethanol. One walking leg from each specimen was detached and kept in 100% ethanol for later molecular studies. All specimens were submitted to a thorough morphological examination, prior to being deposited at different international zoological museums. In addition, older samples from museums, including the holotype of Pretzmann (1976b) were examined morphologically and some genetically. Detailed information concerning the sampling localities is provided in Figure 3.1 and Table 3.1. The studied specimens are deposited in the following collections: Zoology Museum, University of Tehran (ZUTC); Senckenberg Museum, Frankfurt am Main (SMF); Natural History Museum, Wien (NHMW); Natural History Museum, London (NHM); Bavarian State Collection of Zoology, Munich (ZSM); and Netherlands Centre for Biodiversity Naturalis, Leiden (RMNH).

DNA was isolated from muscle tissue extracted from walking legs using a modified Puregene method (Gentra Systems) at the University of Regensburg. Cytochrome oxidase subunit I (Cox1) and a ribosomal RNA gene (16S) were partially amplified using the following primer combinations: 16L29 (5'-CATATTATCTGCCAAAATAG-3') and 16HLeu (5'-YGCCTGTTTATCAAAAACAT-3') (Schubart, 2009) for 16S rRNA, COL6 (5′-TYTCHACAAAYCATAAAGAYATYGG-3′) and COH3 (5′-ATTTTYGSYGGWATTGCHCAYTGATT-3′) (Reuschel & Schubart, 2006) for Cox1. Polymerase chain reaction (PCR) was carried out under the following conditions: denaturation for 45s at 94°C, annealing for 1 min at 48°C, and extension for 1 min at 72°C (40 cycles) for 16S; denaturation for 45s at 94°C, 45s annealing with a touchdown procedure lowering the temperature from 53°C to 50°C for the 15 first cycles prior to keeping at 50°C for another 25 cycles, and extension for 1.25 minutes at 72°C for Cox1. The sequences were run on an ABI Prism 310 Genetic Analyser (Applied Biosystem, Foster City, USA) or outsourced to LGC Genomics, Berlin. Many other and longer sequences of the Cox1 region were obtained in collaboration the Canadian Centre for DNA Barcoding (CCDB) (Ratnasingham & Hebert, 2007). Six additional 16S sequences were retrieved from Genbank (HE963841- 4 and HE963847-8) (Table 3.1). Sequences were corrected manually with BioEdit (version 5.09) and aligned with Mafft (version 6) (Hall, 2001; Katoh *et al.*, 2002). The sequences were deposited at the European Molecular Database EMBL (accession numbers in Table 3.1).

A phylogeny based on a 1306 basepair alignment of combined 16S - Cox1 mtDNA was inferred by Bayesian Inference (BI) and generated with MrBayes (version 3.1.2) (Huelsenbeck & Ronquist, 2001). The appropriate substitution model was previously evaluated using MrModeltest for each gene separately (version 2.3; Nylander, 2004). The best model was selected by the Akaike information criterion (AIC). Four Montecarlo Markov chains (MCMC) were run for 6 million generations until the average standard deviation of split frequencies reached 0.0026 (it approach zero as runs progress). The tree was saved every 500 generations. The first 1000,000 generations were excluded from the analysis as burnin. *Potamon ibericum* Bieberstein, 1808 (ZUTC Pot.1089) was used as outgroup for the phylogenetic reconstructions. This species occurs in close geographic vicinity with *P. elbursi*, in rivers draining north into the Caspian Sea. However it is characterized by a very distinct morphology and was until recently classified in a separate subgenus.

A statistical parsimony network with larger dataset for each gene was individually constructed using the software TCS version 1.21 (Clement, 2000) in order to document the intraspecific range of divergence and relationships among haplotypes as well as the relationship between the new species and *P. persicum*. Gaps in 16S dataset were treated as fifth character state.

Table 3.1. Specimens used for DNA sequencing with collection number, locality, coordinate and GenBank accession number. Abbreviations for museum explained in Experimental Procedures.

Species	Voucher number	Locality	Coordinate	Accession no. of 16S	Accession no. of Cox1	Label
P. ibericum	ZUTC Pot.1089	Iran, Gorgan, Naharkhoran	36°45.68' N54°28.74'E	HE963848	KF227380	-
P. transcaspicum	ZUTC Pot.1088	Iran, Sabzevar, Zardkoohi R.	36°17.29'N 57°23.34'E	HE963841	KF227384	-
P. elbursi	NHMW 25432	Tehran Prv., Kamard, Jajrood R.	35°46.350'N 51°45.263'E	HG321369	-	KM
P. elbursi	RMNH-D 54895	Alborz Prv., Taleghan R.	36°10.137'N 50°45.757'E	HG321388	-	TL
P. elbursi	SMF-39032	Tehran Prv., Tehran, Darakeh R.	35°49.09'N 51°22.85'E	HG321373	-	DR
P. elbursi	ZUTC Pot.1082	Tehran Prv., Tehran, Darakeh R.	35°49.09'N 51°22.85'E	HG321370	KF227379	DR
P. elbursi	ZUTC Pot.1087	Guilan Prv., Roodbar, Sepidrood R.	36°49.060'N 49°25.256'E	HG321368	-	SD
P. elbursi	ZUTC Pot.1084	Qazvin, Molaali R., Trib to Shahrood R.	36°27.74'N 49°30.86' E	HG321372	KF227385	ML
P. elbursi	RMNH-D 54896	Qazvin, Molaali R., Trib to Shahrood R.	36°27.74'N 49°30.86' E	HG321386	-	ML
P. elbursi	ZUTC-1094	Zanjan Prv., Mahneshan, Ghezelozan R.	36°49.19'N 47°37.75'E	HG321381	HG321389	GO
P. elbursi	ZUTC Pot.1086	Ardabil Prv.,5 kn N Khalkhal, Arpachay R.	37°40.926'N 48°30.237'E	HG321387	-	AP
P. elbursi	ZUTC Pot.1085	Tehran, Lavasan, 3km west of Latyan Dam	35°48.774'N 51°36.059'E	HG321379	-	FM
P. elbursi	ZUTC-1090	Road to Bostanabad, 25 km off Miyaneh	37°29.761'N 47°28.123'E	HG321375	-	SC
P. elbursi	ZUTC-1092	25 km N Zanjan, Near to Taham village	36°49.959'N 48°36.173'E	HG321380	-	TM
P. elbursi	NHMW -25502	Kordestan, 33 km N Bijar, Qamchi R.	36°9.952'N 47°37.334'E	HG321376	-	QI
P. elbursi	ZUTC-1093	15 km N of Bijar, Hosseinabad village	35°59.905'N 47°1.442'E	HG321374	-	BJ
P. elbursi	ZUTC-1091	27 km E of Bijar, Telvar R.	35°51.389'N 47°54.006'E	HG321378	-	TV
P. elbursi	SMF-43957	15 km W of ABhar, Kinehvars Dam.	35°51.389'N 47°54.006'E	HG321377	-	KS
P. persicum	NHMW 25426	Ilam Prv., Chardavol R.	33°40.17'N 46°41.48'E	HG321371	-	CV
P. persicum	NHMW 25426	Ilam Prv., Chardavol R.	33°40.17'N 46°41.48'E	HE963842	KF227381	CV
P. persicum	SMF-39031	Isfahan, Zayandehrood R.	32°38.32'N 51°35.97'E	HE963844	KF227383	IF
P. persicum	SMF-39030	Khuzestan Prv., Dezful, Dez R.	32°23.28'N 48°23.67'E	HE963843	KF227382	DZ
P. persicum	ZUTC Pot.1019	Chaharmahal Bakhtiari Prv, Junqan	-	HG321385	-	JN
P. persicum	NHMW 4315	Iran, 50 km SE Khorramabad	-	HG321384	-	KB
P. persicum	ZUTC Pot.1062	Iran, Kohkiluyeh buyerahmad, Sisakht	-	HG321382	-	ST
P. persicum	NHMW 3950	Iran, 3 km NW of Brujerd	-	HG321383	-	BD

Morphology

First gonopods, chelipeds, walking legs, third maxillipeds, thoracic sternites, abdomen and different regions of the carapace were studied using a binocular microscope Leica EZ4. Important characters were drawn with the aid of a camera lucida attached to the dissecting scope. The characteristic terminology follows Brandis *et al*. (2000) and Pretzmann (1976b).

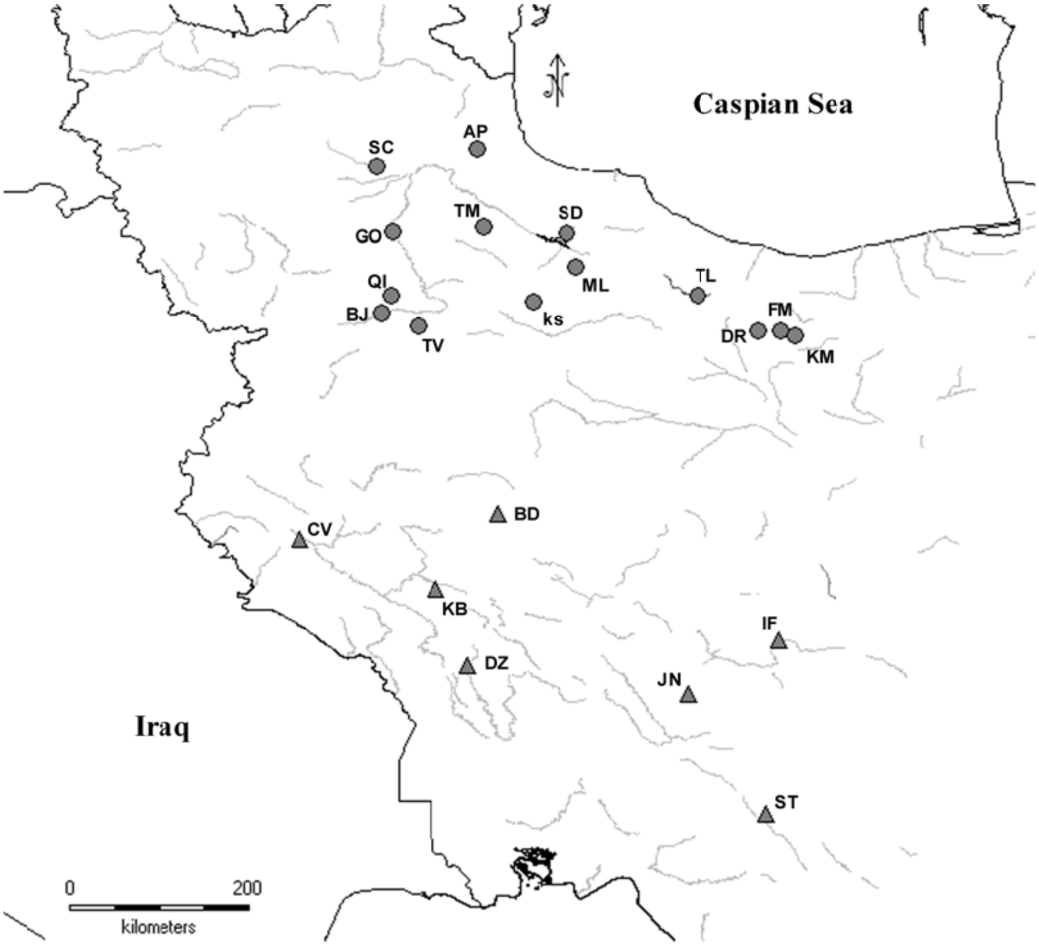

Figure 3.1. Map of Iran with main rivers. Gray circles and triangles represent the sampling sites corresponding to *P. elbursi* and *P. persicum,* respectively. For abbreviations see Table 3.1.

Systematic Account

Family Potamidae Ortmann, 1896

Genus *Potamon* Savigny, 1816

Potamon elbursi Pretzmann, 1962 stat. nov.

(Fig. 3.2a-c)

Potamon (*magnum*) *elbursi* Pretzmann 1962: 222 (elevated to species level)

Potamon (*magnum*) *persicum* Pretzmann 1965: 522 (partially misidentification)

Potamon (*persicum*) *persicum* Pretzmann 1976: 461 (partially misidentification)

Potamon (*persicum*) *elbursi* Pretzmann 1976: 463

Potamon persicum Brandis *et al*. 2000: 37-40 (partially misidentification)

Material examined — Holotype: ♂ (NHMW 3182) Karadj, middle of the Alborz Mountains, leg. Rechinger, 1937.

Iran: 2♂ (SMF-39032), 2♂ (RMNH-D 54893), 3♂ (NHMW 25429), 2♂, 1j (ZUTC Pot.1082), Tehran Province, Tehran, Darakeh, Darakeh River, 35°49.092'N 51°22.855'E, leg. A. Keikhosravi, 28.04.2009; 2♂, 1♀ (RMNH-D 54894), 2♂ (NHMW 25430), 3♂ (SMF-39033), 2♂, 1♀ (ZUTC Pot.1087), Guilan Province, Roodbar, Sepidrood River, 36°49.060'N 49°25.256'E, leg. A. Keikhosravi & M. Houshmand, 23.12.2009; 2♂, 2j (SMF-39034), 1♂, 1j (RMNH-D 54895), 2♂, 2j (ZUTC Pot.1083), Alborz Province, Taleghan, Thaleghan River, 36°10.137'N 50°45.757'E, leg. F. A. Houshmand, 18.08.2009; 3♂, 2♀ (SMF-39035), 4♂, 1♀ (NHMW 25431), 3♂, 2j (RMNH-D 54896), 2♂, 1♀, 1j (ZUTC Pot.1084), 55km northwest of Qazvin, Molaali River, Trib to Shahrood R., 36°27.742'N 49°30.868' E, leg. Y. A. Houshmand & A. Keikhosravi, 03.09.2010; 1♀, 1♂, 1j (SMF-39036), 1♂, 2j (ZUTC Pot.1085), Tehran, Lavasan, 3km west of Latyan Dam 35°48.774'N 51°36.059'E, leg. Y. Houshmand & A. Keikhosravi, 13.11.2009; 2♂, 3♀ (SMF-39037), 3♂, 3♀, 1j (NHMW 25432), 30 km northeast of Tehran, Kamard, Trib to Jajrood River, 35°46.350'N 51°45.263'E, leg. A. Keikhosravi, Y. A. Houshmand & H. Heydari, 01.10.2009; 1♂, 2♀ (SMF-39038), 2♀ (ZUTC Pot.1086), 5 km north of Khalkhal, Arpachay River, 37°40.926'N 48°30.237'E, leg. Y. Houshmand & A. Keikhosravi, 02.09.2010; 1♂ (NHM 1920.2.5.3-4), northwest of Qazvin, leg. P. A. Buxton, R. Gurney, 1920; 1♂ (SMF 2640), Tehran Province, Tehran, leg. F. Bruhns; 1♂, 1♀ (SMF-4157), road of Abu-Ali, 30 km off Tehran, leg. J. Theodorides, 1959; 9♂, 1♀, 2j (NHMW 3915), 26 km SE of Qazvin, leg. Pretzmann, 1970; 1♂, 2♀, 2j (NHMW 3919), ca. 62 km S of Rasht, right tributary of the Sepidrood R., leg. Eiselt & Bilek, 1968; 17♂, 12♀, 2j (NHMW 3920), ca. 62 km S of Rasht, right tributary of the Sepidrood R., leg. Pretzmann & Bilek, 1970; 2♂, 2♀, 4j (NHMW -25502), Kordestan Province, 25 km N Bijar, Qamchi village, Qamchi R., 36°09.960'N 47°37.316'E, leg. A. Keikhosravi & E. Keikhosravi, 22.10.2011; 3♂, 4♀, 1j (ZUTC -1090), Azarbaiejan-e Sharghi, road to Bostanabad, 25 km off Miyaneh, Shahrychi R., 37°29.761'N 47°28.125'E, leg. A. Keikhosravi & E. Keikhosravi, 20.10.2011; 3♂, 4j (SMF-43957), 2♂, 2j (ZSMA20130030), Zanjan Province, 15 km W of Abhar, Kinehvars Dam, 36°09.950'N 49°4.141'E, leg. A. Keikhosravi & E. Keikhosravi, 18.10.2011; 3♂, 3♀, 2j (ZUTC -1091), 2♂, 1♀, (NHMW -25503), Kordestan Province, 27 km E of Bijar, road to Pirtaj, Mehrabad village, Telvar R., 35°51.389'N 47°54.006'E, leg. A. Keikhosravi & E. Keikhosravi, 21.10.2011; 3♂, 3♀, 4j (SMF-43958), 2♂, 2♀ (ZUTC -1092), Zanjan Province, 25 km N Zanjan, Near to Taham village, stream to Galharood, 36°49.959'N 48°36.173'E, leg. A. Keikhosravi & E. Keikhosravi, 19.10.2011; 4♂, 3♀, 3j (ZUTC -1093), 2♂, 2♀ (ZSMA20130031), Kordestan Province, 15 km N Bijar, Hosseinabad Kamarzard village, upsteram of Ghezelozan R., 35°59.905'N 47°31.442'E, leg. A. Keikhosravi & E. Keikhosravi, 21.10.2011; 5♂, 3♀(ZUTC -1094), Zanjan Province, 10 km N Mahneshan, Saraghoon village, Ghezelozan R., 36°49.195'N 47°37.752'E, leg. A. Keikhosravi & E. Keikhosravi, 20.10.2011; 11♂, 9♀ (NHMW 3923), 29 km N of Qazvin, Pretzmann *et al.*, 1972; 6♂, 9♀, 7j (NHMW 3911), 16km West of Delijan, leg. Pretzmann & Bilek, 1970; 25♂, 21♀ (NHMW 3910), Taareh, 43 km W of Arak, leg. Pretzmann & Bilek, 1970; 3♀ (NHMW 3914),169 km SW of Qazvin, leg. Pretzmann, 1970; 6♂, 6♀ (NHMW 3930), 20 km S of Makoo, 39°17.200'N 44°33.517'E, leg. Pretzmann *et al.*, 1972.

Armenia: 1♂ (NHMW 3232), Yerevan, water channel, Zool. Inst. Halle, 1959.

Diagnosis — Terminal segment of G1 nearly triangular; mesial margin in two different morphs: <u>either</u> sinus-shaped, bent outward medially, proximal half bulged and arched, distal portion slightly concave; <u>or</u> straight at 1/5 of proximal part, bent outward at 1/5 of proximal part, second portion (4/5) nearly straight; mesial margin at distal half with long setae; subterminal segment of G1 semi-stout; mesial edge covered by setae, proximally dense, distally sparser, subapically slightly swollen (Fig. 3.3a, b, c).

Redescription — Carapace distinctly broader than long; dorsal surface of carapace almost flat, glabrous, moderately sculptured. Anterolateral region prominent, swollen, with many short rows of wide-based granules (>6), irregularly arranged; anterolateral margin cristate, lined with small granules, posteriorly arched inward; first epibranchial tooth short, blunt; epibranchial denticles short, slightly serrate (Fig. 3.2c). Posterolateral region arch-shaped (Fig. 3.2c); margins moderately converging posteriorly. Cervical groove distinct, deeper anteriorly; H-shape depression relatively deep, with distinct margins. Cephalothoracic region well defined; mesogastric lobe distinct; mesogastric grooves well defined, dorsally elongated; epigastric region well developed, separated from postorbital cristae by shallow groove.

Frontal region structured, flexed downward, granulated, depressed medially, bilobed appearance (Fig. 3.3b, c); frontal margin slightly sinuous, decorated by blunt, visible granules; postfrontal crest sharp, prominent and detached from anterolateral region by deep cervical groove. Supraorbital margin smooth, finely tuberculated at exterior side; infraorbital margin finely tuberculated (Fig. 3.3a). Exorbital tooth triangular, distinct, very blunt, lateral side slightly serrate. Epistome smooth, with a number of short setae; posterior margin distinctly granulated(Fig. 3.3b). Suborbital lobe anteriorly and posteriorly smooth, glabrous, middle region with curved line of tubercles (Fig. 3.3a).

Third maxilliped ischium with deep median sulcus (Fig. 3.3a); exopod with flagellum nearly as long as merus.

Thoracic sternites smooth, sparsely covered with setae, pilose at margins of segments (Fig. 3.3a); second sternal segment prominent. Male pleon long, triangular, smooth; lateral margins with lined short, dense setae; segments 2-6 progressively longer; telson slightly longer than segment 6, tip rounded.

Male chelipeds unequal (Fig. 3.3). right one bigger than left one, Upper margin of merus with short transverse rows of granules, serrate appearance; inner-ventral margin irregularly granular, granules with wide base; outer-ventral margin regularly granular, progressively larger from proximal to distal; outer surface smooth, transverse rows of granules near upper margin; inner surface smooth. Carpus with two large acute spines on upper-inner margin; outer surface with transverse granulated rows, median region depressed distally. Palm with outer surface slightly swollen, nearly smooth, transverse rows of small granules on upper region, becoming larger dorsally; ventral margin completely smooth; dorsal margin denticulate; inner surface of palm smooth. Dactyl slightly shorter than propodal pollex, dorsal longitudinal row of small granules, disappearing at distal half; cutting edges with distinct row of conical teeth.

Pereiopods 2-5 (P2 – P5) relatively long, P3 longest, P5 shortest; dorsal margin of merus, carpus and propodus of P2-5 serrate, dorsal margin of merus with subdistal notch (Fig. 3.3a, c); carpus with longitudinal carina, medially on posterior surface, carpus of P5 without carina; propodus with two rows of spines along ventral margin, spines larger distally; propodus of P2 with three rows of spines; dorsal margin of dactylus with two rows of sharp spines, proximal to distal progressively larger; ventral margin with two rows of spines, proximal to distal progressively larger; spines of dorsal margin slightly larger than those of ventral margin; three rows of short setae along dorsal and ventral margins.

Male first gonopod (G1) sinuous, nearly S shaped (Fig. 3.3a-c); terminal segment nearly triangular, bent outward; lateral margin nearly straight, sparsely covered by short setae; mesial margin in two different morphs: either sinus-shaped, proximal half bulged, arched, distal portion slightly concave (Fig. 3.3a, c; arrows), almost covered by long setae; or straight at 1/5 of proximal part, bent outward at 1/5 of proximal part (Fig. 3.3b; arrow), second portion (4/5) nearly straight, distally covered by long setae. Flexible zone well developed, V-shaped. Subterminal segment semi-stout; mesial edge almost straight, slightly concave, covered by setae, proximally dense, distally sparser, subapically slightly swollen; lateral margin curved (sinus-shaped), with scattered setae near base (Fig. 3.3a, b, c).

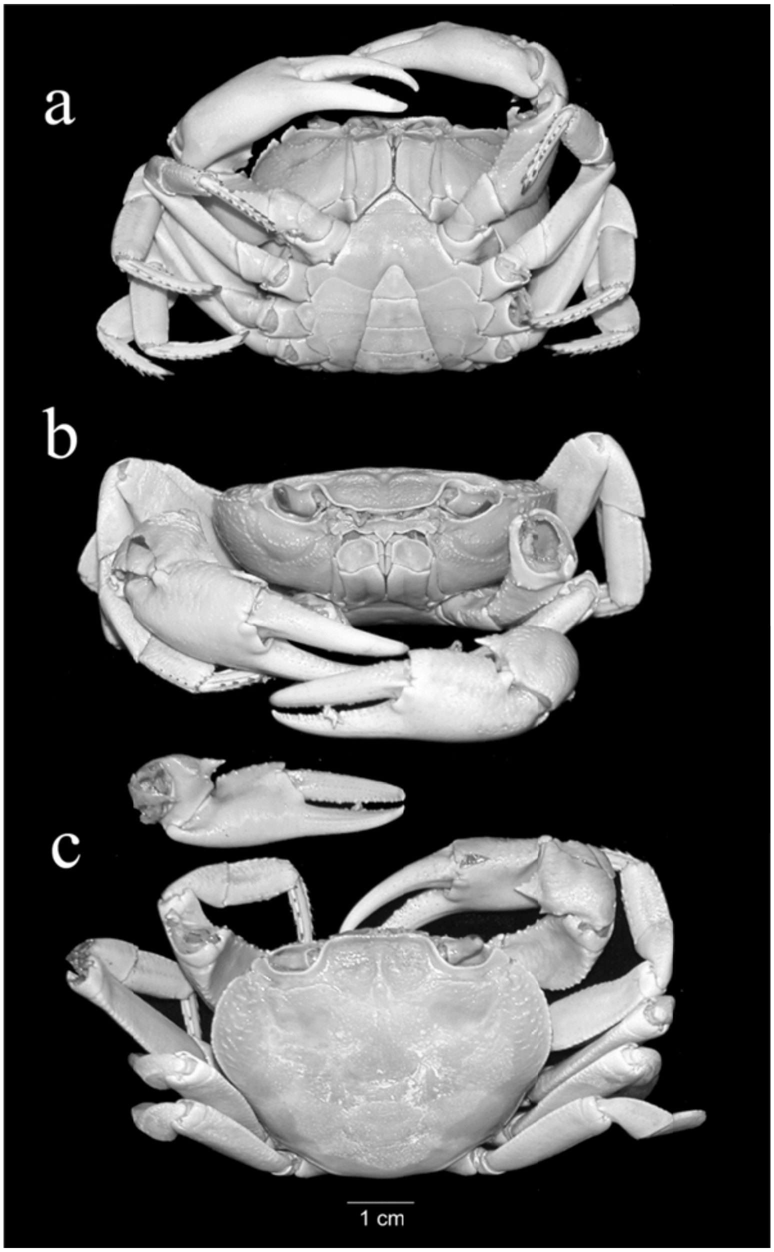

Figure 3.2. Holotype of *Potamon elbursi* (NHMW 3182); a, ventral view; b, frontal view; c, dorsal view.

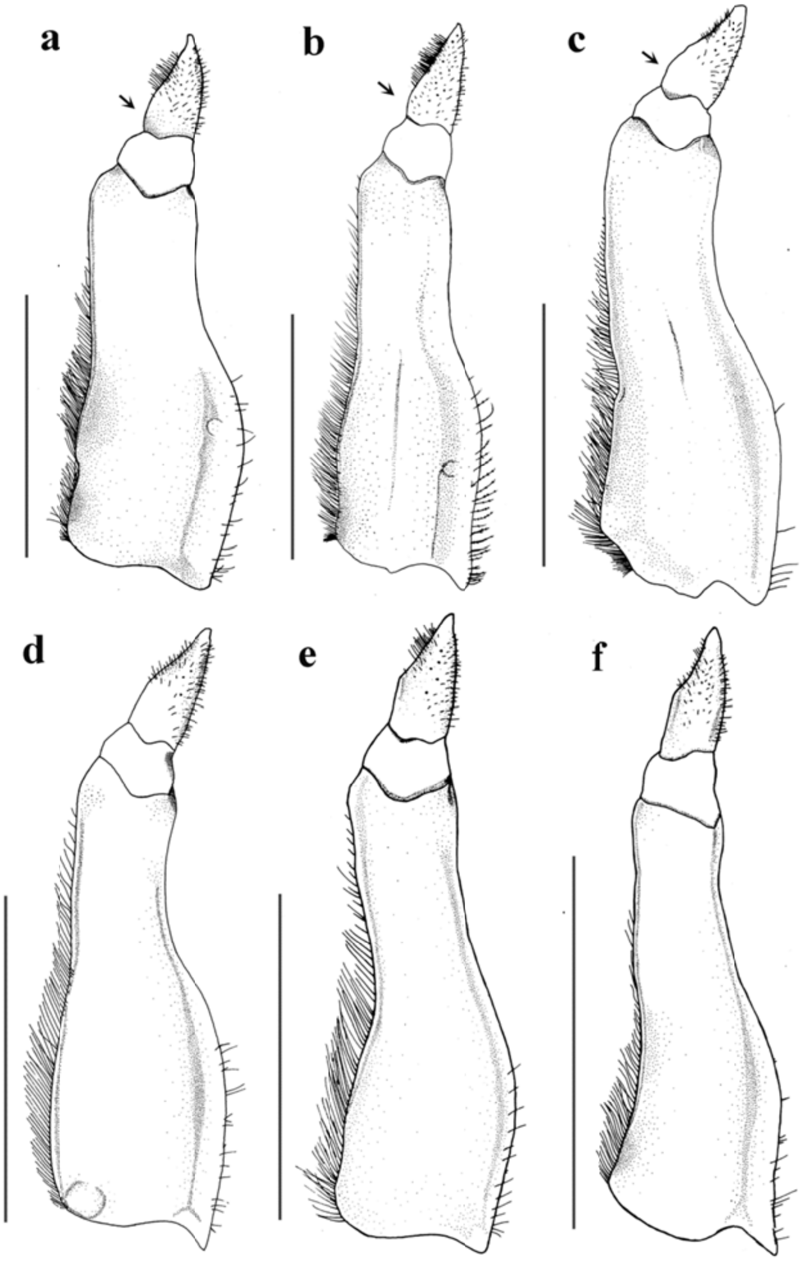

Figure 3.3. Morphological characteristics of the first gonopod (G1) in male specimens of *Potamon elbursi* (a-c) and *Potamon persicum* (d-f). Dorsal view; a, SMF-43958; b, SMF-39038; c, NHMW 3182 (holotype); d, NHMW 3927; e, NHMW 4151; f, NHMW 25428. Scale bars: 5 mm.

Comparative material — *Potamon persicum* Pretzmann 1962, Iran: 3♂, 1j (SMF-39029), 3♂, 1♀ (NHMW 25426), 2♂, 1j (ZUTC Pot.1079), Ilam Province, 25 km West of Ilam, Chardavol River, Shemsheh Strait, 33°40.177'N 46°41.489'E, leg. A. Keikhosravi, B. Fathinia & M. Moradmand, 15.04.2009; 2♂, 1j (NHMW 25427), 2♂, 1j (SMF-39030), 2♂ (ZUTC Pot.1080), Khuzestan Province, Dezful, Dez River, 32°23.282'N 48°23.679'E, leg. A. Keikhosravi & M. Moradmand, 18.04.2009; 4♂, 1♀ (SMF-39031), 3♂ (NHMW 25428), 3♂, 1j (ZUTC Pot.1081), Isfahan Province, Isfahan, Zayandehrood River, 32°38.322'N 51°35.977'E, leg. A. Keikhosravi & M. Moradmand, 19.04.2009; 1♂ (NHMW 3909 II), Isfahan, leg. Pretzmann, Bilek & Raffi, 1970; 1♂ (NHMW 3927), Kermanshah Province, 50 km SE of Shahabad (Eslamabad Gharb), 1300 m, Aspock & Ressl, 1975; 1♂ (NHM 1920.2.5.3-4), northwest of Qazvin, leg. P. A. Buxton, R. Gurney, 1920; 3♂, 2♀, 13j (NHM 1899.10.6.1-5), Azarbaiejan-e Gharbi Province, near Orumiyeh, Northwest of Iran, leg. R. J. Gunther, 1899; 1♂ (ZMUC-CRU-9522), Kermanshah, ca. 600 km W of Tehran, leg. Kaiser, 06.02.1937. [holotype of *Potamon persicum kermanshahi*]. Iraq: 1♂ (NHM 1934.8.29.1-6), stream on Kirkuk road, 16 km west of Sulaymaniyh, 760 m, 35°33'N 45°16'E, leg. MacFadyen, 1933. Turkey: 3♂ (NHMW 13362), SE of Turkey, no further locality information, leg. Eiselt, 1968; 2♂, 1♀ (SMF 5881), Mengene Dağlari, SE of the Van Gölu, Başkale, leg. Lampe, 7. I. 1972; 1♂ (NHMW 4151), 40 km SE of Diyarbakir, leg. Eiselt, 1966.

Remarks — The taxonomic classification of the *P. persicum* complex has been under constant discussion over the past half century. Pretzmann (1962, 1965, 1971, 1973, 1976b) introduced four new species/subspecies (as well as other infraspecific categories) belonging to the *P. persicum* complex. Later, Brandis *et al.* (2000) synonymised them under the priority name of *P. persicum*. The current study re-describes one of Pretzmann's subspecies, *P.* (*persicum*) *elbursi*, and elevates it to full species rank. The best characteristic of distinguishing both species from each other is the G1 structure. *Potamon persicum* has a monotypic shape of the G1with negligible diversity in different populations. The terminal segment of the G1 in *P. persicum* has straight mesial margins in the proximal half and becomes sharply deflected in its distal half. The degree of deflection slightly changes in different populations (Fig. 3.3d, e, f). In contrast, the terminal segment of the G1 in *P. elbursi* can be found in two morphs of the mesial margin with no sharp deflection: It is either sinus-shaped with a bulged proximal half, bent outward medially, and with a distal portion slightly concave (Fig. 3.3a, c); or it can be straight at

1/5 of proximal part, bent outward at 1/5 of proximal part, with the distal section (4/5) being nearly straight (Fig. 3.3b). Both forms can be found within the same population in similar-sized individuals and do not seem to represent any geographical association. The carapace is sculptured in both species. The cervical groove in *P. elbursi* is more distinct (deeper) than in the other species. The subterminal segment of G1 in *P. elbursi* is slightly stouter than in *P. persicum*. Whenever possible, these morphological characteristics should be determined, when having both species in hand and thus the possibility of direct comparison.

Distribution — The species distributes in the Caspian Sea drainage system, but is only present along the southern slopes of the middle and western half of the Alborz Mountains in Iran. The species also occurs in the neighbouring watershed system *i.e*. Namak Lake drainage.

Molecular results

The molecular tree was constructed based on a combination of 16S and Cox1 sequence data, with a total alignment length of 1306 basepairs. GTR +I (with proportion of invariable sites 0.8590 for 16S and 0.7407 for COX1) was chosen as the best evolutionary model of substitution by MrModeltest according to the Akaike criterion. The model was used to reconstruct a phylogenetic tree with eight specimens. Our results discern three main groups with marked genetic differentiations between groups (Fig. 3.4). The well supported clade of *P. elbursi* (Fig. 3.4, H1) is a sister group to the highly supported clade of *P. persicum* from two western localities (Fig. 3.4, H2), which is consistent with their biogeographic distribution, whereas *Potamon persicum* from the type locality in Isfahan (IF) represents a third group (Fig. 3.4, H3), holding a basal position to the clade H1-H2. However, the relationship between these three haplogroups is not resolved since the clade H1-H2 is only supported by a low posterior probability (0.6). There is no evidence for a close relationship between the clades of *P. persicum* H3 and H2, even though they represent morphologically similar populations based on the first gonopod. However, there is also no statistically significant phylogenetic evidence against it (Fig. 3.4).

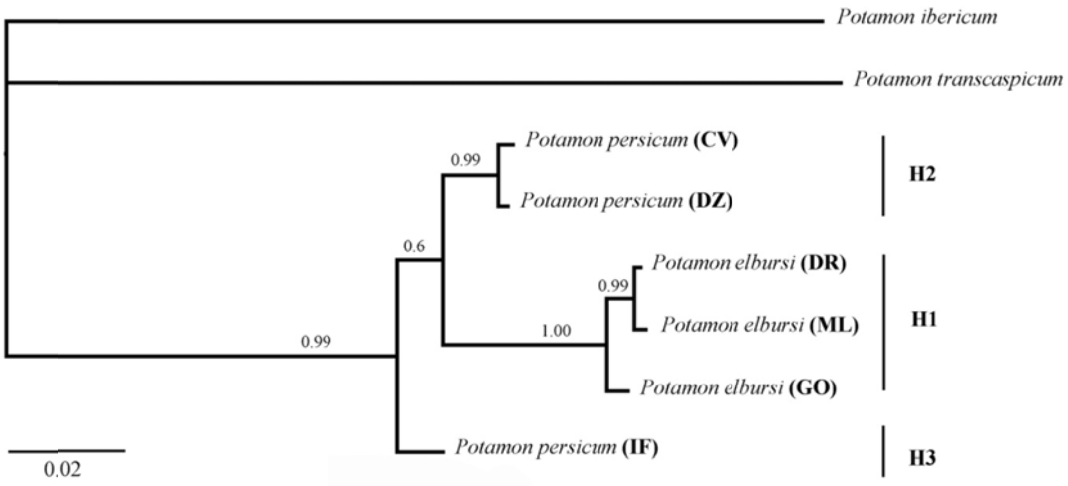

Figure 3.4. Phylogenetic relationships among populations of *Potamon elbursi* and *P. persicum* according to a concatenated tree using Bayesian analysis of a 1306 basepair alignment of combined 16S and Cox1 sequence data. Posterior probability values show support for the corresponding clades. Abbreviations refer to Figure 3.1 and Table 3.1.

The two parsimony networks were congruent with the phylogenetic tree but with different resolution. The haplotype network based on 648 basepairs of 16S showed three groups with short distances, as haplogroups are separated by few steps from each other (Fig. 3.5a). The parsimony network constructed with 658 basepairs of Cox1 demonstrates convincingly that there are three haplogroups and that the distance between *P. elbursi* (H1) and the *P. persicum* complex (H2 & H3) is significantly larger than that of *P. persicum* from Isfahan (IF) and conspecific populations (Fig. 3.5b).

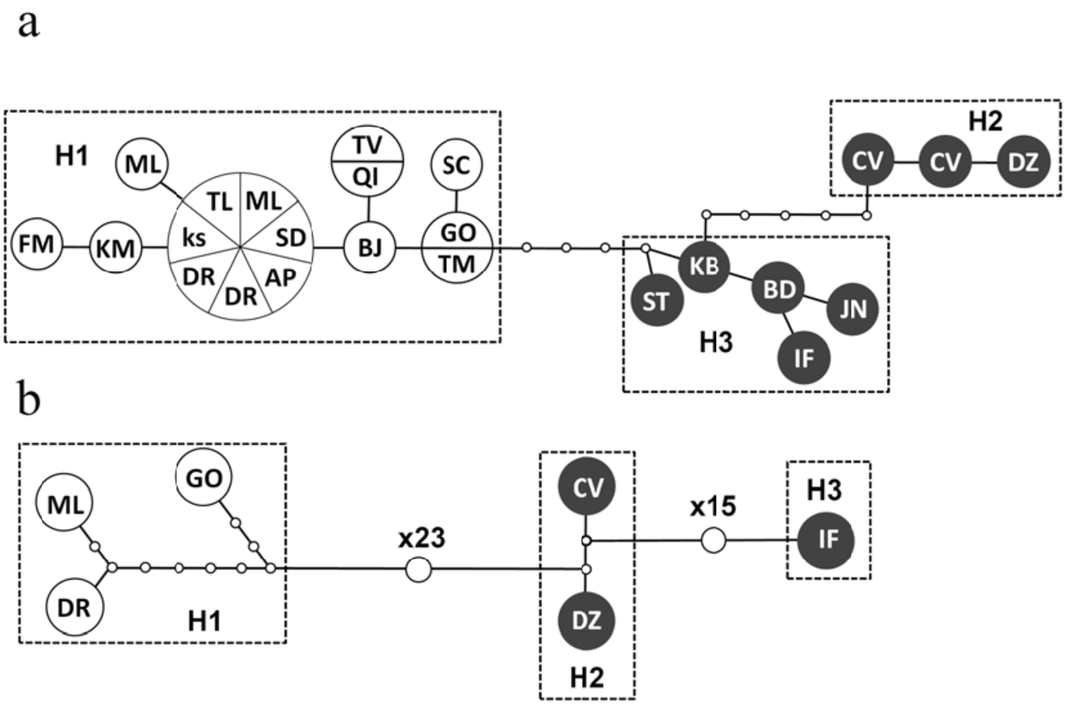

Figure 3.5. Statistical parsimony networks based on 648 basepairs alignment of 16S (a) and 658 basepairs alignment of Cox1 (b). Each line represents one mutation, each dot indicates one missing haplotype and numbers next to large unlabeled nodes indicates the number of missing haplotypes. The size of the haplotypes is proportional to their frequencies. Haplogroups are defined by: H1= *Potamon elbursi*, H2= *Potamon persicum* from Euphrates-Tigris drainage system and H3= *Potamon persicum* from Isfahan (near type locality). For abbreviations of haplotypes and localities see Figure 3.1 and Table 3.1.

Discussion

In an era of human-induced biodiversity decrease, assessing current biological diversity as quickly as possible is of crucial importance. Therefore, understanding species delimitations and solving taxonomic problems in species complexes is an important goal. The current study takes an important step in this direction by redescribing and revalidating the freshwater crab *P. elbursi* based on a combination of new morphological and molecular evidence. It also aims to solve the question, whether morphological features used in taxonomy are congruent with DNA taxonomy and thus the underlying phylogeny.

Phylogenetic and morphological results, showed a noticeable congruence with hydrographic boundaries, either as drainage or subdrainage systems, between *P. persicum* and *P. elbursi*. *Potamon elbursi* is confined to the Caspian Sea watershed (only southern slopes of the middle and western half of the Alborz Mountains) and the endorheic drainage of Namak Lake (Daryacheh-e-Namak). A large water system at the southern slopes of the Alborz Mountains (the Ghezel Ozan Basin) drains to the Caspian Sea via Sepidrood River. The Namak Lake Basin is fed by few rivers from the southern slopes of the Alborz Mountains and some western rivers originating from the Zagros Mountains. The land between these two drainage systems (Caspian Sea and Namak Lake) does not act as a terrestrial barrier for the freshwater crabs since headwaters of both drainage systems are located very close to each other.

The unresolved question here is about morphological similarities and molecular dissimilarities within *P. persicum.* The type locality of *P. persicum* is Isfahan, lying in a small endorheic drainage basin surrounded from three sides by the Zagros Mountains. Beyond these mountains, it is bordered by the Namak Lake Basin to the north, Euphrates-Tigris drainage systems to the west, and the Kavir Plain (Dasht-e-Kavir) drainage to the east (www.feow.org/index.php & www.briancoad.com) (Pretzmann, 1976b). The Isfahan haplogroup (Fig. 3.5a, H3) is shared by specimens from Isfahan and peripheral regions of the neighbouring watershed systems *i.e.* Euphrates-Tigris drainage system, whereas the second haplogroup is found westward beyond this circumferential zone (Fig. 3.5 a-b: H2). The explained mode of distribution raises the question of why these two evolutionary significant lineages partially share the same watershed system. The apparent gene flow between populations of the Isfahan Basin and peripheral regions of the neighbouring watershed system (Euphrates-Tigris) can be explained by the proximity of some headwaters of both drainage systems, as it is generally believed that freshwater crabs are able to cross small land bridges between headwaters when humidity is sufficiently high (Daniels *et al*., 2006a; Yeo *et al*., 2008). As an alternative explanation could serve a series of man-made tunnels (Kuhrang tunnels) redirecting water of the Kuhrang River from the Euphrates-Tigris drainage system towards the main river of the Isfahan drainage (*i.e*. Zayandehrood R.) (www.briancoad.com). However, faunal analysis of Old Stone Age excavations near Isfahan recovered remains of freshwater crabs (Jaubert *et al*., 2010) (Fereidoun Biglari, personal communication, August 23, 2011), demonstarting the existence of viable populations before the construction of the tunnels. At the current point, inadequate geographic sampling precludes any further conclusions concerning occurrence of two evolutionary significant units in the same drainage system. Our mtDNA data show further molecular diversity within *P. persicum* as some of the other old names may have to be revalidated. Therefore, future attention is demanded to investigate genetic and morphologic differentiation of the constituent populations over the entire range of the *P. persicum* to uncover the biodiversity and to resolve the phylogenetic relationships within the *P. persicum* complex.

Acknowledgements

The first author would like to express his special gratitude to Mr. Esmail Keikhosravi who helped to collect specimens. Special thanks is given to the PhD mentors, Prof. Christoph Oberprieler (Universität Regenbsurg) and Dr. Sara Fratini (Universitá di Firenze), due to their beneficial advice. We are grateful to Dr. Peter Dworschak for granting access to the infrastructure of the Vienna collection (NHMW). The first author's visit to NHMW was funded by the "Synthesis" Project of the European Union, which is greatly appreciated. We are grateful to Prof. Michael Türkay (SMF) and Dr. Paul Clark (NHM) for loaning material from their collections. Our thanks also go to Prof. Jürgen Heinze (University of Regensburg) and his staff for the use of the necessary facilities and continuing support, and the Akademisches Auslandsamt of the University of Regensburg for financial support.

Chapter 4

Discerning phylogeographic units and the possible role of glaciations in the freshwater crab *Potamon elbursi* from the Alborz Mountains in northern Iran

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Submitted in: *Journal of Limnology*

In this study, we investigated hydrographically isolated populations of freshwater crab *Potamon elbursi* Pretzmann, 1976, to assess their conservation status and genetic diversity, and to reconstruct the role of Quaternary glaciations in shaping current freshwater diversity in the Alborz Mountains. A total of 61 specimens belonging to six populations were collected from two drainage systems, Namak Lake and Caspian watershed, from the Alborz Mountains. A comparative analysis of partial DNA sequence of Cox1 for the whole dataset revealed relatively high haplotype diversity (0.821) and low nucleotide diversity (0.00409). For four populations, the haplotype network did not show any association with geographical separation. The remaining two populations showed high endemicity that can be the result of strong genetic drift or geographic barriers. F-statistics revealed highly significant differentiation among all populations, regardless of their association to river systems. The only exception was the comparison of two genetically similar populations found in distant rivers belonging to different drainages, which may be attributed to either human-mediated introductions or incomplete lineage sorting. Mismatch distribution analysis gave an idea about a constant population size model, whereas other values support a recent expansion model. Ongoing anthropogenic activities resulting in desiccation, urge to include fresh waters of the Alborz Mountains into immediate conservation plans. After giving evidence for genetic isolation, we propose to consider the studied populations of *P. elbursi* as endemic phylogeographic units and to develop plans for their long term conservation.

Introduction

Although only 0.8 percent of the Earth's surface is occupied by freshwaters, they contain extremely high species diversity (approximately 12% of all species) (Stiassny, 1996; McAllister *et al.*, 1997). The extinction rate of species in freshwater is assumed to be five times higher than the rate for terrestrial fauna. Such accelerating shrink of freshwater's biodiversity is attributed to habitat destruction, the occurrence of exotic species, over exploitation, secondary extinction (extinction of one species because of extinction of another one), organic and chemical pollution, and climate change (Allan & Flecker, 1993; Ricciardi & Rasmussen, 1999). Therefore, freshwater habitats and their biodiversity are among the most threatened ecosystems worldwide (Revenga *et al.*, 2005; Dudgeon *et al.*, 2006). Freshwater crabs are one of the most ecologically important macro-invertebrate groups in freshwaters, because of their relative abundance and high biomass in aquatic ecosystems (Turnbull-Kemp, 1960; Abdallah *et al.*, 2004; Dobson *et al.*, 2007a, b; Marijnissen *et al.*, 2009), their ability to utilize both terrestrial and aquatic energy pathways (Gherardi *et al.*, 1989; Gherardi & Vannini, 1989), and their central position in food webs as primary and secondary consumers (Williams, 1961, 1962; Gherardi *et al.*, 1987). Despite their importance, about one-sixth of all freshwater crab species have an elevated risk of extinction as result of anthropogenic environmental impacts (Cumberlidge *et al.,* 2009). Therefore, freshwater crabs deserve to receive special conservation attention.

Iran is a large country of diverse climates, ecology, flora and fauna (Collins, 2001; Kehl, 2009). Natural environment of Iran is being severely threatened, which can be deduced from more than thousand plant and animal species that have been listed in the IUCN Red List (Khabaronline, 2010). Iranian freshwater ecosystems may be the most endangered of all, as many freshwater fishes have reached alarming population sizes and they deserve to receive more conservation attention (Coad, 1980; Kiabi *et al.*, 1999). Thus, assessing current freshwater diversity of Iran as soon as possible is of crucial importance for obtaining new insights for conservation purposes. This is even more critical, when considering that it has been suggested to categorize Iran as chronic scarcity zone for freshwater by 2025 and by the time mentioned, it will reach the complete limit in the development of their water resources (Cheraghi, 1996; Seckler, 1998; Abrishamchi & Tajrishi, 2005).

Freshwater crabs are highly philopatric (*i.e.* remaining or returning individuals to their point of origin or birthplace) and mostly confined to their provenance freshwaters systems. This is due to their dependence on freshwater and the lack of larval stages. Potamid crabs have direct development, resulting in extended maternal care. In consequence, they must be considered as low dispersal organisms, having a limited tendency or ability to cross terrestrial distances between river basins, and thus biogeographically important. Therefore, freshwater crabs are good model organisms for reconstructing the evolutionary history of drainage systems, the demography of conspecific populations, or the phylogeny of congeneric species (Ng & Rodriguez, 1995; Daniels *et al.*, 2006a; Shih *et al.*, 2006; Yeo *et al.*, 2008; Schubart & Santl, in press).

The Middle East is one of the main distribution areas for the freshwater crab genus *Potamon* Savigny, 1816. Recently, Keikhosravi & Schubart (in press) re-described and revalidated *Potamon elbursi* Pretzmann, 1976 from Iran. The distribution of *P. elbursi* is bordered to the north by the central and southern slopes of the western Alborz Mountains. The southernmost distribution reaches to the sources of two drainage systems, Namak Lake and Caspian Sea drainages. Only at one point, the distribution extends northward through the Alborz Mountains (Sepidrood Valley), reaching to the Caspian Sea (Fig. 4.1). Interestingly, *P. elbursi* lives in different climate zones and different altitudes from 100 to 2400 m above sea level as witnessed during collecting for this study. Its geographically confined area of distribution in

Iran (about 100,000 km^2) accompanied with a diverse topography and climate within its distribution area (Keikhosravi and Schubart, in press), incite to monitor the population genetic structure and diversity in *P. elbursi*.

The Pleistocene climate of Iran was more compartmented than in other countries at similar latitudes (Krinsley, 1968). Pleistocene glaciations exerted great influence on Iran, as the snow line of the higher elevations of the Alborz Mountains (currently around 3800 to 5000 m, e.g. at Damavand Peak) was lowered by about 600 to 1100 m (*i.e.* approx. 3000m) (Bobek, 1937; Ferrigno, 1991). During those periods, the precipitation/evaporation ratio was higher since the mean annual temperature was 5 to 8 °C lower than at present (Krinsley, 1966; Krinsley, 1970; Ferrigno, 1991). Pleistocene glaciations created many ice barriers and blocked connection between populations of freshwater fauna in the Alborz Mountains (Banarescu, 1991; Brandis *et al.*, 2000), which explains the occurrence of a freshwater crab species with a locally highly confined distribution, *Potamon bilobatum* Brandis *et al.*, 2000, in this region.

In this study, we use mitochondrial DNA of six populations of *P. elbursi* from the Central Alborz Mountains with the following aims: (1) to estimate genetic diversity and population connectivity in order to reconstruct past and current genetic structure and differentiation patterns; (2) to track the likely impact of Pleistocene glaciations on the population structure of *P. elbursi*, while proposing a historical demography, and (3) to discuss conservation status of this species based upon possibly discernible phylogenetic units and considering whether they deserve to be protected.

Methods

Sampling strategy and laboratory work

The Alborz Mountains extend from the northwest of Iran to the southern shores of the Caspian Sea and stretch to the borders of Turkmenistan in the northeast (Fig. 4.1). Two distinct climate zones can be encountered on each side of the mountains: The northern slopes face the lowland area of the Caspian region, which is a highly forested area with lots of rainfall, whereas to the south the climate is semi-arid with low precipitation. All six permanent rivers belonging to two drainage systems of the Alborz Mountains were considered in the current study. Two rivers (Darakeh and Jajrood) form part of the Namak Lake drainage and flow southward towards the centre of Iran. The remaining four rivers considered in this study (Taleghan, Sepidrood, Mollali and Arpachai) belong to the Caspian watershed and all drain into the Spidrood River to be finally discharged northward into the Caspian Sea (see Fig. 4.1). A total of 61 specimens of *P. elbursi* were collected during three field trips in 2009 and 2010. In order to not overexploit extant populations of freshwater crabs, a limited number of individuals were removed from the rivers. The specimens were killed on ice and subsequently transferred to 70% ethanol. One walking leg from each individual was removed and preserved in absolute ethanol.

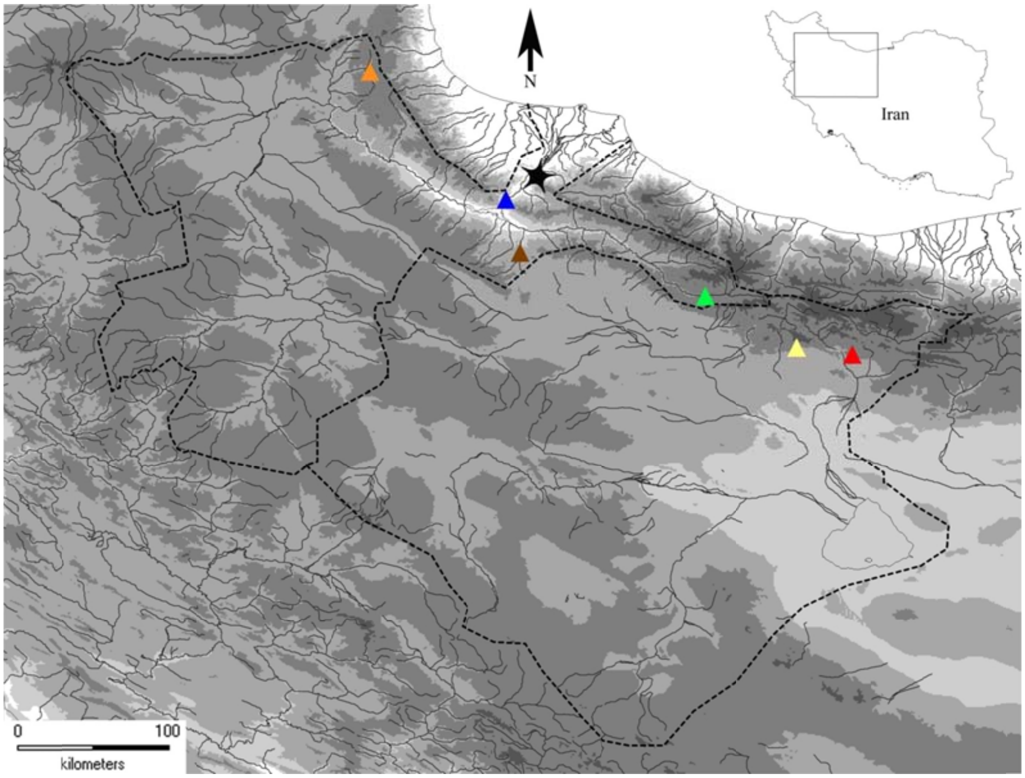

Figure 4.1. Distribution of *Potamon elbursi* in two drainage systems encircled by dashed lines: Namak Lake (right) and South Caspian Sea (left). Asterisk indicates location of the Sepidrood Valley. Higher Alborz elevations are indicated by increasing gray shades. Sampling sites are marked as triangle. JAJ (red); DRK (yellow); TLN (green); MOL (brown); SEP (blue); ARP (orange). See fig. 2 for river labels.

Locality information concerning the sites and sample sizes are shown in table 4.1. Since some areas are inhabited by more than one species of freshwater crab, all populations were examined morphologically beforehand.

Genomic DNA was isolated from the muscle tissue extracted of walking legs using a modified Puregene method (Gentra Systems) at the University of Regensburg. The entire mitochondrial gene cytochrome oxidase subunit I (Cox1, ~1500 basepairs) was amplified with the primer combination COL6 (5′–TYTCHACAAAYCATAAAGAYATYGG–3′) and COH16 (5′–CATYWTTCTGCCATTTTAGA–3′) (Schubart, 2009). Polymerase chain reaction (PCR) was carried out under the following conditions: an initial denaturation step at 95°C for 4 min, 40 cycles consisting of a denaturation step at 94°C for 45s, an annealing step of 45s with a touchdown procedure lowering the annealing temperature from 53°C to 50°C for the 15 first cycles followed by 25 cycles with 50°C, an extension step at 72°C for 90s, and a final extension at 72°C for 8 min. The sequences were outsourced to LGC Genomics, Berlin using COH16 as sequencing primer. They were manually corrected with Chromas Lite 2.01 (Technelysium Pty Ltd, 2007) and aligned with BioEdit (version 5.09, Hall, 2001). The software Artemis (version 14.2.2; Carver *et al*., 2012) was used to test for possible amplification of pseudogenes. The presence of indels or premature stop codons in the open reading frame of a coding gene would indicate the amplification of pseudogene. Sequences are made available at the European Molecular Biological Laboratories database EMBL [accession numbers to be added in proof version].

Table 4.1. Studied specimens with further information including locality details, number of sampled individuals (N) and abbreviations for population.

Sampling site	N	Abbreviation
South Caspian drainage:		
Tehran Prv., Tehran, Darakeh Darakeh R.	10	DAR
30 km NW Tehran, Kamard, Trib to Jajrood R.	12	JAJ
Namak Lake drainage:		
Qazvin Prv., Mollali R., Trib to Shahrood R.	12	MOL
Alborz Prv., Thaleghan R.	10	TLN
Guilan Prv., Roodbar, Sepidrood R.	11	SPD
Ardabil Prv., 5 km north of Khalkhal, Arpachai R.	6	ARP

Statistical analyses

Haplotype diversity (h), number of segregating sites (S) and nucleotide diversity (π) (Nei, 1987) were computed using DnaSP 5.00 (Librado & Rozas, 2009). Two selective neutrality tests; Fu's F_s-test (Fu & Li, 1993) and Tajima's D (Tajima, 1989), were estimated with 1000 simulate replicates using Arlequin 3.5.1.2 (Excoffier & Lischer, 2010). Both tests were used to determine whether sequences of each population depart from expected neutrality. In addition, a negative value of Tajima's D test would suggest an excess of rare alleles or haplotypes that may be due to a recent population expansion.

To test historical demographic trends in the pooled populations an overall mismatch distribution was plotted. The mismatch distribution of populations which passed through a recent expansion or bottleneck would be unimodal, whereas a multimodal distribution carries the signature of a constant population size in the past (Rogers & Harpending, 1992; Schneider & Excoffier, 1999; Hamilton, 2009). Genetic structure and differentiation among subpopulations was estimated using the F_{ST} statistics as implemented in Arlequin 3.5.1.2. A statistical parsimony network analysis was conducted using the program TCS version 1.21 (Clement *et al*., 2000) in order to graphically represent mutation steps between sequences and reconstruct the relationships among all encountered haplotypes.

Results

A partial segment of the 3′ end of the Cox1 gene with readable sequence lengths of at least 840 basepair (bp) was obtained from 61 specimens belonging to six river populations. The sequence alignment was unambiguous and free of stop codons and indels. The length of amplicon of 1500 bp and the lack of stop codons makes it less likely that pseudogenes have been co-amplified, which was confirmed by the software Artemis. The final alignment included 15 variable sites, of which eleven were parsimony-informative and four were singleton variable sites.

Total haplotype diversity was relatively high (0.821) despite the fact that one population (TLN) showed no haplotype diversity. In contrast, total nucleotide diversity is relatively low (0.00409) as the 11 detected haplotypes are closely related. Most specimens (42) share one out of three main haplotypes (Fig. 4. 2; haplotypes T, M, P) and the remaining animals present eight

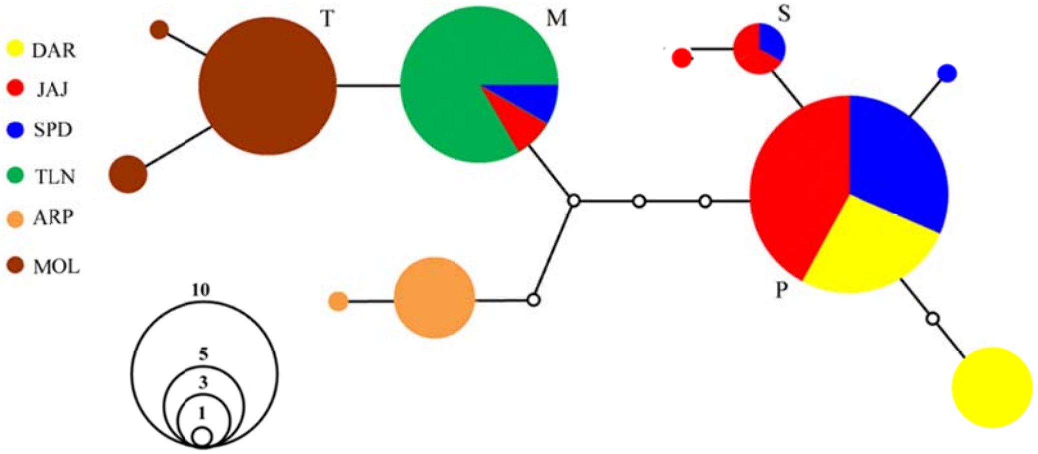

Figure 4.2. Maximum parsimony spanning network constructed with TCS based on a 840 basepair alignment of the Cox1 gene for a total of 61 specimens. Each line represents one substitution and open circles on lines indicate unsampled or missing haplotypes. The open circles with numbers above indicate the number of individuals in each haplotype.

Table 4.2. Sample size (n), number of hyplotypes (Hn), haplotype diversity (Hp), nucleotide diversity (π), Tajima's (D), Fu's (Fs)

River	n	Hn	Hp	π	D	Fs
JAJ	12	4	0.561	0.00149	-1.4289	-0.154
DAR	10	2	0.556	0.00136	1.8442	2.429
TLN	10	1	0.000	0.000	-	-
MOL	12	3	0.439	0.00056	-0.8497	- 0.725
SPD	11	4	0.600	0.00200	-0.7262	0.364
ARP	6	2	0.400	0.00048	-0.933	-0.003
Total	61	11	0.821	0.00409	0.11064	-0.15221

haplotypes with low frequencies. Among them, four haplotypes are represented by single specimens (Table. 4.2, Fig. 4.2).

The haplotype network indicates that there is phylogeographic structure in populations with local endemicity (MOL and ARP, see Fig. 4.2). Other populations did not show clear genetic population subdivision among rivers, even though they belong to two different drainage systems. The populations JAJ and SEP revealed a total of four different haplotypes, of which three are shared by individuals of both populations, even though they are not sharing the same drainage system. The main haplotype (P) is also present in the DAR population, which shares drainage system with the JAJ population. The population TLN revealed only one haplotype (M) which is occasionally shared by SEP and JAJ populations.

F-statistics were run to find possible significant differences among populations (Fst = 0.77, across all samples). The analysis showed an overall significant differentiation ($p < 0.05$) among populations regardless of their association to river systems (Table. 4.3). The only exception is the SPD population which shows a negative Fst value (-0.03) and obviously not significant difference in comparison to the JAJ population. The negative value indicates intra-population variability in this population is higher than inter-population variability. This seems to be an unnatural phenomenon, since both rivers do not belong to the same river system nor do their headwaters originate from the same mountain region. Alternatively, the negative value may indicate lack of sufficient sampling.

Tajima's D and Fu's F_s neutrality tests were not significant for the whole and each single population dataset ($p<0.05$). The positive values of both tests for the DAR population support the assumption that it has undergone a recent bottleneck effect (Table. 4.2). The positive and small negative value of both tests for the entire dataset precluded any conclusive outcome on population expansion. To overcome this problem, a mismatch analysis was applied. The mismatch diagram shows a bi-modal distribution for both demographic and spatial expansion models, implying that the null hypothesis of constant effective population size cannot be rejected (see Fig. 4.3).

Table 4.3. Pairwise Fst values of genetic differentiation among populations. Below diagonal are values of Fst, and their levels of significance are above diagonal

	JAJ	DAR	TLN	MOL	SPD	ARP
JAJ	—	***	***	***	NS	***
DAR	0.29	—	***	***	***	***
TLN	0.83	0.88	—	***	***	***
MOL	0.83	0.87	0.79	—	***	***
SPD	-0.03	0.26	0.74	0.77	—	***
ARP	0.82	0.86	0.96	0.90	0.76	—

*** = $p < 0.01$
NS= Non Significant

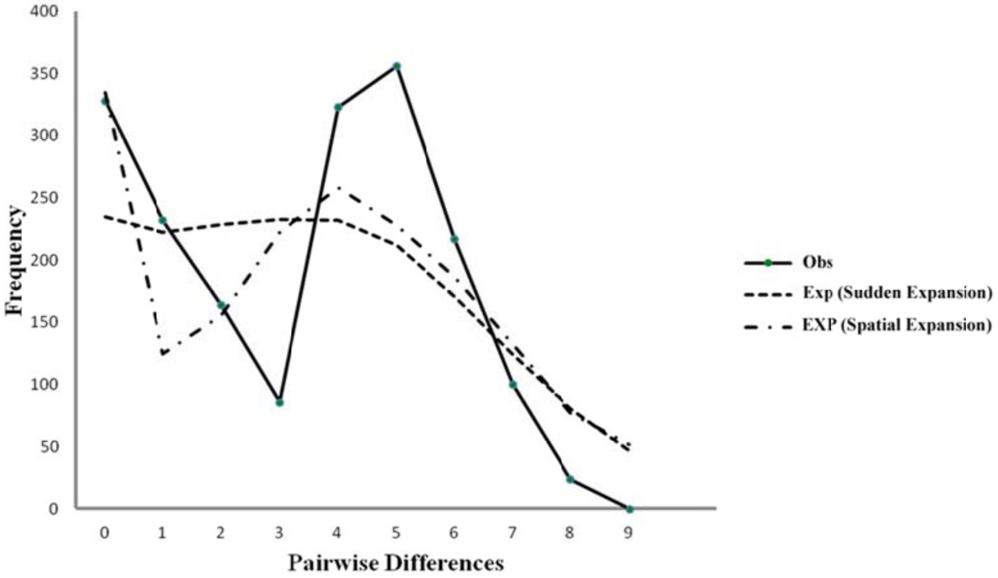

Figure 4.3. Mismatch distribution of the whole dataset. Dotted line represents expected value under the hypothesis of spatial expansion; dashed line indicates expected value under the hypothesis of demographic expansion. Solid lines represent the observed data.

Discussion

This study is the first to document intraspecific genetic differentiation, and thus potential endemism, in a macrozoobenthos organism from the Alborz Mountains. The large degree of genetic differentiation and high local endemicity in some populations shows these populations are well structured, even though it is not associated with geographical separation. According to presence of endemic phylogeographic units and negative impact of human activities on this region, the conservation importance of *P. elbursi* is here stressed. Contradictory results of mismatch analysis and haplotype network topology (and other statistical values) preclude any conclusive statement about the likely impact of the glacial cycles on population structure.

Our results give evidence for significant genetic differentiation among populations of *P. elbursi* resulting intra-drainage endemicity. It is also concluded that current drainage isolation is not a sufficient indicator for predicting genetic differentiation between populations of *P. elbursi* (e.g. between the Caspian and Namak Lake drainages). Occasionally, freshwater crabs may cross short land bridges during periods of heavy rainfall, when headwaters of these populations originate from the same mountain regions (Daniels, 1998, 2006a; Cook *et al.*, 2008; Poettinger *et al.*, 2011; Schubart *et al.* 2011; Keikhosravi & Schubart, 2013). Therefore, shared haplotypes among three populations (TLN, SPD and JAJ) can be attributed to geographic closeness of their headwaters. The finding of a main haplotype in population SPD grouping with those of JAJ and DAR (Fig. 4.2, haplotype P) cannot be explained by gene flow alone, but also may involve incomplete lineage sorting that is not a rare event in animals. The term “incomplete lineage sorting” means persistence of ancestral variation in descendant lineages for some time (Funk & Omland, 2003). Therefore, it can be assumed that both populations (JAJ and SPD) are still maintaining the ancestral haplotype diversity since divergence time. In freshwater crabs, such demographic asymmetry is also triggered by anthropogenic transport, because natural crossing of the land bridge for these populations is highly unlikely (Charmantier, 1992; Noël & Guinot, 2007; Jesse *et al.*, 2009). Jesse *et al.* (2009) stated that the occurrence of *P. fluviatile* in the two relatively small islands of Malta (off southern Italy) could be the result of human translocation. This is not surprising, since freshwater crabs are considered as a food source in ancient and modern Greek culture (Brandis, 1997). One confirmed example of human-mediated introduction is the recent presence of *P. ibericum* in southern France. The species has been presumably transported accidentally to France together with the Turkish crayfish *Astacus leptodactylus*, used for aquaculture in the 1960s and 1970s (Charmantier, 1992; Noël & Guinot, 2007). Recent translocation due to human consumption seems unlikely in the case of *P. elbursi*, since using freshwater crab as food resource is not documented in archaeological studies from this region. Some rivers of the southern Caspian Sea and neighbouring drainage systems (e. g. Sepidrood R. and Jajrood R.) have been used for fish farming and stocking since 1961 (Mehrabi, 2002). Therefore, freshwater crabs may have accidentally been transferred together with fish fingerlings from Jajrood R. to Sepidrood R. or vice versa.

The populations of MOL and ARP are highly endemic and each one has its own private haplotypes. This could be explained by a recent population expansion after a low effective population size caused by bottlenecks (Hou *et al.*, 2012). The low effective population size is also explained by proneness of small populations to genetic drift as a consequence of founder effects under a reduced gene flow and population size model (Whiteley *et al.*, 2004). As mentioned above, freshwater crabs are confined to their birthplace, and therefore the reduced gene flow of these populations can be associated with their regional philopatric behaviour. Apparently, the large degree of genetic differentiation and endemicity in the ARP population can also be attributed to its geographical distance and consequently habitat fragmentation. Endemicity in the MOL River (which is geographically very close to TLN and SEP) can be partly addressed to occurrence of subterranean passages along the river (seen by first author) that could hinder crabs to migrate along the river and thus maintaining the gene flow.

Glacial influences were important in speciation processes and shaping current population demography of organisms in the Alborz Mountains (Brandis *et al.*, 2000; Ahmadzadeh *et al.*, 2012). *Potamon bilobatum*, for example, is assumed to be the consequence of an allopatric speciation event in the same mountains resulting from such extreme Pleistocene glaciations (Brandis *et al.*, 2000). In spite of this, glaciations may not have severely impacted the overall distribution pattern of Alborz's flora (Ferrigno, 1991, Noroozi *et al.* 2011). The hypothesis is supported by high endemicity in subnival-nival flora of the Alborz Mountains, indicating the absence of extensive Pleistocene glaciations (Noroozi *et al.*, 2008 & 2011). It seems that the Pleistocene glacial periods had no considerable impact on population structure of

P. elbursi. This is revealed by a bi-modal shape of the mismatch distribution for either expansion models (Fig. 4.3). But, on the other hand, the haplotype network topology supports the idea that most of populations are regionally expanded (except for the case of DAR and TLN populations) (see Fig. 4.2). In addition, high haplotype diversity (Hp = 0.821) and low nucleotide diversity ($\boldsymbol{\pi}$ = 0.00409) of total population is also consistent with a recent expansion model (table. 4.3). The current incomplete geographic sampling precluded any conclusive outcome as neither of hypotheses (expansion or constant population size) can be strongly accepted.

Conservation priority for the southern Caspian Basin is of crucial importance considering that some unique and endemic freshwater fish fauna of the southern Caspian Basin are critically endangered by human anthropogenic activities and desiccation (Kiabi, 1999; Coad, 1980). As detailed in the Introduction, decapod crustaceans are important components of food webs in freshwater ecosystems (Carnevali *et al.*, 2012). The restricted distributions of most of the freshwater crab species pose serious problems for conservation (Yeo *et al*., 2008). *Potamon elbursi* is represented in both local basins, Namak Lake and southern Caspian Sea in Iran. The sampling trips between 2009 and 2011 for this study revealed that beyond any doubt both watersheds surrounding the restricted distribution of *P. elbursi* are extremely suffering from desiccation and dam construction. In addition, this freshwater crab, as well as the rest of the macrozoobenthos, has not received any conservation attention. Therefore, all facts put together justify a prompt and thorough investigation of this species' populations with a wider sampling strategy to uncover more precisely the necessity and level of protection. Herewith we want to make emphasis on the importance of considering the Alborz Mountains for possible future conservation plans, as the studied freshwater crab includes local populations with unique diversification and high endemism (Davies *et al.*, 1993). Occurrence of genetically distinct endemic and isolated populations is of critical importance as highlighted in this study. It is also suggested that inter and intra drainage fish transfer should be carefully considered because cross-stocking could erode genotypic and phenotypic diversity over time (Largiadèr & Scholl, 1995).

Acknowledgement

The first author would like to express his special appreciation to Mr Yadollah Houshmand and Fatollah Houshmand who helped to collect specimens. Special thank to Nicole Rivera, Nicolas Thiercelin, Nicole Rivera and Jürgen Trettin for their great help with statistical analysis. Our thanks also go to Prof. Jürgen Heinze (University of Regensburg) and his staff for the use of the necessary facilities and continuing support and the Akademisches Auslandsamt of the University of Regensburg for financial support.

Chapter 5

General discussion

The current study shows that many areas of the entire distribution range of *Potamon* in the Middle East, in particular Iran, are still unexplored. *P. ilam* from the westernmost slopes of the Zagros Mountains is an example of such lack of knowledge, since it was not sampled in the last studies (e.g. Pretzmann 1962, 1965, 1966a, 1976a; Brandis *et al*., 2000, see Chapter 2). The present work also takes an important step in understanding species phylogenetic delimitations by solving taxonomic problems in species complexes and discovering evolutionary significant units (ESUs) with molecular tools. As an example, the species complex *P. persicum* consists of three separated lineages: the first corresponding to the species itself from the type locality, the second is *P. elbursi*, a revalidated species, and the third is an evolutionary significant unit from western Iran. The development of the Zagros Mountain Belt favored an increase in biodiversity (Hoorn *et al*., 2013). This study shows the great influence of the Zagros Mountains on freshwater crabs' diversity by determining the structure of river systems in the Middle East (see Chapters 2 and 3). Intraspecific scale analysis of *P. elbursi* populations from the Alborz Mountains indicates a phylogeographic structure with some populations showing high endemicity. Ongoing anthropogenic activities, in addition to local populations with unique diversification urge to include this freshwater crab from the Alborz Mountain into immediate conservation.

The Zagros and Alborz Mountains are pointed as one of the main reasons for diverse topography and climate variability in the Middle East resulting from mountain uplifting and consequences on isolation and climate (Kehl, 2009). Hence, such different topography and climate regimes explain the current varied and unique biological diversity of Iran (Ahmadzadeh *et al*., 2012; Hoorn *et al*., 2013; Rajaei Sh *et al*., 2013; see Chapter 2 and 3).

Western and southwestern Iran and adjacent areas in Iraq exclusively include many endemic plant forms, the majority with Irano-Turanian origins (a floristic region including central, east and southeast Anatolia). This phytogeographic evidence of high endemicity classifies the region as "biogeographically important area" (Rechinger, 1972; Rechinger & Lack, 1991; Akhani, 2004). However, adjacent phytochoria (area with a relatively homogeneous composition of plant species) do not usually have a distinct boundary, and they are defined by a transitional area in which many species from both areas overlap. In our study, two species of *Potamon*, *P. mesopotamicum* and *P. ilam*, from this Iraq/Iran area are phylogenetically closer to each other than both are to *P. persicum* (Fig. 5.1). Both species have almost the same distribution as Irano-Turanian vegetations and do not extend their range over the Zagros Mountains to the East. Hence, this study along with other herein cited studies highlights the need to emphasize more on the biogeographical uniqueness of the western region of the Zagros Mountains. The local endemism is most likely the result of geological activity in this region, viz., the current uplift of Zagros Mountains. The description of *P. ilam* (see Chapter 2) lacks molecular dating, but nucleotide divergence between the two groups, *P. persicum* + *P. elbursi* and *P. ilam* + *P. mesopotamicun* of 3.03% (uncorrected pairwise distance) supports an allopatric event in separating ancestor populations around 3.6 million years ago based on a substitution rate of 0.65% per million years for non-endemic Jamaican freshwater crab (Schubart *et al*., 1998) (see Chapter 2; Fig. 2.5). The separation time between these taxa is almost in accordance with orogenic activity of the Zagros Mountains. It is roughly estimated that a rate of 1 mm/year is shaping the current uplift of the Zagros Mountains since 4-5 mya (Rangzan & Iqbaluddin, 1995; Agard *et al*., 2011). Therefore, it can be postulated that speciation processes resulting in the formation of both species, *P. ilam* and *P. mesopotamicum*, as in the fish and lizards, can be the result of uplifting of the Zagros Mountains (Zagros Simply Folded Belt) (Macey *et al*., 1998; Rastegar-Pouyani & Nilson, 2002; Hrbek *et al*., 2006). *Potamon ilam* and *P. mesopotamicum* share a large drainage system (Euphrates-Tigris drainage system), but they are not encountered together in the same river. All the rivers occupied by *P. ilam* originate from the westernmost slopes of the Zagros Mountains and flow into the Tigris River in eastern Iraq. In contrast, *P. mesopotamicum* is distributed in the Khabur River, adjacent to Euphrates River, and based on a recent report, in Al-Huwaizah marshes, Iraq (downstream of Tigris River) (Brandis *et al.*, 1998; Naser, 2009). Occupation of upstream Tigris by *P. magnum* and *P. persicum*, suggests limited distribution potential for *P. ilam* within this river system. To uncover the ambiguities about possible occurrence of other species together with *P. ilam*, it is necessary to evaluate genetic variation and hidden local genetic diversities by help of wider sampling and assessing the behavioural ecology of each species.

Although one lineage of *P. persicum* complex from Isfahan (type locality) is a genetically distinct clade (Fig. 5.1), it does not show any consistent morphological character to distinguish it from its sister clade from the western Iran. The third lineage, formerly named as *P. persicum kermanshahi* (see Chapter 3 for detailed discussion). Likewise, a freshwater fish species, *Aphanius isfahanensis*, was described from the same locality (Isfahan) only based upon molecular results and the authors even emphasize that the colour pattern used in the description is not valid to identify it from the closely related species *A. sophiae* and *A. persicus* (Hrbek *et al.*, 2006). The shared results of both studies are probably due to morphological stasis or, in other words, lack of enough time/environmental change for morphological changes (Schubart *et al.*, 2000; Hrbek *et al.*, 2006). Therefore, it still must be considered a cryptic lineage but uncovering other likely significant morphological features for discrimination is demanded.

Pretzmann (1962) described *P. elbursi* from the middle Alborz Mountains. Later, Brandis *et al*. (2000) synonymised the species under *P. persicum*, giving priority to the latter species. In Chapter 3, *P. elbursi* is revalidated and redescribed using morphological and molecular evidence. Two hypotheses can be formulated for the separation between *P. persicum* and *P. elbursi*. The first hypothesis is related to the drying of the big desert, Dasht-e Kavir, in Central Iran. It is claimed that Dasht-e Kavir was partially considered a shallow lake in the Pliocene to Lower Pleistocene evidenced by the presence of thick brown silt and clay layers deposited under quasi-permanent lake environment (Huber, 1960; Bobek, 1963; Kehl, 2009). Therefore, it can be deduced that some water systems must have drained to the lake. In addition, climate was slightly more humid than today which is favoured by freshwater crabs (Bobek, 1963). These conditions enable dynamic freshwater organisms to have genetic exchanges via land crossing and river connections. This connection disappeared, because of drier climate condition and shrinking of the lake by the beginning of the Pleistocene (Kehl, 2009). The second hypothesis is that the type locality of *P. persicum*, Isfahan, lies in a small endorheic drainage basin (Gavkhouni), mainly surrounded by mountains. This basin is considered a freshwater eco-region and as a consequence, it may have distinct and unique freshwater biodiversity from that of rivers belonging to nearby water systems (an eco-region is defined by World Wildlife Fund as a large unit of land or water containing a geographically distinct assemblage of species, natural communities, and environmental conditions). The latter reason emphasizes the consequence of the diverse topography caused by the Zagros Mountain Belt. In conclusion, the results suggest that *P. persicum* radiated recently and, with the course of time, became restricted to different watersheds.

It is proposed that Quaternary cold periods have had a dramatic influence on most organisms in temperate regions (Hewitt, 1996). Direct physical evidence of multiple glacial progressions have been reported for several parts of the Iranian Plateau (Wright, 1962; Kuhle, 2004), but the timing and its possible consequence of climate change during the glacial periods on Iranian biota is poorly studied (e.g. Ferrigno, 1991; Djamali *et al.*, 2008a, b; Noroozi *et al.*, 2011, Ahmadzadeh *et al.*, 2012; Rajaei Sh *et al.*, 2013). A palynological study from Lake Urmia in northwestern Iran showed ample vegetation dynamics through the glacial and interglacial periods (Djamali *et al.*, 2008a). In spite of such extensive influence of glacial periods on plant coverage in northwestern Iran, it may not have severely impacted the overall distribution pattern of high altitude flora (Ferrigno, 1991; Noroozi *et al.*, 2011). In other words, the high endemicity in subnival-nival flora of the Alborz Mountains is considered a result of the absence of extensive Pleistocene glaciations (Noroozi *et al*., 2008, 2011). In contrast, Brandis *et al*. (2000), states that *Potamon bilobatum* is the result of an allopatric speciation event in the Alborz Mountains caused by extreme Pleistocene glaciations, since it is supposed that ice-barriers occurred in the Alborz Mountains during the glacial periods (Thienemann, 1950; Banarescu, 1992). Likewise, the possible explanation for the fragmented distribution of the genus of lizard *Iranolacerta*, present in the northwest of Iran and the Central Zagros, is most likely the past climate fluctuations according to Ahmadzadeh *et al.* (2012). Rajaei Sh *et al*. (2013) discuss the occurrence of two sympatric moth species that were introduced into several possible refugia (especially the Zagrosians) during the LGM, implies a noticeable effect of glacial periods on biota in Iran. Clearly, studies on freshwater organisms must be somehow different, in particular when the demographic history of the organism is mainly linked to the geological history of drainage systems, but at least either limnic or terrestrial organisms might have shared the same history, while facing past climate fluctuations. Unlike the moth and lizard examples

(Ahmadzadeh *et al*., 2012; Rajaei Sh *et al*., 2013), the bi-modal shape of the mismatch distribution for populations of *P. elbursi* explains that the null hypothesis of constant population size cannot be rejected. In other words, the cold periods probably had no significant impact on the population structure of *P. elbursi*. On the contrary, high endemicity and starburst phylogeographic pattern (Avise, 2000) in some of inhabitant populations justifies a sort of expansion after a cold period. Therefore, a larger sampling is needed to resolve population demography of the species during the glacial and interglacial periods. The current study puts more emphasis on the importance of considering the Alborz Mountains for future conservation plans, as the studied populations of freshwater crab *P. elbursi*, displays local populations with unique diversification and high endemism (Davies *et al.*, 1993).

Unpublished results

The following section includes the preliminary results of unpublished data that help to have a better understanding about the phylogenetic and phylogeographic relationships of the genus *Potamo*n in the Middle East.

Preliminary results of an expanded study on populations of *P. elbursi* show that the upstream population of this species (Ghezel Ozan Basin) is distantly connected to the populations from downstream, i.e. the Alborz Mountains (Fig. 5.2; haplotype GON). It also indicates a high endemicity in upstream population, whereas downstream populations stay closer to populations belonging to different drainage systems. This may be because of the philopatric behavior (Daniels *et al*., 2006a), the long drainage system (666 km), or drought that stops the crabs from contacting each other. The current incomplete number of sequences from further populations precluded any conclusive outcome.

In another phylogeographic approach we studied *Potamon ibericum*. This species is distributed from the southeastern Balkan Peninsula to the Black Sea Coast (except the northern coast) and at the east end reaches to the tributaries of the south Caspian Sea. *P. ibericum* is exclusively distinctive because of consistency of its morphological characters - the first gonopod shape in particular- that virtually shows no intraspecific or regional variation (Brandis *et al.*, 2000). Nevertheless, populations of *P. ibericum* can be considered several isolated lineages (evolutionary significant units) (see Fig. 5.1 and Fig. 5.3).

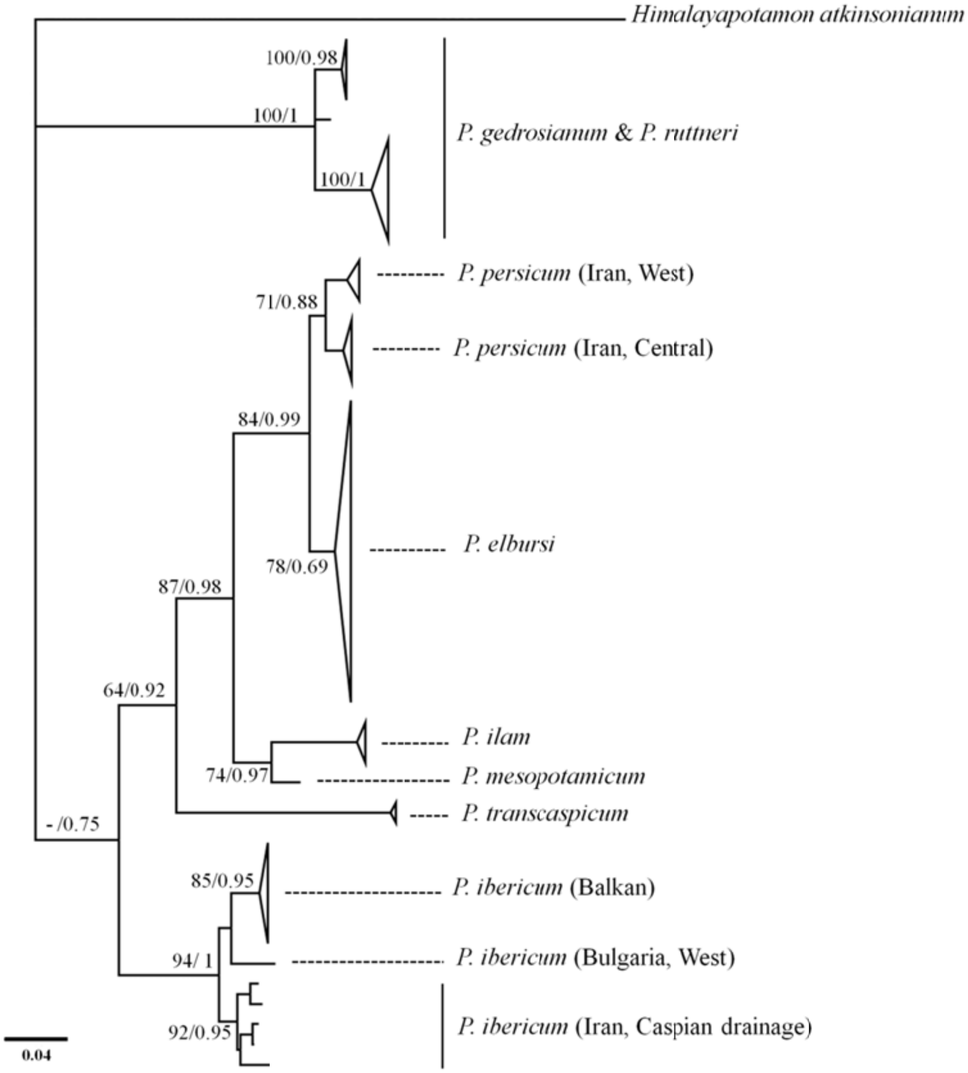

Figure 5.1. Phylogenetic relationship of eight species of *Potamon*. According to a concatenated tree of 2116 basepair alignment of combined 16S and Cox1 sequence data. 1000 bootstraps and 6×10^4 generations were used for running MrBayes and RAxML, respectively. Bootstrap values/posterior probabilities expressing support for the corresponding clades. Values < 50% are not shown.

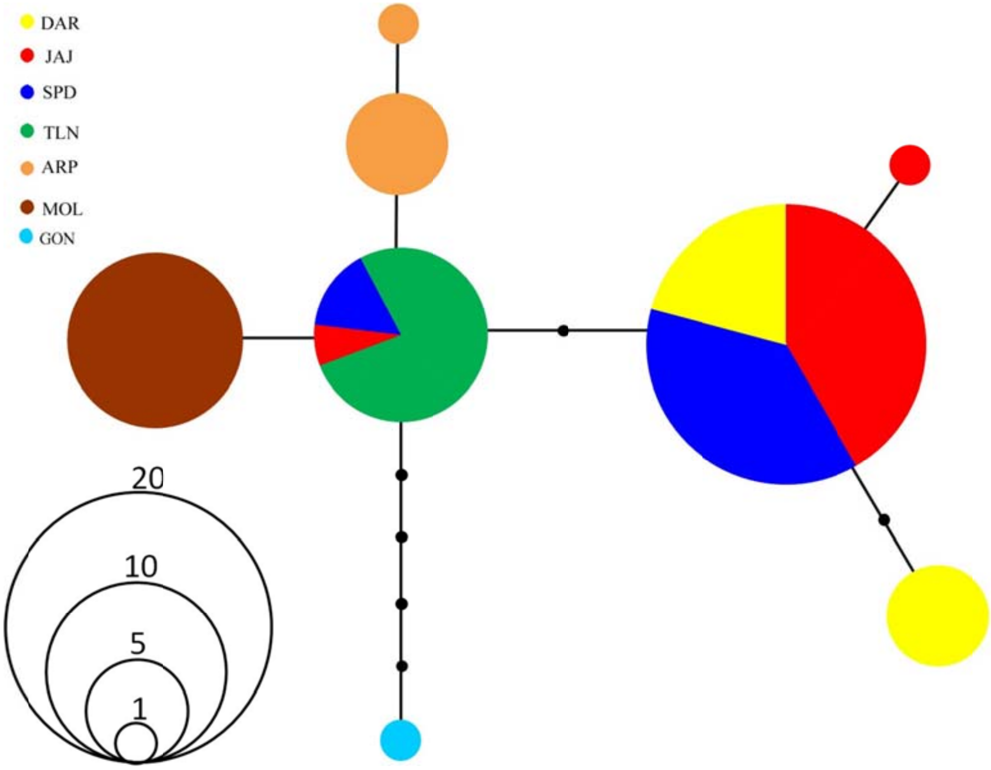

Figure 5.2. Maximum parsimony spanning network constructed with TCS of populations of *Potamon elbursi* based on 62 individuals and a 467 basepair alignment of Cox1. Each line represents one substitution and filled circles on lines indicate unsampled or extinct haplotypes. The open circles with numbers above indicate the number of individuals in each haplotype. See table 4.1 for haplotype labels. GON, upstream population.

Populations from southwestern Bulgaria and northern Greece from rivers draining into the northern Aegean Sea appear to be distinct from populations of the Black Sea drainage system (Fig. 5.1 and 5.3). Thus, it is a divergent cryptic lineage requiring further taxonomic attention (see also Jesse *et al*., 2011). The remaining populations from central-eastern Bulgaria and an introduced French population have high sequence similarity and low endemicity (Fig. 5.1 and Fig. 5.3). Such impoverished genetic diversity can be explained by remains of a strong population decline (or bottleneck) during glacial period, followed by a rapid expansion of populations or founder effect from a refugium (Hewitt, 1999). The strong population decline could be witnessed by an archeology-based study by Klaus & Gross (2010). They propose

Potamon antiquum, an extinct population of *P. ibericum* from Hungary, represents evidence for the northern distribution limit of *P. ibericum* (Pannonian Basin at the Gerecse Mountains) during phases of warmer climate (Klaus & Gross, 2010). Therefore, it is here suggested that this colonization could be an expansion from southern Carpathians (considering a blocked expansion route via Turkey by more northerly neighbours) (Taberlet *et al*., 1998; Hewitt, 1999). In addition, this expansion is also explained by colonization from western Anatolia (west Turkey) to the Balkan Peninsula via Thrace (Taberlet *et al*., 1998; Hewitt, 1999; Dubey *et al*., 2006; Ahmadzadeh *et al*., 2013). The current study also proposes that the Greek and Bulgarian (SW) populations remained in their distinct refugia, Greece where they were blocked in their regions by more northerly neighbours (King & Ferris, 1998; Cooper *et al*., 1998; Hewitt, 1999). Jesse *et al*. (2011) demonstrate that the divergence event between this *P. ibericum* and the Greek lineage happened about 1 million years ago. Therefore, all facts put together justify the current diversity between populations of *P. ibericum* in the Balkan Peninsula.

The strong observed genetic differentiation between the Caspian and Black-Aegean Sea group (Fig. 5.3), in accordance with the same results acquired by other phylogeographic studies, introduces the southeast of the Caucasus Mountains and southern coast of the Caspian Sea as an apparent hotspot of refugia during the Pleistocene glaciations (e.g. Hewitt, 1999; Dubey *et al*., 2006, Gvozdík *et al*., 2011). Surprisingly, populations from the Caspian drainage are highly differentiated from each other and comprise three distinct lineages with high haplotype diversity (Fig. 5.1 and Fig. 5.3). In contrast, we expected to have the minimal endemicity and maximal homogeneity for these populations, since humid and rainy conditions are permanent climate characteristics of the southern Caspian lowlands. It is assumed that freshwater crabs can cross short terrestrial distances between rivers especially in humid and rainy conditions (Gherardi *et al*., 1988b; Gherardi *et al*., 1988a; Morris & Van Aardt, 1998; Daniels, 2003; Daniels *et al*., 2006a). Therefore the question arises: why is such strong endemicity and genetic divergence present between populations of *P. ibericum* in the northern face of the Alborz Mountain, where we expect high dispersal ability and minimal genetically structured populations similar to those from the Black Sea? This is more evident when populations of *P. elbursi* from the southern slopes are showing more homogeneity even though they do not share the same water system (see Chapter 4), and climate in the region is semi-arid with low precipitation. From the ecological point of view, this prompts us to give more attention to populations of both species

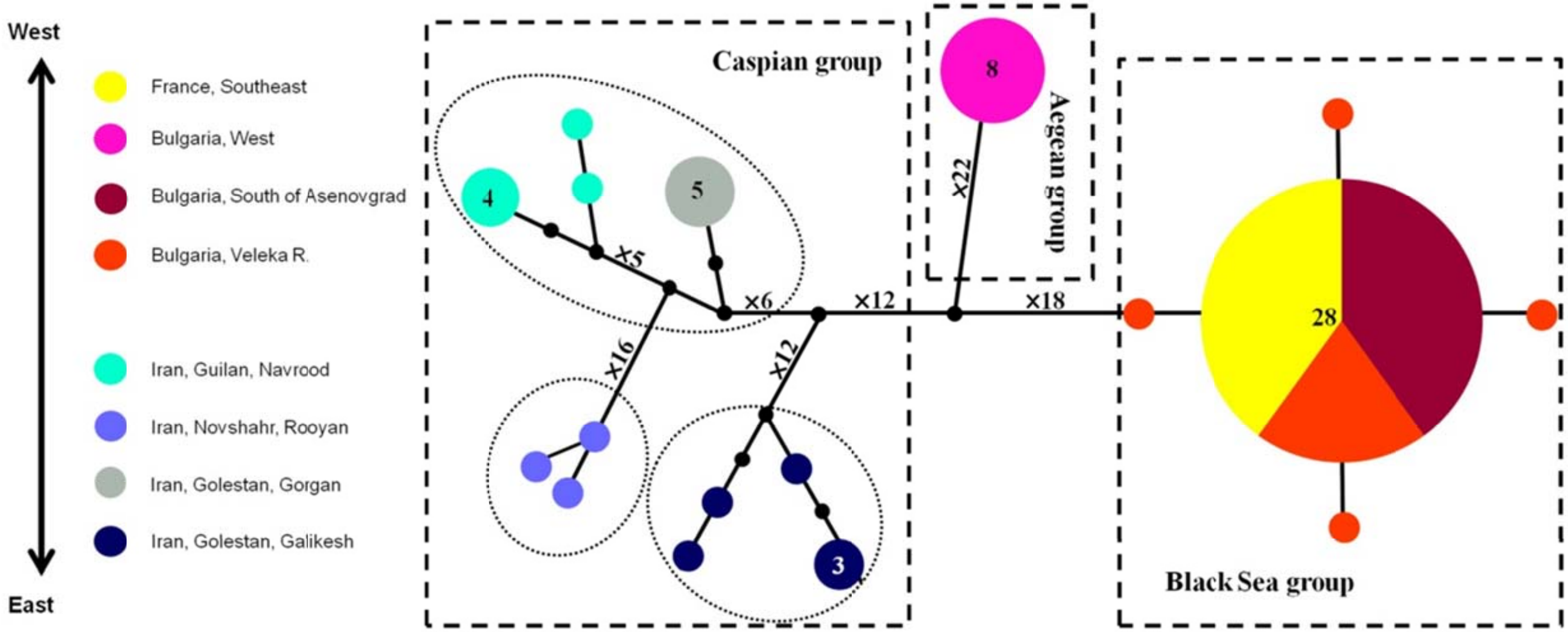

Figure 5.3. Maximum parsimony spanning network constructed with TCS of *Potamon ibericum* based on 60 individuals and a 911 basepair alignment of Cox1. Numbers next to connection lines correspond to mutation steps. Each line represents one substitution and filled circles on lines indicate unsampled or extinct haplotypes.

on either side of the mountains using different markers and wider sampling (work in progress). In summary, this remarkable diversity within *P. ibericum* combined with the diverse range in topography, suggests additional uncovered diversity. The current incomplete geographic sampling (i.e. Turkey, Georgia and Azerbaijan), lack of sequences from more specimens, and the need of further taxonomic attention precluded any conclusive outcome.

The easternmost distribution of *Potamon* in the Middle East is a region is in need of more concern for understanding diversity of temperate freshwater crabs, where the distribution extends to the western tributaries of the Indus River. Some species and subspecies were described from this region, e.g. *Potamon fluviatile* Latreille, 1909, *Potamon gedrosianum lindbergi* Bott, 1967; *Potamon gedrosianum torbenwolffii* Bott, 1967; *Potamon gedrosianum waziristanis* Pretzmann, 1965; *Potamon gedrosianum ruttneri* Bott, 1967 (Alcock, 1909; Pretzmann, 1962, 1965, 1966a; Bott, 1967, 1970). Later, Brandis *et al*. (2000) synonymised them and ranked them at species level. Based on the latter study, there are two species of *Potamon* known from this region, *P. gedrosianum* Alcock, 1909 and *P. ruttneri*. Preliminary genetic data show that some populations are very divergent as three distinct lineages can be distinguished (Figure 5.1 and Figure 5.4). This questions the current classification and the actual boundaries between the two species. Therefore, overall species diversity in this region seems to be considerably higher than is currently recognized and some synonymised species deserve to be revalidated. The genetic results also show that there is confusion in the morphological recognition of two formerly valid species. This prompts us to examine further material using both techniques, molecular and morphological, for disentangling the issue.

P. transcaspicum was formerly supposed to have a distribution in eastern tributaries draining to the Caspian Sea. Our morphological and molecular studies showed that the species has a wider distribution to the East, i.e. western tributaries of the Indus River in western Afghanistan. Therefore, the species has a distributional rage partially overlapped with that of *P. gedrosianum*.

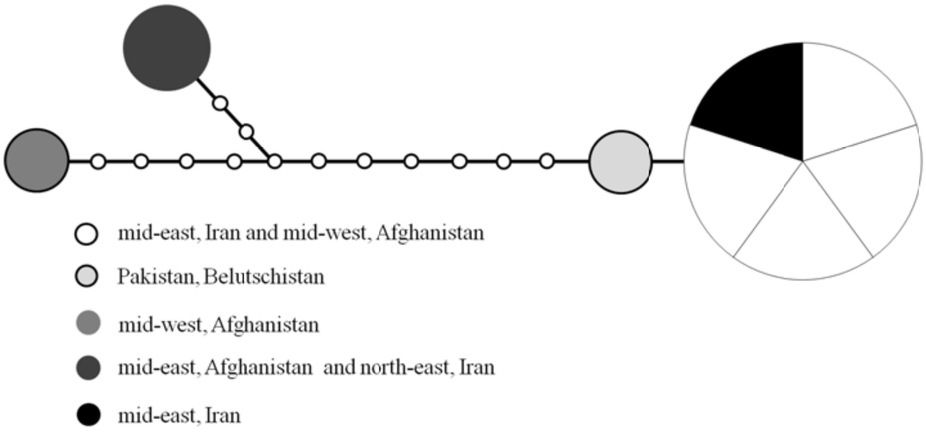

Figure 5.4. Parsimony haplotype network of *Potamon ruttneri* (Black and darkest grey) and *P. gedrosianum* based on 9 individuals and 607 basepair alignment of 16S. Each line represents one substitution and open circles on lines present unsampled or extinct haplotypes.

Conclusions

Resolving the phylogenetic relationships among the species of *Potamon* and discovering evolutionary significant units of species with different techniques will provide an insight into the biogeography and diversity within this genus. The distribution of the species of *Potamon* in the Middle East, which is known as a connection between the Palaearctic and Oriental faunas, also reflects underlying geotectonic and zoogeographic patterns and processes. Therefore, to answer the raised questions, a larger study including more species of *Potamon* is demanded in order to reveal the biogeographic history of freshwater crabs in the Middle East. Additionally, morphology- based phylogeny of *Potamon* has been source of disagreement in the Middle East (i.e. Bott, 1967, 1970; Pretzmann 1962, 1965, 1966a, 1971, 1976a, b; Brandis *et al*., 1998, 2000). Therefore it seems necessary to apply higher resolution methods for these purposes like the ones provided by molecular techniques (Jesse *et al*., 2011; and Chapters 2 and 3).

Some interesting questions raised by this study are from an ecological perspective. First, occurrence of different species in the same river system (e.g. *P. ilam*, *P. persicum*, *P. mesopotamicum and P. magnum*) and second, showing genetic pattern contradictory to behavioural ecology and biology of the species (*P. elbursi* and *P. ibericum*): considering the climate and drainage specificities, populations of *P. ibericum* must be more capable to cross terrestrial barriers and expectedly show more genetic homogeneity and low endemicity along the northern face of the Alborz Mountains. On the contrary, they show a large extent of heterogeneity and endemicity. In the case of *P. elbursi*, it was expected to have high heterogeneity and endemicity, but the reverse pattern is observed, since they show mild heterogeneity and endemicity along the southern face of the same mountains.

Freshwater organisms receive less attention and legal protection than terrestrial organisms, even if, such small area of habitat contains extremely high species diversity. It is roughly estimated that about 12,000 species of freshwater invertebrates are now endangered or have been extinct by human activities (Stiassny, 1996; McAllister *et al.*, 1997; Strayer, 2006). Iran’s freshwater systems receive even less attention than those in developed countries. Diverse ecology in Iran is threatened by desertification caused by anthropogenic activities (e.g. excessive water exploitation and pollution). Since 1950, as an example, 643 dams have been constructed and many more are under construction and planned (Cheraghi, 1996; *http://daminfo.wrm.ir/fa/dam/stats*).

The sampling trips between 2009 and 2011 for our study also revealed that beyond any doubt Iranian watersheds are suffering terribly from desiccation and dam construction. More than thousands of species in Iran are on the brink of extinction (Khabaronline, 2010). This is even more critical, when considering that firstly, Iran is suggested to qualify as chronic scarcity zone for freshwater by 2025. Secondly, the above mentioned changes are fragmenting the freshwater habitats and hampering aquatic organisms to disperse across the fragmented landscape to adjust their geographic range, while they are exposed to different conditions (Strayer, 2006). These conditions are undoubtedly problematic and call an immediate action for conservation of freshwater invertebrates in Iran. Therefore, assessing current biodiversity of Iran as soon as possible is of crucial importance, since measuring biodiversity within species will also open a new window for conservation purposes before a complete loss of highly endangered species. The current study takes an important step in these directions by describing one new and revalidating another freshwater crab based on a combination of new morphological and molecular evidences and also by assessing the genetic richness of the populations of the selected species.

General outlook

The presented results may seem very specific for this group of freshwater crabs. However they can probably be extrapolated to other freshwater organisms with limited distribution capacities in Iran and other regions worldwide. Thus, freshwater crabs are introduced as good model organisms for testing patterns of evolution and paleobiogeographic hypotheses in freshwater systems. I also show that they can be used for understanding speciation patterns and cryptic speciation. Measuring overall biodiversity and prediction of human impact on biodiversity is another important goal highlighted by this study.

References

Abdallah, A., Mazancourt, C.d., Elinge, M., Graw, B., Grzesiuk, M., Henson, K., Kamoga, M., Kolodziejska, I., Kristersson, M. & Kuria, A. (2004) Comparative studies on the structure of an upland African stream ecosystem. *Freshwater Forum*, 21, 27-47.

Abrishamchi, A. & Tajrishi, M. (2005) Interbasin water transfer in Iran. *Water conservation, reuse, and recycling: proceeding of an Iranian American workshop.* pp. 252-271.

Adams, A.M. (2006) Foodborne Trematodes. *Foodborne Parasites* (ed. by Y.R. Ortega), pp. 161-196. Springer.

Agard, P., Omrani, J., Jolivet, L., Whitechurch, H., Vrielynck, B., Spakman, W., Monie, P., Meyer, B. & Wortel, R. (2011) Zagros orogeny: a subduction-dominated process. *Geological Magazine*, 148, 692-725.

Ahmadzadeh, F., Carretero, M.A., Rödder, D., Harris, D.J., Freitas, S.N., Perera, A. & Böhme, W. (2012) Inferring the effects of past climate fluctuations on the distribution pattern of Iranolacerta (Reptilia, Lacertidae): Evidence from mitochondrial DNA and species distribution models. *Zoologischer Anzeiger-A Journal of Comparative Zoology*, 252, 141-148.

Ahmadzadeh, F., Flecks, M., Rödder, D., Böhme, W., Ilgaz, Ç., Harris, D.J., Engler, J.O., Üzüm, N. & Carretero, M.A. (2013) Multiple dispersal out of Anatolia: biogeography and evolution of oriental green lizards. *Biological Journal of the Linnean Society*, 110, 398-408.

Akhani, H. (2004) A new spiny, cushion-like *Euphorbia* (Euphorbiaceae) from south-west Iran with special reference to the phytogeographic importance of local endemic species. *Botanical Journal of the Linnean Society*, 146, 107-121.

Alcock, A. (1909) Diagnosis of new species and varieties of freshwater crabs. Nos. 1–3. *Records of the Indian Museum*, 3, 243-253.

Allan, J.D. & Flecker, A.S. (1993) Biodiversity conservation in running waters. *Bioscience*, 43, 32-43.

Avise, J.C. (2000) *Phylogeography*: *the history and formation of species*. Harvard University Press.

Bahir, M.M., Ng, P.K.L., Crandall, K. & Pethiyagoda, R. (2005) A conservation assessment of the freshwater crabs of Sri Lanka. *Raffles Bulletin of Zoology*, 12, 121-126.

Banarescu, P. (1991) *Zoogeography of fresh waters, Volume 1. General distribution and dispersal of freshwater animals*. Aula-Verlag, Wiesbaden.

Banarescu, P.M. (1992) *Zoogeography of fresh waters. Volume 2: distribution and dispersal of freshwater animals in North America and Eurasia*. Aula-Verlag, Wiesbaden.

Bieberstein, M. De. (1808) Notice sur queiques Insectes su Caucase. Mem. Soc. Imp. Natural. Moscou, 2, 3-5.

Blair, D., Xu, Z.B. & Agatsuma, T. (1999) Paragonimiasis and the Genus *Paragonimus*. *Advances in parasitology*, 42, 113-222.

Bliss, D.E. (1968) Transition from water to land in decapod crustaceans. *American Zoologist*, 8, 355-392.

Bobek, H. (1937) Die Rolle der Eiszeit in Nordwestiran. *Zeitschr. Gletscherk*, 130-183.

Bobek, H. (1963) Nature and implications of Quaternary climatic changes in Iran. *Changes of Climate*, 20, 403-413.

Bott, R. (1955) Die Süßwasserkrabben von Afrika (Crustacea, Decapoda) und ihre Stammesgeschichte. *Annalen van het Koninkljk Museum van Belgisch Congo*, 1, 209-352.

Bott, R. (1967) Potamidae (Crustacea, Decapoda) aus Afghanistan, Westasien und dem Mittelmeerraum. *Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening*, 130, 7-43.

Bott, R. (1969) Präadaption und Besiedlungsgeschichte der süßwasserkrabben nach der Sammlung des naturhistorischen der Erde. *Nat. Museum.*, 99, 266-275.

Bott, R. (1970) Die Süßwasserkrabben von Europa, Asien und Australien und ihre Stammesgeschichte. *Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft*, 526, 1-338.

Bott, R. (1972) Stammesgeschichte und geographische Verbreitung der Süßwasserkrabben. *Nat. Museum.*, 102, 63-77.

Brandis, D. (1997) *Untersuchungen zur Morphologie und Systematik altweltlicher Potamidae* (*Brachyura*, *Decapoda*, *Crustacea*). Dissertation, Ruprecht-Karls-Universität Heidelberg.

Brandis, D., Storch, V. & Türkay, M. (1998) The status of the freshwater crab populations of the Khabur River (Syria). *Journal of Natural History*, 32, 1439-1445.

Brandis, D., Storch, V. & Türkey, M. (2000) Taxonomy and zoogeography of the freshwater crabs of Europe, North Africa, and the Middle East. *Senckenbergiana Biologica*, 80, 5-56.

Carnevali, R.P., Collins, P.A. & Poi de Neiff, A.S. (2012) Trophic ecology of the freshwater prawn, *Pseudopalaemon bouvieri* (Decapoda: Palaemonidae) in Northeastern Argentina, with remarks on population structure. *Revista de Biologia Tropical*, 60, 305-16.

Charmantier, G. (1992) Occurrence of Fresh-Water Crabs, Genus *Potamon*, in Southern France. *Journal of Crustacean Biology*, 12, 620-626.

Cheraghi, S.A.M. (1996) Iran, country report. In: *Status and new developments on the use of brackish water for agricultural production in the Near East* (ed. M.K. Halim), pp. 1-81. United Nations Food and Agriculture Organization, regional office for the Near East (RNE), Cairo.

Clement, M., Posada, D. & Crandall, K.A. (2000) TCS: a computer program to estimate gene genealogies. *Molecular Ecology*, 9, 1657-9.

Coad, B.W. (1980) Environmental change and its impact on the freshwater fishes of Iran. *Biological Conservation*, 19, 51-80.

Collins, B.J. (2001) *A history of the animal world in the ancient Near East*. Brill, Boston.

Cook, B.D., Pringle, C.M. & Hughes, J.M. (2008) Phylogeography of an Island endemic, the Puerto Rican Freshwater Crab (*Epilobocera sinuatifrons*). *Journal of Heredity*, 99, 157-64.

Cooper, S., Ibrahim, K. & Hewitt, G. (1995) Postglacial expansion and genome subdivision in the European grasshopper *Chorthippus parallelus*. *Molecular Ecology*, 4, 49-60.

Cox, B. (2001) The biogeographic regions reconsidered. *Journal of Biogeography*, 28, 511-523.

Crosskey, R.W. (1990) *The natural history of blackflies*. John Wiley & Sons Ltd.

Cumberlidge, N. (1986) Ventilation of the branchial chambers in the amphibious West African freshwater crab, *Sudanonautes* (*Convexonautes*) *aubryi monodi* (Balss, 1929) (Brachyura, Potamonautidae). *Hydrobiologia*, 134, 53-65.

Cumberlidge, N. (1991) The respiratory system of *Globonautes macropus* (Rathbun, 1898), a terrestrial freshwater crab from Liberia (Gecarcinucoidea, Gecarcinucidae). *Crustaceana*, 61, 69-80.

Cumberlidge, N. & Daniels, S.R. (2008) A conservation assessment of the freshwater crabs of southern Africa (Brachyura : Potamonautidae). *African Journal of Ecology*, 46, 74-79.

Cumberlidge, N. & Ng, P.K.L. (2009) Systematics, Evolution, and Biogeography of Freshwater Crabs. *Crustacean Issues: Decapod Crustacean Phylogenetics* (ed. by J.W. Martin, K.A. Crandall and D.L. Felder), pp. 491-508. Taylor & Francis Group, CRC Press, New York.

Cumberlidge, N., Von Sternberg, R.M. & Daniels, S.R. (2008) A revision of the higher taxonomy of the Afrotropical freshwater crabs (Decapoda: Brachyura) with a discussion of their biogeography. *Biological Journal of the Linnean Society*, 93, 399-413.

Cumberlidge, N., Ng, P.K.L., Yeo, D.C.J., Magalhaes, C., Campos, M.R., Alvarez, F., Naruse, T., Daniels, S.R., Esser, L.J., Attipoe, F.Y.K., Clotilde-Ba, F.L., Darwall, W., McIvor, A., Baillie, J.E.M., Collen, B. & Ram, M. (2009) Freshwater crabs and the biodiversity crisis: Importance, threats, status, and conservation challenges. *Biological Conservation*, 142, 1665-1673.

Daniels, S., Stewart, B. & Gibbons, M. (1998) Genetic and morphometric variation in the potamonautid river crab *Potamonautes parvispina* (Decapoda: Potamonautidae) from two Western Cape rivers, South Africa. *Journal of Natural History*, 32, 1245-1258.

Daniels, S.R. (2003) Examining the genetic structure among populations of the common Cape river crab *Potamonautes perlatus* from river systems in South Africa reveals hydrographic boundaries. *Journal of Crustacean Biology*, 23, 936-950.

Daniels, S.R., Gouws, G. & Crandall, K.A. (2006a) Phylogeographic patterning in a freshwater crab species (Decapoda: Potamonautidae: *Potamonautes*) reveals the signature of historical climatic oscillations. *Journal of Biogeography*, 33, 1538-1549.

Daniels, S.R., Cumberlidge, N., Perez-Losada, M., Marijnissen, S.A. & Crandall, K.A. (2006b) Evolution of Afrotropical freshwater crab lineages obscured by morphological convergence. *Molecular Phylogenetics and Evolution*, 40, 227-35.

Davies, B.R., O'keeffe, J. & Snaddon, C.D. (1993) *A synthesis of the ecological functioning, conservation and management of South African river ecosystems*. Water Research Commission.

Diesel, R., Schubart, C.D. & Schuh, M. (2000) A reconstruction of the invasion of land by Jamaican crabs (Grapsidae: Sesarminae). *Journal of Zoology*, 250, 141-160.

Djamali, M., Kürschner, H., Akhani, H., de Beaulieu, J-L., Amini, A., Andrieu-Ponel, V., Ponel, P. & Stevens, L. (2008a) Palaeoecological significance of the spores of the liverwort Riella (Riellaceae) in a late Pleistocene long pollen record from the hypersaline Lake Urmia, NW Iran. *Review of palaeobotany and palynology*, 152, 66-73.

Djamali, M., de Beaulieu, J.L., Shah-hosseini, M., Andrieu-Ponel, V., Ponel, P., Amini, A., Akhani, H., Leroy, S.A., Stevens, L. & Lahijani, H. (2008b) A late Pleistocene long pollen record from Lake Urmia, NW Iran. *Quaternary Research*, 69, 413-420.

Dobson, M. (2004) Freshwater crabs in Africa. *Freshwater Forum*, 21, 3-26.

Dobson, M., Magana, A.M., Mathooko, J.M. & Ndegwa, F.K. (2007a) Distribution and abundance of freshwater crabs (*Potamonautes* spp.) in rivers draining Mt Kenya, East Africa. *Fundamental and Applied Limnology*, 168, 271-279.

Dobson, M., Magana, A.M., Lancaster, J. & Mathooko, J.M. (2007b) Aseasonality in the abundance and life history of an ecologically dominant freshwater crab in the Rift Valley, Kenya. *Freshwater Biology*, 52, 215-225.

Dubey, S., Zaitsev, M., Cosson, J.F., Abdukadier, A. & Vogel, P. (2006) Pliocene and Pleistocene diversification and multiple refugia in a Eurasian shrew (*Crocidura suaveolens* group). *Molecular Phylogenetics and Evolution*, 38, 635-647.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z.I., Knowler, D.J., Leveque, C., Naiman, R.J., Prieur-Richard, A.H., Soto, D., Stiassny, M.L.J. & Sullivan, C.A. (2006) Freshwater biodiversity: importance, threats, status and conservation challenges. *Biological Reviews*, 81, 163-182.

Excoffier, L. & Lischer, H.E. (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular ecology resources*, 10, 564-567.

Ferrigno, J.G. (1991) Glaciers of Iran. *Glaciers of the Middle East and Africa: Satellite Image Atlas of Glaciers of the World* (ed. by J. Ferrigno and R.S. Williams), pp. G31-G47. United States Government Printing Office.

Fu, Y.X. & Li, W.H. (1993) Statistical tests of neutrality of mutations. *Genetics*, 133, 693-709.

Funk, D.J. & Omland, K.E. (2003) Species-level paraphyly and polyphyly: frequency, causes, and consequences, with insights from animal mitochondrial DNA. *Annual Review of Ecology, Evolution, and Systematics*, 397-423.

Gherardi, F. & Vannini, M. (1989) Spatial-Behavior of the Fresh-Water Crab, *Potamon fluviatile*: a Radio-Telemetric Study. *Biology of Behaviour*, 14, 28-45.

Gherardi, F., Tarducci, F. & Micheli, F. (1989) Energy Maximization and Foraging Strategies in *Potamon fluviatile* (Decapoda, Brachyura). *Freshwater Biology*, 22, 233-245.

Gherardi, F., Tarducci, F. & Vannini, M. (1988a) Locomotor activity in the freshwater crab *Potamon fluviatile*: the analysis of temporal patterns by Radio-telemetry. *Ethology*, 77, 300-316.

Gherardi, F., Messana, G., Ugolini, A. & Vannini, M. (1988b) Studies on the locomotor activity of the freshwater crab, *Potamon fluviatile*. *Hydrobiologia*, 169, 241-250.

Gherardi, F., Guidi, S. & Vannini, M. (1987) Behavioural ecology of freshwater crab, *Potamon fluviatile*: Preliminary observation. *Investigacion Pesquera.*, 51, 389-402.

Greenaway, P., Taylor, H.H. & Bonaventura, J. (1983) Aerial gas exchange in Australian freshwater/land crabs of the genus *Holthuisana*. *Journal of experimental biology*, 103, 237-251.

Gvoždík, V., Moravec, J., Klütsch, C. & Kotlík, P. (2010) Phylogeography of the Middle Eastern tree frogs (*Hyla*, Hylidae, Amphibia) as inferred from nuclear and mitochondrial DNA variation, with a description of a new species. *Molecular Phylogenetics and Evolution*, 55, 1146-1166.

Hall, T. (2001) BioEdit: a user-friendly biological sequence alignment editor and analysis, version 5.09. *Department of Microbiology, North Carolina State University, Raleigh, NC*,

Hamilton, M. (2009) *Population genetics*. Blackwell-Wiley, New York.

Hewitt, G.M. (1996) Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society*, 58, 247-276.

Hewitt, G.M. (1999) Post-glacial re-colonization of European biota. *Biological Journal of the Linnean Society*, 68, 87-112.

Hill, M. & O'Keeffe, J. (1992) Some aspects of the ecology of the freshwater crab (*Potamonautes perlatus* Milne Edwards) in the upper reaches of the Buffalo River, Eastern Cape Province, South Africa. *Southern African Journal of Aquatic Science*, 18, 42-50.

Hoorn, C., Mosbrugger, V., Mulch, A. & Antonelli, A. (2013) Biodiversity from mountain building. *Nature Geoscience*, 6, 154-154.

Hou, F., Zhang, X., Zhang, X., Yue, B. & Song, Z. (2012) High intra-population genetic variability and inter-population differentiation in a plateau specialized fish, *Triplophysa orientalis*. *Environmental biology of fishes*, 93, 519-530.

Hrbek, T., Keivany, Y. & Coad, B.W. (2006) New species of *Aphanius* (Teleostei, Cyprinodontidae) from Isfahan Province of Iran and a reanalysis of other Iranian species. *Copeia*, 2, 244-255.

Huber, H. (1960) the Quaternary deposits of the Darya-i-Namak, Central Iran, Iranaian oil Company. *Geological Magazine*, Note 51

Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, 17, 754-755.

Jaubert, J., Biglari, F., Crassard, R., Mashkour, M., Rendu, W. & Shidrang et, S. (2010) Paléolithique moyen récent de la grotte de Qaleh Bozi 2 (Esfahan, Iran): premiers résultats de la campagne 2008. *Iranian Archaeology*, 1, 21-31.

Jesse, R., Grudinski, M., Klaus, S., Streit, B. & Pfenninger, M. (2011) Evolution of freshwater crab diversity in the Aegean region (Crustacea: Brachyura: Potamidae). *Molecular Phylogenetics and Evolution*, 59, 23-33.

Jesse, R., Pfenninger, M., Fratini, S., Scalici, M., Streit, B. & Schubart, C.D. (2009) Disjunct distribution of the Mediterranean freshwater crab *Potamon fluviatile* -natural expansion or human introduction? *Biological Invasions*, 11, 2209-2221.

Jesse, R., Schubart, C.D. & Klaus, S. (2010) Identification of a cryptic lineage within *Potamon fluviatile* (Herbst) (Crustacea: Brachyura: Potamidae). *Invertebrate Systematics*, 24, 348-356.

Kabish, K. (1968) zur lebensweise und haltung der süßwasserkrabbe *Potamon ibericum tauricum*. *Aquar. Terrar. Mschr. Ornith. Vivar.*, 15, 378-379.

Katoh, K., Misawa, K., Kuma, K. & Miyata, T. (2002) MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, 30, 3059-3066.

Kehl, M. (2009) Quaternary Climate Change in Iran - the State of Knowledge. *Erdkunde*, 63, 1-17.

Keikhosravi, A. & Schubart, C.D. (in press) Description of a new freshwater crab species of the genus *Potamon* (Decapoda, Brachyura, Potamidae) from Iran, based on morphological and genetic characters. *Custaceana Monogaraphs*.

Keikhosravi, A. & Schubart, C.D. (online first, 2013) Revalidation and redescription of *Potamon elbursi* Pretzmann, 1976 (Brachyura, Potamidae) from Iran, based on morphology and genetics. *Central European Journal of Biology*,

Khabaronline (2010) *Thousands of Iran's native flora and fauna in the red list*. Available at: http://www.khabaronline.ir/detail/63332 (accessed 2012, June).

Kiabi, B.H., Abdoli, A. & Naderi, M. (1999) Status of the fish fauna in the south Caspian Basin of Iran. *Zoology in the Middle East*, 18, 57-65.

King, A.R. & Ferris, C. (1998) Chloroplast DNA phylogeography of *Alnus glutinosa* (L.) Gaertn. *Molecular Ecology*, **7**, 1151-1161.

Kinzelbach, R. (1980) Hydrobiologie am Orontes. *Natur und Museum*, 110, 9-18.

Klaus, S., Brandis, D., Ng, P.K.L., Yeo, D.C.J. & Schubart, C.D. (2009) Phylogeny and biogeography of Asian freshwater crabs of the family Gecarcinucidae (Brachyura: Potamoidea). *Crustacean Issues: Decapod Crustacean Phylogenetics* (ed. by J.W. Martin, K.A. Crandall and D.L. Felder), pp. 509-531. CRC, Florida.

Klaus, S. & Gross, M. (2010) Synopsis of the fossil freshwater crabs of Europe (Brachyura: Potamoidea: Potamidae). *Neues Jahrbuch für Geologie und Paläontologie-Abhandlungen*, 256, 39-59.

Klaus, S., Schubart, C.D. & Brandis, D. (2006) Phylogeny, biogeography and a new taxonomy for the Gecarcinucoidea Rathbun, 1904 (Decapoda: Brachyura). *Organisms Diversity & Evolution*, 6, 199-217.

Klaus, S., Schubart, C.D., Streit, B. & Pfenninger, M. (2010) When Indian crabs were not yet Asian-biogeographic evidence for Eocene proximity of India and Southeast Asia. *BMC Evolutionary Biology*, 10, 1-9.

Klaus, S. & Streit, B. (2011) Pathways for freshwater fauna into Wallacea. In: *Status and Future of Tropical Biodiversity* (ed. by M. Niekisch and B. Streit), p. 205, Frankfurt am Main.

Klaus, S., Yeo, D.C.J. & Ahyong, S.T. (2011) Freshwater crab origins-Laying Gondwana to rest. *Zoologischer Anzeiger*, 250, 449-456.

Krinsley, D.B. (1966) The palaeoclimatic significance of the Iranian Playas. *Palaeoclimatology of Africa*, 6, 114-120.

Krinsley, D.B. (1968) Geomorphology of three kavirs in northern Iran (field study of geomorphology and climatological effects of three kavirs playas in northern Iran). *Playa surface morphology*: *Miscellaneous investigations* (ed. by J.T. Neal), pp. 105-130. U. S. Air Force, Office of Aerospace Research.

Krinsley, D.B. (1970) A geomorphological and palaeoclimatological study of the Playas of Iran: U.S. Geological Survey Interagency Report. United States Government Printing Office: Washington, DC.

Kuhle, M. (2004) The high Glacial (pre-LGM and LGM) glaciation of SE-Iranian mountains exemplified by Kuh-i-Jupar massif in the Zagros, Part II. *Territory*, 1, 1-15.

Largiadèr, C. & Scholl, A. (1995) Effects of stocking on the genetic diversity of brown trout populations of the Adriatic and Danubian drainages in Switzerland. *Journal of Fish Biology*, 47, 209-225.

Librado, P. & Rozas, J. (2009) DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics*, 25, 1451-1452.

Macey, J.R., Schulte II, J.A., Ananjeva, N.B., Larson, A., Rastegar-Pouyani, N., Shammakov, S.M. & Papenfuss, T.J. (1998) Phylogenetic Relationships among Agamid Lizards of the *Laudakia caucasia* Species Group: Testing Hypotheses of Biogeographic Fragmentation and an Area Cladogram for the Iranian Plateau. *Molecular Phylogenetics and Evolution*, 10, 118-131.

Maleewong, W. (2003) *Paragonimus* species. *International handbook of foodborne pathogens* (ed. by M. Miliotis and J. Bier), pp. 601-611. Marcel Dekker, CRC Press, New York.

Marijnissen, S.A., Michel, E., Cleary, D.F. & McIntyre, P.B. (2009) Ecology and conservation status of endemic freshwater crabs in Lake Tanganyika, Africa. *Biodiversity and Conservation*, 18, 1555-1573.

McAllister, D.E., Hamilton, A.L. & Harvey, B. (1997) Global freshwater biodiversity: striving for the integrity of freshwater ecosystems. *Sea Wind*: *Bulletin of Ocean Voice International*. 11(3):1-140.

McMahon, B.R. & Wilkens, J.L. (1983) Ventilation, perfusion and oxygen uptake. *The biology of Crustacea* (ed. by L.H. Mantel), pp. 289-372. Academic Press, New York.

Mehrabi, Y. (2002) Cold water aquaculture in Iran. In: *Cold water fisheries in the trans-Himalayan countries. FAO Fisheries Technical Paper* (ed. T. Petr and S.B. Swar), pp. 63-70, Rome.

Micheli, F., Gherardi, F. & Vannini, M. (1990) Growth and reproduction in the freshwater crab, *Potamon fluviatile* (Decapoda, Brachyura). *Freshwater Biology*, 23, 491-503.

Morris, S. & Van Aardt, W.J. (1998) Salt and water relations, and nitrogen excretion, in the amphibious African freshwater crab *Potamonautes warreni* in water and in air. *Journal of experimental biology*, 201, 883-893.

Naderloo, R., Turkay, M. & Chen, H.L. (2010) Taxonomic revision of the wide-front fiddler crabs of the *Uca lactea* group (Crustacea: Decapoda: Brachyura: Ocypodidae) in the Indo-West Pacific. *Zootaxa*, 1-38.

Naser, M.D. (2009) First record of the freshwater crab, *Potamon mesopotamicum* Brandis, Storch & Turkay, 1998 (Decapoda, Brachyura, Potamidae) from the Al-Huwaizah Marshes, Iraq. *Crustaceana*, 82, 1599-1602.

Nei, M. (1987) *Molecular evolutionary genetics*. Columbia University Press, New York.

Ng, P.K.L., Guinot, D. & Davie, P.J.F. (2008) Systema Brachyurorum: Part I. An Annotated Checklist of Extant Brachyuran Crabs of the World. *Raffles Bulletin of Zoology*, 17, 1-286.

Ng, P.K.L., Števčić, Z. & Pretzmann, G. (1995) A revision of the family Deckeniidae Ortmann, 1897 (Crustacea: Decapoda: Brachyura: Potamoidea), with description of a new genus (Gecarcinucidae: Gecarcinucoidea) from the Seychelles, Indian Ocean. *Journal of Natural History*, 29, 581-600.

Ng, P.K.L. & Rodriguez, G. (1995) Freshwater crabs as poor zoogeographical indicators: a critique of Bănărescu (1990). *Crustaceana*, 636-645.

Ng, P.K.L. & Yeo, D.C.J. (2007) Malaysian freshwater crabs: conservation prospects and challenges. *Status of Biological Diversity in Malaysia and Threat Assessment of Plant Species in Malaysia* (ed. by L.S.L. Chua, L.G. Kirton and L.G. Saw), pp. 95-120. Chua.

Noël, P.Y. & Guinot, D. (2007) Non-indigenous freshwater crabs in France: a new occurrence of a potamid near Nice. *Biological invaders in inland waters: Profiles, distribution, and threats* (ed. by F. Gherardi), pp. 77-90. Springer, Dordrecht, The Netherlands.

Noroozi, J., Akhani, H. & Breckle, S.W. (2008) Biodiversity and phytogeography of the alpine flora of Iran. *Biodiversity and Conservation*, 17, 493-521.

Noroozi, J., Pauli, H., Grabherr, G. & Breckle, S.W. (2011) The subnival–nival vascular plant species of Iran: a unique high-mountain flora and its threat from climate warming. *Biodiversity and Conservation*, 20, 1319-1338.

Nwokolo, C. (1974) Endemic paragonimiasis in Africa. *Bulletin of the World Health Organization*, 50, 569-571.

Nylander, J. A. A., 2004. MrModeltest v2. Evolutionary Biology Centre, Uppsala University, Uppsala, Sweden http://www.abc.se/~nylander/mrmodeltest2/mrmodeltest2.html.

Poettinger, T. & Schubart, C.D. (2013) Molecular diversity of freshwater crabs from Sulawesi and the sequential colonization of ancient lakes. *Hydrobiologia*, DOI 10.1007/s10750-013-1643-1

Poettinger, T., Wehrtmann, I.S. & Schubart, C.D. (2011). Genfluss in Flussoberläufen des zentralen Hochlands von Costa Rica? Vorläufige Ergebnisse bei Süßwasserkrabben (Pseudothelphusidae). In: Extended Abstracts of the Annual Meeting 2010. Deutsche Gesellschaft für Limnologie, Hardegsen, p. 242-246, ISBN 978-3-9813095-0-8.

Pretzmann, G. (1962) Die mediterranean und vorderasiatischen Süsswasserkrabben. *Annalen des Naturhistorischen Museums in Wien*, 65, 205-240.

Pretzmann, G. (1963) Weiterer Bericht über die mediterranen und vorderasiatischen Potamoniden. *Annalen des Naturhistorischen Museums in Wien*, 66, 373-380.

Pretzmann, G. (1965) Die Süßwasserkrabben des Mittelmeers und Vorderasiens des British Museums of Natural History, London. *Annalen des Naturhistorischen Museums Wien*, 519-525.

Pretzmann, G. (1966a) Potamoniden (Crustacea Brachyura) aus Afghanistan. *Annalen des Naturhistorischen Museums in Wien*, 69, 297-298.

Pretzmann, G. (1966b) Süsswasserkrabben aus dem westlichen Himalayagebiet. *Annalen des Naturhistorischen Museums in Wien*, 299-303.

Pretzmann, G. (1971) Ergebnisse einiger Sammelreisen nach Vorderasien: I. Teil: Zwei neue Unterarten von Süßwasserkrabben. *Annalen des Naturhistorischen Museums Wien*, 473-475.

Pretzmann, G. (1972) Die Pseudothelphusidae (Crustacea Brachyura). *Zoologica*, 120, 1-182.

Pretzmann, G. (1976a) Ergebnisse einiger Sammelreisen nach Vorderasien: 6. Die Süßwasserkrabben Persiens. *Annalen des Naturhistorischen Museums in Wien*, 457-472.

Pretzmann, G. (1976b) Ergebnisse einiger Sammelreisen nach Vorderasien: 5. weitere neue Süßwasserkrabben. *Annalen des Naturhistorischen Museums Wien*, 453-456.

Pretzmann, G. (1987a) Versuch einer historischen Deutung des Verbreitungsbildes der mediterranen und europäischen Süßwasserdekapoden. *Sitzungsbericht der Österreichischen Akademie der Wissenschaften Mathematisch Naturwissenschaftlich Klasse*, 196, 1-4.

Pretzmann, G. (1987b) a contribution to a historic of mediterranian freshwater decapod chorology. *Inv pesq*, 17-25.

Rajaei Sh, H., Rödder, D., Weigand, A.M., Dambach, J., Raupach, M.J. & Wägele, J.W. (2013) Quaternary refugia in southwestern Iran: insights from two sympatric moth species (Insecta, Lepidoptera). *Organisms Diversity & Evolution*, 1-15.

Rangzan, K. & Iqbaluddin (1995) Morpho-tectonic study of Zagros structural belt of SW Iran using remote sensing techniques. *Journal of the Indian Society of Remote Sensing*, 23, 211-224.

Rastegar-Pouyani, N. & Nilson, G. (2002) Taxonomy and biogeography of the Iranian species of *Laudakia* (Sauria: Agamidae). *Zoology in the Middle East*, 26, 93-122.

Rathbun, M. J. (1904-1906) Les crabes d’eau douce (Potamonidae). Archives du Muséum d’Histoire naturelle Paris, 6(4), 7(4), 8(4): 225-312, 159-321, 33-122.

Ratnasingham, S. & Hebert, P.D. (2007) BOLD: The Barcode of Life Data System (http://www. barcodinglife. org). *Molecular ecology notes*, 7, 355-364.

Rechinger, K.H. (1972) *Flora Iranica*, Volume 90: Cousinia (ed. by K.H. Rechinger). Akademische Druck-und Verlagsanstalt., Graz.

Rechinger, K.H. & Lack, W. (1991). *Flora Iranica*, Volume 168: Dipsacaceae (ed. by K.H. Rechinger). Akademische Druck-und Verlagsanstalt., Graz.

Reuschel, S. & Schubart, C.D. (2006) Phylogeny and geographic differentiation of Atlanto–Mediterranean species of the genus *Xantho* (Crustacea: Brachyura: Xanthidae) based on genetic and morphometric analyses. *Marine Biology*, 148, 853-866.

Revenga, C., Campbell, I., Abell, R., De Villiers, P. & Bryer, M. (2005) Prospects for monitoring freshwater ecosystems towards the 2010 targets. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 360, 397-413.

Ricciardi, A. & Rasmussen, J.B. (1999) Extinction rates of North American freshwater fauna. *Conservation Biology*, 13, 1220-1222.

Rodríguez, G. (1986) Centers of radiation of freshwater crabs in the Neotropics. *Crustacean Issues*, 4, 51-67.

Rodríguez, G. & Magalhães, C. (2005) Recent advances in the biology of the Neotropical freshwater crab family Pseudothelphusidae (Crustacea, Decapoda, Brachyura). *Revista Brasileira de Zoologia*, 22, 354-365.

Rogers, A.R. & Harpending, H. (1992) Population growth makes waves in the distribution of pairwise genetic differences. *Molecular Biology and Evolution*, 9, 552-569.

Savigny, J. (1816) *Mémoires sur les animaux sans vertèbres: Première Partie: Description et classification des animaux invertébrés et articulés, connus sous les noms de Crustacés, d'Insectes, d'Annélides, etc.* G. Dufour, Paris.

Schneider, P. (1971) Zur Biologie der afghanischen Flußkrabbe *Potamon gedrosianum* Alcock 1910. I. Lebensraum, Verbreitung, Morphologie und Systematische Stellung. *Bonn zoological Bulletin*, 22, 305-321.

Schneider, S. & Excoffier, L. (1999) Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. *Genetics*, 152, 1079-89.

Schubart, C.D., (2009) Mitochondrial DNA and decapod phylogenies: the importance of pseudogenes and primer optimization. *Crustacean Issues: Decapod Crustacean Phylogenetics* (ed. by J.W. Martin, K.A. Crandall and D.L. Felder), pp. 47-65. CRC Press, Taylor & Francis Group., New York.

Schubart, C.D., & Santl, T. (2013, in press) Differentiation within a river system: ecology or geography driven? Examples from evolutionary significant units in Jamaican freshwater crabs. *Crustaceana Monographs.*

Schubart, C.D., Neigel, J. & Felder, D.L. (2000) Molecular phylogeny of mud crabs (Brachyura: Panopeidae) from the northwestern Atlantic and the role of morphological stasis and convergence. *Marine Biology*, 137, 11-18.

Schubart, C.D., Diesel, R. & Hedges, S.B. (1998) Rapid evolution to terrestrial life in Jamaican crabs. *Nature*, 393, 363-365.

Schubart, C.D., Rivera, N.T., Crandall, K.A. & Santl, T. (2011) Shallow phylogeographic structure of Puerto Rico freshwater crabs: an evolutionary explanation for low species diversity compared to Jamaica. *Phylogeography and Population Genetics in Crustacea* (ed. by C. Held, S. Koenemann and C.D. Schubart), pp. 345-365. Taylor & Francis, Boca Raton, Florida.

Seckler, D.W. (1998) World water demand and supply, 1990 to 2025: Scenarios and issues. In: International Water Management Institute, Research report 19, Sri Lanka.

Shih, H.T., Hung, H.C., Schubart, C.D., Chen, C.L.A. & Chang, H.W. (2006) Intraspecific genetic diversity of the endemic freshwater crab *Candidiopotamon rathbunae* (Decapoda, Brachyura, Potamidae) reflects five million years of the geological history of Taiwan. *Journal of Biogeography*, 33, 980-989.

Shih, H.T., Yeo, D.C. & Ng, P.K. (2009) The collision of the Indian plate with Asia: molecular evidence for its impact on the phylogeny of freshwater crabs (Brachyura: Potamidae). *Journal of Biogeography*, 36, 703-719.

Shine, R. (1978) Propagule Size and Parental Care: The "Safe-Harbor" Hypothesis. *Journal of Theoretical Biology*, 75, 417-424.

Silk, N. & Ciruna, K. (2005) *A practitioner's guide to freshwater biodiversity conservation*. Island Press.

Sodhi, N.S., Koh, L.P., Brook, B.W. & Ng, P.K.L. (2004) Southeast Asian biodiversity: an impending disaster. *Trends in Ecology & Evolution*, 19, 654-660.

Somers, M.J. & Nel, J.A.J. (1998) Dominance and population structure of freshwater crabs (*Potamonautes perlatus* Milne Edwards). *South African Journal of Zoology*, 33, 31-36.

Stiassny, M.L. (1996) An overview of freshwater biodiversity: with some lessons from African fishes. *Fisheries*, 21, 7-13.

Strayer, D.L. (2006) Challenges for freshwater invertebrate conservation. *Journal of the North American Benthological Society*, 25, 271-287.

Taberlet, P., Fumagalli, L., Wust-Saucy, A.G. & Cosson, J.F. (1998) Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology*, **7**, 453-464.

Tajima, F. (1989) Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, 123, 585-595.

Takin, M. (1972) Iranian geology and continental drift in the Middle East. *Nature*, 235, 147-150.

Technelysium Pty Ltd. (2007) Chromas lite version 2.01. Available from: http://www.technelysium.com.au/chromas_lite.html

Thienemann, A. (1950) *Verbreitungsgeschichte der Süßwassertierwelt Europas, Versuch einer historischen Tiergeographie der europäischen Binnengewässer*. Schweizerbart, Stuttgart.

Toscano, C., Yu, S.H., Nunn, P. & Mott, K.E. (1994) Paragonimiasis and Tuberculosis-diagnostic confusion: A review of the literature. *Tropical Diseases Bulletin*, 92, R1–R26.

Turnbull-Kemp, P.S.J. (1960) Quantitative Estimations of Populations of the River Crab, *Potamon* (*Potamonautes*) *perlatus* (M. Edw.), in Rhodesian Trout Streams. *Nature*, 13, 481.

von Sternberg, R. & Cumberlidge, N. (2001) Notes on the position of the true freshwater crabs within the brachyrhynchan Eubrachyura (Crustacea: Decapoda: Brachyura). *Hydrobiologia*, 449, 21-39.

von Sternberg, R., Cumberlidge, N. & Rodriguez, G. (1999) On the marine sister groups of the freshwater crabs (Crustacea: Decapoda: Brachyura). *Journal of Zoological Systematics and Evolutionary Research*, 37, 19-38.

Whiteley, A.R., Spruell, P. & Allendorf, F.W. (2004) Ecological and life history characteristics predict population genetic divergence of two salmonids in the same landscape. *Molecular Ecology*, 13, 3675-3688.

Whiting, M.F., Carpenter, J.C., Wheeler, Q.D. & Wheeler, W.C. (1997) The Strepsiptera problem: phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. *Systematic Biology*, 46, 1-68.

WHO (1995) Control of foodborne trematode infections: report of a WHO study group. p. 849

Williams, T.R. (1961) The diet of freshwater crabs associated with *Simulium neavei* in East Africa. I. Crabs from West and East Uganda collected by the Cambridge East African expedition, 1959. *Annals in Tropical Medicine and Parasitology*, 55, 128-131.

Williams, T. (1962) The diet of freshwater crabs associated with *Simulum neavei* in East Africa. II. The diet of *Potamon berardi* from Mount Elgon, Uganda. *Ann Trop Med Parasitol*, 56, 362-367.

Williams, T. (1964) *The freshwater crabs of East Africa and their relation to* Simulium neavei *and onchocerciasis*. M. Sc. Thesis, University of Liverpool, UK,

Williams, T.R. (1991) Freshwater crabs and *Simulium neavei* in east Africa. III. Morphological variation in *Potamonautes loveni* (Decapoda: Potamidae). *Annals in Tropical Medicine and Parasitology*, 85, 181-188.

Wolcott, T.G. (1992) Water and solute balance in the transition to land. *American Zoologist*, 32, 428-437.

Wright, H.E. (1962) Pleistocene glaciation in Kurdistan. *Eiszeitalter und Gegenwart*, 12, 131–164.

Yeo, D.C. & Ng, P.K. (2004) Recognition of two subfamilies in the Potamidae Oertman, 1896 (Brachyura, Potamidae) with a note on the genus *Potamon* Savigny, 1816. *Crustaceana*, 76, 1219-1235.

Yeo, D.C.J., Ng, P.K.L., Cumberlidge, N., Magalhaes, C., Daniels, S.R. & Campos, M.R. (2008) Global diversity of crabs (Crustacea: Decapoda: Brachyura) in freshwater. *Hydrobiologia*, 595, 275-286.