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Cent. Eur. J. Biol. • 9(1) • 2014 • 114-123
DOI: 10.2478/s11535-013-0203-z

Central European Journal of **Biology**

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

Research Article

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Received 15 October 2012; Accepted 12 May 2013

Abstract: 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 first gonopods in males. 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.

Keywords: *Mitochondrial genes • Morphological features • Species complex*

1. 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 [1]. Currently, there are a total of twenty-one recognized species in the genus *Potamon* [2]. 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 [3-9] 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*. [10,11] 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*. [10] 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 More recently, A. Keikhosravi and C.D. Schubart (unpublished data) documented noticeable diversity within *P. persicum* based on molecular and morphological evidence, that is probably related to the diverse topography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 [3] requires revalidation and elevation to species rank, *i.e*. *Potamon elbursi* Pretzmann, 1962. Therefore, these specimens are here redescribed and the species revalidated as the eighth species of *Potamon* from Iran.

2. 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

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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 [8] were examined morphologically and some genetically. Detailed information concerning the sampling localities is provided in Figure 1 and Table 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, Vienna (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’) [12] for 16S rRNA, COL6 (5′-TYTCHACAAAYCATAAAGAYATYGG-3′) and COH3 (5′- ATTTTYGSYGGWATTGCHCAYTGATT-3′) [13] for Cox1. Polymerase chain reaction (PCR) was carried out under the following conditions: denaturation for 45 s at 94°C, annealing for 1 min at 48°C, and extension for 1 min at 72°C (40 cycles) for 16S; denaturation for 45 s at 94°C, 45 s 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 min 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

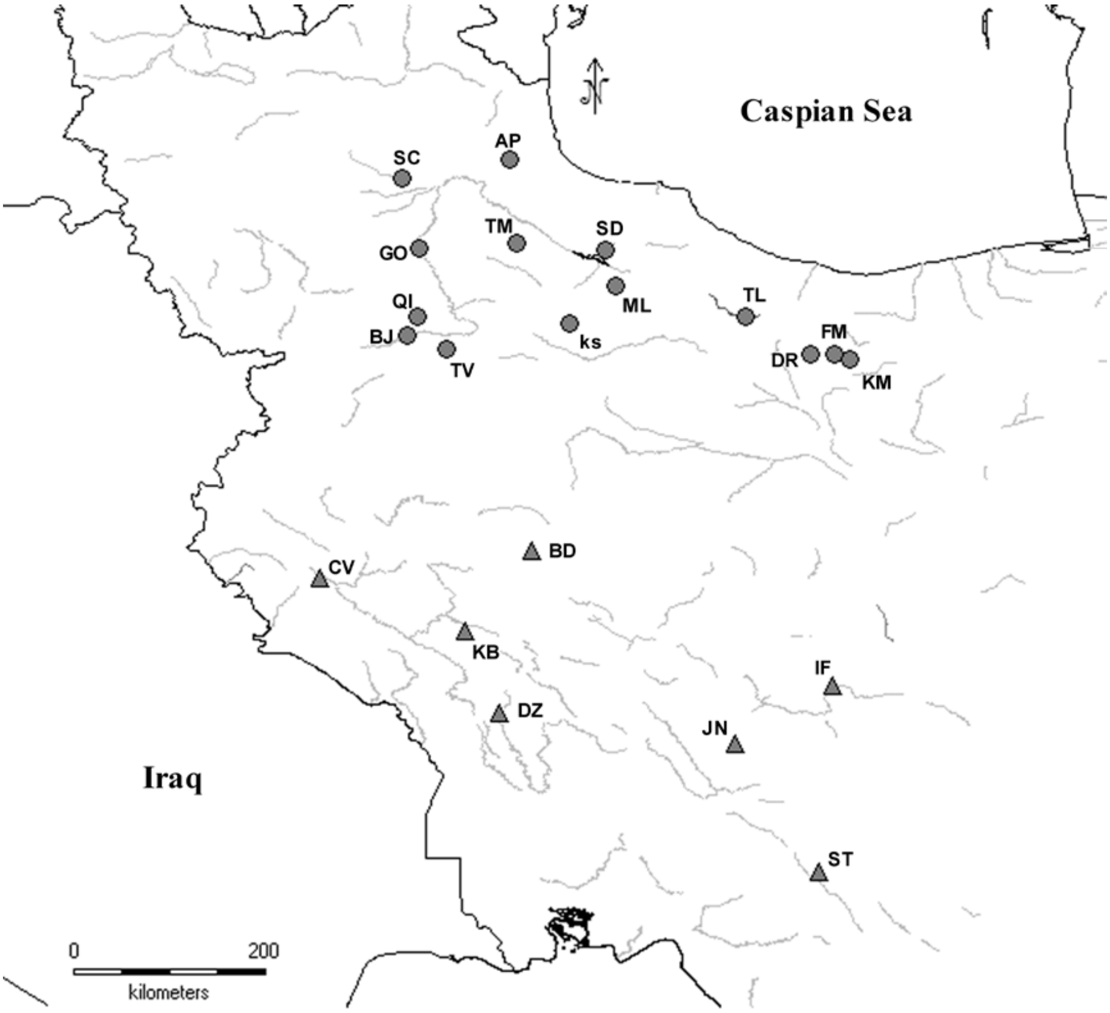

Figure 1. Map of Iran with main rivers. Gray circles and triangles represent the sampling sites corresponding to *P. elbursi* and *P. persicum*, respectively. For abbreviations see Table 1.

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

Table 1. Specimens used for DNA sequencing with collection number, locality, coordinates and GenBank accession number. Abbreviations for museums are explained in Introduction.

were obtained in collaboration the Canadian Centre for DNA Barcoding (CCDB) [14]. Six additional 16S sequences were retrieved from Genbank (HE963841- 4 and HE963847-8) (Table 1). Sequences were corrected manually with BioEdit (version 5.09) and aligned with Mafft (version 6) [15,16]. The sequences were deposited at the European Molecular Database EMBL (accession numbers in Table 1).

A phylogeny based on a 1306 basepairs alignment of combined 16S - Cox1 mtDNA was inferred by Bayesian Inference (BI) and generated with MrBayes (version 3.1.2) [17]. The appropriate substitution model was previously evaluated using MrModeltest for each gene separately (version 2.3) [18]. The best model was selected by the Akaike's 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 1,000,000 generations were excluded from the analysis as burnin. *Potamon ibericum* Bieberstein, 1808 [19] (ZUTC Pot.1089) was used as an 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 [20] 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 the 16S dataset were treated as a fifth character state.

2.1 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*. [10] and Pretzmann [8].

3. Results

3.1 Systematic Account

Systematic Account; Family Potamidae Ortmann, 1896; Genus *Potamon* Savigny, 1816; *Potamon elbursi* Pretzmann, 1962 stat. nov. (Figure 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).

3.2 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,

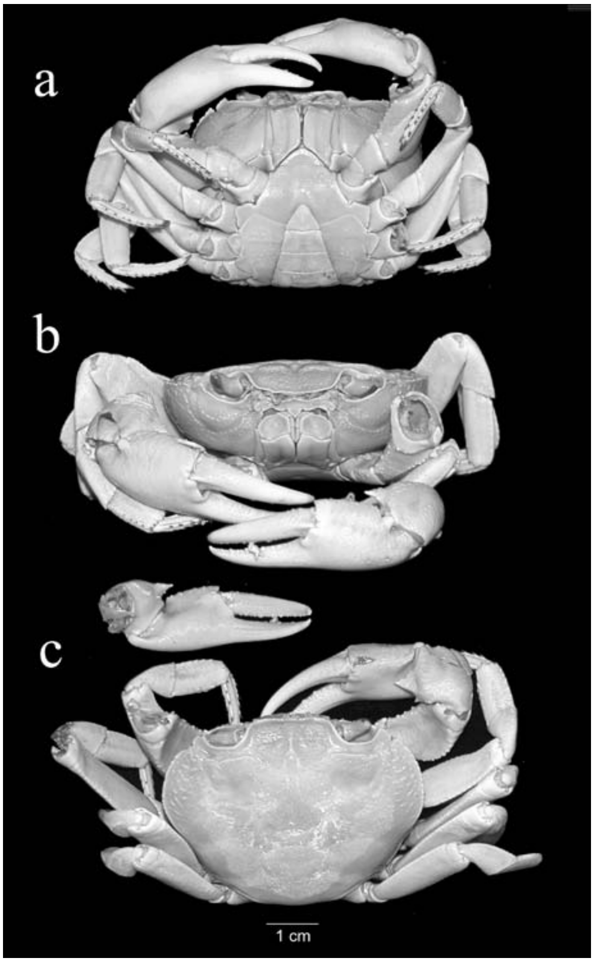

Figure 2. Holotype of *Potamon elbursi* (NHMW 3182); a, ventral view; b, frontal view; c, dorsal view.

18.08.2009; 3♂, 2♀ (SMF-39035), 4♂, 1♀ (NHMW 25431), 3♂, 2j (RMNH-D 54896), 2♂, 1♀, 1j (ZUTC Pot.1084), 55 km 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, Ghamchi village, Ghamchi R., 36°09.960'N 47°37.316'E, leg. A. Keikhosravi & E. Keikhosravi, 22.10.2011; 3♂, 4♀, 1j (ZUTC-1090), Azerbaiejan-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, Hoseinabad 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), 16 km 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.

3.3 Diagnosis

Terminal segment of G1 nearly triangular; mesial margin in two different morphs: either sinus-shaped, bent outward medially, proximal half bulged and arched, distal portion slightly concave; or 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 (Figure 3a, b, c).

3.4 Redescription

Carapace distinctly broader than long; dorsal surface of carapace almost flat, glabrous, moderately sculptured.

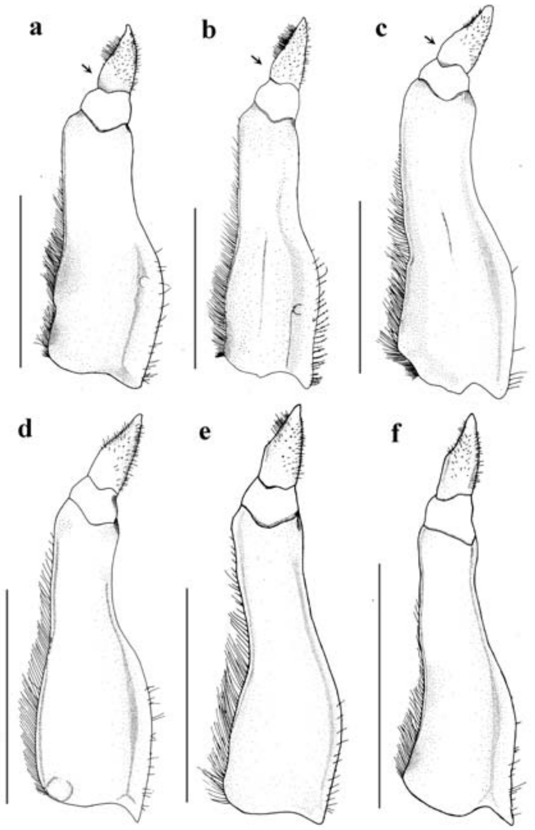

Figure 3. Morphological characteristics of the first gonopod (G1) in male specimens of *Potamon elbursi* (a-c) and *Potamon persicum* (d-f). Dorsal view; a, SMF-43958; b, SMF-39038; c, NHMW 3182 (holotype); d, NHMW 3927; e, NHMW 4151; f, NHMW 25428. Scale bars: 5 mm.

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 (Figure 2c). Posterolateral region arch-shaped (Figure 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 (Figure 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 (Figure 3a). Exorbital tooth triangular, distinct, very blunt, lateral side slightly serrate. Epistome smooth, with a number of short setae; posterior margin distinctly granulated (Figure 3b). Suborbital lobe anteriorly and posteriorly smooth, glabrous, middle region with curved line of tubercles (Figure 3a).

Third maxilliped ischium with deep median sulcus (Figure 3a); exopod with flagellum nearly as long as merus. Thoracic sternites smooth, sparsely covered with setae, pilose at margins of segments (Figure 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 (Figure 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 (Figure 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 (Figure 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 (Figure 3a, c; arrows), almost covered by long setae; or straight at 1/5 of proximal part, bent outward at 1/5 of proximal part (Figure 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 (Figure 3a, b, c).

3.5 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, 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), Azarbayejan-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.

3.6 Remarks

The taxonomic classification of the *P. persicum* complex has been under constant discussion over the past half century. Pretzmann [3,4,6-9] introduced four new species/subspecies (as well as other infraspecific categories) belonging to the *P. persicum* complex. Later, Brandis *et al*. [10] 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 (Figure 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 (Figure 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 (Figure 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 is distributed 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.

3.7 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's 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 (Figure 4). The well supported clade of *P. elbursi* (Figure 4, H1) is a sister group to the highly supported clade of *P. persicum* from two western localities (Figure 4, H2), which is consistent with their biogeographic distribution, whereas *Potamon persicum* from the type locality in

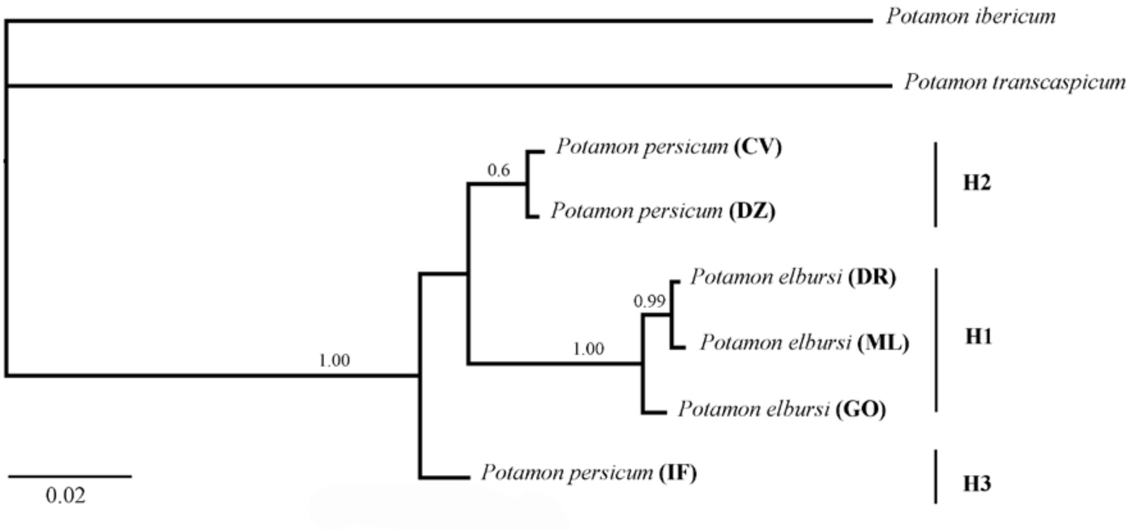

Figure 4. Phylogenetic relationships among populations of *Potamon elbursi* and *P. persicum* according to a concatenated tree using Bayesian analysis of a 1306 basepair alignment of combined 16S and Cox1 sequence data. Posterior probability values show support for the corresponding clades. Abbreviations refer to Figure 1 and Table 1.

Isfahan (IF) represents a third group (Figure 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 (Figure 4).

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 (Figure 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 (Figure 5b).

4. Discussion

In an era of human-induced biodiversity declines, 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

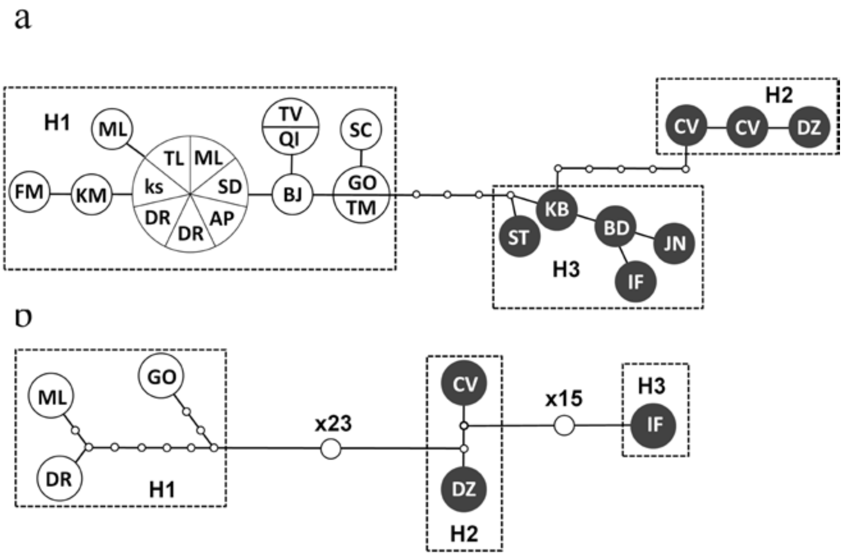

Figure 5. Statistical parsimony networks based on 648 basepairs alignment of 16S (a) and 658 basepairs alignment of Cox1 (b). Each line represents one mutation, each dot indicates one missing haplotype and numbers next to large unlabeled nodes indicate the number of missing haplotypes. The size of the 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 1 and Table 1.

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) [8]. The Isfahan haplogroup 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. 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 [21,22]. As an alternative explanation, 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 could serve this purpose (*i.e.* Zayandehrood R.) (www.briancoad.com). However, faunal analysis of Old Stone Age excavations near Isfahan recovered remains of freshwater crabs [23] (Fereidoun Biglari, personal communication, August 23, 2011), demonstrating the existence of viable populations before the construction of the tunnels. Currently, 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 should investigate genetic and morphologic differentiation of the constituent populations over the entire range of *P. persicum* to uncover biodiversity and 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 Regensburg) 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.

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