Redescription and mitochondrial identification of *Chiromantes boulengeri* (Calman, 1920) (Decapoda: Brachyura: Sesarmidae) based on fresh material from the Persian Gulf, Iran

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Abstract

*Chiromantes boulengeri* (Calman, 1920) is redescribed based on fresh material from Iran. The species is morphologically more similar to *C. dehaani* (H. Milne Edwards, 1853) than both of these species are to the type species of the genus, *C. haematocheir* (De Haan, 1833). Results from mitochondrial DNA, however, propose a closer sister species relationship of the two East Asian species, *C. haematocheir* (De Haan, 1833) and *C. dehaani*.

Key words: Brachyura, Sesarmidae, *Chiromantes*, molecular phylogeny, mtDNA

Introduction

The Persian Gulf is a semi-enclosed sea which forms part of the northwestern Indian Ocean. Harsh environmental conditions result from high evaporation and low fresh water input. Nevertheless, it has several marine habitats with remarkably high biodiversity (Jones, 1985). The main freshwater source of the Persian Gulf is Shat Al Arab River (“Arvandroud” in Iran) which discharges into the northern Persian Gulf, having great influence on the biological and physical factors of the intertidal and subtidal habitats. Two sesarmid crab species, *Parasesarma plicatum* (Latreille, 1806) and *Chiromantes boulengeri* (Calman, 1920), are typical decapod crustacean inhabitants of the river. *Parasesarma plicatum* is a predominant crab of the marshes and mangroves in the Gulf. It is an entirely marine species, but also thrives along the banks of the lower reaches of the river. In contrast, adults of *C. boulengeri* are exclusively freshwater crabs that inhabit the upstream areas of the river, although it remains unclear how far from the sea. *Chiromantes boulengeri* is poorly known and was collected for the first time from the Ashar Creek, Basra (Iraq) about 96 kilometres from the mouth of Shat Al Arab. It was briefly described by Calman (1920) with just one figure of the cheliped of this species, and comparing it mainly to *C. dehaani* (H. Milne Edwards, 1853). During the last nine decades, there was no further record of this species. Apel & Türkay (1999) merely listed this species in their treatment of “grapsoid crabs” of the Persian Gulf. Therefore, more information on the taxonomy and other aspects of this species is clearly needed.

Material and methods

A research program is being undertaken since October 2007 to investigate the biophysical structure of the intertidal habitats and their crustacean decapods along the Iranian coast of the Persian Gulf, with particular emphasis on the Brachyura. In addition to the sea shore, two main rivers discharging into the northern Persian Gulf were targeted. A total of 21 species of brachyuran crabs were collected along the Iranian coast of the Persian Gulf. Two species, *Parasesarma plicatum* and *Chiromantes boulengeri*, were identified.
Gulf are being surveyed and decapods collected in three localities along the eastern banks (Iranian side) of the Shat Al Arab and two localities of the Bahmanshir River (fig. 1). The here examined material of *Chiromantes boulengeri* originates from the latter river. Specimens were mainly collected by hand, using sieves for fine sediments, or digging in muddy substrates. Crustacean organisms were preserved in ethanol 70%; but some in ethanol 80% to be used for genetic analyses. All illustrations were drawn with the aid of a camera lucida. Detailed descriptions and appropriate drawings of fresh material are provided. The abbreviations CL and CB are used for the carapace length and carapace breadth, respectively, and G1 for first male gonopods. Collected specimens are deposited in the Zoology Museum, University of Tehran (ZUTC), and the Senckenberg Museum, Frankfurt am Main (SMF).

**FIGURE 1.** Major rivers discharging on the northern Persian Gulf: 1, Shat Al Arab River; 2, Karun River; 3, Bahmanshir River. Arrows indicate the sampling sites of *C. boulengeri* at the Bahmanshir River.

One specimen of the type series of Calman (1920) from the British Museum of Natural History (NHM 2002.298) and one new specimen from the Bahmanshir River (Abadan, Pole Tanki Abolhassan) deposited in the Senckenberg Museum Frankfurt (SMF 33818) were used for tissue extraction and DNA isolation. Mitochondrial DNA (mtDNA) sequences from these individuals were obtained at the University of Regensburg in 2002 and 2008, respectively. In addition, mtDNA was amplified from sesarmid species for which incomplete 16S sequences were already present in the genetic database: *Chiromantes dehaani* (H. Milne Edwards, 1853) and *Sesarmops intermedium* (De Haan, 1835). Specimens used are listed in the comparative material section. Selective amplification of part of the mitochondrial large ribosomal subunit gene 16S rRNA was achieved by PCR-amplifications with four minutes denaturation at 94°C, 40 cycles with 45s 94°C, 1 min 48°C, 1 min 72°C and 10 min final denaturation at 72°C and the primers 16L2 or 16L29 and 1472 or 16H10 (primer information see Schubart, 2009). PCR products were purified with Quick-Clean (Bioline) and subsequently sequenced with the ABI BigDye terminator mix followed by electrophoresis in an ABI Prism 310 Genetic Analyzer (Applied Biosystem, Foster City, USA). New sequence data were submitted to the European molecular database EMBL (accession numbers: FN296219 to FN296222). In addition, the following sequences archived in molecular databases were included in our analyses: *Chiromantes haematocheir* (De Haan, 1833) (AJ308414), *Chiromantes dehaani* (AY151822), *Chiromantes eulimene* (De Man, 1895) (AJ784017), *Chiromantes ortmanni* (Crosnier, 1965) (AJ784016), *Bresedium brevipes* (De Man, 1889) (AM180685), *Sesarmops intermedium* (AB057811), *Armasces cinereum* (Bosc, 1802) (AJ784010),
Sesarma reticulatum Say, 1817 (AJ130799). Sequences were aligned with xESEE version 3.2 (Cabot & Beckenbach 1989). Sesarma reticulatum was selected as outgroup.

The model of DNA substitution that fitted our data best was determined using the software MODELTEST 3.6 (Posada & Crandall 1998). Phylogenetic inference was carried out by Bayesian analysis (BI) as implemented in MrBayes v. 3.0b4 (Huelsenbeck & Ronquist 2001) and using the suggested model of evolution. The Bayesian analysis was run with four MCMC (Markov chain Monte Carlo) chains for 5,000,000 generations, saving a tree every 500 generations (with a corresponding output of 10,000 trees). The –lnL converged on a stable value between 5,000 and 10,000 generations (burn-in phase). The first 100,000 generations were later excluded; the posterior probabilities of the phylogeny being determined by constructing a 50% majority-rule consensus of the remaining trees with the “sumpt” option of MrBayes. In addition, a Maximum Composite Likelihood / minimum evolution analysis was carried out as implemented in MEGA4 (Tamura et al. 2007) with 5,000 bootstrap pseudoreplicates and the gamma correction as estimated by MODELTEST.

Family Sesarmidae Dana, 1851

Chiromantes Gistel, 1848

Chiromantes boulengeri (Calman, 1920)

Sesarma boulengeri Calman, 1920: 63–65, fig. A.  

Type locality: Shat Al Arab (Basra), Iraq.

New material. 3 males (ZUTC Brach 1151), Iran, Bahmanshir River, summer 2006, coll. E. Gholinezhad; 1 male, 1 female (ZUTC Brach 1153), same data; 18 males, 27 females (16 ovigerous), 3 juv. (SMF-33818), Iran, Abadan, Bahmanshir River, Pole Tanki Abolhassan, 30° 21' 01.5"N, 48° 18' 35.9"E, 20.05.2008, coll. R. Naderloo, A. Kazemi (1 male DNA voucher).

Comparative material. 


Redescription. Carapace squarish (almost quadrate) (Figs. 2a, 3a), slightly broader than long (CL/CB about 1.17–1.20), greatest breadth slightly posterior to exorbital teeth, no anterolateral teeth; slightly convex (in particular at posterior region); surface smooth, nearly glabrous, some short setae scarcely scattered on carapace, particularly on posterolateral regions; regions well defined, arched prominent groove separating gastric from cardiac region, one pair of very small tubercles in middle of groove; two other arched, less distinct grooves in gastric region. Intestinal region distinctly depressed, separated from cardiac region by rather shallow, wide groove. Oval groove, more or less prominent, in hepatic region, just behind of orbit. Eight smooth ridges with different length on lateral region, with two short ones in between.

Front strongly deflexed, 4 lobes in frontal region, 2 middle ones larger than lateral ones; frontal edge
gently curved upwards, sinuous, nearly bilobed; region between frontal lobes, frontal edge concave; low transverse elevation, somewhat fragmentary in front of middle frontal lobes, lateral angles of front rounded (Fig. 3b).

Basal segments of antennae, antennules separated by distinct segment (Fig. 3b), long setae cover three proximal segments of antenna, antennule; flagellum of antenna very short, extends shortly beyond orbital hiatus, not reaching cornea of eye.

FIGURE 2. Chiromantes boulengeri (Calman, 1920). Male (CL 17.3, CB 20.7 mm), Bahmanshir River (SMF-33818). a, dorsal view; b, ventral view.
FIGURE 3. *Chiromantes boulengeri* (Calman, 1920). Bahmanshir River (SMF-33818). a, carapace of male; b, frontal region of male; c, outer surface of right cheliped of male; d, upper surface of right male cheliped; e, anterior thoracic sternites (setae removed) of male; f, abdomen of male; g, right G1 (dorsal view); h, apical part of right G1 (ventral view); i, apical part of right G1 (dorsal view); j, genital opening of female. Scale: a–f 1cm, g–h 1mm.

Ischium of third maxilliped almost same size as merus, with submedian shallow groove; merus suboval, posterior surface with elevated ridge extending from inner angle of anterior margin to median part of posterior margin, with wide sulcus lateral to ridge, relatively deep groove on lateral part. Inner margins of ischium,
merus with long setae; lateral margin of ischium with dense short setae, that of merus with scarce short setae; surface of merus with short bristles, that of ischium smooth without pubescence; exopod slender.

Chelipeds equal, sometimes subequal, distinctly large; ischium smooth with relatively large tooth on inner margin; outer surface of merus with finely granulated transverse ridges, inner surface smooth with longitudinal line of brown setae near inner margin, inner margin dentate on two-thirds of proximal part, subdistally roundly expanded, distally smooth, lower margin dentate, outer margin serrate. Carpus with spine-like tooth on inner margin, outer surface with granulated short ridges, with L-shaped depression on distal part. Palm (Fig. 3c) massive, oblique granule ridges on lower margin, on proximal part of outer surface; outer surface with small granules on lower half, large granules on middle area, sometimes 5, 6 in longitudinal row, smaller ones on upper part of outer surface; upper surface (Fig. 3d) with prominent 2, 3 small longitudinal granular ridges; inner surface with small granules on lower half, an oblique line of relatively large granules extending from base of immovable finger towards upper part, 2, 3 large granules on upper part. Fingers with small gap in between, widening distally; movable finger slender, relatively arched, curving gently inwards, longer than palm, small granules on dorsal margin, proximally numerous, extending distally into a row, while becoming smaller, lower. Cutting edge of movable finger with 3 large teeth: 1 proximal, 1 distal, 1 middle; cutting edge of immovable finger with 4 large teeth: 3 proximal (with distal first 2 fused), 1 distal, some low teeth between them. Tips of fingers pectinated, scalloped, that of movable finger nearly tridentate. Cheliped of female small, more narrow, otherwise possessing all features of that of male.

Walking legs medium-sized, third, second pairs longest; merus with granular transverse ridges on upper surface, upper surface of last walking legs nearly smooth with few ridges on anterior part, anterior margin serrated, with subdistal tooth, posterior margin smooth. Carpus with 2 carinae on posterior surface, 1 on anterior surface; anterior margin of carpus, propodus densely covered with short brown setae, with some large bristles in between. Propodus slightly longer than carpus, with one carina on posterior, anterior surfaces. Dactylus slender, of nearly same length as propodus, with setae on anterior, posterior margins.

Anterior margin of first thoracic sternites (Fig. 3e) densely covered with setae, row of relatively long setae along suture of second, third sternites; fused third, fourth sternites relatively narrow, abdominal cavity near suture of second, third sternites, reaching to coxae of chelipeds.

Male abdomen (Fig. 3f) widely triangular, margin of segments with short dense setae; sixth segment longer than fifth, with arched anterodistal margin; telson clearly longer than sixth segment, lateral margin proximally nearly straight, becoming strongly convex distally, forming wide triangular tip, with scarce setae.

G1 (Fig. 3g) straight, clear longitudinal depression on ventral surface near lateral margin; inner margin distally roundly expanded; terminal pectinated part gently bent outwards, with concave apical margin, small pectinated process (Fig. 3h), nearly triangular, laterally to terminal part. Genital opening (Fig. 3i) subdistal on ventral surface of terminal pectinated part, long setae covering terminal part, not fully concealing it, short setae along outer margin.

Female gonopore (Fig. 3j) in depression on anterior edge of sternite 5, attached to posterior margin of sternite 4, with elevated small operculum, directed inwards, sternite 5 scarcely covered with plumose setae. Colour. Live animals dark grey on dorsal surface of carapace; chelipeds, walking legs slightly lighter colour than carapace, ventral surface light, abdomen distinct by its light brown colour. Chelae white to light brown from anterior view. Granules on palm, fingers distinctly white, lower, inner portions of palm sometimes pale orange.

Habitat. The species was found in muddy banks of the Shat Al Arab and Bahmanshir rivers, where it digs burrows. Type material was collected from Ashar Creek near Basra (the Euphrates River) about 96 km from the sea in fresh water (Calman, 1920). Apel & Türkay (1999) examined several other specimens deposited in the British Museum of Natural History in London, which were found together with Parasesarma plicatum in the same jar labelled as collected from “FAO” (M. Apel, personal communication). Fao is official pronunciation of Al Faw, a city about 20 km from the sea. Apel & Türkay (1999) mentioned that at this station which located at the mouth of the river, C. boulengeri coexists with P. plicatum. All recently collected adults of C. boulengeri, however, show complete independence from the sea, and it would not be possible to explain
its range extension down these rivers. So far, it has been clear that this species shows no coexistence with *P. plicatum*, which lives in the downstream areas of the river.

**Molecular results.** The molecular dataset consisted of 615 aligned characters of which 479 were conserved, 99 variable, and 53 parsimony-informative. GTR+G+I with a gamma shape of 0.468 and an invariable proportion of 0.538 was selected as the best-fitting evolutionary model by MODELTEST and implemented for subsequent Bayesian analysis. The tree topology was in agreement with the one of the Maximum Composite Likelihood which is shown in Fig. 4 together with posterior probabilities and bootstrap values exceeding 50%. According to this phylogeny, the genus *Chiromantes* is a paraphyletic assemblage including in close relationship representatives of the genera *Sesarmops* and *Bresedium*. Most derived seem to be the East African species *C. eulimene* and *C. ortmanni*. This confirms the need of revision of this large genus (see Ng & Liu, 1999; Ng & Schubart, 2003; Ng et al., 2008; Ng & Schubart, in progress). The species *Chiromantes boulengeri* currently holds an outgroup position to a clade comprised of *C. haematocheir* (type species of the genus), *C. dehaani*, and *Sesarmops intermedium*. Apparently, no closely related species to *C. boulengeri* nor to *Bresedium brevipes* was among the here sampled taxa. Thus, there is no evidence for a close genetic relationship between the morphologically similar species *C. boulengeri* and *C. dehaani*, but rather a clustering according to geography of all the East Asian species, where a radiation of crabs from mangroves and estuaries may have taken place. This will be analysed further with additional representatives. The two sequence of *C. boulengeri* are genetically identical, confirming conspecific status of Calman's (1920) type from Basra (Iraq) and the new material from Iran and the lack of genetic differentiation between river systems, probably due to the marine larval dispersal within the Persian Gulf.

**FIGURE 4.** Reconstruction of the phylogenetic relationships of selected species of *Chiromantes* and other Sesarmidae according to a Bayesian analysis (5 my generations) and Maximum Composite Likelihood (5,000 pseudoreplicates) of a 615 basepair alignment of 16S ribosomal mtDNA. Consensus tree shown with bootstrap values / posterior probabilities expressing support for the corresponding clades.

**Taxonomic remarks.** The taxonomic position of *C. boulengeri* (Calman, 1920) is not clear. It was described by Calman (1920) as *Sesarma boulengeri*, but Apel and Türkay (1999) placed it in *Chiromantes* Gistel, 1848, by referring to a comment by Holthuis (1977). A useful account of *Chiromantes* has been provided by Ng & Liu (1999) who argue that the species composition of the genus is heterogeneous. In their paper, they commented that the species can roughly separated into two broad groups, with smaller groupings within each. One subgrouping includes *Sesarma elongatum* A. Milne-Edwards, 1869 which more recently is recognised as a member of the genus *Selatium* Serène & Soh, 1970 (see Hartnoll 1975; Ng et al. 2008;
Schubart et al. in press), with the others in another group. A complete revision of Chiromantes is now being undertaken by P. K. L. Ng and the second author of the present paper.

In view of the genetic information and the upcoming revision of Chiromantes, it seems best to leave Sesarma boulenegeri in the genus for the moment (cf. Ng et al. 2008).

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References


