ABSTRACT

Many species and subspecies of Potamon have been described from the easternmost distribution of the genus in the western tributaries of the Indus River. Most of them were synonymised subsequently under the two names of currently valid species known from the region: Potamon gedrosianum Alcock, 1909 and Potamon ruttneri Pretzmann, 1962. Genetic and morphological information, based on mitochondrial 16S rDNA and the first male gonopod (G1), were gathered in the course of the present study. The corresponding results suggest the occurrence of four groups and question the taxonomic status of both species. We also revise the distribution range of both species, in particular that of P. gedrosianum, with a new record from Iran. Overall, the study reveals the need for a major revision using further morphological and molecular data. Because of the complexity of this necessary revision and the incomplete sampling, we here refrain from proposing any taxonomic conclusions.

ZUSAMMENFASSUNG


4) Corresponding author; e-mail: A.Keikhosravi@hsu.ac.ir

© Koninklijke Brill NV, Leiden, 2016 DOI 10.1163/15685403-00003512
INTRODUCTION

Freshwater crabs of the genus *Potamon* Savigny, 1816 find their easternmost distribution in the western tributaries of the Indus River. After examining the collection of freshwater crabs from the Indian Museum in the early twentieth century, Alcock (1909) described a new variant of *Potamon fluviatile* Latreille, i.e., *P. f. gedrosianum* from Pakistan. In later studies, an additional species, *Potamon ruttneri* Pretzmann, 1962, and several subspecies, i.e., *Potamon gedrosianum waziristanis* Pretzmann, 1965, *Potamon gedrosianum lindbergi* Pretzmann, 1966, *Potamon gedrosianum lindberglundi* Bott, 1967 and *Potamon gedrosianum torbenwolffii* Bott, 1967, were described from the region. In the most recent taxonomic revision, Brandis et al. (2000) synonymised these newer subspecies and ranked *P. gedrosianum* and *P. ruttneri* at species level. According to the latter study, *P. gedrosianum* is distributed from Afghanistan to northwestern Pakistan, whereas *P. ruttneri* mainly occurs in neighbouring areas ranging from northeastern Iran to northwestern Afghanistan. These two species are morphologically distinct from each other in terms of the structure of the first male gonopod (G1) and carapace features. Brandis et al. (2000) also showed that the gonopod morphology is constant or has very limited variation at intraspecific level in both species.

In recent years, molecular techniques are being used together with morphological re-investigations to shed new light on the diversity within *Potamon*. These surveys resulted in the recognition of additional diversity within the genus, as a description of new species, revalidation of synonymised species, and introduction of evolutionary significant units (ESUs) (Jesse et al., 2010, 2011; Keikhosravi & Schubart, 2014a, b). As consequence of its diverse topography and potential endemisms, the Middle East is of particular interest in terms of species diversity of the genus *Potamon* (see Brandis et al., 2000; Keikhosravi & Schubart, 2014a, b).

In the present study, we provide genetic data for *P. gedrosianum* and *P. ruttneri* from different museum collections in order to determine whether the current classification reflects actual species boundaries and if hidden diversity can be revealed. A second aim is to determine how reliable the currently used morphological characters are, in particular the G1, in separating different taxa within the species complex in accordance with the genetic information.

MATERIAL AND METHODS

Morphological traits

First male gonopods (G1) from the studied museum vouchers (table I) were investigated using light microscopy and digitally photographed with a camera-equipped stereomicroscope. Localities corresponding to the examined material are
<table>
<thead>
<tr>
<th>Map label</th>
<th>Species</th>
<th>Voucher number</th>
<th>Locality</th>
<th>Remark</th>
<th>G1 label</th>
<th>Haplotype label</th>
<th>16S accession no.</th>
</tr>
</thead>
<tbody>
<tr>
<td>KLT</td>
<td><em>Potamon ruttneri</em></td>
<td>ZUTC-5907</td>
<td>Iran, 55 km N Mashad, road to Kalat</td>
<td>–</td>
<td>a</td>
<td>M</td>
<td>LT 158587</td>
</tr>
<tr>
<td>MRD</td>
<td><em>P. ruttneri</em></td>
<td>ZUTC-5908</td>
<td>Iran, Khorasan Razavi Prv., Nishaboor, Mirabad R.</td>
<td>–</td>
<td>a</td>
<td>M</td>
<td>LT 158588</td>
</tr>
<tr>
<td>BHN</td>
<td><em>P. ruttneri</em></td>
<td>SMF-4150</td>
<td>Iran, Khorasan Razavi Prv., W Bijistan</td>
<td><em>P. ruttneri</em> (paratype)</td>
<td>a</td>
<td>M</td>
<td>LT 158589</td>
</tr>
<tr>
<td>ERK</td>
<td><em>P. ruttneri</em></td>
<td>SMF-24267</td>
<td>Iran, Khorasan Razavi Prv., Eresk, Kal-e-Jaffri</td>
<td>–</td>
<td>a</td>
<td>S</td>
<td>LT 158590</td>
</tr>
<tr>
<td>OBH</td>
<td><em>P. ruttneri</em></td>
<td>NHMW 17027</td>
<td>Afghanistan, Herat Prv., Owbeh</td>
<td><em>P. gedrosianum lindbergi</em> Pretzmann, 1966 (type)</td>
<td>a</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>ZBL</td>
<td><em>Potamon gedrosianum</em></td>
<td>ZUTC Pot-1076</td>
<td>Iran, Sistan va Baluchestan Prv., Zabul</td>
<td>New record</td>
<td>e</td>
<td>S</td>
<td>LT 158591</td>
</tr>
<tr>
<td>ZBL</td>
<td><em>P. gedrosianum</em></td>
<td>ZUTC-pending</td>
<td>Iran, Sistan va Baluchestan Prv., Zabul</td>
<td>New record</td>
<td>–</td>
<td>S (n = 3)</td>
<td>LT 158592</td>
</tr>
<tr>
<td>RSA</td>
<td><em>P. gedrosianum</em></td>
<td>MZUF-C3701</td>
<td>Afghanistan, Rabat Sacha</td>
<td>–</td>
<td>a</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>FRU</td>
<td><em>P. gedrosianum</em></td>
<td>SMF-2623</td>
<td>Afghanistan, Farah Prv., Harut R.</td>
<td>Female</td>
<td>–</td>
<td>M</td>
<td>LT 158593</td>
</tr>
<tr>
<td>HEL</td>
<td><em>P. gedrosianum</em></td>
<td>SMF-24263</td>
<td>Afghanistan, Helmand Prv., Gawargin, Helmand R.</td>
<td>–</td>
<td>e</td>
<td>S</td>
<td>LT 158594</td>
</tr>
<tr>
<td>PZA</td>
<td><em>P. gedrosianum</em></td>
<td>ZMUC-MOK</td>
<td>Afghanistan, Kandahar Prv., Pirzada</td>
<td><em>P. gedrosianum torbenwolffi</em> (type)</td>
<td>d</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Map label</td>
<td>Species</td>
<td>Voucher number</td>
<td>Locality</td>
<td>Remark</td>
<td>G1 label</td>
<td>Haplotype label</td>
<td>16S accession no.</td>
</tr>
<tr>
<td>-----------</td>
<td>---------------------</td>
<td>----------------</td>
<td>---------------------------------</td>
<td>----------------------</td>
<td>----------</td>
<td>-----------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>QEA1</td>
<td><em>P. gedrosianum</em></td>
<td>NHM 81.10</td>
<td>Pakistan, Beluchistan Prv., Quetta</td>
<td>–</td>
<td>e</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>QEA2</td>
<td><em>P. gedrosianum</em></td>
<td>SMF-24261</td>
<td>Pakistan, Beluchistan Prv., Quetta</td>
<td>–</td>
<td>e</td>
<td>R</td>
<td>LT 158595</td>
</tr>
<tr>
<td>KAM</td>
<td><em>P. gedrosianum</em></td>
<td>NHMW 17021</td>
<td>Afghanistan, Paktia Prv., Kurram R.</td>
<td>–</td>
<td>d</td>
<td>W</td>
<td>LT 158596</td>
</tr>
<tr>
<td>KML</td>
<td><em>P. gedrosianum</em></td>
<td>SMF-2887</td>
<td>Afghanistan, Paktia Prv., road Khost-Mangall</td>
<td>–</td>
<td>b</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>HRT</td>
<td><em>P. gedrosianum</em></td>
<td>SMF-2622</td>
<td>Afghanistan, Herat</td>
<td><em>P. gedrosianum lindbergi</em> Bott, 1967 (type)</td>
<td>d</td>
<td>T</td>
<td>LT 158597</td>
</tr>
<tr>
<td>AZT</td>
<td><em>P. transscaspicum</em></td>
<td>NHMW 3226</td>
<td>Afghanistan, Zabul Prv., Kalat</td>
<td>This specimen labelled as <em>P. gedrosianum</em></td>
<td>c</td>
<td>K</td>
<td>LT 158598</td>
</tr>
<tr>
<td>ZRD</td>
<td><em>P. transscaspicum</em></td>
<td>ZUTC Pot. 1088</td>
<td>Iran, Sabzevar, Zardkoohi R.</td>
<td>–</td>
<td>–</td>
<td>K</td>
<td>HE963841</td>
</tr>
</tbody>
</table>

Specimens without accession number were not examined molecularly. Map, G1 and haplotype labels correspond to fig. 1, fig. 2 and fig. 3, respectively. NHM, Natural History Museum, London, UK; NHMW, Naturhistorisches Museum, Vienna, Austria; SMF, Senckenberg Forschungsinstitut und Museum, Frankfurt am Main, Germany; ZUTC, Zoology Museum, University of Tehran, Tehran [= Teheran], Iran.
showed in fig. 1 and table I. The terminology used for description of the G1 follows Brandis et al. (1999).

**Molecular characterization**

Tissue samples were extracted from legs of the same specimens that were used for morphological analyses. DNA was isolated using a modified Puregene method (Gentra Systems) at the University of Regensburg. A fragment of the mitochondrial 16S rRNA gene was amplified using the primer pair 16L29 and 16HLeu (Schubart, 2009). Polymerase chain reaction (PCR) was carried out under the following conditions: 94°C 45 s/48°C 1 min/72°C 1 min (40 cycles) with initial denaturation of 4 min at 95°C and final extension of 7 min at 72°C. PCR products were purified using the Sure Clean protocol and sequences were obtained with an ABI Prism 310 Genetic Analyser (Applied Biosystems) or by outsourcing to LGC Genomics using 16HLeu as sequencing primer. DNA-sequences were corrected manually with BioEdit (version 5.09; Hall, 2001) and aligned with Mafft version 6 (Katoh et al., 2002). Sequences of the different haplotypes were submitted to the European Molecular Database EMBL (for accession numbers see table I).

A statistical parsimony network based on a 606 bp (base pair) alignment of the 16S rRNA was inferred using the software TCS version 1.21 (Clement et al., 2000)
in order to document the inter- and intraspecific range of divergence and relationships among haplotypes, and to quantify genetic distances among the single specimens and populations. The analysis was run with 50 steps connection limit, and gaps were treated as fifth character state. A sequence of the same fragment, belonging to *Potamon transcaspicum* Pretzmann, 1962 (accession number HE963841), was used as outgroup for the network construction. The genetic distances, as the percentages of the nucleotide differences, were also calculated among haplogroups using the software MEGA version 6 (Tamura et al., 2013).

**RESULTS**

**Morphological findings**

The morphological examination of the G1 was mainly based on the terminal joint and flexible zone, as these regions normally show highest variability among the different taxa. In addition, the distal section of the subterminal joint was considered, since our examinations show a large intraspecific variability of this segment. According to this morphological study, four distinct gonopod morphotypes can be distinguished:

**Morphotype-1.**— Terminal joint of first gonopod elongated, conical, mesial part proximally straight, 2/3 of mesial part distally slightly convergent; subterminal joint distally with significant projection at mesial edge; flexible zone V-shaped, mesial side elongated (fig. 2d). This morphotype is predominant in eastern and central Afghanistan (northern group).

**Morphotype-2.**— Terminal joint of first gonopod short, stout, conical or subconical, mesial side slightly convex; subterminal joint distally with deep notch at lateral side, distally gently deflected at mesial edge, with no significant projection; flexible zone well-developed, mesial side elongated (fig. 2a). This morphotype is typical for the *P. ruttneri* group.

**Morphotype-3.**— Terminal joint of first gonopod elongated, triangular or conical; subterminal joint distally bent outward; flexible zone asymmetrically bilobed, mesial lobe larger (fig. 2e). This morphotype can be found in the south of Afghanistan, north of Pakistan, and mid-east Iran (southern group).

**Morphotype-4.**— Terminal joint of first gonopod not elongated, conical; mesial part straight, with small depression medially at mesial side, lateral part noticeably curved; flexible zone not bilobed, mesial side elongated (fig. 2b). This morphotype was found in a single examined specimen from eastern Afghanistan (eastern group) and the corresponding animal was not included in the genetic analysis.

The fifth gonopod morphotype represents the one of the outgroup *P. transcaspicum* and is shown in fig. 2c.
Fig. 2. Comparison of the distal part of the first male gonopod (G1, dorsal view) in different populations and species of freshwater crabs from the eastern range of *Potamon* Savigny, 1816. a, *P. ruttneri* group; b, eastern group of *Potamon gedrosianum* Alcock, 1909; c, *P. transcaspicum* group; d, northern group of *P. gedrosianum*; e, southern group of *P. gedrosianum*. Scale bars = 2 mm. For detailed information and abbreviations see table I.

Molecular results.— The parsimony network analysis, based on a 606 basepair alignment of the 16S mtDNA consists of four haplogroups and six haplotypes. There is no evidence for a close relationship among the haplogroups, as they are separated by at least nine steps from neighbouring haplogroups. The first haplogroup (haplotype M in fig. 3) includes all specimens belonging to *P. ruttneri* from Iran and northwestern Afghanistan and one *P. gedrosianum* from western
Afghanistan. The second haplogroup (haplotypes S, R in fig. 3) contains all specimens belonging to *P. gedrosianum* from Afghanistan, including mid-east Iran as a new record for Iran (southern group), and surprisingly one specimen morphologically belonging to *P. ruttneri* (fig. 2a, ERK) from Iran. The third haplogroup (haplotype T, W in fig. 3) corresponds to the northern group and includes specimens from eastern and central Afghanistan. The fourth haplogroup (haplotype K in fig. 3) includes the outgroup, *P. transscaspicum* and one presumed *P. gedrosianum* from western Afghanistan. The distances between haplotype K and the three other groups range from 7 to 7.5%; otherwise genetic distances among all three “ingroups” range between 1.2 and 2%.

**DISCUSSION**

The results of this study suggest that the analysed populations of the *Potamon ruttneri* and *P. gedrosianum* species complex can be classified in four different gonopod morphotypes and, to date, in three mitochondrial haplogroups (no available sequences for one of the morphotypes). Their allocation is mixed, therefore questioning the current taxonomic classification into two species.

Alcock (1909) described *P. gedrosianum* merely based on carapace features. Later studies included the G1 structure in description and diagnosis of the species and infraspecific categories, but carapace features remained the dominant character.
used to distinguish the corresponding species and (now synonymized) subspecies (see Pretzmann, 1962, 1965, 1966; Bott, 1967). As a consequence, several taxa have been described according to the carapace features and without G1 distinction (e.g., *P. gedrosianum waziristanis* and *P. gedrosianum gedrosianum*), or using G1, but merely emphasizing the carapace structures. The revision of the genus *Potamon* by Brandis et al. (2000) resulted in synonymy of the subspecies giving priority to the species *P. gedrosianum* and *P. ruttneri*. These authors considered the G1 structure as the only consistent feature for defining the species delimitation and suggested that carapace characters are very variable. The data presented here reveal that, although three haplogroups can be diagnosed that are in line with morphological characters, neither of them can yet be considered as valid species. Our results also put into question some findings of older studies, for example in the case where type specimens of different subspecies fall into the same morphological group (e.g., *P. gedrosianum lindberglundi* and *P. gedrosianum torbenwolffi*). Another interesting question raised by this study concerns the occurrence of a gonopod type and a 16S mtDNA haplotype corresponding to *P. transcaspicum* in a specimen from eastern Afghanistan (fig. 1, AZT). This may be a labelling mistake, as it is far-fetched to believe in the occurrence of *P. transcaspicum* from this region, since its distribution is largely disconnected by the here studied species’ habitats. The unexpected appearance of one *P. ruttneri* and one *P. gedrosianum* in different haplogroups (fig. 3; haplotypes S, M), and the unforeseen occurrence of a *P. transcaspicum* haplotype from eastern Afghanistan (fig. 3; haplotype K) justify the necessity of a wider re-sampling to clarify the current uncertainties.

The general congruence of morphological and molecular results suggests that previous studies relied overproportionally on variable morphological characters (e.g. overall shape and carapace features) and did not consider gonopod characters enough (i.e., Alcock, 1909; Pretzmann, 1962, 1965, 1966; Bott, 1967, 1970). In addition, and regarding the fact that the gonopod structures of brachyuran crabs is only a sufficient, but not a necessary modification for species delimitation (Jesse et al. 2010), using other trustworthy morphological characters accompanied by further molecular markers is here recommended for uncovering the real diversity. For the moment, the current incomplete geographic sampling, lack of sequences from more specimens, and the need of further taxonomic and molecular work precludes any further taxonomic conclusions.

ACKNOWLEDGEMENTS

The first author’s visits to London (NHM) and Vienna (NHMW) were funded by the “Synthesys” Project of the European Union, which is greatly appreciated,
including the important help of Miranda Lowe, Paul Clark, and Peter Dworschak. We are grateful to Prof. Alireza Sari for access to the infrastructure of the Zoology Museum of the University of Tehran (ZUTC). 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 to the Akademisches Auslandsamt of the University of Regensburg for financial support. This study was presented as a poster contribution during the 8th International Crustacean Congress in Frankfurt am Main (August 2014), and we like to thank the late Prof. Michael Türkay (†) for organizing this great event and for all his support during many years.

REFERENCES


