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The enigmatic Marmorkrebs (marbled crayfish) is the parthenogenetic form of *Procambarus fallax* (Hagen, 1870)

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Abstract

A mysterious parthenogenetic cambarid crayfish (the Marmorkrebs) has been spreading across the globe for the past decade. We compare this crayfish directly to two other cambarids, *Procambarus fallax* and *P. alleni*, that have been suggested to be related or even identical to the Marmorkrebs. Using external morphology and sequences of two mitochondrial genes we show clear correspondences between Marmorkrebs and *P. fallax*, a species found natively throughout peninsular Florida, USA. Based on these congruent results we suggest that the Marmorkrebs is the parthenogenetic form of *P. fallax*. This finding has potential evolutionary and ecological implications at several levels. The Marmorkrebs might be a type of geographical parthenogenesis, but a natural population in the wild is so far unknown. Furthermore, challenges arise in regard to the respective species status of the Marmorkrebs. Taxonomically we suggest that the Marmorkrebs is treated as 'parthenogenetic form' of *P. fallax*. Last but not least, the identity of this animal and its ecology has an impact for considering potential spread and effects of this species across the globe.

Contents

Introduction

In 2003 Scholtz *et al*. reported that an unidentified crayfish in Germany was reproducing parthenogenetically in an aquarium. This was the first record of a parthenogenetic decapod crustacean, and while its identity was unknown it was dubbed Marmorkrebs (marbled crayfish) for its marbled carapace.

Based on molecular evidence, on morphological characters, and on characteristics of the postembryonic development, it was clear that the Marmorkrebs belongs to the large group of the North American Cambaridae (Scholtz *et al*., 2003; Vogt *et al*., 2004). Unfortunately, however, a precise determination of the position of the Marmorkrebs within the cambarids has not been possible so far. Because of its thelytokous parthenogenesis, only females are known to date. Thus, all existing keys to this group, based on characters of the male gonopods (*e.g*. Hobbs, 1972, 1989) cannot be used.

Due to its very similar coloration and general appearance *Procambarus fallax* (Hagen, 1870) (Fig. 1) has been included in the initial molecular study on Marmorkrebs' phylogenetic relationships (Scholtz *et al*., 2003). Indeed, this analysis based on mitochondrial data has resolved the Marmorkrebs as the closest relative of *Procambarus fallax* (Scholtz *et al*., 2003). However, the putative identity between *P. fallax* and Marmorkrebs has not been tested. Subsequently, several authors have considered the Marmorkrebs to be a parthenogenetic *Procambarus alleni* (Faxon, 1884) (see Vogt, 2008). This is an unsatisfying situation since the Marmorkrebs is currently being established as a laboratory model organism and over the past 7 years research and publications on the development of female clones of Marmorkrebs have been growing (*e.g*. Vogt *et al*., 2004; Seitz *et al*., 2005; Alwes and Scholtz, 2006;

Martin *et al*., 2007; Vogt, 2008; Vogt *et al*., 2008). Meanwhile the Marmorkrebs has been spreading over the globe, ostensibly via the aquarium trade and specimens have been found in the wild in Germany, the Netherlands, Italy, Japan, and Madagascar (*e.g*. Holdich *et al*., 2009; Jones *et al*., 2009; Kawai *et al*., 2009; Martin *et al*., 2010; Kawai and Takahata, 2010) (Table 1). The purpose of this article is to definitively identify the species affinity of the Marmorkrebs through comparative morphological and genetic analyses.

Table 1. Countries where Marmorkrebs (parthenogenetic *P. fallax*) are reported to be found in the wild.

country	citations
Germany	Marten *et al*., 2004; Souty-Grosset *et al*., 2006; Martin *et al*., 2010
Netherlands	Koese and Vletter, 2008; Souty-Grosset *et al*., 2006
Italy	Marzano *et al*., 2009
Madagascar	Jones *et al*., 2009; Kawai *et al*., 2009
Japan	Kawai and Takahata, 2010

Material and methods

Animal choice

Based on the studies of Scholtz *et al*. (2003) and Vogt *et al*. (2004) it is clear that the Marmorkrebs is a member of the North American Cambaridae. This group consists of about 400 species showing a great diversity of forms and lifestyles (*e.g*. Lukhaup, 2003). This makes the determination of the closest relative or even the species of origin of the Marmorkrebs comparable to a search for a needle in a haystack. However, the study of Seitz *et al*. (2005) indicates that the Marmorkrebs appears to be adapted to a relatively warm environment which excludes species occurring in the northern parts of the United States or in Canada. Furthermore, crayfish species with a notably similar appearance to the Marmorkrebs are not so common. For instance, according to Hobbs (1942, 1981) none of the other *Procambarus* crayfish forms in the south-eastern region of the United States area has a similar morphology. This is the reason why the two species from

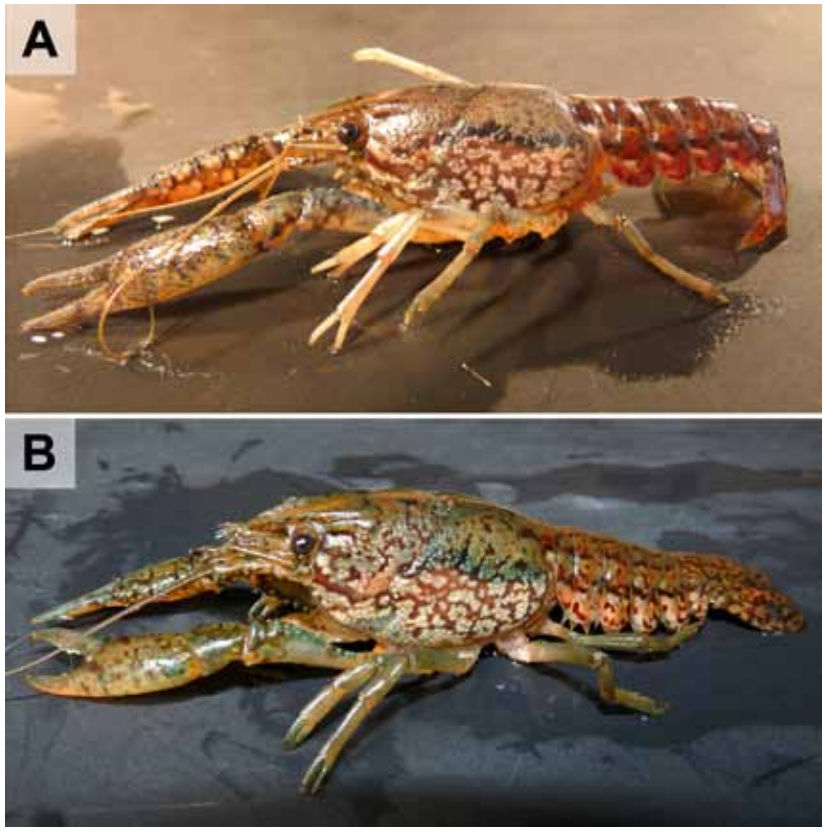

Fig. 1. Similar appearance of A) *Procambarus fallax* (male) from the aquarium trade and B) Marmorkrebs (female) from HU-stock.

Florida, *Procambarus fallax* (Slough Crayfish) and *P. alleni* (Everglade Crayfish) have been suggested to be either closely related or even identical to the latter (Scholtz *et al*., 2003; Vogt, 2008; Jones *et al*., 2008; Kawai *et al*., 2009). Here we follow and test these previous suggestions.

Morphological comparisons

Procambarus fallax and *P. alleni* occur in the southeast of the USA (Hobbs, 1981, 1989; Lukhaup, 2003). As part of past and ongoing population studies in south Florida both species are found in a diversity of habitats including forested and open wetlands, ponds, and ditches (Hendrix and Loftus, 2000; Dorn and Trexler, 2007; Dorn and Volin, 2009).

While the first two pairs of male pleopods, the gonopods are generally considered necessary for identifying crayfish (*e.g*. Hobbs, 1972, 1981, 1989), the ecological studies in south Florida require the identification of preserved specimens including males and females. A key feature for the differentiation of females of these two species even at small (< 10 mm carapace length, CL) sizes is the morphology of the sperm receptacle, the *annulus ventralis* (Dorn and Trexler, 2007). For this study the *annulus ventralis* of preserved *P. fallax* (n = 12) and *P. alleni* (n = 10) from Florida was compared with that of preserved Marmorkrebs (n > 18 of specimens from Berlin).

There are important coloration differences in live *P. fallax* and *P. alleni* that were noted by Hendrix and Loftus (2000). Accordingly, in this study we compare the overall coloration patterns and some more detailed aspects between *P. fallax*, *P. alleni*, from Florida and Marmorkrebs specimens from the Humboldt-Universität zu Berlin and from the aquarium trade in Japan based on living individuals and on the images published in the scientific literature (*e.g*. Scholtz *et al*., 2003, Seitz *et al*., 2005, Kawai *et al*., 2009) and online (various sites).

Molecular study

For our molecular genetic analysis we used partial sequences of the mitochondrial protein coding cytochrome oxidase subunit I gene (COI) and the mitochondrial 12S ribosomal RNA gene, which are well established markers for comparisons at the species level including freshwater crayfish (*e.g*. Hebert *et al*., 2003; Munasinghe *et al*., 2004; Sinclair *et al*., 2004; Balitzki-Korte *et al*., 2005; Schubart and Huber, 2006; Chu *et al*., 2006; Braband *et al*., 2007; Costa *et al*., 2007; Ferri *et al*., 2009; Toon *et al*., 2009; Filipová *et al*., 2010). Total DNA was extracted by using a DNA extraction kit (DNeasy Blood and Tissue Kit, Qiagen) from muscle tissue of walking legs of several specimens of each, *P. fallax* collected from different areas of the Water Conservation Areas of the Everglades (South Florida, USA.) and *P. alleni* sampled from wetlands near Tampa Bay (West Florida) and from areas of the Big Cypress National Preserve (South Florida), respectively (Table 2). For amplifying the COI fragment we used the universal primer pair LCO1490/HCO2198 designed by Folmer *et al*. (1994) following the slightly modified protocol described by the same authors. PCR was performed in a final volume of 25 µl with 10 to 100 ng of total DNA,

specimen	sex	origin	species	accession number	
				COI	12S
mc-HU	female	HU stock	Marmorkrebs	HM358010	HM358014
mc-Sax	female	Saxony	Marmorkrebs	HM358011	HM358015
pal-1	female	Florida	*P. alleni*	HQ171449	HQ171460
pal-3	female	Florida	*P. alleni*	HQ171451	HQ171461
pal-4	female	Florida	*P. alleni*	HQ171452	HQ171462
pal-10	male	Florida	*P. alleni*	HQ171450	HQ171463
pal-HU	male	Aquarium trade	*P. alleni*	HM358013	HM358017
pfx-5	female	Florida	*P. fallax*	HQ171455	HQ171464
pfx-6	female	Florida	*P. fallax*	HQ171456	HQ171465
pfx-7	female	Florida	*P. fallax*	HQ171457	HQ171466
pfx-8	male	Florida	*P. fallax*	HQ171458	HQ171467
pfx-9	female	Florida	*P. fallax*	HQ171459	HQ171468
pfx-11	female	Florida	*P. fallax*	HQ171453	HQ171469
pfx-12	female	Florida	*P. fallax*	HQ171454	HQ171470
pfx-HU	male	Aquarium trade	*P. fallax*	HM358012	HM358016

Table 2. Details of the analysed specimens and their GenBank® accession numbers of the COI and 12S sequences.

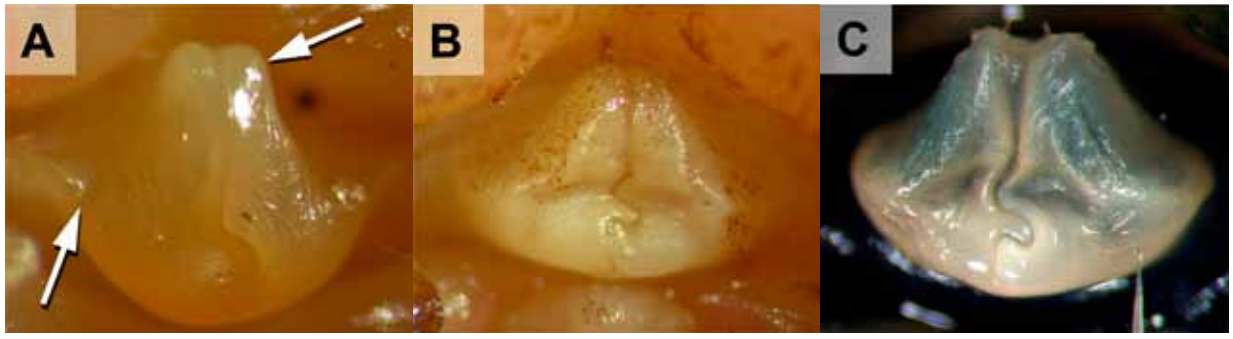

Fig. 2. Annulus ventralis of A) *Procambarus alleni*, B) *Procambarus fallax* from south Florida wetlands, and C) Marmorkrebs from Germany. All crayfish were >28 mm carapace length (>20 mm post orbital carapace length). The arrows point to the differences between the *annulus ventralis* of *P. alleni* with the peaked anterior portion and the scooped lateral wings and that of *P. fallax*/Marmorkrebs which lack these characteristics.

1× $(NH_4)2SO_4$ buffer, 3 mM $MgCl_2$, 0.2 mM of each dNTP, 0.2 µM of each forward and reverse primer, and 0.6 U Taq DNA polymerase. Amplification commenced at 94°C for 2 min followed by five cycles of 1 min at 96°C, 1.5 min at 45°C and 1.5 min at 72°C, afterwards succeeded by 35 cycles of 93°C for 1 min, 50°C for 1.5 min, 72°C for 1.5 min, and finished finally with a 5-min extension at 72°C. 12S rRNA fragment was amplified using the primers CF12FOR (5'-AMATGARAGC-GACGGGCGAT) and CF12REV (5'-AWCAAYTAG-GATTAGATACC) designed by Braband *et al*. (2007) according to a standard PCR protocol with 40 cycles of 94°C for 30 s, 40°C for 30 s, and 72°C for 40s, and with a final extension of 72°C for 5 minutes. All PCR products were purified by the MinElute® PCR Purification Kit (Qiagen) and sense and antisense strand of the fragments were sequenced by the sequencing service company LGC Genomics Berlin, Germany. For the phylogenetic analysis, the produced sequences were compared with a data set obtained from another study (Martin *et al*., 2010) of two Marmorkrebs specimens from our laboratory culture (mc-HU) and from Saxony (south-east Germany; mc-Sax) and one male of *P. fallax* (pfx-HU) and *P. alleni* (pal-HU) from the aquarium trade. For tree reconstruction further data of other *Procambarus* species and the cambarid *Orconectes limosus* (Rafinesque, 1817) were added from GenBank® (Accession numbers see Fig. 4). The COI and the 12S datasets were aligned using the ClustalW Multiple alignment application (Thompson *et al*., 1994) integrated in the program BioEdit version 7.0.9.0. for Windows (Ibis Biosciences, USA; Hall 1999) and subsequently pruned by using the program Gblocks 0.91b (Castresana, 2000) with the following parameters: minimum number of sequences for a conserved position (COI/12S) 10/11, minimum number of sequences for a flanking position (COI/12S) 16/18, maximum number of contiguous non conserved positions (COI/12S) 4/4, minimum length of a block (COI/12S) 10/10, allowed gap positions (COI/12S) none/none. In addition, the COI alignment was carefully checked as recommended by Buhay (2009) to avoid data corruption through pseudogenes such as 'COI-like' nuclear mitochondrial DNA (numt). A maximum-likelihood (ML) tree consisting of the sequenced specimens and additional species was computed from a combination of both the COI and 12S sequences with the program TREEFINDER (Jobb, G. TREEFINDER, version Oct. 2008, Munich, Germany. Distributed by the author at www.treefinder.de) using the substitution models J1+G (TA=TG and CA=CG; complexity 3+3) for COI partition and the HYK+G for 12S partition estimated by the program-internal model proposer based on the Akaike Information Criterion (AICc), with five rates categories, and with 1000 replicates for bootstrap analysis. The tree was rooted at *Orconectes limosus*. Pairwise nucleotid divergences between *P. fallax*, *P. alleni* and Marmorkrebs were calculated separately for COI and 12S with the program MEGA4.0.2 by using the Tamura-Nei (TN) model (Tamura *et al*., 2007) estimated by the TREEFINDER model proposer.

Results

Morphological comparisons reveal detailed similarities between Procambarus fallax *and Marmorkrebs*

Concerning morphological characters the Marmorkrebs identifies closely with *P. fallax* and is quite distinct

from *P. alleni* (Figs 2-3). The Marmorkrebs *annulus ventralis* lacks the characteristic scooped 'wings' on the lateral parts and the anterior portion is not peaked like the *annulus ventralis* of *P. alleni* (Fig. 2A vs. B-C). In contrast, the Marmorkrebs *annulus ventralis* closely resembles the flatter, bell-shaped *annulus ventralis* of *P. fallax* (see also Hobbs, 1942; Kawai *et al*., 2009) (Fig. 2). Furthermore, the Marmorkrebs *annulus ventralis* does not closely resemble that of any other North American members of the genus *Procambarus* (Hobbs, 1989). The overall coloration pattern of *P. fallax* (Fig. 3), including a 'marbled' carapace and lateral dark stripes, is consistent with the coloration of Marmorkrebs from the Berlin laboratory culture, in published photos (Scholtz *et al*., 2003; Seitz *et al*., 2005; Vogt *et al*., 2008; Kawai *et al*., 2009), and with those on the internet. The dark lateral horizontal stripes through the cephalothorax and abdomen on *P. fallax* (Fig. 3B) can vary in intensity between individuals (or populations), but a distinct stripe is a good indication that the crayfish is *P. fallax* (Fig. 3B) rather than *P. alleni* (Fig. 3A). Specimens of *Procambarus alleni* have conspicuous dark spots ventral to the bases of the eye stalks and anterior to the oral cavity (Fig. 3A) while those of *P. fallax* do not (Fig. 3B). This was first pointed out by Hendrix and Loftus (2000) and after seeing thousands of individuals of these species we can further attest to the uniformity of this difference in south Florida (N. Dorn, pers. obs.). All the Marmorkrebs individuals studied in the laboratory culture in Berlin lack these conspicuous spots (Fig. 3C). The absence of these spots can also be clearly seen in Kawai *et al*. (2009) from Marmorkrebs from the Japanese aquarium trade (Fig. 3D).

Molecular analysis shows a high degree of DNA sequence correspondence between Procambarus fallax *and Marmorkrebs*

The molecular analysis shows distances of the COI sequences (total length 675 base pairs) between *P. alleni* and *P. fallax* ranging from 6.40 to 7.24% (mean 6.88 ± 1.01%), between *P. alleni* and Marmorkrebs from 6.75 to 7.24% (mean 6.88 ± 1.05%), and between *P. fallax* and Marmorkrebs from 0.60 to 0.75 (mean 0.67 ± 0.24%). Within the species, the values are 0.00-1.36% (mean 0.79 ± 0.25%) in *P. alleni* and 0.00-1.05% (mean 0.57 ± 0.19%) in *P. fallax*. The 12S sequences (381 base pairs) of *P. fallax* and Marmorkrebs are identical. This is also true for the *P. alleni* specimens, where uniform sequences are found except in specimen pal-10, which shows a single substitution. The distance between the *P. fallax* / Marmorkrebs complex and the *P. alleni* population ranges from 2.95 to 3.27% (mean 3.02 ± 0.92%). The two Marmorkrebs specimens are identical in the COI and 12S sequences over a total length of 1057 base pairs (see also Martin *et al*., 2010).

For tree reconstruction, a total data set consisting of the combined COI and 12S sequences of the specimens listed in Table 2 and a selection of several species from GenBank® of 927 nucleotides was available after pruning the DNA alignments with the program Gblocks 0.91b. The resulting tree shows that the two Marmorkrebs specimens nest clearly within the *P. fallax* representatives (Fig. 4). The same result was obtained if each gene alone was used for tree reconstruction (not shown).

Discussion

The relationship of the Marmorkrebs to Procambarus fallax

Our comparison of morphological aspects and our analysis of molecular sequence data show a congruent result revealing the close affinities between Marmorkrebs and *Procambarus fallax*.

The shape of the *annulus ventralis* is clearly identical between *P. fallax* and Marmorkrebs to the exclusion of *P. alleni* and other cambarid crayfish species (Hobbs, 1942). The body coloration, despite some variability (see Hobbs, 1989), points strongly to the same direction. Pigmentation can be affected by coloration of the background environment (Thacker *et al*., 1993) and we observe differences in the size of the marbled spots and darkness of pigments in wild caught animals in Florida wetlands and between individuals of Marmorkrebs (Vogt *et al*., 2008; Martin *et al*., 2010). The close resemblance of *P. fallax* and the Marmorkrebs speaks against the possibility that the latter originated from a hybridisation event (see below).

Furthermore, the analysis of the two mitochondrial genes convincingly supports the assumption of a close relationship between Marmorkrebs and *P. fallax*. Regarding the 12S marker, the sequences of both groups are identical. In contrast, clear differences occur between them and *P. alleni*. In comparison to the 12S gene, divergences exist in the COI data within *P. fallax* and *P. alleni*, but these values were almost ten times lower than the average value between both species. Differences are also found between Marmorkrebs and

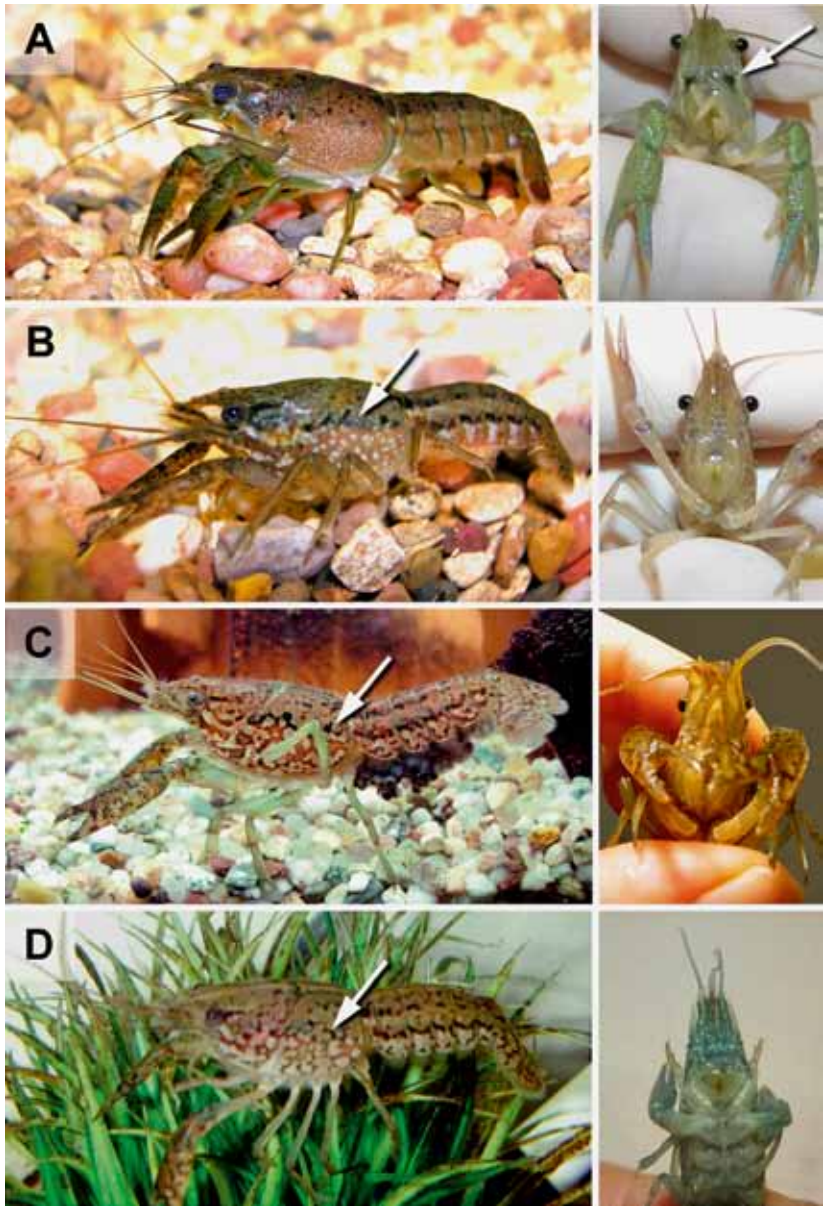

Fig. 3. Photographs of live A) *Procambarus alleni* and B) *Procambarus fallax* from south Florida, USA and C) Marmorkrebs from the lab cultures in Germany and the aquarium trade in Japan D). In every case the lateral overall view (left) and the facial ventral view (right) is shown. The arrows in the left pictures mark the dark lateral horizontal stripes through the cephalothorax and abdomen in *P. fallax* and Marmorkrebs (compare with Fig. 1). The arrow in the top right picture points at the facial dark spots characteristic for *P. alleni*.

P. fallax. However, the values remain in the range of the calculated distances within *P. fallax*. Furthermore, the divergence of Marmorkrebs to *P. alleni* is on average ten times higher, and thus corresponding to that between *P. fallax* and *P. alleni*. All this implies that the Marmorkrebs shows indeed very close affinities to *P. fallax*.

The result of the divergence analysis coincides well to the reconstructed ML tree, which shows that the two Marmorkrebs specimens are deeply nested within the individuals of *P. fallax*. Furthermore, the *P. fallax*/Marmorkrebs branch is strongly supported by the bootstrap value of 100%, which is another indication for the very close affinities or even identity of Marmorkrebs and *P. fallax*.

Taken together, all this reveals that the Marmorkrebs is a parthenogenetic form of *P. fallax*.

While our conclusion that Marmorkrebs is a parthenogenetic *P. fallax* agrees with tentative suggestions of Scholtz *et al*. (2003) and Kawai *et al*. (2009) this is the first direct morphological comparison between each species and Marmorkrebs and it is the first genetic

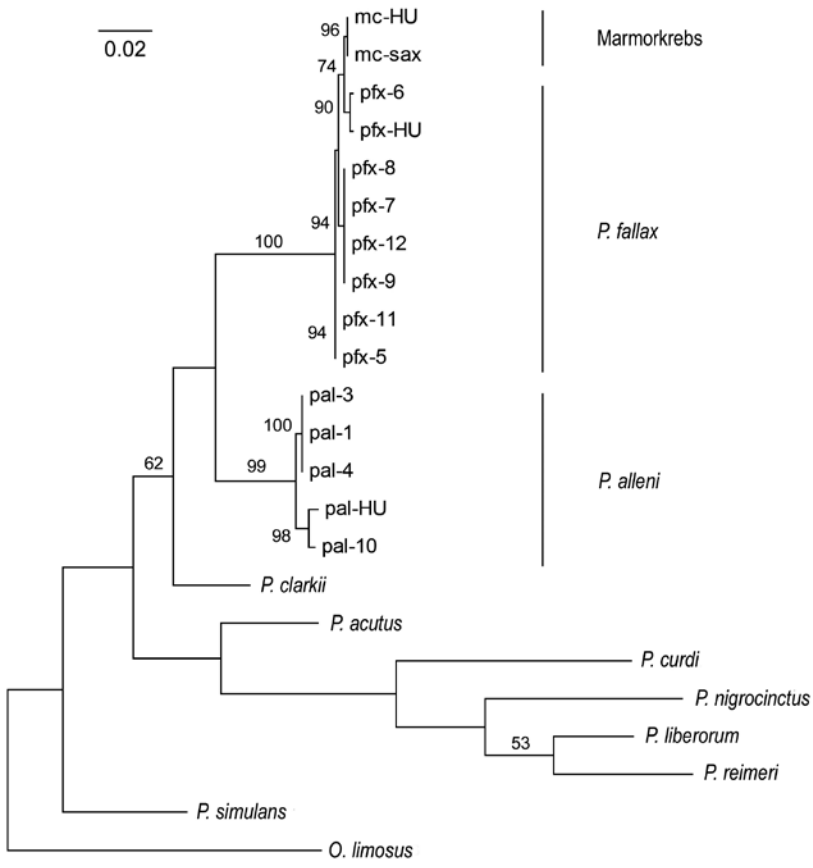

Fig. 4. Maximum-likelihood analysis of Marmorkrebs, several *Procambarus* species, and *Orconectes limosus* as outgroup. The tree was estimated from combined nucleotide sequence data sets of COI and 12S mitochondrial genes calculated under the J1+G and the HYK+G model, respectively, with five rates categories. Numbers above branches are values in percent obtained from the bootstrap analysis of 1,000 replicates (bootstrap support values lower than 50% are not shown). The scale bar indicates the evolutionary distances in substitutions per site. Accession numbers of the used sequences from the GenBank© are (COI /12S) (÷ = sequence not available): *P. clarkii* AY701195/EF 012280, *P. acutus* AF474366/FJ619794, *P. curdi* Reimer, 1975 ÷/EF012281, *P. liberorum* Fitzpatrick, 1978 ÷/EF012311, *P. nigrocinctus* Hobbs, Jr., 1990 ÷/EF012282, *P. reimeri* Hobbs, 1979 ÷/EF012283, *P. simulans* (Faxon, 1884) EU583575/÷, *O. limosus* AY701199/AY151531.

study including a comparison with both species. All molecular analyses performed prior to this study had confirmed that Marmorkrebs is a cambarid and that it is neither closely related to *P. clarkii* (Girard, 1852) (Scholtz *et al*., 2003) nor to *P. acutus* (Girard, 1852) (Marzano *et al*., 2009). In contrast to our conclusion, Vogt (2008) and Jones *et al*. (2009) suggested that the Marmorkrebs is probably *P. alleni* based on molecular analyses, but their studies did not include *P. fallax*.

Marmorkrebs is only known from aquaria and from secondary releases to the wild in various countries (Martin *et al*., 2010). Other crustaceans, like the widespread European terrestrial isopod species *Trichoniscus pusillus* Brandt, 1833 exhibit both biparental and parthenogenetic unisexual populations in a patchwork distribution (Vandel, 1928, 1938). Differences in reproductive mode of the populations may be related to micro-habitat conditions (Fussey, 1984). While we do not see a patchwork distribution of uni- and bi-sexual populations of *P. fallax*, indeed most populations have a close to a 1:1 sex ratio (N. Dorn, unpublished data); parthenogenetic abilities among *P. fallax* females would not require the existence of completely unisexual populations.

Our molecular data reveal that the Marmorkrebs origi nated from within the population of *Procambarus fallax*. This raises the question of its taxonomic treatment, *i.e*. should it be considered a new species, or a subspecies, or a form of *P. fallax*? It is evident that this is not trivial because it touches the general problem of species concepts (see Sudhaus and Rehfeld, 1992; Wheeler and Meier, 2000). For instance, according to the biological species concept, a species is defined by its capacity of sexual reproduction in combination with genetic isolation to other species. A biological species is thus a reproductively cohesive assemblage of populations (Mayr, 2000). Accordingly, parthenogenetic lineages are no longer part of the original species since they are genetically isolated from its species of origin. But by Mayr's own admission the biological species concept does not apply to asexual organisms (Mayr, 2000) and is therefore unhelpful in dealing with the Marmorkrebs. According to the Hennigian species concept (Meier

and Willmann, 2000), the switch of a subpopulation of a species to uniparental reproduction can be seen as a sort of speciation event resulting in the occurrence of two daughter lineages: a new species and an agamotaxon (Meier and Willmann, 2000). Due to these conceptual difficulties, there are numerous contradictory suggestions how to treat parthenogenetic lineages taxonomically (*e.g.* Enghoff, 1976; Suomalainen *et al.*, 1987; Frost and Wright, 1988; Sudhaus and Rehfeld, 1992).

There are a number of aspects that speak against the establishment of a new species for the Marmorkrebs. First, we do not know whether the Marmorkrebs had a single origin or whether it arose (arises) repeatedly from *P. fallax*. Second, the Marmorkrebs is morphologically not distinct from *P. fallax*. Third, since the natural range of Marmorkrebs is unknown to date we cannot argue with specific ecological requirements of the Marmorkrebs which would allow the application of the ecological species concept (*e.g.* Van Valen, 1976; Sudhaus and Rehfeld, 1992). In particular, the latter point argues also against the consideration of the Marmorkrebs as a subspecies of *P. fallax*.

However, a scientific name for the Marmorkrebs is strongly demanded. Thus, we have decided to take up the recommendations of Enghoff (1976) and Suomalainen *et al.* (1987) who propose that a parthenogenetic lineage which derived from a bisexual species should neither be regarded as a separate species nor as a subspecies but as a 'parthenogenetic form' of the bisexual species. Hence, we recommend '*Procambarus fallax* (Hagen, 1870) f. *virginalis*' as a proper name for articles dealing with Marmorkrebs. Although 'forma' is not accepted by the International Code of Zoological Nomenclature (ICZN) (1999) it appears appropriate for the time being. If additional data should clarify some of the problematic issues (*e.g.* confirmation of a single origin and/or the detection of regional populations of the Marmorkrebs in the wild) it should be easy to establish a new species using '*virginalis*' as epithet.

The origin of parthenogenesis in crayfish

It is interesting to consider the mode of the switch to obligate parthenogenesis in the Marmorkrebs (Suomalainen *et al.*, 1987; Normark, 2003; Simon *et al.*, 2003; Kearney, 2005; Lundmark and Saura, 2006). However, the occurrence of a thelytokous lineage from *P. fallax* is difficult to explain because Marmorkrebs reproduce apomictically (*i.e.* the eggs do not undergo meiosis; Martin *et al.*, 2007). Little is known about the origin of apomictic parthenogenesis from a bisexual ancestor (White, 1973; Suomalainen *et al.*, 1987; Normark, 2003; Schwander *et al.*, 2009). One possible explanation could be the occurrence of the so called tychoparthenogenesis. This is a relatively widespread type of parthenogenesis in sexual invertebrates in which females show a certain capability to parthenogenetic reproduction (White, 1973; Suomalainen *et al.*, 1987). Tychoparthenogenesis might be advantageous in an environment such as the Everglades or other wetlands in south Florida with considerable inter- and intra-annual variation in water depths. Here a genotype capable of asexual reproduction stands a better chance of locally persisting through a series of years of unfavourable conditions when overall densities are low and sexual reproduction is unlikely (Ball, 2002; Sekiné and Tojo, 2010). However, almost all tychoparthenogens known to date reproduce automictically, *e.g.* by meiotic parthenogenesis, (White, 1973; Suomalainen *et al.*, 1987; Ball, 2002; Schwander *et al.*, 2009) and hence it is doubtful that thelytoky in the apomictic Marmorkrebs originated this way. In addition, if such parthenogenetic capability were common in decapods, more thelytokous species than just the Marmorkrebs have to be expected. According to Yue *et al.* (2008) some of the introduced Chinese populations of the American cambarid freshwater crayfish *Procambarus clarkii* demonstrate a pattern of molecular markers (microsatellites) that suggests the occurrence of genetically uniform populations. However, some doubts remain whether this is indeed an indirect evidence for an apomictic parthenogenetic reproduction. The occurrence of identical genotypes with a high degree of heterozygosity is not necessarily caused by parthenogenesis. It can also arise, according to Mendel's law, in the F_1 generation when the parents are homozygous in genes with different alleles. This is often found in populations with inbreeding effects caused by introduction of only few specimens.

A further possibility for the switch from sexual to obligate parthenogenetic reproduction can be an infection with the intracellular bacterium *Wolbachia pipientis* Hertig, 1936 (Stouthamer *et al.*, 1999) or by mating of parthenogenetically produced males with sexual females (contagious origin; Simon *et al.*, 2003). However, these two origins are unlikely because *Wolbachia*-like bacteria could not be detected in Marmorkrebs and parthenogenetically produced males are not known in Astacida (Vogt *et al.*, 2004).

Another possible route towards obligate parthenogenetic lineages is the hybridisation of two closely

related and sympatric cambarid species such as *P. fallax* and *P. alleni*. Since mitochondria are almost exclusively inherited from the maternal lineage (Perry *et al*., 2001a), based on mitochondrial genes alone we cannot exclude the possibility that the Marmorkrebs and its parthenogenesis are the product of a hybridization event. However, our comparative morphological results contradict this possibility. The morphology of the Marmorkrebs presents no blend of two species but clearly resembles that of *P. fallax* alone. Hybrids, even between closely related cambarid species, are clearly recognizable because of their intermediate morphological characters as shown in *Orconectes* by Capelli and Capelli (1980) and Perry *et al*. (2001b). Furthermore, the experimental hybridisation of the two Australian freshwater crayfish species, *Cherax rotundus* Clark, 1941 and *C. albidus* Clark, 1936 did not result in parthenogenesis (Lawrence and Morrissy, 2000).

Finally, it cannot be excluded that the origin of a parthenogenetic lineage from *P. fallax* was a unique event, a 'macromutation' (White, 1973), which led to apomixis directly from amphimixis (*i.e*. sexuality).

Ecological implications

The determination of the identity of the Marmorkrebs is important for considering its invasive potential. In the Everglades and other parts of Florida *P. fallax* rarely ever exceed 45 mm carapace length and also its parthenogenetic form becomes only slightly larger (carapace length normally less than 50 mm; Kawai *et al*., 2009; Pöckl, 2009). Thus, one might think that this should make it a relatively poor candidate for consumption-based aquaculture. Nevertheless, Marmorkrebs has probably been introduced to Madagascar for this reason (Jones *et al*., 2009; Kawai *et al*., 2009; Kawai and Takahata, 2010).

Due to its reproduction mode, the parthenogenetic form of *P. fallax* is regarded as a particularly effective invader because only a single individual is able to found a new population (Marten *et al*., 2004; Jones *et al*., 2009; Jimenez and Faulkes, 2010). Furthermore, Marmorkrebs is a potential transmitter of the crayfish plague *Aphanomyces astaci* Schikora, 1906 (Culas, 2003), a highly contagious disease which causes mass mortalities in non-North-American crayfish populations (Oidtmann *et al*., 1999). Once introduced, these two features make Marmorkrebs a special threat for the indigenous crayfish species.

We already know a number of lifestyle aspects about each of these procambarids from studies in their native wetlands in south Florida (Hendrix and Loftus, 2000; Dorn and Trexler, 2007; Dorn and Volin, 2009). In particular, *P. fallax* is the smaller of the two species, it is competitively inferior and it grows more slowly (Dorn and Trexler, 2007). *P. fallax* is a tertiary burrowing species, only burrowing under extreme conditions, and it does not burrow effectively in dense clay or sand substrates (Dorn and Trexler, 2007; Dorn and Volin, 2009). It is generally more abundant in permanent water bodies or in temporary wetlands (drying briefly most years) with lightweight organic soils.

Considering the native distribution of *P. fallax* in peninsular Florida and southern Georgia (approx. 30.45° N latitude) (Hobbs, 1981) the average minimum winter air temperatures fall to around 6° C (Myers and Ewel, 1990) in the northern part of the range. While populations of the Marmorkrebs seem well established in Madagascar because of its similar climatic conditions, we wonder how well *P. fallax* could persist in cold winters, for instance in northern or central European lakes or streams where temperatures drop to ≤ 4° C under the ice for months. The observation that single specimens are able to survive under an ice cover (Pfeiffer, 2005) and the laboratory results of Seitz *et al*. (2005) suggest Marmorkrebs has a substantial cold tolerance, but the latter study also showed that their temperature optimum is rather high (18-25 °C). Therefore, more investigations are necessary to evaluate the ability of the parthenogenetic form of *P. fallax* to colonize cold water environments.

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