The marbled crayfish (Decapoda: Cambaridae) represents an independent new species

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Abstract

Marbled crayfish are a globally expanding population of parthenogenetically reproducing freshwater decapods. They are closely related to the sexually reproducing slough crayfish, Procambarus fallax, which is native to the southeastern United States. Previous studies have shown that marbled crayfish are morphologically very similar to P. fallax. However, different fitness traits, reproductive incompatibility and substantial genetic differences suggest that the marbled crayfish should be considered an independent species. This article provides its formal description and scientific name, Procambarus virginalis sp. nov.

Key words: parthenogenesis, annulus ventralis, genetic analysis, mitochondrial DNA

Introduction

Marbled crayfish were first described in 2001 as the only known obligatory parthenogen among the approximately 15,000 decapod crustaceans (Scholtz et al., 2003). The animals were first described in the German aquarium trade in the late 1990s (Scholtz et al., 2003) and became widely distributed in subsequent years under their German name "Marmorkrebs". Stable populations have developed from anthropogenic releases in various countries including Madagascar, Germany, Czech Republic, Hungary, Croatia and Ukraine (Chucholl et al., 2012; Jones et al., 2009; Kawai et al., 2009; Liptak et al., 2016; Lokkos et al., 2016; Novitsky & Son, 2016; Patoka et al., 2016). On Madagascar, marbled crayfish form an aggressively expanding population that has invaded a large variety of habitats (Jones et al., 2009). Due to their predicted ability to found large populations from single animals (Feria & Faulkes, 2011), marbled crayfish introductions have a significant potential to endanger indigenous crayfish species through competition or pathogen transmission (Chucholl & Pfeiffer, 2010; Jimenez & Faulkes, 2011; Keller et al., 2014).

Several important characteristics of marbled crayfish have been described in detail, including morphology (Kawai et al., 2009), embryonic development (Alwes & Scholtz, 2006; Seitz et al., 2005), life history (Seitz et al., 2005; Vogt, 2008; Vogt et al., 2004), parthenogenetic reproduction (Martin et al., 2007; Scholtz et al., 2003; Vogt et al., 2008) and a triploid karyotype (Martin et al., 2016). Using morphological characters and molecular markers, Martin et al. identified the sexually reproducing slough crayfish Procambarus fallax from Florida as the closest relative of marbled crayfish and suggested the provisional name Procambarus fallax forma virginalis (Martin et al., 2010). However, the International Code of Zoological Nomenclature (ICZN, 1999) excludes "forma" for names published after 1960 (Article 15.2). Consequently, the provisional name for marbled crayfish was also considered "unavailable" in a recent taxonomic summary of freshwater crayfish (Crandall & De Grave, 2017).

Importantly, recent findings have supported the notion that marbled crayfish should be considered as an independent species, for which the scientific name Procambarus virginalis was suggested (Vogt et al., 2015). These findings are summarized below and complemented by a formal new species description.
PROCAMBARUS VIRGINALIS NEW SPECIES

FIGURE 1. Comparison of morphological characters between marbled crayfish and *P. fallax*. (A) Annulus ventralis from exuvia of marbled crayfish, *P. fallax* and *P. alleni*. (B) Different life history traits in populations of ovigerous marbled crayfish (red) and *P. fallax* females (blue). Horizontal bars indicate ranges and vertical lines indicate mean values (m) and lower and upper range limits. (C) Parentage analysis in crossbreeding experiments of marbled crayfish and *P. fallax*. Colors indicate specific genotypes, as determined by microsatellite analysis. (D) Phylogenetic tree representing genetic relationships between marbled crayfish, *P. fallax*, *P. alleni* and *P. clarkii*. The tree is based on complete mitochondrial genome sequences, numbers indicate bootstrap values. All data adapted from (Vogt et al., 2015) with technical details provided in the reference.

Results

Morphological characters and life history traits. Marbled crayfish are morphologically similar to *P. fallax* and to *P. alleni* (Kawai et al., 2009; Martin et al., 2010). A discrimination according to the taxonomically highly relevant first pleopods of breeding (Form1) males (Hobbs, 1972, 1989) is not possible as there are no male marbled crayfish (Scholtz et al., 2003). However, marbled crayfish/*P. fallax* females can be discriminated from *P. alleni* by the
annulus ventralis (Kawai et al., 2009; Martin et al., 2010). This important morphological character of cambarid females (Hobbs, 1972, 1989) is bell-shaped with an S-shaped sinus in marbled crayfish and *P. fallax*, but differently shaped in *P. alleni* (Fig. 1A). The specific morphology of the annulus ventralis is highly conserved among marbled crayfish from various sources (Kawai et al., 2009; Martin et al., 2010; Vogt et al., 2015; Vojkovska et al., 2014). Specific morphological characters that discriminate marbled crayfish from *P. fallax* are not known, which is likely related to the very recent speciation of marbled crayfish 20–30 years ago (Vogt et al., 2015).

Their morphological similarities notwithstanding, life history traits have been found to be markedly different between marbled crayfish and *P. fallax* (Fig. 1B). Specifically, mean carapace length and clutch size were found to be significantly higher in adult marbled crayfish than in *P. fallax* (Vogt et al., 2015). These findings suggest important phenotypic differences between marbled crayfish and *P. fallax* that are independent of the taxonomically relevant morphological characters.

**Reproductive incompatibility.** The marbled crayfish genome is triploid, which represents a major cytogenetic roadblock for meiotic chromosome segregation (Martin et al., 2016; Vogt et al., 2015). As such, most triploid organisms are apomorphic parthenogens (Saura et al., 1993) and thus reproductively isolated from sympatric sexually reproducing relatives. In crossbreeding experiments, marbled crayfish females and *P. fallax* males showed typical courtship behavior and mating (Vogt et al., 2015). However, offspring of marbled crayfish females that had been mated with *P. fallax* males were exclusively female and exclusively showed the microsatellite markers of the marbled crayfish mother (Fig. 1C). These findings are in agreement with the notion that the *P. fallax* male did not contribute to the genome of the offspring and that the progeny is the product of apomorphic parthenogenesis in the marbled crayfish mother. Taken together, the data suggest that marbled crayfish are reproductively isolated from *P. fallax* (Vogt et al., 2015).

**Genetic differences.** Genetic analysis can be used to reveal independent species in the absence of distinctive morphological features (Bickford et al., 2007). Furthermore, convergent evolution of morphological characters appears to be common in cambarids (Breinholt et al., 2012), which further illustrates the value of genetic information for crayfish taxonomy (Owen et al., 2015). Complete mitochondrial genome sequencing of marbled crayfish from diverse sources revealed their genetic homogeneity and strongly suggested a single origin of the known marbled crayfish population (Vogt et al., 2015). In addition, mitochondrial genome sequence analysis identified considerable genetic differences between marbled crayfish and *P. fallax* (Fig. 1D). This was further supported by comparative whole-genome sequencing of marbled crayfish and their closest relatives, *P. fallax* and *P. alleni* (Gutekunst et al., submitted). Altogether, these findings are consistent with the notion that marbled crayfish represent an independent species.

**Procambarus virginalis**, new species
http://zoobank.org/NomenclaturalActs/CFEE97E8-558A-4A86-8496-85CF86A4716C

Marbled crayfish
Figures 2–3
Tables 1–2

**Diagnosis.** Body pigmented, eyes well developed. Rostrum with marginal spine and lacking median carina. Carapace with cervical spine. Areola 5 to 7 times as long as wide, constituting 30 to 35 percent carapace length (Tab. 1). Suborbital angle obtuse and weak. Postorbital ridge well developed with cephalic spine. Hepatic area punctate. Antennal scale approximately 2.5 times as long as wide, widest at midlength. Annulus ventralis bell-shaped, about 1.6 times as broad as long, bisected by narrow furrow leading caudally into median depression. Sinus originating on median line, continuing longitudinally on anterior half, then curving dextrally before curving caudally before continuing and terminating on median line. Sternum immediately cephalic to annulus with no tubercles or projections and not overhanging annulus. Unadorned bell-shaped postannular sclerite with central longitudinal furrow, width similar to annulus. First pleopods present. Mitochondrial DNA with guanine and cytosine at positions 8754 and 8783, respectively (GenBank accession number KT074364, see Tab. 2 for a list of discriminatory genetic variants).
**TABLE 1.** Morphometric parameters of *P. virginalis.*

<table>
<thead>
<tr>
<th></th>
<th>Holotype</th>
<th>Paratype 1</th>
<th>Paratype 2</th>
<th>Paratype 3</th>
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All measurements are indicated in mm.

**Holotypic female.** See Fig. 2. Cephalothorax ovate, slightly depressed. Abdomen narrower than cephalothorax (23 and 24 mm, respectively). Carapace slightly higher than wide at caudodorsal margin of cervical groove (Fig. 3A, B). Areola 6 times as long as wide, its length comprising 34% of the carapace length. Surface of carapace punctate. Rostrum slightly deflected ventrally with converging slender margins. Very short acumen, nearly reaching distal end of ultimate segment of antennular peduncle. Base of acumen marked by small marginal spines. Dorsal surface concave with punctations and no median carina. Postorbital ridge well developed, bearing spine at cephalic extremity. Suborbital angle weak and obtuse. Cervical spine twice the size of neighboring ones on caudal flank of cervical groove.

Abdomen slightly shorter than carapace (51 and 53 mm, respectively). Pleura of abdominal segments subtruncate. Telson and uropod with terminal setae. Telson with characteristic lateral spine and shallow lateral depressions. Mesial ramus with median ridge terminating in spine.

Antenna with small spiniform tubercle on ischium; flagellum reaching slightly beyond caudal margin of telson. Antennal scale (Fig. 3B) 2.5 times as long as wide, widest at midlength; lamellar area 2 times as wide than thickened lateral part. Third maxillipod extending cephalically to level of penultimate podomere of antennule, ventral surface of basal podomere densely covered with setae.

Right chela (Fig. 3C) elongate and depressed. Palm punctate and longer than wide (16 and 11 mm, respectively); mesial ridge with row of 8 small and subacute tubercles. Surface of fingers setiferous dentations. Opposable margin of fixed finger with 9 tubercles. Most distal tubercle at distal 1/4 of fixed finger larger and conormous. Surface of dactyl punctate, mesial margin with 4 small and subacute tubercles in proximal 1/4. Opposable margin of dactyl with row of 8 tubercles on proximal 2/3. Tip of dactyl elongate and pointed. Carpus of cheliped longer than broad with distinct oblique furrow dorsally, tuberculate mesially, dorsomesially and ventromesially. Ventral surface with 2 spines on distal margin. Merus with few weak spines dorsally, two larger ones distally. Mesial surface punctate, ventral surface with mesial row of 15 spines and lateral row of 13 spines. Ischium with dorsolateral tubercle and ventromesial row of 4 tubercles.

Annulus ventralis (Fig. 3D) as described in "Diagnosis".
FIGURE 2. *Procambarus virginalis* new species holotype (A) lateral and (B) dorsal views. Bar: 1 cm.
**FIGURE 3.** (A) Dorsal view of the carapace. (B) Lateral view of the carapace. (C) Dorsal view of the antennal scale. (D) Dorsal view of the right chela. (E) Ventral view of the annulus ventralis. Bars: 1 cm (A-D), 1 mm (E).

**Color notes.** The marmorated coloration of the carapace, which prompted the informal names "marbled crayfish" or "Marmorkrebs", is particularly prominent in aquarium stocks. It is often less pronounced in wild animals, which usually present in dark shades of brown or green with spotted patterns on the lateral cephalothorax and pleon (Fig. 2).
TABLE 2. Genetic markers for the discrimination of *P. virginalis*.

<table>
<thead>
<tr>
<th>Position (KT074364)</th>
<th><em>P. virginalis</em> (n=9)</th>
<th><em>P. fallax</em> (n=5)</th>
<th><em>P. alleni</em> (n=1)</th>
<th><em>P. clarkii</em> (n=1)</th>
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<td>13366</td>
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<td>C</td>
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Single nucleotide variants were determined by mapping of high-coverage Illumina sequencing data to the *P. virginalis* mitochondrial genome sequence (GenBank accession number KT074364). Numbers of analyzed animals are indicated below the species name.

**Type locality.** Lentic lake at Reilingen, Germany (Reilinger See, N49.296893, E8.544591). The holotype was selected from >150 animals that were collected using handnets and traps in August 2017. The lake has a size of 12 ha and a maximum depth of 20 m. The presence of the animals has been known by local fishermen for several years.

**Disposition of types.** The holotype (SMF 50818) and the paratypes 1 and 2 (SMF 50819) are deposited at the Senckenberg Natural History Museum (Frankfurt, Germany). The paratypes 3 and 4 remain in the author's collection.

**Etymology.** The name *Procambarus virginalis* is derived from the preliminary designation *Procambarus fallax* forma *virginalis* (Martin et al., 2010) and reflects the species’ unique parthenogenetic mode of reproduction.

**Remarks.** The oldest known record of *P. virginalis* is from a German biologist and hobby aquarist. In a personal conversation with the author (February 2017), he recalled obtaining an uncharacterized batch of "Texas crayfish" from a pet trader specializing in American insects and other invertebrates, at a trade fair in Frankfurt (Germany) in 1995. Animal numbers of this original stock increased rapidly and animals were subsequently distributed to other German aquarists, eventually reaching commercial traders and pet stores. The absence of male animals and the parthenogenetic mode of reproduction were soon recognized among aquarists and subsequently confirmed in the first scientific description of marbled crayfish (Scholtz et al., 2003).

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