**Rapid Communication**

# A voyage into the Levant: the first record of a marbled crayfish *Procambarus virginalis* (Lyko, 2017) population in Israel

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## Abstract

The marbled crayfish, *Procambarus virginalis* (Lyko, 2017) is a novel parthenogenetic freshwater species that has rapidly colonized diverse habitats in Europe and Madagascar. Here we describe the first wild population of marbled crayfish in the Middle East. Numerous specimens, including gravid females, were collected in the waters of a spring on Mt. Carmel, Israel. The results from phenotypic, morphometric and genetic analyses showed the close relationship with other known populations, consistent with the clonal structure of the global marbled crayfish population. Our findings represent a considerable expansion of the known distribution range and further illustrate the role of anthropogenic activities in the dispersal of marbled crayfish.

**Key words:** invasive species, freshwater crayfish, freshwater ecology, anthropogenic dispersal

## Introduction

Numerous freshwater crayfish invasions have attracted the attention of scientists and the general public due to their negative impact on the biomass and biodiversity of native freshwater ecosystems (Jeschke et al. 2014; Krieg et al. 2020). A notorious example is the global anthropogenic distribution of the red swamp crayfish *Procambarus clarkii* (Girard, 1852) (Hernandez et al. 2008; Kouba et al. 2014). In the last two decades, several European countries have identified and taken measures to prevent the spread of invasive crayfish species (Henttonen and Huner 2017; Krieg et al. 2020; Souty-Grosset et al. 2016).

The marbled crayfish, *Procambarus virginalis* (Lyko 2017), represents a relatively new addition to this complex problem. Marbled crayfish were originally introduced into Germany in 1995, as an ornamental aquarium pet from the USA (Lyko 2017). Based on morphological and genetic markers, the slough crayfish, *Procambarus fallax* (Hagen, 1870) has been suggested as the parent species of *P. virginalis* (Martin et al. 2010). *Procambarus fallax* are sexually reproducing diploids that are endemic to peninsular Florida and southern Georgia (Hobbs 1942). Indeed, a recent phylogeographic analysis of *P. fallax* showed that both parental haplotypes of the *P. virginalis* were inherited from the Everglades subpopulation of *P. fallax* (Gutekunst et al. 2021).

In contrast *to P. fallax*, marbled crayfish reproduce parthenogenetically and a single female is sufficient to found a population. This unique feature, together with their overall robustness, established the animals as popular aquarium pets. As a consequence, following their initial emergence in the German aquarists' network, marbled crayfish became rapidly distributed through the pet trade (Chucholl 2015; Scholtz et al. 2003). Their ability to quickly populate aquaria with their offspring has resulted in numerous anthropogenic releases and the subsequent formation of stable wild populations.

Stable wild populations of marbled crayfish have been documented in diverse habitats across Europe (Chucholl et al. 2012; Maiakovska et al. 2021). A mark-recapture analysis of a small (9 ha) lake in Germany resulted in a population size estimate of > 20,000 sexually mature animals (Maiakovska et al. 2021), thus illustrating the fecundity of the animals and their resilience to challenging habitat and climate conditions (Tönges et al. 2021a). In addition to Europe, marbled crayfish are also known to be highly abundant in the freshwater networks of Madagascar (Gutekunst et al. 2018; Jones et al. 2009; Kawai et al. 2009). However, beyond Europe and Madagascar, the distribution of marbled crayfish remains poorly documented. This paper provides the first record of marbled crayfish in the Levant. We describe a population from Mount Carmel in Haifa, Israel and provide relevant ecological, phenotypic and genetic data.

## Materials and methods

### *Sampling, water analysis and total length measurements*

Samples were collected at Ein Meshotetim, Haifa (32.801N; 34.974E), a spring situated in a valley that descends from the top of the Carmel ridge to the sea. The spring flows from a small cave into a stone-bordered shallow pool. The pool is adjacent to the ancient (13$^{th}$ century) remains of the first church built by order of the Brothers of the Blessed Virgin Mary of Mount Carmel. On October 16, 2022, 84 specimens were collected by O. Kolodny (SMNH AR30237); on October 23, 2022, 63 specimens collected by O. Kolodny and M. Truskanov (SMNH AR30239). Animals were collected by handnets and the specimens are preserved in the National Collections at the Steinhardt Museum of Natural History, Tel Aviv University. The presence of the animals was confirmed on March 06, 2023 and a water sample from the pool was obtained for chemical analysis (Raiffeisen-Laborservice, Ormont, Germany). Water parameters for the type locality (Lake Reilingen, Germany) were taken from published data (Tönges et al. 2021b). Total length (rostrum to uropods) was measured using digital Vernier calipers (Mitutoyo 500-196-30 AOS). Data was plotted in histograms using GraphPad Prism 9.

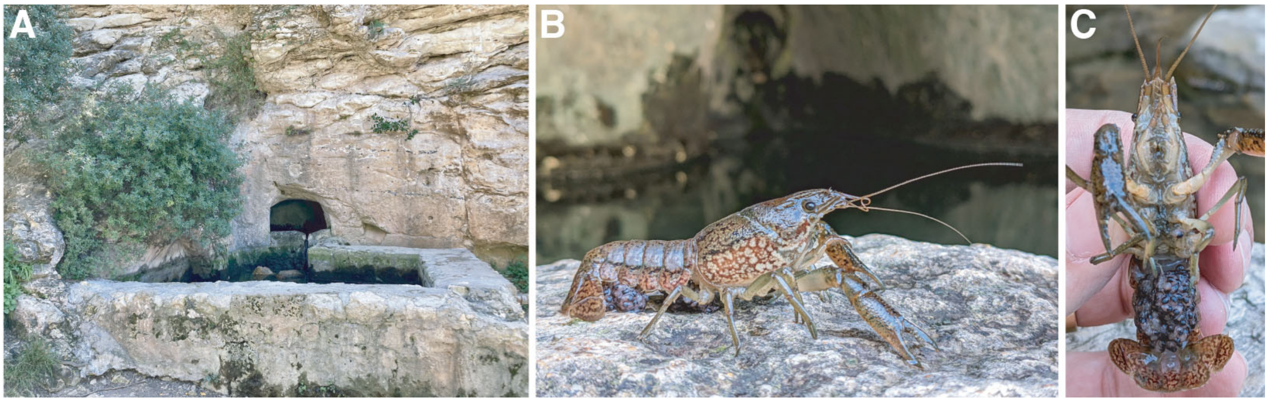


**Figure 1.** Location of a newly described marbled crayfish population at Ein Meshotetim. (A) The collection site Ein Meshotetim. (B) *Procambarus virginalis* berried specimen, collected at the spring (C) Ventral view of same specimen, presenting juveniles attached to its pleopods. Photos by F. Lyko (A) and V. Coutinho Carneiro (B, C).

*PCR genotyping*

Genomic DNA was isolated and purified from abdominal muscle tissue using a Tissue Ruptor (Qiagen), followed by proteinase K digestion and isopropanol precipitation. The quality of isolated genomic DNA was assessed via agarose gel electrophoresis and/or TapeStation (Agilent). PCR genotyping of 3 specimens was performed using the following primer pairs (Gutekunst et al. 2021): Cytb (FWD CAGGACGTGCTCCGATTCATG and REV GACCCAGATAACTTCATCCCAG), Dnmt1 (FWD GCTTTCT GGTCTCGTATGGTG and REV CTGCACACAGCCTAAGATGC), Cox1 (FWD CTGCTATTGCTCATGCAGGT and REV TGCCCGAGTATCTA CATCCA). Amplicons were verified by agarose gel electrophoresis and Sanger sequencing. Sequencing results were analysed with SnapGene v5.2.5 software. GenBank accession numbers: GCA_020271785.1 (complete *P. virginalis* genome sequence) (Gutekunst et al. 2021) and the geneIDs for CytB – WNGS01164437, DNMT1 – GenBank KM453737.1 , and Cox1 – WNGS0 1066681, can be used to retrieve the nucleotide sequences.

## Results

One hundred forty-seven crayfish specimens were collected in October 2022 at the Ein Meshotetim pool of Wadi Siah in Haifa, Israel (Figure 1A). Closer inspection revealed 17 gravid specimens and 5 with juveniles attached to their abdominal pleopods (Figure 1B and C), suggesting active reproduction of the population. Chemical analysis of a water sample revealed a slightly basic pH (7.6) and a high mineral content (Table 1), which is generally considered as supportive for crayfish survival.

Visual inspection of the collected specimens suggested that they were marbled crayfish (Figure 1B). Total lengths of the collected animals ranged between 17 and 79 mm (Figure 2), consistent with results from other known populations (Andriantsoa et al. 2019; Tönges et al. 2021a). For genetic authentication, PCR amplicons were sequenced and aligned with the partial

**Table 1.** Main water parameters of the collection site.

| Parameter | Measurement |
|---|---|
| pH | 7.6 |
| Conductivity (mS/cm) | 1194 |
| Hardness (°dH) | 24.2 |
| Nitrate (mg/l) | 27.3 |
| Chloride (mg/l) | 171.8 |
| Calcium (mg/l) | 73.3 |
| Potassium (mg/ml) | 1.3 |
| Magnesium (mg/l) | 60.6 |
| Sodium (mg/l) | 88.0 |

The Table lists all dissolved solids that were detected at a concentration of > 1 mg/l.

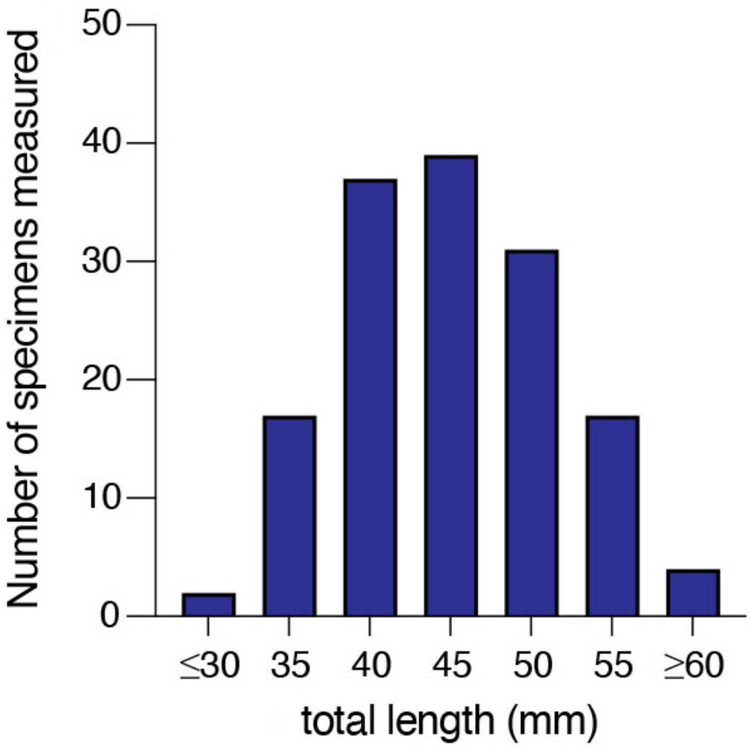


**Figure 2.** Total length distribution of the 147 marbled crayfish collected at Ein Meshotetim. Total lengths were determined for individual specimens and then sorted into bins, as indicated.

**Table 2.** Genetic analysis of collected specimens

| Specimen | CytB | Cox1 | Dnmt1 |
|---|---|---|---|
| #1 | 274/274 | 553/553 | 334/334 |
| #2 | 274/274 | 553/553 | 334/334 |
| #3 | 274/274 | 553/553 | 334/334 |

Numbers represent the numbers of sequenced bases that are identical with the reference sequence of *P. virginalis*, and the number of bases from the reference sequence of *P. virginalis*.

sequences of two mitochondrial genes (cytochrome B: 274 bp and Cox1: 553 bp) and one nuclear gene (DNMT1: 334 bp) from the marbled crayfish reference genome sequence. The results showed 100% sequence identity with marbled crayfish reference genome for all three analyzed specimens (Table 2), thus confirming their taxonomic classification.

## Discussion

The marbled crayfish is an invasive species with a high level of adaptivity, capable of colonizing a wide range of habitats. This process is driven by a combination of unusual factors: asexual reproduction allows the formation of a population from a single animal (Chucholl et al. 2012). At the same time, inbreeding-like deleterious effects are mitigated by the considerable genetic heterogeneity of the marbled crayfish genome (Gutekunst et al. 2018). Further fitness advantages may stem from epigenetic differences between marbled crayfish populations (Carneiro and Lyko 2020; Tönges et al. 2021b), which may function to rapidly adapt them to newly invaded habitats. We describe and genetically validate the first population of marbled crayfish in the Levant and thus provide important further evidence for the increasing distribution of this species. Additionally, we document adult specimens bearing eggs and juveniles, indicating active reproduction, a requirement for successful colonization of the new environment. As there are no known natural sources of marbled crayfish in the Levant, our results strongly suggest that this population was founded by local people, probably through the release of animals that were originally acquired as aquarium pets. This is consistent with the known role of anthropogenic releases in the formation of marbled crayfish colonies (Chucholl et al. 2012).

Aggressive water management policies have greatly diminished Israel's natural freshwater bodies, particularly ephemeral or low-flowing springs (Tal and Katz 2012). These collapsing aquatic ecosystems face an increasing number of introduced plants, invertebrates and fish species (Gasith et al. 2011; Golani 2000; Goren and Ortal 1999; Roll et al. 2007; Roll et al. 2009; Yanai et al. 2017). Crayfish are the largest invasive alien invertebrates recorded in Israel's inland waters. A large-sized individual of the Australian redclaw crayfish, *Cherax quadricarinatus* (von Martens, 1868), was captured in the Sea of Galilee (Lake Tiberias) in 2011. The species had been introduced to Israel from the USA in the early 1990s by the Department of Fisheries, Ministry of Agriculture, with no assessment of its potential to establish feral populations. It has since been raised in agricultural research, in academic facilities and in commercial farms (Snovsky and Galil 2011). More recently, specimens were also imported directly from Australia (Davidovich et al. 2022). While *C. quadricarinatus* failed to establish known breeding populations in Israel, *P. clarkii* has done so. The first record of this notoriously invasive species 'in the wild' was based on individuals, including gravid females and juveniles, collected in groundwater-filled pits in an abandoned quarry on the central coastal plain in 2008 (Wizen et al. 2008). Since 2015 a large population is known to inhabit the National Park at the headwaters of the Yarkon river. More recently the species was also recorded in additional locations, as countermeasures were initiated only fifteen years after the first *P. clarkii* population was reported.

It will be important to monitor Israeli waterbodies for a potential spread of marbled crayfish. However, it has also been argued that the impact of invasive species is often assessed with an unjustified focus on negative effects (Boltovskoy et al. 2022) and the ecological impact of marbled crayfish remains to be understood. In Madagascar, marbled crayfish have spread over more than 100,000 km² within a decade (Gutekunst et al. 2018). This process has largely been driven by anthropogenic propagation, as the species has become a significant source of protein that is widely consumed and represents an important product in the local economy (Andriantsoa et al. 2020; Andriantsoa et al. 2019). While marbled crayfish aquaculture in open systems should be prohibited due to its significant ecological risks, closed-system approaches could exploit the inherent robustness and effectivity of the animals, particularly in arid regions (Tönges et al. 2021a).

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## Authors' contribution

Conceptualization, BG, FL; investigation and data collection, VCC, BG, FL; data analysis and interpretation, VCC, BG, FL; manuscript writing, review and editing, VCC, BG, FL

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