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A NEW THREAT TO GROUNDWATER ECOSYSTEMS: FIRST OCCURRENCES OF THE INVASIVE CRAYFISH *PROCAMBARUS CLARKII* (GIRARD, 1852) IN EUROPEAN CAVES

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Abstract: The American red swamp crayfish, *Procambarus clarkii*, is today the alien species most widespread in European water bodies. This invasive crayfish was found for the first time in some caves of Europe, specifically in Portugal and Italy. The presence of *P. clarkii* in caves is noteworthy, representing a new threat for the groundwater ecosystems due to the possible negative impacts on the native communities.

Introduction

The subterranean environment hosts unique biological communities of remarkable diversity that, however, are still understudied in large parts of Europe (Gibert and Culver, 2009). Groundwater biodiversity has attracted much attention in recent years for two important aspects: the distinctiveness of its fauna, whose composition is highly different from that of surface freshwater fauna, and its narrow endemism (Deharveng et al., 2009). This particular habitat is vulnerable to local extinctions caused by anthropogenic impacts such as groundwater pollution, water abstraction, and habitat deterioration (Danielopol et al., 2003). Biological invasions, one of the most significant components of human-induced environmental change, could also imperil this environment (Lodge et al., 2000; Sala et al., 2000). Specifically, invasive species exert particularly heavy impacts in inland waters (Sala et al., 2000), where they threaten biodiversity, leading to extinction of indigenous species (Lodge, 2001; Gherardi, 2007) and altering the structure and function of ecosystems (Mack and D'Antonio, 1998; Strayer, 1999). Invasive species colonizing the subterranean environment by way of rivers have already been reported in Europe, e.g., the Asiatic clam *Corbicula fulminea* (Müller, 1774) in France (Callot-Girardi et al., 2012) and the New Zealand mudsnail *Potamopyrgus antipodarum* (Gray, 1843) in Italy (Bodon et al., 2009).

One of the most successful and best known invasive species of aquatic ecosystems in Europe is the North American red swamp crayfish *Procambarus clarkii* (Girard, 1852), now widely distributed in the world due to its biological features such as plastic life cycle, high fecundity, ability to rapidly disperse in the habitat, and tolerance to environmental extremes (Gherardi, 2006). This successful invader, that can cause a large variety of impacts (see Savini et al., 2010), was first introduced in Portugal in the 1970s and then in Italy in the 1980s, and it has now colonized most of the surface freshwater systems of both countries (Correia, 2003; Aquiloni et al., 2010). Here we report the first occurrences of this invasive crayfish in some cave waters of Portugal and Italy and briefly discuss the implications of this discovery for this unique and complex aquatic environment.

Materials and Methods

Procambarus clarkii was collected in July 2007 in the Sicó massif (Anços spring: 39°58′42.41″N 8°34′22.67″W) and observed since August 2007 in the Estremenho massif (Olhos de Água do Alviela: 39°26′44.25″N 8°42′43.63″W), both caves being located in central Portugal. It was also collected in September 2011, October 2012, and February 2013 in pools inside of the adjoining Grotta del Leone, Grotta del Lago, Buca della croce di Agnano n. 2, Buca dell'acqua presso la Buca di Agnano, and Buca dei Ladri in the Monti Pisani area: 43°44′17.7″N 10°28′24.8″E, municipality of San Giuliano Terme, Pisa Province, Tuscany Region, Central Italy (Fig. 1). The Portuguese caves are located in two karst massifs and are up to 110-m deep and 700-m long. In Italy, Monte Pisano includes a group of hills, rich in karst caves and quarries, extending from northwest to southeast between the Lucca and Pisa plains in northwestern Tuscany.

In Portugal, specimens were collected by active search with dip-nets, while in Italy also by baited traps left

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Figure 1. Approximate locations of sampled caves with *Procambarus clarkii* in Europe.

overnight. In Italy, sampling also was conducted in the nearby epigean channel by both methods. For each crayfish, sex was determined and the length of the cephalothorax (CL), from the tip of the rostrum to the posterior edge of the carapace, was measured using a caliper with 0.1 mm precision. The number of individuals partially or totally depigmented was recorded.

The associated aquatic fauna was also sampled and recorded using dip-nets and small baited traps. Portuguese material is deposited at the Museu Nacional de História Natural, Lisbon, and the Italian samples at the Department of Biology, Florence.

During the surveys, the following water parameters were recorded with a multiparameter sonde: temperature, pH, dissolved oxygen, conductivity, and NH_4^+. Frequency data were analyzed by G test with Williams correction (statistic G). Differences in CL between the sexes were compared by t-test for independent samples (statistic t).

Results

Since 2007, six specimens of *Procambarus clarkii* have been observed in the groundwater of the Anços and Alviela springs in Portugal. Both caves are active resurgences, although the Rio Anços may dry up in the peak of summer. The individuals found inside the caves are totally depigmented, and one male was collected at 40-m depth in the Anços spring (CL=33.1 mm; Fig. 2). Pigmented adults of the species are abundant (two individuals per meter) in the corresponding surface streams.

In Italy, a total of fifteen specimens of *P. clarkii* were collected from Grotta del Leone (one female: CL = 34 mm), Grotta del Lago (two males: CL = 33.9± 0.05 mm and one female: CL = 23.1 mm), Buca della croce di Agnano n. 2 (one male: CL = 26.4 mm and three females: CL = 24±5.5 mm), Buca dell'acqua presso la Buca di Agnano (five males: CL = 23.5±2.1 mm and one female: CL = 33.7 mm) and Buca dei Ladri (one male: CL =3 8.8 mm). About 53% of the crayfish had pereopods and cephalothorax bluish-whitish in color. The pools inside the caves were also inhabited by several species of Crustacea, the most abundant invertebrates in groundwater (Botoşăneanu, 1986), such as many species of stygobiotic copepods of the genera *Acanthocyclops* and *Diacyclops* and several specimens of amphipods belonging to *Salentinella angelieri pisana* Ruffo, 1953 (Salentinellidae, endemic to Monti Pisani karstic massif), *Niphargus* sp. group *speziae* Schellenberg, 1936, and *Niphargus* sp. prope *stefanellii* Ruffo and Vigna-Taglianti, 1968. Both Nipharg-idae are probably species new to science, according to F. Stoch and J.F. Flot. In Grotta del Lago, we also found three adult specimens of the European eel *Anguilla anguilla* (Linnaeus, 1758).

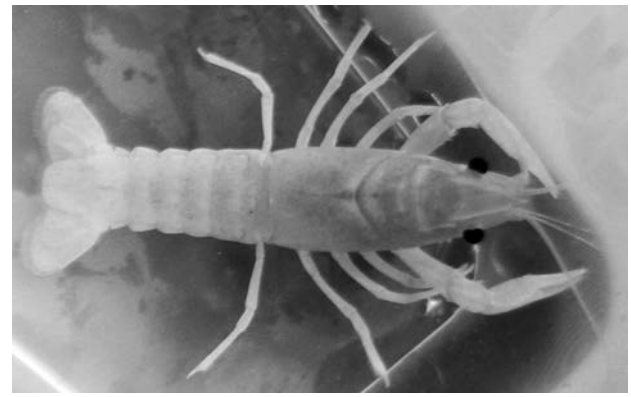

Figure 2. Depigmented specimen of *Procambarus clarkii* found in Anços spring, Portugal.

In the nearby epigean channel we collected fifty-six individuals of *P. clarkii* with a balanced sex-ratio (males = 32; females = 24; G = 1.14, df = 1, $p > 0.1$) and a similar mean size (CL males = 35.7±2.2 mm; CL females = 39.1±2.4 mm; t = 1.03, df = 54, $p = 0.31$). All the crayfish were pigmented. The invasive mosquitofish *Gambusia holbrooki* Girard, 1859, the crucian carp *Carassius carassius* (Linnaeus, 1758) and the black bullhead *Ameiurus melas* (Rafinesque, 1820) were also abundant in this channel. Concerning the water parameters, the Portuguese and Italian caves had similar temperatures (nearly constant during the year), pH, conductivity, and NH_4^+. Only the epigean channel showed a wide range of temperatures (Table 1).

Discussion

To the best of our knowledge, these are the first documented occurrences of the invasive crayfish *Procambarus clarkii* in European caves. In Europe, crayfish occasionally found in subterranean habitats have been almost unknown or rarely reported, and only identified native species, such as *Austropotamobius torrentium* (Schrank, 1803) (Koutrakis et al., 2005). The only large

Table 1. Waterparameter ranges recorded in the Portuguese and Italian study areas.

Sites	Water Temperature, °C	pH	Oxygen, mg L^{-1}	Conductivity, µS cm^{-1}	NH_4^+, mg L^{-1}
Anços	15.8–16.4	7	8.5–9.5	497–540	0
Alviela	15.6–17.5	7	7.6	443–508	0
Epigean channel	11–31	6	5.4	482–500	0.4
Grotta del Leone	16–17	6	4.3	524–540	0
Grotta del Lago	14.5–17.7	6	4.3	560–585	0
Buca della croce di Agnano n. 2	12–19	7	1.4–2.5	695–730	0.6
Buca dell'acqua presso la Buca di Agnano	15–23	6.5	3.9	503–515	0
Buca dei Ladri	14.5–18	6.5	5	498–516	0

stygobiotic decapod in Europe is represented by the blind prawn genus *Typhlocaris* Calman, 1909, present in Italy (Apulia) with the species *T. salentina* Caroli, 1923. The family Cambaridae is represented in the troglobiotic American fauna with four genera, three of which have epigean members as well (*Procambarus* Ortmann, 1905, *Orconectes* Cope, 1872, and *Cambarus* Erichson, 1846: Hobbs et al. 1977; Hobbs, 1988). For example, in Florida, thirteen of the fifty-two known species and subspecies are troglobites, while in southern Indiana six species or subspecies inhabit the subterranean caves in the karst region (Hobbs, 1988; Hogger, 1988). There are also cases among the Cambaridae of stigoxenic (largely confined to surface water) and stygophilic (able to spend part of their life in ground waters but without or with limited specialization to subterranean life) species. Specifically, *P. clarkii* has been found in caves of Texas (Hobbs et al., 1977), probably due to the displacement of populations living in epigean environments. In Portugal, the individuals found in ground waters were totally depigmented, while in Italy pigmentation of cave-dwelling individuals of *P. clarki* occurs. The presence of mostly pigmented crayfish inside the Italian caves could indicate a more recent invasion; an external source of carotenoids, which can occur in caves providing a source of food for crayfish as mentioned in Hogger (1988) and in Koutrakis et al. (2005), even if it is probably not abundant, as evidenced by the presence of bluish individuals; or movement from the epigean channel to the caves and vice versa, due to the locomotory activity of crayfish. The individuals found in Monti Pisani area can be undoubtedly classified as stygoxenic, while specimens from Portugal show a certain degree of stygobization. In both areas, the caves showed similar water conditions favorable for the species; only the oxygen differed substantially among sites, but *P. clarkii* can tolerate a wide range of oxygen content, being capable of air breathing (Huner, 2002).

The presence of *Procambarus clarkii* in caves is noteworthy, due to the possible negative impacts on native communities. This species is recognized as one of the most invasive crayfish in Europe (Tricarico et al., 2010) and, thanks to its plasticity, it can prey on many endemic cave species, since it feeds on diverse items in proportion to their availability. The European groundwater fauna comprises more than 1,800 stygobitic species (Stoch and Galassi, 2010), most of them endemic to restricted areas or single karstic massifs in southern countries. At least sixty-seven groundwater-dwelling species are endemic to Portugal (Reboleira et al., 2011, 2013), and *P. clarkii* could be an important threat to some of these species, such as asellid isopods like *Proasellus lusitanicus* (Frade, 1938), as well as amphipods belonging to the genus *Pseudoniphargus* Chevreux, 1901. Groundwater biodiversity is even greater in Italy, with more than 320 stygobiotic species reported in recent catalogues (Ruffo and Stoch, 2006, and Fauna Europaea Web Service, 2013), around 300 of them being endemic. In the karstic area where it was found, *P. clarkii* can feed on the amphipods of the genus *Niphargus* (two species yet to be described) and *Salentinella angelieri pisana*, all endemics to the restricted area of Monti Pisani. However, in Italy, the eels in the pools could control the population of *P. clarkii*; indeed *Anguilla anguilla* actively preys on crayfish juveniles (Aquiloni et al., 2010).

Further studies are necessary to investigate more deeply the status of this species in caves and to assess how the species arrived and the possible impacts on this particular habitat.

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