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The allochthonous crayfish *Procambarus clarkii* (Girard, 1852) (Crustacea Cambaridae) from the subterranean stream of the Ausi cave (Latium, Italy): the second documented case of cave invasion

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ABSTRACT In this paper, data on the second case in Europe of hypogean environment invasion by the crayfish *Procambarus clarkii* (Girard, 1852) (Crustacea Cambaridae) are reported. Sixteen specimens were collected and measured during spring and late summer 2017 and other numerous specimens were observed from the entrance until 500 meters inside the cave. The presence of at least two shelter tunnels dug on the clayey stream bank suggests a stable colonization of the cave. Many individuals collected were characterized by bluish chaele indicating sexual activity. The omnivorous diet of this crayfish, its resistance to adverse environmental conditions, its high reproduction rate and dispersal capability allow us to predict that this species could have a strong and negative impact on the aquatic and terrestrial cave communities.

KEY WORDS Cambaridae; *Procambarus*; groundwater; colonisation; cave habitat.

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INTRODUCTION

Cave habitats are characterized by unique biological communities of remarkable diversity. Hypogean environments are still scarcely known, but the studies on this fields are numerous and ongoing in many parts of the world. In particular, groundwater biodiversity has attracted the attention of many researchers in recent years, mainly for two aspects: the peculiarity of its fauna, whose composition is different compared to the surface freshwater fauna, and its high level of endemisms (Deharveng et al., 2009; Gibert & Culver, 2009). The species characterizing these particular habitats are vulnerable to local extinctions caused by human impacts, such as groundwater pollution, water extraction and habitat deterioration (Danielopol et al., 2003). Furthermore, in the last decades the increase in allochthonous species invasions in Europe represents a new risk for the delicate equilibrium of the cave ecosystem. In fact, the presence of alien species strongly affects the endemic ones, causing the reduction in their population size and, in some case, local extinctions. The effects of the alien species on endogenous species are particularly magnified in the fragile cave environments, even if only few data on

cave invasion by alien species are available in literature, e.g. Asiatic clam *Corbicula fulminea* (Müller, 1774) in France (Callot-Girardi et al., 2012), the New Zealand mudsnail *Potamopyrgus antipodarum* (Gray, 1843), the American spider *Psilochorus simoni* (Berland, 1911) and the Japanese diplopod *Oxidus gracilis* (Koch, 1847) in Italy (Zapparoli, 2008; Bodon et al., 2009; Sparacio et al., 2017).

One of the most successful and best known invasive species of aquatic ecosystems in Europe is the North American red swamp crayfish *P. clarkii* (Girard, 1852), nowadays 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 a wide range of environmental conditions (Gherardi, 2006). In Europe, this successful invader, was first introduced in Spain in the 1970s and then in the north of Italy in the 1980s, and now it has colonized most of the surface freshwater systems from the north to the central Italy (Aquiloni et al., 2010). The first occurrence of crayfish *P. clarkii* in hypogean water bodies in Europe (Portugal and Italy) is reported by Mazza et al. (2014).

In this paper, we report the second case of a recent invasion of a subterranean environment in Italy by *P. clarkii* (Fig. 1). The possible implications of the cave colonization is briefly discussed. Although presently there are no evidences of direct impact on the local subterranean aquatic environment, the massive occurrence of this crayfish in the cave is presented and discussed in relation to its potential role in the deterioration of the subterranean aquatic and terrestrial community.

MATERIAL AND METHODS

Our study has been carried out in the Ausi cave (cadaster number 342La, 41°30’33” N 13°16’27”E) since summer 2016, as a part of a more extensive faunistic investigation of the the Ausoni Mountains caves. The cave, located at 55 meters above sea level in Fornaro hill locality near Prossedi (Latina, Latium, Central Italy) has been declared Site of Community Importance (code n. IT6040001) under the European Commission Habitats Directive (92/43/EEC) since 2016, for the occurrence of seven bat species which use this cave as a site of reproduction and hibernation (Bollettino Ufficiale della Regione Lazio, 2016).

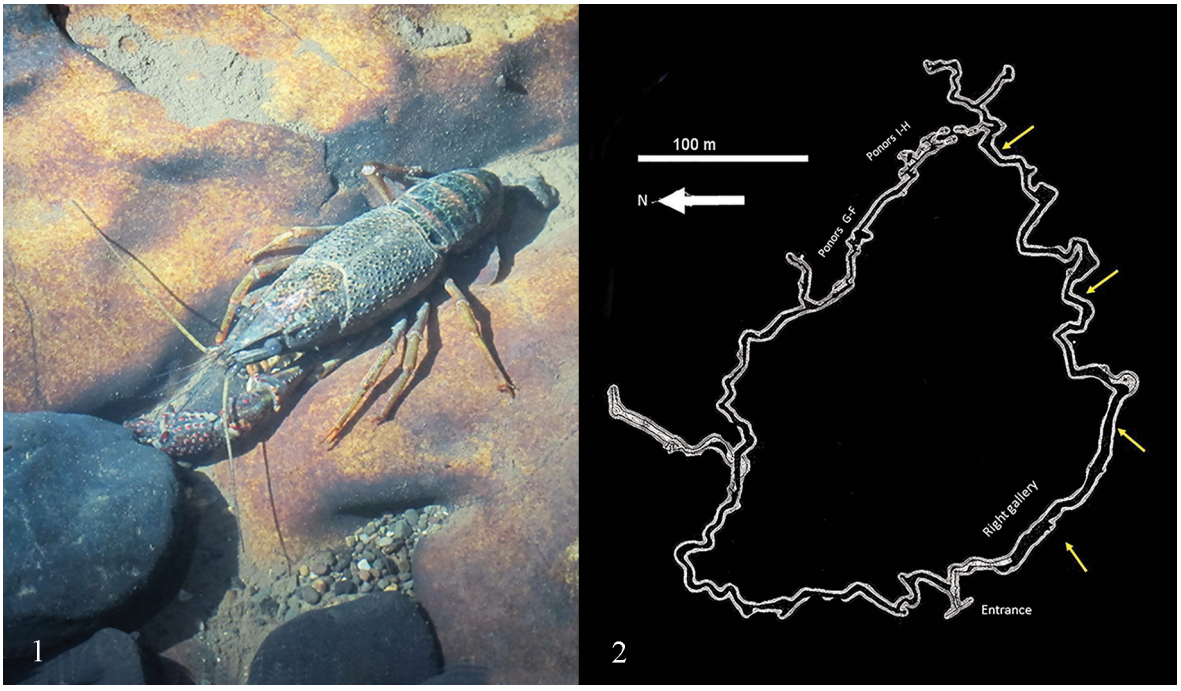

Figure 1. Adult specimen of *Procambarus clarkii* in the Ausi cave (Latium, Italy).
Figure 2. Map of the Ausi cave (Latium, Italy). Yellow arrows indicate the sites of crayfishes samplings.

The cave is 1,505 meters long and it is composed by two galleries of about equal length, starting few meters from the big entrance and functioning as a resurgence connected with the near Amaseno river. The right gallery, 530 meters long, is crossed by a perennial stream characterized by a variable flow depending on the season. In summer, the water flow is often very low and in the first 300 meters of the cave the stream is reduced to a series of pools of different deepness. The left gallery, which is approximately 510 meters, shows only a sporadic water flow while it is permanently occupied by ponds deep even a meter along its entire length. The stream goes into the cave at the points I, H and G-F, as shown in the cave map (Fig. 2). These points correspond to the upper entrances of the subterranean system, draining most of the surface water of the plateaux above the cave that extends until Villa Santo Stefano village (Mecchia et al., 2003).

Specimens of *P. clarkii* were actively captured using a fishery net only in the right gallery of the cave. Each specimen was measured according to three morphological parameters: TL=total length, CL=carapax length and ChL= chaele length, using a digital calliper (accuracy 0.1 mm) and weighed with a portable digital balance (accuracy 0.1 g). Water temperature was recorded using a digital thermometre (accuracy 0.1 °C). For the identification of the species was used Mazzoni et al. (2004), the sex of mature individuals was determined by the occurrence of modified pleiopods in males and of the annulus ventralis in females. The species composition of the aquatic zoocenose was evaluated using Latella (1995) and data from our three recent samplings (16 July 2016, 10 November 2016 and 03 June 2017). A survey on the Amaseno river and on the little streams connected with the superior entrance was conducted on 1st November 2017 in order to verify the occurrence of *P. clarkii* in the epigean habitat surrounding the cave.

RESULTS

In the first two sampling dates no specimens of *P. clarkii* were observed and only from April 2017 numerous individuals of the crayfish started to be found in the cave. In Table 1, sampling dates, occurrence of *P. clarkii* and water temperature are re-

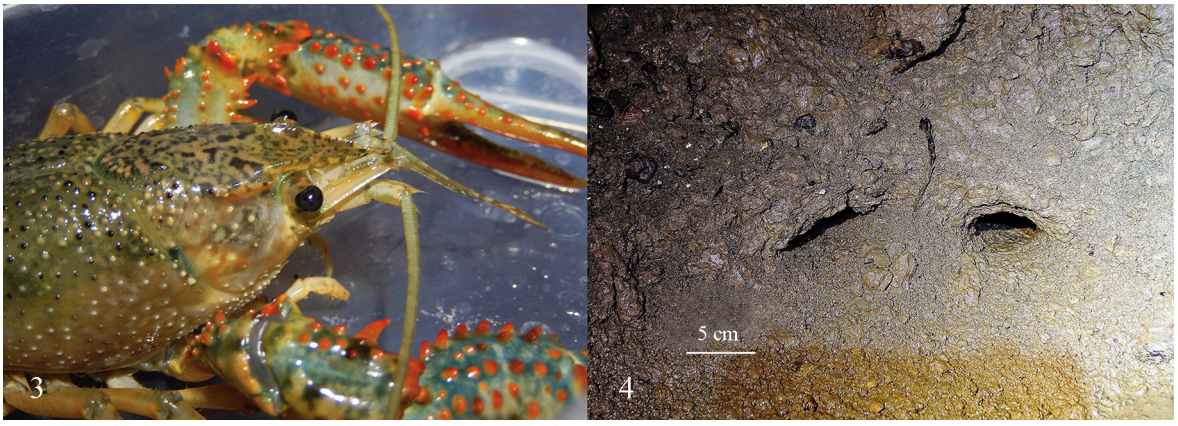

Figure 3. *Procambarus clarkii* showing the bluish color of the chaele indicating sexual activity.
Figure 4. *Procambarus clarkii* shelter tunnels in the Ausi cave (Latium, Italy).

Date	Specimens observed	Specimens collected	T °C
8.IV.2017	15	--------	/
14.IV.2017	20	5 (2M,1F and 2 juveniles)	13.4
17.IX.2017	30	11 (6M and 5F)	15

Table 1. Specimens observed and collected and temperature recorded in each sampling date.

ported. The survey conducted in the epigean habitats confirmed the occurrence of this allochthonous crayfish both in the Amaseno river and in the little streams draining water on the calcareous plateaux above the cave.

The crayfish were observed along all the right underground stream, about 30 meters from the entrance up to 500 meters inside the cave, mainly in the deep pools formed by the river (Fig. 2).

In total, 16 individuals were collected, five on 14th April 2017 and eleven on 17th September 2017. In males, TL ranged between 8.4 and 11.2 cm, CL between 6 and 4.5 cm, ChL between 6 and 2.7 cm and the weight between 17 and 44 g. In females, TL ranged between 9.3 and 11.4, CL between 4.8 and 5.8 cm, ChL between 1.9 and 4.0 cm and the weight between 30 and 40 g.

All the captured specimens, both adults and immatures were almost pigmented. Some males individuals captured on 17th September 2017 showed the typical bluish chelae colouration that indicates sexual activity, as reported in De Luise (2010) (Fig. 3). In the same sampling date, two shelter tunnels were observed on the bank of the underground stream about 300 meters inside the cave. The tunnels were dug in the clayey river bank, few centimeters above the water level (Fig. 4). For an evaluation of the possible impact on subterranean ecosystem, the aquatic biocenose composition is reported. On the whole, at least 21 taxa were identified (Table 2). Arthropods are the main group represented in the water bodies with the Crustaceans Amphipods *Niphargus longicaudatus* (A. Costa, 1851) and *Gammarus elvirae* Iannilli et Ruffo, 2002, the Isopod *Proasellus coxalis* (Dollfus, 1892) and Copepods *Bryocamptus* sp. and *Canthocamptus* sp.

Among Vertebrata the amphibians *Bufo bufo* (Linnaeus, 1758) and *Rana italica* Dubois,1987 occur in the cave and use the stream water to lay eggs. Finally fishes as *Squallius* sp. and *Alburnus* sp. were captured in the deep pools also 500 meters inside the cave while *Anguilla anguilla* (Linnaeus, 1758), reported in literature (Latella, 1995), was not observed during our study.

DISCUSSION

Out of the 15 families of decapods (excluding crabs) inhabiting subterranean water of the world, two shrimps and one crayfish family include the vaste majority of species (Hobbs III, 1998). In particular, the family Atyidae is represented by 49 stygobitic species and the family Cambaridae is depicted by 40 stygobitic and 35 stigophilic and stygoxenic species. Cambaridae family is a typical representant of 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). In particular, *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 Europe, Atyidae are represented by at least 5 species of the genus *Troglocaris* Dormitzer, 1853, occuring in caves from Balkans and Transcaucasia (Franjevic et al. 2010), while Palaemonidae by the blind prawn genus *Typhlocaris* Calman, 1909, present in Southern Italy (Apulia) with the species *T. salentina* (Caroli, 1923) (Hobbs III, 1998). On the other hand, crayfish are occasionally found in European subterranean habitats and they have been almost unknown or rarely reported. Only identified native species, such as the Astacidae *Austropotamobius torrentium* (Schrank, 1803) collected in the underground river from Divaca and Rakek in Slovenia and from Alistrati cave in Macedonia is so far reported (Hobbs III, 1998; Koutrakis et al., 2005).

Regarding the studied area, the first record of *P. clarkii* in the water bodies of the province of Latina, where the Ausi cave is located, dates back to 2002 (Gelosi & Colombari, 2004; Monaco, 2014).

In this preliminary study we can confirm the presence of the crayfish *P. clarkii* in the subterranean stream of the Ausi cave. This occurrence, in spite of our repeated samplings conducted in 2016, can be considered very recent, starting not until spring 2017.

Two ways of cave invasion can be supposed, the first throughout the big resurgence at the bottom of the cave connected with the Amaseno river. The second throughout the upper entrances placed on the overlying calcareous plateaux. Both these ways are subjected to periodical floods due to intense rainfalls as occurred during the winter and the spring 2017. In fact in this period the rainfall repertoire documents a total of 9 days with rainfall exceeding 15 mm for Prossedi locality (www. 3Bmeteo.com).

All the captured specimens show morphometric parameters according to the size variation typical of the species as indicated in Mazzoni et al. (2004).

Taxa	Latella (1995)	Our collections
ANELLIDA		
LUMBRICIDAE indet.		*
HIRUDINEA indet.		*
GASTROPODA		
Ancylus sp.		*
Bythynia sp.		*
CRUSTACEA		
COPEPODA		
Bryocamptus sp.	*	
Canthocamptus sp.	*	
AMPHIPODA		
Niphargus longicaudatus	*	*
Gammarus elvirae		*
ISOPODA		
Proasellus coxalis	*	*
INSECTA		
ODONATA		
Calopterix sp.	*	*
EPHEMEROPTERA		
Habrophlebia sp.	*	*
Coenis sp.	*	
Baetis sp.	*	*
TRICOPTERA		
Hydroptila sp.	*	
Rhyacophyla rougemonti	*	
VERTEBRATA		
PISCES		
CIPRINIDAE		
Squallius sp.		*
Alburnus sp.		*
ANGUILLIDAE		
Anguilla anguilla	*	
AMPHIBIA		
BUFONIDAE		
Bufo bufo		*
RANIDAE		
Rana italica		*

Table 2. List of aquatic species from Ausi underground stream. Data are obtainded from Latella (1995) and our collections (2016–2017).

Moreover, as expected in the case of recent cave colonization, no observed or collected individuals resulted depigmented. The presence of two shelter tunnels dugged on the river banks, can suggest a stable colonization of the underground river while the occurrence of males showing bluish pigmentation of the chelae could indicate sexual activity in the cave. Our observations seem to indicate a good tolerance of *P. clarkii* to relatively low temperature of the cave water that in all the sampling dates ranged between 13.4 and 15.0 °C. This range of temperature is close to the limit of 12.0°C beneath which the species stops its growth (Ackerfors, 1999). The lack of direct data on the diet doesn't allow us to extimate the impact of the local population of *P. clarkii* on the aquatic biocenose of the Ausi cave. However, as discussed by Mazza et al. (2014), the potential strong disturbing activity on the native aquatic community is highly predictable. In fact, although adults of *P. clarkii* are usually considered plant eater, young individuals are active predators of invertebrates (De Luise, 2010). This feeding behaviour, in absence of natural competitors and predators like carnivore fishes, birds and mammals, could result in a magnification of the negative impact on the aquatic biocenose, composed in the cave by endemic species, often limited to a single subterranean habitat and reprensented by small populations (Culver, 1982; Culver & Pipan, 2009). In the case of Ausi, the invasion of *P. clarkii* could represent a new threat for the Amphipods *Niphargus longicaudatus* and *Gammarus elvirae*, the first widespread mostly in southern-central Italy and in the Tyrrhenian islands (Karaman, 1993) and the second ones widespread in the central part of the Apennines, between Marche and Campania and recently collected in Capodacqua, the main spring of Amaseno river (Iannilli & Ruffo, 2002).

Finally, being *P. clarkii* capable to stay for a long time outside the water, as reported by many authors (see Barbaresi & Gherardi, 2000), we can strongly suppose its impact also on the terrestrial cave community.

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