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SHORT RESEARCH NOTE

The new threat to Italian inland waters from the alien crayfish "gang": the Australian *Cherax destructor* Clark, 1936

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Abstract Biological invasions inflict damage to the ecology, economy, and human health, and pose serious threats to the native communities. Among the many invasive taxa, crayfish have attracted much attention by scientists and policy makers. Recently, an established population of an alien species of crayfish, new for Italy, was found in the Natural Preserve of "Laghi di Ninfa" (central Italy). Based on morphological and genetic evidence (molecular barcoding of *COI* and *16S rDNA*), we classified it at subspecies level as the Parastacidae *Cherax destructor destructor*, native to Australia. Its introduction possibly occurred at the end of the 1980s but the species seems to be still confined in the preserve. The low temperature of the adjacent waters may be a barrier against its natural spreading but not against its intentional translocation into natural waters by man. Because of the invasive history of *C. destructor*, eradication of this population is urgent and still economically profitable.

Keywords Biological invasions · Alien crayfish species · Parastacidae · Barcoding · *COI* mtDNA · Italy

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The introduction of alien species is considered today as one of the most serious threats to biodiversity (Mack et al., 2000). This is particularly true for freshwater ecosystems due to their higher vulnerability to biological invasions than terrestrial biomes (Ricciardi & Rasmussen, 1999; Sala et al., 2000; Rodríguez et al., 2005; Gherardi, 2007a).

The invasions by alien crayfish have attracted much attention by scientists and policy makers worldwide (Horwitz, 1990; Gherardi & Holdich, 1999; Lodge et al., 2000). Today, most European countries have at least one alien crayfish species (Souty-Grosset et al., 2006); all of the introduced species pose considerable threats to native species, as a consequence of their tolerance to extreme environmental conditions, their rapid spread and diffusion, and, in the case of the North American species, their capability to transmit the crayfish plague to the

European crayfish (Souty-Grosset et al., 2006; Gherardi, 2007b). Among the other European countries, Italy seems to be of particular concern since the introduced species quickly establish self-sustaining populations and rapidly spread becoming invasive (Gherardi et al., 2008a). Populations of *Astacus leptodactylus* Eschscholtz, 1823, *Orconectes limosus* (Rafinesque, 1817), *Pacifastacus leniusculus* (Dana, 1852), and, mostly, *Procambarus clarkii* (Girard, 1852) inhabit many Italian basins (Gherardi et al., 2008b).

In September 2008, for the first time in Italy, we recorded an established wild population of an alien crayfish that we first morphologically assigned to the Australian Parastacidae species *Cherax destructor* (Electronic supplementary material). A dense population constituted by 0.28 females/m^2 and 0.24 males/m^2 (Scalici et al., in preparation) was found in five abandoned cultivation ponds (40 × 6 m, depth: about 0.6 m, with muddy bottom covered by submerged macrophytes) in the Natural Preserve of "Laghi di Ninfa" (Province of Latina, central Italy) (Electronic supplementary material). Crayfish were collected by hand-nets and traps for a morphological identification and 30 individuals were transported to the laboratory for genetic analyses.

The genus *Cherax* is the most widespread and diverse of the nine crayfish genera native to Australia. It is one of the principal cultured freshwater crayfish in Australia, contributing 73% of the total freshwater crayfish production between 1996 and 2000 (Piper, 2000). It is widely stocked into artificial waterbodies by farmers and commonly used as bait by recreational fishers (Nguyen, 2005). Thus, *C. destructor* is highly vulnerable to translocation, and consequently feral or cultured stocks of this species have become established outside its natural range in Tasmania (Elvey et al., 1996) and Western Australia. Three centers of this genus' diversity have been identified: the southwest of Western Australia, the southeast of Queensland, and the Cape York region in the north (Austin, 1996; Austin & Knott, 1996). The genus also occurs in the southern New Guinea.

The eastern group includes the *Cherax destructor* lineage, which is composed of four subspecies (*C. d. destructor*, *C. d. albidus*, *C. d. esculus*, and *C. d. davisi*) according to Souty-Grosset et al. (2006). However, there is some debate on the correct taxonomy since previous mitochondrial gene sequences recognized only two or three taxa (Austin et al., 2003; Munasinghe et al., 2004a, b; Nguyen et al., 2004; Nguyen & Austin, 2005). Nevertheless, the complex is highly morphologically variable and systematic questions are still unsolved (Munasinghe et al., 2004a, b; Souty-Grosset et al., 2006).

To better classify the crayfish found in "Laghi di Ninfa," high molecular weight genomic DNA was extracted from the muscle tissues obtained from the pereopods and chelae of 15 individuals fixed in absolute ethanol and stored at −20°C. DNA was extracted either according to the classical SDS-proteinase K and phenol–chloroform technique or using the Aquapure genomic DNA kit (Biorad) as tested in a previous study (Chiesa, 2009). DNA quality was assessed with 1% agarose gel in TAE buffer. *COI* gene fragment was amplified using primers HCO 2198 and LCO 1490 (Folmer et al., 1994; Trontelj et al., 2005; Dawnay et al., 2007). A 25 µl reaction volume, containing 1 U of GoTaq DNA Polymerase (Promega), Mg^{2+} 2.5 mM, and $dNTP_s$ 0.2 mM, was used to perform PCR amplification at the following conditions: 40 cycles of 30 s at 95°C, 45 s at 45°C, and 1 min at 72°C, after an initial 10 min denaturation step at 95°C and final amplification at 72°C for 10 min. PCR products were purified by elution from a 2.5% agarose gel, then precipitated with 3 volumes of 100% ethanol, and washed with 70% ethanol. They were sequenced using FW or RV *COI* primers by CEQ™ DTCS-Quick Start Kit (Beckman Coulter) on "CEQ™ 8000 DNA Analysis System" (Beckman Coulter). An amplified region of about 650 bp of *COI* was thus obtained.

The obtained sequences were compared with those available from genomic databases using Blast; multiple alignments of sense and antisense sequences were conducted using Clustal X (Thompson et al., 1997) and Sequencer 4.2 (Gene Code Corporation). Correctness of the alignment was verified at the amino acid level. *COI* sequences of analyzed specimens aligned with those of *Cherax destructor* (Accession number: AY383557).

Within the species *C. destructor*, four subspecies have been previously described. To identify the subspecific level of our sample, we amplified *16S* rDNA gene using specific *Cherax* primers 1471 and 1472, and the proposed amplification conditions by Munasinghe et al. (2004a). Amplicons of 531 bp

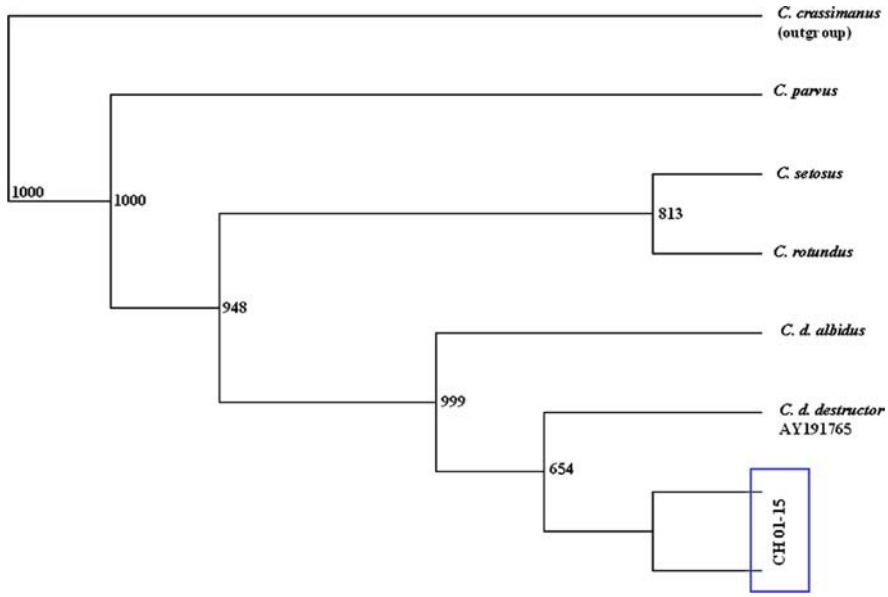

Fig. 1 Neighbor Joining tree representing barcoding based on *16S* rDNA polymorphisms (CH01–CH15: sampled crayfish 1–15)

length were obtained and sequenced by means of CEQ^{TM} DTCS-Quick Start Kit.

Alignment with the GenBank sequences (Accession numbers: AF492805; AY191757; AY191765; AY191767-68; AY191770) of the "eastern group" of the *Cherax* genus (Munasinghe et al., 2004a) showed that the specimens from "Laghi di Ninfa" belong to the subspecies *C. destructor destructor* (Fig. 1). Two polymorphic sites (positions 85 and 183) were identified with respect to *C. d. destructor* sequences (100% identity) as reported in GenBank (Accession numbers: AY191764-66), but no within-population variation emerged among the 15 specimens of our sample. Interestingly, we identified an amount of genetic variation (12 polymorphic sites) greater than the one found in *C. d. albidus* (Accession number AY191767).

This is the second European country in which established populations of *C. destructor* have been found, the first being Spain (in the Provinces of Zaragoza, Aragón, and Navarra; Souty-Grosset et al., 2006). *Cherax destructor destructor* was possibly introduced into "Laghi di Ninfa" at the end of the 1980s to foster an experimental aquaculture. Although the real distribution of this crayfish in Italy is still unknown, it seems likely that the low temperature of the surrounding waters acts as a barrier against the natural spreading of crayfish; however, barriers against their intentional translocation into natural waters are weak. In fact, the species is considered a delicacy, being sold live for restaurants in several European countries, such as Switzerland, Germany, and England (Souty-Grosset et al., 2006). Although being susceptible to the crayfish plague, this species has a well-known history of invasion, being ranked as a high-risk species for the Italian ecosystems (Tricarico et al., 2009). For these reasons, eradicating the population in "Laghi di Ninfa" should be considered as a priority. In Spain, four populations of *C. destructor* were eradicated using crayfish plague, either directly or with infected signal crayfish (J. Dieguez-Uribeondo, pers. comm. in Souty-Grosset et al., 2006). Nevertheless, the eradication of alien crayfish by spreading of crayfish plague could represent a serious risk of infection and disease for native populations of *Austropotamobius pallipes.*

Eradication may be expensive and its cost should be weighed against the likely benefits (Manchester & Bullock, 2000; Genovesi, 2005; Pimentel et al., 2005). This is one of the few cases for invasive crayfish in which, due to the still confined distribution of *C. destructor* and the enormous costs that this species might inflict to the society in the near future, eradication is still feasible and economically profitable.

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