Research Article

An update of the known distribution and status of *Cherax* spp. in Italy (Crustacea, Parastacidae)

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Abstract

To date, only two *Cherax* species have been reported to occur in Italy, i.e., *C. destructor* Clark, 1936 and *C. quadricarinatus* (von Martens, 1868), both in the wild and in aquaculture farms. Therefore, we aimed to update their current status and distribution in Italian mainland and Sicily. In addition, we investigated the origin of their known populations, and their possible routes of invasion. In order to genetically characterize the *Cherax* populations occurring in Italian inland waters and aquaculture facilities, the barcode region of the mtDNA gene cytochrome oxidase subunit I was sequenced in the available specimens originating from an aquaculture facility and a museum collection. The sampling campaigns conducted in 2021 did not lead to the collection of any *Cherax* individuals in the sites where the species was formerly reported to occur. The recent failures to detect *Cherax* spp. from Italian inland waters might be due to the inability of the species to cope with the Italian climate, which is likely for *C. quadricarinatus* but less so for *C. destructor*, to the possible impact of the alien red swamp crayfish *Procambarus clarkii* (as well as its indirect impact i.e., the spread of the crayfish plague for which yabby is susceptible), which occurs in both the sites where *C. destructor* was reported in Italy, or to the burrowing habits of the species, which might lead to overlook their presence when present at low densities. In the light of the well-known impact of invasive crayfish and considering the scant knowledge about the current distribution and status of *Cherax* species in Italy, a regular monitoring of their possible presence is recommended.

Key words: aquaculture, biological invasions, cytochrome c oxidase subunit I, local extinction, ornamental trade, *Procambarus* impact

Introduction

The occurrence of invasive alien species (IAS) negatively affects natural ecosystems and threats biological diversity worldwide (Ricciardi and MacIsaac 2011). Negative ecological impacts of IAS have been well documented globally with mechanisms such as competition, predation, and parasite spill over impacting native species (e.g., Nentwig 2007; Ficetola et al. 2011; Rusch et al. 2020; Vecchioni et al. 2021). It is thus crucial to properly assess the long-term impacts of IAS on native biota and ecosystems, despite sometimes their evaluation might be challenging (e.g., for species dynamic, direct/indirect effects: Kerns et al. 2021) because, due to insufficient information, public awareness, and policy frameworks, the effects of IAS are often underestimated (Early et al. 2016; Madzivanzira et al. 2021).

Introduced crayfish are among the most concerning IAS in inland waters due to their ability to successfully colonise novel water bodies, disperse among them, and play ecological key roles (Gherardi et al. 2011; Lodge et al. 2012; Twardochleb et al. 2013, Marrone and Naselli-Flores 2015). In Europe, several alien crayfish species are known to occur in the wild, mainly introduced for ornamental trade or aquaculture purposes (Kouba et al. 2014), thus leading the European Union to currently include six crayfish species (plus two under consideration) in the list of IAS of Union concern (EU regulation 1143/2014; last update of the list: July 2022). In Italy, six alien crayfish species have been reported to occur in the wild: the North American red swamp crayfish *Procambarus clarkii* (Girard, 1852), the marbled crayfish *Procambarus virginalis* Lyko, 2017, the spinycheek crayfish *Faxonius limosus* (Rafinesque, 1817), the signal crayfish *Pacifastacus leniusculus* (Dana, 1852), the Turkish crayfish *Pontastacus leptodactylus* (Eschscholtz, 1823), and the Australian common yabby *Cherax destructor* Clark, 1936 (Aquiloni et al. 2010; Mazza et al. 2018; Deidun et al. 2018; Sanna et al. 2021).

Cherax destructor, which is native to south-eastern Australia, where it is listed as "vulnerable" by the IUCN red list (Crandall 1996), experienced a rapid human-mediated range expansion, colonizing the eastern drainages of western Australia and Tasmania (Beatty et al. 2005; Lynas et al. 2007; McCormack 2014). Globally, *C. destructor* has been introduced to: South Africa in the 80's for research purposes, without any records in the wild (Nunes et al. 2017); Zambia in early 90's for aquaculture purposes, but this attempt was not successful and no records from the wild are available (Madzivanzira et al. 2020); Europe and Asia for aquaculture purposes (Holdich et al. 2009). In Europe, the species has successfully established viable populations in Spain (cf. Vedia and Miranda 2013 and reference therein) where it was translocated for aquaculture purposes (see Bolea 1996). In 2018, it has been reported in the wild in Brittany, France (probably introduced for aquaculture in ponds connected to open waters; Vigneron et al. 2019; Collas 2020), and in southern Ireland where it was probably introduced in 2010 (Julian Reynolds, *pers. comm.*, 2021). In Italy, *C. destructor* was reported to occur in Latium (in the "Monumento Naturale Regionale – Giardino di Ninfa", see Scalici et al. 2009a, b) and in Sicily (Costanzo Stream, Syracuse, see Deidun et al. 2018). However, four years after its finding, *C. destructor* disappeared in Latium possibly due to the crayfish plague, caused by the pathogen *Aphanomyces astaci* Schikora, 1906 carried by *P. clarkii* (Mazza et al. 2018), to which the common yabby is vulnerable (but see Mrugała et al. 2016).

Although the red-claw crayfish *Cherax quadricarinatus* (von Martens, 1868) is bred in Italian aquaculture facilities (D'Agaro et al. 1999; Vecchioni et al.

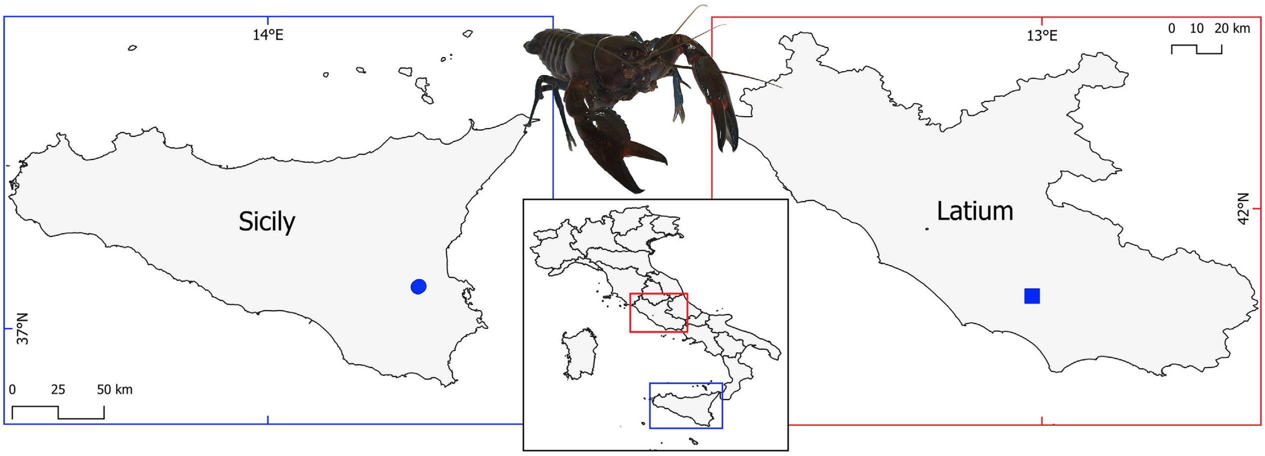

Figure 1. Location of the former Italian occurrence localities of *Cherax destructor* (blue dot and square). The blue dot in Sicily represents the location site sampled during the present study.

2021), no records are to date reported for the species in the wild. Conversely, *C. quadricarinatus* individuals have already been observed in the wild in other European countries (i.e., Malta, Deidun et al. 2018; Hungary, Weiperth et al. 2019; northern Spain, Arias and Torralba-Burrial 2021). Moreover, a self-sustaining population of the species is known from thermal springs in Slovenia (Kouba et al. 2014). Negative impacts caused by *C. destructor* and *C. quadricarinatus*, as reduction of macroinvertebrates and macrophytes (Lynas et al. 2004; Beatty 2006; Coughran and Daily 2012; Cerato et al. 2019; Haubrock et al. 2021), changes in ecosystem services for their digging activity, and damages to fishery (Withnall 2000; Coughran and Daily 2012; Haubrock et al. 2021), have been already reported in their invaded range outside Europe.

Despite their potential invasiveness, both *Cherax destructor* and *C. quadricarinatus* are widely used in ornamental trade and aquaculture due to their economic value (D'Agaro et al. 1999; Saoud et al. 2013; Haubrock et al. 2021), and their escape in the wild is thus an actual risk. We here update the status of *C. destructor* in the study area and provide some novel data on the molecular characterization of both *Cherax* species from Italian waters and aquaculture facilities, with the aim to verify their identity, and to investigate their origin and possible invasion routes. Possible establishment and impacts of the species in Italy are also discussed.

Materials and methods

Samplings aimed at collecting invasive crayfish species were performed by hand and using hoop traps (three hoop traps were used in each sampling session) baited with mackerel fillet and beef liver in Costanzo stream (WGS84 geographical coordinates: 37.252467°N; 14.912453°E), a shallow, slow-flowing stream characterised by the occurrence of hygrophilous vegetation on the banks, where the occurrence of *Cherax destructor* was reported by Deidun et al. (2018, see also Figure 1). Overall, ten trapping

Table 1. Origin and GenBank Accession Numbers (A.N.) of the analysed *Cherax* spp. Geographical coordinates are expressed in terms of decimal degrees (Map Datum: WGS84).

Taxon	Location	Habitat type	Coordinates	A.N.
Cherax quadricarinatus	Fiumefreddo di Sicilia (Sicily, Catania)	Acquaculture farm	37.784986–15.215430	OM728391–OM728394
C. destructor	Monumento Naturale Regionale – Giardino di Ninfa (Latium, Latina)	Cultivation ponds	41.583020–12.954234	OM728395
C. destructor	Fiumefreddo di Sicilia (Sicily, Catania)	Acquaculture farm	37.784986–15.215430	OM728396–OM728397

sessions of three days each were carried out between April and October 2021, when the monthly average temperature ranges between 15.7 (April) and 27.6 °C (August). No sampling was carried out in Latium, since that *C. destructor* population is now considered extinct (Mazza et al. 2018) and up to now no new records of the species have been reported (A. Monaco, *pers. comm*., 2022). However, four individuals of *C. destructor* sampled in Latium before the extinction of the local population were retrieved from the Zoology Section "La Specola" of the Natural History Museum of the University of Florence (Italy). Furthermore, ten specimens of *C. quadricarinatus* and *C. destructor* from an aquaculture farm located in Fiumefreddo di Sicilia (Contrada Vignagrande, province of Catania, Sicily) were obtained (Table 1).

In the light of the occurrence of cryptic species among parastacid decapod crustaceans (Dawkins et al. 2017), the morphological identification of the studied *Cherax* specimens was supplemented with their molecular characterization. One pereopod (walking leg) was excised from each obtained individual, fixed *in situ* in 96% ethanol and then stored in freezer at −20 °C. The barcode fragment of the gene encoding the cytochrome oxidase subunit I (COI) was amplified in seven specimens of *Cherax* spp. (see Table 1) using the primers COL6b (5′-ACA AAT CAT AAA GAT ATY GG-3′) and COH6 (5′-TAD ACT TCD GGR TGD CCA AAR AAY CA-3′), described by Schubart and Huber (2006), following the procedure described in Vecchioni et al. (2019a). The obtained PCR products were sequenced with an ABI 3130xL sequencer (Applied Biosystems) by Macrogen Inc. (Madrid, Spain; https://dna.macrogen.com/eng/) and uploaded to the public database GenBank under Accession Numbers, A.N., OM728391–OM728397. In addition to our new sequences, 79 *Cherax* spp. Sequences were downloaded from GenBank and included in the analysis (see Figure 2 for their A.N.). The analysis of the sequences was performed following Marrone et al. (2019) and Vecchioni et al. (2019c).

Results

No crayfish were caught in the wild during the sampling campaign (Figure 1). Moreover, no sign of the presence of any crayfish species was found at the sampling site.

Overall, four novel COI sequences belonging to *Cherax quadricarinatus*, and three belonging to *C. destructor* were produced (Table 1). The inferred

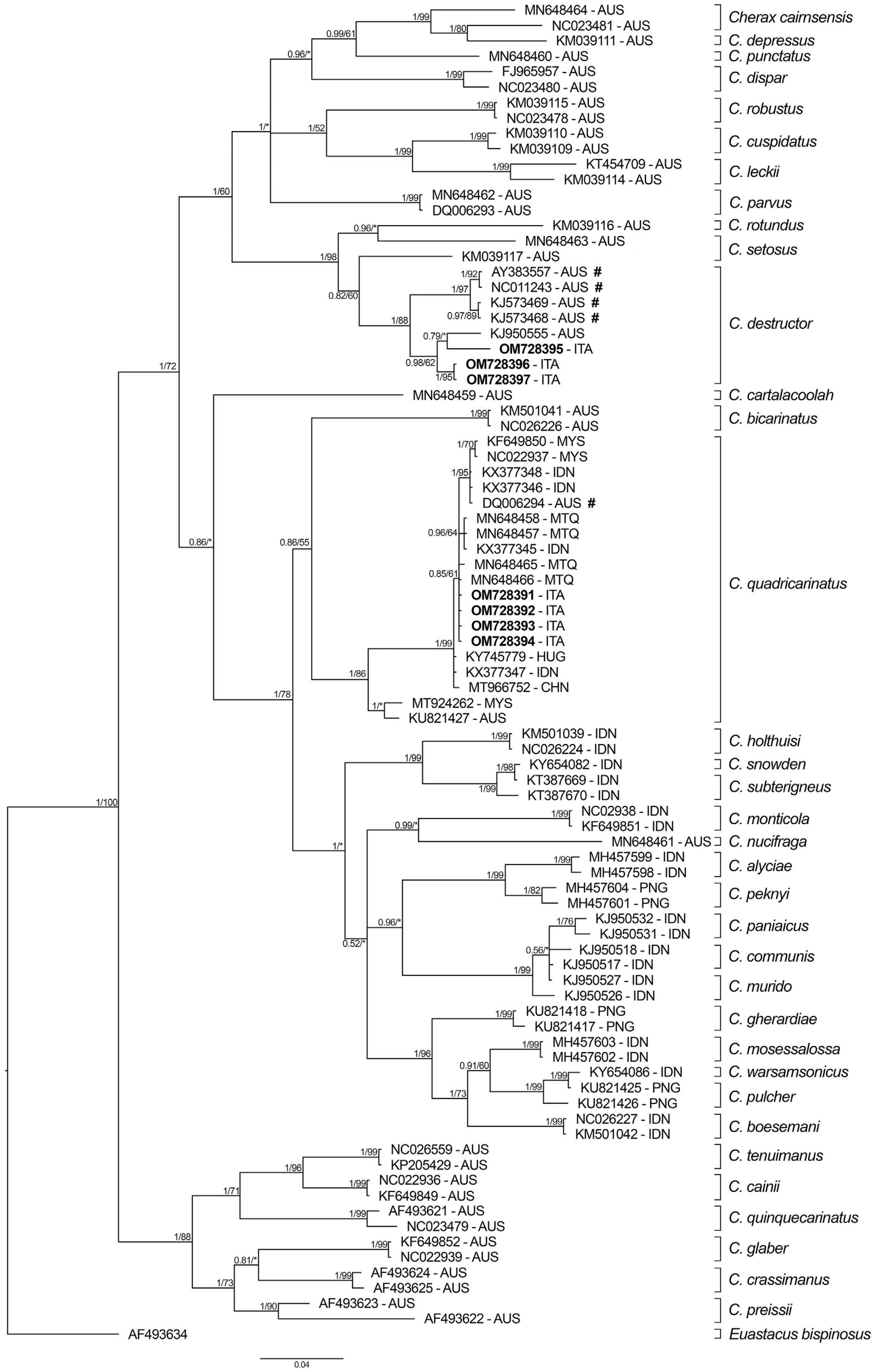

Figure 2. Bayesian phylogram of *Cherax* spp. based on the 558 bp fragment of the mtDNA COI. The Parastacidae *Euastacus bispinosus* Clark 1941 was used as outgroup. Node statistical support is reported as nodal posterior probabilities (Bayesian Inference of phylogeny, BI)/bootstrap values (Maximum Likelihood, ML). *, Nodal statistical supports < 0.50. Square brackets group the samples according to the current taxonomy of the genus. Novel sequences are reported in bold. AUS, Australia; CHN, China; HUG, Hungary; IDN, Indonesia; ITA, Italy; MTQ, Martinique; MYS, Malaysia; PNG, Papua New Guinea. The hashtags represent those sequences that belong to the native populations of the species.

phylogenetic trees (Bayesian Inference, BI and Maximum likelihood, ML, Figure 2), based on a 558 base-pair COI fragment included both new and published sequences and rooted on the Parastacidae *Euastacus bispinosus*

Clark, 1941 (A.N. AF493634), showed congruent and well-supported topologies at the nodes grouping the analysed sequences of *C. quadricarinatus* and those of *C. destructor* in monophyletic clades, thus confirming their morphology-based identification (Figure 2). Furthermore, most of the 35 species belonging to the genus *Cherax* included in the analyses proved to be monophyletic with few exceptions; a paraphyletic status was observed for the following species: *C. cairnsensis* Riek, 1969, *C. setosus* Riek, 1951, *C. subterigneus* Patoka, Bláha and Kouba, 2015, *C. murido* Holthuis, 1949 and *C. pulcher* Lukhaup, 2015 (Figure 2).

Discussion

Our study did not confirm the presence in the wild of *Cherax destructor* in Sicily.

The populations of *C. destructor* from Latium and Sicily (Deidun et al. 2018; Mazza et al. 2018, and present data) seem thus to be locally extinct. As mentioned in the introduction, the disappearance of the Latium *C. destructor* population was ascribed by Mazza et al. (2018) to the spread of deadly pathogens (i.e., the oomycete *Aphanomyces astaci*) mediated by *Procambarus clarkii*. *Procambarus clarkii* and *C. destructor* co-existed in both the sites where the common yabby is now reported to be disappeared (Deidun et al. 2018; Mazza et al. 2018). The possible role of the crayfish plague in the local extinction of *C. destructor* populations is supported by a mass mortality of the congeneric *C. quadricarinatus* due to *Procambarus*-mediated fungi reported in an aquaculture farm in Sicily (Marino et al. 2014). Thus, the widespread presence of *P. clarkii* in Italy (Sicily included: Aquiloni et al. 2010; Faraone et al. 2017; Lo Parrino et al. 2020) could limit the establishment and spread of *Cherax* spp. However, Mrugała et al. (2016) in laboratory experiments found that *C. destructor* individuals exposed to the least virulent *A. astaci* strain (genotype group A) can partly survive and that not all the individuals died after being infected with the two more virulent strains (genotype B and E). Based on these results, the authors suggested that under favourable conditions (e.g., fluctuations of spore concentration), *C. destructor* may survive and contribute to crayfish plague spread, which is conversely lethal to native European crayfish, such as the white clawed crayfish *Austropotamobius pallipes* complex. Moreover, even if no evident signs of burrows were found, we cannot discard the possibility that the burrowing habits of *C. destructor,* being able to dig up to two meters in the ground, could have led to overlook its presence in Sicily. Thus, further work including the use of eDNA analyses, to integrate the traps that could not be effective when species abundance is very low, is necessary to confirm its disappearance (e.g., Baudry et al. 2021).

The molecular analysis revealed a clear clustering of the sequences, which is mostly congruent with the current taxonomy of the analysed species of the genus *Cherax*. However, the phylogenetic trees showed some paraphyletic relationships that could be due either to the existence of taxonomical inconsistencies (i.e., the presence of invalid species) or linked to mitonuclear discordance phenomena that make such single-marker phylogenetic inferences unreliable (e.g., Vecchioni et al. 2019b; Belaiba et al. 2019). Accordingly, the status and taxonomy of the paraphyletic *Cherax* species reported in Figure 2 should be desirably verified with dedicated studies. The *C. quadricarinatus* sequences produced in the frame of this work cluster with one sequence (A.N., DQ006294) from Queensland, Australia, i.e., from an unknown population occurring in the native distribution area of the species (see Shull et al. 2005). Regarding the novel *C. destructor* sequences, these are clustered together with one sequence that belongs to a specimen from the North Territory, Australia (i.e., originating from the non-native range of the species). Accordingly, the origin of the Italian specimens could not be traced.

Further introductions of the two *Cherax* species could happen as the pathways of introductions are still active (pet trade, aquaculture) and the species are not banned. Originally, it was believed they were not capable of tolerating European climates (Mrugała et al. 2016 and reference therein), being native to the semiarid and arid part of the Australian continent (*C. destructor*) and to temperate and tropical climate (*C. quadricarinatus*) (Horwitz and Knott 1995; Curtis and Jones 1995), respectively. However, based on the results reported by Veselý et al. (2015), the common yabby has good chances to survive under the European regimes of winter temperatures as proved by the occurrence of self-sustaining populations of the species in France (Vigneron et al. 2019) and Spain (Vedia and Miranda 2013), even if management actions to eradicate them are ongoing: J. Dieguez-Uribeondo *pers. comm*., 2021), thus posing some concerns to native biota (Tricarico et al. 2010). Moreover, *C. destructor* can colonize a wide range of habitats, tolerate salinity, hypoxia, and a broad spectrum of temperatures, and with the forecasted increase of temperatures, in the future more suitable areas will be available for its establishment (Withnall 2000; Souty-Grosset et al. 2006; Veselý et al. 2015).

In Sicily, for example, where no native crayfish are present, the common yabby *Cherax destructor* (Deidun et al. 2018) was able to survive in the wild. In addition, the white yabby *Cherax albidus* was also previously bred in a pilot aquaculture facility near Siculiana (province of Agrigento) (Coccia et al. 2010). This facility is no longer active and there is no evidence of the species occurrence on the island. However, even in the absence of native crayfish, a possible establishment of a further *C. destructor* populations would threaten other native decapods, e.g., the river crab *Potamon fluviatile* (Herbst, 1785) (Vecchioni et al. 2017; Marrone et al. 2020). Similarly, concerns are also already being raised in Southern Africa with regards to the potential impacts of *Cherax* spp. on native river crabs through mainly competition for resources and predation (see Madzivanzira et al. 2021, 2022). The establishment of *Cherax* spp. might have an impact on the populations of endemic taxa as the Sicilian pond turtle *Emys trinacris* Fritz et al. 2005 (see Vecchioni et al. 2022, and references therein), whose isolated populations are recognized as Independent Management Units due to their noteworthy small-scale genetic fragmentation (Vecchioni et al. 2020). On the contrary, its impact on the diverse biota of Sicilian temporary waters is likely to be negligible (Naselli-Flores and Marrone 2019).

With regards to the red-claw crayfish, the species seems not able to endure European climate, and therefore it should not be considered a threat to our ecosystems, at least in the near future (Veselý et al. 2015). In fact, to date, the only confirmed established *Cherax quadricarinatus* population known in Europe is reported for thermal springs in the Slovenian lake Topla (Jaklič and Vrezec 2011; Kouba et al. 2014).

In the light of the results reported here, *Cherax quadricarinatus* could be considered a "low-risk alien species" in Italy due to its limited potential of invasion in European climates. On the contrary, although no individuals belonging to *C. destructor* were found during our sampling activities in Sicily and no other report of the species occurred in the Italian peninsula, greater attention should be placed on this last species considering its ability to adapt to the Italian climate. Therefore, due to also the paucity of available information, further studies and regular monitoring are required to confirm the absence of *Cherax* species in the study area.

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Authors' contribution

MA and VA planned and headed the project. PC and LV carried out the field work. LV and FM carried out the laboratory work and the analyses. LV, FM and ET wrote a first draft of the manuscript, which was discussed and improved by all of the authors.

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