New records of *Austropotamobius pallipes* (Decapoda: Astacidae) relict populations from the Ticino River

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Abstract - Based on available literature, the white-clawed crayfish *Austropotamobius pallipes* complex, a species of Community interest according to the EU Habitats Directive, has not been reported in the Lombard Park of the Ticino Valley (Northern Italy) since almost two decades, with the last findings dating back to 2005. In 2021, we found two small populations in the northernmost area of the park (Sesto Calende, province of Varese). These observations support the urge for implementing actions focused on the monitoring and conservation of this endangered species in the Lombard Park of the Ticino Valley in order to avoid its local extinction.

Key words: *Austropotamobius pallipes*, conservation, Lombard Park of the Ticino Valley, species of Community interest.

Riassunto - Nuove segnalazioni di popolazioni relitte di *Austropotamobius pallipes* (Decapoda: Astacidae) nel fiume Ticino.

Sulla base delle informazioni bibliografiche e dati inediti, il gambero di fiume *Austropotamobius pallipes* complex, specie di interesse comunitario presente nella Direttiva Habitat, risulta non più segnalato nel Parco Lombardo della Valle del Ticino da circa due decadi, con l'ultima osservazione datata 2005. Nel 2021 abbiamo scoperto due piccole popolazioni site nel comune di Sesto Calende (Varese), nella parte più settentrionale del parco regionale. Queste osservazioni evidenziano la necessità di attuare progetti di monitoraggio e conservazione del gambero di fiume italiano all'interno del Parco Lombardo della Valle del Ticino, per scongiurarne l'estinzione a livello locale.

Parole chiave: *Austropotamobius pallipes*, conservazione, Parco Lombardo della Valle del Ticino, specie di interesse comunitario.

The Italian white-clawed crayfish, sometimes referred to as *Austropotamobius italicus* (Faxon 1914), is a freshwater crustacean, ascribed to the *Austropotamobius pallipes* (Lereboullet 1858) species complex (Souty-Grosset *et al*., 2006). This freshwater crustacean (Fig. 1) has been reported for five Sites of Community Interest (SCI) within the Lombard Park of the Ticino Valley (Casale *et al*., 2008). Recent genetic typification indicate *A. italicus* as occurring in the Prealpine areas of the Varese province, with the subspecies *A. i. carinthiacus* (Bernini *et al*., 2016). In the last decades, the white-clawed crayfish has shown a dramatic decreasing population trend in Europe mainly caused by the competition with alien crayfish species, e.g. *Procambarus clarkii* (Girard 1852), *Orconectes limosus* (Rafinesque 1817) and habitat modifications. Consequently, the International Union for the Conservation of Nature lists the species as Endangered (Füreder *et al*., 2010) and the European Union considers it a "species of Community interest" in Annex II and V of the EU Habitats Directive. Given its concerning vulnerability, the white-clawed crayfish conveyed particular attention by the local institutions, which addressed specific actions for its conservation at both the regional and national levels.

At the beginning of the current century, the species was considered still present in the Ticino River (Furlanetto, 2002), although threatened by the co-occurrence of alien crayfish species. However, the last record of *A. pallipes* in the park dates back to 2005 (Nardi *et al*., 2005b; Zaccara *et al*., 2005). At that time, it was found in three sites located in the municipalities of Sesto Calende and Vergiate (province of Varese) and in one site in the province of Pavia. These authors, while suggesting that proper attention should be paid to native populations of white-clawed crayfish through restocking, at the same time warned of the risk related to the possible co-occurrence of alien crayfish species in the areas individuated for these conservation actions. The alien species distribution is fairly well known due to their invasiveness and the damage they cause to Italian ecosystems (Fea *et al*., 2006; Lo Parrino *et al.,* 2020).

In 2021, while rescuing toads and others amphibians attempting to cross roads during their seasonal migrations, volunteers from the association 'Tutela Anfibi Basso Verbano' spotted a crayfish belonging to the *A. pallipes* species complex in the municipality of Sesto Calende. Following this accidental observation, in order to provide an update on the distribution of the *A. pallipes* complex in the northernmost part of the Ticino Park, six sites were monitored in 2021: five in Sesto Calende and one in Vergiate (province of Varese). Among the sites investigated, three were already known from a previous report (GRAIA,

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Fig. 1 - Adult of white-clawed crayfish found in this study. / Adulto di gambero di fiume osservato nel corso della presente indagine (Photo/foto: Milo Manica).

2003). In total, five field survey sessions were carried out, two in daytime (25 April and 26 August), and three during the night (12 August, 31 August and 1 September).

The sampling methodology followed the recommendations of the Action Plan for the conservation of the white-clawed crayfish in Italy (Ghia *et al*., 2014). Specifically, during the daytime inspections we looked for crayfish by moving stones and looking under shelters along selected sections of the streams. An exuvia was collected to obtain genetic material for positive taxonomic confirmation through barcoding procedures. During the night, the same transects were covered by walking slowly along the banks of the stream for 60 min. To avoid the transmission of pathogens from one site to another, the crayfish were manipulated wearing disposable gloves that were changed at each site.

The white-clawed crayfish were found in three out of the six survey sites (one already reported in 2003; Fig. 2). Two populations are included in a Special Area of Conservation (SAC 'Sorgenti del Rio Capricciosa'), while the other one lays outside the SAC.

All the populations discovered occupy small streams located on the morainic hills of Sesto Calende.

In conclusion, the observations carried out during this study can be summarized as follows:

- Population A is located in a stream running in a fairly well preserved wood. The site is at 290 m a.s.l. During the first field session, one adult male was found, while during the nighttime inspection forty-four crayfish (25 males, 12 females and 7 juveniles) were observed distributed along 315 m of the entire course of the stream.

- Population B is located in a stream with a stony substrate in the SAC 'Sorgenti del Rio Capricciosa' at 260 m a.s.l. Twenty-two specimens (7 males, 10 females and 5 juveniles) were found during nighttime inspections.

- Population C is located in a muddy stream in the SAC 300 m a.s.l. Seventy-seven specimens (34 males, 15 females, 27 undetermined and 1 juvenile) were found during nighttime survey. The specimens that could not be captured were not sexed.

To the best of our knowledge, the populations of *A. pallipes* investigated during this survey are the only ones present in the northernmost part of the Ticino Park and those located at the lowest altitude in the province of Varese.

Noteworthy, two crayfish belonging to the alien species *Procambarus clarkii* were found 416 m downstream of population A. The spread of Non-Indigenous Crayfish Species (NICS) populations and other threats, like pollution or habitat alteration, probably limit the occurrence of Indigenous Crayfish Species (ICS) populations in this area, as well as in their entire range (Manenti *et al.,* 2014).

To date, no information about the occurrence and distribution of white-clawed crayfish in the Varese province have been published, even if the species occurrence is already known in some Natura 2000 sites, such as Campo dei Fiori Regional Park (Bernini *et al*., 2016).

The isolation of the populations found in this survey and the spread of alien crayfish species in nearby sites are concerning factors for the long-term persistence of the white-clawed crayfish in the Park.

Future investigations and eDNA-based studies could allow to find new undetected populations in other watercourses within the investigated area, as well as to assess the presence of pathogens (e.g. the oomycete *Aphanomyces astaci*) that represent the main conservation risks for *A. pallipes* (Cammà *et al.,* 2010). Species-specific e-DNA probes are under development at the University of Milano-Bicocca. Genetic studies could also shed light on the genetic structure and isolation of such populations and could contribute to disentangle the degree of isolation and genetic drift patterns to properly address future conservation actions.

Given the ecological value of this species, guidelines to improve its conservation are developed at the national and European level (Nardi *et al*., 2004, 2005a; Palazzini *et al*., 2011; Ferrante *et al*., 2018; Biasetti *et al*., 2021). With this work, we aim to encourage the design of specific conservation projects for the white-clawed crayfish in the Ticino Park, in order to avoid its local extinction.

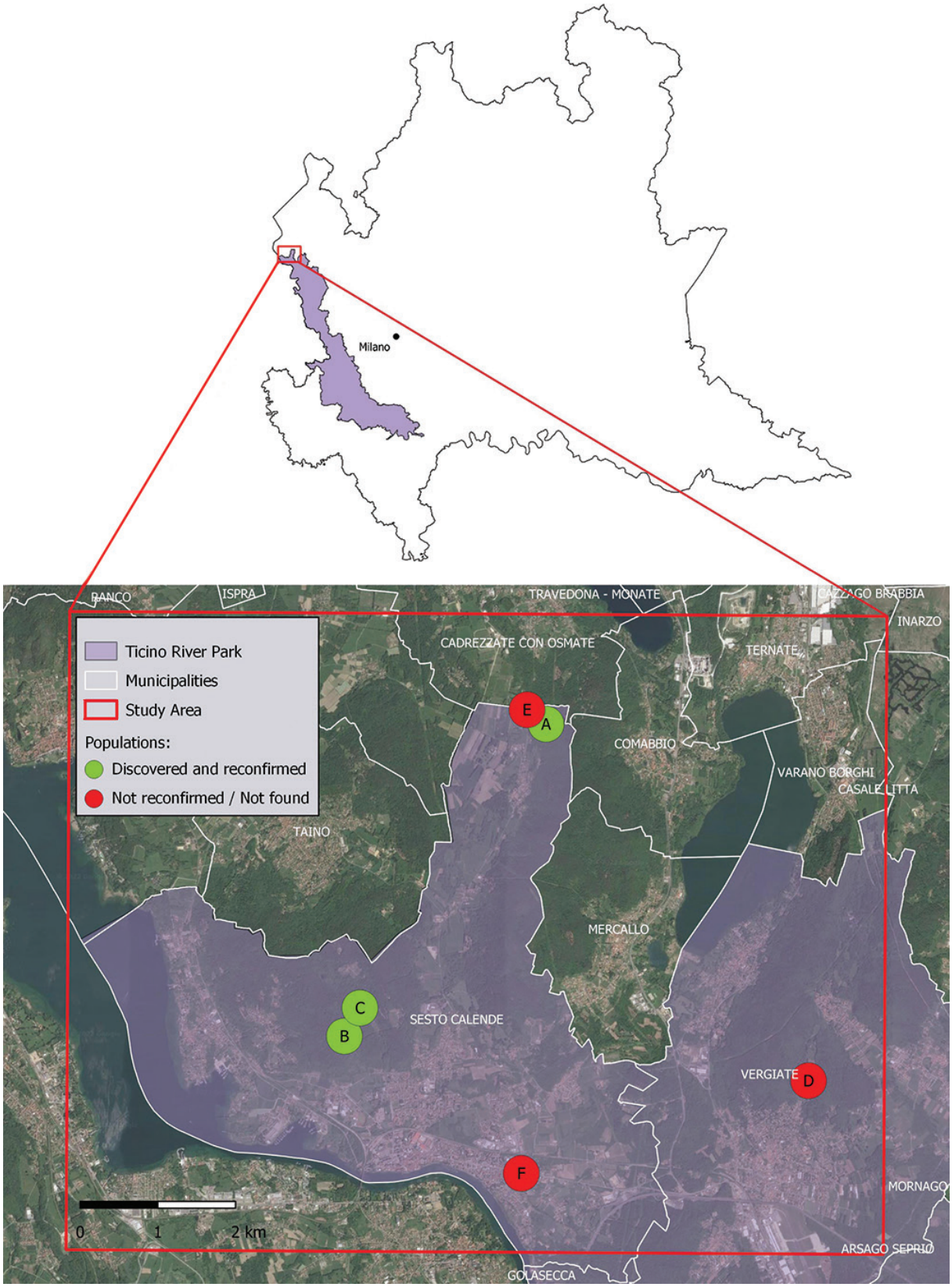

Fig. 2 - Study area and its location in Lombardy and in the Lombard Park of the Ticino Valley. In green, the locations of the white-clawed crayfish populations. Populations A and B were discovered in 2021. C is a population already known and reconfirmed after 16 years. In red, the populations (D, F) known until 2005 but not confirmed in the 2021 surveys. In site E, the species was not found. / Area di studio e sua localizzazione in Lombardia e nel Parco Lombardo della Valle del Ticino. Le località dove sono state osservate popolazioni di gambero di fiume sono indicate in verde. Le popolazioni A e B sono state scoperte nel 2021. C'è una popolazione precedentemente nota e la cui presenza è stata riconfermata dopo 16 anni. In rosso sono indicate le popolazioni (D, F) note fino al 2005 ma la cui presenza non è stata confermata nel corso del 2021. Nel sito E la specie non è stata trovata.

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