

Molecular evidences confirm a late Pleistocene origin of the Italian and Maltese populations of the freshwater crab, *Potamon fluviatile* (Herbst, 1785) (Malacostraca, Decapoda).



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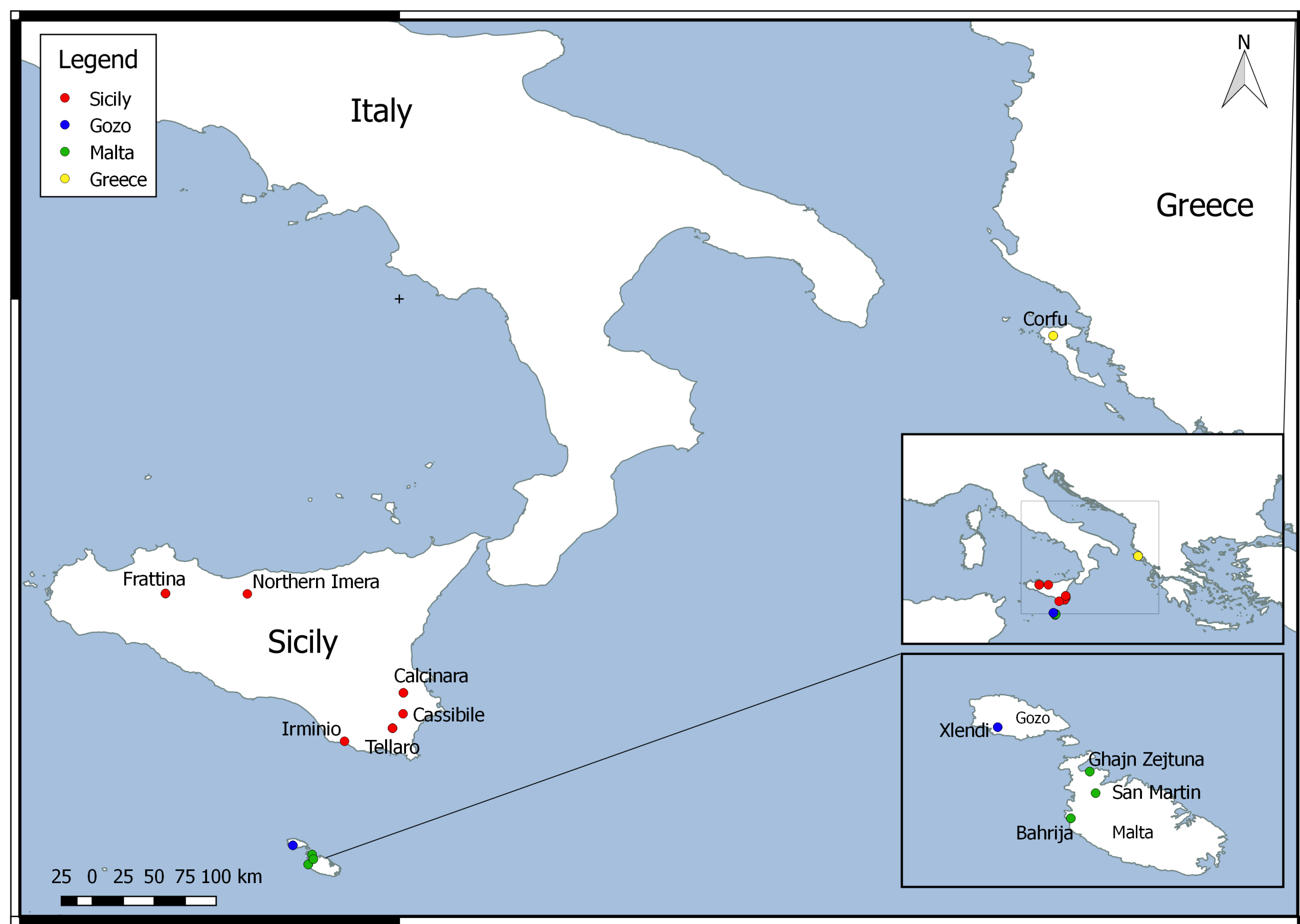


Figure 1: Map of the sampling sites.

In total, 40 specimens (1-7 per site) were analysed. 33 individuals were collected from 10 sampling sites in Malta, Gozo and the Sicilian mainland, whilst seven individuals were collected from a sampling site in Corfu (Greece) (Fig. 1). In addition, 21 sequences, corresponding to 104 individuals, were downloaded from GenBank and analysed along with our sequences. Genomic DNA was extracted using the Real Genomics “Genomic DNA Extraction Kit” (RBC BioScience) following the manufacturer’s protocol. The selective amplification of a 502 bp-long fragment from the gene encoding for the cytochrome oxidase subunit I (COI) was carried out by the polymerase chain reaction (PCR) 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). A haplotype network for the available sequences was constructed using the Median-joining method (Fig. 2). Past population demography was inferred using a Bayesian skyline plot (BSP) model, as implemented in BEAST 1.8.0 (Fig. 3).

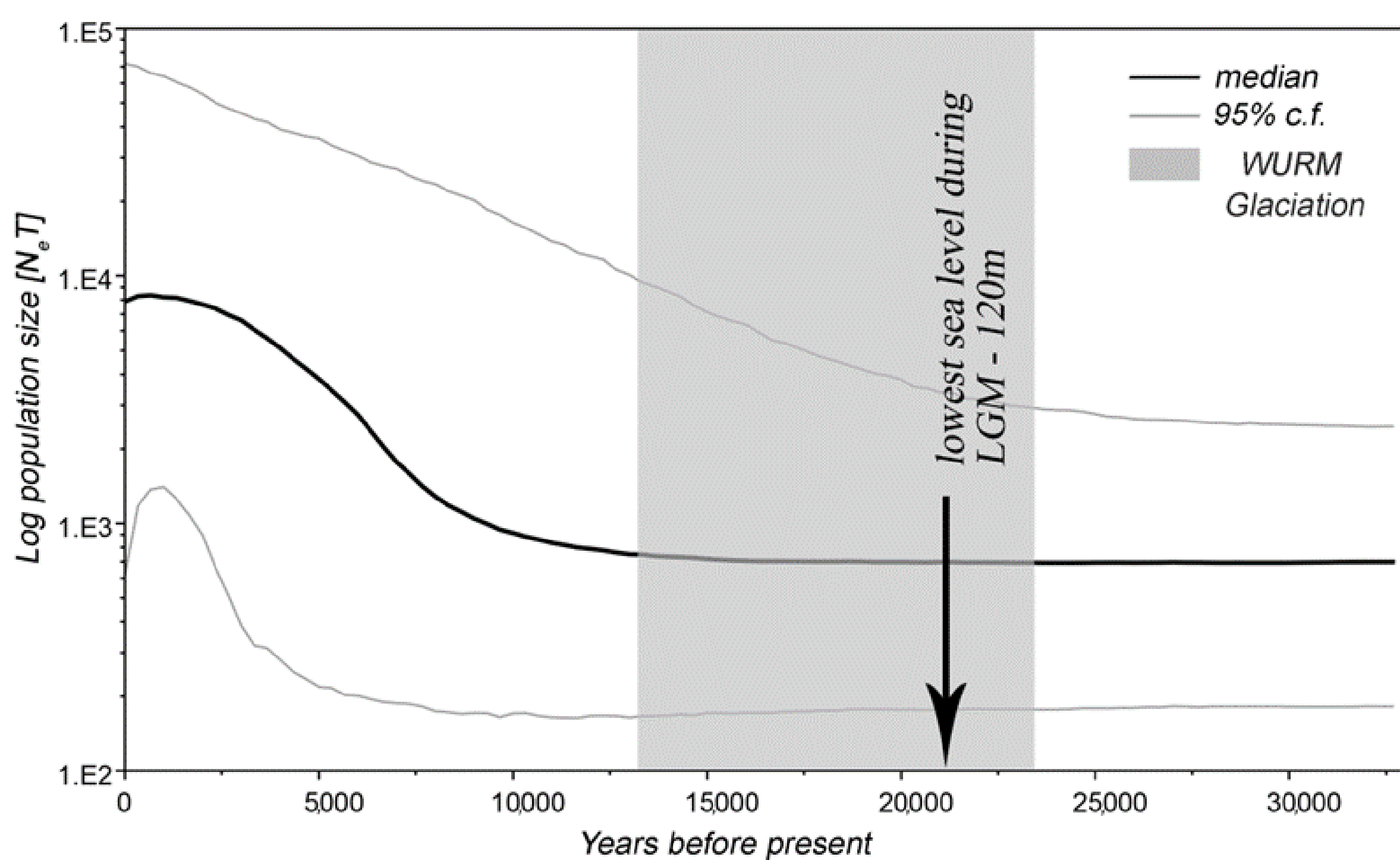


Figure 3: Bayesian skyline plots: evolution of population sizes through time based on Bayesian coalescence.

In the last decade, several studies were conducted on *Potamon fluviatile* in order to investigate its phylogenetic relationships with the other species in the subgenus *Euthelphusa*, and to elucidate its phylogeography (see Jesse et al. 2010, and references therein). Based on molecular data, Jesse et al. (2009) proposed a late Pleistocene origin from the Balkans for the Italian and Maltese populations of the species. However, it is also well-known that, during the Pleistocene glaciations, southern Italy and Sicily acted as refugia for species which persisted *in situ*, with a resulting genetic differentiation of these “southern” strains, both for aquatic and terrestrial taxa. The purpose of our study was thus to test the possible role played by southern Italy and Malta as refugia for the freshwater crab during the Pleistocene, through the implementation of an expanded sampling of the genetic diversity of different populations of the species in Sicily and the Maltese archipelago.

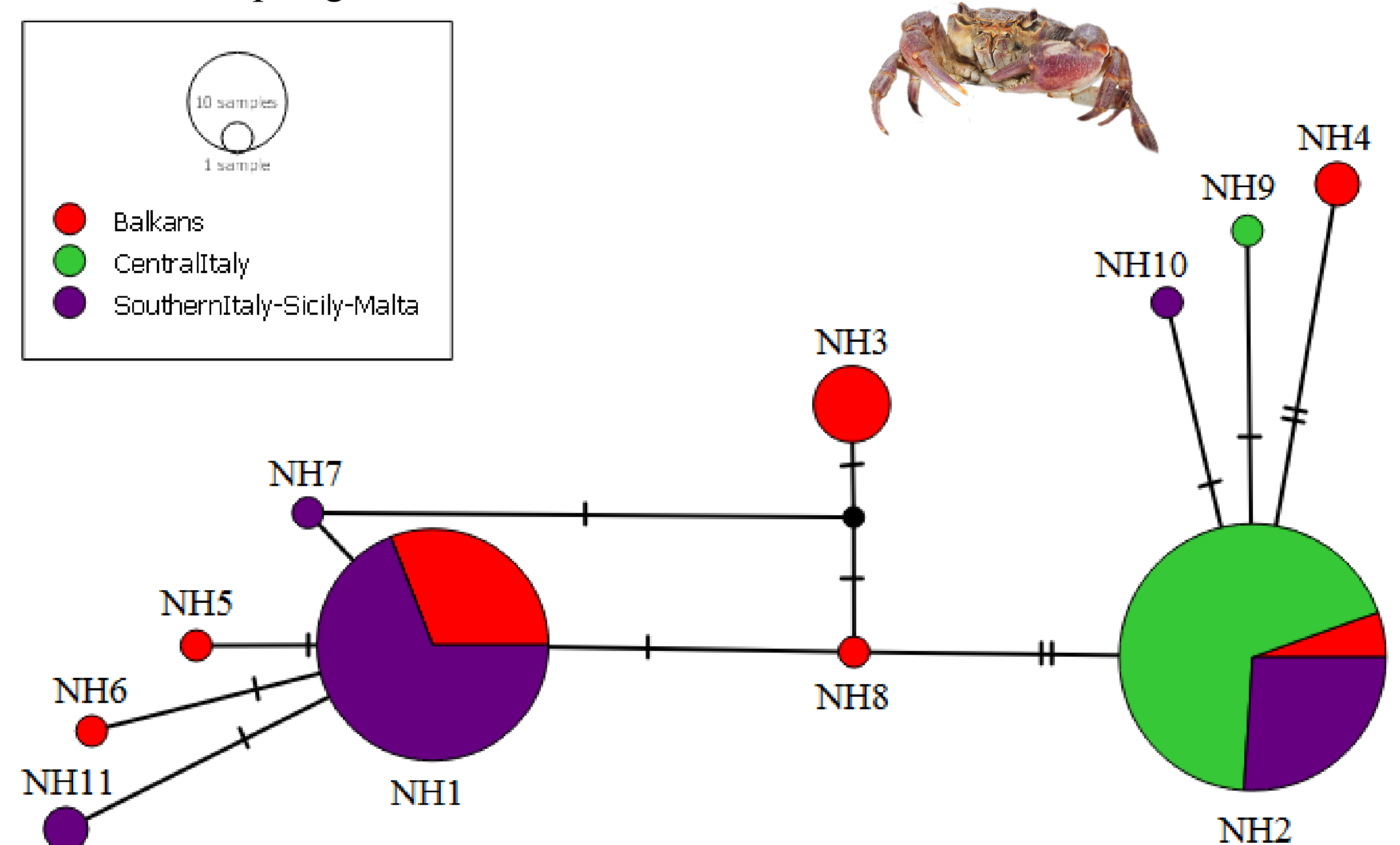


Figure 2: Median-joining Haplotype Network based on a 502 bp-long fragment of mtDNA COI.

The obtained haplotype network indicates that there are two common and widespread haplotypes shared by all the three groups, plus some rarer haplotypes shared by a single or few individuals which are restricted to single areas (Fig. 2). No obvious associations between observed haplotypes and their geographic locations was found – i.e. individual haplotypes could not be ascribed to exclusive geographical locations.

The estimated change in effective population size over time as computed with the BSP analysis is shown in Fig. 3. As pointed out by the BSP analysis, the demographic expansion began approximately 12,000 years before present (BP) with a 95% confidence interval ranging from 6,000 years BP to 24,000 years BP. In particular, the upper 95% confidence interval suggests that this inferred demographic expansion could have started even before the last glacial maximum (LGM), when the freshwater crab is supposed to have reached southern peninsular Italy, and then Sicily and the Maltese archipelago, through a trans-Adriatic route (cf. Jesse et al. 2009).

The performed analyses suggest that the studied *Potamon fluviatile* populations are not geographically structured, that the highest haplotype diversity is present in the Balkan area, and that the freshwater crab is in a phase of demographic expansion. Our results are thus in agreement with the hypothesis formulated by Jesse et al. (2009), i.e. that southern Italy and the Maltese Islands during the Pleistocene did not act as refugia where *P. fluviatile* local populations survived the glaciation period, but rather they proved to be a “land of conquest” for the Balkan *P. fluviatile* populations, which could colonize these new lands further south by virtue of the marine regressions linked with Pleistocene glaciations.

References:

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