

ROOTED IN THE PAST: GENETIC TRACES OF *PINNA NOBILIS* ACROSS A CENTURY.

Azzena, I.¹; Locci, C.^{1,2}; Pascale, N.^{1,2}; Deplano, I.²; Senigaglia, R.¹; Batistini, E.³; Chiantore, M.^{4,5}; Ciriaco, S.^{3,6}; Ferranti, M.P.^{4,5}; Grech, D.⁷; Liconti, A.⁸; Pitacco, V.⁹; Segarich, M.³; Scarpa, F.²; Casu, M.¹; Sanna, D.²

¹ Department of Veterinary Medicine, University of Sassari, Via Vienna 2, 07100 Sassari, Italy; ² Department of Biomedical Sciences, University of Sassari, Viale San Pietro 43b, 07100 Sassari, Italy; ³ Shoreline Soc. Coop., AREA Science Park, Località Padriciano 99, 34149 Trieste, Italy; ⁴ DiSTAV (Department of Earth, Environment and Life Sciences), University of Genoa, Corso Europa, 26, 16132 Genova, Italy; ⁵ National Biodiversity Future Center (NBFC), Palermo, Italy; ⁶ WWF Fondazione, Area Marina Protetta di Miramare, Via Beirut 2/4, 34151 Trieste, Italy; ⁷ IMC - International Marine Centre, Loc. Sa Mardini, Torregrande - 09170 Oristano, Italy; ⁸ OutBe, Via Nino Bixio 19, 16043, Chiavari, Genova, Italy; ⁹ Marine Biology Station Piran, National Institute of Biology, Fornače 41, SI-6330 Piran, Slovenia.

INTRODUCTION

Pinna nobilis, the noble pen shell, is a Mediterranean endemic bivalve closely associated with *Posidonia oceanica* seagrass meadows. Historically, it was highly valued for both its shell and the production of byssus (or sea silk), a rare and precious textile material. This long-standing exploitation contributed to a significant population decline, prompting its legal protection under European legislation in 1992 (Directive 92/43/EEC). However, since 2016, *Pinna nobilis* has been facing a new and severe threat in the form of Mass Mortality Events (MMEs), driven by a combination of pathogens and environmental stressors. These events have drastically reduced its distribution, leaving only a few remnant populations in isolated refuge areas. In response, several European conservation projects have been launched, including the LIFE PINNA program, alongside various local efforts. Within this context, the present study aims to: 1) reconstruct the population dynamics of *Pinna nobilis* from the Pleistocene to the present, and 2) assess the impact of environmental and anthropogenic stressors on the species' recent history.



Figure 1. *Pinna nobilis*

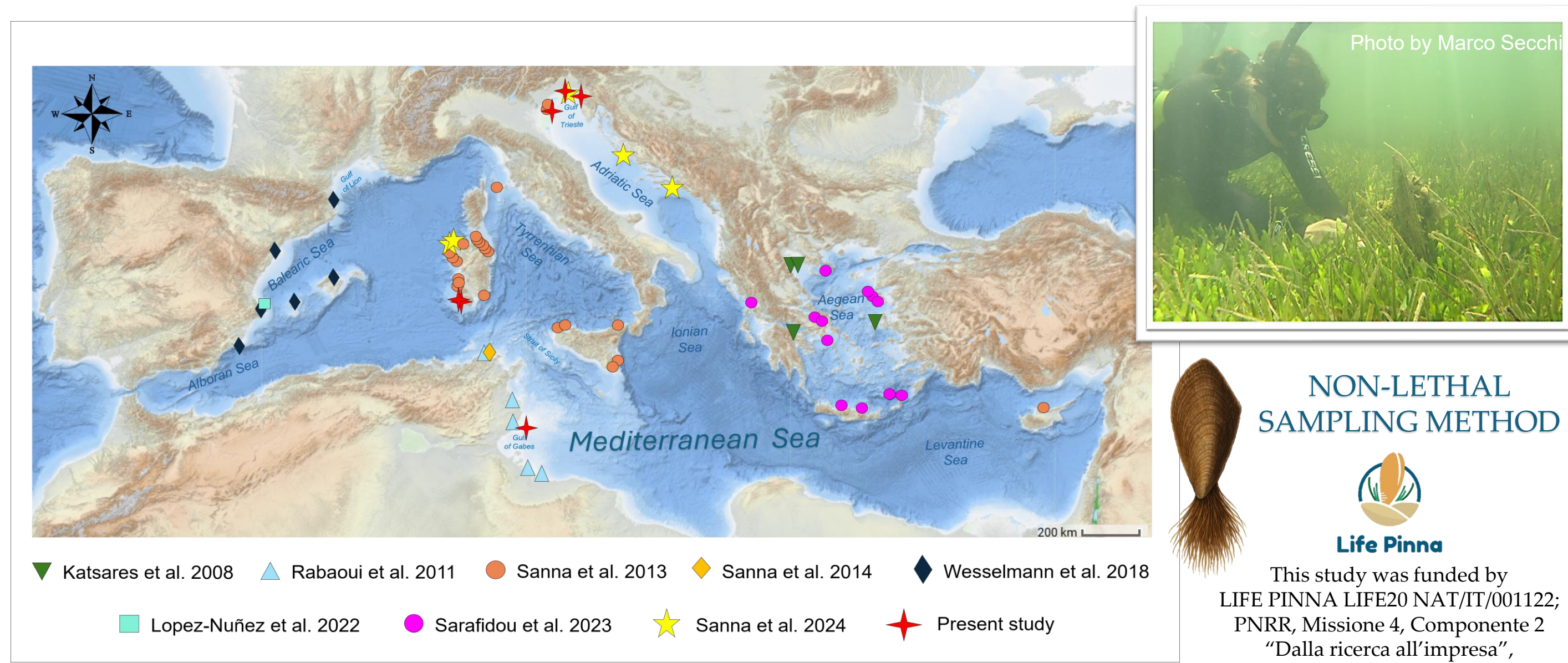


Figure 2. Sampling Map

MATERIALS AND METHODS

A total of 667 mitochondrial COI gene sequences were analysed, including 119 newly obtained in this study from specimens collected between the 1700s and 2024. Thanks to the standardisation of a DNA extraction protocol applied to byssus gland fragments, we successfully retrieved genetic material from historical samples sourced from private and museum collections around Sant'Antioco Island (Sardinia, Western Mediterranean). This allowed us to investigate the evolutionary history of *Pinna nobilis*, assessing the impact of major stressors such as overharvesting, pollution, and climate change on its genetic makeup over time.

The dataset was divided into three temporal cohorts: ancient (1700s, 1920s, 1970s, 1990s), modern (early 2000s, pre-MMEs), and surviving (post-MMEs).

RESULTS AND DISCUSSION

Genetic analysis revealed that ancient populations exhibited the lowest levels of genetic variability (Table 1), whereas modern and surviving populations displayed comparably high diversity, with a slight reduction observed in the latter group. Based on the mitochondrial COI gene analyses, a total of 100 haplotypes were identified and subsequently used to generate a time-calibrated phylogenetic tree (Figure 3), which revealed two major clades:

- **CLADE A** originating around 860 kya, consists of ancient lineages derived from early *Pinna nobilis* populations. These lineages are now nearly extinct and seems to be confined to the Adriatic and western Mediterranean basins;
- **CLADE B** dating back to approximately 1.55 mya, represents the first major adaptive radiation of the species in the Mediterranean and accounts for over 90% of the mitochondrial diversity observed.

Most haplotypes detected in 20th century specimens originated between 900 and 500 kya, indicating that present-day mitochondrial diversity has deep evolutionary roots. The prevalence of a limited number of haplotypes (Figure 4), presumably resilient, suggests an association with adaptive genetic traits (possibly nuclear?) that have undergone positive selection during successive glacial cycles, thereby enhancing the species' capacity to withstand environmental stressors. Notably, haplotypes **H1** and **H2**, found across all temporal cohorts, appear to have played a crucial role in the long-term survival of the species.

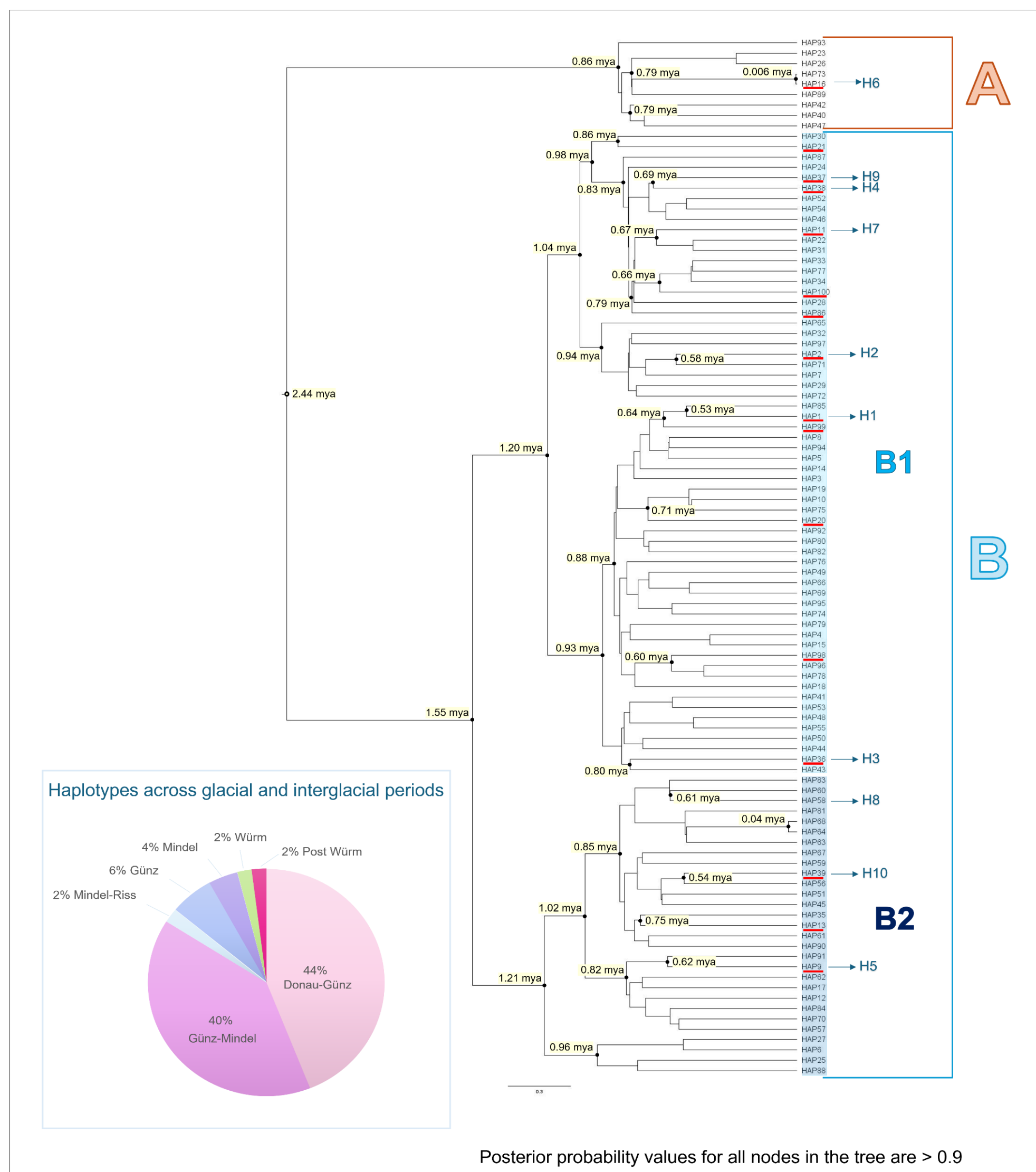


Figure 3. Time-calibrated phylogenetic tree of the 100 *Pinna nobilis* haplotypes

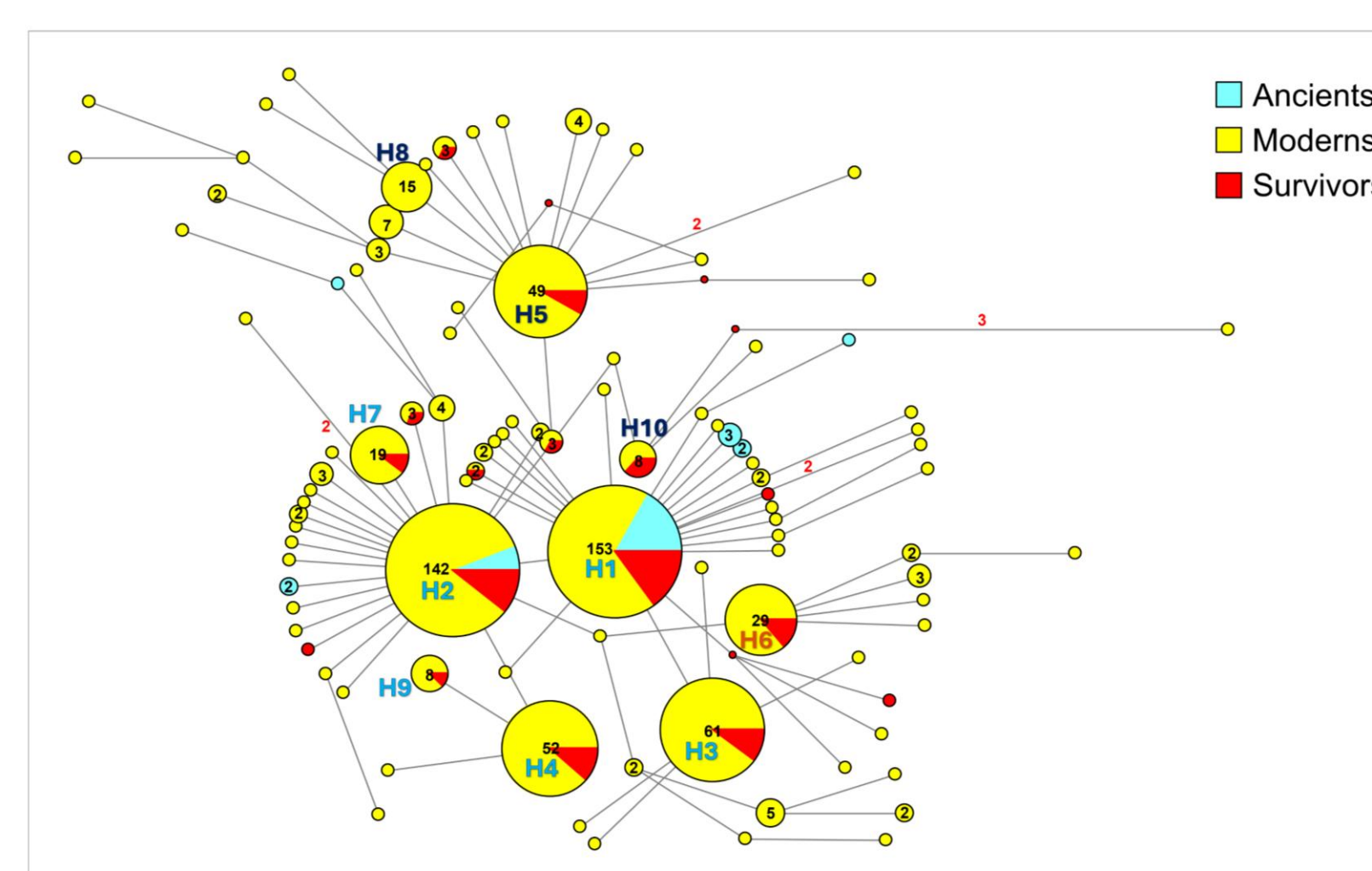


Figure 4. Network analysis

The observed genetic structure appears to primarily reflect ancient evolutionary dynamics, linked to the adaptive radiation during the Pleistocene.

Samples	N	S	H	h	π
Ancients - Sant'Antioco Island (Western Mediterranean)					
1920'	17	2	3	0.412	0.00131
1970'	20	6	6	0.721	0.00366
1990'	6	2	3	0.733	0.00276
Total Ancients	44*	7	7	0.613	0.00263
Moderns					
Total Moderns	552	55	92	0.892	0.00675
Survivors					
Total Survivors	71	13	16	0.837	0.00525

CONCLUSIONS

Despite strong environmental and anthropogenic pressures, including recent MMEs, the mitochondrial genetic variability of *Pinna nobilis* has remained consistently high over time. While natural bottlenecks may promote the selection of adaptive genetic traits, those of anthropogenic origin randomly reduce genetic variability, potentially leading to the accidental loss of traits associated with the species' resilience. The presence of resilient haplotypes and the high mitochondrial genetic variability observed among surviving individuals highlight the urgent need to protect even small remnant populations to ensure the survival of the species.