GENETIC CHARACTERIZATION OF AN ENDANGERED SPECIES OF SICILIAN INLAND WATERS: THE MEDITERRANEAN TROUT (Salmo cettii)

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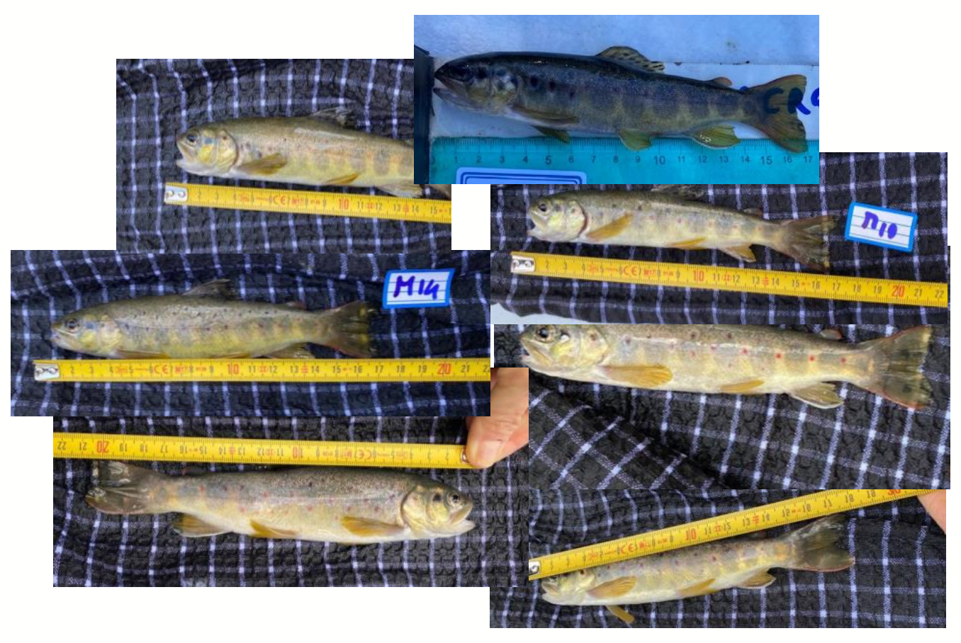
1. INTRODUCTION

Mediterranean trout (*Salmo cetti*), is endemic species belonging to *Salmo trutta* group which is widespread exclusively in Sardinia, Sicily on a few areas of the Italian Tyrrhenian side. Following repopulation carried out with Atlantic trout, its presence on the Italian territory has undergone a strong contraction due to hybridization and introgression effects. At the moment, *Salmo cettii* is on the IUCN Red List, rated as Critically Endangered (CR). The selection of pure breeders with high genetic variability and the storage of their sperm in a cryobank become important tools to make the management of the conservation of endangered species more efficient. Recent pieces of evidence indicated the presence of a specific haplotype in the Iblei Mountains of Sicily suggesting the presence of some conserved areas with the endemic populations (Segherloo et al., 2021). The objective of the work was to genetically characterize a population of Mediterranean trout (Salmo cettii) caught in different waterways of the Iblei Mountains of Sicily in order to verify its level of introgression with the allochthonous Northern Atlantic haplotypes and evaluate the possibility to use this population as a precious resource for future restocking plans.

2. MATERIAL AND METHODS

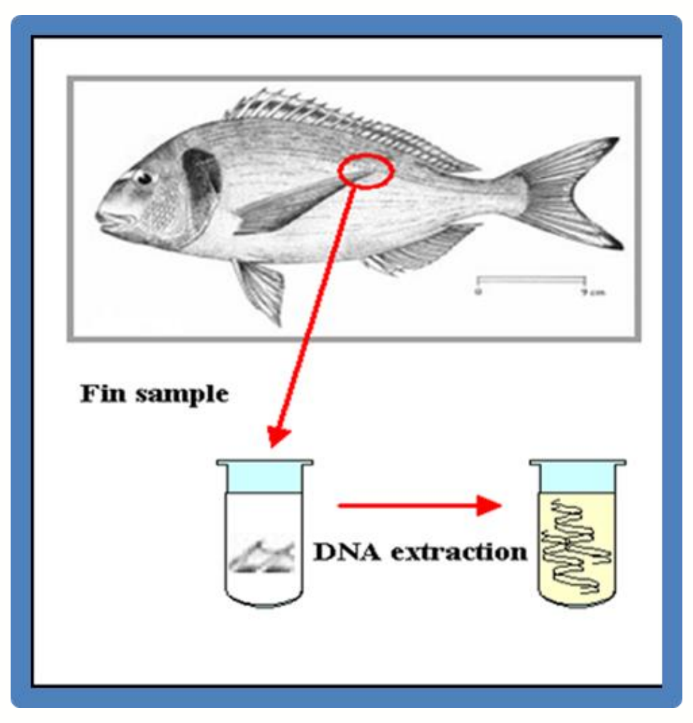


SICILY MEDITERRANEAN TROUT
2 fishing excursions:
- **Torrent Cardinale: 38 individuals;**
- **Torrent Anapo: 19 individuals**

Total N of wild trout : 57

NORTHERN ATLANTIC TROUT:
2 Morgex (Val d’Aosta) : 29 individuals;
Fiumelatte –(Como): 12 individuals
Total N of individuals : 41

Fin CLIP collection and DNA extraction



Genotypic and Phenotypic investigation

D-Loop: PCR, sequencing and alignment with haplotypes in GeneBank

Score	Expect	Identities	Gaps	Strand
549 bits(297)	3e-161	299/300(99%)	0/300(0%)	Plus/Plus

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Query  1    TGAGCCCGCCAGGAACAAGGTGACAGTGGTGGGCGTCGGCCAGGTGGGCATGGCCTGTGC  60
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1    TGAGCCCGCCAGGAACAAGGTGACAGTGGTGGGCGTCGGCCAGGTGGGCATGGCCTGTGC  60

Query  61   TGTCAGTATCCTGCTCAGGGAGCTGGCTGATGAGCTGGCCTTGGTGGATGTGATGGAGGA  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  61   TGTCAGTATCCTGCTCAGGGAGCTGGCTGATGAGCTGGCCTTGGTGGATGTGATGGAGGA  120

Query  121  CAAGTTGAAGGGAGAGATGATGGATCTGCAGCATGGCAGCCTCTTCCTCAAAACGCCCAA  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  121  CAAGTTGAAGGGAGAGATGATGGATCTGCAGCATGGCAGCCTCTTCCTCAAAACGCCCAA  180

Query  181  GATAGTCGCCAGCAAAGACTACTCTGTGACGGCGAACTCTCGTATCGTCGTGGTAACAGC  240
            |||||||||||||||| |||||||||||||||||||||||||||||||||||||||||||
Sbjct  181  GATAGTCGCCAGCAAAGGCTACTCTGTGACGGCGAACTCTCGTATCGTCGTGGTAACAGC  240

Query  241  GGGCGTTCGTCAGCAGGAGGGAGAGAGCAGGTTGAACCTGGTTCAGCGGAACGTCAACAT  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  241  GGGCGTTCGTCAGCAGGAGGGAGAGAGCAGGTTGAACCTGGTTCAGCGGAACGTCAACAT  300
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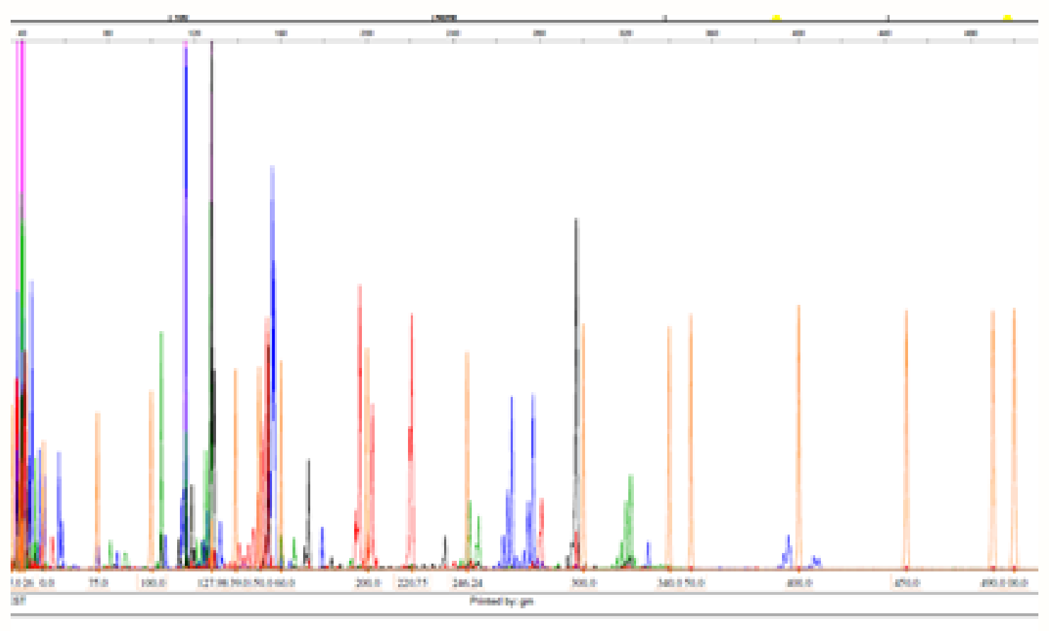
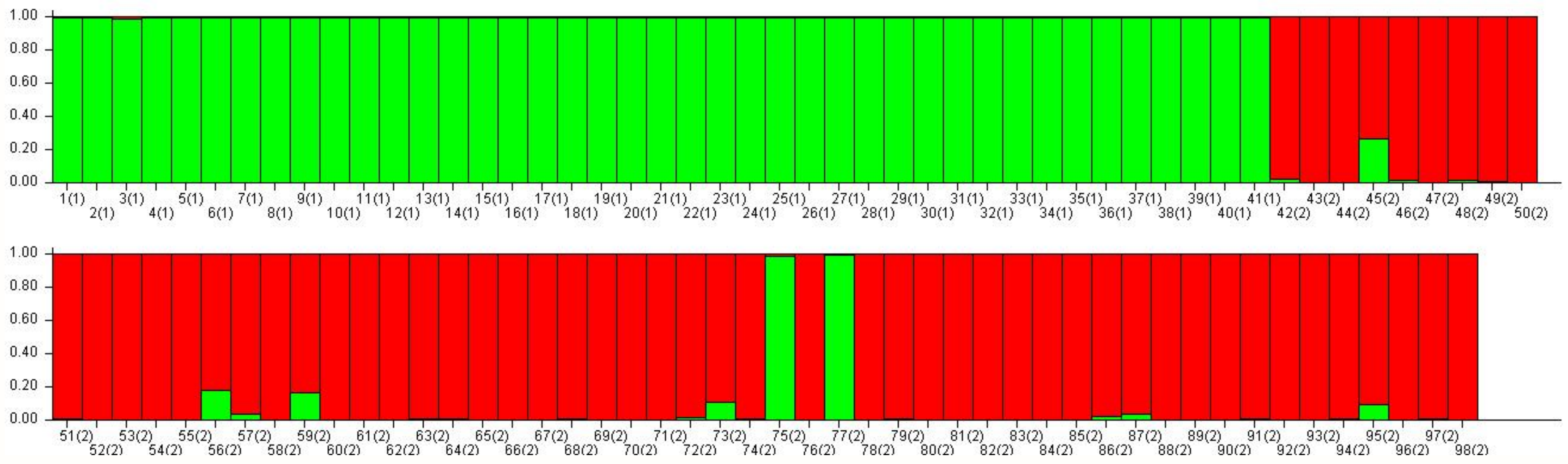
LDH-C1: Diagnostic alleles for the Atlantic (* 90 -guanine allele) and Mediterranean (* 100 -adenine allele) populations of Salmo sp.



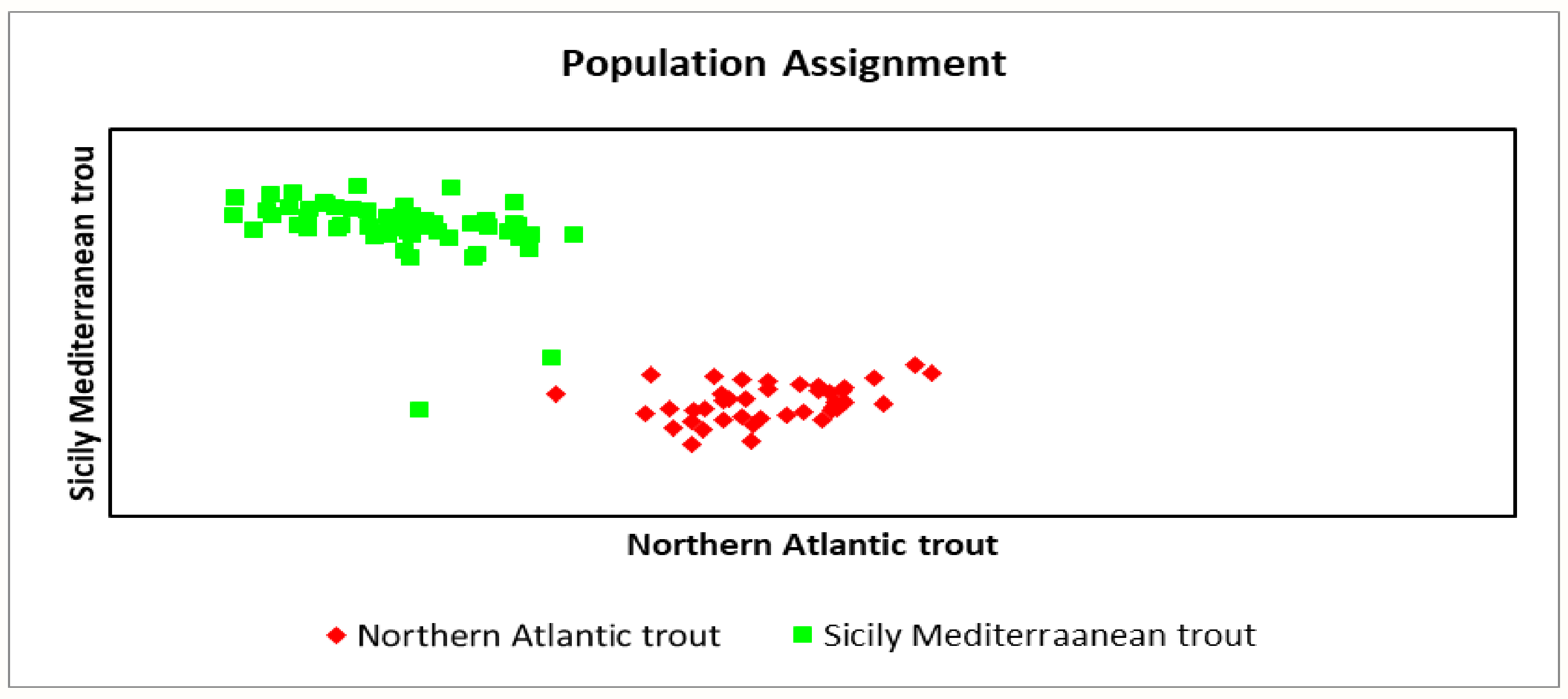
16 loci STRs loci amplified in multiplexes + SDY E2S: sex marker

3. RESULTS

The analysis of the SDY-ES2 locus revealed that, out of the total Sicily Mediterranean trout, 42 were males and 15 females. The expected and observed heterozygosity (He: 0,630 and Ho: 0,627, respectively) was quite low, as was the actual number of alleles (Ne: 2,70). A deviation from a Hardy-Weinberg equilibrium was found for 9 out of 16 loci (p <0.05). STRUCTURE and GenAlEx 6.5 analysis clearly revealed the presence of 2 distinct populations, with few misassignments in the Northern Atlantic populations. The Locus D-loop mtDNA analysis showed that the Sicily Mediterranean population 100% matched with the haplotype sequence present in the database for *Salmo cetti (*GenBank MW251439.1, Segherloo et al., 2021), except for 3 individuals. The screening of the LDH-C1 Locus revealed the presence of 3 individuals with allele LDH-C1 * 90. The hybridization index indicated the 87% of individuals belonging to Class VI (zero potential of hybridization), 6% to Class V and only 8% to Class II (high potential of hybridization) (Pensierini et al., 2006).



Barplots of individual, STR-based admixture as obtained through the Bayesian clustering analysis in STRUCTURE



GenAlEx assignment plot showing distinct clustering of all 2 sampled groups

4. DISCUSSION AND CONCLUSIONS

The results obtained in this study indicated that the Mediterranean trout population sampled in some waterways of Sicily Iblei Mountains showed a very low level of introgression with the Norther Atlantic lineage, despite the numerous releases of the allochthonous individuals occurred in the past. Probably, the extreme conditions of the waterways and the permanence to which they are forced make difficult the survival of the North Atlantic haplotype and the relative hybrids. At the same time, the geographic isolation of individuals, due to the characteristics of the waterways, represent a limit to the genetic variability of the population, which was found quite low. The genetic results obtained in this preliminary study suggests a potential use of the Sicily Iblei Mountains trout population as a precious resource for planning native fish restocking activities.

5. REFERENCE

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