

Multiple introductions and first record of *Phoxinus phoxinus* in the Douro Basin revealed by molecular data

Aina Garcia-Raventós¹, Filipa MS Martins^{1,2}, Maria Filomena Magalhães³, José Pedro Ramião³, Mário Ferreira^{1,4}, Sara Carona³, Franciso Carvalho⁵, Ronaldo Sousa⁵, Elsa Froufe⁶, Amilcar Teixeira⁷, Simone Varandas⁸, Manuel Lima^{1,6}, Pedro Beja^{1,4} & Ana Filipa Filipe^{1,4}

1 CIBIO/InBIO, Centro de Investigação em Biodiversidade e Recursos Genéticos da Universidade do Porto, Vairão, Portugal. 2 Departamento de Biologia, Faculdade de Ciências da Universidade do Porto, Rua do Campo Alegre s/n, 4169-007 Porto, Portugal. 3 cE3c, Centro de Ecologia, Evolução e Alterações Ambientais, Faculdade de Ciências, Universidade de Lisboa, 1749-016 Lisboa, Portugal. 4 CEABN/InBio, Centro de Ecologia Aplicada "Professor Baeta Neves", Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal. 5 CBMA, Centre of Molecular and Environmental Biology, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal. 6 CIIMAR/CIMAR, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Rua dos Bragas 289, 4050-123 Porto, Portugal. 7 CIMO-IPB, Centro de Investigação de Montanha, Instituto Politécnico de Bragança, Campus de Santa Apolónia, 5300-253 Bragança, Portugal. 8 CITAB-UTAD-Centre for Research and Technology of Agro-Environment and Biological Sciences, Forestry Department, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

Our question

Is it possible to know the origin and pathway of biological introductions using molecular data?

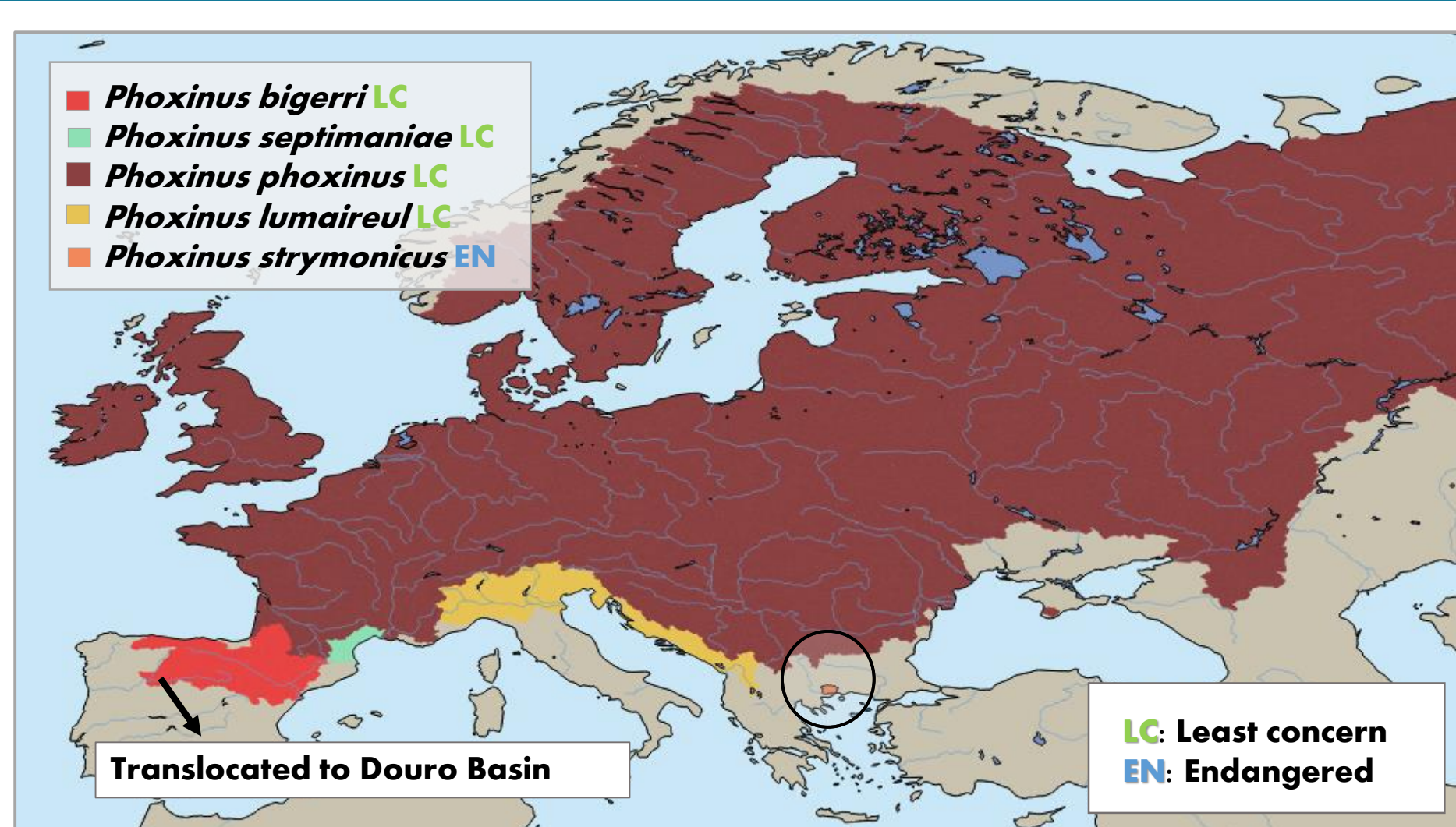
Study aims

1. To identify the species of *Phoxinus* sp. found in Portugal (Sousa stream)
2. To examine the origin of the introduction
3. To discuss the possible pathway of the introduction

Biological invasions are a major threat to global biodiversity. Freshwater ecosystems are particularly vulnerable to anthropogenic introductions.

In the Iberian Peninsula the number of introduced species has been increasing during the last decades. This is the case of the minnows (*Phoxinus* genus), which have been translocated and used as a live bait since the 1900s.

Study case: *Phoxinus* genus



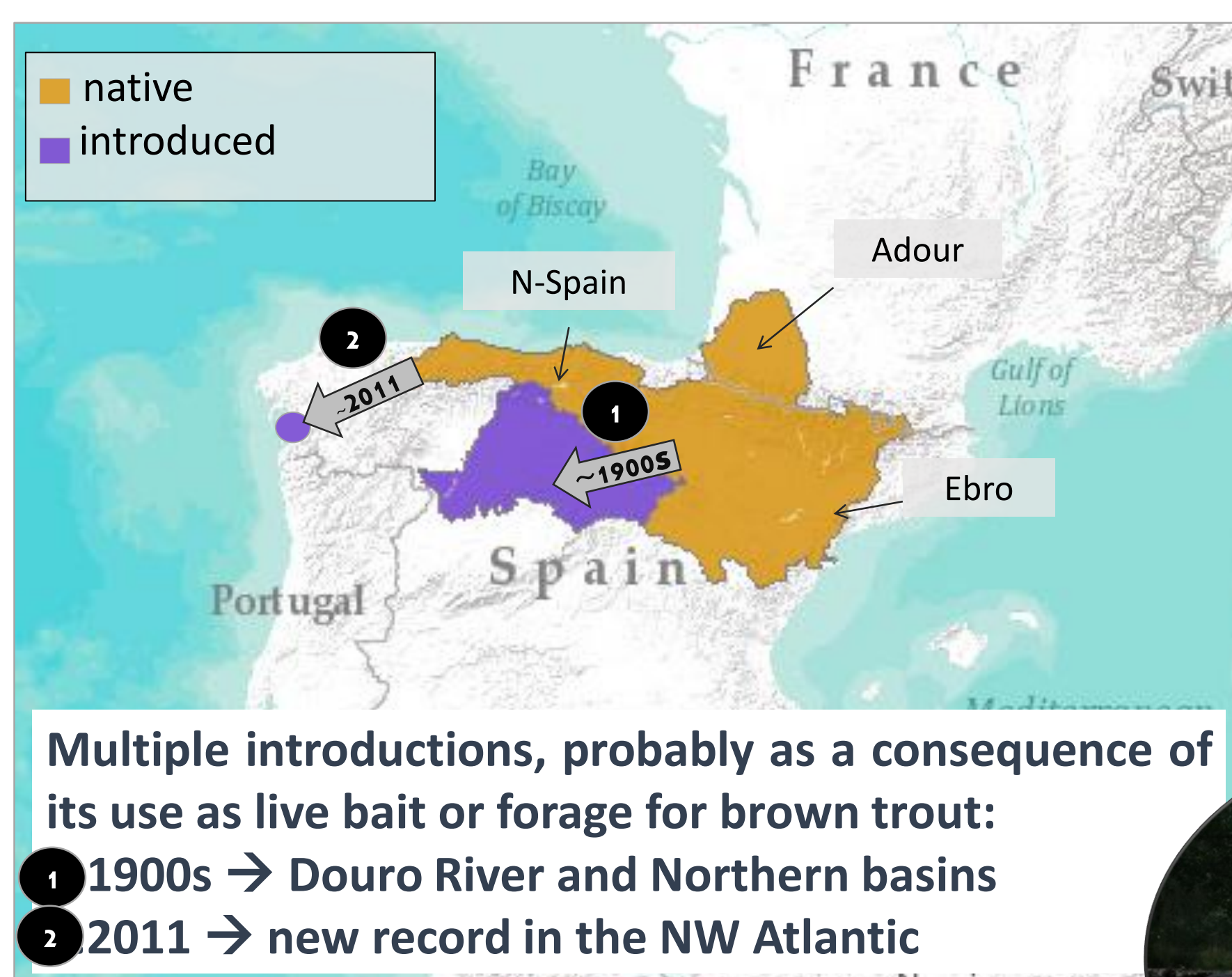
The European minnow *Phoxinus phoxinus* (Cyprinidae) is a species complex previously thought as having a wide-ranging Palearctic distribution.

New species were described recently.

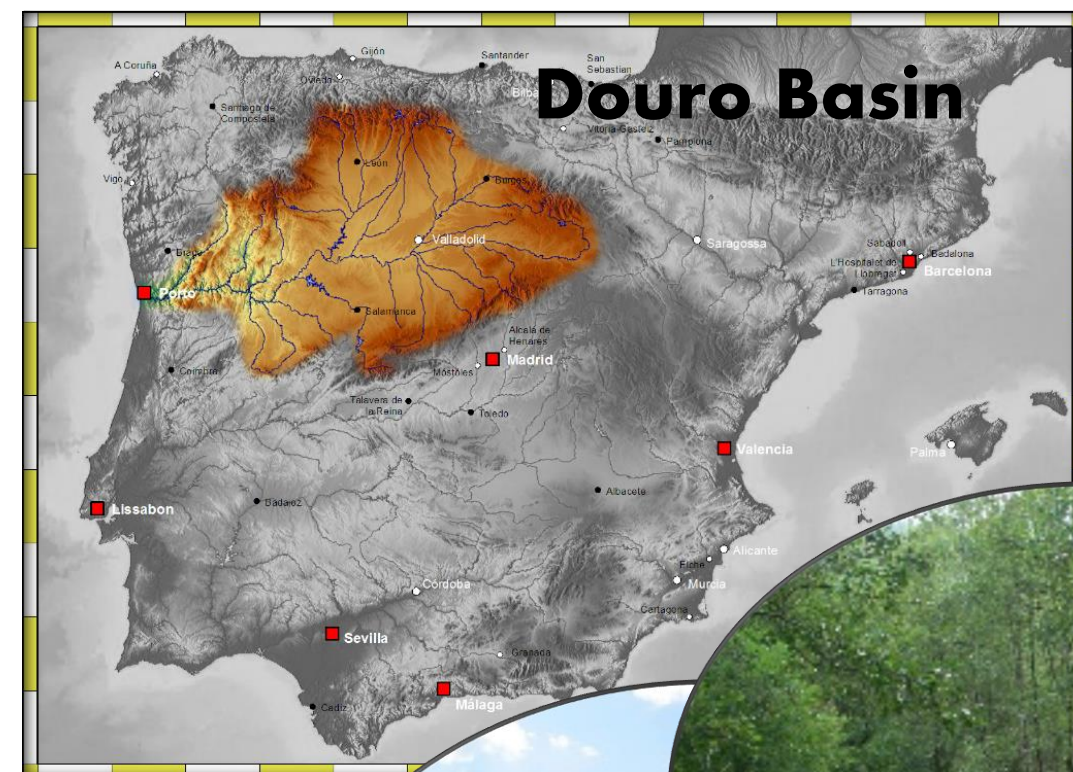
But there is a plasticity in body shape dependent upon habitat which influences some of the diagnosing characters.

Molecular studies point to the existence of 18 cryptic lineages in the genus *Phoxinus*.

Phoxinus bigerri (Kottelat, 2007)



Field and molecular methods

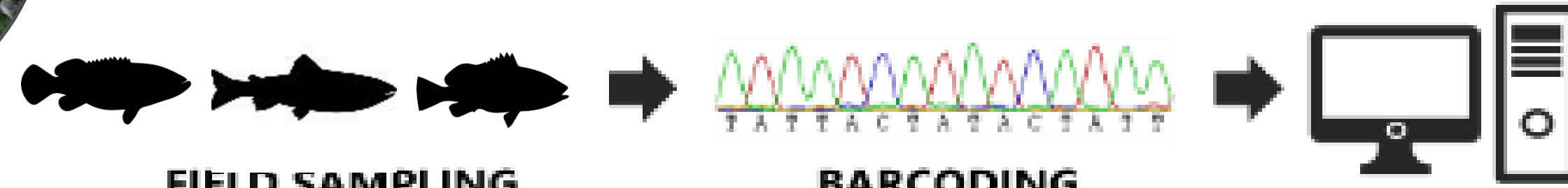


- Electrofishing
- 3 tissue samples (pelvic fin) per species from geographically distant sampling sites

Freshwater fish species detected:

- 14 Native (*Cobitis vettonica* and *Achondrostoma salmantinum* confirmed with molecular data)
- 9 Introduced (*Phoxinus phoxinus* detected with molecular data)

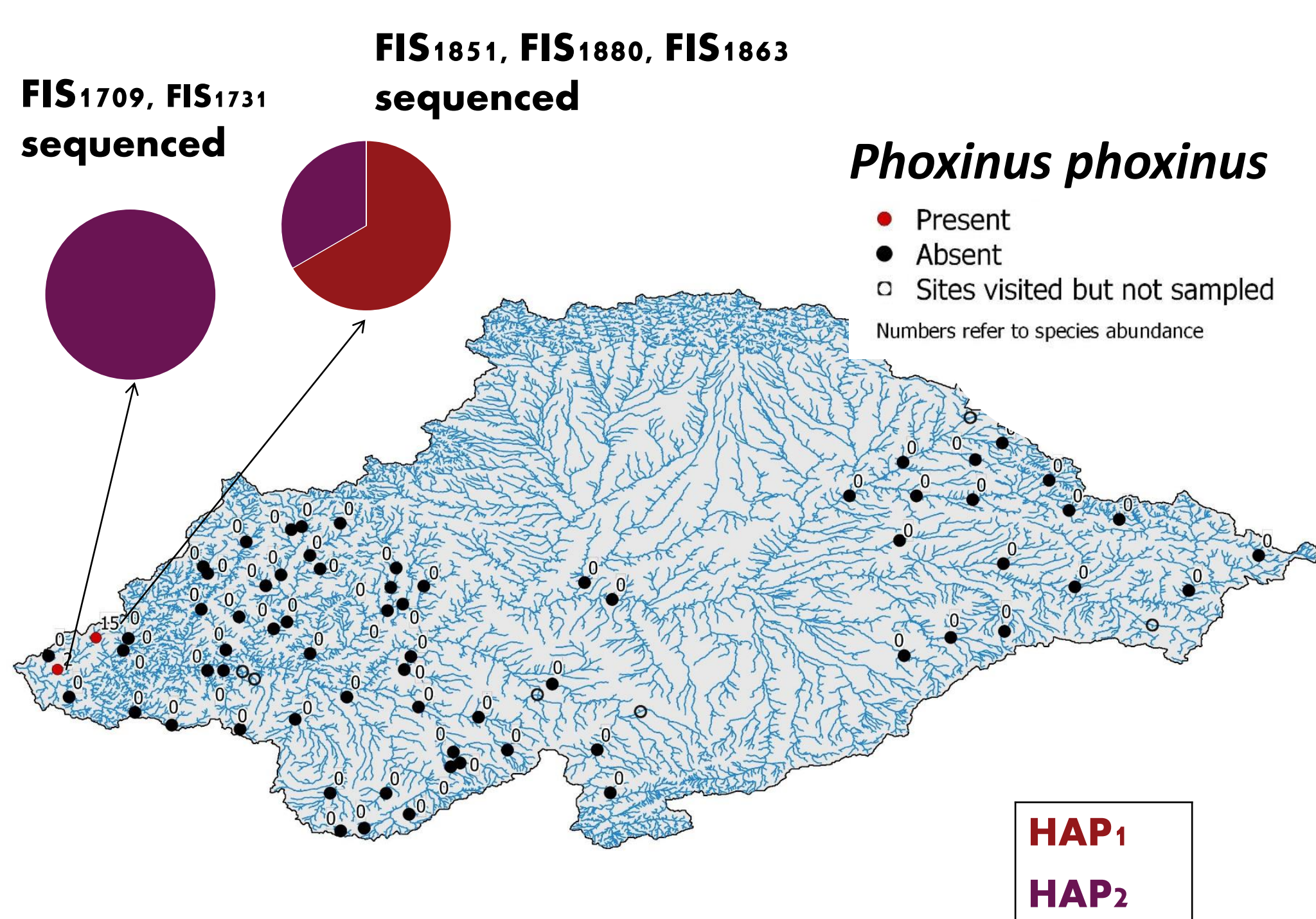
DNA Extraction, Amplification and Sanger Sequencing:



Markers used for species identification: COI (cytochrome c oxidase subunit I) and CYTB (cytochrome B)

Results

Haplotypes



First record *Phoxinus phoxinus* (Douro Basin)

1. The unknown species found in Douro Basin (Portugal) is *Phoxinus phoxinus*
2. The origin of the introduction is Charente Basin (France, closest phylogeny 99%)
3. Pathway of introduction might be related with human activities (sport fisheries by Portuguese immigrants living in France) rather than geographical proximity as in the case of *P. bigerri*

Currently a established population might occur (1 exclusive haplotype occur, individuals with distinct size)

Barcoding can provide great help in distinguishing cryptic and introduced species overlooked by morphology

The study highlights the value of using molecular approaches for detecting new introductions and tracking spread histories

Phylogenetic tree (COI)

