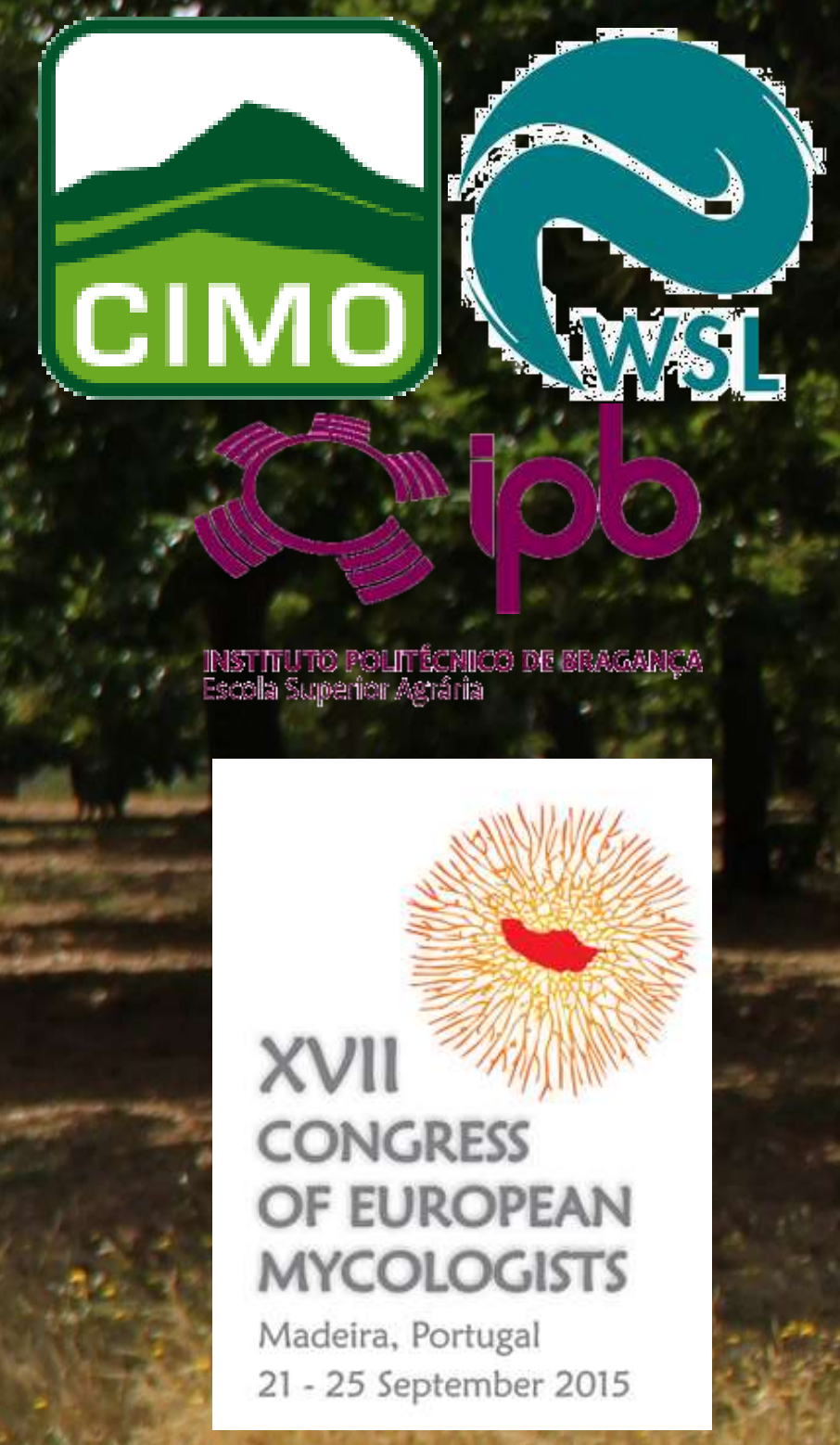


# Genetic diversity of the chestnut blight fungus *Cryphonectria parasitica* and its associated hypovirus in Portugal



Eric Pereira<sup>1</sup>, Simone Prospero<sup>2</sup>, Daniel Rigling<sup>2</sup> e Eugénia Gouveia<sup>1</sup>

<sup>1</sup>CIMO/School of Agriculture, Polytechnic Institute of Bragança, Campus de Santa Apolónia, Apartado 1172, 5301-854 Bragança, Portugal. \*e-mail: eric@ipb.pt  
<sup>2</sup>WSL/Swiss Federal Institute for Forest, Snow and Landscape Research, Zuercherstrasse 111, CH-8903 Birmensdorf, Switzerland



## Introduction

The European chestnut (*Castanea sativa* Mill.) agro-ecosystem has been of high social, economic, and landscape importance in Portugal. Chestnut blight caused by the fungus *Cryphonectria parasitica* is considered a major cause of the decline of chestnut trees across Europe. *C. parasitica* is an ascomycete (Diaporthales) that is native to eastern Asia. Infection of chestnut trees with this pathogen is typically associated with extensive bark necrosis (so-called cankers) on stems and branches, resulting in the subsequent death of the part of the tree above the infection point. Chestnut blight in Portugal was first reported in 1992 and since expanding in distribution. Here, we investigated the invasion history of *C. parasitica* and its associated hypovirus in Portugal.

## Objectives

Characterization of isolates in chestnut stands for virus-infection, vegetative compatibility (vc) type, mating type and microsatellite haplotype. In Portugal.

## Methods and Results

### 1. Chestnut groves



### 2. Isolation, purification and identification

Tissue samples with chestnut blight were collected from all the Chestnut trees (one sample per Chestnut). 133 isolates of *Cryphonectria parasitica* were obtained (38 from Vilar de Peregrinos, 28 from Parada, 28 from Rio Bom and 39 from Sendim).



### 3. Morphological and physiologic characterization of *C. parasitica* population

Were characterized 133 isolates for virus-infection, vegetative compatibility (vc) type and mating type. Four vc-types were observed, although the Portuguese *C. parasitica* population is currently dominated by a single vc type (EU-11). *Cryphonectria* hypovirus 1 (CHV-1) was found in two populations. Genetic analysis of the CHV-1 isolates revealed that three viral strains belong to the Italian subtype and three to the French subtype.

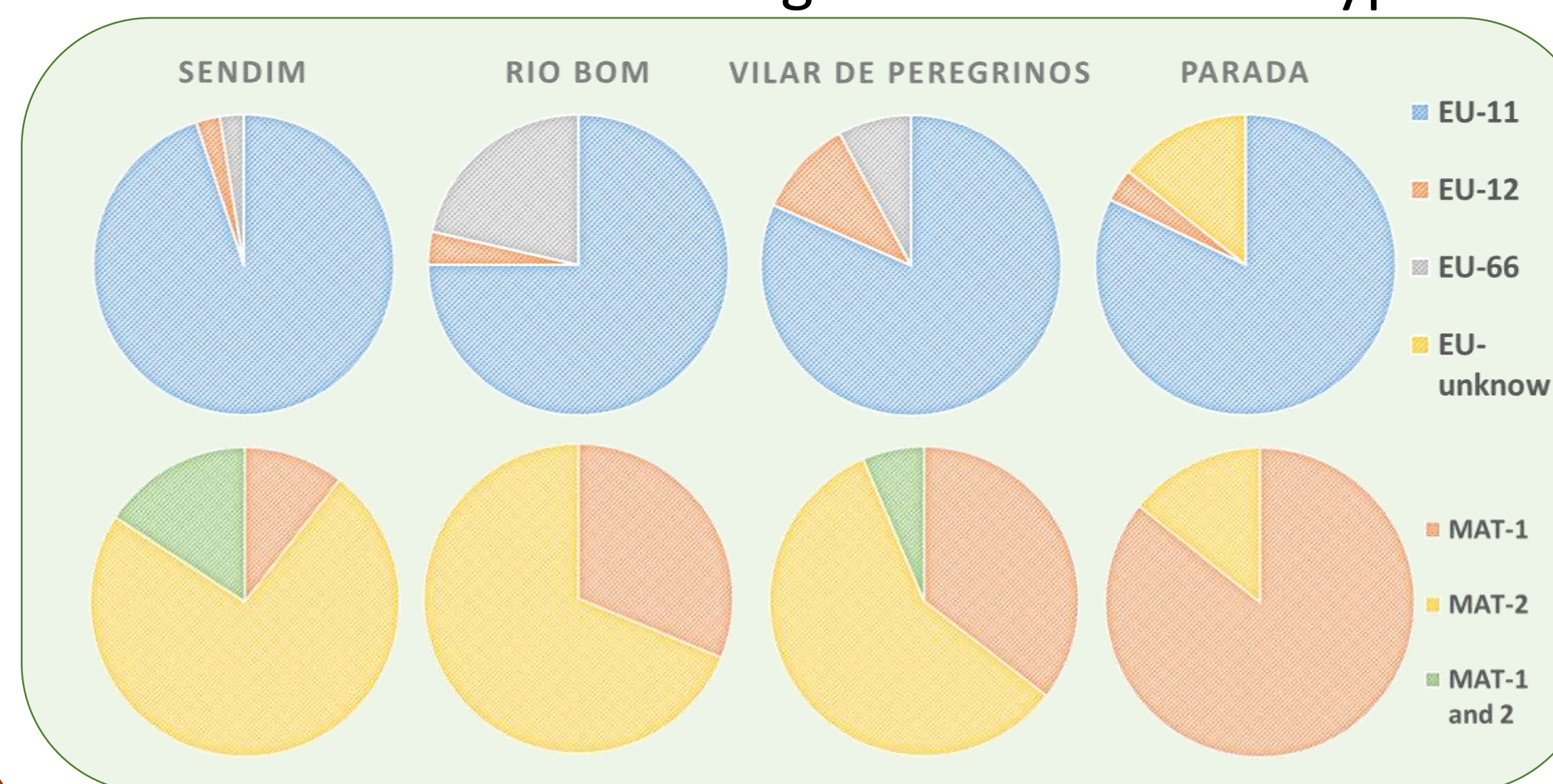


Fig. 1 - Vc-type and mating type

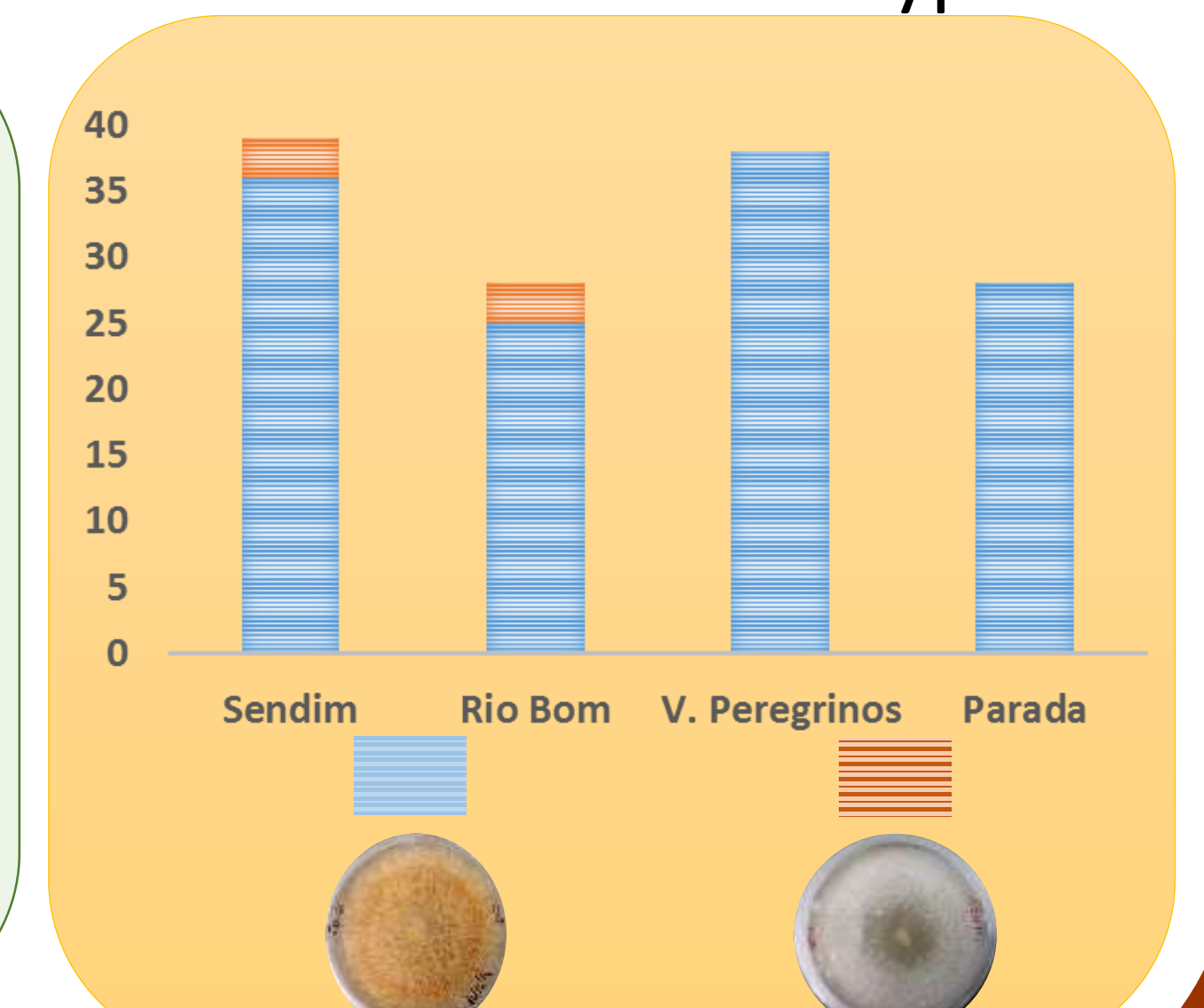


Fig. 2- Isolates and virus-infection

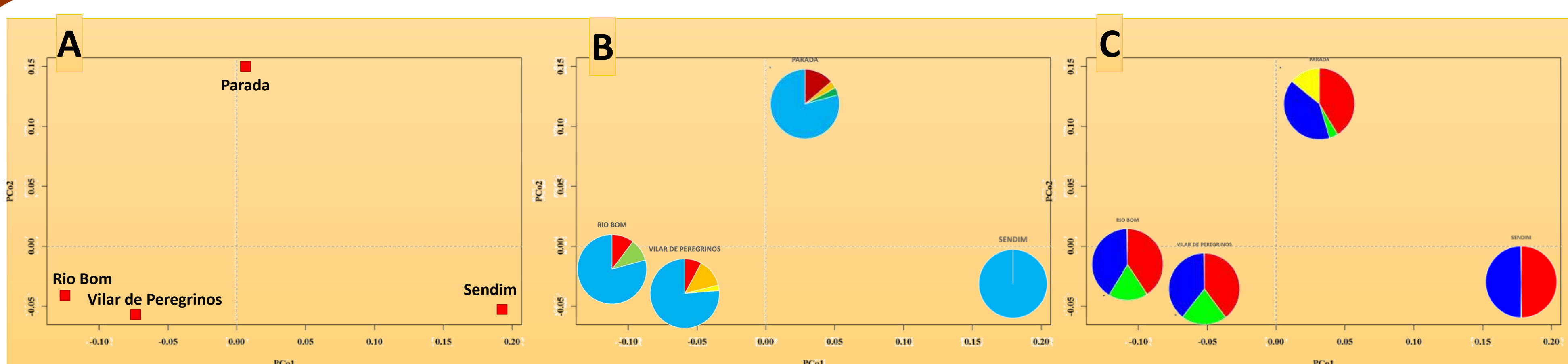


Fig. 3 – Populational analyses of *C. parasitica* (Analyses for principal coordinates; B – Haplotype variation; C – Genotypic variation)

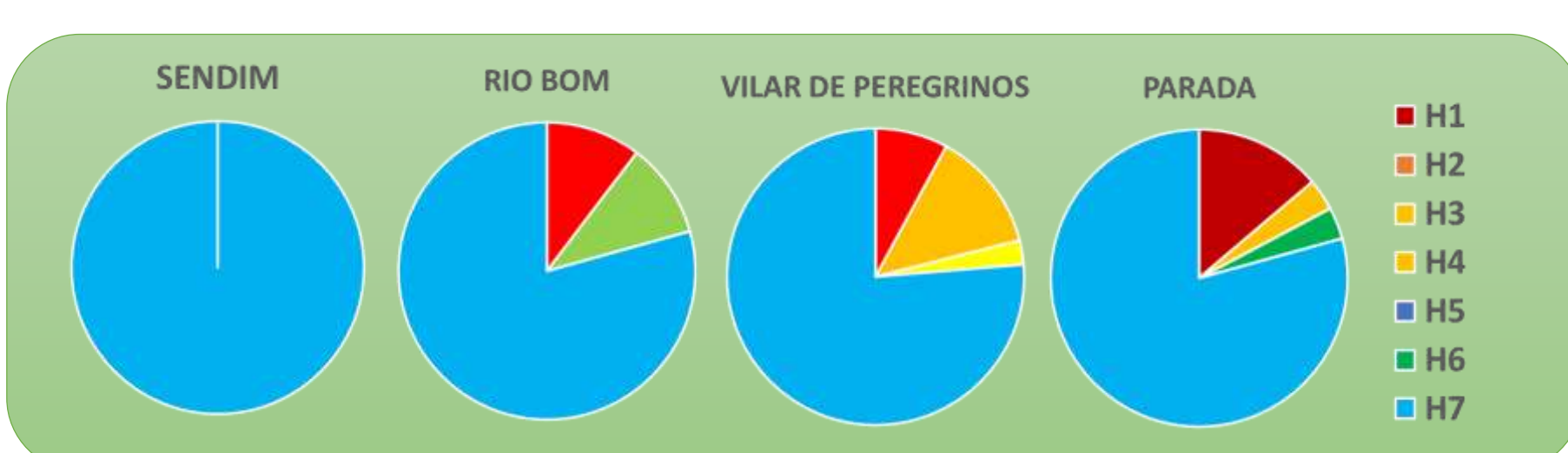


Fig. 4 – Haplotypic distribution for *C. parasitica*

According with the Evanno test, the most probable genetic group (K) is 2, identifying Sergude as a population source. Analyzing structure K=4, it is possible to differentiate populations of Parada relatively to Vilar de Peregrinos and Rio Bom, coinciding with the PCO analysis, and likewise haplotype variation.

### 4. Genetic diversity of *C. parasitica*

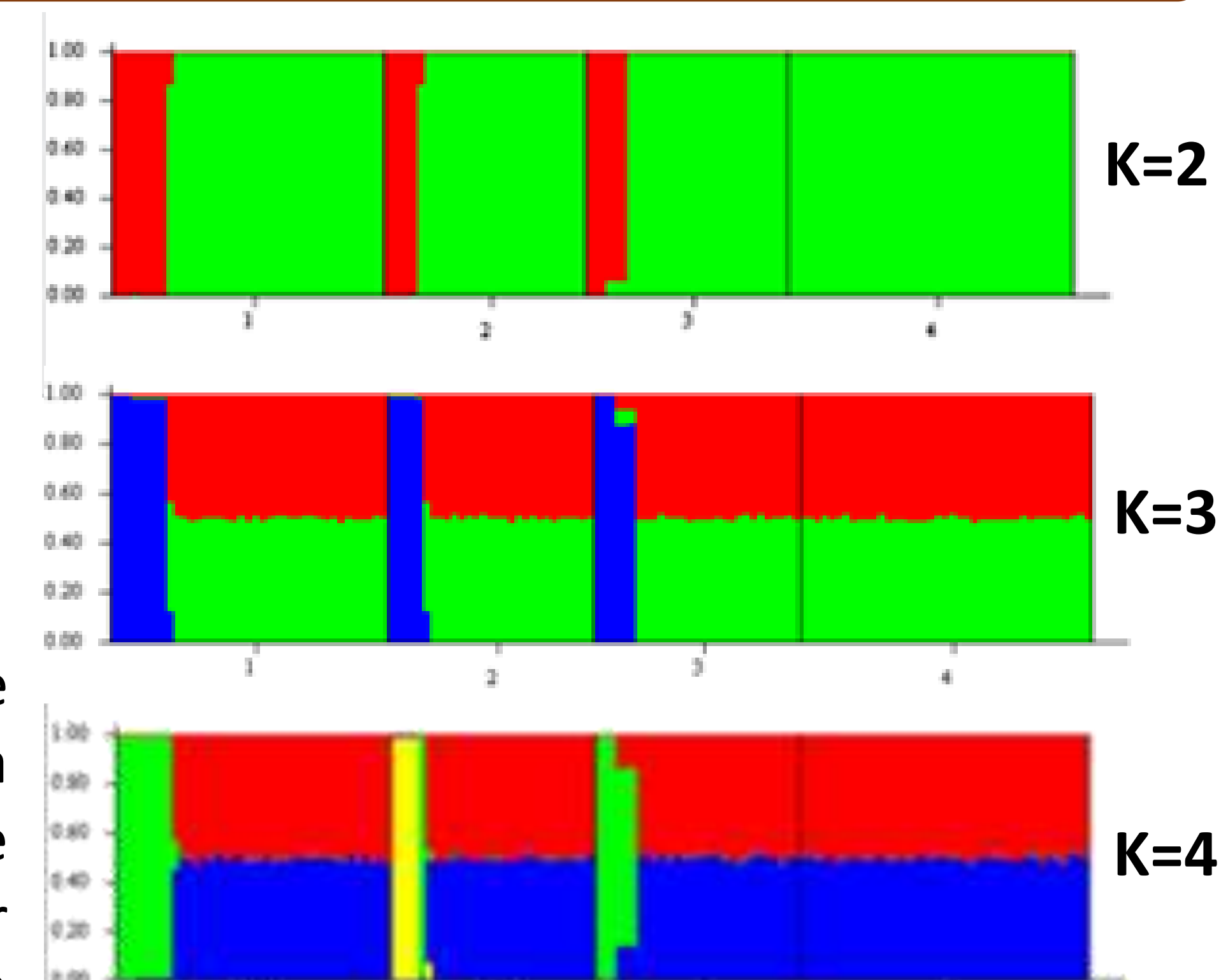


Fig. 5 - Structure graphic presenting the genetic composition the *C. parasitica* for K=2-4

**Conclusion** This study shows that the genetic diversity of the Chestnut blight fungus *C. parasitica* has increased in Portugal. This genetic diversification is mainly due the further natural ou human-mediated introduction of new haplotypes, but in part also to sexual reproduction and recombination of the fungus *in situ*. The diversity of the hypoviruses has different in different regions, suggesting that they have different ancestor.