

New ploidy levels and genome size estimates in wild *Beta* taxa using flow cytometry

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Introduction

It is widely recognized that the conservation of the **Crop Wild Relatives** (CWR) in their wild habitats, where they can continue to adapt and evolve along with their natural surroundings, is fundamental to ensure the continuous supply of the novel genetic material critical for future crop improvement (Prescott-Allen and Prescott-Allen 1988).

The genus *Beta* L. (Amaranthaceae) is an important source of crops, primarily for sugar production. Within a broader research project aiming to select appropriate wild accessions and identify priority localities in which to establish genetic reserves of the wild *Beta* species occurring in Portugal, the goal of this study was to **assess the cytogenetic diversity of wild *Beta* populations**.

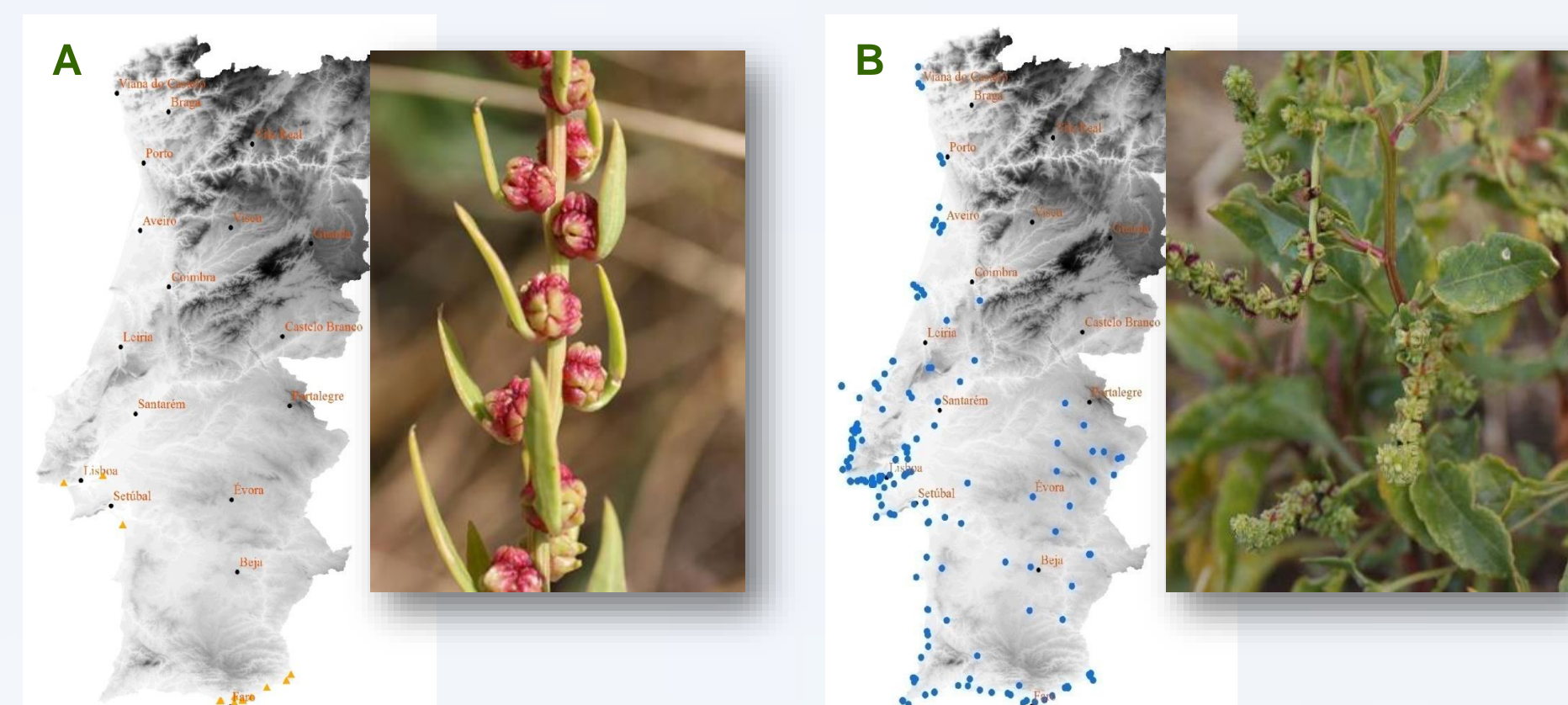


Figure 1. Distribution of the studied *Beta* species in Portugal mainland: **A.** *B. macrocarpa* (mainly in the south); **B.** the widespread *B. vulgaris* ssp. *maritima*.

Results

Flow cytometric analyses

Large scale sampling of natural populations of wild *Beta* species (Fig. 1) was performed across Portugal and genome size and ploidy level estimated using flow cytometry.

Fresh leaves of up to 10 plants were sampled in 16 and 7 populations of *B. vulgaris* ssp. *maritima* and *B. macrocarpa*, respectively. Galbraith *et al.* (1983) procedure was followed and at least 3000 nuclei were analysed per sample. *Solanum lycopersicum* (2C = 1.96 pg DNA) and *Zea mays* (2C = 5.43 pg DNA) were used as internal standards.

- Most populations were ploidy homogenous and diploid (**Fig. 2 and 3**);
- Still, **cryptic diversity** was observed, with three populations bearing several cytotypes (**Fig. 3**);
- One population of *B. vulgaris* subsp. *maritima* had both 2x and 4x plants, two populations of *B. macrocarpa* had two or three cytotypes (2x, 4x and/or 6x);
- Leaves of both species presented variable levels of **endopolyploidy** (**Fig. 2**).

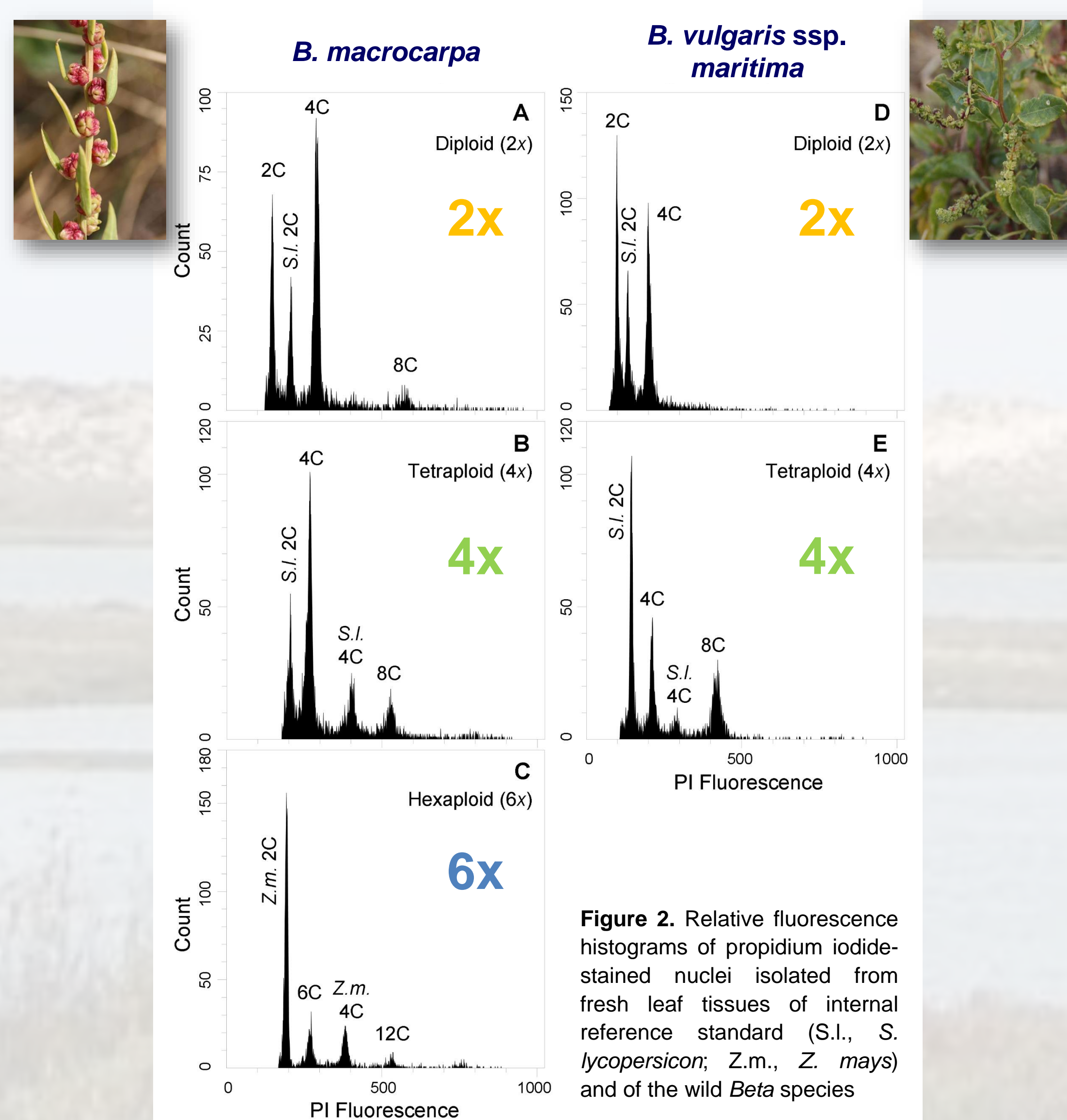


Figure 2. Relative fluorescence histograms of propidium iodide-stained nuclei isolated from fresh leaf tissues of internal reference standard (S.l., *S. lycopersicum*; Z.m., *Z. mays*) and of the wild *Beta* species

Table 2. Holoploid (2C) and monoploid (1Cx) genome size (pg) of wild *Beta*. Values are given as mean and standard deviation of the mean; no. of plants analysed (n) is also provided. Different letters reveal statistically significant differences at $P < 0.05$ after a Tukey's test.

Species	DNA ploidy level	n	Genome size (pg)	
			2C value	1Cx value
<i>Beta macrocarpa</i>	2x	27	1.41 ± 0.027 ^a	0.704 ± 0.013 ^a
	4x	4	2.80 ± 0.048 ^c	0.700 ± 0.012 ^{ab}
	6x	2	4.23 ± 0.049 ^e	0.705 ± 0.008 ^{ab}
<i>Beta vulgaris</i> subsp. <i>maritima</i>	2x	111	1.44 ± 0.035 ^b	0.721 ± 0.018 ^b
	4x	3	2.90 ± 0.053 ^d	0.724 ± 0.013 ^{ab}
Statistical test			$F_{4,146} = 5774.81$, $P < 0.001$	$F_{4,146} = 7.31$, $P < 0.001$

Distribution of ploidy levels

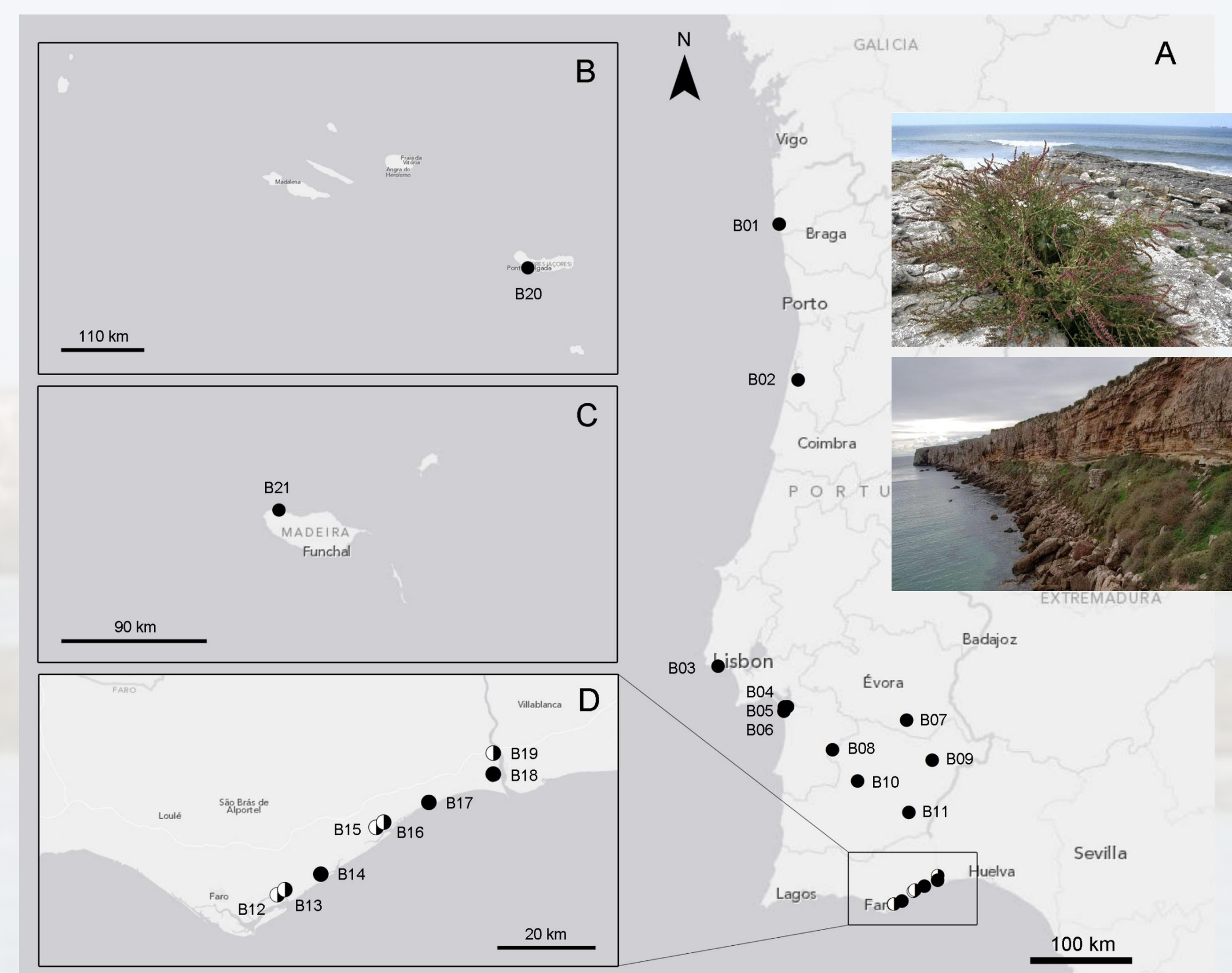


Figure 3. Populations of *Beta macrocarpa* (white) and *B. vulgaris* subsp. *maritima* (black) sampled in Portugal mainland (**A**) and in Azores (**B**) and Madeira archipelagos (**C**). The region of Algarve is enlarged to show the sampling in a clearer view (**D**). Insets provide images of the habitat.

Discussion and Conclusions

The value of crop wild relatives has long been acknowledged and this wild resource has been used to improve crop performance with clear economic benefits. The results obtained in this study provide novel insights on the cytogenetic diversity of *Beta* wild relatives, with the detection of **cytological diversity**, namely 4x and 6x individuals that may constitute potential sources for crop improvement of cultivated beets. Considering the importance of wild crop relatives, it is of pivotal importance that germplasm banks and conservation programs develop direct efforts focused in the conservation of the geographical regions encompassing the mixed-ploidy populations.

References

- Galbraith D.W., Harkins K.R., Maddox J.M., Ayres N.M., Sharma D.P., Firoozabady E.. **1983.** Rapid flow cytometric analysis of the cell cycle in intact plant tissues. *Science* 220 1049–1051.
Prescott-Allen C., Prescott-Allen R. 1988. Genes from the Wild: Using Wild Genetic Resources for Food and Raw Materials, International Institute for Environment and Development, London.

Acknowledgments

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