

Evolutionary and biogeographic history of the Macaronesian *Beta-Patellifolia* species (Amaranthaceae): combining phylogenetic and ecological studies

Maria M. Romeiras^{1,2}, M. Cristina Duarte^{1,3}, Arnaldo Santos-Guerra⁴, Mónica Moura³, Dora Batista^{1,5}, Ana Vieira⁵ & Octávio S. Paulo⁵

¹IICT, Tropical Research Institute, Trav. Conde da Ribeira, 9, 1300-142 Lisbon
²BioFIG, University of Lisbon, Faculty of Sciences, Campo Grande, 1749-016 Lisbon
³CIBIO, Polo das Azores, Dep. of Biology, University of the Azores, 9501-801 Ponta Delgada
⁴ICIA, Unidad de Botánica-Jardín de Aclimatación de La Orotava, Tenerife, Spain
⁵CBA, University of Lisbon, Faculty of Sciences, Campo Grande, 1749-016 Lisbon

Methodology:

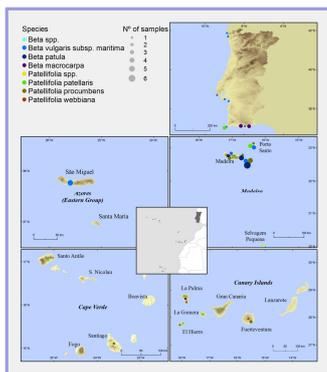
1. Sampling:

Portugal and Macaronesian Islands

43 samples of *Beta*



25 samples of *Patellifolia*



Three species are endemic to the Macaronesian Islands:

- *Patellifolia webbiana* in Canary Islands
- *Beta patula* in Madeira (incl. Porto Santo and Desertas)
- *Patellifolia procumbens* in Madeira; Selvagens; Canary and Cape Verde Islands

2. Molecular Methods:

PCR were performed to amplify the ITS and four cpDNA regions namely:

- *rbcl* DNA barcodes for plants
- *matK*
- *trnL-F*
- *trnH-psbA*

All the samples were sequenced for ITS region (68), but only a subset (27 samples) was selected from each species for sequencing using the cpDNA markers, due to the low variability shown during the first preliminary lab studies

List of primers used for DNA amplification and sequencing

DNA Region	Forward	Reverse	Ta	Source
ITS	TCC TCCGATATGATGAC	GGAGCAAAACCTGACAGG	55	Whitehead et al. (198)
matK	CGACATGATGATGATTC	CTACAGCAGCAGACGAGAT	50	Gutierrez et al. 2002
rbcl	ATGTACACGACACGACGACGAC	CTAAGACGACGACGACGAC	55	Kron & Ibrahim 2007
trnL-F	GCTTAAACGCGGACGAC	ATTGATGACGACGACGAC	58	Tabatake et al. (199)
trnH-psbA	CGCCGCGCGCGGATGACGAC	GTTATGATGACGACGAC	65	Kron et al. (2005)

- Products were sequenced on automatic genetic analyser (ABI Priss310).
- Sequences were aligned (ClustalX v2.0.12, Thompson *et al.*, 1997) and corrected manually (BioEdit, Hall, 1999).
- Data matrices format conversions and concatenations were performed (Concatenator, Pina-Martins & Paulo, 2008).

3. Phylogenetic analyses:

- Different phylogenetic methods were performed: Neighbor Joining (NJ), Maximum Parsimony (MP) and Maximum Likelihood (ML) using PAUP* 4.0b10 and Bayesian Inference using MrBayes 3.1.2.
- ML and BI models were selected according to the AIC [Modeltest 3.7 and MrModeltest 2.2., respectively] in association with PAUP*.
- ML and BI were used to reconstruct phylogenies from the separate (ITS, *rbcl*, *matK*, *trnL-F*, *trnH-psbA*) and combined datasets (all the cpDNA markers).

OUTGROUP:

- Sequences of three representatives of section Corollinae (*Beta nana*; *B. corolliflora*; *B. trigyna*) were obtained from GenBank, as well as sequences of *Aphanisma*, *Habitiza*, and *Oreobliton*, that were used as outgroup

4. Molecular data was complemented with:

Ecology and Taxonomy

- Distribution and ecology of *Beta* species. Characterization of habitats and defining conservation policies
- Taxonomic studies of *Beta* and *Patellifolia* species
- Conservation and management of the targeted populations

Introduction

Understanding the origins and diversification of oceanic island flora has long fascinated evolutionary biologists, ever since Darwin. The Macaronesian Islands, in the North-East Atlantic Region, are considered a hot-spot of biodiversity and contain a large proportion of endemic taxa. Recent molecular studies are providing insights into the patterns of colonization and radiation within the endemic plant species, while the relationships between the wild (i.e. endemics and natives) and the crop species have not been suitably addressed from an evolutionary perspective.

The aim of this study is: (1) to understand the evolutionary and biogeographic history of the seven native species of Macaronesian *Beta-Patellifolia* species complex (Subfamily Betoideae, Amaranthaceae), which are ancestral species of the cultivated sugarbeet species (*Beta vulgaris* subsp. *vulgaris*); and (b) to compare the patterns of diversification identified for Macaronesia with those of the Mediterranean Region to assess to what extent patterns may be generalized across regions.

Results

All datasets of cpDNA, resulting from the analysis of over 2280bp, recover a split between the *Beta* and *Patellifolia* genera

The results of cpDNA revealed low genetic variability for the regions *matK* and *rbcl* (plant DNA barcoding), having a limited value in species delimitation, within *Beta* and *Patellifolia* genera.

The analyses of *trnL-F* and *trnH-psbA* did not add any information to what was already provided by the *matK* + *rbcl* datasets.

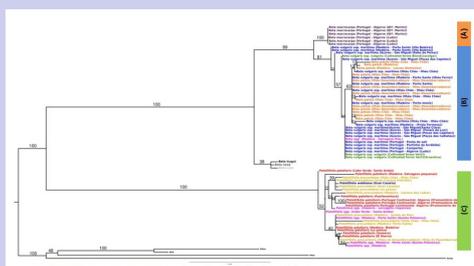


Fig. 3. Maximum Likelihood tree based on 71 ITS sequences of the *Beta* and *Patellifolia*



Fig. 2. Maximum Likelihood tree based on 27 *rbcL* + *matK* sequences of the *Beta* and *Patellifolia* (based on 1330bp)

The nuclear DNA has a more discrimination power than the cpDNA. The ITS dataset (590 pb) show a clear and very well supported division between each of the species:

- (A) *Beta macrocarpa* (from Portugal mainland)
- (B) *Beta vulgaris* subsp. *vulgaris*; *B. vulgaris* subsp. *maritima*; *B. patula*
- (C) *Patellifolia* (*P. patellaris*; *P. procumbens*; *P. webbiana*)

The analysis of the sequencing data from ITS and cpDNA regions indicate that *Patellifolia* genera (formerly included in Section Procumbentes of the genus *Beta*) should be separated from the genus *Beta*.

Our results confirm a close relationship between *Beta vulgaris* subsp. *maritima* samples collected in Madeira arquipelago and *B. patula*, endemic in Madeira.

Beta patula showed low levels of polymorphism, that could be the consequence of a recent divergence from *B. vulgaris* subsp. *maritima* within a limited part of the species distribution area (Madeira Islands).

The phylogenetic trees could be the signature of a recent divergence of *Beta vulgaris* subsp. *maritima* starting from *B. patula* in the Madeira archipelago (including Madeira, Porto Santo and Desertas), and/or contemporary hybridization between both species.

Maximum Likelihood and Bayesian inference analyses produced similar tree topologies that strongly support the ancestral split between *Beta* and *Procumbentes* sections

Despite extensive sampling, low levels of sequence variation are consistently found throughout *Beta* spp., thus preventing complete resolution among taxa

Beta vulgaris subsp. *maritima* shows the highest levels of diversity within the section *Beta*. This is probably associated with its long evolutionary history and its wide geographical distribution, which would have allowed the accumulation of genetic variation

This pattern of low genetic diversification especially found among the Macaronesian endemic taxa may indicate a rapid radiation and/or contemporary hybridization

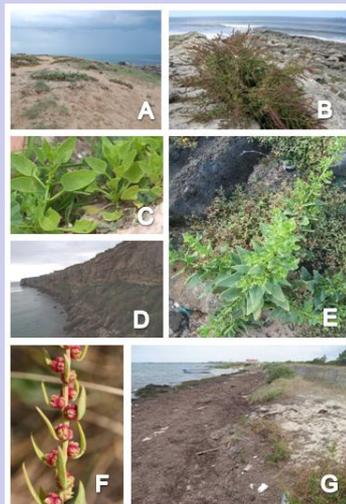


Fig. 4. *Beta-Patellifolia* species from Portugal and Macaronesian Islands. (A–B). *Beta vulgaris* subsp. *maritima* (Portugal mainland); (C–D). *Patellifolia patellaris* (Sagres, Portugal Mainland); (E). *P. procumbens* (Cabo Verde); (F–G). *B. macrocarpa* (Tavira, Portugal mainland).

Conclusions

- Phylogenetic analyses based on nuclear (ITS) and plastid (*rbcl*, *matK*, *trnH-psbA*, *trnL-F*) DNA markers produced similar topologies that suggest a recent colonization event in the Macaronesian Region. Despite extensive sampling, low levels of sequence variation are consistently found throughout *Beta* spp., thus preventing complete resolution among taxa.
- *Beta vulgaris* subsp. *maritima* sampled populations may be a good starting point to track ecologically important candidate genes (e.g. drought and salt tolerance), for a future introgression in marker-assisted selection in the breeding sugarbeets programs

Acknowledgements

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