Leaf shape variation in two sympatric rupicolous species of *Helichrysum* (Asteraceae) from the Balearic Islands, assessed by geometric morphometry

Miquel À. Conesa[1*], Maurici Mus[1], Josep A. Rosselló[2,3]

* ma.conesa@uib.es
[1] Departament de Biologia, Universitat de les Illes Balears, E-07122 Palma de Mallorca, Balearic Islands, Spain.
[2] Jardí Botànic, Universitat de València, c/Quart 80, E-46008, València, Spain.
[3] Marimurtra Bot. Garden, Carl Faust Fdn., PO Box 112, E-17300 Blanes, Catalonia, Spain.

INTRODUCTION

Selection of useful traits to discern among closely related species is not always succeeded, especially when qualitative characters are scarce. This is the case of the western Mediterranean *Helichrysum* sect. *Stoechadina*.

In this work, morphological variation between the two Balearic rupicolous *Helichrysum* species, the endemic *H. crassifolium* and the sympatric widespread Mediterranean *H. pendulum* (Fig. 1), was investigated by linear and geometric morphometric methods. Its correlation was tested against several environmental variables.

Our aims were to detect the causes underlying in the observed leaf variation among both species, and to ascertain to what extent leaf shape is a good taxonomic feature to discriminate between these rupicolous species.

Fig.1. The the two rupicolous *Helichrysum* species inhabiting the Balearic Islands: the endemic *H. crassifolium* (left) and the Mediterranean distributed *H. pendulum* (right).

MATERIAL & METHODS

Geometric morphometrics: On scanned leaves from all plants and populations (Fig. 2 left), 16 landmarks (LMs, Fig. 2 right) were positioned using tpsDIG v. 2.05 (Rohlf, 2006). A Generalized Procrustes analysis (GPA), followed by a thin-plate splines analysis (TPS; Bookstein, 1991), was performed with tpsRELW v. 1.45 (Rohlf, 2007). Dimensionality was reduced by principal component analysis (i.e., relative warp analysis, RWA; Bookstein, 1991).
Environmental parameters: 8 climatic parameters estimated with Cliba2 software (v.3, Guijarro, 1996-99) for each1x1 UTM (Table 1). Altitude, exposition and sea influence were recorded at the sampling sites.
Correlation between RWs data and abiotic parameters: A two-block partial least squares analysis (2B-PLS) was performed with tpsPLS v. 1.18 (Rohlf, 2006).
Isoline representation of leaf parameters: Surfer v. 9.11 (Golden Software Inc., 2010) was used to create isoline maps for the variables explaining most of the leaf variation observed in the sampling.

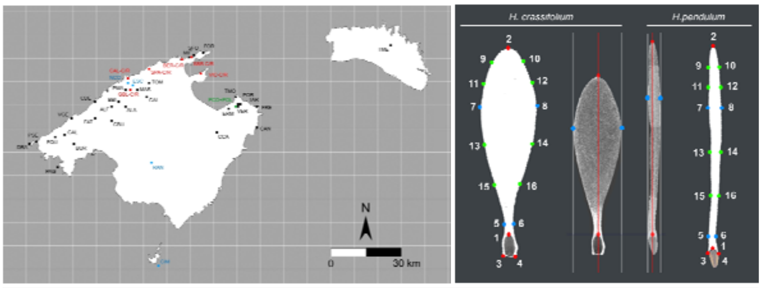

Fig. 2. Left: sampled populations in the Gymnesic islands; *H. crassifolium* in black, *H. pendulum* in blue, both species in red, and two *H. crassifolium* populations in the same 1x1 UTM in green. Right: 16 LMs configuration on representative leaves of both species. Red points correspond to the type I LMs, blue points to the type II LMs, and green points to the type III LMs, according to Bookstein (1991).

RESULTS

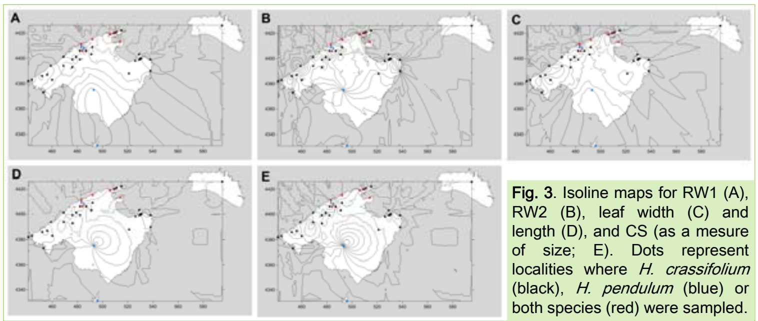

Fig. 3. Isoline maps for RW1 (A), RW2 (B), leaf width (C) and length (D), and CS (as a mesure of size; E). Dots represent localities where *H. crassifolium* (black), *H. pendulum* (blue) or both species (red) were sampled.

	H. crassifolium			*H. pendulum*		
Parameters / Dimensions	D1	D2	D3	D1	D2	D3
Altitude	-0.36391	-0.08257	0.11107	**0.54459**	-0.09647	0.06531
Exposition	-0.09690	0.28878	0.15566	0.00559	0.05406	**0.95268**
Sea influence	**-0.44187**	-0.12828	**0.81330**	**0.44025**	-0.16514	-0.18873
Annual precipitation	-0.36914	**0.64639**	-0.23705	0.21425	-0.09291	0.21085
Average anual temp.	0.33479	0.11030	0.17134	-0.16352	0.30598	0.00027
Daily min temp.	0.36919	-0.08821	0.17776	-0.31091	0.22328	-0.03894
Daily maximum temp.	0.18562	0.37782	0.18063	0.29627	**0.48369**	0.01612
Minimum monthly temp.	0.33731	-0.03349	0.25051	-0.29218	0.23097	0.00428
Evaporation (Linacre)	0.10551	0.37244	0.19041	0.24160	**0.46128**	-0.05059
Potential evapotranspiration (Linacre)	0.08814	0.39591	0.17243	0.27796	**0.46795**	-0.04733
Potential evapotranspiration(Thornthwaite)	0.33337	0.12739	0.15319	-0.17745	0.29888	-0.03839
Covariance	0.0473	0.0098	0.0059	0.0324	0.0239	0.0055
Singular value (%)	**93.8 ***	4.0	1.5	**63.1**	**34.3**	1.8
Cum. singular value (%)	**93.8 ***	97.8 *	99.3 *	63.1	**97.4**	99.2
Correlations	**0.4082 ***	0.1760 *	0.1514 *	0.3602 *	**0.3652 ***	0.1915 *

Table 1. Results of the 2B-PLS analysis. Coefficients of standardized variables are shown for the first three dimensions obtained (D1, D2, D3). Significance values (p) were calculated from 999 random permutation tests (* means p < 0.01).

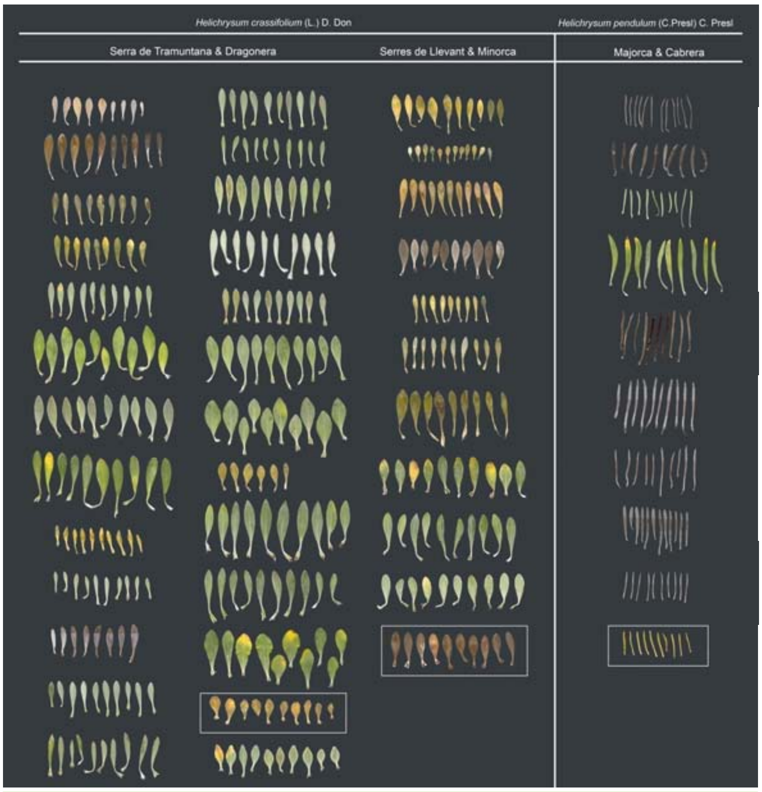

Fig. 4. Leaf variability in *H. crassifolium* and *H. pendulum*. Each 10 leaves correspond to a single plant, and a plant per population is shown. White frames represent the most isolated populations (left to right: *H. crassifolium* from Dragonera islet and Minorca, and *H. pendulum* from Cabrera island).

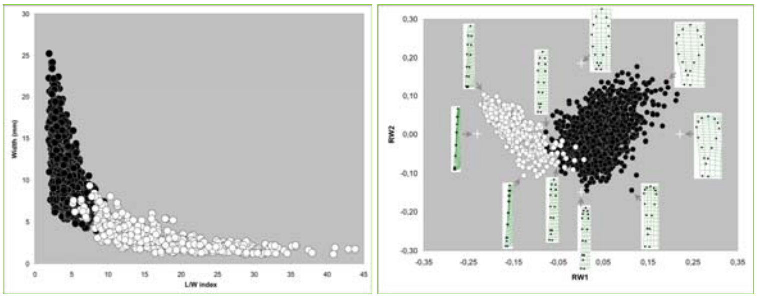

Fig. 5. Leaf measurements in *H. crassifolium* (white) and *H. pendulum* (black). Five leaves per plant and 2-17 plants per population are represented, thus 2085 leaves (up to 1665 for *H. crassifolium* and 420 for *H. pendulum*). Left: linear measurements: leaf width vs. length/width index. Right: geometric morphometric measurements. Axes correspond to the two main principal components (RW1 63.5%, and RW2 16.4% of total variation explained). Green grids arround the plot represent deformations of leaf shape along the axes, corresponding to real leaves, or to the extremes of the axes (white crosses).

CONCLUSIONS

- *H. crassifolium* and *H. pendulum* show different leaf shapes with a high degree of variability, especially in the endemic species (Figs. 4 and 5).
- There is a continuous range of variation between both species leaf shapes (Fig. 5), with plants of difficult adscription based on leaf size and shape found in many locations corresponding neither to locations where both species inhabit nor to climatic or location-related parameters (Table 1).
- Maximum variability between species is detected by RW1 and leaf width (W). However, RW2, leaf length (L) and centroid size (CS, as a mesure of size in geometric morphometrics) show maximum variation within rather than between populations (Fig. 3), suggesting that intra-population events are responsible of such high variability.
- Thus, the wide variation range detected in both rupicolous species better responds to ancient and recurrent natural hybridization events between both species along their ranges; as also suggested by DNA molecular data (unpublished). However, processes related to lineage sorting, or homoplasy, could also be invoked.
- The remaining *Helichrysum* species inhabiting in Majorca and Minorca islands (the non-rupicolous *H. italicum* subsp. *microphyllum* and *H. stoechas*) seem to be involved in such hybridization events and thus, could be responsible of the leaf size and shape variation detected in the rupicolous species, and especially in the endemic *H. crassifolium*.
- Conservation of the endemic *H. crassifolium* should with no doubt be linked to the conservation of the remaining *Helichrysum* species in the Balearic Islands.

References. **Bookstein, F.L.** 1991. Cambridge Univ. Press. **Guijarro, J.A.** 1996—99. http://webs.ono.com/climatol/. **Rohlf, F.J.** (2006, 2007). Department of Ecology and Evolution, State Univ. of New York at Stony Brook.

Acknowledgements. This work has been funded by the projects REN2001-3506-C02-02 and CGL2007-60550/BOS from the Ministerio de Ciencia y Tecnología of the Spanish Government.