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Morphological Variation within and Between taxa of the *Santolina rosmarinifolia* L. (Asteraceae: Anthemideae) Aggregate

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Abstract—Morphological variation within and between taxa of the *Santolina rosmarinifolia* L. aggregate were studied. This work demonstrates that polyploidy and hybridization may be effective evolutionary mechanisms of speciation, promoting the persistence and survival of new species, and ultimately increasing the diversity of plant species. The patterns of morphological variation of the *S. rosmarinifolia* aggregate indicate a recent diversification process for these taxa; as a consequence they are poorly differentiated. The intriguing taxonomic complexity of the taxa of the *S. rosmarinifolia* aggregate can probably be explained to a large degree by recurrent hybridization and subsequent interbreeding of the resulting genotypes, and by the absence of karyotypic divergence and of spatial isolation (except for *S. impressa*) between diploid taxa. Quantitative and qualitative data support two evolutionary lines that are not yet strongly differentiated in this aggregate. On one hand are the diploid and tetraploid cytotypes of *S. pectinata* and *S. ageratifolia*, and on the other the remaining taxa. Two new subspecies, ***S. rosmarinifolia*** subsp. ***castellana*** and ***S. pectinata*** subsp. ***montiberica***, are described from the Iberian Peninsula.

Keywords—Cluster analysis, hybridization, logistic regression analysis, multidimensional scaling, nested ANOVA, stepwise discriminant analysis.

Hybridization and polyploidy generate new races and species, some of which become adapted to new conditions. The ecological conditions are potentially significant in the maintenance, morphological differentiation, diversity, and evolution of the species, which grows in localities determined by historical factors and by its ecology (Thompson et al. 2005).

The *Santolina rosmarinifolia* aggregate comprises nine taxa. Three of them (*S. rosmarinifolia* subsp. *rosmarinifolia* and *S. canescens*, Rivero-Guerra 2009; and *S. pectinata*, Rivero-Guerra 2008a) have two cytotypes: diploid and tetraploid, and the others are diploid (*S. impressa*, Rivero-Guerra 2010; *S. oblongifolia*, *S. semidentata* subsp. *semidentata*, and *S. semidentata* subsp. *melidensis*, Rivero-Guerra 2009), tetraploid (*S. rosmarinifolia* subsp. *arrabidensis*, Rivero-Guerra 2008b) and hexaploid (*S. ageratifolia*, Rivero-Guerra 2008c). *Santolina rosmarinifolia* subsp. *rosmarinifolia* is located in the central Iberian Peninsula, running northwards in the Peninsula in the Occidental and Central System of the Iberian Peninsula (Rivero-Guerra 2008b). The remaining species of the aggregate are located towards the periphery of its distribution. The polyploids of this aggregate have a disjunct distribution, in "islands" in the extreme west and east of the Iberian Peninsula, and a recent polyploidization process occurs in the center and south of the Iberian Peninsula. However, diploid taxa characterize the entire range of the aggregate and show a broader ecological spectrum than that of polyploids (Rivero-Guerra 2008a).

Ellstrand et al. (1996) found that the Asteraceae are one of the most important families in which intrageneric hybridization occurs. Despite the high degree of sympatry and a conservative tendency towards maintaining the general structure of the karyotype in the *S. rosmarinifolia* aggregate's diploid taxa, the species are not separated by strong structural barriers, so that introgressive hybridization occurs (Rivero-Guerra 2009). Hybrid zones interest evolutionary biologists because they constitute natural experiments that can be used to study phenomena related to adaptation and speciation (Freeman et al. 1991). In the center of the distribution of this aggregate, *S. oblongifolia* and *S. rosmarinifolia* subsp. *rosmarinifolia* coexist within the same territory where introgressive hibridization occurs and the hybrid zones spread throughout the Central System of the Iberian Peninsula. As a consequence, several botanists have granted various taxonomic ranks to the populations of the Central System of the Iberian Peninsula, for example, Willkomm and Cutanda, in Willkomm (1859), Willkomm in Willkomm and Lange (1870), Jordan and Fourreau (1870), and Guinea (1970). The taxonomic chaos of the *S. rosmarinifolia* aggregate reflects three problems: first, our limited knowledge of its patterns of variability; second, the multiple definitions of species (de Queiroz 1998; Ereshefsky 2002), and third, the lack of definition provided by rank-based nomenclature (Laurin 2005, 2008). This study attempts to solve the first problem.

The presence of multivalent configurations in diakinesis, bridges and chromosome association in anaphase suggest introgressive hybrid origin for *S. semidentata* subsp. *semidentata*, *S. semidentata* subsp. *melidensis*, *S. canescens*, and for the populations of *S. rosmarinifolia* subsp. *rosmarinifolia* of Toledo, Salamanca, and Zamora provinces (HET populations or central populations in the text) (Rivero-Guerra 2009). In contrast, diploid populations of *S. pectinata* and the remaining populations of *S. rosmarinifolia* subsp. *rosmarinifolia* (ROS populations in the text) show normal meiosis (Rivero-Guerra 2008b,c). Cytogenetic studies in all these taxa (Rivero-Guerra 2008a,b,c, 2009, 2010) indicate that structural changes by translocation and chromosome inversions, and local speciation through autopolyploidy and introgressive hybridization, are the main processes of evolution and diversification in these taxa. They result in a complex mosaic of closely related taxa characterized by a high degree of sympatry, providing an excellent opportunity to investigate the effect of polyploidy and hybridization on morphological variation under natural conditions.

Rieseberg and Ellstrand (1993) and Rieseberg (1995) have demonstrated that hybridization does not always result in morphological intermediacy. The general hypotheses of this work are that *S. semidentata* subsp. *semidentata*, *S. semidentata* subsp. *melidensis*, *S. canescens*, and central populations of *S. rosmarinifolia* subsp. *rosmarinifolia* show morphological signs of hybridization. The study of morphological differentiation between populations is a first step in determining the identity and relative importance of the evolutionary forces promoting or preventing differentiation (Domínguez et al. 1998). Population differentiation may be promoted either by natural selection or by genetic drift. Intense natural selection may

favor different phenotypes in each population in response to differences in selective regimes between localities (Domínguez et al. 1998). Analysis of phenotypic variation of morphological characteristics can be useful in understanding how development of the pleiotropic process, linkage disequilibrium, the environment, genetic drift, and natural selection may generate patterns of character variation within species (Armbruster 1991; Endler 1995). This paper examines, for the first time in the genus *Santolina* L., the relationship between quantitative and qualitative morphological data within and between taxa and within and between groups, and documents the partition of their phenotypic variability. Furthermore, it is an attempt to clarify the taxonomic status of these taxa, determine the degree of intraspecific differentiation in *S. pectinata* and in *S. rosmarinifolia* subsp. *rosmarinifolia*, determine a set of morphological characteristics by which the taxa of *S. rosmarinifolia* aggregate can be separated, and finally, the origin of these taxa is discussed.

Five questions are addressed here: (1) How is morphological variation partitioned between and within taxa, and between and within groups? (2) Are there differences in morphological data between closely related taxa of *Santolina*? (3) Are differences between taxa associated with ploidy level? (4) Does intraspecific variation in *S. rosmarinifolia* subsp. *rosmarinifolia* and in *S. pectinata* support their intraspecific delimitation? (5) Do *S. semidentata* subsp. *semidentata*, *S. semidentata* subsp. *melidensis*, *S. canescens*, and central populations of *S. rosmarinifolia* subsp. *rosmarinifolia* show morphological signs of hybridization?

Materials and Methods

Sampling—The study sampled 38 ROS populations (458 individuals) and 18 HET populations (187 individuals) of *S. rosmarinifolia* subsp. *rosmarinifolia*, 44 of *S. canescens* (507 individuals), 41 (236 individuals) and 20 (173 individuals) of diploid and tetraploid cytotypes of *S. pectinata* respectively, six of *S. impressa* (87 individuals), two of *S. ageratifolia* (62 individuals), four of *S. oblongifolia* (96 individuals), 25 of *S. semidentata* subsp. *semidentata* (185 individuals), one of *S. semidentata* subsp. *melidensis* (26 individuals), two of *S. rosmarinifolia* subsp. *arrabidensis* (55 individuals), and eight of the hybrid complex, where *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. oblongifolia*, and their putative hybrids grow together or one parent grows together with the presumed hybrids or the populations are formed exclusively by presumed hybrids (referred to as 'hybrid complex' in the text; 251 individuals). These are detailed in Appendix S1. The populations were sampled in the summers of 1995–1999. Figure 1 shows the approximate geographical distribution of the taxa studied. All the specimens were collected by the author.

Morphometry of the Natural Populations—Quantitative and qualitative characteristics studied are shown in Appendices S2 and S3 respectively. They were selected according to their common use in *Santolina* taxonomy, and variability within and between taxa was observed. Plant diameter and plant height were measured in the field, in natural populations. The lobes were defined as each segment or division of the leaf's limb. Leaf width, involucral bract width, interseminal bract width, and apical width of the appendage of the involucral bracts were measured at the widest point. Lateral width of the appendage of the involucral bracts was measured at the midpoint of the bracts.

The characteristics were evaluated in relation to the position of (1) the leaves on flowering and sterile stems: basal (which arise from the base of the flowering and sterile stems), lower, middle, upper, and fascicular (which arise from the axils of the cauline leaves of the sterile stems); (2) involucral bracts (outer, middle, and the two well-defined inner rows), and interseminal bracts; and (3) the flowers and achenes on the involucre: peripheral and central. The involucral bracts, flowers, and achenes were chosen at equidistant points around the capitulum.

For each characteristic (quantitative and qualitative), except for plant diameter and plant height, three observations were made on each individual. For each individual, the average of the three quantitative measurements and the mean of the frequency of the each qualitative characteristic were determined. The observations and measurements were performed under a binocular microscope, and measurements were made with a digital calibrator. The terminology of Stearn (1996) was used.

Each individual (specimen) measured was treated as an independent operational taxonomic unit (OTU) for the entire statistical test, although dissimilarity between groups of OTUs (taxa, population, and individuals) was also measured.

Statistical Methods—Quantitative and qualitative characteristics were studied jointly and separately to assess the different behaviour of both sorts of data (Greimler et al. 2004). First, resemblances between OTUs were quantitative using Gower's coefficient for mixed data (Gower 1971). A principal component analysis (PCA) and multidimensional scaling were employed to explore the correlation structure of the quantitative and qualitative characteristics, respectively, and to assess the relative importance of each characteristic to dissimilarity between taxa. Both procedures allowed the variance within the phenotypic characteristics to be considered simultaneously (Sargent et al. 2004).

The nested ANOVA technique was employed to analyze the variation within and between taxa and within and between groups of the PCA factors and of the dimensions provided by multidimensional scaling. The nested MANOVA technique was also employed to analyze the partition of variance of each of the quantitative characteristics within and between taxa, and the post hoc test was carried out using the Bonferroni method.

Stepwise discriminant analysis was performed to determine (1) the group each individual belonged to with the highest probability, (2) dissimilarity between groups, and (3) the importance of each quantitative characteristic for taxon differentiation. The contribution of the qualitative characteristics to taxon differentiation was established by means of a logistic regression analysis (a Bonferroni correction was applied).

Finally, the relationships among all populations studied were explored by means of cluster analysis. Cluster analysis was performed to represent the relationships among OTUs (populations) using the complete survey of the characteristics and with the characteristics of greatest contribution to taxon differentiation. Three different sorting algorithms were used to distinguish between data-dependent and method-dependent features of the results (following Dickinson and Phipps 1985): single linkage, complete linkage, and the unweighted pair-group method with arithmetic averaging (UPGMA; Sokal and Michener 1958) were used, with Euclidean distances as the criterion for clustering for quantitative data (Appendix S4), for the mean of the frequency of the qualitative data (Appendix S5), and for the characteristics of greatest contribution to taxon differentiation (qualitative and quantitative) based on Gower's coefficient. Some minor differences were found among them, and only UPGMA dendograms are presented (the phenograms show all populations but the number of OTU labels is only about one-half of the number of OTUs). Futhermore, the cophenetic value matrix was compared with the initial similarity matrix by Mantel's test correlation coefficient.

The techniques were applied after ensuring that requirements regarding data distribution were met for (1) multivariate (MANOVA) or univariate (ANOVA) normality by means of the Kolmogorov-Smirnov and Shapiro-Wilk contrast; (2) homogeneity of variance by means of the Barlett-Box contrast in the multivariate models, the Kaiser-Meyer-Olkin (KMO) test prior PCA analysis (Almeida-Pinheiro de Carvalho et al. 2004), and the Levene test in the univariate models (Dytham 2003; Grafen and Hails 2003); and (3) the presence of rare values or outliers, which were detected graphically, MANOVA being especially sensitive to them. The quantitative characteristics were square-root-transformed prior to the analysis to increase the homogeneity of variance, whereas the comparison with the results obtained from the original characteristics indicated only minor differences.

The statistical packages STATISTICA version 6.0 (StatSoft, Tulsa, Oklahoma), and SPSS version 14.0 (SPSS, Chicago, Illinois), were used. The correlation coefficient was considered high when $r \geq 0.75$, moderate when $0.50 \leq r < 0.75$, and low when $r < 0.50$. Results were deemed significant if the probability of the null hypothesis was less than 0.05.

Results

Descriptive Statistics—Quantitative Characteristics—The variation coefficient of the vegetative characteristics is higher than that of the flower characteristics (Table 1). This coefficient is lower in *S. ageratifolia* and *S. impressa* for vegetative and flower characteristics, respectively than in the other taxa. The polyploid taxa present a coefficient of variation of the vegetative characteristics lower than those of the diploids (Table 1).

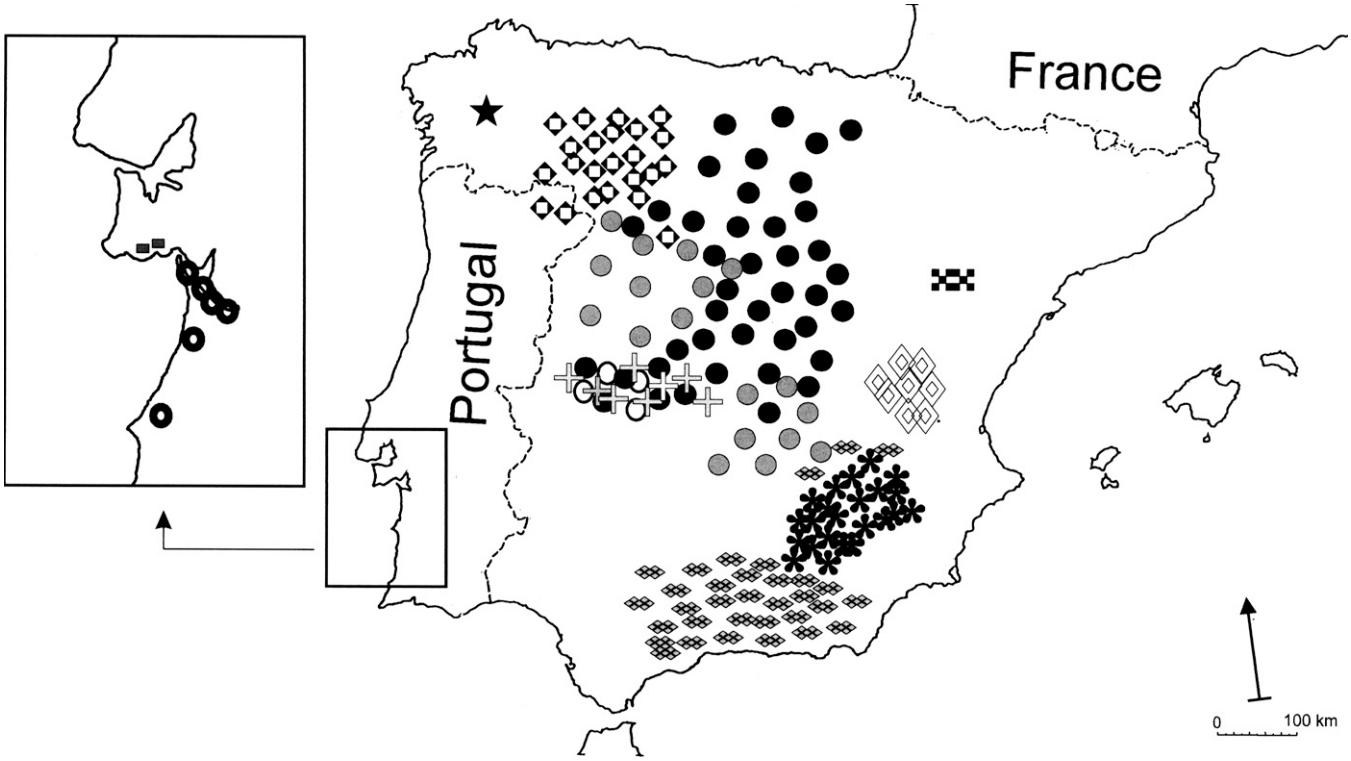

FIG. 1. Approximate geographical distribution of the taxa studied: ROS (solid circle) and HET (gray circle) populations of *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. rosmarinifolia* subps. *arrabidensis* (square), *S. oblongifolia* (thin-bordered circle), *S. semidentata* subsp. *semidentata* (diamond), *S. semidentata* subsp. *melidensis* (star), *S. canescens* (flower), diploid (asterisk) and tetraploid (bordered diamond) cytotypes of *S pectinata*, *S. ageratifolia* (multiplacation sign), *S. impressa* (bold-bordered circle), and the hybrid complex of *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. oblongifolia* and their putative hybrids (plus sign).

Individual diameter is greater in *S. impressa* than in the other taxa. Plant height is greater in *S. semidentata* subsp. *semidentata* and lesser in *S. semidentata* subsp. *melidensis*, S. *ageratifolia*, *S. oblongifolia*, and in diploid and tetraploid cytotypes of *S. pectinata* than in the other taxa. Length of the flowering stems is greater in *S. impressa* and lesser in *S. semidentata* subsp. *melidensis* and S. *ageratifolia* than in the other taxa (Fig. 2A). Sterile stems show little variation between taxa, and are lower in *S. semidentata* subsp. *melidensis* and in *S. ageratifolia* than in the other taxa. Stem peduncle is shorter in *S. impressa* than in the other taxa (Fig. 2B). Number of primary branches and diameter of the flowering stems show little variability between taxa (Fig. 2B). Diameter of the capitulum is smaller in *S. semidentata* subsp. *melidensis* than in the other taxa (Fig. 2C). Capitulum height is greater in diploid and tetraploid cytotypes of *S. pectinata* and in *S. ageratifolia* than in the other taxa, and lesser in *S. semidentata* subsp. *melidensis*, *S. semidentata* subsp. *semidentata*, and in *S. oblongifolia* than in the other taxa (Fig. 2C). Receptacle diameter is smaller in *S. semidentata* subsp. *melidensis* than in the other taxa (Fig. 2C). Receptacle height is slightly greater in *S. rosmarinifolia* subsp. *arrabidensis* and *S. ageratifolia* than in the other taxa (Fig. 2C).

Leaf length (except that of upper leaves) is greater in *S. rosmarinifolia* subsp. *arrabidensis* than in the other taxa (Fig. 3A). Length of the leaves of the flowering and sterile stems is less in *S. semidentata* subsp. *melidensis* and in tetraploid cytotypes of *S. pectinata* than in the other taxa (Fig. 3A). Width of the lower and middle leaves of the flowering and sterile stems is greater in *S. oblongifolia* and in diploid cytotypes of *S. pectinata* than in the other taxa (Fig. 3B). Width of the basal and fascicular leaves is greater in *S. oblongifolia* and in the hybrid complex than in the other taxa (Fig. 3B). Lobe number of the leaves is higher in *S. impressa* and lower in *S. oblongifolia* and in the hybrid complex than in the other taxa (Fig. 4A). Lobe number of the leaves is higher for basal and fascicular leaves in all taxa (Fig. 4A).

TABLE 1. Means of variation coefficient of the vegetative and reproductive characteristics of ROS and HET populations of *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. rosmarinifolia* subsp. *arrabidensis* (ARR), *S. semidentata* subsp. *semidentata* (SEM), *S. semidentata* subsp. *melidensis* (MEL), *S. impressa* (IMP), diploid (PEC-18) and tetraploid (PEC-36) cytotypes of *S. pectinata*, *S. canescens* (CAN), *S. ageratifolia* (AGE), *S. oblongifolia* (OBL) and the hybrid complex of *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. oblongifolia*, and their putative hybrids (ROB).

	Characteristic	
Taxa	Vegetative	Reproductive
ROS	47.97	15.27
ARR	44.83	10.72
HET	67.92	16.85
CAN	66.50	14.14
SEM	58.54	15.95
MEL	65.61	19.05
IMP	43.22	10.08
AGE	28.22	11.44
PEC-18	35.47	14.38
PEC-36	33.79	12.42
OBL	63.26	15.00
ROB	63.34	15.02

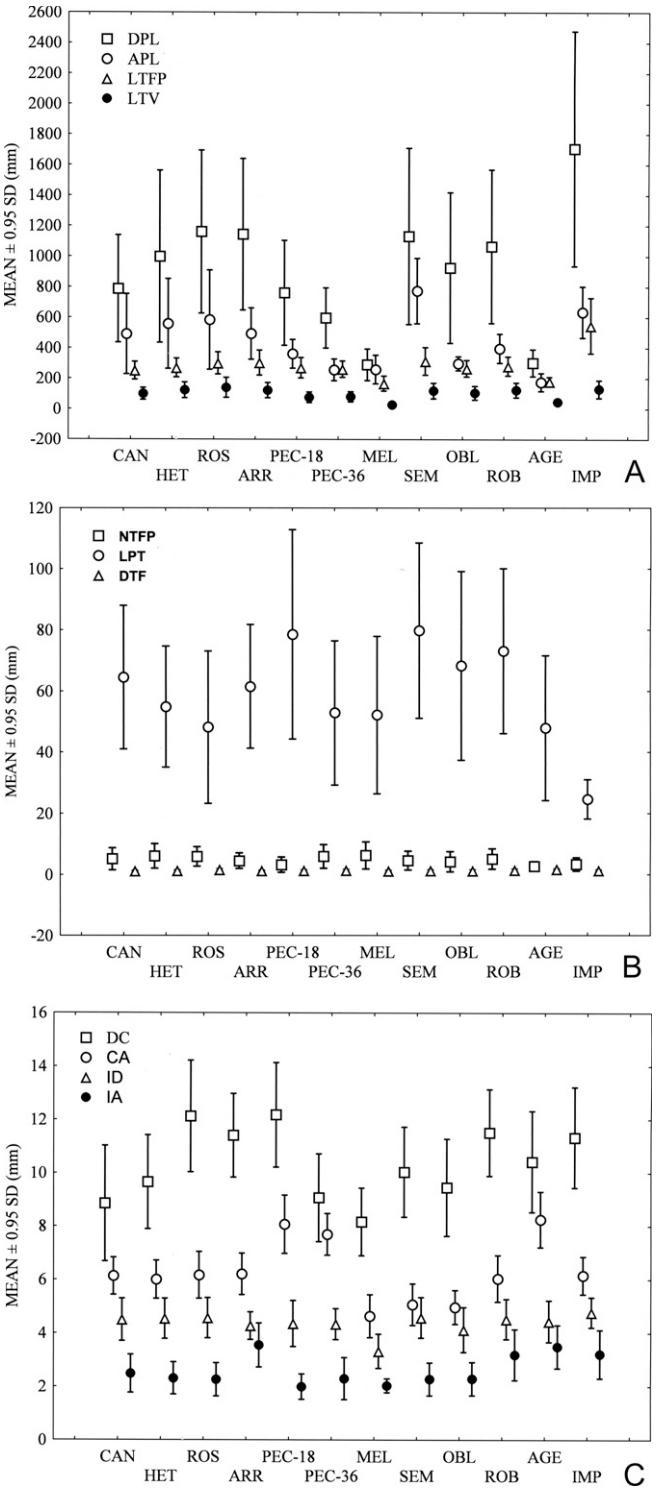

FIG. 2. Box plots for: A: DPL, Individual diameter; APL, plant height; LTFP, length of flowering stems; LTV, length of sterile stems; B: NTFP, number of primary branches; LPT, length of stem peduncle; DTF, diameter of flowering stems; C: CD, capitulum diameter; CA, capitulum height; ID, receptacle diameter; IA, receptacle height. For taxon codes see Table 1.

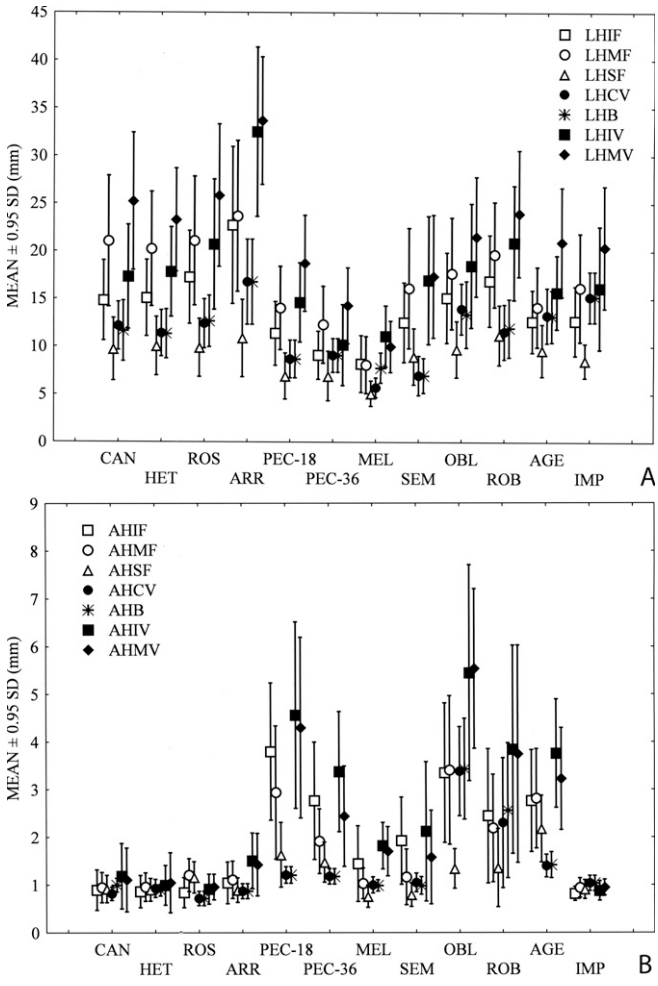

FIG. 3. Box plots for: A: Leaves of the flowering stem: LHB, basal leaf length; LHIF, lower leaf length; LHMF, middle leaf length; LHSF, upper leaf length; Leaves of the sterile stem characteristics: LHIV, lower leaf length; LHMV, middle leaf length; LHCV, fascicular leaf length; B: Leaves of the flowering stem: AHB, basal leaf width; AHIF, lower leaf width; AHMF, middle leaf width; AHSF, upper leaf width; Leaves of the sterile stem: AHIV, lower leaf width; AHMV, middle leaf width; AHCV, fascicular leaf width. For taxon codes see Table 1.

Lobe length of the lower and middle leaves of the flowering and sterile stems is greater in diploid cytotypes (PEC-18 populations) of *S. pectinata* and in *S. oblongifolia* than in the other taxa (Fig. 4B). For each position of the leaves on the flowering stems, the mean of lobe length is similar in value in HET and ROS populations of *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. rosmarinifolia* subsp. *arrabidensis*, *S. canescens*, and in *S. ageratifolia*; the contrary occurs in the remaining taxa (Fig. 4B).

In general, leaf characteristics indicate that (1) there is high similarity between *S. canescens*, HET and ROS (except for lobe numbers of the leaves) populations of *S. rosmarinifolia* subsp. *rosmarinifolia*, and *S. rosmarinifolia* subsp. *arrabidensis*, (2) diploid and tetraploid cytotypes of *S. pectinata*, *S. semidentata* subsp. *melidensis* and *S. semidentata* subsp. *semidentata* show different patterns of variation, and (3) basal and fascicular leaf characteristics show similar mean values.

All taxa show four rows of involucral bracts except for *S. oblongifolia* and most individuals of *S. semidentata* subsp. *melidensis* that show three rows of the involucral bracts. This difference should be enhanced by the loss of the inner bract row, remaining rows surely being homologous. The individuals of *S. oblongifolia* that live above 1,800 m in the Central System of the Iberian Peninsula show three rows of involucral bracts, while those that live below 1,800 m show three or four rows of involucral bracts.

Figures 5 and 6 A-B show that (1) the base length of the second row of inner bracts is greater than that of the remaining

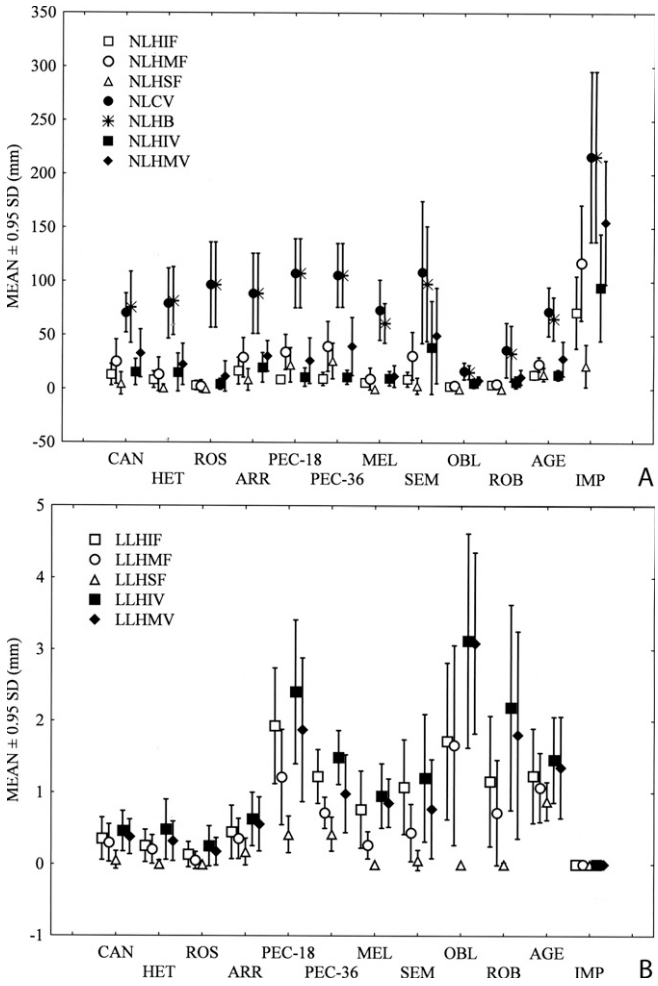

FIG. 4. Box plots for: A: Leaves of the flowering stem: NLHB, number of basal leaf lobes; NLHIF, number of lower leaf lobes; NLHMF, number of middle leaf lobes; NLHSF, number of upper leaf lobes; Leaves of the sterile stem: NLHIV, number of lower leaf lobes; NLHMV, number of middle leaf lobes; NLHCV, number of fascicular leaf lobes; B: Leaves of the flowering stem: LLHIF, length of lower leaf lobes; LLHMF, length of middle leaf lobes; LLHSF, length of upper leaf lobes; Leaves of the sterile stem: LLHIV, length of lower leaf lobes; LLHMV, length of middle leaf lobes. For taxon codes see Table 1.

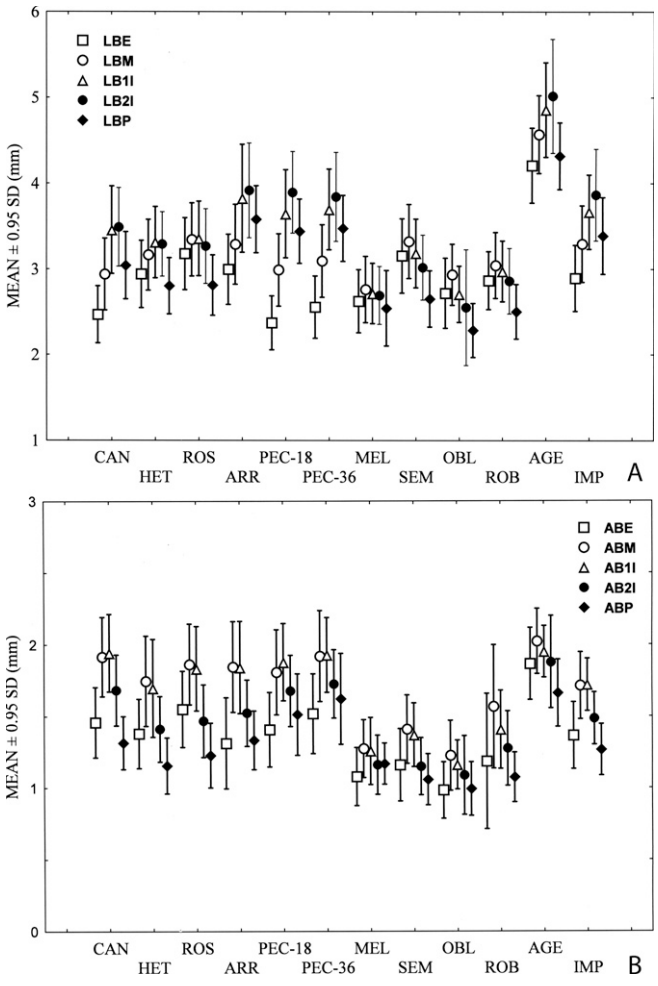

FIG. 5. Box plots for: A: LBE, base length of outer bracts; LBM, base length of middle bracts; LB1I: base length of the first row of inner bracts; LB2I, base length of the second row of inner bracts; LBP, base length of the interseminal bracts; B: ABE, base width of outer bracts; ABM, base width of middle bracts; AB1I, base width of the first row of inner bracts; AB2I, base width of the second row of inner bracts; ABP: base width of the interseminal bracts. For taxon codes see Table 1.

involucral bracts in diploid and tetraploid cytotypes of *S. pectinata*, *S. impressa*, *S. ageratifolia*, and *S. rosmarinifolia* subsp. *arrabidensis*; (2) the mean of the base length of the first and second rows of inner bracts is similar in *S. canescens* and in HET populations of *S. rosmarinifolia* subsp. *rosmarinifolia*; the same is true for the middle and first rows of inner bracts of ROS populations of *S. rosmarinifolia* subsp. *rosmarinifolia* and *S. rosmarinifolia* subsp. *melidensis*; (3) the mean of the base lengths of outer, middle, inner, and interseminal (only in *S. semidentata* subsp. *melidensis*) bracts are similar in value in HET and ROS populations of *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. semidentata* subsp. *semidentata*, *S. semidentata* subsp. *melidensis*, *S. oblongifolia*, and the hybrid complex; (4) the base length of outer, middle, inner, and interseminal bracts shows a similar pattern of variation with regard to the position in the involucre except for the outer bracts in *S. rosmarinifolia* subsp. *arrabidensis*, diploid and tetraploid cytotypes of *S. pectinata*, and *S. impressa*; (5) the interseminal bracts are smaller than the other bracts in HET and ROS populations of *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. semidentata* subsp. *semidentata*, *S. semidentata* subsp. *melidensis*, *S. oblongifolia*, and the hybrid complex; (6) the involucral bracts are smaller in *S. semidentata* subsp. *semidentata*, *S. semidentata* subsp. *melidensis*, *S. oblongifolia*, and the hybrid complex than in the other taxa, and show a similar pattern of variation with regard to their position in the involucre; (7) the involucral bracts are larger in *S. ageratifolia*, except for appendage length of the inner bracts; (8) the length and apical width of the appendage of the involucral bracts are greater for inner bracts than for the other bracts; (9) the base width is greater for the middle and first rows of involucral bracts than for the other bracts; (10) the first and second rows of inner bracts show a similar pattern of variation, except for base width; and (11) *S. canescens* and diploid and tetraploid cytotypes of *S. pectinata* show similar patterns of variation. The lateral width of the appendage varied between 0–0.2(0.4) mm in ROS populations of *S. rosmarinifolia* subsp. *rosmarinifolia*, whereas the remaining taxa varied between (0)0.2–0.5(0.9) mm, but the appendage of *S. pectinata* and *S. ageratifolia* was always decurrent.

Flower and achene characteristics show similar mean values in all taxa (Fig. 7 A-C), but anther length of the peripheral

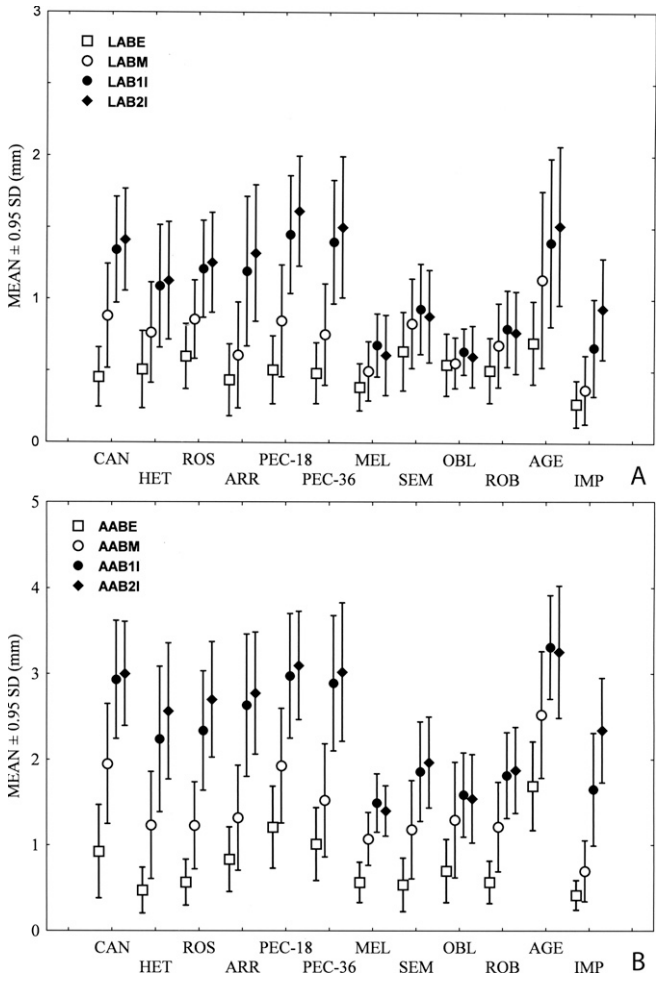

FIG. 6. Box plots for: A: LABE, appendage length of outer bracts; LABM, appendage length of middle bracts; LAB1I, appendage length of the first row of inner bracts; LAB2I, appendage length of the second row of inner bracts; B: AABE, apical width of the outer bract appendage; AABM, apical width of the middle bract appendage; AAB1I, apical width of the first row of the inner bract appendage; AAB2I, apical width of the second row of the inner bract appendage. For taxon codes see Table 1.

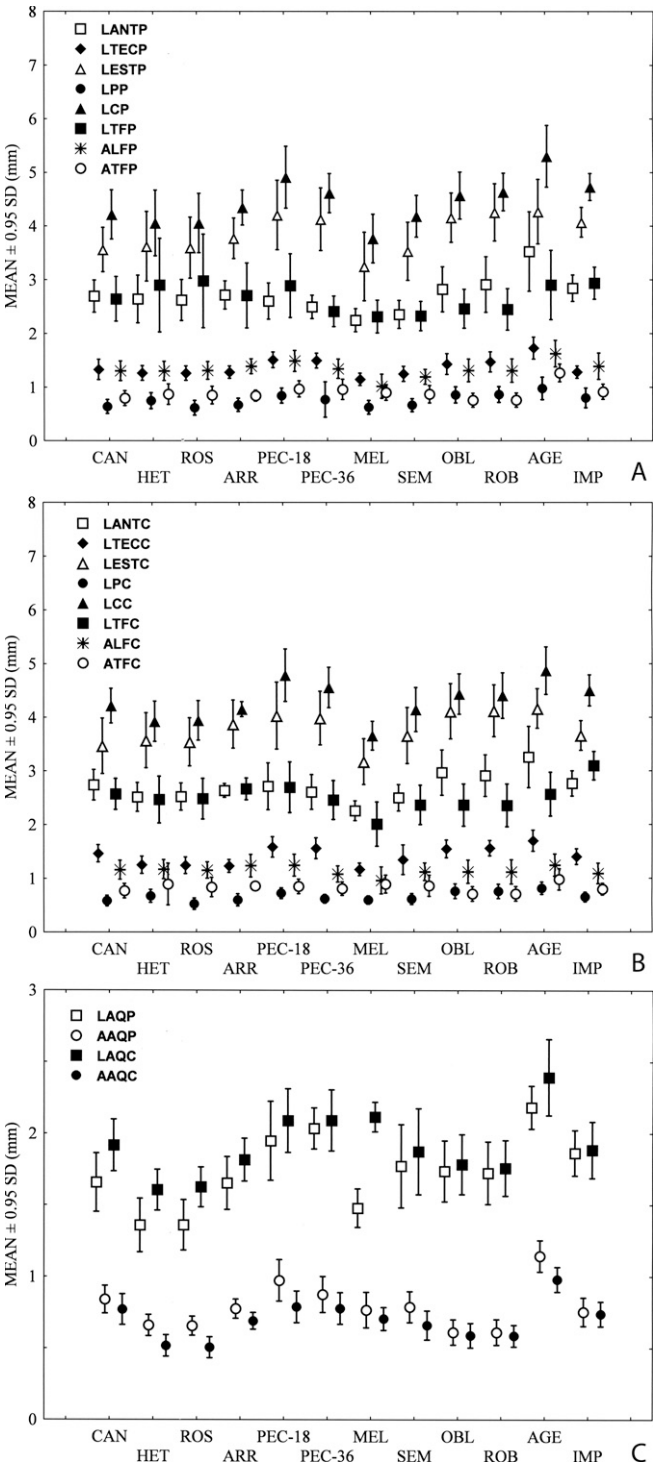

FIG. 7. Box plots for: A: Peripheral flowers: LANTP, anther length; LTECP, theca length; LESTP, style length; LPP, corolla lobe length; LCP, corolla length; LTFP, corolla tube length; ALFP, corolla aperture; ATFP, corolla tube aperture; B: Central flowers: LANTC, anther length; LTECC, theca length; LESTC, style length; LPC, corolla lobe length; LCC, corolla length; LTFC, corolla tube length; ALFC, corolla aperture; ATFC, corolla tube aperture; C: Peripheral achene: LAQP, achene length; AAQP, achene width; NCQP, number of peripheral achene ribs; Central achene: LAQC, achene length; AAQC, achene width. For taxon codes see Table 1.

flowers, and length and width of the peripheral and central achenes, are greater in *S. ageratifolia* than in the other taxa.

QUALITATIVE CHARACTERISTICS—Plant color, plant indumentum, plant habit, viscose plant covering; leaf apex; lobe shape; leaf shape and incision of the basal and fascicular leaves; capitulum base; receptacle shape; leaf margin; color, texture of the appendage of the involucral bracts; apex shape of the involucral bracts; shape and keel insertion; receptacle shape; and position of the peripheral and central flowers show little variation within the taxa (Table S1). The same is true of flowering and vegetative stem characteristics, but this is the first time that these characteristics have been studied in *Santolina* (Table S1). However, leaf shape, leaf incision (except for *S. impressa*), lobe insertion (except for *S. impressa*), capitulum shape, involucral bract shape, insertion of the appendage of the involucral bracts, apex of the involucral bracts, and indumentum of the interseminal bracts are variable within each taxon (Table S1).

The high frequency of lanceolate leaves and characteristics of the involucral bracts in diploid and tetraploid cytotypes of *S. pectinata* and in *S. ageratifolia* indicate strong relationships between these taxa. This is also true of *S. pectinata* and *S. canescens* (see shape of the lower and middle leaves of the flowering and sterile stems and the appendage insertion of the involucral bracts).

TABLE 2. Variation within and between populations in each taxon (except in *S. semidentata* subsp. *melidensis*), by means of nested ANOVA. For taxon codes see Table 1. APP, between populations; AIP, between individuals within each populations; *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.0001$; dfe, degrees of freedom of the effect; dfr, degrees of freedom of the error. Variance components (%) in brackets.

Taxa	Sources of variation	dfe	Factor 1	Factor 2	Factor 3
ROS (dfr = 437)	APP	35	43.47*** (19.70)	74.45*** (20.00)	-
	AIP	401	10.38*** (66.20)	17.98*** (71.60)	-
ARR (dfr = 70)	APP	1	11.41** (2.10)	34.91*** (39.80)	2.85 ns (4.30)
	AIP	38	1.90* (22.20)	1.45 ns (8.60)	1.80* (21.40)
HET (dfr = 187)	APP	17	190.94*** (58.80)	33.77***(29.80)	97.05*** (55.70)
	AIP	169	11.42*** (31.80)	5.48*** (48.50)	6.06*** (31.80)
CAN (dfr = 425)	APP	37	62.80*** (33.60)	0.83 ns	-
	AIP	386	8.69*** (52.70)	1.82*** (29.20)	-
SEM (dfr = 184)	APP	24	9.14*** (32.80)	5.61*** (12.40)	7.12***(29.80)
	AIP	159	1.23 ns (7.00)	2.25*** (33.80)	0.98 ns (2.20)
IMP (dfr = 87)	APP	5	249.80*** (38.40)	187.23*** (39.50)	-
	AIP	81	24.85*** (56.80)	17.65*** (54.00)	-
AGE (dfr = 124)	APP	1	231.13*** (37.70)	6.52* (7.70)	78.68*** (11.90)
	AIP	60	10.11*** (46.90)	1.03 ns (1.00)	13.94*** (71.60)
PEC-18 (dfr = 245)	APP	34	2,427.20*** (77.40)	1.07 ns (4.60)	4.01*** (13.40)
	AIP	210	108.39*** (22.20)	0.40 ns (0.75)	1.41* (14.70)
PEC-36 (dfr = 173)	APP	18	356.82*** (62.00)	250.03*** (87.20)	0.28 ns (1.3)
	AIP	154	22.02*** (34.70)	3.05*** (6.50)	0.06 ns (0.10)
OBL (dfr = 95)	APP	3	501.55*** (64.10)	26.38*** (15.80)	155.10*** (60.40)
	AIP	92	12.31*** (30.50)	4.65*** (54.50)	3.83*** (23.30)
ROB (dfr = 251)	APP	3	211.34*** (28.80)	176.61*** (29.50)	69.61*** (25.30)
	AIP	92	15.76*** (62.70)	12.60*** (60.10)	5.57*** (52.00)

***Variation Within Taxa*—QUANTITATIVE CHARACTERISTICS**—The correlation of the characteristics with PCA factors is usually low-to-moderate and rarely strong (Table S2); and the percentage of total variance of the factors in each taxon is not high (Table S3). Nested ANOVA (Table 2) shows that the variation is higher within than between populations in all taxa, except for (1) factor 2 of *S. rosmarinifolia* subsp. *arrabidensis*; (2) factors 1 and 3 of HET populations of *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. semidentata* subsp. *semidentata*, and *S. oblongifolia*; (3) factor 1 of diploid cytotypes of *S. pectinata*; and (4) factors 1 and 2 of tetraploid cytotypes of *S. pectinata*.

QUALITATIVE CHARACTERISTICS—The percentage of accumulated variance of the first six factors ranged between 10.12% and 29.86% among taxa, indicating that the characteristics are poorly correlated within each taxon, and thus there is variability among taxa.

***Variation Between Taxa*—QUANTITATIVE CHARACTERISTICS**—Principal component analysis shows that the first three factors accounted for an eigenvalue of 28.89% and 38.91% of the variance. Peripheral and central flower characteristics ($-0.75 \leq r \leq -0.86$), capitulum height ($r = -0.83$), peripheral achene length ($r = -0.58$), and length ($r = -0.61$) and width ($r = -0.61$) of the central achene show strong-to-moderate correlation with factor 1. Apical ($r = -0.63$) and lateral ($r = -0.66$) width of the outer bracts appendage, apical ($r = -0.50$) and lateral ($r = -0.67$) width of the middle bracts appendage, base length of the first row of inner bracts ($r = -0.56$), apical ($r = -0.53$) and lateral ($r = -0.53$) width of the first row of inner bracts appendage, base length of the second row of inner bracts ($r = -0.55$), and base length ($r = -0.62$) and width ($r = -0.58$) of the interseminal bracts show moderate correlation with factor 2. Lobe number and lobe length of the leaves of the flowering and sterile stems ($-0.51 \leq r \leq -0.66$), and lobe number of basal ($r = -0.61$) and fascicular ($r = -0.58$) leaves, show moderate correlation with factor 3. The nested ANOVA (Table 3) indicates that the quantitative characteristics show significant differences for all the sources of variation analysed, and extensive variation occurs between taxa.

The nested MANOVA (the results are not presented here) reveals that all the characteristics analysed show significant variation ($p < 0.0001$) within and between taxa, except for the number of central achene ribs within taxa. The number of secondary flowering branches, and the number of peripheral and central achene ribs are the characteristics with the lowest variation between taxa (3.50%, 8.30% and 4.30% of the total variance, respectively). Central achene width, lobe number of the lower and middle leaves of the flowering and sterile stems, capitulum height, width of the lower leaves of the flowering

TABLE 3. Variation within and between taxa of the factor loading of the PCA and dimensions of the multidimensional scaling by means of nested ANOVA. ATX, between taxa; APT, between populations within each taxon; dfe, degrees of freedom of the effect; dfr, degrees of freedom of the error; VCP, variance components (%).

		Quantitative data (dfr = 4832; $p < 0.0001$)			Qualitative data (dfr = 4987; $p < 0.0001$)			
Source of Variation	dfe	Factor 1 F (VCP)	Factor 2 F (VCP)	Factor 3 F (VCP)	Dimension 1 F (VCP)	Dimension 2 F (VCP)	Dimension 3 F (VCP)	Dimension 4 F (VCP)
ATX	11	2,197.66 (83.40)	1,409.57 (78.60)	645.40 (61.30)	13,337.49 (97.00)	16,266.53 (97.40)	6,455.66 (94.60)	53,158.43 (99.30)
APT	197	13.42 (5.80)	9.27 (5.70)	10.65 (11.50)	12.18 (1.00)	14.24 (1.00)	11.83 (1.90)	6.88 (0.20)
Error		(10.70)	(15.70)	(27.20)	(2.00)	(1.70)	(3.50)	(0.50)

stems, base length of the interseminal bracts, peripheral achene length, lateral width of the middle bracts appendage, width of the lower leaves of the sterile stems, and peripheral achene width, account for a higher variance between taxa. A post hoc test (the results are not presented here) indicates that differences are statistically significant ($p < 0.0001$) between all the taxa.

The discriminant analysis (Fig. S1) shows three groups. Group I is formed by HET and ROS populations of *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. canescens*, *S. rosmarinifolia* subsp. *arrabidensis*, *S. semidentata* subsp. *semidentata*, *S. semidentata* subsp. *melidensis*, *S. oblongifolia*, and the hybrid complex of *S. oblongifolia*, *S. rosmarinifolia* subsp. *rosmarinifolia*, and their putative hybrids; Group II is formed by diploid and tetraploid cytotypes of *S. pectinata* and *S. ageratifolia* and Group III is formed by *S. impressa*. The classification matrix (Table 4) indicates that all individuals of *S. semidentata* subsp. *melidensis*, *S. impressa*, *S. ageratifolia*, and *S. canescens* are well classified. The remaining taxa show more than 90% of the individuals as well classified, except in *S. oblongifolia* and in the hybrid complex, where 18.75% of the individuals of *S. oblongifolia* are classified within the hybrid complex and 17.93% of the individuals of the hybrid complex are classified as *S. oblongifolia*. In addition, 6.68%, 5.39%, 0.98% and 0.005% of the individuals of the hybrid complex are classified as HET populations of *S. rosmarinifolia*, *S. semidentata* subsp. *semidentata*, *S. canescens*, and *S. semidentata* subsp. *melidensis* respectively.

Squared Mahalanobis distances (Table 5) indicate that all taxa are significantly different but the distances between them are not high, except for (1) *S. impressa* with all taxa, except with *S. rosmarinifolia* subsp. *arrabidensis*, *S. semidentata* subsp. *melidensis* and with the hybrid complex; (2) *S. ageratifolia* with *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. canescens*, *S. oblongifolia*, and the hybrid complex; (3) diploid cytotypes of *S. pectinata* with *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. canescens*, *S. semidentata* subsp. *semidentata*, *S. impressa*, and the hybrid complex; (4) tetraploid cytotypes of *S. pectinata* with *S. rosmarinifolia* subsp. *rosmarinifolia* and *S. impressa*; and (5) ROS populations of *S. rosmarinifolia* subsp. *rosmarinifolia* with *S. canescens* and *S. oblongifolia*. Furthermore, the distances between taxa of Group I are short (Table 5), especially between ROS and HET populations of *S. rosmarinifolia* subsp. *rosmarinifolia*, between *S. semidentata* subsp. *semidentata* and *S. semidentata* subsp. *melidensis* and between HET populations of *S. rosmarinifolia* subsp. *rosmarinifolia* and *S. canescens*, respectively.

The nested ANOVA shows that the variance between groups (Factor 1: $F_{2,2303} = 3{,}967.02$, 84.70% of the total variance; Factor 2: $F_{2,2303} = 329.83$, 10.40% of the total variance; Factor 3: $F_{2,2303} = 1{,}172.32$, 69.20% of the total variance) is higher than the variance within groups (Factor 1: $F_{9,2303} = 344.99$, 8.80% of the total variance; Factor 2: $F_{9,2303} = 802.84$, 81.09% of the total variance; Factor 3: $F_{9,2303} = 124.36$, 12.20% of the total variance) except for Factor 2. The discriminant analysis indicates that all individuals of each group are well classified. Squared Mahalanobis distances (SMD) indicate that the groups are significantly ($p < 0.0001$) different (Groups I-II: SMD = 49.68, $F_{59,2254} = 304.92$; Groups I-III: SMD = 111.80, $F_{59,2254} = 153.14$, Groups II-III: SMD = 153.85, $F_{59,2254} = 186.69$), but the distance between groups I and II is not high.

All functions are significantly different for all levels analysed (Table S4). The characteristics with greatest contribution to the differentiation: between taxa, between Groups, within Group I, and within Group II (*S. pectinata* from *S. ageratifolia*) are shown in Table S5 in bold. Within the Group I, Function 1 differentiate HET and ROS populations of *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. rosmarinifolia* subsp. *arrabidensis*, and *S. canescens*, whereas function 2 differentiate *S. semidentata* subsp. *semidentata*, *S. semidentata* subsp. *melidensis*, *S. oblongifolia*, and the hybrid complex of *S. oblongifolia*, *S. rosmarinifolia* subsp. *rosmarinifolia* and their putative hybrids, however function 3 differentiates *S. impressa* from the remaining taxa. Within Group II, function 1 differentiates *S. ageratifolia* from *S. pectinata*, whereas function 2 differentiates diploid and tetraploid cytotypes of *S. pectinata*.

Partial discriminant analysis were carried out within Group I. The characteristics with greatest contribution to differentiate: (1) *S. semidentata* subsp. *melidensis* from *S. semidentata* subsp. *semidentata* are: LTV (r = 0.41), ID (r = 0.36), LTFP (r = 0.29), LHSF (r = 0.29), LBM (r = 0.28), LBE (r = 0.28), LHMV (r = 0.26), DC (r = 0.24), LHIV (r = 0.23), LAQP (r = 0.23), NBI (r = 0.23), NLHMV (r = 0.23), NLHMF (r = 0.22), LHMF (r = 0.21), LB2I (r = 0.21), AAB2I (r = 0.21), ABM (r = 0.20), LB1I (r = 0.19), NLHIV (r = 0.18), LAB2I (r = 0.18), IA (r = 0.18), LAQC (r = -0.18), LPT (r = 0.15), LHIF (r = 0.15), AAB1I (r = 0.15), AHS (r = 0.14), AB1I (r = 0.14), LABM (r = 0.13), LAB1I (r = 0.12), NLHB (r = 0.11), NLCV (r = 0.10), NLHSF (r = 0.10), LLHMF (r = 0.10), LLHSF (r = 0.10), and ABE (r = 0.10); (2) *S. semidentata* subsp. *melidensis* and *S. semidentata* subsp. *semidentata* from the remaining taxa of the Group I (SMD = 15.64; $F_{39,1804} = 73.11$) are: CA (r = 0.30), LHMV (r = 0.28), ABM (r = 0.27), AB1I (r = 0.27), LHIF (r = 0.20),

Table 4. Classification matrix, by means of discriminant analysis. For taxon codes see Table 1.

Taxa	Percent	ROS	ARR	HET	CAN	SEM	MEL	IMP	AGE	PEC-18	PEC-36	OBL	ROB
ROS	98.01	443	1	8	-	-	-	-	-	-	-	-	-
ARR	98.18	-	54	-	1	-	-	-	-	-	-	-	-
HET	90.91	14	-	170	1	1	-	-	-	-	-	-	1
CAN	100	-	-	-	505	-	-	-	-	-	-	-	-
SEM	98.36	-	-	3	-	180	-	-	-	-	-	-	-
MEL	100	-	-	-	-	-	26	-	-	-	-	-	-
IMP	100	-	-	-	-	-	-	87	-	-	-	-	-
AGE	100	-	-	-	-	-	-	-	62	-	-	-	-
PEC-18	96.18	-	-	-	-	-	-	-	-	227	9	-	-
PEC-36	97.10	-	-	-	-	-	-	-	-	5	168	-	-
OBL	81.25	-	-	-	-	-	-	-	-	-	-	78	18
ROB	81.74	-	-	14	2	11	1	-	-	-	-	38	186
Total	95.14	457	55	195	509	192	27	87	62	232	177	116	204

TABLE 5. Squared Mahalanobis distances and test of their significance. F (in brackets) test with 77 degrees of freedom of the effect and 2,226 degrees of freedom of the error, $p < 0.0001$. For taxon codes see Table 1.

Taxa	ROS	ARR	HET	CAN	SEM	MEL	IMP	AGE	PEC-18	PEC-36	OBL
ARR	32.62 (20.09)	-									
HET	12.19 (20.25)	42.99 (22.95)	-								
CAN	38.93 (116.62)	39.21 (24.43)	29.99 (51.40)	-							
SEM	39.96 (65.37)	62.78 (33.34)	31.34 (36.41)	46.89 (79.10)	-						
MEL	52.86 (16.32)	79.58 (17.64)	38.61 (11.07)	48.19 (14.97)	28.22 (8.07)	-					
IMP	217.12 (198.93)	190.15 (80.47)	186.16 (138.81)	165.71 (154.44)	171.24 (126.80)	197.78 (49.72)	-				
AGE	204.98 (140.35)	156.08 (57.13)	213.81 (125.03)	167.67 (116.27)	170.78 (99.32)	186.66 (42.94)	335.39 (103.04)	-			
PEC-18	127.62 (248.49)	113.41 (63.53)	110.31 (144.53)	66.73 (134.78)	83.66 (108.30)	90.66 (26.67)	216.31 (172.68)	117.72 (72.52)	-		
PEC-36	99.71 (156.66)	93.29 (48.89)	85.45 (96.43)	52.29 (84.63)	62.23 (69.51)	68.88 (19.55)	196.46 (142.82)	113.64 (65.14)	18.82 (23.52)	-	
OBL	95.87 (95.34)	112.83 (49.55)	77.95 (62.10)	92.45 (110.54)	66.67 (52.72)	80.84 (20.77)	253.03 (145.02)	217.81 (103.04)	114.50 (98.12)	102.23 (79.27)	-
ROB	53.02 (107.45)	68.52 (38.82)	39.98 (53.82)	52.50 (96.66)	41.28 (54.87)	58.72 (17.37)	192.82 (12.88)	200.15 (120.19)	100.50 (147.37)	83.68 (97.93)	192.49 (90.75)

and LLHIF (r = -0.19); (3) ROS and HET populations of *S. rosmarinifolia* subsp. *rosmarinifolia* are: DC (r = -0.38), NLHMF (r = 0.35), NLHIV (r = 0.32), LLHMF (r = 0.32), NLHIF (r = 0.29), NLHMV (r = 0.24), LLHIV (r = 0.22), LLHMV (r = 0.22), AHMF (r = 0.23), and LHMV (r = -0.18); and (4) *S. oblongifolia* (SMD = 191.95; $F_{18,1574} = 951.79$) from the remaining taxa of the Group I are: AHCV (r = 0.59), AHB (r = 0.55), AHMV (r = 0.41), LLHMV (r = 0.39), AHIV (r = 0.31), AHMF (r = 0.30), AHIF (r = 0.21), LB2I (r = 0.19), and LLHMF (r = 0.19).

The factors' structures show that no quantitative characteristics strongly differentiate taxa, groups, or taxa within each group. The multivariate combination of all these characteristics allows recognition of taxa and groups.

Cluster analysis of the quantitative data (Fig. 8) distinguishes two groups: a small group comprises *S. impressa*, and a large group (G2) formed by three subgroups. One of them comprises *S. oblongifolia* and some populations of the hybrid complex (S1), another comprises diploid and tetraploid cytotypes of *S. pectinata*, *S. ageratifolia* and one population of *S. rosmarinifolia* (S2), while the third comprises the remaining taxa and two populations of the hybrid complex, three populations of diploid cytotypes of *S. pectinata*, and one population of tetraploid cytotypes of *S. pectinata*.

QUALITATIVE CHARACTERISTICS—Qualitative characteristics that show strong to moderate correlation with the first four dimensions of the multidimensional scaling are shown in Table S6 in bold. The nested ANOVA shows that variation between taxa is the greatest (Table 3).

Multidimensional scaling (Fig. S2) distinguishes four groups: (I) ROS and HET populations of *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. rosmarinifolia* subsp. *arrabidensis*, *S. canescens*, *S. semidentata* subsp. *semidentata*, *S. semidentata* subsp. *melidensis*, *S. oblongifolia*, and the putative hybrids between *S. oblongifolia* and *S. rosmarinifolia* subsp. *rosmarinifolia*; (II) diploid and tetraploid cytotypes of *S. pectinata*; (III) *S. impressa*, and (IV) *S. ageratifolia*. This analysis shows that: (1) *S. impressa* and *S. ageratifolia* are strongly differentiated from the other taxa; (2) diploid and tetraploid cytotypes of *S. pectinata* are poorly differentiated; (3) *S. canescens* and the diploid cytotype of *S. pectinata* are well differentiated; and (4) HET and ROS populations of *S. rosmarinifolia* subsp. *rosmarinifolia*, S. *rosmarinifolia* subsp. *arrabidensis*, *S. canescens*, *S.semidentata* subsp.*semidentata*, *S.semidentata* subsp.*melidensis*, *S. oblongifolia*, and the hybrid complex are poorly differentiated, indicating strong relationships between them.

The nested ANOVA (Table 6A) indicates that the variance between groups is significantly higher than the variance within groups, except for D3 for the groups provided by multidimensional scaling. All characteristics are significantly different between taxa and between groups (Table S6). Logistic regression analysis (the results are not shown here) indicates that all characteristics show statistical heterogeneity ($p < 0.0001$) within each Group, except for (1) PRT, TVZ, MHBT, FLH, and MHMF in Group I; and (2) PCL, PUB, PRT, TFQ, TFZ, TVZ, BFLS, BFLG, MHBF, MIA, FLH, APHIMF, APHIMV, CPS, CPU, FAPBE, FAPBM, FAPBI1, APC, BEAD, BMAD, BI1AD, BI2AD, BEAQ, BMAQ, BI1AQ, BI2AQ, and FLPS in Group II.

However, cluster analysis of the qualitative data (Fig. 9) distinguishes two groups, and the relationships of the taxa are different from those found in the discriminant analysis. Group I (G1) is formed by four subgroups: *S. canescens* (S1),

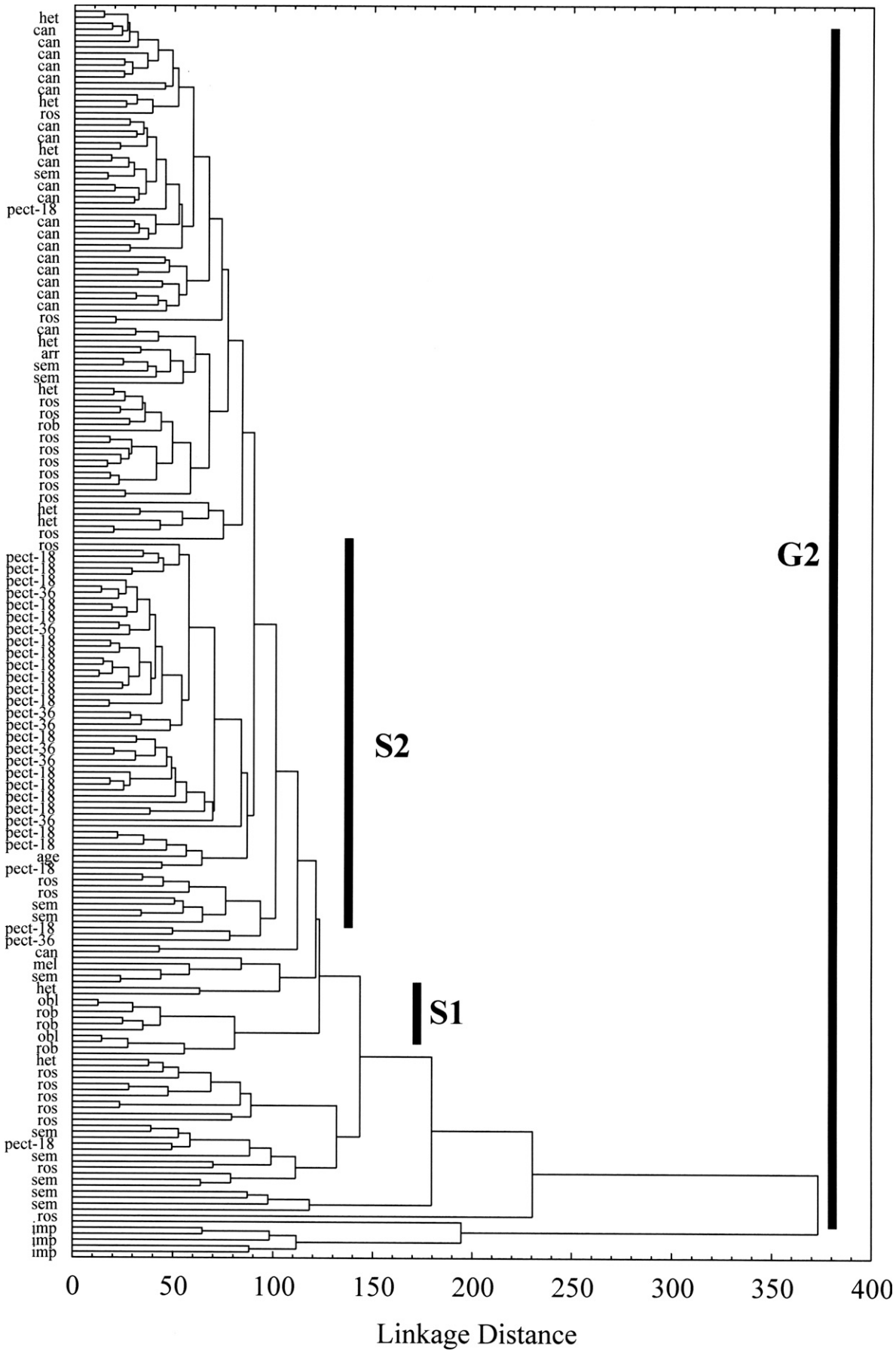

FIG. 8. UPGMA dendrogram constructed with the complete quantitative data set. For taxon codes see Table 1.

TABLE 6. Variation within and between groups of the dimension loading of the multidimensional scaling (A) and of cluster analysis (B) by means of nested ANOVA (dfr = 5,095, $p < 0.0001$). BGR, between groups; BTX (GR): between taxa within each group; dfe, degrees of freedom of the effect; dfr, degrees of freedom of the error. Variance components (%) in brackets.

Source of Variation	dfe	D1	D2	D3	D4
A					
BGR	3	30,328.77 (75.30)	38,782.92 (86.30)	5,525.06 (21.10)	15,1229.74 (94.90)
BTX (GR)	8	6,110.68 (23.10)	3,854.82 (12.30)	5,779.64 (73.40)	5,449.70 (4.70)
B					
BGR	1	62,716.71 (73.40)	3,895.07 (1.60)	310.71 (2.10)	102,666.01 (28.60)
BTX (GR)	10	5,762.85 (24.90)	13,055.89 (97.20)	7,629.06 (93.30)	49,929.90 (70.80)

diploid (S2), and tetraploid (S3) cytotypes of *S. pectinata* and *S. ageratifolia* (S4). Group II (G2) is formed by six subgroups: HET populations of *S. rosmarinifolia* subsp. *rosmarinifolia* (S1), *S. oblongifolia* and the hybrid complex (S2), *S. semidentata* subsp. *semidentata* and *S. semidentata* subsp. *melidensis* (S3), *S. impressa* (S4), ROS populations of *S. rosmarinifolia* subsp. *rosmarinifolia* (S5), and *S. rosmarinifolia* subsp. *arrabidensis* (S6) [Fig. 9]. A strong relationship was found between HET populations of *S. rosmarinifolia*, *S. oblongifolia*, and the hybrid complex and between *S. semidentata* subsp. *semidentata*, *S. semidentata* subsp. *melidensis* and *S. impressa*. The Mantel cophenetic correlation coefficient obtained was high (r = 0.91), indicating a good fit of the cluster analysis performed with the initial similarity matrix. The nested ANOVA (Table 6B) indicates that the variance between taxa within each groups is significantly higher than the variance between groups, except for D1.

QUANTITATIVE AND QUALITATIVE CHARACTERISTICS—The UPGMA dendrogram constructed using Gower's similarity measure for mixed data produced three groups (Fig. 10). Group I (G1) is formed by seven subgroups: *S. canescens* (S1), HET (S2) and ROS (S3) populations of *S. rosmarinifolia* subsp. *rosmarinifolia*, *S. rosmarinifolia* subsp. *arrabidensis* (S4), *S. semidentata* subsp. *melidensis* (S5), *S. semidentata* subsp. *semidentata* (S6), *S. oblongifolia*, and the hybrid complex (S7). Strong relationships were found between *S. semidentata* subsp. *melidensis* and *S. semidentata* subsp. *semidentata*, *S. oblongifolia* and the hybrid complex, and HET and ROS populations of *S. rosmarinifolia* and *S. rosmarinifolia* subsp. *arrabidensis*. Group II (G2) is formed by three subgroups: diploid (S1) and tetraploid (S2) cytotypes of *S. pectinata* and the Ródenas population of *S. ageratifolia* (S3). Group III (G3) is formed by two subgroups: *S. impressa* and the San Gines population of *S. ageratifolia*. The Mantel cophenetic correlation coefficient obtained was high (r = 0.85), indicating a good fit of the cluster analysis performed with the initial similarity matrix.

DISCUSSION

***Mean, Coefficients of Variation and Variance*—**This work confirms that the mean of morphological characteristics (length of the leaves, length of the lobes of the leaves [except fascicular leaves], number of lobes of the lower and middle leaves of the flowering and sterile stems, and length of the inner and interseminal bracts) is generally increased by polyploidy in *S. rosmarinifolia* subsp. *arrabidensis* (tetraploid) [Rivero-Guerra 2008c]. Furthermore, the mean of the number of lobes of the middle leaves of the flowering and sterile stems is increased by polyploidy in *S. pectinata*. The coefficient of variation of the vegetative characteristics is higher in diploid than in polyploid taxa in the *S. rosmarinifolia* L. aggregate. In agreement with the results of Schwaegerle and Schaal (1979) in *Sarracenia*, Coyle et al. (1982) in *Betula*, Schnabel and Hamrick (1990) in *Gleditsia*, Jain et al. (1981) in *Avena*, and Thomas et al. (2001) in *Oryza malampuzhaensis*, the polyploids of the *S. rosmarinifolia* L. aggregate show significantly lower phenotypic variation than do diploid taxa.

High coefficients of variation, for most of the characteristics, indicate wide variation between individuals of *S. rosmarinifolia* L. aggregate. Vegetative characteristics are more variable than floral characteristics, as shown by Herrera (1990, 1993) in *Viola cazorlensis*, Conner and Via (1993) in *Raphanus raphanistrum*, Herrera (2001) in various species of the tribe Genisteae, Urbaniak et al. (2003) in *Pinus sylvestris*, and Klimko et al. (2004) in *Juniperus oxycedrus* subsp. *macrocarpa*. The opposite occurs in *Solidago canadensis* (Weber 1997) and *Rhizophora mangle* (Domínguez et al. 1998), where various flower characteristics show higher coefficients of variation than vegetative characteristics. Usually, vegetative characteristics are more affected by the environment than are floral characteristics (Herrera 2001).

***Taxonomy and Ecology*—**Variation in the qualitative characteristics of the appendage of the involucral bracts is probably an adaptation to xeric conditions and constitutes a phenotypic response to annual rainfall regimen and latitude. It probably confers defense or protection as described by Stuessy and Spooner (1988). Similar results were found by Sapir et al. (2002) for flower, stem, and leaf size traits of *Oncocyclus*. In contrast, Pimentel and Sahuquillo (2007) found that pubescence and pruinose leaves in *Anthoxanthum amarum* were strongly related to the mean and minimum temperature, whereas the presence of bulbils or stolons was associated with the presence or absence of a drought period. In addition, Domínguez et al. (1998) reported that flower characteristics of *R. mangle* do not exhibit continuous clinal variation in Mexico, whereas Lefèbvre and Vekemans (1995) found an overall decrease in size for most vegetative organs in relation to increase in latitude. Mayer (1991) and Weber (1997) found that several vegetative and floral characteristics exhibited positive clinal variation among populations. Nevertheless, these correlations were not observed in the *S. rosmarinifolia* aggregate except for the insertion of the appendages of the involucral bracts. Diploid (latitude 37°–38°, annual rainfall 661.73 ± 285.67 mm) and tetraploid (latitude 39°–40°, annual rainfall 497.38 ± 174.93 mm) cytotypes of *S. pectinata*, *S. ageratifolia* (latitude 40°, annual rainfall 360.96 ± 109.95 mm), and *S. canescens* (latitude 36°–38°, 591.56 ± 222.47 mm) show a continous scarious appendage from the apex to the base, whereas the remaining taxa (latitude 38°–42°, annual rainfall 702.03 ± 283.22 mm) have fimbriate involucral-bract margins (except

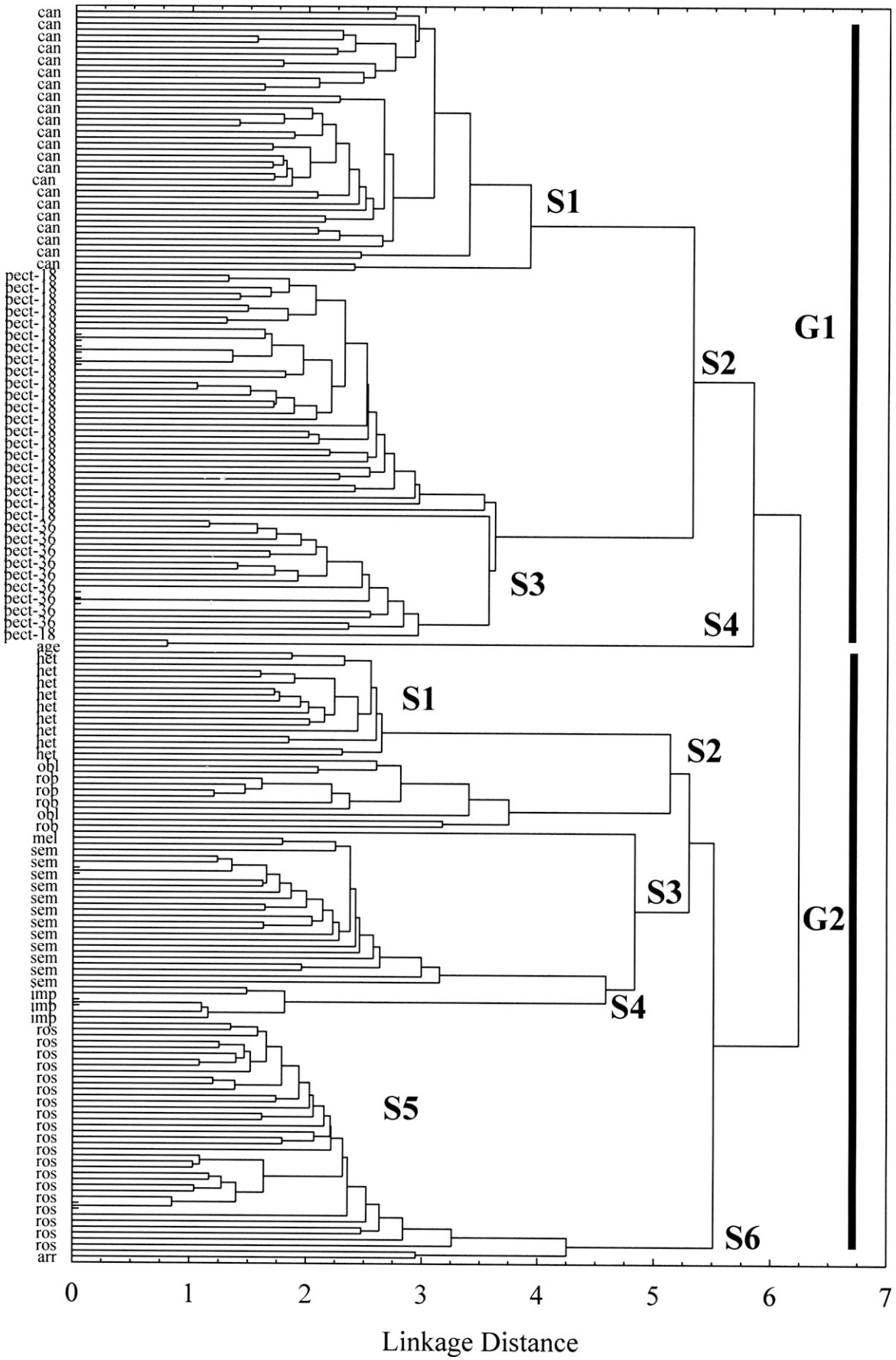

FIG. 9. UPGMA dendrogram constructed with the complete qualitative data set based on the frequency of the qualitative characteristics per individual. For taxon codes see Table 1.

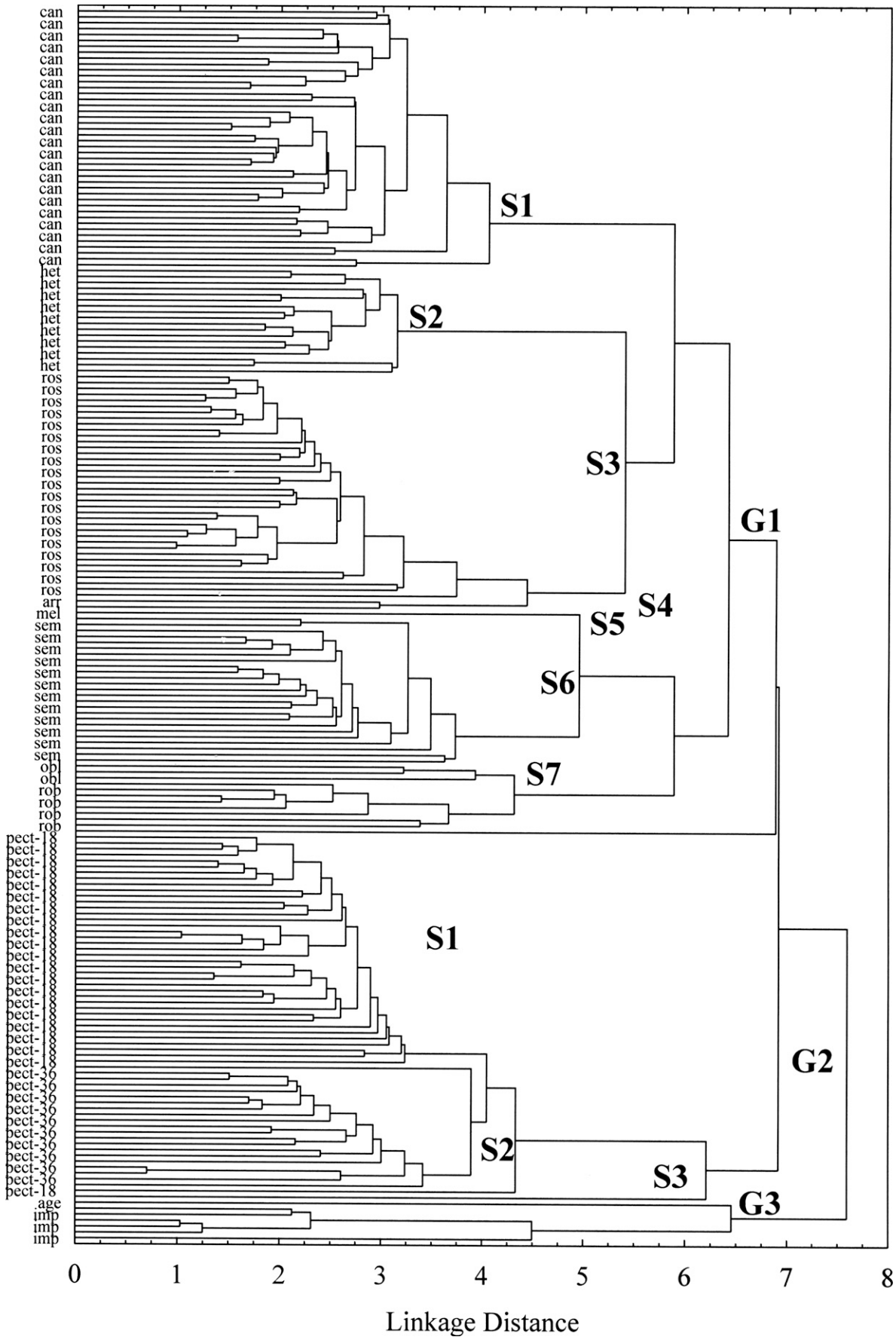

FIG. 10. UPGMA dendrogram constructed with the complete quantitative and qualitative data set based on the Gower's similarity coefficient for the mixed data. For taxon codes see Table 1.

S. rosmarinifolia subsp. *rosmarinifolia*). Nevertheless, common garden experiments would give more conclusive results.

Taxonomic Characteristics—Multivariate methods of phenetic analysis as well as classical biometrical methods have been applied to *Santolina* data for first time. The two-stage approach used here, multivariate analysis of data and explicit application of morphological, biological, and ecological species concepts, leads to a more realistic and certainly more scientific estimate of taxonomic diversity than traditional herbarium methods. Qualitative data are important for taxon differentiation. The mostly quantitative and qualitative characteristics represent overlap between the taxa.

The morphological characteristics used to discriminate among these taxa are not always constant at population and individual level. Leaf shape, leaf incision (Guinea 1970; Guinea and Tutin 1976; Rodríguez-Oubiña and Ortiz 1993; López Udías et al. 1997) and lobe length (Guinea and Tutin 1976) were used by earlier authors. Rodríguez-Oubiña and Ortiz (1993) were the first to consider the lobe number of the leaf as a good taxonomic characteristic, but they used this characteristic exclusively to differentiate *S. semidentata* from *S. melidensis*. However, incision of the leaves (except for *S. impressa*) and lobe insertion (except for *S. impressa*) have little taxonomic value except for basal and fascicular leaves. This work demonstrated that leaf width, lobe number of the leaf, lobe length, and flower characteristics are good taxonomic characteristics.

Guinea and Tutin (1976) included for the first time the presence/absence of glandular-viscid, and presence/absence of peduncle thickeness as diagnostic characteristics, but Rodríguez-Oubiña and Ortiz (1993) and López Udías et al. (1997) did not include these characters. All of these authors included indumentum characters either of the leaves or the involucral bracts.

The results show that several characters have strong taxonomic value. These include: plant color, plant indumentum, viscose gland covering, plant habit, peduncle shape, insertion, color and texture of the appendage of the involucral bracts, apex shape of the involucral bracts, leaf margin, leaf apex, lobe shape, and receptacle shape. The same occurs for fragility of flowering stems, whether stems are solid or hollow, and number of involucral bracts, but this is the first time that these characteristics have been used in the genus *Santolina*.

This work demonstrated that the quantitative characteristics of the involucral bracts and achene size have essential contribution to the taxonomy of the *S. rosmarinifolia* aggregate, contrary to Pau's (1922) idea the involucral bract shape is not a good taxonomic characteristic for differentiating *S. rosmarinifolia* from *S. pectinata*. The same is true in the genus *Enceliopsis* (Sanders and Clark 1987).

López Udías et al. (1997) differentiated *S. rosmarinifolia* and *S. semidentata* from *S. ageratifolia* and *S. pectinata* based on the decurrence of the involucral bract appendage. They indicated that in the former the appendage is not decurrent, whereas in the latter it is narrowly decurrent to the base. This work demostrates that *S. rosmarinifolia* subsp. *rosmarinifolia* has 10.17%, 67.26%, 80.75%, and 62.39% of the outer, middle and first and second rows of the inner bracts with the appendage narrowly decurrent to the base (lacerate to lacerate-denticulate or lacerate to erose from the apex to the base) whereas *S. semidentata* subsp. *semidentata* has 84.24%, 64.67%, 69.57% and 71.74% of the outer, middle and first and second rows of the inner bracts with the appendage lacerate or lacerate to fimbriate in the apex or in the upper 1/3 and slight fimbriate to the base or in the upper 2/3. However, all individuals of *S. pectinata* and *S. ageratifolia* have the appendage of the involucral bracts decurrent to the base.

Taxonomic Proposals and Evolutionary Hypotheses—Multivariate analysis of morphological data from the *S. rosmarinifolia* aggregate delimits 11 taxa. This number is greater than the five, six, and seven taxa recognized by Rodríguez-Oubiña and Ortiz (1993), López Udías et al. (1997), and Greuter (2008), respectively. The reasons for this increase appears to be based on two factors, data and methodology.

According to the morphological species concept (Cronquist 1978: 15), all of these taxa in *Santolina* can potentially be recognized as species. One possibility would be to apply the biological species concept strictly, with absolute reproductive isolation (Mayr 1969) and a specific niche in nature (Mayr 1982) required for species status. This species concept is strongly related to the ecological species concept (Van Valen 1976: 273). However, for de Queiroz (1998), each species is an independent lineage which occupies a segment of an evolutionary tree delimited by two nodes.

Interspecific hybridization is common in many groups of plants (Heiser 1949, 1973), which might seem to vitiate the criterion of reproductive isolation (Stuessy 1990). Close affinity was observed between: (1) ROS and HET populations of *S. rosmarinifolia* with *S. canescens* and between these taxa with *S. rosmarinifolia* subsp. *arrabidensis*, (2) *S. semidentata* subsp. *semidentata* and *S. semidentata* subsp. *melidensis*, and (3) diploid and tetraploid cytotypes of *S. pectinata* with *S. ageratifolia*. Cytogenetic studies (Rivero-Guerra 2009), together with the morphological analysis suggest that the hybridization between *S. rosmarinifolia* subsp. *rosmarinifolia* and *S. oblongifolia* (diploid) in the Central System of the Iberian Peninsula, and the extensive introgression of the putative hybrids with both parentals, has formed an ample spectrum of phenotypes in the populations. They include plants such as *S. semidentata* subsp. *semidentata* (diploid), *S. semidentata* subsp. *melidensis* (diploid), plants with characteristics intermediate between *S. rosmarinifolia* subsp. *rosmarinifolia* and *S. semidentata* subsp. *semidentata*, and a high frequency of phenotypes with a high degree of similarity to *S. rosmarinifolia* subsp. *rosmarinifolia* and *S. oblongifolia*. These results support hypothesis of Rieseberg and Ellstrand (1993) and Rieseberg (1995) that hybridization does not always result in morphological intermediacy. An ongoing seedling development study (Rivero-Guerra unpubl. data) demonstrates that all of the taxa (except *S. rosmarinifolia*) show narrowly spatulate leaves. Their frequency is variable among the taxa, being lower in diploid cytotypes of *S. pectinata* and in *S. ageratifolia*. However, these two taxa together with *S. impressa* and *S. rosmarinifolia* subsp. *arrabidensis* do not show intermediate characteristics. *Santolina impressa* probably is derived from *S. rosmarinifolia* subsp. *arrabidensis* prior to the polyploidization process in the latter. Several individuals of the hybrid complex show sterile stems with lanceolate leaves, but these leaves were not observed on the flowering stems, while the leaves of diploid cytotypes of *S. pectinata* are mostly lanceolate. However, tetraploid cytotypes of *S. pectinata* have a low frequency of spatulate lower leaves.

The presence of a meiotic configuration above quadrivalent and hexavalent levels in tetraploid cytotypes of *S. pectinata* (Rivero-Guerra 2008b) and *S. ageratifolia* (Rivero-Guerra 2008a) and above bivalent level in *S. semidentata* subsp. *semidentata*,

S. semidentata subsp. *melidensis*, *S. canescens*, HET populations of *S. rosmarinifolia* subsp. *rosmarinifolia* (Rivero-Guerra 2009), *S. impressa* (Rivero-Guerra 2010), and in the hybrid complex (*S. rosmarinifolia* subsp. *rosmarinifolia*, *S. oblongifolia*, and their putative hybrids; Rivero-Guerra 2009) suggest hybrid origins for these taxa. Moreover, the presence of chromosome bridges in anaphase, as well as the moderate to high pollen stainability in these taxa and in the hybrid complex suggest a recombinational hybrid speciation mechanism, where the hybrids probably differ by two or more chromosomal rearrangements from their parental species. However, the extensive introgression with one or both parental taxa restored pollen stainability and a new genotype with a new chromosomal balance was formed.

Several authors consider that the species periphery is one the most active regions of speciation (Simpson 1944; Carson 1959; Mayr 1963; Levin 1970, 1983; Lesica and Allendorf 1995). The spatial distance of the peripheral species with regard to *S. rosmarinifolia* L. is narrow, so the gene flow between them is potentially high, except for *S. rosmarinifolia* subsp. *arrabidensis*, *S. impressa*, and *S. ageratifolia*. The degree of interspecific differentiation is greater than the degree of intraspecific differentiation, reflecting the high level of total phenotypic diversity among taxa, but the Mahalanobis distances indicate that the degree of differentiation between taxa is not high. The patterns of morphological variation of the *S. rosmarinifolia* aggregate indicate a recent diversification process for these taxa, as a consequence they are poorly differentiated. Population differentiation of the *S. rosmarinifolia* aggregate may be promoted either by natural selection or by genetic drift. Intense natural selection may favor different phenotypes in each population in response to differences in selection. While the differences between individuals within populations may be due to the expression of genetic variance and/or phenotypic plasticity, they may allow further changes through natural selection (Williams 1992; Domínguez et al. 1998). In general, morphometric data alone cannot yield precise estimates of the duration of population differentiation (Anderson 1993).

As in Hawaiian *Wikstroemia* (Mayer 1991), continous phenotypic variation occurs in several taxa of the *S. rosmarinifolia* aggregate. Stebbins (1950) suggests that the continuous variation in widespread species is probably due to ecotypic adaptation, where clines or character gradients occur as responses to changes in habitats. The results of this work do not support the suggestion of López Udías et al. (1997) that all the populations in the south of Spain of this aggregate are *S. pectinata*. *Santolina canescens* Lag. was cited as *S. rosmarinifolia* subsp. *canescens* (Lag.) Nyman by Valdés-Bermejo and Antúnez (1981) for two populations of central of the Iberian Peninsula, whereas López Udías et al. (1997) and Greuter (2008) recognized this taxon as a species. However, Lagasca y Segura (1816) cited *S. canescens* for the south of the Iberian Peninsula. *Santolina canescens* and HET populations are strongly related to ROS populations of *S. rosmarinifolia* subsp. *rosmarinifolia*. The morphological and cytogenetic (Rivero-Guerra 2009) variation does not support species status for the populations of the central (Madrid, Salamanca, Soria, Toledo, and Valladolid) and northern Iberian Peninsula (Burgos and Logroño) as López Udías et al. (1997) suggested. The results of this study support the differentiation at subspecies status of HET populations of *S. rosmarinifolia* subsp. *rosmarinifolia*, and support the species status for *S. canescens*.

Two theories may explain the origin of the populations of *S. canescens*. One is that central populations of *S. rosmarinifolia* subsp. *rosmarinifolia* have dispersed from Toledo and Ciudad Real to the south of the Iberian Peninsula, developing a higher number of lobes per leaf, a continuous scarious appendage from the apex to the base, increasing the base width and the length of the appendage of the inner bracts, the base width of the interseminal bracts, and the width of the appendage of the involucral bracts for adapting to the rigorous summers. This theory is supported by the continuous gradual latitudinal increase, from 42° to 36° of these characteristics together with the slight increase in the lobe number of the lower and middle leaves of the flowering and sterile stems. The second theory is based on a suggestion by Valdés-Bermejo and López (1977): *S. canescens* is a result of hybridization between *S. rosmarinifolia* subsp. *rosmarinifolia* and *S. pectinata*. This theory is supported first because *S. canescens* shows a combination of characteristics found in central populations of *S. rosmarinifolia* subsp. *rosmarinifolia* (leaf shape, presence of entire leaves, presence of leaves with involute appressed margins [except for the populations from Sierra de la Pandera in Jaén Province], lobe number and length and width of the leaf of the flowering and sterile stems, presence of solid flowering stems, capitulum shape, and receptacle shape) and *S. pectinata* (plant color, peduncle shape, presence of lanceolate leaves, apex of the outer and middle bracts, appendage insertion of the outer and middle bracts, and keel of the middle bracts). Second, *S. canescens* and *S. pectinata* show similar ecological preferences and are not reproductively isolated; in the contact zone between them, the individuals are indistinguishable by qualitative leaf and involucral bract characteristics, but the specimens of *S. pectinata* can be differentiated by the presence of non-solid flowering stems.

The hybrid zone between *S. rosmarinifolia* subsp. *rosmarinifolia* and *S. oblongifolia* (in granite substrate) is active, large, and stable; the contact zone between HET populations of *S. rosmarinifolia* subsp. *rosmarinifolia* and diploid cytotypes of *S. pectinata* is another important center of speciation and dispersion, but this zone is small and unstable. Hybridization between them could generate individuals capable of persistence in their respective environments and with phenotypes suitable for colonizing habitats divergent from either parent, without an increase in ploidy (homoploid speciation or hybrid speciation). For hybridization to contribute to adaptation some hybrid genotypes within a hybrid population must be able to gain a comparable high fitness which can also involve the colonization of new habitats (Barton 2001; Rieseberg 2001; Baack and Rieseberg 2007). The colonization of ecological niches, divergent from the niches of the parental species may result in ecological isolation if hybrid genotypes attain a higher fitness in the new niche than do the parental genotypes (Buerkle et al. 2000). The introgressive hybrids (with a high degree of similarity to *S. rosmarinifolia* subsp. *rosmarinifolia*) have spread to the northwest (Salamanca, Valladolid, and Zamora) and center (Toledo and Ciudad Real) of the Iberian Peninsula. They established monotypic populations or cohabit with *S. rosmarinifolia* subsp. *rosmarinifolia* in Salamanca, Valladolid, Zamora, and Toledo provinces on basic substrate, or with this taxon and *S. semidentata* subsp. *semidentata* in Zamora province. Probably *S. semidentata* subsp. *semidentata* and *S. semidentata* subsp. *melidensis* found their optimum in slate substrates in Zamora and León provinces in the former and serpentine substrates in La Coruña province in the latter, establishing

monotype populations without competion from *S. rosmarinifolia* subsp. *rosmarinifolia*.

Santolina semidentata Hoffmanns. & Link was cited as *S. rosmarinifolia* L. subsp. *semidentata* (Hoffmanns. & Link) Valdés-Bermejo by Valdés-Bermejo and Antúnez (1981). Rodríguez-Oubiña and Ortiz (1993) agree with this combination. However, López Udías et al. (1997), Rodríguez-Oubiña and Ortiz (1998), and Greuter (2008) recognised this taxon at the species level. The results of this work support this conclusion. As Rodríguez-Oubiña and Ortiz (1993, 1998) suggest, *S. semidentata* subsp. *melidensis* is more closely related to subsp. *semidentata* than to other subspecies. López Udías et al. (1997) cited *S. rosmarinifolia* L. subsp. *melidensis* Rodríguez-Oubiña & Ortiz as *S. semidentata* Hoffmanns. & Link subsp. *melidensis* (Rodríguez-Oubiña & Ortiz) López Udías, Fabregat & Mateo, whereas Rodríguez-Oubiña & Ortiz (1998) cited it as *S. melidensis* (Rodr. Oubiña & S. Ortiz) Rodr. Oubiña & S. Ortiz. Greuter (2008) also recognized *S. melidensis* as a species. Rodríguez-Oubiña and Ortiz (1998) mentioned that various crossing tests show that *S. semidentata* subsp. *melidensis* is reproductively isolated from *S. semidentata* subsp. *semidentata* and from *S. rosmarinifolia* subsp. *rosmarinifolia*, but they do not show any experimental data that support this statement. The involucral bract and leaf characteristics, as well as seedling development (Rivero-Guerra unpubl. data) support the hypothesis that *S. semidentata* subsp. *semidentata* and *S. semidentata* subsp. *melidensis* are derived from the same lineage. Both taxa are significantly differentiated but the degree of differentiation is not high. The same is true of the other two taxa in Group I. In addition, the voucher specimens from Mirantes de Luna (León province; MA 473487), Golada (Pontevedra province; MA 454633), Palas del Rey (Lugo province; MA 508497, 454632), and Velilla del Río Carrión (Palencia province; MA 560236) show that there is no sharp boundary between these taxa. The results of the present work support treating this taxon as *S. melidensis*, as proposed by Rodríguez Oubiña and Ortiz (1998).

Levin (1970) considered that small peripheral isolate populations will be subjected sooner or later to severe environmental stress to which they may respond by rapid evolution or extinction. *Santolina impressa* (diploid), *S. rosmarinifolia* subsp. *arrabidensis* (tetraploid), and *S. ageratifolia* (hexaploid) are "islands" in the extreme west and east of the Iberian Peninsula (Rivero-Guerra 2008a). *Santolina ageratifolia* lives on sandstone and red limolite, and quartzite, whereas *S. impressa* and *S. rosmarinifolia* subsp. *arrabidensis* live on dunes and marl-limestone and sandstone and limestone conglomerate respectively. The tetraploid cytotypes of *S. pectinata* (Rivero-Guerra 2008b) extend to spurs of the Iberian System, occupying mainly the southern part of Cuenca Province. Two tetraploid individuals were found in two diploid populations (Parador Nacional of Sierra de Cazorla and Orcera). These occupy a more restricted and more disturbed area with great human impact towards the north-east of the distribution range, frequently growing on the embankments of highways, in a narrower altitude range. They show a less diverse ecological preference and are located on soils derived from limestone, marl, gypsiferous marl, and clay. The disjunctive distribution of the polyploids arose from fragmentation or contraction of the species range, preventing gene flow between them (Richardson et al. 2003) and allowing fixation of chromosomal, ontogenetic, and morphological changes, which favored differentiation and allopatric speciation (Marlowe and Hufford 2007; Rivero-Guerra 2008c). In addition, the results suggest that the variation between taxa is not associated with ploidy level because all taxa are significantly different from each other. The results suggest strong relationships between *S. pectinata* (diploid and tetraploid cytotypes) and *S. ageratifolia* (hexaploid), but the morphological differences between these two taxa are stronger than those within the former. The results support the differentiation at subspecies status of diploid and tetraploid cytotypes of *S. pectinata*.

As opposed to the situation found in *Gaillardia* by Marlowe and Hufford (2007), hybridization and polyploidization play an important role in speciation in *Santolina*. The intriguing taxonomic complexity of the *S. rosmarinifolia* aggregate taxa can probably be explained to a large extent by recurrent hybridization and subsequent interbreeding of the resulting genotypes, and by the absence of karyotypic divergences and of spatial isolation (except for *S. impressa*) between diploid taxa. Probably, the Central System of the Iberian Peninsula is the center of the origin and dispersion of the taxa of this aggregate. The results suggest that the introgression of advantageous alleles (Kim and Rieseberg 1999) is probably the "escape" mechanism for these taxa. Quantitative and qualitative data support two evolutionary lines in this aggregate that are not yet strongly differentiated. On one hand are the diploid and tetraploid cytotypes of *S. pectinata* and *S. ageratifolia*, and on the other the remaining taxa. *Santolina impressa*, with a peripheral distribution and reproductive isolation, is a taxon with higher potential for differentiation from the remaining taxa. These results support the hypothesis that the more disjunct taxa are, the more divergent they are likely to be (Lesica and Allendorf 1995).

Taxonomic Implications—Multivariate analysis of the of morphological data of 2,323 individuals from 209 populations of the *S. rosmarinifolia* aggregate delimit 11 taxa within it: three species and six subspecies, two of them with two varieties each. The taxa are: (1) *S. oblongifolia* Boiss., (2) *S. ageratifolia* Barnades ex Asso, (3) S. *impressa* Hoffmanns. & Link, (4) *S. canescens* Lag., (5) *S. semidentata* Hoffmanns. & Link, (6) *S. melidensis* (Rodríguez-Oubiña & Ortiz) Rodríguez-Oubiña & Ortiz, (7) *S. rosmarinifolia* L. subsp. *rosmarinifolia*, (8) *S. rosmarinifolia* L. subsp. *arrabidensis* Rivero-Guerra, (9) *S. rosmarinifolia* L. subsp. *castellana* Rivero-Guerra, (10) *S. pectinata* Lag. subsp. *pectinata*, and (11) *S. pectinata* Lag. subsp. *montiberica* Rivero-Guerra.

Taxonomic Treatment

Santolina rosmarinifolia L. subsp. **castellana** Rivero-Guerra, subsp. nov.—TYPE: SPAIN: Salamanca Province: Castellanos de Villiquera, 41°02′65″N 5°40′52″W, 800 m, on limestone and quartzite, 10 July 1998, *A. O. Rivero-Guerra s. n.* (holotype: SEV 239491).

A *Santolinae rosmarinifolia* L. s. s. praesertim differt colore viridi-olivaceo vel vivide viridi; indumento tomentoso aut tomentosa vel glabrescenti, raro glabra; caule florifero 0.7–1.9(–2.1) mm diametri, plerumque solido, raro infla capitulum fistuloso, foliis inferioribus dentatis, squamoso-denticulatis, pinnatifidis vel pinnatisectis, (0–)2–32(–48) lobulos ferentibus, lobulis (0–)0.1–0.7(–2) mm longis, foliis mediis dentatis, squamoso-denticulatis, integris vel pinnatifidis,

0–70(–92) lobulos ferentibus, lobulis (0–)0.1–0.7(–2) mm longis; caulibus sterilibus foliis inferioribus pinnatifidis, dentatis, squamoso-denticulatis, pinnatipartitis raro integris, (0–)2–60(–90) lobulos ferentibus, lobulis (0–)0.1–1.5(–3.9) mm longis; foliis mediis ut in caule florifero; pedunculo leviter incrassato; capitulo 4.9–13.8 mm diametri, plerumque semigloboso vel hemisphaerico; involucri phylliis in appendicem scariosam productis, appendice in 1/3 superiore lacerata vel lacerato-fimbriata, plerumque decurrenti, in 2/3 inferioribus leviter fimbriata vel lacerato-denticulata, raro non decurrenti.

Plant usually bright olive-green or bright dark-green, usually tomentose or becoming glabrescent, rarely glabrous. Flowering stem 0.7–1.9(–2.1) mm in diameter, usually solid, rarely hollow near the insertion with the capitulum; lower leaves with (0–)2–32(–48) lobes of (0–)0.1–0.7(–2) mm long, usually dentate, scaly-dentate, pinnatifid or pinnatisect; middle leaves with 0–70(–92) lobes of (0–)0.1–0.7(–2) mm long, usually dentate, scaly-dentate, entire or pinnatifid. Sterile stem with lower leaves having (0–)2–60(–90) lobes of (0–)0.1–1.5 (–3.9) mm long, pinnatifid, pinnatipartite, dentate, or scaly-dentate, rarely entire; middle leaves same as the middle leaves of the flowering stem. Peduncle slightly thickened above. Capitulum 4.9–13.8 mm diameter, usually subglobose or hemispherical. Involucral bracts with scarious appendages lacerate to lacerate-fimbriate 1/3 upper, usually decurrent, slightly fimbriate or lacerate-denticulate to fimbriate in the lower 2/3, rarely not decurrent.

Chromosome Number—$2n = 2x = 18$ and $2n = 4x = 36$ (Rivero-Guerra 2009)

Distribution—Central Iberian Peninsula, occupying Ciudad Real, Salmanca, Toledo, and Zamora Provinces.

Habitat—Located in disturbed areas with great human impact, frequently growing on the embankments of highways, in a narrow altitude range of 363–853 m, on soils derived from limestone; slate and quartzite; limestone and quartzite; conglomerates, sand, sandstone, lime and clay; marl, marl-limestone, and limestone; and limestone and marl.

Phenology—Flowering and fruiting from July to August.

Additional Specimens Examined—SPAIN. Ciudad Real: 6 km from Manzanares, towards Cuenca, 39°01′87″N 3°18′41″W, 666 m, limestone, (SEV 249111); between Herencia and Puerto Lápices, 39°21′08″N 3°23′98″W, 665 m, limestone, (SEV 249112); Sierra Madrona, Solana del Pino, 38°27′51″N 40°04′57″W, 722 m, slate and quartzite, (SEV 249113); idem, San Lorenzo de Calatrava, 38°28′32″N 3°48′15″W, 808 m, slate and quartzite, (SEV 249114). Salamanca: Calzada de Valdunciel, 41°04′67″N 5°41′62″W, 807 m, granites, (SEV 249254); Castellanos de Villiquera, 41°02′65″N 5°40′52″W, 800 m, limestone and quartzite; Santiz, 41°13′43″N 5°49′58″W, 897 m, limestone, (SEV 249115). Toledo: Mocejón, 39°56′34″N 3°54′29″W, 475 m, sand, clay, gypsum and limestone, (SEV 249255); Puebla de Montalbán, 39°50′65″N 4°23′81″W, 420 m, sand, clay and limestone, (SEV 249116); Azucaica, 39°52′87″N 3°59′33″W, 458 m, limetone, clay and sandstone, (SEV 249117); between Talavera de la Reina and Calera y Chozas, 39°55′20″N 4°54′55″W, 363 m, conglomerates, sand, sandstone, lime and clay, (SEV 249118). Valladolid: Olmedo, 41°18′25″N 4°41′0.8″W, 800 m conglomerates, sandstone, sand, clay and granites, (SEV 249256). Zamora: El Cubo de la Tierra del Vino, 41°16′32″N 5°42′17″W, 853 m, limestone, (SEV 249119); Peleas de Arriba, 41°19′30″N 5°43′44″W, 835 m, marl, marl-limestone and limestone, (SEV 249120); Morales del Vino, 41°27′66″N 5°43′17″W, 681 m, marl, marl-limestone and limestone, (SEV 249121); Corrales, 41°22′81″N 5°43′18″W, 739 m, marl, marl-limestone and limestone, (SEV 249122); Sayago, 41°18′97″N 5°56′99″W, 820 m, limestone and marl, (SEV 249123); Cubillos, 41°34′97″N 5°45′81″W, 700 m, limestone, (SEV 249257).

Santolina pectinata Lag. subsp. **montiberica** Rivero-Guerra, subsp. nov.—TYPE: SPAIN: Cuenca Province: Olmeda del Rey, 39°48′53″N 2°4′22″W, 910 m, on marl, 1 July 1998, *A. O. Rivero-Guerra s. n.* (holotype: SEV 239492).

A *Santolinae pectinata* Lag. sensu stricto praesertim differt magnitudine 23–90 cm diametri, 14–40 cm alte; caule florifero foliis inferioribus (4.5–)5.2–12.9(–13.6–17.2) mm longis, (0.9–)1.2–5.8(–10.2) mm latis, lobulis 0.6–2.1 mm longis, foliis mediis 6.2–19.3(–20.2–31.5) mm longis, (0.8–)1.1–2.7(–3–5) mm latis, linearibus, anguste ellipticis atque ambae superficiebus leviter canaliculatis, vel lanceolatis, lobulis 0.2–0.9(–1.5) mm longis, foliis superioribus plerumque linearis vel lanceolatis; caulibus sterilibus foliis inferioribus (0.9–)5.5–17.6(–20.3) mm longis, 1.1–6.6(–8.7) mm latis, lobulis 0.7–2.8 mm longis, foliis mediis (7.3–)8.8–19.8(–20–30.5) mm longis, (0.9–)1.1–4.8(–5–7.8) mm latis, (6–)8–136(–160) lobulos ferentibus, lobulis 0.3–3(–6.2) mm longis; pedunculus (2.2–6.8–)13.1–99(–103–128.8) mm longis, leviter incrassatus; capitulo 5.4–10.7(–11–14.7) mm diametri, 6.1–9.9(–10.9) mm alte.

Plant 23–90 cm in diameter and 14–40 cm tall. Flowering stem with lower leaves (4.5–)5.2–12.9(–13.6–17.2) mm long and (0.9–)1.2–5.8(–10.2) mm wide, lobes 0.6–2.2 mm long; middle leaves 6.2–19.3(–20.2–31.5) mm long and (0.8–)1.1–2.7 (–3–5) mm wide, linear, narrowly elliptical, slightly grooved on both sides, or lanceolate, lobes 0.2–0.9(–1.5) mm long; upper leaves usually linear or lanceolate. Sterile stem with lower leaves (0.9–)5.5–17.6(–20.3) mm long and 1.1–6.6 (–8.7) mm wide, lobes 0.7–2.8 mm long; middle leaves (7.3–) 8.8–19.8(–20–30.5) mm long and (0.9–)1.1–4.8(–5–7.8) mm wide, with (6–)8–136(–160) lobes 0.3–3(–6.2) mm long. Peduncle (2.2–6.8–)13.1–99(–103–128.8) mm long, slightly thickened above. Capitulum 5.4–10.7(–11–14.7) mm in diameter and 6.1–9.9(–10.9) mm high.

Chromosome Number—$2n = 4x = 36$ (Rivero-Guerra 2008b).

Distribution—This taxon grows on spurs of the Iberian System, mainly in the southern part of Cuenca Province (Rivero-Guerra 2008b).

Habitat—Located in disturbed areas with great human impact, frequently growing on the embankments of highways, in a narrow altitude range from 740–1,140 m, on soils derived from limestone, marl, gypsiferous marl, and clay (Rivero-Guerra 2008b).

Phenology—Flowering and fruiting from July to August.

Additional Specimens Examined—SPAIN. Cuenca: Almodóvar del Pinar, 39°44′1″N 1°54′46″W, 920 m, limestone, *Rivero-Guerra* (SEV 249235); between Almodóvar del Pinar and Puerto de Tórdigas, 39°47′26″N 1°56′34″W, 1,000 m, limestone, (SEV 249236); between Puerto de Tórdigas and Cuenca, 40°8′32″N 2°20′44″W, 1,140, limestone, (SEV 249237); La Almarcha, 2 km from La Almarcha, 39°49′29″N 2°21′8″W, 890 m, clay, (SEV 249238); idem, 5 km from La Almarcha, 39°42′41″N 2°22′29″W, 920 m, clay, (SEV 249239); between Cuenca and Ciudad Real, 16 km from Villa Escusa de Haro, 39°38′5″N 2°34′50″W, 880 m, gypsiferous marl, (SEV 249240);

between Almarcha and Cuenca, at Belmontejo crossroads, 39°43′55″ N 2°21′25″W, 850 m, clay, (SEV 249241); Mota del Cuervo, 5 km from Mota del Cuervo towards Cuenca, 39°30′58″N 2°50′47″W, 740 m, gypsiferous marl, (SEV 249242); Cuenca to Ciudad Real road, after the detour towards La Almarcha, 39°41′46″N 2°22′33″W, 840 m, gypsiferous marl, (SEV 249243); between Villar de Olalla and San Lorenzo de la Parrilla, 39°52′7″N 2°20′6″W, 910 m, limestone, (SEV 249244); between Cuenca and Almodóvar del Pinar, at the Olmeda del Rey crossroads, 39°50′6″N 2°0′56″W, 1,090 m, gypsiferous marl, (SEV 249245); Olmeda del Rey, 39°48′53″N 2°4′22″W, 910 m, marl, (SEV 249246); Valeria, 39°48′55″N 2°8′24″W, 880 m, marl, (SEV 249247). Valverde de Júcar, 39°43′43″N 2°13′10″W, 820 m, limestone and marl, (SEV 249248); Olivares de Júcar, 39°45′47″N 2°20′39″W, 850 m, marl, (SEV 249249); Huete, 40°8′18″N 2°41′27″W, 840 m, limestone, (SEV 249250); Los Pozuelos, Barchín del Hoyo, 39°39′50″N 2°4′28″W, 1,000 m, limestone, (SEV 249251); Tarancón, 39°59′23″N 3°0′32″W, 808 m, limestone, (SEV 249252); Villarejo de Fuentes, 39°47′19″N 2°41′36″W, 900 m, limestone, (SEV 249253).

Conservation Value—Species conservation depends on protecting the genetic variability present throughout the range of the species (Lesica and Allendorf 1995; Lihová et al. 2004). Rivero-Guerra (2008a) explained in detail why the preservation of the genetic diversity of the endemic species and endangered species of the genus *Santolina*, in situ, should be a high priority. This work, together with other studies in this aggregate (Rivero-Guerra 2008a-c, 2009), provide important information with which to evaluate the conservation value of these taxa and so justify their protection. For example: the habitat of *S. impressa* has suffered alteration through human activity. In 1998, a large population (43 km) of *S. impressa* inhabited an area from Alcaçer do Sal to Troia on dunes, whereas in 2007, only isolated individuals were found. The soil has been flattened and the dunes have been destroyed. The present work provides justification for including this species in the IUCN red list, since it is an endangered species. The creation of a natural reserve in the zone would be ideal for its conservation.

Hybridization may, however, endanger the parent species. The pure populations of *S. rosmarinifolia* subsp. *rosmarinifolia* and *S. oblongifolia*, principally *S. oblongifolia*, are endangered in the Central Systems of the Iberian Peninsula as a result of hybridization between the two taxa. This phenomenon may lead to the extinction of the genetically pure parent forms through their "dilution" by swarms of the hybrid forms (Adamowski 1995, Cozzolino et al. 2006). I strongly advocate the investment of resources in the protection of these pure parent forms.

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Literature Cited

Adamowski, W. 1995. Phenotypic variation of *Epipactis helleborine* × *E. atrorubens* hybrids in anthropogenic conditions. *Acta Societatis Botanicorum Poloniae* 64: 303–312.

Almeida-Pinheiro, de Carvalho, M. A., C. C. Wilcock, T. M. Marques dos Santos, I. C. Vale-Lucas, J. F. Teixeira-Ganança, E. Franco, D. Thangadurai, D. Muralidhra-Rao, and D. Freitas-Sousas. 2004. A review of the genus *Semele* (Ruscaceae) systematics in Madeira. *Botanical Journal of the Linnean Society* 146: 483–497.

Anderson, S. 1993. Population differentiation in *Crepis tectorum* (Asteraceae): patterns of correlation among characters. *Biological Journal of the Linnean Society. Linnean Society of London* 49: 185–194.

Armbruster, W. S. 1991. Multilevel analysis of morphometric data from natural plant populations: insights into ontogenetic, genetic, and selective correlations in *Dalechampia scandens*. *Evolution* 45: 1229–1244.

Baack, E. J. and L. H. Rieseberg. 2007. A genomic view of introgression and hybrid speciation. *Current Opinion in Genetics & Development* 17: 513–518.

Barton, N. H. 2001. The role of hybridization in evolution. *Molecular Ecology* 10: 551–568.

Buerkle, C. A., R. J. Morris, M. A. Asmussen, and L. H. Rieseberg. 2000. The likelihood of homoploid hybrid speciation. *Heredity* 84: 441–451.

Carson, H. L. 1959. Genetic conditions which promote or retard the formation of species. *Cold Spring Harbor Symposia on Quantitative Biology* 24: 87–105.

Conner, J. and S. Via. 1993. Patterns of phenotypic and genetic correlations among morphological and life-history traits in wild radish, *Raphanus raphanistrum*. *Evolution* 47: 704–711.

Coyle, B. F., T. L. Sharik, and P. O. Feret. 1982. Variation in leaf morphology among disjunct and continuous populations of river birch (*Betula nigra* L.). *Silvae Genetica* 31: 122–125.

Cozzolino, S., A. M. Nardellaa, S. Impagliazzoa, A. Widmerb, and C. Lexerc. 2006. Hybridization and conservation of Mediterranean orchids: Should we protect the orchid hybrids or the orchid hybrid zones? *Biological Conservation* 129: 14–23.

Cronquist, A. 1978. Once again, what is a species? Pp. 3–20 in *Biosystematics in agriculture*, ed. L. V. Knutson. Montclair: Allenheld Osmun.

Dickinson, T. A. and J. B. Phipps. 1985. Studies in *Crataegus* L. (Rosaceae: Maloideae). XIII. Degree and pattern of phenotypic variation in *Crataegus* sect *Crus-galli* in Ontario. *Systematic Botany* 10: 322–337.

Domínguez, C. A., L. E. Eguiarte, J. Nuñez-Farfán, and R. Dirzo. 1998. Flower morphometry of *Rhizophora mangle* (Rhizophoraceae): geographical variation in Mexican populations. *American Journal of Botany* 85: 637–643.

Dytham, C. 2003. *Choosing and using statistics. A biologist's guide*, 2nd ed. Oxford, U. K.: Blackwell.

Ellstrand, N. C., R. Whitkus, and L. H. Rieseberg. 1996. Distribution of spontaneous plant hybrids. *Proceedings of the National Academy of Sciences USA* 93: 5090–5093.

Endler, J. A. 1995. Multiple-trait coevolution and environmental gradients in guppies. *Trends in Ecology & Evolution* 10: 22–29.

Ereshefsky, M. 2002. Linnaean ranks: Vestiges of a bygone era. *Philosophy of Science* 69: S305–S315.

Freeman, D. C., W. A. Turner, E. D. Mcarthur, and J. H. Graham. 1991. Characterization of a narrow hybrid zone between two subspecies of big sagebrush (*Artemisia tridentata*: Asteraceae). *American Journal of Botany* 78: 805–815.

Gower, J. C. 1971. A general coefficient of similarity and some of its properties. *Biometrics* 27: 857–871.

Grafen, A. and R. Hails. 2002. *Modern statistics for the life sciences*. Oxford: Oxford University Press.

Greimler, J., B. Hermanowski, and C. C. Jang. 2004. A reevaluation of morphological characters in European *Gentianella* section *Gentianella* (Gentianaceae). *Plant Systematics and Evolution* 248: 142–169.

Greuter, W. 2008. *Santolina*. Pp. 697–698 in *Med-Checklist. A critical investory of vascular plants of the circum-mediterranean countries. II. Dicotyledones (Compositae)*. Palermo: OPTIMA Secretariat. Genèva: Med-Checklist Trust of OPTIMA. Berlin: Euro+Med Plantbase Secretariat.

Guinea, E. 1970. *Santolina* europaeae. *Anales del Instituto Botánico A. J. Cavanilles* 27: 29–44.

Guinea, E. and T. G. Tutin. 1976. *Santolina*. Pp. 144–145 in *Flora Europea*, vol. 4, eds. T. G. Tutin, V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine, S. M. Walter, and D. A. Webb. Cambridge: Cambridge University Press.

Heiser, C. B. Jr. 1949. Natural hybridization with particular reference to introgressions. *Botanical Review* 15: 645–687.

Heiser, C. B. Jr. 1973. Introgression re-examined. *Botanical Review* 39: 645–687.

Herrera, C. M. 1990. The adaptedness of the floral phenotype in a relict endemic, hawkmoth-pollinated violet. 1. Reproductive correlates of floral variation. *Biological Journal of the Linnean Society* 40: 263–274.

Herrera, C. M. 1993. Selection of floral morphology and environmental determinants of fecundity in a hawkmoth-pollinated violet. *Ecological Monographs* 63: 251–275.

Herrera, J. 2001. The variability of organs differentially involved in pollination, and correlations of traits in Genisteae (Leguminosae: Papilonoideae). *Annals of Botany* 88: 1027–1037.

Jahandiez, E. and R. Maire. 1934. *Catalogue des plantes du Maroc. III*. Imprimerie Minerva.

Jain, S. K., K. N. Rai, and R. S. Singh. 1981. Population biology of *Avena* XI. Variation in peripheral isolates of *A. barbata*. *Genetica* 56: 213–215.

Jordan, A. and U. J. Fourreau. 1869. *Icones ad Floram Europae novo fundamento instaurandam spectantes. II.* Paris: Apud Savy, Bibliopolam.

Kim, S. C. and L. H. Rieseberg. 1999. Genetic architecture of species differences in annual sunflowers: implications for adaptive trait introgression. *Genetics* 153: 965–977.

Klimko, M., K. Boratyńska, A. Boratyńska, and K. Marcysiak. 2004. Morphological variation of *Juniperus oxycedrus* subsp. *macrocarpa* (Cupressaceae) in three Italian localities. *Acta Societatis Botanicorum Poloniae* 73: 113–119.

Lagasca y Segura, M. 1816. *Elenchus plantarum, quae in horto Region Botanico Matritensis*. Madrid: Matriti ex Typographia Regia.

Laurin, M. 2005. The advantages of phylogenetic nomenclature over Linnean nomenclature. Pp. 67–97 in *Animal Names*, eds. A. Minelli, G. Ortalli, and G. Sanga. Venice: Instituto Veneto di Scienze, Lettere ed Arti.

Laurin, M. 2008. The splendid isolation of biological nomenclature. *Zoologica Scripta* 37: 223–233.

Lefèbvre, C. and X. Vekemans. 1995. A numerical taxonomic study of *Armeria maritima* (Plumbaginaceae) in North America and Greenland. *Canadian Journal of Botany* 73: 1583–1595.

Lesica, P. and F. W. Allendorf. 1995. When are peripheral populations valuable for conservation? *Conservation Biology* 9: 753–760.

Levin, D. A. 1970. Developmental instability and evolution in peripheral isolates. *American Naturalist* 104: 343–353.

Levin, D. A. 1983. Polyploidy and novelty in flowering plants. *American Naturalist* 122: 1–25.

Lihová, J., K. Marhold, A. Tribsch, and T. F. Stuessy. 2004. Morphometric and AFLP re-evaluation of tetraploid *Cardamine amara* (Brassicaceae) in the Mediterranean. *Systematic Botany* 29: 134–146.

López Udías, S., C. Fabregat, and G. Mateo. 1997. *Santolina ageratifolia* Barnades ex Asso (Compositae) y el agregado *S. rosmarinifolia* L. *Anales del Jardin Botanico de Madrid* 55: 285–296.

Marlowe, K. and L. Hufford. 2007. Taxonomy and biogeography of *Gaillardia* (Asteraceae): a plylogenetic analysis. *Systematic Botany* 32: 206–226.

Mayer, S. S. 1991. Morphological variation in Hawaiian *Wikstroemia* (Thymeleaceae). *Systematic Botany* 16: 693–704.

Mayr, E. 1963. *Animal species and evolution*. Cambridge: Harvard University Press.

Mayr, E. 1969. The biological meaning of species. *Biological Journal of the Linnean Society* 1: 311–320.

Mayr, E. 1982. *The growth of biological thought: diversity, evolution, and inheritance*. Cambridge: Harvard University Press.

Nyman, C. F. 1879. *Conspectus florae Europaeae*. Orebro Suecia typis Officinae Bohlinianae.

Pau, C. 1922. *Nueva contribución al estudio de la Flora de Granada*. Memòries del Museu de Ciències Naturals de Barcelona. I, n° 1, sèrie Botánica. Barcelona: Museu de Ciéncies Naturals Barcelona. Ajuntament de Barcelona I Mancomunitat de Barcelona.

Pimentel, M. and E. Sahuquillo. 2007. Infraspecific variation and phylogeography of the high-polyploid Iberian endemic *Anthoxanthum amarum* Brot. (Poaceae; Pooideae) assessed by random amplified polymorphic DNA markers (RAPDs) and morphology. *Botanical Journal of the Linnean Society* 155: 179–192.

Queiroz, de. K. 1998. The general lineage concept of species, species criteria, and the rocess of speciation: A conceptual unification and terminological recommendations. Pp. 57–75 in *Endless forms: Species and speciation*, eds. D. J. Howard, and S. H. Berlocher. New York: Oxford University Press.

Rieseberg, L. H. 1995. The role of hybridization in evolution: old wine in new skins. *American Journal of Botany* 82: 944–953.

Rieseberg, L. H. 2001. Chromosomal rearrangements and speciation. *Trends in Ecology & Evolution* 16: 351–358.

Rieseberg, L. H. and N. C. Ellstrand. 1993. What can morphological and molecular markers tell us about plant hybridization? *Critical Reviews in Plant Sciences* 12: 213–241.

Richardson, J. E., M. F. Fay, Q. C. B. Cronk, and M. W. Chase. 2003. Species delimitation and the origin of populations island representatives of *Phylica* (Rhamnaceae). *Evolution* 57: 816–827.

Rivero-Guerra, A. O. 2008a. Cytogenetics, geographical distribution and pollen fertility of diploid and tetraploid cytotypes of *Santolina pectinata* Lag. (Asteraceae: Anthemideae). *Botanical Journal of the Linnean Society* 156: 657–667.

Rivero-Guerra, A. O. 2008b. Phenotypic differentiation of peripheral populations of *Santolina rosmarinifolia* L. (Asteraceae: Anthemideae). *Botanical Journal of the Linnean Society* 158: 650–668.

Rivero-Guerra, A. O. 2008c. Cytogenetics, biogeography and biology of *Santolina ageratifolia* Barnades ex Asso (Asteraceae: Anthemideae). *Botanical Journal of the Linnean Society* 157: 797–807.

Rivero-Guerra, A. O. 2009. Cytogenetics, geographical distribution, pollen stainability and reproductive fitness of five diploid taxa of *Santolina rosmarinifolia* L. aggregate (Asteraceae: Anthemideae). *Plant Systematics and Evolution* 281: 17–34.

Rivero-Guerra, A. O. 2010. Cytogenetics, geographical distribution, pollen stainability and fecundity of *Santolina impressa* Hoffmanns. & Link (Asteraceae: Anthemideae). *Folia Geobotanica* 45: 95–109.

Rodríguez-Oubiña, J. and S. Ortiz. 1993. A new subspecies of *Santolina rosmarinifolia* L (Asteraceae) from serpentine soils in Central Galicia Iberian Peninsula). *Botanical Journal of the Linnean Society* 111: 457–462.

Rodríguez-Oubiña, J. and S. Ortiz. 1998. Unha nova combinación en *Santolina* L. (Compositae). *Nova Acta Científica Compostelana (Biología)* 8: 299–300.

Sanders, D. L. and C. Clark. 1987. Comparative morphology of the capitulum of *Enceliopsis* (Asteraceae: Heliantheae). *American Journal of Botany* 74: 1072–1086.

Sapir, Y., A. V. I. Shmida, O. R. I. Fragman, and H. P. Come. 2002. Morphological variation of the Oncocyclus irises (*Iris*: Iridaceae) in the southern Levant. *Botanical Journal of the Linnean Society* 139: 369–394.

Sargent, D. J., M. Geibel, J. A. Hawkins, M. J. Wilkinson, N. H. Battey, and D. W. Simpson. 2004. Quantitative and qualitative differences in morphological characters revealed between diploid *Fragaria* species. *Annals of Botany* 94: 787–797.

Schnabel, A. and J. L. Hamrick. 1990. Organization of genetic diversity within and among populations of *Gleditsia triacanthos* (Leguminosae). *American Journal of Botany* 77: 1060–1069.

Schwaegerle, K. E. and B. A. Schaal. 1979. Genetic variability and founder effect in the pitcher plant *Sarracenia purpurea* L. *Evolution* 33: 1210–1218.

Simpson, G. G. 1944. *Tempo and mode in evolution*. New York: Columbia University Press.

Sokal, R. R. and C. D. Michener. 1958. A statistical method for evaluating systematic relationships. *University of Kansas Science Bulletin* 28: 1409–1438.

Stearn, W. T. 1996. *Botanical Latin*, 4th ed. Kew: David and Charles.

Stebbins, G. L. 1950. *Variation and evolution in plants*. New York: Columbia University Press.

Stuessy, T. F. 1990. *Plant taxonomy. The systematic evaluation of comparative data*. New York: Columbia University Press.

Stuessy, T. F. and D. M. Spooner. 1988. The adaptive and phylogenetic significance of receptacular bracts in the Compositae. *Taxon* 37: 114–126.

Thomas, G., J. Sreejayan, L. Joseph, and P. Kuriach. 2001. Genetic variation and population structure in *Oryza malampuzhaensis* Krish. et Chand. endemic to Western Ghats, South India. *Journal of Genetics* 80: 141–148.

Thompson, J. D., S. Lavergne, L. Afree, M. Gaudel, and M. Debussche. 2005. Ecological differentiation of Mediterranean endemic plants. *Taxon* 54: 967–976.

Urbaniak, L., L. Karliński, and R. Popielarz. 2003. Variation of morphological needle characters of Scots pine (*Pinus sylvestris* L.)

populations in different habitats. *Acta Societatis Botanicorum Poloniae* 72: 37–44.

Valdés-Bermejo, E. and C. Antúnez. 1981. Estudios cariológicos en especies españolas del género *Santolina* L. (Compositae). *Anales del Jardin Botanico de Madrid* 38: 127–144.

Valdés-Bermejo, E. and G. López. 1977. Aportaciones a la Flora Española. *Anales del Instituto Botánico Cavanilles* 34: 170–173.

Van Valen, L. 1976. Ecological species, multispecies, and oaks. *Taxon* 25: 233–239.

Weber, E. 1997. Morphological variation of the introduced perennial *Solidago canadensis* L. *sensu lato* (Asteraceae) in Europe. *Botanical Journal of the Linnean Society* 123: 197–210.

Williams, G. C. 1992. *Natural selection domains, levels, and challenges.* Oxford Series in Ecology and Evolution. Oxford: Oxford University Press.

Willkomm, M. 1859. Pugillus plantarum novarum peninsulae pyrenaicae. *Linnaea* 30: 83–142.

Willkomm, M. and J. Lange. 1870. *Prodromus Florae Hispanicae.* II. Stuttgartiae: Typis e sumtibus librariae E. Schweizerbart.