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Phylogenetic analysis of the *Santolina rosmarinifolia* aggregate (Asteraceae: Anthemideae: Santolininae) based on morphological characteristics

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The phylogenetic relationships between taxa of the *Santolina rosmarinifolia* aggregate were studied using TNT and PAUP parsimony analyses of a morphological data matrix that encompasses 2516 individuals. Two major clades can be distinguished: clade 1 comprises *S. semidentata*, *S. melidensis*, *S. impressa*, *S. orocarpetana* and *S.* ×*oblongifolia*; clade 2 comprises *S. ageratifolia, S. canescens* and the subspecies *arrabidensis*, *rosmarinifolia*, *castellana*, *pectinata* and *montiberica*. No qualitative characteristics or groups of characteristics clearly differentiate these clades. Monophyly of the *S. rosmarinifolia* aggregate is supported. Most populations appear highly polyphyletic. *Santolina impressa*, *S. melidensis*, *S. ageratifolia* and the *S. rosmarinifolia* subspecies *rosmarinifolia* and *arrabidensis* form a monophyletic group whereas *Santolina orocarpetana* is polyphyletic and *Santolina oblongifolia*, *S. canescens*, *S. semidentata* and the subspecies *castellana*, *pectinata* and *montiberica* are all paraphyletic. The internal branches have an average length of 20.1 steps, with a standard deviation of 8.5 steps. The basal branch of several taxa shows a much higher number than average (20.1 steps) over the tree, which suggests that reasonably good clade support is present for these taxa. *Santolina semidentata* is the most variable taxon of this aggregate. The presence of a capitulum with three rows of involucral bracts is the ancestral condition in the aggregate. The results suggest that this aggregate arose from an ancient polyploid. A key to the taxa is provided.

Speciation plays a major role in modern evolutionary biology (Rieseberg and Willis 2007). The role of speciation for phenotypic and genotypic divergence is well known, but there is also growing evidence that molecular (Venditti and Pagel 2010) and phenotypic (Cubo 2003) evolution accelerates during speciation. Thus, it is not surprising that many evolutionary biologists have focused on the speciation process. Experimental, field, and theoretical work suggests that reproductive isolation, a prerequisite of speciation, may arise through chromosomal repatterning, hybridation, ecological divergence, and/or spatial separation (Grant 1981, McCarthy et al. 1995, Buerkle et al. 2000, Rieseberg et al. 2003, Karrenberg et al. 2007).

Barton (2001) argued that hybridisation has played a crucial role in evolution, and that it is an important force that contributes to adaptive evolution and speciation, especially in angiosperms. However, hybridisation as a process in the evolution of closely related lineages remains poorly understood. Hybrid genotypes may become established through diploid hybrid speciation (Grant 1981, Rieseberg 1997, Gross et al. 2003, 2007, Rieseberg and Willis 2007, Abbott et al. 2010), which involves hybrid establishment. In this process several forces (fertility selection, phenotypic selection and the selection of ecologically relevant traits) act simultaneously (Karrenberg et al. 2007). Hendry et al. (2007) emphasized the importance of the occupation of a new ecological niche for the establishment of a new homoploid hybrid (without change in chromosome number). However, in the absence of reproductive barriers, homoploid hybrids may still become established despite the possibility of backcrossing with their parental species (Turelli et al. 2001, Buerkle and Rieseberg 2008, Abbott et al. 2010).

Hybridisation introduces new allelic combinations that may be neutral, deleterious or advantageous (Barton 2001, Sapir et al. 2007), affecting multiple aspects of the phenotype, genotype, and physiological response to abiotic conditions, among others (Whitney et al. 2010). New gene combinations may enable hybrids to colonize new habitats where they have better chances of becoming genetically stabilized (Kirkpatrick and Barton 1997, Rieseberg et al. 2007, Donovan et al. 2010, Li et al. 2010).

Speciation is often associated with changes in ploidy in angiosperms. This may explain why polyploids are common in that clade. Wood et al. (2009) found that 35.04%

of the taxa of *Asterids* usually ranked at the infrageneric level include polyploids, and 12.45% of the speciations in that clade involved polyploidisation. The *Santolina rosmarinifolia* aggregate (endemic to the Iberian Peninsula and northern Africa) is a good model system to better understand the role of polyploidisation and hybridisation in the speciation process. Indeed, multiple hybridisation events and the recurrent formation of polyploids may have resulted in complex evolutionary patterns in the *S. rosmarinifolia* aggregate (Rivero-Guerra 2008a, 2008b, 2008c, 2009, 2010, 2011). Both mechanisms appear to be important in the evolution of this aggregate, as in *Helianthus* (Rieseberg 2001), polyploid plants (Leitch and Leitch 2008), *Ranunculus auricomus* (Hörandl et al. 2009), *Senecio* (Brennan et al. 2009), and *Pinus* (Zhou et al. 2010). Recent studies by Rivero-Guerra (2011) based on cytogenetic, morphological and ecogeographical characters showed that several of the 11 taxa of the *S. rosmarinifolia* aggregate are polyploids. Three of them (*S. rosmarinifolia* subsp. *castellana* called subsp. *castellana* below, *S. canescens* and *S. pectinata*) have two cytotypes each: diploid and tetraploid (Rivero-Guerra 2008c, 2009). Some of the other taxa of this aggregate are diploid, such as *S. rosmarinifolia* subsp. *rosmarinifolia* (called subsp. *rosmarinifolia* below), *S. impressa*, *S. orocarpetana* (Rivero-Guerra 2008a, 2009, 2010), *S. semidentata*, and *S. melidensis* (Rivero-Guerra 2009). Two taxa, *S. rosmarinifolia* subsp. *arrabidensis* (called subsp. *arrabidensis* below) and *S. pectinata* subsp. *montiberica* (called subsp. *montiberica* below) are tetraploid (Rivero-Guerra 2008a, 2008c). Finally, *S. ageratifolia* is hexaploid (Rivero-Guerra 2008b). Two of the taxa consist exclusively of polyploids, showing multivalent configurations above quadrivalent and hexavalent levels in the meiosis. Seven diploid taxa show a multivalent configuration in the meiosis (Rivero-Guerra 2008a, 2008b, 2008c, 2009, 2010). Most of the polyploids, except *S. ageratifolia,* appear to have arisen multiple times, and cytogenetic and morphological studies support an autopolyploid origin for all of then (Rivero-Guerra 2008a, 2008b, 2008c, 2009). The hybridisation between two morphologically distinct taxa, *S. orocarpetana* and subsp. *rosmarinifolia*, and the bi-directional introgression of the hybrids with the parentals are potentially significant in the maintenance, morphological differentiation, diversity, and evolution of the taxa within this aggregate (Rivero-Guerra 2009, 2011). The frequent occurrence of hybridisation in the *S. rosmarinifolia* aggregate results from: 1) sympatry between diploid taxa, 2) absence of morphological karyotypic divergence, 3) overlap in flowering period, and 4) recent divergence (Rivero-Guerra 2009, 2011).

The nomenclature and systematics of *Santolina* has been throughly revised recently. Rivero-Guerra (2012) examined the lectotype of *S. oblongifolia* Boiss. (Boissier 1856), suggesting that it does not match the current usage of the name. Thus, among populations previously attributed to *S. oblongifolia*, she erected the species *S. orocarpetana* for populations of *Santolina* that occur on the top of the Gredos massif, whereas the populations that grow at altitudes below 1800 m were referred to the nothospecies *S.* ×*oblongifolia*. She also argued that the hybrid swarms between *S. orocarpetana* and subsp. *rosmarinifolia* (*S.* ×*oblongifolia*) are associated to humid areas on granite substrate, and that they should not be recognized as a distinct species.

Rivero-Guerra (2011) proposed a possible homoploid hybrid origin of *S. semidentata*, *S. melidensis* and subsp. *castellana* from the same two parental taxa (*S. orocarpetana* and subsp. *rosmarinifolia*). A biometric study also revealed that *S. canescens* is phenotypically intermediate between subsp. *castellana* and *S. pectinata*, suggesting homoploid hybrid origin for this taxon. However, the intermediate characteristics are more patent in *S. canescens* than in the other taxa. This supports the hypothesis of Rieseberg and Ellstrand (1993) and Rieseberg (1995) that hybridisation does not always result in morphological intermediates. Many other species that have originated by the same biological process are not phenotypically intermediate (Rieseberg 1997, Brochmann et al. 2000, Gross et al. 2003), although *Cirsium* forms an exception (Segarra-Moragues et al. 2007).

According to the patterns of variation of the *S. rosmarinifolia* aggregate described above, we predicted a complex phylogeny of this aggregate. We test this hypothesis below.

Recent investigations have shown the affinity between *Santolina* and other anthemids. Oberprieler (2002, 2005) demonstrated the monophyly of the genus *Santolina*, based on two representative taxa (one population per taxon) of this genus: *S. rosmarinifolia* and *S. africana*. He also showed that *Rhetinolepis* and *Mecomischus* form the sister group of *Santolina*. Guo et al. (2004) showed that *Anacyclus*, *Tanacetum*, *Brocchia*, *Aaronsohnia* and *Santolina* form the sister group of *Achillea*. Oberprieler et al. (2007a) proposed a new subtribal classification of *Asteraceae–Anthemideae*, according to which *Santolininae* formed a monophyletic subtribe, together with *Glebionidinae*, *Leucanthemopsiinae* and *Leucantheminae*. Himmelreich et al. (2008) showed that *Cladanthus arabicus*, *Chamaemelum nobile* and *S. chamaecyparissus* form a clade. Oberprieler et al. (2009) used data on nrDNA ITS, biogeography, evolution of base chromosome number, evolution of embryo sac developmental type, and evolution of receptacle type (paleas absent vs present) in the tribe Anthemideae to show that *Chamemelum*, *Cladanthus*, *Mecomischus*, *Rhetinolepis* and *Santolina* form a clade that occurs in northern Africa, in the Mediterranean area, and in southern Europe. Furthermore, the same authors suggest that *Santolina*, *Mecomischus* and *Rhetinolepis* form a clade based on the evolution of indument type (basifixed vs medifixed hairs). The phylogenetic tree presented by Oberprieler et al. (2009) confirmed previous results of Oberprieler et al. (2007a, 2007b) concerning the tribe Anthemideae.

Most phylogenetic studies of closely related species have focused on a few individuals per populations, and few populations per species. For instance, Rüber et al. (2003) produced a time-calibrated molecular phylogeny of about 70 individuals of 55 species of gobies (Teleostei) to test monophyly of various taxa and hypotheses about the tempo of speciation in the clade. Steinfartz et al. (2007) produced a time-calibrated molecular phylogeny of 38 salamandrid species based on 162 individuals to test the monophyly of various taxa and study the evolution of various reproductive

characters. Díaz-Pérez et al. (2008) studied 215 individuals of 36 populations of four species of *Festuca* (Gramineae) through AFLP (amplified fragments length polymorphism) to study insular speciation and colonization. Few studies have extensively sampled several individuals per population, and several populations per species of a few closely related species. This is precisely the approach that we have taken to study the speciation and phylogeny of the *Santolina rosmarinifolia* aggregate, which contains many polyploid taxa.

Morphological variation, the apparently wide interfertility limits, a complex evolutionary history with hybridisation and polyploidisation events, and the limitations inherent in rank-based nomenclature (de Queiroz and Gauthier 1990, Pleijel and Rouse 2003, Laurin 2005, 2008) cause taxonomic problems in this aggregate. The present work discusses the implications of the phylogeny for the nomenclature in this aggregate.

The following questions are addressed here: 1) What are the phylogenetic relationships between taxa of the *S. rosmarinifolia* aggregate? 2) do biogeographic patterns of speciation emerge? 3) are there patterns of variation between clades? and 4) does the recently proposed nomenclature of the *S. rosmarinifolia* aggregate (Rivero-Guerra 2011) reflect the phylogenetic relationships?

Material and methods

Sampling

This study samples 38 populations (458 individuals) of *S. rosmarinifolia* subsp. *rosmarinifolia* L. (Linnaeus 1753), 18 populations (187 individuals) of *S. rosmarinifolia* subsp. *castellana* Rivero-Guerra (Rivero-Guerra 2011), 2 populations (55 individuals) of *S. rosmarinifolia* subsp. *arrabidensis* Rivero-Guerra (Rivero-Guerra 2008a), 44 populations (507 individuals) of *S. canescens* Lag. (Lagasca 1816), 6 populations (87 individuals) of *S. impressa* Hoffmanns. & Link (Hoffmansegg and Link 1820), 2 populations (62 individuals) of *S. ageratifolia* Barnades ex Asso (Asso 1784), 4 populations (96 individuals) of *S. orocarpetana* Rivero-Guerra (Rivero-Guerra 2012), 25 populations (185 individuals) of *S. semidentata* Hoffmanns. & Link (Hoffmansegg and Link 1820), 1 population (26 individuals) of *S. melidensis* (Rodr. Oubiña & S. Ortiz) Rodr. Oubiña & S. Ortiz (Rodríguez Oubiña and Ortiz 1998), 41 populations (236 individuals) of *S. pectinata* Lag. subsp. *pectinata*, 20 populations (173 individuals) of *S. pectinata* Lag. subsp. *montiberica* Rivero-Guerra (Rivero-Guerra 2011), and 8 populations (251 individuals) of the hybrid swarm (*S.* × *oblongifolia*). The localities in which these were collected are detailed in Supplementary material Appendix 1. All samples were collected by one of us (ARG) in the summers of 1995–1999. Figure 1 in Rivero-Guerra (2011) shows the approximate geographical distribution of the studied taxa.

Morphometry

Quantitative and qualitative characteristics studied are explained in Supplementary material Appendices 2 and 3. They were selected according to their common use in *Santolina* taxonomy and variability within and between taxa. 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 limb. Leaf width, involucral bracts width, interseminal bracts 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 characters used in the phylogenetic analyses concern 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 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 frequency mean of 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 measured individual (specimen) was treated as an independent operational taxonomic unit (OTU) in most analyses, although dissimilarity between groups of OTUs (taxa, population and individuals) was also computed. As outgroup, we used *S. chamaecyparissus* L., a species closely related to the *S. rosmarinifolia* aggregate based on morphology, cytogenetic and seedling development. The 2516 OTUs represent individuals (SOM 1) belonging to several populations per species or subspecies.

Phylogenetic and statistical methods

TNT (Goloboff et al. 2003, 2008) is able to analyze quantitative characters directly, but we decided to discretize them, for several reasons. First, Goloboff et al. (2006) argued that continuous characters should be entered as ranges reflecting the mean plus or minus one (or two) standard deviations, to enable the software to use only significant differences. However, our main analysis includes individual organisms as OTUs, and therefore, there are no standard deviations and no simple way to assess the significance of differences. Second, because other phylogenetic analysis programs cannot analyze continuous characters directly, it would not be possible to compare results with other programs, and we wished to perform at least exploratory searches in PAUP* (Swofford 2003), and export the trees to Mesquite (Maddison and Maddison 2008) for further analysis.

We scaled each character from 0 to 9 (ten states) no matter how many discrete states were initially present (in discrete characters), or irrespective of the total value span of continuous characters. That way, an equal weight was effectively given to all characters, and transitions were

weighted according to their magnitude. We used the new technology search methods of TNT, namely ratchet (Nixon 1999), tree fusing, drift, and sectorial searches (Goloboff 1999). Several searches were necessary because a few crashes occurred when the program ran out of memory. Thus, once some of the earliest searches had run for over 130 h, they were interrupted and the resulting trees were saved. Then, the random seed number was changed, and these trees were used as starting trees for additional searches. After a few replicates, once the tree length got down to 213 707 steps, all searches completed within a few hours at most, typically yielding additional trees of the same length (most frequently) or shorter trees (once in a while). At each step, the most parsimonious trees available from the previous search were used as starting trees. More than 240 searches were thus performed, which enabled TNT to find much shorter trees, over a period of more than six weeks. In the late part of the search, when the tree length had dropped to 213 595 steps, and until it reached 213 495 steps, it took between one and twenty-three searches to find shorter trees. We stopped the search when TNT found trees of 213 494 steps thirty consecutive times. We also launched a second search, from the shortest (but clearly suboptimal) trees (213 639 steps) obtained from a series of 28 independent searches (not from the series evoked above) conducted by P. Goloboff on his cluster computer in an attempt to discover other tree islands. However, after weeks of work (and 170 searches), the length (213 559 steps) was still far from optimal (213 494 steps), so that search was abandoned after a total of about two months of intensive search. A difference of 65 steps out of more than 213 000 steps may seem small, but in this case, the true pattern of relationships between most OTUs is reticulation rather than divergent because many individuals per population have been sampled. This no doubt greatly inflates the noise, so a small difference in length may be significant.

For a typical search, the following settings were used: for sectorial searches, using a separate matrix-buffer for sectors with up to 1258 terms, recursion (user-defined searches) disabled, exclusive sector selections dividing the tree into 14–25 chunks, cycling through entire tree 4 times, sectors of size below 75 analyzed with 3 RAS (random addition sequences) + TBR (tree bisection–reconnection; 3 extra starts were used if the first 3 produced score differences), not fusing starting trees for small sectors, sectors of size 75 or more analyzed with tree-drifting (8 cycles), doing global TBR every 4 cycles, accepting equally good subtrees; for tree drifting, 8 iterations, 200 substitutions (no more than 200 tree-rearrangements accepted in perturbation phase), maximal absolute fit difference of 2, maximal relative fit difference of 0.20, rejection factor for suboptimal trees 3.00, two autoconstrained cycles, stopping when 99% of perturbation phase was completed; for ratchet, 12 iterations, 210 substitutions (no more than 210 tree-rearrangements accepted in perturbation phase), equally weighted cycle enabled, probability of up-weighting and down-weighting 3, 3 autoconstrained cycles, stopping when 99% of perturbation phase was completed; for tree fusing, 3 rounds of tree fusing, accepting exchanges of equal score and all exchanges that improve initial score, starting from best tree, swapping trees with TBR after fusion. We experimented by varying the above settings for several searches, but this did not seem to greatly affect search performance.

Given that two months were necessary to find the optimal trees, we could not conduct bootstrap, jackknife, or Bremer index analyses because these require much more computing time, which, in this case, would necessarily imply years of searches. Thus, we cannot assess the robustness of our results, but future generations of systematists will be able to test our conclusions using the data matrix (Supplementary material Appendix 4). Nevertheless, to assess clade support of the taxa, we looked at the number of steps on the branches. Clade support should be approximately proportional to the number of steps on their basal branch, although this measure does not account for convergence, contrary to the Bremer index and bootstrap. Thus, this measure of support is not as good, but it is the only one that we can provide on this large dataset with the current technology.

Stepwise discriminant analysis was performed to determine dissimilarity between clades. An optimal scaling (categorical principal component) was employed to explore the correlation structure of the qualitative characteristics, and to assess the relative importance of each characteristic in creating dissimilarity between clades.

These techniques were applied after ensuring that requirements regarding data distribution were met for 1) multivariate 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 of Almeida-Pinheiro de Carvalho et al. (2004), and the Levene test in the univariate models (Dytham 2003, Grafen and Hails 2002), and 3) the presence of outliers, which were detected graphically. The quantitative characteristics were square-root-transformed prior to the analysis to increase the homogeneity of variance, although the comparison with the results obtained from the original characteristics indicated only minor differences.

The statistical packages STATISTICA ver. 6.0 and SPSS ver. 14.0 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

Parsimony analysis

The most parsimonious trees were 213 494 steps long. The various algorithms for large datasets in TNT obviously are vastly superior to the swapping algorithms of PAUP, because the latter found 800 trees (the maximal number that could be stored in the allocated memory) of 215 217 steps after 334 h (that search stopped because PAUP ran out of memory to store new trees). By comparison, TNT had found trees of 214 201 steps in 21 min, and of 213 757 steps (1460 fewer steps) in less than 44 h. This study is based on the strict consensus of the 11 most parsimonious

trees found (213 494 steps). We cannot be absolutely certain that these are the shortest trees, because throughout the search, additional search time yielded shorter trees, but to reduce tree length by a given number of steps, the additional time required increased steadily, especially at the end of the search.

S. chamaecyparissus sensu amplo and the *S. rosmarinifolia* aggregate both appear monophyletic, even though in TNT, only individual 2516 (the last of 200 individuals of *S. chamaecyparissus*) was used as the outgroup and no topological constraint was enforced. Thus, monophyly of *S. chamaecyparissus* and the *S. rosmarinifolia* aggregate was not ensured by this procedure, which required re-rooting the tree between these two clades. Therefore, this is a result, rather than a rooting constraint (if the ingroup were not monophyletic with respect with *S. chamaecyparissus*, no rooting would have resulted in both sets of OTUs forming clades). Five taxaare monophyletic (Fig. 1; Supplementary material Appendix 4). *Santolina orocarpetana* is polyphyletic. *Santolina* ×*oblongifolia*, *S. canescens*, *S. semidentata*, subsp. *castellana*, subsp. *pectinata*, and subsp. *montiberica* are paraphyletic.

Two major clades (Fig. 1; Supplementary material Appendix 4) can be distinguished in the aggregate as a result of the parsimony analysis: clade 1 comprises *S. semidentata*, *S. melidensis*, *S. impressa*, *S. orocarpetana* and *S.* ×*oblongifolia*; clade 2 comprises *S. ageratifolia, S. canescens* and the subspecies *arrabidensis, rosmarinifolia, castellana, pectinata* and *montiberica*.

Within clade 1, only two taxa are monophyletic (Fig. 1; Supplementary material Appendix 4): *S. impressa* and *S. melidensis*. These two taxa are nested within *S. semidentata. Santolina orocarpetana* is polyphyletic and is nested within *S.* ×*oblongifolia*.

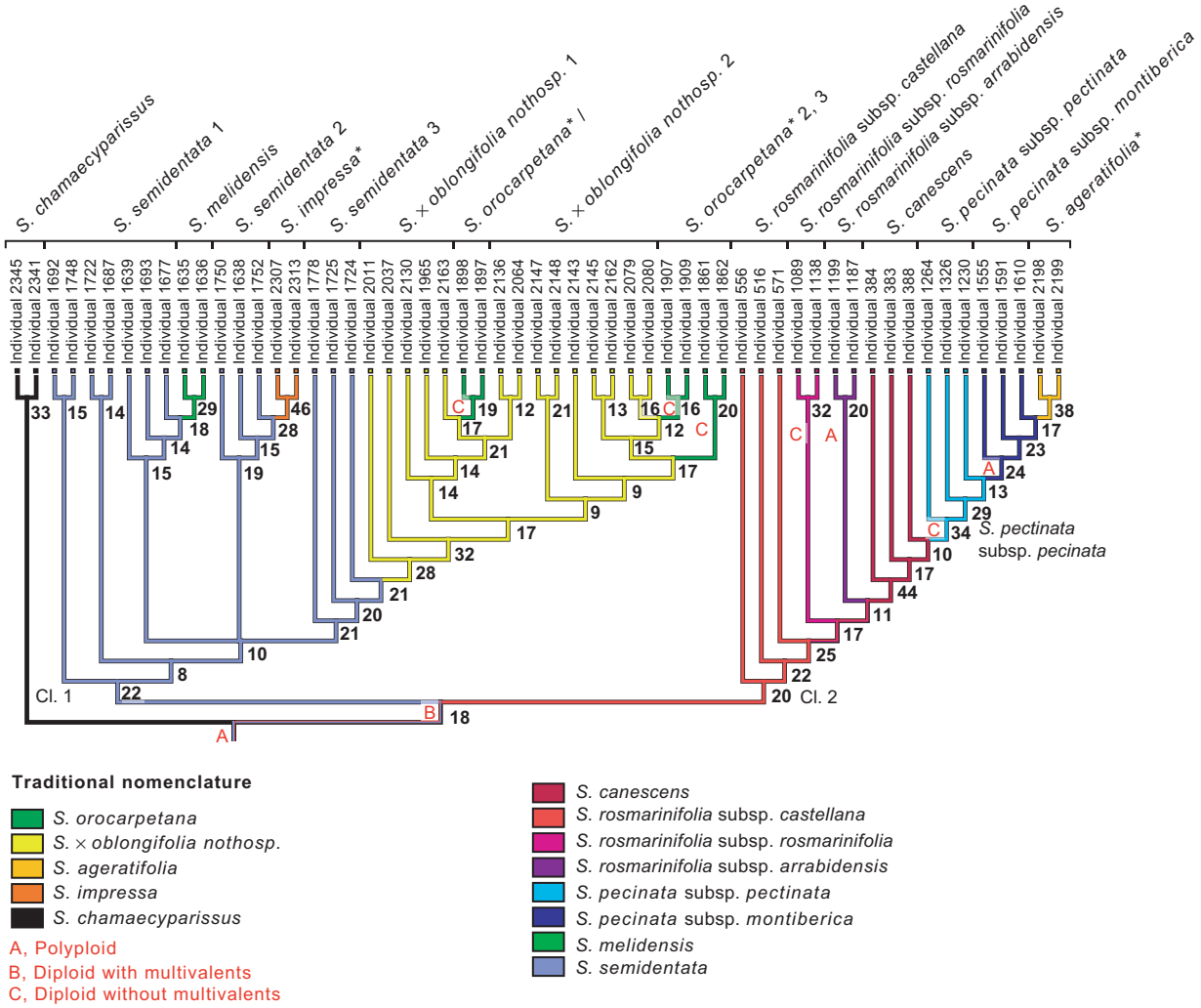

Figure 1. Relationships between subspecies of *Santolina* assessed using all 2516 individuals. The tree was simplified (to be legible) by retaining only enough individuals per clade or grade to show the general pattern of relationships. The number of steps on each internal branch is shown to the left using MacClade ver. 4.08, setting polytomies to hard (otherwise, no changes are shown on branches participating to polytomies), and showing almost all possible changes (approximate maximum number of changes). The average number of steps per branch is 20.1 and the standard deviation, 8.5. For more detailed information, see Supplementary material Appendix 1. Note that several species appear to be paraphyletic, like *S. semidentata*, and *S. canescens*. This also applies to some subspecies, such as *S. rosmarinifolia* subsp. *castellana* and both subspecies of *S. pectinata*, under the nomenclature proposed by Rivero-Guerra (2010), shown immediately above the tree. An alternative nomenclature, that includes no reversals of the Linnaean hierarchy, is proposed above.

Within clade 2, three taxa are monophyletic (Fig. 1; Supplementary material Appendix 4): *S. ageratifolia* and the subspecies *rosmarinifolia* and *arrabidensis*. The subspecies *castellana* is at the base of this clade and is paraphyletic. *Santolina ageratifolia* is deeply nested within subsp. *montiberica*, which is itself nested within the subsp. *pectinata*, and the latter is nested within *S. canescens*. That whole clade is the sister-group of subsp. *arrabidensis*, whereas the latter, along with the *S. canescens*–*S. ageratifolia* clade, is the sister-group of the subsp. *rosmarinifolia*. All of them are nested within the subsp. *castellana*.

The populations of *S.* × *oblongifolia* are very similar to one of the parental species (*S. orocarpetana*), and this is reflected by the position of the latter within the hybrid form in the consensus tree (Fig. 1). The subsp. *castellana* is another hybrid taxon and shows close relationships with the other parent (subsp. *rosmarinifolia*) in the phylogenetic tree, and like *S.* × *oblongifolia*, it appears to contain its parental species (which is a rather counter-intuitive result).

Most populations of all taxa appear highly polyphyletic (Fig. A1, Supplementary material Appendix 4) in all trees examined as well as in the strict consensus tree.

Clade differentiation

Squared Mahalanobis distances (17.23) indicate that the two main clades within this aggregate are significantly different ($F_{51, 2262} = 153.55$, $p < 0.0001$) from each other, but the distances between them are very short. The discriminant function is statistically significant (eigenvalue = 3.46, R canonical = 0.88, Wilk's $\lambda = 0.22$, $\chi^2 = 3$, 419.73, $p < 0.0001$). The factor structure shows that no quantitative characteristic strongly differentiate between these clades. The correlation coefficient varies between 0.15 and 0.35 among the characters with high contributions to clade differentiation. The characters that discriminate between clades are: leaf width of the sterile stems, width of basal and cauline leaves, leaf lobe number of the sterile stems, length and width of the involucral and interseminal bracts, and length and width of the appendage of the involucral bracts. The multivariate combination of all these characteristics allows for taxon and clade recognition. The classification matrix shows that the two clades are well differentiated (94.09% and 99.40% of the individuals are correctly classified in the clades 1 and 2). The same prevails within each clade, except for *S. orocarpetana* in clade 1.

The relationships between the taxa that are included within clade 1, except for *S. impressa*, are based, mostly, on the characteristics of the involucral bracts. The nested relationship between *S. impressa* and *S. semidentata* is based on quantitative characteristics of flowering and sterile stem leaves. The characteristics of the involucral bracts of *S. impressa* are similar to those of the subspecies of *S. rosmarinifolia* (clade 2). The discriminant analysis also shows that leaf width is another discriminant characteristic between clades. Leaf width is greater in *S. orocarpetana* and the hybrid swarms than in the remaining taxa of the aggregate; the range of variation in leaf width for *S. semidentata*, *S. melidensis*, *S. impressa*, *S. canescens* and the subspecies of *S. rosmarinifolia* is similar. Therefore, a group of quantitative and/or qualitative characteristics that supports the phylogeny shows a modest differentiation of the clades.

The analysis of variation patterns of each qualitative character in the phylogeny, as well as the results of the optimal scaling analysis, indicate that no qualitative characteristic or group of characteristics clearly differentiates between the two major clades. The results show that *S. semidentata* is the most variable taxon of this aggregate, which is consistent with its paraphyletic status, which includes four other taxa. The presence of some individuals of *S. semidentata* and of subsp. *castellana* within the hybrid populations indicates close similarity with *S. oblongifolia*. The results also show close similarities between *S. semidentata*, *S. melidensis*, and between the three subspecies of *S. rosmarinifolia* (*rosmarinifolia*, *arrabidensis* and *castellana*), except for: leaf margin, presence of fragile flowering stem from the apex to the base, plant pubescence, plant colour, peduncle shape, shape of the middle leaf of the sterile stem, incision of the lower leaf of the flowering and sterile stems, lobe insertion of the lower leaf of the sterile stem, leaf apex, capitulum shape, shape of the middle and inner bracts, shape of the apex of the outer and middle bract (except for subsp. *castellana*), insertion of the appendage of the middle and inner bracts, and hair characteristics of the interseminal bracts.

The following characteristics of *S. orocarpetana* are present in subsp. *castellana*: 1) spatulate lower leaf of the flowering and sterile stems, 2) external bract with acuminate apex, 3) middle bract ovate, with appendage not decurrent, 4) first and second rows of the inner bracts with the appendage decurrent in the upper 1/3, 5) interseminal bract with simple and modified hairs, and with villous indument, and 6) presence of individuals with three and four rows of involucral bracts.

The following qualitative characteristics of subsp. *rosmarinifolia* are present in populations of *S.* × *oblongifolia*: 1) bright dark green or yellowish–green stems with dark green leaves, sterile stem and leaf of the sterile stem usually greyish-glaucous or dark green and glabrous, 2) flowering stem fragile from the apex to the base, and not solid near the capitulum, with peduncle strongly thickened above, 3) solid sterile stem, 4) lower and upper leaves of the flowering stems and lower and middle leaves of the sterile stems linear, 5) middle leaf of the flowering stem dentate or entire, 6) lower and middle leaves of the sterile stem pinnatifid and dentate, with lobes along upper 1/3 or 1/2, 7) lower and middle leaves with acute mucronate or obtuse mucronate apex, 8) leaf with thickened and involute-appressed margin, 9) external bract with acuminate apex, 10) interseminal bract glabrous, outer and middle bracts strongly carinate, and 11) presence of four rows of involucral bracts.

Santolina canescens is most similar to *S. rosmarinifolia* regarding the characteristics of the leaf, whereas it matches *S. pectinata* and *S. ageratifolia* in the characteristics of the involucral and interseminal bracts. The presence of hollow flowering stems in *S. orocarpetana*, *S.* × *oblongifolia*, and subspecies *pectinata* and *montiberica* reflects two independent appearances of this character (one in each pair of taxa).

The basal branch of several taxa shows a much higher number of steps than the average (20.1 steps, with a standard deviation of 8.5 steps) over the tree (Fig. 1). These include *S. chamaecyparissus* (33 steps), *S. melidensis* (29 steps), *S.* ×*oblongifolia* (28 steps), *S. impressa* (46 steps), *S. rosmarinifolia* subsp. *rosmarinifolia* (32 steps), *S. canescens* (44 steps), *S. pectinata* subsp. *pectinata* (34 steps), and *S. ageratifolia* (38 steps). On the other hand, a few other taxa do not appear to be associated with more than the background level of apomorphies, such as *S. semidentata* (22 steps), *S. rosmarinifolia* subsp. *castellana* (20 steps), and *S. orocarpetana* (16 to 20 steps depending on the various clades), or to have a barely longer basal branch, such as *S. pectinata* subsp. *montiberica* (24 steps).

Character evolution

Parsimony inference of the ancestral states onto the phylogeny reveals that the following characteristics are ancestral for this aggregate: 1) plant decumbent, tomentose, olive green, with viscose glands, 2) flowering stem fragile near the base and not solid near the capitulum, with peduncle not thickened above or slightly thickened above, 3) solid sterile stem, 4) lower leaf of the flowering and sterile stems spatulate, 5) middle and upper leaves of the flowering stem and middle leaf of the sterile stem linear, 6) basal and fascicular leaf elliptical, grooved and impressed-tuberculate-denticulate, 7) lower leaf of the flowering stem pinnatisect to pinnatifid or pinnatipartite to pinnatifid, with lobes along upper 1/2, 8) middle leaf of the flowering stem dentate or scaly-dentate, with lobes along upper 1/2, 9) upper leaf of the flowering stem linear, without lobes, 10) lower leaf of the sterile stem pinnatisect or pinnatisect to dentate, with lobes along upper 1/3 or 1/2, 11) middle leaf of the sterile stem pinnatifid, basal and cauline leaf with lobes along upper 1/2 or 2/3, 12), leaf with obtuse mucronate or/and acute mucronate apex, 13) lobes elliptical with obtuse mucronate apex, 14) capitulum hemispherical or campanulate, not umbilicate, 15) receptacle hemispherical, 16) outer bract triangular, with apex not acuminate, with non-decurrent appendage, and strongly carinate from the apex to the base, 17) middle bract ovate-triangular, with apex not acuminate, with non-decurrent appendage, and strongly carinate from the apex to the base, 18) first row of the inner bract elliptical, with appendage decurrent in the upper 1/3, and carinate from the apex to the base, 19) second row of the inner bract elliptical, ovate or ovate-triangular, with appendage decurrent in the upper 1/3, 20) interseminal bract elliptical, without decurrent appendage, with hairs modified or/and simple, 21) involucral bracts in four rows, with appendage hyaline and not fragile, 22) interseminal bracts pilose, and 23) flowers erect, or with peripheral flowers with corolla tube at an angle of 90°. This aggregate thus probably originated from an ancestor with the morphological characteristics cited above.

The phylogeny suggests that the presence of a capitulum with three rows of involucral bracts is the ancestral condition in the aggregate, but this condition may diagnose a more inclusive clade because the closely related *S. elegans* and *S. viscosa* also display it.

Discussion

Hypotheses on the origin of the *S. rosmarinifolia* aggregate

Polyploidy is frequent in angiosperms, as emphasized in the introduction, and it is attested in the *S. rosmarinifolia* aggregate by the presence of quadrivalents in diakinesis, bridges and chromosome association in anaphase in the diploids *S. semidentata*, *S. melidensis*, *S. canescens*, *S. impressa*, subsp. *castellana*, *S.* × *oblongifolia*, *S. chamaecyparissus* and *S. viscosa* (Rivero-Guerra 2009, 2010, unpubl.). It is further suggested by the presence of multivalent configurations above the quadrivalent and hexavalent levels in tetraploids (Rivero-Guerra 2008a, 2008b, 2009) and hexaploids (Rivero-Guerra 2008b). Two hypotheses can be proposed to explain the evolution of these taxa.

The first hypothesis suggests that the aggregate arose from an ancient polyploid by means of somatic chromosome number reduction, taxonomic diversification and geographic range expansion in the Iberian Peninsula, and that these gave rise to *Santolina*. As a consequence of the diversification and expansion, two or more taxa coexisted and hybridised, giving rise to the present hybrid populations. The diploid taxa originated later, through chromosome reduction and/or differentiation, and they spread through the entire geographical range of the aggregate (latitude 42°–36°N), perhaps displacing previously-established polyploid taxa. They also show a broader ecological spectrum than polyploids. The polyploids have a restricted habitat distribution (Rivero-Guerra 2008b) that may be relictual, according the phylogeny (Fig. 1) and this hypothesis. The second hypothesis suggests that the ancestor of the aggregate was diploid, and that structural changes by translocation and chromosome inversions, local speciation through auto- or allopolyploidy, and homoploid hybrid speciation explain the karyotipic diversity in the aggregate. Both parsimony optimization of ploidy (with ordered states) on the phylogeny (Fig. 1) and cytogenetic study in the genus *Santolina* (Rivero-Guerra unpubl.) strongly corroborate the first hypothesis that this species aggregate arose from an ancient polyploid.

Studies in *Helianthus* demostrates that hybridization leads to increased geographical range, ecological amplitude, and/or the colonization of new habitats (Rieseberg et al. 2007). Rivero-Guerra (2011) suggested that the 'center system' of the Iberian Peninsula is the centre of origin of this aggregate, where the introgression of advantageous alleles is a major mechanism of diversification of these taxa. Parsimony optimization (with unordered states) on the phylogeny does not fully resolve the ancestral range of the aggregate, but clearly indicates that the basal dichotomy between clades 1 and 2 reflects a range segregation between the northwest of the Iberian Peninsula (clade 1) and the centre (clade 2), either of which may represent the ancestral range of the aggregate. The centre–west, centre–east, and

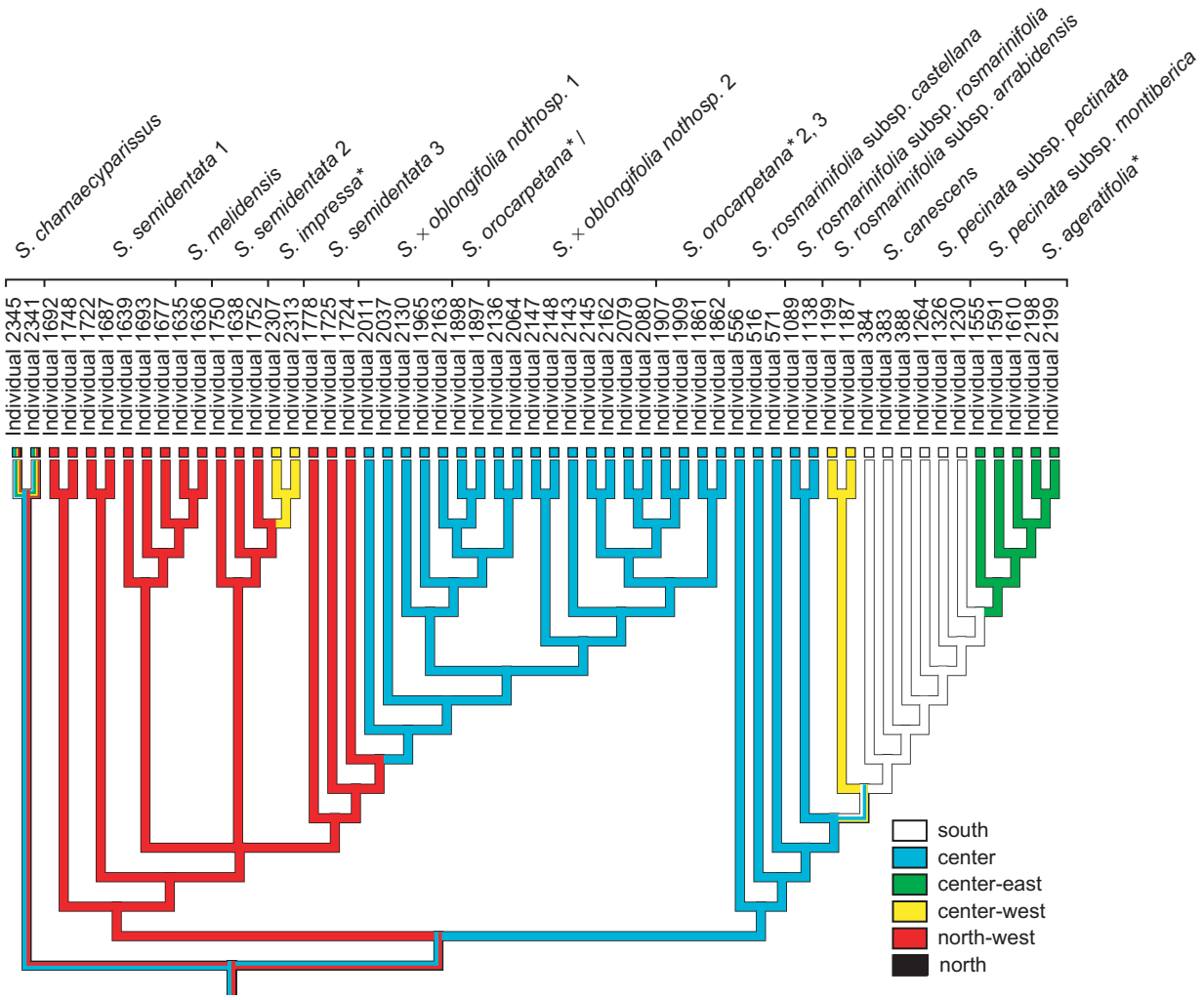

Figure 2. Biogeographic analysis of the *S. rosmarinifolia* aggregate.

possibly the south of the Iberian Peninsula may have been colonized later (Fig. 2).

Reticulate evolution

Homoploid reticulate evolution is relatively common in angiosperms (Rieseberg 1991, Rieseberg and Noyes 1998, Baumel et al. 2002, Guo et al. 2004, Cron et al. 2008), and it raises problems in the phylogenetic analysis (Rieseberg and Morefield 1995). The evolution of this aggregate includes hybridization (Rivero-Guerra 2008a, 2008b, 2008c, 2009, 2010, 2011), rather than exclusively dichotomous branching patterns, which complicates interpretation of the phylogenetic tree. Analogous conclusions have been published by Rieseberg (1991) on the genus *Helianthus*. Rivero-Guerra (2011) discussed in detail the homoploid hybrid origin of subsp. *castellana*, as well as the importance of hybrization in promoting the formation of introgressive races in *Santolina*. The position of subsp. *castellana* at the base of clade 2 thus presumably reflects the somewhat intermediate phenotype of the hybrid, compared with its presumed parental taxa (*S. orocarpetana* and *S. rosmarinifolia*). Rieseberg et al. (2007) suggested that "hybridization may promote the persistence, aggressiveness and ecological amplitude of invasive plant populations". The range extension, ecological amplitude and aggressiveness of this taxon is lower than that of the parental taxon *S. rosmarinifolia*, but higher than that of the other parent (*S. orocarpetana*), contrary to the suggestion by Rieseberg et al. (2007). The introgressive hybridization of the hybrids with *S. rosmarinifolia* (invasive) probably contributed to increase invasiveness in the former. However, the results of Scascitelli et al. (2010) in two species of *Helianthus* do not support the theory of Currat et al. (2008) that the introgression trend is mostly in the direction of the colonizing species or invader.

Rivero-Guerra (2011) inferred a close affinity between *S. rosmarinifolia*, *S. semidentata*, and *S. melidensis*, as well as between the last two taxa, *S. orocarpetana* and *S. ×oblongifolia*. She discussed the hypothesis that *S. semidentata* and subsp. *castellana* are derived from hybridization of the same parental species, *S. rosmarinifolia* subsp. *rosmarinifolia* and *S. orocarpetana*. Apparently, the present phylogeny does not corroborate these results, perhaps

because the reticulate evolution present in this aggregate obscures some cladogenetic patterns. However, our phylogeny suggests that *S. semidentata* gave rise to *S. impressa* (Fig. 1), and seedling development (Rivero-Guerra unpubl.) supports this hypothesis. The phylogeny also suggests that *S. melidensis* is derived from *S. semidentata*, which corroborates the close relationships between these taxa inferred by Rivero-Guerra (2011).

Hypotheses on the origin of taxa within the *S. rosmarinifolia* aggregate

Santolina pectinata is tomentose, with a hollow flowering stem, involucral bracts with broad appendages decurrent to the base, and lanceolate leaves. A previous study of *Santolina* (Rivero-Guerra 2011) suggests that *S. pectinata* is derived from a phenotype with lanceolate leaves, hollow flowering stems, four rows of involucral bracts and tomentose indument. A phenotype with these characteristics occurs within the hybrid swarms (*S.* ×*oblongifolia*). The two subspecies of *S. pectinata*, together with *S. orocarpetana* and the hybrid swarms (*S.* ×*oblongifolia*) are the only taxa of the genus with hollow flowering stems. However, within the hybrid swarm, the appendage of the involucral bracts is never decurrent to the base. The phylogeny does not corroborate the hypothesis of Rivero-Guerra (2011) because it suggests that *S. pectinata* is derived from *S. canescens.* Our results suggest that the precursors of *S. pectinata* probably occured in the centre or northwest of the Iberian Peninsula.

The phylogeny suggests that the precursors of *S. ageratifolia* already occurred in the eastern part of the Iberian Peninsula. *Santolina ageratifolia* is hexaploid, located in the extreme east (Teruel province) of the distribution of this aggregate, growing on sandstone and red limolite, and quartzite (Rivero-Guerra 2008b). It is geographically and reproductively isolated from the remaining taxa of this aggregate; however, it coexists with *S. chamaecyparissus* in this area. *Santolina ageratifolia* shows high similarity with subsp. *pectinata* in leaf shape and in appendage incision; thus, Rivero-Guerra (2008b, 2011) suggested that this species probably arose from *S. pectinata*, a hypothesis confirmed by our phylogeny.

Two hypotheses regarding the origin of *S. canescens* were discussed by Rivero-Guerra (2011). One is that subsp. *castellana* has dispersed from the center to the south of the Iberian Peninsula. This hypothesis is based on the gradual increase along a north–south gradient of the following characteristics demonstrated by Rivero-Guerra (2011): number of lobes per leaf, a continuous scarious appendage from the apex to the base, base width and the length of inner bract appendage, the base width of the interseminal bracts, and the width of involucral bract appendage. These characteristics may be advantageous for adapting to the warm and dry summers in the southern Iberian Peninsula. The other hypothesis is that *S. canescens* arose from hybridisation between subsp. *castellana* and *S. pectinata*. This is based on similarities between *S. canescens* and these potential parents. *Santolina canescens* has involucral bracts like *S. pectinata* and a leaf morphology that matches subsp. *castellana*. The phylogeny is compatible with both hypotheses because it indicates that a range expansion from the center to the south occurred with the origin of *S. canescens* (first hypothesis), and because both presumed parental species appear closely related to *S. canescens* (second hypothesis).

The cytogenetic and morphometric analyses, as well as the phylogeny (Fig. 2) suggest that the presence of polyploids in the center–west and in the center–east regions must have appeared fairly recently and results from two independent range extensions, one in *S. impressa* and one in subsp. *arrabidensis*.

Nomenclatural implications

According to the generalized lineage species concept (de Queiroz 1998), species rank should be given to distinct evolutionary lineages that are more or less reproductively isolated, through intrinsic or extrinsic causes. Unfortunately, no experimental study or field study has directely estimated gene flow among taxa of *Santolina*. Therefore, ranking taxa within this species aggregate is a subjetive exercise, whether one uses the biological species concept (Mayr 1982), the generalized lineage species concept, or any other. Phylogenetic nomenclature requires taxa to be monophyletic, although the 'PhyloCode' (Cantino and de Queiroz 2010) explicitly excludes species names, precisely because several currently recognized species are paraphyletic and because many species concepts imply that species are not necessarily clades. Our phylogeny suggests that several currently recognized taxa in *Santolina* are paraphyletic. Under rank-based nomenclature, as implemented in the 'International Code of Botanical Nomenclature' (Greuter et al. 2000, McNeill et al. 2006), all taxa should be of a rank inferior to the next most inclusive taxon. This second principle is apparently blatantly violated by the current nomenclature, in which *S. melidensis* is nested in *S. semidentata* and *S. ageratifolia* is nested in subsp. *montiberica* of *S. pectinata.* Under rank-based nomenclature, this is no problem if paraphyletic taxa are recognized, which is the solution adopted here. It would not be practical to propose a phylogenetic nomenclature in the *S. rosmarinifolia* aggregate because of apparent paraphyly of several recognized taxa and the preliminary nature of our phylogeny. Extensive nomenclatural revisions should better await molecular phylogenetic studies of the aggregate and are beyond the scope of the present study.

Santolina rosmarinifolia subsp. *melidensis* was erected by Rodriguez-Oubiña and Ortiz (1993). López Udías et al. (1997) attributed this subspecies to *S. semidentata*, whereas Greuter (2008) raised *S. melidensis* to the species level, as first proposed by Rodriguez-Oubiña and Ortiz (1998). Rodriguez-Oubiña and Ortiz (1998) mentioned that various crossing tests show that *S. melidensis* is reproductively isolated from subsp. *semidentata* (species *semidentata* in our nomenclature) and from subsp. *rosmarinifolia*, but they did not provide any experimental data to support this statement. Close affinity was inferred between *S. semidentata* and *S. melidensis*, and they indeed show few differences (Rivero-Guerra 2009, 2011). They have parapatric distributions and are both grow in northwestern Iberian Peninsula.

The phylogeny suggests that *S. melidensis* is nested within *S. semidentata*, which supports the hypothesis of Rivero-Guerra (2011) that the former is derived from the latter and is more compatible with the nomenclature proposed by López Udías et al. (1997) than by that of Rodriguez-Oubiña and Ortiz (1993, 1998).

The polyphyly of the various populations (Fig. A1, Supplementary material Appendix 4) is consistent with the persistence of gene flow between populations of a single species (Li et al. 2010, Zhou et al. 2010). Thus, there is little point in discussing these relationships in detail.

The taxa of this aggregate form a complex of taxa that can be considered as species and subspecies, some of which can be considered microspecies (e.g. *S. impressa*, *S. melidensis*, *S. orocarpetana* and *S. ageratifolia*) with restricted habitat distribution. The poorly differentiated patterns of morphological, cytogenetic, biosystematic, ecological and seedling development variation in taxa of the *S. rosmarinifolia* aggregate suggest a recent speciation and diversification process. The phylogeny supports this hypothesis to the extent that some of the recognized taxa are paraphyletic or even appear polyplyletic, a result that should be verified using other types of data. Our results nevertheless provide strong evidence for the existence of several lineages in the aggregate, as recently suggested by Rivero-Guerra (2011).

The following key to taxa in the *S. rosmarinifolia* aggregate is proposed by Rivero-Guerra:

1. Plant glaucous and sericeous; leaves spatulate, basal and cauline leaf 1.5–6.6 mm wide; capitulum with three rows of involucral bracts; flowers covering the capitulum ………..…....…..…........... *S. orocarpetana*
   – Plant usually bright dark green or bright olive green, rarely yellowish–green or greyish-glaucous; glabrous, tomentose, tomentose-to-glabrescent, or glabrescent; leaves usually linear, elliptical, lanceolate or subterete, rarely spatulate; basal and caulinar leaves 0.3–2.2 mm wide; capitulum with four (rarely three) rows of involucral bracts; flowers erect or with the peripheral flowers with the corolla tube at an angle of 90 degrees ………….… 2
2. Inner bracts (3.6–)4.0–6.6 mm long; appendage of the involucral bracts dark copperish and fragile; receptacle conical; plant decumbent-rooting, thickly perennial woody basal stems or decumbent-rooting and ascending with scarce lignification; flowers and interseminal bracts with viscose glands ……………………..… *S. ageratifolia*
   – Inner bracts 2.1–4.6(–5.6) mm long, appendage of the involucral bracts hyaline and not fragile; receptacle usually hemispherical, lenticular or conical; plant decumbent (flowering stems ascending, erect-patent and erect), or procumbent (flowering stems patent and divergent); glandulose ………………………………… 3
3. Flowering stem hollow; receptacle usually lenticular …. ………………………………………………..… *S. pectinata*
   – Flowering stem solid or holow only near the insertion with the capitulum; receptacle usually hemispherical or conical …………………………………………….… 5
4. Peduncle (0–4.6–)10.6–156(–180) mm, strongly thickened above; middle leaf of the flowering stem lanceolate; upper leaf usually lanceolate or linear; lobes of the lower and middle leaves of the flowering stem of 0.4–4.3(–5.0–6.8) mm and (0.3–)0.4–3.8(–6.2) mm long, respectively; lobes of the lower and middle leaves of the sterile stem (0.7–0.9–)1.1–4.8(–5.0–6.4) mm and (0.1–)0.4–3.9(–4.2–5.5) mm long, respectively; lower leaf of the flowering stem 5.2–22.5(–29.3) × 1.2–8.9 mm; lower leaf of sterile stem (6.5–)7.1–19.4(–20.1–28.6) × (1.3–)1.5–7.5(–10.7–15.3) mm, middle leaf (7.0–)8.0–22.3(–23.0–34.5) × (1.1–) 1.6–7.7(–9.4–11.3) mm; capitulum 7.2–12.9(–20.2) × 5.9–9.4(–11.8) mm; plant 20–176 × 20–70 cm …..… ……..………..………..………..…………. subsp. *pectinata*
   – Peduncle (2.2–6.8–)13.1–99.0(–103.0–128.8) mm long, slightly thickened above; middle leaf of the flowering stem linear, narrowly elliptical, slightly grooved on both sides, or lanceolate; upper leaf usually linear or lanceolate; lobes of lower and middle leaves of the flowering stem 0.6–2.1 mm and 0.2–0.9(–1.5) mm long, respectively; lobes of lower and middle leaves of the sterile stem 0.7–2.8 mm and 0.3–3.0(–6.2) mm long, respectively; lower leaf of the flowering stem (4.5–)5.2–12.9(–13.6–17.2) × (0.9–)1.2–5.8 (–10.2) mm; lower leaf of sterile stem (0.9–)5.5–17.6(–20.3) × 1.1–6.6(–8.7) mm; middle leaf (7.3–) 8.8–19.8(–20.0–30.5) × (0.9–)1.1–4.8 (–5.0–7.8) mm; capitulum 5.4–10.7(–11.0–14.7) × 6.1–9.9 (–10.9) mm; plant 23–90 × 14–40 cm …..…… subsp. *montiberica*
5. Number of lobes per leaf (23)35–362 rounded; lower and middle leaf of the flowering and sterile stems strongly grooved on both sides, tuberculate with lobes appressed to the limb from the apex to the base on both sides, apexrounded ………………………… *S. impressa*
   – Number of lobes per leaf 0–187(–239) linear or elliptical; lower and middle leaf of the flowering and sterile stems slightly grooved on both sides, entire, dentate, scaly-dentate, pinnatifid, pinnatipartite or pinnatisect, apex obtuse mucronate or acute mucronate ………… 6
6. Plant 17–64 × 9–45 cm, green or green to reddish–brown, procumbent; glabrescent; flowers orange yellow; flowering stem 105–255(–285) mm, patent and divergent; basal and fascicular leaves imbricate-scaly-dentate; capitulum 6.2–10.8 mm in diameter, slightly umbilicate, with three or four rows of involucral bracts; outer and middle bracts carinate; receptacle 2.3–5.1 mm in diameter ………………………………… *S. melidensis*
   – Plant 16–230 × 20–357 cm, usually bright olive green or bright dark green or with yellowish–green flowering stem and dark green leaves, sterile stem and leaves of the sterile stem usually greyish-glaucous or dark green, decumbent; flowers yellow; flowering stem (80–)100–610 mm, ascending, erect-patent and erect; basal and fascicular leaves impressed-tuberculate-denticulate; capitulum 4.8–22.8 mm in diameter, usually not umbilicate or strongly umbilicate, with four rows of involucral bracts; outer and middle bracts strongly carinate; receptacle 2.3–6.9 mm in diameter ……… 7
7. Flowering stem fragile near the base, sterile stem not fragile; leaf without thickened and involute-appressed margin; middle leaf of the sterile stem lanceolate, narrowly

elliptical slightly grooved on both sides, linear or spatulate; lower and middle leaves of the flowering and sterile stems 0.6–5.9(–7.6–8.7) mm wide; lower and middle leaves of the sterile stem with 2–187(–239) lobes; lobes of the lower and middle leaves of the flowering and sterile stems 0.1–3.6(–4.0–5.3) mm long; capitulum 2.6–10.7 mm diameter; base of the involucral bracts 0.6–1.9(–2.0–2.3) mm wide; appendage of involucral bracts 0.2–1.5(–1.7–2.0) × 0.3–2.8 (–3.0–3.5) mm ..... *S. semidentata*
– Flowering and sterile stems fragile; leaf with thickened and involute-appressed margin, usually linear, rarely lanceolate; lower and middle leaves of the flowering and sterile stems 0.3–1.9(–2.0–5.6) mm wide, with 0–90(–100–152) lobes; lobes 0–0.9(1.0–3.9) mm long; capitulum 4.8–17.8(–22.8) mm in diameter; base of the involucral bracts (0.8–)1.0–2.9 mm wide; appendage of the involucral bracts 0.1–2.5(–2.8–4.0) × 0.1–5.5 mm 8

8. Outer and middle bracts carinate with appendage usually lacerate to lacerate-denticulate or lacerate to fimbriate from the apex to the base; outer bracts non-acuminate; appendage of the middle bracts 0.3–3.9(–4.0–4.5) mm wide; plant tomentose; lower and middle leaves with 0–90(–100–152) lobes ..... *S. canescens*
– Outer and middle bracts strongly carinate with appendage lacerate and non decurrent, lacerate to fimbriate, lacerate-denticulate, or lacerate to erose from the apex to the base, or lacerate along upper 1/3 or 1/2; outer bract non-acuminate or acuminate; appendage of the middle bracts 0.2–2.0(–2.1–3.3) mm wide; plant glabrous or tomentose; lower and middle leaves with 0–95 lobes 9
9. Receptacle (1.7–)2.5–4.9 mm height, conical; interseminal bracts (2.6–2.8–)3.1–4.5 mm long; peduncle usually not thickened or slightly thickened above; outer bracts usually non-acuminate, rarely acuminate; appendage usually decurrent upper 1/3 or not decurrent, rarely erose decurrent narrowly to the base or upper 1/2; lobes of the lower and middle leaves along upper 1/3 to 2/3 on both sides to the margin; middle leaf usually scaly-dentate, pinnatifid to dentate, dentate, tuberculate-dentate or pinnatipartite, rarely entire; plant usually bright olive green or with yellowish–green stem and bright olive green leaves; glabrous or tomentose; lower and middle leaves of the sterile stem (9.6–17.5–)21.0–41.0(–45.0–51.0) mm long, the same of the flowering and sterile stem with (0)5–60(–78) and (0–9–)10–48(–50–71) lobes, respectively ..... *S. rosmarinifolia* subsp. *arrabidensis*
– Receptacle 1.1–2.9(–3.0–4.7) mm height, hemispharical; interseminal bracts 2.0–3.9(–4.3) mm long; peduncle strongly or slightly thickened above; outer bracts usually acuminate or non-acuminate; appendage non-decurrent, decurrent along upper 1/3 or 1/2, erose or fimbriate decurrent to the base; lobes of the lower and middle leaves along upper 1/3 to 1/2 or 2/3; middle leaf entire, dentate, scaly-dentate, pinnatifid, or pinnatipartite; plant usually bright dark green, bright olive green or with yellowish–green flowering stem; sterile stem greyish-glaucous, dark green or bright olive green; leaf dark green or bright olive green; glabrous, tomentose or tomentose-to-glabrescent; lower and middle leaves (6.4–9.8)10.4–40.0(–41.0–58.4) mm long, with 0–60(–70–92) lobes 10
10. Plant bright olive-green or bright dark green on occasion with the vegetative stem glaucous; usually tomentose or tomentose-to-glabrescent; flowering stem usually solid; peduncle slightly thickened above; middle leaf of the flowering stem with 0–40(–44–80) lobes, the same leaf of the sterile stem with 0–60(–70–92) lobes, usually dentate, scaly-dentate, entire, pinnatifid, or pinnatipartite; lower and middle leaf with lobes usually along upper 1/3 to 1/2 or 2/3 on both sides to the margin; capitulum usually subglobose or hemispherical; appendage of the outer and middle bracts usually lacerate, lacerate to fimbriate, lacerate to lacerate-denticulate to slightly fimbriate to the base or non-decurrent exclusively in the middle bracts; basal and fascicular leaves subterete, elliptical or obovate, grooved on both sides ..... *S. rosmarinifolia* subsp. *castellana*
– Plant usually bright dark green or with yellowish–green flowering stem and sterile stem dark green or greyish-glaucous; usually glabrous, rarely tomentose to glabrescent; flowering stem not solid near the insertion with the capitulum; peduncle strongly thickened above; middle leaf of the flowering stem with 0–12 (–26–52) lobes, the same leaf of the sterile stem with 0–30(–32–95) lobes, usually entire, scaly-dentate or dentate, rarely pinnatifid; lower and middle leaf with lobes along upper 1/3 to 1/2 in the margin; capitulum usually hemispherical or subglobose; appendage of the outer bracts usually lacerate non-decurrent or decurrent along upper 1/3 to 1/2, rarely erose, decurrent narrowly to the base, middle bracts with appendage usually lacerate to lacerate-denticulate or lacerate to erose from the apex to the base or lacerate along upper 1/3 to 1/2; basal and fascicular leaves subterete or elliptical, grooved on both sides ..... *S. rosmarinifolia* subsp. *rosmarinifolia*

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Supplementary material (available as Appendix NJB1382 at <www.oikos.ekol.lu.se>). Appendices 1–4.

Note to supplementary material: Figure A1. Relationships between all 2516 individuals of *Santolina* studied here. Because of the high number of taxa, the tree is legible only at high magnification, on-screen.

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