Molecular systematics of *Descurainia* (Brassicaceae) in the Canary Islands: biogeographic and taxonomic implications

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Descurainia Webb & Berthel. (Brassicaceae) comprises approximately 45 species distributed throughout temperate areas of the world. In contrast to the small-flowered herbaceous taxa which constitute the majority of the genus, the seven species endemic to the Canary Islands are relatively large-flowered woody perennials. A molecular-based phylogeny of Canarian *Descurainia* was constructed using DNA sequences from nuclear ribosomal internal transcribed spacer (ITS) and seven non-coding chloroplast regions. The results of parsimony and Bayesian analyses suggest that species of *Descurainia* in the Canary Islands are recently derived via a single colonization event. The closest continental relative is *D. tanacetifolia*, a perennial herb from the mountains of southwestern Europe. Chloroplast data suggest that both intra-island adaptive radiation and inter-island colonization have played a prominent role in the evolution of *Descurainia* in the Canary Islands. The most likely ancestral location of the island progenitor was the lowland scrub zone on Tenerife.

KEYWORDS: biogeography, *Brassicaceae*, *Descurainia*, Canary Islands, Macaronesia, oceanic islands.

INTRODUCTION

The Canary Islands comprise seven islands and several islets located in the eastern Atlantic Ocean near northwest Africa. The islands are volcanic in origin and range in age from 0.8 to 21 million years old (Carracedo, 1994). The native flora, which is allied to that of the surrounding Macaronesian region and in many cases to the Mediterranean area, exhibits a high degree of endemicity (Francisco-Ortega & al., 2000).

The Canarian flora, along with that of Macaronesia, has been the subject of a number of recent molecular studies involving colonization and adaptive radiation on islands. Many of these studies have focused on the high prevalence of woodiness in Macaronesian endemics. This trait has been proposed as evidence of a relict origin for the island flora, suggesting that Macaronesian species are descended from woody continental ancestors extirpated from Europe during Pleistocene glaciation and that herbaceous continental relatives derive from subsequent recolonization of the mainland (Bramwell, 1972; Sunding, 1979; Cronk, 1992). Molecular studies have revealed that some island endemics are probably relictual (e.g., *Lavatera phoenicea* Vent. [Malvaceae; Ray, 1995; Fuertes-Aguilar & al., 2002]; *Plocama pendula* Aiton [Rubiaceae; Bremer, 1996; Andersson & Rova, 1999]; and *Tolpis* Adanson [Asteraceae; Moore & al., 2002]), but most groups appear to be recently derived from herbaceous continental ancestors (e.g., the Macaronesian

clade of Crassulaceae [Mort & al., 2002]; the *Bencomia* Webb & Berthel. [Rosaceae] alliance [Helfgott & al., 2000]; *Echium* L. [Boraginaceae; Böhle & al., 1996]; *Sideritis* L. [Lamiaceae; Barber & al., 2000]; and the *Sonchus* L. [Asteraceae] alliance [Kim & al., 1996]). Other issues of interest concern patterns of diversification within the islands, such as colonization routes, direction of habitat shifts, and, in particular, the relative contribution of inter-island colonization compared to intra-island adaptive radiation in the evolution of the insular flora. The Canary Islands feature several distinct ecological zones arising from varied elevations and the influence of northeastern trade winds (Bramwell, 1972; Francisco-Ortega & al., 1996; Juan & al., 2000). Since similar ecological zones are present on different islands, inter-island colonization may have played an important role in the evolution of the Canarian flora. Molecular studies suggest that while intra-island adaptive radiation appears to be the dominant mode of species diversification in *Sideritis* (Barber & al., 2000) and one introduction of *Teline* Medik. (Fabaceae; Percy & Cronk, 2002), several Macaronesian groups may have speciated primarily via inter-island colonization (e.g., *Adenocarpus* DC. [Fabaceae; Percy & Cronk, 2002]; *Aeonium* Webb & Berthel. [Crassulaceae; Mes & t'Hart, 1996]; *Argyranthemum* Sch. Bip. [Asteraceae; Francisco-Ortega & al., 1996]; *Bystropogon* L´Hèr. [Lamiaceae; Trusty & al., 2005]; and *Lotus* L. [Fabaceae; Allan & al., 2004]). Because well-resolved multigene phylogenies for many
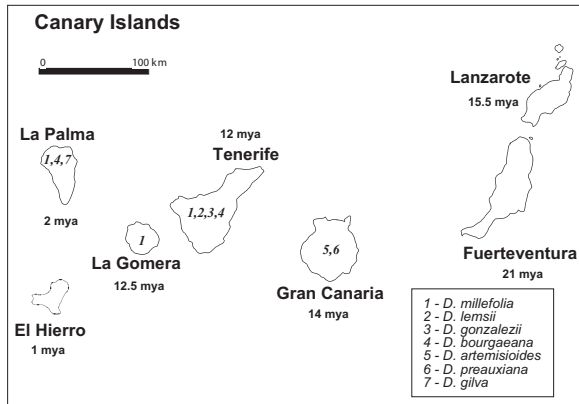
Fig. 1. Map and distribution of *Descurainia* in the Canary Islands. Approximate age of each island is given in millions of years (mya) following Carracedo (1994).

insular groups have not yet been acquired, however, the overall picture of evolution within the islands is still emerging. In this study we explore several of these issues in the context of evolution of *Descurainia* Webb & Berthel. (Brassicaceae) in the Canary Islands.

Descurainia includes approximately 45 species distributed throughout temperate areas of the world. Members of this taxonomically complex genus are characterized by dendritic trichomes, pinnate to tripinnate leaves, yellow or whitish flowers, narrow siliques with seeds that are mucilaginous when wet, and, in many cases, unicellular clavate glands (Schulz, 1924; Al-Shehbaz, 1988; Rollins, 1993). In the only comprehensive treatment of the genus, Schulz (1924) divided *Descurainia* into two clearly-delineated sections: sect. *Descurainia* (published as sect. *Seriphium* O. E. Schulz) and sect. *Sisymbriodendron* (Christ) O. E. Schulz. Section *Descurainia*, consisting of small-flowered herbaceous annuals, biennials, and perennials, comprises the majority of species in the genus. Generally weedy and wide-ranging, members of this section are restricted to the New World, with the exception of *D. kochii* (eastern Turkey and the Caucasus) and *D. sophia* (originally Eurasian, now world-wide). Species in sect. *Sisymbriodendron* are self-incompatible perennial shrubs, possessing relatively large flowers, conspicuous nectaries, and slightly winged seeds, and are endemic to the Canary Islands.

Recent molecular work (Al-Shehbaz & Price, 2001) supports the inclusion of an additional genus—*Hugueninia* Rchb.—in *Descurainia*. This genus constitutes a single species, *H. tanacetifolia*, which is a perennial herb distributed in the mountains of northern Spain, the Pyrenees, and the southwestern Alps. It shares many morphological features with *Descurainia*, including branched trichomes, pinnate leaves, and fruit comprised of siliques. This similarity led Prantl (1891) to include

the species in *Descurainia*, placing it in its own section (sect. *Hugueninia*), and recently Appel & Al-Shehbaz (2003) have also placed it in synonymy with *Descurainia*.

As circumscribed by Bramwell (1977), there are seven species in sect. *Sisymbriodendron*. These species are restricted to four of the five westernmost Canary Islands (Fig. 1) where they variously occupy lowland scrub, pine forest, and high altitude desert ecological zones (Bramwell & Bramwell, 1990; Francisco-Ortega & al., 1996; Juan & al., 2000). Subtropical lowland scrub, occurring at altitudes of 250–600 m on the five westernmost islands, is frequently partitioned into humid and arid sub-zones based on whether the area falls under the influence of humid northeastern trade winds. Pine forest, in which *Pinus canariensis* C. Sm. (Pinaceae) is dominant, is primarily found on the islands of Gran Canaria, Tenerife, La Palma, and El Hierro on southern-facing slopes at elevations of 600–2000 m and northern-facing slopes at elevations of 1200–2000 m. At elevations above 2000 m on Tenerife, Gran Canaria, and to a lesser extent La Palma, a subalpine shrub community occupies high altitude desert.

The only widespread insular species of *Descurainia*, *D. millefolia*, inhabits lowland scrub on Tenerife, La Gomera, and La Palma, extending into pine forest on the latter island. Two *Descurainia* species are endemic to lowland scrub on Gran Canaria: *D. artemisioides* in shady ravines and cliffs of the Guayedra Massif in western Gran Canaria and *D. preauxiana* on cliffs in the southern and central regions of the island. *Descurainia lemsii* is restricted to Tenerife, where it is locally frequent on high northern slopes at the upper limit of the pine forest. Another Tenerife endemic is *D. gonzalezii*, inhabiting pine forest, and very rarely, the high altitude desert of Las Cañadas del Teide. *Descurainia bourgaeana* also occupies Las Cañadas del Teide on Tenerife and has recently been discovered in similar habitat on the island of La Palma. *Descurainia gilva*, which is morphologically similar to *D. lemsii*, occurs in the upper limits of pine forest near the rim of Caldera de Taburiente in the north central region of La Palma. Because *Descurainia* has speciated into separate habitats on several of the islands, a molecular study of this genus can provide valuable insights into colonization patterns in the Canarian flora.

In this paper, we examine the origin and evolution of *Descurainia* in the Canary Islands using molecular-based phylogenies constructed from nuclear and chloroplast DNA markers. The objectives of this study were to: (1) identify the closest continental relative of the island taxa; (2) determine whether the island taxa are relictual or derived compared to continental relatives; and (3) investigate the dominant pattern of colonization within the islands.

■ MATERIALS AND METHODS

Sampling. — We sampled all seven members of *Descurainia* sect. *Sisymbriodendron*. For each species, we obtained material from each island and habitat (i.e., lowland scrub, pine forest, or high altitude desert) on which it was reported, except for *D. millefolia* from pine forest on La Palma.

Because a comprehensive molecular phylogeny for *Descurainia* has not been published, our choice of additional taxa for inclusion is based on the following considerations. Warwick & al. (2004) included three species of *Descurainia* in their analysis of *Smelowskia* C. A. Mey and related genera. They found that *Descurainia* formed a well-delineated group which was related to *Smelowskia*. We are currently completing a molecular systematic study of *Descurainia*. We also find that *Descurainia* is closely related to *Smelowskia*. *Robeschia schimperi* O. E. Schulz, a Middle Eastern monotypic genus which was not included in the Warwick & al. (2004) study, is sister to *Descurainia* (B. Goodson & al., unpubl.). Our unpublished results indicate that both sect. *Sisymbriodendron* and New World *Descurainia* are monophyletic. Consequently, for this analysis we included the remaining Old World congeners (*D. kochii*, *D. sophia*, *D. tanacetifolia*) and two New World representatives (*D. depressa*, *D. incisa*). *Smelowskia americana* and *Arabidopsis thaliana* were used as outgroups. Sources of plant material used in this study, along with voucher information and GenBank accession numbers, are in the Appendix.

Leaf material was field-collected and dried over silica, or harvested from cultivated plants grown in the greenhouse at the University of Texas at Austin (seed provided by César Gómez-Campo from the Escuela Técnica Superior de Ingenieros Agrónomos seedbank). Total DNA was extracted using the CTAB method of Doyle & Doyle (1987) followed by purification using cesium chloride and ethidium bromide gradients. Material was also obtained from herbarium specimens, and the DNA isolated following the protocol in Loockerman & Jansen (1996).

PCR amplification and DNA sequencing. — Seven non-coding chloroplast DNA regions and the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA repeat (ITS1, 5.8S rRNA, ITS2; Kim & Jansen, 1994) were utilized as phylogenetic markers in this study. The non-coding chloroplast regions were the *rps16* intron (Oxelman & al., 1997) and *trnD*GUC-*trnE*UUC (Demesure & al., 1995), *trnE*UUC-*trnT*GGU (Demesure & al., 1995), *psbZ-trnfM*CAU (Demesure & al., 1995), *rpoB-trnC*GCA (Shaw & al., 2005), *ndhF-rpl32*, and *ndhC-trnV*UAC intergenic spacers. Primers for *ndhF-rpl32*   (*ndhF-F*:   5'-ACTGGAAGTGGAAT-

GAAAGG-3'; *rpl32-R*: 5'-GCTTTCAACGATGTC-CAATA-3') and *ndhC-trnV* (*ndhC-F*: 5'-TGC-CAAAACAGGAATAGCAC-3'; *trnV-R*: 5'-TTTACC-GAGCAGGTCTACGG-3') were designed based on the *Arabidopsis thaliana* chloroplast genome (GenBank accession number NC_000932).

DNA regions were amplified via the polymerase chain reaction (PCR) in 50 μL volumes containing 5 μL of 10X buffer, 4 μL of 25 mM $MgCl_2$, 4 μL of 0.25 μM dNTPS, 0.5 μL of a 100 μM solution of each primer, 0.5 μL of *Taq* polymerase and 1 μL of unquantified DNA template. For ITS amplifications, reaction conditions were as follows: one round of amplification consisting of denaturation at 96°C for 3 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min, followed by 35 cycles of 95°C for 1 min, 50°C for 1 min, and 72°C for 45 sec, with a final extension step of 72°C for 7 min. Chloroplast regions were amplified using the following conditions: denaturation at 96°C for 3 min, followed by 35 cycles of 94°C for 35 sec, 50°C for 45 sec, and 72°C for 1 min, with a final extension of 72°C for 12 min. Following amplification, PCR products were cleaned with Qiagen spin columns following the manufacturer's protocols. Sequencing reactions were carried out using Big Dye Terminator chemistry. The sequencing products were cleaned with Centri-cep columns and sequenced on either an MJ Research BaseStation or ABI Prism 3730 automated sequencer.

Phylogenetic analyses. — Sequences were edited with Sequencher 4.1.2 (Gene Codes Corp., 2000) and aligned with ClustalX (Thompson & al., 1997) followed by manual adjustments. Indels that were potentially phylogenetically informative were coded as binary (presence/absence) characters and appended to the alignment. All sequences were deposited in GenBank and GenBank accession numbers are included in the appendix.

Parsimony analyses were performed on each dataset with PAUP* 4.0b10 (Swofford, 2002). For each dataset, heuristic searches were conducted using 10,000 random addition sequence replicates, holding 10 trees at each step, and with tree-bisection-reconnection (TBR) branch swapping, characters equally weighted, and gaps treated as missing. Support for internal nodes was assessed using bootstrap analysis (Felsenstein, 1985) of 500 replicates with 100 random additions per replicate and holding 10 trees at each step.

Separate Bayesian analyses were carried out on the ITS dataset and a combined chloroplast dataset using MrBayes 3.1 (Ronquist & Huelsenbeck, 2003). Evolutionary models were selected based on the hierarchical likelihood ratio test implemented in MrModeltest 2.2 (Nylander, 2004). The model chosen for the ITS dataset was SYM+G (fixed equal state frequencies, six substitution types, and across-site rate variation according to a

gamma-shaped distribution). For the combined chloroplast dataset, separate models were applied to the two data partitions with all parameters unlinked except for topology and branch length; the F81+G model (one substitution type and gamma-shaped rate variation) was applied to the nucleotide partition and the BINARY model (with coding bias set to variable) was applied to the coded indels. Two independent analyses were performed on each dataset. Each analysis was run for 1,000,000 generations with four Markov chains (three heated and one cold) and trees saved every 100 generations. Trees were checked for stationarity by plotting log likelihood values vs. generation, and trees from the burn-in period were discarded. A 50% majority-rule consensus tree was constructed in PAUP* from the remaining trees.

Topological incongruence was assessed using the incongruence length difference (ILD) test as implemented in PAUP* (partition homogeneity test of Farris & al., 1994). Each test consisted of 100 replicates, with 500 random additions per replicate, and the MULTREES option set to off.

Character optimizations. — To elucidate patterns of colonization within the islands, redundant taxa, as well as non-insular *Descurainia* and a putative recent hybrid of *D. gonzalezii × D. bourgaeana* (see discussion), were removed from the combined chloroplast dataset. Parsimony analysis of the reduced dataset was conducted with *Arabidopsis thaliana* and *Smelowskia americana* as outgroups. Island distribution and ecological zone were then mapped separately onto the phylogenetic tree using MacClade 4.0 (Maddison & Maddison, 2000). Fitch parsimony (unordered characters and unweighted character state changes) was employed as the optimality criterion.
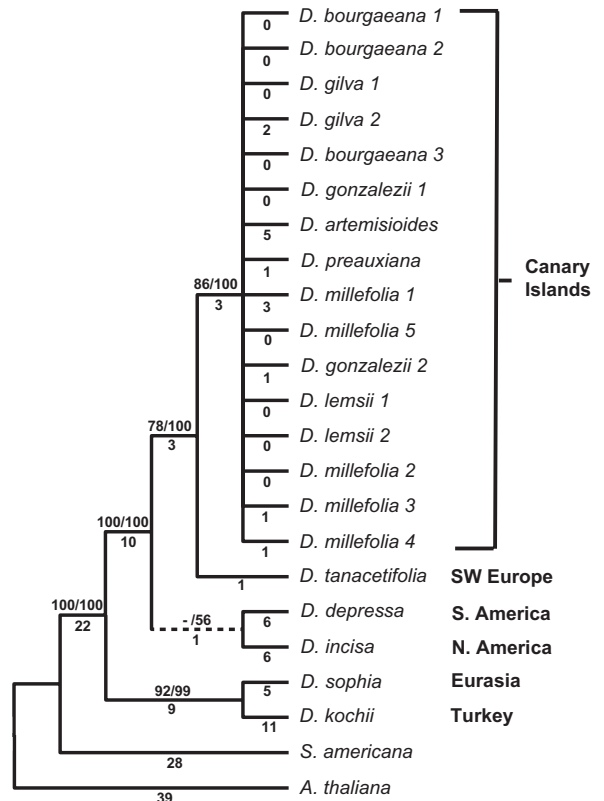

Fig. 2. One of 28 most parsimonious trees derived from nuclear ITS data. Dashed lines indicate branches that collapse in the strict consensus tree. Bootstrap values > 50 % / Bayesian posterior probabilities are indicated above branches; branch lengths below. Generic names are abbreviated as follows: *A. = Arabidopsis, D. = Descurainia, H. = Hugueninia,* **and** *S. = Smelowskia.*

RESULTS

Analysis of ITS data. — The ITS dataset was readily alignable, and comprised 621 nucleotide positions including gaps (1.9%) and missing (0.1%) characters. There were no phylogenetically informative gaps. Three characters were polymorphic for most of the Canary Island taxa and were excluded from the analysis. Of the remaining 618 characters, 128 (20.7%) were variable and 47 (7.6%) were parsimony informative.

Parsimony analysis of the ITS data for all 23 taxa yielded 28 most parsimonious trees of 158 steps (CI [excluding uninformative characters] = 0.79, RI = 0.85) (Fig. 2). The Canary Island taxa form a well-supported clade (bootstrap value [BV] = 86%) which is sister to *Descurainia tanacetifolia* in the strict consensus tree. The node joining the island taxa with *D. tanacetifolia* is moderately well-supported, with a BV = 78%. The remaining Old World *Descurainia* (*D. kochii* and *D. sophia*) form a clade (BV = 92%) which is strongly supported (100%) as sister to the rest of the genus. Phylogenetic relationships within the island are completely unresolved.

Bayesian analysis of the ITS dataset recovered a consensus tree with the same topology as parsimony. Posterior probabilities (PP) are 100% for all nodes which received bootstrap support > 50% in the parsimony tree, with the exception of the branch joining *D. kochii* with *D. sophia* (99% support). While analysis of the ITS data does not allow us to make any inferences about colonization within the Canary Islands, the data strongly support the monophyly of the island taxa and identify *D. tanacetifolia* as the closest continental relative.

Analysis of chloroplast data. — Sequence characteristics for the various chloroplast regions are listed in Table 1. Trees obtained from parsimony analysis of the individual chloroplast datasets (not shown) were congruent, which is expected as the chloroplast is inherited as a single unit and thus all genes should be linked. The indi-
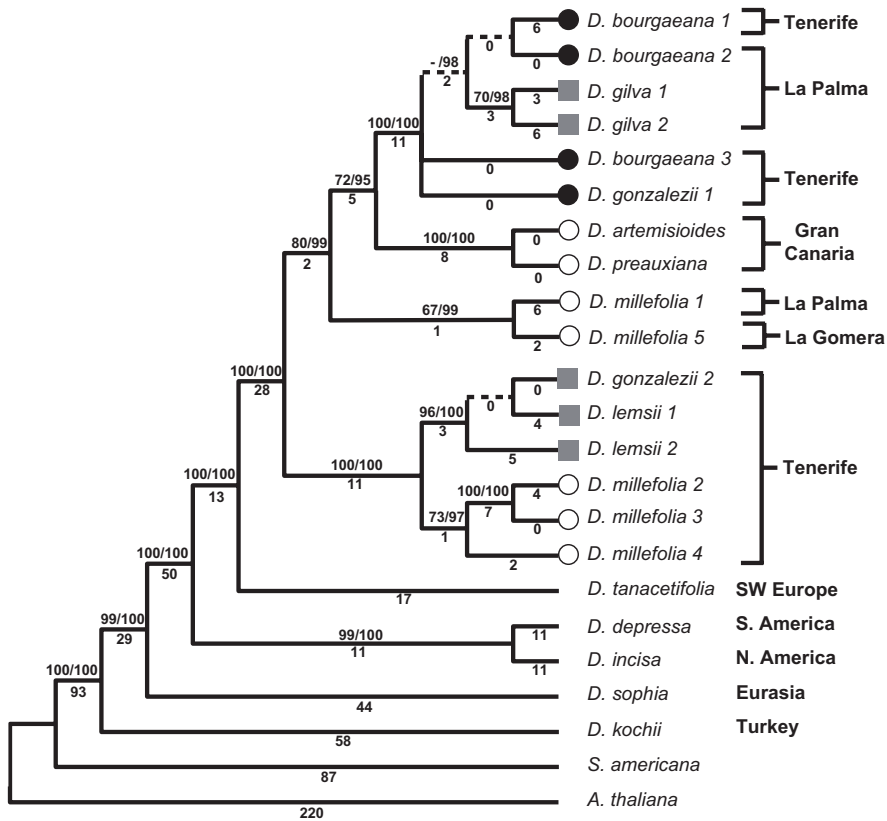
Fig. 3. One of 42 most parsimonious trees derived from combined *rps16, trnD-trnE, trnE-trnT, psbZ-trnfM, ndhF-rpl32, rpoB-trnC*, and *ndhC-trnV* chloroplast sequence data. Dashed lines indicate branches that collapse in the strict consensus tree. Bootstrap values > 50 % / Bayesian posterior probabilities are indicated above branches; branch lengths below. Generic names are abbreviated as follows; *A. = Arabidopsis, D. = Descurainia, H. = Hugueninia*, and *S. = Smelowskia*. Ecological zones are indicated as follows: open circle = lowland scrub, filled square = pine forest, and filled circle = high altitude desert.

vidual chloroplast datasets were consequently combined into a single dataset and subjected to further analyses. The combined dataset contained 6029 nucleotide positions including gaps (8.6%) and missing (0.04%) characters. Fourteen indels, ranging in length from 5 to 160 base pairs, were binary-coded and appended to the dataset, resulting in 6043 characters, of which 668

(11.1%) were variable and 239 (4.0%) were parsimony informative.

Parsimony analysis of the combined chloroplast data for 23 taxa generated 42 trees of 762 steps (CI [excluding uninformative characters] = 0.81, RI = 0.89). One of the most parsimonious trees is shown in Fig. 3. The tree strongly supports (BV = 100%, PP = 100%) the mono-

Table 1. Sequence characteristics of DNA regions used in this study. MPTs = most parsimonious trees.

	trnD-trnE	*trnE-trnT*	*psbZ-trnfM*	*ndhF-rpl32*	*rpoB-trnC*	*ndhC-trnV*	*rps16* intron	Combined chloroplast	ITS
Seq. length (bp)	521–537	592–759	656–731	849–922	1008–1170	824–884	782–815	5478–5637	592–611
Alignment length	553	790	754	968	1202	913	849	6029	618
No. of non-autapo-morphic indels	0	1	2	3	2	2	4	14	0
No. informative characters (%)*	22 (3.9)	27 (3.4)	23 (3.3)	44 (4.5)	37 (3.1)	45 (4.9)	41 (4.8)	239 (4.0)	47 (7.6)
No. informative characters (%)**	4 (0.72)	3 (0.38)	4 (0.53)	6 (0.62)	7 (0.58)	10 (1.1)	10 (1.2)	44 (0.73)	0
No. of MPTs	1	1	>50,000	>50,000	156	900	1382	42	28
Length of MPTs	61	90	99	163	128	122	95	762	158
Consistency index***	0.87	0.83	0.69	0.82	0.82	0.81	0.88	0.82	0.79
Retention index***	0.93	0.91	0.72	0.90	0.91	0.91	0.94	0.89	0.85

*incl. outgroups and indels
**incl. only island taxa and indels
*** excluding uninformative characters

phyly of the Canary Island taxa and a sister relationship to *D. tanacetifolia*. The island taxa resolve into two well-supported clades. One clade (BV and PP = 100%) comprises three species from Tenerife: *D. millefolia* and *D. lemsii + D. gonzalezii*. The other major clade (BV = 80%, PP = 99%) includes exemplars of six of the seven island species. Within this group are three clades: (1) *D. millefolia* samples collected from La Palma and La Gomera (BV = 67%, PP = 99%); (2) *D. artemisioides + D. preauxiana* (BV and PP = 100%) from Gran Canaria; and (3) a well-supported (BV and PP = 100%) but poorly-resolved group comprising *D. bourgaeana* (Tenerife and La Palma), *D. gilva* (2 samples from La Palma), and *D. gonzalezii* (Tenerife). The latter two clades appear to be sister, but the node joining them is only moderately supported (BV = 72%, PP = 95%). In contrast to the ITS tree, *D. sophia* is more closely related to the rest of the genus than *D. kochii*, but the Old World taxa still do not comprise a monophyletic group.

The strict consensus tree generated by Bayesian analysis was similar to the parsimony tree. An additional branch was recovered uniting the two *D. gilva* exemplars with two *D. bourgaeana* samples. Bayesian posterior probabilities range from 95–100% for each node in the tree.

Analysis of combined data. — When the ITS and chloroplast datasets were combined, the ILD test indicated they were not homogeneous (p = 0.03). Following removal of either *D. kochii* or *D. sophia* from the analysis, no significant heterogeneity was detected (p = 1.0). Not unexpectedly (given that the ITS dataset contains no parsimony-informative characters within the Canary Island taxa), parsimony and Bayesian analysis of the combined datasets (excluding *D. kochii*) generated results essentially identical to those generated by analysis of the chloroplast dataset alone. As a consequence, inferences regarding diversification within the islands can only be made based on the results of the chloroplast analysis.

Character optimizations. — Parsimony analysis of the reduced chloroplast dataset generated one most parsimonious tree of 534 steps (CI [excluding uninformative characters] = 0.95, RI = 0.96) (not shown). This tree was identical to that obtained by pruning taxa from the original strict consensus tree except that *D. bourgaeana* from La Palma was sister to *D. gilva* rather than *D. bourgaeana* from Tenerife. This sister relationship is most likely an artifact of the short branch lengths between these taxa; when *D. bourgaeana* samples from both islands were constrained to monophyly, a single most parsimonious tree only one step longer and otherwise identical in topology was generated. This slightly less parsimonious tree was used in the character optimizations. Island distribution and ecological zone were
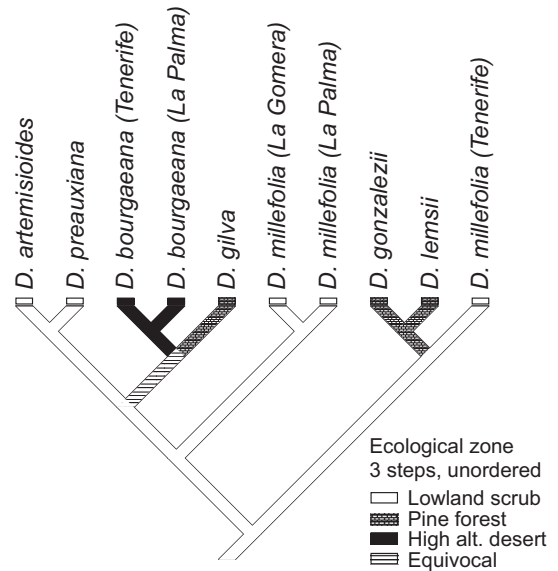

Fig. 4. Character state reconstruction generated from optimization of ecological zone on the single most parsimonious tree obtained from phylogenetic analysis of the reduced chloroplast DNA data set with *D. bourgaeana* constrained to monophyly. The state of the branch joining *D. gilva* with *D. bourgaeana* is equivocal; assignment of either lowland scrub, pine forest, or high altitude desert to this branch yield equally parsimonious reconstructions.

traced separately on the reduced tree (Figs. 4–5). Outgroup taxa (*Arabidopsis thaliana* and *Smelowskia americana*) were not scored because the characters optimized on the tree (ecological zone and island distribution) were not applicable.

Optimization of ecological zone on the reduced tree yielded three most parsimonious reconstructions (Fig. 4). These reconstructions suggest that the ancestral habitat of *Descurainia* in the Canary Islands was located in lowland scrub, and that there have been at least three ecological shifts into pine forest and high altitude desert zones.

When island distribution was traced on the reduced tree, two most parsimonious reconstructions were generated (Fig. 5). One reconstruction implies that the original location of Canarian *Descurainia* was on the island of La Palma and the other optimization identifies Tenerife as the ancestral island.

◼ DISCUSSION

Taxonomic implications. — The results of our ITS and chloroplast analyses clearly demonstrate that the Canary Island species are monophyletic. Phylogenetic analysis of the chloroplast data reveals two major island lineages. One lineage (the "Tenerife" clade) is restricted
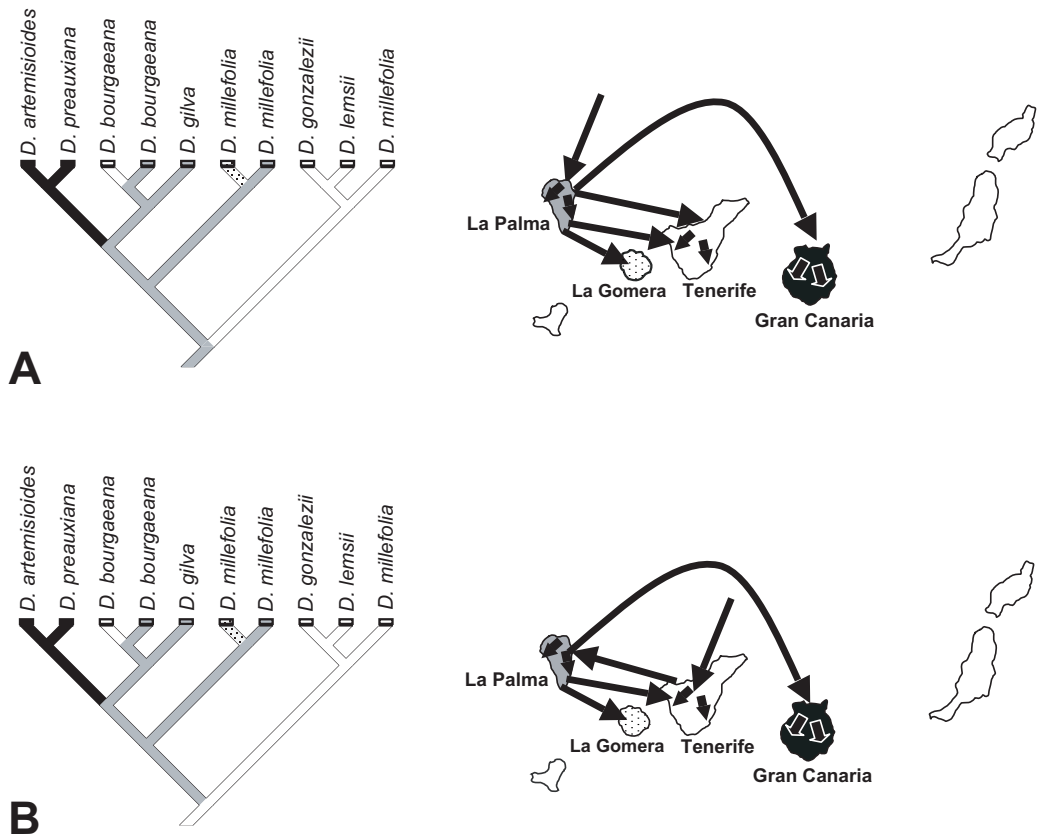
Fig. 5. Most parsimonious reconstructions resulting from optimization of island distribution on the single most parsimonious tree obtained after phylogenetic analysis of the reduced chloroplast DNA data set when *D. bourgaeana* is constrained to monophyly. Arrows between islands on the map indicate direction of dispersal; arrows within an island represent intra-island adaptive radiation. A, reconstruction suggesting La Palma as ancestral island for Canarian *Descurainia*; B, reconstruction suggesting Tenerife as ancestral island.

to Tenerife (*D. gonzalezii*, *D. lemsii*, *D. millefolia*), whereas the other lineage (the "mixed" clade) includes taxa from four islands: *D. bourgaeana* (Tenerife and La Palma), *D. artemisioides* and *D. preauxiana* (Gran Canaria), *D. gilva* (La Palma), and *D. millefolia* (La Palma and La Gomera).

There are two unusual placements in the tree. The first of these is the apparent polyphyly of *D. millefolia*. While three samples of *D. millefolia* collected on Tenerife group together in the "Tenerife" clade, *D. millefolia* from La Palma and La Gomera are well-supported as part of the "mixed" clade. *Descurainia millefolia* is the most widespread of the island species and is morphologically quite variable. Several varieties and forms have been described, including *D. millefolia* f. *brachycarpa* (Schulz, 1924) from western La Palma, *D. millefolia* var. *sabinalis* (Schulz, 1924) from Tenerife and *D. millefolia* var. *macrocarpa* from La Gomera, Tenerife, and La Palma (Pitard & Proust, 1908). Many of the characters that distinguish these varieties, however, are reportedly not constant in cultivation (Bramwell, 1977). Our samples from La Gomera and eastern La Palma,

however, are morphologically similar to the Tenerife samples (all of which were collected from the Teno region of Tenerife). Whether the divergent position of these specimens is due to an ancient hybridization event or simply represents morphological convergence cannot be ascertained without more extensive sampling and development of a better-resolved nuclear phylogeny than that provided by the ITS data. The other unusual feature in the chloroplast phylogeny is the placement of one sample of *D. gonzalezii* (from Las Cañadas) in the "mixed" clade and one sample (from Vilaflor) in the "Tenerife" clade. Except for a unique 18-bp insertion, the Las Cañadas sequence is identical to one of the *D. bourgaeana* samples, yet in morphology it appears to be *D. gonzalezii*. Pérez de Paz (1981) has noted the two reported locations of *D. gonzalezii* and hypothesized that the high altitude desert Las Cañadas population is derived from the population in the pine forest at Vilaflor. Chromosome counts (Borgen, 1969; Bramwell, 1977) for *D. gonzalezii* collected at Las Cañadas, where it is rare and sympatric with *D. bourgaeana*, have detected triploid ($3n = 21$) and tetraploid ($4n = 28$) individuals in

addition to diploids. Chromosome counts of all other Canary Island species have been exclusively diploid (2*n* = 14) (Borgen, 1969; Bramwell, 1977; Suda & al., 2003). On the basis of this chromosomal evidence, it appears likely that *D. gonzalezii* from Las Cañadas is hybridizing with another species, and *D. bourgaeana* is the geographically closest and therefore most likely candidate. Consequently, we believe that the unusual phylogenetic position of this *D. gonzalezii* collection and its short branch length reflects recent chloroplast capture from *D. bourgaeana*.

In addition to the putative *D. gonzalezii* × *D. bourgaeana* hybrid identified in our analysis, other natural *Descurainia* hybrids have also been reported in the Canary Islands. These include *D. artemisioides* × *D. preauxiana* on Gran Canaria (Hansen & Sunding, 1993), *D. bourgaeana* × *D. lemsii* on Tenerife (A. Santos, unpublished), and *D. gilva* × *D. bourgaeana* on La Palma (A. Santos, unpublished). Interspecific hybridization within groups that have radiated following a single introduction has been reported for many Macaronesian taxa (reviewed in Francisco-Ortega & Santos-Guerra, 2001). Many of these hybrids have arisen recently as previously isolated taxa come into close contact through human disturbance (Levin & al., 1996).

In his revision of *Descurainia* in the Canary Islands, Bramwell (1977) identified several characters, such as growth form, distribution and density of the indumentum, leaf shape, petal shape, and size and orientation of siliques, which have taxonomic utility for delineating species boundaries. There appears, however, to be little correlation between these morphological characters and the chloroplast phylogeny. Based on their morphological similarity, for example, Bramwell suggested that *D. gilva* and *D. lemsii* are closely related. This similarity is likely due to convergence: the chloroplast tree indicates that *D. lemsii* is in fact sister to *D. gonzalezii* and not closely related to *D. gilva*. On the other hand, Bramwell's assertion that *D. gilva* might be considered as a local vicariant of *D. bourgaeana* is consistent with their sister relationship in the chloroplast tree. From a morphological point of view, however, the growth habit and fruit of *D. bourgaeana* and *D. gilva* are not very similar.

Biogeography. — Single colonization events into Macaronesia can be inferred from molecular phylogenies of over two dozen Macaronesian endemic genera, including, to name just a few, *Argyranthemum* (Francisco-Ortega & al., 1996, 1997), *Bystropogon* (Trusty & al., 2005), *Cheirolophus* Cass. (Asteraceae; Susanna & al., 1999), *Crambe* L. (Brassicaceae; Francisco-Ortega & al., 2002), *Echium* (Böhle & al., 1996), *Isoplexis* (Lindl.) Loud. (Scrophulariaceae; Bräuchler & al., 2004), *Lotus* (Allan & al., 2004), *Micromeria* Benth. (Lamiaceae; Bräuchler & al., 2005), *Pericallis* D. Don (Asteraceae;

Panero & al., 1999; Swenson & Manns, 2003), and *Sideritis* (Barber & al., 2000). Molecular studies have also uncovered examples of multiple independent introductions, but in almost every case these have involved genera with very few Macaronesian representatives (e.g., *Asteriscus* Miller [Asteraceae; Goertzen & al., 2002], *Hedera* L. [Araliaceae; Vargas & al., 1999], *Ilex* L. [Aquifoliaceae; Cuénoud & al., 2000], *Lavatera* L. [Malvaceae; Fuertes-Aguilar & al., 2002; Ray, 1995], *Plantago* L. [Plantaginaceae; Rønsted & al., 2002], and *Solanum* L. [Solanaceae; Bohs & Olmstead, 2001]. The explanation for why groups arising from single introductions have radiated more spectacularly than those which have arrived repeatedly is currently being debated (Herben & al., 2005; Saunders & Gibson, 2005; Silvertown & al., 2005); one possibility is that niche preemption by initial colonists has prevented successful establishment of later-arriving congeners (Silvertown, 2004).

Both the ITS and chloroplast datasets strongly support the monophyly of *Descurainia* in the Canary Islands and hence the idea that there was a single colonization of the islands. The closest continental relative of the insular taxa is *D. tanacetifolia* which is distributed in the mountains of southwestern Europe. Like the island taxa, *D. tanacetifolia* is a perennial with relatively large flowers and a diploid chromosome number of 2*n* = 14. In contrast, the other European *Descurainia*, *D. sophia*, is a small-flowered annual or biennial with a chromosome number of 2*n* = 28.

Recent molecular studies have demonstrated that many Macaronesian groups, rather than being relictual, are recently derived from herbaceous continental ancestors. The monophyly of the island clade and its sister relationship to *D. tanacetifolia* is consistent with a derived position for *Descurainia* in the Canary Islands. The low sequence divergence among the island species lends support for a recent introduction. In contrast to groups in which woodiness and the perennial habit appear to have been acquired after arrival in the islands (e.g., *Aichryson* Webb & Berthel. [Crassulaceae; Fairfield & al., 2004]; *Argyranthemum* [Francisco-Ortega & al., 1997]; and *Echium* [Böhle & al., 1996]), both characteristics may have been present in the continental ancestor of Canarian *Descurainia*. The closest continental relative, *D. tanacetifolia*, is a perennial, and one of its two subspecies, *D. tanacetifolia* ssp. *suffruticosa*, is suffrutescent near the base (Schulz, 1924). *Descurainia* sect. *Sisymbriodendron* is not the only recently derived insular group whose continental ancestors may have been woody perennials. While there has undeniably been an increase in insular woodiness in many Macaronesian groups, in many cases the closest continental relatives of these endemics are reported to be suffrutescent perennials or shrubs. Among such groups,

which also have annual and/or herbaceous members, are the *Bencomia* alliance (Helfgott & al., 2000), *Convolvulus* L. (Convolvulaceae; Carine & al., 2004), *Isoplexis* (Bräuchler & al., 2004), *Plantago* (Rønsted & al., 2002), the *Sonchus* alliance (Kim & al., 1996), and possibly *Pericallis* (Swenson & Manns, 2003; but see Panero & al., 1999).

Character optimizations suggest that *Descurainia* first arrived in the Canary Islands on one of two islands (Fig. 5). One most parsimonious reconstruction (Fig. 5A) implies that the original location of Canarian *Descurainia* was on the island of La Palma with one dispersal to Gran Canaria, one dispersal to La Gomera, and two dispersals to Tenerife. The other optimization (Fig. 5B) points to Tenerife as the ancestral island. It suggests that there has subsequently been a single dispersal from Tenerife to La Palma, followed by dispersal from La Palma to Gran Canaria and La Gomera and back-dispersal from La Palma to Tenerife (and thus that *D. bourgaeana* arose from *D. gilva*). We feel that initial introduction onto Tenerife is more likely because Tenerife is older, larger, and closer to the continent than La Palma. While both scenarios imply that taxa on Gran Canaria arose from introductions from La Palma, it should be noted that reconstructions that support dispersal to Gran Canaria from adjacent Tenerife require only one additional step.

Few molecular studies have addressed origin and direction of colonization within the Canary Islands. Each island, except El Hierro (the youngest), has been proposed at least once as the location of an initial introduction, but the two most common patterns reported to date involve dispersal from the eastern to western islands and dispersal from Tenerife. The easternmost, and oldest, islands of Fuerteventura and/or Lanzarote have been identified as the ancestral location for *Androcymbium* Willd. (Colchicaceae; Caujapé-Castells & al., 2001), *Aichryson* (Fairfield & al., 2004), and two of the three independent introductions of *Asteriscus* (Goertzen & al., 2002). In the case of *Androcymbium*, and possibly *Aichryson*, subsequent dispersal to the five westernmost islands proceeded via La Palma. Percy & Cronk (2002) proposed that La Gomera was the location of two separate introductions of *Teline*. One colonization of *Asteriscus* (Goertzen & al., 2002) appears to have taken place on Gran Canaria. In addition to *Descurainia*, genera for which Tenerife is proposed as either the island of first introduction or as an important center of dispersal include *Lotus* (Fairfield & al., 2004), *Crambe* (Francisco-Ortega & al., 2002), and *Sonchus* (Kim & al., 1996).

Ecological diversification. — When ecological zones are mapped onto the chloroplast tree (Fig. 4), all reconstructions agree that the most likely ancestral habitat of *Descurainia* in the Canary Islands was lowland scrub with subsequent shifts into pine forest and high altitude desert. This pattern of radiation from lower elevation zones to higher elevation zones has also been observed in *Crambe* (Francisco-Ortega & al., 2002).

Several modes of species diversification have been identified within the Canary Islands. One such pattern is intra-island adaptive radiation, in which speciation is accompanied by habitat shifts within the same island. Speciation may also be facilitated by inter-island colonization, either between similar ecological zones or accompanied by ecological shifts. Molecular studies of several Macaronesian groups have sought to examine the relative importance of these modes of evolution. It should perhaps be emphasized that in all of the groups studied, one pattern may dominate, but both processes appear to have contributed in varying degrees. Inter-island colonization has been the primary mode of diversification in most Macaronesian groups, including *Aeonium* (Mes & t'Hart, 1996), *Argyranthemum* (Francisco-Ortega & al., 1996), *Crambe* (Francisco-Ortega & al., 2002), *Lotus* (Allan & al., 2004), *Pericallis* (Panero & al., 1999), and the *Sonchus* alliance (Kim & al., 1996), while intra-island adaptive radiation has dominated the evolutionary history of the *Gonospermum* Less. (Asteraceae) alliance (Francisco-Ortega & al., 2001), *Sideritis* (Barber & al., 2000) and the *Teline monspessulana* group (Percy & Cronk, 2002). In *Bystropogon* (Trusty & al., 2005), intra-island adaptive radiation has contributed to the evolution of one major clade but inter-island dispersal has been common in the other.

Both intra-island adaptive radiation and inter-island dispersal have occurred in *Descurainia*. At least two species, and possibly a third, have arisen on Tenerife through adaptive radiation (i.e., *D. gonzalezii*, *D. lemsii*, and perhaps *D. bourgaeana*). On the other hand, several cases of inter-island dispersal can be inferred as well. Character state reconstructions suggest inter-island dispersal has taken place from lowland scrub on Tenerife or La Palma to similar habitat on Gran Canaria, giving rise to *D. artemisioides* and *D. preauxiana*. Furthermore, two dispersals must have occurred between La Palma and Tenerife, although the direction of colonization is equivocal. In these cases, inter-island dispersal has been accompanied by at least one habitat shift.

Conclusions. — Canary Island species of *Descurainia* appear to be recently descended from continental ancestors via a single colonization event. The closest continental relative is *D. tanacetifolia*, which is clearly nested within *Descurainia*. Our chloroplast data suggest that intra-island adaptive radiation and inter-island colonization have both played a prominent role in the evolution of *Descurainia* in the Canary Islands, and that the most likely ancestral location of the island progenitor was the lowland scrub zone on Tenerife.

By utilizing several rapidly-evolving non-coding chloroplast DNA regions, we were able to construct a highly-resolved phylogenetic history for *Descurainia* in the Canary Islands. In contrast, the ITS tree is uninformative. Given the lack of resolution in the ITS tree and the evidence for hybridization in the chloroplast phylogeny, a better-resolved nuclear-based phylogeny is needed to confirm the patterns detected in this study.

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Appendix. Plant material used in this study. Seed source for all cultivated plants was the Escuela Técnica Superior de Ingenieros Agrónomos de Madrid crucifer seedbank, Universidad Politécnica de Madrid, Spain.

Taxon; Location, collector and DNA voucher (herbarium); Insular habitat; GenBank accessions (ITS, *trnD-trnE, trnE-trnT, psbZ-trnfM, ndhF-rpl32, rpoB-trnC, ndhC-trnV, rps16*)

Arabidopsis thaliana (L.) Heynh.; GenBank; –; NC_000932; *Descurainia artemisioides* Svent.; Gran Canaria: Berrazales, cultivated, (TEX) Lowland scrub; DQ418708, DQ418554, DQ418576, DQ418598, DQ418620, DQ418642, DQ418664, DQ418686; *D. bourgaeana* Webb ex O. E. Schulz #1; Tenerife: Cañadas del Teide, El Portillo, *A. Santos s. n.* (ORT); High altitude desert; DQ418709, DQ418555, DQ418577, DQ418599, DQ418621, DQ418643, DQ418665, DQ418687; *D. bourgaeana* Webb ex O. E. Schulz #2; La Palma: Los Andenes, La Caldera National Park, *A. Santos s. n.* (ORT); High altitude desert; DQ418711, DQ418557, DQ418579, DQ418601, DQ418623, DQ418645, DQ418667, DQ418689; *D. bourgaeana* Webb ex O. E. Schulz #3; Tenerife: Las Cañadas, cultivated, (TEX); High altitude desert; DQ418710, DQ418556, DQ418578, DQ418600, DQ418622, DQ418644, DQ418666, DQ418688; D. depressa (Phil.) Reiche; Bolivia: Patarani, *Goodson 1505* (TEX); –; DQ418712, DQ418558, DQ418580, DQ418602, DQ418624, DQ418646, DQ418668, DQ418690; *D. gilva* Svent. #1; La Palma: Las Manchas, cultivated (TEX); Pine forest; DQ418713, DQ418559, DQ418581, DQ418603, DQ418625, DQ418647, DQ418669, DQ418691; *D. gilva* Svent. #2; La Palma: Cumbres de Puntallana, *A. Santos s. n.* (ORT); Pine forest; DQ418714, DQ418560, DQ418582, DQ418604, DQ418626, DQ418648, DQ418670, DQ418692; *D. gonzalezii* Svent. #1; Tenerife: Las Cañadas, cultivated, (TEX); High altitude desert; DQ418561, DQ418583, DQ418605, DQ418715, DQ418627, DQ418649, DQ418671, DQ418693; *D. gonzalezii* Svent. #2; Tenerife: Vilaflor, Carretera a Madre de Agua, *A. Santos s. n.* (ORT); Pine forest; DQ418562, DQ418584, DQ418606, DQ418716, DQ418628, DQ418650, DQ418672, DQ418694; *D. incisa* (Engelm. ex A. Gray) Britton; USA: Eagle Co., Colorado, *Goodson 1502* (TEX); –; DQ418717, DQ418563, DQ418585, DQ418607, DQ418629, DQ418651, DQ418673, DQ418695; *D. kochii* (Petri) O. E. Schulz; Turkey: Kastamonu, *A. A. Dönmez 11793* (TEX); –; DQ418718, DQ418564, DQ418586, DQ418608, DQ418630, DQ418652, DQ418674, DQ418696; *D. lemsii* Bramwell #1; Tenerife: La Crucita, cultivated, (TEX); Pine forest; DQ418719, DQ418565, DQ418587, DQ418609, DQ418631, DQ418653, DQ418675, DQ418697; *D. lemsii* Bramwell #2; Tenerife: Cumbres de la Orotova, *A. Santos s. n.* (ORT): Pine forest; DQ418720, DQ418566, DQ418588, DQ418610, DQ418632, DQ418654, DQ418676, DQ418698; *D. millefolia* (Jacq.) Webb & Berthel. #1; La Palma: Barranco del Rio, *A. Santos s. n.* (ORT); Lowland scrub; DQ418721, DQ418567, DQ418589, DQ418611, DQ418633, DQ418655, DQ418677, DQ418699; *D. millefolia* (Jacq.) Webb & Berthel. #2; Tenerife: Buenavista del Norte, *Panero 6987* (TEX); Lowland scrub; DQ418723, DQ418569, DQ418591, DQ418613, DQ418635, DQ418657, DQ418679, DQ418701; *D. millefolia* (Jacq.) Webb & Berthel. #3; Tenerife: El Fraile, *A. Santos s. n.* (ORT); Lowland scrub; DQ418724, DQ418570, DQ418592, DQ418614, DQ418636, DQ418658, DQ418680, DQ418702; *D. millefolia* (Jacq.) Webb & Berthel. #4; Tenerife: Buenavista, cultivated, (TEX); Lowland scrub; DQ418722, DQ418568, DQ418590, DQ418612, DQ418634, DQ418656, DQ418678, DQ418700; *D. millefolia* (Jacq.) Webb & Berthel. #5; La Gomera: Chejelipes, *leg. ign. AAU71-7533* (MO); Lowland scrub; DQ418725, DQ418571, DQ418593, DQ418615, DQ418637, DQ418659, DQ418681, DQ418703; *D. preauxiana* (Webb) Webb ex O. E. Schulz; Gran Canaria: Ayacata, cultivated, (TEX); Lowland scrub; DQ418726, DQ418572, DQ418594, DQ418616, DQ418638, DQ418660, DQ418682, DQ418704; *D. sophia* (L.) Webb ex Prantl; USA. Park Co., Colorado: *Goodson 1463* (TEX); –; DQ418727, DQ418573, DQ418595, DQ418617, DQ418639, DQ418661, DQ418683, DQ418705; *D. tanacetifolia* (L.) Prantl ssp. *tanacetifolia*; Italy: Piemonte. *Pistarino 2027* (NY); –; DQ418728, DQ418574, DQ418596, DQ418618, DQ418640, DQ418662, DQ418684, DQ418706; *Smelowskia americana* (Regel & Herder) Rydb.; U.S.A. Park Co., Colorado: *Goodson 1462* (TEX); –; DQ418729, DQ418575, DQ418597, DQ418619, DQ418641, DQ418663, DQ418685, DQ418707.