The Dissertation Committee for Barbara Elizabeth Goodson certifies that this is the approved version of the following dissertation:

Molecular Systematics and Biogeography of *Descurainia* Webb & Berthel. (Brassicaceae)

Committee:

Robert K. Jansen, Supervisor

C. Randal Linder

Alan M. Lloyd

Beryl B. Simpson

Tandy J. Warnow

Molecular Systematics and Biogeography of *Descurainia* Webb & Berthel. (Brassicaceae)

by

Barbara Elizabeth Goodson, B.S., M.C.S., M.S

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Molecular Systematics and Biogeography of *Descurainia* Webb & Berthel. (Brassicaceae)

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Barbara Elizabeth Goodson, Ph.D.

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Supervisor: Robert K. Jansen

Descurainia is a genus in the Brassicaceae distributed throughout temperate areas of the Old and New World. The genus is well-known for its taxonomic complexity, especially within New World species, on account of its numerous intergrading forms coupled with circumscriptions dependent upon inconsistent and overlapping characters. *Descurainia* is most diverse in western North America and western South America, with a smaller center of distribution in the Canary Islands and three additional Old World species. This distribution makes the genus well-suited for addressing biogeographical issues related to New World intercontinental dispersal and evolution in island systems.

A molecular-based analysis of *Descurainia* was conducted using DNA sequences from nuclear ribosomal internal transcribed spacer (ITS), single-copy nuclear Target of Rapamycin (TOR), and non-coding chloroplast regions. The genus, with the inclusion of the monotypic genera *Hugueninia* and possibly *Robeschia*, is strongly supported as monophyletic, and appears to be of Old World origin with recent diversification within

the Canary Islands and the New World. A phylogeny recovered from combined ITS and chloroplast data is not well-resolved with respect to relationships between some major New World lineages, but suggests that multiple independent dispersals of *Descurainia* have taken place between North and South America. Substantial incongruence between ITS, chloroplast, and TOR phylogenies, as well as the presence of mixed ITS and TOR sequences, point to a complex evolutionary history involving extensive gene flow and hybridization for North American *Descurainia*. The molecular data highlight possible problems with current species circumscriptions, especially within North American taxa such as *D. incisa*, *D. obtusa*, and *D. pinnata*. ITS and chloroplast data indicate that species of *Descurainia* in the Canary Islands are derived via a single colonization event, most likely from southwestern Europe onto the lowland scrub zone on Tenerife. Both intra-island adaptive radiation and inter-island colonization have played a prominent role in the evolution of this genus in the islands. The results presented in this dissertation represent the first comprehensive molecular study of *Descurainia,* and may serve as a phylogenetic framework for future research on the genus as well as phenomena such as speciation and hybridization in recently-evolved groups.

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Chapter 1: Introduction

Descurainia Webb & Berthel. is a genus in the Brassicaceae with centers of diversity in the Canary Islands (7 spp.), North America (*ca*. 12 spp.), and western South America (*ca*. 21 spp.) (Fig. 1.1; Table 1.1). Only three species are not wholly confined to these areas, occupying instead portions of Eurasia or both arctic North America and northern Siberia. Most species of *Descurainia* are small-flowered herbaceous annuals or biennials with small nectaries, although the Canarian species are suffrutescent perennials with relatively large flowers and conspicuous nectaries (Schulz, 1924; Al-Shehbaz, 1988).

Descurainia was last monographed in its entirety by Schulz (1924), with treatments of North American (Detling, 1939) and Canary Island (Bramwell, 1977) species appearing more recently. Although one of the principal genera in the Brassicaceae (Mabberley, 1997) on the basis of species diversity, there have been no detailed molecular systematic studies of *Descurainia* and no hypotheses have been put forth regarding phylogenetic relationships within the genus. This paucity of critical studies is largely due to the taxonomic complexity of *Descurainia*, especially within New World species that comprise the majority of the genus. Extensive morphological variation exists within numerous species, many wide-ranging and widely-overlapping taxa appear to intergrade endlessly, and descriptions are frequently based on inconsistent and overlapping diagnostic characters. Polyploidy is widespread, at least within North American species, with tetraploid and hexaploid populations reported for many taxa. This ploidal level variation, coupled with the presence of morphologically intergrading forms, suggests the possibility of frequent hybridization, complicating classification efforts.

Although the genus is in need of revision, attempts to circumscribe correctly many species are likely to fail without insights provided by a molecular study.

Many of the morphological characters used to delimit taxonomic boundaries in the Brassicaceae are homoplastic, and molecular systematic studies are demonstrating that most traditional tribal and subtribal classifications are highly artificial (Al-Shehbaz & al., 2006 and Bailey & al., 2006 and references therein). Generic boundaries in the family are also problematic, and many genera have been discovered to be non-monophyletic (e.g., Koch & al., 1999a, 2000; Crespo & al., 2000; Sweeney & Price, 2000; Warwick & al., 2002, 2004b; O'Kane & Al-Shehbaz, 2003; Koch & Al-Shehbaz, 2004; Beilstein & al., 2006). Consequently, in addition to clarifying species circumscriptions within *Descurainia*, molecular data are also needed to confirm the monophyly of the genus and its relative position within the Brassicaceae.

On account of its distribution, *Descurainia* has potential as a model system for addressing several broad biogeographical questions. One such issue concerns the frequency and pattern of dispersals between North and South American temperate zones. North American species of *Descurainia* are separated from their congeners in South America by a distance of approximately 2200 km. This pattern of disjunction is well-known, having been observed for a great number of species, species-pairs, and genera, many of which are more widely separated then *Descurainia*. These disjunctions are generally thought to have arisen via long-distance dispersal, most likely by migrating birds (Raven, 1963, 1972; Cruden, 1966; Solbrig, 1972; Carlquist, 1983; Simpson & Neff, 1985). In the case of taxa such as *Descurainia* which have a more continuous distribution along the western Cordillera, some authors have suggested that dispersal may have proceeded in a stepping-stone fashion, rather than by way of a single long-distance event (Raven, 1963; Cruden, 1966; Thorne, 1972). Considering the large number of New

World temperate zone disjuncts, surprisingly few molecular phylogenetic investigations have been carried out on such taxa. Although a few broadly-sampled and well-resolved phylogenies of genera speciose on both continents have recently appeared (e.g., Bell & Donoghue, 2005; Simpson & al., 2005; Blattner, 2006; Moore & al., 2006), the majority of relevant studies have focused on groups comprising many species on one continent and only one or two species on the other (e.g., Wallace & Jansen, 1990; Vargas & al., 1998; Morrell & al., 2000; Chan & al., 2001; Lia & al., 2001; Soltis & al., 2001; Bleeker & al., 2002; Lee & al., 2003; Beier & al., 2004). *Descurainia*, with its moderate size and centers of diversity on both continents, is well-suited for studying the origins of New World temperate zone disjunctions.

Other biogeographical questions which can be addressed using *Descurainia* are related to the Canary Islands, whose flora and fauna, together with that of the surrounding Macaronesian region, has been the focus of a number of recent studies involving colonization and adaptive radiation on islands (see Juan & al., 2000). It has been suggested that the Macaronesian endemic flora is a relict of a Tertiary flora which spread from the Mediterranean basin before the first glaciation of Europe (Bramwell, 1977; Sunding, 1979; Cronk, 1992). Recent molecular studies, however, suggest that while some Macaronesian species may be relictual in nature (e.g., Ray, 1995; Mes & al., 1996; Fuertes-Aguilar & al., 2002; Moore & al., 2002), most groups appear to be recently derived from herbaceous continental ancestors (e.g., Böhle & al., 1996; Kim & al., 1996; Francisco-Ortega & al., 1997; Barber & al., 2000; Helfgott & al., 2000; Mort & al., 2002). Several recent studies of the Macaronesian flora have also investigated diversification patterns within the islands, such as the relative importance of inter-island colonization between similar ecological zones compared to adaptive radiation within each island. Molecular studies suggest that while intra-island adaptive radiation appears to be

the dominant mode of species diversification in a few groups (e.g., Barber & al., 2000; Percy & Cronk, 2002), others may have speciated primarily via inter-island colonization (e.g., Francisco-Ortega & al., 1996; Mes & t'Hart, 1996; Percy & Cronk, 2002; Allan & al., 2004; Trusty & al., 2005). Because well-resolved multigene phylogenies for many insular groups have not yet been acquired, however, the overall picture of evolution within the islands is still emerging. Development of a phylogeny for Canary Island *Descurainia* and identification of continental relatives will provide additional evidence concerning the origin and colonization patterns of the Macaronesian flora.

A major goal of the research described in this dissertation was construction of a molecular-based phylogeny for the genus *Descurainia*. Additional aims were to use the resulting phylogeny to test the monophyly of *Descurainia* and elucidate its relationship to other members of the Brassicaceae, estimate evolutionary relationships among New World *Descurainia*, with an emphasis on dispersal patterns between North and South America and the origin of the New World disjunction, and examine the origin and evolution of the Canary Island species. To accomplish these goals, DNA was isolated from multiple accessions of most described *Descurainia* species as well as several close relatives and suitable outgroups. Phylogenetic analyses were conducted using DNA sequences obtained for the nuclear ITS ribosomal repeat region (ITS1, 5.8S rRNA, ITS2 [Kim & Jansen, 1994]), several non-coding chloroplast regions, and, for a subset of taxa, a portion of the single-copy nuclear Target of Rapamycin (TOR) gene. The remaining three chapters of this dissertation outline these molecular studies, the results, and their taxonomic and biogeographic implications.

Chapter 2 of this dissertation presents a phylogeny of *Descurainia* and related taxa generated from parsimony and Bayesian analyses of the ITS and chloroplast molecular data. These results indicate that *Descurainia* is monophyletic with the

inclusion of monotypic genera *Hugueninia* Rchb. and possibly *Robeschia* O. E. Schulz and *Ianhedgea* Al-Shehbaz & O'Kane. These genera are sister to a clade consisting of the European genus *Hornungia* Rchb. and the New World genus *Tropidocarpum* Hook.. On the basis of this study and other reports (e.g., Beilstein & al., 2006), Al-Shehbaz & al. (2006) have recognized these genera and *Trichotolinum* O. E. Schulz as constituents of a new tribe, the Descurainieae.

Phylogenies based on ITS and non-coding chloroplast data reveal that Old World species *D. kochii* and *D. sophia* are the earliest diverging lineages in the genus, and that the Canary Island species and *Hugueninia* are sister to all the New World taxa. Within the New World clade, several major lineages are identified, but their relationships to one another are not completely resolved.

Molecular clock dating suggests a recent origin in the Irano-Turanian region of the Old World for *Descurainia*, with subsequent diversification during the late Pliocene or early Pleistocene into Europe and into the New World. Species in the Canary Islands are monophyletic, implying a single colonization event into the islands, and are most closely related to European *Hugueninia*. Following introduction into the New World, most likely from Eurasia into North America, there have been at least two, and possibly more, dispersals between North and South America. Incongruence between ITS and chloroplast trees, as well as mixed ITS types observed for some North American accessions, provide evidence of hybridization in North American *Descurainia*. The recent origin of the genus and frequent hybridization are probably responsible for most of the taxonomic complexity which plagues efforts to classify *Descurainia* in North and South America.

In Chapter 3, the results of a molecular analysis based on sequences of a portion of the single-copy nuclear gene Target of Rapamycin (TOR) are presented. Parsimony

and Bayesian analyses of the TOR sequence data strongly support New World *Descurainia* as a monophyletic lineage which is most closely related to *Hugueninia* and sister to Canary Island taxa. The position of *Hugueninia* with respect to Canary Island and New World taxa thus differs from the results of Chapter 2. Although most major New World clades identified by ITS and chloroplast data are present in the TOR phylogeny, their position with respect to one another is largely unresolved. Extensive incongruence between ITS, chloroplast, and TOR phylogenies, as well as the presence of mixed ITS and TOR sequences, suggests a complex evolutionary history for *Descurainia*. As a recently-diverged genus, processes such as hybridization and lineage sorting complicate efforts to develop an accurate taxonomy for the group. Despite the substantial incongruence observed between ITS, chloroplast and TOR phylogenies as well as the need for more extensive taxon sampling, some general trends with regards to North American *Descurainia* species taxonomy can nonetheless be discerned. Although the molecular data are consistent with many existing species concepts, problems with the circumscriptions of taxa such as *D. incisa*, *D. obtusa* and *D. pinnata* are highlighted.

Finally, in Chapter 4, the origin and evolution of the Canary Island species is examined. A molecular-based phylogeny of Canarian *Descurainia* was constructed using DNA sequences from ITS and 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 *Hugueninia 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.

Table 1.1. Species concepts for *Descurainia* and related taxa initially recognized in this study[1]. Taxa sampled in this study are marked with an * (see Table 2.2).

Taxon	Distribution	Reported chromosome numbers[14]	
		n =	2*n* =
SECTION *SISYMBRIODENDRON* (CHRIST) O. E. SHULZ (Canary Islands)			
D. artemisioides Svent.*	Gran Canaria	--	14[a,b]
D. bourgaeana Webb. ex. O. E. Schulz*	Tenerife	7[b,c]	14[a]
D. gilva Svent.*	La Palma	--	14[d]
D. gonzalezi Svent.*	Tenerife	7[b], 14[b]	14[e], 21[a,b]
D. lemsii Bramwell*	Tenerife	7[b]	14[b]
D. millefolia (Jacq.) Webb & Berthel.*	Tenerife, La Palma, La Gomera	--	14[bc]
D. preauxiana (Webb) Webb ex O. E. Schulz*	Gran Canaria	--	14[ab]
SECTION *DESCURAINIA*			
Eurasia –			
D. kochii (Petri) O. E. Schulz*	Turkey, Caucasus region	--	--
D. sophia (L.) Webb*	Europe, Asia (except SE), N Africa (& New World)	14[x]	28[f]
North America –			
D. californica (A. Gray) O. E. Schulz*	W U.S.	7[j]	14[j]
D. hartwegiana (E. Fourn.) Britton[2†]	Central Mexico?	--	--
D. impatiens (Cham. & Schltdl.) O. E. Schulz*	Central & S Mexico	7[k]	--
D. incana (Bernh. ex Fisch. & C. A. Mey.) Dorn[3]*	Canada, N U.S. incl. Alaska	7[l]	14[n], 28[m]

Table 1.1. Continued.

D. incisa (Engelm. ex A. Gray) Britton[3]			
ssp. *filipes* (A. Gray) Rollins*	W Canada, W U.S.	--	14[n,m], 28[l], 42[l]
ssp. *incisa**	W Canada, W U.S.	--	--[13]
ssp. *paysonii* (Detling) Rollins*	W U.S.	--	--
ssp. *viscosa* (Rydb.) Rollins*	W U.S.	7[o]	14[p]
D. obtusa (Greene) O. E. Schulz			
ssp. *adenophora* (Wooton & Standley)*	SW U.S., N Mexico	21[p]	--
ssp. *brevisiliqua* Detling[4]*	N Arizona, N New Mexico	--	42[m]
ssp. *obtusa**	Arizona, New Mexico, N Mexico	--	14[m]
D. paradisa (A. Nels. & Kenn.) O. E. Schulz			
ssp. *nevadensis* Rollins*	Nevada, Oregon, California	--	--
ssp. *paradisa**	Nevada	--	--
D. pinnata (Walter) Britton			
ssp. *brachycarpa* (Richardson) Detling*	Canada, N & central U.S.	7[q]	28[n]
ssp. *glabra* (Wooton & Standl.) Detling*	SW U.S., N Mexico	--	28[m]
ssp. *halictorum* (Cockerell) Detling*[9]	Central & W U.S., N Mexico	7[r,j]	14[r],28[n], 42[m]
ssp. *intermedia* (Rydb.) Detling*[9]	W Canada, W U.S.	--	28[m]
ssp. *menziesii* (DC.) Detling*	S California, Baja California	--	28[h]
ssp. *nelsonii* (Rydb.) Detling*	W Canada, W U.S.	--	14[m]
ssp. *ochroleuca* (Wooton) Detling*	SW U.S., N Mexico	14[s]	--
ssp. *pinnata**	SE U.S.	7[j]	--
D. ramosissima Rollins[5†]	Saguache Co., Colorado	--	--
D. sophioides (Fischer ex Hook.) O. E. Schulz*	N Canada, Alaska, N Siberia[10]	--	14[n,t]
D. streptocarpa (E. Fourn.) O. E. Schulz*[11]	Central Mexico, Guatemala	14[l]	--
D. torulosa Rollins[6†]	W Wyoming	--	--
D. virletii (E. Fourn.) O. E. Schulz*	Central Mexico	14[j]	--
South America –			
D. adpressa Boelcke	Prov. San Juan, Argentina	--	--
D. altoandina Romanczuk	Prov. Neuquén, Argentina	--	--
D. antarctica (E. Fourn.) O. E. Schulz			
var. *antarctica*	S Argentina, S Chile	--	--
var. *bonarelli* O. E. Schulz*	S Argentina	--	--
var. *patagonica* (Speg.) O. E. Schulz*	S Argentina, S Chile	--	--

Table 1.1. Continued.

D. appendiculata (Griseb.) O. E. Schulz*	Central & NW Argentina, Uruguay, S Bolivia	--	--
D. argentea O. E. Schulz[7†]	Prov. Chubut, Argentina	--	--
D. argentina O. E. Schulz*	Central & NW Argentina	--	--
D. athrocarpa (A. Gray) O. E. Schulz* (includes *D. gilgiana* Muschl., *D. macbridei* O. E. Schulz, and *D. urbaniana* Muschl.)	Peru, Bolivia, N Chile	--	--
D. brevifructa Boelcke ex Mart.-Laborde	Prov. San Juan, Argentina	--	--
D. cumingiana (Fisch. & C. A. Mey.) Prantl		7[u]	14[h]
var. *cumingiana*	Chile, W Argentina	--	--
var. *glabrescens* (Speg.) Speg.	Chile, S Argentina	--	--
var. *tenuissima* (Phil.) Reiche*	Chile, S Argentina	--	--
D. depressa (Phil.) Reiche*	Bolivia, Peru, N Chile, NW Argentina		
var. *depressa*[8]†		--	--
var. *pflanzii* (Muschl.) O. E. Schulz[8]†		--	--
D. erodiifolia (Phil.) Prantl ex Reiche*	Chile	--	--
D. glaucescens (Phil.) Prantl ex Reiche*	Chile, W Argentina	--	--
D. heterotricha Speg.*	Argentina	--	--
D. latisiliqua (Muschl.) O. E. Schulz	S Bolivia	--	--
D. leptoclada Muschl. ex O. E. Schulz*	Bolivia, Peru, NW Argentina, N Chile	--	14[v]
D. myriophylla (Willd. ex DC.) R. E. Fr.* (includes *D. perkinsiana* Muschl. and *D. pulcherrima* Muschl.)	Colombia, Ecuador, Peru, Bolivia, N Chile, NW Argentina	7[w]	14[h,v,w] (28 in some cells)[h]
D. nana Romanczuk	Prov. Santa Cruz, Argentina	--	--
D. nuttallii (Colla) O. E. Schulz	Chile	--	--
D. pimpinellifolia (Barnéoud) O. E. Schulz*	Chile, W Argentina	--	--
D. stricta (Phil.) Reiche* var. *minutiflora* (Phil.) O. E. Schulz var. *rubescens* (Phil.) O. E. Schulz var. *stricta*	N Chile	--	--

Table 1.1. Continued.

var. *florida* (Phil.) O. E. Schulz			
D. titicacensis (Walpers) Lillo	Peru, Bolivia, NW Argentina	--	--
RELATED TAXA			
Hugueninia tanacetifolia (L.) Rchb.[12]		7[g]	14-16[h]
ssp. *suffruticosa* (L.) Prantl	Pyrenees, N Spain	--	14[i]
ssp. *tanacetifolia**	SW Alps	--	--
Ianhedgea minutiflora (Hook. f & Thoms.) Al-Shehbaz & O'Kane*	Central & SW Asia	--	28[y]
Robeschia schimperi (Boiss.) O. E. Schulz*	Middle East	8[z]	--

Notes:
[1]Authors frequently disagree in regards to species and subspecific concepts and no classification is satisfactory. I have generally followed the Brassicaceae checklist of Warwick & al. (2006) in conjunction with Schulz (1924), regional treatments, and personal observations of herbarium material. In my view, the validity of several named species and/or subspecies is questionable (including but not limited to taxa marked with a †).
[2]Most likely mislabelled South American collections (*cf*. Rollins 1993a).
[3]Rollins (1993a) and Holmgren & al. (2005) include ssp. *filipes* and *paysonii* in *D. incisa*, whereas Detling (1939) and Welsh & al. (1993) consider them subspecies of *D. pinnata*. Detling (1939) includes the remainder of *D. incisa* in *D. incana* (= *D. richardsonii* [Sweet] O. E. Schulz).
[4]Included by Rollins (1993a) in *D. obtusa* ssp. *obtusa*.
[5]Known only from the type locality (Saguache Co., CO) and probably an intermediate form between *D. pinnata* and *D. incana*.
[6]Studies by Bricker & al. (2000) suggest that *D. torulosa* is not sufficiently distinct from *D. incana* to merit taxonomic recognition.
[7]The species description (Schulz 1924) is apparently based on an immature specimen without fruit. Almost no herbarium material exists that has been considered to be *D. argentea*.
[8]These two varieties occur together and differ only in the degree of silique pubescence.
[9]Holmgren & al. (2005) included ssp. *halictorum* and ssp. *intermedia* in var. *osmiarum* (Cockerell) Shinners.
[10]There is also one specimen at MO! collected by Dr. F. V. Hayden 12 June 1860 from "Jacksons Hole on Snake River [Wyoming] during the expedition of Capt. W. F. Reynolds to the head waters of the Missouri and Yellowstone Rivers 1859-60." Plant material from that expedition and many others was processed by George Engelmann and added to his 94000+ specimen herbarium (inherited by MO); perhaps a label or sample mix-up occurred with Canadian or Alaskan material.
[11]Rzedowski & Rzedowski (2001) include *D. streptocarpa* in *D. impatiens*.
[12]A second species, *H. balearica* (Porta) O. E. Schulz, has been transferred to *Diplotaxis* (as *D. catholica* [L.] DC.).
[13]Reports of $2n = 42$ for *D. incisa* are based on a sample which is identifiable as *D. obtusa* ssp. *brevisiliqua*.
[14]References for reported chromosome numbers: [a]Borgen, 1969; [b]Bramwell, 1977; [c]Larson, 1960; [d]Suda & al., 2003; [e]Polatschek, 1983; [f] Manton, 1932 (56 in some cells); Baldwin & Campbell, 1940; Löve & Löve, 1956; Mulligan, 1961; Taylor & Mulligan, 1968; Podlech & Dieterle, 1969; Gadella & Kliphuis, 1973; Ancev, 1981, 1983; Murín, 1974; Aryavand, 1977, 1978; Dvorák & al., 1981; Dvorák & Dadáková, 1984; Parfenov & Dmitrieva, 1988; Yang & al., 1996; Dobeš & Hahn, 1997; Pogan & al., 1980; Lövkvist &

Hultgård, 1999; (also one report of $2n = 12$ [Saidabadi & Garenflot, 1975] and three reports of $2n = 14$ [Bochantzeva, 1972; Krasnoborov & al., 1980; Krasnikov & Lomonosova, 1990]); [g]Delay, 1971; [h]Manton,

Table 1.1. Continued.

1932; [i]Ortiz, 1993; [j]Rollins & Rüdenberg, 1977; [k]Beaman & al., 1962; [l]Reported in Rollins, 1993a but source not cited; [m]Baldwin & Campbell, 1940; [n]Mulligan, 1961; [o]Rodman, 1978; [p]Rollins & Rüdenberg, 1979; [q]Easterly, 1963; [r]Rollins & Rüdenberg, 1971; [s]Ward, 1983; [t]Mulligan, 1961, Packer, 1964; Mulligan, 1970; Zhukova & al., 1973, 1977; Löve & Löve, 1982; Yurtsev & Zhukova, 1982; Petrovsky & Zhukova, 1983; Zhukova & Petrovsky, 1984 (also one report of $2n = 26$ [Zhukova, 1966]); [u]Jaretzky, 1932; [v]Diers, 1961; [w]Huynh, 1965; [x] Jaretzky, 1932; Tischler, 1935; Rohweder, 1937; Rodman, 1978; Arohonka, 1982; Mulligan, 1984; Lan & Cheo, 1989; Khatoon & Ali, 1993; Hill, 1995 (one report of $n = 7$ [Ghaffari & Chariat-Panahi, 1985] and one report of $n = 10$ [Maassoumi, 1980]); [y]Polatschek, 1971; [z]Aryavand, 1975.

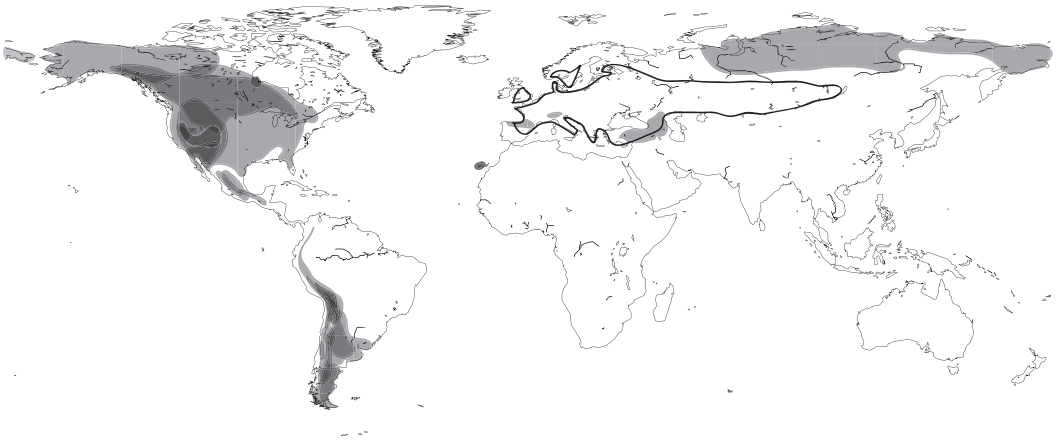


Fig. 1.1. World-wide distribution of *Descurainia* and *Hugueninia*. Darker areas correspond to regions of greatest species diversity. The primary range of widely-introduced *D. sophia*, based on Anderberg & Anderberg (1997), is shown in black outline.

Chapter 2: Molecular systematics of *Descurainia* Webb & Berthel. (Brassicaceae) based on nuclear ITS and non-coding chloroplast DNA

INTRODUCTION

Descurainia Webb & Berthel. is a genus in the Brassicaceae comprising approximately 40 – 45 species distributed throughout temperate areas of the Old and New World (Table 1.1; Fig. 1.1). The vast majority of *Descurainia* species are concentrated in three regions of the world, namely the Canary Islands (7 spp.), North America (*ca*. 12 spp.), and western South America (*ca*. 21 spp.). Only three species are not wholly located within these centers of distribution: *D. sophioides* is found in northern Siberia as well as arctic North America, *D. kochii* is distributed throughout Turkey and the Caucasus, and *D. sophia* is a now widely-introduced Eurasian weed. An additional genus included in *Descurainia* by some authors (Prantl, 1891; Appel & Al-Shehbaz, 2003) – *Hugueninia* Rchb. – is restricted to the mountains of southwestern Europe.

Morphological characteristics of *Descurainia* include the presence of minute dendritic trichomes, pinnate to tripinnate leaves, small yellow or whitish flowers with spathulate petals, filiform fruiting pedicels, and narrow siliques containing seeds that are mucilaginous when wet (Schulz, 1924; Al-Shehbaz, 1988; Rollins, 1993a). Many species of *Descurainia* also possess unicellular clavate glands, a feature not found in other members of the Brassicaceae (Al-Shehbaz & al., 2006). The genus name is derived from *Descurea*, the name applied to currently-termed *Descurainia sophia* by Guettard (1747) in honor of his grandfather François Descurain, a French apothecary and botanist (Webb & Berthelot, 1836; Holmgren & al., 2005). Although *Descurainia* is one of the principal genera in the Brassicaceae (Mabberley, 1997) in terms of species richness, the genus has not been extensively studied and estimates of the number of species vary. The only

comprehensive treatment of the genus has been that of Schulz (1924), who divided *Descurainia* into two sections. Section *Sisymbriodendron* (Christ) O. E. Schulz comprises the seven species endemic to the Canary Islands. These species are self-incompatible suffrutescent perennials, possessing relatively large flowers, conspicuous nectaries, and slightly winged seeds. Except for *D. millefolia*, each of the Canarian species is narrowly restricted in respect to habitat and island distribution. Section *Descurainia* (= sect. *Seriphium* O. E. Schulz), consisting of small-flowered herbaceous annuals, biennials, or rarely perennials with inconspicuous nectaries, comprises the majority of species in the genus. The reproductive biology of this section has not been studied thoroughly, but several species (i.e., *D. californica*, *D. pinnata*, *D. richardsonii* [= *D. incana* or *D. incisa*], and *D. sophia*) are known to be autogamous (Rollins and Rüdenberg, 1971; Best, 1977; Bramwell, 1977; Wiens, 1984; Wiens & al., 1987). Excluding *D. sophioides*, which extends westward from northern Canada into Siberia, only two members of this section are found in the Old World. In contrast to sect. *Sisymbriodendron*, taxa in sect. *Descurainia* are generally weedy and wide-ranging.

Section *Descurainia* has successfully radiated throughout the northern and southern hemispheres of the New World. A presence in both North and South America is uncommon in the Brassicaceae, being true for fewer than a dozen (Rollins, 1993a; Al-Shehbaz & Price, 2001) out of the approximately 338 genera in the family (Al-Shehbaz & al., 2006).

Most North American species of *Descurainia* are distributed in the western United States, with the greatest concentration of taxa located south and east of the Great Basin. Only two species extend their ranges east of the Mississippi: *D. incana* stretches into Canada as far east as the Great Lakes, *D. pinnata* ssp. *brachycarpa* reaches into the Ohio Valley, New England, and Quebec, and *D. pinnata* ssp. *pinnata* is an inhabitant of

the southeastern U. S. coastal plain. Three to four species are endemic to Mexico. In a major revision of North American *Descurainia*, Detling (1939) recognized nine native species (*D. californica*, *D. hartwegiana*, *D. impatiens*, *D. obtusa*, *D. pinnata*, *D. richardsonii*, *D. sophioides*, *D. streptocarpa*, and *D. virletii*) and 18 subspecies. He reclassified many species of Schulz and other authors and recognized 11 subspecies of the wide-ranging and morphologically variable *D. pinnata*, dividing them between southern and northern complexes. More recently, Rollins (1993a) addressed species concepts in the genus as part of a comprehensive taxonomic treatment of North American Cruciferae. While agreeing with many of Detling's classifications, he made a number of changes, including naming two new species (*D. ramosissima* and *D. torulosa*) and separating *D. richardsonii* into *D. incana* and *D. incisa*. He also transferred several subspecies of *D. pinnata* to *D. incisa*, and raised one subspecies to the species level (*D. paradisa*). Detling (1939) and Rollins (1983, 1993a,b), as well as many authors of regional treatments (e.g., Welsh and Reveal, 1977; Goodrich & Neese, 1986; Al-Shehbaz, 1988; Welsh & al., 1993; Holmgren & al., 2005) have commented on the taxonomic complexity of North American *Descurainia* and the unsatisfactory nature of the diagnostic characters used to interpret relationships. This has been particularly problematic within – and between – *D. pinnata* and *D. incisa*. Furthermore, in a study assessing the taxonomic status of *D. torulosa* (Bricker & al., 2000), six accessions of *D. pinnata*, *D. incana*, and *D. incisa*, all from Wyoming, were included along with *D. sophia* in a molecular analysis. While this sampling was extremely limited, the results uncovered evidence suggesting that *D. pinnata* and *D. incana* as currently recognized may not be monophyletic.

No comprehensive revision of South American *Descurainia* has been produced since Schulz' (1924) monograph of the entire genus, although checklists and regional

floras have appeared, most notably in Argentina (Romanczuk, 1984a; Boelcke & Martínez-Laborde, 1994; Prina, 1995; Zuloaga & Morrone, 1996) and Peru (Macbride, 1938; Brako & Al-Shehbaz 1993). In the absence of an updated comprehensive study, the number of South American *Descurainia* species is poorly understood. Roughly 20 – 25 species and 10 – 15 subspecies are generally recognized, although the actual number is probably much smaller (Al-Shehbaz, pers. omm..). Like their North American congeners, the South American species are marked by a high degree of taxonomic complexity. This problem is particularly acute within several wide-ranging complexes centered around species such as *D. appendiculata* and *D. myriophylla*. The center of diversity for South American *Descurainia* is northwestern Argentina, and there is a clear morphological and geographical division in the group. Species distributed along the Andes from Columbia to northern Argentina are characterized by the appearance of fruit strongly appressed to the rachis and the possession of fruit valves dehiscing from the apex to the base. This group comprises about a third of South American *Descurainia*, including such species as *D. athrocarpa*, *D. depressa*, and *D. myriophylla*, and is least diverse, in terms of number of species, in the northern part of its range. The remaining South American species feature fruit that is erect, spreading, or reflexed, but never appressed to the rachis. These taxa are distributed throughout various (mainly low- to mid-elevation) portions of Argentina and Chile, with wide-ranging *D. appendiculata* present in Uruguay and southernmost Bolivia as well as Argentina.

As alluded to above, it is difficult to identify many North and South American *Descurainia* specimens with confidence using current species concepts, and much confusion exists regarding some taxonomic boundaries. According to Brassicaceae expert Ihsan Al-Shehbaz (pers. omm..), *Descurainia* is the most taxonomically complicated genus in a family which is itself noted for its taxonomic complexity. Several factors

contribute to problems with the taxonomy of *Descurainia*. First, as a result of inadequate sampling, normal intra- and inter-populational variation is poorly understood. For many named taxa, material has not been collected from enough populations, and, of those populations which have been sampled, the only representative is frequently a single exemplar on an isolated herbarium sheet. A second confounding element is the nature of the characters used to delimit taxa. Many diagnostic characters used in *Descurainia*, such as degree of pubescence or flower color hue, are highly subjective. Others, including silique length and orientation of pedicels and siliques, are heavily dependent upon the stage of maturity and often overlap in diagnostic keys. Because inheritance of characters and the molecular basis of morphological variation is still not fully understood in the Brassicaceae (Bowman, 2006), it is also uncertain to what extent diagnostic characters such as glandulosity, pubescence, leaf morphology and silique shape are subject to convergence. Finally, the extent of hybridization in the genus is unknown, but it has probably been a more significant process than previously recognized. Gene flow between populations has undoubtedly been facilitated by the ready dispersability of the small mucilaginous seeds, range contractions and expansions during previous glacial cycles, and the effects of human activity and disturbance over past centuries.

Chromosome numbers (x = 7) have been reported for many taxa (Table 1.1). Species of the Canary Islands are diploid ($2n$ = 14), while the widely-distributed Eurasian *D. sophia* is generally tetraploid ($2n$ = 28). North American species surveyed are either diploid, tetraploid, or hexaploid ($2n$ = 14, 28, 42), with different ploidy levels reported between some subspecies and even between populations. This variation in ploidy level, coupled with the morphological variation found in many wide-ranging and widely-overlapping species, provides support for the idea that hybridization may be a factor in the evolution of North American *Descurainia*. In contrast, only three South American

species have been examined and all three are diploid. This sampling is insufficient, however, to develop a comprehensive picture of ploidy levels in South American *Descurainia*.

In his treatments of the Brassicaceae, Schulz (1924, 1936) included *Descurainia* as the largest genus in Sisymbrieae subtribe Descurainiinae, comprising *Descurainia*, *Hugueninia*, *Redowskia* Cham. & Schltdl., *Robeschia* O. E. Schulz, *Sophiopsis* O. E. Schulz, *Smelowskia* C. A. Mey, and *Trichotolinum* O. E. Schulz. Molecular evidence (Koch & al., 2001, 2003a; Warwick & al., 2002; Beilstein & al., 2006) has clearly established that the Sisymbrieae, like many other tribes in the Brassicaceae, is not a natural grouping, and several recent studies have uncovered different relationships with *Descurainia* from those suggested by Schulz and indicate that the Descurainiinae may not be monophyletic. In an ITS- and *trnL*-based investigation of *Smelowskia* and related genera, Warwick & al. (2004b) found that *Redowskia* and *Sophiopsis*, as well as several other small genera, are more appropriately considered as part of *Smelowskia* than as distinct genera. They determined that *Descurainia*, although related, was distinct from *Smelowskia*, but they only included three *Descurainia* species (Eurasian *D. sophia* and North American *D. californica* and *D. pinnata*) in their analysis. Unpublished molecular data (cited in Koch & al., 2003a) indicate that *Hugueninia* is not distinct from *Descurainia* and it has been placed in synonymy with *Descurainia* (Appel & Al-Shehbaz, 2003). Two genera not previously included in the Descurainiinae, *Ianhedgea* Al-Shehbaz & O'Kane and *Hornungia* Rchb., have been found to be closely allied to *D. sophia* (the only *Descurainia* sampled in the *ndhF* study of Beilstein & al., [2006]). Three species of North American *Tropidocarpum* Hook. were also found to be distinct but related to *Descurainia* and *Hugueninia* (R. A. Price, unpublished data cited in Al-Shehbaz, 2003). Ma & Zhao (1979) placed the Chinese genus *Yinshania* Y. C. Ma & Y. Z. Zhao in the

Descurainiinae, but it has been determined, however, not to be closely related (Koch & Al-Shehbaz, 2000; Bailey & al., 2006). Bailey & al. (2006) recently published the results of a broad survey of 146 genera and 461 species in the Brassicaceae based on ITS sequence data. They found that *Descurainia*, *Ianhedgea*, and more distantly *Hornungia* formed a well-supported lineage whose relationship to *Smelowskia* and related genera was unresolved. Their sampling included 2 – 4 accessions of only four *Descurainia* species, namely *D. sophia* and North American *D. californica*, *D. incana*, and *D. pinnata*. On the basis of those studies (as well as results reported in this dissertation), Al-Shehbaz & al. (2006) have tentatively proposed new tribal classifications for the Brassicaceae. Among them is the tribe Descurainieae Al-Shehbaz, Beilstein & E. A. Kellogg which comprises *Descurainia* (including *Hugueninia*), *Hornungia*, *Ianhedgea*, *Robeschia*, *Trichotolinum*, and *Tropidocarpum*. Most of the other members of the Descurainiinae have been incorporated into another new tribe – Smelowskieae Al-Shehbaz, Beilstein & E. A. Kellogg.

The goals of the research presented in this chapter were two-fold. The first objective was to establish generic boundaries and confirm the taxonomic position of *Descurainia* by a more thorough sampling of the genus with respect to the Descurainieae, Descurainiinae and other related taxa. To accomplish this goal, DNA sequences from the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA repeat (ITS1, 5.8S rRNA, ITS2; Kim & Jansen, 1994) and from the chloroplast *trnL* intron (Taberlet & al., 1991) were utilized. Because these markers have been widely used within the Brassicaceae for investigating generic level relationships, published sequences were available for incorporation along with data generated by this study. The second major focus of the research presented in this chapter was the construction of a molecular-based phylogeny for *Descurainia* to assess infrageneric relationships and, given sufficient

resolution, investigate New World biogeography, especially the timing, number, and direction of New World dispersal events. To accomplish this goal, the genus was widely sampled and seven non-coding chloroplast DNA regions and the nuclear ITS region were employed as phylogenetic markers.

MATERIALS AND METHODS

Sampling.—To assess the monophyly of *Descurainia*, six representative *Descurainia* species, seven close relatives (i.e., *Hornungia alpina*, *H. petraea*, *H. procumbens*, *Hugueninia tanacetifolia*, *Ianhedgea minutiflora*, *Robeschia schimperi*, and *Tropidocarpum gracile*), and a selection of representatives from other tribes (Al-Shehbaz & al., 2006; Bailey & al., 2006) were included in the analysis (Table 2.1). The accessions of *Descurainia* were selected to represent major lineages identified from preliminary phylogenetic analysis of a broader data set. *Arabis alpina*, *Brassica rapa*, and *Sisymbrium altissimum* were included as outgroups.

For the broader study of relationships within *Descurainia*, an attempt was made to include multiple accessions of each named species and subspecies. Due to collecting limitations and unavailability of satisfactory herbarium material, DNA was not obtained for every taxon. The sampling in this study, however, is a strong representation of morphological and geographical diversity in *Descurainia*. DNA was isolated from 135 *Descurainia* samples, representing all ten Old World species (including *Hugueninia*) (25 accessions), ten of 13 named North American species (71 accessions), and 12 of 21 named South American species (39 accessions), and from putative congeners *Ianhedgea minutiflora* (1 accession) and *Robeschia schimperi* (2 accessions). On the basis of preliminary analyses of the data in this study and published studies (Warwick & al.,

2004b; Al-Shehbaz & al., 2006; Bailey & al., 2006) *Arabidopsis thaliana*, *Sisymbrium altissimum* and *Smelowskia americana* were included as outgroups. Sources of plant material used in this study and voucher information are in Table 2.2.

Leaf material was field-collected and dried over silica, or harvested from cultivated plants grown from seed in the greenhouse at the University of Texas at Austin. 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 (with permission) and the DNA isolated following the protocol in Loockerman & Jansen (1996). DNA from *Ianhedgea minutiflora*, one accession of *Robeschia schimperi*, and one accession of *D. sophia* was provided by Mark Beilstein of the University of Missouri – St. Louis.

Because the entire genus was last monographed in 1924, the species concepts of a variety of authors were followed when classifying and identifying plant material. Classification of material from the Canary Islands is according to Bramwell (1977) with identifications confirmed by A. Santos-Guerra, an expert on the Canarian flora. North American identifications are mainly based on Rollins (1993a) except for *D. obtusa* which follows the ideas of Detling (1939). For South American taxa, sources consulted included Romanczuk (1984a; Patagonia), Boelcke & Martínez-Laborde (1994; northwestern Argentina), Schulz (1924; South America), and a tentative draft key for South American species designed by Al-Shehbaz (1999, pers. omm..). Due to the widely-noted and endless intergrading variation in species such as *D. pinnata*, determinations can be notoriously difficult, especially at the subspecies level. Consequently, identifications of some *D. pinnata* specimens are necessarily approximate but are shown because to do otherwise would obscure the general morphological picture.

PCR amplification and DNA sequencing. — To ascertain the phylogenetic position of *Descurainia*, the chloroplast *trnL*^{UAA} intron (Taberlet & al., 1991) and the nuclear ITS region were amplified for six *Descurainia* taxa and three close relatives. Published and unpublished ITS and *trnL* sequences of other closely-related taxa and a selection of representatives from other tribes (Al-Shehbaz & al., 2006; Bailey & al., 2006) were also incorporated into the data set (Table 2.1). Although no *trnL* sequence was available for *Tropidocarpum gracile*, the ITS sequence was provided by Robert A. Price and included in the data set.

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 the broader 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), *trnC*^{GCA}-*ycf6* (Shaw & al., 2005), *ycf6-psbM* (Shaw & al., 2005), and *ndhF-rpl32* intergenic spacers. Primers for *ndhF-rpl32* (*ndhF*-F: 5'-ACTGGAAGTGGAATGAAAGG-3'; *rpl32*-R: 5'-GCTTTCAACGATGTCCAATA-3'; internal sequencing primers *ndhF*-iF: 5'-CGTGTAAATCTTTGTTCTAT-3'; *rpl32*-iR: 5'-ATAGAACAAAGATTTACACG-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 25mM $MgCl_2$, 4 µL of 0.25µM dNTPS, 0.5 µL of a 100µM solution of each primer, 0.5 µL of *Taq* polymerase and 1 µL of unquantified DNA template. For some difficult-to-amplify samples extracted from herbarium material, PCR amplifications were accomplished in 25 µL volumes containing 0.25 µL of a 100µM solution of each primer, 0.25 µL of *Taq* polymerase, 0.5 – 1 µL of

unquantified DNA template, and 12.5 µL of FailSafe PCR 2x Premix A, D, E or H (Epicentre). 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.

Direct sequencing of the ITS region for 10 accessions of *D. antarctica*, *D. incisa*, and *D. pinnata* generated traces exhibiting double peaks at multiple nucleotide positions. These samples were cloned with a TOPO TA kit (Invitrogen with vector pCR 2.1-TOPO) using 1/3 the recommended reaction volumes. For each cloning reaction, 5 – 10 positively transformed colonies were amplified and products sequenced. PCR amplification of clones containing ITS inserts were carried out as follows: 94˚C for 10 min, followed by addition of *Taq* polymerase while the reactions were held at 72˚C for 5 – 10 min, and then 37 cycles of 94˚C for 1 min, 48˚C for 1 min, and 72˚C for 1 min, followed by a final extension step of 72˚C for 12 min. For each cloned sample, sequencing of transformed colonies generated two distinct sequence types, i.e., clones for a given type either formed a monophyletic group or, if not forming a monophyletic group of clones, were identical or differed by a single autapomorphic nucleotide substitution from other clones of that type. Inclusion of the entire set of cloned sequences in the

complete data set would have increased analysis run times with slight or no information gain; consequently, for each sample, two representative sequences (one for each putative parental type) were selected for inclusion. There were also five sequences (from three accessions) which upon visual inspection were clearly chimeric and probably an artifact of PCR-mediated recombination (Bradley & Hillis, 1997; Cronn & al., 2002) or the result of incomplete concerted evolution following *in vivo* recombination (Álvarez & Wendel, 2003; Buckler & al., 1997). These were excluded along with the redundant cloned sequences. During final phylogenetic analyses (after the conclusion of lab work) it was discovered that the only clone corresponding to one parental type of the accession C19 (*D. pinnata* spp. *intermedia* type 1) was also chimeric; although it would have been possible to reconstruct the "missing" type from an examination of the original heterogeneous sequence trace, this chimeric sequence was dropped from the data set without substitution because the presence of the chimeric sequence and absence of the easily-inferred missing sequence had no topological effect on the outcome of phylogenetic analyses.

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 following Simmons and Ochoterena (2000) and appended to the alignment.

Parsimony analyses were performed on each data set with PAUP* 4.0b10 (Swofford, 2002). For the ITS, *trnL*, and combined ITS-*trnL* Brassicaceae data sets, 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. For the larger ITS, chloroplast, and combined ITS-chloroplast *Descurainia* data sets, 20 independent parsimony ratchet (Nixon, 1999) runs of 200 iterations each were carried out in PAUP* using batch files generated by PAUPRat (Sikes & Lewis, 2001). Support for internal nodes was assessed using bootstrap analysis (Felsenstein, 1985). For the Brassicaceae data sets, 500 bootstrap replicates were conducted with 100 random additions per replicate, holding 10 trees at each step; for the *Descurainia* data sets, this entailed 100 bootstrap replicates of 10 random additions each, holding one tree at each step and saving no more than 500 trees of length greater than or equal to 200 steps in each replicate. Bootstrap support was categorized as strong (> 85%), moderate (70 – 85%), weak (50 – 69%), or unsupported (< 50%).

Bayesian analyses were carried out separately on individual and combined data sets using MrBayes 3.1 (Ronquist & Huelsenbeck, 2003). Best-fit models of evolution for each data set were selected in MrModeltest 2.2 (Nylander, 2004) based on the Akaike information criterion (Akaike, 1974; Posada & Buckley, 2004). For those data sets containing both nucleotide and coded indels, separate evolutionary models were applied to the data partitions with all parameters unlinked except for topology and branch length; the model(s) selected by MrModeltest were applied to each 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 data set. Each analysis was run for 2 – 6 million 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. Branches with

posterior probabilities ≥ 95% were considered to be strongly-supported, with posterior probabilities < 95% indicating weak support.

To explore alternative hypotheses regarding some New World relationships, a 95% credible set of trees (Huelsenbeck & Rannala 2004) was constructed as follows from the phylogenies recovered by Bayesian analysis of a combined ITS-chloroplast data set. The "sumt" command in MrBayes was used to identify the 95% credible set of trees and generate a .trprobs file containing trees sorted by posterior probability. Trees corresponding to the 95% credible set were then imported from the .trprobs file into PAUP*. The "filter constraints" command in PAUP* was subsequently used to search this 95% credible set for topologies consistent with alternative hypotheses of interest.

Maximum likelihood (ML) analyses were conducted on selected data sets, excluding coded gap characters, using PAUP* or GARLI 0.95 (Zwickl, 2006; http://www.bio.utexas.edu/faculty/antisense/garli/Garli.html). GARLI is a genetic-algorithm-based program which is able to perform rapid ML searches on large data sets and published studies making use of it are beginning to appear in the literature (Brady & al., 2006; Schultz & al., 2006). For ML analyses using GARLI, the tree with the best likelihood score was chosen from the results of 10 – 15 independent runs. ML bootstrapping was conducted in GARLI using 300 replicates. Substitution models for both PAUP* and GARLI ML searches were chosen based on the Akaike information criterion calculated in ModelTest 3.7 (Posada & Crandall, 1998). Shimodaira-Hasegawa (SH) tests (Shimodaira & Hasegawa, 1999; Goldman & al., 2000) of alternative hypotheses were conducted on constrained and unconstrained ML trees as implemented in PAUP* with 1000 RELL bootstrap replicates (one-tailed test).

The incongruence length difference (ILD) test as implemented in PAUP* (partition homogeneity test of Farris & al., 1994) was used to assess global topological

incongruence. Each test consisted of 100 replicates, with 10 random additions per replicate, holding 20 trees per step. For ITS vs. combined chloroplast data sets, if the ILD test indicated significant data heterogeneity, conflicting clades were identified by means of visual inspection and degree of partitioned branch (Bremer) support (Baker & DeSalle, 1997; Baker & al., 1998). Taxa which appeared to contribute to the observed incongruence were removed until the ILD test indicated no significant conflict. Partitioned branch support indices were calculated using the program TreeRot.v2 (Sorenson, 1999).

To explore possible New World dispersal patterns in *Descurainia*, continent of distribution (i.e., North America or South America) was mapped onto topologies representing phylogenetic relationships between major New World lineages recovered from phylogenetic analysis of the combined ITS-chloroplast data set. This was accomplished using MacClade 4.0 (Maddison & Maddison, 2000) with Fitch parsimony optimization (unordered characters and unweighted character state changes).

Estimates of divergence times. – Absolute divergence times were calculated from the ITS and chloroplast data. To eliminate zero- or near zero-length terminal branches (Sanderson, 2004) and expedite computation, redundant taxa were removed by pruning identical or nearly-identical sequences from the full data sets. Sequence data for *Rorippa indica* were added to the ITS data set for calibration purposes. To eliminate arbitrary zero-length branches at the root of the tree in PAUP*, *Aethionema grandiflorum* (GenBank accession DQ452067), representing a basal genus in the Brassicaceae (Zunk & al., 1996, 1999; Galloway & al., 1998; Hall & al., 2002; Koch, 2003a; Beilstein & al., 2006), was also incorporated as an extra outgroup. The best ML tree was generated from the resulting 27-taxon ITS data set under the SYM+I+Γ model of evolution using GARLI

0.95. Rate heterogeneity was assessed using a likelihood ratio test (Felsenstein, 1981; Huelsenbeck & Rannala, 1997) in PAUP* to compare ML trees generated with and without enforcing a molecular clock. For the ITS data, a molecular clock could be rejected at the p = 0.01 level but not at p = 0.05. For this data set, divergence times were consequently estimated under both the assumption of a molecular clock and using a relaxed molecular clock model.

Two calibration points were employed for the ITS data set. Because fossil pollen assignable to *Rorippa* has been identified from Pliocene deposits (2.5-5.0 million years ago [mya]; Mai, 1995), the node joining *Rorippa* to the closely-related genus *Cardamine* (Franzke & al., 1998) was constrained to a minimum age of 5 million years. Based on mitochondrial *nad4* sequence data and the estimated divergence time between corn and wheat (Yang & al., 1999), nuclear *Chs* and chloroplast *matK* sequence data (Koch & al., 2001), nuclear *Chs* and *Adh* sequence data and *Rorippa* fossils (Koch & al., 2000), and comparative chromosome painting (Lysak & al., 2006), the divergence of *Arabidopsis* from *Brassica* has been dated to approximately 20 mya; the age of that node was accordingly fixed at 20 mya in the analyses.

Based on the above calibration points, the program r8s 1.71 (Sanderson, 2004) was used to estimate divergence times on the reduced ML tree. The distant outgroup *Aethionema* was pruned from the tree, and divergence times were first calculated in r8s under the assumption of a molecular clock using the Langley-Fitch (LF) method (Langley & Fitch, 1974) with the truncated Newton (TN) algorithm. Divergence times were also estimated using penalized likelihood rate-smoothing (Sanderson, 2002). The cross-validation procedure in r8s was used to determine an optimal smoothing level of 1000, and divergence times of selected nodes were calculated with this smoothing factor using

the PL method with the TN algorithm. All solutions were evaluated for correctness using the checkGradient option.

Confidence intervals on divergence dates were generated using a parametric bootstrapping approach as recommended by Sanderson (2004). SG Runner, a graphical user interface to Seq-Gen 1.3.2 (Rambaut & Grassly, 1997), was used to generate 100 bootstrapped data sets based on the reduced ML tree, its branch lengths, and the model parameters selected by ModelTest for the original data set. ML trees with different branch lengths but the same topology as the original tree were then generated in PAUP* from these data sets. These trees were imported into r8s, divergence times were estimated for nodes of interest, and the "profile" command was used to summarize rate and time information for each node across the collection of trees.

It was of interest to estimate divergence times on the less optimal ML tree obtained from constraining New World *Descurainia* to monophyly, because a Shimodaira-Hasegawa test indicated that topology could not be rejected. When the constrained ML tree was generated from the reduced ITS data set, however, several zero-length internal branches joining some New World clades resulted. Because r8s collapses zero-length internal branches when calculating divergence times, some relevant dates (e.g., the age of the most recent common ancestor of New World taxa) could not be generated. Divergence times were therefore estimated using the chloroplast data where monophyly of New World taxa is strongly supported. A date of 14.7 mya, estimated from the ITS data for the last common ancestor of *Arabidopsis* and *Descurainia*, was used as a fixed calibration point. Using a calibration date obtained from another molecular analysis is not the best approach (Magallón, 2004), but it was the only option available because no sequence data for these non-coding chloroplast markers exist for *Rorippa* or *Brassica*. A ML tree was generated using GARLI 0.95 under a GTR+Γ model from a reduced

chloroplast data set of 25 taxa. A likelihood ratio test to assess rate constancy strongly rejected a molecular clock ($p << 0.001$). Divergence times were calculated in r8s using the PL method with the TN algorithm and a smoothing factor of 1000, and confidence intervals were estimated as described for the ITS data.

RESULTS

Analysis of ITS and *trnL* data to assess the monophyly of *Descurainia*. – The ITS data set for 36 taxa was 647 characters in length, including 644 nucleotide positions and 3 coded indels, with 5.9% gaps and 0.5% missing characters. 304 characters (50.0%) were variable and 212 (32.8%) were parsimony informative. Parsimony analysis of the ITS data set recovered 553 trees of 953 steps (CI [excluding informative characters] = 0.44; RI = 0.60) (Fig. 2.1). Bayesian analysis (SYM+I+Γ, 4 million generations) resulted in a strict consensus tree (Fig. 2.1) which was essentially identical in topology to that generated from the parsimony analysis. The strict consensus tree from parsimony and Bayesian inference is congruent with proposed tribal classifications (Al-Shehbaz & al., 2006) based in part on the *ndhF* Brassicaceae study of Beilstein & al. (2006). Taxa recognized by Al-Shehbaz & al. (2006) as constituting the tribe Descurainieae (except *Trichotolinum*, which was not sampled in this study) are well-supported as a distinct group (bootstrap value [BV] = 91%, posterior probability [PP] = 100%). The Descurainieae are partitioned into two lineages: 1) *Hornungia* and *Tropidocarpum* (BV = 70%, PP = 96%) and 2) *Descurainia*, *Hugueninia*, *Ianhedgea*, and *Robeschia* (BV = 86%, PP = 100%). Within the latter clade, *Ianhedgea* is sister to a polytomy (BV = 71%, PP = 99%) composed of *D. kochii* + *D. sophia* (BV = 84%, PP = 100%), *Robeschia*, and a strongly-supported (BV = 100%, PP = 100%) New World-Canary Island-*Hugueninia*

clade. The Bayesian tree places *Robeschia* at the base of this polytomy, but support is weak (PP = 66%).

The aligned *trnL* intron data set was 554 base pairs in length, including gaps (15.1%) and missing (0.84%) data. In addition, eight autapomorphic indels were binary-coded and appended to the data set. Of the 562 characters in the resulting data set, 134 (23.8%) were variable and 60 (10.7%) were parsimony-informative. The same taxa were included in the *trnL* data set as in the ITS data set, excluding *Tropidocarpum gracile*, for which a *trnL* intron sequence was not available. Parsimony analysis of the *trnL* data set for 35 taxa generated 8734 most parsimonious trees (length = 185 steps, CI = 0.717, RI = 0.843) (Fig. 2.2). Compared to the ITS tree, the strict consensus tree from parsimony and Bayesian (GTR+I, 3 million generations) analyses is not very well-resolved, although it is generally congruent. Like the ITS results, a strongly-supported (BV = 97%, PP = 100%) *Descurainia* New World-Canary Island-*Hugueninia* clade is present, but its relationship to other members of the Descurainieae, and most of the other included taxa, is unresolved. In contrast to the ITS tree, where *D. sophia* is sister to *D. kochii*, the *trnL* tree joins *D. sophia* with *Ianhedgea*. Support for this relationship, however, is weak (BV = 71%, PP = 90%). As in the ITS results, *Hornungia alpina* is strongly supported (BV and PP = 100%) as sister to *H. petraea*, but additional relationships within Descurainieae and between Descurainieae and other tribes is largely absent. The Bayesian phylogeny weakly supports (PP = 89%) a polytomy comprising various members of the Descurainieae and a strongly-supported (BV = 94%, PP = 99%) Smelowskieae. This latter tribe contains taxa such as *Smelowskia*, *Redowskia*, and *Sophiopsis* which were included by Schulz in his subtribe Descurainiinae.

The ITS and *trnL* data were combined into a single data set comprising 1198 nucleotide bases (1.9% gaps and 9.9% missing) and 11 coded indels. The high percentage

of missing data was due to the absence of the *trnL* sequence for *Tropidocarpum gracile*. Because the exclusion of *T. gracile* did not appreciably affect the outcome of preliminary phylogenetic analyses, this taxon was retained in the combined data set. Of the 1209 characters in the combined data set, 438 (36.2%) were variable and 272 (22.5%) were parsimony informative. Visual comparison of trees from the separate data sets, as well as results from the larger-scale study to be described later, suggested incongruence due to the varying placement of *D. sophia*. The ILD test (p = 0.18) indicated that the two partitions were not heterogeneous, however, and parsimony analysis of the combined data set with and without *D. sophia* gave essentially identical results. Parsimony analysis of the combined data set (with *D. sophia* included) generated 41 most parsimonious trees of 1149 steps (CI = 0.470, RI = 0.627) (Fig. 2.3). The Descurainieae are strongly supported (BV = 96%) as a distinct group comprised of *Hornungia* and *Tropidocarpum* (BV = 77%) as a sister lineage to *Descurainia*, *Hugueninia*, *Robeschia* and *Ianhedgea* (BV = 89%). Within the latter clade, New World and Canary Island *Descurainia*, along with *Hugueninia*, form a strongly-supported clade (BV = 100%), but resolved relationships among *D. sophia*, *D. kochii*, *R. schimperi*, and *I. minutiflora* are largely absent (BV ranging from < 50% to 55%). The Descurainieae are sister to the Smelowskieae, but the support is weak (BV = 53%) and conclusions regarding the relationship between the two tribes is not warranted because of very limited taxon sampling of other tribes.

Bayesian analysis (SYM+I+Γ for the ITS partition, GTR+I for *trnL,* and BINARY for *trnL* indels, 6 million generations) of the combined data set generated a tree of similar topology to that of parsimony, but the presence or absence of *D. sophia* affected which taxon diverges first within the *Descurainia*/*Ianhedgea*/*Robeschia* lineage. When *D. sophia* is included, *Robeschia* is placed at the base of the clade (PP = 90%) and

D. kochii is strongly supported (PP = 99%) as sister to *D. sophia* + *Ianhedgea* (PP = 100%). When *D. sophia* is excluded from the analysis, *Ianhedgea* is placed at the base of the clade (PP = 99%) with *Robeschia* next most basal (PP = 99%) followed by *D. kochii* (PP = 88%). Regardless of whether *D. sophia* is included or not, Bayesian analyses join the Descurainieae and Smelowskieae as sister tribes (PP = 96 – 97%) in this data set.

Analysis of ITS data to assess relationships within *Descurainia*. – The ITS data set for 150 accessions and representative cloned samples was easily alignable, comprising 627 nucleotide positions including gaps (2.5%) and missing (0.1%) characters. 228 characters (36.4%) were variable and 127 (20.3%) were parsimony informative (Table 2.3). Excluding outgroups, uncorrected pairwise sequence divergence ("p" distance in PAUP*) ranged from 0 – 10.8%, with an average of 2.40% (Table 2.4).

Parsimony analysis of the ITS data set using the parsimony ratchet generated 4020 most parsimonious trees of 388 steps (CI [excluding uninformative characters] = 0.63; RI = 0.93) (Figs. 2.4, 2.5). Bayesian analysis of the ITS data set (SYM+Γ, 3 million generations) produced a consensus tree (Fig. 2.5) which recovered the same major clades as parsimony. In contrast to the analysis to assess the monophyly of *Descurainia*, these results place *Robeschia* as sister to *Descurainia* with moderate to strong support (BV = 73%, PP = 100%). This support for a sister relationship between *Robeschia* and *Descurainia* appears to be sensitive to the presence of *Hornungia* and *Tropidocarpum*; when those taxa were added to the analysis, bootstrap support for the branch uniting *Robeschia* with *Descurainia* dropped from 73% to 55%. Within *Descurainia*, *D. sophia* and *D. kochii* form a clade (BV = 91%, PP = 100%) which is sister to the remainder of the genus. This well-supported clade (BV = 100%, PP = 100%) comprises a polytomy with four distinct lineages. Lineage "A" (BV = 86%, PP = 100%) is exclusively North

American and includes five species (*D. californica*, *D. incana*, *D. incisa*, *D. sophioides*, and *D. streptocarpa*) with definite morphological affinities to each other. The taxon *D. obtusa* ssp. *brevisiliqua* and several accessions with morphology resembling *D. pinnata* are also found in lineage A. Lineage "B" (BV = 81 %, PP = 100%) is primarily North American, consisting of *D. paradisa*, *D. incisa* ssp. *filipes*, some accessions and clones of *D. pinnata*, and interestingly, half of the South American *D. antarctica* clones. The third main lineage, clade "C", is very strongly supported (BV = 99%, PP = 100%). It includes all South American species sampled (except two *D. antarctica* clones) and North American taxa *D. obtusa* ssp. *obtusa*, *D. obtusa* ssp. *adenophora*, *D. impatiens*, *D. virletii*, some accessions and clones of *D. pinnata*, and an undetermined specimen (C44) similar to *D. streptocarpa* and possibly of hybrid origin. Of the four lineages, C had the greatest sequence divergences, ranging up to 2.46%. There is little resolution within lineage C, but a few weakly supported clades consistent with geography and morphology are evident. One of these (clade C-I) encompasses *D. pinnata* and the Mexican endemic *D. virletii* (BV = 57%, PP = 99%); another (C-II) comprises all South American taxa characterized by fruit spreading away from the rachis (BV = 61%, PP = 93%). Every accession of *D. pinnata* exhibiting sequence polymorphism yielded clones in both lineage B and lineage C. The fourth lineage, "D", is weakly supported (BV = 53%, PP = 93%), but includes all the species from the Canary Islands, and is coupled, weakly to strongly (BV = 55%, PP = 99%), with *Hugueninia tanacetifolia* ssp. *suffruticosa*. Surprisingly, the clade comprising D and *Hugueninia* is joined in a trichotomy with not only another accession of *Hugueninia* (ssp. *tanacetifolia*) but also with New World clade B. Bootstrap support for the branch leading to this trichotomy is very weak, although it is strongly-supported in the Bayesian topology (BV = 57%, PP = 100%). To further evaluate support for this relationship, an SH test was conducted comparing this topology to one where

New World *Descurainia* species (clades A, B and C) were constrained to monophyly. The outcome of the SH test (p = 0.12) indicated that a tree with New World taxa monophyletic could not be rejected as being significantly less likely than the tree which joins lineage D and *Hugueninia* with New World lineage B.

Chloroplast data. – To assess incongruence, the ILD test was applied to all possible pairings of the seven chloroplast data sets. All data combinations were supported as homogeneous at the p = 0.01 level, but were rejected at the p = 0.05 level for the combinations *trnD-trnE* vs. *rps16* and *psbZ-trnfM* vs. *ndhF-rpl32* (p = 0.04 and 0.02, respectively). Since the topologies generated from the individual chloroplast partitions (not shown) do not appear to seriously conflict, and the chloroplast genome is inherited uniparentally as a single unit and does not usually undergo recombination, this borderline significant heterogeneity is assumed to be a type I error (i.e., inference of incongruence where none exists) to which the ILD test has been shown to be highly susceptible (Dolphin & al., 2000; Barker & Lutzoni, 2002; Darlu & Lecointre, 2002; Dowton & Austin, 2002). The data from the seven non-chloroplast coding regions were consequently combined into a single data set. Sequence characteristics for the individual chloroplast regions and combined data set are found in Table 2.3. The combined chloroplast data set for 135 accessions contained 5351 nucleotide positions including gaps (12.8%) and missing (0.8%) characters. (The missing data includes five sequences that are absent due to unsuccessful PCR amplification: the *ndhF-rpl32* region of *D. antarctica* D52, *D. incisa* ssp. *viscosa* D21, and *D. incisa ssp. filipes* B195 and the *rps16* intron of *D. antarctica* D52 and *D. pinnata* ssp. *brachycarpa* F11). Thirty-eight indels, ranging in length from 4 to 278 base pairs, and one 5-bp inversion, were binary-coded and appended to the data set. The resulting data set comprised 5390 characters, of which

1107 (20.5%) were variable and 581 (10.8%) were parsimony informative. When outgroups were excluded, uncorrected pairwise ("p") sequence differences ranged from 0 to 4.42%, with an average divergence of 1.04%.

Parsimony analysis of the combined chloroplast data set for 135 accessions yielded 3419 most parsimonious trees from the 4020 trees produced using the parsimony ratchet (length = 1538, CI = 0.713, RI = 0.918) (Figs. 2.6, 2.7). The chloroplast data place *Robeschia schimperi* sister to *Descurainia* with moderate support (BV = 81%). In contrast to the ITS tree where *D. sophia* and *D. kochii* form a separate clade sister to the remainder of the genus, the chloroplast tree strongly supports *D. kochii* as sister to the rest of the genus (BV = 96%). Within the rest of the genus, *D. sophia* is sister to the remaining taxa (BV = 100%). In respect to the four lineages A – D described earlier, the chloroplast phylogeny is generally consistent with the results obtained from the ITS data set. The Canary Island taxa (lineage D) are strongly supported as monophyletic (BV = 100%) and sister (BV = 100%) to European *Hugueninia tanacetifolia* ssp. *suffruticosa* C6. This clade in turn is sister to the other subspecies of *Hugueninia tanacetifolia*, ssp. *tanacetifolia* B111 (BV = 100%). The Canarian/European lineage is sister with strong support (BV = 100%) to New World *Descurainia*. Within the New World clade, lineages A (*D. incana* + *D. incisa* + *D. obtusa* ssp. *brevisiliqua*, etc.) and B (*D. pinnata* + *D. paradisa* + *D. antarctica*) are still present (BV = 100% and 82%, respectively) and form a strongly-supported clade (BV = 91%) along with one accession of *D. incisa* which is found in clade A in the ITS phylogeny. The relationship of the remainder of the New World taxa (which are primarily found in clade C in the ITS tree) to the A+B clade is unresolved, but provides further support for clades C-I (*D. pinnata* + *D. virletii*, BV = 99%) and C-II (South American spreading fruit, BV = 100%). Clade C-II is grouped with *D. sophioides*, *D. obtusa* ssp. *obtusa*, *D. obtusa* ssp. *adenophora*, and one sample of *D.*

californica, but support for this clade is extremely weak (BV = 55%). In addition, all South American taxa with fruit appressed to the rachis are strongly supported in two lineages (henceforth designated as C-III and C-IV, BV = 100% in both).

The 50% majority-rule consensus tree generated from Bayesian analysis (GTR+I+Γ, 4 million generations) is nearly identical to the parsimony tree, with support values for most of the branches recovered under parsimony generally above 98%. The only significant topological difference between the trees recovered from the two methods is that all unresolved "clade C" taxa (i.e., *D. californica*, *D. sophioides*, *D. obtusa* ssp. *obtusa*, *D. obtusa* ssp. *adenophora*, *D. pinnata* B12A, C47, D23, and *D. incisa* D21, D57) are placed by Bayesian inference in a strongly-supported (PP = 100%) clade that also includes C-II (PP = 100%).

Maximum sequence divergence within lineages A (0.44%) and B (0.43%) are similar, and about half of that found within D (1.22%). Sequence divergences within clade C range from 0 – 1.15% with C-I and C-III having the greatest sequence diversity, up to 0.49% and 0.45%, respectively. Maximum sequence diversity within C-II (0.16%) and C-IV (0.086%) is very low.

Sequencing of ITS clones revealed that 10 accessions possessed both clade B and clade C (either C-I or C-II) types. In the chloroplast phylogeny, all but one of these accessions are placed in clade B. The exception, *D. pinnata* ssp. *intermedia* C19, is found in clade C-I in the chloroplast tree.

While there are many similarities between the chloroplast and ITS trees, there are a number of obvious incongruences, especially within North American taxa (summarized as part of Table 2.5). For example, *D. californica*, *D. sophioides*, two *D. incisa* accessions (D57 and D21), and *D. pinnata* C12 are found in clade A in the ITS tree but in clade C in the chloroplast tree. Conversely, *D. impatiens* and *D. obtusa* ssp. *adenophora*

are located in clade C in the ITS tree but in clade A in the chloroplast tree. *Descurainia paradisa* ssp. *nevadensis* C48 is found in clade B (ITS) or in clade A (chloroplast), *D. pinnata* ssp. *nelsonii* accessions C47 and D23 are in either clade B (ITS) or clade C (chloroplast), and *D. incana* D25 is found in clade A in the ITS phylogeny and its position is unresolved with respect to clades A and B in the chloroplast tree. In addition, there is also considerable incongruence *within* clade A between the two trees. Outside of North America, the only major incongruence is that of *D. sophia*; in the ITS phylogeny it is sister to *D. kochii*, whereas in the chloroplast phylogeny it is sister to *Hugueninia* and species of the New World and Canary Islands.

Tests for incongruence. – Before combining ITS and chloroplast data, incongruent and redundant taxa were identified and removed as described under Materials and Methods (*cf.* Table 2.5). This process was straightforward with two exceptions. First, the considerable incongruence within lineage A could be resolved by removal of varying sets of taxa, so that the choice of accessions remaining in that clade in the final combined data set represents only one alternative among several. Fortunately, which set of taxa was chosen did not affect the relationship of major lineages in the combined topology. Secondly, the ILD test detected significant incongruence when clade D was included ($p = 0.05$ with compared to $p = 0.17$ without). Clade D (the Canary Island/*Hugueninia* clade) is strongly supported as monophyletic and sister to all New World taxa. While the topology of the most parsimonious ITS tree conflicts with such a relationship, the SH test discussed previously supports it as an equally likely alternative. With respect to clade D and New World species, the phylogeny based on the combined data set seems reasonable based on morphology and geography. Based on these observations, and noting that phylogenetic accuracy does not always depend on

congruent data sets (Hipp & al., 2004), clade D was accordingly retained in the combined ITS-chloroplast data set.

Analysis of combined data. – The combined ITS and chloroplast data set for 74 accessions included 5894 nucleotide positions, with 0.32% missing characters and 10.9% gaps. Twenty-three indels were coded as binary characters and appended to the combined data set. The resulting data set comprised 5917 characters, of which 1159 (19.6%) were variable and 441 (7.5%) were parsimony informative. The ITS partition contributed 20.6% (91) and the chloroplast partition 79.4% (350) of the parsimony informative characters.

Parsimony analysis of the combined data set was carried out with *Arabidopis thaliana*, *Sisymbrium altissimum*, and *Smelowskia americana* as outgroups. For the Bayesian analysis, a mixed model analysis was conducted (2 million generations) with the SYM+Γ, GTR+I+Γ, and BINARY models applied to the ITS, chloroplast, and indel partitions, respectively. The parsimony ratchet recovered 4020 most parsimonious trees of 1534 steps (CI = 0.709, RI = 0.907) (Fig. 2.8). The strict consensus tree generated by the Bayesian analysis was identical to that recovered by parsimony except for one weakly supported branch described below. The phylogeny obtained for the combined ITS-chloroplast data set provides strong support for the major clades previously observed in the trees from the separate data sets. *Robeschia schimperi* is placed as sister (BV = 92%, PP = 100%) to *Descurainia*, with *D. kochii*, in the absence of *D. sophia*, sister to the rest of the genus (BV = 100%, PP = 100%). Canary Island taxa and *Hugueninia* (BV = 100%, PP = 100%) are sister (BV = 100%, PP = 100%) to the New World species. New World *Descurainia* are strongly supported (BV =100%, PP = 100%) as a monophyletic group composed of the clades A, B and C previously discussed (all with BV and PP = 100%).

Clade C is sister to a lineage (BV = 89%, PP = 100%) comprising clades A and B. Within clade C are the North and South American sub-lineages C-I (North American *D. pinnata* and *D. virletii)*, C-II (South American spreading fruit + North American *D. obtusa*), C-III (South American appressed fruit), C-IV (South American appressed fruit), and C-V (South American *D. cumingiana* var. *tenuissima*), all of which have bootstrap support values ranging from 97 – 100% and Bayesian posterior probabilities of 100%. The relationship among these lineages to one another is largely unresolved: parsimony analysis weakly (BV = 64%) joins clades C-I, C-II, and C-III in a polytomy and places C-IV and C-V in a sister relationship (BV = 58%). Bayesian analysis (PP = 94 – 95%) supports these same relationships, and weakly (PP = 87%) implies that clades C-I and C-III are most closely related. Most of the remaining sampled Bayesian trees that differ from this topology place C-II, rather than C-III, as sister to C-I.

Optimization of New World distribution on phylogenies. – An examination of the 4020 most parsimonious trees recovered from parsimony analysis of the combined ITS-chloroplast data set revealed only two topologies present with respect to relationships between major New World lineages (Figs. 2.9, 2.10). These two topologies were also present in a 95% credible set of trees constructed from the trees sampled during the Bayesian analysis. The topologies differ in the placement of North American clade C-I and South American clade-III with respect to South American C-II + North American *D. obtusa*. The first topology (Fig. 2.9), representing 35% of most parsimonious trees and 84% of the set of 95% credible Bayesian trees, places clade C-III sister to C-II + *D. obtusa*. The second topology (Fig. 2.10), representing 65% of most parsimonious trees and 10% of 95% credible Bayesian trees, groups clade C-I with C-II + *D. obtusa*.

When New World continental distribution was traced onto simplified trees representing these two topologies, five most parsimonious reconstructions were recovered for the first topology and seven reconstructions were generated for the second. All reconstructions are consistent with four separate dispersals of *Descurainia* between North and South America. Nine of these reconstructions are illustrated in Figs. 2.9 and 2.10. The remaining three reconstructions are not shown because they are inconsistent with a North American origin for one parent of *D. antarctica* strongly suggested by the molecular data.

Divergence time estimates. – Divergence times obtained from the ITS data were virtually identical regardless of which dating method (LF or PL) was employed (Table 2.6) (Fig. 2.11). Calculated divergence dates (from PL) are as follows: *Hornungia-Ianhedgea* 11.03 +/- 0.98 mya; *Robeschia-Descurainia* 6.75 +/- 0.82 mya; *D. kochii*-New World/Canary Island/*Hugueninia* 5.47 +/- 0.70 mya; *Hugueninia*-Canary Island 1.00 +/- 0.33 mya; and the last common ancestor of Canary Island taxa 0.75 +/- 0.27 mya. *Hornungia procumbens* is estimated to have diverged from its congeners 10.24 +/- 0.98 mya and *H. alpina* and *H. petraea* last shared a common ancestor 6.09 +/- 0.90 mya. Using *Rorippa* fossil data and ITS sequences, Kropf & al. (2003) estimated under the assumption of a molecular clock the same splits as occurring 6.1 – 13.2 mya and 3.4 – 7.4 mya, respectively. The dates calculated from this study thus appear to be consistent with their calculations. The rate of sequence evolution – 6.9 +/- 0.5 x 10^{-9} substitutions/site/year – is similar to rates reported for the ITS region in other annual and perennial herbs including crucifers (Richardson & al., 2001; Koch & al., 2006).

Divergence times estimated from the chloroplast data are: *Robeschia-Descurainia* 8.67 +/- 0.48 mya; *D. kochii*-New World/Canary Island/*Hugueninia* 7.27 +/- 0.44 mya;

the most recent common ancestor of New World *Descurainia* 1.93 +/- 0.17 mya; *Hugueninia*-Canary Island 2.21 +/- 0.26 mya; and the last common ancestor of Canary Island *Descurainia* 0.77 +/- 0.21 mya. It may be noted that these dates are, in most cases, somewhat older than the corresponding dates calculated from ITS sequence evolution. The overall sequence evolution rate for these chloroplast regions was calculated to be 2.2 +/- 0.1 x 10^{-9} substitutions/site/year. This rate is comparable to typical rates of evolution for other non-coding chloroplast regions (Richardson & al., 2001). A summary of these estimates and the phylogenetic tree on which they are based are in Table 2.6 and Fig. 2.12, respectively.

DISCUSSION

Taxonomic position and monophyly of *Descurainia*. – The results of this study provide strong support for the monophyly of the recently-designated tribe Descurainieae (Al-Shehbaz & al., 2006). With the exception of monotypic *Trichotolinum*, for which only a few very old collections exist and which is most likely a reduced *Descurainia* (Al-Shehbaz, pers. omm..), all putative members of Descurainieae were included and form a monophyletic group (Fig. 2.3). As described by Al-Shehbaz & al. (2006), species in this tribe are primarily annual or perennial herbs possessing petiolate, 1 – 3 pinnatisect leaves which are non-auriculate at the base, ebracteate racemes, dendritic or rarely forked trichomes, predominately yellow flowers, fruits in usually glabrous terete siliques or silicles, often numerous tiny mucilaginous seeds in one or two rows, incumbent cotyledons, and, with some exceptions, a base chromosome number of $x = 7$. As can be seen from a comparison of various characters given in Table 2.7, however, not many of these characteristics are universally present throughout Descurainieae. The tribe is

morphologically most similar to another newly-proposed tribe, Smelowskieae (Al-Shehbaz & al., 2006), into which have been placed the remainder of Schulz's Descurainiinae, namely *Smelowskia*, *Sophiopsis*, and *Redowskia*, along with several other small genera (*Hedinia* Ostenf., *Sinosophiopsis* Al-Shehbaz, *Gorodkovia* Botsch. & Karav., and *Ermania* Cham. ex Botsch.). Members of the Smelowskieae are predominantly white-flowered perennials with non-mucilaginous seeds and a base chromosome number of $x = 6$, but these characteristics, as well as others noted by Al-Shehbaz & al. (2006), are also found in some members of Descurainieae and there are no morphological characters which uniquely distinguish the two tribes from each other. Such convergence of characters has confounded attempts to accurately classify many members of the Brassicaceae solely on the basis of morphology (Koch, 2003a; Mitchell-Olds & al., 2005; Al-Shehbaz & al., 2006). The results of this project (although very limited in tribal scope) as well as the broad *ndhF* study of Beilstein & al. (2006) weakly support a sister relationship between Descurainieae and Smelowskieae (Fig. 2.3). In contrast, phylogenetic analysis of ITS sequences from 146 genera (Bailey & al., 2006) did not find support for a sister relationship, although intertribal relationships in their trees were very poorly resolved. An effort to sequence many nuclear genes for representatives throughout the entire family is in the organizational stages (C. Pires [Brassicaceae Phylogeny Working Group], pers. omm..) and will hopefully elucidate the relationship between Descurainieae and Smelowskieae in the near future.

The results of this study indicate that there are two distinct lineages within Descurainieae (Fig. 2.3). The first is composed of two genera: *Hornungia* and *Tropidocarpum*. *Hornungia* is centered in Europe, although one species, the widespread *H. procumbens* Hayek, also extends into Asia and western North America. *Tropidocarpum* is a genus of four New World species. The only common and relatively

widespread species is *T. gracile* Hook., which is distributed from Baja California north into central California (Al-Shehbaz, 2003); two other species are found in California and one in central Chile. In addition to its disjunct distribution between California and Chile, the genus is of interest due to its extreme inter-specific variation in fruit morphology accompanied by essentially identical vegetative and floral morphology and very limited sequence divergence. Unpublished ITS and *ndhF* data (Price, cited in Al-Shehbaz, 2006 and Mitchell-Olds & al., 2005) indicate that sequences of the species differ by only one or two base pair substitutions. Based on the present study, *Tropidocarpum* appears to be most closely related to *Hornungia procumbens*. While fruit morphology varies widely in the *Hornungia/Tropidocarpum* clade, in cross-section the fruits are all angustiseptate, i.e., flattened at right angles to the septum. This morphological feature distinguishes the *Hornungia/Tropidocarpum* clade from the second Descurainieae lineage, in which the fruits are terete, quadrangular or rarely slightly latiseptate (*D. sophioides*).

The second lineage in the Descurainieae comprises *Descurainia*, *Hugueninia*, *Ianhedgea*, and *Robeschia*. The molecular data from this study indicate that *Hugueninia* is clearly embedded in *Descurainia*, confirming the preliminary results of Price (unpublished, cited in Koch & al., 2003a) and conclusions of Appel & Al-Shehbaz (2003). (To minimize confusion, the designation *Hugueninia* is retained throughout the remainder of the dissertation). The taxonomic position of monotypic genera *Ianhedgea* and *Robeschia* with respect to *Descurainia* is unclear; their placement is affected by the inclusion or exclusion of *D. sophia* and *Hornungia* in the analyses. When *D. sophia* or *Hornungia* are excluded, *Ianhedgea*, which is distributed in central and southwest Asia, is placed at the base of this clade, and *Robeschia*, a native of the Middle East, is sister to the remaining taxa. Both of these genera share more features with *Descurainia* than differences. *Robeschia* primarily differs from *Descurainia* by its tapering siliques,

thickened fruiting pedicels, and a base chromosome number of $x = 8$ instead of 7. Both *Ianhedgea* and *Robeschia* have white or pinkish flowers; most species of *Descurainia* have yellowish flowers although a few New World taxa have white flowers. Approximately two-thirds of recognized genera in the Brassicaceae consist of one to three species (Koch & Kiefer, 2006), and Al-Shehbaz & al. (2006) suggest that the vast majority of these should be united with larger genera. Appel & Al-Shehbaz (2003) in fact considered *Robeschia* to be encompassed within *Descurainia*, but the genus has not been formally transferred to *Descurainia*. Regardless of the exact position of *Ianhedgea* and *Robeschia*, the molecular data presented here would support the inclusion of these two genera within *Descurainia*. The final genus in the Descurainieae, *Trichotolinum*, is very similar to *Descurainia*, from which it is separated mainly by basally-pubescent anther filaments and an elongated style (Schulz, 1936; Romanczuk, 1984c). This rare species was not included in the analysis, however, and until molecular data can be obtained, its position is open to speculation.

Relationships within *Descurainia*. – ITS and chloroplast phylogenies offer mixed support for Schulz's sectional classifications. In particular, sect. *Descurainia*, which was considered to include all the non-Canary Island species, is polyphyletic. New World species are clearly separated in the tree from *D. kochii* and *D. sophia*, the Old World members of this section. Section *Sisymbriodendron*, which comprises the Canary Island taxa, is monophyletic, although it is nested within *Hugueninia* which is now confirmed to belong in *Descurainia*.

Descurainia kochii and *D. sophia* are sister to the remainder of the genus (Figs. 2.5, 2.7). While *D. kochii* has a relatively narrow distribution (Turkey and Caucasia), *D. sophia* is wide-ranging throughout most of Europe and temperate Asia and is an

introduced weed in other temperate areas of the world. The successful colonization, vigor and weediness of *D. sophia* compared to its Old World congeners is consistent with a hybrid origin (Grant, 1981; Doyle & al., 1999; Rieseberg & al., 2007), which can be deduced for *D. sophia* from its tetraploid chromosome number ($2n = 28$) and differing placements in the ITS and chloroplast phylogenies (as well as results to be presented in Chapter 3). The paternal ancestor of *D. sophia* is presumably extinct, because there are no other described *Descurainia* species occupying the same phylogenetic position as *D. sophia* in the ITS tree. *Descurainia kochii* appears to be closely related to the maternal parent of *D. sophia*. As chloroplast DNA is inherited maternally in most angiosperms, including crucifers such as *Brassica* (Johannessen & al., 2005) and *Arabidopsis* (Martínez & al., 1997), it is assumed in *Descurainia* that the chloroplast phylogeny is reflective of maternal ancestry.

Species of *Descurainia* in the Canary Islands comprise a monophyletic lineage (Fig. 2.8), suggesting that these woody perennials are descended from a single colonization of the islands. Relationships within the island taxa, based on the results reported in this chapter and additional molecular data, are the subject of Chapter 4. The Canary Island species are, unsurprisingly, most closely related to their nearest continental neighbor, *Hugueninia tanacetifolia*. Based on the very limited sampling in this study, *H. tanacetifolia* ssp. *suffruticosa* is more closely related to the Canarian species than to ssp. *tanacetifolia*. A range disjunction exists between these two subspecies of *Hugueninia*, with ssp. *suffruticosa* restricted to the Pyrenees and mountains of northern Spain and ssp. *tanacetifolia* distributed in the Italian and Swiss Alps. Morphologically, the two subspecies are more similar to each other than to any of the Canary Island taxa, differing only in minor details such as degree of pubescence, number of leaf lobes or teeth, fruiting pedicel length, and a tendency to woodiness, or lack thereof, at the base of the stem

(Schulz, 1924; Ball, 1964; Ortiz, 1993). The genetic differentiation and present-day distribution of *Hugueninia* most likely reflect range disruption during Pleistocene glaciation and subsequent evolution in isolation in Iberian and Italian glacial refugia (Hewitt, 1996; Taberlet & al., 1998). If additional sampling confirms the pattern observed in this study, reclassification of ssp. *suffruticosa* as a distinct species may be warranted.

Although ITS sequence data is equivocal regarding the monophyly of New World *Descurainia*, chloroplast data strongly support New World *Descurainia* as monophyletic and sister to *Hugueninia* and Canary Island species (Fig. 2.7). Within the New World, there are three major, well-supported groups: clade A is exclusively North American, clade B is North American with the exception of the maternal type of one South American species, and clade C contains a mixture of North and South American taxa.

While there is a good deal of incongruence between ITS and chloroplast phylogenies, it is possible to discern four major North American lineages (Figs. 2.5, 2.7, 2.8). Clade A is distributed along the Rocky Mountains and Sierra Madre Oriental, and includes *D. incana*, *D. incisa* (ssp. *incisa*, *paysonii*, and *viscosa*), *D. streptocarpa*, and (as recognized by Detling [1939]) *D. obtusa* ssp. *brevisiliqua*. In addition, *D. sophioides*, *D. californica* and *D. impatiens* are placed by either ITS or chloroplast data (but not both) in clade A. With the exception of four specimens that are morphologically more similar to *D. pinnata* ssp. *halictorum*, the collection dates range from late June to August, and the sampled plants are tall, generally branched above, with simply pinnate or pinnate-pinnatifid leaves, yellow sepals, and more-or-less linear fruit. Seeds in the siliques are arranged in a single row except for those specimens identified as *D. incisa* ssp. *paysonii*, *D. obtusa* ssp. *brevisiliqua*, or cf. *D. pinnata* ssp. *halictorum*, all of which exhibit a biseriate seed arrangement. The common characteristic of fruit appressed to the rachis, which is shared by *D. incana* and some of the South American species, must be due to

morphological convergence, because there is no close phylogenetic relationship between clade A and any South American taxa.

Based on the sampling of this study, clade B has its center of distribution in the Great Basin region of the western United States, and extends from southern California at least to Wyoming (hybrids between this clade and clade C are also found north to Montana and east to Minnesota). When putative hybrids with other clades are removed, this group includes *D. paradisa* ssp. *paradisa*, *D. incisa* ssp. *filipes*, *D. pinnata* ssp. *menziesii*, and one representative each of *D. pinnata* ssp. *nelsonii* and *D. pinnata* ssp. *halictorum*. This clade is morphologically heterogeneous, with no shared characteristics other than the plants tending to be relatively short compared to those in clade A. Dates of collection of these specimens – May through June – are earlier than for clade A. The maternal ancestor of the Patagonian species *D. antarctica* is also found in clade B, and appears to be most closely related to *D. incisa* ssp. *filipes* (Fig. 2.7).

There are two distinct North American lineages which are part of Clade C (Figs. 2.5, 2.7, 2.8). The first of these comprises a single species, *D. obtusa*, which is distributed in the mountains and plateau regions of New Mexico, Arizona, northern Baja California and northern Chihuahua. It is well-supported as a sister species to a group of South American taxa distributed in Argentina and Chile. The second lineage, referred to as clade C-I, is composed of the Mexican endemic *D. virletii* and most of Detling's southern subspecies complex of *D. pinnata*, particularly ssp. *pinnata*, *glabra*, *ochroleuca*, and *halictorum*. The *D. pinnata* specimens in this group range from the coastal plains of the southeastern United States into Arizona, New Mexico, and northern Mexico. In contrast to clade B, the members of clade C-I generally share a number of morphological characters, including pinnatifid or bipinnate lower leaves, purplish or rose-tipped sepals, distinctly elongated racemes, usually wide-spreading fruiting pedicels, clavate siliques, a

biseriate seed arrangement, and flowering time from March to April. A more northern subspecies, *D. pinnata* ssp. *brachycarpa*, may also belong in this clade, but chloroplast sequence data is missing for all but one of the four specimens sequenced or obtained from GenBank. Based on ITS cloning, the one specimen for which chloroplast data was obtained appears to be a clade C x clade B hybrid.

Although the ITS and chloroplast trees are in general agreement regarding major lineages in North America (Figs. 2.5, 2.7), the degree of incongruence is striking (Table 2.5 and Fig. 2.13). Of the 71 sampled North American accessions, 22 (31%) either differ between the two trees in major clade placement or possess mixed ITS types for different major clades. Discordance between nuclear and plastid phylogenies is often seen as evidence of past hybridization events, although other processes, such as lineage sorting (especially in recently-diversified groups) can also give rise to conflicting topologies (Wendel & Doyle, 1998; Comes & Abbott, 2001; Linder & Rieseberg, 2004).

While both processes may have contributed to the observed conflict, a good deal of the incongruence is probably due to hybridization. Virtually nothing is known about the reproductive biology of North American *Descurainia*, but widely-noted infra-specific morphological variation and confusing taxonomic boundaries (e.g., Detling, 1939; Rollins, 1993a,b; Welsh & al., 1993; Rzedowski & Rzedowski, 2001; Holmgren & al., 2005), overlapping ranges, and the occurrence of possible intermediate forms (Detling, 1939; pers. obs.) suggest that inter-populational and interspecific gene flow is occurring. Polyploidy is also relatively common – 15 out of the 29 reported North American chromosome counts (excluding *D. sophioides*) are tetraploid or higher (Table 1.1), although whether these cases represent allopolyploids or autopolyploids is unknown. Hybridization is an extremely common phenomenon in the Brassicaceae (Marhold & Lihová, 2006), and hybrid polyploid complexes have been extensively characterized and

studied in genera such as *Biscutella* (Tremetsberger & al., 2002), *Boechera* (e.g., Koch & al., 2003b; Schranz & al., 2005; Sharbel & al., 2005), *Brassica* (Osborn. 2004 and references therein), *Cardamine* (Urbanska & al., 1997; Franzke & Hurka, 2000; Marhold & al., 2002a,b, 2004), *Cochlearia* (Koch & al., 1999b), *Draba* (Brochmann, 1992; Koch & Al-Shehbaz 2002), *Thlaspi* (Koch & al., 1998), and *Yinshania* (Koch & Al-Shehbaz, 2000). The eight North American accessions with mixed ITS types (seven clade C x clade B and one *vice versa*) are presumably allopolyploids arising from relatively recent hybridization events: with one exception, they belong to taxa which have known tetraploid populations and whose ranges occur in areas where members of the two clades are sympatric. The detection of ITS additive sequences is often considered strong evidence for a recent hybrid origin, especially when hybridization has previously been suspected for the taxa under investigation (e.g., Kim & Jansen, 1994; Sang & al., 1995; Whittall & al., 2000; Alice & al., 2001; Tate & Simpson, 2003; Guggisberg & al., 2006). Some of the samples with polymorphic sequences (e.g., *D. pinnata* ssp. *brachycarpa* F11) are identifiable according to standard taxonomic treatments, but others (e.g., accession C4) defy easy classification and exhibit characteristics of several named subspecies, consistent with a hybrid origin. Sequences of different cloned accessions within a given clade are not all identical, and they do not tightly cluster together geographically (nor morphologically); several are from the Great Basin region of Utah and Nevada, one from central Arizona, two from southern California and northern Baja California, and one from Minnesota. If these accessions represent hybrid populations, then such geographic and morphological disparity suggests several independent hybridization events have taken place.

No ITS additivity was observed for the 14 remaining strongly incongruent accessions. In contrast to the samples with mixed ITS sequences, for all but two

accessions (i.e., *D. pinnata* ssp. *nelsonii* C47 and D23: ITS clade B vs. chloroplast clade C) the incongruence exhibited in this category is between clades A and either C or B. Most involve taxa for which only diploid chromosome counts have been reported (i.e., *D. sophioides* [both B112 and F13: A x C], *D. californica* [both C9 and D12: A x C], *D. impatiens* [both C40 and C42: C x A], and *D. incisa* ssp. *viscosa* [D21: A x C]) or none are known (i.e., *D. incisa* ssp. *incisa* [D25: A x B?; D57: A x C]. In the absence of additional molecular or cytological information, it is difficult to distinguish between potential processes responsible for the incongruence observed in these cases. Some, such as *D. pinnata* ssp. *nelsonii* C47, might be recently-derived polyploids like the accessions possessing mixed types, but this history has been obscured by either complete concerted evolution leading to fixation of the paternal type or by preferential PCR amplification of one parental type. For the species exhibiting incongruence between clades A and C, the ITS phylogeny is much more consonant with morphology than the chloroplast phylogeny, a pattern which has been observed in other groups with similar phylogenetic discordance and often attributed to cytoplasmic introgression (e.g., Soltis & Kuzoff, 1995; Hardig & al., 2000; Ferguson & Jansen, 2002).

In addition to between-clade incongruence, 21 other accessions (30%) (Table 2.5) exhibit conflicting placements within a given clade but possess few if any polymorphic loci. In many of these cases branch lengths are very short and terminal clade support in a given tree is very weak (Figs. 2.4 – 2.7). Such conflicts could equally well reflect within-clade gene flow or the effects of lineage sorting, or even represent "soft incongruence" arising as an artifact of rapid or recent diversification (Wendel & Doyle, 1998). In the case of clade A where the majority of this conflict resides, the within-clade incongruence is suggestive of chloroplast introgression. The ITS topology resolves into four weakly-supported sublineages which, in contrast to the chloroplast tree, are strongly correlated

with morphology and existing species concepts. If the conflict is indeed due to chloroplast capture, however, one would expect to see a relationship between geography and chloroplast haplotypes and this is not the case. If anything, there is more geographical structure in the ITS tree, with northern and southern components evident. Geographic distribution of North American *Descurainia* accessions, along with their inferred parental lineages, is shown in Fig. 2.14.

As in *Boechera*, where diversification and hybridization in a Great Basin refugial area followed by migration to the north and northeast has been inferred (Dobeš & al., 2004), it is tempting to correlate the distribution of North American *Descurainia* lineages and their putative hybrids with evolution in and expansion from southwestern and southeastern Pleistocene refugia. Given the complicating effects of overlapping glacial cycles, human activity over past centuries, and ready dispersal of seeds and pollen, however, any attempt to do so for *Descurainia* without much more extensive population sampling would be premature.

Based on ITS and chloroplast data, several preliminary comments can be made regarding taxonomic issues in North American *Descurainia*. First, it is clear that *D. pinnata*, as currently circumscribed, is polyphyletic, and comprises at least two distinct species complexes. The first complex, henceforth designated as *D. pinnata s.s.*, is centered around *D. pinnata* ssp. *pinnata* and probably encompasses the majority of ssp. *glabra* and *ochroleuca* as well as *D. virletii*. In the western part of its range this complex undergoes extensive hybridization with other taxa. The other species complex appears to consist of *D. incisa* ssp. *filipes* [= *D. longipedicellata* O. E. Schulz] which encompasses or intergrades with *D. pinnata* ssp. *intermedia* and *D. pinnata* ssp. *nelsonii*. *Descurainia paradisa* is affiliated with this group, but appears to be sufficiently distinct to merit continued recognition at the species level. *Descurainia pinnata* ssp. *menziesii* is also

associated with this second complex, which is surprising because morphologically it is much more similar to *D. pinnata s. s*. The placement of *D. pinnata* ssp. *brachycarpa* cannot be ascertained with certainty based on the results of this study, but preliminary results suggest it is more closely allied with *D. pinnata s.s*. than with the "*D. longipedicellata*" complex. Some subspecies, particularly (but not limited to) *D. pinnata* ssp. *halictorum*, most probably represent hybrid populations of polytopic or polyphyletic origin, and their continued taxonomic recognition may not be justified. A second major discovery is that Detling's *D. obtusa* ssp. *brevisiliqua* should not be included in *D. obtusa* ssp. *obtusa* where Rollins placed it, and in fact this taxon does not even belong in *D. obtusa*. It is clearly more closely related to *D. incisa*. Because data from an additional nuclear marker (TOR) further inform relationships within *Descurainia*, a detailed discussion of species concepts in North American *Descurainia* will be deferred until the end of the next chapter (Chapter 3).

Based on morphology and geography, South American *Descurainia* comprise two major divisions – 1) high Andean species with appressed fruit that range from Colombia to northern Argentina and northern Chile and 2) species with spreading fruit that occupy mid-level elevations throughout most of Argentina and Chile. The most widespread species of this latter group, *D. appendiculata*, also extends into Uruguay and southern Bolivia. Unlike their North American congeners, there is very little range overlap between the two morphological divisions. ITS and chloroplast molecular data resolve South American *Descurainia* into four strongly-supported lineages that generally correlate well with major morphological divisions (Figs. 2.5, 2.7, 2.8). There are conflicts between the two trees, but the incongruence is not as marked nor as extensive as that seen in North America.

The monophyly of the first South American lineage, designated clade C-II, is supported by both ITS and chloroplast data. This clade includes all of the South American spreading-fruit species that were sampled (i.e., 8 out of 13 named species) except for *D. cumingiana* var. *tenuissima*. There is very little phylogenetic structure within the group in either tree, and the overall sequence divergence is low (maximum of 0.66% and 0.15% divergence for ITS and chloroplast, respectively, with most ITS sequences identical or only differing by one base pair). While it is fairly easy to sort many C-II specimens into more-or-less distinct morphological categories, such as *D. appendiculata*, *D. pimpinellifolia*, and *D. antarctica*, the specific identification of some samples is extremely difficult due to intermediate morphology, overlapping characters and sometimes differing interpretations by various authors. Moreover, many rarely-collected but widely-dispersed species, such as *D. argentea* and *D. heterotricha*, basically differ from major taxa only in minor details (e.g., petal length, degree of glandulosity) and possibly represent hybrid forms or variation between populations. This group is strongly in need of a more detailed morphological and molecular study with additional sampling to clarify species concepts.

As mentioned previously, at least some populations of *D. antarctica*, which is distributed throughout Patagonia, appear to be a product of hybridization between a member of this South American clade C-II and a presumed dispersant from North American clade B. All four accessions of *D. antarctica*, which were collected at different times from various locations in eastern Chubut or Santa Cruz, form a monophyletic group which is strongly supported as part of clade B in the chloroplast phylogeny (Fig. 2.7). Mixed ITS types were detected for three of these four samples; when two of them were cloned, the resulting sequences were placed in both clade B and clade C-II. Inspection of the additive ITS sequence for the third sample revealed polymorphisms consistent with

the same two clades as well. A fifth accession from Chubut (Correa & al. 4949 BAA) which was not cloned nor included in the final data set also had the same set of polymorphisms. As described earlier, clade B is the North American clade which contains *D. paradisa*, *D. incisa* ssp. *filipes* (*D. longipedicellata*), and several subspecies of *D. pinnata s. l.* Romanczuk (1984a,b) claims that *D. pinnata* is adventive in Patagonia, citing four specimens collected from sandy, ruderal areas and river banks in Neuquén, Río Negro, and Chubut. If *D. pinnata* is present in Patagonia, it might be the clade B maternal parent of *D. antarctica*. Upon examination of several specimens notated as *D. pinnata* from one of the collections cited (Fisher 62 [SI! NY!]), however, these specimens appear to be *D. appendiculata* or *D. argentina* (both South American), not *D. pinnata*. Material from five other collectors, although not cited in Romanczuk, were sent by Romanczuk's collaborator O. Boelcke to R. C. Rollins at Harvard for comparison with known *D. pinnata* taxa; Rollins' opinion, recorded in November 1984 on the sample label at GH, was that they did not match *D. pinnata*, and he assigned them to *D. appendiculata*. An additional specimen annotated as *D. pinnata* by C. Romanczuk in 1984 (*Paladini s. n.* from Mendoza [BAA!]) also appears to be *D. appendiculata.* DNA sequences were obtained from this specimen (D47) and they are found only in clade C-II in both ITS and chloroplast trees. The presence of *D. pinnata* in Argentina appears doubtful; nevertheless, without examining the other cited specimens, it is not possible to be certain that *D. pinnata* has not been collected in Argentina.

The other sampled South American species of *Descurainia* with spreading fruit is *D. cumingiana*. This species, and *D. nuttalli* of Chile which was not sampled, are rather morphologically distinct from the species in clade C-II, being easily distinguished from the latter by elegantly tripinnatisect leaves and long narrow siliques. The four accessions of *D. cumingiana* (all var. *tenuissima* from Chile and Argentina) are well-supported by

molecular data as forming a distinct monophyletic lineage (C-V) that is not part of clade C-II. The species is distributed throughout central Chile and also scattered across central Patagonian regions of Argentina, where it has been reported to hybridize with *D. antarctica* (Romanczuk, 1984a). Reports of *D. cumingiana* var. *cumingiana* in Mendoza and northern Neuquén provinces of Argentina (Romanczuk, 1984a; Zuloaga & Morrone, 1994) appear to be based on an overly-broad species concept for *D. cumingiana*. Examination of specimens from Neuquén and Mendoza annotated as *D. cumingiana* by C. Romanczuk (including *Vallerini 393* [BAA!] cited in Romanczuk 1984a) reveals, for example, a leaf morphology (2- rather than 3-pinnatisect) and seed arrangement (biseriate instead of uniseriate) that differs from the concepts of other authors (e.g., Schulz, 1924). Morphologically these samples seem to have closer affinity to taxa such as *D. antarctica* and *D. pimpinellifolia*, but do not key out cleanly to any specific species. Two such specimens (D34 and D39), one annotated as *D. cumingiana* by C. Romanczuk and the other unidentified, were sequenced. ITS data for D34, and ITS and chloroplast data for D39, definitely place them in clade C-II, not with *D. cumingiana*.

Morphologically, the high Andean species constituting the other two South American lineages (denoted as C-III and C-IV) are united by distinct characters such as fruit appressed to the rachis and valves of the fruit dehiscing from the apex to the base. These characters are absent from other South American *Descurainia* species. While the 17 accessions representing five of the eight appressed-fruit species recognized by Brako & Al-Shehbaz (1993) are basically unresolved with respect to each other and other species of lineage C in the ITS phylogeny (Fig. 2.5), the chloroplast data provides resolution within these two lineages that is somewhat correlated with existing species concepts (Fig. 2.7). Lineage C-III unites one *D. myriophylla* accession with an unresolved clade consisting of three branches (Fig. 2.7), one joining both *D. athrocarpa*

accessions, another uniting two *D. depressa* accessions, and a third including all four *D. leptoclada* accessions and the two *D. stricta* samples. Lineage C-IV includes two *D. depressa* and four *D. myriophylla* accessions. The only anomalous aspects of these placements are that one *D. myriophylla* accession is in C-III rather than C-IV, and that some accessions of *D. depressa* are in C-III while others are in C-IV with *D. myriophylla*. These placements might be a result of hybridization. The two species grow in similar disturbed habitats and what appear to be intermediate forms have been seen where the two species were growing side by side (pers. obs.).

Compared to the confusing muddle in clade C-II, the species of C-III and C-IV are mostly easily distinguishable. There is some taxonomic difficulty regarding *D. stricta*, however. This species is restricted to the Atacama region of Chile, and it has been suggested that it may represent a variety of *D. myriophylla* with pilose fruits or a variety of *D. leptoclada* (A. Prina to S. Perfetti, pers. omm..; Al-Shehbaz, pers. omm..). The situation is not helped by the fact that one named variety of *D. stricta* (var. *florida*) has glabrous fruits, and that the type specimen of *D. stricta* is so fragmentary that it is hard to tell what it represents (Al-Shehbaz, pers. omm..). If one considers *D. myriophylla* to include *D. perkinsiana* and *D. pulcherrima*, following Brako & Al-Shehbaz (1993), most of the distinguishing features of *D. stricta* are encompassed within *D. myriophylla*. Two *D. stricta* accessions collected from northern Chile, B38 and D45, could be considered as *D. myriophylla*. The fact that they are more closely related to *D. leptoclada* than *D. myriophylla*, however, suggests a different determination. For the purposes of this study, they are recognized as *D. stricta*, but, clearly, a better sampling is needed to gain an accurate understanding of this taxon.

Origins and biogeography. – Divergence time estimates support an Upper Miocene origin (5 – 11 mya) for *Descurainia* and related species. *Ianhedgea* (central and southwest Asia), *Robeschia* (Middle East), and *D. kochii* (Turkey and Caucasia) are in a basal position with respect to the remainder of the genus. Assuming that present-day distributions reflect ancestral areas, this suggests that the genus arose in the Irano-Turanian region posited by Hedge (1976) as a likely center of origin for the Brassicaceae.

Geographic expansion of *Descurainia* out of ancestral areas appears to have begun in the early Pliocene with diversification accelerating during the late Pliocene or early Pleistocene. This period was marked by dramatic climate changes which opened up new niches for speciation, and by the final uplift of Eurasian and American mountain systems which could serve as corridors for migration (Simpson, 1975, 1983; Agakhanjanz & Breckle, 1995; Hewitt, 1996, 2000; Hewitt & Ibrahim 2001). The genus spread into Europe giving rise to *H. tanacetifolia* as well as an unknown (or now extinct) taxon which hybridized with *D. kochii* about 2 – 3 mya to form *D. sophia*. Pleistocene glacial cycles have had a profound effect on the composition and distribution of species in Europe, and one of the early glacial cycles may have abetted the extirpation of the maternal parent of *D. sophia* as well as contributed to the expansion of *D. sophia* throughout Eurasia. Such an event has been reported, for example, in *Paeonia* L. (Paeoniaceae), where European populations of present-day Asian species appear to have been completely replaced by their hybrids during the Pleistocene (Sang & al., 1997a). Excluding a few probable misreads in one "messy" GenBank sequence with numerous ambiguous positions, it is interesting that ITS sequences from *D. sophia* collected in New Mexico, Colorado, and Argentina as well as sequences reported in GenBank from Canada (AY230619 and AY230618) and Wyoming (AF205587 and AF118860) are identical. While this limited sampling may simply reflect introduction of *D. sophia* into the New

World from a single Old World source, low genetic diversity is a hallmark of populations derived from post-glacial expansion (Hewitt, 1996, 2000). *Descurainia* species in the Canary Islands are closely related to *H. tanacetifolia* of the Pyrenees and northern Spain. These island taxa are clearly not Tertiary relicts as postulated by authors such as Bramwell (1972), since they arrived in the Canary Islands 500,000 – 750,000 years ago, probably from the Iberian peninsula, a refugial area during Pleistocene glacial maxima (Hewitt, 1996; Taberlet & al., 1998).

New World species of *Descurainia* are of late Pliocene/Pleistocene origin, with molecular clock calculations from chloroplast data estimating a date of 1.8 – 2.1 mya for the last common ancestor of all New World taxa, and ITS data suggesting an origin of approximately 1 mya for each of the three major New World clades. Biogeographic reconstructions with MacClade (Figs. 2.9, 2.10) are equivocal regarding whether *Descurainia* was first introduced into North or South America, although most of the reconstructions support initial introduction into North America. Although approximately twice as many species have been recognized in South America compared to North America, maximum ITS sequence divergence within North American taxa (5.1%) , and even among many western North American taxa alone (e.g., clades A+B, 3.0%), is much greater than for all of South America (1.3%); chloroplast data reveal a similar trend (Table 2.4). This greater genetic diversity within North America relative to South America argues for North America as the continent of initial establishment, and is consistent with the general distribution pattern observed in New World Brassicaceae.

Assuming a North American origin for *Descurainia* in the New World, the ancestor of North American *Descurainia* could have conceivably migrated either westward from Europe or eastward from Eurasia. The close relationship between New World *Descurainia* species and those of the Canary Islands and Europe (i.e., *Hugueninia*)

would be congruent with dispersal from the European continent. Any introduction from Europe via a North Atlantic land bridge, however, can be ruled out; such a land connection is believed to have been broken by the early Eocene (Tiffney, 1985a; Tiffney & Manchester, 2001) which considerably predates the origin of the genus. More recent long-distance dispersal from Europe to North America is a possibility though; examples of such trans-Atlantic dispersals, while not common, include South American *Hypochaeris* arriving from northwest Africa during the Pliocene or Pleistocene (Tremetsberger & al., 2005) and various amphi-Atlantic arctic species whose North American populations are of late Quaternary origin (Brochmann & al., 2003).

Eastern North America is not the likely ancestral area for the genus in North America, because the area of greatest species and sequence diversity for North American *Descurainia* is the Great Basin region of the western United States. While disjunctions between southwest North America and the Mediterranean/European flora are known (cited in Coleman & al., 2003), their origins date from times much earlier than the Pliocene. Migration from Eurasia, rather than Europe, into western North America is therefore the most plausible route of introduction of *Descurainia* into the Americas. Immigration from central Asia to western North America via the Bering land bridge, which served as a glacial refugium and corridor for migration of temperate taxa during the late Tertiary and Quaternary (Tiffney, 1985b; Colinvaux, 1996; Hewitt, 2000), has been invoked to explain the distribution of a number of genera in the Brassicaceae, such as *Braya* Sternb. & Hoppe, *Eutrema* R. Br., *Parrya* R. Br., *Stroganowia* Karelin & Kir., and *Thellungiella* O. E. Schulz (Rollins, 1982). Moreover, many recent molecular studies have uncovered evidence of dispersal from Asia (especially southwest Asia and Eurasia) to North America during the Pliocene/Pleistocene, either by long-distance dispersal or via Beringia. Examples from the Brassicaceae include *Braya* (Warwick & al., 2004a), *Draba*

L. (Koch & Al-Shehbaz 2002), *Lepidium* (Mummenhoff & al., 2001), *Noccaea* Kuntz (Koch & Al-Shehbaz, 2004) and *Smelowskia* C. A. Mey (Warwick & al., 2004b); representatives from other families are *Androsace* L. (Primulaceae; Schneeweiss & al., 2004), *Gentianella* Moench (Gentianaceae; Hagen & Kadereit, 2001), *Halenia* Borkh. (Gentianaceae; Hagen & Kadereit, 2003), *Hordeum* (Poaceae; Blattner, 2006), and *Senecio mohavensis* (Asteraceae; Coleman & al., 2003). A Eurasian—North American link within *Descurainia* is exemplified in the present day by arctic/subarctic *D. sophioides*, which is distributed from western Canada (with outlier populations around Hudson Bay) across Alaska and northern Siberia westward almost to the Ural Mountains. This species occupies a derived phylogenetic position with respect to New World *Descurainia*, however, and its current range may represent expansion from Beringia after the last glacial maximum rather than a relictual connection between Asia and western North America. The most recent common ancestor of European and New World *Descurainia* could have been eliminated from the mountains of northern Asia during a period of rapid glaciation in the Pleistocene, as has been considered for *Androsace* (Schneeweiss & al., 2004).

Regardless of whether or not one assumes a North American origin for New World *Descurainia*, it is clear that there have been several independent dispersals between North and South America. The exact number, and direction, of all dispersal events is difficult to infer, unfortunately, because the major North/South American clade (clade C) is not resolved with respect to some lineages in the parsimony tree (Fig. 2.8). While the topology (Fig. 2.8) recovered by Bayesian inference is well-resolved, the posterior probabilities joining some of the clade C lineages are low, suggesting uncertainty in the placement of those branches. The inability to obtain a well-supported

resolution for these lineages suggests a period of rapid diversification with some dispersals occurring nearly simultaneously.

Figures 2.9 and 2.10 illustrate most parsimonious reconstructions of New World continental distribution (North or South America) traced onto simplified trees representing the two topologies recovered from parsimony analysis and 94% of the 95% credible set of trees from Bayesian analysis of the combined ITS-chloroplast data set. All of these optimizations suggest that there have been multiple dispersals of *Descurainia* between North and South America. The majority of reconstructions support a New World origin in North America followed by three or four independent dispersals to South America. The general trend of relatively-recent colonization of South America from North America is consistent with that seen in many genera (e.g., *Chrysosplenium* [Saxifragaceae; Soltis & al., 2001], *Draba* [Koch & Al-Shehbaz, 2002], *Fagonia* L. [Zygophyllaceae; Beier & al., 2004], *Gentianella* [Hagen & Kadereit, 2001], *Gilia* [Polemoniaceae; Morell & al., 2000], *Lasthenia* [Asteraceae; Chan & al., 2001], *Lepidium* [Mummenhoff & al., 2001], and *Microseris* D. Don [Asteraceae; Wallace & Jansen, 1990]). Several studies have uncovered evidence of multiple independent dispersals from North to South America, such as in *Halenia* (Gentianaceae; Hagen & Kadereit, 2003), *Osmorhiza* Raf. (Apiaceae; Wen & al., 2002), *Sanicula* L. (Apiaceae; Vargas & al., 1998), *Tiquilia* Pers. (Boraginaceae; Moore & al., 2006) and Valerianaceae (Bell & Donoghue, 2005). Although multiple dispersals are also suggested in *Lycium* L. (Solanaceae; Fukuda & al., 2001; Levin & Miller, 2005), the direction of dispersal is uncertain. *Hoffmannseggia* (Simpson & al., 2005) also illustrates a history of multiple dispersals, but the dispersal direction has been from South to North America.

Several of the scenarios in Figs. 2.9 and 2.10 that support a North American origin for *Descurainia* also indicate re-dispersal from South America to North America

has occurred. They suggest that *D. obtusa*, which is distributed in the mountains and plateaus of the southwest U. S. and northern Mexico, arose from a common ancestor of clade C-II, which comprises all of the species (except *D. cumingiana*) with spreading fruit ranging primarily throughout Argentina and parts of Chile. Such a situation would not be without precedent – Blattner (2006) has detected evidence that after introduction into South America from the north ca. 2 mya, *Hordeum* re-dispersed to North America on two separate occasions. It is equally or more likely, however, that South American clade C-II arose from long-distance dispersal of *D. obtusa* or a close relative or ancestor from North America to South America. It is not possible to determine which of these two scenarios is correct from the current data.

With the exception of the branch joining South American clade C-II with North American *D. obtusa*, the branches resolving relationships between major lineages in clade C, on which the above scenarios are based, are not well-supported (Fig. 2.8). Generation of trees with alternative arrangements of these weakly-supported branches (not shown) requires only one or two additional steps compared to the shortest trees recovered from the parsimony analysis. A few of these alternate topologies are also found in the 95% set of credible trees generated from Bayesian analysis. Nonetheless, optimization of continental distribution on any of these alternate topologies yields most parsimonious reconstructions (not shown) supporting three or four dispersals between North and South America similar to those illustrated in Figs. 2.9 and 2.10.

As first proposed by authors such as Cruden (1966) and Carlquist (1983), adhesion of seeds or fruits to (or ingestion by) migrating birds has been suggested as the mechanism for long-distance dispersal in many studies (e.g., Vargas & al., 1998; Ballard & Sytsma, 2000; Morrell & al., 2000; Fukuda & al., 2001; Mummenhoff & al., 2001; Bleeker & al., 2002; Wen & al., 2002; Levin & Miller, 2005; Simpson & al., 2005;

Blattner, 2006). Because *Descurainia* has seeds which are mucilaginous when wet, it is easy to envision such a bird-mediated transport over tropical areas into temperate regions of South America (and *vice versa*). Mummenhoff & al. (1992) cite several reports of *Lepidium* and *Capsella* seeds – both crucifers with mucilaginous seeds – found attached to birds. Long-distance transport of seed from North to South America, whether by birds or otherwise, would be consistent with the origin of *D. antarctica* in Patagonia, since maternal alleles would only be dispersed by seed.

Relative taxonomic utility of non-coding chloroplast markers. – In addition to the seven non-coding regions which were sequenced and incorporated into the chloroplast data set, other non-coding chloroplast regions were screened for taxonomic utility during the course of this study. The additional regions tested included the *trnG*^{UUC} intron (Shaw & al., 2005), the *trnL*^{UAA} intron (Taberlet & al., 1991), and *rpoB-trnC*^{GCA} (Shaw & al., 2005), *psbM-trnD*^{GUC} (Demesure & al., 1995), *rps11-rps8*, *ndhC-trnV*^{UAC}, *rbcL-accD*, *accD-psaI*, *trnS*^{UGA}*-psbZ* (Demesure & al., 1995), *rpl32-trnL*^{UAG}, *trnS*^{GCU}*-trnG*^{UUC}, *trnT*^{UGU}*-trnL*^{UAA} (Taberlet & al., 1991), *atpF-atpH*, *petA-psbJ*, *rps16-psbK*, *psbA-trnH*^{GUG} (Sang & al., 1997a [*psbA*-F]; Tate & Simpson, 2003 [*trnH*-R]), and *trnL*^{UAA}*-trnF*^{GAA} (Taberlet & al., 1991) intergenic spacers. (Primer sequences for regions without cited references were designed from the *Arabidopsis thaliana* genome and are in Table 2.8). Screening of some regions was abandoned before many sequences were obtained because either there was essentially no variation between distant taxa (e.g., *atpF-atpH*, *psbA-trnH*) or due to difficulty in achieving consistent PCR amplification (e.g., *petA-psbJ*, *rbcL-accD*, *rps16-psbK*, *trnS-psbZ*, *trnS-trnG*). Histograms comparing pairwise sequence divergence (uncorrected "p" distance), number of parsimony informative characters, and degree of phylogenetic resolution between representative

Descurainia species are shown in Fig. 2.15. The four most divergent regions, without considering indels, were the *trnG* intron and *trnD-trnE*, *trnE-trnT*, and *ycf6-psbM* intergenic spacers.

Several recent studies have compared the potential phylogenetic utility of various non-coding chloroplast regions. Shaw & al. (2005, 2007) compared non-coding regions across major angiosperm lineages, while the investigations of Daniell & al. (2006) and Timme & al. (2007) were confined to the Solanaceae and Asteraceae, respectively. Mort & al. (2007) recently surveyed several regions reported by Shaw & al. (2005), but focused on additional criteria, such as parsimony informative characters, in addition to the metric used by Shaw & al. (number of variable characters). For *Descurainia* (also taking into account the less-fully screened regions mentioned previously), the results are broadly consistent with the observations of Shaw & al. (2005, 2007). Some regions which were potentially very informative in those studies, however, were not very variable within *Descurainia* (e.g., *ndhC-trnV*, *petA-psbJ*, and *atpF-atpH* [half of their *atpI-atpH*]) and vice versa (e.g., *trnG* intron). Although not illustrated in Fig. 2.15, some regions were variable within certain lineages (e.g., Canary Island taxa), but provided little resolution within other major lineages (e.g., New World taxa). The most taxonomically-useful regions identified in *Descurainia* varied considerably from many regions of potential high utility in the Solanaceae and Asteraceae studies. The most useful non-coding regions will most likely be highly idiosyncratic to the lineage under investigation. For closely-related taxa like *Descurainia*, where sequence variation is low, the wisest approach is to screen a large number of potentially useful non-coding regions before embarking on an extensive regime of PCR amplification and sequencing for many samples.

CONCLUSIONS

The genus *Descurainia* is supported as a member of the recently-designated tribe Descurainieae. The placement of *Hugueninia* within *Descurainia* is strongly corroborated, and the possible expansion of *Descurainia* to include *Robeschia* and *Ianhedgea* is suggested. Phylogenies based on ITS and non-coding chloroplast data suggest a recent origin in the Irano-Turanian region of the Old World for *Descurainia*, with subsequent diversification during the late Pliocene or early Pleistocene into Europe and into the New World. Species in the Canary Islands are monophyletic, implying a single colonization event into the islands, and are most closely related to European *Hugueninia*. Following introduction into the New World, most likely from Eurasia into North America, multiple independent dispersals of *Descurainia* appear to have taken place between North and South America.

Incongruence between ITS and chloroplast trees, as well as mixed ITS types observed for some North American accessions, provide strong evidence for substantial reticulation within North American *Descurainia*. The recent origin of the genus and frequent hybridization are probably responsible for most of the taxonomic complexity which plagues efforts to classify *Descurainia* in North and South America.

The molecular data indicate several problems with current species concepts, especially in regard to *Descurainia pinnata*. To obtain a clear picture of species limits and confirm the patterns suggested by this study, much more extensive sampling needs to be carried out, preferably with the addition of one or more low-copy nuclear markers. These results are necessarily preliminary, but represent the first major molecular investigation of *Descurainia* and will thus serve as a important phylogenetic framework for future studies.

Table 2.1. Plant material used to assess the monophyly of *Descurainia* and its relationship to other genera. Seed source for cultivated plants designated as follows: [ETSIA] = Escuela Técnica Superior de Ingenieros Agrónomos de Madrid crucifer seedbank, Universidad Politécnica de Madrid, Spain; [B&T] = B&T World Seeds, Paguignan, France.

Taxon: Location, date, collector; DNA voucher (herbarium) or GenBank accession numbers (ITS, trn*L*)
Arabidopsis thaliana* (L.) Heynh**.: NC_000932; ***Arabis alpina* L.**: AF137559, AY034180; ***Boechera holboellii* (Hornem.) Á. Löve & D. Löve**: AY457932, DQ013055; ***Brassica rapa* L.**: AF531563, AY236217; ***Cardamine amara* L.**: AY260584, AF266633; ***Descurainia argentina* O. E. Schulz var. *brachysiliqua* (Chodat & Wilczek) O. E. Schulz**: Cultivated, seed [ETSIA 240-5886-81] from Argentina (TEX); ***D. gilva* Svent.**: Spain: Canary Islands, *A. Santos s. n.* (ORT); ***D. incisa* (Engelm. ex A. Gray) Britton ssp. *incisa: USA: Colorado, *Goodson 1502* (TEX); ***D. kochii* (Petri) O. E. Schulz**: Turkey: Çankiri, *A. Dönmez 11789* (TEX); ***D. pinnata* (Walter) Britton ssp. *glabra* (Wooton & Standley) Detling**: USA: Arizona, *R. C. Haberle 177* (TEX); ***D. sophia* (L.) Webb ex Prantl**: USA: New Mexico, *Beilstein 01-19* (MO); ***Ermania parryoides* (Cham.) Botsch.**: AY230625, AY230540; ***Gorodkovia jacutica* Botsch. & Karav.**: AY230606, AY230548; ***Halimolobos elatus* (Rollins) Al-Shehbaz & C. D. Bailey**: DQ336388, DQ336387; ***Hedinia tibetica* (Thomson) Ostenf.**: AY230627, AY230551; ***Hornungia alpina* (L.) O. Appel**: DQ310527, DQ310515; ***H. petraea* (L.) Reichenbach**: AJ440308, AY015905; ***H. procumbens* (L.) Hayek**: AJ440309, AY015903; ***Hugueninia tanacetifolia* (L.) Prantl ssp. *suffruticosa***: Cultivated from seeds [B&T] (TEX); ***Ianhedgea minutiflora* (Hook. *f.* & Thoms.) Al-Shehbaz & O'Kane**: Tajikistan: Badakhson, *Solomon et al. 21646* (MO); ***Lepidium campestre* (L.) R. Br.**: AF055197, AY015845; ***L. virginicum* L.**: AY662280, AY015902; ***Mancoa bracteata* (S. Wats.) Rollins**: AF307633, AF307556; ***Nasturtium officinale* R. Br.**: AY254531, AY122457; ***Nerisyrenia linearifolia* (S. Wats.) Greene**: AF055200, AF055267; ***Nevada holmgrenii* (Rollins) N. H. Holmgren**: AY230589, AY230555; ***Physaria fendleri* (A. Gray) O'Kane & Al-Shehbaz**: AF055199, AF055266; ***Polyctenium fremontii* (S. Wats) Greene**: AY230614, AY230614; ***Redowskia sophiifolia* Cham. & Schltdl.**: AY230608, AY230542; ***Robeschia schimperi* (Boiss.) O. E. Schulz**: Iran: Prov. Esfahan, *American-Iranian Botanical Delegation 33719* (TUH); ***Sinosophiopsis bartholomewii* Al-Shehbaz**: AY230609, AY230550; ***Sisymbrium altissimum* L.**: USA: Colorado, *Goodson 1460* (TEX); ***Smelowskia americana* (Regel & Herder) Rydb.**: USA: Colorado, *Goodson 1462* (TEX); ***Smelowskia calycina* (Stephen) C. A. Mey**: AY230576, AY230523; ***Sophiopsis sisymbrioides* (Regel & Herder) O. E. Schulz**: Tajikistan: Pil'doni-Poyen, *Chukavina 352* (GH); ***Tropidocarpum gracile* Hook.**: ITS seq. from R. A. Price.

Table 2.2. Plant material used to examine phylogenetic relationships within *Descurainia*. Seed source for cultivated plants designated as follows: [ETSIA] = Escuela Técnica Superior de Ingenieros Agrónomos de Madrid crucifer seedbank, Universidad Politécnica de Madrid, Spain; [B&T] = B&T World Seeds, Paguignan, France.

Taxon; Location, date, collector and DNA voucher (herbarium)

***Arabidopsis thaliana* (L.) Heynh.:** GenBank; NC_000932;

***Descurainia antarctica* (Fourn.) O. E. Schulz: var. *bonarelli* O. E. Schulz – D37**: Argentina: Cráter Oreja de Burro, Ea. Monte Aymond, Dept. Güer Aike, Prov. Santa Cruz, 13 February 1980, *O. Boelke et al. 16806* (BAA); **var. *patagonica* (Speg.) O. E. Schulz – D52**: Argentina: RN 40, a 30 km NE de Esquel, Dept. Cushamen, Prov. Chubut, 2 February 1975, *O. Boelcke 16038* (BAA); **E2**: Cultivated, seed collected by B. Goodson, 7 January 2005, roadside, RN 3, between Florentino Ameghino and Uzcudun, Dept. Florentino Ameghino, Prov. Chubut, Argentina (TEX); **F15**: Cultivated, seed collected by B. Goodson, 7 January 2005, roadside, RN3, between Uzcudun and Commodoro Rivadavia, Dept. Florentino Ameghino, Prov. Chubut, Argentina (TEX);

***D. appendiculata* (Griseb.) O. E. Schulz: B126**: Cultivated, seed collected by B. Goodson, 27 Dec 2001, on side of gravel road ca. 1.4 km E of Universidad Católica de Salta, Dept. Capital, Prov. Salta, Argentina (TEX); **C25**: Cultivated, seed collected by B. Goodson, 31 Dec 2001, along RP 307 (S 26º52'38.0" W 65º41'35.9"), Dept. Tafí del Valle, Prov. Tucumán, Argentina (TEX); **D47**: Argentina: Vivero Sur, Dept. Godoy Cruz, Prov. Mendoza, 1 October 1984, *Paladina s. n.* (BAA);

***D. argentina* O. E. Schulz: var. *brachysiliqua* (Chodat & Wilczek) O. E. Schulz – B37**: Cultivated, seed [ETSIA 240-5886-81] collected from General Acha, Dept. Ultracan, Prov. La Pampa, Argentina (TEX); **var. undet. – B96**: Cultivated, seed [ETSIA 239-5908-81] collected from roadside, Uspallata, Dept. Las Heras, Prov. Mendoza, Argentina (TEX);

***D. artemisioides* Svent.: B36**: Cultivated, seed collected [ETSIA 241-4201-76] by G. Kunkel from Berrazales, Gran Canaria, Canary Islands, Spain (TEX);

***D. athrocarpa* (A. Gray) O. E. Schulz: B94**: Peru: trail to Lago Ishinca, Huascarán National Park, Prov. Carhuaz, Dept. Ancash, 12 February 1985, *D. N. Smith et al. 9450* (MO); **C27**: Bolivia: slope above road to Valle del Zongo (S 16º16'51" W 68º7'21"), Prov. Murillo, Dept. La Paz, 5 March 2004, *B. Goodson 1506* (TEX);

Table 2.2. Continued.

***D. bourgaeana* Webb ex O. E. Schulz: B14**: Spain: El Portillo, Cañadas del Teide, Tenerife, Canary Islands, *A. Santos s. n.* (ORT); **B171**: Cultivated, seed [ETSIA 242-1629-68] collected by J. Esquinas from Las Cañadas, Tenerife, Canary Islands, Spain (TEX); **D7**: Spain: Los Andenes, La Caldera National Park, La Palma, Canary Islands, *A. Santos s. n.* (ORT);

***D. californica* (A. Gray) O. E. Schulz: C9**: USA: East Creek campground, Humboldt National Forest (N 39º29'43" W 114º39'13"), White Pine Co., NV, 22 May 2003, *B. Goodson 1493* (TEX); **D12**: USA: Cedar Creek Campground, Dixie National Forest (N 37º35'28" W 112º53'53"), Iron Co., UT, 19 August 2001, *B. Goodson 1466* (TEX);

***D.* cf. *erodiifolia* (Phil.) Reiche: D50**: Argentina: pié de Paramillo de Cuevas, Dept. Las Heras, Prov. Mendoza, 28 December 1981, *Roig 10766* (BAA);

***D. cumingiana* (Fisch. & C. A. Mey): var. *cumingiana* – D34**: Argentina: Ea. Fortin Chacabuco, Dept. Los Lagos, Prov. Neuquén, 1 December 1966, *Abadie-Speck 7* (BAA); **D39**: Argentina: S de Mina Escondida, alrededores del Río Carranza, Dept. Añelo, Prov. Neuquén, 13 October 1982, *M. N. Correa et al. 8699* (BAA); **var. *tenuissima* (Phil.) Reiche – B103**: Chile: Prov. Huasco, Atacama (Region III), 2 November 1991, *M. Muñoz et al. 2930* (MO); **D38**: Argentina: SE de Pico Oneto, Dept. Sarmiento, Prov. Chubut, 22 October 1976, *Irisarri 180* (BAA); **D43**: Argentina: 60 km de Jacobacci subiendo a la meseta, Dept. 25 de Mayo, Prov. Rio Negro, 8 November 1966, *Abadie-Vallerini 1020* (BAA); **D49**: Chile: chacra del Sr. Benjamin Olivares C., San Felipe, Prov. Los Andes, Valparaíso (Region V), 5 October 1962, *A. Garaventa 8072* (BAA);

***D. depressa* (Phil.) Reiche: C26**: Bolivia: Patarani (S 17º14'54" W 67º59'59"), Prov. Aroma, Dept. La Paz, 3 March 2004, *B. Goodson 1505* (TEX); **C37**: Bolivia: fallow field along road between Sajama to Patacamaya, ca. 3 km W of Puerto Japones (17º22'02" W 68º13'27"), Prov. Pacajes, Dept. La Paz, 15 March 2004, *B. Goodson 1520* (TEX); **D17**: Bolivia: road from Tiwanaku to La Paz, ca. 5 miles E of Tiwanaku (S 16º35'08" W 68º35'00"), Prov. Ingavi, Dept. La Paz, 11 March 2004, *B. Goodson 1510* (TEX); **D31**: Argentina: entre Tres Cruces y Iturbe, Dept. Humahuaca, Prov. Jujuy, 25 January 1964*, L. Giusti et al. 558* (BAA);

***D. gilva* Svent: B22**: Spain: Cumbres de Puntallana, La Palma, Canary Islands, May 2001, *S. Santos s. n.* (ORT); **B163**: Cultivated, seed collected [ETSIA 243-4055-76] by A. Santos from Las Manchas, La Palma, Canary Islands, Spain (TEX);

Table 2.2. Continued.

***D. glaucescens* (Phil.) Prantl ex Reiche: D20**: Chile: Prov. Copiapó, Region III(Atacama), January 1926, *E. Werdermann 971* (MO);

***D. gonzalezi* Svent.: B19**: Spain: Carretera a Madre de Agua, Vilaflor, Tenerife, Canary Islands, May 2001, *A. Santos s. n.* (ORT); **B160**: Cultivated, seed [ETSIA 244-3172-74] collected from Las Cañadas, Tenerife, Canary Islands, Spain (TEX);

***D. heterotricha* Speg.: B124**: Cultivated, seed collected by B. Goodson, December 2001, weedy field in El Salto, Dept. Luján de Cuyo, Prov. Mendoza, Argentina (TEX);

***D. impatiens* (Cham. & Schlecht.) O. E. Schulz: C40**: Mexico: 3 km S of Neverías, Mun. Miahuatlán, Oaxaca, 3 August 1996, *G. B. Hinton et al. 26690* (TEX); **C42**: Mexico: orilla de camino, Mun. Perote, Veracruz, 22 June 1970, *F. Ventura A. 1338* (TEX);

***D. incana* (Bernh. ex Fischer & C. A. Meyer) Dorn: B109**: USA: open meadow at end of Price Peet Road, Beaverhead Co., MT, 27 July 1979, *P. P. Lowrey 2693* (MO); **C2**: USA: near US Hwy 10A, 21.5 miles west of Anaconda, Granite Co., MT, 18 July 1983, *R. C. & K. W. Rollins 83308* (GH); **D29**: USA: N side of Galena Summit area, between Stanley and Galena, Blaine Co., ID, 27 June 1986, *R. C. & K. W. Rollins 86118* (TEX);

***D. incisa* (Engelm. ex A. Gray) Britton: ssp. *filipes* (A. Gray) Rollins – B195**: USA: Falls Canyon, W of Paradise Peak, Humboldt Co., NV, 28 May 1987, *A. Tiehmn 11104* (GH); **C21**: USA: Flaming Gorge Overlook, Hwy 44, Flaming Gorge NRA (N 40º54'28" W 109º41'54"), Daggett Co., UT, 29 June 2003, *B. Goodson 1499* (TEX); **C45**: USA: 3 miles SE of North Battle Mountain on road to Stony Point, Lander Co., NV, 21 May 2002, *A. Tiehm 12845* (TEX); **D14**: USA: FR 221, Ashley National Forest (N 40º56'22" W 110º00'12"), Daggett Co., UT, 29 June 2003, *B. Goodson 1500* (TEX); **ssp. *incisa* – C24**: USA: McKenzie Gulch Trail, White River National Forest, Eagle Co., Colorado, 2 July 2003, *B. Goodson 1502* (TEX); **D6**: USA: Snowbird Ski Resort, Salt Lake Co., UT, 4 August 2004, *B. Goodson 1528* (TEX); **D25**: USA: steep bank off state Hwy 75, 6.6 miles from Stanley near the Salmon River, Custer Co., ID, 25 June 1986, *R. C. & K. W. Rollins 86101* (TEX); **D56**: USA: near road to Lower Lagunitas Lakes campground, Rio

Table 2.2. Continued.

Arriba Co., NM, 2 August 1998, *J. McGrath 157* (UNM); **D57**: USA: Upper Frijoles Meadow, Los Alamos Co., NM, 19 July 1982, *T. Dunbar 609* (UNM); **ssp. *paysonii* (Detling) Rollins – D28**: USA: Browns Park, just NE of Gates of Lodore above Vermilion drainage, Moffat Co., CO, 26 June 1965, *W. A. Weber & P. Salamun 12649* (TEX); **D73**: USA: Navajo site T 17N, R 16W, Sec. 4, McKinley Co., NM, 25 May 1976, *W. L. Wagner 1932* (UNM); **ssp. *viscosa* (Rydb.) Rollins – D21**: USA: Crystal Reservoir, Laramie Co., WY, 7 July 1966, *Porter & Porter 10187* (TEX); **D24**: USA: Big Lake, Apache National Forest, Apache Co., AZ, 16 August 1973, *A. R. & H. N. Moldenke 27885* (LL);

***D. kochii* (Petri) O. E. Schulz: D2**: Turkey: Karaören Köyü (N 40º30'02" E 33º14'47"), Sabanözü, Çankiri, June 2004, *A. Dönmez 11789* (TEX); **D3**: Turkey: Eskihisar Köyü çevresi (N 40º51'11" E 33º26'21"), Kastamonu, June 2004, *A. Dönmez 11793* (TEX); **D18**: Turkey: Koçubaba Kasabasi (N 39º59'27" E 32º51'31"), Baliseyh, Kirikkale, 15 June 2004, *A. Dönmez 11928* (TEX);

***D. lemsii* Bramwell: B23**: Spain: Cumbres de la Orotova, Tenerife, Canary Islands, April 2001, *A. Santos s. n.* (ORT); **B170**: Cultivated, seed [ETSIA 245-3094-74] collected from La Crucita, Tenerife, Canary Islands, Spain (TEX);

***D. leptoclada* Muschl.: C33**: Bolivia: W-facing bank of Río Sururia (S 18º11'08" W 68º53'48"), Parque Nacional Sajama, Prov. Sajama, Dept. Oruro, 14 March 2004, *B. Goodson 1514* (TEX); **C34**: Bolivia: above village of Sajama (S 18º07'51" W 68º56'49"), Parque Nacional Sajama, Prov. Sajama, Dept. Oruro, 14 March 2004, *B. Goodson 1515* (TEX); **C36**: Bolivia: E side of Río Tomarapi, ca. 2 km E of Cosapa (S 18º05'27" W 68º44'06"), Prov. Sajama, Dept. Oruro, 15 March 2004, *B. Goodson 1516* (TEX); **D46**: Argentina: ladera entre Molino y Mina Aguilar, Dept. Humahuaca, Prov. Jujuy, 2 March 1983, *J. H. Hunziker et al. 10531* (BAA);

***D. millefolia* (Jacq.) Webb & Berthel.: B24**: Spain: Barranco del Rio, La Palma, Canary Islands, April 2001, *A. Santos s. n.* (ORT); **B38**: Cultivated, seed [ETSIA 246-1073-67] collected from Buenavista, Tenerife, Canary Islands, Spain (TEX); **D1**: Spain: Buenavista del Norte, Tenerife, Canary Islands, *J. Panero & J. Francisco-Ortega 6987* (TEX); **D5**: Spain: El Fraile, Tenerife, Canary Islands, *A. Santos s. n.* (ORT); **F1**: Spain: W of San Sebastian, along road to Langrero, La Gomera, Canary Islands, *leg. ign. AAU71-7259* (MO); **F2**: Spain: Chejelipes, La Gomera, Canary Islands, *leg. ign. AAU71-7533* (MO);

Table 2.2. Continued.

***D. myriophylla* (Willdenow ex DC.) R. E. Fries: C29**: Bolivia: Laguna Apaña, Ovejuyo (S 16º32'52" W 68º00'48"), Prov. Murillo, Dept. La Paz, 7 March 2004, *B. Goodson 1508* (TEX); **C52**: Peru: Cuzco, 17-18 May 1989, *Tupayachi 1065* (MO); **D9**: Bolivia: La Paz Montículo (S 16º30'27" W 68º07'38"), Prov. Murillo, Dept. La Paz, 7 March 2004, *B. Goodson 1507* (TEX); **D13**: Bolivia: ca. 2 km W of Patacamaya (S 17º13'48" W 67º56'17"), Prov. Aroma, Dept. La Paz, 13 March 2004, *B. Goodson 1511* (TEX); **D16**: Bolivia: ca. 2 km W of Patacamaya (S 17º13'48" W 67º56'17"), Prov. Aroma, Dept. La Paz, 13 March 2004, *B. Goodson 1512* (TEX);

***D. obtusa* (E. L. Greene) O. E. Schulz: ssp. *adenophora* (Wooton & Standley) – D61**: USA: adjacent to FS 111, Gila National Forest, Grant Co., NM, 19 July 1995, *C. A. Huff & D. Stevens 2310* (UNM); **D62**: USA: Laguna Lake, Hualpai Indian Reservation, Coconino Co., AZ, 7 July 1936, *W. N. Anderson A201* (UNM); **ssp. *brevisiliqua* Detling – D58**: USA: vicinity of Water Canyon, Socorro Co., NM, 26 July 1973, *B. Hutchins 4450* (UNM); **D59**: Datil Mountains, Catron Co., NM, 1 August 1976, *Fletcher 823* (UNM); **D72**: USA: junction of Forest Roads 234 and 46, Socorro Co., NM, 28 July 1974, *B. Hutchins 5099* (UNM); **D4 (cf. ssp. *brevisiliqua*)**: USA: VLA radio telescope observatory, Socorro Co., NM, 15 July 2004, *B. Goodson 1527* (TEX); **ssp. *obtusa* – B26**: USA: slopes along NM Hwy 159, 5 miles E of junction with US Hwy 180 (N 33º23'16" W 108º49'58"), Catron Co., NM, 10 August 2001, *T. Chumley 7359* (TEX); **D63**: USA: Canon del Alamito, N side of Ladrons, Socorro Co., NM, 15 August 1965, *O. Baca 262* (UNM); **D64**: USA: Lower Indian Creek Canyon, Hidalgo Co., NM, 22 August 1975, *W. Wagner 1157* (UNM); **D65**: USA: Sawmill Peak area, Sierra Co., NM, 12 August 1982, *B. Hutchins 10245* (UNM);

***D. paradisa* (A. Nels. & Kenn.) O. E. Schulz: ssp. *nevadensis* Rollins – C8**: USA: valley floor, W of NV Hwy 95 and N of Walker Lake (N 38º48'47" W 118º45'59"), Mineral Co., NV, 21 May 2003, *B. Goodson 1492* (TEX); **C48**: USA: 1.1 miles SE of main dirt road to Mina, Dunlap Canyon, Mineral Co., NV, 12 May 1988, *A. Tiehm 11582* (TEX); **ssp. *paradisa* – C7**: USA: NV Hwy 445 (MM 27), ca. 2 miles SW of Pyramid Lake Indian Reservation (N 39º52'07" W 119º38'16"), Washoe Co., NV, 21 May 2003, *B. Goodson 1490* (TEX); **C46**: USA: 2.8 miles S of Wheeler Reservoir road on main N-S road to Double Hot Springs, Humboldt Co., NV, 14 May 2002, *A. Tiehm 13794* (TEX);

***D. pimpinellifolia* (Barnéoud) O. E. Schulz: D11**: Argentina: RP 52, ca. 34 km from Uspallata (S 32º30'10" W 69º03'26"), Dept. Las Heras, Prov. Mendoza, 15 December 2001, *B. Goodson 1475* (TEX); **D42**: Argentina: Puesto Agua del Godo, Reserva San Guillermo, Dept. Iglesia, Prov. San Juan, 13 Jan 1983, *Nicora 8466* (BAA); **D51**: Argentina: Chacras de Coria, Dept. Luján de Cuyo, Prov. Mendoza, 10 October 1972, *Roig 7409* (BAA); **E3**: Argentina: Prov. San Juan, 9 January 1997, *R. Kiesling 8760* (SI);

Table 2.2. Continued.

***D. pinnata* (Walter) Britton: ssp. *brachycarpa* (Richardson) Detling – F11**: USA: banks of Mississippi River, Winona Co., MN, 13 June 1975, *S. D. Swanson 490* (MO); **F12**: USA: limestone cliffs downstream from Dycusburg, overlooking the Cumberland River, Crittenden Co., KY, 10 April 1969, *R. Athey 536* (MO); **ssp. *glabra* (Wooton & Standley) Detling – B144**: USA: 2 mi SW of courthouse, Prescott, Yavapai Co., AZ, 18 June 2002, *R. C. Haberle 177* (TEX); **D27**: Mexico: canyon of Rio Guararáy, ca. 0.5 km upstream from Los Aguaros, Mun. Alamos, Sonora, 16 March 1994, *R. S. Felger 94-88* (TEX); **C10**: USA: ca. 3 miles N of northern entrance to Joshua Tree National Park (N 34º05'26" W 116º02'12"), San Bernardino Co., CA, 1 May 2003, *T. Chumley 7434* (TEX); **ssp. *halictorum* (Wooton) Detling – C12**: USA: Spring Valley along Hwy 93 ca. 8 miles S of Majors Place (N 38º56'27" W 114º30'46"), White Pine Co., NV, 6 May 2003, *T. Chumley 7437* (TEX); **C14**: USA: BLM road to Mormon Mountains, ca. 22 miles N of junction with I-15 (N 37º01'25" W 114º18'56"), Lincoln Co., NV, 7 May 2003, *T. Chumley 7440* (TEX); **D10**: USA: Hwy 67, 40 miles N of Alpine (N 30º43'03" W 103º11'56"), Pecos Co., TX, 9 April 2004, *B. Goodson 1521* (TEX); **D19**: USA: Hwy 67, ca. 9.6 miles S of Marfa (N 30º10'54" W 104º04'43"), Presidio Co., TX, 9 April 2004, *B. Goodson 1523* (TEX); **D67**: USA: Chiracahua Mountains, Cochise Co., AZ, 14 March 1984, *M. Kurzius 84-5* (UNM); **D69**: USA: Petroglyph National Monument lowlands, Bernalillo Co., NM, 19 April 2001, *A. C. Cully & M. Medrano s. n.* (UNM); **D71**: USA: Cochiti Lake site along Rio Grande, Sandoval Co., NM, 5 April 1975, *G. Tierney A84575* (UNM); **ssp. *intermedia* (Rydb.) Detling – C19**: USA: Red Canyon Lodge Horse Stables, Hwy 44, Flaming Gorge NRA (N 40º52'22" W 109º32'35"), Daggett Co., UT, 29 June 2003, *B. Goodson 1498* (TEX); **ssp. *menziesii* (DC.) Detling – B35**: Cultivated, seed [ETSIA 248-1725-69] collected from Oakzanitas, San Diego Co., CA, USA (TEX); **C3**: Mexico: RN 1, ca. 5 miles E of El Aquajito (N 30º04'20" W 115º22'41"), Mun. Ensenada, Baja California Norte, 9 March 2003, *T. Chumley 7429* (TEX); **D53**: USA: 0.5 mile W of Aguanga, San Diego Co., CA, Riverside Co., CA, 29 March 1990, *E. LaRue s. n.* (TEX); **D55**: USA: Anzo-Borrego State Park, San Diego Co., CA, 24 April 1976, *A. L. & H. N. Moldenke 30653* (TEX); **ssp. *nelsonii* (Rydb.) Detling – C17**: USA: McCarty Canyon Road (N 41º22'39" W 107º18'46"), Carbon Co., WY, 26 June 2003, *B. Goodson 1495* (TEX); **C47**: USA: dome just N of Kobeh Valley Hot Springs, Eureka Co., NV, 7 June 2002, *A. Tiehm 13911* (TEX); **D23**: USA: Lemhi Pass, Beaverhead Mountains, between Grant, MT and Tondoy, ID, Beaverhead Co., MT, 3 July 1986, *R. C. & K. W. Rollins 86185* (TEX); **ssp. *ochroleuca* (Wooton) Detling – D8**: USA: junction of Hwy 17 and county road 112, ca. 16 miles S of Pecos (N 31º11'12" W 103º34'42"), Reeves Co., TX, 10 April 2004, *B. Goodson 1524* (TEX); **D26:** Mexico: Santa Rosa, Mun. Guadalupe y Calvo, Chihuahua, 3 June 1960, *C. W. Pennington 325* (TEX); **ssp. *pinnata* – B12a**: USA: Fly Gap division of Double Helix Ranch, Mason Co., TX, 14 April 2001, *B. Goodson 1457* (TEX); **D15**: USA: picnic area on Hwy 90, 5 miles W of Alpine (N 30º19'22" W 103º44'35"), Brewster Co., TX, 9 April 2004, *B. Goodson 1522* (TEX); **F5**: USA:

Table 2.2. Continued.

St. Catherine's Island, Liberty Co., GA, 29 March 1986, *S. B. Jones 24758* (MO); **F6**: USA: FL 232 ca. 4 miles W of Gainesville, Alachua Co., FL, 25 March 1970, *M. R. Crosby 4844* (MO); **F17**: USA: I-75 rest area N of Tampa (N 28º12'50" W 82º22'25"), Pasco Co., FL, 5 March 2006, *B. Goodson 1616* (TEX); **ssp. undet. – C4**: USA: exit 390 I-10 (N 32º13'47" W 109º03'18"), Cochise Co., AZ, 8 March 2003, *T. Chumley 7427* (TEX); **C15**: USA: BLM road to Mormon Mountains, ca. 0.5 miles E of junction with road to Lyman's Crossing (N 37º08'41" W 114º23'01"), Lincoln Co., NV, 7 May 2003, *T. Chumley 7439* (TEX); **D68**: USA: lower La Cueva Canyon, Socorro Co., NM, 19 April 1989, *T. Maddux & S. Loftin 12* (UNM); **D70**: USA: Sevilleta Wildlife Refuge, Socorro Co., NM, 27 April 1990, *T. Maddux 327* (UNM);

***D. preauxiana* (Webb) Webb ex O. E. Schulz: B117**: Cultivated, seed [ETSIA 249-4135-76] collected by G. Kunkel, Ayacata, Gran Canaria, Canary Islands, Spain (TEX);

***D. sophia* (L.) Webb ex Prantl: B20**: USA: Hwy 160 (N 38º15'17" W 105º57'13"), Saguache Co., CO, 17 August 2001, *B. Goodson 1461* (TEX); **E6**: Argentina: RN 25, Quichara, Dept. Languiñeo, Prov. Chubut, 18 Jan 2005, *B. Goodson 1560* (TEX); **MB3**: USA: New Mexico, *Beilstein 01-19* (MO);

***D. sophioides* (Fischer) O. E. Schulz: B112**: Canada: Yukon Territory, 15 July 1978, *Cooper 715* (NY); **F13**: Cultivated, seed collected by J. McKendrick, 17 August 1990, Dalton Highway MP 398.7, Prudhoe Bay, North Slope Co., AK, USA (TEX);

***D. streptocarpa* (Fourn.) O. E. Schulz: B33**: Mexico: road to summit of Cofre de Perote, Mun. Perote, Veracruz, 8 July 1980, *B. F. Hansen & M. Nee 7702* (MO); **C44 (*D.* cf. *streptocarpa*)**: Mexico: Rancho de la Tinaja (N 29º42'30" W 107º35'30"), Chihuahua, 30 August 1989, *M. H. Mayfield et al. 206* (TEX);

***D. stricta* (Phil.) Reiche: var. undet. – C38**: Chile: km 90 on the Arica-Putre road, Prov. Arica, Tarapacá (Region I), *J. L. Panero & B. S. Crozier 8435* (TEX); **D45**: Chile: Zapahuira/Putre, Prov. Parinacota, Tarapacá (Region I), *s. d.*, *C. Villagrán 2457* (BAA);

***D. virletii* (Fourn.) O. E. Schulz: B108**: Mexico: Laguna de Zumpango, Mun. Zumpango, Mexico, 3 December 1978, *I. Piña E. 100* (MO); **C39**: Mexico: Tuul Ja', 6 km al E de la cabecera municipal de Amatenango del Valle, Chiapas, 10 February 1988, *J. Pérez 266* (TEX);

Table 2.2. Continued.

***Hugueninia tanacetifolia* (L.) Prantl: ssp. *suffruticosa* – C6**: Cultivated from seeds [B&T] (TEX); **ssp. *tanacetifolia* – B111**: Italy: Piemonte, 10 July 1988, *Pistarino 2027* (NY);

***Ianhedgea minutiflora* (Hook. *f.* & Thoms.) Al-Shehbaz & O'Kane: MB2**: Tajikistan: Badakhson, *Solomon et al. 21646* (MO);

***Robeschia schimperi* (Boiss.) O. E. Schulz: B106**: Iran: Prov. Kerman, 27 April 1948, *K. H. & F. Rechinger 3076* (MO); **MB1**: Iran: Prov. Esfahan, ca. 10 km past Khansar, on road to Golpayegan, 21 May 2004, *American-Iranian Botanical Delegation 33719* (TUH);

***Sisymbrium altissimum* L.: B21**: USA: Hwy 160, 1.7 miles W of Huerfano Co. line (N 37º33'06" W 105º17'05"), Costilla Co., Colorado, 17 August 2001, *B. Goodson 1460* (TEX);

***Smelowskia americana* (Regel & Herder) Rydb.: B146**: USA: Mt. Sherman, Park Co., Colorado, 18 August 2001, *B. Goodson 1462* (TEX);

***Sophiopsis sisymbrioides* (Regel & Herder) O. E. Schulz: B194**: Tajikistan: South Altai Mountains, 5 km from Pil'doni-Poyen, in mixed-grass area, 10 July 1963, *Chukavina 352* (GH).

Table 2.3 Sequence characteristics of DNA regions used in this study for the 150 sample set.

	trnC-ycf6	*ycf6-psbM*	*trnD-trnE*	*trnE-trnT*	*psbZ-trnfM*	*ndhF-rpl32*	*rps16* intron	Combined chloroplast	ITS
Seq. length (bp)	519-585	579-619	517-541	534-775	647-731	656-940	787-828	4423-4857	596-614
# taxa	137	137	137	135	136	133	134	135††	150
Alignment length	613	647	573	820	788	1044	866	5351	627
% missing/% gaps	0/7.5	0/7.8	0/8.0	0/26.1	0.2/15.5	0.3/15.0	0.2/6.3	0.8/12.8	0.1/2.5
No. of non-autapomorphic indels	9	5	3†	1	9	9	3	39†	0
No. informative characters (%)*	73 (11.7%)	62 (9.5%)	62 (10.7%)	75 (9.1%)	88 (11.0%)	134 (12.7%)	87 (10.0%)	581 (10.8%)	127 (20.3%)
No.informative characters (%)**	68 (10.9%)	45 (6.9%)	47 (8.2%)	51 (6.2%)	62 (7.8%)	89 (8.5%)	59 (6.8%)	505 (9.4%)	103 (16.4%)
No. of MPTs	--	--	--	--	--	--	--	(3419)	(4020)
Length of MPTs	--	--	--	--	--	--	--	1538	338
Consistency index ***	0.835	0.753	0.745	0.740	0.653	0.754	0.765	0.713	0.630
Retention index	0.962	0.948	0.948	0.935	0.886	0.921	0.918	0.918	0.925

*incl. outgroups and indels; ** incl. only ingroups and indels; *** excluding informative characters; †including one inversion; ††data missing for *ndhF-rpl32* (*D. antarctica* D52, *D. incisa* D21, & *D. incisa* ssp. *filipes* B195) and *rps16* (*D. antarctica* D52 & *D. pinnata* F11).

Table 2.4. Maximum sequence divergences (based on uncorrected "p" distances) within major *Descurainia* lineages.

Clade	ITS %	chloroplast %
New World - A	1.16	0.44
New World - B	1.63	0.43
New World - A+B	2.95	1.02
New World - C	2.46	1.15
C-I (NA *D. pinnata* + *D. virletii*)	1.80	0.49
C-II (SA spreading-fruit)	0.66	0.16
C-III (SA appressed fruit)	0.67[†]	0.45
C-IV (SA appressed fruit)	0.33[†]	0.09
C-V (*D. cumingiana*)	0.17	0.00
D. obtusa ssp. *obtusa* (NA)	0.49[†]	0.11
New World - A+B+C	5.08	1.18
All North American accessions	5.08	1.18
All South American accessions (excluding *D. antarctica* type 2)	1.31	0.83
D (Canary Is. + *Hugueninia*)	--	1.22
Canary Is.	1.16	0.54
A+B+C+D	5.08	1.94

[†]Not present as distinct clade in ITS; members unresolved as part of clade C.

Table 2.5. Accessions pruned from combined ITS-chloroplast data set due to incongruence, redundancy, or absence of chloroplast sequence data.

Accession	Position in ITS tree	Position in chloroplast tree
Cloned ITS type not present in chloroplast tree [included type in brackets]		
D. antarctica D37 type 1	C [B]	-- [B]
D. antarctica E2 type 1	C [B]	-- [B]
D. incisa ssp. filipes D14 type 1	C [B]	-- [B]
D. paradisa ssp. *nevadensis* C8 type 1	C [B]	-- [B]
D. pinnata ssp. *brachycarpa* F11 type 1	C [B]	-- [B]
D. pinnata C4 type 1	C [B]	-- [B]
D. pinnata ssp. *glabra* C10 type 1	C [B]	-- [B]
D. pinnata ssp. *halictorum* C14 type 1	C [B]	-- [B]
D. pinnata ssp. *menziesii* C3 type 1	C [B]	-- [B]
D. pinnata ssp. *intermedia* C19 type 2	B [C]	-- [C]
Between-clade incongruence		
D. californica C9	A	C
D. californica D12	A	C
D. incisa ssp. incisa D57	A	C
D. incisa ssp. viscosa D21	A	C
D. pinnata ssp. *halictorum* C12	A	C
D. sophioides B112	A	C
D. sophioides F13	A	C
D. impatiens C40	C	A
D. impatiens C42	C	A
D. cf. *streptocarpa* C44	C	A
D. incisa ssp. incisa D25	A	polytomy with A & B
D. paradisa ssp. *nevadensis* C48	B	A
D. pinnata ssp. *nelsonii* C47	B	C
D. pinnata ssp. *nelsonii* D23	B	C
D. sophia B20	basal	basal
D. sophia E6	basal	basal
D. sophia MB3	basal	basal
Within-clade incongruence		
D. incana C2	A	A
D. incisa ssp. paysonii D28	A	A
D. incisa ssp. paysonii D73	A	A
D. obtusa ssp. *brevisiliqua* D58	A	A
D. obtusa ssp. *brevisiliqua* D59	A	A
D. obtusa ssp. *brevisiliqua* D72	A	A
D. cf. *obtusa* ssp. *brevisiliqua* D4	A	A

Table 2.5. Continued.

Accession	Position in ITS tree	Position in chloroplast tree
D. pinnata D68	A	A
D. pinnata D70	A	A
D. pinnata ssp. *halictorum* D19	A	A
D. pinnata ssp. *halictorum* D71	A	A
D. streptocarpa B33	A	A
D. pinnata ssp. *brachycarpa* F11 type 2	B	B
D. pinnata ssp. *nelsonii* C17	B	B
D. athrocarpa B94	C	C
D. depressa C37	C	C
D. depressa D31	C	C
D. leptoclada D46	C	C
D. myriophylla C29	C	C
D. myriophylla D13	C	C
D. myriophylla D16	C	C
D. pinnata ssp. *ochroleuca* D26	C	C
D. obtusa ssp. *adenophora* D61	C	C
D. obtusa ssp. *adenophora* D62	C	C
D. pinnata ssp. *glabra* B144	C	C
D. pinnata ssp. *halictorum* D69	C	C
D. pinnata ssp. *ochroleuca* D8	C	C
D. pinnata ssp. *pinnata* B12A	C	C
Not present in chloroplast tree (some regions would not amplify)		
D. cumingiana var. *cumingiana* D34	C	--
D. cumingiana var. *tenuissima* B103	C	--
D. cumingiana var. *tenuissima* D38	C	--
D. millefolia F1	D	--
D. pinnata ssp. *brachycarpa* F12	C	--
D. pinnata ssp. *pinnata* F6	C	--
Redundant		
D. antarctica D52	C	B
D. antarctica F15	C	B
D. bourgaeana B171	D	D
D. gilva B22	D	D
D. gonzalezi B160	D	D
D. kochii D3	basal	basal
D. kochii D18	basal	basal
D. lemsii B170	D	D
D. leptoclada C34	C	C
D. millefolia D1	D	D
D. millefolia D5	D	D
D. millefolia F2	D	D
D. obtusa ssp. *obtusa* D65	C	C
R. schimperi B106	basal	basal

Table 2.6. Estimated node ages with standard deviations (from parametric bootstrapping) calculated using penalized likelihood in r8s, based on branch lengths from reduced ITS and chloroplast maximum likelihood trees (Figs. 2.11 and 2.12). Ages marked with an * were fixed in the analysis. MRCA = most recent common ancestor.

Clade or Node	Age (mya) estimated from ITS (Fig. 2.11)	Age (mya) estimated from from chloroplast (Fig. 2.12)
MRCA *Arabidopsis*, *Brassica*	20.0*	--
MRCA *Arabidopsis*, *Descurainia*	17.4	17.4*
Descurainieae	11.03 +/- 0.98	--
MRCA *Hornungia*, *Tropidocarpum*	10.24 +/- 0.98	--
MRCA *H. alpina*, *H. procumbens*	6.09 +/- 0.90	--
MRCA *Robeschia*, *Descurainia*	6.75 +/- 0.82	8.67 +/- 0.48
MRCA *D. kochii*, New World *Descurainia*	5.47 +/- 0.70	7.27 +/- 0.44
MRCA *D. sophia*, NW & Canary Is.	--	5.56 +/- 0.43
MRCA *Cardamine*, *Rorippa*	≥ 5.0*	--
NW *Descurainia* – Canary Is. – *Hugueninia*	2.72 +/- 0.38	2.92 +/- 0.26
MRCA *D. kochii*, *D. sophia*	2.52 +/- 0.57	--
New World *Descurainia*	--	1.93 +/- 0.17
New World clade B	1.07 +/- 0.25	--
New World clade C	1.07 +/- 0.25	--
New World clade A	0.87 +/- 0.31	--
MRCA Canary Is., *Hugueninia*	1.00 +/- 0.33	2.21 +/- 0.26
Canary Is.	0.75 +/- 0.27	0.77 +/- 0.21

Table 2.7. Comparison of *Descurainia* and related genera. Information compiled from Spegazzini (1893), Schulz (1924, 1936), Detling (1939), Ball (1964), Jafri (1973), Bramwell (1977), Romanczuk (1984a), Bramwell & Bramwell (1990), Ortiz (1993), Rollins (1993a, c), Al-Shehbaz (1999, 2003), Al-Shehbaz & O'Kane (1999), Boulos (1999), and Appel & Al-Shehbaz (2003). A = annual, B = biennial, and P = perennial.

Character	*D. sophia*	*D. kochii*	New World	Canary Is.	*Hugueninia*	*Robeschia*	*Ianhedgea*	*Hornungia*	*Tropidocarpum*	*Trichotolinum*
Duration	A	A	A, B, P	P	P	A	A	A, P	A	P
Habit	Herbs	Herbs	Herbs	Suffrutescent herbs	Herbs	Herbs	Herbs	Herbs	Herbs	Suffrutescent herbs
Unicellular glands	Absent	Absent	Present or absent	Present or absent	Absent	Absent	Absent	Absent	Absent	Absent
Leaf divisions	2- or 3-pinnatisect	2-pinnatisect	2- or 3-pinnatisect or rarely pinnate	1- to 3-pinnatisect or pinnate	1- to 2-pinnatisect	2-pinnatisect	Finely pinnatisect or trisect	Pinnatisect, dentate or entire	Pinnatisect to pinnatifid (cauline lvs only)	Pinnatifid to pinnatisect (basal lvs only)
Racemes bracteate	Absent	Absent	Rarely basally	Absent	Absent	Absent	Absent	Absent	Present throughout	Absent
Petal length (mm)	2 - 2.5	3	1 - 4	3 - 6	2 - 4	4 - 5	1 - 1.8	0.6 - 1.2	1.6 - 5	3 - 4
Petal color	Yellow	Intense yellow or orange	Yellow or white	Yellow	Yellow	White or pale lilac	White or pink	White	Yellow or yellowish	White
# stamens	6	6	6	6	6	6	6	4 or 6	6	6
Style	Short or obsolete	Short	Obsolete or rarely prominent	Prominent to obsolete	Very short	Obsolete	Absent or obsolete	Absent or obsolete	Prominent to obsolete	Very prominent
Locules per ovary	2	2	2	2	2	2	2	2	2 or 4	2

Table 2.7. Continued.

Character	*D. sophia*	*D. kochii*	New World	Canary Is.	*Hugueninia*	*Robeschia*	*Ianhedgea*	*Hornungia*	*Tropidocarpum*	*Trichotolinum*
Ovules per ovary	20 - 40	8-15	4-85	6-32	6-11	22 - 28	(6-)10-20	4-20	4-70	10-14
Fruit shape	Linear	Linear	Linear, oblong, clavate, or elliptic	Linear or oblong	Oblong	Linear, somewhat tetragonal, tapering above	Linear	Elliptic to ovate or lanceolate	Linear, oblong, elliptic, or obdeltoid	Linear
Fruit compression	Nearly terete	Quadrangular	Terete or rarely quadrangular	Quadrangular	Quadrangular	Terete	Terete	Angustiseptate	Angustiseptate	Terete
Fruit pubescence	Glabrous	Glabrous	Glabrous or pubescent	Glabrous	Glabrous	Usually densely hairy	Glabrous or minutely dendritic	Mostly glabrous	Retrorsely or antrorsely pubescent, rarely glabrous	Glabrous
Fruit orientation	Divaricately ascending	Pedicels patent or slightly reflexed; fruit erect	Erect to widely spreading	Patent, erect or ascending	Ascending	Ascending or suberect	Divaricate or appressed to rachis	Ascending to divaricate	Ascending to divaricate	Erect to ascending
Thickened fruiting pedicels	Absent	Absent	Absent	Absent	Absent	Present	Absent or Present	Absent	Absent	Absent
Seed arrangement	Uniseriate	Uniseriate	Biseriate or uniseriate	Uniseriate or biseriate	Uniseriate	Uniseriate	Uniseriate	Biseriate or aseriate	Uniseriate	Uniseriate
Seed mucilage	Present	Present	Present	Present	Absent	Present	Absent	Absent or Present	Present	Not reported
Winged seeds	Absent	Absent	Absent	Present	Absent	Absent	Absent	Absent	Absent	Not reported
Cotyledons	Incumbent	Incumbent	Incumbent	Incumbent	Incumbent	Not reported	Incumbent	Incumbent or accumbent	Incumbent	Not reported

Table 2.7. Continued.

Character	*D. sophia*	*D. kochii*	New World	Canary Is.	*Hugueninia*	*Robeschia*	*Ianhedgea*	*Hornungia*	*Tropidocarpum*	*Trichotolinum*
# of species	1	1	ca 30 - 35	7	1	1	1	3	4	1
Distribution	Eurasia (introduced elsewhere)	Turkey, Caucasia	NA & SA	Canary Islands	Europe	Middle East	C & SW Asia	Europe (1 sp. into Asia & W NA)	California, Baja California, (1 sp. Chile)	Patagonia
Chromosome numbers	$x = 7$ ($2n = 28$)	Not reported	$x = 7$ ($2n = 14,28,42$)	$x = 7$ ($2n = 14$)	$x = 7$ ($2n = 14$)	$x = 8$	$x = 7$ ($2n = 28$)	$x = 6$ ($2n = 12,24$)	$x = 8$	Not reported

Table 2.8. Primer sequences for non-coding chloroplast markers designed in this study but not employed in the phylogenetic analysis.

Region	Primer	Sequence
rps11-rps8	*rps11*-F	5'-GTATTGTTGAAACTTGCTTGAAC-3'
	rps8-R	5'-CGACTTCTCAAGGTATAATGAC-3'
ndhC-trnV	*ndhC*-F	5'-TGCCAAAACAGGAATAGCAC-3'
	trnV-R	5'-TTTACCGAGCAGGTCTACGG-3'
rbcL-accD	*rbcL*-F	5'-GCTGCTGCTTGTGAAGTATGG-3'
	accD-R	5'-AACTATCCATTGCTTTACTTAGC-3'
accD-psaI	*accD*-F	5'-AGCGAGTTATTTCAGCTCCATGC-3'
	psaI-R	5'-GGTAAGTTATTGAAAGTTGTC-3'
rpl32-trnL	*rpl32*-F	5'-CATTAGGGAAATCACTTT-3'
	trnL-R	5'-GCGTGTCTACCAATTTCACC-3'
rps16-psbK	*rps16*-F	5'-CGTTGCTTTCTACCACATCG-3
	psbK-R	5'-CGCATAACATCTACGATTGG-3'
trnS-trnG	*trnS*-F	5'-CAATCCAACGCTTTAGTCCAC-3'
	trnG-R	5'-ATCGTTAGCTTGGAAGGC-3'
atpF-atpH	*atpF*-F	5'-GACCCAAGAAACGAAAGAATCGG-3'
	atpH-R	5'-GCCTGGTTGTAGCATTAGC-3'
petA-psbJ	*petA*-F	5'-GAGAAGGTTCAATTATCCGAAATG-3'
	psbJ-R	5'-GATTAGGTTCATCCCTGTAG-3'

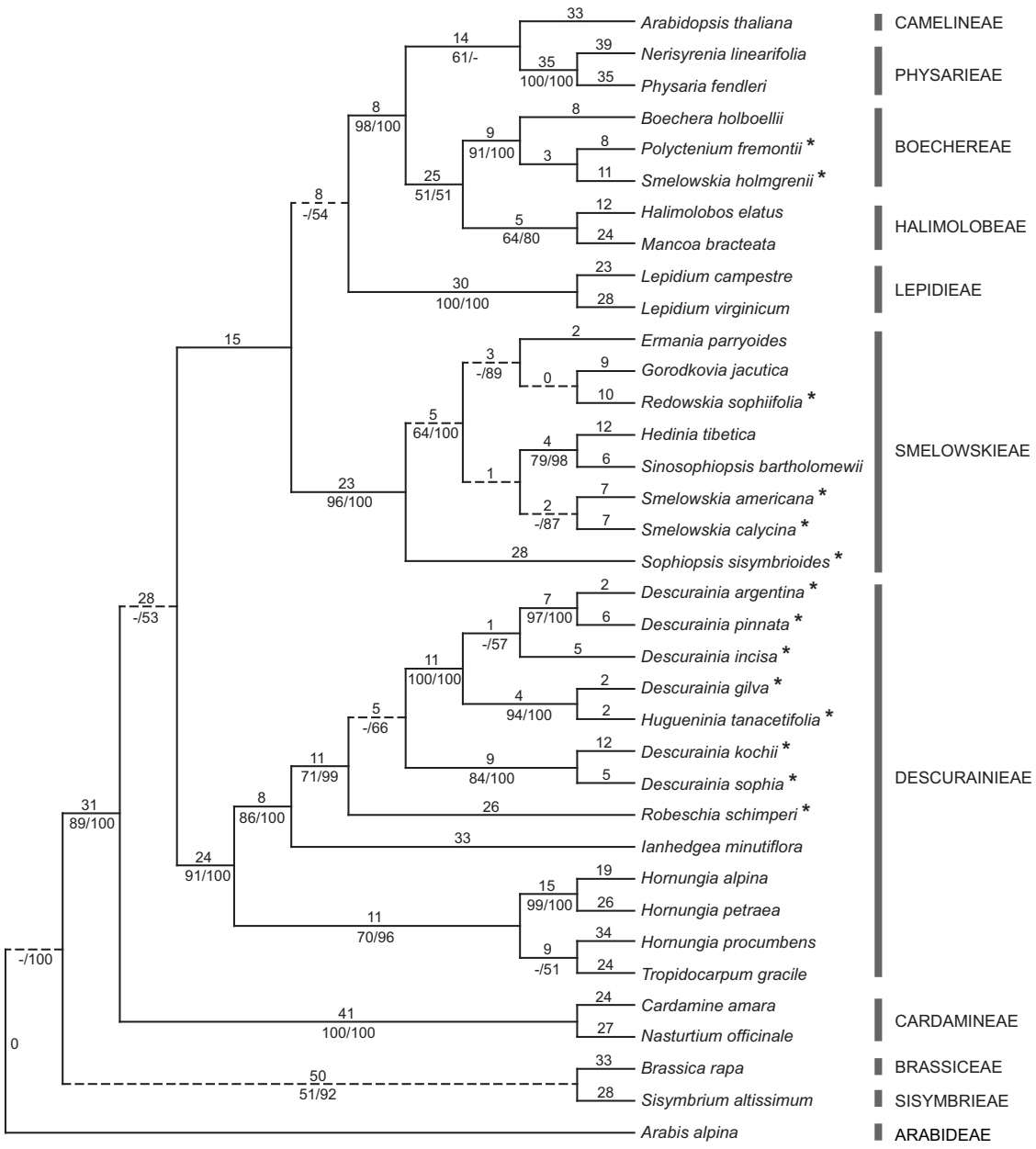


Fig. 2.1. One of 553 most parsimonious trees generated from nuclear ITS data to assess the monophyly of *Descurainia*. Branch lengths are indicated above branches; bootstrap values > 50%/Bayesian posterior probabilities are below branches. Dashed lines indicate branches that collapse in the strict consensus tree. Species tagged with an * belong to the subtribe Descurainiinae as circumscribed by Schulz (1924); tribal classifications on the right-hand side are those proposed by Al-Shehbaz & al., 2006.

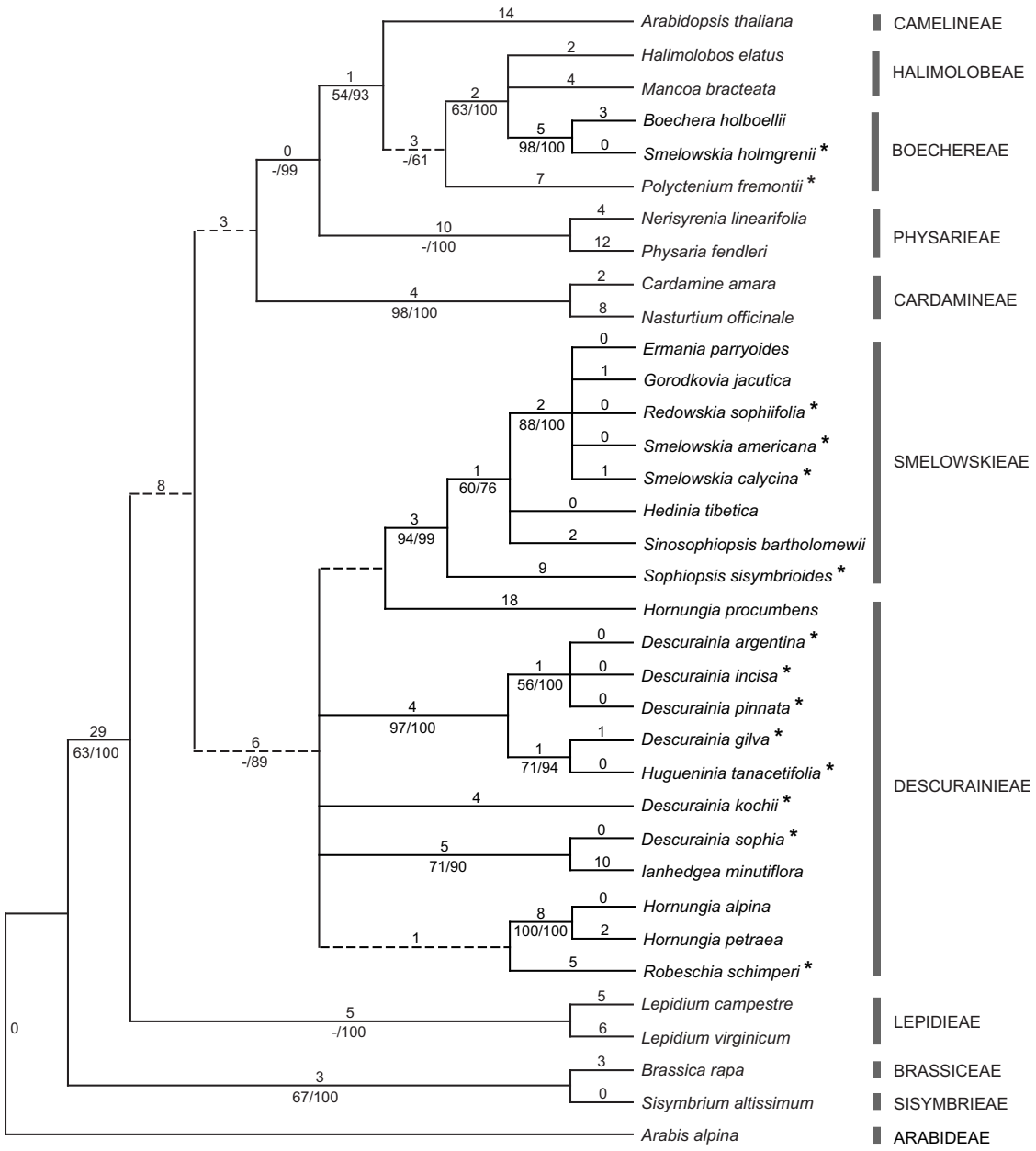


Fig. 2.2 One of 8734 most parsimonious trees derived from chloroplast *trnL* data to assess the monophyly of *Descurainia*. Branch lengths are indicated above branches; bootstrap values >50%/Bayesian posterior probabilities are below branches. Dashed lines indicate branches that collapse in the strict consensus tree. Species tagged with an * belong to the subtribe Descurainiinae as circumscribed by Schulz (1924); tribal classifications on the right-hand side are those proposed by Al-Shehbaz & al., 2006.

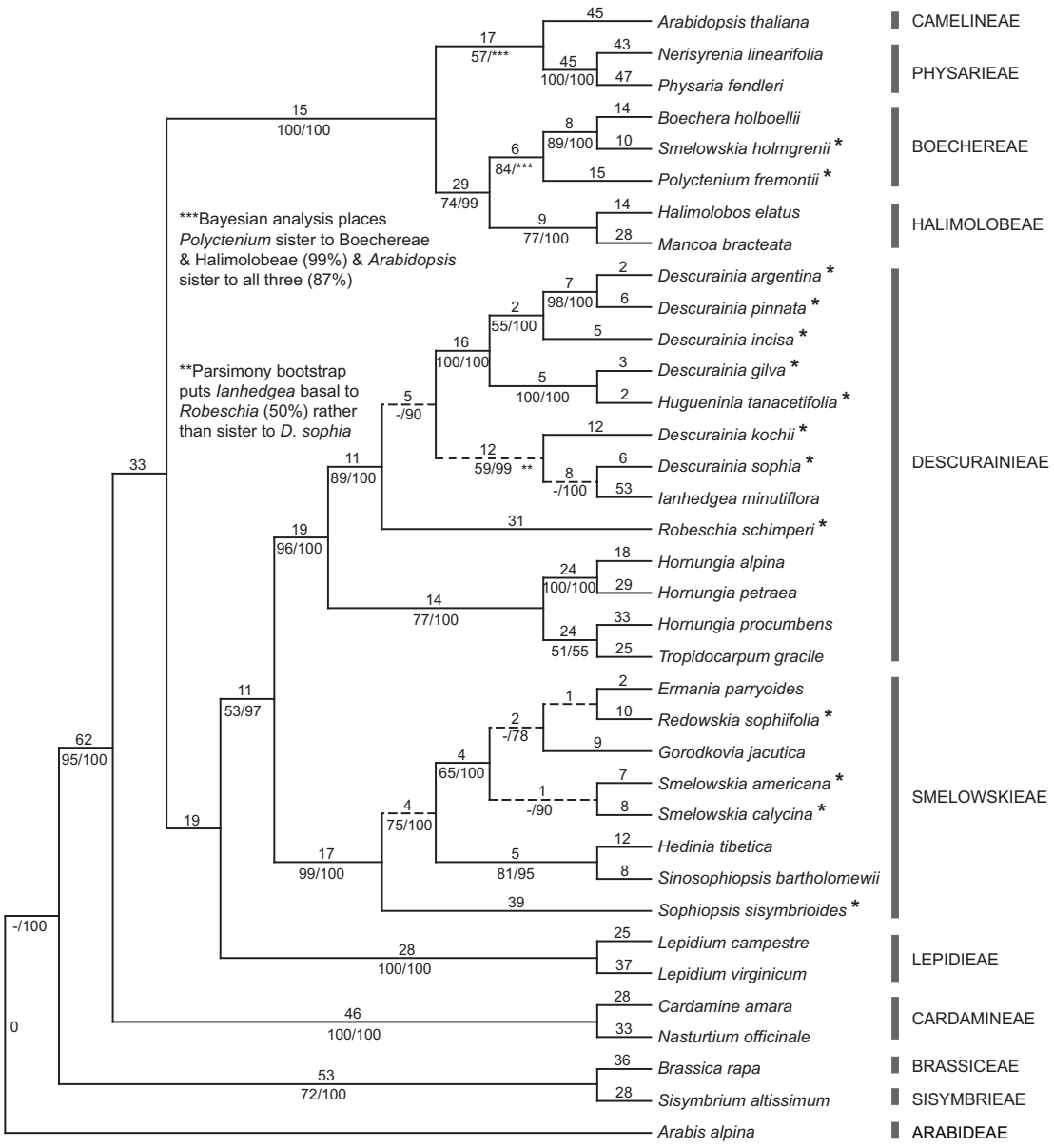
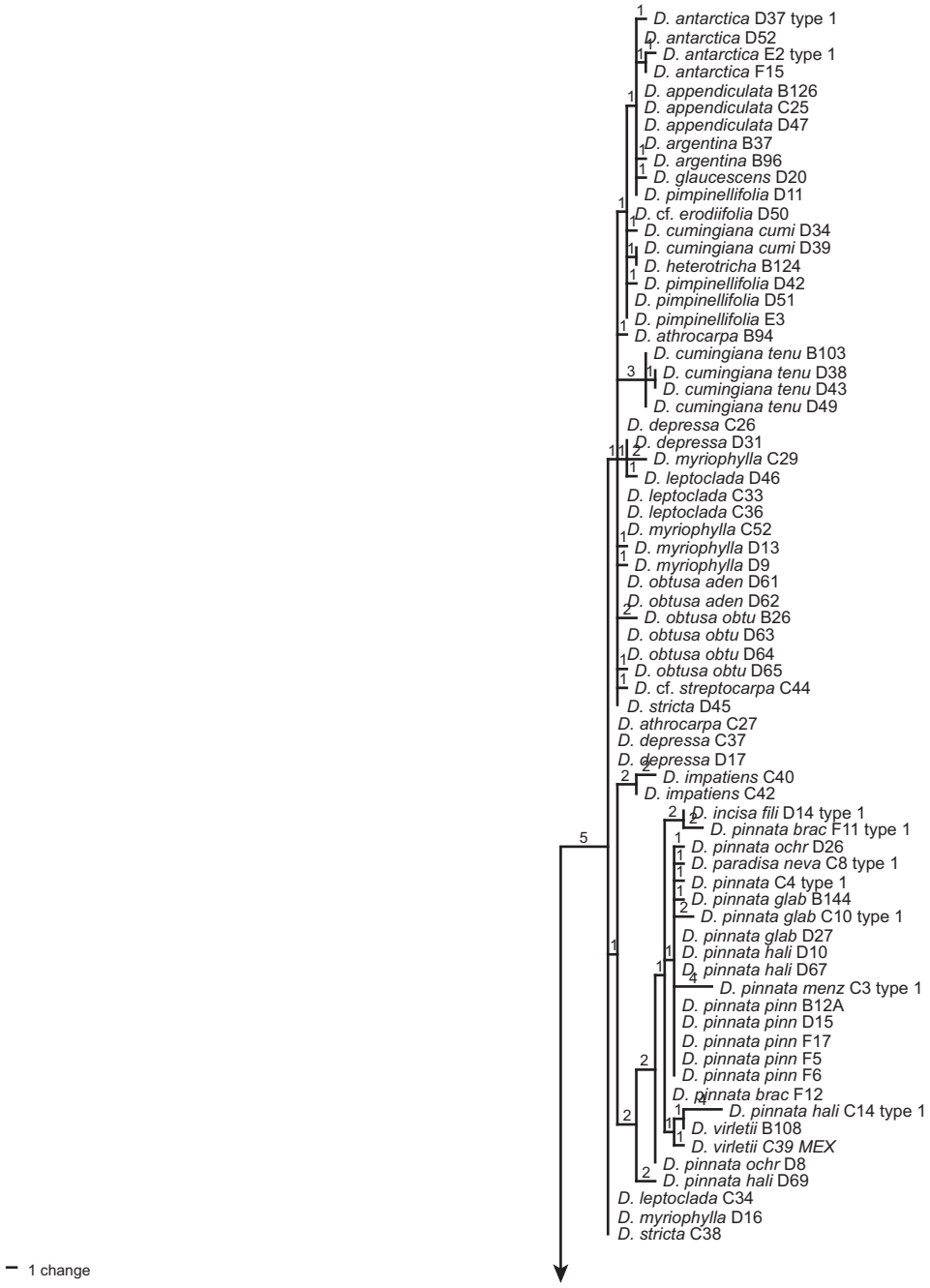


Fig. 2.3. One of 41 most parsimonious trees derived from combined ITS-*trnL* data to assess the monophyly of *Descurainia*. Branch lengths are indicated above branches; bootstrap values >50%/Bayesian posterior probabilities are below branches. Dashed lines indicate branches that collapse in the strict consensus tree. Species tagged with an * belong to the subtribe Descurainiinae as circumscribed by Schulz (1924); tribal classifications on the right-hand side are those proposed by Al-Shehbaz & al., 2006.

Fig. 2.4. One of 4020 most parsimonious trees recovered from ITS data to assess relationships within *Descurainia* using the parsimony ratchet. Branch lengths are indicated above branches. Generic names are abbreviated as follows: *A*. = *Arabidopsis*, *D*. = *Descurainia*, *H*. = *Hugueninia*, *I*. = *Ianhedgea*, *R*. = *Robeschia*, and *S*. = *Sisymbrium* (*altissimum*) or *Smelowskia* (*americana*).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D. cf. erodiifolia D50
D. cumingiana cumi D34
D. cumingiana cumi D39
D. heterotricha B124
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D. pimpinellifolia E3
D. athrocarpa B94
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D. cumingiana tenu D38
D. cumingiana tenu D43
D. cumingiana tenu D49
D. depressa C26
D. depressa D31
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D. leptoclada D46
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D. leptoclada C36
D. myriophylla C52
D. myriophylla D13
D. myriophylla D9
D. obtusa aden D61
D. obtusa aden D62
D. obtusa obtu B26
D. obtusa obtu D63
D. obtusa obtu D64
D. obtusa obtu D65
D. cf. streptocarpa C44
D. stricta D45
D. athrocarpa C27
D. depressa C37
D. depressa D17
D. impatiens C40
D. impatiens C42
D. incisa fili D14 type 1
D. pinnata brac F11 type 1
D. pinnata ochr D26
D. paradisa neva C8 type 1
D. pinnata C4 type 1
D. pinnata glab B144
D. pinnata glab C10 type 1
D. pinnata glab D27
D. pinnata hali D10
D. pinnata hali D67
D. pinnata menz C3 type 1
D. pinnata pinn B12A
D. pinnata pinn D15
D. pinnata pinn F17
D. pinnata pinn F5
D. pinnata pinn F6
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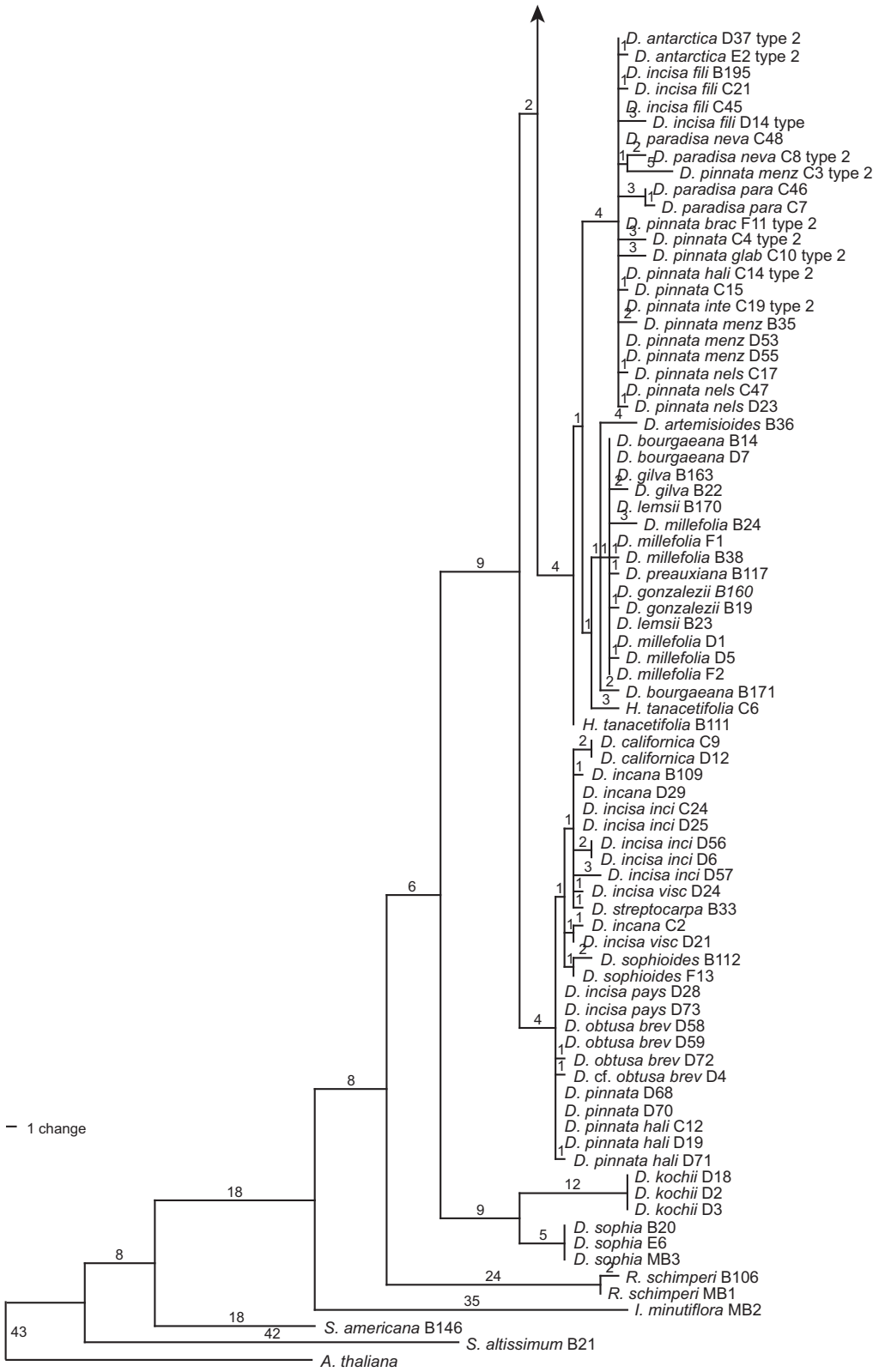
D. myriophylla D16
D. stricta C38
1 change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D. paradisa neva C8 type 2
D. pinnata menz C3 type 2
D. paradisa para C46
D. paradisa para C7
D. pinnata brac F11 type 2
D. pinnata C4 type 2
D. pinnata glab C10 type 2
D. pinnata hali C14 type 2
D. pinnata C15
D. pinnata inte C19 type 2
D. pinnata menz B35
D. pinnata menz D53
D. pinnata menz D55
D. pinnata nels C17
D. pinnata nels C47
D. pinnata nels D23
D. artemisioides B36
D. bourgaeana B14
D. bourgaeana D7
D. gilva B163
D. gilva B22
D. lemsii B170
D. millefolia B24
D. millefolia F1
D. millefolia B38
D. preauxiana B117
D. gonzalezii B160
D. gonzalezii B19
D. lemsii B23
D. millefolia D1
D. millefolia D5
D. millefolia F2
D. bourgaeana B171
H. tanacetifolia C6
H. tanacetifolia B111
D. californica C9
D. californica D12
D. incana B109
D. incana D29
D. incisa inci C24
D. incisa inci D25
D. incisa inci D56
D. incisa inci D6
D. incisa inci D57
D. incisa visc D24
D. streptocarpa B33
D. incana C2
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D. obtusa brev D58
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D. obtusa brev D72
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D. pinnata D68
D. pinnata D70
D. pinnata hali C12
D. pinnata hali D19
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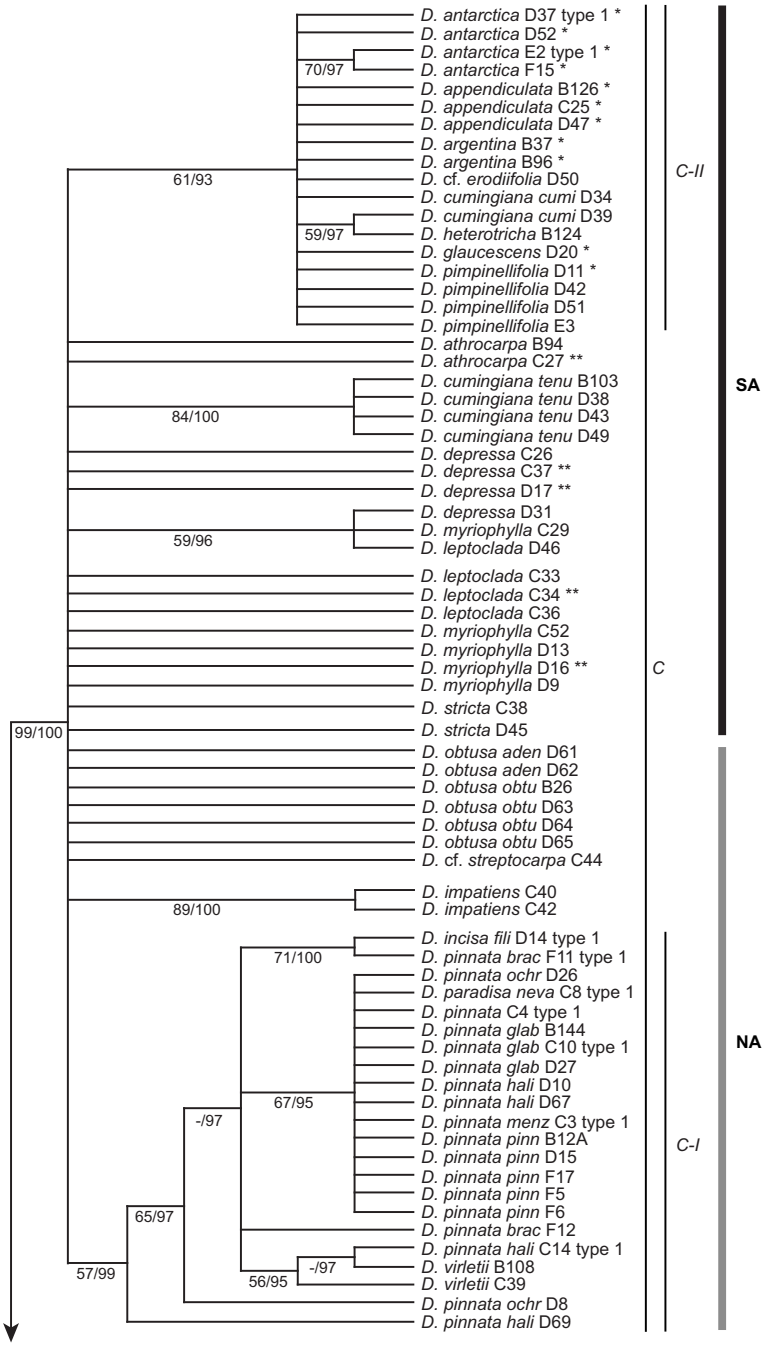
D. kochii D18
D. kochii D2
D. kochii D3
D. sophia B20
D. sophia E6
D. sophia MB3
R. schimperi B106
R. schimperi MB1
I. minutiflora MB2
S. americana B146
S. altissimum B21
A. thaliana
1 change

Fig. 2.5. Strict consensus of 4020 most parsimonious trees derived from ITS data to assess relationships within *Descurainia* using the parsimony ratchet. Bootstrap values > 50%/Bayesian posterior probabilities are indicated below branches. Generic names are abbreviated as follows: *A*. = *Arabidopsis*, *D*. = *Descurainia*, *H*. = *Hugueninia*, *I*. = *Ianhedgea*, *R*. = *Robeschia*, and *S*. = *Sisymbrium* (*altissimum*) or *Smelowskia* (*americana*). The designations A, B, C, C-I, C-II, and D refer to clades described in the text. Distributions are abbreviated as follows: NA = North America, SA = South America, CI = Canary Islands, and EU = Europe, Eurasia and/or Middle East. Additional clades recovered from parsimony bootstrap or Bayesian analysis which do not appear in the strict consensus tree are marked by: * (PP = 81%); ** (all members of clade C *except* these accessions) (PP = 72%); *** (BV = 63%; PP = 81%). Bayesian analysis also groups H. tanacetifolia B111 as sister to clade D (55%).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D. appendiculata D47 *
D. argentina B37 *
D. argentina B96 *
D. cf. erodiifolia D50
D. cumingiana cumi D34
D. cumingiana cumi D39
D. heterotricha B124
D. glaucescens D20 *
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D. pimpinellifolia E3
D. athrocarpa B94
D. athrocarpa C27 **
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D. depressa C26
D. depressa C37 **
D. depressa D17 **
D. depressa D31
D. myriophylla C29
D. leptoclada D46
D. leptoclada C33
D. leptoclada C34 **
D. leptoclada C36
D. myriophylla C52
D. myriophylla D13
D. myriophylla D16 **
D. myriophylla D9
D. stricta C38
D. stricta D45
D. obtusa aden D61
D. obtusa aden D62
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D. obtusa obtu D63
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D. pinnata ochr D26
D. paradisa neva C8 type 1
D. pinnata C4 type 1
D. pinnata glab B144
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D. pinnata glab D27
D. pinnata hali D10
D. pinnata hali D67
D. pinnata menz C3 type 1
D. pinnata pinn B12A
D. pinnata pinn D15
D. pinnata pinn F17
D. pinnata pinn F5
D. pinnata pinn F6
D. pinnata brac F12
D. pinnata hali C14 type 1
D. virletii B108
D. virletii C39
D. pinnata ochr D8
D. pinnata hali D69
70/97
61/93
59/97
84/100
59/96
99/100
89/100
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-/97
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57/99
-/97
56/95
C-II
C
C-I
SA
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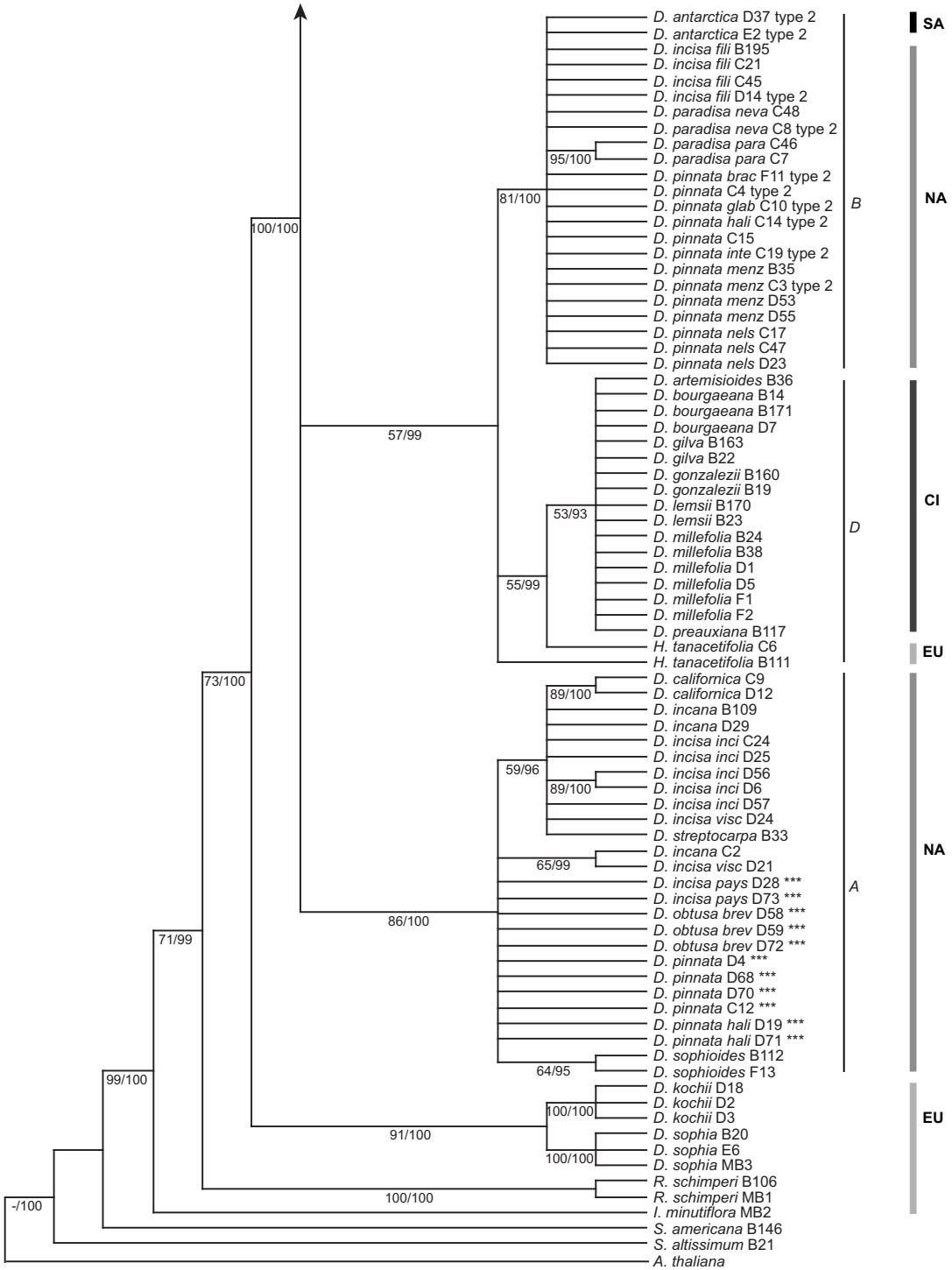
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D. paradisa neva C48
D. paradisa neva C8 type 2
D. paradisa para C46
D. paradisa para C7
D. pinnata brac F11 type 2
D. pinnata C4 type 2
D. pinnata glab C10 type 2
D. pinnata hali C14 type 2
D. pinnata C15
D. pinnata inte C19 type 2
D. pinnata menz B35
D. pinnata menz C3 type 2
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D. bourgaeana B14
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D. gilva B163
D. gilva B22
D. gonzalezii B160
D. gonzalezii B19
D. lemsii B170
D. lemsii B23
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D. millefolia B38
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D. millefolia D5
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D. millefolia F2
D. preauxiana B117
H. tanacetifolia C6
H. tanacetifolia B111
D. californica C9
D. californica D12
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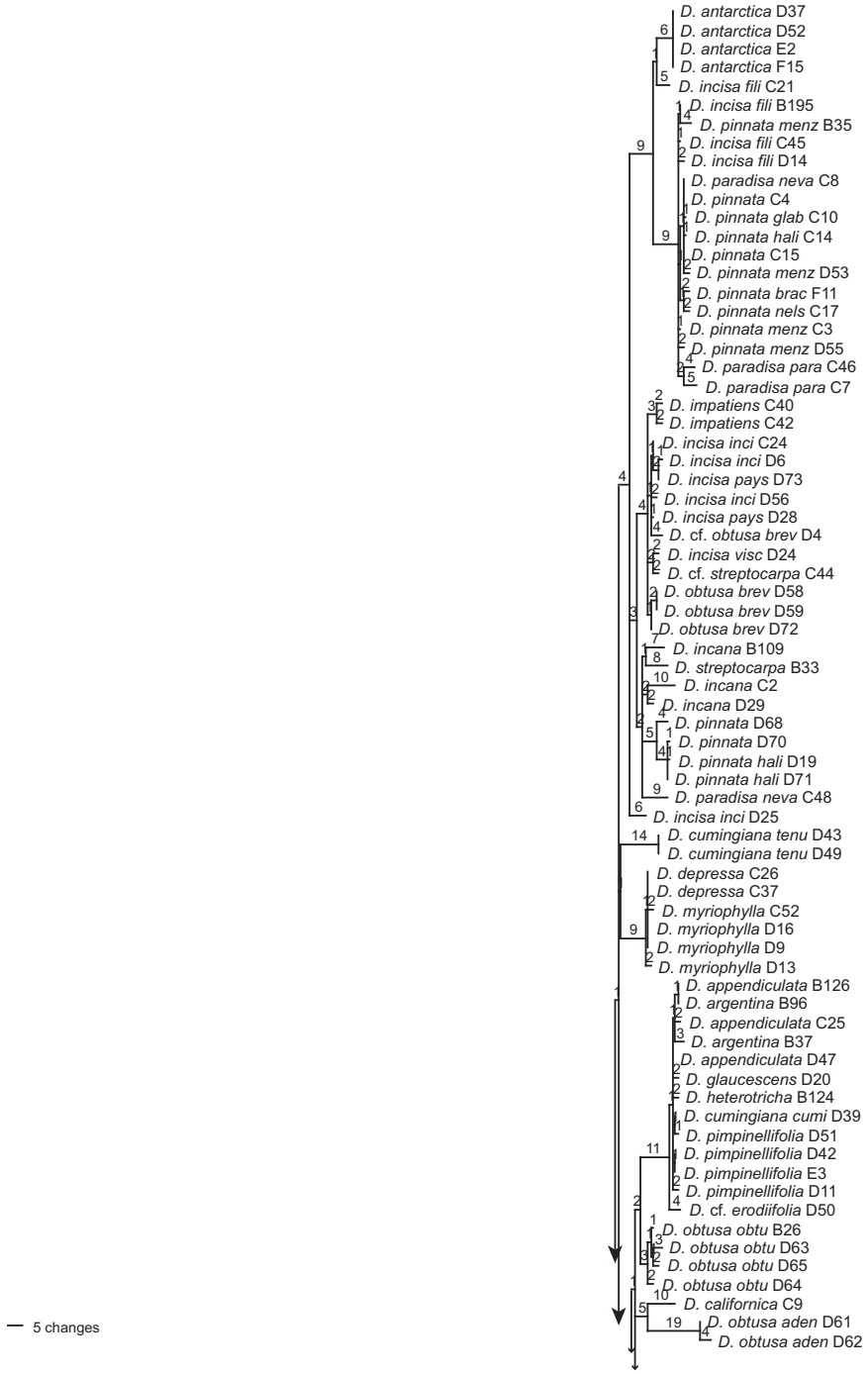
D. incisa inci D56
D. incisa inci D6
D. incisa inci D57
D. incisa visc D24
D. streptocarpa B33
D. incana C2
D. incisa visc D21
D. incisa pays D28 ***
D. incisa pays D73 ***
D. obtusa brev D58 ***
D. obtusa brev D59 ***
D. obtusa brev D72 ***
D. pinnata D4 ***
D. pinnata D68 ***
D. pinnata D70 ***
D. pinnata C12 ***
D. pinnata hali D19 ***
D. pinnata hali D71 ***
D. sophioides B112
D. sophioides F13
D. kochii D18
D. kochii D2
D. kochii D3
D. sophia B20
D. sophia E6
D. sophia MB3
R. schimperi B106
R. schimperi MB1
I. minutiflora MB2
S. americana B146
S. altissimum B21
A. thaliana
95/100
81/100
100/100
57/99
53/93
55/99
89/100
59/96
89/100
65/99
86/100
64/95
100/100
91/100
100/100
73/100
71/99
99/100
-/100
100/100
B
D
A
SA
NA
CI
EU
NA
EU

Fig. 2.6. One of 3419 most parsimonious trees recovered from combined chloroplast data to assess relationships within *Descurainia* using the parsimony ratchet. Branch lengths are indicated above branches. Generic names are abbreviated as follows: *A*. = *Arabidopsis*, *D*. = *Descurainia*, *H*. = *Hugueninia*, *I*. = *Ianhedgea*, *R*. = *Robeschia*, and *S*. = *Sisymbrium* (*altissimum*) or *Smelowskia* (*americana*).


D. antarctica D37
D. antarctica D52
D. antarctica E2
D. antarctica F15
D. incisa fili C21
D. incisa fili B195
D. pinnata menz B35
D. incisa fili C45
D. incisa fili D14
D. paradisa neva C8
D. pinnata C4
D. pinnata glab C10
D. pinnata hali C14
D. pinnata C15
D. pinnata menz D53
D. pinnata brac F11
D. pinnata nels C17
D. pinnata menz C3
D. pinnata menz D55
D. paradisa para C46
D. paradisa para C7
D. impatiens C40
D. impatiens C42
D. incisa inci C24
D. incisa inci D6
D. incisa pays D73
D. incisa inci D56
D. incisa pays D28
D. cf. obtusa brev D4
D. incisa visc D24
D. cf. streptocarpa C44
D. obtusa brev D58
D. obtusa brev D59
D. obtusa brev D72
D. incana B109
D. streptocarpa B33
D. incana C2
D. incana D29
D. pinnata D68
D. pinnata D70
D. pinnata hali D19
D. pinnata hali D71
D. paradisa neva C48
D. incisa inci D25
D. cumingiana tenu D43
D. cumingiana tenu D49
D. depressa C26
D. depressa C37
D. myriophylla C52
D. myriophylla D16
D. myriophylla D9
D. myriophylla D13
D. appendiculata B126
D. argentina B96
D. appendiculata C25
D. argentina B37
D. appendiculata D47
D. glaucescens D20
D. heterotricha B124
D. cumingiana cumi D39
D. pimpinellifolia D51
D. pimpinellifolia D42
D. pimpinellifolia E3
D. pimpinellifolia D11
D. cf. erodiifolia D50
D. obtusa obtu B26
D. obtusa obtu D63
D. obtusa obtu D65
D. obtusa obtu D64
D. californica C9
D. obtusa aden D61
D. obtusa aden D62
5 changes

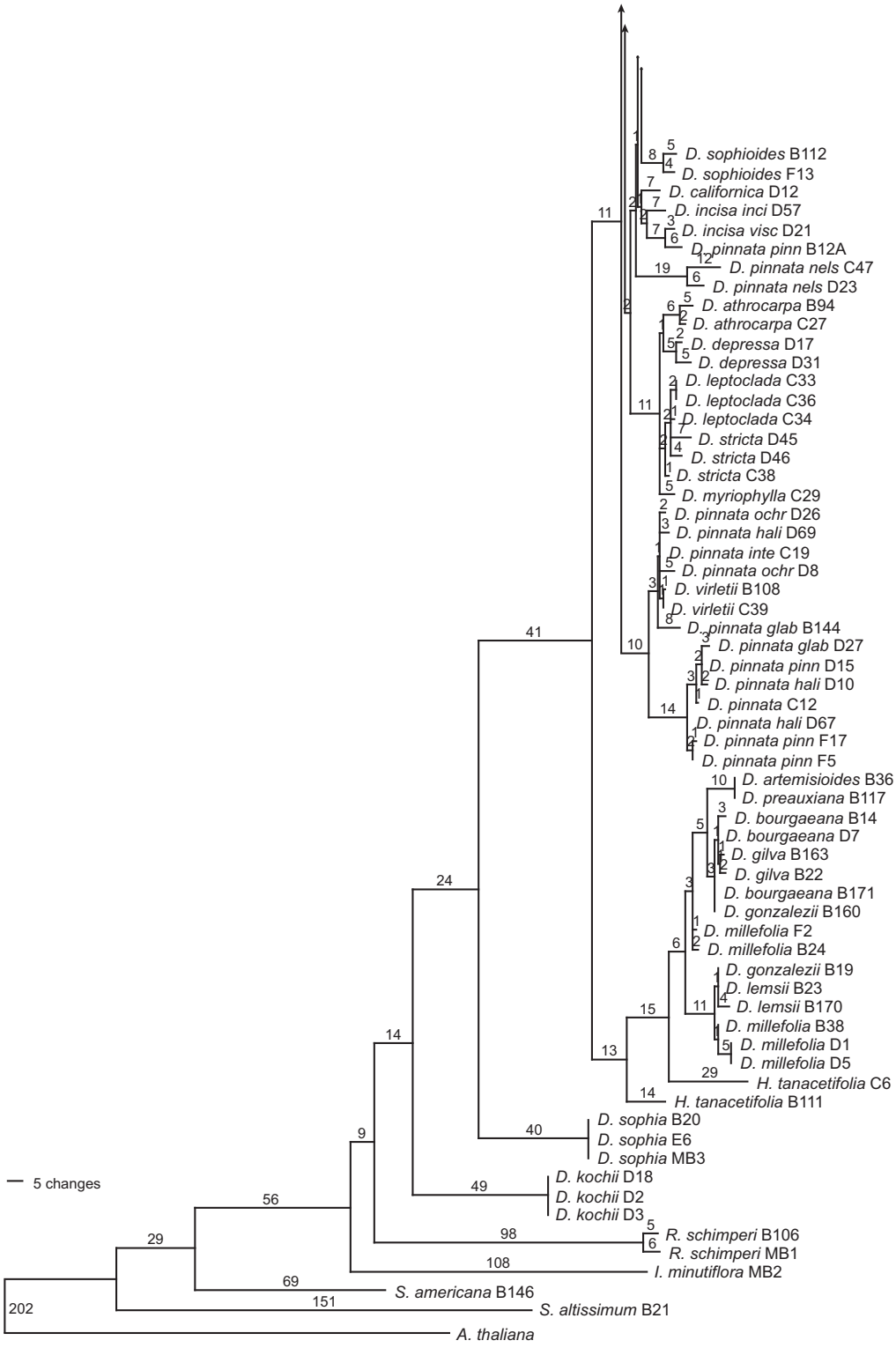
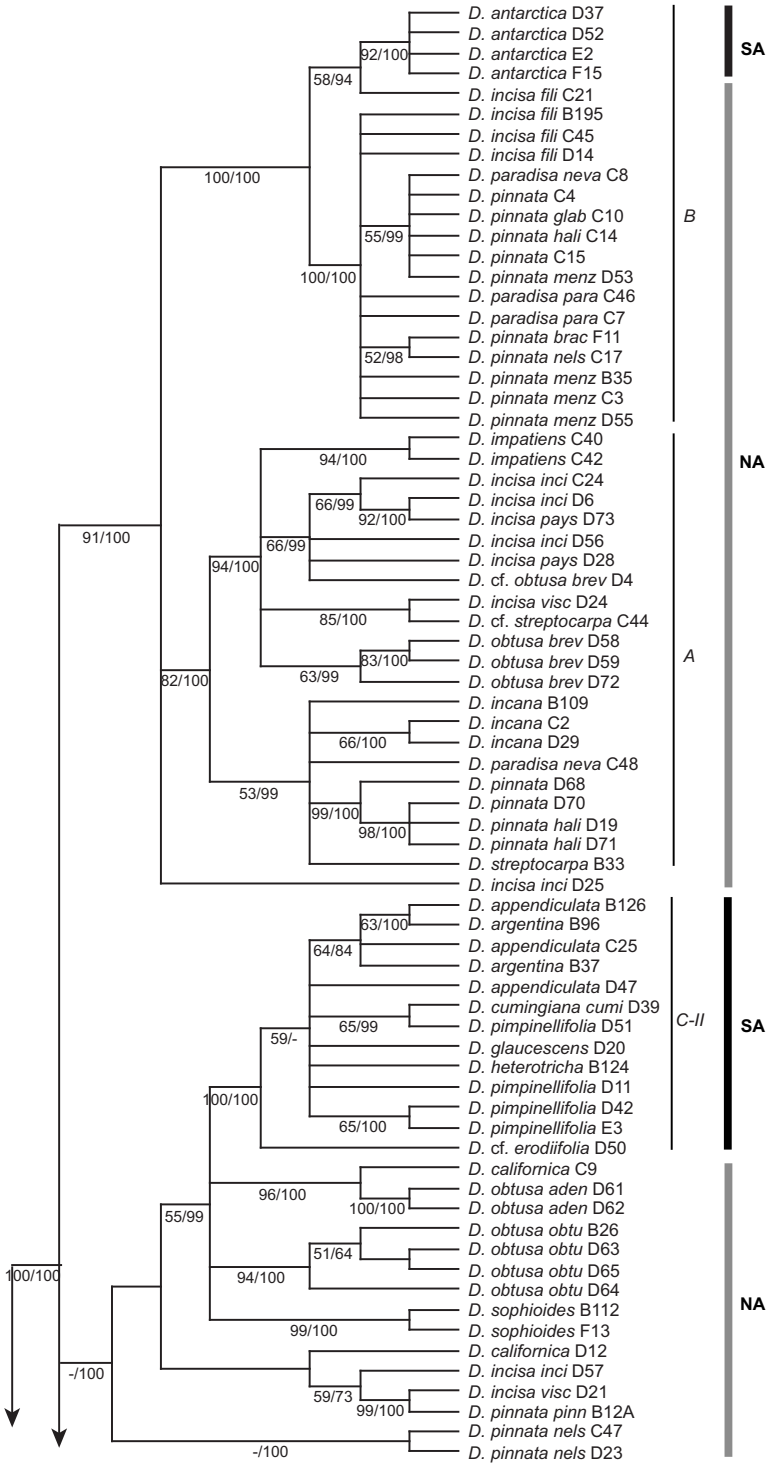

5 changes
D. sophioides B112
D. sophioides F13
D. californica D12
D. incisa inci D57
D. incisa visc D21
D. pinnata pinn B12A
D. pinnata nels C47
D. pinnata nels D23
D. athrocarpa B94
D. athrocarpa C27
D. depressa D17
D. depressa D31
D. leptoclada C33
D. leptoclada C36
D. leptoclada C34
D. stricta D45
D. stricta D46
D. stricta C38
D. myriophylla C29
D. pinnata ochr D26
D. pinnata hali D69
D. pinnata inte C19
D. pinnata ochr D8
D. virletii B108
D. virletii C39
D. pinnata glab B144
D. pinnata glab D27
D. pinnata pinn D15
D. pinnata hali D10
D. pinnata C12
D. pinnata hali D67
D. pinnata pinn F17
D. pinnata pinn F5
D. artemisioides B36
D. preauxiana B117
D. bourgaeana B14
D. bourgaeana D7
D. gilva B163
D. gilva B22
D. bourgaeana B171
D. gonzalezii B160
D. millefolia F2
D. millefolia B24
D. gonzalezii B19
D. lemsii B23
D. lemsii B170
D. millefolia B38
D. millefolia D1
D. millefolia D5
H. tanacetifolia C6
H. tanacetifolia B111
D. sophia B20
D. sophia E6
D. sophia MB3
D. kochii D18
D. kochii D2
D. kochii D3
R. schimperi B106
R. schimperi MB1
I. minutiflora MB2
S. americana B146
S. altissimum B21
A. thaliana

Fig. 2.7. Strict consensus of 3419 most parsimonious trees derived from combined chloroplast data to assess relationships within *Descurainia* using the parsimony ratchet. Bootstrap values > 50%/Bayesian posterior probabilities are indicated below branches. Generic names are abbreviated as follows: *A.* = *Arabidopsis*, *D.* = *Descurainia*, *H.* = *Hugueninia*, *I.* = *Ianhedgea*, *R.* = *Robeschia*, and *S.* = *Sisymbrium* (*altissimum*) or *Smelowskia* (*americana*). The designations A, B, C, C-I, C-II, C-III, C-IV, C-V and D refer to clades described in the text. Distributions are abbreviated as follows: NA = North America, SA = South America, CI = Canary Islands, and EU = Europe, Eurasia and/or Middle East. Two additional branches within clade B recovered from Bayesian analysis with very weak support (PP < 72%) are not shown.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D. antarctica D52
D. antarctica E2
D. antarctica F15
D. incisa fili C21
D. incisa fili B195
D. incisa fili C45
D. incisa fili D14
D. paradisa neva C8
D. pinnata C4
D. pinnata glab C10
D. pinnata hali C14
D. pinnata C15
D. pinnata menz D53
D. paradisa para C46
D. paradisa para C7
D. pinnata brac F11
D. pinnata nels C17
D. pinnata menz B35
D. pinnata menz C3
D. pinnata menz D55
D. impatiens C40
D. impatiens C42
D. incisa inci C24
D. incisa inci D6
D. incisa pays D73
D. incisa inci D56
D. incisa pays D28
D. cf. obtusa brev D4
D. incisa visc D24
D. cf. streptocarpa C44
D. obtusa brev D58
D. obtusa brev D59
D. obtusa brev D72
D. incana B109
D. incana C2
D. incana D29
D. paradisa neva C48
D. pinnata D68
D. pinnata D70
D. pinnata hali D19
D. pinnata hali D71
D. streptocarpa B33
D. incisa inci D25
D. appendiculata B126
D. argentina B96
D. appendiculata C25
D. argentina B37
D. appendiculata D47
D. cumingiana cumi D39
D. pimpinellifolia D51
D. glaucescens D20
D. heterotricha B124
D. pimpinellifolia D11
D. pimpinellifolia D42
D. pimpinellifolia E3
D. cf. erodiifolia D50
D. californica C9
D. obtusa aden D61
D. obtusa aden D62
D. obtusa obtu B26
D. obtusa obtu D63
D. obtusa obtu D65
D. obtusa obtu D64
D. sophioides B112
D. sophioides F13
D. californica D12
D. incisa inci D57
D. incisa visc D21
D. pinnata pinn B12A
D. pinnata nels C47
D. pinnata nels D23
92/100
58/94
100/100
55/99
100/100
52/98
94/100
66/99
92/100
66/99
91/100
94/100
85/100
83/100
63/99
82/100
66/100
53/99
99/100
98/100
63/100
64/84
65/99
59/-
100/100
65/100
96/100
100/100
55/99
51/64
100/100
94/100
99/100
-/100
59/73
99/100
-/100
B
A
C-II
SA
NA
SA
NA

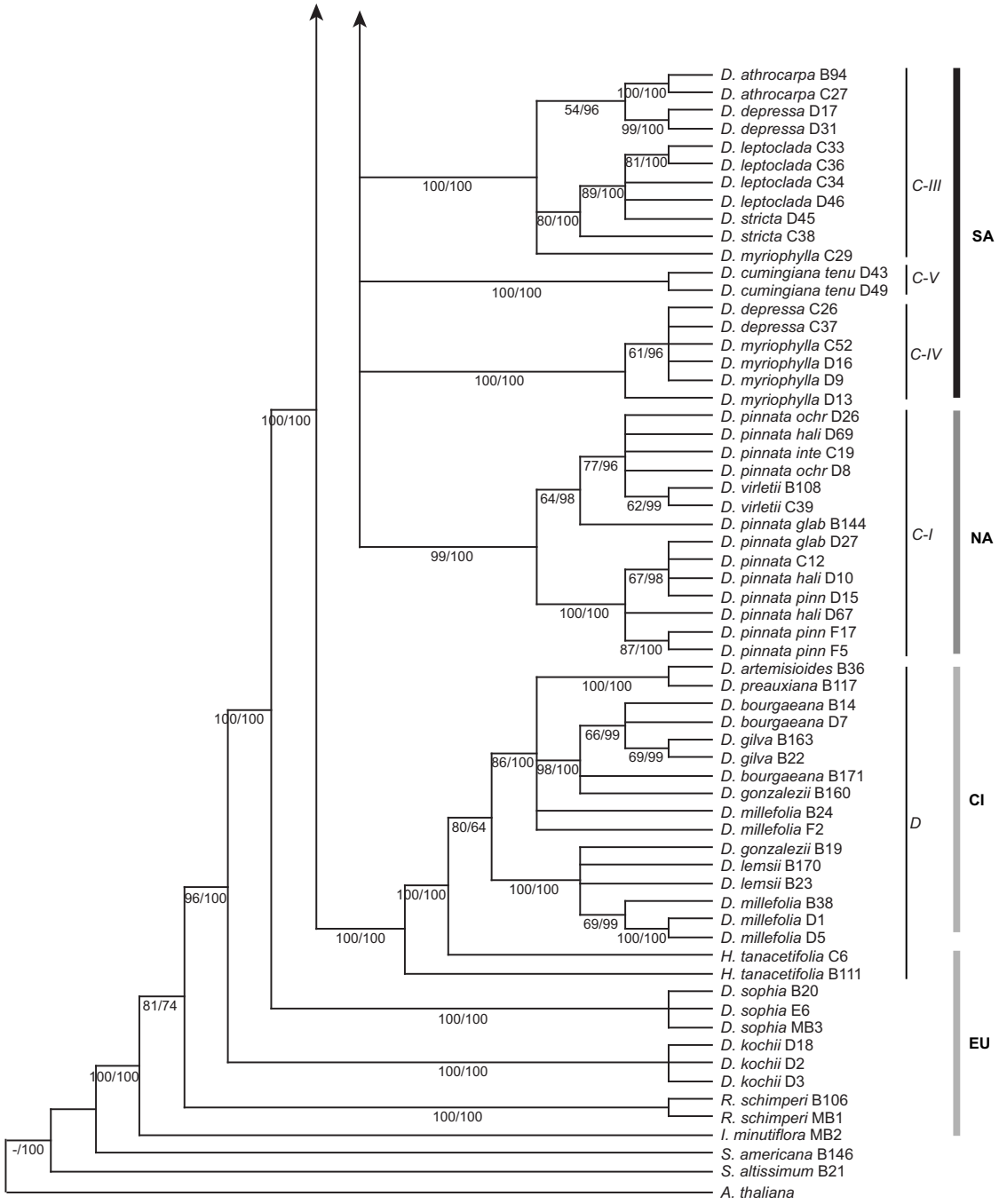
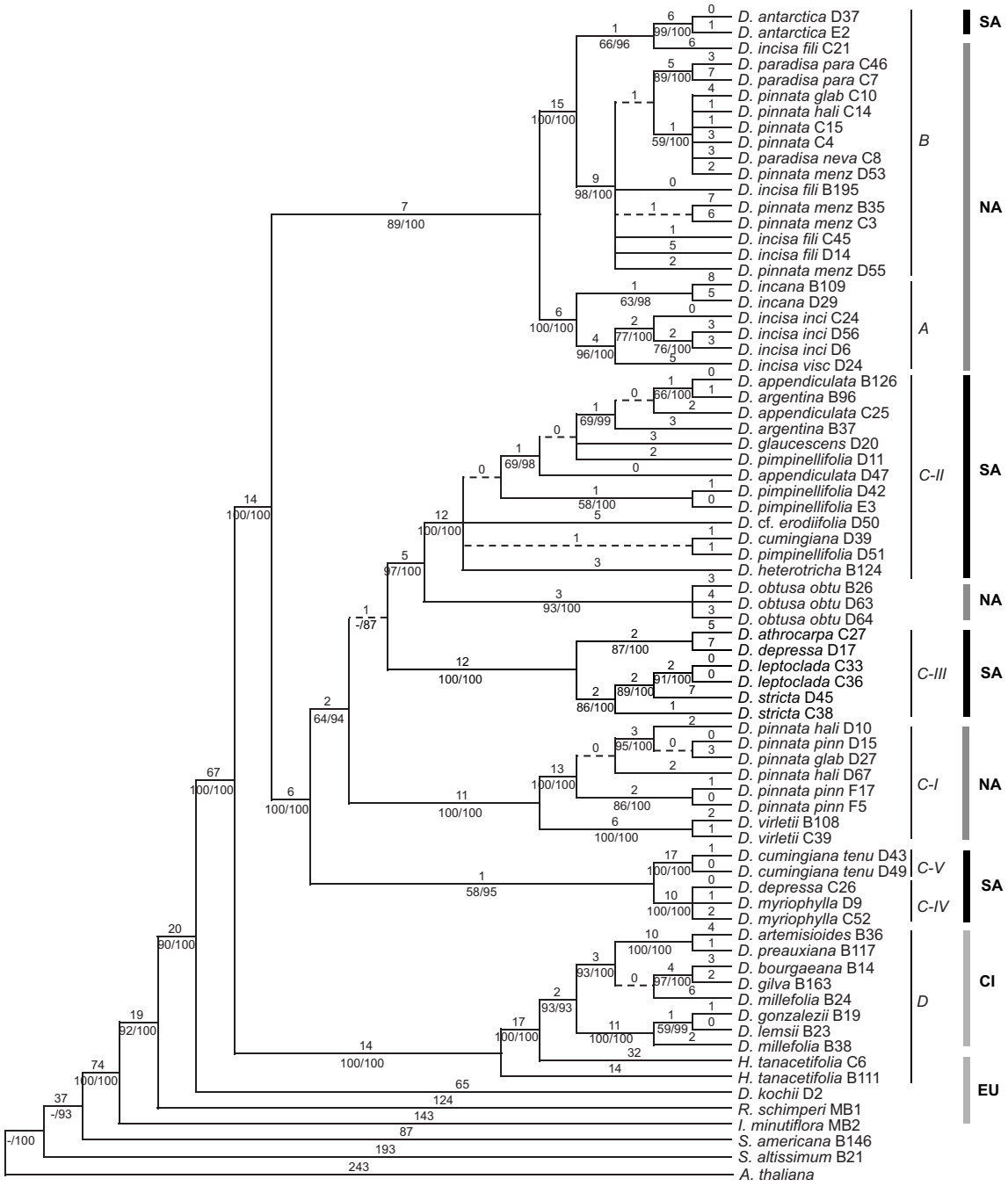

D. athrocarpa B94
D. athrocarpa C27
D. depressa D17
D. depressa D31
D. leptoclada C33
D. leptoclada C36
D. leptoclada C34
D. leptoclada D46
D. stricta D45
D. stricta C38
D. myriophylla C29
D. cumingiana tenu D43
D. cumingiana tenu D49
D. depressa C26
D. depressa C37
D. myriophylla C52
D. myriophylla D16
D. myriophylla D9
D. myriophylla D13
D. pinnata ochr D26
D. pinnata hali D69
D. pinnata inte C19
D. pinnata ochr D8
D. virletii B108
D. virletii C39
D. pinnata glab B144
D. pinnata glab D27
D. pinnata C12
D. pinnata hali D10
D. pinnata pinn D15
D. pinnata hali D67
D. pinnata pinn F17
D. pinnata pinn F5
D. artemisioides B36
D. preauxiana B117
D. bourgaeana B14
D. bourgaeana D7
D. gilva B163
D. gilva B22
D. bourgaeana B171
D. gonzalezii B160
D. millefolia B24
D. millefolia F2
D. gonzalezii B19
D. lemsii B170
D. lemsii B23
D. millefolia B38
D. millefolia D1
D. millefolia D5
H. tanacetifolia C6
H. tanacetifolia B111
D. sophia B20
D. sophia E6
D. sophia MB3
D. kochii D18
D. kochii D2
D. kochii D3
R. schimperi B106
R. schimperi MB1
I. minutiflora MB2
S. americana B146
S. altissimum B21
A. thaliana
C-III
C-V
C-IV
C-I
D
SA
NA
CI
EU

Fig. 2.8. One of 4020 most parsimonious trees recovered from combined ITS-chloroplast data to assess relationships within *Descurainia* using the parsimony ratchet. Bootstrap values > 50%/Bayesian posterior probabilities are indicated below branches. Dashed lines indicate branches that collapse in the strict consensus tree. Generic names are abbreviated as follows: *A*. = *Arabidopsis*, *D*. = *Descurainia*, *H*. = *Hugueninia*, *I*. = *Ianhedgea*, *R*. = *Robeschia*, and *S*. = *Sisymbrium* (*altissimum*) or *Smelowskia* (*americana*). The designations A, B, C, C-I, C-II, C-III, C-IV, C-V and D refer to clades described in the text. Distributions are abbreviated as follows: NA = North America, SA = South America, CI = Canary Islands, and EU = Europe, Eurasia and/or Middle East.


D. antarctica D37
D. antarctica E2
D. incisa fili C21
D. paradisa para C46
D. paradisa para C7
D. pinnata glab C10
D. pinnata hali C14
D. pinnata C15
D. pinnata C4
D. paradisa neva C8
D. pinnata menz D53
D. incisa fili B195
D. pinnata menz B35
D. pinnata menz C3
D. incisa fili C45
D. incisa fili D14
D. pinnata menz D55
D. incana B109
D. incana D29
D. incisa inci C24
D. incisa inci D56
D. incisa inci D6
D. incisa visc D24
D. appendiculata B126
D. argentina B96
D. appendiculata C25
D. argentina B37
D. glaucescens D20
D. pimpinellifolia D11
D. appendiculata D47
D. pimpinellifolia D42
D. pimpinellifolia E3
D. cf. erodiifolia D50
D. cumingiana D39
D. pimpinellifolia D51
D. heterotricha B124
D. obtusa obtu B26
D. obtusa obtu D63
D. obtusa obtu D64
D. athrocarpa C27
D. depressa D17
D. leptoclada C33
D. leptoclada C36
D. stricta D45
D. stricta C38
D. pinnata hali D10
D. pinnata pinn D15
D. pinnata glab D27
D. pinnata hali D67
D. pinnata pinn F17
D. pinnata pinn F5
D. virletii B108
D. virletii C39
D. cumingiana tenu D43
D. cumingiana tenu D49
D. depressa C26
D. myriophylla D9
D. myriophylla C52
D. artemisioides B36
D. preauxiana B117
D. bourgaeana B14
D. gilva B163
D. millefolia B24
D. gonzalezii B19
D. lemsii B23
D. millefolia B38
H. tanacetifolia C6
H. tanacetifolia B111
D. kochii D2
R. schimperi MB1
I. minutiflora MB2
S. americana B146
S. altissimum B21
A. thaliana
B
A
C-II
C-III
C-I
C-V
C-IV
D
SA
NA
SA
NA
SA
NA
SA
CI
EU

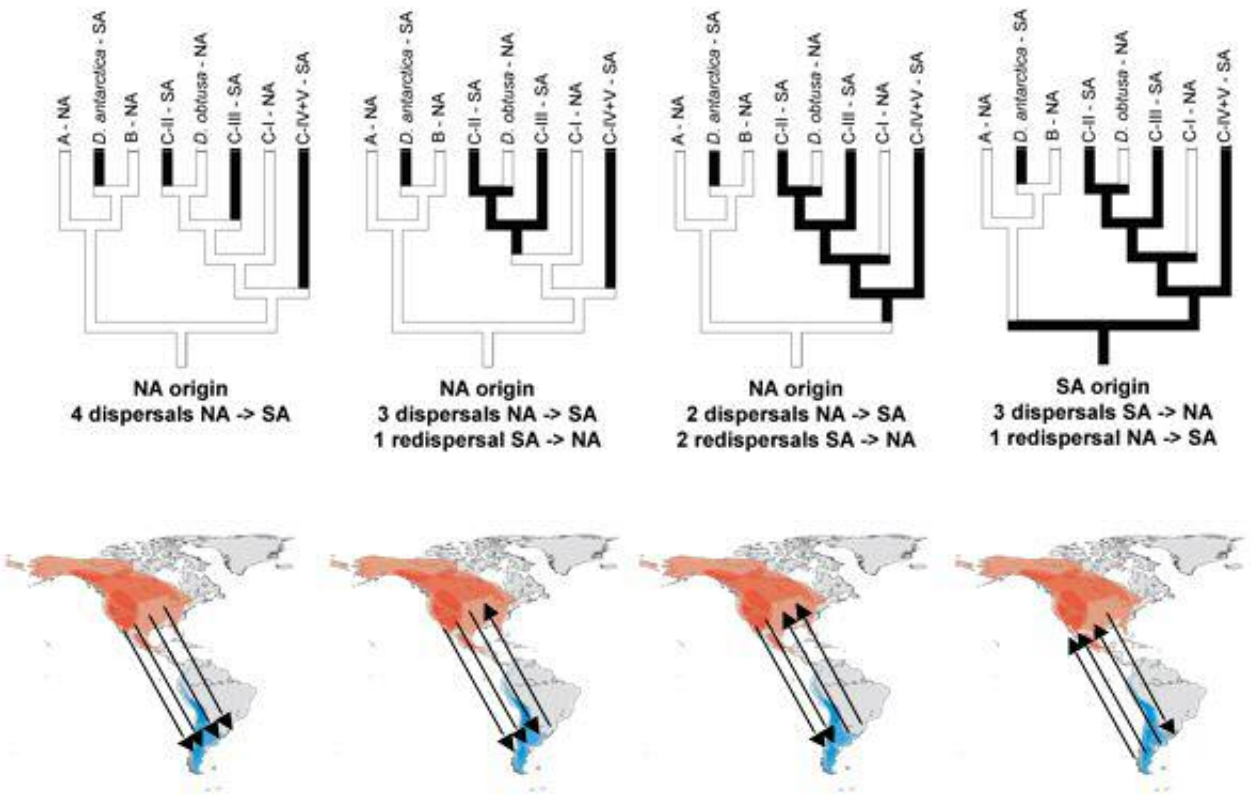


Fig. 2.9. Most parsimonious reconstructions from optimization of New World *Descurainia* continental distribution (North America [NA] or South America [SA]) on the topology found in 35% of most parsimonious trees and 84% of the Bayesian 95% credible set of trees from phylogenetic analysis of the combined ITS-chloroplast data set. Outgroups and Old World taxa are not shown because they have no effect on the outcome of the reconstructions. (One additional reconstruction, which is inconsistent with the recent introduction of *D. antarctica* to SA seen in molecular data, is not illustrated.

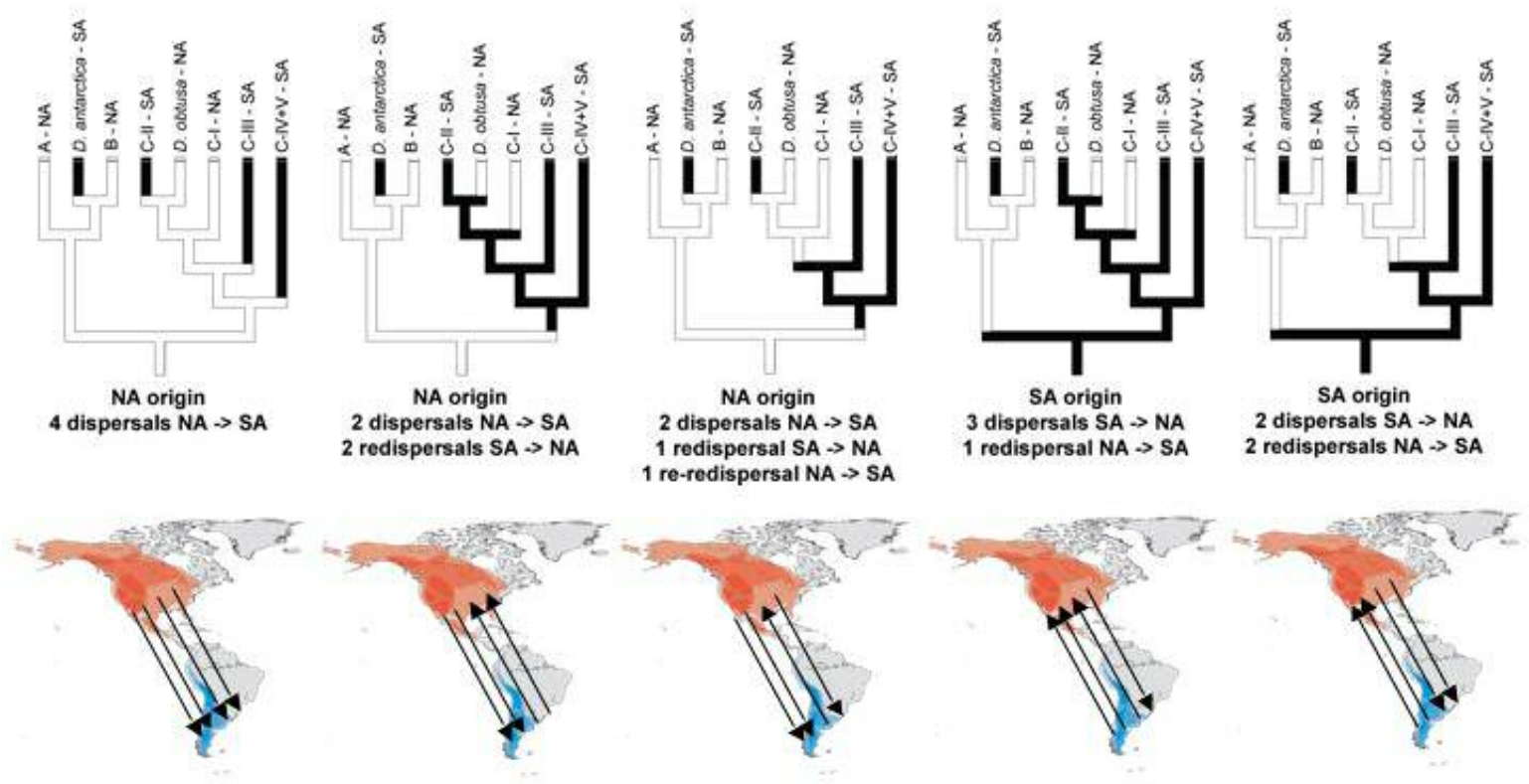


Fig. 2.10. Most parsimonious reconstructions from optimization of New World *Descurainia* continental distribution (North America [NA] or South America [SA]) on the topology found in 65% of most parsimonious trees and 10% of the Bayesian 95% credible set of trees from phylogenetic analysis of the combined ITS-chloroplast data set. Outgroups and Old World taxa are not shown because they have no effect on the outcome of the reconstructions. (Two additional reconstructions, inconsistent with the recent introduction of *D. antarctica* to SA seen in molecular data, are not illustrated.

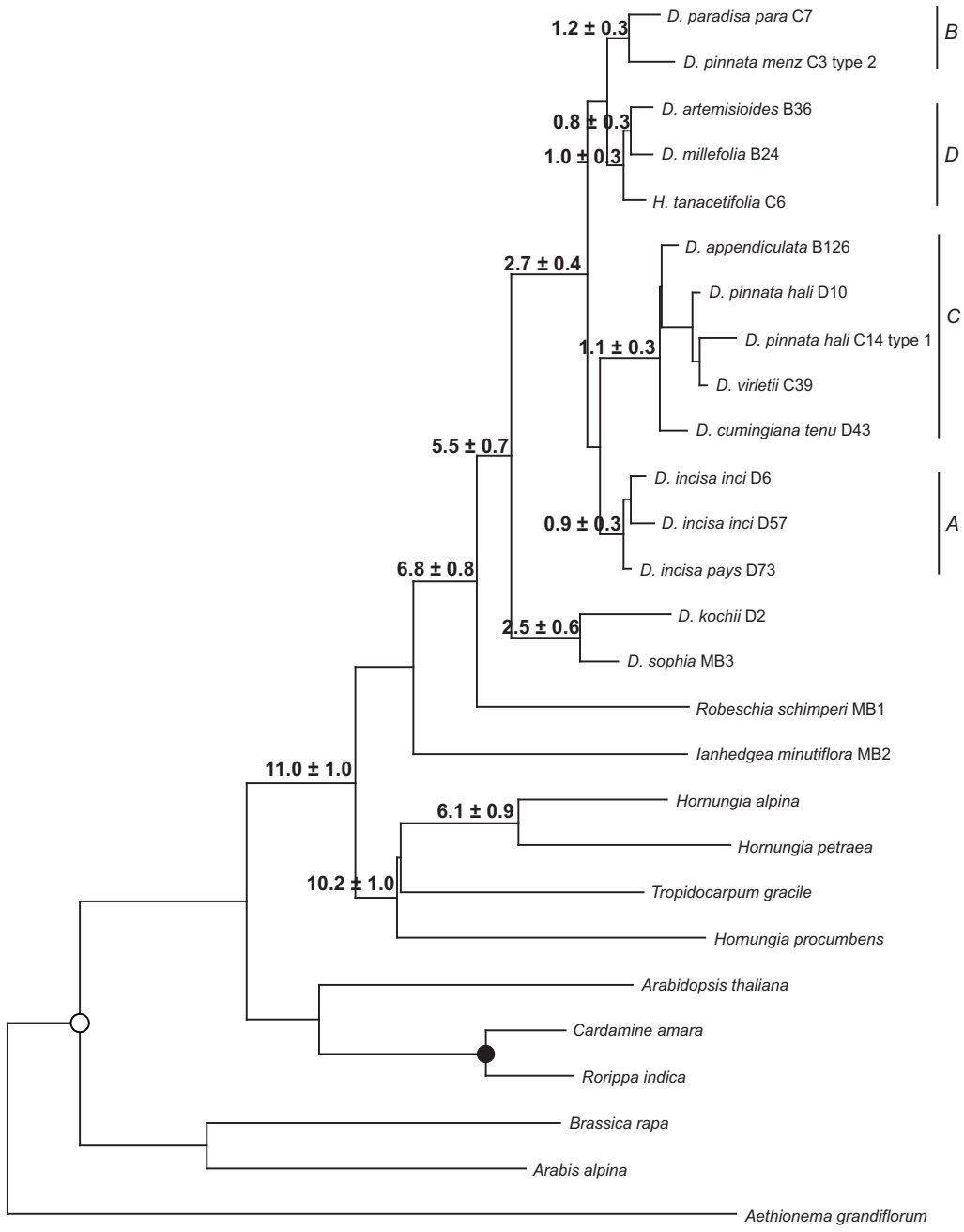


Fig. 2.11. The maximum likelihood tree (lnL = -3845.573) derived from a reduced ITS data set used for divergence time estimates in r8s. Calculated divergence times for labeled nodes are in million years before present (mya). Calibration nodes are marked with a filled circle (5 mya, MRCA of *Rorippa* and *Cardamine*) and open circle (20 mya, MRCA of *Arabidopsis* and *Brassica*). Generic names are abbreviated as follows: *D.* = *Descurainia* and *H.* = *Hugueninia*.

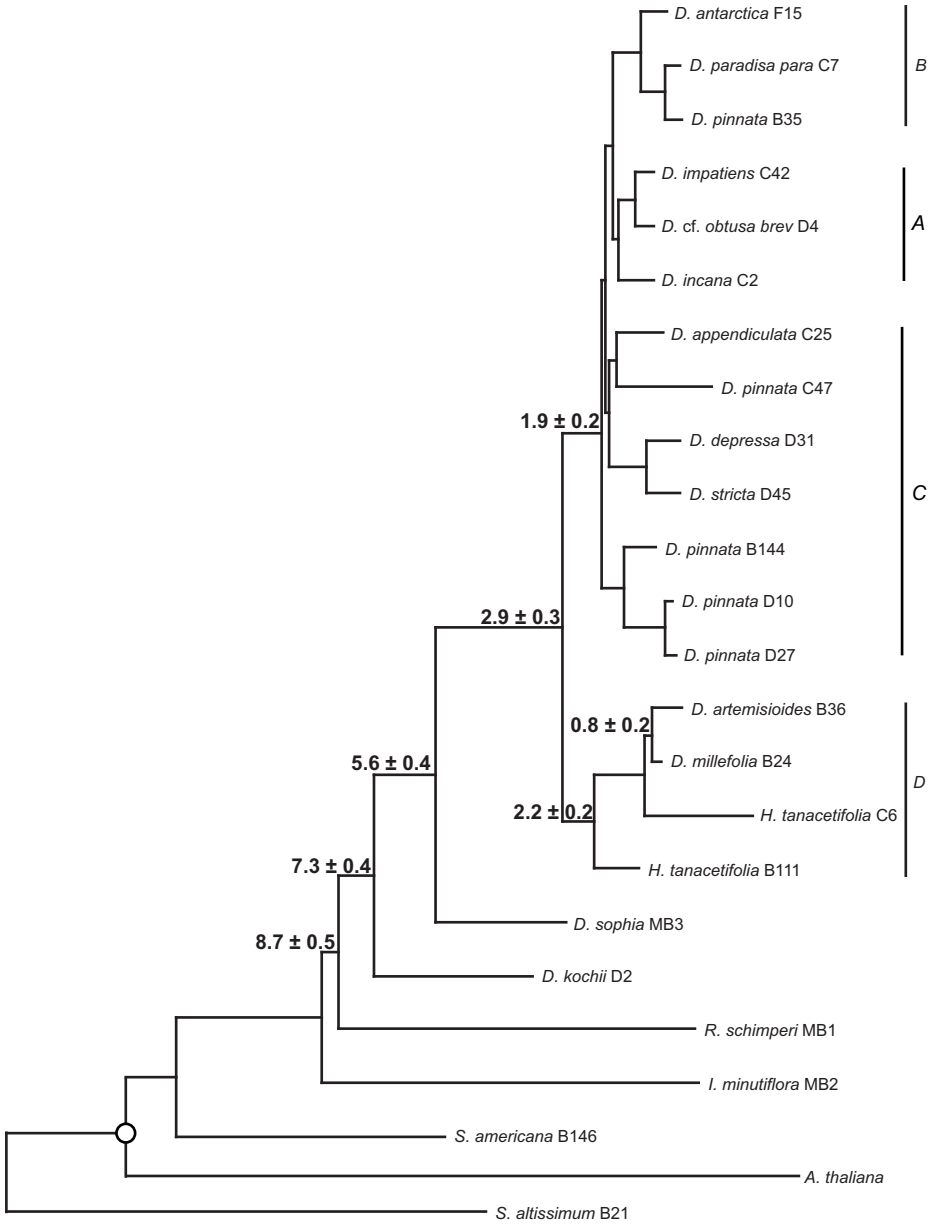


Fig. 2.12. The maximum likelihood tree (lnL = -13776.947) based on a reduced chloroplast data set used for divergence time estimates in r8s. Calculated divergence times for labeled nodes are in million years before present (mya). The estimated age of the MRCA of *Arabidopsis* and *Descurainia* (17.4 mya) calculated from the ITS data was used for calibration and is marked with an open circle. Generic names are abbreviated as follows: *A.* = *Arabidopsis*, *D.* = *Descurainia*, *H.* = *Hugueninia*, *I.* = *Ianhedgea*, *R.* = *Robeschia*, and *S.* = *Sisymbrium* (*altissimum*) or *Smelowskia* (*americana*).

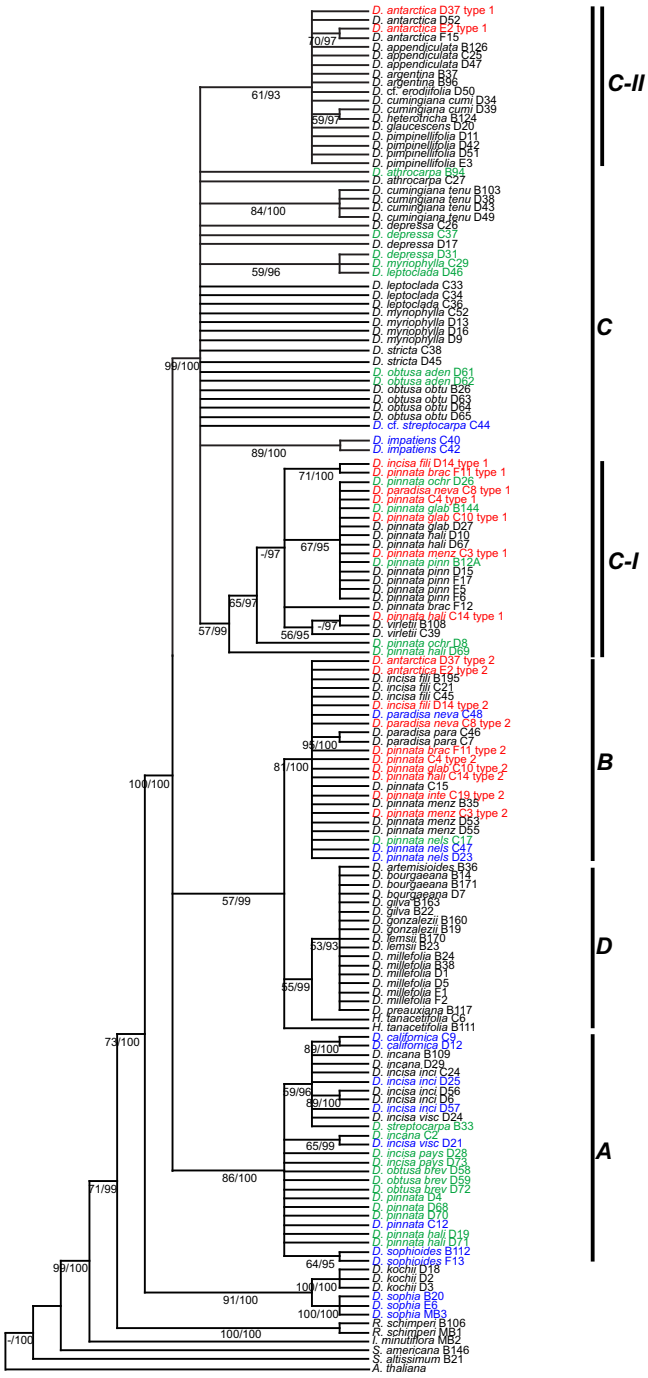


Fig. 2.13. Incongruence between ITS and chloroplast topologies illustrated using the ITS strict consensus tree. Accessions are highlighted according to the type of incongruence exhibited (see text): 1) red – mixed ITS types; 2) blue – between-clade; 3) green – within-clade.

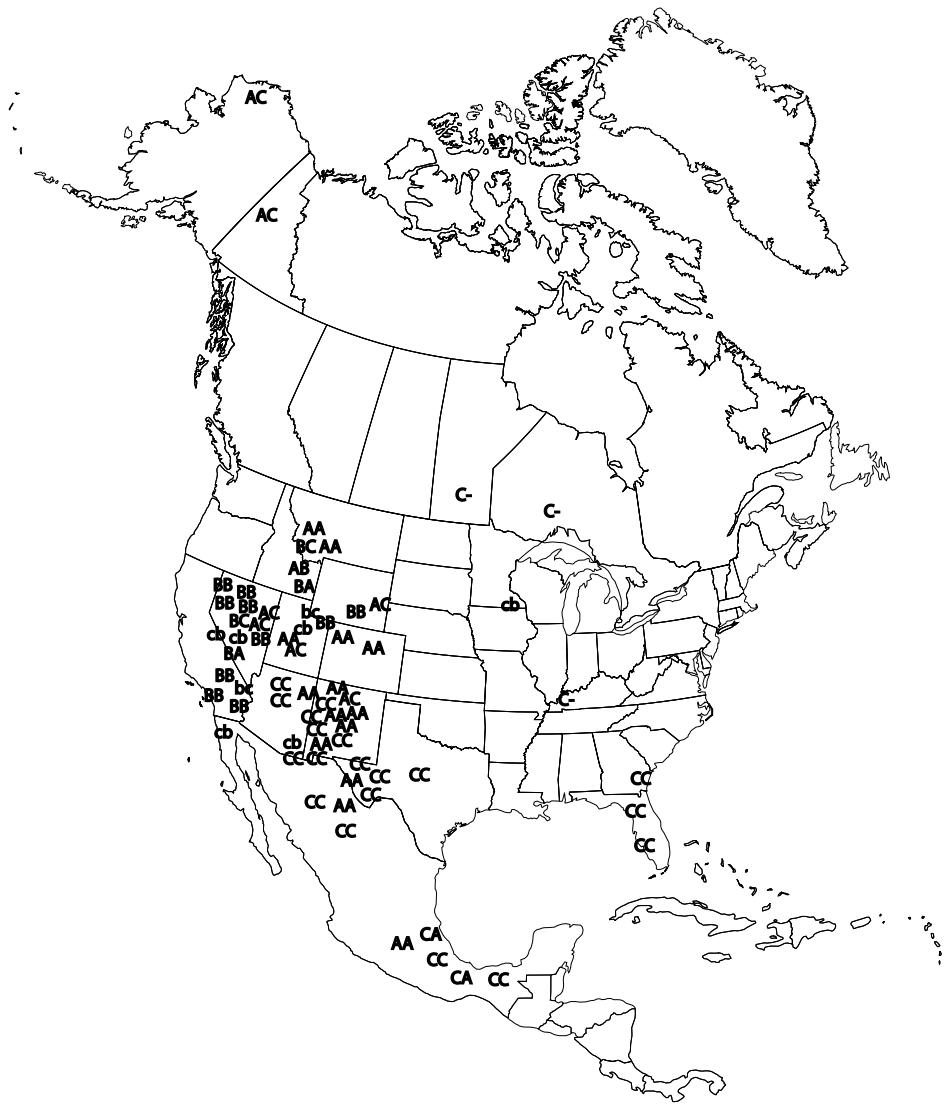


Fig. 2.14. Distribution of North American *Descurainia* accessions according to parental lineages (clades A, B or C) inferred from placement in ITS and chloroplast phylogenies. The first letter in each pair refers to the paternal lineage (ITS) and the second to the maternal lineage (chloroplast). Accessions where both maternal and paternal lineages were present in ITS mixed types are in lowercase; a dash (-) indicates that chloroplast data is missing for that accession. For clarity, several accessions in central New Mexico (AA and CC) are not shown. Two Canadian specimens (both C-) reported in Warwick & al., 2004 are also included.

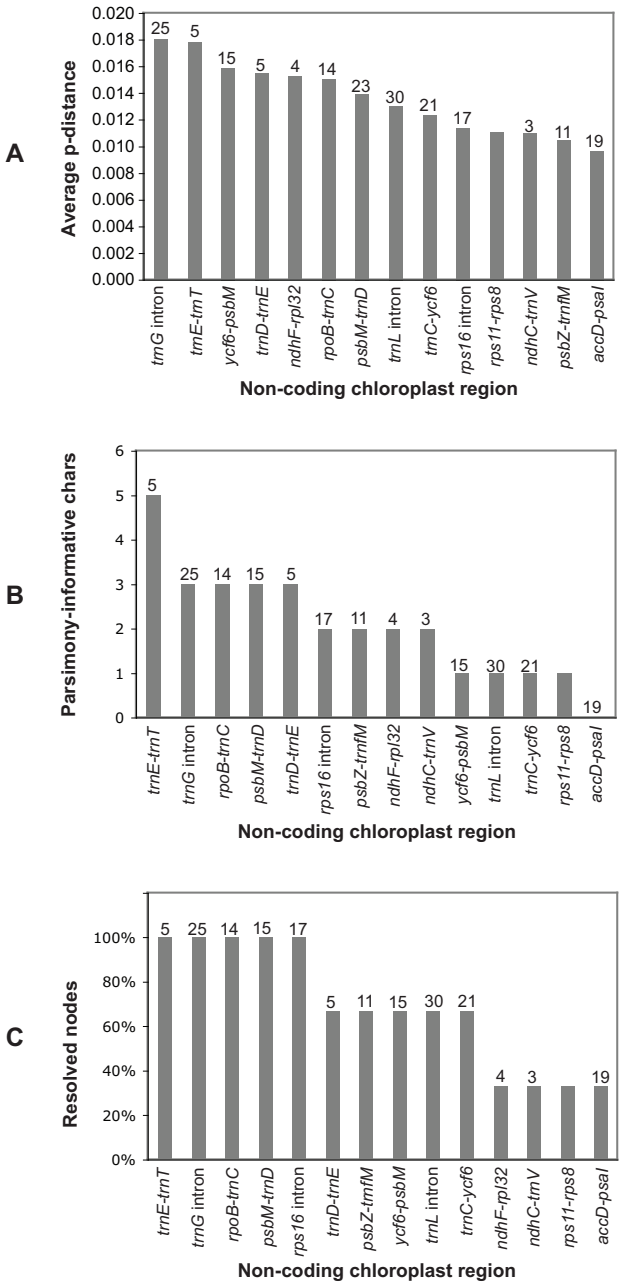


Fig. 2.15. Relative taxonomic utility of non-coding chloroplast markers using five accessions of *Descurainia* (*D. sophia* and one exemplar each from clades A, B, C and D). A) Average p-distance; B) number of parsimony informative characters; C) percent of nodes resolved in strict consensus tree with *D. sophia* as outgroup. Numbers above bars refer to the ranking reported in Shaw & al., 2007 for 34 non-coding chloroplast regions. In that study, *trnD-trnE* and *trnE-trnT* were grouped together and *psbZ-trnfM* was grouped with *trnS-psbZ.*

Chapter 3: Insights into the systematics of New World *Descurainia* Webb & Berthel. (Brassicaceae) based on single-copy nuclear Target of Rapamycin (TOR)

INTRODUCTION

Descurainia Webb & Berthel. is a genus in the Brassicaceae consisting of approximately 40 – 45 species distributed in many temperate areas of the Old and New World (Table 1.1; Fig. 1.1). The genus is most diverse in western North America and western South America, with a smaller center of distribution in the Canary Islands and three additional Old World species. *Descurainia* is well-known for its taxonomic complexity, especially within New World species, on account of its numerous intergrading forms coupled with descriptions based on inconsistent and overlapping characters.

The results of a molecular analysis of *Descurainia* based on ITS and seven non-coding chloroplast regions were reported in Chapter 2. The genus, with the inclusion of the monotypic genera *Hugueninia* and possibly *Robeschia*, was strongly supported as monophyletic, and appears to be of Old World origin with recent diversification within the Canary Islands and the New World. The phylogeny recovered from the combined ITS and chloroplast data was not well-resolved with respect to some New World lineages and therefore equivocal, but suggested that multiple independent dispersals of *Descurainia* have taken place between North and South America.

Substantial incongruence between ITS and chloroplast trees, as well as mixed ITS types observed for several North American accessions, pointed to extensive gene flow and hybridization within North American *Descurainia*. Lineage sorting could not be ruled out as a possible explanation for some incongruence, however, especially within

major clades. Possible problems with current species circumscriptions were highlighted, especially within North American *D. pinnata* and *D. obtusa*.

As the first comprehensive molecular study of *Descurainia*, the phylogeny recovered by the ITS and chloroplast data represents a significant advance in our understanding of the genus. Because deep nodes joining some New World lineages are poorly resolved, however, the results are not entirely satisfying. When confronted with insufficient resolution in such a situation, there are several approaches which might be employed to increase the amount of usable variation (Hughes & al., 2006). These alternatives include adding more non-coding chloroplast data, incorporating other nrDNA sequences such as the external transcribed spacer (ETS) region, making use of PCR-based fragment length characters from AFLPs, ISSRs, or RAPDs, or employing one or more low-copy nuclear markers. In the case of *Descurainia*, the application of a low-copy nuclear marker would be advantageous for several reasons. In contrast to data from ETS or additional chloroplast regions, sequences from a low-copy nuclear gene would constitute an independent data set for corroborating the phylogeny inferred from ITS and chloroplast DNA. Because low-copy nuclear regions are inherited bi-parentally, but are less frequently subject to concerted evolution than ITS or ETS (Small & al., 2004), a low-copy nuclear marker would aid reconstruction of possible past hybridization events suggested by the ITS and chloroplast data. Finally, unlike DNA fingerprinting methods such as AFLPs and RAPDs, procedures for sequencing a low-copy nuclear region would require minimal development before data could be generated and assessment of homology was expected to be straightforward. Consequently, to confirm and expand on the patterns suggested by ITS and chloroplast results, the addition of sequence data from a low-copy nuclear marker for a subset of taxa was the chosen approach.

The acquisition of sequence data from low-copy nuclear regions is becoming increasingly popular as a supplement to that obtained from chloroplast and nrDNA. Markers which have been successfully applied to inter- and infraspecies level problems include *Adh* (Sang & al., 1997b; Small & al., 1998; Sang & Zhang, 1999; Small & Wendel, 2000; Ferguson & Sang, 2001), GBSSI (*waxy*) (Miller & al., 1999; Peralta & Spooner, 2001; Small, 2004; Levin & al., 2005; Moore & al., 2006), *LEAFY* (Feng & al., 2005), ncpGS (Emshwiller & Doyle, 1999), *Pep*C (Malcomber, 2002; Olson, 2002; Helfgott & Mason-Gamer, 2004; Weeks & Simpson, 2004), *PgiC* (Ford & Gottlieb, 2002; Kamiya & al., 2005; Ford & al., 2006), *phyA* (Ohi-Toma & al., 2006), *pistillata* (Bailey & al., 2002), and RPA2, RPB2, and RPD2 (Goetsch & al., 2005; Popp & al., 2005).

Screening of several of these low- or single-copy markers reported in the literature, as well as a survey of some promising markers designed by Padolina & al. (2004), failed to identify a suitable region for use in *Descurainia*. Regions that amplified consistently were either insufficiently variable or possessed multiple gene copies which, given the known presence of polyploidy in the genus, would have necessitated extensive (and hence expensive) cloning for every sampled accession. Consequently, the genome of *Arabidopsis thaliana*, a fairly close relative of *Descurainia*, was examined for regions which might be better applicable.

For reconstructing relationships at or below the species level, especially in recently diverged groups, the most useful gene regions appear to be those that are single-copy and contain large introns and/or high proportions of intronic DNA (Sang, 2002). Primers for five regions meeting these criteria were designed based on the *A. thaliana* genome and screened for variability, ease of amplification, and absence of multiple copies. From this survey, a ~2300 bp portion of the nuclear gene Target of Rapamycin

(TOR) was selected for use as an independent nuclear marker in *Descurainia*. The TOR gene, which controls cell growth and proliferation (Robaglia & al., 2004), is single-copy in *Arabidopsis thaliana* (Menand & al., 2002). The gene and its protein products have been extensively investigated in organisms as diverse as plants, fungi, insects and mammals (e.g., Hay & Sonenberg, 2004; Thomas & al., 2004 & references therein; Mahfouz, 2006). It has not previously been used in phylogenetic studies. In *Descurainia*, nearly 80% of the portion of TOR sequenced consists of several large introns (Fig. 3.1), which amplified strongly when DNA was of good quality and gave no evidence of gene duplication in initial screening.

A major objective in using TOR sequence data was to resolve further relationships between North and South American taxa to clarify dispersal patterns between the two continents. Secondary goals were to confirm the existence of the major New World clades recovered from the ITS and chloroplast study, and to acquire additional insights into the causes of incongruence observed between the two data sets. A final aim was to bring together information from TOR, ITS and chloroplast phylogenies, geography, and general morphology as a starting point for addressing North American species concepts.

MATERIALS AND METHODS

Sampling. — DNAs from 56 (Table 3.1) of the 145 accessions isolated in the ITS and chloroplast investigation (Chapter 2) were used for this study. The major criterion used to select samples, subject to PCR amplifiability, was representation with multiple accessions of each major New World lineage recovered in the ITS-chloroplast phylogeny. The subset comprised samples sharing congruent positions in the two trees as well as

some exhibiting conflicting placements or mixed ITS types, and included 45 New World *Descurainia* accessions, three Canary Island species, two accessions of European *Hugueninia*, and one accession each of *D. kochii* (Turkey and Caucasia), *D. sophia* (Eurasia), *Robeschia schimperi* (Middle East), and the close relative and possible congener *Ianhedgea minutiflora* (Central Asia). Based on the results of Chapter 2, *Arabidopsis thaliana* and *Smelowskia americana* were used as outgroups.

PCR amplification and DNA sequencing. — The region between exons 22 – 27 of the nuclear gene Target of Rapamycin (TOR) was employed as a phylogenetic marker. This region, excluding *ca*. 80 base pairs of exon 25 which were not sequenced due to non-overlapping internal sequencing primers, comprised approximately 2340 base pairs, including five introns ranging in length from ~120 to ~600 base pairs (Fig. 3.1). PCR and internal sequencing primers are listed in Fig. 3.1.

The TOR region between exons 22 and 27 was amplified via the polymerase chain reaction (PCR) in 25 µL volumes containing 0.25 µL of a 100µM solution of each primer, 0.25 µL of *Taq* polymerase, 0.5 – 1 µL of unquantified DNA template, and 12.5 µL of FailSafe PCR 2x Premix D, E or H (Epicentre). Reaction conditions were: 3 min of denaturation at 96˚C, followed by 40 cycles of 94˚C for 35 sec, 50 - 54˚C for 45 sec, and 72˚C for 3 min, with a final extension of 72˚C for 15 min. Agarose gel electrophoresis of individual reaction mixtures yielded in every case a single band on the gel corresponding to the expected length of the TOR product. Following amplification, PCR products were cleaned with Qiagen spin columns according to 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 an ABI Prism 3730 automated sequencer.

Direct sequencing of TOR regions generated traces exhibiting multiple polymorphisms or overlapping sequences for 18 accessions. These samples were cloned with a TOPO TA kit (Invitrogen with vector pCR 2.1-TOPO) using 1/3 the recommended reaction volumes. For each cloning reaction, 4 - 6 positively transformed colonies were amplified and products sequenced. PCR amplification of clones was carried out as follows: 94˚C for 10 min, followed by addition of *Taq* polymerase while the reactions were held at 72˚C for 5 – 10 min, and then 37 cycles of 94˚C for 1 min, 48˚C for 2 min, and 72˚C for 1 min, followed by a final extension step of 72˚C for 15 min. From each cloned accession, with one exception, sequencing of transformed colonies recovered a maximum of two distinct types, i.e., clones for a given type either formed a monophyletic group or, if not forming a monophyletic group of clones, were identical or differed by a single autapomorphic nucleotide substitution from other clones of that type. Because the exclusion of redundant clones did not alter the outcome of phylogenetic analyses, two representative sequences (one for each putative parental type) were retained in the final data set for each sample exhibiting more than one distinct type. For one accession of *D. pinnata* ssp. *halictorum* (D19), three distinct types were observed and three representative sequences, one for each type, were consequently retained in the final data set.

The TOR region proved especially difficult to amplify strongly for four accessions extracted from herbarium specimens (*D. cumingiana* var. *tenuissima* D43, *D. impatiens* C40, *D. incisa* ssp. *incisa* D25, and *H. tanacetifolia* B111). For these accessions, this region was therefore amplified in two portions. Because some of the primers used for these amplifications were originally designed solely as internal sequencing primers (TORX25R and TORX25F; Fig. 3.1) and were not expected to anneal exclusively to the region of interest, a “nested-PCR”-type approach was taken:

following an initial round of PCR to amplify the entire TOR region between exons 22 and 27, the reaction mixture was cleaned and subjected to a second round of amplification with the annealing temperature lowered to 46ºC using either the primer pairs TORX22F and TORX25R (to amplify exons 22 – 25 ["TOR-I"]) or TORX25F and TORX27R (to amplify exons 25 – 27 ["TOR-II"]). Direct sequencing of TOR-I or TOR-II of accessions D25 and D43 resulted in traces exhibiting multiple polymorphisms or overlapping sequences, and these products were therefore cloned and sequenced as described previously. Because transformed colonies for a given accession, however, contained only TOR-I or TOR-II inserts instead of the entire TOR region, it was then necessary to determine which resulting TOR-I sequence corresponded to which (if any) TOR-II sequence type. Since only two types each were identified for D25 and D43, it was possible to piece them together by reference to their phylogenetic placement in trees including only TOR-I or TOR-II regions and inspection of the traces obtained from direct sequencing.

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 following the simple gap coding method of Simmons and Ochoterena (2000) and appended to the alignment.

Parsimony analyses were performed on the aligned data set by means of 20 independent parsimony ratchet (Nixon, 1999) runs of 200 iterations each in PAUP* 4.0b10 (Swofford, 2002) using batch files generated by PAUPRat (Sikes & Lewis, 2001). Support for internal nodes was assessed using bootstrap analysis (Felsenstein, 1985). One hundred bootstrap replicates of 10 random additions were performed, holding one tree at

each step and saving no more than 500 trees of length greater than or equal to 200 steps in each replicate. Bootstrap support categories were strong (> 85%), moderate (70 – 85%), weak (50 – 69%), or unsupported (< 50%).

Bayesian analyses were carried out on the TOR data set using MrBayes 3.1 (Ronquist & Huelsenbeck, 2003), with the best-fit model of evolution determined by the Akaike information criterion (Akaike, 1974; Posada & Buckley, 2004) as calculated in MrModeltest 2.2 (Nylander, 2004). Separate models were applied to the nucleotide and indel data partitions with all parameters unlinked except for topology and branch length; the model selected by MrModeltest (GTR+Γ) 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 the data set. Each analysis was run for three million 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. Strongly-supported branches were considered to be those with posterior probabilities greater than or equal to 95%, with posterior probabilities less than 95% indicating weak support.

Phylogenetic trees generated from TOR, ITS and chloroplast data sets were explored visually for conflicts, as well as with the incongruence length difference (ILD) test as implemented in PAUP* (partition homogeneity test of Farris & al., 1994). Each test consisted of 100 replicates, with 10 random additions per replicate, holding 20 trees per step.

RESULTS

Analysis of TOR data. – The TOR data set was easily alignable, consisting of sequences from 56 accessions; including unique clones, the data set contained a total of 75 sequences. The aligned data set was 2341 base pairs in length, including gaps (8.9%) and missing (0.2%) data. In addition, 17 indels, ranging in length from 4 – 25 base pairs, were binary-coded and appended to the combined data set. Of 1015 variable characters in the resulting data set, 415 (17.6%) were parsimony-informative. This level of variation is quite similar to that observed for the ITS data (20.3%). Nearly equal levels of variation for introns from nuclear genes compared to ITS is atypical – most such introns exhibit smaller (often much smaller) percentages of parsimony-informative characters than for ITS (Hughes & al., 2006). With outgroups excluded, uncorrected pairwise sequence ("p") distances ranged up to 9.74%, with an average of 2.11%.

Parsimony analysis of the TOR data set using the parsimony ratchet generated 4020 most parsimonious trees of 1463 steps (CI = 0.69, RI = 0.83) (Fig. 3.2). Bayesian analysis of the data set recovered a tree which was identical to the strict consensus parsimony tree except for the addition of a few poorly-supported branches (Fig. 3.2). While the TOR-based phylogeny is generally less resolved than those recovered from ITS and chloroplast data, it nonetheless confirms many patterns detected in the other data sets and provides new insights. As in the other phylogenies, the TOR tree strongly supports (BV = 100%, PP = 100%) *Ianhedgea* as sister to *Robeschia* and *Descurainia*; the position of *Robeschia* with respect to *Descurainia*, however, is unresolved. The TOR phylogeny reveals an interesting situation in regards to *D. sophia*. Sequencing of cloned PCR products uncovered two *D. sophia* types; one is sister (BV = 88%, PP = 99%) to *D. kochii*, and the other type is sister (BV = 87%, PP = 99%) to the remainder of the genus

excluding *D. kochii* and *Robeschia*. These positions correspond to the conflicting placements of *D. sophia* in the ITS vs. chloroplast trees, respectively, and provide strong evidence of an allopolyploid origin for this tetraploid species.

Within the remainder of the genus, the Canary Island taxa are strongly supported (BV = 100%, PP = 100%) as sister to a clade divided into two lineages: 1) both subspecies of *Hugueninia tanacetifolia* (BV = 71%, PP = 77%) and 2) New World taxa (BV = 100%, PP = 100%). A sister relationship between *Hugueninia* and New World taxa, instead of *Hugueninia* sister to Canary Island species, is in strong conflict with the chloroplast data. The ITS data, however, was equivocal on this point.

New World *Descurainia* species are strongly supported as monophyletic. Most New World clades identified from the ITS and chloroplast results (Fig. 2.8) are also observed in the TOR phylogeny but their relative positions are unresolved (Fig. 3.2). These include clade B (BV = 84%, PP = 100%) and clade C-I (BV = 99%, 100%) and, as qualified below, clades C-II, C-III, and C-IV. The position of a few accessions (*D. sophioides*, *D. pinnata* ssp. *halictorum* D69, *D. pinnata* ssp. *ochroleuca* D8, and one clone each of *D. pinnata* ssp. *intermedia* C19 and *D. pinnata* ssp. *nelsonii* C17) are unresolved within New World *Descurainia*. With the exception of one clone of *D. pinnata* ssp. *halictorum* D19 and *D. incisa* ssp. *incisa* D25, the remaining North American samples (all in clade A in the ITS and/or chloroplast phylogenies) group, depending on the analysis method, weakly (BV <50%) or strongly (PP = 98%) with some C-II and North American taxa as discussed below. Some of these clade A accessions (*D. californica* C9 and D12 and *D. incisa* ssp. *incisa* D6, D25 type 1, and D56) form a monophyletic group (BV = 75%, PP = 100%), while others (e.g., *D. incisa* ssp. *incisa* C24 and *D. pinnata* ssp. *halictorum* D19) are completely unresolved with respect to this group.

Several new pieces of information were also uncovered within the New World group. First, the members of the two South American appressed-fruit clades, C-III and C-IV, are strongly supported (BV = 96%, PP = 100%) as one lineage. Except for *D. stricta* C38, which may be a hybrid between *D. depressa* and *D. myriophylla*, this lineage neatly resolves according to existing species concepts, with *D. myriophylla* sister to *D. athrocarpa*, *D. depressa*, and *D. leptoclada*. In the Bayesian phylogeny, *D. cumingiana* var. *tenuissima* (clade V) is placed sister to this appressed-fruit lineage, but the support is too weak (PP = 50%) to be meaningful. Secondly, in addition to corroborating the mixed parentage of several putative hybrids identified in the ITS and chloroplast studies (i.e., *D. incisa* ssp. *incisa* D25 [clade A x clade B], *D. pinnata* ssp. *glabra* C10 [B x C-I, but not cloned and hence not included in the data set], *D. pinnata* ssp. *menziesii* C3 [B x C-I], and *D. paradisa* ssp. *nevadensis* C8 [B x C-I]), some other taxa also appear to have experienced hybridization based on multiple types found in the TOR sequences. These include *D. pinnata* C15 (B x C-I), *D. pinnata* ssp. *menziesii* B35 and B53 (B x C-I), *D. pinnata* ssp. *nelsonii* C17 (clade B x A?), and *D. stricta* C38 (clade C-III x C-IV). Surprisingly, South American *D. cumingiana* var. *tenuissima* D43 also possess two types, one in North American clade B and the other unresolved. In contrast to ITS and chloroplast results, where a maternal parent from clade B was strongly suggested, no clones of *D. antarctica* E2 were placed in clade B. An inspection of the direct sequencing trace for *D. antarctica* E2 also did not reveal the presence of any clade B types contributing to the observed polymorphism. Five clade A accessions which possess a biseriate seed arrangement were sequenced (*D. pinnata* D68, and D70, *D. pinnata* ssp. *halictorum* D19, *D. obtusa* ssp. *brevisiliqua* D60, and *D.* cf. *obtusa* ssp. *brevisiliqua* D4). Although only two of these accessions were cloned successfully and hence included in the TOR data set (D4 and D19), all five accessions exhibited mixed types upon direct

sequencing. Cloning or careful inspection of the sequence traces suggest, however, that none of these types resemble those found in North American clades other than clade A. With one exception (*D. pinnata* ssp. *halictorum* D19), no more than two distinct types of sequences (other than minor allelic variants as described under Materials and Methods) were uncovered from any cloned *Descurainia* accession exhibiting sequence additivity, although it is possible that more exhaustive cloning would have revealed additional types.

The most unusual aspect of the New World phylogeny concerns the five species of the South American spreading-fruit clade C-II included in the analysis. Sequences of all five were overlapping and polymorphic at many nucleotide positions; when they were cloned and placed into the phylogeny, two separate but incongruent clades containing all five species were discovered. One of the C-II clades (C-IIa) (BV = 58%, PP = 98%) was associated with moderate to strong support (BV = 73%, PP = 100%) with North American taxa *D. impatiens*, *D. obtusa* ssp. *obtusa*, and *D. obtusa* ssp. *adenophora*, and possibly with North American clade A (BV < 50%, PP = 98%). The relative position of the other C-II clade (C-IIb) (BV = 82%, PP = 100%), which is weakly to strongly sister (BV = 53%, PP = 100%) to one clone of *D. pinnata* ssp. *halictorum* D19, is unresolved.

Combined TOR, ITS and chloroplast data. – Relationships between major New World clades are unresolved in the TOR phylogeny. Combination of the TOR sequence data with non-conflicting ITS and chloroplast data from Chapter 2 might assist in resolving relationships between these clades. Disregarding minor incongruence within New World groups and the conflicting position of *Hugueninia*, however, there are two factors which hamper attempts to combine data sets. The TOR phylogeny contains two sets of clade C-II, and it is not clear which clade C-II group in the TOR tree is orthologous to that uncovered by the ITS and chloroplast data. Relationships within one

of the clade C-II groups (labeled C-IIa in Fig. 3.2), as well as a sister relationship to *D. obtusa*, are however strongly congruent with the pattern seen in the combined ITS and chloroplast phylogenies, and thus C-IIa might justifiably be included in a combined data set. More problematically, though, it is clear from visual comparison of strict consensus trees as well as the ILD test ($p < 0.05$) that clade A occupies conflicting positions within the TOR and chloroplast phylogenies. The three data sets were therefore not combined.

DISCUSSION

General relationships within *Descurainia*. – The phylogeny reconstructed from analysis of TOR data, while not as well resolved as either the ITS or chloroplast trees, is consistent in many respects with the results obtained from those data sets. *Robeschia*, *D. kochii*, and *D. sophia* occupy a basal position in the tree, New World species are strongly supported as a monophyletic group, and further evidence for a hybrid origin for *D. sophia* is provided. As in the ITS and chloroplast study, the inclusion of *Hugueninia* in *Descurainia* is strongly supported. In contrast to chloroplast – and to some extent ITS – phylogenies, both accessions of *Hugueninia* are placed in a well-supported sister relationship to New World rather than Canary Island *Descurainia*. The explanation for the differing gene histories with respect to *Hugueninia* is unclear. One possibility is an ancient hybridization or allopolyploidization event. Although *Hugueninia* is diploid ($2n = 14$) and no evidence of multiple types was detected in ITS or TOR sequences, processes such as diploidization (Grant 1981; Comai 2005) or concerted evolution could have obscured such an origin. An alternative explanation involves differential sorting of ancestral polymorphisms (i.e., lineage sorting) (Wendel & Doyle 1998). Additional independent nuclear markers will be required to distinguish between these possibilities

for *Hugueninia*. Regardless of which topology correctly reflects organismal history with respect to *Hugueninia*, the phylogeny generated from the TOR data set is still congruent with a common European or Eurasian ancestor for New World, southwestern European, and Canary Island *Descurainia*. While the two *Hugueninia tanacetifolia* accessions group together, they are only moderately supported as monophyletic (BV = 71%, PP = 77%). The pairwise sequence divergence (2.09%) between these two geographically-separated subspecies (ssp. *suffruticosa* from the Pyrenees and northern Spain and ssp. *tanacetifolia* from the southwest Alps) is almost as large as the maximum divergence within all New World accessions (2.33%).

Although most of the major New World clades identified in Chapter 2 are strongly supported in the TOR phylogeny, with few exceptions they form a polytomy suggesting rapid initial diversification within the New World. With respect to the major clades revealed by ITS and chloroplast data, there are two notable differing placements. The first difference is a sister relationship for clades C-III and C-IV, which are joined with strong support. This relationship is consistent with morphology and geography, because these two lineages comprise all sampled Andean species with appressed fruit. Combined ITS and chloroplast data suggest a different relationship, but the bootstrap support is very weak and thus those data may not actually conflict with the TOR topology. Furthermore, a *pistillata* intron screening study to be mentioned later also joined these two clades with strong support. The second significant departure from the results of Chapter 2 concerns North American clade A with respect to clade C, and especially South American clade C-II. This situation, graphically summarized in Fig. 3.3, is difficult to interpret. Without exact chromosome counts and more detailed sampling, identifying the competing processes giving rise to such a complicated pattern of relationships is challenging.

The phylogenetic trees generated using ITS and chloroplast sequence data suggested numerous cases of hybridization between North American clades B and C-I presumably arising from recent secondary contact following speciation. This hybridization was inferred from the occurrence of additive ITS sequences which upon cloning revealed parental types from both clades. In addition to confirming the mixed parentage of several of these putative hybrids, cloning of TOR sequences uncovered additional instances of presumed past hybridization between clades B and C-I and other lineages. Given the extent of reticulation uncovered, it is little wonder that species boundaries are so difficult to define in *Descurainia*.

A major aim of incorporating TOR sequence data into the study was to further resolve relationships between North and South American clades. Because the TOR data could not be combined with ITS and chloroplast data sets due to incongruence, it is not possible to make many additional inferences regarding dispersal patterns between North and South America. As with ITS and chloroplast data, however, sequence diversity within North American taxa is greater than in South America. This is consistent with the earlier suggestion that New World *Descurainia* was first introduced into North rather than South America.

Hybridization and dispersal in South American *Descurainia*. – Molecular data from Chapter 2 uncovered three to four South American lineages that correlate well with morphology and geography, and also indicated that *D. antarctica* arose from a separate and presumably recent dispersal from North America. TOR sequence data do not necessarily contradict these observations, but there are several interesting features that bear addressing.

One accession of *Descurainia cumingiana* (var. *tenuissima*) was included in the TOR data set. Direct sequencing of this sample generated a polymorphic, overlapping trace. When PCR products were cloned, two sequence types were recovered. One was resolved with respect to all other New World taxa, and this result is not incongruent with previous data which placed *D. cumingiana* as a distinct lineage within clade C. The second *D. cumingiana* TOR clone, however, is included with strong support within the North American B clade – a placement not observed in either ITS or chloroplast trees. This might indicate that the species *D. cumingiana* arose from a clade B x C ancestor whose clade B paternal line was subsequently lost from ITS regions as a result of concerted evolution. If so, the hybrid ancestor was probably not polyploid – there have been two chromosome counts for *D. cumingiana* and both are diploid ($2n = 14$) (Jaretzky 1932; Manton 1932). There is an alternative explanation for these results. In Argentina, from where the sampled *D. cumingiana* accession was collected, *D. cumingiana* var. *tenuissima* and *D. antarctica* sometimes co-occur, and specimens have been cited that are believed to be hybrids (Romanczuk, 1984a). Many Argentinean exemplars, including the sequenced accession, do indeed bear a stronger resemblance to *D. antarctica* compared to Chilean material. If *D. cumingiana* in Argentina has at some point undergone hybridization with *D. antarctica*, the clade B type uncovered in the TOR analysis of the *D. cumingiana* accession could reflect the clade B maternal ancestor of introgressed *D. antarctica*.

Molecular data in Chapter 2 reveal that *Descurainia antarctica* is a probable recent hybrid of an undetermined South American clade C-II member and a maternal parent from North American clade B. This was inferred from the presence of ITS sequences that were additive for clades C-II and B and placement of *D. antarctica* in clade B in the chloroplast phylogeny. It is thus surprising that no evidence of clade B

ancestry was detected for the *D. antarctica* accession included in the TOR data set, with the only two types recovered corresponding to different C-II sequence groups. Without more extensive amplification and cloning, it cannot be ascertained if the clade B TOR type has been eliminated from *D. antarctica*. This is quite possible; rapid loss of low-copy DNA sequences following hybridization or polyploidization has been documented, for example, in wheat (Feldman & al., 1997; Ozkan & al., 2001; Shaked & al., 2001) and *Brassica* (Song & al., 1995). The *D. antarctica* clade B TOR copy could also have been eliminated due to backcrossing with C-II parents, for example as seen in homoploid hybridization between allotetraploids in *Paeonia* (Ferguson & Sang, 2001).

ITS and chloroplast sequence data indicate that the South American spreading-fruit lineage C-II is monophyletic and possesses a low level of sequence variation. In contrast, the TOR data reveal two incongruent, monophyletic clades, each comprising one of the two sets of distinct types generated for each C-II accession cloned and sequenced (Fig. 3.2). Possible explanations for these patterns include retention of ancestral polymorphisms, polyploidization, or a recent gene duplication event prior to diversification of the C-II lineage with subsequent lineage sorting. Of these, polyploidization is the most likely explanation for the following reason. Although no polymorphic ITS sequences were observed for any clade C-II accessions, mixed types were, like TOR, observed for these taxa with another low-copy nuclear marker. While screening potential nuclear markers, sequences of the *pistillata* intron (Bailey & Doyle, 1999) were generated for 17 representative accessions (including *D. sophia*, Canary Islands, and New World clades A, B, C-I, C-II, C-III, and C-IV). Direct sequencing of 12 of these accessions, as in the TOR study (except for *D. sophia*), resulted in non-polymorphic sequences whose phylogenetic placement (not shown) was identical to their positions in the TOR phylogeny. Direct sequencing of the remaining five accessions,

consisting of three C-II samples (*D. argentina* B96, *D. heterotricha* B124, and *D. pimpinellifolia* D11), *D. pinnata* D19, and *D. pinnata* ssp. *menziesii* B35, generated polymorphic, overlapping traces as in the TOR study. These *pistillata* products were not cloned and it is therefore difficult to determine the exact composition of the constituent types, but there are at least two distinct types in each case. Because the C-II accessions were polymorphic for both TOR and *pistillata*, which are located on different chromosomes (1 and 5, respectively, in *Arabidopsis thaliana*), a genome-wide event, such as polyploidization, is a more compelling explanation for the observed pattern than either gene duplication or noncoalescence of ancestral alleles. Furthermore, although it by no means excludes the possibility of gene duplication, and would need to be confirmed for *Descurainia* by techniques such as Southern blot hybridization (Small & Wendell, 2000), the TOR gene is highly conserved and single-copy in a wide variety of organisms (except for two copies in yeast) (Menand & al., 2002).

If the TOR gene was therefore not independently duplicated in the most recent common ancestor of these taxa (or duplicated less recently with one copy then selectively lost in other descendants), this result could reflect genome duplication due to allopolyploidization in a C-II common ancestor. Such an event could have taken place prior to dispersal from North America, and, based on molecular data, most likely involved Mexican *D. impatiens* or a close relative as one of the participants. (See Mummenhoff & Franzke [in press] for discussion and examples of allopolyploidization preceding intercontinental dispersal). Unfortunately, because no chromosome counts have been reported for any C-II species, cytology cannot be used to support or reject this hypothesis. Furthermore, a broader sampling of populations in the southwestern United States and especially Mexico is required to identify more precisely the closest North American relative(s) of the C-II taxa.

While gene flow seems to be occurring within South American clade C-II (spreading-fruit) and within South American clades C-III and C-IV (appressed-fruit), neither ITS, chloroplast, nor TOR sequence data have uncovered any evidence of hybridization between clade C-II and the two appressed-fruit clades. From the standpoint of geography, this is not unexpected, because the two morphological groups occupy different habitats and their ranges do not extensively overlap. Given the weedy nature of many of these taxa and the wide-ranging extensive hybridization within their North American congeners, however, it may be that some other reproductive barrier, yet to be discovered, is in fact responsible for the genetic isolation between these clades.

Phylogenetic utility of the TOR region. – Although the sequenced TOR regions were not able to resolve most deep nodes joining New World lineages, they were able to corroborate some information obtained from the ITS and chloroplast study as well as provide some additional insights. The number of parsimony informative characters and resolved nodes was nearly comparable to ITS and combined chloroplast data (Table 3.2). For *Descurainia*, the degree of resolution provided by these TOR regions also appears to be similar to that obtainable from the first intron of *pistillata*, a low-copy nuclear region successfully employed in several other Brassicaceae studies (Bailey & Doyle, 1999; Bailey & al., 2002; Lee & al., 2002). Unlike some other low-copy nuclear markers which have been used in recent phylogenetic studies, the TOR gene has been found to be single-copy in a wide variety of organisms. In *Arabidopsis thaliana*, it is 17370 bp long, including 56 exons. About half of this gene consists of introns, with many ranging in size between 300 – 750 bp. The results of this study, as well as the features of the TOR gene, suggest that it might have good applicability as an independent nuclear marker for members of the Brassicaceae and related families.

North American taxonomic implications based on ITS, chloroplast, and TOR phylogenies. – Although TOR sequence data shed little additional light on New World biogeography, these data, when considered along with that obtained from ITS and chloroplast data, permit some observations regarding how North American species are currently delimited. This discussion is limited to North America rather than all New World species for two reasons: 1) taxon sampling was much more extensive in North America than in South America, and 2) the results of these phylogenetic analyses and this discussion will inform a treatment of *Descurainia* being prepared with Ihsan Al-Shehbaz for an upcoming volume of *Flora of North America*. Summaries of the phylogenetic position of all North American accessions with respect to major clades in the three data sets are shown in Fig. 3.4 and Table 3.3.

Given the limits imposed by taxon sampling, the findings of this study regarding North American *Descurainia* are preliminary, but point to some general trends subject to confirmation by additional research. As recognized by Rollins (1993a), there are 13 native and one introduced North American species of *Descurainia*. Multiple populations of eleven of these species were sampled, and several major lineages were consistently identified across different data partitions. With the exception of *D. pinnata*, most existing North American species concepts appear to be broadly accurate. Based on the molecular data and/or examination of herbarium specimens, there are perhaps ten native species: *D. californica*, *D. impatiens*, *D. incana* (including *D. incisa* and *D. obtusa* ssp. *brevisiliqua*), *D. longipedicellata* (= *D. incisa* ssp. *filipes*), *D. obtusa*, *D. paradisa*, *D. pinnata*, *D. sophioides*, *D. streptocarpa*, and *D. virletii*. Molecular dating in the previous chapter demonstrated that the genus *Descurainia* is of recent origin. As a consequence, on-going diversification and widespread gene flow (due to easy dispersability and presumed absence of reproductive barriers), as well as hybrid formation when diverged species

come into secondary contact, are responsible for blurred species boundaries. Taxonomic assignments for some herbarium and field specimens may be difficult in the absence of sequence data from those specific populations.

***D. californica*.** On account of its short fusiform siliques tipped with a prominent style, profusely branched racemes subtended by leafy bracts, and bright yellow flowers, *D. californica* is one of the most easily identifiable species in the genus. As noted by several authors, this biennial species bears a morphological resemblance to taxa such as *D. impatiens*, *D. streptocarpa*, and *D. incisa* (Detling, 1939), especially *D. incisa* ssp. *incisa* (Welsh & Reveal, 1977; Welsh & al., 1993). Not surprisingly, the two accessions of *D. californica* included in this study are associated in the ITS and TOR phylogenies with *D. incisa* and related taxa (e.g., *D. incana*, *D. streptocarpa*) (clade A). Although well-supported as sister to each other in the ITS tree (Fig. 2.5), they are not, however, sister in the TOR phylogeny (Fig. 3.2). If ITS sequences of two *D. californica* samples reported by Warwick & al., 2004b (GenBank accessions AY230616 and AY230617) are incorporated into the data set, the new sequences are also placed in clade A. They do not, however, form a monophyletic group with the *D. californica* accessions of the present study, weakly clustering instead with *D. streptocarpa* (BV = 63%, tree not shown). In contrast to the nuclear data, the two *D. californica* accessions in this study are placed in the chloroplast tree in clade C, where they are not monophyletic, but both associated with taxa such as *D. obtusa* and *D. sophioides*, among others (Fig. 2.7). There has been one chromosome count reported in the literature – a specimen from Nye Co., Nevada (Beatley & al. 11484 GH!), was found to be diploid ($2n = 14$) (Rollins & Rüdenberg, 1977). Despite the diploid chromosome count and distinctive morphology, it seems likely that gene flow and/or lineage sorting is on-going in *D. californica*.

***D. hartwegiana*.** This species, which was not sampled nor seen, is known only from the type collection (Hartweg 38), comprising two specimens from two different locations in central Mexico. It closely resembles Andean appressed-fruit species such as *D. stricta* or *D. leptoclada*, and Rollins (1993a) suggests that it in fact represents mislabeled material from one of Hartweg's South American collecting trips. In the absence of other Mexican material or clear evidence that Hartweg indeed collected the specimens from Mexico, it is debatable whether *D. hartwegiana* should be included as part of the North American flora.

***D. impatiens*.** *Descurainia impatiens* is a diploid species ($2n = 14$) (Beaman & al., 1962) distributed in the high mountains of southern Mexico. Plants of this species are tall annuals, with linear fruit and pinnate leaves that are usually sharply incised or toothed. In overall aspect it resembles species such as *D. incisa* and *D. sophioides* more than, for example, *D. pinnata*. Both accessions of *D. impatiens* included in this study are strongly supported as sister in the ITS and chloroplast phylogenies, where they occupy an unresolved position in clade C (Fig. 2.5), or are placed with most *D. incisa* samples in clade A (Fig. 2.7), respectively. The single accession of *D. impatiens* sequenced for the TOR study indicates a sister relationship with South American clade C-IIa but also suggests that *D. impatiens* is closely related to *D. obtusa* (ssp. *obtusa* and *adenophora*) (Fig. 3.2).

***D. incana* and *D. incisa*.** There have been several different ideas, most prominently those of Detling (1939) and Rollins (1993a), put forth regarding the classification of these taxa. Detling recognized a single species, *D. richardsonii*, comprising four subspecies: ssp. *typica* (= ssp. *richardsonii*), ssp. *procera*, ssp. *incisa*, and ssp. *viscosa*. All four subspecies occupy mainly cool, mountainous habitats primarily in the western United States. They are biennials with stems generally branched above,

and possess pinnate to pinnate-pinnatifid leaves, linear siliques, and a uniseriate seed arrangement. In two subspecies, ssp. *richardsonii* and ssp. *procera*, the pedicels and siliques are closely appressed to the axis of the raceme, with these two subspecies further delineated by degree of pubescence and silique length. The other two subspecies, ssp. *incisa* and ssp. *viscosa*, are marked by spreading or ascending pedicels and siliques. Plants of ssp. *viscosa* are glandular, whereas those of ssp. *incisa* are eglandular. As noted by Dorn (1988) and Kartesz & Gandhi (1991), the name *Descurainia incana* has priority over *D. richardsonii*, and recent authors who follow Detling's classification scheme have employed nomenclature updated accordingly. In contrast, as in the classification scheme of Schulz (1924), Rollins (1993a) segregated ssp. *richardsonii* and ssp. *procera*, along with another minor variety (var. *alpestris* [Cockerell] O. E. Schulz), from ssp. *incisa* and *viscosa*, merging the former three subspecies into an undifferentiated *D. incana*. The remaining subspecies (ssp. *incisa* and ssp. *viscosa*) were considered to belong to *D. incisa,* to which Rollins added as additional subspecies Detling's *D. pinnata* ssp. *filipes* and *D. pinnata* ssp. *paysonii* (*cf.* Table 1.1).

Molecular data for ssp. *paysonii*, at least for the two accessions included in this study, agree with its placement in a close relationship with *D. incisa* and *D. incana*. Subspecies *filipes*, on the other hand, is shown to be part of an entirely different lineage and hence belonging to neither *D. incisa* nor *D. pinnata* (see later discussion). While twelve accessions identifiable as *D. incana* or *D. incisa* (excluding ssp. *filipes*) were included in the molecular study, the resulting phylogenies are not able to confirm the circumscriptions of these taxa. They mostly group together in clade A, but intra-clade relationships differ between trees, some accessions appear to have experienced introgression of chloroplast DNA from other lineages, and morphologically similar taxa, such as *D. californica*, *D. impatiens*, *D. obtusa* ssp. *brevisiliqua*, and *D. streptocarpa*, are

sometimes intermixed or sister to *D. incisa* or *D. incana* to one extent or another. This is clearly a complicated and difficult group. A much broader molecular study is needed to better understand this *D. incana – D. incisa* complex.

***D. obtusa*.** *Descurainia obtusa* is a coarse, strict, canescent biennial restricted to the mountains and high plateaus of the arid southwestern United States and Northern Mexico. Two to three subspecies have been recognized. *Descurainia obtusa* ssp. *obtusa* and *D. obtusa* ssp. *adenophora* possess narrow, linear siliques, ranging up to 15 – 20 mm long, and leaves with obtuse tips. They differ in several relatively minor characteristics, including glandulosity, silique pubescence, seed arrangement, and flower size. *Descurainia obtusa* ssp. *obtusa* is generally confined to the mountain and plateau regions of New Mexico and Arizona, whereas ssp. *adenophora* has a more southern and western desert distribution, ranging from northwestern Chihuahua and western New Mexico to the mountains of southern California and Baja California. Chromosome number reports (Baldwin & Campbell, 1940) indicate that ssp. *obtusa* is diploid ($2n = 14$) (based on Detling 2381 OSC!) while ssp. *adenophora* is hexaploid ($2n = 42$) (Baldwin & Campbell, 1940). A third subspecies, *D. obtusa* ssp. *brevisiliqua*, was described by Detling (1939). Found in *Juniperus* forests of northern New Mexico and Arizona, it differs considerably from the other two subspecies, with which it does not co-occur. *Descurainia obtusa* ssp. *brevisiliqua* is characterized by short glabrous siliques reminiscent of those in southern species of *D. pinnata,* a tall strict growth habit with numerous short branches in the upper part of the plant, mostly linear leaf segments, and stems that are usually but not always purple. Detling encountered specimens in Arizona which he felt were morphologically intermediate between ssp. *brevisiliqua* and ssp. *obtusa*, which was apparently the basis for his placement of ssp. *brevisiliqua* as part of *D. obtusa.* Rollins (1993a) did not recognize ssp. *brevisiliqua* as a distinct subspecies, but included it as part of ssp. *obtusa*.

Chromosome counts for two *D. obtusa* ssp. *brevisiliqua* exemplars (Detling 2380 OSC! and Detling 2375 OSC) were both hexaploid ($2n = 42$) (Manton, 1932).

Four accessions of *D. obtusa* ssp. *obtusa* were included in the present study. They are strongly supported as monophyletic in the combined ITS-chloroplast phylogeny. The phylogenetic position of the single accession of *D. obtusa* ssp. *obtusa* sampled for the TOR phylogeny is identical to its location in the ITS-chloroplast tree. All evidence supports *D. obtusa* ssp. *obtusa* as a well-marked and distinct taxon. Although obviously closely related, polyploidy and conflicting phylogenetic placements for accessions of *D. obtusa* ssp. *adenophora* suggest that morphological differences from ssp. *obtusa* could be due to hybridization with other taxa.

In contrast to the first two subspecies, molecular data indicate that *D. obtusa* ssp. *brevisiliqua* does not belong in *D. obtusa*. Three exemplars of *D. obtusa* ssp. *brevisiliqua* which closely match Detling's type specimens were sampled, as well as several other similar accessions which differ from the original description in some respects (e.g., presence of glands). None of these accessions share a close relationship with *D. obtusa*, but instead are strongly supported as part of the clade A *D. incana – D. incisa* complex in both the ITS (Fig. 2.5) and chloroplast (Fig. 2.7) trees. When the TOR regions of an accession of *D. obtusa* ssp. *brevisiliqua* (D60) were sequenced, polymorphic, overlapping sequence traces were generated. Cloning of this region for the *D. obtusa* ssp. *brevisiliqua* sample was unfortunately unsuccessful. Careful examination of the mixed sequence traces, however, did not detect evidence of a contribution from *D. obtusa*, but instead that they comprised different types apparently corresponding to those uncovered for undetermined specimens morphologically similar to ssp. *brevisiliqua*, e.g., D4. The evidence suggests that *D. obtusa* ssp. *brevisiliqua* is an allopolyploid derived from

hybridization within the *D. incisa* group and that specimens matching its description should thus be classified under *D. incisa*.

***D. paradisa*.** *Descurainia paradisa* is quite well-marked, characterized by a low, bushy habit and unmistakable short, rounded, few-seeded siliques. This annual species ranges throughout most of Nevada as well as portions of southeastern Oregon and eastern central California. Detling (1939) classified it as a subspecies of *D. pinnata*, but most other botanists have considered it to be a distinct species. Rollins (1993a) separated *D. paradisa* into two subspecies, naming a new subspecies – ssp. *nevadensis* – which is distinguished from ssp. *paradisa* by its eglandular nature and prominent styles. Molecular data support *D. paradisa* as a good species: both accessions of *D. paradisa* ssp. *paradisa* included in this study are in a strongly-supported sister group relationship which is part of lineage B and distinct from *D. pinnata*. Taxonomic recognition of ssp. *nevadensis*, however, may not be justified. Examination of a number of such specimens, including several of Rollins' paratypes, calls his subspecies concept into question. Many of the so-designated specimens resemble *D. pinnata* ssp. *nelsonii* to some degree or another, and some were actually previously annotated as that taxon. Others, especially from southern Nevada, seem to represent *D. paradisa* intergrading with *D. pinnata* ssp. *glabra*, an observation which was also made by Detling (1939). The possibility of intergradation of *D. paradisa* with other taxa to yield the entity considered as ssp. *nevadensis* is supported by the molecular data. Three accessions of *D. paradisa* ssp. *nevadensis* (one of which [C47] I consider actually closer to *D. pinnata* ssp. *nelsonii*) were sequenced. In all three cases, the molecular data suggest that genetic material from either clade A, in the case of C47, or clade C, for C8 and C48, has been introduced into these populations.

***D. pinnata*.** *Descurainia pinnata* exhibits the widest distribution of the genus in North America. As currently circumscribed, members of this species are annuals

possessing (usually) bipinnate lower leaves and siliques which are clavate, subclavate, or rarely broadly linear. The species is highly polymorphic, with approximately 7 – 10 extensively overlapping and intergrading subspecies, many of which are marked by the presence of polyploidy (i.e., $2n = 28, 42$). On the basis of morphology and geography, Detling (1939) proposed the existence of two distinct complexes within the species. The first complex consisted of subspecies occupying hot, arid regions of the southwest (including northern Mexico), with one subspecies (ssp. *pinnata*) ranging eastward along the southern Gulf Coastal Plain to the south Atlantic states. As noted by Detling, compared to the second complex, these subspecies tend to be more canescent and bushier, with the pedicels and siliques more widely-spreading and the flowers smaller and/or paler. The subspecies included within this southern complex were ssp. *glabra*, *halictorum*, *menziesii*, *ochroleuca*, *paradisa*, and *pinnata*, with ssp. *halictorum* exhibiting the widest distribution and greatest range of morphological variation. The second *D. pinnata* complex, encompassing ssp. *brachycarpa*, *filipes*, *intermedia*, *nelsonii*, and *paysonii*, was centered around the northern Rocky Mountains, Pacific Northwest, and adjacent Nevada and Utah, with ssp. *brachycarpa* extending into the northern and central plains of the United States and Canada and sporadically to New England. In contrast to the southern complex, these subspecies are taller and stricter, subglabrous or moderately pubescent, with more erect pedicels and siliques and generally larger and more brightly yellow flowers. Detling pointed out that there was extensive intergradation both within and between the two complexes where the various subspecies come into contact.

Thirty-six accessions of *D. pinnata,* representing all subspecies recognized by Detling or Rollins, were sequenced for most or all of the markers in this study. Molecular results from Chapter 2 broadly confirmed the observations of Detling regarding two distinct lineages (clades B and C) within taxa circumscribed as *D. pinnata*. Because the

complexes were not sister lineages, however, *D. pinnata* would be polyphyletic. More specifically, while there were many hybrids, accessions of *D. pinnata* ssp. *pinnata*, ssp. *ochroleuca*, and in part ssp. *glabra* and *halictorum*, were placed in clade C, whereas *D. pinnata* ssp. *menziesii* was placed together with *D. paradisa* (Detling's *D. pinnata* ssp. *paradisa*) and *D. incisa* / *pinnata* ssp. *filipes* in clade B. When TOR sequences were obtained for 21 of the 36 *D. pinnata* samples, many of the same clade placements were confirmed. For others, however, such as three *D. pinnata* ssp. *menziesii* accessions, previously undetected evidence of past hybridization between clades B and C was uncovered. The picture that is thus beginning to emerge is that *D. pinnata* is too broadly defined. Based on the molecular data, *D. pinnata* strictly speaking (*D. pinnata s. s.*) might comprise a complex consisting of *D. pinnata* ssp. *pinnata* and in part ssp. *ochroleuca*, *glabra*, and *halictorum*. This group corresponds to those accessions found in lineage C-I in the molecular phylogenies. As indicated in Chapter 2, sampled members of this complex share a similar morphology, including pinnatifid or bipinnate lower leaves, purplish or rose-tipped sepals, distinctly elongated racemes, wide-spreading fruiting pedicels, clavate siliques, a biseriate seed arrangement, and a spring flowering time. Two other taxa are associated with this group, and further research might either support their inclusion in *D. pinnata s. s.* or maintenance and/or elevation as separate species. One is the Mexican endemic *D. virletii*, which is morphologically similar but distinguishable from *D. pinnata s. s.* The second related taxon is *D. pinnata* ssp. *brachycarpa*, which has a more northern and western distribution. Morphologically, it is distinct enough to have been considered as a separate species by earlier botanists (e.g., Richardson, 1823; Kuntze, 1891; Britton, 1901; Schulz, 1924). ITS data is available for four accessions of *D. pinnata* ssp. *brachycarpa* (two from this study and two from GenBank generated by Warwick & al., 2004b), but chloroplast data was only obtained for one of them and TOR

for none. The accession for which both ITS and chloroplast data was generated, F11 from Minnesota, has a clade B maternal ancestor. One cloned ITS type for F11 is also placed in clade B. ITS data for the other cloned type of this sample, as well as ITS sequences for the other three *brachycarpa* accessions (from Kentucky, Manitoba [AY230622], and Ontario [AY230621]), place them in clade C-I with *D. pinnata s. s*. and *D. virletii*. (The tree incorporating the GenBank sequences is not illustrated). No evidence of ITS additivity was observed for these latter three sequences (pers. obs.; C. Sauder, pers. comm.). While the Kentucky accession is unresolved within clade C-I, the other two accessions, one cloned type of the Minnesota sample, and one cloned type of a *D. incisa* ssp. *filipes* sample from Utah form a monophyletic group in C-I (Fig. 2.5). Two chromosome counts have been reported for *brachycarpa*; one from Ohio is diploid ($2n$ = 14) and one from Alberta (western Canada) is tetraploid ($2n$ = 28) (Mulligan, 1961; Easterly, 1963). Detling notes that *brachycarpa* intergrades in the northern Rocky Mountains with *D. pinnata* ssp. *intermedia*. On the basis of the current molecular, cytological, and morphological evidence, it thus appears that *brachycarpa* represents either a distinct species closely related to *D. pinnata*, or a well-defined subspecies of that taxon, which hybridizes in the western parts of its range with a species complex centered around *D. incisa / pinnata* ssp. *filipes* (= *D. longipedicellata*) (see below).

Three subspecies included in *D. pinnata* by Detling and some other authors clearly represent different taxa. *Descurainia pinnata* ssp. *paradisa* is correctly considered a distinct species, *D. paradisa*. *Descurainia pinnata* ssp. *paysonii* is, as surmised by Rollins (1993a), more closely related to *D. incisa*. *Descurainia pinnata* ssp. *filipes* is neither a subspecies of *D. pinnata* nor of *D. incisa*. This latter subspecies (ssp. *filipes*) was in fact considered by Schulz (1924), among others, to be a distinct species, *D. longipedicellata*. The morphology of this taxon, and that of ssp. *intermedia*, *nelsonii*, and

D. paradisa, differs from *D. pinnata s. s*. in many respects. For example, leaves of these taxa tend to be less highly divided and less rounded, especially in the upper portion of the plant where they are noticeably more linear. Siliques are not clavate, but are broadly or narrowly linear, or, in the case of *D. paradisa*, fusiform. There appear to be other subtle differences as well, such as yellow calyx color, in addition to the more erect pedicels, less canescent nature of the plants, and other characteristics of the northern *D. pinnata* complex which were noted by Detling.

Accessions of the remaining named subspecies of *D. pinnata* – *intermedia*, *menziesii*, and *nelsonii* – as well as some accessions identified as ssp. *glabra* and *halictorum*, largely appear to be hybrids between clades C-I and B. The molecular data do not provide sufficient resolution to precisely identify parental species. In the case of *intermedia* and *nelsonii*, as noted in the previous paragraph, morphology suggests that they are possibly most closely related to ssp. *filipes* (= *D. longipedicellata*). It is possible that the morphology of *intermedia* and *nelsonii* might represent variation within a *filipes*-like species complex which is undergoing diversification. Alternatively, these forms could have arisen largely as the result of hybridization events between distantly related but geographically overlapping taxa. The reported tetraploid ($2n = 28$) (Baldwin & Campbell, 1940) chromosome count for ssp. *intermedia* would be consistent with the latter scenario. Because DNA from a limited number of populations was utilized, more extensive sampling is clearly needed to work out the evolutionary history of this group.

ITS and chloroplast data do not distinguish *D. pinnata* ssp. *menziesii* from the rest of clade B, but morphologically and geographically, it appears to be more closely aligned with *D. pinnata s. s.* of clade C. Restricted almost entirely to southern California, it shares the bipinnate leaves, wide-spreading clavate siliques and biseriate seed arrangement found in these southern subspecies of *D. pinnata*. When the ITS and non-

coding chloroplast regions of four accessions of *D. pinnata* ssp. *menziesii* were sequenced, it was therefore rather surprising that three of them were placed in clade B in both trees. The fourth accession, from northern Baja California, had ITS types of both B and C-I, and a clade B chloroplast haplotype. When TOR introns were sequenced for three of these accessions, the explanation for morphological similarity to clade C was apparent: upon cloning, all three accessions possessed types corresponding to both clade B and clade C-I. Given the reported tetraploid ($2n = 28$) (Manton, 1932) chromosome count and the rather different morphology compared to most other clade B members, it appears that an independent allopolyploidization event has occurred giving rise of some or all populations of *D. pinnata* ssp. *menziesii*. Pending further sampling, it is unclear how this taxon should be classified.

***D. ramosissima*.** This annual species was described by Rollins (1984) based on plants growing intermixed with *D. pinnata* ssp. *halictorum* in Saguache Co., Colorado. It possesses a distinctive growth habit, with numerous branches arising from the base giving the plant a very bushy appearance. *Descurainia ramosissima* was not sampled in this study, but probably is part of the *D. incisa – D. incana* (clade A) complex discussed previously. It is mentioned here, even though not included in the molecular study, so that this discussion of species concepts will be comprehensive for North America.

***D. sophia*.** *Descurainia sophia* is not native to the New World, but is widely-distributed throughout North America where it has become established as an important agricultural weed (Best, 1977). During the course of this project, it was frequently encountered in disturbed areas along road margins and cultivated fields. Hultén (1968) reports hybrids of *D. sophia* x *D. sophioides* occurring around Alaskan villages and elsewhere, but the characteristic highlighted – both glandular and stellate trichomes – is considered part of the normal range of variation within *D. sophioides* by other authors

(e.g., Schulz, 1924; Detling, 1939; Rollins, 1993a). (*D. sophia* is stellate and eglandular, whereas these characters vary in *D. sophioides*). Best (1977) states that no hybrids between *D. sophia* and other congeners are known in Canada, and no other authors report hybrids. Molecular data from this study indicate that *D. sophia* is not closely related to North American species, and no evidence of gene flow was detected between *D. sophia* and any New World taxa. It appears that the species have diverged to such an extent that hybridization is unlikely.

***D. sophioides*.** Two accessions of *D. sophioides* were included in this study. Although their placement differs between phylogenies derived from ITS and chloroplast data sets, they are sister to each other in both trees (Figs. 2.5 and 2.7). Only one accession was sequenced for the TOR portion of the study; no polymorphic loci were observed and in that tree (Fig. 3.2), its position is unresolved within the New World species. Compared to many New World *Descurainia*, *D. sophioides* is morphologically fairly uniform, and this diploid ($2n = 14$), typically annual, species is readily identified from its subumbellate inflorescence which is overtopped by the developing siliques. There is very little range overlap between arctic *D. sophioides* and other North American species, although it may come in contact in the southern part of its Canadian range with *D. incana* and *D. pinnata* ssp. *brachycarpa*. Its relatively isolated geographical distribution probably accounts for the lack of taxonomic complexity in this species compared to many of its congeners.

***D. streptocarpa*.** *Descurainia streptocarpa* is the southernmost ranging species of the genus in North America, distributed in the high mountain regions of central and southern Mexico and Guatemala. This annual species differs mainly from *D. impatiens* of southern Mexico by virtue of its wide-spreading, often slightly deflexed pedicels and narrow siliques less than 1 mm wide. In contrast, in *D. impatiens* the siliques are generally broader and the pedicels are ascending, but this latter character is somewhat

dependent on plant maturity. Rzedowski and Rzedowski (2001) include *D. streptocarpa* within a very variable *D. impatiens*, but in the present study they do not form a monophyletic group. One *D. streptocarpa* accession, B33 collected in Veracruz, is placed in varying positions within the clade A *D. incana – D. incisa* complex in ITS and chloroplast phylogenies (Figs. 2.5 and 2.7). Another accession (C44 from central Chihuahua) may also represent *D. streptocarpa*, but the determination is difficult because it also resembles out-of-range *D. incisa* ssp. *viscosa*. A complex ancestry is indicated by conflicting lineages in the ITS (unresolved in clade C) and chloroplast (clade A) trees. This accession is not included in the TOR data set because only the TOR-II portion (i.e., exons 25 – 27) was successfully amplified and sequenced, but that sequence portion groups C44 most closely with *D. impatiens*. *Descurainia streptocarpa* is reportedly tetraploid ($2n = 24$) (Rollins, 1993a).

***D. torulosa*.** *Descurainia torulosa*, as first characterized by Rollins (1983), is distinguished by a short, low branching habit, extremely short pedicels, and closely-appressed but flaring torulose siliques. The taxon is of conservation interest, being restricted to a few populations in western Wyoming (Bricker & al., 2000). In a morphological analysis of all known specimens, however, Bricker & al. (2000) found a wide range of variation and failed to identify any characters that clearly separate *D. torulosa* from *D. incana*. They also conducted a brief ITS molecular survey and found *D. torulosa* to be weakly monophyletic (BV = 52%), but their analysis was inadequate from a geographic and taxonomic sampling standpoint, consisting of four *D. torulosa* accessions along with two *D. sophia*, three *D. incana*, and three *D. pinnata* specimens all collected from the vicinity of Laramie, Wyoming. DNA from *D. torulosa* was not sampled in the present study, but the four *D. torulosa* ITS sequences generated by Bricker & al. (2000) were retrieved from GenBank. When incorporated into the ITS data

set and analyzed, they are clearly placed in clade A (not illustrated) but not as a monophyletic group. Owing to several ambiguous nucleotide positions and some obvious polymorphisms between sequences, the exact placement of any given *D. torulosa* sequence, and its effect on the topology of clade A, is influenced by which other *D. torulosa* accessions are included. Without examining the original sequence traces, it is impossible to determine whether the individual sequences were polymorphic, but the published sequences seem to suggest this might be the case. Regardless of the taxonomic validity of *D. torulosa*, the data clearly support inclusion of these accessions within the complicated and confusing *D. incana – D. incisa* species complex which remains to be satisfactorily interpreted.

***D. virletii*.** ITS and chloroplast regions were sequenced for two accessions of this Mexican endemic. They are strongly supported as sister in the combined ITS-chloroplast tree when incongruent taxa are removed. Based on this limited sampling, *D. virletii* is closely related to southern species of *D. pinnata*, from which it is chiefly distinguished by shorter pedicels which on the lower portions of the racemes are subtended by bracts. Like them, this tetraploid species ($2n = 28$) (Rollins & Rüdenberg, 1977) is annual in habit. Additional sampling might in fact indicate that *D. virletii* should be considered a subspecies of *D. pinnata s. s*.

CONCLUSIONS

TOR sequence data strongly support New World *Descurainia* as a monophyletic lineage which is most closely related to *Hugueninia* and sister to Canary Island taxa. Although most major New World clades identified by ITS and chloroplast data are present in the TOR phylogeny, their position with respect to one another is largely unresolved. Extensive incongruence between ITS, chloroplast, and TOR phylogenies, as

well as the presence of mixed ITS and TOR sequences, suggests a complex evolutionary history for *Descurainia*. As a recently-diverged genus, processes such as hybridization and lineage sorting complicate efforts to develop an accurate taxonomy for the group.

The results reported in this chapter, combined with those in Chapter 2, have established a phylogenetic framework for future research, which will be critical as almost every aspect of this genus warrants further investigation. To confirm and extend the results of this study, chromosome counts need to be obtained, more thorough sampling at the population level must take place, and the addition of sequences from other single-copy nuclear regions or data from DNA fingerprinting techniques such as ISSRs should be considered. Informed by present and future molecular data, a thorough and statistically rigorous morphological study and revision will also be required for the development of an accurate taxonomy for *Descurainia*. Further molecular and morphological work may lead to establishment of *Descurainia* as a model generic system, like *Boechera, Brassica*, and *Cardamine* (Bailey & al., 2006), for studying phenomena such as speciation and hybridization in recently-evolved groups in the Brassicaceae and other plant families.

Table 3.1. Plant material used to examine phylogenetic relationships within *Descurainia* with TOR sequence data. Seed source for cultivated plants designated as follows: [ETSIA] = Escuela Técnica Superior de Ingenieros Agrónomos de Madrid crucifer seedbank, Universidad Politécnica de Madrid, Spain; [B&T] = B&T World Seeds, Paguignan, France.

Taxon; Location, date, collector and DNA voucher (herbarium)
***Arabidopsis thaliana* (L.) Heynh.:** GenBank; NC_000932;
***Descurainia antarctica* (Fourn.) O. E. Schulz: var. *patagonica* (Speg.) O. E. Schulz – E2**: Cultivated, seed collected by B. Goodson, 7 January 2005, roadside, RN 3, between Florentino Ameghino and Uzcudun, Dept. Florentino Ameghino, Prov. Chubut, Argentina (TEX);
***D. appendiculata* (Griseb.) O. E. Schulz: B126**: Cultivated, seed collected by B. Goodson, 27 Dec 2001, on side of gravel road ca. 1.4 km E of Universidad Católica de Salta, Dept. Capital, Prov. Salta, Argentina (TEX);
***D. argentina* O. E. Schulz: B96**: Cultivated, seed [ETSIA 239-5908-81] collected from roadside, Uspallata, Dept. Las Heras, Prov. Mendoza, Argentina (TEX);
***D. athrocarpa* (A. Gray) O. E. Schulz: C27**: Bolivia: slope above road to Valle del Zongo (S 16º16'51" W 68º7'21"), Prov. Murillo, Dept. La Paz, 5 March 2004, *B. Goodson 1506* (TEX);
***D. bourgaeana* Webb ex O. E. Schulz: B14**: Spain: El Portillo, Cañadas del Teide, Tenerife, Canary Islands, *A. Santos s. n.* (ORT);
***D. californica* (A. Gray) O. E. Schulz: C9**: USA: East Creek campground, Humboldt National Forest (N 39º29'43" W 114º39'13"), White Pine Co., NV, 22 May 2003, *B. Goodson 1493* (TEX); **D12**: USA: Cedar Creek Campground, Dixie National Forest (N 37º35'28" W 112º53'53"), Iron Co., UT, 19 August 2001, *B. Goodson 1466* (TEX);
***D. cumingiana* (Fisch. & C. A. Mey): var. *tenuissima* (Phil.) Reiche – D43**: Argentina: 60 km de Jacobacci subiendo a la meseta, Dept. 25 de Mayo, Prov. Rio Negro, 8 November 1966, *Abadie-Vallerini 1020* (BAA);

Table 3.1. Continued.

***D. depressa* (Phil.) Reiche: C26**: Bolivia: Patarani (S 17º14'54" W 67º59'59"), Prov. Aroma, Dept. La Paz, 3 March 2004, *B. Goodson 1505* (TEX); **C37**: Bolivia: fallow field along road between Sajama to Patacamaya, ca. 3 km W of Puerto Japones (17º22'02" W 68º13'27"), Prov. Pacajes, Dept. La Paz, S 15 March 2004, *B. Goodson 1520* (TEX); **D17**: Bolivia: road from Tiwanaku to La Paz, ca. 5 miles E of Tiwanaku (S 16º35'08" W 68º35'00"), Prov. Ingavi, Dept. La Paz, 11 March 2004, *B. Goodson 1510* (TEX):

***D. heterotricha* Speg.: B124**: Cultivated, seed collected by B. Goodson, December 2001, weedy field in El Salto, Dept. Luján de Cuyo, Prov. Mendoza, Argentina (TEX);

***D. impatiens* (Cham. & Schlecht.) O. E. Schulz: C40**: Mexico: 3 km S of Neverías, Mun. Miahuatlán, Oaxaca, 3 August 1996, *G. B. Hinton et al. 26690* (TEX);

***D. incisa* (Engelm. ex A. Gray) Britton: ssp. *filipes* (A. Gray) Rollins – C21**: USA: Flaming Gorge Overlook, Hwy 44, Flaming Gorge NRA (N 40º54'28" W 109º41'54"), Daggett Co., UT, 29 June 2003, *B. Goodson 1499* (TEX); **C45**: USA: 3 miles SE of North Battle Mountain on road to Stony Point, Lander Co., NV, 21 May 2002, *A. Tiehm 12845* (TEX); **ssp. *incisa* – C24**: USA: McKenzie Gulch Trail, White River National Forest, Eagle Co., Colorado, 2 July 2003, *B. Goodson 1502* (TEX); DQ418717; **D6**: USA: Snowbird Ski Resort, Salt Lake Co., UT, 4 August 2004, *B. Goodson 1528* (TEX); **D25**: USA: steep bank off state Hwy 75, 6.6 miles from Stanley near the Salmon River, Custer Co., ID, 25 June 1986, *R. C. & K. W. Rollins 86101* (TEX); **D56**: USA: near road to Lower Lagunitas Lakes campground, Rio Arriba Co., NM, 2 August 1998, *J. McGrath 157* (UNM);

***D. kochii* (Petri) O. E. Schulz: D3**: Turkey: Eskihisar Köyü çevresi (N 40º51'11" E 33º26'21"), Kastamonu, June 2004, *A. Dönmez 11793* (TEX);

***D. lemsii* Bramwell: B23**: Spain: Cumbres de la Orotova, Tenerife, Canary Islands, April 2001, *A. Santos s. n.* (ORT);

***D. leptoclada* Muschl.: C34**: Bolivia: above village of Sajama (S 18º07'51" W 68º56'49"), Parque Nacional Sajama, Prov. Sajama, Dept. Oruro, 14 March 2004, *B. Goodson 1515* (TEX); **C36**: Bolivia: E side of Río Tomarapi, ca. 2 km E of Cosapa (S 18º05'27" W 68º44'06"), Prov. Sajama, Dept. Oruro, 15 March 2004, *B. Goodson 1516* (TEX);

***D. millefolia* (Jacq.) Webb & Berthel.: B24**: Spain: Barranco del Rio, La Palma, Canary Islands, April 2001, *A. Santos s. n.* (ORT);

Table 3.1. Continued.

***D. myriophylla* (Willdenow ex DC.) R. E. Fries: C29**: Bolivia: Laguna Apaña, Ovejuyo (S 16º32'52" W 68º00'48"), Prov. Murillo, Dept. La Paz, 7 March 2004, *B. Goodson 1508* (TEX); **D9**: Bolivia: La Paz Montículo (S 16º30'27" W 68º07'38"), Prov. Murillo, Dept. La Paz, 7 March 2004, *B. Goodson 1507* (TEX);

***D. obtusa* (E. L. Greene) O. E. Schulz: ssp. *adenophora* (Wooton & Standley) – D61**: USA: adjacent to FS 111, Gila National Forest, Grant Co., NM, 19 July 1995, *C. A. Huff & D. Stevens 2310* (UNM); **cf. ssp. *brevisiliqua* Detling – D4**: USA: VLA radio telescope observatory, Socorro Co., NM, 15 July 2004, *B. Goodson 1527* (TEX); **ssp. *obtusa* – B26**: USA: slopes along NM Hwy 159, 5 miles E of junction with US Hwy 180 (N 33º23'16" W 108º49'58"), Catron Co., NM, 10 August 2001, *T. Chumley 7359* (TEX);

***D. paradisa* (A. Nels. & Kenn.) O. E. Schulz: ssp. *nevadensis* Rollins – C8**: USA: valley floor, W of NV Hwy 95 and N of Walker Lake (N 38º48'47" W 118º45'59"), Mineral Co., NV, 21 May 2003, *B. Goodson 1492* (TEX); **ssp. *paradisa* – C7**: USA: NV Hwy 445 (MM 27), ca. 2 miles SW of Pyramid Lake Indian Reservation (N 39º52'07" W 119º38'16"), Washoe Co., NV, 21 May 2003, *B. Goodson 1490* (TEX); **C46**: USA: 2.8 miles S of Wheeler Reservoir road on main N-S road to Double Hot Springs, Humboldt Co., NV, 14 May 2002, *A. Tiehm 13794* (TEX);

***D. pimpinellifolia* (Barnéoud) O. E. Schulz: D11**: Argentina: RP 52, ca. 34 km from Uspallata (S 32º30'10" W 69º03'26"), Dept. Las Heras, Prov. Mendoza, 15 December 2001, *B. Goodson 1475* (TEX);

***D. pinnata* (Walter) Britton: ssp. *glabra* (Wooton & Standley) Detling – D27**: Mexico: canyon of Rio Guararáy, ca. 0.5 km upstream from Los Aguaros, Mun. Alamos, Sonora, 16 March 1994, *R. S. Felger 94-88* (TEX); **ssp. *halictorum* (Wooton) Detling –D10**: USA: Hwy 67, 40 miles N of Alpine (N 30º43'03" W 103º11'56"), Pecos Co., TX, 9 April 2004, *B. Goodson 1521* (TEX); **D19**: USA: Hwy 67, ca. 9.6 miles S of Marfa (N 30º10'54" W 104º04'43"), Presidio Co., TX, 9 April 2004, *B. Goodson 1523* (TEX); **D69**: USA: Petroglyph National Monument lowlands, Bernalillo Co., NM, 19 April 2001, *A. C. Cully & M. Medrano s. n.* (UNM); **ssp. *intermedia* (Rydb.) Detling – C19**: USA: Red Canyon Lodge Horse Stables, Hwy 44, Flaming Gorge NRA (N 40º52'22" W 109º32'35"), Daggett Co., UT, 29 June 2003, *B. Goodson 1498* (TEX); **ssp. *menziesii* (DC.) Detling – B35**: Cultivated, seed [ETSIA 248-1725-69] collected from Oakzanitas, San Diego Co., CA, USA (TEX); **C3**: Mexico: RN 1, ca. 5 miles E of El Aquajito (N 30º04'20" W

Table 3.1. Continued.

115º22'41"), Mun. Ensenada, Baja California Norte, 9 March 2003, *T. Chumley 7429* (TEX); **D53**: USA: 0.5 mile W of Aguanga, San Diego Co., CA, Riverside Co., CA, 29 March 1990, *E. LaRue s. n.* (TEX); **ssp. *nelsonii* (Rydb.) Detling – C17**: USA: McCarty Canyon Road (N 41º22'39" W 107º18'46"), Carbon Co., WY, 26 June 2003, *B. Goodson 1495* (TEX); **ssp. *ochroleuca* (Wooton) Detling – D8**: USA: junction of Hwy 17 and county road 112, ca. 16 miles S of Pecos (N 31º11'12" W 103º34'42"), Reeves Co., TX, 10 April 2004, *B. Goodson 1524* (TEX); **ssp. *pinnata* – B12a**: USA: Fly Gap division of Double Helix ranch, Mason Co., TX, 14 April 2001, *B. Goodson 1457* (TEX); **D15**: USA: picnic area on Hwy 90, 5 miles W of Alpine (N 30º19'22" W 103º44'35"), Brewster Co., TX, 9 April 2004, *B. Goodson 1522* (TEX); **F17**: USA: I-75 rest area N of Tampa (N 28º12'50" W 82º22'25"), Pasco Co., FL, 5 March 2006, *B. Goodson 1616* (TEX); **ssp. undet. – C15**: USA: BLM road to Mormon Mountains, ca. 0.5 miles E of junction with road to Lyman's Crossing (N 37º08'41" W 114º23'01"), Lincoln Co., NV, 7 May 2003, *T. Chumley 7439* (TEX);

***D. sophia* (L.) Webb ex Prantl: MB3**: USA: New Mexico, *Beilstein 01-19* (MO);

***D. sophioides* (Fischer) O. E. Schulz: F13**: Cultivated, seed collected by J. McKendrick, 17 August 1990, Dalton Highway MP 398.7, Prudhoe Bay, North Slope Co., AK, USA (TEX);

***D. stricta* (Phil.) Reiche: var. undet. – C38**: Chile: km 90 on the Arica-Putre road, Prov. Arica, Tarapacá (Region I), *J. L. Panero & B. S. Crozier 8435* (TEX);

***Hugueninia tanacetifolia* (L.) Prantl: ssp. *suffruticosa* – C6**: Cultivated from seeds [B&T] (TEX); **ssp. *tanacetifolia* – B111**: Italy: Piemonte, 10 July 1988, *Pistarino 2027* (NY);

***Ianhedgea minutiflora* (Hook. *f.* & Thoms.) Al-Shehbaz & O'Kane: MB2**: Tajikistan: Badakhson, *Solomon et al. 21646* (MO);

***Robeschia schimperi* (Boiss.) O. E. Schulz: MB1**: Iran: Prov. Esfahan, ca. 10 km past Khansar, on road to Golpayegan, 21 May 2004, *American-Iranian Botanical Delegation 33719* (TUH);

***Smelowskia americana* (Regel & Herder) Rydb.: B146**: USA: Mt. Sherman, Park Co., Colorado, 18 August 2001, *Goodson 1462* (TEX); DQ418729.

Table 3.2. Comparative phylogenetic utility of ITS, combined chloroplast, and TOR regions based on a 34-accession data set.

Characteristic	ITS	Combined chloroplast	TOR
Length of aligned data set (bp)	627	5351	2341
Number of non-autapomorphic indels	0	20	12
CI (excluding uninformative characters)	0.709	0.794	0.806
RI	0.877	0.901	0.898
Tree length	245	1444	984
Number of MPTs	6	36	45
Number of variable characters	180	1162	782
Parsimony informative characters (%)	72 (11.5%)	425 (7.9%)	296 (12.6%)
Number of resolved nodes in strict consensus tree	8	17	16
Number of nodes with bootstrap values > 85%	6	16	13
Number of nodes with posterior probabilities > 94%	15	20	19

Table 3.3. Summary of ITS, chloroplast, and TOR placements for North American *Descurainia* accessions. Chromosome numbers are from literature reports for that taxon and do not represent actual counts of sequenced accessions.

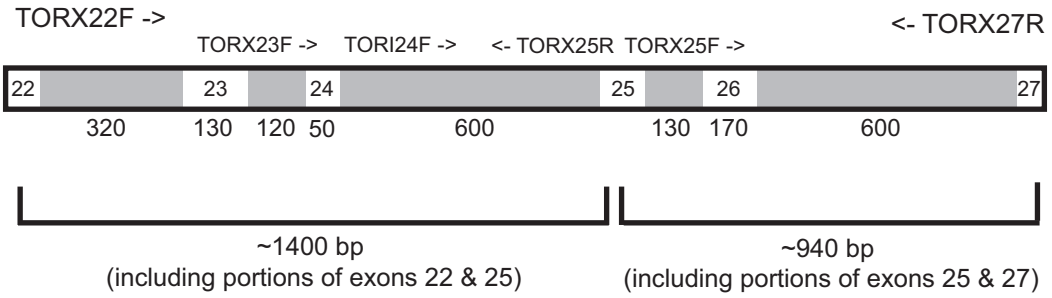
Taxon	Accession	Location	ITS	cp	TOR	Chromosome counts ($2n$ =)
D. californica	C9	White Pine Co., NV	A	C	A	14
D. californica	D12	Iron Co., UT	A	C	A	14
D. impatiens	C40	Oaxaca, MEX	C	A	C-IIa	14
D. impatiens	C42	Veracruz, MEX	C	A	--	14
D. incana	B109	Beaverhead Co., MT	A	A	--	14, 28
D. incana	C2	Granite Co., ID	A	A	--	14, 28
D. incana	D29	Blaine Co., ID	A	A	--	14, 28
D. incisa ssp. *filipes*	B195	Humboldt Co., NV	B	B	--	14, 28, 42
D. incisa ssp. *filipes*	C21	Daggett Co., UT	B	B	B	14, 28, 42
D. incisa ssp. *filipes*	C45	Lander Co., NV	B	B	B	14, 28, 42
D. incisa ssp. *filipes*	D14	Daggett Co., UT	BxC	B	--	14, 28, 42
D. incisa ssp. *incisa*	C24	Eagle Co., CO	A	A	A	--
D. incisa ssp. *incisa*	D25	Custer Co., ID	A	A or B	B	--
D. incisa ssp. *incisa*	D56	Rio Arriba Co., NM	A	A	A	--
D. incisa ssp. *incisa*	D57	Los Alamos Co., NM	A	C	--	--
D. incisa ssp. *incisa*	D6	Salt Lake City Co., UT	A	A	A	--
D. incisa ssp. *paysonii*	D28	Moffat Co., CO	A	A	--	--
D. incisa ssp. *paysonii*	D73	Mckinley Co., NM	A	A	--	--
D. incisa ssp. *viscosa*	D21	Laramie Co., WY	A	C	--	14
D. incisa ssp. *viscosa*	D24	Apache Co., AZ	A	A	--	14
D. obtusa ssp. *adenophora*	D61	Grant Co., NM	C	C	C-IIa	42
D. obtusa ssp. *adenophora*	D62	Coconino Co., AZ	C	C	--	42
D. obtusa ssp. *brevisiliqua*	D58	Socorro Co., NM	A	A	--	42
D. obtusa ssp. *brevisiliqua*	D59	Catron Co. NM	A	A	--	42

Table 3.3. Continued.

Taxon	Accession	Location	ITS	cp	TOR	Chromosome counts ($2n$ =)
D. obtusa ssp. *brevisiliqua*	D72	Socorro Co., NM	A	A	--	42
D. cf. obtusa ssp. *brevisiliqua*	D4	Socorro Co., NM	A	A	A	42
D. obtusa ssp. *obtusa*	B26	Catron Co., NM	C	C	C-IIa	14
D. obtusa ssp. *obtusa*	D63	Socorro Co., NM	C	C	--	14
D. obtusa ssp. *obtusa*	D64	Hidalgo Co., NM	C	C	--	14
D. obtusa ssp. *obtusa*	D65	Sierra Co., NM	C	C	--	14
D. paradisa ssp. *nevadensis*	C48	Mineral Co., NV	B	A	--	--
D. paradisa ssp. *nevadensis*	C8	Mineral Co., NV	BxC-I	B	BxC-I	--
D. paradisa ssp. *paradisa*	C46	Humboldt Co., NV	B	B	B	--
D. paradisa ssp. *paradisa*	C7	Washoe Co., NV	B	B	B	--
D. pinnata ssp. *menziesii*	D53	Riverside Co., CA	B	B	BxC-I	28
D. pinnata ssp. *menziesii*	D55	San Diego Co., CA	B	B	--	28
D. pinnata	C15	Lincoln Co., NV	B	B	BxC-I	14
D. pinnata	C4	Cochise Co., AZ	BxC	B	--	14
D. pinnata	D68	Socorro Co., NM	A	A	A?	14
D. pinnata	D70	Socorro Co., NM	A	A	A?	14
D. pinnata ssp. *brachycarpa*	F11	Winona Co., MN	BxC	B	--	14, 28
D. pinnata ssp. *brachycarpa*	F12	Crittenden Co., KY	C	--	--	14, 28
D. pinnata ssp. *glabra*	B144	Yavapai Co., AZ	C-I	C-I	--	28
D. pinnata ssp. *glabra*	C10	San Bernadino Co., CA	BxC-I	B	B x C	28
D. pinnata ssp. *glabra*	D27	Sonora, MEX	C-I	C-I	C-I	28
D. pinnata ssp. *halictorum*	C12	White Pine Co., NV	A	C-I	--	14, 28, 42
D. pinnata ssp. *halictorum*	C14	Lincoln Co., NV	BxC	B	--	14, 28, 42
D. pinnata ssp. *halictorum*	D10	Pecos Co. TX	C-I	C-I	C-I	14, 28, 42
D. pinnata ssp. *halictorum*	D19	Presidio Co., TX	A	A	A?	14, 28, 42
D. pinnata ssp. *halictorum*	D67	Cochise Co., NM	C-I	C-I	--	14, 28, 42
D. pinnata ssp. *halictorum*	D69	Bernalillo Co., NM	C	C-I	unres	14, 28, 42
D. pinnata ssp. *halictorum*	D71	Sandoval Co., NM	A	A	--	14, 28, 42
D. pinnata ssp. *intermedia*	C19	Daggett Co., UT	BxC	C-I	unres x B	28

Table 3.3. Continued.

Taxon	Accession	Location	ITS	cp	TOR	Chromosome counts ($2n$ =)
D. pinnata ssp. *menziesii*	B35	San Diego Co., CA	B	B	BxC-I	28
D. pinnata ssp. *menziesii*	C3	Baja California, MEX	BxC-I	B	BxC-I	28
D. pinnata ssp. *nelsonii*	C17	Carbon Co., WY	B	B	unres x B	14
D. pinnata ssp. *nelsonii*	C47	Eureka Co., NV	B	C	--	14
D. pinnata ssp. *nelsonii*	D23	Beaverhead Co., MT	B	C	--	14
D. pinnata ssp. *ochroleuca*	D26	Chihuahua, MEX	C-I	C-I	--	28
D. pinnata ssp. *ochroleuca*	D8	Reeves Co., TX	C	C-I	unres	28
D. pinnata ssp. *pinnata*	B12A	Mason Co., TX	C-I	C	C-I	14
D. pinnata ssp. *pinnata*	D15	Brewster Co., TX	C-I	C-I	C-I	14
D. pinnata ssp. *pinnata*	F17	Pasco Co., FL	C-I	C-I	C-I	14
D. pinnata ssp. *pinnata*	F5	Liberty Co., GA	C-I	C-I	--	14
D. pinnata ssp. *pinnata*	F6	Alachua Co., FL	C-I	--	--	14
D. sophioides	B112	Yukon Terr., CAN	A	C	--	14
D. sophioides	F13	North Slope Co., AK	A	C	unres	14
D. streptocarpa	B33	Veracruz, MEX	A	A	--	28
D. cf. *streptocarpa*	C44	Chihuahua, MEX	C	A	--	28
D. virletii	B108	Mexico DF, MEX	C-I	C-I	--	28
D. virletii	C39	Chiapas, MEX	C-I	C-I	--	28



Name	Primer sequence
TORX22F	5'-AAC TTA TCT TCC AGT CAT CCT TCC-3'
TORX27R	5'-CTC ATC CAT TCC TCC CAA TC-3'
internal sequencing primers:	
TORX23F	5'-TGA CAA GAG TAA TCC CGT GTG-3'
TORI24F	5'-AGG CAT TGT GTC ACT ATC TGG-3'
TORX25R	5'-GTG AAG TCC TCT CCA AGT GC-3'
TORX25F	5'-GCA CTT GGA GAG GAC TTC AC-3'

Fig. 3.1 Diagram of TOR region amplified and sequenced, with a list of PCR and sequencing primers. Exons are numbered; shaded regions represent introns. Numbers below exons and introns represent approximate size (in base pairs) of sequenced region in *Descurainia*.

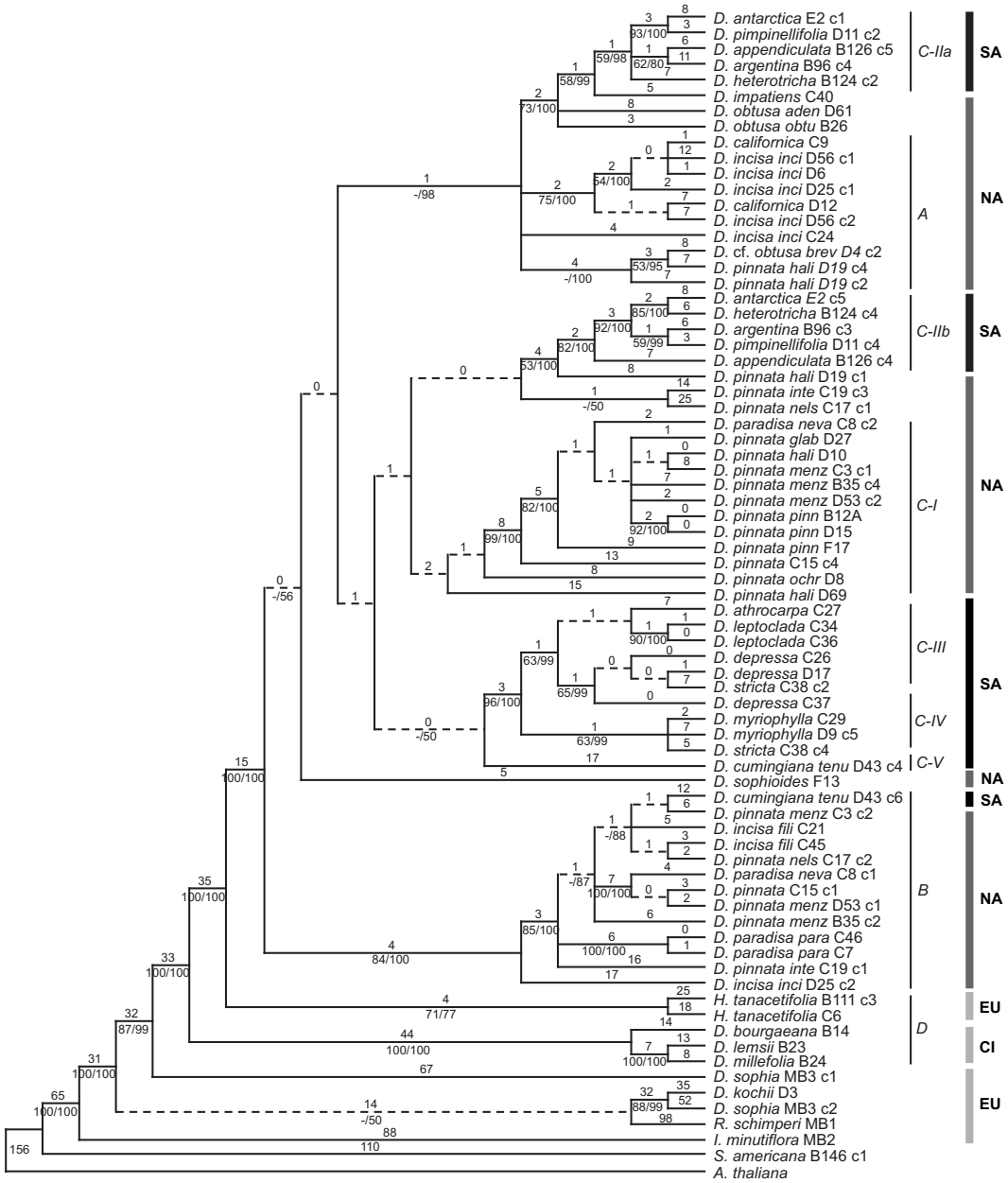

D. antarctica E2 c1
D. pimpinellifolia D11 c2
D. appendiculata B126 c5
D. argentina B96 c4
D. heterotricha B124 c2
D. impatiens C40
D. obtusa aden D61
D. obtusa obtu B26
D. californica C9
D. incisa inci D56 c1
D. incisa inci D6
D. incisa inci D25 c1
D. californica D12
D. incisa inci D56 c2
D. incisa inci C24
D. cf. obtusa brev D4 c2
D. pinnata hali D19 c4
D. pinnata hali D19 c2
D. antarctica E2 c5
D. heterotricha B124 c4
D. argentina B96 c3
D. pimpinellifolia D11 c4
D. appendiculata B126 c4
D. pinnata hali D19 c1
D. pinnata inte C19 c3
D. pinnata nels C17 c1
D. paradisa neva C8 c2
D. pinnata glab D27
D. pinnata hali D10
D. pinnata menz C3 c1
D. pinnata menz B35 c4
D. pinnata menz D53 c2
D. pinnata pinn B12A
D. pinnata pinn D15
D. pinnata pinn F17
D. pinnata C15 c4
D. pinnata ochr D8
D. pinnata hali D69
D. athrocarpa C27
D. leptoclada C34
D. leptoclada C36
D. depressa C26
D. depressa D17
D. stricta C38 c2
D. depressa C37
D. myriophylla C29
D. myriophylla D9 c5
D. stricta C38 c4
D. cumingiana tenu D43 c4
D. sophioides F13
D. cumingiana tenu D43 c6
D. pinnata menz C3 c2
D. incisa fili C21
D. incisa fili C45
D. pinnata nels C17 c2
D. paradisa neva C8 c1
D. pinnata C15 c1
D. pinnata menz D53 c1
D. pinnata menz B35 c2
D. paradisa para C46
D. paradisa para C7
D. pinnata inte C19 c1
D. incisa inci D25 c2
H. tanacetifolia B111 c3
H. tanacetifolia C6
D. bourgaeana B14
D. lemsii B23
D. millefolia B24
D. sophia MB3 c1
D. kochii D3
D. sophia MB3 c2
R. schimperi MB1
I. minutiflora MB2
S. americana B146 c1
A. thaliana
C-IIa
A
C-IIb
C-I
C-III
C-IV
C-V
B
D
SA
NA
EU
CI

Fig. 3.2. One of 4020 most parsimonious trees recovered from TOR sequence data using the parsimony ratchet. Bootstrap values > 50%/Bayesian posterior probabilities are indicated below branches. Dashed lines indicate branches that collapse in the strict consensus tree. Cloned sequences are indicated by the letter "c" followed by the colony number (e.g., c5). Generic names are abbreviated as follows: *A*. = *Arabidopsis*, *D*. = *Descurainia*, *H*. = *Hugueninia*, *I*. = *Ianhedgea*, *R*. = *Robeschia*, and *S*. = *Smelowskia*. The designations A, B, C, C-I, C-IIa, C-IIb, C-III, C-IV, C-V and D refer to clades described in the text. Distributions are abbreviated as follows: NA = North America, SA = South America, CI = Canary Islands, and EU = Europe, Eurasia and/or Middle East.

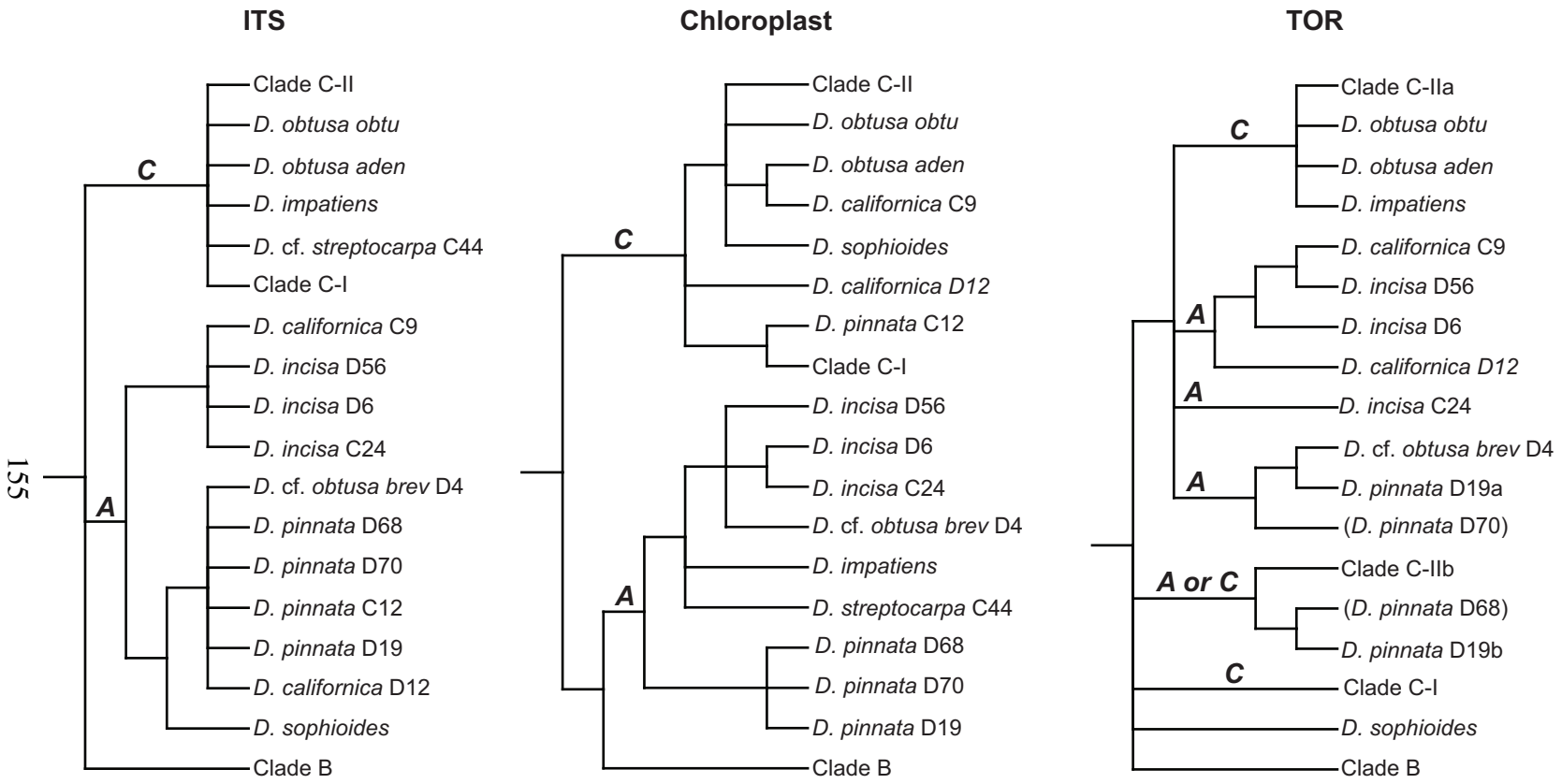


Fig. 3.3. Illustration of some aspects of conflict between ITS, chloroplast and TOR phylogenies with respect to clades A and C. Placement of accessions D68 and D70 in the TOR tree inferred from examination of polymorphic sequence traces.

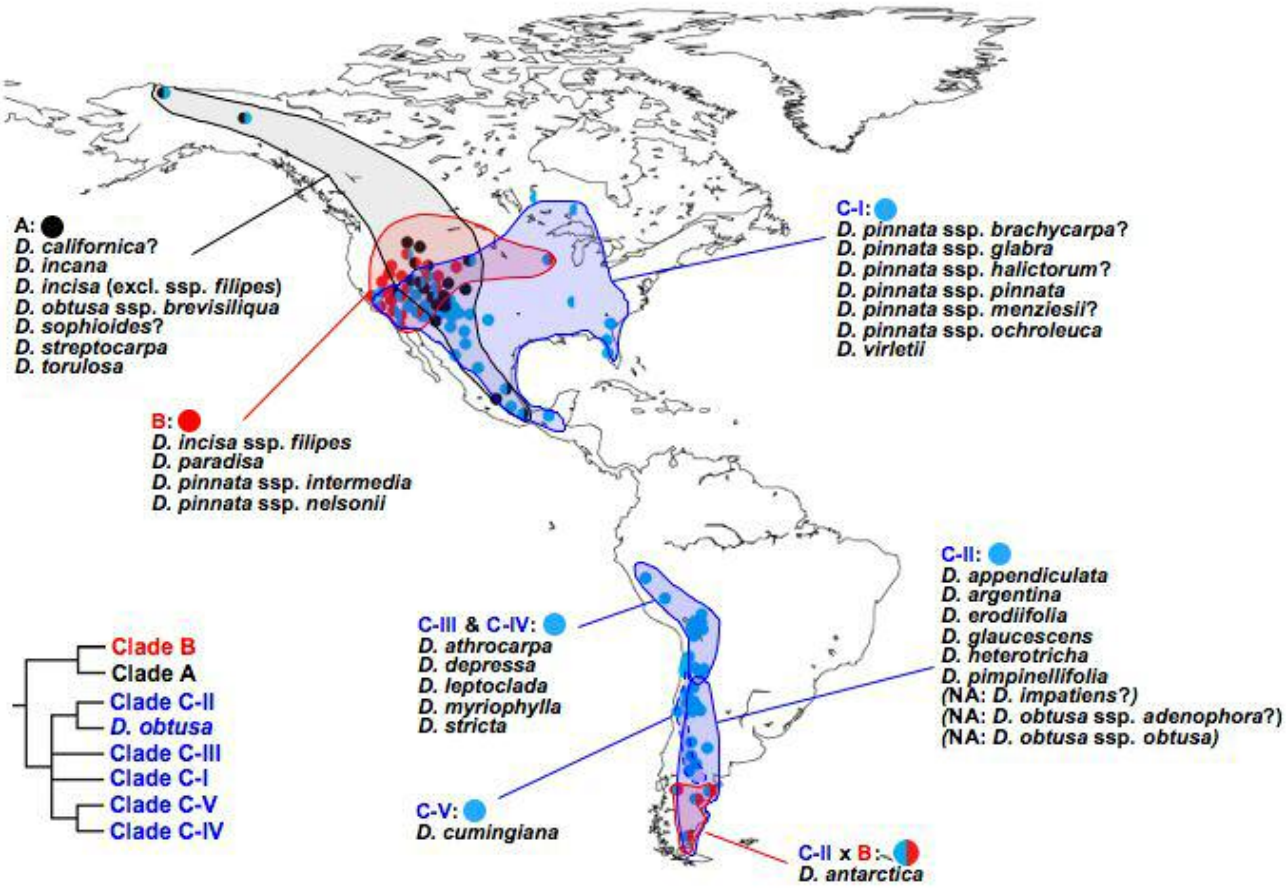


Fig. 3.4. New World *Descurainia* species concepts inferred from ITS, TOR, and chloroplast molecular data, and distribution of accessions with parental lineages. Left side of each colored circle indicates inferred paternal lineage (from ITS and/or TOR) and the right side the inferred maternal lineage (from chloroplast). The tree in the lower left shows lineage relationships based on the topology generated from combined ITS-chloroplast molecular data.

Chapter 4: Molecular systematics of *Descurainia* (Brassicaceae) in the Canary Islands: biogeographic and taxonomic implications

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 insular groups have not yet been acquired, however, the overall picture of evolution within the islands is still emerging. In this study several of these issues are explored 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, 1993a). 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 (cited in Koch & al., 2003a) and the results reported in Chapters 2 and 3 of this dissertation support 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. 4.1) 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. gonzalezi*, 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 chapter, the origin and evolution of *Descurainia* in the Canary Islands is examined 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. — All seven members of *Descurainia* sect. *Sisymbriodendron* were sampled. For each species, material was obtained 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 molecular data of Chapter 2 support the monophyly of both sect. *Sisymbriodendron* and New World *Descurainia*, for this analysis the remaining Old World congeners (*D. kochii*, *D. sophia*, and *H. tanacetifolia*) and two New World representatives (*D. depressa* and *D. incisa*) were included. *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 Table 4.1.

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. 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* (Demesure & al., 1995), *rpoB-trnC*^{GCA} (Shaw & al., 2005), *ndhF-*

rpl32, and *ndhC-trnV*^{UAC} intergenic spacers. Primers for *ndhF-rpl32* (*ndhF*-F: 5'-ACTGGAAGTGGAATGAAAGG-3'; *rpl32*-R: 5'-GCTTTCAACGATGTCCAATA-3') and *ndhC-trnV* (*ndhC*-F: 5'-TGCCAAAACAGGAATAGCAC-3'; *trnV*-R: 5'-TTTACCGAGCAGGTCTACGG-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 25mM $MgCl_2$, 4 µL of 0.25µM dNTPS, 0.5 µL of a 100µM 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 1min, 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 Table 4.1.

Parsimony analyses were performed on each data set with PAUP* 4.0b10 (Swofford 2002). For each data set, 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 data set and a combined chloroplast data set 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 data set was SYM+Γ. For the combined chloroplast data set, separate models were applied to the two data partitions with all parameters unlinked except for topology and branch length; the F81+Γ model 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 data set. 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. gonzalezi* X *D. bourgaeana* (see discussion), were removed from the combined chloroplast data set. Parsimony analysis of the reduced data set 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.

RESULTS

Analysis of ITS data. — The ITS data set 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 (Table 4.2).

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. 4.2). The Canary Island taxa form a well-supported clade (bootstrap value [BV] = 86%) which is sister to *Hugueninia tanacetifolia* in the strict consensus tree. The node joining the island taxa with *H. 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 data set 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 *H. tanacetifolia* as the closest continental relative.

Analysis of chloroplast data. — Sequence characteristics for the various chloroplast regions are listed in Table 4.2. Trees obtained from parsimony analysis of the individual chloroplast data sets (not shown) were congruent, which is expected as the chloroplast is inherited as a single unit and thus all genes should be linked. The individual chloroplast data sets were consequently combined into a single data set and subjected to further analyses. The combined data set 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 data set, 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. 4.3. The tree strongly supports (BV = 100%, PP = 100%) the monophyly of the Canary Island taxa and a sister relationship to *H.*

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. gonzalezi*. 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. gonzalezi* (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 data sets 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 data set contains no parsimony-informative characters within the Canary Island taxa), parsimony and Bayesian analysis of the combined data sets (excluding *D. kochii*) generated results essentially identical to those generated by analysis of the chloroplast data set 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 data set 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 traced separately on the reduced tree (Figs. 4.4 and 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. 4.4). 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, two into pine forest and one into high altitude desert zones.

When island distribution was traced on the reduced tree, two most parsimonious reconstructions were generated (Fig. 4.5). 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 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 to Tenerife (*D. gonzalezi*, *D. lemsii*, and *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). The samples in this study 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. gonzalezi* (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. gonzalezi*. Pérez de Paz (1981) has noted the two reported locations of *D. gonzalezi* 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. gonzalezi* collected at Las Cañadas, where it is rare and sympatric with *D. bourgaeana*, have detected triploid ($2n = 21$) and tetraploid ($2n = 28$) individuals in addition to diploids. Chromosome counts of all other Canary Island species have been exclusively diploid ($2n = 14$) (Borgen, 1969; Bramwell, 1977; Suda & al., 2003). On the basis of this chromosomal evidence, it appears likely that *D. gonzalezi* from Las Cañadas is hybridizing with another species, and *D. bourgaeana* is the geographically closest and therefore most likely candidate. Consequently, the unusual phylogenetic position of this *D. gonzalezi* collection and its short branch length most likely reflects recent chloroplast capture from *D. bourgaeana*.

In addition to the putative *D. gonzalezi* X *D. bourgaeana* hybrid identified in the analysis, other natural *Descurainia* hybrids have also been reported in the Canary Islands. These include *D. artemisioides* X *D. preauxiana* on Gran Canaria (Hansen & Sunding, 1993), *D. bourgaeana* X *D. lemsii* on Tenerife (A. Santos, unpublished), and *D. gilva* X *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. gonzalezi* 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 data sets 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 *H. tanacetifolia* which is distributed in the mountains of southwestern Europe. Like the island taxa, *H. tanacetifolia* is a perennial with relatively large flowers and a diploid chromosome number of $2n = 14$. In contrast, the other European *Descurainia*, *D. sophia*, is a small-flowered annual or biennial with a chromosome number of $2n = 28$.

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 *H. tanacetifolia* is consistent with a derived position for *Descurainia* in the Canary Islands. The low sequence divergence among the island species, and molecular dating reported in Chapter 2, lend 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, *H. tanacetifolia*, is a perennial, and one of its two subspecies, *H.*

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. 4.5). One most parsimonious reconstruction (Fig. 4.5A) 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. 4.5B) 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*). 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. 4.4), 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. gonzalezi*, *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 *H. tanacetifolia*, which is clearly nested within *Descurainia*. The 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, it was possible 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.

Table 4.1. Plant material used in the Canary Island 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 (DNA accession): 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 B14**: Tenerife: Cañadas del Teide, El Portillo, *A. Santos s. n.* (ORT); High altitude desert; DQ418709, DQ418555, DQ418577, DQ418599, DQ418621, DQ418643, DQ418665, DQ418687; **D7**: La Palma: Los Andenes, La Caldera National Park, *A. Santos s. n.* (ORT); High altitude desert; DQ418711, DQ418557, DQ418579, DQ418601, DQ418623, DQ418645, DQ418667, DQ418689; **B171**: Tenerife: Las Cañadas, cultivated, (TEX); High altitude desert; DQ418710, DQ418556, DQ418578, DQ418600, DQ418622, DQ418644, DQ418666, DQ418688*;*
***D. depressa* (Phil.) Reiche**: Bolivia: Patarani, *B. Goodson 1505* (TEX); – ; DQ418712, DQ418558, DQ418580, DQ418602, DQ418624, DQ418646, DQ418668, DQ418690;
***D. gilva* Svent. B22**: La Palma: Cumbres de Puntallana, *A. Santos s. n.* (ORT); Pine forest; DQ418714, DQ418560, DQ418582, DQ418604, DQ418626, DQ418648, DQ418670, DQ418692*;* **B163**: La Palma: Las Manchas, cultivated (TEX); Pine forest; DQ418713, DQ418559, DQ418581, DQ418603, DQ418625, DQ418647, DQ418669, DQ418691;
***D. gonzalezi* Svent. B19**: Tenerife: Vilaflor, Carretera a Madre de Agua, *A. Santos s. n.* (ORT); Pine forest; DQ418562, DQ418584, DQ418606, DQ418716, DQ418628, DQ418650, DQ418672, DQ418694*;* **B160**: Tenerife: Las Cañadas, cultivated, (TEX); High altitude desert; DQ418561, DQ418583, DQ418605, DQ418715, DQ418627, DQ418649, DQ418671, DQ418693;
***D. incisa* (Engelm. ex A. Gray) Britton**: USA: Eagle Co., Colorado, *B. Goodson 1502* (TEX); – ; DQ418717, DQ418563, DQ418585, DQ418607, DQ418629, DQ418651, DQ418673, DQ418695*;*

Table 4.1. Continued.

***D. kochii* (Petri) O. E. Schulz**: Turkey: Kastamonu, *A. Dönmez 11793* (TEX); – ; DQ418718, DQ418564, DQ418586, DQ418608, DQ418630, DQ418652, DQ418674, DQ418696*;*

***D. lemsii* Bramwell B23**: Tenerife: Cumbres de la Orotova, *A. Santos s. n.* (ORT): Pine forest; DQ418720, DQ418566, DQ418588, DQ418610, DQ418632, DQ418654, DQ418676, DQ418698*;* **B170**: Tenerife: La Crucita, cultivated, (TEX); Pine forest; DQ418719, DQ418565, DQ418587, DQ418609, DQ418631, DQ418653, DQ418675, DQ418697;

***D. millefolia* (Jacq.) Webb & Berthel. B24**: La Palma: Barranco del Rio, *A. Santos s. n.* (ORT); Lowland scrub; DQ418721, DQ418567, DQ418589, DQ418611, DQ418633, DQ418655, DQ418677, DQ418699; **B38**: Tenerife: Buenavista, cultivated, (TEX); Lowland scrub; DQ418722, DQ418568, DQ418590, DQ418612, DQ418634, DQ418656, DQ418678, DQ418700; **D1**: Tenerife: Buenavista del Norte, *J. Panero & J. Francisco-Ortega 6987* (TEX); Lowland scrub; DQ418723, DQ418569, DQ418591, DQ418613, DQ418635, DQ418657, DQ418679, DQ418701; **D5**: Tenerife: El Fraile, *A. Santos s. n.* (ORT); Lowland scrub; DQ418724, DQ418570, DQ418592, DQ418614, DQ418636, DQ418658, DQ418680, DQ418702; **F2**: 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 B117**: Gran Canaria: Ayacata, cultivated, (TEX); Lowland scrub; DQ418726, DQ418572, DQ418594, DQ418616, DQ418638, DQ418660, DQ418682, DQ418704*;*

***D. sophia* (L.) Webb ex Prantl**: USA. Saguache Co., Colorado: *B. Goodson 1461* (TEX); – ; DQ418727, DQ418573, DQ418595, DQ418617, DQ418639, DQ418661, DQ418683, DQ418705;

Hugueninia tanacetifolia* (L.) Prantl ssp. *tanacetifolia: Italy: Piemonte. *Pistarino 2027* (NY); – ; DQ418728, DQ418574, DQ418596, DQ418618, DQ418640, DQ418662, DQ418684, DQ418706;

***Smelowskia americana* (Regel & Herder) Rydb.**: USA. Park Co., Colorado: *B. Goodson 1462* (TEX); – ; DQ418729, DQ418575, DQ418597, DQ418619, DQ418641, DQ418663, DQ418685, DQ418707.

Table 4.2. Sequence characteristics of DNA regions used in the Canary Island study.

	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-autapomorphic indels	0	1	2	3	2	2	4	14	0
No. inf. chars. (%) incl. outgroups and indels	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. inf. chars. (%) incl.only island taxa and indels	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 excl. uninf. chars.	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

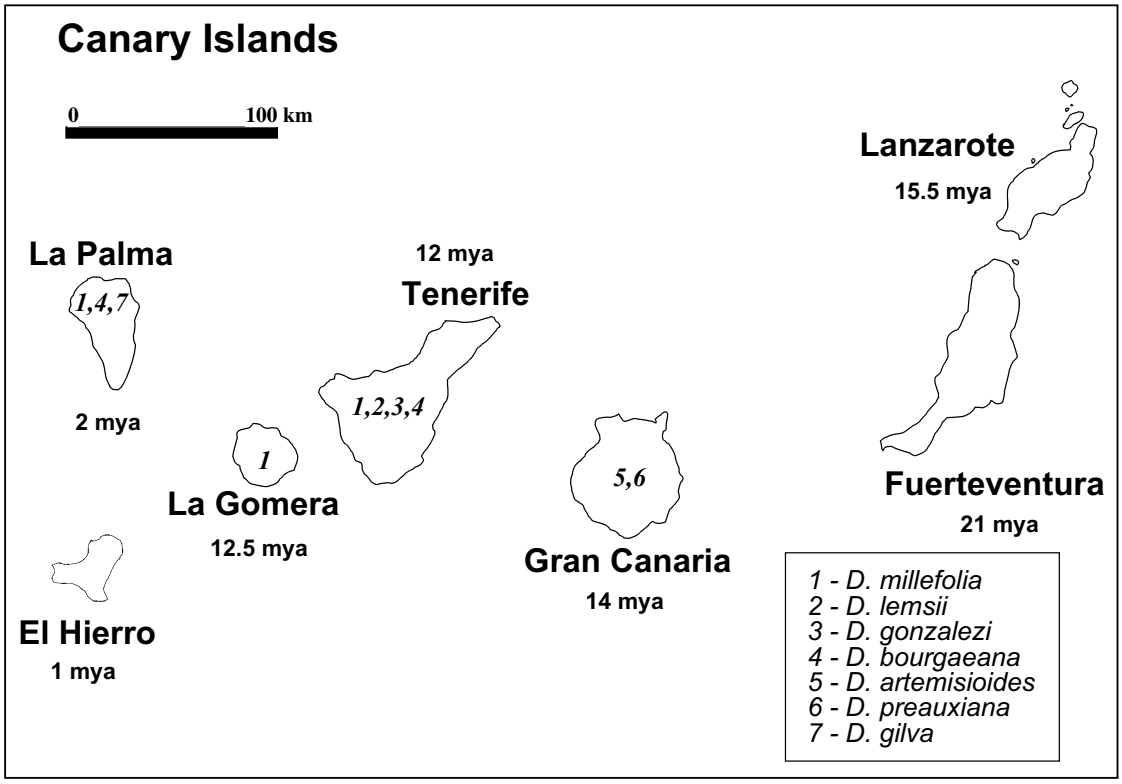


Fig. 4.1. Map and distribution of *Descurainia* in the Canary Islands. Approximate age of each island is given in millions of years (mya) following Carracedo (1994).

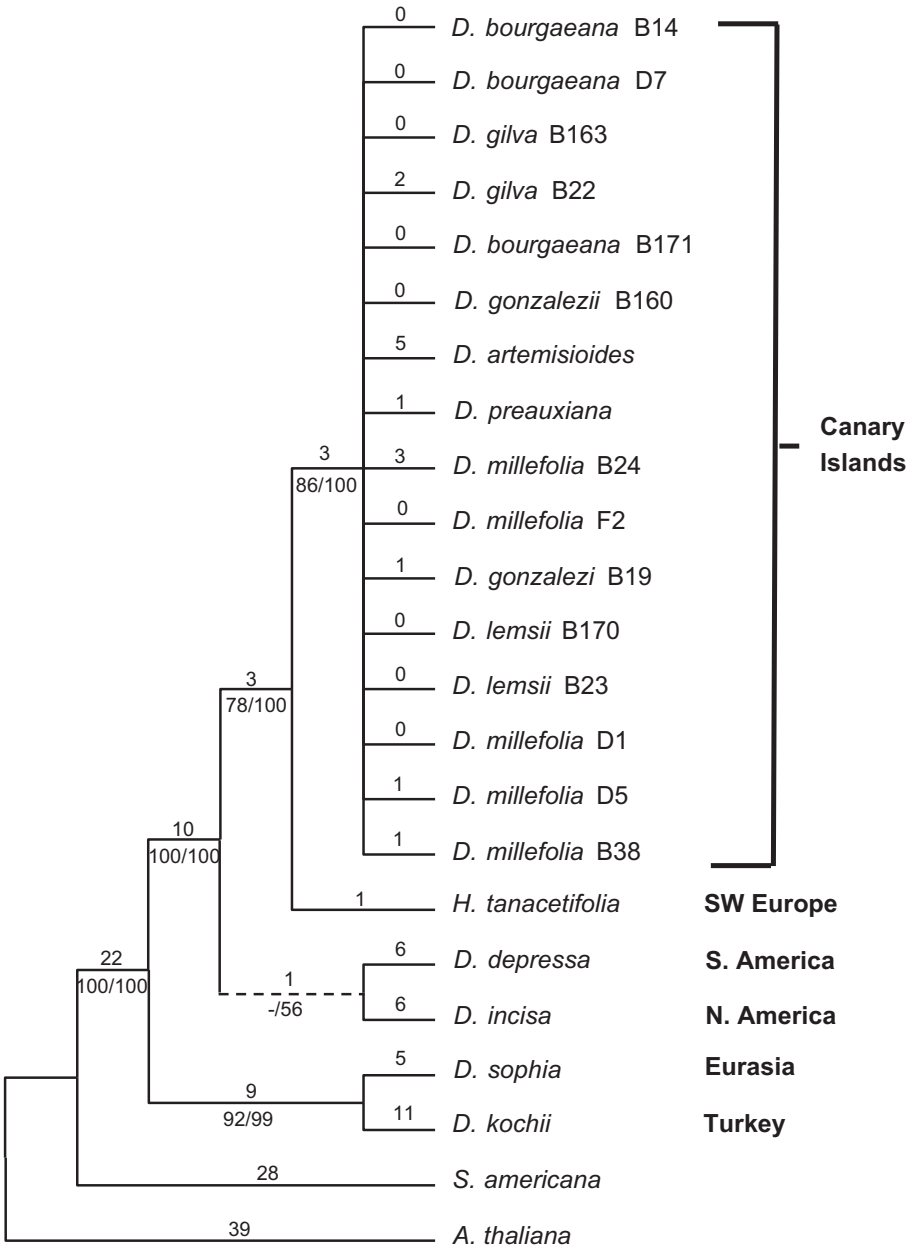


Fig. 4.2. One of 28 most parsimonious trees derived from nuclear ITS data. Dashed lines indicate branches that collapse in the strict consensus tree. Branch lengths are indicated below branches; bootstrap values > 50 % / Bayesian posterior probabilities are indicated below. Generic names are abbreviated as follows: *A.* = *Arabidopsis*, *D.* = *Descurainia*, *H.* = *Hugueninia*,and *S.* = *Smelowskia*.

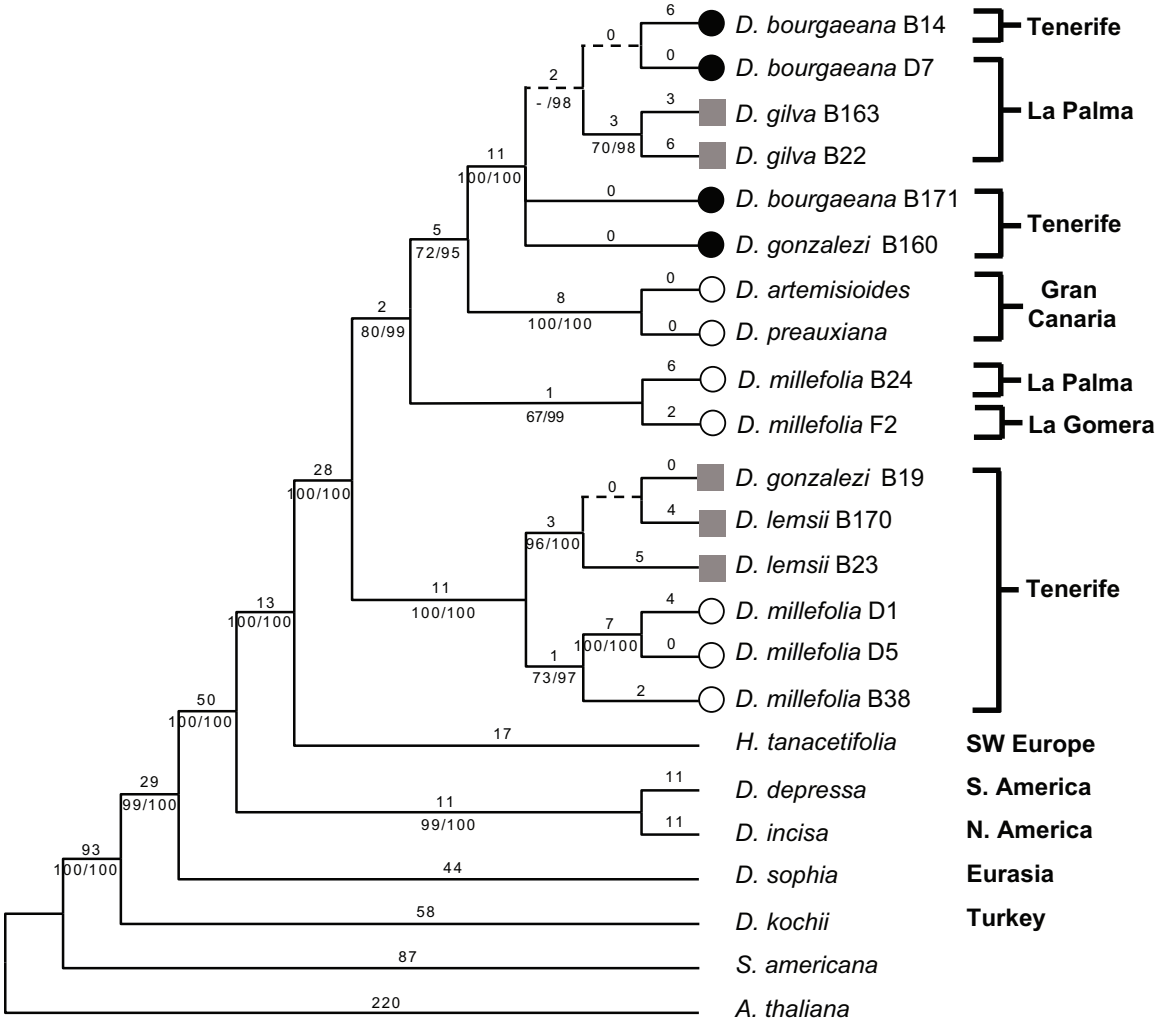


Fig. 4.3. One of 42 most parsimonious trees derived from combined *rps16*, *trnD-trnE*, *trnE-trnT*, *psbZ-trnfM, ndhF-rpl32*, *rpoB-trnC*, and *ndhC-trnV* chloroplast sequence data. Dashed lines indicate branches that collapse in the strict consensus tree. Bootstrap values > 50 % / Bayesian posterior probabilities are indicated below branches; branch lengths above. 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, gray square = pine forest, and filled circle = high altitude desert.

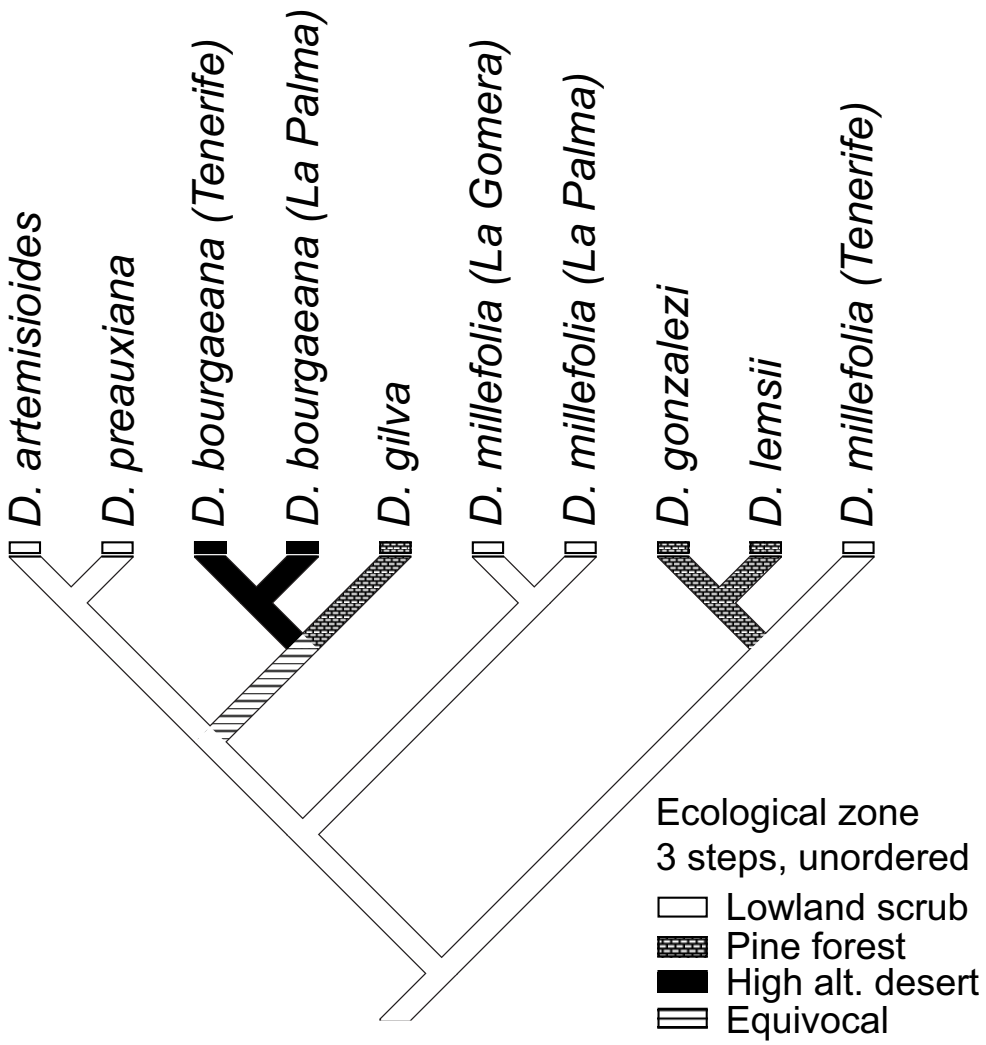


Fig. 4.4. Character state reconstruction generated from optimization of ecological zone on the single most parsimonious tree obtained from phylogenetic analysis of the reduced chloroplast DNA data set with *D. bourgaeana* constrained to monophyly. The state of the branch joining *D. gilva* with *D. bourgaeana* is equivocal; assignment of either lowland scrub, pine forest, or high altitude desert to this branch yield equally parsimonious reconstructions.

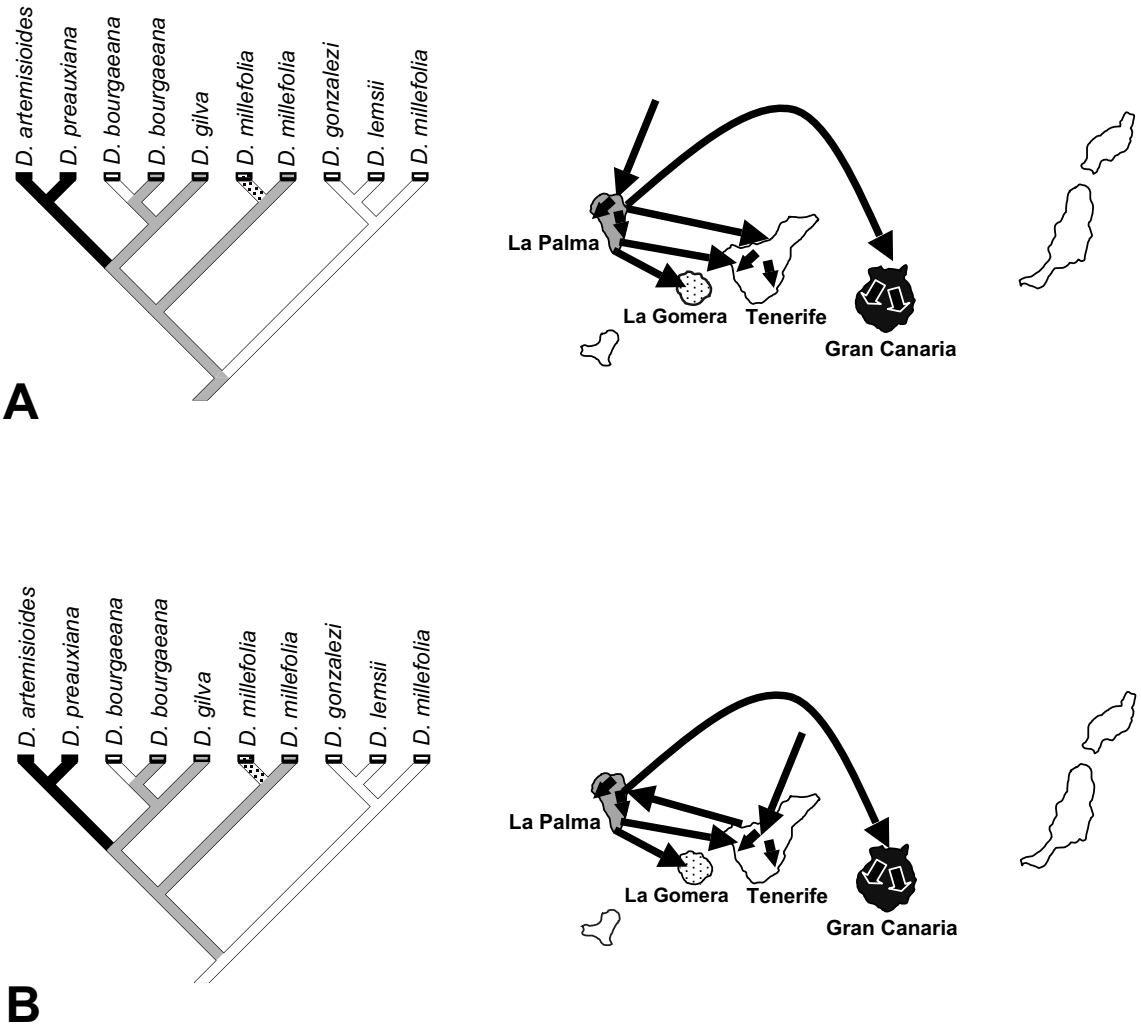


Fig. 4.5. Most parsimonious reconstructions resulting from optimization of island distribution on the single most parsimonious tree obtained after phylogenetic analysis of the reduced chloroplast DNA data set when *D. bourgaeana* is constrained to monophyly. Arrows between islands on the map indicate 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.

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Vita

Barbara Elizabeth Goodson was born 13 June 1959 in Homestead, Florida, the daughter of John and Hazel Goodson. She received a B. S. in chemistry, with a minor in Russian, from the University of Alabama in 1981, and a master's degree (M. C. S.) in computer science from the University of Illinois in 1989. From 1990 until 1997 she was employed in the computer center at Tennessee Technological University.

After developing an interest in gardening with native plants and the Tennessee local flora, she enrolled in the biology program at Tennessee Technological University in January 1998. Her thesis topic was the Vascular Flora of the Clear Fork and New River Gorges of the Big South Fork National River and Recreation Area, for which she was awarded an M. S. in biology in May 2000. In August 2000 she enrolled in the graduate botany program at the University of Texas in Austin.

Permanent address: 2051 Clearview Dr., Cookeville, TN 38506

This dissertation was typed by the author.