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Ribosomal DNA evidence for the diversification of *Tropaeolum* sect. *Chilensia* (Tropaeolaceae)

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Abstract. Maximum parsimony and Bayesian likelihood analysis of ribosomal DNA internal transcribed spacer (ITS) sequences from 55 samples representing 25 species, subspecies, and/or hybrids of *Tropaeolum* sect. *Chilensia* yielded a tree with a large proportion of well-supported nodes at the interspecific and intersubspecific level. However, the tree shows samples from two species, *T. azureum* and *T. brachyceras*, arising in two different positions. Samples representing subspecies of *T. hookerianum* and *T. leptophyllum* suggest that these species are polyphyletic. The data corroborate evidence for hybridization between *T. brachyceras* and *T. tricolor*. Consideration of interfertility data, past and present Chilean ecology, and empirical evidence for the behavior of phenotypic and genotypic characters in known hybrids suggest a high likelihood that reticulate evolution has played a role in the diversification of *T.* sect. *Chilensia*. This reticulate evolution may explain the discordance between the ITS tree and the conventional taxonomy. High divergence in ITS sequences between *T.* sect. *Chilensia* and other members of *Tropaeolum* prohibits reliable outgroup-rooting, but midpoint rooting places the root at a partition comprising taxa whose distribution conforms to a relictual eastern-western South American disjunction described for other taxa. Within the Chilean taxa, the analysis suggests that biogeographic diversification has been from the mesophytic southern habitats northward to central mediterranean and northern arid zones.

Key words: *Tropaeolum*, Tropaeolaceae, hybridization, phytogeography, South America, Chile.

Introduction

Tropaeolum sect. *Chilensia* Sparre *sensu* Andersson and Andersson (2000), including *T.* sect. *Chymocarpus* D. Don *sensu* Sparre and Andersson 1991, *Tropheastrum* Sparre, and *Magallana* Cav., represents a putative clade comprising 22 species and six subspecies out of the total of ca. 90 species of *Tropaeolum* (cf. Sparre and Andersson 1991). *Tropaeolum* sect. *Chilensia* is distributed in temperate South American, primarily in Chile, and the remaining species of *Tropaeolum* are distributed in the American tropics. Phylogenetic analysis of DNA sequences (Andersson and Andersson 2000) of the chloroplast DNA *rbc*L and *rps*16 intron and nuclear ribosomal DNA (nrDNA) internal transcribed spacer (ITS) sequences yielded strong evidence for monophyly of the sampled taxa of *T.* sect. *Chilensia* and the sister relation of these to

T. sect. *Tropaeolum*, which comprises the remaining species of *Tropaeolum*. The latter section previously had been divided into eight sections (Sparre and Andersson 1991). Hernández-Pellicer (2003) undertook a preliminary phylogenetic analysis of the nrDNA-ITS of 35 samples of *T.* sect. *Chilensia*: 26 newly generated sequences, along with nine ingroup sequences from Andersson and Andersson (2000). The objective of this work was to evaluate whether the relative rarity/abundance of Chilean species of *Tropaeolum* was related to their relative degree of relictuality. That study found that different individuals of two species, *T. brachyceras* and *T. azureum*, emerged in two different clades in the ITS trees. In one clade, the taxa emerged as sisters, and, in another, *T. azureum* was sister to an unresolved clade comprising samples of *T. brachyceras*, *T. tricolor*, and morphological hybrids between these known as *T.* x *tenuirostre*. However, the PCR reactions yielded no evidence of intragenomic ITS polymorphism.

In order to clarify the nature of the apparent taxonomic conflicts uncovered in the preliminary analysis (Hernández-Pellicer 2003), the present work analyzes sequences from a total of 55 samples representing *T.* sect. *Chilensia*: 24 new samples, along with the 26 samples of Hernández-Pellicer (2003) and five from Andersson and Andersson (2000). An additional outgroup sequence from Andersson and Andersson (2000) was used in analysis of rooting. Most of the 26 samples used in the preliminary analysis were resequenced in order to resolve ambiguities and to be sure that the taxonomic conflicts did not result from error. Most of the new samples represent *T. azureum*, *T. brachyceras*, and *T. tricolor*. The present analysis also takes into consideration evidence for hybridization discussed by Sparre and Andersson (1991). Finally, the present work presents a perspective on the phytogeography of *T. sect. Chilensia* in the context of broader evidence for Chilean phytogeographic history.

Materials and methods

Taxon sampling. Plant materials (Table 1) for the present work include specimens collected in the field and preserved in silica gel, and herbarium specimens. For the field collections, above-ground parts (variously, leaves, stems, flower buds, but not open flowers) from one or a few individuals, free from insect or other apparent damage or infection, were washed thoroughly with tap water and dried in clean paper towels prior to silica preservation. Herbarium specimens were prepared at the same time. Specimens were identified according to Sparre and Andersson (1991) and Watson and Flores (2000) and were also compared with collections and data in major Chilean herbaria (CONC, SGO, ULS). The analysis incorporated five of nine of the previously published ITS sequences from *T.* sect. *Chilensia* (Andersson and Andersson 2000). Two of these represent taxa not available for the present study (*T. porifolium* and *T. patagonicum*), one for a taxon for which only one sample was available (*T. pentaphyllum* subsp. *pentaphyllum*), and two for taxa here considered problematic (*T. azureum* and *T. brachyceras*). Except for some putatively spurious deletions (see below), the remaining four published sequences were identical to those from the corresponding taxa that were sampled more than once in the present study. In addition to the above, the published sequence of *T. magnificum* was also used in some analyses. The published sequences exclude up to 65 bases of the 5′ end of the ITS sequence. Unavailable for the present study were *T. myriophyllum* (Poepp. and Endl.) Sparre, which is known only from type material, as well as *T. trialatum* (Suess.) L. Andersson and S. Andersson and two of the three subspecies of *T. pentaphyllum*.

Molecular methods. DNA was extracted using the DNeasy Mini protocol (Quiagen, Inc.) and quantified using agarose gels. In some cases, additional purification was necessary and accomplished via either PEG precipitation or by a silica suspension (Boyle and Lew 1995). The PEG protocol used equal volumes of sample and a fresh (< 2 weeks at 4C) solution of 20% PEG 8000 (Sigma)/2.5M NaCl. Following mixing, the solution is maintained at 37 °C for ca. 20 min, followed by high-speed centrifugation, removal of the supernatant, three washes of the pellet with ca. 80% EtOH, drying, and resuspension in water or AE

Table 1. Taxa, specimens, acronyms, and collection data or references for plant materials used in this study. Locality data provided are as complete as are available. Acronyms with the "GB" designation signify samples/sequences published previously (Andersson and Andersson 2000). Locality data include country, first political division, and usually second political division. Except for the Metropolitana Region, main political divisions of Chile are indicated with roman numerals. Superscripts adjacent to the acronyms refer to DNA extraction procedure: [1] extracted from herbarium material, [2] extracted from silica-dried or frozen tissue, [3] ITS sequences determined from more than one independent extraction

Taxon	Acronym	Collection data or reference	GenBank accession
Tropaeolum. sect. *Chilensia* Sparre			
T. azureum Miers ex Colla	azureum 8[2]	CHILE. **V:** Chacabuco, Cuesta Chacabuco, south-facing side near the peak, S 32 57, W 71 41, 20 VIII 2000, *Hernández-Pellicer 8* (CONC)	DQ007252
	azureum 22[2]	CHILE. **IV:** Elquí, Andacollo, S 30 10, W 71 07, 5 X 2000, *Hernández-Pellicer 22* (CONC)	DQ007250
	azureum 49[2]	CHILE. **IV:** Limarí, Combarbalá, road between Montepatria and Combarbalá, just south of Chañaral Alto, S 29 21 08.76, W 071 01, 10 VIII 2002, *Hernández-Pellicer 49* (CONC)	DQ007251
	azureum 992117[2]	CHILE. **Metropolitana:** Chacabuco, Rungue, S 33 03, W 70 52, 750 m, 20 IX 1999, *Arroyo 99-2117* (CONC)	DQ007253
	azureum 119103[1]	CHILE. **Metropolitana:** Santiago, Santiago, Cerro Lo Chena, 580 m, 10 X 1950, *Recabarren 44* (CONC)	DQ007248
	azureum 119178[1]	CHILE. **V:** Quillota, Cerro La Campana, 1500 m, XII 1969, *Gunckel s.n.* (CONC)	DQ007249
	azureum GB	L. Andersson and S. Andersson (2000)	AF253993
T. beuthii Klotzsch	beuthii 11[2]	CHILE. **II:** Antofagasta, Reserva Nacional de Paposo, Quebrada Los Yales, east slope, S 25 00, W 70 26, 8 IX 2000, *Hernández-Pellicer 11* (CONC)	DQ007255
T. brachyceras Hook. & Arn.	brachyceras 13[2]	CHILE. **Metropolitana:** Melipilla, Curacaví, S 33 24, W 71 09, 29 IX 2000, *Hernández-Pellicer 13* (CONC)	DQ007256
	brachyceras 50[2]	CHILE. **IV:** Choapa, Los Vilos, hacia el norte pasado Totoralillo, S 32 03 42, 39, W 071 31, 64 m, 11 VIII 2002, *Hernández-Pellicer 50* (CONC)	DQ007258

Table 1. (continued)

Taxon	Acronym	Collection data or reference	GenBank accession
	brachyceras 37060[1]	CHILE. **Metropolitana:** Santiago, Renca, Cerro Colorado, 600 m, 8 X 1969, *Mahu s.n.* (CONC)	DQ007257
	brachyceras GB	L. Andersson and S. Andersson (2000)	AF253996
T. ciliatum Ruiz & Pav. subsp. *ciliatum*	ciliatum_ciliatum 28[2]	CHILE. **VI:** Carenal Caro, road between Alcones and Pichilemu, 29 XI 2000, S 34 26, W 71 44, *Hernández-Pellicer 28* (CONC)	DQ007259
T. ciliatum Ruiz & Pav. subsp. *septentrionale* Sparre	ciliatum_ septentrionale 34[2]	CHILE. **V:** Quillota, Cerro La Campana, S 32 59, W 71 10, 17 XII 2000, *Hernández-Pellicer 34* (CONC)	DQ007280
T. hookerianum Barnéoud subsp. *hookerianum*	hookerianum_ hookerianum 9[2]	CHILE. **IV:** Elquí, mouth of the Río Limarí, S 30 44, W 71 42, 27 VIII 2000, *Hernández-Pellicer 9* (CONC)	DQ007262
T. hookerianum Barnéoud. subsp. *austropurpureum* J.M. Watson & A.R. Flores	hookerianum_ austropurpureum 15[2]	CHILE. **IV:** Limarí, road between the Panamerican Highway and Canela Baja, 4.5 km from Panamerican Highway, S 31 25, W 71 28, 30 IX 2000, *Hernández-Pellicer 15* (CONC)	DQ007246
	hookerianum_ austropurpureum 47[2]	CHILE. **IV:** Choapa, road between Tunga Sur and road connection Illapel and the Panamerican Highway, S 31 40, W 71 18, 17 IX 2001, *Hernández-Pellicer 47* (CONC)	DQ007247
	hookerianum_ austropurpureum 992341[2, 3]	CHILE. *Arroyo 99-2341*	DQ007254
T. hookerianum Barnéoud subsp. *pilosum* J.M. Watson & A.R. Flores	hookerianum_ pilosum 46[2]	CHILE. **IV:** Elquí, road between Andacollo and road connecting La Serena and Ovalle, S 30 10, W 71 07, 17 IX 2001, *Hernández-Pellicer 46* (CONC)	DQ007275
T. incisum (Speg.) Sparre	incisum 36[2]	CHILE. **VII:** Talca, international road to Laguna del Maule, S 35 58, W 70 33, 24 I 2001, *Hernández-Pellicer 36* (CONC)	DQ007263
	incisum 41[2]	CHILE. **IX:** Malleco, international road between Lonquimay and Liucura, S 38 29, W 71 13, 30 I 2001, *Hernández-Pellicer 41* (CONC)	DQ007264
	incisum 37[2]	CHILE. **VII:** Talca, international road to Laguna del Maule, S 35 07, W 70 29, 25 I 2001, *Hernández-Pellicer 37* (CONC)	DQ007268

Table 1. (continued)

Taxon	Acronym	Collection data or reference	GenBank accession
T. jilesii Sparre	jilesii 263[2]	CHILE. **IV:** Elquí, road between Hurtado and Pabellón near Pabellón, S 30 26, W 70 32, 9 X 2000, *Hershkovitz 00-263* (SGO)	DQ007265
T. kingii Phil.	kingii 16[2]	CHILE. **IV:** Elquí, road between Marquesa and Condoriaco, Cuesta de La Viñita, S 29 52, W 70 50, 1 X 2000, *Hernández-Pellicer 16* (CONC)	DQ007266
T. leptophyllum G. Don subsp. *gracile* (Hook. & Arn.) Sparre	leptophyllum_ gracile 1[2, 3]	CHILE. **VII:** Cauquenes, experiment station of the Instituto de Investigaciones Agropecurias (INIA), S 35 56, W 72 16 , 22 XI 1999, *Hernández-Pellicer 1* (CONC)	DQ007260
	leptophyllum_ gracile 26[2,3]	CHILE. **VIII:** Ñuble, road between Chillán and Portezuelo, S 36 35, W 72 11, *Hernández-Pellicer 26* (CONC)	DQ007261
T. leptophyllum G. Don subsp. *leptophyllum*	leptophyllum_ leptophyllum 30[2]	CHILE. **VII:** Curicó, international road from Los Queñes 12 km before the border with Argentina, S 35 58, W 70 33, 3 XII 2000, *Hernández-Pellicer 30* (CONC)	DQ007267
	leptophyllum_ leptophyllum 5[2]	CHILE. **VI:** Colchagua, Termas del Flaco, S 34 55, W 70 28, 23 XI 1999, *Hernández-Pellicer 5* (CONC)	DQ007269
	leptophyllum_ leptophyllum 6[2]	CHILE. **VI:** Colchagua, Termas del Flaco, S 34 54, W 70 30, 23 XI 1999, *Hernández-Pellicer 6* (CONC)	DQ007270
T. looseri Sparre	looseri 17[2]	CHILE. **IV:** Elquí, road between Marquesa and Condoriaco, Cuesta de La Viñita, S 29 52, W 70 50, 1 X 2000, *Hernández-Pellicer 17* (CONC)	DQ007272
	looseri 0299[2]	CHILE. **III:** Huasco, road between Vallenar and Los Morteros 10 km past Los Morteros, S 28 39, W 7026, 2293 m, *Hershkovitz 02-99* (SGO)	DQ007265
T. nuptae-jacundae Sparre	nuptae-jacundae 27[2]	CHILE. **IX:** Malleco, road between Collipulli and Mininco 3 km from Mininco, along railroad tracks, S 37 49, W 72 27, 26 X 2000, *Hernández-Pellicer 27* (CONC)	DQ007273
T. patagonicum Speg.	patagonicum GB	L. Andersson and S. Andersson (2000)	AF253984

Table 1. (continued)

Taxon	Acronym	Collection data or reference	GenBank accession
T. pentaphyllum Lam. subsp. *pentaphyllum*	pentaphyllum 58476[1]	ARGENTINA. **Buenos Aires:** Punta Lara, 18 VIII 1946, *W. Partridge,* (BA)	DQ007274
	pentaphyllum GB	L. Andersson and S. Andersson (2000)	AF254035
T. polyphyllum Cav.	polyphyllum 45[2, 3]	CHILE. **Metropolitana:** Cordillera, road to Cajón del Maipo, S 33 40, W 70 04, 3 II 2001, *Hernández-Pellicer 45* (CONC)	DQ007276
	polyphyllum 48[2, 3]	CHILE. **Metropolitana:** Cordillera, Valle Nevado, *Hernández-Pellicer 48* (CONC)	DQ007277
T. porifolium (Cav.) L. Andersson & S. Andersson	porifolium GB	L. Andersson and S. Andersson (2000)	AF253981
T. rhomboideum Lem.	rhomboideum 23[2]	CHILE. **Metropolitana:** Cordillera, 10 marked curves before Refugio Lagunillas, S 33 36, W 70 17, 22 X 2000, *Hernández-Pellicer 23* (CONC)	DQ007279
T. rhomboideum × *T. tricolor*	rhomboideumX-tricolor 24[2]	CHILE. **Metropolitana:** Cordillera, 10 marked curves before Refugio Lagunillas, S 33 36, W 70 17, 22 X 2000, *Hernández-Pellicer 24* (CONC)	DQ007278
T. sessilifolium Poepp. & Endl.	sessilifolium 32[2]	CHILE. **Metropolitana:** Cordillera, Santuario Nacional Yerba Loca, S 33 20, W 70 20, 13 XII 2000, *Hernández-Pellicer 32* (CONC)	DQ007281
	sessilifolium 35[2, 3]	CHILE. **V:** Petorca, road to Laguna Alicahue, S 32 17, W 70 32, 28 XII 2000, *Hernández-Pellicer 35* (CONC)	DQ007282
T. speciosum Poepp. & Endl.	speciosum 40[2]	CHILE. **IX:** Malleco, Termas de Tolhuaca, S 38 16, W 71 45, 29 I 2001, *Hernández-Pellicer 40* (CONC)	DQ007283
T. tricolor Sweet	tricolor 10[2]	CHILE. **II:** Antofagasta, Paposo, Quebrada de los Yales, S 25 00, W 70 26, 8 IX 2000, *Hernández-Pellicer 10* (CONC)	DQ007286
	tricolor 12[2]	CHILE. **Metropolitana:** Cordillera, Lagunillas, S 33 38, W 70 20, 27 IX 2000, *Hernández-Pellicer 12* (CONC)	DQ007287
	tricolor 14[2]	CHILE. **Metropolitana:** Mellipilla, Colliguay, S 33 16, W 71 11, 29 IX 2000, *Hernández-Pellicer 14* (CONC)	DQ007288

Table 1. (continued)

Taxon	Acronym	Collection data or reference	GenBank accession
	tricolor 20[2]	CHILE. **IV:** Elquí, road between La Serena and de Caleta Hornitos, S 29 38, W 71 18, 1 X 2000, *Hernández-Pellicer 20* (CONC)	DQ007289
	tricolor 21[2]	CHILE. **IV:** Elquí, road between Andacollo and road connecting La Serena and Ovalle, S 30 10, W 71 07, 5 X 2000, *Hernández-Pellicer 21* (CONC)	DQ007290
	tricolor 25[2]	CHILE. **Metropolitana:** Cordillera, 10 marked curves before Refugio Lagunillas, S 33 36, W 70 17, 22 X 2000, *Hernández-Pellicer 25* (CONC)	DQ007291
	tricolor 31[2, 3]	CHILE. **VII:** Curicó, international road from Los Queñes 12 km before the border with Argentina, S 35 07, W 70 29, 3 XII 2000, *Hernández-Pellicer 31* (CONC)	DQ007292
	tricolor 992022[2, 3]	CHILE. **Metropolitana:** Mellipilla, Cuesta Lo Prado, S 33 27, W 70 58, 400 m, 18 IX 1999, *Arroyo 99-2022* (CONC)	DQ007293
	tricolor 992345[2]	CHILE. **V: Valparaiso**, S 31 46, W 71 19, 615 m, 25 IX 1999, *Arroyo 99-2345* (CONC)	DQ007294
	tricolor 994488[2]	CHILE. **Metropolitana:** Cordillera, Reserva Nacional Río Clarillo, S 33 49, W 70 25, 1800 m, 24 XI 1999, *Arroyo 99-4488* (CONC)	DQ007295
T. × *tenuirostre* Steud.	tenuirostre 992024[2]	CHILE. **Metropolitana:** Mellipilla, Cuesta Lo Prado, S 33 27, W 70 58, 470 m, 18 IX 1999, *Arroyo 99-2024* (CONC)	DQ007285
	tenuirostre 2538[1, 3]	CHILE. **Metropolitana:** Talagante, hills around, S 33 25, W 70 54, 400 m, IX 1935, *Montero 2358* (CONC)	DQ007284
T. sect. *Tropaeolum*			
T. magnificum Sparre		L. Andersson and S. Andersson (2000)	AF254018

buffer from the DNeasy kit. To minimize the risk of cross contamination, especially by PCR products, extraction supplies and reagents, as well as PCR reagents, were maintained separately from those of post-PCR procedures. In addition, all surfaces (including equipment, pipettors, and reagent containers) contacted during extraction and PCR reaction preparation were thoroughly washed with 10% chlorine bleach solution prior to performing these procedures. In some cases, specimens were

extracted more than once in order to confirm sequencing results (Table 1).

Amplification of ITS for sequencing generally followed Hershkovitz and Zimmer (2000), i.e., double-strand amplification followed by separate asymmetric amplification of each strand using primers internal to the first. The amplification and sequencing primers are listed in Table 2. The amplification products were sequenced according to the Big-Dye Dye Terminator (Applied Biosystems) or DYEnamic ET Terminator (Pharmacia) cycle sequencing protocols. Most specimens were sequenced in both directions. Exceptions were made for chromatograms that were especially clean in a single direction and which matched completely those of samples sequenced in both directions.

Alignment. Alignment proved to be unproblematic for the ingroup sequences. Excluding eight single-base indels characteristic of all of the published sequences, only four length variations were found, three of them uninformative. The deletions in the published sequences are presumably erroneous, because they occur in positions conserved in all of the sequences generated here, including representatives of most of the same species sampled among the published sequences. The published sequences and corresponding sequences generated here are otherwise identical. Alignment of a representative outgroup sequence (*T. magnificum*) was performed manually using conserved ITS and 5.8S regions (Hershkovitz and Zimmer 1996, Hershkovitz et al. 1999) as anchors.

Phylogenetic analysis. Phylogenetic analyses were performed using PAUP4.0 (Swofford 2002) version b10 and Mr. Bayes version 2.01 (Huelsenbeck and Ronquist 2001). Unweighted maximum parsimony (MP) and associated bootstrap analysis (1000 replicates) were applied with stepwise addition and tree bisection-reconnection. Bootstrap replicates held 10 trees at each addition step with maxtrees at 100 trees per replicate. Gaps were scored as separate characters in the data matrix. Minimum evolution (ME) analyses and associated bootstraps were performed using a variety of distance corrections, as described in the results. Bayesian likelihood analysis using Mr. Bayes specified a general time-reversible (GTR) substitution model (nst = 6) assuming gamma-distributed rate heterogeneity (rates = gamma) with four rate categories (ncat = 4) and with substitution probabilities varying according to Bayesian likelihood estimates of equilibrium base frequencies (basefreq = est). Preliminary experimentation (Hernández-Pellicer 2003) using hierarchical likelihood ratio tests had determined that the substitution and base frequency ratios and gamma rate parameter were all significantly different from one (the equivalent of "no parameter"). These tests were not repeated using the 55-sequence alignment, because the additional samples added relatively few substitutions to the alignment, and because comparison of the present and preliminary results (see below) indicated that the incorporation of numerous parameters had little effect on the tree topology. In other words, it appears that, for these particular data, a one-parameter model does not provoke serious statistical inconsistency, at least for nodes of interest to the present discussion. Estimates and variances for these parameters were derived from the Bayesian analysis. The Bayesian analysis applied 500,000 Markov Chain – Monte Carlo (MCMC) generations, saving trees each 100 generations, four simultaneous chains, with default acceptance/rejection and chain swapping parameters. Posterior probabilities of clades and parameters were calculated using a "burn in" of variously 1000 and 2000 of the 5001 trees generated. In addition to the above, particular analytical procedures described by Andersson and Andersson (2000) and Hernández-Pellicer (2003) were repeated with the present alignment, as noted in the results.

Rooting presented a problem for the present analysis. Combined analysis of Tropaeolaceae chloroplast DNA *rps*16 intron and nrDNA-ITS sequences rooted with Bretschneideraceae (Andersson and Andersson 2000) indicated that *T.* sect.

Table 2. Amplification and sequencing primers. The primers ITS6 and ITS7 were used to amplify the ITS region. The primers N18L18 and ITS4 lie internal to the amplification primers. Each was used to produce single-strand DNA from the double-stranded PCR product and as the primer to sequence single-stranded DNA produced with the other

Name	Sequence
ITS6	TTTCTTTTCCTCCGCTTA
ITS7	GAAGGAGAAGTCGTAACAAG
N18L18	AAGTCGTAACAAGGTTTC
ITS4	TCCTCCGCTTATTGATATGC

Chilensia is sister to *T.* sect. *Tropaeolum* (99% jackknife support in the combined analysis, greater than 50% support in each separate analysis). Within *T.* sect. *Tropaeolum*, *T. magnificum* formed the basal partition (93% jackknife support), followed by a clade comprising *T. cuspidatum* and *T. kuntzeanum*. The present study repeated the jackknife procedure of L. Andersson and S. Andersson using the alignment of *T.* sect. *Chilensia* plus *T. magnificum*. The present work also demonstrates that ITS region sequences of *T.* sect. *Chilensia* are highly diverged from those of *T.* sect. *Tropaeolum*, and that ITS sequences of the latter (including those of *T. magnificum*, *T. cuspidatum* and *T. kuntzeanum*) are much more similar to each other than to those of *T.* sect. *Chilensia* (see below). Thus, in addition to attempting outgroup rooting using *T. magnificum*, midpoint and biogeographic evidence was considered.

Results

Sequence characteristics of ITS in *T.* sect. *Chilensia*. The aligned length of the ITS region is 654 bases, including a relatively long ITS1 sequence (ca. 275 bases; cf. Hershkovitz et al. 1999), the 5.8S sequence (162 bases), and the ITS2 sequence (ca. 217 bases). The alignment includes 157 variable and 118 informative sites, including four length variations, one of which was informative at the interspecific level. As noted, the five previously published ITS sequences incorporated into the analysis (Table 1) introduced additional and presumably spurious gaps at a total of eight positions in the alignment. Because bases at the putatively spurious gaps are invariant, ignoring the spurious gaps would not appear to alter the results relative to those using our sequences alone. This is barring the possibility that *T. porifolium* and *T. patagonicum*, which we did not sample, actually share synapomorphies at these sites. Inclusion of the putatively spurious gaps as informative characters, however, would indicate a closer phylogenetic relationship among the published sequences relative to our samples.

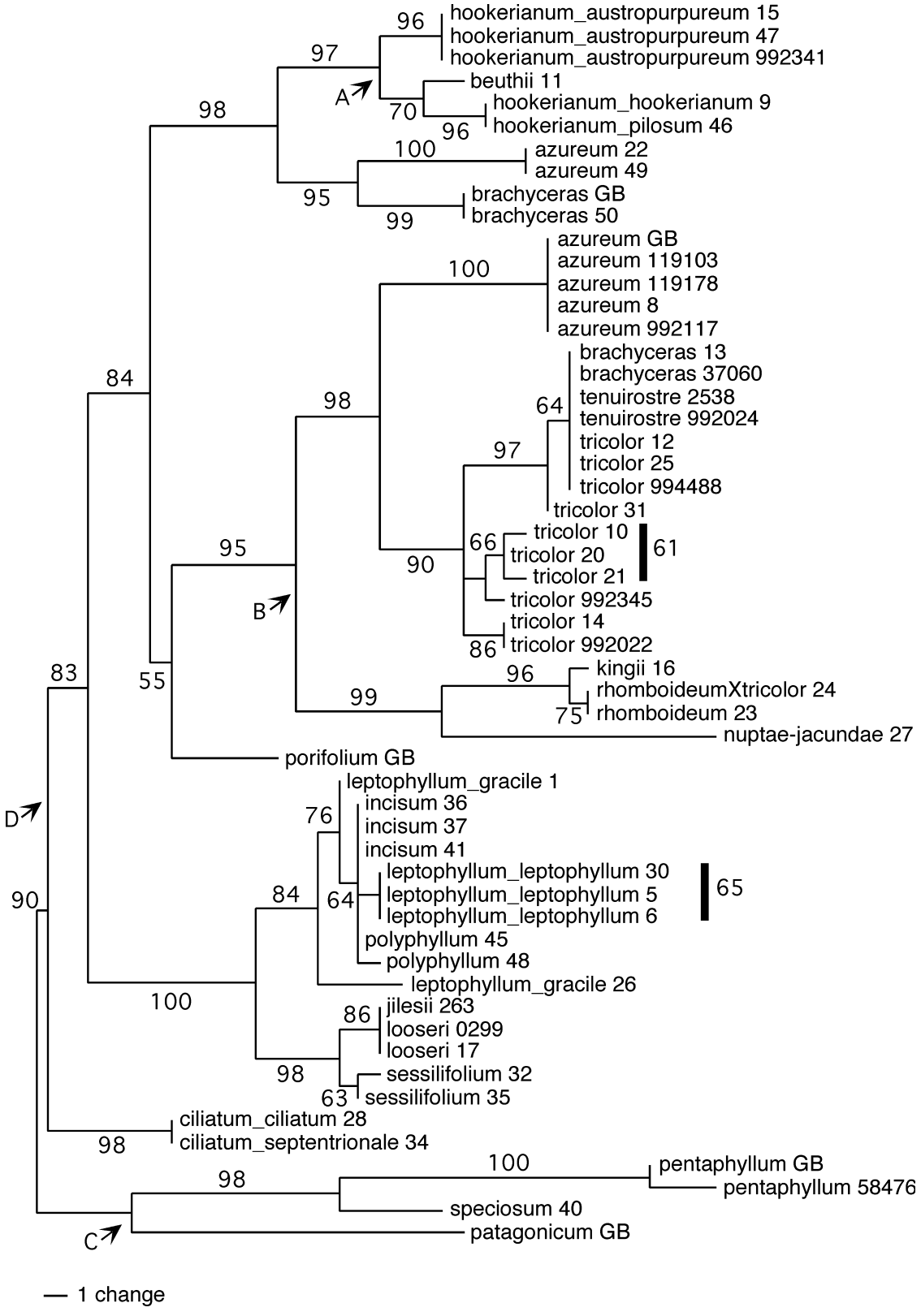
GC content of the sequences appears to be relatively stable, averaging 48% and ranging from 46–51%. However, at informative positions, the spread is much greater, averaging 49% but ranging from 39–62%.

For the present data, the parameter means for substitution bias derived in the Bayesian analysis varied slightly depending on how many initial trees were discarded, but the final 4000 trees yielded relative rates of approximately: dCT = 11, dAG = 5, dAT = dAC = 3, and dCG = dGT = 1. These biases appear to be typical of those found in ITS sequences (e.g. Hershkovitz and Zimmer 2000). However, the 95% confidence interval for each of the estimates (except dGT, which is fixed at 1) spans a five-fold difference, e.g. dCT ranges from 6 to 26. The 95% confidence interval of the gamma rate parameter was much narrower, ranging from 0.047 to 0.052.

Only one clear case of ITS polymorphism was uncovered. One of the *T. tricolor* samples, tricolor_21, was dimorphic. It has a strong signal for a sequence identical to that of the tricolor_20 sample but with a unique three-base insertion toward the 5′ end of ITS1, and a weaker signal for a sequence without this insertion. The sequence with the insertion was included in the analysis. We cannot determine without cloning whether the sequence lacking the insertion is otherwise the same as that in the strong signal.

Rooting of *T.* sect. *Chilensia*. In the MP analysis with outgroup rooting of *T.* sect. *Chilensia* using *T. magnificum*, the root attached within the *T. hookerianum*/*T. beuthii* partition (Fig. 1; see also below), but the jackknife support (following the procedure of Andersson and Andersson 2000) was only 51%. Analysis of the ITS1 sequences alone produced the same result. Using the ITS2 sequences, however, the outgroup attached to the *T. porifolium* branch, but this rooting was equivocal and supported by less than 50% jackknife support. Some of the MP trees placed the root near the midpoint rooting (Fig. 1; see below), while others place the root in the same position as outgroup rooting for the entire sequence.

The high ITS divergence between *T.* sect. *Chilensia* and *T.* sect. *Tropaeolum* possibly


hookerianum_austropurpureum 15
hookerianum_austropurpureum 47
hookerianum_austropurpureum 992341
beuthii 11
hookerianum_hookerianum 9
hookerianum_pilosum 46
azureum 22
azureum 49
brachyceras GB
brachyceras 50
azureum GB
azureum 119103
azureum 119178
azureum 8
azureum 992117
brachyceras 13
brachyceras 37060
tenuirostre 2538
tenuirostre 992024
tricolor 12
tricolor 25
tricolor 994488
tricolor 31
tricolor 10
tricolor 20
tricolor 21
tricolor 992345
tricolor 14
tricolor 992022
kingii 16
rhomboideumXtricolor 24
rhomboideum 23
nuptae-jacundae 27
porifolium GB
leptophyllum_gracile 1
incisum 36
incisum 37
incisum 41
leptophyllum_leptophyllum 30
leptophyllum_leptophyllum 5
leptophyllum_leptophyllum 6
polyphyllum 45
polyphyllum 48
leptophyllum_gracile 26
jilesii 263
looseri 0299
looseri 17
sessilifolium 32
sessilifolium 35
ciliatum_ciliatum 28
ciliatum_septentrionale 34
pentaphyllum GB
pentaphyllum 58476
speciosum 40
patagonicum GB
A
B
C
D
61
65
— 1 change

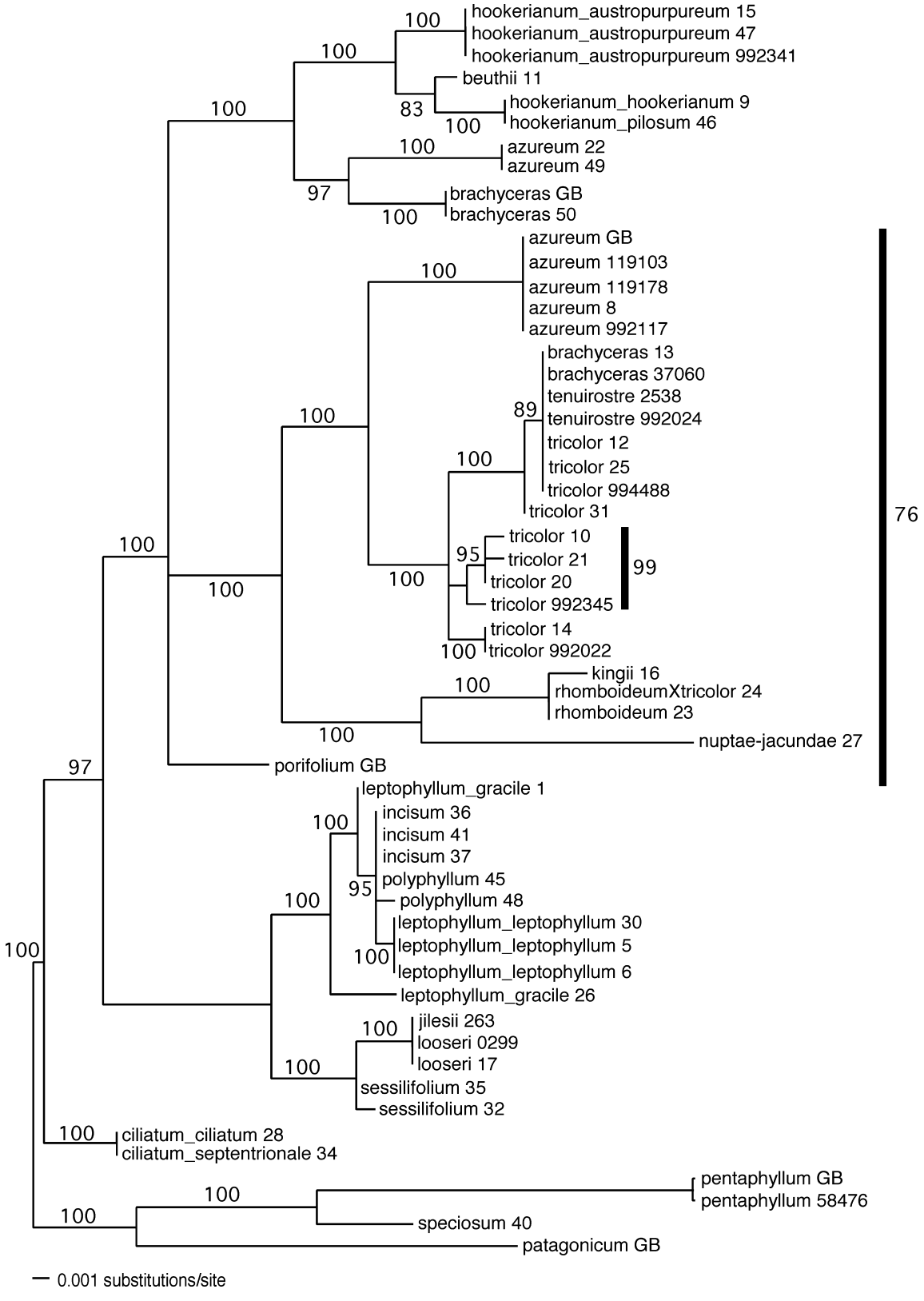
misleads outgroup rooting. Maximum divergences among the ingroup sequences, whether uncorrected or corrected using GTR plus gamma and the parameter values estimated in the Bayesian analysis (see above) were approximately 8%. The uncorrected and corrected distances of *T.* (sect. *Tropaeolum*) *magnificum* from the ingroup sequences were, respectively, 23–25% and 49–56%. A BLAST search (Altschul et al. 1990) using the complete ITS2 sequence of *T. magnificum* recovered all of the previously published sequences of *T.* sect. *Tropaeolum* with E values in the range of e^{-25} $-e^{-40}$ and bit scores ranging from 123–170. The search recovered only 5/9 of the published sequences of *T.* sect. *Chilensia* with E values on the order of e^{-4} and bit scores of 54, i.e. only some of the sequences produced significant matches and the match values were much worse than for sequences of *T.* sect. *Tropaeolum*. In fact, sequences of species of *Nothofagus* produced better matches, with E values on the order of e^{-9} and bit scores of 68. Using the published *T.* (sect. *Tropaeolum*) *kuntzeanum* ITS2 sequence, the BLAST search did not recover any of the sequences of *T.* sect. *Chilensia*.

Because of the high outgroup sequence divergence, midpoint rooting was also evaluated. Midpoint rooting placed the root at a branch between the *T. pentaphyllum*/*T. patagonicum*/*T. speciosum* partition and the remaining species of *T.* sect. *Chilensia* (Fig. 1). The position of the short internal branch shown adjacent to the *T. ciliatum* branch reflects the midpoint calculation. Midpoint rooting of ITS1 sequences alone produced the same root as for the complete sequences. Midpoint rooting of ITS2 sequences, however, placed the root in a different position (Fig. 1). The phylogram representing the final tree of the Bayesian analysis placed the midpoint in the same position as for MP analysis (Fig. 2). ME analysis using a Kimura 2-parameter plus gamma model placed the midpoint one node higher on the more ramified side of the tree (Fig. 1).

Notwithstanding the logic of preferring midpoint rooting for the present data, it should be noted that the midpoint root shown in Figs. 1 and 2 is consistent with the placement of the root in the analysis Tropaeolaceae of Andersson and Andersson (2000), based on both ITS and *rps*16. That analysis showed a basal trichotomy for *T.* sect. *Chilensia*, one solution of which is the midpoint root of the present analysis.

MP bootstrap *versus* Bayesian posterior probabilities for phylogenetic partitions in *T.* sect. *Chilensia*. The bootstrap and posterior probability partitions of the MP (Fig. 1) and Bayesian (Fig. 2) analysis are very similar to the equivalent partitions obtained in the preliminary analysis (Hernández-Pellicer 2003) using a larger number of methodological variations but with fewer samples and with more sequence positions scored as ambiguities. These ambiguities were resolved in the present work by resequencing the same samples. Both consenses show comparatively high support for relations among the sequences. In the bootstrap analysis, partitions with less than 70% bootstrap support are mainly those separating highly similar sequences. The exception is the partition involving *T. porifolium*. The position indicated in both trees is more strongly supported in the Bayesian analysis. As expected, the Bayesian posterior probabilities are equal to or greater than the bootstrap proportions (see discussion). Only three parti-

Fig. 1. Midpoint-rooted MP tree for *Tropaeolum* sect. *Chilensia* ITS sequences. One of two trees, length 198, rescaled consistency index 0.79, retention index 0.96. Taxon acronyms are defined in Table 1. Numbers adjacent to the branches or bold lines to the right of taxon names are bootstrap proportions (1000 replicates). The midpoint rooting is consistent with the outgroup rooting indicated in Andersson and Andersson (2000). Letters adjacent to branches refer to alternative rootings as following: *A* MP, entire sequence, outgroup rooting; *B* MP, ITS2 only, midpoint rooting; *C* MP, ITS2 only, equally parsimonious outgroup rooting; *D* Kimura distance plus gamma, entire sequence, midpoint rooting


hookerianum_austropurpureum 15
hookerianum_austropurpureum 47
hookerianum_austropurpureum 992341
beuthii 11
hookerianum_hookerianum 9
hookerianum_pilosum 46
azureum 22
azureum 49
brachyceras GB
brachyceras 50
azureum GB
azureum 119103
azureum 119178
azureum 8
azureum 992117
brachyceras 13
brachyceras 37060
tenuirostre 2538
tenuirostre 992024
tricolor 12
tricolor 25
tricolor 994488
tricolor 31
tricolor 10
tricolor 21
tricolor 20
tricolor 992345
tricolor 14
tricolor 992022
kingii 16
rhomboideumXtricolor 24
rhomboideum 23
nuptae-jacundae 27
porifolium GB
leptophyllum_gracile 1
incisum 36
incisum 41
incisum 37
polyphyllum 45
polyphyllum 48
leptophyllum_leptophyllum 30
leptophyllum_leptophyllum 5
leptophyllum_leptophyllum 6
leptophyllum_gracile 26
jilesii 263
looseri 0299
looseri 17
sessilifolium 35
sessilifolium 32
ciliatum_ciliatum 28
ciliatum_septentrionale 34
pentaphyllum GB
pentaphyllum 58476
speciosum 40
patagonicum GB
0.001 substitutions/site
76
99

tions are supported with less than 90% posterior probability. Bootstrap proportions using ME analysis with two different distance corrections (Jukes-Cantor with no among site rate variation and GTR plus gamma) produced partitions (not shown) similar to each other and to those in the MP analysis. The main difference was that bootstrap support was 60–65% for the attachment of *T. porifolium* to the clade adjacent (sister) to that in the MP and Bayesian analysis.

Polyphyly of sequences representing four species. The samples of *T. azureum* and *T. brachyceras* from the northern Chilean desert region (Region IV; Table 1) emerge as sisters in a clade that is, in turn, sister to the *T. beuthii*/*T. hookerianum* clade. The samples of *T. brachyceras* from the mediterranean zone (Regions V and Metropolitana; Table 1) have ITS sequences identical or similar to samples of *T. tricolor* and *T.* x *tenuirostre*. The latter are hybrids of these two species from the mediterranean zone (Sparre and Andersson 1991). These *T. brachyceras*/*T. tricolor*/*T.* x *tenuirostre* samples are, in turn, sister to a clade of additional *T. tricolor* sample. Collectively, these samples are, in turn, sister to the mediterranean zone samples of *T. azureum*.

The sequences of two samples of *T. leptophyllum* ssp. *gracile* are different from those of the three samples of *T. leptophyllum* ssp. *leptophyllum* and from each other. The divergence of the samples of *T. leptophyllum* ssp. *gracile* is approximately equal to that of the maximum divergence among samples of *T. tricolor*. Both the MP and Bayesian consenses show strong support for polyphyly of *T. leptophyllum* ssp. *gracile* with respect to the other three taxa. The ITS sequence shared by three samples of *T. hookerianum* ssp. *austropurpureum* is divergent from that shared by the other two subspecies, and the consensus trees indicate that *T. hookerianum* is polyphyletic. The present data also include a morphologically-diagnosed hybrid, *T. rhomboideum x tricolor*. The sequence of this sample is the same as for the sample of *T. rhomboideum*, and no polymorphism was detected in the chromatogram.

Discussion

A principal objective of the present analysis was to examine more critically and with more samples the preliminary finding (Hernández-Pellicer 2003) of dual relations of samples of *T. brachyceras*, and *T. azureum*. It also sought to examine additional discrepancies between the ITS data and the taxonomy and to generate explanations that might reconcile these discrepancies. Finally, the present discussion considers the historical phytogeography of *T.* sect. *Chilensia* in view of southern South American paleoecological evidence.

Comparison of the ITS results with previous conjectures of relationships. Sparre and Andersson (1991) and Watson and Flores (2000) presented relatively few conjectures of relationships among the members of *T.* sect. *Chilensia*, but these can be compared with those suggested by the ITS tree. Presumably, the previous authors considered subspecific taxa to be each other closest relatives. The ITS results in this respect have been presented above. Sparre and Andersson's (1991) discussion includes conjectures of close relationships of:

1. *T. patagonicum* with the *T. polyphyllum* group;
2. *T. porifolium* with *T. pentaphyllum*;
3. *T. incisum* with *T. looseri*;
4. *T. myriophyllum* as a "transition between" *T. incisum* and *T. leptophyllum*;
5. *T. leptophyllum* with *T. polyphyllum*;

Fig. 2. Final topology (tree 5001) of the Bayesian Markov Chain – Monte Carlo procedure. Taxon acronyms are defined in Table 1. Rooting is as in Fig. 1. Numbers adjacent to the branches or bold lines to the right of taxon names are posterior probabilities over the final 4000 trees

6. *T. jilesii* with *T. looseri* "on the one hand" and *T. polyphyllum* and *T. leptophyllum* "on the other;"
7. *T. nuptae-jucundae* with *T. brachyceras* and *T. rhomboideum*;
8. *T. beuthii* with *T. brachyceras* and *T. hookerianum*;
9. *T. brachyceras* with *T. tricolor* "in spite of morphological differences."

The first two conjectures find no particular support in the ITS data. Conjectures 3–6 appear to define a group of closely interrelated species that is evident in the ITS tree, although the details of closest relationships among these differ. Conjecture 7 appears partially correct. A relationship of *T. nuptae-jucundae* with *T. rhomboideum*, less so with *T. brachyceras*, is evident in the ITS tree (but note conjecture 9). Conjectures 8 and 9 can be considered consistent with the ITS data, noting that *T. brachyceras* appears in two places in the ITS tree.

Discordance between the ITS data and the taxonomy of *T.* sect. *Chilensia*. The preliminary analysis (Hernández-Pellicer 2003) sought primarily to produce a provisional phylogeny with which hypotheses on the evolution of species rarity could be tested. The unexpected finding of dual relations of samples *T. brachyceras* and *T. azureum*, among other discrepancies between the ITS data and the conventional taxonomy, proved problematic for this objective. In particular, the analytical parameters in the analysis of rarity included species geographic range size, frequency of individuals within a range, and phylogenetic position. Even if the divergent samples of each of the two taxa were treated as independent OTUs, the geographic ranges and frequency of these OTUs remain unknown. While a scenario of cryptic taxa might explain the dual positions of two taxa, examination of the ITS data in light of conventional data suggest that hybridization may have played an important role not only in the evolution of *T. azureum* and *T. brachyceras*, but of *T.* sect. *Chilensia* more broadly. Hybridization would further confound comparative analyses that assume strict cladogenesis and would have additional implications for the study and management of the Chilean flora.

The discordance between the ITS trees and the conventional taxonomy evoke three possible evolutionary scenarios or a combination of them, as well as the possibility of analytical artifact. One possibility is that the true species have undergone a process of simple cladogenesis, but that the gene tree does not reflect the species tree. In other words, the gene tree possibly reflects genetic/genomic-level processes such as lineage sorting of ancient ITS polymorphisms, selective extinction of ITS paralogs, or selective amplification of functional and degenerate (pesudogene) paralogs (Mayol and Rossello 2001, Tank and Sang 2001, Kimball et al. 2003, Smedmark et al. 2003). A less complicated possibility is that the true species have undergone a process of simple cladogenesis, but that the existing taxonomy is "defective," i.e. does not reflect the true species. The third possibility is that the taxa have a history of reticulate evolution. Finally, such artifacts as branch attraction resulting in misrooting of subclades might explain discordance of phylogenetic trees and taxonomy. It is axiomatic that these possibilities could be evaluated more reliably with additional genomic and taxonomic sampling, but also that no amount of data can reconstruct history with empirical certainty. In the meantime, the likelihood of each of the possibilities can be evaluated with the data at hand.

The gene tree versus the species tree. The possibility that the discordance between the ITS tree and the taxonomy reflects genomic-level processes appears unlikely. Paralogy, lineage sorting, and pseudogenes might be expected to occur in the evolution of ITS sequences, especially because ribosomal genes occur in hundreds to thousands of copies and often at multiple loci. Moreover, the copies are normally subject to infragenomic recombination, gene conversions, and other potentially obfuscating mechanisms that remain poorly understood at the molecular genetic level.

Nonetheless, the genomic scenarios that could explain the present results would tend to leave molecular genetic signatures that should be uncovered with sufficient sampling. For example, pseudogenic ITS paralogs can sometimes be detected by the presence of abnormalities in conserved sequence motifs (Hershkovitz and Zimmer 1996, Hershkovitz et al. 1999, Mayol and Rossello 2001). The similarity some of *T. azureum* and *T. brachyceras* sequences to those of the many *T. tricolor* samples suggests no *a priori* reason to believe that these sequences are pseudogenic. Examination of the other *T. azureum* and *T. brachyceras* samples do not reveal the sort of abnormalities found by Mayol and Rossello (2001) in certain *Quercus* ITS sequences, e.g. the absence of one of the conserved motifs reported by Hershkovitz and Zimmer (1996). Most of the substitutions in the *Tropaeolum* sequences are transitions, most are at sites that vary in other species, and many are homoplasious. The sequences do not appear to produce abnormally long branches in the tree, as might be expected in pseudogenic sequences that evolve without functional constraints. At the same time, it must be stressed that these criteria are useful only when severe abnormalities occur. It is possible that as little as a single substitution could render a ribosomal gene nonfunctional, and this would not be obvious from either the sequence or its putative secondary structure (Hershkovitz et al. 1999).

The possibility that the results can be explained by sorting of functional alleles or paralogs, is not inconceivable, but it would be unexpected then to find no evidence of polymorphism in any of the samples except for the autapomorphic insertion in the tricolor_21 sample. It appears that the persistence of functional ITS paralogs through speciation events is conserved in the descendent taxa and is related to properties shared in the descendents, e.g., allopolyploidy (Campbell et al. 1997). Thus, assuming none of the present ITS sequences are pseudogenes, the alternative possibility that the ITS tree reflects selective extinction of functional paralogs seems incredible. This scenario would require that the functional paralogs diverged prior to the divergence of the common ancestor of the divergent *T. azureum* and *T. brachyceras* samples, which is the common ancestor to a total of 10–11 taxa. The paralogs would have to have coexisted through the eventual divergence of *T. azureum* and *T. brachyceras*, and then, through remarkable coincidence, become selectively and completely extinguished in a manner that separated clades of desert and mediterranean zone taxa (see also below). Finally, the peculiar extinction of paralogs in the absence of polymorphism would cast doubt on the reliability of ITS trees generally.

The species tree versus the traditional taxonomy. The possibility that the discordance between the ITS data and the conventional taxonomy reflects "defects" in the taxonomy also appears *a priori* unlikely. *Tropaeolum* sect. *Chilensia* is one of the best-studied supraspecific taxa in the Chilean flora. The monograph of Sparre and Andersson (1991) culminated more than 30 years of research on the genus, and this work cites two previously published monographs and numerous additional taxonomic publications. In contrast, of four primarily Chilean taxa of Portulacaceae (*Calandrinia* Kunth, *Cistanthe* Spach, *Montiopsis* Kuntze, and *Calyptridium* subg. *Philippiamra* Hershkovitz, ined. = *Cistanthe* sect. *Amarantoideae* Hershkovitz plus *Cistanthe* sect. *Philippiamra* Hershkovitz), only one subgenus of *Montiopsis* has ever been monographed. Comparison of the monography of *T.* sect. *Chilensia* with that of *Montiopsis* subg. *Montiopsis* (Ford 1992) is revealing in this respect, because these taxa have comparable numbers of taxa and their taxonomic histories involve many of the same collectors and floristicians. For 25 recognized nonhybrid taxa of *T.* sect. *Chilensia* (*sensu* Andersson and Andersson 2000), Sparre and Andersson (1991) listed a total of 12 validly published nonhomotypic synonyms based on wild collections. For 15 recognized taxa of *M.* subg. *Montiopsis*, Ford (1992) listed 36. This suggests that, aside from being more thoroughly monographed, morphological taxonomic

species of *T.* sect. *Chilensia* have been more easily delimited and taxonomically stable.

Perhaps the only notable oversights of Sparre and Andersson's monograph are the three subspecies of *T. hookerianum* subsequently recognized by Watson and Flores (2000). Sparre and Andersson (1991) noted that *T. hookerianum* was "sometimes finely puberulous," a characteristic considered by Watson and Flores (2000) diagnostic of *T. hookerianum* subsp. *pilosum*. Sparre and Andersson (1991) did not, however, note the presence of blue flowers in this species, a characteristic considered by Watson and Flores (2000) diagnostic of *T. hookerianum* subsp. *austropurpureum*. The ITS tree shows this subspecies as rather divergent from the other two and, in fact, it makes the species polyphyletic. Thus, the ITS data suggest that this subspecies might be better considered as a distinct species. Certainly the distinction must be considered as more than a mere color polymorphism. The oversight of the blue flowers by Sparre and Andersson (1991) seems puzzling, because these flowers are conspicuous along certain well-traveled roads of southern Region IV of Chile. One wonders whether these blue-flowered plants were assumed to represent the sympatric *T. azureum*, as many herbarium specimens are so identified. However the flowers of the latter are a much deeper violet and with strikingly contrasting white throats, and the species are otherwise distinct in several characteristics.

Sparre and Andersson's (1991) discussion of *T. azureum* and *T. brachyceras* are, at best, only slightly suggestive of taxonomic uncertainty regarding the former. In the case of *T. brachyceras*, they comment that it "is well circumscribed and little variable." The only segregates that have been proposed are two varieties described in the mid-19th century from material cultivated in Europe. In the case of *T. azureum*, Sparre and Andersson (1991) listed in synonymy a yellow-flowered taxon that they otherwise considered "hardly worthy of taxonomic recognition." In addition, they listed *T. lepidium* Philippi ex Buchenau from Limarí Province in Region IV, but they remarked that its diagnostic leaf and petal morphology was "within the limits of the not very polymorphic *T. azureum*." Notwithstanding, the name *T. lepidium* may be significant in the context of the present discussion, given the geographic origins of the two clades of *T. azureum* samples. In particular, one clade includes the two more northerly samples from Limarí and Elquí Provinces and the other includes samples from Region V, where *T. azureum* originates, and the Metropolitana Region. Thus, regardless of whether the dual positions of *T. azureum* specimens reflect cryptic species or hybridization (see below), *T. lepidium* may prove to be an appropriate name for the northern plants.

The gene tree versus analytical artifacts. Analytical artifacts do not appear to be a major source of discrepancy between the ITS trees and the taxonomy. This is most obvious in the case of *T. azureum* and *T. brachyceras*. The polyphyly of both sets of samples is well-supported in the MP and Bayesian analysis, and the branch lengths do not suggest any artificial attraction phenomenon. The subclade that includes the subspecies of *T. leptophyllum* cannot be rerooted in a manner that makes this species monophyletic. It should be noted also that the divergence of the two samples of *T. leptophyllum* subsp. *gracile* is surprisingly high for samples of the same species, let alone subspecies. *Tropaeolum hookerianum* could become monophyletic by rerooting this clade at *T. beuthii*. This possibility should be considered given the somewhat more modest bootstrap and posterior probability support for polyphyly of *T. hookerianum*.

Possibly excessive posterior probabilities appear in the Bayesian tree relative to the MP tree, but these cannot explain the discrepancies between the ITS trees and the taxonomy. Bootstrap proportions have a known tendency to be conservative (when high) or liberal (when low) measures of statistical support in the context of phylogenetic analysis (e.g. Li and Zharkikh 1995), irrespective of whether bootstrapping is considered valid in this context (Sanderson 1995). Bayesian posterior proba-

bilities tend to be excessively liberal, especially in the case of very short branches, where the paucity or absence of contradictory evidence can yield overconfidence in clades where there is little or no supporting evidence (Suzuki et al. 2002; Cummings et al. 2003; P. Lewis, oral comm. 2003). This tendency is evident in Fig. 2, where there are high posterior probabilities for branches that are essentially zero-length. However, the nodes that reflect discordance between the ITS tree and the taxonomy are all supported with 70–100% bootstrap support in the MP trees.

The gene tree and hybridization. As a preface to consideration of the possible role of reticulate evolution (gene flow between historically individuated lineages that nominally merit taxonomic distinction) as an explanation for the discordance between the taxonomy and the ITS results, it should be emphasized that reticulate evolution cannot be considered *a priori* an *ad hoc* assumption. As discussed in Hershkovitz (unpubl. data), the likelihood of hybridization is the probability that the observed data would be produced by hybridization in relation to the probabilities of other possible generative processes given some prior likelihood of each possible process. We presume that the alternative process is cladogenesis without hybridization. As with now popular phylogenetic tree-building methods, the likelihoods are conditioned by the likelihoods of the underlying parameters, e.g. interfertility and sympatry (current and historical) among taxa, current and historical ecological conditions that would tend to promote or inhibit intertaxon gene flow, etc. It is further tempered by inheritance properties of the phenotypic and genotypic characteristics under consideration, i.e. the expected genotypes and phenotypes of hybrids versus nonhybrids. In the absence of knowledge sufficient to establish or at least conjecture the value of any parameter, the prior probability of that parameter must be considered "flat," i.e. it must be set at a value that *a priori* neither favors, nor refutes, hybridization.

In the light of evidence for interfertility and sympatry among species of *T.* sect. *Chilensia*, as well as the historical and prevailing ecological conditions, reticulate evolution must be considered as highly likely in the diversification of this group. This conclusion is independent of the results of the ITS analysis, but discordance between the ITS tree and the taxonomy reinforces the likelihood. Sparre and Andersson (1991) implicate nine taxa as parentals of interspecific hybrids. From among these parentals, they list five types as having hybrid origin, two suspected and three confirmed. Most relevant to the present discussion are recurring spontaneous hybrids between *T. brachyceras* and *T. tricolor*, known as *T.* x *tenuirostre*. Spontaneous hybrids between *T. brachyceras and T. azureum* have occurred in cultivation, and Sparre and Andersson (1991) commented that such hybrids might be expected in the wild. Hernández-Pellicer and Moreira (2001) reported the spontaneous natural hybrid between *T. rhomboideum* and *T. tricolor*. Sparre and Andersson (1991) further noted an artificial hybrid of *T. leptophyllum* and *T. polyphyllum*, a possible spontaneous hybrid of *T. nuptae-jacundae* and *T. tricolor*, the possible origin of *T. myriophyllum* as *T. incisum* x *T. leptophyllum*, and the possible origin of *T. jilesii* as *T. looseri* x *T. polyphyllum* or *T. sessilifolium*. Although Sparre and L. Andersson regarded natural hybridization between *T. leptophyllum* and *T. polyphyllum* as unlikely because of elevational differences, the local instability and heterogeneity in Chilean habitats could facilitate contact at any given time (see below).

Ecological evidence probably favors a hybridization scenario. Chile may represent one of the most hybrid-favorable environments in the world. Ecological and historical conditions that promote hybridization and success of hybrids include high degrees of sympatry, in turn promoted by habitat heterogeneity/mosaicism They also include conditions that create unoccupied niches, such as historical ecological instability and extreme environments (Rieseberg 1997). Chile is dominated by mountainous terrain, providing altitudinal gradients of commonly 3000–

4000 m over relatively short distances. The precipitation gradient ranges from absolute desert to rain forest, and temperature regimes range from subtropical to boreal and maritime to continental. The combination of high relief and temperate latitudes provide sharp climatic differences on opposing polar- and equatorial-facing slopes. Chile has experienced extreme ecological instability, especially in the period of ca. 10 million–10,000 ybp (Arroyo et al. 1988, Villagrán 1995, Villagrán and Hinojosa 1997, Hinojosa 2005, Hinojosa and Villagrán 2005). During this period, Chile's extreme alpine and desert habitats and its mediterranean type climate developed, these representing novelties for South America. The vegetation zones were substantially and repeatedly perturbed by advancing and retreating glaciers during the Pleistocene. Climatic instability persists today, influenced by the Southern Oscillation, which generates cycles of significant climate differences approximately every 5–10 years.

Paradoxically, the phenotypic and genotypic characteristics of the discordant samples do not per se support nor refute a hybridization scenario. The characteristics fit a cladistic and hybridization model equally well. It is only in the context of the evidence for interfertility, lack of evidence for restriction of gene flow, and the prevailing ecological conditions that the character patterns favor hybridization. In particular, considerable data indicate that, while particular character patterns reveal hybrids, hybrids have no particular phenotypic or genotypic signature (McDade 1995, Rieseberg and Welch 2002, Morjan and Rieseberg 2004, Rieseberg et al. 2004). Phenotypes of hybrids may appear more similar to one or the other parental type, as intermediates, or as novel forms. Likewise, substantial portions of the genome appear to be labile to introgression without immediate and/or obvious effect on the phenotype. Perhaps the strongest evidence of hybridization is conflicting character patterns not easily explained by homoplasy, e.g. conflict between nuclear and chloroplast DNA sequences or nuclear polymorphisms representing divergent clades. However, hybrids may exist in the absence of such obvious conflicts. For example, elimination in hybrids of divergent parental ITS forms appears to take place over the course of relatively few generations, so that the inheritance is essentially uniparental in evolutionary time (Kotseruba et al. 2003, Song et al. 1995, Smedmark et al. 2003, Kovarik et al. 2005). Thus, hybrids commonly may not exhibit the character patterns considered diagnostic of hybrids. In the present study, it is the conflict between morphology and ITS sequences that may be indicative of hybridization, viz., highly divergent ITS relations of apparently morphologically indistinguishable samples representing different geographic regions. A cladistic process would imply that the morphology of both taxa is highly convergent. Convergence might be expected if the different regions are ecologically are similar, but they are not: one is semi-desert and the other is mediterranean. Again, with the character patterns *per se* fitting cladistic and hybridization models equally well, the ecological circumstances favor the hybridization model.

Superficially, it may seem overly ambitious to infer, based on the present data, the cause of discordance between the ITS data and the taxonomy of *T.* sect. *Chilensia*. It might seem more appropriate to focus efforts on additional tests of the present hybridization hypothesis, e.g. more samples and more genomic loci. However, the rigor of a test of a hypothesis does not depend upon the quality or quantity of particular data, but rather on the estimation of the likelihood of models that explain the *existing* data better or worse than alternative models (cf. de Queiroz and Poe 2003). Additional data permit estimation of model parameters with greater precision. For example, the addition of samples in the present analysis appears to clarify, relative to the preliminary analysis (Hernández-Pellicer 2003), the geographic distribution of ITS genotypes in *T. azureum* and *T. brachyceras*. In particular, the present analysis supports the existence of a northern Chilean clade of species, including the northern Chilean samples of *T. azureum*

and *T. brachyceras*. This clade was less evident in the preliminary analysis, which included a total of five sequences of these two species, two of which were the published sequences derived from samples of cultivated plants of unspecified geographic origin (Andersson and Andersson 2000). Also, the present analysis considers additional (prior) observations, e.g. interfertility in *T.* sect. *Chilensia* and between *T. azureum* and *T. brachyceras* in particular, current and historical ecology, and the empirical behavior of phenotype and ITS characters in known hybrids. These observations refine the estimate of the prior probability of hybridization. Likely models of evolution of *T.* sect. *Chilensia* are, in turn, useful in the parameterization of models for other taxa.

Although the present discussion attempts to validate a reticulate evolution framework to explain the ITS data for *T.* sect. *Chilensia*, the data must be considered too meager to evaluate specific hybridization hypotheses. There are many more reticulograms than cladograms that might explain given data, and hybridization might occur more than once in the history of a lineage. Moreover, there does not appear to be any robust methodology for comparing, specifically, the likelihood of reticulation versus cladogenesis, or, more specifically, distinguishing between homoplasy and lateral transfer. Current methodology appears to be limited to evaluation of data conflict, which could arise via either process (cf. Linder and Rieseberg 2004). In any case, the simplest scenario for the *T. brachyceras* results would appear to be introgression from *T. tricolor* into the mediterranean zone plants. However, *T. tricolor* shows considerable ITS diversity, and the mediterranean zone samples of *T. brachyceras* and *T.* x *tenuirostre* form part of a distinct cluster. Thus, the mediterranean plants of *T. brachyceras* might represent an historically individuated lineage that is introgressing into *T. tricolor*. The divergence between the northern and southern samples of *T. brachyceras* and *T. azureum* might be explained by ancient introgression with the *T. tricolor* clade followed by backcrossing to maintain the *T. brachyceras* and *T. azureum* phenotypes. This speculation would predict that trees for genes related to phenotype should show each species as monophyletic. Thus, a test of the hypothesis is conceivable, if not immediately practical.

This discussion is timely given the state of molecular biosystematic studies in Chile and other Latin American countries (Michelangeli et al. 2004) and the recognition of the potential effect of phylogeny on emergent ecological patterns in of Chile (e.g. Arroyo et al. 1995b, Hernández-Pellicer 2003). Note that for the present study, sampling of single individuals of *T. azureum* and *T. brachyceras*, or even multiple individuals from the same geographic region, would have produced very different phylogenetic conclusions, hence different conclusions in derivative comparative analyses. However, the implications of the present phylogenetic results extend beyond evolutionary studies and into more ecological and conservation concerns. For example, regardless of whether hybridization or convergence better explains the discordance uncovered, the results suggest caution in the interpretation of such parameters as rarity and endemism of morphological taxa, likewise in the interpretation of biotic inventories.

Phytogeography of *T.* sect. *Chilensia*. Even without consideration of rooting, several partitions in the tree delimit recognized phytogeographic regions. As noted above, the samples of *T. azureum* and *T. brachyceras* from the semi-desert region are sister to a partition of coastal to lowland semi-desert to desert taxa (*T. beuthii* and the subspecies of *T. hookerianum*). The desert clade is part of a larger partition whose remaining members include most of the taxa associated with mediterranean-zone vegetation (*T. tricolor*, *T. rhomboideum*, and the mediterranean zone samples of *T. azureum* and *T. brachyceras*). *Tropaeolum tricolor* is most common in the mediterranean zone, although its range extends further to the north and south (Sparre and Andersson 1991). The exceptional taxa in this partition are *T. kingii*, which occurs inland in the semi-desert to desert zone (Regions III and IV) and the Argentinean taxa

T. porifolium and *T. trialatum*. Both of the last two taxa occur in xerophytic vegetation primarily in the northern Patagonian Andes (at mediterranean zone latitudes), but *T. porifolium* extends to coastal southern Patagonia. The next major partition in the ITS tree circumscribes a group of cordilleran taxa (*T. incisum*, *T. polyphyllum*, *T. sessilifolium*, *T. jilesii*, *T. looseri*, and the subspecies of *T. leptophyllum*). These taxa occur primarily at mediterranean zone latitudes, but *T. jilesii* and *T. looseri* occur in the semi-desert to desert zone. The remaining partitions include the subspecies of *T. ciliatum*, which together extend from the cordillera of central Chile to the Valdivian rainforest, and a cluster of disjunct taxa, *T. speciosum*, *T. patagonicum*, and the subspecies of *T. pentaphyllum*. *Tropaeolum speciosum* is the only species endemic to the Valdivian rain forest. *Tropaeolum patagonicum* has a broad range in Argentinean Patagonia, but is restricted to humid sites (Sparre and Andersson 1991). *Tropaeolum pentaphyllum* subsp. *megapatelum* (Buchenau) Sparre occurs in Bolivia, *T. p.* subsp. *metapetaloides* Sparre in southeastern Brazil, and *T. p.* subsp. *pentaphyllum* from southeastern Brazil to northeastern Argentina.

Notwithstanding the suggestions above of problematic rooting and reticulate evolution, the ITS results for *T.* sect. *Chilensia* appear to corroborate phytogeographic patterns suggested by current and historical ecology, as well as the phytogeography of other taxa. Important historical events in the development of the modern Chilean flora (from Arroyo et al. 1988, Villagrán 1995, Villagrán and Hinojosa 1997, Hinojosa 2005, Hinojosa and Villagrán 2005) include the following:

1. During the Eocene, a "mixed" vegetation (i.e. both Neotropical and Antarctic woody elements present, Hinojosa and Villagrán 2005) replaced a late Cretaceous Gondwanan tropical vegetation in the southern cone of South America.
2. During the upper Eocene to Oligocene, the Andes remained a relatively low mountain range, and the area of the modern Atacama Desert was occupied by humid tropical forest. The mixed forest became restricted to what is now central Chile and Argentina, and Patagonia was dominated by Antarctic elements.
3. During the lower Miocene, South America and Antarctica separated and seas inundated southeastern South America. Aridification of the Atacama region began. The mixed and Antarctic vegetations persisted in, respectively, central and southern Chile, but these vegetations no longer extended to Argentina, which was inundated.
4. During the middle to upper Miocene, the Atacama region was desertified. A subtropical mesic forest extended from southern Bolivia to central Argentina and into a portion of central Chile. With the subsiding of the seas in Argentina, the mixed and Antarctic vegetations of central and southern Chile reextended to the modern Atlantic shore. The Humboldt current developed, bringing cold currents to the coast of Chile and beginning the development of the mediterranean type climate.
5. During the Pliocene, the Andes rose to their current stature and the modern phytogeography of southern South America became established. An arid vegetation developed in central Argentina in the Andes rain shadow, and the earlier subtropical mesic forest in this region receded to the modern limits in north and northeastern Argentina. This left a disjunction between forests that were once continuous between Chile and eastern South America.

Molecular dating suggests that Tropaeolaceae had diverged by the end of the late Cretaceous (Hall et al. 2004, Sytsma et al. 2004), at which time South America, Antarctica, and Australia were still connected. If present at that time in South America, Tropaeolaceae would have been part of the tropical vegetation that dominated the entire continent. The divergence between the temperate *T.* sect. *Chilensia* and the tropical *T.* sect. *Tropaeolum* could be aligned with the development of the

mixed forest in southern South America and the subsequent development of the Antarctic vegetation. In this regard, it is noteworthy that midpoint rooting of the ITS tree of *T.* sect. *Chilensia* delimits a partition of taxa that occupy the ecological descendents of precisely these vegetations. As noted above, *T. speciosum* is the only species of the genus endemic to the Valdivian forest, and *T. pentaphyllum* occurs in the subtropical vegetation of southeastern Brazil to northeastern Argentina and also in Bolivia. Thus, these taxa occur in vegetations that are thought to harbor numerous relicts of a mixed vegetation that once connected these regions (Arroyo et al. 1995a, Villagrán and Hinojosa 1997, Hinojosa and Villagrán 2005). Villagrán and Hinojosa (1997) did not list *Tropaeolum* sect. *Chilensia* among several examples of taxa having this relictual distribution, but at that time, there was no phylogenetic evidence for the monophyly of the modern circumscription of *T.* sect. *Chilensia*, nor of its high divergence from the tropical *T.* sect. *Tropaeolum*. It is also noteworthy that *T. patagonicum* shares its range with the xerophytic *T. porifolium,* but is mesophytic. This is consistent with relictuality from an earlier mesic Antarctic vegetation. In any case, the taxonomic frequency of the presumed relictual Valdivian forest-southeast Brazil-Bolivia disjunction pattern and the correlation with paleoecological data suggesting that the relictual taxa of *T.* sect. *Chilensia* should be more mesophytic yield a higher prior probability that the partition produced by midpoint rooting is approximately or precisely correct. It also should be noted that midpoint rooting is accurate to the degree that maximum sequence divergence is balanced on either side of the true root. In other words, given a reasonably balanced divergence pattern, midpoint rooting should place the root at the true root or at least at a proximal internode. An excessively divergent outgroup sequence approaches that of a random sequence, whose probability of attaching to the correct branch in the tree is proportional to the number of branches (cf. Graham et al. 2002). However, to the degree that the divergence pattern is balanced and the outgroup is not excessively divergent, midpoint and outgroup rooting should converge on the same root. Finally, it should be noted that the present rooting and consequent biogeographic scenario is fully consistent with the results of Andersson and Andersson (2000) based on fewer (nine) taxa of *T.* sect. *Chilensia*, but on two gene sequences.

With the rooting described above, the phytogeography of the more derived taxa is reasonably consistent with the paleoecological history. Specifically, *T. ciliatum* subsp. *ciliatum* is a mesophytic mixed forest taxon. The other subspecies, *T. c.* subsp. *septentrionale*, occurs in the mediterranean zone, but only in the montane belt that harbors other austral taxa. The cordilleran taxa diverge subsequently, followed by the lowland mediterranean and desert taxa. These habitats are all more recent than the mixed forest, and the phylogeny appears to correlate with increasing aridity. As noted, *T. porifolium* and *T. trialatum* share their range with *T. patagonicum* but are associated with more xerophytic vegetation (Sparre and Andersson 1991). Although the precise phylogenetic position of *T. porifolium* is unstable in the ITS tree, all methods strongly support its membership in the clade of mediterranean zone and desert taxa.

Finally, it should be noted that the rooting and diversification scenario is consistent with divergence dates calculated using a typical herbaceous dicot ITS divergence rate (5×10^{-9} substitutions/site/year; Hershkovitz and Zimmer 2000). Using the uncorrected and corrected distances between the two *Tropaeolum* sections (see above), this divergence would date at, respectively, 50 and 100 mybp, or lower-upper Cretaceous boundary to the Paleocene-Eocene boundary. Using the maximum distances among sequences of *T.* sect. *Chilensia*, the diversification of this section would have begun ca. 16 mybp, or mid-Miocene.

The alternative rootings appear less likely, because they root at taxa that occupy vege-

tation types that developed most recently. A root among the desert taxa as suggested by outgroup rooting would imply that *Tropaeolum* was absent from the mixed and Antarctic vegetations of Eocene-Oligocene-Miocene. The taxa that occupy the ecological descendents of these vegetations would have evolved more recently. One problem with this scenario is that an ITS divergence rate an order of magnitude faster than the typical rate would be required to date the sectional divergence as mid-Miocene, and a further rate increase would be required to explain the consequential branch length to *T. pentaphyllum*. Another scenario is that *Tropaeolum* was present in the mixed and Antarctic vegetations of Eocene-Oligocene-Miocene, but that those occupants are now extinct. This would account for the high intersectional ITS divergence. The establishment of modern *Tropaeolum* taxa in these habitats would be secondary. In either scenario, the similarity of the distribution of *T. speciosum*/*T. patagonicum*/*T. pentaphyllum* to a relictual Miocene distribution would be coincidence, and the disjunction of *T. pentaphyllum* would be via dispersal. Thus, it appears that the midpoint rooting produces the phytogeographic pattern expected on the basis of modern distributions and paleoecological evidence. As always in likelihood, maximizing the likelihood of the model (the phytogeographic history) also maximizes the likelihood of the parameters (e.g. the rooting).

As a final comment regarding rooting, Sparre and Andersson (1991) remarked that "obviously, *Tropaeolum* has spread from the Andes to SE Brazil in relatively recent times." This implies the scenario of recent long distance dispersal here doubted. Although it is not clear from the discussion, Sparre and Andersson's comment may have been directed towards relationships of disjunct northern Andean and Brazilian taxa of *T.* sect. *Tropaeolum*. In any case, Sparre and Andersson (1991) did not discuss evidence for vicariance between the floras southeastern Brazil and Chile.

Conclusions

Analysis of ITS sequences from samples of *T.* sect. *Chilensia* yield a well-supported but problematic gene tree, especially with respect to the relations of *T. azureum* and *T. brachyceras*. In light of evidence for interfertility, theoretical and empirical evidence for the behavior of plant species under conditions prevalent in Chile, and empirical evidence for the behavior of phenotypic and genotypic characteristics in hybrids, a scenario of reticulate evolution emerges as a likely explanation for discordance between the gene tree and the conventional taxonomy. The likelihood of reticulate evolution can be reevaluated in light of additional sampling of individuals and their genomes, but future studies should perceive the need to test (reject) reticulate evolution rather than assume cladogenesis. The present results should also influence approaches to the analysis of the diversification of other Chilean taxa. Likewise, the present results should influence approaches to the conservation and management of Chilean plants. Finally, despite the discordance between the ITS tree and the taxonomy, the ITS tree is generally consistent with paleoecological evidence for diversification of the Chilean flora.

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References

Altschul S. F., Gish W., Miller W., Myers E. W., Liplan D. J. (1990) Basic local alignment search tool. J. Molec. Biol. 215: 403–410.

Andersson L., Andersson S. (2000) A molecular phylogeny of Tropaeolaceae and its systematic implications. Taxon 49: 721–736.

Arroyo M. T. K., Squeo F. A., Armesto J. J., Villagrán C. (1988) Effects of aridity on plant diversity in the northern Chilean Andes: results

of a natural experiment. Ann. Missouri Bot. Gard. 75: 55–78.

Arroyo M. T. K., Riveros M, Peñaloza Q. A., Cavieres L., Faggi A. M. (1995a) Phytogeographic relationships and regional richness patterns of the cool temperate rainforest flora of southern South America. In: Arroyo M. T. K., Zedler P. H., Fox M. D. (eds.) Ecology and biogeography of mediterranean ecosystems in Chile, California, and Australia. Springer, New York, pp. 134–172.

Arroyo M. T. K., Cavieres L., Marticorena C., Muñoz-Schick M. (1995b) Convergence in mediterranean floras in Central Chile and California: insights from comparative biogeography. In: Arroyo M. T. K., Zedler P. H., Fox, M. D. (eds.) Ecology and biogeography of mediterranean ecosystems in Chile, California, and Australia. Springer, New York, pp. 43–88.

Boyle J. S., Lew A. M. (1995) An inexpensive alternative to glassmilk for DNA purification. Trends Genet. 11: 8.

Campbell C. S., Wojciechowski M. F., Baldwin B. G., Alice L. A., Donoghue M. J. (1997) Persistent nuclear ribosomal DNA sequence polymorphism in the *Amelanchier* agamic complex (Rosaceae). Molec. Biol. Evol. 14: 81–90.

Cronn R. C., Small R. L., Haselkorn T., Wendel J. F. (2002) Rapid diversification of the cotton genus (*Gossypium*: Malvaceae) revealed by analysis of sixteen nuclear and chloroplast genes. Amer. J. Bot. 89: 707–725.

Cummings M. P., Handley S. A., Myers D. S., Reed D. L., Rokas A., Winka K. (2003) Comparing bootstrap and posterior probability values in the four-taxon case. Syst. Biol. 52: 477–487.

de Queiroz K., Poe S. (2003) Failed refutations: further comments on parsimony and likelihood methods and their relationship to Popper's degree of corroboration. Syst. Biol. 52: 352–367.

Ford D. I. (1992) Systematics and evolution of *Montiopsis* subg. *Montiopsis* (Portulacaceae). PhD Dissertation, Washington University, St. Louis.

Graham S. W., Olmstead R. G., Barrett S. C. H. (2002) Rooting phylogenetic trees with distant outgroups: a case study from the commelinoid monocots. Molec. Biol. Evol. 19: 1769–1781.

Hall J. C., Iltis H. H., Sytsma K. J. (2004) Molecular phylogenetics of core Brassicales, placement of orphan genera *Embligia*, *Forchammeria*, *Tirania*, and character evolution. Syst. Bot. 29: 654–669.

Hernández-Pellicer C. C. (2003) Relación entre distribución y posición filogenética en las especies del género *Tropaeolum*, sección *Chilensia*. Masters thesis, Facultad de Ciencias, Universidad de Chile.

Hernández-Pellicer C., Moreira C. (2001) Nota sobre *Tropaeolum rhomboideum* Lemaire, (Tropaeolaceae), una interesante y escasa especie, endémica de Chile Central. Chloris Chilensis 4 (1). http://www.chlorischile.cl.

Hershkovitz M. A., Hahn W. J., Zimmer E. A. (1999) Ribosomal DNA and angiosperm evolution. In: Hollingsworth P. M., Bateman R. M., Gornall R. (eds.) Molecular systematics and plant evolution. Cambridge University Press, Cambridge, pp. 268–326.

Hershkovitz M. A., Zimmer E. A. (1996) Conservation patterns in angiosperm ITS2 sequences. Nucl. Acids Res. 24: 2857–2867.

Hershkovitz M. A., Zimmer E. A. (2000) Ribosomal DNA evidence and disjunctions of western American Portulacaceae. Molec. Phylogenet. Evol. 15: 419–439.

Hinojosa F. (2005) Cambios climáticos y vegetacionales inferidos a partir de paleofloras cenoziocas del sur de Sudamérica. Rev. Geol. Chile. 32: 95–115.

Hinojosa F., Villagrán C. (1997) Historia de los bosques del sur de Sudamérica, I: antecedentes paleobotánicos, geológicos y climáticos del Terciario del cono sur de América. Rev. Chil. Hist. Nat. 70: 225–239.

Hinojosa F., Villagrán C. (2005) Did South America mixed paleofloras evolve under thermal equability or in the absence of an effective Andean barrier during the Cenozoic? Palaeogeog. Palaeoclim. Palaeoecol. 217: 1–23.

Huelsenbeck J. P., Ronquist F. (2001) Mr. Bayes: Bayesian inference of phylogenetic trees. Bioinformatics 17: 754–755.

Kimball R. T., Crawford D. J., Smith E. B. (2003) Evolutionary processes in the genus *Coreocarpus*: insights from molecular phylogenetics. Evolution 57: 52–61.

Kotseruba V., Gernand D., Meister A., Houben A. (2003) Uniparental loss of ribosomal DNA in the allotetraploid grass *Zingeria trichopoda* (2n = 8). Genome 46: 156–163.

Kovarik A., Pires J. C., Leitch A., Lim K. Y., Sherwood A., Matyasek R., Rocca J., Soltis D., Soltis P. (2005) Rapid concerted evolution of nuclear ribosomal DNA in two *Tragopogon* allopolyploids of recent and recurrent origin. Genetics 169: 931–944.

Li W.-H., Zharkikh A. (1995) Statistical tests of DNA phylogenies. Syst. Biol. 44: 49–63.

Linder C. R., Rieseberg L. H. (2004) Reconstructing patterns of reticulate evolution in plants. Amer. J. Bot. 91: 1700–1708.

Mayol M., Rossello J. A. (2001) Why nuclear ribosomal DNA spacers (ITS) tell different stories in *Quercus*. Molec. Phylogenet. Evol. 19: 167–176.

McDade L. A. (1995) Hybridization and phylogenetics. In: Hoch P. C., Stephenson A. G. (eds.) Experimental and molecular approaches to plant biosystematics. Missouri Botanical Garden, St. Louis, pp. 305–331.

Michelangeli F. A., Francisco-Ortega J., González D., Jaramillo A. Ruiz E., Santiago E., Vovides A. P. (2004) Plant molecular systematics in Latin America: status, realities, and perspectives. Taxon 53: 263–265.

Morjan C. L., Rieseberg L. H. (2004) How species evolve collectively: implications of gene flow and selection for the spread of advantageous alleles. Molec. Ecol. 13: 1341–1356

Rieseberg L. H. (1997) Hybrid origins of plant species. Annual Rev. Ecol. Syst. 28: 359–389.

Rieseberg L. H., Church S. A., Morjan C. L. (2004) Integration of populations and differentiation of species. New Phytol. 161: 59–69.

Rieseberg L. H., Welch M. E. (2002) Gene transfer through transgressive hybridization: history, evolutionary significance, and phylogenetic consequences. In: Syvanen M., Kado C. (eds.) Horizontal gene transfer, 2nd edn. Chapman and Hall, New York, pp. 193–210.

Sanderson M. J. (1995) Objections to bootstrapping phylogenies: a critique. Syst. Biol. 44: 299–320.

Smedmark J. E. E., Eriksson T., Evans R. C., Campbell C. S. (2003) Ancient allopolyploid speciation in Geinae (Rosaceae): evidence from nuclear granule-bound starch synthase (GBSSI) gene sequences. Syst. Biol. 52: 374–385.

Song K., Lu, P., Tang K., Osborn T. C. (1995) Rapid genome change in synthetic polyploids of *Brassica* and its implications for polyploid evolution. Proc. Natl. Acad. Sci. USA 92: 7719–7723.

Sparre B., Andersson L. (1991) A taxonomic revision of the Tropaeolaceae. Opera Bot. 108: 1–139.

Suzuki Y., Glazko G. L., Nei M. (2002) Overcredibility of molecular phylogenies obtained by Bayesian phylogenetics. Proc. Natl. Acad. Sci. (USA): 99: 16138–16143.

Swofford D. L. (2002) PAUP*: phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer Associates, Sunderland, Massachusetts.

Sytsma K. J., Litt A., Zihra M. L., Pires C., Nepokroeff M., Conti E., Walker J., Wilson P. G. (2004) Clades, clocks and continents: historical and biogeogrpahical analysis of Myrtaceae, Vochysiaceae, and relatives in the southern hemisphere. Int. J. Pl. Sci 165 (4 Suppl.): S85–S105.

Tank D. C., Sang T. (2001) Phylogenetic utility of the glycerol-3-phosphate acyltransferase gene: evolution and implications in *Paeonia* (Paeoniaceae). Molec. Phylogenet. Evol. 19: 421–429.

Villagrán C. (1995). Quaternary history of the mediterranean vegetation in Chile. In: Arroyo M. T. K., Zedler P. H., Fox M. D. (eds.) Ecology and biogeography of mediterranean ecosystems in Chile, California, and Australia. Springer, New York, pp. 3–20.

Villagrán C., Hinojosa F. (1997) Historia de los bosques del sur de Sudamérica, II: Análisis fitogeográfico. Rev. Chil. Hist. Nat. 70: 241–267.

Watson J. M., Flores A. R. (2000) Two new subspecies of *Tropaeolum* (Tropaeolaceae) from the IV Region (Coquimbo), Chile. Gayana Bot. 57: 61–66.

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