

Phylogeny and evolution of *Perezia* (Asteraceae: Mutisieae: Nassauviinae)

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Abstract A molecular phylogenetic analysis of most of the species of *Perezia* reveals that, as traditionally defined, the genus is not monophyletic with two species more closely related to *Nassauvia* than to *Perezia*. In addition, our results show that *Burkartia* (*Perezia*) *lanigera* is related to *Acourtia* and is the only member of that clade in South America. The remaining species are monophyletic and show a pattern of an early split between a western temperate and an eastern subtropical clade of species. Within the western clade, the phylogeny indicates a pattern of diversification that proceeded from southern, comparatively low-elevation habitats to southern high-elevation habitats, and ultimately into more northern high-elevation habitats. The most derived clades are found in the high central Andes, where significant radiation has occurred.

Key words Andes, biogeography, Mutisieae, Nassauviinae, *Perezia*.

Perezia Lag., a genus of 30–35 primarily high-elevation species, occurs exclusively in South America, primarily in the central and southern Andes, and thus constitutes a useful model for examining the evolution of high-elevation floras in South America. Considered until 1970 (Vuilleumier, 1970) to be the nominate section of a larger genus that also included *Perezia* section *Acourtia* (D. Don) A. Gray, a North American taxon, *Perezia* is now known to be distinct from *Acourtia* D. Don and more closely related to South American genera. Species of *Perezia* occur from sea level in Chile and eastern Argentina to over 4000 m above sea level (a.s.l.) in Bolivia and Peru and from Tierra del Fuego to Colombia. Species range across most of the high Andean habitats, except true páramo, with plant habit often correlated with habitat. The genus has historically included large foliose species that occur in the *Nothofagus* forests of the southern cone and in the Paraguay–southern Brazil–Uruguay basin, as well as tiny rosettes that grow in the very high-elevation puna of central Bolivia.

Perezia is a member of the Nassauviinae, a subtribe of the Mutisieae with 25–27 exclusively America genera (Hind, 2007). *Perezia* is the fifth largest genus in the subtribe (Crisci, 1980). Vuilleumier (1970) monographed the group (as *Perezia* sect. *Perezia*; the other

section, *Acourtia*, having been monographed previously by Bacigalupi in 1931). In the 1970 monograph, Vuilleumier recognized 30 species. Since that work was published, four species have been added (or re-added) to the genus based on morphology, namely *P. lanigera* Hook. and Arn. (but see below), *P. eryngioides* (Cabrera) Crisci & Marticorena (described as a *Trixis* but transferred to *Perezia* by Crisci and Marticorena), *P. catharinensis* Cabrera, and *P. volcanensis* Cabrera. Two species placed by Vuilleumier (1970) in synonymy under *P. purpurea* (i.e. *P. atacemensis* Phil. and *P. burkartii* Cabrera) were treated as distinct species by Cabrera (1978). Of these taxa, *Perezia lanigera* Hook. & Arn. has engendered the most controversy. The entity was originally described as a *Perezia* but, in her monograph, Vuilleumier (1970) excluded it from the genus because of its aspect and the presence of wooly trichomes in the leaf axils. No *Perezia* species has this type of trichome. However, Cabrera (1971) considered these trichomes of little importance and consistently treated the species as a *Perezia*. Crisci (1976), like Vuilleumier (1970), considered the taxon distinct and erected the monotypic genus *Burkartia* Crisci for it, with the comment that *Lophopappus* Rusby was its closest relative.

In 1970, Vuilleumier suggested a potential relationship between *Perezia* and *Leucheria* Lag. based on morphology. Cassini (1825) had earlier placed *Perezia* with *Holocheilus* Cass., *Leucheria*, and *Trixis* P. Browne in a subgroup of one of his three sections of the Nassauviinae, but Crisci (1974) was the first to address relationships of the genera within the subtribe

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in a rigorous way. He scored a wide array of discrete characters, five geographical and 85 morphological (including pollen shape and exine patterning), for 26 taxa that he used in a numerical taxonomic study. In that study, he used various combinations of two different scoring methods and two different measures of similarity to generate three cladograms (Crisci, 1974). The results were, in general, concordant with one another and allowed him to draw several conclusions. One of the conclusions was that the two sections of *Perezia* were not “close” to one another and should be treated as distinct genera (as suggested previously by Vuilleumier 1970). Second, he commented that *Nassauvia* and *Triptilion* were very closely “related”, as well as that *Perezia lanigera* was more closely “related” to a cluster containing *Proustia* Lag., *Lophopappus* Rusby, *Acourtia*, and *Gochnatia glomeriflora* A. Gray than to *Perezia*. Finally, the results suggested that *Perezia* and *Leucheria* were each rather isolated within the subtribe, possibly as a “result of the great spectrum of types presented by the two genera” (Crisci, 1974). Shortly after Crisci’s study, Reveal and King (1973) formally re-elevated *Acourtia* to generic status and provided the necessary new combinations for the caulescent species. A few years later, Turner (1978) moved the North American scapiform *Perezia* species into *Acourtia*.

Six years after his numerical taxonomic study, Crisci (1980) used the same data set to produce phylogenetic hypotheses of relationships in the Nassauviinae. Character polarity was determined based on several criteria and trees were constructed using a Wagner tree algorithm. Three trees were produced, each with a different outgroup. With a hypothetical outgroup, *Perezia* was sister to *Panphalea* DC. and *Holocheilus* was sister to this pair. This same relationship was produced when *Trixis* was used as the outgroup. With *Dolichlasium* Lag. as the outgroup, *Perezia* was sister to a clade consisting of *Panphalea* Lag., *Moscharia* Ruíz & Pavón, *Polyachyrus* Lag., *Calopappus* Meyen, *Nassauvia* Comm. ex Juss., and *Triptilion* Ruíz & Pavón.

Within the past 10 years, there have been two published molecular studies that included several members of the subtribe Nassauviinae. Using *ndhF* sequence data and including representatives of *Acourtia*, *Adenocaulon* Hook., *Jungia* L.f., *Leucheria*, *Nassauvia*, *Perezia*, and *Triptilion*, Kim et al. (2002) reported that the Nassauviinae had only weak support as a clade but that within the subtribe there were several well-supported subclades, one of which included *Perezia* as sister to a *Nassauvia/Triptilion* clade. *Acourtia* was sister to a clade containing *Trixis* and *Proustia* (although this relationship collapsed in the strict consensus tree). *Leucheria* was in a third clade, sister to *Jungia*. In a more re-

cent study, Katinas et al. (2008) used internal transcribed spacer (ITS) and *trnL-F* sequence data to generate a phylogeny that they used to infer the evolution of secondary heads in Nassauviinae. Their phylogeny included 12 of the 25 genera of the tribe: *Ameghinoa* Speg., *Dolichlasium* Lag., *Holocheilus* Cass., *Jungia*, *Leucheria*, *Moscharia*, *Nassauvia*, *Panphalea*, *Perezia* (four species), *Polyachyrus* Lag., *Proustia* Lag., and *Triptilion*. Their results showed *Perezia* as sister to *Panphalea* and this clade sister to a clade of *Nassauvia* plus *Triptilion*. In a paper circumscribing a segregate genus of *Perezia*, namely *Calorezia* Panero, Panero (2007), stated that his unpublished data showed that *Perezia nutans* Less. (and, by association, *P. prenanthoides* Less.) was more closely related to *Calopappus* Meyen, *Nassauvia*, and *Triptilion* than to the rest of *Perezia* and, hence, necessitated a new genus. Panero (2007) considered these three genera along with *Panphalea* and *Perezia* to form a “*Perezia* clade”, called the *Perezia* group in our discussions.

Within *Perezia*, only Vuilleumier (1970) has made inferences about species relationships. In her 1970 revision, she conducted a numerical taxonomic study of the genus using 24 numerical and 23 non-numerical characters that were measured or scored for over 1200 herbarium specimens. Each species was usually represented by several different “populations” (two or more specimens from the same locality). Each of these populations was designated as a terminal taxonomic unit, with the numerical characters of each unit represented by a mean and variance (calculated from the cluster of specimens measured in the population). These values were used to generate dendrograms based on a Mahalanobis’ generalized distance matrix. The dendrograms were rooted by the a priori placement of *P. pungens* (H. & B.) Less. as the first taxonomic unit. *Perezia nutans* and *P. prenanthoides* and *P. multiflora* (H. & B.) Less. and similar leafy species tended to be at the base of the dendrogram, but most of the remainder of the terminal units (populations) came out stepwise with terminal taxonomic units of several variable species scattered across the dendrogram. Although there were some cohesive clusters, the dendrograms did not provide easily interpretable relationships among the terminal taxonomic units included.

Using the generalized distances as a rough guide and combining them with the morphology underlying them, Vuilleumier (1970) suggested six species groups shown in Fig. 1 with distributions indicated in Fig. 2: A, B. Assessing these groups together with paleoecological data, Vuilleumier (1970) postulated that the genus arose early in the Tertiary in the warm open forests that covered extratropical South America. Specifically, she

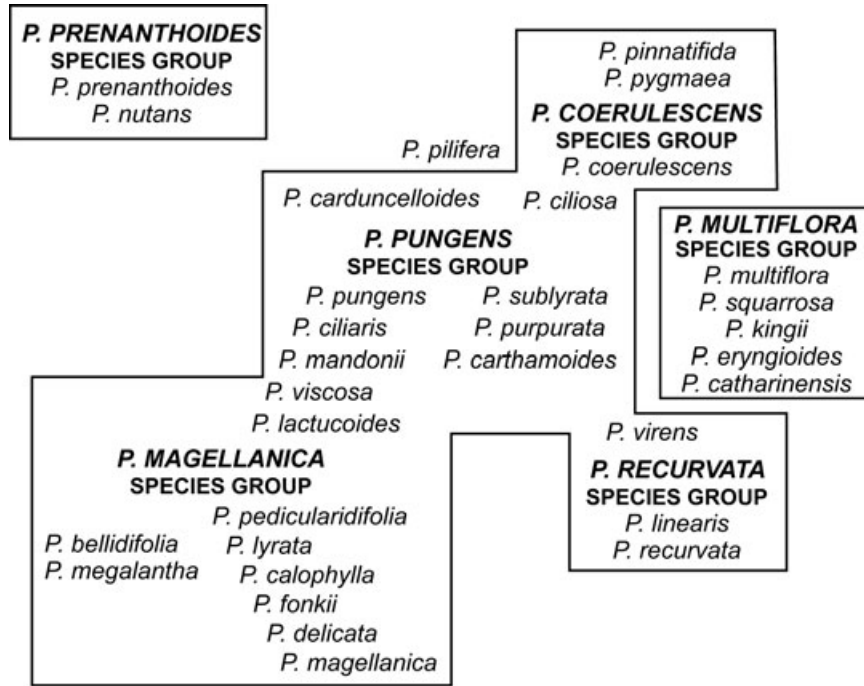


Fig. 1. Species groups modified from Vuilleumier (1970). Note that the prenanthoides and the multiflora groups were considered quite distinct, whereas taxa such as *Perezia pilifera* and *Perezia carduncelloides* could not be placed with certainty.

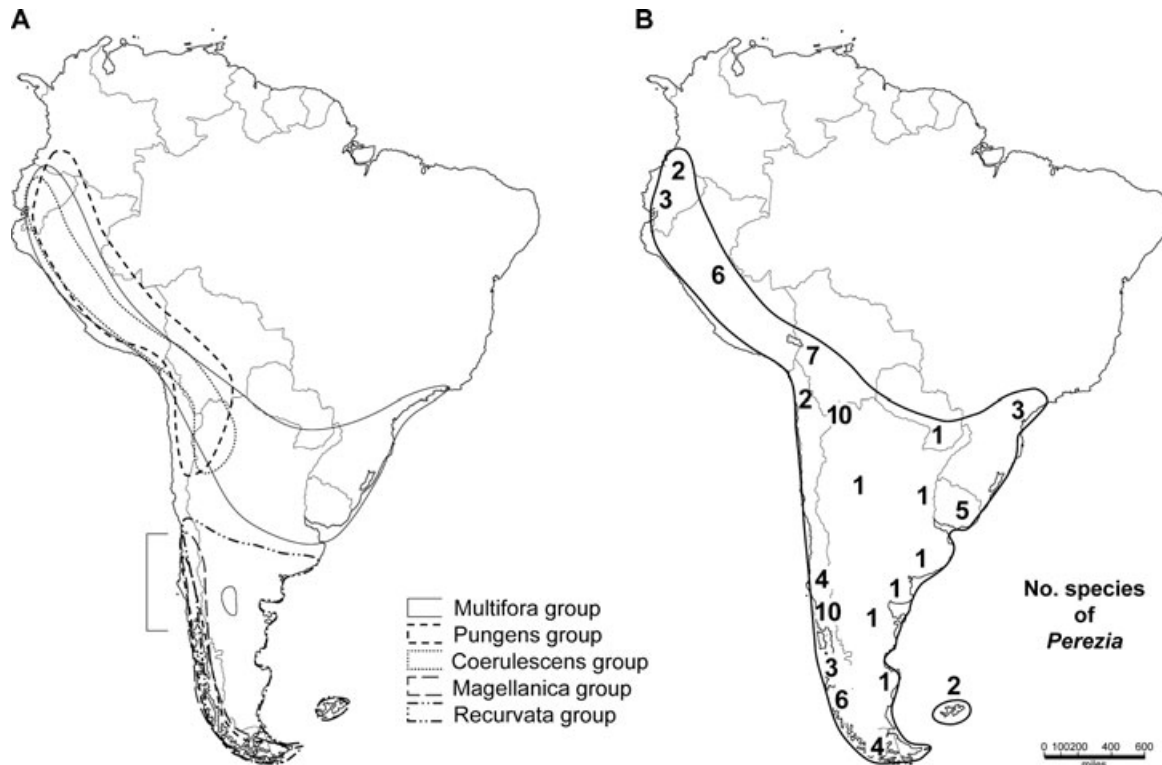


Fig. 2. Distribution of the species groups of *Perezia* (A) as delineated by Vuilleumier (1970) and the number of species in various parts of the generic distribution (B). The species of the former prenanthoides group are excluded, but their distribution is indicated by the brackets that show the northern and southern limits of their distribution in the *Nothofagus* forests of southern South America. The additional species of the multiflora group added after 1970 are included in both A and B.

suggested that the ancestral *Perezia* was similar in habit to *Perezia pungens* and inhabited mid-to-low elevation montane habitats of what is now the central Andes. As drying began in the mid-Tertiary, Vuilleumier (1970) postulated a split leading to the ancestor of the multiflora group in southern Brazil–eastern Argentina, the ancestor of the prenanthoides group in the *Nothofagus* forest, and the ancestor of the remaining species initially at mid altitudes in the central Andean region. She suggested that this last group radiated in the high Andes and southern Patagonia with speciation linked to drying in the late Tertiary, the uplift of the Andes, and Pleistocene climatic fluctuations.

Our purpose here is to generate hypotheses of in-frageneric species relationships of *Perezia* using molecular data in order to assess the directions of change in habit and habitat, and to test the patterns of relationships suggested by Vuilleumier (1970). To establish a phylogeny that allows us to examine these patterns, we have included most of the species of *Perezia*. Based on the studies of Kim et al. (2002), Panero (2007), and Katinas et al. (2008), we have included species of *Acourtia*, *Nassauvia*, *Panphalea*, and *Triptilion* (all previously linked with *Perezia* in various ways), as well as *Adenocaulon* and *Lophopappus*, two other genera in the Nassauviinae.

1 Material and methods

1.1 Material

Material was obtained from herbarium specimens (with permission) or collected in the field. Vouchers and GenBank numbers are listed in Table 1. Included in the ingroup were 28 of the 30–35 species of *Perezia*, two accessions of *Burkartia* (*Perezia lanigera*), the two former species of *Perezia* now placed in *Calorezia*, seven species of *Acourtia*, five species of *Nassauvia*, three species of *Adenocaulon*, and one species each of *Leucheria*, *Panphalea*, and *Triptilion*. *Leucheria* was designated as the outgroup for the purposes of rooting.

1.2 Methods

1.2.1 DNA Sequencing DNA was isolated from herbarium specimens or silica-dried leaf material using a modified cetyltrimethylammonium bromide (CTAB) protocol (Loockerman & Jansen, 1996). A PCR was used to amplify the ITS region using primers P1 and P4 of Kim and Jansen (1994), with internal primers (P2 and P3) also used for amplification or sequencing when necessary.

The chloroplast intergenic spacers *rpl32–ndhF* and *trnL(UAG)–rpl32* were chosen based on their rates of evolution and degree of phylogenetic utility as reported

by Shaw et al. (2007) and Timme et al. (2007). *Perezia*-specific internal primers were designed for the present study to assist with amplification and sequencing when necessary. The sequences of the primers used for these regions are given in Table 2.

The PCR reactions contained 2.5 μL of 10 \times PCR buffer; 2 μL of a 10 mmol/L stock solution of combined dNTPs, 2–4 μL of 25 mmol/L MgCl_2 , 0.25 μL of a 25 $\mu\text{mol/L}$ stock solution of each forward and reverse primer, 2 μL of 3.3% (w/v) bovine serum albumin (BSA), 1 unit Taq polymerase, 2–8 μL of 1:10 diluted template DNA extract, and water to a final volume of 25 μL . For amplification of ITS, 1.25 μL dimethylsulfoxide (DMSO) was added to the reaction. Annealing temperatures ranged between 45 $^\circ\text{C}$ and 52 $^\circ\text{C}$ depending on primers and template. The PCR reaction products were purified using exonuclease I and shrimp alkaline phosphatase to degrade unincorporated primers and dNTPs (Werle et al., 1994), and then sequenced via BigDye (v. 3.1) Terminator Cycle Sequencing (Applied Biosystems, Foster City, CA, USA) at the Institute for Cell and Molecular Biology Core Facility at the University of Texas (Austin, TX, USA).

Forward and reverse sequence reads were assembled into contigs and edited in Sequencher 4.5 (GeneCodes Corp., 2005), whereas the chloroplast sequences were aligned manually in MacClade 4.08 (Maddison & Maddison, 2005), and ITS sequences were aligned using default settings in MUSCLE (Edgar, 2004), followed by manual adjustments in MacClade.

1.2.2 Phylogenetic analyses The three data matrices were analyzed separately and in combination using maximum parsimony (MP) with PAUP* 4.0b10 (Swofford, 2002), Bayesian inference (BI) using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001), and maximum likelihood (ML) with GARLI (Zwickl, 2006). Areas of uncertain alignment were excluded from all analyses, as were autapomorphic insertions. The sequenced portions of the 18S and 25S ribosomal subunits were also excluded from the ITS matrix.

In PAUP, all characters were treated as equally weighted and unordered, with gaps treated as missing data. The MP tree searches were conducted using the heuristic search option with 1000 random-addition replicates and tree bisection–reconnection (TBR) branch swapping, with no limitations placed on the number of trees swapped to completion, and all other settings at defaults. For MP bootstrapping, 500 bootstrap replicates were performed using the heuristic search option with one random addition replicate and swapping to completion on a maximum of 10 000 trees. The incongruence length difference (ILD) test (Farris et al., 1995), implemented in PAUP as the

Table 1 Sources of material

Taxon	Voucher	Origin	Herbarium	GenBank No.		
				ITS	<i>trnL-rpl32</i>	<i>rpl32-ndhF</i>
<i>Acourtia coulteri</i> (A. Gray) Reveal & R.M. King	H.H. Iltis 30784	Tamaulipas, Mexico	TEX	FJ979680	FJ979729	FJ979781
<i>A. microcephala</i> DC.	T. Ross 6616	California, USA	TEX	FJ979679	N/A	FJ979780
<i>A. nana</i> (A. Gray) Reveal & R.M. King	B.L. Turner 7-22-07	Texas, USA	TEX	FJ979682	FJ979732	FJ979784
<i>A. purpusii</i> (Brandege) Reveal & R.M. King	G.B. Hinton 23607	Nuevo Leon, Mexico	TEX	FJ979681	FJ979730	FJ979782
<i>A. scapiformis</i> (Bacigalupi) Reveal & R.M. King	J. Calzada 21592	Oaxaca, Mexico	TEX	FJ979683	FJ979733	FJ979785
<i>A. runcinata</i> (D. Don) B. L. Turner	W. M. Turner 76	Texas, USA	TEX	FJ979684	FJ979734	FJ979786
<i>A. wrightii</i> (A. Gray) Reveal & R.M. King	E.J. Lott 4829	Texas, USA	TEX	N/A	FJ979731	FJ979783
<i>Adenocaulon bicolor</i> Hook.	G. Helmkamp K-17	Idaho, USA	TEX	FJ979672	FJ979722	FJ979773
<i>A. chilense</i> Less.	Ricardi & Marticorena 1923	Malleco, Chile	F	FJ979674	FJ979724	FJ979775
<i>A. lyratum</i> S.F. Blake	D. E. Breedlove 13424	Chiapas, Mexico	F	FJ979673	FJ979723	FJ979774
<i>Calopappus acerosus</i> Meyen	J. Panero & B. Crozier 8457	Los Andes, Chile	TEX	FJ979685	FJ979735	FJ979787
<i>Leucheria bridgesii</i> Hook. & Arn.	W. D. Clark 1350	Santiago, Chile	TEX	FJ979675	FJ979725	FJ979776
<i>Lophopappus foliosus</i> Rusby	Sanders et al. 3325	Jujuy, Argentina	TEX	FJ979676	FJ979726	FJ979777
<i>Nassauvia aculeata</i> Poepp. & Endl.	Marticorena et al. 74	Curico, Chile	F	FJ979688	FJ979738	FJ979790
<i>N. digitata</i> Wedd.	K. Gengler et al. 11	Reg. XIII, Chillan, Chile	TEX	FJ979690	FJ979740	FJ979792
<i>N. heterophylla</i> (Phil.) Reiche	C.P. Cowan 4250	Farellones, Chile	TEX	FJ979687	FJ979737	FJ979789
<i>N. lagascae</i> F. Meigen	C.P. Cowan 4238	Farellones, Chile	TEX	FJ979686	FJ979736	FJ979788
<i>N. pinnigera</i> D. Don	C.P. Cowan 4239	Farellones, Chile	TEX	FJ979691	FJ979741	FJ979793
<i>Panphalea cardaminifolia</i> Less.	M. Dias de Moraes 832	Santa Catarina, Brazil	TEX	FJ979669	FJ979719	FJ979770
<i>Perezia atacamensis</i> (Phil.) Reiche*	M.K. Arroyo et al. 94014	Reg. II Atacama, Chile	CONC	FJ979657	FJ979707	FJ979758
<i>P. calophylla</i> (Phil.) Reiche	B. S. Vuilleumier 189	Rio Negro, Argentina	GH	N/A	FJ979700	FJ979751
<i>P. carduncelloides</i> Griseb.	B. B. Simpson 6-II-00-1	Tucuman, Argentina	TEX	FJ979655	FJ979705	FJ979756
<i>P. carthamoides</i> (D. Don) Hook. & Arn.	E. Wall s.n	Mendoza, Argentina	GH	FJ979641	FJ979692	FJ979742
<i>P. ciliaris</i> Hook. & Arn.	St. Beck et al. 18088	Cochabamba, Bolivia	LPB	FJ979644	FJ979694	FJ979745
<i>P. ciliosa</i> (Phil.) Reiche	St. Beck 26317	Arequipa, Peru	LPB	FJ979645	FJ979695	FJ979746
<i>P. cirsiifolia</i> Wedd.*	I. Henson 830	Cochabamba, Bolivia	LPB	FJ979646	FJ979696	FJ979747
<i>P. coerulescens</i> Wedd.	E. Garcia 886	La Paz, Bolivia	LPB	FJ979649	FJ979699	FJ979750
<i>P. fonkii</i> (Phil.) Reiche	Weigend et al. 6824	Rio Negro, Argentina	NY	FJ979660	FJ979710	FJ979761
<i>P. integrifolia</i> Wedd.*	X. Menhofer X-1900	La Paz, Bolivia	LPB	FJ979654	FJ979704	FJ979755
<i>P. kingii</i> Baker	Rosengurth PE5334	Florida, Uruguay	GH	FJ979667	FJ979717	FJ979768
<i>P. lactuoides lactuoides</i> (Vahl) Less.	O. Dollenz 648	Magellanes, Argentina	GH	FJ979659	FJ979709	FJ979760
<i>P. lactuoides</i> ssp. <i>palustris</i> (Phil.) Vuill.	B. Vuilleumier 204	Rio Negro, Argentina	GH	FJ979642	N/A	FJ979743
<i>P. lanigera</i> Hook. & Arn.** = <i>Burkartia lanigera</i> (Hook. & Arn.) Crisci	S. Albert 8-XI-2006-1 S. Albert 8-XI-2006-2	Santa Cruz, Argentina Santa Cruz, Argentina	TEX TEX	FJ979677 FJ979678	FJ979727 FJ979728	FJ979778 FJ979779
<i>P. linearis</i> Less.	Pirion 3499	Aisen, Chile	GH	FJ979664	FJ979714	FJ979765
<i>P. lyrata</i> (Remy) Wedd.	Marticorena et al. 194	Reg. VII Talca, Chile	CONC	FJ979666	FJ979716	FJ979767
<i>P. magellanica</i> (L.f.) Less.	O. Dollenz 708	Isla Wollaston, Argentina	GH	FJ979661	FJ979711	FJ979762
<i>P. mandonii</i> Rusby	I. Henson 1505	Cochabamba, Bolivia	LPB	FJ979647	FJ979697	FJ979748
<i>P. megalantha</i> Speg.	E. Pisano V. 5602	Cerro Corona, Argentina	GH	FJ979651	FJ979702	FJ979753
<i>P. multiflora</i> (H. & B.) Less.	M. Madison 1044	Cuzco, Peru	GH	FJ979652	N/A	N/A
<i>P. (Calorezia) nutans</i> Less.*	J. Wen 7472	Chile	F	FJ979671	FJ979721	FJ979772
<i>P. pedicularidifolia</i> Less.	F. Jaffuel 3795	Chillan, Chile	GH	FJ979662	FJ979712	FJ979763
<i>P. pilifera</i> (D. Don) Hook. & Arn.	M.T.K. Arroyo 20680	Yerba Loca, Chile	CONC	FJ979658	FJ979708	FJ979759
<i>P. pinnatifida</i> (Humb. & Bonpl.) Wedd.	Hutchison 4250	Lima, Peru	GH	FJ979650	FJ979701	FJ979752
<i>P. pungens</i> (Humb. & Bonpl.) Less.	S. King et al. 285	Urubamba, Peru	GH	FJ979653	FJ979703	FJ979754
<i>P. (Calorezia) prenanthoides</i> Less.**	G. Seijo 1671	Neuquen, Argentina	NY	FJ979670	FJ979720	FJ979771
<i>P. purpurata</i> Wedd.	St. Beck 31111	Oruro, Bolivia	LPB	FJ979643	FJ979693	FJ979744

Table 1 Continued

Taxon	Voucher	Origin	Herbarium	GenBank No.		
				ITS	<i>trnL-rpl32</i>	<i>rpl32-ndhF</i>
<i>P. recurvata</i> (Vahl) Less.	E. Pisano V. 4045	Patagonia, Argentina	GH	FJ979663	FJ979713	FJ979713
<i>P. squarrosa</i> ssp. <i>cubaetensis</i> (Less.) Vuill.	O.S. Ribas et al. 2152	Parana, Brazil	TEX	FJ979668	FJ979718	FJ979769
<i>P. sublyrata</i> Domke	J. L. Luteyn & L. Door 13773	La Paz, Bolivia	TEX	FJ979656	FJ979706	FJ979757
<i>P. virens</i> (D. Don) Hook. & Arn.	E. Wall 29.XII.1946	Aconcagua, Chile	NY	FJ979648	FJ979698	FJ979749
<i>P. viscosa</i> Less.	G. Montero O. 1304	Cautin, Chile	GH	FJ979665	FJ979715	FJ979766
<i>Triptilion spinosum</i> Ruiz & Pavon	Para & Rodriguez 109	Concepcion, Chile	F	FJ979689	FJ979739	FJ979791

*Vuilleumier (1970) placed *P. atacamensis* in *P. purpurata* and *P. cirsiifolia* and *P. integrifolia* in *P. coreulescens*. Specimens referable to these synonyms are included here because of doubt expressed by Vuilleumier about their placement.

**These species have been moved to the genera indicated in parentheses. The authorities listed here are for the original description in *Perezia*. In the monograph of the genus.

N/A, not applicable.

Table 2 Primers used to amplify and sequence chloroplast intergenic spacers

Region	Primer	Sequence (5'-3')	Reference	
<i>ndhF-rpl32</i>	<i>ndhF</i>	GAA AGG TAT KAT CCA YGM ATA TT	Shaw et al., 2007	
	316A	GAG CAA GGA TAA AAA ATT AC	Present study	
	316B	GTA ATT TTT TAT CCT TGC TC	Present study	
	602A	CRT ATC CTT TAA CAG ATT K	Present study	
	602B	MAA TCT GTT AAA GGA TAY G	Present study	
	906A	GAG AGA TAA AGA ACG AGA AY	Present study	
	906B	RTT CTC GTT CTT TAT CTC TC	Present study	
	<i>rpl32-R</i>	CCA ATA TCC CTT YYT TTT CCA A	Shaw et al., 2007	
	<i>rpl32-trnL</i>	<i>rpl32-F</i>	CAG TTC CAA AAA AAG GTA CTT C	Shaw et al., 2007
		432A	CCC ATC GAC CTT TAC AAT AA	Present study
432B		TTA TTG TAA AGG TCG ATG GG	Present study	
534A		GAA ATT CAT TGA TTC CAT G	Present study	
534B		CAT GGA ATC AAT GAA TTT C	Present study	
649A		GCY CAA AAC AGA ACT TAA TAG	Present study	
649B		CTA TTA AGT TCT GTT TTG RGC	Present study	
<i>trnL</i> (UAG)		CTG CTT CCT AAG AGC AGC GT	Shaw et al., 2007	

partition homogeneity test, was used to assess conflict between the chloroplast and nuclear data, with 100 replicates performed using the heuristic search option under the same constraints as used for MP bootstraps.

In GARLI, 100 bootstrap replicates were performed for each marker and for the combined matrix, using default settings and the GTR+G+I model. Bootstrap values were determined from a 50% majority rule consensus of the best trees found in each bootstrap replicate.

Prior to analysis in MrBayes, the number of substitution types and applicability of gamma rate heterogeneity (G) or invariant sites (I) for each marker were determined with the MrModelTest (Nylander, 2004) using the Akaike Information Criterion (AIC). In MrBayes, each marker was subjected to four million generations of MCMC sampling, with tree topology, estimated model parameters, and likelihood score saved every 100 generations and the automated diagnostic statistic comparing the parameters from two simultaneous runs every ten-thousandth generation (with the first 25% of generations excluded as "burn-in"). Chain heating and priors for model parameters were kept at default values, ex-

cept for the nucleotide frequency prior, which was set to a dirichlet distribution. For the combined data analysis in MrBayes, the matrix was partitioned to apply the appropriate substitution model (G) and I to each marker; partition model parameters were unlinked and allowed to vary independently for each partition, except for branch length and topology. All Bayesian analyses were terminated at four million generations as long as the automated diagnostic statistic (average standard deviation of split frequencies) was below 0.01 by that time. Runs were also checked for stationarity in the post-burn-in sample by graphical plotting of $-\ln L$ scores against generation time. Clade posterior probabilities were determined from a 50% majority rule consensus of the post-burn-in sample of 60 000 trees (30 000 sampled during stationarity from each simultaneous run).

2 Results

2.1 Sequence characteristics

Sequences of the ITS could not be obtained from *Perezia calophylla* and *Acourtia wrightii*. *Perezia*

Table 3 Parameters for the DNA markers used in the present study

Marker	Aligned length	Included bp	Variable sites	Informative sites	Mean GC content (%)	Substitution model
<i>rpl32</i>	1049	829	265	168	24.14	GTR+G
<i>ndhF-rpl32</i>	1229	991	284	154	23.69	GTR+G
Combined CPL	2278	1820	549	322	25.03	N/A
ITS	762	599	315	238	56.15	GTR+I+G
CPL+ITS	3040	2419	864	560	33.17	N/A

N/A, not applicable; ITS, internal transcribed spacer; CPL, chloroplast loci.

lactuoides subsp. *palustris* and *Acourtia microcephala* were lacking sequence from the *rpl32-trnL* intergenic spacer, and *Perezia multiflora* was missing sequence from both chloroplast markers. Table 3 provides descriptive statistics from parsimony analyses of the data matrices assembled for the present study and the ML substitution model type selected. Figure 3: A, B shows the majority rule consensus trees for the Bayesian analyses.

The parsimony-based ILD test indicated significant conflict between the chloroplast and ITS data partitions ($P = 0.01$). Topological incongruences between the chloroplast and ITS (Fig. 3: A, B), mostly affecting placement of outgroup taxa, are discussed below. Consequently, we combined our data to generate the phylogeny shown in Fig. 4.

2.2 Phylogenetic results

Figure 3 shows a comparison between the phylogenies generated with the combined chloroplast markers and the sequences from ITS 1 and 2. Considering *Perezia*, several discrepancies should be noted. First, in the chloroplast (cp) DNA tree (Fig. 3: A), *P. lactuoides* is sister to *P. megalantha*, whereas the ITS data (Fig. 3: B) show this species in a polytomy with the majority of the species of the genus. However, we were not able to obtain one of the chloroplast sequences from *P. lactuoides* subsp. *palustris*, which probably led to this difference. Second, *P. virens* in the chloroplast tree (Fig. 3: A) is part of a polytomy with members of the high Andean clade; however, in the ITS tree (Fig. 3: B) it branches much lower in the cladogram and is sister to *P. linearis*. Third, *P. ciliosa* is in an unresolved clade with *P. cirsiifolia* and *P. atacamensis* in the chloroplast tree (Fig. 3: A) but is sister to a clade of *P. integrifolia*, *P. pinnatifida*, and *P. purpurata* in the ITS tree (Fig. 3: B). Fourth, *P. viscosa* is part of a clade with *P. linearis*, *P. pilifera*, and *P. recurvata* in the chloroplast tree (Fig. 3: A) and in a clade with *P. lyrata* and *P. pedicularidifolia* in the ITS tree (Fig. 3: B). The latter placement seems more reasonable in terms of morphology. Finally, *P. coeruleascens* is in a completely unresolved clade of central Andean species in both analyses, but the members of that clade differ in the two trees (Fig. 3: A, B).

Common to both analyses is the position of the *P. multiflora* group as sister to or in a basal polytomy with the remaining members of the genus (Fig. 3: A, B). Similarly, in the cp analysis (Fig. 3: A) there is a basal grade of comparatively low elevation, generally humid habit, southern South American species (*P. calophylla* and *P. fonkii*) subsequent to the *P. multiflora* group, whereas in the ITS tree (Fig. 3: B) the multiflora group and several southern South American species (*P. fonkii*, *P. magellanica*, and *P. megalantha*) form a polytomy basal to the remaining species.

The preanthoides group (= *Calorezia*) is distinct from *Perezia*, although the chloroplast data (Fig. 3: A) place it as sister to a clade of *Calopappus*, *Nassauvia*, and *Triptilion* and the ITS (Fig. 3: B) places it as sister to the entire *Perezia* clade. In both analyses, *Perezia lanigera* (= *Burkartia*) shows a strong relationship with *Acourtia*, with the chloroplast data indicating it is sister to *Acourtia* and the ITS data suggesting that it is embedded within *Acourtia*. Both show *Nassauvia* paraphyletic with respect to *Triptilion*. Although other genera were not sampled thoroughly, our data suggest that *Panphalea* is always strongly supported as sister to *Perezia*.

The combined tree (Fig. 4) strongly supports the multiflora group as sister to the rest of *Perezia* (Fig. 4: a; note, the lowercase letters refer to labeled clades in Fig. 4). It also shows a grade (Fig. 4: b, b') of low elevation, humid habitat southern South American species (*P. fonkii*, *P. magellanica* + *P. megalantha*) that is sister to a clade (Fig. 4: c) containing the remaining species of the genus. This large clade (Fig. 4: c) consists of a basal polytomy of southern (south of approximately 40°S, except for *P. pilifera*, which has a distribution that extends from 30° to 55°S latitude) species (*P. lyrata* + *P. pedicularidifolia*, a recurvata clade plus *P. viscosa* (Fig. 4: d), *P. calophylla*, *P. lactuoides*) sister to a clade of central Andean species (Fig. 4: e). *Perezia virens* and subsequently *P. carthamoides*, the basal members of this clade, occur relatively further south (~35°S) than the other members of this clade. All members of the most derived clade (Fig. 4: f) occur from northwest Argentina to Ecuador and all occur in high Andean habitats.

In the combined analysis, *Panphalea* is sister to *Perezia*, *Perezia (Burkartia) lanigera* is sister to

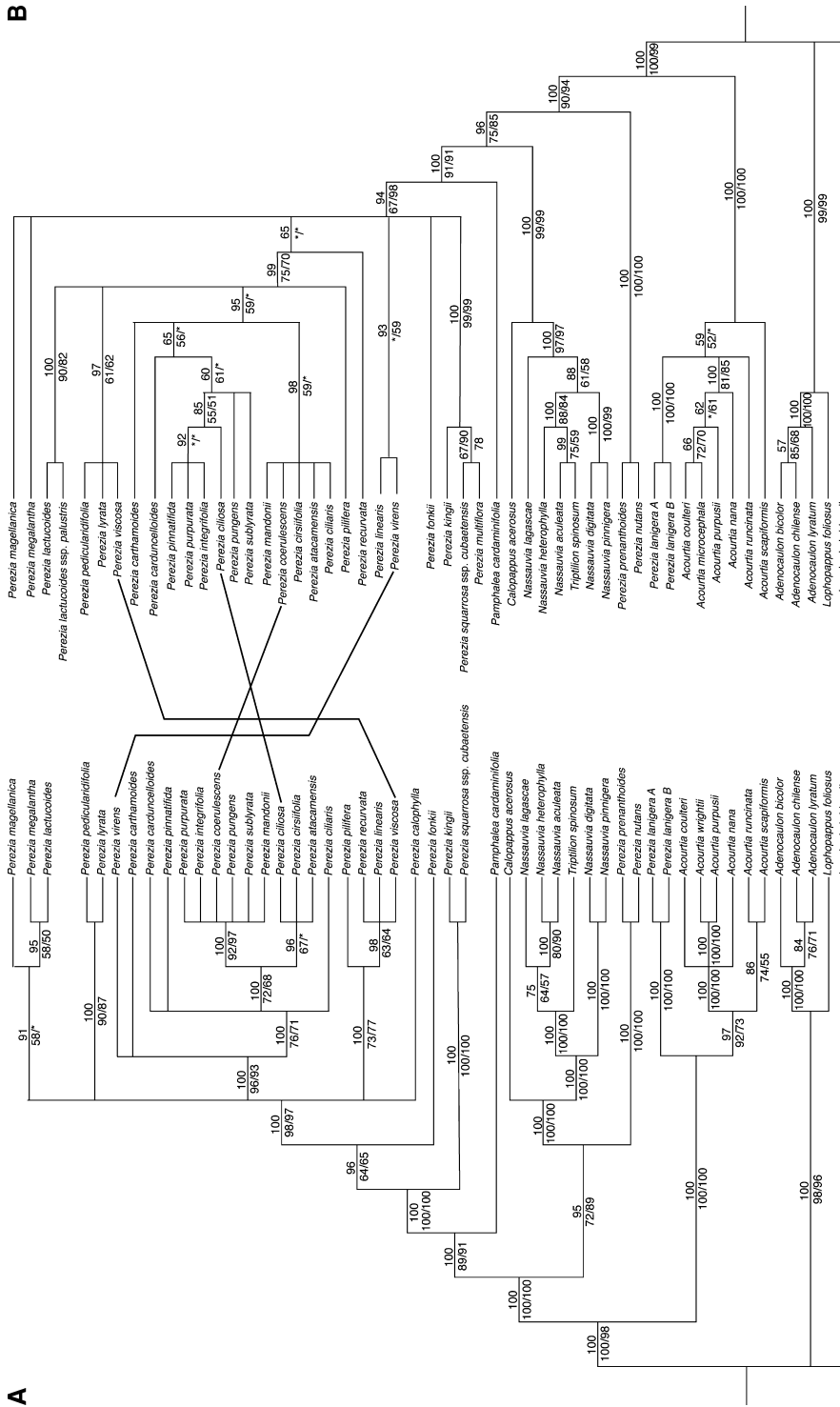


Fig. 3. Cladograms from the Bayesian majority rule consensus constructed using (A) combined chloroplast DNA data and (B) internal transcribed spacer (ITS) data. The *Perezia* group is the clade containing *Acaurtia*, *Burkartia*, *Calopappus*, *Nassauvia*, *Perezia*, *Pamphalea*, and *Tripitilon*. Numbers above branches are the Bayesian posterior probabilities. Numbers below the lines are the maximum likelihood/maximum parsimony bootstrap values.

Acourtia, the prenanthoides group (*Calorezia*) is sister to a clade of *Calopappus*, *Nassauvia*, and *Triptilion*, and *Triptilion* is embedded within *Nassauvia*.

3 Discussion

3.1 Phylogenetic relationships among species of *Perezia*

Our finding that *Perezia prenanthoides/nutans* do not cluster with the remainder of *Perezia* was not surprising given the fact that the two differ in habit and habitat from all other *Perezia* species and were considered “isolated within the genus” by Vuilleumier (1970). They are large (to 84 cm) branched, soft-leaved, caulescent species that occur in the *Nothofagus* forest of southern Chile, reaching the subalpine in central Chile. They also have an “*Acourtia*” type of style branches (Crisci, 1974), unlike the species of *Perezia*. Thus, the segregation of this group into the separate genus *Calorezia* (Panero, 2007) is justified, but our data (not shown) for several samples of both species suggest that only one species rather than two may be involved. *Calopappus* (often treated as a synonym of *Nassauvia*; Hind, 2007), one of the genera closest to *Calorezia* according to Panero (2007), occurs above the treeline in the central Chilean Andes. *Nassauvia* itself is primarily southern South American. Although we sampled only five of the 38+ species, our data suggest that *Nassauvia* is paraphyletic with respect to *Triptilion* and that the latter should be included within *Nassauvia*.

Our data confirm that *Burkartia* (*Perezia*) *lanigera*, as suggested by Vuilleumier (1970), is not a *Perezia*. Moreover our data indicate that it should either be considered a monophyletic genus (*Burkartia*, cf. Crisci, 1976) sister to the North American genus *Acourtia* or perhaps a member of *Acourtia* because, according to some of our phylogenetic reconstructions, *Acourtia* is paraphyletic with respect to *Burkartia* (Fig. 3: B). Morphological characters that *B. lanigera* shares with *Acourtia* but not with *Perezia* include a shrubby habit, subsessile capitula, capitula with six to 14 florets, pubescent corolla, wooly trichomes, and a *Trixis* Lag. type of pollen exine (Crisci, 1974). Until *Acourtia* is more fully studied, we retain *Burkartia* as a monophyletic genus, but note that this is the only clade in the *Perezia* group that has a New World amphitropical disjunct distribution.

These new data enable the clarification of the evolution of *Perezia*. Contrary to Vuilleumier’s (1970) suggestions that the *Perezia pungens* group is “basal” (= sister) to the rest of the genus, the *Perezia multiflora* group is clearly the sister to the rest of *Perezia* s.s.

Table 4 Chromosome numbers of members of the *Perezia* clade

Taxon	Chromosome number	Source*
<i>Acourtia belizeana</i> B. L. Turner	n = 18	1
<i>A. carpholepis</i> (A. Gray) Reveal & R. M. King	~27 pairs	1
<i>A. nana</i> (A. Gray) Reveal & R. M. King	n = 27	1
<i>A. microcephala</i> DC.	2n = 54	3
<i>A. rigida</i> DC.	n = 26	1
<i>A. scapiformis</i> (Bacig.) B.L. Turner	~27 pairs	2
<i>A. thurberi</i> (A. Gray) Reveal & R. M. King	2n = 54	1
<i>A. wrightii</i> (A. Gray) Reveal & R. M. King	n = 27	1
<i>Nassauvia aculeata</i> var. <i>robusta</i> (Cabrera) Cabrera	n = ~44	1
<i>N. axillaris</i> D. Don	n = 11	1
<i>N. chubutensis</i> Speg.	n = 11	1
<i>N. darwinii</i> (Hook. & Arn.) O. Hoffm. & Dusen	n = 11	1
<i>N. gaudichaudii</i> Cass.	2n = 22	2
<i>N. glomerulosa</i> D. Don	n = 11	1
<i>N. lagascae</i> F. Meigen	n = 11	1
<i>N. magellanica</i> J. F. Gmel.	n = 11	2
<i>N. pygmaea</i> (Cass.) Hook. f.	n = 11	1
<i>N. revoluta</i> D. Don	n = 11	1
<i>N. serpens</i> d’Urv.	n = 11	2
<i>N. uniflora</i> (D. Don) Hauman	n = 11	1
<i>Panphalea bupleurifolia</i> Less.	n = 8	1
<i>Perezia calophylla</i> (Phil.) Reiche	2n = 24	3
<i>P. carduncelloides</i> Griseb.	2n = 24	3
<i>P. ciliaris</i> Hook & Arn.	2n = 24	3
<i>P. ciliosa</i> (Phil.) Reiche	2n = 24	2
<i>P. coeruleascens</i> Wedd.	2n = 24	3
<i>Perezia magellanica</i> (L.f.) Lag.	n = 24	2
<i>P. multiflora</i> (Humb. & Bonpl.) Less.	n = 8	1, 3
<i>Perezia “nivalis”</i> Wedd.	2n = 24	4
<i>P. pedicularidifolia</i> Less.	2n = 24	1
<i>P. pilifera</i> (D. Don. & Arn.) Hook.	n = 16	1
<i>P. pungens</i> (Humb. & Bonpl.) Less.	2n = 28	1, 3
<i>P. recurvata</i> (Vahl) Less.	2n = 24	1, 3
<i>P. squarrosa</i> ssp. <i>cubaetensis</i> (Less.) Vuill.	n = 4	2
<i>Triptilion gibbosum</i> Remy	2n = 20	1

*Sources for the information as are follows: 1, Index to Plant Chromosome Numbers (IPCN; available at <http://mobot.mobot.org/W3T/Search/ipcn.html>, accessed 15 September 2008) and/or batch files at that URL; 2, Crisci (1974); 3, Vuilleumier (1970); 4, Bolkhovskikh et al. (1969).

(Fig. 4). This placement is consistent with the chromosome numbers (Table 4) known for species of this group: $2n = 8$ for *P. squarrosa* subsp. *cubaetensis* and $2n = 16$ for *P. multiflora* compared with $2n = 24$ for the remaining species studied. Like most members of the multiflora group, species of *Panphalea* (the sister genus to *Perezia*) are also large (20–100 cm tall), have leafy stems, and inflorescences of numerous small heads (Cabrera, 1953). In addition, the only chromosome count made for that genus (*Panphalea bupleurifolia*) is $n = 8$ (Table 4).

Most of the *Perezia* species (those sister to the multiflora group) form a clade with either a grade of species of southern humid and alpine habitats (*P. fonkii*, followed by a clade of *P. magellanica* plus *P. megalantha*) sister to the remaining species (Fig. 3: A) or with

a basal polytomy of southern species (Fig. 3: B). Thus, most of the species of the magellanica group of Vuilleumier (1970) constitute a grade rather than a group or clade. The combined analysis supports the recurvata group (Fig. 4: d) of Vuilleumier (1970) plus *P. viscosa*. The members of the recurvata group are small loose cushion-forming, narrow-leaved (often spiny) plants (Vuilleumier, 1970), with *P. viscosa* anomalous morphologically in this group with its basal rosette of broad oblanceolate leaves reminiscent of other members found in *Nothofagus* forest. This clade and several other taxa placed in the magellanica and pungens groups (Fig. 1) form a polytomy with a large clade of species that occur from northwestern Argentina to Ecuador. This northern clade contains a mixture of species placed in the pungens and coerulescens groups by Vuilleumier (1970).

3.2 Biogeographic implications

Considering the results as a whole, the confirmation that the prenanthoides group is sister to *Calopappus* and *Nassauvia* plus *Triptilion* strongly suggests that the *Perezia* group (the clade of *Calopappus*, *Nassauvia*, *Panphalea*, *Perezia*, and *Triptilion*) arose in southern, probably southwestern, South America. However, *Panphalea*, the sister genus to *Perezia*, occurs in eastern Argentina and adjacent Uruguay and Paraguay. The species of the multiflora clade, sister to the majority of *Perezia* also occur predominantly in southeastern South America, with *P. kingii* occurring in northeastern Argentina and Uruguay, *P. squarrosa* Less. in extreme southeastern Brazil and Uruguay, and both *P. eryngioides* and *P. catharinensis* in Santa Catarina, Brazil. Within this group of five species, only *P. multiflora* itself extends its distribution westward into dry high regions of the central Andes (and accounts for the seemingly broad distribution of this group; Fig. 2: A). Therefore, it would appear that following the origination of the *Perezia* group, there was a spread or dispersal into eastern subtropical South America with either a recolonization of the southwestern Andes or a later radiation of an ancestral stock in southwestern South America. Although the cladogram in Fig. 4 suggests the former, it is possible that inclusion of more species of both *Panphalea* and the multiflora group will show these to be sister and consistent with the second pattern.

The three species that branch sequentially to the multiflora clade form a grade of taxa that occur in relatively low-elevation moist forest or humid steppe habitats south of 30°S. Although there is little resolution among the high Andean species (Fig. 4: c), it is evident that the high-elevation species are the most derived and that the genus is of temperate South American

origin, radiating in western South America from south to north and from low to high elevation (Fig. 4).

As far as we can tell, the pattern we found in *Perezia* does not completely match that of any temperate/Andean plant genus studied phylogenetically to date. The most similar pattern to that of *Perezia* was shown in a recent study by Hershkovitz et al. (2006a) for the 20 species of *Tropaeolum* L. sect. *Chilensia* Sparre (Tropaeolaceae). Although plagued by problems of uncertain rooting, the data of Hershkovitz et al. (2006a) also indicate an east–west split in a basal clade (eastern Argentina, southern Chile). The remaining species are primarily Chilean and show a pattern of diversification from southern mesophytic areas to central Mediterranean scrub habitats to northern deserts. However, it should be noted that species of this section rarely reach the elevations of many *Perezia* taxa (over 4000 m a.s.l.) and, unlike *Perezia*, *Tropaeolum* consists of 90 species, most of which (70 species) occur in tropical America.

A molecular study of *Chaetanthera* Ruíz & Pavón, an Andean/Patagonian genus of the Mutisieae (Hershkovitz et al., 2006b), found some high-elevation species to be derived from lowland stock, whereas others were interpreted to be relictual, migrating upward on account of increasing aridity in the central Andes. In addition, a number of the lower-elevation species in south-central Chile were found to be more derived. Regardless of differences, *Chaetanthera*, like *Perezia*, underwent a major species radiation in high-elevation habitats in the more arid areas of the central Chilean Andes and puna. In the small Andean genus *Schizanthus* (Solanaceae), lowland species were shown to have diverged more recently than the few alpine species in that genus (Perez et al., 2006). Yet another pattern is seen in *Hamadryas*, a small dioecious, predominantly alpine genus of four species in the Patagonian component of the Ranunculus grade of Ranunculaceae. Its closest relatives are found in the alpine of southern South America, Asia, North America, and South Africa (Hoot et al., 2008). Finally, a study of *Ourisia* (Plantaginaceae) by Meudt (2006) and Meudt and Simpson (2007) indicated an origin in south-central America (~34°S) at mid elevation (800–2400 m a.s.l.) and a subsequent spread both south and north in the Andes as well as to New Zealand, where a subsequent significant radiation occurred.

Clearly, rigorous phylogenies of many more groups need to be performed before we can say whether the *Perezia* or one of the other patterns is most commonly found in genera with a predominance of species in temperate and high Andean regions of South America. It is possible that the evolutionary patterns of radiation are related to the location of the ancestors of the groups

studied. Genera (clades) with tropical Andean ancestors may commonly show the basal members of the clade to be mesophytic with lowland xerophytic species derived, whereas groups originating in temperate areas may commonly show patterns of late radiation in the very high supraforest habitats of the tropical Andes.

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