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ORIGINAL ARTICLE

Phylogenetic reconstruction of the South American genus *Leucheria* Lag. (Asteraceae, Nassauvieae) based on nuclear and chloroplast DNA sequences

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Abstract The genus *Leucheria* Lag. (Asteraceae Bercht. and J. Presl, tribe Nassauvieae Cass.) comprises 45 species and three infraspecific taxa distributed in the Andean region from southern Chile and Argentina to Peru. Six species are annual herbs. The genus has had a long taxonomic history involving the transference of species described originally under many different genera. The main objectives of this paper were to determine the phylogenetic relationships of species of *Leucheria*, examine the hypothesis that the ancestor of *Leucheria* would have originated in a forested habitat and examine the validity of nine morphologically defined evolutionary lines recognized in earlier work on the genus. Additionally we investigated whether the annual species of *Leucheria* are derived. We extracted DNA from leaf material for 45 taxa (94%) of *Leucheria*. We used Bayesian inference and plastid and nuclear genes to construct a phylogenetic hypothesis. Results show that *Leucheria* is monophyletic and is comprised of two main clades. One clade comprises perennial acaulescent/subacaulescent species, all with a solitary capitulum. We recognized three lineages in the second clade comprised of caulescent species that exhibit multiple capitula. Optimization of life-form over the phylogeny showed that five of the six annual species studied are derived in our tree. We conclude that the appearance of the annual habit is associated with the colonization of arid conditions in the winter rainfall coastal desert of northern Chile. Our result shows that species of *Leucheria* from forested habitats are derived. Discrepancies with previously recognized morphologically defined evolutionary lines were detected.

Keywords Annual life-form · Caulescent · Evolutionary lines · *Leucheria* · Phylogeny

Introduction

Southern South America houses many genera and tribes of Asteraceae, the second most species rich and a widely cosmopolitan family of angiosperms (Stevens 2001; Panero and Crozier 2016). A Middle Eocene fossil from northwestern Patagonia and climatic reconstruction of the fossil-bearing locality suggest that Asteraceae may have formed part of a diverse tropical to subtropical vegetation assemblage in southern South America (Wilf et al. 2005; Barreda et al. 2010). The fossil documents the divergence of Mutisioideae s.l. and Carduoideae from Barnadesioideae at least 47.5 Ma (Barreda et al. 2010). Here we undertake a molecular phylogenetic reconstruction of the genus

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Leucheria Lag. belonging to the tribe Nassauvieae Cass. of the large predominantly South America subfamily Mutisioideae (Cass.) Lindl. Tribe Nassauvieae contains 25 genera (Katinas et al. 2008b). However, to date, molecular phylogenies for the tribe Nassauvieae have only been constructed for *Perezia* (Simpson et al. 2009) and *Nassauvia* (Maraner et al. 2012).

Leucheria is exclusively South American, occurring from 11°N in Peru to 54°S (55) in Tierra del Fuego (Fig. 1), where it is found in forests, Mediterranean-type climate vegetation, winter rainfall coastal desert, Andean vegetation, Magellanic tundra and Patagonian steppe. The great majority of the species occur in the high Andes and in the Mediterranean-type climate area of central Chile. All *Leucheria* species are herbaceous, with both annual (six species) and perennial (39 species) species represented. Species are acaulescent/subacaulescent with a single large capitulum or caulescent with numerous, small capitula disposed in cymose or paniculate-like cymose inflorescences (Crisci 1976).

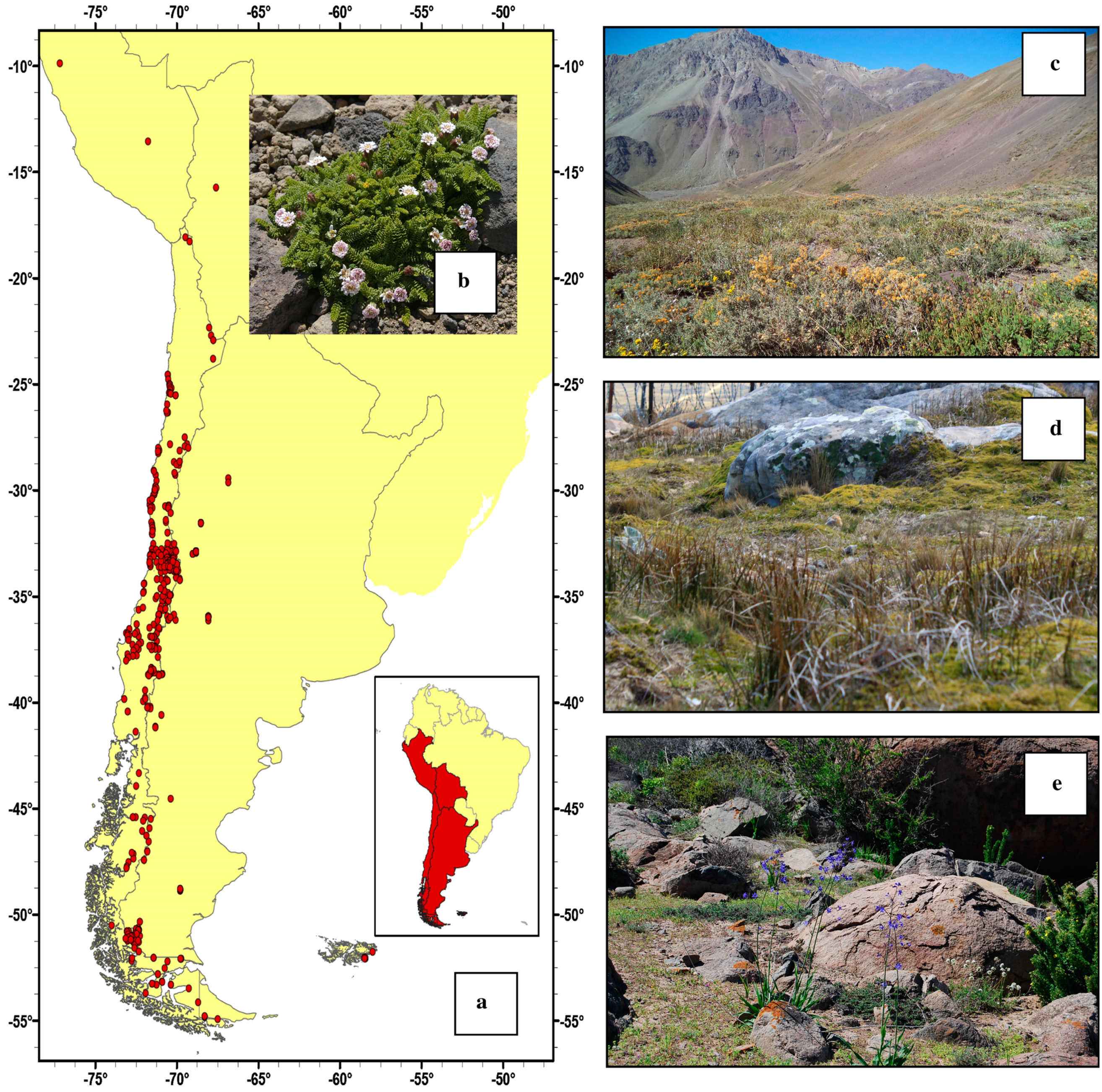

Fig. 1 Distribution of *Leucheria* in southern South America (**a**). **b** *L. scrobiculata* (image courtesy of Sebastián Teillier). Habitat types occupied by species of *Leucheria*: **c** Andean, **d** alpine tundra, **e** winter rainfall coastal desert

The genus *Leucheria* has a long history involving transferences of numerous currently unrecognized genera. In his comprehensive monograph of *Leucheria*, Crisci (1976) recognized 46 species and one subspecies. Katinas et al. (2008b, c) recognized 47 species, and Moreira-Muñoz et al. (2012) recognized 43 species and two subspecies for Chile. Currently 45 species, two subspecies and one variety are recognized in the flora of the Southern Cone (Zuloaga et al. 2008, and it is including Ratto et al. 2014).

The ancestor of *Leucheria* according to Crisci (1976) would have been a large caulescent forest-dwelling species, with the leaves distributed along the entire stem and bearing a paniculate-cymose inflorescence with large capitula. This ancestor gave rise to smaller herbaceous plants that subsequently colonized a wide range of drier habitats in South America. Crisci (1976) assigned species of the genus to nine evolutionary lines based mainly on characteristics of the stem, inflorescence morphology, capitulum size and distributional area.

Phylogenetic studies show that annual species are derived from a perennial ancestor (Datson et al. 2008; Cruz-Mazo et al. 2009). However, transitions in the reverse direction have also been reported. For example, Bena et al. (1998) showed that perennial species of *Medicago* (Fabaceae) were derived from an annual lineage, Tank and Olmstead (2008) showed that the perennial clade of *Castilleja* (Orobanchaceae) with 160 species was derived from an annual ancestor, and Eastwood et al. (2008) and Drummond (2008) showed the perennial species of *Lupinus* were derived. If *Leucheria* arose in a forested habitat as suggested by Crisci (1976), the annual species of *Leucheria* are likely to have evolved later as the genus spread northward into more arid conditions and thus be derived.

The main objectives of this paper were to determine the phylogenetic relationships of species of *Leucheria* as presently circumscribed, test the hypothesis that the ancestor of *Leucheria* evolved in a forested habitat as proposed by Crisci (1976) and examine whether the nine lineages proposed by this author based on morphological characters are monophyletic. To answer these questions, we constructed a molecular phylogeny of *Leucheria* based on the ITS of the rDNA and the *trn*L intron and *trn*L-*trn*F spacer and *rpl*32 intron of the cpDNA, identified clades within the genus and compared the results with Crisci's (lines) informal infrageneric phylogeny. We investigated where the perennial and annual species are placed in the phylogeny.

Methodology

Taxon sampling

We collected material of *Leucheria* species and outgroups for DNA extraction in the field. Samples were stored in silica. Vouchers are deposited in the herbaria HULS, CONC and SGO. For some species of *Leucheria*, we also sampled herbarium specimens in the collections of the herbaria listed above. We also downloaded sequences for some species from GenBank. The total number of *Leucheria* taxa considered was 45, representing 94% of all currently recognized taxa (Table 1). We also sampled 18 representatives of the tribe Nassauvieae found in other studies to be closely related to *Leucheria* (Kim et al. 2002; Katinas et al. 2008a; Panero and Funk 2008; Gruenstaeudl et al. 2009). Given the sister relationship to Asteraceae with Calyceraceae (Kim and Jansen 1995; Lundberg and Bremer 2003), *Nastanthus scapigerus* was chosen as outgroup.

DNA extraction, amplification and sequencing

Genomic DNA was extracted with the DNeasy Plant Kit (Qiagen, Valencia, CA, USA). We amplified the DNA using the primers listed in Table 2. PCR used a final volume of 30 µL, which contained 4 µl DNA (25 ng/µl), 8.35 µl distilled water, 3 µl MgCl2 (25 mM), 6 µl buffer, 2.4 µl of dNTP (1 mM), 1.8 µl of each primer (10x), 2.4 µl BSA (25 mM) and 0.25 µl GoTaq (5 U/µl). DNA was denatured at 95 °C for 5 min, followed by 35 amplification cycles of 45 s at 94 °C, annealing for 1 min at 50 °C, elongation for 1.5 min at 72 °C and a final extension of 7 min at 72 °C. Samples were sent to Macrogen (Seoul, South Korea) for purification and sequencing. Sequences were loaded, edited and aligned using ChromasPro 2.33 (Technelysium, Brisbane, Australia) and BioEdit 7.0 (Hall 1999) and have been deposited in GenBank (Table 1).

Phylogenetic analysis, character reconstruction and evolutionary lines

We performed a combined analysis for sequences of the nuclear gene ITS (White et al. 1990) and the two chloroplast genes, *trn*L-*trn*F (Taberlet et al. 1991) and *rpl*32 (Shaw et al. 2007) (Table 2). Bayesian inference analyses were performed with MrBayes (Ronquist and Huelsenbeck 2003). For the combined analysis with Bayesian inference, three partitions were used corresponding for each genes (ITS, *trn*L-*trn*F and *rpl*32), in which evolutionary models for each one were: GTR+G in ITS; GTR+I+G in *trn*L-*trn*F; and GTR+I in *rpl*32. The Tracer program (v1.6, Rambaut et al. 2003–2013) was used to visualize output parameters in order to prove stationarity and if there are duplicated runs or not to converged on the same mean likelihood. Runs appeared stationary prior to 20^6 generations, and we conservatively excluded the first 2.0×10^6 generations of each run as burn-in. The effective sample size (ESS) value was greater than 200 in a range

Table 1 Collection localities, herbarium voucher numbers and GenBank accession numbers of ingroup and outgroup taxa

Taxon	Authority	FV	Localities		Collection No.	Source material	Main habitat	GenBank No.		
			Latitude °S	Longitude °W				ITS	*trn*L-*trn*F	*rpl*32
Leucheria										
L. achillaeifolia	Hook. & Arn.	PH	47° 08′	72° 40′	CONC 166564	H	Patagonian steppe/ Andean	KY010381	KY223757	KY223808
L. amoena	Phil.	PH	35° 37′	72° 21′	CONC 147768	H	Patagonian steppe/ Mediterranean	KY010387	KY223762	KY223814
L. apiifolia	Phil.	PH	35° 10′	70° 35′	CONC 44744	H	Andean	KY010392	KY223766	
L. bridgesii	Hook. & Arn.	PH	32° 50′	70° 08′	CONC 164989	H	Andean	KY010363	KY223742	KY223791
*L. candidissima**	Gillies & D.Don	PH	25° 12′	70° 31‘	CONC 181242	F	Andean	KY010399	KY223773	KY223824
L. cerberoana	J.Rémy	AH	28° 05′	71° 09′	HULS 6904	H	Winter-rainfall coastal desert	KY010395	KY223769	KY223820
L. coerulescens	J.Rémy	PH	41° 13′	71° 55′	SGO 148897	H	Forest	KY010377	KY223754	KY223805
L. congesta	D.Don	PH	33° 14′	70° 16′	CONC 163109	H	Andean	KY010364	KY223743	KY223792
L. cumingii	Hook. & Arn.	AH	23° 49′	71° 18′	HULS 14014	F	Winter-rainfall coastal desert	KY010404	KY223778	KY223830
*L. daucifolia**	(D.Don) Crisci	PH	18° 04′	69° 29′	CONC 117424	H	Andean	KY010398	KY223772	KY223823
*L. diemii var. diemii**	Cabrera	PH	47° 00′	71° 49′	Ratto et al. 107	F	Andean	KY010402	KY223776	KY223827
*L. diemii var. purpurea**	Ratto, M.Bello & Adr.Bartoli	PH	47° 39′	71° 37′	Ratto et al. 352	F	Andean	KY010403		KY223828
*L. eriocephala**	Speg.	PH	52° 04′	69° 44′	CONC 158094	H	Patagonian steppe/ Andean	KY010365	KY223744	KY223793
L. floribunda	DC.	PH	32° 36′	70° 20′	CONC 156432	H	Andean	KY010382	KY223758	KY223809
L. garciana	J.Rémy	PH	35° 12′	70° 31′	CONC 181258	F	Andean	KY010401	KY223775	KY223826
L. gayana	(J.Rémy) Reiche	PH	33° 46′	70° 03′	CONC 156581	H	Mediterranean/ Andean	KY009611	KY223741	KY223790
L. gilliesii	Hook. & Arn.	PH	36° 00′	70° 30′	CONC 21218	H	Andean	KY010388	KY223763	KY223815
L. glacialis	Reiche	PH	35° 30′	71° 11′	SGO 144413	H	Andean	KY010391	KY223765	KY223817
L. glandulosa	D.Don	PH	33° 21′	70° 20′	CONC 163104	H	Mediterranean	KY010366	KY223745	KY223794
*L. hahnii**	Franch.	PH	51° 10′	72° 50′	CONC 128451	H	Andean/Patagonian steppe	KY010394	KY223768	KY223819
L. hieracioides	Cass.	PH	33° 51′	71° 07′	CONC 149053	H	Mediterranean/ Andean	KY010367	KY223746	KY223795
*L. leontopodioides**	(Kuntze) K.Schum.	PH	50° 43′	72° 19′	CONC 93089	H	Patagonian steppe/ Andean	KY010383	KY223759	KY223810
L. lithospermifolia ssp. lithospermifolia	Reiche	PH	35° 11′	70° 30	CONC 181222	F	Mediterranean/ Andean	KY010400	KY223774	KY223825

Table 1 continued

Taxon	Authority	FV	Localities		Collection No.	Source material	Main habitat	GenBank No.		
			Latitude °S	Longitude °W				ITS	*trn*L-*trn*F	*rpl*32
L. lithospermifolia ssp. integrifolia	(Phil.) Grau & Zinnecker	PH	35° 09′	70° 29′	CONC 181251	F	Mediterranean/ Andean	KY010393	KY223767	KY223818
L. magna	Phil.	PH	38° 34′	71° 38′	CONC 77669	H	Forest	KY010389		
L. menana	J.Rémy	AH	33° 10′	71° 33′	SGO 61991	H	Winter-rainfall coastal desert/ Mediterranean	KY010378	KY223755	KY223806
*L. millefolium**	Dusén & Skottsb.	PH	38° 39′	70° 54′	CONC 26087	H	Andean	KY010390	KY223764	KY223816
L. multiflora	Phil.	PH	33° 16′	71° 39′	CONC 170914	H	Mediterranean	KY010368		KY223796
*L. nutans**	(J.Rémy) Reiche	PH	38° 39′	70° 54′	CONC 181226	F	Patagonian steppe/ Mediterranean	KY010397	KY223771	KY223822
L. oligocephala	J.Rémy	AH	33° 26′	70° 30′	CONC 157381	H	Mediterranean	KY010384	KY223760	KY223811
L. paniculata	Poepp. ex Less.	PH	37° 28′	72° 21′	CONC 13268	H	Mediterranean/ Andean	KY010379		
*L. papillosa**	Cabrera	PH	47° 48′	73° 06′	CONC 169242	H	Andean	KY010369	KY223747	KY223797
L. polyclados	(J.Rémy) Reiche	PH	28° 40′	70° 11′	CONC 166845	H	Andean	KY010370		KY223798
*L. pteropogon**	(Griseb.) Cabrera	PH	22° 55′	67° 46′	CONC 139328	H	Andean	KY010371	KY223748	KY223799
*L. purpurea**	Hook. & Arn.	PH	51° 01′	72° 50′	CONC 162014	H	Patagonian steppe/ Andean	KY010372	KY223749	KY223800
L. rosea	Poepp. ex Less.	PH	33° 20′	70° 21′	CONC 163098	H	Mediterranean	KY010373	KY223750	KY223801
L. runcinata	D.Don	PH	33° 12′	70° 56′	CONC 170377	H	Andean	KY010374	KY223751	KY223802
*L. salina ssp. salina**	(J.Rémy) Dusén	PH	33° 16′	70° 15′	CONC 163106	H	Andean	KY010375	KY223752	KY223803
*L. scrobiculata**	Gillies & D.Don	PH	34° 13′	69° 49′	CONC 166158	H	Andean	KY010385	KY223761	KY223812
L. senecioides	Hook. & Arn.	PH	36° 45′	73° 02′	CONC 134250	H	Mediterranean	KY010376	KY223753	KY223804
*L. suaveolens**	(d'Urv.) Skottsb.	PH	51° 67′	57° 81′	CONC 181261	F	Alpine tundra	KY085964	KY223777	KY223829
L. tenuis	Less.	AH	33° 21′	70° 22′	CONC 163082	H	Mediterranean	KY006586	KY223740	KY223789
L. thermarum	Reiche	PH	36° 54′	71° 24′	CONC 181224	F	Forest	KY010396	KY223770	KY223821
L. tomentosa	(Less.) Crisci	PH	32° 47′	71° 32′	CONC 93777	H	Mediterranean	KY010380	KY223756	KY223807
L. viscida	(Bertero ex Colla) Crisci	PH	34° 24′	72° 02′	CONC 105587	H	Andean/ Mediterranean	KY010386		KY223813
Representatives of the Nassauvieae tribe										
Acourtia coulteri	(A.Gray) Reveal & R.M.King	PH				GB		FJ979680		FJ979729
Ameghinoa patagonica	Speg.	SH				GB		EF530223	EF530269	

Table 1 continued

Taxon	Authority	FV	Localities		Collection No.	Source material	Main habitat	GenBank No.		
			Latitude °S	Longitude °W				ITS	*trn*L-*trn*F	*rpl*32
Burkartia lanigera	(Hook. & Arn.) Crisci	SH				GB				FJ979728
Calopappus acerosus	Meyen	PH	33° 21′	70° 15′	SGO 160730	F		KY010360	KY223737	KY223786
Calorezia nutans	(Less.) Panero	PH	36° 30′	71° 11′	CONC 164923	H		KY009609	KY210140	KY223781
Dolichlasium lagascae	Gillies ex D.Don	SH				GB		EF530259	EU385062	
Holocheilus brasiliensis	(L.) Cabrera	PH				GB		EF530247	EF530293	
Jungia floribunda	Less.	PH				GB		EF530233	EF530279	
Leunisia laeta	Phil.	SH	32° 05′	70° 34′	HULS 13393	H		KY010357	KY223734	KY223783
Macrachaenium gracile	Hook.f.	PH	53° 33′	72° 18′	CONC 172103	H		KY009610		KY223782
Marticorenia foliosa	(Phil.) Crisci	SH	32° 50′	70° 08′	CONC 164979	H		KY006584	KY223733	KY223780
Moscharia pinnatifida	Ruiz & Pav.	AH				GB		EF530218	EF530264	
Nassauvia aculeata	Poepp. & Endl.	PH	38° 33′	71° 11′	SGO 159014	F		KY010361	KY223738	KY223787
Perezia lyrata	Wedd.	PH	36° 54′	71° 24′	CONC 181225	F		KY010359	KY223736	KY223785
Phamphalea heterophylla	Less.	AH				GB		EF530248	EF530294	
Pleocarphus revolutus	D.Don	SH	30° 42′	71° 00′	HULS 13772	F		KY006585	KY210141	
Proustia ilicifolia	Hook. & Arn.	SH	28° 56′	71° 09′	HULS 1630	H		KY010358	KY223735	KY223784
Triptilion spinosum	Ruiz & Pav.	PH	33° 43′	70° 28′	SGO 161136	F		KY010362	KY223739	KY223788
Representatives de the Calyceraceae										
Nastanthus scapigerus	Miers	PH	33° 48′	70° 14′	SGO 161140	F		KY009608	KY223732	KY223779

Information on stem type and number of capitula is given for all taxa

FV life-form (*AH* annual herbs, *HP* perennials herbs, *SH* shrubs)

Source of material (*H* herbarium, *F* field, *GB* GenBank)

* Indicate taxa with an acaulous/subacaulous habit and solitary capitulum

Table 2 Primers used to amplify and sequence rDNA and cpDNA

Primer	Sequence (5′–3′)	References
ITS 4	TCCTCCGCTTATTGATATGC	White et al. (1990)
ITS 5	GGAAGTAAAAGTCGTAACAAGG	White et al. (1990)
*trn*L	CGAAATCGGTAGACGCTACG	Taberlet et al. (1991)
*trn*F	ATTTGAACTGGTGACACGAG	Taberlet et al. (1991)
*rpl*32-F	CAGTTCCAAAAAAACGTACTTC	Shaw et al. (2007)
*trn*L(UAG)	CTGCTTCCTAAGAGCAGCGT	Shaw et al. (2007)

between 8076 and 32,250. Nodes with ≥0.95 were considered to be supported for posterior probabilities (Ronquist and Huelsenbeck 2003).

Life-form reconstruction was performed on the combined Bayesian tree using parsimony and maximum likelihood methods as implemented in Mesquite 2.74 (Maddison and Maddison 2008). Life-form character was scored as annual or perennial according to information given in Crisci (1976) (Table 1). To analyze the evolutionary lines proposed by Crisci (1976), the nine evolutionary lines were compared with the molecular phylogenetic relationships obtained in this study (Online Resource 1).

Results

The data matrix contained 3631 nucleotide characters (945 ITS, 1139 *trn*L-*trn*F and 1547 *rpl*32). The pattern indicated in this study was maintained in all cases. The few differences with the combined analysis are due to: (a) genes with different evolutionary history (maternal and bi-parental inherence); (b) not all trees were constructed with the same number of species, since not all the genes could be amplified in some samples; (c) the robustness level of many nodes in each of the trees was low, but these strengthened when information from genes was combined; and (d) in the combined analysis secondary signals appeared that were not observed in the analysis of the genes separately. Topologies based on individual markers are shown in online resources 2, 3 and 4. The combined analysis shows that the genus *Leucheria* is monophyletic with respect to the groups considered (Fig. 2). Consequently, all species belonging to genera synonymized under *Leucheria* are part of *Leucheria* as originally circumscribed by Crisci (1976). Included here are *L. floribunda* and *L. nutans* originally described in *Eizaguirrea* and *Clybatis,* respectively, and which according to Crisci (1976) are somewhat atypical in the genus.

The topology revealed a primary division between acaulescent/subacaulescent species with solitary capitula (Clade A) and caulescent species with numerous capitula (Clade B). The only exception to this observation is *L. achillaeifolia*, which although caulescent and its inflorescence has multiple capitula, is part of Clade A. Clade A contains 16 taxa of perennial herbs. Three well-supported lineages were identified in Clade B. Subclade I comprises two species (*L. coerulescens* and *L. floribunda*), both of which are perennial herbs; Subclade II contains 13 taxa, of which three are annuals; Subclade III contains 14 taxa, including two annual species (Fig. 3). These last three lineages unlike A and B cannot be separated by any obvious morphological characteristic.

Table 1 shows the vegetation type for each species of *Leucheria*. The three forest species are found in different subclades (Subclade I: *L. coerulescens;* Subclade III: *L magna* and *L. thermarum*) and not sister to all other species of *Leucheria*. Species sister to the forest dwellers are found predominantly in non-forested habitats. Thus, it is unlikely that the *Leucheria* clade arose from an ancestor that inhabited forest.

We found incongruences between the evolutionary lines proposed by Crisci (1976) and our phylogenetic results (Fig. 3). Species of the *L. thermarum* line are dispersed throughout Subclade I and III. Species in each of the *L. glacialis, L. amoena* and *L. cerberoana* lines are found in Subclade II and III. Species of the *L. salina* and *L. candidissima* line are all found in Clade A, but are not monophyletic. The *L. achillaeifolia* and *L. nutans* lines, each with a single species, are also in Clade A, whereas the *L. floribunda* line, also with a single species, is part of Clade B, Subclade I (Fig. 3).

Reconstruction of life-form showed that the annual herbs in *Leucheria,* found in Subclade II and III are derived (Figs. 3, 4). Two of the five annuals included in the phylogeny form a single clade within Subclade II, indicating that while the annual habit arose from the perennial habit, some annuals in *Leucheria* have given rise to other annual species. These particular annual species inhabit the coastal winter rainfall desert in northern Chile (*L. cerberoana* and *L. cumingii*) as *L. menana*. The remaining annuals (*L. tenuis* y *L. oligocephala*) form a clade in Subclade III and occur at low to mid-elevations in the Mediterranean-type climate area of central Chile (Figs. 3, 4).

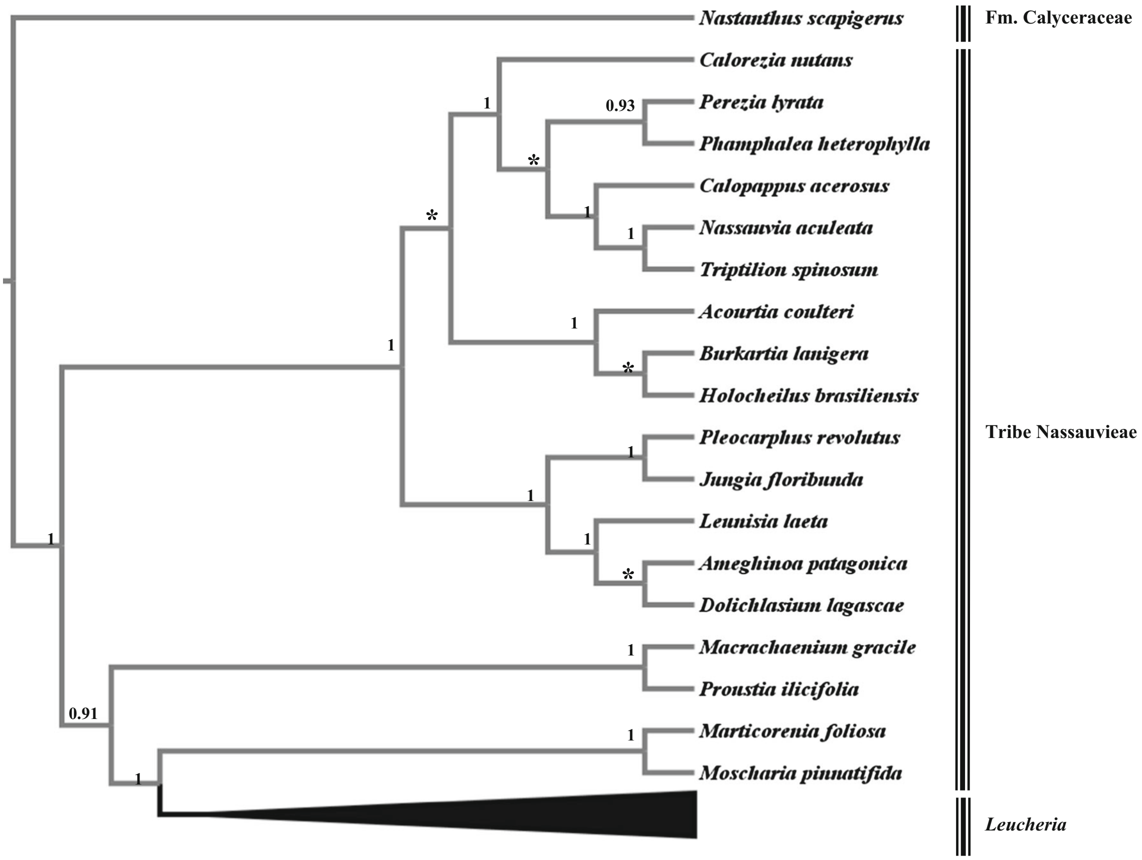

Fig. 2 Bayesian inference phylogenetic tree based on combined analysis (rDNA and cpDNA) for taxa of the genus *Leucheria* and 19 taxa belong to the Calyceraceae and the tribe Nassauvieae. Posterior probability values are found above the branches. *Asterisks* indicate nodes without support (<0.90). *Gray lines* indicate the external group, and *black lines* the internal group

Discussion

According to our results, the sister group to *Leucheria* according to the outgroups used, is a clade formed by monotypic *Marticorenia* and *Moscharia* (with two closely related species). *Leucheria's* closest relatives according to Crisci (1976) are *Holocheilus* and *Macrachaenium.* Kim et al. (2002) using cpDNA concluded that the sister group of *Leucheria* was *Jungia*. Gruenstaeudl et al. (2009) using cpDNA placed *Leucheria* in a clade with *Perezia* and *Nassauvia.* According to Katinas et al. (2008a), based on cpDNA, the sister group to *Leucheria* is *Polyachyrus.* We were unable to extract quality DNA from *Polyachyrus,* which prevented us from verifying Katinas et al. (2008a) findings. Panero and Funk (2008) using cpDNA concluded that *Leucheria* is sister to a clade formed by *Acourtia, Dolichlasium, Jungia, Nassauvia, Perezia* and *Trixis.* Resolution of this important issue for understanding the evolutionary history of tribe Nassauvieae Cass., subfamily Mutisioideae clearly requires consideration of all genera recognized in the Nassauvieae tribe.

Crisci (1976) proposed that *Leucheria* descended from a forest-dwelling ancestor. In the topology presented here, the three forest species of *Leucheria* (*L. magna*, *L. coerulescens*, *L. thermarum*) appear in two derived subclades. Thus, it is unlikely that the ancestor of *Leucheria* grew in a forested habitat. The great majority of species in *Leucheria* are found in open, non-forested habitats. From there, species have evolved into forests on at least two occasions.

Our results suggest *Leucheria* underwent a major split between acaulescent plants with solitary capitula (Clade A) and caulescent plants with multiple capitula (Clade B comprised of three subclades). In *Leucheria*, the acaulescent habitat is probably primitive, but more work and dating are required to ascertain this conclusion. Species in Clade A are found principally in high Andean vegetation from the extreme southern part of the South American continent to southern Peru. The acaulescent habitat is common in high elevation species (Fabbro and Körner 2004), where plants of short stature are favored under the harsh environmental conditions. Species belonging to the three caulescent lineages (Subclades I, II, III) are distributed principally in sub-Andean vegetation,

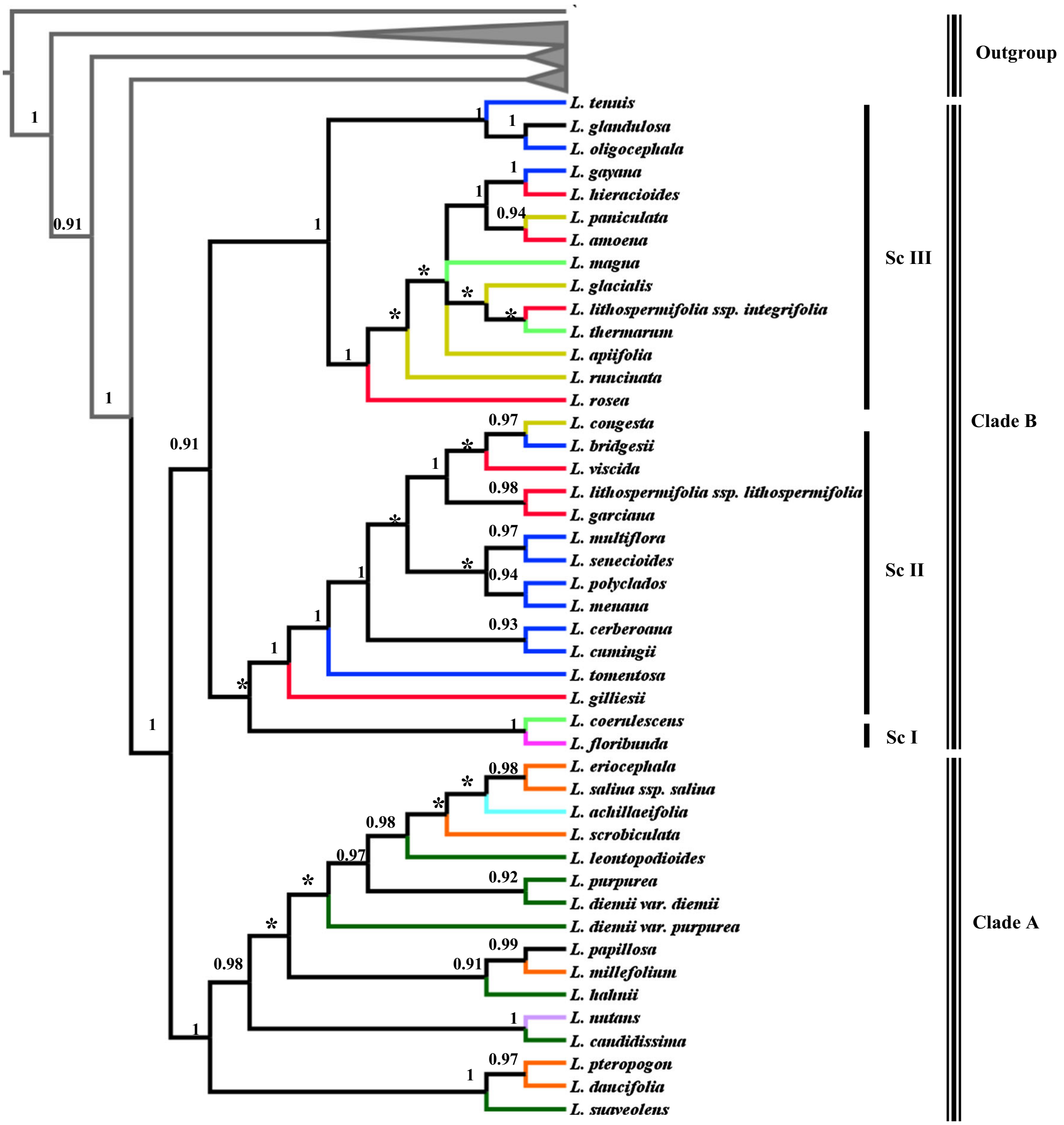

Fig. 3 Bayesian inference phylogenetic tree based on combined analysis (rDNA and cpDNA) for 45 taxa of the genus *Leucheria* and taxa belong to the Calyceraceae and the Tribe Nassauvieae. Posterior probability values are found above the branches. *Asterisks* indicate nodes without support (<0.90). *Gray lines* indicate the external group, and *black lines* the internal group. The principal clades are denoted by *letter A* (acaulescent/subacaulescent species with solitary capitula) and *B* (caulescent species with numerous capitula). Subclades (Sc) are denoted by roman numerals (I, II, III). The *colors* of the *lines* indicate the nine evolutionary lines recognized by Crisci (1976): *light green* Line *L. thermarum*; *yellow* Line *L. glacialis*; *red* Line *L. amoena*; *blue* Line *L. cerberoana*; *dark green* Line *L. candidisima*; *orange* Line *L. salina*; *cyan* Line *L. achillaeifolia*; *violet* Line *L. nutans*, *pink* Line *L. floribunda* and *black* taxa not considered by Crisci (1976)

Mediterranean-type vegetation and coastal desert (Table 1). Species found at relatively lower elevations, as are the species in these three subclades are likely to have evolved well-developed stems because of competition for light with other species in taller vegetation.

Molecular analysis of cpDNA suggests that the caulescent state is basal in the genus *Rheum* (Sun et al. 2012). In the genus *Streptocarpus*, the basal species are caulescent species, but shifts to the acaulescent habit have also occurred (Möller and Cronk 2001). In *Viola*, derived clades can include both caulescent and acaulescent species (Ballard and Sytsma 2000). Evolutionary transitions between the caulescent and acaulescent state will depend on the sequence of habitats colonized in evolutionary time. For example, acaulescent species are common in high alpine environments. When a lineage descends from high elevation, it is likely to acquire a caulescent habit; to the contrary, if a linage colonizes into the high altitude

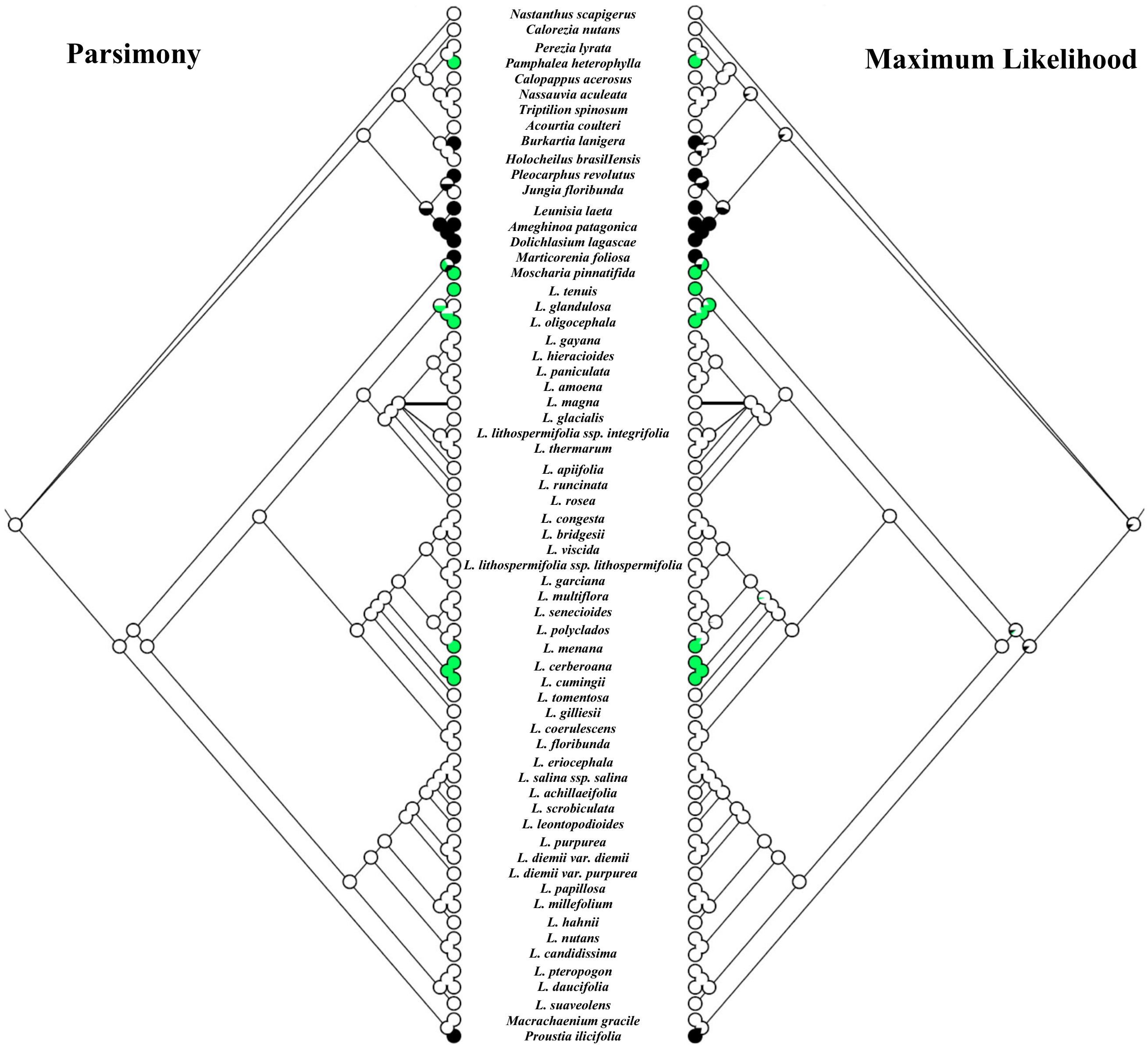

Fig. 4 Reconstruction of life-form for 45 taxa of *Leucheria* on the Bayesian tree using parsimony (*left*) and maximum likelihood (*right*). *Green* annual herbs; *white* perennial herbs; *black* shrubs. The outgroup included 19 taxa belonging to the Calyceraceae and the Tribe Nassauvieae

environment from lower elevation, the direction of evolution is likely to be from caulescent to acaulescent.

The discrepancies found between the nine morphologically defined evolutionary lines recognized by Crisci (1976) and the lineages identified in this study are mainly due to the types of characters that were used to categorize the nine lines. Morphological characteristics, such as amount of pubescence or plant size, can be highly homoplastic (Álvarez et al. 1999) and show similarities without an ancestor–descendent relationship. Consequently, morphological and molecular characters commonly show incongruences (Hillis and Wiens 2000). Moreover, characteristics such as plant size can be affected by environmental factors (Goyenechea and Contreras-Ramos 2007). Crisci (1976) omitted two species from his nine evolutionary lines (*L. papillosa* and *L. glandulosa*). In the topology presented here, these species are found in Clade A and Subclade III, respectively.

The results showed that the annual habit in *Leucheria* is a derived condition that evolved on two independently occasions in Subclades II and III. The three annual herbs in Subclade II (*L. cerberoana, L. cumingii* and *L. menana*) inhabit the Chilean coastal winter rainfall desert characterized by extremely low and unpredictable rainfall. Rainfall varies from 100 to 120 mm/year in the southern part of the winter rainfall desert to 14 mm/year in the northern sector near Antofagasta. Annuals in Subclade III (*L. tenuis* and *L. oligocephala*) are found in the northern part of central Chile with a Mediterranean-type climate, where rainfall is less than 250–700 mm/year. In general, annuals are favored in desert and Mediterranean-type climates. Annuals can avoid long dry seasons and unpredictable rainfall as seed banks and have the capacity for high growth rates during the humid season (Schaffer and Gadgil 1975; Evans et al. 2005; Jara et al. 2006; Rivero et al. 2007). Annuals are favored in deserts also because of the abundance of open space, which enhances seedling survival (Schaffer and Gadgil 1975; Crawley 1997; Dimmitt 2000; Silvertown and Charlesworth 2001; Evans et al. 2005). In central Chile, the frequency of annual species in the vascular plant flora increases with the degree of aridity (Arroyo et al. 1995), and it is known that the annual species have longer lived seed banks than perennial species in *Chaetanthera* (Arroyo et al. 2006). Although no experimental work has been undertaken to detect seed banks in *Leucheria,* it is very likely that the annual species will turn out to have persistent seed banks.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Information on Electronic Supplementary Material

Online Recourse 1. Evolutionary lines in *Leucheria* recognized by Crisci (1976).
Online Recourse 2. Bayesian Inference phylogenetic tree based on rDNA ITS.
Online Recourse 3. Bayesian Inference phylogenetic tree based on cpDNA *trn*L–*trn*F.
Online Recourse 4. Bayesian Inference phylogenetic tree based on cpDNA *rpl*32.
Online Recourse 5. Alignment used to produce phylogenie.

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