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Phylogenetics of *Escallonia* (Escalloniaceae) based on plastid DNA sequence data

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Escallonia (Escalloniaceae) is a New World genus of *c*. 39 species distributed mainly in the South American highlands. Plastid DNA sequence data from the intergenic spacers *trnS-trnG* and *3′ trnV-ndhC* and the *ndhF* gene for 32 species were used to examine the relationships among species and related genera and to analyse the relationship between phylogeny and the geographical distribution of the species. Maximum parsimony and Bayesian inference were employed to analyse the data. The sister relationship of *Escallonia* to *Forgesia* and *Valdivia* was corroborated. We recovered five strongly supported clades that are geographically structured, suggesting that the evolutionary history of the genus may be linked to historical processes, including the uplift of mountainous systems in South America. *Botanical Journal of the Linnean Society*, 2013, **173**, 442–451.

ADDITIONAL KEYWORDS: Bayesian inference – maximum parsimony – *ndhF* – South American highlands – *trnS-trnG* – *trnV-ndhC*.

INTRODUCTION

Escallonia Mutis ex L.f. is the most diverse genus of Escalloniaceae R.Br. ex Dumort., with 39 species distributed in the South American highlands, excluding the Guyana Shield. Most species occur along the Andes from Costa Rica to Tierra del Fuego, with the highest species diversity in the southern Andes (Chile–Argentina). Some species also occur in southern Brazil, Paraguay, Uruguay and north-eastern and central Argentina, and one species occurs in the Juan Fernández archipelago (off the coast of central Chile) (Sleumer, 1968). Most plants in the genus are found near mountains, freshwater streams, open forests and coastal areas; however, a few species can occur at elevations of > 3000 m, in Colombia, Ecuador, Peru and Bolivia (Brako & Zarucchi, 1993; Saldias-Paz, 1993; Jørgensen & León-Yañez, 1999). Morphologically, all *Escallonia* spp. are shrubs or trees characterized by simple, serrate leaves that are spirally arranged. The flowers can be solitary or arranged in inflorescences; they are always pentamerous with free petals, and they have inferior ovaries. The flowers or inflorescences are visited by generalist insects, and selfing is possible (Anderson *et al*., 2001; Valdivia & Niemeyer, 2006), but our knowledge about the pollination biology of the genus is limited. A few species have been studied cytologically and have the same chromosome number ($2n = 24$) (Zielinski, 1955; Sanders, Stuessy & Rodríguez, 1983). Based on a limited sampling and using sequence data from the plastid genes *atpB*, *ndhF* and *rbcL* with morphological data, Lundberg (2001) suggested that *Escallonia* could be paraphyletic with respect to *Valdivia* J.Rémy and *Forgesia* Comm. ex Juss. Other previously published phylogenetic analyses (Soltis *et al*., 2000; Winkworth, Lundberg & Donoghue, 2008; Tank & Donoghue, 2010) included few *Escallonia* spp.

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Recently, new evidence based on nuclear DNA sequence data and a broad sampling corroborated the monophyly of *Escallonia* (Zapata, 2013).

The remaining members of Escalloniaceae include *Eremosyne* Endl. and *Anopterus* Labill. from Australia, *Polyosma* Blume from Australasia, *Forgesia* from La Réunion Island in the Indian Ocean and the Patagonian genera *Valdivia* and *Tribeles* Phil. (APG III, 2009). Previous phylogenetic analyses based on nuclear and plastid DNA sequence data revealed that Escalloniaceae is monophyletic (Soltis *et al*., 2000; Lundberg, 2001; Winkworth *et al*., 2008; Tank & Donoghue, 2010); however, the relationship of Escalloniaceae to other campanulids (Asterales, Dipsacales and Apiales) still remains uncertain (Tank & Donoghue, 2010). Here, we reconstruct a phylogeny of *Escallonia* using plastid DNA sequence data from three regions and a broad taxonomic sampling to examine the relationships among species and related genera. Furthermore, we analyse the relationship between phylogeny and the geographical distribution of the species.

MATERIAL AND METHODS

Taxon sampling

We included 32 of the 39 *Escallonia* spp. in this study (Table 1). Whenever possible, we included more than one accession/individual per species. We also included *Anopterus*, *Eremosyne*, *Forgesia*, *Polyosma*, *Tribeles* and *Valdivia* in the analyses (see Lundberg, 2001; Winkworth *et al*., 2008; Tank & Donoghue, 2010).

DNA isolation, amplification and sequencing

Genomic DNA was isolated from silica-dried leaf tissue following a cetyltrimethylammonium bromide (CTAB) protocol (Doyle & Doyle, 1987) and from herbarium material with the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). The plastid intergenic spacers *trnS-trnG* (primers $trnS^{GCU}$ and 5´trnG2S; Shaw *et al*., 2005), *3´trnV-ndhC* (primers $trnV^{(UAC)}$ × 2 and ndhC; Shaw *et al*., 2007) and the *ndhF* gene [primers 5F and 972R; Olmstead & Sweere, 1994; and 1230F (5′-ACCCCTTGCTTGTTTTTGG-3′) and 2357R (5′-CTCTTGTGACCCTTCTTTTCG-3′)] were amplified with a profile consisting of 94 °C for 3 min, followed by 30 cycles of 94 °C for 1 min, 52 °C for 1 min and 72 °C for 1 min. Polymerase chain reactions (PCRs) were performed in a final volume of 25 μL with 50–100 ng of DNA template, 0.2 μM of each primer, 25 μM deoxynucleotide triphosphates (dNTPs), 5 mM $MgCl_2$, 1 × Taq buffer and 1.5 units of Taq polymerase provided by Invitrogen Life Technologies. Automated sequencing was performed by Macrogen Inc. (South Korea). We edited and assembled electropherograms in BioEdit 5.0.9 (Hall, 1999). All sequences were deposited in GenBank (Table 1).

Sequence alignment and phylogenetic analysis

For sequence alignment, we used MAFFT version 6 (Katoh & Toh, 2008) available online (http://mafft.cbrc.jp/alignment/server/), with default settings. Indels were coded as binary characters using simple indel coding (Simmons & Ochoterena, 2000) as implemented in SeqState 1.4 (Müller, 2005). The three plastid regions were concatenated into a single matrix, which has been deposited in TreeBASE (study number 12841).

We used TNT v1.1 (Goloboff, Farris & Nixon, 2008) for phylogenetic analyses of the individual and combined datasets under the maximum parsimony criterion. All characters were considered to be unordered, and parsimony-uninformative characters were excluded from the analyses. The search strategy consisted of heuristic searches performed using 1000 series of random addition sequences, followed by tree bisection–reconnection (TBR) branch rearrangements retaining 10 trees per series. These trees were saved in memory, and additionally swapped with TBR, retaining a maximum of 10 000 total trees. A strict consensus tree was generated from the set of most parsimonious trees. Branch support was calculated by jackknifing (JK) (Farris *et al*., 1996), with a character removal probability of 36%, performing 10 000 replicates and a heuristic search strategy of five addition sequences swapped with TBR with one tree saved per replication. We rooted the tree in *Eremosyne*, guided by previous molecular phylogenetic analyses (Winkworth *et al*., 2008; Tank & Donoghue, 2010).

We used the Akaike information criterion (Akaike, 1974) as implemented in jModeltest v0.1.1 (Posada, 2008) for model selection. The GTR + I + G model of sequence evolution was chosen for Bayesian inference. We also analysed the combined matrix using a Bayesian Markov chain Monte Carlo (MCMC) approach (Yang & Rannala, 1997) as implemented in MrBayes 3.2 (Ronquist & Huelsenbeck, 2003). We ran MrBayes for 2 000 000 generations with two independent simultaneous runs each with four linked chains (one cold, three heated with a temperature of 0.2), using default priors (all topologies equally probable, branch lengths modelled under an exponential distribution with parameter 10, a uniform distribution for the gamma shape parameter, a beta distribution for the transition and transversion rates, a uniform distribution for the proportion of invariable sites and a flat Dirichlet distribution for the state frequencies). Trees were sampled every 100 generations. We used Tracer v1.5 (Rambaut & Drummond, 2007) to assess the convergence of the independent

Table 1. Voucher information (collection locality, collection number, herbarium acronym) and GenBank accession numbers of the species included in this study. A dash indicates that the sequence was not obtained. Previously published sequences used in this study are indicated by an asterisk

Species	Collection number	Geographical location	*trnS-trnG*	*trnV-ndhC*	*ndhF*
Escallonia alpina Poepp. ex DC.	*S. Sede 215* (SI)	Argentina, Neuquén, Las Ovejas	JX896272	JX896226	JX896184
Escallonia alpina Poepp. ex DC.	*S. Sede 259* (SI)	Argentina, Chubut, Lago Fontana	JX896310	JX896262	–
Escallonia alpina Poepp. ex DC.	*F. Zapata 331* (MO)	Chile, Región Metropolitana	JX896311	JX896263	–
Escallonia alpina Poepp. ex DC.	*F. Zapata 440* (MO)	Chile, Región IX	JX896312	JX896264	–
Escallonia angustifolia C.Presl	*F. Zapata 324* (MO)	Peru, Arequipa	JX896273	JX896227	JX896185
Escallonia bifida Link & Otto	*F. Zuloaga 9915* (SI)	Argentina, Misiones, San Pedro	JX896274	JX896228	JX896186
Escallonia callcottiae Hook. & Arn.	*F. Zapata* 127A (MO)	Chile, Juan Fernández archipelago	JX896275	JX896229	JX896187
Escallonia cordobensis (Kuntze) Hosseus	*S. Sede 210* (SI)	Argentina, San Luis, Merlo	JX896276	JX896230	JX896188
Escallonia discolor Vent.	*F. Zapata 128* (MO)	Colombia, Boyacá, Duitama	JX896277	JX896231	JX896189
Escallonia discolor Vent.	*F. Zapata 84* (MO)	Colombia, Cundinamarca, Subachoque	JX896278	JX896232	JX896190
Escallonia farinacea A.St.-Hil.	*L. Fonseca 10* (SPF)	Brazil, Parana, Piraquara	JX896279	JX896233	JX896191
Escallonia florida Poepp. ex DC.	*F. Zapata 431* (MO)	Chile, Región IX	JX896280	JX896234	JX896192
Escallonia herrerae Mattf.	*F. Zapata 190* (MO)	Peru, Cusco, Anta	–	JX896235	JX896193
Escallonia hypoglauca Herzog	*F. Zuloaga 10330* (SI)	Argentina, Jujuy, Valle Grande	JX896281	JX896236	JX896194
Escallonia hypoglauca Herzog	*F. Zuloaga 10434* (SI)	Bolivia, Tarija	JX896313	JX896265	–
Escallonia hypoglauca Herzog	*F. Zapata 304* (MO)	Bolivia, Santa Cruz, M. M. Caballero	JX896314	JX896266	–
Escallonia illinita C.Presl	*F. Zapata* 539 (MO)	Chile, Región Metropolitana	JX896282	JX896237	JX896195
Escallonia illinita C.Presl					AJ419706*
Escallonia laevis (Vell.) Sleumer	*L. Fonseca 87* (SPF)	Brazil, Paraná, Campina Grande do Sul	JX896283	JX896238	JX896196
Escallonia ledifolia Sleumer	*L. Fonseca 59* (SPF)	Brazil, Santa Catarina	JX896284	JX896239	JX896197
Escallonia leucantha Remy	*S. Sede 271* (SI)	Argentina, Chubut, Arroyo Los Hitos	JX896286	JX896241	JX896199
Escallonia leucantha Remy	*S. Sede 277* (SI)	Argentina, Chubut, PN Lago Puelo	JX896287	JX896242	JX896200
Escallonia leucantha Remy	*F. Zapata 383* (MO)	Chile, Región VIII	JX896285	JX896240	JX896198
Escallonia micrantha Mattf.	*F. Zapata 242* (MO)	Peru, Cajamarca, San Miguel	JX896288	JX896243	JX896201
Escallonia millegrana Griseb.	*F. Zuloaga 10362* (SI)	Argentina, Salta, Santa Victoria	JX896289	JX896244	JX896202

Table 1. *Continued*

Species	Collection number	Geographical location	*trnS-trnG*	*trnV-ndhC*	*ndhF*
Escallonia millegrana Griseb.	*F. Zuloaga 10413* (SI)	Bolivia, Tarija	JX896290	JX896245	JX896203
Escallonia myrtilloides L.f.	*F. Zapata 318* (MO)	Bolivia, La Paz, Murillo	JX896291	JX896246	JX896204
Escallonia myrtoidea Bertero ex DC.	*F. Zapata 126* (MO)	Chile, Región Metropolitana	JX896292	JX896247	JX896205
Escallonia myrtoidea Bertero ex DC.					AJ419707*
Escallonia paniculata (Ruiz & Pav.) Roem. & Schult.	*F. Zapata 245* (MO)	Peru, Piura, Huancabamba	JX896293	JX896248	JX896206
Escallonia pendula (Ruiz & Pav.) Pers.	*F. Zapata 244* (MO)	Peru, Cajamarca, San Miguel	JX896294	–	JX896207
Escallonia petrophila Rambo & Sleumer	*L. Fonseca 42* (SPF)	Brazil, Santa Catarina	JX896295	JX896249	JX896208
Escallonia piurensis Mattf.	*F. Zapata 239* (MO)	Peru, Cajamarca, Contumaza	JX896296	JX896250	JX896209
Escallonia polifolia Hook.	*F. Zapata 224* (MO)	Peru, Amazonas	JX896297	JX896251	JX896210
Escallonia pulverulenta (Ruiz & Pav.) Pers.	*F. Zapata 95* (MO)	Chile, Región IV	–	JX896252	JX896211
Escallonia resinosa (Ruiz & Pav.) Pers.	*F. Zapata 182* (MO)	Peru, Cusco, Urubamba	JX896298	JX896253	JX896212
Escallonia resinosa (Ruiz & Pav.) Pers.	*F. Zapata 310* (MO)	Bolivia, Tarija	JX896299	JX896254	JX896213
Escallonia reticulata Sleumer	*F. Zapata 299* (MO)	Bolivia, Santa Cruz, Florida	JX896300	JX896255	JX896214
Escallonia revoluta (Ruiz & Pav.) Pers.	*F. Zapata 359* (MO)	Chile, Región VIII	JX896301	JX896256	JX896215
Escallonia revoluta (Ruiz & Pav.) Pers.	*F. Zapata 491* (MO)	Chile, Región VII	JX896315	JX896267	–
Escallonia rosea Griseb.	*S. Sede 270* (SI)	Argentina, Chubut, PN Lago Puelo	JX896302	–	JX896216
Escallonia rosea Griseb.	*S. Sede 283* (SI)	Chile, Región X	JX896303	–	JX896217
Escallonia rosea Griseb.	*F. Zapata 379* (MO)	Chile, Región VIII	JX896316	JX896268	–
Escallonia rubra (Ruiz & Pav.) Pers.	*S. Sede 263* (SI)	Argentina, Chubut, Corcovado	JX896304	JX896257	JX896218
Escallonia rubra (Ruiz & Pav.) Pers.	*O. Morrone 5700* (SI)	Argentina, Neuquén, PN Nahuel Huapi	JX896317	JX896269	–
Escallonia schreiteri Sleumer	*F. Zuloaga 10403* (SI)	Bolivia, Tarija	JX896305	JX896258	JX896219
Escallonia tucumanensis Hosseus	*F. Zuloaga 10003* (SI)	Argentina, Tucumán, Tafí	JX896306	JX896259	JX896220
Escallonia tucumanensis Hosseus	*F. Zuloaga 10377* (SI)	Argentina, Salta, Santa Victoria	JX896307	JX896260	JX896221
Escallonia virgata (Ruiz & Pav.) Pers.	*S. Sede 261* (SI)	Argentina, Chubut, Lago Fontana	JX896308	–	JX896222
Escallonia virgata (Ruiz & Pav.) Pers.	*O. Morrone 5690* (SI)	Argentina, Río Negro, PN Nahuel Huapi	JX896309	JX896261	–

Table 1. *Continued*

Species	Collection number	Geographical location	*trnS-trnG*	*trnV-ndhC*	*ndhF*
Anopterus macleayanus F.Muell.					AJ292984*
Eremosyne pectinata Endl.					AJ236272*
Forgesia racemosa J.F.Gmel.					AJ419701*
Forgesia racemosa J.F.Gmel.	*J. F. 425* (MO)	La Réunion	JX896271	JX896224	–
Polyosma cunninghamii Benn.					AJ429122*
Tribeles australis Phil.	*S. Sede 289* (SI)	Chile, Región X	JX896270	JX896223	–
Tribeles australis Phil.					AJ429123*
Valdivia gayana Remy					AJ419703*
Valdivia gayana Remy	*F. Zapata 99* (MO)	Chile, Región X, Valdivia	–	JX896225	–

Table 2. Summary information for the aligned data matrices and parsimony analyses

	trnS-trnG	*trnV-ndhC*	*ndhF*	Combined
Taxa	48	47	47	56
Aligned length (bp)	745	778	2730	4253
Potentially parsimony-informative characters	42	30	174	246
Number of coded indels	6	3	2	11
Missing data (%)	1.39	1.93	14.91	23.27
Number of most parsimonious trees/length (bp)	322/61	64/59	1224/294	>10 000/419
Main clades recovered	(C,(D,E),(A,B))	(A,B,C,(D,E))	(A,B,C,(D,E))	(C,(D,E),(A,B))
Consistency index/retention index (CI/RI)	0.72/0.93	0.67/0.87	0.75/0.85	0.73/0.87

runs. The first 3000 trees from each run were discarded as burn-in. We summarized the posterior distribution of trees with a majority-rule consensus tree showing average branch lengths and posterior probabilities (PP) for each clade.

RESULTS

The individual matrices, *trnS-trnG* (48 terminals and 745 aligned characters), *trnV-ndhC* (47 terminals and 778 characters) and *ndhF* (47 terminals and 2730 characters), contained 1.39%, 1.93% and 14.91% missing data, respectively. Eleven gaps (six from *trnS-trnG*, three from *trnV-ndhC* and two from *ndhF*) were binary coded (Table 2).

The combined three-region dataset consisted of 56 terminals and 4253 characters. The matrix contained 246 potentially parsimony-informative characters and 23.27% missing data, as only one or two regions were included for 20 accessions (Tables 1 and 2). We removed two autapomorphic insertions from this matrix, one in *Tribeles australis* Phil. (122 bp, *trnV-ndhC*) and one in *Anopterus macleayanus* F.Muell. (412 bp, *ndhF*), and several regions with ambiguous alignment and gaps in positions: 895–1270, 1414–1426, 1451–1456, 1490–1495 from *ndhF*; 3103–3120 from *trnV-ndhC*; and 3614–3628, 3764–3768, 3840–3845, 3870–3883, 3966–3973, 4210–4242 from *trnS-trnG*. Eleven species were represented by two or more accessions.

Maximum parsimony (Fig. 1) and Bayesian inference (Fig. 2) resulted in similar topologies. Maximum parsimony analysis of the combined dataset yielded > 10 000 trees [L = 419, consistency index (CI) = 0.73, retention index (RI) = 0.87]. The strict consensus tree was similar to the topologies when each dataset was analysed independently (topologies not shown; see Table 2 for details). Maximum parsimony and Bayesian inference corroborated the monophyly of *Escallonia* with moderate support (JK 77, PP 0.75). *Valdivia* and *Forgesia* formed a clade (JK 75, PP 0.93) sister to *Escallonia* (JK 93, PP 1). There was no phylogenetic

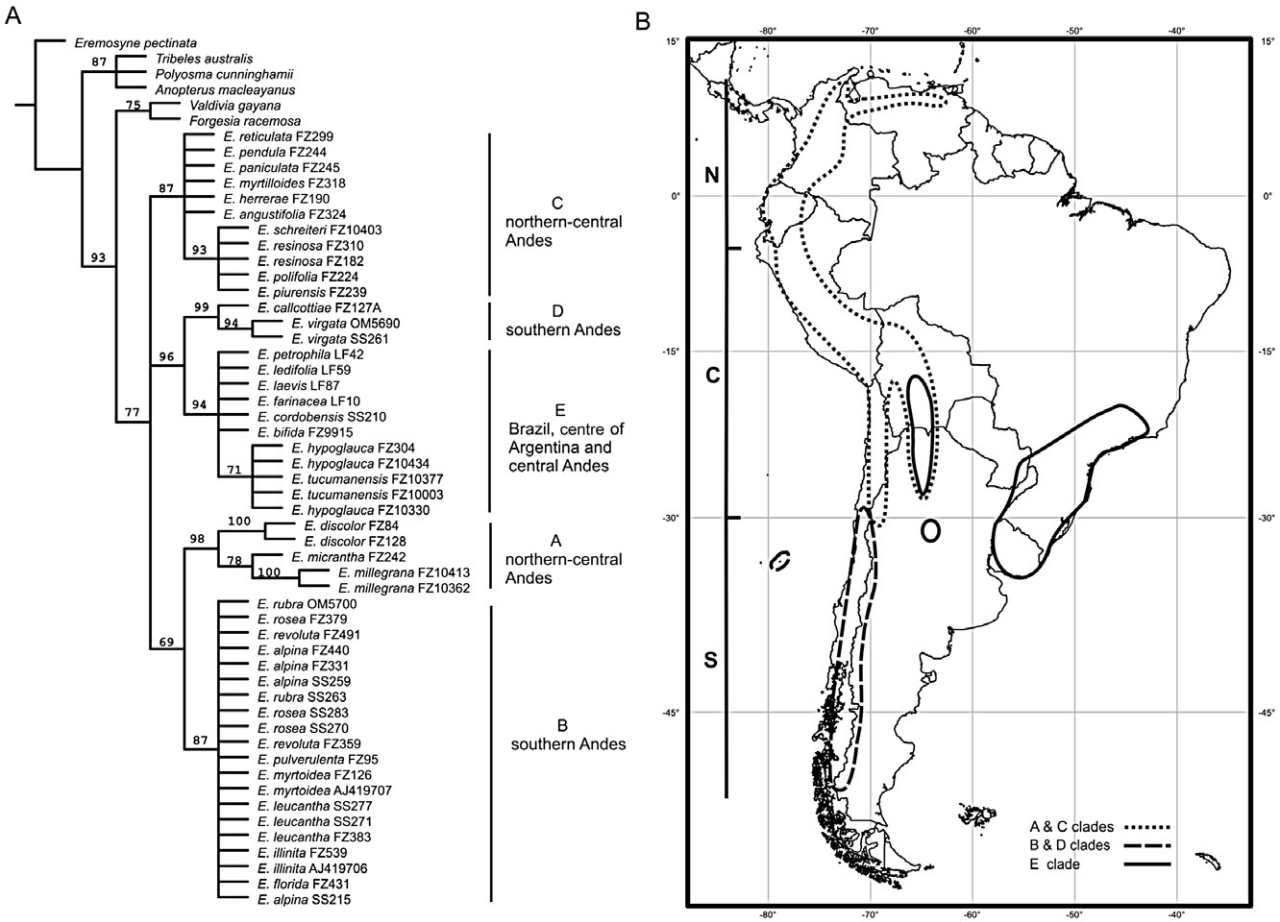

Figure 1. A, Strict consensus of 10 000 most parsimonious trees resulting from the analysis of the combined matrix (*trnS-trnG*, *trnV-ndhC* and *ndhF*). Numbers above the branches refer to jackknife values. Bars indicate clades as discussed in the text. B, Distribution of major clades of *Escallonia* derived from the phylogenetic analyses. Bars indicate major divisions of the Andean region: N, north; C, centre; S, south.

resolution at the base of *Escallonia*; however, we recovered five well-supported clades (Table 2; Figs 1, 2, clades A–E). In clade A (JK 98, PP 1), *E. discolor* Vent. was sister to *E. micrantha* Mattf. and *E. millegrana* Griseb. These three species are restricted to the northern and central Andes, with *E. discolor* occurring in central Colombia, *E. micrantha* occurring in southern Ecuador and northern Peru, and *E. millegrana* occurring in Bolivia and north-western Argentina. In clade B, *E. alpina* Poepp. ex DC., *E. florida* Poepp. ex DC., *E. illinita* C.Presl, *E. leucantha* Remy, *E. myrtoidea* Bertero ex DC., *E. pulverulenta* (Ruiz & Pav.) Pers., *E. revoluta* (Ruiz & Pav.) Pers., *E. rosea* Griseb. and *E. rubra* (Ruiz & Pav.) Pers. formed a polytomy in the maximum parsimony consensus tree (JK 87, PP 1). These species are restricted to the southern Andes (from *c*. 30°S to 53°S); most occur on both slopes of the Andes, except for *E. florida*, *E. illinita*, *E. pulverulenta* and *E. revoluta* which occur exclusively in Chile. Clades A and B formed a clade (JK 69, PP 0.92). In clade C (JK 87, PP 1), *E. schreiteri* Sleumer, *E. resinosa* (Ruiz & Pav.) Pers., *E. polifolia* Hook. and *E. piurensis* Mattf. formed a well-supported polytomy in the maximum parsimony consensus tree (JK 93), which, in turn, was part of a larger polytomy also including *E. reticulata* Sleumer, *E. pendula* (Ruiz & Pav.) Pers., *E. paniculata* (Ruiz & Pav.) Roem. & Schult., *E. myrtilloides* L.f., *E. herrerae* Mattf. and *E. angustifolia* C.Presl. All species in clade C are restricted to the northern and central Andes, with *E. myrtilloides* ranging from Colombia to north-western Argentina, *E. pendula* from Venezuela to Peru, *E. paniculata* from Venezuela to Bolivia, *E. resinosa* from Ecuador to Bolivia, *E. angustifolia* from southern Peru to central Chile and Argentina (*c*. 30°S) and *E. schreiteri* from Peru to Argentina. *Escallonia herrerae*, *E. polifolia* and *E. piurensis* occur exclusively in Peru, and

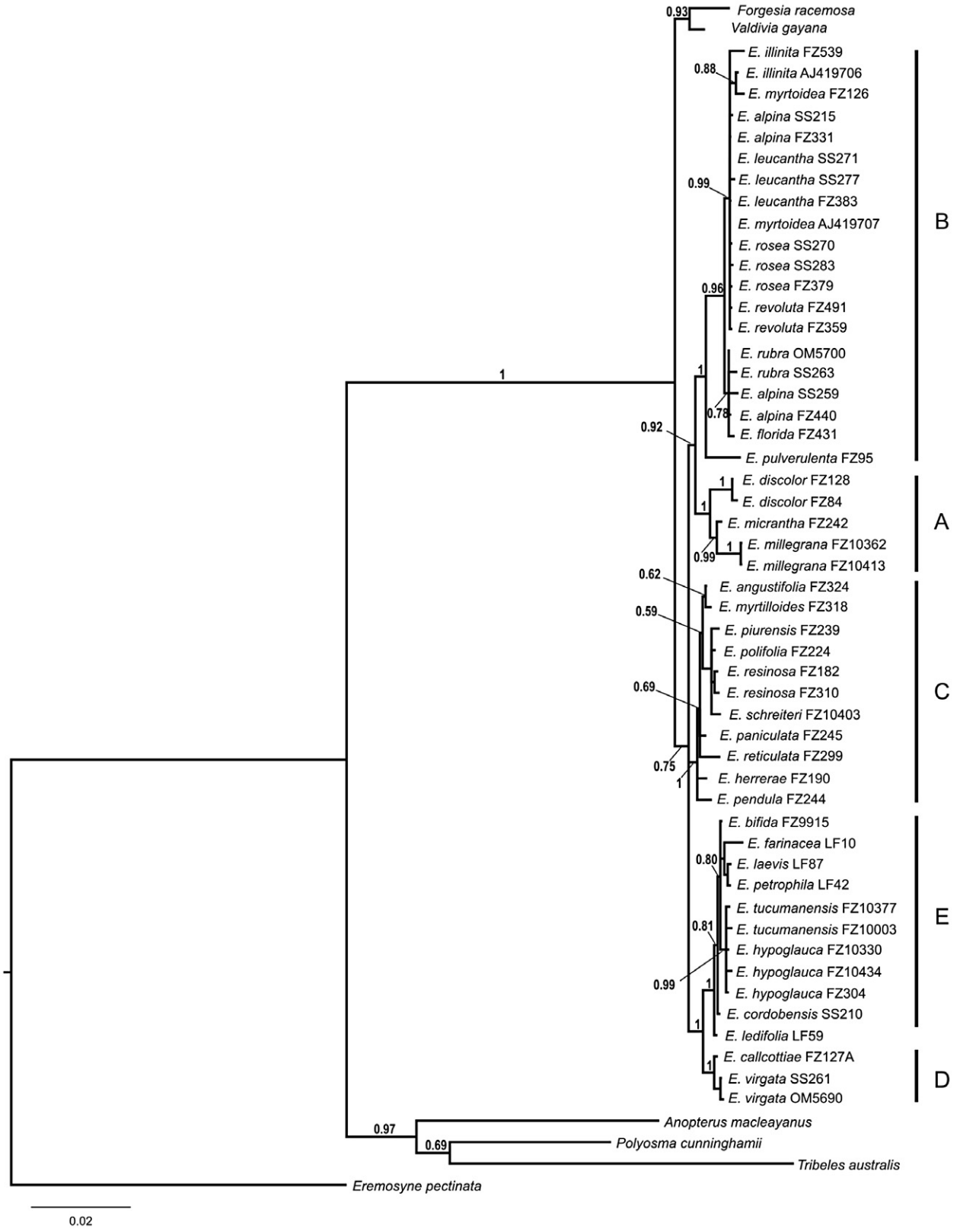

Figure 2. Majority-rule consensus tree from the Bayesian inference of the combined matrix (*trnS-trnG*, *trnV-ndhC* and *ndhF*). Numbers above the branches indicate posterior probabilities. Bars indicate principal groups as discussed in the text.

E. reticulata is restricted to Bolivia. In clade D (JK 99, PP 1), *E. callcottiae* Hook. & Arn., endemic to the Juan Fernández archipelago (Chile), was sister to *E. virgata* (Ruiz & Pav.) Pers., a species restricted to the southern Andes, from 34°S to 53°S. In clade E (JK 94, PP 1), *E. hypoglauca* Herzog and *E. tucumanensis* Hosseus formed a clade (JK 71, PP 1), which, in turn, was part of a polytomy in the maximum parsimony tree, including *E. petrophila* Rambo & Sleumer, *E. ledifolia* Sleumer, *E. farinacea* A.St.-Hil., *E. bifida* Link & Otto, *E. cordobensis* (Kuntze) Hosseus and *E. laevis* (Vell.) Sleumer. Most species in clade E occur in south-eastern Brazil: *E. farinacea*, *E. laevis*, *E. ledifolia*, *E. petrophila* and *E. bifida* (*E. bifida* extends into north-eastern Argentina, Paraguay and Uruguay). *Escallonia cordobensis* is restricted to the highlands in central Argentina, and *E. hypoglauca* and *E. tucumanensis* range from Bolivia to north-western Argentina. The sister relationship of clades D and E was strongly supported (JK 96, PP1).

DISCUSSION

The main goal of our study was to reconstruct a molecular phylogenetic tree for *Escallonia* derived from plastid DNA sequence data. These data supported the monophyly of the genus, in agreement with the taxonomic hypothesis proposed by Sleumer (1968) and a recent phylogenetic analysis based on nuclear DNA sequence data (Zapata, 2013). Moreover, the sister relationship between *Valdivia–Forgesia* and *Escallonia*, proposed by Tank & Donoghue (2010) and Zapata (2013), was corroborated.

The lack of phylogenetic resolution at the basal node of *Escallonia* and shallower nodes was caused mainly by the lack of sequence divergence in the plastid regions used (Table 2). Although the deepest relationships in *Escallonia* were not completely resolved, we recovered five main well-supported clades (Figs 1, 2). These clades are all geographically restricted to different Andean regions: the northern (Colombia, Venezuela and Ecuador) and central (Peru, Bolivia, Argentina and Chile up to *c*. 30°S) Andes or the southern Andes (Argentina and Chile from *c*. 30°S to 54°S), with the exception of clade E, which includes species from the central Andes, central Argentina and south-eastern Brazil (Figs 1, 2). None of these geographical clades is characterized by unique macro-morphological characters (or combinations thereof), suggesting that high levels of homoplasy may characterize the morphological diversification of *Escallonia*. For instance, in all clades, there are species with tree or shrub habit, species with elliptic or obovate leaves, species with paniculate inflorescences and species with red and white petals. This suggests a pattern of repeated morphological convergence among regions.

Species from the northern-central Andes occurred in two separate clades. One of these clades (clade A) is sister to a group including most of the species from the southern Andes (clade B). This phylogenetic pattern between the northern and southern Andean region is shared by various plant and animal groups (*Solanum tuberosum* L., Castillo & Spooner, 1997; birds, García-Moreno, Arctandera & Fjeldså, 1999; *Oxalis tuberosa* alliance, Emshwiller, 2002; lizards, Doan, 2003; *Fuchsia* L., Berry *et al*., 2004; nematodes, Picard, Sempere & Plantard, 2008). The connection between *E. discolor–E. micrantha–E. millegrana* and the southern Andean clade is intriguing: first, because only this clade, and not the remaining species from the northern Andes, are closely related, and, second, because the geographical distribution of these three allopatric species overlaps with other northern Andean species, but they do not form a clade with other species from the northern Andes (Figs 1, 2).

Species from south-eastern Brazil, central and north-western Argentina and Bolivia formed a well-supported clade (clade E). This geographical connection had already been suggested by Villagrán & Hinojosa (1997) and is also consistent with the topologies obtained by Zapata (2013) using nuclear DNA sequence data. The group of Brazilian species is distributed in cool and high-altitude regions of south-eastern Brazil (Brazilian planalto), one species endemic to the Sierras de Comechingones (Sierras Pampeanas) in central Argentina and two species from north-western Argentine and Bolivian forests. All of these regions are characterized by high diversity and high levels of endemism (Bianco *et al*., 2001; Scarano, 2002; Ortiz-Jaureguizar & Cladera, 2006), and various examples from many distantly related plant families exhibit the same disjunct distribution (*Crinodendron* Molina, *Azara* Ruiz & Pav., *Blepharocalyx* O.Berg and *Myrceugenia* O.Berg, among others; Villagrán & Hinojosa, 1997).

Most species from the southern Andes grouped together, except for *E. virgata* and *E. callcottiae*, which formed a separate clade (clade D). It is surprising that they are not closely related to the remaining species from the southern Andes (clade B). The sister relationship of clades D and E is well supported, consistent with the affinity among the flora of temperate forests of southern Brazil, eastern Bolivia and north-western Argentina (Villagrán & Hinojosa, 1997; Kreier *et al*., 2008) with austral-Antarctic floristic elements (e.g. *Fuchsia*, *Griselinia* J.R.Forst. & G.Forst., *Araucaria* Juss., *Drimys* J.R.Forst. & G.Forst.) (Zinsmeister, 1987; Berry, 1989; Katinas, Morrone & Crisci, 1999; Berry *et al*., 2004).

The geographically structured and well-supported phylogenetic topology suggests that historical processes, such as the uplift of mountainous systems in South America, have played an important role in the isolation and diversification of the genus by providing new ecological opportunities during the formation of different environments and microclimates. The phylogenetic analysis using plastid data presented here contributes towards the understanding of the species relationships in a morphologically complex and biogeographically interesting genus. The inferred phylogenetic hypothesis lays the foundations for future analyses that will focus on geographically restricted clades to disentangle the evolutionary processes leading to plant species diversification in the mountains of South America. In order to evaluate hypotheses on the mechanisms of speciation in these regions, we will expand the infraspecific sampling and carry out phylogeographical and population genetic analyses in the near future.

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