



Phylogenetics of *Darwiniothamnus* (Asteraceae: Astereae) – molecular evidence for multiple origins in the endemic flora of the Galápagos Islands

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ABSTRACT

Aim The aims of this study were (1) to investigate whether the two growth forms of *Darwiniothamnus* Harling (Asteraceae) originated from the colonization of a single ancestor, (2) to identify the closest relative(s) of *Darwiniothamnus*, and (3) to review molecular phylogenies from other plant groups to infer the origin of Galápagos endemics.

Location *Darwiniothamnus* is endemic to the Galápagos Islands.

Methods All putative relatives of *Darwiniothamnus* plus 38 additional species were included. Nucleotide sequences of the internal transcribed spacers of the nuclear ribosomal DNA were used for Bayesian and parsimony analyses.

Results *Darwiniothamnus* is polyphyletic. Two species (*D. lancifolius* (Hook. f.) Harling and *D. tenuifolius* (Hook. f.) Harling) are woody shrubs that usually grow to 1–2 m in height; they belong to a clade composed of species otherwise restricted to the Caribbean. These two species are sister to *Erigeron bellidistroides* Griseb., a herbaceous species endemic to Cuba. The third species (*D. alternifolius* Lawesson & Adersen) is a perennial herbaceous plant, woody at the base and reaching only up to 50 cm in height. It is sister to two Chilean (Coquimbo–Valparaiso region) species that also have a perennial herbaceous habit: *E. fasciculatus* Colla and *E. luxurians* (Skottsb.) Solbrig. They are placed in an assemblage restricted to South America. The review of previous molecular phylogenetic studies revealed that two of the endemic genera and endemic species of three non-endemic genera have their closest relatives in South America. Endemic species belonging to three non-endemic genera have sister species in North America or the West Indies. One endemic genus and endemic species in three non-endemic genera have sister taxa with a widespread continental distribution, or their molecular phylogenies yielded equivocal results.

Main conclusions The flora of Galápagos has affinities with both North America (including the Antilles) and South America. *Darwiniothamnus* exhibits both patterns: two species of this genus are sister to a taxon endemic to Cuba, supporting a connection between the Cocos plate and the West Indies; the third species, *D. alternifolius*, provides a link with the Coquimbo–Valparaiso region, suggesting a biogeographical connection between the Nazca plate and southern South America.

Keywords

Caribbean Islands, critically endangered species, *Darwiniothamnus*, DNA markers, Neotropics, oceanic islands, Pacific Islands, phylogeography, trans-oceanic dispersal.

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INTRODUCTION

Ever since Hooker (1847) published the first extensive account of the plants of the Galápagos Islands, there has been great interest in determining the continental affinities of the flora of the archipelago (Robinson, 1902; Stewart, 1911; Svenson, 1935, 1942, 1946; Porter, 1979, 1983, 1984a,b). These early studies concluded that, although the majority of plants endemic or native to the Galápagos seem to come from South America, a small component (*c.* 8% of the endemics and *c.* 6% of the native species) has a North American (mostly Mexican) or Caribbean origin. These conclusions have been confirmed by more recent historical biogeography studies (Grehan, 2001a,b; Lanteri, 2001).

Located along the equator *c.* 965 km west of continental Ecuador, the Galápagos archipelago has provided some of the best-known examples of speciation through adaptive radiation. Importantly, these islands have also been the focus of molecular phylogenetic studies of vertebrates (e.g. Caccone *et al.*, 1999, 2002; Sato *et al.*, 1999, 2001; Kizirian *et al.*, 2004; Arbogast *et al.*, 2006; Bollmer *et al.*, 2006), invertebrates (e.g. Lovejoy *et al.*, 2006; Parent & Crespi, 2006) and seed plants (e.g. Schilling *et al.*, 1994; Moore *et al.*, 2006).

The plant genus *Darwiniothamnus* Harling (Asteraceae, Astereae) is endemic to the Galápagos Islands. It was erected in 1962 for two endemic species formerly included in *Erigeron* L. (Hooker, 1847; Solbrig, 1962). To the two original species, *D. lancifolius* (Hook. f.) Harling and *D. tenuifolius* (Hook. f.) Harling, a third was added with the discovery of *D. alternifolius* Lawesson & Adersen (1987). The genus is restricted to the

islands of Fernandina, Floreana, Isabela, Pinta, Pinzón, Santiago and Santa Cruz, and some of their offshore islets, i.e. predominantly the western half of the archipelago (Lawesson & Adersen, 1987). *Darwiniothamnus alternifolius* is known only from small populations on the two southern volcanoes of Isabela Island and it is considered Critically Endangered (Tye, 2007; P. Jaramillo, unpublished data). The three described subspecies of *D. lancifolius* are restricted to Fernandina and Isabela. *Darwiniothamnus tenuifolius* (with at least two subspecies) has the broadest distribution, including these two islands and five additional ones (Fig. 1). Morphological patterns of infraspecific variation are not still well understood, and a definitive taxonomic study at lower levels is needed for the genus (Lawesson & Adersen, 1987).

Two major growth forms can be distinguished within the genus. The first form is that of *D. lancifolius* and *D. tenuifolius*, which are woody shrubs that usually grow to 1–2 m in height and occasionally reach up to 3.5 m (Fig. 2). Their leaves are condensed in terminal whorls with very short internodes (Harling, 1962). This kind of life-form is also displayed by other Asteraceae species endemic to oceanic islands or sky islands. Good examples are the silversword alliance (*Argyroxiphium* DC. – *Dubautia* Gaudich. – *Wilkesia* A. Gray) in the Hawaiian Islands, the woody *Sonchus* L. species from the Macaronesian Islands, *Scalesia* Arn. in the Galápagos, and *Dendrosenecio* (Hauman ex Hedberg) B. Nord. on the East African mountains (Carlquist, 1965, 1974). The second form is exhibited by *D. alternifolius*: plants of this species are perennial herbaceous, woody at the base and only reach up to 50 cm in height (Fig. 2). This species has

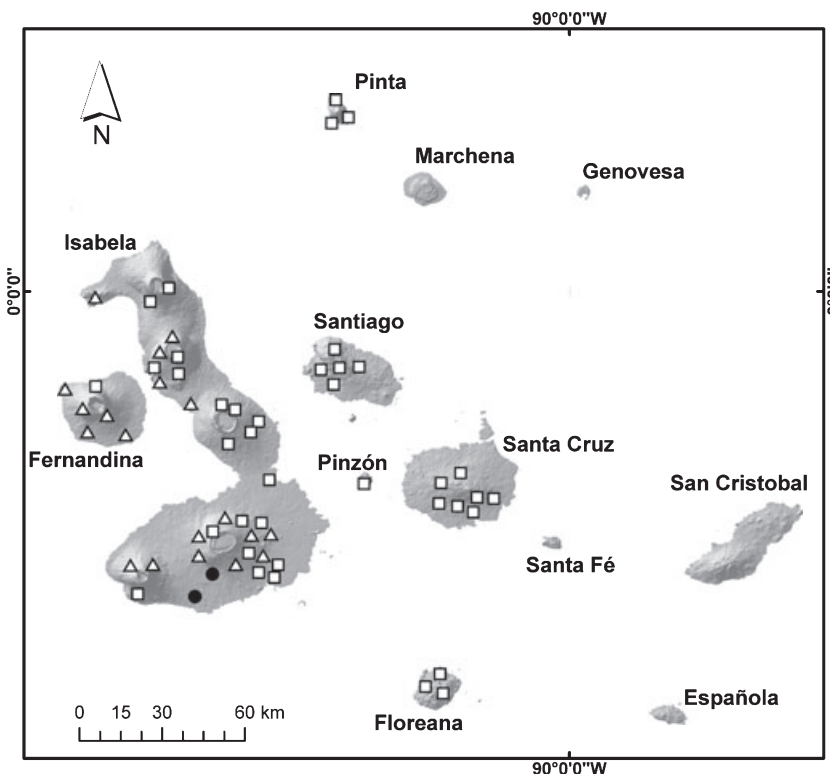


Figure 1 Geographical distribution of the three species of *Darwiniothamnus* Harling in the Galápagos Islands. Black circles, *D. alternifolius* Lawesson & Adersen; white triangles, *D. lancifolius* (Hook. f.) Harling; white squares, *D. tenuifolius* (Hook. f.) Harling.

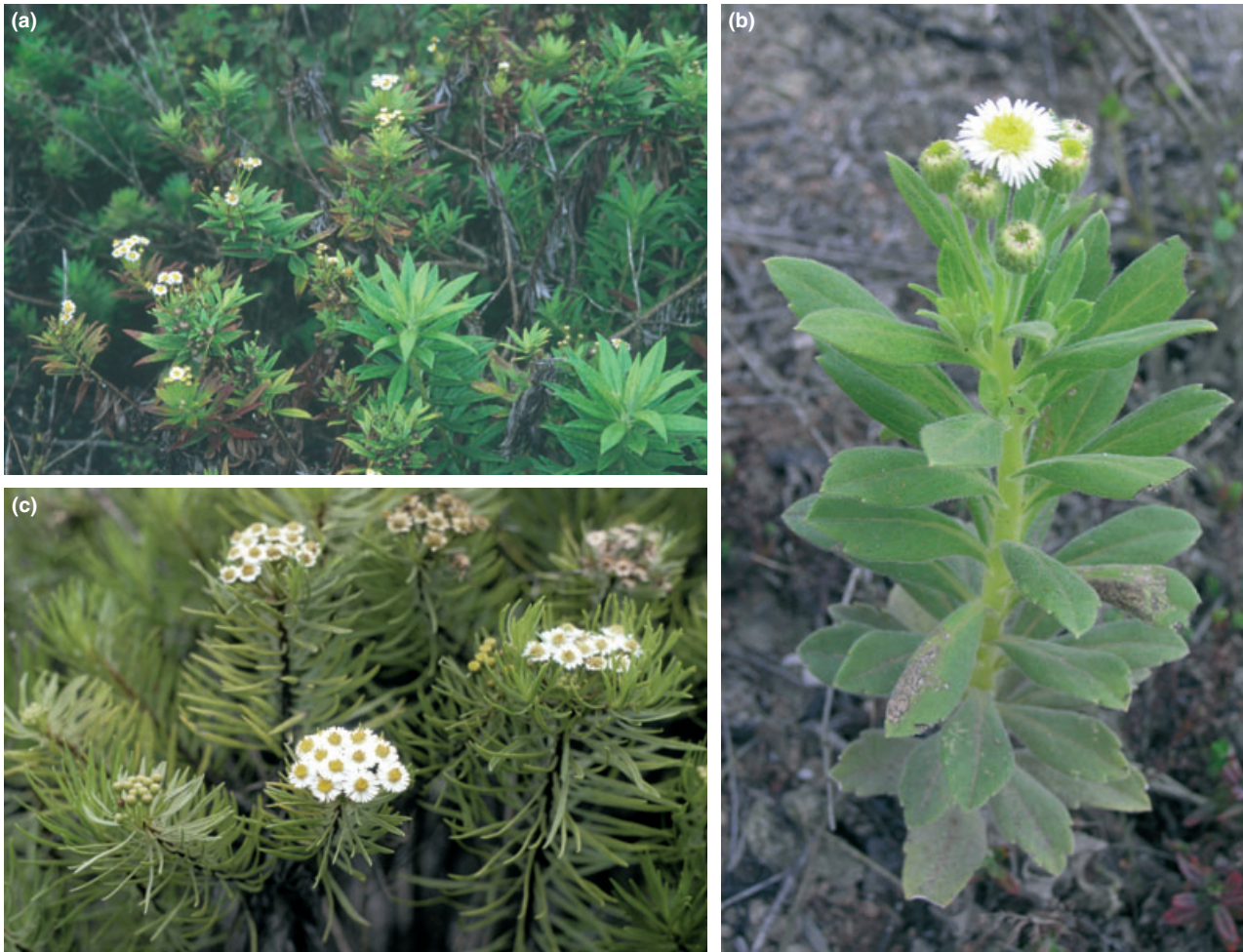


Figure 2 Photographs of the three species of *Darwiniothamnus* Harling in the Galápagos Islands. (a) *D. lancifolius* (Hook. f.) Harling (photograph by N.A.), (b) *D. alternifolius* Lawesson & Adersen (photograph by P.J.), (c) *D. tenuifolius* (Hook. f.) Harling (photograph by P.J.).

alternate leaves that are not condensed in terminal whorls and that have long internodes (Lawesson & Adersen, 1987). Despite these differences, the three species were considered to belong to a single genus because they possess aromatic scent and have many imbricate phyllaria of unequal length (Lawesson & Adersen, 1987).

Darwiniothamnus has been considered to be closely related to *Erigeron sensu lato* (Harling, 1962; Lawesson & Adersen, 1987; Nesom, 1989), a large genus (c. 400 species) of mostly herbaceous species that are accommodated in 34 sections within the subtribe Conyzinae (Nesom, 2008). Nesom (1989) earlier noted that there is little morphological reason to consider *Darwiniothamnus* as distinct, suggesting that the only trait that separates it from *Erigeron* is its shrubby habit. In addition, Nesom (2000; 2008) has noted that *D. alternifolius* is different from the two other species of the genus not only in life-form but also in its capitulescence (heads not in a condensed-corymbiform cluster among the leaves), achenes (not dimorphic) and ray florets (not tightly coiling). Despite these differences, most recent studies of the Galápagos flora

and the tribe Astereae accept *Darwiniothamnus* as a valid monophyletic endemic genus (Cronquist, 1970, 1971; Wiggins & Porter, 1971; McMullen, 1999; Nesom & Robinson, 2007).

There have been several hypotheses regarding the closest relative of *Darwiniothamnus*. Harling (1962) suggested two shrubby species of *Erigeron* endemic to coastal areas of the Coquimbo–Valparaiso region, central Chile [i.e. *E. fasciculatus* Colla (as *E. berterianus* DC.) and *E. luxurians* (Skotts.) Solbrig (as *E. litoralis* (Phil.) Skotts.)] (Marticorena *et al.*, 2001) or any of the six species endemic to the Juan Fernández Islands, a small archipelago located c. 600 km off the Chilean coast. Although the Coquimbo–Valparaiso region covers a relatively small area it has a number of unique biogeographical patterns, including the maximum generic diversity for Asteraceae in Chile (Moreira-Muñoz & Muñoz-Schick, 2007). Valdebenito *et al.* (1992) suggested that the Juan Fernández endemic species of *Erigeron* form a monophyletic group. Lawesson & Adersen (1987) also considered *Darwiniothamnus* as closely related to the Juan Fernández endemics and suggested that *D. alternifolius* is more similar to

E. fernandezianus (Colla) Solbrig than to the other species of *Darwiniothamnus*.

Johnston (1931) & Nesom (1989), in contrast, suggested that the species presently included in *Darwiniothamnus* might have their closest relatives in the Northern Hemisphere. *Erigeron crenatus* Eastwood and *E. socorrensis* Brandegee from the Revillagigedo Islands were suggested to be closely related to *Darwiniothamnus* because they share similar growth forms (Johnston, 1931). These islands lie in the Pacific Ocean c. 390 km off the Baja California coast and have two endemic species of *Erigeron* (Nesom, 1990a). Nesom (1989, 2000) indicated that the reduced number of disc florets and small achenes found in *Darwiniothamnus* also occur in two groups of Caribbean and primarily Mexican *Erigeron*, and suggested that the closest relationship of *Darwiniothamnus* lay with these species.

In our study we used nucleotide sequences of the internal transcribed spacer (ITS) region of the nuclear ribosomal DNA to perform phylogenetic reconstructions for *Darwiniothamnus*. This molecular marker has proved its phylogenetic utility within *Erigeron* (Noyes, 2000). Although Noyes did not include *Darwiniothamnus* and the majority of its putative relatives, his study revealed six major phylogenetic groups within *Erigeron*, with the majority of the South American species forming a distinct clade. In addition, this study supports the hypothesis of a North American origin for *Erigeron*.

The major aims of our study were: (1) to investigate if the two growth forms exhibited in *Darwiniothamnus* result from a single introduction followed by insular speciation or from two independent introductions, and thereby to test the monophyly of *Darwiniothamnus*, (2) to identify the closest relative(s) of *Darwiniothamnus*, and (3) to infer additional biogeographical links between the Galápagos archipelago and continental source areas.

MATERIALS AND METHODS

Plant material

We retrieved 29 ITS sequences of the nuclear ribosomal DNA from GenBank: five of them were previously published by Noyes & Rieseberg (1999), 21 were developed by Noyes (2000), and the remaining three were developed by Urbatsch *et al.* (2003). Seventeen of the ITS sequences presented in this study were developed by us (Table 1). The ingroup had 44 species and we sampled across the six groups within *Erigeron* that were identified by Noyes (2000). We included six accessions for the three recognized species of *Darwiniothamnus*. Importantly, the ingroup had ITS sequences from all the species that previous studies have considered as putative close relatives of *Darwiniothamnus*, including *E. fasciculatus* and *E. luxurians* from mainland Chile, *E. fernandezianus* from Juan Fernández, *E. socorrensis* from Revillagigedo, four Caribbean Basin species of *Erigeron* with very small capitula (i.e. *E. bellidiastroides* Griseb., *E. bellioides* DC., *E. cuneifolius* DC. and *E. thrincioides* Griseb.), and two larger-headed but closely related species

primarily from Mexico–Central America and the USA–Mexico–Gulf of Mexico region (*E. longipes* DC. and *E. procumbens* (Mill.) G. L. Nesom, respectively). The outgroup comprised *Chrysopsis* (Nutt.) Elliott and *Heterotheca* Cass.; these two genera are in the same tribe (Astereae) as *Erigeron* but belong to a different subtribe, Chrysopsidinae (Nesom, 1990b), and also were selected as the outgroup in a previous molecular phylogenetic study of *Erigeron* (Noyes, 2000).

DNA extraction, polymerase chain reaction amplification, and sequencing

Total DNA was isolated from herbarium specimens or silica-gel-dried material using the DNeasy Plant Mini Kit (Qiagen, Inc., Valencia, CA, USA) followed by an extra purification with the GeneClean Kit (Bio 101, Vista, CA, USA). The entire ITS1–5.8S–ITS2 region was amplified by polymerase chain reaction (PCR) following the protocols of Francisco-Ortega *et al.* (1999). Sequences were obtained for both strands using the procedures outlined by Andrus *et al.* (2004). Cycle sequencing products were separated in the DNA Core Facility of Florida International University (FIU).

Data analysis

Sequences obtained by us were assembled and edited using Sequencher version 3.2 (Gene Codes Inc., Ann Arbor, MI, USA). All the sequences available for the study were aligned using Clustal X (Thompson *et al.*, 1997). The aligned data matrix was adjusted visually using Se-Al (Rambaut, 1995). Phylogenetic parsimony analysis was conducted by means of Fitch parsimony (equal weights, unordered; Fitch, 1971) using PAUP* (Swofford, 2002). Heuristic searches with 1000 random addition replicates were made, to look for multiple optimal tree islands (Maddison, 1991). These searches were performed with the tree bisection–reconnection (TBR) and multiple parsimony (MULPARS) options. Phylogenetic support for each clade was assessed by bootstrap analysis (Felsenstein, 1985) of 100,000 replicates with one random sequence addition per replicate; however, owing to computer memory limitations, 100 trees were saved per replicate. Both the consistency index (CI; Kluge & Farris, 1969) and the retention index (RI; Farris, 1989) were also calculated using PAUP*.

Constraint analysis was performed with the six accessions of *Darwiniothamnus*, to test the hypothesis that the genus is monophyletic. Search strategies for these additional parsimony analyses were the same as described above. The Kishino–Hasegawa test was performed, as implemented in PAUP*, to generate a significance value for this hypothesis (Kishino & Hasegawa, 1989).

Phylogenetic reconstructions based on Bayesian analysis (Huelsenbeck *et al.*, 2001) were performed with the program MrBayes version 3.1.2 (Ronquist & Huelsenbeck, 2003). The model of nucleotide evolution was assessed with ModelTest version 3.7 (Posada & Crandall, 1998) with the Akaike information criterion (Akaike, 1974) to select the best-fit

Table 1 Plant material included in the phylogenetic study, geographical distribution and voucher location (herbaria indicated in parentheses, institutions coded following Holmgren & Holmgren (1998), GenBank numbers included).

Taxon	Distribution	Voucher	GenBank
<i>Aphanostephus skirrhobasis</i> (DC.) Trel.	USA, NE Mexico	A. Brant 1974 (MO)	AF118512*
<i>Conyza bonariensis</i> (L.) Cronquist	South America, adventive elsewhere	R.D. Noyes 1182 (IND)	AF118513*
<i>Conyza canadensis</i> (L.) Cronquist	North America, adventive elsewhere	C. Ochs 248 (MO)	AF046987†
<i>Darwiniothamnus alternifolius</i> Lawesson & Adersen	Galápagos Islands	Isabela Island, N. Andrus 16.7/ND (FTG)	AF511573
<i>Darwiniothamnus alternifolius</i>	Galápagos Islands	Isabela Island, N. Andrus 16.9/ND (FTG)	AF511575
<i>Darwiniothamnus lancifolius</i> (Hook. f.) Harling subsp. <i>glandulosus</i> Harling	Galápagos Islands	Isabela Island, N. Andrus 7.1/3.1 (FTG)	AF511574
<i>Darwiniothamnus lancifolius</i> subsp. <i>glabriusculus</i> (A. Stewart) Lawesson & Adersen	Galápagos Islands	Isabela Island, N. Andrus 10.2/67.2 (FTG)	AF511577
<i>Darwiniothamnus tenuifolius</i> (Hook. f.) Harling	Galápagos Islands	Pinta Island, N. Andrus 5.1/81.1 (FTG)	AF511576
<i>Darwiniothamnus tenuifolius</i>	Galápagos Islands	Santiago Island, A. Tye 4/25/00 (CDS)	AF511578
<i>Erigeron arenarioides</i> (D. C. Eaton) Rydb.	W USA	J.S. Tuhy 3619 (MO)	AF118528*
<i>Erigeron argentatus</i> A. Gray	W USA	M.A. Franklin 7062 (MO)	AF118506*
<i>Erigeron bellidiastroides</i> Griseb.	Cuba	Pinar del Río, A. Urquiola <i>et al.</i> Junio 2001 (HPPR)	AF511580
<i>Erigeron bellidiastrum</i> Nutt.	W USA	Karaman 8 (LSU)	AF477644†
<i>Erigeron bellioides</i> DC.	Cuba, Hispaniola, Puerto Rico	C. Taylor 11705 (MO)	AF118522*
<i>Erigeron coronarius</i> E. Greene	W Mexico	R. M. King 9896 (MO)	AF118520*
<i>Erigeron cuneifolius</i> DC.	Greater Antilles, widely adventive elsewhere	Isla de La Juventud 12 Octubre 2000 (HAC)	AF511583
<i>Erigeron dissectus</i> Urb.	Hispaniola	Los Cacaos, San Cristobal, Dominican Republic, Jiménez, Peguero & Francisco-Ortega 3/01 (JBSD)	AF511581
<i>Erigeron dryophyllus</i> A. Gray	NE Mexico	G.B. Hinton 136 (MO)	AF118524*
<i>Erigeron ecuadoriensis</i> Hieron.	Colombia, Ecuador	Ximena Aguirre s.n. (QCNE)	AF511584
<i>Erigeron fasciculatus</i> Colla	Chile	Los Vilos, C. Maticorena <i>et al.</i> 9788 (CONC)	EU486856
<i>Erigeron fernandezianus</i> (Colla) Solbrig	Juan Fernández Islands	T. Stuessy 11441 (OS)	AF118515*
<i>Erigeron grandiflorus</i> Hook.	W USA, Canada	R. Hartman 55581 (RM)	AF118494*
<i>Erigeron jamaicensis</i> L.	Greater Antilles	Cordillera Central, Dominican Republic, Zanoni <i>et al.</i> 477598 (JBSD)	EU486857
<i>Erigeron lepidopodus</i> (B. L. Robins. & Fernald) G. L. Nesom	NW Mexico	R. Spellenberg 9185 (NMC)	AF118545*
<i>Erigeron longipes</i> DC.	Mexico, Central America	G. Carmona 800 (GCD)	AF511579
<i>Erigeron luxurians</i> (Skottsb.) Solbrig	Chile	P. Jorge (S)	AF511582
<i>Erigeron maximus</i> DC.	Brazil	R. Wasum 8044 (MO)	AF118509*
<i>Erigeron philadelphicus</i> L.	E USA, adventive elsewhere	R.D. Noyes 1165 (IND)	AF046989†
<i>Erigeron pinnatus</i> Turcz.	Ecuador, Peru	B. Lojtnant 13865 (MO)	AF118517*
<i>Erigeron pinnatisectus</i> (A. Gray) A. Nelson	W USA	R.D. Noyes 1146 (IND)	AF118501*
<i>Erigeron podophyllus</i> G. L. Nesom	NW Mexico	G. Nesom 5438 (MO)	AF118542*
<i>Erigeron procumbens</i> (Mill.) G. L. Nesom	SE USA, E Mexico	Westphal 2121 (LSU)	AF477645‡
<i>Erigeron pygmaeus</i> (A. Gray) Greene	W USA	K. Ake 215 (MO)	AF118526*
<i>Erigeron quercifolius</i> Lam.	SE USA, Bahamas	S. Hill 17962 (MO)	AF11825*
<i>Erigeron rosulatus</i> Wedd.	Bolivia, Peru	D.N. Smith 9460 (MO)	AF118512*
<i>Erigeron socorrensis</i> Brandegeee	Revillagigedo Islands	R. Morán 25455 (LL)	AF511587
<i>Erigeron stanfordii</i> G. L. Nesom	NE Mexico	Tamaulipas, M. Martínez 1725 (LL)	AF511585
<i>Erigeron subtrinervis</i> Porter & Britton	W USA	Karaman 29 (LSU)	AF477647‡
<i>Erigeron tenuis</i> Torr. & A. Gray	SE USA	G. Nesom 7224 (MO)	AF118488*
<i>Erigeron thrincoides</i> Griseb.	Cuba	Pinar del Río, R. Oviedo & E. Gamboa 8/18/00 (HAC)	AF511586
<i>Erigeron tweedyi</i> Canby	W USA	J. Grimes 2243 (MO)	AF118529*

Table 1 Continued.

Taxon	Distribution	Voucher	GenBank
<i>Erigeron uniflorus</i> L.	Alaska, Canada, Greenland, Scandinavia	R.D. Noyes 1190 (IND)	AF046988†
<i>Erigeron ursinus</i> D.C. Eaton	W USA	L.M. Schultz 3711 (MO)	AF118491*
<i>Erigeron veracruzensis</i> G. L. Nesom	NE Mexico	G. Nesom 5945 (MO)	AF118523*
Outgroup:			
<i>Chrysopsis gossypina</i> (Michx.) Elliott	SE USA	M. Merello 416 (MO)	AF046993†
<i>Heterotheca villosa</i> (Pursh) Shinnars	W USA	B. Stein 416 (MO)	AF046994†

Geographical distribution compiled from Cronquist (1947), Liogier (1962, 1996), Correll & Correll (1982), Noyes (2000) and Urbatsch *et al.* (2003).

*Sequences published by Noyes (2000).

†Sequences published by Noyes & Rieseberg (1999).

‡Sequences published by Urbatsch *et al.* (2003).

substitution model. Two separate runs with four simultaneous Markov chains each were computed for 1,000,000 generations. In each run, trees were sampled every 100 generations with a burn-in value of 2500. Chain convergence was assessed by looking at the values of the Potential Scale Reduction Factor (PSRF) (Gelman & Rubin, 1992). These values reached 1.0 before 200,000 iterations for each of the two runs. Trees found before PSRF stabilized were discarded, and the remaining trees were used to obtain a 50% majority rule consensus tree.

RESULTS

Parsimony analyses

The final aligned data matrix had 651 characters and included 62 gaps. There were 252 variable characters, and 143 of them (57%) were parsimony-informative. One hundred and sixty equally most-parsimonious trees of 521 steps each (CI = 0.630; RI = 0.690) were obtained after these analyses. The strict consensus tree does not support the monophyly of *Darwiniothamnus* (Fig. 3). The two accessions of *D. alternifolius* formed a clade together with two species from the Coquimbo–Valparaíso region (Chile), *E. luxurians* and *E. fasciculatus* (Fig. 4). This clade was nested within a lineage composed of five additional species of *Conyza* Less. and *Erigeron* from South America (i.e. *C. bonariensis* (L.) Cronquist, *E. ecuadoriensis* Hieron., *E. maximus* DC. and *E. rosulatus* Wedd.) or the Juan Fernández Islands (i.e. *E. fernandezianus*).

The two remaining species of *Darwiniothamnus* formed a monophyletic assemblage (bootstrap support of 63%) that was sister to the Cuban endemic *E. bellidiastroides*, supported by a bootstrap value of 85% (Fig. 4). This clade was sister to a lineage with five species, two of them restricted to the Caribbean Islands (i.e. *E. bellioides* and *E. thrincioides*). *Erigeron cuneifolius* also belongs to this lineage; this species was originally an endemic to the West Indies, although it has become a pantropical weed in historical times. This lineage was nested within a monophyletic assemblage that also included the distinctive genus *Aphanostephus* DC. and six additional North American–Antillean species of *Erigeron*. The 16 species

belonging to this assemblage are herbaceous, with the exception of the two species of *Darwiniothamnus*.

The analysis constraining *Darwiniothamnus* as monophyletic resulted in trees that are 21 steps longer. The results of the Kishino–Hasegawa test ($P < 0.0001$) caused us to reject the hypothesis of *Darwiniothamnus* monophyly.

Bayesian analyses

The best-fitting model of nucleotide evolution was GTR+I+G with a gamma-distribution shape parameter of 0.7783, with $R[A-C] = 1.75$, $R[A-G] = 2.03$, $R[A-T] = 2.71$, $R[C-G] = 0.623$, $R[C-T] = 5.12$ and $R[G-T] = 1.0$, and with base frequencies set to 0.25, 0.22, 0.25 and 0.28 for A, C, G and T, respectively. The resulting tree from the Bayesian analysis resolved four of the polytomies detected in the parsimony strict consensus (Fig. 3). In addition, clade credibility measured as posterior probability was much higher than bootstrap support values from parsimony; indeed, 28 of the 39 nodes had posterior probability values higher than 90%. This tree was identical to one of the most parsimonious trees yielded by the parsimony analysis. The sister relationship between *D. alternifolius* and the two species of *Erigeron* from Chile received a posterior probability value of 92%. In addition, the clade composed of the remaining species of *Darwiniothamnus* and *E. bellidiastroides* had 100% credibility. The group composed almost exclusively of South American species was supported by a posterior probability value of 92%. Likewise, the clade that had *D. lancifolius*, *D. tenuifolius*, *E. longipes* DC. and five additional Caribbean endemics had 92% credibility.

DISCUSSION

Morphological evolution and phylogenetics

The ITS phylogeny partially supports previous taxonomic hypotheses concerning the placement of *Darwiniothamnus* within *Erigeron*, although the Galápagos genus is polyphyletic whereas *Erigeron* is paraphyletic. Clearly, given its polyphyly, a



Figure 3 One of the 160 most parsimonious trees from the analysis of ITS nucleotide sequence data illustrating *Darwiniothamnus* Harling as a polyphyletic genus (521 steps, CI = 0.630; RI = 0.690). This tree was identical to the 50% majority rule consensus tree inferred from the Bayesian analysis. Branches that collapse in the strict consensus tree yielded by the parsimony analyses are shown with solid diamonds. Bootstrap values (> 50%) from parsimony analyses are below branches; clade credibility values from Bayesian analysis are above branches. The number of steps along each branch is indicated in italics.

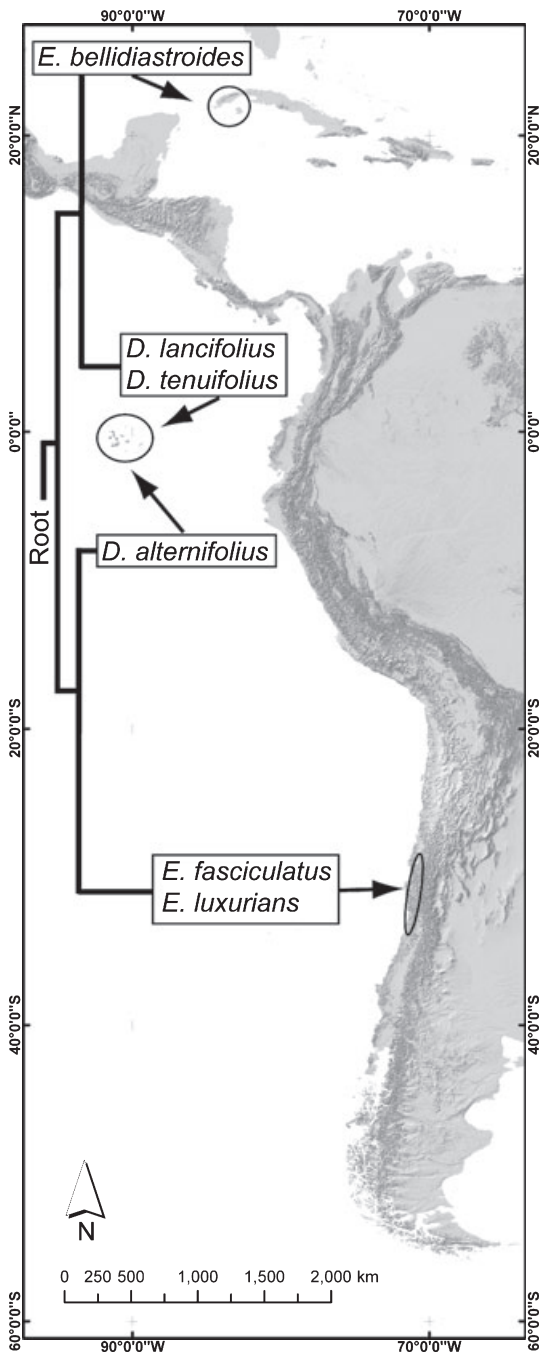


Figure 4 Geographic distributions and phylogenetic relationships of species of *Darwiniothamnus* and their allied *Erigeron* species. *Erigeron fasciculatus* and *E. luxurians* are endemic to the Coquimbo–Valparaiso region (Chile), whereas *E. bellidiastroides* is restricted to the Pinar del Rio and Isla de la Juventud provinces (Cuba).

recognition of *Darwiniothamnus* as a distinct taxonomic entity for the three traditional species is unwarranted.

Our study provides additional support for the existence of a clade composed of species from South America previously identified by Noyes (2000). Importantly, *D. alternifolius* belongs to this clade, and the two Chilean species

(*E. fasciculatus* and *E. luxurians*) that are sister to *D. alternifolius* also are perennials with a woody base. These two South American species were suggested by Harling (1962) to be close relatives of the other two species of *Darwiniothamnus*, although our results suggest that this is not the case.

Erigeron has c. 22 species native or endemic to the Caribbean Islands (including the widespread, weedy *E. cuneifolius*), and preliminary morphological data suggest that 13 of them should be placed together in an endemic infrageneric taxon, *Erigeron* sect. *Microcephalum* G. L. Nesom. Eight of the other Caribbean Island endemics belong to *Erigeron* sect. *Quercifolium* G. L. Nesom, which is confined to North America and the Caribbean Islands and has 17 species. *Erigeron* sect. *Lamprocaules* G. L. Nesom is the third morphological group represented by Caribbean Island endemism: the Hispaniolan *E. tuerckheimii* Urb. is placed in this section, which otherwise is Mexican. We studied six species of *Erigeron* sect. *Quercifolium*, which formed a clade with a posterior probability of 83%. The four species of *Erigeron* sect. *Microcephalum* included in our study (i.e. *E. bellidiastroides*, *E. bellioides*, *E. cuneifolius* and *E. thrincioides*) formed a monophyletic group that also contained two North American species of *Erigeron* (i.e. *E. longipes* and *E. procumbens* from *Erigeron* sect. *Cincinnatiactis* G. L. Nesom, which includes three or perhaps four other species, all from Mexico), plus *D. lancifolius* and *D. tenuifolius*. All species of sect. *Microcephalum* and sect. *Cincinnatiactis* have toothed leaves, nodding buds, filiform rays tightly coiling at maturity, and small, oblong achenes with prominent orange ribs; all except one (a taprooted annual) are herbaceous perennials from short, fibrous-rooted rhizomes. Similarities in ray florets and achene nervation led Nesom (1989, 2000) to suggest that the two large woody species of *Darwiniothamnus* were closely related to these species. Both the morphological data and our ITS topology support this hypothesis. *Darwiniothamnus alternifolius*, however, does not have the tightly coiling ray florets and possesses a suite of features absent in the two other species of *Darwiniothamnus*.

It is noteworthy that, even though the ITS topology shows *E. bellidiastroides* to be sister to two species of *Darwiniothamnus*, this Cuban taxon is confined to the western provinces of Pinar del Rio and the Island of Youth, and clearly is an unexceptional member of *Erigeron* sect. *Microcephalum*. Our study did not sample all the species of *Erigeron* belonging to the sections *Microcephalum* (Caribbean Islands) and *Cincinnatiactis* (primarily Mexican and Central American), and future studies with more extensive sampling may further clarify whether the immediate ancestral relationships of the large woody species of *Darwiniothamnus* are with groups from the Caribbean Islands or from continental North America.

Our results therefore suggest that the woody and shrubby habit found in *D. lancifolius* and *D. tenuifolius* might have originated on the islands following long-distance dispersal of a herbaceous ancestor. This pattern is well known for many of the woody species found in insular environments worldwide, which originated *in situ* from ancestors that were annuals or perennial herbs (Givnish, 1998). Within the Galápagos

Asteraceae, shifts from continental herbaceous ancestors to endemic taxa with woody habit seem to have occurred in *Lecocarpus* Decne. (Asteraceae) and *Macraea* Hook. f. (Asteraceae) (Panero *et al.*, 1999b; Rauscher, 2002).

The perennial herbaceous species *D. alternifolius* belongs to a clade of South American taxa that has only one species with a shrubby habit and extensive wood development: *E. fernandezianus* from the Juan Fernández Islands. *Darwiniothamnus alternifolius* shares the habit of its sister species of the South American mainland, *E. luxurians* and *E. fasciculatus*, which also are perennial herbs with a woody base. Two of the other species in this clade also are perennial herbs, but they are high-elevation species with little or no wood development (*E. rosulatus* and *E. ecuadoriensis*). This clade also includes an annual species, *Conyza bonariensis*. An optimization of these four habit stages (i.e. annual herbaceous, perennial herbaceous without wood development, perennial herbaceous with wood development, and woody shrub) along the topology obtained after the Bayesian analyses using the parsimony method implemented in MacClade (Maddison & Maddison, 2000) suggested that the immediate ancestor of *D. alternifolius* was a herbaceous taxon with woody development at the base (data not shown). The ITS topology shows that *D. alternifolius* belongs to a lineage that does not have other representatives in the Galápagos Islands; clearly this is an isolated taxon and should be of high conservation priority.

Thirty-three of the native angiosperm plant genera found in the Galápagos Islands have more than one endemic species each (c. 34% of the genera with endemic species), and they represent putative examples of speciation through adaptive radiation following a single introduction. However, molecular data suggest that at least the two endemic species of *Cuscuta* L. (Convolvulaceae) are the result of two independent introductions (Stefanovic *et al.*, 2007). ‘*Darwiniothamnus*’ and *Opuntia* Mill. (Cactaceae) are the only Galápagos genera having endemic species within which a divergent growth habit has evolved. This pattern, although unusual in oceanic islands, has also been reported for Macaronesian island endemics belonging to the *Aeonium* Webb & Berthel. alliance (Crassulaceae), *Convolvulus* L. (Convolvulaceae), *Echium* L. (Boraginaceae), and the woody *Sonchus* alliance (reviewed by Panero *et al.*, 1999a). It also occurs in the Hawaiian archipelago for the endemic genus *Schiedea* Cham. & Schtldl. (Caryophyllaceae) (Wagner *et al.*, 2005). These radiative groups have both herbaceous and woody species; molecular phylogenies suggest that the herbaceous condition is plesiomorphic, and therefore that the woody shrub habit also evolved *in situ* on these islands.

Biogeographical implications

The molecular phylogeny of *Darwiniothamnus* provides valuable insights into the origin of the Galápagos endemics. Panbiogeographical studies for the Galápagos biota conducted by Grehan (2001a,b) suggested the existence of an ‘East Pacific’ track for the archipelago. This track connects the Galápagos

with low-elevation areas of Chile, Ecuador and Peru. Indeed, Porter (1984a,b) indicated that c. 56% of the endemic plants have their origin in western South America. The closest geographical sources for the Galápagos are found in the arid coastal and Andean regions of Ecuador. The phylogenetic relationship detected for *D. alternifolius* provides additional support for an ‘East Pacific’ biogeographical track for the Galápagos. The closest relatives of this species are not found in Ecuador but occur in the Coquimbo–Valparaíso region of Chile.

In contrast, *D. lancifolius* and *D. tenuifolius* are sister to the Cuban endemic *E. bellidistroides*. Strong links between the floras of the Galápagos and Caribbean were suggested early on by Hooker (1847). Indeed, Takhtajan (1986) in his account of global plant biogeography considered the Galápagos together with coastal areas of Colombia and Ecuador to be part of a ‘Caribbean Region’. Likewise, Croizat (1958) proposed a biogeographical track joining the archipelago with the Caribbean Islands and Central America. Molecular phylogenies for Galápagos vertebrates provide additional support for associations between the Galápagos and the Northern Hemisphere. Historical biogeographical analyses for Darwin’s finches strongly support a Caribbean link (Burns *et al.*, 2002). In addition, a DNA phylogeny for the endemic species of iguana support a monophyletic group that is sister to a clade of two genera restricted to Central America (Rassmann, 1997). The direct connection between the Galápagos and the Caribbean Islands found for *Darwiniothamnus* has also been reported in other studies. For instance, Grehan (2001a,b) found such direct links for isopods (*Nesophilosia* (Galápagos) and *Trogophilosia* (Cuba)), snakes (*Antillophis* in Cuba, Hispaniola and Galápagos) and marine sponges (*Rhabderemia destituta* (Galápagos) and *R. mona* (Puerto Rico)). More recently, Arbogast *et al.* (2006) found that the Galápagos mockingbird genus *Nesomimus* is sister to *Mimus gundlachi*, a species restricted to Jamaica.

Seamounts along the Carnegie and Cocos ridges are associated with the Galápagos Geological Hotspot (O’Connor *et al.*, 2007). The Carnegie Ridge runs from the east of the archipelago towards the coast of Ecuador, and the Cocos Ridge joins the north-western section of the Galápagos platform to oceanic areas near Costa Rica. These seamounts are much older than the present Galápagos archipelago (Christie *et al.*, 1992; Werner *et al.*, 1999; Harpp *et al.*, 2005; O’Connor *et al.*, 2007) and were probably above sea level long before the existence of the current Galápagos Islands (Christie *et al.*, 1992; Werner *et al.*, 1999). Radiometric dating suggests that islands have occupied the Cocos Ridge during at least the last 15 Myr (Werner *et al.*, 1999; O’Connor *et al.*, 2007). However, the hotspot has been active for the last 90 Myr, and it is likely that the region has had oceanic islands since the Turonian–Coniacian in the late Cretaceous.

Several authors (e.g. Christie *et al.*, 1992; Collins *et al.*, 1996; Rassmann, 1997; Werner *et al.*, 1999) have suggested that the existence of these drowned islands has major biogeographical implications for the region. First, it is likely that an important

Table 2 Plant molecular phylogenies relevant for biogeographical relationships between the Galápagos Islands and the continent.

Galapagos taxon*	Sister taxon/clade†	Distribution of sister taxon/clade and notes	Reference
<i>Cuscuta acuta</i> Engelm. (Convolvulaceae)	<i>C. umbellata</i> Kunth var. <i>reflexa</i> Yunck. (175/96)‡	North America	Stefanovic <i>et al.</i> (2007)
<i>C. gymnocarpa</i> Engelm.	<i>C. campestris</i> Yunck (175/96)‡	North America	Stefanovic <i>et al.</i> (2007)
<i>Encelia hispida</i> Anderss. (Asteraceae)	<i>E. canescens</i> Lam. (19/22)	South America	Fehlberg & Ranker (2007)
<i>Euphorbia equisetiformis</i> Stewart (Euphorbiaceae)	A clade composed of <i>E. aaron-rossii</i> A. H. Holmgren & N. H. Holmgren, <i>E. bicolor</i> Engelm. & A. Gray, <i>E. cassythoides</i> Boiss., <i>E. innocua</i> L. C. Wheeler, and <i>E. ipecacuanhae</i> L. (192/2,000)§	West Indies and North America	Steinmann & Porter (2002)
<i>Gossypium klotzschianum</i> Anderss. (Malvaceae)	<i>G. davidsonii</i> Kellogg (13/13)¶	North America	Álvarez <i>et al.</i> (2005)
<i>Lecocarpus lecocarpoides</i> (B. L. Rob. & Greenm.) Cronquist & Stuessy (Asteraceae) (3)††	Polytomy with <i>Acanthospermum australe</i> (Loefl.) Kuntze and <i>A. microcarpum</i> B. L. Rob	Both species are from South America but <i>Acanthospermum</i> Schrank (six species) occurs from Central to South America	Rauscher (2002)
<i>Lycopersicon cheesmanii</i> Riley (Solanaceae)	Polytomy with <i>L. esculentum</i> Mill., <i>L. peruvianum</i> (L.) Mill., and <i>L. pimpinellifolium</i> Mill. (9/9)	South America	Peralta & Spooner (2001)
<i>Macraea laricifolia</i> Hook. (Asteraceae) (1)††	<i>Sphagneticola trilobata</i> (L.) Pruski	Pantropical	Panero <i>et al.</i> (1999b)
<i>Nolana galapagensis</i> (Christoph.) I. M. Johnst. (Solanaceae)	<i>N. arenicola</i> I. M. Johnst. (63/89)	South America	Dillon <i>et al.</i> (2007)
<i>Phaseolus mollis</i> Hook. f. (Fabaceae)	Nested in a clade with six accessions of <i>P. lunatus</i> L. (49/50)	North, Central and South America	Delgado-Salinas <i>et al.</i> (1999)
<i>Polygala galapageia</i> Hook. f. (Polygalaceae)	Forms a clade with the Galápagos endemic <i>P. sancti-georgii</i> , this assemblage is sister to <i>P. paniculata</i> L. (47/300)**	West Indies and North, Central and South America	Forest <i>et al.</i> (2007)
<i>P. sancti-georgii</i> Riley	See entry for <i>P. galapageia</i>		
<i>Scalesia pendulata</i> Hook. f. (Asteraceae) (15)††	A clade with eight species of <i>Pappobolus</i> S. F. Blake	South America	Schilling <i>et al.</i> (1994)
<i>Tiquilia darwinii</i> (Hook. f.) A. T. Richardson (Boraginaceae)	Resulting phylogenies have ambiguous sister relationships with <i>T. cuspidata</i> (I. M. Johnst.) A. T. Richardson, and <i>T. paronychioides</i> (Phil.) A. Richardson. The three Galápagos endemics appear to form a monophyletic group (27/30)	<i>T. cuspidata</i> (North America), <i>T. paronychioides</i> (South America)	Moore & Jansen (2006); Moore <i>et al.</i> (2006)
<i>T. galapagoa</i> (Howell) A. T. Richardson	See entry for <i>T. darwinii</i>		
<i>T. nesiotica</i> (Howell) A. T. Richardson	See entry for <i>T. darwinii</i>		

The number of species belonging to endemic genera are indicated in parentheses.

*Figures in parentheses indicate the approximate number of species in the endemic genus.

†Figures in parentheses indicate the approximate number of species in the non-endemic genus included in the phylogeny/total number of species in the genus.

‡Study included representatives of all sections and subsections of *Cuscuta* L. subgenus *Grammica* (Lour.) Yunck. This subgenus has c. 150 species.

§Study included representatives of all sections of *Euphorbia* L.

¶Study included representatives of all species of *Gossypium* L. subgenus *Houzingenia* Fryxell.

**Study included representatives of all sections of *Polygala* L.

††Genera endemic to the Galápagos Islands.

component of the Galápagos biota pre-dates the present islands and speciated on some of these seamounts before they subsided below sea level. Phylogenetic studies of the endemic iguanas support this (Rassmann, 1997). Second, it is possible that these ancient islands facilitated dispersal to the Galápagos from Central America and North America. Cocos-Carnegie Ridge islands existed prior to the Late Pliocene, long before the closure of the Panama Isthmus, and therefore it is likely that they acted as stepping stones from the proto-Greater Antilles (Iturralde-Vinent, 2006). Such dispersal could then have been facilitated by the oceanic palaeocurrent known as the Circum-tropical Current, which, until the formation of the Panama Isthmus, ran through the Tropical American Seaway from the Proto-Caribbean Sea towards the Pacific Ocean (Collins, 1996; Collins *et al.*, 1996; Droxler *et al.*, 1998).

A likelihood-ratio test (Felsenstein, 1981) did not support a molecular clock for the ITS sequences included in our study (data not shown); in addition, we can find no evidence of a fossil record for *Erigeron* or *Conyza*. Therefore, methods to date relevant nodes would require the use of ITS substitution rates from other taxa, which would not provide an accurate estimate of divergence times for *Darwiniothamnus* and its closest continental relatives. We thus decided not to date speciation events in our study.

However, the phylogenetic patterns of *Darwiniothamnus* suggest relatively recent origins for the genus. Clearly, the ITS topology does not place the three species of *Darwiniothamnus* in any of the early branches; indeed, they belong to some of the most recently divergent lineages. These results indicate that long-distance dispersal after the closure of the Panama Isthmus (c. 3 Ma) may account for the origin of the two *Darwiniothamnus* species that are sister to the Cuban *E. bellidiastroides*. Unfortunately, the history of the seamounts found along the Cocos and Carnegie ridges is poorly understood, and it is unknown how recently these ancient islands subsided below sea level. Therefore, it remains uncertain if any of these palaeo-islands might have hosted ancestral forms of *Darwiniothamnus* prior to their arrival to the current islands.

Our results suggest that some of the connections between the archipelago and the mainland can be explained by recent long-distance dispersal events without the involvement of the Circumtropical Current or the drowned seamounts along the Cocos and Carnegie ridges. We anticipate that future studies of Galápagos plants focusing on divergence ages from ancestral forms will find that a significant proportion of the endemic flora is relatively recent and does not pre-date the current Galápagos Islands.

Some floristic studies have suggested affinities between the floras of the Galápagos and Revillagigedo islands (Johnston, 1931; Svenson, 1946), and even that the Revillagigedo Islands are those with the closest phytogeographic links with the Galápagos (Svenson, 1946). However, our study does not support Johnston (1931), who suggested that *Darwiniothamnus* (then included with *Erigeron*) represents an example of these floristic affinities. The Revillagigedo endemic *E. socorr-ensis* is sister in the molecular analysis to the North American

native (and widespread weedy) species *Conyza canadensis* (L.) Cronquist and belongs to neither of the two major clades in which the *Darwiniothamnus* species are placed. Previous studies have alternatively indicated that *Darwiniothamnus* represents a floristic association between the Galápagos and Juan Fernández archipelagos. Our study partially supports this suggestion, as *D. alternifolius* and the Juan Fernández endemic *E. fernandezianus* belong to the same major clade. However, there is not yet any strong evidence demonstrating a direct affinity of the Galápagos flora with that of the Juan Fernández archipelago.

With the inclusion of data from our *Darwiniothamnus* study, molecular phylogenies (pertinent for the origin of the endemic flora) for a total of 37 endemic species (21% of the endemic flora) (Table 2) provide some preliminary insights concerning the origin of the flora, confirming that the endemic flora of the Galápagos has closest relatives in both the Northern and Southern Hemispheres.

Two of the endemic genera, *Lecocarpus* and *Scalesia*, are sister to or belong to clades restricted primarily to South America (Panero *et al.*, 1999a), whereas *Macraea* is sister to a widespread genus and therefore it is unclear where its closest affinities lie. Among the non-endemic genera, Galápagos species of *Encelia* Adans. (Asteraceae), *Lycopersicon* Mill. (Solanaceae) and *Nolana* L. f. (Solanaceae) are sister to South American species (Peralta & Spooner, 2001; Dillon *et al.*, 2007; Fehlberg & Ranker, 2007), whereas the four endemic species in *Cuscuta*, *Euphorbia* L. (Euphorbiaceae) and *Gossypium* L. (Euphorbiaceae) have their sister species in North America or the West Indies (Steinmann & Porter, 2002; Álvarez *et al.*, 2005; Stefanovic *et al.*, 2007). The six endemics in *Phaseolus* L. (Fabaceae), *Polygala* L. (Polygalaceae) and *Tiquilia* Pers. (Boraginaceae) have sister taxa with a widespread continental distribution, or their molecular phylogenies yielded equivocal results (Delgado-Salinas *et al.*, 1999; Moore & Jansen, 2006; Moore *et al.*, 2006; Forest *et al.*, 2007).

The Galápagos Islands are located at the boundary between the Nazca and Cocos plates, and the two clades of *Darwiniothamnus* show biogeographical affinities that reflect this. *Darwiniothamnus alternifolius*, a species confined to the southwestern part of the archipelago, forms a lineage that links the Nazca plate with the biodiversity-rich area of the Coquimbo–Valparaíso region in Chile. In contrast, the two remaining species of the genus that are restricted to the western islands link the Cocos plate with the Caribbean Islands.

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BIOSKETCH

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