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Biogeographic diversification in *Nolana* (Solanaceae), a ubiquitous member of the Atacama and Peruvian Deserts along the western coast of South America

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Abstract The present paper reconstructs the biogeographic diversification for *Nolana* L.f. (Solanaceae), a genus of 89 endemic species largely restricted to fog-dependent desert *lomas* formations of coastal Peru and Chile. Previous efforts have reconstructed a phylogenetic estimate for *Nolana* using a combination of molecular markers. Herein, we expand on those results to examine hypotheses of biogeographic origins and diversification patterns. *Nolana* occupies habitats within a continuous coastal desert and forms a terrestrial archipelago of discrete "islands" unique in size, topography, and species composition. Each locality contains at least one *Nolana* species and many contain multiple species in sympatry. The genus has a Chilean origin, with the basal clades confined to Chile with wide geographic and ecological distributions. Peru contains two strongly supported clades, suggesting two introductions with subsequent radiation. A Chilean clade of shrubby, small-flowered species appears to have had its origins from the same ancestors of the second line that radiated in Peru and northern Chile. *Nolana galapagensis* is endemic to the Islas Galápagos, with origins traced to Peruvian taxa with a divergence time of 0.35 mya. Rates of diversification over the past 4.02 mya in *Nolana*, in one of the driest habitats on Earth, suggest rapid adaptive radiation in several clades. Success in *Nolana* may be attributed to characters that confer a competitive advantage in unpredictable and water-dependent environments, such as succulent leaf anatomy and ecophysiology, and the reproductive mericarp unique to *Nolana*. The processes affecting or shaping the biota of western South America are discussed.

Key words Atacama Desert, biogeography, chloroplast DNA, *LEAFY* second intron, *Nolana*, Peruvian Desert, Solanaceae, South America.

For nearly 3500 km along the south-western coast of South America (7–28°S latitude), the Atacama and Peruvian Deserts form a continuous, hyper-arid belt, broken only by occasional river valleys from the Andean Cordillera. Although this is one of the driest places on Earth, it is home to an extraordinary type of vegetation termed "*lomas* formations" (Dillon, 1997; Dillon & Hoffmann-J, 1997; Rundel et al., 1991, 2007). These highly endemic communities occur in near-shore locations where ocean fog provides sufficient moisture for the development of plant communities. Each locality is an island of vegetation among a virtual ocean of desert, and over 100 such localities have been identified from northern Peru to north-central Chile. The size and topography of individual formations varies greatly, as does the distance between formations. Some species are not restricted to *lomas* formations and exhibit ranges throughout the desert, especially cacti and other stem and leaf succulents.

Aridity is maintained by several factors, including the South Pacific anticyclone (Trewartha, 1961), which results in a constant temperature inversion generated by the cold, north-flowing Humboldt or Peruvian current, moderate onshore temperatures, and a rain shadow created by the Andean Cordillera (Rundel et al., 2007). All of western South America is isolated from weather patterns to the east by the Andean Cordillera, which reaches an average height of over 3500 m (Garzione et al., 2008). The timing of the final uplift is debatable, but most authors cite 10 Ma as a minimum age (Hoke et al., 2007).

The result is a uniform coastal climate where rains are rare and sporadic, but there is the regular formation of thick stratus clouds below 1000 m from September

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to December that provides moisture for plant growth (Prohaska, 1973).

Although aridity is continuous between northern Peru and central Chile and prevailing climates comparable, the non-uniform distribution of species suggests strong partitioning into at least three distinct coastal regions: (i) a northern Peru region (7–13°S); (ii) a southern Peru region (13–18°S); and (iii) a northern Chilean region (20–28°S). These regions exhibit high distributional fidelity with minimal overlap in their floristic components, suggesting independent histories. Combined north and south Peru have only 93 non-introduced or native species in common with the northern Chilean region, suggesting that the hiatus between 18° and 20°S has inhibited active exchange between what is modern-day Chile and Peru. This portion of the northern Chilean coast (18–20°S) lacks the proper near-shore upland topography and/or prevailing winds to allow the development of *lomas* vegetation typical further south (Cereceda et al., 2007).

In addition to seasonal fogs, the coastal region is also influenced by periodic and recurrent El Niño Southern Oscillation (ENSO) events. The frequency of strong El Niño events today is approximately every 30–50 years, with minor events every 3.5–7 years (Quinn & Neil, 1987). Two of the strongest El Niño events ever recorded occurred in 1982/3 (Dillon & Rundel, 1990) and 1997/8 (Vidiella et al., 1999). The El Niño phenomenon is complex and a complete explanation is beyond the scope of the present paper (Allan et al., 1996) but, simply put, El Niño conditions prevail when the normally cold waters off the coast of western South America are displaced by warmer water originating in the western Pacific. These conditions stimulate brief periods of heavy rainfall and relatively high temperatures. The increase in available moisture has profound effects upon the *lomas* formations and undoubtedly shapes their composition and structure. This is manifested in the stimulation of massive blooming events that replenish seed banks for annual and perennial plants. During a non-El Niño year, the terrestrial vegetation is essentially dormant, with perennials completely deciduous. It is difficult to imagine what the coastal vegetation would resemble in the prolonged absence of El Niño events. One effect may be the reduction in levels of floristic diversity and elimination of migration and establishment within and between formations.

Data relating to the historical onset of El Niño conditions suggest that the phenomenon can be dated to between 5000 and 15 000 years ago (Fontuge et al., 1999; Rodbell et al., 1999). Longer-term records of El Niño events are more difficult to establish, but results from fossil coral suggest that El Niño-like conditions may have existed for 124 000 years (Hughen et al., 1999) or longer (Kilbourne et al., 2004). Authors have argued that the present extent of coastal aridity of the Atacama and Peruvian deserts has great antiquity (Hartley & Chong, 2002; Hartley et al., 2005; Wara et al., 2005), but has been influenced by pluvial cycles (Alpers & Brimhall, 1988; Rundel et al., 1991; McKay et al., 2003). Regardless of their age, recurrent El Niño events have surely helped shape the coastal communities present today (Dillon & Rundel, 1990; Holmgren et al., 2001).

The vascular flora of the *lomas* formations is varied in composition and structure, with 108 families, 520 genera, and approximately 1400 species of vascular plants recorded (Dillon, 2009). This includes a few pan-tropical or weedy species, a few Northern Hemisphere desert disjunctions, many more Andean disjunctions, and a complement of *lomas* endemics at the level of genus and species. The patterns recognized here fall into broad categories, with both allodisjunctions and autodisjunctions in origin (Turner, 1972). Allodisjunctions are two or more closely related populations (i.e. more closely related to each other than either is to yet some other taxon) that are widely separated spatially, the various elements of which have been derived through phyletic divergence from populations now extinct. Autodisjunctions are defined as two or more morphologically similar populations that are widely separated spatially, the more remote elements having become isolated through the dissemination of appropriate colonizers from some extant population or gene pool. The latter type of disjunction is typically a long-distance dispersal event, but vicariant events can accomplish the same distribution pattern.

Endemics can be in several *lomas* formations or restricted to a single locality. Endemism in the overall flora often exceeds 40% in individual communities and typically includes endemic genera from an array of families (Duncan & Dillon, 1991). In a detailed analysis of the flora, only *Nolana* was found throughout the range of the *lomas* formations, with 40 species in Peru, 44 species in Chile, and one oceanic island endemic, *N. galapagensis* (Christoph.) I. M. Johnst. Only four *Nolana* species have distributional records on both sides of 18°S or both in Peru and Chile. *Nolana*, with its 89 species, is the most specious genus in the flora; no other genus has 25% its diversity.

There are few species-level phylogenetic studies for taxa represented within the *lomas* formations, and fewer still with good time-calibrated molecular phylogenies. Although reported results do not point to any clear community-level pattern, it is useful to examine the putative biogeographic implications and timing. In the Boraginaceae, *Tiquilia* Pers. (Moore & Jansen, 2006),

an amphitropic inhabitant of North and South American desert environments, was postulated to have split from its extant relatives in the Paleocene or Eocene (59–48 mya). Diversification in *Heliotropium* L. (Luebert & Wen, 2008) led to the split of sect. *Cochranea*, a primarily Atacama desert group, dating from middle Miocene (approximately 14 mya). In the Fabaceae, *Hoffmannseggia* Cav. (Simpson et al., 2005) was suggested to have reached its amphitropic distribution via two separate long-distance dispersals, but the study did not explicitly date the group. In *Poissonia* Baill. (Lavin et al., 2003), the crown clade was stated to be at least 18 million years old, with the potential vicariant events involving the single *lomas* endemic to be approximately 3 million years of age. Studies in *Prosopis* L. (Catalano et al., 2008) suggested that aridity-driven diversification occurred simultaneously in North and South America between the Miocene and the Pleistocene. *Astragalus* L. (Scherson et al., 2008) was cited as one of a growing list of recent, rapid radiations, perhaps as short as 0.98 mya for a clade containing 15 desert species. In contrast, another South American clade has been dated to 1.89 mya and contains 90 species. In the Malvaceae, *Palaua* Cav. (Huertas et al., 2007), with 15–16 species, was thought to have undergone diversification between 3 and 4 mya, and it was suggested the *lomas* environment was no older than the genus.

Rapid rates of diversification are reported in lineages outside of the desert environments being discussed here. For example, the Aizoaceae from South Africa has rates of 0.77–1.45 species per million years (spp/my; Klak et al., 2004), *Gentianella* Moench from South America has rates of 1.48–1.71 spp/my (von Hagen & Kadereit, 2001), *Valeriana* L. from the Andean páramos has rates of 1.71–3.2 spp/my (Bell & Donoghue, 2005), *Lupinus* L. from the tropical high Andes has rates of 2.49–3.72 spp/my (Hughes & Eastwood, 2006), and Neo-*Astragalus* from North and South America has a rate of 1.48 spp/my (Wojciechowski et al., 1999).

1 Material and methods

1.1 Sampling and molecular procedures

Sequences in the phylogenetic reconstructions included all sequences of the *LEAFY* second intron and four chloroplast markers (*ndh*F, *trn*C–*psb*M, *trn*H–*psb*A, and *rps*16–*trn*K) in our previous study (Tu et al., 2008), as well as those from 29 newly sequenced samples representing 18 species, of which eight species were studied for the first time. As a whole, 70 species of *Nolana* plus two outgroups were included in the phylogenetic analysis. This represents 78% of all members of the genus collected over a decade of intensive exploration, especially during El Niño events. A full list of taxa and voucher information are provided in Tu et al. (2008), with a few additional samples listed here in Table 1. The distribution and range of each species was mapped using 1′ intervals of latitude and longitude and generated from a database of over 1200 georeferenced records from herbarium accessions from all major herbaria and collections by MOD et al. (i.e. B, BM, CGE, CONC, F, FI, GH, HAO, HUSA, HUT, K, M, MO, NY, SGO, UC, US, USM). The database and a large number of digitized herbarium sheets are available via web access (Dillon, 2009). Three chloroplast genes (*ndh*F, *rbc*L and *atp*B) were used to estimate the divergence time of *Nolana* in a broad phylogenetic framework, including taxa from Solanaceae and Convolvulaceae. Sequences of the three genes are newly sequenced or obtained from GenBank. Samples and GenBank accession numbers are listed in Table 2.

Methods for DNA extraction, PCR amplification, cycle sequencing and/or cloning for obtaining sequences of the *LEAFY* second intron, *ndh*F, *trn*H–*psb*A, *trn*C–*psb*M, and *rps*16–*trn*K were as described by Tu et al. (2008). The *rbc*L for dating analysis was amplified using the primer pair 26F (5′-GTGCACCACAAACAGAGACTAAAGC-3′) and 60123R (5′-TGAGTTCTTTCTCCTTTATCCTTC-3′) and the *atp*B gene was amplified using the primer pair 56292F (5′-TCAGTACACTAAGATTTAAGGTCAT-3′) and 57472R (5′-TGCTCGGAGAACCTGTTGATAA-3′). The amplification reactions for *rbc*L and *atp*B were conducted for 3 min at 95 °C, followed by 35 cycles of 30 s at 94 °C, 1 min at 50 °C, and 2 min at 72 °C, with a 10-min extension at 72 °C, ending with a 4 °C hold.

1.2 Phylogenetic analysis

For each region, sequences from all primers were edited and aligned using Sequencher v. 4.8 (Gene Codes, Ann Arbor, MI, USA). All sequences for each genomic accession were initially aligned with ClustalX version 1.83 (Thompson et al., 1997), followed by manual adjustments using the program Se-Al v2.0a11 (Rambaut, 2007).

Both parsimony and Bayesian analyses of *Nolana* were performed with the *LEAFY* second intron sequences (Fig. 1) and the combined data of four chloroplast markers (*ndh*F, *trn*H–*psb*A, *trn*C–*psb*M, and *rps*16–*trn*K; Fig. 2). The parsimony analysis was performed with PAUP 4.10b (Swofford, 2003), using a heuristic search with 100 random sequences addition replicates, tree bisection–reconnection (TBR)

Table 1 Samples and GenBank accession numbers of *Nolana* included in the phylogeny reconstruction in the present analysis not listed in Tu et al. (2008)

Species	Collection voucher (herbarium)	LEAFY	*ndh*F	*trn*H–*psb*A	*rps*16–*trn*K	*trn*C–*psb*M
N. sessiliflora	*M. Dillon 9052* (F)	FJ914094	FJ913999	FJ914065	FJ914182	FJ914036
N. onoana	*M. Dillon 9050* (F)	FJ914095	FJ914000	FJ914066	FJ914183	FJ914037
N. humifusa	*S. Leiva 4135* (HAO)	FJ914096	FJ914001	FJ914067	FJ914184	FJ914038
N. humifusa	*S. Leiva 4124* (HAO)	FJ914097	FJ914002	FJ914068	FJ914185	FJ914039
N. pallidula	*V. Quipuscoa 3413* (HUSA)	FJ914098	FJ914003	FJ914069	FJ914186	FJ914040
N. revoluta	*V. Quipuscoa 3597*(HUSA)	FJ914099	FJ914004	FJ914070	FJ914187	FJ914041
N. tovariana	*V. Quipuscoa 3580* (HUSA)	FJ914100	FJ914005	FJ914071	FJ914188	FJ914042
N. willeana	*V. Quipuscoa 3493* (HUSA)	FJ914101	FJ914006	FJ914072	FJ914189	FJ914043
N. spathulata	*V. Quipuscoa 3594* (HUSA)	FJ914102	FJ914007	FJ914073	FJ914190	FJ914044
N. stenophylla	*M. Dillon 9064* (F)	FJ914103	FJ914008	FJ914074	FJ914191	FJ914045
N. tovariana	*V. Quipuscoa 3533* (HUSA)	FJ914104	FJ914009	FJ914075	FJ914192	FJ914046
N. pallidula	*V. Quipuscoa 3430* (HUSA)	FJ914105	FJ914010	FJ914076	FJ914193	FJ914047
N. tovariana	*V. Quipuscoa 3517* (HUSA)	FJ914106	FJ914011	FJ914077	FJ914194	FJ914048
N. pilosa	*V. Quipuscoa 3411* (HUSA)	FJ914107	FJ914012	FJ914078	FJ914195	FJ914049
N. cerrateana	*V. Quipuscoa 3467* (HUSA)	FJ914108	FJ914013	FJ914079	FJ914196	FJ914050
N. tovariana	*V. Quipuscoa 3520* (HUSA)	FJ914109	FJ914014	FJ914080	FJ914197	FJ914051
N. tovariana	*V. Quipuscoa 3525* (HUSA)	FJ914110	FJ914015	FJ914081	FJ914198	FJ914052
N. arequipensis	*V. Quipuscoa 3529* (HUSA)	FJ914111	FJ914016	FJ914082	FJ914199	FJ914053
N. plicata	*V. Quipuscoa 3532* (HUSA)	FJ914112	FJ914017	FJ914083	FJ914200	FJ914054
N. coronata	*M. Dillon 8813* (F)	FJ914113	FJ914018	FJ914084	FJ914201	FJ914055
N. humifusa	*V. Quipuscoa 3396* (HUSA)	FJ914114	FJ914019	FJ914085	FJ914202	FJ914056
N. humifusa	*V. Quipuscoa 3397* (HUSA)	FJ914115	FJ914020	FJ914086	FJ914203	FJ914057
N. lycioides	*M. Weigend 8414* (F)	FJ914116	FJ914021	FJ914087	FJ914204	FJ914058
N. gracillima	*M. Weigend 8396* (F)	FJ914117	FJ914022	FJ914088	FJ914205	FJ914059
N. humifusa	*V. Quipuscoa 3396A* (HUSA)	FJ914118	FJ914023	FJ914089	FJ914206	FJ914060
N. humifusa	*V. Quipuscoa 3397* (HUSA)	FJ914119	FJ914024	FJ914090	FJ914207	FJ914061
N. spergularioides	*V. Quipuscoa 3644* (HUSA)	FJ914120	FJ914025	FJ914091	FJ914208	FJ914062
N. gracillima	*V. Quipuscoa 3693* (HUSA)	FJ914121	FJ914026	FJ914092	FJ914209	FJ914063
N. lezamae	*S. Leiva 4443* (HAO)	FJ914122	FJ914027	FJ914093	FJ914210	FJ914064

swapping, collapse of zero-length branches, multiple tree option in effect, and character state changes equally weighted in the analysis. The maximum number of trees in memory was limited to 10 000 during each of 100 random sequence addition replicates. Gaps were treated as missing data. Bootstrap values (BS; Felsenstein, 1985) of the internal nodes were obtained with 500 replicates. In each replicate, we performed 10 random sequence addition replicates, followed by the TBR swapping algorithm and keeping no more than 1000 trees per replicate. Bayesian inference was performed with MrBayes v. 3.1.2 (Ronquist & Huelsenbeck, 2003), using 2 000 000 generations per run with four chains and sampling trees every 100 generations. Burn-in values were set to 100 000 generations.

1.3 Estimation of divergence time and biogeographic analysis

The phylogenetic tree (Fig. 3) for the estimation of the divergence time of *Nolana* was derived from the combined data of three chloroplast genes (*ndh*F, *rbc*L, and *atp*B). Sequences of 35 species from Convolvulaceae and Solanaceae (including eight species of *Nolana*) were obtained from GenBank or newly sequenced in the present study. A few taxa were coded as missing data for one or two of the markers (*ndh*F, *rbc*L, or *atp*B) when the sequences were not available. Taxa with GenBank accession numbers for the three genes are listed in Table 2.

Likelihood ratio tests (LRTs; Felsenstein, 1988) were performed with PAUP and were used to determine whether the sequence data conformed to the expectation of a molecular clock. The likelihood scores of the trees with and without the enforcement of a clock were compared. Significant differences between these two evolutionary models were calculated by comparing two times the difference in log likelihoods to a χ^2 distribution with $n - 2$ degrees of freedom (d.f.), where n is the number of sequences in the data matrix. Because the LRTs strongly rejected the molecular clock for the data (LR $= 2 \times (14\ 254.90 - 13\ 866.71) = 776.38$; $P < 0.001$, 33 d.f.), we performed a Bayesian method using the program BEAST v1.8 (Drummond & Rambaut, 2007) under a relaxed clock model with branch-specific rates following a log normal distribution and a GTR+I+G model of nucleotide substitution (Drummond et al., 2006). The analyses were completed with a randomly generated starting-tree topology. Posterior estimates were obtained by sampling every 1000 Markov chain Monte Carlo (MCMC) steps from a total

Table 2 Samples and GenBank accessions included in the analysis of divergence time

Species	Collection voucher or source	*ndh*F	*rbc*L	*atp*B
Nolana acuminata	*M. Dillon 8100* (F)	EU742307	FJ914163	FJ914144
Nolana adansonii	*M. Dillon 8974* (F)	EU742308	FJ914164	FJ914145
Nolana baccata	*M. Dillon 8612* (F)	EU742314	FJ914165	FJ914146
Nolana coelestis	*Miller 0498* (F)	EU741321	FJ914166	FJ914147
Nolana galapagensis	*M. Dillon 8504* (F)	EU742329	FJ914167	FJ914148
Nolana sessiliflora	*M. Dillon 8644A* (F)	EU742358	FJ914168	FJ914149
Nolana tomentella	*M. Dillon 8784* (F)	EU742365	FJ914169	FJ914150
Nolana werdermannii	*M. Dillon 8665* (F)	EU742370	FJ914170	FJ914151
Lycium chinense Mill.	*T. Tu 0660* (KUN)	FJ914028	FJ914171	FJ914152
Lycium deserti Phil.	*M. Dillon 8545* (F)	EU742305	FJ914172	FJ914153
Lycianthes sp.	*J. Wen et al. 2111* (US)	FJ914029	FJ914173	FJ914154
Solanum pennellii Correll	*M. Dillon 8779A* (F)	FJ914030	FJ914174	FJ914155
Solanum chilense Dunal	*M. Dillon 8717* (F)	FJ914031	FJ914175	FJ914156
Phrodus microphyllus	*M. Dillon 8643* (F)	EU742442	FJ914176	FJ914157
Grabowskia glauca (Phil.) I. M. Johnst.	*M. Dillon 8581* (F)	EU742303	FJ914177	FJ914158
Atropa belladonna L.	*H. Sun 9040* (KUN)	FJ914032	FJ914178	FJ914159
Withania somnifera (L.) Dunal	*T. Tu 0650* (KUN)	FJ914033	FJ914179	FJ914160
Physalis peruviana L.	*T. Tu 0661* (KUN)	FJ914034	FJ914180	FJ914161
Physalis peruviana	*S. Volis s.n.* (KUN)	FJ914035	FJ914181	FJ914162
Physalis alkekengi L.	Olmstead & Sweere 1994	U08927	U08617	–
Sclerophylax adnatifolia Fulvio	Olmstead et al., 2008	EU126016	–	–
Witheringia solanacea L'Hér.	Bohs & Olmstead, 1997	U72755	–	–
Witheringia meiantha(Donn. Sm.) Hunz.	Olmstead et al., 2008	EU126020	–	–
Nicotiana sylvestris Speg.	Yukawa et al., 2006	AB237912	AB237912	AB237912
Nicotiana tomentosiformis Goodsp.	Yukawa et al., 2006	AB240139	AB240139	AB240139
Salpiglossis sinuata Ruiz & Pav.	Olmstead & Sweere 1994	U08928	U08618	–
Schizanthus pinnatus Ruiz & Pav.	Stefanovic et al., 2002	–	AY101063	AY100851
Wilsonia humilis R.Br.	Stefanovic et al., 2002	–	AY101020	AY100811
Wilsonia backhousei Hook.f.	Stefanovic et al., 2002	–	AY101021	AY100812
Ipomoea purpurea (L.) Roth	McNeal et al., 2007	EU118126	EU118126	EU118126
Cressa depressa Goodd.	Stefanovic et al., 2002	–	AY101016	AY100807
Dinetus truncatus (Kurz) Staples	Stefanovic & Olmstead, 2005	–	AY101053	AY100841
Humbertia madagascariensis Lam.	Stefanovic et al., 2002	–	AY101062	AY100850
Porana velutina Hallier f.	Stefanovic et al., 2002	–	AY101027	AY100816
Petrogenia repens I. M. Johnst.	Stefanovic et al., 2002	–	AY101134	AY100814

of 20 000 000 steps with a burn-in of 10% generations. The divergence times are given as the mean and the 95% highest posterior density (HPD) intervals in millions of years (my).

Two fossils were used as the calibration points in the estimation of divergence time. According to the *Physalis*-like seed fossils found in the bottom of the upper Tortonian in the middle Miocene from Silesia in central Europe (Szafer, 1961), a normally distributed prior for the stem age of *Physalis* L. as suggested by the manual of BEAST was assumed to be 8.6 my (SD = 1.0). The pollen fossils with affinities to *Wilsonia* R. Br. of Convolvulaceae from southern Australia were dated back to the late Eocene (Martin, 2000, 2001). Thus, we assigned 37 my (SD = 3.0) as the stem age of *Wilsonia*. We constrained the root age of the Solanaceae–Convolvulaceae clade as 86 my (SD = 5.0) based on the estimates from Bremer et al. (2004).

We used dispersal-vicariance (DIVA) analysis (Ronquist, 1997) to infer the biogeographic diversification of *Nolana* based on a simplified most parsimonious tree (MPT) generated by PAUP using a subset of the *LEAFY* second intron sequences (Fig. 1). This tree (Fig. 4) includes one outgroup species and 14 species of *Nolana*, which represent the major clades of the *LEAFY* second intron tree in Fig. 1. Most species in each major clade in the *LEAFY* second intron tree are restricted to Peru or Chile; thus, we designated Peru and Chile each as an area of endemism for *Nolana*. *Nolana galapagensis* is endemic to the Islas Galápagos. We therefore circumscribed the Islas Galápagos as the third area of endemism for the *Nolana* analysis. The DIVA analysis was implemented using the program DIVA version 1.1 (Ronquist, 1996).

2 Results and Discussion

2.1 Phylogenetic analysis

The alignment of sequences of the *LEAFY* second intron has 4830 positions, of which 1173 are variable (24.3%) and 448 are parsimony informative (9.3%). Treating gaps as missing data, the parsimony analysis yielded 140 006 MPTs with a tree length of 2163

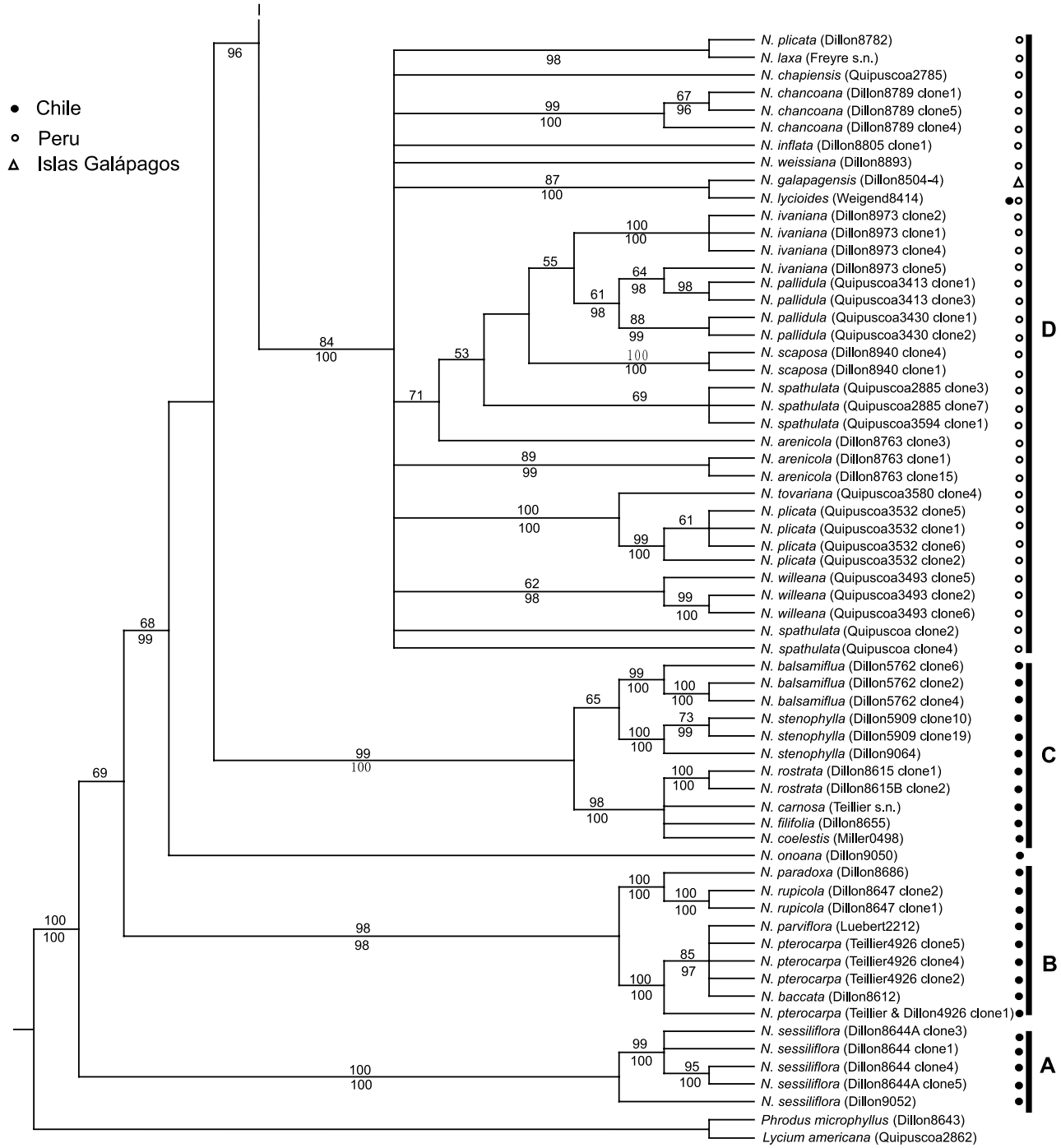

Fig. 1. The strict consensus tree of *Nolana* based on sequences of the *LEAFY* second intron (tree length of 2163 steps; consistency index = 0.67; retention index = 0.89). Bootstrap values of 1000 replicates are above the branches and Bayesian posterior probabilities are below the branches. Bootstrap values < 50% and Bayesian values < 95% are not shown. A, B, C, D, E, F, and G represent the major clades in the tree. Open circles, solid circles, and triangle represent taxa from Peru, Chile, and the Islas Galápagos, respectively.

steps, a consistency index (CI) of 0.67, a CI excluding uninformative characters of 0.58, and a retention index (RI) of 0.89. The topology of the tree in the Bayesian analysis is similar to that in the parsimony analysis. All nodes with high BS values (> 90%) also had high posterior probability (PP) values. Seven major clades with medium to high BS and PP values were recovered in this study, a finding consistent with our previous study

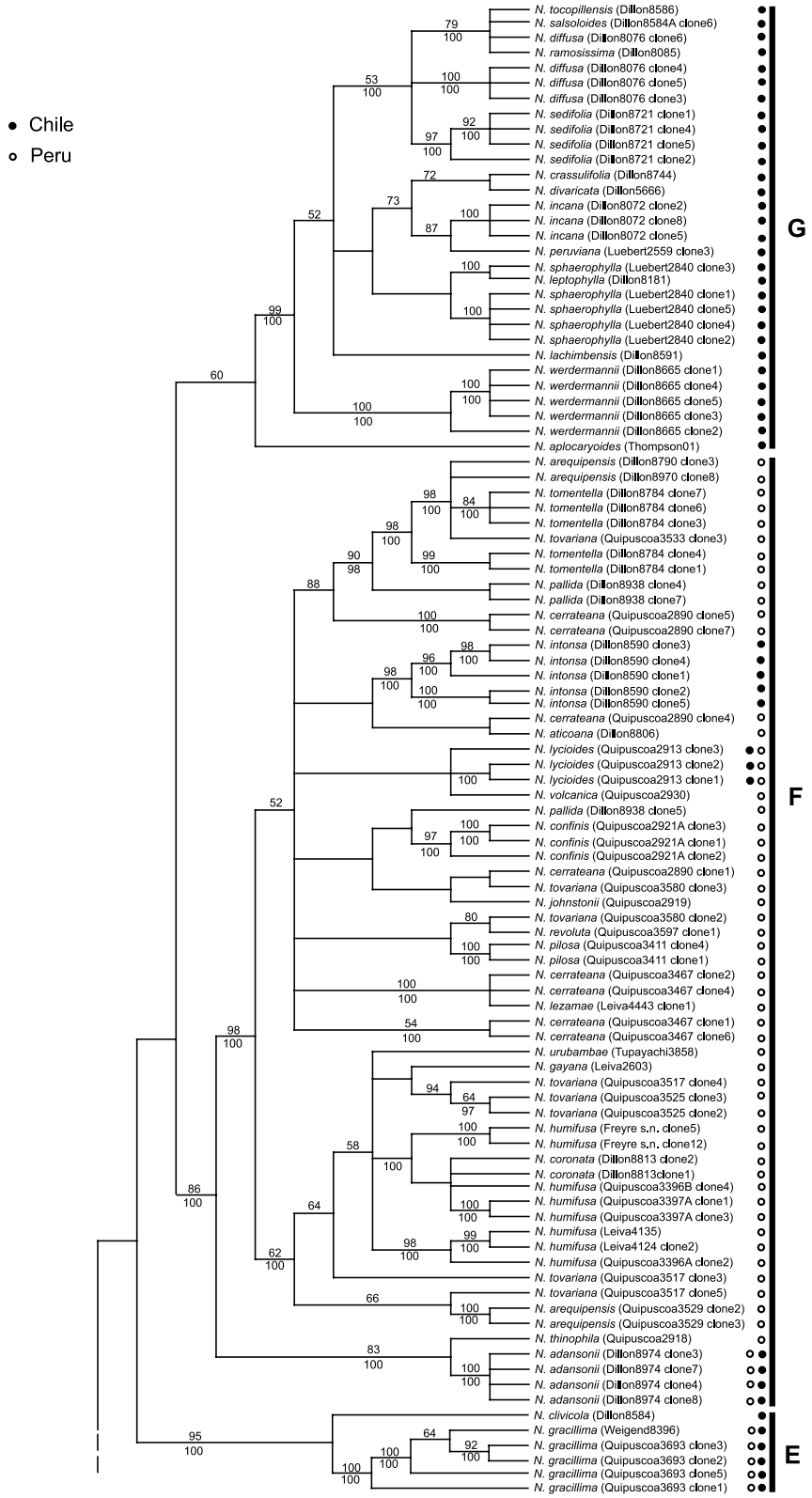

Fig. 1. Continued.

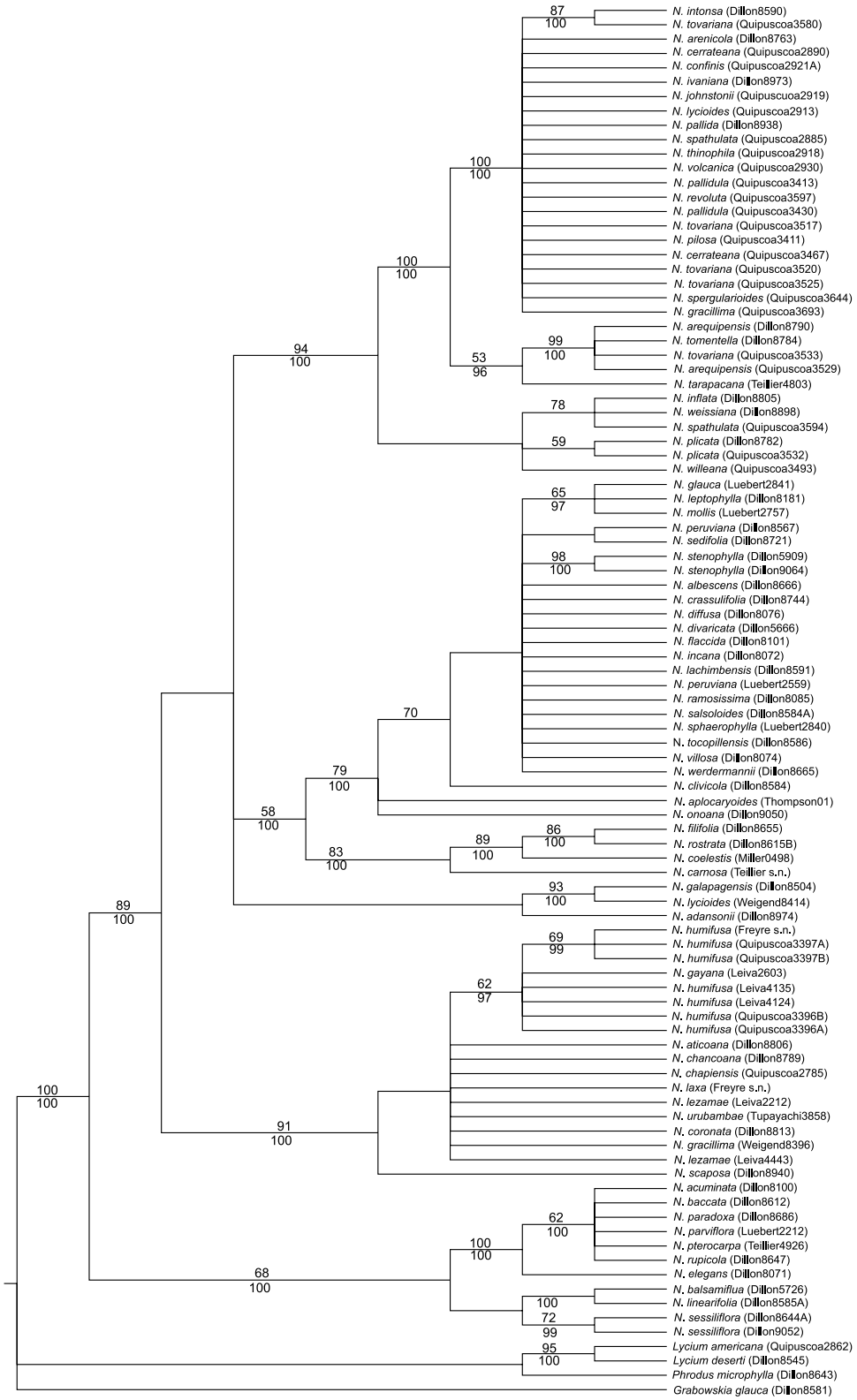

Fig. 2. The strict consensus tree from the combined sequences of four chloroplast markers (*ndh*F, *trn*C–*psb*M, *trn*H–*psb*A, and *rps*16–*trn*K; tree length 306 steps; consistency index = 0.87; retention index = 0.95). Bootstrap values of 1000 replicates are above the branches and Bayesian posterior probabilities are below the branches. Bootstrap values < 50% and Bayesian values < 95% are not shown.

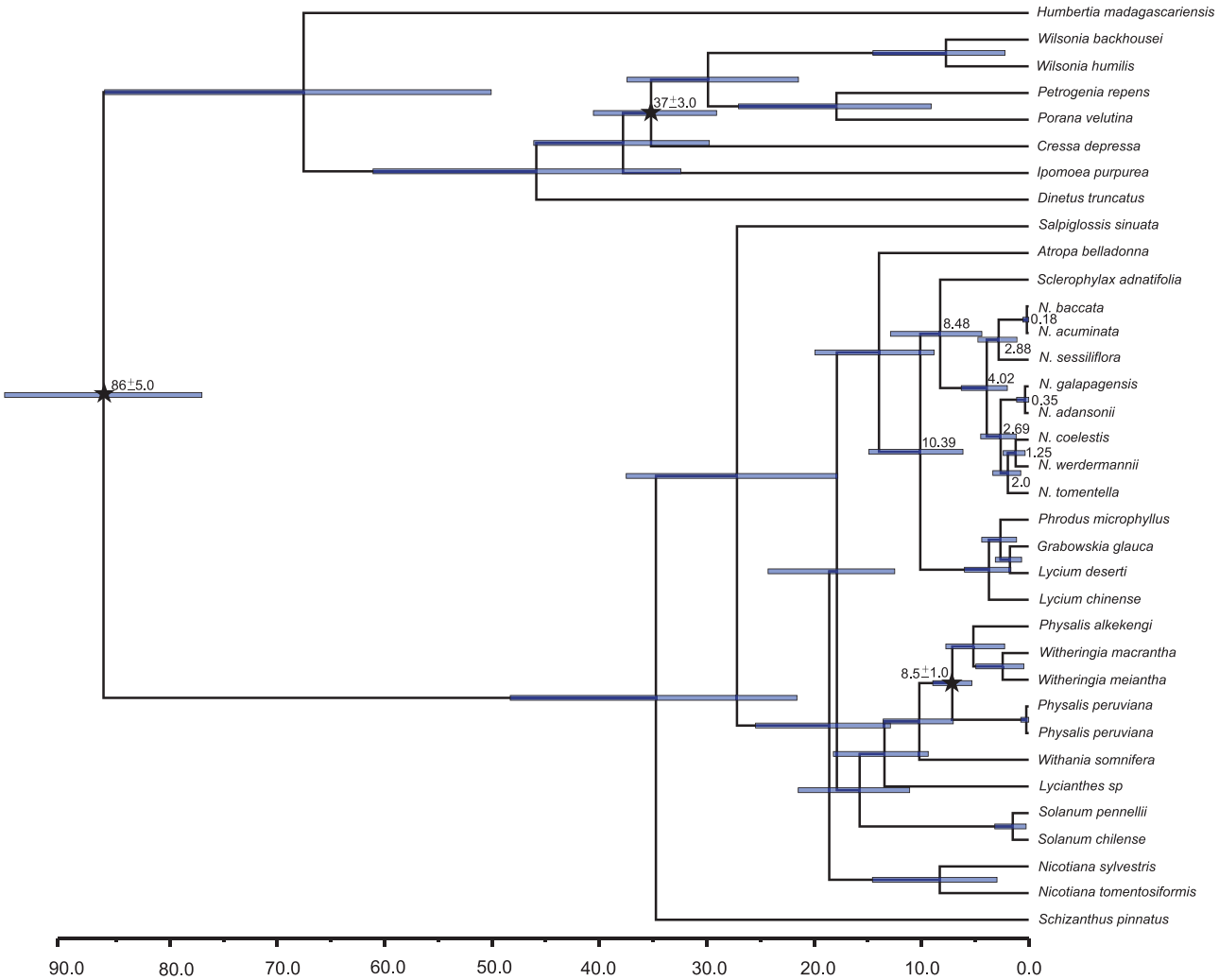

Fig. 3. Bayesian divergence time estimates of *Nolana* based on the combined sequence data from three chloroplast genes (*ndh*F, *rbc*L, and *atp*B), two fossil calibration points, and one estimated age for the crown group of Convolvulaceae and Solanaceae by Bremer et al. (2004) (stars). The blue bars on the nodes indicate 95% highest posterior density (HPD) intervals.

(Tu et al., 2008). The strict consensus topology of the parsimony analysis with BS and PP for each clade is shown in Fig. 1.

The combined matrix of four chloroplast DNA markers comprised 5174 positions, of which 243 are variable (4.6%) and 163 are parsimony informative (3.2%). When gaps were treated as missing data, 53 965 equal MPTs were generated with a tree length of 306 steps, a CI of 0.87, a CI excluding uninformative characters of 0.75, and an RI of 0.95. The Bayesian analysis produced the same overall topology as the parsimony analysis. All clades with high BS values in the parsimony analysis also had high PP values in the Bayesian analysis. The strict consensus topology of the parsimony analysis with BS and PP for each clade is shown in Fig. 2.

2.2 Chronogram

Using two fossil calibration points from Convolvulaceae and Solanaceae and one estimated age point for the crown group of Convolvulaceae and Solanaceae, we estimated the divergence time of *Nolana* from its sister *Sclerophylax* Miers to be 8.48 mya and the crown age of *Nolana* to be 4.02 my (Fig. 3). The divergence of the *N. sessiliflora* Phil.–*N. acuminata* Miers–*N. baccata* (Lindl.) Dunal node is dated at 2.88 mya. The divergence time between *N. galapagensis* (Islas Galápagos, Ecuador) and *N. adansonii* (Roem. & Schult.) I. M. Johnst. from mainland Peru was estimated to be 0.35 mya. The node for the divergence of *N. tomentella* Ferreyra is 2.0 mya and the divergence of *N. coelestis* (Lindl.) Miers ex Dunal and *N. werdermannii* I. M. Johnst. is 1.25 mya. The dated nodes and

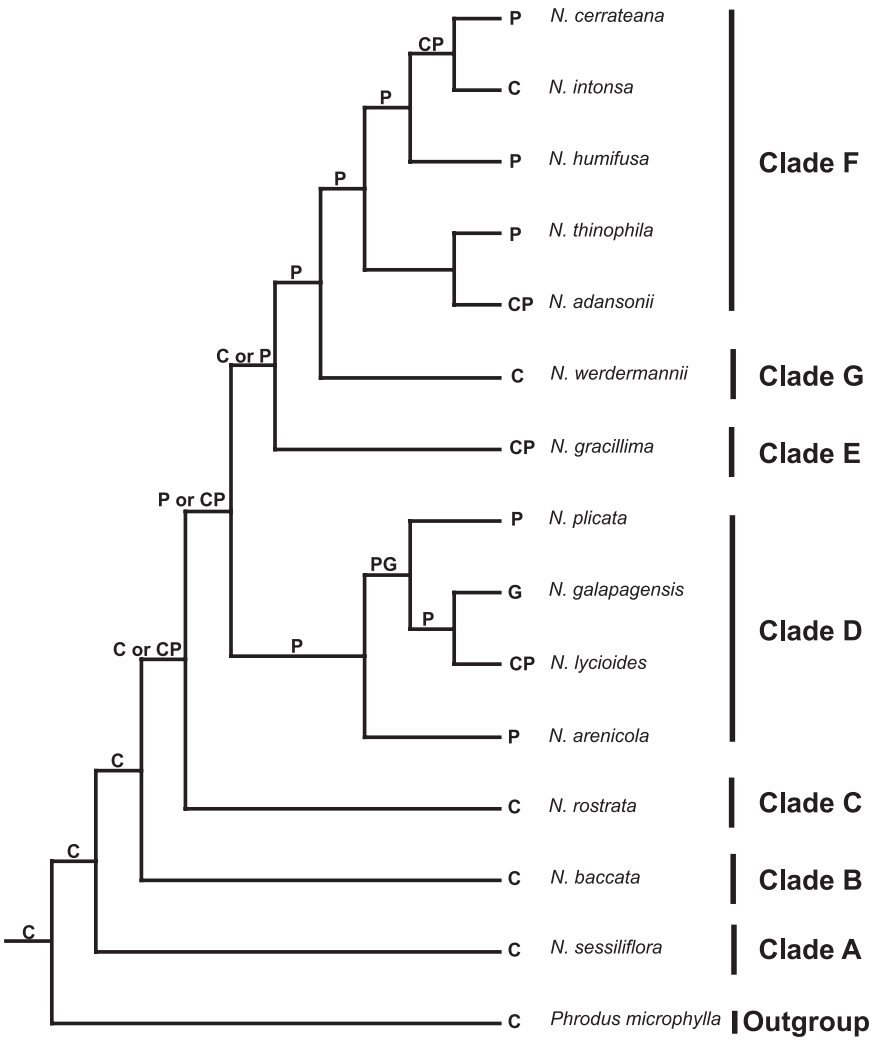

Fig. 4. Dispersal-vicariance analysis of *Nolana*. C, Chile; P, Peru; G, Islas Galápagos, Ecuador.

implications of these results are discussed below in relation to the major clades.

2.3 Distribution of species and major clades

Previous studies (Dillon et al., 2007; Tu et al., 2008) have elucidated a robust phylogeny for *Nolana* that supports the monophyly of the genus and has identified several strongly supported clades with geographic and morphological fidelity. The present analysis includes species not investigated in previous analyses and/or additional samples of taxa sampled previously. The *LEAFY* second intron analysis suggests that *Phrodus microphyllus* (Miers) Miers is sister to *Lycium* L., which is in turn sister to *Nolana* (Fig. 1). The floral morphology and white corolla color are similar to that of *N. sessiliflora* (cf. Dillon et al., 2007), and *P. microphyllus* and *N. sessiliflora* occur sympatrically in the interior, 120 km east of the coast and near Potrerillos, Chile (26°26′S, 69°36′W). *Sclerophylax*, an Argentine genus, was included in the Bayesian divergence time estimates based upon chloroplast genes (*ndh*F, *rbc*L, and *atp*B), and was sister to *Nolana* and closer than either *Phrodus* or *Lycium*. The divergence estimate of 8.48 mya (Miocene) would have been coincident with the final uplift of the Frontal Cordillera and Precordillera in Chile (Hoke & Garzione, 2008) to progressively shut off the eastern precipitation and generally a time of global drying and cooling. Consequently, aridity along the coast of western South America was established for the entirety of the evolutionary history of *Nolana*, only interrupted by wetter cycles of variable periods.

The analysis found the crown group for *Nolana* dated at 4.02 mya (Pliocene), when western South America was responding to increasing aridity

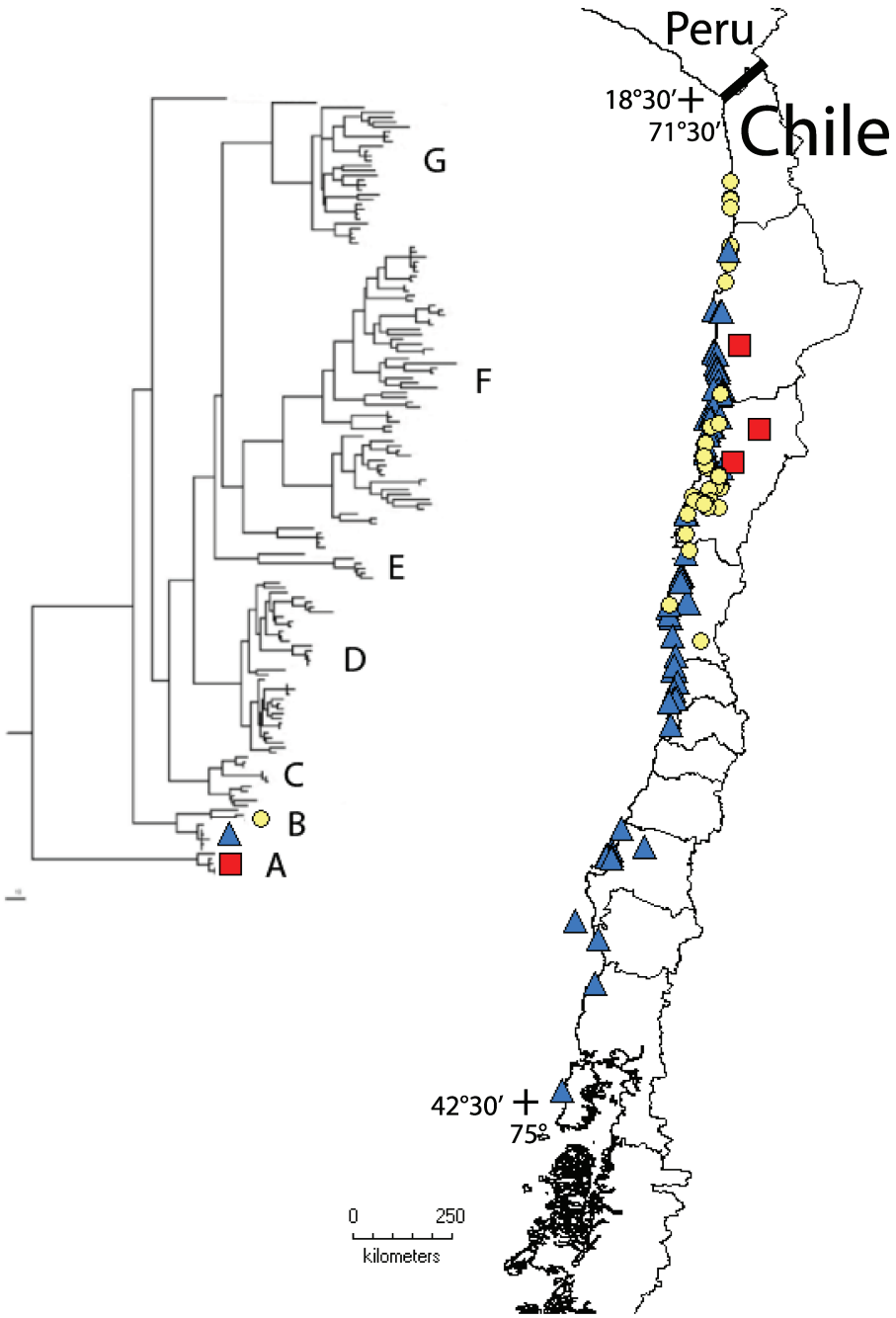

Fig. 5. Distribution of Chilean *Nolana* species. Clade A, *N. sessiliflora* (red squares); Clade B, *Sorema* (blue triangles = round mericarps; yellow circles = winged mericarps).

(Wara et al., 2005). The divergence of other genera, such as *Prosopis* (Catalano et al., 2008), *Astragalus* (Scherson et al., 2008), and *Palaua* (Huertas et al., 2007), has been reported to have occurred at the beginning of the Pliocene. Our analyses recovered *Nolana sessiliflora* (Clade A) as the taxon sister to the remainder of the genus (Fig. 1) with 100 BS and PP support and congruent with its position suggested by previous analyses (Dillon et al., 2007; Tu et al., 2008). This species is restricted to a few hyperarid inland localities (Fig. 5) over 100 km from the coast and at elevations over 2500 m, well outside the range of coastal fogs. In the Bayesian divergence estimate, *N. sessiliflora* was sister to *N. acuminata–N. baccata* (Clade B), which were, in

turn, sister to the remainder of *Nolana* (Clades C, D, F, and G; Fig. 3).

The divergence node of clade B (*N. acuminata* and *N. baccata*) was dated at 2.88 mya (the Pliocene–Pleistocene border) and was congruent with the results of the *LEAFY* analysis (Fig. 1), with Clade B (BS = 98, PP = 98) strongly supported. This clade has been recovered by all other markers, including internal transcribed spacer (ITS), *mat*K, and *waxy* (Tago-Nakazawa & Dillon, 1999; Dillon et al., 2007; Tu et al., 2008). The group can be expanded to nine species (*N. acuminata*, *N. elegans* (Phil.) Reiche, *N.* parviflora (Phil.) Phil., *N. pterocarpa* Phil. ex Wettst., *N. baccata, N. jaffuelii* I. M. Johnst., *N. rupicola* Gaudich. *N. paradoxa* Lindl., and *N. reichei* M. O. Dillon & Arancio) and contains a suite of morphological characters that circumscribe the clade. This clade has been recognized as a segregate genus, *Sorema* Lindl., a group not recognized above the sectional rank (Tago-Nakazawa & Dillon, 1999). Although it is not included in the present analysis, *N. jaffuelii* clearly belongs to this clade by virtue of its overall similar morphology. Our analysis further recovered two groups within the strongly supported clade (BS = 100, PP = 100). First, the *N. paradoxa–N. rupicola* subclade (BS = 100, PP = 100) is typified as rosette-forming, taprooted, plants with long decumbent stems, larger flowers, and more spherical to rounded mericarps lacking prolonged wings (Fig. 5, blue triangles). Second, the *N. pterocarpa–N. baccata–N. parviflora* clade (BS = 100, PP = 100) is diagnosed as erect annuals with slightly smaller flowers and angular mericarps, at times prolonged into wings (Fig. 5, yellow circles). The Bayesian divergence estimates suggest a divergence time between *N. acuminata* (round mericarps) and *N. baccata* (winged mericarps) at 0.18 mya (late Pleistocene), a time of highly variable climatic conditions in the Atacama. The two groups have largely overlapping geographic distributions and ecological preferences (Fig. 5).

The position of *N. onoana* M. O. Dillon & M. Nakaz. as the sister species to the remainder of the genus is not congruent with its position as sister to species in Clade G in previous studies (Tu et al., 2008). The clear morphological affinities of *N. onoana* with *N. aplocaryoides* (Gaudich.) I. M. Johnst. and other species in Clade G may reflect reticulate evolution in this taxon and will be investigated further.

Clade C has recovered a monophyletic group (BS = 99, PP = 100) comprised of species traditionally classified as *Alona* Lindl. (Lindley, 1844), accepted by Johnston (1936) at the generic level and at sectional status by Miers (1845) and Tago-Nakazawa and Dillon (1999). The strong support for this clade has not been universal among markers; however, the included species are all large-flowered shrubs with linear, terete or tubular leaves, and gynoecia with apical stigmas arising from the apex of the fused mericarps, and all are confined to Chile, between the Río Loa (21°26′S) and the Río Limarí (30°44′S; Fig. 6). The group consists of six species: *N. carnosa* (Lindl.) Miers ex Dunal, *N. coelestis* (Lindl.) Miers ex Dunal, *N. filifolia* (Hook. & Arn.) I. M. Johnst., *N. rostrata* (Lindl.) Miers ex Dunal, *N. stenophylla* I. M. Johnst., and *N. balsamiflua* (Gaudich.) Mesa. The Bayesian divergence estimates (Fig. 3) suggest that *N. coelestis* and *N. werdermannii* are members of sister clades with a divergence time of 1.25 mya (Pleistocene). These results are difficult to interpret owing to the conflicts between various markers.

Clade D has recovered a well-supported group (BS = 84, PP = 100) consisting of 14 Peruvian species ranging from central to southern Peru (*N. arenicola* I. M. Johnst.–*N. spathulata* Ruiz & Pav.–*N. pallidula* I. M. Johnst.–*N. scaposa* Ferreyra–*N. ivaniana* Ferreyra–*N. weissiana* Ferreyra–*N. willeana* Ferreyre–*N. inflata* Ruiz & Pav.–*N. chancoana* M. O. Dillon & Quipuscoa–*N. chapiensis* M. O. Dillon & Quipuscoa–*N. lycioides* I. M. Johnst.–*N. laxa* (Miers) I. M. Johnst.–*N. plicata* I. M. Johnst.–*N. tovariana* Ferreyre) and *N. galapagensis* sharing relationships with *N. lycioides* (Figs. 1, 3, 6). In the *waxy* analysis (Dillon et al., 2007), *N. adansonii* had been suggested as sharing relationships with both *N. arenicola* and *N. galapagensis*. In the chloroplast analysis (Fig. 2.), *N. galapagensis* has sister species relationships with *N. lycioides*, a species that shares the former's shrubby habit, floral size and shape, and mericarp structure. The geographic distribution of *N. lycioides* extends into northern Chile; however, all accessions in this analysis have come from Peru. The occurrence of Clone 4 of *Quipuscoa 3580*, identified as *N. tovariana*, is notable, given that Clones 2 and 3 are found associated in Clade F with other Peruvian species, specifically *N. cerrateana* Ferreyra, *N. johnstonii* Vargas, and *N. pilosa* I. M. Johnst. *Nolana tovariana* must be examined in light of these results and alternative positions for accessions of *N. lycioides* need to be examined.

The Bayesian divergence estimates (Fig. 3) dated the node between *N. galapagensis* and its sister taxon *N. adansonii* and the remainder of the genus at 2.69 mya and the subsequent divergence between *N. galapagensis* and *N. adansonii* at 0.35 mya. This is a much different and more recent estimate than prior analysis had suggested (Tago-Nakazawa & Dillon, 1999).

In Clade E, *Nolana clivicola* (I. M. Johnst.) I. M. Johnst. is supported as sister to *N. gracillima* (I. M. Johnst.) I. M. Johnst. (BS = 95, PP = 100). In the *waxy* analysis, *N. clivicola* was in an unresolved

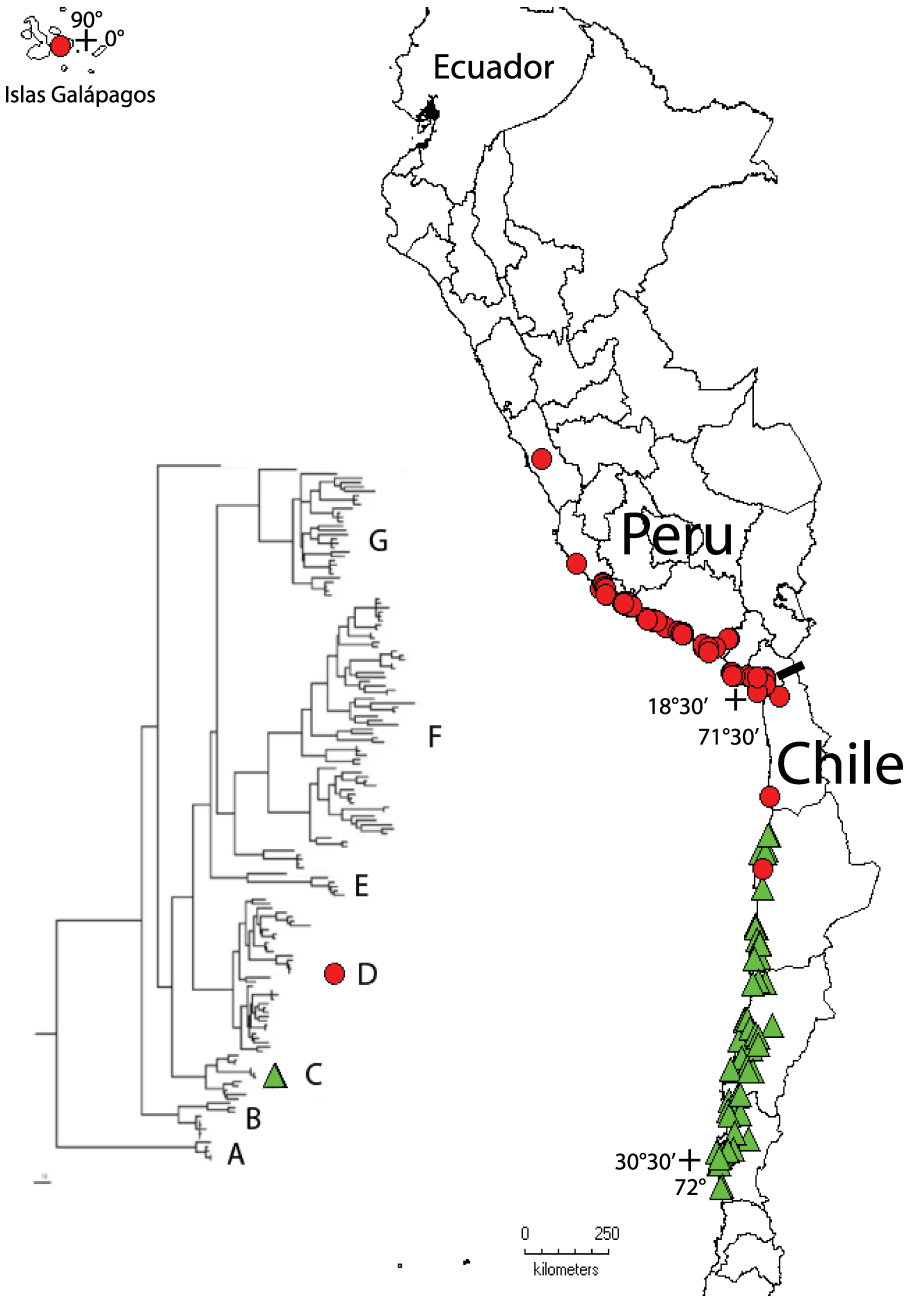

Fig. 6. Distribution of *Nolana* species. Clade C, *Alona*, is strictly Chilean (green triangles) and does not extend north of the Rio Loa (21°26′S) or south of the Rio Limarí (30°44′S). Clade D (red circles) is largely Peruvian, with a few disjunctions into northern Chile and to the Islas Galápagos, Ecuador.

polytomy with other Chilean taxa. Its overall morphology and ecological preferences would suggest that its relationships are within the Chilean taxa found in Clade G. In the waxy analysis, *N. spergularioides* Ferreyra and *N. tarapacana* (Phil.) I. M. Johnst., two species not included in the present study, were related to *N. gracillima* and share morphological similarity. The latter three species are found in interior localities at elevations over 2000 m and well beyond the influence of coastal fogs, but bloom by winter rains common to the area (Fig. 7).

Clade F has strong support (BS = 98, PP = 100) and is comprised of 18 Peruvian species (*N. arequipensis* M. O. Dillon & Quipuscoa–*N. tomentella*–*N. tovariana*–*N. pallida*–*N. cerrateana*–*N. aticoana* Ferreyra–*N. lycioides*–*N. volcanica* Ferreyra–*N.*

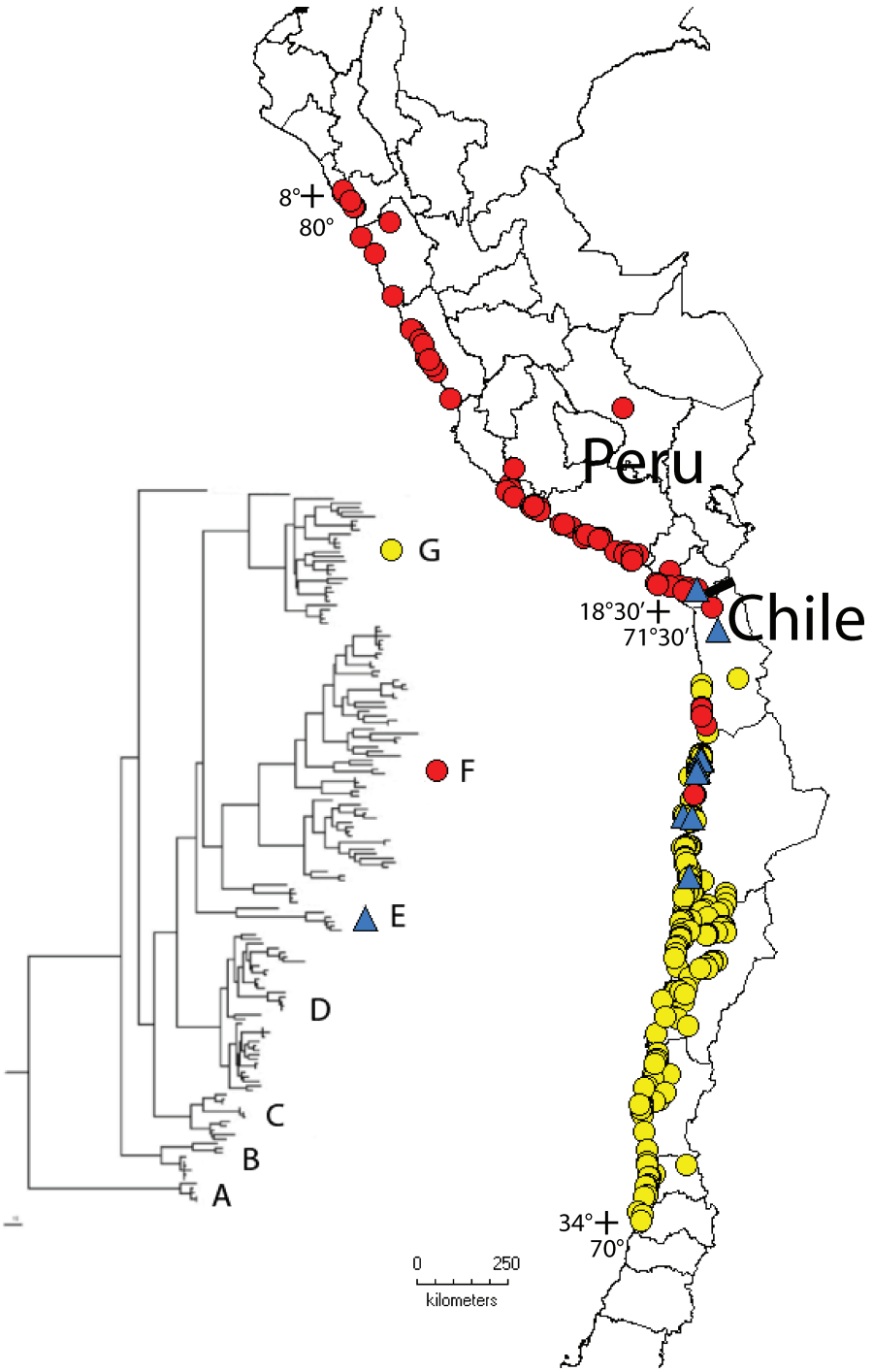

Fig. 7. Distribution of *Nolana* species. Clade E (blue triangles) contains species from southern Peru and northern Chile; Clade F (red circles) is primarily Peruvian, but with a few northern Chilean disjunctions; and Clade G (yellow circles) contains only Chilean species, north to 20°15′S and south to 33°20′S.

revoluta Ruiz & Pav.–*N. pallida* I. M. Johnst.–*N. confinis* I. M. Johnst.–*N. johnstonii*–*N. pilosa*–*N. lezamae* M. O. Dillon, S. Leiva, & Quipuscoa–*N. urubambae* Vargas–*N. gayana* (Gaudich.) Koch–*N. humifusa* (Gouan) I. M. Johnst.–*N. coronata* Ruiz & Pav.) and one Chilean species, *N. intonsa* I. M. Johnst. (Figs. 4, 7). The recovery of a clade comprised of *N. urubambae* and *N. humifusa* is congruent with the *waxy* results; however,

the inclusion of *N. gayana* is unexpected given its suggested relationships with the *waxy* marker and overall morphological similarities. The position of the various clones of *N. cerrateana* is notable, with clones found in three positions on the cladogram in Clade E. *Nolana intonsa* is a northern Chilean species that shares strong morphological resemblance to southern Peruvian species, such as *N. pallida*. Support for the *N. adansonii–N. thinophila* I. M. Johnst. clade is moderate (BS = 83, PP = 100), but they share a few characters, including habitat preference of near-ocean habitats in southern Peru. The weak internal support for the clade overall makes it difficult to discuss biogeographic implications. The Bayesian divergence estimates (Fig. 3) found the *N. tomentella* (Clade F) node dated at 2.0 mya (Pleistocene) and suggested a second diversification event in Peru.

Clade G has recovered (BS = 60, PP < 95) a strictly Chilean group of 13 species represented by small to moderate shrubs and annuals, all with highly reduced corollas, often white or yellowish (Fig. 7). *Nolana aplocaryoides* is the sister taxon to the strongly supported (BS = 99, PP = 100) remainder of the clade (*N. tocopillensis* (Phil.) I. M. Johnst.–*N. salsoloides* (Lindl.) I. M. Johnst.–*N. diffusa* I. M. Johnst.–*N. ramosissima* I. M. Johnst.–*N. sedifolia* Poepp.–*N. crassulifolia* Poepp.–*N. divaricata* (Lindl.) I. M. Johnst.–*N. incana* (Phil.) I. M. Johnst.–*N. peruviana* (Gaudich.) I. M. Johnst.–*N. sphaerophylla* (Phil.) Mesa ex M. O. Dillon–*N. leptophylla* (Miers) I. M. Johnst.–*N. lachimbensis* M. O. Dillon & Luebert–*N. werdermannii*). A few taxa that are clear members of Clade G were absent from this analysis, but their positions are not in question and are supported by the results of the waxy marker (e.g. *N. albescens* (Phil.) I. M. Johnst., *N. villosa* (Phil.) I. M. Johnst., *N. mollis* (Phil.) I. M. Johnst., *N. glauca* (I. M. Johnst.) I. M. Johnst., and *N. flaccida* (Phil.) I. M. Johnst.). With the plausible addition of these taxa, Clade G would contain no fewer than 18 species from northern to central Chile (Fig. 7) and not extending south of 33°20′S. Others that occur further north, such as *N. deflexa* (I. M. Johnst.) I. M. Johnst., *N. dianae* M. O. Dillon, *N. inconspicua* (I. M. Johnst.) I. M. Johnst., *N. philippiana* M. O. Dillon & Luebert, and *N. rhombifolia* Martic. & Quezada, are yet to be incorporated into any analyses. The sister taxon relationship between *N. incana* and *N. peruviana* is supported by their very close resemblance and they share sympatric geographic ranges. The position of *N. diffusa* Clone 6 (*Dillon 8076*) with *N. ramosissima*, *N. salsoloides*, and *N. tocopillensis* may suggest some gene flow in these taxa. The Bayesian divergence estimates (Fig. 3) find *N. werdermannii* (Clade G) and its sister taxon *N. coelestis* (clade C) dated at 1.25 mya and suggest a second diversification event in Chile.

The analysis of four chloroplast markers (*ndh*F, *trn*C–*psb*M, *trn*H–*psb*A, and *rps*16–*trn*K), as shown in Fig. 2, indicated lower resolution than the *LEAFY* second intron analysis. The analysis recovered some of the same clades; for example, Clade B (*Sorema*) has strong support, but the clade lacks resolution and did not recover the two subclades representing rounded and winged mericarps. The analysis recovered two distinct Peruvian clades, each with taxa from Clades D and F in the *LEAFY* analysis. Further, a large weakly supported clade containing all Chilean species was composed of species found in Clades C and F. The relationship between *N. galapagensis* and *N. lycioides* is recovered as strongly supported sister species, a relationship found in the *LEAFY* analysis. Clearly, the relationships suggested by the four chloroplast markers (Fig. 2) are congruent on some level, but do not appear to be the most parsimonious hypothesis. There appears to be a lack of congruence with morphology and the relationships suggested by the *LEAFY* second intron analysis. Chloroplast genes are inherited maternally as a single molecule and conflicting molecular phylogenies can be explained in terms of reticulate evolution and sorting of ancient polymorphisms during speciation.

2.4 Geographic partitioning and sympatry

Geographic isolation is usually considered as one of the primary processes in speciation (Perret et al., 2007). Throughout their ranges within the geographic subregions, many *Nolana* species occur sympatrically. An examination of the geographic ranges of species at 1′ intervals suggests that sympatry is the norm throughout Peru and Chile, with no fewer than 23 geographic points in Peru with three or more species present and nearly 80 geographic points in Chile where there are three or more *Nolana* species recorded. The highest number of species occurring sympatrically is to be found in southern Peru (16°14′S), where nine species are recorded (i.e. *N. adansonii* (Clade F), *N. arequipensis* (Clade F), *N. aticoana* (Clade F), *N. cerrateana* (Clade D), *N. chancoana* (Clade D), *N. inflata* (Clade D), *N. pallida* (Clade F), *N. scaposa* (Clade D), and *N. spathulata* (Clade D)). In northern Chile (25°24′S), 11 species can be found in the same immediate geographic area, namely *N. acuminata* (Clade B), *N. aplocaryoides* (Clade G), *N. diffusa* (Clade G), *N. flaccida* (Clade G), *N. mollis* (Clade G), *N. peruviana* (Clade G), *N. rostrata* (clade C), *N. rupicola* (Clade B), *N. salsoloides* (Clade G), *N. sedifolia* (Clade G), and *N. villosa* (Clade G). *Nolana* species grow in various degrees of sympatry (i.e. congeners

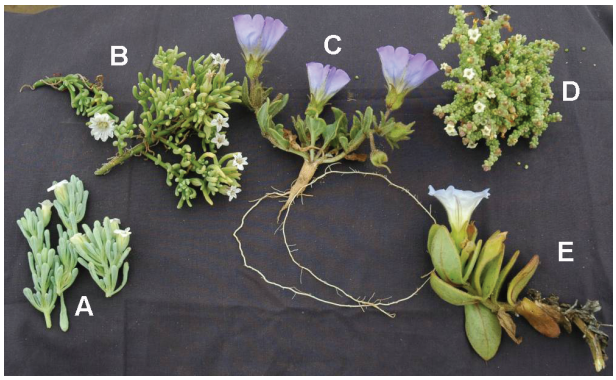

Fig. 8. Sympatric *Nolana* species gathered in a small area near Caleta El Toro, central Chile. **A,** *N. crassulifolia* (Clade G); **B,** *N. werdermannii* (Clade G); **C,** *N. reichei* (Clade B); **D,** *N. sedifolia* (Clade G); **E,** *N. rupicola* (Clade B).

can be found in the same general areas and are separated by slight differences in microecological ranges or through position effects, or sometimes species are found growing interspersed and actually touching).

Although the data at hand do not allow elucidation of detailed species relationships or speciation patterns, it is possible to examine the sympatric taxa in relation to their phylogenetic relationships. An examination of the phylogeny suggests co-occurring combinations of taxa are typically derived from different major clades and species from within the same clade. For example, at a site near Fray Jorge, five sympatric *Nolana* species were gathered within 20 m of each other at Caleta El Toro (Fig. 8) and included *N. reichei* and *N. rupicola* from Clade B and *N. sedifolia*, *N. crassulifolia*, and *N. werdermannii* from Clade G (Fig. 1). Given Bayesian divergence estimates (Fig. 3), it appears that the timing of cladogenesis was not simultaneous and clades reached current geographic ranges at different times. Multiple colonization events can account for the mixtures of *Nolana* species found in any one locality (i.e. secondary sympatry).

2.5 DIVA analysis and biogeographic patterns

The DIVA analysis suggests that *Nolana* had its origins in Chile (Fig. 4) and more than one dispersal from Chile into Peru (S → N), with subsequent radiation (Clades D, F), with 18 and 14 species, respectively. The pattern in Chile also suggests a south-to-north dispersal and radiation, beginning with Clade B (*Sorema*), Clade C (*Alona*), and ultimately Clade G, the small-flowered northern Chilean group. These display a south-to-north dispersal pattern with highest diversity found around 25°25′S latitude.

The observed patterns in our DIVA analysis suggest a south-to-north pattern for *N. galapagensis*, with Peru as the geographic origin or source of Peruvian *N. lycioides* (Fig. 1), a species perhaps preadapted to sandy habitats prior to its dispersal to the island chain. The Bayesian divergence estimates yielded a divergence time of 0.35 mya (Fig. 3).

Not all distributions follow the S → N pattern; in the case of *N. adansonii*, *N. gracillima*, and *N. lycioides*, each of these are autodisjunctions (Dillon, 2005) with the directionality of dispersal from north to south. Similarly, both *N. intonsa* (Fig. 4) and *N. tarapacana* are Chilean endemics (allodisjunction) with obvious phylogenetic connections with the largely Peruvian Clade F that includes *N. lycioides* (pro parte), *N. volcanica*, and *N. cerrateana* (Figs. 1, 7). Whether these are the product of vicariance or long-distance dispersal has not been resolved. However, it is evident that short-term climatic fluctuations, such as El Niño events (5–50 year cycles), and longer-term climatic changes associated with glacial cycles (13 000–200 000 year cycles) have been influential in expansions and contractions in the floras of the Andean Cordillera (Dillon et al., 1995). Pleistocene glacial cycles have caused significant sea level fluctuations, with estimates of lowering between 120 and 230 m, which would have significantly changed the position of the shoreline in relation to that of the present day. The northern Peruvian continental shelf would have been exposed and this event would have most certainly displaced plant communities, especially from 5° to 13°S latitude (Dillon et al., 2003). These events provided opportunities for interchange of flora and fauna of the adjacent Andean Cordillera and the coastal desert.

2.6 Character evolution

The authors are cognizant of calls to abandon the common practice of invoking “key adaptations” to explain the high diversity of groups (Slowinski & Guyer, 1993). With over fivefold the species diversity of any other genus in the coastal deserts, *Nolana* is notable for its success and geographic range. On a local scale, *Nolana* can be important members of their respective environments. For example, Luebert et al. (2007) recorded no fewer than 16 species of *Nolana* from the region of Tocopilla (~22°S), an area of approximately 4000 km^2. This flora contains 146 species in total, so nearly 11% of the flowering plant species there are within a single genus. This is more than twice the diversity of any other genus within the flora of that region; recorded species include *N. elegans* and *N. jaffuelii*, both members of Clade B, *N. balsamiflua* and *N. stenophylla*, both members of Clade C, *N. clivicola* and *N. gracillima*, both in Clade E, and *N. aplocaryoides*, *N. deflexa*, *N. diffusa*, *N. inconspicua*, *N. leptophylla*, *N.*

linearifolia Phil., *N. peruviana*, *N. salsoloides*, *N. sedifolia*, and *N. tocopillensis*, all in Clade G (Figs. 1, 7).

The distribution of morphological characters in relation to phylogenetic estimates was discussed in previous studies (Tago-Nakazawa & Dillon, 1999; Dillon et al., 2007; Tu et al., 2008). Characters that potentially provide *Nolana* with a competitive advantage (Schluter, 2000) and may account for successful radiations within arid habitats include habit diversity, leaf succulence, and salt glands and trichomes that capture water and/or retard transpiration. Important ecophysiological adaptations include high water use efficiencies (WUE) in *Nolana*, as measured by the $^{13}C/^{12}C$ ratio (M. Nakasawa, pers. comm., 1999). Although only 21 species of *Nolana* have been analyzed, the species with the most positive values (i.e. the highest WUE) were from Chile, whereas those with the least positive values (i.e. lowest WUE) were from Peru. It appears that this high WUE capacity spans several clades, but essentially the Chilean species have the highest WUE values recorded.

Modifications of the ovary have been used to define the internal classification of *Nolana*. Although only a few species have been studied in detail, floral anatomy and development in representatives have been investigated. Species with carples united into 3–5-sulcate, multiseeded mericarps, which are broadly affixed to the receptacle and joined to one another laterally, have been recovered in Clade C (*Alona*; Fig. 6). The style appears apical due to the large amount of receptacular tissue that engulfs the mericarps, as in *N. filifolia* with fused mericarps surrounded by receptacle. In the remainder of *Nolana*, including *N. sessiliflora*, the carpels are more deeply lobed and composed of between two and 30 1–7-seeded mericarps attached to the receptacle and individual mericarps are free, or practically so. The condition of apical (vs. gynobasic) styles is not completely distinct. A comparable apical position of the style is found in the Peruvian *N. plicata*, a species in Clade D and distinct in all other characters of habit and leaf morphology.

Although the mericarp is the one character that distinguishes *Nolana* from all other members of the Solanaceae, it is of limited use in circumscribing internal groups. Overall, the number and size of mericarps do not appear to be a reliable criterion for defining groups, but trends can be observed. The amount of receptacle tissue has undergone a reduction, with the most being retained in Clade C (*Alona*). The total number of mericarps per flower has undergone a reduction, with the greatest number of > 30 being found in members of Clade B (*Sorema*). The reduction to five or fewer mericarps is most prevalent in Clades D, E, F, and G and occurs in both Peru and Chile.

3 Conclusion

The *lomas* formations of coastal Peru and Chile are dynamic areas for detailed evolutionary and biogeographic studies. The continuous deserts appear to be partitioned and phyletic differences are reflected in different histories for different regions of coastal Peru and Chile. *Nolana* stands out as the most widespread genus, with no fewer than 71 coastal endemics found from northern Peru to southern Chile and another 17 species in interior Andean sites. The results from the analysis of four chloroplast markers (*ndh*F, *trn*C–*psb*M, *trn*H–*psb*A, and *rps*16–*trn*K) lacked the resolution of the *LEAFY* second intron analysis, but were used in the Bayesian divergence time estimates. The 80 (of a total of 89) species of *Nolana* investigated here using *LEAFY* second intron are arrayed in seven clades. There appears to be a significant barrier to dispersal along the coast between 18° and 20°S latitude and that barrier is reflected in the present day distribution and phylogeny of *Nolana* species. Given past climate changes in northern Peru, the pattern strongly suggests the possibility of vicariant distributions; however, long-distance dispersal from the adjacent Andean Cordillera cannot be ruled out on the basis of these results. The *lomas* formations in southern Peru (13°–18°S) are home to extensive radiation, with 30 species of *Nolana* recorded from the Department of Arequipa constituting two clades, namely D and F. *Nolana galapagensis* appears to have been derived from a Peruvian ancestor (Clade D) and may have reached the Islas Galápagos at ca. 0.35 mya. Northern Chilean endemics (Clade G), the Peruvian endemics (Clade F), and Clade E with Chilean and Peruvian species have more distant relationships with southern taxa (i.e. Clade A (*Nolana sessiliflora*), Clade B (*Sorema*), and Clade C (*Alona*)). Although the age of the aridity in coastal Peru and Chile is still contentious, the rapid diversification in such environments has been demonstrated in separate clades between 2.69 and 2.88 mya. Our results suggest that the crown group in *Nolana* is approximately 4.02 mya and the observed species radiations in *Nolana* are the most specious in the Peruvian and Atacama Deserts. The DIVA analysis suggests a Chilean origin for the genus. The stunning diversity in form and function in *Nolana* represents radiation of several monophyletic groups into the same geographic areas and environments, but at different times. Partitioning environments has been accomplished with the development of different habits, leaf forms, pubescence types,

ecophysiology, and phenology reflected in secondary sympatry. Finer resolution of species relationships and testing potential reasons for diversification must await additional data, but reticulate evolution may explain some patterns of relationship. Further, subgeneric classification will await ongoing investigations at lower taxonomic levels.

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