Phylogeny of *Nolana* (Nolaneae, Solanoideae, Solanaceae) as inferred from granule-bound starch synthase I (GBSSI) sequences

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The phylogenetic relationships of *Nolana* (Nolaneae, Solanaceae) were constructed using partial sequences (ca. 891 bp) of the granule-bound starch synthase I (GBSSI) or the *waxy* gene. *Nolana*, with 89 species, is primarily distributed in coastal Chile (49 spp.) and Peru (43 spp.), and of these, four species are recorded in Peru and Chile, and another from the Galápagos Islands, Ecuador. Our phylogenetic analysis, utilizing a sampling of 63 of the 89 species, supports the monophyly of *Nolana* and recovered three clades with 95%–100% bootstrap support. *Nolana sessiliflora* is the sister taxon to the remainder of the genus. Two large, highly supported clades are evident; one containing taxa from Chile, Peru and the Galápagos Islands, and another containing taxa from Chile and Peru. *Nolana galapagensis*, an endemic to the Galápagos Islands, is suggested to be sister to *N. arenicola* in a clade that also includes *N. adansonii* from southern Peru and northern Chile. These two species differ substantially in habitat preference, habit, leaf shape, and mericarp morphology. The monophyly is confirmed for a morphologically cohesive group composed of *N. acuminata*, *N. baccata*, *N. elegans*, *N. reichei*, *N. parviflora*, *N. pterocarpa*, and *N. paradoxa*, a clade of essentially Chilean species.

KEYWORDS: GBSSI, *Nolana*, phylogeny, Solanaceae, *waxy*

INTRODUCTION

The *lomas* formations of coastal Peru and northern Chile are isolated and strictly delimited ecosystems, unique within the context of South American floristic composition and ecological preferences. These highly endemic communities exist as a linear, terrestrial archipelago of vegetation islands within a 3,500 km sea of hyper-arid desert. Long-term aridity, dating from the late Jurassic, makes this the oldest and driest desert on Earth (Alpers & Brimhall, 1988; Hartley & Chong, 2002; Hartley, 2003; Hartley & al., 2005). Communities are composed of floristic elements from various biogeographic sources (Dillon, 1997; Dillon & Hoffmann, 1997; Rundel & al., 2007). *Nolana* L. (Nolaneae, Solanaceae) with 89 species stands out as the most wide-ranging and conspicuous floristic element of these formations.

Frequent habitats for *Nolana* include coastal sites from sea-level, up to 1,000 m, but a few rare inland or upland species exist at elevations to 4,000 m. The majority of species are narrow endemics, with small, restricted geographic ranges and specific ecological requirements. The ecological preferences of *Nolana* are essentially arid and semi-arid habitats (Fig. 1). The greatest concentration of species is in localities between 50 and 600 meters elevation and within a few kilometers of the ocean. Habitats range from highly saline beach dunes (e.g., *N. carnosa*, Fig. 1B) and near ocean sites (e.g., *N. stenophylla*, Fig. 1D; *N. adansonii*, Fig. 1H; *N. spathulata*, Fig. 1E) or inland habitats (e.g., *N. sessiliflora*, Fig. 2A; *N. lycioides*, Fig. 1F; *N. plicata*, Fig. 1G). Two concentrations or centers of species diversity are obvious, one in the southern Peruvian Department of Arequipa where no fewer than 27 species are recorded and another with 30 species in the northern Chile from Región II (Antofagasta) and Región III (Atacama).

Nolana species are essentially herbaceous with woodiness varying from nearly absent in annuals to moderate in shrubs (Carlquist, 1987). Most species can be considered succulent with leaves sometimes swollen or inflated and

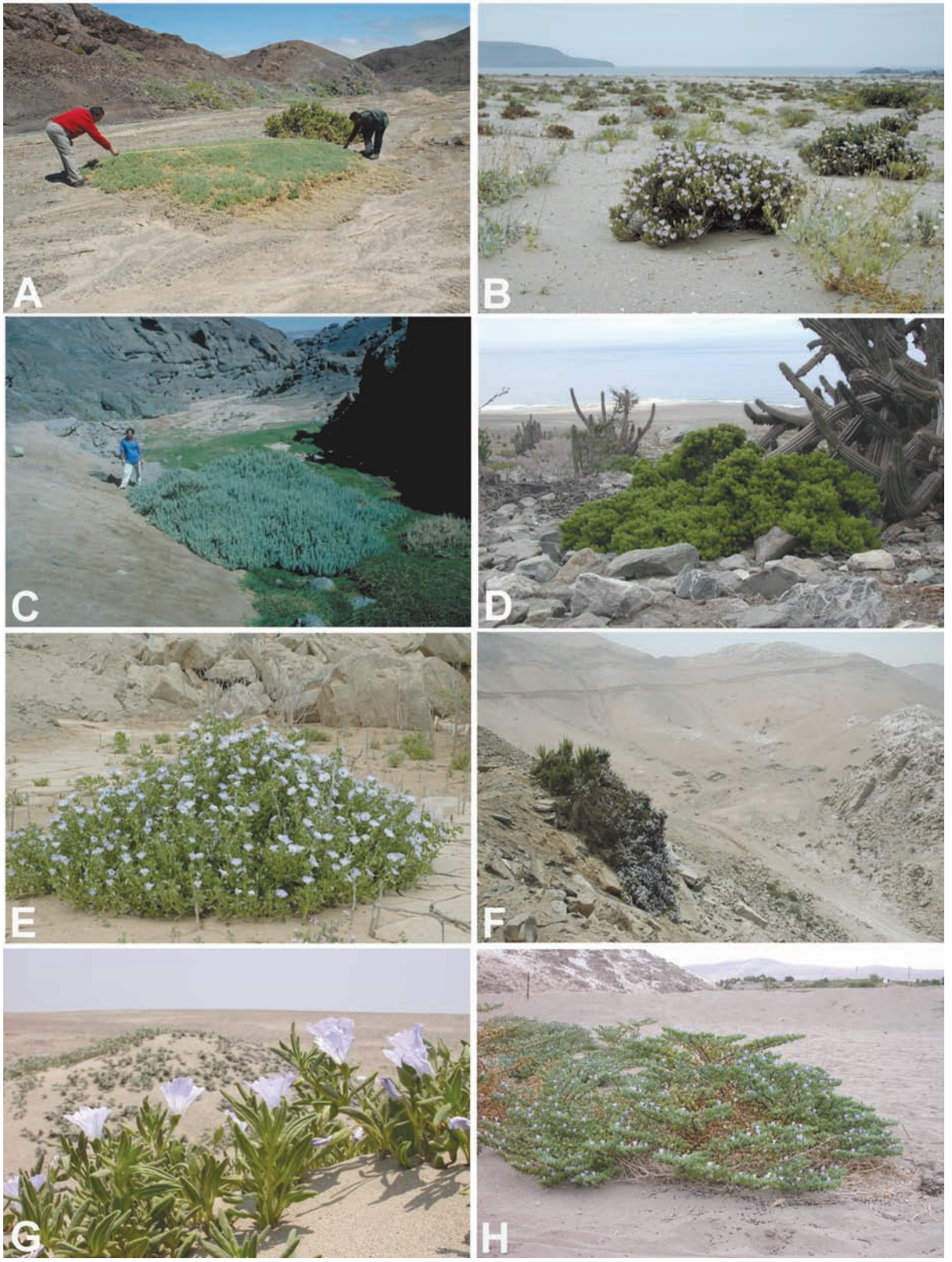

Fig. 1. *Nolana* habits and habitats. A, *N. villosa*, near La Brea, northern Chile; B, *N. carnosa*, Bahia Inglesa, near Caldera; C, *N. incana*, Quebrada Bandurrias, northern Chile; D, *N. stenophylla*, north of Paposo, northern Chile; E, *N. spathulata*, coastal southern Peru; F, *N. lycioides*, above Mollendo, southern Peru; G, *N. plicata*, Lomas de Jahuay, southern Peru; H, *N. adansonii*, Catarindo, southern Peru.

with three-dimensional structure. If allowed to mature with a continuing supply of water, some rosette-forming species form a central stem that elongates and leads to another rosette of leaves. Moisture provided by infrequent rains or proximity to underground sources, termed *aguadas*, allows for sustained growth and uninterrupted flowering. With unlimited moisture, individuals can grow to over 3 m in diameter (e.g., *N. villosa*, *N. incana*, Fig. 1 A, C). Corollas are regular, or more commonly irregular to weakly zygomorphic, with primary modifications in the size, shape and coloration patterns (Fig. 2C–Q). The most common color is lavender to blue, with considerable variation in the bands of colors or colored veins within the throats. Within populations of species that typically possess blue or lavender corollas, a few individuals may possess white corollas; this has been observed as occurring at a low frequency in some species, for example, *N. humifusa*, *N. paradoxa*, *N. pallida* and *N. rupicola*.

The early taxonomic history of *Nolana* was reviewed by Johnston (1936) and Mesa (1981). Both Don (1838) and Dunal (1852) suggested that the Nolanaceae be included within the Solanaceae, but *Nolana* has been recognized at the familial rank, Nolanaceae Dumort. by Cronquist (1981) and Takhtajan (1980) or placed within the Solanaceae at subfamilial rank, Nolanoideae Kostel., or tribal rank, Nolaneae Rchb. as treated by Dahlgren (1980), D'Arcy (1979, 1991) and Thorne (1983). Regardless of rank or hierarchical position, *Nolana* s.l. has been recognized as a cohesive group largely due to their unique, 5-merous gynoecium forming mericarps with multi-seeded, hard-coated segments (Fig. 2R–X). While variable in size, form, and degree of fusion, the mericarp is a strong and compelling synapomorphy for *Nolana*. Over a decade ago, the familial status of *Nolana* was tested by Olmstead & Palmer (1992) using plastid DNA restriction site mapping. Their studies supported the recognition of the *Nolana* clade within the Solanaceae and suggested it was deeply nested within the subfamily Solanoideae (Solanaceae) and related to *Lycium* L., *Grabowskia* Schlecht, *Hyoscyamus* L. and *Atropa* L. (e.g., Olmstead & Palmer, 1992; Olmstead & al., 1999). While some authors have refused to accept *Nolana* in the Solanaceae (Hunziker, 2001), it is clear that the diagnosis of the family has changed and *Nolana*, with 5-merous gynoecium and mericarps, is now generally accepted (Olmstead & al., 1999; Knapp, 2002). It is noteworthy that with the placement of *Nolana* within the Solanaceae, its 89 species makes it one of the larger genera in the family, e.g., *Solanum* (1,000 spp.), *Lycianthes* (200 spp.), *Cestrum* (175 spp.), *Lycium* (83 spp.) and *Nicotiana* (75 spp.) (Hunziker, 2001).

The number of genera and species accepted in *Nolana* has been highly controversial, largely due to the fact that many are succulents and lose characters when rendered flat in herbarium collections. Workers that have studied *Nolana* in the field have generally tended to recognize more genera and species. Lindley (1844) accepted 19 species in five genera, four proposed by him; *Nolana* L. f., *Alonia* Lindl., *Dolia* Lindl., *Sorema* Lindl., and *Aplocarya* Lindl. Dunal (1852) accepted the group as a tribe in the Solanaceae and recognized 33 species in five genera; *Nolana*, *Dolia*, *Alibrexia* Miers, *Aplocarya*, and *Bargemontia* Gaud. Bentham & Hooker (1873) recognized 27 species in four genera, *Alona*, *Nolana*, *Dolia*, and *Bargemontia*, in tribe Nolaneae of the Convolvulaceae. Johnston (1936) provided the first modern monograph treating 63 species in two genera, *Alona* and *Nolana*. Mesa (1981, 1986) interpreted the group narrowly, accepting only 18 species in a single genus, *Nolana*, containing two sections, *Nolana* sect. *Alona* (Lindl.) Miers with 5 species and *Nolana* sect. *Nolana* with 13 species in two subsections, subsect. *Bargemontia* (Gaud.) Mesa (7 spp.) and subsect. *Nolana* (6 spp.). D'Arcy (1991) recognized 22 species in two genera, *Alona* and *Nolana* in the tribe Nolaneae (Solanoideae). Recently, Mesa (1997; & al., 1998) has expanded his species concepts to accept 70 species. Tago-Nakazawa & Dillon (1999) recognized 84 species in a single genus, *Nolana*, with two subgenera, *Alona* and *Nolana*. Dillon (2005) recognized 86 species and subsequent field studies have led to the discovery of additional taxa with 89 species currently accepted (Dillon & al. 2007a, b). Segregate genera and previously proposed subgeneric categories need to be tested within a robust phylogenetic framework.

An initial attempt to construct a phylogeny for *Nolana*, using ITS sequence data and a much smaller sampling of 37 species, was not successful in resolving relationships (Tago-Nakazawa & Dillon, 1999). The ITS results did recover a few weakly supported clades, but overall the lack of resolution made interpretation difficult. In the current study, we recover a more fully resolved phylogeny of *Nolana* using sequences of the granule-bound starch synthase I (GBSSI) or the *waxy* gene utilizing 63 of the 89 recognized species. The GBSSI gene is about 3 Kb long and contains 12 introns in *Solanum tuberosum* (van der Leij & al., 1991). It occurs as a single copy in *Capsicum* (Walsh & Hoot, 2001) and has been shown to be an informative phylogenetic marker in *Solanum* sect. *Lycopersicon* (Peralta & Spooner, 2001) and testing subgeneric categories in *Solanum* in combination with additional markers (Weese & Bohs, 2007). The phylogeny provides a framework to begin testing the previous classification schemes with the recognition of sister group relationships and hypotheses of broad-scale biogeographic patterns within the deserts of coastal Peru and Chile. *Nolana galapagensis*, an endemic species to the Galápagos Islands, was included in the analysis and results allow us to test hypotheses of its origin among the continental species.

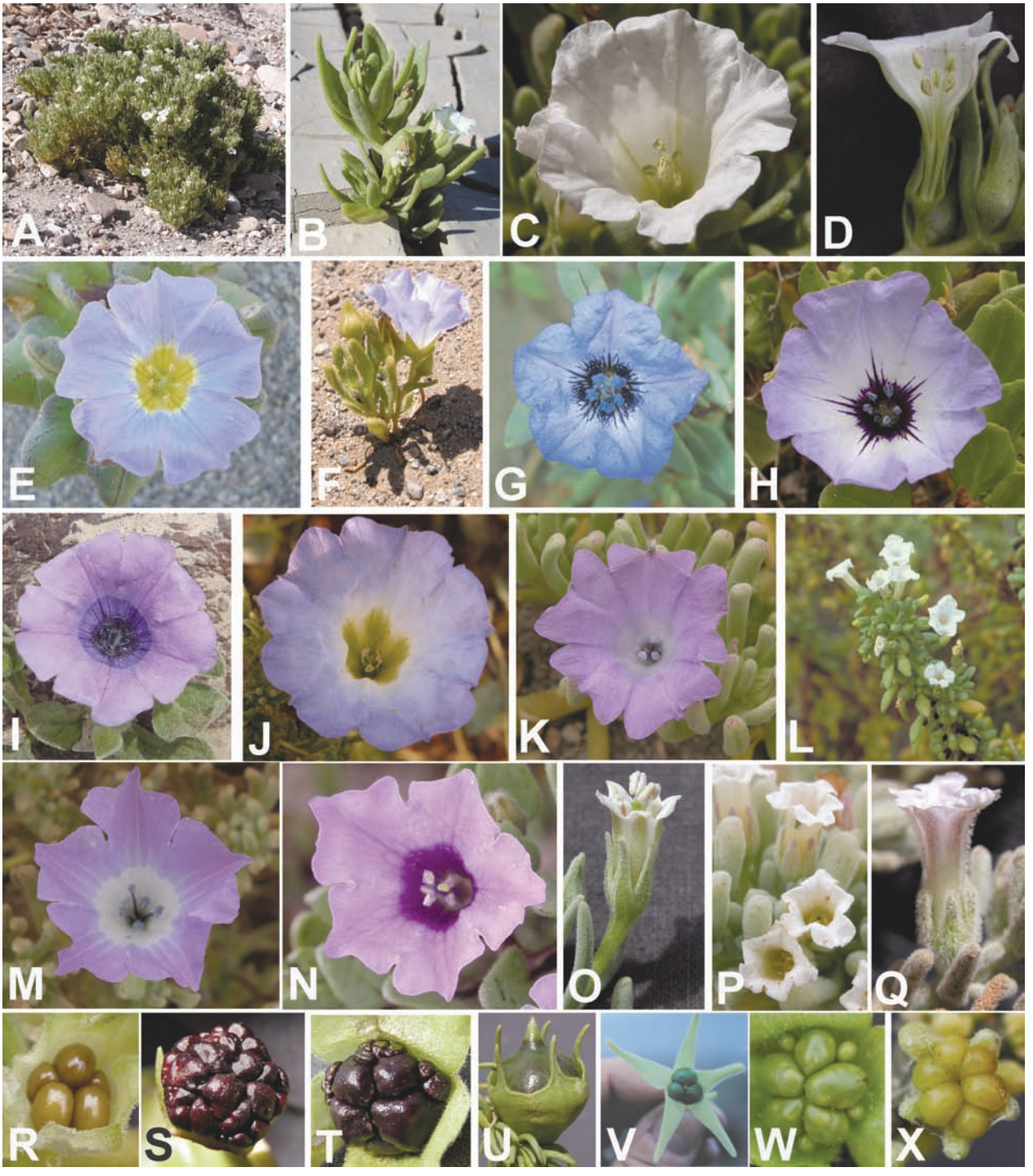

Fig. 2. Diversity in *Nolana*, illustrating species included in the molecular analysis. A–D, *N. sessiliflora*: A, shrubby habit; B, seedling; C, corolla; D, corolla dissected to reveal anthers. E–Q, *Nolana* corollas: E, *N. paradoxa*; F, *N. baccata* seedling in flower; G, *N. humifusa*; H, *N. spathulata*; I, *N. weissiana*; J, *N. coelestis*; K, *N. linearifolia*; L, *N. galapagensis* apical branch with small succulent leaves and white tubular corollas; M, *N. stenophylla*; N, *N. adansonii*; O, *N. werdermannii*; P, *N. crassulifolia*; Q, *N. villosa*. R–X, Mericarps: R, *N. sessiliflora*; S, *N. paradoxa*; T, *N. spathulata*; U, *N. filifolia*; V, *N. stenophylla*; W, *N. weissiana*; X, *N. peruviana*.

MATERIALS AND METHODS

Taxon sampling. — Sequences of the GBSSI gene were obtained from 63 species (76 accessions) of *Nolana*, including 37 species from Chile, 25 species from Peru, and one species, *N. galapagensis*, from the Galápagos Islands, Ecuador. This sampling represents approximately 70% of the specific diversity in *Nolana* and most of the species which remain un-sampled are narrow endemics, only known from type specimens collected in the late 1800s and the beginning of the last century (e.g., *N. foliosa* (Phil.) I.M. Johnst., *N. insularis* (I.M. Johnst.) I.M. Johnst., *N. pearcei* I.M. Johnst., *N. platyphylla* (I.M. Johnst.) I.M. Johnst., *N. polymorpha* Gaud., *N. weberbaueri* I.M. Johnst.), and others that are likely extinct due to habitat destruction (e.g., *N. minor* Ferreryra). The species sampled included annual and perennial herbs and shrubs from a variety of ecological preferences and representatives from both coastal and inland habitats, such as *N. urubambae* from 3,000 m and 350 km from the ocean.

In an analysis using several molecular markers, R. Olmstead (pers. comm.) recovered a well-supported clade in the Solanaceae containing *Grabowskia*, *Lycium*, *Nolana*, *Phrodus*, and *Sclerophylax*, and of those, the tribal name with priority is the Nolaneae Rchb. (Reichenbach, 1837). Four species from three of the closely related genera, *Grabowskia glauca*, *Lycium americana*, *L. deserti*, and *Phrodus microphyllus* were thus employed as outgroups for our *Nolana* analysis.

All samples were collected by the authors, unless otherwise noted. Vouchers are housed at Field Museum (F) and the Smithsonian Institution (US), and duplicates are deposited at the Museo Nacional de Historia Natural, Santiago, Chile (SGO) and Herbario Antenor Orrego, Trujillo, Peru (HAO), Herbario de Universidad Nactional de San Agustín, Arequipa, Peru (HUSA), and the Charles Darwin Research Station, Santa Cruz, Galápagos Islands (CDS). All sequences have been deposited in GenBank.

Molecular methods. — Total DNA was extracted from 15 mg silica-gel-dried leaf material using the Dneasy (QIAGEN) extraction kits or the modified CTAB (hexadecyltrimethylammonium bromide) extraction method (Doyle & Doyle, 1987). The GBSSI gene was amplified with primers B and C_R, and was sequenced with primers B, D_R and C_R of Peralta & Spooner (2001). All PCR reactions were performed in 25 µl reaction-mixture volumes using reagents and manufacturer's instructions for the *Taq* polymerase (JumpStart RED Accutaq DNA Polymerase, Sigma, St. Louis, MO). The amplified products were then purified using the polyethylene glycol (PEG) precipitation following the manufacturer's protocols. Cycle sequencing was conducted using BigDye 3.1 reagents with an ABI 3100 automated sequencer (Applied Biosystems, Foster City, California, U.S.A.). Sequences were then aligned with ClustalX version 1.83 (Thompson & al., 1997), followed by manual adjustments. The aligned sequences cover intron 3 through exon 8 of the GBSSI gene.

Directly sequencing the PCR products of eight species (*N. plicata*, *N. weissiana*, *N. coelestis*, *N. adansonii*, *N. arenicola*, *N.arequipensis*, *N. pallida*, *N. werdermannii*) produced ambiguous sequences. The PCR products of samples of these taxa were cloned using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA, U.S.A.). Five to eight clones for each sample were selected and sequenced.

Phylogenetic analysis. — All sequences were deposited in GenBank (see Appendix for accession numbers). Phylogenetic analyses of the data set was performed with PAUP* (Swofford, 2001) using maximum parsimony (Swofford & al., 1996) and the Bayesian method (Rannala & Yang, 1996; Mau & Newton, 1997; Mau & al., 1999). Parsimony analysis was performed using a heuristic search with 100 random taxon addition replicates, tree bisection-reconnection (TBR) branch swapping, collapse of zero-length branches, and character state changes equally weighted in the analysis. Gaps were treated either as missing data or as new characters. The amount of support for monophyletic groups revealed in the maximally parsimonious tree(s) (MPTs) was examined with 500 bootstrap replicates (Felsenstein, 1985) with the random addition and the heuristic search options.

Bayesian inference was conducted using MrBayes version 3.0 (Huelsenbeck & Ronquist, 2001) with the model as estimated above. The Markov Chain Monte Carlo algorithm was run for 2,000,000 generations with four (4) incrementally heated chains, starting from random trees and sampling one out of every 100 generations. The first 2,000 to 5,000 trees were discarded as burn-in, depending on when chains appeared to have become stationary, and the remaining trees were used to construct Bayesian consensus trees. Internodes with posterior probabilities ≥ 95% were considered statistically significant.

RESULTS

Of the 891 total aligned characters in the GBSSI data, 660 characters are constant, 137 variable characters are parsimony-informative. Treating gaps as missing data, the parsimony analysis generated 1,193,600 most parsimonious trees (MPTs) with a tree length of 304 steps, a consistency index (CI) of 0.87, a CI excluding uninformative characters of 0.82, and a retention index (RI) of 0.97. The strict concensus tree is presented in Fig. 3. When gaps were treated as new characters, the strict consensus tree of the parsimony analysis had nearly identical topology as in Fig. 3, except that the following three subclades became unresolved: (1) (*N. lezamae*, *N. humifusa*), (2) ((*N. lezamae*, *N. humifusa*) *N. aticoana*), and (3) (((*N. lez-*

amae, *N. humifusa*) *N. aticoana*) *N. urubambae*). These three subclades were only weakly supported in Fig. 3. With *Grabowskia glauca*, *Lycium americana*, *L. deserti*, and *Phrodus microphylla* as outgroups, *Nolana* is well-supported to be monophyletic (BS = 100, PP = 100) with *Nolana sessiliflora* basal to the remainder of *Nolana*, and two well-supported clades designated Clade I ~ A–E (BS = 98) and Clade II ~ F–H (BS = 98) in Fig. 3.

Subclade A has strong bootstrap support (>90%) and contains a morphologically cohesive group composed of *N.*

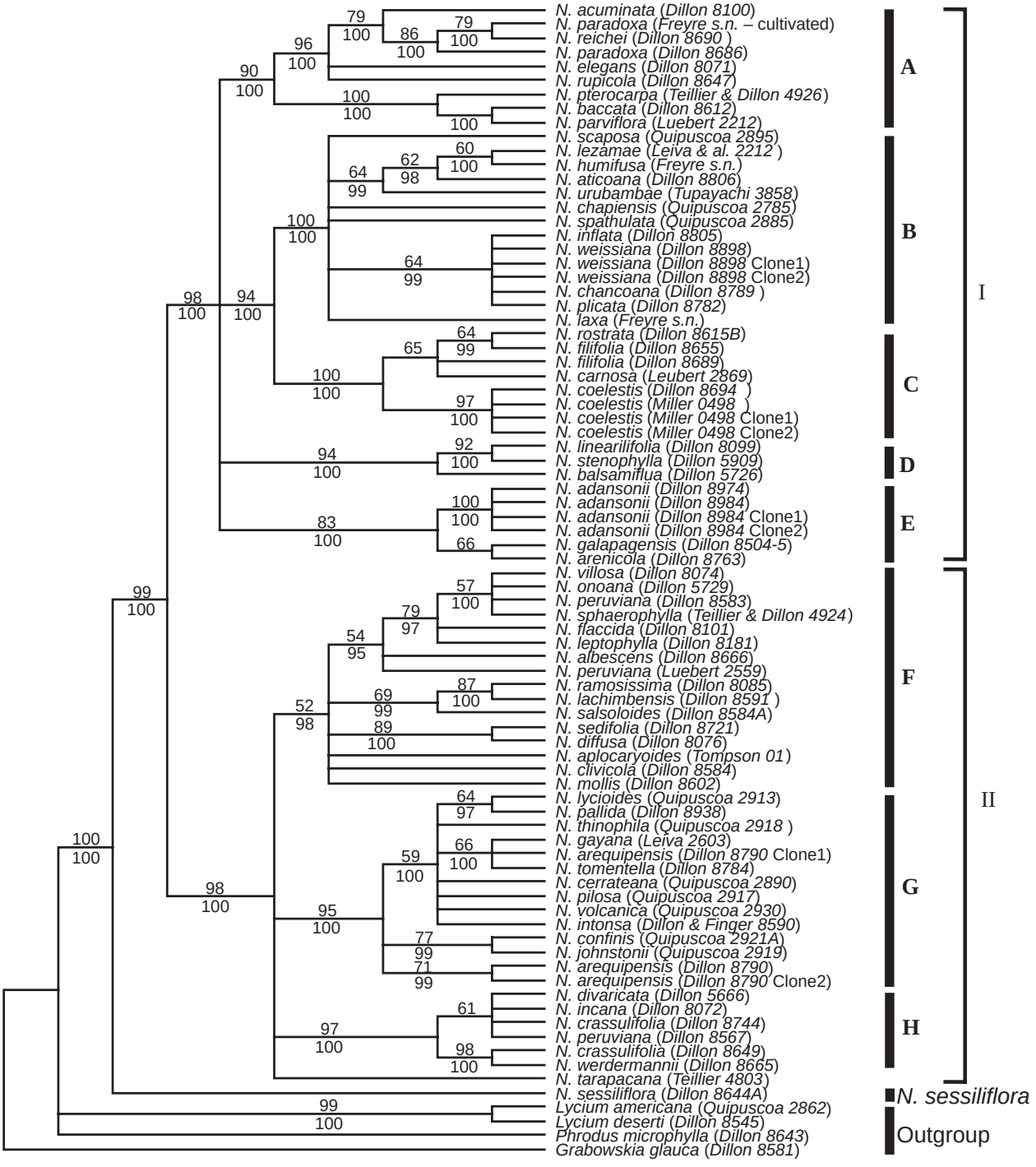

Fig. 3. Strict consensus cladogram of the most parsimonious trees of *Nolana* based on GBSSI sequences with bootstrap values > 50% above the clades and the Baysean values below the clades. Major clades are annotated I and II, and subclades A–H.

acuminata, *N. baccata*, *N. elegans*, *N. reichei*, *N. parviflora*, *N. pterocarpa*, and *N. paradoxa*. This clade is essentially a Chilean group and was also recovered by previous studies by ITS and *matK* sequence data generated by Tago (1999). Subclade B-C with strong support (BS = 94) with two groups, subclade B (BS = 100), a strictly Peruvian group with *N. scaposa*, *N. lezamae*, *N. humifusa*, *N. aticoana*, *N. urubambae*, *N. chapiensis*, *N. spathulata*, *N. inflata*, *N. weissiana*, *N. chancoana*, *N. plicata*, and *N. laxa*. Clade C (BS = 100), is a strictly Chilean group containing, *N. rostrata*, *N. filifolia*, N. carnosa, and *N. coelestis*. Subclade D (BS = 94) is a strictly Chilean group containing *N. linearifolia*, *N. stenophylla*, and *N. balsamiflua*. Subclade E (BS = 83) is a group containing *N. adansonii*, *N. arenicola*, both from Peruvian populations, and *N. galapagensis*.

Subclade F (BS = 52) is a weakly supported (< 90%) group, composed entirely of Chilean species, *N. villosa*, *N. onoana*, *N. peruviana*, *N. sphaerophylla*, *N. flaccid*, *N. leptophylla*, *N. albescens*, *N. ramosissima*, *N. lachimbensis*, *N. salsoloides*, *N. sedifolia*, *N. diffusa*, *N. aplocaryoides*, *N. clivicola*, and *N. mollis*. Subclade G (BS = 95) is composed of the Peruvian species, *N. lycioides*, *N. pallid*, *N. thinophila*, *N. gayana*, *N. arequipensis*, *N. plicata*, *N. cerrateana*, *N. pilosa*, *N. volcanica*, *N. confinis*, and *N. johnstonii*. Also grouped here is *N. intonsa* found in northern Chile, but sharing morphological characters with this group. Subclade H (BS = 100) contains Chilean species, *N. divaricata*, *N. incana*, *N. crassulifolia*, and *N. werdermannii*. The placement of an accession of *N. peruviana* in this clade was not expected. *Nolana tarapacana*, a Chilean species from the interior of northern Chile, is part of the unresolved polytome with subclades F–H.

DISCUSSION

Phylogenetic relationships. — *Nolana* is well-supported as a monophyletic group (Fig. 3), with *N. sessiliflora* as the sister to the clade consisting of the remainder of the genus. *Nolana sessiliflora* (Fig. 2A–D) occurs in hyper-arid environments at elevations approaching 2,500 meters and approximately 120 km east from the Pacific coast. Its position as sister taxon to the remainder of the genus is of interest given its ecological preferences are in an extremely barren habitat above 2,500 m and far from the effects of coastal fogs.

Two large clades (I, II) are recovered in the remainder of the genus, each with strong support and forming polytomies with groups with varying degrees of support (Fig. 3). Clade I of the polytomous subclades A, B, C, D, E is recovered with a high degree of support (BS = 98, PP = 100), and Clade II of the polytomous subclades F, G, H, and *N. tarapacana* is recovered with strong support (BS = 98, PP = 100).

The species in subclade A (Fig. 3) form a well-supported, monophyletic group diagnosed by distinctive morphological features, including basal rosettes, thick taproots, large showy corollas and 10–20 mericarps. As mentioned previously, this group of species was recovered as a monophyletic group by other molecular markers, including ITS and *matK* (Tago-Nakazawa & Dillon, 1999). The distribution of this group essentially covers the entire coast of Chile from ca. 20° to 42° S, a distance of over 3,000 km. *Nolana jaffuelii*, a species with a northern Chilean distribution with one report from extreme southern Peru (18° S), was not available for the present analysis. It is here associated with subclade A by virtue of overall morphological similarity. Most of the species (*N. acuminata*, *N. baccata*, *N. elegans*, *N. jaffuelii*, *N. parviflora*, *N. pterocarpa*, *N. rupicola*) exhibit broadly overlapping geographic distributions in coastal sites from 22°05′ to 32°00′ S. *Nolana paradoxa* (Fig. 2E, S) has an extended distribution of over 1,200 km, from central Chile (32°00′ S) to as far south as Isla Chiloé (42°30′ S) and Isla Talcán (42°45′ S) in near shore or beach habitats (0–100 m). Another central Chilean species, *N. crassulifolia*, is a prostrate shrub often covering rocks just above high tide, possessing small, white tubular corollas (Fig. 2P) and only distantly relationed to *N. paradoxa* (Fig. 3). Lindley (1844) described *Sorema* and included five species, including *N. acuminata*, *N. elegans*, *N. paradoxa*, *N. parviflora*, and *N. rupicola*. As a subgeneric group, the members are monophyletic and easily diagnosed morphologically and should support be found in additional markers it may merit sectional status. Mesa (1981) treated the eight species accepted here in clade A as synonyms of *N. paradoxa*.

A subclade consisting of B and C has strong support (BS = 94) and the individual subclades B and C are strongly supported (BS = 100). Subclade B lacks internal resolution and forms a large polytomy, but it does recover a group of species strictly Peruvian in distribution and morphologically variable. While the distribution of most are considered coastal (e.g., *N. scaposa*, *N. humifusa*, *N. spathulata*, *N. inflata*, *N. plicata*, *N. weissiana*, *N. chancocana*), some species are distributed exclusively outside of the *lomas* formations in upland interior localities at elevations of over 2,500 m, e.g., *N. lezamae*, *N. laxa*, *N. urubambae*, and *N. chapiensis*. The clade recovering *N. lezamae*, *N. humifusa* (Fig. 3G), *N. aticoana*, *and N. urubambae* has relatively low support (BS = 64) and similarities in overall morphology also suggested their relationships. This clade collapsed when gaps were treated as new characters. This relationship clearly needs to be tested in our further studies.

The species in subclade C (Fig. 3) form a well-supported monophyletic group (BS = 100) with a strictly Chilean distribution and diagnosed by morphological features, including erect woody or shrubby habits, large

showy flowers, and highly fused mericarps with apical stigmas. The clones of *N. coelestis* all cluster together, and are sister to *N. carnosa*, *N. rostrata*, and a paraphyletic *N. filifolia*. These species share the synapamorphy of the modification of the ovary into 3–5-sulcate, multi-seeded mericarps, broadly affixed to the receptacle and joined to one another laterally (Fig. 2U). Johnston (1936) relied heavily upon the strength of the position of the style to diagnose *Alona* Lindl. (Johnston, 1936; Lindley, 1844), and it has been treated at the rank of subgenus (Mesa, 1986). Johnston (1936) also included *N. balsamiflua* and *N. stenophylla* (subclade D) in *Alona*, both Chilean species that share the character of fused mericarps (Fig. 2V) and apical stigmas. One unexpected result was the strong support (BS = 94) for the inclusion of *N. linearifolia* in subclade D (Fig. 3) in spite of its non-apical style and mericarp morphology distinct from that of *Alona* with 4–6, deeply lobed, spherical mericarps attached basally to the receptacle and free, or practically so. *Nolana linearifolia* (Fig. 2K) is distributed in the same general region in northern Chile as *N. stenophylla* (Fig. 2M), but it has not been recorded as actually sympatric. In the herbarium, the two have been confused, but the former species does not share the mericarp morphology peculiar to *Alona* and is a weakly lignified, prostrate perennial herb. Among the various genera that have been proposed for taxa within *Nolana*, only a portion of subgenus *Alona* Lindl. and the aforementioned, *Sorema* Lindl., are monophyletic in this analysis.

Subclade E has moderate support (BS = 83) and contains *N. adansonii* as sister to a weakly supported clade (BS = 66) with *N. arenicola* and *N. galapagensis*. The various clones of *N. adansonii* strongly cluster together (BS = 100), and while this species has been recorded for northern Chile, only Peruvian accessions were included in this analysis. This result was not anticipated, given the lack of morphological similarity between these three taxa. The shrubby habit, small succulent leaves, and white tubular corollas found in *N. galapagensis* (Fig. 2L) are dissimilar from those of either *N. arenicola* or *N. adansonii*, both essentially facultative annuals with laminar leaves and much larger corollas (Fig. 2N). Overall morphology suggested that *N. arenicola* and *N. spathulata* share relationships, a result not reflected in this analysis. Further studies involving additional markers will be necessary to test this hypothesis of relationships. Likewise, the lack of resolution for *N. aticoana*, a Peruvian species most closely resembling *N. spathulata*, must be investigated.

The second large polytomous clade of subclades F, G, H, and *N.tarapacana* is well-supported (BS = 97) and contains clades of strictly Chilean or Peruvian species. Subclade F has only weak support (BS = 52) but is a large polytomy of 16, morphologically variable species, typically erect shrubs with moderately small, white to blue or lavender corollas. They all occupy habitats in northern Chile, either from near coast (e.g., *N. ramosissima*, *N. lachimbensis*, *N. diffusa*, *N. mollis*, *N. clivicola*, *N. salsolioides*, *N. aplocaryioides*, *N. sedifolia*, *N. peruviana*) or from interior environments (e.g., *N. onoana*, *N. albescens*), and some species distributions span both of these (e.g., *N. leptophylla*, *N. villosa*, *N. flaccida*, *N. sphaerophylla*).

Subclade G has strong support (BS = 95) and contains twelve Peruvian species, and *N. intonsa*, a northern Chilean endemic. Based upon overall morphological similarity, *N. intonsa* more closely resembles species restricted to southern Peru that shared its dense villous pubescence and corollas with dark purple throats. Johnston (1936) related *N. intonsa* to *N. gayana*, but overall similarity is with *N. pallida* or *N. cerrateana*, two species who share the dark purple corolla throats and villous pubescence. Studies currently underway utilizing additional markers should help to resolve this large polytomy. *Nolana lycioides* has its greatest distributional range in southern Peru, but one outlier population in northern Chile has been reported (Mesa & al., 1998). These two species, and *N. adansonii*, suggests some connections between southern Peru and populations in the *lomas* formations near Iquique, Chile. The timing of this floristic interchange or connections in the past has not been established. The accession of *N. lycioides* used in this analysis was from Peru, and we are seeking material from the Chilean population.

Subclade H has strong support (BS = 97) and reveals a group of strictly Chilean, shrubby species with small, tubular, often white, corollas, e.g., *N. divaricata*, *N. incana*, *N. werdermannii*, and *N. crassulifolia*. *Nolana divaricata* is a glabrous species, often confused with *N. salsoloides*, a species that has a more southern distribution and densely puberulent foliage. *Nolana incana* is often confused with *N. albescens*, which has a more southern distribution, and differ in habit and details of the floral structure. *Nolana crassulifolia* (Fig. 2P) and *N. werdermannii* (Fig. 2O) have often been confused and assumed to be conspecific, but they have quite different ecological preferences and distinct floral and foliar morphology. *Nolana crassulifolia* occurs in extremely close proximity to the ocean, its prostrate branches often cascading over rocks, whereas, *N. werdermannii* is usually in some distance from the ocean and its habit a more erect, globose shrub.

The lack of resolution for *N. tarapacana* must be investigated further since its overall morphology does not suggest any close relationships among other Chilean species in this analysis. It possesses small corollas with ovaries of only two mericarps and inhabits arid sites above 1,400 m in extreme northern Chile. It may be that its relationships are with the morphologically similar *N. gracillima*, a species not available for this analysis.

Implications for infrageneric classification. — Of all the segregate genera proposed for species generally accorded to *Nolana*, none of the following were clearly revealed as monophyletic groups in the present analysis, and include *Alibrexia* Miers, *Aplocarya* Lindl., *Bargemontia* Gaud., *Dolia* Lindl., *Gubleria* Gaud., *Leloutrea* Gaud., *Neudorfia* Adan., *Osteocarpus* Phil., *Rayera* Gaud., *Teganium* Schmidel, *Tula* Adans., *Velpeaulia* Gaud., and *Zwingera* Hofer.

As mentioned previously, the taxa attributed to *Sorema* Lindl. (= *Periloba* Raf.) do form a cohesive group that may eventually warrant subgeneric recognition. This clade was also recovered in the analysis of Tago-Nakawaza & Dillon (1999) using ITS data. There are no other groups that would warrant recognition of subgenera or sections at this time. The potential acceptance of *Alona* Lindl. must await further analysis given that the results here with subclades C and D both containing species considered members of *Alona*. The phylogeny-based classification scheme for *Nolana* will be proposed when results from additional markers are available.

Biogeographic patterns. — With the results from only one molecular marker (GBSSI), it is premature to rigorously test major biogeographic hypotheses. However, it is useful to pose the most obvious questions and discuss current hypotheses. Patterns of similarity within the overall flora of the *lomas* formations suggest non-uniform distribution of taxa (Rundel & al., 1991; Dillon & al., 2003). Three primary floristic sectors have been proposed within the coastal deserts of western South America based upon analysis of presence and absence data a north Peru unit (7°55′ to 12° S latitude), a south Peru unit (12° to 18° S), and a north Chile unit (20° to 28° S) (Duncan & Dillon, 1991; Rundel & al., 1991). Most endemics are centered either in southern Peru or northern Chile, a pattern we find in *Nolana* endemics as well. Southern Peru and the Department of Arequipa, with no fewer than 27 species, represent a concentration of species; however, the composition is a mixture of taxa from several subclades in Clade I (B, E) and Clade II (G). In the current analysis, we find twelve Peruvian species in Clade I and an equal number in Clade II. Northern Peru has four endemic species, three of which are included in this analysis and found in subclade B of Clade I (*N. lezamae*, *N. humifusa*) and subclade G of Clade II (*N. gayana*). Ostensibly, this represents two, independent events, either long-distance dispersal or a vicariant event, with subsequent radiation. Another area with 30 species in the northern Chile in Región II (Antofagasta) and Región III (Atacama) likewise contain taxa from Clade I and Clade II.

These three major areas of endemism may be refined, and further divided but at this time three areas of endemism have been recognized in *Nolana*: north Peru, south Peru, north Chile. It is apparent that extreme northern Chile has been a barrier to dispersal (Dillon & al., 2003). With only ca. 6% of the entirety of the vascular floras having distributions on either side of the Peru/Chile border (~ 18° S latitude), it appears that this region has acted as a strong barrier to dispersal. The area between 18° to 20° S latitude is marked by the coastal range pulling away from the shoreline and an absence of suitable topography for capture of fog. The fact that our results imply at least two, asynchronous, migrations from Chile into Peru and followed by subsequent radiation, indicates that dispersal has obviously played a role in the distribution of the genus.

While the vast majority of *Nolana* species occupy habitats in the near coastal fringe of the deserts of coastal Peru and Chile, there are several species that have distributions far from the coast and at higher elevations, well outside of the influences of the coastal fogs. As discussed previously, *N. sessiliflora*, the sister taxon to the remainder of *Nolana*, is distributed well outside of the fog zone at nearly 2,500 m and over 100 km from the coast. It is morphologically distinct and how it derives its moisture in this severe hyper-arid region is not currently known, but the vegetative surfaces are covered with dense, glandular trichomes. The shrubby habit with glandular pubescence may be a shared plesiomorphic character found in the outgroups, *Phrodus* and *Lycium* as well. The hypothesis, that ancestral *Nolana* were shrubby, high-elevation elements that migrated to coastal and near-coastal environments, and subsequently diversified there will be tested with additional marker data.

In Peru, three species are distributed well outside the coastal influences. *Nolana urubambae* is restricted to a single locality south of the city of Urubamba (Dept. Cusco), more than 350 km from the coast and at +3,000 m. The entire population encompasses only a few hectares and contains perhaps a hundred individuals. *Nolana chapiensis* is restricted to a locality southeast of Arequipa (Dept. Arequipa) over 50 km inland and over 2,000 m. *Nolana lezamae* is restricted to a locality near the village of Corongo (Dept. Ancash) nearly 100 km from the coast and over 2,000 m. Initial results suggest that these three, high elevation, inland species share relationships, and these are most closely related to *N.humifusa*, a strictly coastal species with small isolated populations ranging from Lima north to Trujillo. *Nolana laxa* of the central coast occurs over 50 km from the ocean and at elevations above 500 m.

That coastal species were derived from upland species has been suggested in the evolution of diversity in *Jaltomata* (T. Mione, pers. comm.) and *Nicotiana* (Clarkson & al., 2004). Alternatively, the upland or high-elevation species may have radiated after derivation from coastal ancestors. Testing hypotheses of speciation patterns must await more fully resolved estimates of relationships.

Origin of Nolana galapagensis. — Upon purely comparative morphological criteria, *N. galapagensis* compares favorably with *N. sedifolia* of coastal Chile. Both robust shrubs with highly reduced, succulent leaves, each with small white, tubular corollas, and overall similarity of mericarp shape and number. For this reason, the inferred relationships of *N. galapagensis* with Peruvian species, *N. adansonii* and *N. arenicola*, was unexpected. *Nolana galapagensis* (Clade I, subclade E) and *N. sedifolia* (Clade II, subclade F) are suggested to be quite distantly related (Fig. 3). Perhaps the morphology exhibited in *N. galapagensis* is plesiomorphic and not unexpected, but the gross morphological differences which exist between *N. adansonii*, *N. arenicola*, and *N. galapagensis* must be examined in light of additional data. Until results imply otherwise, we must entertain the possibility that the progenitors of *N. galapagensis* reached the archipelago from southern Peru. *Nolana galapagensis* occurs on no fewer than six islands in the chain; however, only one accession of *N. galapagensis* was available for this study.

Based upon *matK*, Tago (1999) estimated the divergence time of *N. galapagensis* from the remainder of the genus ca. 4 mya and came to a much older age of 8.1 mya based upon ITS data. Geological evidence points to an age for the current Galápagos archipelago at 4–5 million years, but underwater seamounts may date to 15–20 million years (Grehan, 2001). Molecular clock calibrations for determining colonization events may provide estimates that exceed the age of the modern islands. Given its morphology, it appears that *N. galapagensis* was pre-adapted to the arid, coastal beach environments prior to its dispersal to the island chain (Tago-Nakazawa & Dillon, 1999). We will estimate the divergence time of *N. galapagensis* once its phylogenetic position has been tested with additional molecular markers.

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Appendix. List of the taxa used in the GBSSI analysis, geographic origins, voucher numbers, and GenBank accession numbers of the investigated taxa.

Taxon, origin (country, department or región), voucher (herbarium), GenBank #

Grabowskia glauca (Phil.) I.M. Johnst., Chile (Antofagasta), *Dillon 8581* (F), **EU051857**. *Lycium americana* Jacq., Peru (Arequipa), *Quipuscoa 2862* (F), **EU051858**. *L. deserti* Phil., Chile (Antofagasta), *Dillon 8545* (F), **EU051859**. *Nolana acuminata* (Miers) Miers ex Dunal, Chile (Región II), *Dillon 8100* (F), **EU051828**. *N. adansonii* (Roem. & Schult.) I.M. Johnst., Peru (Arequipa), *Dillon 8974* (F), **EU051884**; *Dillon 8984* (F), **EU051885**, clone1, **EU051886**, clone2, **EU051887**. *N. albescens* (Phil.) I.M. Johnst., Chile (Región III), *Dillon 8666* (F), **EU051908**. *N. aplocaryoides* (Guadich.) I.M. Johnst., Chile (Región II), *Thompson 01* (F), **EU051849**. *N. arenicola* I.M. Johnst., Peru (Arequipa), *Dillon 8763* (F), **EU051904**. *N. arequipensis* M.O. Dillon & Quipuscoa, Peru (Arequipa), *Dillon 8790* (F), **EU051890**, clone1, **EU051889**, clone2, **EU051891**, *N. aticoana* Ferreyra, Peru (Arequipa), *Dillon 8806* (F), **EU051896**. *N. baccata* (Lindl.) Dunal, Chile (Región III), *Dillon 8612* (F), **EU051865**. *N. balsamiflua* (Gaudich.) Mesa, Chile (Región II), *Dillon 5726* (F), **EU051905**. *N. carnosa* (Lindl.) Miers ex Dunal, Chile (Región III), *Luebert 2869* (F), **EU051892**. *N. cerrateana* Ferreyra, Peru (Arequipa), *Quipuscoa 2890* (F), **EU051850**. *N. chancoana* M.O. Dillon & Quipuscoa, Peru (Arequipa), *Dillon 8789* (F), **EU051893**. *N. chapiensis* M.O. Dillon & Quipuscoa, Peru (Arequipa), *Quipuscoa 2785* (F), **EU051854**. *N. clivicola* (I.M. Johnst.) I.M. Johnst., Chile (Región II), *Dillon 8584* (F), **EU051862**. *N. coelestis* (Lindl.) Miers ex Dunal, Chile (Región IV), *Dillon 8694* (F), **EU051875**; *Miller 0498* (US), **EU051881**, clone1, **EU051882**, clone2, **EU051883**. *N. confinis* I.M. Johnst., Peru (Moquegua), *Quipuscoa 2921A* (F), **EU051860**. *N. crassulifolia* Poepp., Chile (Región III), *Dillon 8744* (F), **EU051888**; *Dillon 8649* (F), **EU051869**. *N. diffusa* I.M. Johnst., Chile (Región II), *Dillon 8076* (F**)**, **EU051907**. *N. divaricata* (Lindl.) I.M. Johnst., Chile (Región II), *Dillon 5666* (F), **EU051844**. *N. elegans* (Phil.) Reiche, Chile (Región II), *Dillon 8071* (F), **EU051847**. *N. filifolia* (Hook. & Arn.) I.M. Johnst., Chile (Región III), *Dillon 8655* (F), **EU051870**; *Dillon 8689* (F), **EU051873**. *N. flaccida* (Phil.) I.M. Johnst., Chile (Región II), *Dillon 8101* (F), **EU051841**. *N. galapagensis* (Christoph.) I.M. Johnst., Ecuador (Galápagos Islands), *Dillon 8504* (F), **EU051895**. *N. gayana* (Gaudich.) Koch, Peru (Ancash), *Leiva 2603* (F), **EU051846**. *N. humifusa* (Gouan) I.M. Johnst., Peru, *Freyre s.n.* (F), **EU051900**. *N. incana* (Phil.) I.M. Johnst., Chile (Región II), *Dillon 8072* (F), **EU051845**. *N. inflata* Ruiz & Pav., Peru (Arequipa), *Dillon 8805* (F), **EU051877**. *N. intonsa* I.M. Johnst., Chile (Región I), *Dillon & Finger 8590* (F), **EU051876**. *N. johnstonii* Vargas, Peru (Moquega), *Quipuscoa 2919* (F), **EU051861**. *N. lachimbensis* M.O. Dillon & Luebert, Chile (Reigón II), *Dillon 8591*, **EU051863**; *N. laxa* (Miers) I.M. Johnst., Peru (Lima), *Freyre s.n.* (F), **EU051897**. *N. leptophylla* (Miers) I.M. Johnst., Chile (Región II), *Dillon 8181* (F)**, EU051842**. *N. lezamae* M.O. Dillon, S. Leiva & Quipuscoa, Peru (Ancash), *Leiva 2212* (F), **EU051836**. *N. linearifolia* Phil., Chile (Región II), *Dillon 8099* (F), **EU051832**. *N. lycioides* I.M. Johnst., Peru (Arequipa), *Quipuscoa 2913* (F), **EU051831**. *N. mollis* Phil., Chile (Región II), *Dillon 8602* (F), **EU051864**. *N. onoana* M.O. Dillon & M. Nakazawa, Chile (Región II), *Dillon 5729* (F), **EU051838**. *N. pallida* I.M. Johnst., Peru (Arequipa), *Dillon 8938* (F), **EU051898**. *N. paradoxa* Lindl., Chile (greenhouse cultivated), *Freyre s.n.* (F), **EU051848**; Chile (IV), *Dillon 8686* (F), **EU051872**. *N. parviflora* (Phil.) Phil., Chile (Región III), *Luebert 2212* (F), **EU051906**; *N. peruviana* (Gaudich.) I.M. Johnst., Chile (Región II), *Luebert 2559* (F), **EU051909**; *Dillon 8567* (F), **EU051902**; *Dillon 8583* (F), **EU051839**. *N. pilosa* I.M. Johnst., Peru (Arequipa), *Quipuscoa 2917* (F), **EU051851**. *N. plicata* I.M. Johnst., Peru (Arequipa), *Dillon 8782* (F), **EU051899**. *N. pterocarpa* Phil., Chile (Región III), *Teillier & Dillon 4926* (F), **EU051833**. *N. ramosissima* I.M. Johnst., Chile (Región II), *Dillon 8085* (F), **EU051837**. *N. reichei* M.O. Dillon & Arancio, Chile (Región IV), *Dillon 8690* (F), **EU051874**. *N. rostrata* (Lindl.) Miers ex Dunal, Chile (Región III), *Dillon 8615*B (F), **EU051866**. *N. rupicola* Gaudich., Chile (Región II), *Dillon 8647* (F), **EU051868**. *N. salsoloides* (Lindl.) I.M. Johnst., Chile (Región II), *Dillon 8584*A (F), **EU051853**. *N. scaposa* Ferreyre, Peru (Arequipa), *Quipuscoa 2895* (F), **EU051829**. *N. sedifolia* Poepp., Chile (Región II), *Dillon 8721* (F), **EU051843**. *N. sessiliflora* Phil., Chile (Región II), *Dillon 8644A* (F), **EU051903**. *N. spathulata* Ruiz & Pav., Peru (Arequipa), *Quipuscoa 2885* (F), **EU051856**. *N. sphaerophylla* (Phil.) Mesa ex Dillon, Chile (Región III), *Teillier & Dillon 4924* (CONC), **EU051840**. *N. stenophylla* I.M. Johnst., Chile (Región II), *Dillon 5909* (F), **EU051834**. *N. tarapacana* (Phil.) I.M. Johnst., Chile (Región I), *Teillier 4803* (F), **EU051901**. *N. thinophila* I.M. Johnst., Peru (Arequipa), *Quipuscoa 2918* (F), **EU051835**. *N. tomentella* Ferreyre, Peru (Arequipa), *Dillon 8784* (F), **EU051894**. *N. urubambae* Vargas, Peru (Cusco), *Tupayachi 3858* (F), **EU051855**. *N. villosa* (Phil.) I.M. Johnst., Chile (Región II), *Dillon 8074* (F), **EU051830**. *N. volcanica* Ferreyra, Peru (Arequipa), *Quipuscoa 2930* (F), **EU051852**. *N. weissiana* Ferreyra, Peru (Arequipa), *Dillon 8898* (F), **EU051878**, clone1, **EU051879**, clone3, **EU051880**. *N. werdermannii* I.M. Johnst., Chile (Región IV), *Dillon 8665* (F), **EU051871**. *Phrodus microphylla* (Miers) Miers, Chile (Región II), *Dillon 8643* (F), **EU051867**.