MOLECULAR PHYLOGENETIC STUDIES IN NYCTAGINACEAE: PATTERNS OF DIVERSIFICATION IN ARID NORTH AMERICA

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Dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor
of Philosophy in the Department of
Biology in the Graduate School
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ABSTRACT

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Abstract

The Four O'clock Family (Nyctaginaceae) has a number of genera with unusual morphological and ecological characters, several of which appear to have a "tendency" to evolve repeatedly in Nyctaginaceae. I present a molecular phylogeny for the Nyctaginaceae, consider taxonomic implications, biogeographic patterns, and the evolution of cleistogamy and gypsophily. These characters have each evolved multiple times in the xeric-adapted genera of the family. Further progress towards understanding these phenomena requires specific investigation of the ecology of pollination and gypsum tolerance. In the genus *Boerhavia*, an intensively sampled phylogeny based on internal transcribed spacer (ITS) and nitrate reductase (NIA) sequences provides new insights into relationships among species in the genus, and identifies a clade of annual species centered in the Sonoran Desert. Phylogeographic patterns are present in the genus that may reflect both relatively ancient vicariant events as well as the post-Pleistocene expansion of the Sonoran Desert. Many species in this group are found to be genetically cohesive, however two annual species complexes are found which species were nonmonophyletic. Since several mechanisms can potentially lead to the finding of nonmonophyletic species, Amplified Fragment Length Polymorphisms (AFLPs) were used to examine the structure of genetic variation in the two complexes. These data show that in these two groups, different evolutionary

mechanisms are needed to explain the distribution of genetic diversity within and among populations. A complex comprised of *Boerhavia spicata* and *B. xanti* shows little evidence of genetic divergence between the species in Sonora, a pattern which may indicate recent contact between two very closely related forms. In contrast, high genetic structure between populations is found in the other complex, which contains the species with umbellate inflorescences. This complex includes several nominal species with highly restricted distributions, whose evolution may have been facilitated by low gene flow among populations. Little evidence was found for associations of inbreeding within populations, and floral traits which might be expected to influence outcrossing rates.

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1. Molecular phylogeny of Nyctaginaceae: taxonomy, biogeography, and characters associated with a radiation of xerophytic genera in North America

1.1 INTRODUCTION

Nyctaginaceae Juss. is a family of 28-31 genera and 300-400 species, that contains the familiar cultivated Four O'clocks (*Mirabilis jalapa*) and Bougainvillea (*Bougainvillea* spp.). Nyctaginaceae have long been known to be one of the core group of families of Caryophyllales (Centrospermae) on the basis of the presence of betalain pigments, free-central placentation, p-type sieve tube elements, and the presence of perisperm, as well as molecular evidence (Bittrich and Kühn, 1993; Bremer et al., 2003). Within this group, the modern consensus is that Nyctaginaceae are closely related to certain monocarpellate members of a paraphyletic Phytolaccaceae, especially subfam. Rivinoideae (Rodman et al., 1984; Rettig et al., 1992; Downie and Palmer, 1994; Behnke, 1997; Downie et al., 1997; Cuenoud et al., 2002), although *Sarcobatus* (Sarcobataceae) has also been implicated as a close relative of this group (Behnke, 1997; Cuenoud et al., 2002).

Nyctaginaceae have a uniseriate petaloid perianth, usually interpreted as being sepalous in origin (Rohweder and Huber, 1974). In most taxa the lower part of the

perianth is fleshy or coriaceous and encloses the superior ovary, giving it the appearance of an inferior ovary. This accessory fruit is persistent and accrescent around the mature achene. While technically a diclesium (Bogle, 1974; Spellenberg, 2003), it is typically referred to as an "anthocarp".

Most genera can be recognized on the basis of fruit structure alone. In *Boldoa*, *Cryptocarpus*, and *Salpianthus*, the perianth is persistent but not accrescent, and thus these taxa lack the anthocarp (Bittrich and Kühn, 1993). In *Andradea*, *Leucaster*, and *Reichenbachia*, the perianth is variously accrescent but is not expanded (Bittrich and Kühn, 1993). However, in the remaining genera the anthocarp completely encloses the fruit and takes many forms (Willson and Spellenberg, 1977; Bittrich and Kühn, 1993). In taxa in which anthocarps are ribbed, the 3-10 ribs can be elaborated into wings (*Phaeoptilum*, *Grajalesia, Tripterocalyx*, *Abronia*, and some *Colignonia*, *Acleisanthes*, and *Boerhavia*), covered by viscid glandular hairs or warts (*Pisonia*, *Pisoniella*, *Cyphomeris*, *Commicarpus*, some *Boerhavia* and *Acleisanthes*), or unelaborated, to leave an essentially gravity-dispersed fruit (*Mirabilis*, *Anulocaulis*, *Nyctaginia*, some *Colignonia* and *Boerhavia*). Fleshy anthocarps are probably bird-dispersed in *Neea* and *Guapira*. They are also found in *Okenia*, though this genus is geocarpic and the seeds generally germinate at the spot where they are "planted" by the maternal individual (N. Douglas, personal observation). The unusual anthocarps of *Allionia* are boat-shaped, with two rows of

inward-pointing teeth lining the concave side, suggesting possible exozoochory or wind dispersal, though no observations on this are available. In herbaceous taxa, at least, species-level characters are often found in this structure (Willson and Spellenberg, 1977; Spellenberg, 2003).

The family was treated by Heimerl in *Die Natürlichen Pflanzenfamilien* (Heimerl, 1889, 1934) and by Standley in several papers (Standley, 1909, 1911, 1918, 1931a, b) by which time most of the currently recognized genera had been described. Standley (1931b) formally transferred *Oxybaphus* L'Hér. ex Willd., *Hesperonia* Standl., *Quamoclidion* Choisy, and *Allionella* Rydb. into *Mirabilis*, though this has been overlooked in some floras (e.g. Kearney and Peebles, 1960). Heimerl (1934) synthesized the family as it was known, including in his classification genera which had been recently described by Standley (i.e. *Pisoniella*, *Cuscatlania*). He based his supergeneric classification on a combination of plant habit, indumentum, linear vs. capitate stigma, straight vs. curved embryo, sex distribution, pollen grain morphology, and the occurrence of bracts or involucre (Heimerl, 1934; Bittrich and Kühn, 1993). Bittrich and Kühn (1993) provided the most recent summary of the classification at the tribal and subtribal level (Table 1). Their treatment broadly followed that of Heimerl (1934), adjusting ranks and incorporating genera described after 1934, i.e. *Caribea*. It recognized six tribes, two of

which, Pisonieae and Nyctagineae, contain the majority of genera and species, (Table 1, Pisonieae: six genera, ca. 200 spp.; Nyctagineae: 14 genera, ca. 100 spp.).

Whereas the bulk of diversity of Pisonieae resides in three highly similar arborescent genera with poorly-differentiated species, Nyctagineae sensu Bittrich and Kühn (1993) is a diverse, mainly herbaceous, group recognized largely on the basis of very large (100-200 μm), pantoporate pollen grains, among the largest known in angiosperms (Stevens, 2001). The original formulation of tribe Mirabileae subtribe Boerhaaviinae (Heimerl, 1934), the antecedent of tribe Nyctagineae, was partly diagnosed by the presence of pantoporate pollen grains. Of four currently recognized subtribes, the Nyctagininae comprises those taxa with involucres, which may be of connate or distinct bracts. In contrast, the largest subtribe, Boerhaviinae, is composed of eight genera united primarily by their lack of involucral bracts. Four of these (*Boerhavia*, *Anulocaulis*, *Cyphomeris*, *Commicarpus*) have occasionally been treated as a single *Boerhavia* (Fosberg, 1978). This seems merely to reflect a preference for fewer large genera, as the four segregate genera are as distinct from each other as any other given pair of genera in the herbaceous group. The others, *Caribea*, *Okenia*, and *Acleisanthes*, *Selinocarpus*, (including *Ammocodon*), were placed in Boerhaviinae on the basis of pollen morphology and the absence of involucres subtending flowers or inflorescences (though small subtending bracts may be present). The remaining two subtribes, Colignoniiae

and the monospecific Phaeoptilinae, have aberrant morphology compared to Nyctagininae and Boerhaviinae, for example, pollen grains in *Colignonia* and *Pisoniella* are dramatically smaller and in *Phaeoptilum* they are pantocolpate. In *Pisoniella*, the embryo is straight, typical of Pisoneae, instead of a hooked embryo that encircles the perisperm found in the remaining Nyctagineae (Bittrich and Kühn, 1993). Additionally, the shrubby, scandent or lianoid growth habits of these taxa are rare in the other subtribes, which are mostly perennial herbs. Though Heimerl placed *Colignonia* in a monogeneric tribe Colignonieae, Bittrich and Kühn (1993) include subtribe Colignoniinae (including *Pisoniella*) in tribe Nyctagineae, uniting in one tribe all taxa with pantoporate grains, and *Phaeoptilum*.

Two major centers of distribution have been noted for the Nyctaginaceae (Standley, 1909): the first in the Neotropics and Caribbean, characterized by arborescent genera such as *Neea*, *Guapira*, *Pisonia*, and *Bougainvillea*, as well as the herbaceous *Colignonia* and *Salpianthus*. The second is in arid western North America, where several herbaceous or suffrutescent genera are native, including *Boerhavia*, *Mirabilis*, *Abronia*, *Acleisanthes* sensu Levin (2002), and *Commicarpus*. A few genera are widespread in tropical and subtropical regions of the world (*Boerhavia*, *Commicarpus*, *Pisonia*); *Mirabilis* is present in North and South America with one species in Asia; *Acleisanthes* contains the disjunct *Acleisanthes somalensis* from Somalia. *Mirabilis* (*M. jalapa*, *M. oxybaphoides*) and

Bougainvillea (*B. glabra*, *B. spectabilis*, *B. peruviana* and numerous hybrid cultivars) are naturalized in many parts of the world. Only one genus is restricted to the Old World, the monospecific *Phaeoptilum* of southwestern Africa.

The first molecular phylogenetic study of Nyctaginaceae was presented by Levin (2000). The focus was on species in certain genera of tribe Nyctagineae sensu Bittrich and Kühn (1993), including genera in subtribes Nyctagininae (*Allionia*, *Mirabilis*) and Boerhaviinae (*Acleisanthes*, *Selinocarpus*, *Boerhavia*), as well as *Abronia* and *Pisonia*. The study justified the formal combination of *Acleisanthes*, *Selinocarpus*, and *Ammocodon* (Levin, 2002; Spellenberg and Poole, 2003) though due to limited sampling of genera, it was not possible to evaluate the monophyly of the subtribes of Nyctagineae (Levin, 2000). The Flora of North America treatment of Nyctaginaceae (Spellenberg, 2003), while not referring to tribal classification, reflected these and other taxonomic changes for the genera and species which occur in North America north of Mexico (Table 1).

In the herbaceous taxa of Nyctaginaceae found in the deserts of North America, several unusual characters occur with notable frequency. As indicated by the common name for the family, species in several genera (*Anulocaulis*, *Cyphomeris*, *Acleisanthes*, *Mirabilis*, *Abronia*, and *Tripterocalyx*) flower in the evening and are adapted to moth pollination (Baker, 1961; Grant, 1983; Grant and Grant, 1983; Hernández, 1990; Hodges, 1995; Levin et al., 2001). Internodal bands of viscid secretions, which may discourage

aphid colonization (McClellan and Boecklen, 1993), are present in *Anulocaulis*, *Cyphomeris*, and some species of *Boerhavia*. As mentioned above, anthocarp morphology is also variable, with wings and viscid glands being common modifications.

As these characters are often polymorphic at the generic level, they would seem to represent evolutionary "tendencies". Sanderson (1991) discussed evolutionary tendencies in explicit phylogenetic terms: a tendency is a concentrated distribution of homoplasy within a tree. The main objection to the study of tendencies is the difficulty in defining the taxonomic scope at which they operate, in other words, it is "…biologically inappropriate [when investigating a hypothesized tendency] to include taxa that cannot under any circumstances exhibit the states of interest" (Sanderson, 1991). Thus, when considering whether a character exhibits a tendency to evolve, it is first necessary to evaluate the range of taxa in which it could potentially appear. In some cases, it may be possible to identify another character upon which the evolution of the character of interest is dependent. If this other trait is itself uniquely derived, its occurrence will define the group in which the tendency may conceivably exhibit itself. If the independent character is itself derived multiple times, then the problem is pushed back so that the challenge is first to explain the tendency for the *independent* character to evolve in the group.

In the case of tendencies in Nyctaginaceae, is not immediately obvious what sorts of traits may be required to enable, for instance, a shift to nocturnal pollination or the development of viscid bands on stem internodes. There are two traits, however, that seem to have a tendency to evolve in Nyctaginaceae, and that we can reasonably assume are contingent on other traits: the evolution of cleistogamy is improbable without prior self-compatibility, and lineages that specialize on gypsum are unlikely to have arisen from lineages with no latent or expressed gypsum tolerance.

Cleistogamous (closed, self fertilizing) flowers are produced in addition to chasmogamous (open) flowers in four genera of Nyctaginaceae: *Acleisanthes*, *Cyphomeris*, *Nyctaginia*, and some *Mirabilis* (Cruden, 1973; Spellenberg and Delson, 1974; Fowler and Turner, 1977; Levin, 2002). Though species with cleistogamous flowers have evolved in a number of angiosperm families, this trait is found in as many genera only in much larger families, e.g., Poaceae, Fabaceae, and Malpighiaceae (Lord, 1981). Despite a long awareness of this phenomenon generally (Darwin, 1884), the evolution of this character has only rarely been investigated with phylogenetic methods (Desfeux et al., 1996; Bell and Donoghue, 2003).

Second, as in many caryophyllid families, e.g. Amaranthaceae and Portulacaeae, there is a propensity in many Nyctaginaceae to be tolerant of, or specialists of, gypseous soils. Outcrops of gypsum (hydrous calcium sulfate) are quite common in arid North

America, especially in the Chihuahuan Desert. These areas have a flora characterized by gypsophiles which never occur on other substrates, and gypsum-tolerant species, which are found on both gypseous and non-gypseous soils (Waterfall, 1946; Parsons, 1976; Meyer, 1986). In the United States and Mexico, Nyctaginaceae are well-represented in gypsum communities (Parsons, 1976). At least 25 species in seven genera are known to occur on gypsum. Of these, roughly half are known gypsophiles, found only on gypsum soils (Johnston, 1941; Waterfall, 1946; Fowler and Turner, 1977; Turner, 1991; Spellenberg, 1993; Turner, 1993; Mahrt and Spellenberg, 1995; Harriman, 1999; Levin, 2002; Spellenberg, 2003).

Although gypsum soils support a distinct flora, the evolution of gypsophily is not understood as well as other cases of edaphic endemism. Gypsum is not an inherently poor substrate for plants in the same way as soil with, for instance, toxic levels of heavy metals (Cockerell and Garcia, 1898; Johnston, 1941; Loomis, 1944; Parsons, 1976; Meyer, 1986; Oyonarte et al., 2002). Recent experimental work has pointed toward mechanical, rather than chemical, factors to explain the limited flora of gypsum soils: seedlings of non-gypsophiles are unable to penetrate the hard crust typical of gypseous soils. This indicates that adaptations of gypsum-tolerant taxa primarily act to enhance survival in the establishment stage (Meyer, 1986; Meyer et al.,

1992; Escudero et al., 1997; Escudero et al., 1999; Escudero et al., 2000; Romao and Escudero, 2005).

Edaphic endemic species are sometimes found to be related to species that are merely tolerant: in the case of a serpentine endemic *Layia* (Asteraceae), populations of the non-endemic progenitor species were found to tolerate serpentine soils (Baldwin, 2005). Thus, even in the case of highly toxic soils, saltational speciation (Antonovics, 1971; Kruckeberg, 1986) is not required to explain edaphic endemism. These lines of evidence, and the fact that around half of the species of Nyctaginaceae found on gypsum are not restricted to it, makes it reasonable to assume that an underlaying ability to survive in gypsum soils is an early stage in the evolution of this type of edaphic endemism in Nyctaginaceae.

In principle, for both of these examples, the evolution of both the independent and contingent characters can be reconstructed on a phylogeny. With an understanding of the distribution of homoplasy in Nyctaginaceae, we will have a more robust framework for asking questions about character evolution and adaptation to xeric environments. In this phylogenetic study we comprehensively sample the genera of Nyctaginaceae, with the following goals: (1) to evaluate the existing classification of Bittrich & Kühn; (2) to understand the biogeographic history of the family; and (3) to have a basis for understanding the evolutionary history of characters of historical

taxonomic importance, and potential adaptive significance as manifested in their "tendency" to evolve repeatedly in lineages occurring in the deserts of North America.

1.2 MATERIALS AND METHODS

1.2.1 Sampling

Fifty-one species representing twenty-five genera of Nyctaginaceae were sampled. Taxa, voucher information and GenBank numbers are given in Appendix 1. Our sampling is nearly comprehensive at the generic level, with representative species of every genus except *Neeopsis*, *Cephalotomandra*, *Grajalesia*, *Cuscatlania*, *Boldoa*, and *Cryptocarpus*. The genera omitted are monotypic, rarely collected, or of dubious distinction. For example, *Boldoa purpurascens* is often included in *Salpianthus* (Pool, 2001). All tribes and subtribes recognized by Bittrich and Kühn (1993) are included. Since different taxa have been found to be sister to Nyctaginaceae (Rettig et al., 1992; Behnke, 1997; Downie et al., 1997; Cuenoud et al., 2002), outgroups were selected from both Phytolaccaceae and Sarcobataceae. More distantly related taxa in the "core Caryophyllales", i.e., Aizoaceae, Molluginaceae, and Stegnospermataceae (Cuenoud et al., 2002), were also included, to enable us to test the monophyly of Nyctaginaceae and to identify which taxa are sister to the family. For four species, data were obtained from

two different accessions, for two, GenBank sequences were used for some loci. "*Phytolacca*" is a composite of one GenBank sequence from *P. acinosa* and three new sequences from *P. americana*.

1.2.2 Molecular Data

Genomic DNA was extracted from fresh, silica-dried, or air-dried (herbarium) leaf tissue using either Qiagen DNAeasy Plant Mini Kits or a modified CTAB method (Doyle and Doyle, 1987). Internal transcribed spacer (ITS) sequences were obtained using primers ITS4 and ITS5a (White et al., 1990; Stanford et al., 2000), which amplifies ITS1, 5.8s, and ITS2. Chloroplast *ndh*F sequences were obtained as two overlapping fragments using primers Nyct-ndhF1, ndhF972, Nyct-ndhF13R, and Nyct-ndhF22R. With the exception of ndhF972 (Olmstead and Sweere, 1994), these were designed based on GenBank *ndh*F sequences for Nyctaginaceae and Phytolaccaceae. Many samples, especially those from herbarium materials, were recalcitrant to PCR of long (>1kb) fragments due to DNA degradation; for these, four additional primers (Nyct-ndhF6F, Nyct-ndhF8R, Nyct-ndhF13F, and Nyct-ndhF16R) were designed, based on sequences for Nyctaginaceae and Phytolaccaceae, and used in conjunction with the aforementioned primers, so that the gene was amplified in four overlapping fragments. The chloroplast intron *rps*16 was amplified using primers rpsF and rps2R (Oxelman et al., 1997), and *rpl*16 was obtained using primers F71 and R1661 (Jordan et al., 1996). Primer sequences

and references are given in Table 2. PCR products were cleaned with Qiaquick columns (Qiagen, Valencia, California, USA). Cycle sequencing was performed using the BigDye® Terminator v3.1 Cycle Sequencing Kit and sequences were determined with an ABI 3700 DNA Analyzer (Applied Biosystems, Foster City, California, USA) in the Genetic Analysis facility in the Department of Biology at Duke University. Raw chromatograms were edited and assembled in Sequencher 4.1 (Gene Codes Corporation, Ann Arbor, Michigan, USA). Sequence alignment was performed either by eye (*ndh*F) or in ClustalX (Thompson et al., 1997) (other regions) followed by manual adjustment in Se-Al (Rambaut, 1996). Across the entire dataset, ITS1 and ITS2 were too variable to be confidently aligned, though the 5.8s region was highly conserved. Ambiguously aligned regions were excluded from further analyses of the entire dataset, though they were used in analyses of more restricted taxon sets (see below).

Caribea littoralis Alain, a Cuban endemic, has been collected only once. The morphology of the plant is difficult to interpret as it is highly distinct from any other member of the Nyctaginaceae. The collection locality is in southeastern Cuba in a dry coastal habitat. Few details are clearly visible on the specimen, though the description appears to have been based on fresh material (Alain, 1960). Due to the age of the collection, only about 25% of an *ndhF* sequence was obtainable. This sequence was unique and a BLAST search found that this sequence fragment was most similar to an

existing *Bougainvillea ndhF* sequence (GenBank #AF194825). Preliminary phylogenetic analysis (see *Data Analysis*) placed this taxon as sister to either *Pisoniella* or *Belemia*. The latter are not closely related to each other, resulting in substantial loss of resolution in the part of the tree between these taxa. Therefore, *Caribea* was excluded from all further analysis, and while this result confirms that this enigmatic taxon belongs in Nyctaginaceae, further study must await rediscovery of this species. Unfortunately, repeated attempts to relocate the population at the type locality in Cuba have proved unsuccessful (D. Stone, personal communication).

1.2.3 Data Analysis

Initial maximum parsimony (MP), maximum likelihood (ML), and Bayesian analyses were performed for each of the four loci. 5.8s, not surprisingly, had low variation and produced poorly resolved trees; however, examination of the support values for the topology favored by each locus revealed no supported nodes in conflict, therefore the datasets were combined for further analyses.

MP analysis was performed using PAUP* 4.0b10 (Swofford, 2002). A heuristic search was performed, with 1000 replicates of 10 random-addition sequences, TBR branch swapping, MAXTREES set to autoincrease, MULTREES=yes. Support was evaluated using 1000 bootstrap replicates of 10 random addition sequences, TBR branch swapping, MULTREES=YES.

For the ML analysis, the dataset was first examined using ModelTest 2.0 (Posada and Crandall, 1998), which selected a complex model of evolution (GTR+I+ Γ). Ten random-addition replicates (TBR, MAXTREES set to autoincrease, MULTREES=yes) were run in PAUP*. Maximum-likelihood bootstrap support values were obtained by 100 replicates of single random-addition sequences, TBR branch swapping, MULTREES=YES.

Bayesian analysis was performed using MrBayes 3.1 (Ronquist and Huelsenbeck, 2003). To explore the effect of different models for different partitions of the data, best-fit models for each partition were estimated in MrModelTest (Nylander, 2004), which selects the best-fit model from those available in MrBayes. The partitions were as follows: 1, all loci together; 2, nuclear 5.8s; 3, all chloroplast loci; 4, *rpl*16; 5, *rps*16; 6, *ndh*F; and 7, 8, 9, first, second, and third positions of *ndh*F, respectively. The models selected by MrModelTest for each partition are given in Table 3. Bayesian searches were then performed on the entire dataset using four partition/model combinations: "B1", Single model for all partitions, (1); "B2", nuclear & chloroplast, (2 & 3); "B4", all loci, (2, 4, 5, & 6); and "B6", all loci with separate models for each codon position of *ndh*F (2, 4, 5, 7, 8, & 9). For each combination, we executed four independent runs of 1 X 10^6 generations each, sampling every 100^{th} tree. After discarding trees from the burn-in (determined by visualizing the plateau in –lnL scores, approximately after 50,000 generations), the

posterior tree sets from each run were compared by computing a 50% majority rule tree in PAUP*. No strongly supported topological differences (at posterior probability ≥ 95%) were found between the four runs of each model set. Therefore, the four posterior treefiles for each set of models were combined into a single posterior treefile for purposes of assessing support values yielded by each set of models. These preliminary analyses were conducted including the partial *ndh*F sequence for *Caribea*; however, the B6 analysis was repeated without this sequence.

1.2.3.1 Sensitivity Analyses

Due primarily to the inclusion of GenBank sequences for outgroup taxa and the failure of certain loci to amplify (mostly from herbarium material), approximately 17.7% of the data matrix is coded as "missing". The potential impact of this was investigated by deleting from the analysis 18 taxa (Appendix 1) for which one or more sequences were entirely missing, and combining sequences from *Bougainvillea glabra* and *B. infesta* into a composite OTU "*Bougainvillea*". "*Phytolacca*" and *Rivina humilis* were the only remaining outgroups in this analysis, which allowed us to examine the effect of including distant outgroups. MP, ML, and corresponding bootstrap searches were performed with the same settings as in the analysis of the full matrix. The resulting trees were compared to the topology from the full analysis to see whether the exclusion of

missing data led to a preferred topology that differed substantively from the topology or levels of support in the analysis of the full matrix.

1.2.3.2 Restricted Analyses

In order to gain resolution within and between closely related genera, our selection of loci encompassed a large range of sequence variation. Due to questionable alignment, both the ITS1 and ITS2 regions had to be excluded from the analysis of the complete dataset, though the highly conserved 5.8s region was kept in the full matrix. Therefore, following the analysis of the full dataset, two restricted datasets were constructed to allow us to increase the number of included characters (Table 3) by reducing the taxon sampling to two distinct clades found in the full analysis. These restricted datasets comprised all included nucleotide positions in the full dataset, plus sites that were unalignable across the breadth of taxa included in the full dataset, but that were alignable within the restricted sets of taxa. The first restricted analysis group was comprised of North American herbs representing all taxa in the sister group to *Allionia*, whereas the second corresponded to the Pisonieae, *Bougainvillea*, *Belemia*, and *Phaeoptilum* (the "B&P" clade from the full analysis). MP, ML, and corresponding bootstrap analyses were performed in the same fashion as in the full matrix and sensitivity analyses, with the exception that the ML models were reestimated in ModelTest.

1.2.4 Character Data

The historical taxonomic significance given to pollen morphology and involucral bracts led us to examine these characters in a phylogenetic context. Pollen data follows the scheme of Nowicke, who identified four types in Nyctaginaceae (Nowicke, 1968; Nowicke, 1970; Nowicke and Luikart, 1971; Nowicke, 1975; Reyes-Salas and Martinez-Hernandez, 1982; Chavez et al., 1998). Pollen type was coded as a multistate, unordered character. In many cases, the exact species included in our study were not examined in the published studies. When there was no indication of within-genus pollen polymorphism, that pollen type was assigned to all species in this analysis. However, multiple pollen types were recorded within *Neea* and *Pisonia*. Thus, only *Neea psychotrioides*, which was examined by Nowicke, was coded unambiguously; other species of *Neea* and *Pisonia* were coded as polymorphic (states "1&3" and "1&4", respectively) to reflect this uncertainty in the assignment of ancestral states. The presence of involucral bracts was scored as present/absent. If only small subtending bracteoles (common in many taxa) this character was coded as "absent", mirroring the usage of this character in defining subtribe Nyctagininae. The occurrence of cleistogamous flowers was scored based primarily on literature sources (Spellenberg and Delson, 1974; Bittrich and Kühn, 1993; Levin, 2002; Spellenberg, 2003). Gypsophilic taxa were identified in literature sources (Waterfall, 1946; Parsons, 1976; Fowler and

Turner, 1977; Turner, 1991; Harriman, 1999; Levin, 2002; Spellenberg, 2003) and personal observation of N. Douglas, R. Spellenberg, and P. Hernández-Ledesma. Taxa were identified as full gypsophiles (recorded only from gypseous soils), gypsum tolerant (recorded from both gypseous and non-gypseous soils), or non-gypsophilic. Taxa which do not occur in areas with gypsum outcrops were considered to be non-gypsophilic. It is unlikely that transitions to or from full gypsophily could evolve with no intermediate gypsum-tolerant step, therefore, this character was analyzed both as an unordered, and as an ordered, character, with two steps required between non-gypsophily and full gypsophily. Parsimony ancestral states of all characters were reconstructed in Mesquite 1.6 (Maddison and Maddison, 2006). Those terminals which were not assigned a single state, and branches which are not unambiguously resolved, are depicted as "equivocal".

1.3 RESULTS

1.3.1 Data matrix

The entire data matrix (Table 3) had a length of 5505 bp, of which 1771 were excluded due to ambiguous alignment, mainly due to the presence of length variation in ITS1 and ITS2 and in the two chloroplast introns, *rpl16* and *rps16*. Of the remaining 3734 characters, 652 were parsimony informative.

1.3.2 Phylogenetic analysis of the complete dataset

The MP analysis resulted in 36 shortest trees (length: 2287, consistency index [CI]: 0.657, retention index: 0.809, rescaled CI: 0.531); however, the strict consensus (tree not shown) resolved all but two ingroup nodes. Thirty-nine nodes were supported with parsimony bootstrap values (MPBS) ǂ 70.

The best-fit model as determined by ModelTest (Table 3) using both a heiarchical liklihood ratio test (HLRT) and the Akaike Information Criterion (AIC) was a general-time-reversible model with a proportion of invariant sites and a gamma shape parameter (GTR+I+Γ). The ML search returned a single ML tree, which was nearly identical to the MP topology, except in the placement of the genus *Colignonia*. This taxon is placed as sister to the large clade containing *Acleisanthes* and *Boerhavia* in the MP analysis (MPBS = 80) and is not resolved with strong support in any ML or Bayesian analysis. Overall, 38 nodes in the ML analysis were supported with likelihood bootstrap values (MLBS) ǂ 70.

Models determined by MrModelTest for each data partition in the Bayesian analyses are given in Table 3. On the basis of our preliminary examination of partitioned models, the signal in the data set apparently is strong and the topology is not contingent on model selection: the tree topologies produced by the Bayesian B1, B2, B4, and B6 searches were consistent. The principal difference between them is in the level of

support for the topology, with 37, 39, 40, and 40 nodes, respectively, supported by posterior probabilities (PP) ‡ 95%. Deletion of *Caribea* led to the resolution of two additional nodes in the repeated B6 search with support for a total of 42 nodes supported at greater than 95% PP. The topology of this Bayesian B6 consensus tree is identical to the ML tree. All further Bayesian support values refer to the B6 analysis.

The Nyctaginaceae are supported as monophyletic by ML (MLBS = 71) and Bayesian (PP = 100) analyses (Fig. 1). Interestingly, in the MP bootstrap analysis of this matrix, the monophyly of the Nyctaginaceae is not supported. Despite the inclusion of several outgroups, no single sister lineage emerges with strong support.

Leucastereae, a tribe of four South American genera (*Andradea*, *Leucaster*, *Ramisia*, and *Reichenbachia*), is supported as the earliest branching lineage in Nyctaginaceae (Fig. 1) followed by Boldoeae, represented by *Salpianthus*. A clade containing largely neotropical trees and shrubs, and the African genus *Phaeoptilum*, receives support from MP and B6 analyses, though not from ML. We will refer to this group as the Bougainvilleeae and Pisonieae ("B&P") clade, (Fig. 2), recognizing that it also includes *Phaeoptilum* and *Pisoniella*, which are currently classified in Nyctagineae. *Bougainvillea*, *Belemia*, and *Phaeoptilum* form a clade within this group, which is sister to a clade containing the Pisonieae and the genus *Pisoniella*. Within the Pisonieae, *Neea* and *Guapira* together form a clade but neither genus appears to be monophyletic.

Strong support is found in all analyses for a clade including mostly North American xerophytic genera. For the purposes of this paper, we refer to it as the North American Xerophytic ("NAX" clade) (Fig. 2). The NAX clade is well defined by geography, habit, and habitat, but it has never been recognized formally. The earliest branch within this clade leads to *Acleisanthes* sensu Levin (2000). It is followed by a clade representing *Abronia* and *Tripterocalyx* (tribe Abronieae). Phylogenetic relationships of the remaining genera in the NAX clade are mostly well resolved, with the exception of low support values for the placement of *Commicarpus* and *Allionia*. Two pairs of genera in this clade are not resolved as monophyletic. *Anulocaulis* includes *Nyctaginia*, and *Boerhavia* includes *Okenia*, though support in both of these cases is weak or lacking. Examination of the branch lengths (Fig. 2) makes it clear that *Anulocaulis* and *Nyctaginia* are at least very closely related.

The position of *Colignonia* is not resolved in the ML and Bayesian analyses. The ML analysis resolves *Colignonia* sister to the B&P and NAX clades but with weak support. A position sister to only the NAX clade is supported in the MP analysis.

1.3.3 Sensitivity analyses

The deletion of taxa with significant missing data resulted in a matrix of 39 taxa with only 3.1% missing data, as compared to 58 taxa with 17.7% missing data in the full analysis (See Appendix1). The MP/ML analyses of this matrix yielded trees (not shown)

that had no well-supported nodes conflicting with the topology of the tree from the full matrix. The support for the monophyly of the Nyctaginaceae increased to 94/95 MPBS/MLBS, from −/71 in the analysis of the full data set. The high level of support found in this analysis for the monophyly of Nyctaginaceae indicates that the inclusion of many outgroups in the full matrix, including the quite distant *Stegnosperma*, may have affected the level of support in the MP analysis. Alternatively, high levels of missing data in the full data set may be responsible for low support values at this key node. Support for the placement of *Cyphomeris* decreased to 70/66 relative to the full analysis. *Commicarpus* and *Allionia* increased to 73/67 and 87/77, respectively; these nodes had not received strong support in any analysis of the full data set. The remainder of the comparable nodes were similarly supported between the full and sensitivity analyses.

1.3.4 Restricted analyses

For the two restricted analysis groups, 122 and 76 additional informative characters were gained with the inclusion of ITS1 and ITS2, respectively (Table 3). A small number of additional sites were gained from the chloroplast introns *rps16* and *rpl16* (<5 characters in either data set). ModelTest 3.7 selected a GTR+I+Γ model for each (Table 3) data set. For the first group (all taxa in the sister group to *Allionia*), MP and ML analyses produced a tree (Fig. 3) with improved resolution in the *Anulocaulis* +

Nyctaginia clade. Though the placement of *A. annulatus* differs between the full matrix topology and the restricted analysis, the monophyly of *Anulocaulis* ss well supported with bootstrap values of 72/89 MPBS/MLBS. Similarly, the full analysis resolves *Okenia* within a paraphyletic *Boerhavia* with low MP and ML bootstrap support, but 97% Bayesian posterior probability, yet the restricted analysis found *Boerhavia* strongly supported as monophyletic and sister to *Okenia* with high support (100/100). *Boerhavia* consists of two clades, corresponding to annual and perennial species, that were also found in the full analysis.

The restricted analysis of the B&P clade produced a tree (not shown) that did not conflict with the topology of this clade in the full matrix analysis. Support values were generally slightly lower, probably due to the concentration of missing data in this group and the lower number of additional characters from the ITS region. Support values remained high for the nodes uniting *Guapira eggersiana* and *Neea hermaphrodita*, for the placement of *G. discolor* in the clade sister to *Neea psychotrioides*, and for the monophyly of *Neea* + *Guapira* (MPBS/MLBS bootstrap support of 64/73, 82/94, and 94/92, respectively).

1.3.5 Character reconstructions

For each character reconstructed (Fig. 4), multiple state transitions are inferred. Tricolpate-spinulose pollen (Fig. 4a) appears to be the ancestral condition in the group,

transitioning to a pantoporate-spinulose condition subsequent to the divergence of *Salpianthus* from the main lineage. The latter condition is found in nearly all members of the NAX clade, yet appears to predate that group. At least eight transitions among the four pollen types have occurred in the Nyctaginaceae. Considering the small number of *Neea* and *Pisonia* examined and the polymorphism exhibited by these genera, the number of transitions could be higher. Reconstruction of involucral bracts shows five gain/loss steps. This character is fixed within genera, thus this interpretation is likely to be affected only by the future inclusion of the remaining genera in the family. Only the inclusion of *Cuscatlania*, which has an involucre, could conceivably change the number of steps required. Cleistogamous flowers are uniquely derived in four genera. Gypsophily requires nine or 13 steps to explain, depending on whether it is considered to be an unordered or an ordered character. Reconstructions were performed only on the ML topology from the full analysis. Adjusting the positions of *Okenia* and *Nyctaginia* to reflect the topology from the restricted analysis (Fig. 3) results in the branches leading to *Nyctaginia* + *Anulocaulis* and *Nyctaginia* + *Anulocaulis* + *Okenia* + *Boerhavia* being resolved as non-gypsophilic. Treating gypsophily as an unordered character has the same result. Otherwise, the alternative topology has no substantive effect on the conclusions we make regarding the degree of homoplasy shown by the remaining three characters shown in Fig. 4.

1.4 DISCUSSION

1.4.1 Phylogeny of Nyctaginaceae

The earliest branching lineage in Nyctaginaceae, the Leucastereae (Fig. 1), had been previously recognized as a natural group on the basis of arborescence, a stellate indumentum, and tricolpate pollen (Heimerl, 1934; Bittrich and Kühn, 1993). The Boldoeae, an herbaceous group native from the Galapagos to northwestern Mexico and the Caribbean, are represented in this study by *Salpianthus*. These two lineages had been predicted to be basal or outside of Nyctaginaceae on the basis of apparent pleisomorphies such as alternate leaves and bisexual flowers (Bittrich and Kühn, 1993). The anthocarp structure is absent in Leucastereae and Boldoeae, although the unexpanded perianth does persist around the fruit. Persistent tepals are also found in many Phytolaccaceae. However, the perianth consists of free tepals in most Phytolaccaceae and all of subfamily Rivinoideae (except *Hilleria*, in which three of four tepals are partially fused, (Rohwer, 1993)). In Nyctaginaceae, including Leucastereae and Boldoeae, tepals are fully connate.

Within the B&P clade (Fig. 2), *Phaeoptilum* is found to be sister to *Belemia*, rendering the Bougainvilleeae paraphyletic. The Pisonieae are found to be sister to *Pisoniella*, which had been included in that tribe by Heimerl (1934) but was removed to subtribe Colignoniinae by Bittrich and Kühn (1993) following the suggestion of Bohlin

(1988). The reasoning behind this move is mysterious, and in light of our results, it appears to have been unwarranted. *Pisoniella* possesses a straight embryo like other Pisonieae, and the large coriaceous anthocarps are provided with viscid glands along the ribs, much like those in *Pisonia* (Heimerl, 1934).

Within Pisonieae, *Neea* and *Guapira* form a clade (Fig. 2). These genera are distinguished primarily by whether the stamens are included (*Neea*) or exserted (*Guapira*). Our sampling is extremely limited in these two large genera, with only five accessions to represent ca. 150 species, though we were able to include accessions from geographically disparate locales. Neither genus forms a monophyletic group. This conclusion has been occasionally anticipated, (e.g. Pool, 2001). It is unclear whether our sampling simply happened to include misclassified species in otherwise good genera, or whether this paraphyly is representative of *Neea* and *Guapira* generally. Much more intensive sampling is clearly needed to understand the relationships of the species in these genera, and it would be imprudent to attempt to reclassify them until a more detailed study is made including phylogenetic, morphological, and distributional data. Unfortunately, collections of these dioecious trees often do not include individuals of both sexes. Also, the tendency of many Pisonieae to oxidize when dried, has left many descriptions lacking crucial information, especially concerning the color of fruits. Therefore, the taxonomic literature is quite confused and species limits are known not

much better than when Standley wrote of these genera, "I know of few groups of plants in which specific differences are so unstable and so baffling." (Standley, 1931b). Finally, in this study we did not attempt to infer the ages of lineages, yet it appears that the branch lengths in the *Neea* + *Guapira* clade are comparatively short, especially considering that this clade can be expected to accommodate as many as 150 species (Fig. 2). A similar pattern has been noted in other radiations of Neotropical trees, e.g. *Inga* (Fabaceae) (Richardson et al., 2001). If the pattern of relatively short branches inferred between species was upheld with the inclusion of a larger sample of taxa and more rapidly evolving markers, it would point to this clade as another example of rapid diversification in the Neotropics.

Tribe Nyctagineae is broadly paraphyletic. As mentioned, *Pisoniella* and *Phaeoptilum* are not found in this study to be closest relatives of any other Nyctagineae. Based on pollen morphology, it has been suggested (Bohlin, 1988) that *Colignonia* (subtribe Colignoniinae) has affinities to the tribe Mirabileae of Heimerl (1934), which roughly corresponds to Tribe Nyctagineae and the NAX clade. *Colignonia* may in fact be sister to the NAX clade as suggested by the MP analysis, or to the NAX + B&P clade as suggested by the ML analysis (Fig. 1). Tribe Nyctagineae also does not include *Abronia* or *Tripterocalyx* (Tribe Abronieae, Fig. 1). There are certain characters of the Abronieae which are anomalous within the Nyctagineae (and the NAX clade), and which justified

recognition at a higher taxonomic level, namely, tricolpate pollen and linear stigmas. The two genera in the tribe have long been thought to be a natural group, and are often synonymized (Heimerl, 1934; Bittrich and Kühn, 1993), though most authors have maintained the two genera (Galloway, 1975; Spellenberg, 2003). Additional morphological synapomorphies for the *Abronia* + *Tripterocalyx* clade include an umbellate inflorescence of salverform flowers with included stamens and style, a well-developed involucre, anthocarps with typically well-developed wings or lobes, and a mature embryo with a single well-developed cotyledon.

Anulocaulis and *Nyctaginia* are classified in different subtribes in the classification of Bittrich and Kühn (1993), presumably based on the presence of an involucre in the latter. Both genera are succulent perennial herbs, and the turbinate fruits with umbonate apices of *Nyctaginia* capitata strongly resemble those of *Anulocaulis* eriosolenus. They differ in many characters, including flower color (red-orange in *Nyctaginia* vs. white to pink in *Anulocaulis*) and flowering time (flowers of *Nyctaginia* are open during the day, while in *Anulocaulis* anthesis is at sunset or later and flowers wilt in the morning). While the full matrix ML tree (Fig. 1) indicates that *Anulocaulis* may not be monophyletic, this relationship is poorly supported (MPBS/MLBS/PP = 64/55/63). In the restricted MP and ML analysis (Fig. 3), however, a monophyletic *Anulocaulis* is more

strongly supported (MPBS/MLBS = 72/89). Therefore, we see no compelling reason to question the taxonomic status of *Anulocaulis*.

Anulocaulis + *Nyctaginia* are sister to a strongly-supported clade containing *Boerhavia* and *Okenia*. Like the previous instance, *Okenia* resolves within *Boerhavia* in the full matrix ML topology (Fig. 1), but support for this relationship is only moderately significant in the Bayesian analysis of the full dataset (PP = 97) and weakly supported by MPBS and MLBS (67/69). Conversely, *Boerhavia* is strongly supported as a monophyletic group in the MP and ML analyses of the restricted dataset (MPBS/MLBS = 100/100, Fig. 3). Vegetatively, *Okenia* strongly resembles most *Boerhavia* in its decumbent habit, and subequal opposite leaves with crenulate or wavy margins. The flowers of *Okenia*, though larger, are similar in color to some perennial *Boerhavia* from the Chihuahuan Desert. Finally, *Okenia* is annual, a condition found in one clade of *Boerhavia*. However, *Okenia* is strikingly different than *Boerhavia* in its unique reproductive biology: it produces aerial flowers, but the large, spongy fruits are geocarpic, with peduncles elongating greatly post-fertilization and the fruits maturing several cm belowground. The relationship between these two genera is deserving of more study.

1.4.2 Biogeographical patterns

The basal lineages of Nyctaginaceae (Boldoeae, Leucastereae, *Colignonia*, Bougainvilleeae, and Pisonieae (including *Pisoniella*), are fundamentally South American. Though some taxa have representatives or populations in (sub)tropical North America, (*Salpianthus*, *Neea*, *Guapira*, *Pisonia*, *Pisoniella*), their distributions all include the Neotropics and phylogenetically they are interspersed with Neotropical endemics. The widespread tropical genus *Pisonia* possesses extremely viscid anthocarps which aid dispersal, frequently by seabirds (Burger, 2005). The sole genus not native to the Americas is *Phaeoptilum*, endemic to arid southwestern Africa. This monospecific genus is closely related to *Belemia* and *Bougainvillea*, both from eastern and southern South America. *Phaeoptilum* is morphologically quite distinct from its sister taxon *Belemia*, though vegetatively it resembles the xeric-adapted *Bougainvillea spinosa*. The early Cretaceous date (130-90 Ma) for the opening of the south Atlantic (Smith et al., 1994) makes vicariance an unlikely explanation for this disjunction. Dispersal seems more likely, and while there is no specialized dispersal structure on the anthocarp of *Belemia*, both *Bougainvillea* and *Phaeoptilum* show compelling (albeit different) adaptations for wind dispersal. *Phaeoptilum* produces winged anthocarps highly similar to those found in *Tripterocalyx* and some species of *Acleisanthes*. In *Bougainvillea*, most species display three showy bracts, each fused to a solitary flower. In fruit each

involucral bract remains fused to a fruit and acts as a wing, the structure functioning as a unit of dispersal (Ridley, 1930).

The North American Xerophytic Clade has diversified in the deserts of the southwestern United States and northwestern Mexico. Every genus is confined to, or has representatives in, this region. Widespread taxa in this clade, namely *Commicarpus* and *Boerhavia*, possess glandular fruits which have most likely aided bird-dispersal in a manner similar to that of *Pisonia*. Two red-flowered *Boerhavia*, *B. coccinea* and the similar *B. diffusa* are widespread in most tropical and subtropical areas. *Boerhavia diffusa* appears to have naturally dispersed from the Americas, though the confused taxonomy of this species and *B. coccinea* in regional floras makes this difficult to evaluate, and both of these species are frequently transported by human activity. The "repens" complex in *Boerhavia* (*B. repens* and related species) is widespread in coastal habitats throughout the tropical Pacific and Indian Oceans to the Arabian Peninsula, along with *B. dominii* from Australia. Like the red-flowered perennial *Boerhavia* mentioned above, these species also have viscid glandular anthocarps. *Okenia* is found in deep sand dune habitat along the Pacific and Caribbean coasts of Mexico and Central America, with a disjunct population in southern Florida. Other authors (Heimerl, 1934; Fowler and Turner, 1977; Thulin, 1994; Levin, 2002; Spellenberg and Poole, 2003) have discussed the remarkable disjunctions of *Acleisanthes somaliensis* and *Mirabilis himalaicus* from east Africa, and

southern Asia, respectively. These appear to be attributable to long-distance dispersal events, due to their derived position within otherwise exclusively American clades (Levin, 2000; Douglas, unpublished data).

1.4.3 Pollen and involucre evolution

Tribal and subtribal classifications (Table 1) of the Nyctaginaceae have relied heavily on a few characters, such as pollen morphology and the development of an involucre. However, divisions based on these characters are not supported by our results, as these characters show a high degree of homoplasy among genera.

Parsimony reconstruction of pollen type across Nyctaginaceae (Fig. 4a) shows that substantial homoplasy exists (11 changes), involving three of the four types diagnosed by Nowicke (Nowicke, 1970; Nowicke and Luikart, 1971; Nowicke, 1975). Pantocolpate grains may constitute a synapomorphy for *Belemia* + *Phaeoptilum*. It has been noted that desiccation-resistant large pantoporate pollen grains, equipped with pore plates, were found primarily in the herbaceous desert taxa (Nowicke and Luikart, 1971). Specific correlations between large and/or polyaperturate grains and habitat in angiosperms has not been adequately investigated. In one study of a ecological correlates of pollen morphology in a wide selection of angiosperms (Lee, 1978), the association between pore number and width, and "temperature" was extremely weak. According to our reconstructions, the origin of pantoporate-spinulose pollen predates

the major radiation of desert taxa in the NAX clade. However, *Colignonia* and *Pisoniella* have much smaller grains than do the remaining taxa with pantoporate-spinulose pollen (*Colignonia* = 25-35 μM, *Pisoniella* = 30-37 μM, Nowicke and Luikart, 1971; Douglas, unpublished data). Therefore, it would seem best to consider grain size as a variable separate from grain shape and exine structure.

Within Nyctagineae, the subtribes Nyctagininae and Boerhaviinae were separated by the presence or absence of an involucre subtending the inflorescence. In subtribe Nyctagininae, the involucre of *Mirabilis* is comprised of fused bracts; the remaining genera possess involucres of distinct bracts. The involucre in *Bougainvillea* is distinctive; fruits of *Bougainvillea* retain a large involucral bract discussed above. Involucres have no known dispersal function in any of the other taxa; it is likely they merely serve to protect the flower buds and developing fruits or discourage nectar robbing insects (Cruden, 1970). Parsimony reconstruction of this character on the molecular topology (Fig. 4b) indicates that, for involucres, there are at least five gain/loss steps in the family, four in the NAX clade, which contains the members of the Nyctagineae-Nyctagininae, Nyctagineae-Boerhaviinae, and Abronieae, reflecting the artificial nature of this classification. In this analysis, the character was treated in a very simplistic fashion, reflecting nothing more than taxonomic convention. Comparative developmental studies may shed light on deeper homologies or convergences, especially

as they relate to the subtending bracts found in many genera. The selective benefits involved in the expression of this structure could be revealed by appropriate ecological investigations.

1.4.4 Self-compatibility and cleistogamy

The production of obligately selfing flowers is obviously contingent on the ability of plants to self-pollinate and produce fertile progeny. Our incomplete knowledge of reproductive systems in Nyctaginaceae means that an unambiguous reconstruction of self-compatibility is not currently possible. However, several studies have addressed mating systems in select Nyctaginaceae: Sporophytic self-incompatibility (SI) is known in *Bougainvillea* (Zadoo et al., 1975; López and Galetto, 2002). Some *Mirabilis* (sect. *Quamoclidion*), and *Abronia macrocarpa* fail to set seed when self-pollinated (Cruden, 1973; Williamson et al., 1994), but the basis for incompatibility is not known in these genera. The Pisonieae are usually dioecious and are thus self-incompatible, although in these genera there are occasional monoecious or hermaphroditic species (e.g. *Pisonia brunoniana*) for which mating system has not been studied (Sykes, 1987). Evidence suggests that many genera in the NAX clade are self-compatible: in addition to the production of cleistogamous flowers in four genera, *Boerhavia* and some *Mirabilis* are known to have a delayed self-pollination mechanism whereby the style curls and encounters the anthers as the flower wilts (Chaturvedi, 1989;

Hernández, 1990; Spellenberg, 2000). Finally, flowers protected from pollinators have set viable seed in *Abronia umbellata* Lam. (McGlaughlin et al., 2002) and *Colignonia* (Bohlin, 1988).

Reasoning from these data, certain inferences regarding the evolution of mating systems in Nyctaginaceae are possible. Explanations for current distribution of mating systems family must incorporate one, or some combination of both, of the following scenarios. Which one is preferred depends on the likelihood of self-compatible lineages giving rise to lineages with an inability to self-fertilize, and the implications of either scenario are interesting.

One scenario, and the most parsimonious given our current knowledge, is that there have been at least three independent derivations of SI from a self-compatible ancestor. A single change can account for the Pisonieae and *Bougainvillea*, one for the derived *Mirabilis* sect. *Quamoclidion*, and one for *Abronia macrocarpa*. It is often assumed that outcrossing species are not derived from selfing ancestors and that selfing lineages are an evolutionary "dead end" (Fisher, 1941; Stebbins, 1974; Lande and Schemske, 1985). In the case of Nyctaginaceae, however, the question is whether it is possible that *self-incompatible* species have arisen from *self-compatible* ancestors. It would seem that populations making this transition would be subject to most of the forces which affect the balance of selfing and outcrossing in self-compatible populations. A recent study of

s-locus polymorphism in Solanaceae (Igic et al., 2006) has shown that losses of SI are irreversible in that family. The "cost" of developing the complex genetic systems necessary for SI would be added to the transmission advantage of alleles promoting self-fertilization (Uyenoyama et al., 1993); these factors must count against a hypothesis of multiple transitions to SI in one family.

Conversely, if we assume that SI is ancestral and has been lost repeatedly, transitions from SI to self-compatibility have occurred a minimum of six times (in *Colignonia*, *Acleisanthes*, some *Abronia*, two or more times in *Mirabilis*, and finally in the clade sister to *Mirabilis*). This represents a doubling of the number of evolutionary steps required to explain the distribution of known Nyctaginaceae mating systems. Other authors have discussed the merits of parsimony weighting schemes or maximum-likelihood approaches to testing the irreversibility of selfing (Barrett et al., 1996; Bena et al., 1998; Takebayashi and Morrell, 2001). It may not be possible to escape a circular argument employing *only* phylogenetic evidence, as a weighting scheme favoring losses of SI assumes the conclusion. In Solanaceae (Igic et al., 2006), additional evidence from the distribution of variation in the incompatibility locus was required to demonstrate the irreversibility of the loss of SI. In our case, the most convincing resolution will come when SI is characterized in *Mirabilis* sect. *Quamoclidion* and SI *Abronia*. If in these taxa, and any others which may yet be discovered to be self-incompatible, it is possible to

identify the genetic basis for SI, assessments of homology could be made and the functionality of underlying mechanism could be tested.

Assuming the derived state is self-compatibility, of these six lineages, three have given rise to cleistogamous/chasmogamous lineages, and four gains of cleistogamy are required to explain the distribution of the character in Nyctaginaceae (Fig. 4c). Interestingly, the cleistogamous genera are all perennial, which should be less susceptible to selection pressure for reproductive assurance than annuals (Barrett et al., 1996). Alternatively, cleistogamous flowers can function to maximize seed set when resources, rather than pollinators, are limiting (Schemske, 1978). These hypotheses are both applicable to the cleistogamous Nyctaginaceae, though distinguishing between them may be difficult, as pollinators in desert environments tend to be scarce when water is scarce. Spellenberg and Delson (1974) found that *Acleisanthes* (*Ammocodon*) *chenopodioides*, with a generalized flower morphology and a diurnal pollinator fauna, produced roughly equal numbers of seeds from cleistogamous and chasmogamous flowers, and did not show a strong seasonal pattern in the production of cleistogamous flowers. In contrast, *Acleisanthes longiflora*, a species with large, specialized hawkmoth-pollinated flowers, produced the majority of a season's seeds from cleistogamous flowers produced preferentially in the dry early summer when sphingid moths are less

active. This may suggest that cleistogamy in this genus is insurance against reproductive failure due to the absence of pollinators in some years.

1.4.5 Gypsophily

Parsimony reconstruction of gypsophily in Nyctaginaceae (Fig. 4d) indicates that gypsophiles and gypsum-tolerant species are widely dispersed in the NAX clade. With the current sampling, the ancestor of this clade is inferred to be non-gypsophilic (whether or not the character is considered "ordered"), indicating that gypsum tolerance is derived multiple times. This conclusion is tenuous for two reasons. First, gypsum outcrops are common in the Chihuahuan Desert but less so in other parts of the ranges of the NAX genera. We are unable to rule out the possibility that taxa coded in this analysis as "non-gypsophilic" are actually gypsum-tolerant, but simply do not occur in areas with gypsum soils.

Second, there are two *Mirabilis* (*M. nesomii* Turner and *M. linearis* (Pursh) Heimerl) which are gypsophilic (Turner, 1991) and gypsum-tolerant, (R. Spellenberg, personal communication), respectively. These species, both in section *Oxybaphus*, are close relatives of the oxybaphoid *M. albida*, a non-gypsophile included in this study. It is possible to add gypsophilic taxa as sisters to *M. albida* on our topology, so that the resolution of the ancestor of the NAX clade becomes equivocal, with ACCTRAN

reconstruction as gypsum-tolerant, and DELTRAN as non-gypsophilic. The same reconstruction would be made for the ancestors of *Commicarpus* and *Abronia* +*Tripterocalyx*. The sensitivity of the reconstruction at these key nodes to sampling artifacts indicates that in order to reconstruct the history of gypsophily in this clade, it will be necessary to undertake more intensive phylogenetic sampling at the species level, investigating an appropriate sample of non-gypsophilic taxa closely related to known gypsophiles.

Even if we cannot know the gypsum tolerance of the ancestor of the NAX clade based on existing data, it is evident that there are at least four instances of strong gypsophily evolving in the family. It would be profitable to investigate the ecology of these gypsophytes and their relatives in the NAX clade. An experimental approach investigating whether or not seedlings of non-gypsphiles have the latent ability to establish on gypseous crusts would disentangle the expression of gypsum tolerance from biogeographic complications, clarify the phylogenetic distribution of gypsum tolerance and perhaps reveal the nature of the adaptation(s) involved.

It is possible that establishment on gypsum is facilitated by some sort of modification to the radicle. Alternatively, since germination in a desert environment is always risky, adaptations to gypsum soils may differ little from germination strategies of desert taxa generally. Possible strategies could serve to optimize the timing of

germination, minimize the risk of all seedlings perishing, or increase the length of time a seedling has to establish itself. These could include high germination rate at low temperatures and various forms of bet-hedging, such as seed heteromorphism and variable seed dormancy (Escudero et al., 1997). The production of mucilage upon wetting by the seed coat presumably increases the local availability of water and upon drying, anchors the seed (Romao and Escudero, 2005). Some of these traits are known in Nyctaginaceae. For instance, production of mucilage by the anthocarp is common in both gypsophilic and non-gypsophilic taxa in the NAX clade (Spellenberg, 2003), and fruit/seed heteromorphism is known in *Abronia* and *Tripterocalyx* (Wilson, 1974).

Understanding when in their history Nyctaginaceae became gypsum-tolerant will clarify whether homoplasy is best explained by answering the question "how do species become gypsum-tolerant?" or, "why are certain species found only on gypsum?" If it turned out that gypsum tolerance was ancestral in the NAX clade, then experiments may reveal the reasons full gypsophiles do not occur on more typical soils.

The tendency of Nyctaginaceae to evolve cleistogamy and gypsophily has been shown to the extent that we have demonstrated that the high level of homoplasy for these traits is restricted to the NAX clade. In neither case are we able to conclusively identify the largest group capable of evolving the trait. Largely due to the phylogenetic position of *Acleisanthes* (with gypsophilic, cleistogamous species), we infer that it is

possible that the ancestor of the entire NAX clade was predisposed to evolve these traits. In the case of cleistogamy, the topology indicates either that SI mechanisms develop easily in Nyctaginaceae, or that once self-compatibility emerges, there is a high chance of cleistogamy following. If the latter situation is correct, the ultimate explanation for the large number of cleistogamous species in the NAX clade must ultimately rely on explaining the frequent loss of SI, though the proximate cause is more likely related to resource or pollinator limitation in xeric environments. With gypsophily, it remains to be seen what trait(s) allow for tolerance of gypsum soils and when they evolved, and what factors act exclude to gypsophiles from non-gypsum soils.

The present study is the first to provide a comprehensive genus-level examination of the phylogeny of Nyctaginaceae. Though sampling of *Caribea*, *Cuscatlania*, *Cephalotomandra*, *Grajalesia*, and *Neeopsis* would be desirable, the current level of sampling is sufficient to draw several useful conclusions with bearing on future studies of the family. Aside from providing a framework for future taxonomic revisions, it raises interesting evolutionary questions regarding biogeography, reproductive biology, and edaphic endemism. To a degree, this work may be considered a case study into the practical issues that may arise in an investigation of tendencies in character evolution. New insights will be gained with a combination of phylogenetic work at

finer taxonomic scales and experimental data to better understand the natural history of individual species, especially those in the xerophytic clade.

Table 1: Classification of Nyctaginaceae and estimates of species number.

Classification scheme according to Bittrich and Kühn (1993) and estimates of species number. For those genera treated in Flora of North America (Spellenberg, 2003), species number reflects newly described species and taxonomic readjustments. SA= South America, CA=Central America, NA=North America. * as 20 spp. *Abronia* & 4 spp. *Tripterocalyx*. ** including *Selinocarpus* & *Ammocodon* (Levin, 2002).

Tribe Subtribe	Genus	Species number	Spellenberg (2003), if different	Distribution
Leucastereae	*Leucaster* Choisy	1		SA
	Reichenbachia Spreng.	2		SA
	Andradea Fr. Allemão	1		SA
	Ramisia Glaz. ex Baillon	1		SA
Boldoeae	*Boldoa* Cav. ex Lagasca	1		SA, CA
	Salpianthus Humb. & Bonpl.	1		CA
	Cryptocarpus H.B.K.	1		SA
Abronieae	*Abronia* Juss. (incl. *Tripterocalyx* Hook. ex. Standl.)	33	24*	NA
Nyctagineae				
Colignoniinae	*Colignonia* Endl.	6		SA
	Pisoniella (Heimerl) Standl.	1		SA, CA
Boerhaviinae	*Boerhavia* L.	20	ca. 40	Pan-tropical/ subtropical
	Anulocaulis Standl.	4-5	5	NA
	Cyphomeris Standl.	2		NA
	Commicarpus Standl.	25	30-35	Pan-tropical/ subtropical
	Caribea Alain	1		Cuba
	Acleisanthes A. Gray	7	17**	NA
	Selinocarpus A. Gray (incl. *Ammocodon* Standl.)	10	—	NA, Africa
	Okenia Schldl. & Cham.	1-2		NA, CA
Nyctagininae	*Mirabilis* L.	54	ca. 60	NA, Asia
	Cuscatlania Standl.	1		CA
	Allionia L.	2		NA, CA, SA
	Nyctaginia Choisy	1		NA
Phaeoptilinae	*Phaeoptilum* Radlk.	1		Africa
Bougainvilleeae	*Bougainvillea* Comm. Ex Juss.	18		SA
	Belemia Pires	1		SA
Pisonieae	*Pisonia* L.	40	10-50	Pan-tropical/ subtropical
	Guapira Aubl.	70	10-50	SA, CA
	Neea Ruiz & Pavon	83		SA, CA
	Neeopsis Lundell	1		CA
	Cephalotomandra Karst. & Triana	1-3		SA
	Grajalesia Miranda	1		CA

Table 2: Primer sequences used and original publication.

Region Primer name	Sequence	Reference
ITS		
ITS4	TCCTCCGCTTATTGATATGC	White et al., 1990
ITS5a	CCTTATCATTTAGAGGAAGGAG	Stanford et al., 2000
ndhF		
Nyct_ndhF1F	TGCCTGGATTATACCCTTCA	This study
NdhF972F	ATGTCTCAATTGGGTTATATGATG	Olmstead and Sweere, 1994
Nyct_ndhF13R	CAFCBGGATTACYGCATTT	This study
Nyct_ndhF22R	CTTGTAACGCCGAAACCATT	This study
Nyct_ndhF6F	AACGGGBAGTTTYGARTTTG	This study
Nyct_ndhF8R	AGTAGGCCCCTCCATAGCAT	This study
Nyct_ndhF14F	TCAATCGTTGCAATCCTTCT	This study
Nyct_ndhF16R	TTTCCGATTCATGAGGATATGA	This study
*rps*16		
rpsF	GTGGTAGAAAGCAACGTGCGA	Oxelman et al., 1996 (modified)
Rps2R	TCGGGATCGAACATCAATTGCAAC	Oxelman et al., 1996
*rpl*16		
F71	GCTATGCTTAGTGTGTGACTCGTTG	Jordan et al., 1996
R1661	CGTACCCATATTTTTCCACCACGAC	Jordan et al., 1996

Table 3: Summary of sequence statistics by partition for the molecular matrix.

Maximum-likelihood model estimated by ModelTest (Posada and Crandall, 1998): Full, Entire; Sensitivity, Entire; Restricted I & II, Entire. ML model for remaining partitions (used in Bayesian analyses "B2", "B4", and "B6", see text) estimated with MrModelTest (Nylander, 2004). Numbers in parentheses are number of informative characters gained from the inclusion of ITS1 & ITS2 in restricted analyses.

	Full Analysis									Sensitivity Analysis	Restricted Analysis I
Partition	*ndh*F (entire)	*ndh*F (1st pos.)	*ndh*F (2nd pos.)	*ndh*F (3rd pos.)	*rps*16	*rpl*16	5.8s	Entire	Chloroplast	Entire	Entire
#Taxa Included	58	58	58	58	58	58	58	58	58	41	19
Aligned Length	2193	731	731	731	1237	1367	157	5505	4797	5359	4887
Analyzed Length	2205	669	668	668	780	792	157	3734	3577	3396	4278
Constant	1348	474	532	342	520	552	136	2556	2420	2467	3883
Uninformative	272	83	69	120	110	139	5	526	521	493	160
Parsimony-informative	385	112	67	206	150	101	16	652	636	436	235 (122)
ML Model	GTR+I+Γ	GTR+I+Γ	GTR+I+Γ	GTR+Γ	GTR+Γ	GTR+Γ	SYM+I+Γ	GTR+I+Γ	GTR+I+Γ	GTR+I+Γ	GTR+I+Γ

Figure 1: Maximum-likelihood (ML) topology from the analysis of the entire data set.

Parsimony bootstrap/ML bootstrap support values above branches, Bayesian posterior probability from the "B6" analysis below branches, "-" indicates bootstrap support value <50. Tribes of Nyctaginaceae according to Bittrich and Kühn (1993) are in bold. "-" before unbold name signifies a subtribe of Tribe Nyctagineae.

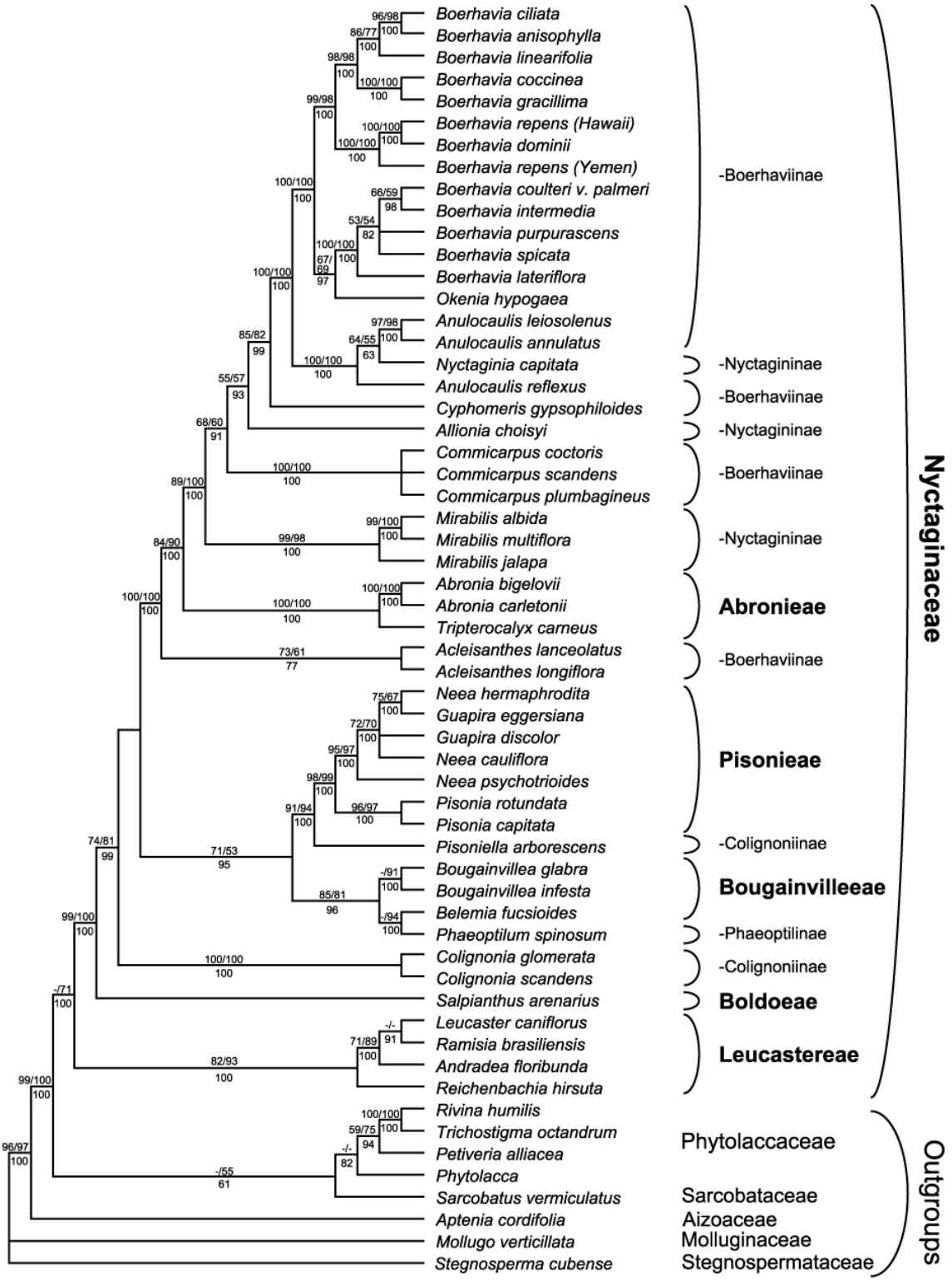

Boerhavia ciliata
Boerhavia anisophylla
Boerhavia linearifolia
Boerhavia coccinea
Boerhavia gracillima
Boerhavia repens (Hawaii)
Boerhavia dominii
Boerhavia repens (Yemen)
Boerhavia coulteri v. palmeri
Boerhavia intermedia
Boerhavia purpurascens
Boerhavia spicata
Boerhavia lateriflora
Okenia hypogaea
Anulocaulis leiosolenus
Anulocaulis annulatus
Nyctaginia capitata
Anulocaulis reflexus
Cyphomeris gypsophiloides
Allionia choisyi
Commicarpus coctoris
Commicarpus scandens
Commicarpus plumbagineus
Mirabilis albida
Mirabilis multiflora
Mirabilis jalapa
Abronia bigelovii
Abronia carletonii
Tripterocalyx carneus
Acleisanthes lanceolatus
Acleisanthes longiflora
Neea hermaphrodita
Guapira eggersiana
Guapira discolor
Neea cauliflora
Neea psychotrioides
Pisonia rotundata
Pisonia capitata
Pisoniella arborescens
Bougainvillea glabra
Bougainvillea infesta
Belemia fucsioides
Phaeoptilum spinosum
Colignonia glomerata
Colignonia scandens
Salpianthus arenarius
Leucaster caniflorus
Ramisia brasiliensis
Andradea floribunda
Reichenbachia hirsuta
Rivina humilis
Trichostigma octandrum
Petiveria alliacea
Phytolacca
Sarcobatus vermiculatus
Aptenia cordifolia
Mollugo verticillata
Stegnosperma cubense
-Boerhaviinae
-Nyctagininae
-Boerhaviinae
-Nyctagininae
-Boerhaviinae
-Nyctagininae
Abronieae
-Boerhaviinae
Pisonieae
-Colignoniinae
Bougainvilleeae
-Phaeoptilinae
-Colignoniinae
Boldoeae
Leucastereae
Phytolaccaceae
Sarcobataceae
Aizoaceae
Molluginaceae
Stegnospermataceae
Nyctaginaceae
Outgroups

Figure 2: Phylogram of the maximum-likelihood topology from Fig. 1.

Major clades referred to in text are highlighted.

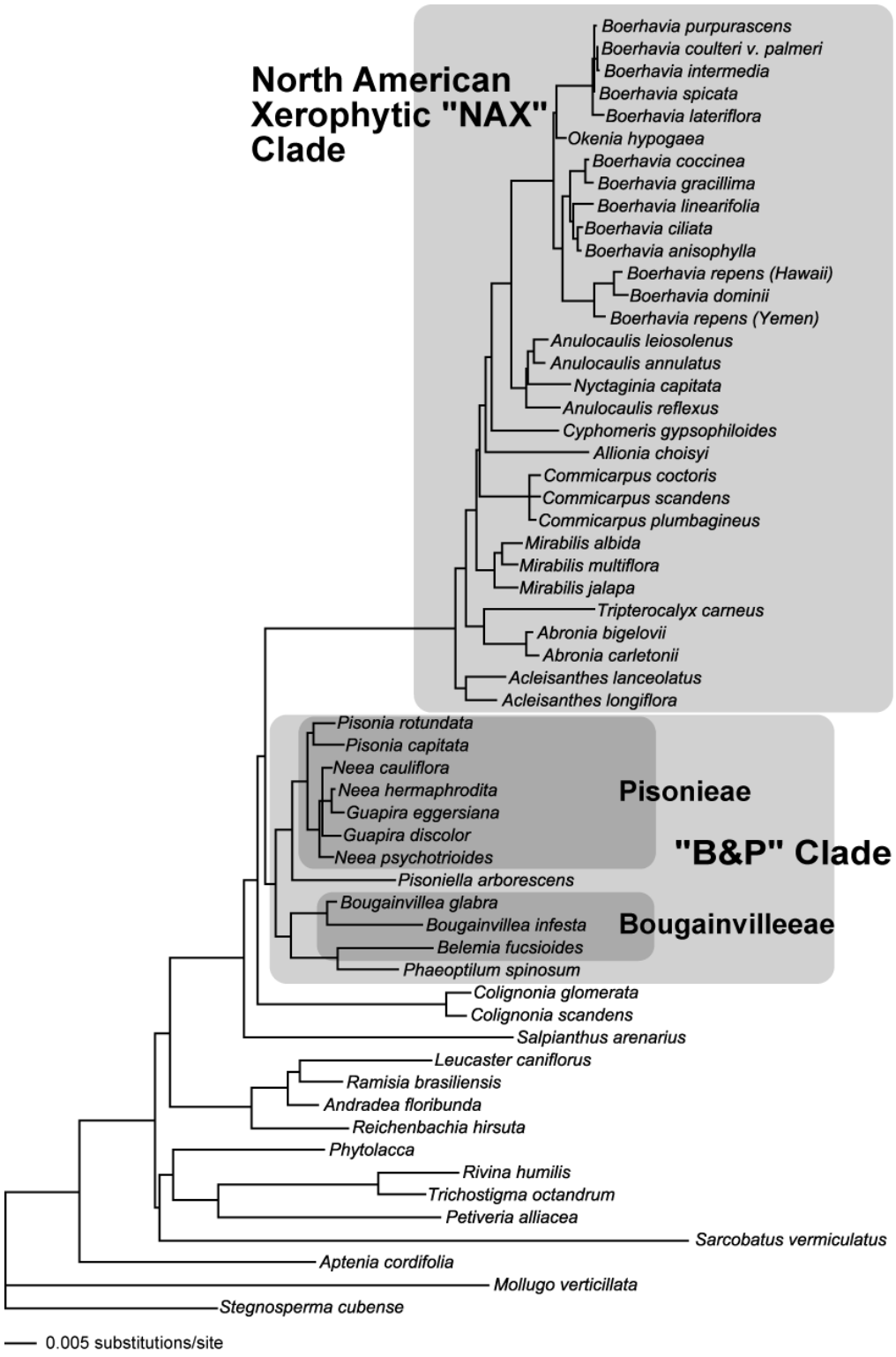

North American Xerophytic "NAX" Clade
Boerhavia purpurascens
Boerhavia coulteri v. palmeri
Boerhavia intermedia
Boerhavia spicata
Boerhavia lateriflora
Okenia hypogaea
Boerhavia coccinea
Boerhavia gracillima
Boerhavia linearifolia
Boerhavia ciliata
Boerhavia anisophylla
Boerhavia repens (Hawaii)
Boerhavia dominii
Boerhavia repens (Yemen)
Anulocaulis leiosolenus
Anulocaulis annulatus
Nyctaginia capitata
Anulocaulis reflexus
Cyphomeris gypsophiloides
Allionia choisyi
Commicarpus coctoris
Commicarpus scandens
Commicarpus plumbagineus
Mirabilis albida
Mirabilis multiflora
Mirabilis jalapa
Tripterocalyx carneus
Abronia bigelovii
Abronia carletonii
Acleisanthes lanceolatus
Acleisanthes longiflora
Pisonia rotundata
Pisonia capitata
Neea cauliflora
Neea hermaphrodita
Guapira eggersiana
Guapira discolor
Neea psychotrioides
Pisonieae
"B&P" Clade
Pisoniella arborescens
Bougainvillea glabra
Bougainvillea infesta
Belemia fucsioides
Phaeoptilum spinosum
Bougainvilleeae
Colignonia glomerata
Colignonia scandens
Salpianthus arenarius
Leucaster caniflorus
Ramisia brasiliensis
Andradea floribunda
Reichenbachia hirsuta
Phytolacca
Rivina humilis
Trichostigma octandrum
Petiveria alliacea
Sarcobatus vermiculatus
Aptenia cordifolia
Mollugo verticillata
Stegnosperma cubense
0.005 substitutions/site

Figure 3:MP bootstrap/ML bootstrap support values are shown.

Anulocaulis and *Boerhavia* are each supported as monophyletic.

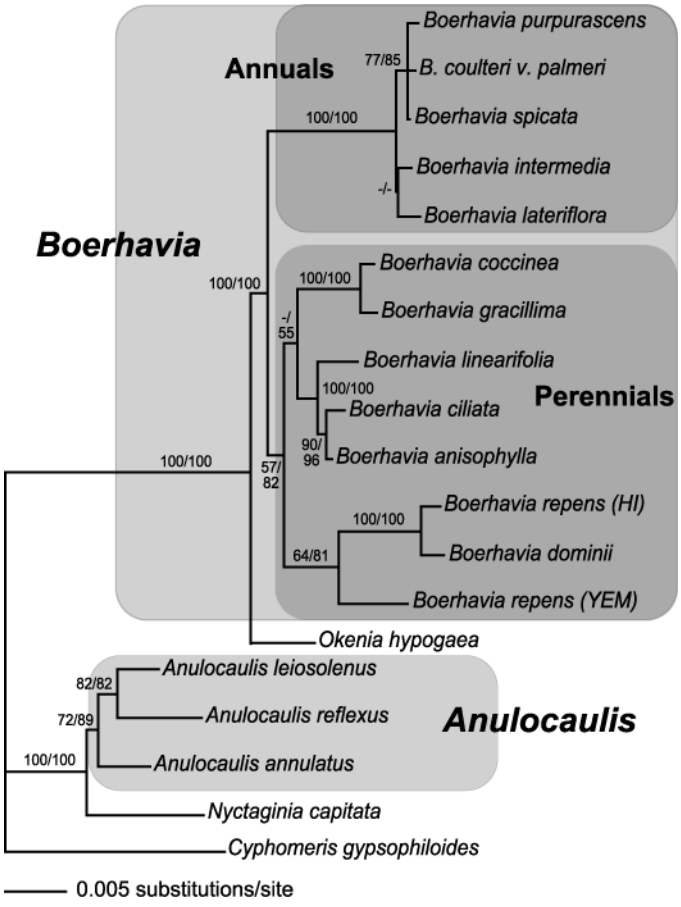
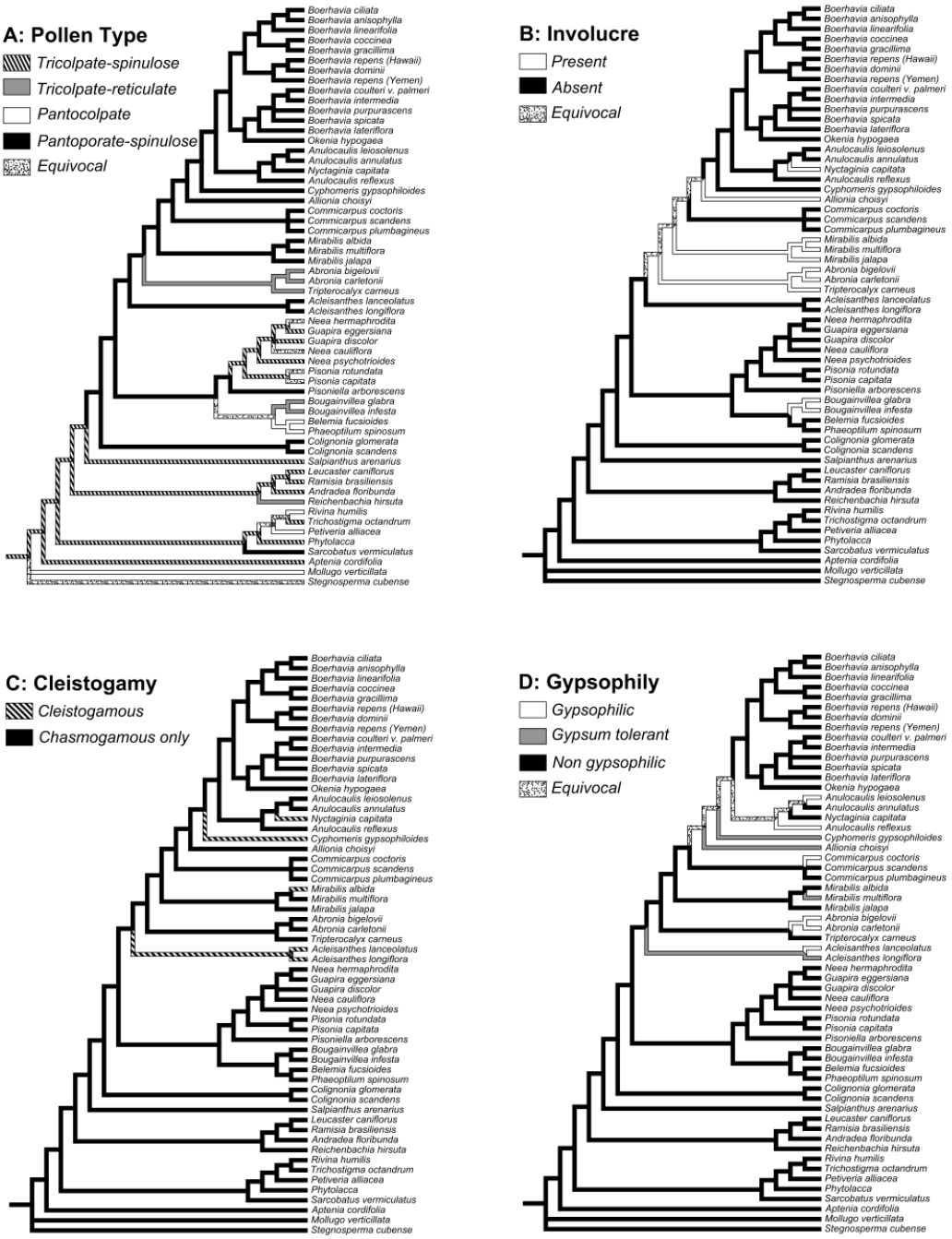


Figure 4: Parsimony reconstruction of (A) pollen morphology, (B) involucre presence, (C) cleistogamous flowers, and (D) gypsophilic habit (based on ordered characters).


A: Pollen Type
Tricolpate-spinulose
Tricolpate-reticulate
Pantocolpate
Pantoporate-spinulose
Equivocal
B: Involucre
Present
Absent
Equivocal
C: Cleistogamy
Cleistogamous
Chasmogamous only
D: Gypsophily
Gypsophilic
Gypsum tolerant
Non gypsophilic
Equivocal
Boerhavia ciliata
Boerhavia anisophylla
Boerhavia linearifolia
Boerhavia coccinea
Boerhavia gracillima
Boerhavia repens (Hawaii)
Boerhavia dominii
Boerhavia repens (Yemen)
Boerhavia coulteri v. palmeri
Boerhavia intermedia
Boerhavia purpurascens
Boerhavia spicata
Boerhavia lateriflora
Okenia hypogaea
Anulocaulis leiosolenus
Anulocaulis annulatus
Nyctaginia capitata
Anulocaulis reflexus
Cyphomeris gypsophiloides
Allionia choisyi
Commicarpus coctoris
Commicarpus scandens
Commicarpus plumbagineus
Mirabilis albida
Mirabilis multiflora
Mirabilis jalapa
Abronia bigelovii
Abronia carletonii
Tripterocalyx carneus
Acleisanthes lanceolatus
Acleisanthes longiflora
Neea hermaphrodita
Guapira eggersiana
Guapira discolor
Neea cauliflora
Neea psychotrioides
Pisonia rotundata
Pisonia capitata
Pisoniella arborescens
Bougainvillea glabra
Bougainvillea infesta
Belemia fucsioides
Phaeoptilum spinosum
Colignonia glomerata
Colignonia scandens
Salpianthus arenarius
Leucaster caniflorus
Ramisia brasiliensis
Andradea floribunda
Reichenbachia hirsuta
Rivina humilis
Trichostigma octandrum
Petiveria alliacea
Phytolacca
Sarcobatus vermiculatus
Aptenia cordifolia
Mollugo verticillata
Stegnosperma cubense

2. Diversification patterns in the Sonoran Desert: insights from an intensively sampled species-level nuclear phylogeny of *Boerhavia* (Nyctaginaceae).

2.1 INTRODUCTION

2.1.1 Development of the Sonoran Desert

Plants of arid environments have long attracted the attention of evolutionary biologists. While the extremity of desert environments has provoked an overwhelming number of studies focusing on floristics, physiology, and ecology, their history has been given somewhat less attention. The foremost authority on paleoarid environments, D.I. Axelrod, wrote a series of papers concerning the age of desert vegetation in North America, and reached the conclusion that arid zones were much more localized in the Tertiary and regional deserts like those in North America only developed between 5 ma and 100 ka (Axelrod, 1975, 1978, 1979; Axelrod and Raven, 1985). At the time of these papers, a recent origin of regional deserts ran counter to the conventional wisdom, which held that deserts were ancient (Johnston, 1940; Blair et al., 1976).

As summarized by Van Devender, (1990), modern desert plant communities fluctuated dramatically in the Pleistocene and Holocene. At lower elevations community assemblages differed from those found today. In the last glacial period,

woodland habitats were widespread in areas of the present Sonoran Desert now home to scrub vegetation, and summer emphemeral species were uncommon. Since then the climate has become more arid overall, but summer monsoonal rains have become relatively more important. This has led to the establishment of a diverse assemblage of species that respond to the winter and summer precipitation that characterizes the modern Sonoran Desert. Most elements of the modern regional flora were present during this period, though their distribution and associations were often dramatically different.

Investigations into the assembly of modern desert vegetation have profited greatly from the work of investigators studying subfossil plant remains in rodent middens, (Betancourt et al., 1990). In these studies, though, resolution is obtained spanning tens of thousands of years. To understand the *origin* of species that comprise modern desert floras, a deeper perspective is needed. For those spectacular oddities thought to be paleoendemics of the Peninsular desert of Baja California, Mexico, e.g. *Fouquieria columnaris* (Wiggins, 1960; Schultheis and Baldwin, 1999), moister periods were thought to have been spent in isolated rain shadows, or more southerly xeric areas (i.e. the "Hidalgan desert"). Some of these taxa were (as are many now) part of tropical dry forest habitats (Martin et al., 1998), which have existed in western Mexico since the early Miocene (Becerra, 2005). On the other hand, it has long been thought that there is a

tendency for speciation to be accelerated in arid environments (Stebbins, 1952). Many phylogenetically isolated lineages have produced radiations in which modern species are likely to be recently derived in North American deserts; for example, *Krameria* (Simpson et al., 2004), *Fouquieria* (Schultheis and Baldwin, 1999), *Fagonia* (Beier et al., 2004), and *Tiquilia* (Moore and Jansen, 2006). Thus, while xeric-adapted lineages in general may have a reasonably long history in North America, the modern representatives are often of recent origin. Furthermore, many have only attained their current distributions in the past 4,000-5,000 years (Betancourt et al., 1990). While biogeographic and phylogeographic patterns reflecting older vicariant events have been found in many taxa, mostly animals, from the Baja California peninsula (Wiggins, 1960; Riddle et al., 2000a; Riddle et al., 2000b; Nason et al., 2002; Zink, 2002; Clark-Tapia and Molina-Freaner, 2003), rapid regional changes in vegetation are likely to have left a genetic signature on constituent species (Hewitt, 1996). Thus, in addition to phylogeographic patterns reflecting divergence in allopatry, patterns may also be found that reflect the recent, rapid expansion of desert vegetation (Nason et al., 2002).

2.1.2 Species-level phylogenetic studies of recent radiations

It is increasingly recognized that the study of many macroevolutionary patterns is dependent on the resolution of phylogeny at the species level (Barraclough and Nee, 2001). Species-level phylogenetic studies are here taken to mean those studies in which

most or all of the species in a particular clade are included in the analysis, preferentially with multiple accessions per species. Species-level phylogenies are optimal for investigation of evolutionary trends involving ecological, physiological, or morphological traits (Beardsley et al., 2004). Additionally, studies in which focus on historical process (i.e. phylogeography, lineage sorting, introgression) attempt to discern the present and historical population structures that have affected diversification at the species level. Such work, especially phylogeographic, in animal systems has a long history in molecular systematics owing to the early development of mitochondrial DNA as a marker (Avise, 2000). Similar studies in plants have lagged behind, owing to the much slower rate of sequence evolution in plant mitochondria and chloroplasts (Soltis et al., 2006).

There are advantages to using organellar genomes for the estimation of species-level phylogenies, such as a lack of conflict due to recombination, smaller effective population size. Thus, sequences of mtDNA, and in certain cases chloroplast introns, extremely high resolution is obtainable (Small et al., 1998; Shaw et al., 2005; Shaw et al., 2007). There are also drawbacks to these genomic compartments as a source of data. Depending on the question, the lack of recombination can be a curse in addition to a blessing. In some cases, commonly referred to as "chloroplast capture", organellar trees can be misleading with respect to a species phylogeny. Additionally, the lack of

recombination means that the entire organellar sequence acts as a single locus, meaning that, in the absence of other data, trees built on organelle sequences are relatively insensitive tools for detecting hybridization, introgression, and lineage sorting (Doyle, 1992). This problem is compounded by low intraspecific sampling (Omland et al., 1999).

Species level phylogenies in plants are *de facto* based on comparative sequencing of chloroplast introns, often in combination with the rapidly evolving ribosomal internal transcribed spacer (ITS). Though the application of this technique is common, it is often the case that conclusions are limited by a lack of phylogenetic resolution in the available markers due to low amounts of informative variation (Hughes et al., 2006). These constraints have led researchers to use rapidly-evolving nuclear sequences for phylogeny reconstruction at the species level. Single- or low-copy nuclear introns in protein coding genes are a favored target for such studies due to relatively large amounts of detectable variation (even to the point of homo- and heterozygosity at a locus). The genes in which such introns are located are often characterized in a variety of species, facilitating exon-anchored primer construction based on published sequences (Hughes et al., 2006). Outside of model organisms and their close relatives, this technique has been slower to develop, since with increasing phylogenetic distance from a known sequence it becomes more difficult to design primers to amplify intron regions. Also, introns are regularly gained or lost (Nielsen et al., 2004), or their size changes to

become too small to contain useful information or too large to easily amplify. However, the growth of published sequences in GenBank and other databases as made it easier than ever to develop single-copy nuclear introns and other highly resolving markers in non-model systems (Whittall et al., 2006).

The increasing availability of molecular data seems to have led to a recent quieting of the debate over the "species problem". The copious literature on species definitions has come to no resolution, rather, most researchers would prefer to have data that reflects an ambiguous reality than endless discussion of definitions (Hey, 2001, 2006). In particular, the opportunity is now here to evaluate patterns of diversification in non-model systems, especially those of relatively recent origin. As more studies have been published with species-level phylogenies it has become apparent that, in plants, species non-monophyly is a common phenomenon, occurring in about 35% of the species in studies included in a recent review (Syring et al., in press). In these surveyed studies, it was not necessarily the intent of the researchers to engage the question of species non-monophyly, but it was discovered nevertheless. Reasons for this are numerous. Particularly in plant systems, where mating systems are more varied than in animals, hybridization, and polyploidy can play a major role in controlling the distribution of alleles within and among species (Rieseberg, 1997; Linder and Rieseberg, 2004). More general causes are frequent, ranging from imperfect or incomplete

taxonomic knowledge related to polymorphism, and various forms of phenotypic plasticity, to historical or ongoing processes that involve recent origin and cryptic speciation (Funk and Omland, 2003).

When problematic taxonomy is recognized, we refer to "species complexes" in which variation is not cleanly partitioned into discrete taxonomic units; these are common in plants. Though a species complex could arise from a number of different causes, it is easiest to explain in a group undergoing active diversification, and it is in such a group that patterns of reticulation are predicted to be most acute (Linder and Rieseberg, 2004). However, following a recent speciation event, a period of paraphyly is expected, potentially producing a similar pattern in gene trees (Rieseberg and Brouillet, 1994). While biologists are attracted to such systems where they can observe "evolution in action", it has been historically difficult or impossible to factor out genealogical factors. Very few studies have targeted species level questions, especially in recent radiations, with intraspecific sampling of single- or low-copy nuclear markers.

2.1.3 Boerhavia as a system to examine genetic patterns in a recently radiated clade

The North American xeric Nyctaginaceae are in all likelihood another example of the "relictual lineages & recent radiations" pattern found, for example, in *Tiquilia* (Moore and Jansen, 2006). This family has produced a number of genera of xeric-adapted

species in the deserts of North America (Douglas and Manos, 2007). Several genera of desert Nyctaginaceae, for example *Cyphomeris* and *Allionia*, are morphologically unusual and have low species diversity, suggesting that they may be relictual. Some genera, have undergone substantial recent speciation: *Commicarpus*, *Acleisanthes*, *Mirabilis*, and *Abronia*. Each of these genera present taxonomic difficulties at the species level. However, these genera have radiated most dramatically in the Old World (*Commicarpus*), the Chihuahuan Desert (*Acleisanthes*), or are not confined to single biogeographic region or habitat (*Mirabilis, Abronia*). The genus *Boerhavia*, on the other hand, is most abundant and diverse in the Sonoran and Peninsular deserts. Within the genus both annual and perennial species occur. The Chihuahuan has three distinct perennial taxa and the annual *B. torreyana*, but otherwise fewer nominal species of *Boerhavia*, most of which are also found in the Sonoran.

The perennial taxa in general are widespread and morphologically diverse, with two widespread complexes being well-known as problematic. The *Boerhavia coccinea*/*B. diffusa* group comprises species with small, usually deep purple to red, flowers and glandular fruits. Infloresence architecture varies widely, with long-pedunculate axillary cymes to diffuse panicles of cymes to solitary flowers. Species in this group have frequently been combined as a single taxon, *B. diffusa*, e.g. (Porcher, 1978), although it is now more frequent to separate the two on the basis of whether inflorescences are

paniculate or axillary (Whitehouse, 1998; Spellenberg, 2003). The "*repens* group" (Fosberg, 1978) comprises an indeterminate number of species of typically coastal perennial plants also with glandular fruits and white to pink flowers, distributed mainly on islands in the tropical Pacific and Indian oceans, and in Australia, north Africa, and Arabia. This group has received limited attention since Fosberg's treatment (Meikle and Hewson, 1984; Fosberg, 1988).

Though *Boerhavia* has a history of being a difficult genus to circumscribe, (Fosberg, 1978; Spellenberg, 2003; Douglas and Manos, 2007), the greater difficulty in the genus lies in the delimitation of species. Sereno Watson and Paul Standley, who worked on the taxonomy of Nyctaginaceae at the time that botanical exploration was expanding in southwestern North America, were responsible for many early descriptions of taxa occurring in this region (Watson, 1889; Standley, 1909, 1911, 1918). Their tendency, common at the time, was to be liberal with the application of names. More recent authors have tended to lump species of *Boerhavia*. For example, *B. watsonii* and *B. intermedia* have been reduced to varietal status of related species (Spellenberg, 2002; Spellenberg, submitted).

A distinctive group of *Boerhavia*, characterized by a summer-annual life cycle, is mainly confined to the deserts of North America where there are 16 taxa currently recognized. A few of these have become widespread, e.g. *Boerhavia erecta*, which is

found in warmer areas worldwide, not exclusively in deserts. In the annual group, taxa are recognized by a combination of traits and occasional unique characters. Infloresence architecture can be either umbellate, subumbellate, capitate, racemose, or spicate. Flower color varies slightly, from reddish-pink is some *B. erecta* to pale purple in *B. purpurascens* and *B. traubae*, and pale pink to white in the remaining annuals. The size of the flowers is highly variable, as are other floral traits, such as the number and size of the stamens, and the relative position of the stamens and stigma with respect to the perianth and the anthers. In contrast to the flowers, which provide little in the way of distinguising taxonomic characters, the morphology of the fruit is often distinctive. Fruits can be clavate, obovoid, or obpyramidal. Furthermore, they can vary in the number of ribs and the degree of expansion of ribs. Ribs are smooth or rugose, and can be slightly to prominately winged, and the sulci can be narrow to broad, and smooth, rugose, or papillate. Table 4 presents a summary of floral and fruit characters for the 16 taxa of North American annuals. Examination of these measurements shows that there is considerable overlap among the traits between taxa. However, overall variation is high, with the length of the perianth tube, for example, varying by a factor of nearly six within the annuals (this figure becomes much higher when perennial taxa are included). Large amounts of variation are evident within taxa as well (Table 4). Differing taxonomic philosophy and simple confusion has led to the creation of more than 180

binomials for species of *Boerhavia* (Solomon, 2007) whereas informed estimates of the actual number of species fall between 20 and 40 (Bittrich and Kühn, 1993; Spellenberg, 2003). Taken together, the high level of variation within and among species of *Boerhavia*, the historical taxonomic difficulty the genus has presented, and the concentration of annual species in the geologically young Sonoran desert, strongly suggest that this radiation of species in the Sonoran is likely to be quite young. Indeed, Axelrod (1979) included *Boerhavia* among genera that began to appear in the Sonoran desert in the Quaternery, less than 2 MA.

The objective of this study is to investigate the phylogeny of *Boerhavia* at the species level using nuclear sequence data, as a first step in understanding the recent radiation of species. I focus especially on the annual group and include multiple samples of nearly all morphospecies from across the geographic range of each. The perennial taxa are sampled with less intensity owing to their broader geographic distribution, however, I have attempted to follow a similar philosophy of including multiple accessions of species from geographically disparate regions. With these data it is possible to evaluate a suite of existing taxonomic hypotheses pertaining to relationships within and among species and put to rest some long-standing controversies in the genus as a whole and within the annual group (Codd, 1966;

Whitehouse, 1998; Spellenberg, 1999, 2000, 2002; Spellenberg, submitted). I identify those taxa for which molecular sequence data support recognition as independent evolutionary entities and thus represent "good" species. For taxa that are not supported with molecular data, I explore the strength of the evidence and biological reasons for the discordance between morphologically based taxa and molecular data. Finally, I investigate phylogeographic trends in the radiation of the genus, especially in the context of a recent, rapid expansion of the Sonoran Desert.

2.2 MATERIALS AND METHODS

2.2.1 Sampling

Samples of *Boerhavia* (Appendix 2) were obtained primarily as collections made by the author and/or R. Spellenberg. Material for DNA extraction was also obtained from the following herbaria: DUKE, MEXU, MO, NMC, and NY. I included representative collections from each continent where *Boerhavia* is found. Pacific and Indian insular populations, are poorly sampled due to the paucity of collections suitable for molecular work. Since the primary focus of this study relates to the diversification of summer annuals, the distribution of sampling is heavily biased towards the southwestern United States and northern Mexico. DNA was extracted for 191 accessions of *Boerhavia* using either a modified CTAB method (Doyle and Doyle, 1987) or with

Qiagen DNAEasy Plant Mini Kits (Qiagen, Valencia, CA). Two close relatives of *Boerhavia*, *Nyctaginia capitata* and *Okenia hypogaea* (Douglas and Manos, 2007), were also included as outgroups.

2.2.2 Molecular data

ITS sequences were generated with the same protocol as was used in Chapter 1 (Douglas and Manos, 2007). Primers which amplified the third intron of nitrate reductase (NIA) were developed using the published degenerate primers NIA3F and NIA3R (Howarth and Baum, 2002). These primers frequently amplified two fragments in *Boerhavia*, one approximately 400-600 bp, the other 1.3-1.6 kb. From a sample of taxa, these fragments were excised, cloned, and sequenced. The larger fragment was found to represent roughly 100 bp with strong homology to the 3′ end of intron 3 of NIA in *Spinaca*. The remainder of the sequence produced no significant alignments in BLAST searches, though it corresponded to the approximate size of the third intron of NIA from *Spinaca*. The 3′ reverse primer is sited on the first 24 bases of intron 4. Sequences from the smaller fragment were also alignable in the exon region, but the remainder produced no significant alignment to any sequence in GenBank. The smaller fragment was not investigated further. A primer (NyctNR3Fa: 5′-TCTSAACACCCAACCTGAGAAGC-3′) was designed using *Boerhavia* sequences for the longer fragment. It is sited near the end of exon 3. A less-degenerate reverse primer, located in the same site as NIA3R, was

constructed using the published *Spinaca* NIA sequence for exon 4 (NyctNR4Ra: 5′-GAACCAGCAGTTGTTCATCATSCC-3′). The length of the amplified fragment required the development of two internal sequencing primers located in a conserved region of the intron: NyctNR3Fb: 5′-CGTAAGGCGGCGTTAAAGCGCAAC-3′ and NyctNR4Rb: 5′-GATGATATGATGCGAGGACCCATG-3′. Use of these primers resulted in double-stranded sequence coverage for most accessions. In the case of double-peaks (rare in high-quality reads) IUPAC ambiguity codes were inserted in the sequence. Heterogeneity in sequence length was not detected, although a long poly-T region occasionally caused the sequencing reaction to fail. Sequences were edited in Sequencher (Gene Codes, Ann Arbor, MI). Alignment was performed manually using Se-Al (Rambaut, 1996). Regions of questionable alignment (in the NIA dataset) were excluded from further analysis.

2.2.3 Data analysis

Preliminary parsimony analyses and likelihood analyses were carried out on the two datasets independently. Maximum-parsimony (MP) searches were conducted with 1000 replicates of 10 random addition sequences, TBR branch swapping, MaxTrees set to auto-increase, saving, per replicate, 100 trees greater than or equal to 800 steps. Parsimony bootstrapping was performed with 1000 replicates of 10 random-addition sequences, TBR branch swapping, with steepest descent and a reconnection limit of 8,

and a limit of 10^6 rearrangements per replicate. Maximum likelihood (ML) searches were conducted in Garli (Zwickl, 2006) using the default parameters, which correspond to a General-Time Reversible model with a proportion of invariant sites and a gamma shape parameter (GTR+I+G).

Overall support levels in the ITS dataset were low, and only one instance of supported topological incongruence was found between the ITS and NIA topologies (regarding the placement of *B. verbenacea*); thus, I analysed the combined dataset as above. I also computed ML bootstrap values with 1000 replicates, in Garli, and performed a Bayesian analysis. For this analysis, MrModelTest (Nylander, 2004) was first used to estimate a best-fit model for the following data partitions: NIA, (GTR+I+G); ITS1 (GTR+I); 5.8s (Jukes-Cantor). As a conserved secondary structure has been found for ITS2 (Coleman, 2003), three representative sequences were folded using Mfold (Zuker et al., 1999). Locations of stems and loops were identified in these sequences and the alignment of ITS2 was divided into two partitions, "stem" and "loop". Nucleotides which are paired in the folded structure were then specified. The "doublet" model, which was designed to account for non-independence in compensatory base changes in folded RNAs, was applied to the stem partition. The HKY model was favored by MrModelTest for both the stem and loop partitions of ITS2. Prior to implementing the doublet model in the full analysis, a preliminary Bayesian analysis was conducted on

ITS data alone. A slight improvement was noted in the number of nodes supported at the 95% posterior probability level when the doublet model was applied. The doublet model has seen limited use in animal studies with 18s rDNA sequences, e.g. (Kjer, 2004), but I am unaware of any studies which have applied it in either plant systems or to the ITS2 region. Analysis of the full dataset with the full complement of five models entailed 4 replicated runs of 10^6 generations. Stationarity was apparent following 10^5 generations. Consensus trees were computed for the four runs independently. The number of clades supported in the four replicates differed somewhat, but in no case were conflicting clades supported. Thus, I conservatively report Bayesian support values as average support across the four runs.

In certain cases alternative topologies were tested using the Templeton (1983) and Shimodaira-Hasegawa (1999) (SH) tests as implemented in PAUP* (Swofford, 2002) by constructing appropriate constraint trees, generating up to 10 optimized alternative topologies consistent with each constraint, then conducting the resampling using PAUP* and comparing the resultant distributions of ranks or log-likelihoods and associated p-values to that of the preferred ML topology.

Morphological and distributional data was assembled for the taxa in this study from disparate sources; for North American species, species accounts in Flora of North America (Spellenberg, 2003) were used, except in the case of Mexican endemics, for

which Standley (1918) and Spellenberg (1999) reflect the most relevant taxonomic understanding. For species in Australia, Meikle and Hewson (1984) provided descriptions. Fosberg (1978) described some taxa from Pacific and Indian ocean islands. Southern African species are treated in Codd (1966). The taxonomy of *B. cordobensis* and *B. pterocarpa* as they were recognized in Argentina and southern Africa were clarified by Stirton (1982) and López (1998). In certain instances I used original descriptions (Standley, 1909, 1911, 1918; Killip, 1926; Standley, 1931a, b) and/or original observations from collections in this study.

2.3 RESULTS

2.3.1 Dataset

ITS was readily amplified from the 154 samples (Appendix 2). Few instances of heterogeneous sequences were found, in these cases, the position was coded with the appropriate IUPAC ambiguity code at that site. Alignment of ITS was trivial. Complete or partial NIA sequences were obtained for 123 samples. Complete sequences ranged from 1430 bp to 1690 bp (Table 5). The region either failed to amplify or anomolous fragments were amplified in all nearly all samples of *Boerhavia spicata*, *linearifolia*, and *ciliata*. Sequenced fragments from *Boerhavia traubae* and some *B. purpurascens* were unalignable to the rest of the dataset, even using the published degenerate primers, thus

I was not able to include *B. spicata* or *B. traubae* in the nitrate reductase dataset. For *B. purpurascens*, only a subset of "normal" sequences are included. NIA was found to have numerous indels, including a 248 bp insertion in *B. gracillima*. Details of the alignment are given in Table 5.

2.3.2 Phylogeny of *Boerhavia* based on combined data

Results from phylogenetic analyses of the combined dataset (Figure 5) were robust to the optimality criterion employed. No cases of strongly-supported conflicting nodes were found between the MP, ML, and Bayesian analyses. Twenty-two nodes were supported at the 70% level in the parsimony bootstrap, in the MLBS and Bayesian analyses 27 nodes were supported at the 70% and 95% level, respectively.

Boerhavia was not found to be monophyletic: *Okenia* was placed as the sister taxon to the red-flowered clade in analyses of both loci indepedently and combined, although without bootstrap support except in the maximum-likelihood bootstrap (MLBS) and Bayesian analyses of the combined dataset. However, the monophyly of *Boerhavia* requires one step more than the preferred ML tree (and two steps more than in the shortest parsimony trees found). Trees with *Boerhavia* constrained to be monophyletic are not significantly worse than the ML tree using the either the Templeton test with parsimony scores, or the SH test comparing likelihoods.

Perennial taxa were not found to be monophyletic in this analysis, in contrast to the topology found in the "full" and "restricted" analyses presented in Douglas and Manos (2007). Note that the support values for a monophyletic perennial clade decreased dramatically in the restricted analysis I (Figure 7). This re-examination of the data increased the number of informative characters in *Boerhavia* and closely related genera (Table 3), particularly from the ITS region, which is also included in the present analysis. However, the NIA dataset yields a backbone topology which is nearly identical to that from ITS. Unfortunately, it remains unclear at this point whether or not *Okenia* is derived from within *Boerhavia*.

Among the perennial taxa, three main groups are found, each with high support (Figure 5). These clades also were recovered with support in the family-level analyses in Douglas and Manos (2007). The first, the "Chihuahuan Perennials", is a clade comprising *B. anisophylla*, *linearifolia*, and *ciliata*. This is a group of purple-flowered species endemic to the Chihuahuan Desert, and Tamaulipan thornscrub in northeastern Mexico and southern Texas. *Boerhavia coccinea*, *B. diffusa*, *B. gracillima*, and *B. hereroensis* form a strongly supported clade, the "red-flowered perennials." *B. gracillima* is not supported as a monophyletic group, though all accessions have a large insertion in the NIA sequence. Within *B. coccinea*, two accessions from Yemen and one from India are found to be a clade sister to *B. coccinea* from the Americas. Note that one sample

(*Spellenberg 7183*) was originally determined to be *B. repens* whereas *Spellenberg 7153* was called *B. coccinea* (both by R. Fosberg). Both plants were recorded to have pink to lavender flowers rather than red. *Boerhavia diffusa* (with the similar *B. hereroensis*) is found to represent the earliest diverging lineage in the red-flowered clade; this analysis includes samples from North America, Hawaii, and South Africa. One clade of perennial taxa was found to not be monophyletic with the other perennial clades. It will be referred to as the "*repens* complex". Taxa in this group include *B. repens* from South Africa, Sri Lanka and, *B. repens* (*B. herbstii*?) from Hawaii. Also included are the Australian species *B. paludosa*, *B. gardneri*, *B. schomburgkiana*, *B. dominii*, and *B. repleta*, as well as *B. verbenacea*, an annual species from Peru.

A strongly-supported clade of annual taxa is found sister to the *repens* complex. This clade is further subdivided into two clades with differing inflorescence structures. One clade of comprises species with spicate to racemose inflorescences, although within this clade subumbellate (*B. erecta*) or capitate (*B. purpurascens*) architectures may appear umbellate at first glance. High support is found for the cohesiveness of populations of *B. purpurascens*, *B. torreyana*, and *B. erecta*. Significant support is low or absent for the relationships of the other taxa in the spicate clade. The ML topology indicates that *B. coulteri* comprises three groups, one of which achieves some support (parsimony bootstrap =74). This group contains mostly *B. coulteri* var. *palmeri*. The other two groups

are mostly comprised of *B. coulteri* var. *coulteri*. *Boerhavia xanti* is recovered as a paraphyletic unit, out of which is found *B. wrightii*. SH tests in which populations of *coulteri* var. *coulteri*, *coulteri* var. *palmeri*, and *xanti* were each constrained to be monophyletic were not significant (Table 6) though the Templeton test rejected the monophyly of *xanti*.

Sister to the spicate clade is a clade of taxa with umbellate infloresence architecture. This clade is consistently recovered in all analyses, but achieves high support only in the Bayesian analysis of the combined dataset. Found within this clade are representatives of seven taxa: *B. intermedia*, *B. triquetra*, *B. maculata*, *B. lateriflora*, *B. alata*, *B. pterocarpa*, and *B. megaptera*. Despite deep phylogenetic structure in the clade, species-level monophyly is not found for any species with multiple accessions with the exception of *B. pterocarpa*. Three species in this clade have winged fruits; Templeton and SH tests significantly rejected trees in which either the winged or the non-winged species were constrained to monophyly (Table 6).

2.3.3 Noteworthy results from single-locus topologies

The ITS dataset is more complete than the NIA dataset, albeit less well resolved (Figure 7). The inclusion of *B. spicata* and *B. traubae* in the analysis indicated that these taxa both belong to the spicate annual clade. However, the resolution of *B. spicata* is poor, with sequences placed throughout the spicate clade. The two populations of *B.*

traubae (separated by only 2.5km) have different ITS alleles. It is also notable that two accessions of *B. intermedia* (*Douglas 2109* and *Spellenberg 13319*) are placed within the spicate clade, whereas a sample of *B. spicata*, *2171*, appears to have an umbellate ITS allele. In the NIA topology (Figure 6), *Douglas 2109* and *Spellenberg 13319* are placed in the umbellate clade as would be expected, and *Douglas 2171*, being a sample of *B. spicata*, was not amplified. These results were not considered to be in conflict as there were essentially no supported relationships within the annual clade with ITS, the only exception involving an accession for which a NIA sequence was not available. There was no indication of large-scale incongruence in which umbellate and spicate NIA alleles failed to be properly placed in their respective clades, indicating that the anomolous results with ITS are either the result of an actual biological process in which introgressant alleles are maintained and amplified, or more likely, that the result reflects too little information in the ITS region of annual *Boerhavia*s for reliable topologies to be inferred. With the NIA topology, parsimony bootstrap support values in excess of 70% were obtained for some clades that were not recovered in the combined analysis. For example, the "Umbellate I" clade was supported in this analysis (72%).

2.4 DISCUSSION

2.4.1 Major clades of Boerhavia

The Chihuahuan perennial clade (Figure 5) comprises two species that are found throughout the Chihuahuan Desert region, *B. anisophylla* and *B. linearifolia*, and a third, *B. ciliata*, which is found in drier areas of Tamaulipan thornscrub from San Luis Potosí to Mathis, in south-central TX. These species have large, characteristically rich pink to purple flowers that are similar to many Nyctaginaceae in the North American Xerophytic clade, i.e. *Okenia*, *Mirablis*, and *Allionia*. Fruits, unlike other perennial taxa, are not glandular.

The red-flowered perennial taxa (Figure 5) are recovered as a monophyletic group with strong support in all analyses. One species, *B. gracillima*, is found in the Sonoran and Chihuahuan deserts. Though it is not recovered as monophyletic in this study, it is characterized by extremely large flowers for a *Boerhavia*, (often >4mm across, with stamens in excess of 5mm long). *Boerhavia coccinea*, which has variously been split into smaller taxa e.g. (Standley, 1918; Fosberg, 1978; Whitehouse, 1998) or combined with *B. diffusa* e.g. (Long and Lakela, 1971; Wunderlin, 2003), is supported as a distinct lineage. Spellenberg (2003) recognized both *B. coccinea* and *B. diffusa* in the United States, and it appears from these results that *coccinea* and *diffusa* indeed represent two distinct lineages. Whereas *B. diffusa* has been typically recognized from Pacific, African

and Asian localities, in addition to Caribbean and Central American sites, *B. coccinea* has been thought of as a primarily New World species (Fosberg, 1978; but see Whitehouse, 1998). According to these results, *Boerhavia coccinea* is primarily a North American species, however, it is related to a similar group which is to be found at least near the tropical Indian Ocean, and which does not exclusively bear red flowers. In contrast, *B. diffusa* is found from Florida, USA and Hawaii, and is related to *B. hereroensis* of South Africa, which may or may not be a truly distinct taxon (Codd, 1966).

The *repens* clade (Figure 5) is composed of mainly pale pink flowered species with a distribution from Africa, around the Indian Ocean including Australia, into the tropical Pacific, including plants referred to as *B. repens* and varieties. These were last reviewed by Fosberg, who referred to the "*repens* group" as a "protean species most of whose varieties have not been clearly distinguished" (Fosberg, 1978). In his conception of the *repens* group, he was not including the Australian endemic species now implicated as part of this clade by the present study, though later he speculated that several species from coastal habitats were allied to the *repens* group (Fosberg, 1988). Additionally, it is now clear that this group is distinct from the other two perennial *Boerhavia* lineages that have entered the paleotropics, the Old World *B. coccinea* clade, and the *B. diffusa* group.

Use of rapidly evolving nuclear loci for phylogenetic analysis has revealed that species of *Boerhavia* are rampantly nonmonophyletic (Table 7). While the relatively slowly evolving ITS region fails to recover monophyletic species due to a lack of informative variation between haplotypes, the NIA dataset illustrates that the genealogical history of alleles does not, in many cases, conform to current conceptions of morphological taxa. That half of the species with multiple accessions in this analysis are recovered as monophyletic is on par with other studies of this nature (Syring et al., in press), though idiosyncracies of sampling and loci employed between studies precludes any direct comparison of absolute levels of species nonmonophyly.

In this study, the most intensive collection and sampling effort was directed at the North American annual group (Figure 5). Most of the species in this clade have recently been reviewed (Spellenberg, 1999, 2002; Spellenberg, 2003). Though inflorescence architecture has long been a character of taxonomic importance, the phylogenetic significance it bears in this clade has not been previously appreciated, due in part to the fact that the fruits of the umbellate *B. intermedia* strongly resemble those of *B. erecta*, and inflorescences of *B. erecta* are typically subumbellate, adding to the resemblance. *Boerhavia erecta* is, as the name suggests, an erect plant, in contrast to the more decumbent *B. intermedia*. The former is usually found in more mesic microsites

and has been widely introduced in warmer areas of the tropics and subtropics. The two species are not closely related.

Boerhavia purpurascens is supported in this analysis as a member of the spicate annual clade. This species is distinctive among annual *Boerhavia* in having pink to purple flowers and fruits with broad, smooth sulci between the straight ribs. It is similar to the edaphic endemic species *B. traubae* from the Sierra Madre Occidental in Sonora in both flower color and fruit shape. The ITS topology indicates that three accessions of *B. traubae* each share an ITS haplotype with a population of *B. purpurascens*, however, *purpurascens* does not exhibit monophyletic haplotypes for ITS and the populations sister to *B. traubae* samples are not related. NIA was difficult to amplify for *purpurascens* and *traubae*, and though some "normal" sequences were obtained from *B. purpurascens*, many sequenced products were not clearly alignable to other *Boerhavia* NIA sequences. In distribution *B. purpurascens* is clearly a Madrean species, found at somewhat higher elevations (typically 1300-1800 m) than its congeners (Spellenberg, 2003).

Boerhavia torreyana is recovered as a 'good' species in all analyses. This is somewhat surprising given that it was generally confused with *B. spicata* or *B. coulteri* var. *palmeri* until recently (Spellenberg, 2002). However, papillate sulci appear to be diagnostic, as five samples show are found to be monophyletic in NIA. These samples are from Texas and New Mexico; no samples from populations in northern Arizona

were included in this study. *B. torreyana*, *purpurascens*, and *erecta* are all distributed outside of the Sonoran desert and show evidence of being exclusive lineages. That these three represent the three earliest diverging spicate annual species seems to be consistent with a relatively long period of isolation from the remaining spicate annuals, as opposed to being recently derived taxa. This would imply that annual *Boerhavia* have persisted in the Sierra Madre and Chihuahuan Desert for a comparatively long period.

Recognition of the identity of *B. torreyana*, based on the previously unnoticed papillae in the sulcus of the fruits, eased the recognition of the remaining spicate taxa and allowed Spellenberg (2002) to clarify the taxonomy. Four additional species and a variety were treated in this paper: *B. wrightii*, *B. spicata*, and *B. coulteri* and its variety *palmeri*. To this list of taxa should be added *B. xanti*, which is apparently not established in the United States though it has been collected in Pima County, AZ (*Douglas 2113*). Numerous accessions of these species are included in the present analysis. These taxa are collectively found to represent a clade in the combined analysis, but without significant support from either bootstrap analysis or Bayesian posterior probability. However, in the NIA topology (Figure 6), the clade is supported. This most likely results from a lack of resolution in the ITS topology.

In the combined analysis, the next clades to diverge are comprised mostly of *B. coulteri* and its variety, *B. coulteri* var. *palmeri* (formerly *B. watsonii*). This species is

distributed in the Sonoran desert, though perhaps only the var. *palmeri* occurs in southern Baja California. In the ITS analysis (Figure 7), alleles from the species (not the variety) fall out in three groups: One, NMSU17, was collected in Sonora, along the Rio Yaqui (*AL Reina G. 98-834*) and is placed closest to a sample of *B. purpurascens* from SE Arizona (*Spellenberg 13261*). The second group, which is completely identical at the sequence level to all ITS sequences from *B. coulteri* var. *palmeri* (and two accessions of *B. intermedia*), is distributed in Arizona and New Mexico, but this haplotype was not found in *B. coulteri* var. *coulteri* in Mexico. The third group is also widespread throughout the range of *B. coulteri* var. *coulteri*, but also includes sequences from some Sonoran *B. spicata* and *B. xanti*. In contrast, in the NIA topology (Figure 6), a clade containing exclusively *B. coulteri* var. *palmeri* is supported, as are two clades of var. *coulteri*. The variety is morphologically distinctive, with small (≤ 1mm), well-spaced flowers and small (≤ 2.5mm) fruits with narrow sulci and rounded apices. The stems, especially along the infloresence branches, are highly viscid. This clade represents a thorough sample of this taxon from across its range from southern Baja California through Sonora to southern Arizona. A single accession of this taxon, *Spellenberg 13372*, is found in one of the var. *coulteri* clades. However, it is a sample from a roadside in Las Cruces, NM, where *coulteri* var. *coulteri* is known to occur. In an ideal situation for observing hybridization, *Douglas 2126* and *2127* were taken from a closely intermixed population of the species

and the variety in Arizona, yet the samples do not have "incorrect" alleles of either NIA or ITS. Though support was found for two clades of var. *coulteri* and one of var. *palmeri* in the NIA analysis, in the combined tree constraining either variety to monophyly was not significantly worse given the present data (Table 6).

Boerhavia wrightii is a morphologically distinctive spicate species which is distributed widely in the Sonoran and Chihuahuan deserts, into parts of the Mojave. It has 4-angled fruits with correspondingly wide, rugose, sulci, and the flowers are subtended by relatively large persistent bracts. Sampling of this species included two Chihuahuan samples from NM and TX, and two Sonoran samples, from AZ and Baja California. These formed a clade (not highly supported) in the combined analysis. Though they were not well resolved in the ITS tree, they shared an identical sequence. In the NIA topology (Figure 6), the three included samples form a supported clade related to a paraphyletic *B. xanti*.

B. spicata and *B. xanti* are morphologically similar taxa which differ primarily in flower size, with *B. xanti* referring to large-flowered plants in Baja California and Sonora, and *B. spicata* referring to smaller-flowered plants which occur throughout the Chihuahuan and Sonoran Deserts. In the ITS tree, neither group is well resolved. Three related haplotypes are found for *xanti*, and *spicata* samples are recovered in four haplotypes which are resolved in very different areas of the spicate annual clade. *B.*

xanti, on the other hand, gives the appearance of being a clade, in spite of its potential status as the progenitor of *B. wrightii* (according to NIA) or sharing ITS alleles with some populations of *B. coulteri* var. *coulteri*. In the constraint analysis, a hypothesis of a monophyletic is *B. xanti* is rejected with the Templeton test but not with the SH test.

B. spicata samples, interestingly, were either not amplifiable for NIA, or PCR produced a short fragment of unknown identity. It is not simply that the DNA was of poor quality, since ITS was amplified and sequenced without difficulty. Polyploidy or hybridization could result in a heterogeneous pool of PCR products for a single-copy locus such as NIA, making sequencing difficult, however, no products of the correct size were observed in any sample. It is possible that the loss of a priming site is responsible. If this is the case, it is perhaps a molecular character which is associated with the species, though the sequence diversity found at the ITS locus would not lead me to expect a such clean-cut molecular character to be diagnostic for what is in every respect a variable species (Spellenberg, 2002; Spellenberg, 2003).

The other major clade within the annual *Boerhavia*s contains seven species with strictly umbellate infloresences. For convienence, the two major clades of umbellate annuals are referred to as Umbellate I and II (Figure 5). Three are well marked morphologically (Table 4) by the presence of wings on the fruits and by their highly restricted distributions: *B. alata* is endemic to the area of Guaymas, Sonora; *B. pterocarpa*

to south central Arizona and perhaps adjacent Sonora, and *B. megaptera* to south central Arizona.

B. alata has recently been collected only from a small island in the harbor of Guaymas, Sonora, which may be the type locality (Watson, 1889). In the NIA and combined analyses, it is placed with support within a clade which contains some accessions of *intermedia*, *maculata*, *lateriflora*, and *triquetra*. It does not have the identical NIA or ITS allele as *B. megaptera*, the species it most closely resembles morphologically (Standley, 1909), but the two species are both found in the Umbellate annuals II (Figure 5). Since *B. alata* and *B. megaptera* are not sympatric, are morphologically distinct from one another, and do not share alleles, there is no reason to doubt their specific status.

Both of these species are clearly distinct, based on morphological and molecular data, from the third winged species, *B. pterocarpa*, which is broadly sympatric with *B. megaptera*. It apparently prefers sandy or loamy alluvial soils, whereas *B. megaptera* typically occurs on rocky slopes. *Boerhavia pterocarpa* is a species that has highly reduced flowers, <1mm in diameter, with included stamens. It is presumably highly autogamous. The species has not been frequently collected and its distribution is restricted to local areas in southern Arizona and perhaps northern Sonora. It is placed, with support in the NIA topology, as a member of the Umbellate I clade (Figure 5). While there is no direct evidence one way or the other concerning the reproductive

isolation of the winged species from the widespread, non-winged *B. intermedia* complex (described next), the winged taxa are fully sympatric with non-winged umbellates and no indication of hybridization has been noted.

The four remaining taxa in this clade, *B. intermedia*, *B. maculata*, *B. lateriflora*, and *B. triquetra*, are members of a complex of difficult-to-distinguish nominal species (Table 4). *Boerhavia intermedia* is widespread in the Chihuahuan, Sonoran, and Peninsular deserts, the others are confined to the Sonoran desert and Baja California near the Gulf of California. *Boerhavia lateriflora* was distinguished from the similar *B. intermedia* by slightly larger flowers and the production of numerous lateral infloresences (Standley, 1911) though in (Standley, 1918) the description was refined to include broad sulci as compared to narrow as in *B. intermedia*. In this study, two accessions (and one possible hybrid) resemble *B. lateriflora*. One specimen is placed in each clade of umbellate annuals according to the NIA topology (Figure 6), despite the fact that the collections were made within 10 km of each other.

B. maculata, as described by (Standley, 1909, 1911) has large flowers for an annual *Boerhavia* (6-7mm) on long (up to 10mm) pedicels (Table 4), and has large fruit nearly 4mm in length with narrow wings. The collection of *B. maculata* in this study (NMSU23) conforms well to this description (Spellenberg, pers. comm.) and shares an ITS haplotype with the accession of *B. alata* (Figure 7). However, this plant has a divergent

NIA allele. With only a single accession, it is only possible to place it in the Umbellate II clade, without making inferences as to its distinctness.

Samples of *B. intermedia* and *B. triquetra* are found in Umbellate I and II (Figure 5). These two species are poorly distinguished (Spellenberg, submitted). In the eastern part of the range, fruits are exclusively five-angled. In coastal Sonora, southern California and Baja California, fruits range from five- to three-angled, with exclusively four- and three-angled plants being found primarily in parts of Baja California, where plants with more typical five-angled fruits are common as well (Spellenberg, 2003, submitted). This morphological intergradation is reason to be suspicious of the distinction between the species. In this study, the majority of samples of "*triquetra*" are found in the Umbellate II clade. Samples of the Umbellate I clade essentially overlap this range (except perhaps in the state of Baja California). All of the samples of *B. intermedia* from Arizona, New Mexico, Texas, Chihuahua and Coahuila fall within the Umbellate I clade, making it the more broadly distributed clade.

From a taxonomically agnostic perspective, the topology from the NIA analysis (Figure 6) reveals two phylogroups: the Umbellate II clade is completely restricted to coastal Baja California, Sonora, and Sinaloa, with the sole exception of *B. megaptera* in southern Arizona. The Umbellate I clade is much more broadly distributed across the entire distribution of umbellate annuals. As for the non-winged umbellate species, there

is a strong indication that the winged morphospecies are all derived from within the clade. Regardless of whether *intermedia*, *triquetra*, *maculata* and *lateriflora* are kept as separate taxa, or they are treated as a single species, it is unlikely that a taxonomic adjustment will be able to preserve a monophyletic non-winged umbellate species that is diagnosible, at the same time maintaining the status of the three highly distinct winged species.

2.4.2 Nonmonophyletic species

Confronted with the reality that phylogenetic studies at the species level often do not recover multiple accessions of morpological species as monophyletic groups, several factors have been offered as possible explainations (Funk and Omland, 2003; Syring et al., submitted). Insufficient phylogenetic resolution is perhaps the least interesting cause of this pattern as the solution, in principle, is simply to add data. It is also likely to be one of the more common causes of species-level nonmonophyly, since variable markers are harder to come by at the species level. Paralogous sequences resulting from gene duplication can cause the appearance of species nonmonophyly, but in species-level phylogenetic analyses, this is likely to be a relatively easy error to detect, since the orthologous sequences are likely to remain without being greatly altered, leading to multiple products in the PCR pool. Imperfect taxonomy can also result in discordance between molecular trees and morphospecies, in a variety of ways ranging to

oversplitting or overlumping on the basis of polymorphic traits, unperceived variation, cryptic species, or geographically structured variation in interbreeding populations (Funk and Omland, 2003). This may be part of the explanation for the rampant nonmonophyly found in the umbellate annual clade. Nonmonophyly issues that result from imperfect taxonomy will have causes ranging from the banal to the interesting, but these will be ultimately be idiosyncratic to the taxonomic group (and the group of taxonomists) involved.

Imperfect taxonomy means merely that one or more of the taxa included in the analysis are, for whatever reason, incorrectly labeled in a species tree. There is another class of causes of nonmonophyly that are not based on observational error or mistakes in interpretation, but on biological processes. These lead gene trees to be imperfect estimators of the real history of species (or lead them to reflect the variable genetic affinities of individuals or populations, depending on how one's species concept accomodates the temporal aspects of speciation). These biological phenomena are introgression (ongoing or old) and lineage sorting. Discriminating among these two hypotheses is not straightforward or even always possible. When hybridization is recent and restricted, the expectation is that the geographic distribution of introgressed alleles in the recipient species would be proximate to their source in the donor species. Two potential cases of this may be seen in this study: in the ITS dataset, where two

samples of *B. traubae* (and a nearby sample of *B. purpurascens*) appears in two areas of the spicate tree; and in the NIA topology, where a sample of *B. coulteri* var. *palmeri* from outside its natural range (*Spellenberg 13372*) has an allele otherwise found only in *B. coulteri* var. *coulteri*. Conflicting signal provided by other loci, especially organellar, may highlight introgressed alleles in special cases (Arnold, 1997).

Lineage sorting, the preservation of ancestral allelic diversity in modern populations, is a function of historical genealogical and demographic patterns. Qualitatively it is expected that subsequent to a speciation event, samples of alleles from the daughter species should show first polyphyly, then paraphyly, and finally reciprocal monophyly (Neigel and Avise, 1986; Rieseberg and Brouillet, 1994). The rate at which this occurs is directly related to, among other factors, the effective population size, N_e. In peripatric models of speciation, e.g. (Eldredge and Gould, 1972), the initial populations are not of equal size. Under neutral conditions, populations with smaller N_e will have all of their alleles descend from a common ancestor more recently (Rosenberg, 2003). Narrowly endemic species in the umbellate group would be, for instance, predicted to maintain less genetic diversity than their widespread sister taxa, however, opportunites to test this are limited by sampling in this study.

In the ITS topology (Figure 7), nonmonophyly is widespread within the annual clade. Due to overall low levels of variation in the ITS sequence, it is very difficult to say

whether the pattern of species nonmonophyly is simply an artifact. Essentially no nodes are strongly supported with ITS data alone. Within the annual *Boerhavia*s there are two groups that show supported nonmonophyly of species in the NIA topology (Figure 6). The first is the *B. coulteri* + *B. xanti* + *B. wrightii* clade of spicate annuals. *B. wrightii* occupies a derived position with respect to *B. xanti*, which in turn is derived with respect to *B. coulteri*. That the two varieties of *B. coulteri* are phylogenetically separate in the NIA analysis argues for some historical isolation, although it is not clear that there is reproductive isolation given the one sample of var. *palmeri* (*Spellenberg 13372*) that was placed with var. *coulteri*. *Boerhavia coulteri* var. *palmeri*, it should be noted, is widespread in Baja California, whereas var. *coulteri* is absent. One possible explanation is that the origin of the Gulf of California may predate the divergence of var. *coulteri* and have served as an allopatric isolating mechanism. In the context of an expanding Sonoran Desert, var. *palmeri* could have spread northward and eastward. Of all annuals, *coulteri* var. *palmeri* has the most viscid, finely branched infructesence, lending credence to any hypothesis of dispersal.

Boerhavia xanti is placed within a paraphyletic *B. coulteri* in the NIA topology, but without strong support. However, within *B. xanti* there are strongly-supported subclades that indicate that this species harbors substantial genetic diversity. *B. xanti* is restricted to the area surrounding the Gulf of California. The early-diverging *xanti*

alleles (*Douglas 2254* and *2239*) are from the Baja Peninsula, but the derived clade is comprised of samples from Baja, the Sonoran Desert, and *B. wrightii*. Again, this appears to be a situation in which high genetic diversity in Baja is carried eastward, as far as Sonora in the form of *B. xanti* but also much farther (to the Chihuahuan Desert) as the genetically monomorphic *B. wrightii*. This may have been quite recent, since *B. wrightii*, with as large a geographic range of as any annual, maintains a high level of genetic (and morphological) cohesiveness. It is also possible that there may be intrinsic barriers to genetic exchange, either a highly selfing mating system, or reproductive isolation. It is interesting to note that the distinctive 4-angled fruits of *B. wrightii* make it one of the only species of *Boerhavia* identified in packrat middens; it has been reported, along with other unidentified species, more or less consistently in the northern Sonoran Desert of Arizona since 10.4 ka (McAuliffe and Van Devender, 1998).

In the umbellate group, a geographic pattern also exists. The Umbellate I clade (Figure 5) contains all umbellate samples from east of Sierra Madre Occidental. However, it also contains a number of samples from Baja California and Sonora. The Umbellate II clade is restricted to sites around the Gulf in Sonora and Baja California, with the exception of the Arizona species *B. megaptera*. This represents yet another instance of larger amounts of phylogenetic diversity being maintained near the Gulf of California. In this instance, two of the lineages that do not occur in the coastal area, *B.*

pterocarpa and *B. megaptera*, have developed winged fruits, like the third winged lineage, *B. alata*, which is endemic to coastal Sonora. Each of these lineages is geographically restricted in comparison to the nonwinged species, thus it would be expected that due to a lower population size, samples would achieve monophyly much faster than the nonwinged widespread umbellate annuals.

2.4.3 Biogeographical patterns in *Boerhavia*

The genus *Boerhavia*, like the remainder of the Nyctaginaceae, is probably of American origin. While the exact order of divergence is not established in either (Douglas and Manos, 2007) or the present study, all early diverging lineages including *Okenia*, the outgroups *Anulocaulis* and *Nyctaginia*, and the red-flowered and Chihuahuan perennial clades are all found in the Americas. The finding that *B. verbenacea*, a Peruvian species, is part of the Indo-Pacific "*repens* complex" is interesting because it is the only member of this group native to the Americas, and furthermore, it is an annual (Killip, 1926). The NIA tree places it in a different clade (Figure 6), though this is based on a partial NIA sequence. If either result is validated, it indicates that there are potentially two origins of the annual habit in two separate continental arid zones.

Among the three lineages of *Boerhavia* which have "escaped" North America naturally, it is noteworthy that all bear viscid glands on their fruits, a condition not

found in the Chihuahuan perennial clade or either group of annuals. Similarly, other genera in the Nyctaginaceae, namely *Commicarpus* and *Pisonia*, have achieved a wide distribution including the paleotropics and subtropics, and South America most likely due to their viscid anthocarps (Burger, 2005). The annual species often present a similar viscid exudate along the internodes of the stem, but it seems that this has not resulted in continental-scale dispersal.

Finally, it is possible to interpret the phylogenetic results in light of paleoecological and paleoclimatic inferences about the origin of the Sonoran Desert. First, the earliest diverging lineages of the spicate annual clade are "good" species that are not restricted to the Sonoran: only the widespread *B. erecta* occurs in the desert proper (albeit in slightly mesic microsites), *B. purpurascens* is a higher elevation species, and *B. torreyana* is essentially a Chihuahuan species. Among the remaining spicate species, and the umbellate species, there is a common pattern in which 1) distinctive morphological species, namely *B. wrightii*, *B. pterocarpa*, *B. megaptera*, and *B. alata*, are highly restricted geographically, genetically, or both. 2) these taxa are derived from within poorly defined species complexes. 3) These progenitor species complexes have a geographical structure in the distribution of alleles that includes high diversity in Baja California and/or coastal Sonora, and reduced diversity away from this area, indicating that populations have spread eastward, most likely as the Sonoran Desert became

established as a regional desert, and as the Chihuahuan Desert developed from an arid savannah even more recently (Wells, 1974).

2.4.4 Conclusions

This study represents one of the most intensively sampled phylogenetic studies to date that includes both multiple accessions for many species *and* takes advantage of the high resolution of a single- or low-copy nuclear locus. While this work is descriptive in nature, it highlights the utility of such markers for studies at fine geographic and taxonomic scales. In particular, this study has attempted to take advantage of a poorly understood radiation of species to bridge the gap between traditional taxonomy, molecular phylogenetics, and phylogeography. Though only two loci contributed to the inferences reached in this study, it is evident that multiple processes are shaping the distribution of genealogical history among the taxa in this genus. Deep phylogenetic structure is evident separating the five major clades of *Boerhavia*. Examples are found of introgression and lineage sorting, particularly in the annual clade, in addition to species that are cohesive across a large geographic scale, for instance *B. coccinea*, *B. diffusa*, and annuals such as *B. torreyana* and *B. wrightii*. It has delivered insights into possible geographic patterns of diversification within the American deserts and serves as a source of hypotheses to be tested regarding dispersal, refugia, and the process of speciation in this floristically unique region.

Table 4: Morphological information and taxonomic conception of species of annual *Boerhavia*

Table 1

	Species						
	B. intermedia	***B. triquetra***	***B. maculata***	***B. lateriflora***	***B. alata***	***B. megaptera***	***B. pterocarpa***
Author	Jones 1902	Watson 1889	Standley 1909	Standley, 1911	Watson 1889	Standley 1909	Watson 1882
Range[1]	S	S, P	S	C,S,P	S	S	S
Inflorescence	Umbellate	Umbellate	Umbellate	Umbellate	Umbellate	Umbellate	Umbellate
L pedicel, mm	0.5-3.2	1-2	*2-10*	sessile	2-15	1-2	0.3-0.6
L flower, mm	0.7-1.2	1	*3-3.5 (width = 6-7mm)*	*2-3*	3	1-1.5	0.4-1.0
# stamens	2-3	2	5	3	5	3-4	1-3
L stamens, relative	included, equal or exserted	included	exserted	exserted	included	equal	included
L fruit, mm	2-2.8	2-2.5	*3.5-4,*	2.5	4	3-4	2.9-3.4
Fruit shape	narrowly obpyramidal	*obpyramidal, 3-4 angled*	obpyramidal	narrowly obpyramidal	*5-winged, round base*	*5 winged, truncate base*	*3-4 winged, apex truncate*
sulcus width	narrow	narrow	narrow	narrow	broad-very broad	broad-very broad	very broad
Rugosity							
angles	smooth	smooth	smooth	slight	smooth	smooth	smooth
sulcus	yes	strong	yes	strong	strong	smooth	strong

Table 1, cont.

	Species								
	B. erecta	***B. purpurascens***	***B. traubae***	***B. wrightii***	***B. spicata***	***B. xanti***	***B. torreyana***	***B. coulteri var. coulteri***	***B. coulteri var. palmeri***
Author	Linneaus 1753	Gray 1853	Spellenberg 1999	Gray 1853	Choisey 1849	Watson 1889	(S. Watson) Standley 1909	(Hooker) S. Watson	(S. Watson) Spellenberg 2002
Range[1]	S,C,P, tropics, subtropics worldwide	C,S	S	C,S,P	C,S,(P?)	S,P	C (and Colorado Plateau)	(C),S	(C),S,P
Inflorescence	*Subumbellate*	*Capitate*	*Soliatary-spicate*	Spicate-racemose	Spicate-racemose	Spicate-racemose	Spicate-racemose	Spicate-racemose	Spicate-racemose
L pedicel, mm	0.3-2.5	0.8-2.5	1	<1	0.4-2.3	1	0.1-1.7	0.2-1.6	0.1-1.1
L flower, mm	1-1.5	2.5-4	2	1.5	*1-1.3*	*3*	1-1.3	*1-2*	*0.7-1*
# stamens	2-4	3-4	3	2-4	*2-3*	*3-4*	2	2-3	2
L stamens, relative	exserted	equal to exserted	equal to exserted	included	included-exserted	exserted	exserted	*exserted*	*included to equal*
L fruit, mm	2-2.5	2.5-3	2.5-2.8	2.1-2.5	1.9-2.4	2.5	2.2-3	2.5-3.2	2-2.4
Fruit shape	narrowly obpyramidal	obovoid	obovoid	*ovoid, usually 4-angled*	*obovoid*	*obovoid*	*obovate-clavate*	*obpyramidal-clavate*	*obovoid-clavate*
sulcus width	narrow	*very broad*	very broad	very broad	*narrow*	*narrow*	narrow	*narrow*	*narrow*
Rugosity									
angles	slight	*smooth*	smooth	smooth	slight	slight	strong	slight	slight
sulcus	strong	slight	*smooth*	yes	slight	yes	*papillate*	slight	smooth

Data taken from Spellenberg (2003), Standley (1918) and Douglas, (pers. obs.) "Narrow" indicates sulcus width is less than width of rib base, "broad" is 1-2X the width of rib base, "very broad" is in excess of 2X width of rib base. Italicized characters, in combination with inflorescence type, are especially useful in determination.

[1] S: Sonoran desert (continental), C: Chihuahuan desert, P: Peninsular desert

Table 5: Sequence statistics for the ITS, NIA, and combined datasets and partitions

Partition	ITS1 (Bayesian)	5.8s (Bayesian)	ITS2 stem (Bayesian)	ITS2 loop (Bayesian)	ITS (MP)	NIA	Combined
#Taxa Included	154	154	154	154	154	123	145
Aligned Length	262	167	86	170	685	2436	3121
Analyzed Length	244	167	86	139	636	1546	2181
Constant	151	162	60	101	474	1126	1600
Uninformative	39	2	9	22	72	217	289
Parsimony-informative	54	3	17	16	90	202	292
ML Model	GTR+I	J-C	HKY	HKY	GTR+I+Γ	GTR+I+Γ	GTR+I+Γ

Table 6: Results of tests of constraint trees

Monophyly constrained	Templeton test [1]		Shimodaira-Hasegawa test [2]		
	L	P	-lnL	Diff -lnL	P
Boerhavia	793	0.75	8137.3713	9.9789	0.95
B. xantii	799	0.05*	8170.5956	43.2032	0.22
B. coulteri v. coulteri	797	0.18	8161.3922	33.9998	0.40
B. coulteri v. palmeri	795	0.37	8156.9912	29.5988	0.45
B. intermedia+lateriflora+maculata+triquetra	805	0.01*	8197.7889	70.3965	0.06
B. alata+pterocarpa+megaptera	808	<0.01*	8203.9828	76.5904	0.01*
shortest parsimony trees	791	1.00	8130.6797	3.2874	0.97

[1] L = length of shortest trees consistent with constraint, P = P-value associated with Templeton (Wilcoxon signed-ranks) test for significant

[2] -lnL = likelihood of best trees consistent with constraint, difference in likelihoods between best tree and best constraint tree, and P-value

Table 7: Summary of species-level monophyly found in phylogenetic analyses

	ITS		NIA		Combined	
Species Name	accessions	Monophyly?	accessions	Monophyly?	accessions	Monophyly?
alata	1		1		1	
anisophylla	3	n	2	y*	3	n*
ciliata	1				1	
coccinea	14	n	17	y	17	y*
cordobensis	1					
coulteri v. coulteri	11	n	7	n	9	n*
coulteri v. palmeri	11	n[1]	11	n	11	n*
crispifolia	1				1	
diffusa	4	n	4	y*	4	n[1]
dominii	1		1		1	
erecta	7	n	5	y*	6	y*
gardneri	1				1	
gracillima	6	n	5	n	6	n
heronensis	1				1	
intermedia	23	n*	23	n*	20	n*
lateriflora	2	n[1]	2	n*	2	n*
linearifolia	2	n			2	n*
maculata	1		1		1	
megaptera	2	n[1]	1		2	n[1]
paludosa	1				1	
pterocarpa	2	y	2	y*	2	y*
purpurascens	7	n	3	y*	3	y*
repens	3	n	1		3	n*
repleta	1					
schomburgkiana	1				1	
spicata	10	n			2	y*
torreyana	5	y	5	y*	5	y*
traubae	3	n				
triquetra	3	n	9	n*	9	n*
verbenacea	1		1		1	
wrightii	4	n[1]	3	y*	4	y
xantii	17	n	13	n*	17	n*
TOTALS						
# of species	32		22		29	
# of singletons	12		6		10	
# multiple accessions	20		16		19	
# (%) nonmonophyletic**	14	(70%)	7	(43.75%)	10	(52.63%)

n[1]: Accessions shared identical sequences, but other taxa were also found with these haplotypes.

* indicates that monophyly or nonmonophyly is supported by bootstrap values ≥ 70% and/or Bayesian posterior probabilities ≥ 95%

** percent nonmonophyletic species does not include species with identical but non-exclusive haplotypes

Figure 5: ML topology from analysis of combined NIA and ITS dataset

Clades referred to in text are highlighted.

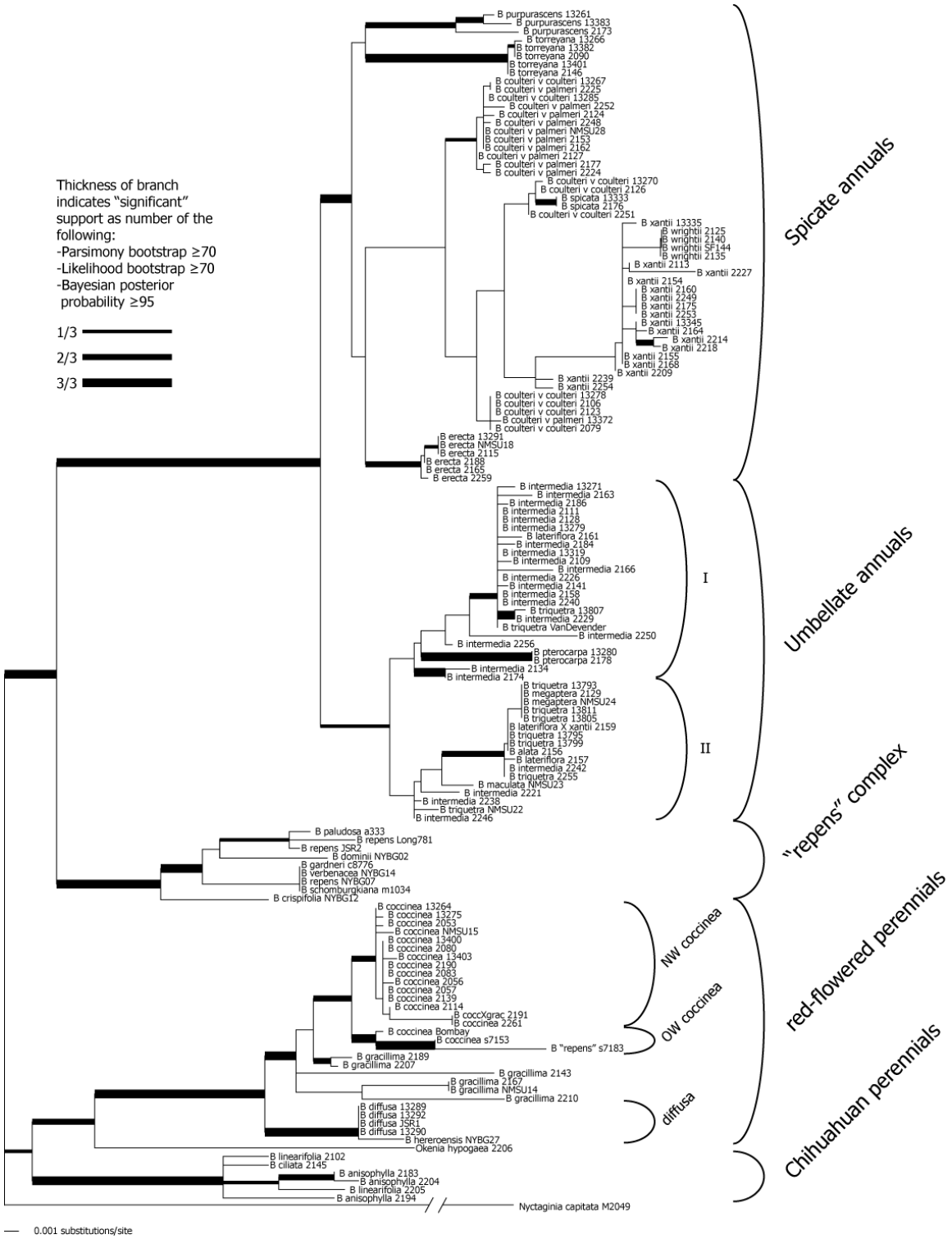

Thickness of branch indicates "significant" support as number of the following:
-Parsimony bootstrap ≥70
-Likelihood bootstrap ≥70
-Bayesian posterior probability ≥95
1/3
2/3
3/3
B purpurascens 13261
B purpurascens 13383
B purpurascens 2173
B torreyana 13266
B torreyana 13382
B torreyana 2090
B torreyana 13401
B torreyana 2146
B coulteri v coulteri 13267
B coulteri v palmeri 2225
B coulteri v coulteri 13285
B coulteri v palmeri 2252
B coulteri v palmeri 2124
B coulteri v palmeri 2248
B coulteri v palmeri NMSU28
B coulteri v palmeri 2153
B coulteri v palmeri 2162
B coulteri v palmeri 2127
B coulteri v palmeri 2177
B coulteri v palmeri 2224
B coulteri v coulteri 13270
B coulteri v coulteri 2126
B spicata 13333
B spicata 2176
B coulteri v coulteri 2251
B xantii 13335
B wrightii 2125
B wrightii 2140
B wrightii SF144
B wrightii 2135
B xantii 2113
B xantii 2227
B xantii 2154
B xantii 2160
B xantii 2249
B xantii 2175
B xantii 2253
B xantii 13345
B xantii 2164
B xantii 2214
B xantii 2218
B xantii 2155
B xantii 2168
B xantii 2209
B xantii 2239
B xantii 2254
B coulteri v coulteri 13278
B coulteri v coulteri 2106
B coulteri v coulteri 2123
B coulteri v palmeri 13372
B coulteri v coulteri 2079
B erecta 13291
B erecta NMSU18
B erecta 2115
B erecta 2188
B erecta 2165
B erecta 2259
B intermedia 13271
B intermedia 2163
B intermedia 2186
B intermedia 2111
B intermedia 2128
B intermedia 13279
B lateriflora 2161
B intermedia 2184
B intermedia 13319
B intermedia 2109
B intermedia 2166
B intermedia 2226
B intermedia 2141
B intermedia 2158
B intermedia 2240
B triquetra 13807
B intermedia 2229
B triquetra VanDevender
B intermedia 2250
B intermedia 2256
B pterocarpa 13280
B pterocarpa 2178
B intermedia 2134
B intermedia 2174
I
B triquetra 13793
B megaptera 2129
B megaptera NMSU24
B triquetra 13811
B triquetra 13805
B lateriflora X xantii 2159
B triquetra 13795
B triquetra 13799
B alata 2156
B lateriflora 2157
B intermedia 2242
B triquetra 2255
B maculata NMSU23
B intermedia 2221
B intermedia 2238
B triquetra NMSU22
B intermedia 2246
II
B paludosa a333
B repens Long781
B repens JSR2
B dominii NYBG02
B gardneri c8776
B verbenacea NYBG14
B repens NYBG07
B schomburgkiana m1034
B crispifolia NYBG12
B coccinea 13264
B coccinea 13275
B coccinea 2053
B coccinea NMSU15
B coccinea 13400
B coccinea 2080
B coccinea 13403
B coccinea 2190
B coccinea 2083
B coccinea 2056
B coccinea 2057
B coccinea 2139
B coccinea 2114
B coccXgrac 2191
B coccinea 2261
B coccinea Bombay
B coccinea s7153
B "repens" s7183
B gracillima 2189
B gracillima 2207
B gracillima 2143
B gracillima 2167
B gracillima NMSU14
B gracillima 2210
B diffusa 13289
B diffusa 13292
B diffusa JSR1
B diffusa 13290
B hereroensis NYBG27
Okenia hypogaea 2206
B linearifolia 2102
B ciliata 2145
B anisophylla 2183
B anisophylla 2204
B linearifolia 2205
B anisophylla 2194
Nyctaginia capitata M2049
0.001 substitutions/site
Spicate annuals
Umbellate annuals
"repens" complex
red-flowered perennials
Chihuahuan perennials
NW coccinea
OW coccinea
diffusa

Figure 6: ML topology from analysis of nitrate reductase (NIA) intron dataset

Clades referred to in text are highlighted. Branches in bold supported ≥ 70% in parsimony bootstap.

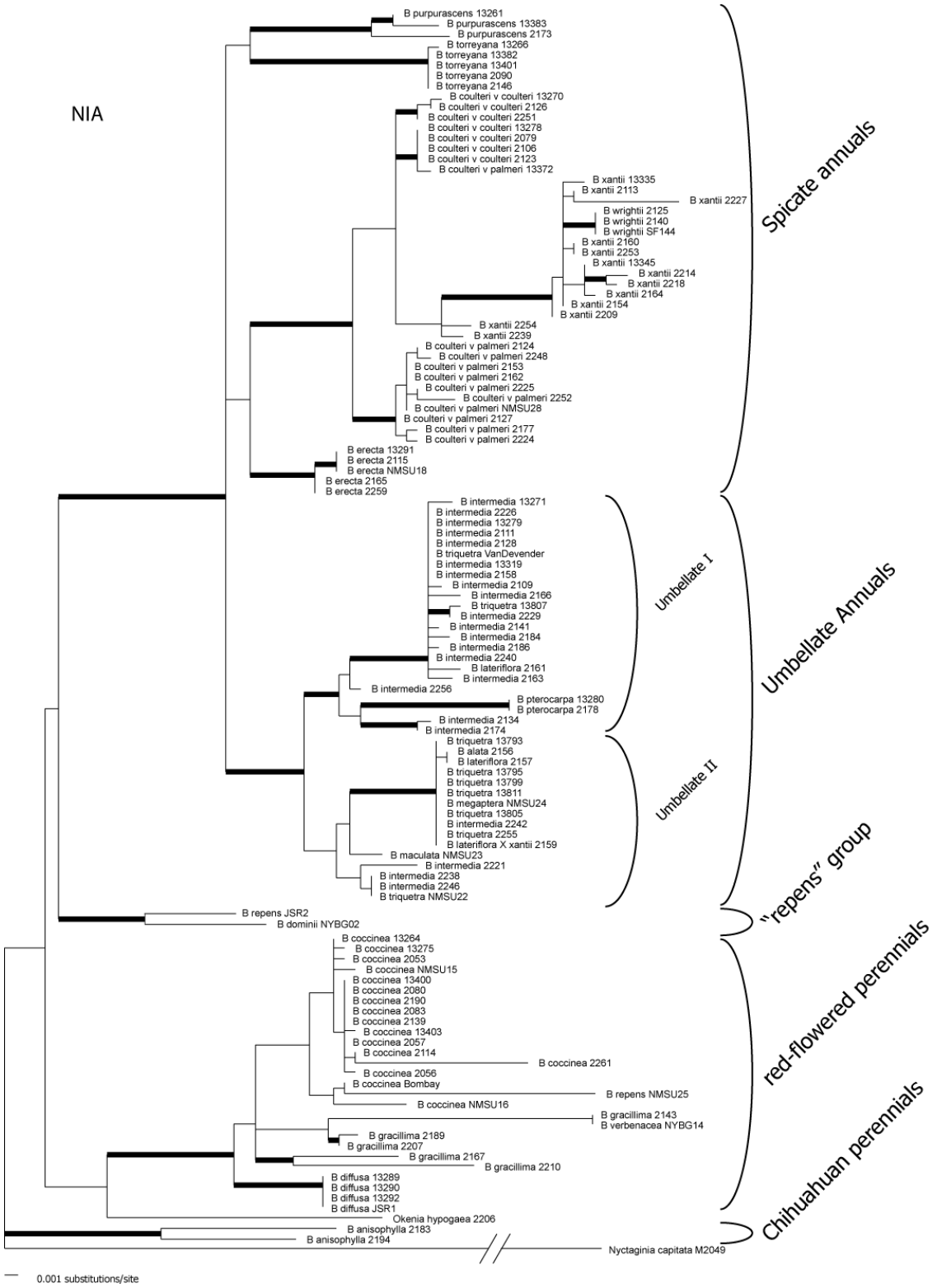
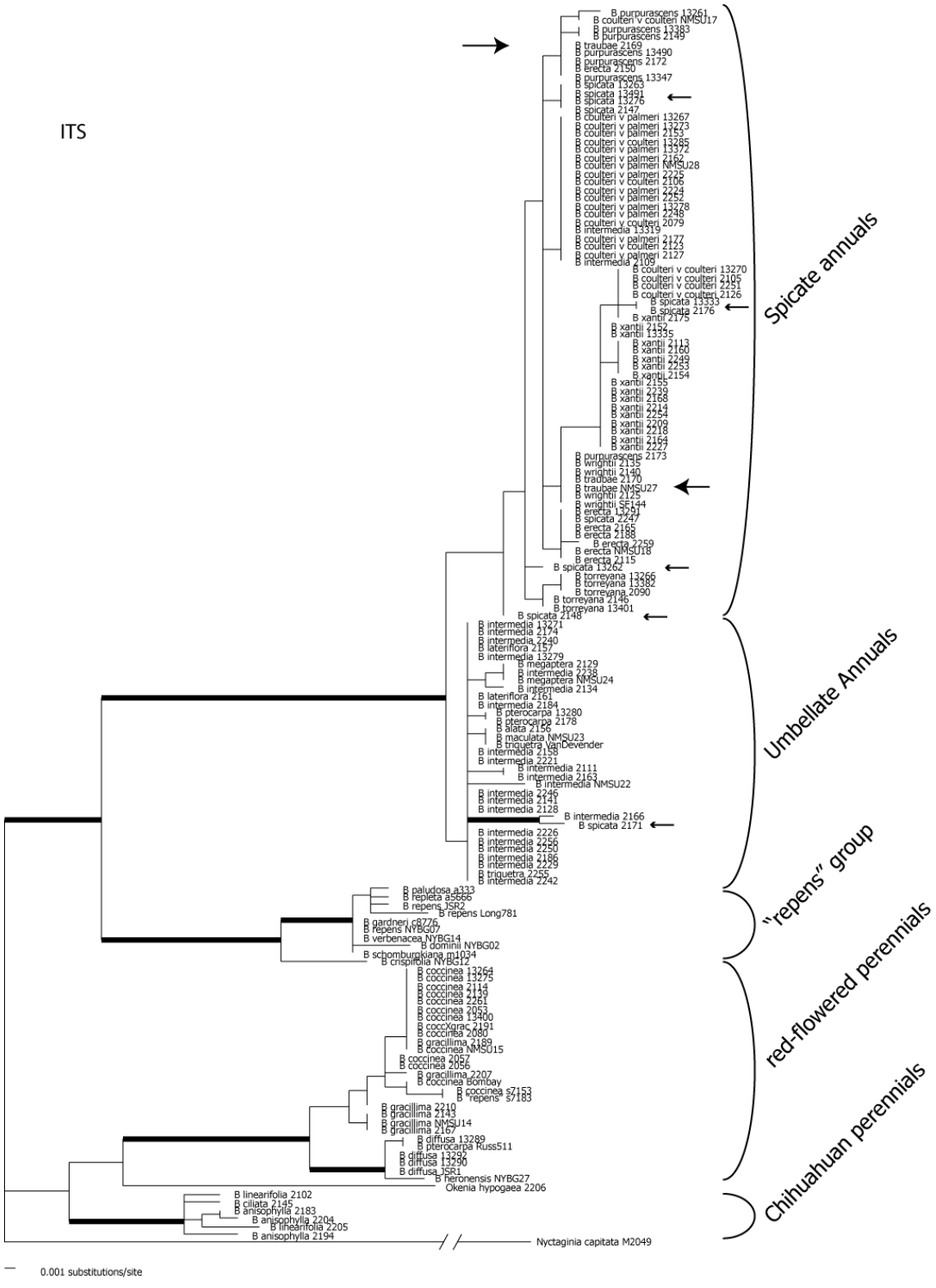

NIA
B purpurascens 13261
B purpurascens 13383
B purpurascens 2173
B torreyana 13266
B torreyana 13382
B torreyana 13401
B torreyana 2090
B torreyana 2146
B coulteri v coulteri 13270
B coulteri v coulteri 2126
B coulteri v coulteri 2251
B coulteri v coulteri 13278
B coulteri v coulteri 2079
B coulteri v coulteri 2106
B coulteri v coulteri 2123
B coulteri v palmeri 13372
B xantii 13335
B xantii 2113
B xantii 2227
B wrightii 2125
B wrightii 2140
B wrightii SF144
B xantii 2160
B xantii 2253
B xantii 13345
B xantii 2214
B xantii 2218
B xantii 2164
B xantii 2154
B xantii 2209
B xantii 2254
B xantii 2239
B coulteri v palmeri 2124
B coulteri v palmeri 2248
B coulteri v palmeri 2153
B coulteri v palmeri 2162
B coulteri v palmeri 2225
B coulteri v palmeri 2252
B coulteri v palmeri NMSU28
B coulteri v palmeri 2127
B coulteri v palmeri 2177
B coulteri v palmeri 2224
B erecta 13291
B erecta 2115
B erecta NMSU18
B erecta 2165
B erecta 2259
B intermedia 13271
B intermedia 2226
B intermedia 13279
B intermedia 2111
B intermedia 2128
B triquetra VanDevender
B intermedia 13319
B intermedia 2158
B intermedia 2109
B intermedia 2166
B triquetra 13807
B intermedia 2229
B intermedia 2141
B intermedia 2184
B intermedia 2186
B intermedia 2240
B lateriflora 2161
B intermedia 2163
B intermedia 2256
B pterocarpa 13280
B pterocarpa 2178
B intermedia 2134
B intermedia 2174
B triquetra 13793
B alata 2156
B lateriflora 2157
B triquetra 13795
B triquetra 13799
B triquetra 13811
B megaptera NMSU24
B triquetra 13805
B intermedia 2242
B triquetra 2255
B lateriflora X xantii 2159
B maculata NMSU23
B intermedia 2221
B intermedia 2238
B intermedia 2246
B triquetra NMSU22
B repens JSR2
B dominii NYBG02
B coccinea 13264
B coccinea 13275
B coccinea 2053
B coccinea NMSU15
B coccinea 13400
B coccinea 2080
B coccinea 2190
B coccinea 2083
B coccinea 2139
B coccinea 13403
B coccinea 2057
B coccinea 2114
B coccinea 2261
B coccinea 2056
B coccinea Bombay
B repens NMSU25
B coccinea NMSU16
B gracillima 2143
B verbenacea NYBG14
B gracillima 2189
B gracillima 2207
B gracillima 2167
B gracillima 2210
B diffusa 13289
B diffusa 13290
B diffusa 13292
B diffusa JSR1
Okenia hypogaea 2206
B anisophylla 2183
B anisophylla 2194
Nyctaginia capitata M2049
0.001 substitutions/site
Spicate annuals
Umbellate I
Umbellate II
Umbellate Annuals
"repens" group
red-flowered perennials
Chihuahuan perennials

Figure 7: ML topology from analysis of internal transcribed spacer (ITS) dataset

Branches in bold supported ≥ 70% in parsimony bootstap. In general this topology is in agreement with that of nitrate reductase (NIA), although there is no support for relationships among taxa in the annual clade. Large arrows indicate the position of samples of *B. traubae*, small arrows signify samples of *B. spicata*. NIA was not amplified from these taxa.


ITS
B purpurascens 13261
B coulteri v coulteri NMSU17
B purpurascens 13383
B purpurascens 2149
B traubae 2169
B purpurascens 13490
B purpurascens 2172
B erecta 2150
B purpurascens 13347
B spicata 13263
B spicata 13491
B spicata 13276
B spicata 2147
B coulteri v palmeri 13267
B coulteri v palmeri 13273
B coulteri v palmeri 2153
B coulteri v coulteri 13285
B coulteri v palmeri 13372
B coulteri v palmeri 2162
B coulteri v palmeri NMSU28
B coulteri v palmeri 2225
B coulteri v coulteri 2106
B coulteri v palmeri 2224
B coulteri v palmeri 2252
B coulteri v palmeri 13278
B coulteri v palmeri 2248
B coulteri v coulteri 2079
B intermedia 13319
B coulteri v palmeri 2177
B coulteri v coulteri 2123
B coulteri v palmeri 2127
B intermedia 2109
B coulteri v coulteri 13270
B coulteri v coulteri 2105
B coulteri v coulteri 2251
B coulteri v coulteri 2126
B spicata 13333
B spicata 2176
B xantii 2175
B xantii 2152
B xantii 13335
B xantii 2113
B xantii 2160
B xantii 2249
B xantii 2253
B xantii 2154
B xantii 2155
B xantii 2239
B xantii 2168
B xantii 2214
B xantii 2254
B xantii 2209
B xantii 2218
B xantii 2164
B xantii 2227
B purpurascens 2173
B wrightii 2135
B wrightii 2140
B traubae 2170
B traubae NMSU27
B wrightii 2125
B wrightii SE144
B erecta 13291
B spicata 2247
B erecta 2165
B erecta 2188
B erecta 2259
B erecta NMSU18
B erecta 2115
B spicata 13262
B torreyana 13266
B torreyana 13382
B torreyana 2090
B torreyana 2146
B torreyana 13401
B spicata 2148
B intermedia 13271
B intermedia 2174
B intermedia 2240
B lateriflora 2157
B intermedia 13279
B megaptera 2129
B intermedia 2238
B megaptera NMSU24
B intermedia 2134
B lateriflora 2161
B intermedia 2184
B pterocarpa 13280
B pterocarpa 2178
B alata 2156
B maculata NMSU23
B triquetra VanDevender
B intermedia 2158
B intermedia 2221
B intermedia 2111
B intermedia 2163
B intermedia NMSU22
B intermedia 2246
B intermedia 2141
B intermedia 2128
B intermedia 2166
B spicata 2171
B intermedia 2226
B intermedia 2256
B intermedia 2250
B intermedia 2186
B intermedia 2229
B triquetra 2255
B intermedia 2242
B paludosa a333
B repleta as666
B repens JSR2
B repens Long781
B gardneri c8776
B repens NYBG07
B verbenacea NYBG14
B dominii NYBG02
B schomburgkiana m1034
B crispifolia NYBG12
B coccinea 13264
B coccinea 13275
B coccinea 2114
B coccinea 2139
B coccinea 2261
B coccinea 2053
B coccinea 13400
B coccXgrac 2191
B coccinea 2080
B gracillima 2189
B coccinea NMSU15
B coccinea 2057
B coccinea 2056
B gracillima 2207
B coccinea Bombay
B coccinea s7153
B "repens" s7183
B gracillima 2210
B gracillima 2143
B gracillima NMSU14
B gracillima 2167
B diffusa 13289
B pterocarpa Russ511
B diffusa 13292
B diffusa 13290
B diffusa JSR1
B heronensis NYBG27
Okenia hypogaea 2206
B linearifolia 2102
B ciliata 2145
B anisophylla 2183
B anisophylla 2204
B linearifolia 2205
B anisophylla 2194
Nyctaginia capitata M2049
0.001 substitutions/site
Spicate annuals
Umbellate Annuals
"repens" group
red-flowered perennials
Chihuahuan perennials

3. An investigation into pattern and process of diversification in two species complexes in the annual *Boerhavia* clade.

3.1 INTRODUCTION

Powerful insights into the process of diversification can be gained from detailed examinations of species complexes. However, at the species or population level, individual gene trees provide an incomplete representation of organismal history, for a number of reasons discussed in Chapter 2. One potentially powerful approach is to sample markers from across the genome. While not immune to systemic processes which can effect topological incongruence ranging from introgression, hybridization, and lineage sorting, it would be predicted that trees generated from a large number of markers would be less susceptible to the stochastic effects that may be observed when sampling only a single locus (Despres et al., 2003). A large number of studies of genetic diversity in plants have used arbitrarily amplified PCR fragment markers such as randomly amplified polymorphic DNAs (RAPDs) (Welsh and McClelland, 1990), inter simple sequence repeats (ISSR) (Zietkiewicz et al., 1994) and amplified fragment length polymorphisms (AFLP) (Vos et al., 1995). Researchers interested in systematic questions at a taxonomic scale below that for which informative DNA sequences are available

have used fragment based marker systems with varying levels of success (e.g. Despres et al., 2003; Koontz et al., 2004; Spooner et al., 2005; Albach et al., 2006; Tremetsberger et al., 2006); in general, fragment based topologies have been found to be largely congruent with sequence-based trees (Koopman, 2005) provided that the individuals are not too divergent (Bussell et al., 2005). Alternatively, within-species questions are frequently addressed with fragment markers (Despres et al., 2003; Stuessy et al., 2003; Brouat et al., 2004; Whittall et al., 2004; Castillo-Cardenas et al., 2005; Guo et al., 2005; Albach et al., 2006). The ease with which large numbers of AFLP markers can be generated makes this technique a leading choice for population genetic studies despite clear disadvantages in terms of homology assessment and marker dominance. In microsatellite and isozyme studies relatively few co-dominant alleles are assumed to be homologous; these marker systems are used in situations where relationships are assumed to be tokogenetic and identical alleles are assumed to be identical by descent, i.e. within biological species. In contrast, the evolution of sets of fragment markers is modeled (imperfectly) by assuming that restriction sites are easier to lose than to gain (e.g. Nei and Li, 1979). Therefore, when supplied with a large number of markers, it is possible to estimate genetic distance across species boundaries. Especially in recently derived lineages of plants, an approach that considers species limits to be hypothetical

provides an appropriate framework in which to ask certain questions regarding divergence.

These marker systems are also generally variable enough to be useful for genotyping individuals. The dominant nature of AFLP markers necessitates a different approach to calculating many population genetic estimators developed for codominant data, but progress on this front has been rapid (Lynch and Milligan, 1994; Hardy and Vekemans, 2002; Holsinger et al., 2002; Vekemans, 2002; Hardy, 2003).

Difficulty in the taxonomic delimitation of *Boerhavia* species, combined with a center of diversity in a geologically recent environment, prompted an investigation of the genus with DNA sequences in Chapter 2. The high level of resolution provided especially by the nuclear gene, nitrate reductase, enabled, for the first time, a well-resolved phylogenetic hypothesis to be advanced. With these data, it was possible to infer species relationships. Furthermore, intraspecific sampling made it possible to establish the cohesiveness or distinctness of certain taxonomic species, some of which had endured decades of taxonomic controversy.

However, poor resolution was obtained among accessions of two groups: the spicate annuals *Boerhavia spicata* and *B. xanti*, and the umbellate annual species. In the former case, NIA was unamplifiable for *B. spicata* and ITS sequences resolve samples of *B. spicata* in several groups. In the case of the umbellate annuals, several

morphologically divergent species have been recognized from coastal Sonora and southern Arizona, but NIA and ITS sequence data indicate that these segregates are derived from within a larger, widespread complex comprised mostly of *B. intermedia* (and *B. triquetra*). In both of these groups, there is significant (though not complete) geographic overlap among the taxa. This sympatry leads to questions of whether the taxa are reproductively isolated, and whether there are signals of historical vicariance or range expansions that may reflect Pleistocene contractions and expansions of the Sonoran Desert.

In this chapter, I employ AFLP data to examine the distribution of genetic diversity within the genus, and especially within these two complexes of annual *Boerhavia* identified in Chapter 2. With a combination of distance-tree methods and ordination techniques I examine the relationships of individuals and populations in each group. Isolation-by-distance analyses provide a complementary perspective to assess the importance of geography in controlling the distribution of variation across the range of the taxa. The fine-scale resolution provided by AFLP data allows the use of analyses of molecular variation (AMOVA) (Excoffier et al., 1992) to quantify the amount of genetic variation explained by taxonomic categories. Also, with AFLP data, it is possible to estimate expected levels of genetic variation present within populations. Since this is expected to be partly affected by processes which operate at the level of individuals such

as mating patterns, it allows me to evaluate evidence for the hypothesis that differences in floral morphology among populations and species have impacts on genetic diversity within populations, a factor which could potentially relate to speciation.

3.2 MATERIALS AND METHODS

3.2.1 Sampling

Populations included in this study represent a subset of those samples included in Chapter 2. In general, populations of *Boerhavia* were sampled from the United States, and mainland Mexico, not including the Baja California peninsula. The widespread taxa *B. diffusa* and *B. erecta* are represented by some additional samples from Central America and Hawaii. Localities and voucher information for the accessions included in this study are included in Appendix 3. Populations are represented by 1-10 individuals.

3.2.2 Molecular data

To obtain high-quality DNA for AFLP, embryos were dissected from individual seeds. Following disruption in a shaker, DNA was extracted following Kang et al. (1998) with slightly reduced volumes to facilitate the use of a 96-well extraction plate. The resulting extractions were quantified by eye on an ethidium-bromide stained gel and DNA concentrations were adjusted to be approximately equal. The AFLP fingerprints

(Vos et al., 1995) were generated as follows: simultaneous restriction-ligation reactions were performed in which approximately 100ng of genomic DNA was digested with two restriction enzymes, EcoRI and MseI, at 37°C for 12 h. Double-stranded adapters, with sticky ends complementary to those produced on the genomic fragments cleaved by the restriction enzymes, are ligated with T4 ligase. Subsequently, products are diluted 1:2.5, and the dilution is used for two PCR reactions. The first, the preselective amplification, uses primers complementary to each of the adapters, plus a single selective base each. After a second dilution, the second selective amplification is run using primers with two additional selective bases each. The selective primer complementary to the EcoRI adapter sequence is labeled with a fluorophore which enables the fragments to be visualized and sized via capillary separation on an ABI 3730 DNA Sequencer. Varying the two selective bases and their associated fluorophores allows many markers to be produced from the same preamplification product. In this study, three selective primer combinations were used: Eco-GTT X Mse-TGC, Eco-GAG X Mse-TGG, and Eco-GTC X Mse-TCC

Electropherograms were interpreted using GeneMarker 1.41 (SoftGenetics, 2005). After standardization of signal strength, peaks <10% of the maximum intensity at that allele size were considered to be absent. In the case of a reaction failure for one selective primer pair, missing data were coded as "?"; if two or three primer pairs were not

readable, the individual was excluded from further consideration. Fingerprints were assembled into a final matrix of 282 individuals, for 485 loci.

3.2.3 Data Analysis

Phenetic analysis of fingerprint data was conducted in PAUP* 4.10b (Swofford, 2002) under the Nei-Li distance criterion (Nei and Li, 1979) which was developed for restriction site data. Heuristic searching entailed 100 random-addition-sequence replicates with nearest-neighbor branch swapping and steepest-descent. Missing data for some individuals produced anomalous results. These fingerprints were excluded and the analysis was repeated with the final matrix including 222 individuals. Within the *B. spicata* + *B. xanti* group, and the umbellate group, similar analyses were conducted except that composite fingerprints (with each allele coded as present, absent, or polymorphic) were composed for those populations with multiple individuals.

To visualize the distribution of variation in *B. spicata* + *B. xanti*, and the umbellate species, non-metric multidimensional scaling (NMS) of individual fingerprints of both groups, was performed in PC-ORD (McCune et al., 2002). The "autopilot" mode was employed to select the most appropriate dimensionality for each ordination.

I tested for the presence of isolation-by-distance (IBD) using the software program SPAGeDi (Hardy and Vekemans, 2002). This program estimates a kinship parameter between individuals from dominant data (Hardy, 2003). This estimator

requires a user-supplied inbreeding coefficient. In this dataset, qualitative differences were not noted between runs in which the coefficient was given as 0.1 and 0.9; all reported values were generated with a coefficient of 0.5. The kinship matrix is compared to a geographic distance matrix. Significance is assessed by permutation of localities, equivalent to a Mantel test (Hardy and Vekemans, 2002).

Independently, for each population with > 4 individuals, expected heterozygosity or Nei's gene diversity (Hj) was estimated using AFLP-SURV (Vekemans, 2002), implementing the unbiased statistic of Lynch and Milligan (1994) which accounts for the presence of unscored homozygous recessives and the indistinguishability of heterozygous and homozygous dominants in AFLP data. Finally Arlequin 3.1 was used to conduct an AMOVA analysis (Excoffier et al., 1992).

Floral morphology was quantified for the majority of the samples in this study. From either field-fixed flowers or rehydrated herbarium specimens, the following measurements were made: length of perianth limb, diameter of perianth, number of stamens, length of longest stamen, length of shortest stamen, and length of stigma. From these data, additional variables were computed, including the relative length of stamens and stigma to the perianth (exsertion) and the relative position of the stigma to the stamens. The basal angle of the perianth was computed treating the diameter of the flower as the base of a cone and the length of the perianth as the length of a side:

Basal angle = ($\tan^{-1}$((diameter of perianth/2)/length of perianth) *(180/2π)))

Correlations of floral morphological variables with population genetic parameters were examined in JMP (SAS Institute, 2005)

3.3 RESULTS

Phenetic analysis of the entire AFLP dataset produced a dendrogram (Figure 8, L=6.336) in which fingerprints from individual populations formed clades, or occasionally were intermixed with other populations of the same taxon. Exceptions were found in *Boerhavia coulteri*, which sometimes exhibited terminal clades containing both the species and the variety *palmeri*. Like previous analyses of sequence data in Chapter 2, the tree showed monophyly of *B. purpurascens*, *B. wrightii*, *B. torreyana*. Notable differences include the monophyly of *B. coulteri* and the nonmonophyly of *B. erecta*, in which a population from Puerto Rico is resolved sister to *B. torreyana*. As was found in Chapter two, AFLP data resolved *B. xanti* as a non-monophyletic species, although in this study, samples of *B. spicata* were available and thus this species is implicated as another member of the *B. xanti* + *B. wrightii* group. In the umbellate group, AFLP data were unavailable for the populations of *B. triquetra*, or *B. intermedia* from Baja California. Nevertheless, *B. intermedia* is not recovered as a monophyletic group, rather

samples of this species are intermixed with those of *B. pterocarpa*, *B. lateriflora*, and *B. megaptera*, similar overall to results of sequence data.

Ordination of individuals in these two complexes (Figure 9a, 9c) showed that, in general, species were not separated in distinct clusters but rather represented trends in mostly continuous distributions of variation. Overlay of geographic region on these ordinations (Figure 9b, 9d) showed that, in the *B. spicata* + *B. xanti* group, relatively low amounts of variation are found in the United States, compared to Mexican *B. spicata* and *B. xanti*.

In the spicate group, phenetic analysis at the population level (Figure 10) showed evident trends in which northern populations of *B. spicata* were most similar to each other. Likewise, southern populations represented a group, this one comprised of *B. xanti* and most southern populations of *B. spicata*.

Similarly, NMS ordination of the umbellate group shows that *B. intermedia* from the Chihuahuan Desert of Texas and Coahuila represents a small subset of the total variation found among umbellate species from the Sonoran Desert. However, unlike the spicate group, no geographic pattern is evident in the population-level phenogram (Figure 11). Even the three eastern populations are revealed to belong to two distinct groups. A strong pattern of isolation-by-distance (IBD) was found in the spicate group (Figure 12, $R^2 = 0.121$, $P < 0.0001$). Conversely, no evidence of IBD was seen in the

umbellate group (R^2 = 0.0001, P = 0.2537). AMOVA analysis (Table 8) showed significant differences in the partitioning of genetic variation in the two groups, with most variation being found within populations in the spicate group, and among populations of *B. intermedia*.

Estimates of expected heterozygosity (H_j) for 34 populations ranged from 0.08 – 0.16; across the genus, the value for H_j was not correlated with any of the primary measurements of floral dimensions. Two computed floral variables, the basal angle and the length of the stigma relative to the length of the shortest stamen, displayed non-significant trends (Figure 13). Between the spicate and umbellate groups, there was a significantly different relative stigma length (spicate relative length = 1.35, umbellate = 1.09, P=0.044, two-tailed value of t-test assuming unequal variances). However, this was not associated with a difference in mean values of H_j.

3.4 DISCUSSION

3.4.1 AFLP Phylogeny of Boerhavia

Though the sampling in the present study is not totally comparable to that in the sequence-based study detailed in Chapter 2 (Figure 5), there are similar conclusions to be made at the population level: those species that have monophyletic sets of NIA and ITS alleles tend to be found as monophyletic groups in the AFLP analysis, and vice

versa. The AFLP topology (Figure 8) did not show strong bootstrap support values (not shown; highest support values tend to be found for groups of populations within species). Remarkably, the analysis did yield sister clades of spicate and umbellate annuals, red-flowered perennials, and Chihuahuan perennials. Within the spicate annuals there were differences between the AFLP topology and the sequence topology. Most notably, in the spicate group where *B. erecta* is found to be polyphyletic, and *B. purpurascens* and *B. torreyana* are not found to be sister taxa. *Boerhavia coulteri* and *B. coulteri* var. *palmeri* are together found to be monophyletic but neither forms a clade, in contrast to the results from NIA (Figure 6). In a recently diversified group, the whole-genome sampling of AFLPs should present a more thorough picture of overall similarity than a single-locus topology, which may or may not track species relationships with fidelity for reasons discussed previously. However, AFLPs have their own shortcomings ranging from non-homology of scored fragments, to incompletely understood evolutionary dynamics (though in this study, I used genetic distance measures that take into account some of the biological processes believed to affect restriction site-based dominant data, making losses of fragments more probable that gains). These problems are believed to be more pronounced at higher taxonomic levels (Koopman, 2005). This, combined with generally low support above the species level in

this tree lead me to avoid making overall conclusions about the phylogeny of the genus based solely on these data.

In some cases, however, sister-taxa relationships that would be predicted based on morphology are in fact recovered in this analysis. *Boerhavia purpurascens* is placed next to *B. traubae*, which was not well-resolved in the ITS topology (Figure 7) and for which NIA was not amplifiable. These two are so similar in flower, fruit, and vegetative morphology such that *B. traubae* was initially identified as a diminutive *B. purpurascens* (Spellenberg, 1999). More interestingly, the AFLP topology recovers a group containing *B. coulteri* and *B. coulteri* var. *palmeri*. These are reported to intergrade (Spellenberg, 2002) and they are interspersed with each other in this analysis. In Baja California, *B. coulteri* var. *coulteri* is absent and var. *palmeri* is abundant. Unfortunately, it was not possible to include in this analysis samples of *B. coulteri* var. *palmeri* from Baja California. The NIA topology, in contrast, recovered the two varieties as mostly separate clades paraphyletic with respect to *B. xanti* and *B. wrightii* (Figure 6).

3.4.2 Contrasting patterns of diversity in the Boerhavia spicata + B. xanti complex and umbellate annual complex

This study has revealed that in both the *Boerhavia spicata* + *B. xanti* complex and the umbellate annual complex, taxonomic names do not fully correspond with the phenetic groups revealed by AFLP data, and that the two complexes each possess strong

evidence of nonmonophyletic taxonomic species. This conclusion is in line with those reached in the study of sequence data presented in chapter two. In that case, I found that *B. xanti* alleles were ancestral to those found in *B. wrightii*. The inclusion of *B. spicata*, for which NIA was unamplifiable, in this study, has confirmed the close relationship between this species and *B. xanti*, which was suspected based on fruit morphology and the well-developed glandular pubescence usually found on the lower stem (also found in *B. wrightii*). Interestingly, neither *B. spicata* nor *B. xanti* form monophyletic groups (Figure 8).

The umbellate annual group was found, with NIA, to comprise two clades, neither of which contained a monophyletic set of morphospecies except for trivial cases. With AFLP data, the situation is the same (considering sampling differences). This topology also does not include monophyletic species, except when only a single population of a restricted endemic (*B. pterocarpa*, *B. megaptera*) was included. The seemingly deep split in dividing Umbellate I and Umbellate II (Figure 5) was not seen in this dataset. This could be due simply to the absence of Baja California samples in the AFLP dataset, or any number of possible factors which preserve older diversity in NIA.

The high levels of variation provided by AFLP fingerprints allow deeper insights to be gained into the pattern of diversification in these two groups. With respect to the *Boerhavia spicata* + *B. xanti* complex, it is evident from the analysis of AFLPs across the

genus that these two taxa are indeed closely related, and that one or the other is ancestral to the widespread but seemingly cohesive *B. wrightii*. The distinctness of the latter based on morphology as well as genetic data indicates that there is no need to consider it further. *Boerhavia spicata* and *B. xanti*, which morphologically are distinguished based on flower size, are poorly differentiated with genetic data, whether with ordination (Figure 9a) or in distance-based trees. However, the pattern is not simple. In fact, there is a strong geographic pattern: *Boerhavia xanti*, which is restricted to Mexico (including Baja California, though only Sonoran samples are included in this study) is found exclusively in one clade in the phenogram (Figure 10). Conversely, most samples of *B. spicata* are found in the adjacent clade. The isolation-by-distance analysis (Figure 12), which was highly significant despite explaining a relatively low amount of variation, is consistent with two interpretations. One is that gene flow is ongoing among populations, albeit at relatively short distances, which would be a satisfactory explanation for the pattern seen in Figure 9*B*. Alternately, strong IBD is also consistent with the existence of a northern and southern group, such that northern populations are each others' closest relatives, as are the southern populations. This is the situation suggested by the phenogram (Fig 10). If it were the case that there were two biological species, wholly or partly sympatric, no geographic pattern would be predicted in an analysis of IBD, as co-occurring individuals would be likely to be unrelated genetically.

This is decidedly not the case, and in fact two of three populations of *B. spicata* from Sonora are placed within the *B. xanti* clade. Further evidence of this is seen in the results of the AMOVA analysis (Table 8), in which only 2.6% of the variance was seen between taxonomic species. Finally, an astounding 80.1% of the genetic variation was found to occur *within* populations, indicating that genetic structuring is not high in this group. In Sonora, both species frequently co-occur in mixed populations. In one instance, from a parental plant identified as *B. spicata*, multiple seeds were genotyped, and fingerprints from the individual seeds were all found to contain a mixture of *B. spicata* and *B. xanti* alleles (N. Douglas, data not shown). Thus, it seems that there is little evidence for reproductive isolation between these two species. *Boerhavia xanti* is found adjacent to the Gulf of California in Baja California, Sonora, and Sinaloa. *Boerhavia spicata*, occurs in Arizona, New Mexico, and Texas, but is apparently absent from Baja California. Considering the morphological divergence between the two, it is possible that *B. xanti* represents a distinct group in Baja California, and that the area of sympatry in Sonora may be a zone of contact between two incipient species with some history of allopatry related to the origin of the Gulf of California or more recent climate shifts which affected the composition of the Sonoran Desert. Recent genetic studies of Sonoran Desert columnar cacti have shown patterns reflecting dispersal as well as relatively ancient vicariance (Nason et al., 2002; Clark-Tapia and Molina-Freaner, 2003). To resolve the

question of allopatry and dispersal in *Boerhavia*, future work should focus on including samples of *B. xanti* from Baja California, and developing sequence markers which can be amplified in both *B. xanti* and *B. spicata*.

The umbellate clade of annual *Boerhavia*, which represents up to seven species, is another poorly-understood complex. Three species, *B. triquetra*, *B. maculata*, and *B. alata*, are not included in this study, though they are unambiguously part of the clade according to sequence data. In fact, *B. triquetra* and *intermedia* may not be distinct according to either sequence data or morphological data (see Chapter 2, and Spellenberg, submitted). The three species that have broadly winged fruits are rare. Only *B. megaptera* and *B. pterocarpa*, from Arizona, are included in this study, represented by single populations. The remaining species, *B. lateriflora* and *B. intermedia*, exhibit similarities to *B. xanti* and *B. spicata*: *B. lateriflora* represents a large-flowered Sonoran form otherwise very similar to the more widely distributed, small-flowered, *B. intermedia*. The genus-wide distance analysis (Figure 8) recovers the umbellate species as a clade. Within this group, the populations of *B. intermedia* are found to be polyphyletic. Individuals of the winged species are separated in the NMS ordination (Figure 9c), but this does not appear to be the case with the similar *B. intermedia* and *B. lateriflora*. When geographic region is overlaid (Figure 9d), eastern populations of *B. intermedia* occupy a restricted central position in the graph, whereas samples of the four

species from Arizona and Sonora exhibit a much greater range of variability. The population-level phenogram (Figure 11) gives no obvious pattern according to either taxonomic group or geographic range. Samples of *B. intermedia* from two distinct groups are found in Texas and Coahuila. A similar instance of distantly related populations in close geographic proximity is seen in Arizona, and a third is seen in Sonora. Not surprisingly, there is no evidence whatsoever of isolation-by-distance (Figure 12). A very high percentage of the total genetic variation is to be found between populations of *B. intermedia* (the segregate species were not included in this AMOVA). This indicates that, in stark contrast to the *B. xanti* + *B. spicata* group, very little gene flow is occurring in this species.

3.4.3 Trends in flower morphology and genetic diversity in populations

One possible explanation for the strong difference in F_{st} values may be that outcrossing is rarer in the umbellate group. *Boerhavia* flowers open in the morning and are visited by many species of potentially pollinating insects (Spellenberg, 2000). As the day warms, the flowers wilt and are generally closed by early- to mid-afternoon. However, there is a delayed self-pollination mechanism in which the stamens and style curl and are brought into contact as the perianth wilts (Chaturvedi, 1989; Spellenberg, 2000). Spellenberg (2000) noted that larger flowered species receive more attention from

pollinating insects. This raises the possibility that the size of the flower in a given species would be associated with the levels of outcross pollination and thus with the levels of inbreeding in populations. However, across the genus, no indication was found of an association between floral dimensions related to size and levels of expected heterozygosity in populations. On the other hand, there was a non-significant trend for higher heterozygosity to be associated with 1) a greater basal angle, and 2) a shorter stigma length with respect to that of the shortest stamen (relative herkogamy). The first factor makes intuitive sense, in that more open flowers offer a greater display to insects and less restricted access to nectar and included stamens and stigmas. Indeed, flower size has been demonstrated to be correllated with outcrossing rates in several species (Feliner, 1991; Herrera, 1992; Armbruster et al., 2002; Elle and Hare, 2002). The association with herkogamy is in accord with many studies which have shown an association with this trait and higher outcrossing rates (Karron et al., 1997; Luijten et al., 1999; Armbruster et al., 2002; Elle and Hare, 2002; Takebayashi et al., 2006). In *Boerhavia* there is no significant difference between H_j and the simple presence of herkogamy; there is only a trend if the stigma length is less than the smallest stamen- "reverse herkogamy" (Webb and Lloyd, 1986). If, in *Boerhavia*, this association reflects a real phenomenon, it might be understood in light of the self-pollination mechanism: as the flower wilts, if the stigma is already shorter than the shortest stamen, it has a "head

start" and may be less likely to actually contact a stamen. If this conjecture is true, it would make the self-pollination mechanism is less effective and relatively more pollinations would thus be outcrossed. Unfortunately for this hypothesis, the umbellate clade actually has shorter stigmas than the *B. spicata* + *B. xanti* group (stigma L : shortest stamen L ratios are 1.09 and 1.35, respectively), and comparisons of H_j between the spicate and umbellate groups were not significantly different (P = 0.37, t-test, assuming unequal variance). Therefore, while there is a slight indication that these factors may be operating among species across the genus, it is not an adequate explanation for the disparity in the partitioning of genetic diversity between the two groups. Another hypothesis may relate geitonogamy to inflorescence architecture itself: it is possible that the crowded flowers in umbellate species are subject to greater levels of self-pollination than are the spicate species. However, there is little reason to expect differences in selfing rates for different architectures, unless there is also some form of separation of male and female function (Jordan and Harder, 2006).

Regardless of the mechanism responsible for the low levels of gene flow, the umbellate group presents a stark contrast to the *B. spicata* + *B. xanti* group. It is interesting that this clade has produced a large number of isolated, morphologically distinct, and/or endemic species or varieties, considering the apparently very high genetic structure evident in the most widespread species. If high F_{st} value is typical of

umbellate species generally, this may represent adequate isolation to allow local, microallopatric, speciation to have occurred, giving rise to the large amount of morphological diversity present in the clade.

Table 8: Summary of genetic analyses in two complexes of annual *Boerhavia*

"Clean" signifies that clouds of points in NMS are non-overlapping; * indicates that result is significant either at a bootstrap level ≥ 70% or P ≤ 0.05.

Taxon	Monophyletic in AFLP?	Distinguished in NMS?	Geographic pattern			AMOVA		
			NMS	Phenogram	IBD	within pop.	between pop.	between spp.
spicata	no	mostly clean	Northern pops. have low variation	Samples nested in *xanti* clade are in Sonora	yes*	80.1%	17.3% Fst=0.20	2.6%
xanti	no		no	no latitudinal pattern in Sonora				
intermedia	no	mostly clean	Chihuahuan pops. have low variation	Chihuahuan pops. sister, no pattern in Sonora	no	4.9%	95.1% Fst=0.95	*n.a.*
lateriflora	no	no						
megaptera	yes	clean						
pterocarpa	yes*	clean						

* significant result

Figure 8: Phylogeny of *Boerhavia* based on AFLP data

Nei-Li phenogram based on 222 individual samples for 485 polymorphic AFLP bands. Triangles indicate monophyletic clusters of the same taxonomic species. Number in parenthesis is the number of populations represented in that cluster. Absence of triangle simply indicates that the terminal was represented by a single accession.

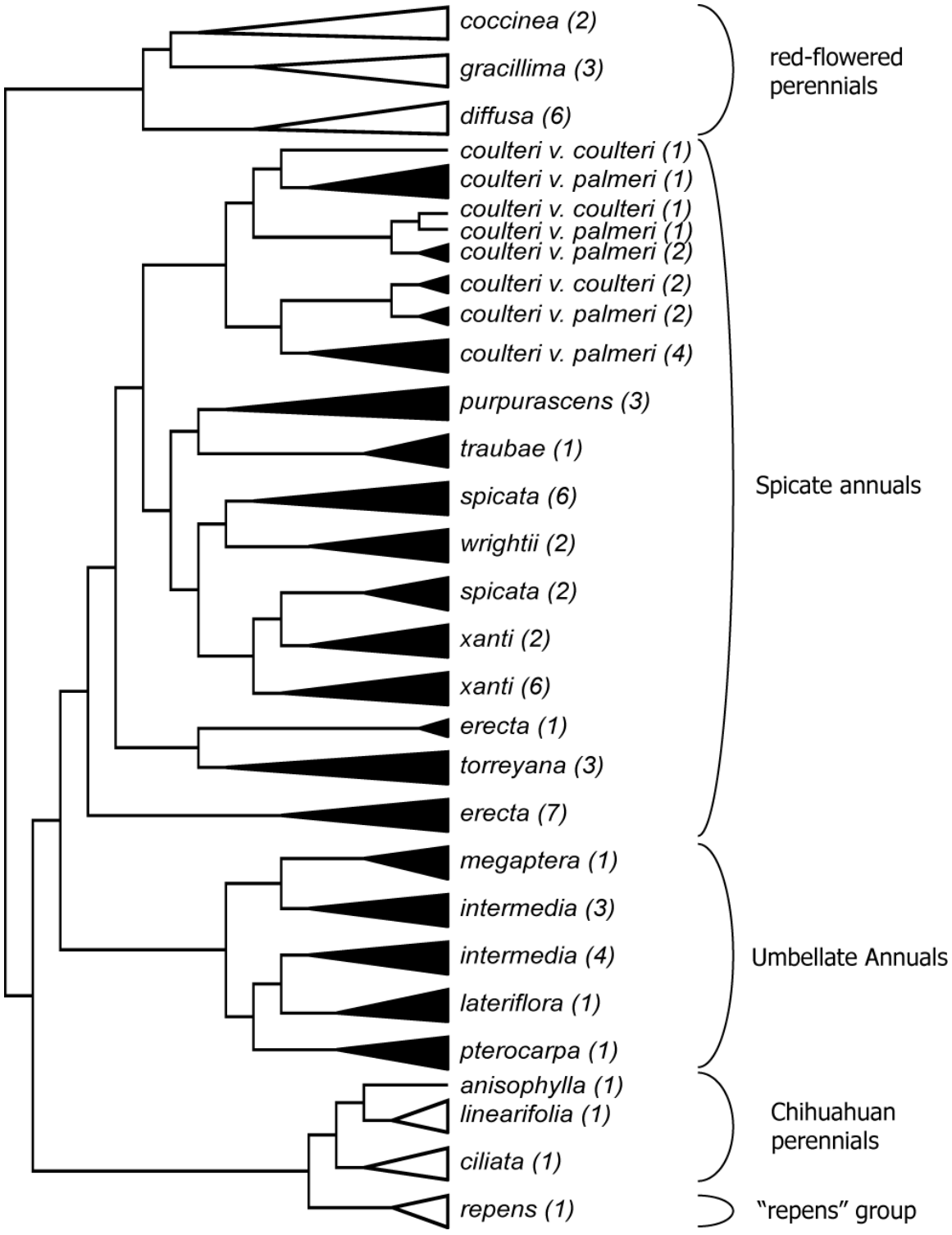

coccinea (2)
gracillima (3)
diffusa (6)
red-flowered perennials
coulteri v. coulteri (1)
coulteri v. palmeri (1)
coulteri v. coulteri (1)
coulteri v. palmeri (1)
coulteri v. palmeri (2)
coulteri v. coulteri (2)
coulteri v. palmeri (2)
coulteri v. palmeri (4)
purpurascens (3)
traubae (1)
spicata (6)
wrightii (2)
spicata (2)
xanti (2)
xanti (6)
erecta (1)
torreyana (3)
erecta (7)
Spicate annuals
megaptera (1)
intermedia (3)
intermedia (4)
lateriflora (1)
pterocarpa (1)
Umbellate Annuals
anisophylla (1)
linearifolia (1)
ciliata (1)
Chihuahuan perennials
repens (1)
"repens" group

Figure 9: Non-metric multidimensional scaling (NMS) ordination of individuals in the spicate and umbellate complexes.

A: Taxonomic identity of samples in the spicate group; B: identical graph as A with points labelled according to geographic origin. C: Taxonomic identity of samples in the umbellate group; D: identical graph as C with points labelled according to geographic origin.

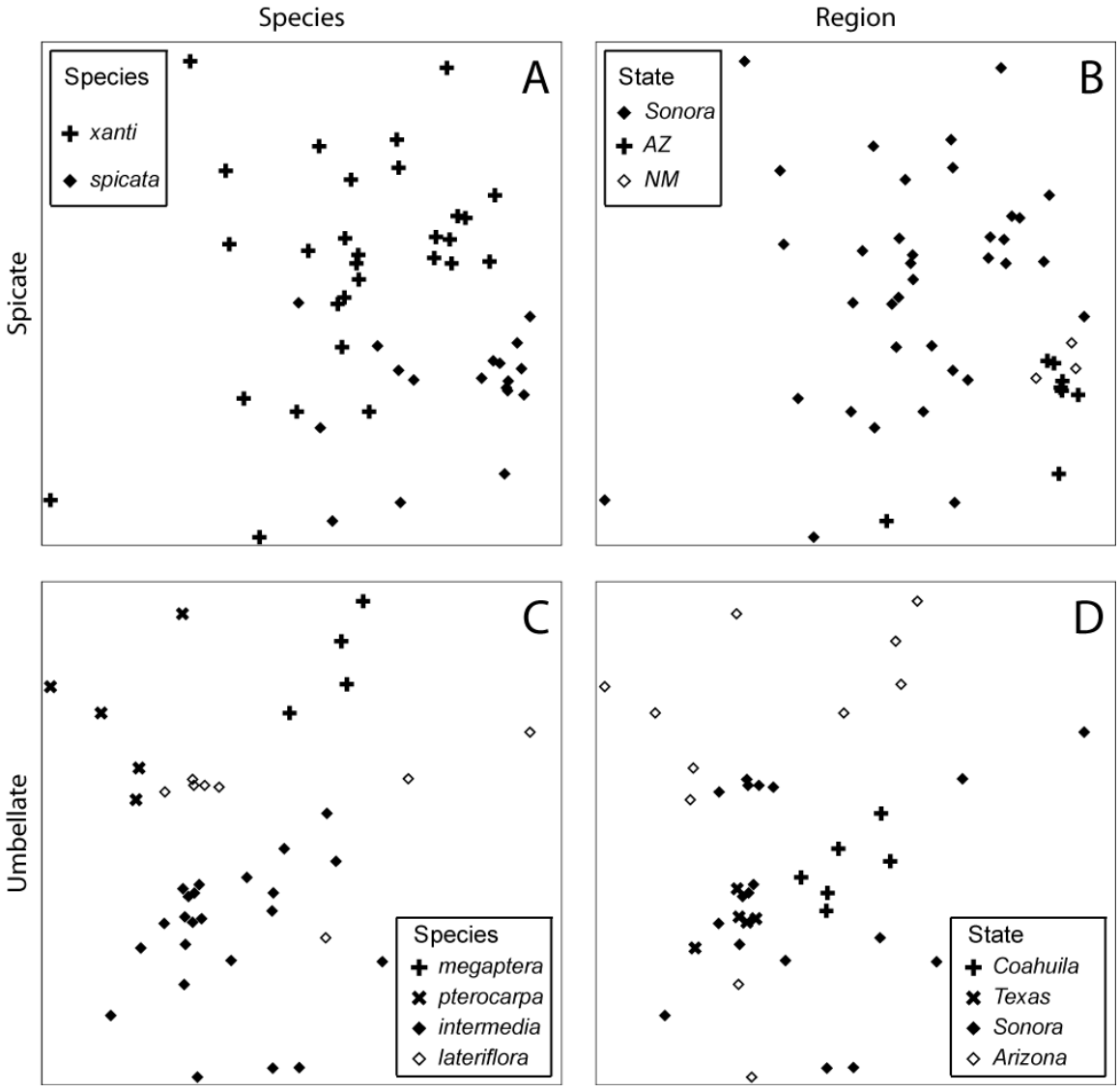

Species
Region
Spicate
Umbellate
Species
xanti
spicata
A
State
Sonora
AZ
NM
B
C
Species
megaptera
pterocarpa
intermediata
lateriflora
D
State
Coahuila
Texas
Sonora
Arizona

Figure 10: Phenogram of populations of *Boerhavia spicata* and *B. xanti* with location of populations

Most samples of *B. spicata* are found in Arizona and New Mexico. Samples from Sonora, Mexico, are mostly *B. xanti*. 2 of 3 samples of *B. spicata* from Sonora cluster with *B. xanti.*

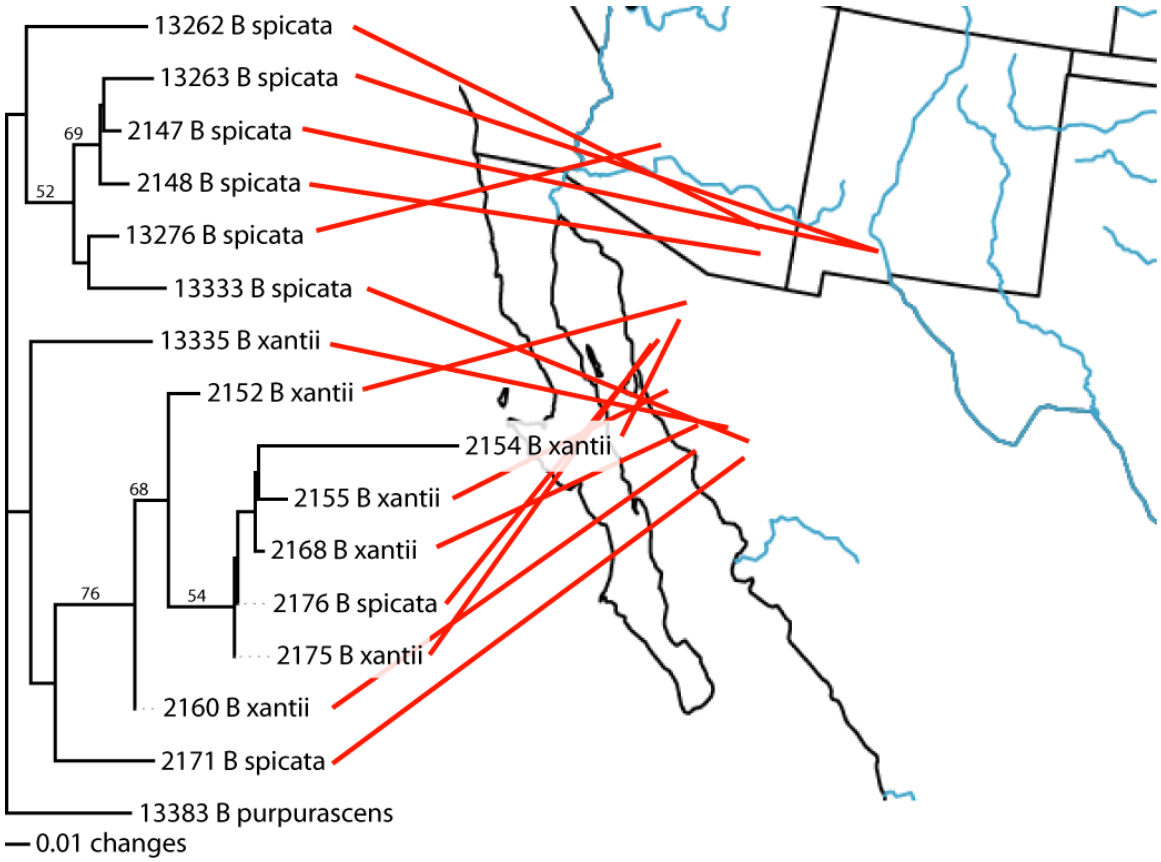


Figure 11: Phenogram of populations of umbellate species of *Boerhavia* with location of populations

No geographic structure is apparent with populations of *B. intermedia*.

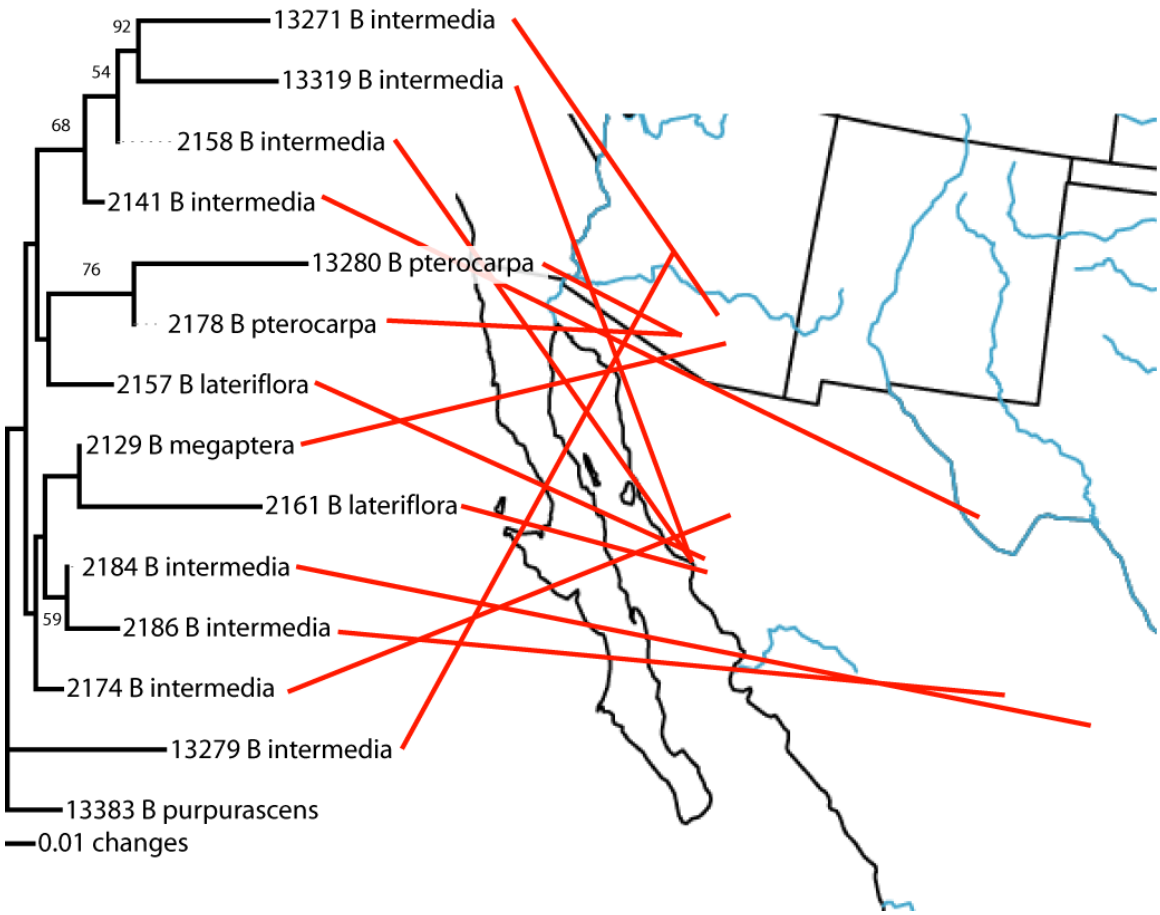
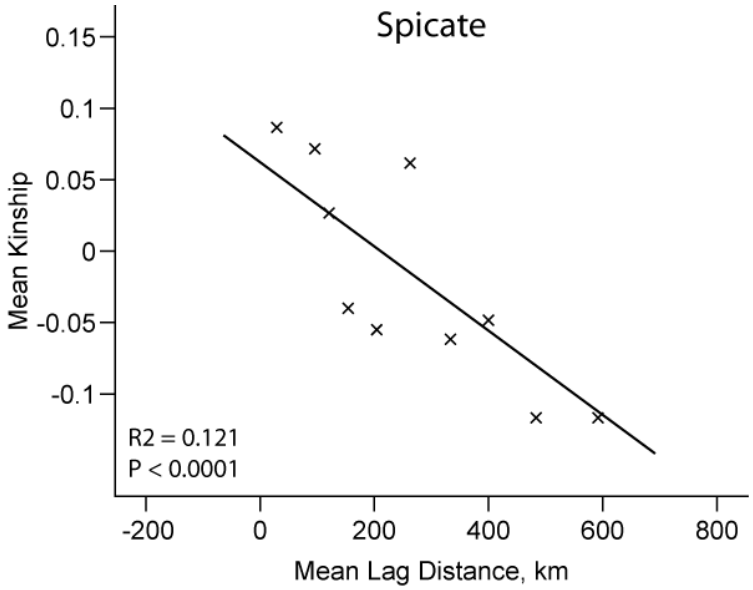


Figure 12: Plots of isolation-by-distance (IBD) for spicate and umbellate complexes

Strong evidence for IBD is seen only in the spicate annuals.


Spicate
0.15
0.1
0.05
0
-0.05
-0.1
Mean Kinship
R2 = 0.121
P < 0.0001
-200
0
200
400
600
800
Mean Lag Distance, km

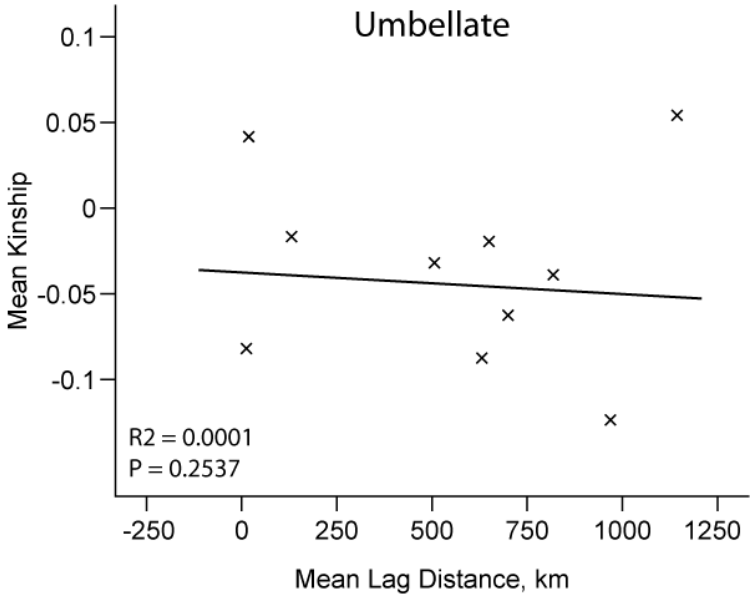
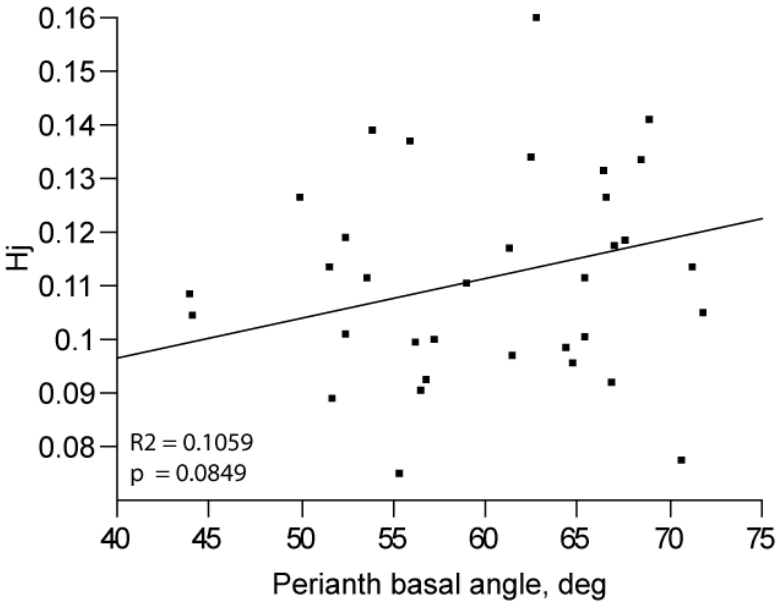
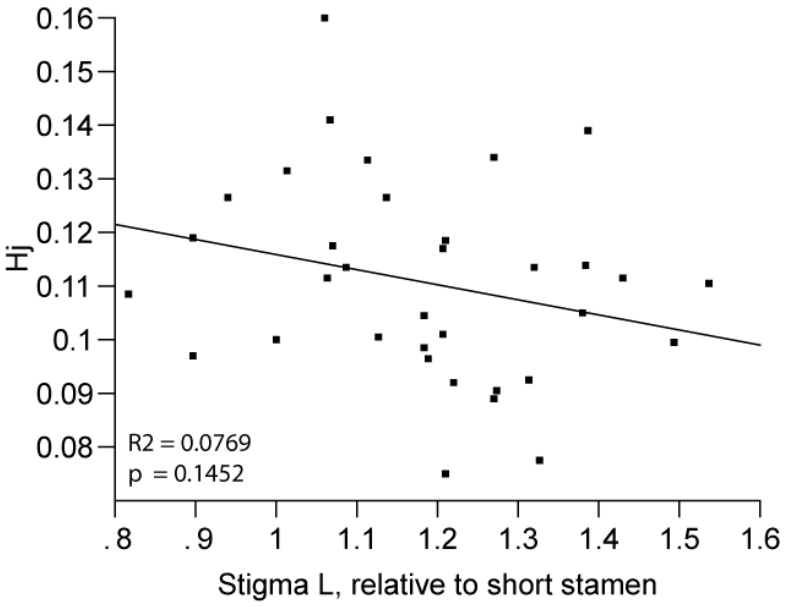

Umbellate
0.1
0.05
0
-0.05
-0.1
Mean Kinship
R2 = 0.0001
P = 0.2537
-250
0
250
500
750
1000
1250
Mean Lag Distance, km

Figure 13: Linear regression of expected heterozygosity (H_j) against floral traits

Weak trends are seen for increased H_j with flowers that are more open (top) and with greater negative herkogamy (bottom), however neither factor is significant.


0.16
0.15
0.14
0.13
0.12
0.11
0.1
0.09
0.08
Hj
R2 = 0.1059
p = 0.0849
40
45
50
55
60
65
70
75
Perianth basal angle, deg


0.16
0.15
0.14
0.13
0.12
0.11
0.1
0.09
0.08
Hj
R2 = 0.0769
p = 0.1452
.8
.9
1
1.1
1.2
1.3
1.4
1.5
1.6
Stigma L, relative to short stamen

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Appendix 1: Samples used in Chapter 1, and GenBank accession numbers

Species Name	Author	ITS	*ndhF*	*rpl16*	*rps16*	Collector	Locality, Year, Voucher location
Abronia bigelovii	Heimerl	EF079455	EF079510	EF079564	EF079606	*Douglas 2088*	New Mexico, USA, 2001, DUKE
Abronia carletonii	J.M. Coult. & Fisher	EF079456	EF079511	EF079565	EF079607	*Douglas 2091*	New Mexico, USA, 2001, DUKE
Acleisanthes lanceolatus	(Wooton) R.A. Levin	EF079454	EF079509	EF079563	EF079605	*Douglas 2072*	New Mexico, USA, 2001, DUKE
Acleisanthes longiflora	A. Gray	EF079457	EF079512	-	EF079608	*Douglas 2098*	New Mexico, USA, 2001, DUKE
Allionia choisyi	Standl.	EF079467	EF079519	EF079574	EF079618	*Douglas 2187*	Coahuila, Mexico, 2002, DUKE
Andradea floribunda	Allemão	EF079491	EF079545	-	EF079639	*Amorim 2294*	Brazil, 1998, NY
Anulocaulis annulatus	(Coville) Standl.	EF079503	EF079557	EF079599	EF079650	*Spellenberg 3162*	California, USA, 1993, NMC
Anulocaulis leiosolenus v. leiosolenus	(Torr.) Standley	EF079464	EF079517	-	EF079615	*Douglas 2122*	Arizona, USA, 2002, DUKE
Anulocaulis reflexus	I.M. Johnst.	EF079468	EF079520	-	-	*Douglas 2192*	Chihuahua, Mexico, 2002, DUKE
		-	-	EF079586	EF079629	*Spellenberg 10739*	Chihuahua, Mexico, 1990, NMC
Belemia fucsioides	Pires	EF079488	EF079542	-	-	*Belem 3796*	Brazil, 1968, NY
Boerhavia anisophylla	Torr.	EF079469	EF079521	EF079575	EF079619	*Douglas 2194*	Durango, Mexico, 2002, DUKE
Boerhavia ciliata	Brandegee	EF079465	-	EF079572	EF079616	*Douglas 2145*	Texas, USA, 2002, DUKE
Boerhavia coccinea	Mill.	EF079472	EF079525	EF079579	EF079622	*Spellenberg 13275*	Arizona, USA, 2001, DUKE
Boerhavia coulteri	Spellenb.	EF079471	EF079524	EF079578	EF079621	*Spellenberg 13273*	Arizona, USA, 2001, DUKE
Boerhavia dominii	Meikle & Hewson	EF079487	EF079540	EF079594	EF079638	*Smyth 42*	Australia, 1997, MO
Boerhavia gracillima	Heimerl	EF079479	EF079533	EF079587	EF079630	*Spellenberg 12447*	Texas, USA, 1997, NMC
Boerhavia intermedia	M.E. Jones	EF079474	EF079527	EF079581	EF079624	*Spellenberg 13279*	Arizona, USA, 2001, DUKE
Boerhavia lateriflora	Standl.	EF079466	EF079518	EF079573	EF079617	*Douglas 2161*	Sonora, Mexico, 2002, DUKE
Boerhavia linearifolia	A. Gray	EF079459	EF079514	EF079567	EF079610	*Douglas 2102*	New Mexico, USA, 2001, DUKE
Boerhavia purpurascens	A. Gray	EF079470	EF079523	EF079577	EF079620	*Spellenberg 13261*	Arizona, USA, 2001, DUKE
Boerhavia repens	L.	EF079480	EF079534	EF079588	EF079631	*Spellenberg 7183*	Sana, Yemen , 1983, NMC
		EF079477	EF079531	EF079584	EF079627	*Rose 2*	Oahu, Hawaii, USA, 2001, DUKE
Boerhavia spicata	Choisy	EF079473	EF079526	EF079580	EF079623	*Spellenberg 13276*	Arizona, USA, 2001, DUKE
Bougainvillea glabra	Choisy	EF079463	-	EF079571	EF079614	*Douglas 2121*	North Carolina, USA*, 2002, DUKE
Bougainvillea infesta	Griseb.	EF079498	EF079551	-	EF079644	*Nee 51442*	Bolivia, 2000, NY
Caribea litoralis	Alain	-	EF079530	-	-	*A. H. Liogier 7013*	Cuba, 1959, NY
Colignonia glomerata	Griseb.	EF079495	EF079549	-	EF079642	*Nee 52523*	Bolivia, 2003, NY
Colignonia scandens	Benth.	EF079502	EF079556	EF079598	EF079648	*Grantham 63*	Lojas, Ecuador**, 2003, DUKE
Commicarpus coctoris	N.A. Harriman	EF079481	EF079535	EF079589	EF079632	*Spellenberg 12883*	Oaxaca, Mexico, 1998, NMC
Commicarpus plumbagineus	(Cav.) Standl.	EF079504	EF079558	EF079600	EF079651	*Spellenberg 7374*	Ta'izz, Yemen, 1983, NMC
Commicarpus scandens	(L.) Standl.	EF079482	EF079536	EF079590	EF079633	*Spellenberg 12887*	Puebla, Mexico, 1998, NMC
Cyphomeris gypsophiloides	Standl.	EF079458	EF079513	EF079566	EF079609	*Douglas 2100*	New Mexico, USA, 2001, DUKE
Guapira discolor	(Spreng.) Little	EF079476	EF079529	EF079583	EF079626	*Spellenberg 13294*	Florida, USA, 2001, DUKE
Guapira eggersiana	(Heimerl) Lundell	EF079496	EF079550	-	EF079643	*Mori 25542/40*	French Guiana, 2003, NY
Leucaster caniflorus	(Mart.) Choisy	-	EF079541	-	-	*Pirani 3602*	Brazil, 1995, NY
		EF079497	-	-	-	*Hatschbach 50421*	Brazil, 1993, NY
Mirabilis albida	(Walter) Heimerl	EF079451	EF079506	EF079560	EF079602	*Douglas 2035*	Arizona, USA, 2001, DUKE
Mirabilis jalapa	L.	EF079461	EF079515	EF079569	EF079612	*Douglas 2119*	North Carolina, USA*, 2002, DUKE

Continued

Species Name	Author	ITS	*ndhF*	*rpl16*	*rps16*	Collector	Locality, Year, Voucher location
Mirabilis multiflora	(Torr.) A. Gray	EF079452	EF079507	EF079561	EF079603	*Douglas 2037*	Arizona, USA, 2001, DUKE
Neea cauliflora	Heimerl	EF079493	EF079547	-	-	*Schanke S15106*	Peru, 2002, NY
Neea hermaphrodita	S. Moore	EF079489	EF079543	-	-	*Nee 51426*	Bolivia, 2000, NY
Neea psychotrioides	Donn. Sm.	EF079505	EF079559	EF079601	EF079652	*Wilbur 63654*	Heredia, Costa Rica, 1995, DUKE
Nyctaginia capitata	Choisy	EF079478	EF079532	EF079585	EF079628	*McIntosh 2049*	New Mexico, USA, 1992, NMC
Okenia hypogaea	Schltdl. & Cham.	EF079483	-	-	EF079634	*VanDevender 92-*	Sonora, Mexico, 1992, NMC
		-	EF079522	EF079576	-	*Douglas 2206*	Veracruz, Mexico, 2002, DUKE
Phaeoptilum spinosum	Radlk.	EF079490	EF079544	-	-	*Seydel 4077*	Namibia, 1964, NY
Pisonia capitata	(S. Watson) Standl.	EF079484	EF079537	EF079591	EF079635	*193)*	Sonora, Mexico, 2000, NMC
Pisonia rotundata	Griseb.	EF079475	EF079528	EF079582	EF079625	*Spellenberg 13293*	Florida, USA, 2001, DUKE
Pisoniella arborescens	(Lag. & Rodr.) Standl.	EF079485	-	EF079592	EF079636	*LeDuc 231*	Oaxaca, Mexico, 1992, NMC
		-	EF079539	-	-	*Anderson 13522*	Oaxaca, Mexico, 1988, NY
Ramisia brasiliensis	Oliv.	EF079492	EF079546	-	EF079640	*Jardim 1507*	Brazil, 1998, NY
Reichenbachia hirsuta	Spreng.	EF079494	EF079548	EF079595	EF079641	*Nee 51972*	Bolivia, 2002, NY
Salpianthus arenarius	Humb. & Bonpl.	EF079486	EF079538	EF079593	EF079637	*Spellenberg 12903*	Michoacan, Mexico, 1999, NMC
Tripterocalyx carneus	(Greene) L.A. Galloway	EF079453	EF079508	EF079562	EF079604	*Douglas 2060*	New Mexico, USA, 2001, DUKE
Outgroups							
Aptenia cordifolia	(L. F.) Schwantes	-	*AF194824	-	-		
Mollugo verticillata	L.	-	*AF194827	-	-		
		-	-	-	EF079649	*Wilbur 77788*	North Carolina, USA, 2004, DUKE
Petiveria alliacea	L.	EF079499	EF079552	-	-	*AL Reina G. 98-2048*	Sonora, Mexico, 1998, NY
Phytolacca americana	Roxb.	EF079460	-	EF079568	EF079611	*Douglas 2118*	North Carolina, USA, 2002, DUKE
Phytolacca acinosa	L	-	*AF194828	-	-	*;*	
Rivina humilis	L.	EF079462	EF079516	EF079570	EF079613	*Douglas 2120*	North Carolina, USA*, 2002, DUKE
Sarcobatus vermiculatus	(Hook.) Torr.	EF079501	EF079555	EF079597	EF079647	*Spellenberg 13312*	Nevada, USA, 2002, DUKE
Stegnosperma cubense	A. Rich.	EF079500	EF079554	EF079596	EF079646	*Salas-M. 2649*	Oaxaca, Mexico, 1999, NY
Trichostigma octandrum	(L.) H. Walter	-	EF079553	-	EF079645	*5447*	Virgin Islands, USA 1993, NY

Appendix 2: Samples used in Chapter 2

Species	Accession	ITS	NIA	Voucher	Locality	Deposition
B. alata	2156	yes	yes	*Douglas 2156*	Sonora, Mexico	DUKE
B. anisophylla	2183	yes	yes	*Douglas 2183*	Coahuila, Mexico	DUKE
B. anisophylla	2194	yes	yes	*Douglas 2194*	Durango, Mexico	DUKE
B. anisophylla	2204	yes		*Douglas 2204*	Coahuila, Mexico	DUKE
B. ciliata	2145	yes		*Douglas 2145*	Texas	DUKE
B. coccinea	13264	yes	yes	*Spellenberg 13264*	New Mexico	NMC, DUKE
B. coccinea	13275	yes	yes	*Spellenberg 13275*	Arizona	NMC, DUKE
B. coccinea	13400	yes	yes	*Spellenberg 13400*	New Mexico	NMC, DUKE
B. coccinea	13403		yes	*Spellenberg 13403*	Nevada	NMC, DUKE
B. coccinea	2053	yes	yes	*Douglas 2053*	Arizona	DUKE
B. coccinea	2056	yes	yes	*Douglas 2056*	Arizona	DUKE
B. coccinea	2057	yes	yes	*Douglas 2057*	Arizona	DUKE
B. coccinea	2080	yes	yes	*Douglas 2080*	New Mexico	DUKE
B. coccinea	2083		yes	*Douglas 2083*	New Mexico	DUKE
B. coccinea	2139	yes	yes	*Douglas 2139*	Texas	DUKE
B. coccinea	2190		yes	*Douglas 2190*	Chihuahua, Mexico	DUKE
B. coccinea	2261	yes	yes	*Douglas 2261*	Sinaloa, Mexico	DUKE
B. coccinea	Bombay	yes	yes	*s.n.*	Bombay, India	
B. coccinea	NMSU15	yes	yes	*Spellenberg 12894*	Michoacan, Mexico	NMC
B. coccinea	NMSU16	yes	yes	*Spellenberg 7153*	Ibb, Yemen	NMC
B. coccinea X gracilima	2191	yes		*Douglas 2191*	Chihuahua, Mexico	DUKE
B. cordobensis	Russ511	yes		*Russell 511*	South Africa	MO
B. coulteri v. coulteri	13267	yes		*Spellenberg 13267*	New Mexico	NMC, DUKE
B. coulteri v. coulteri	13270	yes	yes	*Spellenberg 13270*	Arizona	NMC, DUKE
B. coulteri v. coulteri	13278	yes	yes	*Spellenberg 13278*	Arizona	NMC, DUKE
B. coulteri v. coulteri	13285	yes		*Spellenberg 13285*	New Mexico	NMC, DUKE
B. coulteri v. coulteri	2079	yes	yes	*Douglas 2079*	New Mexico	DUKE
B. coulteri v. coulteri	2105	yes		*Douglas 2105*	Arizona	DUKE
B. coulteri v. coulteri	2106	yes	yes	*Douglas 2106*	Arizona	DUKE
B. coulteri v. coulteri	2123	yes	yes	*Douglas 2123*	Arizona	DUKE
B. coulteri v. coulteri	2126	yes	yes	*Douglas 2126*	Arizona	DUKE
B. coulteri v. coulteri	2251	yes	yes	*Douglas 2251*	Sonora, Mexico	DUKE
B. coulteri v. coulteri	NMSU17	yes		*Reina G. AL 98-834*	Sonora, Mexico	NMC
B. coulteri v. palmeri	13273	yes		*Spellenberg 13273*	Arizona	NMC, DUKE
B. coulteri v. palmeri	13372	yes	yes	*Spellenberg 13372*	New Mexico	NMC, DUKE
B. coulteri v. palmeri	2124		yes	*Douglas 2124*	Arizona	DUKE
B. coulteri v. palmeri	2127	yes	yes	*Douglas 2127*	Arizona	DUKE
B. coulteri v. palmeri	2153	yes	yes	*Douglas 2153*	Sonora, Mexico	DUKE
B. coulteri v. palmeri	2162	yes	yes	*Douglas 2162*	Sonora, Mexico	DUKE
B. coulteri v. palmeri	2177	yes	yes	*Douglas 2177*	Sonora, Mexico	DUKE
B. coulteri v. palmeri	2224	yes	yes	*Douglas 2224*	Baja California Sur, Mexico	DUKE
B. coulteri v. palmeri	2225	yes	yes	*Douglas 2225*	Baja California Sur, Mexico	DUKE
B. coulteri v. palmeri	2248	yes	yes	*Douglas 2248*	Sonora, Mexico	DUKE
B. coulteri v. palmeri	2252	yes	yes	*Douglas 2252*	Sonora, Mexico	DUKE
B. coulteri v. palmeri	NMSU28	yes	yes	*Spellenberg 12961*	Arizona	NMC
B. crispifolia	NYBG12	yes		*Fosberg 48976*	Aldabra	NY
B. diffusa	13289	yes	yes	*Spellenberg 13289*	Florida	NMC, DUKE
B. diffusa	13290	yes	yes	*Spellenberg 13290*	Florida	NMC, DUKE
B. diffusa	13292	yes	yes	*Spellenberg 13292*	Florida	NMC, DUKE
B. diffusa	2114	yes	yes	*Douglas 2114*	Puerto Rico	DUKE
B. diffusa	JSR1	yes	yes	*Rose 1*	Hawaii, Oahu	DUKE
B. dominii	NYBG02	yes	yes	*Smyth 42*	Australia	NY
B. erecta	13291	yes	yes	*Spellenberg 13291*	Florida	NMC, DUKE
B. erecta	2115	yes	yes	*Douglas 2115*	Puerto Rico	DUKE
B. erecta	2150	yes		*Douglas 2150*	Arizona	DUKE
B. erecta	2165	yes	yes	*Douglas 2165*	Sonora, Mexico	DUKE
B. erecta	2188	yes		*Douglas 2188*	Coahuila, Mexico	DUKE
B. erecta	2259	yes	yes	*Douglas 2259*	Sonora, Mexico	DUKE

Continued

Species	Accession	ITS	NIA	Voucher	Locality	Deposition
B. erecta	NMSU18	yes	yes	*Spellenberg 12900*	Michoacan, Mexico	NMC
B. gardneri	c8776	yes		*Cowin 8776*	Australia	DNA
B. gracillima	2143	yes	yes	*Douglas 2143*	Texas	DUKE
B. gracillima	2167	yes	yes	*Douglas 2167*	Sonora, Mexico	DUKE
B. gracillima	2189	yes	yes	*Douglas 2189*	Chihuahua, Mexico	DUKE
B. gracillima	2207	yes	yes	*Douglas 2207*	Coahuila, Mexico	DUKE
B. gracillima	2210	yes	yes	*Douglas 2210*	Baja California Sur	DUKE
B. gracillima	NMSU14	yes		*Spellenberg 12447*	Texas	NMC
B. heronensis	NYBG27	yes		*Seydel 4323*	Namibia	NY
B. intermedia	13271	yes	yes	*Spellenberg 13271*	Arizona	NMC, DUKE
B. intermedia	13279	yes	yes	*Spellenberg 13279*	Arizona	NMC, DUKE
B. intermedia	13319	yes	yes	*Spellenberg 13319*	Sonora, Mexico	NMC, DUKE
B. intermedia	2109	yes	yes	*Douglas 2109*	Arizona	DUKE
B. intermedia	2111	yes	yes	*Douglas 2111*	Arizona	DUKE
B. intermedia	2128	yes	yes	*Douglas 2128*	Arizona	DUKE
B. intermedia	2134	yes	yes	*Douglas 2134*	New Mexico	DUKE
B. intermedia	2141	yes	yes	*Douglas 2141*	Texas	DUKE
B. intermedia	2158	yes	yes	*Douglas 2158*	Sonora, Mexico	DUKE
B. intermedia	2163	yes	yes	*Douglas 2163*	Sonora, Mexico	DUKE
B. intermedia	2166	yes	yes	*Douglas 2166*	Sonora, Mexico	DUKE
B. intermedia	2174	yes	yes	*Douglas 2174*	Sonora, Mexico	DUKE
B. intermedia	2184	yes	yes	*Douglas 2184*	Coahuila, Mexico	DUKE
B. intermedia	2186	yes	yes	*Douglas 2186*	Coahuila, Mexico	DUKE
B. intermedia	2221	yes	yes	*Douglas 2221*	Baja California Sur, Mexico	DUKE
B. intermedia	2226	yes	yes	*Douglas 2226*	Baja California Sur, Mexico	DUKE
B. intermedia	2229	yes	yes	*Douglas 2229*	Baja California Sur, Mexico	DUKE
B. intermedia	2238	yes	yes	*Douglas 2238*	Baja California Sur, Mexico	DUKE
B. intermedia	2250	yes	yes	*Douglas 2250*	Sonora, Mexico	DUKE
B. intermedia	2256	yes	yes	*Douglas 2256*	Sonora, Mexico	DUKE
B. lateriflora	2157	yes	yes	*Douglas 2157*	Sonora, Mexico	DUKE
B. lateriflora	2161	yes	yes	*Douglas 2161*	Sonora, Mexico	DUKE
B. laterifolia X xantii	2159		yes	*Douglas 2159*	Sonora, Mexico	DUKE
B. linearifolia	2102	yes		*Douglas 2102*	New Mexico	DUKE
B. linearifolia	2205	yes		*Douglas 2205*	Coahuila, Mexico	DUKE
B. maculata	2240	yes	yes	*Douglas 2240*	Baja California Sur, Mexico	DUKE
B. maculata	2242	yes	yes	*Douglas 2242*	Baja California	DUKE
B. maculata	2246	yes	yes	*Douglas 2246*	Baja California	DUKE
B. maculata	NMSU23	yes	yes	*Reina G. AL 98-2075*	Sinaloa, Mexico	NMC
B. megaptera	2129	yes		*Douglas 2129*	Arizona	DUKE
B. megaptera	NMSU24	yes	yes	*Spellenberg 13096*	Arizona	NMC
B. paludosa	a333	yes		*Allan 333*	Australia	DNA
B. pterocarpa	13280	yes	yes	*Spellenberg 13280*	Arizona	NMC, DUKE
B. pterocarpa	2178	yes	yes	*Douglas 2178*	Arizona	DUKE
B. purpurascens	13261	yes	yes	*Spellenberg 13261*	Arizona	NMC, DUKE
B. purpurascens	13347	yes		*Spellenberg 13347*	New Mexico	NMC, DUKE
B. purpurascens	13383	yes	yes	*Spellenberg 13383*	New Mexico	NMC, DUKE
B. purpurascens	13490	yes		*Spellenberg 13490*	Arizona	NMC, DUKE
B. purpurascens	2149	yes	yes	*Douglas 2149*	Arizona	DUKE
B. purpurascens	2172	yes		*Douglas 2172*	Sonora, Mexico	DUKE
B. purpurascens	2173	yes	yes	*Douglas 2173*	Sonora, Mexico	DUKE
B. repens	JSR2	yes	yes	*Rose 2*	Oahu, Hawaii	DUKE
B. repens	Long781	yes		*Long 781*	South Africa	MO
B. repens	NMSU25	yes	yes	*Spellenberg 7183*	Sana, Yemen	NMC
B. repens	NYBG07	yes		*Nowicke 351*	Sri Lanka	NY
B. repleta	a5666	yes		*Albrecht 5666*	Australia	DNA
B. schomburgkiana	m1034	yes		*Mitchell 1034*	Australia	DNA
B. spicata	13262	yes		*Spellenberg 13262*	Arizona	NMC, DUKE

Continued

Species	Accession	ITS	NIA	Voucher	Locality	Deposition
B. spicata	13263	yes		*Spellenberg 13263*	New Mexico	NMC, DUKE
B. spicata	13276	yes		*Spellenberg 13276*	Arizona	NMC, DUKE
B. spicata	13333	yes		*Spellenberg 13333*	Sonora, Mexico	NMC, DUKE
B. spicata	13491	yes		*Spellenberg 13491*	Arizona	NMC, DUKE
B. spicata	2147	yes		*Douglas 2147*	New Mexico	DUKE
B. spicata	2148	yes		*Douglas 2148*	Arizona	DUKE
B. spicata	2171	yes		*Douglas 2171*	Sonora, Mexico	DUKE
B. spicata	2176	yes		*Douglas 2176*	Sonora, Mexico	DUKE
B. spicata	2247	yes		*Douglas 2247*	Sonora, Mexico	DUKE
B. torreyana	13266	yes	yes	*Spellenberg 13266*	New Mexico	NMC, DUKE
B. torreyana	13382	yes	yes	*Spellenberg 13382*	New Mexico	NMC, DUKE
B. torreyana	13401	yes	yes	*Spellenberg 13401*	Texas	NMC, DUKE
B. torreyana	2090	yes	yes	*Douglas 2090*	New Mexico	DUKE
B. torreyana	2146	yes	yes	*Douglas 2146*	Texas	DUKE
B. traubae	2169	yes	yes	*Douglas 2169*	Sonora, Mexico	DUKE
B. traubae	2170	yes	yes	*Douglas 2170*	Sonora, Mexico	DUKE
B. traubae	NMSU27	yes		*Fishbein 2479*	Sonora, Mexico	NMC
B. triquetra	13793		yes	*Spellenberg 13793*	Baja California	NMC, DUKE
B. triquetra	13795		yes	*Spellenberg 13795*	Baja California	NMC, DUKE
B. triquetra	13799		yes	*Spellenberg 13799*	Baja California	NMC, DUKE
B. triquetra	13805		yes	*Spellenberg 13805*	Baja California	NMC, DUKE
B. triquetra	13807		yes	*Spellenberg 13807*	Baja California	NMC, DUKE
B. triquetra	13811		yes	*Spellenberg 13811*	Baja California	NMC, DUKE
B. triquetra	2255	yes	yes	*Douglas 2255*	Sonora, Mexico	DUKE
B. triquetra	NMSU22	yes	yes	*de la Luz 9409a*	Baja California Sur, Mexico	NMC
B. triquetra	VanDevender	yes	yes	*Van Devender*	Sonora, Mexico	NMC
B. verbenacea	NYBG14	yes	yes	*Sanchez-Vega 6182*	Peru	NY
B. wrightii	2125	yes	yes	*Douglas 2125*	Arizona	DUKE
B. wrightii	2135	yes		*Douglas 2135*	New Mexico	DUKE
B. wrightii	2140	yes	yes	*Douglas 2140*	Texas	DUKE
B. wrightii	SF144	yes	yes	*Fuentes 144*	Baja California Sur, Mexico	DUKE
B. xantii	13335	yes	yes	*Spellenberg 13335*	Sonora, Mexico	NMC, DUKE
B. xantii	13345		yes	*Spellenberg 13345*	Sonora, Mexico	NMC, DUKE
B. xantii	2113	yes	yes	*Douglas 2113*	Arizona	DUKE
B. xantii	2152	yes		*Douglas 2152*	Sonora, Mexico	DUKE
B. xantii	2154	yes	yes	*Douglas 2154*	Sonora, Mexico	DUKE
B. xantii	2155	yes		*Douglas 2155*	Sonora, Mexico	DUKE
B. xantii	2160	yes	yes	*Douglas 2160*	Sonora, Mexico	DUKE
B. xantii	2164	yes	yes	*Douglas 2164*	Sonora, Mexico	DUKE
B. xantii	2168	yes		*Douglas 2168*	Sonora, Mexico	DUKE
B. xantii	2175	yes		*Douglas 2175*	Sonora, Mexico	DUKE
B. xantii	2209	yes	yes	*Douglas 2209*	Baja California Sur, Mexico	DUKE
B. xantii	2214	yes	yes	*Douglas 2214*	Baja California Sur, Mexico	DUKE
B. xantii	2218	yes	yes	*Douglas 2218*	Baja California Sur, Mexico	DUKE
B. xantii	2227	yes	yes	*Douglas 2227*	Baja California Sur, Mexico	DUKE
B. xantii	2239	yes	yes	*Douglas 2239*	Baja California Sur, Mexico	DUKE
B. xantii	2249	yes		*Douglas 2249*	Sonora, Mexico	DUKE
B. xantii	2253	yes	yes	*Douglas 2253*	Sonora, Mexico	DUKE
B. xantii	2254	yes	yes	*Douglas 2254*	Sonora, Mexico	DUKE
Outgroups						
Nyctaginia capitata	M2049	yes	yes	*McIntosh 2049*	New Mexico	NMC
Okenia hypogaea	2206	yes	yes	*Douglas 2206*	Veracruz, Mexico	DUKE
Total		154	123			

Appendix 3: Samples used in Chapter 3

Accession Number	Species	# individuals	Locality	Voucher[1]
2194	*B. anisophylla*	1	Durango	*Douglas 2194*
2145	*B. ciliata*	6	Texas	*Douglas 2145*
13275	*B. coccinea*	1	Arizona	*Spellenberg 13275*
13400	*B. coccinea*	10	New Mexico	*Spellenberg 13400*
B24122	*B. coccinea*	1	Chiapas	*Breedlove 24122*
13267	*B. coulteri* var.. *coulteri*	1	New Mexico	*Spellenberg 13267*
13270	*B. coulteri* var.. *coulteri*	1	Arizona	*Spellenberg 13270*
13278	*B. coulteri* var.. *coulteri*	1	Arizona	*Spellenberg 13278*
13285	*B. coulteri* var.. *coulteri*	1	New Mexico	*Spellenberg 13285*
2126	*B. coulteri* var.. *coulteri*	5	Arizona	*Douglas 2126*
13272	*B. coulteri* var.. *palmeri*	10	Arizona	*Spellenberg 13272*
13273	*B. coulteri* var.. *palmeri*	1	Arizona	*Spellenberg 13273*
2127	*B. coulteri* var.. *palmeri*	5	Arizona	*Douglas 2127*
2162	*B. coulteri* var.. *palmeri*	8	Sonora	*Douglas 2162*
2177	*B. coulteri* var.. *palmeri*	5	Sonora	*Douglas 2177*
13289	*B. diffusa*	1	Florida	*Spellenberg 13289*
13290	*B. diffusa*	1	Florida	*Spellenberg 13290*
13292	*B. diffusa*	1	Florida	*Spellenberg 13292*
JSR1	*B. diffusa*	4	Hawaii	*Rose1*
W24133	*B. diffusa*	1	Panama	*Wilbur 24133*
W32838	*B. diffusa*	1	Puntarenas, Costa Rica	*Wilbur 32838*
13291	*B. erecta*	1	Florida	*Spellenberg 13291*
2115	*B. erecta*	3	Puerto Rico	*Douglas 2115*
2150	*B. erecta*	6	Arizona	*Douglas 2150*
2165	*B. erecta*	5	Sonora	*Douglas 2165*
W26546	*B. erecta*	1	Guanacaste, Costa Rica	*Wilbur 26546*
W30690	*B. erecta*	1	Limon, Costa Rica	*Wilbur 30690*
W32856	*B. erecta*	1	Puntarenas, Costa Rica	*Wilbur 32856*
2167	*B. gracillima*	7	Sonora	*Douglas 2167*
2189	*B. gracillima*	5	Chihuahua	*Douglas 2189*
2207	*B. gracillima*	5	Coahuila	*Douglas 2207*
13271	*B. intermedia*	1	Arizona	*Spellenberg 13271*
13279	*B. intermedia*	1	Arizona	*Spellenberg 13279*
13319	*B. intermedia*	1	Sonora	*Spellenberg 13319*
2141	*B. intermedia*	5	Texas	*Douglas 2141*
2158	*B. intermedia*	5	Sonora	*Douglas 2158*
2174	*B. intermedia*	5	Sonora	*Douglas 2174*
2184	*B. intermedia*	8	Coahuila	*Douglas 2184*
2186	*B. intermedia*	4	Coahuila	*Douglas 2186*
2157	*B. lateriflora*	5	Sonora	*Douglas 2157*
2161	*B. lateriflora*	5	Sonora	*Douglas 2161*
2159	*B. laterifolia* X *xantii*	1	Sonora	*Douglas 2159*
2205	*B. linearifolia*	5	Coahuila	*Douglas 2205*
2129	*B. megaptera*	5	Arizona	*Douglas 2129*
13280	*B. pterocarpa*	1	Arizona	*Spellenberg 13280*
2178	*B. pterocarpa*	5	Arizona	*Douglas 2178*
13261	*B. purpurascens*	1	Arizona	*Spellenberg 13261*
13347	*B. purpurascens*	5	New Mexico	*Spellenberg 13347*
13383	*B. purpurascens*	10	New Mexico	*Spellenberg 13383*
2173	*B. purpurascens*	5	Sonora	*Douglas 2173*
JSR2	*B. repens*	3	Hawaii	*Rose 2*
13262	*B. spicata*	1	Arizona	*Spellenberg 13262*
13263	*B. spicata*	1	New Mexico	*Spellenberg 13263*

cont.

Accession Number	Species	# individuals	Locality	Voucher[1]
13276	*B. spicata*	1	Arizona	*Spellenberg 13276*
13333	*B. spicata*	1	Sonora	*Spellenberg 13333*
2147	*B. spicata*	4	New Mexico	*Douglas 2147*
2148	*B. spicata*	6	Arizona	*Douglas 2148*
2171	*B. spicata*	5	Sonora	*Douglas 2171*
2176	*B. spicata*	6	Sonora	*Douglas 2176*
13382	*B. torreyana*	10	New Mexico	*Spellenberg 13382*
13401	*B. torreyana*	8	Texas	*Spellenberg 13401*
2146	*B. torreyana*	6	Texas	*Douglas 2146*
2169	*B. traubae*	7	Sonora	*Douglas 2169*
2135	*B. wrightii*	7	New Mexico	*Douglas 2135*
2140	*B. wrightii*	5	Texas	*Douglas 2140*
13335	*B. xantii*	1	Sonora	*Spellenberg 13335*
13345	*B. xantii*	1	Sonora	*Spellenberg 13345*
2152	*B. xantii*	4	Sonora	*Douglas 2152*
2154	*B. xantii*	5	Sonora	*Douglas 2154*
2155	*B. xantii*	6	Sonora	*Douglas 2155*
2160	*B. xantii*	5	Sonora	*Douglas 2160*
2168	*B. xantii*	5	Sonora	*Douglas 2168*
2175	*B. xantii*	5	Sonora	*Douglas 2175*

[1]Voucher specimens housed at DUKE, except Spellenberg collections, housed at DUKE and NMC

Biography

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Publications

Douglas, N.A., submitted. Tripterocalyx carneus (Nyctaginaceae) is self-compatible, *Southwestern Naturalist*.

Douglas, N.A. & P.S. Manos. 2007. Molecular phylogeny of Nyctaginaceae: Taxonomy, biogeography, and characters associated with a radiation of xerophytic genera in North America, *American Joural of Botany* 94(5).

Conference Presentations and Posters

Douglas, N.A. Understanding species limits and phylogeny of the vexing genus *Boerhavia* (Nyctaginaceae): AFLP and nuclear ITS data. Paper presented at Botany 2006, Chico CA, July 28-August 2, 2006.

Chau, J.H. & Douglas, N.A. Do endophytes in *Magnolia grandiflora* leaves produce false bands in AFLP fingerprints from leaf DNA extractions? Poster presented at Botany 2005, Austin TX, Aug 13-17 2005.

Douglas, N.A. A molecular phylogeny of the four-o'clock family, Nyctaginaceae. Paper presented at Botany 2005, Austin TX, Aug 13-17, 2005.

Douglas, N.A. & Manos, P.S. Phylogenetics and selfing in *Boerhavia* (Nyctaginaceae). Paper presented at Botany 2003, Mobile AL, July 26-31, 2003.

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