

MOLECULAR SYSTEMATICS OF THE TEXAS GENERA OF NYCTAGINACEAE

THESIS

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in Partial Fulfillment of
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For the Degree
Master of Science

By

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2002

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ABSTRACT

MOLECULAR SYSTEMATICS OF THE TEXAS GENERA OF NYCTAGINACEAE

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For a family well known for its confusing morphological taxonomy, surprisingly little molecular phylogenetic work has been published for the Nyctaginaceae (four o'clock family). This plant group is important in Texas because it includes the endangered endemic *Abronia macrocarpa*. Elucidating phylogenetic relationships, especially sister group relationships, offers insight into areas like speciation and character evolution. With endangered taxa, this insight may help guide management strategies. Approximately twelve genera (ca. 68 species) of Nyctaginaceae occur in Texas. A molecular phylogenetic investigation was undertaken to determine the relationships among eleven of these genera. DNA sequences of the ITS nrDNA were used to reconstruct relationships via maximum parsimony, distance, and maximum likelihood methods. Phylogenetic relationships within Tribe Nyctagineae do not agree with any of the current classifications. Other relationships within the family, as well as recommendations for needed taxonomic changes, are discussed.

INTRODUCTION

Nyctaginaceae (four o'clock family) comprises 400 species across 31 genera distributed throughout all warmer parts of the world, but mainly in the New World tropics and subtropics (Bittrich and Kühn 1993). Its two centers of distribution are tropical and subtropical South America including the Antilles and the southwestern United States including northern Mexico (Bittrich and Kühn 1993). Approximately twelve genera (ca. 68 species) occur in Texas.

Taxonomic delineations in this family (seemingly at all intrafamilial levels) have been unstable since the original description of Nyctaginaceae by A. L. de Jussieu in 1789. Botanists have called this family “sorely neglected” (Rydberg 1902), its specific differences “baffling” and “unstable” (Standley 1931), its taxonomy “very confused” (Duke 1961), and its systematics “problematical” (Bogle 1974). The latter two seem like understatements; Spellenberg and Tijerina (2001) report “*Mirabilis laevis* and its component taxa have a tortuous taxonomic history.” Although recent work in several fields generally (pollen morphology [Nowicke 1970, 1971], pollination biology [Cruden 1970; del Rio and Búrquez 1986; Williamson et al. 1994], breeding system [Williamson and Bazeer 1997], population genetics [Williamson and Werth 1999], anthocarp anatomy [Willson and Spellenberg 1977]) and on several genera specifically (Galloway 1975; Smith 1976; Fowler and Turner 1977; Pilz 1978; Spellenberg 1993; Turner 1993; Turner 1994; Le Duc 1995; Mahrt and Spellenberg 1995; Harriman 1999; Spellenberg and

Tijerina 2001; Levin 2002) has been done, the most recent familial treatment, Bittrich and Kühn (1993) (see Table 1), is based on Heimerl's (1934) work (Levin 2000). This may change pending the publication of the Flora of North America treatment.

Bittrich and Kühn's (1993) treatment is compared to other classifications in Table 2. A few examples illustrative of the scale of the problems assist in supporting the need for phylogenetic evaluations of the family. The status of three tribes (Abronieae, Bougainvilleae, Colignonieae) remain obscure, and *Cryptocarpus* and *Reichenbachia* have been individually ascribed to different tribes by different authors (see Table 2). Generic delineations are confused as well. Mabberley's (1997) *Boerhavia* includes *Anulocaulis*, *Cyphomeris*, and *Commicarpus*, although taxonomic treatments exist for each of the latter three genera (e.g., Spellenberg 1993; Mahrt and Spellenberg 1995; Harriman 1999). *Tripterocalyx* is included in *Abronia* by some authors (e.g., Wilson 1972, 1976; Correll and Johnston 1979; Bittrich and Kühn 1993), while others recognize both genera (e.g., Nowicke 1970; Galloway 1975; Kartesz and Kartesz 1980; Spellenberg 1993; Jones et al. 1997). Taxonomic uncertainty also persists at the species level. A "taxonomic addendum", reporting name changes for taxa in *that paper*, concludes Spellenberg's (2000) *Boerhavia* paper. Hence, having a publication at hand that disentangles the accepted names from the synonyms is a delight; in some cases, experts on this family recognize taxa but without currently available publications; for example, B. L. Turner now recognizes *Abronia nealleyi* instead of *A. carletonii*, the more generally accepted name (D. J. McHenry pers. obs. 26 Jul 2002). In fact, the family name itself is based on *Nyctago* Juss., an illegitimate synonym of *Mirabilis* L. (Nowicke 1970)! As a

TABLE 1. Family Nyctaginaceae according to Bittrich and Kühn (1993). Asterisks indicate genera included in this study. Format follows Levin (2000). ^a *Selinocarpus* was transferred to *Acleisanthes* by Levin (2002). ^b Includes *Tripterocalyx*. Galloway (1975) recognizes both genera; I follow his treatment.

Genus	# spp	Geographic Range
Tribe Boldoeae Heimerl 1889		
<i>Boldoa</i>	1	from Mexico and West Indies to Venezuela and Colombia
<i>Salpianthus</i>	1	Mexico
<i>Cryptocarpus</i>	1	Galapagos Islands, Peru, Bolivian, Ecuador
Tribe Leucastereae Benth & Hook 1880		
<i>Andradea</i>	1	SE Brazil
<i>Ramisia</i>	1	SE Brazil
<i>Leucaster</i>	1	Brazil
<i>Reichenbachia</i>	2	Argentina, Bolivia, Brazil, Paraguay
Tribe Nyctagineae Horan 1847		
Subtribe Colignoninae Heimerl 1889		
<i>Colignonia</i>	6	Andes of Columbia, Ecuador, Peru, Bolivia, Argentina
<i>Pisoniella</i>	1	Mexico, Bolivia, Argentina
Subtribe Boerhaviinae Benth & Hook 1880		
<i>*Boerhavia</i>	about 20	in almost all tropical and subtropical areas of the world
<i>*Anulocaulis</i>	4-5	C and southern USA, Mexico
<i>*Cyphomeris</i>	2	Texas to Mexico
<i>Commucarpus</i>	25	mainly in Africa, except the extreme N and S, but also in warm regions of Europe (S Spain), Asia (Burma, Malaysia, S China), Australia, N and S America
<i>Caribea</i>	1	Cuba
<i>*Acleisanthes</i>	7	from Texas to California, and in Mexico
<i>Selinocarpus</i> ^a	about 10	C to W USA, Mexico
<i>Okenia</i>	1 or 2	from Florida to Mexico and Nicaragua
Subtribe Nyctaginae		
<i>*Mirabilis</i>	about 54	almost exclusively American, C and W USA, C America, N South America, one sp in Western Himalaya, one sp in Europe (an escape from cultivation)
<i>Cuscatlania</i>	1	El Salvador
<i>*Nyctaginia</i>	1	from Texas to South Mexico
<i>*Allionia</i>	2	C and W USA, Antilles, and from Venezuela to Chile and Argentina
Subtribe Phaeoptilinae Heimerl 1934		
<i>Phaeoptilum</i>	1	endemic to SW Africa and Botswana

TABLE 1 continued.

Tribe Abronieae (Benth. & Hook.) Heimerl 1897		
* <i>Abronia</i> ^b	about 33	from southwestern USA to N Mexico
Tribe Bougainvilleae Choisy 1849		
* <i>Bougainvillea</i>	18	S America, cultivars of <i>B. peruviana</i> , <i>B. glabra</i> and <i>B. spectabilis</i> in all warmer regions of the world
<i>Belemia</i>	1	E Brazil (Espírito Santo)
Tribe Pisonieae Meisner 1841		
* <i>Pisonia</i>	about 40	tropical and subtropical regions predominantly of America and SE Asia, with a few spp. in Africa and Australia
<i>Guapira</i>	about 70	C and tropical S America, the Antilles
<i>Neea</i>	about 83	S Florida to C America and Bolivia, Antilles
<i>Neeopsis</i>	1	Guatemala
<i>Cephalotomandra</i>	1-3	Colombia
<i>Grayalesia</i>	1	Mexico

result, the name of the tribe including *Mirabilis* (Nyctagineae Horan. 1847 = Mirabileae Meisner 1841) is dubious (see Nowicke 1970; Bogle 1974) (see Table 2). Further, Bittrich and Kühn (1993) divided Subtribe Boerhaviinae (Benth. & Hook. 1880) (= Mirabilinae [and = Nyctagininae according to Bogle 1974!]) into Subtribes Boerhaviinae and Nyctagininae; insuring that future taxonomic confusion is imminent.

No within-family molecular study had been attempted until Levin's (2000) recent work involving phylogenetic reconstructions among three genera — *Acleisanthes*, *Mirabilis*, and *Selinocarpus* (*Selinocarpus* was transferred to *Acleisanthes* by Levin [2002]). Levin's (2000) work remains the only molecular treatment of this family. Some genera have been included in higher level molecular phylogenetic studies (e.g., Rettig et al. 1992; Chase et al. 1993; Downie and Palmer 1994; Soltis et al. 1997; Hershkovitz and Zimmer 1997; Downie et al. 1997; Savolainen et al. 2000; Applequist and Wallace 2001; Cuénoud et al. 2002) but with little attention within the family.

TABLE 2. The current classification of Nyctaginaceae according to Bittrich and Kühn (1993), with comparisons to other classifications. Asterisk indicates a taxon is placed in another tribe, dash a taxon is not listed by the author(s). Parentheses enclose # of spp. ^a Includes *Ammocodon*. ^b Includes *Tripterocalyx*. ^c No tribe given.

Bittrich and Kuhn 1993	Mabberley 1997	Bentham & Hooker 1880	Eckardt 1964
Tribe Boldoeae Heimerl 1889	Tribe Boldoeae	<u>Subtr. Boldoeae of Tr. Mirabilieae</u>	Tribe Boldoeae
<i>Boldoa</i> (1)	<i>Boldoa</i> (1)	<i>Boldoa</i> (2)	<i>Boldoa</i> (1)
*	*	<i>Reichenbachia</i> (1)	-
<i>Cryptocarpus</i> (1)	<i>Cryptocarpus</i> (1)	*	-
<i>Salpianthus</i> (1)	<i>Salpianthus</i> (1)	-	-
*	-	not used	Tribe Colignonieae
*	*	<i>Collignonia</i> (6)	<i>Colignonia</i> (11)
Tribe Leucastereae Benth. & Hook. 1880	Tribe Leucastereae	Tribe Leucastereae	Tribe Leucastereae
<i>Leucaster</i> (1)	<i>Leucaster</i> (1)	<i>Leucaster</i> (1)	-
<i>Andradea</i> (1)	<i>Andradea</i> (1)	<i>Andradea</i> (1)	-
*	*	<i>Cryptocarpus</i> (2)	-
<i>Reichenbachia</i> (2)	<i>Reichenbachia</i> (2)	*	<i>Reichenbachia</i> (1)
<i>Ramusia</i> (1)	<i>Ramusia</i> (1)	-	-
Tribe Nyctagineae Horan. 1847	Tribe Nyctagineae	Tribe Mirabilieae Meisner 1841	Tribe Mirabileae
<u>Subtribe Colignoninae</u>	-	not used	-
<i>Colignonia</i> (6)	<i>Colignonia</i> (6)	*	*
<i>Pisoniella</i> (1)	<i>Pisoniella</i> (1)	-	-
<u>Subtribe Boerhaviinae Benth. & Hook. 1880</u>	-	<u>Subtr. Boerhaavieae of Tr. Mirabilieae</u>	-
<i>Boerhavia</i> (about 20)	<i>Boerhavia</i> (c 50)	<i>Boerhaavia</i> (ca 30)	<i>Boerhaavia</i> (ca 20)
<i>Anulocaulis</i> (4 to 5)	not used, = <i>Boerhavia</i>	-	-
<i>Cyphomeris</i> (2)	not used, = <i>Boerhavia</i>	-	-
<i>Commucarpus</i> (25)	not used, = <i>Boerhavia</i>	-	-
<i>Caribea</i> (1)	<i>Caribea</i> (1)	-	-
<i>Acleisanthes</i> (7)	<i>Acleisanthes</i> (7)	<i>Acleisanthes</i> (6)	-
<i>Selinocarpus</i> (about 10) [in 2 sections] ^a	<i>Selinocarpus</i> (10)	<i>Selinocarpus</i> (3)	-

TABLE 2 continued.

<i>Okenia</i> (1 or 2)	<i>Okenia</i> (1 or 2)	<i>Okenia</i> (1)	-
-	-	<i>Senkenbergia</i> (4)	-
<u>Subtribe Nyctagininae</u>	-	-	-
<i>Mirabilis</i> (about 54) [in 4 sections]	<i>Mirabilis</i> (54)	<i>Mirabilis</i> (ca 10) [in 2 sections]	<i>Mirabilis</i> (60)
not used, = <i>Mirabilis</i>	not used, = <i>Mirabilis</i>	<i>Oxybaphus</i> (ca 20)	-
-	<i>Quamoclidion</i> (1) ^c	not used, = <i>Mirabilis</i>	<i>Quamoclidion</i> (1)
-	not used, = <i>Mirabilis</i>	<i>Hermidium</i> (1)	-
<i>Cuscutlania</i> (1)	<i>Cuscutlania</i> (1)	-	-
<i>Nyctaginia</i> (1)	<i>Nyctaginia</i> (1)	<i>Nyctaginia</i> (1)	-
<i>Allionia</i> (2)	<i>Allionia</i> (2)	<i>Allionia</i> (1)	-
<u>Subtribe Phaeoptulinae Heimerl 1934</u>	-	-	-
<i>Phaeoptilum</i> (1)	<i>Phaeoptilum</i> (1)	-	<i>Phaeoptilum</i> (1)
Tribe Abronieae (Benth. & Hook.) Heimerl 1897	Tribe Abronieae	<u>Subtr Abronieae of Tr Mirabilieae</u>	not used
<i>Abronia</i> (about 33) [in 2 sections] ^b	<i>Abronia</i> (33)	<i>Abronia</i> (ca 10)	<i>Abronia</i> (50)
Tribe Bougainvilleae Choisy 1849	Tribe Bougainvilleae	<u>Subtr Bougainvilleae of Tr Mirabilieae</u>	not used
<i>Bougainvillea</i> (18)	<i>Bougainvillea</i> (18)	<i>Bougainvillea</i> (7-8)	<i>Bougainvillea</i> (14)
<i>Belemia</i> (1)	<i>Belemia</i> (1)	-	-
-	not used, = <i>Bougainvillea</i>	<i>Tricycla</i> (1)	-
Tribe Pisonieae Meisner 1841	Tribe Pisonieae	Tribe Pisonieae	Tribe Pisonieae
<i>Pisonia</i> (about 40)	<i>Pisonia</i> (40)	<i>Pisonia</i> (ca. 60)	<i>Pisonia</i> (30)
<i>Neea</i> (about 83)	<i>Neea</i> (83)	<i>Neea</i> (ca. 30)	<i>Neea</i> (30)
-	-	<i>Timeroya</i> (2)	-
<i>Cephalotomandra</i> (1-3)	<i>Cephalotomandra</i> (1-3)	<i>Cephalotomandra</i> (1)	-
<i>Guapira</i> (about 70)	<i>Guapira</i> (ca 70)	-	-
<i>Neeopsis</i> (1)	<i>Neeopsis</i> (1)	-	-
<i>Grajaesia</i> (1)	<i>Grajaesia</i> (1)	-	-

The lack of phylogenetic certainty for the family is a problem, but also serves to exacerbate the difficulties in evaluating the endangered species within it. The current study began as a phylogenetic study of the Texas species in the genus *Abronia* to determine the sister taxon to the endangered *A. macrocarpa*. However, due to the considerable phylogenetic confusion at the genus level, the scope required expansion. Five *Abronia* species, out of about 30 in the genus, occur in Texas: *A. ameliae* Lundell, *A. angustifolia* Greene, *A. carletoni* Coult. & Fisher, *A. fragrans* Nutt. ex Hook., and *A. macrocarpa* L. A. Galloway (Williamson et al. 1994). Two, *A. ameliae* and *A. macrocarpa*, are endemic to the state (Galloway 1975; Williamson et al. 1994). Of these five Texan *Abronia* species, none are or ever were synonymous with any *Tripterocalyx* species. Although *A. macrocarpa* and *A. ameliae* are not considered synonymous by Correll and Johnston (1979), they are lumped together by those authors. The easternmost limit, according to Correll and Johnston (1979), of *A. ameliae* (Leon County, Texas) is actually a population of *A. macrocarpa* (P. S. Williamson, pers. comm. 30 Jan 2002). The two taxa are considered distinct by most authors (Galloway 1975; USFWS 1992; Williamson et al. 1994; Williamson and Bazeer 1997; Williamson and Werth 1999). While *A. macrocarpa* was described only recently (Galloway 1972) and its evolutionary history is currently unknown, its listing as Federally endangered in 1988 (U.S. Fish and Wildlife Service 1992) catalyzed recent interest in the species. Williamson et al. (1994) investigated its pollination biology, Williamson and Bazeer (1997) its breeding system, and Williamson and Werth (1999) used allozyme electrophoresis to examine the population genetics of *A. macrocarpa*, but as yet the molecular variation has not been

investigated. Thus, a phylogenetic investigation seems a next logical step in describing and documenting this endangered taxon.

Conservation biology of endangered and endemic organisms requires the integration of many biological disciplines including ecology, genetics, and reproductive biology. Molecular genetics, in particular, has become increasingly important as a tool in conservation biology. Molecular phylogenetics and population genetics provide insight into introgression, taxonomic boundaries, historical relationships, and biogeography at the species level, and gene flow, inbreeding, and diversity at the population level (Ellstrand and Elam 1993). While a large variety of genetic analyses are now possible and technically achievable, foremost in conservation is defining the evolutionary relationships among endangered fauna or flora and their sister groups. In identifying a taxon's sister group, phylogenetic analysis can contribute information about the evolution and classification of a particular group. It offers otherwise unknowable aspects of character evolution (Miles and Dunham 1993), speciation, biogeography (Page 1993, 1994), and co-speciation (Page 1991; Chapela et al. 1994; Hinkle et al. 1994). Despite the value of such analyses many endangered taxa have not yet been characterized for molecular markers.

Genes Available. The inherent nature of plant genetics provides a large potential group of molecular markers. Within the cells of most plants, three independent genomes are present: mitochondrial DNA (mtDNA), chloroplast DNA (cpDNA), and nuclear DNA (nDNA) (Graur and Li 2000). In species and genus level phylogenetic reconstruction, most molecular markers are from nDNA or cpDNA. Mitochondrial DNA in plants is not widely used in phylogenetic reconstruction at any taxonomic level due to the particularly

slow rate of plant mtDNA sequence evolution (Bachmann 1994; Laroche et al. 1997). The internal transcribed spacer (ITS) region of the 18S-26S nuclear ribosomal DNA (nrDNA) is widely used in plant phylogenetic systematics at lower taxonomic levels (Baldwin et al. 1995). For a full review of the utility of the ITS region see Baldwin et al. 1995.

Phylogenetic Analyses Available. The ultimate goal in phylogenetic systematics is to reconstruct the true phylogeny for the taxa of interest. Many analyses are available to do so; the three main tree reconstruction methods are maximum parsimony (MP), distance, and maximum likelihood (ML) (Graur and Li 2000). In each, assessment criteria and assumptions are made, or model parameters are estimated, using the data, in an attempt to converge on the true tree (although the true tree may never be determined). However, in any model based study, assumption and estimation are by nature potentially misleading.

A MP tree, reconstructed from DNA sequence data, is a topology with the fewest number of nucleotide substitutions (evolutionary changes) required to explain the data (Swofford et al. 1996; Graur and Li 2000). Some assumptions involved in reconstructing a MP topology are the order of character-state change, importance of one type of character over another (leads to differentially weighted characters), and the algorithm used to search for optimal trees.

In distance based methods using DNA sequence data, trees are constructed based on the number of nucleotide substitutions (distances) among all pairs of taxa (Graur and Li 2000). Distance based tree reconstructions methods include neighbor-joining (NJ) (Saitou and Nei 1987) and the unweighted pair-group method with arithmetic means

(UPGMA). Neighbor-joining seeks to correct for substitution biases using probabilistic evaluation of the actual DNA pairwise differences. It may incorporate a correction algorithm (e.g., Jukes-Cantor, Kimura 2-parameter); these are generally based on the type of base frequencies (i.e., equal or unequal) and the types of substitutions in the data (i.e., one type of substitution, two types [transitions (ti) and transversions (tv)], or three types [ti and two classes of tv or tv and two classes of ti]).

Maximum likelihood attempts to find the most probable tree that best explains the data under an assumed model of evolution. The model of evolution depends on many parameters including substitution rates (e.g., all rates equal, ti rate $\neq$ tv rate, general time reversible), base frequencies, gamma distribution shape parameter for variable sites, and the proportion of invariant sites. A good model of evolution includes only as many parameters as necessary to correctly explain the data (Posada and Crandall 2001), although ML methods have been found to be fairly robust to violations of assumptions (Huelsenbeck 1995; Schöniger and von Haeseler 1995).

Objectives. The objectives of this study were to employ DNA sequence data of the ITS region of the 18S-26S nrDNA to 1) determine relationships within the genus *Abronia*; and 2) determine relationships among the Texas genera in Nyctaginaceae.

MATERIALS AND METHODS

Taxon Sampling. The taxa sampled are listed in Table 3. Leaf tissue samples (5-10 were preferable due to vagaries in sample success and potential intrapopulational variation) were obtained during the spring and summer of 2002. Geographically disparate populations were sampled when possible. Samples were stored in sealable plastic bags or 2 ml Nunc™ CryoTubes™ on ice while transported to the laboratory where they were then stored at -80°C. Voucher specimens were also collected from each population sampled and deposited in the herbarium and the M. Forstner Catalog at Southwest Texas State University (SWTSU). Sample numbers were prefixed with my initials. DJM sample numbers 001-179 correspond to MF sample numbers 7583-7761 in the M. Forstner Catalog at SWTSU. Seed or leaf tissue was collected from herbarium specimens on deposit at SWT (see Appendix 1); these herbarium specimens were annotated accordingly. Seed tissue was also collected from purchased seeds (see Appendix 1 for company names). *Rivina humilis* L. (Rivinioideae: Phytolaccaceae) and *Phytolacca americana* L. (Phytolacchoideae: Phytolaccaceae) were included as outgroups; Phytolaccaceae has been shown to be a very close, if not the closest, relative of Nyctaginaceae at the family level (Rettig et al. 1992; Bittrich 1993; Chase et al. 1993; Downie and Palmer 1994; Soltis et al. 1997; HersHKovitz and Zimmer 1997; Downie et al. 1997; Savolainen et al. 2000; Applequist and Wallace 2001; Cuénoud et al. 2002).

TABLE 3. Taxa, DJM sample #, voucher specimens, and localities for individuals included in phylogenetic analyses; every genotype/taxon is represented. Herbarium vouchers are deposited at SWTSU. DJM sample # 001-179 correspond to MF sample # 7583-7761 in the M. Forstner Catalog at SWTSU. See Appendix 1 for complete collection information. Sample size (n) is indicated for genotypes with n > 1. *Taxonomic circumscription follows: ^a Galloway 1975, ^b Tillett 1967, ^c Levin 2002, ^d Smith 1976, ^e Turner 1994, ^f Spellenberg 1993, ^g Correll and Johnston 1979, ^h Mahrt and Spellenberg 1995, ⁱ Turner 1993.

Taxon*	DJM#	Herbarium Voucher	Locality
<i>Abronia ameliae</i> Lundell 1945 ^a n = 9	050	DJM051	Brooks Co., TX
<i>Abronia angustifolia</i> Greene 1898 ^a	173	JMM-020413-2	Hudspeth Co , TX
<i>Abronia fragrans</i> Nutt. ex Hook 1853 ^a n = 5	081	none	unknown
<i>Abronia fragrans</i> Nutt ex Hook 1853 ^a n = 6	002	none	Hutchinson Co., TX
<i>Abronia macrocarpa</i> L A Galloway 1972 ^a n = 15	008	DJM047	Leon Co , TX
<i>Abronia umbellata</i> Lam 1791 ^b n = 5	144	none	unknown
<i>Abronia villosa</i> S Wats 1873 ^a n = 6	080	none	unknown
<i>Abronia villosa</i> var <i>aurita</i> (Abrams) Jepson 1914 ^a n = 6	154	none	unknown
<i>Acleisanthes chenopodioides</i> (A Gray) R. A Levin 2002 ^c n = 2	115	S & G Jones 7635	Brewster Co., TX
<i>Acleisanthes longiflora</i> A Gray 1853 ^{cd} n = 3	138	DJM138	Brewster Co., TX
<i>Allionia incarnata</i> L 1759 ^e n = 2	132	DJM132	Brewster Co., TX
<i>Anulocaulis eriosolenus</i> (A. Gray) Standl 1909 ^f	137	DJM137	Brewster Co., TX
<i>Anulocaulis leiosolenus</i> (Torr) Standl 1909 ^f	174	Worthington 13843	Hudspeth Co , TX
<i>Boerhavia coccinea</i> Mill 1768 ^g	141	DJM141	Brewster Co , TX
<i>Boerhavia erecta</i> L 1753 ^g	073	DJM073	Hays Co., TX
<i>Boerhavia gracillima</i> Heimerl 1889 ^g	136	DJM136	Brewster Co , TX
<i>Boerhavia intermedia</i> M E Jones 1902 ^g	134	DJM134	Brewster Co , TX
<i>Boerhavia spicata</i> Choisy in D C. 1849 ^g n = 2	133	DJM133	Brewster Co., TX
<i>Bougainvillea</i> sp ^g	172	none	unknown
<i>Cyphomeris gypsophiloides</i> (Mart. & Gal.) Standl 1911 ^h	176	D E Lemke 4284b	Kinney Co , TX
<i>Mirabilis jalapa</i> L 1753 ⁱ	001	none	Guadalupe Co., TX
<i>Mirabilis jalapa</i> L 1753 ⁱ	003	none	Oldham Co., TX
<i>Mirabilis jalapa</i> L 1753 ⁱ n = 2	071	DJM071	Hays Co , TX
<i>Mirabilis longiflora</i> L 1755 ⁱ n = 7	076	none	unknown
<i>Mirabilis multiflora</i> (Torr) Gray 1859 ⁱ n = 8	066	DJM066	Culberson Co , TX
<i>Nyctaginia capitata</i> Choisy ^g n = 3	121	DJM121	Val Verde Co , TX
<i>Phytolacca americana</i> L ^g	179	C S. Berkhouse 025	Smith Co., TX
<i>Pisonia aculeata</i> L ^g	177	D. E. Lemke 1492	Cameron Co , TX
<i>Rivina humilis</i> L ^g	178	Tom Starbuck 2436	Robertson Co , TX
<i>Tripterocalyx carnea</i> (Greene) Galloway 1975 ^a n = 4	060	DJM 060	El Paso Co , TX
<i>Tripterocalyx mucranthus</i> (Torr) Hook 1853 ^a n = 4	099	none	Saguache Co , CO

DNA Extraction. DNA extractions were performed via two protocols (see Appendix 1).

MODIFIED SAMBROOK ET AL. 1989 PROTOCOL. A modified cell lysis and DNA extraction protocol of Sambrook et al. (1989) was used. Leaf tissue (about 0.3 g) was homogenized in 1.0 ml (1.5 ml for herbarium specimen tissue) lysis solution (0.5% SDS, 150 mM NaCl, 15 mM sodium citrate dihydrate powder, 1 mM EDTA disodium dihydrate) in a 1.5 ml microcentrifuge tube on ice. The mixture was centrifuged (Eppendorf Centrifuge 5415D) at room temperature for 1 min at 12,000 rpm and the supernatant was collected. Centrifugation and collection were repeated until no supernatant formed.

To 500 µl supernatant, 500 µl chloroform were added. This mixture was hand-shaken for 5 min and then centrifuged at room temperature for 1 min at 12,000 rpm. The aqueous phase was collected and to it, 500 µl chloroform were added. Again, this mixture was hand-shaken for 5 min and then centrifuged at room temperature for 1 min at 12,000 rpm. The aqueous phase was collected and to it, 500 µl chloroform were added. For a third time, the mixture was hand-shaken for 5 min and then centrifuged at room temperature for 1 min at 12,000 rpm. The aqueous phase was collected and to it, 500 µl chloroform were added. For a fourth time, the mixture was hand-shaken for 5 min and then centrifuged at room temperature for 1 min at 12,000 rpm. The aqueous phase was collected.

To precipitate the DNA, an equal volume of ice cold 100% EtOH was added to the DNA solution. The solution was stored for 12 min (or less) at -80°C, without allowing the DNA solution to freeze. The DNA solution was centrifuged at room

temperature for 5 min at 13,000 rpm to pellet the precipitant. The supernatant was collected and discarded. The pelleted DNA was dried under vacuum centrifugation (Savant Speed Vac® SC110). The DNA pellet was reconstituted in 50-400 µl ddH₂O. Successful extractions were judged visually under UV light by staining with ethidium bromide after electrophoresis in 1% agarose gels.

MODIFIED KANG ET AL. 1998 PROTOCOL. Extraction from seed tissue (and some leaf tissue, see Appendix 1) followed a modified protocol of Kang et al. (1998). Fruit wall (and seed coat when possible) was removed. The whole seed (or less than 0.1 g leaf tissue) was placed in a 1.5 ml microcentrifuge tube along with 400 µl lysis solution (0.5% SDS, 150 mM NaCl, 15 mM sodium citrate dihydrate powder, 1 mM EDTA disodium dihydrate) and 10 µl 20 mg/ml Proteinase K solution (Qiagen DNeasy® Tissue Extraction Kit Cat. No. 69506). This solution was incubated at 37°C for 1 hr. After incubation, the tissue was homogenized in the 1.5 ml tube. To the solution, 500 µl 25:24:1 phenol:chloroform:isoamyl alcohol were added. This mixture was hand-shaken for 5 min and then centrifuged at room temperature for 10 min at 12,000 rpm. The supernatant was transferred to a new 1.5 ml microcentrifuge tube. Two-thirds volume isopropanol was added, and this solution was incubated at room temperature for 10 min to precipitate DNA. Following centrifugation at room temperature for 5 min at 12,000 rpm, the supernatant was discarded, and the DNA pellet was washed with 70% ethanol. The pelleted DNA was dried under vacuum centrifugation (Savant Speed Vac® SC110). The DNA pellet was reconstituted in 40-300 µl TE buffer (10 mM Tris-HCl [pH 8.0], 0.1 mM EDTA). Successful extractions were judged visually under UV light by staining with ethidium bromide after electrophoresis in 1% agarose gels.

Polymerase Chain Reaction (PCR). PCR amplification of the ITS region of the 18S-26S nrDNA was performed in 100 (or 50) μ l volumes with 20 μ l 5x Buffer F (pH = 9.0, includes 20 mmol/l $MgCl_2$), 1 μ l DMSO, 1 μ l dNTPs, 1 μ l 0.01 mM forward primer, 1 μ l 0.01 mM reverse primer, 0.5 μ l *Taq* polymerase, and 1 μ l template DNA. The ITS region was amplified using N-nc18S10 (Wen and Zimmer 1996) as the forward primer and C26A (Wen and Zimmer 1996) as the reverse primer (see Table 4 for oligonucleotide sequences and relative positions within the fragment). PCR was performed using the GeneAmp® PCR System 9700 (PE Applied Biosystems) and 35 cycles, each consisting of denaturing at 95°C for 30 sec, annealing at 50°C for 1 min, and extension at 72°C for 1 min. A final extension period of 72°C for 5 min terminated the PCR. Positive and negative (no DNA) controls were included. PCR products were judged visually under UV light by staining with ethidium bromide after electrophoresis in 1% agarose gels.

TABLE 4. Primers used in amplification of ITS nrDNA. Oligonucleotide sequences are given 5' to 3'. Numbers in parentheses at ends of sequences represent nucleotide positions relative to the sequence of *Rivina humilis* DJM178 (see Appendix 2). N-nc18S10 and C26A are located just outside the sequences presented in Appendix 2.

Name	Sequence	Source
N-nc18S10	(-17) 5'-AGGAGAAGTCGTAACAAG-3' (0)	Wen and Zimmer 1996
C26A	(753) 5'-GTTTCTTTTCTCCGCT-3' (737)	Wen and Zimmer 1996
N58S	(380) 5'-ATCGAGTCTTTGAACGCA-3' (397)	Suh et al. 1993
5 8SR	(446) 5'-GTGACGCCCAGGCAGACGTG-3' (427)	This paper

Cycle Sequencing. The PCR product included excess primer, unincorporated nucleotides, and salts which need to be removed prior to sequencing. This was done with the CONCERT™ Rapid PCR Purification System (Cat. No. 11458-023, GIBCOBRL®) or the Rapid PCR Purification System (Cat. No. 11458-023, Marligen Bioscience Inc.). Cleaned PCR products were electrophoresed in 2% agarose gels along with pGEM®

3Zf(+)-sequencing standard (PE Applied Biosystems 2000) to estimate template concentration.

Cleaned PCR products were cycle sequenced in both 5' to 3' and 3' to 5' directions using the BigDye™ (version 2.0) Terminator Cycle Sequencing Ready Reaction Kit (Cat. No. 4314415 PE Applied Biosystems). Cycle sequencing reactions were performed in 9 µl volumes with varying amounts of ddH₂O, 0.5 µl 0.01 mM sequencing primer, varying amounts of template PCR product, and 3 µl Big Dye™. Forward primers were N-nc18S10 and N58S (Suh et al. 1993). Reverse primers were C26A and 5.8SR (McHenry 2002). See Table 4 for oligonucleotide sequences and relative positions within the fragment. Cycle sequencing was performed using the GeneAmp® PCR System 9700 (PE Applied Biosystems) and conditions were 25 cycles of 96°C for 30 sec, 50°C for 1 min, and 60°C for 4 min. Extraneous components in reactions, including unincorporated ddNTPs and primers, were removed by centrifugation through 0.05 g Sephadex G-50 suspended in 800 µl ddH₂O within CentriSep columns (Princeton Separations) and then dried under vacuum centrifugation. After rehydration in a 1:5 ABI loading buffer:formamide solution and denaturation, cleaned cycle sequence products were analyzed on ABI PRISM™ 377 XL DNA Sequencer.

Phylogenetic Analyses. Chromatograms resulting from the ABI PRISM® Sequencing Analysis Version 3.4.1 (PE Applied Biosystems) were inspected visually and ambiguous bases called manually. A consensus sequence for each individual was made in Sequencher™ Version 4.1.2 (GeneCodes Corp.). Resultant sequences were aligned algorithmically using Sequencher™ Version 4.1.2 and adjusted manually. Boundaries of

subunit and spacer regions were determined by comparison with those of *Daucus carota*, *Vicia faba* (Yokota et al. 1989), and *Nicotiana rustica* (Venkateswarlu and Nazar 1991), by visual inspection of the data matrix in PAUP* 4.0b10 (Swofford 2002), and by examination of substitution rates across the region for all taxa in MacClade 3.05 (Maddison and Maddison 1992). The boundaries of mature 18S, 5.8S, and 25S rRNAs were originally defined by S1 nuclease mapping in Yokota et al. (1989).

The subsequent data matrix was analyzed in PAUP* 4.0b10 (Swofford 2002). Gaps were treated as missing data. Combined analysis of the ITS gene regions has been shown to provide more accurate estimates of phylogeny (Baldwin et al. 1995), therefore a partition homogeneity test (Farris et al. 1995), with uninformative characters excluded, was performed in PAUP* 4.0b10 (Swofford 2002), using 100 replicates or until memory was exhausted, to determine if the data matrix should be analyzed as one dataset or as subsets (e.g., ITS1, 5.8S). Maximum parsimony (MP), distance, and maximum likelihood (ML) analyses (Hillis et al. 1996) were performed on the entire data matrix; additionally, separate analyses (MP and distance) were performed for the individual subsets: 18S, ITS1, 5.8S, ITS2, and 26S.

To test for phylogenetic signal, the g_1 statistic was evaluated from a distribution of 10,000 random trees reconstructed from the entire data matrix under MP with equally weighted characters. A dataset with little phylogenetic signal has a bell-shaped distribution, while the shape of the distribution becomes more left-skewed with increasing signal (Huelsenbeck 1991). The degree of skewness, and hence signal, is measured by the g_1 statistic.

MP topologies were generated using equal character weighting, Fitch parsimony, ACCTRAN optimization, heuristic search, random stepwise addition sequence (2500 replicates or until memory was exhausted), tree bisection-reconnection (TBR) branch swapping, and MulTrees. Multiple equally parsimonious trees were summarized using strict consensus. To account for transition saturation and/or bias within the data, a weighted MP analysis was also performed. The transition:transversion ratio (ti/tv) was estimated from the observed data in MacClade 3.05 (Maddison and Maddison 1992) and PAUP* 4.0b10 (Swofford 2002), and that value was subsequently used to weight transversions over transitions in the aforementioned weighted MP analysis.

The distance matrix was constructed using the Kimura 2-parameter distance correction (Kimura 1981) and analyzed using neighbor-joining in PAUP* 4.0b10 (Swofford 2002). The correction algorithm was determined from the base frequencies and types of transitions and transversions determined from the data. Base frequencies were empirically determined in PAUP* 4.0b10 (Swofford 2002). Types of transitions and transversions were determined by graphing the substitution matrix in MacClade 3.05 (Maddison and Maddison 1992).

To find a ML (Felsenstein 1981) tree in PAUP* 4.0b10 (Swofford 2002), a heuristic search with tree bisection-reconnection (TBR) branch swapping on the NJ topology found previously and MulTrees was performed with an appropriate rate substitution model, appropriate base frequencies, an appropriate proportion of invariable sites, and an appropriate shape parameter (α) for the gamma distribution for rates among variable sites. Due to time constraints, these parameters are often estimated using an iterative approximation method (Swofford et al. 1996); this was done so here. In PAUP*

4.0b10 (Swofford 2002), an initial ML analysis was performed using a heuristic search (the starting tree was the neighbor-joining tree found using the entire data matrix, branches were swapped via TBR, MulTrees) and the following defaults, unless that parameter was being estimated: ti rate $\neq$ tv rate (“2ST”) set to 2; base frequencies (as determined in PAUP* 4.0b10 [Swofford 2002], see above) set to A= 0.21562, C = 0.27602, G = 0.2854, T = 0.22296; proportion of invariable sites set to 0; and equal rates for among-site rate variation. Each search was aborted after a score was found. Another ML analysis was performed using a heuristic search (the starting tree was the neighbor-joining tree found using the entire data matrix, branches swapped via TBR, MulTrees) and the four parameters estimated in the previous four ML analyses. This search was allowed to continue until completion. The resulting tree was saved to memory. Again, four ML analyses were performed using a heuristic search (*the starting tree was the one saved from the analysis using estimated parameters*, branches swapped via TBR, MulTrees) and the “defaults” unless that parameter was being estimated. In these estimations the defaults were determined from the resultant tree from the analysis using estimated parameters. Each search was aborted after a score was found. Another ML analysis was performed using a heuristic search (*the starting tree was the one saved from the analysis using estimated parameters*, branches swapped via TBR, MulTrees) and the four parameters estimated in the previous four ML analyses. This search was allowed to continue until completion. The resulting tree was saved to memory and was used as the starting point for another search. This process was repeated until the parameter values stabilized. Due to time constraints, the iterative estimation was stopped, with sixteen iterations completed, before values stabilized.

Parameters necessary to find a ML tree were also determined using Modeltest 3.06 (Posada and Crandall 1998). A model of evolution, with its attendant parameters, was selected using Modeltest 3.06 (Posada and Crandall 1998), which implements two approaches to comparing models. Likelihood scores, calculated for 56 models in PAUP* 4.0b10 (Swofford 2002) from the NJ tree derived from all characters, were compared in Modeltest 3.06 (Posada and Crandall 1998) via hierarchical likelihood ratio tests (hLRTs) (Huelsenbeck and Crandall 1997). The other approach was the Akaike information criterion (AIC). For a full review, see Posada and Crandall 2001. Values for parameters determined in Modeltest 3.06 (Posada and Crandall 1998) were then used as input to the ML settings in PAUP* 4.0b10 (Swofford 2002) to find the ML tree (see above).

Bootstrapping (Felsenstein 1985) and jackknifing were used to assess support for individual nodes on MP and NJ topologies. Bootstrap (BS) and jackknife (JK) values were estimated from 2500 replicates (or until memory was exhausted) using Fitch parsimony, ACCTRAN optimization, heuristic search, random stepwise addition sequence (1 replicate), TBR branch swapping, and MulTrees in PAUP* 4.0b10 (Swofford 2002). Uninformative characters were excluded, due to time constraints, from the 5.8S bootstrap, 5.8S jackknife, and ITS2 jackknife analyses. Decay indices (DI) were calculated, via AutoDecay 4.0.1 (Eriksson 1998) and PAUP* 4.0b10 (Swofford 2002) from 100 replicates, to assess support for individual clades on the equal weights MP topologies (Bremer 1988). Quartet puzzling was performed to assess support for clades on the ML topology (Strimmer and von Haeseler 1996); 2500 puzzling steps were used in PAUP* 4.0b10 (Swofford 2002). Bootstrapping was used as secondary support measure on the ML topology; BS values were estimated from 2500 replicates using a heuristic

search with tree bisection-reconnection (TBR) branch swapping on the NJ topology found previously and MulTrees was performed with an appropriate rate substitution model, appropriate base frequencies, an appropriate proportion of invariable sites, and an appropriate shape parameter (α) for the gamma distribution for rates among variable sites in PAUP* 4.0b10 (Swofford 2002).

Where appropriate, tests to determine if two trees were significantly different and to determine if one tree was significantly favored over another were performed. The compare-2 permutation test was performed with 2500 replicates using Fitch parsimony, ACCTRAN optimization, heuristic search, random stepwise addition sequence (1 replicate), TBR branch swapping, and MulTrees in PAUP* 4.0b10 (Swofford 2002). The Kishino-Hasegawa test was also performed in PAUP* 4.0b10 (Swofford 2002).

RESULTS

DNA Extraction and PCR. DNA was extracted successfully from 143 samples, 38 via the modified Sambrook et al. (1989) and 105 via the modified Kang et al. (1998) protocol (see Table 5). One hundred twenty-one successful amplifications were performed. Sambrook-extractions were viscous and green to light brown in color, while Kang-extractions were less viscid and clear in color. DNA extracted via Sambrook et al. (1989) appeared more degraded than DNA extracted via Kang et al. (1998) because little, if any, high molecular weight DNA was visualized (see Fig. 1). This degradation seemed to lead to difficulty in amplification; however, PCR was reattempted (many times in some cases) for troublesome Sambrook-extracted DNA samples. In many instances, this persistence was rewarded, and, as a result, the success rate for PCR of Sambrook-extracted DNA (92.1%, see Table 5) may be inflated. Reattempting PCR for troublesome Kang-extracted DNA was constrained by time. Amplification reactions using a small volume of DNA (0.1 μ l in 50 μ l total volume) generally resulted in greater PCR success.

Cycle Sequencing. Successful sequences from all four primer reads were obtained for 103 individual plants; see Table 5 for numbers of individuals sampled per taxon. Out of these 103 individuals, 31 were subsequently aligned and analyzed; that is, where all sequences in a taxon were identical, only one was analyzed. A genotype occurred in more

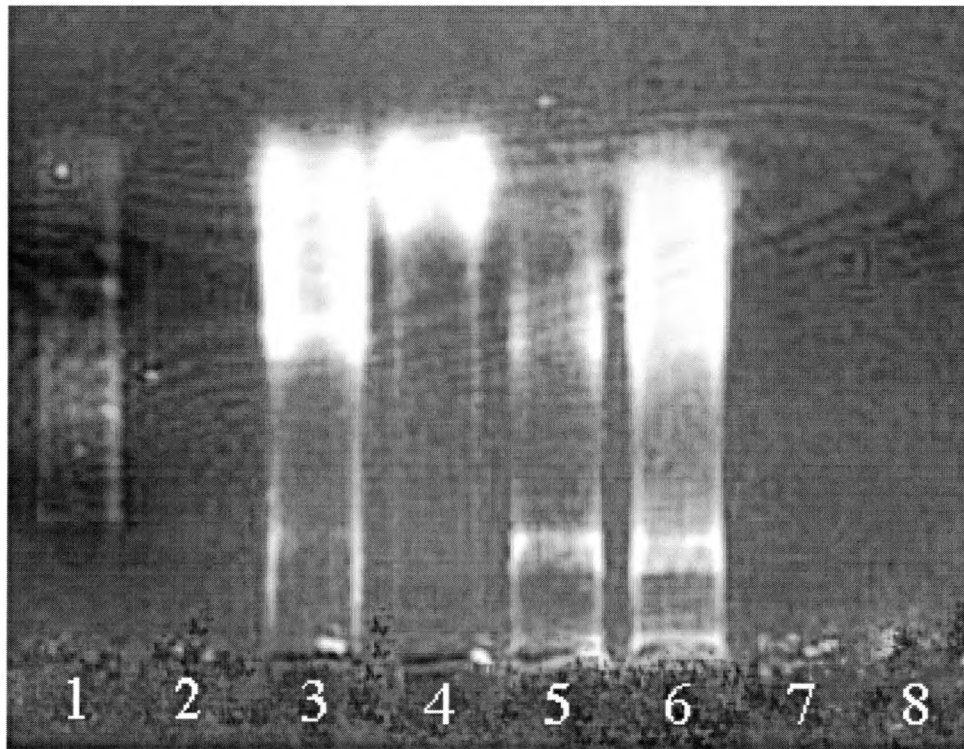


FIG. 1. Photograph of agarose mini-gel under UV light after electrophoresis. Numbers represent lane numbers. Lane 1: DNA size marker; Lanes 2 & 8: empty; Lanes 3 & 4: DNA extracted via modified Sambrook et al. 1989 protocol; Lanes 5-7: DNA extracted via modified Kang et al. 1998 protocol; Lanes 3, 4, & 7: DNA extracted from leaf tissue; Lanes 5 & 6: DNA extracted from seed tissue. All DNA samples on this gel, including that in Lane 7, amplified successfully. High molecular weight DNA is visible in Lanes 5 & 6.

than one species in two instances. The genotypes of *A. angustifolia*, *A. macrocarpa*, and one *A. fragrans* (DJM081) were identical. The genotypes of the *Abronia umbellata*, *A. villosa*, and *A. villosa* var. *aurita* individuals were identical. In the final alignment and phylogenetic analyses, all genotypes and taxa were represented; see Table 3 for numbers of individuals sequenced per genotype/taxon. In all, 28 unique genotypes were detected.

Phylogenetic Analyses. Aligned length of the data matrix was 736 nucleotides. Consensus sequences ranged from 673 to 695 nucleotides in length. See Table 6 for a summary of statistics of the data matrix and subsets. The data matrix included 400

TABLE 5. List of taxa sampled, taxon abbreviations, number of successful extractions by extraction protocol, number of successful amplifications by extraction protocol (Sambrook et al. 1989 or Kang et al. 1998), and number of individuals sequenced. Percent success of PCR is also given. Numbers after abbreviations indicate DJM sample # (see Appendix 1).

Taxon	Abbreviation	Extracted		Amplified		Sequenced
		Sambrook	Kang	Sambrook	Kang	
<i>Abronia ameliae</i>	Abr ame 050	9	1	9	1	9
<i>Abronia angustifolia</i>	Abr ang 173	n/a	1	n/a	1	1
<i>Abronia fragrans</i>	Abr fra 081, 002	5	12	5	11	11
<i>Abronia macrocarpa</i>	Abr mac 008	18	1	17	1	15
<i>Abronia umbellata</i>	Abr umb 144	n/a	10	n/a	5	5
<i>Abronia villosa</i>	Abr vil 080	n/a	17	n/a	12	6
<i>Abronia villosa</i> var <i>aurita</i>	Abr vil aur 154	n/a	8	n/a	7	6
<i>Acleisanthes chenopodioides</i>	Acl che 115	n/a	2	n/a	2	2
<i>Acleisanthes longiflora</i>	Acl lon 138	n/a	3	n/a	3	3
<i>Alltonia incarnata</i>	All inc 132	n/a	2	n/a	2	2
<i>Anulocaulis eriosolenus</i>	Anu eri 137	n/a	1	n/a	1	1
<i>Anulocaulis leiosolenus</i>	Anu lei 174	n/a	1	n/a	1	1
<i>Boerhavia coccinea</i>	Boe coc 141	n/a	1	n/a	1	1
<i>Boerhavia erecta</i>	Boe ere 073	n/a	1	n/a	1	1
<i>Boerhavia gracilluma</i>	Boe gra 136	n/a	1	n/a	1	1
<i>Boerhavia intermedia</i>	Boe int 134	n/a	1	n/a	1	1
<i>Boerhavia spicata</i>	Boe spi 133	n/a	2	n/a	2	2
<i>Bougainvillea</i> sp	Bou sp 172	n/a	1	n/a	1	1
<i>Cyphomeris gypsophiloides</i>	Cyp gyp 176	n/a	1	n/a	1	1
<i>Mirabilis jalapa</i>	Mir jal 001, 003, 071	5	1	3	1	4
<i>Mirabilis longiflora</i>	Mir lon 076	n/a	7	n/a	7	7
<i>Mirabilis multiflora</i>	Mir mul 066	1	7	1	7	8
<i>Nyctaginia capitata</i>	Nyc cap 121	n/a	3	n/a	3	3
<i>Phytolacca americana</i>	Phy ame 179	n/a	1	n/a	1	1
<i>Pisonia aculeata</i>	Pis acu 177	n/a	1	n/a	1	1
<i>Rivina humilis</i>	Riv hum 178	n/a	1	n/a	1	1
<i>Tripterocalyx carnea</i>	Tri car 060	6	n/a	6	n/a	4
<i>Tripterocalyx mucranthus</i>	Tri mic 099	n/a	11	n/a	4	4
Totals		38	105	35	86	103
		143		121		
PCR success				92 1%	81 9%	
				84 6%		

constant characters; 254 characters were parsimony informative. Uncorrected and Kimura 2-parameter pairwise distances are given in Table 7; uncorrected distances ranged from 0% to 26.3% for all taxa pairs and 0%-24.0% between ingroup members. Interspecific pairwise distances were 0%-1.6% for *Abronia* (average 0.9%), 3.7% *Acleisanthes*, 2.2% *Anulocaulis*, 0.3%-5.9% *Boerhavia* (average 3.8%), 0.7%-2.8% *Mirabilis* (average 1.9%), and 0.1% *Tripterocalyx*. The average interspecific distance was 1.7%. The treelength distribution from 10,000 random trees is shown in Fig. 2; it was left skewed with a g_1 of -0.58932 ($P < 0.01$). This suggests the distribution of all trees differs significantly from a distribution derived from random data, and hence the dataset has phylogenetic signal (Hillis and Huelsenbeck 1992). The partition homogeneity test was aborted after five replicates due to memory constraints; results from this test ($P = 1$) indicate that the subsets were congruent and, thus, should be analyzed as one dataset. Nonetheless, subset analyses were performed to examine putative effects of each gene region.

Maximum Parsimony Analyses. Equal weights maximum parsimony (MP) analysis of all characters resulted in three equally parsimonious topologies, each with a treelength (TL) of 795. For each tree, the consistency index (CI) was 0.648, the retention index (RI) was 0.827, and the rescaled consistency index (RC) was 0.536. The strict consensus tree of these three topologies is presented in Fig. 3a; values from bootstrap (BS), jackknife (JK), and decay index (DI) analyses are presented on that topology (see Fig. 3 legend for explanation). A plot of uncorrected pairwise distances versus absolute distances (Fig. 4) indicated that saturation of transitions occurred at higher pairwise distances; the transition: transversion ratio (ti/tv) was about 1.5. Ti/tv weighted

TABLE 6. Summary statistics for the entire data matrix of the ITS region of nrDNA and gene subsets (18S, ITS1, 5.8S, ITS2, and 26S). ^a Determined using the equal weights MP strict consensus tree. ^b Parentheses enclose value for ti/tv weighted MP.

	Entire data matrix	18S	ITS1	5.8S	ITS2	26S
Character positions in matrix	1-736	1-33	34-284	285-448	449-685	686-736
Range of raw length	673-695	33	220-231	162-164	204-219	51
Aligned length	736	33	251	164	237	51
# of constant sites	400 (54.3%)	33	86	150	86	45
# of variable sites	336 (45.7%)	0	165	14	151	6
# of parsimony informative sites	254 (34.5%)	0	130	9	110	5
Base frequencies						
A	0.21562	0.21212	0.22370	0.24896	0.17201	0.25237
C	0.27602	0.18182	0.29299	0.26377	0.28331	0.27135
G	0.28540	0.33333	0.28129	0.27423	0.29763	0.25870
T	0.22296	0.27273	0.20202	0.21303	0.24704	0.21758
G + C content	56.142%	51.515%	57.428%	53.800%	58.094%	53.005%
T ₁ (minimum) ^a	451	n/a	n/a	n/a	n/a	n/a
T _v (minimum) ^a	344	n/a	n/a	n/a	n/a	n/a
T ₁ /t _v	1.5	n/a	n/a	n/a	n/a	n/a
Uncorrected pairwise distances	0%-26.3%	0%-0%	0%-41.6%	0%-6.1%	0%-39.7%	0%-7.8%
Length of most parsimonious tree(s) ^b	795 (957.0)	0	429	22	328	7
# of equally parsimonious trees ^b	3 (6)	1	8	6	18	5
Consistency index ^b	0.648 (0.646)	n/a	0.634	0.818	0.668	0.857
Retention index ^b	0.827 (0.828)	n/a	0.815	0.892	0.849	0.933
Rescaled consistency index ^b	0.536 (0.535)	n/a	0.517	0.730	0.567	0.800
<i>g</i> ₁	-0.58932	n/a	n/a	n/a	n/a	n/a

(weighting transversions over transitions according to transition saturation [$t_i = 1$ and $t_v = 1.5$]) MP analysis resulted in six equally parsimonious trees (TL = 957, CI = 0.646, RI = 0.828, RC = 0.535); the strict consensus tree is shown in Fig. 5a. Ti/tv weighted jackknife analysis was aborted after 2407 replicates due to memory constraints. Values from ti/tv weighted BS and JK analyses are shown on Fig. 5a (see legend for explanation).

TABLE 7. Distance matrix constructed from analysis of 736 nucleotides of ITS nrDNA. Uncorrected distances are below the diagonal and Kimura 2-parameter distances are above. Abbreviations of taxa are (see also Table 5): Abr ame = *Abronia ameliae*, Abr ang = *A. angustifolia*, Abr fra = *A. fragrans*, Abr mac = *A. macrocarpa*, Abr umb = *A. umbellata*, Abr vil = *A. villosa*, Abr vil aur = *A. villosa* var. *aurita*, Acl che = *Acleisanthes chenopodioides*, Acl lon = *A. longiflora*, All inc = *Allionia incarnata*, Anu eri = *Anulocaulis eriosolenus*, Anu lei = *A. leiosolenus*, Boe coc = *Boerhavia coccinea*, Boe ere = *B. erecta*, Boe gra = *B. gracillima*, Boe int = *B. intermedia*, Boe spi = *B. spicata*, Bou sp. = *Bougainvillea* sp., Cyp gyp = *Cyphomeris gypsophiloides*, Mir jal = *Mirabilis jalapa*, Mir lon = *M. longiflora*, Mir mul = *M. multiflora*, Nyc cap = *Nyctaginia capitata*, Phy ame = *Phytolacca americana*, Pis acu = *Pisonia aculeata*, Riv hum = *Rivina humilis*, Tri car = *Tripterocalyx carnea*, Tri mic = *T. micranthus*, Numbers following taxa indicate DJM sample numbers.

	1	2	3	4	5	6	7	8	9	10	11
1 Riv hum 178	-	0.214	0.219	0.255	0.309	0.280	0.277	0.277	0.275	0.276	0.299
2 Phy ame 179	0.185	-	0.151	0.188	0.294	0.294	0.292	0.283	0.278	0.280	0.285
3 Pis acu 177	0.189	0.136	-	0.139	0.256	0.243	0.245	0.238	0.239	0.240	0.213
4 Bou sp 172	0.215	0.165	0.125	-	0.290	0.285	0.288	0.281	0.286	0.285	0.257
5 All inc 132	0.252	0.239	0.214	0.237	-	0.164	0.164	0.160	0.156	0.156	0.117
6 Boe ere 073	0.233	0.242	0.207	0.235	0.146	-	0.003	0.010	0.061	0.062	0.144
7 Boe spi 133	0.231	0.241	0.208	0.237	0.146	0.003	-	0.010	0.061	0.062	0.144
8 Boe int 134	0.231	0.234	0.204	0.232	0.143	0.010	0.010	-	0.057	0.059	0.138
9 Boe gra 136	0.229	0.231	0.204	0.235	0.139	0.058	0.058	0.055	-	0.007	0.133
10 Boe coc 141	0.231	0.233	0.205	0.235	0.139	0.059	0.059	0.056	0.007	-	0.134
11 Cyp gyp 176	0.246	0.234	0.184	0.215	0.108	0.128	0.128	0.124	0.119	0.121	-
12 Anu eri 137	0.247	0.234	0.196	0.222	0.114	0.136	0.136	0.133	0.129	0.131	0.052
13 Anu lei 174	0.254	0.241	0.198	0.219	0.120	0.145	0.145	0.143	0.136	0.138	0.058
14 Nyc cap 121	0.249	0.239	0.202	0.224	0.123	0.139	0.139	0.135	0.131	0.133	0.053
15 Acl che 115	0.251	0.234	0.193	0.216	0.153	0.169	0.169	0.168	0.160	0.162	0.103
16 Acl lon 138	0.252	0.236	0.196	0.224	0.147	0.159	0.159	0.160	0.155	0.156	0.097
17 Abr fra 081	0.260	0.231	0.199	0.236	0.168	0.180	0.183	0.181	0.172	0.172	0.131
18 Abr ame 050	0.258	0.230	0.199	0.236	0.170	0.181	0.184	0.183	0.173	0.173	0.133
19 Abr ang 173	0.260	0.231	0.199	0.236	0.168	0.180	0.183	0.181	0.172	0.172	0.131
20 Abr mac 008	0.260	0.231	0.199	0.236	0.168	0.180	0.183	0.181	0.172	0.172	0.131
21 Abr fra 002	0.261	0.233	0.198	0.234	0.167	0.181	0.184	0.183	0.173	0.173	0.130
22 Abr umb 144	0.261	0.231	0.205	0.238	0.176	0.185	0.188	0.187	0.176	0.176	0.141
23 Abr vil 080	0.261	0.231	0.205	0.238	0.176	0.185	0.188	0.187	0.176	0.176	0.141
24 Abr vil aur 154	0.263	0.233	0.207	0.240	0.177	0.186	0.189	0.187	0.177	0.177	0.141
25 Tri car 060	0.262	0.239	0.198	0.233	0.174	0.172	0.172	0.174	0.175	0.176	0.134
26 Tri mic 099	0.262	0.239	0.198	0.233	0.173	0.171	0.174	0.172	0.173	0.175	0.133
27 Mir jal 001	0.240	0.222	0.190	0.224	0.148	0.152	0.152	0.148	0.148	0.150	0.101
28 Mir jal 003	0.242	0.220	0.192	0.222	0.150	0.154	0.154	0.149	0.150	0.151	0.102
29 Mir jal 071	0.242	0.222	0.187	0.221	0.148	0.155	0.155	0.151	0.151	0.153	0.104
30 Mir lon 076	0.239	0.224	0.189	0.225	0.147	0.155	0.155	0.151	0.145	0.147	0.099
31 Mir mul 066	0.235	0.228	0.183	0.220	0.144	0.157	0.157	0.153	0.153	0.153	0.092

TABLE 7 continued.

	12	13	14	15	16	17	18	19	20	21	22	23	24
1	0.302	0.313	0.305	0.310	0.310	0.320	0.318	0.320	0.320	0.323	0.323	0.323	0.325
2	0.284	0.294	0.292	0.286	0.289	0.279	0.276	0.279	0.279	0.281	0.279	0.279	0.281
3	0.230	0.232	0.237	0.225	0.230	0.234	0.234	0.234	0.234	0.231	0.241	0.241	0.243
4	0.267	0.262	0.270	0.259	0.271	0.287	0.287	0.287	0.287	0.284	0.290	0.290	0.292
5	0.125	0.132	0.136	0.173	0.166	0.192	0.194	0.192	0.192	0.190	0.203	0.203	0.204
6	0.153	0.165	0.157	0.195	0.182	0.208	0.210	0.208	0.208	0.210	0.216	0.216	0.216
7	0.153	0.165	0.157	0.195	0.182	0.212	0.214	0.212	0.212	0.214	0.220	0.220	0.221
8	0.149	0.163	0.151	0.193	0.183	0.210	0.212	0.210	0.210	0.212	0.218	0.218	0.219
9	0.145	0.154	0.148	0.183	0.176	0.198	0.200	0.198	0.198	0.200	0.203	0.203	0.204
10	0.146	0.156	0.149	0.184	0.177	0.197	0.199	0.197	0.197	0.199	0.203	0.203	0.203
11	0.054	0.061	0.056	0.111	0.104	0.145	0.147	0.145	0.145	0.144	0.157	0.157	0.158
12	-	0.022	0.027	0.111	0.110	0.162	0.164	0.162	0.162	0.160	0.173	0.173	0.173
13	0.022	-	0.030	0.113	0.112	0.164	0.166	0.164	0.164	0.162	0.173	0.173	0.173
14	0.027	0.030	-	0.121	0.116	0.161	0.163	0.161	0.161	0.160	0.172	0.172	0.172
15	0.103	0.104	0.111	-	0.038	0.146	0.148	0.146	0.146	0.148	0.151	0.151	0.151
16	0.102	0.103	0.107	0.037	-	0.123	0.125	0.123	0.123	0.125	0.130	0.130	0.130
17	0.145	0.146	0.144	0.132	0.113	-	0.001	0.000	0.000	0.001	0.015	0.015	0.015
18	0.147	0.148	0.146	0.134	0.115	0.001	-	0.001	0.001	0.003	0.016	0.016	0.016
19	0.145	0.146	0.144	0.132	0.113	0.000	0.001	-	0.000	0.001	0.015	0.015	0.015
20	0.145	0.146	0.144	0.132	0.113	0.000	0.001	0.000	-	0.001	0.015	0.015	0.015
21	0.144	0.145	0.143	0.134	0.115	0.001	0.003	0.001	0.001	-	0.016	0.016	0.016
22	0.153	0.153	0.153	0.136	0.119	0.015	0.016	0.015	0.015	0.016	-	0.000	0.000
23	0.153	0.153	0.153	0.136	0.119	0.015	0.016	0.015	0.015	0.016	0.000	-	0.000
24	0.154	0.153	0.153	0.137	0.119	0.015	0.016	0.015	0.015	0.016	0.000	0.000	-
25	0.145	0.145	0.141	0.128	0.112	0.067	0.068	0.067	0.067	0.068	0.070	0.070	0.070
26	0.144	0.144	0.140	0.126	0.110	0.065	0.067	0.065	0.065	0.067	0.068	0.068	0.068
27	0.107	0.115	0.109	0.104	0.100	0.140	0.141	0.140	0.140	0.141	0.143	0.143	0.143
28	0.105	0.113	0.108	0.106	0.102	0.141	0.143	0.141	0.141	0.143	0.145	0.145	0.145
29	0.110	0.117	0.112	0.107	0.102	0.141	0.143	0.141	0.141	0.143	0.145	0.145	0.145
30	0.105	0.111	0.107	0.101	0.099	0.140	0.141	0.140	0.140	0.141	0.143	0.143	0.143
31	0.097	0.107	0.101	0.101	0.100	0.138	0.140	0.138	0.138	0.139	0.140	0.140	0.141

Distance Analysis. The appropriate distance correction algorithm was determined empirically from the data to meet the Kimura 2-parameter measure. Neighbor-joining (NJ) analysis of the Kimura 2-parameter distance matrix resulted in the topology shown in Fig. 3b (TL = 801, CI = 0.643, RI = 0.824, RC = 0.530); values from BS and JK analyses are presented on Fig. 3a (see Fig. 3 legend for explanation).

TABLE 7 continued.

	25	26	27	28	29	30	31
1	0.324	0.324	0.291	0.294	0.294	0.289	0.283
2	0.291	0.291	0.266	0.264	0.266	0.270	0.276
3	0.232	0.232	0.221	0.223	0.217	0.220	0.212
4	0.284	0.284	0.268	0.266	0.264	0.271	0.263
5	0.202	0.200	0.168	0.170	0.168	0.166	0.162
6	0.199	0.197	0.172	0.174	0.176	0.177	0.180
7	0.199	0.201	0.172	0.174	0.176	0.177	0.180
8	0.201	0.199	0.167	0.169	0.170	0.171	0.174
9	0.203	0.201	0.167	0.169	0.171	0.163	0.173
10	0.204	0.202	0.169	0.171	0.172	0.165	0.173
11	0.150	0.148	0.108	0.110	0.112	0.107	0.098
12	0.163	0.161	0.116	0.114	0.119	0.114	0.105
13	0.163	0.161	0.125	0.123	0.128	0.121	0.116
14	0.158	0.157	0.118	0.117	0.122	0.117	0.109
15	0.141	0.139	0.112	0.114	0.116	0.109	0.109
16	0.122	0.120	0.108	0.110	0.110	0.106	0.108
17	0.070	0.069	0.155	0.157	0.157	0.155	0.153
18	0.072	0.070	0.157	0.159	0.159	0.157	0.155
19	0.070	0.069	0.155	0.157	0.157	0.155	0.153
20	0.070	0.069	0.155	0.157	0.157	0.155	0.153
21	0.072	0.070	0.157	0.159	0.159	0.157	0.155
22	0.074	0.072	0.160	0.162	0.161	0.160	0.156
23	0.074	0.072	0.160	0.162	0.161	0.160	0.156
24	0.074	0.072	0.160	0.162	0.162	0.160	0.156
25	-	0.001	0.148	0.150	0.150	0.148	0.144
26	0.001	-	0.146	0.148	0.148	0.146	0.142
27	0.133	0.132	-	0.001	0.003	0.007	0.026
28	0.135	0.133	0.001	-	0.004	0.009	0.027
29	0.135	0.133	0.003	0.004	-	0.010	0.029
30	0.133	0.132	0.007	0.009	0.010	-	0.024
31	0.130	0.129	0.025	0.027	0.028	0.024	-

Maximum Likelihood Analyses. The Akaike information criterion (AIC) and hierarchical likelihood rates tests (hLRTs) as implemented in Modeltest 3.06 (Posada and Crandall 1998) selected different models of evolution: GTR+I+G (general time reversible plus invariable sites and gamma distribution) and TrN+I+G (Tamura and Nei plus invariable sites and gamma distribution) respectively. Maximum likelihood (ML) heuristic searching from the Kimura 2-parameter NJ tree using the GTR+I+G model and using the TRN+I+G model were performed. According to a compare-2 permutation test

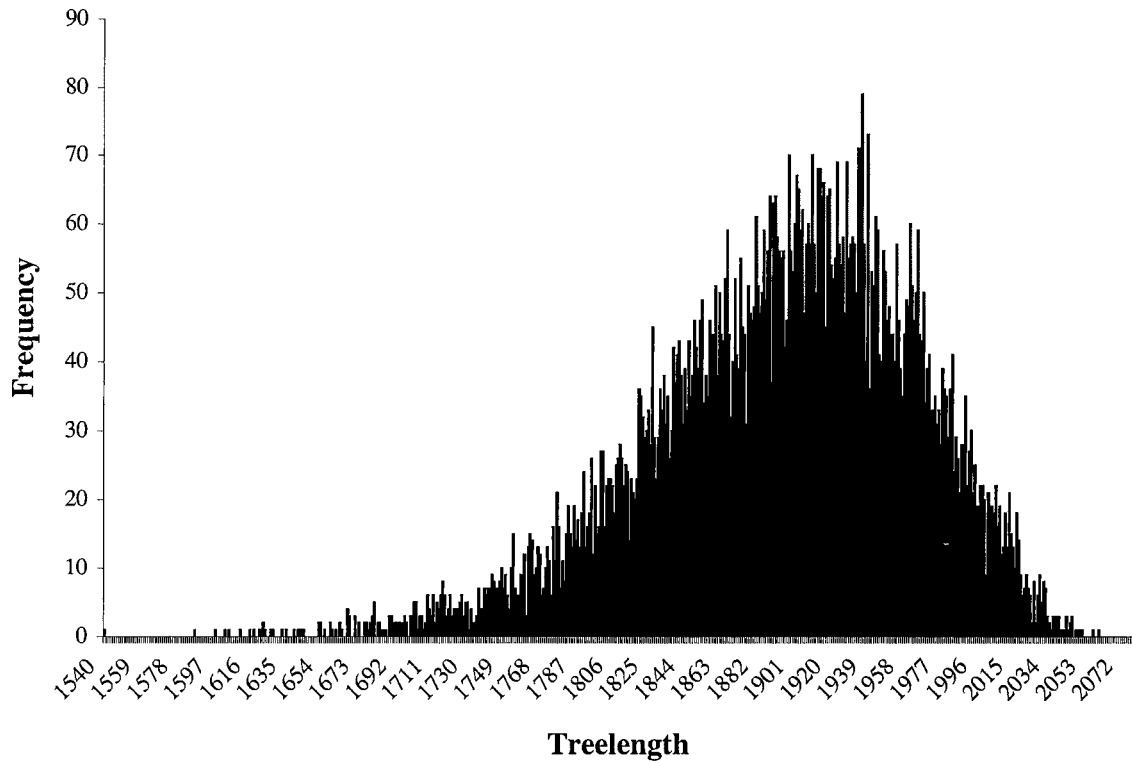


FIG. 2. The treelength distribution of 10,000 random trees generated from the entire data matrix. $g_1 = -5.8932$, $P < 0.01$.

($P = 0.979600$), these topologies were not significantly different. The TrN+I+G model was chosen as the model of evolution for this dataset for several reasons. Posada and Crandall (2001) report that when the gamma distribution shape parameter is small, most sites are evolving slowly, as in the data presented here. The TrN+I+G model had a smaller gamma shape parameter ($\alpha = 1.9550$; GTR+I+G $\alpha = 2.5270$). The ML method generally finds one tree for any given dataset; here, three topologies were found using the GTR+I+G model of evolution and one using the TrN+I+G model. Posada and Crandall (2001) report that in their study, the AIC seemed to slightly favor more complex models; here, the AIC selected the GTR+I+G model of evolution. Lastly, according to the Kishino-Hasegawa test, the TrN+I+G tree was a more appropriate tree than the

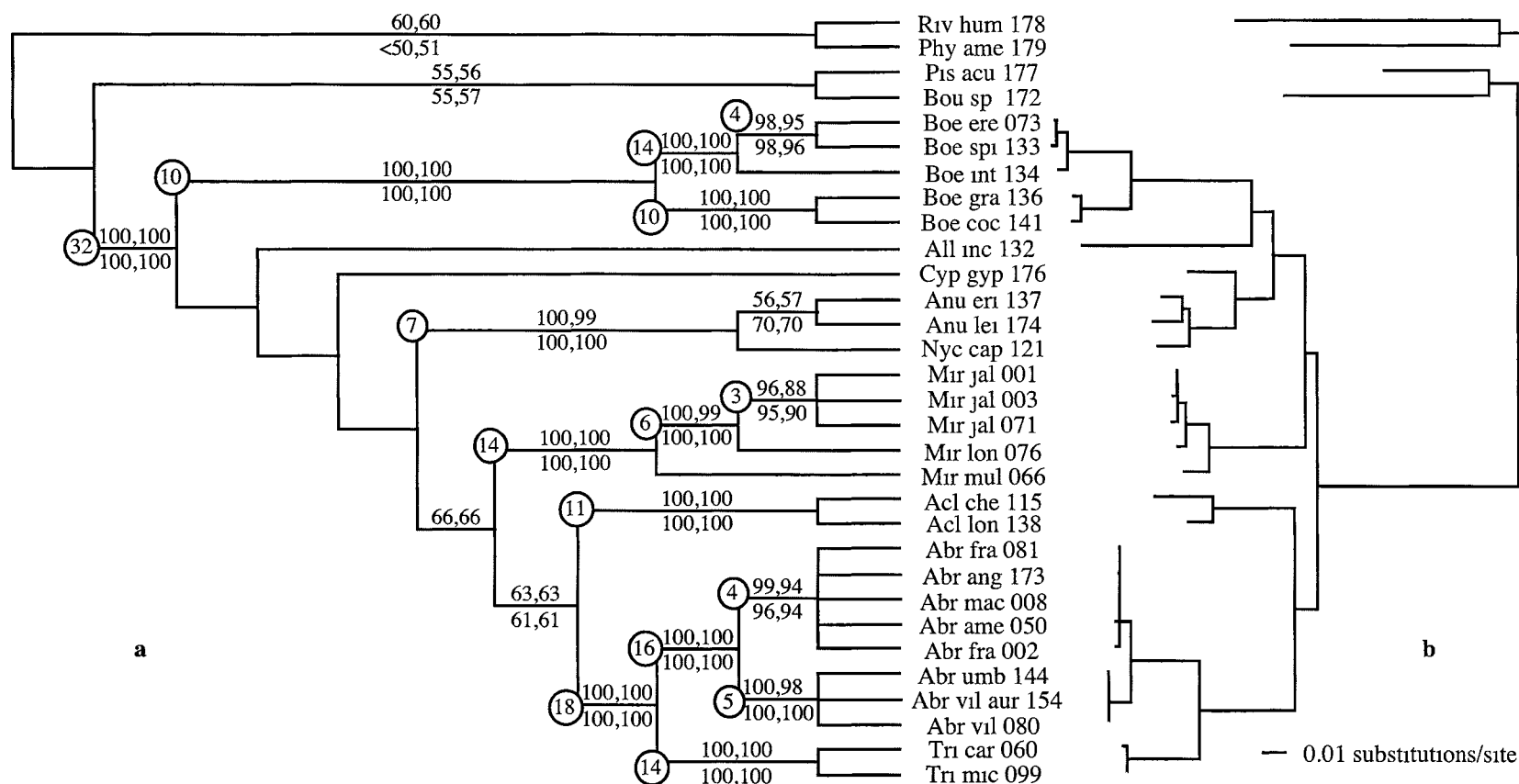


FIG. 3. (a) Equal weights maximum parsimony (MP) strict consensus tree; 2500 random-addition sequence replicates and TBR branch swapping were used in a heuristic search. Numbers above branches indicate bootstrap (BS), jackknife (JK) values from 2500 replicates of heuristic searching. Decay index (DI) values are shown in circles. (b) Neighbor-joining (NJ) tree using Kimura 2-parameter distance matrix. Branch lengths are proportional to number of nucleotide substitutions. BS, JK values from 2500 replicates of NJ are below branches on the MP strict consensus tree (a). Abbreviations of taxa are (see also Table 5). Abr ame = *Abronia ameliae*, Abr ang = *A. angustifolia*, Abr fra = *A. fragrans*, Abr mac = *A. macrocarpa*, Abr umb = *A. umbellata*, Abr vil = *A. villosa*, Abr vil aur = *A. villosa* var. *aurita*, Acl che = *Acleisanthes chenopodioides*, Acl lon = *A. longiflora*, All inc = *Allionia incarnata*, Anu eri = *Anulocaulis eriosolenus*, Anu lei = *A. leiosolenus*, Boe coc = *Boerhavia coccinea*, Boe ere = *B. erecta*, Boe gra = *B. gracillima*, Boe int = *B. intermedia*, Boe spi = *B. spicata*, Bou sp = *Bougainvillea* sp., Cyp gyp = *Cyphomeris gypsophiloides*, Mir jal = *Mirabilis jalapa*, Mir lon = *M. longiflora*, Mir mul = *M. multiflora*, Nyc cap = *Nyctaginia capitata*, Phy ame = *Phytolacca americana*, Pis acu = *Pisonia aculeata*, Riv hum = *Rivina humilis*, Tri car = *Tripterocalyx carnea*, Tri mic = *T. mucranthus*. Numbers following taxa indicate DJM sample numbers.

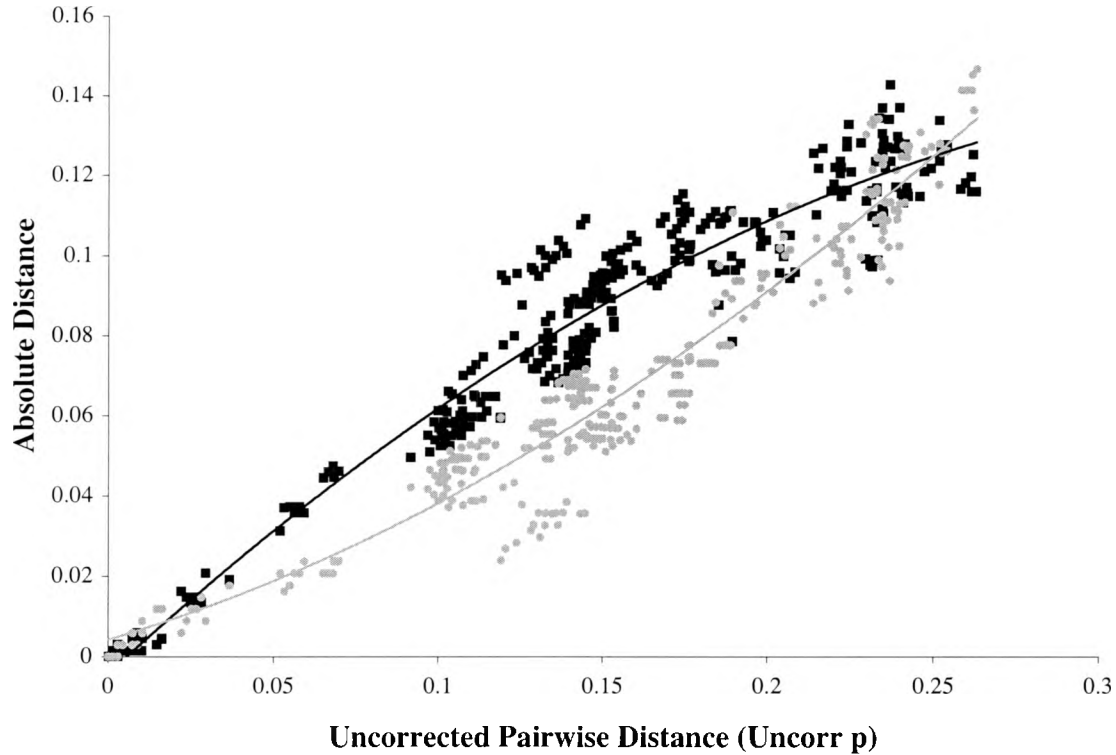


FIG. 4. Uncorrected transitions (dark squares) and transversions (pale circles) plotted against absolute distance. Saturation of transitions is observable at pairwise distances greater than 0.2. Saturation of transversions does not occur.

GTR+I+G tree ($P = 0.0252$). The base frequencies associated with the TrN+I+G model of evolution as determined in Modeltest 3.06 (Posada and Crandall 1998) were $A = 0.2181$, $C = 0.2680$, $G = 0.2776$, and $T = 0.2363$. The rate matrix was $R(a) [A-C] = 1.0000$, $R(b) [A-G] = 1.9785$, $R(c) [A-T] = 1.0000$, $R(d) [C-G] = 1.0000$, $R(e) [C-T] = 5.2482$, and $R(f) [G-T] = 1.0000$. The proportion of invariable sites was 0.4046, and the gamma distribution shape parameter (α) was 1.9550. ML heuristic searching from the Kimura 2-parameter NJ tree and using these parameter values of the TRN+I+G model of evolution resulted in the topology ($-\ln L = 4647.65796$, $TL = 797$, $CI = 0.646$, $RI = 0.826$, $RC = 0.534$) presented in Fig. 5b and Fig. 6. BS and quartet puzzling (QP) values are shown on

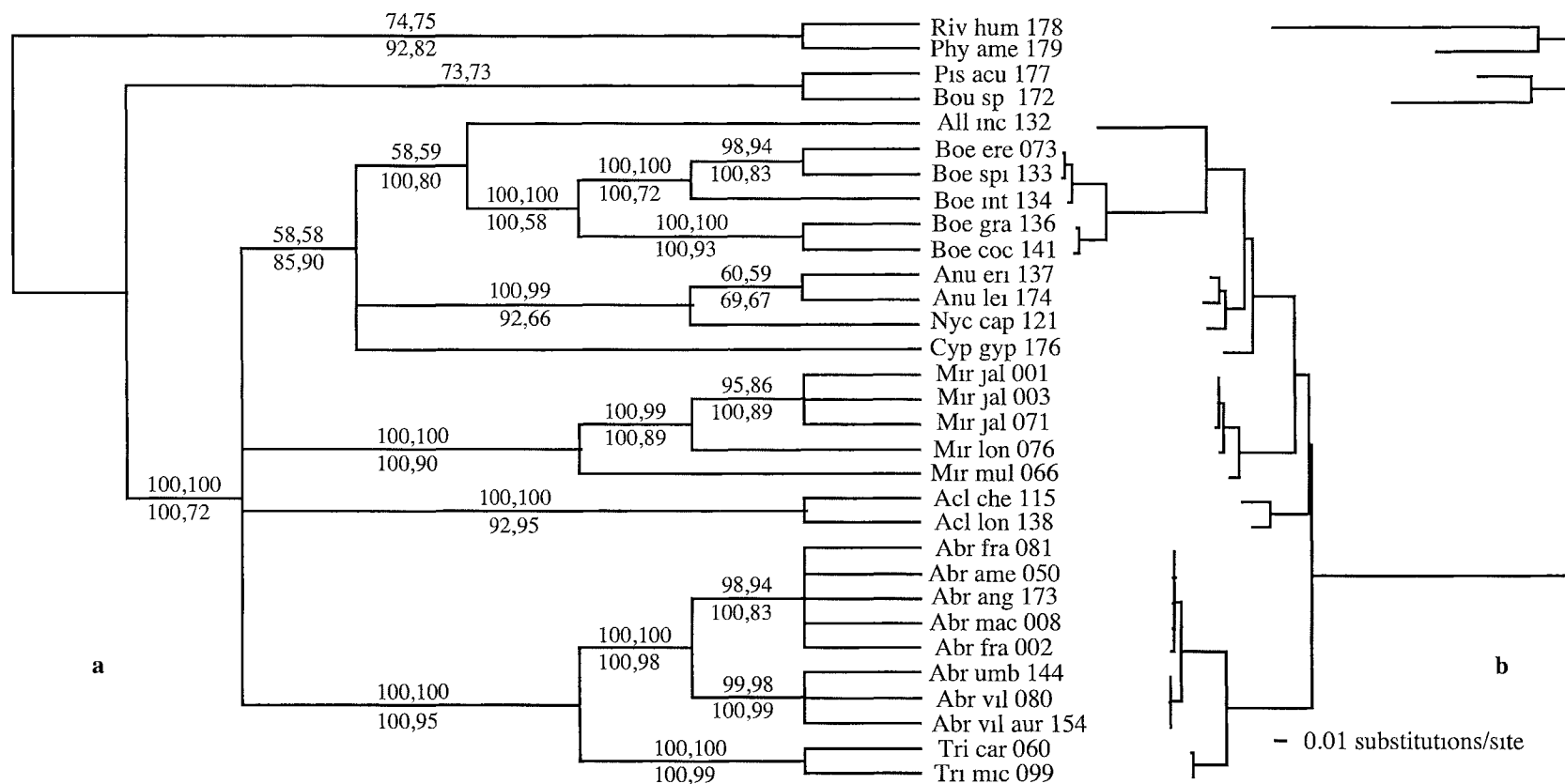


FIG. 5. (a) T_i/t_v weighted maximum parsimony (MP) strict consensus tree, 2500 random-addition sequence replicates and TBR branch swapping were used in a heuristic search. Numbers above branches indicate bootstrap (BS), jackknife (JK) values from 2500 replicates of heuristic searching. (b) Maximum likelihood (ML) tree using TrN+I+G model of evolution. Branch lengths are proportional to number of nucleotide substitutions. BS, quartet puzzling (QP) values from 100 replicates of heuristic searching, 2500 puzzling steps are below branches on the MP strict consensus tree. (a) Abbreviations of taxa are (see also Table 5) Abr ame = *Abronia ameliae*, Abr ang = *A. angustifolia*, Abr fra = *A. fragrans*, Abr mac = *A. macrocarpa*, Abr umb = *A. umbellata*, Abr vil = *A. villosa*, Abr vil aur = *A. villosa* var. *aurita*, Acl che = *Acleisanthes chenopodioides*, Acl lon = *A. longiflora*, All inc = *Allionia incarnata*, Anu eri = *Anulocaulis eriosolenus*, Anu lei = *A. leiosolenus*, Boe coc = *Boerhavia coccinea*, Boe ere = *B. erecta*, Boe gra = *B. gracillima*, Boe int = *B. intermedia*, Boe spi = *B. spicata*, Bou sp = *Bougainvillea* sp., Cyp gyp = *Cyphomeris gypsophuloides*, Mir jal = *Mirabilis jalapa*, Mir lon = *M. longiflora*, Mir mul = *M. multiflora*, Nyc cap = *Nyctaginia capitata*, Phy ame = *Phytolacca americana*, Pis acu = *Pisonia aculeata*, Riv hum = *Rivina humilis*, Tri car = *Tripterocalyx carnea*, Tri mic = *T. micranthus*. Numbers following taxa indicate DJM sample numbers.

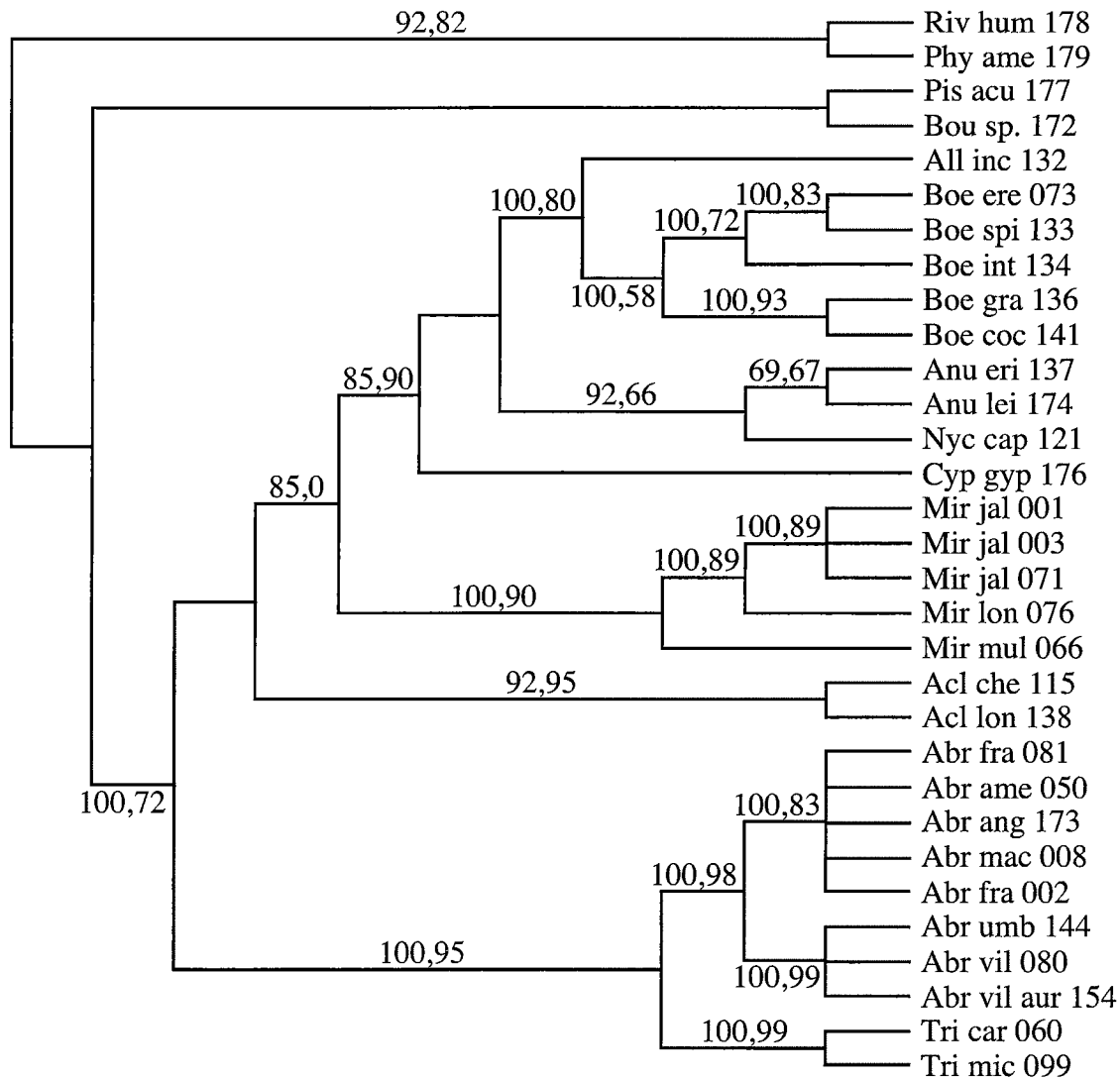


FIG. 6. Maximum likelihood (ML) cladogram derived from the TrN+I+G model of evolution. Bootstrap (BS), quartet puzzling (QP) values from 100 replicates of heuristic searching, 2500 puzzling steps are above or below branches. Abbreviations of taxa are (see also Table 5): Abr ame = *Abronia ameliae*, Abr ang = *A. angustifolia*, Abr fra = *A. fragrans*, Abr mac = *A. macrocarpa*, Abr umb = *A. umbellata*, Abr vil = *A. villosa*, Abr vil aur = *A. villosa* var. *aurita*, Acl che = *Acleisanthes chenopodioides*, Acl lon = *A. longiflora*, All inc = *Allionia incarnata*, Anu eri = *Anulocaulis eriosolenus*, Anu lei = *A. leiosolenus*, Boe coc = *Boerhavia coccinea*, Boe ere = *B. erecta*, Boe gra = *B. gracillima*, Boe int = *B. intermedia*, Boe spi = *B. spicata*, Bou sp. = *Bougainvillea* sp., Cyp gyp = *Cyphomeris gypsophiloides*, Mir jal = *Mirabilis jalapa*, Mir lon = *M. longiflora*, Mir mul = *M. multiflora*, Nyc cap = *Nyctaginia capitata*, Phy ame = *Phytolacca americana*, Pis acu = *Pisonia aculeata*, Riv hum = *Rivina humilis*, Tri car = *Tripterocalyx carnea*, Tri mic = *T. micranthus*. Numbers following taxa indicate DJM sample numbers.

Fig. 5a (see legend for explanation) and Fig. 6. ML bootstrap analysis was aborted after 100 replicates due to time constraints.

Analyses of Entire Data Matrix. Several relationships were common to all four topologies (Figs. 3, 5, and 6). Tribes Nyctagineae (*Acleisanthes*, *Allionia*, *Anulocaulis*, *Boerhavia*, *Cyphomeris*, *Mirabilis*, and *Nyctaginia*) and Abronieae (*Abronia* and *Tripterocalyx*) together formed a monophyletic clade. Neither Subtribe Nyctagininae (*Allionia*, *Mirabilis*, and *Nyctaginia*) nor Subtribe Boerhaviinae (*Acleisanthes*, *Anulocaulis*, *Boerhavia*, and *Cyphomeris*) in Tribe Nyctagineae sensu Bittrich and Kühn 1993 was monophyletic. However, Tribe Abronieae was monophyletic. All respective genera were monophyletic. *Nyctaginia* was the sister group to *Anulocaulis*. *Boerhavia erecta* was most closely related to *B. spicata*. *Boerhavia intermedia* was the nearest neighbor to the *B. erecta*-*B. spicata* clade. *Boerhavia gracillima* was most closely related to *B. coccinea*. *Mirabilis jalapa* was the sister species to *M. longiflora*. *Mirabilis multiflora* was the closest relative of the *M. jalapa*-*M. longiflora* clade. Two distinct clades occurred in *Abronia*. Branch lengths in both the NJ and ML phylograms (Figs. 3b and 5b, respectively) indicate that the genera *Abronia* and *Tripterocalyx* are strongly divergent.

Equal Weights MP vs. Ti/tv Weighted Parsimony. The equal weights MP topology (Fig. 3a) and the ti/tv weighted MP topology (Fig. 5a) were significantly different according to the compare-2 permutation test and the Kishino-Hasegawa test ($P = 0.0021$, equal weights MP strict consensus tree was more appropriate). Specifically, enhanced resolution was found in the weighted topology and hence conflicts between these trees exist. *Allionia* was a supported, although weakly, sister group to *Boerhavia* by

weighted MP; this relationship remained unresolved under equal weights MP. A monophyletic clade of *Allionia*, *Boerhavia*, *Anulocaulis*, *Nyctaginia*, and *Cyphomeris* was resolved by weighted MP; equal weights MP did not recover this node with any support.

Conflicts and Unresolved Relationships. Only the equal weights MP topology and the ti/tv weighted MP topology were found to be significantly different from one another by compare-2 permutation test. All other tree pairings were statistically similar; however, topological conflicts, as well as unresolved clades, exist among the results from the four analyses of the entire data matrix.

No analysis recovered a well supported position for three groups: *Acleisanthes*, Tribe Abronieae, and the *Allionia-Boerhavia-Anulocaulis-Nyctaginia-Cyphomeris-Mirabilis* clade; that is, a polytomy was formed by the branches leading to these clades.

Only ML resolved the position of *Mirabilis* with strong support. Bootstrapping supported *Mirabilis* as the nearest neighbor to the *Allionia-Boerhavia-Cyphomeris-Anulocaulis-Nyctaginia* clade (BS = 85, see Fig. 6) while quartet puzzling very weakly supported a sister group relationship of the *Acleisanthes-Abronia-Tripterocalyx* and *Allionia-Boerhavia-Cyphomeris-Anulocaulis-Nyctaginia* clades (not shown, QP = 38).

Equal weights MP and NJ failed to resolve the monophyletic clade of *Allionia*, *Boerhavia*, *Anulocaulis*, *Nyctaginia*, and *Cyphomeris*. Ti/tv weighted MP and ML resolved and supported this clade (see Fig. 5).

Only equal weights MP failed to recover a sister group relationship of *Allionia* to *Boerhavia*. Not only did all other analyses resolve this relationship (see Figs. 3, 5, and 6), but the relationship was supported (some weakly, see Figs. 3, 5, and 6), even by equal

weights MP bootstrap and jackknife analyses, although very weakly (not shown, BS = 51, JK = 51).

Placement of *Cyphomeris* was not determined. ML analyses resolved *Cyphomeris* as the nearest neighbor to the *Allionia-Boerhavia-Anulocaulis-Nyctaginia* clade, but bootstrapping did not support this (not shown, it resulted in a polytomy of *Cyphomeris*, *Allionia-Boerhavia*, and *Anulocaulis-Nyctaginia* branches) and quartet puzzling weakly supported *Cyphomeris* as the sister group to the *Anulocaulis-Nyctaginia* clade (not shown, QP = 51). Neighbor-joining resolved *Cyphomeris* as the sister group to the *Anulocaulis-Nyctaginia* clade (not shown, BS = 98, JK = 98). All MP analyses resulted in a polytomy of *Cyphomeris*, *Allionia-Boerhavia*, and *Anulocaulis-Nyctaginia* branches.

Acleisanthes was the nearest genus to Tribe Abronieae according to equal weights MP and NJ, but with little support (see Fig. 3a). Ti/tv weighted MP resulted in polytomy that included the *Acleisanthes* branch (see Fig. 5a); however, in ti/tv weighted bootstrap and jackknife analyses, *Acleisanthes* was very weakly supported as the nearest neighbor to Tribe Abronieae (not shown, BS = 52, JK = 52). The ML tree (Fig. 5b) indicated a sister group relationship between *Acleisanthes* and the *Allionia-Boerhavia-Anulocaulis-Nyctaginia-Cyphomeris-Mirabilis* clade, but this relationship was unsupported by subsequent bootstrapping and quartet puzzling. In fact, quartet puzzling weakly supported *Acleisanthes* as the sister group to Tribe Abronieae (not shown, QP = 44).

Analyses of Subsets. Jackknife analysis of the ITS1 region was aborted after 2422 replicates due to memory constraints. Bootstrap analysis of the ITS2 region was aborted after 240 replicates due to time constraints. Topologies resulting from MP and NJ analyses of 18S and of 26S were compatible with results from analyses of the entire data

matrix, but were less resolved (not shown, see Appendix 3 Trees 26-31 and 82-92). NJ bootstrap and jackknife analyses of ITS1 and of ITS2 supported *Cyphomeris* as the nearest neighbor to the *Anulocaulis-Nyctaginia* clade (BS = 73-92, JK = 73-93, not shown, see Appendix 3 Trees 32-45 and 58-81); ML also resolved and supported this relationship (see above). Additionally, many subclades, common to all analyses of the entire data matrix, were also resolved and supported by subset analyses (not shown, see Appendix 3 Trees 26-92).

Topologies conflicting with those of analyses of the entire data matrix were found in subset analyses. MP and NJ analysis of 5.8S resulted in *Bougainvillea* as the nearest neighbor to the Tribe Nyctagineae-Tribe Abronieae clade (BS = 82-88, JK = 74-81, not shown, see Appendix 3 Trees 46-57); NJ bootstrap and jackknife analyses of 5.8S support *Phytolacca* as the sister group to this *Bougainvillea*-Tribe Nyctagineae-Tribe Abronieae clade (BS = 87, JK = 76, not shown, see Appendix 3 Trees 46-57). NJ bootstrap and jackknife analyses of ITS2 supported a clade of *Phytolacca*, *Pisonia*, and *Bougainvillea* (BS = 72, JK = 71, not shown, see Appendix 3 Trees 58-81); these analyses also weakly supported the sister group relationship of *Boerhavia spicata* and *B. intermedia* (BS = 72, JK = 64, not shown, see Appendix 3 Trees 58-81).

DISCUSSION

Approximately 68 species (9.5% of all species) in 12 genera (38.7% of all genera) of Nyctaginaceae occur in Texas. I sampled 91.7% of the Texas genera (35.5% of worldwide) and 30.9% of the Texas species (5.3% of worldwide). *Commicarpus* was the only Texas genus not sampled in this study; however, this genus is often included in *Boerhavia* (Bittrich and Kühn 1993). The only species occurring in Texas is *Commicarpus scandens*.

In generating the molecular dataset, Sambrook-extractions seemed more difficult to amplify. This may have been due to impurities not removed during the extraction, which not only gave the extraction a thicker texture and more color, but also may have impeded primer annealing and DNA extension during PCR. PCR products, for many Sambrook-extractions, were eventually produced by decreasing the amount of DNA, and thus concentration of impurities, added to an amplification reaction and by persistence (reattempting PCR). This doggedness was unnecessary for most Kang-extractions because these included fewer impurities that impeded PCR.

The ITS region of the nrDNA consists of two spacers, ITS1 and ITS2, which flank the highly conserved 5.8S subunit (Sper-Whitis et al. 1992; Baldwin et al. 1995). The small size of this region (< 700 bp in angiosperms) is suitable for molecular analysis (Baldwin et al. 1995). Its high copy number within a genome eases detection, amplification, and sequencing (Baldwin et al. 1995). Rapid concerted evolution of this

gene family results in homogeneity of repeat units within a genome (Baldwin et al. 1995). Concerted evolution also results in uniformity of repeat units within an interbreeding population which may induce a reduction in sample size of such a population (Baldwin et al. 1995). The spacers are bounded by conserved sequences so they are amplified easily (Sper-Whitis et al. 1992; Clegg 1993). ITS length is conserved (Baldwin et al. 1995) which facilitates alignment. Site substitutions rather than insertion-deletion events (indels) predominate in the spacers, and conserved and variable sites are interspersed in the spacers; both qualities aid ITS sequence alignment (Baldwin et al. 1995). As they do not encode rRNA subunits, the spacers have higher sequence variation than the 5.8S region (Ritland et al. 1993; Suh et al. 1993; Bogler and Simpson 1994). These variable evolutionary rates among the subunit and spacers of the ITS region allow investigation of different levels of divergence using different parts of the region (Suh et al. 1993; Campbell et al. 1995). ITS nrDNA has been used at high taxonomic levels (Baldwin et al. 1995); however, due to the moderate to high levels of sequence variability within the spacers, it is more appropriately used at lower levels (e.g., intrafamilial [Baldwin et al. 1995], intergenic [Baldwin 1993; Bogler and Simpson 1994], and interspecific [Suh et al. 1993; Baldwin et al. 1995]). Levin (2000) used ITS nrDNA to evaluate the phylogenetic relationships among *Mirabilis* and *Acleisanthes* in Nyctaginaceae.

Pairwise distances in Nyctaginaceae ranged from 0% to 24.0% (see Table 7). Interspecific pairwise distances ranged from 0% (in *Abronia*) to 5.9% (in *Boerhavia*) (see Table 7) with an average of 1.7%. Levin (2000) reported pairwise distances of 0.152%-17.41% for 19 taxa in Nyctaginaceae; exactly which taxa are unclear, but most likely they included *Abronia fragrans*, *Mirabilis* species from section *Quamoclidion*, *Acleisanthes*

species, *Selinocarpus* (as it was called then) species, and *Pisonia capitata*. Unfortunately, a pairwise distance table was not included by Levin (2000), so precise interspecific distances were not obtained. In other angiosperm families, interspecific distances range from 0% to 21.4%: 0%-5.0% for *Amelanchier* (Rosaceae) (Campbell et al. 1997), 0%-21.4% for *Arceuthobium* (Viscaceae) (Nickrent et al. 1994), 0.6%-7.9% for *Krigia* (Asteraceae) (Kim and Jansen 1994), 0.7%-8.9% for *Panax* (Araliaceae) (Wen and Zimmer 1996), and 0%-6.8% for *Robinsonia* (Asteraceae) (Sang et al. 1995). These values may be difficult to compare, because authors define “ITS region” differently. In the present study (and Levin 2000), ITS nrDNA includes 33 (25) nucleotides of the 18S gene, ITS1, the 5.8S gene, ITS2, and 51 (65) nucleotides of the 26S gene. Some authors use only ITS1 and ITS2 in their analyses (Sang et al. 1995; Campbell et al. 1997), while others use ITS1, 5.8S, and ITS2 (Kim and Jansen 1994; Nickrent et al. 1994; Wen and Zimmer 1996). Overall though, the interspecific distances found here are comparable to those reported in Nyctaginaceae and in other plant families.

Although the ITS nrDNA region is generally too invariable to be used at the population level, intraspecific pairwise distances have been reported, and they range from 0% (*Panax* spp. [Wen and Zimmer 1996]) to 3.7% (*Calycadenia truncata* [Asteraceae] [Baldwin 1993]). Here, only two species had any intraspecific variability: *Mirabilis jalapa* (0.1%-0.4%, see Table 7) and *Abronia fragrans* (0.1%, see Table 7). These findings are also comparable to the reported distances. Several trends within the genus *Abronia* were unexpected. *Abronia ameliae*, *A. angustifolia*, *A. fragrans*, and *A. macrocarpa* demonstrated little, if any, DNA sequence divergence (0%-1.6%, see Table 7); more explicitly, individuals of *A. angustifolia*, *A. macrocarpa*, and one *A. fragrans*

(DJM081) had identical genotypes. This *A. fragrans* genotype was extracted from seed purchased from Plants of the Southwest in Sante Fe Co., New Mexico (<http://www.plantsofthesouthwest.com>). However, a unique *A. fragrans* genotype (DJM002), representing specimens collected in Texas, was found. *Abronia umbellata*, *A. villosa*, and *A. villosa* var. *aurita* have identical DNA sequences for this region (0%, see Table 7). Given the reported interspecific pairwise distance values for other plants, these findings were unexpected.

Phytolaccaceae was chosen as the outgroup because it has been shown to be a very close, if not the closest, relative of Nyctaginaceae (Rettig et al. 1992; Bittrich 1993; Chase et al. 1993; Downie and Palmer 1994; Soltis et al. 1997; Hershkovitz and Zimmer 1997; Downie et al. 1997; Savolainen et al. 2000; Applequist and Wallace 2001; Cuénoud et al. 2002). *Rivina humilis* and *Phytolacca americana* were specifically included as outgroups because seed tissue was available from herbarium specimens of these taxa at the SWTSU herbarium. *Pisonia* and *Bougainvillea* appear to be less divergent from *Rivina* (18.9%-21.5%) and *Phytolacca* (13.6%-16.5%) than from the other members of Nyctaginaceae (18.3%-23.8%) (see Table 7). These low sequence divergences suggest a close relationship between Nyctaginaceae and Phytolaccaceae; hence outgroup selection here was appropriate.

Subset Analyses. Although subset analyses sometimes resulted in topologies conflicting with those resulting from analyses of all characters, these conflicts may be ignored. Graybeal (1998) found that more characters improved phylogenetic accuracy in computer simulation. The number of informative characters in a subset is most likely too small to provide accurate estimates of phylogeny. Also, partitions in the traditional sense

are separate datasets, for example, a nuclear molecular marker vs. a mitochondrial molecular marker vs. a set of behavioral characteristics; here, the partitions were different regions within a DNA sequence fragment that is widely used as one cohesive dataset. Lastly, combined analysis of the regions in ITS nrDNA has been shown to provide more accurate phylogenies (Baldwin et al. 1995). It has also been argued that combining homogeneous data sets is appropriate (Bull et al. 1993); the partitions in this study were found to be homogeneous (partition homogeneity test, $P = 1$).

Phylogenetic Hypothesis for Nyctaginaceae. My current hypothesis for the phylogenetic relationships of the Texas genera of Nyctaginaceae is the ML reconstruction, depicted in Figs. 5b and 6. Its treelength was very close to the optimal equal weights MP treelength, and its branch lengths were very similar to the neighbor-joining branch lengths (see Figs. 3b and 5b). This reconstruction confirms some proposed relationships but refutes others; additionally, it delimits relationships never before observed.

Branch lengths showed that Tribe Abronieae (*Abronia*, *Tripterocalyx*) is deeply separated from other clades (see Fig. 5b); bootstrap (100) and quartet puzzling (95) values provided support for the monophyly of this tribe (see Fig. 6).

Based on the molecular data, Bittrich and Kühn's (1993) division of Subtribe Boerhaviinae into Subtribes Boerhaviinae (*Acleisanthes*, *Anulocaulis*, *Boerhavia*, *Cyphomeris*) and Nyctagininae (*Allionia*, *Mirabilis*, *Nyctaginia*) is unwarranted due to paraphyly. *Allionia* (Subtribe Nyctagininae sensu Bittrich and Kühn [1993]) was the closest relative of *Boerhavia* (Subtribe Boerhaviinae sensu Bittrich and Kühn [1993]). *Nyctaginia* (Subtribe Nyctagininae sensu Bittrich and Kühn [1993]) was the nearest

neighbor of *Anulocaulis* (Subtribe Boerhaviinae sensu Bittrich and Kühn [1993]). Levin (2000) also found these subtribes to be paraphyletic. Although Bittrich and Kühn (1993) recognize *Anulocaulis* and *Cyphomeris* as genera separate from *Boerhavia*, others do not (Mabberley 1997; Levin 2000). Given that *Anulocaulis* and *Cyphomeris* were not the closest relatives of *Boerhavia* nor of each other, they should both receive recognition at the generic level.

Many works include *Tripterocalyx* in *Abronia* (Wilson 1972, 1976; Correll and Johnston 1979; Bittrich and Kühn 1993). Support values (see Fig. 6) and branch lengths (see Fig. 5b) indicated that *Tripterocalyx* is monophyletic and deeply divergent from *Abronia*, and thus recognition at the generic level is warranted.

Mirabilis jalapa and *M. longiflora* are recognized as members of *Mirabilis* section *Mirabilis* (Le Duc 1995; Levin 2000). Support values (see Fig. 6) and branch lengths (see Fig. 5b) bore out this circumscription. *Acleisanthes* and *Mirabilis* have been found to be sister genera, but with moderate (Levin's Fig. 1, BS = 69, DI = 1) or no (Levin's Fig. 2) support (Levin 2000). Here, *Mirabilis* was the nearest neighbor to the *Allionia-Boerhavia-Anulocaulis-Nyctaginia-Cyphomeris* clade, with *Acleisanthes* basal to *Mirabilis*. Although the present study included more genera, the placement of *Mirabilis* remained obscure.

Levin (2002) transferred *Selinocarpus* to *Acleisanthes* based on molecular and morphological data; results here supported this. Again, *Acleisanthes* and *Mirabilis* have been shown to have a weakly supported sister group relationship (Levin 2000). In this study, many analyses resulted in a weakly supported sister group relationship of *Acleisanthes* and Tribe Abronieae, while the ML topology (Figs. 5b and 6) indicated an

unsupported sister group relationship between *Acleisanthes* and the *Allionia-Boerhavia-Anulocaulis-Nyctaginia-Cyphomeris-Mirabilis* clade. Unfortunately, the phylogenetic placement of *Acleisanthes* as a genus was not clarified by the analyses performed here. These conflicting topologies may indicate that *Acleisanthes* is an intermediate between Tribes Nyctagineae (*Acleisanthes*, *Allionia*, *Anulocaulis*, *Boerhavia*, *Cyphomeris*, *Mirabilis*, and *Nyctaginia*) and Abronieae (*Abronia* and *Tripterocalyx*).

The Genus *Abronia*. The lack of resolution among the Texas species of *Abronia* (*A. ameliae*, *A. angustifolia*, *A. fragrans*, and *A. macrocarpa*), the shared genotype among three of these species (*A. angustifolia*, *A. fragrans*, and *A. macrocarpa*), and the intraspecific variability in *A. fragrans* require explanation. Under the assumption of a strict phylogenetic species concept (Mishler and Theriot 2000), the Texas species sampled here (*A. ameliae*, *A. angustifolia*, *A. fragrans*, and *A. macrocarpa*) represent one lineage and thus one species. I feel strongly that to propose taxonomic changes for this group at present is inappropriate, because this study encompasses the genetic variability of only one molecular marker; moreover, this study to my knowledge, is the only phylogenetic study incorporating these taxa. Reliable molecular phylogenies are derived from multiple data sets, often incorporate morphological and ecological data, and are as taxa-inclusive as computationally feasible. Although these *Abronia* species are recognized primarily by anthocarp morphology (Galloway 1975), characteristics of reproductive structures are widely accepted as species-delineating morphological markers.

Lineage sorting may also explain some trends found in *Abronia*. Genotypes appearing to be more closely related to those of different biological species than to those

of conspecifics have been reported (Neigel and Avise 1986); one explanation of this has been hybridization and introgression between species (Neigel and Avise 1986; Doyle 1992). Neigel and Avise (1986) used computer simulation to evaluate lineage sorting as another explanation. They found that in recently separated species, genotype distribution may not always reflect species boundaries as a consequence of ancestral polymorphism and random differential gene survivorship beyond speciation events (Neigel and Avise 1986; Doyle 1992). Given the close relationships of the Texas species of *Abronia* (indicated by short branches on the phylograms depicted in Figs. 3b and 5b), lineage sorting may explain the trends observed in the ITS nrDNA sequences of *Abronia ameliae*, *A. angustifolia*, *A. fragrans*, and *A. macrocarpa*. Figure 7 depicts a lineage sorting and extinction scenario for *A. angustifolia*, *A. fragrans*, and *A. macrocarpa*.

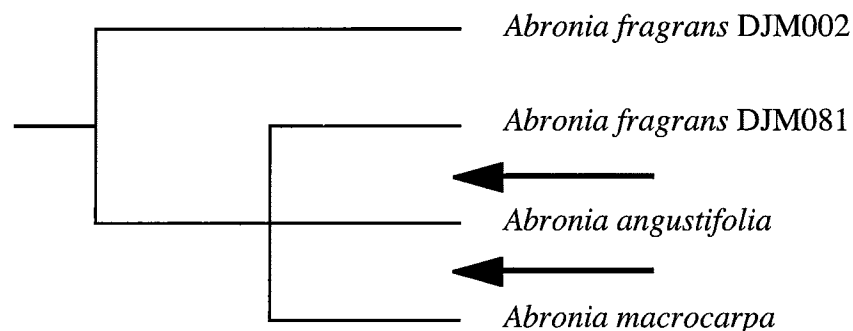


FIG. 7. Gene tree depicting lineage sorting in *Abronia angustifolia*, *A. fragrans*, and *A. macrocarpa*. *A. fragrans* DJM081, *A. angustifolia*, and *A. macrocarpa* had identical ITS nrDNA genotypes. *A. fragrans* DJM002 represents genotypes of Texas specimens, while *A. fragrans* DJM081 represents genotypes of specimens purchased from a seed company in Sante Fe. Co., New Mexico. Black arrows indicate speciation events. Modified from Neigel and Avise (1986).

Another explanation for these trends is that, for the genus *Abronia*, the ITS nrDNA region is insufficiently variable. Recent speciation may preclude ample accumulation of substitutions; consequently too few substitutions results in small

numbers of informative characters in a phylogenetic dataset. However, given the resolution found in other genera in Nyctaginaceae, here and in Levin (2000), recent speciation may more appropriately explain the lack of resolution found in the *Abronia* taxa.

More questions were raised rather than answered concerning the genus *Abronia* during this study. First, what is the sister taxon to the endangered Texas endemic, *Abronia macrocarpa*? Due to very limited ITS nrDNA sequence variability in the Texas species of *Abronia*, this was not determined. Second, given the limited variability within the Texas species and within the *Abronia umbellata*/*A. villosa* complex, is limited genetic variation universal in this genus? The corollary to this question is, do thirty genetically distinct species of *Abronia* truly exist?

Future Investigation. To further refine the evolutionary relationships within Nyctaginaceae, several ambiguous nodes must be clarified. The phylogenetic positions of both *Mirabilis* and *Acleisanthes* remain obscure and merit further investigation. Verification of the monophyly of the *Allionia-Boerhavia-Anulocaulis-Nyctaginia-Cyphomeris-Mirabilis* clade is needed. The position of *Acleisanthes* most assuredly requires investigation. This genus has long been recognized as belonging to Tribe Nyctagineae; while the data presented here do not dispute this assignment, they did indicate that *Acleisanthes* retains molecular sequence affinities allying to Tribe Abronieae as well as with genera in Tribe Nyctagineae.

Elucidating these systematic complexities will require a larger sampling of taxa, both genera and species, across their geographic ranges. Other molecular markers should be evaluated for their utility in this family. For example, a finer-scale, hypervariable

marker may clarify the phylogenetic relationships of the Texas species of *Abronia*. Also, genes from mtDNA and cpDNA should be investigated in order to test the evolutionary history proposed here against other independent datasets. Finally, additional phylogenetic analyses seeking to refine character state change optimality should be considered. For example, in this study, nucleotide substitution bias with respect to stems and loops (secondary structures occurring in the genes and in the spacers) was ignored. Nucleotide substitutions in stems are nonindependent, due to the complementary base pairing that occurs in stems, while nucleotides in loops may evolve freely (Wheeler and Honeycutt 1988; Kraus et al. 1992). The paired substitutions observed in stem regions may overwhelm substitutions at positions in loop regions, because a substitution in a stem region has a compensatory substitution in the other member of the base pair (Wheeler and Honeycutt 1988; Kraus et al. 1992). Phylogenetic analyses, like downweighting stem substitutions in maximum parsimony analysis, are available to cope with these conditions. While ML models can account for significant rate heterogeneity, it may be possible to bring the resolution of MP analyses (Fig. 3a) to accordance with the ML hypothesis (Figs. 5b and 6) by completing more refined MP weighting schemes.

As the first attempt at a comprehensive investigation into the molecular systematics of Nyctaginaceae, this work represents a solid preliminary hypothesis of the evolutionary relationships within an understudied yet fascinating group of plants.

LITERATURE CITED

- APPLEQUIST, W. L. and R. S. WALLACE. 2001. Phylogeny of the portulacaceous cohort based on *ndhF* sequence data. *Systematic Botany* 26: 406-419.
- BACHMANN, K. 1994. Tansley Review No. 63: Molecular markers in plant ecology. *New Phytologist* 126: 403-418.
- BALDWIN, B. G. 1993. Molecular phylogenetics of *Calycadenia* (Compositae) based on ITS sequences of nuclear ribosomal DNA: chromosomal and morphological evolution reexamined. *American Journal of Botany* 80: 222-238.
- , M. J. SANDERSON, J. M. PORTER, M. F. WOJCIECHOWSKI, C. S. CAMPBELL, and M. J. DONOGHUE. 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Garden* 82: 247-277.
- BENTHAM, G. and J. D. HOOKER. 1880. *Genera plantarum ad exemplaria imprimis in Herbariis kewensibus servata definita*, Volume 3. London: 1. Reeve & Co.
- BITTRICH, V. 1993. Introduction to Centrospermae. Pp. 13-19 in *The families and genera of vascular plants*, Vol. II, eds. K. Kubitzki, J. G. Rohwer, and V. Bittrich. New York: Springer-Verlag.
- BITTRICH, V. and U. KUHN. 1993. Nyctaginaceae. Pp. 473-486 in *The families and genera of vascular plants*, Vol. II, eds. K. Kubitzki, J. G. Rohwer, and V. Bittrich. New York: Springer-Verlag.
- BOGLE, A. L. 1974. The genera of Nyctaginaceae in the southeastern United States. *Journal of the Arnold Arboretum* 55: 1-37.
- BOGLER, D. J. and B. B. SIMPSON. 1994. Sequence alignment, gap weights and phylogenetic inference: analysis of ITS rDNA in the Agavaceae and Liliaceae. *American Journal of Botany* 81: 143.
- BREMER, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* 42: 795-803.

- BULL, J. J., J. P. HUELSENBECK, C. W. CUNNINGHAM, D. L. SWOFFORD, and P. J. WADDELL. 1993. Partitioning and combining data in phylogenetic analysis. *Systematic Biology* 42: 384-397.
- CAMPBELL, C. S., M. J. DONOGHUE, B. G. BALDWIN, and M. F. WOJCIECHOWSKI. 1995. Phylogenetic relationships in Maloideae (Rosaceae): evidence from sequences of the internal transcribed spacers of nuclear ribosomal DNA and its congruence with morphology. *American Journal of Botany* 82: 903-918.
- CAMPBELL, C. S., M. F. WOJCIECHOWSKI, B. G. BALDWIN, L. A. ALICE, and M. J. DONOGHUE. 1997. Persistent nuclear ribosomal DNA sequence polymorphism in the *Amelanchier* agamic complex (Rosaceae). *Molecular Biology and Evolution* 14: 81-90.
- CHAPELA, I. H., S. A. REHNER, T. R. SCHULTZ, and U. G. MUELLER. 1994. Evolutionary history of the symbiosis between fungus-growing ants and their fungi. *Science* 266: 1691-1694.
- CHASE, M. W., D. E. SOLTIS, R. G. OLMSTEAD, D. MORGAN, D. L. LES, B. D. MISHLER, M. R. DUVAL, R. A. PRICE, H. G. HILLS, Y-L. QIU, K. A. KRON, J. H. RETTIG, E. CONTI, J. D. PALMER, J. R. MANHART, K. J. SYTSMA, H. J. MICHAELS, W. J. KRESS, K. G. KAROL, W. D. CLARK, M. HEDRÉN, B. S. GAUT, R. K. JANSEN, K-J. KIM, C. F. WIMPEE, J. F. SMITH, G. R. FURNIER, S. H. STRAUSS, Q-Y. XIANG, G. M. PLUNKETT, P. S. SOLTIS, S. M. SWENSEN, S. E. WILLIAMS, P. A. GADEK, C. J. QUINN, L. E. EGUIARTE, E. GOLENBERG, G. H. LEARN, JR., S. W. GRAHAM, S. C. H. BARRETT, S. DAYANANDAN, and V. A. ALBERT. 1993. Phylogenetics of seed plants: an analysis of nucleotide sequences from the plastid gene *rbcL*. *Annals of the Missouri Botanical Garden* 80: 528-6580.
- CLEGG, M. T. 1993. Chloroplast gene sequences and the study of plant evolution. *Proceedings of the National Academy of Sciences of the United States of America* 90: 363-367.
- CORRELL, D. S. and M. C. JOHNSTON. 1979. *Manual of the vascular plants of Texas*. Dallas: University of Texas at Dallas.
- CRUDEN, R. W. 1970. Hawkmoth pollination of *Mirabilis* (Nyctaginaceae). *Bulletin of the Torrey Botanical Club* 97: 89-91.
- CUÉNOUD, P., V. SAVOLAINEN, L. W. CHATROU, M. POWELL, R. J. GRAYER, and M. W. CHASE. 2002. Molecular phylogenetics of Caryophyllales based on nuclear 18S rDNA and plastid *rbcL*, *atpB*, and *matK* DNA sequences. *American Journal of Botany* 89: 132-144.
- DEL RIO, C. M. and A. BÚRQUEZ. 1986. Nectar production and temperature dependent pollination in *Mirabilis jalapa* L. *Biotropica* 18: 28-31.

- DOWNIE, S. R. and J. D. PALMER. 1994. A chloroplast DNA phylogeny of the Caryophyllales based on structural and inverted repeat restriction site variation. *Systematic Botany* 19: 236-252.
- , D. S. KATZ-DOWNIE, and K-J. CHO. 1997. Relationships in the Caryophyllales as suggested by phylogenetic analyses of partial chloroplast DNA ORF2280 homolog sequences. *American Journal of Botany* 84: 253-273.
- DOYLE, J. J. 1992. Gene trees and species trees: molecular systematics as one-character taxonomy. *Systematic Botany* 17: 144-163.
- DUKE, J. A. 1961. Flora of Panama. Part IV. Fascicle 4. Nyctaginaceae. *Annals of the Missouri Botanical Garden* 48: 51-65.
- ECKARDT, T. 1964. Nyctaginaceae. Pp. 84-86 in *Syllabus Der Pflanzenfamilien*, ed. A. Engler. Berlin: Gebrüder Borntraeger.
- ELLSTRAND, N. C. and D. R. ELAM. 1993. Population genetic consequences of small population size: implications for plant conservation. *Annual Review of Ecology and Systematics* 24: 217-242.
- ERIKSSON, T. 1998. AutoDecay ver. 4.0 (program distributed by the author). Department of Botany, Stockholm University, Sweden.
- FARRIS, J. S., M. KALLERSJO, A. J. KLUGE, and C. BULT. 1995. Testing significance of congruence. *Cladistics* 10: 315-319.
- FELSENSTEIN, J. 1981. Evolutionary trees from DNA sequences: a maximum-likelihood approach. *Journal of Molecular Evolution*. 17: 368-376.
- . 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*: 783-791.
- FOWLER, B. A. and B. L. TURNER. 1977. Taxonomy of *Selinocarpus* and *Ammocodon* (Nyctaginaceae). *Phytologia* 37: 177-208.
- GALLOWAY, L. A. 1972. *Abronia macrocarpa* (Nyctaginaceae): a new species from Texas. *Brittonia* 24: 148-149.
- GALLOWAY, L. A. 1975. Systematics of the North American desert species of *Abronia* and *Tripterocalyx* (Nyctaginaceae). *Brittonia* 27: 328-347.
- GRAUR, D. and W-H. LI. 2000. *Fundamentals of molecular evolution*, 2nd edition. Sunderland: Sinauer Associates Inc., Publishers.

- GRAYBEAL, A. 1998. Is it better to add taxa or characters to a difficult phylogenetic problem? *Systematic Biology* 47: 9-17.
- HARRIMAN, N. A. 1999. Synopsis of New World *Commicarpus* (Nyctaginaceae). *Side* 18: 679-684.
- HEIMERL, A. 1934. Nyctaginaceae. Pp. 86-134 in *Die Natürlichen Pflanzenfamilien*, 2nd edition, 16c, eds. A. Engler and K. Prantl. Leipzig: Engelmann.
- HERSHKOVITZ, M. A. and E. A. ZIMMER. 1997. On the evolutionary origins of the cacti. *Taxon* 46: 217-232.
- HILLIS, D. M. and J. P. HUELSENBECK. 1992. Signal, noise, and reliability in molecular phylogenetic analyses. *Journal of Heredity* 83: 189-195.
- HILLIS, D. M., B. K., MABLE, A. LARSON, S. K. DAVIS, and E. A. ZIMMER. 1996. Nucleic acids IV: sequencing and cloning. Pp. 321-381 in *Molecular systematics*, 2nd ed., eds. D. M. Hillis, C. Moritz, and B. K. Mable. Sunderland: Sinauer Associates Inc., Publishers.
- HINKLE, G., J. K. WETTERER, T. R. SCHULTZ, and M. L. SOGIN. 1994. Phylogeny of the attine ant fungi based on analysis of small subunit ribosomal RNA gene sequences. *Science* 266: 1695-1697.
- HUELSENBECK, J. P. 1991. Tree-length distribution skewness: an indicator of phylogenetic information. *Systematic Zoology* 40: 257-270.
- HUELSENBECK, J. P. 1995. The robustness of two phylogenetic methods: four-taxon simulations reveal a slight superiority of maximum likelihood over neighbor joining. *Molecular Biology and Evolution* 12: 843-849.
- HUELSENBECK, J. P. and K. A. CRANDALL. 1997. Phylogeny estimation and hypothesis testing using maximum likelihood. *Annual Review of Ecology and Systematics* 28: 437-466.
- JONES, S. D., J. K. WIPFF, and P. M. MONTGOMERY. 1997. *Vascular plants of Texas: a comprehensive checklist including synonymy, bibliography, and index*. Austin: University of Texas Press.
- KANG, H. W., Y. G. CHO, U. H. YOON, and M. Y. EUN. 1998. A rapid DNA extraction method for RFLP and PCR analysis from a single dry seed. *Plant Molecular Biology Reporter* 16: 1-9.
- KARTESZ, J. T. and R. KARTESZ. 1980. *A synonymized checklist of the vascular flora of the United States, Canada, and Greenland*. Chapel Hill: The University of North Carolina Press.

- KIM, K.-J. and R. K. JANSEN. 1994. Comparisons of phylogenetic hypotheses among different data sets in dwarf dandelions (*Krigia*, Asteraceae): additional information from internal transcribed spacer sequences of nuclear ribosomal DNA. *Plant Systematics and Evolution* 190: 157-185.
- KIMURA, M. 1981. Estimation of evolutionary distances between homologous nucleotide sequences. *Proceedings of the National Academy of Sciences of the United States of America* 78:454-458.
- KRAUS, F., L. JARECKI, M. M. MIYAMOTO, S. M. TANHAUSER, and P. J. LAIPIS. 1992. Mispairing and compensational changes during the evolution of mitochondrial ribosomal RNA. *Molecular Biology and Evolution* 9: 770-774.
- LAROCHE, J., P. LI, L. MAGGIA, and J. BOUSQUET. 1997. Molecular evolution of angiosperm mitochondrial introns and exons. *Proceedings of the National Academy of Sciences of the United States of America* 94: 5722-5727.
- LE DUC, A. 1995. A revision of *Mirabilis* section *Mirabilis* (Nyctaginaceae). *Sida* 16: 613-648.
- LEVIN, R. A. 2000. Phylogenetic relationships within Nyctaginaceae tribe Nyctagineae: evidence from nuclear and chloroplast genomes. *Systematic Botany* 25: 738-750.
- LEVIN, R. 2002. Taxonomic status of *Acleisanthes*, *Selinocarpus*, and *Ammocodon* (Nyctaginaceae). *Novon* 12: 58-63.
- MABBERLEY, D. J. 1997. *The plant-book: a portable dictionary of the vascular plants*, 2nd ed. Cambridge: Cambridge University Press.
- MADDISON, W. P. and D. R. MADDISON. 1992. MacClade Release Version 3.05. Sunderland: Sinauer Associates Inc., Publishers.
- MAHRT, M. and R. SPELLENBERG. 1995. Taxonomy of *Cyphomeris* (Nyctaginaceae) based on multivariate analyses of geographic variation. *Sida* 16: 679-697.
- MISHLER, B. D. and E. C. THERIOT. 2000. The phylogenetic species concept (sensu Mishler and Theriot): monophyly, apomorphy, and phylogenetic species concepts. Pp. 44-54 in *Species concepts and phylogenetic theory*, eds. Q. D. Wheeler and R. Meier. New York: Columbia University Press.
- MILES, D. B. and A. E. DUNHAM. 1993. Historical perspectives in ecology and evolutionary biology: the use of phylogenetic comparative analyses. *Annual Review of Ecology and Systematics* 24: 587-619.

- NEIGEL, J. E., and J. C. AVISE. 1986. Phylogenetic relationships of mitochondrial DNA under various demographic models of speciation. Pp. 515-534 in *Evolutionary processes and theory*, eds. S. Karlin and E. Nevo. Orlando: Academic Press Inc.
- NICKRENT, D. L., K. P. SCHUETTE, and E. M. STARR. 1994. A molecular phylogeny of *Arceuthobium* (Viscaceae) based on nuclear ribosomal DNA internal transcribed spacer sequences. *American Journal of Botany* 81: 1149-1160.
- NOWICKE, J. W. 1970. Pollen morphology in the Nyctaginaceae I. Nyctagineae (Mirabileae). *Grana* 10: 79-88.
- NOWICKE, J. W. and T. J. LUIKART. 1971. Pollen morphology of the Nyctaginaceae II. Colignonieae, Boldoeae, and Leucastereae. *Grana* 11: 145-150.
- PAGE, R. D. M. 1991. Clocks, clades, and cospeciation: comparing rates of evolution and timing of cospeciation events in host-parasite assemblages. *Systematic Zoology* 40: 188-198.
- . 1993. Genes, organisms, and areas: the problem of multiple lineages. *Systematic Biology* 42: 77-84.
- . 1994. Maps between trees and cladistic analysis of historical associations among genes, organisms, and areas. *Systematic Biology* 43: 58-77.
- PILZ, G. E. 1978. Systematics of *Mirabilis* subgenus *Quamoclidion* (Nyctaginaceae). *Madroño* 25: 113-132.
- POSADA, D. and A. CRANDALL. 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics* 14: 817-818.
- POSADA, D. and A. CRANDALL. 2001. Selecting models of nucleotide substitution: an application to human immunodeficiency virus 1 (HIV-1). *Molecular Biology and Evolution* 18: 897-906.
- RETTIG, J. H., H. D. WILSON, and J. R. MANHART. 1992. Phylogeny of the Caryophyllales - gene sequence data. *Taxon* 41: 201-209.
- RITLAND, C. E., K. RITLAND, and N. A. STRAUS. 1993. Variation in the ribosomal internal transcribed spacers (ITS1 and ITS2) among eight taxa of the *Mimulus guttatus* species complex. *Molecular Biology and Evolution* 10: 1273-1288.
- RYDBERG, P. A. 1902. Studies on the Rocky Mountain Flora-IX. Nyctaginaceae of the Rocky Mountain region. *Bulletin of the Torrey Botanical Club* 29: 680-693.

- SAITOU, N. and M. NEI. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4: 406-425.
- SAMBROOK, J., E. F. FRITSCH, and T. MANIATIS. 1989. *Molecular cloning: a laboratory manual*, Book 3. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- SANG, T., D. J. CRAWFORD, and T. F. STUESSY. 1995. ITS sequences and the phylogeny of the genus *Robinsonia* (Asteraceae). *Systematic Botany* 20: 55-64.
- SAVOLAINEN, V., M. W. CHASE, S. B. HOOT, C. M. MORTON, D. E. SOLTIS, C. BAYER, M. F. FAY, A. Y. DE BRUIJN, S. SULLIVAN, and Y-L. QIU. 2000. Phylogenetics of flowering plants based on combined analysis of plastid *atpB* and *rbcL* gene sequences. *Systematic Biology* 49: 306-362.
- SCHONIGER, M. AND A. VON HAESELER. 1995. Performance of the maximum likelihood, neighbor joining, and maximum parsimony methods when sequence sites are not independent. *Systematic Biology* 44: 533-547.
- SMITH, J. M. 1976. A taxonomic study of *Acleisanthes* (Nyctaginaceae). *Wrightia* 5: 261-276.
- SOLTIS, D. E., P. S. SOLTIS, D. L. NICKRENT, L. A. JOHNSON, W. J. HAHN, S. B. HOOT, J. A. SWEERE, R. K. KUZOFF, K. A. KRON, M. W. CHASE, S. M. SWENSEN, E. A. ZIMMER, S-M. CHAW, L. J. GILLESPIE, W. J. KRESS, and K. J. SYTSMA. 1997. Angiosperm phylogeny inferred from 18S ribosomal DNA sequences. *Annals of the Missouri Botanical Garden* 84: 1-49.
- SPELLENBERG, R. 1993. Taxonomy of *Anulocaulis* (Nyctaginaceae). *Sida* 15: 373-389.
- SPELLENBERG, R. 2000. Blooming "behavior" in five species of *Boerhavia* (Nyctaginaceae). *Sida* 19: 311-323.
- SPELLENBERG, R. and S. R. R. TIJERINA. 2001. Geographic variation and taxonomy of North American species of *Mirabilis*, section *Oxybaphoides* (Nyctaginaceae). *Sida* 19: 539-570.
- SPER-WHITIS, G. L., S. B. GRAU, D. V. JOBES, and K. G. WILSON. 1992. DNA sequence variability of the ITS1 portion of the ribosomal RNA genes as a genetic tool for determining variation within individuals and between species of *Mandevilla*. *American Journal of Botany* 79: 95.
- STANDLEY, P. C. 1931. The Nyctaginaceae and Chenopodiaceae of northwestern South America. *Field Museum of Natural History, Botanical Series* 11: 71-126.

- STRIMMER, K. and A. VON HAESELER. 1996. Quartet puzzling: a quartet maximum-likelihood method for reconstructing tree topologies. *Molecular Biology and Evolution* 13: 964-969.
- SUH, Y., L. B. THIEN, H. E. REEVE, and E. A. ZIMMER. 1993. Molecular evolution and phylogenetic implications of internal transcribed spacer sequences of ribosomal DNA in Winteraceae. *American Journal of Botany* 80: 1042-1055.
- SWOFFORD, D. L. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (* and Other Methods). Version 4. Sunderland: Sinauer Associates Inc., Publishers.
- SWOFFORD, D. L., G. J. OLSEN, P. J. WADDELL, and D. M. HILLIS. 1996. Phylogenetic inference. Pp. 407-514 in *Molecular systematics*, 2nd ed., eds. D. M. Hillis, C. Moritz, and B. K. Mable. Sunderland: Sinauer Associates Inc., Publishers.
- TILLET, S. S. 1967. The maritime species of *Abronia* (Nyctaginaceae). *Brittonia* 19: 299-327.
- TURNER, B. L. 1993. Texas species of *Mirabilis* (Nyctaginaceae). *Phytologia* 75: 432-445.
- TURNER, B. L. 1994. Revisionary study of the genus *Allionia* (Nyctaginaceae). *Phytologia* 77: 45-55.
- U. S. FISH AND WILDLIFE SERVICE. 1992. Large-fruited Sand-verbena (*Abronia macrocarpa*) Recovery Plan. U. S. Fish and Wildlife Service, Albuquerque, New Mexico. 37 pp.
- VENKATESWARLU, K. and R. NAZAR. 1991. A conserved core structure in the 18-26S rRNA intergenic region from tobacco, *Nicotiana rustica*. *Plant Molecular Biology* 17: 189-194.
- WEN, J. and E. A. ZIMMER. 1996. Phylogeny and biogeography of *Panax* L. (the ginseng genus, Araliaceae): inferences from ITS sequences of nuclear ribosomal DNA. *Molecular Phylogenetics and Evolution* 6: 167-177.
- WHEELER, W. C. and R. L. HONEYCUTT. 1988. Paired sequence difference in ribosomal RNAs: evolutionary and phylogenetic implications. *Molecular Biology and Evolution* 5: 90-96.
- WILLIAMSON, P. S. and S. K. BAZEER. 1997. Self-incompatibility in *Abronia macrocarpa* (Nyctaginaceae). *The Southwestern Naturalist* 42: 409-415.
- and C. R. WERTH. 1999. Levels and patterns of genetic variation in the endangered species *Abronia macrocarpa* (Nyctaginaceae). *American Journal of Botany* 86: 293-301.

- , L. MULIANI, and G. K. JANSSEN. 1994. Pollination biology of *Abronia macrocarpa* (Nyctaginaceae), an endangered Texas species. *The Southwestern Naturalist* 39: 336-341.
- WILLSON, J. and R. SPELLENBERG. 1977. Observations on anthocarp anatomy in the subtribe Mirabilinae. *Madroño* 24: 104-111.
- WILSON, R. C. 1972. *Abronia*: I. Distribution, ecology, and habit of nine species of *Abronia* found in California. *Aliso* 7: 421-437.
- WILSON, R. C. 1976. *Abronia*: IV. Anthocarp dispersibility and its ecological implication for nine species of *Abronia*. *Aliso* 8: 493-506.
- YOKOTA, Y., T. KAWATA, Y. IIDA, A. KATO, and S. TANIFUJI. 1989. Nucleotide sequences of the 5.8S gene and internal transcribed spacer regions in carrot and broad bean ribosomal DNA. *Journal of Molecular Evolution* 29: 294-301.

APPENDIX 1

Tissue sample and voucher information.

Unless otherwise noted, tissue samples and vouchers were collected by Diana J. McHenry.

DJM #	MF #	Genus	Species	Date Collected	County	State
DJM 001	MF 7583	<i>Mirabilis</i>	<i>jalapa</i>	08-Aug-01	Guadalupe	TX
DJM 002	MF 7584	<i>Abronia</i>	<i>fragrans</i>	14-Aug-01	Hutchinson	TX
DJM 003	MF 7585	<i>Mirabilis</i>	<i>jalapa</i>	26-Aug-01	Oldham	TX
DJM 004	MF 7586	<i>Mirabilis</i>	<i>jalapa</i>	26-Nov-01	Hays	TX
DJM 005	MF 7587	<i>Mirabilis</i>	<i>jalapa</i>	26-Nov-01	Hays	TX
DJM 006	MF 7588	<i>Mirabilis</i>	<i>jalapa</i>	23-Feb-02	Hays	TX
DJM 007	MF 7589	<i>Abronia</i>	<i>macrocarpa</i>	23-Feb-02	Leon	TX
DJM 008	MF 7590	<i>Abronia</i>	<i>macrocarpa</i>	23-Feb-02	Leon	TX
DJM 009	MF 7591	<i>Abronia</i>	<i>macrocarpa</i>	23-Feb-02	Leon	TX
DJM 010	MF 7592	<i>Abronia</i>	<i>macrocarpa</i>	23-Feb-02	Leon	TX
DJM 011	MF 7593	<i>Abronia</i>	<i>macrocarpa</i>	23-Feb-02	Leon	TX
DJM 012	MF 7594	<i>Abronia</i>	<i>macrocarpa</i>	23-Feb-02	Leon	TX
DJM 013	MF 7595	<i>Abronia</i>	<i>macrocarpa</i>	23-Feb-02	Leon	TX
DJM 014	MF 7596	<i>Abronia</i>	<i>macrocarpa</i>	23-Feb-02	Leon	TX
DJM 015	MF 7597	<i>Abronia</i>	<i>macrocarpa</i>	23-Feb-02	Leon	TX
DJM 016	MF 7598	<i>Abronia</i>	<i>macrocarpa</i>	?	?	TX
DJM 017	MF 7599	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 018	MF 7600	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 019	MF 7601	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 020	MF 7602	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 021	MF 7603	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 022	MF 7604	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 023	MF 7605	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 024	MF 7606	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 025	MF 7607	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 026	MF 7608	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 027	MF 7609	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 028	MF 7610	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 029	MF 7611	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 030	MF 7612	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 031	MF 7613	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 032	MF 7614	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 033	MF 7615	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 034	MF 7616	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 035	MF 7617	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 036	MF 7618	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 037	MF 7619	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 038	MF 7620	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 039	MF 7621	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 040	MF 7622	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 041	MF 7623	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 042	MF 7624	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 043	MF 7625	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 044	MF 7626	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 045	MF 7627	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 046	MF 7628	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 047	MF 7629	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 048	MF 7630	<i>Abronia</i>	<i>macrocarpa</i>	14-Apr-02	Leon	TX
DJM 049	MF 7631	<i>Boerhavia</i> ?	sp?	18-Apr-02	Kenedy	TX
DJM 050	MF 7632	<i>Abronia</i>	<i>ameliae</i>	18-Apr-02	Brooks	TX
DJM 051	MF 7633	<i>Abronia</i>	<i>ameliae</i>	18-Apr-02	Brooks	TX
DJM 052	MF 7634	<i>Abronia</i>	<i>ameliae</i>	18-Apr-02	Brooks	TX
DJM 053	MF 7635	<i>Abronia</i>	<i>ameliae</i>	18-Apr-02	Brooks	TX
DJM 054	MF 7636	<i>Abronia</i>	<i>ameliae</i>	18-Apr-02	Brooks	TX
DJM 055	MF 7637	<i>Abronia</i>	<i>ameliae</i>	18-Apr-02	Brooks	TX
DJM 056	MF 7638	<i>Abronia</i>	<i>ameliae</i>	18-Apr-02	Brooks	TX
DJM 057	MF 7639	<i>Abronia</i>	<i>ameliae</i>	18-Apr-02	Brooks	TX
DJM 058	MF 7640	<i>Abronia</i>	<i>ameliae</i>	18-Apr-02	Brooks	TX

DJM #	MF #	Genus	Species	Date Collected	County	State
DJM 059	MF 7641	<i>Abronia</i>	<i>ameliae</i>	18-Apr-02	Brooks	TX
DJM 060	MF 7642	<i>Tripterocalyx</i>	<i>carnea</i>	15-May-02	El Paso	TX
DJM 061	MF 7643	<i>Tripterocalyx</i>	<i>carnea</i>	15-May-02	El Paso	TX
DJM 062	MF 7644	<i>Tripterocalyx</i>	<i>carnea</i>	15-May-02	El Paso	TX
DJM 063	MF 7645	<i>Tripterocalyx</i>	<i>carnea</i>	15-May-02	El Paso	TX
DJM 064	MF 7646	<i>Tripterocalyx</i>	<i>carnea</i>	15-May-02	El Paso	TX
DJM 065	MF 7647	<i>Tripterocalyx</i>	<i>carnea</i>	15-May-02	El Paso	TX
DJM 066	MF 7648	<i>Mirabilis</i>	<i>multiflora</i>	15-May-02	Culberson	TX
DJM 067	MF 7649	<i>Abronia</i>	<i>fragrans</i>	16-May-02	Ward	TX
DJM 068	MF 7650	<i>Abronia</i>	<i>fragrans</i>	16-May-02	Ward	TX
DJM 069	MF 7651	<i>Abronia</i>	<i>fragrans</i>	16-May-02	Ward	TX
DJM 070	MF 7652	<i>Abronia</i>	<i>fragrans</i>	16-May-02	Ward	TX
DJM 071	MF 7653	<i>Mirabilis</i>	<i>jalapa</i>	8-Jun-02	Hays	TX
DJM 072	MF 7654	<i>Abronia</i>	<i>fragrans</i>	?	Cottle	TX
DJM 073	MF 7655	<i>Boerhavia</i>	<i>erecta</i>	12-Jun-02	Hays	TX
DJM 074	MF 7656	<i>Mirabilis</i>	<i>multiflora</i>	?	Grant & Socorro	NM
DJM 075	MF 7657	<i>Mirabilis</i>	<i>multiflora</i>	?	Grant & Socorro	NM
DJM 076	MF 7658	<i>Mirabilis</i>	<i>longiflora</i>	?	?	NM
DJM 077	MF 7659	<i>Mirabilis</i>	<i>longiflora</i>	?	?	NM
DJM 078	MF 7660	<i>Abronia</i>	<i>villosa</i>	?	?	?
DJM 079	MF 7661	<i>Abronia</i>	<i>villosa</i>	?	?	?
DJM 080	MF 7662	<i>Abronia</i>	<i>villosa</i>	?	?	?
DJM 081	MF 7663	<i>Abronia</i>	<i>fragrans</i>	?	?	?
DJM 082	MF 7664	<i>Abronia</i>	<i>fragrans</i>	?	?	?
DJM 083	MF 7665	<i>Abronia</i>	<i>fragrans</i>	?	?	?
DJM 084	MF 7666	<i>Mirabilis</i>	<i>longiflora</i>	?	Grant	NM
DJM 085	MF 7667	<i>Mirabilis</i>	<i>longiflora</i>	?	Grant	NM
DJM 086	MF 7668	<i>Mirabilis</i>	<i>longiflora</i>	?	Grant	NM
DJM 087	MF 7669	<i>Mirabilis</i>	<i>longiflora</i>	?	Grant	NM
DJM 088	MF 7670	<i>Mirabilis</i>	<i>longiflora</i>	?	Grant	NM
DJM 089	MF 7671	<i>Mirabilis</i>	<i>multiflora</i>	?	Fremont & Huerfano	CO
DJM 090	MF 7672	<i>Mirabilis</i>	<i>multiflora</i>	?	Fremont & Huerfano	CO
DJM 091	MF 7673	<i>Mirabilis</i>	<i>multiflora</i>	?	Fremont & Huerfano	CO
DJM 092	MF 7674	<i>Mirabilis</i>	<i>multiflora</i>	?	Fremont & Huerfano	CO
DJM 093	MF 7675	<i>Mirabilis</i>	<i>multiflora</i>	?	Fremont & Huerfano	CO
DJM 094	MF 7676	<i>Tripterocalyx</i>	<i>micranthus</i>	?	Saguache	CO
DJM 095	MF 7677	<i>Tripterocalyx</i>	<i>micranthus</i>	?	Saguache	CO
DJM 096	MF 7678	<i>Tripterocalyx</i>	<i>micranthus</i>	?	Saguache	CO
DJM 097	MF 7679	<i>Tripterocalyx</i>	<i>micranthus</i>	?	Saguache	CO
DJM 098	MF 7680	<i>Tripterocalyx</i>	<i>micranthus</i>	?	Saguache	CO
DJM 099	MF 7681	<i>Tripterocalyx</i>	<i>micranthus</i>	?	Saguache	CO
DJM 100	MF 7682	<i>Tripterocalyx</i>	<i>micranthus</i>	?	Saguache	CO
DJM 101	MF 7683	<i>Tripterocalyx</i>	<i>micranthus</i>	?	Saguache	CO
DJM 102	MF 7684	<i>Tripterocalyx</i>	<i>micranthus</i>	?	Saguache	CO
DJM 103	MF 7685	<i>Tripterocalyx</i>	<i>micranthus</i>	?	Saguache	CO
DJM 104	MF 7686	<i>Tripterocalyx</i>	<i>micranthus</i>	?	Saguache	CO
DJM 105	MF 7687	<i>Abronia</i>	<i>fragrans</i>	?	Chaffee & Fremont	CO
DJM 106	MF 7688	<i>Abronia</i>	<i>fragrans</i>	?	Chaffee & Fremont	CO
DJM 107	MF 7689	<i>Abronia</i>	<i>fragrans</i>	?	Chaffee & Fremont	CO
DJM 108	MF 7690	<i>Abronia</i>	<i>fragrans</i>	?	Chaffee & Fremont	CO

DJM #	MF #	Genus	Species	Date Collected	County	State
DJM 109	MF 7691	<i>Abronia</i>	<i>fragrans</i>	?	Chaffee & Fremont	CO
DJM 110	MF 7692	<i>Abronia</i>	<i>fragrans</i>	?	Chaffee & Fremont	CO
DJM 111	MF 7693	<i>Abronia</i>	<i>fragrans</i>	?	Chaffee & Fremont	CO
DJM 112	MF 7694	<i>Abronia</i>	<i>fragrans</i>	?	Chaffee & Fremont	CO
DJM 113	MF 7695	<i>Abronia</i>	<i>fragrans</i>	?	Chaffee & Fremont	CO
DJM 114	MF 7696	<i>Abronia</i>	<i>fragrans</i>	?	Chaffee & Fremont	CO
DJM 115	MF 7697	<i>Acleisanthes</i>	<i>chenopodioides</i>	03-Sep-91	Brewster	TX
DJM 116	MF 7698	<i>Abronia</i>	<i>villosa</i>	?	?	?
DJM 117	MF 7699	<i>Abronia</i>	<i>villosa</i>	?	?	?
DJM 118	MF 7700	<i>Abronia</i>	<i>villosa</i>	?	?	?
DJM 119	MF 7701	<i>Abronia</i>	<i>villosa</i>	?	?	?
DJM 120	MF 7702					
DJM 121	MF 7703	<i>Nyctaginia</i>	<i>capitata</i>	23-Jul-02	Val Verde	TX
DJM 122	MF 7704	<i>Nyctaginia</i>	<i>capitata</i>	23-Jul-02	Val Verde	TX
DJM 123	MF 7705	<i>Nyctaginia</i>	<i>capitata</i>	23-Jul-02	Val Verde	TX
DJM 124	MF 7706	<i>Macrosiphonia</i>	<i>macrosiphon</i> ?	23-Jul-02	Brewster	TX
DJM 125	MF 7707	<i>Macrosiphonia</i>	<i>macrosiphon</i> ?	23-Jul-02	Brewster	TX
DJM 126	MF 7708	<i>Macrosiphonia</i>	<i>macrosiphon</i> ?	23-Jul-02	Brewster	TX
DJM 127	MF 7709	<i>Ipomopsis</i> ?	?	23-Jul-02	Brewster	TX
DJM 128	MF 7710	<i>Ipomopsis</i> ?	?	23-Jul-02	Brewster	TX
DJM 129	MF 7711	<i>Acleisanthes</i>	<i>longiflora</i>	23-Jul-02	Brewster	TX
DJM 130	MF 7712	<i>Quincula</i>	<i>lobata</i>	23-Jul-02	Brewster	TX
DJM 131	MF 7713	Unknown	?	23-Jul-02	Brewster	TX
DJM 132	MF 7714	<i>Allionia</i>	<i>incarnata</i>	24-Jul-02	Brewster	TX
DJM 133	MF 7715	<i>Boerhavia</i>	<i>spicata</i>	24-Jul-02	Brewster	TX
DJM 134	MF 7716	<i>Boerhavia</i>	<i>intermedia</i>	24-Jul-02	Brewster	TX
DJM 135	MF 7717	<i>Boerhavia</i>	<i>spicata</i>	24-Jul-02	Brewster	TX
DJM 136	MF 7718	<i>Boerhavia</i>	<i>gracillima</i>	24-Jul-02	Brewster	TX
DJM 137	MF 7719	<i>Anulocaulis</i>	<i>ertosolenus</i>	24-Jul-02	Brewster	TX
DJM 138	MF 7720	<i>Acleisanthes</i>	<i>longiflora</i>	24-Jul-02	Brewster	TX
DJM 139	MF 7721	<i>Allionia</i>	<i>incarnata</i>	25-Jul-02	Brewster	TX
DJM 140	MF 7722	<i>Anulocaulis</i>	sp	25-Jul-02	Brewster	TX
DJM 141	MF 7723	<i>Boerhavia</i>	<i>coccinea</i>	26-Jul-02	Presidio	TX
DJM 142	MF 7724	<i>Acleisanthes</i>	<i>chenopodioides</i>	26-Jul-02	Presidio	TX
DJM 143	MF 7725	<i>Acleisanthes</i>	<i>longiflora</i>	25-Jul-02	Brewster	TX
DJM 144	MF 7726	<i>Abronia</i>	<i>umbellata</i>	?	?	?
DJM 145	MF 7727	<i>Abronia</i>	<i>umbellata</i>	?	?	?
DJM 146	MF 7728	<i>Abronia</i>	<i>umbellata</i>	?	?	?
DJM 147	MF 7729	<i>Abronia</i>	<i>umbellata</i>	?	?	?
DJM 148	MF 7730	<i>Abronia</i>	<i>umbellata</i>	?	?	?
DJM 149	MF 7731	<i>Abronia</i>	<i>umbellata</i>	?	?	?
DJM 150	MF 7732	<i>Abronia</i>	<i>umbellata</i>	?	?	?
DJM 151	MF 7733	<i>Abronia</i>	<i>umbellata</i>	?	?	?
DJM 152	MF 7734	<i>Abronia</i>	<i>umbellata</i>	?	?	?
DJM 153	MF 7735	<i>Abronia</i>	<i>umbellata</i>	?	?	?
DJM 154	MF 7736	<i>Abronia</i>	<i>villosa</i>	?	?	?
DJM 155	MF 7737	<i>Abronia</i>	<i>villosa</i>	?	?	?
DJM 156	MF 7738	<i>Abronia</i>	<i>villosa</i>	?	?	?
DJM 157	MF 7739	<i>Abronia</i>	<i>villosa</i>	?	?	?
DJM 158	MF 7740	<i>Abronia</i>	<i>villosa</i>	?	?	?
DJM 159	MF 7741	<i>Abronia</i>	<i>villosa</i>	?	?	?
DJM 160	MF 7742	<i>Abronia</i>	<i>villosa</i>	?	?	?
DJM 161	MF 7743	<i>Abronia</i>	<i>villosa</i>	?	?	?
DJM 162	MF 7744	<i>Abronia</i>	<i>villosa</i>	?	?	?
DJM 163	MF 7745	<i>Abronia</i>	<i>villosa</i>	?	?	?
DJM 164	MF 7746	<i>Abronia</i>	<i>villosa</i>	?	?	?
DJM 165	MF 7747	<i>Abronia</i>	<i>villosa</i>	?	?	?
DJM 166	MF 7748	<i>Abronia</i>	<i>villosa</i>	?	?	?
DJM 167	MF 7749	<i>Abronia</i>	<i>villosa</i>	?	?	?
DJM 168	MF 7750	<i>Abronia</i>	<i>villosa</i>	?	?	?
DJM 169	MF 7751	<i>Abronia</i>	<i>villosa</i>	?	?	?
DJM 170	MF 7752	<i>Abronia</i>	<i>villosa</i>	?	?	?
DJM 171	MF 7753	<i>Abronia</i>	<i>villosa</i>	?	?	?
DJM 172	MF 7754	<i>Bougainvillea</i>	sp	14-Aug-02	Hays	TX

DJM #	MF #	Genus	Species	Date Collected	County	State
DJM 173	MF 7755	<i>Abronia</i>	<i>angustifolia</i>	13-Apr-02	Hudspeth	TX
DJM 174	MF 7756	<i>Anulocaulis</i>	<i>leiosolenus</i>	27-Oct-85	Hudspeth	TX
DJM 175	MF 7757	<i>Bougainvillea</i>	<i>glabra</i>	12-Feb-02	Hidalgo	TX
DJM 176	MF 7758	<i>Cyphomeris</i>	<i>gypsophiloides</i>	24-Nov-94	Kinney	TX
DJM 177	MF 7759	<i>Pisonia</i>	<i>aculeata</i>	27-Mar-88	Cameron	TX
DJM 178	MF 7760	<i>Rivina</i>	<i>humilis</i>	18-Jun-82	Robertson	TX
DJM 179	MF 7761	<i>Phytolacca</i>	<i>americana</i>	27-Jun-87	Smith	TX

DJM #	Locality	GPS
DJM 001	Forstner's backyard flower bed closest to house	
DJM 002	Peacevale Ranch, 15 m SE of 1st ⁷ gate, 6 m E of road, near fence, 2625 ft elev	UTM 14310934 E, 3972981 N
DJM 003	Best Western flower bed, Vega	
DJM 004	502 Lindsey St across from duplex, in ditch, San Marcos	
DJM 005	E of steps by condos, Lindsey St, roadside, San Marcos	
DJM 006	502 Lindsey St across from duplex, in ditch, San Marcos	
DJM 007	Hilltop Lakes Resort, hill facing disturbed area, 448 ft elev	N 31d04 511 ft W 096d15 925 ft
DJM 008	Hilltop Lakes Resort, hill facing disturbed area, 448 ft elev	N 31d04 511 ft W 096d15 925 ft
DJM 009	Hilltop Lakes Resort, hill facing disturbed area, 448 ft elev	N 31d04 511 ft W 096d15 925 ft
DJM 010	Hilltop Lakes Resort, hill facing disturbed area, 448 ft elev	N 31d04 511 ft W 096d15 925 ft
DJM 011	Hilltop Lakes Resort, hill facing disturbed area, 448 ft elev	N 31d04 511 ft W 096d15 925 ft
DJM 012	Hilltop Lakes Resort, hill facing disturbed area, 448 ft elev	N 31d04 511 ft W 096d15 925 ft
DJM 013	Hilltop Lakes Resort, hill facing disturbed area, 448 ft elev	N 31d04 511 ft W 096d15 925 ft
DJM 014	Hilltop Lakes Resort, hill facing disturbed area, 448 ft elev	N 31d04 511 ft W 096d15 925 ft
DJM 015	Hilltop Lakes Resort, hill facing disturbed area, 448 ft elev	N 31d04 511 ft W 096d15 925 ft
DJM 016	?	?
DJM 017	Hilltop Lakes Resort, undisturbed area, 428 ft elev	N 31d04 511 ft W 096d15 925 ft
DJM 018	Hilltop Lakes Resort, undisturbed area, 428 ft elev	N 31d04 511 ft W 096d15 925 ft
DJM 019	Hilltop Lakes Resort, undisturbed area, 428 ft elev	N 31d04 511 ft W 096d15 925 ft
DJM 020	Hilltop Lakes Resort, undisturbed area, 428 ft elev	N 31d04 511 ft W 096d15 925 ft
DJM 021	Hilltop Lakes Resort, undisturbed area, 428 ft elev	N 31d04 511 ft W 096d15 925 ft
DJM 022	Hilltop Lakes Resort, undisturbed area, 443 ft elev	N 31d04 426 ft W 096d11 868 ft
DJM 023	Hilltop Lakes Resort, undisturbed area, 443 ft elev	N 31d04 426 ft W 096d11 868 ft
DJM 024	Hilltop Lakes Resort, undisturbed area, 443 ft elev	N 31d04 426 ft W 096d11 868 ft
DJM 025	Hilltop Lakes Resort, undisturbed area, 443 ft elev	N 31d04 426 ft W 096d11 868 ft
DJM 026	Hilltop Lakes Resort, undisturbed area, 443 ft elev	N 31d04 426 ft W 096d11 868 ft
DJM 027	Winstead/Petty/Carrington Ranch, S end, 437 ft elev, N of Marquez heading N on US 79 turn left onto 1146, go past Historical marker, gate is on right, Petty Ranch sign is too far	N 31d18 669 ft W 096d15 949 ft
DJM 028	Winstead/Petty/Carrington Ranch, S end, 437 ft elev	N 31d18 669 ft W 096d15 949 ft
DJM 029	Winstead/Petty/Carrington Ranch, S end, 437 ft elev	N 31d18 669 ft W 096d15 949 ft
DJM 030	Winstead/Petty/Carrington Ranch, S end, 437 ft elev	N 31d18 669 ft W 096d15 949 ft
DJM 031	Winstead/Petty/Carrington Ranch, S end, 437 ft elev	N 31d18 669 ft W 096d15 949 ft
DJM 032	Winstead/Petty/Carrington Ranch, S end, 437 ft elev	N 31d18 669 ft W 096d15 949 ft
DJM 033	Winstead/Petty/Carrington Ranch, S end, 437 ft elev	N 31d18 669 ft W 096d15 949 ft
DJM 034	Winstead/Petty/Carrington Ranch, S end, 437 ft elev	N 31d18 669 ft W 096d15 949 ft
DJM 035	Winstead/Petty/Carrington Ranch, S end, 437 ft elev	N 31d18 669 ft W 096d15 949 ft
DJM 036	Winstead/Petty/Carrington Ranch, S end, 437 ft elev	N 31d18 669 ft W 096d15 949 ft
DJM 037	Winstead/Petty/Carrington Ranch, N end, 425 ft elev	N 31d18 781 ft W 096d15 936 ft
DJM 038	Winstead/Petty/Carrington Ranch, N end, 425 ft elev	N 31d18 781 ft W 096d15 936 ft
DJM 039	Winstead/Petty/Carrington Ranch, N end, 425 ft elev	N 31d18 781 ft W 096d15 936 ft
DJM 040	Winstead/Petty/Carrington Ranch, N end, 425 ft elev	N 31d18 781 ft W 096d15 936 ft
DJM 041	Winstead/Petty/Carrington Ranch, N end, 425 ft elev	N 31d18 781 ft W 096d15 936 ft
DJM 042	Winstead/Petty/Carrington Ranch, N end, 425 ft elev	N 31d18 781 ft W 096d15 936 ft
DJM 043	Winstead/Petty/Carrington Ranch, N end, 425 ft elev	N 31d18 781 ft W 096d15 936 ft
DJM 044	Winstead/Petty/Carrington Ranch, N end, 425 ft elev	N 31d18 781 ft W 096d15 936 ft
DJM 045	Winstead/Petty/Carrington Ranch, N end, 425 ft elev	N 31d18 781 ft W 096d15 936 ft
DJM 046	Winstead/Petty/Carrington Ranch, N end, 425 ft elev	N 31d18 781 ft W 096d15 936 ft
DJM 047	Hilltop Lakes Resort, undisturbed area, 443 ft elev	N 31d04 426 ft W 096d11 868 ft
DJM 048	Winstead/Petty/Carrington Ranch, S end, 437 ft elev	N 31d18 669 ft W 096d15 949 ft
DJM 049	very S end of Kenedy Co Rest Area on US 77, about 6 mi S of Santa Elementary School	
DJM 050	N end of Brooks Co Rest Area on US 281, on E side, 10.6 mi S of junction of nonbusiness US 281 and State 285, 3-4 individuals next to a smelly big puddle about 1 ft deep	
DJM 051	N end of Brooks Co Rest Area on US 281, on E side, 10.6 mi S of junction of nonbusiness US 281 and State 285, 3-4 individuals next to a smelly big puddle about 1 ft deep	
DJM 052	N end of Brooks Co Rest Area on US 281, on E side, 10.6 mi S of junction of nonbusiness US 281 and State 285, 3-4 individuals next to a smelly big puddle about 1 ft deep	
DJM 053	very N end of Brooks Co Rest Area on US 281 but closer to crossover, 10.6 mi S of junction of nonbusiness US 281 and State 285, 30+ individuals under mesquite?	
DJM 054	very N end of Brooks Co Rest Area on US 281 but closer to crossover, 10.6 mi S of junction of nonbusiness US 281 and State 285, 30+ individuals under mesquite?	
DJM 055	very N end of Brooks Co Rest Area on US 281 but closer to crossover, 10.6 mi S of junction of nonbusiness US 281 and State 285, 30+ individuals under mesquite?	
DJM 056	very N end of Brooks Co Rest Area on US 281 but closer to crossover, 10.6 mi S of junction of nonbusiness US 281 and State 285, 30+ individuals under mesquite?	
DJM 057	very N end of Brooks Co Rest Area on US 281 but closer to crossover, 10.6 mi S of junction of nonbusiness US 281 and State 285, 30+ individuals under mesquite?	
DJM 058	very N end of Brooks Co Rest Area on US 281 but closer to crossover, 10.6 mi S of junction of nonbusiness US 281 and State 285, 30+ individuals under mesquite?	

DJM #	Locality	GPS
DJM 059	very N end of Brooks Co Rest Area on US 281 but closer to crossover, 10 6 mi S of junction of nonbusiness US 281 and State 285, 30+ individuals under mesquite?	
DJM 060	roadside, sand dunes on N side of Ranch Road 793, ~0.7 mi W of junction of 793 and I-10, E of road to Fabens airport	
DJM 061	roadside, sand dunes on N side of Ranch Road 793, ~0.7 mi W of junction of 793 and I-10, E of road to Fabens airport	
DJM 062	roadside, sand dunes on N side of Ranch Road 793, ~0.7 mi W of junction of 793 and I-10, E of road to Fabens airport	
DJM 063	roadside, sand dunes on N side of Ranch Road 793, ~0.7 mi W of junction of 793 and I-10, E of road to Fabens airport	
DJM 064	roadside, sand dunes on N side of Ranch Road 793, ~0.7 mi W of junction of 793 and I-10, E of road to Fabens airport	
DJM 065	roadside, sand dunes on N side of Ranch Road 793, ~0.7 mi W of junction of 793 and I-10, E of road to Fabens airport	
DJM 066	roadside, in asphalt on E side of Ranch Road 1108, ~0.5 mi S of junction of 1108 and Ranch Road 652	
DJM 067	roadside, E side of Park Road 41, sandy area just outside gate to Monahans Sandhills State Park	
DJM 068	roadside, sand dunes on N side of access road to I-20, ~0.7 mi E of junction of Park Road 41 and I-20	
DJM 069	roadside, sand dunes on N side of access road to I-20, ~0.7 mi E of junction of Park Road 41 and I-20	
DJM 070	roadside, sand dunes on N side of access road to I-20, ~0.7 mi E of junction of Park Road 41 and I-20	
DJM 071	502 Lindsey St across from duplex, in ditch, San Marcos	
DJM 072	Matador Wildlife Management Area	
DJM 073	in flower bed, N entrance to SCL, SWT campus, San Marcos	
DJM 074	purchased from Plants of the Southwest seed catalog, Mark Myers	
DJM 075	purchased from Plants of the Southwest seed catalog, Mark Myers	
DJM 076	purchased from Plants of the Southwest seed catalog, Mark Myers, cultivated for a couple years	
DJM 077	purchased from Plants of the Southwest seed catalog, Mark Myers, cultivated for a couple years	
DJM 078	purchased from Plants of the Southwest seed catalog, Mark Myers, purchased from S CA seed company, maybe wild collected there	
DJM 079	purchased from Plants of the Southwest seed catalog, Mark Myers, purchased from S CA seed company, maybe wild collected there	
DJM 080	purchased from Plants of the Southwest seed catalog, Mark Myers, purchased from S CA seed company, maybe wild collected there	
DJM 081	purchased from Plants of the Southwest seed catalog, Mark Myers, original unknown, mine from their property in Sante Fe Co NM	
DJM 082	purchased from Plants of the Southwest seed catalog, Mark Myers, original unknown, mine from their property in Sante Fe Co NM	
DJM 083	purchased from Plants of the Southwest seed catalog, Mark Myers, original unknown, mine from their property in Sante Fe Co NM	
DJM 084	purchased from Western Native Seed catalog, Alex Tonnesen, 4600 ft elev	
DJM 085	purchased from Western Native Seed catalog, Alex Tonnesen, 4600 ft elev	
DJM 086	purchased from Western Native Seed catalog, Alex Tonnesen, 4600 ft elev	
DJM 087	purchased from Western Native Seed catalog, Alex Tonnesen, 4600 ft elev	
DJM 088	purchased from Western Native Seed catalog, Alex Tonnesen, 4600 ft elev	
DJM 089	purchased from Western Native Seed catalog, Alex Tonnesen, 7000 ft elev	
DJM 090	purchased from Western Native Seed catalog, Alex Tonnesen, 7000 ft elev	
DJM 091	purchased from Western Native Seed catalog, Alex Tonnesen, 7000 ft elev	
DJM 092	purchased from Western Native Seed catalog, Alex Tonnesen, 7000 ft elev	
DJM 093	purchased from Western Native Seed catalog, Alex Tonnesen, 7000 ft elev	
DJM 094	purchased from, Western Native Seed catalog, Alex Tonnesen, 6800-8000 ft elev	
DJM 095	purchased from, Western Native Seed catalog, Alex Tonnesen, 6800-8000 ft elev	
DJM 096	purchased from, Western Native Seed catalog, Alex Tonnesen, 6800-8000 ft elev	
DJM 097	purchased from, Western Native Seed catalog, Alex Tonnesen, 6800-8000 ft elev	
DJM 098	purchased from, Western Native Seed catalog, Alex Tonnesen, 6800-8000 ft elev	
DJM 099	purchased from, Western Native Seed catalog, Alex Tonnesen, 6800-8000 ft elev	
DJM 100	purchased from, Western Native Seed catalog, Alex Tonnesen, 6800-8000 ft elev	
DJM 101	purchased from, Western Native Seed catalog, Alex Tonnesen, 6800-8000 ft elev	
DJM 102	purchased from, Western Native Seed catalog, Alex Tonnesen, 6800-8000 ft elev	
DJM 103	purchased from, Western Native Seed catalog, Alex Tonnesen, 6800-8000 ft elev	
DJM 104	purchased from, Western Native Seed catalog, Alex Tonnesen, 6800-8000 ft elev	
DJM 105	purchased from Western Native Seed catalog, Alex Tonnesen, 8400 ft elev	
DJM 106	purchased from Western Native Seed catalog, Alex Tonnesen, 8400 ft elev	
DJM 107	purchased from Western Native Seed catalog, Alex Tonnesen, 8400 ft elev	
DJM 108	purchased from Western Native Seed catalog, Alex Tonnesen, 8400 ft elev	

DJM #	Locality	GPS
DJM 109	purchased from Western Native Seed catalog, Alex Tonnesen, 8400 ft elev	
DJM 110	purchased from Western Native Seed catalog, Alex Tonnesen, 8400 ft elev	
DJM 111	purchased from Western Native Seed catalog, Alex Tonnesen, 8400 ft elev	
DJM 112	purchased from Western Native Seed catalog, Alex Tonnesen, 8400 ft elev	
DJM 113	purchased from Western Native Seed catalog, Alex Tonnesen, 8400 ft elev	
DJM 114	purchased from Western Native Seed catalog, Alex Tonnesen, 8400 ft elev	
DJM 115	1 2 mi N on TX 118 from its jct w/ FR 170, N of Study Butte, 2950 ft elev	
DJM 116	purchased from Plants of the Southwest seed catalog, Mark Myers, cultivated for a couple years	
DJM 117	purchased from Plants of the Southwest seed catalog, Mark Myers, cultivated for a couple years	
DJM 118	purchased from Plants of the Southwest seed catalog, Mark Myers, cultivated for a couple years	
DJM 119	purchased from Plants of the Southwest seed catalog, Mark Myers, cultivated for a couple years	
DJM 120		
DJM 121	roadside on N side of US 90, 20 mi W of Del Rio, 6 mi E of jct US 90 & TX 163, 1412 ft elev, ~20 individuals	N 29d37'18 0" W 101d06'32 3"
DJM 122	roadside on N side of US 90, 20 mi W of Del Rio, 6 mi E of jct US 90 & TX 163, 1412 ft elev, ~20 individuals	N 29d37'18 0" W 101d06'32 3"
DJM 123	roadside on N side of US 90, 20 mi W of Del Rio, 6 mi E of jct US 90 & TX 163, 1412 ft elev, ~20 individuals	N 29d37'18 0" W 101d06'32 3"
DJM 124	roadside on US 90, 99 mi W of jct US 90 & TX 163, 3914 ft elev	N 30d10'30 4" W 102d47'48"
DJM 125	roadside on US 90, 99 mi W of jct US 90 & TX 163, 3914 ft elev	N 30d10'30 4" W 102d47'48"
DJM 126	roadside on US 90, 99 mi W of jct US 90 & TX 163, 3914 ft elev	N 30d10'30 4" W 102d47'48"
DJM 127	roadside on US 90, 2 mi E of jct 118 & US 90, 3931 ft elev	N 30d23'01 6" W 103d33'52 0"
DJM 128	roadside on US 90, 2 mi E of jct 118 & US 90, 3931 ft elev	N 30d23'01 6" W 103d33'52 0"
DJM 129	roadside on US 90, 2 mi E of jct 118 & US 90, 3931 ft elev	N 30d23'01 6" W 103d33'52 0"
DJM 130	roadside on US 90, 2 mi E of jct 118 & US 90, 3931 ft elev	N 30d23'01 6" W 103d33'52 0"
DJM 131	roadside on US 90, 2 mi E of jct 118 & US 90, 3931 ft elev	N 30d23'01 6" W 103d33'52 0"
DJM 132	roadside on E side of N County Road (off of 118, turnoff is 10 mi N of Study Butte) 0.5 mi N of Frenchman's, 3269 ft elev	N 29d27'42 0" W 103d32'58 4"
DJM 133	Lajitas Cemetery on Hwy 170, 2422 ft elev	N 29d15'42 9" W 103d46'30 4"
DJM 134	Lajitas Cemetery on Hwy 170, 2422 ft elev	N 29d15'42 9" W 103d46'30 4"
DJM 135	Lajitas Cemetery on Hwy 170, 2422 ft elev	N 29d15'42 9" W 103d46'30 4"
DJM 136	Lajitas Cemetery on Hwy 170, 2422 ft elev	N 29d15'42 9" W 103d46'30 4"
DJM 137	Lajitas Cemetery on Hwy 170, 2422 ft elev	N 29d15'42 9" W 103d46'30 4"
DJM 138	near parking lot pf Easter Egg Motel near Study Butte on 170, 1 7 mi W of jct 118 & 170, 2422 ft elev	N 29d19'27 9" W 103d32'53 0"
DJM 139	near parking lot pf Easter Egg Motel near Study Butte on 170, 1 7 mi W of jct 118 & 170, 2422 ft elev	N 29d19'27 9" W 103d32'53 0"
DJM 140	in Big Bend National Park	
DJM 141	roadside on 169, 2 mi SE of jct Hwy 169 & US 67, 2580 ft elev	N 30d09'47 8" W 104d02'51 5"
DJM 142	roadside on 169, 2 mi SE of jct Hwy 169 & US 67, 2580 ft elev	N 30d09'47 8" W 104d02'51 5"
DJM 143	1 mi W of Park gate on Hwy 118, 0 25 mi N of road in dry creek bed	
DJM 144	purchased from Theodore Payne Foundation catalog	
DJM 145	purchased from Theodore Payne Foundation catalog	
DJM 146	purchased from Theodore Payne Foundation catalog	
DJM 147	purchased from Theodore Payne Foundation catalog	
DJM 148	purchased from Theodore Payne Foundation catalog	
DJM 149	purchased from Theodore Payne Foundation catalog	
DJM 150	purchased from Theodore Payne Foundation catalog	
DJM 151	purchased from Theodore Payne Foundation catalog	
DJM 152	purchased from Theodore Payne Foundation catalog	
DJM 153	purchased from Theodore Payne Foundation catalog	
DJM 154	purchased from Theodore Payne Foundation catalog	
DJM 155	purchased from Theodore Payne Foundation catalog	
DJM 156	purchased from Theodore Payne Foundation catalog	
DJM 157	purchased from Theodore Payne Foundation catalog	
DJM 158	purchased from Theodore Payne Foundation catalog	
DJM 159	purchased from Theodore Payne Foundation catalog	
DJM 160	purchased from Theodore Payne Foundation catalog	
DJM 161	purchased from Theodore Payne Foundation catalog	
DJM 162	purchased from Theodore Payne Foundation catalog	
DJM 163	purchased from Theodore Payne Foundation catalog	
DJM 164	purchased from Theodore Payne Foundation catalog	
DJM 165	purchased from Theodore Payne Foundation catalog	
DJM 166	purchased from Theodore Payne Foundation catalog	
DJM 167	purchased from Theodore Payne Foundation catalog	
DJM 168	purchased from Theodore Payne Foundation catalog	
DJM 169	purchased from Theodore Payne Foundation catalog	
DJM 170	purchased from Theodore Payne Foundation catalog	
DJM 171	purchased from Theodore Payne Foundation catalog	
DJM 172	Dr Koehn's? houseplant	

DJM #	Locality	GPS
DJM 173	gypsum sand dunes, Salt Basin Dunes, Guadalupe National Park	501560 E, 3531004 N
DJM 174	near Rio Grande 13.5 rd mi (10 air mi) NW of Indian Hot Springs on the Cretaceous Benevides formation of calcareous shale, 3475 ft elev	N 31d56' W 105d27'30"
DJM 175	Holcomb Airfield, W side of FM 490, McCook, rocky soil of Rio Grande Valley	
DJM 176	Kickapoo Cavern State Park, approx 22 mi N of Brackettville on Ranch Rd 674, occasional herbs on open, dry, rocky, limestone soil along dry washes near main park entrance road	
DJM 177	along roadside of Old Port Isabel Rd, 0.5 mi S of jct w/ FM 511, large shrub 2.5 m tall	
DJM 178	SE 1/4 of the NW 1/4 Marquez Quad, USGS 1965, Camp Cooley Ranch, Navasota River woodlands, 4.1 mi N-NE of Ridge	
DJM 179	in abandoned field off Rhone's Quarters Rd, 4.6 mi S of jct w/ Hwy 64, sandy soil	

DJM #	Additional information	Tissue collected	Tissue used in DNA extr	date of DNA extraction	DNA extr protocol used	PCR	Included in alignment
DJM 001		leaf	leaf	28-Feb-02, 21-Mar-02	Sambrook	yes	yes
DJM 002	with sand sagebrush, sunflower, creek plum, ragweed, buckwheat	leaf	leaf	21-Mar-02, 4-Apr-02	Sambrook	yes	yes
DJM 003		leaf	leaf	28-Feb-02, 21-Mar-02	Sambrook	yes	yes
DJM 004		leaf	leaf	09-Apr-02	Sambrook	no	
DJM 005		leaf	leaf	28-Feb-02	Sambrook	yes	
DJM 006		leaf	leaf	09-Apr-02	Sambrook	no	
DJM 007		leaf	leaf	28-Feb-02, 21-Mar-02	Sambrook	yes	
DJM 008		leaf	leaf	28-Feb-02, 21-Mar-02	Sambrook	yes	yes
DJM 009		leaf	leaf	28-Feb-02, 21-Mar-02	Sambrook	yes	
DJM 010		leaf	leaf	28-Feb-02, 21-Mar-02	Sambrook	yes	
DJM 011		leaf	leaf	28-Feb-02	Sambrook	yes	
DJM 012	using as PCR positive	leaf	leaf	28-Feb-02	Sambrook	yes	
DJM 013		leaf	leaf	28-Feb-02, 21-Mar-02	Sambrook	yes	
DJM 014		leaf	leaf	28-Feb-02, 21-Mar-02	Sambrook	yes	
DJM 015		leaf	leaf	09-Apr-02	Sambrook	yes	
DJM 016	herbarium specimen SWT, Rasme y 63, 016821, 0 25 g lf tissue	n/a	leaf	04-Apr-02	Sambrook		
DJM 017		leaf	leaf	19-Apr-02	Sambrook	yes	
DJM 018		leaf	leaf	21-Apr-02	Sambrook	yes	
DJM 019		leaf	leaf	22-Apr-02	Sambrook	yes	
DJM 020		leaf	leaf	23-Apr-02	Sambrook	yes	
DJM 021		leaf	leaf	17-Jul-02	Kang	yes	
DJM 022		leaf					
DJM 023	took photo looking W from GPS spot	leaf					
DJM 024		leaf					
DJM 025		leaf					
DJM 026		leaf					
DJM 027		leaf	leaf	19-Apr-02	Sambrook	yes	
DJM 028		leaf	leaf	21-Apr-02	Sambrook	yes	
DJM 029		leaf	leaf	22-Apr-02	Sambrook	yes	
DJM 030		leaf	leaf	23-Apr-02	Sambrook	yes	
DJM 031		leaf					
DJM 032		leaf					
DJM 033		leaf					
DJM 034		leaf					
DJM 035		leaf					
DJM 036		leaf					
DJM 037	white flowers	leaf					
DJM 038	white flowers	leaf					
DJM 039		leaf					
DJM 040		leaf					
DJM 041		leaf					
DJM 042		leaf					
DJM 043		leaf					
DJM 044		leaf					
DJM 045		leaf					
DJM 046		leaf					
DJM 047	voucher specimen only, no tissue, D J McHenry & J R Mittelhauser collectors	voucher					
DJM 048	voucher specimen only, no tissue, D J McHenry & J R Mittelhauser collectors	voucher					
DJM 049	was mowed, no voucher	leaf					
DJM 050		leaf	leaf	19-Apr-02	Sambrook	yes	yes
DJM 051	voucher specimen, D J McHenry collector	voucher, leaf	leaf	21-Apr-02	Sambrook	yes	
DJM 052		leaf	leaf	22-Apr-02	Sambrook	yes	
DJM 053	~100 m N of smelly puddle population	leaf	leaf	19-Apr-02	Sambrook	yes	
DJM 054		leaf	leaf	21-Apr-02	Sambrook	yes	
DJM 055		leaf	leaf	22-Apr-02	Sambrook	yes	
DJM 056		leaf	leaf	23-Apr-02	Sambrook	yes	
DJM 057		leaf	leaf	23-Apr-02	Sambrook	yes	
DJM 058		leaf	leaf	24-May-02	Sambrook	yes	

DJM #	Additional information	Tissue collected	Tissue used in DNA extr.	date of DNA extraction	DNA extr. protocol used	PCR	Included in alignment
DJM 059		leaf	leaf	17-Jul-02	Kang	yes	
DJM 060	voucher specimen, D J McHenry collector	voucher, leaf	leaf	22-May-02	Sambrook	yes	yes
DJM 061		leaf	leaf	22-May-02	Sambrook	yes	
DJM 062		leaf	leaf	22-May-02	Sambrook	yes	
DJM 063		leaf	leaf	23-May-02	Sambrook	yes	
DJM 064		leaf	leaf	23-May-02	Sambrook	yes	
DJM 065		leaf	leaf	23-May-02	Sambrook	yes	
DJM 066	voucher specimen, D J McHenry collector	voucher, leaf	leaf	23-May-02	Sambrook	yes	yes
DJM 067	voucher specimen, D J McHenry collector	voucher, leaf	leaf	23-May-02	Sambrook	yes	
DJM 068	voucher specimen, D J McHenry collector	voucher, leaf	leaf	23-May-02	Sambrook	yes	
DJM 069		leaf	leaf	24-May-02	Sambrook	yes	
DJM 070		leaf	leaf	24-May-02	Sambrook	yes	
DJM 071	voucher specimen, D J McHenry collector, photos	voucher, leaf	leaf	28-Aug-02	Kang	yes	yes
DJM 072	voucher specimen only, no tissue, K Burke & R Simpson collectors	voucher	leaf	17-Jul-02	Kang	yes	
DJM 073	voucher specimen, D J McHenry collector	voucher, leaf	leaf	17-Jul-02	Kang	yes	yes
DJM 074	1 seed, no voucher, no tissue left, using as PCR positive	n/a	seed	19-Jun-02	Kang	yes	
DJM 075	1 seed, no voucher, no tissue left	n/a	seed	19-Jun-02	Kang	yes	
DJM 076	1 seed, no voucher, no tissue left	n/a	seed	19-Jun-02	Kang	yes	yes
DJM 077	1 seed, no voucher, no tissue left	n/a	seed	19-Jun-02	Kang	yes	
DJM 078	1 seed, no voucher, no tissue left	n/a	seed	19-Jun-02	Kang	yes	
DJM 079	1 seed, no voucher, no tissue left	n/a	seed	19-Jun-02	Kang	yes	
DJM 080	1 seed, no voucher, no tissue left	n/a	seed	19-Jun-02	Kang	yes	yes
DJM 081	1 seed, no voucher, no tissue left	n/a	seed	19-Jun-02	Kang	yes	yes
DJM 082	1 seed, no voucher, no tissue left	n/a	seed	no DNA left	Kang		
DJM 083	1 seed, no voucher, no tissue left	n/a	seed	no DNA left	Kang		
DJM 084	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang	yes	
DJM 085	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang	yes	
DJM 086	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang	yes	
DJM 087	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang	yes	
DJM 088	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang	yes	
DJM 089	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang	yes	
DJM 090	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang	yes	
DJM 091	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang	yes	
DJM 092	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang	yes	
DJM 093	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang	yes	
DJM 094	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang	yes	
DJM 095	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang		
DJM 096	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang		
DJM 097	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang	yes	
DJM 098	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang		
DJM 099	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang	yes	yes
DJM 100	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang		
DJM 101	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang		
DJM 102	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang		
DJM 103	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang		
DJM 104	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang	yes	
DJM 105	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang	yes	
DJM 106	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang		
DJM 107	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang	yes	
DJM 108	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang	yes	

DJM #	Additional information	Tissue collected	Tissue used in DNA extr.	date of DNA extraction	DNA extr protocol used	PCR	Included in alignment
DJM 109	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang	yes	
DJM 110	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang	yes	
DJM 111	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang	yes	
DJM 112	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang	yes	
DJM 113	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang	yes	
DJM 114	1 seed, no voucher, no tissue left	n/a	seed	23-Jun-02	Kang	yes	
DJM 115	herbarium specimen SWT, S & G Jones 7635, took 1 seed 16-Jul-02	n/a	seed	17-Jul-02	Kang	yes	yes
DJM 116	1 seed, no voucher, no tissue left	n/a	seed	17-Jul-02	Kang	yes	
DJM 117	1 seed, no voucher, no tissue left	n/a	seed	17-Jul-02	Kang	yes	
DJM 118	1 seed, no voucher, no tissue left	n/a	seed	17-Jul-02	Kang		
DJM 119	1 seed, no voucher, no tissue left	n/a	seed	17-Jul-02	Kang	yes	
DJM 120							
DJM 121	voucher specimen, D J McHenry & M R J Forstner collectors	voucher, leaf	leaf	28-Jul-02	Kang	yes	yes
DJM 122		leaf, seed	leaf	28-Jul-02	Kang	yes	
DJM 123		leaf, seed	leaf	28-Jul-02	Kang	yes	
DJM 124	voucher specimen, D J McHenry & M R J Forstner collectors, Apocynaceae	voucher, leaf					
DJM 125	Apocynaceae	leaf					
DJM 126	Apocynaceae	leaf, seed					
DJM 127	voucher specimen, D J McHenry & M R J Forstner collectors, yellow flower	voucher, leaf, seed					
DJM 128	yellow flower	leaf, seed					
DJM 129	voucher specimen, D J McHenry & M R J Forstner collectors	voucher, leaf	leaf	28-Jul-02	Kang	yes	
DJM 130	voucher specimen, Solanaceae, puffy fruit, D J McHenry & M R J Forstner collectors	voucher, leaf, seed					
DJM 131	voucher specimen, puffy fruit, D J McHenry & M R J Forstner collectors	voucher, leaf, seed					
DJM 132	voucher specimen, D J McHenry & M R J Forstner collectors	voucher, leaf	leaf	28-Jul-02	Kang	yes	yes
DJM 133	voucher specimen, fr, D J McHenry & M R J Forstner collectors	voucher, leaf	leaf	29-Jul-02	Kang	yes	yes
DJM 134	voucher specimen, fl, D J McHenry & M R J Forstner collectors	voucher, leaf	leaf	29-Jul-02	Kang	yes	yes
DJM 135	same as DJM 133 but is in fl, voucher specimen, D J McHenry & M R J Forstner collectors	voucher, leaf	leaf	28-Aug-02	Kang	yes	
DJM 136	voucher specimen, no tissue?, big fl, D J McHenry & M R J Forstner collectors	voucher	leaf	19-Sep-02	Kang	yes	yes
DJM 137	voucher specimen, D J McHenry & M R J Forstner collectors	voucher, leaf	leaf	28-Jul-02	Kang	yes	yes
DJM 138	voucher specimen, D J McHenry & M R J Forstner collectors	voucher, leaf	leaf	19-Sep-02	Kang	yes	yes
DJM 139	voucher specimen, D J McHenry & M R J Forstner collectors	voucher, leaf	leaf	29-Aug-02	Kang	yes	
DJM 140		leaf	leaf	29-Jul-02	Kang	yes	
DJM 141	voucher specimen, D J McHenry & M R J Forstner collectors	voucher, leaf	leaf	28-Aug-02	Kang	yes	yes
DJM 142	voucher specimen, D J McHenry & M R J Forstner collectors	voucher, leaf	leaf	28-Jul-02	Kang	yes	
DJM 143		leaf	leaf	28-Jul-02	Kang	yes	
DJM 144	var umbellata?, 1 seed, no voucher, no tissue left	n/a	seed	28-Jul-02	Kang	yes	yes
DJM 145	var umbellata?, 1 seed, no voucher, no tissue left	n/a	seed	28-Jul-02	Kang	yes	
DJM 146	var umbellata?, 1 seed, no voucher, no tissue left	n/a	seed	28-Jul-02	Kang	yes	
DJM 147	var umbellata?, 1 seed, no voucher, no tissue left	n/a	seed	28-Jul-02	Kang		
DJM 148	var umbellata?, 1 seed, no voucher, no tissue left	n/a	seed	28-Jul-02	Kang		
DJM 149	var umbellata?, 1 seed, no voucher, no tissue left	n/a	seed	28-Jul-02	Kang		
DJM 150	var umbellata?, 1 seed, no voucher, no tissue left	n/a	seed	29-Jul-02	Kang		
DJM 151	var umbellata?, 1 seed, no voucher, no tissue left	n/a	seed	29-Jul-02	Kang		
DJM 152	var umbellata?, 1 seed, no voucher, no tissue left	n/a	seed	29-Jul-02	Kang	yes	
DJM 153	var umbellata?, 1 seed, no voucher, no tissue left	n/a	seed	29-Jul-02	Kang	yes	
DJM 154	var aurita, 1 seed, no voucher, no tissue left	n/a	seed	29-Jul-02	Kang	yes	yes
DJM 155	var aurita, 1 seed, no voucher, no tissue left	n/a	seed	29-Jul-02	Kang	yes	
DJM 156	var aurita, 1 seed, no voucher, no tissue left	n/a	seed	29-Jul-02	Kang	yes	
DJM 157	var aurita, 1 seed, no voucher, no tissue left	n/a	seed	29-Jul-02	Kang	yes	
DJM 158	var aurita, 1 seed, no voucher, no tissue left	n/a	seed	29-Jul-02	Kang		
DJM 159	var aurita, 1 seed, no voucher, no tissue left	n/a	seed	29-Jul-02	Kang	yes	
DJM 160	var aurita, 1 seed, no voucher, no tissue left	n/a	seed	29-Jul-02	Kang	yes	
DJM 161	var aurita, 1 seed, no voucher, no tissue left	n/a	seed	29-Jul-02	Kang	yes	
DJM 162	1 seed, no voucher, no tissue left	n/a	seed	29-Jul-02	Kang		
DJM 163	1 seed, no voucher, no tissue left	n/a	seed	29-Jul-02	Kang		
DJM 164	1 seed, no voucher, no tissue left	n/a	seed	29-Jul-02	Kang		
DJM 165	1 seed, no voucher, no tissue left	n/a	seed	29-Jul-02	Kang	yes	
DJM 166	1 seed, no voucher, no tissue left	n/a	seed	29-Jul-02	Kang	yes	
DJM 167	1 seed, no voucher, no tissue left	n/a	seed	29-Jul-02	Kang	yes	
DJM 168	1 seed, no voucher, no tissue left	n/a	seed	29-Jul-02	Kang		
DJM 169	1 seed, no voucher, no tissue left	n/a	seed	29-Jul-02	Kang	yes	
DJM 170	1 seed, no voucher, no tissue left	n/a	seed	29-Jul-02	Kang	yes	
DJM 171	1 seed, no voucher, no tissue left	n/a	seed	29-Jul-02	Kang	yes	
DJM 172	no voucher	leaf	leaf	28-Aug-02	Kang	yes	yes

DJM #	Additional information	Tissue collected	Tissue used in DNA extr.	date of DNA extraction	DNA extr. protocol used	PCR	Included in alignment
DJM 173	voucher specimen, JMM-020413-2, several plants, NAD 27 for GPS, James Mueller collector	voucher, leaf	leaf	28-Aug-02	Kang	yes	yes
DJM 174	herbarium specimen SWT, R D Worthington 13843, Ex Herb UTEP, took 1 fruit (did not have persistent floral parts) 18-Sep-02, no tissue left	n/a	seed	19-Sep-02	Kang	yes	yes
DJM 175	herbarium specimen SWT, R R Garcia 012, took 1 lf (1 cm x 1 cm) 18-Sep-02, no tissue left	n/a	leaf	19-Sep-02	Kang	yes	
DJM 176	herbarium specimen SWT, David E Lemke 4284b, took 1 fruit 18-Sep-02, no tissue left	n/a	seed	19-Sep-02	Kang	yes	yes
DJM 177	herbarium specimen SWT, D E Lemke 1492, took part of a lf (1 cm x 1 cm) 18-Sep-02, no tissue left	n/a	leaf	19-Sep-02	Kang	yes	yes
DJM 178	herbarium specimen SWT, Tom Starbuck 2436, took 1 fruit 18-Sep-02, no tissue left	n/a	seed	19-Sep-02	Kang	yes	yes
DJM 179	herbarium specimen SWT, C S Berkhout 025, took 1 fruiting inf (w/ many seeds) 18-Sep-02, outgroup, Phytolaccaceae	n/a	seed	19-Sep-02	Kang	yes	yes

APPENDIX 2.

Final alignment of the thirty-one consensus sequences included in subsequent phylogenetic analyses.

Columns are nucleotide positions, and rows are genotypes/taxa. Nucleotide position numbers are indicated above the sequence for Riv hum 178. Gaps are indicated by “-“. At each position, a nucleotide matching that of Riv hum 178 is indicated by “.”.

Abbreviations of taxa are (see also Table 5): Abr ame = *Abronia ameliae*, Abr ang = *A. angustifolia*, Abr fra = *A. fragrans*, Abr mac = *A. macrocarpa*, Abr umb = *A. umbellata*, Abr vil = *A. villosa*, Abr vil aur = *A. villosa* var. *aurita*, Acl che = *Acleisanthes chenopodioides*, Acl lon = *A. longiflora*, All inc = *Allionia incarnata*, Anu eri = *Anulocaulis eriosolenus*, Anu lei = *A. leiosolenus*, Boe coc = *Boerhavia coccinea*, Boe ere = *B. erecta*, Boe gra = *B. gracillima*, Boe int = *B. intermedia*, Boe spi = *B. spicata*, Bou sp. = *Bougainvillea* sp., Cyp gyp = *Cyphomeris gypsophiloides*, Mir jal = *Mirabilis jalapa*, Mir lon = *M. longiflora*, Mir mul = *M. multiflora*, Nyc cap = *Nyctaginia capitata*, Phy ame = *Phytolacca americana*, Pis acu = *Pisonia aculeata*, Riv hum = *Rivina humilis*, Tri car = *Tripterocalyx carnea*, Tri mic = *T. micranthus*. Numbers following taxa indicate DJM sample numbers.

	1	10	20	30	40	50
Riv hum 178	GTTTCCGTAGGTGAACCTGCGGAAGGATCATTGTCTGAAACCTACCCAGCA					
Phy ame 179	.	.	.	.	.	G.
Pis acu 177	.	.	.	.	.	G.
Bou sp. 172	.	.	.	.	.	G. T.
All inc 132	.	.	.	.	.	G. AT.
Boe ere 073	.	.	.	.	.	G. C.
Boe spi 133	.	.	.	.	.	G. C.
Boe int 134	.	.	.	.	.	G. C.
Boe gra 136	.	.	.	.	.	G. C.
Boe coc 141	.	.	.	.	.	G. C.
Cyp gyp 176	.	.	.	.	.	G. TT.
Anu eri 137	.	.	.	.	.	G. TT.
Anu lei 174	.	.	.	.	.	G. TT.
Nyc cap 121	.	.	.	.	G.	G. TT.
Acl che 115	.	.	.	.	.	G. T.
Acl lon 138	.	.	.	.	.	G. T.
Abr fra 081	.	.	.	.	.	G.
Abr ame 050	.	.	.	.	.	G.
Abr ang 173	.	.	.	.	.	G.
Abr mac 008	.	.	.	.	.	G.
Abr fra 002	.	.	.	.	.	G.
Abr umb 144	.	.	.	.	.	G.
Abr vil 080	.	.	.	.	.	G.
Abr vil aur 154	.	.	.	.	.	G.
Tri car 060	.	.	.	.	.	G. T.
Tri mic 099	.	.	.	.	.	G. T.
Mir jal 001	.	.	.	.	.	G. AT.
Mir jal 003	.	.	.	.	.	G. AT.
Mir jal 071	.	.	.	.	.	G. AT.
Mir lon 076	.	.	.	.	.	G. TT.
Mir mul 066	.	.	.	.	.	G. TT.

	51	60	70	80	90	100
Riv hum 178	GAAAGACC	AGCGAACATGT	--TCATAATT	CAACGGGAGG	-GATGGCCGCG	
Phy ame 179	. . . C	C	--T . . C . CA .	T	- . C - . T . . .	TT
Pis acu 177	. . . C	C	--GCAT . CA .	T	GA . G . T - G .	TC
Bou sp. 172		C . T	--TCAT . A .	TTA	-AGG . . .	GATC
All inc 132	. . . -	C	GC . . GG .	GC GC . CGTTC	-	-A . G . . - . . .
Boe ere 073	. . . -	G	GC . . --	TC - . . G . TC	-	-A . G . . - . . T .
Boe spi 133	. . . -	G	GC . . --	TC - . . G . CC	-	-A . G . . - . . T .
Boe int 134	. . . -	G	TC . . --	TC - . . G . TC	-	-A . G . . - . . T .
Boe gra 136	. . . -	G	G	- - - T - . . G . T . TC		-A . G . . - . . T .
Boe coc 141	. . . -	G	G	- - - T - . . G . T . TC		-A . G . . - . . T .
Cyp gyp 176		C . T	--TGT .	CGTTT	-	-A . G . . - . . T .
Anu eri 137	. - . T	G . T	--T - T . C -	TT . T -	-A . G . . - . . TA	
Anu lei 174	. - . T	G . T	--T - TGC -	TT . T -	-A . G . . - . . TA	
Nyc cap 121	. . . -	G . T	--T - T . C -	TT . T -	-A . G . . - . . TA	
Acl che 115	. . . C	C . G	--TCT .	CGTTTT	- . . G . .	-A . G . . - . . TT
Acl lon 138	. . . C	C . T	--TCT .	CGTTTT	- . . C . .	-A . G . . - . . TT
Abr fra 081	. . . C	C . T	--TC . .	AGATT	- . . - . .	-A . G . . - . . T
Abr ame 050	. . . C	C . T	--TC . .	AGATT	- . . - . .	-A . G . . - . . T
Abr ang 173	. . . C	C . T	--TC . .	AGATT	- . . - . .	-A . G . . - . . T
Abr mac 008	. . . C	C . T	--TC . .	AGATT	- . . - . .	-A . G . . - . . T
Abr fra 002	. . . C	C . T	--TC . .	AGATT	- . . - . .	-A . G . . - . . T
Abr umb 144	. . . C	C . T	--TC . .	AGATT	- . . G . .	-A . G . . - . . T
Abr vil 080	. . . C	C . T	--TC . .	AGATT	- . . G . .	-A . G . . - . . T
Abr vil aur 154	. . . C	C . T	--TC . .	AGATT	- . . G . .	-A . G . . - . . T
Tri car 060	. . . C	C . T	--TCT .	AG . CT	- A .	-A . G . . - . . A . T
Tri mic 099	. . . C	C . T	--TCT .	AG . TT	- A .	-A . G . . - . . A . T
Mir jal 001	. . . C	-	--T . T .	GTTT	-	-A . A . - . . . GA
Mir jal 003	. . . C	-	--T . T .	GTTT	-	-A . A . - . . . GA
Mir jal 071	. . . C	-	--T . T .	GTTT	-	-A . A . - . . . GA
Mir lon 076	. . . C	-	--T . T .	GTTT	-	-A . A . - . . . GA
Mir mul 066	. . . C	-	--TGT . .	-TTTT	-	-A . A . - . . . GA

	101	110	120	130	140	150
Riv hum 178	GCCC	-CTGG	-CCTCGGACCCCTC	-CCGAGCGCTGGG	GAGGGGCGACCCCTG	
Phy ame 179	. . . T	- . GA	- . T . . . C . A . . . T	. . TC	- - TC	. . - - T . . T . CT . . TC
Pis acu 177	T . . AT	. . - - C . . T	- - . . . TA	- - - - - - . T . . . - - - . . . CT	. . G . .	
Bou sp. 172	. T . ATT	. . G . . T	. TC . . T . - - . . AC	. T . T . . . - - . . . TAC	. . TGC	
All inc 132	. . . GTTC	. . - - . . C - - . . GGA	. T . TC . . TC . . - - TTAT	. . T . - . . C		
Boe ere 073	. . . GCTC	. . - - . CATCT	. . GGA . T . CC . . TCC	. - - TCAC	. . . - . . C	
Boe spi 133	. . . GCTC	. . - - . CATCT	. . GGA . T . CC . . TCC	. - - TCAC	. . . - . . C	
Boe int 134	. . . GCTC	. . - - . C . TCT	. . GGA . T . CC . . TCC	. - - TCAC	. . C . - . . C	
Boe gra 136	. . . GT . C	. . - - . . TCT	. . GGA . T . TC . T . TC	. - - CC . C . C . - . . C		
Boe coc 141	. . . GT . C	. . - - . . CT	. . GGAAT . TC . T . TC	. - - CC . C . C . - . . C		
Cyp gyp 176	. . . ATTC	. . - - . . TTT	. T GGA . T . TT . . A . . . - . T-AT	. . T . - . . A		
Anu eri 137	. . . ATTC	. . - - . . TT	. . GGA . T . TT . . AT . . - - T . ATT	. . T . - . . A		
Anu lei 174	. . . ATTC	. . - - . . TT	. . GGA . T . TT . . AT . . - - T . ATT	. . T . - . . A		
Nyc cap 121	. . . ATTC	. . - - . . TTT	. . GGA . T . CT . . AT . . - - T . ATT	. . T . - . . A		
Acl che 115	. . . ATT	. . - - . . TCT	. . TTA . C . TT . TATC	. - - . AT . T . - . . CA		
Acl lon 138	. . . ATT	. . - - . . ATCT	. . TTA . C . TT . ATC	. - - . AT . T . - . . T		
Abr fra 081	. G . AG	. . A . - - . . AC	. T . . GA . T . TTT . ATC	. - - . AT . T . - . . C		
Abr ame 050	. G . AG	. . A . - - . . AC	. T . . GA . T . TTT . ATC	. - - . AT . T . - . . C		
Abr ang 173	. G . AG	. . A . - - . . AC	. T . . GA . T . TTT . ATC	. - - . AT . T . - . . C		
Abr mac 008	. G . AG	. . A . - - . . AC	. T . . GA . T . TTT . ATC	. - - . AT . T . - . . C		
Abr fra 002	. G . AG	. . A . - - . . AC	. T . . GA . T . TTT . ATC	. - - . AT . T . - . . C		
Abr umb 144	. G . AG	. . A . - - . . AC	. T . . GA . T . TT . . ATC	. - - . TTT . T . - . . C		
Abr vil 080	. G . AG	. . A . - - . . AC	. T . . GA . T . TT . . ATC	. - - . TTT . T . - . . C		
Abr vil aur 154	. G . AG	. . A . - - . . AC	. T . . GA . T . TT . . ATC	. - - . TTT . T . - . . C		
Tri car 060	. G . ACT	. . A . - - . . AT	. T . . GA . T . CT . . ATC	. - - . AT . T . - . . C		
Tri mic 099	. G . ACT	. . A . - - . . AT	. T . . GA . T . CT . . ATC	. - - . AT . T . - . . C		
Mir jal 001	T . . ATT	. . - - . . T . T . . GA	. T . TT . . TC . . - - TTAA	. . T . - . . CT		
Mir jal 003	T . . ATT	. . - - . . T . T . . GA	. T . TT . . TC . . - - TTAAAT	. . T . - . . CT		
Mir jal 071	T . . ATT	. . - - . . T . T . . GA	. T . TT . . TC . . - - TTAA	. . T . - . . GCT		
Mir lon 076	T . . ATT	. . - - . . T . T . . GA	. T . TT . T . TC . . - - TTAA	. . T . - . . T		
Mir mul 066	T . . ATT	. . - - . . C . T . . TA	. T . TT . . T . . - - TTAA	. . T . - . . C		

	151	160	170	180	190	200
Riv hum 178	G G G G A -	G T T C C C T C A G C -	C T A A C A A C G A A C C C C C -	G G C G C G G A A C G C G T C A		
Phy ame 179	T . . . T - .	C C T T . C . G . . A - A			T . . . C . .	
Pis acu 177	C . . . C - -	A A A . . C A G A . G T A . . T . . .	A		T . . . C . .	
Bou sp. 172	C . . . T - .	C . A . T C A G - .	A . A	A	C	G . T . . . C . .
All inc 132	. C . . . G . -	A T G T C . G A . G . A . G T . . .	A	T		C
Boe ere 073	. A . . . C . -	G T G G A . G . . G . . C C T . . .	A	A	T	T . . . C . .
Boe spi 133	. A . . . C . -	G T G G A . G . . G . . C C T . . .	A	A	T	T . . . C . .
Boe int 134	. A . . . C . -	G T G G C . G . . G . . C C T . . .	A	A	T	T . . . C . .
Boe gra 136	. A . . . C . -	G T G G . . G A . G . G C C T . . .	A	A	T T	T . . . C . .
Boe coc 141	. A . . . C . -	G T G G . . G A . G . G C C T . . .	A	A	T T	T . . . C . .
Cyp gyp 176	. C . A . T . -	A T G T C . . A . G . A T - T . . .	A	A	T A	T . . . C . .
Anu eri 137	. C . . . T . -	A T G T C . . A . G . A T G T . . .	T	A	T	T . . . C . .
Anu lei 174	. C . . . T . -	A T G T C . . A . G . A T G T . . .	T	A	T	T . . . C . .
Nyc cap 121	. C . . . T . -	A T G . C . . A . G . A T G T . . .	T	A	T	T . . . C . .
Acl che 115	. C . . G C . -	A . G T C . G A T G . A T . T . . .	A	T	T	T . . . C . .
Acl lon 138	. C . - . C . -	A G G T C . G A T G . A T . T . . .	A	T	T	T . . . C . .
Abr fra 081	. - . . . T . -	C T T T C . G A T G . A C T T . . .	A	T	T	T . . . C . .
Abr ame 050	. - . . . T . -	C T T T C . G A T G . A C T T . . .	A	T	T	T . . . C . .
Abr ang 173	. - . . . T . -	C T T T C . G A T G . A C T T . . .	A	T	T	T . . . C . .
Abr mac 008	. - . . . T . -	C T T T C . G A T G . A C T T . . .	A	T	T	T . . . C . .
Abr fra 002	. - . . . T . -	C T T T C . G A T G . A C T T . . .	A	T	T	T . . . C . .
Abr umb 144	. - . . . T . -	C T T T C . G A T G . A C . T	T	T	T	T . . . C . .
Abr vil 080	. - . . . T . -	C T T T C . G A T G . A C . T	T	T	T	T . . . C . .
Abr vil aur 154	. - . . . T . -	C T T T C . G A T G . A C . T	T	T	T	T . . . C . .
Tri car 060	. - . . . C . -	C T A T C . G A T G . A C . T	T	T	T	T . . . C . .
Tri mic 099	. - . . . C . -	C T A T C . G A T G . A C . T	T	T	T	T . . . C . .
Mir jal 001	. T . . . T . - .	T G G C . . A T G . A T G T . . .	A	T	- A	T . . . C . .
Mir jal 003	. T . . . T . - .	T G G C . . A T G . A T G T . . .	A	T	- A	T . . . C . .
Mir jal 071	. T . . . T . - .	T G G C . . A T G . A T G T . . .	A	T	- A	T . . . C . .
Mir lon 076	. T . . . T . - .	T G G C . . A T G . A T G T . . .	A	T	- A	T . . . C . .
Mir mul 066	. T . . . T . -	A T G G C . . A T G . A T G T . . .	A	T	- A	T . . . C . .

	201	210	220	230	240	250
Riv hum 178	A G G A A C A C A A A C A G - - C G G - A T T G - C C - C G C C T G C G C T C G G - G C - T C C C G					
Phy ame 179	 T G T . . . A T - - A . - . G - . A . . C T . C A - T A C A . . T A					
Pis acu 177	 T G C T - - . . - T G . . T - . . . C T . C . . C A - . . . - C T T G . . G .					
Bou sp. 172	 T G C T - - A . - T G . A - . . . - . C T - C . . C . - . . . - C T T G . . G .					
All inc 132	 G . G . G A - - . A . C T G . . T . . A T T . G C T . C T T . C G T . G .					
Boe ere 073	 G . T C C - - . A . - C G . . T . . G T T T G C . . C . C . . . T T . - G					
Boe spi 133	 G . T C C - - . A . - C G . . T . . G T T T G C . . C . C . . . T T . - G					
Boe int 134	 G . T G C - - . A . - C G . . T . . G T T T G C . . C . C . . . G T . - G					
Boe gra 136	 G G . T G C T - - A . - C G . . T . . G T T T G C . . C . C . . . T T . - G					
Boe coc 141	 G G . T G C T - - A . - C G . . T G . G T T T G C . . C . C . . . T T . - G					
Cyp gyp 176	 G . T G C T - - A . - T G . . T . . A T T T G C T . C . C - . . T T T T G . . G .					
Anu eri 137	 G . T T . . T . C T - - A . - C G . . T . . A T T . G C A . C . C T . . T T T C G . . G .					
Anu lei 174	 G . T C . . T . C T - - A . - T G . . T . . A T T T G C A . C . C T . . T T T C G . . G .					
Nyc cap 121	 G . T G . . T . C T - - A . - T G . . T . . A T T . G C A . C . C T . . T T T C G . . G .					
Acl che 115	 G . . C . . A . C T G T A . - T G . . T . . - T T . T - . . T . C T . . T T T T G . . G .					
Acl lon 138	 G . . C . . T . C T G T A . - T G . . T . . - T T . G - . . T . C T . . T T T T G . . G .					
Abr fra 081	 G . T G . . T C C T - - A . - T G . . T . . A A T T G - - . C . C . . . T T T A G . . G .					
Abr ame 050	 G . T G . . T C C T - - A . - T G . . T . . A A T T G - - . C . C . . . T T T A G . . G .					
Abr ang 173	 G . T G . . T C C T - - A . - T G . . T . . A A T T G - - . C . C . . . T T T A G . . G .					
Abr mac 008	 G . T G . . T C C T - - A . - T G . . T . . A A T T G - - . C . C . . . T T T A G . . G .					
Abr fra 002	 G . T G . . T C C T - - A . - T G . . T . . A A T T G - - . C . C . . . T T T A G . . G .					
Abr umb 144	 G . T G . . T C C T - - A . - T G . . T . . A A T T G C C . C . C . . . T T T A G . . G .					
Abr vil 080	 G . T G . . T C C T - - A . - T G . . T . . A A T T G C C . C . C . . . T T T A G . . G .					
Abr vil aur 154	 G . T G . . T C C T - - A . - T G . . T . . A A T T G C C . C . C . . . T T T A G . . G .					
Tri car 060	 G . T G . . T C C T - - A . - T G . . T . . G A C T G - - . T . C . . . T T T A G . . G .					
Tri mic 099	 G . T G . . T C C T - - A . - T G . . T . . G A C T G - - . T . C . . . T T T A G . . G .					
Mir jal 001	 T . T G . . T . C T - T - . - T G A . T . . A T - - G C . . T . C . . . T T T T G					
Mir jal 003	 T . T G . . T . C T - T - . - T G A . T . . A T - - G C . . T . C . . . T T T T G					
Mir jal 071	 T . T G . . T . C T - T - . - T G A . T . . A T - - G C . . T . C . . . T T T T G					
Mir lon 076	 T . T G . . T . C T - T - . - T G . . T . . A T T - - C . . T . C . . . T T T T G					
Mir mul 066	 G . T G . . T . C T - T - . - T G . . T . . A T T A G - - . T . C . . . T T T T G					

	251	260	270	280	290	300
Riv hum 178	GTGCGTAGG	TGCGGCA	GTGACT	-C--	TAACAATGAAACGACTCTCGGCA	
Phy ame 179	TG. A-. G. . C. T. . . .	CCT. AC-	-TTGAGTA.	- . T.		
Pis acu 177	TGTG- CG. TC. . . A. .	CCT. A. G-	ATGG. . T. . - . . .			
Bou sp. 172	AGT- - - . T. . G. . . .	CCT. A. GGTTGA.	. . . C- A. . . T.			
All inc 132	- GAGCGGCA. . . . A. .	CC- GG. - TACG. T. . .	C- . . . -			
Boe ere 073	. GAGC. GCAC. . . . A. .	CCCCA. - . GGG. . . .	C- . . . -			
Boe spi 133	. GAGC. GCAC. . . . A. .	CCCCA. - . GGG. . . .	C- . . . -			
Boe int 134	. GAGC. GCAC. . . . A. .	CCCCA. - . GGG. . . .	C- . . . -			
Boe gra 136	. GAG. . GC. C. . . . A. .	CCCCA. - . GGG. . . .	C- . . . -			
Boe coc 141	. GAG. . GC. C. . . . A. .	CCCCA. - . GGG. . . .	C- . . . -			
Cyp gyp 176	- GAG. . GCA. . . . A. .	CCT- A. - TAGGG. . . .	C-			
Anu eri 137	- GAGC. GCA. . . . A. .	CCC- A. - TAGG. . . .	C-			
Anu lei 174	- GAGCCGCA. . . . A. .	CCC- A. - TAGG. . . .	C- T.			
Nyc cap 121	- GCG. . GCA. . . . A. .	CCC- A. - TAGG. . . .	C- T.			
Acl che 115	- GAGT. GCA. . . . A. .	CCT. A. - . AAGG. . . .	C-			
Acl lon 138	- GAGT. GCA. . . . A. .	CCC. A. - . AAGG. . . .	C-			
Abr fra 081	- GCGT. GC. CTT. . . .	CCT. A. - TAAAG. . . .	C-			
Abr ame 050	- GCGT. GC. CTT. . . .	CCT. A. - TAAAG. . . .	C-			
Abr ang 173	- GCGT. GC. CTT. . . .	CCT. A. - TAAAG. . . .	C-			
Abr mac 008	- GCGT. GC. CTT. . . .	CCT. A. - TAAAG. . . .	C-			
Abr fra 002	- GCGT. GC. CTT. . . .	CCT. A. - TAAAG. . . .	C-			
Abr umb 144	- GCGT. GC. CTT. . . .	CCT. A. - . AAAG. . . .	C-			
Abr vil 080	- GCGT. GC. CTT. . . .	CCT. A. - . AAAG. . . .	C-			
Abr vil aur 154	- GCGT. GC. CTT. . . .	CCT. A. - . AAAG. . . .	C-			
Tri car 060	- GTGT. GC. C.	CCT. A. G- AAAG. . T. - -	 A. .			
Tri mic 099	- GTGT. GC. C.	CCT. A. G- AAAG. . T. - -	 A. .			
Mir jal 001	- GAGT. GCA.	CCT. A. - . AAGG. . . .	C-			
Mir jal 003	- GAGT. GCA.	CCT. A. - . AAGG. . . .	C-			
Mir jal 071	- GAGT. GCA.	CCT. A. - . AAGG. . . .	C-			
Mir lon 076	- GAGT. GCA.	CCT. A. - . AAGG. . . .	C-			
Mir mul 066	- GAGT. GCA.	CCT. A. - . AAGG. . . .	C-			

	301	310	320	330	340	350
Riv hum 178	A C G G A T A T C T T	G G C T C T C G C A T C G A T G A A G A A C G T A G C G A A A T G C G A T A C				
Phy ame 179	.	C.	.	.	.	.
Pis acu 177	.	C.	.	.	.	.
Bou sp. 172	.	C.	.	.	.	.
All inc 132	.	C.	T.	.	.	.
Boe ere 073	.	C.	.	.	.	.
Boe spi 133	.	C.	.	.	.	.
Boe int 134	.	C.	.	.	.	.
Boe gra 136	.	C.	T.	.	.	.
Boe coc 141	.	C.	T.	.	.	.
Cyp gyp 176	.	C.	T.	.	.	.
Anu eri 137	.	C.	T.	.	.	.
Anu lei 174	.	C.	T.	.	.	.
Nyc cap 121	.	C.	T.	.	.	.
Acl che 115	.	C.	T.	.	.	.
Acl lon 138	.	C.	T.	.	.	.
Abr fra 081	.	C.	T.	.	.	.
Abr ame 050	.	C.	T.	.	.	.
Abr ang 173	.	C.	T.	.	.	.
Abr mac 008	.	C.	T.	.	.	.
Abr fra 002	.	C.	T.	.	.	.
Abr umb 144	.	C.	T.	.	.	.
Abr vil 080	.	C.	T.	.	.	.
Abr vil aur 154	.	C.	T.	.	.	.
Tri car 060	.	C.	.	.	.	.
Tri mic 099	.	C.	.	.	.	.
Mir jal 001	.	C.	T.	.	.	.
Mir jal 003	.	C.	T.	.	.	.
Mir jal 071	.	C.	T.	.	.	.
Mir lon 076	.	C.	T.	.	.	.
Mir mul 066	.	C.	T.	.	.	.

	351	360	370	380	390	400
Riv hum 178	TTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTTTTTTGAACGCAAGT					
Phy ame 179	.					C.
Pis acu 177	.					C.
Bou sp. 172	C.	.				
All inc 132	.					C.
Boe ere 073	.					C.
Boe spi 133	.					C.
Boe int 134	.					C.
Boe gra 136	.					C.
Boe coc 141	.					C.
Cyp gyp 176	.					C.
Anu eri 137	.					C.
Anu lei 174	.					C.
Nyc cap 121	.					C.
Acl che 115	.					C.
Acl lon 138	.					C.
Abr fra 081	.					C.
Abr ame 050	.					C.
Abr ang 173	.					C.
Abr mac 008	.					C.
Abr fra 002	.					C.
Abr umb 144	.					C.
Abr vil 080	.					C.
Abr vil aur 154	.					C.
Tri car 060	.					C.
Tri mic 099	.					C.
Mir jal 001	.					C.
Mir jal 003	.					C.
Mir jal 071	.					C.
Mir lon 076	.					C.
Mir mul 066	.					C.

	401	410	420	430	440	450																																																	
Riv hum 178	T	G	C	G	C	C	T	G	A	A	G	C	C	C	T	T	A	G	G	C	C	G	A	A	G	G	C	A	C	G	T	C	T	G	C	C	T	G	G	G	C	G	T	C	A	C	G	C	A	C					
Phy ame 179	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T
Pis acu 177	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Bou sp. 172	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	AA	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T		
All inc 132	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Boe ere 073	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Boe spi 133	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Boe int 134	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Boe gra 136	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Boe coc 141	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Cyp gyp 176	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Anu eri 137	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Anu lei 174	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Nyc cap 121	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Acl che 115	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Acl lon 138	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Abr fra 081	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Abr ame 050	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Abr ang 173	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Abr mac 008	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Abr fra 002	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Abr umb 144	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Abr vil 080	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Abr vil aur 154	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Tri car 060	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Tri mic 099	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Mir jal 001	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Mir jal 003	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Mir jal 071	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Mir lon 076	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	
Mir mul 066	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	

	451	460	470	480	490	500
Riv hum 178	C G C G T C T C C C C C C A C C C C A	- - - -	C A A A G C C G G G	- - - -	A G G G G C G A G G A T G	
Phy ame 179		T	T A - - -	C T . . T G . A . G - G G .		- A G .
Pis acu 177			C C - - -	C T C . . G . . - T A G G .		- A G T
Bou sp. 172		T . . T . .	C C - - -	T T T A T G . . - T T G G .		- A
All inc 132	A . . A C . .	- - . . .	T T C C - T - -	T C C T G C A . T T G G G .		- -
Boe ere 073	A . . A . .		G T C C - -	G - T C T . G . T . A C G T C .		- - C .
Boe spi 133	A . . A . .		G T C C - -	G - T C T . G . T . A C G T C .		- - C .
Boe int 134	A . . A . .		G T C C - -	G - T C T . G . T . A C G T C .		- - C .
Boe gra 136	A . . A . .		G T C C - -	G - T C T . G T A . A C G T C .		- - C .
Boe coc 141	A . . A . .		G T C C - -	G - T C T . G T A . A C G T C .		- - C .
Cyp gyp 176	A . . A . .		A T C - - -	C - T T C T . G . A . A T G T T .		- -
Anu eri 137	A . . A . .		A . - - - T C C C -	C A T T C T T G . A . A T G T .		- -
Anu lei 174	A . . A . .		A . - - - T C C C -	C A T T C T T G . A . A T G T .		- -
Nyc cap 121	A . . A . .		A T C C - T C	A T T C T T G . A . A T G T .		-
Acl che 115	A A . A . .		T A . . T A . C C C T C	- - - C T T G . C . A T G A .		- A
Acl lon 138	A A . A . .		T A T C - - -	- - - C T T G T C . G T G T .		- A
Abr fra 081	A . . A . .		A G T C C - -	A T - C T . G . A . G C G T T .		- A T
Abr ame 050	A . . A . .		A G T C C - -	A T - C T . G . A . G C G T T .		- A T
Abr ang 173	A . . A . .		A G T C C - -	A T - C T . G . A . G C G T T .		- A T
Abr mac 008	A . . A . .		A G T C C - -	A T - C T . G . A . G C G T T .		- A T
Abr fra 002	A . . A . .		A G T C C - -	A T - C T . G . A . G C G T T .		- A T
Abr umb 144	A . . A . .		A G T C C - -	A T - C T . G . A . G C G T T .	T	- A T
Abr vil 080	A . . A . .		A G T C C - -	A T - C T . G . A . G C G T T .	T	- A T
Abr vil aur 154	A . . A . .		A G T C C - -	A T - C T . G . A . G C G T T .	T	- A T
Tri car 060	A . . A . .		A T C G - -	A T - C T T G T A . G C G T .	A	- A T
Tri mic 099	A . . A . .		A T C G - -	A T - C T T G T A . G C G T .	A	- A T
Mir jal 001	A . . A . .		A T T C C - T C	- - - C T T T . T . A G G T .		- A
Mir jal 003	A . . A . .		A T T C C - T C	- - - C T T T . T . A G G T .		- A
Mir jal 071	A . . A . .		A T T C C - T C	- - - C T T T . T . T G G T .		- A
Mir lon 076	A . . A . .		A T T C C - T C	- - - C T T T . T . A G G T .		- A
Mir mul 066	A . . A . .		A T C C - T C	- - - C T T T . T . A G G T .		- A

	551	560	570	580	590	600
Riv hum 178	CCCGGCTATGAGTTG-	CTGCGGGCTATTGGTGGTTGACAAGGCC-	TCGGCC			
Phy ame 179	. T . . . TA - . C G A -					
Pis acu 177	. AA . . . G . C - . C G -					
Bou sp. 172	. AAT . . A . C - T A - . T					
All inc 132	. G . A . TG . . . C . C . . - . C A . . A . T C T . T . . .					
Boe ere 073	. A . A . . G . C . TTC . . - . C A . . A . C TC					
Boe spi 133	. A . A . . G . . . TTC . . - . C A . . A . C TC					
Boe int 134	. A . A . . G . . . TTC . . - . C A . . A . C TC					
Boe gra 136	. A . A . TG . . . C . C . . - . C A . . A . T TC					
Boe coc 141	. A . A . TG . . . C . C . . - . C A . . A . T TC					
Cyp gyp 176	. A . A . TG . . . T . C . . - A . . A . T T . T . . .					
Anu eri 137	. A . A . TG . . . T . C . . - A . . A . T T . T . . .					
Anu lei 174	. A . A . TG . . . T . C . . C A . . A . T T . T . . .					
Nyc cap 121	. A . A . TG . . . T . C . . - . . . T . A . . A . A . T T . T . . .					
Acl che 115	. T . A . TG C . . - A . . A . T T . T . . .					
Acl lon 138	. T . . . TG C . . - A . . A . TC TC . T . . .					
Abr fra 081	TG . . . TG . CT . . C . C . . - T . . . A . . A . T T C . T . . .					
Abr ame 050	TG . . . TG . CT . . C . C . . - T . . . A . . A . T T C . T . . .					
Abr ang 173	TG . . . TG . CT . . C . C . . - T . . . A . . A . T T C . T . . .					
Abr mac 008	TG . . . TG . CT . . C . C . . - T . . . A . . A . T T C . T . . .					
Abr fra 002	TG . . . TG . CT . . C . C . . - T . . . A . . A . T T C . T . . .					
Abr umb 144	TG . . . TG . CT . . C . C . . - T . . . A . . A . T T C . T . . .					
Abr vil 080	TG . . . TG . CT . . C . C . . - T . . . A . . A . T T C . T . . .					
Abr vil aur 154	TG . . . TG . CT . . C . C . . - T . . . A . . A . T T C . T . . .					
Tri car 060	TG . A . TG . CT . . C . . - TC . T . . . A . . A . T T C . T . . .					
Tri mic 099	TG . A . TG . CT . . C . . - TC . T . . . A . . A . T T C . T . . .					
Mir jal 001	. T . . . TG C . . - - - . . . A . . A . T A T . T . . .					
Mir jal 003	. T . . . TG C . . - - - . . . A . . A . T A T . T . . .					
Mir jal 071	. T . . . TG C . . - - - . . . A . . A . T A T . T . . .					
Mir lon 076	. T . . . TG C . . - - - . . . A . . A . T A T . T . . .					
Mir mul 066	. T . A . TG C . . - - - . . . A . . A . T A T . T . . .					

	601	610	620	630	640	650
Riv hum 178	CTGAATT-	-CATCGCGTC	CGTGC	CGGACAT	TGGCACAGG-	GGAGATC-GTT
Phy ame 179	G. - G. - CG.		T. T. C.		T. . CAGTT-	 C. . - . A
Pis acu 177	G. - G. - G.		C. . . .	C. . G. T. T	TCGGTT-	 C. . - . CA
Bou sp. 172	G. - G. - A.		T. C. . . .	T. T. C. TG. T.	TCA-TTT.	C. . C. . - . CA
All inc 132	G. - G. - G.	GAT. TT. T.	C. . A. C. AG.	CATTGGTCA.	..	C. . - . C.
Boe ere 073	GC--G. - G.	G. . . T. . T.	C. . G. C. AG.	C. . TGCTTC.	..	C. . - . C.
Boe spi 133	GC--G. - G.	G. . . T. . T.	C. . G. C. AG.	C. . TGCTTC.	..	C. . - . C.
Boe int 134	GC--G. - G.	G. . . T. . T.	C. . G. C. AG.	C. . TGCTTC.	..	C. . - . C.
Boe gra 136	GC--G. - C-	TG. . . T. . T.	C. . G. C. A. .	CA. TGCTCT.	..	C. . - . C.
Boe coc 141	GC--G. - C-	G. . . T. . T.	C. . G. C. A. .	CA. TGCTCT.	..	C. . - . C.
Cyp gyp 176	G. - G. - G.	... TTT. . T.	C. . A. C. AG.	CATTGGTTA.	..	CC. - . C.
Anu eri 137	G. - G. - G.	G. G. TTT. . T.	C. . A. C. A. .	CATTG. TTA.	..	C. . - . C.
Anu lei 174	G. - G. - G.	G. G. TTT. . T.	C. . A. C. A. .	CATTG. T. A.	..	C. TT. C.
Nyc cap 121	G. - G. - G.	G. G. TTT. . T.	C. . G. C. A. .	CATTG. TTAT.	..	C. . - . C.
Acl che 115	G. - G. - G.	... T. T. . T.	C. . A. C. A. .	CA. CG. AAA.	..	C. . - . CG
Acl lon 138	G. - G. - G.	... T. T. . T.	CT. A. C. A. .	CA. CG. TAA.	..	C. . - . CG
Abr fra 081	G. - GC. T-	... AT. A. CT.	CCT. A. C. A. .	CA. CG. TTA.	..	C. . - . GG
Abr ame 050	G. - GC. T-	... AT. A. CT.	CCT. A. C. A. .	CA. CG. TTA.	..	C. . - . GG
Abr ang 173	G. - GC. T-	... AT. A. CT.	CCT. A. C. A. .	CA. CG. TTA.	..	C. . - . GG
Abr mac 008	G. - GC. T-	... AT. A. CT.	CCT. A. C. A. .	CA. CG. TTA.	..	C. . - . GG
Abr fra 002	G. - GC. T-	... AT. A. CT.	CCT. A. C. A. .	CATCG. TTA.	..	C. . - . GG
Abr umb 144	G. - GC. T-	... AT. A. CT.	CCTCA. C. A. .	CA. CG. TTA.	..	C. . - . CG
Abr vil 080	G. - GC. T-	... AT. A. CT.	CCTCA. C. A. .	CA. CG. TTA.	..	C. . - . CG
Abr vil aur 154	G. - GC. T-	... AT. A. CT.	CCTCA. C. A. .	CA. CG. TTA.	T- C. . - .	CG
Tri car 060	G. - GC. T-	... AT. A. . T.	CC. . A. C. A. .	CA. TGCCTA.	..	C. . - . CG
Tri mic 099	G. - GC. T-	... AT. A. . T.	CC. . A. C. A. .	CA. TGCCTA.	..	C. . - . CG
Mir jal 001	GC--G. . T-	... TTT. . T.	C. . A. C. A. .	T. . TG. TTA.	..	C. . - . CA
Mir jal 003	GC--G. . T-	... TTT. . T.	C. . A. C. A. .	T. . TG. TTA.	..	C. . - . CA
Mir jal 071	GC--G. . T-	... TTT. . T.	C. . A. C. A. .	T. . TG. TTA.	..	C. . - . CA
Mir lon 076	GC--G. . T-	... TTT. . T.	C. . A. C. A. .	T. . TG. TCA.	..	C. . - . CA
Mir mul 066	G. - G. . T-	... TTT. . T.	C. . A. C. A. .	T. . TG. TTA.	..	C. . - . CA

	701	710	720	730	736
Riv hum 178	AGGCGGGGCTACCCGCTGAGTTTAAGCATATCAACA				
Phy ame 179	.	.	.	.	T.
Pis acu 177	.	.	.	.	T.
Bou sp. 172	.	.	.	.	T.
All inc 132	.	A.	.	.	T.
Boe ere 073	.	A. C.	.	.	T.
Boe spi 133	.	A. C.	.	.	T.
Boe int 134	.	A. C.	.	.	T.
Boe gra 136	.	A.	.	.	T.
Boe coc 141	.	A.	.	.	T.
Cyp gyp 176	.	A.	.	.	T.
Anu eri 137	.	A.	.	.	T.
Anu lei 174	.	A.	.	.	T.
Nyc cap 121	.	A.	.	.	T.
Acl che 115	.	AT.	.	.	T.
Acl lon 138	.	AT.	.	.	T.
Abr fra 081	.	A.	.	.	T.
Abr ame 050	.	A.	.	.	T.
Abr ang 173	.	A.	.	.	T.
Abr mac 008	.	A.	.	.	T.
Abr fra 002	.	A.	.	.	T.
Abr umb 144	.	A.	.	.	T.
Abr vil 080	.	A.	.	.	T.
Abr vil aur 154	.	A.	.	.	T.
Tri car 060	.	AT.	.	.	T.
Tri mic 099	.	AT.	.	.	T.
Mir jal 001	.	AT.	.	.	T.
Mir jal 003	.	AT.	.	.	T.
Mir jal 071	.	AT.	.	.	T.
Mir lon 076	.	AT.	.	.	T.
Mir mul 066	.	AT.	.	.	T.

APPENDIX 3.

Topologies from phylogenetic analyses. Tree numbers are indicated on each tree. Numbers above branches indicate branch lengths.

Trees 1-25 resulted from analyses of all characters. Tree 1 is the strict consensus tree of the three most parsimonious trees (Trees 2-4) derived from equal weights maximum parsimony (MP). Tree 5 is the bootstrap consensus tree from equal weights MP. Tree 6 is the jackknife consensus tree from equal weights MP. Tree 7 is the neighbor joining (NJ) topology derived from Kimura 2-parameter distance matrix. Tree 8 is the bootstrap consensus tree from NJ using the Kimura 2-parameter distance matrix. Tree 9 is the jackknife consensus tree from NJ using the Kimura 2-parameter distance matrix. Tree 10 is the maximum likelihood (ML) tree using the TrN+I+G model of evolution. Tree 11 is the quartet puzzling tree using the TrN+I+G model of evolution. Tree 12 is the strict consensus tree of the three ML trees (Trees 13-15) using the GTR+I+G model of evolution. Tree 16 is the quartet puzzling tree using the GTR+I+G model of evolution. Tree 17 is the strict consensus tree of the six most parsimonious trees (Trees 18 -23) derived from ti/tv weighted MP. Tree 24 is the bootstrap consensus tree from ti/tv weighted MP. Tree 25 is the jackknife consensus tree from ti/tv weighted MP.

Trees 26-31 resulted from analyses of the 18S subset (characters 1-33). Tree 26 is the most parsimonious tree derived from equal weights maximum parsimony (MP). Tree 27 is the bootstrap consensus tree from equal weights MP. Tree 28 is the jackknife consensus tree from equal weights MP. Tree 29 is the neighbor joining (NJ) topology derived from uncorrected distances. Tree 30 is the bootstrap consensus tree from NJ using uncorrected distances. Tree 31 is the jackknife consensus tree from NJ using uncorrected distances.

Trees 32-45 resulted from analyses of the ITS1 subset (characters 34-284). Tree 32 is the strict consensus tree of the eight most parsimonious trees (Trees 33-40) derived from equal weights maximum parsimony (MP). Tree 41 is the bootstrap consensus tree from equal weights MP. Tree 42 is the jackknife consensus tree from equal weights MP. Tree 43 is the neighbor joining (NJ) topology derived from the Kimura 2-parameter distance matrix. Tree 44 is the bootstrap consensus tree from NJ using the Kimura 2-parameter distance matrix. Tree 45 is the jackknife consensus tree from NJ using the Kimura 2-parameter distance matrix.

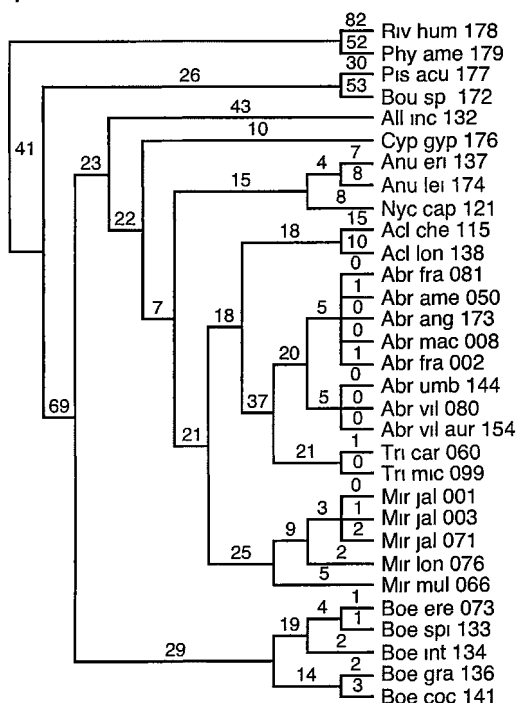
Trees 46-57 resulted from analyses of the 5.8S subset (characters 285-448). Tree 46 is the strict consensus tree of the six most parsimonious trees (Trees 47-52) derived from equal

weights maximum parsimony (MP). Tree 53 is the bootstrap consensus tree from equal weights MP. Tree 54 is the jackknife consensus tree from equal weights MP. Tree 55 is the neighbor joining (NJ) topology derived from the Kimura 2-parameter distance matrix. Tree 56 is the bootstrap consensus tree from NJ using the Kimura 2-parameter distance matrix. Tree 57 is the jackknife consensus tree from NJ using the Kimura 2-parameter distance matrix.

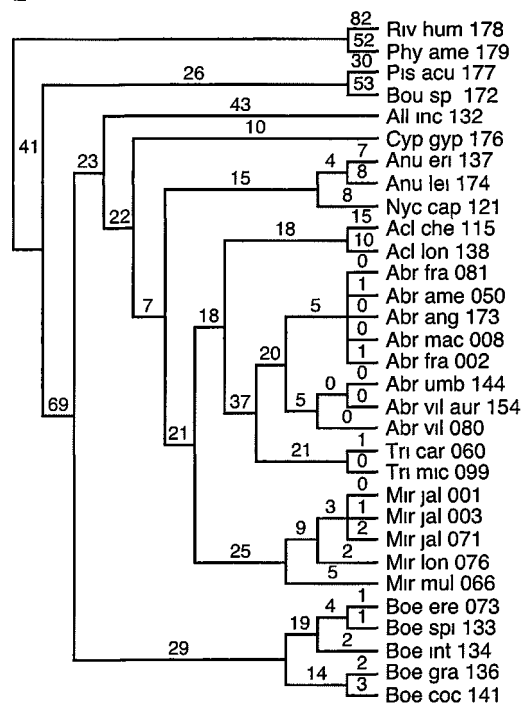
Trees 58-81 resulted from analyses of the ITS2 subset (characters 449-685). Tree 58 is the strict consensus tree of the eighteen most parsimonious trees (Trees 59-76) derived from equal weights maximum parsimony (MP). Tree 77 is the bootstrap consensus tree from equal weights MP. Tree 78 is the jackknife consensus tree from equal weights MP. Tree 79 is the neighbor joining (NJ) topology derived from the Kimura 2-parameter distance matrix. Tree 80 is the bootstrap consensus tree from NJ using the Kimura 2-parameter distance matrix. Tree 81 is the jackknife consensus tree from NJ using the Kimura 2-parameter distance matrix.

Trees 82-92 resulted from analyses of the 26S subset (characters 686-736). Tree 82 is the strict consensus tree of the five most parsimonious trees (Trees 83-87) derived from equal weights maximum parsimony (MP). Tree 88 is the bootstrap consensus tree from equal weights MP. Tree 89 is the jackknife consensus tree from equal weights MP. Tree 90 is the neighbor joining (NJ) topology derived from the Kimura 2-parameter distance matrix. Tree 91 is the bootstrap consensus tree from NJ using the Kimura 2-parameter distance matrix. Tree 92 is the jackknife consensus tree from NJ using the Kimura 2-parameter distance matrix.

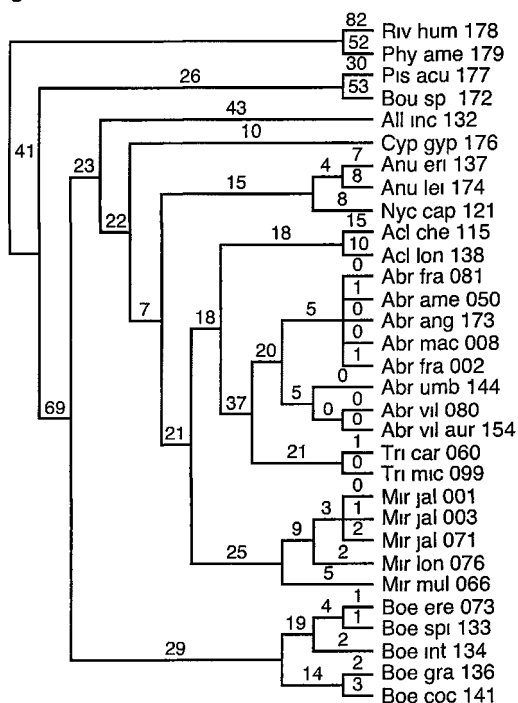
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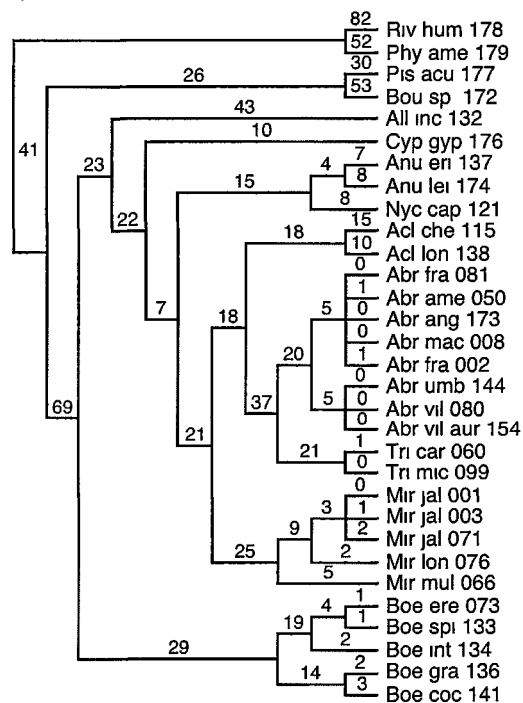
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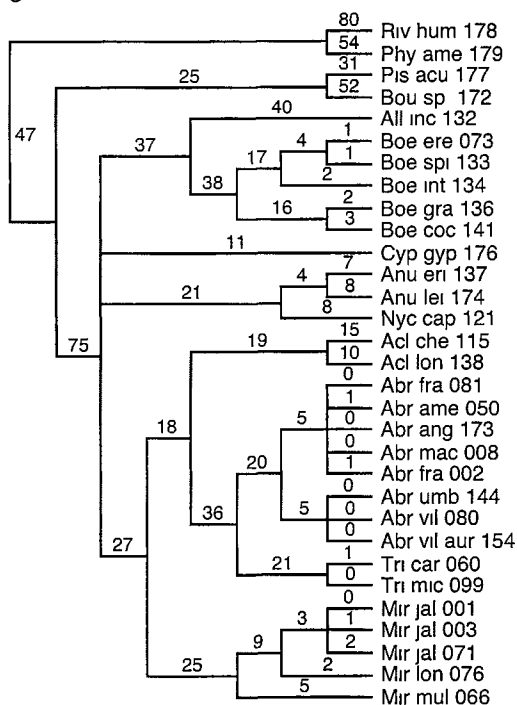
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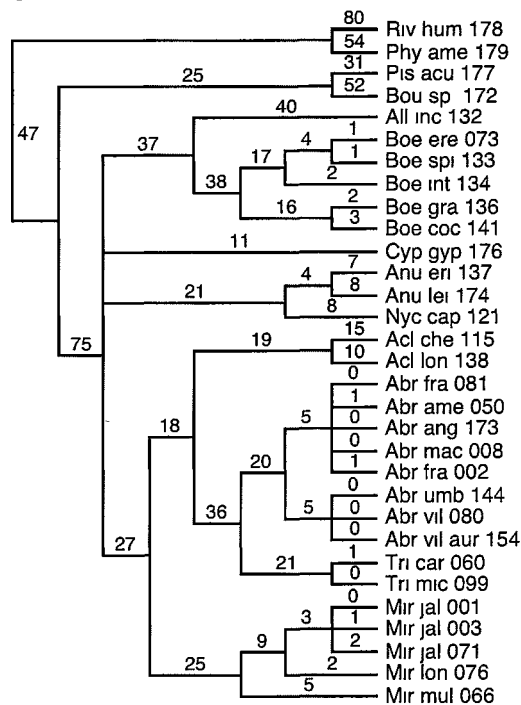
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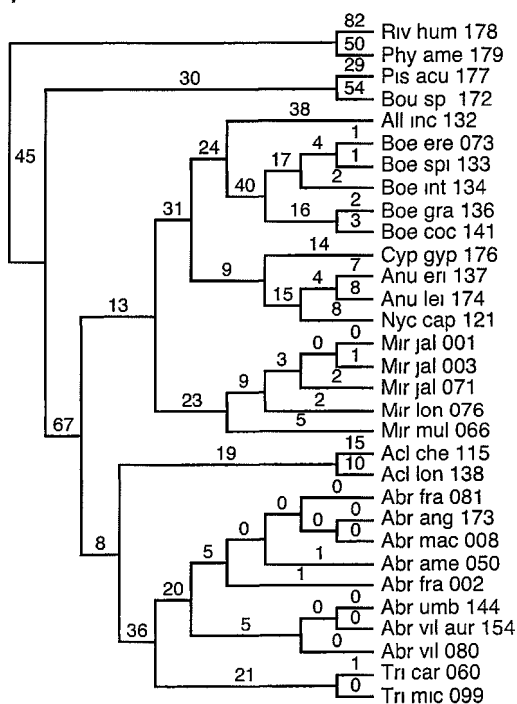
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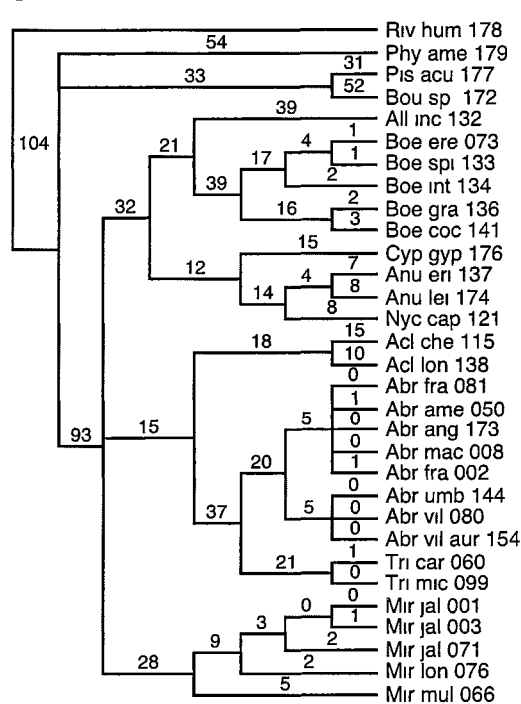
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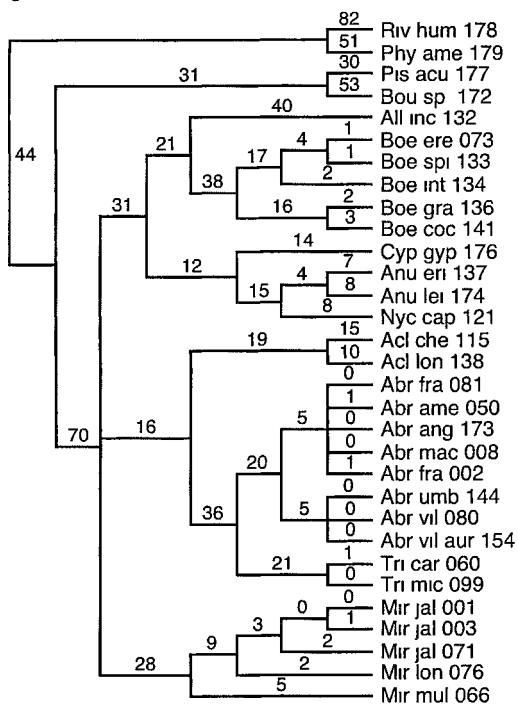
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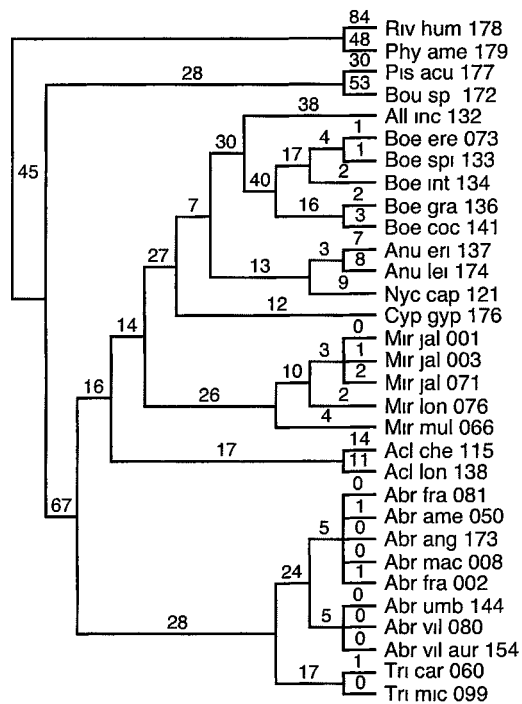
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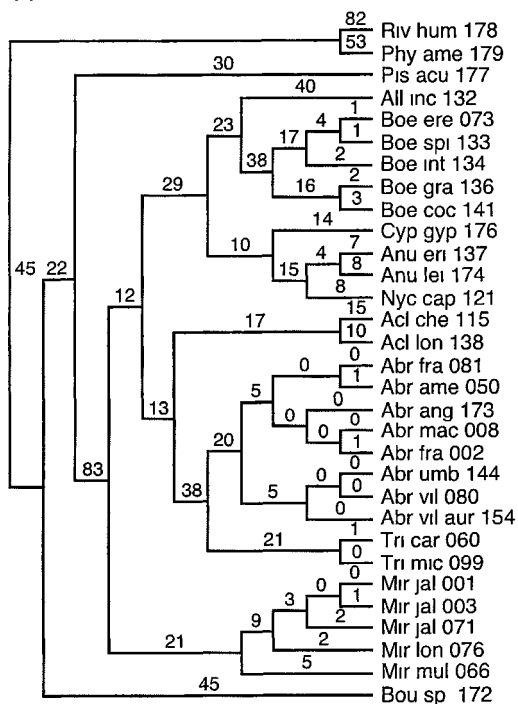
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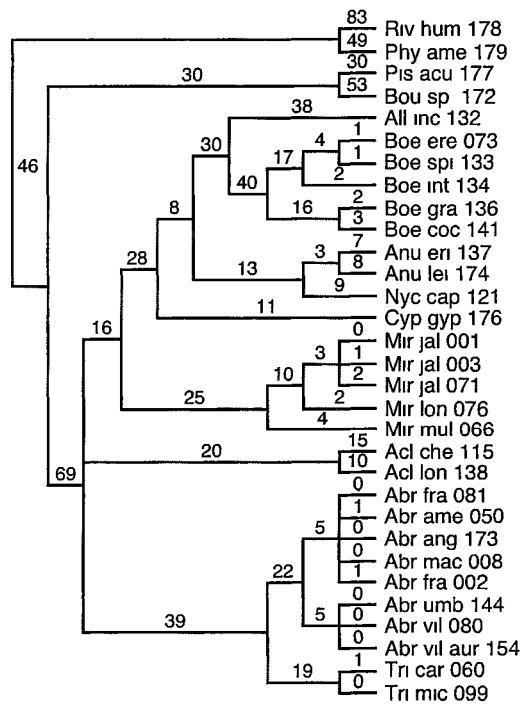
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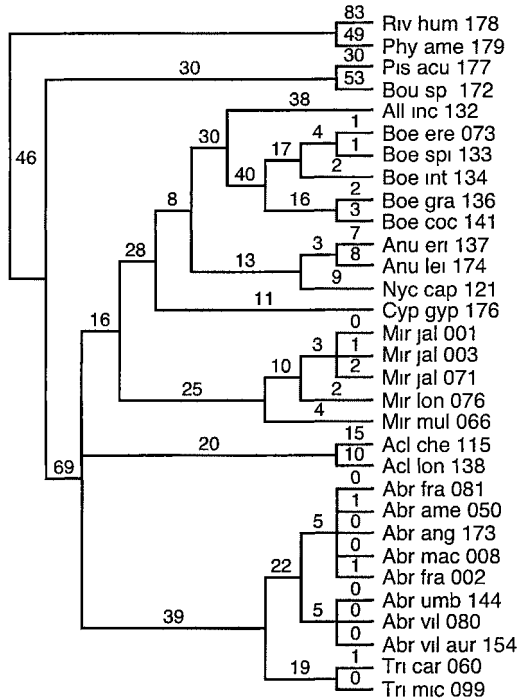
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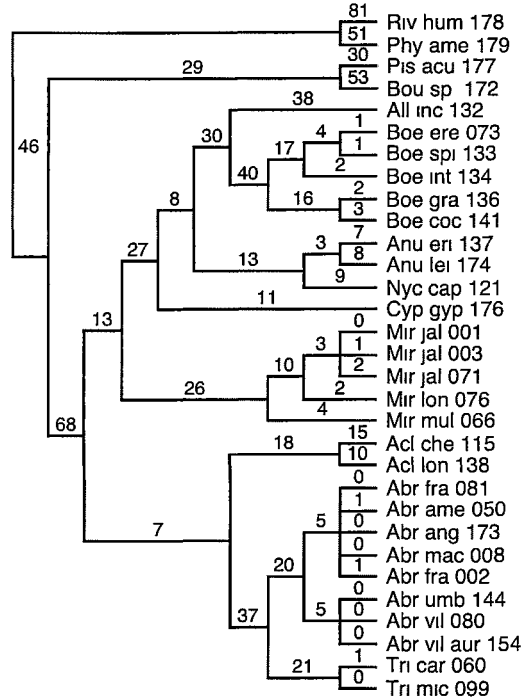
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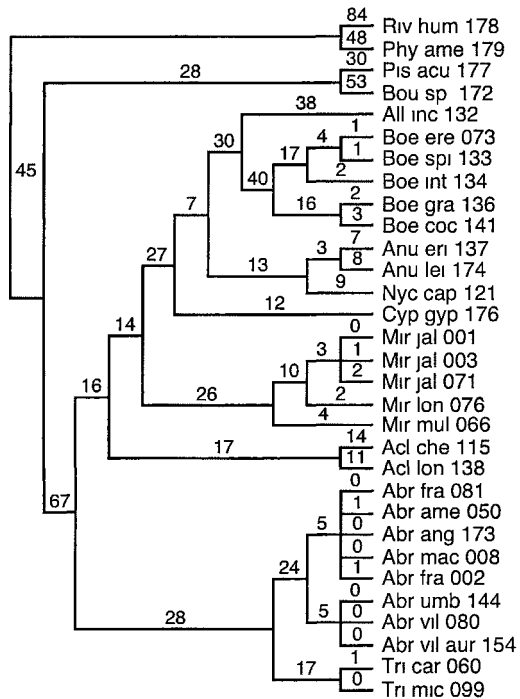
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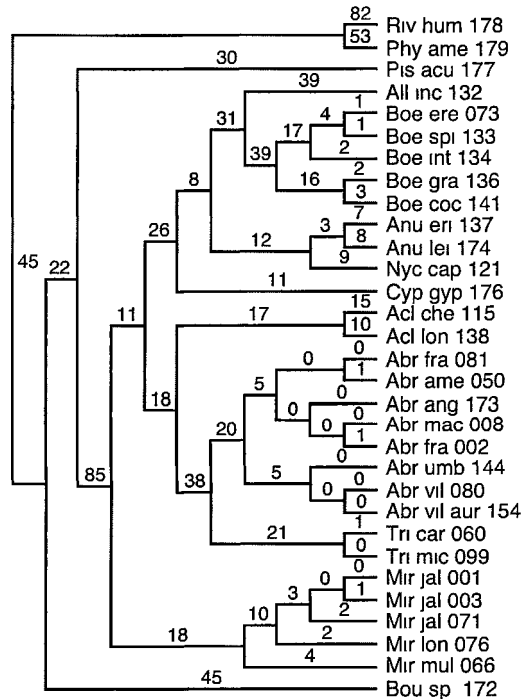
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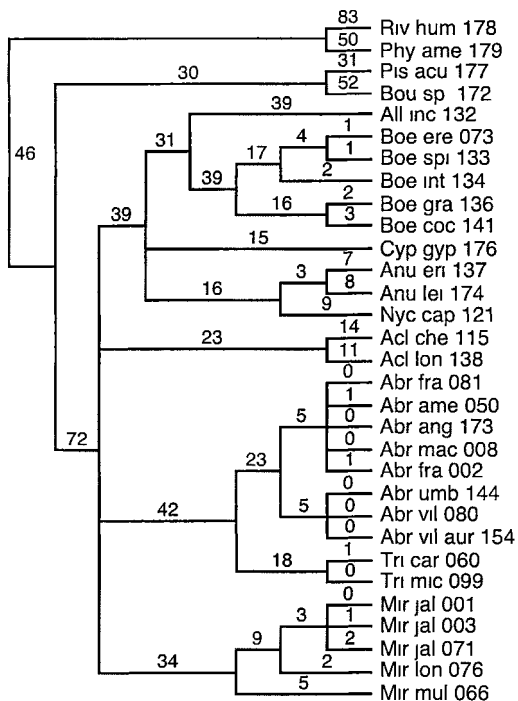
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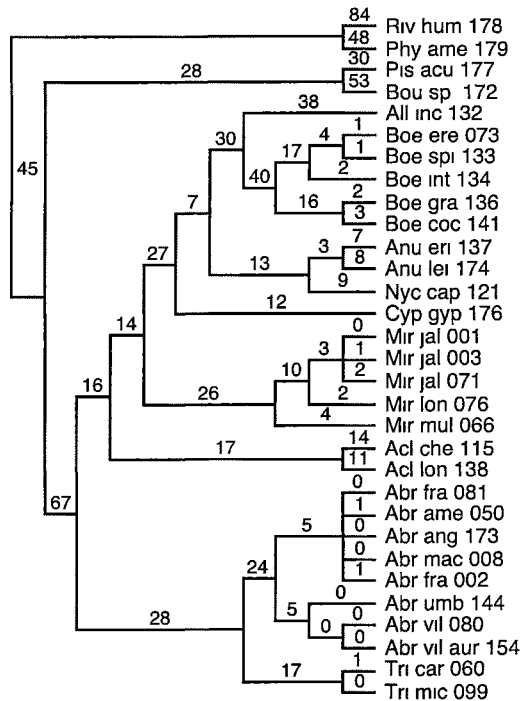
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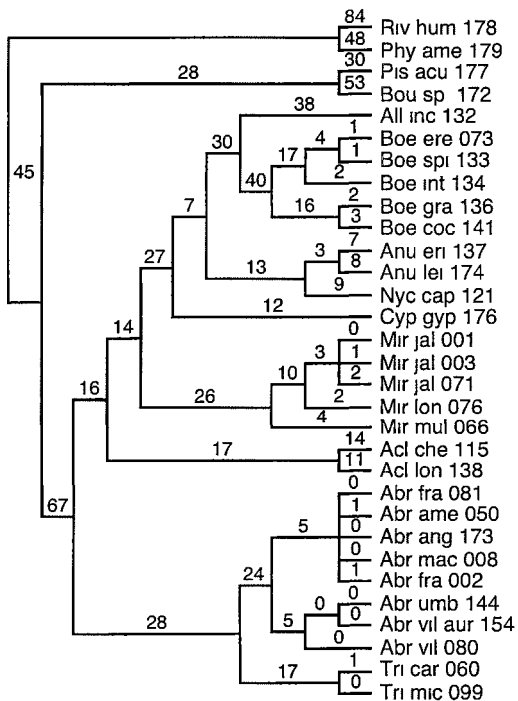
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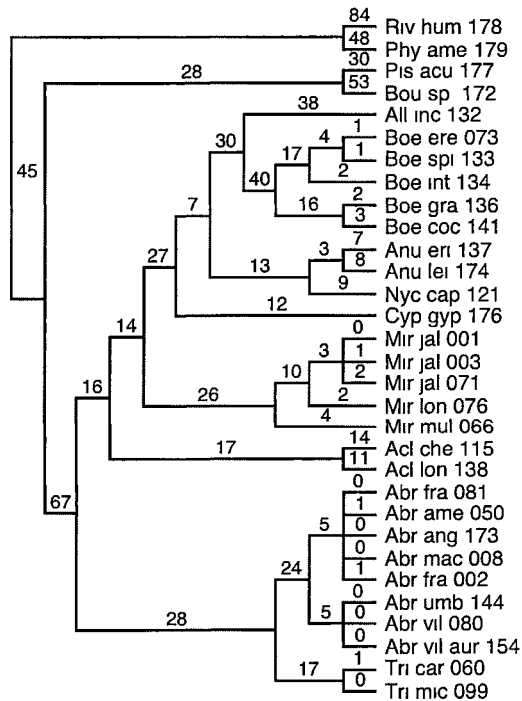
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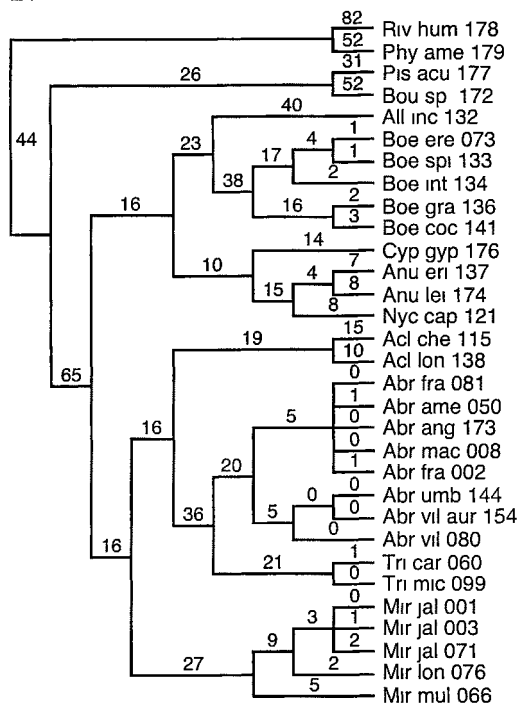
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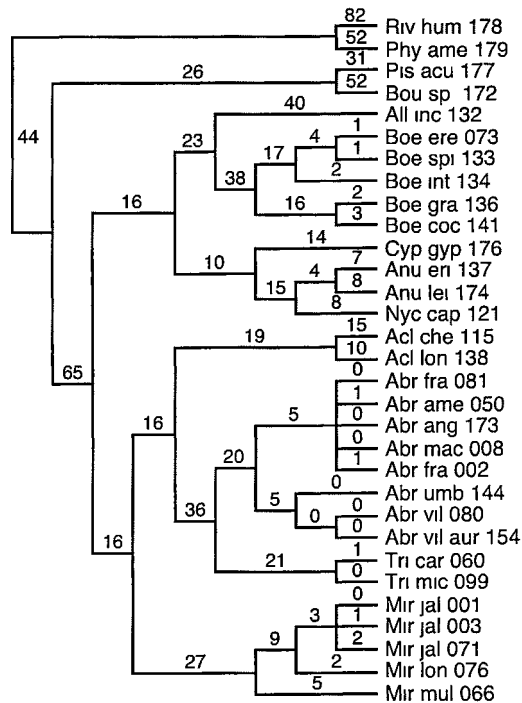
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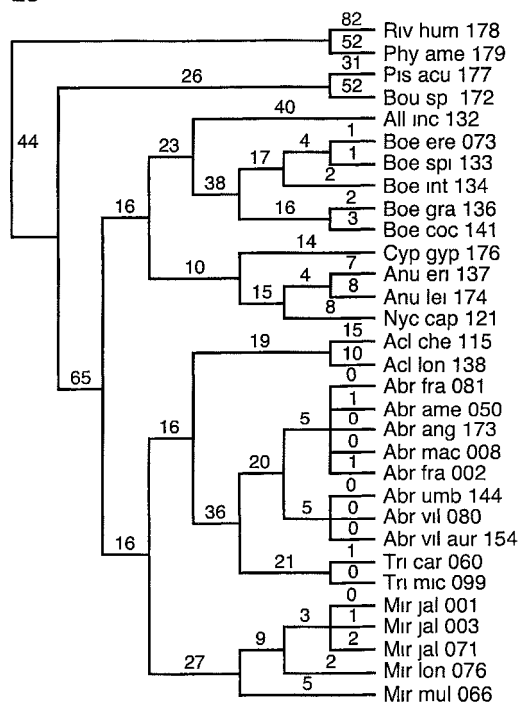
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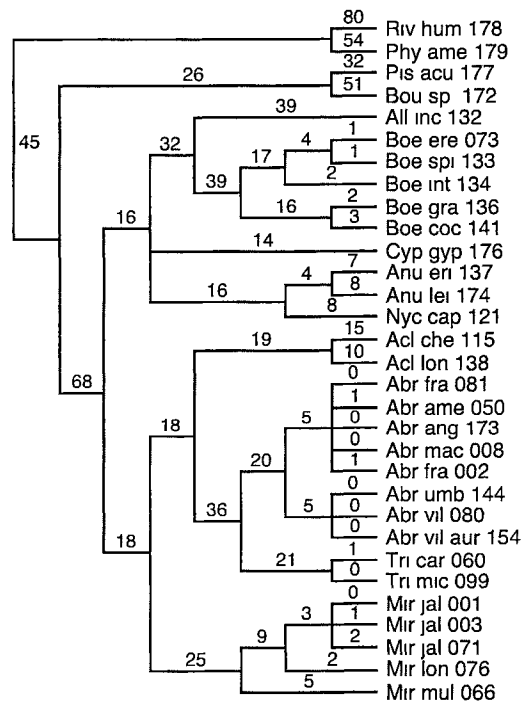
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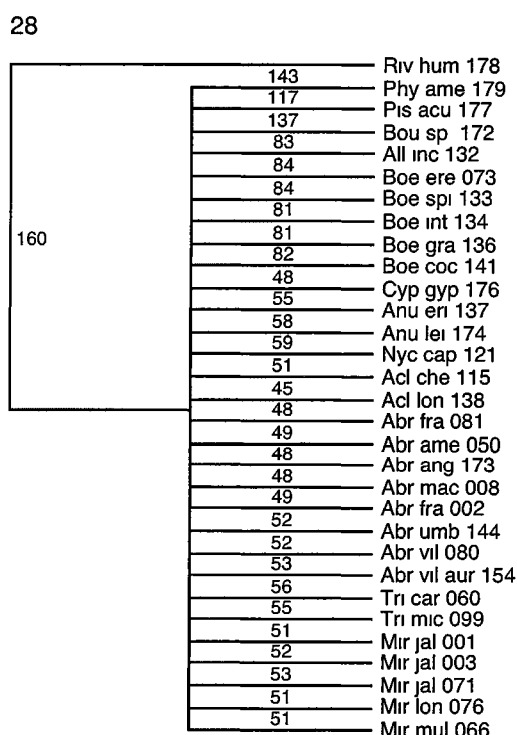


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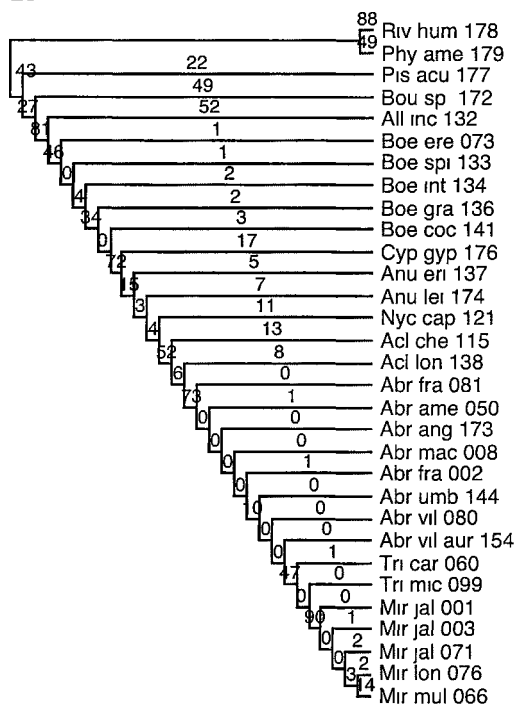


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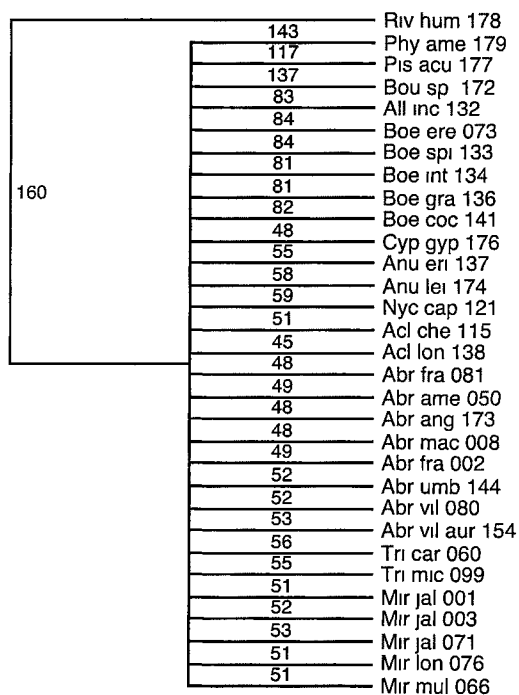




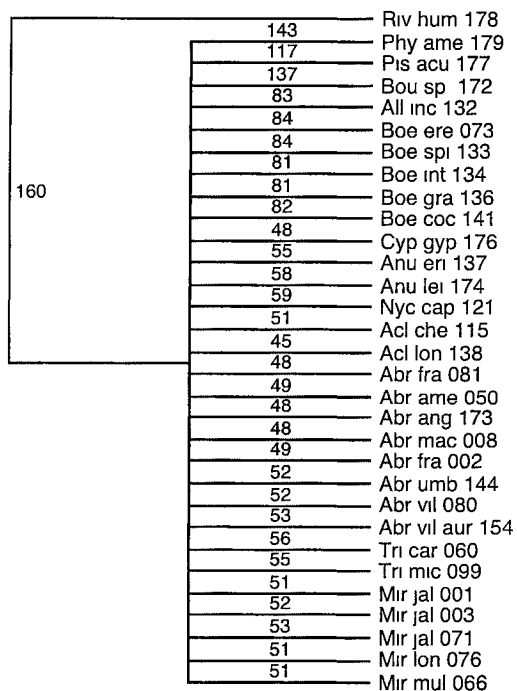
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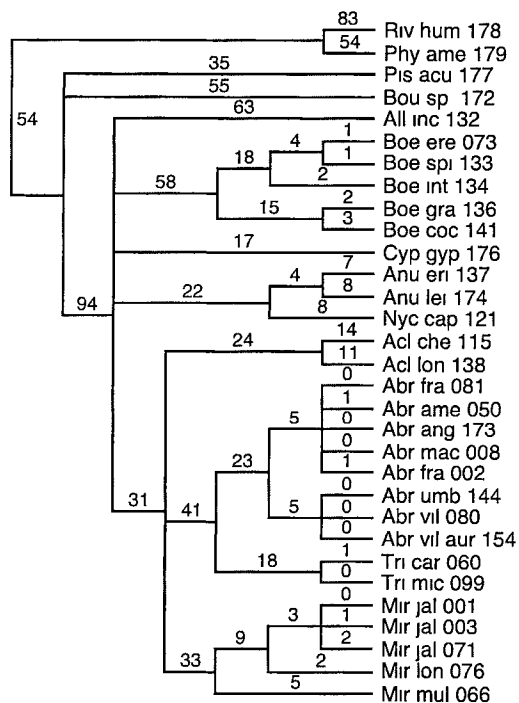
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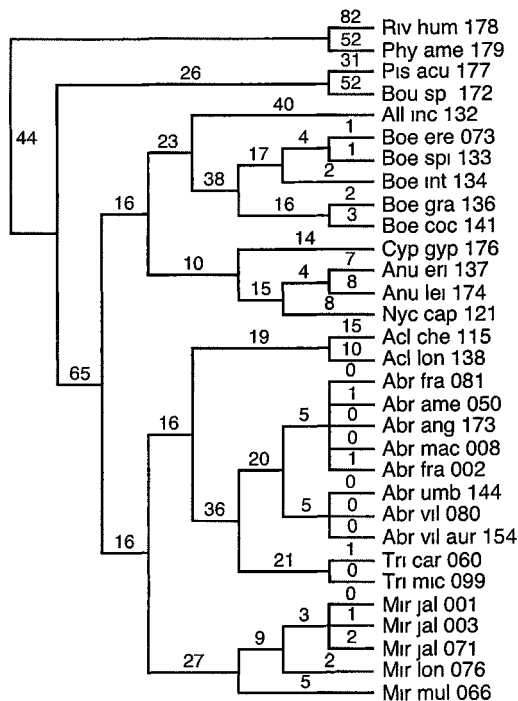
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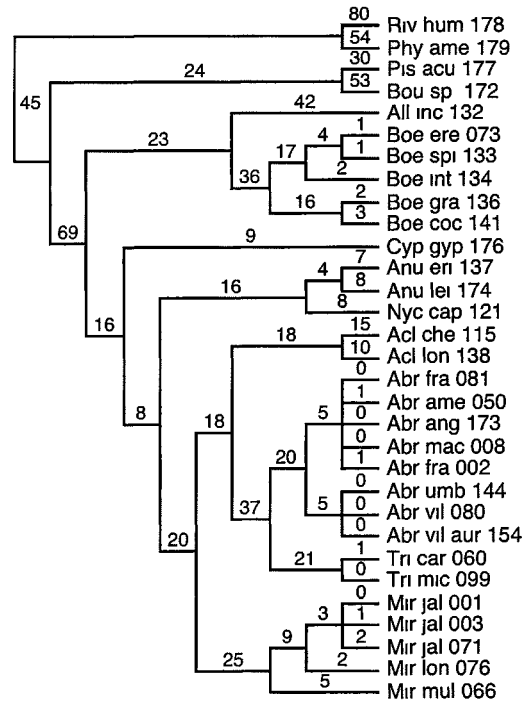
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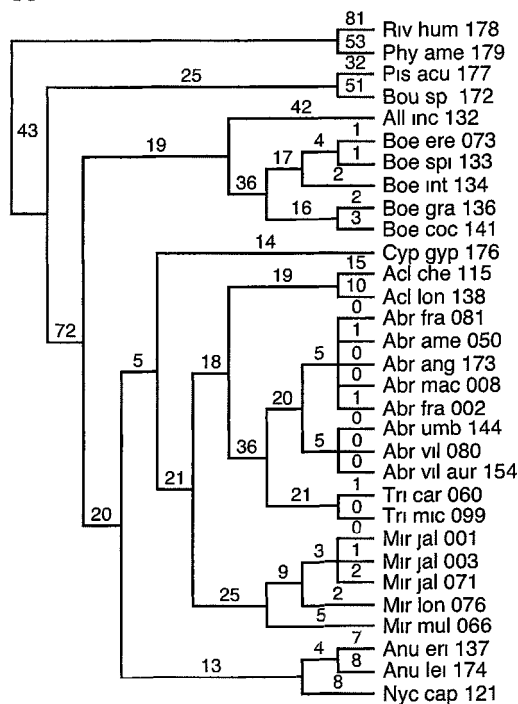
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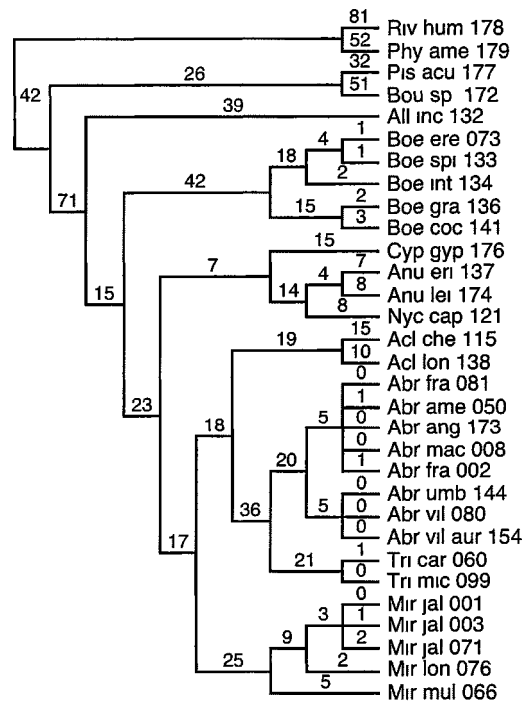
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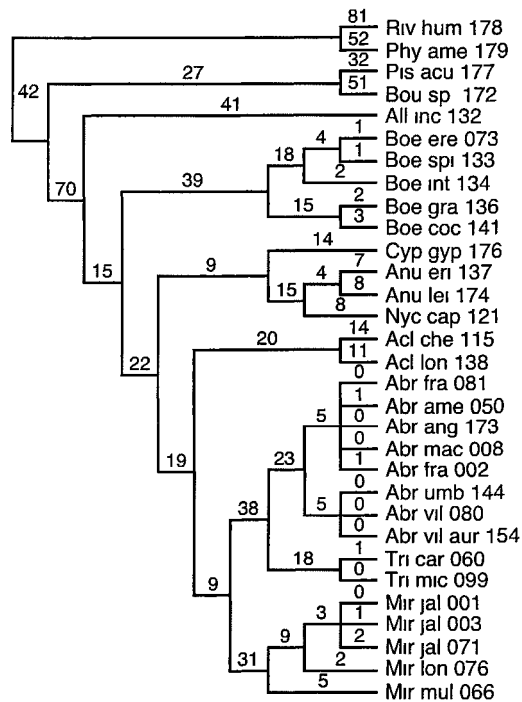
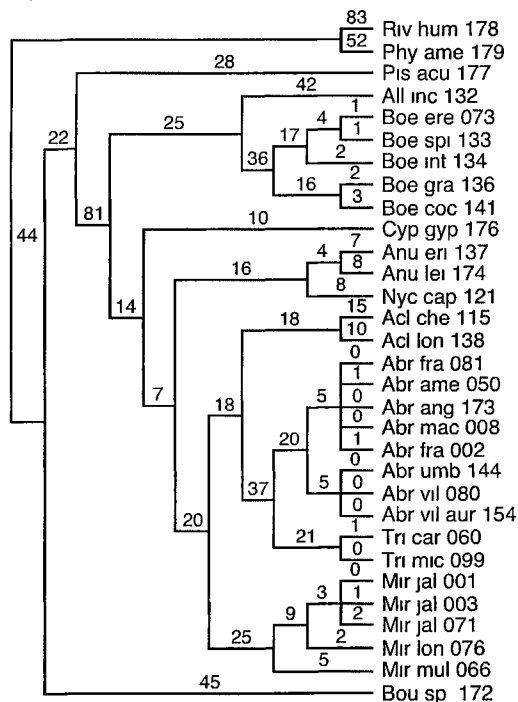
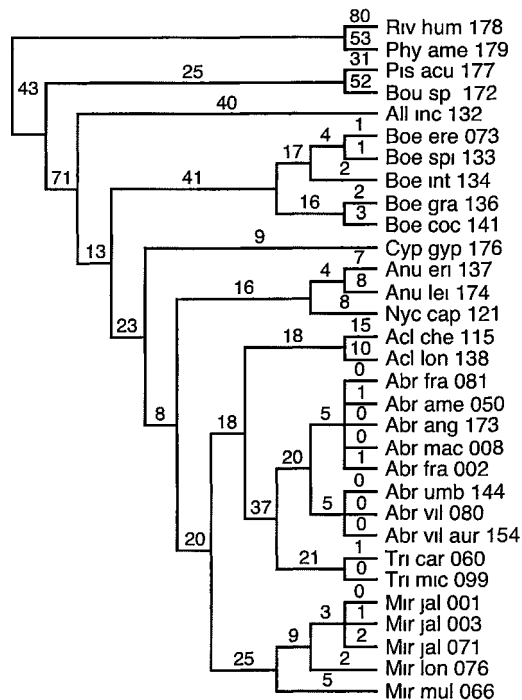
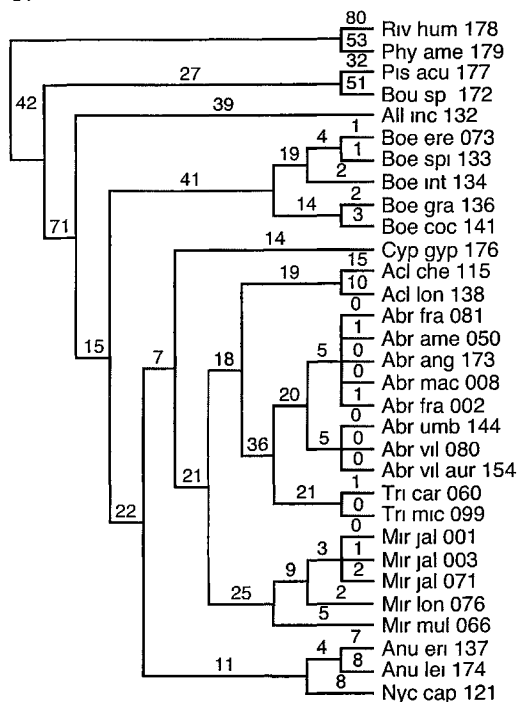


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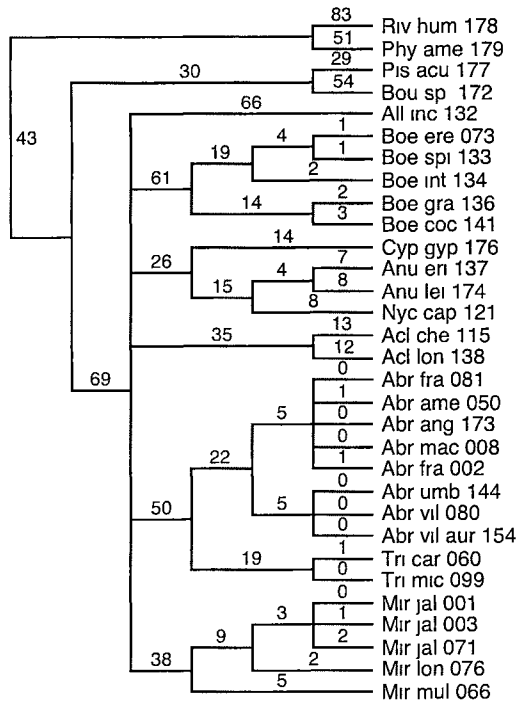


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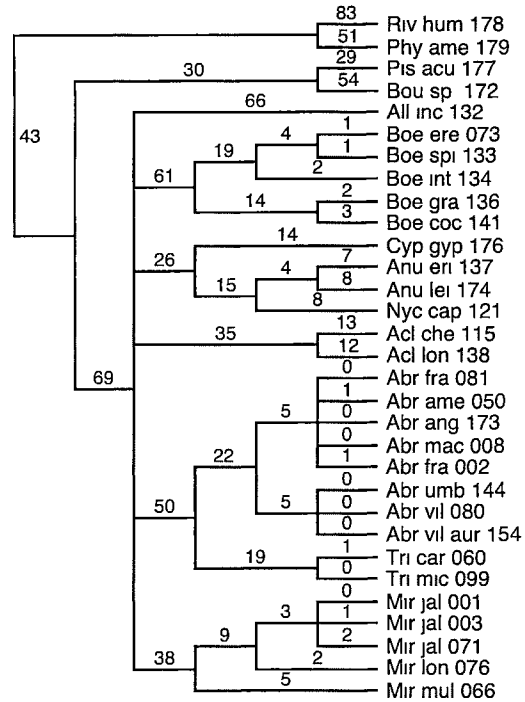




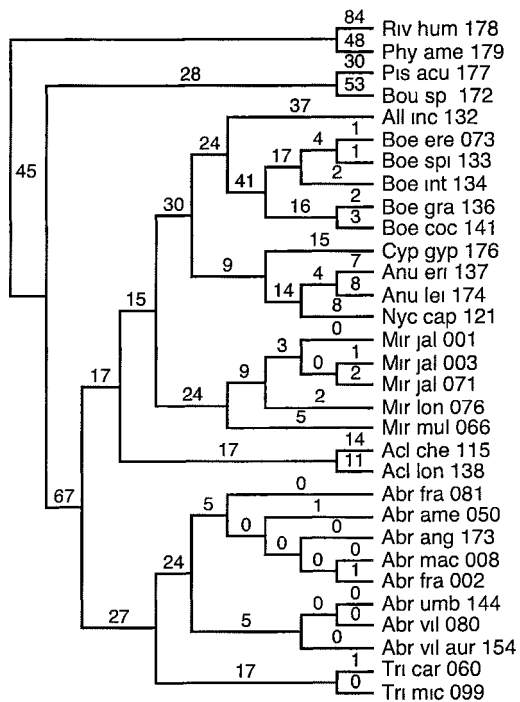
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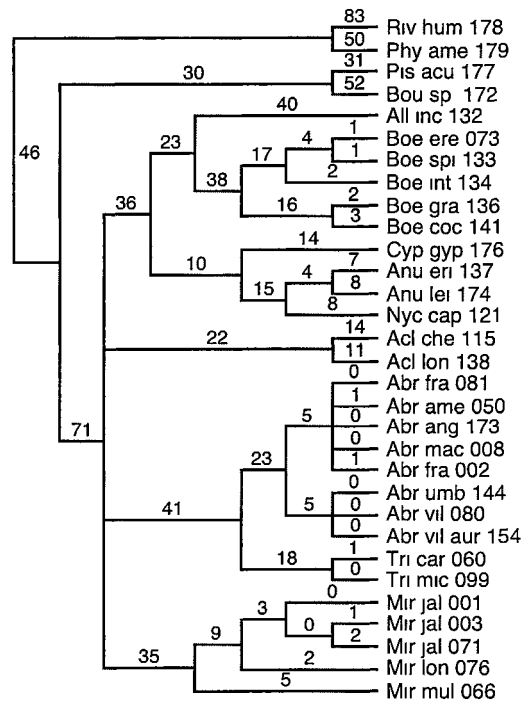
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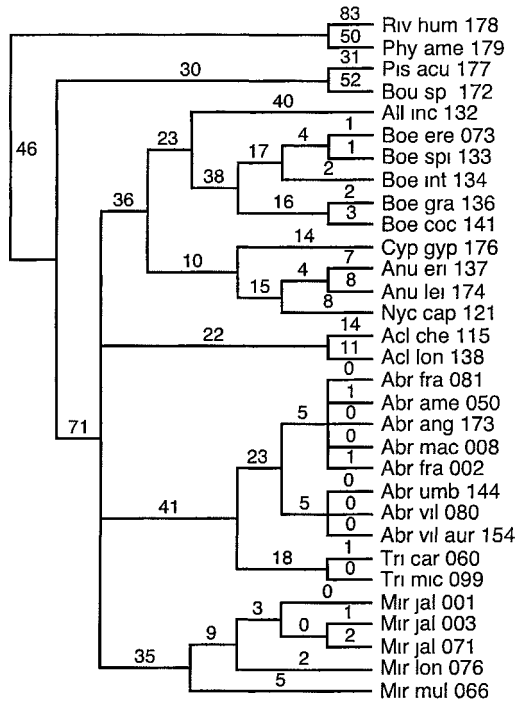
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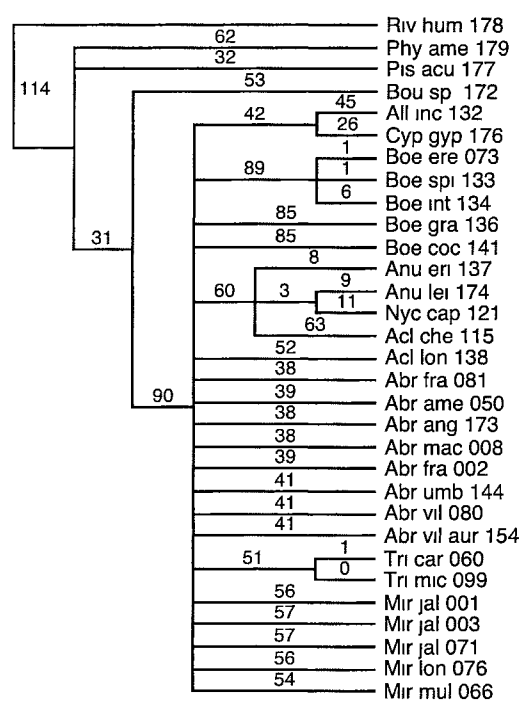
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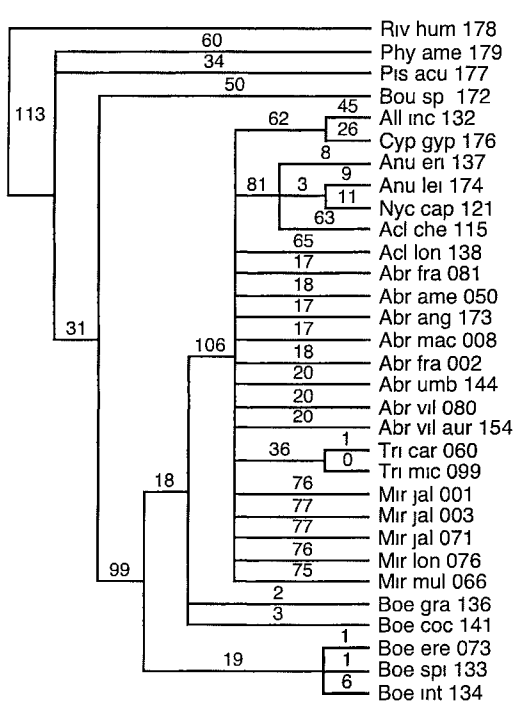
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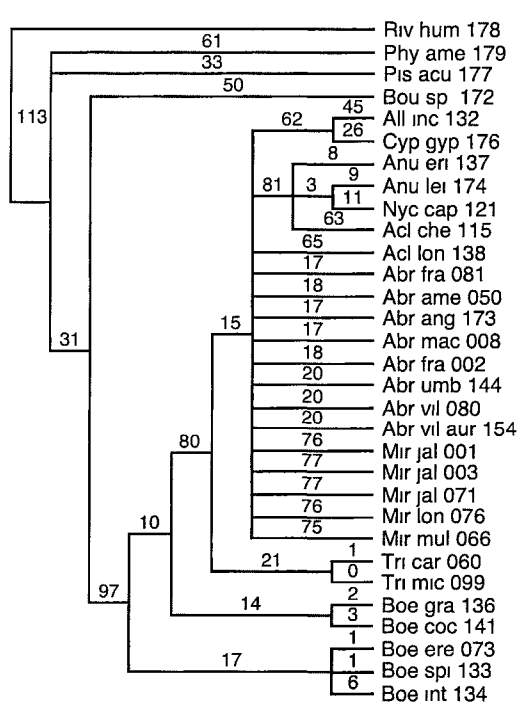
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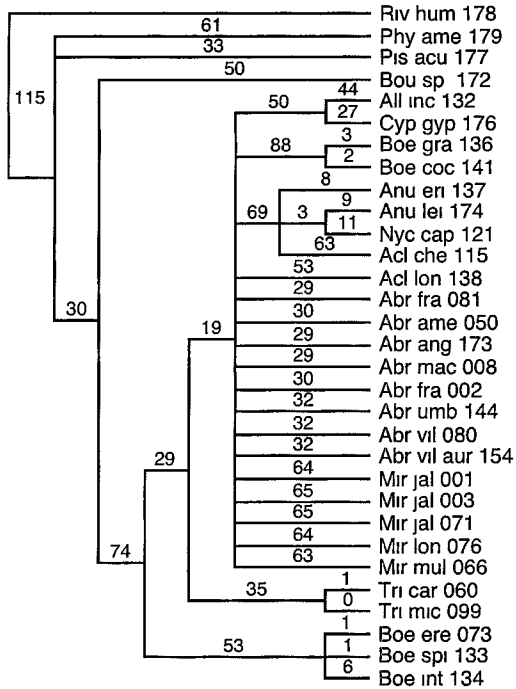
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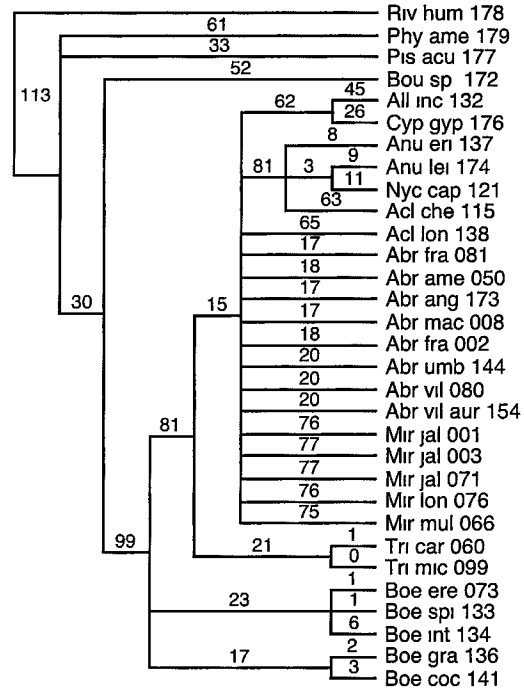
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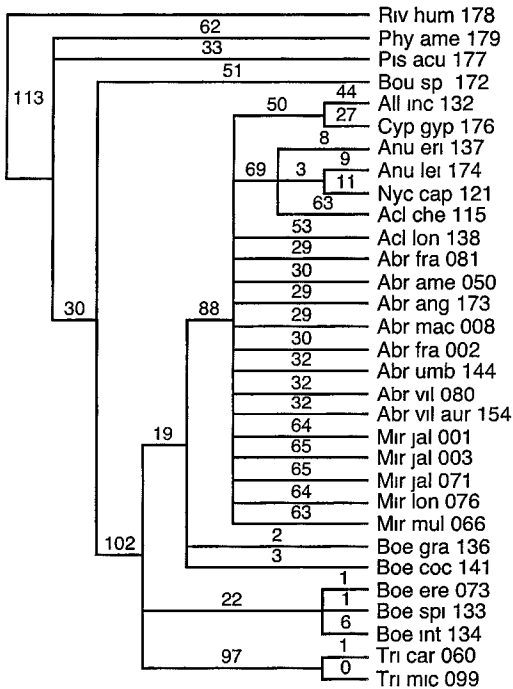
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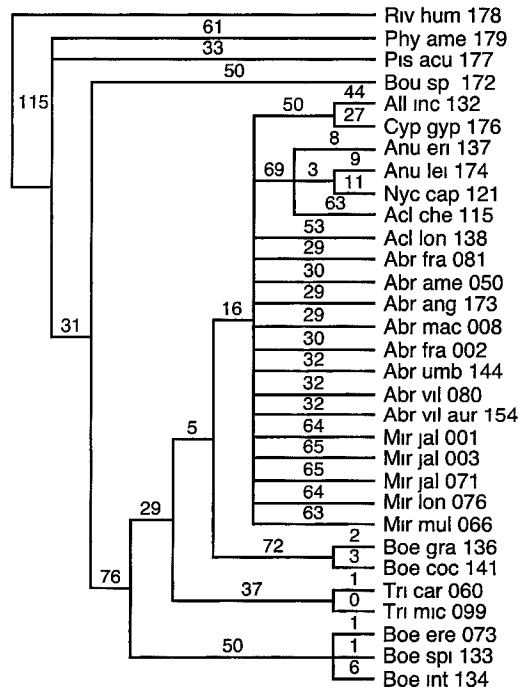
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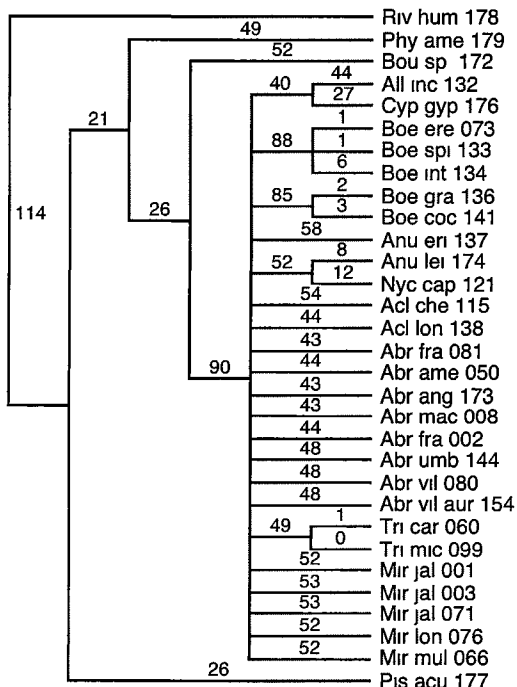


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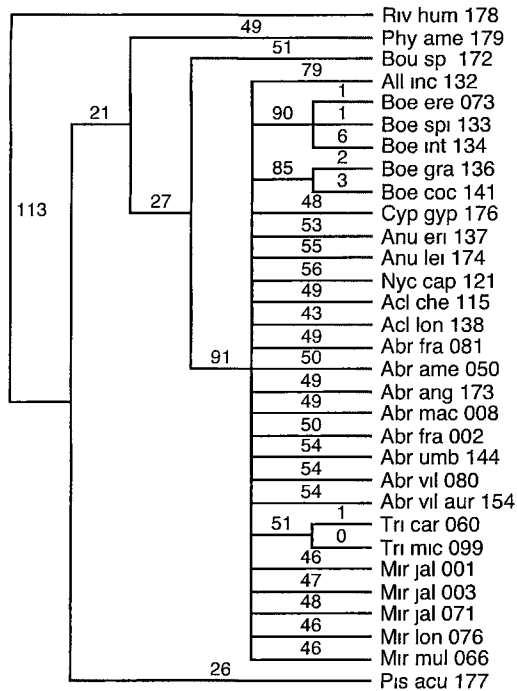


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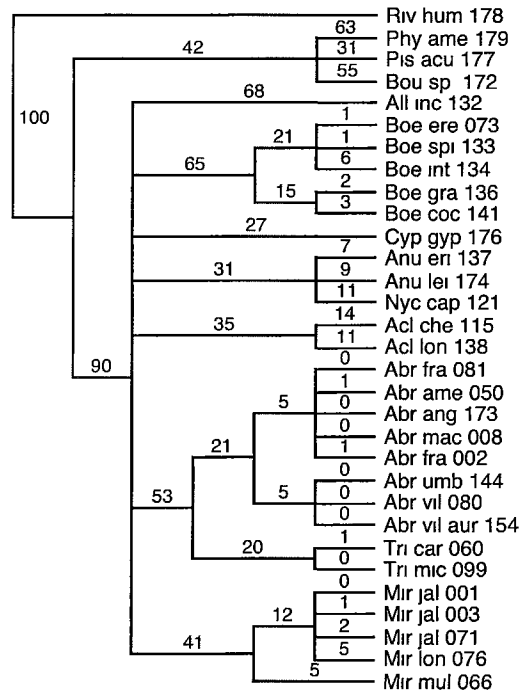




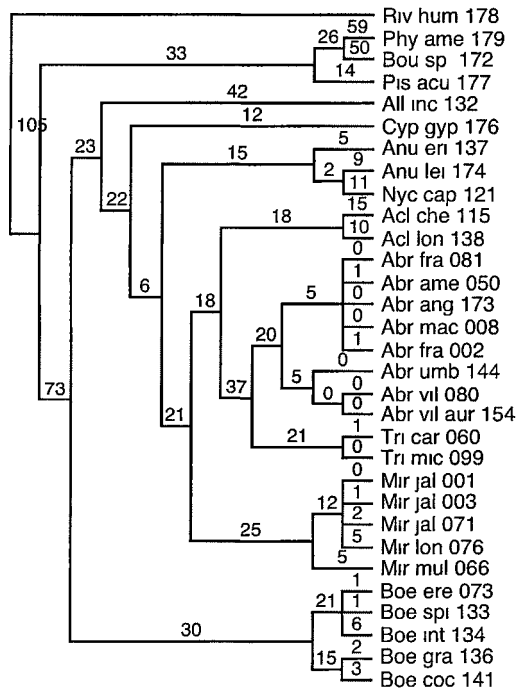
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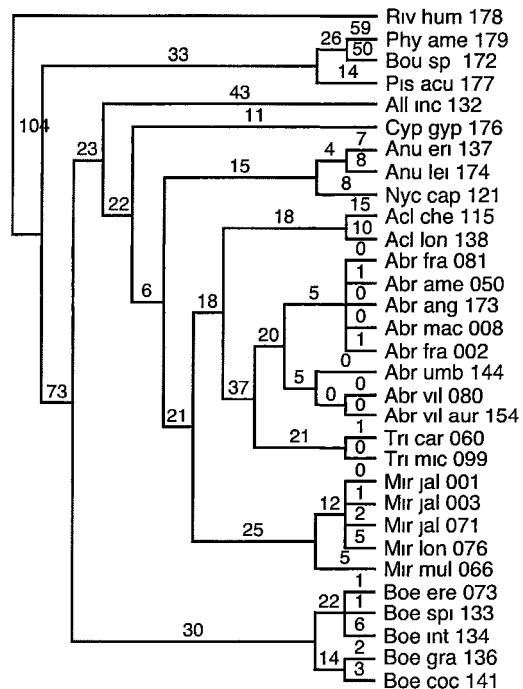
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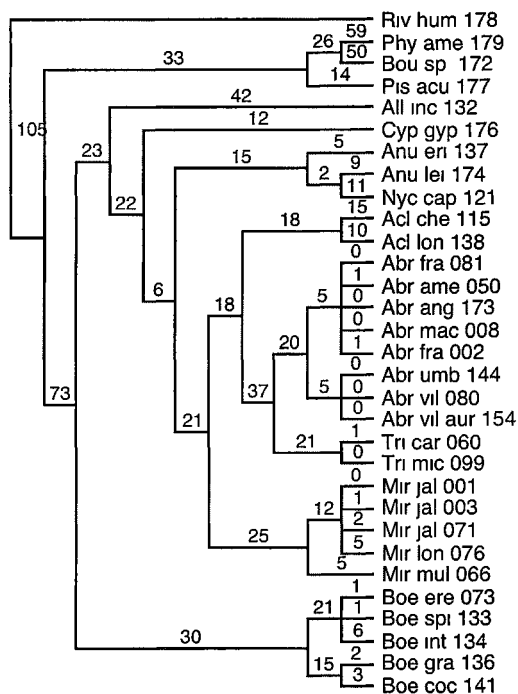
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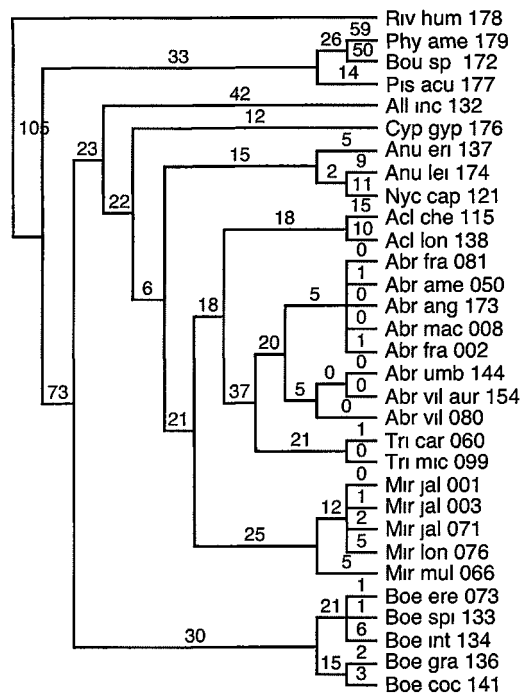
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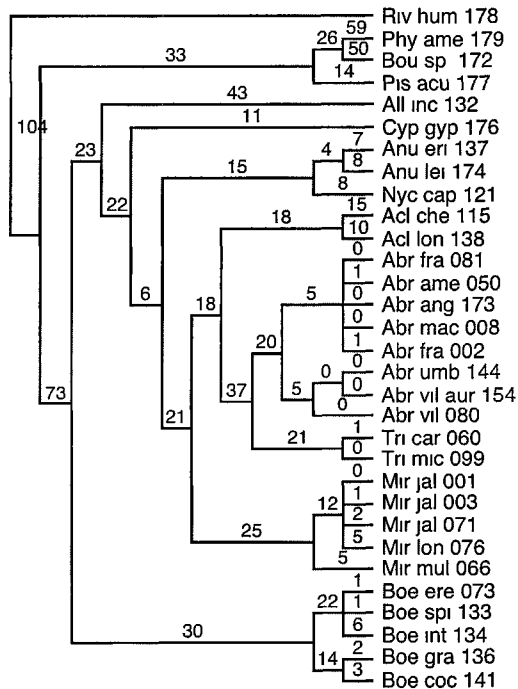
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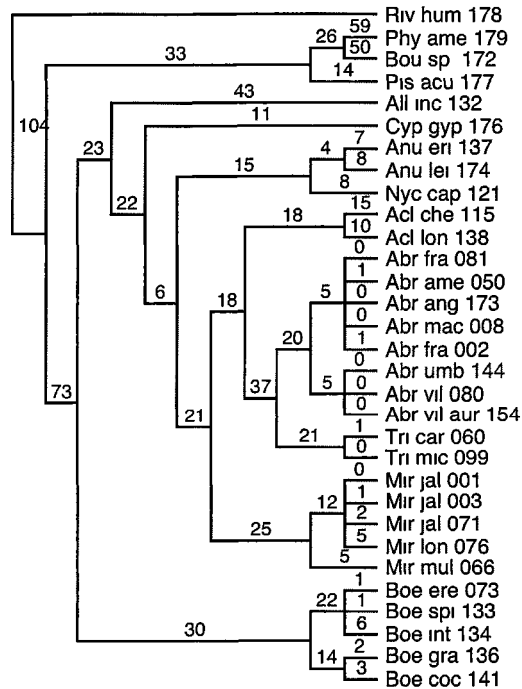
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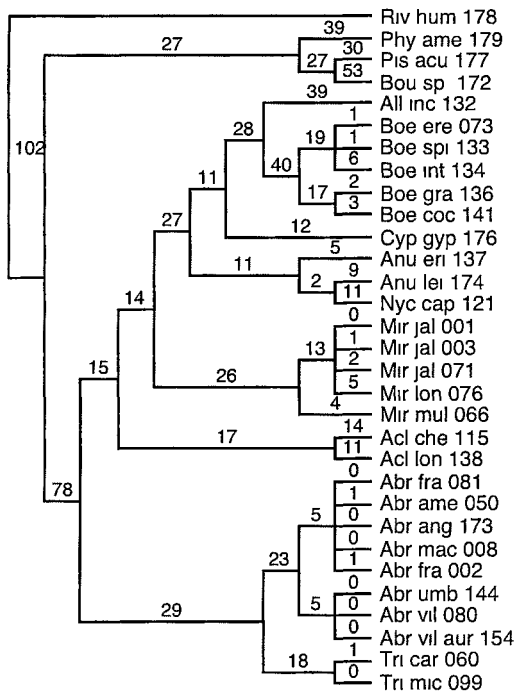
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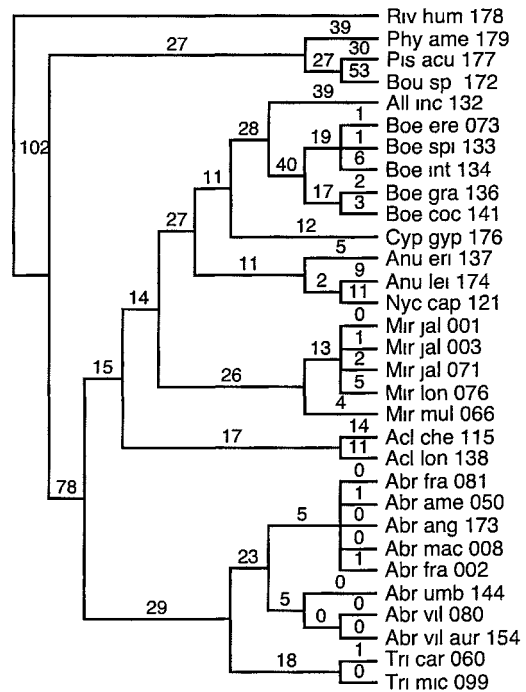
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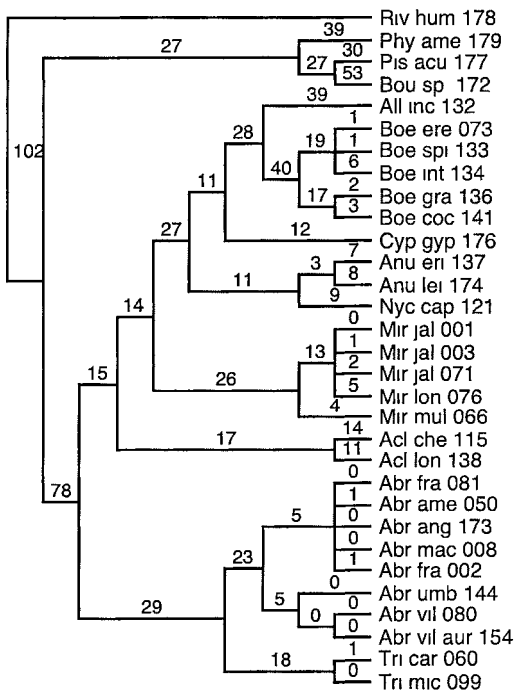
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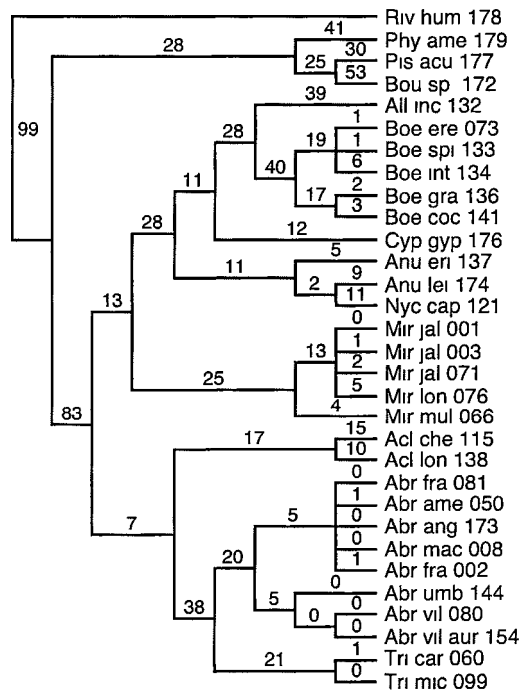
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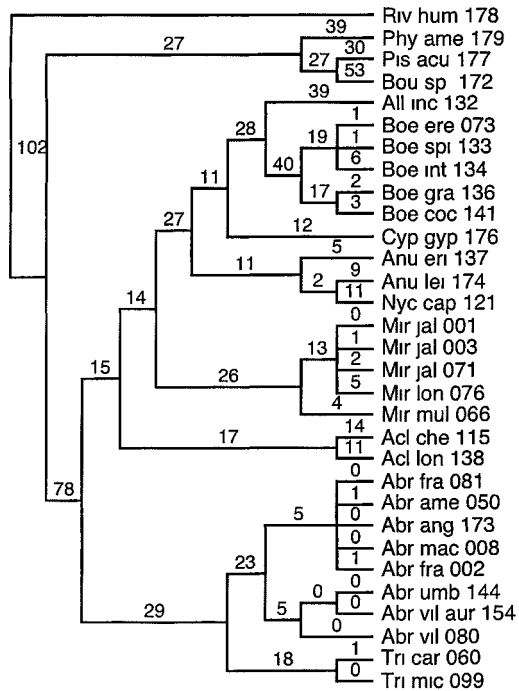
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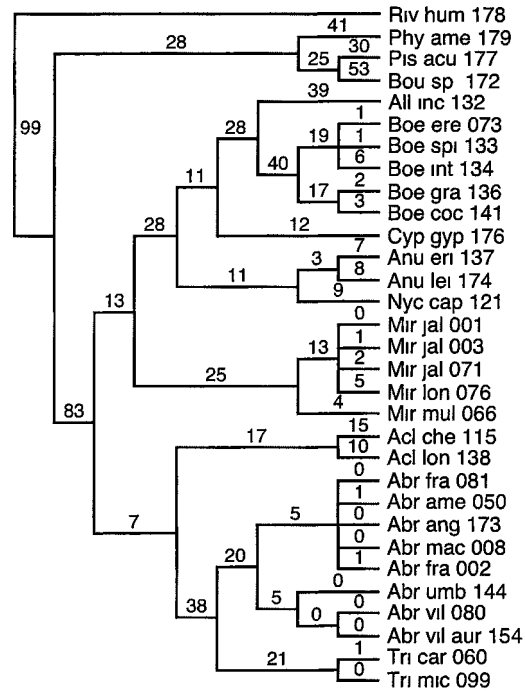
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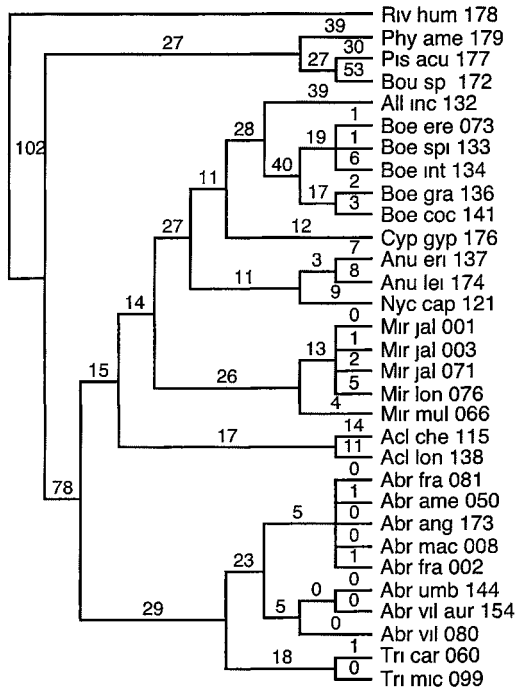
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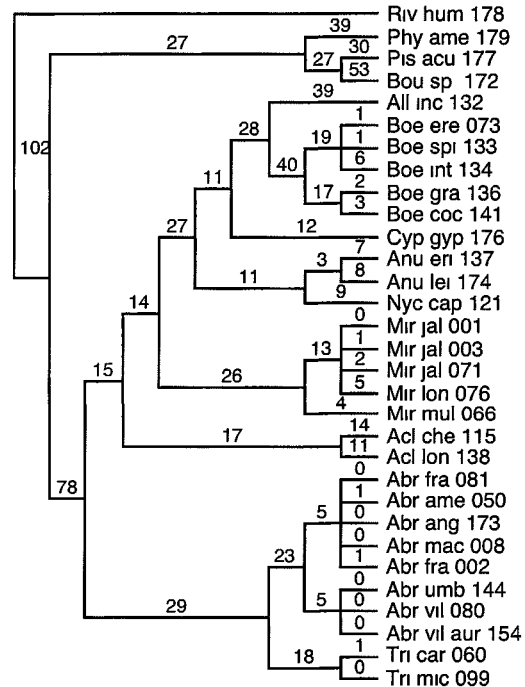
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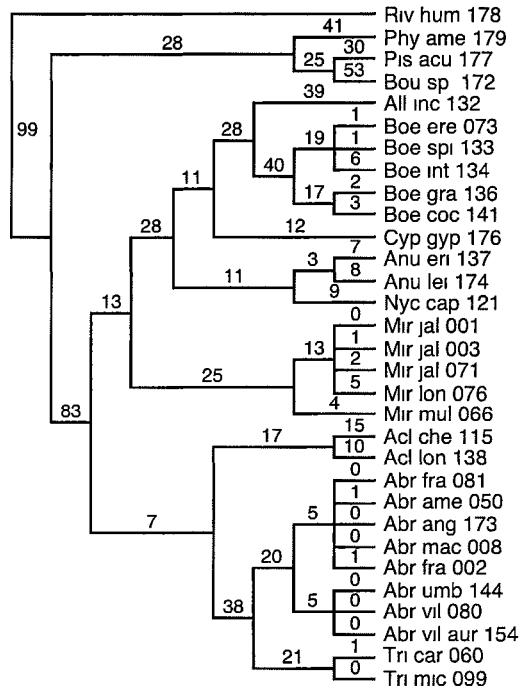


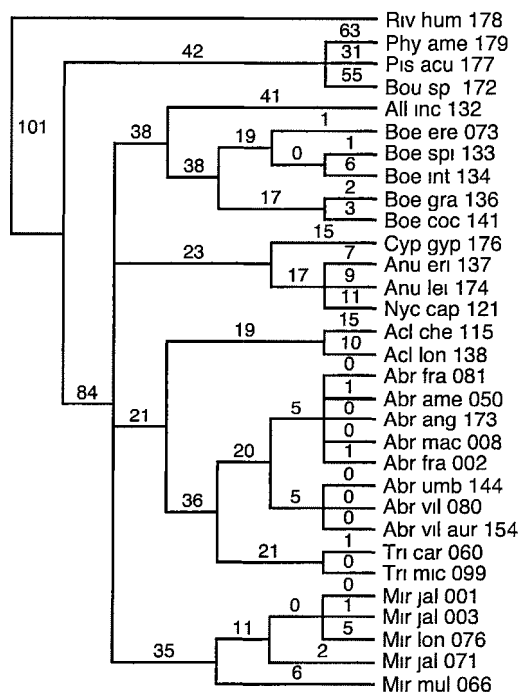
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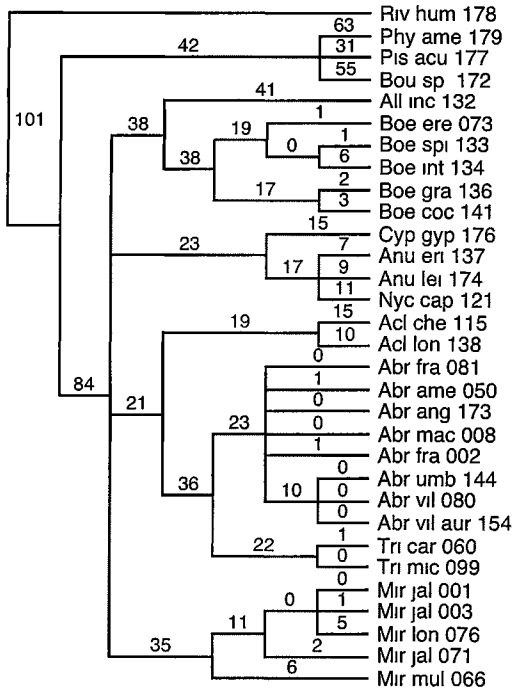
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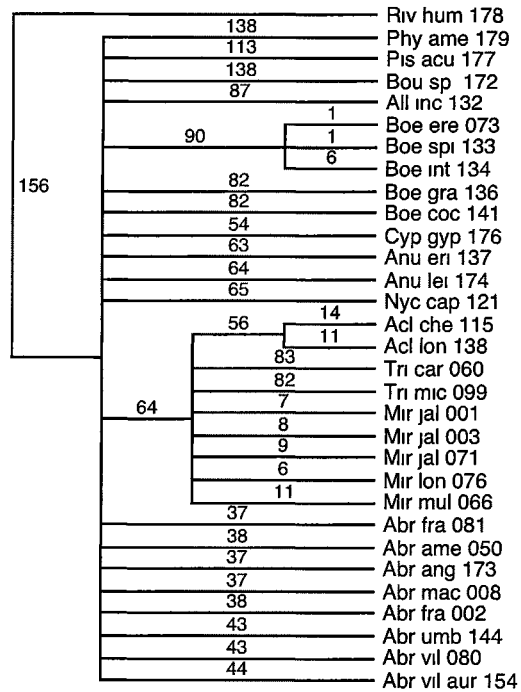




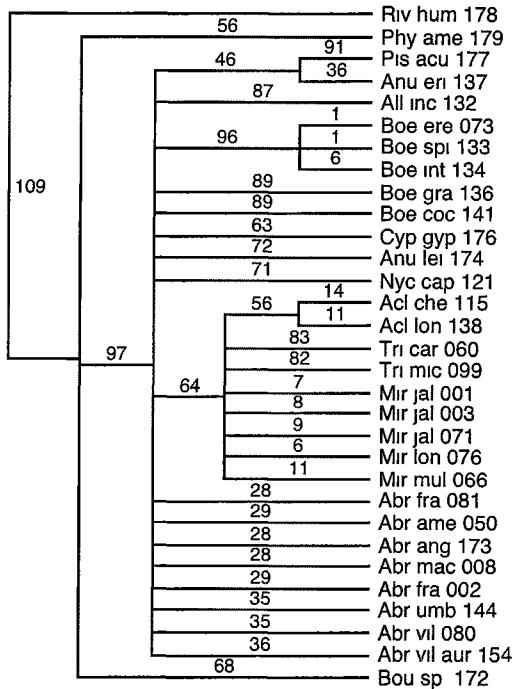
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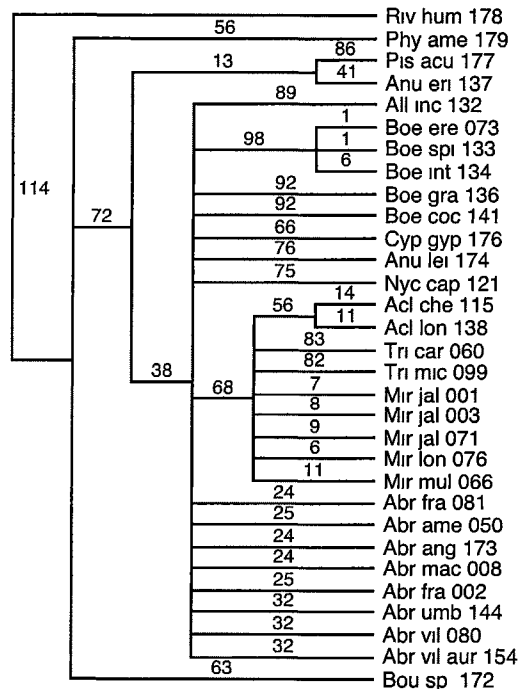
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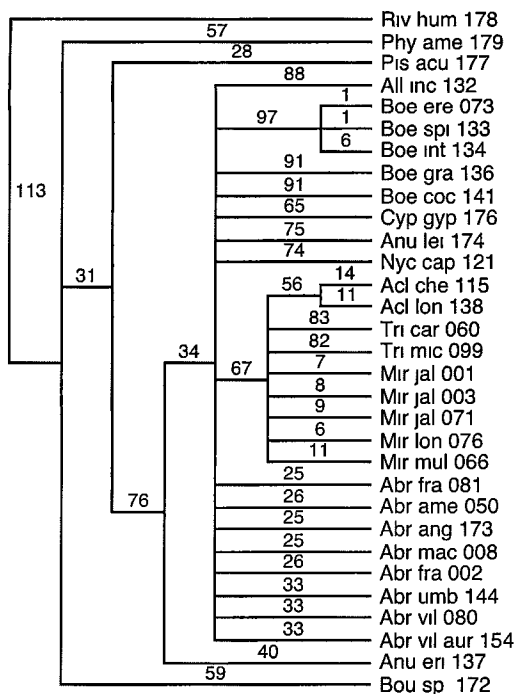
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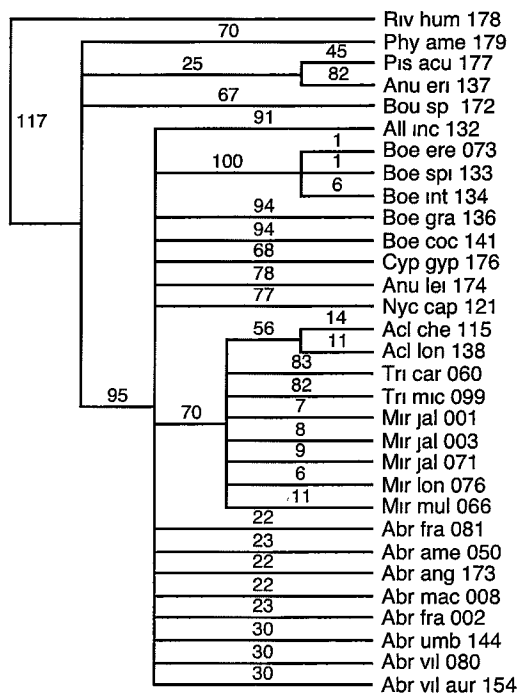
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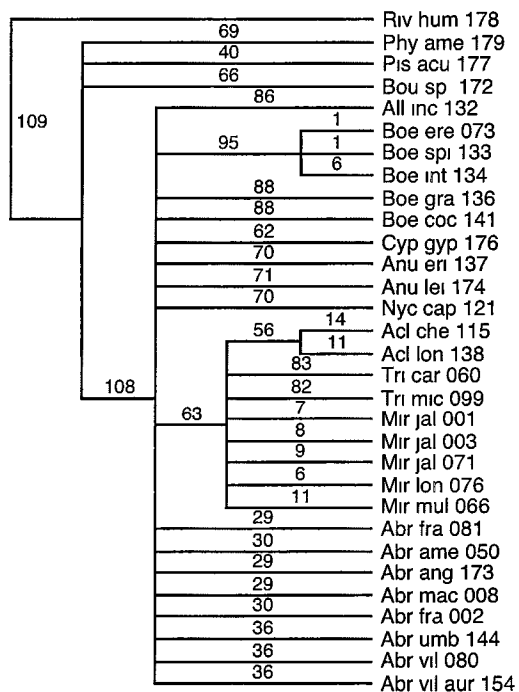
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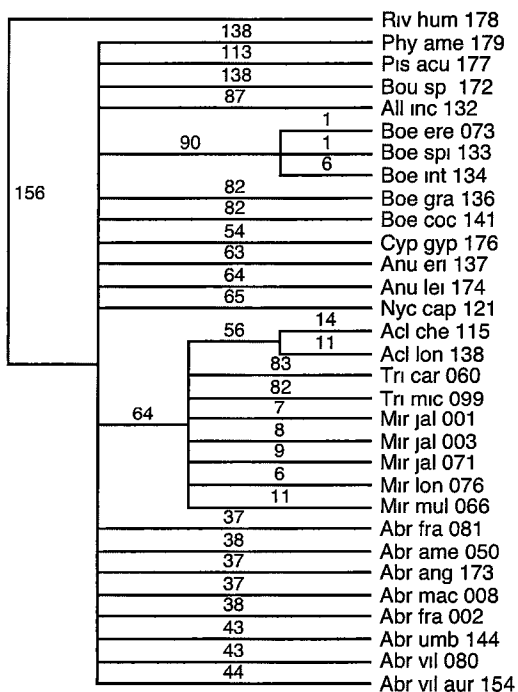
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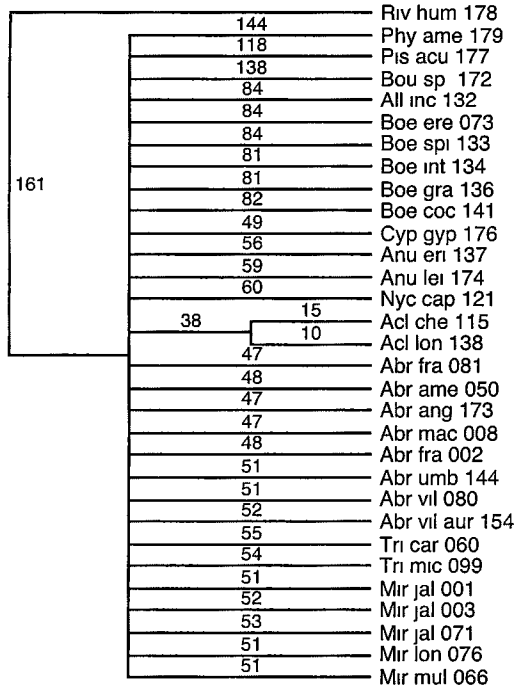
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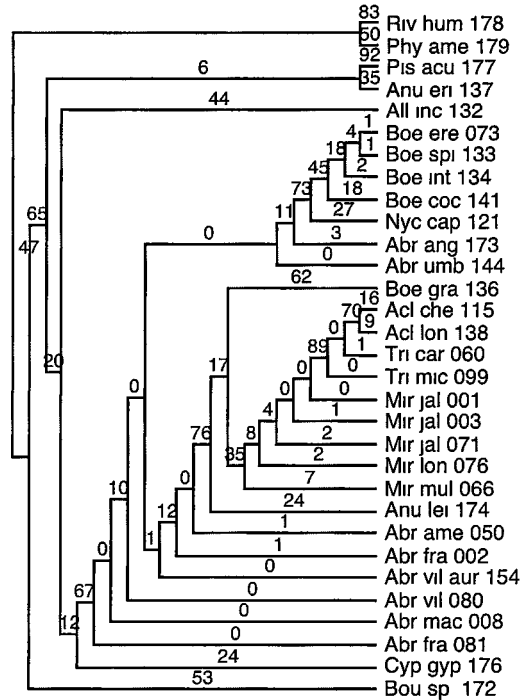
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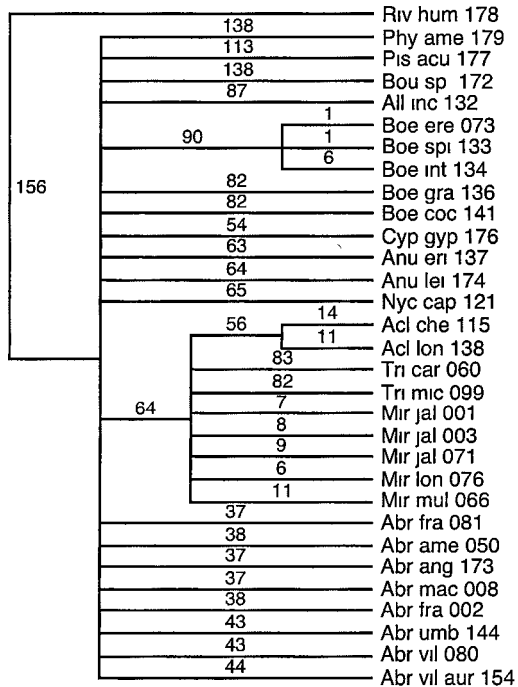
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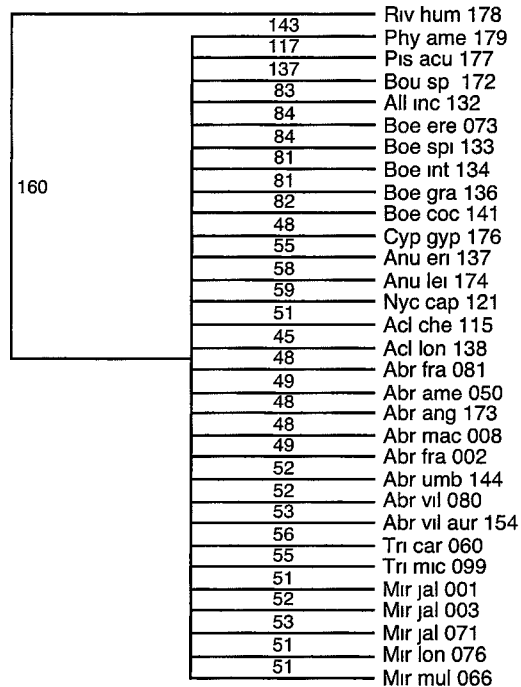
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91



92



APPENDIX 4.

Resultant chromatogram from cycle sequencing performed using the forward external primer N-nc18S10 (cycle sequencing product DM039 of *Abronia macrocarpa* DJM017).

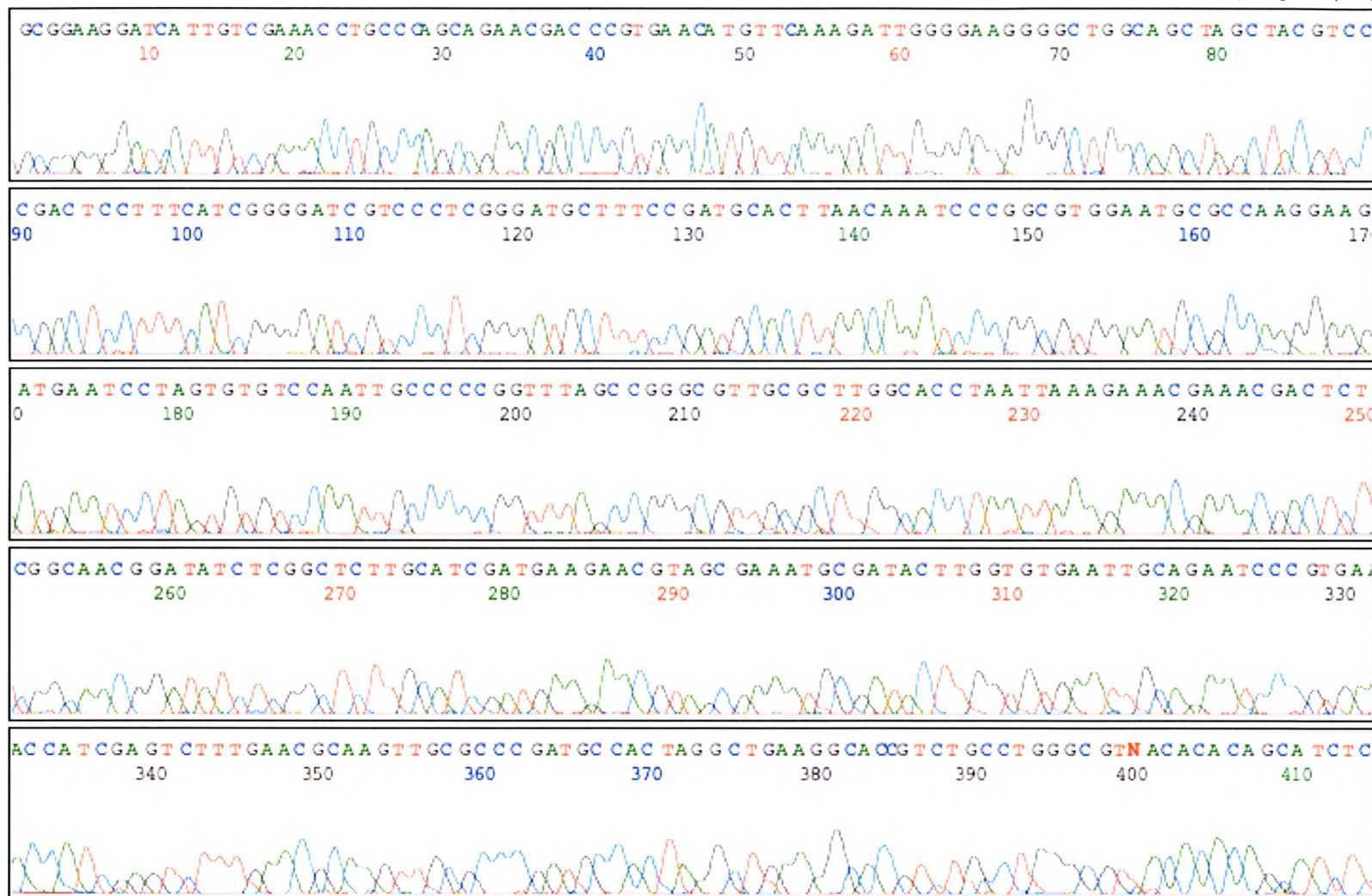


Model 377
Version 3.4.1
Adaptive
Version 3.3.1

12-dm339
dm339
Lane 12

Signal G:82 A:55 T:26 C:42
DT {BD Set Any-Primer}
Big Dye 1dHhodi Matrix
Points 1061 to 7995 (Pk 1 Loc: 1061)

Page 1 of 3
Sat, May 25, 2002 8:40 AM
Fri, May 24, 2002 1:25 PM
Spacing: 9.01(9.01)



VITA

Diana McHenry was born in Brunswick, Maine, on 03 February 1974. She is the daughter of Sandy and Leonard White. After graduating from Great Bridge High School in Chesapeake, Virginia, in June 1992, she attended the College of William and Mary, James Madison University, and Old Dominion University. She earned a Bachelor of Science in Biology from Old Dominion University in May 1997. In 1998, she enrolled as a graduate student in the Biology Department at Southwest Texas State University in San Marcos, Texas. While at Southwest Texas State University, she worked as an instructional assistant for Botany (nonmajors and majors) and Genetics.

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This thesis was typed by Diana McHenry.