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POTENTIAL UTILITY OF CHLOROPLAST *trnL* (UAA) GENE INTRON SEQUENCES FOR INFERRING PHYLOGENY IN SCROPHULARIACEAE¹

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ABSTRACT

Whereas chloroplast DNA-derived sequence data from protein coding regions have been utilized successfully at many taxonomic levels in recent years, sequences which are variable enough to allow for efficient phylogenetic inference (maximum information with relatively low sequencing costs and effort) at the subfamilial level have been few. Sequence data were obtained in this study from a noncoding region, the *trnL* (UAA) gene intron, from a selection of taxa from the Scrophulariaceae and closely related families (representing 41 species in 26 genera). Groups of species from commonly recognized tribes and subtribes were included to determine if these taxa were grouped together by analyses of this sequence. These included seven species from the tribe Cheloneae, 14 species of the tribe Antirrhineae, and four from the tribe Euphraseae or Pedicularae. Also included are representatives of Bignoniaceae (four species, each in a different genus), and the outgroup *Anisacanthus thurberi* of the Acanthaceae. These taxa were examined to estimate the potential utility of this sequence dataset for subfamilial phylogenetic reconstruction. In the majority rule consensus tree, taxa of the tribe Cheloneae (the North American representatives) form a monophyletic clade and generally conform to previous systematic hypotheses from the literature. Sampled taxa of the tribe Antirrhineae (with the exception of *Linaria*) also appear as a potentially monophyletic clade, with support for the subtribes Antirrhinae and Maurandynae. Based on these sequence data we recommend reassessment of some generic placements at the tribal level. The genera *Verbascum* and *Scrophularia* appear on the same, well-supported clade and *Veronica* and *Digitalis* occur together on another well-supported clade. The *trnL* intron sequence comparisons indicate that this relatively short region (about 500 bp) may be useful in studies of phylogeny within Scrophulariaceae and allied taxa at the suprageneric level.

Key words: Antirrhineae, Cheloneae, chloroplast DNA, phylogeny, Scrophulariaceae, *trnL* intron.

INTRODUCTION

Chloroplast DNA sequences have been widely used recently as a source of information on plant phylogeny at various systematic levels. Earlier literature reported the restriction-site variation of the entire chloroplast genome (hybridization studies) or within specific PCR-amplified parts of the chloroplast genome. Wolfe et al. (1997) have recently summarized this literature. While this type of data has proven helpful, it has shortcomings. Because the rate of evolution in the cpDNA genome is thought to be slow and because the portion of variability detected by this technique is rather low, this approach is relatively inefficient. A great deal of work (often using a very large number

of restriction enzymes and several regions of the cpDNA genome) is required to generate a database large enough to allow phylogenetic reconstruction at lower taxonomic levels (Dowling et al. 1996). Further, small differences in fragment size may go undetected. There is even doubt as to whether RFLP data are suitable for phylogenetic analysis (Swofford et al. 1996). All of this leads to the conclusion that DNA primary sequence data are the characters of choice in phylogenetic reconstruction.

Recent technological advances in the ease of DNA sequencing mean that sequence data are more commonly used in the recent literature because of their greater efficiency in detection of variation. These sequences have generally been from coding cpDNA of a relatively few genes, including *rbcl* (e.g., Kim and

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Jansen 1996; Doyle et al. 1997; Morton et al. 1997; Pax et al. 1997; Soltis and Soltis 1997; Wagstaff and Olmstead, 1997), *ndhF* (e.g., Terry et al. 1997), and *matK* (e.g., Plunkett et al. 1996; Hilu and Liang 1997; Kron 1997; Sang et al. 1997). More recently, noncoding sequences of both chloroplast and nuclear DNA have been used in phylogenetic reconstruction. These include intron and intergenic spacer regions in cpDNA and intergenic transcribed spacers of nuclear ribosomal DNA.

Noncoding sequences are not as subject to selective constraints as coding sequences and, therefore, are more variable. Gielly and Taberlet (1994) have estimated that the intron of the chloroplast *trnL* (UAA) gene evolves 2.7 times faster, on the average, than does the *rbcL* sequence. In the genera *Hordeum*, *Triticum*, and *Aegilops* (Poaceae), it was determined that the *trnL* intron evolved 3.74 times faster than *rbcL*. In addition, noncoding regions tend to accumulate insertions/deletions (indels) at rates comparable to nucleotide substitutions (e.g., Wolfe et al. 1987; Zurawski and Clegg 1987; Clegg and Zurawski 1991). These indels can sometimes be successfully used in differentiating between species within a genus (Gielly and Taberlet 1994). Analysis of noncoding chloroplast DNA could, then, be expected to extend the utility of sequence data to lower taxonomic levels than is generally possible with coding regions and require the sequencing of considerably fewer bases.

A few chloroplast introns and intergenic spacers have been used to infer relationships at the interfamilial level and below (e.g., Taberlet et al. 1991; Morton and Clegg 1993; Böhle et al. 1994; Ham et al. 1994; Maner et al. 1994; Mes and Hart 1994). Further, unlike many nuclear DNA genes, cpDNA has the advantage of being a haploid, nonrecombining genome. We chose, therefore, to evaluate the utility of the *trnL* (UAA) intron in reconstructing phylogeny within the Scrophulariaceae and closely related families.

The *trnL* (UAA) intron is a group I (self-splicing) intron composed of seven regions: four conserved in sequence among all *trnL* introns and three nonconserved. It averages about 500 bp in length in most angiosperms (ranging from 350 bp in *Avena* to 600 bp in *Euphorbia*). The nearly two-fold size range of this intron is due mainly to length mutations located in the P8 loop (Gielly and Taberlet 1994). The conserved portions are responsible for the formation of secondary structure which controls folding during the editing process and its catalytic properties (Kuhse et al. 1990; Gielly and Taberlet 1994).

The Scrophulariaceae are a large, cosmopolitan, principally temperate family composed of approximately 3000 species. Both Pennell (1935) and Thieret

(1967) noted that the phylogeny of the family is poorly understood. Based on morphological characters, there have been numerous interpretations of the subfamilial organization, including the recognition of various suites of subfamilies and tribes. Van Tieghem (1903) divided the family into groups determined by staminal number. Bellini (1907) proposed a subfamily classification based on nectary characteristics. After studying New World taxa, Pennell (1935) attempted to reclassify the family on what he believed was a phylogenetic basis. He recognized two subfamilies, Antirrhinoideae (six tribes) and Rhinanthoideae (four tribes) but, due to geographic restrictions, his work was limited in scope. Thieret (1967) recognized six tribes for this family, including one (Selagineae) restricted to the mountains of tropical Africa and Madagascar. Pennell (1935) and Thieret (1967) also recognized different tribal circumscriptions. In summary, this "well-known" family is far from being well known, and a satisfactory tribal classification has not yet been attained. Even the limits of the family itself are not agreed upon (Thieret 1967). The taxonomic history of the family clearly reflects the problem of defining natural groups (Olmstead and Reeves 1995), and our understanding of relationships within this family is imperfect at present.

Since morphological characters have not yielded a consensus phylogeny, the use of DNA data (both restriction fragment length polymorphisms and base sequence) may be of use in the further refinement of our understanding of phylogeny within Scrophulariaceae and between it and closely allied families. Olmstead and Reeves (1995), using data from coding chloroplast DNA sequences (the *rbcL* and *ndhF* genes), hypothesized that the family is polyphyletic, being composed of a "Scroph I" lineage including *Verbascum* and *Scrophularia* and a "Scroph II" lineage including *Antirrhinum*, *Digitalis*, and *Veronica*. While the phylogeny within the Scrophulariaceae has not been extensively investigated using DNA sequence data, Wolfe et al. (1997) used RFLP data to infer relationships especially within the Tribe Cheloneae. Their conclusion was that the North American clade of Cheloneae is monophyletic and includes *Chelone*, *Chionophila*, *Keckiella*, *Nothochelone*, *Penstemon*, and possibly the two genera currently recognized as the Tribe Collinsae (*Collinsia* and *Tonella*).

The major question addressed in this pilot investigation was whether the variability contained in the *trnL* intron sequence is appropriate to phylogenetic investigations within Scrophulariaceae and, if so, at what taxonomic level(s).

The taxa included in this study began as an eclectic sample of 22 species of Scrophulariaceae and later expanded to 41 species. The sampled taxa included at least one representative from most putative tribes of

Scrophulariaceae, but emphasized the New World genera of the tribe Cheloneae (seven species in five genera), the tribe Antirrhineae (14 species in 6 genera), and four species (four species of three genera) of the tribe Euphraseae (e.g., Pennell 1935) sometimes placed in the tribe Pedicularae (e.g., Chuang and Heckard 1991). Also included were four species of Bignoniaceae and *Anisacanthus thurberi* (Acanthaceae) as the outgroup. The sample was, then, intended to determine if the sequence was capable of clustering recognized tribes as monophyletic clades and would be capable of yielding information as to the tribal placement of some problematic taxa. Problematic taxa include the genus *Paulownia*, which has been assigned to the Bignoniaceae by some authors and the Scrophulariaceae by others (e.g., Thieret 1967 and Armstrong 1985), three *Verbascum* species, and three *Scrophularia* species. *Verbascum* and *Scrophularia* have sometimes been treated both as members of the same tribe (Verbasceae) or separately (*Scrophularia* being placed in the tribe Cheloneae).

METHODS AND MATERIALS

Leaf material for total DNA extraction was obtained from several sources (Table 1). Fresh leaf material was collected by CEF from the field. These materials were vouchered at UTEP. Fresh leaf material was also obtained by RS from the Los Angeles State and County Arboretum and Rancho Santa Ana Botanic Garden. Other leaf samples were obtained from the University of California Botanical Garden, Berkeley. Some total DNAs were obtained from herbarium material at UTEP. Additional dried leaf materials of European species of *Verbascum* and *Scrophularia* were obtained from the Canton of Geneva in Switzerland.

Leaf samples (both fresh and dried) were ground in a lysis buffer (e.g., Elu-quik[™] lysis buffer, Schleicher and Schuell, Keene, NH) or DNAzol[™]ES for plant DNA isolation (DN-128, Molecular Research Center, Inc., Cincinnati, OH). The DNA was then precipitated by addition of ethanol and chilling (-70°C for 30 min) and pelleted by centrifugation. After drying, the pellet was dissolved in TE buffer and the DNA purified using the GeneClean[™] protocol (Bio101 Corp., San Diego, CA) or by reprecipitation as needed. This DNA purification procedure was also generally effective in removing sufficient phenolics from the total DNA preparation to allow PCR amplification. When this was not the case, repeated reprecipitations with ethanol gradually reduced phenolics to levels which allowed PCR amplification. When it was difficult to obtain amplifiable DNA (especially from dried leaf material) due to contamination with proteins, an overnight digestion at room temperature

with Proteinase K was almost always sufficient. When not, the DNA was precipitated and retreated with Proteinase K.

PCR primers which amplified both the *trnL* (UAA) intron and *trnL-trnF* intergenic spacer regions were synthesized. These were primers "c" and "f" from Taberlet et al. (1991). The resulting PCR product, consistently about 1050 bp in length in the taxa studied here, was then gel-purified in 0.8% HMP agarose. The excised agarose plug containing the initial PCR product was placed in a 0.5 ml microfuge tube with 10 μl of TE. This product was used as the template for obtaining additional PCR product for sequencing. The sequencing of the *trnL* intron was performed using primers "c" and "d" from Taberlet et al. (1991) and an internal sequencing primer "g" designed by CEF when necessary. The sequence of g primer is 5'-GTGCAGAGACT-CAATGGAAGC-3'. PCR products (double-stranded) were sequenced directly by dideoxy techniques using the Sequenase[™] PCR product sequencing kit (U.S. Biochemical, Cleveland, OH) and labeled internally with ^{35}S -labeled dATP.

All sequence data for analysis began 13 bp downstream from the 5' end of the intron based on the tobacco sequence. Sequences were aligned using the program MAP (Baylor College of Medicine) via the internet (Pedro's Molecular Biology Tools). Minor alignment adjustments were then made manually. The data were subjected to Wagner parsimony and neighbor-joining analysis using PAUP 4.0* Beta (Swofford 1998) using the "gapmode = missing" option. While the maximum parsimony method is the most widely used of the discrete character tree-building methods, it is not always a consistent estimator of the true tree (Kumar et al. 1993). Therefore, a distance method was also used. We chose the neighbor-joining method as an alternative distance-based tree building method (Saitou and Nei 1987). Computer simulations (Nei 1991) have indicated that it is one of the most efficient distance methods in recovering the correct topology. To use the information in the gaps for phylogenetic reconstruction and yet reduce their significance in parsimony analysis, as many gaps (1–26 bp) as possible were removed from the sequence dataset as units. These gaps were inserted into the data set as presence or absence data ("1" or "0"). When an indel was only a single bp, the bases were coded as 0 (gap), 1 (A), 2 (C), 3 (G), or 4 (T). A gap was recorded only once, regardless of the number of bases it contained. The sequences have been deposited in GenBank (Accession numbers AF034862 through AF034890 and AF118786 through AF118806).

Table 1. Sources of materials sampled in this study. UTEP = Univ. of Texas at El Paso (voucher numbers). UCBG = Univ. of California Botanic Garden (accession numbers). LASCA = Los Angeles State and County Arboretum (accession numbers). RSA = Rancho Santa Ana Botanic Garden.

Family and species	bp analyzed	Voucher/accession number	Origin
Acanthaceae:			
<i>Anisacanthus thurberi</i> (Torr.) A. Gray	489	UTEP 56099	cult.
Bignoniaceae:			
<i>Chilopsis linearis</i> (Cav.) Sweet	476	UTEP 56100	TX, El Paso Co.
<i>Kigelia pinnata</i> (Jacq.) DC.	468	LASCA 50-1303-S	cult.
<i>Tabebuia chrysotricha</i> (Mart. ex DC.) Standl.	487	LASCA 79-1276-S	cult.
<i>Tecoma stans</i> (L.) Juss.	476	UTEP 56101	TX, El Paso Co.
Scrophulariaceae:			
Subfamily Antirrhoideae			
Tribe Antirrhineae			
<i>Antirrhinum coulterianum</i> Benth.	476	UTEP 34904	CA, Riverside Co.
<i>Antirrhinum cyanthiferum</i> Benth.	474	UTEP 56311	Mex, Baja Calif.
<i>Antirrhinum filipes</i> A. Gray	473	UTEP 57520	AZ, Mohave Co.
<i>Antirrhinum watsonii</i> Vasey & Rose	473	UTEP 56315	Mex, Baja Calif.
<i>Antirrhinum kelloggii</i> Greene	476	UTEP 50629	CA, San Diego Co.
<i>Antirrhinum majus</i> L.	472	UTEP 56104	cult.
<i>Galvezia speciosa</i> (Nutt.) A. Gray	513	RSA 498102	cult., RSA
<i>Linaria canadensis</i> (L.) Dum. Cours.	464	UTEP 52991	NM, Luna Co.
<i>Mabrya acerifolia</i> (Pennell) Elisens	479	UTEP 56309	AZ, Maricopa Co.
<i>Maurandya antirrhiflora</i> Willd.	485	UTEP 56105	TX, El Paso Co.
<i>Maurandya wislizeni</i> Engelm.	484	UTEP 56828	TX, El Paso Co.
<i>Mohavea breviflora</i> Cov.	474	UTEP 57521	AZ, Mohave Co.
<i>Mohavea confertifolia</i> (Benth.) A. A. Heller	474	UTEP 57522	AZ, Mohave Co.
Tribe Cheloneae			
<i>Chelone obliqua</i> L.	471	UTEP 56102	cult., MBG
<i>Chionophila jamesii</i> Benth.	481	UTEP 44223	CO, El Paso Co.
<i>Keckiella antirrhinoides</i> (Benth.) Straw	472	UTEP 51241	AZ, Maricopa Co.
<i>Nothochelone nemorosa</i> (Lindley) Straw	471	UTEP 54188	WA, Cowlitz Co.
<i>Penstemon centralifolius</i> (Benth.) Benth.	476	UCBG 87-1278	CA, Monterey Co.
<i>Penstemon palmeri</i> A. Gray	477	UTEP 53282	NV, Clark Co.
<i>Penstemon whippleanus</i> A. Gray	483	UTEP 44272	NM, Otero Co.
<i>Scrophularia macrantha</i> Greene ex Stiefelh.	480	UTEP 54192	NM, Grant Co.
<i>Scrophularia nodosa</i> L.	479	UTEP 57503	Switzerland
		RSA 637104	
<i>Scrophularia californica</i> Chamb. & Schldl.	477		cult., RSA
Tribe Colliniseae			
<i>Collinsia heterophylla</i> Buist	476	UTEP 53541	CA, Tulare Co.
Tribe Digitalieae			
<i>Digitalis purpurea</i> L.	478	UTEP 54185	OR, Clatsop Co.
<i>Mimulus lewisii</i> Pursh	469	UTEP 54186	WA, Yakima Co.
<i>Paulownia tomentosa</i> (Thunb.) Steud.	475	LASCA 64-1441-S	cult.
Tribe Leucopylleae			
<i>Leucophyllum minus</i> A. Gray	481	UTEP 51523	TX, Culberson Co.
Subfamily Rhinanthoideae			
Tribe Euphrasieae			
<i>Castilleja angustifolia</i> (Nutt.) G. Don	472	UTEP 55472	WY, Lincoln Co.
<i>Castilleja integra</i> A. Gray	470	UTEP 51729	TX, Presidio Co.
<i>Cordylanthus kingii</i> S. Wats.	465	UTEP 53576	NV, Clark Co.
<i>Pedicularis grayi</i> A. Nels.	469	UTEP 53917	AZ, Graham Co.
Tribe Verbasceae			
<i>Verbascum nigrum</i> L.	479	UTEP 57501	Switzerland
<i>Verbascum phlomoides</i> L.	472	UTEP 57499	Switzerland
<i>Verbascum pulverulentum</i> L.	480	UTEP 57497	Switzerland
Tribe Veroniceae			
<i>Veronica cusickii</i> A. Gray	461	UTEP 54179	WA, Pierce Co.

RESULTS AND DISCUSSION

The alignment obtained from the MAP alignment algorithm (with very minor manual adjustments) is shown in Appendix 1. The consensus length of the aligned sequences was 563 bp. Eighty-four gaps were contained within the sequence alignment used in analysis. The length of individual sequences in the taxa considered is shown in Appendix I but was generally about 480 bp. Numerous gaps were, then, identified in the intron sequence and ranged in size from one to 26 base pairs in length. The largest insertion was found in *Galvezia (Gambelia) speciosa*, making the *trnL* intron sequence of this species the longest of the sample at 506 bp. Most indels were concentrated in the nonconserved regions of the intron (Fagan et al. 1994), especially in the nonconserved region VI. Gaps were, however, found in other regions as well.

The purpose of this study was to determine the utility of this chloroplast DNA sequence in studying phylogeny in the Scrophulariaceae. A total of 190 parsimony informative sites was discovered in the aligned sequences and these were used in an analysis. This analysis of the data generated presented a number of interesting observations.

Wagner parsimony yielded 234 most parsimonious trees. The results of the parsimony analysis is shown in Fig. 1 (strict consensus tree) and Fig. 2 (bootstrap 50% majority rule consensus tree). In addition, the cladogram generated by the neighbor joining algorithm (a clustering procedure) is shown in Fig. 3. This body of data suggests several conclusions concerning phylogeny in Scrophulariaceae and the allied Bignoniaceae. Using the proportions obtained by bootstrapping as estimates of the degree of support of individual clades, we follow the convention of Hillis and Bull (1993) that bootstrap proportions above 70% represent true clades of 95% of the time.

Subfamilies of Scrophulariaceae

The family Scrophulariaceae has sometimes been divided into two subfamilies, Antirrhinoideae and Rhinanthoideae, based on the relative positions of corolla lobes in aestivation (Pennell 1935). Alternatively, Diels (1897), Hallier (1903), and Hartl (1957) believe that this character is a feature of little importance in establishing relationships. The cladograms generated in this study fail to support two distinctive clades recognizable as subfamilies of Scrophulariaceae. The representative genera of two tribes of the Rhinanthoideae (*Digitalis*: Digitaleae and *Veronica*: Veroniceae) do seem closely related. However, *Pedicularis* (Subfamily Rhinanthoideae, tribe Euphasieae) and *Leucophyllum* (Subfamily Antirrhinoideae, tribe Leucophylleae) also appear closely related. These data, therefore, support

the view that the position of corolla lobes in bud are of little value in understanding phylogeny in this family.

Circumscription of Scrophulariaceae and Placement of Paulownia

The cladograms presented here can be interpreted as supporting the recent hypothesis that Scrophulariaceae, as currently circumscribed, may be polyphyletic as suggested by Olmstead and Reeves (1996) and Wolfe et al. (1997). However, with this limited array of taxa, two scroph clades, as suggested by Olmstead and Reeves (1996), are not readily apparent.

Interestingly, the representatives of the Bignoniaceae appear as a clade imbedded deeply within the Scrophulariaceae. The delimitation of Bignoniaceae and Scrophulariaceae historically has been troublesome (e.g., Armstrong 1985) as exemplified by "intermediate" genera such as *Paulownia*. The family Bignoniaceae has been segregated from the Scrophulariaceae largely on the basis of its greater woodiness, seed characters (e.g., the lack of endosperm in the seeds), and placentation. *Paulownia*, an arborescent genus native to eastern China, has been assigned variously to both the Scrophulariaceae and Bignoniaceae since the first half of the nineteenth century. *Paulownia tomentosa* was originally described as *Bignonia tomentosa* Thunb. in 1784 (Campbell 1930) and placed in the Bignoniaceae by Hallier (1903) and by Li (1947). It was subsequently transferred to the Scrophulariaceae because of its slightly endospermous (two or three cell layers) seeds (Millsaps 1936). This character, according to Campbell (1930), is the only one by which the families can be separated. It was included in the Scrophulariaceae by Wettstein (1891), Steenis (1949), and Gleason (1952). More recently Armstrong (1985) placed *Paulownia* among the Scrophulariaceae based on its floral anatomy. Olmstead and Reeves (1995), using sequence data from the chloroplast *rbcL* and *ndhF* genes, concluded that *Paulownia* is isolated near the base of the order Scrophulariales in Bignoniaceae (*rbcL* data) or near Lamiales (*ndhF* data). In both cases, bootstrap values were very low, providing little support for either scenario. They conclude that *Paulownia* does not belong to lineages containing other members of the Scrophulariaceae. The *trnL* sequence data presented here also do not indicate a clear placement for *Paulownia*. *Paulownia* does appear as a basal, weakly supported taxon on the Bignoniaceae clade in both the parsimony strict consensus tree (Fig. 1) and the neighbor joining tree (Fig. 3). However, it is not allied to Bignoniaceae in the bootstrap tree (Fig. 2).

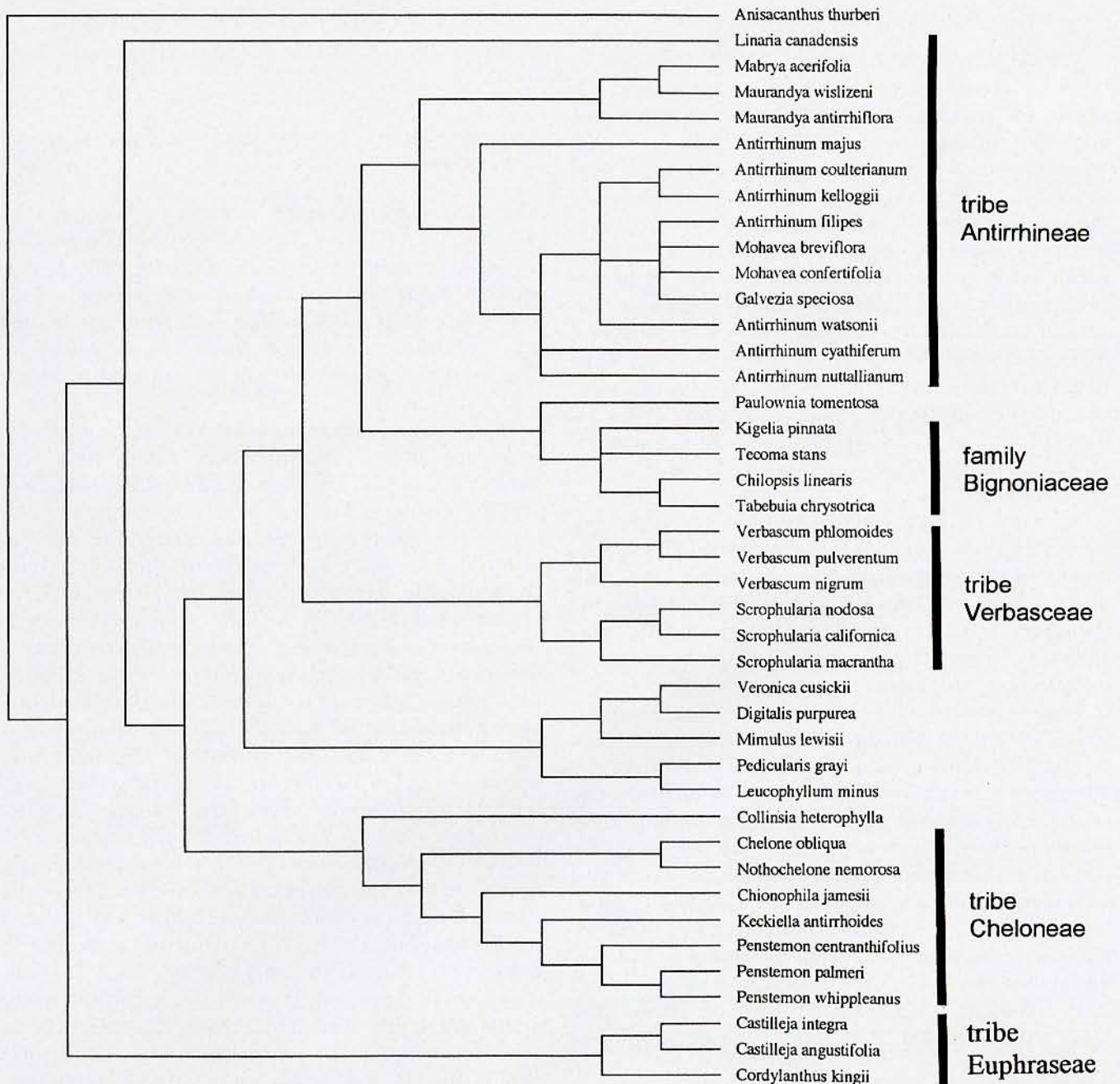


Fig. 1. Parsimony strict consensus tree of 234 trees saved (tree length = 747; CI = 0.6787; RI = 0.7460, RC = 0.5063) based on sequence data of the intron of the chloroplast *trnL* gene.

Thus, the position of *Paulownia* as either Bignon or Scroph remains unclear based on the sequence of this noncoding region, although it seems here to be more closely related to the Bignoniaceae than Scrophulariaceae.

Tribal phylogeny of Cheloneae.—The tribe Cheloneae as sampled here includes representative members from all North American genera of the tribe as presented by Straw (1966). Members include *Chelone obliqua*, *Chionophila jamesii*, *Keckiella antirrhoides*, *Nothochelone nemorosa*, *Penstemon centranthi-*

folius, *P. palmeri*, *P. whippleanus*, and *Scrophularia macrantha*, *S. californica*, and the European *S. nodosa*. With the exception of *Scrophularia* species, these genera appear in the cladograms generated in this study as a highly supported monophyletic assemblage. The three *Penstemon* species appear together at terminal nodes.

The seven species of *Keckiella* were removed from *Penstemon* by Straw (1966) on the basis of their shrubby habit, nectariferous hypogenous discs, and the lack of glandular hairs within their corollas. *Keckiella* here

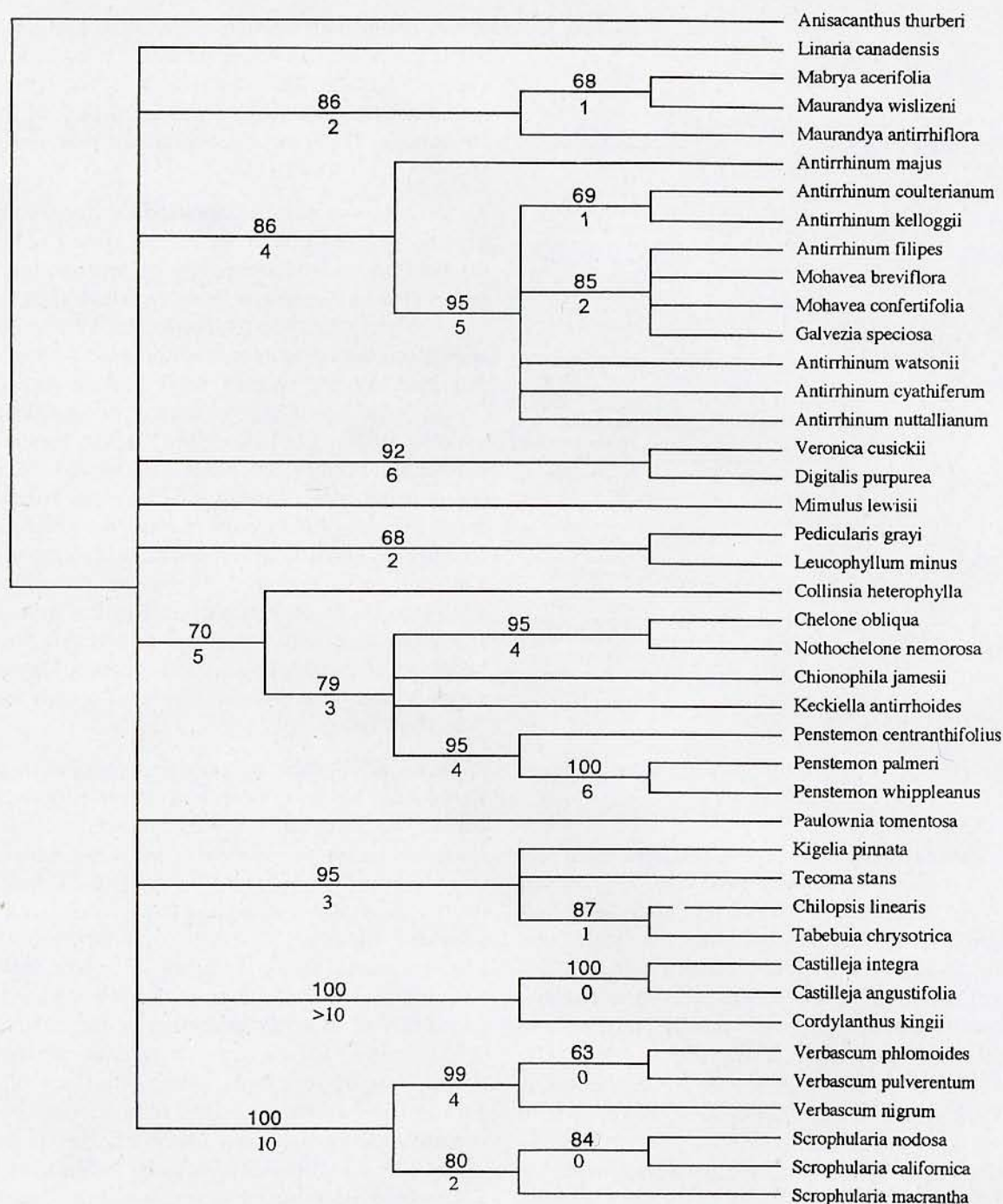


Fig. 2. Parsimony bootstrap majority-rule consensus tree based on sequence data of the intron of the chloroplast *trnL* gene. Bootstrap values are shown above the line, decay indices below the line.

appears to be the sister taxon to *Penstemon* with *Chionophila*, *Chelone*, and *Nothochelone* being more distantly related within the clade. This is surprising because *Penstemon* and *Chionophila* share the unusual character within the Scrophulariaceae of nectaries that are epistaminal trichomes. It was reasonable, then, to expect *Penstemon* and *Chionophila* to appear as sister taxa. *Keckiella*, *Chelone*, and *Nothochelone* have nectaries that are hypogenous disks. The placement of

Keckiella may, therefore, be in error and additional studies are needed.

The cladograms also indicate strongly that *Nothochelone nemorosa* and *Chelone obliqua* are close relatives. Straw (1966) noted that no other species of *Cheloneae* has caused more taxonomic problems than *Nothochelone nemorosa*. It has been transferred repeatedly between *Penstemon* and *Chelone* since its original description. Its somewhat galeate upper lip,

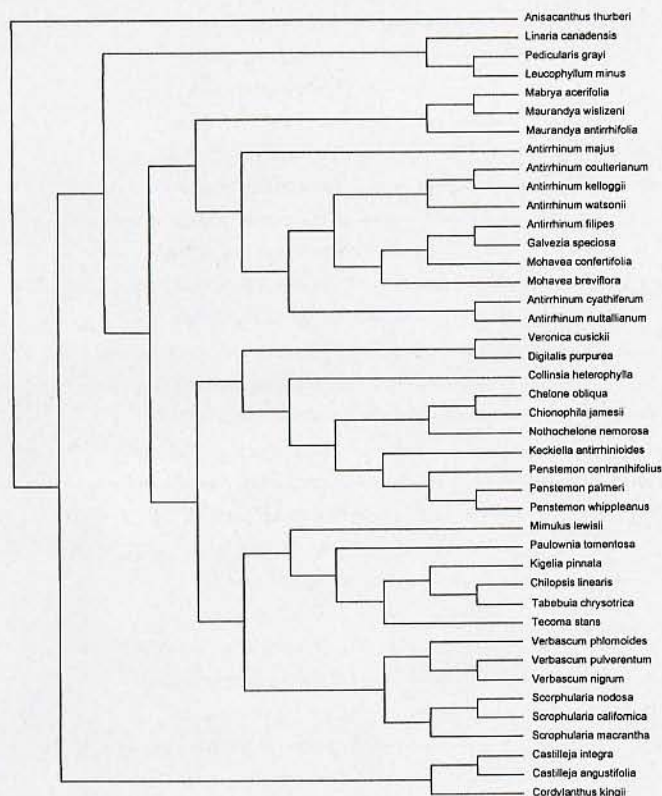


Fig. 3. Neighbor-joining tree.

similarly comose anthers, winged seeds, and moist forest ecology indicate an affinity with *Chelone* (Nelson 1995). With *Penstemon*, especially with subgenus *Dasanthera*, it shares the woolly anthers, a rather open inflorescence, and a more open corolla orifice. Anatomical examination, however, has demonstrated that there are no glandular hairs in the corolla and the functional nectary is a hypogenous disc (Straw 1966). The cladograms (Fig. 1, 2) suggest that *Nothochelone* is indeed much more closely related to *Chelone* than to *Penstemon*.

The topology of our cladograms for tribe Cheloneae is very similar to Fig. 1 of Wolfe et al. (1997) based on combined RFLP data from the *trnK*, *rps2*, and *rbcL* genes. Both this study and Wolfe et al. (1997) suggest that the New World genera of this tribe represent a monophyletic clade. Wolfe et al. also suggest that *Penstemon* and *Keckiella* are most closely related and that *Chionophila* is more closely related to *Chelone* and *Nothochelone*.

Bellini (1907) and Pennell (1935) independently proposed the erection of the tribe Collinseae consisting of *Collinsia* and *Tonella* from the Cheloneae based on distinctive nectary characteristics. The intron sequence data from *Collinsia heterophylla* indicate that Collinseae may be closely related to the Cheloneae but appears basal to it. The Cheloneae

clade, including *Collinsia*, is weakly supported while the traditional Cheloneae genera represent a strongly supported clade. This suggests that the Tribe Collinseae may tentatively be considered part of the Tribe Cheloneae. The same conclusion was reached by Wolfe et al. (1997).

Tribal assignment of *Scrophularia*.—*Scrophularia* has commonly been placed within the tribe Cheloneae on the basis of most species having staminodes, but the genus also has several species in which the staminode is completely lacking (Stiefelshagen 1910). This is in contrast to the other genera of the tribe which are characterized by all species having staminodes. Straw (1966) was unwilling to accept *Scrophularia* as a member of the Cheloneae and cites a personal communication with Dr. Richard J. Shaw who believed the genus to be more closely allied to the Tribe Verbasceae. Thieret (1967) pointed out that the systematic position of *Scrophularia* needs clarification and notes that seed characters of *Scrophularia* are almost identical with those of *Verbascum*. Results from the present study distinctly separate *Scrophularia* from other members of the Cheloneae and this topology suggests strongly that *Scrophularia* has been misplaced within the tribe Cheloneae.

Position of *Verbascum* within Scrophulariaceae.—*Verbascum* has long been considered to have, at least among the Scrophulariaceae, similarities with the Solanaceae based on its nearly actinomorphic corolla, alternate phyllotaxy, and five stamens (Thieret 1967). In this view *Verbascum* has been considered to be a primitive member of the Scrophulariaceae “on the road to acquiring zygomorphism” (Henslow 1893). Robertson (1891) challenged this view based on stamen number in other members of the tribe. Robyns (1931) stated that the type of zygomorphism present is like that of scrophulariaceans and not of the zygomorphic Solanaceae. This idea has not been universally accepted. Thus, other authorities have believed that the similarity between *Verbascum* and the Solanaceae is more apparent than real, but that the position of the genus remained unanswered (Thieret 1967).

Schmid (1906) asserted that *Scrophularia* and *Verbascum* are obviously more closely related than current classification systems indicate, and *Scrophularia* could possibly even belong to the Verbasceae. The topology of a cladogram generated in this study (but not reported here) included the *Nicotiana tabacum* L. sequence for the *trnL* intron. That cladogram indicated that tobacco is not closely related to *Verbascum*. This suggests that the near actinomorphy of *Verbascum* is indeed a derived character, not a primitive one, and the bootstrap value, both by parsimony and neighbor joining, indicates it is almost certainly closely related to

Scrophularia. The close relationship between *Scrophularia* and *Verbascum* has also been detected in the cladograms of Reeves and Olmstead (1998). The *Leucophyllum* species included in this analysis appears quite distinct from the tribe Verbasceae as suggested by Bentham. Wettstein (1891) included *Leucophyllum* in the tribe Verbasceae.

The tribe Antirrhineae.—Though tentative at this point because of our limited sample, the tribe Antirrhineae as commonly circumscribed may or may not form a monophyletic branch. The parsimony strict consensus tree (Fig. 1) includes all taxa, except *Linaria canadensis*, within a single clade. *Linaria canadensis* appears on a branch basal to virtually the entire family. The neighbor joining cladogram (Fig. 3), however, places *Linaria* within the same clade but at a basal position. The algorithm RASA v2.2 (Lyons-Weiler et al. 1996) indicates that the *Linaria* is a borderline long branch and, therefore, of uncertain placement. This possibly indicates that *Linaria* represents a primitive member of the Antirrhineae, and our limited sample did not allow for its inclusion in the clade. Further, the *trnL* intron sequence was capable of distinguishing the subtribes Antirrhinae and Maurandynae (Fig. 1, 3).

Position and composition of the tribe Pedicularae (Euphrasineae).—Taxa of this tribe sampled included *Pedicularis grayi*, *Cordylanthus kingii*, *Castilleja angustifolia*, and *C. integra*. The last three species appear as a very highly supported clade which is very distinct from the rest of the scrophs and basal to it. *Pedicularis*, on the other hand, appears in the Maximum Parsimony tree on a clade which includes *Veronica*, *Digitalis*, *Mimulus*, and *Leucophyllum* (Fig. 1), far removed from the sampled members of the subtribe Castillejinae. In the neighbor tree (Fig. 3), *Pedicularis* appears as more closely related to the tribe Cheloneae. Additional sampling needs to be done to determine which, if either, of the groupings seen here is substantiated.

In conclusion, this small region of about 500 bp has sufficient variability to provide some resolution within a highly diverse assemblage of genera, and the entire intron can usually be manually sequenced with only two reactions, a forward and a reverse. Further, the sequences are variable enough, with numerous gaps, so that alignment is sometimes problematic. The small number of taxa of the Scrophulariaceae and allied taxa sampled in this pilot study is clearly insufficient to draw many well-supported conclusions. However, the data do suggest that *trnL* intron sequences might, as a large database of these sequences accumulates, be helpful in understanding phylogeny in Scrophulariaceae and allied families, especially at

the tribal level and above. It might be useful in other families as well.

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Appendix 1. Aligned *trnL* sequences of the taxa included. Missing are the first 13 bp (5' end).

	10	20	30	40	50	60	70
<i>Anisacanthus thurberi</i>	CTTGGTATGG	AAACCTACTA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAATA	AAAAATGGGCA
<i>Linaria canadensis</i>	CTTGGTATGG	AAACCTACCA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAAGA	AAAAATGGGCA
<i>Mabrya acerifolia</i>	CTTGGTATGG	AAACCTACTA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAATA	AAAAATGGGCA
<i>Maurandya wislizeni</i>	CTTGGTATGG	AAACCTACTA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAATT	AAAAATGGGCA
<i>Maurandya antirrhiflora</i>	CTTGGTATGG	AAACCTACTA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAATA	AAAAATGGGCA
<i>Antirrhinum majus</i>	CTTGGTATGG	AAACCTACTA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAAGA	AAAAATGGGCA
<i>Antirrhinum coulterianum</i>	CTTGGTATGG	AAACCTACTA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAATA	AAAAATGGGCA
<i>Antirrhinum kelloggii</i>	CTTGGTATGG	AAACCTACTA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAATA	AAAAATGGGCA
<i>Antirrhinum filipes</i>	CTTGGTATGG	AAACCTACTA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAATA	AAAAATGGGCA
<i>Antirrhinum watsonii</i>	CTTGGTATGG	AAACCTACTA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAAGA	AAAAATGGGCA
<i>Antirrhinum cyathiferum</i>	CTTGGTATGG	AAACCTACTA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAATA	AAAAATGGGCA
<i>Antirrhinum nuttallianum</i>	CTTGGTATGG	AAACCTACTA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAATA	AAAAATGGGCA
<i>Mohavea breviflora</i>	CTTGGTATGG	AAACCTACTA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAATA	AAAAATGGGCA
<i>Mohavea confertifolia</i>	CTTGGTATGG	AAACCTACTA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAATA	AAAAATGGGCA
<i>Galvezia speciosa</i>	CTTGGTATGG	AAACCTACTA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	GGGAATTAATA	AAAAATGGGCA
<i>Veronica cusickii</i>	CTTGGTATGG	AAACCTACCA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAATC	AAAAATGGGCA
<i>Mimulus lewisii</i>	CTTGGTATGG	AAACCTACCA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAAGA	AAAAATGGGCA
<i>Digitalis purpurea</i>	CTTGGTATGG	AAACCTACCA	AGTGATAACT	TTCAAATTC	ATAGAAACCC	CGGAATTAATA	AAAAATGGGCA
<i>Pedicularis grayi</i>	CTTGGTATGG	AAACCTACTA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAATA	AAAAATGGGCA
<i>Leucophyllum minus</i>	CTTGGTATGG	AAACCTACTA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAATA	AAAAATGGGCA
<i>Collinsia heterophylla</i>	CTTGGTATGG	AAACCTACCA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAATA	AAAAATGGGCA
<i>Chelone obliqua</i>	CTTGGTATGG	AAACCTACCA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATGAATA	AAAAATGGGCA
<i>Nothochelone nemorosa</i>	CTTGGTATGG	AAACCTACCA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATGAATA	AAAAATGGGCA
<i>Chionophila jamesii</i>	CTTGGTATGG	AAACCTACCA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATGAATA	AAAAATGGGCA
<i>Keckiella antirrhiflora</i>	CTTGGTATGG	AAACCTACCA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATGAATA	AAAAATGGGCA
<i>Penstemon centranthifolius</i>	CTTGGTATGG	AAACCTACCA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATGAATA	AAAAATGGGCA
<i>Penstemon palmeri</i>	CTTGGTATGG	AAACCTACCA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATGAATA	AAAAATGGGCA
<i>Penstemon whippleanus</i>	CTTGGTATGG	AAACCTACCA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATGAATA	AAAAATGGGCA
<i>Paulownia tomentosa</i>	CTTGGTATGG	AAACCTACTA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAAGA	AAAAATGGGCA
<i>Kigelia pinnata</i>	CTTGGTATGG	AAACCTACTA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAATA	AAAAATGGGCA
<i>Tecoma stans</i>	CTTGGTATGG	AAACCTACTA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAATA	AAAAATGGGCA
<i>Chilopsis linearis</i>	CTTGGTATGG	AAACCTACTA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAATA	AAAAATGGGCA
<i>Tabebuia chrysotrica</i>	CTTGGTATGG	AAACCTACTA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAAGA	AAAAATGGGCA
<i>Castilleja integra</i>	CTTAGTATGG	AAACCTACTA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	TGGAATTAATA	AAAAATGGGCA
<i>Castilleja angustifolia</i>	CTTAGTATGG	AAACCTACTA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	TGGAATTAATA	AAAAATGGGCA
<i>Cordylanthus kingii</i>	CTTGGTATGG	AAACCTACTA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	TGGAATTAAGA	AAAAATGGGCA
<i>Verbascum phlomoides</i>	CTTGGTATGG	AAACCTACCA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAATA	AAAAATGGGCA
<i>Verbascum pulverentum</i>	CTTGGTATGG	AAACCTACCA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAATA	AAAAATGGGCA
<i>Verbascum nigrum</i>	CTTGGTATGG	AAACCTACCA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAATA	AAAAATGGGCA
<i>Scrophularia nodosa</i>	CTTGGTATGGA	AACCTACTA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAATA	AAAAATGGGCA
<i>Scrophularia californica</i>	CTTGGTATGGA	AACCTACTA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAATA	AAAAATGGGCA
<i>Scrophularia macrantha</i>	CTTGGTATGGA	AACCTACCA	AGTGATAACT	TTCAAATTC	AGAGAAACCC	CGGAATTAATA	AAAAATGGGCA

Appendix 1. Extended.

80	90	100	110	120	130	140	150	160
ATCCTGAGCC	AAATCCTGTT	TT-CGCAAGA	CAAAGGTTCA	GAAAACGA--	-----	AAAGTTGGCT	ACATTGGCAT	TGGTAGAGGA
ATCCTGAGCC	AAATCCTGTT	TT-CTCAAAA	CAAAGGTTCA	GAAAGC----	-----AAA	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTGTC	TT-CTCAAAA	CAAAGGTTCA	GAAAGCGA--	----AAAAA	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTGTC	TT-CTCAAAA	CAAAGGTTCA	GAAAGCGAAA	AGGGAAAAA	AAAGGGATAG	GTGCATATAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTGTC	TT-CTCAAAA	CAAAGGTTCA	GAAATCTAAA	AGCG--AAAA	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTGTT	TT-CTCAAAA	CAAAGGTTCA	GAAAGCGA--	-----AA	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTGTC	TT-CTCAAAA	CAAAGGTTCA	GAAAGC----	-----AAAAA	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTGTC	TT-CTCAAAA	CAAAGGTTCA	GAAAGC----	-----AAAA	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTGTC	TT-CTCAAAA	CAAAGATTCA	GAAAGA----	-----AAAA	AAAGGGATAG	GTGCAGAGAC	TCGATGGAAG
ATCCTGAGCC	AAATCCTGTC	TT-CTCAAAA	CAAAGGTTCA	GAAAGC----	-----AAA	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTGTC	TT-CTCAAAA	CAAAGGTTCA	GAAAGC----	-----AAAA	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTGTC	TT-CTCAAAA	CAAAGGTTCA	GAAAGC----	-----AAAA	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTGTC	TT-CTCAAAA	CAAAGGTTCA	GAAATC----	-----AAAA	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTGTC	TT-CTCAAAA	CAAAGGTTCA	GAAAGC----	-----AAAA	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTGTC	TT-CTCAAAA	CAAAGGTTCA	GAAAGTG----	----AAAAA	AAAGGGATAG	GTGCAGAGAC	TCGATGGAAG
ATCCTGAGCC	AAATCCTCGT	TTT-TCAAAA	GAAAGGTTTA	GAAAACGA--	-----AAA	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTGTC	TT-CTCAAAA	CAAAGGTTCA	GGAAGCTA--	-----A	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTGTT	TT-CTCAAAA	CAAAGGTTCA	GAAAGCGA--	-----A	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTGTT	TT-CTCAAAA	CAAAGGTTCA	GAAAGCGA--	-----AA	AAAGGGATAG	GTGCAGAGAC	TCGATGGAAG
ATCCTGAGCC	AAATCCTATC	TTT-TCAAAA	CAGGGGTTCA	GAAAGCGA--	-----AA	AAGGGGATAG	GTGCAGAGAC	T-AATGGAAG
ATCCTGAGCC	AAATCCTATC	TT-CTCAAAA	CAAAGGTTCA	GAAAGCGA--	-----AA	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTATC	TT-CTCAAAA	CAAAGGTTCA	GAAAGCCA--	-----AA	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTATC	TT-CTCAAAA	CAAAGGTTCA	GAAAGCGA--	-----AA	ACAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTATC	TT-CTCAAAA	CAAAGGTTCA	GAAAGCGA--	-----AAA	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTATC	TTT-TCAAAA	CAAAGGTTCA	GAAAGCGA--	-----AAA	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTATG	CTTCTCAAAA	TAAAGGTTCA	AAAAGCGA--	-----AAA	TAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTATG	CTTCTCAAAA	TAAAGGTTCA	AAAAGCGA--	-----AAA	TAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTGTT	TT-CTCAAAA	CAAAGGTTCA	GAAAACGA--	---AAAAA	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTTTT	TT-CTCAAAA	CAAAGGTTCA	GAAAACGA--	---AAAAA	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTTTT	TT-CTCAAAA	CAAAGGTTCA	GAAAACGA--	-----AAAA	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTTTT	TTTCTCAAAA	CAAAGGTTCA	GAAAACGA--	-----AAAA	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTGTT	TT-CTCAAAA	CAAAGGTTCA	GAAAACGA--	-----AAAA	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTGTT	TT-CTCAAAA	CAAAGGTTCA	GAAAACGA--	---AAAAA	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTGTT	TT-CTCAAAA	CAAAGGTTCA	GAAAACGA--	-----AA	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTATT	TT-CTCAAAA	CAAAGGTTCA	GAAGGCGA--	-----A	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTATT	TT-CTCAAAA	CAAAGGTTCA	GAAGGCGA--	-----A	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTATT	TT-CTCAAAA	CAAAGGTTCA	GAAGGCCA--	-----A	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTGTT	TT-CTCAAAA	CAAAGGTTCA	GAAGGCGA--	-----	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTGTT	TT-CTCAAAA	CAAAGGTTCA	GAAGGCGA--	-----	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG
ATCCTGAGCC	AAATCCTGTT	TT-CTCAAAA	CAAAGGTTCA	GAAGGCGA--	-----A	AAAGGGATAG	GTGCAGAGAC	TCAATGGAAG

Appendix 1. Continued.

	170	180	190	200	210	220	230
<i>Anisacanthus thurberi</i>	ATGGTTCCCC	CGAAATTTAC	GAAA-----	GG-ATAAACG	TATCTATTGA	ATACTATA--	----AGGGTA
<i>Linaria canadensis</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTATGT-	-----TGGTA	GATGAATCTT	TTCATCAAAA	CTTCAGAAAAG
<i>Mabrya acerifolia</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTATGT-	-----TGGTA	GAGGAATCTT	TCCATCGAAA	CTTCAGAAAAG
<i>Maurandya wislizeni</i>	CT-GTTCTAA	CAAAATGTAAT	TGACTGTGT-	-----TGGTA	GAGGAATCTT	TCCATCGAAA	CTTCAGAAAAG
<i>Maurandya antirrhiflora</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGTGT-	-----TGGTA	GAGGAATCTT	TCCATCGAAA	CTTCAGAAAAG
<i>Antirrhinum majus</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGTGT-	-----TGGTA	GAGGAATCTT	TCCATCAAAA	CTTCAGAAAAA
<i>Antirrhinum coulterianum</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGTGT-	-----TGGTA	GAGGAATCTT	TCGATTAAAA	CTTCAGAAAAA
<i>Antirrhinum kelloggii</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGTGT-	-----TGGTA	GAGGAATCTT	TCGATTAAAA	CTTCAGAAAAA
<i>Antirrhinum filipes</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGTGT-	-----TGGTA	GAGGAATCTT	TCGATTAAAA	CTTCAGAAAAA
<i>Antirrhinum watsonii</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGTGT-	-----TGGTA	GAGGAATCTT	TCGATTAAAA	CTTCAGAAAAA
<i>Antirrhinum cyathiferum</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGTGT-	-----TGGTA	GAGGAATCTT	TCGATTAAAA	CTTCAGAAAAA
<i>Antirrhinum nuttallianum</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGTGT-	-----TGGTA	GAGGAATCTT	TCGATTAAAA	CTTCAGAAAAA
<i>Mohavea breviflora</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGTGT-	-----TGGTA	GAGGAATCTT	TCGATTAAAA	CTTCAGAAAAA
<i>Mohavea confertifolia</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGTGT-	-----TGGTA	GAGGAATCTT	TCGATTAAAA	CTTCAGAAAAA
<i>Galvezia speciosa</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGTAT-	-----TGGTA	GAGGAATCTT	TCGATTAAAA	CTTCAGAAAAA
<i>Veronica cusickii</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGCGT-	-----CGGTA	GATGAATCTT	TTCATCGAAA	CTTCAGAAAAG
<i>Mimulus lewisii</i>	CT-ATTCTAA	CAAAATGGAGT	TGACTGCGT-	-----CGGTA	GATGAATCTT	TTCATCGAAA	CTTCAGAAAAG
<i>Digitalis purpurea</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGCGT-	-----TGGTA	GAGGAATCTT	TCCATCGAAA	CTTCAGAAAAA
<i>Pedicularis grayi</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGCGT-	-----TGGTA	GAGGAATCTT	TCCATCGAAA	CTTCAGAAAAG
<i>Leucophyllum minus</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGCGT-	-----TGATA	GAGGAATCTT	TCCATCGAAA	CTTCAGAAAAA
<i>Collinsia heterophylla</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGCAT-	-----TGGTA	AATGAATCTT	TTCATCGAAA	CGTCAGAAAAG
<i>Chelone obliqua</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGCAT-	GGTATTGGTA	GATGAATCTT	TTCATCGAAA	CTTCAGAAAAG
<i>Nothochelone nemorosa</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGCAT-	-----TGGTA	GATGAATCTT	TTCATCGAAA	CTTCAGAAAAG
<i>Chionophila jamesii</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGCAT-	-----TGGTA	GATGAATCTT	TTCATCGAAA	CTTCAGAAAAG
<i>Keckia antirrhiflora</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGCAT-	-----TGGTA	GATGAATCTT	TTCATCGAAA	CTTCAGAAAAG
<i>Penstemon centranthifolius</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGCAT-	-----TGGTA	GATGAATCTT	TTCATCGAAA	CTTCAGAAAAG
<i>Penstemon palmeri</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGCAT-	-----TGGTA	GATGAATCTT	TTCATCGAAA	CTTCAGAAAAG
<i>Penstemon whippleanus</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGCAT-	-----TGGTA	GATGAATCTT	TTCATCGAAA	CTTCAGAAAAG
<i>Paulownia tomentosa</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGCGT-	-----TGGTA	GAGGAATCTT	TCCATCGAAA	CTTCAGAAAAG
<i>Kigelia pinnata</i>	CT-GTTCTAA	CAAAAGGGAGT	TGGCTGCGT-	-----TGGTA	GAGGAATCTT	TCCATCGAAA	CTTCAGAAAAG
<i>Tecoma stans</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGCGT-	-----TGGTA	GAGGAATCTT	TCCATCGAAA	CTTCAGAAAAG
<i>Chilopsis linearis</i>	CT-GTTCTAA	CAAAATGGAGT	TGGCTGCGT-	-----TGGTA	GAGGAATCTT	TCCATCGAAA	CTTCAGAAAAG
<i>Tabebuia chrysotrica</i>	CT-GTTCTAA	CAAAATGGAGT	TGGCTGCGT-	-----TGGTA	GAGGAATCTT	TCCATCGAAA	CTTCAGAAAAG
<i>Castilleja integra</i>	CT-GTTCTAA	TAAATGGAGT	TGATTGCGC-	-----TGATA	GAGGAATG--	-----AAA	CTTCAGATCA
<i>Castilleja angustifolia</i>	CT-GTTCTAA	TAAATGGAGT	TGATTGCGC-	-----TGATA	GAGGAATG--	-----AAA	CTTCAGATCA
<i>Cordylanthus kingii</i>	CT-GTTCTAA	TAAATGGAGT	TGATTGCGC-	-----TGATA	GAGGAATG--	-----AAA	CTTCAGAT--
<i>Verbascum phlomoides</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGCTT-	-----TGGTA	GAGGAATCTT	TCCATCGAAA	CTTCAGAAAAG
<i>Verbascum pulverentum</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGCGT-	-----TGGTA	GAGGAATCTT	TCCATCGAAA	CTTCAGAAAAG
<i>Verbascum nigrum</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGCGT-	-----TGGTA	GAGGAATCTT	TCCATCGAAA	CTTCAGAAAAG
<i>Scrophularia nodosa</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGCGT-	-----TGGTA	GAGAAATCTT	TCCATCGAAA	CTTCAGAAAAG
<i>Scrophularia californica</i>	CT-GTTCTAA	CAAAATGGAGT	TGACTGCGT-	-----TGGTA	GAGAAATCTT	TCCATCGAAA	CTTCAGAAAAG
<i>Scrophularia macrantha</i>	CT-ATTCTAA	CAAAATGGAGT	TGACTGCGT-	-----TGGTA	GAGAAATCTT	TCCATCGAAA	CTTCAGAAAAG

[illegible]

Appendix 1. Continued.

	330	340	350	360	370	380	390
<i>Anisacanthus thurberi</i>	GCTCACATCT	CTAATGGCTC	ACATCTATTT	TATATTTTTT	ATAAGAAAAA	TGGAAGAAT-	-TGGTGTGAA
<i>Linaria canadensis</i>	TGGCCCAAAT	CTGTATCTGT	TTTTT-----	-----T	ATATAAAAAA	TGGAAGAAT-	-TGATGTGAA
<i>Mabrya acerifolia</i>	TGGCCCGAAT	CTGTATCTGT	ATTTTTTT--	-----AT	ATGAAAAA--	TGGAATAAT-	-TGATGTGAA
<i>Maurandya wislizeni</i>	TGGCCCGAAT	CTGTATCTGT	ATTTTTTT--	-----AT	ATGAAAAA--	TGGAATAAT-	-TGATGTGAA
<i>Maurandya antirrhiflora</i>	TGGCCCGAAT	CTGTATCTGT	ATTTTTTT--	-----AT	ATGAAAAA--	TGGAATAAT-	-TGATGTGAA
<i>Antirrhinum majus</i>	TGGCCCAAAT	CTGTATCTGT	ATTTTCT--	-----AT	ATGAAAAA--	TGGAATAAT-	-TGATGTGAA
<i>Antirrhinum coulterianum</i>	TGGCCCGAAT	CTGTATCTGT	ATTTTTTT--	-----AT	ATTAAAAA--	TGGAAGAAT-	-TGGTGTGAA
<i>Antirrhinum kelloggii</i>	TGGCCCGAAT	CTGTATCTGT	ATTTTTTT--	-----AT	ATGAAAAA--	TGGAAGAAT-	-TGGTGTGAA
<i>Antirrhinum filipes</i>	TGGCCCGAAT	CTGTATCTGT	ATTTTTTT--	-----AT	ATGAAAAA--	TGGAAGAAT-	-TGGTGTGAA
<i>Antirrhinum watsonii</i>	TGGCCCGAAT	CTGTATCTGT	ATTTTTTT--	-----AT	ATGAAAAA--	TGGAAGAAT-	-TGGTGTGAA
<i>Antirrhinum cyathiferum</i>	TGGCCCGAAT	CTGTATCTGT	ATTTTTTT--	-----AT	ATGAAAAA--	TGGAAGAAT-	-TGGTGTGAA
<i>Antirrhinum nuttallianum</i>	TGGCCCGAAT	CTGTATCTGT	ATTTTTTT--	-----AT	ATGAAAAA--	TGGAAGAAT-	-TGGTGTGAA
<i>Mohavea breviflora</i>	TGGCCCGAAT	CTGTATCTGT	ATTTTTTT--	-----AT	ATGAAAAA--	TGGAAGAAT-	-TGGTGTGAA
<i>Mohavea confertifolia</i>	TGGCCCGAAT	CTGTATCTGT	ATTTTTTT--	-----AT	ATGAAAAA--	TGGAAGAAT-	-TGGTGTGAA
<i>Galvezia speciosa</i>	TGGCCCGAAT	CTGTATCTGT	ATTTTTTT--	-----AT	ATGAAAAA--	TGGAAGAAT-	-TGGTGTGAA
<i>Veronica cusickii</i>	TGGGCCAAGT	CTGTATCTGT	ATTTTTTT--	-----T	TAGATGAAAA	AATACAAGAC	TTGGTGAAAA
<i>Mimulus lewisii</i>	TAGCCTGAAT	CTGTATCTGT	ATTTTT--	-----T	AAGATGAAAA	ATGAAAGAAT	-TGGTGTGAA
<i>Digitalis purpurea</i>	TGGCCCGAGT	CTGTATCTGT	ATTTTTTTTAG	-----AT	GAAAAAGAGA	AGAATTAGTA	-TGATATGAA
<i>Pedicularis grayi</i>	TGGCCCTAAT	CTGTATCTGT	TTTTT-----	-----	ATATGAAAA	ATGGAAGAAT-	-TGGTATGAA
<i>Leucophyllum minus</i>	TGGCCCTAAT	CTGTATCTGT	ATTTTTTT--	-----	--ATGAAAA	GTGGAAGAAT-	-TGGTATGAA
<i>Collinsia heterophylla</i>	TGCCCCGAAT	CTGGATCGGT	ATTTTTTT--	-----T	ATCTGAAAA	ATGGAAGAAAT-	-TCGTATGAA
<i>Chelone obliqua</i>	TGCCCCGAAT	CTGTATCTGT	TTTTT-----	-----AT	ATGAAAAA--	-TGGAAGAAT-	-TGGTGTGAA
<i>Nothochelone nemorosa</i>	TGCCCCGAAT	CTGTATCTGT	TTTTT-----	-----AT	ATGAAAAA--	-TGGAAGAAT-	-TGGTGTGAA
<i>Chionophila jamesii</i>	TGCCCCGAAT	CTGTATCTGT	ATTTTTTT--	-----AT	ATGAAAAA--	-TGGAAGAAT-	-TGGTGTGAA
<i>Keckiella antirrhiflora</i>	TGCCCTCGAAT	CTGTATCTGG	ATTTTTTT--	-----AT	ATGAAA----	--GAAGAAT-	-TGGTGTGAA
<i>Penstemon centranthifolius</i>	TGCCCCGAAT	CTGTATCTGT	ATTTTTCT--	-----AT	ATGAAAAA--	TGGAAGAAT-	-TGGTGTCAA
<i>Penstemon palmeri</i>	TGCCCCGAAT	CTGTATCTGT	ATTTTTCT--	-----AT	ATGAAAAA--	TGGAAGAAT-	-TGGTGTCAA
<i>Penstemon whippleanus</i>	TGCC--CGAAT	CTGTTTCTGT	ATTTTTCT--	-----AT	ATGAAAAA--	TGGAAGAAT-	-TGGTGTCAA
<i>Paulownia tomentosa</i>	TGGCCCGAAT	CTGTATCTGT	ATTTTTTA--	-----AT	ATGAAAAA--	TGGAAGAAT-	-TGGTGTGAA
<i>Kigelia pinnata</i>	TGGCCCGAAT	CTGTATCTGT	ATTTTTTT--	-----AT	ATGAAAA--	-----	-TGGAAGGAA
<i>Tecoma stans</i>	TGGCCCGAAT	CTGTATCTGT	ATTTTTTT--	-----AT	ATGAAAA--	TGGAAGAAT-	-TGGTGTGAA
<i>Chilopsis linearis</i>	TGGCCCGAAT	CTGTATCGTA	TTTTTTTT--	-----AT	ATGAAAAA--	TGGAAGAAT-	-TAGTGTGAA
<i>Tabebuia chrysotrica</i>	TGGCCCGAAT	CTGTATCTGT	ATTTTTTTTGT	ATTTTTTTAT	ATGAAAAA--	TGGAAGAAT-	-TAGTGTGAA
<i>Castilleja integra</i>	TGGCCCAAAT	CTGTATCTGT	ATTTTT--	-----GAAT	ACGAAAA--G	TGGAAGAAT-	-TGGTGTGAA
<i>Castilleja angustifolia</i>	TGGCCCAAAT	CTGTATCTGT	ATTTTT--	-----GAAT	ACGAAAA--G	TGGAAGAAT-	-TGGTGTGAA
<i>Cordylanthus kingii</i>	TGGCCCAAAT	CTGTATCTGT	ATTTTT--	-----GAAT	ACGAAAA--G	TGGAAGAAT-	-TGGTGTGAA
<i>Verbascum phlomoides</i>	TGGCCCGAAT	CTGTATCTTT	TTTTTTTTTTT	-----AT	ATGAAAAA--	-----	-TGATGTGAA
<i>Verbascum pulverentum</i>	TGGCCCGAAT	CTGTATCTTT	TTTTTTTTTTT	-----AT	ATGAAAAA--	TGGAAGAAATC-	-GATGTGAA
<i>Verbascum nigrum</i>	TGGCCCGAAT	CTGTATCTTT	TTTTTTTTTTT	-----AT	ATGAAAAA--	TGGAAGAAATC-	-GATGTGAA
<i>Scrophularia nodosa</i>	TGGCCCAAAT	CTGTATCTTT	ATTTTTTT--	-----AT	ATGAAAAA--	TAGAAAAAT-	-TGATGTGAA
<i>Scrophularia californica</i>	TGGCCCGAAT	CTGTATCTTT	ATTTTTTT--	-----AT	ATGAAAAA--	TAGAAAAAT-	-TGATGTGAA
<i>Scrophularia macrantha</i>	TGGCCCGAAT	CTGTATCTTT	ATTTTTTT--	-----AT	ATGAAAAA--	TAGAAAAAT-	-TGATGTGAA

Appendix 1. Extended.

[illegible]

Appendix 1. Continued.

	490	500	510	520	530	540	550
<i>Anisacanthus thurberi</i>	ATTAAAGAAG	CGGACGAGAA	TAAAGATAGA	GTCCCATTCC	ACATGT-CAA	TA-----	CTGGCAACAA
<i>Linaria canadensis</i>	CT-GATTAAT	CGGACGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	TCCGCAACAA
<i>Mabrya acerifolia</i>	CT-GATTAAT	CGGACGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	CCGGCAACAA
<i>Maurandya wislizeni</i>	CT-GATTAAT	CGGACGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	CCGGCAACAA
<i>Maurandya antirrhiflora</i>	CTTGATTAAT	CGGACGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	CCGGCAACAA
<i>Antirrhinum majus</i>	CT-GATTAAT	CGGACGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGTCCAA	TA-----	CCGGCAACAA
<i>Antirrhinum coulterianum</i>	CT-GATTAAT	CGGACGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	CCGGCAACAA
<i>Antirrhinum kelloggii</i>	CT-GATTAAT	CGGACGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	CCGGCAACAA
<i>Antirrhinum filipes</i>	CT-GATTAAT	CGGACGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	CCGGCAACAA
<i>Antirrhinum watsonii</i>	CT-GATTAAT	CGGACGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	CCGGCAACAA
<i>Antirrhinum cyathiferum</i>	CT-GATTAAT	CGGACGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	CCGGCAACAA
<i>Antirrhinum nuttallianum</i>	CT-GATTAAT	CGGACGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	CCGGCAACAA
<i>Mohavea breviflora</i>	CT-GATTAAT	CGGACGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	CCGGCAACAA
<i>Mohavea confertifolia</i>	CT-GATTAAT	CGGACGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	CCGGCAACAA
<i>Galvezia speciosa</i>	CT-GATTAAT	CGGACGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	CCGGCAACAA
<i>Veronica cusickii</i>	---GATTAAT	CGGACGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	CCGACAAAAA
<i>Mimulus lewisii</i>	CT-GATTAAT	CGGACGAGAA	TAAAGATAGA	GTCCCGTTCT	ACATGT-CAA	TA-----	CCGGCAACAA
<i>Digitalis purpurea</i>	CT-GATTAAT	CGGACGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	CCGACAAACAA
<i>Pedicularis grayi</i>	CT-GATTAAT	CGGACAAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	TTGGCAACA-
<i>Leucophyllum minus</i>	CT-GATTAAT	CGGACGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	CCGGCAACAA
<i>Collinsia heterophylla</i>	CT-GATTAAT	CGGACAAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	CCGGCAACAA
<i>Chelone obliqua</i>	CT-GATTAAT	CGGACAAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	CCGGCAACAA
<i>Nothochelone nemorosa</i>	CT-GATTAAT	CGGACAAGAA	TAAAGATATA	GTCCCATTCT	ACATGT-CAA	TA-----	CCGGCAACAA
<i>Chionophila jamesii</i>	CT-GATTAAT	CGGACAAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	CCGGCAACAA
<i>Keckiella antirrhiflora</i>	CT-GATTAAT	CGGACAAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	GCGGCAACAA
<i>Penstemon centranthifolius</i>	CT-GATTAAT	CGGACAAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	GCGGCAACAA
<i>Penstemon palmeri</i>	CT-GATTAAT	CGGACAAGAA	TAAAGATAGA	GTCCCATTCT	ACA-GT-CAA	TA-----	CCGGCAACAA
<i>Penstemon whippleanus</i>	CT-GATTAAT	CGGACAAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TATGTCAATA	CCGGCAACAA
<i>Paulownia tomentosa</i>	CT-GATTAAT	CGGACGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	CCGGCAACAA
<i>Kigelia pinnata</i>	CT-GATTAAG	CGGACGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	CCGGNAACAA
<i>Tecoma stans</i>	CT-GATTAAG	CGGACGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	CCGGCAACAA
<i>Chilopsis linearis</i>	CT-GATTAAG	CGGTCGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	CCGGCAACAA
<i>Tabebuia chrysotrica</i>	CT-GATTAAG	CGGACGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	CCGGCAACAA
<i>Castilleja integra</i>	CT-GATTAAT	CGGACGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	CCGGCAACAA
<i>Castilleja angustifolia</i>	CT-GATTAAT	CGGACGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	CCGGCAACAA
<i>Cordylanthus kingii</i>	CT-GATTAAT	CGGACGAGAA	TAAAGATAGA	GTC-CATTCT	ACATGT-CAA	TA-----	CCGGCAACAA
<i>Verbascum phlomoides</i>	CTTGATTAAT	CGGACGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	TCCGCAACAA
<i>Verbascum pulverentum</i>	CT-GATTAAT	CGGACGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	TCCGCAACAA
<i>Verbascum nigrum</i>	CT-GATTAAT	CGGACGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	TCCGCAACAA
<i>Scrophularia nodosa</i>	CT-GATTAAT	CGGACGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----TA	CCGGCAACAA
<i>Scrophularia californica</i>	CT-GATTAAT	CGGACGAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	CCGGCAACAA
<i>Scrophularia macrantha</i>	CT-GATTAAT	CGGACAAGAA	TAAAGATAGA	GTCCCATTCT	ACATGT-CAA	TA-----	CCGGCAACAA

[illegible]