

PHYLOGENY, TAXONOMIC AFFINITIES, AND BIOGEOGRAPHY OF *PENSTEMON* (PLANTAGINACEAE) BASED ON ITS AND CPDNA SEQUENCE DATA¹

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The large and diverse genus *Penstemon* (ca. 271 species) is endemic to North America and has been divided into six subgenera primarily based on anther dehiscence patterns. Species of *Penstemon* are known to be pollinated by a variety of insects (hymenopterans, lepidopterans, dipterans) and hummingbirds. Nucleotide sequence data from ITS and two noncoding regions of chloroplast DNA were used to reconstruct the phylogeny of *Penstemon*. Trees generated from nuclear and chloroplast DNA sequences are incongruent, which is probably the result of hybridization, and not fully resolved, which is likely due to a rapid evolutionary radiation. *Penstemon* represents a recent continental radiation where speciation has resulted primarily from evolutionary adaptations to ecological niches such as pollinator specialization. The results from these analyses show that the current circumscription of subgenera and sections needs revision to reflect more closely the evolutionary relationships of species. Specifically, species in subgenera *Saccanthera*, *Habroanthus*, and *Penstemon* are polyphyletic. These results also confirm the independent origin of hummingbird floral morphology in 10 clades.

Key words: biogeography; Cheloneae; hummingbird floral morphology; ITS; Scrophulariaceae; *trnC-D*, *trnT-L*.

Penstemon Mitchell, with ca. 271 species (Lodewick and Lodewick, 1999), is the largest plant genus endemic to North America. It is distributed from Alaska to Guatemala and from coast to coast. Most of the species occur in the western cordillera with the Intermountain Region representing the center of diversity. Most species have narrow distributions, and more than 100 are endemic to a single state. Straw (1966) proposed that *Penstemon* originated in the central Rocky Mountains and adjacent Columbia Plateau in the late Tertiary. Wolfe et al. (2002) supported this hypothesis in a molecular phylogenetics study of Cheloneae, but the sampling within *Penstemon* was very limited. Wolfe et al. (2002) also proposed a post-Tertiary radiation of the genus given its remarkable floral and vegetative diversity. Much of the diversity within *Penstemon* is associated with evolutionary adaptations such as specialization for pollinators or ecological niches. Thus, *Penstemon* represents a rare example of a continental evolutionary radiation.

The genus has been divided into six subgenera (Lodewick and Lodewick, 1999), two of which are monotypic (subg. *Cryptostemon* and subg. *Dissecti*). The other four subgenera are *Penstemon* (ca. 182 spp.), *Habroanthus* (ca. 50 spp.), *Saccanthera* (ca. 28 spp.), and *Dasanthera* (ca. 9 spp.). There

is considerable morphological diversity within and among subgenera involving habit, floral, leaf, and stem characters. Flowers of most *Penstemon* species attract a wide variety of insect pollinators including bees and wasps, moths, or bee flies (Pennell, 1935; Straw, 1955, 1956a, b, 1963a; Crosswhite and Crosswhite, 1966; Tepedino et al., 1999; Thomson et al., 2000), whereas ca. 40 species have floral syndromes typical of hummingbird pollination (Wilson et al., 2006).

In addition to floral divergence, the architecture of vegetative characters varies dramatically among species (Holmgren, 1984). Stems are woody, suffrutescent, or herbaceous. They are either simple or branched and arise from a woody caudex, from a thick crown subtended by a taproot or from a basal rosette subtended by a taproot. Some species form dense mats, while others have one or few stems above a basal rosette; some are long-lived shrubs, while others are short-lived perennials. Many of these morphological features characterize taxonomic groupings within *Penstemon* (e.g., most of the taxa within subg. *Dasanthera* are suffrutescent, subg. *Saccanthera* has species with a woody caudex, sect. *Ericopsis* subsect. *Caespitosi* consists of diminutive mat formers).

Variable characters in leaf morphology; inflorescence architecture; stem, leaf, and floral vestiture; and numerous floral structures also distinguish species of *Penstemon*. However, many species are differentiated from their closest relatives by only a few characters (Keck, 1932, 1936a, b, 1937a, b, 1938, 1940, 1945, 1957; Pennell, 1935; Straw, 1959, 1962b; Crosswhite, 1965b, c, 1967a, c; Holmgren, 1984), and hybridization has obscured taxonomic differences (Straw, 1955, 1956a, b; Viehmyer, 1958; Every, 1977; Wolfe and Elisens, 1995; Wolfe et al., 1998a, b; Datwyler and Wolfe, 2004).

Penstemon is a member of Cheloneae, a tribe characterized by a cymose inflorescence and flowers having a staminode

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(Wettstein, 1891; Straw, 1966; Thieret, 1967; Wolfe et al., 2002). *Pennellianthus*, *Nothochelone*, and *Keckiella* were segregated from *Penstemon* by Straw (1966). The primary synapomorphies for Straw's (1966) circumscription of *Penstemon* are (1) the presence of epistaminal nectaries as opposed to a nectary consisting of a hypogynous disc, (2) glabrous anther-bearing filaments, (3) presence of a staminode, and (4) seeds without wings. *Chionophila* also has epistaminal nectaries, and Straw (1966) inferred a close relationship of the two genera based on this character.

Penstemon has a range of other dehiscence patterns and morphologies that characterize subgenera. For example, subg. *Saccanthera* is named for the saccate anther morphology possessed by its species. Saccate anthers dehisce across the connective, but not to the distal ends. However, three species not within subg. *Saccanthera* (*P. baccharifolius*, *P. multiflorus*, and *P. dissectus*) also have saccate anther morphology. Subgenus *Habroanthus*, as traditionally circumscribed (Crosswhite, 1967a–c), has species with anthers dehiscing from the distal end of the anther sac but not across the connective. All other species of *Penstemon* have anthers that dehisce across the connective and to the distal ends, including species of subg. *Dasanthera*, which have anther sacs densely covered in woolly hairs. Anther dehiscence patterns play a role in pollination (Thomson et al., 2000; Castellanos et al., 2003, 2006), with bee-pollinated species restricting the pollen presentation to small quantities over a long period, and bird-pollinated species dispensing pollen in larger quantities over a short period.

The staminode of *Penstemon* may be bearded or glabrous, exerted or included, and also plays a role in pollination (Walker-Larsen and Harder, 2001; Dieringer and Cabrera R., 2002). Hummingbird-pollinated species of *Penstemon* tend to have glabrous staminodes that are reduced in size and do not function in pollination (Walker-Larsen and Harder, 2001). In contrast, the staminode of bee-pollinated species varies in size, structure, and pubescence, and contributes to pollination by enhancing contact of floral sexual organs by insects (Walker-Larsen and Harder, 2001; Dieringer and Cabrera R., 2002).

The most distinctive group of species within *Penstemon* is subg. *Dasanthera*. In addition to the woolly anthers characteristic of the group, most species are woody or suffrutescent (except *P. lyallii*), and the flowers have a distinct pleating along the base of the throat and a petal fusion ridge along the top of the flower. The species in the Sierra Nevada and Cascades have evergreen foliage, whereas the species in the Rocky Mountains and Intermountain region have deciduous leaves.

Other taxonomic groups with morphological features that are strikingly distinctive include sect. *Elmigera* of subg. *Habroanthus* and sect. *Fasciculus* and sect. *Ericopsis* subsect. *Caespitosi* of subg. *Penstemon*. Species within sect. *Elmigera* have flowers that are typical of hummingbird pollination (e.g., red, narrow corollas; exerted anthers; reflexed petals). Taxa within *Fasciculus* have fascicles at each stem node, and species of subsect. *Caespitosi* are diminutive wiry mat formers with small leaves and pleated flowers.

The base chromosome number of *Penstemon* is $x = 8$ (Keck, 1945; Straw, 1966). Polyploidy occurs in several sections. The nature of polyploid species has not been extensively studied in *Penstemon*. However, hypotheses of hybrid origin for polyploid species have been made for species in subg. *Saccanthera* and *Penstemon* sect. *Penstemon* (Keck, 1932, 1945). Hybridization and hybrid speciation at the diploid level

TABLE 1. Taxonomy of *Penstemon* as currently recognized by the American Penstemon Society or treated in the most recent field guides (Lodewick and Lodewick, 1999; Nold, 1999). Sampling of species for this study is indicated in the numerator, and abbreviations are those used in Figs. 1–4.

Subgenus	Section	Subsection	Taxon sampling	Abbreviation
<i>Cryptostemon</i>			1/1 species	C
<i>Dasanthera</i>			9/9 species	D
<i>Dissecti</i>			1/1 species	I
<i>Habroanthus</i>	<i>Habroanthus</i>		25/43 species	H1
	<i>Elmigera</i>		3/7 species	H2
<i>Penstemon</i>	<i>Penstemon</i>	<i>Penstemon</i>	9/17 species	P1.1
		<i>Proceri</i>	10/16 species	P1.2
		<i>Humiles</i>	14/18 species	P1.3
		<i>Tubaeiflora</i>	1/1 species	P1.4
		<i>Multiflora</i>	1/1 species	P1.5
		<i>Harbouriani</i>	1/1 species	P1.6
		<i>Gairdneriani</i>	1/2 species	P1.7
		<i>Deusti</i>	1/3 species	P1.8
		<i>Arenarii</i>	1/2 species	P1.9
	<i>Ericopsis</i>	<i>Ericopsis</i>	1/1 species	P2.1
		<i>Caespitosi</i>	6/7 species	P2.2
		<i>Linarioides</i>	2/3 species	P2.3
		<i>Ambigui</i>	2/2 species	P3
		<i>Aurator</i>	10/30 species	P4
		<i>Anularius</i>	16/19 species	P5
	<i>Peltanthera</i>	<i>Peltanthera</i>	11/15 species	P6.1
		<i>Centranthifolia</i>	7/10 species	P6.2
		<i>Havardiani</i>	1/3 species	P6.3
		<i>Petiolati</i>	0/1 species	P6.4
	<i>Fasciculus</i>	<i>Fasciculi</i>	6/12 species	P7.1
		<i>Campanulati</i>	3/8 species	P7.2
		<i>Perfoliati</i>	1/3 species	P7.3
		<i>Racemosi</i>	1/4 species	P7.4
		<i>Baccharifoli</i>	1/1 species	P8
		<i>Chamaeleon</i>	3/4 species	P9
<i>Saccanthera</i>	<i>Saccanthera</i>	<i>Saccanthera</i>	10/21 species	S1.1
		<i>Serrulati</i>	5/6 species	S1.2
		<i>Bridgesiani</i>	1/1 species	S2

have been hypothesized (Straw, 1955, 1956b) and are supported by molecular data (Wolfe et al., 1998a, b).

The objectives of our study were to use nucleotide sequence data to infer the phylogeny of *Penstemon* and to use the resulting phylogeny to examine (1) taxonomic definitions, (2) patterns of morphological and chromosome evolution within *Penstemon*, (3) transitions from insect to bird pollination, and (4) prior biogeographic hypotheses.

MATERIALS AND METHODS

Sampling—Twelve genera comprising 196 taxa were included in our survey (Appendix 1), including all members of Cheloneae (*Chelone*, *Chionophila*, *Collinsia*, *Keckiella*, *Nothochelone*, *Pennellianthus*, *Penstemon*; Wolfe et al., 2002) and representatives from all taxonomic sections of *Penstemon*. The total number of *Penstemon* species surveyed was 163 (Appendix 1, Table 1), 11 of which included two or more varieties for a total of 178 taxa. *Uroskinnera* (Central America), which has traditionally been placed in Cheloneae, and *Tetranema* and *Russelia*, shown to be sister taxa to Cheloneae (Wolfe et al., 2002; Albach et al., 2005; Oxelman et al., 2005), were also included in the survey. *Antirrhinum* and *Linaria* were used as outgroup taxa.

PCR and sequencing protocols—DNA was extracted from either fresh-frozen or silica-gel-dried leaves using a modified CTAB protocol (Wolfe, 2005). PCR products were generated for nrDNA ITS following Wolfe and Randle (2001). Amplifications of cpDNA spacers *trnT-L* and *trnC-D* were

performed using universal primers described in Taberlet et al. (1991) and Demesure et al. (1995), respectively. PCR products were either gel-isolated using the Sephadex kit (Amersham/Pharmacia Biotech, Piscataway, New Jersey, USA) or Concert rapid PCR purification system (Gibco-BRL/Invitrogen, Carlsbad, California, USA).

Di-deoxy termination sequencing was performed using manual and automated techniques. Manual sequencing was performed using the dITP terminators of the USB (Cleveland, Ohio) DNA sequencing kit (Wolfe et al., 2002; Datwyler and Wolfe, 2004). Automated cycle sequencing reactions were performed using the Big Dye terminator chemistry (ABI, Foster City, California, USA), and reactions were run on either an ABI 310 or ABI 3100 automated DNA sequencer. Double-stranded sequences were generated for ITS, *trnT-L*, and *trnC-D* using internal primers appropriate for each locus (Taberlet et al., 1991; Demesure et al., 1995; Wolfe and Randle, 2001).

Data analyses—Sequences were assembled using Sequencher (GeneCodes, Ann Arbor, Michigan, USA). Sequences were aligned in ClustalX (Thompson et al., 1997) with manual adjustments as necessary. Analyses were performed separately for ITS, *trnC-D*, and *trnT-L*, in addition to a combined data set of the plastid loci. Gaps within the ITS matrix and the combined plastid sequence matrix were treated as missing data. Eleven and 22 indel characters were concatenated onto the end of the ITS and plastid sequence matrices, respectively. Of these indels, 12 (three for ITS and nine for *trnC-D/T-L*) were coded as complex gap characters (Simmons and Ochoterena, 2000), and treated as ordered characters with step matrices constructed in MacClade 3.08a (Maddison and Maddison, 1999).

Phylogenetic analyses were conducted using PAUP* version 4.0b10 (Swofford, 2002). All analyses were performed with uninformative characters excluded. Trees were generated using the heuristic search option with 10 random addition sequences (MaxTrees = 2000, Mulpars, tree-bisection-reconnection (TBR) branch swapping, 10 shortest trees held at each step). Our initial analyses were run with MaxTrees set to “automatically increase by 100.” This resulted in 11 500 trees, the limit supported by available computer memory. Strict consensus trees for the entire set of 11 500 trees and subsets of 2000 trees were compared to examine topological congruence among the saved trees. The differences among strict consensus trees were only in the extreme tips so subsequent analyses were done with MaxTrees set to 2000 to expedite the analyses. Support for each node was assessed using 2000 “fast” bootstrap replicates.

Topological incongruence was examined by visually comparing trees from each data set. All nodes resolved in the strict consensus trees were considered in this analysis, and, particularly, nodes with bootstrap values of at least 70% (Kellogg et al., 1996; Mason-Gamer and Kellogg, 1996; Archibald et al., 2005). In addition to the visual analysis, the partition homogeneity test (incongruence length difference test of Farris et al., 1994) in PAUP* was used (100 replicates, TBR branch swapping, 100 replicates of random taxon addition, TBR branch swapping, and MaxTrees = 500).

The ITS and cpDNA consensus trees were used to assess morphological and chromosome evolution in *Penstemon* as well as geographic distribution of taxa. The following traits were mapped onto the consensus trees: Anther morphology, chromosome number(s), and occurrence of bird pollination. In addition, the taxonomic category (subgenus, section, subsection) and geographic distributions were mapped onto the trees.

RESULTS

Analysis of the ITS data set—The aligned sequences and 11 gap characters yielded a matrix with 695 characters, 202 of which were potentially informative, with 0.2% of the matrix cells coded as missing data. The ITS1, 5.8S, and ITS2 sequence lengths ranged from 560 to 618 bp for *Collinsia heterophylla* and *Penstemon californicus*, respectively, with most sequences ranging from 575–605 bp.

From the maximum parsimony analysis, 2000 equally parsimonious trees were saved ($L = 877$, $CI = 0.414$, $RI = 0.726$). The strict consensus tree (Figs. 1, 2) was rooted using *Antirrhinum* and *Linaria* as outgroup taxa. Clades for genera within Cheloneae generally had high bootstrap values, but only

a few terminal lineages of sister taxa had bootstrap values above 70% within the *Penstemon* clade (Figs. 1, 2).

Our study was the first to include a sequence of *Uroskinnera*, which has traditionally been placed in Cheloneae. *Uroskinnera* appears as sister to Cheloneae in this analysis, but without bootstrap support. *Russelia* and *Tetranema* are sister taxa with high bootstrap support in this analysis as observed in previous studies (Wolfe et al., 2002; Albach et al., 2005; Oxelman et al., 2005). The two species of *Chionophila* did not group as sister taxa in the ITS tree. An unresolved trichotomy with *Chionophila tweedyi*, species of subgenera *Dasanthera* and *Cryptostemon* together with the rest of *Penstemon* was found in the ITS strict consensus tree (Fig. 1).

Taxonomic groupings (subgenera, sections, and subsections) within *Penstemon* corresponded sometimes, but certainly not always, to clades (Figs. 1, 2). For example, all of the species in subg. *Dasanthera* group together with *P. personatus* from subg. *Cryptostemon*, all species in subg. *Saccanthera* are found in the basal clades of *Penstemon*, and most species in sections *Ericopsis*, *Aurator*, and *Fasciculus* form clades.

Analysis of the chloroplast data set—The chloroplast data set consisted of 2212 characters, including 22 indel characters coded as simple or complex gaps, with 289 positions being potentially informative and 1.0% of the cells, mostly at the beginning and ends of the aligned sequences, coded as missing data. Nucleotide sequences from two noncoding regions, *trnC-D* and *trnT-L*, were combined for this analysis. The *trnC-D* region yielded an aligned sequence matrix of 663 characters plus 10 gap characters, whereas the *trnT-L* region yielded an aligned sequence matrix of 1527 characters plus 12 gap characters. The number of potentially informative characters was 81 and 186 for *trnC-D* and *trnT-L*, respectively. For either data set, 12.2% of the characters were parsimony-informative. The *trnC-D* sequence lengths ranged 259 to 556 bp for *Tetranema mexicanum* and *Penstemon speciosus*, respectively, with most sequence lengths ranging 500–530 bp. The *trnT-L* sequence lengths ranged from 669 to 1268 bp for *Penstemon aridus* and *Tetranema mexicanum*, respectively, with most sequence lengths in the range of 1150–1200 bp.

From the maximum parsimony analysis, 2000 equally parsimonious trees were saved ($L = 763$, $CI = 0.510$, $RI = 0.803$). The strict consensus tree (Figs. 3, 4) was rooted using *Antirrhinum* and *Linaria* as outgroup taxa. Similar to the results from the ITS study, clades for genera within Cheloneae generally had high bootstrap values, but only a few terminal lineages of sister taxa had bootstrap values above 70 within the *Penstemon* clade (Figs. 3, 4).

The topology of the strict consensus tree resolves the basal nodes of the tree and some clades within *Penstemon*. We were unable to amplify and sequence the chloroplast regions of *Uroskinnera*. However, the basal topologies of the ITS and cpDNA phylogenies agree except for the placement of *Chionophila tweedyi*. The cpDNA tree (Fig. 3) places the two species of *Chionophila* as sister taxa with strong bootstrap support, whereas *C. tweedyi* is placed in an unresolved trichotomy with subg. *Dasanthera* and the rest of *Penstemon* in the ITS analysis (Fig. 1).

Similar to the ITS tree (Figs. 1, 2), subgenera, sections, and subsections within *Penstemon* only sometimes seemed to represent clades (Figs. 3, 4). Most of the species in subg. *Dasanthera* group except for *P. montanus*, which appears as sister to the rest of *Penstemon*, all species in subg. *Saccanthera*

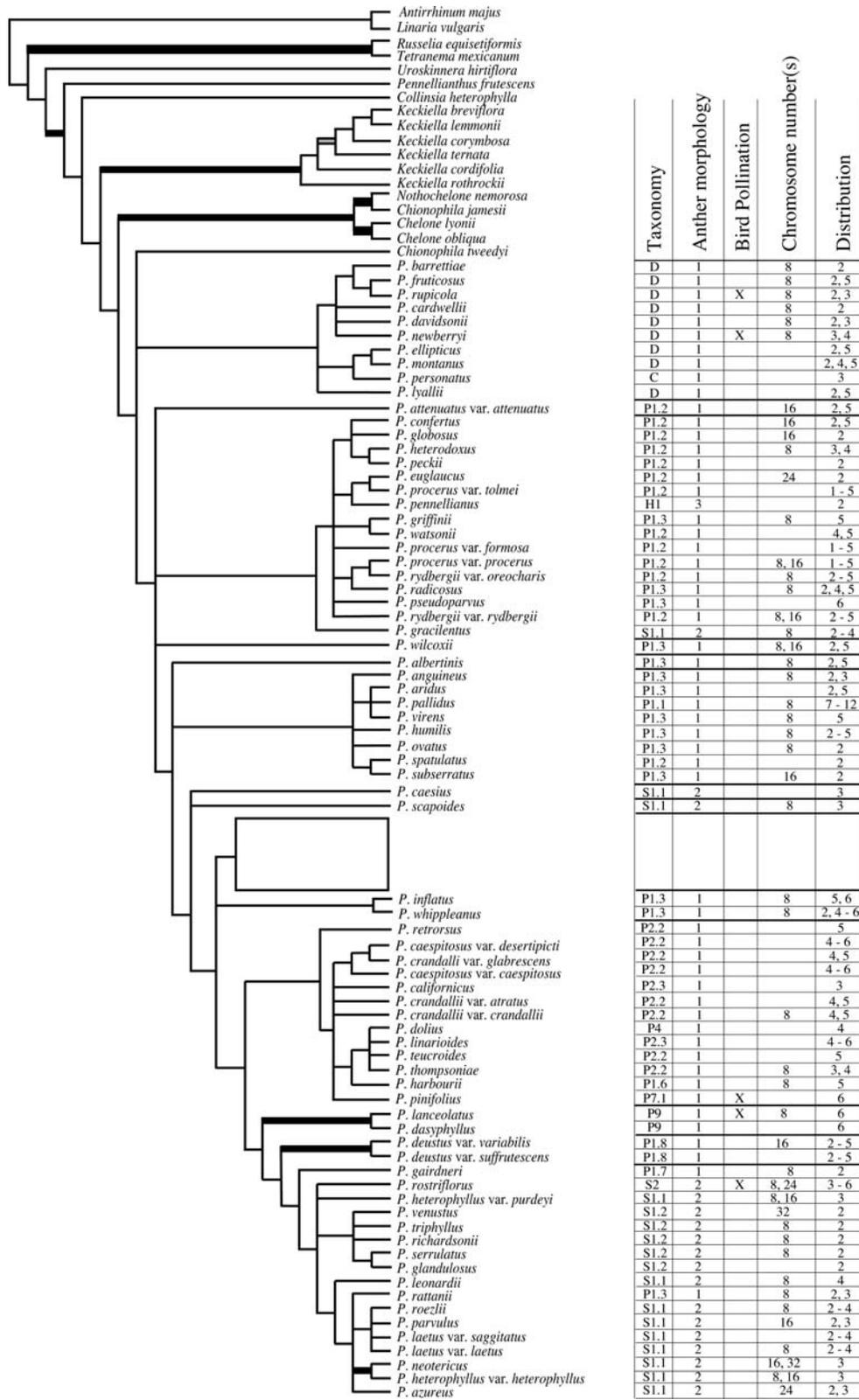


Fig. 1. Strict consensus of 2000 equally parsimonious ITS trees, part I. Outlined box in the tree is shown in Fig. 2. The line thickness shown in the tree reflects bootstrap (BP) values of 70–100%, with BP >90% shown with thick black lines and values 70–80% shown with thick gray lines. Taxon abbreviations are in Table 1, and distribution designations are in Table 2. *P.* = *Penstemon*. Anther morphology: 1 = dehisces across the connective and from end-to-end of the anther sac; 2 = dehisces across the connective but not to the distal end of the anther sac; and 3 = dehisces from the distal end toward the connective, but not across the connective. Bird pollination = hummingbird-pollinated species. Chromosome numbers were obtained from references cited throughout the manuscript.

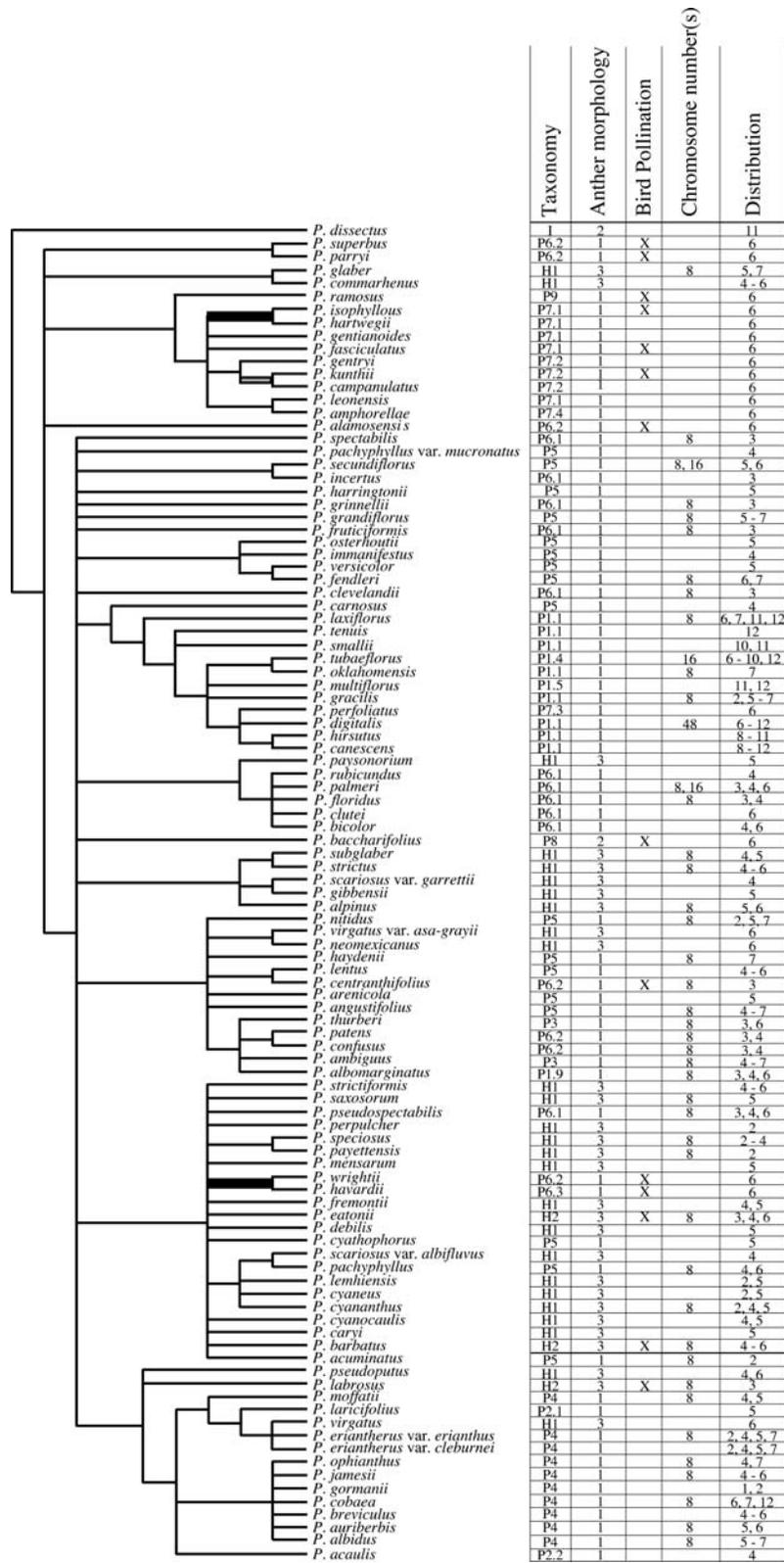


Fig. 2. Strict consensus of 2000 equally parsimonious ITS trees, part II. Abbreviations and designations are the same as in Fig. 1.

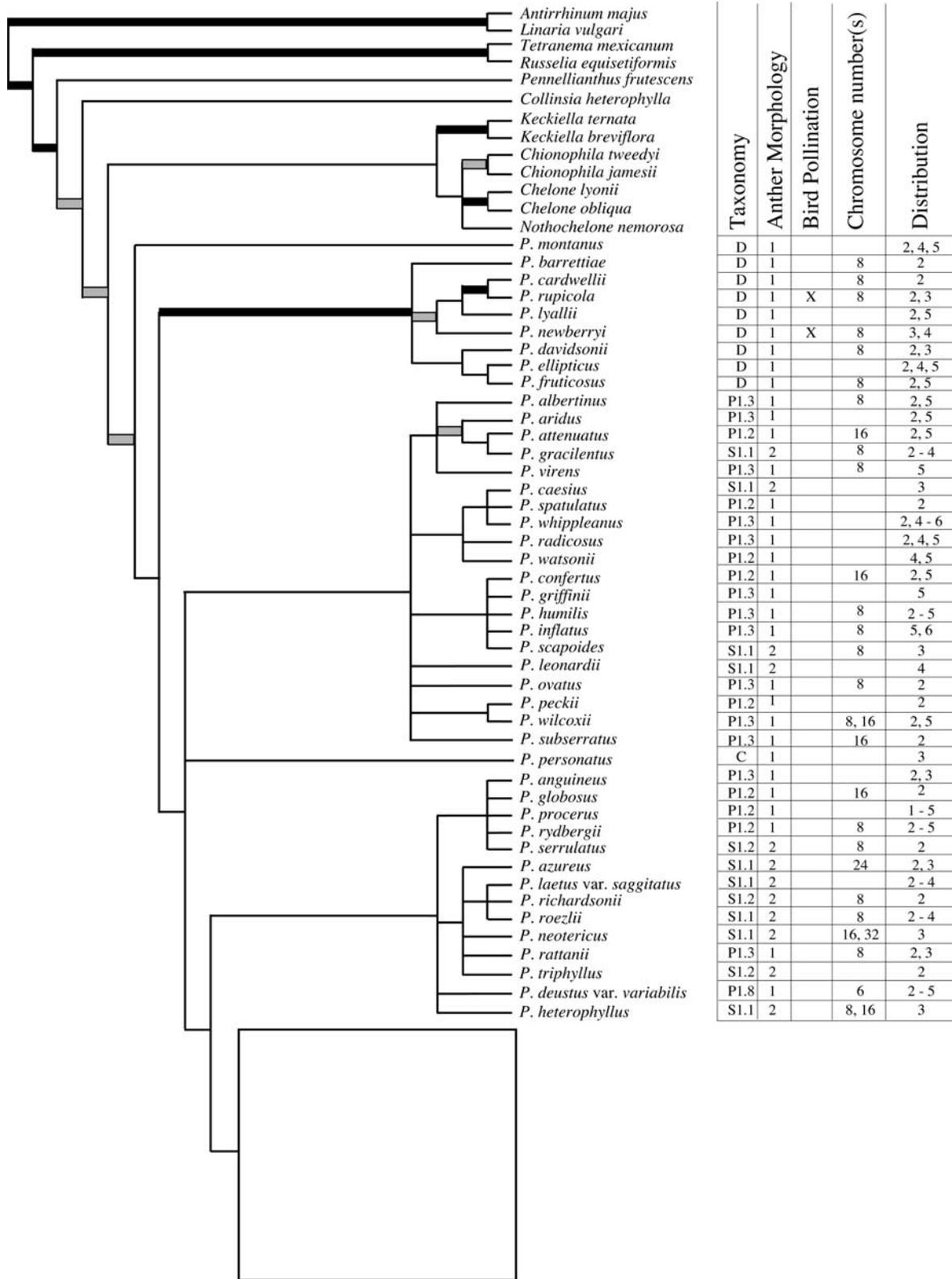


Fig. 3. Strict consensus of 2000 equally parsimonious combined *trnC-D/T-L* trees, part I. Outlined box in the tree is shown in Fig. 4. Abbreviations and designations are the same as in Fig. 1.

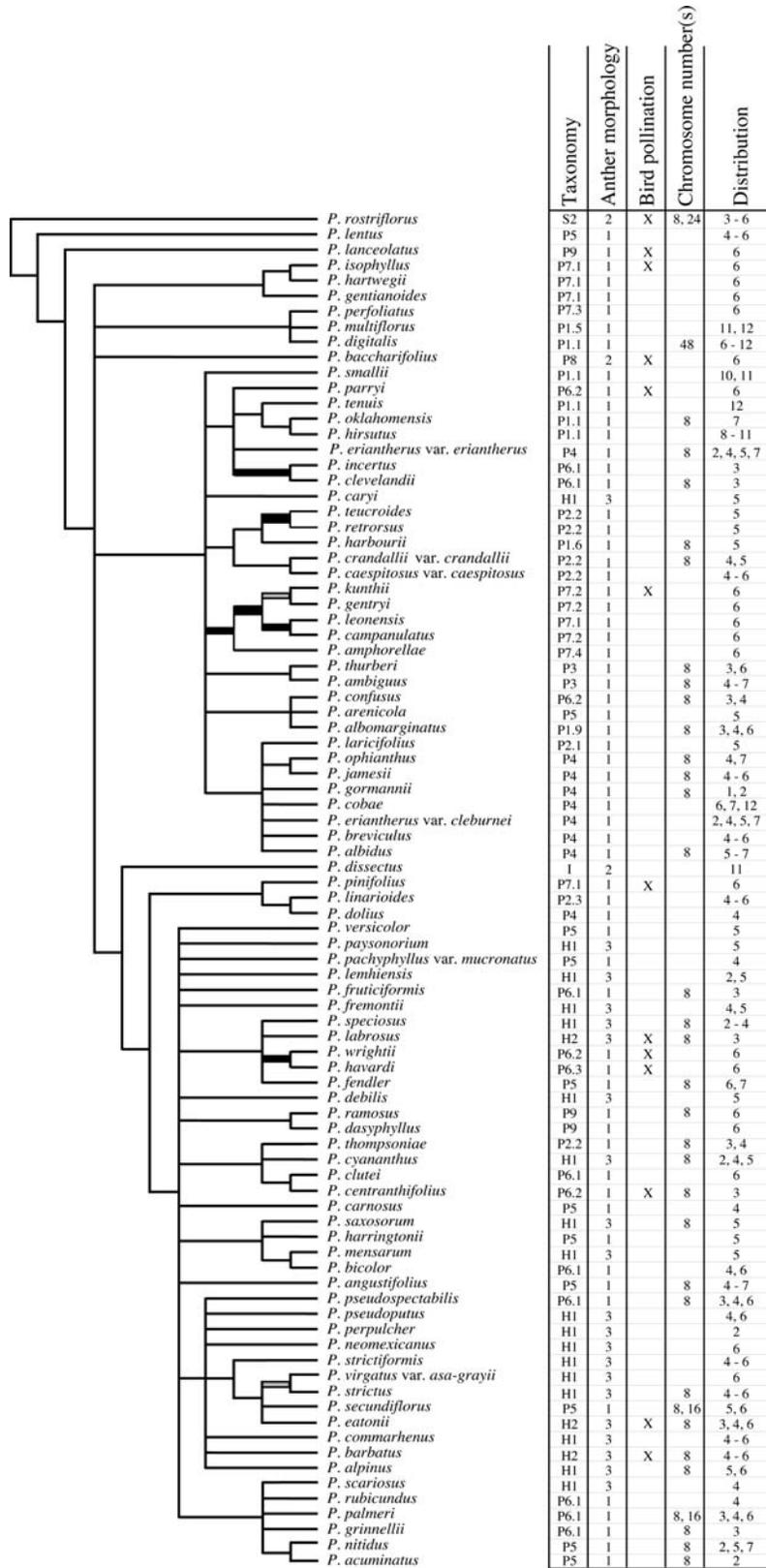


Fig. 4. Strict consensus of 2000 equally parsimonious *trnC-D/T-L* trees, part II. Abbreviations and designations are the same as in Fig. 1.

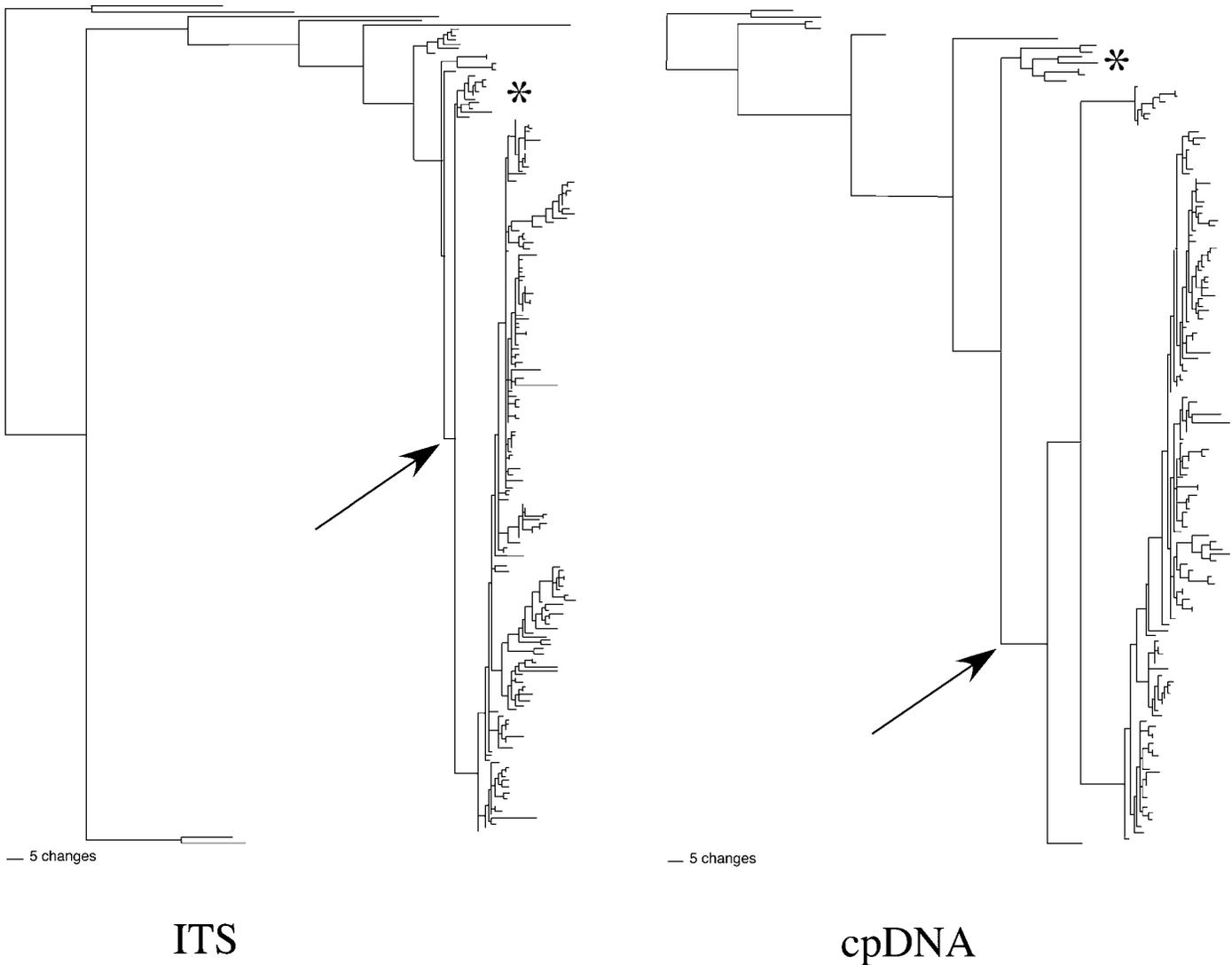


Fig. 5. Phylograms of one of 2000 equally parsimonious trees for ITS (on left) and cpDNA (on right) analyses. The arrows point to the node defining the Cheloneae clade in each analysis. The *Dasanthera* clade of *Penstemon* is marked with an asterisk.

are found in the basal clades of *Penstemon*, and most species in sect. *Aurator* group together in the same clade.

Congruence testing and comparison of ITS and cpDNA trees—Major topological differences observed visually between the ITS and cpDNA trees (Figs. 1–4) include the placement of *Chionophila tweedyi*, *P. campanulatus*, *P. clevelandii*, *P. dasyphyllus*, *P. dissectus*, *P. dolius*, *P. gentryi*, *P. hartwegii*, *P. heterophyllus*, *P. incertus*, *P. isophyllous*, *P. kunthii*, *P. lanceolatus*, *P. leonensis*, *P. linarioides*, *P. montanus*, *P. neotericus*, *P. personatus*, *P. pinifolius*, *P. strictus*, *P. teucroides*, and *P. virgatus* var. *asa-grayii*. Additional topological differences include members of clades containing *P. caespitosus* and *P. gentianoides*.

The partition homogeneity test (ILD) revealed significant incongruence ($P = 0.01$) between the ITS and cpDNA trees. Successive deletions of taxa and a rerun of the analysis did not improve the scores. The incongruence could be caused by a history of introgression and chloroplast capture events (Wolfe

and Elisens, 1993, 1994, 1995; Wolfe et al., 1998a, b; Datwyler and Wolfe, 2004). Thus, we did not combine these data sets.

Despite conflicts in the nuclear and cpDNA data sets, there are elements of MP trees inferred from each data set that are congruent. For example, species in subg. *Dasanthera* group together in a clade that is sister to the rest of *Penstemon* (Figs. 1, 3, 5), and the species in subg. *Saccanthera* are found at the base of the *Penstemon* clade associated with certain species of subg. *Penstemon* sect. *Penstemon*. Species in sections *Aurator*, *Ericopsis*, and *Fasciculus* show similar topological patterns in each analysis in terms of terminal clades and species groupings, which reflects their close relationships within their respective sections. Also, species of subg. *Habroanthus* are mixed in a group with several sections of subg. *Penstemon* toward the tips of the *Penstemon* clade (Figs. 2, 4).

ITS and the cpDNA data did not completely resolve relationships among species of *Penstemon*. Most synapomorphies resolve relationships among genera of Cheloneae.

Relatively few nucleotide character differences were observed among a crown group of *Penstemon* species, and most of the parsimony informative characters were from insertion–deletion events. Figure 5 shows the relative branch lengths in one of 2000 most parsimonious trees for each analysis. Subg. *Dasanthera* has clear differences in molecular characters (e.g., nucleotide and gap characters) from the other taxonomic groups within *Penstemon*, but most of the nodes are very short throughout the *Penstemon* clade in each tree. There appears to have been an explosive radiation above the point marked by the arrows in Fig. 5.

DISCUSSION

Cheloneae and Russeliae—The clade definition of Cheloneae sensu Wolfe et al. (2002) is “the least inclusive clade that contains *Chelone*, *Collinsia*, and *Pennellianthus*.” Synapomorphies for the tribe include a cymose inflorescence, the presence of a staminode, simple hairs, and stems with pith. The previous molecular study by Wolfe et al. (2002) did not include *Uroskinnera*, and it was proposed that this genus was key to elucidating relationships among *Tetranema*, *Russelia*, and Cheloneae. We were able to amplify and sequence ITS from a herbarium specimen of *Uroskinnera*, but were unsuccessful in our attempts to do the same for the noncoding chloroplast regions used in this study. In the ITS phylogeny (Fig. 1), *Uroskinnera* was sister to Cheloneae. The node supporting Cheloneae had high bootstrap support, but there was no support for including *Uroskinnera* as a member of Cheloneae. Burt (1965) proposed that *Uroskinnera* (distributed in Mexico and Guatemala) and *Brookea* (endemic to Borneo) were closest allies. We were unable to sample *Brookea* for this study, but it will be interesting to elucidate the relationships among these genera and Russeliae and Cheloneae when material becomes available for sequencing.

Wolfe et al. (2002) used ITS and *matK* sequences to infer the phylogenetic patterns of Cheloneae and related genera within Plantaginaceae and found strong support for a clade consisting of *Russelia* and *Tetranema* as sister taxa. Two studies since then have used additional plastid genes and independent tissue collections to verify this relationship and the position of this clade as sister, or near to, Cheloneae (Albach et al., 2005; Oxelman et al., 2005). Our current study also reveals a strongly supported clade of *Russelia* and *Tetranema*. Taken together, these results support a redefinition of tribe Russeliae (Pennell, 1920b) to include *Tetranema*.

Chionophila—Straw (1966) hypothesized that *Chionophila* and *Penstemon* were sister taxa based on a shared epistaminal nectary character. The two species of *Chionophila* are morphologically distinct with non-overlapping ranges. *Chionophila jamesii* occurs in the Rocky Mountains of Colorado and Medicine Bow Mountains of Wyoming. It is found at the edges of melting snowfields in the alpine zone. This diminutive plant has an inflorescence of several tightly packed, cream-colored flowers. *Chionophila tweedyi* occurs in subalpine open woodlands in Idaho and Montana, and has a loosely organized raceme of four to 10 lavender flowers.

The placement of *Chionophila tweedyi* differs in the ITS and cpDNA trees (Figs. 1, 3). It occurs within a polytomy within the *Penstemon* clade in the ITS strict consensus tree, but as sister to *Chionophila jamesii* in the cpDNA strict consensus

tree (bootstrap = 70) with this clade grouped with *Chelone*, *Keckiella*, and *Nothochelone*. An analysis of combined data from ITS and *matK* (Datwyler and Wolfe, 2004) using a subset of the taxa found in this study revealed a strongly supported clade of the two species of *Chionophila* (bootstrap = 97, Bremer support = 5). Similar results were reported in Wolfe et al. (2002) using a larger subset of the taxa sampled here. These results are consistent with *Chionophila* being more closely related to other members of Cheloneae rather than as a sister taxon to *Penstemon*. However, further studies are needed to investigate the anomalous pattern in the ITS tree reported here (Fig. 1).

Taxonomic implications for subgenera of Penstemon

This is the first phylogenetic study of *Penstemon* with a thorough sampling of subgenera, sections, and subsections. We were able to sample all but one monotypic subsection for this survey for a total of 163 of 271 species (Table 1). *Penstemon* has not been examined in its entirety, but has been the subject of investigation in regional floras (e.g., Pennell, 1920b, 1935; Holmgren, 1984) or treatments of particular groups of species (Keck, 1932, 1936a, b, 1937a, b, 1938, 1940, 1945; Keck and Cronquist, 1957; Crosswhite, 1965a–c, 1966, 1967a–c, 1970; Straw, 1959, 1962, 1963b). The taxonomy of *Penstemon* has been summarized in publications sponsored by the American Penstemon Society (Lodewick and Lodewick, 1999) and in field and garden guide books (Way and James, 1998; Nold, 1999). However, the genus has not yet been treated as a whole in the scientific literature. Because the Lodewick and Lodewick (1999) publication represents a summary of all the taxonomic literature available for *Penstemon*, we followed the nomenclature and classification scheme presented there.

Our study falls short of providing the phylogenetic basis of a revised taxonomy for *Penstemon*, but does indicate that some infrageneric taxa may be natural groupings while others are hopelessly paraphyletic. Further, it highlights a number of taxonomic anomalies that may be fixed by moving a species from one taxonomic group to another.

Subgenera *Dasanthera* and *Cryptostemon*—Of the six subgenera of *Penstemon*, only subg. *Dasanthera* appears distinctive in the ITS and cpDNA phylogenies (Figs. 1–5). However, *P. personatus* of the monotypic subg. *Cryptostemon* groups with the *Dasanthera* species in the ITS tree (Fig. 1), and *P. montanus* falls outside the clade of *Dasanthera* penstemon in the cpDNA tree (Fig. 3). There are many molecular synapomorphies for the *Dasanthera* clade (Fig. 5), and the morphology of these species is very distinctive compared to the rest of the genus (Datwyler and Wolfe, 2004).

Keck (1936b) discussed the taxonomic affinities of *P. personatus* in terms of its floral morphology. The most striking floral feature of *P. personatus* is the short staminode. Keck (1936b) pointed out that several of the *Dasanthera* species also have short staminodes and that the staminode of *P. rupicola* is shorter than that of *P. personatus*. Morphological synapomorphies for *P. personatus* and species of subg. *Dasanthera* include a short staminode and dense pubescence within the corolla throat. Our results combined with these morphological characters support adding *P. personatus* to subg. *Dasanthera*, and eliminating subg. *Cryptostemon*.

Subgenera *Saccanthera*, *Habroanthus*, and *Dissecti*—The other three subgenera of *Penstemon* identified by distinctive

anther dehiscence patterns are *Saccanthera*, *Habroanthus*, and *Dissecti*. Species in subg. *Saccanthera* and *Dissecti* have anther sacs that dehisce across the connective, but not to the distal ends, and species in subg. *Habroanthus* have anther sacs that dehisce from the distal end toward the connective, but not across the connective. Anther dehiscence patterns and taxonomic affiliations were scored next to the strict consensus trees from each analysis (Figs. 1–4).

Keck (1932) classified all but three species of *Penstemon* with saccate anther morphology as members of sect. *Saccanthera*. Several hypotheses regarding taxonomic affinities were proposed by Keck (1932), including (1) *P. gracilentus* is most closely allied to species in subg. *Penstemon* sect. *Penstemon*; (2) *P. azureus* is a derivative of *P. laetus*; (3) *P. neotericus* is a hybrid between *P. laetus* and *P. azureus*; (4) *P. serrulatus* is closely allied to *P. ovatus* of sect. *Penstemon*; (5) *P. heterophyllus* is a derivative of *P. laetus*, *P. azureus*, or a hybrid derivative of both; (6) *P. triphyllus* is related most closely to *P. richardsonii*; (7) *P. glandulosus* is not closely allied to other species of the section; and (8) *P. rostriflorus* appears to be more closely related to members of sect. *Elmigeria* than to other species of *Saccanthera*. Our results can address most of these hypotheses, beginning with the affinities of subg. *Saccanthera* to other groups of *Penstemon*.

Most of the *Saccanthera* species are found in a single clade in the ITS tree (Fig. 1), with the exception of *P. gracilentus*, which is at the base of the larger clade containing the *Saccanthera* clade. This pattern differs in the cpDNA tree (Figs. 3, 4) in that the *Saccanthera* species do not form a single clade, and the placement of *P. rostriflorus* is very different in each phylogeny. Our results support Keck's (1932) hypothesis 1, that *P. gracilentus* is closely allied with species of sect. *Penstemon*.

Close relationships among most of the *Saccanthera* species (Keck's [1932] hypotheses 2, 3, 5, and 6) are supported by our results, whereas *P. serrulatus* does not appear to be closely related to *P. ovatus* (Keck's [1932] hypothesis 4; Figs. 1, 3). Our results also do not support Keck's (1932) hypotheses 8 about *P. rostriflorus* as more closely related to species in sect. *Elmigeria* than to species in *Saccanthera*, nor is *P. glandulosus* more closely related to *P. ovatus* of sect. *Penstemon* (Keck's [1932] hypothesis 7; Fig. 1).

Crosswhite (1967a) designated *Habroanthus* as a subg. having a synapomorphy of anther dehiscence from the distal end, but not across the connective of the anther sacs. Subg. *Habroanthus* has two sections, *Habroanthus* and *Elmigeria*. The last section is comprised of species with red, tubular corollas. Neither sect. *Habroanthus* nor *Elmigeria* were monophyletic in either of our analyses (Figs. 2, 4). Rather, species in both sections are scattered among species from subg. *Penstemon* sections *Anularius* and *Peltanthera*. The anther dehiscence pattern for subg. *Penstemon* is complete dehiscence, but it is not known whether dehiscence is initiated from the distal end or connective.

The anther dehiscence patterns within *Penstemon* would appear to be insufficient synapomorphies for defining subgenera, although this character has been useful for field identification and grouping species in identification keys. Outside of *Saccanthera*, three species of *Penstemon* have saccate anthers: *P. multiflorus* and *P. baccharifolius* from subg. *Penstemon*, and *P. dissectus* from the monotypic subg. *Dissecti* (Figs. 1–4). None of these three species group with the *Saccanthera* members. *Penstemon dissectus* is an unusual

species, narrowly endemic to granite outcrops in Georgia, and the only species with dissected leaves. Pennell (1935) was unable to define any close relationships of this species with others in eastern North America; and its placement in the ITS and cpDNA tree differs greatly (Figs. 2, 4). However, *P. dissectus* is clearly not of a basal lineage and appears to be sufficiently derived from within subg. *Penstemon* so that its designation as a monotypic subgenus should be questioned.

The taxonomy of *Penstemon* would be improved by revising sections to reflect evolutionary relationships more faithfully. The results from our molecular phylogenetics study indicate the need for a major revision of *Penstemon* to include two expanded subgenera, *Dasanthera* and *Penstemon*, and many taxonomic changes to accommodate new species affiliations. For example, given the lack of monophyly for species in subg. *Saccanthera* together with the convergent evolution of saccate anther morphology in the genus (Figs. 1–4), a revised taxonomy including a reduced and monophyletic subset of *Saccanthera* as a section within subg. *Penstemon* is warranted. Similarly, the taxonomic status of subg. *Habroanthus* should be re-evaluated.

Taxonomic implications for sections of *Penstemon*—With the lack of resolution in the ITS and cpDNA phylogenies, our comments will focus on sections *Penstemon*, *Ericopsis*, *Aurator*, and *Fasciculus* to supplement what we have already discussed regarding the reassignment of subg. *Habroanthus* species within sections *Anularius* and *Peltanthera*. Additional comments on individual species of *Penstemon* and their phylogenetic affinities are in Castellanos et al. (2006).

Sect. *Penstemon* as currently circumscribed has nine subsections (Table 1). Subsections *Proceri* and *Humiles* represent early-branching lineages in both cpDNA and ITS analyses (Figs. 1, 3), with affinities to members of *Saccanthera*. Species in subsections *Deusti* and *Gairdneriani* are in even closer proximity to *Saccanthera* species in both analyses. However, species in subsections *Penstemon*, *Tubae-flori*, *Multiflori*, *Harbouriani*, and *Arenarii* are placed in more terminal clades (Figs. 2, 4) compared to the other four subsections, with the first three grouping together. *Penstemon harbourii* is closely allied to species in sect. *Ericopsis* subsection *Caespitosi* in both analyses (Figs. 1, 4), whereas *P. albomarginatus* of subsection *Arenarii* groups with species from sect. *Ambiguui* (Fig. 2).

Most of the species of sect. *Ericopsis* subsections *Caespitosi* and *Linarioides* form a clade in both analyses (Figs. 1–4); this clade, however, differs in phylogenetic position between trees. In the ITS tree (Fig. 1), this group of species is sister to the *Saccanthera* clade, but is in a larger terminal clade containing species from sect. *Penstemon* subsection *Penstemon*, and sections *Aurator* and *Peltanthera* in the cpDNA tree (Fig. 4). *Penstemon laricifolius* (sect. *Ericopsis* subsection *Ericopsis*) does not group with others in its section in either analysis, but appears more closely related to species in sect. *Aurator* (Figs. 2, 4). *Penstemon acaulis* also appears as more closely related to species in sect. *Aurator* than to species in sect. *Ericopsis* (Fig. 1). Most of the *Aurator* species occur in a clade with species of sect. *Ericopsis* (*P. acaulis* and *P. laricifolius* in the ITS tree and *P. laricifolius* in the cpDNA tree, which did not include *P. acaulis*) in either analysis (Figs. 2, 4). Keck (1937a) and Penland (1958) proposed affinities of sections *Ericopsis* and *Aurator*, primarily through *P. dolius*, which is in clades of *Ericopsis* species in each analysis (Figs. 1, 4). The transfer of

this species to sect. *Ericopsis* seems appropriate, and it would be practical to reexamine the relationships of *P. acaulis* and *P. laricifolius* to species of *Ericopsis* and *Aurator*.

Penstemon pinifolius is another species that should be examined more closely. It is currently placed in sect. *Fasciculus* subsect. *Fasciculi* based on its possession of axillary fascicles (Straw, 1962). However, Straw (1962) hypothesized that *P. pinifolius* was anomalous in this section because its floral characteristics differed greatly from other species in sect. *Fasciculus*. Crosswhite and Crosswhite (1981) hypothesized that *P. pinifolius* has a greater affinity with species of sect. *Ericopsis* than *Fasciculus*. *Penstemon pinifolius* groups with members of sect. *Ericopsis* in both analyses of this study (Figs. 2, 4), and we propose that it be moved from sect. *Fasciculus* to sect. *Ericopsis*.

Incongruence, hybridization, and polyploidy—The topologies of the ITS and cpDNA strict consensus trees (Figs. 1–4) differ in the placement of many taxa. Some of these differences are minor and may reflect the lack of characters to resolve the topologies (Fig. 5). Other differences are apparently large (e.g., the placement of *Chionophila tweedyi* in Fig. 1 vs. Fig. 3, the *Ericopsis* clade in Fig. 1 vs. Fig. 4).

One probable explanation for the differences seen in the ITS and cpDNA trees is hybridization (Viehmeyer, 1958; Straw, 1955, 1956a, b; Crosswhite, 1965a; Wilson and Valenzuela, 2002). Hybrid speciation and introgression have been documented in *Penstemon* (Wolfe and Elisens, 1993, 1994, 1995; Wolfe et al., 1998a, b; Datwyler and Wolfe, 2004), as well as polyploidy (Figs. 1–4). Most of the polyploid taxa are basal lineages within the genus in sections *Penstemon* and *Saccanthera*. Whether these are allopolyploid or autopolyploid has not yet been examined using a molecular approach, but given the ability of many species of *Penstemon* to hybridize, we hypothesize that many of the polyploid species are allopolyploids. Keck (1945) examined the cytology of many species in sections *Penstemon* and *Saccanthera* and hypothesized that *P. attenuatus*, *P. subserratus*, and *P. wilcoxii* are allopolyploids. He (Keck, 1932) also proposed allopolyploidy in the origin of *P. neotericus*, *P. azureus*, and *P. heterophyllus* in his monograph of the *Saccanthera* species.

Penstemon appears to have recently undergone a rapid evolutionary radiation (Fig. 5). Reproductive boundaries between species seem to be imperfect, which allows for hybridization where species occur in sympatry. Chloroplast-capture events have been found in at least one hybrid complex of sect. *Peltanthera* (Wolfe and Elisens, 1995). Thus, it is unsurprising that there is incongruence between the nuclear and plastid gene trees.

Floral evolution in *Penstemon*—Pennell (1935, p. 198) hypothesized that bee pollination was the pleisiomorphic condition in *Penstemon* and that “evolution has clearly progressed from pollination by bees to pollination by moths, butterflies or hummingbirds.” Our results support this hypothesis. Each of the traditional subgenera of *Penstemon*, characterized by different anther dehiscence patterns, has species pollinated by hummingbirds (Figs. 1–4). Many of the taxonomic anomalies resulted from past researchers placing together hummingbird-pollinated species (e.g., sect. *Elmigeria*) or from uncertainty as to what to do with them (e.g., *P. pinifolius*). We scored the occurrence of hummingbird floral morphology onto the ITS and cpDNA trees (Fig. 1–4). Species

of *Penstemon* with this syndrome occur in at least 10 separate clades in each of the phylogenies presented here (Figs. 1–4), and, accounting for incomplete taxon sampling, many more originations are possible. These results are congruent with and an expansion of those reported in Castellanos et al. (2006), and Wilson et al. (2006). Several recent studies (Thomson et al., 2000; Castellanos et al., 2003, 2004, 2006; Wilson et al., 2006) have documented the characteristics of *Penstemon* flowers that accompany the shift from bee- to bird-pollination syndromes. These studies have demonstrated that pollen presentation and pollination efficiency differ in species pollinated primarily by bees vs. birds. Thus, the taxonomic use of anther dehiscence characters is called into question by their apparent convergence.

The majority of *Penstemon* species are pollinated by hymenopterans, and numerous groups of bees are specialized for a limited suite of flowers (Crosswhite and Crosswhite, 1966; Tepedino et al., 1995). Very few species of *Penstemon* have moths as their primary pollinator (e.g., *P. albidus*, *P. guadalupensis*, and *P. tubaeflorus*; Pennell, 1935). We included *P. albidus* and *P. tubaeflorus* in this survey, and they are placed toward the tips of the tree in their respective clades (Figs. 2 and 4). Pennell (1935) hypothesized that the large variety of pollinators in *Penstemon* accounts for the diversity of flowers in the genus. We concur and hypothesize that pollinators, and the selection for particular floral traits, are important driving forces for the diversification of the genus (Wilson et al., 2006).

Biogeography of *Penstemon*—Wolfe et al. (2002) examined the biogeography of Cheloneae, concluding that the North American members of the tribe diversified initially in the Klamath Region with subsequent migration of elements to the Rocky Mountains. These findings were in agreement with Straw’s (1966) hypotheses as to the origins of *Chionophila* and *Penstemon* in the Rocky Mountain/Columbia Plateau region. Datwyler and Wolfe (2004) examined the biogeography of subg. *Dasanthera* and supported a Rocky Mountain origin for this subgenus with a subsequent radiation of species through the Cascade–Sierra Nevada cordillera. Subg. *Dasanthera* appears as a basal lineage in the genus (Figs. 1–5). Thus our biogeographic hypotheses for the genus as a whole will be based on a Rocky Mountain–Cascade–Sierra Nevada radiation of the basal lineage.

Geographic distributions (Table 2) of each species are shown on the ITS and cpDNA strict consensus trees (Figs. 1–4). The basal lineages of *Penstemon* are distributed in the Rocky Mountains, Pacific Northwest, Boreal Northwest, California and Baja California, and the Intermountain Region. Species with distributions into the southwestern USA, Mexico east of the Gulf of California, and Guatemala appear at the base of the terminal clade of *Penstemon* in both analyses, and then scattered throughout the most terminal clades (Figs. 2, 4). Species with distributions east of the Rockies are found predominantly in the most terminal clades of each analysis (Figs. 2, 4). Pennell (1935) considered the species of *Penstemon* in eastern temperate North America to be among the most derived of the genus, and our study supports this hypothesis. We propose the following biogeographic scenario for *Penstemon*: (1) an origin for the genus in the Rocky Mountains with subsequent migration to the Cascade–Sierra Nevada cordillera; (2) radiation of elements throughout North America west of the Cascade–Sierra Nevada cordillera; (3)

TABLE 2. Geographic regions mapped onto phylogenetic trees (Figs. 1–4).

No. on tree	Region	Area included in region
1	Boreal Northwest	USA: Alaska Canada: Yukon, Northwest Territory
2	Pacific Northwest	USA: Idaho, Oregon, Washington Canada: British Columbia
3	California	USA: California Mexico: Baja California
4	Intermountain	USA: Arizona north of Grand Canyon, Nevada, Utah
5	Rocky Mountain	USA: Colorado, Montana, Wyoming Canada: Alberta
6	Southwestern	USA: Arizona south of Grand Canyon, New Mexico, Texas Mexico: east of Gulf of California Guatemala
7	Great Plains	USA: Iowa, Kansas, Minnesota, Missouri, Nebraska, North Dakota, Oklahoma, South Dakota Canada: Saskatchewan, Manitoba
8	Great Lakes	USA: Illinois, Indiana, Michigan, Ohio, Wisconsin Canada: Ontario
9	Northeast	USA: Connecticut, Maine, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Rhode Island, Vermont Canada: Quebec
10	Appalachia	USA: Kentucky, Tennessee, West Virginia
11	Atlantic Coastal Plain	USA: Delaware, Florida, Georgia, Maryland, North Carolina, South Carolina, Virginia
12	Gulf Coastal Plain	USA: Alabama, Arkansas, Louisiana, Mississippi

migration throughout the Intermountain Region and then into southwestern North America; and (4) migration of elements over the Rocky Mountain cordillera into the Great Plains and then throughout eastern North America. The apparent, recent evolutionary radiation (Fig. 5) in *Penstemon*, suggests that this biogeographic scenario is correlated with events in the Pleistocene.

Rapid evolutionary radiations are usually associated with oceanic islands. However, *Penstemon* is an example of a recent continental evolutionary radiation where speciation may have been driven by selective pressure by pollinators in conjunction with ecological adaptations to niches made available by retreating glaciers. Our phylogenetic analyses of ITS, and the combined data sets from *trnC-D* and *trnL-L*, were unable to fully resolve relationships among species of *Penstemon*. Thus, it will be necessary to garner additional molecular data (e.g., low-copy nuclear genes) before we can fully elucidate the evolutionary processes that explain the diversification of this interesting genus.

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APPENDIX. Voucher information and GenBank accession numbers. # = ITS, † = *trnT-L*, and § = *trnC-D* sequences. Underlined names and voucher information indicates that ITS only was sequenced for a particular taxon.

Taxon—Voucher specimen (Herbarium); GenBank nos.

Antirrhinum majus L.—Wolfe *s.n.* (OS); #AF375150, †DQ531193, §DQ531338.
Chelone lyonii Pursh—Nelson 131 (OKL); #AF375163, †DQ531202, §DQ531347. *C. obliqua* L.—Wolfe 586 (OS); #AF375164, †DQ531203, §DQ531348.
Chionophila jamesii Benth.—Wolfe 473 (OS); #AF375167, †DQ531204, §DQ531349. *C. tweedyi* (Canby & Rose) L.F. Hend.—Datwyler 110 (OS); #AF375166, †DQ531205, §DQ531350.
Collinsia heterophylla Graham—Wolfe *s.n.* (OS); #AF375153, †DQ531198, §DQ531343.
Keckiella breviflora (Lindl.) Straw—Wilson 3487 (OS); #AF375161, †DQ531199, §DQ531344. *K. cordifolia* (Benth.) Straw—Wilson 3513 (OS); #AF375162. *K. corymbosa* (Benth.) Straw—Wolfe 437 (OS); #AF375160. *K. lemmonii* (Gray) Straw—Wolfe 436 (OS); #AF375158. *K. rothrockii* (Gray) Straw—Wilson 3512 (OS); #AF375159. *K. ternata* (Torr.) Straw—Valenzuela 43 (OS); #AF375157, †DQ531200, §DQ531345.
Linaria vulgaris P. Mill.—Nickrent *s.n.* (SIU); #DQ531053, †DQ531194, §DQ531139.
Nothochelone nemorosa (Douglas ex Lindl.) Straw—Wolfe 603 (OS); #AF375165, †DQ531201, §DQ531346.
Pennellianthus frutescens (Lambert) Crosswhite—Wolfe *s.n.* (OS); #AF375156, †DQ531197, §DQ531342.
Penstemon acaulis L. O. Williams—Dorn 7961 (OS); #DQ531054. *P. acuminatus* Douglas ex Lindl.—Datwyler 3 (OS); #DQ534923, †DQ531206, §DQ531351. *P. alamosensis* Pennell & Nisbet—Wolfe 813 (OS); #DQ534924. *P. albertinis* Greene—Walker 261 (OS); #DQ531055, †DQ531207, §DQ531352. *P. albidus* Nutt.—Dorn 7949 (OS); #DQ531056, †DQ531208, §DQ531353. *P. albomarginatus* M. E. Jones—Anderson 97-15 (ASU); #DQ531057, †DQ531209, §DQ531354. *P. alpinus* Torr.—Thomson 96-6 (OS); #DQ534925, †DQ531210, §DQ531355. *P. ambiguus* Torr.—Wolfe 837 (OS); #DQ531058, †DQ531211, §DQ531356. *P. amphorellae* Crosswh.—Wolfe 825 (OS); #DQ534926, †DQ531212, §DQ531357. *P. anguineus* Eastw.—Wolfe 537 (OS); #DQ534927, †DQ531213, §DQ531358. *P. angustifolius* Nutt. ex Pursh—Dorn 7947 (OS); #DQ531059, †DQ531214, §DQ531359. *P. arenicola* A. Nels.—Pate and Porter 9002 (RM); #DQ534928, †DQ531215, §DQ531360. *P. aridus* Rydb.—Lutz *s.n.* (OS); #D1531060, †DQ531216, §DQ531361. *P. attenuatus* Dougl. ex Lindl.—Wolfe 914, Wolfe 637 (OS); #DQ531061, †DQ531217, §DQ531362. *P. auriberbis* Pennell—Wolfe 843 (OS); #DQ534929. *P. azureus* Benth.—Wolfe 514 (OS); #AF375178, †DQ531218, §DQ531363.
Penstemon baccharifolius Hook.—Wolfe 839 (OS); #DQ534930, †DQ531219, §DQ531364. *P. barbatus* (Cav.) Roth—Wolfe 509, Wolfe 778 (OS); #AF375177, †DQ531220, §DQ531365. *P. barrettiae* A. Gray—Wolfe 605 (OS); #AY195634, †DQ531221, §DQ531366. *P. bicolor* (Brandeg.) Clokey & Keck—Wolfe 795, Wolfe 796 (OS); #DQ531062, †DQ531222, §DQ531367. *P. breviculus* (Keck) Nisbet & R.C. Jackson—Arft 15-51 (COLO); #DQ531063, †DQ531223, §DQ531368.
Penstemon caesius A. Gray—Wilson 3482 (OS); #DQ531064, †DQ531224, §DQ531369. *P. caespitosus* var. *caespitosus* Nutt. ex A. Gray—Wolfe 782 (OS); #DQ531065, †DQ531225, §DQ531370. *P. caespitosus* var. *desertipicti* Nutt. ex A. Gray—Holmgren 12846 (NY); #DQ531066. *P. californicus* (Munz & Johnston) Keck—Wolfe 915 (OS); #DQ531067. *P. campanulatus* (Cav.) Willd.—Wolfe *s.n.* (OS); #DQ534931, †DQ531226, §DQ531371. *P. canescens* (Britt.) Britt.—Wolfe 854 (OS); #DQ534932. *P. cardwellii* T.J. Howell—Datwyler 11 (OS); #DQ531068, †DQ531227, §DQ531372. *P. carnosus* Pennell—Wolfe 757 (OS); #DQ531069, †DQ531228, §DQ531373. *P. caryi* Pennell—Lutz *Cl.*, Dorn 8005 (OS); #DQ531070, †DQ531229, §DQ531374. *P. centranthifolius* Benth.—Wilson 3521, Wolfe 308 (OS); #DQ531071, †DQ531230, §DQ531375.

P. clevelandii A. Gray—Wilson *s.n.* (OS); #DQ531072, †DQ531231, §DQ531376. *P. clutei* A. Nels.—Wolfe 810 (OS); #DQ531073, †DQ531232, §DQ531377. *P. cobaeva* Nutt.—Wolfe 838 (OS); #DQ534933, †DQ531233, §DQ531378. *P. comarrhenus* A. Gray—Wolfe 812 (OS); #DQ531074, †DQ531234, §DQ531379. *P. confertus* Dougl. ex Lindl.—Wolfe 635 (OS); #DQ534934, †DQ531235, §DQ531380. *P. confusus* M.E. Jones—Wolfe 458, Wolfe 748 (OS); #DQ531075, †DQ531236, §DQ531381. *P. crandallii* var. *atratus* Keck—Holmgren 12824 (NY); #DQ531076. *P. crandallii* var. *crandallii* A. Nels.—Thomson 96-20 (OS); #DQ531077, †DQ531237, §DQ531382. *Penstemon crandallii* var. *glabrascens* (Pennell) Keck—Wolfe 840 (OS); #DQ531078. *P. cyananthus* Hook.—Wolfe 672 (OS); #DQ534935, †DQ531238, §DQ531383. *P. cyaneus* Pennell—Datwyler 65 (OS); #DQ531079. *P. cyanocaulis* Payson—Wolfe 755 (OS); #DQ531080. *P. cyathophorus* Rydb.—Wolfe 774 (OS); #DQ534936.
Penstemon dasyphyllus A. Gray—Freeman 022 (UTEP); #DQ531081, †DQ531239, §DQ531384. *P. davidsonii* Greene—Cultivated, *s.n.*, Datwyler 37 (OS); #AY195637, †DQ531240, §DQ531385. *P. debilis* O'Kane & J. Anderson—McMullen *s.n.* (OS); #AF375180, †DQ531241, §DQ531386. *P. deustus* var. *suffrutescens* L. Henderson—Wilson 3551 (OS); #DQ531082. *P. deustus* var. *variabilis* (Suksdorf) Cronq.—Wolfe 628 (OS); #AF375182, †DQ531242, §DQ531387. *P. digitalis* Nutt. ex Sims—Lindgren *s.n.* (NEB); #DQ531083, †DQ531243, §DQ531388. *P. dissectus* Ell.—Leege *s.n.* (OS); #DQ534937, †DQ531244, §DQ531389. *P. dolius* M.E. Jones ex Pennell—Wolfe 818 (OS); #DQ531084, †DQ531245, §DQ531390.
Penstemon eatonii Gray—Wolfe 508, Holmgren 12841 (OS, NY); #DQ534938, †DQ531246, §DQ531391. *P. ellipticus* Coult. & Fisher—Datwyler 47 (OS); #AF375168, †DQ531247, §DQ531392. *P. eriantherus* var. *cleburnei* (M.E. Jones) Dorn—Hartman 20034 (RM); #DQ531085, †DQ531249, §DQ531394. *P. eriantherus* var. *eriantherus* Pursh—Dorn 7952, Datwyler 64 (OS); #DQ531086, †DQ531248, §DQ531393. *P. euglaucus* English—Wolfe 613 (OS); #DQ531087.
Penstemon fasciculatus A. Gray—Wilson 3590 (OS); #DQ531088. *P. fendleri* Torr. & A. Gray—Wolfe 836 (OS); #DQ531089, †DQ531250, §DQ531395. *P. floridus* Brandeg.—Aldridge and Crandall *s.n.* (OS); #DQ531090. *P. fremontii* Torr. & A. Gray ex A. Gray—Wolfe 788 (OS); #DQ531091, †DQ531251, §DQ531396. *P. fruticiformis* Coville—Wolfe 819 (OS); #DQ531092, †DQ531252, §DQ531397. *P. fruticosus* (Pursh) Greene—Datwyler 29, Datwyler 35 (OS); #AF375171, †DQ531253, §DQ531398.
Penstemon gairdneri Hook.—Wolfe 925 (OS); #DQ531093. *P. gentianoides* (Humboldt, Bonpland & Kunth) Poir.—James 99.0017, Wilson 3606 (OS); #DQ531094, †DQ531254, §DQ531399. *P. gentryi* Standl.—Wilson 3598 (OS); #DQ531095, †DQ531255, §DQ531400. *P. gibbensii* Dorn—Dorn 7953 (OS); #DQ531096. *P. glaber* Pursh—Dorn 8032 (OS); #DQ531097. *P. glandulosus* Dougl.—Wolfe 639 (OS); #DQ531098. *P. globosus* (Piper) Pennell & Keck—Wolfe 643 (OS); #DQ531099, †DQ531256, §DQ531401. *P. gormanii* Greene—Armbruster *s.n.* (ALA); #DQ531100, †DQ531257, §DQ531402. *P. gracilentus* Gray—Wilson 3542 (OS); #DQ531101, †DQ531258, §DQ531403. *P. gracilis* Nutt.—Wolfe 830 (OS); #DQ531102. *P. grandiflorus* Nutt.—Wolfe 852 (OS); #DQ534939. *P. griffinii* A. Nels.—Pate and Porter 10299 (RM); #DQ531103, †DQ531259, §DQ531404. *P. grinnellii* Eastw.—Wolfe 306, Wolfe 299 (OS); #DQ531104, †DQ531260, §DQ531405.
Penstemon harbourii Gray—Thomson *s.n.* (OS); #DQ531105, †DQ531261, §DQ531406. *P. harringtonii* Penl.—Wolfe 784 (OS); #DQ534940, †DQ531262, §DQ531407. *P. hartwegii* Benth.—Wolfe *s.n.* (OS); #DQ531106, †DQ531263, §DQ531408. *P. havardii* Gray—Wolfe 849 (OS); #DQ531107, †DQ531264, §DQ531409. *P. haydenii*

- S. Wats.—*Stubbendeck s.n.* (OS); #DQ531108. *P. heterodoxus* Gray—*Wilson 3502* (OS); #DQ531109. *P. heterophyllus* var. *heterophyllus* Lindl.—*Wilson 3454* (OS); #DQ531110, †DQ531265, §DQ531410. *P. heterophyllus* var. *purdyi* Keck—*Wolfe 574* (OS); #AF375181. *P. hirsutus* (L.) Willd.—*Wolfe s.n.* (OS); #DQ531111, †DQ531266, §DQ531411. *P. humilis* Nutt. ex Gray—*Datwyler 4* (OS); #DQ531112, †DQ531267, §DQ531412.
- Penstemon immanifestus* N. Holmgren—*Wolfe 785* (OS); #DQ531113. *P. incertus* Brandeg.—*Wolfe 465*, *Valenzuela s.n.* (OS); #DQ534941, †DQ531268, §DQ531413. *P. inflatus* Crosswhite—*Wolfe 811* (OS); #DQ531114, †DQ531269, §DQ531414. *P. isophyllous* Robinson—*James 3* (OS); #DQ531115, †DQ531270, §DQ531415.
- Penstemon jamesii* Benth.—*Wolfe 807* (OS); #DQ531116, †DQ531271, §DQ531416.
- Penstemon kunthii* G. Don—*Wolfe 834*, *Wilson 3594* (OS); #DQ531117, †DQ531272, §DQ531417.
- Penstemon labrosus* (Gray) Hook. f.—*Hogue 87.7* (OS); #DQ531118, †DQ531273, §DQ531418. *P. laetus* var. *laetus* Gray—*Wolfe 665* (OS); #DQ531119. *P. laetus* var. *saggitatus* Keck—*Wilson 3550* (OS); #DQ531120, †DQ531274, §DQ531419. *Penstemon lanceolatus* Benth.—*Wilson 3608* (OS); #DQ534942, †DQ531275, §DQ531420. *P. laricifolius* Hook. & Arn.—*Thomson 96-12*, *Dorn 8004* (OS); #DQ531121, †DQ531276, §DQ531421. *P. laxiflorus* Pennell—*Freeman 263* (UTE); #DQ534943. *P. lemhiensis* (Keck) Keck & Cronq.—*Crofts s.n.* (RM); #DQ531122, †DQ531277, §DQ531422. *P. lentus* Pennell—*Wilson 3453* (OS); #DQ531123, †DQ531278, §DQ531423. *P. leonardii* Rydb.—*Wolfe 760* (OS); #DQ531124, †DQ531279, §DQ531424. *P. leonensis* Straw—*James 99.02* (OS); #DQ531125, †DQ531280, §DQ531425. *P. linarioides* Gray—*Lindgren s.n.* (NEB); #DQ531126, †DQ531281, §DQ531426. *P. lyallii* (Gray) Gray—*Datwyler 46*, *Datwyler 44* (OS); #DQ531127, †DQ531282, §DQ531427.
- Penstemon mensarum* Pennell—*Thomson 96-17* (OS); #DQ531128, †DQ531283, §DQ531428. *P. moffatii* Eastw.—*Stermitz 515* (CMML); #DQ531129. *P. montanus* Greene—*Datwyler 51* (OS); #AF375169, †DQ531284, §DQ531429. *P. multiflorus* Chapman ex Benth.—*Wolfe s.n.* (OS); #DQ531130, †DQ531285, §DQ531430.
- Penstemon neomexicanus* Woot. & Standl—*Wolfe 828* (OS); #DQ531131, †DQ531286, §DQ531431. *P. neotericus* Keck—*Aldridge and Crandall s.n.* (OS); #DQ531132, †DQ531287, §DQ531432. *P. newberryi* Gray—*Wilson 3484* (OS); #DQ531133, †DQ531288, §DQ531433. *P. nitidus* Dougl. ex Benth.—*Dorn 7935* (OS); #DQ531134, †DQ531289, §DQ531434.
- Penstemon oklahomensis* Pennell—*Elisens s.n.* (OKL); #DQ531135, †DQ531290, §DQ531435. *P. ophianthus* Pennell—*Wolfe 835* (OS); #DQ531136, †DQ531291, §DQ531436. *P. osterhoutii* Pennell—*Wolfe 781* (OS); #DQ531137. *P. ovatus* Dougl. ex Hook.—*Wolfe 608* (OS); #DQ531138, †DQ531292, §DQ531437.
- Penstemon pachyphyllus* var. *mucronatus* (N. Holmgren) Neese—*Stermitz s.n.* (CMML); #DQ531140. *P. pachyphyllus* var. *pachyphyllus* Gray ex Rydb.—*Arft 1-32* (COLO); #DQ531139, †DQ531293, §DQ531438. *P. pallidus* Small—*Crawford s.n.* (OS); #DQ531141. *P. palmeri* Gray—*Wolfe 747* (OS); #DQ531142, †DQ531294, §DQ531439. *P. parryi* (Gray) Gray—*Wolfe s.n.* (OS); #DQ531143, †DQ531295, §DQ531440. *P. parvulus* (Gray) Krautter—*Wolfe 584* (OS); #DQ531144. *P. patens* (M.E. Jones) N. Holmgren—*Aldridge and Crandall s.n.* (OS); #DQ531145. *P. payettensis* A. Nels. & J.F. Macbr.—*Wolfe 644* (OS); #DQ531146. *P. paysoniorum* Keck—*Dorn 8006* (OS); #DQ531147, †DQ531296, §DQ531441. *P. peckii* Pennell—*Wolfe 617* (OS); #AF375174, †DQ531297, §DQ531442. *P. pennellianus* Keck—*Wolfe 923* (OS); #DQ531148. *P. perfoliatus* Brongniart—*Wolfe 832* (OS); #DQ531149, †DQ531298, §DQ531443. *P. perpulcher* A. Nels.—*Wolfe 822* (OS); #DQ531150, †DQ531299, §DQ531444. *P. personatus* Keck—*Edwards & Carter s.n.* (OKL); #AF375184, †DQ531300, §DQ531445. *P. pinifolius* Greene—*Wolfe 609*, *Wolfe 743* (OS); #AF375175, †DQ531301, §DQ531446. *P. procerus* var. *formosa* (A. Nels.) Cronq.—*Wolfe 648* (OS); #DQ531151. *P. procerus* var. *procerus* Dougl. ex Graham—*Wolfe 616* (OS); #DQ531152. *P. procerus* var. *tolmei* (Hook.) Cronq.—*Olmstead s.n.* (WTU); #DQ531153, †DQ531302, §DQ531447. *P. pseudoparvus* Crosswhite—*Freeman 166* (UTE); #DQ531154. *P. pseudoparvus* (Crosswhite) N. Holmgren—*Wolfe 823* (OS); #DQ531155, †DQ531303, §DQ531448. *P. pseudospectabilis* M.E. Jones—*Wolfe s.n.* (OS); #DQ531156, †DQ531304, §DQ531449.
- Penstemon radicosus* A. Nels.—*Dorn 7962* (OS); #DQ531157, †DQ531305, §DQ531450. *P. ramosus* Crosswhite—*Freeman 024* (UTE); #DQ531158, †DQ531306, §DQ531451. *P. rattanii* Gray—*Wolfe 512* (OS); #DQ531159, †DQ531307, §DQ531452. *P. retrorsus* Payson ex Pennell—*Arft 9-51* (COLO); #DQ531160, †DQ531308, §DQ531453. *P. richardsonii* Dougl. ex Lindl.—*Datwyler 41*, *Wolfe 626* (OS); #DQ534944, †DQ531309, §DQ531454. *P. roezlii* Regel—*Wilson 3539* (OS); #DQ531161, †DQ531310, §DQ531455. *P. rostriflorus* Kellogg—*Walker 255*, *Wilson 3491* (OS); #AF375176, †DQ531311, §DQ531456. *P. rubicundus* Keck—*Wolfe 853* (OS); #DQ531162, †DQ531312, §DQ531457. *P. rupicola* (Piper) T.J. Howell—*Datwyler 14* (OS); #AF375172, †DQ531313, §DQ531458. *P. rydbergii* var. *oreocharis* (Greene) N. Holmgren—*Wilson 3503* (OS); #DQ531163, †DQ531314, §DQ531459. *P. rydbergii* var. *rydbergii* A. Nels.—*Thomson 96-16* (OS); #DQ531164.
- Penstemon saxosorum* Pennell—*Thomson 96-13* (OS); #DQ531165, †DQ531315, §DQ531460. *P. scapoides* Keck—*Aldridge and Campell s.n.* (OS); #DQ531166, †DQ531316, §DQ531461. *P. scariosus* var. *albiflavus* (England) N. Holmgren—*Arft 3-5* (COLO); #DQ531167, †DQ531317, §DQ531462. *P. scariosus* var. *garrettii* (Pennell) N. Holmgren—*Dorn 7988* (OS); #DQ531168. *P. secundiflorus* Benth.—*Thomson 96-3* (OS); #DQ531169, †DQ531318, §DQ531463. *P. serrulatus* Menzies ex Sm.—*Wolfe 610* (OS); #DQ531170, †DQ531319, §DQ531464. *P. smallii* Heller—*Lindgren 8* (NEB); #DQ531171, †DQ531320, §DQ531465. *P. spatulatus* Pennell—*Wolfe 647* (OS); #DQ531172, †DQ531321, §DQ531466. *P. speciosus* Dougl. ex Lindl.—*Wolfe 583*, *Wilson 3486* (OS); #AF375179, †DQ531322, §DQ531467. *P. spectabilis* Thurb. ex Gray—*Wolfe 323* (OS); #DQ531173. *P. strictiformis* Rydb.—*Wolfe 848* (OS); #DQ531174, †DQ531323, §DQ531468. *P. strictus* Benth.—*Lindgren 9* (NEB); #DQ531175, †DQ531324, §DQ531469. *P. subglaber* Rydb.—*Wolfe 779* (OS); #DQ531176. *P. subseratus* Pennell—*Wolfe 590*, *Wolfe 593* (OS); #DQ531177, †DQ531325, §DQ531470. *P. superbus* A. Nels.—*Wolfe s.n.* (OS); #DQ531178.
- Penstemon tenuis* Small—*Wolfe 833* (OS); #DQ531179, †DQ531326, §DQ531471. *P. teucroides* Greene—*Thomson 96-8* (OS); #DQ531180, †DQ531327, §DQ531472. *P. thompsoniae* (Gray) Rydb.—*Wolfe 821* (OS); #DQ531181, †DQ531328, §DQ531473. *P. thurberi* Torr.—*Wolfe 815* (OS); #DQ531182, †DQ531329, §DQ531474. *P. triphyllus* Dougl. ex Lindl.—*Wolfe 651* (OS); #DQ531183, †DQ531330, §DQ531475. *P. tubaeiflorus* Nutt.—*Freeman 104* (UTE); #DQ531184.
- Penstemon venustus* Dougl. ex Lindl.—*Wolfe 638* (OS); #DQ531185. *P. versicolor* Pennell—*Stermitz 527A* (CMML); #DQ531186, †DQ531331, §DQ531476. *P. virens* Pennell ex Rydb.—*Wolfe 770* (OS); #DQ531187, †DQ531332, §DQ531477. *P. virgatus* var. *asagrayii* (Crosswhite) Dorn—*Dorn 8057* (OS); #DQ534945, †DQ531333, §DQ531478. *P. virgatus* var. *virgatus* Gray—*Wolfe 829* (OS); #DQ531188.
- Penstemon watsonii* Gray—*Wolfe 786* (OS); #DQ531189, †DQ531334, §DQ531479. *P. whippleanus* Gray—*Ranker 1739* (COLO); #DQ531190, †DQ531335, §DQ531480. *P. wilcoxii* Rydb.—*Wolfe 645* (OS); #DQ531191, †DQ531336, §DQ531481. *P. wrightii* Hook.—*Wolfe 824* (OS); #DQ531192, †DQ531337, §DQ531482.
- Russelia equisetiformis* Schlecht. & Cham.—*Armstrong 1074* (ISU); #AF375152, †DQ531195, §DQ531340.
- Tetranema mexicanum* Benth.—*Wolfe s.n.* (OS); #AF375151, †DQ531196, §DQ531341.
- Uroskinnera hirtiflora* Hemsl. & R.E. Schult.—*Tenorio 12747* (TEX); #DQ534922.