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Morphological Evolution and Systematics of *Synthyris* and *Besseya* (Veronicaceae): A Phylogenetic Analysis

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ABSTRACT. Phylogenetic analyses are used to examine the morphological diversity and systematics of *Synthyris* and *Besseya*. The placement of *Synthyris* and *Besseya* in Veronicaceae is strongly supported in parsimony analyses of nuclear ribosomal ITS DNA sequences. Parsimony and maximum likelihood (ML) criteria provide consistent hypotheses of clades of *Synthyris* and *Besseya* based on the ITS data. The combination of morphological characters and ITS data resolve additional clades of *Synthyris* and *Besseya*. The results show that *Synthyris* is paraphyletic to *Besseya*. In the monophyletic *Synthyris* clade, *Besseya* forms part of a Northwest clade that also includes the alpine *S. canbyi*, *S. dissecta*, and *S. lanuginosa* and mesic forest *S. cordata*, *S. reniformis*, *S. platycarpa*, and *S. schizantha*. The Northwest clade is the sister of *S. borealis*. An Intermountain clade, comprising *S. ranunculina*, *S. laciniata*, *S. pinnatifida*, and *S. missurica*, is the sister to the rest of the *Synthyris* clade. Constraint topologies are used to test prior hypotheses of relationships and morphological similarities. Parametric bootstrapping is used to compare the likelihood values of the best trees obtained in searches under constraints to that of the best tree found without constraints. These results indicate that topologies in which a monophyletic *Synthyris* is the sister of *Besseya* are significantly worse than the best ML tree in which *Synthyris* is paraphyletic to *Besseya*. Similarly, forcing either the monophyly of all taxa that have deeply incised leaf margins or those that have reniform laminas and broadly rounded apices results in trees that are significantly worse than the best ML tree, in which leaf margin incision and reniform laminas are homoplastic. We propose a new classification for *Synthyris* that emphasizes monophyletic groups. The new combination *Synthyris oblongifolia* is proposed.

Synthyris Benth. and *Besseya* Rydb. are North American members of the *Veronica* L. alliance (Scrophulariaceae tribe Veroniceae or Veronicaceae sensu Olmstead et al. 2001). They are rhizomatous perennials that form rosettes of foliage leaves and axillary, racemose inflorescences annually (Hufford 1992a, b). All *Synthyris* and *Besseya* are distributed in western North America, except for the disjunct *B. bullii* that occurs in north central United States (Pennell 1933). *Synthyris borealis*, which is restricted to unglaciated regions of the Yukon and Alaska (Hultén 1937), is also disjunct from other *Synthyris* and *Besseya* (Pennell 1933).

The systematics of *Synthyris* and *Besseya* has received considerable attention, including three taxonomic revisions in the 20th century. Initially, taxa recognized today as *Synthyris* and *Besseya* were discussed primarily in terms of alternative generic placements and circumscriptions. Bentham (1846) originally circumscribed *Synthyris* as a group of four species that had been placed in *Gymnandra* Pall. (*G. bullii* Eaton, *G. rubra* Douglas), *Veronica* (*V. plantaginea* James), and *Wulfenia* Jacq. (*W. reniformis* Douglas). Greene (1894) preferred to include this group of species in *Wulfenia*. Rydberg (1903) characterized *Synthyris* s. str. as having a corolla like that of *Veronica* and segregated the genus *Besseya*, which he characterized as possessing ''an altogether different corolla, cleft to near the base into two distinct lips'' (p. 278). Rydberg (1903) included also in *Besseya* a group of species (*B. gymnocarpa*, *B. rubra*, and *B. wyomingensis*) that he observed to lack a corolla. Nieuwland (1914, p. 188) regarded presence/absence of a corolla as ''a character deserving generic consideration'' and segregated *B. gymnocarpa*, *B. rubra*, and *B. wyomingensis* as the new genus *Lunellia* Nieuwl.

In the first comprehensive revision of this complex, Pennell (1933) recognized *Wulfenia* as Eurasian and distinct from the North American *Synthyris-Besseya* complex, which he emphasized has flowers and fruits like those of *Veronica*. His approach to generic circumscription largely followed Rydberg's proposal in recognizing *Synthyris* (14 species) and *Besseya* (8 species; including *Lunellia* as a subgenus). Subsequent revisions of the complex, which have contributed to our understanding of character state variation and species circumscriptions, have continued to argue for the recognition of both *Synthyris* and *Besseya* (Hedglin 1959; Schaack 1983). When framed as an evolutionary hypothesis, however, both Pennell (1933) and Schaack (1983) suggested that *Besseya* was derived from *Synthyris*. Our understanding of evolutionary relationships is based on shared-derived features. Hufford (1993) noted that *Synthyris* lacks derived features independent of those it shares with *Besseya*; thus, a provisional hypothesis of their monophyly is warranted. An important objective of phylogenetic systematics is to identify monophyletic groups in classification and to provide revisions that reflect our understanding of monophyly. If *Synthyris* is paraphyletic to *Besseya*, then taxonomic revision to recognize monophyletic groups is warranted. The provisional hypothesis that *Synthyris* and *Besseya* are monophyletic requires testing in a phylogenetic analysis that includes their close relatives.

Synthyris and *Besseya* have consistently been allied to *Veronica* and its relatives, a group recognized as the tribe Veroniceae (Bentham 1846; Pennell 1933, 1935; Thieret 1955). The first explicit evolutionary hypotheses for the relationships of *Synthyris* and *Besseya* were presented by Pennell (1933). He suggested an evolutionary sequence from *Picrorhiza* Royle ex Benth. to *Wulfenia* and, subsequently, to *Veronica* (and the related *Veronicastrum* Heist. ex Fabr. and *Hebe* Comm. ex Juss.; Pennell 1921). He questioned whether *Synthyris* and *Besseya* were more closely related to the more primitive *Picrorhiza* and *Wulfenia* or to *Veronica*; he argued that the closer evolutionary relationship was to the latter. Yamazaki (1957) illustrated *Synthyris* and *Besseya* as equally closely related to *Veronica* and a *Hebe-Detzneria* Schlt. ex Diels clade. Went (1958) suggested that *Synthyris* and *Besseya* were derived directly from New World *Veronica*. In a phylogenetic study of Veroniceae, Hong (1984) placed *Synthyris* as the sister of *Besseya*, and their clade was placed in his *Veronica* group as the sister of a clade comprising *Pseudolysimachion* Opiz, *Veronica*, *Oligospermum* D. Y. Hong (= *Veronica* sect. *Diplophyllum* (Lehm.) Walp.), and *Cochlidosperma* (= *Veronica* sect. *Diplophyllum* subsect. *Cymbalariae* (Benth.) Elenevskij). Hong (1984) recognized the paraphyly of *Veronica* and the problems it can create for a taxonomy based on monophyly. Kampny and Dengler (1997) found *Synthyris* and *Besseya* to be the sister clade of *Veronica*, but their phylogenetic analysis sampled few Veroniceae. Albach and Chase (2001) used sequence data from the internal transcribed spacers (ITS) of nuclear ribosomal DNA to infer phylogenetic relationships in Veroniceae. Their results placed *Synthyris* (*Besseya* was not sampled) as the sister of a clade that included species of *Veronica* and the *Hebe* complex, which bears a striking similarity to the earlier suggestion of Yamazaki (1957). In order to challenge existing hypotheses that *Synthyris* and *Besseya* are monophyletic, selected species of both genera are included here in a phylogenetic analysis of broadly sampled members of Veroniceae that represent major clades found by Wagstaff and Garnock-Jones (1998; Wagstaff et al. 2002) and Albach and Chase (2001).

Synthyris has not been the subject of previous phylogenetic analysis. Hedglin (1959, p. 5) recognized ''three major lines of evolution'' in *Synthyris*, including: 1) *S. reniformis* (including *S. cordata*); 2) *S. schizantha* and *S. platycarpa*, and 3) the other species of the genus. Schaack (1983) proposed detailed phylogenetic scenarios for *Synthyris* and *Besseya*. Hufford (1993) provided a phylogenetic analysis of *Besseya* based on morphological data. The clades found in that analysis had limited robustness; for example, none had a bootstrap proportion greater than 90%. The most parsimonious trees identified a grade at the base of *Besseya* comprising *B. bullii*, *B. rubra*, and *B. wyomingensis*. Trees in which *B. rubra* and *B. wyomingensis* were constrained to be monophyletic (Nieuwland's *Lunellia* and Pennell's subgenus *Lunellia*) were only two steps longer than the most parsimonious. Results of that analysis also identified a monophyletic Southern Rocky Mountain clade that consisted of *B. alpina*, *B. oblongifolia*, *B. plantaginea*, and *B. ritteriana*.

In addition to challenging the hypothesis of monophyly for *Synthyris* and *Besseya* in a broad phylogenetic analysis of Veroniceae, we provide a separate analysis to examine support for major clades and sister species in the two genera, in which we have sampled extensively among species. We examine previous evolutionary hypotheses, especially those for morphological characters. Finally, we include a taxonomy for the *Synthyris-Besseya* complex that emphasizes monophyly, as inferred from our results.

MATERIALS AND METHODS

Taxon Sampling. We examined first whether *Synthyris* and *Besseya* are monophyletic, which required a broad sampling of Veroniceae to test for alternative sister group relationships for the two genera. We used the earlier systematic and phylogenetic studies of Pennell (1921, 1933), Thieret (1955), Hong (1984), Wagstaff and Garnock-Jones (1998), Albach and Chase (2001), and Wagstaff et al. (2002) to guide taxon sampling. We included multiple exemplars from the *Veronica* I-IV clades found by Albach and Chase (2001). We used Wagstaff et al. (2002) to select multiple taxa from the *Hebe*, *Heliohebe* Garn.-Jones, *Parahebe* W.R.B. Oliv., *Chionohebe* B.G. Briggs & Ehrend. A, *Chionohebe* B, *Leonohebe* Heads, and *Derwentia* Raf. s.l. clades identified in their results. For this broad analysis, we sampled five species of both *Synthyris* and *Besseya*. These selected species of *Synthyris* and *Besseya* encompassed the subgeneric groups recognized by Pennell (1933) and Schaack (1983) as well as most of the geographic range and morphological diversity of the two genera. As the outgroups for this broad analysis of Veroniceae, we applied *Aragoa abietina* Kunth, *Erinus alpinus* L., *Globularia salicina* Lam., and *Plantago lanceolata* L., which were placed close to Veroniceae in the phylogenetic studies of Albach and Chase (2001), Wagstaff et al. (2002), and Rønsted et al. (2002) and in the Veronicaceae of Olmstead et al. (2001).

Our second goal was to identify the major clades and sister species in *Synthyris* and *Besseya*. For parsimony analysis we sampled 20 accessions of *Besseya* and 18 accessions of *Synthyris* (Table 1) that encompassed the species recognized in the revisions of Pennell (1933), Hedglin (1959), and Schaack (1983). We sampled multiple accessions of several species of *Synthyris* and *Besseya* (Table 1) to assess the monophyly of species and to examine issues of circumscription raised in those revisions. As outgroups, we used seven other Veroniceae based on the results of our broad analysis of the tribe.

DNA Sequences. New ITS sequences were obtained for all accessions of *Synthyris* and *Besseya* (Table 1). Total DNA was extracted from either herbarium or silica dried specimens of leaves using a standard CTAB procedure (Doyle and Doyle 1987). The ITS region was amplified and sequenced using the primers Nnc18s10 and C26A using a ''touchdown'' profile: 4 min at 94°C, 5 cycles of 1 min at 94°C, 1 min at 52°C and 2 min at 72°C, decreasing the annealing temperature by one degree each cycle, followed by 30 cycles using a 48°C annealing temperature, and ending with a final extension of 5 min at 72°C.

The ITS sequences for Veroniceae other than *Synthyris* and *Besseya* as well as for outgroups outside of Veroniceae were obtained

TABLE 1. Accessions of *Synthyris* and *Besseya* sampled for ITS sequences, with the collection sampled (herbarium voucher or publication) and GenBank accession number.

Besseya alpina (A. Gray) Rydb. - *Hufford 242* (WS), AY483210; *Hufford 244* (WS), AY483179. ***B. bullii*** (Eaton) Rydb. - *Hufford 277* (WS), AY48320; *Hufford 335* (WS), AY483211; *Hufford 609* (WS), AY483184. ***B. oblongifolia*** Pennell - *Hufford 235* (WS), AY483180. ***B. plantaginea*** (E. James) Rydb. - *Hufford 234* (WS), AY483181; *Hufford 241* (WS), AY483208. ***B. ritteriana*** (Eastw.) Rydb. - *Hufford 237* (WS), AY483182. ***B. rubra*** (Douglas) Rydb. - *Hufford 298* (WS), AY483196; *Hufford 451* (WS), AY483201; *Hufford 1691* (WS), AY483183. ***B. wyomingensis*** (A. Nelson) Rydb. - *Hufford 282* (WS), AY483212; *Hufford 1909* (WS), AY483204; *Hufford 1936* (WS), AY483213; *Hufford 2168* (WS), AY483205; *Hufford 2194* (WS) (two individuals from population), AY483203, AY483206; *Hufford 2195* (WS), AY483207; *Hufford 2197* (WS), AY483214.

Synthyris borealis Pennell - *Moran 47* (ALA), AY483177; *Parker 1132* (ALA), AY483188. ***S. canbyi*** Pennell - *Gilbert 3* (MONTU), AY483185; *Stickney 1740* (MONTU), AY483186. ***S. cordata*** (A. Gray) A. Heller - *Schenk 269D* (OSC), AY483197. ***S. dissecta*** Rydb. - *Atwood 14179* (BRY), AY483189. ***S. laciniata*** Rydb. - *Atwood 16148* (BRY), AY483190. ***S. lanuginosa*** (Piper) Pennell and J. W. Thomps. - *McMahon 683* (WS), AY483202. ***S. missurica*** (Raf.) Pennell subsp. ***missurica*** - *Hufford 866* (WS), AY483199; *Hufford 1209* (WS), AY483178. ***S. missurica*** (Raf.) Pennell subsp. ***stellata*** (Pennell) Kartesz & Gandhi - *Hufford 3910* (WS), AY483200. ***S. missurica*** (Raf.) Pennell subsp. ***major*** (Hook.) Pennell - *Hufford 3901* (WS), AY483198. ***S. pinnatifida*** S. Watson - *Huber 1078* (BRY), AY483191; *Brasher and Bates 1907* (BRY), AY483187. ***S. platycarpa*** Gail & Pennell - *Daubenmire 6351* (WS), AY483192. ***S. ranunculina*** Pennell - *Knight 1955* (UNLV), AY483194. ***S. reniformis*** (Douglas) Bentham - *Hufford 435* (WS), AY483195. ***S. schizantha*** Piper - *Hufford 428* (WS), AY483193.

Aragoa abietina Kunth - Bello et al. (2002), AJ459404. ***Chionohebe densifolia*** (F. Muell.) B. G. Briggs & Ehrend. - Wagstaff and Garnock-Jones (1998), AY034849. ***C. thomsonii*** (Buchanan) B. G. Briggs & Ehrend. - Wagstaff and Garnock-Jones (2000), AF229039. ***Derwentia perfoliata*** (R. Br.) Raf. - Wagstaff and Garnock-Jones (2000), AY034850. ***Globularia salicina*** Lam. - Albach and Chase (2001), AF313039. ***Erinus alpinus*** L. - Albach and Chase (2001), AF313032. ***Hebe ciliolata*** (Hook. f.) Cockayne & Allen - Wagstaff et al. (2002), AY034851. ***H. cupressoides*** (Hook. f.) Cockayne & Allen - Wagstaff and Garnock-Jones (1998), AY037378. ***H. formosa*** (R. Br.) Cockayne - Wagstaff and Garnock-Jones (1998), AF037383. ***H. lycopodioides*** (Hook. f.) Cockayne & Allen - Wagstaff and Garnock-Jones (1998), AF037383. ***H. macrantha*** (Hook. f.) Cockayne & Allen - Wagstaff and Wardle (1999), AF069456. ***Heliohebe hulkeana*** (F. Muell.) Garn.-Jones - Wagstaff and Garnock-Jones (1998), AF037379. ***H. raoulii*** (Hook. f.) Garn.-Jones - Wagstaff and Garnock-Jones (1998), AF037380. ***Lagotis angustibracteata*** Tsoong & Yang - Albach and Chase (2001), AF313028. ***L. brachystachya Maxim. - Albach and Chase (2001), AF313027. Paederota lutea*** L. - Albach and Chase (2001), AF313024. ***Parahebe brevistylis*** (Garn.-Jones) Heads - Wagstaff and Garnock-Jones (2000), AF229045. ***H. lyallii*** (Hook. f.) W. R. B. Oliv. - Wagstaff and Garnock-Jones (1998), AF037395. ***H. planopetiolata*** (G. Simpson & J. S. Thomson) W. R. B. Oliv. - Wagstaff and Garnock-Jones (2000), AF229050. ***H. trifida*** W. R. B. Oliv. - Wagstaff and Garnock-Jones (1998), AF037376. ***H. vandewateri*** (Pennell) P. Royen - Wagstaff and Garnock-Jones (2000), AF229052. ***Plantago lanceolata*** L. - Rønsted et al. (2002), AY101898. ***Pseudolysimachion spicatum*** (L.) Opiz - Albach and Chase (2001), AF313022. ***P. dahuricum*** (Steven) T. Yamaz. - Albach and Chase (2001), AF313023. ***Veronica alpina*** L. - Albach and Chase (2001), AF313013. ***V. calycina*** R. Br. - Wagstaff et al. (2002), AY034863. ***V. chamaedrys*** L. - Albach and Chase (2001), AF313003. ***V. fruticulosa*** L. - Albach and Chase (2001), AF313004. ***V. gentianoides*** Vahl - Albach and Chase (2001), AF313018. ***V. montana*** L. - Albach and Chase (2001), AF313014. ***V. peregrina*** L. - Albach and Chase (2001), AF313016. ***V. persica*** Poir - Albach and Chase (2001), AF313001. ***V. saturejoides*** Vis. - Albach and Chase (2001), AF313005. ***Veronicastrum virginicum*** (L.) Farw. - Albach and Chase (2001), AF313030. ***V. stenostachyum*** (Helmsl.) T. Yamaz. - Albach and Chase (2001), AF313031. ***Wulfenia blechicii*** Lakusic - Albach and Chase (2001), AF313026. ***W. carinthiaca*** Jacq. - Albach and Chase (2001), AF313025.

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from GenBank (Table 1). Sequences were aligned manually in Se-Al (Rambaut 1996).

Morphological Characters. Twenty morphological characters (Tables 2, 3) were defined based on the examination of herbarium specimens and data from Hufford (1992a, 1992b, 1993). The Hufford (1992a, 1992b, 1993) data were obtained from dissections of fluid preserved specimens, leaf clearings, preparations of microtomed sections, and scanning electron microscopy.

Phylogenetic Analyses. Data matrices have been accessioned in TreeBASE (study accession number S1099, matrix accession numbers M1879–1881). All phylogenetic analyses were conducted using PAUP* 4.0 (Swofford 2002). Parsimony analyses used heuristic search procedures that included 1000 starting trees built by random taxon addition followed by TBR branch-swapping. All character state transitions were equally weighted. Indels were treated as missing data. Tree statistics and measures of homoplasy were calculated using PAUP* with uninformative characters removed. Multiple most parsimonious trees were combined in PAUP* to construct strict consensus cladograms. The robustness of clades was assessed using bootstrap analysis (Felsenstein 1985) implemented using heuristic procedures in PAUP*, including random taxon addition and TBR branch-swapping for 1000 pseudoreplicates in which Maxtrees was set at 50,000.

A maximum likelihood (ML) analysis was conducted on the ITS data set that included only five outgroup and all 38 ingroup taxa. *Lagotis brachystachya* and *Veronica montana* were excluded to reduce the number of outgroups and because they exhibited many autapomorphies in the parsimony analyses. Modeltest (Posada and Crandall 1998) was used to select among 56 alternative substitution models for maximum likelihood analyses. Models selected using likelihood ratio tests (LRTs) and the Akaike information criterion (AIC) were compared. To facilitate the likelihood tree search, we used the values for model parameters estimated during model selection. To evaluate sensitivity to these parameters, we conducted tree searches using only the models selected by the LRTs and the AIC, optimizing each parameter in the models. Additionally, we used the parameter values as estimated by Modeltest, sought an ML tree, re-estimated the parameter values on that tree, and sought again for a new ML tree. The first and second trees were compared for topology and branch lengths to further assess sensitivity to parameter values.

Alternative Phylogenetic Hypotheses. Hypotheses of taxonomic groups and taxon relationships can be modelled as cladogram topologies. We designed six constraint topologies based on hypotheses of relationships and morphological similarities (Table 4). The hypotheses modelled include the following: (1) *Synthyris* and *Besseya* are both monophyletic and are sister clades, as implied by the classifications of Pennell (1933) and Schaack (1983); (2) taxa that have deeply incised leaf margins, including *S. canbyi*, *S. dissecta*, *S. lanuginosa*, *S. pinnatifida*, are monophyletic; (3) *S. borealis*, *S. canbyi*, *S. dissecta*, *S. laciniata*, *S. lanuginosa*, *S. pinnatifida* are monophyletic as implied by Schaack's (1983) circumscription of

TABLE 2. Morphological characters and character states applied in the phylogenetic analyses of *Synthyris* and *Besseya.* Data and descriptions of character states are presented primarily in Hufford (1992a, 1992b, 1993), although selected data were derived from the examination of herbarium specimens and Pennell (1933), Went (1958), and Schaack (1983).

1. Lamina/petiole juncture: 0, obtuse angle; 1, same plane. This character refers to the angle formed by the lamina and petiole on the lower side of the leaf.
2. Leaf lamina shape: 0, ovate; 1, reniform. The definitions of lamina shapes follow Lawrence (1951).
3. Leaf lamina margin: 0, toothed (not incised to midrib); 1, incised to within 2 mm of midrib.
4. Leaf lamina teeth: 0, compound; 1, simple. Simple teeth have only a single lobe and adjacent teeth have lobes of a similar size and shape; whereas, compound teeth have primary lobe as well as secondary and sometimes tertiary lobes that are smaller and sometimes a different shape from the primary lobe.
5. Adaxial surface of leaf midvein: 0, level adaxial surface of leaf; 1, sunken below surface of leaf. In leaf cross sections, the midvein can be sunken below the plane of the adaxial surface of the lamina or largely flush with the surface (as illustrated in Hufford 1992b)
6. Size of lamina midvein: 0, less than/equal to 1.5%; 1, 2–3%; 2, greater than/equal to 4%. The size of the lamina midvein was measured as a percent of total lamina width at the middle of the lamina, following the definition of Hickey (1979).
7. Diameter of secondaries to midvein: 0, greater than/equal to 80%; 1, 25–80%; 2, less than/equal to 25. The diameters of the lowest secondary vein and the midvein were measured just above the point where they diverge.
8. Secondaries from midvein: 0, four or fewer; 1, six or more.
9. Extent of basal secondaries: 0, distal half of lamina; 1, proximal half of lamina. The basal secondary veins are those that diverged from the midvein in the petiole, and the character refers to whether they extend beyond the middle of the lamina or end in proximal half of the lamina.
10. Inflorescence attitude: 0, erect; 1, reclinate. Inflorescences of *S. cordata* and *S. reniformis* are lax and with the onset of fruiting lie on the surface of the substrate, which contrasts with the inflorescences of other *Synthyris* and *Besseya* that remain stiffly erect from initiation through fruiting.
11. Inflorescence bracts: 0, three or more; 1, two; 2, none. Inflorescences bear either three or more helically arranged bracts, two opposite or subopposite bracts, or no bracts.
12. Peduncle pubescence: 0, pilose-villous; 1, sparse-glabrous
13. Calyx/corolla length: 0, corolla well exserted; 1, corolla slightly exserted; 2, corolla not exserted. At anthesis, flowers can have a corolla in which the lobes are well exserted beyond the calyx, a corolla that is approximately the same length as the calyx and only slightly exserted, or, as in *B. rubra* and *B. wyomingensis,* a highly reduced corolla not exserted beyond the calyx.
14. Corolla throat: 0, open; 1, closed. At anthesis, the lobes of the corolla can either spread, creating an opening to the corolla throat, or they can remain largely erect and loosely imbricate or even tightly furled around the stamen filaments and style (= a closed throat).
15. Corolla lobe margins: 0, entire; 1, laciniate
16. Stamen insertion: 0, corolla tube; 1, receptacle. Stamens can be inserted either on corolla tube or on receptacle as shown in Hufford (1992a).
17. Anther color: 0, reddish purple-blue; 1, yellowish white
18. Fruit base shape: 0, rounded; 1, flat, perpendicular to pedicel; 2, flat, acutely angled to pedicel. Fruits vary in shape, especially in the proximal portion of the fruit, and this influences also the angle formed between the fruit base and the pedicel. Although character states were scored on basis of a survey of herbarium specimens, illustrations of fruit shapes are provided by Schaack (1983).
19. Fruit pubescence: 0, glabrous; 1, sparse or pilose only at margins; 2, villous. The definitions of pubescence states follow Lawrence (1951).
20. Seed number: 0, 6 or fewer; 1, 10–16; 2, 20–40

this group as *Synthyris* section *Dissecta*; (4) taxa that have reniform leaf laminas that have broadly rounded apices, including *S. laciniata*, *S. missurica*, *S. platycarpa*, *S. ranunculina*, and *S. schizantha*, are monophyletic; (5) *S. missurica* subsp. *major* is the sister of all other *Synthyris* and *Besseya*, which models Schaack's (1983) proposal that the most primitive extant species of *Synthyris* is *S. major* and that all other *Synthyris* and *Besseya* are derived from an ancestor most similar to it; and (6) *B. wyomingensis* is monophyletic.

Modelling each of these hypotheses as a cladogram (each with only a few nodes specified), we conducted six constrained ML searches, finding the optimal tree for our data for each alternative. For the constrained analyses, we used SYM+Γ, which was the less parameter-rich model of the two selected by Modeltest, because the unconstrained analyses demonstrated no differences between the results using either model. Likewise, we used the parameter values as originally estimated during model selection because we found no sensitivity to the model parameters in the unconstrained analyses. We also sought the most parsimonious trees under each constraint and compared the results with the likelihood tree searches, primarily to evaluate whether we could use difference in tree length (instead of difference in likelihood, a more consistent but less efficiently obtained measure) as a test statistic in hypothesis testing.

To evaluate the significance in the differences between trees obtained using constraints and the maximum likelihood tree, we conducted parametric bootstrapping (Huelsenbeck and Crandall 1997), also called the SOWH test (Goldman et al. 2000). This test is appropriate for comparing alternatives when one is selected a posteriori (Goldman et al. 2000), as is the ML tree. The null hypothesis is that the alternative tree (i.e., the most likely tree under the constraint) is correct; if so, then we would expect to see the difference in tree scores between the alternative tree and the ML tree frequently. To test this, we simulated data on the alternative tree, sought the best tree under the constraint and the best tree without the constraint, and compared their tree scores. In this way, we apply the same treatment to each simulated data as we did to our observed data. For each alternative tree, we did 500 such simulations. We used Mesquite (v. 0.994, Maddison and Maddison 2003) to simulate the data sets on the alternative trees. To construct the model for data simulation, ten values were estimated from the observed data, corresponding to the GTR+I+Γ model with unequal base frequencies.

TABLE 3. Matrix of morphological character states applied in phylogenetic analyses. Multiple accessions of species sampled for ITS sequence data had the same morphological states, except for *Besseya wyomingensis.* The character state coding for each sampled population of *B. wyomingensis* is provided.

	Characters																			
Taxa	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Synthyris borealis	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1
Synthyris canbyi	0	0	0/1	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	1
Synthyris cordata	0	0	0	0	1	0/1	0	0	0	1	2	1	0	0	0	0	0	1	2	0
Synthyris dissecta	0	0	1	0	0	2	1	0	1	0	0	0	0	0	0	0	0	0	2	1
Synthyris laciniata	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Synthyris lanuginosa	0	0	1	0	0	2	1	0	1	0	0	0	0	0	0	0	0	0	2	1
Synthyris major	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Synthyris missurica	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Synthyris pinnatifida	0	0	1	0	0	2	1	0	1	0	0	0	0	0	0	0	0	0	0	1
Synthyris platycarpa	0	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	2	0	0
Synthyris ranunculina	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1
Synthyris reniformis	0	0	0	0	1	0	0	0	0	1	2	1	0	0	0	0	0	1	1	0
Synthyris schizantha	0	1	0	0	1	0	0	0	0	0	1	0/1	0	0	1	0	0	1	0	0
Synthyris stellata	0	1	0	0	0	0	0	0	0	0	0	0/1	0	0	0	0	0	0	0	1
Besseya alpina	1	0	0	1	0	1	1	0	1	0	0	0	1	1	0	1	0	0	0/2	2
Besseya bullii	1	0	0	1	0	1	1	0	0	0	0	0	1	1	0	0	1	0	2	2
Besseya oblongifolia	1	0	0	1	0	2	2	1	1	0	0	0	1	1	0	1	0	0	2	2
Besseya plantaginea	1	0	0	1	0	2	2	1	1	0	0	0	1	1	0	1	0	0	0	2
Besseya ritteriana	1	0	0	1	0	2	2	1	1	0	0	0	1	1	0	1	1	0	0	2
Besseya rubra	1	0	0	1	0	1	1	0	0	0	0	0	2	?	0	?	0	0	2	2
Besseya wyomingensis 282	1	0	0	1	0	1	1	0	1	0	0	0	?	?	?	?	1	0	2	2
Besseya wyomingensis 1909	1	0	0	1	0	1	1	0	0	0	0	0	?	?	?	?	1	0	2	2
Besseya wyomingensis 1936	1	0	0	1	0	1	1	0	0	0	0	0	?	?	?	?	0	0	2	2
Besseya wyomingensis 2168	1	0	0	1	0	1	1	0	0	0	0	0	?	?	?	?	0	0	2	2
Besseya wyomingensis 2194	1	0	0	1	0	1	1	0	0	0	0	0	?	?	?	?	1	0	2	2
Besseya wyomingensis 2195	1	0	0	1	0	1	1	0	0	0	0	0	?	?	?	?	1	0	2	2
Besseya wyomingensis 2197	1	0	0	1	0	1	1	0	0	0	0	0	?	?	?	?	0	0	2	2

PAUP*4.0 (Swofford 2002) was used to search for the best constrained and unconstrained trees. For this step we used parsimony searches to make possible the evaluation of many data sets and several alternative hypotheses. Although not ideal, we accept the results because the ML tree is also a most-parsimonious tree using the observed data, and this is true for both the constrained and unconstrained analyses. For each alternative tree, we compared the observed difference in treescore (between constrained and unconstrained analyses) to the distribution of differences found using the 500 simulated data sets. The observed difference was considered significantly large if it was found to be higher than most of the values in the distribution. We report P-values (Table 4), calculated as the percentage of the distribution that was equal to or greater than the observed difference and consider a $P < 0.05$ as significant.

Character State Evolution. MacClade 4.0 (Maddison and Maddison 2000) was used to reconstruct the evolution of character states and examine alternatives on the topology from the analysis of the combined ITS and morphological data.

RESULTS

Monophyly of the Synthyris-Besseya Complex. Our parsimony analysis of a broadly sampled set of Veroniceae resulted in 918 trees of 1156 steps that were distributed among three islands (Fig. 1; consistency index [CI] = 0.4376; homoplasy index [HI] = 0.5624; retention index [RI] = 0.6657; rescaled consistency index [RC] = 0.3208). Strong support (bootstrap proportion [BS] = 100%) was found for the monophyly of *Synthyris* and *Besseya* (= *Synthyris* clade). *Synthyris* was found to be paraphyletic to *Besseya*. The *Synthyris* clade was placed in a polytomy with clades of *Veronica*, *Pseudolysimachion*, *Paederota* L., and the *Hebe* complex (sensu Albach and Chase 2001).

Clades of Synthyris and Besseya. The parsimony analysis of ITS data (including 20 accessions of *Besseya*, 18 accessions of *Synthyris*, and seven outgroups) resulted in 728 most parsimonious trees of 485 steps, all of which were in a single island (strict consensus in Fig. 2; phylogram in Fig. 3; CI = 0.5931; HI = 0.4069; RI = 0.7334; RC = 0.5021). The results of the unconstrained ML analysis of the data set with a more limited taxon sampling were entirely consistent with those from the parsimony analysis and are not illustrated.

The analysis of the combined ITS and morphological data resulted in 16 most parsimonious trees of 533 steps, all of which were in the same island (strict consensus in Fig. 4; CI = 0.5896; HI = 0.4104; RI 0.7642; RC = 0.5147). As described below, trees from the analysis of the combined ITS and morphological data resolved several sister group relationships that were not resolved by ITS alone.

The ITS data alone and in combination with morphological data produced trees that have a monophyletic *Besseya* nested in a paraphyletic *Synthyris*. For the

TABLE 4. Tree scores for optimal trees in the unconstrained search and the six constrained searches. All searches were conducted under maximum parsimony (MP) and maximum likelihood (ML); in every case the ML tree(s) was among the MP trees. Parentheses indicate clades specified for the constrained searches. If no subsp. or accession is listed, all were included. Differences are reported between the tree length of the optimal tree without constraints and the optimal tree with contraints. Significance of difference was established using parametric bootstrap with 500 replicate simulated data sets on each constrained tree.

Tree	-ln L	Treelength	Difference in tree length	P
ML, unconstrained	2850.51727	366		
ML, ((all Besseya), (all *Synthyris*))	2862.59981	371	5	0.004
ML, (*S. dissecta, S. canbyi, S. lanuginosa, S. pinnatifida*)	2895.81576	377	11	<0.002
ML, (*S. borealis, S. dissecta, S. canbyi, S. lanuginosa, S. pinnatifida, S. laciniata*)	2902.60284	377	11	<0.002
ML, (*S. missurica, S. platycarpa, S. schizantha, S. laciniata, S. ranunculina*)	2923.49867	383	17	<0.002
ML, (*S. missurica* subsp. *major* H3901, (all other *Synthyris* and *Besseya*))	2870.70848	375	9	<0.002
ML, (all *B. wyomingensis*)	2866.24584	370	4	<0.002

ITS data alone (Fig. 2), *Besseya* consisted of a polytomy of the following four clades: (1) *B. alpina* + *B. ritteriana*; (2) *B. oblongifolia* + *B. plantaginea*; (3) *B. rubra* + *B. wyomingensis* (blue stamen morph); and (4) *B. bullii* + *B. wyomingensis* (white stamen morph). The combination of ITS and morphological data resolved *B. alpina* + *B. ritteriana* as the sister of *B. oblongifolia* + *B. plantaginea* (= southern group). The analysis of the combined data also resolved a northern group in which *B. rubra* + *B. wyomingensis* (blue stamen morph) was the sister of *B. bullii* + *B. wyomingensis* (white stamen morph). The southern group was placed as the sister of the northern group (Fig. 4).

Multiple populations of most species of *Besseya* were sampled, and *B. alpina*, *B. bullii*, and *B. rubra* were found to be monophyletic. In contrast, the two sampled populations of *B. plantaginea* were paraphyletic to *B. oblongifolia*. *Besseya wyomingensis* consisted to two independent clades that correspond to differing floral color morphs, one characterized by blue to purple stamen filaments (= blue stamen morph) and the other by white to slightly yellowish stamen filaments (= white stamen morph). Blue stamen morph populations of *B. wyomingensis* form the sister of *B. rubra*. White stamen morph populations of *B. wyomingensis* are the sister of *B. bullii*. The independent color morph clades of *B. wyomingensis* had good character support (BS = 87% and 94% based on ITS alone).

Based on ITS data alone, *Besseya* formed a polytomy with the following three clades of *Synthyris*: (1) *S. canbyi* + *S. dissecta* + *S. lanuginosa* (= dissected leaf clade); (2) *S. platycarpa* + *S. schizantha* (= laciniate corolla clade); and (3) *S. cordata* + *S. reniformis* (= reclinate inflorescence clade). The monophyletic group comprising these clades of *Synthyris* as well as *Besseya* is here given the informal name 'Northwest clade.' Trees from the analysis of combined ITS and morphological data provided further resolution of relationships in the Northwest clade, including the placement of (1) *Besseya* as the sister of the dissected leaf clade; and (2) the laciniate corolla clade as the sister of the reclinate inflorescence clade. The placement of *S. lanuginosa* as the sister of *S. dissecta* + *S. canbyi* in the analysis based on ITS data alone was not resolved in the trees from the combined data, in which *S. lanuginosa*, *S. dissecta*, and *S. canbyi* were monophyletic but formed a polytomy. The Northwest clade was the sister of *S. borealis*.

The sister of the *S. borealis* + Northwest clade was a monophyletic group, consisting of *S. laciniata*, *S. pinnatifida*, *S. ranunculina*, and the *S. missurica* complex, designated the 'Intermountain clade.' The results based on ITS data alone and the combined data differ in the placement of *S. pinnatifida* relative to the rest of the Intermountain clade. The results from the combined analysis of ITS and morphology place *S. pinnatifida* as the sister of the rest of the Intermountain clade. This reflects the leaf character states that *S. pinnatifida* shares with members of the dissected leaf clade. *Synthyris pinnatifida* and the dissected leaf clade share a syndrome of states associated with leaves that have dissected laminas.

Alternative Phylogenetic Hypotheses. Models selected for the likelihood analyses were as follows: using the hierarchical LRTs, the SYM + Γ model (base frequencies equal, six rate matrix parameters, rate variation among sites) was selected as the least complex yet sufficient model; using the AIC, the model also required the parameter I (percentage of sites invariant). Each of six different tree searches [(1) optimizing model parameters, (2) using parameter values as per Modeltest, and (3) using parameter values estimated on best trees from (2), each run under SYM + Γ and SYM + I + Γ] resulted in the same ML topology, with very few small differences in branch lengths. Therefore, the SYM + Γ model with parameter values as estimated on the ML tree was used for the remainder of the analysis.

Constraining the tree searches according to the six

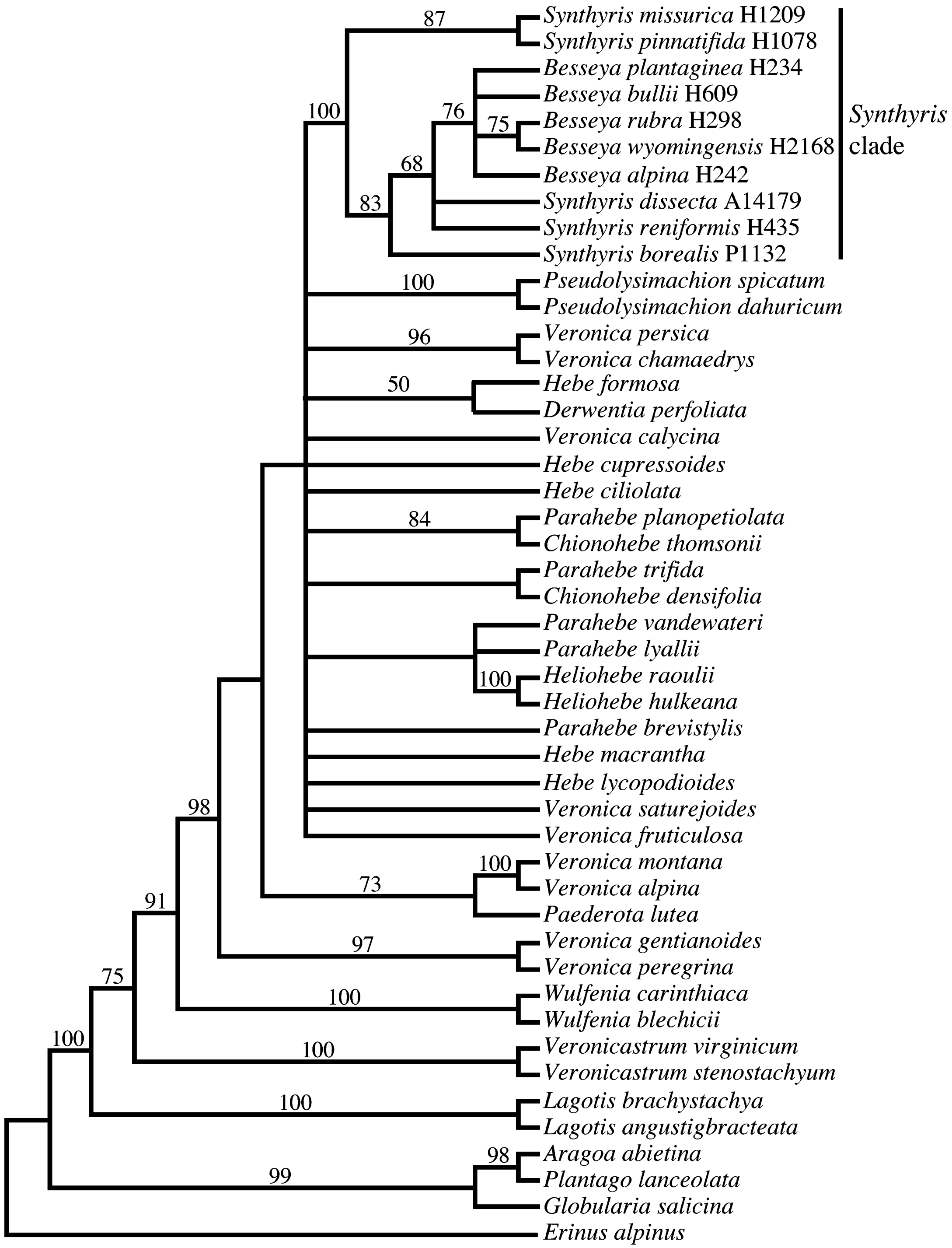

FIG. 1. Strict consensus of 918 most parsimonious cladograms from the broad phylogenetic analysis of Veroniceae based on ITS data. The monophyletic *Synthyris* clade, including taxa recognized as the genera *Synthyris* and *Besseya*, is indicated. Bootstrap proportions above 50% are above clades.

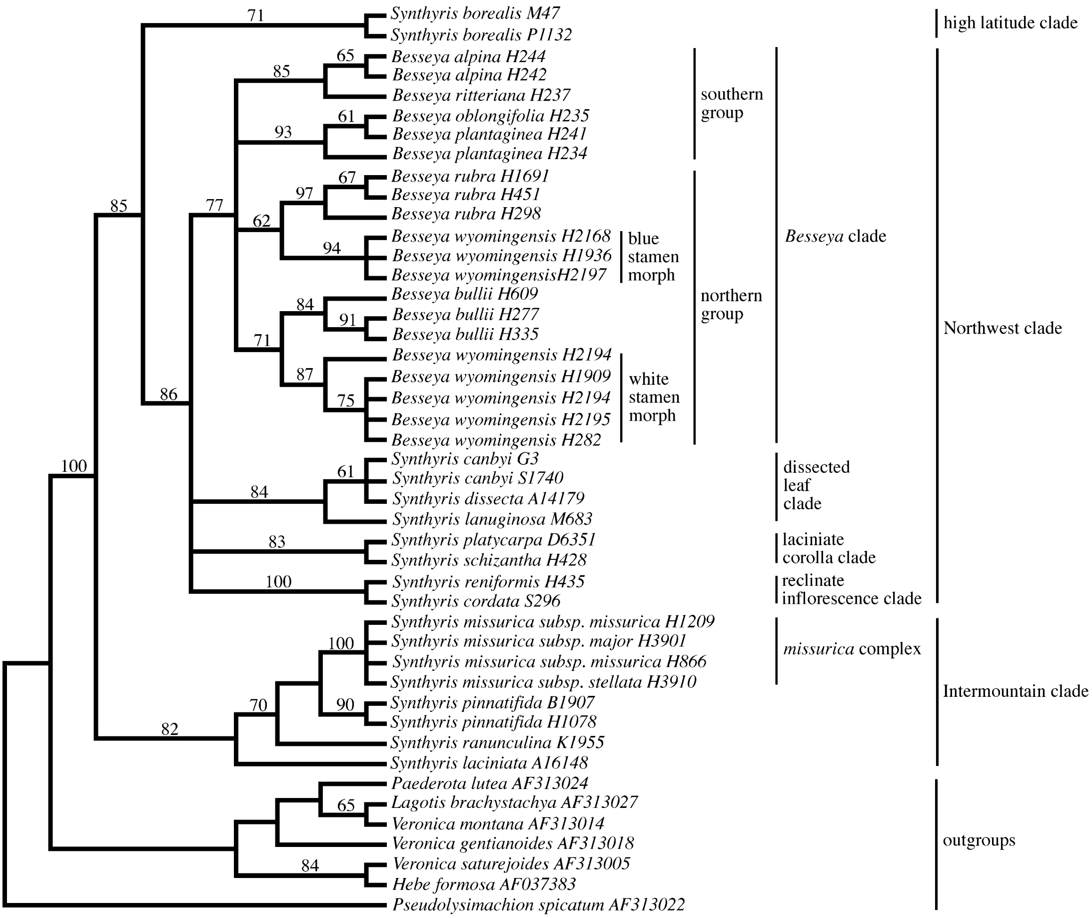

FIG. 2. Strict consensus of 728 most parsimonious cladograms of the *Synthyris* clade, including all species of *Synthyris* and *Besseya*, based on the parsimony analysis of ITS sequences. Bootstrap proportions above 50% are above clades. Informal names for clades are indicated. Accessions sampled are indicated after taxon names by the initials of collectors and collection numbers listed in Table 1.

alternative hypotheses resulted in trees that were less likely (and had longer treelength) than the tree obtained in the unconstrained search (Table 4). All of these trees were found to be significantly worse than the unconstrained optimal tree when we applied the parametric bootstrap (Table 4).

DISCUSSION

Relationship of Synthyris and Besseya. In a broad sampling of Veroniceae our phylogenetic results provide strong support for the monophyly of *Synthyris* and *Besseya* (= *Synthyris* clade) as suggested previously by Pennell (1933), Schaack (1983), Hufford (1993), and Kampny and Dengler (1997). Our *Synthyris* clade is consistent with the group, including elements originally described as *Gymnandra*, *Veronica*, and *Wulfenia*, first circumscribed by Bentham (1846) as *Synthyris*. The placement of the *Synthyris* clade in a polytomy with clades of *Veronica*, *Pseudolysimachion*, *Paederota*, and the *Hebe* complex was similar to the results of Hong (1984) and Albach and Chase (2001). This placement does not support the contention of Greene (1894) that species circumscribed by Bentham (1846) as *Synthyris* were better placed in *Wulfenia* (cf. Kampny 1995). A monophyletic *Wulfenia* diverges earlier in the diversification of Veroniceae than does the *Synthyris* clade in our results and in those of Kampny and Dengler (1997) and Albach and Chase (2001).

The results demonstrate that *Synthyris* is paraphyletic to *Besseya*, providing support for Pennell's (1933) and Schaack's (1983) suggestions that *Besseya* was derived from *Synthyris*. ML trees constrained to force *Besseya* to be the sister of a monophyletic *Synthyris* were significantly worse than the best unconstrained ML tree in our parametric bootstrap results (Table 4).

Synthyris missurica subsp. *major* was central to Schaack's (1983) evolutionary hypotheses for *Synthyris* and *Besseya*. He suggested that it was the most primi-

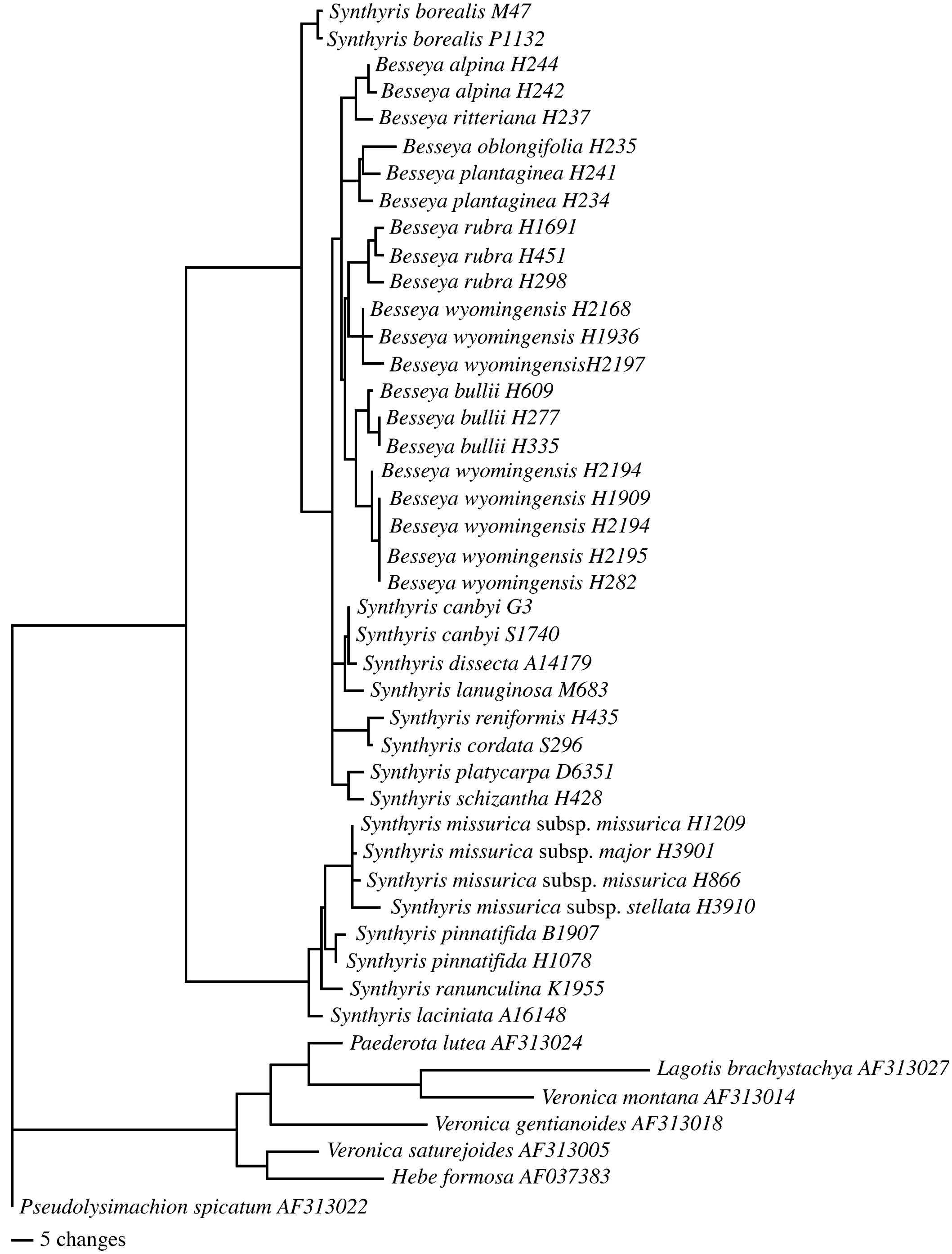

FIG. 3. Phylogram of one most parsimonious tree of the *Synthyris* clade, including all species of *Synthyris* and *Besseya*, from the parsimony analysis of ITS sequences. Accessions sampled are indicated after taxon names by the initials of collectors and collection numbers listed in Table 1.

tive extant *Synthyris* and not only several extant clades of *Synthyris* diverged from it but also *Besseya*. We examined this hypothesis by modelling it as a constraint topology in which *S. missurica* subsp. *major* was placed as the sister of all other *Synthyris* and *Besseya*. The parametric bootstrap analysis demonstrated that the best ML tree under this constraint was significantly worse than the unconstrained ML (Table 4). In contrast

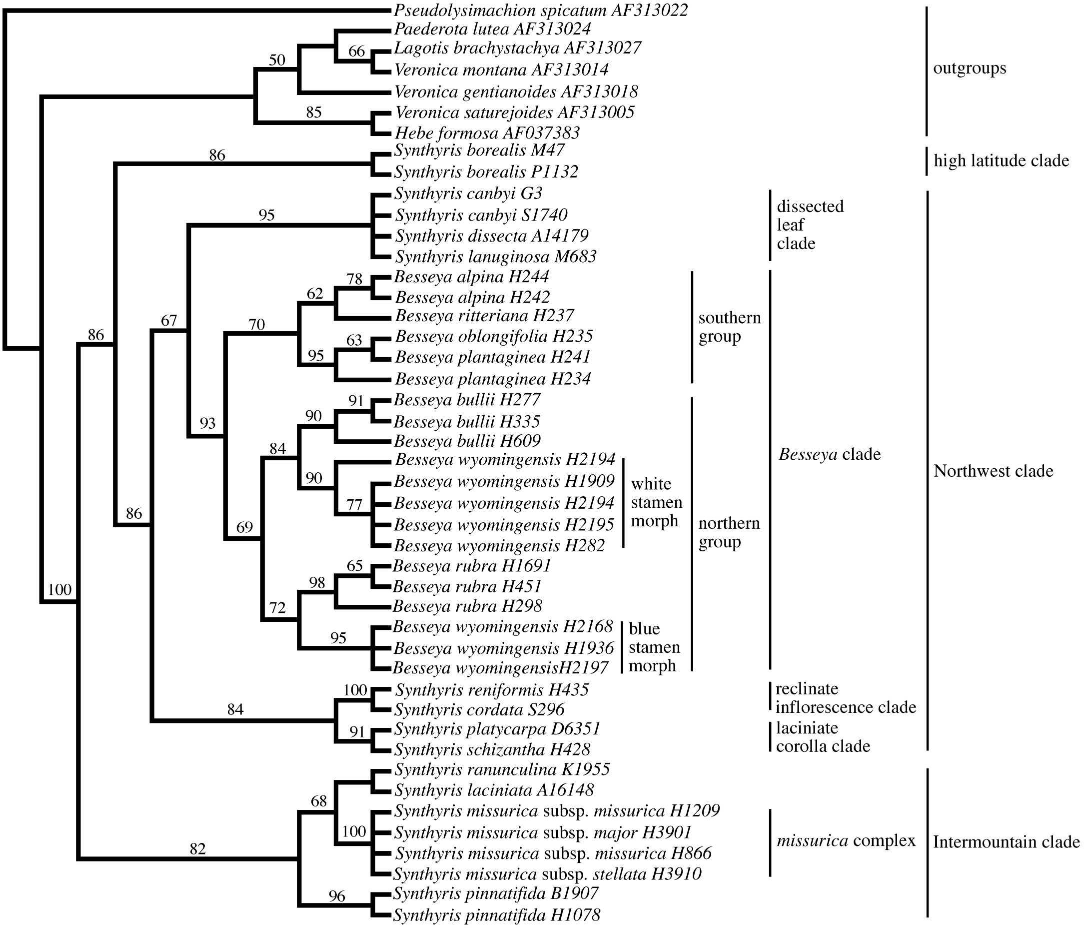

FIG. 4. Strict consensus cladogram of 16 most parsimonious cladograms of the *Synthyris* clade, including all species of *Synthyris* and *Besseya*, based on the parsimony analysis of combined ITS and morphological data. Bootstrap proportions above 50% are above clades. Informal names for clades are indicated. Accessions sampled are indicated after taxon names by the initials of collectors and collection numbers listed in Table 1.

to Schaack's hypothesis, *S. missurica* subsp. *major* forms part of the Intermountain clade and *Besseya* is nested in the Northwest clade of *Synthyris* as the sister of the dissected leaf clade in the analysis of combined ITS and morphological data.

Morphological Diversification. Shoot systems in all taxa of the *Synthyris* clade are very similar. They consist of a stout, subterranean rhizome with a short orthotropic apical zone where a rosette of foliage leaves is produced annually (Fig. 5). Racemes are preformed in the axils of foliage leaves during the season before they extend above ground (Hufford unpublished data). Yamazaki (1957) noted the similarity of these shoot system attributes of *Synthyris* and *Besseya* to those of *Picrorhiza*, *Wulfenia*, and *Lagotis*. Shoot system evolution in Veroniceae has not been investigated, but it is possible that the rosette shoots of *Picrorhiza*, *Wulfenia*, and *Lagotis* as well as *Veronica gentianoides* exemplify the plesiomorphic state for the group. Given the lack of robust nodes between the *Synthyris* clade and the base of the Veroniceae in cladograms of Albach and Chase (2001) and our results (Fig. 1), it is unclear whether the similar rosette shoot morphology characteristic of the *Synthyris* clade is a symplesiomorphy or reversal. Shoot system evolution has been labile in Veroniceae, especially in the *Hebe* complex, and it is possible that rhizomatous rosette shoots have evolved more than once.

Shoot system diversity in the *Synthyris* clade is limited largely to differences in robustness and variation in leaf forms (Went 1958; Hufford 1992b; Fig. 6). Based on the phylogenetic results, we can characterize the leaf plesiomorphies for the *Synthyris* clade to include a petiole and lamina that form an obtuse angle and laminas that are ovate, margins that have compound teeth but are not deeply incised or dissected, basal actinod-

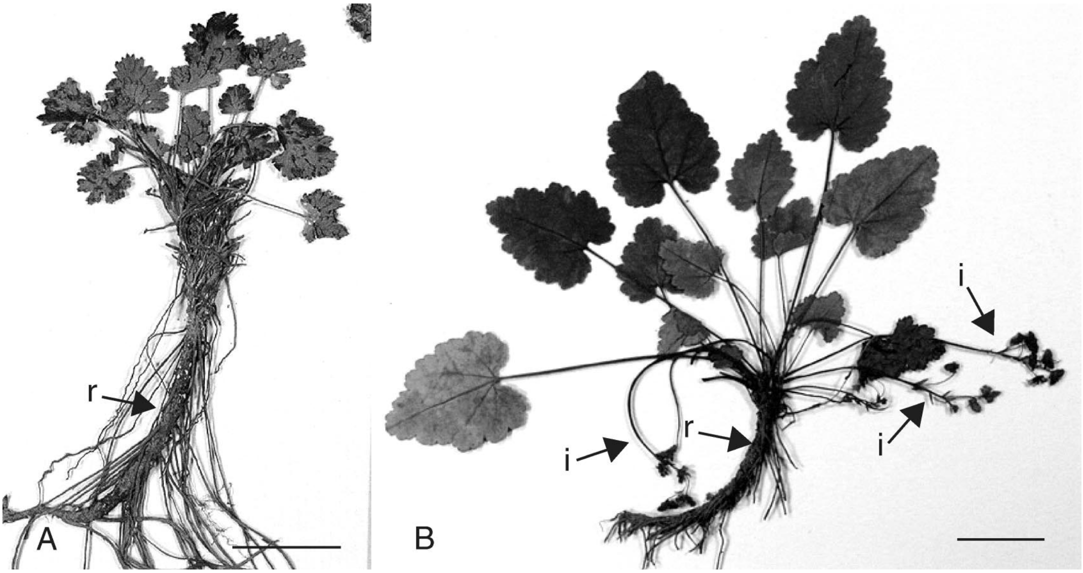

FIG. 5. Shoot systems of *Synthyris*. A. *S. canbyi*. B. *S. cordata*. The reclinate infructescences are indicated (arrows i). r = rhizome. Scale bars = 25 mm.

romous venation that includes a midrib and secondary veins that enter the lamina from the petiole and extend into the distal half of the lamina.

Important leaf transformations involve the incision and dissection of the lamina (Hufford 1992b), which has occurred in parallel in the dissected leaf clade and in *S. pinnatifida* of the Intermountain clade (Fig. 7). In the dissected leaf clade, both *S. dissecta* and *S. lanuginosa* have highly dissected laminas; whereas the laminas of *S. canbyi* range from incised about halfway to the midrib to those that are incised to the midrib in at least the basal part of the lamina. The dissection of the leaf lamina of *S. pinnatifida* parallels that of *S. dissecta* and *S. lanuginosa* (Fig. 7). ML trees constrained to force the monophyly of taxa that have deeply incised margins (*S. canbyi*, *S. dissecta*, *S. lanuginosa*, and *S. pinnatifida*) were significantly worse than the best unconstrained ML in our parametric bootstrap results (Table 4). Associated with the incision and dissection in both *S. pinnatifida* and the dissected leaf clade is a restriction of the extent of the basal secondary veins to the proximal half of the lamina and also an increase in the thickness of the midvein relative to the width of the lamina (Hufford 1992b). Taxa that have deeply incised to dissected leaves are found only in alpine habitats or in the ecotone between subalpine and alpine habitats (Fig. 7). Körner (1999) has noted that overheating, associated the high solar irradiance, is a potential risk for plants in alpine environments. The effectiveness of heat conductance from the leaf surface can be heightened by incision of leaf margins or dissection of laminas (Gurevitch and Schuepp 1990; Schuepp 1993). This may indicate that thermal regulation has played a substantial role in the evolution of the leaf forms of alpine *Synthyris*.

Other leaf transformations were homoplastic, including a shift from ovate to reniform laminas and an associated decrease in the thickness of the midrib relative to that of the basal secondary veins (Hufford 1992b). These changes have occurred in both the Intermountain and laciniate corolla clades (Fig. 7). ML trees constrained to force the monophyly of taxa (*S. laciniata*, *S. missurica*, *S. platycarpa*, *S. ranunculina*, and *S. schizantha*) that share reniform laminas that have broadly rounded apices were significantly worse than the best unconstrained ML tree in our parametric bootstrap results (Table 4). Most of the taxa that have reniform laminas are associated with mesophytic forests of the Pacific Northwest, where they are found at forest margins or gaps in cool, moist valleys (*S. missurica* subsp. *major* and *S. missurica* subsp. *stellata*) or montane slopes (*S. missurica* subsp. *missurica*, *S. platycarpa*, and *S. schizantha*). Leaves that have laminas similar to those above but have apices that are more obtuse or acute than rounded are characteristic of *S. cordata* and *S. reniformis* (Fig. 7), which are also located in mesic forests at low elevations in the Cascade Mountains and coast ranges of Oregon and northern California. Reniform laminas with relatively thin midribs occur also in the Intermountain clade in *S. laciniata* and *S. ranunculina*, which are distributed in partially shaded alpine or subalpine-alpine ecotone communities in the southern Intermountain region (*S. laciniata* in the southern Utah plateaus and *S. ranunculina* in the Spring Mountains of southern Nevada). The reniform laminas of *S. laciniata* and especially *S. ranunculina* are much smaller than

FIG. 6. Habit of *Synthyris* and *Besseya*. A. *S. dissecta*. B. *S. missurica* subsp. *major*. C. *B. wyomingensis* blue stamen morph. Scale bars = 10 mm in A, C; 50 mm in B.

those of the forest-associated *S. missurica* complex or the independently evolved laciniate corolla clade.

The evolution of *Besseya* was also associated with leaf transformations (Fig. 8). Synapomorphic for *Besseya* are simple leaf teeth rather than the compound teeth of other members of the *Synthyris* clade (Hufford 1993). The besseyas tend to have leaf laminas characterized by greater length to width ratios than other members of the *Synthyris* clade (Hufford 1992b). *Besseya oblongifolia*, *B. plantaginea*, and *B. ritteriana* have laminas that are relatively narrow, have thicker midveins, less extensive basal secondary veins, and more pinnately derived secondary veins than other members of *Besseya* (Hufford 1992b; Fig. 8). These states may be homoplastic between the *B. oblongifolia* + *B. plantaginea* clade and *B. ritteriana*, which was placed as the sister of *B. alpina*. *Besseya alpina* lacks the distinctive leaf traits shared by *Besseya oblongifolia*, *B. plantaginea*, and *B. ritteriana*. Schaack (1983) suggested that *B. ritteriana* was an allotetraploid, resulting from the hybridization of *B. alpina* and *B. plantaginea*, which could account for the leaf attributes that it shares with the latter. The hypothesis that *B. ritteriana* had a hybrid origin (Schaack 1983) requires investigation. The distinctive leaf attributes of *B. ritteriana* could simply be associated with an increase in lamina size and be homoplastic with similar lamina attributes of *B. oblongifolia* and *B. plantaginea*, which also have relatively large laminas. In contrast to the dissected leaf clade and Intermountain clade of *Synthyris*, no alpine members of *Besseya* have evolved leaves that have deeply incised margins (Fig. 8).

The diversity of reproductive morphology in the *Synthyris* clade includes variations in inflorescences, flowers, fruits, and seeds (Figs. 9, 10). The plesiomorphic state for the racemose inflorescence is to be stiffly erect and have several (ca. 15–50), densely aggregated flowers (Fig. 9A). Floral pedicels are short before anthesis and elongate through onset of fruiting. Internodes between flowers also elongate noticeably from anthesis through fruiting, resulting in infructescences that are more ''open'' than inflorescences when the fi-

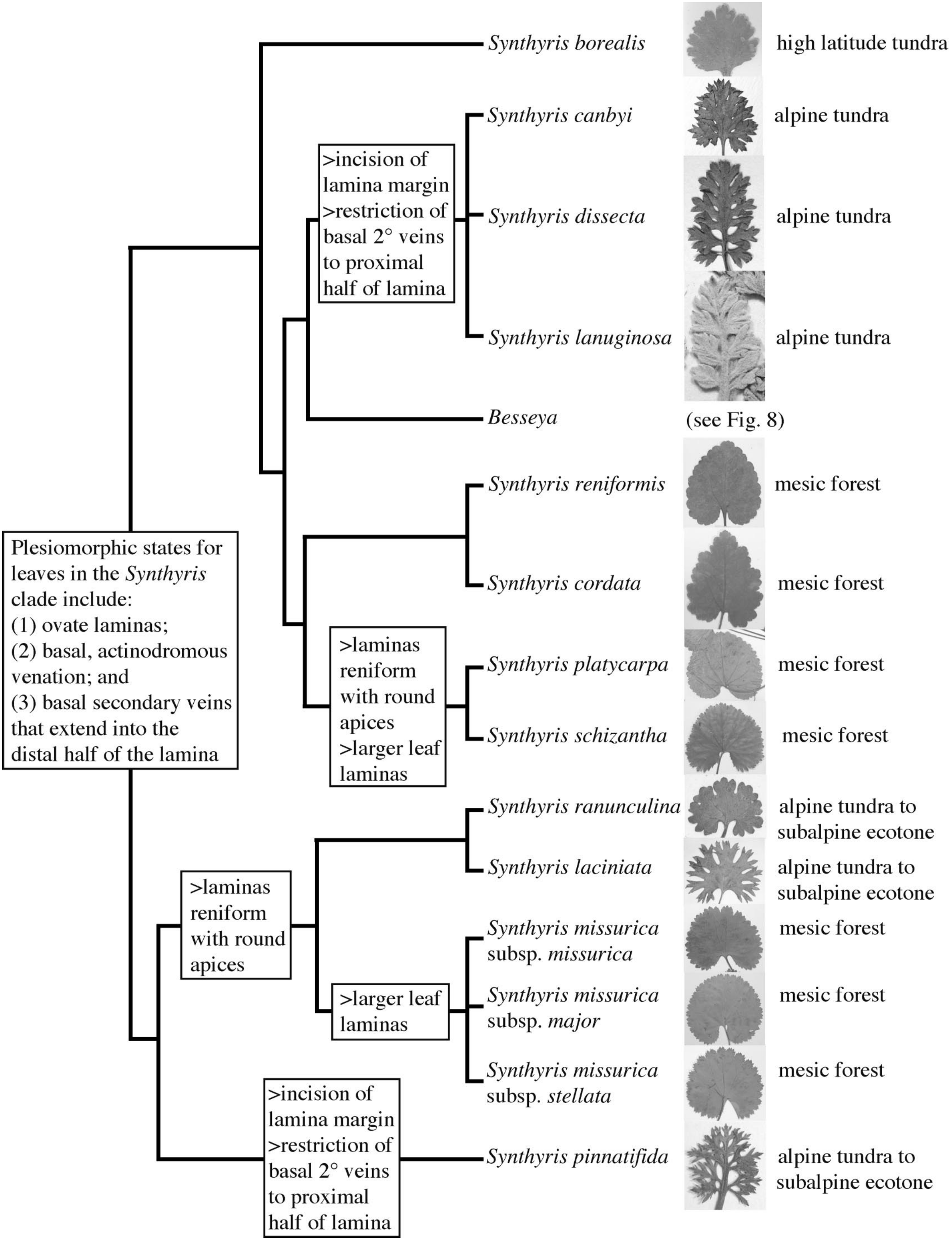

FIG. 7. A synopsis of leaf lamina evolution in the *Synthyris* clade. Representative photographs of leaf laminas and habitats for each species of *Synthyris* are provided. The plesiomorphic states for leaf laminas and apomorphies for clades are shown on part of the strict consensus cladogram from the parsimony analysis of the combined ITS and morphological data.

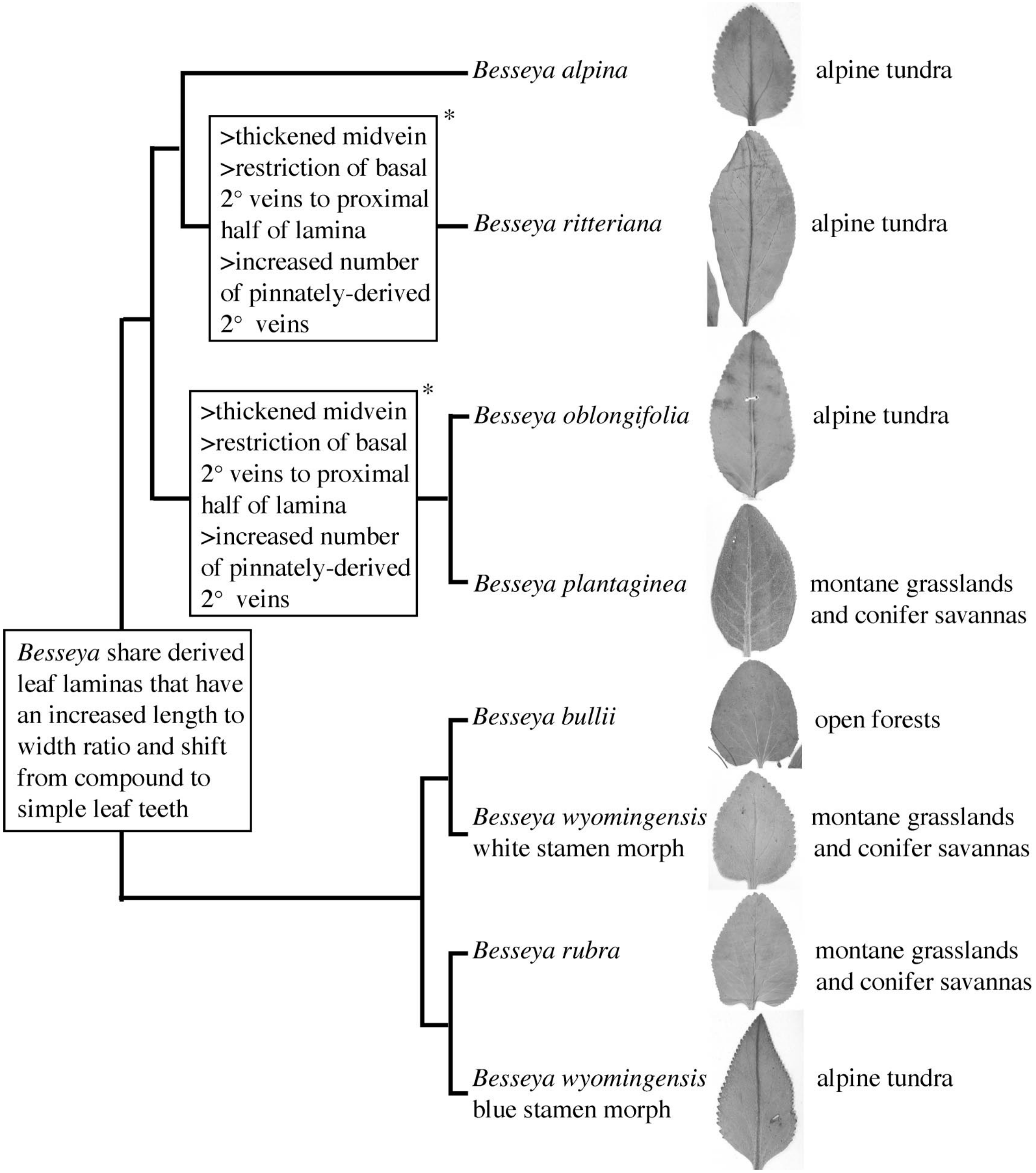

FIG. 8. A synopsis of leaf lamina evolution in *Besseya*. Representative photographs of leaf laminas and habitats for each species of *Besseya* are provided. The synapomorphic leaf lamina states and apomorphies for clades are shown on part of the strict consensus cladogram from the parsimony analysis of the combined ITS and morphological data. Asterisks indicate states shown to have arisen twice, although an equally parsimonious optimization would have had one origin of these states and reversals to plesiomorphic states in *B. alpina*.

nal flowers had reached anthesis. *Besseya* share a derived shift to a higher flower number per inflorescence (more than 100; Hufford 1992a) and pedicels that remain somewhat shorter than among *Synthyris*, giving the impression of more densely packed inflorescences (Fig. 9C–E).

Additional inflorescence changes are centered in the reclinate inflorescence clade. *Synthyris cordata* and *S. reniformis* share a decrease in flower number per inflorescence and also lack the tight packing of flowers characteristic of most other members of the *Synthyris* clade (Figs. 9A, B). The inflorescences of *S. cordata* and *S. reniformis* are erect, but somewhat lax, at anthesis and with fruiting recline to the substrate (Fig. 5B). Correlated with this inflorescence shift is a change in seed morphology. *Synthyris cordata* (Fig. 10A–C) and *S. ren-*

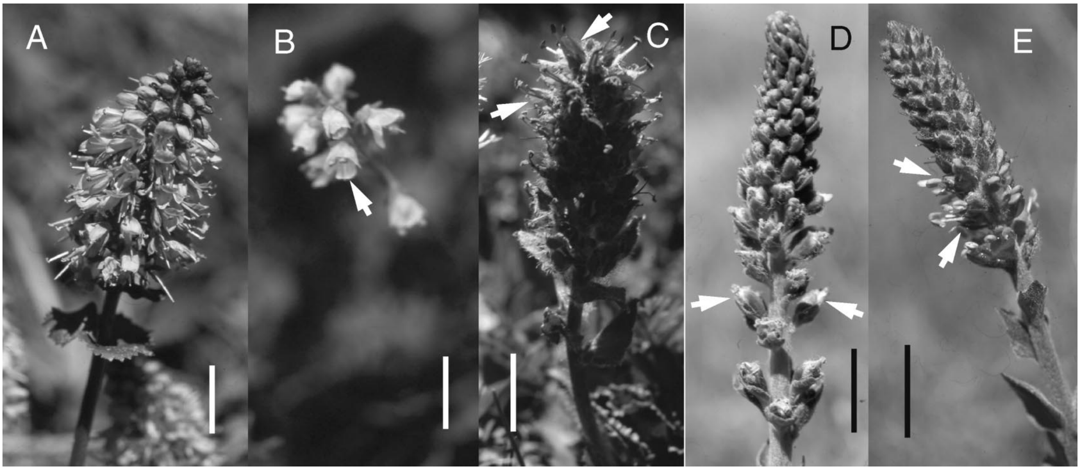

FIG. 9. Inflorescences of *Synthyris* and *Besseya*. Arrows indicate flowers at anthesis. A. *S. missurica* subsp. *major*. B. *S. reniformis*. C. *B. alpina*. D. *B. plantaginea*. E. *B. rubra*. Scale bars = 10 mm.

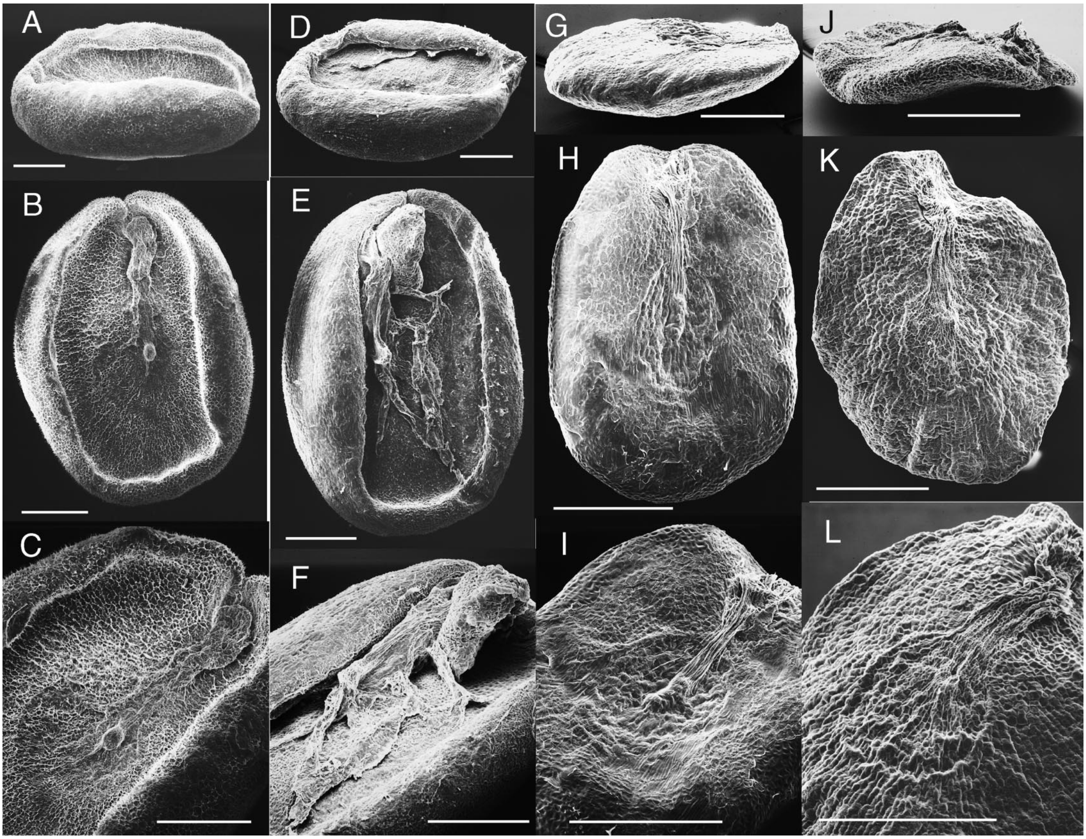

FIG. 10. Scanning electron micrographs of seeds of *Synthyris*. A-C. *S. cordata*. A. Lateral view. B. Ventral view. C. Higher magnification of the ventral surface of the seed. D-F. *S. reniformis*. D. Lateral view. E. Ventral view. F. Higher magnification of the ventral surface of the seed. G-I. *S. platycarpa*. G. Lateral view. H. Ventral view. I. Higher magnification of the ventral surface of the seed. J-L. *S. dissecta*. J. Lateral view. K. Ventral view. L. Higher magnification of the ventral surface of the seed. Scale bars = 0.5 mm.

iformis (Fig. 10D–F) have cochleariform seeds that are unique among *Synthyris* and *Besseya* (Fig. 10G–L). Disc-shaped, dorsiventrally flattened seeds are characteristic of all other examined *Synthyris* and *Besseya* (Thieret 1955; Fig. 10G, H, J, K). Thieret (1955) called attention to a prominent, circular region on the ventral surface of the disc-shaped, dorsiventrally flattened seeds of most *Synthyris* and *Besseya*, which he described as ''raised'' and called an ''endosperm plateau.'' He suggested that this region is less obvious and sometimes lacking in *S. cordata* and *S. reniformis*; instead, he suggested they typically possess a small, knoblike process in this area. In contrast to these observations, we find that the ventral circular feature is more crater-like than a raised plateau and is characteristic of seeds of *S. cordata* (Fig. 10C) and *S. reniformis* as well as other members of the *Synthyris* clade (Fig. 10I, L). A fold of tissue extends from the circular, ventral crater to the hilum (positioned at the distal end of the seed) (Fig. 10H, K), and this can be slightly elaborated in *S. cordata* (Fig. 10B, C) and *S. reniformis* (Fig. 10E, F). This elaboration, as observed by Thieret (1955), often includes a knoblike process in the circular, ventral crater (e.g., Fig. 10B, C). Elaboration of a similar knoblike process in the *Veronica hederaefolia* L. complex has been suggested to provide a reward to ants in association with myrmecochory (Fischer 1975; Juan et al. 1994). The structure of the knoblike process of *S. cordata* and *S. reniformis* has not been investigated, and their seeds appear to lack distinctive specializations for dispersal. Possible myrmecochory in *S. cordata* and *S. reniformis*, especially in light of the reclining inflorescence that places mature fruits on the forest substrate, requires investigation. In addition to the morphological modifications characteristic of the seeds of *S. cordata* and *S. reniformis*, they are also larger than the seeds of other members of the *Synthyris* clade.

Earlier workers called attention to the similarity between flowers of *Synthyris* and those of *Veronica* (Pennell 1933; Went 1958; Hong 1984; Kampny and Dengler 1997). The plesiomorphic floral states for the *Synthyris* clade include (1) a tetramerous, bilaterally symmetrical calyx that has a slight connation between lateral lobes; (2) a tetramerous, bilaterally symmetrical corolla that has a basal corolla tube and four distal lobes, among which the paired lateral, adaxial, and abaxial lobes are morphologically differentiated and spread outward at anthesis; (3) a corolla that extends prominently beyond the calyx at anthesis; (4) an androecium of two stamens inserted on the corolla tube; and (5) a bicarpellate gynoecium that is synstylous and synovarious. Androecial morphology is relatively conserved in the evolution of the clade, although stamen insertion is modified because of changes in the corolla.

Gynoecial evolution is limited to subtle changes in the shape of the ovary from an oval outline in longitudinal section to more ovate in *S. cordata*, *S. reniformis*, and *S. schizantha* (Hufford 1992a). It is unclear whether this change is synapomorphic for the monophyletic reclinate + laciniate clades, because this modified ovary form is less pronounced in *S. platycarpa*, which is also in the laciniate corolla clade. Another gynoecial change shared by the monophyletic reclinate + laciniate clades is a reduction in ovule number per flower (Hufford 1992a). *Synthyris cordata* and *S. reniformis* consistently have four ovules per flower, *S. platycarpa* usually has four, and *S. schizantha* has two to seven per flower. Other *Synthyris* typically have 10–50 ovules per flower and *Besseya* have 17–40 (Hufford 1992a).

Several evolutionary changes have impacted the perianth diversity of *Synthyris* and *Besseya*. Pervasive among the species are subtle allometric differences in the growth of the calyx and corolla, resulting in a range of calyx lobe length to width differences, especially in *Besseya*, and corolla tube to lobe length ratios and lobe length to width ratios, especially in *Synthyris*. Aside from these subtle modifications, there is a more substantial transformation in the laciniate corolla clade, consisting of *S. platycarpa* and *S. schizantha*, in which there has been a change from largely entire or slightly erose corolla lobes to deeply laciniate lobes (Gail and Pennell 1937; Schaack 1983; Hufford 1992a).

A host of perianth transformations have occurred in the evolution of *Besseya* (Hufford 1995). Calyces have been modified in *B. oblongifolia* and *B. wyomingensis*. *Besseya oblongifolia* has a trimerous rather than tetramerous calyx. Hufford (1995) noted that this transformation to trimery involved not only meristic change but also heterotopy. The plesiomorphic developmental trajectory for the calyx begins with the formation of a pair of lateral calyx lobes on the opposing extreme lateral flanks of the floral apical meristem, a primordial rim confluent with the lateral lobes protrudes around the abaxial flank of the apex, and, finally, a pair of abaxial lobes are initiated, each to one side of the sagittal plane of the flower, on the primordial rim. Calyx initiation in *B. oblongifolia* differs only in the initiation of a single abaxial lobe positioned medianly in the sagittal plane.

Calyx initiation in *B. wyomingensis* is more variable. Flowers have been observed to have tetramerous, trimerous, or dimerous calyces, although the latter are most common. Whether meristic and morphological variations are differentiated between blue and white morphs requires investigation; however, meristic and morphologial variation has been observed within inflorescences (Hufford unpublished data). The ontogeny of the calyx has been examined only in populations of the white stamen morph. Calyx ontogeny in the examined specimens diverges from the plesiomorphic trajectory at the beginning of organogenesis (Hufford 1995): calyx inception begins with the formation of a girdling rim that extends from the lateral flanks around the abaxial side of the floral apical meristem. Initiation of the pair of lateral calyx lobes fills the entire expanse of the girdling rim; no space for the initiation of abaxial calyx lobes remains on the abaxial side of the flower. In those flowers that have trimerous or tetramerous calyces, either one or two lobes, respectively, will form as subsidiary lobes on the flanks of the early developing lateral lobes. Abaxial calyx lobes were not observed to form directly from either the floral apical meristem or girdling rim (Hufford 1995).

Hufford (1995) characterized a set of corolla transformations in *Besseya* based on the cladogram from a parsimony analysis of morphological characters; however, the origins of several transformations were equivocal because of extreme juvenilization and the pattern of species relationships. Much of that ambiguity remains, although the cladogram topology that resulted from the combined ITS and morphological data differs slightly from that applied by Hufford (1995).

The corollas of all *Synthyris* extend well beyond the calyx (Figs. 6A, B; 9A, B). In contrast, most species of *Besseya* share the derived state of a corolla that extends to approximately the same length as the calyx lobes or only slightly longer (Fig. 9C–E). Reduction of corolla size is synapomorphic for *Besseya*, but the degree of the size reduction at the origin of *Besseya* remains equivocal (Fig. 11A). In addition to those besseyas that have a corolla that is approximately the same length as the calyx (Fig. 9D), *B. rubra* (Fig. 9E) has an extremely diminutive corolla (generally less than 1 mm in length). Most flowers of *B. wyomingensis* (Fig. 6C) lack a corolla, although some have been found to have a small flange of tissue—less than 1 mm in length—in the position of the abaxial lip of the corolla (Hufford 1992a). It is equally parsimonious to hypothesize either a stepwise reduction in corolla size (Fig. 11A, scenario 1), in which the plesiomorphic state for *Besseya* would be a corolla that is approximately the length of the calyx, or corolla loss at the inception of *Besseya* and three reversals to corolla formation, but with diverse sizes and forms (Fig. 11A, scenario 2). Only one additional character state change is required to hypothesize that a corolla like that of extant *B. rubra* was the plesiomorphic state for *Besseya* and that both corolla loss and corolla elabortion have occurred (Fig. 11A, scenario 3).

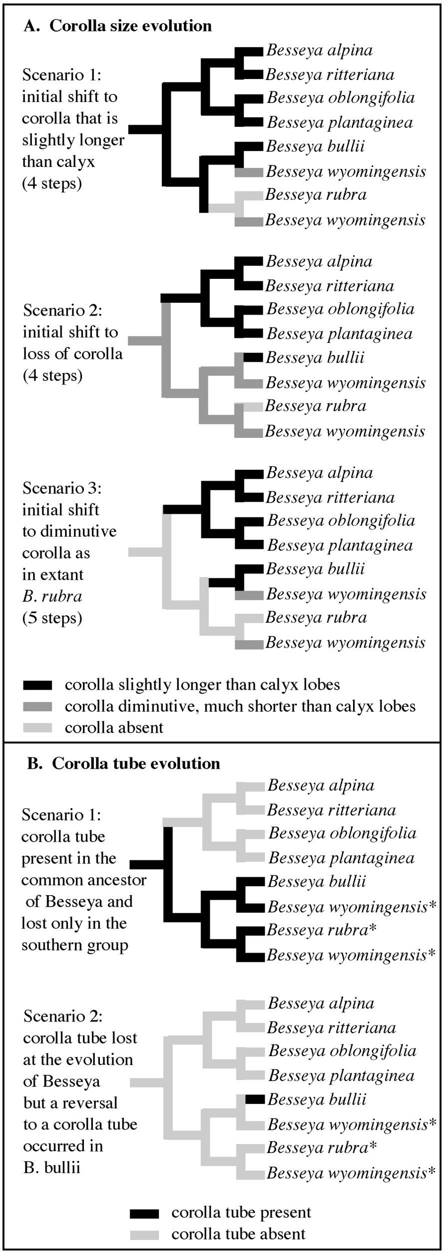

FIG. 11. Alternative scenarios for corolla evolution in *Besseya* based on the relationships of species shown in the strict consensus cladogram from the parsimony analysis of the combined ITS sequence and morphological data. A. Corolla size evolution. Two equally parsimonious optimizations of corolla size states are presented in scenarios 1 and 2. B. Corolla tube evolution. Two equally parsimonious optimizations of corolla tube states are presented in scenarios 1 and 2.

Flowers of all *Synthyris* as well as *B. bullii* have a corolla with a tubular base. A corolla tube is absent in *B. alpina*, *B. oblongifolia*, *B. plantaginea*, and *B. ritteriana* (Hufford 1992a, 1995). The corolla tube may have been lost at the origin of these taxa that constitute a clade (southern group) in the results from the combined ITS and morphology analysis (Fig. 11B, scenario 1), or it may have been lost at the origin of *Besseya* (Fig. 11B, scenario 2). Under the stepwise reduction model of corolla size evolution (Fig. 11A, scenario 1), it is possible that the corolla tube of *B. bullii* is a symplesiomorphy with the condition in *Synthyris* (Fig. 11B, scenario 1). If the corolla was lost at the inception of *Besseya* (Fig.

11A, scenario 2), then there has been a reversal to a corolla that has a basal tube in *B. bullii* (Fig. 11B, scenario 2). The corolla states (or lack of a corolla) of *B. rubra* and *B. wyomingensis* add considerable equivocation to the results: the latter lacks a corolla and the corolla of the former has been juvenilized to a developmental state that would precede the onset of corolla tube growth in those taxa that have a corolla tube (Hufford 1995). The corolla reductions of *B. rubra* and *B. wyomingensis* make it impossible to assess on the basis of our topologies whether the corolla of *B. bullii* is a symplesiomorphy or a reversal.

The corollas of all *Synthyris* have distal lobes that spread at anthesis (Fig. 9A, B). In contrast, *Besseya* corollas do not have spreading distal lobes. The corolla lobes remain more-or-less imbricately furled in *B. alpina* (Fig. 9D) and *B. ritteriana* but are loosely converging against the stamen filaments in *B. bullii*, *B. oblongifolia*, and *B. plantaginea* (Fig. 9D).

The labile evolution of the perianth in *Besseya* follows and may have been facilitated by the increased number of tightly packed flowers per inflorescence in this clade. The visual attraction of pollinators may have shifted from the corolla of individual flowers to the entire inflorescence with the increase in flower number and greater density of flowers in *Besseya* and, consequently, relaxed selection on the corolla, permitting size reduction and the loss of the corolla tube. Little is known, however, about reproductive ecology in the clade. Pollination has been studied only for *B. bullii*, which McKone et al. (1995) reported to be visited most commonly by halictid bees. Given the protogyny of self-compatible flowers in these racemose inflorescences, in which visiting bees usually move upward, they suggested that *B. bullii* could have considerable self-pollination. Protogyny and racemose inflorescences are also characteristic of all other members of the *Synthyris* clade (Hufford 1992a).

Phylogeny and Systematics. Our results recover clades that correspond to alliances discussed by earlier workers. In *Synthyris*, for example, the similar leaf, inflorescence, flower, and fruit forms and overlapping distribution of *S. reniformis* and *S. cordata* led to their alliance by Pennell (1933; as subgenus *Plagiocarpus*), Hedglin (1959), and Schaack (1983; as section *Synthyris*) (Table 5). Similarly, *S. platycarpa* and *S. schizantha*, which share a laciniate corolla, were allied by Gail and Pennell (1937), Hedglin (1959), and Schaack (1983; as section *Platycarpa*) (Table 5). In addition to these readily recognized clades, earlier workers tended to lump the remaining *Synthyris* in a larger, more poorly circumscribed group. For example, Pennell (1933; Table 5) recognized the two subgenera *Plagiocarpus* (including only the monophyletic *S. reniformis* and *S. cordata*) and *Synthyris*, which is paraphyletic in our results. Similarly, Schaack's (1983) subgenus *Synthyris* (consisting only of *S. platycarpa*, *S. schizantha*, *S. cordata*, and *S. reniformis*; Table 5) is supported as monophyletic but subgenus *Missurica*, which includes all other species of the genus, is shown by our results to be paraphyletic. Schaack's (1983) subgenus *Missurica* section *Missurica* (Table 5) corresponds to part of our monophyletic Intermountain clade, but his section *Dissecta* is polyphyletic, consisting of our dissected leaf clade as well as part of the Intermountain clade and *S. borealis*. Schaack (1983) diagnosed section *Dissecta* as characterized by corollas that have limbs shorter than or equal in length to the tube. These corolla proportions may have evolved independently in different clades. ML trees constrained to force the monophyly of Schaack's section *Dissecta* (Table 5) were significantly worse than the best unconstrained ML tree in our parametric bootstrap results (Table 4).

Our dissected leaf clade includes *S. canbyi*, *S. dissecta*, and *S. lanuginosa*. Schaack (1983) suggested that *S. dissecta* was most closely related to *S. canbyi*, which is consistent with the results of our phylogenetic analyses of ITS (Figs. 2, 3). Schaack (1983), however, treated *S. lanuginosa* as a variety of *S. dissecta*; whereas, our results from the analyses of ITS place *S. lanuginosa* as the sister of *S. canbyi* + *S. dissecta*. Because *S. lanuginosa* differs more from *S. dissecta* in its ITS sequence than does *S. canbyi* does from *S. dissecta*, we advocate currently following Pennell's (1933) recognition of these three taxa as independent species. *Synthyris lanuginosa*, an endemic to the Olympic Mountains of northwestern Washington, is disjunct from the contiguous distributions of *S. dissecta* and *S. canbyi* in the Northern Rocky Mountains of western Montana and adjacent Idaho. The whitish, tomentose leaves of *Synthyris lanuginosa* readily distinguish it from both *S. dissecta* and *S. canbyi*, which have leaves that are glabrous to sparsely pubescent.

Only Pennell (1933) has suggested a subgeneric taxonomy for *Besseya* (Table 5). Like Nieuwland (1914), who had advocated segregating *B. wyomingensis* and *B. rubra* as the genus *Lunellia* because of their reduced corollas, Pennell recognized *B. wyomingensis* (his *B. cinerea*) and *B. rubra* as subgenus *Lunellia*. Pennell placed all other species in subgenus *Besseya*. We infer that his subgenus *Besseya* is polyphyletic because *B. bullii* forms a clade with *B. wyomingensis* and *B. rubra* in our results.

Our results indicate that populations included in *B. wyomingensis* may represent two evolutionary lineages. Schaack (1983) called attention to the polymorphism of *B. wyomingensis*, including its blue and white stamen morphs. He emphasized the two-lobed calyx as diagnostic of *B. wyomingensis* as did Pennell (1933); however, Hufford (1992a) noted that calyces of *B. wyomingensis* can have two to four lobes. The type for *B. wyomingensis* is from Albany County, Wyoming, where

TABLE 5. Classification of *Synthyris* and *Besseya*

Pennell 1933	Schaack 1983	Revised classification
Synthyris Subgenus *Eusynthyris* *S. schizantha* Piper *S. stellata* Pennell *S. missurica* (Raf.) Pennell *S. laciniata* Rydb. *S. ranunculina* Pennell *S. canbyi Pennell* *S. lanuginosa* (Piper) Pennell and J. W. Thomps. *S. dissecta* Rydb. *S. hendersonii* Pennell *S. cymopteroides* Pennell *S. paysoni* Pennell and L. Williams *S. pinnatifida* S. Watson Subgenus *Plagiocarpus* *S. cordata (A. Gray) A. Heller* *S. reniformis* (Douglas) Bentham	*Synthyris* Subgenus *Missurica* Section *Missurica* *S. missurica* *S. stellata* *S. major* (Hook.) Heller *S. ranunculina* Section *Dissecta* *S. dissecta* (including *S. cymopteroides, S. hendersonii, S. lanuginosa*) *S. canbyi* *S. borealis* *S. laciniata* *S. pinnatifida* (including *S. paysoni*) Subgenus *Synthyris* Section *Synthyris* *S. cordata* *S. reniformis* Section *Platycarpa* *S. schizantha* *S. platycarpa*	*Synthyris* Intermountain clade (Subgenus *Missurica*) *S. missurica* subsp. *missurica* *S. missurica* subsp. *major* *S. missurica* subsp. *stellata* *S. laciniata* *S. ranunculina* *S. pinnatifida* (including *S. paysoni*) High latitude clade *S. borealis* Northwest clade (subgenus *Synthyris*) Dissected leaf clade (section *Dissecta*) *S. dissecta* (including *S. cymopteroides* and *S. hendersonii*) *S. lanuginosa* *S. canbyi* Laciniate corolla clade (section *Platycarpa*) *S. platycarpa* *S. schizantha* Reclinate inflorescence clade (section *Synthyris*) *S. cordata* *S. reniformis*
Besseya Subgenus *Eubesseya* *B. alpina* (A. Gray) Rydb. *B. bullii* (Eaton) Rydb. *B. ritteriana* (Eastw.) Rydb. *B. oblongifolia* Pennell *B. gooddingii* Pennell *S. borealis* Pennell *B. arizonica* Pennell *B. plantaginea* (E. James) Rydb. Subgenus *Lunellia* *B. rubra* (Douglas) Rydb. *B. cinerea* (Raf.) Pennell	*Besseya* *B. alpina* *B. bullii* *B. oblongifolia* *B. plantaginea* (including *B. arizonica* and *B. gooddingii*) *B. ritteriana* *B. rubra* (=*B. cinerea*) *B. wyomingensis* (A. Nelson) Rydb. (=*B. cinerea*)	Besseya clade (section *Besseya*) *S. alpina* A. Gray *S. bullii* (Eaton) A. Heller *S. oblongifolia* (Pennell) L. Hufford & M. McMahon *S. plantaginea* (E. James) Benth. (including *B. arizonica* and *B. gooddingii*) *S. ritteriana* Eastw. *S. rubra* (Douglas) Benth. *S. wyomingensis* (A. Nelson) A. Heller (=*B. cinerea*)

the populations are characterized by white stamens. Another name applied in the *B. wyomingensis* complex was *B. gymnocarpa* (A. Nelson) Rydb., which was based on a type from Uinta County, Wyoming. This specimen is in fruit, and we can not ascertain its stamen color. Nelson (1898) distinguished *B. gymnocarpa* (as *Wulfenia gymnocarpa* A. Nelson) from *B. wyomingensis* by differences in degree of connation in the calyx. Stamen colors in the western Wyoming populations of *B. wyomingensis* (including the type locality of *B. gymnocarpa*) need to be investigated. Thus, the blue stamen morph populations of *B. wyomingensis* may be an evolutionary lineage distinct from the white stamen morph populations (= *B. wyomingensis* s. str.); however, we prefer to conduct further phylogenetic analyses that include a greater number of populations of both color morphs (including putatively white stamen morph populations from Montana cited by Schaack [1983]), permitting a more comprehensive test of the independent monophyly of the differing stamen color morphs, before proposing taxonomic changes in this complex.

Although Pennell (1933) and Schaack (1983) suggested that *Besseya* was derived from *Synthyris*, each advocated the recognition of both *Besseya* and *Synthyris*. In order to support his position Schaack (1983) indicated that the two genera were distinguished by a variety of characters, there are no taxa intermediate between them, and tradition. We question these criteria. Species that Schaack (1983) recognized as *Besseya* had been treated historically by others as *Synthyris*, *Lunellia*, *Veronica*, and *Wulfenia*; thus, there has been little consistent treatment of the besseyas, at least prior to Pennell's (1933) revision. Whether there are intermediates between Schaack's (1983) or Pennell's (1933) *Synthyris* and *Besseya* depends on the characters that are examined and how one would characterize the paraphyletic *Synthyris*. *Besseya* may be morphologically distinctive relative to any particular set of *Synthyris* species, but this disparity may reflect primarily the set of developmental transformations that occurred at the origin of *Besseya*, a cladogenetic event that was qualitatively different from those that had preceded it in the diversification of *Synthyris* clades. These transformations are also central to Schaack's (1983) contention that the variety of characters that distinguish *Synthyris* and *Besseya* is relevant to their continued recognition as independent genera. The characters that Schaack (1983) and Pennell (1933) used to distinguish the two genera are apomorphies of *Besseya* and plesiomorphies of *Synthyris*.

A central concern is the paraphyly of *Synthyris* in the face of continued recognition of *Besseya* as a coordinate group. We advocate a classification that reflects our knowledge of evolutionary relationships, especially monophyletic groups. Thus, we consider it important that *Besseya* be classified as part of *Synthyris*. The nomenclatural changes required for this are minimal. Most species recognized as *Besseya* by Pennell (1933) and Schaack (1983) had been described originally as *Synthyris* or subsequently combined with *Synthyris*; thus, the only new combination needed is for *B. oblongifolia* (see below). We present a revised taxonomy for the *Synthyris* clade that is centered on monophyletic groups (Figs. 1–4, Table 5). We emphasize descriptive, informal names for the groups, but the names that have priority for each group and a rank are also indicated (Table 5).

TAXONOMY

Synthyris oblongifolia (Pennell) L. Hufford & M. McMahon, comb. nov. Basionym: *Besseya oblongifolia* Pennell, Proc. Acad. Nat. Sci. Phil. 85: 101, 1933.—TYPE: UNITED STATES, New Mexico: White Mountain (Sierra Blanca) Peak, 16 Aug 1897, *Wooton, s. n.* (US).

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