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A Phylogenetic Analysis and Taxonomic Revision of *Bartonia* (Gentianaceae: Gentianeae), Based on Molecular and Morphological Evidence

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Abstract—A phylogenetic study and taxonomic revision of the four currently accepted species of *Bartonia* (Gentianaceae, subtribe Swertiinae) were conducted in order to test species boundaries and interspecific relationships. Species boundaries were examined based on measurements of key quantitative and qualitative morphological characters as given in the original descriptions. Phylogenetic analyses were performed using molecular data from the nuclear internal transcribed spacer region and chloroplast DNA (*trnL* intron through the *trnL-F* spacer), separately and combined using parsimony and Bayesian methodologies, incorporating outgroups from subtribes Swertiinae and Gentianinae. The morphological study revealed that characters of one species, *B. texana*, represent a subset of the morphological variation found within *B. paniculata*, but that *B. paniculata*, *B. verna*, and *B. virginica* could all be separated from one another. The molecular phylogenetic analyses all found *B. texana* to nest in a clade with the two recognized subspecies of *B. paniculata* (subsp. *paniculata* and subsp. *iodandra*), making the latter paraphyletic. *Bartonia texana* is here reduced to subspecific rank, as **Bartonia paniculata** subsp. **texana**. Also, the phylogenetic analyses showed strong support for a sister group relationship between *B. verna* and *B. virginica*, as opposed to between *B. paniculata* and *B. virginica* as has been previously suggested.

Keywords—Gentianinae, internal transcribed spacer, Swertiinae, *trnL-F* spacer, *trnL* intron.

Bartonia Muhl., commonly known as screwstem, is a genus of four diminutive, herbaceous species distributed throughout the eastern United States and Canada. They are presumed to be wholly or partially dependent on mycorrhizal fungi (Holm 1906; Gillett 1959; Nilsson and Skvarla 1969; Wood and Weaver 1982), although this has never been proven. *Bartonia* species have minimal root systems, with little branching and no root hairs. Observations of stained roots of *B. virginica* (L.) BSP have revealed the presence of fungal hyphae on root surfaces and possibly arbuscular formations within root cells (K. Mathews and R. Gualandi, unpubl. data). *Bartonia* species have highly reduced and scale-like leaves, either green or purplish stems, and tend to grow in acidic, low-nutrient, and wet environments, such as sphagnum bogs, vegetation islands on rock outcrops, and sandy or gravelly lake shores. They can also be found in pine barrens, wet thickets, open woods, and ditches.

Four species of *Bartonia* are currently recognized: *B. paniculata* (Michx.) Muhl., *B. texana* Correll, *B. verna* (Michx.) Muhl., and *B. virginica* (Struwe et al. 2002; USDA, NRCS 2007). According to Gillett (1959, 1963), *B. paniculata*, the northernmost-ranging species, contains two subspecies, subsp. *iodandra* (Robins.) Gillett and subsp. *paniculata*. This species ranges along the Atlantic Coastal Plain from Newfoundland, Nova Scotia, and New England south to northern Florida, west to eastern Texas and inland to Oklahoma, Arkansas, and Kentucky. *Bartonia paniculata* subsp. *iodandra* is found in the northernmost part of the range, from Newfoundland to Rhode Island. Disjunct populations of *B. paniculata* subsp. *paniculata* have also been reported from the Muskoka district of Ontario (Reznicek and Whiting 1976) and northern Michigan (Henson 1985).

Bartonia paniculata overlaps with *B. virginica*, which occurs along the coastal plains from Nova Scotia south to central Florida and along the Gulf to eastern Louisiana, as well as inland in the Great Lakes region from southern Ontario and Minnesota south to Arkansas and Tennessee. *Bartonia verna* is confined to the southeastern Gulf and Atlantic coastal plains, ranging from southeastern North Carolina to southern Florida and west to eastern Louisiana, with a disjunct population recorded from False Cape State Park, Virginia (Belden et al. 2004). *Bartonia texana*, which was described in 1966 after Gillett's (1959) revision, has the smallest distribution, being restricted to southeastern Texas and northwestern Louisiana. Distribution dot maps are available in Gillett (1959, 1963) for the three more widespread species, and the distributions of *B. texana* and *B. paniculata* in Texas and Louisiana are shown in Fig. 1.

Correll (1966) acknowledged a strong resemblance between *Bartonia texana* and *B. paniculata*, which overlap in distribution. Yet he described a number of characteristics by which *B. texana* may be distinguished from *B. paniculata*, including its corolla and calyx lobe shapes, capsule shape, shorter calyx, corolla, and style, and capsule usually exceeding the corolla (as opposed to generally being shorter than the corolla in *B. paniculata*). *Bartonia texana* has since been listed as endangered by the Center for Plant Conservation (CPC 2003, 2007), although it is not federally protected. However, its taxonomic distinctness from *B. paniculata* has been called into question (Wood and Weaver 1982).

Gillett (1959) hypothesized *Bartonia virginica* and *B. paniculata* to be the most closely related of the three species he recognized, in part because of the putative hybrids he identified in the field. He believed *B. verna* to be the more "primitive" because of its "poorly developed corolla tube, decurrent stigmas and variable position of leaf scales" (p. 47). Indeed *B. verna* is unique in having a relatively short corolla tube, longer corolla lobes, and a much longer corolla relative to the calyx than any other species of *Bartonia*. However, variations in stigma decurrence onto the ovary and leaf position occur in all *Bartonia* species.

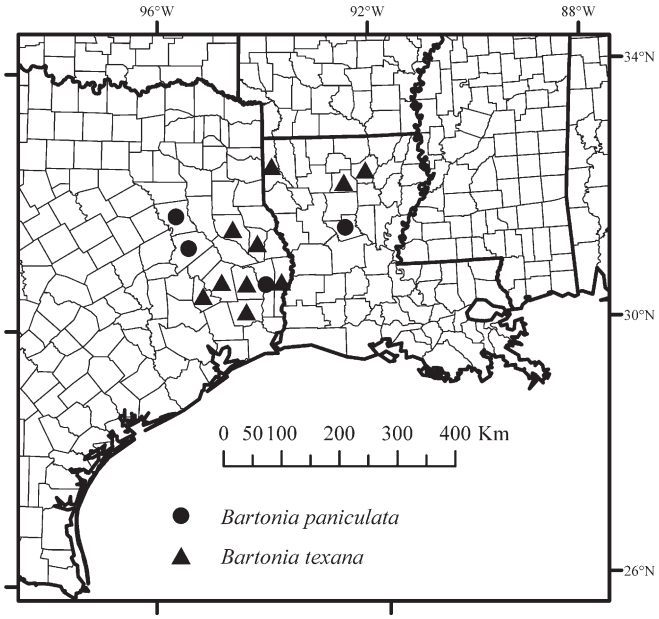

FIG. 1. Distributions of *Bartonia texana* and *B. paniculata* subsp. *paniculata* in Texas and Louisiana based on herbarium specimens examined for this study and from Nixon and Ward (1981).

Bartonia virginica and *B. paniculata* both have chromosome numbers of 2*n*=52 (Wood and Weaver 1982), are sympatric over a large portion of their ranges, and have overlapping flowering times in the late summer to fall (July to September). *Bartonia verna* has 2*n*=44 (Wood and Weaver 1982) and flowers in the winter and spring (November to March), with the southernmost populations flowering earliest. There is no known chromosome count for *B. texana*, which flowers from August to October.

The intergeneric relationships of *Bartonia* are complicated. Tribe Gentianeae and subtribe Swertiinae (14 genera total), to which *Bartonia* belong, are both monophyletic, but relationships among the genera are not completely resolved, and the sister group of *Bartonia* is still unclear (Chassot et al. 2001; von Hagen and Kadereit 2002; Struwe et al. 2002). Based on previous phylogenetic studies, *Bartonia* appears to be an isolated lineage that diverged early in the evolution of the Swertiinae, as evidenced by a long terminal branch and the lack of resolution for any particular sister group relationships (Chassot et al. 2001; von Hagen and Kadereit 2002).

The North American monotypic genus *Obolaria* L. has been regarded as closely related to *Bartonia* because of its purplish stem and leaves and its presumed saprophytic tendencies (Wood and Weaver 1982). von Hagen and Kadereit (2002) found a probable basal assemblage in Swertiinae consisting of *Bartonia*, *Megacodon* (Hemsl.) Harry Sm. (East Asia), and *Obolaria* based on chloroplast (*trnL* intron, *matK*) and nuclear ITS sequences, as well as *Latouchea* Franch. (East Asia) based on morphological similarities. Furthermore, von Hagen and Kadereit hypothesized a sister group relationship between *Bartonia* and *Obolaria* based on the shared morphological characters of imbricate corolla aestivation (vs. convolute) and mycotrophic habit, both of which are unique in the subtribe. In contrast, our preliminary anatomical studies show *Bartonia verna* having contort corolla aestivation rather than imbricate (N. Dunne, this study). Other morphological studies of Gentianaceae have found that *Bartonia* and *Obolaria* share similar specializations not only in vegetative habit but also in placentation (Holm 1897; Lindsey 1940). Both *Obolaria* and *Bartonia* have many small ovules widely-scattered over the interior wall of the unilocular ovary and similar pollen morphology (Nilsson and Skvarla 1969).

Nonetheless, Chassot et al. (2001), also using chloroplast (*trnL* intron, *trnL-F* spacer, *trnS-ycf9* spacer) and nuclear (ITS) markers, found *Obolaria* and *Latouchea* to form a clade sister to the rest of Swertiinae, while *Swertia tashiroi* Makino (East Asia) had a moderately to well supported sister group relationship with *Bartonia* (*Swertia* L. itself is found to be polyphyletic in Chassot et al. [2001]). The position of *Megacodon* within the main Swertiinae clade was unresolved. The present study is different from previous analyses in focusing on interspecific relationships within *Bartonia* and including all recognized *Bartonia* species and subspecies in a phylogenetic analysis instead of only a single representative species.

Our goals were to 1) analyze the species of *Bartonia* morphologically and re-examine Gillett's and Correll's morphology-based hypotheses of species limits and interspecific relationships, 2) determine evolutionary relationships among the species of *Bartonia* using cladistic methods with molecular data from the nuclear and chloroplast genomes, and 3) provide a taxonomic revision of the genus in light of these analyses. In particular, we wanted to know whether *B. texana* is a distinct taxon from *B. paniculata*, and if so, whether these two are sister species; or whether *B. paniculata* and *B. virginica* are sister species; and finally where the affinities of *B. verna* lie.

Materials and Methods

Morphological Analyses—Loans of plant specimens were obtained from BKL, BRIT, CHRB, LL, NYBG, and PH. Approximately 165 herbarium sheets, including all species and subspecies of *Bartonia* and four type specimens, were examined using a dissecting microscope (see Appendix 2 for list of specimens examined). Because *Bartonia* plants are small, most herbarium sheets had multiple dried plants on them (usually in the range of four to seven plants), and all individuals per sheet were examined. Ten key morphological characters used by Gillett (1959) and Correll (1966) to distinguish *Bartonia* species and subspecies were investigated for taxonomic utility. Three quantitative characters were measured: lengths of calyx, corolla, and styles. Seven qualitative characters were examined: shape of corolla lobes, corolla lobe apex, sepal lobes, capsules, style, and stigmas, as well as capsule dehiscence type. Microsoft Excel (version 11.2.3) was used to record, analyze and graph measurements.

Molecular Phylogenetic Analyses—We used sequences from the internal transcribed spacer (ITS) region of nuclear ribosomal DNA and from the chloroplast DNA region containing the *trnL* intron as well as the *trnL-trnF* intergenic spacer. Both of these regions have been used successfully for phylogenetic analysis in tribe Gentianeae (Taberlet et al. 1991; Chassot et al. 2001; von Hagen and Kadereit 2002; Struwe et al. 2002; Chen et al. 2005). Plant material of *Bartonia paniculata* subsp. *paniculata* was obtained from leaves collected in the field and stored in silica gel. Plant material for *B. virginica* and *Gentiana decora* Pollard was collected in the field and stored at -70°C. Plant material of *B. verna*, *B. texana*, *B. paniculata* subsp. *iodandra*, and *Gentianella quinquefolia* Small was obtained from herbarium specimens. All other outgroup sequences and additional sequences for *B. virginica* were obtained from GenBank (Appendix 1). All data sets for phylogenetic analyses were submitted to TreeBASE (study number S2099).

Gentianella quinquefolia, *Latouchea fokienensis* Franch., *Megacodon stylophorus* (C.B.Clarke) Harry Sm., *Obolaria virginica* L., and *Swertia tashiroi* (all subtribe Swertiinae) were included in the analyses as outgroups since the sister group of *Bartonia* is unknown. We also included one outgroup species from subtribe Gentianinae, *Gentiana decora*, and rooted the trees with this taxon. Such a broad sampling of outgroups was used to ensure an accurate rooting of *Bartonia*.

For all *Bartonia* species, both stems and leaves were ground to obtain adequate DNA quantities. Plant tissue was first homogenized either by freezing with liquid nitrogen and grinding with a mortar and pestle or by using the BioMasher™ homogenizing tool and filter (Cartagen,

San Carlos, California), followed by total DNA extraction using the DNeasy Plant Mini Kit (Qiagen, Valencia, California) in accordance with the manufacturer's protocols. Primers 4 and 5 of White et al. (1990) were used for PCR amplification and cycle sequencing of the entire ITS region. Primers c and f of Taberlet et al. (1991) were used for PCR amplification of the entire chloroplast region containing the *trnL* intron and the *trnL-F* spacer along with the intervening coding region. Internal primers e and d of Taberlet et al. (1991) were also used for cycle sequencing to obtain overlapping sequences in both directions for the entire region. PCR reactions contained 10 µl TaqMaster (Applied Biosystems, Foster City, California) adjuvant, 5 µl 10 × buffer, 2 µl dNTPs (10 mM), 2.5 µl $MgCl_2$ (25 mM), 2 µl each primer (10 µM), 0.25 µl Taq polymerase, 1–2 µl template DNA, and sterile distilled water to fill up to 50 µl total volume. PCR parameters for ITS amplification were: 3 min at 95°C followed by 30 cycles of 1 min at 95°C, 1 min at 45°C, and 1 min 20 sec at 72°C, followed by 5 min at 72°C and held at 4°C. Earlier attempts at amplification of the ITS region of *Bartonia* at a higher annealing temperature failed. A gradient PCR reaction found optimal amplification to occur with a 45°C annealing temperature, particularly with DNA extracted from herbarium specimens. A single amplicon band was observed in all PCR products when run on agarose gels. PCR parameters for the *trnL* intron and *trnL-F* spacer region were: 2 min at 95°C followed by 35 cycles of 50 sec at 95°C, 50 sec at 50°C, and 1 min 50 sec at 72°C, followed by 7 min at 72°C and held at 4°C.

PCR products were purified using the QIAquick® PCR Purification Kit (Qiagen) following the manufacturer's protocol and then cycle-sequenced using Big Dye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) following the manufacturer's protocol and PCR parameters, but performing reactions in 10 µl volume (1/2 reactions). Sequencing products were purified using AutoSeq™ MicroSpin G50 (Amersham Biosciences, Piscataway, New Jersey) spin columns containing Sephadex™ and run on a 3130xl Genetic Analyzer (Applied Biosystems). Because the ITS primers used were developed from fungal sequences and because of *Bartonia*'s suspected fungal associations, its sequences were compared to those in GenBank by means of the online BLAST to verify that they were most similar to plant sequences. Sequences were manually edited using Sequencher version 4.6 (Gene Codes Corp., Ann Arbor, Michigan) and automatically aligned in ClustalX version 1.8 (Thompson et al. 1997) using the default alignment parameters. Minor manual adjustments to the alignments were made using the similarity criterion (Simmons 2004).

Branch-and-bound parsimony searches, which guarantee finding the shortest trees, were run in PAUP* v. 4.0 b10 (Swofford 2003) on the ITS and chloroplast data sets separately and then combined following an implementation of the partition-homogeneity test (ILD test; Farris et al. 1994) in PAUP* with 1,000 random repartitions of the data. All gaps (indels) in both data sets were coded as binary (present/absent) characters added to the end of the matrices using the simple gap coding method of Simmons and Ochoterena (2000). One thousand bootstrap (Felsenstein 1985) replicates were run using heuristic searches with starting trees obtained via stepwise addition and random addition sequence with 100 replicates, TBR branch-swapping, and 'MulTrees' option in effect.

A Bayesian MCMC analysis (Yang and Rannala 1997) was also completed using MrBayes ver. 3.1 (Huelsenbeck and Ronquist 2001, 2002) on the combined data matrix partitioned into three sections: ITS, *trnL-F* region, and coded indels. Modeltest ver. 3.7 (Posada and Crandall 1998) called for a GTR model for both nucleotide partitions, and showed that among-site rate variation was best modeled with a gamma correction (Yang 1993). Priors were selected based on the parameters estimated by Modeltest using the Aikaike Information Criterion (AIC; Akaike 1974). Coded indels were treated as restriction data and assigned the Mk model (Lewis 2001). Two independent runs were performed, each of 1 million iterations with four chains (three hot, one cold), sampling one tree every 500 generations. Plots from the MrBayes "sump" command were used to determine the appropriate "burnin." Five hundred samples were discarded as burnin, and the remaining trees were pooled. Majority rule consensus trees were constructed in PAUP*.

Results

Taxonomic Results—The diagnostic characters distinguishing *B. paniculata* (both subspecies), *B. verna*, and *B. virginica* from one another (phyllotaxy, corolla lobe shape and length, flowering time, and capsule dehiscence) were found to be robust. *Bartonia paniculata* had a mainly alternate leaf arrangement, mostly oblong-lanceolate and 2.7–6.3 mm long corolla lobes, a summer/fall flowering period, and apical capsule dehiscence. *Bartonia paniculata* subsp. *paniculata* differed from *B. paniculata* subsp. *iodandra* in its more slender habit, a taller mean height (25.9 cm in subspecies *paniculata* vs. 14.3 cm in subspecies *iodandra*), greater mean leaf number (14 vs. 5.5), stem color (typically green vs. purplish), and shorter mean corolla lobe length (4.1 mm vs. 4.5 mm), although intergradation between the subspecies appeared to be widespread based on intermediacy of characters. *Bartonia verna* had mainly opposite leaf arrangement, spatulate and 4.0–8.0 mm long corolla lobes, a spring flowering time, and medial capsule dehiscence. *Bartonia virginica* also had mainly opposite leaf arrangement and medial capsule dehiscence, but oblong and 2.5–4.0 mm long corolla lobes, and a summer/fall flowering period.

On the other hand, seven key characters for *Bartonia texana* (Correll 1966) were diagnostically unreliable in separating it from *B. paniculata*. Range measurements of the three quantitative diagnostic characters for *B. texana* (calyx, corolla, and style length) all overlapped with range measurements for *B. paniculata*: *Bartonia texana* had sepals 1.1–2.2 mm long (vs. 1.6–2.9 mm in *B. paniculata*), corollas 1.9–3.4 mm long (vs. 2.9–4.5 mm in *B. paniculata*), and styles 0.4–0.8 mm long (vs. 0.4–1.1 mm in *B. paniculata*). The four qualitative characters used to distinguish the two species (capsule exceeding corolla or not, corolla lobe shape, sepal lobe shape, and capsule shape) also overlapped. The diagnostic character for *B. texana* of capsules usually longer than the corolla was unreliable, even among the plants on the holotype sheet, half of which have corollas exceeding the capsule. In addition, there were numerous specimens of *B. paniculata* with capsules exceeding the corolla, especially in the more northern states. The corolla lobes for *B. texana* were primarily ovate with acuminate to apiculate apices. This was different from *B. paniculata* in its southern range (subsp. *paniculata*), which had corolla lobes predominantly oblong-lanceolate with acute to long-acute apices, whereas more northern populations of *B. paniculata* (both subsp. *paniculata* and subsp. *iodandra*) often had ovate corolla lobes with acuminate or apiculate apices. Sepal lobe shape also overlapped in *B. texana* and *B. paniculata*, with both taxa exhibiting triangular-lanceolate to long-lanceolate sepal lobes. To quantify this character, sepal lobe width-to-length ratios were calculated for *B. texana* and *B. paniculata,* and these overlapped between the two species (0.38–0.45 in *B. texana* vs. 0.27–0.61 in *B. paniculata*). Capsule shape on the holotype specimen of *B. texana* varied, being oval, ovate, oblong, or orbicular. Orbicular-shaped capsules are not unique to *B. texana* and can also be found in *B. paniculata* specimens.

We examined a previously unemphasized, relatively stable character trait for each species of *Bartonia*: the shape of the style. In his key to the species, Gillett (1959) distinguished between the style lengths of *B. paniculata* and *B. virginica* as being "short" and "elongate," respectively. Lateral decurrence of the stigmas in all *Bartonia* species often makes it difficult to distinguish style from stigmatic surface. However, for *B. paniculata* (both subspecies) and *B. texana* the style is flared and swollen, but for *B. verna* and *B. virginica* the style is generally slender and elongated (Fig. 2). The style of *B. verna* is the most distinct and slender of all the species and may even be described as filiform. Mean style lengths, as measured from the apex of the ovary to the apex of the pistil, were: *B. verna*: 1.8 mm;

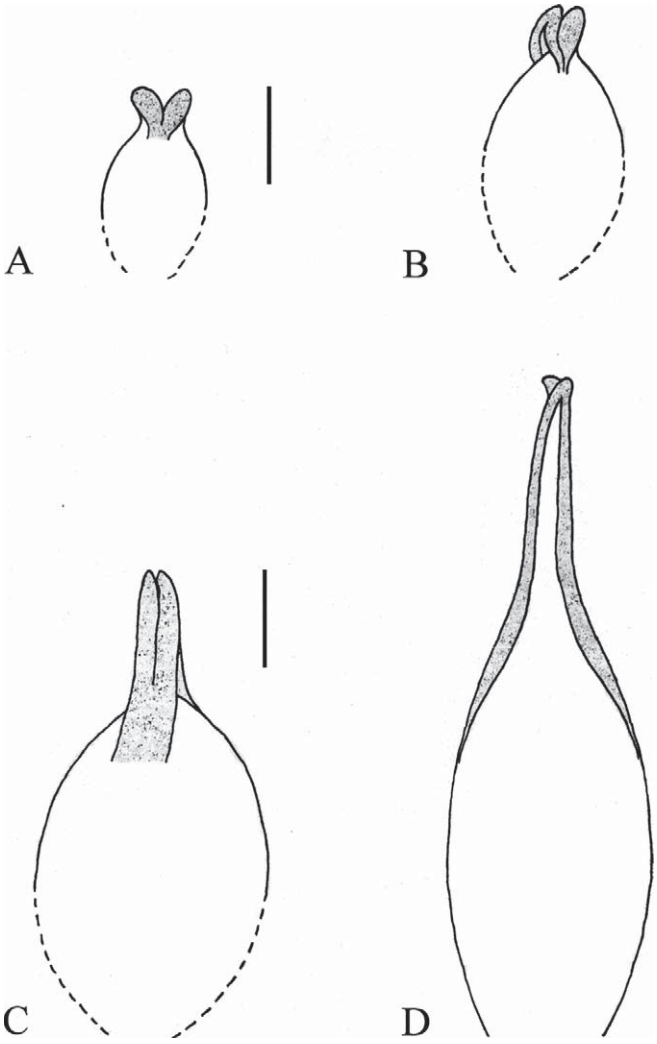

FIG. 2. Style and stigma morphology of *Bartonia* species. Shaded areas represent stigmatic regions. Scale bars represent 1 mm. All figures are drawn to scale. A. *Bartonia texana*. Drawn from *C. Reid 4638* (TEX). B. *Bartonia paniculata* subsp. *paniculata*. Drawn from *S. W. Leonard & A. E. Radford 2164* (WCUH). C. *Bartonia virginica*. Drawn from *J. H. Horton & J. D. Pittillo 7268* (WCUH). D. *Bartonia verna*. Drawn from *R. L. Wilbur & J. Horn 72349* (DUKE). All drawings by K.G.M.

B. virginica: 1.1 mm; *B. paniculata* subsp. *paniculata*: 0.7 mm; *B. p.* subsp. *iodandra*: 0.8 mm; and *B. texana*: 0.6 mm.

We also examined a related character, stigma fusion, first reported for the genus by Godfrey and Wooten (1981). These authors described the dual stigmas of *Bartonia verna* and *B. virginica* as connivent, and those of *B. paniculata* as slightly spreading. (Note: In his protolog of *Bartonia iodandra*, now recognized as *B. paniculata* subsp. *iodandra*, Robinson [1898] mentions the plant's "slightly bilobed" stigma.) Our investigations confirmed their description and also found the stigmas of *B. texana* to be spreading. Figure 2 shows the short styles and spreading stigmas of *B. paniculata* and *B. texana* versus the elongated styles and mostly or completely connivent stigmas of *B. verna* and *B. virginica*. Both characters (style shape and stigma morphology) may be useful for identifying species in the field. They may also help explain the presence of two types of capsular dehiscence in the genus, as noted by Wood and Weaver (1982): medial (capsules splitting medially below a persistent style) for *B. verna* and *B. virginica*, and apical (capsules that split apically into two separate valves) for *B. paniculata* and *B. texana*.

Phylogenetic Results— BLAST searches revealed that *Bartonia* ITS sequences were highly similar to other Gentianaceae ITS sequenes in GenBank and not to fungal sequences. The aligned data matrix of the entire ITS region contained 684 characters, 136 (20%) of which were parsimony-informative. This included 34 coded indels, 14 (41%) of which were informative. The 160 bp 5.8S coding region was missing from four of the sequences downloaded from GenBank, but this region was almost invariant among the remaining taxa (containing one variable character), so it was not removed from the data matrix. This resulted in 8% missing data in the matrix. Two shortest trees were found consisting of 357 steps, with an ensemble consistency index excluding uninformative characters (CI; Kluge and Farris 1969) = 0.71 and the ensemble retention index (RI; Farris 1989) = 0.81. One of the two phylograms is shown in Fig. 3a. The differences in the two trees were found only among outgroup taxa; relationships within *Bartonia* were stable. *Bartonia* was monophyletic with 100% bootstrap support (BS). There was a relatively long branch leading to *Bartonia* containing 47 or 49 changes, depending on the tree, using ACCTRAN optimization in the parsimony searches. *Bartonia verna* and *B. virginica* (both accessions) were supported as sister species (100% BS). Another clade contained *B. texana* and both subspecies of *B. paniculata* (100% BS). Within this clade, *B. texana* and *B. paniculata* subsp. *iodandra* were sister taxa (64% BS). Regarding outgroup relationships, *Swertia tashiroi* was the sister taxon to *Bartonia* (68% BS) in both ITS trees, and *Obolaria* and *Latouchea* formed a clade (64% BS). Only *Megacodon* and *Gentianella* changed position between the two most-parsimonious trees. If one or both of these two taxa were removed from the ITS matrix, a single most-parsimonious tree was found with relationships within *Bartonia* unchanged. There was also a relatively long branch (58–59 changes) leading to *Gentiana decora*. von Hagen and Kadereit (2002) identified potential problems with long-branch attraction (Felsenstein 1978) between *Bartonia* and members of subtribe Gentianinae (here represented by *Gentiana*). However, in our analyses *Bartonia* was not placed at the base of the tree near *Gentiana*, and if *Gentiana* was removed from the analysis, other relationships were unchanged.

The aligned chloroplast data matrix contained 1,000 characters, 103 (10%) of which were parsimony-informative. This included 81 coded indels, 19 (23%) of which were informative. The 50 bp coding region between the *trnL* intron and the *trnL-trnF* spacer was missing from the five sequences downloaded from GenBank, however this region was almost invariant among the remaining taxa (containing two variable characters), so it was not removed from the data matrix. This resulted in 2% missing data in the matrix. A single most-parsimonious tree was found (330 steps, CI = 0.81, RI = 0.89; Fig. 3b). *Bartonia* was monophyletic with 100% bootstrap support. The branch leading to *Bartonia* was relatively long here too, containing 38 changes, but branches leading to *Gentianella* (33 changes) and *Gentiana* (67 changes) were also relatively long. As in the ITS trees, a sister group relationship between *B. virginica* (both accessions) and *B. verna* was supported (100% BS), and a clade containing *B. texana* and both subspecies of *B. paniculata* was recovered (100% BS). However, in the chloroplast tree, there was 100% support for a sister relationship between *B. texana* and *B. paniculata* subsp. *paniculata*, with subsp. *iodandra* sister to these two taxa, which was different

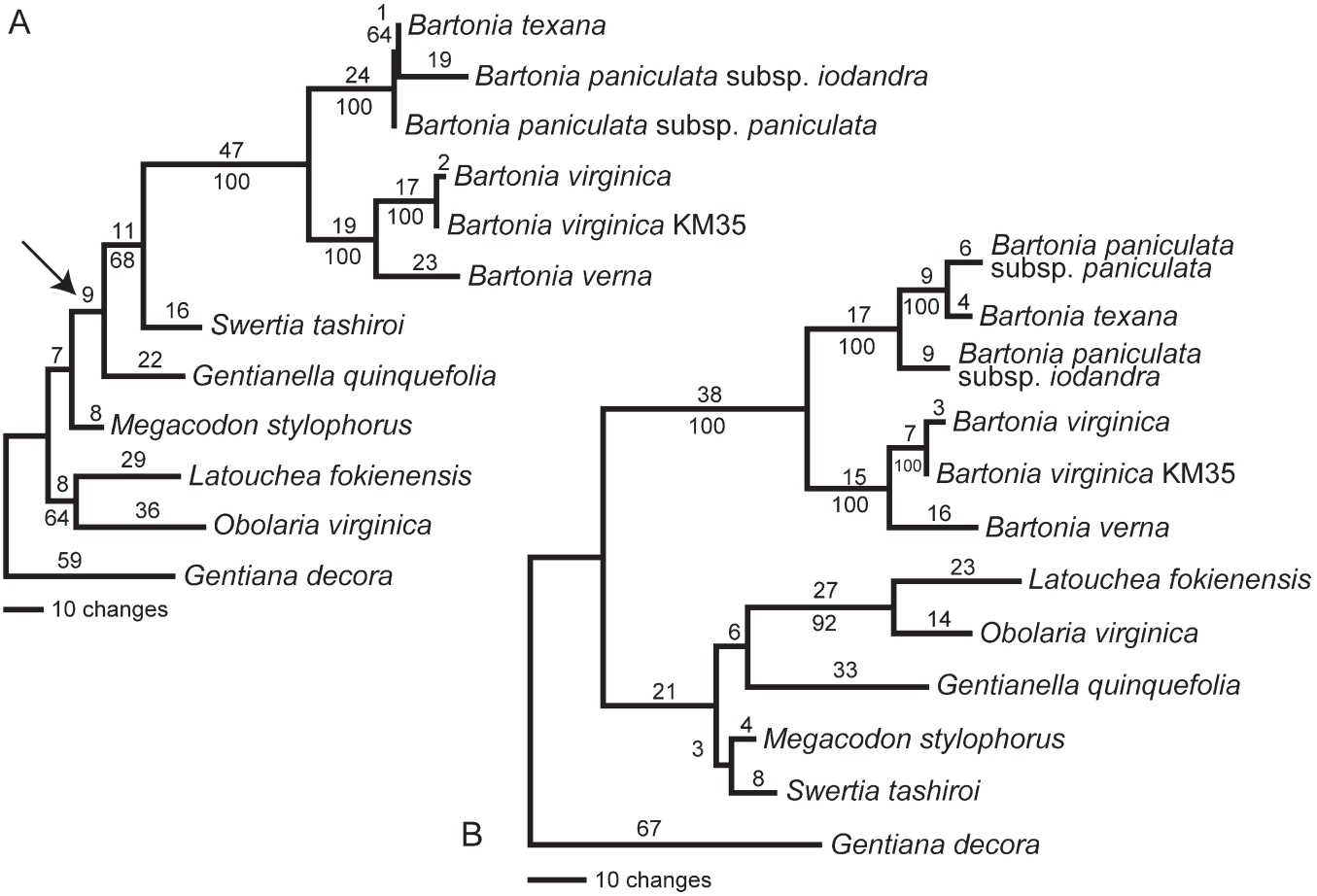

Fig. 3. Phylograms from separate molecular data parsimony analyses. Numbers above branches are branch lengths. Numbers below branches are bootstrap values greater than 50%. A. ITS analysis: one of two most parsimonious trees (length = 357; CI = 0.71; RI = 0.81). The arrow indicates the branch that collapses in the strict consensus tree. B. Chloroplast DNA analysis: single most parsimonious tree (length = 330; CI = 0.81; RI = 0.89).

from the relationships in this clade found in the ITS trees. For outgroup relationships, a clade containing all the outgroups but *Gentiana* was sister to the *Bartonia* clade (41% BS). Within this outgroup clade, *Megacodon* and *Swertia* were sister taxa (48% BS). Also, *Latouchea*, *Obolaria,* and *Gentianella* formed a clade (34% BS), with *Latouchea* and *Obolaria* sister taxa (92% BS) within that grouping.

The partition-homogeneity test returned a *p* value of 0.647, indicating that there was not significant heterogeneity between the two molecular data sets. Additonally, the *Bartonia* relationships were largely concordant between the nuclear and chloroplast results, with the majority of the phylogenetic conflicts being among the outgroups. We therefore conducted a combined data analysis of the two molecular datasets. The combined data matrix contained 1,684 characters, 239 (14%) of which were parsimony-informative. One most-parsimonious tree was found (689 steps, CI excluding uninformative characters=0.74, RI = 0.84; Fig. 4). *Bartonia* was monophyletic (100% BS), *B. verna* was sister to *B. virginica* (both accessions) (100% BS), and *B. texana* and both subspecies of *B. paniculata* again formed a clade (100% BS). *Bartonia texana* was sister to *B. paniculata* subsp. *paniculata* (98% BS), as in the chloroplast results. *Swertia* was sister to *Bartonia* (66% BS) as in the ITS trees, followed by *Megacodon* (35% BS), *Gentianella* (71% BS), and the sister group of *Obolaria* and *Latouchea* (91% BS). The Bayesian analysis tree showed the same topology as the parsimony combined data strict consensus tree (Fig. 4).

A backbone constraint analysis was conducted on the combined molecular data set forcing *B. verna* to be outside of a clade containing *B. virginica* and *B. paniculata*, as hypothesized by Gillett (1959). This resulted in three most-parsimonious trees of 715 steps, 26 steps longer than the shortest tree found in the unconstrained analysis of the same data.

Discussion

The morphological study confirmed the circumscriptions of *Bartonia paniculata* (both subspecies), *B. verna*, and *B. virginica* based on testing the diagnostic characters given in the original descriptions. The phylogenetic results strongly supported the monophyly of *Bartonia* and the sister-group relationship of *B. virginica* + *B. verna*, as opposed to *B. verna* being the earliest diverging species, as was suggested by Gillett (1959). The molecular data show many synapomorphies (19 nuclear, 15 chloroplast) between *B. virginica* and *B. verna*. Morphologically, *B. virginica* and *B. verna* share opposite to subopposite leaves, relatively long, narrow styles with connivent stigmas, and capsular dehiscence below the persistent style ("medial dehiscence," also present in tribe Helieae of Gentianaceae; Struwe et al. 2002). Opposite leaves is presumed to be a symplesiomorphy for these taxa, as most gentians have opposite leaves, including all outgroup taxa in this study. The direction of character state change for the style and stigma traits and capsule dehiscence cannot be assessed at this time,

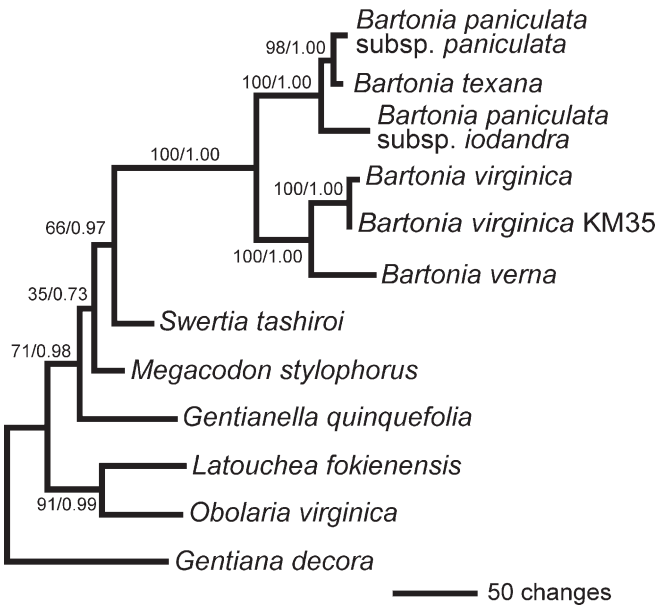

FIG. 4. Single shortest tree from the parsimony analysis of ITS and cpDNA data combined. Numbers at branches are bootstrap values/ Bayesian posterior probability values. (Length = 689; CI = 0.74; RI = 0.84). This tree is in topological agreement with the Bayesian analysis tree.

however, as alternate states occur among outgroup taxa in Swertiinae and Gentianinae. Capsule dehiscence is largely dependent on the persistence or nonpersistence of the style, a character that has been underutilized in classification and descriptions of taxa in the whole family and needs further attention. According to Nilsson and Skvarla (1969), *B. virginica* and *B. verna* also share the same pollen exine morphology (coarse reticulum and less closely-spaced bacules), which differs from that of *B. paniculata* (fine reticulum and closely-spaced bacules). Based on the phylogenetic analyses we conclude that *B. verna* diverged from a common ancestor shared with *B. virginica*, rather than directly from the common ancestor of all *Bartonia* species.

This phylogenetic study also provides genetic evidence for the close relationship of *Bartonia texana* to *B. paniculata*, and its possible derivation from within the latter. *Bartonia texana* nests with the two subspecies of *B. paniculata* in all phylogenetic analyses, supported by 24 shared base-pair changes in the ITS tree and 17 in the chloroplast tree, causing *B. paniculata* to be paraphyetic. This is more likely due to shared ancestry and lack of divergence between *B. texana* and *B. paniculata* rather than intergradation, particularly considering the great geographic distance between our samples of both subspecies of *B. paniculata* and *B. texana* (approximately 2,000 km). Furthermore, as shown in the taxonomic study, morphologically *B. texana* displays a subset of the quantitative traits found in *B. paniculata*, and the two share alternate leaves, short, swollen styles with spreading stigmas, and apical capsule dehiscence, so that *B. texana* is not diagnosable based on unique character states as are other species of *Bartonia*. Thus, using a combination of monophyly (sensu de Queiroz and Donoghue 1988) and diagnosibility (Nixon and Wheeler 1990) to define species boundaries, *B. texana* should not be recognized as a distinct species from *B. paniculata*.

However, since *B. texana* appears to be a geographically well-defined and confined group, perhaps restricted by groundwater seepage from the Fleming Formation (MacRoberts and MacRoberts 1990), we recommend that it be recognized as a subspecies of *B. paniculata* and thereby not completely submerged in *B. paniculata* (see Taxonomic Treatment below). Maintenance of the rank of *B. paniculata* subsp. *iodandra* is supported by evidence of its free intergradation with *B. paniculata* subsp. *paniculata* where the two meet, a phenomenon also noted by Gillett (1959). But the two subspecies also have geographically distinct populations that clearly differ from one another in a number of traits, including mean height, leaf number, habit, and stem color.

The possibility of hybridization between *Bartonia virginica* and *B. paniculata*, which are sympatric over much their ranges, needs further study. In this regard, we examined 20 mixed-species herbarium sheets. Of these, only four contained plants with combinations of traits of both species. A handful of other sheets of individual species also contained plants with combinations of traits of both species. However, no plants with "intermediate" character states were found.

Conclusions about outgroup relationships cannot be made here due to inadequate sampling among outgroup taxa, except that there is no evidence for *Obolaria* being the sister taxon to *Bartonia*. Instead, *Obolaria* consistently forms a clade with *Latouchea*, a monotypic genus endemic to SE and SW China (Ho and Pringle 1995), a relationship also found by Chassot et al. (2001). Our study also confirms the isolated position of *Bartonia* within the subtribe, as shown by the long branch separating it from the outgroup taxa. von Hagen and Kadereit (2002) postulated that large pairwise genetic differences between *Bartonia* and other basal Swertiinae may be due to an increased substitution rate in *Bartonia*, rather than early divergence. If true, this may correlate with the supposedly mycotrophic habit of *Bartonia*. In any case, the two North American genera *Bartonia* and *Obolaria* have clearly diverged separately and possibly in parallel from Swertiinae relatives.

Taxonomic Treatment

Bartonia Muhl. ex Willd. Ges. Naturf. Freunde Berlin Neue Schrift 3: 444. 1801.—TYPE: *Bartonia tenella* Muhl. ex Willd. (= *B. virginica* (L.) BSP.).

Centaurella Michx. Fl. Bor. Amer. 1: 97, 98 t. 12. f. 1 & 2. 1803. *Centaurium* (Michx.) Pers. Syn. Pl. 1: 137; non Hill, 1756. *Andrewsia* (Michx.) Spreng. Syst. Veg. 1: 428. 1825; non *Andreusia*, Vent, 1804.—TYPE: *Centaurella verna* Michx. (= *Bartonia verna* (Michx.) Raf. ex Barton).

Small, inconspicuous, erect (sometimes twining) annual herbs, 4.7–41.0 cm tall, unbranched to sparsely branched. Stems slender, angular, green to purple. Leaves opposite to alternate, reduced to subulate scales, entire, 0.9–4.2 mm long. Inflorescence a simple or compound cyme, sometimes a single flower. Flowers 4-merous, pedicellate. Calyx campanulate, with a short tube or sepals free; sepal lobes subulate to lanceolate. Corolla campanulate, deeply lobed, cream-colored to white or greenish white, marcescent; lobes lanceolate, spatulate, oblong, or ovate; apices obtuse, mucronate, acute, acuminate, or apiculate, sometimes purple; margins entire or sometimes erose; aestivation contort (in *B. verna*). Stamens inserted in sinuses between corolla lobes; filaments short; anthers triangular to oblong, introrse, often deciduous after anthesis, yellow to purple, and with obtuse or apiculate apices. Pistil ovoid to ellipsoid; ovary bicarpellate, placentation parietal, the ovules scattered over the entire surface of the

ovary; style short, stout or slender; stigmas 2, spreading or connivent, decurrent at least to the apex of the ovary, sometimes further. Fruit capsular, ovoid to elliptic or oblong, dehiscing apically or medially. Seeds numerous, minute, brown, ovate.

Key to the Species

1. Leaves generally alternate; style stout with stigmas spreading; capsule dehiscing from the apex ..... 1. *B. paniculata*
1. Leaves generally opposite; style slender with stigmas connivent; capsule dehiscing medially 2
   2. Stems mostly green, purple near the roots; leaf nodes numerous and crowded at base; corolla lobes oblong, 2.5–4.0 mm long; summer- or fall-flowering ..... 2. *B. virginica*
   2. Stems mostly purplish to dark brown; leaf nodes few and arranged evenly; corolla lobes spatulate, 4.0–8.0 mm long; spring-flowering ..... 3. *B. verna*

1. Bartonia paniculata (Michx.) Muhl. Cat. Pl. Amer. Sept. 16. 1813. *Centaurella paniculata* Michx., Fl. Bor. Amer. 1: 98 f. 1. 1803.—TYPE: *Michaux s.n.* (holotype: P, the Richard Herbarium).

Plants 7.1–41.0 (mean 24.0) cm tall. Stems erect, occasionally twining, slender or sometimes stout, green to purple. Leaves usually alternate, but sometimes subopposite, opposite, or mixed, at least 2–24 (mean 13) leaves per plant, scale-like, 0.9–2.0 (mean 1.5) mm long. Flowers in simple or compound cymes, occasionally solitary. Calyx only fused at base, or free; tube 0.0–0.9 (mean 0.4) mm long; sinuses acute or rounded; lobes subulate to lanceolate, 1.6–2.9 (mean 2.2) mm long, 0.5–1.1 (mean 0.8) mm wide. Corolla campanulate, 1.9–6.3 (mean 4.1) mm long, cream-colored to whitish yellow in dried specimens to greenish white in living color (in *B. paniculata* subsp. *texana*); lobes oblong-lanceolate to ovate, 1.2–6.3 (mean 7.5) mm long, 0.6–2.2 (mean 1.6) mm wide, sometimes purple-tipped, and with acute, acuminate, or apiculate apices. Stamens 0.8–1.7 (mean 1.2) mm long; filaments yellow to purple; anthers triangular to oblong, 0.2–0.7 (mean 0.4) mm long, yellow to purple, or sometimes orange, and with obtuse or apiculate apices. Ovary 1.8–5.5 (mean 3.6) mm long, 1.0–2.5 (mean 1.7) mm wide; style stout, 0.3–1.1 (mean 0.7) mm long; stigmas spreading, or very rarely fused, decurrent to the apex of the ovary. Capsule 1.7–4.7 (mean 3.2) mm long (measured below the persistent style), 1.0–3.0 (mean 2.0) mm wide, dehiscing apically.

Key to the Subspecies

1. Plants relatively tall (mean 20.5–25.9 cm); stems usually green and slender; calyx sinuses acute; stamens usually yellowish 2
   2. Stems occasionally twining; corolla lobes usually oblong-lanceolate, 2.9–5.2 (mean 4.1) mm long. ..... 1a. *B. paniculata* subsp. *paniculata*
   2. Stems strictly erect; corolla lobes usually ovate acuminate, 1.9–3.2 (mean 2.6) mm long. ..... 1b. *B. paniculata* subsp. *texana*
1. Plants relatively short (mean 14.3 cm); stems usually purple and often stout; calyx sinuses frequently rounded; stamens frequently purplish. ..... 1c. *B. paniculata* subsp. *iodandra*

1a.) Bartonia paniculata (Michx.) Muhl. subsp. paniculata, Cat. Pl. Amer. Sept. 16. 1813. *Bartonia virginica* L. var. *paniculata* (Michx.) Boivin, Le Naturaliste Canadien 93: 1059. 1966. *Bartonia paniculata* (Michx.) Robinson, Rhodora 10: 35. 1908, superfl. comb.—TYPE: *Michaux s.n.* (holotype: P, the Richard Herbarium).

Bartonia tenella Muhl. ex Willd. var. *brachiata* Wood, Classbook, ed. 2, 586. 1866, ex char. excl. syn. —TYPE: Not designated.

Bartonia lanceolata Small, Fl. S.E. U.S. 932, 1336. 1903.—TYPE: *Chapman s.n.* (holotype: NY, photo!).

Plants 10.7–41.0 (mean 25.8) cm tall. Stems erect, slender, sometimes twining, green, sometimes purple. Leaves usually alternate, but also subopposite, opposite, or mixed, at least 5–23 (mean 14) per plant, 0.9–2.0 (mean 1.5) mm long. Flowers in simple or compound cymes. Calyx fused only at base; tube 0.01–0.5 (mean 0.2) mm long, sinuses mostly acute; lobes subulate to lanceolate, 1.6–2.9 (mean 2.2) mm long, 0.5–1.1 (mean 0.8) mm wide. Corolla 2.9–5.2 (mean 4.0) mm long, cream colored in dried specimens; lobes oblong-lanceolate and with acute apices, or sometimes ovate-acuminate to ovate-apiculate, 1.9–3.5 (mean 2.7) mm long, 0.9–2.2 (mean 1.6) mm wide, occasionally purple-tipped. Stamens 0.9–1.5 (mean 1.2) mm long; filaments yellow, sometimes purple; anthers triangular to oblong, 0.4–0.6 (mean 0.5) mm long, yellow, or sometimes orange or purple, with obtuse or apiculate apices. Ovary 2.1–4.4 (mean 3.3) mm long, 1.2–1.7 (mean 1.4) mm wide; style 0.3–1.1 (mean 0.7) mm long; stigmas spreading. Capsule 2.8–4.2 (mean 3.5) mm long, 1.7–2.5 (mean 2.1) mm wide.

Distribution—*Bartonia paniculata* subsp. *paniculata* is found from Massachusetts southward to Florida, westward to Texas, and northward to Arkansas and Tennessee. It grows in moist woods, bog margins, swamps and swamp borders, low grasslands, pine savannahs, and wet, open thicket in sandy or clayey soils.

Phenology—Plants have been found flowering from August through October and fruiting from September through October.

1b.) **Bartonia paniculata** (Michx.) Muhl. subsp. **texana** (Correll) K. Mathews, Dunne, E. York, & Struwe, stat. nov. *Bartonia texana* Correll, Wrightia 3: 181–191. 1966.—TYPE: U.S.A. Texas: Tyler Co., along Creek, 7.5 miles SE of Colmereil on Rt. # 256, on sphagnum moss forested hills, plants green, 10 Oct 1965, *D.S. Correll 32006* (holotype: LL!).

Plants 15.2–35.7 (mean 20.45) cm tall. Stems erect, slender, green, occasionally purple at the base. Leaves mainly alternate but occasionally subopposite toward the top of stem, at least 8–24 (mean 16) per plant, 0.9–1.9 (mean 1.4) mm long. Flowers in simple or compound cymes. Calyx fused only at base; tube 0.1–0.2 (mean 0.15) mm long; sinuses acute; lobes subulate, 1.1–1.9 (mean 1.5) mm long, 0.6–0.9 (mean 0.75) mm wide. Corolla 1.9–3.2 (mean 2.55) mm long, greenish white (according to herbarium sheet notation); lobes ovate-acuminate to

ovate-apiculate, 1.2–2.0 (mean 1.6) mm long, 0.8–1.4 (mean 1.1) mm wide. Stamens 0.8–1.2 (mean 1.0) mm long; filaments yellow, sometimes purple; anthers triangular to oblong, 0.2–0.6 (mean 0.4) mm long, yellow, with apiculate to obtuse apices. Ovary 1.8–2.7 (mean 2.25) mm long, 1.0–1.8 (mean 1.4) mm wide; style 0.4–0.8 (mean 0.6) mm long; the two stigmas spreading. Capsule 1.7–2.7 (mean 2.2) mm long, 1.0–1.8 (mean 1.4) mm wide.

Distribution—*Bartonia paniculata* subsp. *texana* is endemic to southeastern Texas (including Hardin County, Nacogdoches County, Newton County, Polk County, St. Augustine County, San Jacinto County, and Tyler County) and northeastern Louisiana (including Jackson Parish, Ouichita Parish, and Caddo Parish). It grows in acid seepage swamp forests, small ravine heads, and wet spots on forested hills and cut-over woods.

Phenology—Plants have been found flowering from August through October and fruiting in October.

1c.) Bartonia paniculata subsp. iodandra (Robins.) Gillett, Rhodora 61: 43–62. 1959. *Bartonia iodandra* Robins., Bot. Gaz. 26: 47. 1898. *Bartonia paniculata* var. *iodandra* (Robins.) Fern, Rhodora 23: 288. 1922.—TYPE: CANADA. Newfoundland: near South Arm River, Holyrood, 23 Aug 1894, *Robinson & Schrenk 5* (isotype: NY!).

Bartonia iodandra var. *sabulonensis* Fern, Proc. Bost. Soc. Nat. Hist. 36: 89. 1921. *Bartonia paniculata* var. *sabulonensis* Fern, Rhodora 23: 288. 1922.—TYPE: CANADA. Nova Scotia: East side of Life Saving Station #3, 12 Sep 1913, *St. John 1307* (isotype: NY!).

Bartonia paniculata var. *intermedia* Fern, Rhodora 23: 287. 1922.—TYPE: CANADA. Nova Scotia: Digby Co., along Little River, east of Tiddville, wet peaty hollow in savannahs, 20 Aug 1920, *Fernald & Long 22299* (isotype: NY!).

Plants 7.1–21.4 (mean 14.2) cm tall. Stems erect, slender to stout, usually purple. Leaves mainly alternate, at least 2–9 per plant (mean 5.5), 0.9–1.9 (mean 1.4) mm long. Flowers in simple or compound cymes, occasionally solitary. Calyx fused at base, or unfused; tube 0.0–0.9 (mean 0.45) mm long, sinuses acute to rounded; lobes subulate to lanceolate, 1.9–3.9 (mean 2.9) mm long, 0.5–1.1 (mean 0.8) mm wide. Corolla whitish yellow, 2.7–6.3 (mean 4.5) mm long in dried specimens; the lobes variable, oblong-lanceolate to ovate, 2.7–6.3 (mean 4.5) mm long, 0.9–1.8 (mean 1.35) wide, frequently purple-tipped, and with acuminate to acute apices. Stamens 0.9–1.7 (mean 1.3) mm long; the filaments yellow to purplish yellow; anthers triangular-apiculate to oblong-apiculate, 0.3–0.7 (mean 0.5) mm long, yellow or purple. Ovary 2.8–5.5 (mean 4.1) mm long, 1.5–2.5 (mean 2.0) mm wide. Style 0.7–1.0 (mean 0.85) mm long; stigmas spreading, rarely fused. Capsule 3.5–4.7 (mean 4.1) mm long, 1.5–3.0 (mean 2.25) mm.

Distribution—*Bartonia paniculata* subsp. *iodandra* is found from Newfoundland south to Massachusetts. It grows in wet, peaty hollows, sphagnous bogs, peaty turf, and damp lake shores in sandy soil.

Phenology—Plants have been found flowering from August through September and fruiting in September.

2. Bartonia verna (Michx.) Raf. ex Barton, Fl. Virg. 51. 1812. *Centaurella verna* Michx., Fl. Bor. Amer. 1:98 t. 12. f. 2. 1803. *Centaurium vernum* (Michx.) Pers., Syn. Pl. 1: 137. 1805. *Centaurella vernalis* Pursh, Fl. Amer. Sept. 1: 99. 1814, ex. char. *Centaurella vernalis* var. *uniflora* Pursh. l.c. 100, ex. char. *Andrewsia verna* (Michx.) Spreng., Syst. Veg. 1: 428. 1825.—TYPE: *Michaux s.n.* (holotype: P, Richard Herbarium).

Plants 4.7–22.1 (mean 13.4) cm tall. Stems erect, slender, usually purple. Leaves mainly opposite, sometimes subopposite, at least 7–11 (mean 9) per plant, 0.9–1.9 (mean 1.4) mm long. Flowers usually solitary, occasionally in simple cymes. Calyx unfused or fused only at base; tube 0.0–0.5 (mean 0.25) mm long; sinuses acute; lobes lanceolate to subulate, 1.8–3.5 (mean 2.65) mm long, 0.9–1.5 (mean 1.2) mm wide. Corolla 4.0–8.0 (mean 6.0) mm long, white (according to herbarium sheet annotations); lobes spatulate to obovate, 3.0–6.0 (mean 4.5) mm long, 1.9–2.9 (mean 2.4) mm wide, with obtuse to obtuse-mucronate apices. Stamens 2.1–3.0 (mean 2.55) mm long; the filaments purple to yellow; anthers oblong, sometimes oblong apiculate, 0.8–1.2 (mean 1.0) mm long, primarily yellow. Ovary 3.5–6.9 (mean 5.2) mm long, 1.5–2.5 (mean 2.0) mm wide. Style slender, 1.6–2.1 (mean 1.85) mm long; stigmas connivent and decurrent down to 1/3 of the ovary. Capsule 3.5–5.7 (mean 4.6) mm long (measured below persistent style), 1.9–3.7 (mean 2.8) mm wide, dehiscing medially.

Distribution—*Bartonia verna* is found from Virginia south to Florida and west to eastern Texas. It grows in open bogs, moist pine barrens, flat pine woods, palmetto woods, ditches, savannah marshes, and sphagnous areas in wet, sandy soil.

Phenology—Plants have been found flowering from November through March and fruiting in April.

3. Bartonia virginica (L.) Britton, Sterns & Poggenb., Cat. N.Y. Pl. 36. 1888. *Sagina virginica* L., Sp. Pl. 2: 28. 1753.—TYPE: *Clayton 649* (syntype: BM, photo!).

Bartonia tenella Muhl. ex Willd., Ges. Naturf. Freunde Berlin, Neue Schrif. 3: 444. 1801.—TYPE: *Muhlenberg s.n.* (holotype: B, Willd. Herb.).

Centaurella autumnalis Pursh, Fl. Amer. Sept 1: 100. 1814. *Andrewsia autumnalis* (Pursh.) Spreng., Sys. Veg. 1: 428. 1825.—TYPE: pro parte type based on *S. virginica* L.

Centaurella moseri Steudel & Hochstein ex. Griseb., Gen. et Sp. Gent. 308. 1839. *Bartonia moseri* (Steud. & Hochst. ex Griseb.) Robins. & Schrenk ex Gilg., in Engl. & Prantl. Nat. Pflanzef. 4, abt. 2: 76. 1895.—TYPE: Not designated (see Gillett 1959).

Centaurella autumnalis Pursh var. *brachysepala* Griseb., Gen. et Sp. Gent. 308. 1839. —TYPE: *Drummond s.n.* (location unknown).

Bartonia virginica (L.) Britton , Sterns & Poggenb. forma *abortiva* Vict., Roy. Soc. Can. Proc. & Trans. Ser. III. Sect. V. 13: 113. 1919.—TYPE: *Victorin 19570* (holotype: MT).

Plants 11.8–37.0 (mean 24.4) cm tall. Stems erect, slender, green, usually with purple bases. Leaves opposite to subopposite, often crowded towards the base, at least 13–28 (mean 20.5) per plant, 1.8–4.2 (mean 3.0) mm long. Flowers in simple or compound cymes. Calyx fused only at base; tube 0.01–0.3 (mean 0.15) mm long; sinuses acute; lobes subulate to lanceolate, 1.8–2.7 (mean 2.25) mm long, 0.7–1.0 (mean 0.85) mm wide. Corolla 2.5–4.0 (mean 3.0) mm long, whitish yellow in dried specimens; lobes oblong, 1.8–2.7 (mean 2.25) mm long, 0.6–1.1 (mean 0.85) wide, with obtuse to obtuse-mucronate, occasionally purple apices. Stamens 1.5–2.0 (mean 1.75) mm long; the filaments usually yellow; anthers oblong-apiculate to triangular apiculate, 0.2–0.8 (mean 0.5) mm long, yellow. Ovary 2.3–5.1 (mean 3.7) mm long, 1.2–1.9 (mean 1.55) mm

wide; style slender to tapering, 0.5–1.7 (mean 1.1) mm long; stigmas connivent and decurrent to just below tip of ovary. Capsule 2.8–3.4 (mean 3.1) mm long, 1.5–1.9 (mean 1.7) mm wide, dehiscing medially.

Distribution—*Bartonia virginica* is found from Nova Scotia and Quebec south to Florida and west to Michigan and Louisiana. It grows in wet, open forests, bogs, sphagnous pond margins, swamps, open thickets, dry ledges, and blueberry barrens in sandy, silt-loam, or clay soils.

Phenology—Plants have been found flowering from July through October and fruiting from September through October.

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Appendix 1. DNA vouchers are listed alphabetically with taxon name, locality, collector, herbarium in which deposited, and GenBank accession number (*trnL-F* region or *trnL* intron/*trnL-F* spacer, ITS region or ITS1/ITS2). Taxa for which previously published GenBank accessions were used in this study are noted with an asterisk (voucher information not available). Herbarium abbreviations follow Index Herbariorum.

Bartonia paniculata* subsp. *paniculata. U.S.A. New Jersey: Wharton State Forest, *M. Palmer s.n.* (WCUH), *trnL-F* region (EU834125), ITS region (EU812470). ***Bartonia paniculata* subsp. *iodandra***. Canada. Western Newfoundland: Bonnie Bay, *Fernald et al. 1962* (PH), *trnL-F* region

(EU834126), ITS region (EU812472). ***Bartonia texana***. U.S.A. Louisiana: Caddo Parish, *C. Reid 4638* (TEX/LL), *trnL-F* region (EU834124), ITS region (EU812471). ***Bartonia verna***. U.S.A. North Carolina: Brunswick Co., *Wilbur 72349* (DUKE), *trnL-F* region (EU834128), ITS region (EU812473). ***Bartonia virginica*** **KM35**. U.S.A. North Carolina: Jackson Co., *K. Mathews s.n.* (WCUH), *trnL-F* region (EU834127), ITS region (EU812474). ***Bartonia virginica****, *trnL* intron/*trnL-F* spacer (AJ315185/AJ315231), ITS 1/ITS2 (AJ3060789/AJ306106). ***Gentiana decora***, U.S.A. North Carolina, Jackson Co., *K. Mathews s.n.* (WCUH), *trnL-F* region (EU834122), ITS region (EU812468). ***Gentianella quinquefolia***, U.S.A. North Carolina: Jackson Co., *D. Pittillo 12106* (WCUH), *trnL-F* region (EU834123), ITS region (EU812469). ***Obolaria virginica****, *trnL* intron/*trnL-F* spacer (AJ315201/AJ315247), ITS 1/ITS2 (AJ318549/AJ410328). ***Megacodon stylophorus****, *trnL* intron/*trnL-F* spacer (AJ315200/AJ315246), ITS region (AY858679). ***Latouchea fokienensis****, *trnL* intron/*trnL-F* spacer (AJ318544/AJ410323), ITS1/ITS2 (AJ318544/AJ410323). ***Swertia tashiroi****, *trnL* intron/*trnL-F* spacer (AJ315268/AJ315222), ITS1/ITS2 (AJ318570/AJ410349).

Appendix 2. Specimens examined for the morphological analyses and taxonomic treatment. Taxon name assignments are those accepted in the taxonomic treatment. Herbarium abbreviations follow Index Herbariorum.

Bartonia paniculata subsp. *paniculata*. U.S.A. Alabama: Escambia Co., 1 mile N of Dixonville, *S.L. Orzell & E.L. Bridges 11774* (LL); Mobile Co., 1.8 miles W of U.S. (?) at Chunchula, *S.L. Orzell & E.L. Bridges 12393* (LL). Arkansas: Pulaski Co., Little Rock, *H.E. Hasse s.n.* (NY). Florida: Liberty Co., Apalachicola National Forest, *S.L. Orzell & E.L. Bridges 11987* (LL). Louisiana: Grant Co., Kisatchie National Forest near Tabor Rd. SW of Georgetown, *R.D. Thomas 21593* (LL). Massachusetts: Plymouth Co., *S.F. Blake 4452* (LL); Plymouth Co., sandy shore of Benjamin's Pond, *S.F. Blake 10985* (LL); Plymouth Co., Wareham, *S.F. Blake 11005* (LL). Mississippi: Harrison Co., 1.5 miles N of Riceville, *S.L. Orzell & E.L. Bridges 12592* (LL); Jackson Co., DeSoto National Forest, *S.L. Orzell & E.L. Bridges 8498* (LL); Stone Co., 8.5 miles E of McHenry, *S.L. Orzell & E.L. Bridges 8516* (LL). New Jersey: Atlantic Co., left of road, Hammonton to Batsto, near Jobe Bridge, *S.S. van Pelt s.n.* (PH); Burlington Co., Bass River, Munion Field, N of U.S. Naval Reservation, Warren Grove Target Area, *G. Moore 7561* (BKL); Gloucester Co., stream, 1 mile NE downtown, *F.R. Fosberg 14511* (PH); Ocean Co., Berkeley, *H.K. Svenson s.n.* (BKL). New York: Location unknown: near New York *(Torrey Herbarium) s.n,* (NY); Nassau Co., Bellmore, *W.M.C. Ferguson 5129* (BKL); Nassau Co., Place's Pond, *F.A. Mulford s.n.* (BKL); Orange Co., Cornwall, Blackrock Forest, *K. Barringer 6380* (BKL); Orange Co., West Point Military Reservation, Popolopen Brook, SE of Stillwell Lake, *S. Clemants & K. Barringer 4134* (BKL); Suffolk Co., Cold Spring Harbor, *W.M.C. Ferguson 5347-1/2* (BKL); Suffolk Co., Fisher Island, *C.C. Hanmer 3568* (BKL); Suffolk Co., Long Island, Montauk Point, *W.M.C. Ferguson 4600* (BKL); Suffolk Co., Neapeague, *H.N. Moldenke 23075* (LL); Suffolk Co., Ocean Beach, *L.H. Lightpipe s.n.* (BKL, LL); Suffolk Co., Long Island, *H.K. Svenson 6802* (BKL); Suffolk Co., Long Island, Wading River, Riverhead, *E.S. Miller s.n.* (BKL). Pennsylvania: Berks Co., 2.6 miles NNE of Oley Furnace, near Bieber Creek, *W.C. Brumbach 2631* (PH); Berks Co., 3/4 mile W of Stony Creek Mills, *H. Wilkins 4313* (PH); Bucks Co., 1 mile SE Roelefs, *B. Long 31555* (PH); Bucks Co., 2 miles W of Morrisville, *W.L. Dix s.n.* (PH). Tennessee: Carroll Co., Bruceton, Hollow Rock Jc., *H.K. Svenson 4382* (BKL); Coffee Co., N of Manchester, *H.K. Svenson 9124* (BKL); Grundy Co., between Altamont and Beersheba Springs, *H.K. Svenson 9189* (BKL). Texas: Anderson Co., Andrews Bog, Engeling Wildlife Management Area, *S.L. Orzell & E.L. Bridges 8631* (LL); Anderson Co., N end of EWMA, Chester's Pitcher Plant Bog, *B.R. & M.H. MacRoberts 3688* (LL); Houston Co., Grapeland, *E.J. Palmer 14397* (BKL); Jasper Co., NE corner of Little Rocky Preserve, *W.R. Carr 17042* (LL); Kisatchie National Forest, Beside Johns Hill Road, *R.D. Thomas 21605* (LL).

Bartonia paniculata subsp. *texana*. U.S.A. Louisiana: Jackson Parish, 4 miles NW of Chatham, *R.D. Thomas 21612* (LL); Jackson Parish, *R.D. Thomas & R. Reid 21147* (LL); Ouichita Parish, beside Jim Reeves Rd., 3 miles SW of Luna, *R.D. Thomas 21362* (LL). Texas: Nacogdoches Co., SFA Experimental Forest, about 11 miles S of Nacogdoches, *E.S. Nixon & T.R. Ward 9644* (BRIT); Polk Co., in ravine N of timber rd. and S of Hickory Creek, 0.3 miles W of Tyler Co., *S.L. Orzell & E.L. Bridges 8450* (BRIT, LL); Saint Augustine Co., *S.L. Orzell & E.L. Bridges 8397* (LL); Tyler Co., along creek, 7.5 miles SE of Colmereil on Rt. # 256, on sphagnum moss forested hills, *D.S. Correll 32006* (LL, **holotype of *B. texana***); Tyler Co., baygall at base of slope, on E side of Jacks Creek, *S.L. Orzell & E.L. Bridges 8462* (BRIT, LL).

Bartonia paniculata subsp. *iodandra*. Canada. Newfoundland: Near South Arm River, Holyrood, *Robinson & Schrenk 5* (NY, **isotype)**; Laurentian area at head of Exploits stream system, quarry, *M.L. Fernald & K.M. Wiegand 6082* (NY). Nova Scotia: East side of Life Saving Station #3, *St. John 1307* (NY, **isotype of *B. iodandra* var. *sabulonensis***); Digby Co.: Along Little River, east of Tiddville, wet peaty hollow in savannahs, *Fernald & Long 22299* (NY, **isotype of *B. paniculata* var. *intermedia***); Digby Co., Central Grove, *M.L. Fernald & B. Long 388* (BKL, LL, PH); Lunenburg Co., bordering Rhodeniser Lake, east of Bridgewater, *M.L. Fernald & B. Long 24384* (NY); Lunenburg Co., west of Bridgewater, *M.L. Fernald & B. Long 24390* (NY); Queens Co., *M.L. Fernald & C.H. Bissell 22292* (NY, PH); Shelburne Co., *M.L. Fernald & B. Long 24387* (PH); Shelburne Co., Harper Lake, *M.L. Fernald & B. Long 24379* (NY); Yarmouth Co., *M.L. Fernald et al. 22291* (NY, PH); Yarmouth Co., Galveston, by Tusket Lake, *M.L. Fernald & B. Long 389* (LL, PH); Yarmouth Co., Gavelton, cobble beach of Butler's (Gavelton) Lake, *M.L. Fernald et al. 22303* (NY, PH); Yarmouth Co., sandy shore of Great Pubnico Lake, *M.L. Fernald et al. 22305* (PH); Yarmouth Co., Gavelton, Butler's Lake, *M.L. Fernald et al. 22971* (NY). U.S.A. Massachusetts: Essex Co., Newburyport, *Robinson & Eaton s.n.* (LL); Nantucket Co., Nantucket, *E.P. Bicknell s.n.* (NY).

Bartonia verna. U.S.A. Location unknown, *E.B. Taylor s.n.* (LL). Florida: Location unknown, *A.W. Chapman s.n.* (NY); Alachua Co., *H.B. Meredith s.n.* (PH); Clay Co., Hibernia, *W.M. Canby s.n.* (BKL); Dade Co., *A.A. Eaton 582* (LL); Dade Co., west of Miami, *A.A. Eaton s.n.* (LL); Duval Co., Jacksonville, *A.H. Curtis 2251* (BKL); Duval Co., Jacksonville, *A.H. Curtis s.n.* (PH); Duval Co., S of Jacksonville, *L.H. Lightpipe s.n.* (BKL); Gulf Co., McNeils, *A.L. Moldenke & H.N. Moldenke 26667* (LL); Lee Co., vicinity of Fort Myers, *J.P. Standley 400* (PH); Liberty Co., south of Telogia along Hwy. 65, 0.7 miles S of Hwy. 67, *S. Sandberg with L. Hardison 1974* (LL); Martin Co., Jonathon Dickinson State Park, *D.S. Corell, J. Popenoe 48034* (LL); Orange Co., 3 miles W of Bithlo, *H.N. Moldenke 205* (PH); Osceola Co., Kissimmee, *E.A. Rau s.n.* (PH); Putnam Co., Pilatka, *G. Smith s.n.* (NY, PH); Walton Co., ca 12 miles S of Defuniak Springs, *R.K. Godfrey 54409* (NY). Georgia: Charlton Co., Broad Pile Point, *E.P. Walker 1528* (PH); Liberty Co., Leconte, *A.W. Chapman s.n.* (PH). Louisiana: St. Tammany Parish, E of Abita Springs, W of St. Tammany Airport, *R.D. Thomas 62793* (NY). Mississippi: Harrison Co., Biloxi, *H.S.M. Tracy 4928* (NY). North Carolina: Brunswick Co., 5 miles W of Southport, *R.K. Godfrey 48001* (LL); New Hanover Co., jct. of US 17 and 74 (just E of Wilmington), *H.E. Ahles with J. Haesloop 53161* (LL); New Hanover Co., vicinity of Wilmington, *E.B. Bartram & B. Long 990* (PH); Onslow Co., *S.F. Blake s.n.* (LL). Texas: Location unknown, *T. Drummond 221* (LL); Tyler Co., *G. Watson s.n.* (LL); Tyler Co., on Pitcher Plant Trail, ca. 3.5 km S of FM 1943 on E side of Turkey Creek Unit, Big Thicket National Preserve, *B.R. & M.H. MacRoberts 3757* (LL).

Bartonia virginica. CANADA. Nova Scotia: Queens Co., Port Joli, near Louis Lake, *M.L. Fernald et al. 390* (BKL); Queens Co., Port Mouton, *C.H. Bissell 22294* (NY); Shelbourne Co., N of Jordan Falls, *M.L. Fernald & B. Long 24363* (NY); Yarmouth Co., *M.L. Fernald et al. 22289* (NY). Quebec: Sainte-Therese, compte de Terrebonne, dans la tourbiere, *F.M. Victorin and R. F. Roland-Germain 49713* (LL). U.S.A. Alabama: Mobile Co., *S.L. Orzell & E.L. Bridges 12396* (LL). Florida: Location unknown, *E. Palmer 434* (NY). Georgia: Sumpter Co., near Barlow's Mill, *R.M. Harper s.n.* (BKL). Indiana: Location unknown, Miller's, *L.M. Umbach s.n.* (LL); Steuben Co., 2 miles E of Orland, *R.C. Friesner 6137* (LL). Massachusetts: Barnestable Co., Provincetown, *S.F. Blake 3243* (LL); Dukes Co., Chappaquiddick Island, *E.P. Bicknell 7048* (BKL); Middlesex Co., Natick, *F.F. Forbes s.n.* (LL); Norfolk Co., Stoughton, *S.F. Blake 1992* (LL); Plymouth Co., *S.F. Blake 10991* (LL). Michigan: Barry Co., Rutland Twp., N shore of Otis Lake, *G.W. Parmelee 1633* (LL); Ingham Co., Meridian Twp., *G.W. Parmelee 3252* (LL); St. Clair Co., Near Port Huron, *C.K. Dodge 4409* (BKL). New Jersey: Cumberland Co., along Stathem Neck Rd., *G. Moore 6721* (BKL); Middlesex Co., Cheesequake, *C. Ericson 3359* (BKL); Middlesex Co., Milltown, *H. Dautun s.n.* (BKL); Middlesex Co., South Amboy, *L.H. Lightpipe s.n.* (BKL); Monmouth Co., Lake Como, *L.H. Lightpipe s.n.* (BKL); Monmouth Co., Spring Lake, *L.H. Lightpipe s.n.* (BKL); Monmouth Co., Neptune, *L.H. Lightpipe s.n.* (BKL); Ocean Co., Lakewood, *A.M. Greller 424* (BKL); Ocean Co., *J. Bright 17845* (LL); Ocean Co., *S. Calverley s.n.* (BKL); Pine Barrens, *S. Calverley s.n.* (BKL); Pine Barrens, Tom's River, *L.H. Lightpipe s.n.* (BKL). New York: Nassau Co., Hempstead Plains, 1-1/2 miles northeast of Hicksville, *R.M. Harper s.n.* (BKL); Nassau Co., Hempstead Plains, 1-1/2 miles south of Hicksville, *N. Taylor s.n.* (BKL); Nassau Co., Hicksville, L.I., *G.D. Hulst s.n.* (BKL); Nassau Co., Long Island, Hicksville, *K.O. Fernie s.n.* (BKL); Orange Co., Aleck Meadow Reservoir, Blackrock Forest, *S. Clemants 3775* (BKL); Richmond Co., Staten Island, *R.E. Zaremba 4334* (BKL); Suffolk Co., East Hampton, Montauk Downs Grassland, *R.E. Zaremba 7063* (BKL); Suffolk Co., Ocean Beach, *L.H. Lightpipe s.n.* (BKL); Suffolk Co., Long Island, West Islip, Willet's Creek, *H.K. Svenson 8116* (BKL). Ohio: Jackson Co., Liberty Twp., *F. Bartley & L.L. Pontius 635* (NY). Pennsylvania: Berks Co., about 2 miles SE of Fleetwood, *A. H. Leibelsperger*

421 (PH); Berks Co., 8 mi NW of Scarlets Mill, *H. Wilkins 2678* (PH); Berks Co., S of Princeton, *W.R. Brumbach 3959* (PH); Berks Co., Near E of West Mtn., 1.5 miles NW of Seranton, *S.L. Glowenke 8000* (LL); Bucks Co., 1 mi SE of Roelofs, *B. Long 29907* (PH); Bucks Co., 2 mi W of Morrisville, *W.L. Dix s.n.* (PH); Bucks Co., Bycot, along railroad, 1/2 mi NE of Station, *P.A. Fryer s.n.* (PH); Lehigh Co., vicinity of the South Mtns., 3/4 miles SW by S of Emaus, *H.W. Pretz 6049* (PH). South Carolina: Location unknown, *W.M. Canby s.n.* (NY); Georgetown Co., Prince George Tract, *J.B. Nelson 18461* (NY). Tennessee: Coffee Co., 3 miles S of Manchester, *H.K. Svenson 8782* (BKL); Coffee Co., Manchester, *H.K. Svenson 9149* (BKL); Sequatchie Co., Cumberland Plateau, Spencer-Dunlop Rd., *H.K. Svenson 8752* (BKL). Virginia: Northhampton Co., Eastville, *M.L. Fernald & B. Long 5420* (BKL). West Virginia: Randolph Co., Davis and Elkins College campus, Elkins, *E.E. Hutton, Jr. 935* (BKL, LL).