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Systematics of the *Eupatorium album* Complex (Asteraceae) from Eastern North America

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Abstract*—**Analysis of ITS sequence data showed unexpected complexity for *Eupatorium album* and *E. petaloideum*, a closely related species pair from eastern North America that has been considered to form a single species. The two species consistently differed in ITS sequence by seven to eight bp as well as a one bp indel, and detailed analysis revealed little intraspecific variation. The ITS sequences of other samples that had similar but slightly differing morphology and were pollen sterile gave several patterns involving bp or indel polymorphisms that indicated that they were of hybrid derivation. Based on differences in leaf and phyllary shape and glandular trichome distribution it was possible to delimit the individual hybrid combinations from one another and from their progenitor species. Material previously called *E. album* from the western part of its range is of hybrid origin from *E. album* and *E. lancifolium*, and is described here as ***Eupatorium sullivaniae. Material from the northeastern part of the range previously called *E. album* var. *subvenosum* is a hybrid derivative of *E. petaloideum* and *E. hyssopifolium*, elevated here to ***Eupatorium subvenosum***. Samples having the general morphology previously associated with *E. album* var. *vaseyi* included hybrid derivatives of the combinations *E. petaloideum*/*E. sessilifolium*, here reinstated as *E. vaseyi*, and *E. petaloideum*/*E. sessilifolium*/*E. perfoliatum*, here reinstated as *E. fernaldii*. Other samples gave ITS sequence data consistent with a derivation from *E. album*/*E. hyssopifolium*. The species of hybrid derivation have geographic ranges that extend beyond those of the inferred progenitors, in the case of *E. subvenosum* involving a disjunction of several hundred kilometers, suggesting that there have been considerable differences in species ranges over time.

***Keywords*—**Apomixis, Asteraceae, *Eupatorium*, hybridization, North America.

It has been recognized for some time that the taxonomy of *Eupatorium* L. in eastern North America is complicated by the presence of hybridization, apomixis, and polyploidy (Grant 1953; Sullivan 1972; Sullivan 1976). As typical of apomictic groups, there is an absolute correlation between the occurrence of apomixis and of polyploidy (Sullivan 1972). In *Eupatorium*, there are some species that are entirely diploid and sexual, and others that include a mixture of diploid and sexual as well as apparently autopolyploid and apomictic populations (Sullivan 1972). There are also some species that are entirely apomictic and of allopolyploid origin. Application of nuclear rDNA ITS region (Baldwin et al. 1995) sequence data has proven invaluable in confirming or elucidating the diploid progenitors of two relatively restricted apomictic species, *E. godfreyanum* Cronquist (Siripun and Schilling 2006a) and *E. novae-angliae* (Fernald) V. I. Sullivan ex A. Haines & Sorrie (Schilling et al. 2007), in verifying the hybrid origin of *E.* × *truncatum* Muhl. (McKain et al. 2010), and has also provided evidence that apomictic polyploid populations of *E. sessilifolium* L. are autoploid (Grubbs et al. 2009). The use of ITS as a marker is enhanced in North American *Eupatorium* because data are available for each diploid species, and each is characterized by a distinctive combination of base pair substitutions and sometimes also indels in this region (Schilling et al. 2007).

Eupatorium album L. and *E. petaloideum* Britton are so similar morphologically that they have not been widely recognized to be distinct (Ward 2004; Siripun and Schilling 2006b). The two species differ from all other North American *Eupatorium* by their conspicuously attenuate, white phyllaries in combination with leaves that are relatively long and narrow, although not linear (Siripun and Schilling 2006b). Herbarium specimens of the two have been separated from each other primarily by only a single morphological feature, the presence (*E. album*) or absence (*E. petaloideum*) on the phyllaries of pubescence, including glandular trichomes. As currently interpreted, *E. album* is widespread in eastern North America, whereas *E. petaloideum* is restricted to a relatively limited area in Florida, Georgia, and Mississippi (Sullivan 1972). Where sympatric, the two species appear to differ in habitat preference, with *E. album* occurring in pine flatwood communities with poorly drained soils, whereas *E. petaloideum* is found in more upland sites with scrub oak or oak-hickory and richer and better drained soils (Sullivan 1972). Sullivan (1972) reported both species to be sexual diploids, although a map in a later report (Sullivan 1992) referred to some samples of *E. album* as "autoploid agamospermous races" (the unstated inference is that these specimens were likely to have been found to lack pollen, and thus represent polyploid apomicts). Two taxonomic varieties have been widely recognized within *E. album* in addition to the typical one: *E. album* var. *vaseyi* (T. C. Porter) Cronquist (also sometimes referred to as *E. fernaldii* Godfrey) occurs in the southern Appalachian highlands, and has been hypothesized to be derived by hybridization between *E. album* and *E. sessilifolium* (Sullivan 1972, 1978); *E. album* var. *subvenosum* A. Gray is found in the northeastern pine barrens of Delaware, New Jersey, and New York, and although Sullivan (1972) found that specimens lacked pollen and thus it was likely to be an apomictic polyploid, no hypothesis of relationships has been suggested for it.

The current study developed out of a survey of variability in ITS sequences for species of *Eupatorium* in eastern North America. Initial limited sampling suggested that samples of *E. album* and *E. petaloideum* differ from one another in ITS sequence by seven bp differences and a single one bp indel (Schilling et al. 2007). Further survey of samples labeled *E. album* from across its range, however, revealed some that yielded electropherograms for the ITS region which exhibited a characteristic "stutter" pattern, where an individual is polymorphic for alleles that vary in the presence or absence of an indel and the electropherogram goes "out of phase" at the site of the indel, which is often observed in allopolyploids and hybrids in *Eupatorium*. Similar, though not identical, ITS electropherograms exhibiting "stutter" patterns indicative of the presence of indel polymorphisms were also observed in samples of *E. album* var. *vaseyi* and *E. album* var. *subvenosum*. To elucidate the patterns of sequence and morphological variability, an extensive survey was made of samples of *E. album*, including all of its putative varieties, and *E. petaloideum*. The results showed that genomes from *E. album* and

E. petaloideum can be consistently recognized by their characteristic ITS sequences, and evidence of both can be detected in apomictic polyploids.

Materials and Methods

***Taxonomic Sampling*—**Samples from across the geographic range of *Eupatorium album*, including each of its currently recognized varieties, and *E. petaloideum* were analyzed, utilizing primarily herbarium material supplemented with freshly collected samples (Appendix 1). The samples included 50 identified initially as *E. album* var. *album*; six as *E. album* var. *vaseyi*; two as *E. album* var. *subvenosum*; and four as *E. petaloideum*. A sample of *E. saltuense*, which has been hypothesized to be a hybrid derivative of *E. album* and *E. hyssopifolium* (Sullivan 1978) was also analyzed. Representative sequence data from other species of *Eupatorium* were available from other studies.

***Molecular Methods*—**Protocols for DNA extraction and sequencing and analysis of the ITS region followed Schilling et al. (2007). GenBank accession numbers are provided in Appendix 1. Cloning of the ITS region for selected samples was undertaken to provide confirmatory data on sequences of ITS units inferred from base pair and indel polymorphisms. Purified PCR products were ligated into pGEM-T (Promega, Madison, Wisconsin) according to the manufacturer's instructions. Competent Top10 F′ (Invitrogen, San Diego, California) cells were transformed via electroporation and the resulting colonies were screened for plasmids with inserts by PCR using the original amplification primers. Plasmids were isolated from single recombinant colonies using an alkaline lysis/PEG precipitation protocol (Sambrook et al. 1989). Sequences were obtained from multiple colonies for each sample analyzed.

To determine the probable maternal parental lineage of samples of putative hybrid origin, sequence data for selected samples were obtained for a portion of the plastid *trnC-psbM* intergenic spacer region. Either the entire region was amplified using the *trn*C and *psb*M primer pair (Shaw et al. 2005), or a subset was amplified using the *trnC* and *ycf6R* primers, which worked better for extracts from herbarium specimens; in both cases sequence was obtained using the *ycf6R* primer. A portion approximately 325 bp in length from the *trnC-petN* (*ycf6*) intergenic spacer was utilized for comparisons to sequences previously obtained from other *Eupatorium* species (Grubbs et al. 2009).

***Phylogenetic Analyses*—**Methodology for phylogenetic analysis of ITS sequences followed closely that of Schilling et al. (2007) and Grubbs et al. (2009). Previously published data from GenBank were obtained to include one or more samples of each diploid species of *Eupatorium,* and these were combined with newly obtained sequences from *E. album* and *E. petaloideum* as well as sequences obtained from cloning experiments on DNA samples 2028, 2038, 2039, and 2040. Other samples inferred to be of hybrid origin but not cloned were not included in the phylogenetic analyses. Phylogenetic relationships were analyzed using both maximum parsimony and Bayesian approaches. A sample of the sister genus to *Eupatorium*, *Eutrochium* (*Eupatorium* sect. *Verticillatum*, JoePyeWeeds), was used as the outgroup, based on the results of previous analyses (Schmidt and Schilling 2000; Siripun and Schilling 2006a). Results are submitted to TreeBASE (study number S11680).

Results

Analysis of four samples of *Eupatorium petaloideum* from different parts of its relatively limited range provided confirmation of a slightly but consistently different ITS sequence compared to that of *E. album* (Table 1). Three of the ITS sequences from *E. petaloideum* were identical to one another, and the fourth had two differences, a single bp difference at one position (63) and a fixed bp rather than a polymorphism at a second (137). They each differed from ITS sequences characteristic of *E. album* by seven to eight bp as well as by a single one bp deletion. Among the 31 samples of *E. album* that were analyzed there were a total of three positions (147, 452, 567) in which there was variability between samples, and this involved differences for whether there was a fixed base or a polymorphism at a given position, the latter including the base that was fixed in other samples (Table 1). Each of these samples exhibited the characteristic pattern of morphology of *E. album*, including production of normal pollen (Table 2). Phylogenetic analysis of the ITS sequence data showed that samples of *E. petaloideum* and *E. album* were placed within *Eupatorium* as respectively monophyletic sister groups, each with strong statistical support (Fig. 1).

Other samples that exhibited white phyllaries of the general type characteristic of *Eupatorium album* gave several different patterns of ITS sequences characterized by numerous polymorphisms in bp or length (Table 1), suggesting the presence of more than a single ITS sequence. Comparisons with published ITS sequences for *Eupatorium* species allowed the inference of the combinations of ITS sequences that would produce the observed pattern of polymorphisms. One ITS sequence pattern was observed for the two samples with the morphology of *E. album* var. *subvenosum* which showed a combination of bp and indel polymorphisms that would be expected from a hybrid involving *E. hyssopifolium* with *E. petaloideum* rather than *E. album* (Table 1). There was an indel polymorphism at position 120–124/125, as well as numerous bp polymorphisms (Table 1). One of the ITS sequences was inferred from the pattern of bp polymorphisms (e.g. positions 137, 201, 506, 550, 572 were polymorphic; positions 286, 567 were not polymorphic) to be identical to that of *E. petaloideum* and different from *E. album*. Further confirmation was provided through cloning experiments of one sample, 2040, which recovered individual ITS sequences characteristic of *E. hyssopifolium* and *E. petaloideum* (Fig. 1). Close inspection showed the morphology of samples of *E. album* var. *subvenosum* to be more similar to *E. petaloideum* than to *E. album*, but to be distinct from either in leaf venation as well as presence of phyllary glands (Table 2), and this taxon is elevated here to the species level as *E. subvenosum*.

The samples with the overall morphology of *Eupatorium album* var. *vaseyi* also gave ITS sequences with numerous polymorphisms, but there were two slightly but consistently different patterns that can be associated with previously published species. Some samples, including one from the type locality of *E. vaseyi* on Lookout Mountain, Tennessee, gave ITS sequences with a pattern of polymorphisms that would be expected from a combination of *E. petaloideum* and *E. sessilifolium* (Table 1). Four other samples of similar but slightly different morphology and originating from the piedmont of North Carolina and Georgia where *E. fernaldii* was described, gave ITS sequences with a pattern of polymorphisms that would be characteristic of a combination of not just two but three species: *E. petaloideum*, *E. perfoliatum*, and *E. sessilifolium* (Table 1). Particularly diagnostic was the presence of three distinct peaks at one position (506) where each species has a different base (Table 1). Cloning experiments with sample 2038 recovered individual ITS sequences characteristic of each of these three species (Fig. 1).

Another ITS sequence pattern was observed from 12 samples from the far western part of the geographic range, in Louisiana, Arkansas, and Mississippi, and was characterized by numerous bp polymorphisms and multiple indel polymorphisms (Table 1). Detailed analysis of the bp polymorphisms visible in the sequences showed that the pattern could be derived from a combination of the sequence of *Eupatorium album* with that characteristic of *E. lancifolium*. There were indel polymorphisms in both ITS-1, at positions 120–125, as well as in ITS-2, where *E. lancifolium* has a one bp deletion at position 574 and a further six bp deletion starting at position

TABLE 1. Summary of diagnostic polymorphisms in the DNA sequence output for the ITS regions of samples of *Eupatorium*. Positions numbered starting with the 5′ end of ITS1; bold highlights positions diagnostic between *E. album* and *E. petaloideum*; ambiguities in bold, coded by standard IUPAC symbols; dash symbol shows a deletion relative to other samples; note that because of the direction of sequencing ambiguities within indel polymorphisms were not resolved. The position of indel polymorphisms are indicated with <IP or >IP. Underline used to highlight diagnostic polymorphisms. Base pair positions are relative to the start of ITS-1. Species names abbreviated by first three letters of epithet (alb, album; pet, petaloideum; hys, hyssopifolium; lan, lancifolium; ses, sessilifolium; per, perfoliatum).

ITS-1	16	26	38	**43**	48	63	72	73	100	108	113	114	120	125	**137**	142	147	149	157	188	189	**201**	228	235	239	241	243	253	257	**286**
Direction of sequencing from right to left <																														
Taxon																														
alb	-	C	T	A	T	C	T	T	A	T	G	T	-	C	C	C	A/R	G	C	C	A	G	T	G	T	T	G	G	T	A
pet	-	C	T	-	T	C/T	T	T	A	T	G	T	-	C	G/S	C	A	G	C	C	A	G	T	G	T	T	G	G	T	C
hys	C	C	G	A	C	T	A	T	G	T	G	C	T	G	C	C	A	G	A	A	A	G	C	A	G	C	G	**T**	T	C
lan	C	C	T	A	T	C	T	T	A	T	G	T	T	C	C	C	A	C	A	C	A	G	T	A	T	T	G	G	C	C
ses	C	G	T	-	T	T	T	C	A	T	G	T	-	C	C	G	A	G	A	C	G	G	T	A	T	C	T	G	T	C
per	C	C	T	-	T	T	T	T	A	A	T	T	-	C	C	C	A	G	A	C	A	G	C	A	T	C	G	G	T	C
alb/hys	<IP	<IP	<IP	<IP	<IP	<IP	<IP	<IP	<IP	<IP	<IP	<IP	<IP	**S**	C	C	A	G	M	**M**	A	G	**Y**	**R**	**K**	**Y**	G	**K**	T	**M**
sullivaniae (alb/lan)	<IP	<IP	<IP	<IP	<IP	<IP	<IP	<IP	<IP	<IP	<IP	<IP	<IP	C	C	C	A	**S**	M	C	A	G	T	**R**	T	T	G	G	Y	**M**
subvenosum (pet/hys)	<IP	<IP	<IP	<IP	<IP	<IP	<IP	<IP	<IP	<IP	<IP	<IP	<IP	**S**	**S**	C	A	G	M	**M**	A	**S**	**Y**	**R**	**K**	**Y**	G	**K**	T	C
vaseyi (pet/ses)	<IP	**S**	T	-	T	**Y**	T	**Y**	A	T	G	T	-	C	**S**	**S**	A	G	M	C	**R**	**S**	T	**R**	T	**Y**	**K**	G	T	C
fernaldii (pet/ses/per)	<IP	**S**	T	-	T	**Y**	T	**Y**	A	**W**	**K**	T	-	C	**S**	**S**	A	G	M	C	**R**	**S**	**Y**	**R**	T	**Y**	**K**	G	T	C
ITS-2	452	456	478	**506**	512	516	531	**550**	552	557	564	**567**	**572**	574	575	581	588	589	596	598	609									
Direction of sequencing from left to right >																														
alb	A/R	A	T	T	C	T	A	A	T	T	T	C/Y	C	C	T	C	A	C	T	T	T									
pet	A	A	T	C	C	T	A	G	T	T	T	T	A	C	T	C	A	C	T	T	T									
hys	A	G	C	T	T	T	A	A	A	T	C	T	C	C	G	T	A	T	C	T	C									
lan	T	A	T	T	C	C	A	A	A	G	T	T	C	-	G	T	-	-	T	T	C									
ses	A	A	T	A	C	T	G	A	A	T	T	T	C	C	G	T	A	T	C	C	C									
per	A	A	T	T	C	T	A	A	A	T	T	T	C	C	G	T	G	T	T	T	T									
alb/hys	A	R	Y	T	Y	T	A	A	W	T	Y	Y	C	C	K	Y	A	Y	Y	T	Y									
sullivaniae (alb/lan)	W	A	T	T	C	Y	A	A	W	K	T	Y	C	>IP	>IP	>IP	>IP	>IP	>IP	>IP	>IP									
subvenosum (pet/hys)	A	R	Y	Y	Y	T	A	R	W	T	Y	T	M	C	K	Y	A	Y	Y	T	Y									
vaseyi (pet/ses)	A	A	T	M	C	T	R	R	W	T	T	T	M	C	K	Y	A	Y	Y	Y	Y									
fernaldii (pet/ses/per)	A	A	T	H	C	T	R	R	W	T	T	T	M	C	K	Y	R	Y	Y	Y	Y									

TABLE 2. Comparison of morphology of *Eupatorium album*, *E. petaloideum*, and apomictic hybrids involving them. Measurements shown as mean values for midstem leaves.

Taxon	Leaf length (mm)	Leaf width (mm)	Leaf l/w ratio	Leaf margin teeth	Leaf pubescence	Leaf glands	Inner phyllary shape	Inner phyllary glands	Pollen
album	91.7	21.3	4.3	13	sparse to abundant	abundant	attenuate	basal only	good
album/hyssopifolium	61.7	13.7	4.5	8	abundant	abundant	mucronate	abundant	lacking
sullivaniae	85.1	28.0	3.0	15	abundant	abundant	attenuate	abundant	lacking
petaloideum	48.3	15.0	3.3	9	sparse	sparse	attenuate	lacking	good
subvenosum	59.5	17.8	3.5	7	sparse	sparse	mucronate	abundant	lacking
vaseyi	84.0	26.3	3.2	14	sparse	sparse	mucronate	abundant	lacking
fernaldii	90.0	30.5	3.0	16	sparse	abundant	mucronate	abundant	lacking

587. These 12 samples also exhibited a characteristic pattern of morphology that was distinct from that of *E. album* (Table 2), and because of the relatively widespread contiguous distribution are included as part of a newly described species, *E. sullivaniae*.

Still another ITS sequence pattern was found from four samples originally identified as *Eupatorium album* from localities in Kentucky, Tennessee, and Virginia. These samples had a pattern of polymorphisms characteristic of a combination of *E. album* with *E. hyssopifolium* (Table 1). There was an indel polymorphism in the poly-T region at positions 120–124/125, for which *E. album* has 4-T and *E. hyssopifolium* has 5-T, as well as numerous individual bp polymorphisms (Table 1). The sequences were confirmed through cloning experiments of one sample, 2039, which recovered individual ITS sequences characteristic of *E. album* and *E. hyssopifolium*, respectively (Fig. 1). On close inspection these samples differed from the typical morphology of *E. album* in several features, including a lack of viable pollen (Table 2).

The MAMA approach (Rauscher et al. 2002) to designing sequence-specific primers was utilized to provide a further check on the underlying source of polymorphisms in the ITS sequence data for *Eupatorium subvenosum* and *E. vaseyi*. "Exclusion primers" specific for amplification of the ITS sequences of the ITS types characteristic of *E. hyssopifolium* (5′- AGACCAGTCTCCGCCCACTC -3′ and 5′- CAGCACG TGCCAAGGAAAACA -3′) and *E. sessilifolium* (5′- AGACG ACGCGTTAGGGTACCG -3′ and 5′- CCCTGGATGGCAA AACAACTG -3′) were designed. Individual DNA samples were amplified using the species-specific primers and checked for the presence of amplification using agarose gel electrophoresis. All samples of *E. petaloideum* (2000, 2390, 2391, 2902) gave negative results for both exclusion primer pairs; samples of *E. subvenosum* (2040, 2211) were positive for the *E. hyssopifolium* exclusion primers but negative for the *E. sessilifolium* ones; samples of *E. vaseyi* (2354, 2380, 2477, 2803) were negative for the *E. hyssopifolium* exclusion primers and positive for the *E. sessilifolium* ones. For each species-specific primer pair, a DNA sequence was obtained to confirm that the resulting portion of the ITS sequence matched that expected from the specific primers.

Information on the likely maternal lineage of each putative hybrid combination was sought using partial sequences from the plastid *trnC-petN (ycf6)* intergenic spacer region. Within the initial portion of the region that was sampled, covering ca. 325 bp, there is variability within *Eupatorium* at individual bp positions as well as for a large inversion, and the number of A's and T's in a polyA/T region (Grubbs et al. 2009). There was no variability for 12 samples of *E. album*, all of which matched the GenBank sample (FJ3935175) of this species. All samples of *E. sullivaniae* (2212, 2213, 2215, 2216, 2576, 2579, 2581, 2602) which were analyzed matched the sequence of *E. album*, and differed from that of *E. lancifolium* (for which four additional samples matched completely the sequence of GenBank FJ395178 from this species) by differences at two bp positions as well as the orientation of the inversion. Sequences for *E. petaloideum* matched almost exactly that of *E. album* for the *trnC-petN* region, with three samples identical and the fourth differing for a single bp; all samples of *E. album* and *E. petaloideum* collectively exhibited two apomorphic bp changes relative to all other *Eupatorium* (Grubbs et al. 2009). The sequences from two samples of *E. subvenosum* (2040, 2211) matched those of *E. petaloideum* exactly and differed from those of *E. hyssopifolium* (12 samples, all matching GenBank AY727125 of this species) by two bp differences as well as a difference in the poly A/T region (7/7 vs. 6/8). The sequence of samples of *E. vaseyi* (2354, 2380, 2389, 2703) and *E. fernaldii* (2039, 2672) similarly matched those of *E. petaloideum* and differed from those of *E. sessilifolium* (15 samples, Grubbs et al. 2009) by the orientation of the inversion as well as at least two bp differences; the samples of *E. fernaldii* also differed from those of *E. perfoliatum* (three samples, all matching GenBank FJ395184) by two bp as well as in a difference in the poly A/T region (7/7 vs. 6/9).

A detailed analysis of morphology was undertaken to assess whether the hybrid combinations could be distinguished from their parents and from one another (Table 2). The most promising characters to separate the hybrid combinations involving *E. album* and *E. petaloideum* were related to the size and dimensions of the leaves, the shape of the phyllaries, and the distribution and abundance of the subsessile glandular trichomes (glands; sometimes referred to as resin dots). The distinguishing feature of *E. petaloideum* is that it completely lacks glands on the phyllaries, and they tend to be relatively sparse on the leaves. With *E. album* accurately delimited, it can also be seen that *E. petaloideum* has significantly shorter and somewhat broader leaves with fewer teeth than *E. album* (Table 2). Both *E. album* and *E. petaloideum* are characterized within *Eupatorium* by having inner phyllaries that are both white in color and long attenuate, and hybrids involving other species typically exhibited inner phyllaries that, though also white in color, contrasted by being abruptly mucronate. The sparse leaf pubescence and relative lack of glands characteristic of *E. petaloideum* was also observed in hybrids between it and either *E. sessilifolium* or *E. hyssopifolium* (Table 2). The trihybrid *E. fernaldii* (*E. petaloideum*/*E. sessilifolium*/*E. perfoliatum*) could be separated from the other hybrids involving *E. petaloideum* by the higher abundance of foliar glandular trichomes (Table 2). Specimens of *E. album* had glands on the outer phyllaries, but they were either absent or restricted to

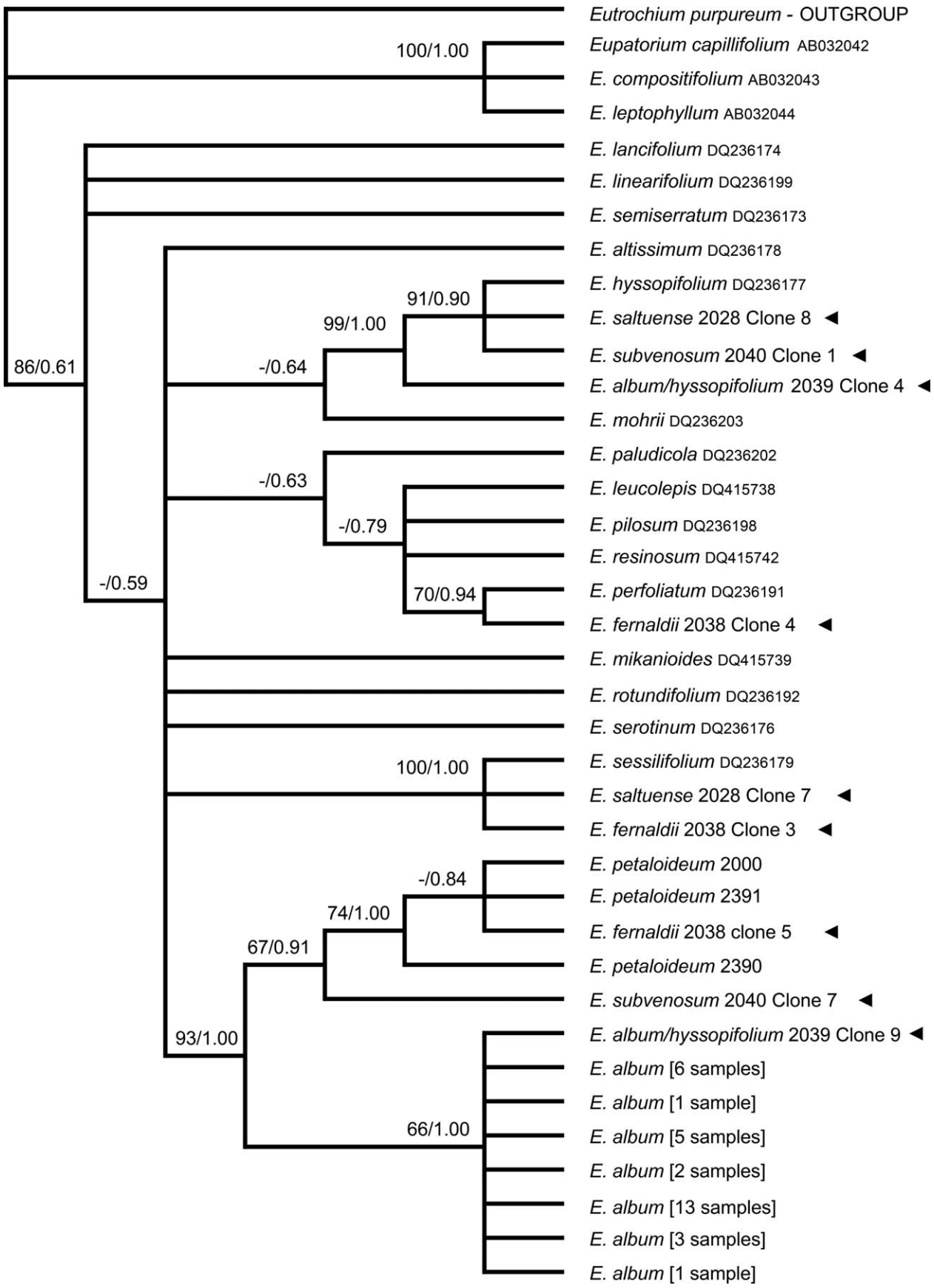

FIG. 1. Tree obtained from Bayesian analysis of ITS data from *Eupatorium* showing relationships of *E. album*, *E. petaloideum*, and selected sequences obtained from cloning experiments on hybrid-derived samples (arrows) to other members of the genus. Samples labeled by GenBank accession number (small font) or DNA numbers from Appendix; numbers above branches: bootstrap percentages (10,000 replicates, FASTSTEP search; -, less than 50%)/ Bayesian posterior probabilities.

the basal half of the innermost phyllaries. In contrast, hybrids of *E. sullivaniae* (*E. album/E. lancifolium*) had glands on all of the phyllaries, in addition to having somewhat broader leaves than *E. album*. Hybrids of the combination *E. album/E. hyssopifolium* had leaves that were shorter and usually narrower than those of *E. album*, and all of the phyllaries had abundant glands (Table 2).

Maps (Fig. 2) were prepared to show the source locations of the samples that were analyzed as well as the general ranges of the inferred progenitor species. The distribution of symbols in Figs. 2A and 2B depicts the approximate overall range of plants that have been identified as *E. album* s. l. based on the Plants Database website of the USDA/NRCS (http://plants.usda.gov, checked 2 November 2010). It can be seen that *E. petaloideum* is relatively restricted, whereas *E. album* samples are widespread, ranging from Louisiana to Florida and north to Tennessee and Virginia (Fig. 2A, B). Samples from the westernmost part of the range in Louisiana and Arkansas were predominantly of *E. sullivaniae* (*E. album/E. lancifolium*), and this is in the area where *E. lancifolium* occurs (Fig. 2C). Plants from the extreme northeastern part of the range in New Jersey were *E. subvenosum* (*E. petaloideum/E. hyssopifolium*; Fig. 2B). *Eupatorium fernaldii* (*E. petaloideum/E. sessilifolium/E. perfoliatum*) was found in the Piedmont of Georgia and North Carolina, whereas *E. vaseyi (E. petaloideum/E. sessilifolium*) occurred in the southern Appalachians and areas to the west and north (Fig. 2B). Finally, the *E. album/E. hyssopifolium* hybrid combination exhibited a somewhat more scattered distribution (Fig. 2A), suggesting the possibility that in some places this may be a first generation hybrid rather than part of a contiguous apomictic species. Both *E. hyssopifolium* and *E. sessilifolium* have widespread distributions, but in both there is a relatively restricted area where sexual diploid populations occur and a much wider distribution of apomictic polyploids (Fig. 2C, D). Because apomictic plants are almost entirely male sterile, it is most likely that hybridization would have involved sexual diploids, and the plastid DNA data indicated that either *E. album* or *E. petaloideum* was consistently the maternal parent. *Eupatorium perfoliatum* is exclusively diploid and sexual, and is widespread across the eastern U. S. A., extending into Canada (not shown).

Discussion

Molecular analyses showed unexpected complexity within material that has often been considered to form a single species, *Eupatorium album*. The ITS sequence data provided evidence that at least six different species of *Eupatorium* have contributed to the formation of a large complex that involves hybridization, apomixis, and polyploidy (although this study relied mostly on herbarium material from which chromosome number determinations could not be made, apomixis and polyploidy show an absolute correlation with each other and with male sterility within *Eupatorium*; Sullivan 1972). In addition to providing support for the recognition that *E. album* and *E. petaloideum* are distinct, four other apomictic species are recognized, each of which has a consistent phenotype associated with a characteristic ITS pattern. Three of those have been described previously as varieties of *E. album*, but molecular data suggested that they were instead derived from *E. petaloideum*.

This study has considerably broadened the sampling to support the conclusion that *Eupatorium album* and *E. petaloideum* are distinct (Siripun and Schilling 2006b). The distinctiveness of these two species has generally not been accepted by taxonomists (e.g. Cronquist 1980; Wunderlin and Hansen 2000), but this may be in part because the morphological distinction is blurred by the alloploid hybrid derivatives involving each species that are documented here. Molecular data showed that the two species consistently differed in ITS sequence, and no intermediates were found (Fig. 1). In contrast, there was not a single fixed bp difference among the 31 samples of *E. album* that were sampled, and only a single one among the four samples of *E. petaloideum* (Table 1). With recognition of which samples are members of either diploid species rather than hybrid derivatives, it can be shown that *E. album* differs from *E. petaloideum* in stem pubescence and leaf size and shape, as well as in the abundance and distribution of glands (Table 2). The two species also differ in habitat preference, and have been documented to maintain their distinctiveness even though they may occur in close proximity to one another (Sullivan 1972).

Molecular data showed that not only are *Eupatorium album* and *E. petaloideum* consistently different from one another in ITS sequences, but also that the sequences characteristic of one or the other can be detected in hybrid, polyploid apomicts. *Eupatorium petaloideum* appears to have contributed uniquely to the formation of *E. subvenosum* (*E. album* var. *subvenosum*), *E. vaseyi* (*E. album* var. *vaseyi*) and *E. fernaldii* (Table 1). In contrast, *E. album* is one of the parents of *E. sullivaniae*, newly described here, from the western portion of its range involving *E. lancifolium* as the second parent, and elsewhere has produced hybrids with *E. hyssopifolium* as the second parent.

There has been widespread recognition that *Eupatorium subvenosum* is a distinct entity, based on leaf venation, but it has been placed as a variety of *E. album*, rather than being associated with *E. petaloideum*. Thus the result from molecular data (Table 1) that *E. subvenosum* is derived from *E. petaloideum* rather than *E. album*, in combination with *E. hyssopifolium,* was somewhat unexpected. Morphological observations also suggested that it is most similar to *E. petaloideum* based on its smaller leaf size and fewer glands on both vegetative and reproductive organs (Table 2). If retained as a variety, *E. subvenosum* would thus need to be placed with *E. petaloideum*, but recent taxonomic practice in the genus has been to recognize apomicts that are widespread and clearly of hybrid origin as species.

The name *Eupatorium vaseyi* has been a source of taxonomic confusion, so perhaps it is appropriate that it is part of a series of apomictic hybrid derivatives. The name was misapplied for many years to a complex of apomictic populations that are now called *E. godfreyanum* (Cronquist 1985) and these have been shown to be derived from hybridization between *E. rotundifolium* and *E. sessilifolium* (Siripun and Schilling 2006a). Plants from the type locality of *E. vaseyi* proved to be of the dihybrid combination, *E. petaloideum/E. sessilifolium*. Morphological data (Table 2) show slight but consistent differences between *E. vaseyi* and *E. album*. In contrast to *E. subvenosum*, the smaller leaf size and more crowded internodes expected from *E. petaloideum* appear to be masked in *E. vaseyi* by the contribution of *E. sessilifolium*, and it is the features of the indument, particularly the glands, that distinguish *E. vaseyi* from *E. album*.

Sullivan (1972, 1978), who was unaware of the correct application of the name *E. vaseyi* (Cronquist 1985), used the name *E. fernaldii* Godfrey (*E. album* L. var. *monardiifolium*

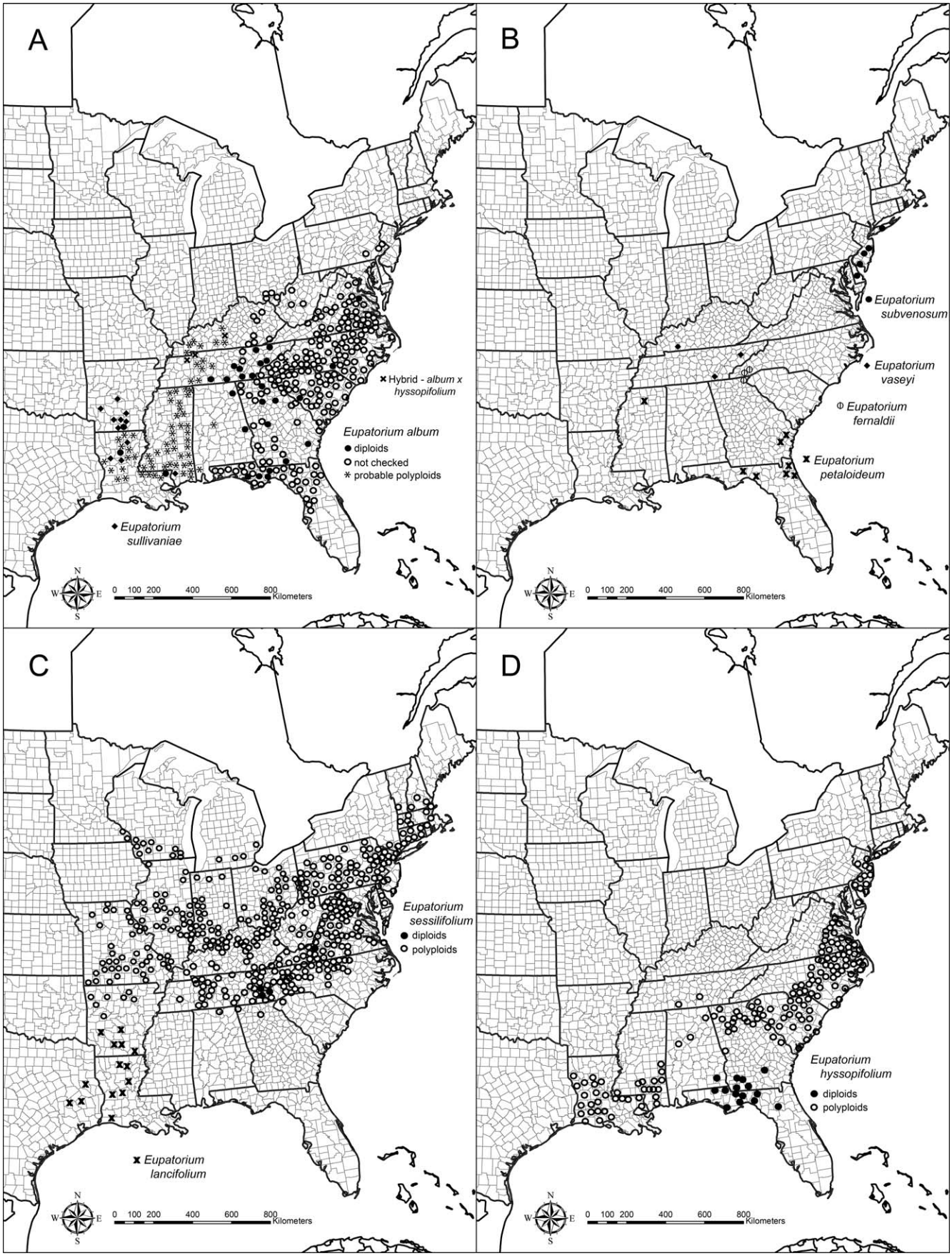

FIG. 2. Maps of the eastern U. S. A. showing the locations of samples of the *Eupatorium album* complex analyzed for ITS sequence data and the general geographical ranges of the putative parental species; symbols centered on county of occurrence. A. *Eupatorium album* and its hybrid derivatives. B. *E. petaloideum* and its hybrid derivatives. C. *E. lancifolium* and *E. sessilifolium*. D. *E. hyssopifolium*. Data on ploidy from Sullivan (1972).

Fernald; Godfrey 1950) for populations from Georgia and North Carolina and presented evidence, primarily morphological, that *E. album* and *E. sessilifolium* were probable progenitors. At least one of her specimens (*Lazor and Sullivan 3811*, Jackson Co., North Carolina), was tested in the present study and proved to be the trihybrid (*E. petaloideum*/*E. sessilifolium*/*E. perfoliatum*) combination, and material from Rabun Co., Georgia, from where she also sampled, showed the same trihybrid genomic combination. Sullivan (1978) reported a tetraploid chromosome count from material from both of these locations, but a sample from another location (Buncombe Co., North Carolina) was reported to be triploid. Note that, as with *E. subvenosum*, if *E. vaseyi* or *E. fernaldii* are recognized as varieties they would be placed more appropriately with *E. petaloideum* rather than *E. album*.

Eupatorium sullivaniae was the only apomictic species of the *E. album* complex that had not been previously recognized taxonomically. This may reflect the morphological similarity between *E. sullivaniae* and *E. album*, but also the lack of detailed study of material from the region where it occurs, in contrast to areas further to the east which have been closely scrutinized by botanists such as Fernald (1937). All specimens of *E. sullivaniae* that were examined were male sterile, in contrast to *E. album* which produces normal pollen, but otherwise the two are not very different (Table 2). The second progenitor, *E. lancifolium*, is endemic to the areas of Arkansas and Louisiana where *E. sullivaniae* occurs (Sullivan 1972; Fig. 2C). *Eupatorium lancifolium* closely resembles the more widespread *E. semiserratum*, but in addition to morphology differs from it by a preference for a relatively dry rather than wet habitat, and it is possible that the underlying differences in physiology have been passed on to *E. sullivaniae* and helped to establish it in areas beyond where *E. album* occurs (Fig. 2A, 3).

The case of whether to provide taxonomic recognition to the populations inferred to be hybrid derivatives of *Eupatorium album/E. hyssopifolium* is not clearcut and will require further study to resolve. At least one of the samples of this combination was a specimen that has been included in *E. saltuense*, a taxon recognized in Virginia and North Carolina (Weakley 2008). However, a specimen from Virginia of *E. saltuense* collected by Fernald, who named the species, gave an ITS sequence that had a pattern of base pair and indel polymorphisms that, though characteristic of a hybrid, showed no evidence of an ITS sequence from either *E. album* or *E. petaloideum*. Rather it was consistent with an additive pattern of sequences from *E. hyssopifolium* and *E. sessilifolium* (data not shown), and cloning experiments using this sample, 2028, recovered individual ITS sequences characteristic of these species (Fig. 1). Thus it is not clear that the name *E. saltuense* is applicable to the *E. album*/*E. hyssopifolium* derivatives. It is also not clear from the scattered geographic origins of the samples of this combination that it represents a contiguous species rather than the results of a number of separate individual hybridization events.

Although the morphological differences that separate the apomictic species from one another and from *Eupatorium album* and *E. petaloideum* are slight, all of these entities can be identified unambiguously from ITS sequence data. Thus, a barcoding type of approach (Kress et al. 2005) could be utilized for identification purposes. The results, however, would have to be interpreted with care to decipher the bp and length polymorphisms that characterize particular apomictic genotypes. The magnitude of this problem for *Eupatorium* can be judged by the fact that almost half (27/62) of the samples examined in this study exhibited at least one indel polymorphism.

For both *Eupatorium album* and *E. petaloideum*, the hybrid derivatives extend beyond the geographic ranges of their diploid progenitors (Fig. 3). This is remarkably so in the case of *E. subvenosum*, which occupies a relatively local area that is at least 700 km away from the northernmost documented occurrence of *E. petaloideum* in Georgia (Fig. 2, 3). Either *E. subvenosum* has migrated far from where it was originally formed, or the range of *E. petaloideum* has contracted severely. Although *E. album* is generally more widespread, it appears that much of the material west of the Mississippi River that has been included under this name is of the hybrid *E. sullivaniae* (Fig. 2, 3). Thus, either *E. sullivania*e has extended beyond the range of *E. album* in Arkansas, or the range of *E. album* has contracted, possibly because it has been competitively excluded by *E. sullivaniae* after its formation.

There has been increasing use of ITS sequence polymorphisms to deduce the hybrid origin of plant samples, although this approach has not been widely applied to apomictic groups. For example Kaplan et al. (2009) and Les et al. (2010) recently report detection of interspecific hybrids in the aquatic genera *Potamogeton* and *Najas*, respectively. Application of this approach to apomictic groups may be limited because of their genetic complexity. Hörandl et al. (2005) did not report polymorphisms in the *Ranunculus auricoma* species group, and Noyes (2000) found potentially informative ITS polymorphisms in only two of six apomictic species of *Erigeron*. Campbell et al. (1997) reported ITS sequence polymorphisms in *Amelanchier* samples, but were only able to narrow the identity of the probable progenitor lineages to multispecific clades. Studies using other molecular approaches have confirmed that groups which exhibit facultative apomixis may be complex genetically, with considerable gene flow and local differentiation (Eugenia et al. 2009; Robertson et al. 2010). As noted by Eriksen (1999) there is considerable variability in the genetic complexity of different apomictic groups, making it impossible to apply a single species concept to each. Particularly in those that exhibit facultative apomixis it is impossible to recognize taxonomically every distinct genetic combination. Thus, the situation reported here for *Eupatorium album* and related species appears to be simpler than in many apomictic groups in two respects. The diploid species are distinct both morphologically and at the DNA sequence level, and there is little or no gene flow through the apomicts, likely because of pronounced male sterility.

Taxonomic Trematment

Key to the Species of the *Eupatorium album* Complex

1. Larger leaves less than 60 mm long; foliar resin dots (subsessile glandular trichomes) sparse 2
   2. All phyllaries lacking resin dots; leaf venation pinnate, or having at least more than one pair of major lateral veins ..... *E. petaloideum*
   2. At least the outer phyllaries with resin dots; leaf venation trinerved, the two major lateral veins diverging at the base of the blade ..... *E. subvenosum*

1. Larger leaves more than 60 mm long (usually more than 80 mm long); foliar resin dots sparse to abundant 3
   3. Leaves sparsely pubescent; pubescence of lower stem typically appressed, hairs less than 1 mm long; phyllaries typically acute and mucronate 4
      4. Foliar resin dots sparse ..... *E. vaseyi*
      4. Foliar resin dots abundant ..... *E. fernaldii*
   3. Leaves moderately pubescent; pubescence of lower stem typically spreading or erect, some hairs 0.5–1 mm long; inner phyllaries long attenuate 5
      5. Leaves lance-ovate to ovate, l/w ratio less than 3; inner phyllaries glandular to near apex ..... *E. sullivaniae*
      5. Leaves lanceolate, l/w ratio more than 3; distal half of inner phyllaries eglandular ..... *E. album*

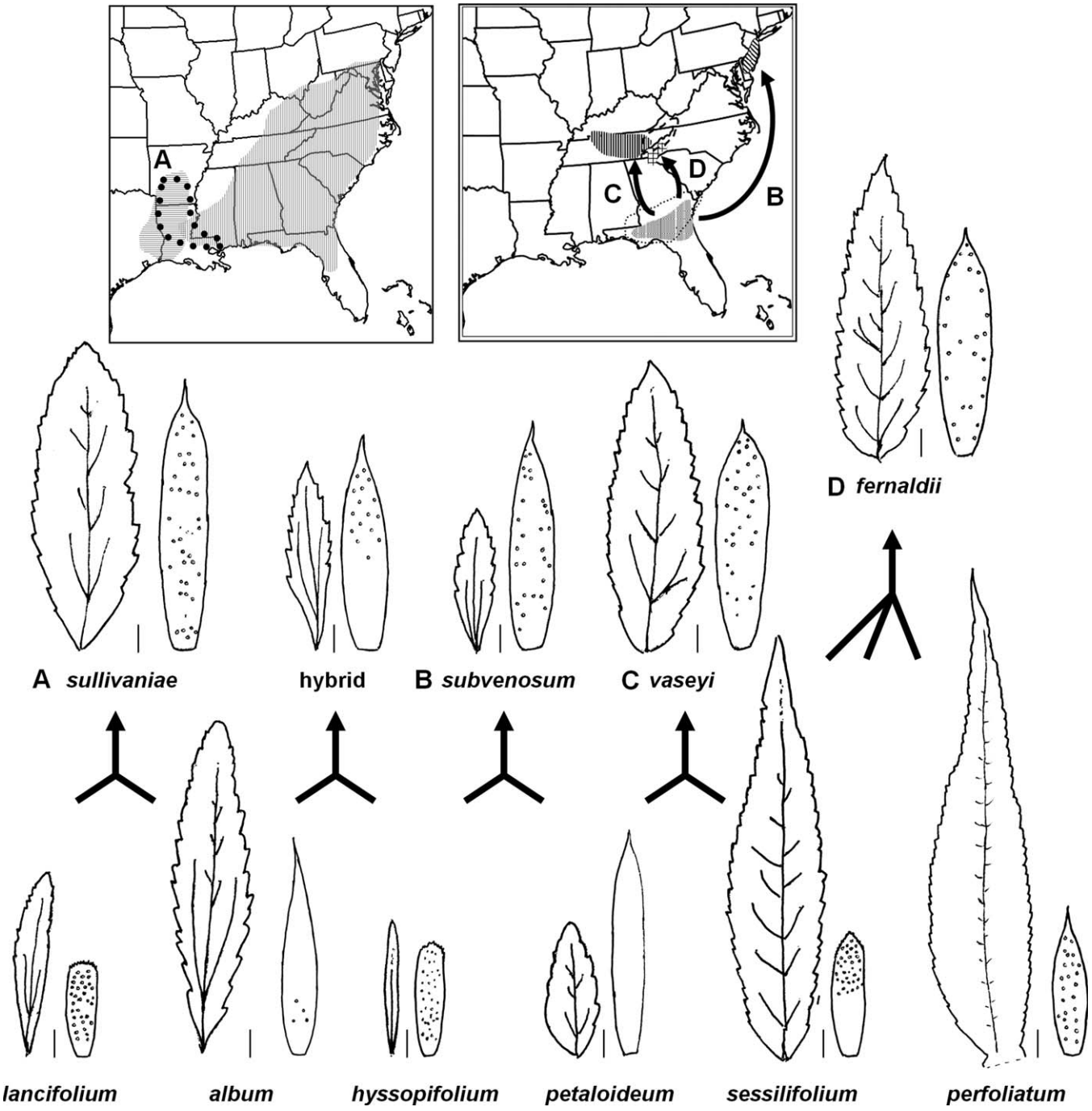

FIG. 3. Summary diagram of relationships inferred from ITS sequence data, key morphological traits, and geographic distributions of members of the *Eupatorium album* complex. For each entity a representative leaf and inner phyllary (with gland distribution) are shown (scale bar 1 cm for leaf; 1 mm for phyllary). Left map shows the geographic distribution of *E. album* (vertical lines), *E. lancifolium* (horizontal lines), and *E. sullivaniae* (dotted line). Right map shows *E. petaloideum* (narrow vertical lines), diploid *E. hyssopifolium* (dotted line), diploid *E. sessilifolium* (dashed line), and *E. subvenosum* (diagonal lines), *E. vaseyi* (wide vertical lines), and *E. fernaldii* (cross-hatched lines). *Eupatorium perfoliatum* occurs throughout most of the area shown in the map.

Eupatorium album L., Mat. Pl., 111. 1767.—TYPE: "Habitat in Pennsylvania, Barthram", *Bartram s. n.*, Herb. Linn. No. 978.5 (Lectotype: LINN, Reveal 1998).

Eupatorium glandulosum Michx. Fl. Bor.-Amer. 2: 98, 1803.

Eupatorium album L. var. *glandulosum* (Michx.) Fernald, Rhodora 39: 451, 1937.

Eupatorium album occurs widely in the southeastern U. S. A., ranging as far north as North Carolina and Pennsylvania and west to Louisiana (Fig. 2), but appears to be most abundant in northern Florida, Alabama, and southern Georgia. In other parts of its range, such as Tennessee and Kentucky, it is encountered more rarely, and many of the specimens from these areas that have been identified previously as *E. album* are probably *E. vaseyi*. As noted by Sullivan (1972), differences in gland color appear to have been the basis for the differentiation of *E. glandulosum*, but they are not taxonomically significant.

Eupatorium fernaldii R. K. Godfrey, J. Elisha Mitchell Soc. 66: 187. 1950. TYPE— U. S. A. Maryland, Prince George's Co., near Chillum, *S. F. Blake 9723* (Holotype: GH).

Eupatorium album L. var. *monardifolium* Fern., Rhodora 39: 451, 1937 non *E. monardifolium* Walp.

Eupatorium fernaldii is the first apomictic species of *Eupatorium* to be identified as having a trihybrid origin (Fig. 1; Table 1). Morphologically it is only slightly different from *E. vaseyi*, with which it shares *E. petaloideum* and *E. sessilifolium* as progenitor species. Godfrey (1950) noted that *E. fernaldii* "differs strikingly" in the piedmont area of Georgia and North Carolina from other varieties of *E. album.* In contrast to *E. vaseyi*, it generally occurs in large continuous populations in open areas (Sullivan 1978), rather than as scattered individuals in woodlands.

Eupatorium petaloideum Britton, Bull. Torrey Bot. Club 24: 492. 1897. TYPE— Florida, *Chapman s. n.* (Lectotype: NY, Ward 2004).

Eupatorium album L. var. *petaloideum* (Britton) R. K. Godfrey ex D. B. Ward, Novon 14: 367, 2004.

Eupatorium petaloideum is restricted to a few sites in Florida, Georgia, Alabama, and Mississippi (Sullivan 1972). It is most readily distinguished from *E. album* by the complete lack of pubescence, including glands, on the phyllaries. As can be seen from the type sheet, which includes material of both species, *E. petaloideum* can also be distinguished from *E. album* by its smaller leaves, more crowded internodes, as well as having shorter pubescence on the lower stem.

Eupatorium subvenosum (A. Gray) E. E. Schill., stat. nov. *Eupatorium album* L. var. *subvenosum* A. Gray, Syn. Fl. N. Amer. Vol. I, part II: 98, 1884.—TYPE: U. S. A. New York: Suffolk Co., 4 Sep 1871, *E. S. Miller s. n.* (Lectotype: GH, here designated).

Eupatorium subvenosum is distinguished from *E. album* and *E. petaloideum* by features of the leaves, the most conspicuous of which is that there are three prominent, major lateral veins that diverge at the base of the blade, and the leaves are apically acute rather than obtuse. Like *E. petaloideum*, the largest leaves are generally shorter than the largest ones in *E. album* and have a lower density of subsessile glandular trichomes ("resin dots"). *Eupatorium album* appears to be absent within the area of geographic distribution of *E. subvenosum* (Fig. 2). The type sheet of *E. subvenosum* includes two different specimens, of which *E. S. Miller s. n.* is here designated as lectotype.

Eupatorium sullivaniae E. E. Schill., sp. nov.—TYPE: U. S. A. Arkansas: Calhoun Co., 8 Sep 2007, *E. E. Schilling 07–51* (Holotype: TENN; Isotypes: BRIT, LSU, MISS, MO, TENN, TEX-LL, UARK, US).

Eupatorio album similis sed phyllareis plus glandulifer et polline destitus vel irregulare.

Plants perennial, from a short stout caudex about 10 mm thick; stems solitary, erect, 4–5 mm wide at base, 60–124 cm tall, longitudinally ribbed, pubescent proximally with appressed to spreading hairs 0.5–1.0 mm long, and densely pubescent distally and within inflorescence with mostly appressed hairs 0.3–0.5 mm long; leaves opposite, simple, sessile, green, flat to somewhat conduplicate, lanceolate to lance-ovate, 8.5–10 cm long by 3–3.5 cm wide; margins toothed; both surfaces sparsely strigose and resinous-punctate; venation pinnate; capitulescences 10–20 cm long by 10–20 cm wide, cymose-corymbiform, branches opposite or alternate, branches and peduncles densely pubescent with appressed to ascending hairs; involucres 9–11 mm long; phyllaries imbricate, outer 1–2 mm long, inner 7–9 mm long, green at base and along midrib with distinct white hyaline margins and apex, acuminate to attenuate, pubescent with spreading hairs, resinous-punctate to near apex; florets 5, corolla 4.5–5 mm long, pappus 4.5–6 mm long; mature cypselae black, 3–3.5 mm long. Figure 4.

Eupatorium sullivaniae is a hybrid apomictic species that combines genomes from *E. album* and *E. lancifolium* (Fig. 1; Table 1). It is morphologically similar to *E. album*, but can be distinguished by the innermost phyllaries, which have resin dots (sessile glandular trichomes) that occur to near the tip, in contrast to those of *E. album* in which the resin dots are absent at the apex of the inner phyllaries. The leaves of *E. sullivaniae* are also a bit broader than those of *E. album*, and the pollen is lacking or malformed. The name honors Dr. Victoria Sullivan, whose extensive work has led to significant advances in our understanding of *Eupatorium*.

Additional Specimens Examined (all TENN)—U. S. A. Arkansas: Ashley Co., along US 425 at Flat Creek about 2 mi S of Drew Co. line, north of Fountain Hill, 4 Oct 1995, *R. D. Thomas 147450*; Bradley Co., along roadbank of Ark 160 between county roads 33 and 64 one mi N Ingalls S of Hermitage, 2 Sep 1994, *R. D. Thomas & C. Amason 142040*; Cleveland Co., right of way of Ark 114 at Kissee Creek 1.2 miles W of Calmer and Ark 15, 18 Sep 1999, *R. D. Thomas 162961*; Ouachita Co., Woods along county road 21 between two streams 0.5–1.0 miles south of Little Missouri River, north of Chidester, 26 Sep 1999, *R. D. Thomas et al. 163193*; North of Chidester, along county road 21 ca 1.5 miles east of junction with Hwy 167, open area between road and woods. 33°45.863 N 93°01.754 W 200 m elev, 8 Sep 2007, *E. E. Schilling 07–47*; Pike Co., corner of AR84/AR8 2 mi W Amity, edge of pine-oak woods, 34°16.043 N 93°29.319 W 170 m. elev., 8 Sep 2007, *E. E. Schilling 07–42*; Saline Co., clearcut pine woods on nearly flat land SW of Ark 229 and Traskwoods, 11 Aug 1993, *R. D. Thomas et al. 136136*; Union Co., roadbank of State Line Road (UC1) in pine woods 2.2 mi W of Junction City and US 167, 14 Jul 1989, *R. D. Thomas 111186*; Louisiana: Grant Par., recently clear cut pine woods at intersection of two dirt roads about 1.3 mi W of US 167, S of Pacton in Tancock's Prairie, 18 Oct 1985, *R. D. Thomas 94648*; Natchitoches Par., Kisatchie Nat. For., dry loblolly pine woods just east of Evergreen Church south of La. 156 and Goldonna, 20 Aug 1998, *R. D. Thomas 157518*; Ouachita Par., pine woods beside road to Cheniere Lake Park, south of La 838. 16 Sep 1971, *R. D. Thomas 25493*; cut over pine woods on dry hillside north of Lapine Rd about 6 mi SW of La 34 and La 3033, 7 Oct 1987, *R. D. Thomas 102601*; Mississippi: Pearl River Co., ca. 0.5 mi. N of Picayune, 8 Aug 1978, *K. E. Rogers 45454.*

Eupatorium vaseyi Porter, Bull. Torrey Bot. Club 19: 128. 1892. TYPE—U. S. A. Tennessee, Hamilton Co, Lookout Mt., 1878, *G. R. Vasey* (Holotype: US).

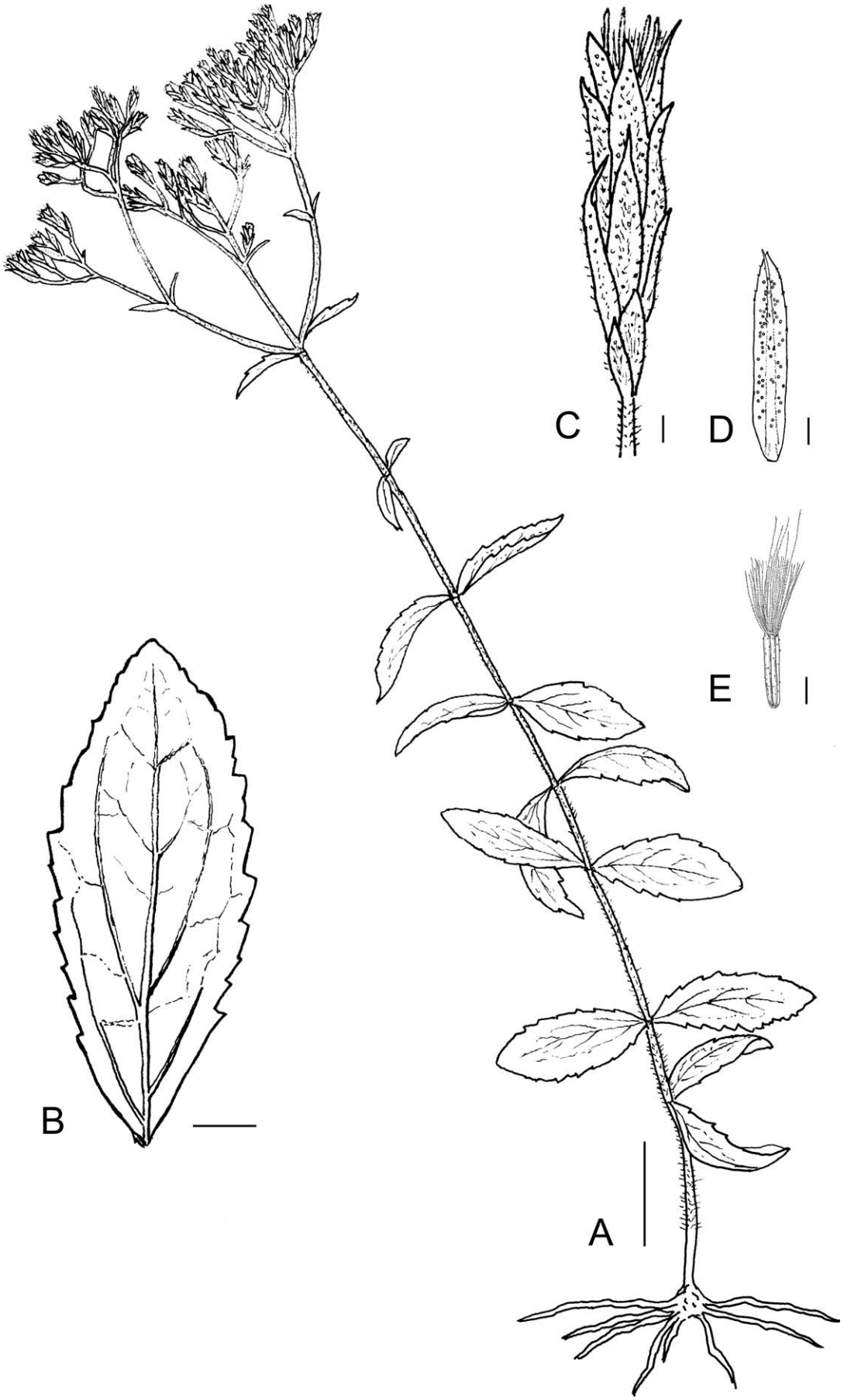

Fig. 4. *Eupatorium sullivaniae*. A. Habit. B. Leaf. C. Head. D. Inner phyllary. E. Cypsela. Scale bars: A, 5 cm; B, 1 cm; C-E, 1 mm. Drawn from *Schilling 07–51*.

Eupatorium album L. var. *vaseyi* (Porter) Cronquist, Brittonia 29: 220. 1977.

Eupatorium sessilifolium L. var. *vaseyi* (Porter) Fern. & Griscom, Rhodora 37: 180, 1935.

As noted by Cronquist (1985) the name *Eupatorium vaseyi* was incorrectly applied for many years. It has thus required both taxonomic and molecular phylogenetic studies to reach an understanding of this species. It occurs in the southern Appalachians and to the north and west in the ridge and valley province of Tennessee and west into Kentucky (Fig. 2, 3). The morphological distinction between *E. vaseyi* and *E. album* is slight (Table 2), but the ITS sequence data (Table 1) suggested that it is derived from *E. petaloideum* rather than *E. album*. The large leaves of *E. vaseyi* which are mostly responsible for its similarity to *E. album* can presumably be traced to the influence of *E. sessilifolium*, although the low amount of pubescence and particularly subsessile glandular trichomes are shared by *E. vaseyi* and *E. petaloideum*.

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Appendix 1. Samples of *Eupatorium* analyzed for ITS sequence data. All vouchers deposited at the University of Tennessee herbarium (TENN). Taxon; locality, collector, DNA number, GenBank accession number.

Eupatorium album L.: **Alabama**, Dekalb Co., *Siripun 02-166*, DNA 985 [EU646468]; Lee Co., *Galloway 176*, DNA 2671 [EU646490]; **Arkansas**, Calhoun Co., *Thomas 141774*, DNA 2582 [EU646485]; **Florida**, Bay Co, *Athey s. n. 8/12/70*, DNA 2668 [EU646489]; Leon Co., *Schilling 09-F03*, DNA 2907 [HQ688790]; Liberty Co., *Schilling 09-F16*, DNA 2904 [HQ688791]; Wakulla Co., *Godfrey 69925*, DNA 2051 [EU646470]; *Schilling 09-F05*, DNA 2906 [HQ688792]; **Georgia**, Crawford Co., *Moore 1274*, DNA 2667 [EU646488]; Dekalb Co., *Moore 2279*, DNA 2050 [EU646471]; Lowndes Co., *Schilling 05-20*, DNA 2198 [EU646469]; Lumpkin Co., *Thomas 171156*, DNA 2005 [DQ236200]; Oconee Co., *Seward 423*, DNA 2666 [EU646487]; Wayne Co., *Schilling 06-17*, DNA 2356 [EU646472]; **Louisiana**, Washington Par., *Thomas 68883*, DNA 2577 [EU646483]; Winn Par., *Thomas 94916*, DNA 2578 [EU646484]; **North Carolina**, Richmond Co., *Fox & Godfrey s.n.* 9/16/50,

DNA 2383 [EU646494]; **Tennessee**, Bledsoe Co., *Fleming & Wofford FCF 1330*, DNA 2369 [EU646474]; Blount Co., *Schilling 07-01*, DNA 2481 [EU646481]; *Schilling 07-02*, DNA 2482 [EU646482]; *Feist et al. 1036*, DNA 2630 [EU646486]; *Thomas s. n. 10/14/65*, DNA 2370 [EU646475]; Claiborne Co., *Patrick 5287*, DNA 2368 [EU646473]; Franklin Co., *Clements 291*, DNA 2374 [EU646478]; Giles Co., *Estes 2828*, DNA 2376 [EU646479]; Hamilton Co., *Deselm 02-134*, DNA 2702 [EU646491]; Hancock Co., *DeSelm 02-232*, DNA 2371 [EU646476]; Polk Co., *Rogers 44037*, DNA 2373 [EU646477]; Sevier Co., *Schilling & Thines s. n.*, DNA 2394 [EU646480]; Van Buren Co., *Shanks et al. 3429*, DNA 2378 [EU646492]; **Virginia**, Caroline Co., *Iltis 2373*, DNA 2382 [EU646493].

***Eupatorium album/Eupatorium hyssopifolium* combination**: **Kentucky**, Hart Co., *Kral 59038*, DNA 2381 [EU646504]; **Tennessee**, Henry Co., *Webb 1094*, DNA 2372 [EU646505]; Stewart Co., *Chester 91-99*, DNA 2377 [EU646506]; **Virginia**, Roanoke Co., *Uttall 6683*, DNA 2039 [EU646501, EU646500].

Eupatorium fernaldii: **Georgia**, Rabun Co., *Duncan 10144*, DNA 2672 [EU646526]; *Harbison et al. 2925*, DNA 2389 [EU646525]; **North Carolina**, Jackson Co, *Lazor & Sullivan 3811*, DNA 2038 [EU646497, EU646498, EU646499]; Macon Co., *Godfrey 52103*, DNA 2388 [EU646524].

Eupatorium petaloideum: **Florida**, Leon Co., *Godfrey 80839*, DNA 2000 [DQ236201]; *Schilling 09-F02*, DNA 2902 [EU646466]; St. Johns Co., *Godfrey 68334*, DNA 2386 [EU646466]; **Georgia**, Wayne Co., *Duncan 30518*, DNA 2387 [EU646467].

Eupatorium subvenosum: **New Jersey**, Cumberland Co., *Morton 2042*, DNA 2040 [EU646503; EU646502]; Cumberland Co., *Morton 2035*, DNA 2211 [EU646519].

Eupatorium sullivaniae: **Arkansas**, Bradley Co., *Thomas 142040*, DNA 2583 [EU646513]; Calhoun Co., *Schilling 07-51*, DNA 2605 [EU646515]; Cleveland Co., *Thomas 162961*, DNA 2049 [EU646507]; Ouachita Co., *Thomas 163193*, DNA 2212 [EU646508]; Pike Co., *Schilling 07-42*, DNA 2602 [EU646514]; Saline Co., *Thomas 136136*. DNA 2213 [EU646509]; Union Co, *Thomas 111186*, DNA 2581 [EU646512]; **Louisiana**, Grant Par., *Thomas 94648*, DNA 2579 [EU646518]; Natchitoches Par., *Thomas 157158*, DNA 2216 [EU646511]; Ouachita Par., *Thomas 102601*, DNA 2576 [EU646517]; *Thomas 25493*, DNA 2214 [EU646510]; **Mississippi**, Pearl River Co., *Rogers 45454*, DNA 2215 [EU646516].

Eupatorium vaseyi: **Kentucky**, Todd Co., *Athey 3586*, DNA 2380 [EU646522]; **Tennessee**, Hamilton Co., *Schilling 07-53*, DNA 2703 [EU646523]; Knox Co., *Schilling 06-27*, DNA 2354 [EU646520]; *Schilling 07-03*, DNA 2477 [EU646521].

Eupatorium saltuense Fernald **(*Eupatorium hyssopifolium/E. sessilifolium* combination)**: **Virginia**, Dinwiddie Co., *Fernald and Long 11166*, DNA 2028 [EU646496, EU646495].