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RELATIONSHIPS OF THE NEW ENGLAND BONESET, *EUPATORIUM NOVAE-ANGLIAE* (ASTERACEAE)

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ABSTRACT. Resolution of the systematic relationships of the New England Boneset, *Eupatorium novae-angliae*, has been elusive. This rare species, known from only 15 sites in Massachusetts and Rhode Island, has been demonstrated to be male-sterile and agamospermous, and thus inferred to be polyploid, but its progenitor diploids have not been identified clearly. In a study that hinged on a combination of fieldwork and morphological study together with molecular analysis, we have demonstrated that *E. novae-angliae* contains ITS repeats characteristic of two sexual diploid species of the genus. One is the widespread *E. perfoliatum*, the second is a previously unrecognized endemic to clay-based Carolina bay and depression meadow habitats in the Carolinas, that had been included in *E. leucolepis* and is now recognized as a separate species, *E. paludicola*. The molecular data highlight the distinctiveness of *E. novae-angliae* and underscore the need for efforts to continue to protect it in its native habitat.

Key Words: *Eupatorium*, Asteraceae, ITS sequences, polyploidy, agamospermy

Despite considerable study, the systematic relationships of the New England Boneset, *Eupatorium novae-angliae* (Fernald) Haines & Sorrie, have remained a puzzle for botanists. This taxon, recognized more widely as *E. leucolepis* (DC.) Torr. & A. Gray var. *novae-angliae* Fernald (Haines 2005), has been the focus of attention because of its relative rarity. It is known from only 15 sites in Massachusetts and Rhode Island (NatureServe 2005; Sorrie 1981), is classified as a Regionally Rare taxon (Ellison 2001), and is listed as State Endangered in Massachusetts (Massachusetts Natural Heritage and Endangered Species Program 2005) and Rhode Island (Enser 2002). Resolution of its genetic relationships and taxonomic status has been considered to be a priority in establishing a conservation plan for it (Ellison 2001).

Previous studies have uncovered important aspects of the biology of New England Boneset. It has been demonstrated to be agamospermous and male-sterile (Sullivan 1992). In *Eupatorium*, there is an absolute correlation between agamospermy and polyploidy (Sullivan 1976), and thus it can be inferred that New England Boneset is polyploid, although no chromosome count has ever been published for it. Morphologically it has been considered to be close to *E. leucolepis*, based on shared features of leaf shape and spacing, and head morphology. New England Boneset differs from *E. leucolepis* in leaf morphology and indumentum (Table 1). The heads often contain more than five florets (Sullivan 1992), which would narrow the possibility for one of its progenitors to the three species of the genus with more than five florets per head. The lack of distinctive perfoliate leaves of *E. perfoliatum* and petiolate leaves of *E. serotinum* has led investigators to suggest that the rare *E. resinosum* was a progenitor. Early attempts were made to apply molecular data to resolving the genetic relationships of New England Boneset (Sullivan 1992; Wiefenbach 1993), but in retrospect these were severely limited by the relatively crude technology available at the time, as well as by an incomplete understanding of variation in the genus.

Advances in two simultaneous research projects set the stage for the current study. A comprehensive assembly of ITS sequences for *Eupatorium* was undertaken by the first author as an outgrowth of a dissertation study on the relationships of diploid and polyploid populations of two species, *E. rotundifolium* and *E. sessilifolium* (Siripun 2004), and preparation of its treatment for the Flora North America project. Fortuitously, ITS sequences within *Eupatorium*

Table 1. Morphological comparison of *Eupatorium novae-angliae* and related taxa.

Character	*E. leucolepis*	*E. paludicola*	*E. novae-angliae*	*E. perfoliatum*
Rhizomes	Absent, or to 2 cm	Present, to 17 cm	Present, stout	Absent or short
Leaf orientation	(Reflexed-) spreading (-ascending)	Ascending to erect-recurved	Spreading to ascending	Spreading
Leaf base	Cuneate	Cuneate	Cuneate to truncate	Perfoliate
Leaf blade	Flat	Conduplicate	Flat	Flat
Leaf venation	Trinerved, laterals diverging from base	Trinerved, laterals diverging from base	Triplinerved, laterals diverging well above base	Pinnate, only midrib prominent
Maximum leaf width (mm)	(5–) 6–13	2–4.5	8–20	11–40
Leaf margin	Strigose-ciliate	Glabrous to sparsely strigose-ciliate	Sparsely strigose	Sparsely strigose
Leaf teeth	Few	Few	Numerous	Numerous
Leaf abaxial surface	Strigose	Glabrous to sparsely strigose	Sparsely strigose	Pilose to villous
Adaxial leaf glands	Abundant	Abundant	Abundant	Sparse to lacking
Involucre length (mm)	5–8	4.0–5.5	5–6	3.5–5.5
Florets/head	5	5	5–7	7–11 (–20)
Corolla length (mm)	2.7–3.8	2.7–3.7	3.1–3.5	2.5–3.5
Pappus length (mm)	(3.3–) 3.9–5.0	2.7–4.1	3.5–4.1	3–3.5
Cypsela length (mm)	2.2–3.5	1.6–2.3	1.8–2.0	1.5–2

are relatively divergent, and with the exception of the dogfennels (*E. capillifolium*, *E. compositifolium*, *E. leptophyllum*), pairwise comparisons show a minimum of 5 base pairs (bp; ca. 5%) differences between species (Siripun and Schilling 2006). At the same time, field studies initiated by the second author and colleagues revealed the existence of a previously unrecognized species, *E. paludicola* (LeBlond et al. 2007). Populations of *E. paludicola* were initially identified as *E. leucolepis* var. *leucolepis*, but appeared to be distinctive both in morphology and also in their restriction to distinctive clay-based Carolina bays and similar wet depressions containing the Cypress Savanna community or its Depression Meadow variant (Schafale and Weakley 1990), equivalent to the *Taxodium ascendens*/*Panicum hemitomon*–*Polygala cymosa* Woodland (NatureServe 2005), and Cypress-Gum Pond and Drawdown Savanna/Meadow communities of Nifong (1998). Molecular studies to compare the wet depression populations to material of *E. leucolepis* var. *leucolepis* produced data that proved critical not only for assessment of their distinctiveness but also for resolution of the genetic relationships of the New England Boneset. The purpose of this paper is to present data on the relationships of *E. novae-angliae*, *E. paludicola*, and *E. leucolepis*, and to discuss the taxonomic implications of the results.

MATERIALS AND METHODS

Plant material. Newly analyzed material for this study came from a combination of field-collected samples and herbarium material (Table 2). Additional ITS sequences needed to perform the phylogenetic analysis of *Eupatorium* were taken from Siripun and Schilling (2006).

Molecular methods. Preparations of total DNA were performed with the DNeasy Plant Mini Kit (Qiagen, Valencia, CA) and typically utilized a portion of a single (ca. 0.1 g) leaf. The crude DNA extracts of some samples required further purification using the Wizard Kit protocol (Promega, Madison, WI). The ITS amplifications were performed in 20 µl reactions using 10–20 ng of genomic DNA, 10× PCR buffer (Promega), 1.8–2.25 mM $MgCl_2$, 0.2 mM each dNTP, 1.25 units of *Taq* polymerase, and 0.2 µM each primer. Primers used were "ITS-4" (5′-TCCTCCGCTTATTGATATGC-3′) and "ITS-5" (5′-GGAAG-

TAAAAGTCGTAACAAGG-3′; White et al. 1990). The following protocol was followed for PCR: 94°C for 2 min.; 40 cycles of 94°C for 1 min., 50°C for 1 min., and 72°C for 1 min.; and a final extension of 72°C for 3 min. All PCR products were checked by agarose gel electrophoresis and purified by the Wizard Kit protocol. Sequences were prepared utilizing the ABI Prism Dye Terminator Cycle Sequencing reaction kit and run at the University of Tennessee Automated Sequencing Facility on an ABI 3100 DNA sequencer (Perkin-Elmer Inc., Foster City, CA). For *Eupatorium*, we have found that use of the amplification primers as the sequencing primers often gave unsatisfactory results because of fungal contamination, and have moved to reliance on internal primers located in the 5.8S coding region that are plant specific; the following pair reliably provide clean sequence with an overlap of about 20 bp: "5.8S 79 for" [5′-GCAGAATCCCGTGAACCATC-3′; listed at Southern Illinois Univ. website: (http://www.science.siu.edu/plant-biology/faculty/nickrent/primer.nuclear.html)] and "ITS-5.8SR" (5′-TGACACCCAGGCAGACGTGC-3′; Small 2004). A chloroplast gene region, *trn*H^{GUG}-*psb*A, was amplified and sequenced to provide information on maternal relationships, using the following primers: trnH^{GUG}: (5′- CGC GCA TGG TGG ATT CAC AAT CC-3′; Tate and Simpson 2003) and psbA: (5′-GTT ATG CAT GAA CGT AAT GCT C-3′; Sang et al. 1997). The initial sequence data text files were edited following comparison with the same data displayed in four-color electropherograms before they were analyzed further. Sequence alignment was performed manually. GenBank accession numbers are provided in Table 2.

Cloning of the ITS region for samples of *Eupatorium novae-angliae* was undertaken to verify the identity of the underlying sequences that were hypothesized to be responsible for the base pair and indel polymorphisms. Purified PCR products were ligated into pGEM-T (Promega, Madison, WI) according to the manufacturer's instructions. Competent Top10 F′ (Invitrogen, San Diego, CA) 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 nine colonies.

Phylogenetic analyses. Previously published data from GenBank were obtained to include at least one sample of each diploid

Table 2. Collections of *Eupatorium novae-angliae* and related taxa analyzed for molecular data. *Voucher at UNC; all other vouchers at TENN. [1]clone 1, [2]clone 6.

Taxon	Reference or DNA# Collector/Number	Location	GenBank #
E. album L.	Siripun and Schilling 2006	Lumpkin Co., GA	DQ236200
E. altissimum L.	Siripun and Schilling 2006	Marion Co., AR	DQ236178
E. capillifolium (Lam.) Small	2082 *Thomas 155641*	Nevada Co., AR	DQ415733
E. hyssopifolium L.	Siripun and Schilling 2006	Orange Co., NC	DQ236177
E. lancifolium Small	Siripun and Schilling 2006	Pike Co., AR	DQ236174
E. leucolepis (DC.) Torr. & A. Gray	2041 *Godfrey 80849*	Wakulla Co., FL	DQ415736
	2044 *Rogers 45923*	George Co., MS	DQ415737
	2199 *Schilling 05-21*	Lowndes Co., GA	DQ415738
E. linearifolium Walter	Siripun and Schilling 2006	Henry Co., AL	DQ236199
E. mikanioides Chapman	2083 *Godfrey 84685*	Wakulla Co., FL	DQ415739
E. mohrii Greene	Siripun and Schilling 2006	Onslow Co., NC	DQ236203
E. novae-angliae (Fernald) Haines & Sorrie	2008 *Sorrie 716**	Plymouth Co., MA	DQ415743[1]
			DQ415744[2]
E. paludicola E.E. Schill. & LeBlond	Siripun and Schilling 2006	Hoke Co., NC	DQ236202
	2009 *LeBlond 5062 & Sorrie*	Florence Co., SC	DQ415734
	2010 *LeBlond 5056 & Sorrie*	Scotland Co., NC	DQ415735
Eupatorium hybrid	2011 *LeBlond 6086*	North Carolina	–
E. perfoliatum L.	Siripun and Schilling 2006	Hot Springs Co., AR	DQ236191
	2209 *Laferriere 3574*	Providence Co., RI	DQ415740
E. petaloideum Britton	Siripun and Schilling 2006	Leon Co., FL	DQ236201
E. perfoliatum L. var. *colpophilum* Fernald & Griscom	2210 *Iltis 3518c*	Quebec, Canada	DQ415741
E. pilosum Walter	Siripun and Schilling 2006	Johnston Co., NC	DQ236198

Table 2. Continued.

Taxon	Reference or DNA# Collector/Number	Location	GenBank #
E. resinosum Torrey *ex* DC.	2007 *Sorrie 8228**	Moore Co., NC	DQ415742
E. rotundifolium L. (993 – diploid)	Siripun and Schilling 2006	Jefferson Co., FL	DQ236192
E. rotundifolium L. (922 – polyploid)	Siripun and Schilling 2006	Richmond Co., NC	DQ236197
E. semiserratum DC.	Siripun and Schilling 2006	Liberty Co., FL	DQ236173
E. serotinum Michx.	Siripun and Schilling 2006	Blount Co., TN	DQ236176
E. sessilifolium L. (859 – diploid)	Siripun and Schilling 2006	Montgomery Co., VA	DQ236179
E. sessilifolium L. (829 – polyploid)	Siripun and Schilling 2006	Franklin Co., VA	DQ236182

species of *Eupatorium*. Phylogenetic relationships were analyzed using both maximum parsimony and Bayesian approaches. Parsimony analysis was implemented using PAUP* 4.0b10 (Swofford 2003), with gaps treated as missing data, using a heuristic search with 1000 random addition replicates and with TBR branch swapping. Bootstrap analysis (Felsenstein 1985) was performed with 10,000 replicates using the FASTSTEP search option. Bayesian analysis was implemented in MRBAYES 3.0B4 (Huelsenbeck and Ronquist 2001) run for ten million generations with four separate chains and trees saved every 100 generations. The number of trees to discard as ''burn-in'' was assessed by plotting likelihoods of trees sampled throughout the run and discarding all trees prior to the stable likelihood plateau (in this case the first 10% were discarded). An appropriate maximum likelihood model of sequence evolution (GTR + I + G; General Time Reversible model with a proportion of invariant sites and gamma distributed rates) for the Bayesian analysis was chosen using Modeltest (Posada and Crandall 1998). Trees were displayed with *E. capillifolium* positioned as the outgroup, based on the results of previous analyses (Schmidt and Schilling 2000; Siripun and Schilling 2006).

RESULTS

The ITS sequences of samples of *Eupatorium leucolepis* and *E. paludicola* obtained by direct sequencing showed no evidence of indel polymorphisms or base pair polymorphisms. Sequences of *E. leucolepis* exhibited an 11 bp deletion in ITS-2 relative to all other *Eupatorium* samples analyzed to date, which include at least one sample of all accepted species. Although the sequences of *E. leucolepis* were similar in sequence to those of *E. paludicola*, they differed consistently at eight positions, for a divergence level of 1.5%. Sequences of ITS obtained for two samples of *E. perfoliatum* from northeastern North America, one of which was of the named var. *colpophilum*, differed by only 1–2 bp from one another and from a previously obtained sequence for this species.

In contrast to the results for *Eupatorium leucolepis* and *E. paludicola*, the ITS sequences of *E. novae-angliae* and of material subsequently identified to be the result of hybridization between *E. paludicola* and *E. mohrii* showed several clear base pair polymorphisms as well as regions with the distorted pattern of peaks produced by indel polymorphisms. Automated readout of the sequencing

results gave numerous assignments of "N" where two peaks overlapped. For both samples, it was possible to interpret the pattern of peaks and distinguish the location of the indel polymorphism accurately, and thus to infer the underlying sequences by reference to those obtained from other species of *Eupatorium*. For the sample of *E. novae-angliae*, cloning was undertaken to confirm that the pattern of polymorphic positions was interpreted accurately.

Cloned samples from *Eupatorium novae-angliae* gave two main types of ITS sequences: (1) a set that closely matched those of *E. paludicola* (1–2 bp different); (2) a set that closely matched those of *E. perfoliatum* (1–3 bp difference). The ITS sequence of *E. paludicola* differed from that of *E. perfoliatum* by two one-bp indels in the ITS-1 region, which explained why direct sequencing produced patterns that were in part difficult to interpret. The ITS sequence of *E. paludicola* also differed at 11 bp positions from that of *E. perfoliatum*, and these were identifiable as base pair polymorphisms in the electropherograms produced by direct sequencing of *E. novae-angliae*. Because *E. resinosum* had been proposed previously to be a progenitor of *E. novae-angliae*, an ITS sequence was obtained for this species to allow comparisons. The two ITS repeats of *E. novae-angliae* differed from that of *E. resinosum* by 10 bp and 2 indels, and by 5 bp, respectively; the ITS sequences of *E. perfoliatum* were identical in length but differed by 4–6 bp from that of *E. resinosum*.

Results of phylogenetic analysis for a data matrix that included ITS data for all diploid species of *Eupatorium* are summarized in Figure 1. The aligned data matrix included 645 characters, of which there were 48 potentially parsimony-informative characters and 52 additional variable but parsimony-uninformative characters. Parsimony analysis produced two minimum length trees of almost identical topology, but there was relatively little statistical support for most of the branches, either from bootstrap values or from Bayesian analyses (which were topologically consistent with those from parsimony analysis.) This is consistent with previous studies (Schmidt and Schilling 2000; Siripun and Schilling 2006) that have shown that ITS sequence data while characterizing individual species, provide little phylogenetic resolution for *Eupatorium*. In considering the status of *E. paludicola* and the relationships of *E. novae-angliae*, it is notable that: (1) the samples of *E. paludicola* and those of *E. leucolepis* were each placed in clades that were

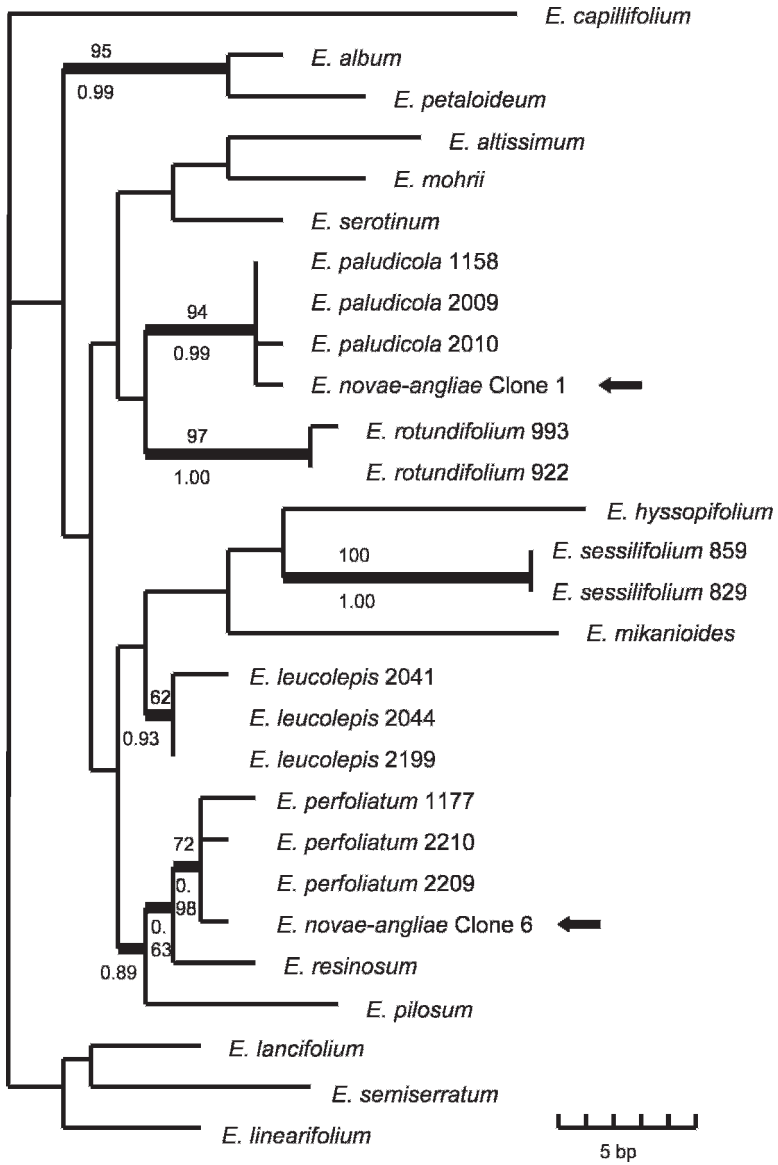

Figure 1. One of two minimum length trees from parsimony analysis of ITS data from *Eupatorium* (149 steps, CI = 0.76, RI = 0.72) showing relationships of *E. novae-angliae*, *E. leucolepis*, and *E. paludicola*. Sample numbers as in Table 2; branches receiving statistical support bolded; numbers above branches

statistically well supported but topologically far separated; (2) the clones of *E. novae-angliae* were placed in strongly supported clades with samples of *E. paludicola* and *E. perfoliatum*, respectively.

Alignment of sequences from the *trn*H-*psb*A chloroplast region produced a data matrix with a length of 534 base pairs. Sequences for all species of *Eupatorium* showed little divergence (not shown), but there were two base pair differences between the sequences obtained for *E. perfoliatum* and for *E. paludicola*. The *trn*H-*psb*A sequence obtained for *E. novae-angliae* matched exactly the sequence from *E. paludicola*, suggesting that the latter was the maternal progenitor.

The ITS sequence of the sample determined to be a hybrid between *Eupatorium paludicola* and *E. mohrii* differed from that of *E. paludicoa* by the presence of bp polymorphisms at 15 positions as well as by the inferred presence of a single one-bp indel polymorphism in ITS-1. The pattern of the polymorphisms matched exactly what would be expected by a mixture of ITS units from *E. paludicola* and *E. mohrii*. Because the two parental species were observed to be present in the immediate vicinity where the hybrid sample was collected, it was not considered necessary to provide further confirmation by cloning.

DISCUSSION

The data from DNA sequence analysis supplements and extends observations from morphology and fieldwork in clarifying the relationships of *Eupatorium novae-angliae*. A key result is the recognition that *E. paludicola* is distinct both morphologically (Table 1) and at the molecular level (Figure 1) from *E. leucolepis*. This allowed the appropriate comparisons to be made to elucidate the progenitors to *E. novae-angliae* as *E. paludicola* and *E. perfoliatum* (Figure 1). Although molecular data do not resolve unambiguously the taxonomic level at which *E. novae-angliae* should be recognized, they make clear its distinctiveness and the desirability of continuing efforts to protect it from extinction.

←

are bootstrap percentages (10,000 replicates, FASTSTEP search) and below branches are Bayesian posterior probabilities. Arrows highlight placement of representative cloned sequences of *E. novae-angliae*.

A first step toward resolving the relationships of *Eupatorium novae-angliae* was the recognition that *E. paludicola* is a separate and distinct entity from *E. leucolepis*. Had sampling been restricted to *E. leucolepis*, the source of one of the ITS sequences present in *E. novae-angliae* would not have been uncovered. The distinction between *E. leucolepis* and *E. paludicola* was based originally on the observation by field botanists undertaking survey work of slight but consistent morphological features (Table 1) as well as on habitat differences. This distinction was supported strongly by consistent differences in the ITS sequences (Figure 1). The description of *E. paludicola* as a novelty in *Eupatorium* is made in an accompanying paper (LeBlond et al. 2007).

The major new result from this study is the evidence that *Eupatorium perfoliatum* and *E. paludicola* are the progenitors of *E. novae-angliae*. This result follows from the demonstration of the presence in *E. novae-angliae* of ITS repeats units characteristic in both sequence and indel pattern of those of *E. perfoliatum* and *E. paludicola*, respectively (Figure 1). Note that because *E. novae-angliae* is an agamospermous polyploid the two different ITS sequence types can be present and not undergo concerted evolution. Considerable confidence can be placed in the ITS based result because the North American species of *Eupatorium* characteristically differ from one another in ITS sequence while showing little or no infraspecific variation (Figure 1; Schmidt and Schilling 2000; Siripun and Schilling 2006; Schilling, unpubl. data). As documented here, one of the most similar species pairs is *E. perfoliatum* and *E. resinosum*, but these species differ by 4–6 bp; the corresponding ITS copy in *E. novae-angliae* differs from that of *E. resinosum* by five differences and from those of *E. perfoliatum* by as little as a single difference. Thus *E. perfoliatum*, rather than *E. resinosum*, can be identified as the species most likely to be one of the progenitors of *E. novae-angliae*.

The morphological data for *Eupatorium novae-angliae* are not inconsistent with its inferred origin. It is important, when assessing such information, to note that distinctive features of parental species are not always expressed in their hybrid progeny (Rieseberg 1995). Thus while lack of the distinctive perfoliate leaf pattern has been cited in the past to exclude *E. perfoliatum* as a progenitor of *E. novae-angliae* (Haines 2005; Sullivan 1992), other information suggests that this feature may be a recessive trait that would not be expressed in the allopolyploid. In other hybrid combinations known

to involve *E. perfoliatum*, including *E.* × *cordigerum* (the hybrid derivative of *E. perfoliatum* and *E. rotundifolium*), and *E.* × *truncatum* (derivative of *E. perfoliatum* and *E. serotinum*; Tucker and Dill 1989), both combinations that we have confirmed with molecular data (Schilling, unpubl. data), the leaves are only truncate or sessile and are not consistently perfoliate. In other features, such as leaf shape and margin, as well as in indument, *E. novae-angliae* is mostly intermediate between *E. paludicola* and *E. perfoliatum* (Table 1).

The data presented also allow insights into the systematics of *Eupatorium leucolepis*. Sullivan (1972) showed that *E. leucolepis*, which is widespread primarily in the coastal plain (Texas to New York), is known primarily from populations that are polyploid and agamospermous. The fact that the ITS sequence of *E. leucolepis* lacked any evidence of base pair or indel polymorphisms (especially in light of its distinctive 11 bp deletion in ITS-2) suggests that the sampled collections of this species are autoploids whose formation did not involve hybridization with other species, such as *E. paludicola*. Sullivan (1972) identified a single collection of *E. leucolepis* from Georgia that appeared to be a sexual diploid, based on the presence of viable pollen. Morphologically this collection is consistent with those of polyploid *E. leucolepis*, and not with *E. paludicola*, but it has not been analyzed for DNA sequence data. Thus, *E. leucolepis* may resemble a number of other species of the genus (e.g., *E. altissimum*, *E. pilosum*, *E. rotundifolium*, *E. sessilifolium*) in consisting of a combination of diploid populations with a relatively restricted geographic distribution and geographically widespread polyploid populations (Sullivan 1976).

Knowledge of the relationships of *Eupatorium novae-angliae* does not resolve unambiguously how it should be treated taxonomically. The taxonomy of apomictic complexes inevitably contains a large arbitrary element, because they include a mixture of self-perpetuating entities that can exhibit nearly continuous genetic variation from one to another (Grant 1981). The apomictic complex within *Eupatorium* is somewhat less complex than those found in other genera, primarily because many apomictic combinations result in plants that are entirely male sterile and also because there is such a strong correlation between apomixis and ploidy level. Nevertheless, reference to two species complexes in the genus can be made to point out the problems in drawing sharp specific boundaries based only on inferred genomic relationships. *Eupatorium hyssopifolium*

var. *laciniatum* has genomes from *E. hyssopifolium* var. *hyssopifolium* and *E. serotinum* (Schilling, unpubl. data) but cannot always be distinguished with confidence from *E. hyssopifolium* var. *hyssopifolium*, and these are thus currently treated as varieties within a species. At an even more extreme level, *E. linearifolium* includes both diploids and perhaps polyploids with only *E. linearifolium* genomes but also includes populations that are not readily distinguished morphologically but have genomes from *E. hyssopifolium* (Schilling, unpubl. data). Although *E. novae-angliae* is clearly distinctive, it is somewhat arbitrary as to whether to recognize it at the species level or to consider it and *E. paludicola* as conspecific varieties because of their morphological resemblance and genetic overlap. Our preference is to treat it as a distinct species, based on the fact that it can be distinguished morphologically and by its occurrence remote from either progenitor species. The molecular data do make clear, however, that it is not accurate to treat it as a variety of *E. leucolepis*.

We would argue that the taxonomic status of *Eupatorium novae-angliae* is not in any case directly relevant to its conservation status. The taxonomic level at which it is recognized may influence how much effort is placed on its preservation, because a species might be considered to warrant more protection than a variety. The argument might also be advanced that as an apomict—and possibly only a single clone—it may not be particularly significant. A very strong counter argument can be made, however, that *E. novae-angliae* represents an evolutionary experiment, and with its distinctive genomic constitution it has been successful at occupying a habitat in a geographic region not exploited by its diploid progenitors. As such, it represents a distinctive model system to make an investigation of the factors that have allowed it to perpetuate and flourish in its relatively limited range. Because its genomic relationships appear to be clear, attempts could be made to reconstitute it by repeating the presumed original cross, and the outcome may shed light on the evolutionary process of hybridization and polyploidy. Thus it is critical to continue to protect this taxon, regardless of the taxonomic level at which it is recognized.

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