Morphological, Molecular, and Biogeographic Evidence for Specific Recognition of *Euthamia hirtipes* and *Euthamia scabra* (Asteraceae, Asteraeae)

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**Abstract**—The number and identity of species within *Euthamia* (Asteraceae, Asteraeae) have varied considerably among taxonomic treatments. *Euthamia graminifolia* (L.) Nutt. is often treated broadly, including plants from the northern and eastern United States and Canada, including the Atlantic and Gulf Coasts. Broad-leaved, largely glabrous plants from New Jersey to the Florida Panhandle have been inconsistently treated as *E. graminifolia, E. graminifolia* var. *hirtipes* (Fernald) C.E.S. Taylor & R.J. Taylor, *E. hirtipes* (Fernald) Sieren, or a hybrid between *E. graminifolia* and *E. caroliniana* (L.) Greene ex Porter & Britton. Similarly, plants from the Florida Panhandle to eastern Louisiana have been incorporated into *E. graminifolia* or *E. graminifolia* var. *hirtipes* with only Greene in 1902 recognizing these plants as a distinct species, *E. scabra* Greene. To determine the identity and proper rank of these entities, morphological and phylogenetic analyses were performed to evaluate relationships within *Euthamia*. Plants from the Atlantic Coast most morphologically resemble Gulf Coast plants which similarly resemble *E. gymnospemoides* Greene. The Gulf Coast plants and *E. gymnospemoides* share similar DNA sequences while the Atlantic Coast plants represent a unique clade. Neither Gulf Coast nor Atlantic Coast plants contain highly polymorphic sequences, indicating that they are not hybrids. Occasional plants found within southernmost Alabama and the Florida Panhandle have polymorphic sequences and intermediate morphology however, suggesting that putative hybrids exist between Gulf and Atlantic Coast plants. This study concludes that both entities deserve specific rank as *E. scabra* Greene for scabrous plants along the central Gulf Coast and *E. hirtipes* (Fernald) Sieren for largely glabrous plants mostly along the Atlantic Coast. Ecological niche modeling indicates that precipitation, especially during summer months, and soils, namely coarse fragments and sand content, drive the distribution of these organisms, largely restricted to either side of the Apalachicola River serving as a distributional barrier.

**Keywords**—Apalachicola, Atlantic Coast, Gulf Coast.

The genus *Euthamia* consists of rhizomatous, suffrutaceous herbs distributed in North America mostly north of Mexico with conflicting taxonomic treatments owing to various interpretations of morphological features. Species of *Euthamia* have been previously included in the genus *Solidago* (Nuttall 1818; Gray 1882; Friesner 1933); however, morphological (Anderson and Creech 1975) and molecular investigations (Urbatsch et al. 2003) support its segregation as a distinct and distantly related genus. Species circumscriptions within *Euthamia* remain problematic though, particularly within the coastal plain of the Gulf Coast and Atlantic Coast regions. The identities of broad-leaved, pubescent plants ranging from southeastern Louisiana into the Florida Panhandle and relatively broad-leaved, largely glabrous plants from Florida northward along the Atlantic Coast have remained ambiguous.

Only one treatment of *Euthamia* recognized these pubescent plants from the central Gulf Coast as a distinct species from *E. graminifolia* (L.) Nutt., though this differentiation was based on a single specimen, the holotype for *E. scabra* Greene (Greene 1902). The lack of duplicate specimens and the use of relatively minor morphological differences to delineate the proposed species perhaps contributed to a lack of recognition for this entity. The name was not taken into synonymy, and the entity was otherwise completely overlooked (Sieren 1970, 1981; Taylor and Taylor 1983; Haines 2006). This treatment encompassed relatively broad-leaved plants in Mississippi and Alabama but did not incorporate the expanded treatment of *E. graminifolia* var. *hirtipes* proposed by Taylor and Taylor (1983) which additionally included Louisiana, Florida, Georgia, the Carolinas, and Virginia. Haines (2006), however, did not accept Taylor’s treatment of *E. graminifolia* var. *hirtipes* and the name was not listed among the synonyms for *E. graminifolia* in FNA (Haines 2006).

The largely glabrous plants known from Florida and extending up the Atlantic Coast have been inconsistently treated as *Euthamia graminifolia* (Friesner 1933; Taylor 1975), *E. graminifolia* var. *hirtipes* (Taylor and Taylor 1983; Johnson 1995), or *E. hirtipes* (Sieren 1970, 1981). Initially this entity was published as *Solidago × hirtipes* Fernald, a probable hybrid of *S. microcephala* (Greene) Bush, now known as *Euthamia*...
caroliniana (L.) Greene ex Porter & Britton, and S. graminifolia var. nuttalii (Greene) Sieren, which is often treated as E. graminifolia (Fernald 1946). As circumscribed by Sieren (1970, 1981) its range included Virginia and the Carolinas, though Taylor and Taylor (1983) included the pubescent Gulf Coast plants in their treatment of E. graminifolia var. hirtipes, expanding its known range from the Carolinas and Virginia to Florida and Louisiana. Since then, no consensus on the taxonomic disposition of the Atlantic Coast plants in recent floristic works has emerged. Thomas and Allen (1996), for instance, recognized E. graminifolia var. hirtipes in the sense of Taylor and Taylor (1983) while Gandhi and Thomas (1989) recognized this entity at the species level as E. hirtipes (Fernald) Sieren. Weakley (2015) used the name E. hirtipes but in a more restricted sense by excluding plants west of the Florida Panhandle.

In order to further understand the status and relationships among the Gulf Coast and Atlantic Coast taxa, plants from these geographic areas were evaluated through morphological, molecular phylogenetic, and biogeographic perspectives. The primary hypothesis tested was whether the taxonomically ambiguous Gulf Coast and Atlantic Coast plants constitute varieties of the E. graminifolia complex, hybrids, or distinct species. Morphological examination of herbarium specimens, phylogenetic analysis of DNA sequence data, species distributions estimated through ecological niche modeling (ENM), and niche comparisons were used to delineate species relationships and characteristics within Euthamia.

**Materials and Methods**

**Taxon Sampling and Morphological Analyses**—Taxon sampling included representatives of currently accepted Euthamia (Haines 2006) species across North America and additional specimens from the coastal regions in eastern North America. Material was primarily obtained from herbarium specimens and supplemented by fresh collections. Field excursions were made throughout the southeastern USA in order to make observations and collect samples for morphological and DNA analyses. Voucher specimens were prepared following standard herbarium procedures and have been deposited in the S. C. Tucker Herbarium (LSU) and the Southern Illinois University Carbondale Herbarium (SIU). Loans of herbarium specimens were used for morphological study and as tissue sources for DNA investigations from the following herbaria: BALT, MICH, NCU, NLU, and NO. On site consultation of herbarium specimens was done at AUA, LL, LSU, MISS, TEX, UNA, US, and UWAL. Data and images were also examined from SERNEC (Denslow et al. 2016; http://sernecportal.org/) and SEINet (SEINET Portal Network 2019; http://svsbiodiversity.org/seinet/collections/list.php).

Morphological measurements and observations were made on field excursions, on freshly collected specimens, and collections from herbaria. Mature, healthy specimens from different geographic regions were selected for analysis. Upper cauline or lower inflorescence branch leaves were measured to retain consistency. Involucrure width was measured at its widest, and dried specimens with artificially spreading phyllaries were excluded from analyses. Measurements and data were obtained from the published literature (Sieren 1970, 1981; Taylor 1975; Taylor and Taylor 1983; Haines 2006). Fifteen to 36 specimens per entity were examined and measured for comparative purposes to supplement data available from published investigations, and five measurements were conducted to identify pairwise differences for individual traits among species. Boxplots based on ANOVAs were subsequently constructed to visualize variability among species. All ANOVAs, Tukey’s HSD tests, and boxplots were conducted in R Software (R Core Team 2019) using the vegan package in the vegan library 2.0.0 (Oksanen et al. 2013) and Tukey-HSD function in the stats package. A distance-based redundancy analysis (dbRDA), comparable to a principal components analysis (PCA) was produced using the vegan package in R using the capscale function (Oksanen et al. 2013) and was visualized in SigmaPlot v. 11.0 (Systat Software, San Jose, CA). The final figures were edited in Adobe Illustrator. The morphological matrix is available in Dryad (Szubryt et al. 2020).

**DNA Extraction, Amplification, and Sequencing**—Total genomic DNA was extracted through a modified CTAB method with an additional silica-column purification step (Doyle and Doyle 1987; Neubig et al. 2014) from herbarium specimens and fresh collections. Two nuclear ribosomal regions, the internal transcribed spacer (ITS 1, 5.8S, and ITS 2) and external transcribed spacer (ETS), were amplified through PCR. Both sequences were aligned for a total of 1164 base pairs with 45 variable sites. The Y4 and Y5 primers were utilized to amplify ITS (Hoshi et al. 2008); the 18S-IGS (Baldo and Markos 1988) and ETS-B (Beardsley and Olmstead 2002) primers amplified the ETS locus. The master mix for ITS amplification included 14.5 μl dH2O, 5.0 μl Phusion buffer, 1.5 μl 25 mM MgCl₂, 0.5 μl dNTPs, 0.05 μl forward 10 μm primer, 0.5 μl reverse 10 μm primer, and 0.2 μl Phusion polymerase for 2.0 μl template DNA per sample. The GoTaq reagent (Promega, Madison, Wisconsin) was used to amplify the ETS region and contained 14.5 μl dH2O, 5.0 μl 5x GoTaq Buffer (Gébèls et al. 2003), 2.0 μl 25 mM MgCl₂, 0.5 μl dNTPs, 0.5 μl 10 μm forward primer, 0.5 μl 10 μm reverse primer, and 0.15 μl Taq polymerase for 1.0 μl template DNA per sample. Cycling conditions for ITS sequence amplification included a warming step of 98°C for 30 s, followed by 35 cycles of 98°C for 10 s, 62°C for 15 s, and 72°C for 30 s, and terminated with 120 s of 72°C before holding at 8°C. Cycling conditions for sequence amplification for ETS included a warming step of 98°C for 120 s, followed by 35 cycles of 95°C for 15 s, 55°C for 15 s, 72°C for 60 s, and terminated with 120 s of 72°C before holding at 8°C. Subsequent PCR products were run on a 1% agarose gel to visualize quality and determine the degree of necessary dilution. PCR products were diluted with water to approximately equivalent concentrations and sequenced by Eurofins Genomics (Louisville, Kentucky) on an ABI3730xl. The matrix used in phylogenetic analyses is available in Dryad (Szubryt et al. 2020).

**Phylogenetic Analyses**—Sequence data were viewed in Geneious R10 (Kearse et al. 2012). Forward and reverse sequence edits were performed to trim low-quality ends and check for improper base calls and polymorphisms for both nuclear ribosomal loci; ends of the loci were trimmed to exclude primer sequences; all newly generated data were deposited in GenBank (Appendix 1). Internal and external transcribed sequence data for outgroup taxa (Urbatsch et al. 2003) were retrieved from GenBank (Appendix 2). These sequences were aligned in SeaView (Galtier et al. 1996; Gouy et al. 2009) using MUSCLE (Edgar 2004). Both ITS and ETS data matrices were concatenated in Geneious R10 (Kearse et al. 2012).

Likelihood model selection of unpartitioned nuclear-ribosomal data was conducted in PAUP* (Swofford 1998; Posada 2003). Model selection algorithms favored the general time reversible (GTR) likelihood model (Waddell and Steel 1997) with gamma (G) and without invariant sites (l). Character states were unweighted in each analysis. Maximum parsimony (MP) analyses were conducted using TBR branch swapping (Fitch 1971; Swofford 1998). Maximum likelihood (ML) analyses were conducted in RAxML v. 8.2.10 (Stamatakis 2014). Bayesian inference (BI) analyses using the Markov chain Monte Carlo (MCMC) algorithm were conducted in MrBayes v. 3.2 (Ronquist et al. 2012) for five-hundred thousand generations to ensure that the average standard deviation of split frequencies fell below 0.01. The BI analyses ran with four chains and two with autocorrelated gene trees using the general time reversible model with both gamma distributions and invariant sites. The BI analyses excluded the first 25% of topologies produced as burn-in which was confirmed by viewing the output files in Tracer v. 1.7 (Rambaut et al. 2018). Phylogenetic trees were visualized in FigTree v. 1.4.3 (Rambaut 2007; http://tree.bio.ed.ac.uk/software/figtree/), and support values were added in Adobe Illustrator. The phylogenetic trees from these analyses are available in Dryad (Szubryt et al. 2020).

**Distribution Models**—Species distributions for the Gulf Coast and Atlantic Coast plants were modeled using MaxEnt (Elith et al. 2006) and parameterized in SDMToolbox (Brown et al. 2017). We evaluated the
performance of various combinations of five feature classes (linear, linear and quadratic; hinge; linear, quadratic, and hinge; and linear, quadratic, hinge, and product), and 3 regularization multipliers (0.5, 1.0, 1.5). Performance of ENMs was evaluated by models built under each combination of model parameters through a geographically structured k-fold cross-validation (i.e. the occurrence records were partitioned into equal geographically clustered subsamples, here k = 3, and the models were trained with three of the groups and then evaluated with the excluded group until all group combinations were run (Shcheglovitova and Anderson 2013). Model fit was assessed through evaluation of the omission error-rate, area under the curve, and model feature class complexity. After optimal model parameters were determined, a final ENM was built with all occurrence sites. Coordinates for specimens were recorded in a CSV file using the WGS84 datum format and projected onto a basemap of the continental United States in ArcMap v. 10.6 (ESRI 2011). All models used 19 standard bioclimatic variables summarizing patterns of precipitation and temperature (Fick and Hijmans 2017), 10 soil variables (Batjes et al. 2015), and one variable corresponding to estimated historical fire frequency and intensity (Ryan and Opperman 2013) environmental data at a 30 arc-second resolution.

Localities were rarefied at 25 km to reduce spatial biases and negative impacts of spatial autocorrelation. A bias file was generated from rarefied points using minimum convex polygons with a 400 km buffer zone from which to select background points in SDMtoolbox (Brown et al. 2017). Response curves, prediction pictures, and jackknifing to measure environmental variable importance were produced additionally for each species modeled. Subsequent model processing produced distribution maps accompanied with estimates of each environmental variables’ percent contribution. The distribution maps were overlain on a map of the United States with the rarefied and collective data points for species evaluated.

Niche overlap and niche divergence tests were performed in the R package ‘humboldt’ (Brown and Carnaval 2019). For these analyses we started with all 19 bioclimatic variables from worldclim.org at 10 arc-minute resolution. These layers were clipped to the extent of both species total distributions and imported into R. Environmental variables used to quantify the species niches were determined by using the ‘humboldt.top.env’ function, which selected variables that contributed more than 5% to a boosted regression model of each species using a learning rate of 0.001. For the niche overlap and niche divergent tests, we performed 200 replicates for each background and equivalence statistic. A matrix of coordinates and specimens used for niche modeling and comparisons is available from Dryad (Szubryt et al. 2020).

Fig. 1. Boxplots of 12 morphological features. Black bars within boxes represent medians, with upper and lower box limits outlining the 25% and 75% quartiles. The dotted lines above and below each box represent 95% confidence intervals. Circles represent outliers (points beyond two standard deviations from the mean). Euthamia gymnospermoïdes has been shortened to E. gymno. for spacing purposes.
**Results**

**Morphological Analyses**—Most Euthamia specimens examined contained leaves with scabrous margins. Virtually all specimens contained resin pits with flagelliform or biseriate trichomes (Supplemental Fig. S1, Szubryt et al. 2020). Each species may have entirely glabrous or at least partially pubescent forms, with trichomes occurring on leaves, stems, inflorescence branches, and peduncles if present. Leaves are frequently linear-lanceolate with either scabrous or hirtellous pubescence and acute-attenuate apices (Fig. S2, Szubryt et al. 2020). Frequently, leaves ascend slightly or spread, only sharply ascending in E. gymnospermoïdes and generally recurved or downward spreading in E. caroliniana. Inflorescence branching can comprise nearly half of a plant’s total height, with cymose clusters of sessile or subsessile heads in E. graminifolia or the Atlantic Coast plants (Fig. S3, Szubryt et al. 2020). Discernible peduncles are common in the southeast Gulf Coast plants and E. gymnospermoïdes, and heads in the latter are less frequently clustered (Fig. S3). Quantitative morphological comparisons via Tukey’s HSD tests are illustrated with the boxplots as letters indicating significantly different groups (Fig. 1). Each of the 12 quantitative characters examined were highly significant ($p < 0.01$) according to Tukey’s HSD across the five species, and E. graminifolia was the most distinct species according to dbRDA analysis (Fig. 2).

Plants from southeastern Louisiana eastward to Bay Co., Florida frequently have relatively broad leaves and decurrent leaf bases. These plants commonly have scabrous stems, inflorescence branches, peduncles, leaf surfaces, bases, veins, and margins. Their leaf surfaces are resin-coated with prominent resin pits visible as white dots (perhaps crystallized resin) or dark markings, adaxially. Most E. gymnospermoïdes are similarly or more prominently resin-coated, producing a shiny varnish on leaves and particularly stems. While E. gymnospermoïdes may be entirely glabrous, particularly in the southeastern portion of its range, plants commonly have sparse scabrous hairs on their abaxial midveins. They may have a prominent line of nearly hirsute hairs on their adaxial midvein, and pubescence may be present on both the upper and lower midvein. Euthamia graminifolia may similarly be wholly glabrous with resin pits to densely hirtellous throughout the entire plant body, often with fewer discernable resin pits. Euthamia graminifolia has the longest and frequently widest leaves of any Euthamia species, considerably larger than plants occurring from the Apalachicola River and peninsular Florida northward along the Atlantic Coast to New Jersey. The latter are primarily glabrous or sparsely hirtellous on leaf surfaces, decurrent leaf bases, and stems. Additionally, the Atlantic Coast plants have prominent resin pits, and pustules may be present. Collectively, plants from the Atlantic Coast are exceedingly morphologically similar, particularly in leaf dimensions, to E. gymnospermoïdes and generally similar to E. caroliniana and the southeastern Gulf Coast plants. (Fig. 2).

**Phylogenetic Analyses**—Thirty new ITS and ETS sequences were obtained for Euthamia species. The total ITS and ETS alignment was 1198 base pairs long with 66 variable sites. Relationships among the five commonly accepted species (E. caroliniana, E. graminifolia, E. gymnospermoïdes, E. leptoechepala (Torr. & A. Gray) Greene ex Porter & Britton, and E. occidentalis Nuttall) and two additional taxa, representing the Gulf Coast and Atlantic Coast plants, were sufficiently resolved using these two nuclear ribosomal loci (Fig. 3). Euthamia caroliniana and northern E. graminifolia, including the conspicuously pubescent variety nuttallii and the largely glabrous varieties, E. graminifolia var. graminifolia and E. graminifolia var. major (Michx.) Moldenke, had similar sequences. Both E. leptoechepala and E. occidentalis possessed markedly distinct DNA sequences. The Gulf Coast populations from southeastern Louisiana eastward to the Apalachicola River were genetically similar to those of the prairie species E. gymnospermoïdes and the southeastern Texas plants sometimes distinguished as E. pulvulenta. The taxonomically ambiguous Atlantic Coast plants ranging from the Apalachicola River extending down peninsular Florida, and northward to coastal New Jersey, formed a unique clade. Three highly polymorphic sequences (Fig. S4, Szubryt et al. 2020) from Mobile Co., Alabama and Wakulla Co., Florida were found which may be hybrids of the Gulf Coast and Atlantic Coast plants and were not included in the final phylogenetic analysis. Bootstrap support values and posterior probabilities for the E. gymnospermoïdes and Gulf Coast plants and the Atlantic Coast plants increased substantially when these hybrid taxa were excluded from the analysis.

**Distribution Models**—The Gulf Coast plants were predicted to occur east of the Mississippi River from southeastern Louisiana to Bay, Taylor, and Wakulla counties in Florida largely within the Gulf Coast. The model predicted the highest habitat suitability throughout eastern Louisiana, southern Mississippi, southernmost Alabama, and the western portion of the Florida Panhandle (Fig. 4). Southeast Louisiana, southern Mississippi, and both coastal counties in Alabama had especially high habitat suitability.

The Atlantic Coast plants occur from Bay Co. in the Florida Panhandle to Lee Co. in peninsular Florida then northward to Ocean Co., New Jersey. The Atlantic Coast taxon’s ENM extends from the coastal regions of New Jersey and Delaware to
Mississippi and Louisiana (Fig. 5). Percentage contributions and permutation importance for each variable tested for both ENMs is available in Dryad (Szubryt et al. 2020) as Supplemental Table 1.

Both niche divergence and niche overlap tests indicate that the Gulf Coast and Atlantic Coast plants inhabit significantly different niches. The niches for both species were significantly different when evaluating the full extent of their ranges (Niche Overlap Test, \( D = 0.08, p = 0.00498 \)) and when solely considering shared analogous environmental space (Niche Divergence Test, \( D = 0.047, p = 0.0099 \)).

**Taxonomic Treatment**

EUTHAMIA SCABRA E.L. Greene, *Pittonia* 5(26C): 80. 1902. Type: USA. Mississippi: Harrison Co. Vicinity of Biloxi, 24 October 1897, S.M. Tracy 1750. (NDG00055!, image viewed in Global Plants JSTOR).

Rhizomatous perennial herbs, 40–120 cm. Stems simple, branched in the inflorescence at \( \frac{1}{4} \) to \( \frac{1}{3} \) below the apex, scabrous distally and on the inflorescence branches, becoming glabrous and woody basally. Leaves usually ascending to spreading, sessile to subsessile; blades 3–5 nerved, rarely 1- or
7-nerved, elliptic to narrowly lanceolate, 40–100 × 2–9 mm, somewhat reduced distally, firm-herbaceous, margins scabrous, apices acute to acuminate, faces abundantly and prominently gland-dotted with resin pits (ca. 50 per mm²), often scabrous, scabrous more so on major veins and decurrent leaf bases, moderately pustular, may be resin coated. **Heads** mostly pedunculate, rarely glomerulate, arrays slightly rounded. **Involucres** obconic, 4–6.5 × 1.9–3.2 mm. **Phyllaries** 3–4 seriate, graduated; often green-tipped, outer ovate, inner linear-oblong, apices obtuse to acute, ± resinous. **Ray florets** 9–16, ligules 1.8–3 × 0.4–0.8 mm. **Disc florets** 3–9; corollas 4.1–5.5 mm, lobes 1–1.7 mm; anthers 1.5–2 mm, appendages 0.4–0.5 mm × 0.15–0.2 mm; style branches 1.2–1.5 mm, appendages 0.7–0.9, stigmatic lines 0.5–0.8 mm. **Achenes** elliptical, 0.9–1 mm, slightly ridged, moderately pubescent, hairs 0.19–0.37 mm. **Pappus** 4.1–5.6 mm. Fig. S2 highlights differences in pubescence from *E. graminifolia* which this species has previously been classified as.

**Distribution and Habitat**—**Euthamia scabra** occurs in southeastern Louisiana, southern Mississippi, southern Alabama, and the western Florida Panhandle from sea level to 130 m. Habitats commonly include open, wet to seasonally dry, sandy areas, occasionally in shallow water, and disturbed sites such as roadsides.

**Notes**—Despite morphological similarities to *Euthamia gymnospermoïdes*, differences in pubescence, leaf dimensions, leaf orientation, nucleotide sequences, habitat, and biogeography support its recognition as a distinct species.

**Euthamia hirtipes** (Fernald) Sieren. *Phytologia* 23:304. 1972. *Solidago × hirtipes* Fernald, *Rhodora* 48:65. 1946. **Type**: USA. Virginia: Sussex Co. roadside thicket ca. 1.5 mi north of Waverly. 13 September 1945. **M. L. Fernald and B. Long** 15015 (GH) (Holotype: GH00012481; Isotypes: (PH), PH00013963; (US), US00127734, images viewed in Global Plants JSTOR).

**Rhizomatous perennial herbs**, 40–150 cm. **Stems** simple or branched in the inflorescence at mid-height and above, glabrous to sparsely hirtellous. **Leaves** usually ascending, lower may be spreading, sessile to subsessile; blades 1–3– rarely 5-nerved, linear to narrowly lanceolate, 45–85(–120) × 1.4–6 mm, somewhat reduced distally, firm-herbaceous, older leaves occasionally curled slightly, margins scabrous, apices acute to acuminate, resin pits abundant and prominent, (approximately 50 per mm²), translucent when viewed in transmitted light with magnification, faces mostly glabrous, major veins and decurrent leaf bases glabrous or sparsely hirtellous, usually resinous; axillary fascicles sometimes present distally and in the inflorescence. **Heads** mostly subsessile, usually glomerate, arrays mostly rounded. **Involucres** obconic, 4.1–6 × 1.7–3.5 mm. **Phyllaries** 3–4 seriate, graduated; often green-tipped, outer ovate, inner linear-oblong, apices obtuse to acute, ± resinous. **Ray florets** 10–15, ligules 1.8–3 × 0.4–0.7 mm. **Disc florets** 4–8; corollas 3.5–5.2 mm, lobes 1.1–1.6 mm; anthers 1.4–1.8 mm, appendages 0.3–0.5 mm × 0.15–0.19 mm; style branches 1.1–1.4 mm, appendages 0.5–0.8 mm, stigmatic lines 0.4–0.6 mm. **Achenes** elliptical, 0.8–1.1 mm, slightly ridged, pubescent, hairs 0.25–0.42 mm. **Pappus** 3.2–4.4 mm. **Distribution and Habitat**—**Euthamia hirtipes** can be found in Florida Panhandle south to Lee Co., Florida and north to New Jersey from sea level to 130 m above. Habitats include open, wet to seasonally dry, sandy areas, occasionally in shallow water, and disturbed regions such as roadsides.

**Notes**—Although designated as a variety of *Euthamia graminifolia* or a hybrid between *E. caroliniana* and *E. graminifolia* by different authors, sequence data clearly differentiate it as a unique species despite morphological similarities to *E. graminifolia* and *E. scabra*. Based on DNA sequence data, the latter and *E. hirtipes* can hybridize in the southwestern portion of Alabama and the western Florida Panhandle, but both species are otherwise distinct morphologically and phylogenetically.
**Key to Euthamia in Eastern North America**

1. Capitula sessile or short pedunculate, possessing 20 or more flowers; leaves and distal stems usually pubescent and often densely so, rarely or only thinly resin coated; resin pits sparse to moderately abundant on the upper and lower leaf surfaces or absent; pustules lacking; found mainly from northern Tennessee and Virginia northward ............................................................................ *E. graminifolia*

1. Capitula pedunculate or subsessile with fewer than 22 flowers; leaves and distal stems glabrous or moderately pubescent, resin coated; numerous, circular resin pits crowding the upper and lower leaf faces; pustules present or absent; distributed along the Gulf or Atlantic Coasts or inland .... 2

2. Leaf surfaces glabrous; resin pits absent; pustules abundant which appear as elliptical raised areas or translucent dots; common throughout eastern Texas, Louisiana, Mississippi, Alabama, western Georgia, and uncommonly in southwestern Tennessee, southeastern Missouri, and southwestern Illinois .... .................................................................................... *E. leptosephala*

2. Leaf surfaces glabrous or pubescent; resin pits abundant; pustules absent or sporadic ................................................................. 3

3. Decurrent leaf bases and leaf faces glabrous except for the margins and or midvein, 2.5–5.5 mm wide, mostly with 1–3 major veins, pustules absent; growing in SW Louisiana and eastern Texas northward throughout the midwestern states ......................... *E. gymnospermoides*

3. Decurrent leaf bases and leaf faces glabrous or sparsely to abundantly pubescent on their margins and or major abaxial veins, 2.4–9 mm wide, (1–)3–5 veined; pustules usually present (best seen with oblique surface lighting and magnification); found along the Gulf or Atlantic coasts .... 4

4. Distributed along the Gulf Coast from southeastern Louisiana to the Apalachicola River in the Florida panhandle; stems, decurrent leaf bases and leaf faces glabrous or hirtellous .............................................................. *E. scabra*

4. Distributed along the Gulf and Atlantic Coasts from the Apalachicola River region and peninsular Florida to New Jersey; stems, decurrent leaf bases and leaf faces glabrous or hirtellous .............................................................. *E. hirtipes*

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Fig. 5. Species distribution map for the Atlantic Coast plants, *Euthamia hirtipes*. The known distribution is limited to coastal regions from western Florida to New Jersey. Red regions indicate higher habitat suitability, and rarefied points are shown as black triangles. *Euthamia hirtipes* and *E. scabra* occur parapatrically along westernmost Florida where hybrid taxa have been discovered. The ecological niche model (ENM) is substantially impacted by the sand content of the soil, mean temperature of the wettest quarter, precipitation of the driest month and warmest quarter.
**Discussion**

**Morphology**—Morphological evaluations suggest that the populations of Gulf Coast plants ranging from southeastern Louisiana to the Florida Panhandle near the coast represent a different taxon from those presented in previous treatments, distinguished most evidently by leaf width (Fig. 1). Specimens from the Gulf Coast from the 1800s were often regarded as *E. graminifolia* (Taylor and Taylor 1983). Similarly, Mohr (1901) reported *Euthamia* (*Solidago*) *graminifolia* in Mobile Co., Alabama. Taylor and Taylor (1983) further noted leaf similarities of the Gulf Coast and Atlantic Coast plants to *E. graminifolia*, denoting both as *E. graminifolia var. hirtipes*. They observed that these southeastern Gulf Coast plants are similar to *E. gymnospermoides* in having pedunculate capitula but seemingly discounted this when suggesting close affinities, perhaps owing to differences in pubescence and leaf dimensions. The many shared features between the Gulf Coast plants and *E. gymnospermoides*, including resin coated leaf faces, prominent and numerous resin pitted leaves, less congested capitulescences, pedunculate heads, and longer involucres and disk corollas, suggest a close affinity between these two taxa.

Despite the resemblance to *E. gymnospermoides*, others have followed the Taylor and Taylor proposal, recognizing the Gulf Coast plants as a variety of *E. graminifolia*, or within a broadened concept of *E. hirtipes* (Gandhi and Thomas 1989; Thomas and Allen 1996; Weakley 2015). While the Gulf Coast and Atlantic Coast plants are morphologically similar in several regards, differences in leaf size, leaf pubescence, floret numbers, floret sizes, capitula sizes, and peduncle length distinguish both entities outside of uncommon hybrid progeny. These putative hybrids plus the morphological variability in pubescence and leaf dimensions likely contributed to their treatment as a single heterogeneous entity. The southeastern Gulf Coast and Atlantic Coast species do not entirely separate out in the dbRDA ordinations (Fig. 2), highlighting that species are highly similar morphologically when cumulatively analyzed.

**Phylogenetics**—Sequence data provide evidence to reject the hypotheses that the Gulf Coast plants are either *Euthamia graminifolia*, the same taxon as the Atlantic Coast plants, or a hybrid between *E. graminifolia* and *E. caroliniana*. The similar sequence data for *E. gymnospermoides* and the Gulf Coast populations indicate that the two are sister taxa and reciprocally monophyletic. The Gulf Coast taxon does not display a hybrid signal, although hybrids between it and the Atlantic Coast taxon were detected owing to highly polymorphic sequences (Fig. S4). The Atlantic Coast taxon similarly does not appear to be of hybrid derivation, nor does it form a clade with *E. graminifolia*, to which it is often taxonomically allied.

**Distribution Models**—The Gulf Coast and Atlantic Coast plants collectively occur along coastal regions from southeastern Louisiana to southwesternmost Alabama and northwestern Florida to New Jersey, respectively. Hybrids have been located near the intersection of their ranges in Mobile Co., Alabama and Wakulla Co., Florida. Each entity evidently can tolerate pulses of salt stress, and salt exposure may be an important factor in their realized niches (Fig. S1). The amount of precipitation appears to primarily drive their distributions, particularly the amount during the driest and warmest quarters for the Gulf Coast and Atlantic Coast plants respectively. Ranges for both taxa are limited by rainfall, which is provided in the coastal environment. Coarse fragments and sand content in the soil are important for the Gulf Coast and Atlantic Coast plants respectively as well, indicating different habitat preferences for these largely coastal species (Fig. S1).

Regarding the Gulf Coast taxon, Taylor and Taylor (1983) mapped the distribution of *E. graminifolia var. hirtipes*, which in their treatment included both the Gulf Coast and Atlantic Coast plants, to include Vermilion Parish, Louisiana, coastal counties in Alabama and Mississippi, and to Pasco Co., Florida but skipping the Mississippi River valley. Our investigations show a similar distribution with few exceptions (Fig. 4). The Gulf Coast plants grow in an area from Lafourche and Livingston Parishes, Louisiana eastward to Bay Co., Florida with a few outlier counties, including Jones Co., Mississippi and Winn Parish, Louisiana west of the Mississippi River. The species occurrence in Winn Parish needs verification in order to rule out a specimen labeling error. However, similar habitat is known in the area that may be suitable for this taxon to thrive. Additional localities have been recorded in other states from second tier coastal counties some distance from the coast. We also have not been able to substantiate the Vermilion Parish record noted in Taylor and Taylor (1983); *Euthamia* recorded for that parish all represent *E. gymnospermoides*. The Winn and Vermilion Parish records are suspect because they are the only ones reported west of the Mississippi River.

The Atlantic Coast plants, which range from Bay and Lee counties in Florida northeast to Ocean County, New Jersey, were projected onto their observed distribution accurately despite some outlier regions. The Atlantic Coast taxon was predicted to occur in coastal Louisiana, Mississippi, and Alabama (Fig. 5), though its presence has not been recorded in these states beyond the Apalachicola River, which serves as a discontinuity for many species distributions (Soltis et al. 2006). The model for the Atlantic Coast plants calculated precipitation was a larger driver of its distribution than soil conditions, hence why this natural barrier may have been undetected. The presence of relatively recent or introgressive hybrids, as inferred from sequence polymorphisms and phylogenetic placement, indicate that this taxon has occurred in westernmost Florida and interbred with the Gulf Coast taxon in the recent past, however.

Niche tests reject the hypothesis that the Gulf Coast and Atlantic Coast plants inhabit identical niches when considering both the entire extent of their distributions and shared accessible environmental space. While both species likely co-occur within the same counties of the Florida panhandle, Niche Divergence and Niche Overlap Tests indicate that both species inhabit distinct niches. While putative hybrids have been noted in the Florida Panhandle, the parent species should not occur syntopically.

**Taxonomy**—A survey of published names in *Euthamia* and *Solidago* and their types concludes that the Gulf Coast populations represent the taxon *E. scabra* Greene. This species was published by Greene (1902) based on Tracy 1750 from the vicinity of Biloxi, Mississippi; that name apparently has been overlooked since its publication. Sieren (1970, 1981) does not mention it, and neither do Taylor and Taylor (1983). Examination of the type image (NDG) in JSTOR Global Plants shows that Arthur Haines annotated it as *E. gymnospermoides* but did not mention that species as occurring in Mississippi (Haines 2006). Collections made by Lowell Urbatsch in the vicinity of Biloxi match the type specimen; sequences and morphologies from those accessions are similar with those plants judged...
to be that taxon. Based on the evidence herein, the Gulf Coast taxon should be recognized as *Euthamia scabra*.

Plants from Wakulla Co. east of the Apalachicola River into peninsular Florida and up the Atlantic Coast to New Jersey share morphological and molecular features with plants judged to be *E. hirtipes*. Sieren (1970, 1981) showed the distribution of that species to include only the Carolinas and Virginia. Likewise, we regard specimen records published by Taylor and Taylor (1983) for *E. graminifolia var. hirtipes* from several counties east of the Apalachicola River to be *E. hirtipes*. Furthermore, our investigations indicate the taxon is more widespread in Florida and also occurs in Delaware, Georgia, Maryland, and New Jersey. *Euthamia hirtipes* appears to grow in similar habitats as *E. scabra*, chiefly sandy, wet areas near the coastal waters of the southern and mid-Atlantic regions. The silt and sand concentrations between the Gulf Coast and Atlantic Coast regions differ considerably however, with the Apalachicola River serving as a common biogeographic barrier that often separates species (Soltis et al. 2006).

Hybrids between *E. hirtipes* and *E. scabra* have been found in southermost Alabama and northwestern Florida sporadically, indicating that the two likely had grown more sympatrically previously. The similarity between the two taxa are likely the result of convergent evolution as sequence differences indicate that *E. hirtipes* and *E. scabra* are not sister species. Interspecific hybridization has been suggested in *Euthamia*, but has not been unequivocally demonstrated (Semple et al. 1984) prior to this study. The proposed hybrid origin for *E. hirtipes* (Fernald 1946) has not been supported in this study, although hybrids between *E. hirtipes* and *E. scabra* were detected herein.

**Acknowledgments**

The authors thank the LSU and SIU herbaria for curation of collections made in this study and AUA, BALT, LI, LSU, MICH, MISS, NCU, NLU, NO, TEX, UNA, US, and UWAL herbaria for access to additional specimens. Also, to Chris Reid, LSU, who made many collections. Additional images of specimens were accessed through SERNEC and SEINet. This work was supported by startup funds from SIU to Kurt Neubig and by funding from NSF DUE-1564969 for scholarship and research funds to Marisa Szubryt. The Botanical Society of America contributed funding towards the phylogenetic aspect of this research. Jason Brown, of Southern Zoology Department, developed the species distribution modeling, and our investigations indicate the taxon is more widespread in Florida and also occurs in Delaware, Georgia, Maryland, and New Jersey. *Euthamia hirtipes* appears to grow in similar habitats as *E. scabra*, chiefly sandy, wet areas near the coastal waters of the southern and mid-Atlantic regions. The silt and sand concentrations between the Gulf Coast and Atlantic Coast regions differ considerably however, with the Apalachicola River serving as a common biogeographic barrier that often separates species (Soltis et al. 2006).

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**Appendix 1.** Plant species, voucher information, herbarium number, and GenBank accession number for ITS and ETS respectively, produced in this study.

**Euthamia caroliniana** (L.) Greene ex Porter & Britton Urbatsch 10780 (LSU:132487; MH794496, MH801440), Urbatsch 10827 (LSU:134814; MH794497, MH801441) Urbatsch 11230 (LSU:176936; MH794498, MH801442). *Euthamia graminifolia* (L.) Nutt. Middleton s.n. (SIU:N/A; AY170945, AY169742).

**Euthamia hirtipes** (Fernald) Sieren Urbatsch 11287 (LSU:176706; MH794476, MH801443), Urbatsch 11288 (LSU:176707; MH794477, MH801444), Urbatsch 11289 (LSU:176708; MH794478, MH801445). *Euthamia scabra* Greene Urbatsch 10098 (LSU:139902; MH794491, MH801435), Urbatsch 10401 (LSU:132475; MH794499, MH801436). *Euthamia hirtipes* (Fernald) Sieren Urbatsch 11287 (LSU:176706; MH794476, MH801443), Urbatsch 11288 (LSU:176707; MH794477, MH801444), Urbatsch 11289 (LSU:176708; MH794478, MH801445). *Euthamia hirtipes* × *scabra* Urbatsch 11231 (LSU:176941; MH794467, MH801431), Urbatsch 11232 (LSU:176942; MH794468, MH801432), Urbatsch 11233 (LSU:176943; MH794469, MH801433). *Euthamia leptocephala* (Torr. & A. Gray) Greene ex Porter & Britton Urbatsch 10401 (LSU:132475; MH794499, MH801443), Urbatsch 11212 (LSU:137674; MH794500, MH801444). *Euthamia occidentalis* Nutt. Urbatsch 7724 (LSU:61862; MH794501, MH801445). *Euthamia scabra* Greene Urbatsch 10780 (LSU:132487; MH794496, MH801440), Urbatsch 10827 (LSU:134814; MH794497, MH801441) Urbatsch 11230 (LSU:176936; MH794498, MH801442). *Euthamia hirtipes* (Fernald) Sieren Urbatsch 11287 (LSU:176706; MH794476, MH801443), Urbatsch 11288 (LSU:176707; MH794477, MH801444), Urbatsch 11289 (LSU:176708; MH794478, MH801445). *Euthamia hirtipes* × *scabra* Urbatsch 11231 (LSU:176941; MH794467, MH801431), Urbatsch 11232 (LSU:176942; MH794468, MH801432), Urbatsch 11233 (LSU:176943; MH794469, MH801433), Urbatsch 11234 (LSU:137674; MH794500, MH801444). *Euthamia leptocephala* (Torr. & A. Gray) Greene ex Porter & Britton Urbatsch 10401 (LSU:132475; MH794499, MH801443), Urbatsch 11212 (LSU:137674; MH794500, MH801444).