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A New Species of Euphorbia subgenus Chamaesyce section Alectoroctonum (Euphorbiaceae) from Limestone Hills of Wayne County, Mississippi

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A New Species of *Euphorbia* subgenus *Chamaesyce* section *Alectoroctonum* (Euphorbiaceae) from Limestone Hills of Wayne County, Mississippi

by

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Abstract

As part of a project to document the vascular flora of Wayne County, Mississippi, an unusual *Euphorbia*, which keys to the *Euphorbia corollata* complex, was encountered in mature hardwood forests in limestone regions. Unlike typical *E. corollata* and *E. pubentissima*, these individuals have long petioles (0.4–1.2 cm), oval to ovate leaves, short stature, small cyathia, small seeds, and a different phenology. In order to test species boundaries, morphological character differences were explored using principal component analysis (PCA), and additional characters were gathered from plastid (*rpL16*) and nuclear (ITS) DNA data of the unusual individuals as well as of *E. corollata*, *E. pubentissima*, and several other species of *Euphorbia* subgenus *Chamaesyce* section *Alectoroctonum*. The PCA indicates that the individuals are morphological outliers, and phylogenetic analyses of the DNA data indicate that the individuals have a unique haplotype different from *E. corollata* or *E. pubentissima* and are rather more closely related to *E. mercurialina*, a species not in the *E. corollata* complex but which occurs in similar mesic habitat in eastern Tennessee and neighboring Alabama, Georgia, and Kentucky. These data support the hypothesis that these unusual individuals represent a new species. Neither the PCA nor the phylogenetic analysis of DNA data reveals any differences between *E. corollata* and *E. pubentissima*.

Key Terms: *Euphorbia* subgenus *Chamaesyce* section *Alectoroctonum*, *Euphorbia* sect. *Tithymalopsis*, limestone, species delimitation, Wayne County, Mississippi

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Chapter 1: Introduction

Euphorbia is a genus of flowering plants in the rubber family (Euphorbiaceae) and is one of the most diverse and speciose genera of plants on Earth, comprising at least 2100 species (Euphorbia PBI, 2012). Euphorbias include plants measuring up to 20 m high and plants that are so short that they resemble groundcover. The genus also includes both herbaceous and succulent plants. One of the unique features of the genus is the cyathium, a tight inflorescence consisting of reduced individual male and female flowers arranged so that the whole structure often appears to be a single flower.

The bracts that are associated with the cyathium are also very diverse. One of the most well-known examples of these bracts is on the poinsettia. The beautiful red parts on these plants that drive millions of dollars of sales annually are actually bracts below the cyathia. Another commonly cultivated member of the genus is the crown-of-thorns (*E. milii*).

Relationships among species of *Euphorbia* have been addressed in the recent *Euphorbia* Planetary Biodiversity Inventory project (see http://www.euphorbiaceae.org/). That project has focused on relationships at a broad scale, but many questions remain at the species level. In the southeastern United States, for example, *Euphorbia* sect. *Tithymalopsis*, now recognized as part of *Euphorbia* subgenus *Chamaesyce* section *Alectoroctonum* (Yang et al., 2012; Zimmermann et al., 2010), is particularly problematic, as two of the commonly recognized species, *E. corollata* and *E. pubentissima*, are very difficult to tell apart. This group of species is commonly known as

the *Euphorbia corollata* "complex." Most authors of floristic treatments have subsequently followed two major taxonomic revisions (Huft, 1979; Park, 1998), perhaps even with the concern that the species that they recognize should be lumped even further. A reassessment of the *E. corollata* complex, focused on morphological variants from the limestone hills of eastern Wayne County, Mississippi, indicates that an overlooked species within the complex should possibly be recognized.

Although Huft's (1979) and Park's (1998) works were remarkably thorough, they did not (1) have DNA data at their disposal or (2) see much material from the prairies and limestone areas of Mississippi or Alabama, which are home to a number of new taxa (e.g., Allison and Stevens, 2001; Sorrie and Weakley, 2001). Because of the ubiquity of the data, DNA data can often provide clues to differences among populations, clarify relationships, and provide an alternative way of looking at plants, especially where morphological / phenotypic variation is confusing. Further, because a survey of vascular plants of Wayne County, Mississippi, is underway (D. McNair, M.S. thesis, USM), locating and studying the unusual populations of *Euphorbia* is timely.

This project has two major goals: (1) to determine if the unusual *Euphorbia* individuals of Wayne County are actually morphological outliers and (2) to determine if DNA markers reveal haplotypes unique to the unusual individuals. The first goal will be accomplished using standard measurements and descriptive terminology and then by comparing to the measurements and descriptions in Huft (1979) and Park (1998). The second goal will be accomplished by testing several plastid and nuclear DNA markers to assess whether any variation occurs among the putative taxa and what that variation indicates about species boundaries and relationships via phylogenetic analysis.

Chapter 2: Materials and Methods

Morphology and Principal Component Analysis

Specimens were collected in the field or borrowed from herbaria (Table 1), and measurements were made from dried, mounted collections. Measurements of greater than 1 cm were made using a transparent ruler; measurements less than 1 cm were made using a caliper. Seeds were viewed using a FEI Quanta 200 environmental scanning electron microscope (ESEM) at 12.5–15 kV after coating with silver. A full description of the unusual individuals—the putative new species—was prepared so that its characteristics could be compared to those in two recent, broad-scale revisions (Huft, 1979; Park, 1998). In addition, characteristics of the putative new species, as well as the closely related species *E. corollata*, *E. pubentissima*, and *E. mercurialina*, were measured for input into principal component analysis (PCA) to determine if the unusual individuals actually represent morphological outliers. Characters used for PCA included: nodes below the first inflorescence branch, plant height, lamina length, lamina width, petiole length, longest peduncle, and cyathia width from appendage tip to appendage tip (across the "corolla"). Measurements of these features were input into an Excel spreadsheet and then transferred to JMP statistical software (SAS, Cary, NC) where a principal component analysis was performed using the character matrix as the Y vectors. Data were not log-transformed. Eigenvalues were obtained through this principal component analysis, and three different score plots and loading plots were produced based on these eigenvalues.

Sampling

Fresh samples of the putative new species were collected from Wayne County, Mississippi, in September, 2014. Fresh samples of *E. pubentissima* were also collected in September, 2014, from both Wayne and Forrest Counties, Mississippi. Additional samples representing geographic variation and other closely related species were obtained from Tennessee, Alabama, Georgia, and Kansas from Dr. Richard Carter (Valdosta State), Dr. Joey Shaw (University of Tennessee–Chattanooga), and Dr. Mark Mayfield (Kansas State). Fragments were taken from each sample for DNA extraction (Table 1).

DNA Extraction, Amplification, and Sequencing

All reagents named in this section can be found in the Qiagen DNeasy Plant Mini Kit (Qiagen, Valencia, CA). The plant fragments were all individually ground with mortar and pestle along with 0.5 mL buffer AP1 until no solid bigger than the size of a needle tip was remaining. The mixture was then incubated for 10 minutes at 65°C, with intermittent flicking to make sure no solid congealed at the bottom of the tube. 0.13 mL Buffer AP2 was added to the lysate, then the solution was mixed thoroughly and incubated on ice for 5 minutes. After incubation the solution was centrifuged at 14,000 rpm for 5 minutes. The lysate was transferred by pipette into the QIAshredder Mini spin column–the column sitting inside a 2 mL collection tube–and was centrifuged again for 2 minutes at 14,000 rpm. The flow-through was transferred to a secondary tube and mixed with 1.5 volumes of Buffer AP3/E by pipetting 0.65 mL of the mixture into a DNeasy Mini spin column–sitting in a 2 mL collection tube–and centrifuged at 8,000 rpm for 1 minute. The flow-through was discarded and the process was repeated once more

including the discarding of flow-through. The spin column was transferred to a new collection tube and 0.5 mL Buffer AW was added, then the mixture was centrifuged at 8,000 rpm for 1 minute and the flow-through discarded. 0.5 mL of Buffer AW was added to the DNeasy Mini spin column again, and the mixture was centrifuged for 2 minutes at 14,000 rpm. The DNeasy Mini spin column was transferred to a new tube and 0.1 mL Buffer AE was pipetted onto the membrane. The mixture incubated at room temperature for 5 minutes and then was centrifuged at 8,000 rpm for 1 minute. The Buffer AE step was repeated once more to ensure full elution of DNA.

PCR was used to amplify the DNA with the following contents in each tube: 0.002 mL template DNA, 0.0025 mL each of forward and reverse primers, 0.008 mL water, 0.01 mL TBT-PAR as an enhancer (Samarakoon et al., 2013), and 0.025 mL Takara *Ex Taq* Premix (Takara Bio, Otsu, Shiga, Japan). Primers 4 and 5 for ITS followed White et al. (1990), and primers for rpL16 followed Small et al. (1998). A Thermo PCR Sprint thermal cycler was used for the reaction with a warm-up temperature of 95° for 3 minutes, then 98° denaturing temperature for 10 seconds, 50° annealing temperature for 30 seconds, 72° extension temperature for 60s, and finally a 72° final extension temperature for 3 minutes. The denaturation, annealing, and extension steps were repeated for 35 cycles. 0.004 mL of each sample product was mixed with 0.002 mL of loading dye, and the mixture was loaded into an agarose gel along with a DNA ladder. A 100 V current was applied to the gel for 20 minutes and the bands were viewed via a UV light. Positive bands were noted, and the sample they corresponded to was prepared for cleaning.

Clean-up of PCR products was accomplished using a Qiagen PCR Purification Kit (Qiagen, Valenica, CA). Reagent names refer to those in the kit. The PCR product was mixed with 5 volumes of Buffer PB and placed into the provided QIAquick column and 2 mL collection tube and centrifuged for 1 minute. The flow-through was discarded and 0.75 mL of Buffer PE was added to the column, and the mixture was centrifuged for 1 minute. The flow-through was discarded and the tube was centrifuged again for 1 minute, and then the column was placed into a clean tube. 0.05 mL Buffer EB was added and the mixture was centrifuged for 1 minute. The tube was allowed to stand for 1 minute and then was centrifuged again for 1 minute. The solution was sent to and sequenced by MWG Eurofins (Louisville, Kentucky).

Phylogenetic Data Analysis

Resulting DNA data were cleaned and checked for accuracy using Sequencher 5.3 (GeneCodes, Ann Arbor, MI) and aligned using ClustalX2 (Thompson et al., 1997). They were then exported to WinClada (Nixon, 2002) and analyzed using parsimony methods (500 heuristic replications, holding 2 trees per replication, saving up to 5000 trees). Bootstrap analyses were performed in WinClada (Nixon, 2002) to assess the stability of clades given these data (500 bootstrap replications, each with 5 replications, holding 2 trees). Character state changes were mapped directly onto the most parsimonious tree, or strict consensus tree.

Table 1. Samples utilized for DNA work.

Taxon (species)	Collector and Number	DNA Sample #	Location
E. pubentissima	McNair 1949-1	342	Gopher Farm, MS
E. pubentissima	McNair 1949-2	343	Gopher Farm, MS
E. sp. nov.	McNair 1944-1	344	Buckatunna, MS
E. sp. nov.	McNair 1944-2	345	Buckatunna, MS
E. pubentissima	McNair 1951-1	354	Lake Thoreau, MS
E. pubentissima	McNair 1951-2	355	Lake Thoreau, MS
E. pubentissima	Floden & Shepard 2012-129	356	Ocoee, TN
E. pubentissima	Shaw 2011-1	357	Parksville, TN
E. pubentissima	Shaw 2011-2	358	Ocoee, TN
E. mercurialina	Anderson 45	359	Marion Co., TN
E. mercurialina	Shaw (2014)	360	HUTC, TN
E. corollata var. *glauca*	Shaw s.n. (13 July 2010)	361	Little Caney, TN
E. discoidalis	Carter 17720	362	Mitchell Co., GA
E. curtisii	Carter 17692	364	Colquitt Co., GA
E. sp. nov.	McNair 1973	366	Shiloh, MS
E. sp. nov.	McNair 1973	367	Shiloh, MS
E. corollata	Mayfield 4083	368	KS
E. pubentissima	McNair 1949-1	369	Gopher Farm, MS
E. sp. nov.	McNair 1944-1	370	Buckatunna, MS
E. sp. nov.	McNair 1973	371	Shiloh, MS
E. corollata	Fishbein 4831	384	Monroe Co., MS

Chapter 3: Results

Morphology

Descriptive assessment of the unusual *Euphorbia* indicated that several features were outside the range or qualitatively different than the closely related species, providing support for the recognition of these unusual individuals as a new species. In particular, the putative new species has pilose petioles and leaf margins, small cyathia, and generally longer petioles and wider leaves (Figures 1–4). The seeds (Figure 5) are the smallest among the eastern North American members of this section (1.6 mm vs. 1.8–3.3 mm long) and are broadly depressed on the hilum side and pitted throughout, much like *E. mercurialina* and unlike *E. corollata* and *E. pubentissima* (cf. Park, 1998). A full description and photos from the field are given below.

Description, Euphorbia sp. nov. (Figures 1–5)

Perennial herb, stems erect, 17–30 cm tall, 0.5–1.1 mm diameter, pink near base, green above, glabrous to sparsely pilose below, sparsely pilose to pilose, especially at the nodes, above, one principal stem per individual, no branching except in the inflorescence, 7–11 nodes, internodes 3.5–4.8 cm at base, 0.5–1.8 cm near apex. Roots cylindrical, mostly erect/vertical, sometimes contorted in other directions, 2.3–4.6 (–7.5) mm diameter. Leaves alternate, petiolate; petioles 4.1–11.5 mm long, 0.1–0.3 (–0.4) mm diameter, pilose, hairs having pink pigment and up to at least 0.7 mm long, light green to pink, straight; scale leaves absent, earliest 2–4 leaves presumably deciduous; leaf blades often flexed at petiole apex, (rotund–) ovate (–ovate-oblong), 1.4–4.4 cm long, 0.5–2 cm

wide, margin moderately pilose, green or with a narrow translucent band, sparsely to moderately pilose abaxially, apex rounded to obtuse, base rounded to obtuse; stipules represented by small glands, ca. 0.1 mm diameter. Stem terminated by cyathia and subtending bracts in a 1–3-rayed umbel. Floral rays branching 1–4 times as a compound dichasium, dichasial branches absent at terminus, each dichasial node subtended by opposite bracts, bracts erect, primary bracts at first node 5 × 3 mm, elliptical, subsessile to petiolate 1 mm, terminal bracts 1 × 0.3 mm, narrowly elliptical, apex acute, often with white tips at apex and infrequently white along the margin. Cyathia bisexual, solitary at node, peduncles green, 1.7–3.5 mm long, 0.1 mm thick, glabrous; involucre campanulate or cupuliform, green, mostly glabrous, but pubescent along upper rim, 1 mm high, 1.3 mm wide. Glands present at the top of the involucre, five, green, elliptical, slightly depressed at the center, 0.1 × 0.2 mm, subtended by petaloid appendages; petaloid appendages white, projecting perpendicular to the peduncle/involucral axis, ligulate to broadly oblong, margin entire, 0.5 × 0.3 mm. Staminate flowers ca. 10; pedicels ca. 0.7 mm long, scattered among about equally-sized filament-like appendages, some densely pubescent, others glabrous; filaments 0.4 mm long, 0.1 mm wide, glabrous; anthers yellow, globose, divergent, 0.3 × 0.2 mm. Pistillate flowers central, surrounded by staminate flowers, green, pedicels ca. 2.8 mm, exerted beyond the involucre, erect to partly reflexed; capsules strongly three-lobed, green, glabrous, 2.5 × 2.1 mm, styles 3, united at base, 1.2 mm long; stigmas bifid, recurved, greenish-yellow. Seeds globose, maroon-brown with a thin silvery coat when dry, ecarunculate, 1.6 × 1.2 mm, shallowly pitted, hilum depressed, apex acute.

Figure 1. *Euphorbia* sp. nov. habit and morphology. Photo courtesy of John Gwaltney.

Figure 2. *Euphorbia* sp. nov. leaf morphology. Note pilose hairs on petiole and leaf margin to the right. Photo courtesy of John Gwaltney.

Figure 3. *Euphorbia* sp. nov. inflorescence morphology. Note white appendages (petal-like structures) and 3-lobed fruits. Photo courtesy of John Gwaltney.

Figure 4. *Euphorbia* sp. nov. cyathia. Note white appendages, green glands, and 3-lobed ovary. Photo courtesy of John Gwaltney.

Habitat. Hardwood forest over limestone. Associates include *Quercus* spp., *Carya* spp., *Juglans nigra*, *Ilex opaca*, *Aesculus pavia*, *Frangula caroliniana*, *Anemone americana*, *Cardamine concatenata*, *Asarum canadense*, *Sanguinaria canadensis*, *Yeatesia viridiflora*, *Trillium cuneatum*, *T. stamineum*, *Phlox* sp., *Viola walteri*, *Podophyllum peltatum*, and *Carex* spp.

Phenology. Based on limited observations, the species appears to flower in late August and early September and fruit in September.

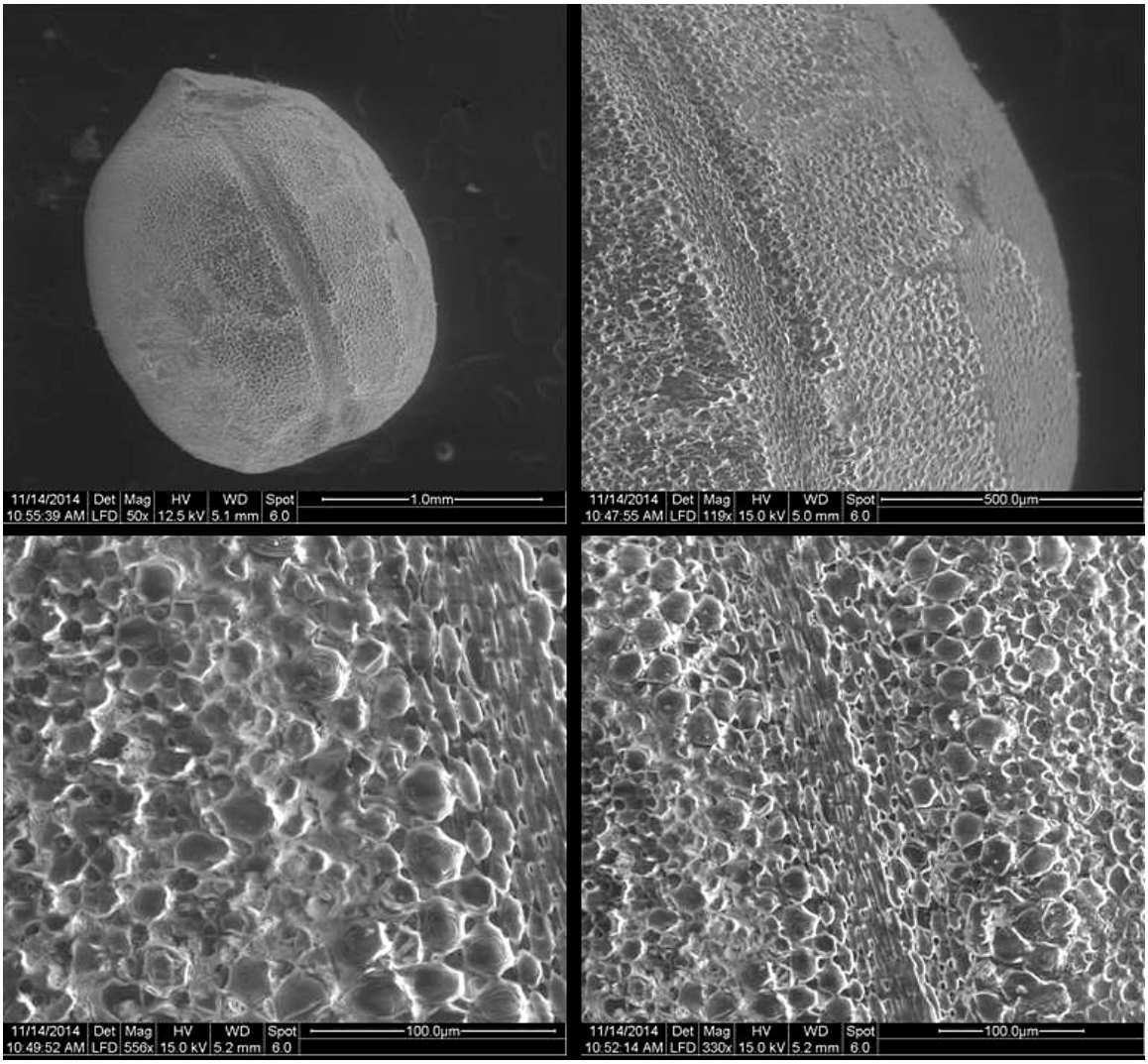

Figure 5. Scanning electron micrographs of seeds of *Euphorbia* sp. nov.

Principal Component Analysis

The principal component analysis indicates that the putative new species is a morphological outlier (Figure 6). Component 1 reveals a difference between a *E. corollata / E. pubentissima* cluster and a *E. mercurialina / E.* sp. nov. cluster. Components 2 and 3 reveal differences between *E. mercurialina* and the putative new species. Component 1 is often interpreted to be the size variable, and indeed, many of the

highly loading features are related to size: plant height, stem width at base, and nodes before first inflorescence branch. One feature was strongly negatively correlated: petiole length, which was also a feature recognized as important for distinguishing the putative species in the descriptive morphological examination. All specimens were collected mature; thus, correlated size differences explain a lot of the variation. Over 80% of the variability between species was accounted for by the first three components. *Euphorbia mercurialina* and the new species came out distinct from the *E. corollata* "complex." These results correspond well with DNA evidence suggesting that these are two separate groups (see *Phylogenetic Analyses* and Figures 8–10 below). As shown in the loading plots provided (Figure 7, Table 2), characteristics that were strong variables in differentiating the putative new species in components 2 and 3 are lamina length, lamina width, petiole length, and peduncle length.

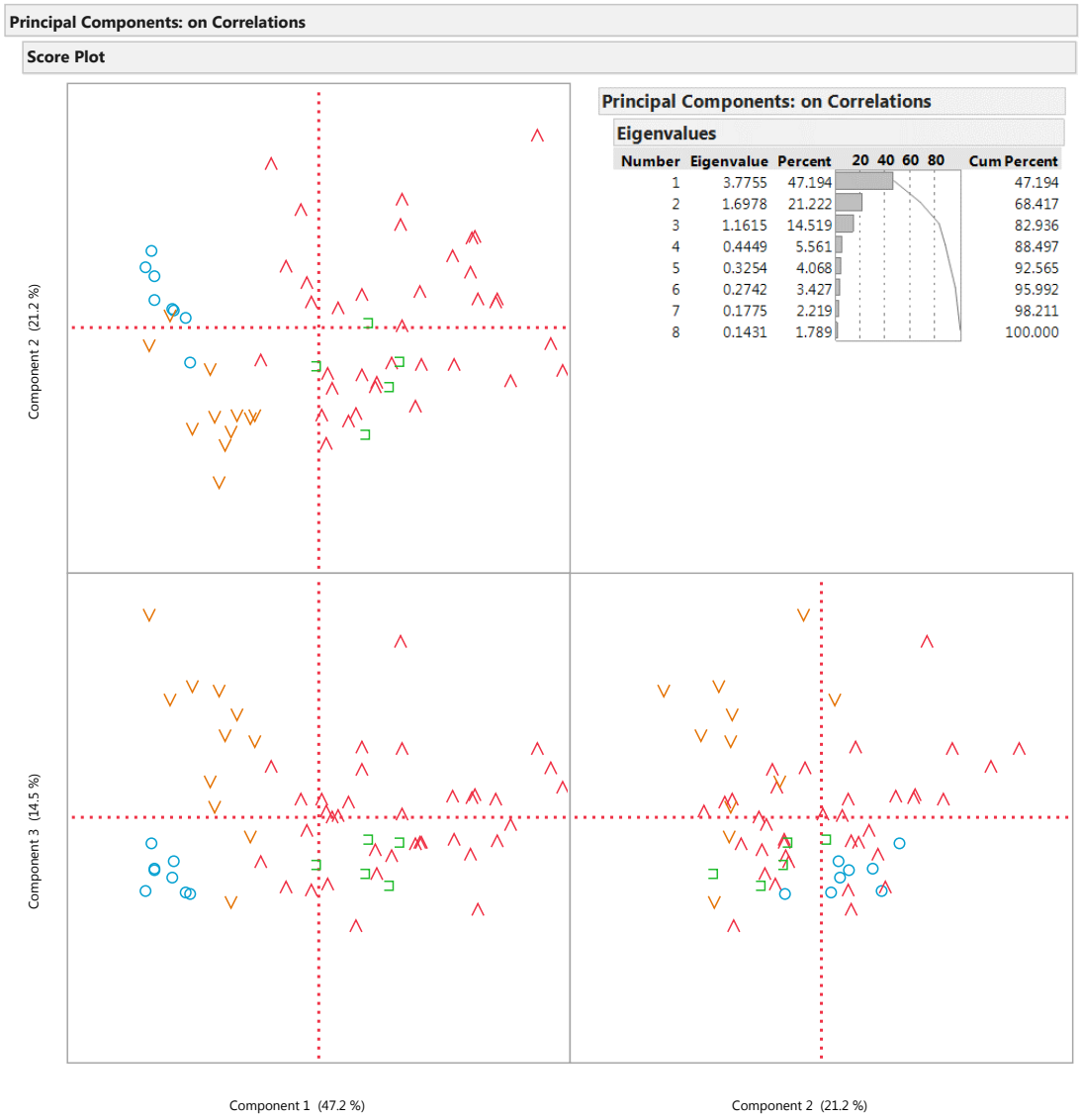

Figure 6. Principal components plots. Blue circles=*Euphorbia* sp. nov., orange triangles=*E. mercurialina*, red triangles=*E. corollata*, green squares=*E. pubentissima*.

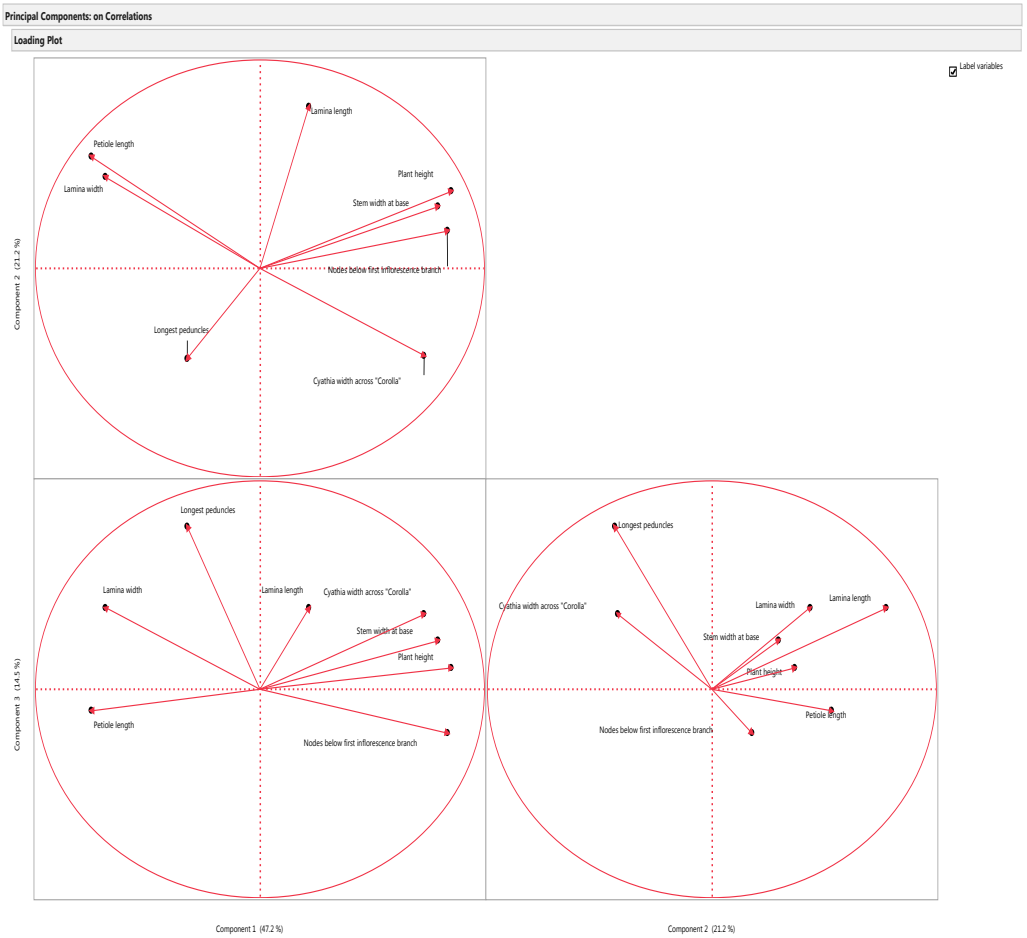

Figure 7. Principal components loading plot. Note that Component 1, as is often the case, is correlated to size measurements (e.g., plant height, stem width at base, and nodes before first inflorescence branch).

Principal Components: on Correlations

Eigenvectors

	Prin1	Prin2	Prin3	Prin4	Prin5	Prin6	Prin7	Prin8
Nodes below first inflorescence branch	0.42934	0.13678	-0.19503	0.12560	0.75449	0.28718	-0.06143	0.29605
Plant height	0.43820	0.28303	0.09472	0.27021	0.05022	-0.15344	0.06166	-0.78487
Stem width at base	0.40889	0.22728	0.21564	0.50174	-0.49906	0.04717	0.08172	0.47427
Lamina length	0.11247	0.59630	0.36097	-0.62169	-0.08816	0.19709	-0.25791	0.04293
Lamina width	-0.35373	0.33541	0.36354	0.22006	0.36705	-0.63992	0.01723	0.19303
Petiole length	-0.38624	0.41061	-0.09597	0.10765	0.02482	0.42325	0.69058	-0.06899
Cyathia width across "Corolla"	0.37651	-0.32070	0.33336	-0.39205	0.09303	-0.22061	0.65057	0.10001
Longest peduncles	-0.16655	-0.33165	0.72366	0.24340	0.16550	0.46435	-0.13709	-0.13242

Table 2. Eigenvectors of principal component analysis. Large positive numbers indicate strong positive correlation to that axis (component); large negative numbers indicate a strong negative correlation to that axis.

Phylogenetic Analyses

Variable DNA data were obtained for plastid *rpL16* and nuclear internal transcribed spacer (ITS) regions, although several other regions were tested (e.g., plastid *trnH-psbA*, nuclear GBSSI), as suggested by Small et al. (1998), Shaw et al. (2014), and others. In both cases, the putative new species of *Euphorbia* had a unique haplotype.

For plastid *rpL16*, 11 individuals were successfully sequenced, yielding sequence lengths of 723–907 bp and an aligned data matrix of 944 bp. Of these, there were only three potentially informative substitutions and 1 potentially informative gap (indel). Phylogenetic analysis of this region using parsimony yielded one most parsimonious tree of length 4, CI of 1.00, and RI of 1.00 (Figure 8). Surprisingly, although these data provided very little resolution among the species in this complex, most of the recovered variation occurred in the putative new species itself. Data from *rpL16* are inadequate for distinguishing among the other species, although two individuals of *E. pubentissima* shared a haplotype.

For nuclear ITS, 14 sequences were downloaded from GenBank, and 21 individuals were successfully sequenced here, yielding sequence lengths of 547–760 bp and an aligned data matrix of 761 bp. Of these, 111 were potentially informative substitutions. Phylogenetic analysis of this region using parsimony yielded 20 most parsimonious trees of length 259, CI of 0.59, and RI of 0.75 (Figures 9–10). These data, too, recover the putative new species distinct from *E. corollata* and *E. pubentissima*. These data also place the new species sister to *E. mercurialina*, which coincides with the results of the PCA, several macromorphological characters (pilose leaf margins, small cyathia, long petioles), and seed characters. These results also indicate that *E. innocua*

and *E. ipacacuanhae* are close relatives, although they were excluded by Park (1998), as they have angular seeds with a mucilaginous seed coat. *Euphorbia aaron-rossii* is also recovered here, a fairly recently described species (Holmgren and Holmgren, 1988) from the southwestern United States that was not treated by Park (1998) or Huft (1979). These results do not correspond to the relationships hypothesized by Park (1998) based on morphological data. He proposed two major clades, one consisting of *E. corollata*, *E. pubentissima*, *E. discoidalis*, and *E. polyphylla*, and another consisting of *E. curtisii*, *E. gracilior*, and *E. mercurialina*. Unfortunately, no samples of *E. curtisii* or *E. gracilior* were obtained for this study.

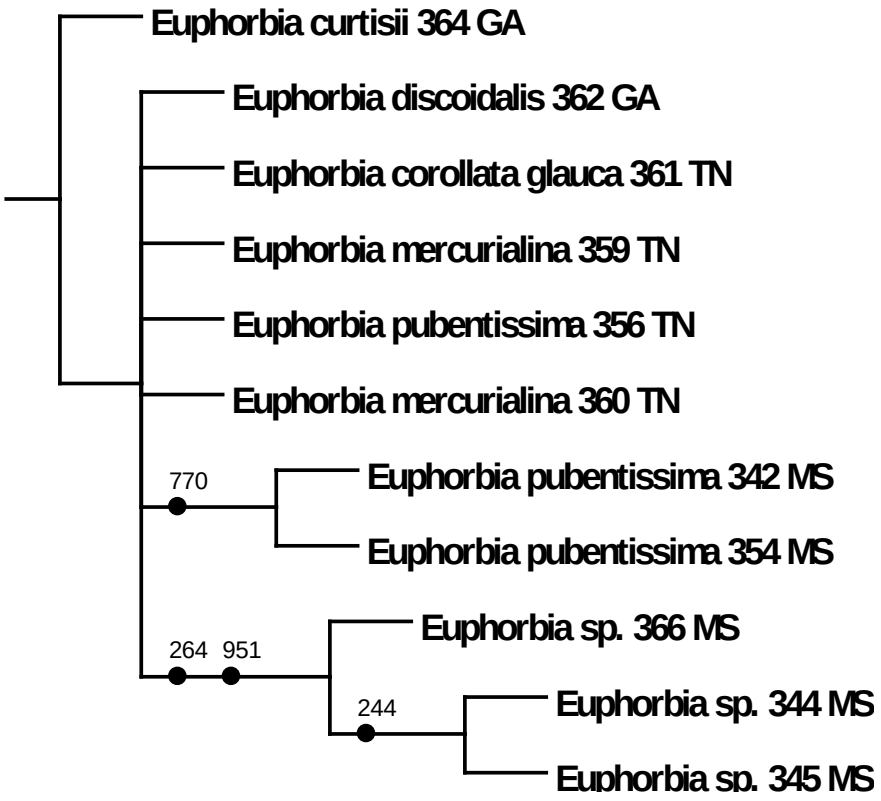

Figure 8. Most parsimonious tree of a phylogenetic analysis of plastid *rpL16* data. Dots indicate DNA substitution changes, and numbers indicate places in the aligned data matrix where those changes occur. L=4, CI=1, RI=1.

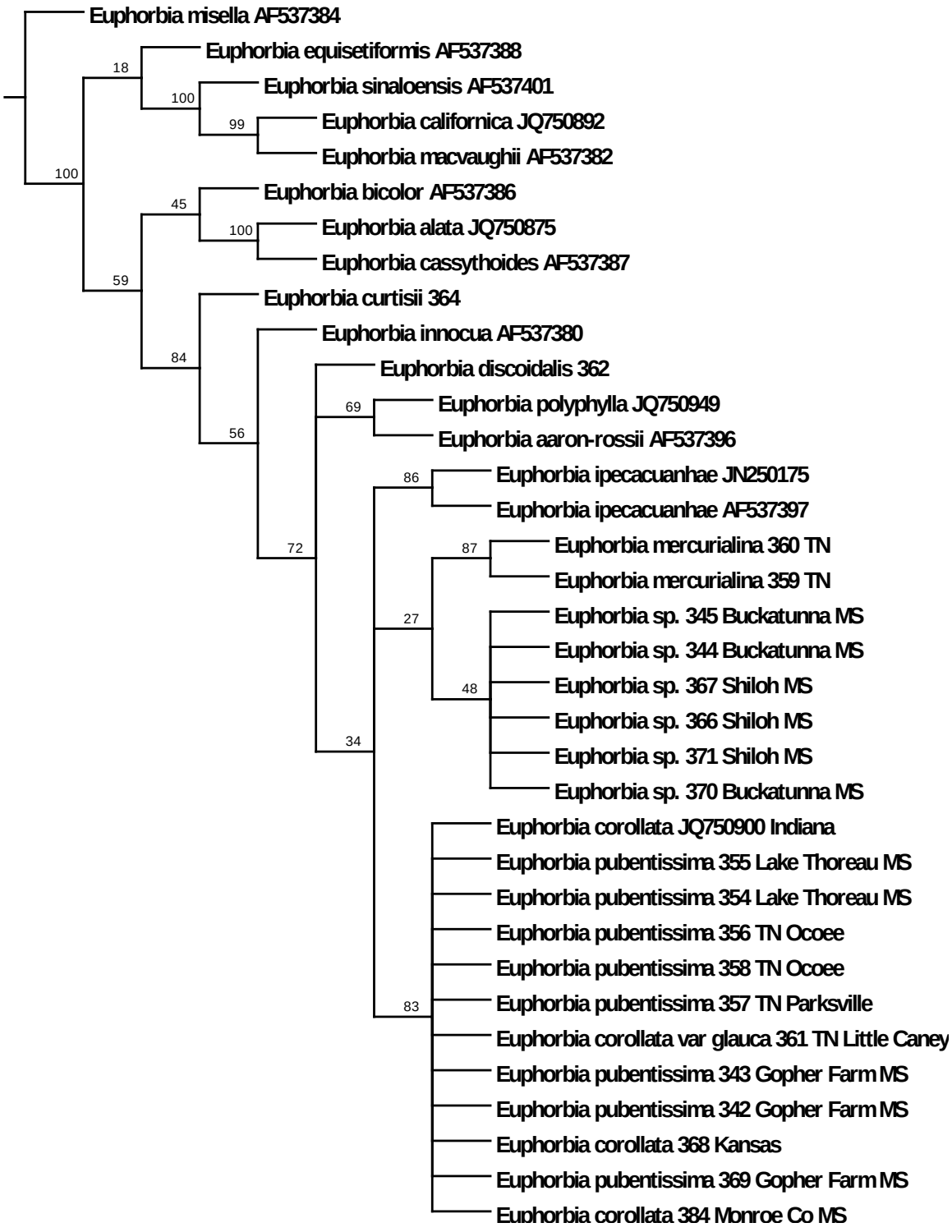

Figure 9. Strict consensus tree of the 20 most parsimonious trees of a phylogenetic analysis of nuclear internal transcribed spacer (ITS) data. Numbers above the branches indicate bootstrap support. L=259, CI=0.59, RI=0.75. Letter-number combinations with species indicate GenBank numbers; data produced here are numbered as in Table 1.

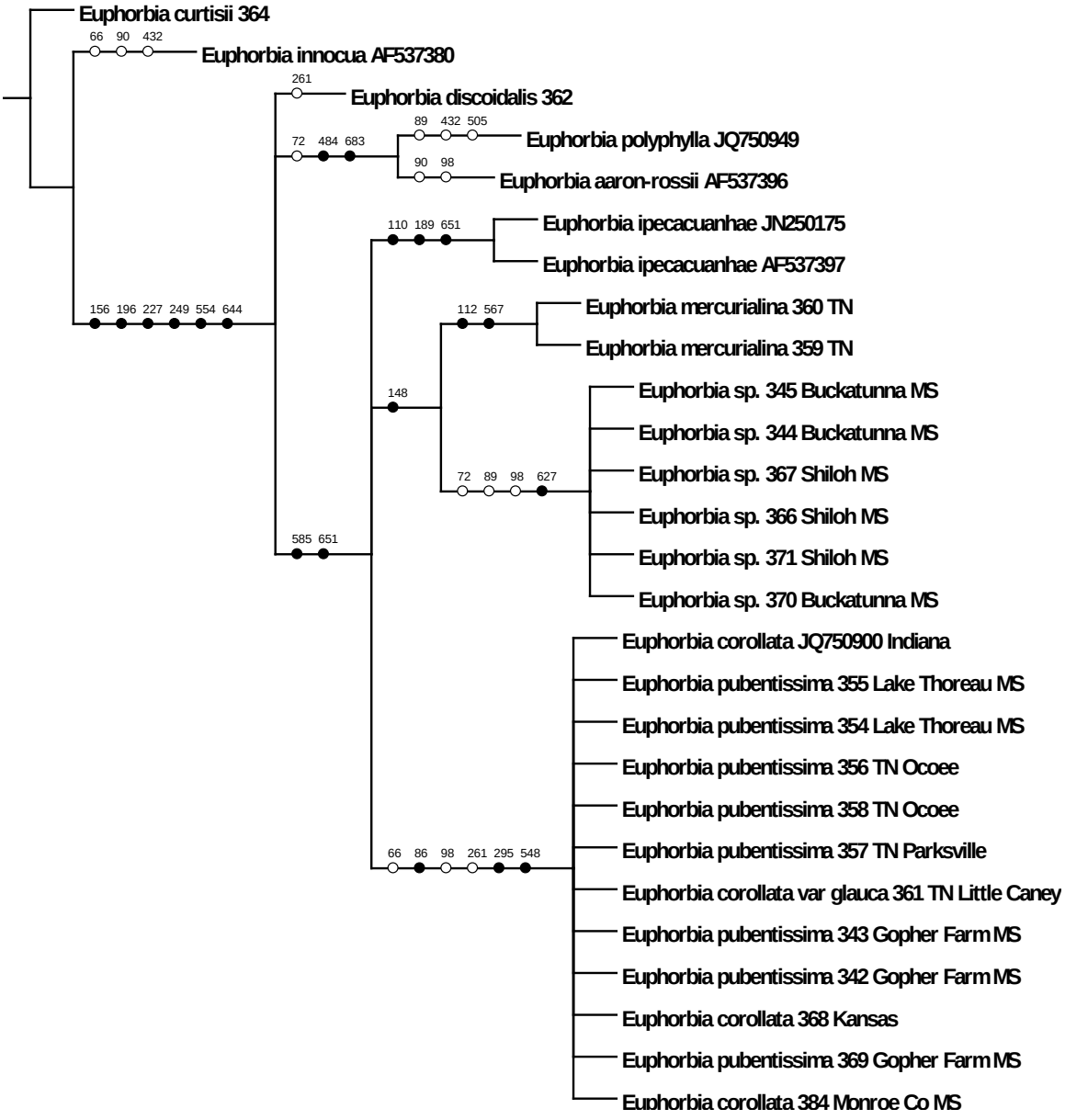

Figure 10. Subset of the strict consensus tree of the 20 most parsimonious trees of a phylogenetic analysis of nuclear internal transcribed spacer (ITS) data showing character state changes. Dots indicate DNA substitution changes, and numbers indicate places in the aligned data matrix where those changes occur. L=259, CI=0.59, RI=0.75. Letter-number combinations with species indicate GenBank numbers; data produced here are numbered as in Table 1.

Chapter 4: Discussion

The morphological and DNA data both indicate that the unusual populations of *Euphorbia* in Wayne County, Mississippi, represent a distinct species. Traditional morphological description indicates that the new species differs from other *Euphorbia* of *Euphorbia* subgenus *Chamaesyce* section *Alectoroctonum* in the eastern United States by its long petioles, abaxially pilose lamina, small cyathia, and small seeds. The new species's small stature coupled with the presence of pilose leaf margins, wide leaves/bracts, and regularly pitted seeds hints at a close relationship to *E. mercurialina*, the only other species in this group that also occurs in mesic hardwood forest over alkaline substrate. The PCA, too, reveals this close relationship between the new species and *E. mercurialina*. Despite their similarities, though, *E. mercurialina* differs from the new species in having scale-like leaves (although inflorescence bracts are broad and leaf-like), having abaxially glabrous leaves (although margins are ciliate), and flowering in April–May, whereas the new species flowers and fruits in late summer, August–September.

DNA data also support recognition of the Wayne County populations as a new species. They exhibit unique haplotypes in both plastid and nuclear DNA, and the relationships inferred from these data do not show as close a relationship to *E. corollata* or *E. pubentissima* as one would expect from taxonomic keys or from the discussions of the variability of those species in Huft (1979) and Park (1998). Rather, the results of the phylogenetic analyses indicate a closer relationship to *E. mercurialina*, corroborating the

results of the morphological analyses here. Surprisingly, the DNA data are ambiguous about species boundaries between *E. corollata* and *E. pubentissima*.

Euphorbia pubentissima is particularly variable morphologically, and several authors have treated some of the regional variation as nomenclatural varieties or even species, especially Small (1898, 1933) and Millspaugh (1898). Some of the morphological variation observed in *E. pubentissima* may be the result of introgression. Some individuals of *E. pubentissima* have long petioles and broader leaves, which was noted by both Huft (1979) and Park (1998) and is a feature of the new entity recognized here. Given that these species of *Euphorbia* can readily hybridize (Park, 1998) and that many of the specimens with long petioles occur in neighboring regions to the new species recognized here (e.g., Jackson Prairie counties of Mississippi), perhaps the characters of this new species (or some progenitor species) were introgressed into *E. pubentissima* or *E. corollata*. In addition, *E. corollata* var. *glauca* (Figure 13) may also be the product of introgression; nuclear DNA sequences of the single sample obtained of this taxon had numerous double-peaks, indicating a high degree of polymorphism or allelic variation. Given its features and geographic distribution in northern Alabama, though, it is more likely a product of hybridization between *E. mercurialina* and *E. corollata* / *E. pubentissima*.

If this new species is recognized, what should it be named? First, names already used must be consulted to determine if someone has already named the entity. Among the potential names are *E. apocynifolia* Small and *E. paniculata* Elliott (Millspaugh, 1898; Small, 1898, 1933). *Euphorbia paniculata* (Figure 12) is an illegitimate name, as it had been used for another species prior to Elliott's use; thus, it is a homonym. That leaves *E.*

apocynifolia (Figure 11), which was originally collected and described from the Apalachicola River basin of Florida, where some limestone areas also occur. The robust size of the plants and the small petiole length argue against it being the same entity, but physical examination of the leaf margins will convincingly determine whether it is the same thing. If so, that name will take priority, and the new species will be called *E. apocynifolia*. If not, a new name will be provided, perhaps *E. buckatunna*, in honor of the species's first collection locality and based on a Choctaw word probably meaning "creek at which there is weaving," or less likely "collected together" (Baca, 2007).

The new species is currently known from only two populations, both in Wayne County, Mississippi, although several botanists have mentioned seeing similar individuals in Mississippi and Alabama. Further research, especially utilizing the herbarium collections at BRIT, MMNS, and VDB, will be needed to resolve its full range and rarity. Even a small range size, though, is not unexpected. The Coastal Plain of the southeastern United States is edaphically diverse, and many of the new species found here in the recent decades have been in these edaphic islands, such as sandhills or outcrops of limestone or dolomite (e.g., Allison and Stevens, 2001; Estill and Cruzan, 2001; Sorrie and Weakley, 2001). A recent analysis of the California floristic province indicates that sister plant species are rather different than sister animal species in that they are often broadly sympatric, very different in size ranges, and are specialized to substrate (Anacker and Strauss, 2014).

To assist in identification of this new taxon, an identification key is given below.

Key to the species of *Euphorbia* subgenus *Chamaesyce* section *Alectoroctonum* in eastern North America, except for *E. innocua* and *E. ipacacuhuanae*

1. Main stem usually branched at the nodes; leaves scale-like (although inflorescence bracts may be leaf-like); inflorescence bracts without white tips at the apex
    2. Leaf/bract margins ciliate; glands green
        3. Leaves/bracts glabrous, except for ciliate margin, 3.7–8.0 × 1.9–4.5 cm; cyathia bisexual .....*E. mercurialina*
        3. Leaves/bracts pubescent, 2.5–5.1 × 0.2–1.0 cm; cyathia unisexual ..... *E. curtisii*
    2. Leaf/bract margins glabrous; glands purple
        4. Involucre purple; appendages rudimentary, purple ..... *E. gracilior*
        4. Involucre green; appendages semicircular, white; perhaps extinct .....*E. exserta*
1. Main stem simple or branched only at the base; leaves with expanded blades; inflorescence bracts usually with white tips at the apex
    5. Leaves usually linear, 1.5–4 (–5) mm wide, margins clearly revolute or involute; glands green or red
        6. Stem not branched from base, usually densely pubescent; appendages entire..... .....*Euphorbia discoidalis*
        6. Stem branching at the base, glabrous; appendages deeply crenate..... .....*Euphorbia polyphylla*
    5. Leaves usually oblong, narrowly elliptic, broadly elliptic, lanceolate, ovate, oblanceolate, or obovate, 4–24 mm wide, margins weakly or not at all revolute or involute; glands green

7. Petioles 5–11 mm long; leaves with ciliate margins; cyathia across the appendages ~2.5 mm; 7–11 nodes below the umbels; shady, mesic hardwood forest over limestone or in sand over limestone ..... *Euphorbia* sp. nov.

7. Petioles sessile, subsessile, or 1–3 (–10) mm long; leaves without ciliate margins; cyathia across the appendages (3.5–) 4–8 (–11) mm; 20–75 nodes below the umbels; habitats various, generally open, drier, and acidic, e.g., pine woods, sandhills, sandstone, ruderal sites

    8. Cyathia (3.5–) 4–5.5 (–6.5) mm across the appendages; longest peduncles 2–3 (–5) mm; nodes below umbel (6–) 18–30 (–41); aerial stems few, 1–2; seeds 1.8–2.3 × 1.4–1.8 mm.....*Euphorbia pubentissima*

    8. Cyathia (5–) 6.5–8 (–11) mm across the appendages; longest peduncles 5–10 mm; nodes below umbels (25–) 35–60 (–75); aerial stems multiple; seeds 2.6–3.2 × 1.9–2.5 mm ..... *Euphorbia corollata*

Figure 11. Holotype specimen of *Euphorbia apocynifolia* Small. Image courtesy of New York Botanical Garden (NY).

Figure 12. Isotype specimen of *Euphorbia paniculata* Elliott, nom. illeg. Image courtesy of Missouri Botanical Garden (MO).

Figure 13. Holotype specimen of *Euphorbia corollata* var. *glauca* Millsp. Image courtesy of the Field Museum (F), Chicago, Illinois.

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