A new variety of Phacelia dubia (Hydrophyllaceae) and a summary of variation and biogeography of the P. dubia complex1

Authors: Levy, Foster, Hou, Zhujun Helen, and Taylor-Bennetts, Stacy

Source: The Journal of the Torrey Botanical Society, 148(4) : 266-284

Published By: Torrey Botanical Society

URL: https://doi.org/10.3159/TORREY-D-21-00017.1

Journal of the Torrey Botanical Society 148(4): 266–284, 2021.

A new variety of *Phacelia dubia* (Hydrophyllaceae) and a summary of variation and biogeography of the *P. dubia* complex[1]

Foster Levy,[1,2] Zhujun Helen Hou,[1] and Stacy Taylor-Bennetts[3]
[1] Department of Biological Sciences, East Tennessee State University, Johnson City, TN 37601
[3] Department of Biological Sciences, University of Augusta, Augusta, GA 30904

Abstract. Occurrences of *Phacelia dubia* in the Piedmont of South Carolina have been taxonomically enigmatic. Prior work had shown that there was partial reproductive isolation when plants from South Carolina were hybridized with any of the other varieties but evidence of morphological differentiation was lacking. In this study, a new morphological analysis showed South Carolina plants differed significantly in corolla lobe size, sepal size, and leaf dissection in comparisons with neighboring varieties, *P. dubia* var. *dubia* and *P. dubia* var. *georgiana*. A preponderance of evidence showing differentiation from all other varieties supports recognition of a new variety, *P. dubia* var. *rionensis*. Field work and an updated analysis of herbarium records showed the new variety is found in nine contiguous counties in the central and northern Piedmont of South Carolina and two counties in the inner Coastal Plain. We posit a hypothesized biogeographic pathway based on migration of a *P. dubia* ancestor from the Great Plains or Mexico to the Nashville Basin cedar glades, then to South Carolina piedmont granite outcrops, followed by a migration south to Georgia and Alabama piedmont granite outcrops and a separate migration north to rocky woodlands in North Carolina.

Key words: biogeography, granite outcrops, morphometric analysis, *Phacelia dubia*, plant taxonomy

Taxonomic status can influence conservation and research priorities because entities with formal names and ranks are monitored by government agencies and included in phylogenetic surveys (Mace 2004, Ely *et al.* 2017, Thomson *et al.* 2018). Invariably, decisions on taxonomic recognition and assignment of rank rest with taxonomists. However, the myriad directions and suites of characters by which divergence can manifest itself (ecological, geographical, molecular, morphological, physiological) preclude strict and sweeping guidelines (Hey *et al.* 2003, Hey 2006). Consequently, taxonomic decisions retain both the appearance of, and an actual degree of subjectiveness (Lee 2003).

With 207 species, *Phacelia* is by far the largest of the 12 genera in the Hydrophyllaceae, a family with a center of diversity in western North America (Ferguson 1998, Walden and Patterson 2012, Walden *et al.* 2014, Vasile *et al.* 2020). *Phacelia* is characterized by pentamerous sympetalous flowers in scorpioid cymes, capsules with four to many seeds, and paired scales on the corolla lobes (Constance 1949, Jeiter and Weigend 2018). Phacelias of eastern North America form a monophyletic clade referable to *Phacelia* subg. *Phacelia* sect. *Cosmantha* (Nolte ex A. de Candolle) Benth. & Hook. f. (≈ subg. *Cosmanthus* (Nolte ex A. de Candolle) A. Gray) and united by morphological (flap-like glandular paired corolla lobe appendages) and cytological (mostly $n = 5$ or 9 chromosomes, but not 11 or higher) synapomorphies (Constance 1949, 1963; Gillett 1968; Walden *et al.* 2014; Vasile *et al.* 2020).

In the cosmanthus phacelias, a set of guidelines has been proposed for taxonomic recognition at the species and subspecific ranks (Levy and Malone 2001, Taylor and Levy 2002, Levy 2021). These guidelines have been applied most notably, in subsect. *Cosmantha* (Nolte ex A. de Candolle) Walden & R. Patt. (comprising *P. fimbriata* Michx. and *P. purshii* Buckl. and known as the "fringed phacelias"), subsect. *Dubiae* (Small) Walden & R. Patt. (comprising *P. dubia* (L.) Trel. and *P. maculata* A. Wood.), and subsect. *Ranunculacea* Walden & R. Patt. (comprising *P. covillei* S. Wats. ex A. Gray and *P. ranunculacea* (Nutt.) Constance;

---

[1] We thank K. Bradley and the South Carolina Department of Natural Resources for advice on localities and permits, curators of herbaria whose specimens are accessible online, A. Brach for images from HUH, J.M. Reichmuth for assistance with field collections, T. McDowell, E. Walker, two anonymous reviewers and the associate editor for invaluable comments on the manuscript, and A. Weakley for the stimulus to complete this work. We were unable to locate K. Malone but we thank her for detailed prior work on *P. dubia* var. *rionensis*.

[2] Author for correspondence: levyf@etsu.edu.

doi: 10.3159/TORREY-D-21-00017.1

Received for publication June 4, 2021, and in revised form August 11, 2021; first published October 18, 2021.

Walden and Patterson 2012). Under the guidelines, the rank of species implies qualitative and quantitative differentiation in morphological characters and complete reproductive isolation as exemplified by each of the species pairs of subsects. *Cosmantha*, *Dubiae*, and *Ranunculacea* (Constance 1949; Murdy 1966; Levy 1991a, 1991b; Sewell and Vincent 2006; Glass and Levy 2011).

Prior to the guidelines, taxon recognition in cosmanthus phacelias was more subjective and less strictly constrained. For example, Small recognized two additional fringed phacelias, *P. bicknellii* Small and *P. boykinii* (A. Gray) Small, both based on quantitative morphological differentiation but lacking evidence of reproductive isolation (Small 1898, 1933). Moreover, both of these taxa were sympatric and embedded within the geographical range of *P. purshii*. These taxa were not accepted by Constance, the most recent monographer of subg. *Cosmanthus*, or by authors of some floras (Constance 1949, Chester *et al.* 2015, Weakley 2020). More recently it was shown that the description of *P. bicknellii* was based on male sterile individuals of *P. purshii* and not deserving of taxonomic recognition (Baker 1961, Levy 2021). Following a morphometric analysis, the description and diagnosis of *P. boykinii* was refined which led to recognition of two varieties of *P. purshii* whose ranges were largely allopatric except for a narrow transition zone (Levy 2021). The absence of evidence of complete reproductive isolation was the rationale for recognition of *P. purshii* var. *boykinii* (Small) Levy at the rank of variety.

The two species in *Phacelia* sect. *Cosmantha* subsect. *Dubiae* (the only species in *Phacelia* with $n = 5$ chromosomes, the lowest number in the genus) are completely reproductively isolated and morphologically distinct—*P. maculata* has a nonglandular inflorescence and larger sepals with spreading marginal bristles, and a larger corolla compared to *P. dubia* with a stipitate-glandular inflorescence, smaller sepals with appressed marginal bristles, and a smaller corolla (Constance 1949; Murdy 1966; Levy 1991a, 1991b). On the basis of greater leaf dissection relative to *P. dubia* var. *dubia*, McVaugh (1943) described *P. dubia* var. *georgiana* McVaugh (Fig. 1). When described, the two varieties of *P. dubia* were allopatric with approximately 240 km separation, the unoccupied region corresponding to the piedmont from southern South Carolina to southern North Carolina. Moreover, the varieties differ ecologically with *P. dubia* var. *dubia* occurring in rocky and floodplain woodlands with a geographic range encompassing the coastal plain, piedmont, and mountains from North Carolina to Pennsylvania, and *P. dubia* var. *georgiana* endemic to piedmont granite outcrops of Alabama and Georgia. Soon after recognition of *P. dubia* var. *georgiana*, Fernald described *P. dubia* var. *interior* Fern. based on ovate (compared to lanceolate–oblong) outer sepals, a shorter fruiting calyx, and shorter flower pedicels (Fernald 1944). *Phacelia dubia* var. *interior* is endemic to middle Tennessee cedar glades, a rocky limestone habitat. When these two new varieties were described in the 1940s, *P. dubia* was virtually unknown from South Carolina having been represented by only two herbarium specimens, both collected in the 1940s. Since then, the species has been found at many sites encompassing counties in the mountains, piedmont and coastal plain and represented by a total of 93 specimens, of which 77 are unique (nonduplicates). However, the varietal identity of these South Carolina collections, notably those from the central and northern piedmont, has been uncertain (Levy 1991a, Levy and Malone 2001).

Historical Summary. By 1947, after the recognition of *Phacelia dubia* var. *georgiana* and *P. dubia* var. *interior*, a tidy picture of the variation and biogeography of *Phacelia dubia* was evident. Soon after, Constance, at Harvard University during 1947–1948 on leave from the University of California–Berkeley, published what stands as the most recent monograph of *Phacelia* subg. *Cosmanthus* (Constance 1949). This extremely thorough and useful work was based on examinations of previously collected herbarium material. Of the six cosmanthus taxa with primary ranges east of the Mississippi River, only five unique collections were collected by Constance, of which two were *P. dubia*. The lack of familiarity with *P. dubia* in the field introduced confusion into the taxonomy as Constance recognized *P. dubia* var. *georgiana* but considered *P. dubia* var. *interior* a phenotypically dwarfed form of *P. dubia* var. *dubia* that “occurs sporadically throughout the range of the species” (Constance 1949, p. 38). This unfortunate statement, proffered in the absence of experimental data, caused many subsequent authors to follow Constance in recognizing only two

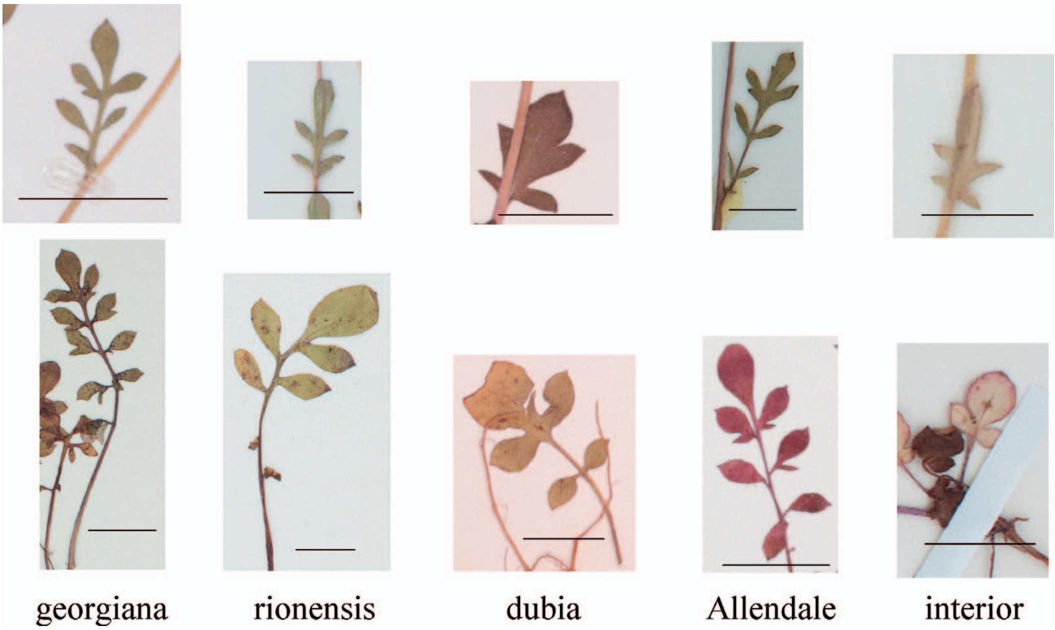

FIG. 1. Images of representative basal (bottom row) and cauline (top row) leaves of (from left to right) *Phacelia dubia* var. *georgiana*, var. *rionensis* (referred to formerly as the "putative variety"), var. *dubia*, plants from Allendale Co., South Carolina, and var. *interior*. Note the multiple pairs of leaflets and additional lobes on *P. dubia* var. *georgiana* and Allendale specimens; the broad terminal leaflet of *P. dubia* var. *interior*; the absence of distinct leaflets in cauline leaves of *P. dubia* var. *dubia* and *P. dubia* var. *interior*. Leaf images from (basal leaf; cauline leaf): *P. dubia* var. *georgiana* (Georgia: Oglethorpe, *Blake and Montgomery s.n.*, DUKE10106409; *Blake and Montgomery s.n.*, HUH); *P. dubia* var. *rionensis* (South Carolina: York, *Levy s.n.* DUKE10106402; *Levy s.n.* DUKE10106112); *P. dubia* var. *dubia* (Tennessee: Knox, *Ruth s.n.* HUH; North Carolina: Haywood, *Fox 2171* HUH); *P. dubia* var. *rionensis* Allendale (South Carolina: Allendale, *Nelson, Pittman, and Rayner 17093* HUH; *Horn 3811* NBYC010292); *P. dubia* var. *interior* (Tennessee: Wilson, *Deam 61295* DUKE1010416; *Kriebel 9230* DUKE1010418). Scale bar = 1 mm.

varieties of *P. dubia* (Baskin and Baskin 1971, Mann *et al*. 1999).

More recent research provided additional morphological as well as genetically based data that confirmed morphological differentiation of the three varieties, showed that each was partially reproductively isolated from the others via reciprocal partial intersterility of both ovules and pollen, demonstrated distinct substrate preferences, and provided evidence of molecular divergence in nuclear and chloroplast genomes. In controlled crosses, hybrids of *P. dubia* var. *dubia* and *P. dubia* var. *georgiana* showed ~ 75% reduction in fertility (Levy 1991a). In backcrosses to each parental variety, fertility segregated as a major factor, that is, plants were either fully fertile or remained 75% sterile. Moreover, in backcrosses, two allozyme marker loci segregated with fertility and showed strong linkage disequilibrium but in crosses among parentals within each variety, these marker loci showed free recombination (Levy 1992, Hou 1995, Levy and Malone 2001). Apparent linkage of two physically unlinked loci in hybrids with a 75% reduction in fertility and the ability to restore fertility in one generation are hallmarks of taxa that differ by two chromosomal structural rearrangements, most likely two reciprocal translocations (Burnham 1962). Hybrids and backcrosses of *P. dubia* var. *dubia* with *P. dubia* var. *interior* and hybrids of *P. dubia* var. *georgiana* with *P. dubia* var. *interior* behaved in a similar manner but the fertility reduction was only ~50% and only one marker locus segregated with fertility, a pattern indicative of pairs of taxa that differ by one reciprocal translocation (Levy 1989, 1991a, 1992; Hou 1995). In sum, the complex is characterized by an entire suite of divergent characters in allopatric forms (Levy 1991a, 1991b; Hou 1995; Levy *et al*. 1996; Levy and Neal 1999; Levy and Malone 2001; Taylor and Levy 2002). Under the proposed guidelines of Glass and Levy (2011), the lack of complete reproductive isolation supported recognition at the varietal rank.

Notwithstanding the misunderstood taxonomic status of *Phacelia dubia* var. *interior*, at the time of Constance's monograph the known geographic range of *P. dubia* was divided into three allopatric regions that corresponded to the three varieties. Virtually the entire state of South Carolina provided geographic separation between *P. dubia* var. *dubia* and *P. dubia* var. *georgiana*. Prior to 1950, there were only two specimens of *P. dubia* from South Carolina: one collected from Charleston Co. (*Ravenels A-8*, CLEMS 0075132), referable to *P. dubia* var. *dubia*, and consistent morphologically with coastal plain occurrences of *P. dubia* var. *dubia* in North Carolina and Virginia; and one from Allendale Co. on the border with Georgia. Moreover, based on morphology, habitat, and geography, plants from McCormick Co., South Carolina were clearly *Phacelia dubia* var. *georgiana*. McCormick Co. is located across the Savannah River from Columbia Co., Georgia, a center of piedmont granite outcrop diversity (McVaugh 1943, Murdy 1968). Each of the nine herbarium sheets from McCormick Co. was collected in proximity to Clarks Hill, the site of a granite outcrop. Similarly, and also based on morphology, habitat, and geography, plants from Oconee Co., South Carolina can be assigned to *P. dubia* var. *dubia*. These specimens were collected from the northwest portion of Oconee Co., in or on the margin of the Blue Ridge physiographic province.

In the 50+ years between Constance's monograph and Levy and Malone's experimental studies, the number of herbarium sheets of *P. dubia* from South Carolina increased from 2 to 71. Because many of these collections were located in smaller herbaria (Appendix), Levy and Malone examined only the 17 piedmont specimens housed in four large herbaria but they visited the many new sites found by J. Allison, F. Levy, and others (Levy and Malone 2001). The identity of these piedmont plants, however, was not readily discernable because morphologically, populations appeared either *dubia*-like or *georgiana*-like but not unique (Levy 1991b). Moreover, there was partial reproductive isolation when plants from the South Carolina piedmont were crossed with either *P. dubia* var. *dubia* or *P. dubia* var. *georgiana* (Levy 1991b, Malone 1997, Levy and Malone 2001). The South Carolina plants were therefore referred to as putative variety "imitator."

The more extensive genetic work of Levy and Malone (2001) showed that all crosses within and between populations of the putative variety resulted in fully fertile plants; F_1 progeny of these crosses were also fully fertile as were all field collected plants. These breeding experiments showed that the putative variety is not a recent hybrid (partial sterility would have been expected) and that there are no segregating sterility factors in these plants (Levy 1991a, Malone 1997, Levy and Malone 2001). Hybrids of the putative variety with *P. dubia* var. *dubia* and *P. dubia* var. *georgiana* had reduced pollen fertility (mean = 63% and 45%, respectively) (Levy 1991a, Levy and Malone 2001). Moreover, backcrosses progenies had bimodal fertilities with one peak at F_1 hybrid levels and one peak at full fertility. The potential to restore fertility in one generation and a strong association of fertility with allozyme-encoding loci and RAPD markers, was evidence of a major segregating fertility factor which was likely caused by varietal differences in one or more reciprocal translocations (Burnham 1962). In contrast, hybrids of the putative variety with *P. dubia* var. *interior* varied from highly fertile (14 of 16 cross combinations had > 80% pollen fertility) to levels typical of *dubia-interior* and *georgiana-interior* F_2 hybrids (2 of 16 cross combinations had 45–55% pollen fertility) and in the latter, sterility occurred in only one direction of reciprocal crosses (Levy and Malone 2001). Differences between reciprocal crosses suggested either a cytotypic polymorphism in *P. dubia* var. *interior* or a nuclear-based polymorphism within the putative variety at a gene locus that interacts with the *P. dubia* var. *interior* cytotype. Although Levy and Malone (2001, p. 143) declined formal taxonomic recognition of the putative variety element as an entity that was not morphologically unique, they also noted that the putative variety should not be considered "a component of one the recognized varieties."

A subsequent experimental test of substrate preferences showed that each variety (including the putative variety) grew most vigorously on a potting mix, and each of the three formally recognized varieties performed similarly well on soil from their respective habitats. There were no significant differences between growth on potting mix compared to home habitat soil, but there was

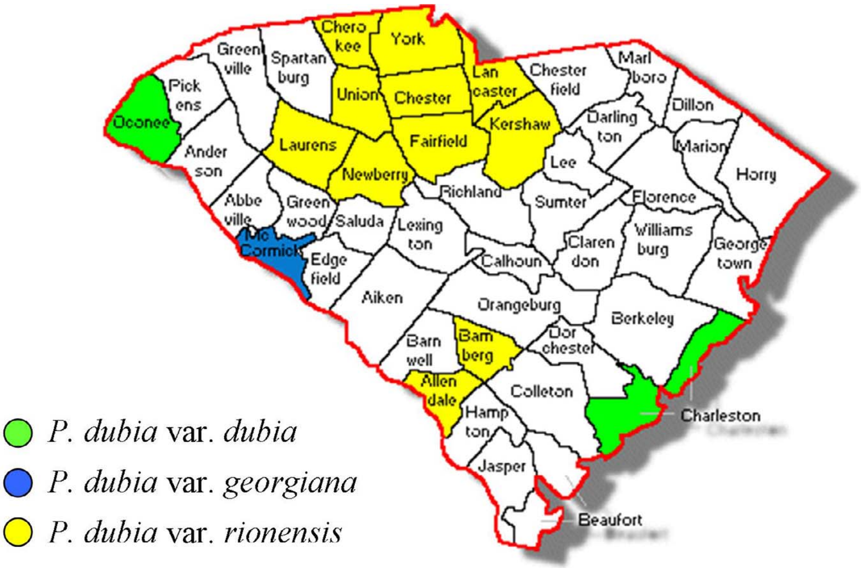

FIG. 2. Map of South Carolina showing county distribution of three varieties of *Phacelia dubia*.

significantly poorer growth on soil from the two non-home habitats (Taylor and Levy 2002). *Phacelia dubia* var. *georgiana* performed exceptionally poorly on the limestone-derived soil (collected from Davidson Co., TN). On the three native soils, the putative variety performed best on the granite outcrop soil (collected from a *P. dubia* var. *georgiana* site in Columbia Co., GA), but unlike the other varieties, its growth was significantly less than on potting soil, and it had more tolerance for the limestone-derived cedar glade soil compared to *P. dubia* var. *georgiana*. Further, when challenged on a serpentine-derived soil (collected from Yancy Co., NC), only *P. dubia* var. *georgiana* was capable of surviving beyond the cotyledon stage; the inviability on serpentine soil of the putative variety was similar to *P. dubia* var. *dubia* and *P. dubia* var. *interior*. These results suggest that the putative variety has its own soil specificity but none of the tests by Taylor and Levy included soil from the putative variety site, so this remains speculative.

The passage of 20+ years since Levy and Malone's experimental studies and an evolution of opinion on cryptic species (Bickford *et al*. 2007, Duminil and Di Michele 2009, Christenhusz *et al*. 2015, Fišer *et al*. 2018, Struck *et al*. 2018, Coughlan *et al*. 2020) provide a motivation to revisit varietal biogeography in *Phacelia dubia* and to formally describe the putative variety as an additional variety. Importantly, several sources of new data have become available. These include the electronic accessibility of herbarium records via Internet, which has uncovered a relative wealth of *P. dubia* specimens from South Carolina (SERNEC Data Portal 2021). Of the 77 unique specimens, 18 have been collected since Levy and Malone (2001) first clarified the range of *P. dubia* in South Carolina. Further, specimens could be accessed electronically from 12 herbaria that were not consulted by Levy and Malone resulting in a total of 55 sheets of central and northern piedmont collections and 22 collections from Allendale and Bamberg counties available for examination (SERNEC Data Portal 2021; Appendix). These new "virtual herbarium" records show a geographical distribution in South Carolina comprising nine contiguous counties of the central and northern piedmont (an increase of four counties since Levy and Malone) and two counties in the inner coastal plain (Fig. 2). It should be noted that the online databases include numerous misidentifications, often mistaking *P. maculata* for *P. dubia*.

GOALS. The purpose of this study is to update the taxonomy and biogeography of the *Phacelia dubia* complex by providing: A summary of the history and basis for subspecific taxon recognition; a description of the nature and extent of differentiation among taxa; a morphometric analysis of variation within the complex; and an update to the geographical distribution and identity of elements in South Carolina. This will culminate in description of a new variety from South Carolina and conclude with a synthesis of the manifestations of differentiation to develop a hypothetical evolutionary and migration pathway in which the new variety plays a key role.

Methods. MORPHOLOGY. For corolla characters (corolla lobe length and width), fresh flowers were collected from 20 plants in three populations of *Phacelia dubia* var. *dubia* (Bull's Gap, Buncombe Co., NC; Red Hill, Mitchell Co., NC; Warriors Path State Park, Sullivan Co., TN), one population of *P. dubia* var. *georgiana* (Evans, Columbia Co., GA), and four populations of the putative variety (all in South Carolina: Allendale, Allendale Co.; Olar, Bamberg Co.; Rion, Fairfield Co.; Clover, York Co.). Fresh flowers were collected and immediately pressed under clear tape (Supplemental Data). In addition, corolla measurements were taken from 20 herbarium specimens from the type locality of *P. dubia* var. *georgiana* (Echols Mill, Oglethorpe Co., GA) (this population could not be visited but the wealth of specimens from that site allowed careful selection of pressed flowers).

Leaf and sepal characters were measured from online high-resolution images of herbarium specimens from the following herbaria (herbaria acronyms followed by an asterisk house South Carolina specimens): ANHC, BOON, BRIT, CLEMS*, CM*, DUKE*, EKY*, FMUH*, FSU*, FUGR, GA*, GEO, HBSH, HUH*, IND, NBYC*, NCU, SALK*, TENN, UNA, UNCC*, USCH*, USCS*, USF*, WINU* (SERNEC Data Portal 2021). On each plant, the following data were recorded from an upper rosette or lowest cauline leaf and the third cauline leaf below the most basal flower: number of leaflets below the terminal leaflet and total number of lobes on those leaflets; number of pinnae or lobes on the terminal leaflet; terminal leaflet lobe length and width (measured from the uppermost sinus to the leaf tip; Fig. 1). A leaflet is considered a leaf segment that is constricted to, or close to the midrib, while a lobe is taken to be a secondary constriction of a leaflet. For sepals, length and width were recorded from a flower at anthesis and a flower with a maturing capsule. Although some character correlations were to be expected among this suite of characters, all were included in analyses because the goal was to find the most effective phenotypic approach to identification rather than to estimate phylogenetic distance. Because herbarium-based data are from field plants, phenotypes were subject to environmental influences and are not directly comparable to data from plants grown in controlled environments. Instead, these data were used to first, determine the efficacy of identifying plants from natural populations, and second, examine the morphologic affinities of the plants referred to as "*P. dubia* putative var. imitator," and referred to from here forward as the putative variety (Levy 1991a, 1991b; Levy and Malone 2001).

Leaf and sepal measurements were taken from 30 plants from each of three taxa; *Phacelia dubia* var. *dubia*, *P. dubia* var. *georgiana*, and the putative variety (Appendix). *Phacelia dubia* var. *interior* was not included because its geographical range is far distant from the parapatric complex of the other varieties, and morphologically, it is more extreme than *P. dubia* var. *dubia* in having larger sepals, larger corolla lobes, and less lobed leaves, all character states that trend away from the *P. dubia* var. *georgiana* and the putative variety phenotypes (Levy 1991b). Since plants from the periphery of the putative variety range were most likely to be confused with *P. dubia* var. *georgiana* to the south and *P. dubia* var. *dubia* to the north, the respective samples were restricted to those regions; that is, *P. dubia* var. *georgiana* plants from the piedmont of Georgia and neighboring Alabama, and *P. dubia* var. *dubia* plants from North Carolina. The putative variety sample included 30 specimens from the piedmont Charlotte belt with its granitic plutons (Wagener and Howell 1973; Fullagar and Butler 1979) and 15 specimens from Allendale and Bamberg Counties, SC, on the inner coastal plain.

For each corolla, leaf, and sepal character, univariate ANOVAs were conducted followed by least squares comparisons of means using the GLM procedure in SAS (SAS Institute Inc. 2016). To identify a suite of leaf and sepal characters that provided the maximum separation between *Phacelia dubia* var. *dubia* and *P. dubia* var. *georgiana*, stepwise discriminant analysis, using the STEPDISC procedure in SAS, was conducted on samples from only those taxa. Incomplete leaf and sepal data for many specimens (usually caused by specimens with only immature capsules or past flowering stage) reduced the sample sizes. This resulted in a set of four characters (number of leaflets on rosette/lowest leaf, number of leaflets on

Table 1. Results of ANOVAs comparing corolla lobe length and width in varieties (var) and populations within varieties [pop(var)] of *Phacelia dubia* with variety means, standard deviations, and ranges (mm). For corolla lobe length and width, means differed significantly from each other ($P < 0.0001$ for each comparison). Populations sampled: *P. dubia* var. *dubia*: Warriors Path State Park, Sullivan Co., TN; Red Hill, Mitchell Co., NC; Blue Ridge Parkway, Buncombe Co., NC. *P. dubia* var. *georgiana*: Evans, Columbia Co., GA; Echols Mill, Oglethorpe Co., GA. Putative variety: Allendale, Allendale Co., SC; Olar, Bamberg Co., SC; Old Carriage Road, Clover, York Co., SC; Rion, Fairfield Co., SC.

Calculation	Values			
ANOVA	Source	d.f.	*F*	*P*
Corolla lobe length	Var	2	404.2	< 0.0001
	Pop(var)	7	14.6	< 0.0001
Corolla lobe width	Var	2	352.4	< 0.0001
	Pop(var)	7	25.0	< 0.0001
Means	Variety	*N*	Mean (SD)	Range
Corolla lobe length	*P. dubia* var. *dubia*	60	6.14 (0.69)	4.5–7.4
	P. dubia var. *georgiana*	40	3.78 (0.34)	2.9–4.3
	Putative variety	100	4.67 (0.46)	3.4–5.5
Corolla lobe width	*P. dubia* var. *dubia*	60	4.75 (0.55)	3.4–6.0
	P. dubia var. *georgiana*	40	2.65 (0.44)	2.0–4.1
	Putative variety	100	3.97 (0.55)	2.7–5.1

the cauline leaf, length of the terminal lobes on rosette/lowest leaf, sepal length of maturing capsule) that were then used in classification discriminant analyses (using the DISCRIM procedure in SAS), first including only *P. dubia* var. *dubia* and *P. dubia* var. *georgiana* to serve as a training data set, and then the putative variety specimens were included to determine the proportion of each sample assigned to *P. dubia* var. *dubia* versus *P. dubia* var. *georgiana*. This was followed by an analysis using all three taxa in the training and assignment data sets. A similar pair of discriminant function analyses were conducted using corolla length and width. The flower and sepal/leaf data sets could not be combined because sample plants differed.

Soils. Chemical properties and pH were determined for soils from three locales supporting populations of the putative variety in South Carolina (in proximity to granite outcrops in Clover, York Co. and Rion, Fairfield Co.; and from a roadside near Olar, Bamberg Co.) and one population of *P. dubia* var. *georgiana* in proximity to a granite outcrop in Appling, Columbia Co., GA. Samples were collected from the upper 15 cm of soils and oven-dried for 24 hr at 60° C. The pH of each sample was measured using a calibrated pH meter (Denver Instrument™, Arvada, CO) and a 1:1 soil:diH_2O ratio. For element analyses, samples were sent to the University of Georgia Agricultural and Environmental Services Laboratories for acid digestion and determinations using the inductively coupled plasma technique.

Results. Morphology. Corolla lobe length and width differed significantly among populations within varieties and among varieties (Table 1). For both characters, *Phacelia dubia* var. *dubia* had the largest and *P. dubia* var. *georgiana* had the smallest corolla lobes with an intermediate size in the putative variety. In 11 of the 13 leaf and sepal characters, mean expression in *P. dubia* var. *dubia* and *P. dubia* var. *georgiana* differed significantly from each other (Table 2). In contrast, the putative variety showed mixed affinities; it was unique in three characters (Table 2A), similar to *P. dubia* var. *dubia* in one (Table 2B), similar to *P. dubia* var. *georgiana* in six (Table 2C), and similar to both *P. dubia* var. *dubia* and *P. dubia* var. *georgiana* in one character (Table 2D). For all except one character, the phenotypic mean in the putative variety was between those of *P. dubia* var. *dubia* and *P. dubia* var. *georgiana* (often not a mid-value but nearer to one of the other varieties; Table 2).

The accuracy of assignment of individual plants into varieties using discriminant function analyses was similar for corolla size and leaf characters. In each case, when only *Phacelia dubia* var. *dubia* and *P. dubia* var. *georgiana* were classification options, plants of those two varieties were correctly assigned (> 97% for each) but the putative variety sample was split between the two historical varieties (Table 3A, 4A). When the

Table 2. Comparisons of means (standard deviations in parentheses) of leaf and sepal characters among three varieties of *Phacelia dubia*, sorted by the pattern of significant differences, based on results of univariate analyses of variance and least squares means comparisons. Sample sizes: *P. dubia* var. *georgiana* = 20–30; *P. dubia* var. *dubia* = 15–30; Putative variety = 39–45. Length and width values are in mm.

		Variety, mean (SD)		
Pattern of differences	*P*	*P. dubia* var. *georgiana*	*P. dubia* var. *dubia*	Putative variety
A. *georgiana* ≠ *dubia* ≠ putative variety				
No. leaflets, rosette/lowest cauline leaf	< 0.0001	5.76 (1.43)	2.30 (1.32)	4.64 (1.64)
No. lobes, rosette/lowest cauline leaf	< 0.0001	1.83 (1.51)	0.10 (0.40)	1.02 (1.60)
Sepal length, fruiting	< 0.0001	2.87 (0.38)	3.95 (0.84)	3.43 (0.65)
B. *georgiana* ≠ *dubia* = putative variety				
Sepal length, flowering	0.03	2.33 (0.51)	2.59 (0.44)	2.54 (0.50)
C. *georgiana* = putative variety ≠ *dubia*				
No. leaflets, cauline leaf	< 0.0001	3.50 (1.41)	1.53 (1.38)	2.96 (1.24)
Terminal leaflet length, rosette/lowest cauline leaf	< 0.001	5.39 (1.84)	7.97 (3.07)	5.70 (2.73)
Terminal leaflet width, rosette/lowest cauline leaf	< 0.001	3.73 (1.08)	5.28 (1.73)	4.96 (1.82)
Terminal leaflet length, cauline leaf	< 0.01	4.94 (2.22)	7.01 (3.27)	5.28 (1.97)
Terminal leaflet width, cauline leaf	< 0.0001	2.23 (0.93)	3.54 (1.52)	2.51 (1.01)
Sepal width, fruiting	< 0.01	0.95 (0.22)	1.36 (0.65)	1.03 (0.35)
D. Putative variety = *dubia* ≠ *georgiana* = putative variety				
Sepal width, flowering	0.03	0.72 (0.26)	0.90 (0.25)	0.77 (0.22)
E. No significant differences				
No. lobes, terminal leaflet, rosette/lowest cauline leaf	0.72	1.16 (1.05)	1.20 (0.96)	1.36 (1.26)
No. lobes, terminal leaflet, cauline leaf	0.88	1.83 (0.53)	1.73 (1.08)	1.78 (0.63)

putative variety was added as a classification option, correct assignment of the putative variety sample increased to 88% with minimal loss of accuracy in assignment of *P. dubia* var. *dubia* and *P. dubia* var. *georgiana*. Nearly all cases of misclassification involved placement to or from the putative variety (Table 3B, 4B).

SOILS. All of the soil samples had a similar and acidic pH, with a range of 5.4–5.6 (Table 5). Fertility of all of these soils would be considered low as concentrations of each of the plant macronutrients (calcium, magnesium, potassium, phosphorus, and sulfur) were lower than recommendations for crop production (Smith *et al.* 2005). The soil from Bamberg Co., SC, with the lowest concentrations of most macro- and micronutrients, was particularly low in magnesium, potassium, and boron (Table 5). Other than the nutrient-poor soil from Bamberg Co., SC, there were no evident differences between soils of the Georgia and South Carolina granite outcrops and their environs.

Table 3. Classification of herbarium specimens as either *P. dubia* var. *georgiana* or *P. dubia* var. *dubia* resulting from discriminant function analysis in three varieties of *Phacelia dubia*. For each variety, the number of specimens assigned to each variety is shown (percentages in parentheses).

A. By analysis of flower characters

From:	To:	
	P. dubia var. *georgiana*	*P. dubia* var. *dubia*
P. dubia var. *georgiana*	39 (97.5)	1 (2.5)
P. dubia var. *dubia*	1 (1.7)	59 (98.3)
Putative variety	69 (69.0)	31 (31.0)

B. By analysis of sepal and leaf characters

From:	To:	
	P. dubia var. *georgiana*	*P. dubia* var. *dubia*
P. dubia var. *georgiana*	21 (100.0)	0 (0)
P. dubia var. *dubia*	0 (0)	13 (100)
Putative variety	15 (60.0)	10 (40.0)

Table 4. Classification of herbarium specimens as *P. dubia* var. *dubia*, *P. dubia* var. *georgiana*, or the putative variety resulting from discriminant function analysis in three varieties of *Phacelia dubia*. For each variety, the number of specimens assigned to each variety is shown (percentages in parentheses).

A. By analysis of flower characters

From:		To:	
	P. dubia var. *georgiana*	*P. dubia* var. *dubia*	Putative variety
P. dubia var. *georgiana*	38 (95.0)	0 (0)	2 (5.0)
P. dubia var. *dubia*	0 (0)	51 (85.0)	9 (15.0)
Putative variety	10 (10.0)	2 (2.0)	88 (88.0)

B. By analysis of sepal and leaf characters

From:		To:	
	P. dubia var. *georgiana*	*P. dubia* var. *dubia*	Putative variety
P. dubia var. *georgiana*	15 (71.4)	0 (0)	6 (28.6)
P. dubia var. *dubia*	0 (0)	12 (92.3)	1 (7.7)
Putative variety	1 (4.0)	2 (8.0)	22 (88.0)

Taxonomic Recognition of *Phacelia dubia* var. *rionensis*. Plants from the central and northern piedmont and the adjoining inner coastal plain of South Carolina merit recognition as a variety of *Phacelia dubia* on the basis of partial reproductive isolation from the three other varieties, a morphology that differs from the other varieties in its combination of characters (but not in possessing unique characters), and its geographical distribution that is allopatric to the other varieties. Consequently, the entity previously referred to as putative variety "imitator," is here formally named and described.

Phacelia dubia* (L.) Trel. var. *rionensis Levy, Hou, and Taylor-Bennetts var. nov. (Fig. 3).

"*Phacelia dubia* putative var. imitator," a *nomen nudum* published by Levy, Rhodora 93:14. 1991.

Type. UNITED STATES, South Carolina, York: 0.5 mi W on Old Carriage Road from county route 422, scattered throughout granite outcrop and adjoining field, *F. Levy s.n.*, 13 April 1987, Holotype: DUKE10106112 (Fig. 3). Paratypes: UNITED STATES, South Carolina, York: roadside outcrop on Route 422 about ¼ mile N of junction with Route 1321, *F. Levy s.n.*, 15 May 1987 (DUKE10106404); roadside outcrop on Route 422, 0.1 mi S of Beaverdam Creek, *F. Levy s.n.*, 15 May 1987 (DUKE10106406); east side of Old Carriage Road (CR-1321) north of SR 422, 2.8 mi northeast of Clover, SC, *Bradley 4022*, 11 April 2016 (USCH0015737, USF295721); south side of Carriage Road Secondary Road 1321, midway between junction Carriage Road and secondary Road 422 and Highway 321, northeast of Clover, *Newberry and Hague 6244*, 9 May 1986 (USCS0014695); About 10.5 mi NNE of York, ca. 2.75 mi NE of Clover, county road 472, ca. 0.6 mi by car south of junction with county road 27, *Allison 2702*, 9 May 1986 (GA210092)

Description. Winter annual; stem with afflexed hairs and capitate glands; basal leaves pinnate-pinnatafid with 2–6 pairs of leaflets but usually no additional lobes, terminal lobe approximately 5.5 × 4.0 mm; cauline leaves (third below inflorescence) with 2–4(6) leaflets or pinnae; corolla (white) pale to dark lavender, lobes 3.5–5.3 × 3.5–5.2 mm, glabrous on the abaxial surface; ovary with 2–4 ovules per placenta.

Diagnosis. Distinguished from other varieties of *P. dubia* by more dissected leaves than *P. dubia* var. *dubia*, flowers with wider corolla lobes than *P. dubia* var. *georgiana*, and narrower corolla lobes than *P. dubia* var. *interior*; habitat of granite outcrops and associated peripheral and ruderal areas (Fig. 4).

Habitat. Granite outcrops and environs including roadsides, with a preference for acidic soil.

Distribution. Piedmont of central and northern South Carolina, usually in the Charlotte belt, and Allendale and Bamberg Co., SC in the inner coastal plain (Fig. 2). Possibly in Anson Co., NC.

Etymology. The variety name *Phacelia dubia* var. *rionensis* refers to the hamlet of Rion, located in Fairfield Co., SC, near the center of the geographical range of the new variety.

FIG. 3. Image of the holotype of *Phacelia dubia* (L.) Trel. var. *rionensis* Levy, Hou, & Taylor-Bennetts (*Levy s.n.*, DUKE10106112).

Table 5. Soil pH and element concentrations (ppm) in samples collected from three populations of the putative variety in South Carolina and one population of *P. dubia* var. *georgiana* in Georgia.

Town, county, state	pH	Ca	Mg	K	P	Fe	B	S	Zn	Al
Clover, York, SC	5.6	2,122	2,111	1,376	439	12,843	18.13	551	45	13,339
Rion, Fairfield, SC	5.4	1,665	1,686	800	288	5,824	9.00	563	62	5,571
Olar, Bamberg, SC	5.5	1,307	224	132	197	1,817	3.00	255	31	2,640
Appling, Columbia, GA	5.4	950	2,821	2,359	354	9,529	12.96	155	46	12,462

Key to the Varieties of *Phacelia dubia*:

1a. Upper rosette/lowest cauline leaves more pinnately dissected with 4–8 distinct leaflets and a total of an additional 2–4 lobes on those leaflets, and a narrow terminal leaflet 4–8 mm × 3–4 mm; cauline leaves (third below inflorescence) with 2–6 leaflets and those usually with some additional lobes; corolla small, lobes < 4.3 mm × < 3.5 mm; granite outcrops and peripheral regions, abundant on some roadsides and lawns; piedmont of Alabama and Georgia north to McCormick Co., SC. . . . . . . ***P. dubia* var. *georgiana***

1b. Upper rosette/lowest cauline leaves usually less dissected (< 6 leaflets) and rarely with additional lobing, and a wider terminal leaflet 4–10 mm × 3–7 mm; cauline leaves (third below inflorescence) with 1–4 leaflets, rarely with additional lobes; corolla larger, lobes usually > 4 mm × > 3 mm 2

  2a. Corolla more dish-shaped; that is, shallowly concave (best viewed on live specimens), lobes > 4.5 mm wide; upper rosette/lowest cauline leaves usually with < 4 leaflets + lobes, terminal leaflet wider than other leaflets; cedar glades and surrounding areas of the Nashville Basin . . . . ***P. dubia* var. *interior***

  2b. Corolla bowl-shaped or more deeply concave, lobes usually < 5.5 mm wide; upper rosette/lowest cauline leaves usually with > 4 leaflets + lobes; terminal leaflet equal to or wider than other leaflets; east of the Cumberland Plateau 3

    3a. Upper rosette/lowest cauline leaves usually with 2–6 leaflets sometimes with a few additional lobes, terminal leaflet smaller (approximately 5.5 mm × 4.0 mm); terminal leaflet of cauline leaf (third below inflorescence) usually smaller (approximately 5.0 mm × 2.5 mm); corolla lobes 3.5–5.2 mm × 3.0–4.8 mm; granite outcrops and peripheral areas including roadsides; piedmont of South Carolina (and adjacent North Carolina?) and Allendale and Bamberg Co., SC. . . . .***P. dubia* var. *rionensis***

    3b. Upper rosette/lowest cauline leaves usually with 0–4 leaflets, very rarely with additional lobes, terminal leaflet larger (approximately 7.0 mm × 5.0 mm); terminal leaflet of cauline leaf (third below inflorescence) usually larger (approximately 7.0 mm × 3.5 mm); corolla lobes > 4.5 mm long and > 4.0 mm wide; rocky woods, floodplain forests, shale barrens, and ruderal areas including roadsides and railroad embankments; Blue Ridge mountains, piedmont, coastal plain; Pennsylvania and south, in the uplands to north Georgia, and coastal plain to Charleston Co., SC . . . . ***P. dubia* var. *dubia***

Discussion. VARIETAL STATUS. With the onset of biosystematic studies of the *Phacelia dubia* complex, it was evident that plants from South Carolina did not comfortably fit in any of the recognized varieties with their less dissected leaves and larger corollas compared to *P. dubia* var. *georgiana*, and the granite outcrop habitat atypical of *P. dubia* var. *dubia*. Consequently plants from the piedmont of South Carolina were referred to as a putative variety (Levy 1991a). When a series of studies showed the putative variety element was partially reproductively isolated from other varieties (Levy 1991b, Levy and Malone 2001), the problem associated with creating a morphologically undiagnosable taxon inhibited formal recognition.

With the ascent and wider application of molecular-based systematics, it has become increasingly clear that there exist many genetically distinct biological groups that are not readily apparent to the human eye (Bickford *et al.* 2007). However, cryptic species are more likely to be accepted if additional lines of evidence supplement molecular data (Fišer *et al.* 2018; Struck *et al.* 2018). Unlike the cryptic species that have been identified via molecular studies and justified on the basis of the phylogenetic species concept, the recognition of *P. dubia* var. *rionensis* more closely aligns with the biological and ecological species concepts in that it shows bidirectional hybrid sterility with *P. dubia* var.

FIG. 4. Photo of the type locality of *Phacelia dubia* var. *rionensis* at Old Carriage Road, York Co., SC. (A) Granite outcrop showing vegetation mats in rock depressions, the one in the background with *Diamorpha smallii* Brit. ex Small (red-colored stems) and the one in the foreground with yellow-flowered *Packera tomentosa* (Michx.) C. Jeffrey. (B) Colony of *P. dubia* var. *rionensis*. Colonies of *P. dubia* var. *rionensis* occur on vegetation mats and along the periphery of the outcrop as well as along adjoining roadsides. Photo from April 14, 2021 by S. Taylor-Bennetts.

dubia and with *P. dubia* var. *georgiana*, and has a major habitat difference and complete allopatry with *P. dubia* var. *interior* (with which it is less reproductively isolated). Moreover, variable levels of hybrid intersterility and the diversity of genetic mechanisms underlying sterility are also consistent with the speciation continuum concept (Stankowski and Ravinet 2021).

Molecular data showed a somewhat different view of the differentiation continuum; the chloroplast genome type in *P. dubia* var. *rionensis* was one of the two types found in both *P. dubia* var. *dubia* and *P. dubia* var. *georgiana*, but these differed from the type fixed in *P. dubia* var. *interior* (Levy *et al*. 1996; Levy and Neal 1999). Since all intervarietal hybrid combinations are viable and backcrosses can restore fertility, introgression of plastid genomes is possible and may explain the shared plastome types among *P. dubia* vars. *dubia*, *georgiana*, and *rionensis* (Duminil and Di Michele 2009). We hypothesize this chloroplast pattern of differentiation can be attributed to a more recent common ancestor shared by the three more eastern taxa, *P. dubia* vars. *dubia*, *georgiana*, and *rionensis*.

Phacelia dubia has adapted to a broad range of novel ecological communities. Of the three rock outcrop habitats with the largest geographical extent and the highest numbers of endemic plant taxa in the eastern United States (cedar glades, granite outcrops, shale barrens), *P. dubia* has evolved specializations to piedmont granite outcrops and Nashville Basin cedar glades. Similarly, in several other genera (*Cyperus* L., *Phemeranthus*

Raf., *Sedum* L.), pairs of closely related species have adapted to different substrates on eastern North American rock outcrops (Crow and Ware 2007, Ware *et al.* 2011). In addition, *P. dubia* var. *dubia* also inhabits some mid-Appalachian shale barrens, this in the absence of evident differentiation (Henry 1954, Tyndall 2015), so it may be considered to have some level of preadaptation to that community. However, while several western American *Phacelia* taxa are serpentine endemics (Safford and Miller 2020), none of the forms of *P. dubia* (or taxa from the aforementioned genera) are endemic to the sparsely distributed eastern serpentine sites and none showed any level of tolerance to serpentine-derived soils (Taylor and Levy 2002).

Soil analyses showed that granite outcrops from South Carolina and Georgia have acidic soils with low concentrations of essential nutrients. Similarly low concentrations of plant nutrients were also observed in acidic, granite-derived soils in Georgia (Taylor and Levy 2002), Missouri (Reinhard and Ware 1989), and China (Hao *et al.* 2015). The Allendale/Bamberg locations for *Phacelia dubia* var. *rionensis* on the inner coastal plain rather than the piedmont are somewhat anomalous, but most of those collections were from roadsides where they may represent migrations from an ecotype with weedy tendencies. Particularly low concentrations of some elements (magnesium, potassium, boron) were observed in the roadside soil from Bamberg Co., a not unexpected finding for a soil from a highly disturbed roadside environment. Thus, both *P. dubia* var. *rionensis* and *P. dubia* var. *georgiana* appear to have adapted to low concentrations of nutrients on granite outcrops, which has likely functioned as a preadaptation to facilitate their widespread occurrence and abundant populations in highly disturbed, nutrient-poor roadside areas in South Carolina and Georgia. In Columbia Co., Georgia, a weedy ecotype of *P. dubia* var. *georgiana* has been long observed and in common garden experiments, these plants flower earlier and are genetically dwarfed compared to plants from nearby, more natural and long-established sites (Taylor-Bennetts, unpublished data).

Although characters in *Phacelia dubia* var. *rionensis* overlap those of *P. dubia* var. *dubia* and *P. dubia* var. *georgiana*, this study has shown significant corolla lobe size, sepal size, and leaf dissection differences among all three varieties. However, specimens from Allendale and Bamberg Counties, SC were problematic in morphology and physiography and their location on the inner coastal plain is geologically distinct. Morphologically, flowers were typical of those of *P. dubia* var. *dubia* but some plants had the extreme leaf dissection typical of *P. dubia* var. *georgiana* (Fig. 1). We opted to provisionally treat the Allendale and Bamberg Co. populations as *Phacelia dubia* var. *rionensis* but molecular and intervarietal hybridization data would further clarify their status.

HYPOTHESIZED MIGRATION PATHWAYS. A synthesis of past and present work on the forms and magnitudes of differentiation in the *Phacelia dubia* complex can guide the development of hypothesized evolutionary and migration pathways. The vast majority of *Phacelia* taxa are from open habitats in western North America with some species ranging into, and others entirely within, Mexico, and a very small number of species in South America (Walden and Patterson 2012, Vasile *et al.* 2020). The taxa in Mexico include several gypsophiles and a few species of sect. *Cosmantha* (Atwood and Pinkava 1977; Turner 2011, 2015).

Molecular phylogenies indicate the genus *Phacelia* originated near the current center of diversity in western and south-central North America and Mexico and that sect. *Cosmantha* is a more recently derived taxon (Ferguson 1998, Walden *et al.* 2014, Vasile *et al.* 2020). Consequently, migration to a habitat somewhat similar to those in western North America can be envisioned as a first entry to eastern North America. Based on geographic proximity to sect. *Cosmantha* species in Arkansas, Missouri, and Texas, the Nashville Basin cedar glades are a likely ancestral location for the eastern lineage leading to subsect. *Dubiae* (Fig. 5). In addition, the Tennessee cedar glades support a host of species typical of the mid to southern Great Plains (Baskin and Baskin 2003). The relatively weak reproductive incompatibility between *P. dubia* var. *interior* and *P. dubia* var. *rionensis*—unidirectional and in only some cross combinations—suggests the next step was migration east to South Carolina granite outcrops (Fig. 5; Levy 1989, 1991a; Levy and Malone 2001). From South Carolina granite outcrops, migration to Georgia granite outcrops would involve a relatively short geographical distance and little to no ecological adaptation, but based on genetic markers, this transition would have been accompanied or followed by a chromosomal rearrange-

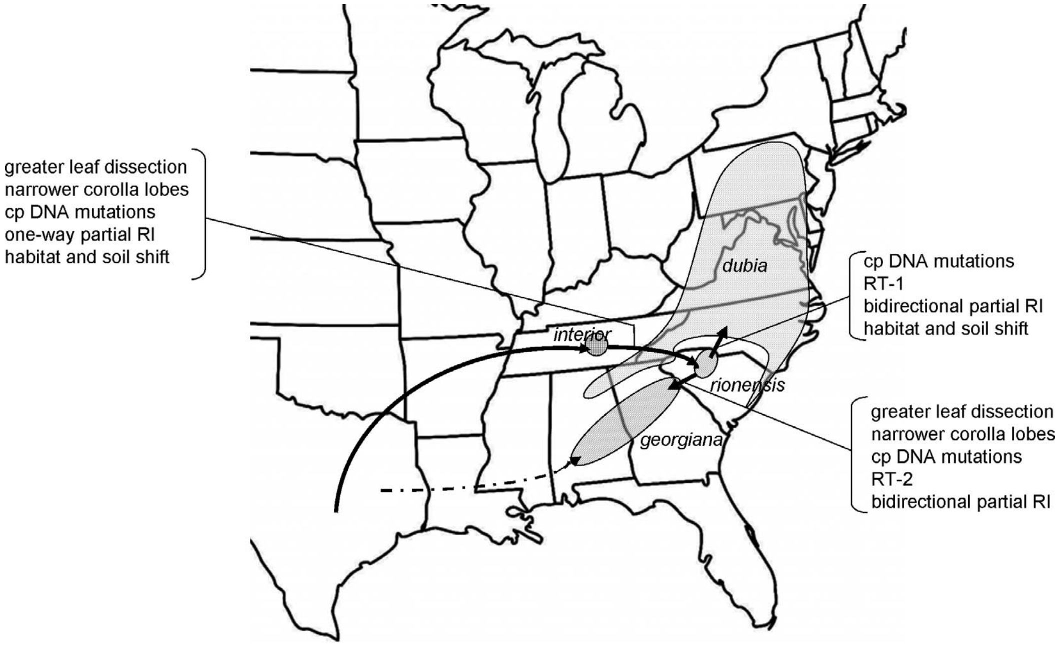

FIG. 5. Map showing hypothesized migration pathway and differentiation pattern of the *Phacelia dubia* complex. Solid line originating in Texas shows a first migration (heavy line) from the southern Great Plains or Mexico to the Nashville Basin cedar glades (*P. dubia* var. *interior*). The hypothesized origin of the complex is based on molecular phylogenetic studies in *Phacelia* showing that sect. *Cosmantha* is derived from western North American ancestors. This was followed by migration to South Carolina granite outcrops (*P. dubia* var. *rionensis*) and from there, migration south to Georgia (*P. dubia* var. *georgiana*) and north to North Carolina (*P. dubia* var. *dubia*). Dotted line shows an alternative migration scheme from the southern Great Plains of the US or directly from Mexico to Alabama/Georgia piedmont granite outcrops, followed by progression to South Carolina, and then followed by separate migrations, one west to the cedar glades and the other north to North Carolina and beyond. Text associated with each migration summarizes the ecological, genetic, and morphological changes associated with each transition based on prior and current work documenting partial sterility in all intervarietal hybrids; two chromosomal rearrangements in the complex (RT-1; RT-2); and differences in cpDNA plastomes, leaf and flower morphology, habitat, and soil preferences. RI = reproductive isolation.

ment (to result in an ~ 50% fertility reduction in F_1 hybrids), mutations in cpDNA, and a morphological transition to more dissected leaves (McVaugh 1943; Levy 1989, 1991a, 1991b, 1992; Levy *et al.* 1996; Hou 1995; Levy and Neal 1999; Levy and Malone 2001). Similarly, a migration north of *P. dubia* var. *rionensis* would also have been accompanied by a (different) chromosomal rearrangement as well as a shift in habitat and soil preference (Fig. 5; Levy 1991a, 1992; Hou 1995; Taylor and Levy 2002). These migrations from *P. dubia* var. *rionensis* in opposite directions would result in *P. dubia* var. *dubia* and *P. dubia* var. *georgiana* differentiated by two chromosomal rearrangements. Those chromosomal rearrangements would explain the restricted recombination among different genetic markers and the ~ 75% reduction in fertility in *P. dubia* var. *dubia* and *P. dubia* var. *georgiana* F_1 hybrids as well as patterns of fertility restoration in backcrosses and in three-way hybrids (Levy 1991a, 1992; Hou 1995; Levy and Malone 2001).

An alternative migration scheme (and one with an equal number of steps) would begin by positing migration from the southern Great Plains of the US or directly from Mexico to Alabama/Georgia piedmont granite outcrops, followed by progression to South Carolina, and then followed by separate migrations, one west to the cedar glades and the other north to North Carolina and beyond (Fig. 5). Under either migration scenario, *P. dubia* var. *dubia*, the most geographically widespread variety and the one that may have been considered less specialized because of its woodland habitat, may be the most recently evolved and most ecologically and cytologically derived. Several arguments support placing the cedar glades as an initial entry point for the *P. dubia* complex: (1) cedar glades have a limestone-derived substrate and floristic affinities to communities of the southern Great Plains and Mexico compared to piedmont granite outcrops whose floristic affinities are more allied with the Atlantic coastal plain, (2) *Phacelia* taxa from just west of the Mississippi River and from Mexico include gypsophiles and calciphiles (Turner 2011, Locklear 2017) and are thus adapted to similar substrates as found in cedar glades, and (3) the morphological transition through *P. dubia* var. *rionensis* would be more gradual than a *P. dubia* var. *georgiana* to *P. dubia* var. *interior* transition. Thus, an origin via the Nashville Basin cedar glades is considered the strongest hypothesis for the biogeographic pathway for the origin of the *P. dubia* complex. The hypothesized migration pathway and its implicit statements of relationship comprise a hypothesis that could be tested with a molecular phylogeny of the complex.

CONSERVATION IMPLICATIONS. Clarification of the taxonomy and biogeography of the *Phacelia dubia* complex in South Carolina impacts species conservation targets. Of the three varieties in the state, the newly described *P. dubia* var. *rionensis* has the broadest geographical distribution (Fig. 2). Moreover, in some counties it can be found at several sites, and at many sites, populations are large. In contrast, *P. dubia* var. *dubia*, previously considered widespread in the state, has been collected only in Oconee Co. in the Blue Ridge (four collections, all in the vicinity of Brasstown Creek) and Charleston Co. in the outer coastal plain (a site that could not be relocated: Appendix). With current known occurrences in only one area of one county, *P. dubia* var. *dubia* should be considered uncommon or rare in South Carolina. *Phacelia dubia* var. *georgiana* has been collected only from McCormick Co., no specimens more recently than the 1970s. With some of the McCormick Co. specimens collected in proximity to a granite outcrop, this site may represent the historic occurrence of the taxon in the state.

Conclusions. Taxonomic recognition of a new variety of *P. dubia* from the piedmont of South Carolina is merited because of partial reproductive isolation from all other varieties of *P. dubia*, an allopatric distribution, and new evidence of differentiation in corolla size, sepal size, and leaflet dissection. The new variety, *P. dubia* var. *rionensis*, is associated with granitic plutons and is found in nine contiguous counties of the Charlotte belt of the piedmont and two counties in the inner coastal plain. The new variety occupies a key transitional position in a hypothesized migration pathway that has given rise to the current *P. dubia* complex.

Literature Cited

ATWOOD, N. D. AND D. J. PINKAVA. 1977. A new gypsophilous species of *Phacelia* (Hydrophyllaceae) from Coahuila, Mexico. Madroño 24: 212–214.

BAKER, M. D. 1961. Experimental and descriptive studies of the *Phacelia fimbriata* complex (Hydrophyllaceae). American Journal of Botany 48: 544.

BASKIN, J. M. AND C. C. BASKIN. 1971. Germination ecology of *Phacelia dubia* var. *dubia* in Tennessee glades. American Journal of Botany 58: 98–104.

BASKIN, J. M. AND C. C. BASKIN. 2003. The vascular flora of cedar glades of the southeastern United States and its phytogeographical relationships. Journal of the Torrey Botanical Society 130: 101–118.

BICKFORD, D., D. J. LOHMAN, N. S. SODHI, P. K. NG, R. MEIER, K. WINKER, K. K. INGRAM, AND I. DAS. 2007. Cryptic species as a window on diversity and conservation. Trends in Ecology and Evolution 22: 148–155.

BURNHAM, C. R. 1962. Discussions in Cytogenetics. Burgess Publishing Company, Minneapolis, MN. 375 pp.

CHESTER, E. W., B. E. WOFFORD, J. SHAW, D. ESTES, AND D. H. WEBB. 2015. Guide to the Vascular Plants of Tennessee. University of Tennessee Press, Knoxville, TN. 813 pp.

CHRISTENHUSZ, M. J., M. S. VORONTSOVA, M. F. FAY, AND M. W. CHASE. 2015. Results from an online survey of family delimitation in angiosperms and ferns: Recommendations to the Angiosperm Phylogeny Group for thorny problems in plant classification. Botanical Journal of the Linnean Society 178: 501–528.

CONSTANCE, L. 1949. A revision of *Phacelia* subgenus *Cosmanthus* (Hydrophyllaceae). Contributions from the Gray Herbarium of Harvard University 168: 1–48.

CONSTANCE, L. 1963. Chromosome number and classification in Hydrophyllaceae. Brittonia 15: 273–285.

COUGHLAN, J. M., M. W. BROWN, AND J. H. WILLIS. 2020. Patterns of hybrid seed inviability in the *Mimulus guttatus* sp. complex reveal a potential role of parental conflict in reproductive isolation. Current Biology 30: 83–93.

CROW, S. E. AND S. WARE. 2007. Soil type tolerance in rock outcrop plants: Species of non-calcareous substrates. Southwestern Naturalist 52: 120–125.

DUMINIL, J. AND M. DI MICHELE. 2009. Plant species delimitation: A comparison of morphological and molecular markers. Plant Biosystems 143: 528–542.

Ely, C. V., S. A. de Loreto Bordignon, R. Trevisan, and I. I. Boldrini. 2017. Implications of poor taxonomy in conservation. Journal for Nature Conservation 36: 10–13.

Ferguson, D. M. 1998. Phylogenetic analysis and relationships in Hydrophyllaceae based on *ndh*F sequence data. Systematic Botany 23: 253–268.

Fernald, M. L. 1944. Overlooked species, transfers and novelties in the flora of eastern North America (continued). Rhodora 46: 32–57.

Fišer, C., C. T. Robinson, and F. Malard. 2018. Cryptic species as a window into the paradigm shift of the species concept. Molecular Ecology 27: 613–635.

Fullagar, P. D. and J. R. Butler. 1979. 325 to 265 M.Y.-old granitic plutons in the Piedmont of the southeastern Appalachians. American Journal of Science 279: 161–185.

Gillet, G. W. 1968. Systematic relationships in the Cosmanthus *Phacelias* (Hydrophyllaceae). Brittonia 20: 368–364.

Glass, P. M. and F. Levy. 2011. Correspondence of morphology, phylogeny, and reproductive barriers in *Phacelia* subgenus Cosmanthus (Hydrophyllaceae). Journal of the Torrey Botanical Society 138: 348–352.

Hao, Z., Y. Kuang, and M. Kang. 2015. Untangling the influence of phylogeny, soil and climate on leaf element concentrations in a biodiversity hotspot. Functional Ecology 29: 165–176.

Henry, L. K. 1954. Shale-barren flora in Pennsylvania. Proceedings of the Pennsylvania Academy of Science 28: 65–68.

Hey, J. 2006. On the failure of modern species concepts. Trends in Ecology and Evolution 21: 447–450.

Hey, J., R. S. Waples, M. A. Arnold, R. K. Butlin, and R. G. Harrison. 2003. Understanding and confronting species uncertainty in biology and conservation. Trends in Ecology and Evolution 18: 597–603.

Hou, Z. 1995. Fertility effects of genes causing reproductive isolation inferred from three-way hybrids in *Phacelia*. M.S. thesis. East Tennessee State University, Johnson City, TN. 96 pp. https://dc.etsu.edu/

Jeiter, J. and M. Weigend. 2018. Simple scales make complex compartments: Ontogeny and morphology of stamen–corolla tube modifications in Hydrophyllaceae (Boraginales). Biological Journal of the Linnean Society 125: 802–820.

Lee, M. S. 2003. Species concepts and species reality: Salvaging a Linnaean rank. Journal of Evolutionary Biology 16: 179–188.

Levy F. 1989. Mechanisms of speciation in *Phacelia*. Ph.D. dissertation. Duke University, Durham, NC. 221 pp.

Levy, F. 1991a. A genetic analysis of reproductive barriers in *Phacelia dubia*. Heredity 67: 331–345.

Levy, F. 1991b. Morphological differentiation in *Phacelia dubia* and *P. maculata*. Rhodora 93: 11–25.

Levy, F. 1992. Linkage of two allozyme loci with hybrid sterility in *Phacelia dubia*. Plant Genome I, International Conference on the Plant Genome, November 9–11, 1991, San Diego, CA. p. 36

Levy, F. 2021. Evaluation of the taxonomy of *Phacelia purshii* (Hydrophyllaceae). Journal of the Torrey Botanical Society 148: 1–16.

Levy, F. and K. A. Malone. 2001. *Phacelia dubia* in South Carolina: The interface of morphology, genetics, and taxonomy. Castanea 66: 134–144.

Levy, F. and C. L. Neal. 1999. Spatial and temporal genetic structure in chloroplast and allozyme markers in *Phacelia dubia* implicate genetic drift. Heredity 82: 422–431.

Levy, F., J. Antonovics, J. E. Boynton, and N. W. Gillham. 1996. A population genetic analysis of chloroplast DNA in *Phacelia*. Heredity 76: 143–155.

Locklear, J. H. 2017. Endemic plants of the Central Grassland of North America: Distribution, ecology, and conservation status. Journal of the Botanical Research Institute of Texas 11: 193–234.

Mace, G. M. 2004. The role of taxonomy in species conservation. Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 359: 711–719.

Malone, K. 1997. Reproductive isolation within *Phacelia dubia*: Investigating the position of putative variety "imitator" in the *Phacelia dubia* complex. M.S. thesis. East Tennessee State University, Johnson City, TN. 81 pp.

Mann, L. K., A. W. King, V. H. Dale, W. W. Hargrove, R. Washington-Allen, L. R. Pounds, and T. L. Ashwood. 1999. The role of soil classification in geographic information system modeling of habitat pattern: Threatened calcareous ecosystems. Ecosystems 2: 524–538.

McVaugh, R. 1943. The vegetation of the granitic flat-rocks of the southeastern United States. Ecological Monographs 13: 119–166.

Murdy, W. H. 1966. The systematics of *Phacelia maculata* and *P. dubia* var. *georgiana*, both endemic to granite outcrop communities. American Journal of Botany 53: 1028–1036.

Murdy, W. H. 1968. Plant speciation associated with granite outcrop communities of the southeastern Piedmont. Rhodora 70: 394–407.

Reinhard, R. T. and S. Ware. 1989. Adaptation to substrate in rock outcrop plants: Interior Highlands *Talinum* (Portulacaceae). Botanical Gazette 150: 449–453.

Safford, H. and J. E. Miller. 2020. An updated database of endemism in the California flora. Madroño 67: 85–104.

SAS Institute Inc. 2016. SAS/STAT® 9.4. SAS Institute, Inc., Cary, NC.

SERNEC Data Portal. 2021. Southeast Regional Network of Expertise and Collections. https://sernecportal.org/portal/collections/index.php. Retrieved March 15, 2021.

Sewell, M. and M. A. Vincent. 2006. Biosystematics of the *Phacelia ranunculacea* complex (Hydrophyllaceae). Castanea 71: 192–209.

Small, J. K. 1898. Studies in the botany of the southern United States-XIII. Bulletin of the Torrey Botanical Club 25: 134–151.

Small, J. K. 1933. Manual of the Southeastern Flora. J. K. Small, New York, NY. 1554 pp.

Smith, D. B., W. F. Cannon, L. G. Woodruff, R. G. Garrett, R. Klassen, J. E. Kilburn, J. D. Horton, H. D. King, M. B. Goldhaber, and J. M. Morrison. 2005.

Major- and trace-element concentrations in soils from two continental-scale transects of the United States and Canada. U.S. Geological Survey, Washington, DC.

Stankowski, S. and M. Ravinet. 2021. Defining the speciation continuum. Evolution 75: 1256–1273.

Struck, T. H., J. L. Feder, M. Bendiksby, S. Birkeland, J. Cerca, V. I. Gusarov, S. Kistenich, K.-H. Larsson, L. H. Liow, M. D. Nowak, B. Stedje, L. Bachmann, and D. Dimitrov. 2018. Finding evolutionary processes hidden in cryptic species. Trends in Ecology and Evolution 33: 153–163.

Taylor, S. I. and F. Levy. 2002. Responses to soils and a test for preadaptation to serpentine in *Phacelia dubia* (Hydrophyllaceae). New Phytologist 155: 437–447.

Thomson, S. A., R. L. Pyle, S. T. Ahyong, M. Alonso-Zarazaga, J. Ammirati, J. F. Araya, J. S. Ascher, T. L. Audisio, V. M. Azevedo-Santos, N. Bailly, W. J. Baker, M. Balke, M. V. L. Barclay, R. L. Barrett, R. C. Benine, J. R. M. Bickerstaff, P. Bouchard, R. Bour, T. Bourgoin, … H.-Z. Zhou. 2018. Taxonomy based on science is necessary for global conservation. PLoS Biology 16: e2005075.

Turner, B. L. 2011. A new gypsophilic *Phacelia* (Hydrophyllaceae) from Coahuila, México. Phytologia 93: 88–93.

Turner, B. L. 2015. Taxonomy of the *Phacelia infundibuliformis* complex (Hydrophyllaceae). Phytologia 97: 175–178.

Tyndall, R. W. 2015. Restoration results for a Maryland shale barren after pignut hickory management and a prescribed burn. Castanea 80: 77–94.

Vasile, M. A., J. Jeiter, M. Weigend, and F. Luebert. 2020. Phylogeny and historical biogeography of Hydrophyllaceae and Namaceae, with a special reference to *Phacelia* and *Wigandia*. Systematics and Biodiversity 18: 757–770.

Wagener, H. D. and D. E. Howell. 1973. Granitic plutons of the central and eastern piedmont of South Carolina. Carolina Geological Society 34th Annual Meeting, Pageland, SC. Division of Geology, South Carolina Development Board, Columbia SC. 12 pp.

Walden, G. K. and R. Patterson. 2012. Nomenclature of subdivisions within *Phacelia* (Boraginaceae: Hydrophylloideae). Madroño 59: 211–222.

Walden, G. K., L. M. Garrison, G. S. Spicer, F. W. Cipriano, and R. Patterson. 2014. Phylogenies and chromosome evolution of *Phacelia* (Boraginaceae: Hydrophylloideae) inferred from nuclear ribosomal and chloroplast sequence data. Madroño 61: 16–47.

Ware, S., S. E. Crow, and B. A. Waitman. 2011. Mode of substrate adaptation in rock outcrop plants: *Cyperus aristatus* Rottb. and *Cyperus granitophilus* McVaugh. Castanea 76: 377–385.

Weakley, A. S. 2020. Flora of the Southeastern United States. University of North Carolina Herbarium, North Carolina Botanical Garden, Chapel Hill, NC. 1848 pp.

Appendix

Specimens Examined. All specimens were examined online or via a transmitted image (HUH). All specimens from Alabama, Georgia, and North Carolina were included in the morphological analysis. For South Carolina specimens, an asterisk after the barcode number denotes specimens included in the morphological analysis and the number symbol after the herbarium code denotes vouchers for plants from the *Phacelia dubia* var. *dubia* populations used to collect flowers.

***Phacelia dubia* var. *dubia*. U.S.A. North Carolina: Alamance**: *Clark 725x* 30 Apr 1964 (FUGR0012367); *Spongberg 6740* 11 Apr 1967 (IND 0101921); **Alexander**: *Allison 3760* 2 May 1989 (GA210080); *Keever 284* 27 Apr 1941 (DUKE10106350); *McMillan 2116* 29 Mar 1997 (NCU00023028): **Anson**: *Radford 6844* 5 Apr 1953 (NCU00023215); **Ashe**: *Goodman and Goodman s.n.* 27 May 1973 (UNCC_08233); **Beaufort**: *Shelburne s.n.* 16 Apr 1972 (DUKE10106352); **Buncombe**: *Basinger s.n.* 14 May 2014 (UNCC_46773); *Levy and Walker 21041a–d* 1 May 2021 (ETSU$^{\#}$); **Caldwell**: *Mitchell s.n.* 7 May 1978 (BOON12230); **Carteret**: *Angerman s.n.* 16 Mar 1982 (USCH001577); **Chatham**: *Ahles 53252* 25 Apr 1960 (UNCC_02423); *Radford 42785* 24 Apr 1960 (NCU00023221); **Clay**: *Smith 9277* 14 Apr 1949 (GEO10728); **Davie**: *Radford 10925* 11 May 1956 (FUGR0012373); **Durham**: *Wilbur 45792* 16 Apr 1988 (DUKE10106353); **Forsyth**: *Davis s.n.* 26 Apr 1958 (NCSC00091284); *Schallert 893* 1 May 1911 (DUKE10106355); **Graham**: *Sharp 20584* 4 May 1956 (TENN-V-0182453); **Halifax**: *Chase s.n.* n.d. (DUKE10106358); **Haywood**: *Fox, Godfrey, and Woods 2171* 14 Apr 1949 (HUH); **Jackson**: *Buchanon 52* 15 Apr 1965 (BOON12218); *Fisher s.n.* 24 Apr 1966 (HBSH0002011); *Godfrey 51237* 6 Jun 1951 (DUKE10106356); **Mitchell:** *Levy and Walker 21008–21011* 16 Apr 2021 (ETSU$^{\#}$); **Montgomery**: *Wells 2818* 9 May 1970 (NCU00023100); **Onslow**: *Leonard 7141* 20 Mar 1978 (NCU00023202); **Rockingham**: *Bell 18430* 13 Apr 1966 (NCSC00091289); **Swain**: *Lewis 6575* 13 Apr 1965 (DUKE10106359); **Warren**: *Bell 2896* 24 May 1956 (NCU00023210); **Watauga**: *Carpenter 71-13* 1 May 1971 (BOON12225); **Wilkes**: *Mathews s.n.* 23 May 1965 (UNCC_00040); **South Carolina:**

Charleston: *Ravenels A-8* 31 Mar 1941 (CLEMS0075132); **Oconee**: *Hanna 89* 10 Apr 1970 (CLEMS0075134); *Hall* 17 Apr 1997 (CLEMS0051456); *Kingsland s.n.* 13 Apr 1985 (CLEMS0075131); *Nelson 227* 11 Apr 1974 (CLEMS0075133); **Tennessee: Knox**: *Ruth 164* 7 May 1899 (HUH); **Sullivan**: *Levy and Walker 21004a–c* 9 Apr 2021 (ETSU#)

***Phacelia dubia* var. *georgiana*. U.S.A. Alabama: Chambers**: *Allison 2408* 30 Mar 1985 (GA210079); *Allison 2406* 30 Mar 1985 (GA210084); **Houston**: *MacDonald 12062* 26 Mar 1999 (DUKE10106419); **Lee**: *Hale 50* 14 Apr 1972 (BRIT156449); *Kral 63363* 18 Apr 1979 (BRIT156447); *Kral 63359* 18 Apr 1979 (BRIT156448); *Schotz 2002* 27 Mar 2006 (UNA00067275); *Underwood s.n.* 15 May 1996 (UNA00039289); **Randolf**: *Allison 2394* 30 Mar 1985 (GA210082); **Tallapoosa**: *Allison 2461* 13 Apr 1985 (GA210085); **Georgia: Butts**: *Allison 2169* 13 May 1984 (GA063691); **Columbia**: *Pryon and McVaugh 2524* 10 Apr 1938 (GA063726); *Kral 57559* 10 Apr 1976 (ANHC003218); *Massey 4542* 6 Apr 1975 (NCU00023234); **Early**: *Faircloth 8739* 31 Mar 1984 (GA063700); *Thorne and Muenscher 7976* 4 Apr 1948 (GEO10727); **Fulton**: *Allison 2110* 5 May 1984 (GA063702); **Glascock**: *Hazelbaker 16* 14 Mar 2018 (GA264612); **Greene**: *Weaver 1888* 12 Apr 1969 (DUKE10106408); **Hancock**: *Kral 57654* 11 Apr 1976 (TENN-V-0182460); **McDuffie**: *Allison 2809* 6 Apr 1987 (GA063722); **Monroe**: *Howel 1160* 8 Apr 1988 (USCH0054083); **Oglethorpe**: *Allison 771* 14 Apr 1979 (GA083025); *Blake and Montgomery s.n.* 15 Apr 1965 (DUKE10106409, FSU000099918, FUGR0012385, GA063709, HUH, NCSU00091316); *Boulware 46* 20 Apr 1968 (CLEMS00051477); *Duncan 7541* 14 May 1947 (GA063676); *Jones 933* 28 Apr 1962 (FSU99988, MISS0073947, VSC0028291); *Montgomery s.n.* 7 Apr 1965 (FSU000099909, GA063723, NCU00023240, TENN-V-0182488); *Pryon and McVaugh 2448* 4 Apr 1938 (GA083826) (ISOTYPE); *Treiber and Nesom 1516* 7 May 1978 (NCU00023239); *Weaver 1886* 12 Apr 1969 (DUKE10106411); *Wilbur 3013* 22 Apr 1953 (FSU000099910, GA063716); **Pike**: *Pryon and McVaugh 2296* 23 Mar 1938 (DUKE10106412); **Richmond**: *Demaree 57727* 28 Apr 1968 (GA063696); **Rockdale**: *Leonard 7314* 24 Apr 1979 (NCU00023242); **Warren**: *Murdy s.n.* 24 Apr 1964 (GEO20455); **South Carolina: McCormick**: *Batson s.n.* 23 Apr 1963 (USCH0054087); *Boufford 12831* 13 Apr 1974 (CM251422); *Knight 208* 15 Apr 1973 (NCU00289787); *MacDougal 132* 16 Apr 1978 (NCU00289789); *Radford 22436* 11 May 1957 (NCU00439022); *Radford 31708* 11 Apr 1958 (NCU00023031, NCU00439023); *Radford s.n.* 11 Apr 1958 (EKY31234100488160); *Sawyer 728* 18 Apr 1964 (USCH0054086)

***Phacelia dubia* var. *interior*. U.S.A. Tennessee: Wilson**: *Deam 61295* 14 Apr 1941 (DUKE1010416); *Kriebel 9230* 14 Apr 1941 (DUKE1010418)

***Phacelia dubia* var. *rionensis*. U.S.A. South Carolina: Allendale**: *Bradley 2917* 31 Mar 2015 (USCH0015738*); *Buker s.n.* 27 Mar 1961 (CM146335); *Civitello 060* 22 Apr 2001 (USCH0015742*); *Fairey et al. 788 78* 18 May 1978 (CLEMS0051454); *Glowenke 10520* 28 Mar 1948 (HUH); *Horn 3811* 12 Mar 1990 (NBYC010292*, USCH0054088); *Horn 3828* 12 Mar 1990 (CLEMS0075137*); *Mathews et al. s.n.* 13 Apr 1996 (UNCC_32200*); *Nelson, Bennett, and Allen 5200* 27 Mar 1987 (CLEMS0075136*, DUKE10106399, FSU000099906, USCH0054085); *Nelson and Moffatt 7316* 31 Mar 1989 (USCH0054067*); *Nelson, Pittman, and Rayner 17093* 13 Apr 1996 (HUH, USCH0054089*); *Nelson 30239* 9 Mar 2012 (USCH0015739*); *Nelson and Bradley 38117* 10 Mar 2017 (HUH, USCH0042246*); **Bamberg**: *Horn 22504* 21 Mar 2017 (NBYC000062*); *Kilpatrick s.n.* 18 Mar 2018 (SALK0000519*); *Nelson and Meyerink 38193* 23 Mar 2018 (USCH0045324*); *Nelson and Meyerink 38194* 23 Mar 2018 (USCH0045325*); **Cherokee**: *Newberry 9838* 25 May 1989 (USCS0014686, USCS0014687*); **Chester**: *Nelson and Borg 6370* 8 Apr 1988 (USCH0054071); *Nelson 38251* 6 Apr 2018 (USCH0042245*); **Fairfield**: *Allison 1355* 19 Apr 1980 (GA210090*, NCU00439024*); *Allison 1385* 19 Apr 1980 (GA210089); *Grant 509* 28 May 1997 (USCH0015740); *Horn 15125* 12 Mar 2004 (NBYC010288*); *Horn 22553* 10 Feb 2017 (NBYC000117*); *Nelson 2063* 2 May 1982 (FSU99912*); *Nelson and Borg 6368* 8 Apr 1988 (USCH0054065); *Nelson, Maybin, and Ray 5164* 11 Feb 1987 (DUKE10106400, USCH0054068); *Radford 43484* 15 Apr 1961 (NCU00439019*); **Kershaw**: *Allison 2693* 8 May 1986 (GA210087); *Kenaleczcyke s.n.* 4 Apr 1974

(USCS0014689); *Levy s.n.* 25 May 1986 (DUKE10106401); *Nelson and Ferral 25335* 29 Apr 2005 (USCH0015741*); *Newberry 266* 7 Apr 1957 (GA153175*); *Newberry 3471* 8 Apr 1984 (USCS0014688); *Radford 20889* 7 Apr 1957 (NCU00439020*); *Rayner 1245* 17 Apr 1981 (USCH0054069, USCH0054070); *Westphal 16* 10 Apr 1984 (USCH0054066*); **Lancaster**: *Ahles 23082* 15 Apr 1957 (NCU00439021, NCU00023029, NCU00023030*); *Allison 3121* 25 Apr 1988 (GA210088*); *Bozeman 8810* 15 Apr 1967 (NBYC010290); *Matthews* 20 Apr 1965 (Emory16153*); *Swails s.n.* 14 Apr 1981 (FMUH0004282); **Laurens**: *Horn 20656* 24 Apr 2014 (CLEMS0060426*, NBYC010287*); **Newberry**: *Horn 1643* 11 May 1987 (NBYC010293); *Horn 14447* 2 May 2003 (NBYC010289); **Union**: *Allison 2707* 9 May 1986 (GA210091*); *Meyer 54* 3 Apr 1990 (USCS0014690); *Newberry 1217* 13 Apr 1982 (USCS0014692*, USCS0014693); *Newberry 2423* 5 May 1983 (USCS0014691, USCS0014694*); *Tremmel TREM 195* 2 Apr 1982 (CLEMS0075138*); **York**: *Allison 2702* 9 May 1986 (GA210092* PARATYPE); *Bradley 4022* 11 Apr 2016 (USCH0015737*, USF295721 PARATYPES); *Newberry 6244* 9 May 1986 (USCS0014695); *Levy s.n.* 13 Apr 1987 (DUKE10106112* HOLOTYPE); *Levy s.n.* 15 May 1987 (DUKE10106406* PARATYPE); *Levy s.n.* 15 May 1987 (DUKE10106402*); *Levy s.n.* 15 May 1987 (DUKE10106404* PARATYPE); *Levy s.n.* 13 Apr 1987 (DUKE10106405*); *Newberry and Hague 6244* 9 May 1986 (USCS0014696* PARATYPE); *Schmidt 1641* 4 Apr 2002 (WINU0000814); *Schmidt 1669* 4 Apr 2002 (WINU0001519); *Schmidt 1723* 18 Apr 2002 (WINU0001518)