ABSTRACT

BIOSYSTEMATICS OF THE *PHACELIA RANUNCULACEA* COMPLEX (HYDROPHYLLACEAE)

by Matthew Sewell

Phacelia ranunculacea sensu lato (subgenus *Cosmanthus*, Hydrophyllaceae) occurs on floodplains and in alluvial hardwood forests throughout eastern North America. It is comprised of herbaceous annuals with alternate, pinnate leaves and white to lavender, tubular-campanulate flowers in terminal scorpioid-like cymes. The distribution of populations displays a disjunct between the Midwest and Atlantic coastal plain, with a chromosome number of $n=6$ associated with the former and $n=14$ allied with the latter. A phenetic analysis of morphological characters was conducted using 350 herbarium specimens examined from 18 herbaria. The resulting phenogram revealed two distinct clusters of specimens on which haploid chromosome number was mapped. Correlation of the clusters and chromosome counts with type specimens resulted in recognition of two taxa: *Phacelia ranunculacea sensu stricto* and *Phacelia covillei*. Results of morphological analyses, a diagnostic key, species descriptions, and lists of representative specimens are provided for these two closely related species.

BIOSYSTEMATICS OF THE *PHACELIA RANUNCULACEA*
COMPLEX (HYDROPHYLLACEAE)

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INTRODUCTION:

The genus *Phacelia* is the largest and most diverse member of the family Hydrophyllaceae. Jussieu (1789) first described *Phacelia* and included it with *Hydrophyllum* and *Ellisia* in the “Ordo Boraginae” (=family Boraginaceae *sensu lato*). Brown (1810) moved the genera into the new order “Hydrophylleae”, segregating it from the “Boraginae” due to lobed leaves, capsular fruits and cartilaginous endosperm. Asa Gray (1875) produced a single large family by merging what then had become Hydrophyllaceae (Lindley, 1836) with genera previously assigned to the Hydroleaceae. Baillon (1890) placed the Hydrophyllaceae within the Boraginaceae until Brand (1913) again elevated it in rank in the most recent treatment of the entire family. Contrasting classifications by Thorne (1976) and Cronquist (1981), the former aligning the Hydrophyllaceae with the Boraginaceae and the latter placing it closer to the Polemoniaceae, illustrate the difficulties in understanding its familial relationships. Despite the differing taxonomic ranks and relationships assigned to members of the Hydrophyllaceae it is generally accepted that they are characterized by sympetalous, pentamerous flowers arranged in helicoid cymes, and are separated from the Polemoniaceae and Boraginaceae by pistil and fruit characters (Cronquist, 1981). Preliminary cladistic analyses of DNA sequence data suggest that the Hydrophyllaceae is not monophyletic when the circumscription includes the genera *Hydrolea* and *Codon* (Ferguson, 1999).

Phacelia is a large and polymorphic assemblage of 150-200 herbaceous annuals and perennials native to the New World (Constance & Chuang, 1982). The genus is best represented in the western United States and northern Mexico, with a few species extending into South America, and others east of the Mississippi River in North America. It is no surprise, given its size and diversity, that *Phacelia* also has the greatest range in chromosome numbers within the Hydrophyllaceae, with haploid counts ranging from $n=5$ to $n=24$. The most common haploid number is $n=11$, found in three times as many taxa as the next most prevalent counts, $n=9$ and $n=12$ (Constance, 1963). In spite of the diversity exhibited by the genus, *Phacelia* constitutes a cohesive group with a circumscription that has had few major changes in the last 150 years. According to *ndhF* sequence data, there is strong support for the monophyly of *Phacelia* (Ferguson, 1999).

Indeed the genetic evidence seems to correlate well with several morphological characteristics of the ovules, seeds, and corolline appendages (intrastaminal scales or glands), features that have historically been used to outline *Phacelia*. Genera such as *Eutoca*, *Microgenetes*, *Cosmanthus*, and *Euphacelia* were submerged under *Phacelia* by Gray (1875), and have served as a basis for more recent subgeneric classifications. These subgeneric classifications were based largely on ovule characteristics and are contradicted by chromosome counts. The classification outlined by Gray (1875), and later adopted by Bentham and Hooker (1876), in addition to those of Brand (1913) and Cronquist et. al. (1984) were based largely on the number and orientation of the ovules and seeds. Constance (1963) argued for a framework based on chromosome number, proposing three subgenera: *Cosmanthus*, *Howellanthus*, and *Phacelia*, based on haploid numbers $n=9$, 8, and 11 respectively, with aneuploid and polyploid series found in *Cosmanthus* and *Phacelia* ranging from $n=5$ to $n=24$. He added:

All the groupings mentioned show considerable internal consistency in vegetative and floral morphology, geographical and ecological distribution, and in cytology, but cut across the traditional arbitrary divisions based on number of ovules. A reduction in ovule number, in fact, appears to have occurred independently in several of the groups.

Phacelia ranunculacea (Nutt.) Constance was included in the subgenus *Cosmanthus* in the most recent revision of the group (Constance, 1949). The subgeneric arrangement established by Constance essentially followed Brand's (1913) section *Cosmanthus*, except for the inclusion of a biennial, *P. bipinnatifida*, in this group of annuals and perennials. Subgenus *Cosmanthus* is an aggregate of 18 species, with an additional 7 infraspecific taxa, and is separated from other subgenera in *Phacelia* based on distributional, morphological, and cytological characteristics (Constance, 1949; Turner, 1991).

The collective distributions of species within *Phacelia* subgenus *Cosmanthus* suggest a northeastern migration from Mexico and Guatemala (five species) to Texas and Oklahoma (three species), to the Ozarks (three species), finally to the Appalachians (six species more or less east and west of the mountains) (Constance, 1949). One character

unique to *Phacelia* subgenus *Cosmanthus* is the number and distribution of ovules amongst the taxa. A third of the species have 6-14 ovules, another third have 4 ovules and the remaining third have 2 ovules. The distribution of ovules across subgenus *Cosmanthus* correlates with the geographic distribution of the taxa, in which the higher ovulate species occur in the southwest and those with fewer ovules are found in the northeast. Therefore there appears to be a trend toward a reduction of ovules to a pair per placenta, in contrast to the consistent number of 4 per placenta observed in other subgenera of *Phacelia* (Constance, 1949). Another character separating the members of subgenus *Cosmanthus* from the rest of *Phacelia* is a lack of the corolla scales found bracketing the base of each stamen and adnate to the corolla tube. Subgenus *Cosmanthus* has an altogether different organ that has been confused with corolla scales. The structure consists of a gland (nectary) or its vestigial remnants found between the stamens, extending distally from the base of the corolla tube to the middle of each corolla lobe and bordered lengthwise by two parallel flaps (Constance, 1949). Cytological investigations of subgenus *Cosmanthus* reveal the absence of a chromosome number of $n=11$, the most common complement found in the remainder of *Phacelia* (Constance, 1949). Of the eleven species with published chromosome data (Cave & Constance 1947, 1950; Constance 1949, 1950, 1963; Gillett 1964, 1968; Murdy 1966) seven species have a haploid number of nine ($n=9$), with $n=5$, 8, and 14 also present in the subgenus. Constance (1963) hypothesized $n=9$ was the ancestral complement for the subgenus because it agreed with his notion that less “highly evolved” perennials provided the “evolutionary stock” for each of the three subgenera.

Just as subgenus *Cosmanthus* is unique amongst *Phacelia*, anomalies in chromosome number, morphology, and distribution have led Gillett (1968) to place *P. ranunculacea* in a class (Group I) by itself and even predicted that it “ultimately will be recognized as a monotypic genus”. Characters such as a particularly small, tubular-campanulate corolla, spherical to near-spherical seeds (i.e. “keelless”), and filaments without hairs distinguish *P. ranunculacea* from the rest of subgenus *Cosmanthus*. Although the corolla glands are apparently functional as nectaries only in *P. bipinnatifida* (Constance, 1949), the adjacent parallel flaps are present in all species of the subgenus. However, in *P. ranunculacea* the parallel flaps (i.e. “nectar flaps”) are often barely

visible, the position of the aborted gland only faintly indicated by two minute ridges at the very base of the corolla tube (Constance, 1949).

Phacelia ranunculacea is found on moist river alluvia in well-shaded habitats of bottomland hardwood forest (Gillett, 1968). Many of these sites undergo seasonal flooding, an adaptation to which is most unusual for a taxon in *Phacelia*. Nuttall (1837) first described *Phacelia ranunculacea* as *Ellisia ranunculacea* Nutt. from the “shady humid alluvial forests of the Arkansas”. Nuttall’s collection is mixed, containing both *Ellisia ranunculacea* Nutt. and *Ellisia microcalyx* Nutt., which led Gray (1875) to consider the names synonymous. As a result the name *Nemophila microcalyx* (Nutt.) Fisch. & Mey. was misapplied to what he thought was one species. Watson (in Gray et. al., 1890) described a strikingly similar species named *Phacelia covillei* S. Wats., found on an island in the Potomac River near Washington, D.C. The synonymy of *P. covillei* and *E. ranunculacea* was later postulated by Constance (1940).

The geographic distribution of *P. ranunculacea* is not only unique amongst subgenus *Cosmanthus* but also compared to almost all other eastern North American taxa by exhibiting populations disjunct in the Midwest and the Atlantic coastal plain (Harvill, 1969) (Figure 1). Populations are scattered, with an apparent separation between Maryland, Virginia, Washington, D.C., and North Carolina east of the Appalachians, and Ohio, West Virginia, Indiana, Illinois, Kentucky, Tennessee, Missouri, Arkansas, and Mississippi west of the Appalachians. *Phacelia ranunculacea* is not federally listed as rare or endangered, but it has been accorded conservation status that varies according to each individual state. The species is “endangered” in Maryland (M.N.H.P., 1988), Indiana (Bacone and Hedge, 1979) and Ohio (O.D.N.R., 2002), “primary proposed” in North Carolina (Sutter et. al., 1983), and recognized in Kentucky (K.S.N.P.C., 2000) and Tennessee (Somers, 1989) as a species of “special concern”.

The chromosome first count for *P. ranunculacea* s.l. ($n=14$) was obtained from material along the Potomac River near Washington, D.C. (Constance, 1949), and later confirmed by a count from a population in central North Carolina (Chuang & Constance, 1977). Despite the attractive possibilities of synthesizing *P. ranunculacea* from others within *Cosmanthus*, for example *P. maculata* or *P. dubia* ($n=5$) with one of the $n=9$ species, it is difficult to explain the morphological characteristics that make *P.*

ranunculacea such a distinctive plant. Further complicating the matter is material collected in the 1960's and 1970's from Kentucky, Illinois, Tennessee and Missouri, in which the counts showed a haploid chromosome number of six ($n=6$) (Chuang & Constance, 1977). For the last quarter century *P. ranunculacea* has been considered a widespread taxon with "eastern" ($n=14$) and "western" ($n=6$) cytotypes.

The purpose of this study is to reexamine the identities of the "eastern" and "western" populations of *Phacelia ranunculacea* s.l., with particular attention paid to populations not sampled by Chuang and Constance (1977) such as those in Ohio, West Virginia, and Indiana. The concept of a single, widespread, cytologically and morphologically diverse taxon is put forward as the null hypothesis. An alternative hypothesis proffered is that what has been historically been called *Phacelia ranunculacea* (Nutt.) Constance represents at least two reproductively and geographically isolated entities. The primary goal of this study is to discern whether any morphological characters consistently correlate with the different chromosome numbers in these disjunct groups of populations. If any correlation can be found between morphological features and chromosome numbers then a clear definition of natural biotic units can be described, and the appropriate nomenclature applied. To this end, aspects of floral and vegetative morphology were measured and analyzed phenetically via several statistical techniques. Additional chromosome counts were obtained from populations without cytological data.

MATERIALS AND METHODS:

This study compared both the macro and micro-morphological features of specimens attributed to *Phacelia ranunculacea* (Nutt.) Constance *sensu lato*. Herbarium sheets were examined from the following herbaria: BRIT/SMU/VDB, EKY, F, GMUF, ILL, IND, KNK, KY, NCU, NY, MO, MU, TENN, UC/JEPS, US, VPI, WKU, and WVA. Out of approximately 350 specimens, 331 were of significant quality that they contained enough measurable vegetative features for analysis. A subset of 55 of the original 331 sheets with adequate floral material was scored for 17 characters. Most specimens contained more than one plant and consequently each individual served as a separate operational taxonomic unit (OTU). Every OTU was assigned a two-letter and four-digit abbreviation as a label to identify it during data analysis (see Appendix B). The letters indicate the state and the first two numbers denote the population from which the specimen was collected. Duplicate specimens, as well as collections made by others at different times, were given the same population number if locations could be precisely correlated using herbarium label data and verified in the field. Assignment of population codes was conservative and any doubt resulted in the application of different numbers. The last two numbers refer to an individual plant. Often measurements were taken from more than one plant on a sheet, resulting in multiple OTU codes for the same specimen. This was especially true for many chromosome vouchers and sheets with flowering material.

Thirty-seven morphological characters were scored for each OTU (Table 1; Figures 2 & 3). The data were grouped into floral, vegetative sets, and a combined dataset. Twenty-two quantitative characters (13 floral and 9 vegetative) were coded as continuous data. Fifteen qualitative characters (11 vegetative and 4 floral) were coded as discrete values, whether the values were binary or multistate positive integers. All sheets used in the morphological analysis were fertile, and sheets used to measure floral characters had six or more “dissectable” flowers. Care was taken to dissect flowers that were approximately the same age in terms of phenology. The character states were recorded as follows (character number in parentheses):

Terminal leaf (uppermost leaf on a stem, directly subtending an inflorescence): Terminal leaflet length (1) and width (2) at the widest point were recorded (Figure 2). Terminal

leaflet width/length ratio (3) was calculated to describe the overall shape of the leaf and to minimize any differences in individual length and width measurements related to leaf maturity and/or the micro-environment effects (i.e. larger leaves in shade). Laminar basal angle (4), to the nearest degree, was recorded (Figure 2). The total number of leaflets was recorded (5). The number of lobes per apical (6) and basal (7) lateral leaflet was recorded (Figure 2). Petiole length (8) was measured, as well as overall indument (9) (0=glabrous; 1=pubescent), and presence or absence of glandular trichomes (10). Petiole width was measured but not entered in the data set due to the varying degrees to which it was folded over on itself, an artifact of pressing. The number of secondary sinuses of the terminal leaflet (11) was tallied. Secondary sinuses were defined as those other than the two primary sinuses formed by the three main lobes of the terminal leaflet. The position and number of veins of the terminal leaflet were examined but not entered in the data set because of an inability to objectify the measurements. This was due to differing levels of maturity of each specimen (veins were obscured in smaller/younger leaves) and micro-environmental factors in habitat that affect leaf size and thickness (veins were more easily distinguished in larger/thinner leaves).

Stem: The stem indument was characterized by describing the differing aspects of the trichomes found along the middle portion of the stem. The section of the stem above the basal rosette of leaves and below but not immediately subtending an inflorescence, corresponding to an area between the second and sixth nodes from the base of the plant, was evaluated as to the type and arrangement of simple, unicellular hairs (12) and glandular trichomes (13). Primarily strigose, with only a few spreading or retorse hairs, was coded as zero. Conversely, straight hairs mostly spreading or pilose, rarely appressed or ascending, was entered as one. Moniliform shaped trichomes with stipitate/capitate glands [a combination of terms from Figure 3.12 in Walters and Keil (1975) and Figure 7.7 in Radford et. al. (1974)] were coded as zero for sparse and short (all <2mm) or one for dense and long (most >2mm).

Leaf surface: The surface vestiture of an upper leaf on the stem was examined. The particular leaf chosen was neither terminal on the stem nor directly subtending an inflorescence. The adaxial (14) and abaxial (15) sides were coded as either glabrous or

pubescent to describe the overall indument. The presence or absence of glandular trichomes on both the adaxial (16) and abaxial (17) surfaces of the leaf was also noted.

Inflorescence: The primary peduncle (18) of the scorpioid cyme was scored as glabrous or pubescent. More specifically the type of pubescence was noted by the presence or absence of glandular trichomes (19). The pedicel indument (20) of the terminal flower on the inflorescence was coded as glabrous or pubescent. Glandular trichomes on the pedicel (21) were scored as present or absent.

For floral measurements, each flower was removed and soaked in 1% Aerosol OT for several minutes to soften the tissue for dissection. The corolla tube was split along one side with a razor blade and removed from the calyx and pistil as one piece. The corolla tube was spread flat on a microscope slide with the inner portion of the corolla tube facing up in order to view the epipetalous stamens. All the floral measurements were made with an ocular micrometer on a Wild model M3 dissecting microscope.

Calyx: The length (22) and width (23) of one sepal was measured (Figure 3). The indument of the sepal (24) and the presence or absence of glandular trichomes (25) was noted. The overall length (26) and the length of the division of the bifid style (27), or style cleft length, were recorded (Figure 3). To quantify the degree to which the style was divided, a ratio of style length to style cleft length (28) was calculated. The indument of the ovary (29) was noted as glabrous or pubescent.

Corolla: Corolla length (30) was measured from the base of the corolla tube to the tip of one of the corolla lobes (Figure 3). Corolla lobe width (31) and corolla lobe length (32) were also recorded. A ratio of the corolla lobe width and corolla lobe length (33) was calculated to describe the overall proportions of the corolla lobe. Filament length (34) was measured from the point of insertion on the corolla tube to the base of the anther. Anther length (35), anther width (36), and filament indument (37) were also recorded. Nectar flap length refers to the parallel flaps of tissue found at the base of the corolla tube (Figure 3).

Data for all variable characters (see Table 1) per OTU were entered into a Microsoft Excel spreadsheet. Initial exploration of the data was accomplished by

constructing histograms for each character (Appendix A). Characters with distributions that appeared bimodal were used to construct bivariate scatter plots to investigate any character correlation patterns. The Excel spreadsheet was imported into NTSYS-pc 2.1 (Rohlf, 2002) for phenetic analysis. A linear transformation of the measurements was performed to standardize the data thus minimizing the effects of the different scales of measurement for some characters. The similarity interval command SIMINT (correlation coefficient code CORR) was used to build a separate similarity matrix for each dataset. The similarity matrices used in the principle components analyses (PCA) were computed by columns, which corresponded to the characters (variables). Eigenvalues and eigenvectors were calculated and plots of the OTUs against the first two principle components were made. The sequential, agglomerative, hierarchical and nested clustering method (SAHN), using the default unweighted pair group method of arithmetic averages (UPGMA), was used to produce phenograms based on the similarity matrices calculated by OTUs (i.e. rows). Cophenetic values, or coefficients of correlation (r), were computed to test how well the phenogram represented the original similarity matrix. The variation in the data explained by the character-loadings for each eigenvector, as illustrated by the first three principle components, and the distributions shown by the histograms were used as a basis for including or excluding characters in successive clustering analyses. Alternatively, characters that displayed a normal distribution and exhibited a low amount of variability according to the eigenvector character-loadings were not regarded as informative and excluded from subsequent analyses because they did not contribute to the separation of OTUs into clusters. The above procedure was repeated for each of the three sets of data and the phenograms were compared. Pairwise comparisons of all characters were conducted in Microsoft Excel using a t-test (two sample-assuming unequal variances) to check for significant differences between the groups of OTUs outlined by the SAHN clustering module.

Chromosome counts were performed on material from populations in the following counties visited during the spring of 2001-2003: Lawrence (Ohio), Wayne (Missouri), and Pike (Indiana) (see Appendix B for location data for all populations visited by the author). Flower buds and seeds were harvested from the field to provide material for counts. Seeds were grown in the Miami University greenhouse to harvest

additional flower buds or root tips. Flower buds and root tips were fixed in a saturated solution of paradichlorobenzene (PDB) for approximately 4-6 hours. The material was transferred to Farmer's solution (3:1; ethanol: glacial acetic acid) for long-term storage. Meiotic counts of anther material followed Belling's Iron-acetocarmine protocol (Johansen, 1940). Mitotic counts of root tip material followed a generalized acetocarmine staining procedure (Sass, 1958). Root tips were hydrolyzed in 1N HCl, rinsed in 95% ethanol, stained with acetocarmine, and destained in glacial acetic acid. Both meiotic and mitotic material was placed on a microscope slide under a cover slip, spread out with a pencil eraser and examined with an oil emersion lens of an Olympus BH-2 light microscope. Specimens collected by the author that correspond to chromosome counts are populational vouchers. Populations with counts, including those performed by others in the past, were mapped onto the phenogram and scatter plots of morphological data for comparison.

RESULTS:

Characters that were not only variable but that could also separate OTUs into groups were deemed as informative; the establishment of which was needed to help evaluate any connection between morphology and chromosome number. The first step in determining the most informative characters was the construction of histograms. Histograms revealed that the distribution of the terminal leaflet basal angle was the most distinctly bimodal of all the characters measured. Some degree of bimodality was seen in two other vegetative characters (3.terminal leaflet length and 5.terminal leaflet length/width ratio) and two floral characters (24.corolla lobe width and 33. corolla width/length ratio) (see Appendix A). Scatter plots of terminal leaflet basal angle versus each of the above characters were made to ascertain if any correlations could be found. The resultant plots showed two clusters of OTUs (Figures 4 & 5). However, out of all the characters, most of the separation was accounted for by the terminal leaflet basal angle. Plots containing characters that represented the proportional shapes of the corolla lobes and terminal leaflets as ratios formed the tightest clusters (Figures 4B & 5A). Mapping the presence/absence stem pubescence data onto the scatter plots reinforced these clusters. The presence/absence stem pubescence data displayed a direct correlation with the two clusters (Figures 4 & 5). Appressed hairs and small/sparse glands corresponded strongly with wider than long corolla lobes, longer than wide terminal leaflets and acute terminal leaflet bases. Conversely, spreading hairs with large/dense glands displayed a match with obtuse terminal leaflet bases, as well as terminal leaflets and corolla lobes with dimensions closer to a 1:1 ratio. Simple hairs and glandular trichomes were scored as separate characters; in no case were plants observed that combined appressed hairs and large/dense glands or spreading hairs with small/sparse glands.

Principle components analysis (PCA) was used to investigate variability in the data and illustrate how the variation was partitioned among each character. Exploration of the floral and vegetative dataset revealed 30.75% of the total variation was accounted for by the first principle component (PC I), with 15.94% and 10.26% of the variation represented by the second (PC II) and third (PC III) principle components respectively (Table 5). An ordination plot of OTUs against the first two principle components (Figure 6) reflected the same morphological separation observed in the bivariate scatter plots.

Cluster analysis of all the floral and vegetative characters produced a phenogram with two groups of OTUs that corresponded to the separation portrayed by the ordination and bivariate scatter plots. The correlation coefficient ($r = 0.83$) calculated from the phenogram based on all the floral and vegetative characters depicted a moderate to good fit of the cluster analysis in terms of representing the original similarity matrix. The next step was to ascertain which, if any, of the characters were more responsible than others in the formation of the two groups of OTUs observed in the floral and vegetative phenogram.

Discerning which were the most informative characters was facilitated by the considerable agreement amongst characters that represented most of the variation and those with bimodal distributions. According to the eigenvector character-loadings, two floral (24.corolla lobe width and 31.sepal length) and three vegetative characters (6.terminal leaflet basal angle, 10.midstem pubescence, and 11.midstem glandular trichomes) contributed most of the variation as expressed by PC I (Table 4). None of the characters appeared to add an appreciable amount of variation to PC II and PC III relative to the first principle component. Although the terminal leaflet length/width ratio and terminal leaflet length exhibited some bimodality, according to PCA neither was appreciably variable. On the other hand, sepal length accounted for a significant amount of variation in PC I (Table 4), yet the character distribution was normal across all OTUs. Corolla lobe width/length ratio, the variable with the sixth highest character-loading according to PCA, had a bimodal distribution and thus was substituted for sepal length in the cluster analysis of the five most informative characters. The resulting phenogram (Figure 8) produced the same separation in OTUs produced by the ordination projection and scatter plots. In addition, the clusters were tighter and correlation coefficient depicted an extremely good fit ($r = 0.97$) compared to the results of clustering on all floral and vegetative characters.

An ordination plot based on a principle component analysis of just the vegetative characters (Figure 7) revealed the same separation in clusters as the plot constructed from the floral and vegetative dataset (Figure 6). The eigenvector character-loadings pointed to the high degree of variation in terminal leaflet basal angle, midstem pubescence and midstem glandular trichomes. These terminal leaflet and stem characters were the same

three most variable vegetative characters indicated by the PCA of the floral and vegetative dataset, and had character-loading values were almost identical. A phenogram (Figure 9) based on the dissimilarity matrix of these characters duplicated the same separation in clusters as the clustering analysis of the five most informative floral and vegetative characters. Principle components analysis of only floral characters failed to demonstrate any separation in OTUs. Furthermore, clustering analyses on all character permutations produced phenograms that were discordant with those generated by the vegetative and floral/vegetative datasets.

Meiotic chromosome counts from populations in Lawrence County, Ohio and Pike County, Indiana revealed a haploid complement $n=14$. Individuals from Lawrence and Pike counties exhibited a morphology consistent with specimens from North Carolina, Virginia, Maryland, Washington, D.C., Illinois, and Missouri (Figures 8 & 9). A haploid complement $n=6$ was found from a population in Wayne County, Missouri. Morphological analysis of the Wayne County material reflected a similarity with other populations in Missouri as well as those in Arkansas, Kentucky, Tennessee, Illinois, Mississippi, and Indiana (Figures 8 & 9). Vouchers supporting previously reported chromosome counts were highlighted in each of the phenograms and correlate precisely with the morphological differences.

The bivariate scatter plots, ordination plots, and phenograms demonstrated the same phenotypic differences between the two groups of OTUs. Just as different chromosome numbers were linked to the differing morphologies in the phenograms (Figures 8 & 9), the same trend was observed when mapped onto the ordination plots of OTUs against the principle components (Figures 6 & 7). In addition to the clusters in all the figures being commensurate, the individual OTUs were equivalent as well. Thus, the solid bar in the phenograms, the darkened triangles in the ordination plots and the darkened triangles in the scatter plots denote the same OTUs. The reverse is also true concerning OTUs depicted by the striped bar and the open circles. Accordingly, the clusters in the scatter plots can also be defined in terms of chromosome number. The per character averages distributed by group can be found in Tables 2 & 3.

DISCUSSION:

A clearer picture of the specific characters that differentiate the groups of OTUs was achieved by comparing the results of PCA and clustering analyses between different datasets. The dataset of vegetative characters produced an ordination plot similar to that of the dataset of both floral and vegetative characters (Figures 6 & 7), whereas a plot using only floral characters had no discernable clusters. Similarly, the phenograms constructed using the vegetative and floral/vegetative data were congruent (Figures 8 & 9), while the clustering on only floral characters produced a phenogram without any cohesive clusters. Thus, the influence of the vegetative characters, particularly attributes of the stem pubescence and terminal leaflet bases, factored predominantly in the separation of OTUs. In fact, these same characters delimit two other species in subgenus *Cosmanthus*. *Phacelia purshii* Buckl. and *P. fimbriata* Michx. were circumscribed based on the arrangement of hairs along the stem and the angle of the lobes on the cauline leaves (Constance, 1949). That is not to say the floral characters were altogether uninformative in *P. ranunculacea* s.l. On the contrary, nine of the 13 floral characters showed a significant difference between the groups outlined by the scatter plots, ordination projections and phenograms (Table 5). Although differences in the floral characters suggest the existence of two separate, biological entities, the usefulness of such characters in a taxonomic framework is restricted. For example, the average style length between the two cytotypes is statistically different, but the distinction between 1.32 and 1.51mm is so small as to be useless in a diagnostic key. The same is true of the vegetative characters where seven of nine are significantly different according to two sample t-tests (Table 5). Although individuals with the “eastern” cytotype had fewer secondary sinuses and more total leaflets per upper cauline leaf compared to the “western” cytotype, distinguishing 1.02 versus 1.59 sinuses and 2.99 versus 2.78 leaflets is impractical. The obvious differences in terminal leaflet basal angle, the lowest p-value among all characters in Table 5, combined with the clear distinction in stem pubescence presence/absence data, demonstrate the usefulness of these three vegetative characters in a diagnostic key. Therefore, appressed hairs with small/sparse glands, acute terminal leaflet bases, and a $n=14$ chromosome number indicate a single entity. Similarly,

spreading hairs with large/dense glands, obtuse terminal leaflet bases, and a haploid complement $n=6$ suggest a separate taxon.

The null hypothesis of this study was that the specimens represented one widespread species, *Phacelia ranunculacea sensu lato*. The alternative hypothesis was that the study sample represented two species, *P. ranunculacea s.s.* and *P. covillei*. Based on differences in morphology and chromosome number, the null hypothesis is rejected and the alternative hypothesis is accepted. In conclusion, the validly published epithet *Phacelia covillei*, a longtime synonym of *P. ranunculacea*, should be recognized as a separate taxon. Diagnostic keys, pertinent type information, species descriptions, phenology, biogeography, representative specimens and notes are provided in the Taxonomic Treatment below.

The placement of the *P. ranunculacea* complex both within *Phacelia* and the Hydrophyllaceae as a whole has been debated for years (Constance, 1949, 1977; Gillett, 1968). Gillett (1968) relied on chromosome number ($n=14$) and the unequal insertion of the stamen filaments on the corolla tube as evidence for suggesting a relationship with the genus *Nama*. The recent discovery of a chromosome number $n=6$ within *P. ranunculacea sensu lato* (Constance, 1977; this study), together with solitary flowers, simple leaves, and many ovules in *Nama* contradicts this idea. Moreover, only about 10% of the flowers dissected for this study had unequally inserted filaments on the corolla (Sewell, pers. obs.). Constance (1940, 1977) pointed out the superficial similarities that *P. ranunculacea s.l.* shares with *Ellisia nyctelea* L. and *Nemophila aphylla* (L.) Brummitt (= *N. microcalyx*) in terms of habit, habitat, and size and shape of the corolla. However, tribal distinctions within the Hydrophyllaceae, tribe Hydrophyleae (*Ellisia*, *Nemophila*) versus tribe Phacelieae (*Phacelia*), preclude placing *P. ranunculacea s.l.* in the former. *Ellisia* and *Nemophila* have corollas contorted in bud, swollen placentae, and a unilocular ovary. The corollas of *P. ranunculacea s.l.* are imbricate in bud and the two narrow placentae project into the ovary and partition it into two locules (without fusing). In addition, *E. nyctelea* has fimbriate corolla scales and solitary flowers whereas *N. aphylla* has cucullate seeds and auriculate calyx sinuses (Constance, 1939), all of which are absent in *P. ranunculacea s.l.* Thus, the nearest affinities are with *Phacelia*, and features of the corolla scales and ovules, as well as

chromosome number and distribution suggest a comfortable placement within subgenus *Cosmanthus*.

Taxonomic Treatment

Key to the species of *Phacelia ranunculacea* s.l.

- 1a. Pubescence along middle portion of stem (main axis of the plant above the basal leaves but not immediately subtending an inflorescence, more or less corresponding to the region between the 2nd and 5th nodes) sparse, of mostly appressed/strigose hairs, rarely spreading, with a few short (<2 mm) moniliform stipitate/capitate glandular trichomes; terminal leaflet of leaves directly subtending an inflorescence with acute (<90°) to cuneate or nearly attenuate bases.....1. *Phacelia covillei*
- 1b. Pubescence along middle portion of the stem of mostly spreading/pilose hairs, some retrorse, with long (most >2 mm) and dense moniliform stipitate/capitate glandular trichomes; terminal leaflet of leaves directly subtending an inflorescence with obtuse (> 90°) to rounded or almost truncate bases.....2. *Phacelia ranunculacea*

1. *Phacelia covillei* S. Watson ex A. Gray, Man. N. Amer. Bot. ed. 6. 360. 1890. TYPE: United States. Maryland: Montgomery County; 12 May 1889, *Coville s.n.* (lectotype, to be designated: GH! (two plants on the upper left of sheet); isotypes: KY!, US!)

Plants annual, (5-) 10-30 cm high at anthesis, simple or branching from near the base, the branches prostrate to erect. Stems strigose at base, the middle portions strigose, glandular with a sparse mixture of short (<2 mm) moniliform stipitate/capitate trichomes. Cotyledons petiolate, persistent at anthesis, ovate to orbicular, 0.5-5.0 cm long, 0.5-1.5 cm wide, tinged with purple, strigulose above, glabrous below. Basal leaves petiolate, pinnate, 3.0-8.0 cm long, (0.5-) 1.0-2.5 cm wide; lateral leaflets 2-6, paired, orbicular to oval, lobed or entire; terminal leaflet larger, obovate, trilobed, often with several smaller lobes. Cauline leaves petiolate, pinnate, (1.0-) 3.0-10.0 (-15.0) cm long, (0.5-) 1.5-3.0 cm wide; lateral leaflets 1-6, usually paired, orbicular to lanceolate, entire or with one to several smaller lobes; terminal leaflet larger, trilobed, often with several smaller lobes, lamina strigose on both surfaces; terminal leaflet of uppermost leaves with acute to cuneate or nearly attenuate bases. Inflorescence terminal, cymose, weakly scorpioid, 2.0-5.0 cm, 1-6 flowered, hirsutulous, glandular with moniliform stipitate/capitate trichomes; mature pedicels (5-) 8-15 mm long, spreading-reflexed to pendent. Sepals linear-lanceolate (2.5-) 3.5-5.0 mm long, 0.5-1.5 mm wide, subequal, acute, strigose on abaxial and adaxial surfaces, sparsely glandular with moniliform/capitate trichomes near base. Corolla pale violet, tubular campanulate, 3.0-5.0 mm long, 2.0-4.0 mm wide; limbs erect, 0.8-2.0 mm long, 1.0-2.6 mm wide, oblong to obovate, entire, glabrous; gland flaps inconspicuous, 0.25-0.45 mm long, reduced to two minute ridges at the very base of the tube. Stamens included, 1.0-2.5 mm long, anthers oblong, 0.3-0.65 mm long, 0.3-0.55 mm wide, intorse, filaments glabrous. Style included in flower, when mature 0.8-2.5 mm long, cleft from 1/3 to 2/3, glabrous; ovary hirsute, ovules 2 per placenta. Capsule depressed-globose, 4.0-6.0 mm in diameter when mature, markedly distended by the seeds; seeds 2-4, globose-ovoid, 2.0-2.5 mm long, brown, reticulate. Chromosome number: $n=14$ (Chuang & Constance 1977; Constance, 1949; this study).

Habitat and Distribution (Figure 13). *Phacelia covillei* is found on the shaded rich soils of floodplains and the adjacent slopes of alluvial woods. The range includes several disjunct areas, including the upper Potomac River drainage (Washington, D.C., northern Virginia, and south-central Maryland), drainages of the Cape Fear and Tar Rivers (central North Carolina), and parts of the Ohio River drainage (southeastern Ohio and western West Virginia). Weakly (2003) reports this species from the Roanoke River drainage in south central Virginia, though no specimens documenting this have been seen by the author. Another unconfirmed record for *P. covillei* has been reported from western Illinois in Adams county (Gleason, 1903). Excluding the populations in Ohio and West Virginia, the above distribution has been referred to as ‘Appalachian’ (Cronquist, 1988; Gleason & Cronquist, 1991). Even with the inclusion of the Ohio and West Virginia populations, the term would be appropriate save for the occurrence of several *P. covillei* populations in the mid-west. The drainage of the Wabash River in southern Indiana and southern Illinois has yielded several populations and another has been located in Texas County, Missouri. The identity of the populations in Texas County may be questioned without an accompanying chromosome count, nevertheless the morphology of plants from this population undoubtedly agrees with that of *P. covillei*. The basal angle measurements of the terminal leaflets range from 66° to 81° and the stem pubescence precisely matches the morphology specimens with a $n=14$ chromosome count.

Phenology. *Phacelia covillei* flowers in late March to mid April, and occasionally at the beginning of May in the northern part of the range, with fruit set immediately following. The seeds fall soon thereafter, upon desiccation and dehiscence of the capsule.

Notes. Several factors point to a need for lectotypification of *P. covillei*. Watson indicated neither a collection number nor a date in his protologue. Furthermore, the original sheet at the Gray Herbarium contains material of two collections, both made by Coville at the same locality but on different dates, 5 May and 12 May 1889. The word “type” is written in an unknown hand above the label with the 12 May date. Indeed, in his revision of subgenus *Cosmanthus*, Constance (1949) did specify the 12 May collection as the type for *P. covillei*. However, Constance was not explicit in his lectotypification, presumably since he subsumed *P. covillei* under *P. ranunculacea*. To add to the confusion, the material on the GH sheet appears to have been switched when

mounted, with what appears to be younger material associated with the label containing the later date. In fact the duplicate deposited at KY is also a mixed collection but with the younger material associated with the label giving the earlier date. Despite the aforementioned uncertainty the two plants above the 12 May label and associated with the word “type” will be designated as the lectotype as this seems the most conservative option and follows the precedent set by Constance.

Although *P. covillei* is rare, it is often locally abundant, and the author has found several populations for which there were no previous records. That is not to say that the conservation status afforded *P. covillei* (as *P. ranunculacea*) is unjustified. On the contrary, the microhabitat shade and moisture requirements seem very specific, resulting in concentrated populations with well-defined boundaries, sometimes occurring on only one side of a river. *Phacelia covillei* (as *P. ranunculacea*) had been categorized as “endangered” and “primary proposed” in Maryland (M.N.H.P., 1988) and North Carolina (Sutter et. al., 1983) respectively, but is absent any such designation in Virginia and West Virginia (see notes under *P. ranunculacea* for discussion of populations in Indiana, Illinois and Missouri).

2. *Phacelia ranunculacea* (Nutt.) Constance, Rhodora 42: 39. 1940. *Ellisia ranunculacea* Nutt. Trans. Amer. Philos. Soc. N. S. 5: 191. 1837. TYPE: United States. Arkansas, *s.d.*, *Nuttall s.n.* (lectotype, to be designated: BM; photo: UC!; isotype: K; photo: UC!, GH!)

Plants annual, (5-) 10-25 cm high at anthesis, simple or branching from near the base, the branches prostrate to erect. Stems strigose at base, the middle portion pilose, glandular with a dense mixture of long (>2 mm) moniliform stipitate/capitate trichomes. Cotyledons petiolate, persistent at anthesis, ovate to orbicular, 1.0-5.0 cm long, 0.5-1.5 cm wide, tinged with purple, sparsely strigulose above, glabrous below. Basal leaves petiolate, pinnate, oblong to ovate 3.0-10.0 cm long, (0.5-) 1.0-2.5 cm wide; lateral leaflets 2-6, paired, orbicular to oval, lobed or entire; terminal leaflet larger, obovate, trilobed, often with several smaller lobes. Cauline leaves petiolate, pinnate, (1.0-) 3.0-10.0 cm long, 1.0-4.5 cm wide; lateral leaflets 1-6, usually paired, orbicular to lanceolate, entire or with one to several smaller lobes; terminal leaflet larger, trilobed, often with several smaller lobes, lamina strigose on both surfaces; terminal leaflet of uppermost leaves with obtuse to rounded or almost truncate bases. Inflorescence terminal, cymose, weakly scorpioid, 1.5-5.0 cm, 1-6 flowered, hirsutulous, glandular with moniliform stipitate/capitate trichomes; mature pedicels 8-15(-21) mm long, spreading-reflexed to pendent. Sepals linear-lanceolate 2.0-5.0 mm long, 0.5-1.5 mm wide, subequal, acute, strigose on abaxial and adaxial surfaces, sparsely glandular with moniliform stipitate/capitate trichomes near base. Corolla pale violet to white, tubular campanulate, 2.5-5.5 mm long, 2.0-4.0 mm wide; limbs erect, 0.8-2.1 mm long, 0.8-2.0 mm wide, ovate to obovate, entire, glabrous; gland flaps inconspicuous, 0.2-0.4 mm long, reduced to two minute ridges at the very base of the tube. Stamens included, 1.0-2.5 mm long, anthers oblong, 0.25-0.5 mm long, 0.25-0.45 mm wide, intorse, filaments glabrous. Style included in flower, when mature 1.0-2.0 mm long, cleft from 1/3 to 2/3, glabrous; ovary hirsute, ovules 2 per placenta. Capsule depressed-globose, 4.0-6.5 mm in diameter when mature, markedly distended by the seeds; seeds 2-4, globose-ovoid, 1.8-2.8 mm long, light brown, finely reticulate. Chromosome number: $n=6$ (Chuang & Constance, 1977; this study).

Habitat and Distribution (Figure 13). *Phacelia ranunculacea* is found on the shaded rich soils of floodplains and the adjacent slopes of alluvial woods. The range is centered about southeastern Missouri and includes western Kentucky, western Tennessee, southern Illinois, southern Indiana, northeastern Mississippi, and most of Arkansas. Reports of *P. ranunculacea* from Jackson County, Illinois, Stewart County, Tennessee, and Clark and Poinsett counties in Arkansas (U.S.D.A., 2002) are included in the overall distribution of the complex (Figure 1). However, without specimens to verify the identifications they are omitted from the distribution map of the two species (Figure 13). Phenology. *Phacelia ranunculacea* flowers in late March to mid April, with fruit set immediately following. The seeds fall soon thereafter, upon desiccation and dehiscence of the capsule.

Notes. The collection by Nuttall is a mixture of *Ellisia ranunculacea* (= *Phacelia ranunculacea*) and *Ellisia microcalyx* (= *Nemophila microcalyx*), and the resulting confusion is understandable considering the remarkable resemblance of these two species. This led to the application of *Nemophila microcalyx* to both taxa (Gray, 1875) until Constance (1940) transferred *Ellisia ranunculacea* to the genus *Phacelia*. *Phacelia ranunculacea* is nearly identical to *P. covillei* except for several subtle but uniform characters. There are differences in the type and arrangement of hairs/glandular trichomes along the stem that are most obvious on the portion approximately halfway up the main axis of the plant. *Phacelia covillei* has mostly appressed/strigose hairs with a sparse arrangement of short (<2mm) glandular trichomes, compared with *P. ranunculacea* which has spreading/pilose hairs with more dense configuration of longer glandular trichomes. The shape of the terminal leaflet on the upper cauline leaves also exhibits minute differences. *Phacelia covillei* has acute leaf bases that range from cuneate to attenuate in shape, while the leaf bases of *P. ranunculacea* are obtuse and rounded to almost truncate. The terminal leaflets of some of the specimens examined had basal angles very close to 90°, even a couple a few degrees smaller. This was most notable for individuals from populations in the following counties: Hickman (KY), Montgomery (TN), Independence (AR), and Hempstead (AR). Remarkably the terminal leaflets of these specimens were twice as long as wide and lacked any secondary sinuses,

both characteristics that deviate considerably from the averages for the $n=6$ cytotype. Nonetheless, designating these individuals as *P. ranunculacea* is appropriate given the unambiguous stem pubescence and the fact that two collections from the aforementioned counties served as chromosome vouchers (see Appendix B). Though two taxa may share the same habitat and occur in the same state, the chromosome number discrepancies and allopatric distribution seem to suggest the two have been separate entities for a substantial amount of time.

As is the case for *P. covillei*, the overall scarcity and narrow microhabitat parameters suggest the conservation status given to *P. ranunculacea* is appropriate. The local abundance of some populations and the addition of two county records during the course of this study belie the need for continued monitoring of *P. ranunculacea* throughout its range. *Phacelia ranunculacea* is listed as a species of “special concern” in Kentucky (Warren et. al., 1986) and Tennessee (Somers, 1989). Conservation efforts will need to be focused on states in which *P. ranunculacea* and *P. covillei* both occur. Indiana lists *P. ranunculacea* as endangered (Bacone & Hedge, 1979) based on populations in Vanderburgh, Pike, and Knox counties. However, the populations studied in Pike and Knox counties are *P. covillei* and will need to be assessed separately from the Vanderburgh county populations. Neither Illinois nor Missouri list *P. ranunculacea* as threatened or endangered, which may be warranted given the number of populations relative to other states, but a single occurrence of *P. covillei* in each state highlights the need for further investigation.

LITERATURE CITED:

- Bacone, J.A. & C.L. Hedge. 1979. A preliminary list of endangered and threatened vascular plants in Indiana. *Proceedings of the Indiana Academy of Science*. 89: 359- 371.
- Baillon, H.E. 1890. Hydrophyllaceae. *In: Histoire des plantes*. L. Hatchette & Co., Paris. 10:397-402.
- Bentham, G. & J.D. Hooker. 1876. *Genera plantarum*, vol. 2. Williams and Norgate, London.
- Brand, A. 1913. Hydrophyllaceae. *In: Engler, A. Das Pflanzenreich*. 59 (IV: 251): 1-210.
- Brown, R. 1810. *Prodromus florae novae hollandiae*, vol. 1. J. Johnson & Co., London.
- Cave, M. & Constance, L. 1947. Chromosome numbers in the Hydrophyllaceae: III. *University of California Publications in Botany*. 18: 449-465.
- Cave, M. & Constance, L. 1950. Chromosome numbers in the Hydrophyllaceae: IV. *University of California Publications in Botany*. 23: 363-382.
- Chuang, T. & Constance, L. 1977. Cytogeography of *Phacelia ranunculacea* (Hydrophyllaceae). *Rhodora*. 79(817): 115-122.
- Constance, L. 1939. The genera of the tribe Hydrophyllae of the Hydrophyllaceae. *Madroño*. 5(1): 28-33.
- Constance, L. 1940. The genus *Ellisia*. *Rhodora*. 42: 33-39.
- Constance, L. 1949. A revision of *Phacelia* subgenus *Cosmanthus* (Hydrophyllaceae). *Contributions to the Gray Herbarium*. 168: 1-48.
- Constance, L. 1950. Some interspecific relationships in *Phacelia* subgenus *Cosmanthus*. *Proceedings of the American Academy of Arts and Science*. 78: 135-147.
- Constance, L. 1963. Chromosome number and classification in Hydrophyllaceae. *Brittonia*. 15: 273-285.
- Constance, L. & T.I. Chuang. 1982. SEM survey of pollen morphology and classification in Hydrophyllaceae (Waterleaf Family). *American Journal of Botany*. 69: 40-53.
- Cronquist, A. 1981. *An integrated system of classification of flowering plants*. Columbia University Press, New York.

- Cronquist, A. 1988. *The Evolution and Classification of Flowering Plants*, 2nd edition. The New York Botanical Garden, Bronx.
- Cronquist, A., A.H. Holmgren, N.H. Holmgren, J.L. Reveal & P.K. Holmgren. 1984. *Intermountain Flora- vascular plants of the intermountain West, U.S.A.* Vol. 4. The New York Botanical Garden, Bronx.
- Ferguson, D.M. 1999. Phylogenetic Analysis of Relationships in Hydrophyllaceae based on *ndhF* sequence data. *Systematic Botany*. 23(3): 253-268.
- Gillett, G.W. 1964. Genetic barriers in the *Cosmanthus phacelias* (Hydrophyllaceae). *Rhodora*. 66: 359-368.
- Gillett, G.W. 1968. Systematic relationships in the *Cosmanthus phacelias* (Hydrophyllaceae). *Brittonia*. 20: 368-374.
- Gleason, H.A. 1903. A second Illinois station for *Phacelia covillei* Watson. *Bulletin of the Torrey Botanical Club*. 3: 89-90.
- Gleason, H.A. & A. Cronquist. 1991. *Manual of the vascular plants of northeastern United States and adjacent Canada*, ed. 2. The New York Botanical Garden, Bronx.
- Gray, A. 1875. A conspectus of the North American Hydrophyllaceae. *Proceedings of the American Academy of Arts and Sciences*. 10: 312-332.
- Gray, A., S. Watson & J.M. Coulter. 1890. *Manual of the botany of the northern United States...*, ed. 6. American Book Company, New York.
- Harvill, A.M. 1969. Virginia species with disjunct populations in the Middle West. *Castanea*. 34: 225-229.
- Johansen, D.A. 1940. *Plant Microtechnique*. McGraw-Hill Co., Inc., New York.
- Jussieu, A.L. De. 1789. *Genera plantarum secundum ordines naturales disposita*, 1st ed. Herissant and Barrois, Paris.
- K.S.N.P.C.(Kentucky State Nature Preserves Commission). 2000. Rare and extirpated biota of Kentucky. *Journal of the Kentucky Academy of Science*. 61(2): 115-132.
- Lindley, J. 1836. *A natural system of botany*, 2nd edition. Longman, Rees, Oram, Green, and Longman, London.
- M.N.H.P.(Maryland Natural Heritage Program). 1988. Rare, threatened, and endangered plants of Maryland. Department of Natural Resources, Annapolis, MD.

- Murdy, W.H. 1966. The systematics of *Phacelia maculata* and *P. dubia* var. *georgiana*, Both endemic granite outcrop communities. *American Journal of Botany*. 53: 1028-1036.
- Nuttall. 1837. *Ellisia ranunculacea*. *Transactions of the American Philosophical Society*, N.S. 5: 191.
- Ohio Division of Natural Areas and Preserves. 2002. Rare native Ohio plants: 2002-03 status list. Ohio Department of Natural Resources, Columbus, OH.
- Radford, A.E., W.C. Dickinson, J.R. Massey & C.R. Bell. 1974. *Vascular Plant Systematics*. Harper & Row Publ., New York.
- Rohlf, F.J. 2002. *NTSYS-pc version 2.11p Numerical Taxonomy and Multivariate Analysis System version 2.11p*. Exeter Software, Setauket, NY.
- Sass, J.E. 1958. *Botanical Microtechnique*. Iowa State College Press, Ames, Iowa.
- Solbrig, O. 1970. *Principles and methods of plant biosystematics*. MacMillen, New York.
- Somers, P. 1989. Revised list of the rare plants of Tennessee. *Journal of the Tennessee Academy of Science*. 64: 179-184.
- Sutter, R.D., L. Mansberg & J. Moore. 1983. Endangered, threatened, and rare plant species of North Carolina: A revised list. *Bull. Assoc. Southeastern Biol.* 30: 153-163.
- Thorne, R.F. 1976. A phylogenetic classification of the angiosperms. *Evolutionary Biology*. 9: 35-106.
- Turner, B.L. 1991. New species of Mexican *Phacelia*, Subgenus *Cosmanthus* (Hydrophyllaceae). *Phytologia*. 71(6): 445-452.
- U.S.D.A., N.R.C.S. 2002. The PLANTS Database, Version 3.5. National Plant Center Data Center, Baton Rouge, LA 70874-4490 U.S.A. (<http://plants.usda.gov>).
- Walters, D.R. & D.J. Keil. 1975. *Vascular Plant Taxonomy*, 4th edition. Kendall Hunt Publ. Co., Dubuque, Iowa.
- Weakley, A.S. Flora of the Carolinas, Virginia, and Georgia. Working draft: September 26, 2003 (http://www.herbarium.unc.edu/weakley_flora).

Characters	Abbreviation	Character states
Vegetative characters		
1.number of leaflets per terminal leaf	numlfts	number
2.number of secondary sinuses of terminal leaflet	numsintlft	number
3.terminal leaflet length	tlftlgh	(mm)
4.terminal leaflet width	tlftwid	(mm)
5.terminal leaflet length/width ratio	lghwdratio	ratio (decimal to hundredth's)
6.terminal leaflet basal angle	basalang	to nearest whole degree
7.terminal leaflet petiole length	petlgh	(mm)
8.number of lobes per apical pinnatifid leaflet	numaplob	number
9.number of lobes per basal pinnatifid leaflet	numbalob	number
10.midstem pubescence	midstmpub	0=strigose; 1=pilose/retorse
11.midstem glandular trichomes	midstmglnd	0=short/sparse; 1=long/dense
12.terminal leaflet petiole indument*	petind	0=glabrous; 1=pubescent
13.terminal leaflet petiole glands*	petgland	0=absent; 1=present
14.adaxial laminar indument*	adlind	0=glabrous; 1=pubescent
15.adaxial laminar glands*	adlagland	0=absent; 1=present
16.abaxial laminar indument*	ablind	0=glabrous; 1=pubescent
17.abaxial laminar glands*	ablagland	0=absent; 1=present
18.primary peduncle indument*	pedunind	0=glabrous 0=pubescent
19.primary peduncle glands*	pedungland	0=absent; 1=present
20.pedicel indument*	pedind	0=glabrous; 1=pubescent
21.pedicel glandular trichomes*	pedgltr	0=absent; 1=present
Floral characters		
22.style length	stylgh	(mm)
23.style division length	clflgh	(mm)
24.corolla lobe width	corlobwid	(mm)
25.corolla lobe length	corloblgh	(mm)
26.corolla length	corlgh	(mm)
27.filament length	fillgh	(mm)
28.anther length	anthlgh	(mm)
29.anther width	anthwid	(mm)
30.nectar flap length	ncflplgh	(mm)
31.sepal length	seplgh	(mm)
32.sepal width	sepwid	(mm)
33.corolla lobe width/length ratio	loberatio	ratio (to nearest hundredth's)
34.style length/division ratio	styclfratio	ratio (to nearest hundredth's)
35.sepal indument*	sebind	0=glabrous; 1=pubescent
36.sepal glandular trichomes*	sepgltr	0=absent; 1=present
37.filament indument*	filind	(mm)
38.ovary indument*	ovaind	0=glabrous; 1=pubescent

Table 1. Morphological characters, acronyms, and the values assigned to each character state used in the NTSys-PC 2.1 analysis of *Phacelia ranunculacea* s.l. (*characters with an asterisk were not variable and not entered in the dataset).

"Eastern" n=14 cytotype	22. stylgh	23. cflgh	24. corlobwid	25. corloblgh	26. corlgh	27. fillgh	28. anthlgh	29. anthwid	30. ncflplgh	31. seplgh	32. sepwid	33. loberatio	34. styclfratio
Mean	1.5081	0.7203	1.74677	1.42193	4.3035	1.8387	0.4484	0.3903	0.36452	3.7997	0.7568	1.23	2.16258
Standard Error	0.0505	0.0344	0.05729	0.03779	0.0845	0.0447	0.0123	0.0111	0.00905	0.0985	0.0208	0.02829	0.07544
Median	1.5	0.7	1.76	1.41	4.41	1.87	0.43	0.37	0.36	3.72	0.76	1.22	2.16
Mode	1.77	0.82	1.97	1.24	4.02	1.79	0.42	0.36	0.39	3.3	0.83	1.27	2.23
Standard Deviation	0.281	0.1915	0.31897	0.21041	0.4703	0.2489	0.0683	0.062	0.05039	0.5484	0.1156	0.15752	0.42003
Sample Variance	0.0789	0.0367	0.10174	0.04427	0.2212	0.062	0.0047	0.0038	0.00254	0.3007	0.0134	0.02481	0.17643
Range	1.27	0.96	1.5	0.87	1.74	0.96	0.28	0.21	0.2	2.06	0.47	0.6	2.09
Minimum	0.93	0.37	1.02	0.96	3.24	1.26	0.36	0.32	0.29	2.62	0.47	1.03	1.5
Maximum	2.2	1.33	2.52	1.83	4.98	2.22	0.64	0.53	0.49	4.68	0.94	1.63	3.59
Sum	46.75	22.33	54.15	44.08	133.41	57	13.9	12.1	11.3	117.79	23.46	38.13	67.04
Count	31	31	31	31	31	31	31	31	31	31	31	31	31

"Western" n=6 cytotype	22. stylgh	23. cflgh	24. corlobwid	25. corloblgh	26. corlgh	27. fillgh	28. anthlgh	29. anthwid	30. ncflplgh	31. seplgh	32. sepwid	33. loberatio	34. styclfratio
Mean	1.32	0.7913	1.33625	1.41416	4.0208	1.7754	0.3654	0.3417	0.28682	3.0408	0.8367	0.94958	1.78083
Standard Error	0.0318	0.0398	0.05304	0.05322	0.1111	0.0513	0.0114	0.0084	0.00886	0.1072	0.0323	0.01912	0.08425
Median	1.325	0.785	1.325	1.425	4.045	1.805	0.35	0.345	0.29	2.925	0.805	0.965	1.75
Mode	1.33	0.61	1.15	1.43	3.97	1.85	0.33	0.34	0.31	2.58	0.69	1	1.75
Standard Deviation	0.1556	0.1947	0.25985	0.26075	0.5441	0.2513	0.0558	0.0409	0.04156	0.5251	0.1584	0.09369	0.41277
Sample Variance	0.0242	0.0379	0.06752	0.06799	0.2961	0.0631	0.0031	0.0017	0.00173	0.2757	0.0251	0.00878	0.17038
Range	0.61	0.83	1.23	1.23	2.7	1.13	0.25	0.18	0.17	2.31	0.6	0.42	1.82
Minimum	1.07	0.5	0.88	0.83	2.76	1.3	0.25	0.26	0.19	1.95	0.6	0.78	1.12
Maximum	1.68	1.33	2.11	2.06	5.46	2.43	0.5	0.44	0.36	4.26	1.2	1.2	2.94
Sum	31.68	18.99	32.07	33.94	96.5	42.61	8.77	8.2	6.31	72.98	20.08	22.79	42.74
Count	24	24	24	24	24	24	24	24	22	24	24	24	24

Table 2. Summary statistics of each floral character segregated into the two groups suggested by the results of the scatter plots, principle component analysis (PCA), and cluster (UPGMA) analyses.

"Eastern" n=14 cytotype	1. numlfts	2. numsintlft	3. tlftlgh	4. tlftwid	5. lghwidratio	6. basalang	7. petlgh	8. numaplob	9. numbalob	10. midstmpub	11. midstmgl
Mean	2.994949	1.020202	15.49495	10.31667	1.510859	69.69192	6.566061	1.635417	1.443299	0	0
Standard Error	0.033964	0.077109	0.404318	0.267779	0.012262	0.556093	0.245989	0.060556	0.058441	0	0
Median	3	1	14.4	9.75	1.51	70	5.85	1	1	0	0
Mode	3	0	18.5	10.9	1.51	74	6	1	1	0	0
Standard Deviation	0.477913	1.085014	5.689261	3.767989	0.172547	7.824927	3.461374	0.839094	0.813985	0	0
Sample Variance	0.228401	1.177255	32.36769	14.19774	0.029772	61.22948	11.98111	0.704079	0.662571	0	0
Range	4	4	29.8	18.2	1.14	36	21.8	5	3	0	0
Minimum	1	0	5.4	3.2	1.08	52	1.2	0	0	0	0
Maximum	5	4	35.2	21.4	2.22	88	23	5	3	0	0
Sum	593	202	3068	2042.7	299.15	13799	1300.08	314	280	0	0
Count	198	198	198	198	198	198	198	192	194	198	198

"Western" n=6 cytotype	1. numlfts	2. numsintlft	3. tlftlgh	4. tlftwid	5. lghwidratio	6. basalang	7. petlgh	8. numaplob	9. numbalob	10. midstmpub	11. midstmgl
Mean	2.781955	1.593985	17.0203	13.49398	1.282857	107.7895	9.165414	1.679389	1.560606	1	1
Standard Error	0.048135	0.11094	0.49798	0.437297	0.015245	0.858133	0.431424	0.085514	0.092049	0	0
Median	3	2	15.3	12.1	1.27	107	8	1	1	1	1
Mode	3	2	13.8	11.6	1.24	96	6	1	1	1	1
Standard Deviation	0.555119	1.279427	5.74299	5.043159	0.175817	9.896474	4.975425	0.978753	1.057561	0	0
Sample Variance	0.308157	1.636933	32.98193	25.43345	0.030911	97.94019	24.75486	0.957957	1.118436	0	0
Range	2	6	27.9	25.8	0.9	47	25.1	4	5	0	0
Minimum	1	0	5.7	4.9	0.81	91	2.4	0	0	1	1
Maximum	3	6	33.6	30.7	1.71	138	27.5	4	5	1	1
Sum	370	212	2263.7	1794.7	170.62	14336	1219	220	206	133	133
Count	133	133	133	133	133	133	133	131	132	133	133

Table 3. Summary statistics of each vegetative character segregated into the two groups suggested by the results of the scatter plots, principal component analysis (PCA), and the cluster (UPGMA) analyses.

<i>Eigenvalues</i>	7.3806	3.8263	2.4621
<i>Component loadings</i>	<i>C1</i>	<i>C2</i>	<i>C3</i>
1.numlfts	0.4835	0.1176	-0.5468
2.numslntlft	-0.1423	0.4376	-0.3396
3.tlftlgh	-0.3475	0.5233	0.2800
4.tlftwid	-0.4963	0.5215	0.3452
5.lghwidratio	0.4435	-0.1817	-0.2849
6.basalang	-0.7973	0.2355	-0.0532
7.petlgh	-0.4515	0.5058	0.2828
8.numaplob	0.1912	0.3048	-0.7580
9.numbalob	0.1298	0.4021	-0.6864
10.midstmpub	-0.8587	0.2588	-0.1006
11.midstmgl	-0.8587	0.2588	-0.1006
22.stylgh	0.5359	0.6019	0.2096
23.cflgh	-0.0195	0.5860	-0.2051
24.corlobwid	0.8316	0.3172	0.2082
25.corloblgh	0.4135	0.6587	0.0064
26.corlgh	0.6079	0.6236	0.1185
27.fillgh	0.3779	0.6251	0.0642
28.anthlgh	0.6759	-0.1831	-0.0002
29.anthwid	0.5569	-0.1674	-0.3320
30.ncflplgh	0.6696	-0.0624	0.2376
31.seplgh	0.8069	0.3528	0.1146
32.sepwid	-0.0768	0.2776	0.2872
33.loberatio	0.6960	-0.2136	0.2259
34.styclfratio	0.4194	-0.0996	0.3993
<i>Percent of total variance explained</i>	30.7527	15.9429	10.2588

Table 4. Eigenvectors and eigenvalues for the first three axes from Principal Components Analysis of *P. ranunculacea s.l.* The values were computed from floral and vegetative variables (both qualitative and quantitative).

<i>Character</i>	<i>Test statistic</i>	<i>degrees of freedom (df)¹</i>	<i>Probability value</i>
1.Number of leaflets**	3.6155	254	0.0004
2.Number of 2° sinuses**	4.2469	251	3.0549E-5
3.Terminal leaflet length*	2.3780	281	0.0181
4.Terminal leaflet width**	6.1964	228	2.6741E-9
5.Terminal leaflet length/width ratio**	11.6536	280	7.5001E-26
6.Basal angle**	37.2570	238	2.7161E-101
7.Petiole length**	5.2340	216	3.9208E-7
8.Lobes per apical leaflet	0.4196	250	0.6751
9.Lobes per basal leaflet	1.0759	232	0.2831
22.Style length**	3.1537	49	0.0028
23.Style cleft length	1.3494	49	0.1834
24.Corolla lobe width**	5.2582	53	2.6584E-6
25.Corolla lobe length	0.1190	44	0.9058
26.Corolla length	2.0260	46	0.0486
27.Filament length	0.9302	49	0.3568
28.Anther length**	4.9580	53	7.6968E-6
29.Anther width**	3.4953	52	0.0010
30.Nectar flap length**	6.1344	50	1.3523E-7
31.Sepal length**	5.2132	51	3.3937E-6
32.Sepal width*	2.0794	41	0.0439
33.Corolla lobe ratio**	8.2115	50	7.8470E-11
34.Style/cleft length ratio**	3.3754	50	0.0014

Table 5. Pairwise comparisons of the two groups produced via ordinate and clustering analyses using a two sample t-test based on separate variances. Statistically significant characters indicated at the $\alpha = 0.05$ and $\alpha = 0.01$ levels are based on probabilities of * $p < 0.05$ and ** $p < 0.01$ respectively. (¹Degrees of freedom calculated via Welch/Satterthwaite method.)

Figure 1. Geographic distribution of *Phacelia ranunculacea* s.l.

Figure 2. Diagram of terminal leaf. 3.**tftlgh** is terminal leaflet length and 4.**tftwid** is terminal leaflet width. Basal angle (6.**basalang**) of terminal leaflet: **A.** acute **B.** obtuse.

Figure 3. Floral diagrams (**A.** corolla and androecium and **B.** calyx and pistil) illustrating measurements. Refer to **Table 1** for acronym explanations.

A **B**

Figure 4. Bivariate scatter plots from floral and vegetative morphological analysis of *Phacelia ranunculacea* s.l. A. Terminal leaflet basal angle (6.basalang) versus corolla lobe width (24.corlobwid) (mm). B. Terminal leaflet basal angle (6.basalang) versus corolla lobe width/length ratio (33.loberatio).

Figure 5. Bivariate scatter plots from vegetative morphological analysis of *Phacelia ranunculacea* s.l. A. Terminal leaflet basal angle (6.basalang) versus terminal leaflet length/width ratio (5.lghwidratio). B. Terminal leaflet basal angle (6.basalang) versus terminal leaflet length (3.tlftlgh) (mm).

Figure 6. Plot of scores from principal component axes I and II computed from floral and vegetative data.

Figure 7. Plot of scores from principal component axes I and II computed from vegetative data.

Figure 8. Phenogram of floral and vegetative morphological data showing two distinct groups corresponding to the chromosome counts. Cophenetic correlation coefficient (r) = .9715; * = chromosome voucher, # = type population of *Phacelia covillei* Wats.

Figure 9. Phenogram of vegetative morphological data showing two distinct groups corresponding to the different chromosome numbers with the OTU codes for the voucher specimens noted (# = OTUs from the type population of *Phacelia covillei* Wats). Cophenetic correlation coefficient (r) = .9819.

Figure 10. Meiotic chromosomes from a population in Pike County, Indiana (Sewell 421). Scale bar 1 μ m.

Figure 11. Meiotic chromosomes from a population in Wayne County, Missouri (Sewell 413). Scale bar 1 μ m.

Figure 12. Meiotic chromosomes from a population in Lawrence County, Ohio (Sewell 370). Scale bar 1 μ m.

Figure 13. Distribution of *P. ranunculacea* and *P. covillei* with chromosome vouchers indicated by county.

Appendix A. Histograms of characters.

Appendix B. Specimens examined (collector numbers and OTU codes w/ **chromosome vouchers in bold**, # = Type population, % = different collections on the same sheet).

Phacelia covillei Wats. ($n = 14$)

State	County	Collector	Collector no.	OTU codes	Date	Herbaria
North Carolina	Chatham	Ahles, H.E.	53249	NC 01 01-17	April 25, 1960	WVU, TENN, IND, ILL, BRIT(2), NCU, NY(2), KU, GH, US, UC/JEP
North Carolina	Chatham	Radford, A.E.	42786	NC 02 01	April 24, 1960	NCU
North Carolina	Chatham	Sewell, M.	330	NC 13 01	April 14, 2001	MU
North Carolina	Chatham	Leonard, S.W.	8360	NC 14 01	April 22, 1984	BRIT
North Carolina	Chatham	Basinger	3972	NC 15 01	June 7, 1991	ILL
North Carolina	Harnett	Radford, A.E.	7850	NC 03 01-05	April 10, 1954	VPI, BRIT, NCU(2), GH
North Carolina	Lee	Sewell, M.	335	NC 04 01	April 14, 2001	MU
North Carolina	Lee	Radford, A.E.	5590	NC 05 01-06	April 27, 1951	VPI, US, NCU, NY, GH, UC/JEP
North Carolina	Lee	Radford, A.E.	5577	NC 06 01	April 27, 1951	NCU
North Carolina	Alamance	Chuang, T.I.	6851	NC 07 02	April 5, 1971	UC/JEP
North Carolina	Alamance	Radford, A.E. & W. Hight	5570	NC 08 01-05	April 23, 1951	NCU, NY, GH, UC/JEP, US
North Carolina	Alamance	Bradley, T.	248	NC 09 01	April 17, 1963	GMU
North Carolina	Alamance	Logue, J.F. & Spongberg, S.	964	NC 10 01,02,04	April 11, 1967	NCU(2), Wku
North Carolina	Moore	Sewell, M.	337	NC 11 03	April 14, 2001	MU
North Carolina	Moore	Legrand, H.E.	s.n.	NC 11 04	April 10, 1988	NCU
North Carolina	Vance	Legrand, H.E.	s.n.	NC 12 02	April 12, 1985	NCU
North Carolina	Vance	Mansberg, L.	442	NC 12 04	April 12, 1985	NCU
North Carolina	Vance	Legrand, H.E.	s.n.	NC 12 05	April 4, 1985	NCU
Washington, D.C.		Constance, L.	3018	DC 01 02&05-09	May 2, 1943	F, WVU, US, TENN, BRIT, MO, UC/JEP, PH, NY
Maryland	Montgomery	Sewell, M.	343	MD 01 01&02	April 21, 2001	MU
Maryland	Montgomery	Sewell, M.	345	MD 02 01	April 21, 2001	MU
Maryland	Montgomery	Constance, L.	3021	MD 03 04-09&11	May 14, 1944	F, WVU, US, ILL, BRIT, TENN, MO, NY, UC/JEP, PH
Maryland	Montgomery	Hermann & Martin	56	MD 04 01-10	May 10, 1939	F(2), VPI, WVU, US, TENN, NY, KU, PH(2)
Maryland	Montgomery	Kearney, T.H.	s.n.	MD 05 04	April 23, 1897	NY, NCU
Maryland	Montgomery	Kearney, T.H.	s.n.	MD 06 01	April 23, 1897	NY
Maryland	Montgomery	Kearney, T.H.	s.n.	MD 07 01	April 23, 1897	NY
Maryland	Montgomery	Kearney, T.H.	s.n.	MD 08 01	April 23, 1897	MO
Maryland	Montgomery	Kearney, T.H.	s.n.	MD 09 01	May 19, 1895	NY
Maryland	Montgomery	Kearney, T.H.	s.n.	MD 10 01	May 19, 1895	NCU
Maryland	Montgomery	Chase, A.	2172	MD 11 01&03-05	May 13, 1904	F, ILL(4)
Maryland	Montgomery	Blake, S.F.	s.n.	MD 12 02-04	April 27/May 5, 1935	VPI, NCU, GH
Maryland	Montgomery	Blake, S.F.	8126	MD 13 01	May 4, 1920	GH
Maryland	Montgomery	Blake, S.F.	9360	MD 14 01	May 2, 1926	UC/JEP
Maryland	Montgomery	Pollard, C.L.	155	MD 15 01-03	May 8, 1895	US, UC/JEP(2)
Maryland	Montgomery	Pollard, C.L.	s.n.	MD 16 01	April 23, 1897	F
Maryland	Montgomery	Hermann, F.J.	10275	MD 17 01&03	May 10, 1939	F, MO

State	County	Collector	Collector no.	OTU codes	Date	Herbaria
Maryland	Montgomery	Sargent, F.H.	s.n.	MD 18 02	April 29, 1951	BRIT
Maryland	Montgomery	Churchill, J.A.	s.n.	MD 19 02	May 20, 1971	BRIT
Maryland	Montgomery	Bartram, E.B.	s.n.	MD 20 01	April 17, 1909	PH
Maryland	Montgomery	Painter, J.H.	1336	MD 21 01	May 25, 1905	MO
Maryland	Montgomery	Painter, J.H.	s.n.	MD 22 01	17-May-03	MO
Maryland	Montgomery	Batchelder, C.F.	2932	MD 23 01	May 10, 1911	GH
Maryland	Montgomery	Batchelder, C.F.	2933	MD 23 02	May 10, 1911	GH
Maryland	Montgomery	House, H.D.	841	MD 24 01&04	May 25, 1905	US, MO, NY
Maryland	Montgomery	Hill, S.R.	16707	MD 25 01-05	May 4, 1986	ILL, MO, NCU, NY, GH
Maryland	Montgomery	Maxon & Standley	337	MD 26 02	May 20, 1916	US
Maryland	Montgomery	Leonard, E.C.	1784	MD 27 02	April 3, 1921	US
Maryland	Montgomery	Sargent, F.H.	s.n.	MD 28 01	May 7, 1950	WVU
Maryland	Montgomery	McVaugh, R.	4920	MD 29 02	May 28, 1940	UC/JEP
Maryland	Montgomery	Wurdack, J.J.	2632	MD 30 01	May 2, 1970	UC/JEP
Maryland	Montgomery	Hunnewell, F.W.	5902	MD 31 01	April 19, 1919	GH
Maryland	Montgomery	Long, B.	s.n.	MD 32 01	April 17, 1908	VPI
Maryland	Montgomery	Killip, E.P.	7457	MD 33 03	April 3, 1921	MO
Maryland	Montgomery	Gandoger, M.	s.n.	MD 34 01	1902	MO
Maryland	Montgomery	Britton, N.L.	s.n.	MD 35 01	May 21, 1902	NY
Maryland	Montgomery	Rose, J.N.	1146	MD 36 01	April 23, 1897	NY
Maryland	Montgomery	Pennell, F.W.	14991	MD 37 01-02	May 4, 1930	NY, PH
Maryland	Montgomery	Greene, E.S.	s.n.	MD 38 01	May 2, 1898	NY
Maryland	Montgomery	Terrell, E.E.	4082	MD 39 01	April 27, 1968	US
Maryland	Montgomery	Steele, E.S.	s.n.	MD 40 01	April 30, 1897	GH
Maryland	Montgomery	Steele, E.S.	s.n.	MD 41 01	April 30, 1897	WVU
Maryland	Montgomery	Steele, E.S.	s.n.	MD 42 01	April 30, 1897	MU
Maryland	Montgomery	Steele, E.S.	8319	MD 43 01	April 30, 1897	GH
Maryland	Montgomery	Steele, E.S.	s.n.	MD 44 01	April 24, 1916	US
Maryland	Montgomery	Waite, M.B.	s.n.		May 19, 1895	GH
Maryland	Montgomery	Williamson, C.S.	s.n.	MD 46 01	April 21, 1908	PH
Maryland	Montgomery	Williamson, C.S.	s.n.		May 30, 1909	PH
Maryland	Montgomery	Williamson, C.S.	s.n.		May 29, 1909	PH
Maryland	Montgomery	Williamson, C.S.	%s.n.	%MD 48 02	May 29, 1909	PH
Maryland	Montgomery	Williamson, C.S.	%s.n.	%MD 49 01	May 1897	PH
Maryland	Montgomery	Williamson, C.S.	%s.n.	%MD 50 01	April 17, 1908	PH
Maryland	Montgomery	Williamson, C.S.	s.n.	MD 50 02	April 17, 1908	PH
Maryland	Montgomery	Williamson, C.S.	s.n.	MD 51 01	May	PH
Maryland	Montgomery	Coville, F.V.	#s.n.	#MD 52 01	May 5/12, 1889	KU
Maryland	Montgomery	Coville, F.V.	#s.n.	#MD 52 02	May 12, 1889	US
Maryland	Montgomery	Coville, F.V.	#s.n.	#MD 52 03,05,06	May 5/12, 1889	GH

State	County	Collector	Collector no.	OTU codes	Date	Herbaria
Maryland	Montgomery	Coville, F.V.	#s.n.		May 5, 1889	NY
Virginia	Fairfax	Sewell, M.	347	VA 01 01	April 21, 2001	MU
Virginia	Fairfax	Fleming, G.P.	6377	VA 02 01-03	May 7, 1992	VPI, GMU(2)
Virginia	Fairfax	Fleming, G.P.	6382	VA 03 01	May 7, 1992	GMU
Virginia	Fairfax	Dowell, P.L.	1341	VA 04 01	May 4, 1902	GH
Virginia	Fairfax	Palmer, W.	s.n.		May 5, 1901	VPI
Virginia	Fairfax	Maxon, W.R.	513	VA 06 01	May 3, 1902	US
Virginia	Arlington	Allard, H.A.	21311	VA 07 01-03	April 23, 1953	WVU, US, PH
Virginia	Arlington	Allard, H.A.	4513	VA 07 04-09	April 26, 1938	VPI, MO, NY, GH, F, UC/JEP
Virginia	Arlington	Allard, H.A.	281	VA 07 10-15	May 2, 1935	F, VPI, US, MO, NY, GH
Virginia	Arlington	Allard, H.A.	2534	VA 08 01-04	April 22, 1937	US, NY, VPI, GH
Ohio	Lawrence	Sewell, M.	364	OH 01 01	May 5, 2001	MU
Ohio	Lawrence	Sewell, M.	370	OH 02 01-02	May 5, 2001	MU
Ohio	Lawrence	McCormac, J.	5778	OH 03 01	May 16, 1997	MU
Ohio	Lawrence	Sewell, M.	373	OH 04 01	May 5, 2001	MU
Ohio	Lawrence	McCormac, J.	6493	OH 05 01	April 28, 1998	MU
West Virginia	Roane	Bush, E.	s.n.	WV 01 01	May 5, 1991	WVU
West Virginia	Roane	Bush, E.	s.n.	WV 02 01	May 5, 1990	WVU
West Virginia	Roane	Bush, E.	s.n.	WV 03 01	May 5, 1990	UC/JEP
West Virginia	Roane	Rossbach, G.B.	12106	WV 04 01	May 9, 1996	WVU
West Virginia	Roane	Bush, E.	s.n.	WV 05 01	May 5, 1988	WVU
West Virginia	Jackson	Richardson, R.	s.n.	WV 06 01	May 5, 1969	WVU
West Virginia	Jackson	Richardson, R.	s.n.	WV 07 01	May 15, 1969	WVU
West Virginia	Jackson	Sewell, M.	377	WV 08 01	May 6, 2001	MU
West Virginia	Jackson	Richardson, R.	s.n.	WV 09 01	April 28, 1974	WVU
Indiana	Knox	Deam, C.C.	44032	IN 01 01-05	April 19, 1927	IND, GH, UARK, US
Indiana	Knox	Deam, C.C.	42896	IN 02 01-04	May 23, 1926	IND(2), NY, PH
Indiana	Knox	Deam, C.C.	s.n.	IN 03 01	May 26, 1926	MO
Indiana	Knox	Schneck, J.	s.n.	IN 04 01	May 16, 1896	IND
Indiana	Knox	Schneck, J.	s.n.	IN 05 01	April 20, 1903	IND
Indiana	Knox	Trelease, W. & J. Schneck	s.n.	IN 06 01	May 8, 1888	MO
Indiana	Knox	Blatchley, W.S.	s.n.	IN 07 01	April 23, 1903	IND
Indiana	Pike	Sewell, M.	421	IN 10 01	April 16, 2003	MU
Indiana	Pike	Sewell, M.	422	IN 11 01	April 16, 2003	MU
Illinois	Wabash	Schneck, H.	s.n.	IL 13 01	May 19, 1896	NY
Missouri	Texas	Sewell, M.	396		March 30, 2002	MU
Missouri	Texas	Steyermark, J.A.	73166	MO 01 02-06	April 28, 1952	F, MO, NY, US, UC/JEP

Phacelia ranunculacea (Nutt.) Constance ($n=6$)

State	County	Collector	Collector no.	OTU codes	Date	Herbaria
Indiana	Vanderburgh	Sewell, M.	404	IN 08 01&02	April 12, 2002	MU
Indiana	Vanderburgh	Schaust, J.	s.n.		May 1983	IND
Illinois	Alexander	Sewell, M.	411	IL 01 01&02	April 14, 2002	MU
Illinois	Alexander	Basinger	5014	IL 02 01	May 9, 1993	ILL
Illinois	Alexander	Ozment & Windler	5	IL 03 01	April 10, 1964	NCU
Illinois	Alexander	Phillippe	23489	IL 04 01	April 12, 1994	ILL
Illinois	Johnson	Winterringer, G.S.	9003	IL 05 01	May 14, 1952	ILL
Illinois	Pulaski	Sewell, M.	406	IL 06 01	April 13, 2002	MU
Illinois	Pulaski	Hill, S.R.	27804	IL 07 01-03	May 7, 1996	ILL, NY, MU
Illinois	Union	Sewell, M.	410	IL 08 01	April 13, 2002	MU
Illinois	Union	Pepoon, H.S.	s.n.	IL 09 01	May, 1932	ILL
Illinois	Union	Sewell, M.	409	IL 10 01	April 13, 2002	MU
Illinois	Union	Phillippe	18569	IL 10 02	April 10, 1991	ILL
Illinois	Union	Chuang, T.I.	7240	IL 11 01	April 28, 1973	UC/JEP
Illinois	Union	Chuang, T.I.	7240	IL 11 02	April 28, 1973	UC/JEP
Illinois	Union	Chuang, T.I.	6608	IL 12 01&02	25-Apr-69	UC/JEP
Illinois	Union	Chuang, T.I.	6608		May 1969	UC/JEP
Illinois	Washington	French, G.H.	11	IL 14 01	1873	US
Missouri	Stoddard	Sewell, M.	399		March 31, 2002	MU
Missouri	Stoddard	Chuang, T.I.	6758	MO 02 05	March 27, 1970	UC/JEP
Missouri	Stoddard	Steyermark, J.A.	80911	MO 02 03&04	April 7, 1956	ILL, MO
Missouri	Stoddard	Steyermark, J.A.	5074	MO 03 01-03	April 25, 1938	F, MO, UC/JEP
Missouri	Wayne	Bush, B.F.	1458	MO 04 01&02	April 29, 1902	NY, MO
Missouri	Wayne	Sewell, M.	413	MO 05 01&02	April 14, 2002	MU
Missouri	Wayne	Hudson, S.	s.n.	MO 05 03	May 26, 1992	MO
Missouri	Butler	Sewell, M.	397		March 31, 2002	MU
Missouri	Butler	Summers, B.	8531	MO 07 01	May 3, 1998	MO
Missouri	Butler	Steyermark, J.A.	80823	MO 08 01&02	April 5, 1956	MO, GH
Missouri	Butler	Hudson, S.	855		February 13, 1996	MO
Missouri	Butler	Hudson, S.	665	MO 09 02	April 9, 1995	MO
Missouri	Pemiscot	Steyermark, J.A.	80876	MO 10 01&02	April 6, 1956	GH, MO
Missouri	Pemiscot	Steyermark, J.A.	5121	MO 11 01&02	April 26, 1938	F, MO
Missouri	Scott	Steyermark, J.A.	4995	MO 12 01&03	April 23, 1938	F, MO
Missouri	Dunklin	Trelease, W.	496		April 24, 1897	MO
Missouri	Dunklin	Kellogg, J.H.	25921	MO 14 01&03	May 23, 1932	MO(2)
Missouri	Dunklin	Palmer, E.J.	39064	MO 15 01	April 24, 1931	GH
Missouri	Dunklin	Bush, B.F.	1024		May 23, 1892	MO
Missouri	?	James(Dr.)	s.n.		s.d.	NY

State	County	Collector	Collector no.	OTU codes	Date	Herbaria
Tennessee	Obion	Sewell, M.	349	TN 01 01	April 28, 2001	MU
Tennessee	Obion	Guthrie, M.	723	TN 03 01	April 10, 1986	BRIT
Tennessee	Obion	Guthrie, M.	726	TN 04 01&02	April 10, 1986	BRIT(2)
Tennessee	Obion	Guthrie, M.	750	TN 05 02	April 13, 1986	NY, BRIT
Tennessee	Obion	Guthrie, M.	775	TN 06 01&02	April 16, 1986	TENN, BRIT
Tennessee	Obion	Guthrie, M.	811	TN 07 01-03	April 26, 1986	BRIT(2), TENN
Tennessee	Obion	Pyne, M. G.	1672	TN 08 01&02	April 9, 1987	TENN, BRIT
Tennessee	Obion	Sharp, A.J., et. al.	12286	TN 09 02&03	April 30, 1949	TENN, BRIT, NCU
Tennessee	Obion	Chuang, T.I.	6855	TN 10 02&03	April 8, 1971	UC/JEP(2)
Tennessee	Montgomery	Somers, P.	1030	TN 11 01	April 19, 1978	BRIT
Tennessee	Montgomery	Wofford, B.E.	4	TN 12 01&02	April 28, 1993	TENN, NY
Tennessee	Montgomery	Chuang, T.I.	6854	TN 13 01&03	April 7, 1971	TENN, UC/JEP
Tennessee	Montgomery	Clebsch, A.	s.n.	TN 14 01	April 23, 1949	TENN
Tennessee	Montgomery	Clebsch, A.	110	TN 15 02	April 10, 1948	TENN, NCU
Tennessee	Montgomery	Fox, H.	s.n.	TN 16 01	April 16, 1917	PH
Tennessee	Montgomery	Chester, W.	1996	TN 17 01&03	April 16, 1968	TENN, BRIT
Tennessee	Montgomery	Brown, F. & Clebsch, A.	s.n.	TN 18 02	April 7, 1957	NCU
Tennessee	Shelby	Pyne, M.	12	TN 19 01&02	April 4, 1992	TENN, BRIT
Tennessee	Shelby	Bates, V.M.	1753	TN 20 01	May 17, 1980	TENN
Tennessee	Shelby	Bates, V.M.	2154		April 16, 1981	TENN
Tennessee	Shelby	Fendler, A.	s.n.	TN 22 02	March 29, 1896	MO
Tennessee	Shelby	Moore, C.	7	TN 23 01	April 1932	MO
Tennessee	Lake	Jones, R.L.	s.n.	TN 24 01	April 8, 1990	EKU
Tennessee	Lauderdale	Keiran, P.	249	TN 25 01	March 31, 1972	TENN
Kentucky	Christian	McKinney, L.E.	6124	KY 01 01	April 7, 1994	TENN
Kentucky	Christian	Athey, R.	4017	KY 02 01	May 19, 1978	BRIT
Kentucky	Christian	Athey, R.	3941	KY 02 02	April 23, 1978	BRIT
Kentucky	Henderson	Sewell, M.	401	KY 03 01	April 12, 2002	MU
Kentucky	Henderson	Wharton, M.E.	9825	KY 04 01-03	May 4, 1956	EKU(2), KU
Kentucky	Henderson	Marx, P.	691	KY 05 01	April 22, 1973	NCU
Kentucky	Ballard	Athey, R.	3279	KY 06 01&02	April 23, 1976	BRIT, WKU
Kentucky	Fulton	Sewell, M.	360	KY 07 01	April 28, 2001	MU
Kentucky	Fulton	McKinney, L.E.	4068	KY 08 01	April 16, 1990	BRIT
Kentucky	Fulton	Athey, R.	1307	KY 09 01-03	May 1, 1971	NCU, NY, GH
Kentucky	Carlisle	Sewell, M.	362	KY 10 01	April 28, 2001	MU
Kentucky	Carlisle	Wilson, L.M.	287	KY 11 01	May 3, 1975	NCU
Kentucky	Carlisle	Athey, R.	1287	KY 12 01-04	April 10, 1971	NCU, NY, GH, WKU
Kentucky	Hickman	Beck, J.	11	KY 13 01	April 19, 1997	EKU
Kentucky	Hickman	Bryan, C.	153	KY 14 02	May 2, 1975	NCU

State	County	Collector	Collector no.	OTU codes	Date	Herbaria
Kentucky	Hickman	Chuang, T.I.	6856	KY 15 01	April 8, 1971	UC/JEP
Arkansas	Pope	Mathis, G.	27	AR 01 01	April 16, 1970	UC/JEP
Arkansas	Pope	Tucker, G.	8478	AR 02 02	April 26, 1971	UC/JEP
Arkansas	Pope	Luers, M.	59	AR 03 01	April 1, 1976	UARK
Arkansas	Pope	Thompson, M.	43	AR 04 01	April 5, 1977	UARK
Arkansas	Pope	Bourne, T.F.	52	AR 05 01	April 5, 1980	BRIT
Arkansas	St. Francis	McDaniel, S.	722	AR 06 01	April 14, 1957	UARK
Arkansas	St. Francis	McDaniel, S.	10465	AR 07 01&02	April 14, 1968	BRIT(2)
Arkansas	Cleburne	Demaree, D.	64458	AR 08 01	April 8, 1972	BRIT
Arkansas	Crawford	Smith, E.B. & A.S. Sherif	14	AR 09 02&03	March 27, 1980	UARK(2)
Arkansas	Crawford	Hayes, C.	1238	AR 10 01	April 12, 1975	UARK
Arkansas	Franklin	Barber, G.	604	AR 11 01	April 23, 1978	UARK
Arkansas	Hempstead	Sundell, E.	12087	AR 12 01	April 23, 1997	UARK
Arkansas	Independence	Board, V.V.	s.n.	AR 13 01	April 29, 1980	UARK
Arkansas	Jefferson	Locke, M.	2128	AR 14 01	April 8, 1977	UARK
Arkansas	Jefferson	Locke, M.	1300	AR 15 01	March 17, 1974	UARK
Arkansas	Johnson	Moore, D.M.	330042	AR 16 01	April 13, 1933	UARK
Arkansas	Searcy	Beer, M.A.	54	AR 17 01	April 11, 1992	UARK
Arkansas	Stone	Beer, M.A.	55	AR 18 01	April 26, 1993	UARK
Arkansas	Van Buren	Beer, M.A.	69	AR 19 01	March 28, 1991	UARK
Arkansas	Montgomery	Kral, R.	77551	AR 20 01	May 12, 1990	BRIT
Arkansas	Montgomery	Demaree, D.	55109	AR 21 01	April 5, 1967	NKU
Arkansas	?	Nuttall	s.n.		s.d.	UC/JEP(photo of ISOTYPE @ K)
Arkansas	?	Nuttall	s.n.		s.d.	UC/JEP(photo of HOLOTYPE @ BM)
Arkansas	?	Nutthall	s.n.		s.d.	GH(photo of ISOTYPE @ K)
Mississippi	Bolivar	Bryson, C.T.	10551	MS 01 01	April 3, 1991	BRIT
Mississippi	DeSoto	McDaniel, S.	10477	MS 02 01	April 15, 1968	BRIT