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Taxonomy, Distribution, and Lectotypification of Two Rare, Southern Appalachian Saxifrages, *Micranthes careyana* and *M. caroliniana*

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ABSTRACT

Taxonomists have traditionally distinguished two very similar Southern Appalachian endemic herbs, *Micranthes careyana* and *M. caroliniana*, by differences in four floral characters: sepal orientation, filament shape, petal coloration, and fruit length. Yet identification in the field and the herbarium has proven difficult, which is problematic for monitoring populations and determining rarity. The goal of this study was to examine these characters to clarify the differences between these species and their distribution, and to look for molecular differences in DNA sequences. Morphological variation was examined in the field and the herbarium, while leaf material was collected in the field for molecular analyses. Two of the four reported floral characters proved to be useful in species identification: sepal orientation and filament shape. Other key characters were not diagnostic to species. Fixed differences in floral characters were correlated with fixed differences in nuclear and chloroplast DNA sequences, supporting their distinction as unique species in accordance with the diagnosability species concept. In molecular phylogenetic analyses, *M. caroliniana* and *M. careyana* accessions are reciprocally monophyletic and may not be sister species. Both are shown to be closely related to *M. virginiensis*, a widespread and variable taxon. We present a key to identifying *M. careyana*, *M. caroliniana*, and *M. virginiensis* in the Southern Appalachians and lectotypify *M. careyana* and *M. caroliniana*, names based on Asa Gray basionyms.

Key words: Floral morphology, *Micranthes*, molecular phylogenetics, Southern Appalachians, species delimitation

INTRODUCTION

Micranthes careyana (A. Gray) Small, Carey's saxifrage, and *M. caroliniana* (A. Gray) Small, Carolina saxifrage (Saxifragaceae), are two morphologically similar species in the Southern Appalachians. They also grow in similar habitats—shady, moist rocks and cliffs, seepage slopes, damp, moss-covered boulders and rock faces, and along streambanks. Both species are listed as Globally Vulnerable (G3) due to their limited distributions and small known population sizes (NatureServe 2018). However, *M. caroliniana* is considered to be a narrow endemic threatened by potential habitat disturbance and, thus, monitored by state Natural Heritage Programs (NatureServe 2018).

However, conservation botanists from state and federal agencies have expressed difficulty in telling the two species apart (E. Schwartzmann, D. Rankin, J. Kelly, pers.comm.), be it in the field or from pressed specimens, and thus are unsure of the geographic distribution of each. Some State Natural Heritage Program species occurrence records and herbarium sheets from multiple herbaria examined for this project appeared to be *M. careyana* incorrectly identified as *M. caroliniana*. In her taxonomic revision, Lord (1960) noted that the "*Saxifraga* [Micranthes] *careyana*–*Saxifraga* [Micranthes] *caroliniana* complex has long been a puzzle to those working with the Southern Appalachian saxifrages" (p. 57).

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In addition, the widespread and variable *Micranthes virginiensis* (Michx.) Small is also similar in appearance to the two aforementioned taxa, particularly in leaf morphology, although it appears to be rare in the mountains. During this study, we discovered both living populations and herbarium specimens that were difficult to distinguish among these three taxa using existing keys. Because of its widespread distribution, a thorough study of the variability encompassed by *M. virginiensis* is outside the scope of this study; as such, we included only samples of *M. virginiensis* located in the Southern Appalachians to meet our goals.

Gray (1841) first described *Saxifraga careyana* in honor of one of its discoverers, John Carey (1791–1880). Gray cultivated plants collected in the fall of 1843, during another botanical excursion to the mountains of North Carolina, and noted consistent differences in the floral characters of some plants the following spring when they flowered. Realizing he had collected two different species, Gray then (1848) described *Saxifraga caroliniana*, based on differences in sepal orientation, filament shape, and fruit length. These species were later placed in the segregate genus *Micranthes* Haw. by Small (1903). Future authors adopted a broader interpretation and replaced these species in *Saxifraga* L. sensu lato, including Fernald (1950), Lord (1961), Radford et al. (1968), Wofford (1989), and Weakley (2007). Small (1933) also recognized and described yet another similar species, *M. tennesseensis* Small (Golden-eye Saxifrage), known only from the bluffs of the Tennessee River in Knox County, TN. This name was later treated as synonymous with *S. careyana* by Lord (1961), who noted that the two taxa shared many characters including overlapping measurements in petal and fruit length (e.g., fruit length 3.5–5 vs. 4.5–5 mm). Lord retained *S. careyana* and *S. caroliniana* as distinct species based on differences in sepal orientation and filament shape, noting fruit length and petal coloration were not significantly different and therefore not informative characters for identification.

Recent molecular phylogenetic analyses, which validated Small's view, showed that *Micranthes* should be recognized as a genus distinct from *Saxifraga* (Soltis et al. 1993, Johnson and Soltis 1994, 1995, Soltis et al. 1996, Mort and Soltis 1999, Soltis et al. 2001); recent floristic treatments (e.g., Brouillet and Elvander 2009; Weakley 2015) followed suit. Each of these taxonomic treatments have retained *M. careyana* and *M. caroliniana* as distinct species based on differences in floral characters, flowering times, and geographical distributions.

We set out to determine whether the morphological character states used to distinguish *M. careyana* and *M. caroliniana* in the aforementioned treatments were fixed within species and were being correctly applied, as well as to describe more precisely the phenology and distribution of these species. We also wanted to see if we could correlate morphological differences with fixed molecular character states. The goals of this study were to determine whether the taxonomic status of these similar species could be clarified using field and herbarium observations of morphology, flowering times, and geographical distribution, and whether phylogenetic analyses based on DNA sequences could be used to distinguish the species, especially if morphology could not. For these goals, we adopted the diagnosability species concept (Cracraft 1989, Nixon and Wheeler 1990), which defines a species as the smallest cluster of populations or lineages displaying a unique set of fixed character states.

Finally, lectotypification of both names, *M. careyana* and *M. caroliniana*, is necessary because Gray did not cite specimens nor designate a holotype in the protologue for either. Lord (1960) did not select lectotypes in her study, nor did Brouillet and Gornall (2007) lectotypify *Micranthes* taxa for which combinations were previously made by Small; therefore, we herein designate lectotype specimens for both *M. careyana* and *M. caroliniana*.

MATERIALS AND METHODS

Plant Material

Leaf material from each species was collected in the field between April and October of 2008 and 2009, and preserved either as frozen (-70°C) or silica gel-dried tissue, along with whole plant specimens that were deposited as herbarium vouchers (Table 1). Voucher specimens were deposited at Western Carolina University Herbarium (WCUH). Leaf material was collected from at least one

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Table 1. New accessions of *Micranthes* (Saxifragaceae) made for this study and four outgroup sequences obtained from Genbank or from D. Soltis (unpubl.). Species determinations for new accessions were confirmed at the end of this study. Numbers in front of population names correspond to numbered accessions in the phylogenetic trees. All voucher specimens collected for this study are deposited at WCUH. Voucher information for outgroup sequences was obtained from Soltis et al. (2001).

Taxon	Population Name	County/State	Voucher #	Genbank # ITS	Genbank # *trn*L-F spacer
M. careyana	Nantahala Gorge 1	Swain/NC	Mathews s.n. (2007)	MK676016	
	Nantahala Gorge 2	Swain/NC	ML 015	MK676015	MK682306
	Slickrock Trail	Graham/NC	ML 030	MK676013	
	Elkhollow Branch	Avery/NC	ML 032	MK676025	
	Cullasaja Falls	Macon/NC	ML 034	MK676019	
	Crow Creek	Macon/NC	ML 035	MK676018	
	Clawhammer Mtn.	Transylvania/NC	ML 037	MK676033	
	Ivy River Bluffs	Madison/NC	ML 053	MK676042	
	Pigeon River Gorge	Haywood/NC	ML 054	MK676031	MK682317
	Ijam's Nat. Center 1	Knox/TN	ML 057	MK676044	
	Ijam's Nat. Center 2	Knox/TN	ML 059	MK676028	
	Little River Gorge	Blount/TN	ML 060	MK676043	
	Grotto Falls	Sevier/TN	Stoehrel s.n. (2009)	MK676014	
	Cliffridge	Macon/NC	ML 064	MK676035	
	Gouges Creek Falls	Mitchell/NC	ML 067	MK676030	MK682310
	Profile Trail	Avery/NC	ML 072	MK676041	
	Linville Gorge	McDowell/NC	ML 074	MK676032	MK682311
M. caroliniana	Mt. Jefferson	Ashe/NC	ML 024	MK676022	MK682305
	New River St. Park	Alleghany/NC	ML 025	MK676036	MK682315
	Shady Valley	Johnson/TN	ML 044	MK676021	MK682314
	Howard Creek Falls	Watauga/NC	ML 070	MK676040	
M. micranthidifolia	(1) Fisher Creek	Jackson/NC	ML 2008a	MK676020	MK682307
	(2) Elkhollow Branch	Avery/NC	ML 2008b	MK676039	
M. pensylvanica	Tater Hill Bog	Watauga/NC	ML 022	MK676038	MK682308
M. petiolaris	(1) Silver Run Falls	Jackson/NC	ML 013	MK676027	
	(2) Cedar Rock Mtn.	Pickens/SC	ML 017	MK676026	
	(3) Mt. Jefferson	Ashe/NC	ML 023	MK676029	
	(4) Whiteside Mtn.	Macon/NC	ML 026	MK676024	MK682304
M. virginiensis	Village Creek	Jefferson/AL	ML 045	MK676034	MK682309
	Melrose	Polk/NC	ML 055	MK676023	MK682312
	Wadakoe Mtn.	Pickens/SC	ML 018	MK676037	MK682313
Unknown sp.	Gap Creek Rd.	Greenville/SC	ML 056	MK676017	MK682316
M. integrifolia	n/a	Pacific NW of North America	Soltis & Soltis 2253	D. Soltis et al., unpubl.	AF374801
M. punctata	n/a	Pacific NW of North America	Soltis & Soltis 2217	D. Soltis et al., unpubl.	AF374800
M. stellaris	n/a	Pacific NW of North America	Horandl 2703	AF374827 (ITS1)/ AF374828 (ITS2)	AF374802
M. tolmiei	n/a	Pacific NW of North America	WS 32167	D. Soltis et al., unpubl.	AF374799

population of each presumed species in the following counties: Alleghany, Ashe, Avery, Graham, Haywood, Macon, Madison, McDowell, Mitchell, Polk, Transylvania, Watauga (North Carolina); Greenville and Pickens (South Carolina); Blount, Knox, Johnson, and Sevier (Tennessee). A total of 21 accessions representing 17 *M. careyana* populations and four *M. caroliniana* populations were

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obtained from the field (Table 1). In addition, numerous herbarium specimens of the two species from the following herbaria were examined either from specimen loans or digital image files: CLEMS, GH, NCSC, NCU, NY, TENN, UTCH, VPI, WCUH, WILLI, and WVA (Appendix).

Morphology and Phenology

The states of three floral characters (sepal orientation, filament shape and petal coloration) were observed for each population of *M. careyana* and *M. caroliniana* visited in the field (Table 1, Figure 1). We observed that in some populations, sepal orientation ranged from curved upward between the petals to curved downward, but never fully reflexed backward against the pedicel. These populations were scored as "sepals: spreading." In other populations, open flowers had fully reflexed sepals, i.e., pressed backward against the pedicel. These populations were scored as "sepals: reflexed." We defined subulate filaments as tapering from a broader base to a very fine point, with the broadest part at the proximal end, and clavate filaments as having a noticeable thickening toward the distal end of the filament before tapering to a very fine point. The distal, clavate thickening is most visible under 10x or greater magnification. Either all petals of a flower were white with no spots, or all petals had two yellow spots near the base. In addition to these floral characters, fruit length was measured to the nearest mm with a metric ruler using a stereo microscope on 21 herbarium specimens (Appendix). Five capsules per specimen were measured and lengths per specimen were averaged. A two-tailed t-test (assuming equal variance) was conducted to determine if there was a significant difference in the means of fruit size.

Herbarium specimens were also used to document population county localities and to determine flowering times by recording label information and the phenophase visible on the specimen. For phenophase observations, non-duplicate specimens of *M. careyana* and *M. caroliniana* were used (Appendix), and specimens bearing open flowers with petals present were recorded as flowering on the collection date given. The number of specimens flowering was then plotted against the date.

DNA Isolation, Amplification, and Sequencing

Leaf tissue from fresh, frozen, silica gel-dried materials or herbarium vouchers was ground to powder in liquid nitrogen using either a mortar and pestle or the BioMasher (Omni Intl., Kennesaw, GA) mini-pestle with a hand-held electric drill, and total DNA was isolated using the DNeasy Plant Mini Kit (Qiagen, Germantown, MD). Total DNA concentrations achieved were quantified using a NanoDrop ND-1000 Full-spectrum UV/Vis spectrophotometer and run on a 1% w/v agarose gel including 3 µl of ethidium bromide (10 mg/ml) for visualization on a UV light box. The nrITS DNA region was amplified using external primer pairs N-nc18S10 and C26A (Wen and Zimmer 1996) following Johnson and Soltis (1998). The cpDNA *trn*L-*trn*F intergenic spacer region was amplified using external primer pairs *trn*L-F*f* and *trn*L-F*c* following the protocol of Soltis et al. (2001). Fifty-microliter PCR reactions were prepared using 1x TaqMaster PCR Enhancer (Eppendorf, Hauppauge, NY), 1x buffer, 0.4 mM dNTP, 1.25 mM $MgCl^2$, 0.4 µM each primer and 0.025 U Taq polymerase to which was added 1 µl of diluted (1/10, 1/50, or 1/100) DNA template with concentration ≤ 30 ng/µl.

Amplifications for the ITS region were performed as follows: one denaturing cycle of 3 minute at 95°C; 30 cycles of 1 minute denaturing at 95°C, 1 minute annealing at 45°C, and a 1 minute and 20 second extension at 72°C; followed by a final extension of 5 minutes at 72°C. Amplifications for the *trn*L-*trn*F region were performed as follows: one denaturing cycle of 2 minutes at 95°C; 30 cycles of 50 seconds denaturing at 95°C, 50 seconds annealing at 50°C, and a 1 minute and 50 second extension at 72°C; followed by a final extension of 7 minutes at 72°C.

PCR products were again visualized on a 1% w/v agarose gel containing ethidium bromide and a DNA ladder for sizing, then cleaned and prepared for sequencing using the QIAquick PCR Purification Kit (Qiagen). Concentrations of PCR products were quantified using NanoDrop ND-1000 to determine the amount of DNA template to be used in sequencing reactions. Ten µl sequencing reactions were prepared with 1 µl DNA template with a concentration ≤ 30 ng/µl, 4 µl Big Dye premix (Applied Biosystems, Foster City, CA), 3.2 µl of a 1 µM solution of each primer (the same

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Figure 1. Morphological characters distinguishing *Micranthes careyana* (A. Gray) Small and *M. caroliniana* (A. Gray) Small (Saxifragaceae). (a, b) *Micranthes careyana*, with spreading sepals and subulate filaments (image of living plant from Nantahala Gorge, Swain Co., NC, and of herbarium specimen from K.I. Miller & I.W. Carpenter 1390 [WILLI]). (c, d) *Micranthes caroliniana*, with reflexed sepals and clavate filaments (image of living plant from New River State Park, Alleghany Co., NC, and of herbarium specimen from G.P. Fleming 7959 [WILLI]). Arrows indicate the widest portion of the filament.

primer pairs were used for sequencing as for amplification) and 1.8 µl ddH_2O. Cycle-sequencing reactions were performed with an initial denaturing cycle of 60 seconds at 96°C; followed by 24 cycles of denaturing for 10 seconds at 96°C; annealing for 5 seconds at 50°C; extension for 4 minutes at 60°C; followed by an indefinite hold at 4°C. Reactions were purified by an EtOH-NaOAc precipitation or Sephadex columns (Illustra GE Healthcare, Marlborough, MA) and dried using a vacuum centrifuge. Dried sequencing reactions were resuspended in 10 µl of Hi-Di formamide (Applied Biosystems, Foster City, CA) by vortexing for 10 seconds. Reaction tubes were incubated at 95°C for five minutes to denature DNA. Tubes were then snap-chilled in ice for at least two minutes to prevent re-annealing of the DNA strands. The entire 10 µl volume of denatured samples was loaded into a 96-well reaction plate and electrophoresed on a 4-capillary 3130 Genetic Analyzer (Applied Biosystems, Foster City, CA). Resulting chromatograms were visualized using the Sequencing Analysis software (Applied Biosystems, Foster City, CA) and downloaded for further analysis.

Sequence Alignments and Phylogenetic Analyses

Forward and reverse primer sequences for each sample were uploaded into and viewed in the sequence editing program Sequencher (GeneCodes Corp., Ann Arbor, MI) to compare and confirm the

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sequences of the opposite complementary strands of each sample and combine into a consensus sequence. All sequences have been deposited in GenBank (**www.ncbi.nlm.nih.gov**; Table 1). A large ITS data set was created using all sequences of Southern Appalachian species generated for this study as well as outgroup sequences obtained from GenBank (Table 1). A reduced-taxon data set was generated with cpDNA data from among the same accessions chosen to represent the range of variation found in the ITS results for *M. careyana* (4/17 accessions) and three accessions each of *M. caroliniana* (3/4) and *M. virginiensis* (3/3), as well as one accession of each outgroup taxon. Sequences were loaded into ClustalX (Thompson et al. 1997) for a complete alignment and generation of a Nexus file of aligned sequences and gaps.

The FindModel web implementation (**http://hiv.lanl.gov/content/sequence/findmodel/findmodel.html**) based on ModelTest (Posada and Crandall 1998) was used to determine the best fit model of molecular evolution for each dataset. Maximum parsimony phylogenetic analyses were performed by loading all sequences into PAUP* (Swofford 2003) to run a heuristic parsimony search on the data using 100 replicates of random taxon addition and TBR branch swapping. One hundred bootstrap (Felsenstein 1985) replicates were run to obtain measures of confidence for the clades recovered.

Bayesian phylogenetic analyses were run using MrBayes 3.2.6 (Ronquist and Huelsenbeck 2003) on the CIPRES Science Gateway v. 3.3 server (Miller et al. 2010). using six simultaneous runs of Metropolis-coupled Markov Chain Monte Carlo (MC3). Model settings for the ITS dataset were Nst=2 and Rates=gamma, corresponding to HKY+G; model settings for *trn*L-*trn*F were Nst=6 and Rates=gamma, corresponding to GTR+G (see Results below). For the combined data analysis, data were not partitioned, and model settings corresponded to GTR+G. All other parameters were left on the default settings. Each chain was run in parallel for six million generations, saving one tree each 1,000th generation, keeping a default "temperature" parameter value of 0.2. The MC3 runs were repeated twice, and the first 10% of the saved trees were discarded as burn-in after checking for (i) stationarity on the log-likelihood curves; (ii) similarity of the respective majority-rule topologies and final likelihood scores; and (iii) the value of the potential scale reduction factor (close to 1). The remaining trees were used to produce a majority-rule consensus tree and to calculate the posterior probability (pp) values.

RESULTS

Morphology

Field observations of *M. careyana* and *M. caroliniana* populations support diagnostic differences in only two of the four reported floral/fruit characters: sepal orientation and filament shape. Populations in Avery, Graham, Haywood, Macon, Madison, McDowell, Mitchell, Polk, Swain, and Transylvania Counties in NC and Blount, Knox, and Sevier Counties in TN, exhibit a spreading sepal orientation and subulate filaments (Figure 1a, b). In contrast, populations in Alleghany, Ashe, and Watauga Counties of NC and Johnson County, TN exhibit a reflexed sepal orientation and clavate filaments (Figure 1c, d). All of the populations examined possessed flowers with two yellow spots on each petal.

Mean fruit size, as measured on herbarium specimens, did differ slightly between species (*M. careyana*=3.61 mm; *M. caroliniana*=4.14 mm; t=0.54; p=0.04). However, the ranges observed for both species was completely overlapping (3–5 mm), with size variation present within individuals and populations (Figure 2). Therefore fruit size is not a reliable character for discriminating these taxa in the field.

Phenology

Forty-eight specimens of *M. careyana* and 14 specimens of *M. caroliniana* were recorded as flowering. There appears to be a difference in peak flowering times between the two, but with overlap (Figure 3). *Micranthes careyana* starts flowering earlier and has a longer bloom period, with flowering specimens from March 25 through July 12. This corresponds to its more southern and broader distribution (Figure 6). The three latest flowering specimens, in June–July, were also in

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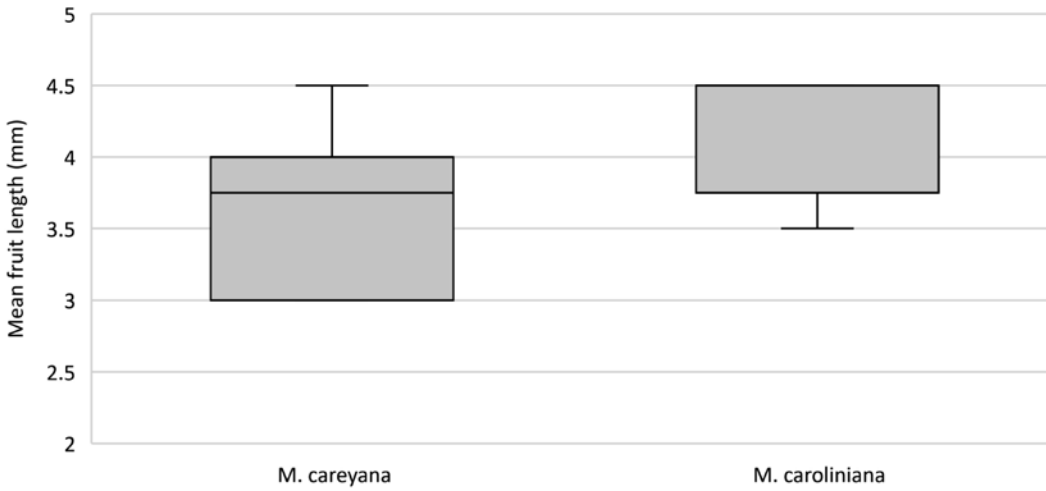

Figure 2. Mean fruit length per herbarium specimen for *Micranthes careyana* (A. Gray) Small and *M. caroliniana* (A. Gray) Small. Species identifications were based on distinguishing floral characters identified in this study.

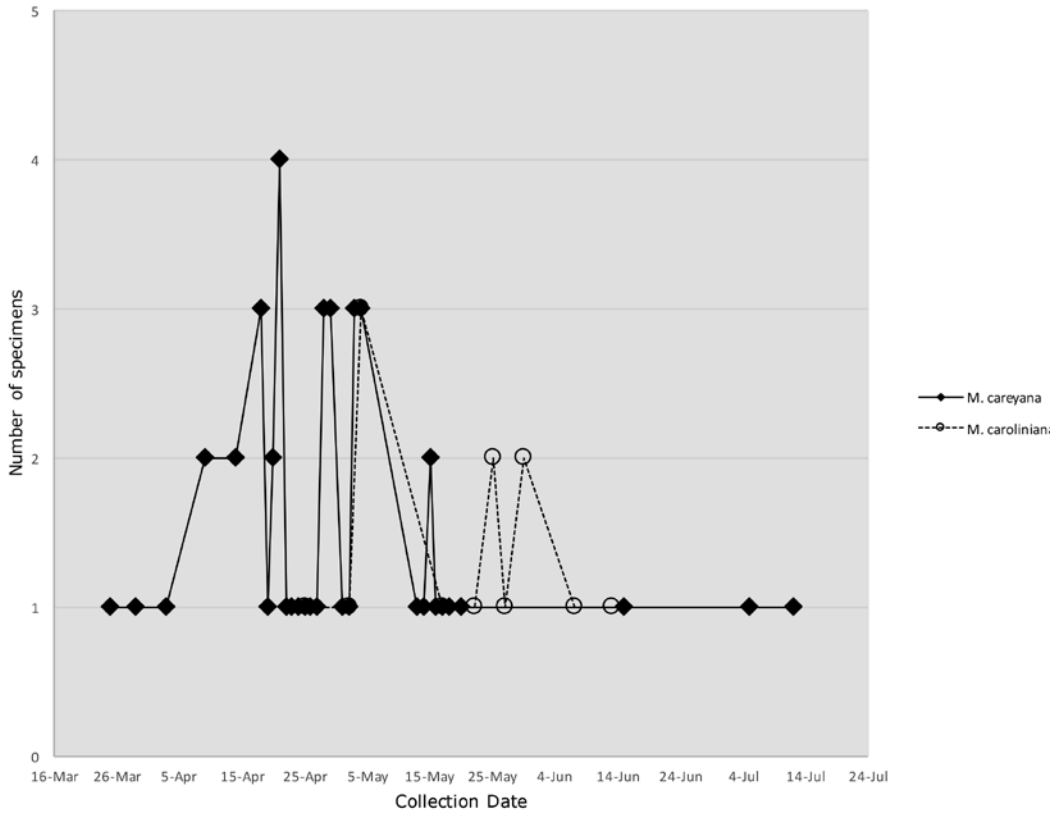

Figure 3. Flowering times recorded from herbarium specimens of *Micranthes careyana* (A. Gray) Small and *M. caroliniana* (A. Gray) Small. Species identifications were based on distinguishing floral characters identified in this study.

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fruit and were from some of the highest elevations in its distribution. *Micranthes caroliniana* has a shorter bloom period that starts later, with flowering specimens from April 25 to June 13. This corresponds to its more northern, narrower distribution.

Phylogeny

***nrITS*—**The aligned ITS data matrix contained 36 taxa and 711 total characters, 214 of which were parsimony informative (30%). Parsimony analysis of the large ITS data set yielded 144 shortest trees of length 739 (CI excluding uninformative characters = 0.5946; RI = 0.7692). The model selected for the nrITS dataset was HKY+G (LnL = -2501.753109, AIC = 5013.506218). The maximum parsimony strict consensus tree is shown in Figure 4. Both the MP and Bayesian analyses showed strong support for the reciprocal monophyly of *M. careyana* populations (BS=90%, pp=1) and *M. caroliniana* populations (BS=100%, pp=1). One accession from Greenville, SC ("Gap Creek Rd") was sister to the *M. caroliniana* populations, with moderate support (BS=73%, pp=0.9); this population is outside of the expected range of *M. caroliniana* and was unidentifiable at the time of collection. There was moderate support for the monophyly of the *M. virginiensis* populations (BS=62%, pp=0.98); both the Pickens Co., SC ("Wadakoe Mtn.") and Greenville Co., SC ("Melrose") populations were also difficult to identify in the field, but consistently grouped with typical *M. virginiensis* from Jefferson Co., AL ("Village Creek"). Finally, there was strong support (BS=95%, pp=1) for the monophyly of *M. careyana*, *M. caroliniana* and *M. virginiensis* together, but relationships among the three taxa could not be resolved in the Bayesian analysis and a sister group of *M. caroliniana* and *M. virginiensis* reconstructed in the maximum parsimony analysis had less than 50% bootstrap support.

***trn*L-F—**The aligned *trn*L-F data matrix contained 18 taxa and 1,585 total characters, 279 of which were parsimony informative. Parsimony analysis yielded two shortest trees of length 1011 (CI excluding uninformative = 0.6726; RI = 0.6558). The model selected for the *trn*L-F dataset was the 3-rate Tamura-Nei+Gamma model (LnL = -3392.425573, AIC = 6796.851146). The maximum parsimony strict consensus tree is shown in Figure 5. There was moderate to strong support for the monophyly of *M. careyana* populations (BS=79%, pp=1), but not for the *M. caroliniana* populations. Rather, the Ashe Co., NC ("Mt. Jefferson") population was resolved as sister to a clade of all other accessions of *M. caroliniana* + *M. careyana* + *M. virginiensis* with strong support (BS=99%, pp=1), while the other two accessions of *M. caroliniana* formed a strongly-supported clade (BS=100%, pp=1). The latter was sister to *M. careyana* + *M. virginiensis* + the unidentified "Gap Creek Rd" accession, but with low support (BS=68%, pp=0.83). The monophyly of the *M. virginiensis* populations was moderately to strongly-supported (BS=85%, pp=1). The parsimony analysis could not resolve relationships among *M. careyana*, *M. virginiensis* and "Gap Creek Rd," while the Bayesian analysis showed *M. careyana* and *M. virginienesis* to be sister taxa with low support (pp=0.8; not shown in Figure 5) to the exclusion of the "Gap Creek Rd" population.

***Combined dataset*—**The well-supported monophyly of all accessions of *M. caroliniana* in the ITS tree was not recovered by the *trn*L-F dataset, and a weakly-supported sister group relationship between *M. careyana* and *M. virginiensis* found in the *trn*L-F tree was not recovered by the ITS dataset. As these were not conflicting results but rather lack of evidence in one dataset for results found in the other, we combined the datasets and repeated the analyses. The aligned *nr*ITS+*trn*L-F data matrix contained the same 18 taxa as the *trn*L-F dataset and 1,721 total characters, 315 of which were parsimony informative (18%). Parsimony analysis yielded three shortest trees of length 1071 (CI excluding uninformative = 0.6629; RI = 0.6226). The combined data consensus tree topology is the same as the *trn*L-F tree (Figure 5). In both parsimony and Bayesian analyses, *M. caroliniana* + *M. careyana* + *M. virginiensis* form a clade with moderate to strong support (BS=88%, pp=1). The Bayesian analysis recovers a clade of all *M. caroliniana* accessions, but with weak support (pp=0.6, not shown in Figure 5) which is sister to the others (pp=1), while the parsimony analysis shows two accessions of *M. caroliniana* as sister to the others (BS=54%), to the exclusion of the Mt. Jefferson population. The monophyly of both *M. careyana* and *M. virginiensis* are supported in both analyses (BS=64%, pp=1; BS=79%, pp=1, respectively). The Bayesian analysis recovers a

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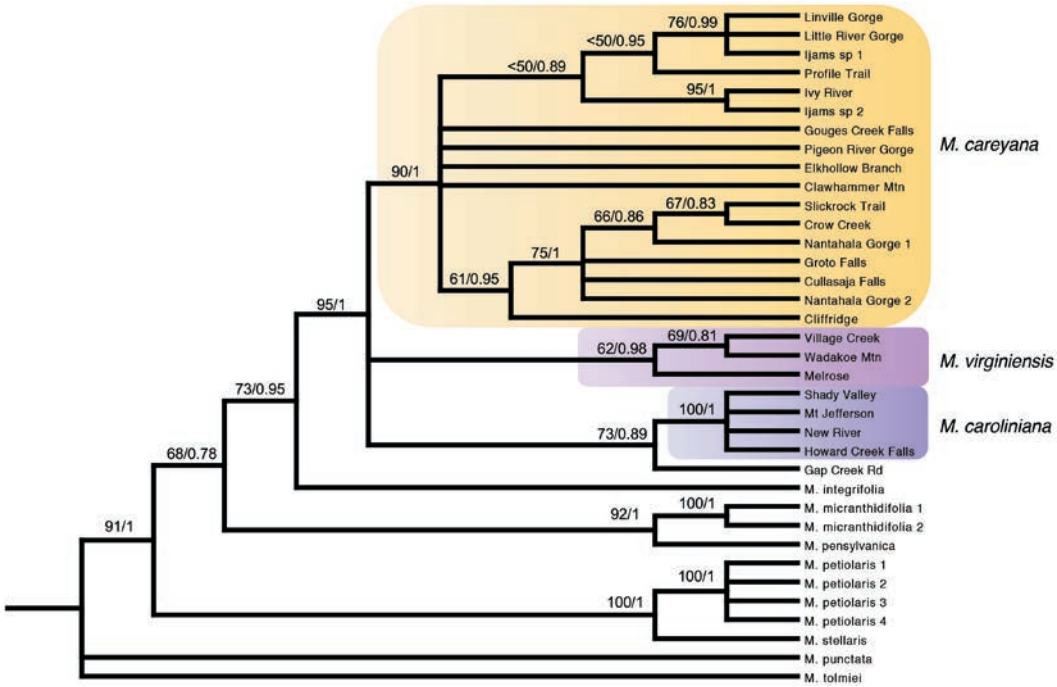

Figure 4. Phylogenetic results based on nrITS sequences of the large dataset of *Micranthes careyana* (A. Gray) Small, *M. caroliniana* (A. Gray) Small, *M. virginiensis* (Michx.) Small and outgroups representing the maximum parsimony strict consensus of 144 trees. Numbers above branches are parsimony bootstrap (bs) percentages/Bayesian posterior probabilities (pp). Branches supported by both less than 50% bs and less than 0.80 pp were collapsed.

weakly-supported sister-group relationship between *M. careyana* and *M. virginiensis* to the exclusion of the "Gap Creek Rd" unknown species (pp=0.59, not shown in Figure 5).

DISCUSSION

Taxonomic Boundaries

The presence of both morphological diagnostic characters and monophyly based on molecular data (ITS) for the "Shady Valley," "Mt. Jefferson," "New River" and "Howard Creek Falls" populations, determined to represent *M. caroliniana*, support the status of this taxon as distinct from *M. careyana* in accordance with the diagnosability species concept. Completely reflexed sepals at anthesis and clavate filaments can be used as morphological characters to identify *M. caroliniana*, vs. spreading sepals and subulate filaments in *M. careyana*. Petal coloration and fruit size are not informative characters for distinguishing these two species. Misidentification of these two species seems to stem from misinterpretation of the characters used in dichotomous keys, rather than the characters themselves being unreliable. *Micranthes careyana* can exhibit a downward-curving sepal orientation during late anthesis that is often interpretated as "reflexed" and subsequently leads to its misidentification as *M. caroliniana*. In addition, these two species are geographically discrete and phenologically divergent, with *M. caroliniana* peak flowering at a slightly later date.

Phylogeny

Analyses of DNA sequence data also support the close relationship of *M. careyana* and *M. caroliniana* with *M. virginiensis*, while sister-group relationships among these three taxa are still unclear. In a molecular phylogenetic study encompassing the entire genus *Micranthes* and using the same two gene regions, with one accession each of *M. careyana*, *M. caroliniana* and *M. virginiensis*, Tkach et al. (2015) also found a tritomy of these three taxa.

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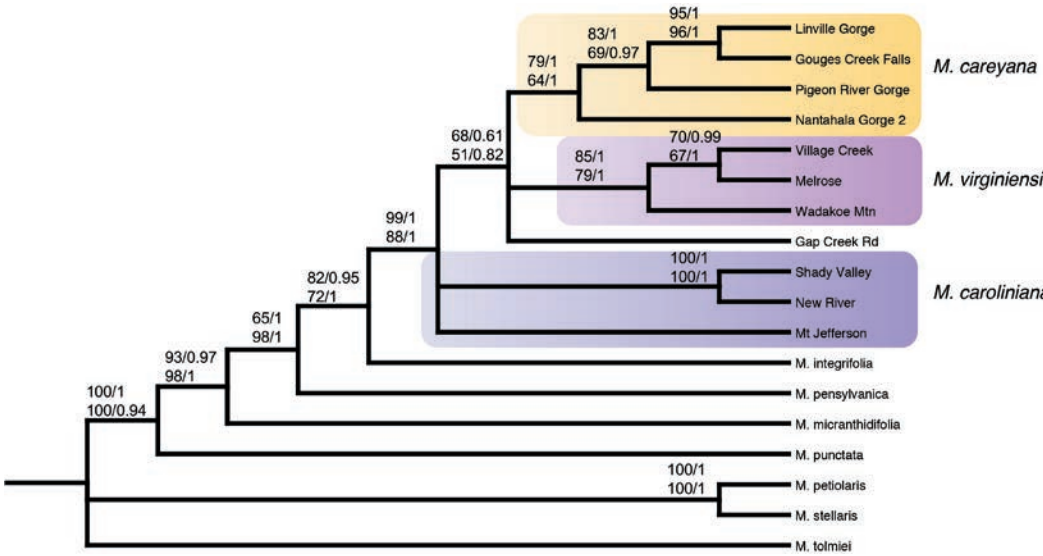

Figure 5. Phylogenetic results based on *trn*L-F cpDNA sequences of the reduced dataset of *Micranthes careyana* (A. Gray) Small, *M. caroliniana* (A. Gray) Small, *M. virginiensis* (Michx.) Small and outgroups representing the maximum parsimony (MP) strict consensus of 2 trees. Numbers above branches are parsimony bootstrap (bs) percentages/ Bayesian posterior probabilities (pp), with the *trn*L values above the combined data values. Branches supported by both less than 50% bs and less than 0.80 pp were collapsed.

Micranthes virginiensis is a broadly distributed taxon in eastern North America, possibly containing cryptic species, and in need of revision. It can be found in the north from New Brunswick west to Manitoba, and south to central Georgia, west to Louisiana and Alaska (Weakley 2015). *Micranthes virginiensis* was not the focus of this study; however, during fieldwork, Lanning noticed unusual floral characteristics in populations from the three adjacent fall-line counties of Polk Co., NC, and Greenville Co. and Pickens Co., SC. Flowers from individuals of the Polk Co., NC ("Melrose") population have unfused sepals, reflexed petals, and nodding flowers, while typical *M. virginiensis* displays partially fused sepals that form a short hypanthium and upright flowers. Otherwise, the "Melrose" population exhibits typical *M. virginiensis* characteristics compared to the other taxa, including longer petals, shorter and subulate filaments, and lack of yellow petal spots. Both the South Carolina populations ("Gap Creek Rd" and "Wadakoe Mt.") were previously identified as *M. virginiensis* (P. McMillan, pers. comm.), but were difficult to confirm at the time of collection due to a late observation of the flowering state. Flowers of both populations showed spreading sepals, subulate filaments, long stamens (ca. 3–3.5 mm vs. 1–1.5 mm in typical *M. virginiensis*), and large fruit size (4–4.5 mm), as in *M. careyana*, but lacked petal spots, as in *M. virginiensis*. Petal spots are difficult to use to identify herbarium specimens, as the spots often fade upon drying. Also, the hypanthium of *M. virginiensis* can be inconspicuous on herbarium specimens, as the flowers spread upon pressing, but there is usually a visible transverse ridge on the calyx tube indicating the adnation point. The "Wadakoe Mt." accession clusters with typical *M. virginiensis* in the phylogenetic analyses, but the "Gap Creek Rd" population does not. Rather, it groups with *M. caroliniana* accessions in the ITS analysis and inside the *M. careyana* + *M. virginiensis* clade in the cpDNA and combined analyses. These results suggest the possibility of a hybrid origin for the "Gap Creek Rd" population. All the populations of supposed *M. virginiensis* in this geographic area deserve further study. Only the Jefferson Co., AL ("Village Creek") population was observed to possess all the typical *M. virginiensis* morphological characteristics in this study.

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Distribution

Both *M. careyana* and *M. caroliniana* species are contained within the Southern Appalachian mountains and are peripatric in distribution, with both occurring in Watauga Co., NC, but otherwise not overlapping (Figure 6). *Micranthes careyana* extends from Murray Co., GA to Scott Co., VA, with most populations found in western North Carolina and eastern Tennessee (Blue Ridge, Valley and Ridge and Cumberland Plateau physiographic provinces), where it is found over granitic or sedimentary rock formations. It ranges from 335–1,280 m elevation, with outliers up to 1,768 m (Craggy Dome, Buncombe Co., NC) and 1,920 m (Roan Mountain, Mitchell Co., NC). Murray County is the only known *M. careyana* locality in northern Georgia (seepy boulderfield, Fort Mountain State Park; Appendix); additional populations may be discovered in similar habitats in northern Georgia, northeast Alabama, and northwestern South Carolina.

Micranthes careyana's range abuts that of *M. caroliniana* to the north/northeast. *Micranthes caroliniana* is endemic to the Blue Ridge mountains of Alleghany Co., Ashe Co., and Watauga Co., NC, Johnson Co., TN, eight counties in western Virginia, and McDowell and Wyoming Co., WV. In North Carolina, it is associated with the mountainous region known as the Amphibolites, characterized by amphibolite-gneiss bedrock (rich in hornblende) and circumneutral soils, as opposed to the acidic, gneiss-derived soils more typical of the Blue Ridge.

In the Southern Appalachians, *M. virginiensis* is most common in the Ridge and Valley physiographic province both east and west of the Blue Ridge and is associated with richer, limestone-derived soils, as well as the amphibolite bedrock found on Wadakoe Mountain in Pickens Co., SC.

Micranthes careyana is currently designated as a Watch List species in North Carolina, and Rare in Virginia, and should remain at the species status, which should ensure its long-term protection. *Micranthes caroliniana* is currently designated as a U.S. Species of Concern, Significantly Rare in North Carolina, Endangered in Tennessee, and a Watch List species in Virginia, and is here confirmed at the species status, which should also benefit its conservation management. Results indicate this species may be even more rare than previously known due to the large number of *M. careyana* populations misidentified as *M. caroliniana* discovered during this study, including seven of the populations included here (Cliffridge, Slickrock, Ivy River, Pigeon River Gorge, Gouges Creek Falls, Elkhollow Branch, and Profile Trail; see Appendix for specimen annotation).

Revised Identification Key and Table of Diagnostic Characters

The following dichotomous key and table of diagnostic characters (Table 2) were constructed based on the results of this study. We included all three taxa discussed here to facilitate the distinction between the two rare species and *M. virginiensis* where it comes into peripatry with the other two.

1. Sepals completely reflexed at anthesis; filaments clavate (use 10x).....***M. caroliniana***
1. Sepals spreading at anthesis; filaments subulate (use 10x)
   2. Hypanthium present; petals not spotted; stamens included, shorter than the petals (1.0–1.5mm); anthers yellow .....***M. virginiensis***
   2. Hypanthium absent; petals each with two yellow spots; stamens exserted, equalling or exceeding the petals (2.5–3.5 mm long); anthers orange.....***M. careyana***

Lectotypification

Saxifraga careyana Gray was published in "Notes of a botanical excursion to the mountains of North Carolina ..." (1841), in which Gray describes finding the new saxifrage on Grandfather Mountain, in Ashe County on July 9, 1841, in the company of Mr. John Carey. No specimens are cited by Gray. There are six specimens in GH whose labels indicate they were collected by Gray and Carey on Grandfather Mountain, NC, in 1841. These have all been designated as possible types in their online database (designator unknown). We have selected the representative specimen below as the lectotype:

Micranthes careyana (Gray) Small, Fl. S.E. U.S. 501. 1903. *Saxifraga careyana* A. Gray, Amer. J. Sci. Arts 42(1): 32, adnot. 1841. TYPE: In monte Grandfather dicto, Carolinae Septentrionalis,

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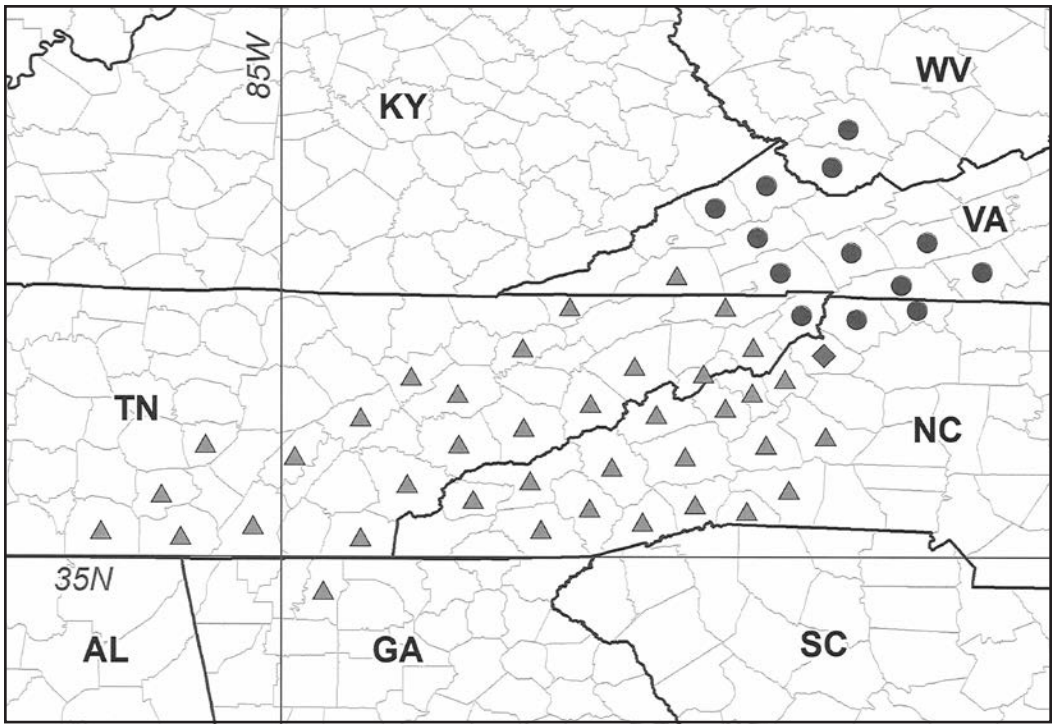

Figure 6. Geographic distributions of *Micranthes careyana* and *M. caroliniana* based on the results of this study. Circles = *M. caroliniana,* triangles = *M. careyana*, diamond = both species (Watauga Co., NC).

Table 2. Diagnostic morphological characters used to distinguish among *Micranthes careyana* (A. Gray) Small, *M. caroliniana* (A. Gray) Small and *M. virginiensis* (Michx.) Small.

Taxon	Hypanthium	Sepal Orientation	Petal Coloration	Stamen Position	Filament Shape	Anther Color
M. careyana	absent	spreading	2 yellow spots	exserted	subulate	orange
M. caroliniana	absent	reflexed	2 yellow spots	exserted	clavate	orange
M. virginiensis	present	spreading	No spots	included	subulate	yellow

Julio 1841, *A. Gray & J. Carey s.n.* (lectotype, here designated: GH [HUH digital image online!], HUH barcode 00043076).

Saxifraga caroliniana Gray was published in 1848, in a paper documenting plants brought into cultivation at the Botanic Garden of Harvard University, Cambridge, MA (Gray indicates it was "communicated to the Academy, January 27th, 1846" p. 1). In the description, Gray states that living plants were gathered by himself in the Alleghany [Appalachian] Mountains of North Carolina in the autumn of 1843. There are three "Hort. Cantab." (referring to the garden in Cambridge) specimens of this species in GH, designated as possible types in their online database (designator unknown), which, according to their labels, were made between 1844–1846. We have selected the representative specimen below as the lectotype:

Micranthes caroliniana (Gray) Small, N. Amer. Fl. 22: 146. 1905. *Saxifraga caroliniana* A. Gray, Mem. Amer. Acad. Arts, n.s., 3:39–40, adnot. 1848. TYPE: Hort. Cantab. Anno 1846 (4–6) (lectotype, here designated: GH [HUH digital image online!], HUH barcode 00043079).

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APPENDIX

Specimens of *Micranthes careyana* (Gray) Small and *M. caroliniana* (Gray) Small examined for this study. Asterisks (*) indicate the specimens used for fruit length measurements; daggers (†) indicate specimens recorded as flowering in the phenology study.

Micranthes careyana (Gray) Small Carey's Saxifrage

GEORGIA. Murray County: *T.E. Govus 1694*, 23 May 2013 (NCU); *T.E. Govus 1695*, 23 May 2013 (GA). **NORTH CAROLINA. Avery County:** †*W.W. Ashe*, 29 Ap 1893 (NCU); *M. Lanning* 032, 7 Aug 2008 (WCUH); *M. Lanning 033*, 7 Aug 2008 (WCUH). **Burke County:** *Campbell, D. s.n.*, 15 Apr 2007 (UNCC); †*D. Pittillo 9187*, 20 Apr 1986 (WCUH). **Buncombe County:** †*A.E. Radford, C.E. Wood, & B.M. Taylor 6947*, 03 May 1953 (NCSC); *A.E. Radford et al. 6947*, 03 May 1953 (NCU); *T. Govus 336 & D. Pittillo*, 5 Sep 1977 (WCUH); †*T. Govus 91 & D. Pittillo*, 8 Jun 1977 (WCUH); †*G. Smathers s.n.*, 2 May 1959 (WCUH). **Graham County:** **A.E. Radford 11893*, 29 May 1956 (NCU); †*D. Pittillo s.n.,* 29 Mar 1981 (NCU); †*R. Johnson 19*, 20 Apr 1985 (WCUH); *†*D. Pittillo 10148*, 22 Apr 1989 (WCUH); †*K. Lathrop 26*, 03 Apr 1993 (WCUH). **Haywood County:** **D. Pittillo & S. McCall 6560*, 15 May 1974 (WCUH); *†*D. Pittillo & B. Dellinger 10247*, 15 May 1989 (WCUH). **Henderson County:** **A.E. Radford & J.G. Haesloop 7116*, 05 Jun 1953 (NCU); †*D. Pittillo 70*, 13 May 1956 (NCU). **Jackson County:** *D. Pittillo & S. Hagar 10719*, 01 Dec 1990 (WCUH). **Macon County:** *†*J.F. Mathews et al. s.n.*, 29 Apr 1967 (NCU); **D. Pittillo 7529*, 12 Jun 1977 (WCUH); †*M.S. Lanning 016*, 9 Apr 2008 (WCUH). **Madison County:** †*H.E. Ahles w/ J.A. Duke 38922*, 26 Apr 1958 (NCU); †*D. Sather 994*, 18 Apr 1980 (NCU); *†*D. Sather 1264*, 04 Jun 1981 (NCU); †*K. Caldwell* 36, 20 May 2006 (WCUH). **McDowell County:** **T. Govus & D. Pittillo 112*, 08 Jun 197 (NCU). **Mitchell County:** †*J.W. Chickering Jr. s.n.*, 05 Jul 1880 (NCU, TENN); **R. Brown 566515*, 22 Jun 2000 (NCU); *unknown coll.*, 04 May (NCU); †*T. Govus 74 & D. Pittillo*, 16 May 1977 (WCUH). **Polk County:** *W.W. Ashe*, 23 April 1916 (NCU); *D. Pittillo 7652*, 27 July 1978 (WCUH). **Rutherford County:** †*H.A. Ahles w/ C.R. Bell 11256*, 21 Apr 1956

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(NCU); †*D. Pittillo 6749*, 25 Apr 1975 (WCUH); †*H. McIver 5*, 27 Apr 1981 (NCU); †*H. McIver* 7, 28 Apr 1981 (NCU). **Swain County:** **A.E. Radford & J.G. Haesloop 7232*, 07 Jun 1953 (NCU); †*D. Pittillo 2898*, 21 Apr 1968 (WCUH); †*T. Kiser III 159*, 18 Apr 1971 (WCUH); †*D. Pittillo* 3582, 18 Apr 1971 (WCUH); †*H.R. Ramsey 040*, 1 May 1971 (WCUH); †*D. Pittillo 11789*, 14 Apr 1995 (WCUH); *D. Pittillo & B. Dellinger 10286*, 18 May 1989 (WCUH); †*K. Carlson 28*, 29 Apr 1995 (WCUH); †*M.S. Lanning 015*, 9 Apr 2008 (WCUH). **Transylvania County:** *M. Lanning 037 & D. Pittillo*, 16 Oct 2008 (WCUH). **Watauga County:** †*H.A. Ahles w/ R.P. Ashworth 39514*, 04 May 1958 (NCU); †*K.I. Miller & I.W. Carpenter 1390*, 03 May 1968 (NCU, WCUH, WILLI). **Yancey County:** †*D. McLeod 1187*, 28 Apr 1971 (NCU). **TENNESSEE. Anderson County:** *W.H. Ellis 28738*, 15 Jun 1961 (TENN). **Blount County:** *J.B. Kinsey*, 07 May 1932 (TENN); *A.J. Sharp 386*, 29 Apr 1934 (TENN); *S.A. Cain & W.H. Duncan 165-2*, 25 Apr 1934 (TENN); *H.M. Jennison 376*, 29 Apr 1934 (TENN); *A.J. Sharp 402*, 13 May 1934 (TENN); *A.J. Sharp 13451*, 08 May 1950 (TENN); *R. Athey s.n.*, 23 Apr 1969 (TENN); L.*R. Phillippe 2629*, 14 Apr 1974 (TENN); *B.E. Wofford & D.K. Smith 98-6*, 26 Mar 1998 (TENN). **Carter County:** †*E. Schell*, 02 May 1984 (TENN). **Cocke County:** *A.J. Sharp, E. Sharp & H. Silva 17256*, 18 Apr 1953 (TENN); †*R.D. Thomas et. al 22918*, 21 Apr 1971 (NCU). **Franklin County:** *H.K. Svenson 7633*, 29 Apr 1936 (TENN); *R.E. Shanks 1847*, 20 May 1946 (TENN); †*D.W. Krickbaum s.n.*, 23 Apr 1959 (NCU). **Grainger County:** *V. Cook M-23*, 09 May 1934 (TENN). **Greene County:** *B.E. Wofford & B. Boom 79-43*, 17 Apr 1979 (TENN); *B.E. Wofford 79-92*, 10 May 1979 (TENN). **Grundy County:** **R.C. Clark 1805*, 15 May 1965 (NCU). **Hamilton County:** *J.R. Churchill*, 08 May 2006 (TENN); †*E. McGilliard s.n.*, 14 Apr 1933 (UCHT); †*S. Huskins 517*, 25 Mar 2007 (UCHT.) **Hancock County:** *A.J. Sharp, G. Ramsey & D. Stone 32673*, 04 May 1964 (TENN). **Knox County:** †*A. Ruth 50*, 10 Apr 1885 (TENN); May 1894 (TENN); *G. Harrill 1*, 1896 (TENN); *S.C. Fain*, 22 Mar 1925 (TENN); *M.B. Wilson*, 25 Apr 1931 (TENN); *D.C. Bain 5*, 29 Mar 1933 (TENN); *D.C. Bain 15*, 02 Apr 1933 (TENN); *V. Cook 16*, 11 Apr 1934 (TENN); *M. Dickson 10*, 11 Apr 1934 (TENN); *N. Hodges 1*, 11 Apr 1934 (TENN); *N. McCarroll M3*, 11 Apr 1934 (TENN); *M.B. Wilson & H.M. Jennison 255*, 11 Apr 1934 (TENN); *A. Morrison 37-49*, 28 Mar 1937 (TENN); *L. Lawhorne*, 12 Apr 1939 (TENN); *A.J. Williams*, 27 Apr 1943 (TENN); *C. Anderson*, 29 Apr 1943 (TENN); *M. Case & P. Kolter*, 28 Apr 1949 (TENN); *F.W. Woods, A.J. Sharp, R.E. Shanks, S.A Dow & F.H. Norris 15159*, 06 Jun 1950 (TENN); *S. Bayston*, 21 Apr 1951 (TENN); *A.A. Ichida 6*, 15 Apr 1954 (TENN); *G.C. Jones, C.R. Varner & J.S. Pringle 27822*, 13 Apr 1961 (TENN); *G. Bresowar 36*, 15 Apr 2004 (TENN). **Marion County:** *J.K. Underwood 710*, 06 May 1934 (TENN); *H.K. Svenson 10064*, 13 May 1939 (TENN); †*R.C. Clark w/ L. Mays 1703a*, 14 May 1965 (NCU); *A.M. Evans & E. Schell 4319*, 22 May 1984 (TENN); *J. Beck 6130*, 2004 (TENN); †*J. Beck 3162*, 19 Apr 1999 (UCHT); †*J. Beck 1597*, 24 Apr 1998 (UCHT); *E. Bridges 304 & P. Somers*, 25 May 1983 (UCHT). **Monroe County:** *A.J. Sharp & E. Sharp 20548*, 06 May 1956 (TENN); *B.E. Wofford, B. Boom & M. Whitten 79-35*, 10 Apr 1979 (TENN); *B.E. Wofford & E.E. Clebsch 98-15*, 13 May 1998 (TENN). **Polk County:** *A.J. Sharp & S.A. Cain 275*, 19 Mar 1939 (TENN); *L. Lord & C.W. James 20428*, 30 May 1956 (TENN); *A.J. Sharp, D. Smith, H. Webster & B. Hattaway*, 14 Mar 1972 (TENN); *B.E. Wofford, B. Boom & M. Whitten 79-34*, 10 Apr 1979 (TENN). **Rhea County:** *A.J. Sharp, E. Clebsch & R.E. Shanks 4357*, 27 Jun 1947 (TENN); *V.E. McNeilus 88-279*, 15 Apr 1988 (TENN). **Roane County:** *S.F. Hale & E.E. Clebsch 47520*, 13 April 1974 (TENN); *B.E. Wofford & K.D. McFarland 84-27*, 05 May 1984 (TENN); *B.E. Wofford & P. Kalla 84-6*, 14 Apr 1984 (TENN). **Sevier County:** *A. Ruth*, May 1897 (NCU); *J.K. Underwood & A.J. Sharp*, 25 Jun 1933 (TENN); *S.A. Cain & W.H. Duncan 315-2*, 12 May 1934 (TENN); *J.K. Underwood 669*, 13 May 1934 (TENN); *S.A. Cain 579*, 10 Jun 1934 (TENN); *T. Jones*, 04 May 1935 (TENN); †*L. Stewart s.n.*, 15 Jun 1936 (NCU); *A.J. Sharp & H. Iltis 1722*, 17 May 1942 (TENN); †*A.J. Sharp & H.H. Iltis 1725*, 17 May 1943 (NCSC); †*F. Bartley s.n.*, 28 April 1956 (NCU); *L.P. Lord 20600*, 13 May 1956 (TENN); *R.E. Shanks 23558*, 03 Jun 1958 (TENN); †*D. Pittillo 8936 & S. Pittillo*, 12 Jul 1983 (WCUH). **Sullivan County:** *H.M. Jennison, A.J. Sharp & J.K. Underwood 795*, 19 May 1934 (TENN); *J.K. Underwood*, 20 May 1934 (TENN); *A.J. Sharp 1456*, 16 May 1941 (TENN); *E. Schell*, 11 May 1990 (TENN). **Unicoi County:** *R.L. James 17347*, 04 May 1953 (TENN); *C. Lyle 19276*, 26 Mar 1955 (TENN); *B.E. Wofford & B. Boom 79-54*, 18 Apr 1979 (TENN). **Van Buren County:** *H.H. Iltis & N.H. Russel 3153*, 02 May 1947 (TENN); *A.J. Sharp 18255*, 25 Apr 1954 (TENN); *C.A. Fleming FCF-105*, 20 Apr 2001 (TENN). **VIRGINIA. Scott County:** *J.F. Townsend*, 10 May 2006 (VPI).

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NORTH CAROLINA. Alleghany County: *A.E. Radford 32669*, 02 May 1958 (NCU); M.S. Lanning 025, 30 May 2008 (WCUH). **Ashe County:** **A.E. Radford 43954*, 23 June 1961 (NCU); *S. Spongberg 67-111*, 25 April 1967 (NCU); **A.E. Radford 45390*, 03 June 1967 (WCUH); *J. W. Hardin 13257*, 23 May 1968 (NCSC); *G.L. Nesom 1015*, 04 May 1968 (NCSC); *D.B. Poindexter 05-325*, 25 May 2005 (NCU); **D.B. Poindexter 05-855*, 26 June 2005 (NCU); M.S. Lanning 024, 30 May 2008 (WCUH). **Watauga County:** *Lanning 070*, 22 May 2009 (WCUH). **TENNESSEE. Johnson County:** *D.W. Ogle*, 27 May 1978 (TENN); *B.E. Wofford, M. Evans & B. Boom 79-67*, 04 May 1979 (TENN); *B.E. Wofford & B. Boom 79-188*, 20 June 1979 (TENN); *J.T. Donaldson 3736*, 25 May 1998 (TENN); R. Kral 63878, 16 June 1979 (TENN). **VIRGINIA. Buchanan County:** *T.F. Wieboldt, G.D. Rouse & R. Keen 6099*, 20 June 1986 (VPI). **Carroll County:** *R. McComb s.n.*, 19 May 1993 (VPI). **Dickenson County:** *K. Markley*, 30 April

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1986 (VPI). **Grayson County:** *C.E. Stevens 12711*, 13 June 1976 (VPI); *C.E. Stevens & B. Davenport 2113*, 17 June 1970 (VPI). **Russell County:** *D.W. Ogle*, 14 April 1983 (VPI); *D.W. Ogle*, 30 April 1986 (VPI). **Smyth County:** **J.K. Small*, 13 June 1892 (NCSC); **N.L. & E.G. Britton & A.M. Vail*, June 1892 (NCU); R. Kral 58425, 29 June 1976 (TENN); *T.F. Wieboldt & A.B. Davenport 3704*, 13 June 1980 (VPI); *G.P. Fleming 7959*, 17 May 1993 (WILLI). **Washington County:** **A.M. Harrill 18580*, 11 June 1968 (NCU); *A.R. Shields*, 04 May 1958 (TENN); *D.W. Ogle*, June 1976 (VPI); J. Harris s.n., 25 May 1985 (WILLI). **Wythe County:** *T.F. Wieboldt*, 06 May 2004 (VPI). **WEST VIRGINIA. McDowell County:** *W.N. Grafton*, 23 April 1975 (WVA 126646); *W.N. Grafton*, May 1981(WVA); *S.J. Norris*, 06 April 2000 (WVA); *S.J. Norris*, 27 April 2001 (WVA 108927). **Wyoming County:** *W.N. Grafton*, 09 June 1984 (WVA).

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