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The Systematics of the *Spiranthes cernua* Species Complex (Orchidaceae): Untangling the Gordian Knot

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Abstract—Two major obstacles to quantifying biodiversity are reticulate evolution and the evolution of genetically distinct but morphologically overlapping cryptic species. The *Spiranthes cernua* species complex (Orchidaceae) has defied satisfactory species delineation, often described as intractable, due to morphological variability within species, overall morphological similarity between species, possible cryptic speciation, and suspected hybridization. We utilized low copy nuclear, nuclear ribosomal, and chloroplast molecular phylogenetic datasets, in addition to expansive field and herbarium research, to clarify long-standing questions regarding species boundaries within the *S. cernua* species complex. Our results justify: 1) narrowing the concept of *S. cernua*; 2) the description of a new cryptic species, ***Spiranthes arcisepala***; 3) the description of a new geographically restricted species of cryptic and ancient hybrid origin, ***Spiranthes niklasii*** (*S. cernua* × *S. ovalis*); 4) a new combination for a biogeographically specific cryptic species of ancient hybrid origin, ***Spiranthes incurva*** (*S. cernua* × *S. magnicamporum*); and 5) the description of a new localized hybrid, ***Spiranthes ×kapnosperia*** (*S. cernua* × *S. ochroleuca*). We also propose formal synonymization of federally endangered *Spiranthes parksii* under *S. cernua* s. s. Our research clarifies species boundaries within this challenging group, and is the first to use molecular phylogenetic data to support hybridization as an evolutionary force within the *S. cernua* species complex.

Keywords—Appalachian Mountains, hybrid speciation, morphological variability, Ouachita Mountains, species tree.

Although challenging, the effort to understand species boundaries and biodiversity remains one of biology's most fundamental and urgent tasks (Soltis and Gitzendanner 1998; Pettengill and Neel 2011; Paul et al. 2013; Fennessy et al. 2016); how species are delineated impacts the allocation of limited conservation funds, designation of official protection status, the selection of individuals used for conservation programs, and the general public's perception of systematics and conservation. The evolution of cryptic species that are unequivocally genetically distinct and even reproductively isolated, but are morphologically overlapping or nearly indistinguishable from other species, represent a major challenge in addressing these issues (e.g. Bickford et al. 2007; Adams et al. 2014; Shirley et al. 2014; Grbic et al. 2015). The wider occurrence of species complexes (assemblages of closely related often cryptic species that defy easy delimitation) also present a perplexing but important window into the process of speciation and diversification, adaptation, gene flow, and reticulate evolution. Achieving a balance between recognition of infraspecific phenotypic variability, identification of cryptic species, taxonomic over-splitting, and conservation is a contentious and fiercely debated topic, particularly in charismatic groups such as orchids, primates, and birds (e.g. Pillon and Chase 2007; Dueck and Cameron 2008b; Hopper 2009; Sangster 2009; Markolf et al. 2011; Zachos et al. 2013; Swarts et al. 2014). This complicated set of issues is further obfuscated by the lack of a universally accepted species concept. The task faced by systematic and conservation biologists is how to best reflect evolutionary pattern and process while simultaneously recognizing diagnosable species, developing useful classifications, and protecting the greatest amount of biodiversity. This challenge is intensified when dealing with putative species that present large degrees of phenotypic variation and plasticity.

The genus *Spiranthes* Rich. (Orchidaceae) has long presented a significant taxonomic and identification challenge: many of its currently accepted 35 species exhibit marked phenotypic variation at both local and continental scales, and hybridization has traditionally been thought to be pervasive. Furthermore, cryptic speciation has been documented in the group (Brown et al. 2008; Dueck and Cameron 2008a; Pace and Cameron 2016, Pace et al. 2017), a few species exhibit polyploidy and apomixis (Catling 1982; Sheviak 1982), the species status of some taxa is questionable (Walters 2005; Dueck and Cameron 2008b), incomplete lineage sorting is likely a reality in the group (Dueck et al. 2014), and taxonomic inflation by certain authors is a troublesome issue (e.g. Brown 1999a, 1999b). The group that best encompasses all of these issues is the *Spiranthes cernua* (L.) Rich. species complex (Figs. 1–4): a true Gordian Knot frequently described as "intractable" (Sheviak 1982, 1991; Sheviak and Brown 2002).

Taxonomic History—Linnaeus' original description of *Ophrys* [*Spiranthes*] *cernua* L. is minimalistic (e.g. lacking details such as measurements) and the type location is imprecise: "Virginia, Canada" (Linnaeus 1763). The lectotype (*Kalm s.n.* (LINN) and drawings of lectotype flowers by A. Gray in Sheviak and Catling 1980, p. 531) displays two plants 25.5–36.1 cm tall with linear to linear-lanceolate leaves and strongly nodding flowers 5.5–7.8 mm long in profile. The labellum is bluntly acute (as denoted in the original description) and slightly dilated near the apex, with two prominent basal callosities (nectar glands). As indicated by 18th and early 19th century collections, the name *S. cernua* was broadly applied to any autumnal flowering *Spiranthes* in eastern North America (e.g. *Muhlenberg 180* (LINN) represents what are now recognized as five distinct species).

The species *S. casei* Catling & Cruise, *S. magnicamporum* Sheviak, *S. ochroleuca* (Rydb.) Rydb., *S. odorata* (Nutt.) Lindl., and *S. parksii* Correll (Figs. 3, 4), were proposed to accommodate much of the morphological variation expressed by the autumn-flowering *Spiranthes*. All of these autumnal North American *Spiranthes* traditionally have been thought to be very closely related to one another and to *S. cernua*, and most have been referred to, or placed under synonymy with *S. cernua*. This group came to be known collectively as the *Spiranthes cernua* species complex sensu Sheviak (Sheviak 1982, 1991; Sheviak and Brown 2002). Even with this added knowledge of species-level diversity among the autumn-flowering *Spiranthes*, *S. cernua* sensu traditum encapsulates a wide degree of morphological and reproductive variability from within-population to biome level scales. The floral and reproductive phenomena of

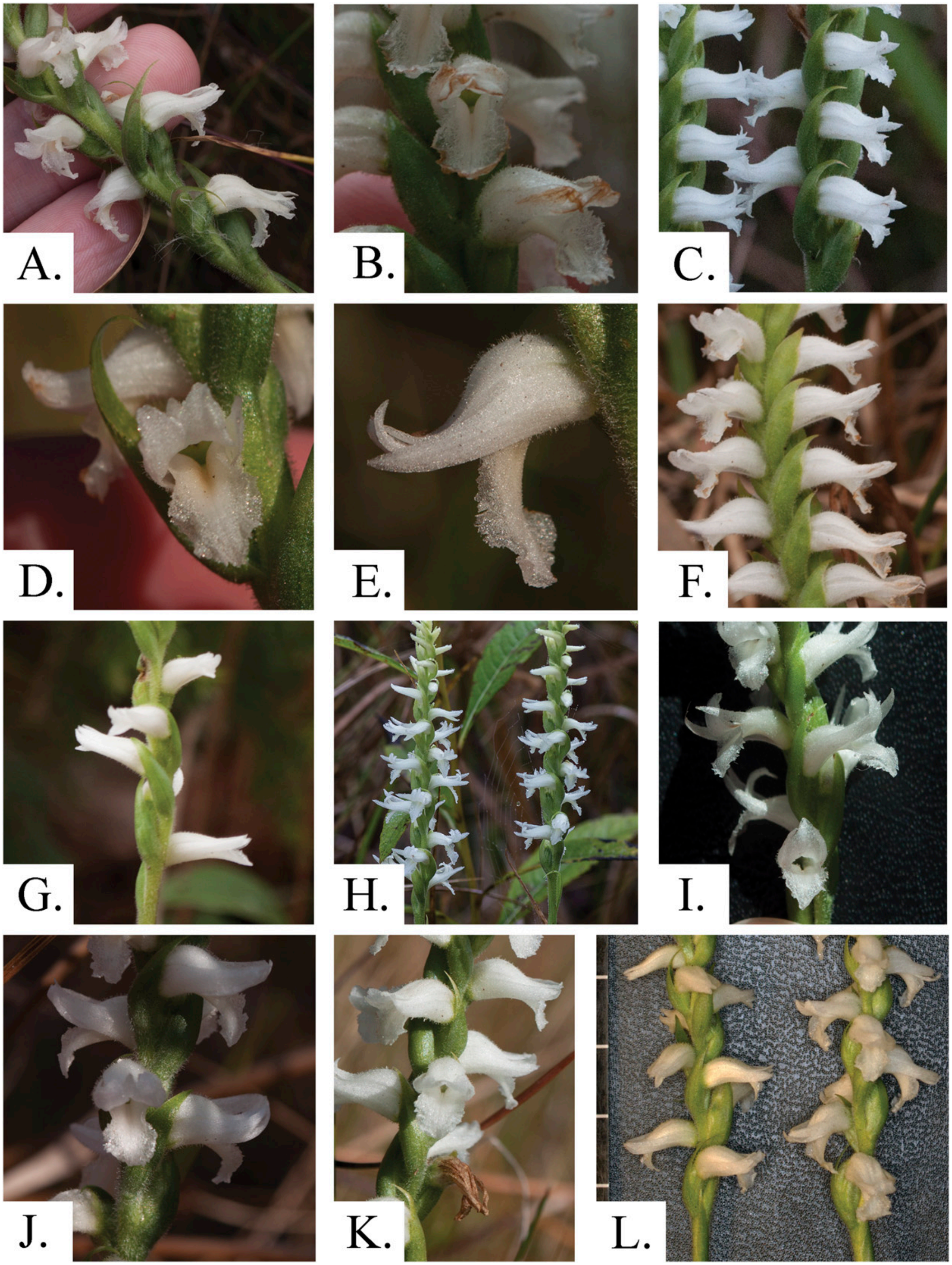

Fig. 1. *Spiranthes cernua* s. s. A. New Castle Co., Delaware, sample sc1. B. Worchester Co., Maryland, sample sc8d. C. Wayne Co., Georgia, sample sc15. D–E. Sussex Co., Delaware. F. Cape May Co., New Jersey, sample sc6. G. Scott Co., Arkansas, sample sc29; flowers reduced (sub-peloric) and partially closed. H. Towns Co., Georgia; photo: James Fowler, used with permission. I. James City Co., Virginia, sample sc9. J. Lexington Co., South Carolina. K. Pike Co., Alabama. L. Co-occurring *S.* ×*kapnosperia* (L) and *S. cernua* (R), Jackson Co., North Carolina; scale bars denote cm.

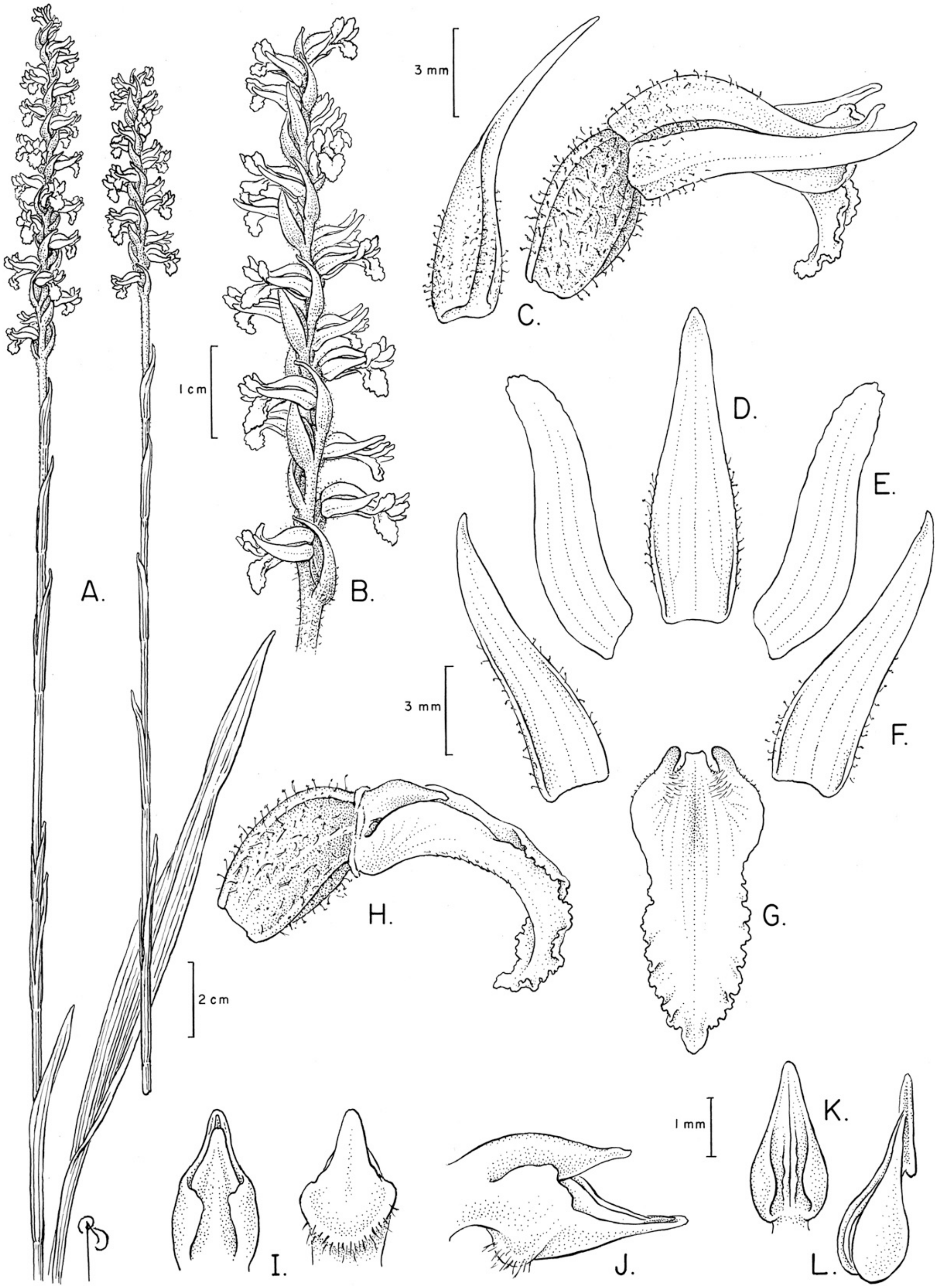

Fig. 2. *Spiranthes cernua* s. s. A. Habit. B. Inflorescence. C. Flower and floral bract in profile. D–G. Flower, dissected view. D. Dorsal sepal. E. Dorsal petal. F. Lateral sepal. G. Labellum. H. Labellum, column, and ovary in natural position. I–K. Column. I. Dorsal and ventral views. J. Profile. K. Anther. L. Pollinia. Drawn from *Pace 612* and *Pace 615* by Bobbi Angell.

peloria, cleistogamy, and apomixis are common within *S. cernua*, contributing to this variability (Sheviak 1982, 1991). Some peloric individuals of *S. cernua*, particularly in the central and southern Great Plains, are very robust in stature, approaching *S. magnicamporum* in overall appearance. In some regions, only cleistogamous individuals may be found in particular populations; occasionally peloric and non-peloric, cleistogamous and open, and apomictic and sexual individuals may all be

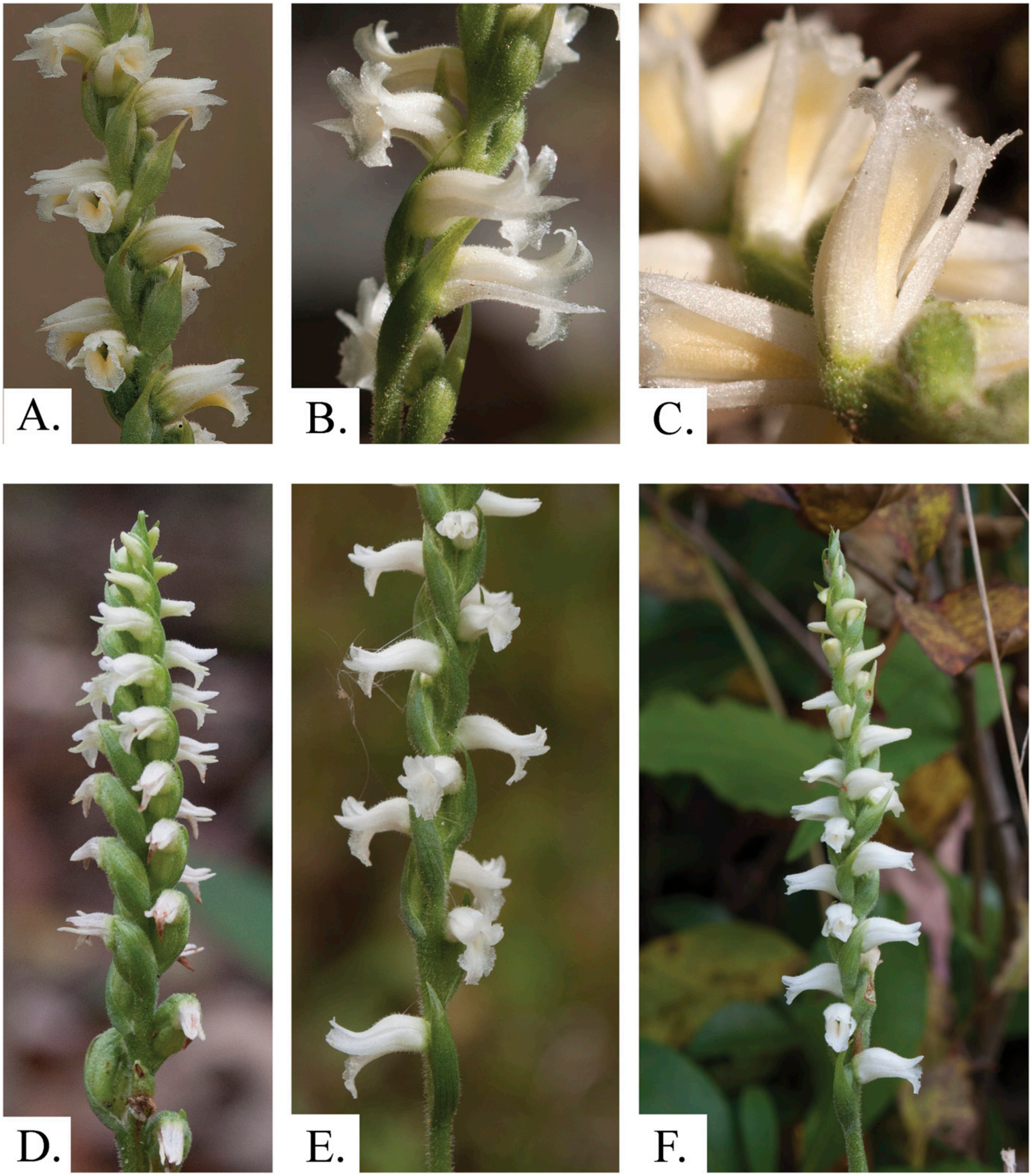

FIG. 3. A. *Spiranthes casei*, Michigan; photo: Aaron Strouse, used with permission. B–C. *Spiranthes ochroleuca*, Rutland Co., Vermont. C. Characteristic ochroleucous-yellow coloration on abaxial labellum surface. D. *Spiranthes ovalis* var. *erostellata* Catling, Waukesha Co., Wisconsin, sample so2. E. *Spiranthes niklasii*, Yell Co., Arkansas (type location). F. *Spiranthes* ×*kapnosperia*, Transylvania Co., North Carolina (type location).

found within a single population or populations separated by only a few kilometers. Based on the work of Catling and Sheviak (Catling 1982, 1983; Catling and Brown 1983; Sheviak 1982, 1991; Sheviak and Brown 2002), *S. cernua* is traditionally understood to be a facultatively agamospermic polyploid 'compilospecies' ($2n = 45, 60$; sensu Harlan and deWet 1963) that freely hybridizes with all other species in the *S. cernua* species complex. Sheviak (1973) went so far as to state "a 'typical' *S. cernua* is difficult to define because of the species' intrinsic hybrid nature." Building upon this hypothesis, Homoya (1993) wrote that hybridization has led to at least five habitat-specific "genetic races" (i.e. ecological morphs) occurring within

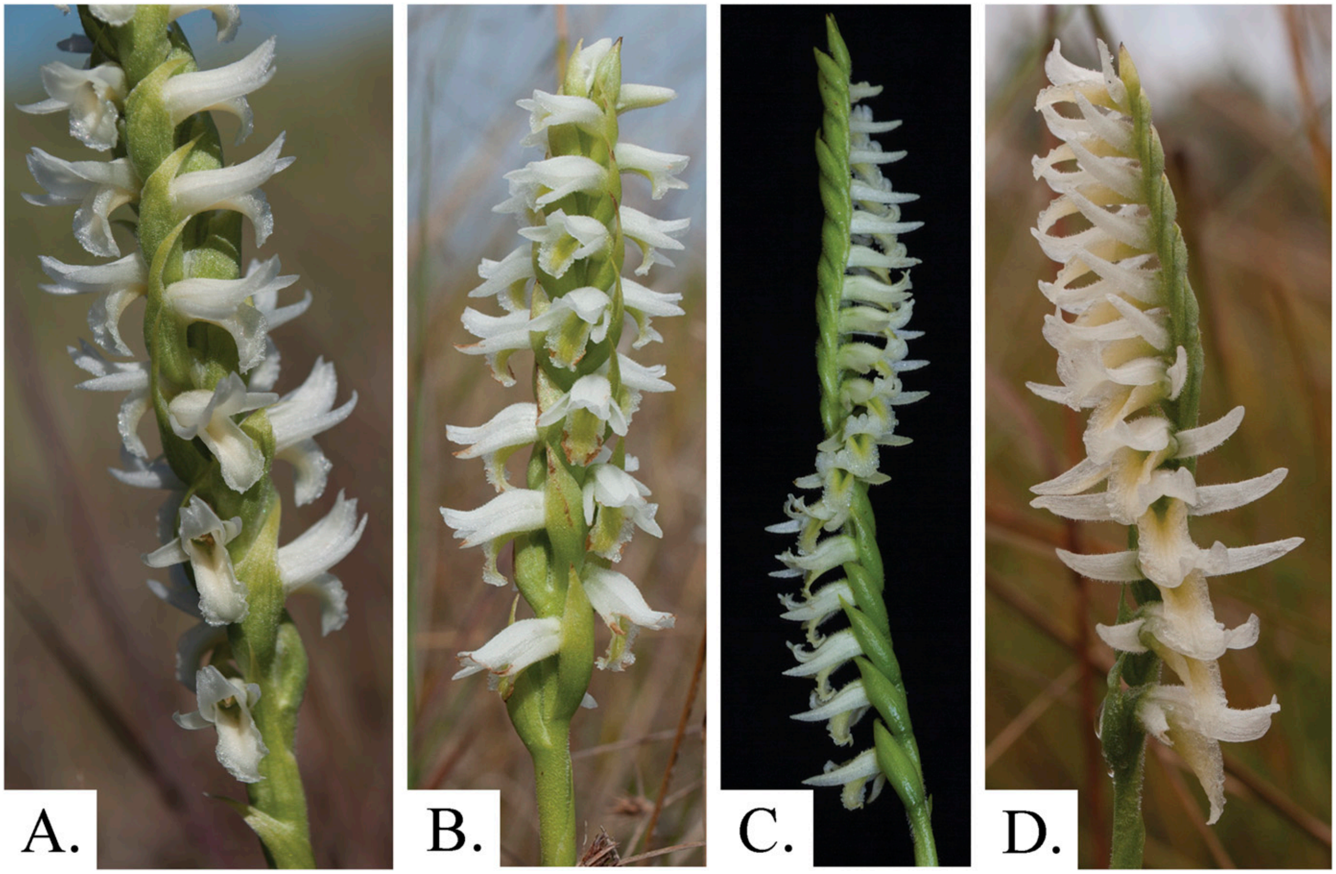

Fig. 4. A. *Spiranthes magnicamporum*, Guadalupe Co., New Mexico, sample sm7h. B. *Spiranthes triloba*, Polk Co., Florida. C. *Spiranthes igniorchis*, Polk Co., Florida; photo: Dave Briley, used with permission. D. *Spiranthes longilabris*, Brunswick Co., North Carolina, sample location of 13b.

Indiana alone. Despite its hypothesized pervasiveness, the morphological identity of potential hybrids is unclear, and no primary hybrids involving *S. cernua* have been formally named; the dubious *S.* ×*borealis* P.M. Br. (*S. casei* × *S. ochroleuca*) is the only named hybrid in the complex. Hybridization has come to be hypothesized as the only, or at least major, source of morphological variability within this complex, with little attention paid to other potential evolutionary forces that might favor the evolution of widespread phenotypic heterogeneity or the possibility of cryptic speciation in combination with or in the absence of hybridization.

Repeated attempts have been made to clarify species relationships and species status within the *Spiranthes cernua* complex, with different methods favored by various authors including breeding systems (Catling 1981), morphological discriminant analysis (Sheviak and Catling 1980; Catling 1981; Sheviak 1982), and cytology (Sheviak 1982, 1991). Most recently, DNA sequences (Dueck and Cameron 2007, 2008b; Dueck et al. 2014; Pace and Cameron 2016; Pace et al. 2017) and AFLPs and microsatellites (Walters 2005, Manhart and Pepper 2007) have been utilized to understand the systematics of these orchids. We suggest that viewing morphological variation through the integrated context of molecular phylogenetic relationships and morphometric analysis offers an attractive and promising path forward.

Phylogenetic Background—Recent molecular phylogenetic research investigating taxonomic confusion in portions of the *S. cernua* species complex (Dueck et al. 2014, Pace and Cameron 2016, Pace et al. 2017) reveal that circumscription of the *Spiranthes cernua* complex sensu Sheviak (1982; Sheviak and Brown 2002) does not properly reflect evolutionary history. Specifically, *S. odorata* does not belong in this grouping since it is distantly related to *S. cernua*, instead being sister to the clade containing most of the Eastern North American species. Additionally, S. *triloba* (Small) Schum., a species lost under synonymy of both *S. cernua* and *S. odorata* for more than a century, is a distinct species, forming a sister relationship with *S. magnicamporum*. Together, Dueck et al. (2014), Pace and Cameron (2016), and Pace et al. (2017) demonstrate that the *Spiranthes cernua* complex sensu nov. includes *S. casei*, *S. cernua*, *S. magnicamporum*, *S. parksii*, and *S. ochroleuca* (the traditionally included species), plus *S. triloba*, in addition to the unexpected *S. longilabris* Lindl., *S. igniorchis* M.C. Pace, and *S. ovalis* Lindl. (Figs. 3, 4). Furthermore, Dueck et al. (2014) found that *S. cernua* itself is likely polyphyletic, and *S. casei* is broadly embedded within *S. ochroleuca*. Although support values for individual taxon clades were typically high (PP > 0.90; often 1), the exact relationship among clades was not well resolved, particularly within the *Spiranthes cernua* complex s. s.: the *S. cernua* clades, *S. parksii*, and *S. ochroleuc*a + *S. casei*. Additionally, the morphological limits of these potential clades were not explored. This occasional deficiency of resolution has been used to advance opposing positions as exemplified by the debate over the species status of federally endangered *S. parksii*: Dueck and Cameron (2008b) hypothesized that its sub-peloric morphology, coupled with a lack of molecular resolution between *S. cernua* and *S. parksii* (and the fact that recognition of the latter would render the former paraphyletic), indicates the two are the same species, whereas Jacobsen et al. (2009) strongly rejected Dueck and Cameron (2008b), attributing the unresolved relationship to the neutral nature of the sampled DNA markers.

To resolve the long-standing problems of species relationships and species status within this complex, we present an expanded sampling of taxa and loci, with integrated morphological and biogeographic data. Working under an integrated history-bound phylogenetic species concept (Baum and Donoghue 1995; Dayrat 2005) in which monophyly is emphasized in concert with supporting morphological and ecological data, these data support the division of '*S. cernua*' into five distinct but morphologically cryptic and variable species and hybrids.

Methods

Taxonomic Sampling—Approximately 700 herbarium specimens of species in the *Spiranthes cernua* species complex were carefully reviewed from AMES, BH, CLEM, CM, F, FLAS, FSU, LSU, MO, NY, NYS, SEL, UARK, US, USF, WILLI, WIS, and WVA (herbarium acronyms from Thiers 2017; Appendix 1). Individual flowers from the lowermost quarter of the inflorescence were rehydrated for morphological examination from select individual specimens. Fieldwork based on georeferenced herbarium specimens was conducted by M. Pace in Alabama, Arkansas, Delaware, Georgia, Florida, Illinois, Indiana, Maryland, New Jersey, New Mexico, New York, North Carolina, Ohio, Oklahoma, Pennsylvania, South Carolina, Texas, Virginia, Vermont, and Wisconsin from 2012–2016. Samples were collected for herbarium and spirit vouchers, morphological measurements, and DNA sequencing. For areas we were unable to visit for fieldwork, 1–10 yr old herbarium specimens were judiciously sampled with permission for inclusion in the molecular phylogenetic analysis.

Morphometrics—To analyze and understand the morphological variability and differentiation of species, boxplots were created for selected species. These plots were visualized in R Studio (R Development Core Team 2012) using rehydrated labella from herbarium samples, scaled drawings in published literature (Sheviak 1982, 1991), and whole herbarium specimens. Measurements of rehydrated labella included labellum length, width below callosities, width at constriction, width of median point below constriction, and callosity height. Measurements of whole herbarium specimens include height of the plant, leaf length, leaf width at widest point, lateral sepal length, lateral sepal width at midpoint, and labellum length.

Molecular and Phylogenetic Methods—Phylogenetic analyses incorporated and expanded upon the dataset of Dueck et al. (2014), Pace and Cameron (2016), and Pace et al. (2017). For new accessions, 3–4 unopened buds or ca. 1 cm^2 of leaf tissue were collected and silica-gel dried for later extraction of Total gDNA. IBI plant isolate kits (Peosta, Iowa) and Maxwell® 16 LEV plant DNA kits (Madison, Wisconsin) were used for all newly collected samples. All accessions were amplified for the chloroplast gene regions *matK*, *ndhJ*, *trnL* intron, *trnS-fM*, and *ycf1* 3′, nuclear ribosomal ITS (internal transcribed spacers 1 and 2, and the 5.8S subunit; "nrITS"), and the low-copy nuclear region *ACO* (Appendix 2). PCR amplification protocols used were as follows: chloroplast (except *ycf*1) and nrITS: following Dueck et al. (2014); *ACO*: following Guo et al. (2012); *ycf*1: following Neubig et al. (2009). The PCR products were purified using ExoSap-It (Cleveland, Ohio), and cycle sequencing products were cleaned using Agencourt CleanSeq (Beverly, Massacusetts) magnetic beads. Direct sequencing of cleaned cycle sequencing products was performed at the University of Wisconsin – Madison Biotechnology Center. Resulting chromatograms were edited and aligned using software modules available in Geneious 7.1 including MUSCLE. Ambiguities in datasets were coded with standard IUPAC-IUB symbols for nucleotide nomenclature (Cornish-Bowden 1985). If samples failed to amplify after repeated attempts for a given locus they were coded as missing data (Table 1). Based on previous research (Dueck et al. 2014, Pace and Cameron 2016), *Spiranthes odorata* was used as the outgroup.

The data were analyzed as: 1) individual loci, 2) combined chloroplast data, 3) combined nuclear data, 4) combined nuclear + chloroplast data, and 5) combined nuclear + chloroplast data using a reduced dataset composed of samples with no missing data and no potential hybrid individuals. Phylogenetic analyses were performed under Bayesian Inference (MrBayes on XSEDE (3.1.2)) implemented through CIPRES Portal v. 3.3 (Miller et al. 2010). Based on Dueck et al. (2014), the GTR+G (general-time-reversible with a gamma distribution) model was implemented for all datasets and partitions. Analyses were run for 10,000,000 generations, with a sample frequency of 100,000, nruns = 2, nchains = 6, temp = 0.2, and a burn-in of 500,000. Phylogenetic inference of the 50% majority-rule consensus tree was constructed using the "sumt" option based on the remaining trees. The topologies of these trees were visualized in FigTree (Rambaut 2014). The combined nuclear + chloroplast data was visualized as a network in the program SplitsTreeWindow (Huson and Bryant 2006). To clarify discordance between individual gene trees resulting from possible incomplete lineage sorting, we also took a multispecies coalescent approach, estimating the species tree using *BEAST (Drummond et al. 2012) under a birth-death process. The species tree was visualized in DensiTree v. 2.2 (Bouckaert 2010).

Table 1. Summary table of molecular regions used in the phylogenetic analysis. Two nuclear: *ACO* and nrITS, and five chloroplast: *matK*, *ndhJ*, *trnL-F*, *trnS-M*, and *ycf1* 3′.

Taxon and sample size	*ACO* failed/ amplified	nrITS failed/ amplified	*matK* failed/ amplified	*ndhJ* failed/ amplified	*trnL-F* failed/ amplified	trnS-M failed/ amplified	*ycf1* 3′ failed/ amplified
Spiranthes arcisepala n = 10	5 / 5	2 / 8	0 / 10	1 / 9	0 / 10	0 / 10	0 / 10
Spiranthes casei n = 4	0 / 4	0 / 4	0 / 4	0 / 4	0 / 4	0 / 4	0 / 4
Spiranthes cernua s. s. n = 12	0 / 12	0 / 12	2 / 10	1 / 11	0 / 12	0 / 12	2 / 10
Spiranthes igniorchis n = 8	0 / 8	0 / 8	0 / 8	0 / 8	0 / 8	0 / 8	0 / 8
Spiranthes incurva n = 8	2 / 6	0 / 8	0 / 8	0 / 8	0 / 8	0 / 8	0 / 8
Spiranthes ×kapnosperia n = 3	0 / 3	0 / 3	1 / 2	0 / 3	0 / 3	0 / 3	0 / 3
Spiranthes longilabris n = 3	0 / 3	0 / 3	0 / 3	0 / 3	0 / 3	0 / 3	0 / 3
Spiranthes magnicamporum n = 11	3 / 8	2 / 9	2 / 9	6 / 5	2 / 9	0 / 11	1 / 10
Spiranthes nikalsii n = 12	0 / 12	0 / 12	3 / 9	0 / 12	0 / 12	0 / 12	2 / 10
Spiranthes ochroleuca n = 11	4 / 7	0 / 11	0 / 11	0 / 11	0 / 11	0 / 11	1 / 10
Spiranthes odorata n = 9	3 / 6	0 / 9	0 / 9	3 / 6	0 / 9	0 / 9	0 / 9
Spiranthes ovalis var. *erostellata* n = 4	2 / 2	0 / 4	0 / 4	2 / 2	0 / 4	0 / 4	0 / 4
Spiranthes ovalis var. *ovalis* n = 1	1 / 0	0 / 1	0 / 1	1 / 0	0 / 1	0 / 1	0 / 1
Spiranthes parksii n = 4	1 / 3	1 / 3	0 / 4	0 / 4	0 / 4	0 / 4	1 / 3
Spiranthes triloba n = 8	3 / 5	1 / 8	0 / 8	2 / 6	0 / 8	0 / 8	1 / 7

Results

Phylogenetics—Individual gene trees generally found *S. cernua* to be polyphyletic (trees not shown). Only the reconstruction of nrITS failed to support the polyphyly of *S. cernua*, and it failed to resolve the relationships between *S. ochroleuca* and *S. cernua* s. l., whereas *ycf1* recovered weak to moderate support for the polyphyly of *S. cernua* s. s. within a broader polytomy of *S. ochroleuca* and *S. casei*. The phylogenetic hypothesis based on combined nuclear data mostly lacks resolution along the backbone of the phylogeny among otherwise moderately to fully supported clades of individual species, whereas the combined chloroplast hypothesis provides more well-supported resolution among strongly to fully supported clades of species (Fig. 5). These reconstructions, however, find *S. cernua* as it has traditionally been circumscribed to be strongly polyphyletic (with these polyphyletic a priori *S. cernua* samples now labeled as *S. arcisepala*, *S. incurva*, and *S. niklasii* in the trees). Discordance between chloroplast and nuclear topologies was observed, with the nuclear tree recovering *S. arcisepala* in a strongly supported (PP = 0.98) polytomy with *S. cernua* s. s. and *S. incurva*, and the chloroplast tree fully supporting (PP = 1) a sister relationship to *S. ochroleuca* + *S. casei* in (Fig. 5). When samples with missing data and presumed hybrids were excluded, the sister relationship between *S. arcisepala* and *S. ochroleuca* + *S. casei* is preserved, although it is weakly supported (PP = 0.64) (Fig. 6). Discordance between nuclear and chloroplast trees was also observed in the relationships of *S. magnicamporum* and *S. triloba*, and *S. ovalis* in relation to the *S. cernua* species complex s. s. (Fig. 5). Additional instances of discordance that indicate hybridization are discussed below.

In nearly all of our phylogenetic hypotheses, *S. parksii* is consistently recovered embedded within the *S. cernua* s. s. clade in an unresolved relationship. The only phylogenetic reconstruction to find any resolution between *S. parksii* and *S. cernua* s. s. is the reduced sampling combined nuclear + chloroplast tree (Fig. 6), which only found weak support (PP = 0.60) for a sister relationship between a clade of *S. cernua* s. s. as distinct from a fully supported *S. parksii* clade.

Evidence for, and Instances of, Hybridization—Based on the samples and molecular regions included here, we recovered molecular evidence for geographically specific hybrid speciation. Hybridization was inferred by samples that

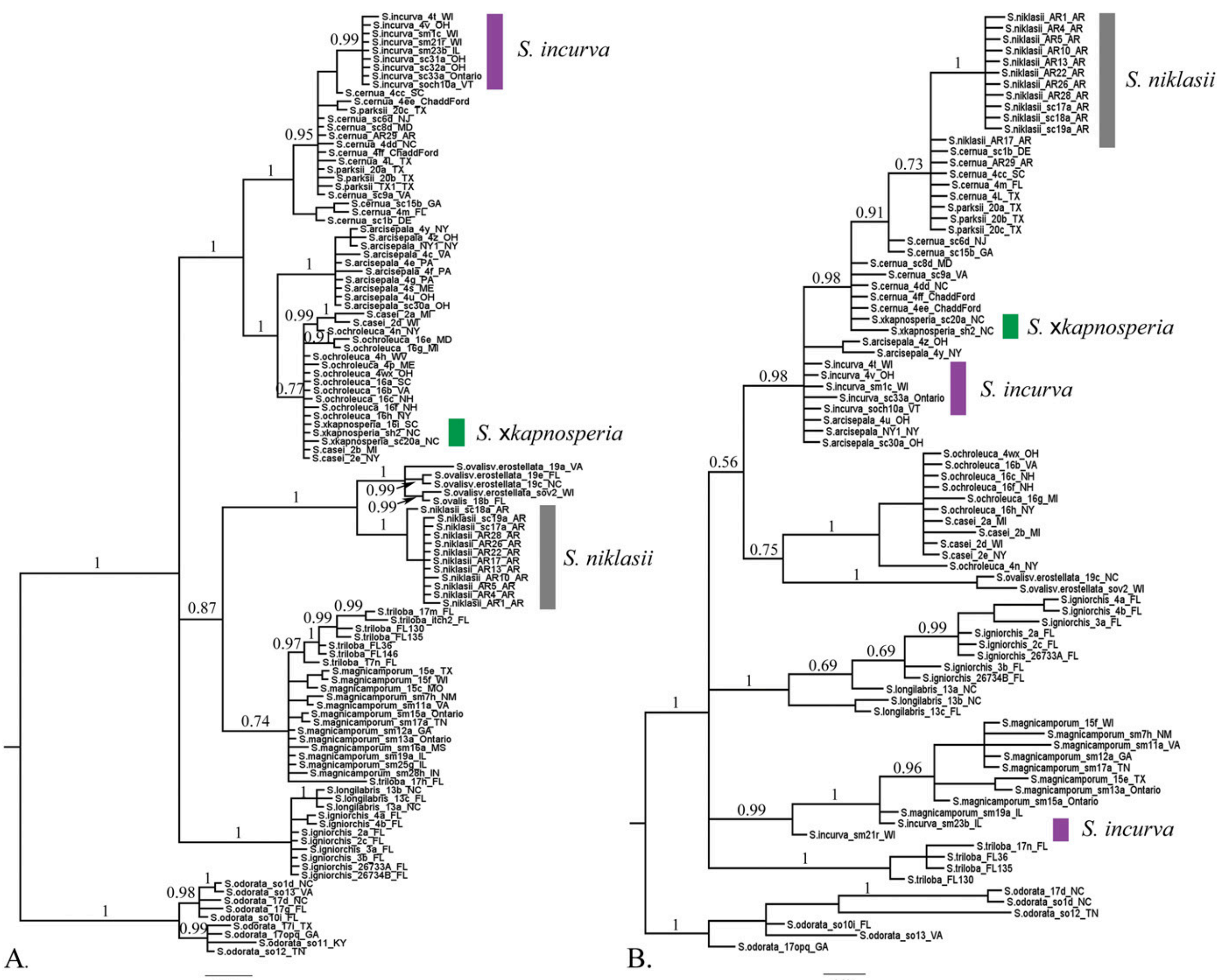

Fig. 5. Bayesian phylogenetic tree reconstructions. Posterior probabilities within species clades are only indicated if they are > 0.90. The discordant positions of the three hybrid taxa are highlighted; colors the same as in Fig. 7. A. Bayesian phylogenetic tree reconstruction based on the combined five-locus chloroplast data. B. Bayesian phylogenetic tree reconstruction based on the combined *ACO* + ITS nuclear data.

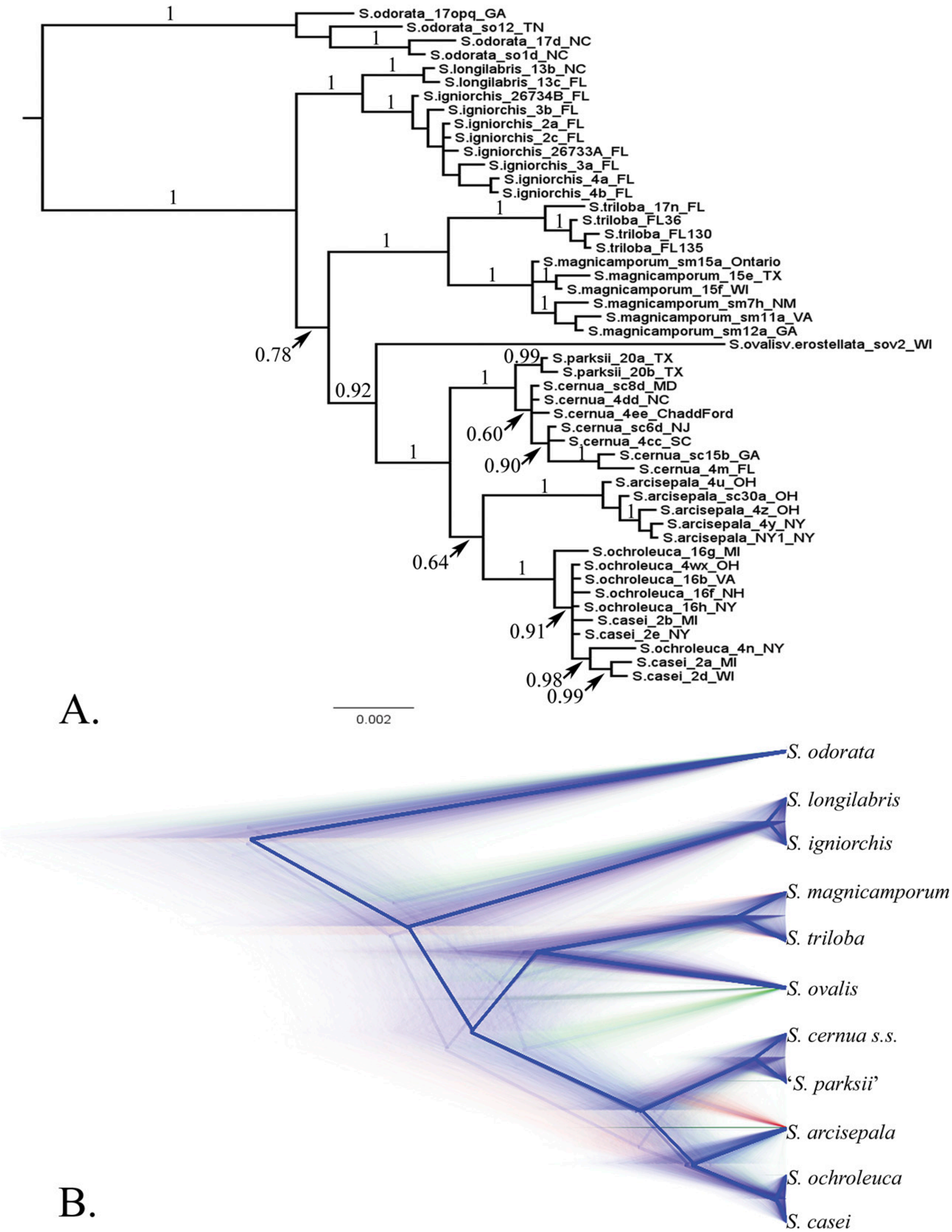

FIG. 6. A. Bayesian phylogenetic tree reconstruction based on the reduced sampling nuclear + chloroplast dataset after removing hybrid individuals and individuals with missing data. Posterior probabilities within species clades are only indicated if they are > 0.90. B. Coalescent species tree based on the reduced sampling nuclear + chloroplast dataset after removing hybrid individuals and individuals with missing data. All trees are shown. The root canal is highlighted in dark blue and the consensus trees are highlighted in faded colors.

display discordant topologies within otherwise strongly supported clades, display strong connections to other species in the network analysis, and have ambiguities in nuclear chromatograms in regions that correspond to nucleotide differences between inferred parental species (Figs. 5, 7, and 8). Cases of probable hybridization are seen in three instances: 1) samples from across the northern Interior Lowlands, Great Lakes Basin, and western and northern Appalachian Highlands which display discordance between *S. cernua* s. s. and *S. magnicamporum*, and are described below as *Spiranthes incurva* comb. nov.; 2) samples from the Ouachita Mountains, Boston Mountains, and Crowley's Ridge in Arkansas displaying a ridge of papillae along the labellum midvein, which switch clades between *S. cernua* s. s. (nuclear) and a sister clade to *S. ovalis* (chloroplast), described below as *Spiranthes niklasii* sp. nov.; and 3) samples from the southern Smoky Mountains that switch clades between *S. cernua* s. s. (nuclear) and *S. ochroleuca* + *S. casei* (chloroplast), and are described below as *Spiranthes* ×*kapnosperia* nothsp. nov.

When we observed our nuclear data for samples of *S. incurva*, we found multiple base pair ambiguities in both loci; these sites of nucleotide ambiguity essentially corresponded perfectly to sites of molecular differentiation between *S. cernua* s. s. and *S. magnicamporum* (Fig. 8; e.g. an A in *S. cernua*, a G in *S. magnicamporum*, and an R – representing either A or G – in *S. incurva*). In nearly all cases, examination of the nuclear chromatographs found essentially identical overlapping peaks of either possible nucleotide. For *S. niklasii*, all chloroplast loci examined had nucleotide mutations that were unique to this species in addition to those shared with its closest relative, *S. ovalis*. These plants only displayed nucleotide ambiguities in the nrITS dataset (corresponding to nucleotide differences between *S. cernua* and *S. ovalis*), whereas in the ACO dataset some regions shared nucleotide differences with *S. cernua*, and in other segments of the same locus they shared nucleotide differences with *S. ovalis* (Fig. 8). Individuals of *S.* ×*kapnosperia* (identified a priori as *S. ochroleuca*) clustered with *S. ochroleuca* in the chloroplast datasets, but shared several nucleotide changes with *S. cernua*, and clustered with *S. cernua* in nuclear analyses. The incongruence and connection to other species of these hybrid species is also depicted in our network analysis (Fig. 7).

Species Tree—Our species tree reconstruction (using the reduced combined nuclear + chloroplast dataset after removing potential hybrid individuals and individual samples with missing data) found that *S. arcisepala* is most closely related and sister to a clade of *S. ochroleuca* + *S. casei*, with this grouping in turn sister to *S. cernua* + the former *S. parksii* (Fig. 6). Overall, the species tree reconstruction recovered sister relationships between *S. igniorchis* and *S. longilabris*, *S. magnicamporum* and *S. triloba*, *S. ochroleuca* + *S. casei* and *S. arcisepala*, and hypothesizes a gradation of relationships between species of the *S. cernua* species complex s. l.: ((*S. igniorchis*, *S. longilabris*), (*S. ovalis*, (*S. magnicamporum*, *S. triloba*)), (*S. cernua* + *S. parksii*, (*S. arcisepala*, *S. ochroleuca* + *S. casei*))). Although this topology is broadly congruent with our reduced combined nuclear + chloroplast dataset gene tree, there are differences in the relationship of *S. ovalis* and the sister pair of *S. magnicamporum* and *S. triloba*. Some uncertainty in the reconstruction of overall relationships along the backbone, particularly in the topological placement of *S. arcisepala* and *S. ovalis*, is also apparent in the visualization of the background consensus trees.

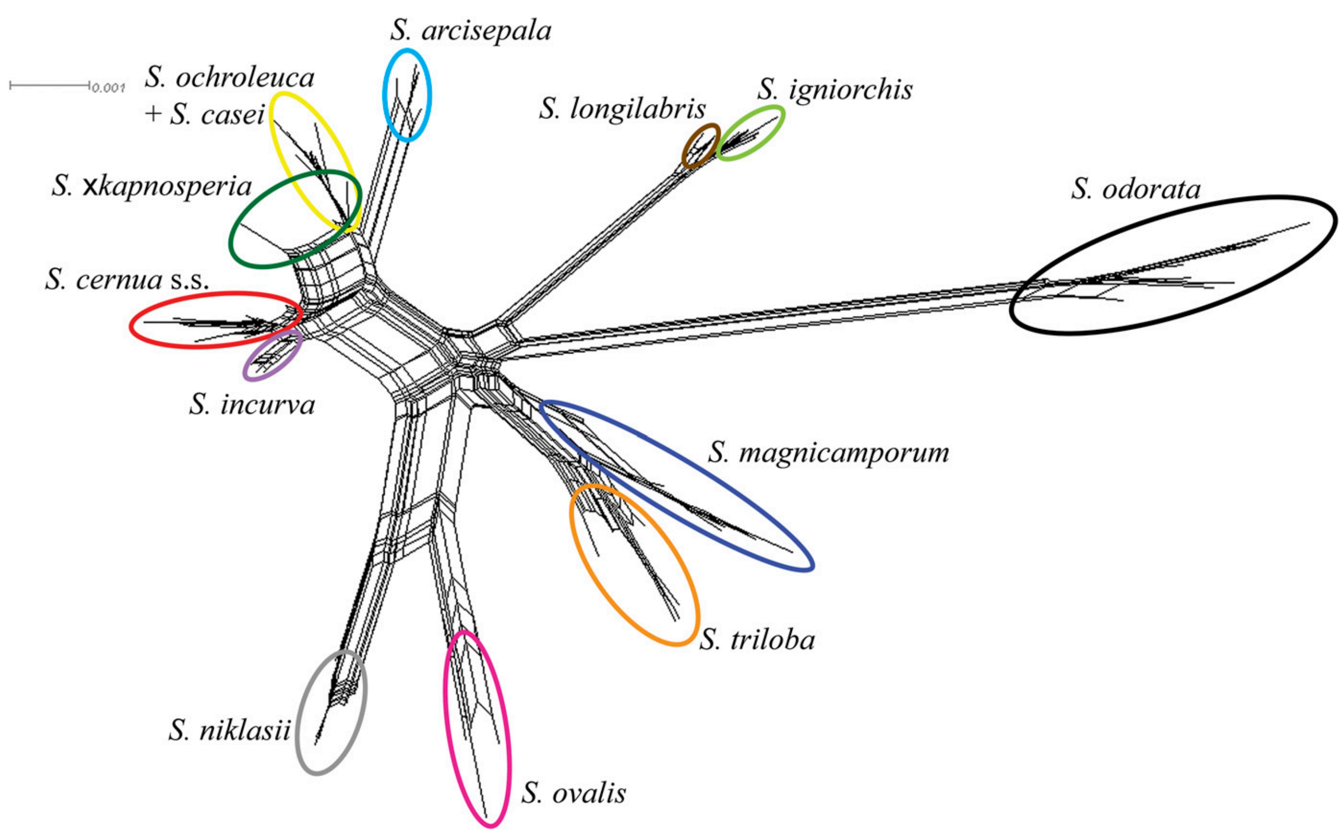

Fig. 7. Phylogenetic network from NeighborNet analysis of the *S. cernua* species complex s. l. plus *S. odorata*, using the nuclear + chloroplast dataset, including all samples of both hybrid and non-hybrid taxa. The position of species is indicated by colored ovals.

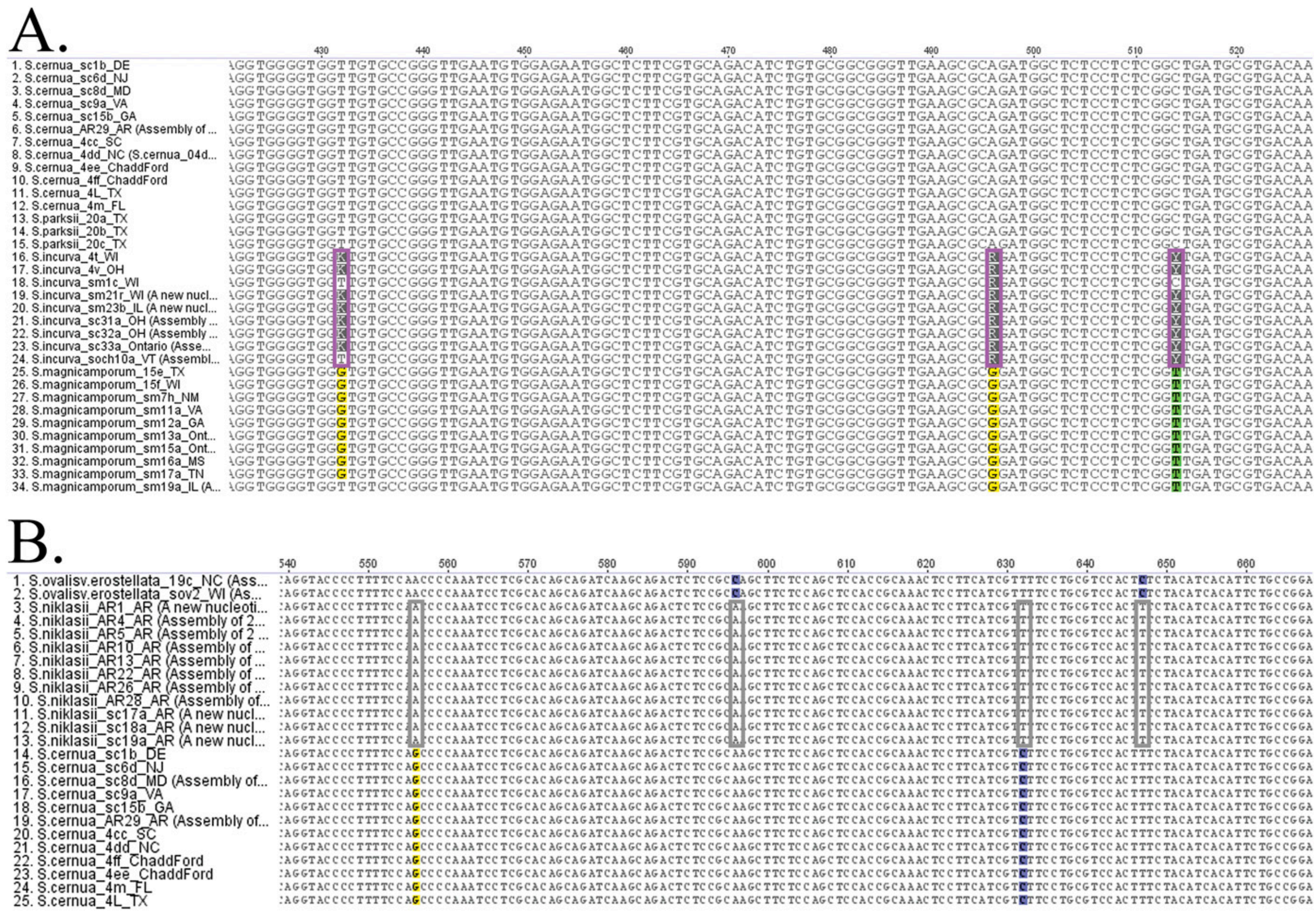

Fig. 8. Examples of nucleotide alignments highlighting regions supporting hybridization between species. A. ITS. *Spiranthes incurva* highlighted in purple, with nucleotide ambiguities corresponding to differences between *S. cernua* s. s. (upper) and *S. magnicamporum* (lower). B. *ACO*. *Spiranthes niklasii* highlighted in gray, displaying shared nucleotide changes with both *S. ovalis* (upper, e.g. position 556) and *S. cernua* s. s. (lower, e.g. position 596).

Morphometrics—Our boxplots of the labella of *S. arcisepala*, *S. cernua*, *S. incurva*, *S. ×kapnosperia*, *S. magnicamporum*, *S. niklasii*, and *S. ochroleuca* yield largely overlapping plots (Fig. 9), thus highlighting the cryptic nature of these species and the confusion many botanists encounter when endeavoring to identify them. Based on the sampling we included, *S. arcisepala* is the least variable species, however the measured features entirely overlap the space and variability of *S. cernua* s. s. Similarly, the variation of *S. ochroleuca* is also contained within *S. cernua* s. s.. *S. arcisepala*, *S. cernua*, and *S. ochroleuca* in turn overlap with the morphological variability of *S. incurva*. Thus morphological measurements alone cannot clearly separate a priori groupings. A separate analysis (not shown) including measurements of the sepals, labella, leaves, and whole plants found a slight difference between a priori taxa, however all taxa were widely overlapping, essentially similar or overlapping in all of the features we measured.

Qualitative characters appear to be more useful in confidently distinguishing members of the *S. cernua* species complex sensu nov. The lateral sepals of *S. arcisepala* are typically downwardly falcate (Figs. 10, 11), whereas the lateral sepals of *S. incurva* typically sweep upward and occasionally outward at the apices (Figs. 12, 13); similarly, the lateral sepals of *S. cernua* usually sweep upwards or are more or less parallel to the ground (i.e. within the plane of the dorsal sepal and petals) (Figs. 1, 2). The flowers of *S. arcisepala* are also generally slightly smaller than *S. cernua* and *S. incurva*, and the flowers of *S. cernua*, as indicated by the specific epithet, often become strongly nodding as the inflorescence matures, whereas the flowers of *S. arcisepala* and *S. incurva* are more commonly held parallel to the ground, or only slightly nodding (the flowers of *S. incurva* are occasionally slightly ascending).

Taxonomic Treatment

Spiranthes cernua (L.) Rich. sensu stricto, Sp. Pl. 2: 946. 1753.—TYPE: U. S. A. "Virginia, Canada" s.d., *Kalm s.n.* (lectotype: LINN!). *Ophrys cernua* L.; *Neottia cernua* (L.) Sw; *Gyrostachys cernua* (L.) Kuntze; *Ibidium cernuum* (L.) Bouse; *Spiranthes annua* Lesq. ex Brandeger and Coville (a misprint of *S. cernua*, see Branner and Coville 1891); *Triorchis cernuus* (L.) Nieuwl.

Limodorum autumnale Walter, Fl. Carol.: 221. 1788.—TYPE: unknown location, s.d., *Walter Herbarium no. 722* (BM).

Spiranthes parksii Correll, Amer. Orchid Soc. Bull. 16: 400, f. 1–6. 1947.—TYPE: U. S. A. Texas: Brazos County, Democrat Bridge, Navasota River, collected 19 October 1945, *Parks s.n.* (holotype: AMES!).

Spiranthes cernua is most similar to *S. arcisepala*, *S. incurva*, and *S. ochroleuca*. It can be distinguished from these species by its upward sweeping lateral sepals (vs. downwardly arching in *S. arcisepala*), centrally thinner labellum, distribution along

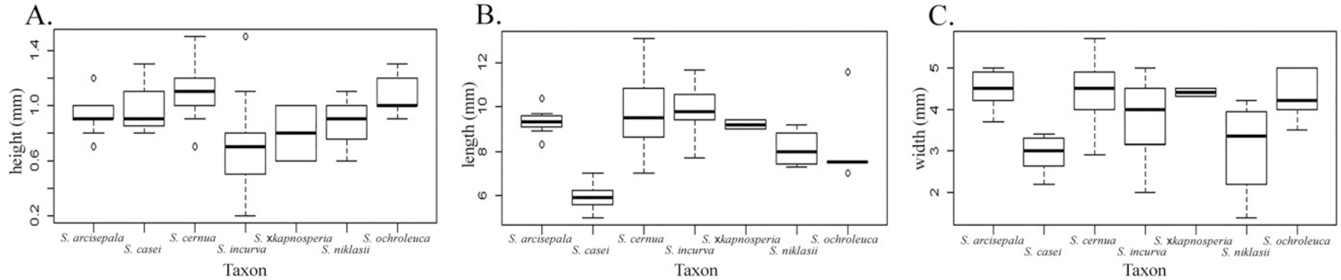

FIG. 9. Boxplot comparisons of select labellum characters for the *Spiranthes cernua* species complex s. s. and hybrids. A. Callosity (nectar gland) height (mm). B. Labellum length (mm). C. Width of lower labellum (mm).

the Coastal Plain and southern Appalachian Mountains (vs. centrally thickened labellum and occurrence in the Interior Lowlands and northern Appalachian Mountains in *S. incurva*), and white to pale-yellow abaxial labellum coloration and abaxial surface with conical, highly reduced glands (vs. abaxial yellow to golden coloration and abaxial surface with spherical glands in *S. ochroleuca*).

Terrestrial, acaulescent, deciduous herb, to ca. 100 cm tall. Roots fasciculate, fleshy, slender. Leaves 1–5, basal, held upright, remaining until after anthesis (occasionally fugacious at anthesis), withering shortly thereafter, linear-lanceolate to lanceolate, 5–22 cm long, 5–8 mm wide, bluntly acuminate, leaf base tapered and decurrent. Peduncle glabrous, 1–3 small leafy cauline bracts occasionally present (frequently absent), quickly reducing to adpressed, clasping, lanceolate, acute bracts; spike a single row of flowers in an open to tightly coiled spiral (appearing as 1–4 'ranks'), moderately to densely pubescent with blunt-tipped septate trichomes to 0.5 mm long. Floral bracts pubescent, lanceolate, acuminate; concave around the ovary, 7–14 mm long. Flowers campanulate, slightly to strongly nodding (more so with age), white to pale ivory, lightly fragrant with a general floral odor or not fragrant (some coastal populations exhibit strong general floral fragrance). Sepals free, moderately to densely pubescent with blunt-tipped capitate septate trichomes. Dorsal sepal slightly convex, slightly to strongly recurved near the tip, lanceolate, bluntly acuminate, 6–12 mm long, 3 mm wide when flattened. Lateral sepals lanceolate, acute, straight to just barely falcate, angled slightly outward and upward, the tips often incurved, surpassing the dorsal sepal and petals, 6–12 mm long, 2 mm wide. Dorsal petals slightly concave, lanceolate, bluntly acute, slightly to strongly recurved at tips, with the dorsal sepal appearing stellate, 6–12 mm long, 3 mm wide when flattened. Labellum shortly clawed, free but clasping the column, keeled/concave for its length, recurved strongly downward at about 1/3 the distance from the claw to labellum apex, centrally glabrous, margin entire to very slightly undulating from the base until the area of recurvature, below point of recurvature margin becoming shallowly laciniate and crisped, white but rarely centrally pale yellow, 7–13 mm long, 3–6 mm wide below the callosities, 2–6 mm wide at the area of recurvature when flattened, apex acuminate; 2 basal callosities/nectar glands, white to pale yellow, conical, upright, 1–2 mm tall, with long, dense papillae at the base. Column protandrous, slightly rhombic, green, 4.1–6 mm long, 2–2.5 mm wide, with a fringe of minute glands or papillae in a thin crescent just below the stigmatic surface, with a pair of upright flaps or wings at each side and clasping the column, the wings green basally; column foot glabrous; rostellum well-developed, white to ivory, tapering to thin acute membranes at the apex, 1.2–1.5 mm long; stigmatic surface glabrous, shiny, 1–2 mm long, 1.5–2.5 mm wide; anther triangular-ovoid; pollinium attached to a well-developed viscidium; viscidium linear, immersed in the rostellum, leaving behind a narrow V-shaped rostellar remnant after removal, 1–1.8 mm long. Ovary moderately to densely pubescent with septate trichomes. Fruit a light brown upright ovoid capsule. Figures 1 and 2.

In its new strict sense, *S. cernua* (Figs. 1, 2) occurs from the Coastal Plain to the eastern and southern Appalachian Mountains, southern Interior Lowlands, and Cumberland Plateau (Fig. 14). Within *S. cernua* s. s., we here formally synonymize *S. parksii* under *S. cernua* as a localized sub-peloric form promulgated through apomixis, supporting the work of Dueck and Cameron (2007, 2008b), Dueck et al. (2014), Pace and Cameron (2016), and Pace et al. (2017). As exemplified by this synonymization, *S. cernua* s. s. is still a morphologically variable species (Figs. 1, 9), although less so than previously defined. Some populations, such as the former *S. parksii*, exhibit small-sized reduced flowers in an open spiral, whereas others, particularly in the Mid-Atlantic region from southern New York to coastal Virginia, display large flowers nearly 1 cm in length held in a very tight spiral (appearing as 3–4 separate ranks), with a complete gradation between these two extremes. In contrast to this morphological variability, *S. cernua* s. s. is consistent in its habitat preferences, occurring in essentially wet, short-statured, open graminoid-cyperoid locations: mossy seeps, maritime dune swales, *Sphagnum* L. dominated lake and pond edges, wet meadows, roadsides, and open savannas (Fig. 15). *Spiranthes cernua* s. s. is typically faintly fragrant with a general floral odor, although some populations are strongly fragrant, whereas others appear to entirely lack a perceivable fragrance.

Spiranthes arcisepala M.C. Pace, sp. nov.—TYPE: U. S. A. New York: Hamilton County, just east of Long Lake, town of Long Lake, north of Shaw Pond in wet roadside ditch and *Sphagnum* seep under a power-line cut along Newcomb Road / 28N, collected 4 September 2014, *Pace 636* (holotype: NY; isotypes: AMES, BH, CM, K, US, RENZ, WIS).

Spiranthes arcisepala is most similar to *S. cernua* s. s. and *S. ochroleuca*. It can be distinguished from *S. cernua* s. s. by its more open spiraled inflorescence, smaller flowers, and rounded labellum apex. It can be distinguished from *S. ochroleuca* by its white colored labellum, and can be distinguished from both *S. cernua* s. s. and *S. ochroleuca* by its downward arching lateral sepals.

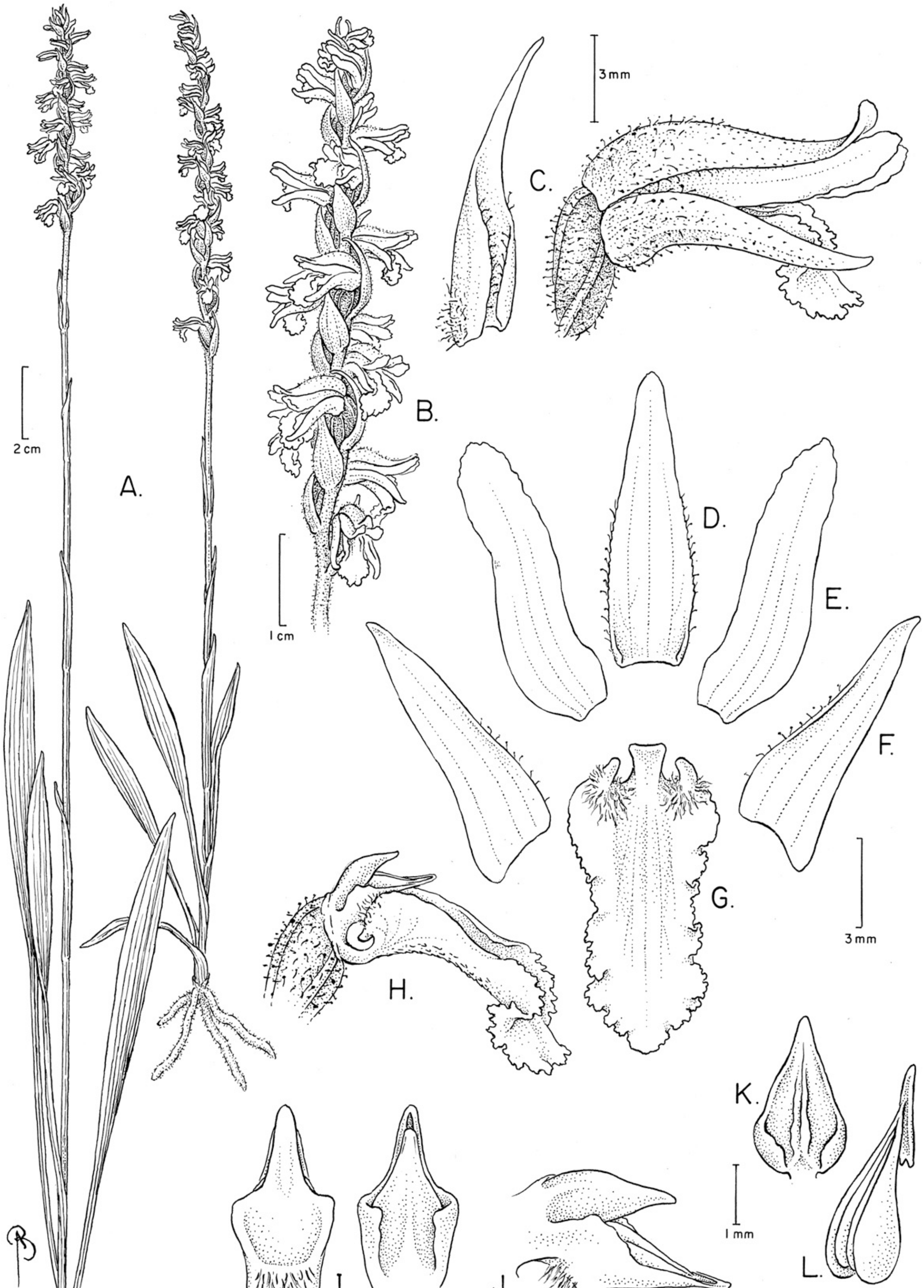

Fig. 10. *Spiranthes arcisepala*. A. Habit. B. Inflorescence. C. Flower and floral bract in profile. D–G. Flower, dissected view. D. Dorsal sepal. E. Dorsal petal. F. Lateral sepal. G. Labellum. H. Labellum, column, and ovary in natural position. I–K. Column. I. Dorsal and ventral views. J. Profile. K. Anther. L. Pollinia. Drawn from *Pace 1005* and *Pace 1008* by Bobbi Angell.

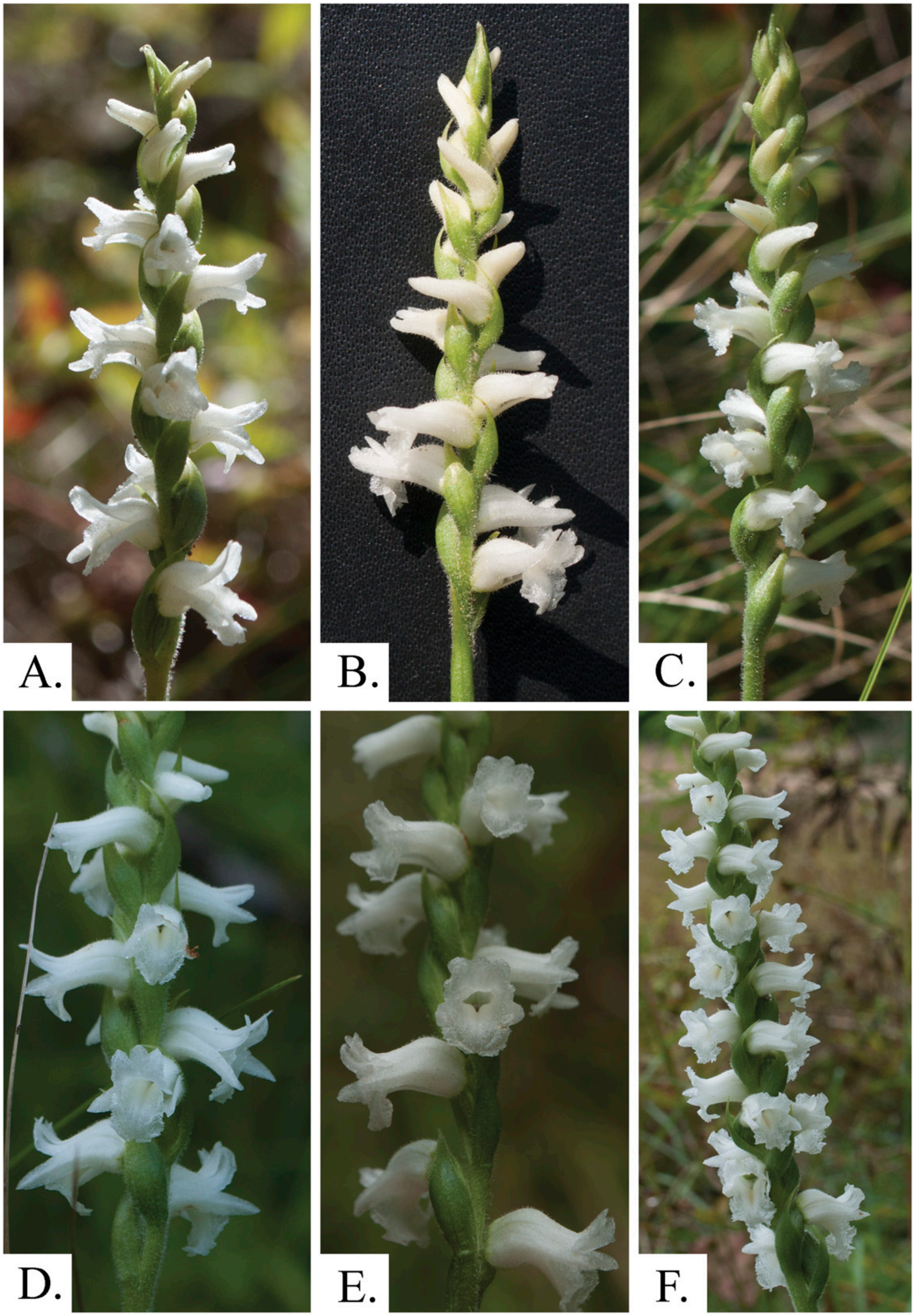

FIG. 11. *Spiranthes arcisepala.* A. Type location, Long Lake, Hamilton Co., New York. B. Delaware Co., New York, sample NY1. C. Indian Lake, Hamilton Co., New York. D. Wells, Hamilton Co., New York. E. Morris Co., New Jersey. F. Fayette Co., Pennsylvania.

Terrestrial, acaulescent, deciduous herb, to ca. 46 cm tall. Roots fasciculate, fleshy, slender. Leaves 1–4, basal, held upright, remaining until after anthesis, withering shortly thereafter, linear-lanceolate to slightly lanceolate, bluntly acuminate, leaf base tapered and decurrent. Peduncle glabrous, 1–2 small leafy cauline bracts occasionally present (frequently absent), quickly reducing to adpressed, clasping, lanceolate, acute bracts; spike a single row of flowers in an

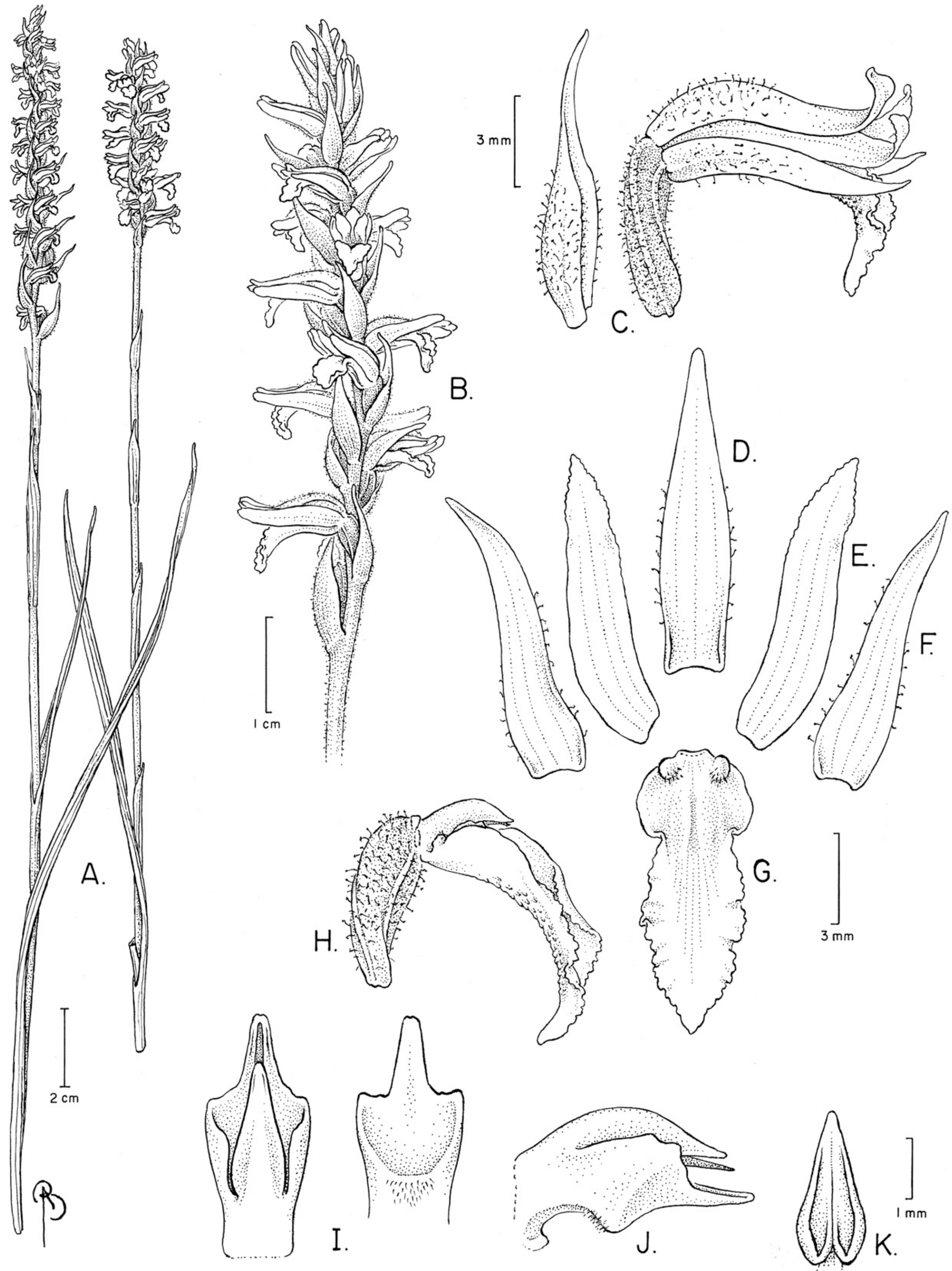

Fig. 12. *Spiranthes incurva*. A. Habit. B. Inflorescence. C. Flower and floral bract in profile. D–G. Flower, dissected view. D. Dorsal sepal. E. Dorsal petal. F. Lateral sepal. G. Labellum. H. Labellum, column, and ovary in natural position. I–K. Column. I. Dorsal and ventral views. J. Profile. K. Anther. Drawn from *Pace 629*, *Pace 631*, and *Pace 1007* by Bobbi Angell.

open to slightly tightly coiled spiral (typically appearing as 1 distinct 'rank'), moderately to densely pubescent with blunt-tipped septate trichomes 0.5 mm long. Floral bracts densely pubescent, narrowly lanceolate, acuminate, concave around the ovary, to 12.3 mm long. Flowers resupinate, campanulate, slightly to moderately nodding and becoming more open with age, white, faintly to moderately fragrant with a general floral scent. Sepals free, moderately to densely pubescent with blunt-

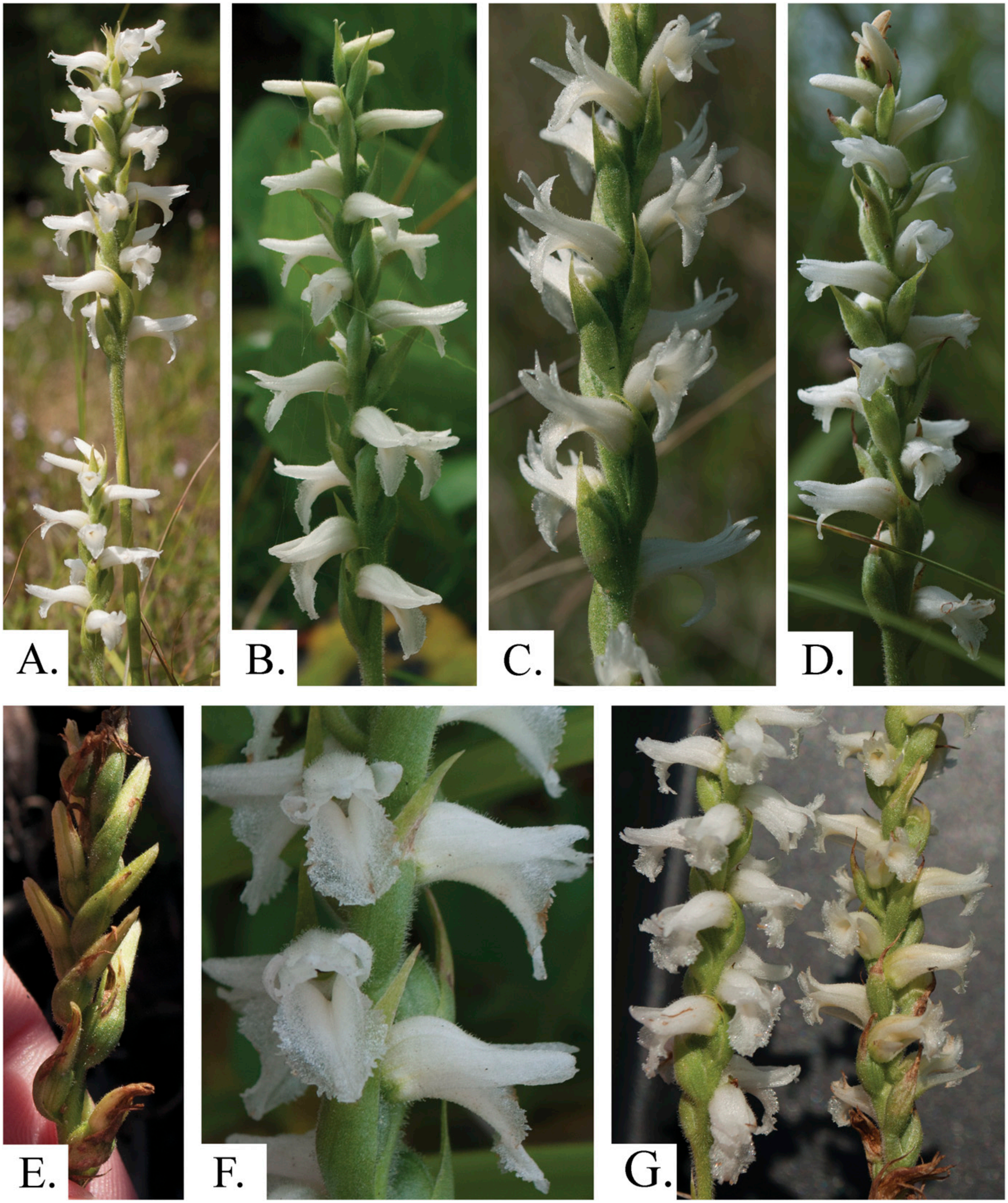

FIG. 13. *Spiranthes incurva*. A. Hamilton Co., New York. B. Portage Co., Ohio, sample sc31. C. Orleans Co., Vermont, sample soch10a. D. Dane Co., Wisconsin. E. Cleistogamous, entirely apomictic population, Lake Co., Indiana. F. Potter Co., Pennsylvania. G. Co-occurring *S. incurva* (L) and *S. ochroleuca* (R) (artificially arranged), Warren Co., New York.

tipped capitate septate trichomes. Dorsal sepal slightly convex, slightly recurved to moderately upwardly reclined distally, lanceolate, bluntly acuminate, 8.3–10.6 mm long, 2–2.9 mm wide when flattened. Lateral sepals lanceolate, acute, slightly to strongly downwardly falcate from about 1/3 to 1/2 of their length, the tips often surpassing the lower labellum margin in profile, 8.3–9.7 mm long, 1.4–2.4 mm wide. Dorsal petals slightly concave, lanceolate, bluntly acute, slightly to strongly

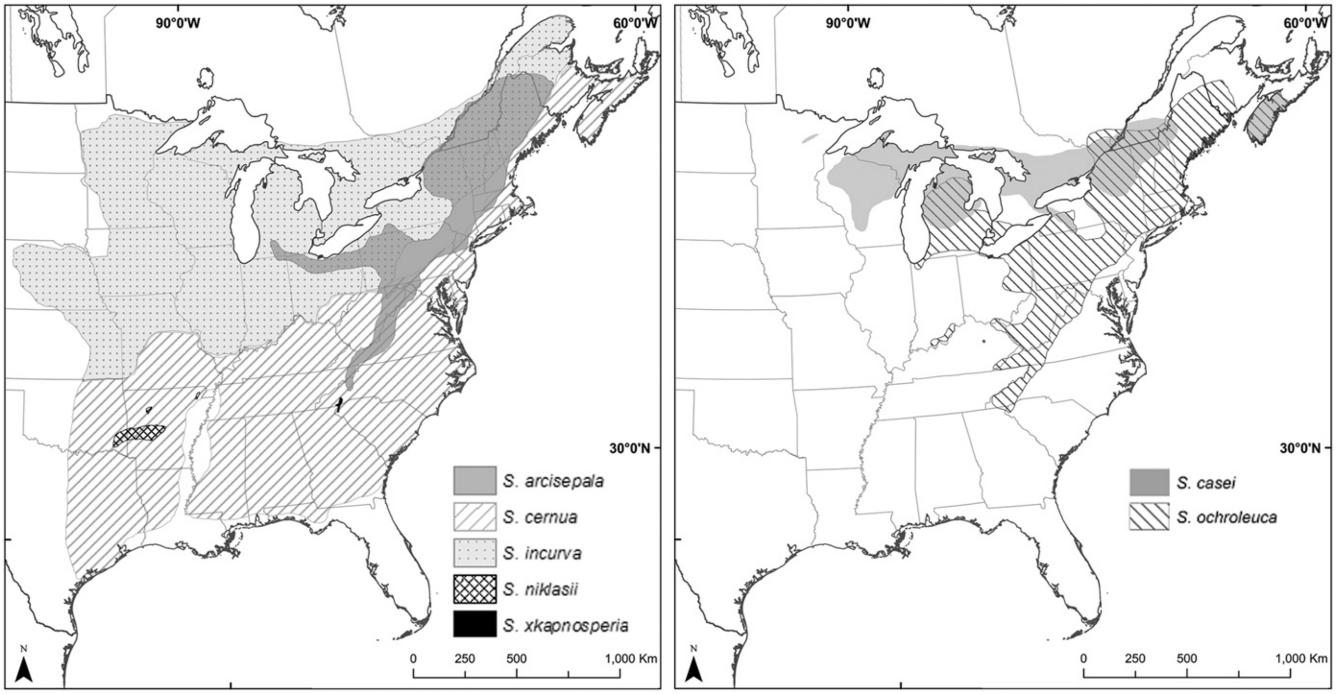

FIG. 14. Distribution maps of the *S. cernua* species complex s. s., based on herbarium specimens and phylogenetic sampling. A. *S. arcisepala*, *S. cernua* s. s., *S. incurva*, *S. ×kapnosperia*, *S. niklasii*. B. *S. casei* and *S. ochroleuca*. Maps produced by Elizabeth Kiernan, New York Botanical Garden GIS Lab.

recurved at tips, with the dorsal sepal appearing stellate, 8.3–10.7 mm long, 2.1–2.5 mm wide when flattened. Labellum shortly clawed, free but clasping the column, keeled/concave for its length, recurved strongly downward at about 1/2 the distance from the claw to labellum apex, constricted near the recurvature and then dilating below, centrally glabrous and thickened, margin entire to very slightly undulating from the base until the area of recurvature, below point of recurvature margin becoming ruffled, margin white, central area of labellum white to extremely pale yellow back in the throat, 7.2–10.1 mm long, 4.4–5 mm wide below the callosities, 3.2–3.8 mm wide at the area of recurvature when flattened, 4.1–5.2 mm wide at widest point below recurvature; 2 basal callosities/nectar glands, white to pale yellow, conical, upright, 0.9–1.2 mm tall, with long, dense papillae at the base. Column protandrous, slightly rhombic, green, 3.6–5 mm long, with a fringe of minute glands or papillae in a thin crescent just below the stigmatic surface and with a pair of upright flaps or wings at each side and clasping the column, the wings green basally; column foot glabrous; rostellum well-developed, white to ivory, tapering to thin acute membranes at the apex; stigmatic surface glabrous, shiny; anther triangular-ovoid; pollinium attached to a well-developed viscidium; viscidium linear, immersed in the rostellum, leaving behind a narrow V-shaped rostellar remnant after removal, 1.6–1.8 mm long. Ovary moderately to densely pubescent with septate trichomes, green. Fruit a light brown upright ovoid capsule. $2n = 45$. Figures 10 and 11.

Etymology—Latin, *'arcisepala'* is a combination of 'arcus' (arching) and 'sepalorum' (sepals), referring to the downwardly arching lateral sepals of this species, serving as a relatively constant diagnostic morphological character. "Appalachian ladies' tresses" is a suggested common name, indicating the main distribution of this species.

Spiranthes arcisepala (Figs. 10, 11) is a newly described and long overlooked cryptic sister species to *S. ochroleuca* + *S. casei*. It is primarily restricted to the mid- and northern Blue Ridge and Northern Highlands, Ridge and Valley, Great Valley, Appalachian Plateau, and Adirondack systems of the Appalachian Highlands in Nova Scotia, east-southeast Ontario, Quebec, Connecticut, Maine, Massachusetts, New Hampshire, New Jersey, New York, Pennsylvania, Virginia, Vermont, and West Virginia, and the eastern Interior Lowlands of northern Ohio, northeastern Indiana, and southern Michigan (Fig. 14). The distribution of *S. arcisepala* is essentially similar to *S. ochroleuca* apart from the occurrence of the latter species further westward into the Great Lakes Basin and southward along the spine of the Appalachian Mountains to Kentucky, North Carolina, and Tennessee. *Spiranthes arcisepala* corresponds to one of the New England races of Sheviak (1982) and to the "fen ecotype" and "old field ecotype" of Homoya (1993). One of the key features distinguishing *S. arcisepala* is its downwardly falcate lateral sepals. This feature is relatively constant across populations, however occasional individuals and populations have lateral sepals that are just barely falcate (e.g. Fig. 11B, C). In these instances, the flowers are still smaller than *S. cernua* s. s., *S. incurva*, and *S. ochroleuca*, and are essentially wholly white. *Spiranthes arcisepala* is typically found in wet, short-statured graminoid-cyperoid habitats including fens, bogs, mossy (often *Sphagnum*) and lichen-covered seeps, and wet roadsides (Fig. 15), and can occasionally be found growing interspersed with *S. incurva*. The flowers of *S. arcisepala* possess a faint general floral fragrance, perceivably similar to *S. cernua* s. s.

Spiranthes incurva (Jenn.) M.C. Pace, comb nov. [ancient *Spiranthes cernua* × *Spiranthes magnicamporum*], *Ibidium incurvum* Jenn., Ann. Carnegie Mus. 3: 483. 1906.—TYPE: U. S. A. Pennsylvania: Erie County, Presque Isle. Sandy margins of pond near Fog Whistle, collected 26 August 1905, *Jennings s.n.* (Lectotype: CM!; isolectotypes: *Jennings*

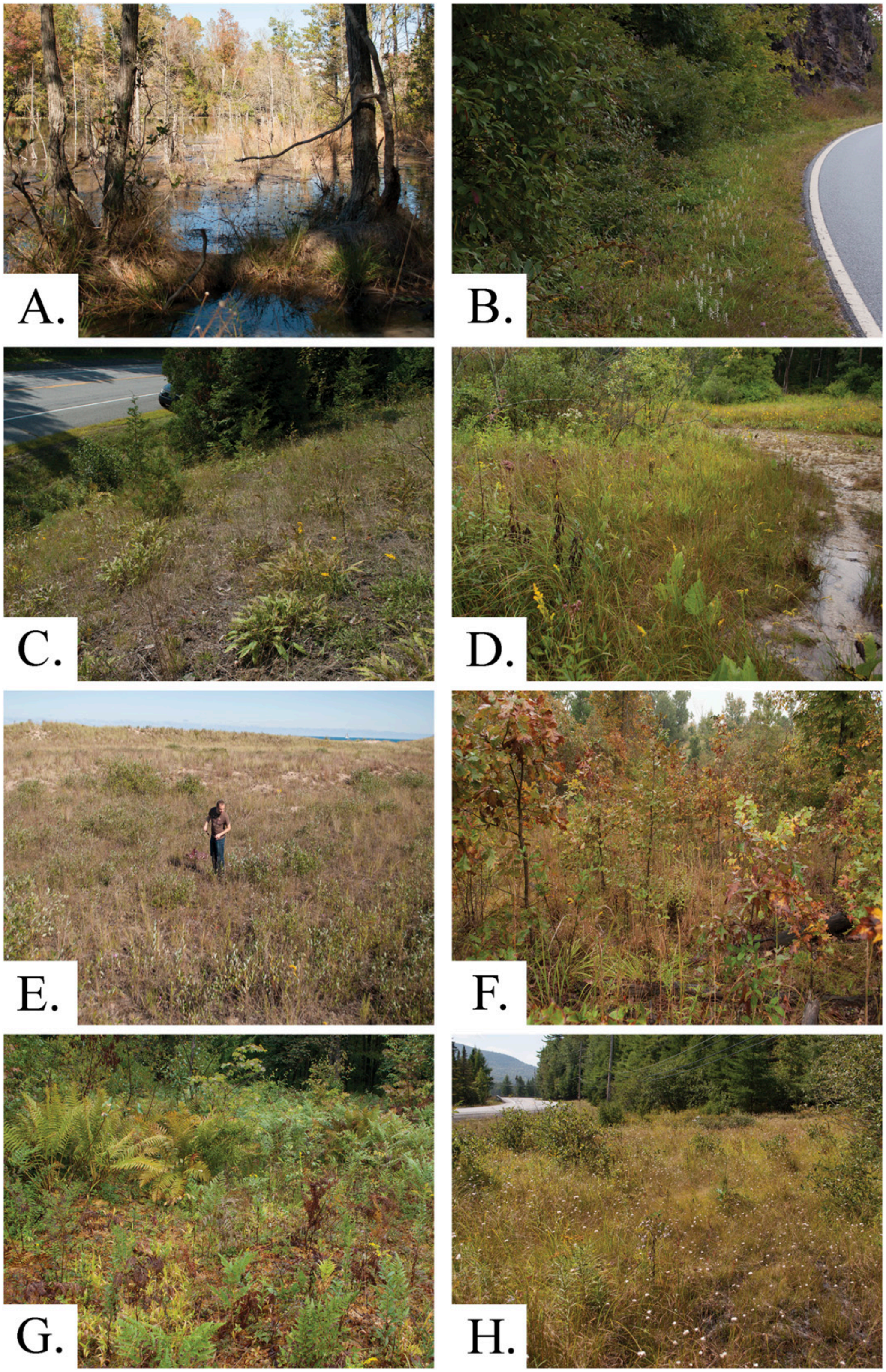

Fig. 15. A–B. Habitat of *S. cernua* s. s. A. Marshy pond edge, with *Nyssa sylvatica* Marshall, *Chamaecyparis thyoides* (L.) Britton, Sterns & Poggenb., *Sarracenia* L., and *Eriocaulon* L., Lexington Co., South Carolina. B. Seeping, graminoid-cyperoid and brushy roadside, mixed with *S.* ×*kapnosperia* (type location), Transylvania Co., North Carolina. C–E. Habitat of *S. incurva*. C. Xeric roadside slope, with *Solidago nemoralis* Aiton and *Andropogon gerardi* Vitman, Orleans Co., Vermont; sample location of soch10. D. High diversity fen, Clark Co., Ohio, sample location of sc32a. E. Sandy, xeric dune swales and back dunes, co-occurring with *S. magnicamporum* (with Mr. S. Martella), Lake Co., Illinois. F. Habitat of *S. niklasii*; xeric, prairie-like opening, Greene Co., Arkansas, sample location of AR1. G–H. Habitat of *S. arcisepala*. G. Seeping fen, McKean Co., Pennsylvania. H. Type location; roadside ditch and *Sphagnum* seep with *Eriophorum virginicum* L. under a power-line cut, Hamilton Co., New York.

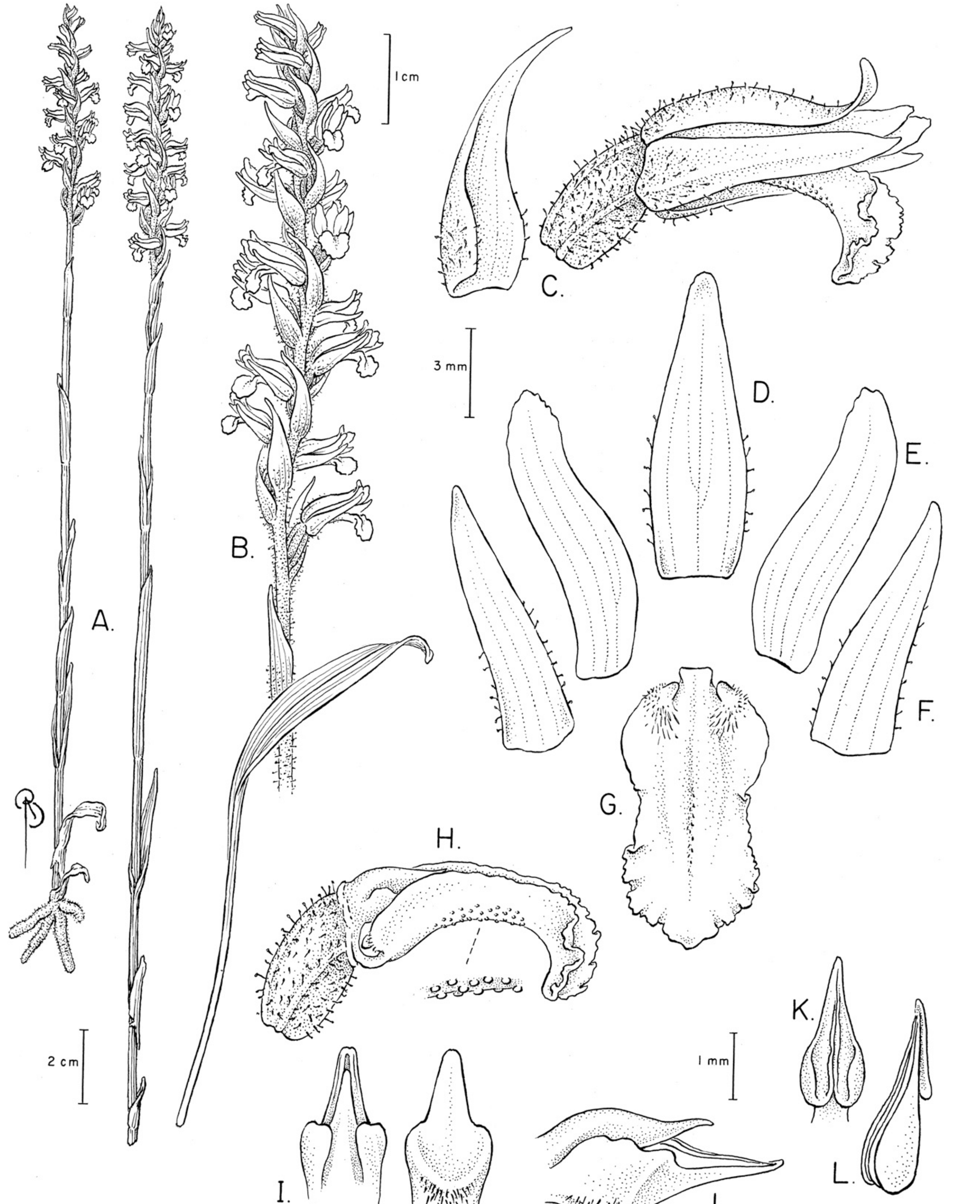

Fig. 16. *Spiranthes* ×*kapnosperia*. A. Habit. B. Inflorescence. C. Flower and floral bract in profile. D–G. Flower, dissected view. D. Dorsal sepal. E. Dorsal petal. F. Lateral sepal. G. Labellum. H. Labellum, column, and ovary in natural position, with detail of spherical abaxial labellum glands. I–K. Column. I. Dorsal and ventral views. J. Profile. K. Anther. L. Pollinia. Drawn from *Pace 1024* and *Pace 1030* by Bobbi Angell.

s.n., *18*, 26 August 1905, CM!, NY!; syntypes: *Jennings s.n.*, 24 Aug 1905, *Jennings s.n.*, *18*, *9780*, 25 August 1905, MICH!, MIN!, NYS!, PH!; paratypes included by Jennings (1906): *Shafer 29, 9781* 9–11 September 1900, CM!, MUHW!, PH!, *Gutenberg s.n.* 16 August 1880, CM!). Note: Catling, via an annotation label, designated *Jennings s.n.*, 26 Aug 1905, as the "holotype." Since Jennings selected a suite of specimens, "Aug. 24–26, 1905", housed at CM as

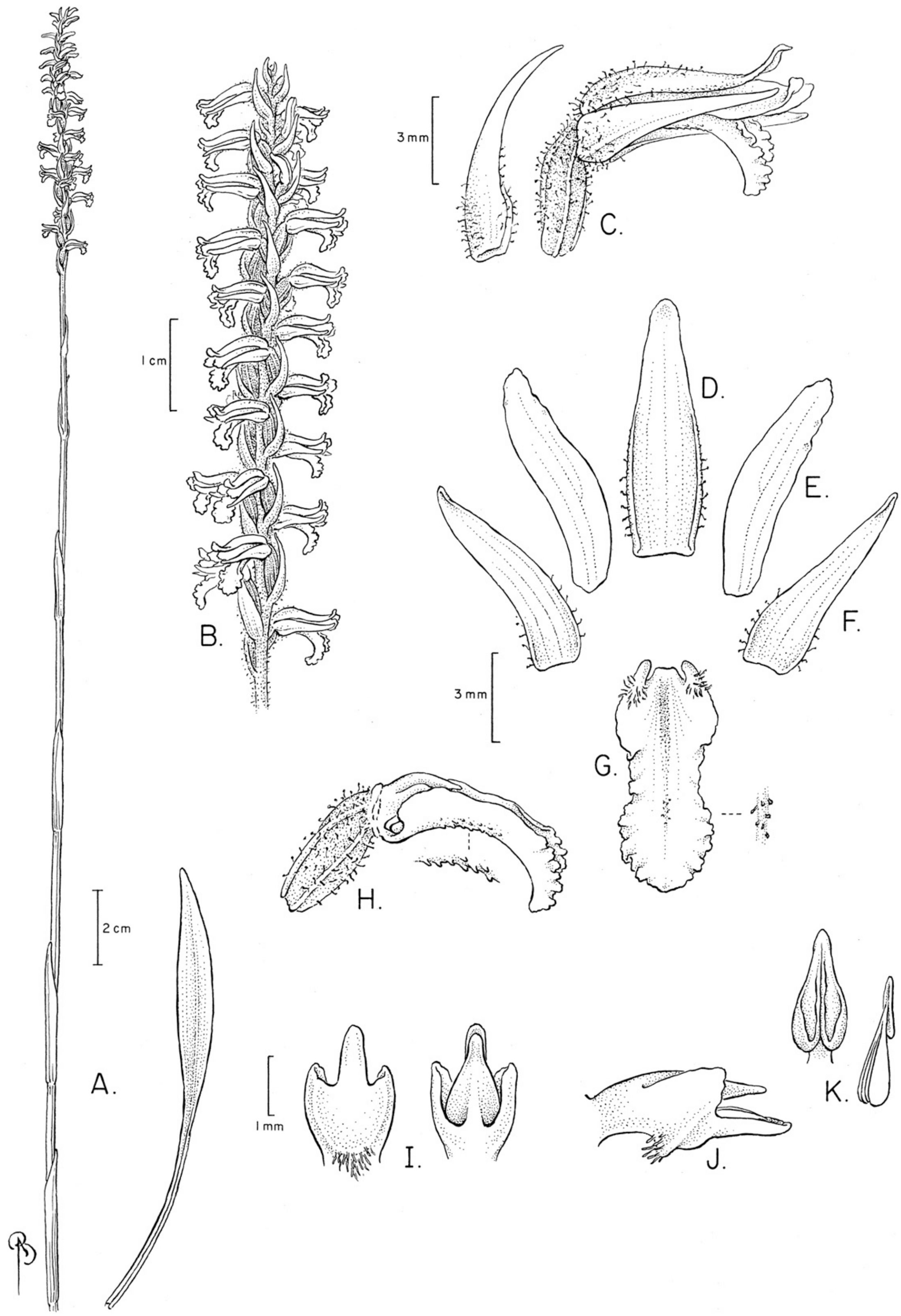

Fig. 17. *Spiranthes niklasii*. A. Habit. B. Inflorescence. C. Flower and floral bract in profile. D–G. Flower, dissected view. D. Dorsal sepal. E. Dorsal petal. F. Lateral sepal. G. Labellum, with detail of central adaxial papillae. H. Labellum, column, and ovary in natural position, with detail of abaxial labellum surface. I–K. Column. I. Dorsal and ventral views. J. Profile. K. Anther and pollinia. Drawn from *Pace 1036* by Bobbi Angell.

"the type specimens", and not a specific specimen, collection number, or sheet, the specimen designated by Catling is more properly designated as the lectotype. All other specimens collected on Aug. 26, 1905 must then be isolectotypes, and all other specimens collected within "the type specimens" collection range designated as syntypes, as above. Jennings' collection number 18 appears multiple times on differing days.

Spiranthes incurva is most similar to its parental species: *S. cernua* s. s. and *S. magnicamporum*. It can be distinguished from *S. cernua* s. s. by its thickened central labellum, more narrowly lanceolate floral parts, frequently more stellate and ascending flowers, and more northern and western distribution, and it can be distinguished from *S. magnicamporum* by its larger callosities, slightly earlier flowering period, and non-papillate, paler labellum.

Terrestrial, acaulescent, deciduous herb, ca. 40 cm tall. Roots fasciculate, fleshy, slender to slightly tuberous. Leaves 1–5, basal, held upright, occasionally remaining until anthesis and withering shortly thereafter but more frequently absent at anthesis, linear-lanceolate to lanceolate, acuminate, leaf base tapered and decurrent. Peduncle glabrous, 1–2 small leafy cauline bracts occasionally present (frequently absent), quickly reducing to adpressed, clasping, lanceolate, acute bracts; spike a single row of flowers in a moderately to tightly coiled spiral (appearing as 1–4 "ranks"), moderately to densely pubescent with blunt-tipped septate trichomes to 0.5 mm long. Floral bracts moderately to densely pubescent, lanceolate, acuminate, concave around the ovary, 9.5–9.7 mm long. Flowers slightly tubularly campanulate, slightly ascending to moderately nodding, white to pale ivory. Sepals free, moderately to densely pubescent with blunt-tipped capitate septate trichomes. Dorsal sepal slightly convex, slightly to strongly recurved near the tip, lanceolate, bluntly acuminate, 8.6–10.9 mm long, 2–2.6 mm wide when flattened. Lateral sepals lanceolate, acute, straight to just barely upwardly falcate, angled slightly upward, the tips often meeting the dorsal sepal and petals, 7.7–10.7 mm long, 1.7–2.3 mm wide. Dorsal petals slightly concave, lanceolate, bluntly acute, slightly to strongly recurved at tips, with the dorsal sepal appearing stellate, 8.1–10.5 mm long, 1.8–2.2 mm wide when flattened. Labellum minutely clawed, free but clasping the column, keeled/concave for its length, recurved strongly downward at about 1/3 to 1/2 the distance from the claw to labellum apex, centrally glabrous, margin entire to very slightly undulating from the base until the area of recurvature, below point of recurvature margin becoming shallowly laciniate and crisped, margin white, central area of labellum white to very pale yellow, labellum 7.4–9.9 mm long, 3.7–5.4 mm wide below the callosities, 3.3–3.9 mm wide at the area of recurvature when flattened, 1.8–4.5 mm wide at midpoint below recurvature, apex acuminate; 2 basal callosities/nectar glands, white to yellow, very small, conical to rounded mounds, upright, 0.3–0.8 mm tall, with long, dense papillae at the base. Column protandrous, slightly rhombic, green, 3.3–5 mm long, with a fringe of minute glands or papillae in a thin crescent just below the stigmatic surface, with a pair of upright flaps or wings at each side and clasping the column, the wings green basally, becoming white to translucent; column foot glabrous; rostellum well-developed, white to ivory, becoming dark brown with age, tapering to thin acute membranes at the apex, 1.2–1.8 mm long; stigmatic surface glabrous, shiny; anther dark coffee-brown, triangular-ovoid; pollinium attached to a well-developed viscidium, yellow; viscidium linear, immersed in the rostellum, leaving behind a narrow V-shaped rostellar remnant after removal, 1.2–1.5 mm long. Ovary moderately to densely pubescent with septate trichomes. Fruit a light brown ovoid capsule. $2n = 45–60$. Figures 12 and 13.

Etymology—As in Jennings' original description, '*incurva*', from the Latin, refers to the incurved callosities of this species of hybrid origin. This feature is a key character to distinguish this species from the frequently co-occurring *S. magnicamporum*, one of its parental species, which has highly reduced, non-incurved callosities (the callosities of some *S. incurva* may approach the highly reduced callosities of *S. magnicamporum*). We suggest the common name "Sphinx ladies' tresses" for this species. The Sphinx is a hybrid mythological creature prone to enigmatic and intractable questions; similarly, the inclusion of hybrid *S. incurva* within the traditional concept of *S. cernua* has long been a major source of the latter's morphological variation, and strongly contributed to the idea that a proper delimitation of the *S. cernua* species complex was intractable.

Spiranthes incurva (Figs. 12, 13) represents likely ancient *S. cernua* s. s. × *S. magnicamporum*, and entirely replaces *S. cernua* in the middle, northern, and eastern Interior Lowlands, Western and Northern Appalachian Mountains and Adirondacks, Great Lakes Basin, and Prairie Peninsula, from New Brunswick, southern Ontario, and southern Quebec, west to Minnesota, central Nebraska, and eastern Kansas (Fig. 14). In addition to the geographic differentiation, *S. incurva* has a centrally thickened labellum, shorter callosities, a more lanceolate labellum, and narrower leaves, vs. *S. cernua* s. s. which has a labellum which is not centrally thickened, longer callosities, a more oblong labellum, and wider leaves. Due to their morphological similarities and occasional to frequent co-occurrence, *S. incurva* has been confused with *S. magnicamporum* and *S. ochroleuca*. These three species can be distinguished from one another by flowering time, labellum surface texture, and floral shape and color: *S. incurva* displays white, stellate to pseudo-campanulate flowers with smooth labella in full bloom just as *S. magnicamporum* is reaching anthesis with ivory-colored more tubularly-shaped flowers with papillate labella, whereas the abaxial labellum coloration of *S. ochroleuca* is yellow to butterscotch colored, and the ivory-colored flowers are typically strongly pseudo-campanulate (Figs. 3, 13). Although it typically occurs in more xeric habitats than *S. cernua* s. s., *S. incurva* has varied habitat preferences: submerged in shallow lake dune pools, fens, bogs, rocky ice-scour meadows, lake edges, wet to xeric roadsides and prairies, alvar escarpments, and xeric rolling lake dunes composed of pure sand (Fig. 15). The fragrance of *S. incurva* is similar to *S. cernua* s. s. in odor and intensity, lacking the strong vanilla-licorice fragrance of *S. magnicamporum*; occasional populations are slightly malodorous.

Spiranthes ×kapnosperia M.C. Pace, nothosp. nov. [*Spiranthes cernua* × *Spiranthes ochroleuca*]—TYPE: U. S. A. North Carolina: Transylvania County, Great Smoky Mountains, Pisgah National Forest, ca 7.5 km NW of Balsam Grove, north side of 215, below a steep seeping cliff, growing in moss and lichen hummocks, collected 2 October 2016, *Pace 1030* (Holotype: NY; isotypes: NCU, US).

Spiranthes ×kapnosperia is most similar to *S. cernua* s. s. and *S. ochroleuca*. It can be distinguished from *S. cernua* s. s. by smaller, less widely gapping ivory-colored flowers, and spherical glands on the abaxial labellum surface; it can be distinguished from *S. ochroleuca* by its pale-yellow colored abaxial labellum surface (vs. deep golden yellow) and sepal apices that are acuminate vs. linear-lanceolate.

Terrestrial, acaulescent, deciduous herb, to ca. 30 cm tall. Roots fasciculate, fleshy, slender. Leaves 1–2, basal, held upright, remaining until after anthesis, withering shortly thereafter, linear-lanceolate to lanceolate, to 133 mm long, 8.5–10.5 mm wide, narrowly acuminate; leaf base narrowly tapered and decurrent. Peduncle glabrous, to 30 cm, 1–2 small leafy cauline bracts occasionally present, quickly reducing to adpressed, clasping, lanceolate, acute bracts; spike a single row of flowers in an open to moderately tightly coiled spiral (appearing as single rank), moderately to densely pubescent with blunt-tipped septate trichomes to 0.5 mm long. Floral bracts pubescent, broadly lanceolate, acuminate, concave around the ovary, 11.4–13.7 mm long. Flowers resupinate, campanulate, only slight gapping, slightly ascending to slightly nodding, pale ivory to white. Sepals free, moderately to densely pubescent with blunt-tipped capitate septate trichomes. Dorsal sepal slightly convex, slightly to moderately recurved near the tip, lanceolate, acuminate, 7.7–10.2 mm long, 1.6–3.3 mm wide when flattened. Lateral sepals lanceolate, acute, straight to just barely falcate, angled slightly outward and upward, the tips often surpassing the dorsal sepal and petals, 7.6–10 mm long, 1–1.9 mm wide. Dorsal petals slightly concave, lanceolate, bluntly acute, slightly to moderately recurved at tips, 7.4–10 mm long, 1.5–2.3 mm wide when flattened. Labellum shortly clawed, free but clasping the column, keeled/concave for its length, recurved strongly downward at about 2/3 the distance from the claw to labellum apex, centrally glabrous, margin entire to very slightly undulating from the base until the area of recurvature, below point of recurvature margin becoming shallowly ruffled, margin white, central area of labellum yellowish, labellum 9–9.5 mm long, 4.7–4.9 mm wide below callosities, 3.6–4 mm wide at the area of recurvature when flattened, 4.4–4.6 mm wide at widest point below recurvature; 2 basal callosities/nectar glands, conical, upright, 0.6–1 mm tall, with long, dense papillae at the base. Column protandrous, slightly rhombic, green, with a fringe of minute glands or papillae in a thin crescent just below the stigmatic surface, with a pair of upright flaps or wings at each side and clasping the column, the wings green basally; column foot glabrous; rostellum well-developed, white to ivory, tapering to thin acute membranes at the apex; stigmatic surface glabrous, shiny; anther brown, triangular-ovoid; pollinium attached to a well-developed viscidium; viscidium linear, immersed in the rostellum, leaving behind a narrow V-shaped rostellar remnant after removal. Ovary moderately to densely pubescent with septate trichomes. Fruit a light brown ovoid capsule. Figures 3 and 16.

Etymology—From the Greek, '*kapnosperia*' is a combination of 'καπνός' (smoke) and 'σπείρα' (spiral), referring to the greater Smoky Mountain region which is the endemic home of this rare hybrid. The choice of Greek (vs. Latin) is an allusion to the Greek-derived specific epithet of *S. ochroleuca*. A suggested common name is "Smoky ladies' tresses".

Spiranthes cernua s. l. and *S. ochroleuca* have long been hypothesized to hybridize or engage in some level of geneflow, particularly in New York and New England (Sheviak 1982; Sheviak and Brown 2002). A binomial for this crossing, however, was never formally proposed. Based on the research we present here, *S. cernua* s. s. and *S. ochroleuca* do not share an overlapping distribution in much of New York and New England, and thus hybridization is unlikely. Hybrid plants, now described as *S.* ×*kapnosperia* (Figs. 3 and 16), do occur along creeks, wet roadsides, and wet grassy openings in a small area of their shared range in the Southern Appalachian Highlands and greater Smoky Mountains of North and South Carolina (Fig. 14). The question of why this hybrid is so geographically limited compared to its parental species' shared distribution is similar to the situation in *S. niklasii*, and is deserving of continued research. The designation of *S.* ×*kapnosperia* as a nothospecies, indicated by the use of "×", as opposed to a species of hybrid origin (such as *S. incurva* or *S. niklasii*) is twofold: 1) *S.* ×*kapnosperia* does not possess any unique molecular or morphological features based on the data we have collected vs. *S. cernua* s. s. or *S. ochroleuca*; 2) *S.* ×*kapnosperia* is nearly always found with one or both parental species (primarily *S. cernua* s. s.), indicating that it may still be continually formed by ongoing hybridization and introgression, and has not yet coalesced into an independent, self-perpetuating lineage (i.e. species).

Spiranthes niklasii M.C. Pace. sp. nov. [probable ancient *Spiranthes cernua* × *Spiranthes ovalis*]—TYPE: U. S. A. Arkansas: Yell Co., Ouachita National Forest, near Forest Road 86, along Fourmile Creek (mostly dry), in cobbled soil, within a *Liquidambar-Carpinus-Ostrya-Acer* forest, south of the western end of Linn Barker Mountain and the eastern end of Fourmile Mountain, collected 5 October 2016, *Pace 1036* (Holotype: NY; isotypes: ANHC, BH, US).

Spiranthes niklasii is most similar to *S. cernua* s. s. from which it can be distinguished by a central ridge of small papillae on the adaxial surface of the labellum, more strongly campanulate flowers, and usual preference for a more xeric habitat. It can be distinguished from *S. ovalis* by its centrally papillate labellum, flattened lateral sepals (vs. cupped), and upright callosities (vs. strongly incurled). It can be distinguished from both species by its typically fugacious leaves at anthesis.

Terrestrial, acaulescent, deciduous herb, to ca. 41 cm tall. Roots fasciculate, fleshy. Leaves 1–2, basal, held upright, fugacious at anthesis (rarely remaining until anthesis and withering shortly thereafter), linear-lanceolate to lanceolate, acuminate, leaf base tapered and decurrent. Peduncle with adpressed, clasping, lanceolate, acute bracts; spike a single row of flowers in a moderately to tightly coiled spiral (appearing as 1–4 "ranks"), moderately to densely pubescent with blunt-tipped septate trichomes to 0.5 mm long. Floral bracts moderately to densely pubescent, lanceolate, acuminate, concave around the ovary, 10–12.5 mm long. Flowers campanulate, held perpendicular to the inflorescence to moderately nodding, white to pale ivory. Sepals free, moderately to densely pubescent with blunt-tipped capitate septate trichomes. Dorsal sepal slightly convex, slightly to strongly recurved near the tip, lanceolate, bluntly acuminate, 8.3–8.6 mm long, 1.8–2.8 mm wide when flattened. Lateral sepals lanceolate, acute, straight to just barely upwardly falcate, angled slightly upward, the tips often meeting the dorsal sepal and petals, 7–9.4 mm long, 1.3–1.8 mm wide. Dorsal petals slightly concave, lanceolate, bluntly acute, slightly to strongly recurved at tips, with the dorsal sepal appearing stellate, 7.7–9.2 mm long, 8.3–8.6 mm wide when flattened. Labellum minutely clawed, free but clasping the column, keeled/concave for its length, recurved strongly downward at about 1/3 to 1/2 the distance from the claw to labellum apex, with a central ridge or patch of small papillae along the midvein, margin entire to very slightly undulating from the base until the area of recurvature, below point of recurvature margin becoming shallowly laciniate and crisped, margin white, central area of labellum white to very pale yellow, 7.3–9.2 mm long, 3.3–4.8 mm wide below the callosities, 2.2–2.6 mm wide at the area of recurvature when flattened, 1.4–3.7 mm wide below the recurvature, lanceolate to oblong, apex acuminate to rounded; 2 basal callosities/nectar glands, white to yellow, prominent, upright, 0.6–1.1 mm tall, with long dense papillae at the base. Column protandrous, slightly rhombic, green, 2.5–3.6 mm long, with a fringe of minute glands or papillate below the stigmatic surface, with a pair of prominent, upright flaps or wings at each side and clasping the column, the wings green basally, becoming white to translucent, column foot glabrous; rostellum well-developed, white to ivory, becoming dark drown with age, tapering to thin acute membranes at the apex, 1.2–1.5 mm long; stigmatic surface glabrous, shiny; anther pale-brown, triangular-ovoid; pollinium attached to a well-developed viscidium, yellow; viscidium linear, slightly sticky, immersed in the rostellum, leaving behind a narrow V-shaped rostellar remnant after removal, 1–1.3 mm long. Ovary moderately to densely pubescent with septate trichomes. Fruit a light brown ovoid capsule. Figures 3 and 17.

Etymology—The specific epithet "*niklasii*" honors Karl J. Niklas, Ph.D. (b. 1948), for his many contributions to botany, paleobotany, and evolutionary biology. Throughout his 43 yr of elegant scholarship, leadership within the botanical community (e.g. President, *Botanical Society of America*, 2008–2009), and dedicated teaching as a professor of Plant Biology at Cornell University, Niklas has mentored and inspired a generation of botanists, including M. Pace. A suggested common name for *S. niklasii* is "Niklas' ladies' tresses".

The discovery of *S. niklasii* (Figs. 3, 17), likely ancient *S. cernua* s. s. × *S. ovalis*, is perhaps one of the more unexpected results of our research. Although previous phylogenetic research found that *S. ovalis* was a member of the *S. cernua* species complex s. l. (Dueck et al. 2014; Pace and Cameron 2016), these two species had never previously been hypothesized to hybridize. Herbarium specimens of *S. niklasii* were originally identified as tentative *S. cernua* s. l., however, close observation found papillae along the central vein of the labellum, a character not present in *S. cernua* s. s. (or *S. ovalis*). This unusual character prompted M. Pace to conduct fieldwork in Arkansas, with an emphasis on the Ouachita Mountains. When samples of these plants were included in our molecular analyses, they displayed strong discordance between nuclear and chloroplast datasets, with the chloroplast datasets hypothesizing a close relationship to *S. ovalis* (Figs. 5, 7).

The labellum shape of *S. niklasii* is somewhat variable, however, overall flower shape is distinctly and strongly campanulate, and the plant is often leafless at flowering; these characters are not typically found in either parental species. Similarly, although *S. niklasii* is often found along streams, these streams are typically dry at anthesis, and many populations grow in xeric graminoid prairie-like clearings and edges within dry *Pinus-Quercus-Acer-Liquidambar-Carpinus-Ostrya* forests (Fig. 15), a habitat somewhat intermediate between the open wet graminoid-cyperoid habitats of *S. cernua* s. s. and the dolomitic oak-savannah to closed-canopy forested habitats of *S. ovalis*.

Spiranthes niklasii is primarily restricted to the Ouachita Mountains of Arkansas and eastern Oklahoma, with small disjunct populations in the south-central Boston Mountains and on Crowley's Ridge in northeastern Arkansas (Fig. 14). Although *S. cernua* s. s. and *S. ovalis* are both found over much of southern North America and potentially share pollinators (Catling 1980), is it unclear why the hybrid species *S. niklasii* displays a restricted and geographically specific distribution. The Ouachita Mountains contain 20 known endemic plant species and are the second most species-rich area within the wider region following southeastern Texas, with ca. 1,500 known plant species (Kartesz 2015); this mountain system is also a known region of species diversity and endemism for North American Plethodontid salamanders (Shepard et al. 2011; Steffen et al. 2014). The Ouachita Mountains are unusual in that they are one of just a few east-west oriented mountain ranges in North America north of Mexico, and they have been hypothesized to have served as glacial refuges during Ice Ages. Additional research is needed to understand how the unusual geologic history of the region might have affected the evolution of its flora and fauna, including the nearly endemic orchid *S. niklasii*.

Discussion

For the first time, the evolutionary relationships and morphological variation of the entire *S. cernua* species complex are put into a comprehensive molecular phylogenetic, biogeographic, and taxonomic context. Our phylogenetic hypotheses indicate that these orchids are recently evolved, very closely related, that speciation via hybridization has occurred, and that incomplete lineage sorting may be a complication to more fully understanding their evolutionary relationships. This conclusion is supported by the presence of short branch lengths and an ambiguous and sometimes discordant gene-tree topology within the *S. cernua* species complex s. l. (Figs. 5, 6, 7). Separate analyses indicate the *S. cernua* species complex s. l. evolved ca. 2–4.5 mya, and the *S. cernua* species complex s. s. shared a common ancestor ca. 1.5–2.5 mya (unpublished data). Pollinators are widely shared across species within the complex (Catling 1982, 1983), and we have documented three instances of hybrid speciation, indicating that speciation has likely occurred due to evolutionary forces other than complete reproductive isolation. It is possible that some of these species may have evolved in response to the glacial cycles of the Quaternary as populations of ancestral species fragmented and/or migrated, and that hybridization likely allowed for newly evolved species to adapt to newly available environments and microhabitats.

Owing to the pioneering work of Catling (1982) and Sheviak (1976, 1982, 1991), *S. cernua* s. l. had traditionally been hypothesized to engage in frequent unidirectional and geographically-specific hybridization as the "gene recipient" with all other members of the traditional *S. cernua* species complex. According to this hypothesis, all individuals of *S. cernua* s. l. that were found within the general range of any other complex member were presumed to be hybrid in origin. Sheviak (1982, 1991) called potential *S. magnicamporum* × *S. cernua* hybrid populations and individuals (here designated as *S. incurva*) triploid and tetraploid forms and "low-prairie races" of *S. cernua* that arose through "adaptive gene flow" whereby ecologically advantageous genes, characteristics, and morphologies were incorporated into *S. cernua* s. l. through repeated backcrossing. Thus *S. cernua* s. l., which in eastern North America inhabits moist habitats, could survive in more xeric and pyrogenic Midwestern prairies. The overarching premise of this hypothesis, that hybridization might confer some evolutionary benefit in a novel environment on the resulting plants vs. non-hybrid *S. cernua* through incorporation of novel genetic information and neofunctionalization, is plausible and cannot be rejected by the molecular phylogenetic data we present here.

However, *S. incurva* grows in a wide variety of habitats, ranging from periodically inundated to xeric (Fig. 15). Furthermore, *S. incurva* does not occur over the entire shared range of *S. cernua* s. l. and *S. magnicamporum* as originally hypothesized by Catling (1982) and Sheviak (1976, 1982, 1991; Sheviak and Brown 2002), and *S. incurva* also occurs outside of the range of *S. magnicamporum* in regions such as Wisconsin north of the Tension Zone, the Keweenaw Peninsula of Michigan, and northern Vermont (Fig. 14). Because all of our samples from the upper Midwest, Great Lakes Basin, Interior Lowlands, western and northern Appalachians, and Saint Lawrence River Valley originally identified as *S. cernua* were recovered as hybrids, it appears likely that all *S. cernua* s. l. (minus those that are *S. arcisepala*) from this region actually represent *S. incurva*. Catling and Brown (1983), working on co-occurring populations of *S. cernua* s. l. and *S. magnicamporum* in the Eastern Peninsula of Southwestern Ontario, identified only three potential individuals of *S. magnicamporum* × *S. cernua*. Although we were unable to incorporate samples of putative *S. cernua* s. l. from that specific area into our study, included accessions of *S. cernua* s. l. from nearby Ohio and Ottawa, Ontario, were recovered as *S. incurva*, and it is likely that all of the plants in Catling and Brown (1983) identified as *S. cernua* were in fact *S. incurva*. Samples we included and identified a priori as *S. magnicamporum* from two stations in southern Ontario were recovered as non-hybrids. Individuals we included of *S. cernua* s. s. from Arkansas and Texas (including *S. parksii*), were also not recovered as hybrids, notwithstanding the occurrence of *S. magnicamporum* throughout those regions. Furthermore, as indicated by the taxonomically re-interpreted breeding work of Sheviak (1982), *S. incurva* is most likely reproductively isolated from *S. magnicamporum* as crosses between these species did not produce seeds, and so introgression back into *S. magnicamporum* appears to be impossible.

We did not recover evidence supporting hybridization between *S. arcisepala* and *S. magnicamporum*, even when these plants grow within pollination range in northern Ohio or western Virginia, nor did we find evidence of hybridization between *S. arcisepala* and *S. incurva* where these taxa are broadly sympatric in Ohio, Pennsylvania, and the Adirondack Mountains (Fig. 14). Future population genetic based approaches may reveal evidence supporting low-levels of introgression that we were unable to detect given the molecular markers we utilized for the present work. We propose, however, that *S. incurva* and *S. arcisepala* are distinct species worthy of recognition.

Taxonomy—Interpretation of published names, particularly older names, in *Spiranthes* is frequently challenging in the absence of accompanying images, well-preserved specimens, and genetic and geographic context. Multiple lines of evidence, however, support the conclusions we reach here. *Spiranthes cernua* can be placed as the correct name for our *S. cernua* s. s. clade based on the morphology of its lectotype. The geographic origin of this specimen cannot be definitively determined ("Virginia, Canada"), as Kalm traveled throughout the ranges of *S. arcisepala*, *S. cernua* s. s., and *S. incurva*, from the greater Philadelphia region, north to Montreal, and west to Niagara Falls. Furthermore, the brief formal description provided by Linnaeus could apply to either *S. cernua* s. s. or *S. incurva* (though it hews more towards *S. cernua*). The predominantly strongly nodding flowers of the lectotype, however, are particularly common features of plants studied and collected for phylogenetic analysis by M. Pace in northern Delaware and the Mid-Atlantic region (e.g. sample sc1), and the labellum is essentially identical to the broadly oblong, dilated labellum of plants from the Coastal Plain (i.e. *S. cernua* s. s.) versus the broadly to narrowly lanceolate labellum of *S. incurva*. The upward to horizontal position of the lateral sepals do not suggest any affinity to *S. arcisepala*. Thus, we are confident in assigning the Kalm lectotype specimen from LINN to our phylogenetic clade as *S. cernua* s. s. As discussed in Sheviak (1982), the type of *Limodorum autumnale* likely conforms to plants recovered within our phylogenetic clade *S. cernua* s. s., and should be placed in synonymy with *S. cernua*. The collection of that specimen from "Carolina" also supports this conclusion (but see Ward (2007), which discusses ambiguities with attributing the "Walter Herbarium" to Thomas Walter, and Ward and Beckner (2011), which does not select a neotype at BM from the Walter/Frasier herbarium for *L. autumnale*).

In contrast to *S. cernua* s. s., the detailed original description (Jennings 1906) and ample type material of *S. incurva* (as *Ibidium* Salisb. ex Small) creates a rich and detailed concept of this species, matching the morphology of individuals we recovered as being hybrid in nature from the prairies and western Appalachians. Combined with its geographic origin from the narrow strip of Central Lowlands along the Lake Erie coast in northwest Pennsylvania, a region where our phylogenetic data indicate *S. cernua* s. s. does not occur, this name confidently and unambiguously represents plants that we found using molecular data to be hybrid in origin between *S. cernua* s. s. and *S. magnicamporum*. As the oldest confidently placed name for hybrid *S. cernua* s. s. × *S. magnicamporum* plants, *Ibidium incurvum* is transferred to *Spiranthes*, serving as the basionym for *S. incurva*.

Ambiguous Names—*Neottia cernua* var. *major* Eaton was described as "stem tall, somewhat leafy: flowers very large" with no geographic origin or type designation (Eaton 1829). Given the brief description and lack of a type, it is possible *N. cernua* var. *major* represents *S. magnicamporum*, *S. incurva*, *S. odorata*, or large flowered *S. cernua* s. s., and this name cannot be placed. Sheviak (1982) tentatively placed *N. cernua* var. *major* in synonymy with *S. cernua* s. l., and suggested that one of his 'New England forms' frequently found in dry to moist sand may represent this variety. Curiously, the illustration paired with this suggestion approaches *S. arcisepala*, however, we would not describe *S. arcisepala* as particularly tall or leafy, and the flowers are typically smaller in all dimensions than *S. cernua*, *S. incurva*, *S. magnicamporum*, and *S. odorata*. We confidently state that *N. cernua* var. *major* should not be applied to *S. arcisepala*, although it cannot be placed in synonymy with any particular name.

The names *S. brevicaulis* Raf., *S. flexuosa* Raf. nom. illeg., and *S. petiolaris* Raf. all lack type specimens (likely destroyed), and their descriptions (Appendix 3) are too brief and ambiguous to assign any one name to a particular morphology or phylogenetic clade with any confidence. Sheviak (1982) reached a similar conclusion, and termed *S. brevicaulis* and *S. flexuosa* "nomina obscura." Based on their formal description and geographic origin *S. brevicaulis* and *S. petiolaris* could represent *S. cernua*, *S. incurva*, or *S. magnicamporum*; all three taxa match aspects of Rafinesque's descriptions, and all three grow in the general vicinity of the Ohio River Valley in Illinois (*S. petiolaris*) and Kentucky (*S. brevicaulis*). The description of *S. flexuosa*, collected in the Appalachian Mountains (no state given), is so vague that it could represent *S. arcisepala*, *S. cernua* s. s., *S. incurva*, *S. ochroleuca*, or *S. ovalis*. As is the case with *N. cernua* var. *major*, although these are validly published names, the destruction of their type specimens and lack of descriptive detail render them unable to be placed, and thus relegated to the side lines of taxonomy in favor or more recently published and confidently placed names.

Spiranthes casei, S. parksii, and the Role of Apomixis in Questions of Species Status and Taxonomic Rank—The *S. cernua* species complex has a long history of study focused on the occurrence of apomixis, most commonly examining the phenomenon in *S. cernua* s. l. (Leavitt 1900, 1901; Schnarf 1929; Swamy 1948; Catling 1982; Sheviak 1982; Schmidt and Antifinger 1992). This literature establishes facultative polyembryony as a frequent occurrence across the geographic ranges of *S. cernua* s. s. (including *S. parksii*), *S. incurva*, *S. ochroleuca*, and *S. casei*. Populations of some taxa, such as *S. cernua* s. s., *S. incurva*, and *S. casei*, are frequently composed entirely of apomicitic individuals (e.g. the former *S. parksii*), and the frequency of apomixis can vary from year to year, possibly in relation to environmental factors. Apomixis is also frequently tied to instances of cleistogamy in *S. incurva* (Fig. 13) and/or degrees of peloria (typically a reduction of the labellum as exhibited in *S. casei*, some populations of *S. incurva*, and the former *S. parksii*).

Apomixis and the accompanying concept of microspecies are important and regionally significant evolutionary forces, particularly when combined with polyploidy and complex patterns of hybridization and reticulate evolution (e.g. Burgess et al. 2014; Dyer et al. 2012). However, we find the use of apomixis to support the species status of micro-endemic, mostly asexually reproducing, and morphologically minutely different individuals and micropopulations (e.g. *S. parksii*; see also *Sorbus* (Ludwig et al. 2013)), as less than ideal, especially when hybridization and reticulation occur between these "species", such that it could be argued these microspecies actually represent an interbreeding meta-population of a single species. Accordingly, we think apomictic populations must also have additional features distinguishing them from other taxa.

Supporting the earlier work of Dueck et al. (2014), our expanded chloroplast and nuclear reconstructions recovered *S. casei* as broadly embedded within a largely unresolved polytomy with *S. ochroleuca* (Fig. 5). Additional analyses combining the chloroplast and nuclear datasets, and eliminating samples with any missing data, did not yield well-supported resolution between these two a priori species (Fig. 6). Based on this phylogenetic topology and the morphological, ecological, and reproductive context we discuss below, we think the evolutionary history of *S. casei* may best be expressed as an ecologically specific subspecies of *S. ochroleuca*. We do not, however, propose any formal changes at this time. *Spiranthes casei* is clearly very closely related to *S. ochroleuca*, and can be distinguished from *S. ochroleuca* by its smaller flowers, non-recurved dorsal sepal and petal apices (Fig. 3), reduced and slightly sub-peloric cordate labellum, predominantly apomictic reproductive mode, and restriction to lichen and bracken barrens of the greater northern Great Lakes Basin and the Canadian Maritimes (Fig. 14). *Spiranthes casei* and *S. ochroleuca*, however, are both variable species, and approach or overlap in several characters, including flower and abaxial labellum color, habitat, and inflorescence spiral tightness. In the case of the former *S. parksii*, all of the available molecular data fail to find any well-supported reciprocally monophyletic cladogenesis or genetic distinction between this purported species and *S. cernua* s. s. from surrounding areas in Texas and the wider North American Coastal Plain (Figs. 5, 6). Furthermore, the plants are highly restricted in distribution, all apparent floral distinctions appear to be tied to mutant peloria (which is particularly common in the short-grass prairies of the Midwest), and there is no ecological or phenological distinction between it and *S. cernua* s. s. As such, we think the evidence strongly supports the position of '*S. parksii*' as an apomictic regional floral morph and synonym of *S. cernua* s. s. In contrast, predominantly apomictic *S. casei* is distributed over a wide area and is mostly morphologically and ecologically distinct from *S. ochroleuca*. It may be possible that *S. casei* has evolved several times as an ecotype specializing on lichen barrens and disturbed open thickets or may represent an extreme within the wider morphological variability of *S. ochroleuca*. Future studies that incorporate next generation DNA sequencing data and methods that address questions at the population genetic level should be conducted in order to continue to evaluate the hypotheses presented here regarding the species status of *S. casei*.

Conclusions—Sheviak (1982, 1991) correctly hypothesized that hybridization has played an important role in the evolution of the *S. cernua* species complex, but the observed patterns of hybridization and evolutionary relationships are often different than he anticipated in both scope and the species involved. Hybridization has occurred between *S. cernua* s. s. and *S. magnicamporum* (*S. incurva*), however, hybridization is unlikely to be ongoing and is confined to a specific biogeographic region. Hybridization has also occurred between *S. cernua* s. s. and *S. ochroleuca* (to form *S. ×kapnosperia*), but only in an extremely limited area, and we present evidence for an unexpected ancient hybrid between *S. cernua* s. s. and *S. ovalis* (*S. niklasii*). Conversely, we did not recover evidence to support hybridization between *S. cernua* s. s. and *S. odorata* or *S. romanzoffiana* Cham., and some of the morphological variation expressed by *S. cernua* s. l. actually represented cryptic speciation in the absence of detectible hybridization events (*S. arcisepala*).

Although the species within the *S. cernua* species complex may still be challenging to identify due to overlapping morphological characters, they represent an intriguing natural group through which to explore evolutionary questions and hypotheses such as how speciation occurs in the presence of ongoing hybridization and geneflow, how to delineate within-species and between-species phenotypical variability, the role of geography in speciation, and the phenomenon of cryptic speciation. Although we have presented solutions to several long-standing systematic problems within the complex through our taxonomic refinement of *S. cernua*, recognition of *S. incurva*, and the description of *S. arcisepala*, *S. niklasii*, and *S. ×kapnosperia*, more population-based research is warranted so that fine-scale levels of hybridization and population connectivity can be illuminated. It is our hope that the information we present here will facilitate conservation of the *S. cernua* species complex while also informing research focused on other orchid genera in which hybridization is hypothesized to be a prominent evolutionary force, such as *Epidendrum* (e.g. Pinheiro et al. 2010), *Platanthera* Rich. (e.g. Wallace 2003), and *Tolumnia* Raf. (e.g. Ackerman and Galarza-Pérez 1991).

Artificial Key to North American *Spiranthes* Occurring East of the Continental Divide

1. Inflorescence with pointed, non-capitate trichomes .... *S. vernalis* Engelm. & A. Gray.
1. Inflorescence with capitate or blunt-tipped trichomes, or glabrous .... 2.
  2. Labellum strongly pandurate; lateral sepals and dorsal petals connivent for most of their length, forming a 'hood' .... *S. romanzoffiana* Cham.
  2. Labellum not pandurate (may be constricted); lateral sepals and dorsal petals not connivent .... 3.
    3. Viscidium ovoid; column white .... *S. lucida* (H.H. Eaton) Ames.
    3. Viscidium linear; column green .... 4.
      4. Labellum centrally green or white with green veining .... 5.
        5. Labellum not papillate .... *S. praecox* (Walter) S. Watson (including *S. sylvatica* P.M. Br.).
        5. Labellum papillate .... 6.
          6. Flowers fragrant .... *S. triloba* (Small) Schum.
          6. Flowers not fragrant .... 7.
            7. Lateral sepals held perpendicular to the stem, not oblique .... *S. lacera* (Raf.) Raf. (including *S. eatonii* Ames ex P.M. Br.).
            7. Lateral sepals slightly downwardly falcate, oblique .... *S. torta* (Thunb.) Garay & H.R. Sweet.
      4. Labellum centrally yellow or entirely white .... 8.
        8. Flowers 4.5 mm long and smaller .... *S. tuberosa* Raf.
        8. Flowers 5 mm long and longer .... 9.
          9. Labellum centrally papillate .... 9.
            10. Leaves basal, ovate .... 11.
              11. Inflorescence densely pubescent .... *S. brevilabris* Lindl.
              11. Inflorescence glabrous .... *S. floridana* (Wherry) Cory.
            10. Leaves upright, linear-lanceolate, or fugacious .... 12.
              12. Callosities highly reduced, rounded mounds .... *S. magnicamporum* Sheviak.
              12. Callosities not reduced, conical and upright .... 13.
                13. Labellum centrally yellow (or green); Florida .... *S. triloba* (Small) Schum.
                13. Labellum centrally white or very pale yellow; Arkansas and Oklahoma .... *S. niklasii* M.C. Pace.
          9. Labellum not centrally papillate .... 14.
            14. Lateral sepals cupped .... 15.
              15. Labellum centrally white .... *S. ovalis* Lindl.
              15. Labellum centrally yellowish .... 16.
                16. Dorsal sepal and petals barely recurved; Great Lakes and Maritimes .... *S. casei* Catling & Cruise.
                16. Dorsal sepal and petals recurved; Coastal Plain .... *S. laciniata* (Small) Ames.

            14. Lateral sepals flattened .... 17.
              17. Labellum margin undulating .... 18.
                18. Flowers fragrant .... 19.
                  19. Callosities reduced and mounded, 0.2–0.6 mm long; leaves usually absent at flowering .... *S. magnicamporum* Sheviak.
                  19. Callosities pronounced, incurved or conical, 1–2.5 mm long; leaves present at flowering .... 20.
                    20. Leaves to 1.5 cm wide; Rocky Mountains and western Great Plains .... *S. diluvialis* Sheviak.
                    20. Leaves to 3.5 cm wide; Coastal Plain and Cumberland Plateau .... *S. odorata* (Nutt.) Lindl.

                18. Flowers lacking fragrance .... 21.
                  21. Lateral sepals widely oblique; flowering Oct–Dec .... *S. longilabris* Lindl.
                  21. Lateral sepals not oblique, held near the flower; flowering Aug–mid-Sep .... *S. igniorchis.* M.C. Pace.

              17. Labellum margin crisped and lacerate .... 22.
                22. Lateral sepals downwardly falcate, apices pointing toward the labellum apex .... *S. arcisepala* M.C. Pace.
                22. Lateral sepals sweeping upward, apices pointing toward dorsal sepal and petals .... 23.
                  23. Labellum abaxially yellow, abaxial glands rounded .... 24.
                    24. Lateral sepal apices linear-lanceolate .... *S. ochroleuca* (Rydb.) Rydb.
                    24. Lateral sepal apices bluntly acuminate .... *S.* ×*kapnosperia*. M.C. Pace.

23. Labellum abaxially white or very pale yellow, abaxial glands conical and reduced . . . . 25.
    25. Labellum essentially white; lateral sepals lanceolate; flowers frequently nodding; essentially to the south and east of the Eastern Continental Divide and Ohio River; rarely cleistogamous . . . . *S. cernua* (L.) Rich.
    25. Labellum centrally yellowish (sometimes faintly); lateral sepals linear-lanceolate; flowers frequently ascending; essentially to the north and west of the Eastern Continental Divide and Ohio River; occasionally peloric or cleistogamous . . . . *S. incurva*. (Jenn.) M.C. Pace.

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Appendix 1. Representative Specimens Examined— Spiranthes arcisepala—CANADA. Quebec: Brome-Missisquoi, Dunborough, 29 Aug 1911, *Edmondson 5285* (NY). U. S. A. Massachusetts: Berkshire Co., Mt. Washington, 30 Aug 1889, *Whitfield s.n.* (NY). New Jersey: Morris Co., Great Swamp National Wildlife Refuge, SE of New Vernon, 4 Sep 2015, *Pace 901* (NY). New York: Cattaraugus Co., gravel road running N from 242 ca. 3.5 mi NE of Ellicottville, just W of Ashford Junction, 17 Sep 1980, *Sheviak s.n.* (NYS). Delaware Co., Town of Hancock, Lordeville Road (paralleling Humphries Brook), Lordville, uphill from the Delaware River, 5 Sep 2014, *Pace 640* (NY). Hamilton Co., Route 8, 7.7 km N of Wells, 4 Sep 2014, *Pace 639* (NY). Durant Lake, 3.1 km E of Blue Mountain Lake, 4 Sep 2014, *Pace 637b* (NY). North Carolina: Yancey Co., Valley of Southtoe River near Mt. Mitchell, 6 Oct 1924, *Beals s.n.* (NYS). Ohio: Lorain Co., Camden Lake, 1 Sep 1895, *Dick s.n.* (WIS). Seneca Co., Springville Marsh, S of Alvada, 31 Aug 2014, *Pace 629* (NY). Summit Co., Singer Lake, Littleton Bog, 2 Sep 1998, *Bissell s.n.* (NYS). Pennsylvania: Centre Co., At foot of mountain 5 miles NW of State College, 17 Sep 1941, *Wahl 1136* (AMES). Fayette Co., pond S of Markleysurg Pond, SW of athletic fields, Henry Clay Township, 18 Sep 2016, *Pace 1015* (NY). Forest Co., Allegheny National Forest, Forest Road 157, 2.5 mi from Marienville, 19 Sep 1999. *Grisez 1645* (CM). Monroe Co., Franklin Hill, 2 mi E of East Stroudsburg. 25 Sep 1943, *Knipp s.n.* (CM). Vermont: Bennington Co., On Rt 9, 0.9 mi E of Searsburg-Woodford line, 10 Sep 1976, *Sheviak 1047* (NYS). Franklin Co., Between Richford and Canadian border, 8 Sep 1911, *Edmondson 5248* (NY). Virginia: Bedford Co., Peaks of Otter, between Mons and Big Spring, 6 Sep 1947, *Freer 1907* (AMES). Bland Co., Kimberling Creek on S side of St. Rt 608 1.25 mi NW of Highway 42 and 4.2 mi NE of Mechanicsburg, 3 Oct 1983, *Wieboldt 4922* (CM). West Virginia: Greenbrier Co., Near White Sulphur Springs, 7 Sep 1903, *Mackenzie 501* (MO). Preston Co., ca. 0.25 mi N of Preston Co 4H camp entrance drive on Co. 3, 24 Sep 1994, *Shriver 319* (CM). ca. 0.5 mi S of SR 7, 1 mi E of Hopemont, 15 Sep 1996. *Shriver 732* (CM).

Spiranthes cernua—CANADA. Nova Scotia: Halifax, Pearl Lake, Kemptville, 2 Sep 1921, *Fernald 23725* (AMES). U. S. A. Alabama: Colbert Co., summit of Sand Mountain off AL 117, 5.2 mi N of Flat Rock, 7 Oct 1969, *Kral 37563* (WILLI). Madison Co., US 431 ca 5 mi S of Huntsville, 27 Sep 1972, *Kral 48625* (MO). Pike Co., Troy. W of Crowes Pond on Elm Street, 8 Nov 2002, *Diamond 13728* (TROY). Arkansas: Bradley Co., Johnsville Prairie, 5.9 mi SW of Johnsville on unnamed county road, 9 Nov 1985, *Leslie 1557* (UARK). Polk Co., About 1.5 mi SSE of Hatfield, near Six Mile Creek, 2 Oct 1955, *McWilliam 619* (AMES). Scott Co., Winding Bend Rd, 14.5 km SE of Parks, Ouachita National Forest, 12 Oct 2014, *Pace 658* (NY). Union Co., N of AR 335 and W of US 167 SW of Calion, 19 Oct 1990, *Thomas 121691* (FLAS). Delaware: Kent Co., N edge of Tubmill Pond, E of RT 1, 1 Oct 2013, *Pace 605* (NY). New Castle Co., Ramsey Road, N of First State National Monument, NW of Wilmington, 29 Sep 2013, *Pace 597* (NY). Saw Mill Road, 1.5 km E of state line, 30 Sep 2013, *Pace 599* (NY). Sussex Co., Shingle Point Road, SE of intersection with RT 30, 1 Oct 2013, *Pace 606* (NY). Georgia: Irwin Co., 1.5 mi N of Berrien-Irwin Co Line on US 129 S of Ocilla, 26 Oct 1968, *Faircloth 5677* (MO). Rockdale Co., Big Haynes Creek, 6 mi SW of Loganville, 18 Oct 1936, *Pyron 1128* (AMES). Wayne Co., E ditch of 38/US84/S 1st street, ca. 6.9 km SW of the center of Jesup, 5 Nov 2013, *Pace 616* (NY). Kentucky: Cumberland Co., Near Cumberland, 28 Sep 1940, *McFarland 100* (AMES, MO). McCreary Co., 3 miles N of Whitley City, 13 Oct 1940, *Rogers 100B* (AMES). Warren Co., Near Indian Creek, Sep 1896, *Price s.n.* (MO). Louisiana: Union Parish, Along Tick Creek in Union Wildlife Management Area, 27 Sep 1981, *Lewis 3436* (FLAS). Maine: Knox Co., Martin's Point, Friendship, 7 Sep 2008, *Haller s.n.* (NYS). York Co., Ocean Park, 28 Aug 1931, *Moldenke 6261* (NY). Maryland: Harford Co., Aberdeen Proving Grounds, Monks Island, just north of the head of Cod Creek and east of Cod Creek Road, 9 Oct 1999, *Steury 991009* (NYS). Worcester Co., Bainbridge Park, Town of Ocean Pines, 23 Oct 2013, *Pace 608* (NY). Massachusetts: Barnstable Co., Crystal Lake, East Orleans, 31 Aug 1901, *Edmondson 2207* (NY). Franklin Co., On RT2, just W of exit for 202, 11 Sep 1976, *Sheviak 1061* (NYS). Nantucket Co., Siasconset, Nantucket Island, 26 Aug 1963, *MacKeever N743* (NYS). Mississippi: Franklin Co., Near Clear Springs Lake, in Homochitto National Forest ca 6 mi SW of Meadville, 8 Nov 1980, *Pruski 1953* (MO). Missouri: Bollinger Co., Blue Pond Natural Area, 6 mi NW of Zalma, 3 Oct 1991, *Summers 4734* (MO). Carter Co., Mark Twain National Forest, S side of Highway 60 at junction with FR 3753, 10 Oct 2009, *Summers 10473* (MO). Howell Co., Thompson Iron Mine on CR 8040, ca. ½ mi N of road, 26 Oct 2002, *Summers 9970* (MO). Pulaski Co., 5 mi SE of Dixon, 7 Oct 1991, *Summers 4750* (MO). St. Francois Co., NE slopes of Simms Mountain, 2 mi SW of Elvins, 29 Sep 1985, *Summers 1546* (MO). Stoddard Co., Holly Ridge CA, ca. 3.5 air mi S of Bloomfield, 1 Oct 2009, *Summers 10481* (MO). New Jersey: Burlington Co., White's Bog, near Browns Mills, 22 Oct 1992, *Larocque s.n.* (NYS). Cape May Co., Cape May Point State Park, blue trail between beach dune and ponds, 19 Oct 2013, *Pace 607* (NY). New York: Bronx Co., Section 13, Pelham Bay Park areas, 6 Sep 1946, *Ahles 1091* (NYS). Van Cortland Park, 11 Oct 1891, *Bicknell s.n.* (NYS). Rockland Co., Tappantown, Sep 1861, *Austin s.n.* (NY). Nassau Co., Jones Pond, Wantagh, 5 Sep 1938, *Muenscher 6834* (BH, NYS). Suffolk Co., Brookhaven, Sunrise Highway margin near exit 59, 9 Sep 1992, *Zaremba 9079* (NYS). Southard's Pond, Belmont State Park, 8 Sep 1939, *Muenscher 6832* (BH). Headwaters of Browns Creek, Patchogue, 8 Sep 1938, *Muenscher 6833* (BH). Orient Point, 16 Sep 1912, *Latham s.n.* (NYS). Westchester Co., North Tarrytown, 25 Sep 1896, *Barnhart 1818* (NY). North Carolina: Brunswick Co, Green Swamp Preserve, The Nature Conservancy, northwest of Supply, 2 Nov 2013, *Pace 610* (NY). Fifty Lakes Drive, just past intersection with George II Highway / 87, SE of Boiling Springs Lake, 3 Nov 2013, *Pace 613* (NY). Buncombe Co., Biltmore Estate, 29 Sep 1899, *Packard s.n.* (MO). Jackson Co., Rt 281, just N of Sumter National Forest, 2 Oct 2016, *Pace 1021* (NY). Swain Co., near base of Thomas Ridge opposite Deep Creek Trail, ca. 2 mi S of Newfound Gap, E side US 441, Great Smokey Mountains National Park, 19 Oct 1983, *Patrick 5059* (NYS). Oklahoma: Bryan Co., South Bennington Bog, 6 mi E and 1 mi S of Bennington on US70. 10 Oct 1981. Magrath 12293 (NYS). Pushmataha Co, Harrison (Doisher) Bog, 5 mi W on SH 3 & 7 of Antlers & 0.5 S, 10 Oct 1981, *Magrath 12277* (NY). Pennsylvania: Franklin Co., 0.4 mi N of Mt. Alton, on E side of PA 4003, 3 Sep 1995, *Shriver 436* (CM). Lancaster Co., About the mouth of the Tucquan, in Eozoio, 20 Sep 1901, *Heller s.n.* (AMES). Lebanon Co., 1.6 mi NW of Twin Grove Park, 16 Sep 1959, *Keener 898* (CM). South Carolina: Kershaw Co., Intersection of 549 and 695, SW of West Mill Pond, 8 mi E of Camden, 20 Sep 1994, *Kirven 19* (AMES). Lexington Co., E bank of Shealy Pond, W of Edmund, 5 Nov 2013, *Pace 615* (NY). Oconee Co., Lake at base of slope below Double Springs Methodist Church, Long Creek Road (co. RD 196), Mountain Rest, 15 Sep 1991, *Hill 22623* (AMES). Tennessee: Blount Co., Tremont Ranger Station, 22 Oct 1977, *Whitten 164* (FLAS). Coffee Co., May Prairie, between Hillsboro and Manchester, 27 Sep 1977, *Bowles s.n.* (NYS). Cumberland Co., Along US 70N at Mayland, 6 Oct 1957, *Rock 1030* (AMES). Johnson Co., Shady Valley, 1 Oct 1950, *Barclay s.n.* (AMES). Van Buren Co., Brockdell Road about 1.3 mi W of Bledsoe Co. line, 3 Oct 1996, *McNeilus 96-1025* (MO). Texas: Angelina Co., 2 mi NW of Bouton Lake along road on way to Rt 69, Angelina National Forest, 30 Nov 1962, *Correll 26888* (AMES). Brazos Co., Jones Bridge, 2 Nov 1945, *Parks s.n.* (AMES). Peach Creek along intermittent tributary to Lick Creek, 140 m SW of Lick Creek parking lot, 24 Oct 1988, *Sheviak 2829* (NYS). Along unnamed tributary to Peach Creek, just W of Panter Branch near large tank and pipeline right of way, ca 3 km NW of Millican, 25 Oct 1986, *Sheviak 2837* (NYS). Kaufman Co., 3.25 air mi SE of Combine, 18 Oct 1946, *Cory 52563* (AMES). Virginia: Fluvanna Co., ¼ mi S of Rt 696, 1 mi S of Rt 250, 10 Oct 1975, Diggs 1119 (WILLI). James City Co., Little Creek Reservoir Park, SW of Toano, 25 Oct 2013, *Pace 609* (NY). Prince Edward Co., 2 mi S of Burks Tavern, 15 Oct 1972, *Harvill 27328* (WILLI). West Virginia: Fayette Co., West side of CR 11 (Old Clifftop Road), ca. 0.3 mi (and across from cemetery) S of the junction of CR 11 and US Route 60, 22 Oct 1995, *Shriver 505* (WVA). McDowell Co., Ballard Harmon Branch of the Tug Fork River, ca. 1.5 mi SW of Jenkinjones, 21 Oct 1995, *Shriver 495* (WVA). Upshur Co., Middle Fork River, w edge of Ellamore, 1 Oct 1955, *Rossbach 796* (CM).

Spiranthes incurva—CANADA. Ontario: Algoma District, Sault Ste. Marie, NW of outskirts of town, 10 Sep 2005, *Oldham 32214* (DAO, NYS). Haldimand Co., NE shore of Long Point, Lake Erie, 22 Aug 1938, *Senn 433* (NY). Lambton Co., Squirrel Island, W of Walpole Island, St. Clair Delta, 28 Sep 1975, *Catling s.n.* (NYS). Muskoka District, 7.2 mi E of Kings Highway 11 junction N side of Kings Highway 118, 24 Aug 1994, *Shriver 300* (CM). Northumberland Co., Presqu'ile Provincial Park, 24 Aug 1978, *Catling s.n*. (FLAS, NYS). Ottawa, Wright's Grove, Prescott Highway, Nepean twp. 21, Sep 1939, *Minshall 1998* (NY). 3 mi SW of old Stittsville, 31 Aug 1950, *Calder 4888* (CM, NY, USF). Quebec: L'Érable, 4.5 km au NW de Lyster, 11 Sep 1991, *Gauthier 91-129* (NY). Memphrémagog, Cherry River near Mt. Orford, 23 Aug 1978, *Catling 91* (WIS). U. S. A. Illinois: Bond Co., Along railroad, Smithboro, 22 Sep 1974, *Shildneck C-7823/17* (NYS). Lake Co., Illinois Beach State Park, 13 Sep 2014, *Pace 644* (NY). Indiana: Harrison Co, Rt 51 N of Ramsey, 8 Sep 1981, *Sheviak 2151* (NYS). Lake Co., Marquette Park, behind dunes S of Lake Michigan, 14 Sep 2014, *Pace 647* (NY). Iowa: Bremer Co., Tripoli, Sweet Marsh Wildlife Management Area, W of the East Fork Wapsipnicon River, 5 Sep 1999, Freeman 14018 (CLEM). Kansas: Crawford Co., 7 mi E and 0.5 mi N of Hepler, 11 Oct 1968, *Magrath 3557* (AMES, NY). Franklin Co., 1.3 mi W & 0.3 mi N of Le Loup, 2 Oct 1975, *Sheviak 962* (NYS). Washington Co., 2.5 mi E of Washington on US36, 21 Sep 1969, *Magrath 4911* (NY, NYS). Michigan: Allegan Co., Lake Michigan N of Ralstons, 5 Sep 1965, *Sanderson s.n.* (FLAS). Chippewa Co., Sugar Island, 1.5 mi WSW of Homestead, 8 Sep 1935, *Hermann 7235* (AMES, NY). Iosco Co., Oscodia, 22 Aug 1906, *Rusby s.n.* (NY). Minnesota: Cook Co., Grand Portage Island, shore of Lake Superior, 12 Aug 1929, Rydberg s.n. (NY). Mower Co., About 2.5 mi NW of LeRoy, 2 Sep 1981, *Smith 5637* (NY). Missouri: Cass Co., Hutton Mound, 10 Oct 1948, *Steyermark 68764* (AMES). Harrison Co., Ca. 2 ¼ mi W of Highway 69 on Highway 46 in the Pawnee Community, 22 Sep 1994, *Summers 7170* (MO). Linn Co., ca. 8 mi N of Brookfield on the W side of Highway M, 11 Oct 2001, *McHale 01-188* (MO). Nebraska: Brown Co., Calamus River, 25 mi S of Ainsworth, 20 Sep 1975, *Sheviak 936* (NYS). Thomas Co., Near Plummer Ford, Dismal River, 23 Aug 1893, *Rydberg 1719* (NY). New Hampshire: Belknap Co., SW shore of Crystal Lake, 28 Sep 1976, *Sheviak 1090* (NYS). Grafton Co., Vicinity of Hanover, 3 Sep 1891, *Jesup s.n.* (NY). New York: Essex Co., HW 18 bordering the Saranac River at S end of Franklin Falls Reservoir ca. 4 mi E of Bloomingdale, 6 Sep 1986, *Pruski 3170* (NY). Genesee Co., 0.5 mi W of Lake Road, Pembroke, 17 Sep 1980, *Sheviak 1967* (BH). Hamilton Co., Durant Lake, 3.1 km E of Blue Mountain Lake, 4 Sep 2014, *Pace 637* (NY). Tompkins Co., Six Mile Creek, Ithaca, 19 Sep 1915, *Eames 3852* (BH). Warren Co., Pack Demonstration Forest, banks of the Hudson River, WNW of Warrensburg, 4 Sep 2016, *Pace 1001* (NY). Ohio: Clark Co., Gallagher Fen State Nature Preserve, 31 Aug 2014, *Pace 625* (NY). Erie Co., Bay Point, 21 Aug 1914, *MacDanniels 283* (AMES, BH, NY). Guernsey Co., Salt Fork State Park, 29 Sep 1996, *Shriver 740* (CM). Mahoning Co., Between W edge of North East River Road and E shore of Lake Milton, 20 Sep 1997, *Shriver 971* (CM). Portage Co., Mogadore Reservoir, W of 43, 31 Aug 2014, *Pace 629* (NY). Pennsylvania: Erie Co., Near E end of Presque Isle, Lake Erie, 30 Aug 1975, *Sheviak 988* (NYS). 2.7 km NNW of Lowville, just W of French Creek, Lowville Fen, 23 Sep 1994, *Wagner 860* (CM). Fayette Co., Between Mil Run and Killarney Park, 24 Sep 1916, *Jennings s.n.* (CM). Potter Co., Lyman Run Reservoir, 17 Sep 2016, *Pace 1003* (NY). Forest Co., Allegheny National Forest, 2.6 km NE of Marienville, 17 Sep 2016, *Pace 1012* (NY). McKean Co., Just E of Allegheny National Forest border, 3.4 km S of Lafayette, 17 Sep 2016, *Pace 1005* (NY). Somerset Co., Buckstown, 1 Oct 1938, *Jennings s.n.* (CM). Vermont: Franklin Co., Lamoille River, Fairfax, 20 Sep 1964, *Seymore 22389* (MO). Orleans Co., US 5, 6.2 km SE of Barton, 2 Sep 2014, *Pace 630* (NY). Windham Co., Jamaica, 10 Sep 1934, *Moldenke 8371* (NY). Windsor Co., Rt 73, 1 mi E of Long Trail, Brandon Gap, 11 Sep 1976, *Sheviak 1053* (NYS). West Virginia: Monogalia Co., 3.5 mi from E end of Cheat Lake bridge, 21 Oct 1961, *Aborn s.n.* (CM). Wisconsin: Adams Co., Highway 82 W of Highway 13 and ¼ mi E of Wisconsin River, 23 Aug 1979, *Catling s. n.* (AMES). Bayfield Co., Pigeon Lake Camp (UW), Pigeon Lake, 24 Aug 1980, *Moran 1286* (NYS). Dane Co., Green Prairie, University of Wisconsin Arboretum, 28 Aug 2014, *Pace 622* (NY). Door Co., Bailey's Harbor-Highway 57 near Heins Creek, 4 Sep 1997, *Mahlberg 29* (WIS). Kenosha Co., Chiwaukee Prairie, just E of Lake Michigan, 11 Sep 2014, *Pace 642* (NY).

Oconto Co., Nauke Road ca. 0.5 mi N of Logan Road, Town of Breed, 15 Sep 1997, *Judziewicz 12576* (WIS).

Spiranthes ×kapnosperia—U. S. A. North Carolina: Jackson Co., Rt 281, just N of Sumter National Forest, 2 Oct 2016, *Pace 1022* (NY). Macon Co., Along Rt 28, near Highland, 27 Sep 2007, *Wen 10003* (US). Transylvania Co., Pisgah National Forest, Silversteen Rd, 2 Oct 2016, *Pace 1024* (NY). Pisgah National Forest, Rt 1326, 3.2 km S of Balsam Grove, 2 Oct 2016, *Pace 1026* (NY). Pisgah National Forest, Rt 215, 4.6 km NW of Balsam Grove, 2 Oct 2016, *Pace 1027* (NY). South Carolina: Oconee Co., Sumter National Forest, Blue Hole Falls, 1 Nov 2003, *Dueck s.n.* (WIS). Poor Mountain Creek just W of Lake Jemika, 27 Sep 1983, *Hodge 445* (CLEM).

Spiranthes niklasii—U. S. A. Arkansas: Garland Co., Ouachita Mountains, Highway 7, Blue Springs, 12 Oct 2014, *Pace 654* (NY). Greene Co., Crowley's Ridge State Park, 9 air miles W of Paragould, 6 Oct 2003, *Nunn 9342* (UARK). Scatter Creek WMA, Crowley's Ridge, 9 air mi NW of Paragould, 8 Oct 2003, *Nunn 9412* (UARK). Johnson Co., 12 mi W of Pelsor, Haw Creek, above Haw Falls, 28 Oct 1991, *Summers 4782* (MO). Montgomery Co., Ouachita National Forest pond on Blowout Mountain Road (Forest Road 274), 4 Oct 2002, *Marsico 4483* (UARK). Pope Co., Ozark National Forest, 5.6 km W of Sand Gap, 6 Oct 2016, *Pace 1045* (NY, UARK). Pulaski Co., Pinnacle Mountain State Park, SE slope of mountain, 11 Oct 2014, *Pace 652* (NY, UARK). Yell Co., Ouachita National Forest, SE of Linn Barker Mountain, W of Skaggs Hollow & Irons Fork, 12 Oct 2014, *Pace 659* (UARK, NY).Oklahoma: Choctaw Co., 0.5 mi N, 0.5 mi E, 3.5 N of Swink (on Pine Creek Road), Oct 1974, *Magrath 8645* (NYS). Le Flore Co., Near AR line on Highway 270, 12 Oct 1958, *Waterfall 15234* (AMES).

Appendix 2. Voucher information is listed as follows: Taxon name, sample number, voucher (herbarium), origin, GenBank accessions (nrITS, *ACO*, *matK*, *ndhJ*, *trnF-L* intron, *trnS-fM*, *ycf1*). An "—" indicates missing data (repeated failed amplification).

Spiranthes arcisepala M.C. Pace, NY1, *Pace 640* (NY), NY, (MF170216, MF460904, MF434693, MF460850, MF434673, MF460938, MF441697); *S. arcisepala*, sc30, *Pace 628* (NY), OH, (MF170215, MF460905, MF434692, MF460851, MF434672, MF460939, MF441698); *S. arcisepala*, 4c, *Bentley s.n.* (WIS), VA, (—, —, KM213782, MF460843, EU384770, EU384709, MF441689); *S. arcisepala*, 4e, *Shriver s.n.* (WIS), PA, (EU384831, —, KM213783, MF460844, EU384772, EU384711, MF441690); *S. arcisepala*, 4f, *Shriver s.n.* (WIS), PA, (KM262277, —, KM213784, —, KM283626, KM283438, MF441691); *S. arcisepala*, 4g, *Shriver s.n.* (WIS), PA, (KM262278, —, KM213785, MF460845, KM283627, KM283439, MF441692); *S. arcisepala*, 4s, *Brown s.n.* (WIS), ME, (—, —, KM213795, MF460846, KM283633, KM283445, MF441693); *S. arcisepala*, 4u, *McCann s.n.* (WIS), OH, (KM262284, MF460901, KM213797, MF460847, KM283635, KM283447, MF441694); *S. arcisepala*, 4y, *Ufford s.n.* (CLEM), NY, (KM262287, MF460902, KM213799, MF460848, KM283638, KM283450, MF441695); *S. arcisepala*, 4z, *Jones s.n.* (CLEM), OH, (KM262288, MF460903, KM213800, MF460849, KM283639, KM283451, MF441696); *Spiranthes casei* Catling & Cruise, 2a, *Case s.n.* (WIS), MI, (KM213852, MF460906, KM213770, MF460852, KM262266, KM283433, MF441699); *S. casei*, 2b, *Case s.n.* (WIS), MI, (KM213853, MF460907, KM213771, MF460853, KM262267, KM283434, MF441700); *S. casei*, 2d, *Knudson s.n.* (WIS), WI, (KM213854, MF460908, KM213772, MF460854, KM262268, KM283435, MF441701); *S. casei*, 2e, *Ufford s.n.* (WIS), NY, (KM213855, MF460909, KM213773, MF460855, KM262269, KM283436, MF441702); *Spiranthes cernua* (L.) Rich., sc1b, *Pace 597* (NY), DE, (MF170213, —, —, MF460858B, MF434670, MF460941, MF441704); *S. cernua*, sc6d, *Pace 607* (NY), NJ, (MF170212, MF460910, MF434691, MF460859, MF434669, MF460942, MF441705); *S. cernua*, sc8d, *Pace 608* (NY), MD, (MF170211, MF460911, MF434690, MF460860, MF434668, MF460943, MF441706); sc9a, *Pace 609* (NY), VA, (KU752296, KU752258, —, KU935561, KU740271, KU935527, KX088325); *S. cernua*, sc15b, *Pace 616* (NY), GA, (KU752297, KU752259, KU752271, KU935562, KU740272, KU935528, KX088326); *S. cernua*, sc29, *Pace 658* (NY), AR, (MF170214, —, —, MF460857, MF434671, MF460940, —); *S. cernua*, 4cc, *Fowler s.n.* (WIS), SC, (KM262291, KU752261, KM213803, KU935558, KM283642, KM283454, KX088322); *S. cernua*, 4dd, *Fowler s.n.* (WIS), NC, (KM262292, KU752260, KM213804, KU935559, KM283643, KM283455, KX088323); *S. cernua* 'Chadd Ford', 4ee, *Dueck s.n.* (WIS), cultivated, (KM262293, KU752262, KM213805, KU935563, KM283644, KM283456, KX088327); *S. cernua* 'Chadd Ford', 4ff, *Patton s.n.* (WIS), cultivated, (KM262294, —, KM213806, —, KM283645, KM283457, MF441703); *S. cernua*, 4L, *Dueck s.n.* (CLEM), TX, (EU384834, —, KM213789, MF460856, EU384776, EU384715, —); *S. cernua*, 4m, *Stewart s.n.* (WIS), FL, (KM262279, KU752257, KM213790, KU935560, KM283628, KM283440, KX088324); *Spiranthes igniorchis* M.C. Pace, 2a, *Orzell & Bridges 26733* (NY), FL, (KX756343, KX793113, KX756352, KX756362, KX756373, KX756389, KX756333); *S. igniorchis*, 2c, *Orzell & Bridges 26733* (NY), FL, (KX756344, KX793114, KX756353, KX756363, KX756374, KX756382, KX756334); *S. igniorchis*, 3a, *Orzell & Bridges 26735* (NY), FL, (KX756345, KX793115, KX756354, KX756364, KX756375, KX756383, KX756335); *S. igniorchis*, 3b, *Orzell & Bridges 26735* (NY), FL, (KX756346, KX793116, KX756355, KX756365, KX756376, KX756384, KX756336); *S. igniorchis*, 4a, *Orzell & Bridges 26734* (NY), FL, (KX756347, KX793117, KX756356, KX756366, KX756377, KX756385, KX756337); *S. igniorchis*, 4b, *Orzell & Bridges 26734* (NY), FL, (KX756348, KX793118, KX756357, KX756367, KX756378, KX756386, KX756338); *S. igniorchis*, 26733A, *Orzell & Bridges 26733* (NY), FL, (KX756349, KX793119, KX756358, KX756368, KX756379, KX756387, KX756339); *S. igniorchis*, 26734B, *Orzell & Bridges 26734* (NY), FL, (KX756350, KX793120, KX756359, KX756369, KX756380, KX756388, KX756340); *Spiranthes incurva* (Jenn.) M.C. Pace, sm1c, *Pace 491* (NY), WI, (MF170207, MF460915, MF434688, MF460866, MF434664, MF460947, MF441712); *S. incurva*, sm21r, *Pace 642* (NY), WI, (MF170206, MF460916, MF434687, MF460867, MF434663, MF460948, MF441713); *S. incurva*, sm23b, *Pace 644* (NY), IL, (MF170205, MF460917, —, MF460868, MF434662, MF460949, MF441714); *S. incurva*, sc31a, *Pace 629* (NY), OH, (MF170210, —, —, MF460863, MF434667, MF460944, MF441709); *S. incurva*, sc32a, *Pace 625* (NY), OH, (MF170209, —, —, MF460864, MF434666, MF460945, MF441710); *S. incurva*, sc33a, *Reddoch s.n.* (WIS), Ontario, (MF170208, MF460914, MF434689, MF460865, MF434665, MF460946, MF441711); *S. incurva*, soch10a, *Pace 630* (NY), VT, (MF170204, MF460918, MF434685, MF460869, MF434661, MF460950, MF441715); *S. incurva*, 4t, *Knudson s.n.* (WIS), WI, (KM262283, MF460912, KM213796, MF460861, KM283634, KM283446, MF441707); *S. incurva*, 4v, *McCann s.n.* (WIS), OH, (KM262285, MF460913, KM213798, MF460862, KM283636, KM283448, MF441708); *Spiranthes ×kapnosperia* M.C. Pace, sc20a, *Wen 10003* (US), NC, (MF170189, —, —, MF460899, MF434645, MF460968, MF441745); *S. ×kapnosperia*, sh2, *Fowler s.n.* (WIS), NC, (MF170188, MF460937, MF434674, MF460900, MF434644, MF460969, MF441746); *S. ×kapnosperia*, 16i, *Dueck s.n.* (CLEM), SC, (EU384830, —, KM213851, MF460898, EU384771, EU384710, MF441744); *Spiranthes longilabris* Lindl., 13a, *Galloway s.n.* (WIS), NC, (EU384844, KU752241, KM213830, KU935570, EU384787, EU384726, KX088334); *S. longilabris*, 13b, *Fowler s.n.* (CLEM), FL, (KM262316, —, KM213831, —, KM283673, KM283485, —); *S. longilabris*, 13c, *Stewart s.n.* (WIS), FL, (EU384845, KU752242, KM213832, KU935571, EU384788, EU384727, KX088335); *Spiranthes magnicamporum* Sheviak, sm7h, *Pace 594* (NY), NM, (KU752300, KU752251, KU752274, KU935577, KU740275, KU935532, KX088340); *S. magnicamporum*, sm11a, *VanAlstine s.n.* (WIS), VA, (KU752301, KU752252, KU752275, KU935578, KU740276, KU935533, KX088341); *S. magnicamporum*, sm12a, *Fowler s.n.* (WIS), GA, (KU752302, KU752253, KU752276, KU935579, KU740277, KU935534, KX088342); *S. magnicamporum*, sm13a, *Reddoch s.n.* (WIS), Ontario, (MF170203, —, —, —, MF434660, MF460951, MF441718); *S. magnicamporum*, sm15a, *Oldham 39307* (NYS), Ontario, (KU752303, KU752254, KU752277, KU935580, KU740278, KU935535, KX088343); *S. magnicamporum*, sm16a, *McCabe s.n.* (NYS), MS, (KU752304,—, KU752278, —, KU740279, KU935536, —); *S. magnicamporum*, sm17a, *Sheviak 7064* (NYS), TN, (KU752305, KU752255, —, KU935581, KU740280, KU935537, KX088344); *S. magnicamporum*, sm19a, *Sheviak 2142* (NYS), IL, (KU752306, —, KU752279, —, KU740281, KU935538, KX088345); *S. magnicamporum*, sm25g, *Pace 645* (NY), IL, (—, —, —, —, —, MF460952, MF441719); *S. magnicamporum*, sm28h, *Pace 648* (NY), IN, (—, —, —, —, —, MF460953, MF441720); *S. magnicamporum*, 15c, *Summers s.n.* (WIS), MO, (EU384849, —, KM213840, MF460870, EU384792, EU384731, MF441716); *S. magnicamporum*, 15e, *Liggio s.n.* (WIS), TX, (EU384851, KU752249, KM213842, KU935575, EU384794, EU384733, KX088338); *S. magnicamporum*, 15f, *Hapeman s.n.* (WIS), WI, (KM262320, MF460919, KM213843, MF460871, KM283678, KM283490, MF441717); *Spiranthes niklasii* M.C. Pace, AR1, *Pace 650* (NY), AR, (MF170202, MF460920, MF434684, MF460872, MF434659, MF460954, MF441721); *S. niklasii* M.C. Pace, AR4, *Pace 651* (NY), AR, (MF170201, MF460921, MF434683, MF460873, MF434658, MF460955, MF441722); *S. niklasii* M.C. Pace, AR5, *Pace 652* (NY), AR, (MF170200, MF460922, MF434682, MF460874, MF434657, MF460956, MF441723); *S. niklasii* M.C. Pace, AR10, *Pace s.n.* (WIS), AR, (MF170199, MF460923, MF434681, MF460875, MF434656, MF460957, MF441724); *S. niklasii* M.C. Pace, AR13, *Pace 653* (NY), AR, (MF170198, MF460924, MF434680, MF460876, MF434655, MF460958, MF441725); *S. niklasii* M.C. Pace, AR17, *Pace 654* (NY), AR, (MF170197, MF460925, MF434679, MF460877, MF434654, MF460959, MF441726); *S. niklasii* M.C. Pace, AR22, *Pace 655* (NY), AR, (MF170196, MF460926, MF434678, MF460878, MF434653, MF460960, MF441727); *S. niklasii* M.C. Pace, AR26, *Pace 656* (NY), AR, (MF170195, MF460927, MF434677, MF460879, MF434652, MF460961, MF441728); *S. niklasii* M.C.

Pace, AR28, *Pace 657* (NY), AR, (MF170194, MF460928, MF434676, MF460880, MF434651, MF460962, MF441729); *S. niklasii* M.C. Pace, sc17a, *Nunn 9342* (UARK), AR, (MF170193, —, —, MF460881, MF434650, MF460963, —); *S. niklasii* M.C. Pace, sc18a, *Nunn 9412* (UARK), AR, (MF170192, —, —, MF460882, MF434649, MF460964, MF441730); *S. niklasii* M.C. Pace, sc19a, *Marsico 4483* (UARK), AR, (MF170191, —, —, MF460883, MF434648, MF460965, —); *Spiranthes ochroleuca* (Rydb.) Rydb., 16a, *Fowler s.n.* (CLEM), SC, (KM262322, —, KM213844, KU935582, KM283680, KM283492, KX088346); *S. ochroleuca*, 16b, Dueck et al. 2014, VA, (KM262323, KU752264, KM213845, KU935583, KM283681, KM283493, KX088347); *S. ochroleuca*, 16c, *Brown s.n.* (CLEM), NH, (KM262324, —, KM213846, MF460888, KM283682, KM283494, —); *S. ochroleuca*, 16e, *Shriver s.n.* (WIS), MD, (KM262325, —, KM213847, MF460889, KM283683, KM283495, MF441735); *S. ochroleuca*, 16f, *Stefanik s.n.* (CLEM), NH, (KM262326, MF460931, KM213848, MF460890, KM283684, KM283496, MF441736); *S. ochroleuca*, 16g, *Case s.n.* (CLEM), MI, (KM262327, MF460932, KM213849, MF460891, KM283685, KM283497, MF441737); *S. ochroleuca*, 16h, *Ufford s.n.* (CLEM), NY, (KM262328, MF460933, KM213850, MF460892, KM283686, KM283498, MF441738); *S. ochroleuca*, 4h, *Shriver s.n.* (CLEM), WV, (—,—, KM213786, MF460884, EU384773, EU384712, MF441731); *S. ochroleuca*, 4n, *Cameron s.n.* (CLEM), NY, (KM262280, MF460929, KM213791, MF460885, KM283629, KM283441, MF441732); *S. ochroleuca*, 4p, *Dueck s.n.* (CLEM), ME, (KM262281, —, KM213792, MF460886, KM283630, KM283442, MF441733); *S. ochroleuca*, 4wx, *Dueck s. n.* (CLEM), OH, (KM262286, MF460930, KM213807, MF460887, KM283637, KM283449, MF441734); *Spiranthes odorata* (Nutt.) Lindl., so1d, *Pace s.n.* (WIS), NC, (KU752307, KU752225, KU752280, KU935588, KU740282, KU935539, KX088352); *S. odorata*, so10i, *Pace 619* (NY), FL, (KU752309, KU752227, KU752281, —, KU740284, KU935541, KX088354); *S. odorata*, so11, *Sheviak 2408* (NYS), KY, (KU752310,—, KU752282, —, KU740285, KU935542, KX088355); *S. odorata*, so12, *Durr s.n.* (NYS), TN, (KU752311, KU752228, KU752283, KU935589, KU740286, KU935543, KX088356); *S. odorata*, so13, *Statler s.n.* (NYS), VA, (KU752312, KU752229, KU752284, —, KU740287, KU935544, KX088357); *S. odorata*, 17d, *Galloway s.n.* (CLEM), NC, (EU384852, MF460934, KM262241, MF460893, EU384795, EU384734, MF441739); *S. odorata*, 17g, *Stewart s.n.* (CLEM), FL, (EU384854, —, KM262244, KU935584, EU384797, EU384736, KX088348); *S. odorata*, 17i, *Liggio s.n.* (WIS), TX, (EU384856, —, KM262246, KU935585, EU384799, EU384738, KX088349); *S. odorata*, 17opq, *Vincent s.n.* (WIS), GA, (KM262333, KU752224, KM262250, KU935586, KM283694, KM283506, KX088350); *Spiranthes ovalis* Lindl., 18b, *Brown s.n.* (CLEM), FL, (KM262343, —, KM262260, —, KM283704, KM283516, KX088358); *Spiranthes ovalis* var. *erostellata* Catling, sov2, *Pace 649* (WIS), WI, (MF170190, MF460935, MF434675, MF460895, MF434647, MF460966, MF441742); *S. ovalis* var. *erostellata*, 19a, *Bentley s.n.* (CLEM), NC, (KM262344, —, KM262261, MF460894, KM283705, KM283517, MF441740); *S. ovalis* var. *erostellata*, 19c, *Fowler s.n.* (CLEM), NC, (KM262346, KU752256, KM262263, KU935590, KM283707, KM283519, KX088359); *S. ovalis* var. *erostellata*, 19e, *Brown s.n.* (CLEM), FL, (KM262348, —, KM262265, —, KM283709, KM283521, MF441741); *Spiranthes parksii* Correll (= *S. cernua* s. s.), TX1, *Pace s.n.* (WIS), TX, (—, —, —, MF460897, MF434646, MF460967, MF441743); *S. parksii*, 20a, *Walters s.n.* (WIS), TX, (EU384861, KU752263, KM262231, KU935591, EU384800, EU384739, KX088361); *S. parksii*, 20b, *Walters s.n.* (WIS), TX, (EU384862, MF460936, KM262232, KU935592, EU384801, EU384740, KX088362); *S. parksii*, 20c, *Walters s.n.* (WIS), TX, (EU384863, —, KM262233, MF460896, EU384802, EU384741, —); *Spiranthes triloba* (Small) Schum. emend. M.C. Pace, FL36, *Pace 561* (NY), FL (KU752313, KU752243, KU752285, KU935598, KU740288, KU935545, KX088372); *S. triloba*, FL130, *Pace s.n.* (WIS), FL (KU752314, KU752244, KU752286, KU935599, KU740289, KU935546, KX088373); *S. triloba*, FL135, *Pace s.n.* (WIS), FL (KU752315, —, KU752287, KU935600, KU740290, KU935547, KX088374); *S. triloba*, FL146, *Pace 618* (NY), FL (KU752316, —, KU752288, —, KU740291, KU935548, KX088375); *S. triloba*, 17h, *Stewart s.n.* (WIS), FL (EU384855, —, KM262245, —, EU384798, EU384737, KX088369); *S. triloba*, 17m, *Brown s.n.* (WIS), FL (—, —, KM262248, KU935596, KM283692, KM283504, KX088370); *S. triloba*, 17n, *Brown s.n.* (WIS), FL (KM262332, KU752247, KM262249, KU935597, KM283693, KM283505, KX088371); *S. triloba*, itch2, *Brown s.n.* (WIS), FL (KM262407, —, KM262493, KU935601, KM283618, KM283592, —).

Appendix 3. Rafinesque descriptions of selected *Spiranthes*.

***Spiranthes brevicaulis* Raf., Herb. Raf.: 45 (1833).** Foliage radicale conica longissima lineari cuneat acuta, caule brevi vix folioso, foliage lanceolate acute spicis flexuosis oblongus laxis pauciflora flora magnis curvis, bract acuminate. Labellum oblong acute. Kentucky. Semi pedalis. 1818.

***Spiranthes flexuosa* Raf. Herb. Raf.: 45 (1833), nom. illeg.** Caule basi folioso flexuoso, foliage ang. lanceolate obtusiuse, spicis densis spiraled flexuosis pubescens, bract acuminate. Flores mediocris curvis. Labellum oblong acute erosum. Appalachian Mountains pedal.

***Spiranthes petiolaris* Raf., Herb. Raf.: 45 (1833).** Foliage radical longe petiolatis cuneato lanceolate acutis, caule basi folioso, fol. lanceolate acuminatis, spicis densis spir-fl. magni curvus, bract acuminate. Labellum oblong acute. Illinois. Pedal. Flowers white as in all *Spiranthes*. 1818.