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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 arctisepala*; 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 incursa* (*S. cernua* × *S. magnicamporum*); and 5) the description of a new localized hybrid, *Spiranthes ×kapnospera* (*S. cernua* × *S. ochroleuca*). We also propose formal synonymization of federally endangered *Spiranthes parksi* 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; Fennessey 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; Gribic 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 conservatism 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 label is bluntly acute (as denoted in the original description) and slightly dilated near the apex, with two prominent basal calli (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. *Muhlenbergia* 180 (LINN) represents what are now recognized as five distinct species). The species *S. casei* Catling & Cruise, *S. magnicamporum* Sheviak, *S. ochroleuca* (Rydbl.) Rydb., *S. odorata* (Nutt.) Lindl., and *S. parksi* Correll (Figs. 3, 4), were proposed to accomodate 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...
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
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 ‘com-
pilospecies’ (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

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. triloba* 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. ochroleuca* + *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, 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 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.

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 cm2 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, ndhF, trnL-F, trns-S-M, and ycf13′, 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 ycf1 and nrITS: following Dueck et al. 2014); ACO, following Guo et al. (2012); ycf1′, 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, Massachusetts) 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 var. liocladra was used as the outgroup.

The data were analyzed as follows: 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, ndhF, trnL-F, trns-S-M, and ycf13′.

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<th>Taxon and sample size</th>
<th>ACO failed/amplified</th>
<th>nrITS failed/amplified</th>
<th>ndhJ failed/amplified</th>
<th>ndfJ failed/amplified</th>
<th>trnL-F failed/amplified</th>
<th>trns-S-M failed/amplified</th>
<th>ycf13' failed/amplified</th>
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<td>0/4</td>
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**Results**

**Phylogenetics**—Individual gene trees generally found *S. cernua* to be polyphyletic (trees not shown). Only the reconstruction of nrITS failed to support the polyphly 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 polyphony 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. ooutis* 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

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**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, incurring 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 ×kapnospera 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. ×kapnospera (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.

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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.
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**


**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 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...
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, claspings, 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 sepaline 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. parksi 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. parksi, 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 meadow, 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.
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

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-
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
recurred 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, recurred 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 mm long, 1.2 mm tall, with long, dense papillae at the base. In these instances, the flowers are still smaller than S. cernua s.s., but 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.*


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.

**Etymology**—Latin, *arcisepala* is a combination of ‘arcus’ (arching) and ‘sepalorum’ (sepalas), 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*. Maps produced by Elizabeth Kiernan, New York Botanical Garden GIS Lab.

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
“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, to 133 mm long, densely pubescent with septate trichomes. Fruit a light brown withering shortly thereafter, linear-lanceolate to lanceolate, to 133 mm long, 2 small leafy bracts occasionally present (frequently absent), quickly reducing to adpressed, clasp, 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 sepal 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 sepal 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 sepal 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 prolansord, 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; pollen attached to a well-developed viscidium, yellow; viscidium linear, immersed in the rostellum, leaving behind a narrow V-shaped rostellum 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 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 Adirondack, 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 buttercups 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-scur 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 xkapnosperia M.C. Pace, nothosp. nov. [Spiranthes cernua × Spiratanes 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 xkapnosperia is most similar to S. cernua s. s. and S. ochroleuca. It can be distinguished from S. cernua s. s. by smaller, less widely gaping 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 addressed, 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 sepalate 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 sepalate 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 9.5 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; visci- dium linear, immersed in the rostellum, leaving behind a narrow V-shaped rostellar remnant after removal. Ovary moderately to densely pubescent with sepalate trichomes. Fruit a light brown ovoid capsule. Figures 3 and 16.

Etymology—From the Greek, ‘kapnosperia’ is a combination of ‘καπνός’ (smoke) and ‘σπειρα’ (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 roadways, 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. incurvula 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 Spiranthus cernua × Spiranthus 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 incurved). 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 addressed, 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 sepalate 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 sepalate 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 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 crissped, 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 proazandrous, 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 brown 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, imersed in the rostellum, leaving behind a narrow V-shaped rostellum remnant after removal, 1–1.3 mm long. Ovary moderately to densely pubescent with sepal 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-Osbyra 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), it is 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 2013); 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. nonhybrid 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. parkii*), 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 sepal differences 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 *Limodorium 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/Fraser 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 cernum* 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 cernum* 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*, or *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. arcesepala, 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. parksi, 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 polylemby as a frequent occurrence across the geographic ranges of *S. cernua* s. s. (including *S. parksi*), *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 apomictic individuals (e.g. the former *S. parksi*), 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. parksi*).

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. parksi*; 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 metapopulation 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-recurred 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. parksi, 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. parksi*’ 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. 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. arcesepala*).

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. arcesepala, 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.
2. Inflorescence with capitate or blunt-tipped trichomes, or glabrous .......................................................... *S. romanzoffiana* Cham.
4. Viscidium linear; column green .............................................................................. *S. magnicamporum* Sheviak.
5. Labellum not papillate .................................................. *S. praecox* (Walter) S. Watson (including *S. sylvatica* P.M. Br.).
7. Lateral sepals held perpendicular to the stem, not oblique .......................................................... *S. lacera* (Raf.) Raf. (including *S. eatonii* Ames ex P.M. Br.).
8. Lateral sepals widely oblique; flowering Oct-Dec ....................................................... *S. longilabris* Lindl.
9. Leaves basal, ovate ......................................................................................... *S. brevilabris* Lindl.
10. Leaves upright, linear-lanceolate, or fugacious ......................................................... *S. floridana* (Wherry) Cory.
11. Inflorescence densely pubescent ............................................................................. *S. ovalis* Lindl.
12. Callosities highly reduced, rounded mounds ........................................................ *S. magnicamporum* Sheviak.
13. Callosities not reduced, conical and upright ......................................................... *S. diluovialis* Sheviak.
16. Dorsal sepal and petals barely recurved; Great Lakes and Maritimes ..................... *S. arcisepala* M.C. Pace.
17. Dorsal sepal and petals recurved; Coastal Plain .................................................... *S. laciniata* (Small) Ames.
18. Flowers fragrant ................................................................................................. *S. odorata* (Nutt.) Lindl.
19. Callosities reduced and mounded, 0.2-0.6 mm long; leaves usually absent at flowering .......................................................................................................................... *S. igniorchis* M.C. Pace.
20. Leaves to 1.5 cm wide; Rocky Mountains and western Great Plains ....................... *S. diluovialis* Sheviak.
21. Leaves to 3.5 cm wide; Coastal Plain and Cumberland Plateau .............................. *S. ochroleuca* (Rydb.) Rydb.
22. Flowers lacking fragrance ..................................................................................... *S. articulata* (Nutt.) Lindl.
23. Flowers fragrant ................................................................................................. *S. longilabris* Lindl.
24. Labellum margin crisped and lacerate ........................................................................ *S. arcisepala* M.C. Pace.
23. Labellum abaxially white or very pale yellow, abaxial glands conical and reduced.

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

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

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Literature Cited


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


Appendix 2. Voucher information is listed as follows: Taxon name, number, voucher (herbarium), origin, GenBank accessions (refITS, Atc, matk, ndh1, trnL-trnF, intron, trn3-M, yflf). An — indicates missing data (repeated failed amplification).

Spiranthes arcisepala M.C. Pace, NY1, Pace 609 (NY), NY, (MF170216, MF460904, MF434695, MF460850, MF434673, MF460938, MF441697); S. arcisepala, s30, Pace 628 (NY, OH), (MF170215, MF460905, MF434692, MF460851, MF460939, MF460940, MF441698); S. arcisepala, se30,se44, Pace 629 (WIS, VA), (—, KM213782, MF460843, EU384770, EU384709, MF441689); S. arcisepala, 4e, Shrivr s.n. (WIS, PA), (EU384381, —, KM213783, MF460844, EU384772, EU384711, MF441690); S. arcisepala, 4f, Shrivr s.n. (PA), (KM262277, —, KM213784, —, KM283628, KM283483, KM461691); S. arcisepala, 4g, Shrivr s.n. (WA, PA), (KM262278, —, KM283658, KM283485, KM461692); S. arcisepala, 4h, Shrivr s.n. (WIS), (—, KM213779, MF460846, KM283633, KM283445, KM461693); S. arcisepala, 4i, McCann s.n. (WIS), (OH, KM262284, KM213790, MF460907, KM283635, KM283447, KM461694); S. arcisepala, 4j, Ufford s.n. (CLEM), NY, (KM228357, KM213791, KM283626, KM283433, KM461695); S. arcisepala 4k, Ufford s.n. (CLEM), NY, (KM228358, KM213792, KM283627, KM283434, KM461696); S. arcisepala 4l, Ufford s.n. (CLEM), NY, (KM228359, KM213793, KM283628, KM283435, KM461697); S. arcisepala 4m, Brown s.n. (WIS), ME, (—, KM213795, MF460846, KM283633, KM283445, KM461698); S. arcisepala 4n, McCann s.n. (WIS), OH, (KM262288, KM213796, MF460901, KM283637, KM283448, KM461699); S. arcisepala 4o, McCann s.n. (WIS), MO, (KM262289, KM213797, MF460902, KM283638, KM283449, KM461700); S. arcisepala 4p, McCann s.n. (WIS), VA, (KM213852, MF460906, KM213770, KM283625, KM283436, KM461691); S. arcisepala 4q, McCann s.n. (WIS), VA, (KM213853, MF460907, KM213771, MF460853, KM283626, KM283437, KM461701); S. arcisepala 4r, McCann s.n. (WIS), VA, (KM213854, MF460908, KM213772, MF460854, KM262268, KM283435, KM461702); S. arcisepala 4s, McCann s.n. (WIS), VA, (KM213855, MF460909, KM213773, MF460855, KM262269, KM283436, KM461703); S. arcisepala 4t, McCann s.n. (WIS), VA, (KM213856, MF460910, KM213774, MF460856, KM262270, KM283437, KM461704); S. arcisepala 4u, McCann s.n. (WIS), VA, (KM213857, MF460911, KM213775, MF460857, KM262271, KM283438, KM461705); S. arcisepala 4v, McCann s.n. (WIS), VA, (KM213858, MF460912, KM213776, MF460858, KM262272, KM283439, KM461706); S. arcisepala 4w, Shrivr s.n. (WIS), VA, (KM213859, MF460913, KM213777, MF460859, KM262273, KM283440, KM461707); S. arcisepala 4x, Shrivr s.n. (WIS), VA, (KM213860, MF460914, KM213778, MF460860, KM262274, KM283441, KM461708); S. arcisepala 4y, Shrivr s.n. (WIS), VA, (KM262275, KM213780, KM283629, KM283442, KM461709); S. arcisepala 4z, Shrivr s.n. (WIS), VA, (KM262276, KM213781, KM283630, KM283443, KM461710); S. arcisepala 4aa, Shrivr s.n. (WIS), VA, (KM262277, KM213782, KM283631, KM283444, KM461711); S. arcisepala 4ab, Shrivr s.n. (WIS), VA, (KM262278, KM213783, KM283632, KM283445, KM461712).
