

Spiranthes bightensis (Orchidaceae), a New and Rare Cryptic Hybrid Species Endemic to the U. S. Mid-Atlantic Coast

MATTHEW C. PACE

New York Botanical Garden, 2900 Southern Blvd., Bronx, New York, 10348, U. S. A.

 mpace@nybg.org;  <https://orcid.org/0000-0002-2333-1924>

Abstract

Recognizing species diversity is challenging in genera that display interspecific similarity and intraspecific variation; hybridization and the evolution of cryptic hybrid species amplifies these challenges. Recent molecular and morphological research focused on the systematics of *Spiranthes* (Orchidaceae) support hybrid speciation as an important driver of species diversity, particularly within the *S. cernua* species complex. Working under an integrated history-bound phylogenetic species concept, new molecular and morphometric data provide evidence for a new and rare cryptic hybrid species resulting from the ancient hybridization of *S. cernua* × *S. odorata*, here described as *S. bightensis*. Although *S. bightensis* is regionally sympatric with *S. cernua* it does not co-occur with that species, and it is allopatric with respect to *S. odorata*. Endemic to a narrow region extending from the Delmarva Peninsula to Long Island, New York, this new species occurs in the shadow of the Northeast megalopolis and appears to have undergone a major population decline over the last 200 years. By recognizing this distinct evolutionary lineage as a new species, this research is the first step towards developing conservation protocols for this rare species and highlights the importance of the North American Geologic Coastal Plain for biodiversity conservation and evolution.

Keywords: evolutionary phylogenetics, North American Geologic Coastal Plain, Northeast megalopolis, *Spiranthes cernua*, *Spiranthes odorata*, species complex

Introduction

Recognition of species diversity is a critically important aspect of biology, particularly as the Earth is increasingly urbanized and species diversity is lost. Fieldwork, specimen collection, and systematic revision are fundamental tools for crafting conservation policy and combating extinction; May's (1990) discussion of “taxonomy as destiny” remains prescient. Yet systematists continue to encounter challenges when attempting to characterize and describe species diversity and evolutionary processes in genera that display interspecific similarity and intraspecific variation, challenges that are amplified when species hybridize, leading to the evolution of cryptic hybrid species. These cryptic hybrid species represent distinct evolutionary lineages worthy of taxonomic recognition, but they are often morphologically intractable and overlooked. Among North American Orchidaceae, *Spiranthes* (Richard 1817: 28–29) contains the largest number of hybrid taxa supported by molecular phylogenetic data (Arft & Ranker 1998, Szalanski *et al.* 2001, Pace & Cameron 2017, 2019), with four accepted species of hybrid origin and three nothospecies; an additional nothospecies occurs in East Asia (Surveswaran *et al.* 2018, Pace *et al.* 2018, Suetsugu *et al.* 2020). More broadly, the integration of molecular phylogenetic data and specimen based morphometric and phenological analyses have recently led to the description or re-recognition of six additional morphologically cryptic non-hybrid *Spiranthes* (Pace & Cameron 2016, 2017, Pace *et al.* 2017, 2018).

Many of the newly described or re-recognized cryptic *Spiranthes* are members of the recalcitrant *S. cernua* (Linnaeus 1753: 946) Richard (1817: 37) species complex (e.g., *S. niklasii* M.C. Pace; Pace & Cameron 2017: 660–661). Composed of 13 taxa, the *S. cernua* species complex displays a primarily autumnal phenology and variously falcate lateral sepals, occurring in moist low-statured graminoid-cyperoid habitats across eastern North America from Nova Scotia to Florida, and the Atlantic Ocean to the headwaters of the North Platte River and eastern Texas. Sheviak (1973, 1982, 1991) and Pace & Cameron (2017) discuss the systematic challenges associated with the complex related

to intraspecific variability, minute morphological differences between species, and patterns of ancient hybridization. *Spiranthes odorata* (Nuttall 1834: 98) Lindley (1840: 467) was previously considered to be a member of the *S. cernua* species complex and was hypothesized to engage in hybridization with *S. cernua*, yet this hypothesis was not supported by previous molecular analyses. Dueck *et al.* (2014) and Pace & Cameron (2016, 2017) recovered *S. odorata* as distantly related to the *S. cernua* species complex, in a sister relationship to the combined sister clades of the *S. cernua* species complex and the clade containing *S. lacera* (Rafinesque 1818: 206) Rafinesque (1833) and its relatives. However, continued molecular and morphometric investigations now allow the hypothesis of inter-clade hybridization between *S. cernua* and *S. odorata* to be more fully examined.

Materials and methods

Taxonomic sampling

The taxonomic literature of the *S. cernua* species complex was reviewed, including all accepted and synonymized names. Herbarium specimens of *a priori* *S. cernua*, *S. odorata*, and indet. *Spiranthes* were reviewed from AMES, BH, BKL, CHRB, CLEM, CM, CONN, F, FSU, MARY, MO, NY, NYS, PH, US, WILLI, and WIS (herbarium acronyms follow Thiers, 2021). Examination of physical herbarium specimens was supplemented by review of digital images provided by the Mid-Atlantic Herbaria Consortium (<https://midatlanticherbaria.org/portal/>), the SouthEast Regional Network of Expertise and Collections (<https://sernecportal.org/portal/>), GBIF (<https://www.gbif.org/>), and verified research-grade observations on the citizen scientist platform iNaturalist (<https://www.inaturalist.org/>). Individual flowers from the lowermost quarter of the inflorescence were rehydrated for morphological examination from select individual specimens. Fieldwork was conducted in Delaware, Maryland, New Jersey, New York, North Carolina, South Carolina, and Virginia from 2012–2019. Samples were collected for herbarium specimens, morphological measurements, and DNA sequencing. Additional silica dried samples from North Carolina were collected for sequencing by citizen scientist Jim Fowler and vouchered by detailed photographs. For areas that I was unable to visit for fieldwork, 1–10-year-old herbarium specimens were judiciously sampled with permission for inclusion in the molecular phylogenetic analyses.

Herbarium specimens and iNaturalist records were georeferenced and approximate area of occupancy distribution estimated in Google Earth Pro 7.3.3.7786 (2020). The species occurrences (points) and estimated ranges (polygons) were imported into ArcMap Desktop 10.6 (Esri 2018) with the GADM United States of America administrative shapefile (GADM 2015). The geographic data were projected to a customized United States East Coast-centric projection (Lambert Azimuthal Equal projection with Central Meridian at -74.300556 and Latitude of Origin at 39.180833) with a World Geodetic System 1984 datum. For the purpose of visualizing distributional changes over time, specimens and observations were grouped into four categories based on their collection or observation date: collected/observed from (pre) 1800–1899, 1900–1949, 1950–1999, and 2000–2020. As more recent collections (1950–2020) were collected/observed from the same general areas as historic locations (1800–1949), the full range of specimens/observations collected/observed from (pre) 1800–2020 were interpreted as representing the full, original distributional extent of *S. bightensis* *sp. nov.*

Morphometrics

Data analysis were performed and the morphospace visualizations were generated in RStudio v 1.0.306 (R Development Core Team 2014) using leaves and rehydrated flowers from herbarium specimens of *S. cernua*, *S. odorata*, and *S. bightensis* *sp. nov.* Comparative measurements included leaf length and width at the widest point, lateral sepal length, labellum length, labellum width at median point below constriction, and floral bract length (Table 1).

Molecular and phylogenetic methods

Phylogenetic analyses incorporated and expanded upon the dataset of Dueck *et al.* (2014), Pace & Cameron (2016), and Pace *et al.* (2017) (see molecular voucher information). 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*, *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 regions ACO and *Xdh*. PCR amplification protocols used were as follows: chloroplast (except *ycf1*) and nrITS: following Dueck *et al.* (2014); ACO: following Guo *et al.* (2012); *Xdh*: following Górniak *et al.* (2010); *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 11.0.3 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 (Appendix 1).

Sanger Sequencing data were analyzed as: 1) individual loci, 2) combined chloroplast data, 3) combined nuclear data, and 4) combined nuclear and chloroplast data. 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 Pace and Cameron (2017), 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 burnin 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). To better contextualize and represent instances of possible hybridization, the combined nuclear and combined chloroplast datasets were visualized as individual networks in the program SplitsTreeWindow (Huson and Bryant 2006).

Species concept

Across my systematic and taxonomic studies of *Spiranthes* (e.g., Pace *et al.* 2017, Pace & Cameron 2017), I implement 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. When hybrid taxa are identified, I elevate those taxa to species status if they meet one or both of the following two criteria: 1) if the hybrid taxon possess unique molecular relationships and/or morphological features based on the data I have collected vs. its progenitor species; 2) if the hybrid taxon is rarely or never found to comingle with one or both progenitor species, indicating probable reproductive isolation. If one or both of these criteria are met, I consider the taxon to have evolved beyond its initial hybridization event(s) and to have coalesced into an independent, self-perpetuating lineage (i.e., species). If the hybrid taxon does not meet one or both of these criteria, then I employ the rank of nothospecies, as indicated by the use of "×". Sheviak (1984: 11–13, 1990: 215–230) and Catling and Sheviak (1993: 78) appear to have employed a similar set of criteria when describing *S. delitescens* Sheviak and *S. diluvialis* Sheviak at the rank of species, and *S. ×simpsonii* Catling & Sheviak at the rank of nothospecies. As both closely and distantly related *Spiranthes* share pollinators and have engaged in hybridization (e.g., Sheviak 1982, 1984, Pace and Cameron 2019), this genus clearly has porous reproductive barriers, and I do not consider total reproductive isolation to be of major importance.

Results

Phylogenetics

The molecular data recovered overall evolutionary relationships that align with previous hypotheses (Fig. 1; Dueck *et al.* 2014, Pace & Cameron 2016, 2017). *Spiranthes odorata* was recovered in all datasets as distantly related to the *S. cernua* species complex. Within the *S. cernua* species complex, *S. cernua* was most closely related to a clade of *S. arcisepala* and *S. ochroleuca*. Although comparisons between nuclear and chloroplast phylogenetic hypotheses did not recover any instances of topographic incongruence along the backbone of the tree, *a priori* *S. cernua* was recovered as non-monophyletic in the *Xdh* and combined nuclear datasets. The ACO, nrITS, and combined chloroplast phylogenetic and network hypotheses recovered *a priori* *S. cernua* samples 4ff, 4ee, sc6d, and sc8d in the *S. cernua* s.s. clade, but the *Xdh* phylogenetic and network hypothesis placed these samples in the *S. odorata* clade, whereas all other *a priori* *S. cernua* samples were recovered in a distantly related clade. Inspection of individual nrITS, ACO, and *Xdh* chromatograms did not reveal any instances of nucleotide ambiguities for discordant samples 4ff, 4ee, sc6d, and

sc8d, including at individual sites of molecular differentiation between *S. cernua* and *S. odorata*; rather samples 4ff, 4ee, sc6d, and sc8d shared the same *Xdh* sequence reads as *S. odorata*. Network analyses of the combined nuclear molecular data (Fig. 1) recover samples 4ff, 4ee, sc6d, sc8d in a discordant topology compared to the other *a priori* *S. cernua* samples; this discordant topographic positioning is similar to the discordant molecular relationships displayed by the previously known hybrid species *S. incurva* (Jenn.) M.C. Pace (Pace & Cameron 2017: 655–659) and *S. niklasii*. However, the network analysis of the combined chloroplast molecular data clearly resolves the discordant samples within the wider *S. cernua* s.l. group (Fig. 1). The discordant topological placement of samples 4ff, 4ee, sc6d, and sc8d between the combined nuclear and combined chloroplast network analyses support a hybrid origin for these samples involving *S. cernua* s.s. and *S. odorata*.

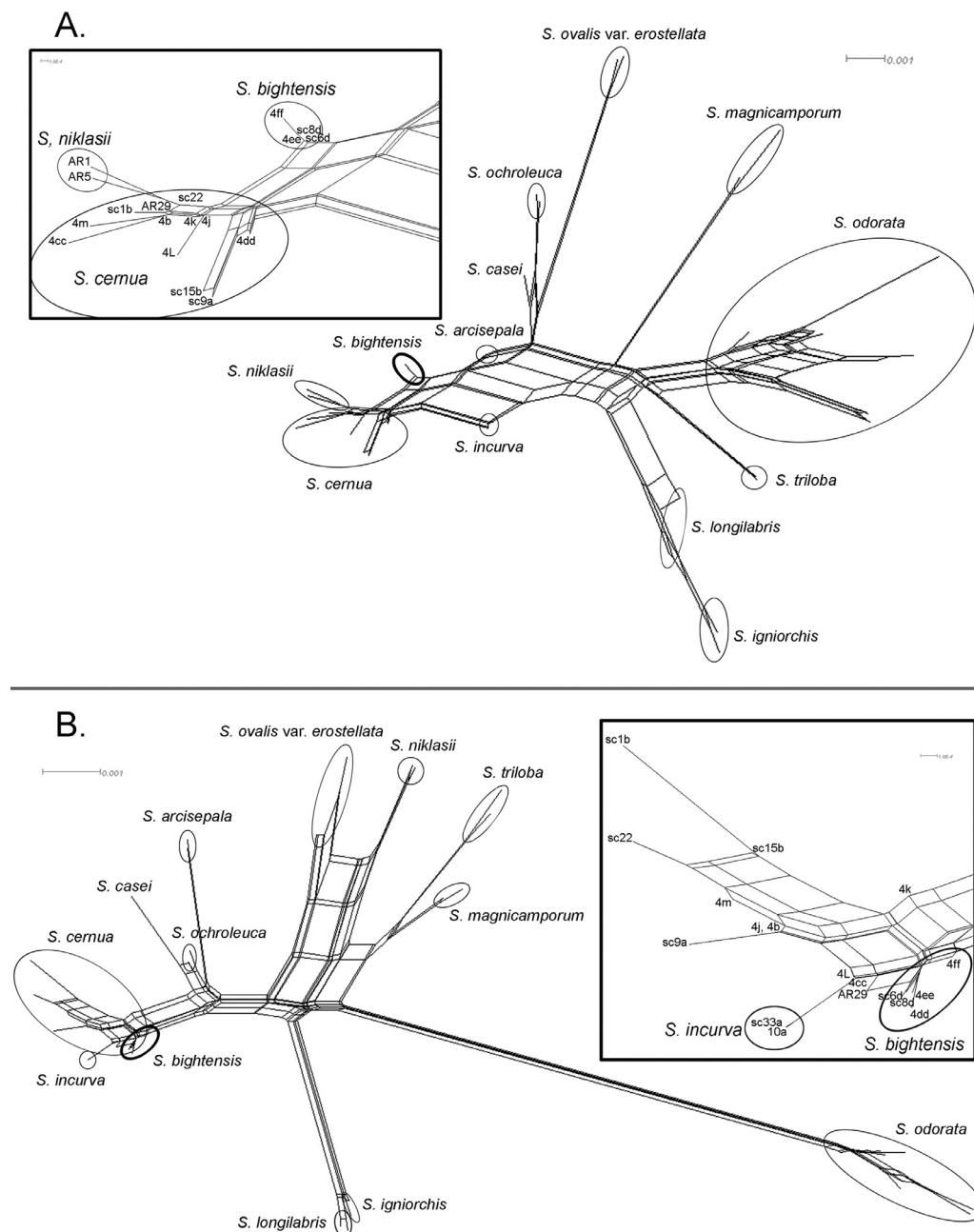


FIGURE 1. Phylogenetic networks from NeighborNet analysis of the *S. cernua* species complex plus *S. odorata*; the position of species is indicated by ovals. A. Combined nuclear dataset (nrITS, ACO, *Xdh*); inset focuses on the relationships between *S. cernua* s.s. and *S. bightensis*, denoting the position of individual samples. B. Combined chloroplast dataset (*matK*, *ndhJ*, *trnL* intron, *trnS-fM*, *ycf1* 3'); inset focuses on the relationships between *S. cernua* s.s. and *S. bightensis*, denoting the position of individual samples (all samples are *S. cernua* s.s. unless otherwise indicated).

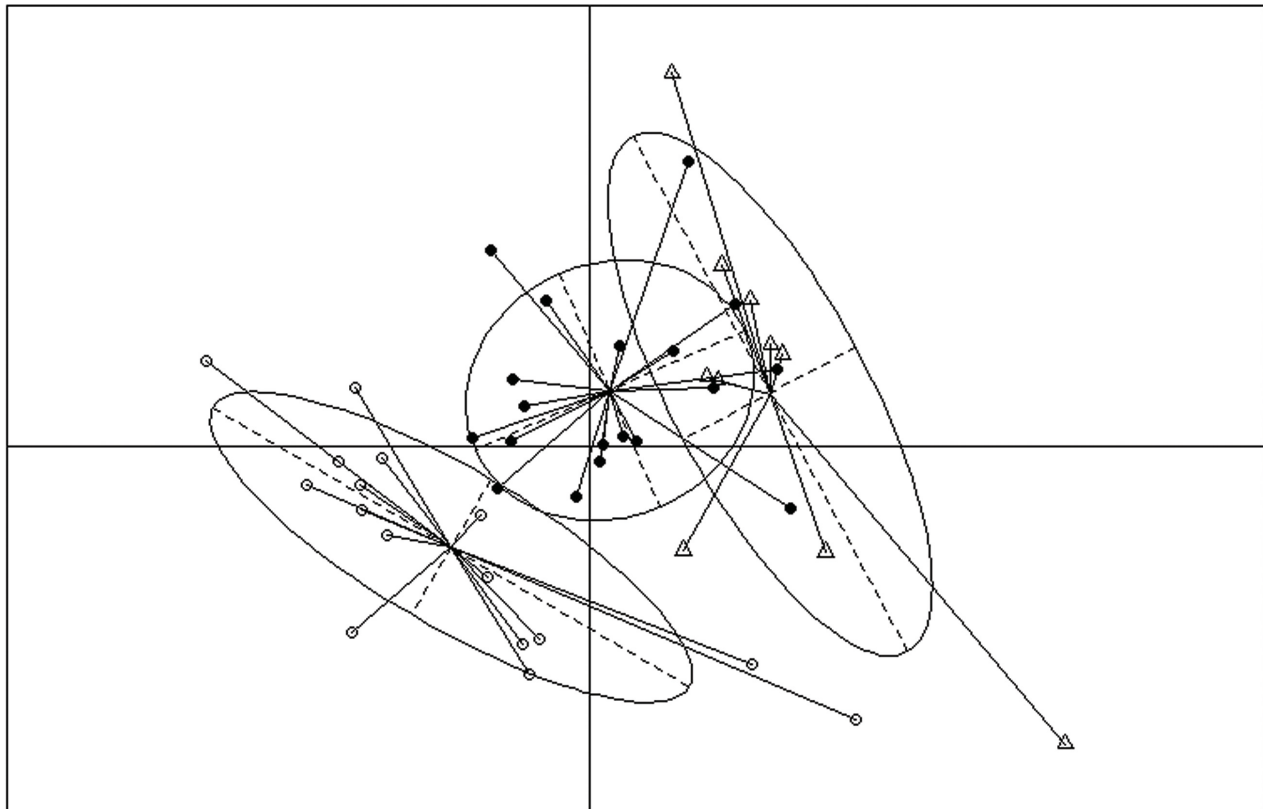


FIGURE 2. Morphospace visualization based on two foliar and four floral characters (Table 1): *S. bightensis* (n = 19, closed circles), *S. cernua* (n = 16, open circles), *S. odorata* (n = 10, triangles).

Morphometrics

The morphometric analysis recovered three groups: *S. cernua* s.s., *S. odorata*, and a third intermediate group (Fig. 2). This morphologically intermediate group includes voucher herbarium specimens of the discordant phylogenetic samples sc6d and sc8d (*Pace* 607, 608), and occupies the morphological space in-between *S. cernua* s.s. and *S. odorata*, supporting a hybrid origin between these two species. This intermediate and phylogenetically hybrid group is described below as *S. bightensis* sp. nov. In general, the features measured here for *S. bightensis* are larger than *S. cernua* but smaller than *S. odorata* (e.g., leaf width; Table 1).

TABLE 1. Morphospace analysis voucher information and measurements.

Species	Voucher (herbarium)	Labellum length (mm)	Lower labellum width (mm)	Sepal length (mm)	Leaf length (mm)	Leaf width (mm)	Bract length (mm)
<i>S. cernua</i>	<i>Kalm</i> s.n. (LINN)	10.0	5.0	8.0	194	4.1	10.2
<i>S. cernua</i>	<i>Pace</i> 605 (NY)	10.8	5.7	9.6	149	10.6	12.3
<i>S. cernua</i>	<i>Pace</i> 606 (NY)	11.2	5.6	12.0	140	11.3	19.5
<i>S. cernua</i>	<i>Pace</i> 616 (NY)	13.1	4.6	14.4	87	10.8	19.1
<i>S. cernua</i>	<i>Muenschner</i> 6834 (NYS)	9.4	4.5	10.0	173	8.7	13.4
<i>S. cernua</i>	<i>Long</i> 15163 (PH)	8.2	3.5	8.7	196	6.8	12.4
<i>S. cernua</i>	<i>Long</i> 2215 (PH)	6.4	2.6	6.5	183	9.9	10.4
<i>S. cernua</i>	<i>Long</i> 29591 (PH)	8.0	3.7	8.6	140	8.8	12.4
<i>S. cernua</i>	<i>Ferguson</i> 1814 (NY)	6.8	—	8.6	126	10.0	13.0
<i>S. cernua</i>	<i>Ferguson</i> s.n. (NY)	7.0	4.5	8.4	201	13.8	11.8
<i>S. cernua</i>	<i>Benedict</i> s.n. (MARY)	9.0	4.4	9.3	209	10.0	—
<i>S. cernua</i>	<i>Bicknell</i> s.n. (NY)	7.4	5.9	7.8	240	10.8	12.4

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TABLE 1 (Continued)

Species	Voucher (herbarium)	Labellum length (mm)	Lower labellum width (mm)	Sepal length (mm)	Leaf length (mm)	Leaf width (mm)	Bract length (mm)
<i>S. cernua</i>	<i>Fry 469</i> (AMES)	9.0	6.7	9.8	222	11.6	13.4
<i>S. cernua</i>	<i>Lippmaa s.n.</i> (TU)	7.47	3.34	9.0	170	9.1	9.2
<i>S. cernua</i>	<i>Hammer 69</i> (BKL)	6.4	3.9	8.2	248	12.0	12.7
<i>S. bightensis</i>	<i>Pace 608</i> (NY)	11.2	4.3	10.9	508	19.6	14.2
<i>S. bightensis</i>	<i>Pace 607</i> (NY)	12.2	4.8	10.2	214	16.9	20.8
<i>S. bightensis</i>	<i>Austin s.n.</i> (NY)	10.2	2.9	11.0	160	15.0	14.9
<i>S. bightensis</i>	<i>Mulford s.n.</i> (NY)	7.7	4.0	8.3	168	12.0	17.7
<i>S. bightensis</i>	<i>Longbottom 20494</i> (USF)	10.7	4.2	8.5	210	17.9	17.3
<i>S. bightensis</i>	<i>Zebryk s.n.</i> (GA)	8.2	4.6	9.7	360	16.0	18.0
<i>S. bightensis</i>	<i>Thompson s.n.</i> (F)	9.9	3.6	10.8	350	15.6	17.3
<i>S. bightensis</i>	<i>Tatnall 2428</i> (PH)	8.9	3.6	9.7	271	12.7	14.8
<i>S. bightensis</i>	<i>Long 53168</i> (PH)	8.1	3.3	9.4	253	10.4	17.8
<i>S. bightensis</i>	<i>Long 32529</i> (PH)	7.9	—	9.6	219	18.8	16.4
<i>S. bightensis</i>	<i>Long 5449</i> (PH)	8.1	3.6	9.2	489	16.5	22.6
<i>S. bightensis</i>	<i>Longbottom 6897</i> (PH)	10.1	3.5	12.7	326	17.5	18.4
<i>S. bightensis</i>	<i>Meredith s.n.</i> (PH)	9.7	—	10.7	209	18.5	20.1
<i>S. bightensis</i>	<i>Fogg 7520</i> (PH)	8.6	5.1	9.8	244	18.5	17.5
<i>S. bightensis</i>	<i>Lighthipe s.n.</i> (BKL)	8.6	3.6	10.7	220	7.9	14.7
<i>S. bightensis</i>	<i>Mulford barcode 68472</i> (BKL)	7.8	4.9	9.3	166	19.2	18.5
<i>S. bightensis</i>	<i>Mulford s.n.</i> (BKL)	8.7	5.4	10.4	320	11.1	15.4
<i>S. bightensis</i>	<i>Hulst s.n.</i> (BKL)	7.9	—	9.5	413	13.9	13.7
<i>S. bightensis</i>	<i>Muenschner 6834</i> (BH)	7.9	4.9	9.2	295	11.9	15.7
<i>S. odorata</i>	<i>Torrey s.n.</i> (NY)	9.8	4.0	9.5	347	27.0	16.7
<i>S. odorata</i>	<i>Pace 611</i> (NY)	10.7	4.0	10.5	309	19.5	17.1
<i>S. odorata</i>	<i>Pace 614</i> (NY)	14.6	5.6	16.3	130	17.8	19.9
<i>S. odorata</i>	<i>Pace 617</i> (NY)	13.5	4.4	11.8	187	20.5	16.2
<i>S. odorata</i>	<i>Wurzlów s.n.</i> (NY)	11.8	4.0	—	500	20.0	—
<i>S. odorata</i>	<i>Chapman s.n.</i> (NY)	9.7	4.6	10.2	284	24.0	16.2
<i>S. odorata</i>	<i>Small 9284</i> (NY)	10.3	4.7	10.0	331	24.0	18.7
<i>S. odorata</i>	<i>Fernald 11308</i> (NY)	11.0	4.4	10.8	323	27.0	—
<i>S. odorata</i>	<i>Wherry s.n.</i> (AMES)	7.1	—	10.1	517	22.4	19.0
<i>S. odorata</i>	<i>McMullen s.n.</i> (NY)	11.3	5.6	10.4	257	17.7	—

Taxonomic treatment

Spiranthes bightensis M.C. Pace, *sp. nov.* [ancient *S. cernua* × *S. odorata*].—Type: U. S. A. Maryland: Worcester County, Bainbridge Park pond, Ocean Pines, off of Beaconhill Rd., ca. 3.5 km west of Isle of Wight Bay, 23 October 2013, *Pace 608* (holotype: NY, isotypes: K, US). Fig. 3.

Diagnosis. *Spiranthes bightensis* is most similar to *S. cernua*, from which it can be distinguished by its stoloniferous roots (vs. non-stoloniferous), typically longer and wider, more lanceolate leaves (vs. linear-lanceolate, 15–21.4 × 1.4–1.7 cm vs. 8.7–20 × 0.4–1.1 cm, Fig. 3, 4, Table 1) commonly fragrant flowers (vs. typically lacking fragrance), and slightly thickened central labellum (vs. centrally membranous). *Spiranthes bightensis* can be distinguished from *S. odorata* by its truncate column to rostellum transition zone, vs. lanceolate, and shorter and narrower leaves (15.0–21.4 × 1.4–1.7 cm vs. 13–51.7 × 1.8–2.7 cm).

To ca. 100 cm tall. Roots slender, stoloniferous. Leaves 1–5, basal, held upright, remaining until after anthesis, lanceolate, 15–21.4 cm long, 1.4–1.7 cm wide. Trichomes capitate and glandular. Spike robust, thickened, a tightly

coiled spiral (appearing as 3–4 ‘ranks’), moderately to densely pubescent. Floral bracts pubescent, 11.8–22.6 mm long. Flowers campanulate, slightly nodding, white to pale ivory, lightly to strongly fragrant with a scent varying from general floral to vanilla-jasmine. Sepals moderately to densely pubescent. Dorsal sepal apically slightly to strongly recurved, concave, lanceolate, 8.3–11.7 mm long when flattened. Lateral sepals lanceolate, acute, very slightly upwardly falcate, slightly ascending, the apices often incurved, surpassing the dorsal sepal and petals, 9.2–11 mm long. Dorsal petals slightly concave, lanceolate, bluntly acute, slightly to strongly recurved at tips, with the dorsal sepal appearing stellate, 9.5–11 mm long when flattened. Labellum recurved strongly downward at about 1/3 the distance from the claw to labellum apex, centrally glabrous, upper margin entire to very slightly undulating becoming shallowly lacinate to lacerate towards the apex, centrally white to pale yellow, 7.7–12.2 mm long, 3.0–5.5 mm wide at the area of recurvature when flattened, apex acuminate; callosities/nectar glands, white to pale yellow, conical, upright, 1–2 mm tall. Column 4.1–6 mm long, apex truncate, column foot stout; rostellum 1.2–1.5 mm long; viscidium linear, 1–1.8 mm long. Ovary moderately to densely pubescent.

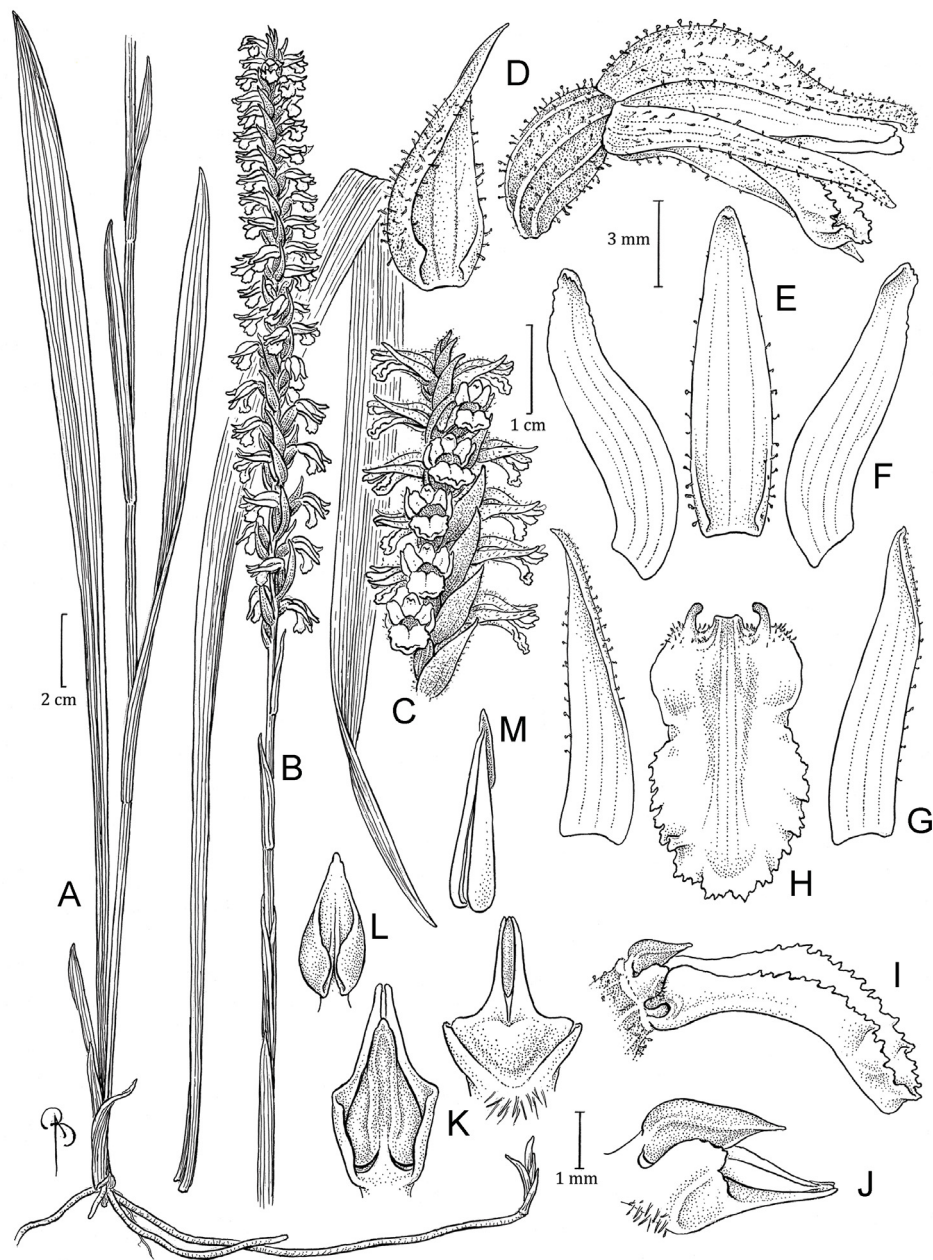


FIGURE 3. Line drawing of *S. bightensis*. A. Habit and leaf detail. B. Inflorescence. C. Inflorescence detail. D. Floral bract and flower. E–M. Dissected flower. E. Dorsal sepal. F. Dorsal petal. G. Lateral sepal. H. Labellum, flattened. I. Labellum and column in natural position. J. Column, profile view. K. Column, ventral and dorsal view. L. Anther. M. Pollinia. Drawn from *Austin s.n.* barcode 01392822 (NY) and *Pace 608* (NY) by Bobbi Angell.

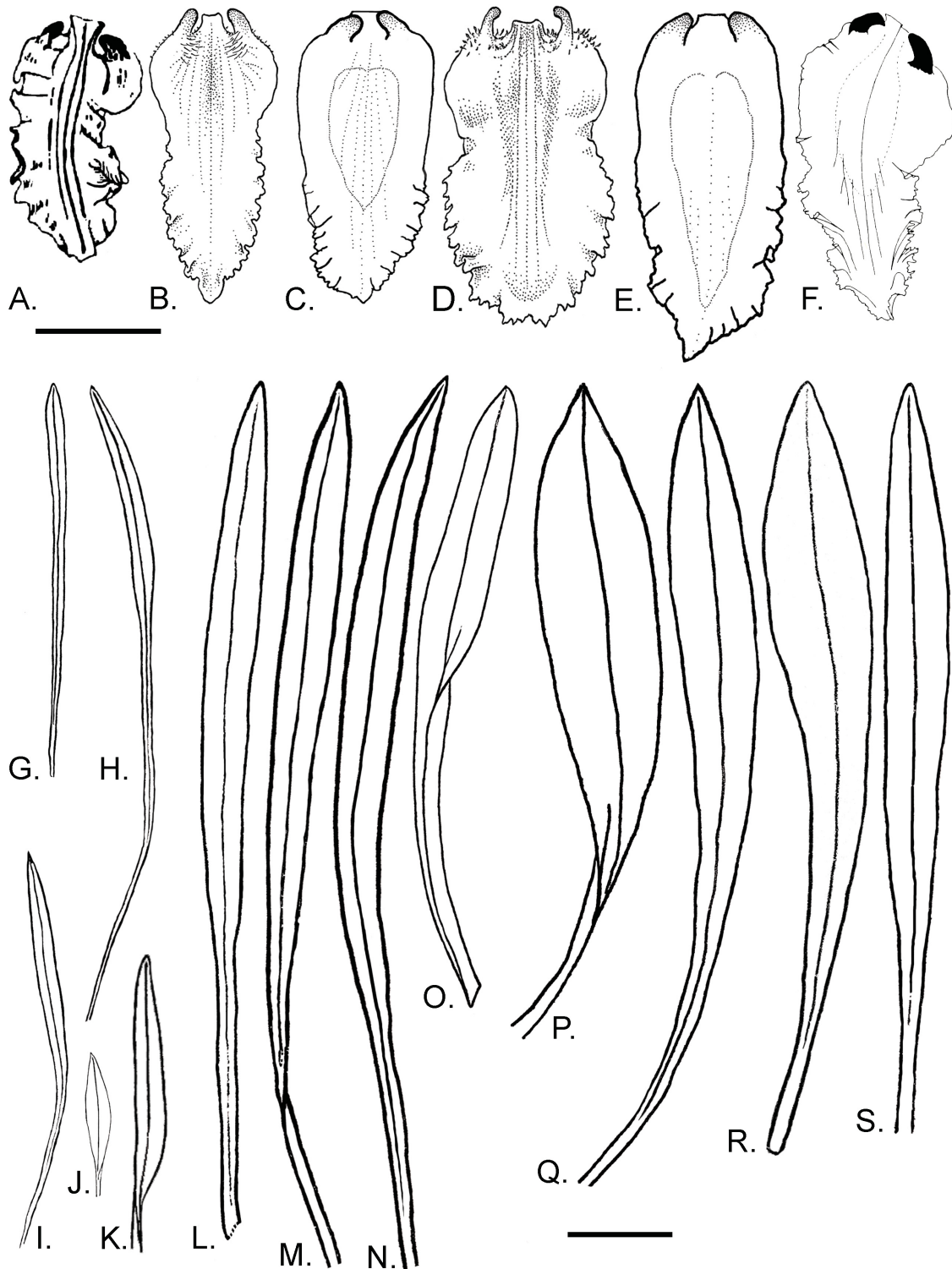


FIGURE 4. Comparative line drawing of *S. cernua* (A, B, G–K), *S. bightensis* (C, D, L–O), and *S. odorata* E, F, P–S) labella (A–F) and leaves (G–S). A. *Pehr s.n.*, lectotype of *Ophrys cernua* (LINN!). B. *Pace 615* (NY!). C. *Pace 607* (NY!). D. *Pace 608*, holotype of *S. bightensis* (NY!). E. *Pace 611* (NY!). F. *Nuttall s.n.*, lectotype of *Neottia odorata* (PH!). G. *Pehr s.n.*, lectotype of *Ophrys cernua* (LINN!). H. *Stone 9462* (PH!). I. *Long 15163* (PH!). J. *Pace 615* (NY!). K. *Pace 605* (NY!). L. *Pace 608* (NY!). M. *Austin s.n.* barcode 01392822 (NY!). N. *Long 5449* (PH!). O. *Pintauro 9*, ‘Chadd’s Ford’ (NY!). P. *Kral 62918* (SAT!). Q. *Small 9284* (NY!). R. *Pace 614* (NY!). S. *Pace 611* (NY!). A. Drawn by A. Gray, published in Sheviak and Catling (1980), used with permission; B. & D. Drawn by Bobbi Angell, used with permission; F. Drawn by P. Catling, attached to *Nuttall s.n.*, used with permission of Philadelphia Herbarium (PH) at The Academy of Natural Sciences of Drexel University; all others drawn by M. Pace.

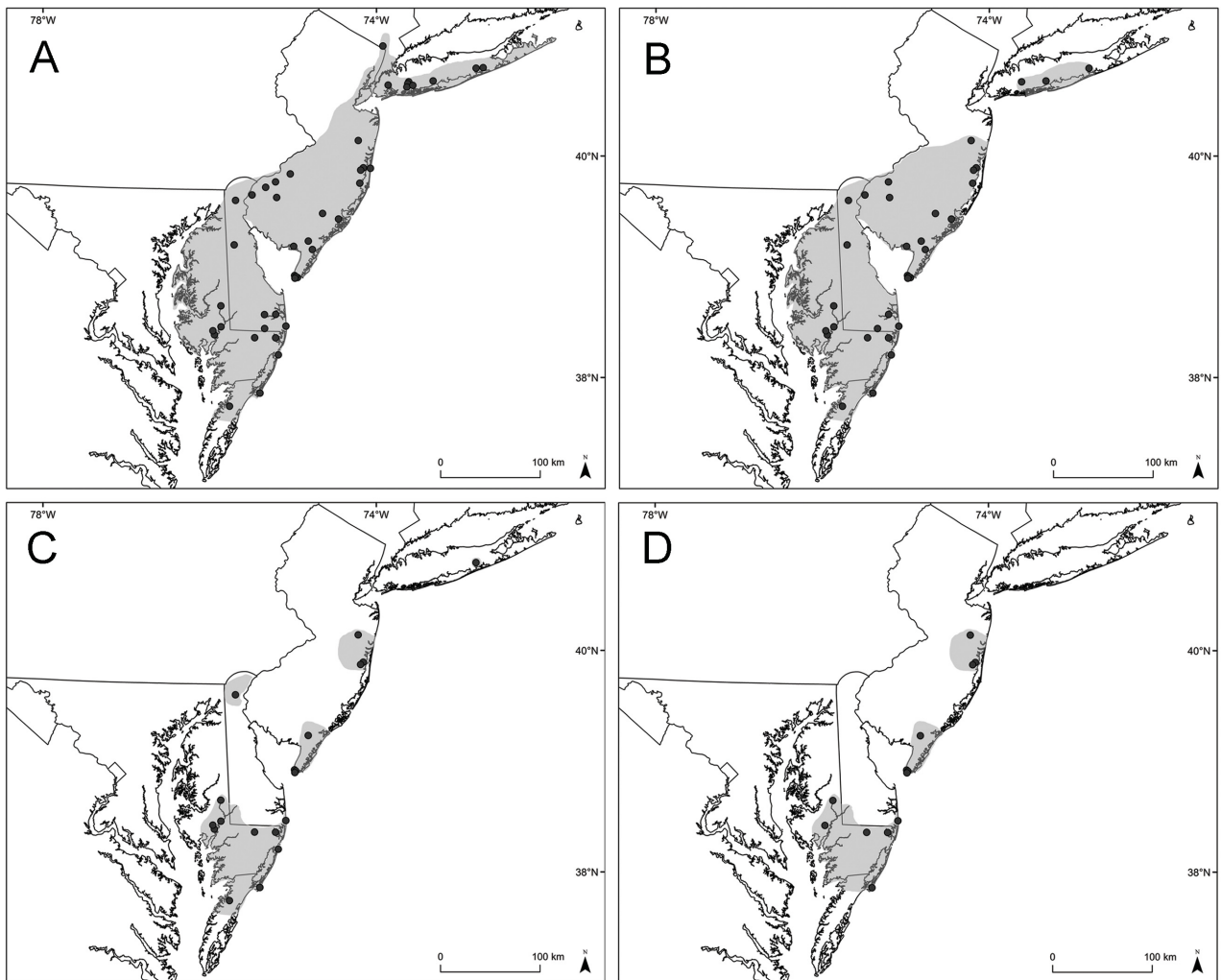


FIGURE 5. Distribution map of *S. bightensis*, highlighting population loss over time. A. Collections made pre-1890's–present (the hypothesized original distribution of *S. bightensis*). B. 1900–present. C. 1950–present. D. 2000–present. Prepared by Elizabeth Gjeli, NYBG GIS Lab.

Etymology:—From the Old English / Anglo-Saxon ‘*byht*’, meaning bend or bay, a bight is a shallowly curved coastline or extremely wide bay; its use here refers to the Mid-Atlantic and New York Bights, which stretches from the Nantucket Shoals off southern New England southward to Cape Lookout, North Carolina. *Spiranthes bightensis* is endemic to the central region of this bight. Atlantic Ladies Tresses is the suggested common name.

Distribution and Habitat:—North American Geologic Coastal Plain endemic, restricted to a narrow region of the Mid-Atlantic Bight and New York Bight from the southern Hudson River estuary and Long Island, New York, to the Delmarva Peninsula of Maryland and Virginia (Fig. 5). The only documented population north of the Fall Line occurred in “bogs” and “boggy places” around Tappantown, New York. The distribution of *S. bightensis* bears many similarities to the “Southeastern Massachusetts to southern New Jersey and adjacent Delmarva Peninsula” endemism pattern described by Sorrie & Weakley (2001), although it is currently unknown from maritime Rhode Island or Massachusetts. *Spiranthes bightensis* is regionally syntopic with *S. cernua*, however it does not co-occur with that species, and it occurs just to northeast of the distributional limit of *S. odorata*. The cultivar ‘Chadds Ford’ is relatively common in cultivation.

Occurring in wet to moist, short-statured, rarely brackish, open graminoid-cyperoid meadows, maritime dune swales, *Sphagnum* Linnaeus (1753: 1106) dominated freshwater lake and pond edges, and roadsides; occasionally/periodically shallowly inundated. Associated species include *Agalinis* Rafinesque (1836: 61–65) spp., *Eutrochium* Rafinesque (1836: 78) spp., *Gentiana* Linnaeus (1753: 227) spp., *Morella pensylvanica* (Mirbel 1804: 190) Kartesz (1999), *Nyssa sylvatica* Marshall (1785: 97–98), *Phragmites australis* (Cavanilles 1799: 100–101) Trinius ex Steudel (1840: 143), *Rhexia* Linnaeus (1753: 346) spp., *Solidago sempervirens* Linnaeus (1753: 87), and *Symphyotrichum*

Nees (1832: 135–136) spp. Although the distribution of *S. bightensis* encompasses the Atlantic Coastal Pine Barrens ecoregion of New Jersey and Long Island, it has not been collected from classic Pine Barrens habitats such as Pine-dominated forests. Rather, *S. bightensis* primarily occurs along the Inner Coastal Plain and Barrier Islands/Coastal Marshes ecoregions, and open wet prairie and meadow-like elements within the Cape Cod/Long Island Pine Barrens ecoregion.

Phenology:—Late September – early November.

Conservation:—Rare and highly localized, although extant populations are often robust and the cultivar ‘Chadds Ford’ is common in cultivation. Apparently never more than ca. 50 km from the Atlantic Ocean coastline, occurring at elevations under ca. 30 m. Although occasionally found in brackish habitats, this species is at major risk of inundation and saltwater intrusion from global warming induced sea-level rise. Its coastal habitat is also under immense pressure from development, urbanization, and invasive species. Poorly timed roadside mowing regimes are an additional threat, as populations are often cut just as they begin to flower, with such mowing regimes appearing to have destroyed at least one recently collected population, *Zaremba 9079* (NYS), which I was unable to relocate 24 years later in 2016 along a very closely cropped highway median. It is important to note that frequent natural disturbances such as fire and hurricanes are critical to maintaining the open habitats favored by *S. bightensis*, and a regional decline in periodic disturbances such as fires may also contribute to the decline of this species in concert with habitat destruction and other potential stresses such as heavy metal deposition in regional soils (Pouyat & McDonnell 1991).

Over the past 200 years, populations of *S. bightensis* appear to have undergone major declines possibly related to the synergistic effects of expanded urbanization and habitat destruction and degradation (Fig 5). Most documented populations from urban centers such as the New York metropolitan area and Philadelphia have not been collected or otherwise observed in at least the past ca. 100–20 years, and a majority of the remaining known populations occur in less densely populated areas of central and southern New Jersey and the central Delmarva peninsula, often in parks or other protected areas. I searched for many of the historic populations that have not been observed in the past 20 years but was unsuccessful in re-locating any. In this regard, the cultivar ‘Chadds Ford’ is illuminating, as it was wild-collected and brought into cultivation from a rural Bear, Delaware, property just before the site was developed into suburban tack housing (Glick 2001). Alarmingly, the known remaining populations of *S. bightensis* are also the most physically close to the ocean, and at an average elevation of 6.5 m above sea-level are more immediately threatened by climate change driven sea-level rise. Furthermore, the Northeastern Megalopolis forms a major physical barrier to inland migration. The largest contraction in distributional area occurred from 1900–1940’s, with the fragmentation of a previously essentially continuous distribution into several smaller regional and discontinuous extant meta-populations. The overall distributional area of these fragmented meta-populations seems to have stabilized from 1950 to the present, however the total number of known populations has continued to decline (Fig. 5). This observed pattern does not appear related to the well-documented decline of North American herbarium collecting (Pranther *et al.* 2004), as recent iNaturalist observations have partially supplemented physical herbarium vouchers, and North American Orchidaceae are rigorously documented by citizen scientists; rather, it is due to actual declines and losses of historic *S. bightensis* populations. The observed range-wide collapse, distributional contraction, and fragmentation of *S. bightensis* fits into a broader trend for Northeastern North America Orchidaceae (Pace 2020) and other phylogenetically diverse taxa (e.g., Willis *et al.* 2008, Duda *et al.* 2020, Zattara & Aizen 2021). Based on the available data, including recent field work, *S. bightensis* appears to be extirpated from New York state. The largest known ex-situ conservation collection of *S. bightensis* is housed as the Mt. Cuba Center, in Hockessin, DE, primarily composed of accessions of the cultivar ‘Chadds Ford’.

Discussion

Spiranthes bightensis was almost named *S. cernua* var. *gigantea* ined. by C. F. Austin (1831–1880), who collected several specimens from Tappantown, NY (e.g., *Austin s.n.*, NY barcode 01392822). However, Austin’s tentative name remained an herbarium name and was never formally published. I also reviewed the validly published name *Neottia cernua* var. *major* Torrey (1826: 320), described without reference to a specimen or specific location as “stem tall, somewhat leafy; flowers very large. Hab. Woods. Sept. Stem 2 ft. high; fl. 3 times as large as in the common variety”. Torrey’s herbarium and types were initially donated to Columbia College (now University) and then transferred to NY in 1895. After reviewing NY’s entire holding of *Spiranthes*, two Torrey Herbarium *S. cernua* s.l. specimens were found (as indicated by stamps added to the sheets when the collection was incorporated into NY): one was collected from New York state (confidently identified as *S. cernua* s.s., NY barcode 01405847), and the other does not have a recorded

collection number, location, or precise date (NY barcode 01392826). Although it lacks collection information, NY barcode 01392826 is accompanied by a descriptive hand-written note:

“This answers the description pretty [illegible] of Mr. Nuttalls ‘*Neottia Odorata*’, this however is not odorous - flowers October & November - truly an aquatic, submerged 6 to 8 months in the year. Roots horizontal & shallow, the tips of them producing new plants. It has 3 ranks of flowers & I had proposed to name it *N. Tristricha* [ined.]. The spike does not become spiral until it is passed flowering - at first you would at a distance from a plant declare that its ranks of flowers decussated, but on close inspection, you see the 3 rows, the flowers of each row exactly perpendicular to each other, giving at a distance the [illegible] appearance of the flowers. It grows in the *Nyssa uniflora* you will find a root entangled in a *N. uniflora* seed.”

This robust specimen, bearing stoloniferous roots, a large lanceolate leaf, and large flowers, could easily be described as “stem tall, somewhat leafy, flowers very large”, as detailed in the description of *N. cernua* var. *major*. Yet the name ‘*N. cernua* var. *major*’ does not appear anywhere on the specimen or note, and Torrey (1826) makes no mention of *N. tristricha* ined. Although there is no collection year indicated on this specimen, *N. odorata*, mentioned in the note, was described in 1834 (Nuttall 1834), postdating the description of *N. cernua* var. *major* by eight years. Of course, this simply means that the note was written during or after 1834, whereas the specimen could have been collected prior to 1826, however the tone and phrasing of the note indicate it was written contemporaneously with the unknown date of collection, leading to the conclusion that this specimen was collected after 1834, and thus after the descriptions of *N. cernua* var. *major* and *N. odorata*. Morphological examination and measurements of Torrey NY barcode 01392826 indicate it should be determined as *S. odorata* (Table 1). The mention of *Nyssa uniflora* Wangenheim (1787: 83) (= *N. aquatica* Linnaeus 1753: 1058) in the note also supports this identification, as *N. aquatica* is restricted to the southeastern U.S., occurring as far north as the Virginia Peninsula of Virginia, in-between the York and James Rivers, an area where *S. odorata* is common, but from which *S. bightensis* is unknown. Therefore, the available evidence indicates this specimen cannot be the missing, unassigned type of *N. cernua* var. *major*.

Torrey (1826) details the vascular plants “found in the United States, north of the Potomac,” a region that encompasses *S. odorata*, *S. cernua*, and *S. bightensis*. However, *S. odorata* is likely extirpated from the Potomac River (e.g., *Wherry s.n.*, AMES barcode 02032524), with the northernmost known extant population occurring just to the south of the Potomac on the Middle Peninsula of Virginia, in-between the Rappahannock and York Rivers. In the absence of a type or applicable specimen available to Torrey for the name *N. cernua* var. *major*, we are left with Torrey’s (1826) twenty-four-word description, which could be applied to *S. odorata*, robust non-hybrid individuals of *S. cernua* (e.g., *Pace 606*, NY), or *S. bightensis*. As such, this name cannot be placed, and must be relegated to the sidelines of taxonomy as an ambiguous name. The inclusion of “woods” as the habitat of *N. cernua* var. *major* lead me to hypothesize that this name is likely to be more closely affiliated with *S. odorata*, as that species commonly occurs in forested habitats, whereas *S. cernua* and *S. bightensis* occur exclusively in open habitats. If a type were to be discovered for *N. cernua* var. *major* and it were found to match the type of *N. odorata*, *N. cernua* var. *major* would have priority over *N. odorata*.

Sheviak, via annotation labels (often employing the phrasing “apparently with strong influence of”, e.g., *Mulford s.n.* (NY)), identified plants he hypothesized to be of hybrid / introgressed origin between *S. cernua* and *S. odorata* scattered along the mid-Atlantic coast. My own observations and collections from this region support many of Sheviak’s observations, and these plants do indeed conform to what one might expect a hybrid between *S. cernua* and *S. odorata* to grossly look like, here described as *S. bightensis*: typically robust and very large in stature (to 1 m tall, flowers ca. 1+ cm in length, herbarium labels often including variations on the phrase “the largest I have seen”, e.g., *Pace 608*, *Zaremba 9079*), with stoloniferous roots, long lanceolate leaves, fragrant flowers, and polyembryonic seeds (indicating the likelihood of apomixis). Based on the data available at the time, Dueck *et al.* (2014) and Pace & Cameron (2017) previously suggested that *S. cernua* and *S. odorata* did not engage in hybridization. However, the newly available molecular data presented here (*Xdh*) indicate a few populations are of (probably ancient) hybrid *S. cernua* × *S. odorata* origin. Additional morphological analysis adds support, helping to identify morphological characters that distinguish between *S. odorata*, *S. bightensis*, and robust non-hybrid *S. cernua* individuals (e.g., *Pace 606*, *Pace 616*; Fig. 2, 4). Given the breadth of sampling and a complimentary review of morphology, I think there is compelling evidence to state that hybridization between *S. cernua* and *S. odorata* is rare and geographically limited, likely occurred in the geologic past and is not ongoing, having occurred somewhere within the region from the southern Hudson River estuary and Long Island, NY, south to the Delmarva Peninsula. Additionally, there is no evidence to suggest that this hybrid species originated in cultivation (e.g., plants previously referred to as *S. cernua* ‘Chadds Ford’), as herbarium specimens from the 19th century clearly show *S. bightensis* existed in the wild prior to its cultivation.

Conclusions

Spiranthes bightensis is one of a limited number of species to be restricted to or have its distribution roughly centered on the mid-Atlantic and New York Bights, including *Carex barrattii* Torrey ex Schweinitz (1824: 361–362), *C. vestita* Willdenow (1805: 263–264), *Morella pensylvanica*, *Prunus maritima* Marshall (1785: 112), *Quercus ×heterophylla* F. Michaux (1812: 87), *Platanthera ×canbyi* (Ames 1908: 70) Luer (1972: 151), *Rhynchospora knieskernii* Carey (1847: 25), *Sagittaria teres* S. Watson (1890: 555), and *Solidago stricta* Aiton (1789: 216) (Kartesz 2015, Naczi *et al.* 2016, Sorrie & Weakley 2001). It is possible the distribution of *S. bightensis* has remained mostly static since its initial evolution, covering essentially the same area as its recent historical distribution in addition to now submerged areas of the continental shelf that were previously exposed during the Last Glacial Maximum, as has been suggested for other regional coastal species (e.g., Ledig *et al.* 2015, Suarez-Gonzalez *et al.* 2015, Wall *et al.* 2010). Furthermore, fossil evidence indicates that many extant plant communities or their close ancient analogs have existed in a relatively stable condition along the North American Geologic Coastal Plain from at least the early Miocene, including bald cypress-black gum and broadleaf wet forests (Berry 1909, Stults & Axsmith 2011), marshes and wet pine forests (Hansen *et al.* 2001), scrub oak dune communities (Berry 1937), and oak-hickory forests (Rachele 1976, Kotthoff *et al.* 2014). I hypothesize that the expanded habitat presented by the now submerged continental shelf may have helped facilitate the initial hybridization of *S. cernua* s.s. and *S. odorata*. The complex geologic history of the North American Geologic Coastal Plain, with likely cryptic and now submerged areas of glacial refugia and the differing inundation histories of embayment areas vs. arches (Bloom 1983, Ward 1992), indicate hypotheses for physiographic relationships and migration patterns for inland taxa may not be applicable to coastal taxa (Sorrie & Weakley 2001). It is extremely interesting that evidence for the hybrid species *S. bightensis* is limited to this area, which is essentially devoid of major topographic change, and is just to the current northern distributional limit of *S. odorata* in Tidewater Virginia, whereas *S. cernua* and *S. odorata* are broadly sympatric and bloom synchronistically across much of the southeastern Coastal Plain of the United States. Much as with other geographically limited hybrid taxa, such as *S. niklasii*, why did hybridization only happen here? What factor(s) led to the evolution of *S. bightensis* and what factor(s) are inhibiting it elsewhere? These questions should direct future research focused on the evolution of *Spiranthes* and will help inform wider biogeographic patterns within North America.

Additional Specimens Examined

Spiranthes cernua—U. S. A. 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!). Maryland: Cecil Co., 2 1/3 mi W of Elkton, 14 Sep 1946, *Stiteler s.n.* (AMES!). Worcester Co., Along MD Rt 611, Stephen Decatur Highway, 1 mi S of MD Rt 376, Assateague Road, 10 Oct 2008, *Longbottom 12334* (NY!). New Jersey: Atlantic Co., Pleasantville, near Atlantic City, 1 Oct 1916, *Tidestrom 7990* (WIS!). Camden Co., Mount Ephraim, 30 Sep 1916, *Long 15163* (PH!). Orchard Station, 12 Oct 1907, *Stone 9462* (PH!). Clementon, 25 Sep 1897, *Jahn s.n.* (AMES!). Cape May Co., Cape May, Bennett Bogs, 1 km. w. of Erma, 24 Sep 1961, *Montgomery s.n.* (CHBR!). Cumberland Co., Streamlet near Bridgeton Junction, NE Bridgeton, 29 Sep 1923, *Long 29591* (PH!). Gloucester Co., Lake Franklinville, 11 Oct 1923, *Meredith s.n.* (PH!). 3/4 mi SW along Mantua Creek, Hurffville, 28 Sep 1919, *Long 22150* (PH!). Mercer Co., Bear Swamp, Lawrence Station, 19 Sep 1913, *Bartram s.n.* (PH!). Middlesex Co., Woodbridge, 21 Sep 1889, *Churchill s.n.* (AMES!). Ocean Co., 1 1/2 miles S of Forked River, 16 Sep 1945, *Koster C12-II-4* (GH!). New York: Bronx Co., Kingsbridge, 6 Sep 1891, *Bicknell s.n.* (NY!). Jerome Park, 26 Sep 1916, *Pennell 9267* (NY!). Mosholu Parkway, New York City, 21 Sep 1904, *Edmondson 3190* (NY!). Nassau Co., Jones Pond, Wantagh, 5 Sep 1938, *Muenschner 6834* (BH!, NYS!). Orange Co., West Point, 23 Sep 1882, *Mearns 4148* (AMES!). Suffolk Co., Camp Hero State Park, on bluff trail close to Point Woods Trail, 1 Sep 2013, *Bustamante 260* (NY!). Wading River, Long Island, 25 Sep 1923, *Ferguson 2648* (NY!). Shinnecock Bay, Jul 1896, *Fry 469* (AMES!). Westchester Co., North Tarrytown, 25 Sep 1896, *Barnhart 1818* (NY!). Virginia: Charles City Co., N of north terminus of Eagles Nest Road in the Chickahominy Wildlife Management Area, 22 Oct 1992, *Stoetzer 53* (WILLI!). James City Co., Little Creek Reservoir Park, SW of Toano, 25 Oct 2013, *Pace 609* (NY!). Prince George Co., about 3 mi SE of New Bohemia, 16 Oct 1936, *Fernald 6803* (AMES!).

Spiranthes bightensis—U. S. A. Delaware: Kent Co., Kenton, s.d., *Thompson s.n.* (F!). Sussex Co., E side of DE 1 (Coastal Highway), 0.5 mi. S of Assawoman Street, York Beach, 12 Oct 2013, *Longbottom 20494* (USF!). Indian River,

Oak Orchard, 10 Oct 1928, *Denslow s.n.* (NYS). Millsboro, Sep 1880, *Canby s.n.* (NY!). Maryland: Dorchester Co., SW of the town of Vienna, along Steele Neck Road at Kraft Neck Road W of the road, 9 Oct 2005, *Longbottom 6897* (PH!). Along Elliott Island Road, 7 mi S of Henry's Crossroads Rd, Oct 14 1990, *Longbottom 1332* (USF!). Wicomico Co., Town of Pittsville, along US Rt 50, Ocean Gateway, between Friendship Road and Sixty Foot Road, in roadside ditch on N side of the road, 14 Oct 2006, *Longbottom 8067* (NCU!). Rt 50 crossing at Nanticoke River, Ferry Point, 12 Oct 1981, *Hill 10866* (AMES!, GA!, NY!). Worcester Co., Town of Ocean Pines, at Bainbridge Park, edge of pond, 12 Oct 2008, *Velsir s.n.* (NY!). Campground, W of dunes and E of road, North Beach campground, National Seashore, Assateague Island, 6 Oct 1984, *Hill 15994* (MARY!). New Jersey: Atlantic Co., On branch of overhead road over PRR about one mile below station, Egg Harbor City, 20 Oct 1920, *Meredith s.n.* (PH!). In marsh by bridge at Oceanville, 3 Oct 1939, *Hynes 1004* (PH!). Cape May Co., Cape May Point State Park, adjacent to the path paralleling the ocean, in-between the parking lot and the "Yellow Trail", 175 m from the ocean, 19 Oct 2013, *Pace 607* (NY!). Gloucester Co., Along Mantua Creek, near the village of Mantua, 29 Oct 1921, *Long 25369* (PH!). Griffith's Swamp, Oct 1865, *Porter s.n.* (CHBR!). Ocean Co., Ocean Twp., Waretown, along Waretown Creek, below Tuckerton RR, 4 Oct 1910, *Long 5449*. (CHRB!, PH!). Salem Co., Boggy border swale along Game Creek, N of Biddles Landing, 3 Oct 1934, *Fogg 7520* (PH!). New York: Nassau Co., Hempstead L.I., 2 Sep 1896, *Mulford s.n.* (NY!). Suffolk Co., Brookhaven, Sunrise Highway margin near exit 59, 9 Sep 1992, *Zaremba 9079* (NYS!). Eastport, 14 Sep 1894, collector unknown (MO!). Rockland Co., Tappantown, Sep 1861, *Austin s.n.* (NY barcode 1392825!). Tappantown, Sep 1861, *Austin s.n.* (NY barcode 1392828!). Tappantown, 1861, *Austin s.n.* (NY barcode 1392822!). Virginia: Accomack Co., Virginia Eastern Shore, Coards Branch Pond, SW of Parksley, 11 Nov 1991, *Zebryk s.n.* (GA!).

Spiranthes odorata—U. S. A. North Carolina: Brunswick Co., Rice Creek, Winnabow, just N of Governors Rd SE / 1521, 2 Nov 2012, *Pace 611* (NY!). Currituck Co., Church's Island, 18–20 Oct 1918, *McAtee 2933* (US!). Pasquotank Co., Outer Banks, N of US 158 bridge N of the Mouth of Albemarle Sound, ca. 30 m west of road on south side of boardwalk at small boat launch site at Duck, 7 Oct 1993, *Stalter 9* (NYS!). Virginia: Charles City Co., Fresh tidal marsh along Kittewan Creek, Weyanoke, 18 Sep 1939, *Fernald 11307* (BH!, DUKE!, F!, MO!, US!, WVA!). Chesapeake Co., Northwest River near Northwest, 17 Oct 1941, *Fernald 11671A* (DUKE!). James City Co., Brackish marsh near footbridge to Visitor's Center - Pitch and Tar Swamp, Jamestown Island, 25 Sep 1983, *North 1192* (WILLI!). King William Co., Fresh tidal shore of Mattaponi River, at Horse Landing, near King William Courthouse, 14 and 16 Oct 1939, *Fernald 11556* (AMES!, US!). Virginia Beach Co., Back Bay NWR, marsh near visitor center, 4 Nov 2006, *Stalter s.n.* (NYS!). Oligohaline wind-tide marsh along SE side of Milldam Creek, 0.25 mi NW of its confluence with the North Landing River, 2.3 mi SW of Creeds, 18 Oct 1995, *Fleming 11540* (GMUF!, VPI!).

iNaturalist records

Spiranthes bightensis—www.inaturalist.org/observations/42801485; www.inaturalist.org/observations/63283830; www.inaturalist.org/observations/62897552; www.inaturalist.org/observations/44760020; www.inaturalist.org/observations/63765635; www.inaturalist.org/observations/62283186; www.inaturalist.org/observations/63675731; www.inaturalist.org/observations/4612702

Molecular voucher information is listed as follows: Taxon name, sample number, voucher (herbarium), origin, GenBank accessions (nrITS, ACO, *Xdh*, *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, MW651936, MF434693, MF460850, MF434673, MF460938, MF441697); *S. arcisepala*, sc30, *Pace 628* (NY), OH, (MF170215, MF460905, MW651937, MF434692, MF460851, MF434672, MF460939, MF441698); *Spiranthes bightensis*, sc6d, *Pace 607* (NY), NJ, (MF170212, MF460910, MW651940, MF434691, MF460859, MF434669, MF460942, MF441705); *S. bightensis*, sc8d, *Pace 608* (NY), MD, (MF170211, MF460911, MW651941, MF434690, MF460860, MF434668, MF460943, MF441706); *S. bightensis* 'Chadd Ford', 4ee, *Dueck s.n.* (WIS), cultivated, (KM262293, KU752262, MW651938, KM213805, KU935563, KM283644, KM283456, KX088327); *S. bightensis* 'Chadd Ford', 4ff, *Patton s.n.* (WIS), cultivated, (KM262294,—, MW651939, KM213806,—, KM283645, KM283457, MF441703); *Spiranthes casei* Catling & Cruise, 2a, *Case s.n.* (WIS), MI, (KM213852, MF460906, MW651942, KM213770, MF460852, KM262266, KM283433, MF441699); *Spiranthes cernua* (L.) Rich., sc1b, *Pace 597* (NY), DE, (MF170213,—,—,—, MF460858B, MF434670, MF460941, MF441704); *S. cernua*, sc9a, *Pace 609* (NY), VA, (KU752296, KU752258,—,—, KU935561, KU740271, KU935527, KX088325); *S. cernua*, sc15b, *Pace 616* (NY), GA, (KU752297, KU752259, MH751566,

KU752271, KU935562, KU740272, KU935528, KX088326); *S. cernua*, 4cc, Fowler s.n. (WIS), SC, (KM262291, KU752261, MW651944, KM213803, KU935558, KM283642, KM283454, KX088322); *S. cernua*, 4dd, Fowler s.n. (WIS), NC, (KM262292, KU752260, MW651945, KM213804, KU935559, KM283643, KM283455, KX088323); *S. cernua*, 4b, Dueck s.n. (CLEM), SC, (EU384829,—, MW651943, KM213781,—, EU384769, EU384708,—); *S. cernua*, 04j, Dueck s.n. (TAMU), TX, (EU384832,—, KM213787,—, EU384774, EU384713,—); *S. cernua*, 4L, Dueck s.n. (CLEM), TX, (EU384834,—, KM213789, MF460856, EU384776, EU384715,—); *S. cernua*, 4m, Stewart s.n. (WIS), FL, (KM262279, KU752257, MW651946, 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*, 3a, Orzell & Bridges 26735 (NY), FL, (KX756345, KX793115,—, KX756354, KX756364, KX756375, KX756383, KX756335); *Spiranthes incurva* (Jenn.) M.C. Pace, sc33a, Reddoch s.n. (WIS), Ontario, (MF170208, MF460914, MW651947, MF434689, MF460865, MF434665, MF460946, MF441711); *S. incurva*, soch10a, Pace 630 (NY), VT, (MF170204, MF460918, MW651948, MF434685, MF460869, MF434661, MF460950, MF441715); *Spiranthes longilabris* Lindl., 13a, Galloway s.n. (WIS), NC, (EU384844, KU752241, MW651949, KM213830, KU935570, EU384787, EU384726, KX088334); *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, MH913324, KU752274, KU935577, KU740275, KU935532, KX088340); *S. magnicamporum*, sm12a, Fowler s.n. (WIS), GA, (KU752302, KU752253, MW651950, KU752276, KU935579, KU740277, KU935534, KX088342); *Spiranthes niklasii* M.C. Pace, AR1, Pace 650 (NY), AR, (MF170202, MF460920,—, MF434684, MF460872, MF434659, MF460954, MF441721); *S. niklasii*, AR5, Pace 652 (NY), AR, (MF170200, MF460922,—, MF434682, MF460874, MF434657, MF460956, MF441723); *Spiranthes ochroleuca* (Rydb.) Rydb., 16b, Dueck s.n. (WIS), VA, (KM262323, KU752264, MW651951, KM213845, KU935583, KM283681, KM283493, KX088347); *S. ochroleuca*, 16g, Case s.n. (CLEM), MI, (KM262327, MF460932, MW651952, KM213849, MF460891, KM283685, KM283497, MF441737); *Spiranthes odorata* (Nutt.) Lindl., sol1d, Pace s.n. (WIS), NC, (KU752307, KU752225, MH751572, KU752280, KU935588, KU740282, KU935539, KX088352); *S. odorata*, so5h, Pace 614 (NY), NC, (KU752308, KU752226, MW651957,—, KU740283, KU935540,—); *S. odorata*, sol10i, Pace 619 (NY), FL, (KU752309, KU752227, MW651958, KU752281,—, KU740284, KU935541, KX088354); *S. odorata*, sol11, Sheviak 2408 (NYS), KY, (KU752310,—, MW651959, KU752282,—, KU740285, KU935542, KX088355); *S. odorata*, sol12, Durr s.n. (NYS), TN, (KU752311, KU752228, MW651960, KU752283, KU935589, KU740286, KU935543, KX088356); *S. odorata*, sol13, Statler s.n. (NYS), VA, (KU752312, KU752229,—, KU752284,—, KU740287, KU935544, KX088357); *S. odorata*, 17d, Galloway s.n. (CLEM), NC, (EU384852, MF460934, MW651953, 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,—, MW651954, KM262246, KU935585, EU384799, EU384738, KX088349); *S. odorata*, 17opq, Vincent s.n. (WIS), GA, (KM262333, KU752224, MW651955, KM262250, KU935586, KM283694, KM283506, KX088350); *S. odorata*, 17z, n.a., FL, (KM262341,—, MW651956, KM262258,—, KM283702, KM283514,—); *Spiranthes ovalis* var. *erostellata* Catling, sov2, Pace 649 (WIS), WI, (MF170190, MF460935, MW651962, MF434675, MF460895, MF434647, MF460966, MF441742); *S. ovalis* var. *erostellata*, 19c, Fowler s.n. (CLEM), NC, (KM262346, KU752256, MW651961, KM262263, KU935590, KM283707, KM283519, KX088359); *Spiranthes triloba* (Small) Schum. emend. M.C. Pace, FL36, Pace 561 (NY), FL, (KU752313, KU752243, MW651963, KU752285, KU935598, KU740288, KU935545, KX088372); *S. triloba*, FL130, Pace s.n. (WIS), FL, (KU752314, KU752244, MW651964, KU752286, KU935599, KU740289, KU935546, KX088373).

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References

- Aiton, W. (1789) *Hortus Kewensis; or, a Catalogue of the Plants Cultivated in the Royal Botanic Garden at Kew*. Vol. 3. London.
<https://doi.org/10.5962/bhl.title.116053>
- Ames, O. (1908) Notes on *Habenaria*. *Rhodora* 10: 70–72.
<https://doi.org/10.1093/nq/s10-X.239.70d>
- Arft, A.M. & Ranker, T.A. (1998) Allopolyploid origin and population genetics of the rare orchid *Spiranthes diluvialis*. *American Journal of Botany* 85: 110–122.
<https://doi.org/10.2307/2446560>
- Baum, D.A. & Donoghue, M.J. (1995) Choosing among alternative “Phylogenetic” species concepts. *Systematic Botany* 20 (4): 560–573.
<https://doi.org/10.2307/2419810>
- Berry, E.D. (1909) A Miocene flora from the Virginia Coastal Plain. *The Journal of Geology* 17: 19–30.
<https://doi.org/10.1086/621583>
- Berry, E.D. (1937) Tertiary floras of Eastern North America. *Botanical Review* 3: 31–46.
- Bloom A.L. (1983) Sea level and coastal changes. *Late-Quaternary Environments of the United States* 2: 42–51.
<https://doi.org/10.5749/j.cttt09b.9>
- Catling, P.M. & Sheviak, C.J. (1993) Taxonomic notes on some North American Orchids. *Lindleyana* 8: 78.
- Carey, J. (1847) Description of Three New Carices, and a new species of *Rhynchospora*. *American Journal of Science and Arts*, ser. 2 4: 22–25.
- Cavanilles, A.J. (1799) *Anales de Historia Natural* 1(2). Madrid.
- Cornish-Bowden, A. (1985) Nomenclature for incompletely specified bases in nucleic acid sequences: recommendations 1984. *Nucleic Acids Research* 13: 3021–3030.
<https://doi.org/10.1093/nar/13.9.3021>
- Dayrat, B. (2005) Towards integrative taxonomy. *Biological Journal of the Linnean Society. Linnean Society of London* 85: 3407–415.
<https://doi.org/10.1111/j.1095-8312.2005.00503.x>
- Duda, M.P., Allen-Mahé, S., Barbraud, C., Blais, J.M., Boudreau, A., Bryant, R. Delord, K., Grooms, C., Kimpe, L.E., Letournel, B., Lim, J.E., Lormée, H., Michelutti, N., Robertson, G.J., Urtizbérrea, F., Wilhelm, S.I. & Smol, J.P. (2020) Linking 19th century European settlement to the disruption of a seabird’s natural population dynamics. *Proceedings of the National Academy of Sciences of the United States of America* 117: 32484–32492.
<https://doi.org/10.1073/pnas.2016811117>
- Dueck, L.A., Aygoren, D. & Cameron, K.M. (2014) A molecular framework for understanding the phylogeny of *Spiranthes* (Orchidaceae), a cosmopolitan genus with a North American center of diversity. *American Journal of Botany* 101: 1551–1571.
<https://doi.org/10.3732/ajb.1400225>
- Glick, B. (2001) *Spiranthes cernua* var. *odorata* ‘Chadds Ford’. *Rock Garden Quarterly* 59: 129–131.
- Google Earth Pro 7.3.3.7786. (2020) United States of America, southern New York to eastern Virginia. Borders and labels; places layers. NOAA, Landsat / Copernicus. Available from: <http://www.google.com/earth/index.html> (accessed 15 January 2021)
- Górniak, M., Paun, O. & Chase, M.W. (2010) Phylogenetic relationships within Orchidaceae based on a low-copy nuclear coding gene, *Xdh*: Congruence with organellar and nuclear ribosomal DNA results. *Molecular Phylogenetics and Evolution* 56: 784–795.
<https://doi.org/10.1371/journal.pone.0038788>
- Guo, Y.-Y., Luo, Y.-B., Liu, Z.-J. & Wang, X.-Q. (2012) Evolution and biogeography of the slipper orchids: Eocene vicariance of the conduplicate genera in the Old and New World tropics. *PLoS One* 7: e3878810.
<https://doi.org/10.1371/journal.pone.0038788>
- Hansen, B.C.S., Grimm, E.C. & Watts, W.A. (2001) Palynology of the Peace Creek Site, Polk County, Florida. *Geological Society of America Bulletin* 113: 682–692.
[https://doi.org/10.1130/0016-7606\(2001\)113<0682:POTPCS>2.0.CO;2](https://doi.org/10.1130/0016-7606(2001)113<0682:POTPCS>2.0.CO;2)
- Huson, D.H. & Bryant, D. (2006) Application of phylogenetic networks in evolutionary studies. *Molecular Biology and Evolution* 23: 254–267.

<https://doi.org/10.1093/molbev/msj030>

- Karetasz, J.T. (1999) *Synthesis of the North American Flora* (version 1.0) Item no. 18 (unpaginated insert with CD-ROM).
- Kartesz, J.T. [The Biota of North America Program (BONAP)] (2015) *North American Plant Atlas*. (<http://bonap.net/napa>). Chapel Hill, North Carolina.
- Kotthoff, U., Greenwood, D.R., McCarthy, F.M.G., Müller-Navarra, K., Prader, S. & Hesselbo, S.P. (2014) Late Eocene to middle Miocene (33 to 13 million years ago) vegetation and climate development on the North American Atlantic Coastal Plain (IODP Expedition 313, Site M0027). *Climate of the Past* 10: 1523–1539.
<https://doi.org/10.5194/cp-10-1523-2014>
- Ledig, F.T., Smouse, P.E. & Hom, J.L. (2015) Postglacial migration and adaptation for dispersal in pitch pine (Pinaceae). *American Journal of Botany* 102: 2074–2091.
<https://doi.org/10.3732/ajb.1500009>
- Lindley, J. (1840) *The genera and species of orchidaceous plants*. Ridgways, London.
- Linnaeus, C.V. (1763) *Species plantarum: Exhibentes plantas rite cognitatas, ad genera relatas, cum differentiis specificis, nominibus trivialibus, synonymis selectis, locis natalibus, secundum systema sexuale digestas*. Editio secunda, aucta.; Holmiae: Impensis Direct, Laurentii Salvii, 1762–63.
<https://doi.org/10.5962/bhl.title.11179>
- Luer, C.A. (1972) *The Native Orchids of Florida*. The New York Botanical Garden, Bronx, NY.
- Marshall, H. (1785) *Arbustrum Americanum*. J. Cruikshank, Philadelphia.
- May, R.M. (1990) Taxonomy as destiny. *Nature* 347: 129–130.
<https://doi.org/10.1038/347129a0>
- Michaux, F.A. (1812) *Histoire des Arbres Forestiers de l’Amerique Septentrionale* 2. L. Haussmann, Paris.
- Miller, M.A., Pfeiffer, W. & Schwartz, T. (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: *Proceedings of the Gateway Computing Environments Workshop (GCE), 14 Nov. 2010, New Orleans*. pp. 1–8.
<https://doi.org/10.1109/GCE.2010.5676129>
- Mirbel, C.F.B. de. (1804) In: Duhamel du Monceau, M. *Traité des Arbres et Arbustes* [Nouvelle edition] 2. Chez H.L. Guerin & L. F. Delatour, Paris.
- Naczi, R.F.C. & Collaborators (2016 onward) *New Manual of Vascular Plants of Northeastern United States and Adjacent Canada*. online edition. NYBG Press, New York.
<https://doi.org/10.1007/s12228-016-9416-6>
- Nees von Esenbeck, C.G.D. (1832) *Genera et Species Asterearum*. Nuremberg: Sumtibus Leonardi Schrag.
- Neubig, K.M., Whitten, W.M., Carlswald, B.S., Blanco, M.A., Endara, L., Williams, N.H. & Moore, M. (2009) Phylogenetic utility of *ycf1* in orchids: A plastid gene more variable than *matK*. *Plant Systematic Evolution* 277: 75–84.
<https://doi.org/10.1007/s00606-008-0105-0>
- Nuttall, T. (1834) A Description of some of the rarer or little known plants indigenous to the United States, from the dried specimens in the herbarium of the Academy of Natural Sciences in Philadelphia. *Journal of the Academy of Natural Sciences of Philadelphia* 7: 61–115.
- Pace, M.C. (2020) The Orchidaceae of northeastern North American: Systematics, evolution, diversity, and conservation. *Memoirs of the Torrey Botanical Society* 29: 156–189.
- Pace, M.C. & Cameron, K.M. (2016) Reinstatement, Redescription, and Emending of *Spiranthes triloba* (Orchidaceae): Solving a 118 year old cryptic puzzle. *Systematic Botany* 41: 924–939.
<https://doi.org/10.1600/036364416X694026>
- Pace, M.C. & Cameron, K.M. (2017) The systematics of the *Spiranthes cernua* species complex (Orchidaceae): Untangling The Gordian Knot. *Systematic Botany* 42: 640–669.
<https://doi.org/10.1600/036364417X696537>
- Pace, M.C. & Cameron, K.M. (2019) The evolutionary and systematic significance of hybridization between taxa of *Spiranthes* (Orchidaceae) in the California Sierra Nevada and Cascade Range. *Taxon* 68: 199–217.
<https://doi.org/10.1002/tax.12034>
- Pace, M.C., Orzell, S.L., Bridges, E.L. & Cameron, K.M. (2017) *Spiranthes igniorchis* (Orchidaceae), a new and rare cryptic species from the south-central Florida subtropical grasslands. *Brittonia* 69: 323–339.
<https://doi.org/10.1007/s12228-017-9483-3>
- Pace, M.C., Giraldo, G., Frericks, J., Lehnebach, C.A. & Camron, K.M. (2018) Illuminating the systematics of the *Spiranthes sinensis* species complex (Orchidaceae): ecological speciation with little morphological differentiation. *Botanical Journal of the Linnean Society* 189: 36–62.
<https://doi.org/10.1093/botlinnean/boy072>

- Pouyat, R.V. & McDonnell, M.J. (1991) Heavy metal accumulations in forest soils along an urban-rural gradient in Southeastern New York, USA. *Water, Air, and Soil Pollution* 57: 797–807.
<https://doi.org/10.1007/BF00282943>
- Pranther, L.A., Alvarez-Fuentes, O., Mayfield, M.H. & Ferguson, C.J. (2004) The decline of plant collecting in the United States: a threat to the infrastructure of biodiversity studies. *Systematic Botany* 29: 15–28.
<https://doi.org/10.1600/036364404772974185>
- RStudio. (2014) RStudio: Integrated development environment for R (Open Source Edition; Version v0.98.932) [Computer software]. Boston, MA. [Retrieved September 25, 2014]
- Rachele, L.D. (1976) Palynology of the Legler Lignite; a deposit in the Tertiary Cohansey Formation of New Jersey, U.S.A. *Review of Palaeobotany and Palynology* 22: 225–252.
[https://doi.org/10.1016/0034-6667\(76\)90004-X](https://doi.org/10.1016/0034-6667(76)90004-X)
- Rafinesque, C.S. (1818) Third decade of new species of North American Plants. *American monthly magazine and critical review* 2: 206–207.
- Rafinesque, C.S. (1833) *Herbarium Rafinesquianum* 44.
- Rafinesque, C.S. (1836) *New flora and botany of North America, or, A supplemental flora, additional to all the botanical works on North America and the United States. Containing 1000 new or revised species*. Philadelphia [Printed for the author and publisher].
<https://doi.org/10.5962/bhl.title.6139>
- Rambaut, A. (2014) FigTree v. 1.4.2, released 2014–07–09. Available from: <http://tree.bio.ed.ac.uk/software/figtree/> (accessed 30 April 2021)
- Richard, L.C.M. (1817) *De Orchideis Europaeis Annotationes, praesertim ad genera dilucidanda spectantes*. Paris: Ex typ. A. Belin.
<https://doi.org/10.5962/bhl.title.15465>
- Schweinitz, L.D. von. (1824) A monograph of the North American Species of *Carex*. *Annals of the Lyceum of Natural History of New York* 1: 283–362.
<https://doi.org/10.1111/j.1749-6632.1825.tb00013.x>
- Sheviak, C.J. (1973) A new *Spiranthes* from the grasslands of central North America. *Botanical Museum Leaflets* 23: 285–297.
- Sheviak, C.J. (1982) Biosystematic study of the *Spiranthes cernua* complex. *Bulletin of the New York State Museum Science Service*, Bulletin Number 448.
<https://doi.org/10.5962/bhl.title.135544>
- Sheviak, C.J. (1984) *Spiranthes diluvialis* (Orchidaceae), a new species from the western United States. *Brittonia* 36: 8–14.
<https://doi.org/10.2307/2806283>
- Sheviak C.J. (1990) A new *Spiranthes* (Orchidaceae) from the cienegas of southernmost Arizona. *Rhodora* 92: 213–231.
- Sheviak, C.J. (1991) Morphological variation in the compliospecies *Spiranthes cernua* (L.) Rich.: ecologically-limited effects of gene flow. *Lindleyana* 6: 228–234.
- Sheviak, C.J. & Catling, P.M. (1980) The identity and status of *Spiranthes ochroleuca* (Rydberg) Rydberg. *Rhodora* 82: 525–562.
- Sorrie, B.A. & Weakley, A.S. (2001) Coastal plain vascular plant endemics: phytogeographic patterns. *Castanea* 66: 50–82.
- Steudel, E.G. von. (1840) *Nomenclator Botanicus. Editio secunda* 1. Stuttgart: Sumptibus J.G. Cottae.
- Stults, D.Z. & Axsmith, B.J. (2011) Filling the gaps in the Neogene plant fossil record of eastern North America: New data from the Pliocene of Alabama. *Review of Palaeobotany and Palynology* 167: 1–9.
<https://doi.org/10.1016/j.revpalbo.2011.07.004>
- Suarez-Gonzalez, A., Sutton, J.T., Trant, A.J., Zamlynny, E. & Good, S.V. (2015) Rethinking refugia: Tree topology, divergence dates, and demographic history trace the distribution of the endangered Plymouth gentian (*Sabatia kennedyana*) from the Pleistocene glaciation to present day. *American Journal of Botany* 10.
<https://doi.org/10.3732/ajb.1400254>
- Suetsugu, K., Lin, J.-Z., Hsu, T.-C. & Hayakawa, H. (2020) Interspecific hybridization between *Spiranthes australis* and *S. sinensis* (Orchidaceae) in southern Taiwan. *Acta Phytotaxonomica et Geobotanica* 71: 177–184.
- Surveswaran, S., Gowda, V. & Sun, M. (2018) Using an integrated approach to identify cryptic species, divergence patterns and hybrid species in Asian ladies' tresses orchids (*Spiranthes*, Orchidaceae). *Molecular Phylogenetics and Evolution* 124: 106–121.
<https://doi.org/10.1016/j.ympev.2018.02.025>
- Szalanski, A.L., Steinauer, G., Bischof, R. & Petersen, J. (2001) Origin and Conservation Genetics of the Threatened Ute Ladies'-Tresses, *Spiranthes diluvialis* (Orchidaceae). *American Journal of Botany* 8: 177–180.
<https://doi.org/10.2307/2657138>
- Thiers, B. (2021) Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. <http://sweetgum.nybg.org/science/ih/> (accessed 2 January 2021)
- Torrey, J. (1826) *A compendium of the flora of the northern and middle states*. New York: Stacy B. Collins.

- Wall, W.A., Douglas, N.A., Xiang, Q-Y., Hoffmann, W.A., Wentworth, T.R. & Hohmann, M.G. (2010) Evidence for range stasis during the latter Pleistocene for the Atlantic Coastal Plain endemic genus, *Pyxidanthra* Michaux. *Molecular Ecology* 19: 4302–4314.
<https://doi.org/10.1111/j.1365-294X.2010.04793.x>
- Wangenheim, F.A.J. von. (1787) *Beytrag zur teutschen holzgerechten Forstwissenschaft, die Anpflanzung nordamericanischer Holzarten, mit Anwendung auf teutsche Forste betreffend, von Friedrich Adam Julius von Wangenheim*. Göttingen, J.C. Dieterich.
- Watson, S. (1890) *A Manual of the Botany of the Northern United States* (ed. 6). New York: American Book Co.
- Ward, L.W. (1992) Tertiary molluscan assemblages from the Salisbury embayment of Virginia. *Virginia Journal of Science* 43: 85–100.
- Willdenow, C.L. von. (1805) *Species Plantarum. Editio quarta* 4: 263–264.
- Willis, C.G., Ruhfel, B., Primack, R.B., Miller-Rushing, A.J. & Davis, C.C. (2008) Phylogenetic patterns of species loss in Thoreau's woods are driven by climate change. *Proceedings of the National Academy of Sciences of the United States of America* 105: 17029–17033.
<https://doi.org/10.1073/pnas.0806446105>
- Zattara, E.E. & Aizen, M.A. (2021) Worldwide occurrence records suggest a global decline in bee species richness. *One Earth* 4: 114–123.
<https://doi.org/10.1016/j.oneear.2020.12.005>