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.

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://sernceportal.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 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 any of these criteria, then I apply 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. arcesepala 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).
A SPECIES OF SPIRANTHES

Phytotaxa 498 (3) © 2021 Magnolia Press • 163

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.

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

......continued on the next page
TABLE 1 (Continued)

<table>
<thead>
<tr>
<th>Species</th>
<th>Voucher (herbarium)</th>
<th>Labellum length (mm)</th>
<th>Lower labellum width (mm)</th>
<th>Sepal length (mm)</th>
<th>Leaf length (mm)</th>
<th>Leaf width (mm)</th>
<th>Bract length (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. cernua</em></td>
<td>Fry 469 (AMES)</td>
<td>9.0</td>
<td>6.7</td>
<td>9.8</td>
<td>222</td>
<td>11.6</td>
<td>13.4</td>
</tr>
<tr>
<td><em>S. cernua</em></td>
<td>Lippmaa s.n. (TU)</td>
<td>7.47</td>
<td>3.34</td>
<td>9.0</td>
<td>170</td>
<td>9.1</td>
<td>9.2</td>
</tr>
<tr>
<td><em>S. cernua</em></td>
<td>Hammer 69 (BKL)</td>
<td>6.4</td>
<td>3.7</td>
<td>8.2</td>
<td>248</td>
<td>12.0</td>
<td>12.7</td>
</tr>
<tr>
<td><em>S. bightensis</em></td>
<td>Pace 608 (NY)</td>
<td>11.2</td>
<td>4.3</td>
<td>10.9</td>
<td>508</td>
<td>19.6</td>
<td>14.2</td>
</tr>
<tr>
<td><em>S. bightensis</em></td>
<td>Pace 607 (NY)</td>
<td>12.2</td>
<td>4.8</td>
<td>10.2</td>
<td>214</td>
<td>16.9</td>
<td>20.8</td>
</tr>
<tr>
<td><em>S. bightensis</em></td>
<td>Austin s.n. (NY)</td>
<td>10.2</td>
<td>2.9</td>
<td>11.0</td>
<td>160</td>
<td>15.0</td>
<td>14.9</td>
</tr>
<tr>
<td><em>S. bightensis</em></td>
<td>Mulford s.n. (NY)</td>
<td>7.7</td>
<td>4.0</td>
<td>8.3</td>
<td>168</td>
<td>12.0</td>
<td>17.7</td>
</tr>
<tr>
<td><em>S. bightensis</em></td>
<td>Lighthipe s.n. (BKL)</td>
<td>10.7</td>
<td>4.2</td>
<td>8.5</td>
<td>210</td>
<td>17.9</td>
<td>17.3</td>
</tr>
<tr>
<td><em>S. bightensis</em></td>
<td>Zebyk s.n. (GA)</td>
<td>8.2</td>
<td>4.6</td>
<td>9.7</td>
<td>360</td>
<td>16.0</td>
<td>18.0</td>
</tr>
<tr>
<td><em>S. bightensis</em></td>
<td>Thompson s.n. (F)</td>
<td>9.9</td>
<td>3.6</td>
<td>10.8</td>
<td>350</td>
<td>15.6</td>
<td>17.3</td>
</tr>
<tr>
<td><em>S. bightensis</em></td>
<td>Tatum 2428 (PH)</td>
<td>8.9</td>
<td>3.6</td>
<td>9.7</td>
<td>271</td>
<td>12.7</td>
<td>14.8</td>
</tr>
<tr>
<td><em>S. bightensis</em></td>
<td>Long 53168 (PH)</td>
<td>8.1</td>
<td>3.3</td>
<td>9.4</td>
<td>253</td>
<td>10.4</td>
<td>17.8</td>
</tr>
<tr>
<td><em>S. bightensis</em></td>
<td>Long 32529 (PH)</td>
<td>7.9</td>
<td>—</td>
<td>9.6</td>
<td>219</td>
<td>18.8</td>
<td>16.4</td>
</tr>
<tr>
<td><em>S. bightensis</em></td>
<td>Long 5449 (PH)</td>
<td>8.1</td>
<td>3.6</td>
<td>9.2</td>
<td>489</td>
<td>16.5</td>
<td>22.6</td>
</tr>
<tr>
<td><em>S. bightensis</em></td>
<td>Longbottom 6897 (PH)</td>
<td>10.1</td>
<td>3.5</td>
<td>12.7</td>
<td>326</td>
<td>17.5</td>
<td>18.4</td>
</tr>
<tr>
<td><em>S. bightensis</em></td>
<td>Longbottom 20494 (USF)</td>
<td>9.7</td>
<td>—</td>
<td>10.7</td>
<td>209</td>
<td>18.5</td>
<td>20.1</td>
</tr>
<tr>
<td><em>S. bightensis</em></td>
<td>Fogg 7520 (PH)</td>
<td>8.6</td>
<td>5.1</td>
<td>9.8</td>
<td>244</td>
<td>18.5</td>
<td>17.5</td>
</tr>
<tr>
<td><em>S. bightensis</em></td>
<td>Lightipe s.n. (BKL)</td>
<td>8.6</td>
<td>3.6</td>
<td>10.7</td>
<td>220</td>
<td>7.9</td>
<td>14.7</td>
</tr>
<tr>
<td><em>S. bightensis</em></td>
<td>Mulford barcode 68472 (BKL)</td>
<td>7.8</td>
<td>4.9</td>
<td>9.3</td>
<td>166</td>
<td>19.2</td>
<td>18.5</td>
</tr>
<tr>
<td><em>S. bightensis</em></td>
<td>Mulford s.n. (BKL)</td>
<td>8.7</td>
<td>5.4</td>
<td>10.4</td>
<td>320</td>
<td>11.1</td>
<td>15.4</td>
</tr>
<tr>
<td><em>S. bightensis</em></td>
<td>Halst s.n. (BKL)</td>
<td>7.9</td>
<td>—</td>
<td>9.5</td>
<td>413</td>
<td>13.9</td>
<td>13.7</td>
</tr>
<tr>
<td><em>S. bightensis</em></td>
<td>Muenscher 6834 (BH)</td>
<td>7.9</td>
<td>4.9</td>
<td>9.2</td>
<td>295</td>
<td>11.9</td>
<td>15.7</td>
</tr>
<tr>
<td><em>S. odorata</em></td>
<td>Torrey s.n. (NY)</td>
<td>9.8</td>
<td>4.0</td>
<td>9.5</td>
<td>347</td>
<td>27.0</td>
<td>16.7</td>
</tr>
<tr>
<td><em>S. odorata</em></td>
<td>Pace 611 (NY)</td>
<td>10.7</td>
<td>4.0</td>
<td>10.5</td>
<td>309</td>
<td>19.5</td>
<td>17.1</td>
</tr>
<tr>
<td><em>S. odorata</em></td>
<td>Pace 614 (NY)</td>
<td>14.6</td>
<td>5.6</td>
<td>16.3</td>
<td>130</td>
<td>17.8</td>
<td>19.9</td>
</tr>
<tr>
<td><em>S. odorata</em></td>
<td>Pace 617 (NY)</td>
<td>13.5</td>
<td>4.4</td>
<td>11.8</td>
<td>187</td>
<td>20.5</td>
<td>16.2</td>
</tr>
<tr>
<td><em>S. odorata</em></td>
<td>Warlow s.n. (NY)</td>
<td>11.8</td>
<td>4.0</td>
<td>—</td>
<td>500</td>
<td>20.0</td>
<td>—</td>
</tr>
<tr>
<td><em>S. odorata</em></td>
<td>Chapman s.n. (NY)</td>
<td>9.7</td>
<td>4.6</td>
<td>10.2</td>
<td>284</td>
<td>24.0</td>
<td>16.2</td>
</tr>
<tr>
<td><em>S. odorata</em></td>
<td>Small 9284 (NY)</td>
<td>10.3</td>
<td>4.7</td>
<td>10.0</td>
<td>331</td>
<td>24.0</td>
<td>18.7</td>
</tr>
<tr>
<td><em>S. odorata</em></td>
<td>Fernald 11308 (NY)</td>
<td>11.0</td>
<td>4.4</td>
<td>10.8</td>
<td>323</td>
<td>27.0</td>
<td>—</td>
</tr>
<tr>
<td><em>S. odorata</em></td>
<td>Wherry s.n. (AMES)</td>
<td>7.1</td>
<td>—</td>
<td>10.1</td>
<td>517</td>
<td>22.4</td>
<td>19.0</td>
</tr>
<tr>
<td><em>S. odorata</em></td>
<td>McMullen s.n. (NY)</td>
<td>11.3</td>
<td>5.6</td>
<td>10.4</td>
<td>257</td>
<td>17.7</td>
<td>—</td>
</tr>
</tbody>
</table>

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 laciniate 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; calli-losities/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](image-url)
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 603* (NY!). L. *Pace 608* (NY!). M. *Austin s.n.* barcode 0139282 (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.
**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.

Neess (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). Alarmingcally, 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 contiguous 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 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 Tappanstown, 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
Phytotaxa
Neottia Odorata

Torrey (1826) details the vascular plants “found in the United States, north of the Potomac,” a region that encompasses S. odorata, S. cerna, 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 S. cerna 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. cerna (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 S. cerna 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. cerna and S. bightensis occur exclusively in open habitats. If a type were to be discovered for S. cerna var. major and it were found to match the type of S. odorata, S. cerna var. major would have priority over S. 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. cerna 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. cerna 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 polyploidic 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. cerna 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. cerna × 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. cerna 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. cerna 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. cerna ‘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 balticca* 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 tere* 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, Kothhoff 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 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,


**iNaturalist records**


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).

**Spiranthesarcisepala** M.C. Pace, NY1, *Page 640* (NY), NJ, (MF170216, MF460904, MW651936, MF434693, MF460850, MF434673, MF460958, MF441697); *S. arcisepala*, sc30, *Page 628* (NY), OH, (MF170215, MF460905, MW651937, MF434692, MF460851, MF434672, MF460939, MF441698); *Spiranthes bightensis*, sc6d, *Page 607* (NY), NJ, (MF170212, MF460910, MW651940, MF434691, MF460859, MF434669, MF460942, MF441705); *S. bightensis*, sc8d, *Page 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, UK935563, 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, *Page 597* (NY), DE, (MF170213,—,—,—, MF460858B, MF434670, MF460941, MF441704); *S. cernua*, sc9a, *Page 609* (NY), VA, (KU752296, KU752258,—,—, UK935561, KU740271, UK935527, KX088325); *S. cernua*, sc15b, *Page 616* (NY), GA, (KU752297, KU752259, MH75166,
Thank you to two anonymous reviewers for their helpful comments. AMES, BH, BKL, CHRB, CLEM, CM, CONN, F, FSU, MARY, MO, NY, NYS, PH, US, WILLI, and WIS sent loans of specimens or images. Specimen digitization was funded by the U.S. National Science Foundation via The Mid-Atlantic Megalopolis: Achieving a greater scientific understanding of our urban world Thematic Collection Network (Award 1601697), the Southeast Regional Network of Expertise and Collections Thematic Collections Network (Award 1410069), and the Digitizing “endless forms”: Facilitating Research on Imperiled Plants with Extreme Morphologies Thematic Collections Network (Award 1802034). Jordan Teisher (PH) provided a high-resolution image of the Neottia odorata lectotype. Bobbi Angell drew Facilitating research on Imperiled Plants with extreme Morphologies (Award 1410069).
the plate, and Elizabeth Gjieli (NY) produced the maps. Robbin Moran (NY) provided helpful feedback in the early stages of manuscript development. Amy Weiss and Lucy Klebieko (NY) helped facilitate access to specimens during COVID-19 pandemic related restrictions to my office.

References


https://doi.org/10.5962/bhl.title.116053


https://doi.org/10.1093/nq/s10-X.239.70d


https://doi.org/10.2307/2446560


https://doi.org/10.1006/sbty.1995.1001


https://doi.org/10.1086/621583


https://doi.org/10.5749/j.ctttt09h.9


https://doi.org/10.1093/nar/13.9.3021


https://doi.org/10.1111/j.1095-8312.2005.00503.x


https://doi.org/10.3732/ajb.1400225


https://doi.org/10.1371/journal.pone.0038788


https://doi.org/10.3732/ajb.1400225


https://doi.org/10.5194/cp-10-1523-2014


https://doi.org/10.3732/ajb.1500009


https://doi.org/10.5962/bhl.title.11179


https://doi.org/10.1038/347129a0


https://doi.org/10.1007/s00606-008-0105-0


https://doi.org/10.1600/s03634416X694026


https://doi.org/10.1600/036364417X696537


https://doi.org/10.1202/taxon.12034


https://doi.org/10.1007/s12228-017-9483-3


https://doi.org/10.1093/botlinnean/boy072


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].


