THE NATIVE ORCHID CONFERENCE JOURNAL

VOLUME 18.3

The Native Orchid Conference, Inc.

P. O. Box 2047 Boone, NC 28607-2047

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THE NATIVE ORCHID CONFERENCE JOURNAL

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THE DIVERSE ORCHIDS IN THE OTTAWA DISTRICT— A COMPARISON

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In 2020, Paul Catling and Brenda Kostiuk published a comprehensive treatment of the orchids of Ontario's Bruce Peninsula in this journal (Catling 2020, Catling and Kostiuk 2020a, 2020b). I thought it worthwhile to compare the orchid populations in "the Bruce" with those 400 km to the east in the Ottawa District (Figure 1).

The Ottawa District, as currently defined, is the area within the circle of 50 km radius centred on the Peace Tower of the Parliament Buildings in Ottawa (45.4251° N, 75.7000° W). It is the study area chosen by The Ottawa Field-Naturalists' Club in 1895 as the common focus for the natural history explorations of its members (Reddoch 1995). During the century and a quarter since then, many significant studies on the flora, fauna and geology of the District have been published by Ottawa Field-Naturalists' Club members, writing in their capacities as government scientists or as serious naturalists. Included among these studies is the 1997 monograph on the orchids (Reddoch and Reddoch 1997) and other orchid articles (e.g. Reddoch and Reddoch 2008, Reddoch, Catling and Reddoch 2013).

Figure 1. Southern Ontario showing the locations of the Bruce Peninsula (highlighted left) and the Ottawa District (highlighted right). Map prepared by Paul Catling.

The Ottawa River flows from west to east across the centre of the District, with the Province of Québec to the north and the Province of Ontario to the south (Figures 2 and 3). The uplands of the Canadian Shield dominate the northern half of the District and also outcrop in the Lowlands to the south. Calcareous bedrocks are common — marble on the Shield and limestone in the Lowlands. There are extensive glacial and postglacial deposits including extensive sandy layers overlying parts of the Lowlands and adjacent Shield. This diverse Ottawa District landscape has many habitats that support orchids: mixed, deciduous and coniferous forests; swamps; fens; bogs; alvars and other rock barrens; sand deposits; and shores and floodplains of streams, lakes and rivers. Half of the Ottawa District orchid species occur in wetlands.

Figure 2. (Above) The Ottawa River and the Lowlands viewed from the 200 m higher Canadian Shield in Gatineau Park, Quebec. Photograph by Joyce M. Reddoch.

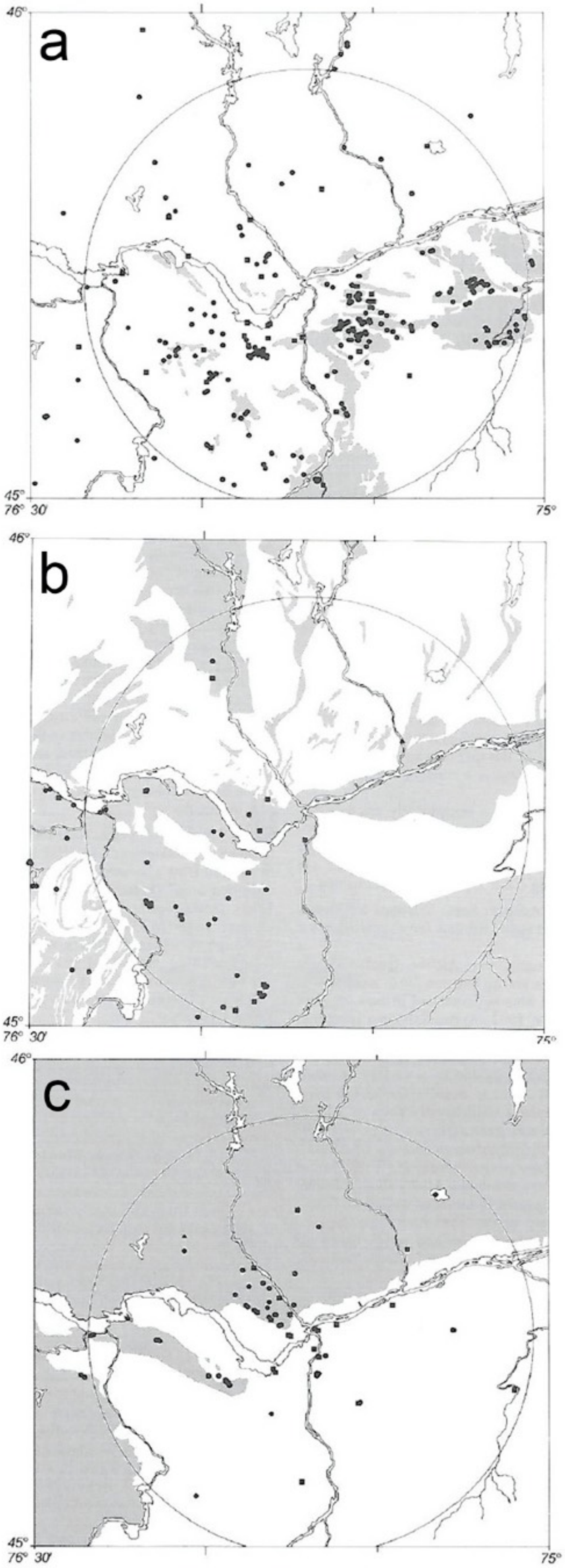

Figure 3. (Right) Distributions of three orchids in the Ottawa District showing relationships to surface geology. a. *Spiranthes incurva* (previously *S. cernua*) showing relationship to open, surface sand deposits, which are shaded on the Lowlands; b. *Cypripedium arietinum* showing relationship to calcareous rocks, including marble and limestone, which are shaded; and c. *Galearis spectabilis* showing its relationship with acidic and neutral substrates on the Canadian Shield (shaded) and in the Lowlands. The maps are taken from Reddoch and Reddoch (1997) and contain all records for each species from the beginning of botanical exploration. Photos next page.

a. *Spiranthes incurva*

b. *Cypripedium arietinum*

c. *Galearis spectabilis*

Table: Orchid Species Lists for the Ottawa District and the Bruce Peninsula.

The species treatments and nomenclature, as well as the list of Bruce Peninsula species, are taken from Catling (2020) and Catling and Kostiuk (2020a). Sources for Ottawa District species are Reddoch and Reddoch (1997) and Reddoch, Catling and Reddoch (2013). Calciphile species are marked with an asterisk (*). A plus sign (+) indicates occurrences less than 100 km from the Ottawa District but not yet known in the District. There have been no records of *Aplectrum hyemale* and *Neottia bifolia* in the District since 1901 and 1902, respectively.

Species /Taxon	**Ottawa District**		**Saugeen Bruce Peninsula**
	Ontario	**Quebec**	
Aplectrum hyemale PUTTYROOT	X	+	X
Arethusa bulbosa DRAGON'S-MOUTH	X	X	X
Calopogon tuberosus TUBEROUS GRASS PINK	X	X	X
Calypso bulbosa var. *americana* CALYPSO *	X	X	X
Coeloglossum viride FROG ORCHID	X	X	X
Corallorhiza maculata varieties SPOTTED CORALROOT	X	X	X
Corallorhiza odontorhiza AUTUMN CORALROOT	+	+	X
Corallorhiza striata varieties STRIPED CORALROOT *	X	X	X
Corallorhiza trifida EARLY CORALROOT	X	X	X
Cypripedium acaule PINK LADY'S-SLIPPER	X	X	X
Cypripedium arietinum RAM'S-HEAD LADY'S-SLIPPER *	X	X	X
Cypripedium parviflorum varieties NORTHERN YELLOW LADY'S-SLIPPER *	X	X	X
Cypripedium reginae SHOWY LADY'S-SLIPPER *	X	X	X

Epipactis helleborine Broad-leaved Helleborine	X	X	X
Galearis (*Amerorchis*) *rotundifolia* Small Round-leaved Orchid *	X	X	X
Galearis spectabilis Showy Orchis	X	X	X
Goodyera oblongifolia Menzies' Rattlesnake-plantain			X
Goodyera pubescens Downy Rattlesnake-plantain	X	X	X
Goodyera repens Dwarf Rattlesnake-plantain	X	X	X
Goodyera tesselata Checkered Rattlesnake-plantain	X	X	X
Liparis loeselii Loesel's Twayblade	X	X	X
Malaxis monophyllos var. brachypoda North American White Adder's-mouth *	X	X	X
Malaxis unifolia Green Adder's-mouth	X	X	X
Neottia (*Listera*) *auriculata* Auricled Twayblade		X	
Neottia (*Listera*) *bifolia* (*L. australis*) Southern Twayblade	X		
Neottia (*Listera*) *convallarioides* Broad-lip Twayblade	+	+	X
Neottia (*Listera*) *cordata* Heart-leaved Twayblade	X	X	X
Neottia (*Listera*) *ovata* Egg-leaved Twayblade			X
Platanthera aquilonis Tall Northern Green Orchid	X	X	X
Platanthera blephariglottis var. *blephariglottis* White Fringed Orchid	X	X	
Platanthera clavellata Club-spur Orchid	X	X	X

Platanthera dilatata var. *dilatata* TALL WHITE BOG ORCHID *	X	X	X
Platanthera flava var. *herbiola* NORTHERN TUBERCLED ORCHID	X	X	X
Platanthera grandiflora GREATER PURPLE FRINGED ORCHID	X	X	
Platanthera hookeri var. *hookeri* HOOKER'S ORCHID	X	X	X
Platanthera huronensis LAKE HURON GREEN ORCHID *	X	X	X
Platanthera hyperborea LEAFY NORTHERN GREEN ORCHID	X	X	X
Platanthera lacera RAGGED FRINGED ORCHID	X	X	X
Platanthera leucophaea EASTERN PRAIRIE FRINGED ORCHID *	X		X
Platanthera macrophylla GREATER ROUND-LEAVED ORCHID	+	X	X
Platanthera obtusata subsp. *obtusata* BLUNT-LEAVED ORCHID	X	X	X
Platanthera orbiculata LESSER ROUND-LEAVED ORCHID	X	X	X
Platanthera psycodes SMALL PURPLE FRINGED ORCHID	X	X	X
Platanthera (*Piperia*) *unalascensis* ALASKA REIN ORCHID *			X
Pogonia ophioglossoides ROSE POGONIA	X	X	X
Spiranthes casei var. *casei* CASE'S LADIES'-TRESSES	X	X	X
Spiranthes incurva (previously *S. cernua s. l.*) INCURVED LADIES'-TRESSES	X	X	X
Spiranthes lacera var. *lacera* NORTHERN SLENDER LADIES'-TRESSES	X	X	X
Spiranthes lucida SHINING LADIES'-TRESSES *	X	X	X

Spiranthes magnicamporum GREAT PLAINS LADIES’-TRESSES *	X		X
Spiranthes romanzoffiana HOODED LADIES’-TRESSES	X	X	X
	46 +2 species		47 species

As shown in the Table, 46 species of orchids have been recorded in the Ottawa District. Forty-four species are on the Ontario side of the Ottawa River, and 42 species are on the Québec side. *Aplectrum hyemale*, *Neottia bifolia*, *Platanthera leucophaea* and *Spiranthes magnicamporum* are only on the Ontario side, while *Neottia auriculata* and *Platanthera macrophylla* are only on the Quebec side. All 44 Ontario species have been observed within the City of Ottawa (Reddoch and Reddoch 1997, Brunton 2005), which is only slightly larger than the Bruce Peninsula. Two additional species are known less than 100 km from the Ottawa District, both in Ontario and Quebec, *Neottia convallarioides* and *Corallorhiza odontorhiza* (Whiting and Catling 1986, Sabourin 1993, Oldham and Consiglio 2018, iNaturalist 2021).

The 47 Bruce Peninsula orchid species are included in the Table for comparison. *Neottia auriculata*, *Neottia bifolia*, *Platanthera blephariglottis* and *Platanthera grandiflora* (Figure 4) are known in the Ottawa District but not in the Bruce, while *Corallorhiza odontorhiza*, *Goodyera oblongifolia*, *Neottia convallarioides* and the non-native *Neottia ovata* have not been recorded within the Ottawa District. As noted above, the first and third species are known from locations close to the District.

Why do the Ottawa District and the Bruce Peninsula have as many orchid species as they do?

Both the Ottawa District and the Bruce Peninsula are at 45° latitude, halfway between the Equator and the North Pole (Figure 1). They are in the Great Lakes - St. Lawrence Forest Region, a region of predominantly deciduous and mixed forests (Rowe 1972, Ressources naturelles Québec 2003, Wester et al. 2018). There are many reasons why the two locations have rich floras, including orchids. Some of these reasons are climate, substrate, and disturbance (Catling and Kostiuk 2020a). As well, location relative to species distributions, and the length of time that the areas have been explored botanically are important factors.

As Catling and Kostiuk (2020a) have shown, the climates of the Ottawa District and the Bruce Peninsula are similar but not identical. These differences are enough to influence the relative occurrences, abundances and distributions of some species in these two areas.

As described above, the Ottawa District has a complex geological history that provides diverse substrates that are the basis for many orchid habitats (Figure 3). Since the withdrawal of the most recent ice sheet about 11,500 years ago, continual changes in climate and the physical landscape have been the norm. Disturbances in the form of floods, earthquakes, landslides, wind storms, tornados and fires are some of the natural events that have altered the landscape for thousands of years. European settlement, beginning in the early 1800s, has also resulted in major and far-reaching changes to the natural landscape. Forest clearing for agriculture and lumbering was followed by widespread fires on both sides of the Ottawa River. The most recent major fire north of the river occurred in 1923. South of the river, a massive fire in 1870 extended from Arnprior, at the western edge of the District, to within a mile of the settlement of Ottawa. The inferno was finally halted when water was released from a dam to fill a wide ravine in front of the fire's path. And, the next year, "once more the land laughed, its harvests and fields were green in the bounty of Nature" (Walker 1968).

The location of a place in relation to the distribution of the species as a whole is, of course, important. Both the Ottawa District and the Bruce Peninsula are well within the distributions of the species that occur there, most of which have Great Lakes - St. Lawrence - eastern North American distributions (Whiting and Catling 1986, Romero-Gonzalez et al. 2002). Both areas are near the southern borders of a few northern, transcontinental species, such as *Calypso bulbosa* and *Galearis rotundifolia*. Additionally, some predominantly western species, such as *Goodyera oblongifolia*, *Platanthera unalascensis*, and the midwestern *Spiranthes magnicamporum*, have disjunct occurrences to the east of their main distributions, perhaps the results of seeds being carried by the prevailing westerly winds in the near or distant past. Thus all three species mentioned are found in the Bruce, but only *Spiranthes magnicamporum* is known in the Ottawa District farther east, where Paul Catling discovered it recently (Reddoch, Catling and Reddoch 2013). The distribution of *Platanthera grandiflor*a (Figure 4) includes the lower Ottawa Valley in its eastern distribution, but does not extend westward to the Bruce Peninsula (Sheviak 2002, iNaturalist 2021). The Eurasian orchid, *Neottia ovata*, just happens to have colonized a few places in southern Ontario including the Bruce Peninsula, likely brought there in the twentieth century by human means.

The Ottawa District has been explored botanically for at least 180 years. The earliest known collection is of an Eastern Red Cedar (*Juniperus virginiana*) gathered by Philip Whiteside Maclagan in 1843 while on a trip on the Rideau Canal from Bytown (Ottawa) to Kingston (Dore 1983). In the 1860s and 1870s four Ottawa orchids, *Calypso bulbosa*, *Cypripedium parviflorum*, *Galearis spectabilis* and *Goodyera repens*, were among the 68 plants that were the subjects of technically excellent watercolour paintings by Elizabeth Keen White (Dore 1965). Most of the plants had been collected by Elizabeth's husband, Lieutenant-Colonel William White, who became the first President of The Ottawa Field-Naturalists' Club in 1879. The earliest herbarium collections of orchid specimens date from 1860 (Queen's University at Kingston, and Agriculture and Agri-Foods Canada herbaria (QK and DAO)). In the 1960s, interested Ottawa Field-Naturalists' Club members began to focus specifically on orchids. Ed Greenwood organized the Native Orchid Location Survey to locate and map accurately the orchid colonies in the Ottawa District (Greenwood 1967, Reddoch and Reddoch 1997). During the decade of its most intense work, the Survey's orchid specialists made over 3500 site records. Needless to say, we did not collect plants at each site for herbarium records.

Figure 4. *Platanthera grandiflora* is an eastern orchid present in Ontario only in the lower Ottawa Valley. In the District it is scattered across the Canadian Shield on acidic and neutral substrates, and in the Lowlands on deep sands. Photograph by Joyce M. Reddoch.

Here is an example of change — the impact of human disturbance, and subsequent succession — during my lifetime. The work of the Native Orchid Location Survey in the 1960s and 1970s coincided with the building of new roads and highways, which resulted in expanses of moist sand being exposed in borrow pits and along roadsides. Several orchids, especially *Platanthera lacera* and *Spiranthes incurva* (Figure 3a), which had previously been very rare in the District, became very common in these newly-created habitats, especially in the Lowlands. In the past 40 years, though, the borrow pits have become forests, and the roadsides have become overgrown. Consequently, both of these species are now uncommon in the District once more.

In summary, then, both the Ottawa District and the Bruce Peninsula are rich in orchid species, and in fact have the same number of native species. This diversity is the result of geology, location, and natural and human disturbances, among many other factors, including climate change. Catling and Kostiuk (2020b) suggest three possible orchids that might move into the Bruce Peninsula: Purple Twayblade (*Liparis liliifolia*), Small White Lady's-slipper (*Cypripedium candidum*), and Northern Oval Ladies'-tresses (*Spiranthes ovalis* var. *erostellata*). These three species are also currently known close to the Ottawa District. (See Figure 5 for background and current status of *Liparis liliifolia* near Montreal.) It might well be that one (or more) of these "close to" species already grows in the District. It's just that no one has discovered it yet.

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Figure 5. (Right) *Liparis liliifolia* is one of the orchids that occurs close to the Ottawa District. It was first discovered in Quebec in 1999 by Bob Barnhurst and Mabel McIntosh at the Morgan Arboretum west of Montreal. They found about a dozen plants in a mostly deciduous swamp. About six years later, Ann Godbout of the Arboretum staff discovered a second group of about 600 plants in similar habitat ½ km farther west. The population was stable through at least 2010 and 2011, but a decade later it is apparent that numbers have declined substantially (Bob Barnhurst, pers. comm. 2021; photographs by Bob Barnhurst).

Acknowledgements

Many thanks to Bob Barnhurst for sharing his knowledge and photographs of the Montreal population of *Liparis liliifolia*, and to Paul Catling and Brenda Kostiuk for their useful suggestions and technical support.

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MINI FIELD TRIP TO LANPHERE NATURE PRESERVE, HUMBOLDT COUNTY, CALIFORNIA

Text and photos by Bill Kress, wckress@gmail.com

Overview

Native Orchid Conference Journal Editor Chelsea Kieffer organized and led a "mini field trip" to the Lanphere Nature Preserve in Arcata, California on Saturday, July 31, 2021. The small group of five NOC members met at The Humboldt Coastal Nature Center and then drove 7 miles to the Lanphere Dunes.

Lanphere: Location, Environment

Mountainous and densely forested, Humboldt County has about 110 miles of coastline and many miles of both public and private parks.

The Lanphere Dunes are surrounded by the Pacific Ocean, the Humboldt Bay, and coniferous forests which grow among the dunes within the Lanphere Unit and surrounding areas. The coastal zone is cooled by a summer ocean temperature in the mid 50's (13℃). Mornings there were foggy and plants we saw were quite wet until dried by the sun around noon. The dunes are about 390 miles north of San Francisco.

Access can be made only by permit – see the Friends of the Dunes website for more information. Chelsea applied for and obtained a written access permit for our visit.

Many different wildflowers were found throughout the dunes and nearby wooded areas. The moist, humid climate enables lots of lichen to grow. Reindeer lichen (*Cladonia rangiferina*) and bear berry manzanita (*Arctostaphylos uva-ursi*), pictured on the left, were common.

Elegant Piperia, *Platanthera elegans* Lindley subsp. *elegans*

Platanthera elegans, also called the Elegant Rein Orchid, is native to California and can be found in several western US states and western Canada. *Platanthera elegans* we saw were about 25 cm high and were found in more open areas.

Hooded Ladies'-tresses, *Spiranthes romanzoffiana* Cham

While we spotted a number of the *Spiranthes romanzoffiana*, we were a week or so late and most of the flowers had brown spots. Since they have been found in several places in Europe, including Ireland and Northern Ireland, they are also referred to as Irish Ladies'-tresses They were less than 20 cm high, and surrounded by the low ground cover, bear berry manzanita, *Arcotstaphylos uva-ursi*

Fat Spurred Piperia, *Platanthera transversa* (Suksdorf) R.M. Bateman

We had been searching for *Platanthera transversa*, and had almost given up. At the end of the day, we decided to try another trail that we earlier bypassed and found just one plant. It was in nearly perfect condition. The spurs of the *P. transversa* are horizontal to the stem, while those of the *P. elegans* hang down against the stem.

Menzies' Rattlesnake-plantain *Goodyera oblongifolia,* Raf.

We saw numerous plants of G*oodyera oblongifolia* along the trails, always growing in the darker, more secluded wooded areas. Most were only in bud and should be blooming mid- to late-August.

Fortunately, we found one with two lower flowers that had just opened and were very fresh.

The Five Hikers

From left to right, Harold Carlson, Chelsea Kieffer, Dorothe Kress, Bill Kress, and Linnea Hanson. Behind us are ropes to enable hikers to climb up the very steep slope of the dune. Climbing this dune was the most difficult part of the entire journey — we were happy when we reached the top.

Left: Chelsea Kieffer photographing the *P. elegans* alongside a Lanphere Dunes trail.
Right: Chelsea Kieffer and Bill Kress photographing a wildflower alongside a Lanphere Dunes trail.

Summary

With COVID-19 concerns, the NOC postponed the annual Symposium and arranged localized field trips with a limited number of participants. This mini field trip worked out very well and we saw four varieties of native terrestrial orchids, had stimulating discussions, a very strenuous dune-walking workout and a wonderful time.

We also found a variety of wildflowers, including the Beach morning glory (*Calystegia soldanella*), Twinberry (*Lonicera involucrata,* pictured above left), Beach buckwheat (*Eriogonum latifolium,* center), Beach evening primrose (*Camissoniopsis cheiranthifolia,* right*)* and Coast goldenrod (*Solidago spathulata*). The Lanphere Dunes have been kept pristine by reducing and controlling human activity.

Post-COVID, the NOC should consider continuing these small, informal field trips, either in conjunction with the Symposium or as extra events throughout the orchid growing season in various parts of the US and Canada.

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Lanphere and Ma-le'l Dunes distinguished as a National Natural Landmark
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A SYSTEMATIC SURVEY OF THE *SPIRANTHES CERNUA* SPECIES COMPLEX (ORCHIDACEAE) IN NEW YORK

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Abstract

Specimens belonging to the *Spiranthes cernua* species complex collected throughout New York, eastern Pennsylvania, and southern New Jersey were identified via genetic sampling and compared against dissected flowers and inflorescence photographs. This, along with revisiting collecting locations, revealed that additional characteristics were needed to satisfactorily separate several newly described taxa. Genetic analyses also show some surprising results not previously reported, including a likely allopolyploid origin for *S. arcisepala* and *S. casei*, in addition to a new cryptic species described here as *S. sheviakii*. Our results suggest that hybridization and possible introgression of *S. ochroleuca* with other members of the complex has played a greater role in diversification of the complex than previously thought, and that accurate identification requires consideration of multiple characteristics.

Orchid enthusiasts in New York have long been perplexed by the morphological diversity of *S. cernua* (L.) Rich, so it was with great interest that we read the paper by Pace and Cameron (2017). In it, the authors describe two new cryptic species occurring in New York which were formerly considered to be *S. cernua*: *S. arcisepala* M.C. Pace and *S. incurva* (Jenn.) M.C. Pace. This raised several new questions including: 1) what is the northern limit of *S. cernua* in New York; and 2) given that *S. ochroleuca* (Rydb.) Rydb. occurs with some populations of *S. cernua* that Charles Sheviak, former Curator of Botany at the New York State Museum, had determined to be of hybrid origin, could *S.* ×*kapnosperia* M.C. Pace occur in the state?

The typical form of *S. arcisepala*, with downwardly falcate sepals, was readily apparent in photographs and examination of plants in the field. *Spiranthes arcisepala* is described as being most similar to *S. cernua* and *S. ochroleuca*, differing from both in having downward arching lateral sepals and smaller flowers, and is distinguished from *S. ochroleuca* in having flowers that are essentially wholly white. *Spiranthes incurva* is said to have a centrally yellowish labellum with conical and reduced abaxial glands, lateral sepals that sweep upwards, and ascending flowers. However, closer examination of the descriptions of these species and comparison to plants in the field revealed limitations in discriminating a number of the specimens we observed.

The given ranges of *S. arcisepala*, *S. cernua*, and *S. incurva* suggested the possibility of the occurrence of all three taxa in central New York, though *S. cernua* was shown to be restricted to extreme southern New York. The downwardly falcate sepals were found to be a distinctive characteristic in many populations of presumed *S. arcisepala*, however many other plants were found with straight or upward curving lateral sepals that otherwise more closely resembled *S. arcisepala* than *S. cernua* or *S. incurva*. We looked to other characteristics, like basal callosity length and the appearance of the abaxial glands, but these proved to be inconsistent. To help resolve these issues of identification, we applied for and received two grants to help fund a genetic study of some confusing members of the complex in New York and surrounding states. We also decided to test material from the type location for *S. incurva* (Jennings 1906) to gain a better understanding of what 'typical' *S. incurva* should look like.

Methods

Taxon sampling

A total of 41 specimens from 7 taxa in the *Spiranthes cernua* complex were collected from sites throughout New York, eastern Pennsylvania, and southern New Jersey (Figure 1). Photographs were taken of the inflorescences and individual flowers, and then specimens were either pressed or samples dried in silica gel. Specimens were mounted and deposited in the SUNY Cortland Herbarium (CORT). Specimens were collected from public right-of-ways, from state land under permit, or with permission from private land.

Flowers of selected plants were dissected and images made of the profile of the flower, abaxial glands of the labellum, flattened labellum, and of the column. Seeds of representative plants were suspended in water and examined using a compound microscope to determine if they were monoembryonic or polyembryonic. After comparing morphological characteristics to the results of the molecular study, photographs of plants posted to iNaturalist (California Academy of Science and National Geographic Society 2021) were examined and assigned to the nearest matching taxon. Those lacking sufficient visible characteristics were discarded.

Specimen photos obtained through the Consortium of Midwest Herbaria (https://midwestherbaria.org/portal/) were also examined and a taxon assigned to each (Appendix). These records were combined with those from the current study and the potential ranges mapped (Figure 2) using the program QGIS (QGIS Development Team 2021).

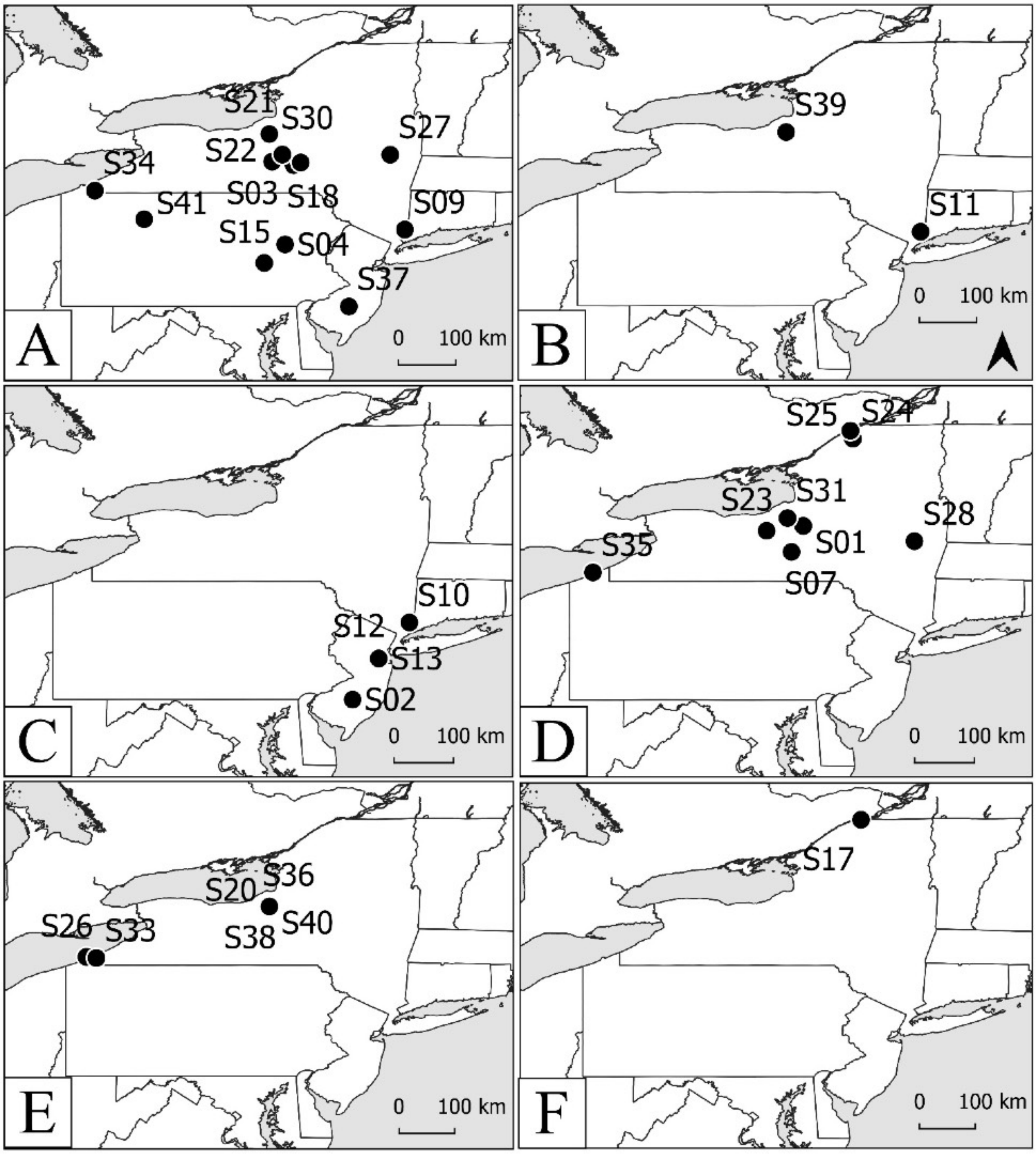


Figure 1. Map of study area including locations and sample names. A. *Spiranthes arcisepala*. B. *Spiranthes ochroleuca*. C. *Spiranthes cernua*. D. *Spiranthes incurva*. E. *Spiranthes sheviakii*. F. *Spiranthes magnicamporum*. Location of *S. odorata* not shown (see McMullen et al. 2021).

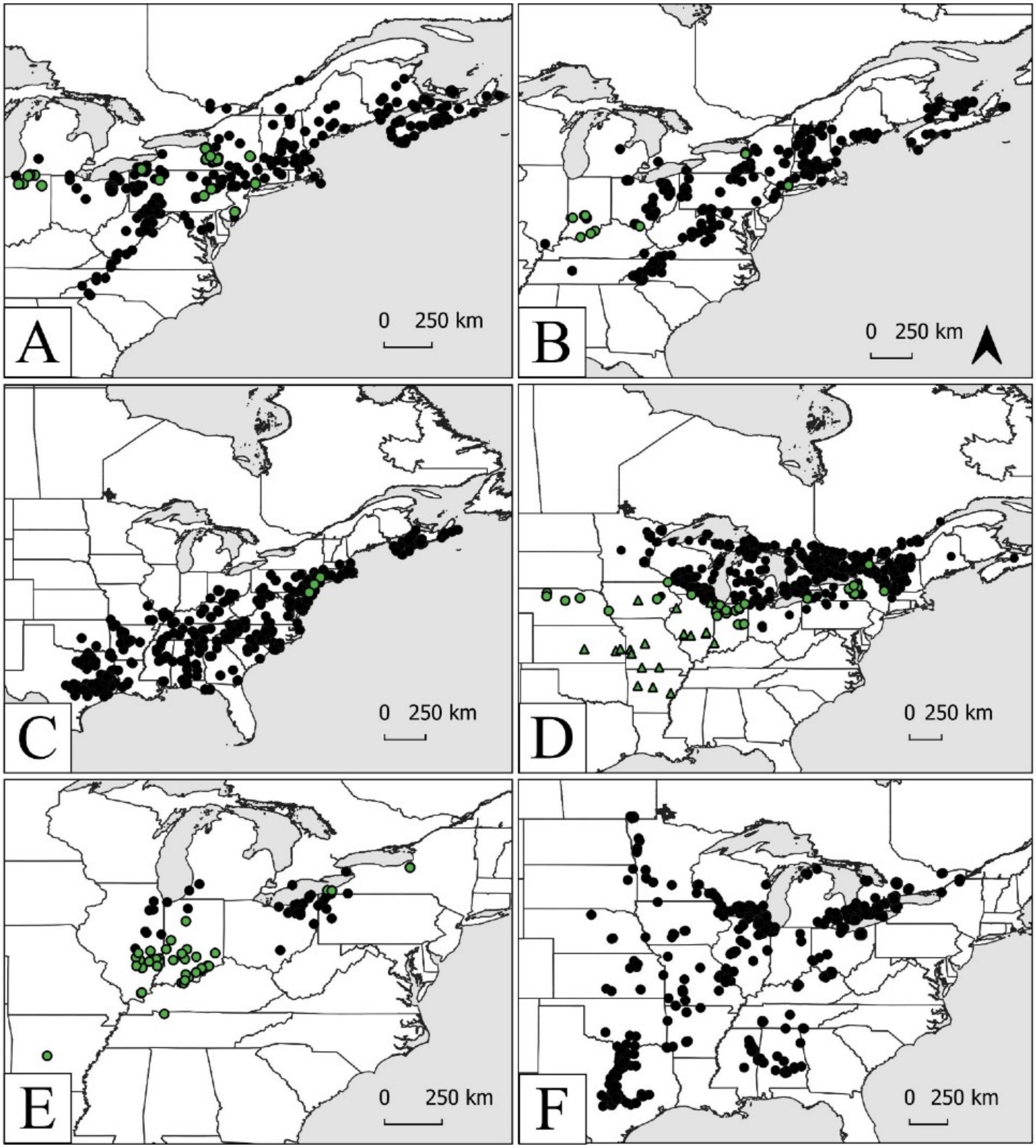


Figure 2. Estimated range maps based on review of iNaturalist observations, examination of specimen photos, and collection locations from this study. It is possible that a given taxon may occur outside of the range indicated on the map. A. *Spiranthes arcisepala*. B. *Spiranthes ochroleuca*. C. *Spiranthes cernua*. D. *Spiranthes incurva* (circles) and southern prairie complex (triangles). E. *Spiranthes sheviakii*. F. *Spiranthes magnicamporum*. Symbols represent specific locations, though some *i*Naturalist data is obscured. Black symbols indicate iNaturalist observations and green symbols indicate vouchered specimens (see Appendix).

Molecular techniques

Genomic DNA samples were obtained from bracts, leaves, or unopened flowers of dried specimens. Approximately 20 mg of dried tissue from one plant per sample was frozen in liquid nitrogen, ground with a mini pestle in 1.5 µl microfuge tubes, and the DNA extracted using a DNeasy Plant Mini Kit (Qiagen, Valencia, California, USA).

Two DNA regions that represent the nuclear and plastid genomes were selected from Pace (2015) to best capture sequence divergence at the interspecific level for the taxa of interest. Samples were amplified using PCR for the low-copy nuclear region ACO (*ACC* oxidase enzyme) following Guo et al. (2012) and for the chloroplast region ndhJ–trnL following Shaw et al. (2007). The PCR products were cleaned using a GeneJET PCR Purification Kit (Thermo Scientific). For ndhJ–trnL, an initial denaturation of 30 second at 94° C was followed by 35 cycles of 30 seconds at 94° C, 1 minute at 55° C, and 1 minute 10 seconds at 72° C, with a final extension of 5 minutes at 68° C.

For ACO, an initial denaturation of 4 minutes at 70° C was followed by 4 cycles of 2 min at 94° C, 30 seconds 57° C, and 5 minutes at 68° C, followed by 36 cycles of 30 seconds at 94° C, 30 seconds at 60° C, and 5 minutes 68° C, with a final extension of 15 minutes at 68° C. This protocol results in a large quantity of the target (~1100 bp) and several extra fainter bands (~1,500+ bp) when visualized on a gel. The target was either gel extracted and purified, or the PCR product purified and diluted to the minimum recommended for sequencing with the sequencing primers. The latter worked well for getting a clean sequence with little or no background noise. Initially a standard PCR protocol was attempted with the sequencing primers but amplification was very poor. We suspect this is because the forward sequencing primer is degenerate and the melting temperatures of the two primers differ considerably. We did not attempt to determine what was causing the extra bands on the gel but they were present for all samples so we suspect this was not contamination but rather the result of suboptimal priming. The primers were designed for a different group of orchids, however in the original paper (Guo et al. 2002) the product was also gel purified suggesting this is a common issue.

Cleaned PCR products were sent to Cornell's Biotechnology Resource Center for sequencing on an ABI 3730xl genetic analyzer (Applied Biosystems, Foster City, California, USA). Phylogenetic analyses incorporated and expanded on the datasets of Dueck et al. (2014) and Pace & Cameron (2017) available through GenBank (NCBI 2019). Alignment of sequences was performed using MUSCLE in MEGA X (Sudhir et al. 2018) and phylogenetic analyses performed using maximum likelihood in RaxML v8.2.4 (Stamatakis 2014). *Spiranthes odorata* was used as the outgroup.

For sites with more than one peak the standard IUPAC codes were used (i.e. an A and G would be coded as R). To aid comparison of electropherograms and for comparison to previously published contigs, the raw reads were aligned in Geneious 10.1.3 (https://www.geneious.com). For the final phylogenetic analyses, the publicly available GenBank sequences (NCBI 2019) for *S. incurva* and *S. arcisepala* were not included because visual inspection of the chromatograms revealed numerous intra-individual site polymorphisms in the ACO traces (i.e. two peaks at a given locus) that are not present in the published sequences from previous studies (Dueck et al 2014; Pace and Cameron 2017), particularly those of *S. arcisepala*. Maximum likelihood was used in RaxML because this software package treats IUPAC codes of DNA base combinations as polymorphisms (e.g. Y represents C or T), whereas other commonly used phylogenetic software packages (BEAST, MrBayes, PAUP*) treat base combinations as uncertain characters or missing data, resulting in these characters being ignored (Potts et al. 2014).

Maximum likelihood bootstrap analyses were performed on all three data sets (nuclear, plastid, and combined) using RaxML as 1000 bootstrap replicates with the GTR + G model under the thorough bootstrap method. Although the resulting nuclear and plastid trees were in strong conflict, and there are good arguments for keeping them separate, we nevertheless performed ML analysis on the combined data in order to see whether a dominant signal might emerge in the phylogenetic reconstruction. To better visualize instances of possible hybridization or introgression, the nuclear and combined (nuclear + plastid) datasets were used to construct individual phylogenetic networks in the program SplitsTree5 (Huson and Bryant 2006) using the Neighbor-Net algorithm.

Results

Examination of seeds supported the finding of previous studies that *S. magnicamporum*, *S. ochroleuca* and *S. odorata* are monoembryonic, while the polyploid members of *S. cernua s.l.* are polyembryonic (Sheviak 1982). The latter includes *S. arcisepala, S. bightensis* M.C. Pace*, S. cernua*, *S. incurva*, and *S. sheviakii* (Figure 3).

Among the species included in this study, the most notable floral differences were with respect to the shape of the labellum, size and orientation of basal callosities, abaxial labellum glands, thickness of the labellum, and the shape, color, and position of the floral bracts (Figures 10, 11, 15). These are explained in detail in the taxonomic treatment and the discussion.

We were only expecting to observe multiple base pair ambiguities in the nuclear electropherograms for the samples of *S. incurva* and possible hybrid taxa such as *S. ×kapnosperia*, however these were found in the samples of *S. arcisepala* and several samples of *S. cernua* as well (Figure 4). When we aligned our electropherograms for *S. arcisepala* with the ACO contigs of Pace and Cameron (2017), we found that the traces had numerous sites with double peaks not reflected in the publicly available contigs deposited in Genbank (NCIB 2020) from previous studies (Figure 5).

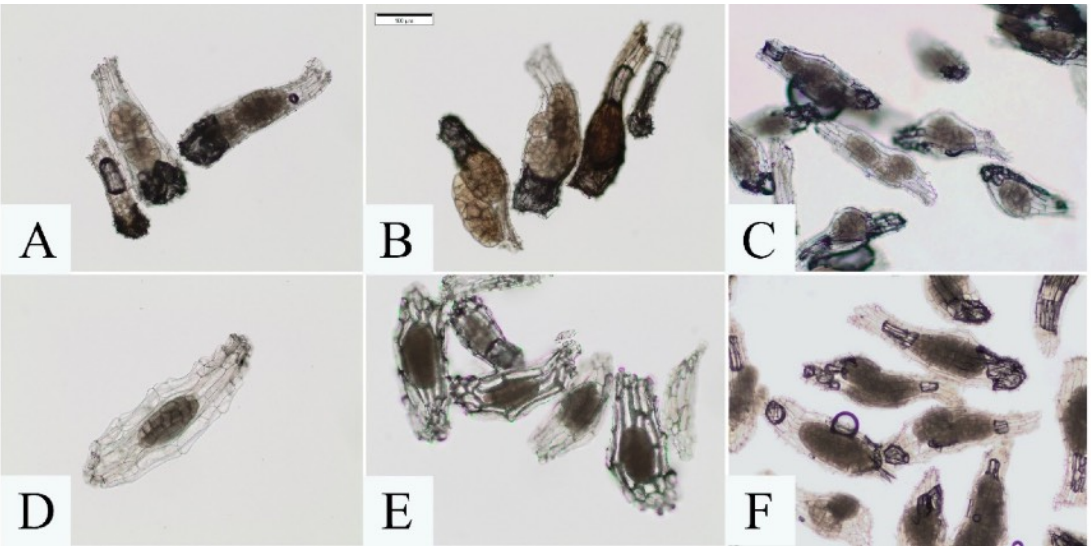


Figure 3. Seeds. A. *Spiranthes arcisepala*, Chenango Co., New York. B. *Spiranthes incurva*, Chenango Co., New York. C. *Spiranthes cernua*, Westchester Co., New York, sample S10. D. *Spiranthes odorata*, Onondaga Co., New York, sample S32. E. *Spiranthes ochroleuca*, Tompkins Co., New York. F. *Spiranthes sheviakii*, Onondaga Co., New York, sample S20.

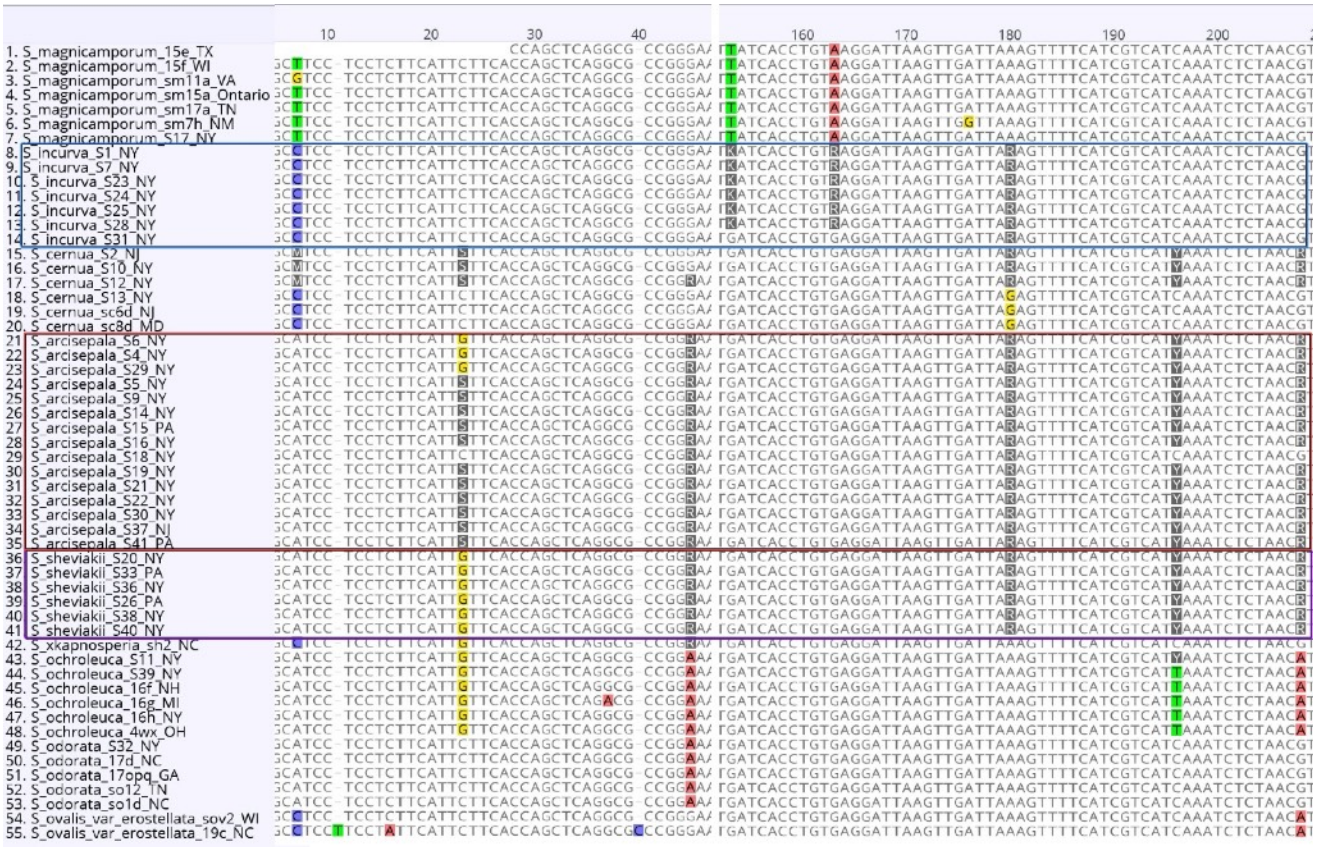


Figure 4 Example portions of ACO nucleotide alignment showing ambiguities (nucleotides in gray) suggesting a hybrid event involving *S. magnicamporum* (*S. incurva*), in addition to hybridization events involving *S. ochroleuca* and/or its descendants (*S. arcisepala*, possibly some *S. cernua*, and *S. sheviakii*). Taxa outlined are *S. incurva* (blue), *S. arcisepala* (red), and *S. sheviakii* (purple).

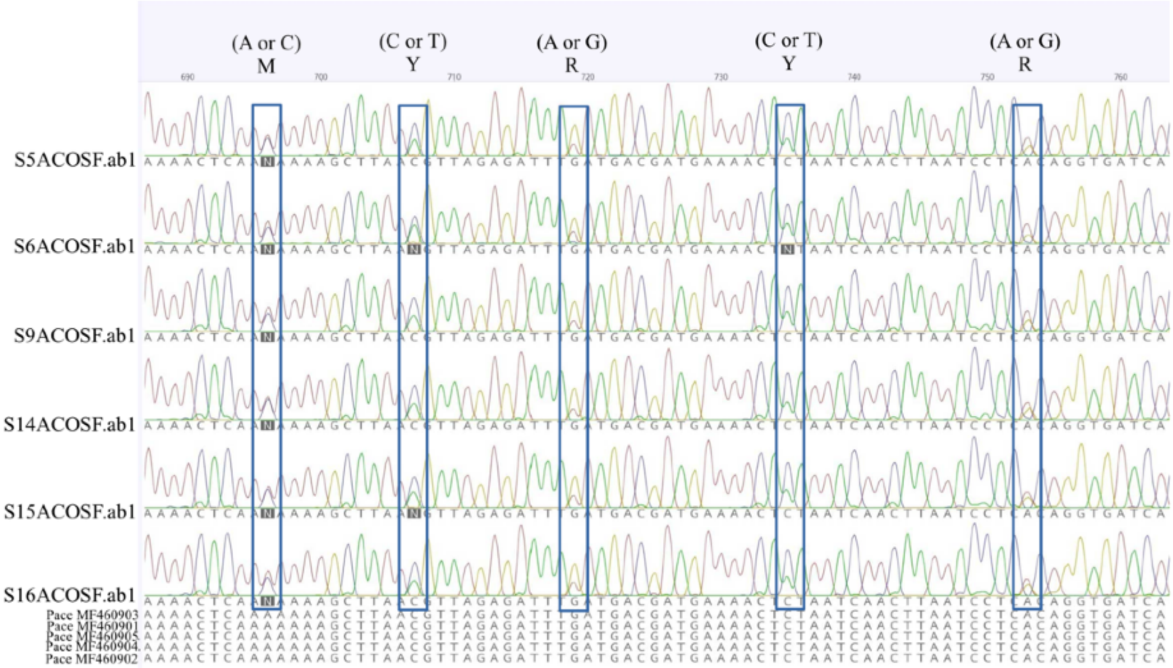


Figure 5. Electropherograms (.ab1) for specimens of *S. arcisepala* obtained from this study for the ACO nuclear region aligned with contigs from Pace and Cameron (2017) obtained from Genbank. Polymorphic sites are outlined in dark blue and standard IUPAC nucleotide codes indicated by multiple peaks are noted above.

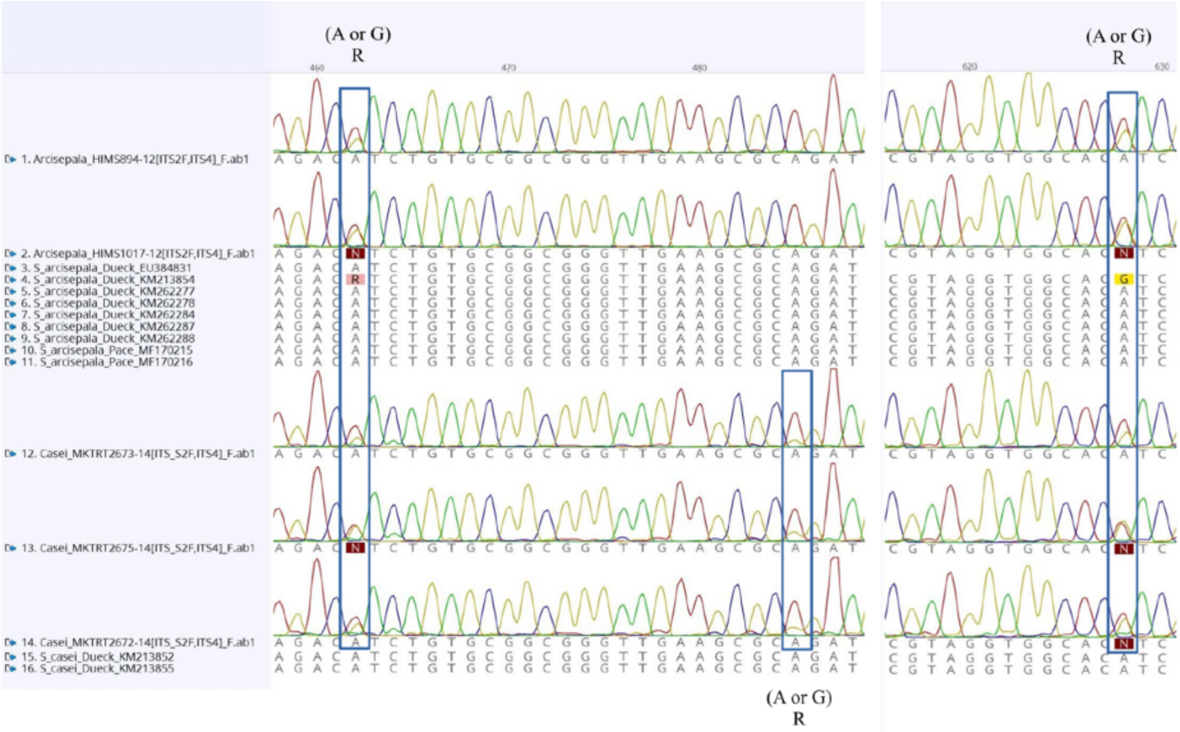


Figure 6. Publicly available nrITS electropherograms (.ab1) obtained from Bold systems (https://www.boldsystems.org) for *S. arcisepala* (as *S. cernua*) and *S. casei* aligned with contigs from Dueck et al. (2014) and Pace and Cameron (2017) obtained from Genbank. Polymorphic sites are outlined in dark blue and standard IUPAC nucleotide codes for multiple peaks indicated above or below regions of similarity.

When we compared publicly available nrITS electropherograms (BOLD 2020) for *S. arcisepala* and *S. casei* Catling & Cruise (determined by examination of specimen photos) to the contigs from Dueck et al. (2014) available on GenBank (NCBI 2019), we found similar differences (Figure 6). The bases exhibited by these two species suggest an allopolyploid origin for both *S. arcisepala* and *S. casei* involving possible ancient hybridization of another species (possibly *S. cernua*) and *S. ochroleuca* (Figure 6). We cannot rule out reticulate evolution based on the methods employed; cloning could yield more accurate determination of homologs that could improve resolution of potential parents. We reached out to Dueck to request nrITS electropherograms but were told that they had likely been discarded. We also requested forward and reverse electropherograms from one of Pace's samples of *S. arcisepala* but did not receive a response. Therefore, we did not include contigs for the polyploid taxa from Dueck et al. (2014) or Pace and Cameron (2017) with the exception of *S. cernua*, which in our data exhibited fewer polymorphic peaks on electropherograms. We suspect the inclusion of these samples

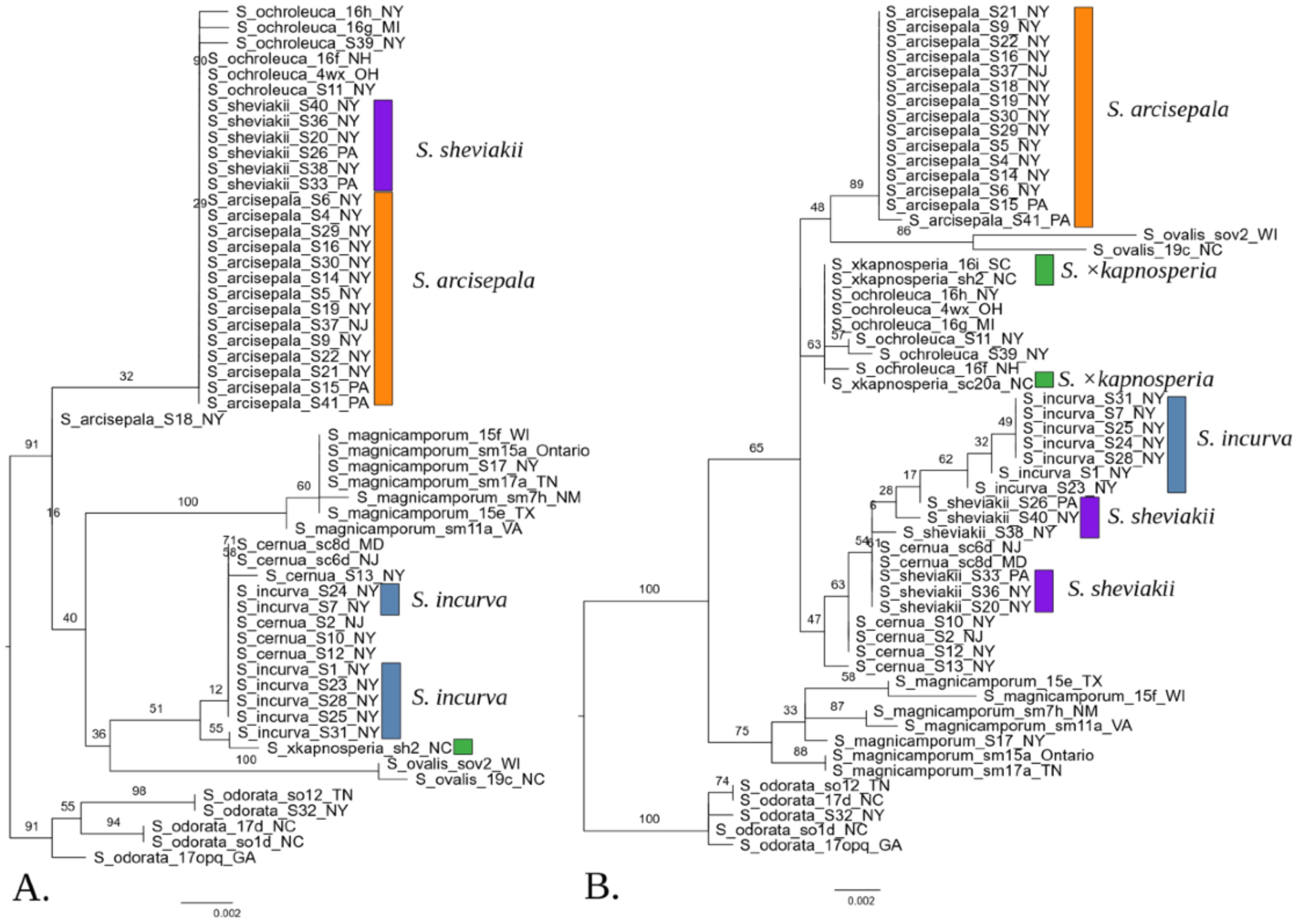


Figure 7. Maximum likelihood phylogenetic tree reconstructions. Branch labels are bootstrap support values. Hybrid taxa highlighted to illustrate discordant positions. A. Phylogenetic tree derived from ACO nuclear data. B. Phylogenetic tree based on ndhJ–trnL chloroplast data.

influenced the results to some extent and could be the cause of the discordant positions of *S. cernua* in the phylogenetic trees (Figure 7) and networks (Figure 8), though it is also possible that *S. cernua* itself is the product of past hybridization events.

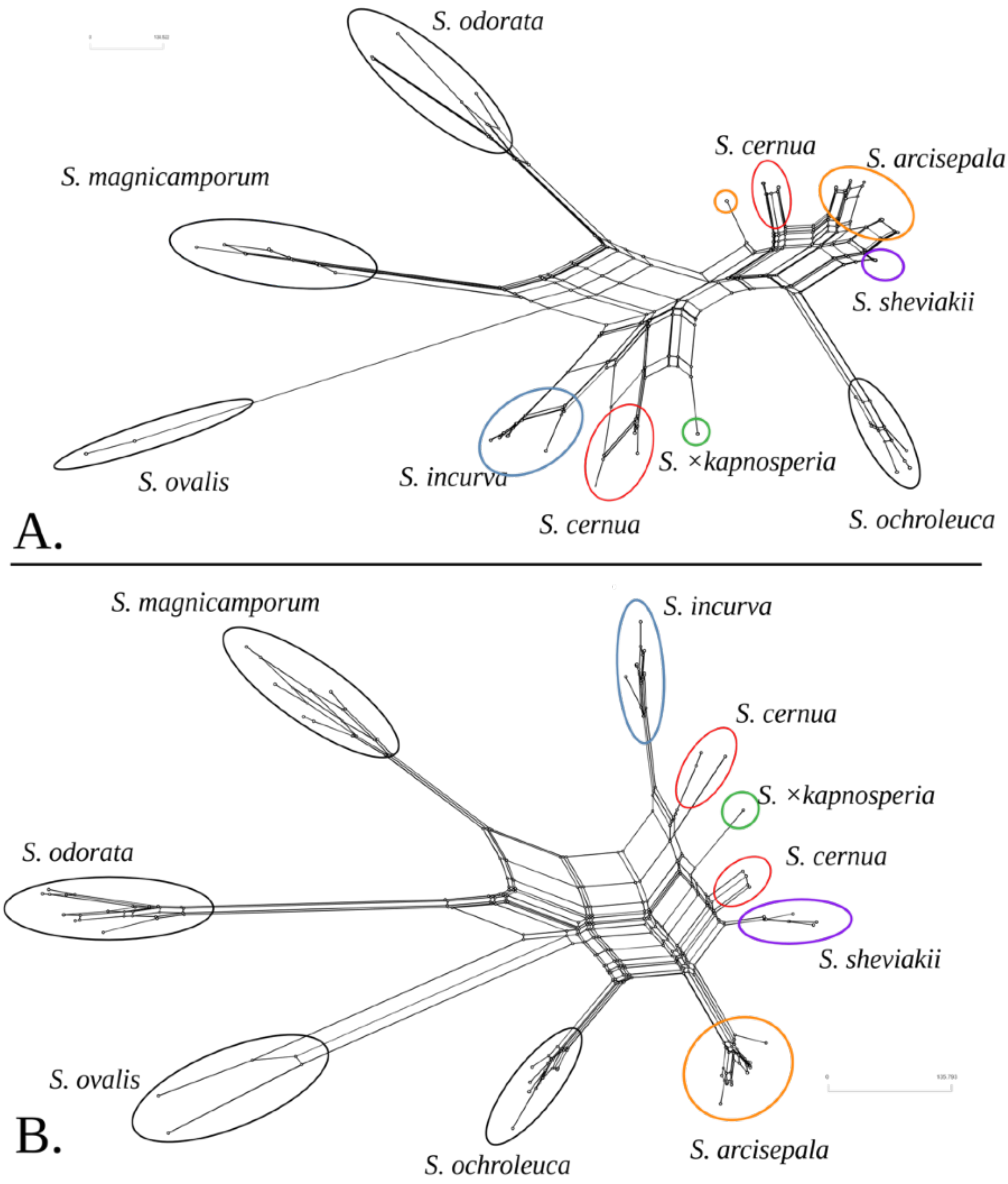


Figure 8. Phylogenetic networks from NeighborNet analysis of the *S. cernua* species complex and *S. odorata*. Hybrid taxa highlighted using the same colors as Figures 8 & 9 with the addition of *S. cernua* in red showing unresolved discordant positions. A. Network produced from nuclear dataset (ACO). B. Network produced from combined chloroplast and nuclear datasets (ACO + ndhJ–trnL).

Of the 18 samples that we originally determined to be *S. incurva* or *S. cernua* (based on upward sweeping lateral sepals and the locations where they were collected) in 2018 and 2019, 12 of them were found to represent *S. arcisepala* through genetic analysis. Two others (one from the interior of Presque Isle, Pennsylvania and the other from a site in central New York) were initially determined to be *S. cernua* × *S. ochroleuca* because they grouped with *S. arcisepala* and *S. ochroleuca* in the nuclear tree (Figure 7A) but grouped with *S. cernua* in the plastid tree (Figure 7B). Additional collections were made in 2020 based on revised morphological concepts of these species derived from photographs of the initial samples, including a sample from Gull Point on Presque Isle and another site about one mile to the south of this location. The genetic identities of these samples were as expected based on floral morphology, with the sample from Gull Point determined to be *S. incurva* and the other sample to be likely *S. cernua* × *S. ochroleuca* (though *S. cernua* was also considered a possibility).

Additional plants recovered as putative *S. cernua* × *S. ochroleuca* were found over an area extending one mile north-south at the site in north central New York, and ca. 350 km further west at two locations in northwestern Pennsylvania, also separated by a distance of about one mile. Although the plants in New York were found co-occurring with *S. ochroleuca*, we were unable to locate a plant corresponding to *S. cernua* in the same area, despite repeated searches of the area and testing of multiple plants, and neither of the two putative parents are present at the other two sites in Pennsylvania. The abundance of plants at these sites suggests that they are not the result of ongoing hybridization, but rather represent an independent, self-perpetuating lineage. They differ genetically from *S.* ×*kapnosperia* in that the maternal parent (plastid donor) is apparently *S. cernua* rather than *S. ochroleuca* (Figure 7B) which suggests they are the product of a different hybridization event. The only other allopolyploid species within the complex where *S. cernua* has been determined to be the maternal parent are *S. incurva* and the more recently described *S. bightensis* M.C. Pace, and these samples clearly do not represent the former taxon (Figures 4, 7, 8, 9. *Spiranthes bightensis* is not a likely identification because it is a more robust plant with stoloniferous roots that is the product of hybridization with *S. odorata* (Pace 2021) rather than *S. ochroleuca*.

When the plastid and nuclear data are combined, these specimens (shown in purple) form a distinct clade positioned between *S. cernua* and *S. incurva* (Figure 9). These plants also form a distinct branch in the combined network analysis (Figure 8B), and a position far removed from *S. incurva* and *S.* ×*kapnosperia* in the network generated from the nuclear dataset (Figure 8A). We believe that these genetic differences, together with differences in morphology, habitat, and an extensive geographic range distinct from that of *S.* ×*kapnosperia* based on a review of herbarium specimens and iNaturalist observations, are ample evidence to support that this is a self-perpetuating lineage representing a distinct allopolyploid species within the *Spiranthes cernua* species complex.

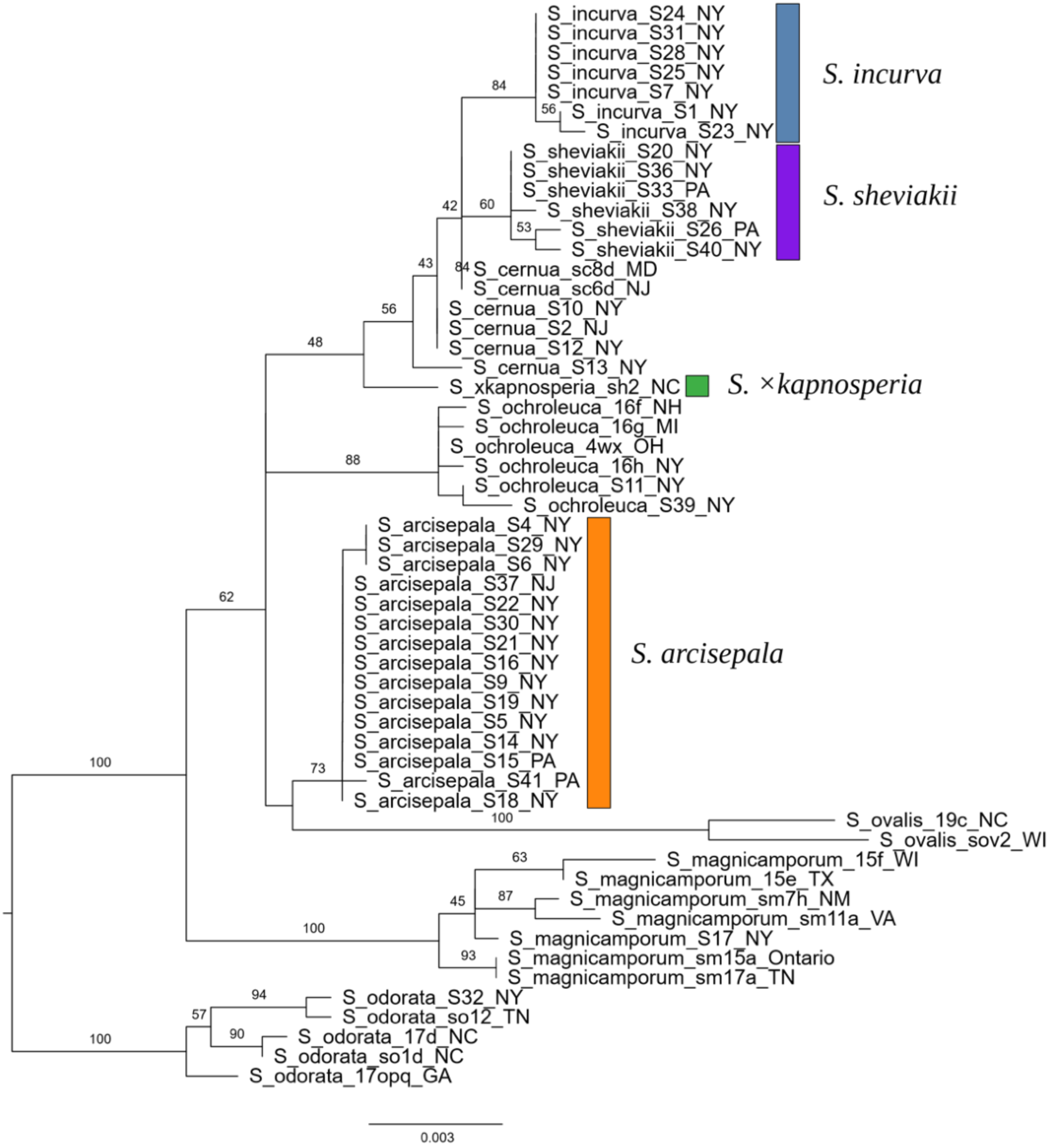


Figure 9. Maximum likelihood phylogenetic tree reconstruction based on combined chloroplast and nuclear data (ndhJ–trnL + ACO). Branch labels are bootstrap support values.

Taxonomic Treatment

Spiranthes sheviakii M. Hough & M.A. Young, sp. nov. Type: U.S.A. New York: Onondaga County, Town of Lysander, Three Rivers WMA, 19 September 2020, *M. Hough & M. Young s.n.* (holotype: BH; isotypes: CORT). Figures 10 and 11.

Diagnosis: *Spiranthes sheviakii* is similar to *S. cernua*, but differs in having a centrally yellow and thickened labellum with spherical abaxial glands (vs. centrally white and membranous, with reduced conical or flattened glands) and preference for dry habitats (vs. wet habitats). The overall color of the flowers appears white but is creamier when compared side-by-side with those of *S. cernua*, *S. arcisepala*, and *S. incurva*. *Spiranthes sheviakii* can be distinguished from *S. ×kapnosperia and S. ochroleuca* by its straight lateral sepals that frequently curve inward at the tips over the top of the flower (vs. straight to somewhat falcate and not incurved at the tips), flowers that typically nod (vs. spreading or slightly ascending), and moderately gaping flowers (vs. not or only slightly gaping). It also has longer lateral sepals (9.3–11.4 mm) than *S. ×kapnosperia* (7.6–10.0 mm), and basal calli that are longer (0.7–1.4 mm) than described for *S. ×kapnosperia* (0.6–1 mm). Like *S. cernua* and *S. ochroleuca*, the labellum is less dilated at the base than it is in *S. incurva* (excluding cleistogamous and peloric forms) and *S. arcisepala*. The leaves of *S. sheviakii* are highly variable and sometimes reduced or senescing prior to anthesis, particularly in plants occurring in open habitats. However, plants in the southern portion of its range growing in upland woods often exhibit larger, sometimes flaccidly spreading basal leaves. The flowers are moderately fragrant, with an odor similar to that of *S. ochroleuca*.

Description: Plants 15-50 cm. Roots few, slender, horizontally spreading to descending, 2–6 mm diameter. Leaves 1–4(–6), erect spreading to flaccidly spreading, persisting until after anthesis or browning shortly before, linear-lanceolate to oblanceolate, rarely obovate, 7–23 cm long, the widest blade to 1–2 cm wide; petiole of lower leaves often slender. Spikes tightly spiraled, 3–4 flowers per cycle, usually not evenly ranked; rachis moderately to densely pubescent with stalked glands. Flowers nearly white to ivory, slightly to strongly nodding, moderately gaping; floral bracts green, stipitate glandular on the abaxial surface and margins, 6–13 mm long, concave, acuminate, incurved over the base of the flower; sepals distinct to the base; lateral sepals 9–11.5 mm × 2–3 mm, appressed, ±straight in profile, the apices typically incurved over the top of the dorsal sepal and petals; dorsal sepal and petals moderately to densely stipitate glandular on the outer surface, linear-lanceolate, 9–11.5 mm, recurved and obtuse at the apex; labellum centrally pale yellow or orange-yellow, oblong or ovate in general outline, slightly constricted in the middle, not or scarcely dilated at the base, 10.0–14.5 × 3.5–6.0 mm, abaxial glands spherical, margins lacerate above the middle, apex obtuse to acute, glabrous; veins several, branches parallel; basal calli usually prominent, 0.7–1.4 mm; viscidia linear; ovary moderately to densely pubescent with stipitate glands, 4.8–6.3 mm. Seeds mostly polyembryonic but some monoembryonic present. 2n=60.

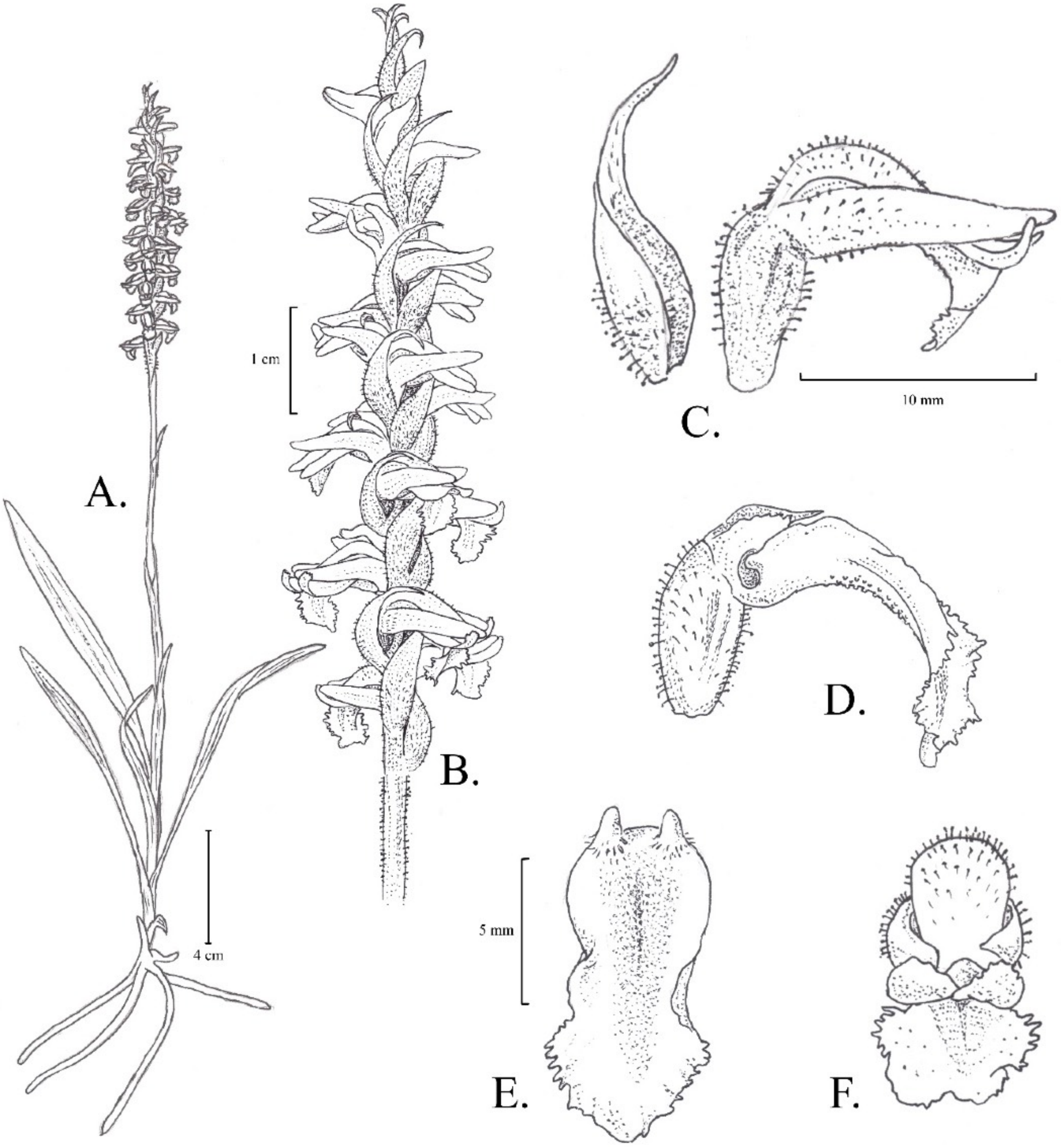


Figure 10. *Spiranthes sheviakii*. A. Habit. B. Inflorescence. C. Profile of flower and floral bract. D. Profile of labellum, column, and ovary. E. Labellum. F. Front view of flower in natural position. Drawn from photographs of living samples S20, S36, S38, S40 by Michael Hough. The habit drawing is a representative composite of these and specimens *Deam 32628*, *Phillippe 1082*, *Ebinger 20709*, and *Ebinger 15567* (EIU).

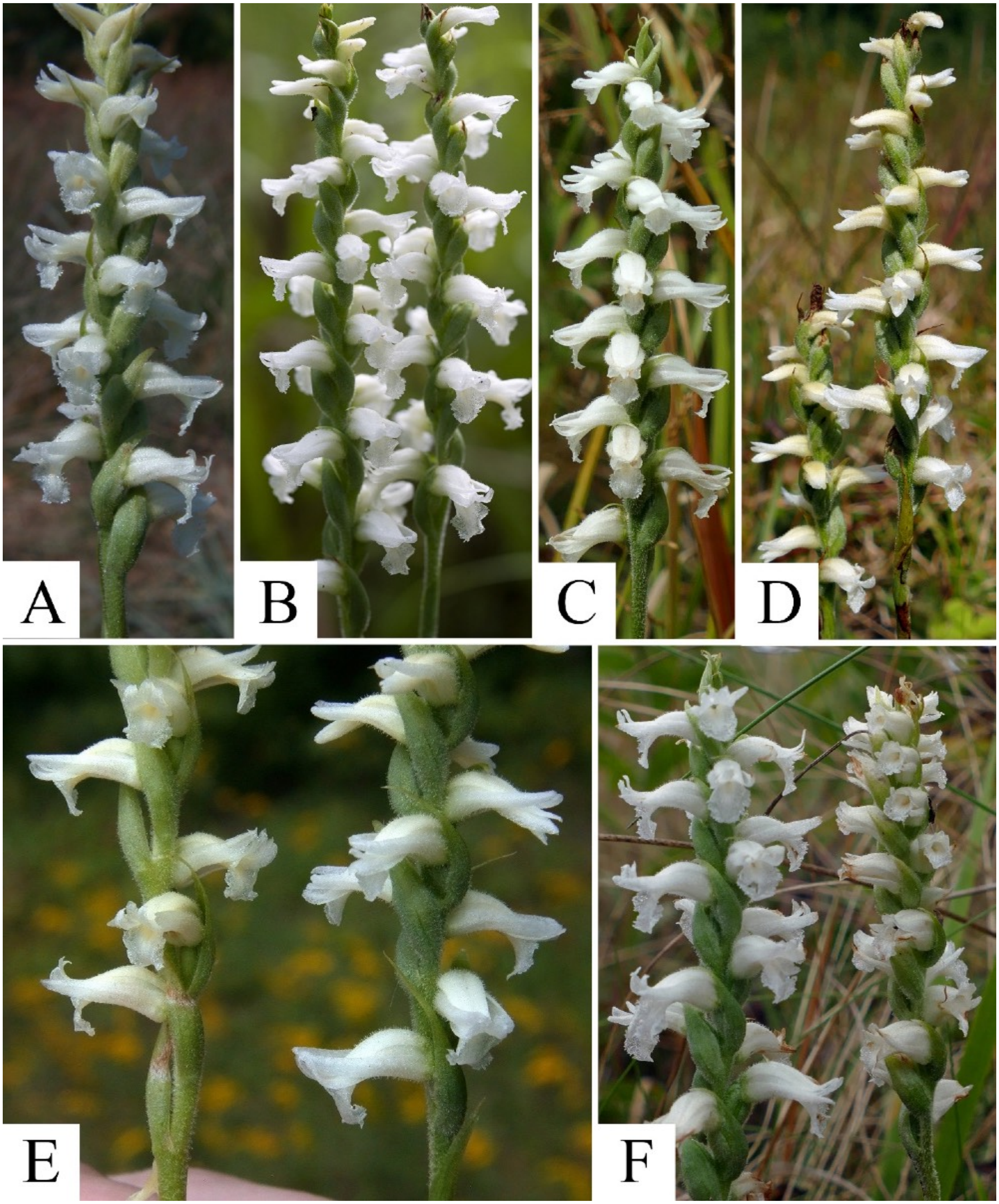


Figure 11. *Spiranthes sheviakii*. A. Presque Isle, Erie Co., Pennsylvania, sample S26. B. Erie Co. Pennsylvania, sample S33, photo by Greg Funka. C. Onondaga Co., New York, sample S36. D. Onondaga Co., New York, sample S38. E. Onondaga Co., New York, sample S20 (right) with co-occurring *S. ochroleuca* (left). F. Onondaga Co., New York, sample S40 (left) with co-occurring *S. ochroleuca*, sample S39 (right).

Etymology: *Spiranthes sheviakii* is named in honor of Charles J. Sheviak, former Curator of Botany at the New York State Museum who, recognizing its unique morphology, range, and habitat preference, originally described this species as the 'old field ecotype' of *S. cernua* s.l. (Sheviak 1982). A suggested common name is "old field ladies' tresses".

Distribution and Habitat: Southeastern corner of Lake Ontario in New York; Lake Erie region in northern Ohio, northwestern Pennsylvania, western New York, and southern Ontario; a few interior sites in Ohio; scattered locations across the sandplains near Lake Michigan from southwestern Michigan to northwestern Indiana, and southward into north-central Illinois; increasing in abundance near the Ohio River valley in southern Illinois and Indiana; range likely extending south into western Kentucky, western Tennessee, and northern Arkansas where it would strongly overlap the range of *S. cernua*.

This species is a colonizer of habitats similar to those of *S. ochroleuca* and sometimes occurring with it; acidic, dry to mesic successional habitats, open barrens, old fields, and thickets undergoing succession to oak-hickory and mixed hardwood-pine forests. It also occurs in dry open woodland on bluff crowns and ravine rims in the southern portion of its range. Soils typically sandy, silty, or clayey.

Phenology: Mid-September to early-October in the northern portion of its range; late-September to late-October in the southern portion of its range.

Discussion

Sheviak considered that his 'old field ecotype' (described here as *S. sheviakii*) could be equivalent to *S. petiolaris* Raf. given that the latter was presumably collected in southern Illinois, though the location was only given as Illinois. The absence of any type specimens, illustration, habitat description, collection date, or a specific location for *S. petiolaris* makes placement of this name with confidence impossible. It is likely that *S. cernua* and *S. ochroleuca*, of which *S. sheviakii* is intermediate in form, occur in southern Illinois presently or at least historically, and Rafinsque's description of *S. petiolaris* is general enough to be applied to these species as well, particularly the description of the flowers as "white as in all Sp.". While the overall appearance of the flowers of *S. sheviakii* is white, they appear off-white or ivory in comparison to *S. cernua*, *S. incurva*, or *S. arcisepala* when observed alongside them, and the labellum is centrally slightly to moderately yellow or orange-yellow. While the flowers of *S. ochroleuca* are more or less ivory in color, they are often overlooked as being white in the field, so it is possible that *S. petiolaris* could have been the same as *S. ochroleuca*.

Given the important role that allopolyploidy has played in the evolution of North American *Spiranthes* (Sheviak 1982; Arft and Ranker 1998; Dueck et al. 2014; Pace and Cameron 2017), understanding hybrid ancestry could prove useful for identifying morphological differences among closely related taxa. *Spiranthes incurva* exhibits several traits in common with *S. magnicamporum*, while *S. arcisepala* and *S. sheviakii* exhibit several traits in common with *S. ochroleuca*. These traits tend to be difficult to quantify, and differentiation requires familiarity with the species that we were only able to acquire through genetic analysis and extensive field work.

Our results support the conclusion of Pace and Cameron (2017) that *S. incurva* is an allopolyploid involving *S. magnicamporum* as one of the parents and possibly *S. cernua* as the other. This species corresponds with the "midwestern subclade" of Dueck et al. (2014), the "sand-prairie ecotype" of Sheviak (1974), and some of the New England races of Sheviak (1982, Figure 18 e-l). Although Homoya (1993) referenced Sheviak's sand prairie ecotype, his photo from Lagrange County, Indiana does not appear to be *S. incurva* and is more likely *S. sheviakii*. Sheviak (1982) described the habitat of the sand-prairie ecotype as "wet to wet-mesic" which comports with our observations of *S. incurva* in New York. At least within the range of this study, we have not observed *S. incurva* growing in xeric sites. The typical habitats appear to be mostly moist to wet and mediacid to calcareous.

Like *S. magnicamporum*, *S. incurva* frequently has flowers arranged in the inflorescence in 3-4 distinct vertical ranks. In addition, the elongate labellum and frequently pointed apex of this structure is typical though somewhat variable. The labellum of *S. incurva*, as in *S. magnicamporum*, exhibits some yellow pigmentation and is noticeably thickened relative to *S. cernua*, though to a lesser degree than in *S. magnicamporum*. The basal callosities are often reduced in size relative to *S. cernua*, but not to the extent of *S. magnicamporum*.

The abaxial glands are usually prominent and spherical on the labellum of *S. magnicamporum*. This was also the case in the majority of samples of *S. incurva* we examined (Figure 12B), in contrast to the original description of *S. incurva* (Pace and Cameron 2017). Although our sample size of *S. cernua* was small, the plants examined mostly exhibited reduced abaxial labellum glands (Figure 12A) as described by Pace and Cameron (2017).

Some aspects of the floral bracts also appear to have been inherited by *S. incurva* from *S. magnicamporum*, and this characteristic appears to be quite useful for identifying many specimens of *S. incurva*. Although we found little mention of this characteristic by previous authors, white-tipped bracts are said to be characteristic of *S. parksii* Correll (Catling and McIntosh 1979). Sheviak (1982) also noted that, within the complex, the longest bracts occur in robust specimens of *S. magnicamporum* and some members of the 'prairie complex', the latter of which may be synonymous with *S. incurva* since Sheviak interpreted these population to have arisen through hybridization of *S. cernua* and *S. magnicamporum*. He also noted Rydberg's description of the bracts of *S. ochroleuca* as being unusually long, and agreed that they do tend to be longer than those of *S. cernua*, but not consistently so.

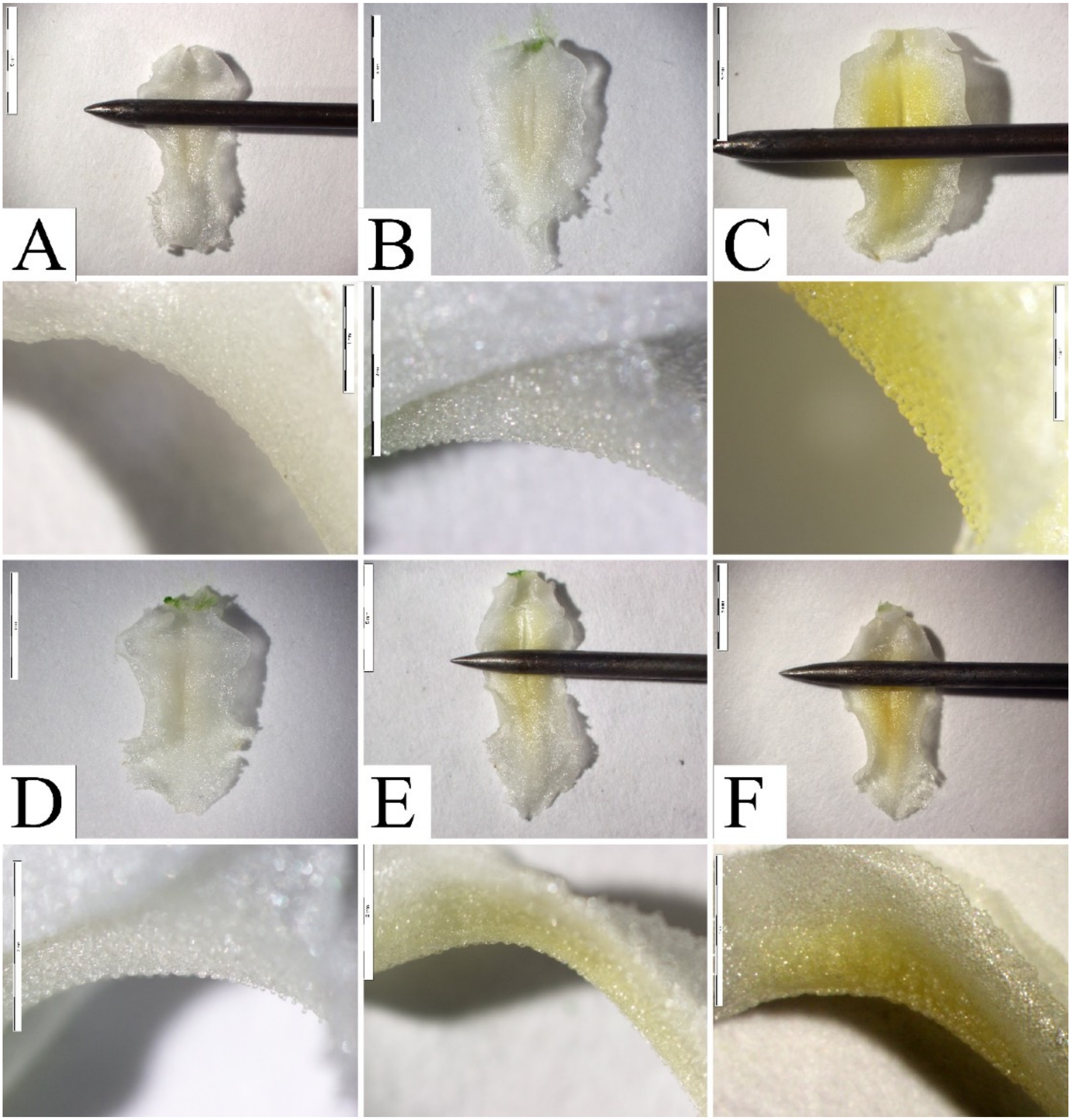


Figure 12. Representative examples of labellum and abaxial glands. A. *Spiranthes cernua*, Richmond Co., New York, sample S12. B. *Spiranthes incurva*, Tompkins Co., New York. C. *Spiranthes magnicamporum*, St. Lawrence Co., New York, sample S17 (note: left side of the base of lip is torn away). D. *Spiranthes arcisepala*, Chenango Co., New York. E. *Spiranthes sheviakii*, Onondaga Co., New York, sample S20. F. *Spiranthes ochroleuca*, Tompkins Co., New York.

In most members of the *S. cernua* species complex the floral bracts are strongly concave, wholly green, and taper abruptly to the apex, with the slender apex curving inward over the base of the flower (Figures 11, 13, 15, and 16B, D). In *S. magnicamporum* (and also *S. romanzoffiana* Cham.) these bracts tend to be less concave, longer, and taper more gradually to the apex and tend to be straighter or less strongly incurved above (Figure 13B). The bracts may also exhibit subtle hyaline margins in *S. magnicamporum* but are more often green. These traits are frequently exaggerated in *S. incurva* to an extent not seen in any other members of the complex except for some specimens of *S. diluvialis* Sheviak (an allopolyploid of *S. magnicamporum* and *S. romanzoffiana*). We have observed many populations of *S. incurva* where the upper bracts have prominent white hyaline margins, or are nearly wholly white, that are strongly spreading or even recurved (Figure 14A, C, E, F, H and Figure 16C). These plants are relatively easy to identify by the bracts alone, and combined with the multi-ranked arrangement of the flowers allows for the identification of some plants pre- and post-anthesis as long as the bracts have not senesced. Another common form of *S. incurva* exhibits bracts that are wholly green but are slenderer and taper gradually to the apex (Figure 16A), and these may be straight and spreading or erect and slightly incurved at the apex. This latter form is less obvious but noticeable when placed side-by-side with other members of the complex (Figure 16). Like *S. magnicamporum*, the flowers of *S. incurva* tend to be held parallel to the ground or be even slightly ascending, whereas those of *S. arcisepala*, *S. cernua*, and *S. sheviakii* are more often somewhat nodding, though not always reliably so.

Given the morphological differences between *S. cernua* and *S. incurva*, in addition to the mostly non-overlapping ranges, it is not difficult to separate these two species when all of the previously mentioned characteristics are considered. The lateral sepals of *S. incurva* tend to be straight throughout, while in *S. cernua* and *S. sheviakii* these are more likely to curve inward at the tips over the top of the flower (Figure 10C, F and Figure 11). This latter orientation may be due to the greater tendency of the flowers to nod in *S. cernua* and *S. sheviakii*, though the tips of the lateral sepals are not normally incurved in *S. arcisepala* which also frequently has flowers that nod.

Phenology also seems to differ considerably among northern members of the complex. In general, we have found that *S. incurva* is the first species to flower in New York, with most plants completely senesced by mid-September, however we did find one specimen of *S. incurva* in full bloom in early October at one site. In contrast, *S. cernua* and *S. sheviakii* seem to mostly begin flowering around mid-September and reach their peak in early October, with *S. cernua* continuing into November and *S. sheviakii* continuing into the latter part of October in the southern portions of their respective ranges. The flowering of *S. arcisepala* tends to fall in between, with flowering beginning in early September, reaching its peak in mid-September, and then falling off in early October.

Our results suggest that *S. arcisepala* is an allopolyploid derived from *S. ochroleuca* and another species that could be *S. cernua,* though the present data is not sufficient to say this with certainty (Figures 4 & 5). This is in contrast to the conclusion of Pace and Cameron (2017) that *S. arcisepala* represents “cryptic speciation in the absence of detectable hybridization events”. Examination of publicly available electropherograms obtained through the Barcode of Life Data System (BOLD 2020) provides supporting evidence for this as well as a likely hybrid origin for *S. casei* also involving *S. ochroleuca* or one of its descendants (Figure 6). This is not unexpected given that *S. arcisepala* and *S. casei* are closely related polyploids with polyembryonic seeds that are sister to the diploid *S. ochroleuca* which produces monoembryonic seeds (Sheviak 1982; Pace and Cameron 2017). In addition to being morphologically distinct, it is likely that *S. arcisepala* and *S. casei* are reproductively isolated given that, at the same latitude, *S. casei* flowers about 2 weeks earlier than *S. arcisepala* (as early as mid-August in central NY), and tends not to occur in the same habitats.

Spiranthes arcisepala corresponds with the “northeastern subclade” of Dueck et al. (2014 Figure 1A), some of the “New England” races of Sheviak (1982, Figures 18 a–d and 19 a–d), and the “fen ecotype” of Homoya (1993). The downwardly falcate lateral sepals of *S. arcisepala* are also sometimes exhibited by *S. ochroleuca* and often by *S. casei*. In these species this trait is variable and in many specimens of *S. arcisepala* it is not evidently expressed (Figure 15; see also Figure 1A in Dueck et al. 2014; Figures 13B, C, & E in Pace 2015, Ch. 3; Figures 11B, C in Pace and Cameron 2017). Like *S. ochroleuca*, the majority of specimens of *S. arcisepala* that we examined had prominent, spherical glands on the lower surface of the labellum (differing from the reduced, conical glands illustrated in Figure 10H of Pace and Cameron 2017), and the labellum was usually more rounded at the apex and slightly shorter relative to *S. incurva* (Figure 16). Both *S. arcisepala* and *S. ochroleuca* are less likely to have flowers arranged in distinct vertical ranks (i.e. multi-ranked) in small to moderately-sized individuals, though exceptionally robust specimens can exhibit this trait. In our experience the typical inflorescence of *S. arcisepala* is usually either single ranked (usually in small specimens, resembling the triploid form illustrated by Sheviak 1982 Figure 18 a–d) or not distinctly ranked (Figure 15, see also Sheviak Figure 19 a–d). The pioneering cytological work of Sheviak suggests that *S. arcisepala* and *S. incurva* are both represented by triploid and tetraploid races, so some morphological variation could be related to these differences in ploidy level.

The labellum of *S. arcisepala* tends to have only slight yellow pigmentation, though occasional, dwarfed plants growing in upland habitats have been observed to approach the pigmentation of *S. ochroleuca*. The labella of both species tend to be rather blunt at the apex. While this structure is typically relatively wider (relative to length) in *S. arcisepala* (Figure 12), a few exceptions have been observed. The callosities of *S. arcisepala* vary in length but are typically shorter (up to 1.3 mm) than those of *S. ochroleuca* which can be up to 2 mm long (Sheviak and Brown 2002) and are usually more strongly directed inward due to the more basally dilated and concave lip (Figure 12D). It should be noted, however, that the callosities may continue to elongate as the flower matures (Sheviak 1982), so their length is therefore variable and perhaps best observed on the larger flowers towards the base of the inflorescence. The labella of both species usually exhibit prominent abaxial glands (Figure 12D, F) except for rare cases of *S. arcisepala*. The latter case, of which we have only observed two examples (samples S15 and S41, both collected in eastern Pennsylvania), are nearly indistinguishable from *S. cernua* except that the callosities are a bit shorter (<1 mm) and, if not for the genetic analysis, would likely have been misidentified.

Spiranthes arcisepala is very similar to *S. cernua* when the former does not exhibit downwardly falcate sepals, a situation that we would describe as more than occasional. Both species frequently exhibit flowers that 'nod' (Figure 13C and Figure 15), especially on the lower portion of the spike and during the later period of anthesis. This poses a major challenge for identification given that the ranges of *S. arcisepala* and *S. cernua* overlap significantly throughout the Appalachian Highlands and along the Atlantic Coastal Plain from Maryland to the Canadian Maritimes (Figure 2A, C).

The flowers of *S. arcisepala* are more likely to appear inflated at the base relative to those of *S. cernua*, *S. incurva*, and *S. sheviakii*, similar to the flowers of *S. ochroleuca*. The flowers of *S. arcisepala* also tend to be more bell-shaped than *S. cernua* — Homoya's description of "a gaping throat" for his "fen ecotype" is an apt description of this. The labellum of *S. arcisepala* usually has prominent rounded abaxial glands, while those of *S. cernua* are usually noticeably reduced (though somewhat pronounced in the cleistogamous form we examined) and those of *S. incurva* are variable (Figure 12). In general, the fresh labellum of *S. arcisepala* is thicker and more concave than that of *S. cernua*, and thus more likely to split when flattened with a probe. The callosities of *S. cernua* can be larger (up to 2 mm long) and more parallel relative to those of *S. arcisepala* which tend to be directed inward. The labella of *S. cernua*, *S. ochroleuca*, and *S. sheviakii* tend to be relatively narrow and less dilated at the base than *S. arcisepala* and *S. incurva* (Figure 12), though some forms of *S. incurva* exhibit varying degrees of peloria and have a much narrower lip (e.g. Sheviak 1982 Figure 18 i–l and the triploid low prairie race Figure 20 a–d). The labellum of *S. incurva* is more likely to be pointed at the apex than that of *S. arcisepala*, and although the measurements overlap, the labellum of *S. incurva* is on average about 1 mm longer than that of *S. arcisepala*.

While the discovery of *S. odorata* in central New York has already been reported (McMullen et al. 2021), the addition of the ACO low-copy nuclear region indicates that this population is genetically most similar to a population in Tennessee (Figure 9). The genetic similarity to a relatively northern, inland, and disjunct population provides further evidence to support that the central New York population of *S. odorata* is native rather than introduced.

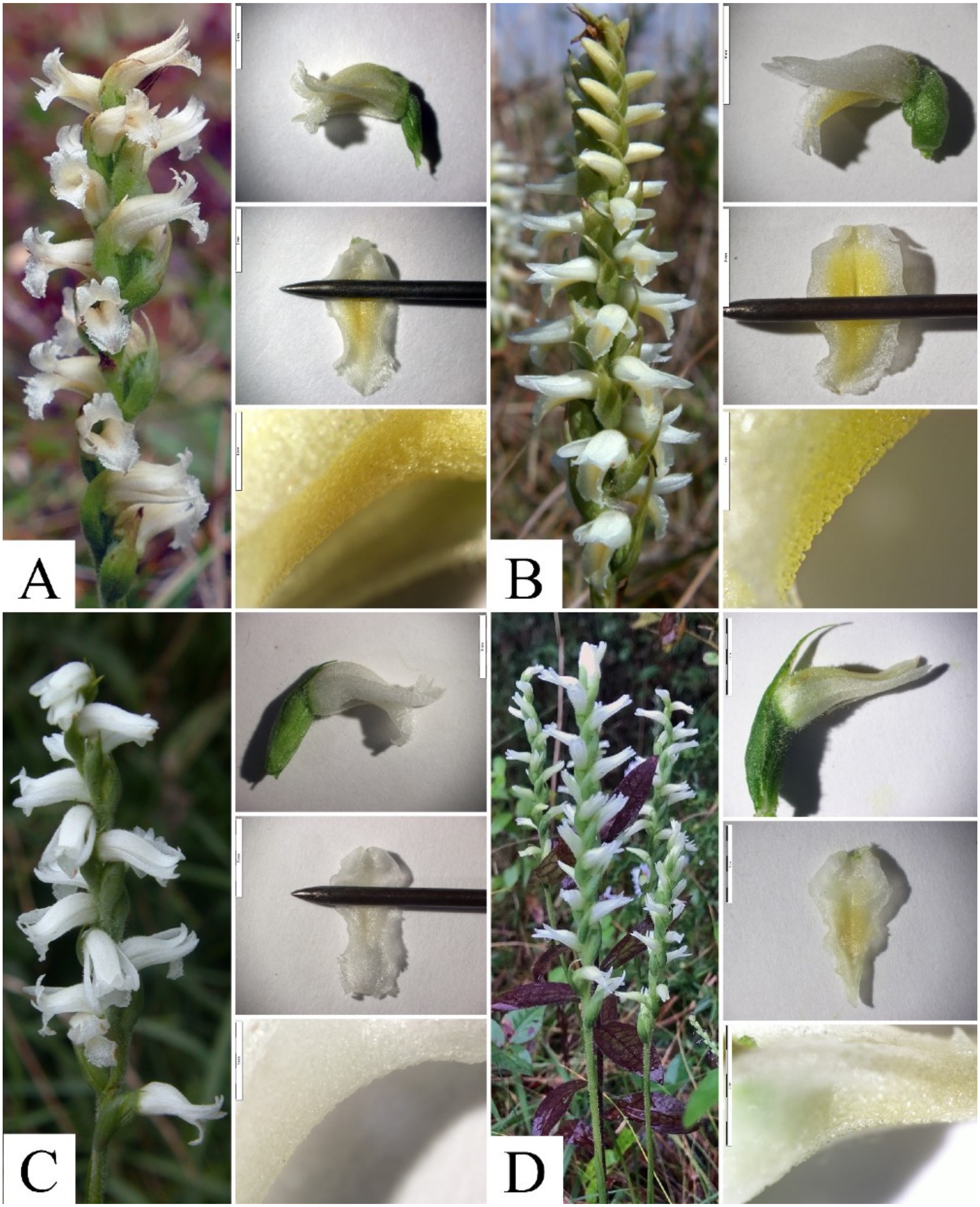


Figure 13. Diploid species and *S. cernua*. A. *Spiranthes ochroleuca*, Onondaga Co., New York, sample S39. B. *Spiranthes magnicamporum*, St. Lawrence Co., New York, sample S17. C. *Spiranthes cernua*, Richmond Co., New York, sample S12. D. *Spiranthes cernua* (cleistogamous form), Burlington Co., New Jersey, sample S8.

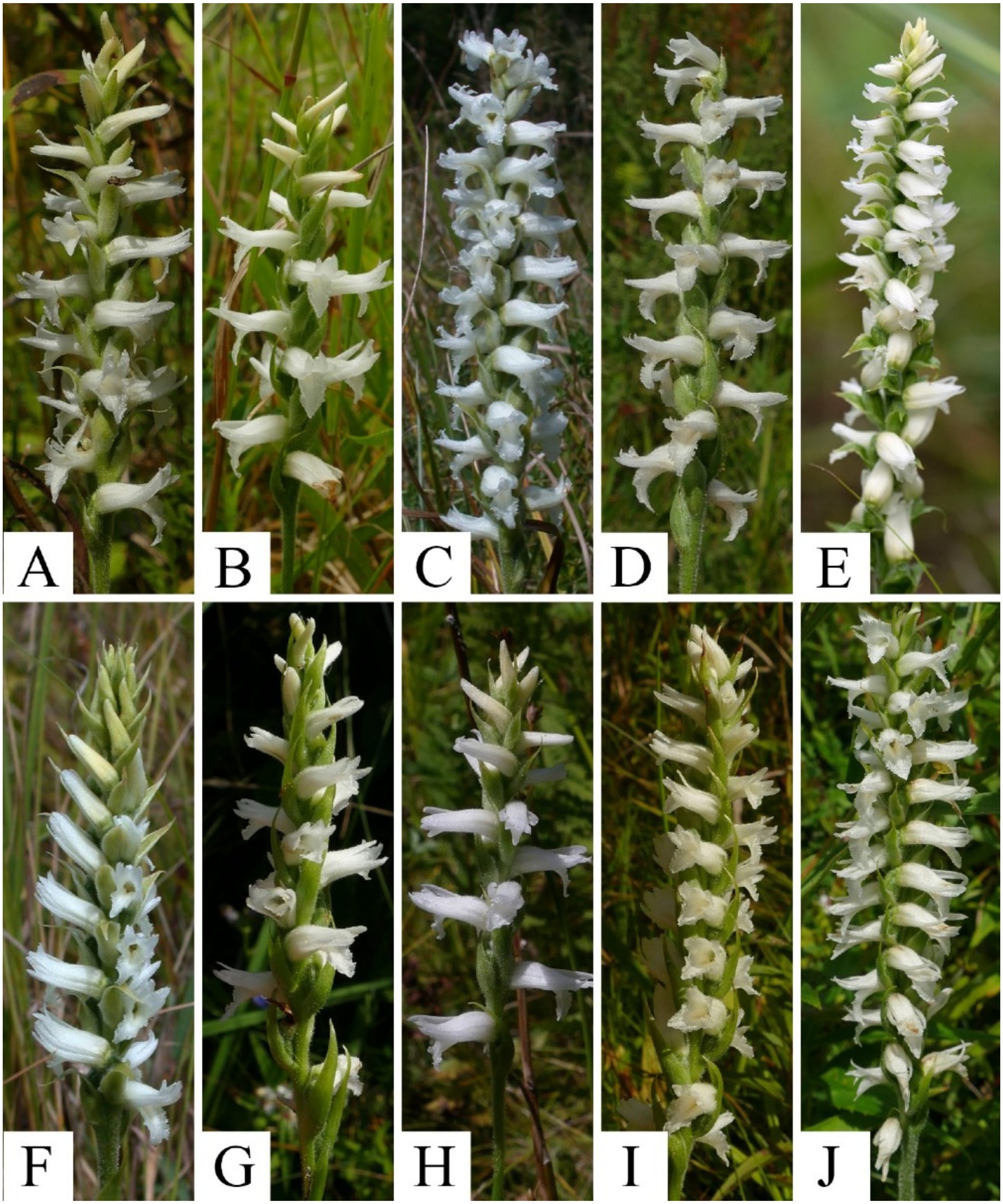


Figure 14. *Spiranthes incurva*. A. Onondaga Co., New York, sample S1. B. Cortland Co., New York, sample S7. C. Cayuga Co., New York, sample S23. D. Onondaga Co., New York, sample S31. E. Presque Isle, Erie Co., Pennsylvania (type location), sample S35, photo by Greg Funka. F. Onondaga Co., New York, site of sample S1. G. Madison Co., New York. H. Tompkins Co., New York. I. Chenango Co., New York. J. Onondaga Co., New York.

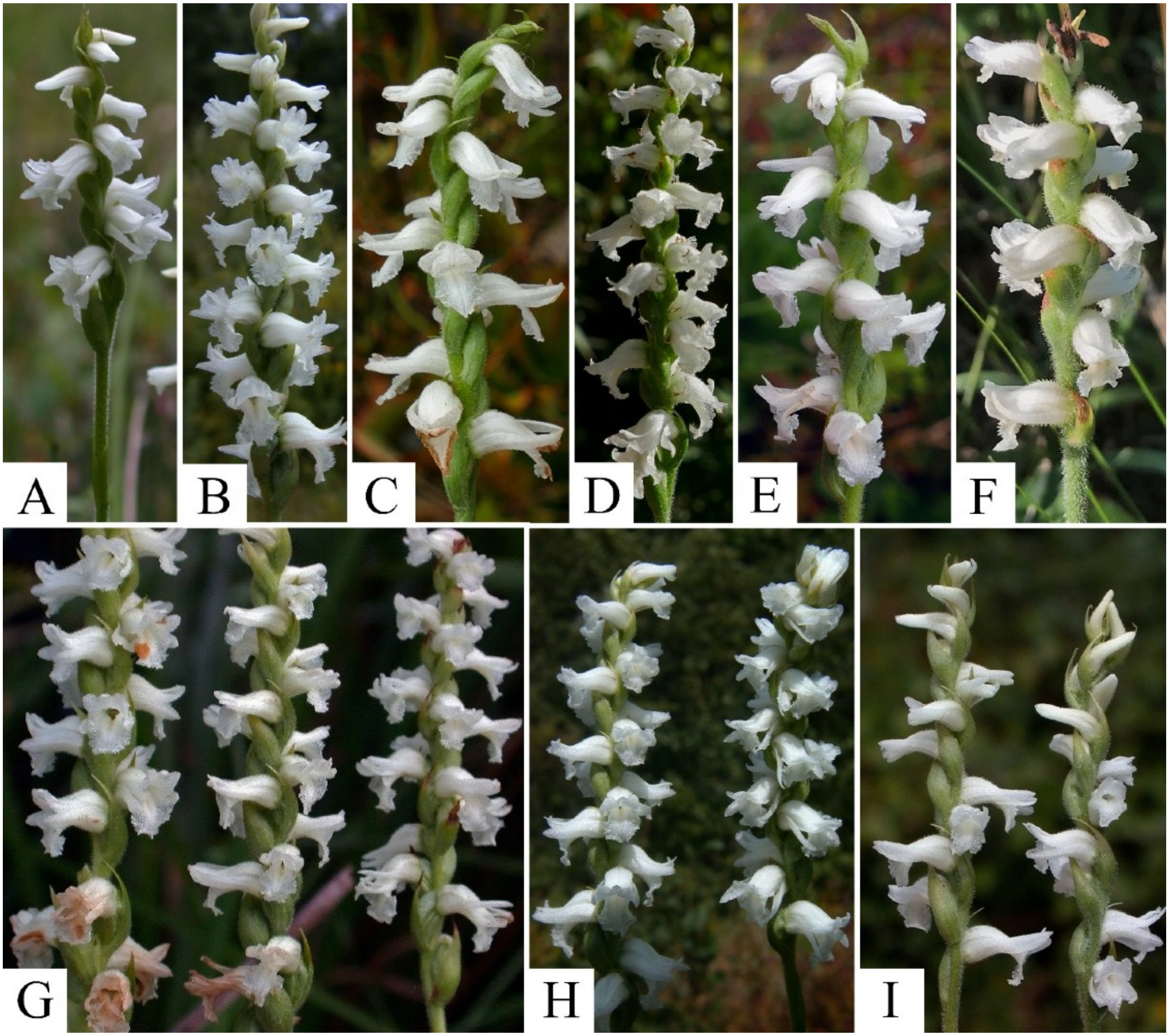


Figure 15. *Spiranthes arcisepala*. A. Oneida Co., New York. B. Chenango Co., New York, sample S16. C. Elk Co., Pennsylvania, sample S41. D. Burlington Co., New Jersey, sample 37. E. Erie Co., Pennsylvania, sample S34.
F. Schenectady Co., New York, sample S27, photo by Sara Martinez. G. Chenango Co., New York, sample S19 (left), Westchester Co., New York, sample S9 (middle), and Luzerne Co., Pennsylvania, sample S4 (right). H. Onondaga Co., New York, sample S22 (left) and sample S21 (right). I. Cortland Co., New York, sample S29 (left).

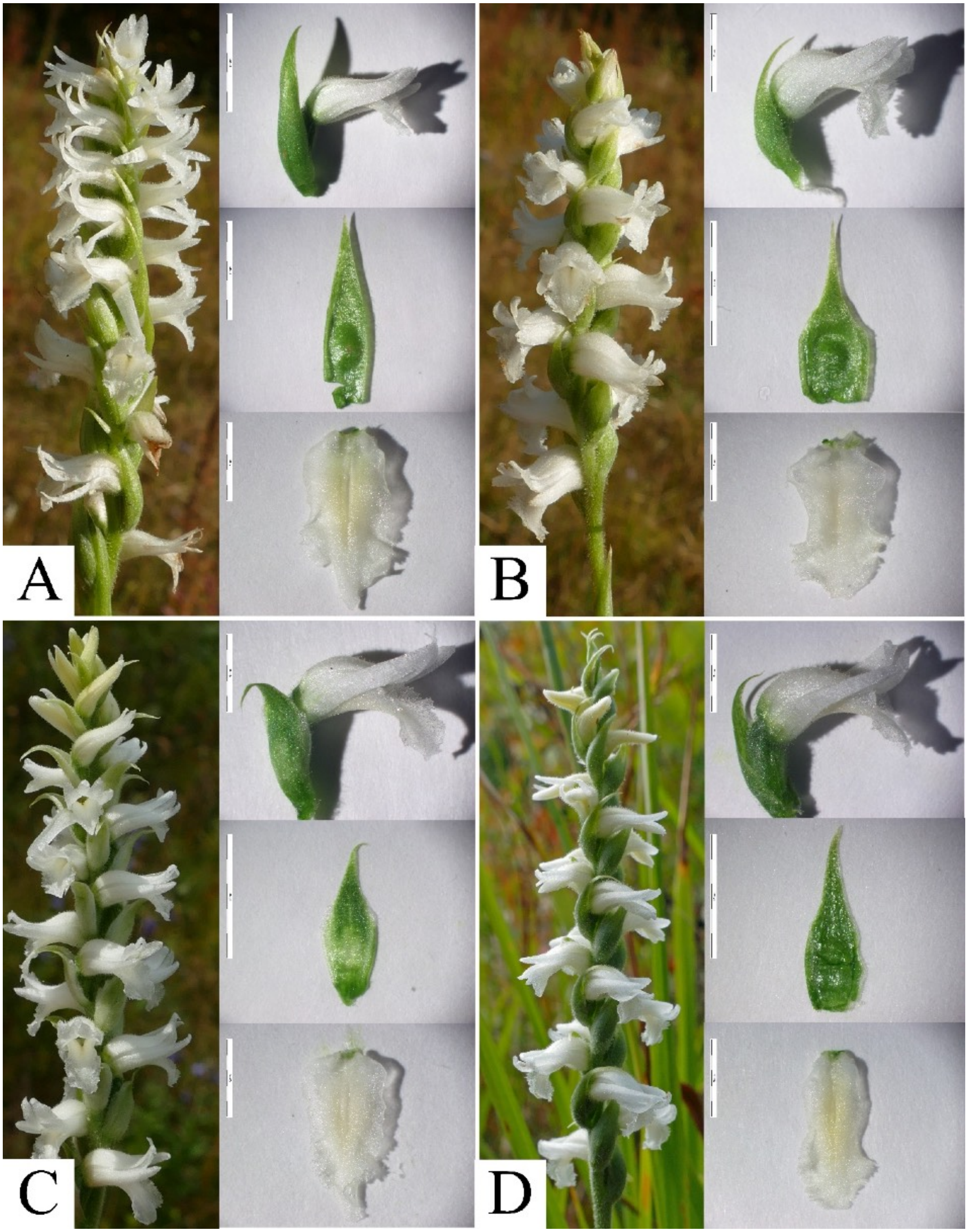


Figure 16. Representative inflorescence, flower, floral bract, and labellum. A. *Spiranthes incurva*, form with elongate bracts, Chenango Co., New York. B. *Spiranthes arcisepala*, typical form, Chenango Co., New York (co-occurring with A). C. *Spiranthes incurva*, form with recurved, hyaline-margined bracts, Tompkins Co., New York. D. *Spiranthes sheviakii*, Onondaga Co., New York, sample S36 (photographed a week prior to collection).

Conclusion

We identified three populations (Figure 1E), one in north-central New York and two in northwestern Pennsylvania, that are apparently the result of hybridization of *S. ochroleuca* with another member of the *S. cernua* species complex. After reviewing herbarium material and carefully considering the genetics and morphology, we have concluded that these plants represent an independent, self-perpetuating lineage that we have described as *S. sheviakii*. One of these populations was found along the Dead Pond Trail in the interior of Presque Isle, about 0.38 miles southwest of the former location of the fog signal, the type location for *S. incurva* (Jennings 1906). Plants that are a genetic match for *S. incurva* were later collected to the east on Gull Point, but this sandspit was scarcely developed back in 1905 when the type of *Ibidium incurvum* Jenn. was collected (Jennings 1909). We were therefore unsure if the name *S. incurva* was valid. We later determined that the Gull Point *S. incurva* (Figure 14E) best matched the holotype for *S. incurva* based on arrangement of flowers in the inflorescence, the shape of the floral bracts, and early bloom date (August 26), thus supporting that this name was correctly applied.

We believe that *S. sheviakii* corresponds with the “old field ecotype” of Sheviak (1974; 1982) and the description (but not the photograph) of this ecotype by Homoya (1993). The plants that we collected (Figure 11) bear a striking similarity to the illustration of this ecotype (Sheviak 1982, Figure 20 e–h), which is said to occur on acidic soils in the forested region to the southeast of the prairies in the Midwest. Pace did not sample plants from southern Illinois and southern Indiana (Pace 2015, Ch. 3, Figure 11) where this ecotype is said to occur and where *S. sheviakii* appears to reach its greatest abundance, so it is not surprising the genetics of this ecotype have not been captured previously. The habitat of this ecotype was described as “dry open woodland on bluff crowns and ravine rims.”, and that it is “a colonizer of old fields undergoing succession to oak-hickory and mixed hardwood-pine forests.” (Sheviak 1982). This is similar to many of the locations from our study (dry, successional, acidic) and the description of this ecotype as having characteristics suggesting *S. ochroleuca* influence, being a “calciphobe” with some specimens exhibiting “decidedly yellowish flowers” (Sheviak 1974), seems fitting. Homoya’s (1993) photograph of the “sand prairie ecotype” from Lagrange County, Indiana more closely matches our photos of *S. sheviakii* than it does *S. incurva*. It is also possible that Homoya’s photograph of the ‘old field ecotype’ is *S. ochroleuca*. In the discussion of *S. ochroleuca* he notes that “sterile, sandy-acid openings in pioneer woodland” matching the habitat of *S. ochroleuca* in Michigan occur in Lagrange and Steuben counties in northern Indiana. He also noted the difficulty of distinguishing the old-field ecotype from *S. ochroleuca* and hypothesized that the former inherited genetic material from *S. ochroleuca* at some time in the past.

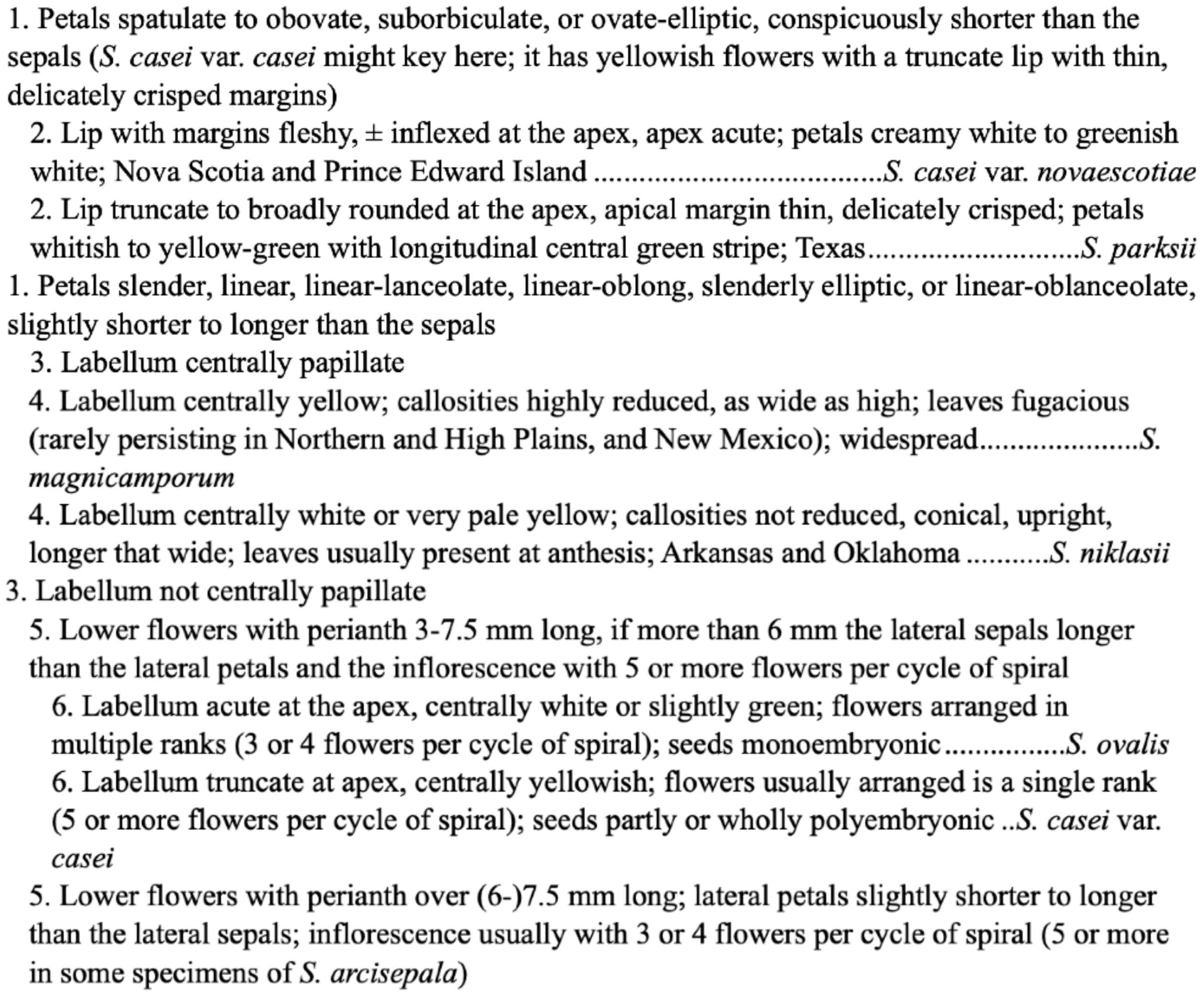
Sheviak (1974) also noted differences in phenology between the sand-prairie ecotype (*S. incurva*) and the old-field ecotype (*S. sheviakii*). Under cultivation the former was found to flower earlier than the latter. In the field he noted that the "sand-prairie ecotype blooms from mid-August to mid-September in the north and as late as early October in its more southern stations", while the other ecotype did not start blooming until "mid-October in the northern portion of its range and late October in the extreme south" (Sheviak 1974). At our study site in north-central New York the first species to flower was *S. incurva* in late August, followed by *S. arcisepala* in early September, and these were mostly past anthesis before the peak bloom of *S. ochroleuca* and *S. sheviakii* from mid-September to mid-October with *S. ochroleuca* being the latest to flower.

Unresolved is the identity of what Sheviak called the 'southern prairie complex' (Sheviak 1982 Figures 21–23). These plants resemble *S. magnicamporum* but with a more membranous lip and are tetraploid or aneuploid plants with polyembryonic seeds, and characterized by a multi-ranked inflorescence, tuberous and descending roots, fugacious leaves, and flowers that are often peloric or cleistogamous. Specimens examined were primarily collected in western and southern Illinois, Missouri, Iowa, eastern Kansas, and northern Arkansas (Figure 2D). The habitat for the majority of specimens is listed as prairie, in contrast to *S. sheviakii* which is associated with upland acid barrens, old fields, and open woods. We cannot rule out that these plants represent *S. incurva* given the clear *S. magnicamporum* influence. However, such characteristics are more pronounced in these plants than in *S. incurva* further to the north and east, in particular the tuberous roots, fugacious leaves, and preference for prairie habitats. The plants also have floral bracts that more closely resemble those of *S. magnicamporum* (relatively broad) than the characteristics previously described for *S. incurva*. It is possible these are derived from the same parents as *S. incurva* but are the product of introgression or a separate hybridization event. As far as we know genetic analyses have not included plants representing this complex, though peloric and cleistogamous plants that exhibit slender roots (rather than tuberous ones) have been determined further to the north (*S. incurva*) and south (*S. cernua*) of this range.

Given that *S. cernua* is apparently an allopolyploid that exhibits many of the same nucleotide ambiguities as *S. arcisepala*, *S. casei*, *S. incurva*, and *S. sheviakii*, as well as the recent description of *S. bightensis* as another genetically similar member of the complex (Pace 2021), we cannot be confident that *S. cernua* is one of the ancient parents or if the other parent could be one of these other allopolyploid species. We can only be reasonably certain that *S. magnicamporum* is a likely progenitor to *S. incurva* and that *S. ochroleuca* or one or more of its allopolyploid decedents are progenitors to the others (*S. arcisepala*, *S. casei*, and *S. sheviakii*). Future work on classification and evolution of this challenging group will benefit from further field and museum work accompanied by robust studies that identify homologues via cloning or next generation sequencing, leading to a better understanding of hybrid ancestry within the genus *Spiranthes*.

Artificial key to the *Spiranthes cernua* species complex

The key assumes one can arrive here with some knowledge of *S. cernua* s.l. When possible multiple plants within a population should be examined and identifications compared with the figures and descriptions in the discussion. Single plants within a population may exhibit atypical traits, particularly if depauperate, damaged, or growing on the margins of the habitat. Plants where the labellum is only slightly or not at all differentiated from the petals (flowers peloric) and/or with buds failing to open (cleistogamous) are not included in the key. In this study these are represented by a plant with leaves present at anthesis thought to represent *S. cernua* (S8). In the upper Midwest Pace and Cameron (2017) determined a plant from one apomictic population in Lake County Indiana to represent *S. incurva* (sm27d), however the genetics on this was limited to the plastid regions matK and ycf1 3'. This specimen is embedded with *S. cernua* and a few other taxa in the individual phylogenetic tree reconstructions for these regions (Pace 2015). A detailed description of this plant is also wanting (e.g. whether or not leaves present). Further work is needed on these and other plants representing the 'low prairie race' and 'southern prairie complex' of Sheviak (1982).



1. Petals spatulate to obovate, suborbiculate, or ovate-elliptic, conspicuously shorter than the sepals (*S. casei* var. *casei* might key here; it has yellowish flowers with a truncate lip with thin, delicately crisped margins)
    2. Lip with margins fleshy, ± inflexed at the apex, apex acute; petals creamy white to greenish white; Nova Scotia and Prince Edward Island ....*S. casei* var. *novaescotiae*
    2. Lip truncate to broadly rounded at the apex, apical margin thin, delicately crisped; petals whitish to yellow-green with longitudinal central green stripe; Texas............................*S. parksii*
1. Petals slender, linear, linear-lanceolate, linear-oblong, slenderly elliptic, or linear-oblanceolate, slightly shorter to longer than the sepals
    3. Labellum centrally papillate
    4. Labellum centrally yellow; callosities highly reduced, as wide as high; leaves fugacious (rarely persisting in Northern and High Plains, and New Mexico); widespread.....................*S. magnicamporum*
    4. Labellum centrally white or very pale yellow; callosities not reduced, conical, upright, longer that wide; leaves usually present at anthesis; Arkansas and Oklahoma ....*S. niklasii*
3. Labellum not centrally papillate
    5. Lower flowers with perianth 3-7.5 mm long, if more than 6 mm the lateral sepals longer than the lateral petals and the inflorescence with 5 or more flowers per cycle of spiral
        6. Labellum acute at the apex, centrally white or slightly green; flowers arranged in multiple ranks (3 or 4 flowers per cycle of spiral); seeds monoembryonic................*S. ovalis*
        6. Labellum truncate at apex, centrally yellowish; flowers usually arranged is a single rank (5 or more flowers per cycle of spiral); seeds partly or wholly polyembryonic ..*S. casei* var. *casei*
    5. Lower flowers with perianth over (6-)7.5 mm long; lateral petals slightly shorter to longer than the lateral sepals; inflorescence usually with 3 or 4 flowers per cycle of spiral (5 or more in some specimens of *S. arcisepala*)

7. Callosities highly reduced, as wide as high, 0.2-0.6 mm long; distal labellum margin undulate and fleshy; leaves absent at anthesis (rarely persisting Northern and High Plains, and New Mexico...*S. magnicamporum*

7. Callosities usually well-developed, (0.6-)0.8-2.5 mm long, longer than wide; distal labellum margin thin, lacerate (undulate and fleshy in *S. odorata*); leaves present or absent at anthesis

8. Seeds monoembryonic; labellum centrally yellow

9. Distal margin of lateral sepals touching, or nearly touching, adjacent margin of dorsal sepal; labellum margin relatively thick, undulate; moist to wet and/or seasonally inundated habitats ....*S. odorata*

9. Distal margin of lateral sepals often separated from adjacent margin of dorsal sepal, the laterals sepals thus appearing to be attached relatively low on the flower; labellum margin thin, translucent, lacerate; dry, upland habitats ....*S. ochroleuca*

8. Seeds partly or wholly polyembryonic; labellum centrally white to yellow

10. Leaves 1.4-1.7 cm wide; roots stoloniferous; Coastal Plain from southern NY (historically) to Virginia ....*S. bightensis*

10. Leaves usually less than 1.4 cm wide; roots not stoloniferous; widespread

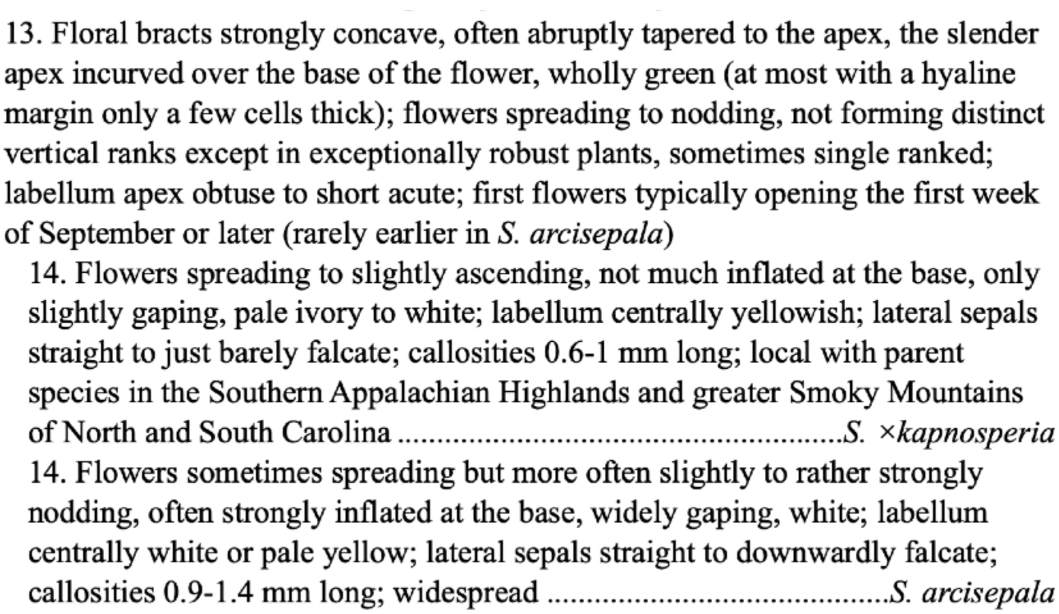
11. Labellum relatively thin and membranous, with reduced, often flattened abaxial glands; lateral sepals curving inward at the tips over the lateral petals and dorsal sepal or the tips similarly upcurved; flowers slender in profile, sometimes not or only faintly fragrant..*S. cernua*

11. Labellum centrally thickened, with spherical abaxial glands; lateral sepals straight to upcurved (often incurved at the tips in *S. sheviakii*, similar to *S. cernua*), or weakly or strongly downwardly falcate, sometimes spreading; flowers slender or stout in profile, usually fragrant

12. Lateral sepals frequently curving inward at the tips ± over the lateral petals and dorsal sepal; flowers slender in profile, usually slightly to rather strongly nodding; acidic, well-drained, relatively dry habitats; leaves often withered by anthesis in open habitats but sometimes large and well-developed in plants growing in upland woods in the southern portion of range...*S. sheviakii*

12. Lateral sepals straight to downwardly falcate, the tips usually not incurved (at most upcurved but remaining parallel to petals); typically in moist or saturated soils ranging from moderately acid to basic, but occasionally drier habitats; flowers spreading to ascending (often nodding in *S. arcisepala*); leaves usually present at anthesis

13. Floral bracts nearly flat to moderately concave, either recurved, straight and spreading, or erect and weakly incurved, green or with prominent white hyaline margins or nearly wholly white; flowers spreading or slightly ascending, typically arranged in the inflorescence in 3-4 distinct vertical ranks; labellum apex typically acuminate; first flowers often opening mid-late August ....*S. incurva*



13. Floral bracts strongly concave, often abruptly tapered to the apex, the slender apex incurved over the base of the flower, wholly green (at most with a hyaline margin only a few cells thick); flowers spreading to nodding, not forming distinct vertical ranks except in exceptionally robust plants, sometimes single ranked; labellum apex obtuse to short acute; first flowers typically opening the first week of September or later (rarely earlier in *S. arcisepala*)

  14. Flowers spreading to slightly ascending, not much inflated at the base, only slightly gaping, pale ivory to white; labellum centrally yellowish; lateral sepals straight to just barely falcate; callosities 0.6-1 mm long; local with parent species in the Southern Appalachian Highlands and greater Smoky Mountains of North and South Carolina ....*S.* ×*kapnosperia*

  14. Flowers sometimes spreading but more often slightly to rather strongly nodding, often strongly inflated at the base, widely gaping, white; labellum centrally white or pale yellow; lateral sepals straight to downwardly falcate; callosities 0.9-1.4 mm long; widespread ....*S. arcisepala*

Acknowledgements

We thank Chuck Sheviak, Paul Catling, Lucy Dueck, Matthew Pace, and Kenneth Cameron for laying much of the theoretical groundwork upon which the research we present is built. We are extremely grateful to the following people who sent specimens, photographs, and/or provided habitat information to include in this study: David Taft, Charles Ufford, David Hand, Greg Funka, Steven Daniel, Steve Young, Sara Martinez, Don Leopold, and April Moore. This study was funded by a grant from the American Orchid Society and a grant from the New York State/United University Professions Joint Labor-Management Committees. We would also like to thank the Pennsylvania Department of Conservation and Natural Resources Bureau of State Parks for permission to collect specimens for testing from Presque Isle State Park. We also thank Bonnie Isaac at the Carnegie Museum of Natural History herbarium for access to photos of some of Jennings' type specimens and Tricia Conklin for the use of her genetics lab at SUNY Cortland. We are grateful to Paul Catling, John Gange, and Kenneth Cameron for their comments on this paper.

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APPENDIX

iNaturalist Records

iNaturalist observation identification numbers for plants identified as *S. sheviakii*. These can be viewed by adding the number to https://www.inaturalist.org/observations/

400447; 4107928; 4147577; 5624187; 5639637; 7897876; 8087479; 16078580; 16205469; 16206693; 16358219; 16539767;16797446; 16992830; 19161673; 32369127; 32612572; 32794039; 32826551; 32895423; 32897373; 32910314; 33008736; 33090989; 33138754; 33173074; 33567635; 35935494; 59282245; 59324748; 59389537; 59906207; 59924040; 59934972; 59935624; 60736334; 61478184; 62186411; 68015616; 33732429; 44922393; 59274535; 59538656; 61148188; 61291573; 32744425; 60270070; 33794815; 33564986; 62815986; 62458915; 61477664; 61477628; 61477592; 33533587.

Representative Specimens Examined

These were primarily used to define the potential range of *S. sheviakii* and the extent of *S. arcisepala* and *S. ochroleuca* in Indiana, but also to distinguish the range of the 'southern prairie complex' from that of *S. incurva*. Specimen voucher information listed as follows: **Taxon name — Country. State.** County: location, date, collector (herbarium acronym).

Spiranthes arcisepala M.C. Pace — **USA**. **Indiana**. Allen Co.: north side of Lake Everett, 23 Sep 1916, *C.C. Deam 22088* (IU); Elkhart Co.: 4 mi. northeast of Middlebury, 2 Sep 1932, *C.C. Deam 52942* (IU); north side of Simonton Lake, 24 Sep 1933, *C.C. Deam 54640* (IU); Marshall Co.: 5 mi. south of Plymouth, 17 Sep 1926, *C.C. Deam 43716* (IU); St. Joseph Co.: south side of St. Joseph River, 24 Sep 1933, *C.C. Deam 54659* (IU); Starke Co.: north shore of Bass Lake, 17 Sep 1915, *E.J. Grimes 2048* (IU).

Spiranthes incurva (Jenn.) M.C. Pace — **USA**. **Illinois**. Winnebago Co.: Laona Township, 19 Aug 1968, *L.J. Musselman 2185* (WIS). **Indiana**. Fulton Co.: north side of Bruce Lake, 21 Sep 1928, *C.C. Deam 46342* (IU); Henry Co.: 2.5 mi. northeast of Springport, 12 Sep 1927, *C.C. Deam 45320* (IU); Kosciusko Co.: south side of Winona Lake, 24 Aug 1905, *C.C. Deam 458* (IU); Lagrange Co.: southwest side of Cedar Lake, 29 Aug 1914, *C.C. Deam 14932* (IU); Lake Co.: U.S. 20 at East Chicago, 29 Aug 1959, *N.C. Henderson s.n.* (FSU); Laporte Co.: 0.5 mi. north of Mill Creek, 25 Aug 1920, *C.C. Deam 32372* (IU); Madison Co.: Mounds Fen, 9 Sep 1956, *H. Starcs 1513* (IU); Newton Co.: 4 mi. southeast of Conrad, 22 Sep 1928, *C.C. Deam 46457* (IU); railroad prairie near Goodland, 5 Oct 1988, *M. Homoya & R. Hedge 88-10-05-107* (IU); Noble Co.: south side of Bear Lake, 13 Sep 1916, *C.C. Deam 21845* (IU); marl prairie bordering Eagle Lake, 6 Oct 1988, *M. Homoya & L. Casebere 88-10-06-115* (IU); Porter Co.: Dune Park, 23 Sep 1916, *B. Shimek s.n.* (BRY); Pulaski Co.: HW 29, 1.1 mi. north of Star City, 26 Sep 1948, *C.C. Deam 1948* (IU); Randolph Co.: Cabin Creek bog, 6 mi. north of Modoc, 5 Sep 1952, *F.B. Buser 1958* (IU); Starke Co.: north shore of Bass Lake, 17 Sep 1915, *E.J. Grimes 2048* (IU); Steuben Co.: south side of Lake Pleasant, 3 Sep 1930, *C.C. Deam 49469* (IU). **Iowa**. Allamakee Co.: French Creek, 5 Sep 1958, *T.G. Hartley & R.F. Thorne 6181* (WIS); Benton Co.: Vinton, nd, *J.J. Davis s.n.* (WIS). **Nebraska**. Antelope Co.: 1 mi. se of Holt County line, 3 Sep 1998, *S.B. Rolfsmeier 14263* (BRY); Cherry Co.: Jumbo Valley Fen, 15 Aug 1992, *J.P. Hardy & J. Phillips 3449* (BRY); Allen Valley fen, 21 Aug 1996, *S.B. Rolfsmeier 12888* (USCH); Douglas Co.: valley sand pits, field, 4 Sep 1948, *E.K. Jones s.n.* (WIS); Loup Co.: Calamus river s of Bassett, 18 Sep 1935, *E.L. Nillsen s.n.* (MIN).

Spiranthes magnicamporum Sheviak — **USA**. **Missouri**. Jasper Co.: Webb City, 25 Sep 1908, *B.F. Bush 5180* (NY).

Spiranthes ochroleuca (Rydb.) Rydb. — **USA**. **Indiana**. Clay Co.: Shakamak State Park, 2 Oct 1931, *C.C. Deam 51382* (IU); Floyd Co.: IUS North Campus beside nature trail, 19 Sep 1984, *F. Roberts 1* (JEF); Harrison Co.: 2 mi. southeast of Corydon, 6 Oct 1920, *C.C. Deam 33461* (IU). Monroe Co.: Rt. 37 ca. 1.5 mi. S Morgan Co. Line, 30 Oct 1961, *A.F. Clewell 1701* (FSU); Morgan-Monroe State Forest, 28 Sep 1932, *W.H. Duncan 225* (GA); Perry Co.: 1.5 mi. east of Tell City, 30 Sep 1929, *C.C. Deam 47958* (IU). **Kentucky**. Greenup Co.: N. of Rt. 784 at Lititia, 26 Sep 1986, *A.W. Cusik 26005* (OSU).

Spiranthes sheviakii M. Hough & M.A. Young — **USA**. **Arkansas**. Saline Co.: Middle Fork Barrens Natural Area, 1 Oct 2008, *T. Witsell 08-482* (ANHC). **Illinois**. Clay Co.: Hanging Rock, 3 mi. sw of Flora, 5 Oct 1977, *J.E. Ebinger 16656* (EIU); 1 mi. se of Edgewood, 10 Oct 2000, *J.E. Ebinger 29299* (EIU); 2 mi. sw of Flora, 5 Oct 1977, *J.E. Ebinger 16694* (EIU); Coles Co.: Fox Ridge State Park, 8 Oct 1974, *J.E. Ebinger 15221* (EIU); Crawford Co.: open dry area, 7 Oct 1972, *L. Phillippe 2180* (EIU); 6 mi. s. of Robinson, 6 Oct 1975, *J.E. Ebinger 15567* (EIU); abandoned field, 14 Sep 1971, *L. Phillippe 1082* (EIU); Effingham Co.: Wildcat Hollow State Forest, 5 Oct 2003, *G.C. Tucker 13586* (EIU); Rock Cave Nature Preserve, 1 Sep 1981, *J.E. Ebinger 20709* (EIU); Jasper Co.: Prairie Ridge State Natural Area, 18 Oct 2005, *G.C.Tucker 14546* (EIU); Lawrence Co.: Red Hills State Park, 10 Oct 1998, *B. Edgin 2006* (EIU); Marion Co.: Stephen A. Forbes State Park, 10 Oct 2000, *J.E. Ebinger 29328* (EIU); Pope Co.: 1.5 mi. southeast of Eddyville, 11 Oct 1968, *B.L. Dolbeare 2680* (EIU); near Lusk Creek canyon, 4 Oct 1970, *J.E. Ebinger 10061* (EIU); Shelby Co.: State Forest, 5 Oct 1968, *D.H. Sickles 212* (EIU). **Indiana**. Bartholomew Co.: road scrape along road near Grandview Lake, 12 Oct 1988, *M. Homoya & C. Hedge 88-10-12-119* (IU); Cass Co.: southwest side of Lake Cicott, 14 Sep 1920, *C.C. Deam 32628* (IU); Clarke Co.: west of tract 28, Clark State Forest, 7 Sep 1910, *C.C. Deam 7575* (IU); Crawford Co.: 3 mi. northwest of Leavenworth, 5 Oct 1920, *C.C. Deam 33416* (IU); ca. 1 mi. east of Taswell, 24 Sep 1932, *C.C. Deam 53389* (IU); Greene Co.: old field, 24 Sep 1985, *T.W. Post & D.B. Abrell 305* (IU); Jefferson Co.: ca. 1.5 mi. southwest of Kent, 10 Oct 1921, *C.C. Deam 35291* (IU); Hanover, Sep 1875, *A.H. Young s.n.* (PH); Monroe Co.: Buckner's Cave, 3 Oct 1981, *J.E. Ebinger 20833* (EIU); Monroe Co.: Cedar Bluffs, 11 Oct 1930, *J.S. Brooks 1356* (IU); Morgan Co.: on top of ridge, 30 Sep 1986, *M. Homoya & H. Huffman 86-09-30-103* (IU); Owen Co.: McCormick's Creek State Park, 4 Oct 1981, *J.E. Ebinger 20897* (EIU); Parke Co.: open woods near Turkey Run State Park, 15 Oct 1975, *J.E. Ebinger 15576* (EIU); Perry Co.: ca. 1.5 east of Tell City, 30 Sep 1929, *C.C. Deam 47985* (IU); ca. 7 mi. east of Cannelton, 2 Oct 1920, *C.C. Deam 33339* (IU); Mogan Ridge, 7 Oct 1988, *M. Homoya et al. 88-10-07-117* (IU); Ripley Co.: old fair ground near Batesville, 11 Oct 1920, *C.C. Deam 33547* (IU); Scott: 2.5 mi. northwest of Underwood, 9 Oct 1920, *C.C. Deam 33518* (IU); Vigo Co.: near ISU Landsbaum Property, north of Terre Haute, 15 Oct 1975, *J.E. Ebinger 15570* (EIU); Washington Co.: Jackson Township, 23 Oct 1990, *G. Emmert 135* (IU). **Tennessee**. Montgomery Co.: Ft. Campbell Military Reservation behind Woodlawn Church, 18 Oct 1974, *E.W. Chester & M. Edwards 2924* (APSC).

Spiranthes 'southern prairie complex' (Sheviak 1982) — **United States**. **Arkansas**. Faulkner Co.: German Lane, S Conway, 10 Nov 1978, *D. Culwell 4588* (UCA); Logan Co.: Magazine Mountain, 17 Oct 1923, *J. Palmer 24170a* (AMES); Phillips Co.: north side of Ark. 1, 20 Oct 1975, *S. Rich 200* (WIS). **Illinois**. Coles Co.: Lincoln Log Cabin State Park, 11 Oct 1969, *B.L. Dolbeare 3257* (EIU); Lakeview Park, Charleston, 29 Sep 1996, *W.B. Davison 79* (EIU); Cook Co.: Riverside, 11 Sep 1900, *L.M. Umbach 12472* (WIS); Macoupin Co.: 4 mi. south of Carlinville, 10 Sep 2010, *J.E. Ebinger 32646* (EIU); dry-mesic cemetery prairie, 21 Sep 1976, *R.W. Nyboer 625* (EIU); Montgomery Co.: 0.5 mi. east of Irving, 16 Sep 1977, *R. Vogel 118* (EIU); Rock Island Co.: Collison Ecological Preserve, east prairie, nd, *J. Siembab s.n.* (AUGIE); Knox Co.: about 5 mi. south of Vincennes, 4 Oct 1931, *C.C. Deam 51452* (IU). **Iowa**. Story Co.: Ames, 20 Aug 1896, *Plants of Iowa 183* (CLEM). **Kansas**. Allen Co.: 4 mi. n Iola, 4 Oct 1941, *W.H. Horr & R.L. McGregor E429* (PH); Linn Co.: 1 mi. N, 1 mi. W Blue Mound, 19 Oct 1999, *C.A. Morse 4156* (CS); Reno Co.: 4 mi. N Hutchinson, 6 Oct 1951, *R.L. McGregor 5238* (NY). **Missouri**. Bates Co.: 1.5 nw of Papinville, 1 Oct 1938, *J.A. Steyermark 9911* (F); Howell Co.: 4.9 mi. southwest of West Plains, 25 Sep 1949, *J.A. Steyermark 69334* (F); Pettis Co.: 3 mi. e of Bahner, 3 Oct 1938, *J.A. Steyermark 21351* (F); Taney Co.: 5 mi. w of Branson, 10 Oct 1974, *P.W. Nelson 432* (MUHW); Vernon Co.: 4-5 mi. w of Vergil City, 28 Sep 1938, *J.A. Steyermark 9693* (F).

Molecular Vouchers

Molecular voucher information listed as follows: **Taxon name — Country. State or province.** County: location, date, collector (herbarium code with barcode number if available), sample number followed by GenBank accessions (ACO, ndhJ–trnL). An "—" indicates missing data.

Spiranthes arcisepala M.C. Pace — **USA**. **New Jersey.** Burlington Co.: Atsion, 18 Sep 2020, *C. Ufford s.n.* (CORT14826), S37 (MW858188, MW858228); **New York.** Chenango Co.: East Pharsalia, 22 Sep 2018, *Hough & Young s.n.* (CORT14790), S16 (MW858170, MW858208); Pitcher, 22 Sep 2018, *Hough & Young s.n.* (CORT14792), S18 (MW858172, MW858210); North Pharsalia, 22 Sep 2018, *Hough & Young s.n.* (CORT14793), S19 (MW858173, MW858211); Cortland Co.: Preble, 7 Sep 2018, *Hough & Young s.n.* (CORT14779), S05 (MW858160, MW858197); Cincinnatus, 25 Aug 2018, *Hough & Young s.n.* (CORT14780), S06 (MW858161, MW858198); 25 Aug 2018, *Hough & Young s.n.* (CORT14788), S14 (MW858168, MW858206); Preble, 7 Sep 2018, *Hough & Young s.n.* (CORT14801), S29 (MW858182, MW858221); Onondaga Co.: Lysander, 14 Sep 2019, *Hough & Young s.n.* (CORT14795), S21 (MW858175, MW858213); 14 Sep 2019, *Hough & Young s.n.* (CORT14796), S22 (MW858176, MW858214); 14 Sep 2019, *Hough & Young s.n.* (—), S30 (MW858183, MW858222); Schenectady Co.: Schenectady, 6 Sep 2018, *S. Martinez s.n.* (—), S27 (—, MW858219); Tompkins Co.: Groton, 12 Sep 2018, *M. Young s.n.* (CORT14777), S03 (MW858158, MW858195); Westchester Co.: Cross River, 22 Sep 2018, *D. Taft s.n.* (CORT14783), S09 (MW858163, MW858201);

Pennsylvania. Elk Co.: Ridgeway, 29 Sep 2020, *A. Moore s.n.* (CORT14822), S41 (MW858192, MW858232); Erie Co.: Verango Twp, 12 Sep 2020, *G. Funka s.n.* (CORT14829), S34 (—, MW858225); Luzerne Co.: Hazleton, 6 Oct 2018, *Hough & Young s.n.* (CORT14778), S04 (MW858159, MW858196); Schuylkill Co.: Tremont, 21 Sep 2018, *D. Hand s.n.* (CORT14789), S15 (MW858169, MW858207).

Spiranthes cernua (L.) Rich. — **USA**. **Maryland.** Worcester Co.: Ocean Pines, 23 Oct 2013, *Pace 608* (NY), sc8d (MF460910, MF460859); **New Jersey.** Burlington Co.: Atsion, 23 Sep 2018, *C. Ufford s.n.* (CORT14776), S02 (MW858157, MW858194); Atsion, 23 Sep 2018, *C. Ufford s.n.* (CORT14782), S08 (—, MW858200); Cape May Co.: Cape May, 19 Oct 2013, *Pace 607* (NY), sc6d (MF460910, MF460859); **New York.** Richmond Co.: Charlseton, 26 Sep 2018, *D. Taft s.n.* (CORT14786), S12 (MW858166, MW858204); Tottenville, 26 Sep 2018, *D. Taft s.n.* (CORT14787), S13 (MW858167, MW858205); Westchester Co.: Bedford, 21 Sep 2018, *D. Taft s.n.* (CORT14784), S10 (MW858164, MW858202).

Spiranthes incurva (Jenn.) M.C. Pace — **USA**. **New York.** Cayuga Co.: Montezuma, 9 Sep 2019, *M. Hough s.n.* (—), S23 (MW858177, MW858215); Cortland Co.: Cortland, 9 Sep 2018, *M. Hough s.n.* (CORT14781), S07 (MW858162, MW858199); Onondaga Co.: East Syracuse, 25 Aug 2018, *Hough & Young s.n.* (CORT14775), S01 (MW858156, MW858193); Lysander, 6 Sep 2020, *M. Hough s.n.* (CORT14831), S31 (MW858184, MW858223); Schenectady Co.: Schenectady, 15 Sep 2019, *S. Young s.n.* (CORT14800), S28 (MW858181, MW858220); St. Lawrence Co.: Gravel Pit, 12 Sep 2019, *S. Daniel s.n.* (CORT14797), S24 (MW858178, MW858216); West of Massena, 16 Sep 2019, *S. Daniel s.n.* (CORT14798), S25 (MW858179, MW858217); **Pennsylvania.** Erie Co.: Presque Isle, 30 Aug 2020, *G. Funka s.n.* (CORT14828), S35 (—, MW858226).

Spiranthes magnicamporum Sheviak — **Canada**. **Ontario.** Lambton Co.: Marthaville, 7 Sep 2011, *Oldham 39307* (NYS), sm15a (KU752254, KU935580); **USA**. **New Mexico.** —: —, 21 Sep 2013, *Pace 594* (NY), sm7h (KU752251, KU935577); **New York.** St. Lawrence Co.: Massena, 29 Sep 2018, *Hough & Young s.n.* (CORT14791), S17 (MW858171, MW858209); **Tennessee.** Franklin Co.: Cowan, 8 Nov 2006, *Sheviak 7064* (NYS), sm17a (KU752255, KU935581); **Texas.** Grimes Co.: —, 31 Oct 2004, *Liggio s.n.* (WIS), 15e (KU752249, KU935575); **Wisconsin.** Walworth Co.: —, 16 Sep 2006, *Hapeman s.n.* (WIS), 15f (MF460919, MF460871).

Spiranthes ochroleuca (Rydb.) Rydb. — **USA**. **Michigan.** Saginaw Co.: —, 8 Sep 2004, *Case s.n.* (CLEM), 16g (MF460932, MF460891); **New Hampshire.** Rockingham Co.: Candia, 13 Sep 2004, *Stefanik s.n.* (CLEM), 16f (MF460931, MF460890); **New York.** Oneida Co.: Camden, 3 Oct 2006, *Ufford s.n.* (CLEM), 16h (MF460933, MF460892); Onondaga Co.: Lysander, 2 Oct 2020, *M. Hough s.n.* (CORT14824), S39 (MW858190, MW858230); Westchester Co.: Bedford, 21 Sep 2018, *D. Taft s.n.* (CORT14785), S11 (MW858165, MW858203); **Ohio.** Lucas Co.: —, 4 Oct 2004, *Dueck s.n.* (CLEM), 4wx (MF460930, MF460887).

Spiranthes odorata (Nutt.) Lindl. — **USA**. **Georgia.** Effingham Co.: —, 2 Dec 2006, *Vincent s.n.* (WIS), 17opq (KU752224, KU935586); **New York.** Onondaga Co.: Liverpool, 12 Sep 2020, *Hough, Young, McMullen s.n.* (CORT14802), S32 (MW858185, MW140015); **North Carolina.** Brunswick Co.: Rice's Creek Bridge, 8 Aug 2004, *Galloway s.n.* (CLEM), 17d (MF460934, MF460893); Carteret Co.: Piney Island, 26 Oct 2013, *Pace s.n.* (WIS), so1d (KU752225, KU935588); **Tennessee.** —: —, 1 Oct 2001, *Durr s.n.* (NYS), so12 (KU752228, KU935589).

Spiranthes ovalis Lindl. **var. erostellata** Catling — **USA**. **Wisconsin.** Waukesha Co.: —, —, *Pace 649* (WIS), sov2 (MF460935, MF460895).

Spiranthes sheviakii M. Hough & M.A. Young — **USA**. **New York.** Onondaga Co.: Lysander, 14 Sep 2019, *Hough & Young s.n.* (CORT14794), S20 (MW858174, MW858212); 19 Sep 2020, *Hough & Young s.n.* (CORT14827), S36 (MW858187, MW858227); 19 Sep 2020, *M. Hough s.n.* (CORT14825), S38 (MW858189, MW858229); 2 Oct 2020, *M. Hough s.n.* (CORT14823), S40 (MW858191, MW858231); **Pennsylvania.** Erie Co.: Presque Isle, 7 Sep 2019, *Hough & Young s.n.* (CORT14799), S26 (MW858180, MW858218); Greenfield Twp, 12 Sep 2020, *G. Funka s.n.* (CORT14820), S33 (MW858186, MW858224).

2021 CASE FUND GRANT RECIPIENTS

The Native Orchid Conference is proud to sponsor a research grant program in memory of Mr. Frederick W. Case, Jr.—teacher, botanist and an internationally acclaimed expert on the North American Orchidaceae, Sarraceniaceae and Trilliaceae. The purpose of the grant is to support basic or applied research on orchids native to North America north of Mexico to university undergraduate or graduate students, or other approved researchers.

This year we are pleased to award Case Grant to two researchers:

Patrick Smallwood

University of Atlanta

Does mycorrhizal specificity vary across the range of Cypripedium acaule (Orchidaceae) and how does this relationship influence the orchid's ability to establish new populations and expand its range?

Mr. Smallwood is using DNA sequencing techniques to study the types of mycorrhizae in *C. acaule* populations. He's comparing them within and between populations to determine which types, if any, seem to be important for the orchids. This information will help guide reintroduction efforts. Funds from the Case Grant funds will be used to pay for the sequencing.

Ellen Garcia

Florida Atlantic University

Conservation of South Florida Mycorrhizal Fungi

Ms. Garcia is studying the populations of fungi on trees in south Florida, both those that have orchids growing on them and those that don't. The information will help guide the reintroduction of epiphytic orchids in parks and along streets in the area. The Case Grant funds will be used to purchase supplies for cryopreservation of the strains she has isolated so that they will be available for future studies.

The Case Fund Grant is made possible by membership fees and donations. For more information contact Doug Martin, dofrma44@gmail.com To make a donation please contact NOC Treasurer, Dick Barmore, rebster61@yahoo.com

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ISSN 1554-1169