

**Final Report—Status, Pollination Biology, and Genetic Diversity of Endangered  
Orchidaceae: *Habenaria distans*, *H. macroceratitis*, and *Spiranthes floridana***

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**Project Introduction:**

The aim of this project was to assess the current status, pollination biology, and genetic diversity of three critically-endangered terrestrial orchids native to Florida—*Habenaria distans*, *H. macroceratitis*, and *Spiranthes floridana*. Information gathered during the investigation of these three species will ultimately lead to the development of an integrated conservation plan for each species. This conservation plan will aid managers of federal, state, county, and private lands better plan and maintain current populations of these three orchids, as well as manage their habitats for the possible future introduction of plant material as a means of species restoration.

While the focus of this project was not to develop an entire integrated conservation plan for these species, the information and data collected throughout the course of the current investigation will directly lead to the development of short-term habitat and species management suggestions. Combined with data from other on-going studies, the information gained during this project will result in an integrated conservation plan for each species. In addition to data relating directly the status, pollination biology, and genetic diversity of these three species, information concerning the seed propagation, growth, and development of each species was developed.

**Plant & Research Site Descriptions:**

*Habenaria distans* Grisebach is a state endangered terrestrial orchid known only from two locations in southern Florida—a moderately-sized population in Collier-Seminole State Park (Collier County) and scattered sites throughout Fakahatchee Strand State Preserve (Collier County). The Collier-Seminole State Park population occurs in a dense, moist, occasionally-inundated tropical hammock. The hammock is dominated by a canopy consisting primarily of *Bursera simaruba* (gumbo limbo), *Sabal palmetto* (sable palm), and *Roystonea elata* (Florida royal palm).

*Habenaria macroceratitis* Willdenow is a proposed endangered terrestrial orchid known from scattered sites in central Florida—primarily surrounding Brooksville, Florida (Hernando County). Populations occur in rich, usually moist hardwood hammocks where

the canopy is dominated by *Sabal palmetto* (sable palm), *Quercus virginiana* (live oak), and *Magnolia grandiflora* (southern magnolia).

*Spiranthes floridana* (Wherry) Cory *emend.* P.M. Brown is a proposed endangered terrestrial orchid endemic to two known sites in northern Florida—near the town of Starke, Florida (Bradford County) and in Jennings State Forest (Duval County). This species is historically known from Highlands and Lee Counties, but are presumed extirpated due to feral hog activity and habitat alteration. The species typically inhabits open, grassy lawns and cemeteries or open scrub pineland with an understory dominated by *Serenoa repens* (saw palmetto).

### **Project Findings—*Habenaria distans***

#### *Current Status & Distribution*

*Habenaria distans* remains state endangered and known from only two public lands in southwestern Florida—Collier-Seminole State Park (CSSP) and Fakahatchee Strand Preserve State Park (FSPSP). While the scattered colonies at CSSP typically consist of nearly 100 plants, only 35 plants could be located in October 2003. The record low number of plants at this location in 2003 was most likely due to excessive water inundation throughout the tropical hammock where the plants are known to exist. Of the plants located in 2003, no plants flowered that year. Observations indicate that only up to 5% of a total *H. distans* population will flower each year. Plants within the FSSPP remain unverified and have yet to be relocated by either the investigators or state park officials. Surveys in 2004 and 2005 were hampered by excessive rains, tropical storms, and hurricanes during peak vegetative plant growth and flowering times. Most notable was the impact of hurricane Wilma during the 2005 hurricane season, which made landfall five miles south of CSSP as a category 3 storm with winds of over 125 mph. The ecological impact of this storm event is still being assessed throughout public lands in southwestern Florida. New surveys based on pre-Wilma observations of known locations within CSSP and FSPSP are being planned in conjunction with state of Florida officials. These surveys will occur over the vegetative growth and flowering times of the species. Other public lands in southwest Florida will be included in the 2006 surveys—Florida Panther National Wildlife Refuge and Big Cypress National Preserve.

#### *Pollination Biology*

Despite the best efforts of both investigators, the pollination mechanism and possible pollinator of *H. distans* remains unknown. Because no plants flowered in 2003 and field time was limited in both 2004 and 2005, a thorough investigation of this species' pollination biology was not possible. Observations and preliminary hand-pollinations of greenhouse grown *H. distans* plants at Marie Selby Botanic Garden (Sarasota, Florida) have shown the species to produce viable seed under the induced autogamic pollination condition. Seeds produced in this manner showed 91.9 % viability based upon the results of a tetrazolium staining test. However, these results may be misleading because the plant used was greenhouse grown, only one plant was used (with multiple

inflorescences), and the plant did not originate from Florida but from Belize. These factors may have contributed to higher-than-expected seed viability because *H. distans* throughout the West Indies, Central America, and South America have shown more frequent flowering and natural reproduction than plants at the northernmost extent of the species' range in southwest Florida.

### *Genetic Diversity*

Leaf material for DNA extraction and purification was collected from 35 plants at CSSP in 2003. Due to the difficulties mentioned previously, repeated and expanded collection at CSSP has not been possible. DNA has been extracted from all 35 leaf samples collected in 2003, and this DNA library has been stored at -80° C in the Plant Environmental Stress Molecular Biology Laboratory at the University of Florida. Further genetic diversity studies using this DNA library have been deferred until a more complete collection of *H. distans* leaf samples can be undertaken throughout southwest Florida. This new round of sampling will occur during the 2006 growing season.

### **Project Findings—*Habenaria macroceratitis***

#### *Current Status & Distribution*

*Habenaria macroceratitis* remains listed as proposed endangered in Florida, and is presently known from scattered populations in Alachua, Citrus, Hernando, Marion, and Orange Counties. Throughout all populations surveyed in Florida, the species was found to have an average height of 30.5 cm, spur length of 13.7 cm, longest leaf length of 13.3 cm, 8.5 leaves per plant, and 5.6 flowers per plant. In Alachua County a sizable (> 50 plants) population occurs within Paynes Prairie Preserve State Park and a privately-owned adjacent land. Citrus and Marion Counties populations occur within the Cross Florida Greenway, with the Marion County population consisting of only 14 plants. Orange County populations are represented by scattered small (< 50 plants) populations existing on a combination of public and private lands. Hernando County populations appear to be the largest and most dynamic populations in Florida, with at least three sites of approximately 200 plants. One particular population in Hernando County (Socash site) on privately-owned land consists of approximately 150 non-flowering plants and 100 flowering plants per year over a three-year study period. Attempts to locate plants in adjacent counties have been unsuccessful to date, although surveys are planned to continue through the 2006 flowering year. At the Socash site, 32.8% of the total population flowers in a given year, and 87.4% of all flowers set a seed capsule. All Florida populations appear to maintain a high level of reproductive fitness evidenced by high seed capsule production per plant per population.

#### *Pollination Biology*

*Habenaria macroceratitis* appears to be general in its pollination mechanism requirements. Seed capsules were set in four of seven pollination mechanism conditions tested—open pollination, induced autogamy, artificial geitonogamy, and artificial

xenogamy. The species does not appear to be self-fertile, agamopermic, or self-pollinating within population. Seed resulting from the pollination mechanism study is currently being germinated under asymbiotic conditions to gain a better perspective on the relative fitness of each pollination mechanism. These data are forthcoming. Tetrazolium staining revealed relatively high seed viability from three of the four pollination mechanism conditions—open pollination (91.0%), induced autogamy (76.8%), and artificial geitonogamy (86.3%). The artificial xenogamy pollination condition produced 50.7% viable seed.

In conjunction with a study of the pollination mechanism of *H. macroceratitis*, a possible pollinator for the species has been identified—*Cocytius antueus*, the giant sphinx moth. The moth was repeatedly seen visiting flowers of *H. macroceratitis* during a 24-hour study of the Socash site population. *Habenaria macroceratitis* was found to have a sweet night scent, and in combination with its white to white-green color and long nectar spur would support pollination by a night-flying sphinx moth. The species produces nectar containing approximately 17.5% soluble sugars.

#### *Genetic Diversity*

Leaf material for DNA extraction and purification was collected from 14 plants at the Marion County population, 7 plants at the Citrus County population, and 93 plants at the Hernando County populations. Sampling of these sites occurred in both 2003 and 2004. DNA has been extracted from all leaf samples, and this DNA library has been stored as previously mentioned. Further genetic diversity studies using this DNA have been deferred until final collections of all three study species have been made. These studies are slated to begin during the summer of 2006.

#### *Other Products*

Two manuscripts are currently in preparation concerning the biological assessment of *H. macroceratitis*. Both manuscripts are planned for submission by the end of summer 2006. Reprints of manuscripts will be provided upon publication.

- 1) Stewart SL & Kane ME (2006) Biological assessment of a rare terrestrial orchid in Florida, *Habenaria macroceratitis* (Orchidaceae). *Plant Ecology* (in preparation).
- 2) Stewart SL & Kane ME (2006) Pollination biology of a rare terrestrial orchid from Florida, *Habenaria macroceratitis* (Orchidaceae). *Plant Ecology* (in preparation).

Two manuscripts are currently in press concerning the asymbiotic and symbiotic seed germination of *H. macroceratitis*. Reprints of manuscripts will be provided upon publication.

- 1) Stewart SL & Kane ME (2006) Asymbiotic seed germination and in vitro seedling development of *Habenaria macroceratitis* (Orchidaceae), a rare Florida terrestrial orchid. *Plant Cell, Tissue and Organ Culture* (in press).
- 2) Stewart SL & Kane ME (2006) Symbiotic seed germination of *Habenaria macroceratitis* (Orchidaceae), a rare Florida terrestrial orchid. *Plant Cell, Tissue and Organ Culture* (in press).

One abstract concerning the asymbiotic seed germination of *H. macroceratitis* has been published.

- 1) Stewart SL & Kane ME (2005) *In vitro* seed germination and seedling development of *Habenaria macroceratitis*, an endangered terrestrial orchid. *In Vitro* 41: 27-A.

One presentation concerning the asymbiotic seed germination of *H. macroceratitis* has been presented.

- 1) Stewart SL (2005) *In vitro* seed germination and seedling development of *Habenaria macroceratitis*, an endangered terrestrial orchid. Society for In Vitro Biology Annual Meeting, Baltimore, Maryland.

One presentation concerning the conservation of *H. macroceratitis* has been presented and another has been prepared for future presentation.

- 1) Stewart SL (2006) Ecology of Florida orchids. Mid-Florida Research and Education Center, Apopka, Florida.
- 2) Stewart SL (2006) Gators, Florida panthers, and orchids. Native Orchid Conference, Ashland Oregon.

## **Project Findings—*Spiranthes floridana***

### *Current Status & Distribution*

*Spiranthes floridana* is currently listed as proposed endangered in Florida, and remains endemic to the state. The species was assumed extirpated from Florida until 1998 when a single extant population of 31 plants was located on property owned by the Rayonier Timber Corporation (Bradford County). Between 1998 and 2003 the number of plants at this site fluctuated slightly, between a high of 31 (1998 and 1999) and a low of 17 (2003). Only eight plants were seen at the Bradford County site in 2004 and seven in 2006. Improperly timed mowing removed all flowering stems from this site in 2005, although no damage was likely done to the plants themselves. Plants at the Bradford County site average 30.2 cm in height, 19.2 flowers per plant, and 1.7 leaves per plant.

In 2004 a second site for *S. floridana* was identified at Jennings State Forest (Duval County). The Duval County population contains an average of 15 individuals per year

(2004-2006), with a high of 18 plants (2006). Plants at this site average 27.2 cm in height, 13.9 flowers per plant, and 2.7 leaves per plant. In 2006 one small seedling was documented from this site.

Currently no other populations are known for *S. floridana* within or outside the state of Florida. Examination of herbarium specimens from Harvard (AMES) and University of Florida Herbarium (FLAS) has revealed the species to be restricted to Florida and historic in 17 counties throughout the state. Presently known from only two populations in two counties, extensive searches for other sites have yielded promising leads but no solid evidence of the species outside northern Florida.

### *Pollination Biology*

*Spiranthes floridana* appears to lack a natural pollinator and reproduce by agamospermy. During the 2003, 2004, and 2005 flowering seasons pollinator observations were made at the Bradford County population (2003 and 2004) and the Duval County population (2004 and 2005). No pollinator visitations were recorded during three separate eight-hour observation periods at each site for each year. However, plants at each site in each year produced seed capsules. Closer examination and dissection of individual flowers revealed the lack of pollinia, helping to confirm agamospermy. Interestingly, as seed capsules matured they appeared to abort just before peak maturity. The reason for this abortion remains unknown, but it may be due to intense inbreeding pressure within the two small, geographically isolated populations. Hopefully genetic diversity studies will elucidate genotypic diversity within the Bradford and Duval County populations, which may demonstrate very low genotypic diversity within these populations. This finding would support the negative effects of inbreeding pressure on reproductive fitness in *S. floridana*.

### *Genetic Diversity*

Left material for DNA extraction and purification was collected from 28 plants at the Bradford County population and 7 plants at the Duval County population. Sampling of these sites occurred in 2003 (Bradford County) and 2006 (Duval County). DNA has been extracted from all leaf samples, and this DNA library has been stored as previously mentioned. Genetic diversity studies are slated to begin during the summer of 2006.

### *Other Products*

One manuscript is currently in preparation concerning the biological assessment of *S. floridana*. The manuscript will be submitted by the end of summer 2006. Reprints of this manuscript will be provided upon publication.

- 1) Stewart SL & Kane ME (2006) Ecology of the Florida endemic terrestrial orchid *Spiranthes floridana* (Orchidaceae)—biological status and pollination biology. *Plant Ecology* (in preparation).

One abstract concerning the mycobionts of *S. floridana* has been accepted.

- 1) Stewart SL & Kane ME (2006) *In vitro* specificity exhibited by fungal mycobionts of *Spiranthes floridana* (Orchidaceae) within the congener *Spiranthes brevilabris*. In Vitro (accepted).

One poster concerning the mycobionts of *S. floridana* will be presented.

- 1) Stewart SL & Kane ME (2006) *In vitro* specificity exhibited by fungal mycobionts of *Spiranthes floridana* (Orchidaceae) within the congener *Spiranthes brevilabris*. Society for In Vitro Biology Annual Meeting, June 3-7, Minneapolis, Minnesota.

One presentation concerning the conservation of *S. floridana* has been presented and another has been prepared for future presentation.

- 3) Stewart SL (2006) Ecology of Florida orchids. Mid-Florida Research and Education Center, Apopka, Florida.
- 4) Stewart SL (2006) Gators, Florida panthers, and orchids. Native Orchid Conference, Ashland Oregon.