

## **Investigators:**

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1) **Species' name:** *Scutellaria floridana* (Florida scullcap) and *Pinguicula ionantha* (Godfrey's butterwort)

2) **Project objective:** delisting

3) **Description, cost, implementing parties:**

a) **Description:** We propose studies for the delisting of two federally threatened plant species: Florida scullcap (*Scutellaria floridana*) and Godfrey's butterwort (*Pinguicula ionantha*). The five-year status reviews from the USFWS Southeast Region identified seed ecology and population genetics as two areas where research is needed for these species. We would like to conduct seed ecology and population genetic studies for both of these species and involve state (e.g., FLDOT) and federal (e.g., BLM and USFS) agencies and local groups (i.e., NGS, private landowners, companies).

#### Proposed studies and rationale:

1) *Seed ecology studies* - The aims of the seed ecology studies will be to gain a better understanding of a) seed germination requirements for *ex situ* conservation and 2) the presence or absence of a persistent seed bank for *in situ* conservation.

2) *Population genetic studies* - The aims of the population genetic studies will be to determine: 1) genetic diversity within and among populations and 2) population genetic similarity. The results of these studies will inform seed collection and reintroduction efforts. The identification of populations with rare alleles or with high levels of genetic diversity will be a focus of these studies, as this will be important for the preservation of these species

#### Protocols:

Seed, plant and soil material collections will be conducted in May for *Pinguicula ionantha* (Godfrey's butterwort) and in July for *Scutellaria floridana* (Florida scullcap). In each county where the species are found material will be collected from multiple populations as identified in the five-year status reviews

from the USFWS Southeast region. State and federal agencies as well as private landowners will be contacted to obtain access and information regarding these populations.

1) *Seed ecology studies*: Two seed ecology studies will be conducted for each species: seed germination and seed bank. In the case of the seed germination studies, seeds will be subjected to different stratification and scarification treatments. In addition to these treatments, two smoke treatments (liquid and gas) will be conducted. The role of smoke on seed germination will be investigated due to the fact that these species are found in fire prone habitats and these treatments could stimulate germination. Smoke solutions will be prepared from on site vegetation. Lastly, in the case of seed bank, soil samples will be collected and placed in the greenhouse to determine presence or absence of a persistent seed bank.

2) *Population genetic studies*: Genetic diversity of populations will be determined using Amplified Fragment Length Polymorphisms (AFLPs). AFLPs are dominantly inherited, genetic markers that are increasingly used in population-level studies. AFLP reactions involve a four-step process. Whole DNA is first cut with two restriction enzymes. Adapter fragments are then attached to the cut ends. These adapter fragments serve as priming sites for two rounds of selective Polymerase Chain Reaction (PCR). The products are then run on a DNA analyzer. An advantage of AFLPs is that they provide a higher number of markers which can be used as fingerprints. Analysis of AFLP data will allow the examination of patterns and levels of population genetic variability in these federally threatened plants.