

March 22, 2016

U.S. Fish and Wildlife Service  
South Florida Ecological Services Office  
1339 20th Street, Vero Beach, FL 32960

Re: Peer Review Comments on *Linum arenicola* and *Chamaecrista lineata* var. *keyensis*

Dear Dave Bender,

Dr. Suzanne Koptur and I have ongoing research with *Chamaecrista lineata* var. *keyensis* and *Linum arenicola*, two of the three species currently proposed for ESA 'endangered' listing status in Florida. We are investigating direct and indirect effects of mosquito insecticide spray on flower visitors and reproductive fitness of these two species throughout protected areas in the Lower Florida Keys. The purpose of this study was to determine if there are associations between degraded pollinator habitat quality (i.e. mosquito adulticide applications) and fitness of rare flowering plants (i.e. fruit set) at the wildland-urban interface. The following are observations of the two species from thesis research work conducted during the summer of 2015; it includes study objectives and outcomes, locations of plant populations, and future research objectives on the breeding system of *Linum arenicola*. This is an ongoing study in which only one of four field seasons has been completed, therefore, results from the first field season are preliminary.

### **Introduction**

Most angiosperms benefit or rely on insect pollinators for reproductive fitness (Tepedino et al. 1996, Buchmann and Nebhan 1996, Kearns et al. 1998). Pollinators promote genetic diversity via sexual reproduction and outcrossing, thereby improving plant fitness, a goal for stabilizing rare populations (Kearns et al. 1998, Johnson and Steneir 2000, Marcot and Molina 2013). Many rare plants have specialized pollination systems, which can make them dependent upon pollinators for reproductive success (Johnson and Steneir 2000, Spira 2001). For example, Tepedino (2000) found that 24 of 26 rare plants in the Southwest US required invertebrates as a pollinating agent for reproduction. Understanding the reproductive strategies of rare plants could assist in management efforts to improve habitat quality for pollinators, thereby reducing threats to plant populations.

The wildland-urban interface introduces a suite of factors that complicate flowering plant species recovery, including pollinator declines from reduced habitat quality (Liu and Koptur 2003, Radeloff 2005). Development of roads and neighborhoods throughout the Refuge have resulted in a matrix of fragmented wilderness with an increased edge exposure to anthropogenic activity (Koptur 2006). Increasing urbanization around the Refuge has increased a demand for mosquito abatement (Hennessey 1992). Mosquito adulticides are applied between spring and late fall in the Lower Florida Keys, via aerial spray and truck fog, throughout neighborhoods and roads adjacent to wilderness refuge. Extensive studies in the Florida Keys suggest that broad spectrum insecticides negatively affect non-target invertebrates, including pollinators (Hennessey 1991, Eliazar and Emmel 1991, Kevan et al. 1997, Salvato 2001, Bargar 2011, Hoang et al. 2011). In addition, pesticides have been shown to drift into adjacent undisturbed habitat that serve as a refuge for native biota (Hennessey 1992, Pierce et al 2005, Zhong et al. 2010, Bargar 2011). These pesticides can be fatal to non-target invertebrates that move between urban and forest habitats, altering ecological processes within forest communities (Kevan and Plowright

1989, 1995, Liu and Koptur 2003). Our hypothesis is that flowers open following an insecticide application will have lower visitation rates and fruit set than flowers open at unsprayed sites.

## Methods

We conducted flower visitor observations for each species throughout the Lower Keys (see attached maps of plant locations) to assess differences in pollinator activity at treated and untreated sites. In addition, indirect effects on reproductive success were measured by marking open flowers and following through reproduction. Reproductive success is considered when open flowers produce fruit that contains at least one seed. Field observations occurred weekly or on dates following spray application at treatment and control sites. Treatment sites are adjacent to insecticide applications routes at least 10m from the road, and control sites are at least 250m in all directions from spray routes.

Knowledge of the reproductive strategies for the model plant species is imperative to determine if insecticide spray can have a negative effect on the species. For species that automatically self-fertilize, decreased pollinator visitors may not have a significant effect in the short term. Model species can be self-compatible as long as pollinator agents are required for pollen movement and fruit set. Selected species must also have one-day flowers to insure that flowers were open only following insecticide spray.

Plant populations were located by USFWS inventory records, population status reports by Hodges and Bradley (2006), and search and encounter (see attached maps of sites). We initially chose three control sites and three potential treatment sites for each species on Big Pine Key and Lower Sugarloaf Key. These islands have a long history of mosquito abatement with adulticides, as well as large areas that are not treated. Outside of the mainland, viable populations of *Linum arenicola* only occur on Big Pine Key and Lower Sugarloaf Key (80 FR 58538), therefore available treatment sites for this species was limited to one site. Sites chosen contained at least 50 open flowers on observation dates from a minimum of 30 individual plants.

### ***Chamaecrista lineata* var. *keyensis***

*C. lineata* var. *keyensis* is endemic to the Lower Florida Keys and occurs within intact pine rockland habitat and occasionally along disturbed roadsides adjacent to pine rockland (80 FR 58538). In the summer of 2015, plants were numerous and reproductive at the few pine rockland sites on Big Pine Key that were not overgrown (i.e. open canopy/fire-prescribed). Our observations also indicate peak flowering season from May until August, which coincides with peak mosquito abatement measures. The breeding system of *C. lineata* was already known from previous work by Liu and Koptur (2003). According to this study, the species is self-compatible, but a 'buzz' pollinating agent is required to release pollen from the poricidal anthers to deposit on the stigmatic surface (Liu and Koptur 2003). The authors also made note of decreased flower visits following an insecticide application, although effects on reproductive success were not considered.

As current agreements between USFWS and Florida Keys Mosquito Control District (FKMCD) stand, *C. lineata* habitat may be buffered from mosquito insecticide drift. Critical habitat was designated in late 2014 for Bartram's scrub-hairstreak (*Strymon acis bartrami*) (79 FR 47222). This designation includes all pine rockland within National Key Deer Refuge where its sole larval host, *Croton linearis*, can potentially occur (79 FR 47222). The designated critical habitat coincides with observed occurrences of *Chamaecrista lineata* var. *keyensis* throughout *C. lineata*'s range. As a result of the critical habitat designation, 'no spray' buffer zones are located in areas occupied by the endangered butterfly. In

addition, adulticide applications are limited in areas where Bartram's scrub-hairstreak has not been observed (unoccupied) but *Croton linearis* may occur. Unoccupied critical-designated habitat buffers limit mosquito insecticide spray to once every 30 days when mosquito landing rates are 'very high' (personal comm. with FKMCD). Regulations also restrict aerial adulticide applications on Big Pine Key to once yearly in the case of emergency situations (personal comm. with FKMCD). During the summer of 2015, FKMCD did not apply adulticides to any critical habitat where *Chamaecrista lineata* var. *keyensis* was observed. Lack of any adulticide treatment in unoccupied critical habitat could have been a result of the unusual drought in the early summer, which decreased FKMCD spray activity throughout the Lower Keys, but further investigation is needed. In summary, critical habitat regulations for Bartram's scrub-hairstreak may possibly extend a benefit to *Chamaecrista lineata* var. *keyensis* populations and its pollinator guild by restricting mosquito insecticide activity to known habitat.

### ***Linum arenicola***

*Linum arenicola* is a small grass-like perennial herb with bright yellow flowers. In the Lower Florida Keys, this species occurs on various habitat types throughout Big Pine Key and along one disturbed road-side on Lower Sugarloaf Key. It has also been documented to occur in very small numbers on other islands in the Lower Keys (80 FR 58538). We observed the species in various habitat types with varying degrees of disturbance; however, all occurrences were in sites with a completely open canopy and sub-canopy. It was frequently found alongside various grasses, *Agalinis fasciculata*, *Chamaecrista lineata* var. *keyensis*, *Rhynchospora colorota*, *Samolus ebracteata*, *Piriqueta cistoides* subsp. *caroliniana* and a few other low growing herbs.

The breeding system of *L. arenicola* was unknown before we started research, but studies of closely related species suggest self-incompatibility and fly pollination (Kearns and Inouye 1994). Our observations indicate that this species flowers year-round but peak flowering season is from April until September. During the summer 2015, we conducted preliminary breeding system analysis of *L. arenicola*, including flower visitor observations. From preliminary pollinator exclusion experiments, *L. arenicola* is apparently automatically self-compatible. Sites that were not treated with adulticides had slightly higher fruit set rates than treated sites and pollinator excluded experimental trials. These results indicate that the species may be facultative for automatic pollination in the absence of efficient pollinating agents. We will be conducting an extensive pollination system analysis beginning the next flowering season, as well as multiple year reproductive studies to assess if inbreeding depression exists in the absence of pollinating agents. Several species of small bees frequented flowers during our observations at untreated sites, while visitation was much less frequent at the treated site. Pollinator populations fluctuate seasonally, yearly, and geographically (Wolda and Roubik 1986, Roubik 2001), so we propose a multi-season study over two years.

The population on Lower Sugarloaf Key is potentially highly vulnerable to multiple threats. This population is the only large population in the Lower Florida Keys outside of the population on Big Pine Key. It occurs on road-side gravel on one side of the road in about a 1 km long strip. Service vehicles frequently pull over and park for short periods of time along this strip. Additionally, the population was completely mowed in early August before peak flowering was finalized. Although yearly mowing may benefit the species when fire is not prescribed, timing of optimum mowing needs to be further investigated.

I hope this review is informative for the listing process. Please feel free to contact Dr. Suzanne Koptur or myself for further information. I will send additional information as my research is complete. Thank you for giving us the opportunity to comment on the listing of these two imperiled species.

Sincerely,

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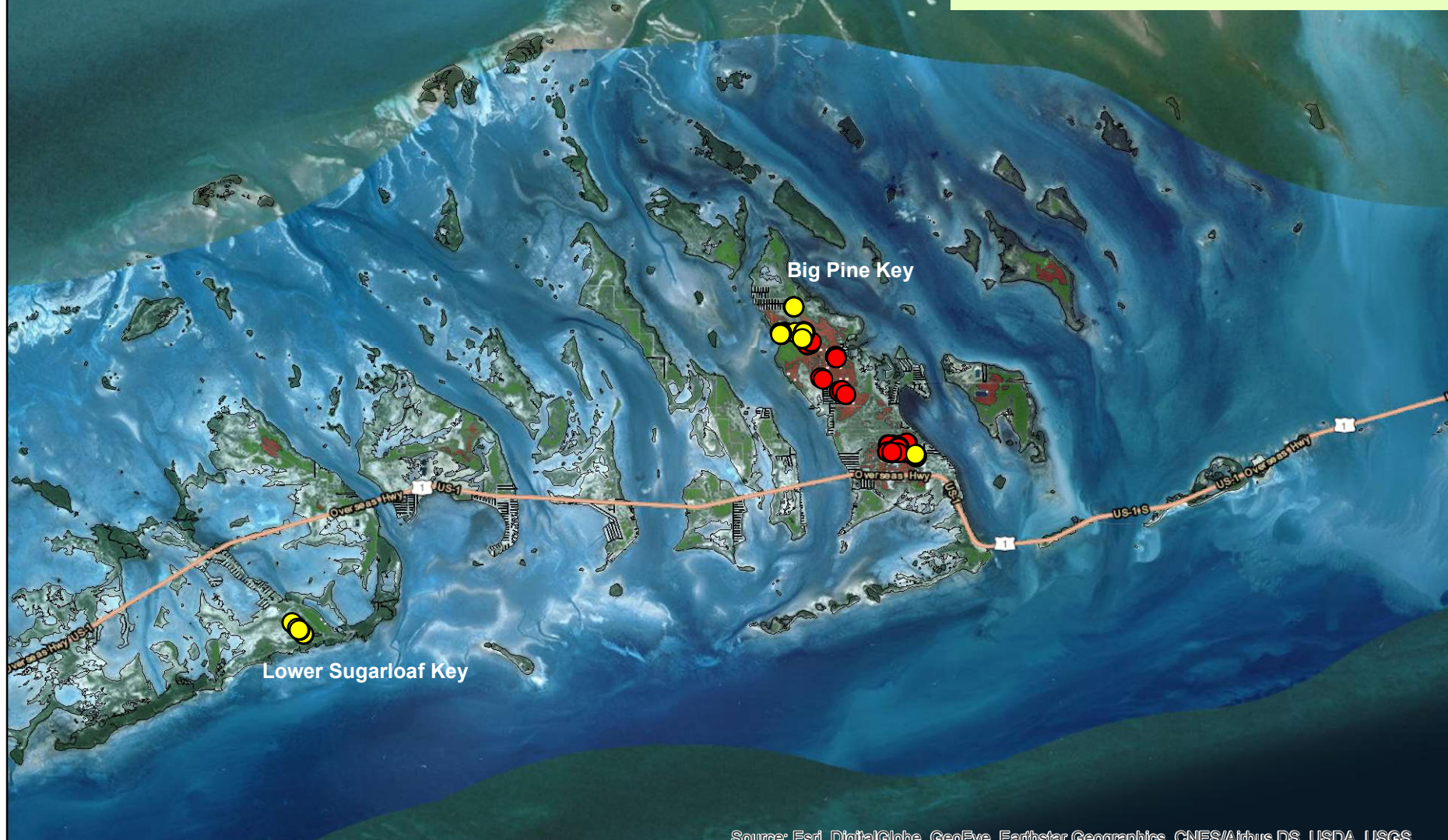
# Lower Florida Keys

## Species

- CHALIN *Harris-Chamaecrista lineata* var. *keyensis*
- LINARE *Harris-Linum arenicola*

## Habitat Type

- Hammock
- Pineland
- IRC\_L.arenicola



Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community, Esri, HERE, DeLorme, MapmyIndia, © OpenStreetMap contributors





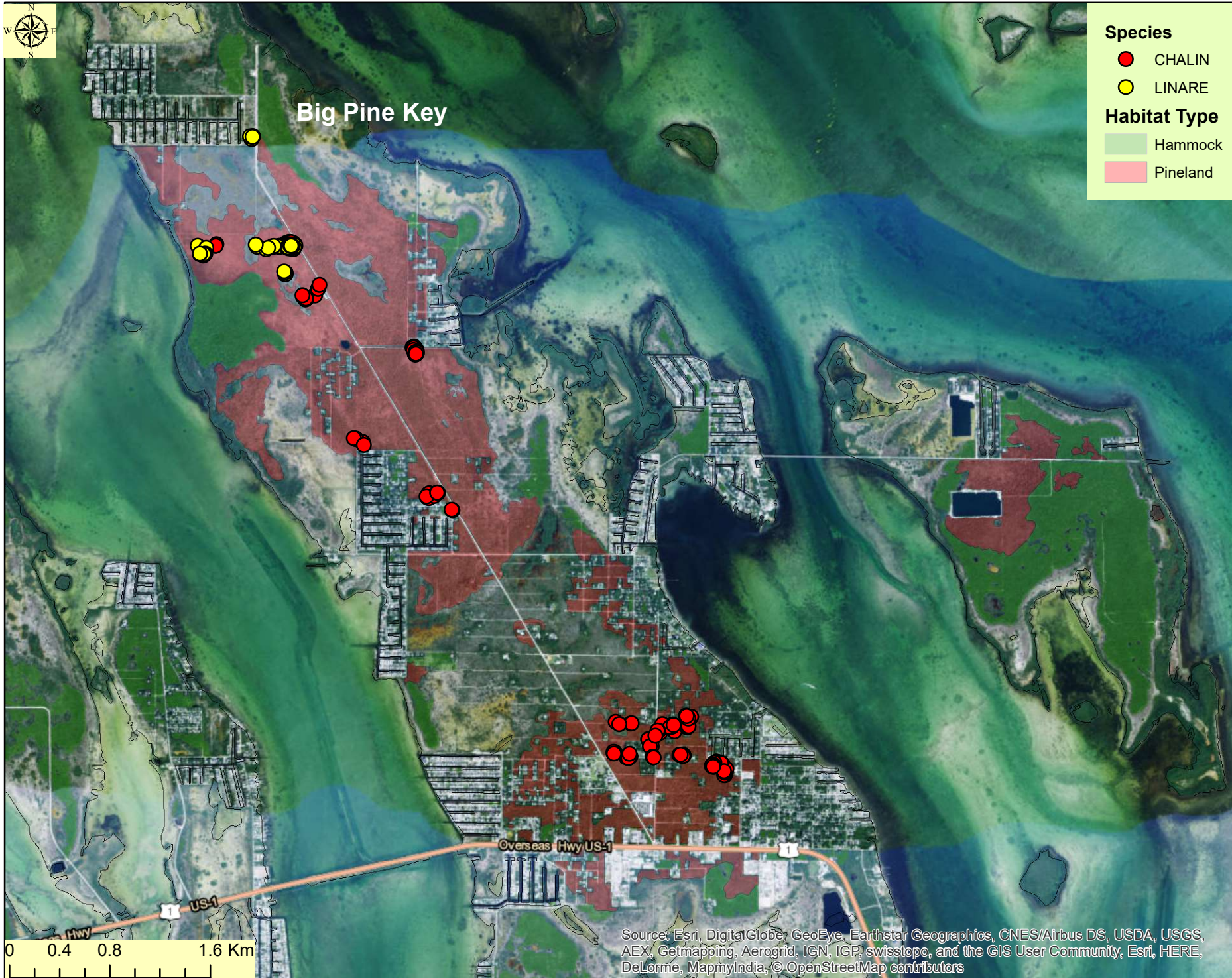
Big Pine Key

**Species**

- CHALIN
- LINARE

**Habitat Type**

- Hammock
- Pineland



Source: Esri, DigitalGlobe, GeoEye, Earthstar Geographics, CNES/Airbus DS, USDA, USGS, AEX, Getmapping, Aerogrid, IGN, IGP, swisstopo, and the GIS User Community, Esri, HERE, DeLorme, MapmyIndia, © OpenStreetMap contributors



# 2015 *Linum arenicola*

