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SYSTEMATICS OF THE UNIFOLIOLATE FLORIDIAN *LUPINUS* CLADE (LEGUMINOSAE: PAPILIONOIDEAE)

EDWIN L. BRIDGES
Botanical and Ecological Consultant
Gig Harbor, Washington 98335
ebridges1@gmail.com

STEVE L. ORZELL
Avon Park Air Force Range
Avon Park Air Force Range, Florida 33825
steve.orzell@us.af.mil

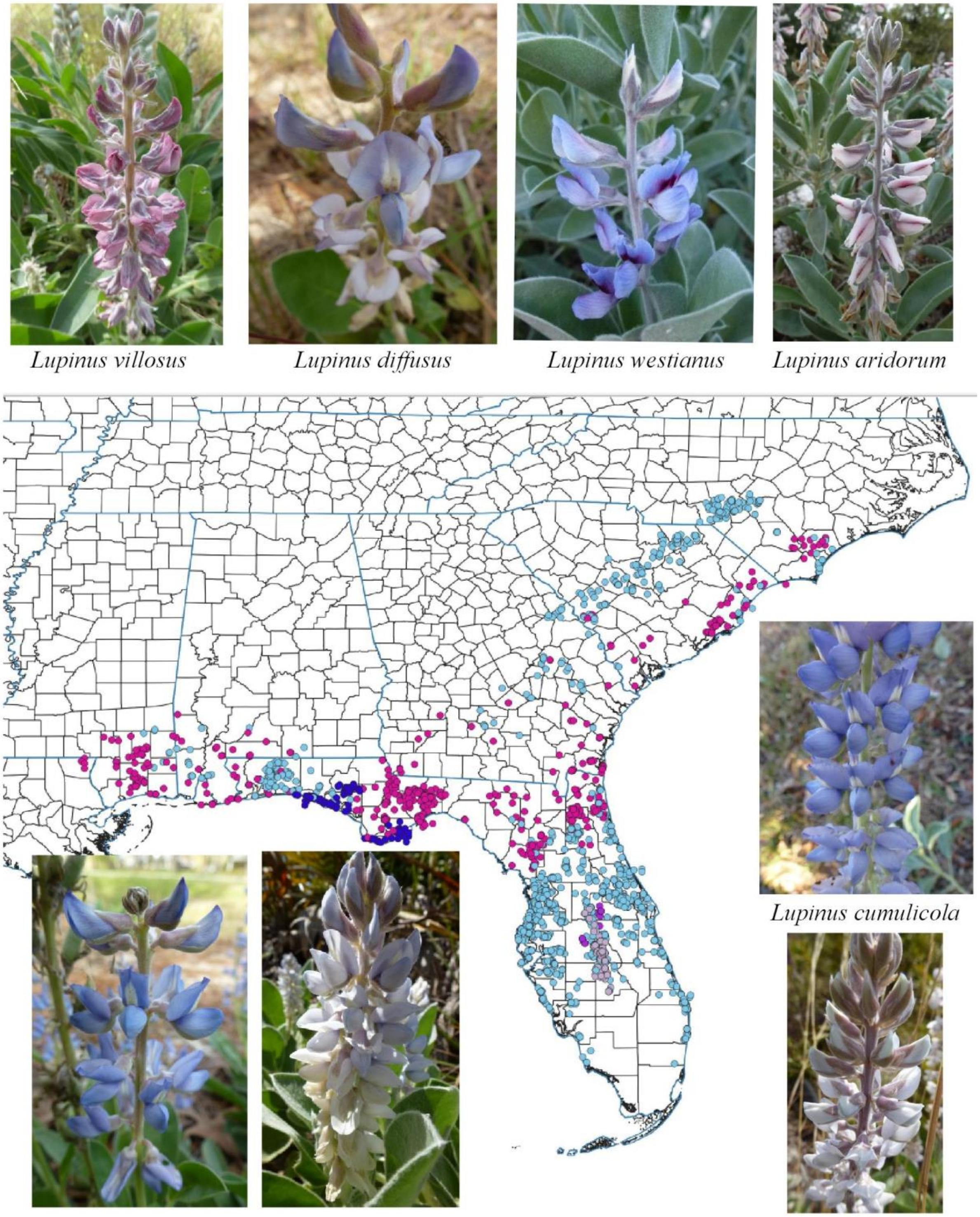
ABSTRACT

Large-scale genome-wide DNA sequence data are enabling reconstruction of much finer-scale relationships among and within species, prompting renewed focus on analyzing species limits and the trajectories of recent evolutionary diversification. We utilized RADseq data as an additional line of evidence for species delimitation within the Florida unifoliolate group of *Lupinus*, a recently diversified clade occurring on xeric sand ridges and uplands within the North American Coastal Plain biodiversity hotspot harboring many narrowly restricted endemic plant species. This clade was previously thought to comprise between three and five taxa, but with no consensus about species limits, which has impacted conservation efforts to protect endangered taxa. Using an integrative taxonomic approach and drawing on evidence from this new phylogeny, morphology, geography, and ecology, we recognize eight species within this clade. *Lupinus aridorum*, previously placed in synonymy or treated as a variety of *L. westianus* by many authors, is recognized as a genetically, geographically, and morphologically distinct species. The former concept of the widespread taxon *L. diffusus* is polyphyletic and is here re-circumscribed in a narrower sense to encompass populations from the Florida Panhandle northward. In peninsular Florida, material previously assigned to *L. diffusus* is here assigned to four species within the *L. cumulicola* complex — *L. cumulicola* and the new species **Lupinus floridanus** Bridges & Orzell, **sp. nov., Lupinus ocalensis** Bridges & Orzell, **sp. nov.**, and **Lupinus pilosior** Bridges & Orzell, **sp. nov.** Although there is some evidence of gene flow and hybridization between these species, they show consistent morphological diversification correlated with geography and ecology. Our study demonstrates the utility of RADseq data to shed light on species limits and relationships within a recently diversifying plant clade. We present a new taxonomic conspectus of the Florida unifoliolate *Lupinus* clade and discuss its evolutionary diversification in relation to the recent geological and biogeographic history of Florida.

Integrative taxonomy (Dayrat 2005) that combines multiple data sources (morphologic, genetic, geographic, and ecologic) to test species hypotheses can provide a more thorough approach to unraveling evolutionary complexities than traditional specimen-based taxonomy. The use of genetic data in a phylogenetic or coalescent context, and inclusion of dense sampling of multiple accessions representing intraspecific diversity across species ranges, is revealing many cases of species non-monophyly and cryptic species via reciprocal illumination of genetic, morphological, ecological and geographical evidence (e.g. Vietes et al. 2009; Dauphin et al. 2014; Pennington and Lavin 2015; McClelland 2023), even in very well-studied clades (e.g. Darwin's finches; Lamichhaney et al. 2015). Recent advances in species delimitation methods and the increasing availability of larger genome-wide DNA sequence data sets using next generation sequencing can be used to explore species limits much more thoroughly and exhaustively than ever before (e.g. Fujita et al. 2012; Wagner et al. 2013; Lamichhaney et al. 2015). In particular, RADseq (Baird et al. 2008) data have renewed interest in integrative taxonomy, offering the potential to quickly generate large genome-wide DNA sequence datasets for large numbers of accessions, thereby contributing robust genetic evidence about species limits and relationships at the species boundary, even with possible incomplete lineage sorting and conflict among individual gene trees (Eaton and Ree 2013; Wagner et al. 2013; Pante et al. 2015a). One advantage of such datasets is the potential to discover monophyletic species clades using very high numbers of loci from across the genome. Wagner et al. (2013) showed that a set of very recently diverged morphologically-defined Lake Victoria cichlid fish species could be robustly resolved as reciprocally monophyletic species clades with dense sampling including multiple accessions of all species. RADseq data thus present new opportunities in taxonomy, but its uptake within botany is quite recent, with efforts so far focusing mainly on resolving species relationships (Eaton and Ree 2013; Mort et al. 2015; Wessinger et al. 2016). Few RADseq studies have sampled large numbers of accessions per species to investigate species limits, and this potential remains largely untested and untapped (but see Wagner et al. 2013; Pante et al. 2015a,b; Herrera and Shank 2016; Atchison et al. 2016; Contreras-Ortiz et al. 2018). With dense sampling of populations and individuals RADseq data, which span large numbers of loci and SNPs scattered across the genome, present opportunities for both phylogenetic / phylogeographic analysis and demographic analysis of geneflow, thus potentially spanning phylogenetic and tokogenetic relationships and boundaries between species and populations.

Unifoliolate leaves have independently evolved twice in the genus *Lupinus*, in the southeastern North American Coastal Plain and in eastern South America. Although Dunn (1971), thought that these somewhat similar species groups may have represented a case of long-distance disjunction, genetic analysis has shown these to be two clearly independently derived lineages (Eastwood et al. 2008, Drummond et al. 2012). The unifoliolate Florida clade of *Lupinus* (Fig. 1) is traditionally taken to comprise between three and five species (Dunn 1971; Beckner 1982; Isely 1986), but the status of most of these has been questioned at one time or another, and there is no current consensus about how many species should be recognized. Furthermore, intensive field collecting over the last decade has revealed evidence for additional geographically structured morphological variation across peninsular Florida (Fig. 2), raising the possibility of recognizing one or more additional species. In previous phylogenetic analyses (Eastwood et al. 2008; Drummond et al. 2012) the Florida unifoliolate clade is robustly supported and is moderately supported as sister to the Old World *Lupinus* clade, except in one study where a single Florida taxon was sampled and found to be nested within the Old World *Lupinus* clade with weak support (Keller et al. 2017). These close relationships to the Old World *Lupinus* taxa are in line with chromosome numbers for Florida *Lupinus*, which at 2n=52, show closer affinity to the Old World *Lupinus albus*, *L. micranthus* and *L. luteus* (2n=50-52) than to the other New World lineages (2n=36/48) (Conterato and Schifino-Wittmann 2006; Eastwood et al. 2008). While the divergence time estimate for the split between the Old World and Florida clades in a recent time-calibrated phylogeny of the genus is c. 10 Ma, diversification of the Florida clade is estimated to be exceptionally recent, with a crown node estimate of 0.9 Ma (Drummond et al. 2012). The lupines of Florida represent one of two independent derivations of unifoliolate from digitately compound leaves within the genus, the other in eastern South America (Eastwood et al. 2008), such that the Florida group is both morphologically and phylogenetically distinct, as well as geographically separated, from almost all other North American *Lupinus*, whose diversity is concentrated in western regions (Drummond et al. 2012).

The species of the Florida unifoliolate clade occur on xeric sands across northern and Peninsular Florida, with two species, *L. diffusus* and *L. villosus*, extending north into Alabama, Georgia, and North and South Carolina, and rarely west to southeastern Louisiana (Fig. 1; Dunn 1971; Isely 1986, 1998). These habitats generally occur on fragmented and isolated areas of predominately xeric upland sands and have notable concentrations of vascular plant endemism (Christman and Judd 1990; Estil and Cruzan 2001; Sorrie and Weakley 2001; Menges et al. 2007), forming regionally distinct hotspots of biodiversity with nationally important unique biota. While



Lupinus villosus *Lupinus diffusus* *Lupinus westianus* *Lupinus aridorum*

Lupinus cumulicola

Lupinus ocalensis *Lupinus pilosior* *Lupinus floridanus*

Figure 1. General distribution of the Floridian unifoliolate clade of *Lupinus* - based on verified records from iNaturalist as of November 16, 2023. *Lupinus aridorum* - purple; *Lupinus westianus* - dark blue; *Lupinus villosus* - magenta; *Lupinus diffusus* - light blue from Florida Panhandle and Georgia northward; *Lupinus cumulicola* (sensu stricto) - lavender; other species of the *Lupinus cumulicola complex* - light blue records in peninsular Florida.

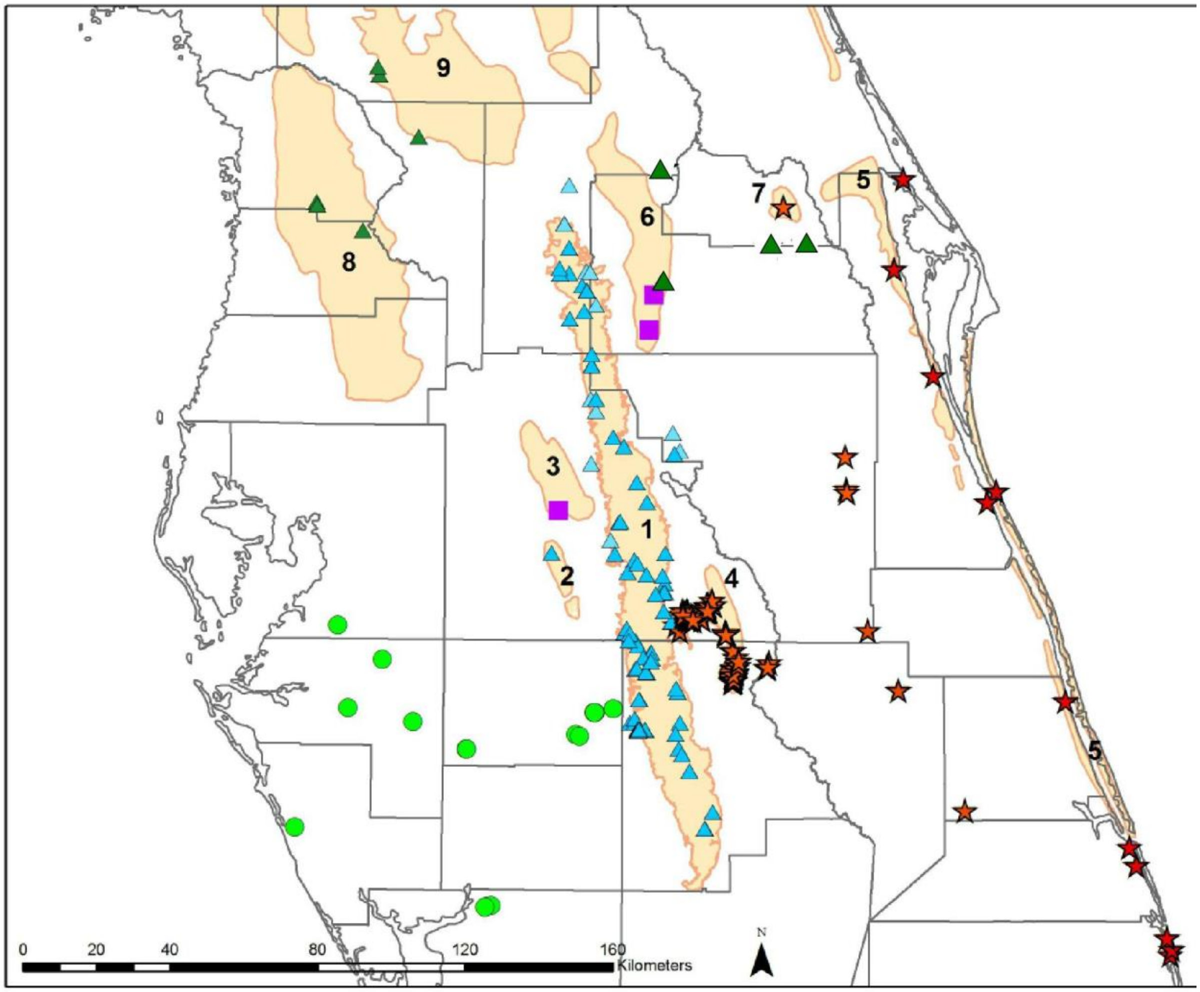


Figure 2. Map of central peninsular Florida showing the distribution of *Lupinus* species in relationship to the major sand ridges of this region, which are numbered as follows: 1 - Lake Wales Ridge; 2 - Lake Henry Ridge; 3 - Winter Haven Ridge; 4 - Bombing Range Ridge; 5 - Atlantic Coastal Ridge (discontinuous); 6 - Mount Dora Ridge; 7 - Geneva Hill; 8 - Brooksville Ridge; 9 - Sumter Upland. *Lupinus* populations sampled – *L. aridorum* (purple squares); *L. cumulicola* (blue triangles); *L. floridanus* (red stars); *L. pilosior* (green circles); *L. ocalensis* (green triangles). No other unifoliolate *Lupinus* species occur within the area depicted.

these endemic-rich inland sand ridges are older than the coastal sand dune systems and habitats, all of these formations are fundamentally recent, reflecting the very mobile shorelines and the dramatic impacts of late Miocene, Pliocene and especially Pleistocene sea-level fluctuations across the very low-lying topography of the Florida peninsula (Fig. 1; Locker et al. 1996; Hine 2013; see Germain-Aubrey et al. 2014: Fig. 1; Krysko et al. 2016: Fig. 3). Through the Pliocene, sea-level highs of 20 meters or more above current levels submerged substantial parts of Florida with the peninsula reduced to a set of islands. Even at the last Pleistocene glacial minimum 125 Kyr ago, sea-level was 6m higher than now, such that virtually the whole of present-day southern Florida and significant coastal areas would have been inundated, generating the coastal dune systems clearly visible today and which also harbor isolated *Lupinus* populations (e.g. Atlantic Coastal Ridge populations – see below). In contrast, at the last glacial maximum ca. 20 Kyr ago, with sea-level ca. 120m lower than today, shorelines were dramatically altered such that Florida was more than twice the area it is today, with the entire modern coastline formed over just the last few 1,000 years as a result of slow sea-level rise, thus allowing for some theoretical connections between formerly isolated populations (Locker et al. 1996; Hine 2013; see Germain-Aubrey et al. 2014, Fig. 1). This recency of many of the habitats where *Lupinus* occurs is in line with the recent divergence time estimate of < 1 Myr for the clade as a whole, and forms the backdrop for understanding patterns of evolutionary diversification within Florida.

This dynamic recent geological history of Florida is correlated with similarly dynamic changes in the extent and distributions of the xeric sand habitats where *Lupinus* occurs. Upland areas of Florida harbor two xeric classes of southeastern USA evergreen ecosystems: sandhill and Florida scrub (i.e. sand pine scrub, low oak scrub, rosemary scrub and scrubby pinelands). Both habitats are pyrogenic grasslands or shrublands with variable pine overstory physiognomy, with scrub communities often dominated by resprouting xeric oaks, decumbent palms, and clonal ericaceous shrubs (Menges and Hawkes 1998). Scrubby pinelands are found on poorly drained spodosols while sandhill and scrub occur on well to excessively drained (sandhill and scrub), nutrient-poor entisols and rarely spodosols (Menges and Hawkes 1998; Myers 1985). Sandhill, with its diverse herbaceous and C4 grass dominated groundcover, is subject to frequent, low-intensity ground fires. In contrast, Florida scrub (sand pine, rosemary, and oak scrub) burn infrequently, yet because of higher fuel build-up, can burn much more intensely, often resulting in stand replacement fires in sand pine (Myers 1985). The past 30 kyr have seen extensive changes in the extent of both these habitats. In addition, sand pine scrub habitat is found along the very recent Pleistocene shoreline sand dune systems (Hine 2013). Recent research to understand the origins of the narrowly endemic Florida sand-ridge scrub biota has attempted to ascertain the sister group relationships and biogeographic affinities of specific endemic sand ridge taxa (Germain-Aubrey et al. 2014), and assess whether they are related to species and lineages in the western USA originating via vicariance associated with mid-Pliocene scrub habitat fragmentation, or instead to eastern USA. species and lineages that diverged more recently during the Pleistocene. The landmark study by Germain-Aubrey et al. (2014) suggested that three of the four narrow Florida sand ridge endemics assessed show affinities to eastern North American species and lineages, and estimated divergence times spanning both the Pliocene and Pleistocene.

Over the last 220 years there have been at least 20 taxonomic and floristic accounts covering *Lupinus* species belonging to the unifoliolate Florida clade (Willdenow 1802; Nuttall 1818; Torrey and Gray 1840; Chapman 1860; Small 1903, 1926, 1933; Phillips 1955; Wilbur 1963, 1968; Dunn 1971; Beckner 1982; Wunderlin 1982; Clewell 1985; Isely, 1986, 1990, 1998; Duncan and McCartney 1992; Wunderlin and Hansen 2008, 2011; Weakley 2023; Sholars and Riggins 2023). The inconsistencies among all these taxonomic accounts are stark. The number of species recognized ranges from one to five (seven in Weakley 2023, two of these unnamed placeholders based on this manuscript), and there is still no consensus today about species limits, the number of species, nor the status of the potentially critically endangered *Lupinus aridorum* McFarlin ex Beckner (Wunderlin and Hansen 2011; Ricono et al. 2015).

The first species of the group to be described was *Lupinus villosus* Willd., in 1802 from a population in Southport, North Carolina (Willdenow, 1802). Shortly after, *Lupinus diffusus* Nutt. was described by Nuttall (1818) from nearby Wilmington in North Carolina, though this species was subsequently reduced to a variety of *L. villosus* by Torrey and Gray (1840), only to be later resurrected as a distinct species by Chapman (1860). In his account of the Flora of the Southeastern United States, Small (1903) followed Chapman in recognizing *L. diffusus* and *L. villosus* as distinct species. Small (1926) later described a third species, *Lupinus westiana* [sic] Small based on material from the St. Andrews Bay region of the Florida Panhandle. In an updated version of his earlier flora, Small (1933) listed the three previously recognized species, *L. diffusus, L. villosus* and *L. westianus*, and described a fourth species, *Lupinus cumulicola* Small from DeSoto County (now Highlands County) in peninsular Florida.

In his revision of North American *Lupinus*, Phillips (1955) reduced the four previously described taxa to a single species — *L. villosus*, recognizing *L. diffusus* as a variety, and *L. cumulicola* and *L. westianus* as conspecific with *L. villosus* var. *villosus*. Then, for the second time in its history, *L. diffusus* was reinstated as a distinct species by Wilbur (1963, 1968) in accounts of *Lupinus* for North Carolina and the Carolinas. Dunn's (1971) treatment of the group followed Small's (1933) delimitation of four species. In 1982, a fifth species, *Lupinus aridorum* McFarlin ex Beckner was described by Beckner, after he saw a manuscript dating from 1935 that gave a short descriptive account but did not validly publish this name (Beckner 1982). Clewell's (1985) Guide to the Vascular Plants of the Florida Panhandle lists three species, *L. diffusus*, *L. villosus* and *L. westianus*. Isely (1986) was the first botanist to reduce *L. aridorum* from species rank to a variety of *L. westianus* because the sole obvious exomorphic difference from *L. westianus* was the flower color. Later in his account of *Lupinus* for the Vascular Flora of the Southeastern United States, Isely (1990) recognized just three species, maintaining *aridorum* as a variety of *L. westianus*, and treating *L. cumulicola* as no more than a peninsular Florida form of the conspecific *L. diffusus*. Subsequently, Duncan and McCartney (1992) disagreed with Isely's (1990) treatment of *L. cumulicola* as conspecific with *L. diffusus*, listing six consistent and significant differences, besides plant height, between the two species. Despite this, Isely (1998) maintained his previous classification of three species for his account in Legumes of the United States, and recent accounts have largely followed Isely's treatment (e.g. Wunderlin and Hansen, 2008; 2011; Sholars and Riggins 2023). Furthermore, alongside these oscillations in the taxonomic history of the group, the original motivation for our study came from extensive and detailed field studies and new field collections that suggested up to nine putative morphological entities with largely non-overlapping distributions (Figs. 1 and 2) and raising the possibility that additional species should be recognized, and the need to test these using molecular data.

This persistent lack of consensus about the taxonomy of this small group of species has confounded assessments of their conservation status, with several contradictory viewpoints depending on what taxonomy is used (U.S. Fish & Wildlife Service 1987; McCoy and Mushinsky 1992; FNAI 2000; Ward et al. 2003; Bibb et al. 2007; Contu 2012; Bupp et al. 2017; Richardson et al. 2014; Nature Serve 2015; Ricono et al. 2015). The scrub lupine, *L. aridorum*, is both federally (Fish & Wildlife Service, 1987) and State listed as Endangered, and *L. westianus* is State listed as Threatened. However, treating these two species as conspecific at varietal rank (Isely, 1986) reduces their conservation ratings (e.g. Contu 2012) and this has resulted in contradictory conservation status assessments, some of which make no reference to taxonomy.

Even when it was first described in 1982 *L. aridorum* was considered to be very rare and nearly extinct due to native habitat loss and disturbance (Beckner, 1982), leading to its federal listing by the US Fish & Wildlife Service (USFWS) as Endangered in 1987. Similarly, McCoy and Mushinsky (1992) make no mention of *L. westianus*, nor treatment of *L. aridorum* at varietal rank, in their assessment of *L. aridorum* as one of the rarest species in the sand pine scrub of Florida. While the US Fish & Wildlife Service (USFWS) has maintained this original listing of *L. aridorum* at the species level as Endangered (Bibb et al. 2007), other conservation assessments have followed Isely's (1986) treatment of *L. aridorum* and *L. westianus* as conspecific varieties (FNAI 2000; Contu 2012). In a global review in 1995 NatureServe listed *L. westianus* var. *aridorum* as critically imperiled, citing the taxon's narrow range, low abundance and pressures on habitats, whereas in 2004, it was changed to Vulnerable (NatureServe 2015). Similarly, despite the USFWS Endangered listing of *L. aridorum* at species level, IUCN categorized *L. westianus* only as Near Threatened (Contu 2012), while acknowledging that the variety has often been considered a distinct species. Christman and Judd (1990) and FNAI (2000) also followed Isely's taxonomy listing *L. westianus* var. *aridorum* as Endangered, as did Ward et al. (2003), who rated *L. westianus* var. *aridorum* as Threatened (as opposed to Endangered if treated as a distinct species), using the numerical ranking system mandated by the Florida legislature. Others studying rare plant species of Florida (e.g. Estill and Cruzan 2001) seem to have chosen to ignore *L. aridorum* altogether. In contrast, conservation researchers and practitioners working on the ground to promote the conservation of *L. aridorum* do not appear to question its status as a distinct species (Bupp et al. 2017; Richardson et al. 2014; Ricono et al. 2015; Peterson et al. 2012; Peterson 2016). However they are treated, *L. aridorum* and *L. westianus* occupy geographically disjunct ranges and both are globally rare, narrowly restricted endemics and are likely to be independently threatened. These conflicting conservation assessments highlight the need to revisit the taxonomy of these species with a more robust and rigorous evidence-based approach.

The very recent diversification of the Florida clade of *Lupinus* across the recent geology of the Florida peninsula provides an excellent test study of the utility of nextRAD DNA sequence data for resolving relationships and delimiting species within a recently evolved clade. We generate a densely sampled phylogeny alongside demographic analyses to estimate geneflow and split times between pairs of putative species (Nevado et al. 2024), and use these to re-examine species boundaries and delimitation in an integrative framework using genetic, morphological and geographical lines of evidence. Building upon this, we present a new taxonomic conspectus for the clade and discuss the implications for assessing the rarity of these species and understanding the evolutionary diversification of xeric sand endemic plants in Florida.

METHODOLOGY

Geographical, ecological, and morphological data were assembled using herbarium collections and fieldwork in order to survey and collect material of living plants throughout Florida. Specimens or digital images of 596 herbarium collections were examined from FTG, FLAS, FSU, MICH, SWF and USF (acronyms follow Thiers [continuously updated]). Specimen data, the Institute for Regional Conservation floristic database covering south Florida, and floristic lists from Brevard County Environmental Areas and Lake Wales Ridge conservation sites were used to map potential lupine populations. Maps of xeric soil map units from NRCS county soil surveys and aerial imagery were used to identify xeric vegetation (sandhill, scrub, and scrubby pinelands). Although we prioritized sites with intact xeric vegetation, we also included disturbed xeric sandy sites, since populations of *Lupinus* often persist in degraded habitats. We made multiple visits to some sites due to fluctuating populations, with plants absent during unfavorable years, followed by episodic mass flowering in subsequent years. We conducted field surveys at over 300 Florida sites in 35 counties, with an emphasis on central peninsular Florida, where we had recognized cryptic morphospecies (groups of biological organisms which differ in one or more morphological traits from similar groups). Most sites were visited during peak flowering, primarily in the late dry season (February to early May). At each population, we collected silica-dried leaf material and voucher specimens, recorded field morphological characters (12 characters from 3-10 plants per population) and ecological/floristic information (surface soil color, habitat, vegetation, associated plants), and photographed plant growth forms, habitats, inflorescences, leaf lamina and indumentum, and stipules. Final site visits during the early wet season allowed us to collect mature fruits and seeds from some populations. We plotted GPS locations using ArcView overlaid onto physiographic and soils data layers to determine the position of our sample sites in relation to Florida xeric ridges and soil series. Three to 34 individuals were sampled from across the geographic range of each of the nine putative morphological entities recognized during fieldwork (see below), producing a total of 106 accessions (Table 1). Leaf samples were collected from wild plants and dried in silica-gel. In addition, the Mediterranean narrow-leafed lupine (*Lupinus angustifolius* L.) was included as an outgroup. Locality details for all nextRAD sequenced individuals are listed in Table 1. The details of methods for the nextRAD preparation and sequencing, assembly, phylogeny, hybrid status assessment, and demographic analysis are provided in Nevado et al. 2024. For conservation status we used the NatureServe global ranking system to determine rarity status of all the unifoliate-leaved *Lupinus*, which are presented under each species in the taxonomic treatment section.

SAMPLE	SPECIES AND PUTATIVE ID	COUNTY	LATITUDE	LONGITUDE	PHYSIOGRAPHIC REGION
F01	Lupinus EFL inland (floridanus)	Highlands	27.56831	-81.25682	Bombing Range Ridge
F02	Lupinus EFL inland (floridanus)	Highlands	27.56594	-81.25857	Bombing Range Ridge
F03	Lupinus EFL inland (floridanus)	Highlands	27.57027	-81.25580	Bombing Range Ridge
F04	Lupinus cumulicola	Polk	27.69177	-81.42891	Osceola Plain
F06	Lupinus NFL (ocalensis)	Polk	27.66954	-81.40775	Osceola Plain
F07	Lupinus cumulicola	Highlands	27.32699	-81.37785	Lake Wales Ridge
F08	Lupinus SWFL (pilosior)	Charlotte	26.99194	-81.93160	Gulf Coastal Lowlands
F09	Lupinus SWFL (pilosior)	Charlotte	26.99386	-81.92037	Gulf Coastal Lowlands
F10	Lupinus SWFL (pilosior)	Charlotte	26.99015	-81.93623	Gulf Coastal Lowlands
F11	Lupinus SWFL (pilosior)	Hardee	27.46787	-81.63859	Desoto Plain
F12	Lupinus SWFL (pilosior)	Hardee	27.47747	-81.58846	Desoto Plain
F13	Lupinus EFL ACR (floridanus)	Palm Beach	26.87337	-80.06298	Eastern Valley
F14	Lupinus EFL ACR (floridanus)	Palm Beach	26.88750	-80.05957	Eastern Valley
F16	Lupinus EFL ACR (floridanus)	Martin	27.09314	-80.15429	Eastern Valley
F17	Lupinus EFL ACR (floridanus)	Martin	27.13826	-80.17301	Eastern Valley
F18	Lupinus EFL ACR (floridanus)	St. Lucie	27.49694	-80.34546	Atlantic Coastal Ridge
F19	Lupinus intermediate Cornwell	Highlands	27.38399	-81.12608	Okeechobee Plain
F20	Lupinus SWFL (pilosior)	Hardee	27.41373	-81.69132	Desoto Plain
F21	Lupinus SWFL (pilosior)	Hardee	27.40979	-81.68069	Desoto Plain
F22	Lupinus SWFL (pilosior)	Hardee	27.46782	-81.64036	Desoto Plain
F23	Lupinus EFL inland (floridanus)	Okeechobee	27.52509	-80.80540	Osceola Plain
F24	Lupinus EFL inland (floridanus)	Osceola	27.67150	-80.88952	Osceola Plain
F25	Lupinus EFL inland (floridanus)	St. Lucie	27.22896	-80.62253	Osceola Plain
F26	Lupinus EFL inland (floridanus)	Highlands	27.59209	-81.16094	Osceola Plain
F27	Lupinus intermediate LHR	Polk	27.81238	-81.63848	Polk Upland
F28	Lupinus cumulicola	Polk	27.85955	-81.76103	Lake Henry Ridge
F29	Lupinus cumulicola	Polk	27.84189	-81.53211	Lake Wales Ridge
F30	Lupinus cumulicola	Polk	27.81323	-81.54915	Polk Upland
F31	Lupinus intermediate LHR	Polk	27.72599	-81.69546	Lake Henry Ridge
F32	Lupinus intermediate LHR	Polk	27.70899	-81.69522	Lake Henry Ridge
F33	Lupinus cumulicola	Osceola	28.31971	-81.65262	Lake Wales Ridge
F34	Lupinus cumulicola	Lake	28.60611	-81.71619	Lake Wales Ridge
F35	Lupinus NFL (ocalensis)	Sumter	28.87600	-82.13698	Sumter Upland
F36	Lupinus NFL (ocalensis)	Marion	29.02657	-82.24758	Sumter Upland
F37	Lupinus NFL (ocalensis)	Marion	29.04645	-82.25219	Cotton Plant Ridge
F38	Lupinus villosus	Levy	29.30333	-82.44900	Western Valley
F39	Lupinus NFL (ocalensis)	Citrus	28.71392	-82.41848	Brooksville Ridge
F40	Lupinus NFL (ocalensis)	Citrus	28.70666	-82.41815	Brooksville Ridge
F41	Lupinus NFL (ocalensis)	Hernando	28.64614	-82.28991	Brooksville Ridge
F42	Lupinus intermediate HHSP	Highlands	27.42841	-81.51595	Desoto Plain
F43	Lupinus intermediate HHSP	Highlands	27.42844	-81.51523	Desoto Plain
F44	Lupinus SWFL (pilosior)	Manatee	27.47516	-82.31790	Desoto Plain
F45	Lupinus SWFL (pilosior)	Manatee	27.44212	-82.13827	Desoto Plain
F46	Lupinus SWFL (pilosior)	Manatee	27.59451	-82.22452	Polk Upland

SAMPLE	SPECIES AND PUTATIVE ID	COUNTY	LATITUDE	LONGITUDE	PHYSIOGRAPHIC REGION
F47	Lupinus SWFL (pilosior)	Hardee	27.37609	-81.99140	Desoto Plain
F48	Lupinus aridorum	Polk	27.96316	-81.74261	Winter Haven Ridge
F50	Lupinus EFL inland (floridanus)	Polk	27.72431	-81.33097	Osceola Plain
F51	Lupinus intermediate LHR	Polk	27.76074	-81.69462	Polk Upland
F52	Lupinus intermediate LHR	Polk	27.87020	-81.67029	Polk Upland
F53	Lupinus cumulicola	Polk	27.66582	-81.55852	Lake Wales Ridge
F54	Lupinus cumulicola	Highlands	27.56827	-81.49815	Intraridge Valley
F55	Lupinus cumulicola	Highlands	27.51999	-81.41148	Lake Wales Ridge
F56	Lupinus SWFL (pilosior)	Sarasota	27.18080	-82.45975	Gulf Coastal Lowlands
F57	Lupinus SWFL (pilosior)	Hillsborough	27.67823	-82.34806	Desoto Plain
F58	Lupinus cumulicola	Highlands	27.18627	-81.33535	Lake Wales Ridge
F59	Lupinus SWFL (pilosior)	Collier	25.93374	-81.67824	Ten Thousand Islands
F60	Lupinus SWFL (pilosior)	Collier	26.02144	-81.73237	Reticulate Coastal Swamps
F61	Lupinus SWFL (pilosior)	Collier	26.10034	-81.77534	Reticulate Coastal Swamps
F62	Lupinus EFL ACR (floridanus)	Brevard	28.77637	-80.78941	Eastern Valley
F63	Lupinus EFL ACR (floridanus)	Brevard	28.55514	-80.81487	Atlantic Coastal Ridge
F64	Lupinus EFL ACR (floridanus)	Brevard	28.29495	-80.70738	Eastern Valley
F65	Lupinus EFL ACR (floridanus)	Brevard	28.01100	-80.53409	Atlantic Coastal Ridge
F66	Lupinus EFL ACR (floridanus)	Brevard	27.98568	-80.55957	Eastern Valley
F67	Lupinus EFL inland (floridanus)	Seminole	28.61982	-81.06234	Osceola Plain
F68	Lupinus NFL (ocalensis)	Lake	28.80458	-81.45266	Marion Upland
F69	Lupinus NFL (ocalensis)	Lake	28.80485	-81.45347	Marion Upland
F70	Lupinus aridorum	Orange	28.40672	-81.49398	Mount Dora Ridge
F71	Lupinus aridorum	Orange	28.49197	-81.48023	Mount Dora Ridge
F72	Lupinus NFL (ocalensis)	Orange	28.52418	-81.46106	Mount Dora Ridge
F73	Lupinus villosus	Levy	29.49298	-82.63926	Brooksville Ridge
F74	Lupinus villosus	Suwannee	29.96812	-82.77463	Gulf Coastal Lowlands
F75	Lupinus villosus	Leon	30.33375	-84.39161	Lake Munson Hills
F76	Lupinus villosus	Wakulla	30.30203	-84.40051	Relict Bar
F77	Lupinus villosus	Wakulla	30.06922	-84.38826	Gulf Coastal Lowlands
F78	Lupinus villosus	Wakulla	30.03154	-84.40844	Gulf Coastal Lowlands
F79	Lupinus villosus	Gulf	29.71635	-85.26779	Gulf Coastal Lowlands
F80	Lupinus villosus	Gulf	29.70468	-85.29215	Gulf Coastal Lowlands
F81	Lupinus westianus coastal	Gulf	29.67976	-85.33976	Gulf Coastal Lowlands
F82	Lupinus westianus coastal	Walton	30.37813	-86.35517	Gulf Coastal Lowlands
F83	Lupinus diffusus Panhandle	Okaloosa	30.46499	-86.72654	Gulf Coastal Lowlands
F84	Lupinus diffusus Panhandle	Okaloosa	30.63631	-86.49290	Western Highlands
F85	Lupinus diffusus Panhandle	Santa Rosa	30.76421	-86.85542	Western Highlands
F86	Lupinus villosus	Okaloosa	30.72397	-86.72532	Western Highlands
F87	Lupinus diffusus Panhandle	Santa Rosa	30.49854	-86.91159	Gulf Coastal Lowlands
F88	Lupinus westianus coastal	Okaloosa	30.38608	-86.40435	Gulf Coastal Lowlands
F89	Lupinus westianus coastal	Walton	30.34317	-86.13231	Gulf Coastal Lowlands
F90	Lupinus westianus coastal	Bay	30.26213	-85.96373	Gulf Coastal Lowlands
F91	Lupinus westianus inland	Bay	30.39383	-85.68226	Greenhead Slope

SAMPLE	SPECIES AND PUTATIVE ID	COUNTY	LATITUDE	LONGITUDE	PHYSIOGRAPHIC REGION
F92	Lupinus westianus inland	Washington	30.51520	-85.65454	Greenhead Slope
F93	Lupinus westianus inland	Washington	30.48314	-85.62923	Greenhead Slope
F94	Lupinus villosus	Calhoun	30.43003	-85.31950	Fountain Slope
F95	Lupinus villosus	Suwannee	30.38092	-83.16144	Gulf Coastal Lowlands
ELB201	Lupinus cumulicola	Osceola	28.09684	-80.95827	Osceola Plain
ELB207	Lupinus cumulicola	Osceola	28.10442	-81.42192	Osceola Plain
AS223	Lupinus villosus	AL - Covington	31.17017	-86.53997	Western Highlands
AS224	Lupinus villosus	AL - Baldwin	30.70925	-87.58953	Western Highlands
AS225	Lupinus villosus	AL - Escambia	31.02219	-87.30211	Western Highlands
DG226	Lupinus diffusus NCSC	NC - Moore	35.30356	-79.67656	Fall Line Sandhills
DG227	Lupinus diffusus NCSC	NC - Moore	35.15081	-79.47119	Fall Line Sandhills
DG228	Lupinus diffusus NCSC	NC - Richmond	34.96447	-79.60753	Fall Line Sandhills
DG229	Lupinus diffusus NCSC	SC - Chesterfield	34.54147	-80.12456	Fall Line Sandhills

Table 1. Locations of samples used in DNA analysis. Sample numbers match those in the text and in Figure 3. Additional abbreviations for Species and Putative ID column: ACR = Atlantic Coastal Ridge, HHSP = Highlands Hammock State Park, LHR = Lake Henry Ridge.

RESULTS

Field and herbarium surveys suggested that there are cryptic morphological differences between populations, particularly among the peninsular Florida populations of the widespread *L. diffusus* group (Table 1; Fig. 2), which are strongly partitioned geographically. Nine morphospecies were initially identified in the field sampling: *L. aridorum*, *L. cumulicola*, *L. diffusus* Carolina, *L. diffusus* North Florida (NFL), *L. diffusus* Panhandle, *L. diffusus* Southwest Florida (SWFL), *L. diffusus* East Florida (EFL), *L. villosus*, and *L. westianus* (Figs. 1 and 2). All of these putative morphospecies were largely allopatric with only limited range overlap (less than 5% of populations) (Figs. 1 and 2). During fieldwork, a number of putative hybrid populations were identified based on morphological intermediacy between the nine putative morphological entities.

NextRAD data assembly – Sequencing yielded an average of 3,226K reads per sample. After filtering and clustering (at 0.85 threshold) the average number of clusters per sample was 275K. The 74-sample dataset for the structure analysis contained 38,244 SNPs. The 94-sample dataset for phylogenetic inference contained 201,230 loci which were reduced to 153,730 loci after removal of paralogs and further filtering. The concatenated matrix was 15,120K base pairs in length and contained 1,099,079 and 326,141 variable and parsimony informative sites respectively. The 94-sample dataset had ca. 90% missing data, while this is high in comparison to traditional phylogenetic datasets, recent research (Huang and Knowles 2014; Eaton et al. 2017) and preliminary tests showed that missing data do not mislead phylogenetic inference and suggested that high levels of missing data were necessary to retrieve sufficient informative sites. The raw sequences are archived in the NCBI sequence read archive (Nevado et al. 2024: Appendix S1).

Hybrid status assessment – The Structure analysis of all accessions excluding *L. aridorum*, *L. westianus* and *L. villosus* revealed multiple accessions showing evidence of genetic admixture. The best supported model with the largest ΔK value was K = 3. Between the *L. diffusus* EFL and *L. diffusus* SWFL groups one sample was found to have partial admixture. The five putative hybrids from Lake Henry Ridge were all found to have genetic admixture with almost equal genetic contributions from the *L. diffusus* SWFL and *L. cumulicola* groups.

Results for K =4 are also presented given that the K = 4 likelihood was close to K = 3. The overall groupings are similar to K = 3 but the samples showing evidence of admixture are somewhat different. Two samples of *L. diffusus* from Northern Florida are shown to be admixed. A smaller group of *L. diffusus* accessions from the Lake Henry Ridge show admixture, and at K = 4, the two putative hybrids from the Desoto Plain (*L. diffusus* EFL. HHSP - F43 & F32) show evidence of admixture between the *L. cumulicola* and *L. diffusus* SWFL/ *L.diffusus* EFL groups.

Phylogeny – The overall topologies recovered are broadly congruent between the maximum-likelihood and Bayesian analyses (Fig. 3 and Nevado et al. 2024: Figs. S1, S2). The *L. diffusus* samples from North and South Carolina, along with those from the Florida Panhandle, form a grade subtending the remainder of the Florida clade or the *aridorum / westianus / villosus* (AWV) subclade, and are placed quite separately from all the *L. diffusus* accessions from peninsular Florida. The AWV subclade comprising *L. aridorum*, *L. westianus*, and *L. villosus* has high internal support and relatively long internal branches with each of the three species recovered as monophyletic with high support. In addition, there is phylogeographic structure within *L. westianus* with three inland accessions (F91, F92, F93) forming a well-supported subclade that is sister to a subclade comprising the coastal accessions. The large subclade comprising *L. cumulicola*, *L. diffusus* NFL, *L. diffusus* SWFL and *L.diffusus* EFL (hereafter referred to as the *L. cumulicola* subclade) is recovered in all three phylogenies, with variable support. This subclade has comparatively weaker internal support and shorter internal branches than the AWV clade, and while accessions of the putative morphological/geographical entities do mostly group together, the support is not as robust (Fig. 3 and Nevado et al. 2024: Figs. S1, S2) and there are weakly supported incongruencies between the different analyses. After reanalysis of morphology, the multiple accessions of *L. diffusus* EFL all group together (Fig. 3) but may show partial phylogeographic structure with differentiation of accessions from the Atlantic Coastal Ridge from those from inland localities, albeit again with only moderate support.

Demographic analyses – Parameter estimates for the best demographic model for each pair of populations (Nevado et al. 2024: Table 1) show that the best model for all comparisons always involves migration, suggesting that there is some geneflow between all the populations and species tested. Migration levels are higher among populations within central peninsular Florida (*L. cumulicola*, *L. diffusus* SWFL and *L. diffusus* EFL) than between *L. villosus* and *L. westianus*, suggesting that the lower phylogenetic support across the *L. cumulicola* subclade is most likely due at least in part to higher levels of geneflow compared to the better resolved AWV subclade. Estimated split times between species / populations (Nevado et al. 2024: Table 2) vary depending on which population is used to calibrate, suggesting that these estimates should be treated with caution. The estimated divergence time between *L. villosus* and the *L. westianus/ L. aridorum* clade is somewhat older (21 - 65 Kyr) than between populations in the *L. cumulicola* subclade which are estimated to range from 14 to 38 Kyr. Even taking into account the uncertainties surrounding these split time estimates, it is clear that species diversification across the entire Florida unifoliolate clade is very recent, most likely occurring in the late Pleistocene.

Morphometric analyses – The morphometric data collected from 1002 leaves from 527 individual plants, representing all species and potential morphospecies sampled was summarized in Excel spreadsheets and basic statistics were derived for each entity. Not all characters measured were determined to be significant, and the significance of other characters was often apparent only between certain species pairs. Graphs of individual characters and combinations were created and used to visualize significant differences. A few of these are presented here as Figures 9-12.

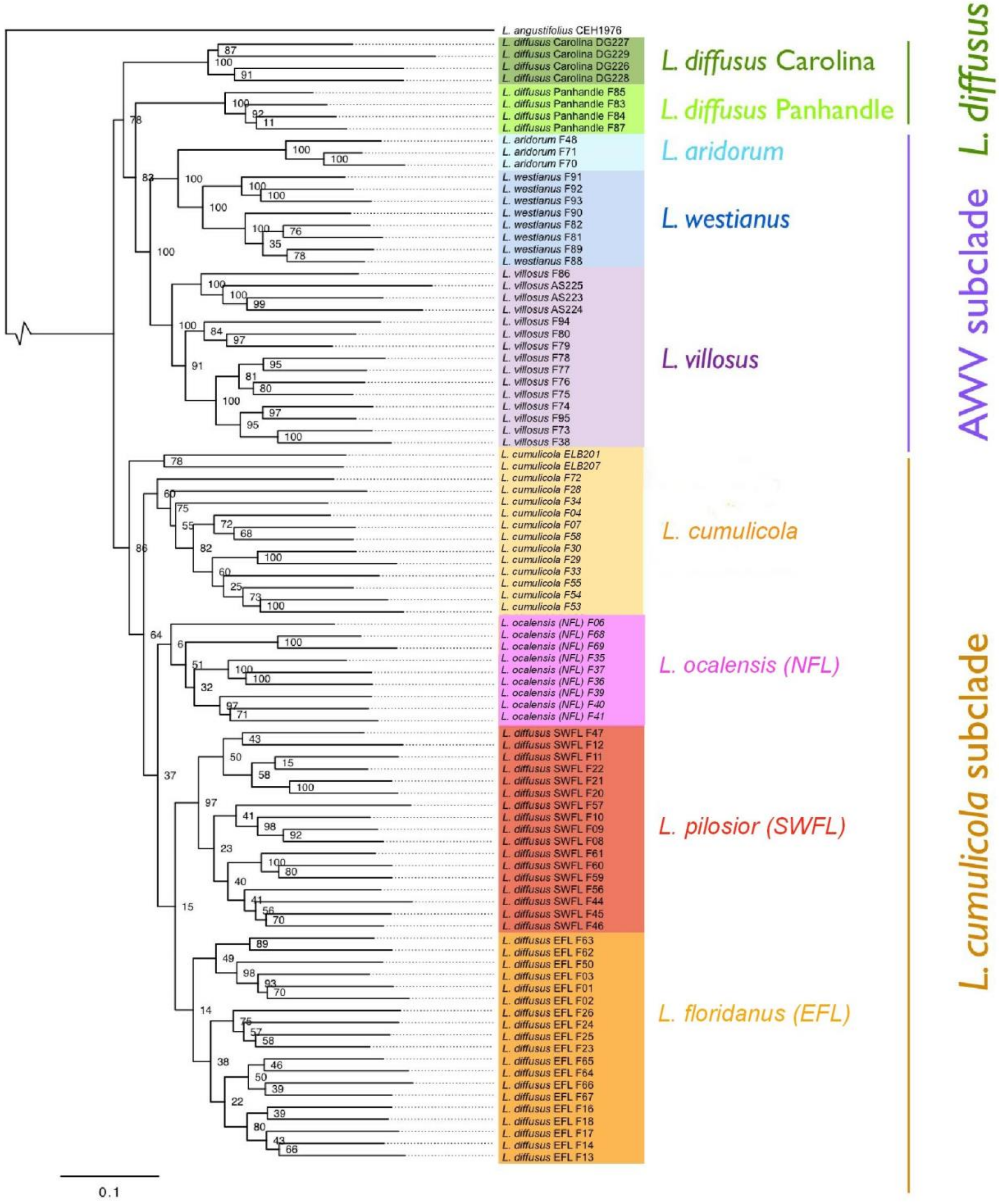


Figure 3. Phylogeny of the unifoliolate Florida *Lupinus* clade constructed under maximum-likelihood, using DNA sequences from 153,730 loci generated from next RADseq data for 94 accessions. Numbers above nodes are bootstrap values and the tree is rooted using *L. angustifolius*, a species from the Old World *Lupinus* clade that is the putative sister group of the Florida clade.

DISCUSSION

Species delimitation and designation of new species serve as the foundation for a wide range of disciplines across pure and applied biology, including biodiversity conservation assessment (Wilson 2004). Without a rigorous approach to species delimitation and designation of new species, we risk basing subsequent downstream analyses and findings on unstable ground. This is particularly true for the Florida unifoliolate lupines, where a historical lack of taxonomic consensus has resulted in the recognition of from one to five species in the clade over the past 200 years. This lack of consensus has resulted in conflicting assessments of the conservation threat status of potentially endangered species in this clade. Furthermore, recent extensive fieldwork in peninsular Florida has revealed cryptic species which are geographically confined to and distributed across a fragmented system of inland and coastal sand ridges and uplands in central peninsular Florida (Figs. 1 and 2). With a more densely sampled phylogeny including multiple accessions of each species, as advocated by Pennington and Lavin (2015), cryptic species within widespread taxa are being discovered, prompting re-evaluation of species limits via reciprocal illumination of new phylogenetic data with that from morphology, ecology and geography (e.g. Bickford et al. 2007; Vietes et al. 2009). Indeed, the utility of densely sampled DNA sequence data to reciprocally illuminate morphologically defined species is such that genetic data are becoming a routinely used source of evidence for species delimitation and discovery. The advantages of using RADseq data are that a large genome-wide set of loci are sequenced providing large numbers of SNPs such that both phylogenetic and demographic analyses can be combined to provide a more complete picture of variation across the species boundary. However, caution is required due to the potential for over-inflation of species numbers by mistakenly equating population-level genetic structure directly with species (Sukumaran and Knowles 2017).

We tested species boundaries across the unifoliate-leaved Florida *Lupinus* clade, including the central Florida geographically confined morphospecies, producing a densely sampled, genome-wide RADseq data set and fully sampled phylogeny for this clade (Nevado et al. 2024). A novel hypothesis of relationships and species limits is presented which does not coincide with any of the previous taxonomic accounts and differs significantly from all previous species delimitations, earlier phylogenies, and genetic studies (Eastwood et al. 2008, Drummond et al. 2012, Keller et al. 2017, Bupp et al. 2017, Ricono et al. 2015). Firstly, there is robust phylogenetic support for the AWV subclade (*L. aridorum*, *L. westianus* and *L. villosus*). Secondly, *L. diffusus*, once thought to occur widely across peninsular Florida, the Florida Panhandle and further north into Alabama, Georgia, South and North Carolina, is non-monophyletic. Thirdly, the populations previously referred to *L. diffusus* from peninsular Florida are here recognized to comprise the *Lupinus cumulicola* complex, with three new species (NFL = *L. ocalensis*, SWFL = *L. pilosior* and EFL = *L. floridanus*) all of which are more closely related to *L. cumulicola* than to the more northerly populations of *L. diffusus*. This relationship between *L. cumulicola* and other populations from peninsular Florida previously assigned to *L. diffusus* sheds new insight on the taxonomic status of these two species (Isely 1990, 1998; Duncan and McCartney 1992). Furthermore, it may help explain Isely's (1998) view that *L. cumulicola* represents a peninsular Florida form of *L. diffusus* and Dunn's (1971) placement of some peninsular Florida populations which others referred to *L. diffusus* within his somewhat broader concept of *L. cumulicola.*

Within the *Lupinus cumulicola* subclade there is evidence of phylogeographic structure corresponding to our field-based morphospecies, and with some re-evaluation, our new species described in this manuscript. Some of these are not strongly supported, so it is possible that some of these putative entities may be non-monophyletic, or that additional evidence may show that their morphologic and geographic limits may need some adjustment. Furthermore, there is morphological and genetic evidence for geneflow and hybridization between some of the species of the *Lupinus cumulicola* complex. These contrasting patterns of resolution and clade support coincide with results from the demographic analysis which show higher levels of geneflow and younger split time estimates within the geographically confined *L. cumulicola* subclade than between *L. westianus* and *L. villosus*. Even though their support may be weak, we feel that the four peninsular Florida entities within the *Lupinus cumulicola* subclade represent at least incipient speciation strongly correlated with the relatively recent geographic isolation of most populations. Alternatively, these could be considered as four varieties of *L. cumulicola*, however there has been increasing recognition within the past decade that incipient species, even if not fully genetically isolated, may merit recognition at the species level when supported by geographic isolation. The large number of populations in our study may have resulted in uncovering more gene flow than would have been revealed in a more limited sample of populations. Some populations which were assigned in the field to a particular morphospecies may actually have some genetic admixture not evidenced by morphology. This is perhaps becoming more common in disturbed landscapes with human-mediated movement of propagules, whether intentional or not. With varying levels of support, these patterns across the Florida *Lupinus* clade provide evidence for delimiting eight species within the clade: *L. diffusus* (re-defined to include only material from northern Florida and adjacent States to the north), *L. aridorum, L. westianus, L. villosus* and four incipient species within the *L. cumulicola* complex (here expanded to include all species from central peninsular Florida except for *L. aridorum*).

Although we did not have strong support for all the geographically confined morphological entities of the *Lupinus cumulicola* subclade across central Florida, and evidence of hybrids between some of these entities is indicative of recency, species status will hopefully stimulate further research into their complex pattern of variation. Even without complete reproductive isolation, and with some gene flow, we here recognize these morphospecies at species rank: NFL as *L. ocalensis*; SWFL as *L. pilosior*; and EFL as *L. floridanus*, with *L. cumulicola* corresponding to *L. cumulicola* as originally described by Small (see Taxonomy below; Table S2). Our treatment of these morphospecies as species rather than as varieties represents a somewhat finer split than in published and forthcoming analyses of RADseq datasets for other *Lupinus* clades. RADseq data resolve all the Old World *Lupinus* species as reciprocally monophyletic with maximal species clade support (Deflorin et al. unpub. data), as well as very recently derived species in the Andes (Atchison et al. 2016; Contreras et al. 2018). This wider context is important to consider in the Florida example because it provides a comparable basis for delimiting species across the genus as a whole.

Lupinus aridorum is strongly differentiated genetically from *L. westianus* with multiple accessions of each of these species forming robustly supported (BS 100%; PP 1.0) sister clades subtended by long branches (especially the branch subtending the *L. aridorum* clade) indicative of substantial genetic divergence. These data strongly support recognition of *L. aridorum* and *L. westianus* as distinct species. These two species occupy disjunct distributions geographically isolated from each other, with *L. westianus* restricted to coastal and inland sand ridges in the Florida Panhandle and *L. aridorum* restricted to inland sand ridges in central peninsular Florida (Fig. 1). A suite of minor but consistent morphological differences in plant stature, branching habit, flower color and leaflet size also support recognition of two distinct species.

Our results clearly show that the biogeographic affinities of *L. aridorum*, as with other *Lupinus* species that occur elsewhere in Florida and the Southeastern United States, align with the predominant pattern of easterly origins of Florida sand ridge endemic plants (Germain-Aubrey et al. 2014). However, in contrast to the four sand ridge Florida endemics studied by Germain-Aubrey et al. (2014), in *Lupinus* there has been in situ diversification of this small clade of eight species centred in Florida, with all of them dating from the Pleistocene. Indeed, there is no evidence that the Florida and south-eastern USA *Lupinus* clade is closely related to the lupines of western North America, but is instead most likely sister to the Old World lupines (Eastwood et al. 2008; Drummond et al. 2012), or even potentially nested within the Old World clade (Keller et al. 2017). In contrast to the general view of south to north Pleistocene migrations from glacial refugia in eastern North America (Soltis et al. 2006), for *Lupinus*, the trajectory of differentiation across the Southeastern United States appears to be from north to south with the peninsular Florida elements derived within the clade.

The three species *Lupinus ocalensis, L. pilosior,* and *L. floridanus,* occupying geographically confined areas within central peninsular Florida, belong to the L. *cumulicola* complex. Despite limited evidence of hybridization and gene flow, we find some support for these entities and intriguing patterns of phylogeographical structure within this subclade. For example, accessions of *L. ocalensis* (NFL) are consistently recovered as a clade albeit with varying levels of support, and within this clade two well supported subclades are found which correspond to the Sumter Upland & Cotton Plant Ridge (F35, F36, F37) and the Brooksville Ridge (F39, F40, F41) respectively. The phylogenetic position of F72 is uncertain and lacks support. This sample originates from the Mount Dora Ridge, and along with F39, shows admixture, suggestive of possible hybridization. Similarly, accessions of *L. pilosior* (SWFL) also form a clade.

The largely allopatric isolation of these morphospecies across central peninsular Florida, with each almost entirely restricted to a few of the major sand ridge systems: Lake Wales ridge (*Lupinus cumulicola*), Atlantic Coastal Ridge, Tenmile Ridge and Bombing Range Ridge (*L. floridanus*), Brooksville Ridge, Cotton Plant Ridge, and Ocala Upland (*L. ocalensis*), and southwest Florida sand ridges (*L. pilosior*), which are separated by largely lupine-free habitats (Fig. 2) is striking. This is very much in line with what might be expected for very recent rapid speciation, where reproductive isolation is still incomplete, and could be compatible with the idea of ephemeral species (Rosenblum et al. 2012; Rabosky 2013) whereby speciation is very common and rapid, but the majority of produced species do not necessarily persist, but instead go extinct or are re-absorbed into parental forms. With tentative age estimates for the splits between these varieties confined to just the last 40 Kyr, plus the weakly supported pattern of differentiation of these species from the older Lake Wales Ridge *L. cumulicola* towards both coasts and including the *L. floridanus* populations on the very recently formed Atlantic Coastal Ridge, we speculate that these more weakly differentiated species diverged in response to very recent late Pleistocene sea-level changes and represent incipient species.

Conservation. The Florida xeric sand ridges and uplands contain one of the highest concentrations of narrowly restricted endemic plants in the south-eastern USA and are considered one of the most threatened habitats in North America (Richardson et al. 2014). Within Florida, 407 plant species have been classified as Endangered (Ward et al. 2003) and within the Southeastern United States, the Florida Panhandle and Central peninsular Florida, 29 endemics are federally listed as Threatened or Endangered (Christman and Judd 1990; Estil and Cruzan 2001; Menges et al. 2007; Richardson et al. 2014). The degradation and destruction of habitat due to encroaching Citrus agriculture, and especially urban development — the suburbanization of central Florida — is a major threat to these globally rare, narrow endemics, including unifoliolate *Lupinus* taxa in Florida. Within these habitats, *Lupinus aridorum* is one of the most critically endangered plant species in Florida, occupying just the Winter Haven and Mount Dora ridges in Polk and Orange counties in central Florida (Fig. 2), with the number of known localities declining from 15 (U.S. Fish & Wildlife Service 1987) to just nine (Peterson et al. 2012; Richardson et al. 2014; Peterson 2016). Furthermore, all populations are small (0.1-1 ha), mostly declining, and mostly unprotected, prompting recent efforts to more closely monitor populations, propagate plants to augment populations (Richardson et al. 2014; Peterson et al. 2012; Peterson 2016), and investigate the conservation genetics of this species (Ricono et al. 2015; Peterson et al. 2012; Peterson 2016).

The lack of consensus surrounding the taxonomic status of *Lupinus aridorum* and its treatment as a variety of *L. westianus* have detracted attention from its endangered red-listing status at both the state and federal levels (U.S. Fish & Wildlife Service 1987; Bibb et al. 2007; Contu 2012). Here we present evidence suggesting that *L. aridorum* is strongly genetically differentiated from *L. westianus*, justifying its treatment as a distinct species and bringing renewed focus to its endangered status and conservation. Furthermore, recognition of *L. aridorum* and *L. westianus* as distinct species also further highlights the threatened status of *L. westianus*. Within *L. westianus*, there is robust support (Fig. 3) for the separation of the inland accessions (F91-F93) from those along the coastal dune systems (F81-F90), indicative of limited dispersal and gene-flow between these sand ridges separated by just 30 km. The genetic distinctiveness of these inland and coastal subclades within *L. westianus* suggests that it will be important to protect both areas to conserve the genetic diversity of this species.

TAXONOMIC TREATMENT

This first well-sampled phylogeny for the Florida unifoliolate clade of *Lupinus*, with a hypothesis for its evolutionary diversification, serves as an additional line of evidence for species delimitations within the clade. This taxonomic treatment adds to the rapidly increasing number of studies which have successfully used RADseq methods (Eaton and Ree 2013; Wagner et al. 2013; Pante et al. 2015a; Eaton et al. 2017) to resolve phylogenetic relationships, even among recently diverged species (Ree and Hipp 2015; Mort et al. 2015; Wessinger et al. 2016) and species delimitation. Using the results of the phylogenetic analysis in an integrative taxonomic approach (Dayrat 2005) we present a new taxonomic conspectus, recognizing eight species, with three new species which are formally named here. For each of the eight species we provide a full species description followed by notes on its geographic range, life history, and ecology. A rarity status is assigned using the NatureServe ranking criteria for global and state rarity. Global rank is based on rarity worldwide, and state rank applies to rarity in Florida unless otherwise specified. Criteria for assigning global and state rarity include estimated number of occurrences including protected occurrences, overall range, threats, and habitat or ecological fragility. Examples of these ranks include G1= Globally Critically Imperiled, G2= Globally Imperiled, G3= Globally Vulnerable, and G4 = Globally Apparently Secure. State rank is assessed using the same criteria as Global rank, except applying only to status within an individual state. A "T" rank is given for infraspecific taxa (subspecies or varieties).

Taxonomic Conspectus of the Florida unifoliolate *Lupinus* clade

Key to species of the Florida unifoliolate *Lupinus* clade

(for additional morphometric differentiation of species in the *L. cumulicola* complex see Table 2).

1. Banner petal with a reddish-purple, maroon, or dark purplish-blue central "eyespot", darker than the surrounding part of the banner.
    2. Plants prostrate or decumbent, the branches at or near ground level; leaf blades narrowly elliptic, mostly more than 10 cm long and 3 to 4 times as long as wide, the ascending to spreading pubescence of the stems and leaves long-villous, free portion of stipules evident, usually 3-5 cm long .... **Lupinus villosus**
    2. Plants erect, the main stem erect and usually branched much above ground level, forming somewhat woody, suffrutescent to "shrubby" plants; leaf blades elliptic to ovate, mostly 4-9 cm long and from 1.5 to 2.5 times as long as wide, the pubescence of the stems and leaves tightly appressed; free portion of stipules absent or rarely present and then less than 1 cm long.
        3. Banner petal pale pink with a dark maroon eyespot; leaf blades mostly 5.0-6.5 cm long and 2.5-3.0 cm wide; robust plants averaging 45-60 cm tall; plants of central peninsular Florida .... **Lupinus aridorum**
        3. Banner petal light to dark blue with a maroon to purplish-blue eyespot; leaf blades mostly 7.5-9.0 cm long and 3.5-4.5 cm wide; robust plants averaging 60-85 cm tall; plants of the Florida Panhandle.. **Lupinus westianus**

1. Banner petal with a white to cream-colored central "eyespot", lighter than the surrounding part of the banner.

   4. Plants erect, the main stem erect and branched much above ground level, forming suffrutescent to somewhat woody "shrubby" plants.

      5. Leaf blades densely silvery-silky appressed pubescent, the hairs totally obscuring the leaf surface, leaf blades flat or nearly so, mostly 3.5-4.5 cm wide, the leaf blade length:width ratio mostly 1.8:1 to 2.1:1 ....**Lupinus cumulicola**
      5. Leaf blades moderately appressed pubescent, the hairs not obscuring the green leaf surface, leaf blades more or less conduplicate, mostly 2.5-3.2 cm wide, the leaf blade length:width ratio mostly 2.4:1 to 2.9:1 .... **Lupinus floridanus**

   4. Plants mat-forming, the main stems prostrate, branched at or near ground level and the branches mostly decumbent, ascending only at their leafy tips.

      6. Plants forming relatively small clumps, 25-80 cm in diameter; leaf petioles mostly 6-8 cm long; leaf blade length:width ratio mostly over 3:1, often 4:1; free portion of stipules mostly 10-16 mm long .... **Lupinus diffusus**
      6. Plants forming relatively large clumps, often 90-130 cm or more in diameter; leaf petioles mostly 3-6 cm long; leaf blade length:width ratio mostly 2:1 to 3:1; free portion of stipules mostly 17-25 mm long.

         7. Plants with dense, short, appressed pubescence, the green surface of the leaves evident; leaf petioles mostly over 5 cm long; mature legumes mostly 2-3 cm long, 6-8 mm wide .... **Lupinus ocalensis**
         7. Plants with dense, spreading, whitish-villous pubescence, mostly obscuring the leaf surface; leaf petioles mostly 3-4 cm long; mature legumes mostly 3.5-4.5 cm long, 10-12 mm wide .... **Lupinus pilosior**

1. LUPINUS VILLOSUS Willd., Sp. Pl. Editio quarta 3(2): 1029. 1802. **TYPE:** “Hab. In Carolina et insula Trinitatus” *Willdenow* (B - destroyed). **NEOTYPE** (Dunn (1971): North Carolina, Southport, *Godfrey & Wiebe 50379* (FSU). Figure 4.

Plants annual to short-lived perennial (mostly semelparous), mat-forming, main stem branches prostrate, forming clumps mostly 25-50 cm in diameter, occasionally larger. Stems ascending, 20-50 cm tall, densely villous with ascending to spreading whitish hairs 1-4 mm long. Leaf petioles (30-) 60-110 (140) mm long, 2-4 mm wide, densely villous with ascending to spreading whitish hairs 2-4 mm long. Stipules fused to the petiole at base, the free tips (20-) 30-50 (-60) mm long, curvate, densely villous with spreading to spreading whitish to tawny hairs 1-3 mm long. Leaf blades lanceolate to lance-ovate, usually broadest below the middle, (80-) 100-140 (-150) mm long, (20-) 25-35 (-45) mm wide, the length:width ratio mostly 3:1 to 5:1, the tips acute to obtuse and mucronate, both leaf surfaces densely villous with appressed white hairs 1-3 mm long, the hairs more ascending-appressed along the midvein, the hairs mostly obscuring the leaf surface. Inflorescence racemose, 10-25 cm long, densely verticillate to subverticillate, 10-15 mm in diameter, bracts subulate, 10-18 mm long, caducous. Pedicels 2-5 mm long at anthesis, elongating in fruit. Calyx densely villous, the lower lip 8-16 mm long, tridentate, the lobes 5-10 mm long, the upper lip 7-12 mm long, bifid. Corolla glabrous, light to bright pink or pinkish-lavender, the central spot on the banner dark maroon to purple, standard clawed, ovate, 12-14 mm long, 9-11 mm wide, wings 12-15 mm long, 5-6 mm wide, the basal clawed portion 2.7-4.5 mm long, keel 3.4-4.6 mm wide. Legumes 25-40 mm long, 12-15 mm wide, very densely villous with tawny hairs up to 5 mm long, ovules 4-8, seeds 3-4 mm in diameter, broadly ellipsoid to nearly orbicular, slightly flattened, light tan with irregular darker brown to gray patches and fine short brown lines.


A
B
C

Figure 4 (A-F). *Lupinus villosus*. A - Small plant in flower, Gulf County, Florida, May 1, 2012. B - Inflorescence in flower, Clay County, Florida, April 6, 2023. C - Large plant in flower, Clay County, Florida, April 6, 2023. D - Single leaf with free stipules, Suwannee County, Florida, May 5, 2012. E - Plant in early flower, Marion County, Florida, April 28, 2017. F - Inflorescence in fruit, Alachua County, Florida, May 1, 2017.

Occasional from southeastern Louisiana, eastward through southern Mississippi, southern Alabama, northern Florida south to Marion and Levy counties, southern Georgia, and northward to the Coastal Plain of South Carolina and southeastern North Carolina. Typically occurring on deep sands of longleaf pine (*Pinus palustris)* dominated sandhills and dry pine savannas, often colonizing adjacent roadsides and disturbed sites (Fig. 18). It is a gap specialist species that occurs in openings with bare exposed soil, scattered lichens, and sparse cover of forbs, usually where there is a lack of continuous grassy cover and with little to no shrub competition.

In parts of the Big Bend region of Florida and the eastern Florida Panhandle this is the only unifoliolate species of *Lupinus* present at sites with suitable habitat. It overlaps in range with *Lupinus ocalensis* in northeast Florida, although they were not found to be sympatric in our field studies in that region. Although both *L. villosus* and *L. diffusus* are present in the western Florida Panhandle and adjacent states, they rarely co-occur, despite no apparent difference in habitat preference. In the South Atlantic Coastal Plain of Georgia and the Carolinas, *L. villosus* is more common on the Outer Coastal Plain, whereas *L. diffusus* is more common in the inner Coastal Plain Fall-Line Sandhills regions.

Based on its relatively wide range and diversity of habitats, *Lupinus villosus* appears to be relatively secure for the recent future, and its current NatureServe rank of G4 (Globally Apparently Secure) seems correct. It is considered as a S1 (State Critically Imperiled) species in North Carolina and S2 (State Imperiled) in Louisiana, at the periphery of its range. Like many of these *Lupinus* species, population levels can fluctuate widely from year to year, based on time since fire and other disturbances, so that single year population assessments may not reflect long-term population sizes or population viability trends. It is a re-seeder, germinating from the seedbank usually post-fire or after other disturbances to the soil enhance germination, with vegetative plants becoming reproductive in a year or more.

2. LUPINUS ARIDORUM McFarlin ex Beckner, Phytologia 50: 209. 1982. **TYPE: Florida**. Orange Co.: Bank of drainage canal, in back of factories in scrub, just S of US 441, on FLA 437, Plymouth, 13 Apr 1970, *Beckner et al. 2375* (holotype: FLAS! 112612; isotypes: FLAS! 112611, FSU, GH, NCU, NY, USF). Figure 5.

Plants short-lived suffrutescent perennials (iteroparous), sometimes monocarpic, with a woody erect main stem and many sympodial ascending lateral branches, mostly from the upper half of the stem, (30-) 45-60 (-80) cm high, (45-) 80-115 (-165) cm wide, the width:height ratio mostly 1.6 to 2.2. Stem branching mostly above the base, most often with the lowest lateral branch 10 to 30 mm above the ground surface, but occasionally branching at the base, or not branching in the lower 25 cm of the stem. Leaves mostly near the branch tips, the lower leaves often caducous, strongly silvery-pubescent with dense appressed hairs, obovate or elliptic, tips rounded and mucronate, the largest leaves (45-) 55-65 (-80) mm long, (20-) 25-30 (-36) mm wide, the leaf length:width ratio typically 2.1 to 2.3. Leaf petioles (16-) 26-39 (-52) mm long, 2-3 mm wide, densely appressed-pubescent. Stipules fused to the petiole for most or all of their length, rarely (in less than 5% of leaves) with free stipule tips 1-2 (-16) mm long. Inflorescence racemose, mostly 12-15 cm long, densely flowered, bractlets absent or subtending only the lowermost flowers. Calyx 2-lipped, the upper lip 9-10 mm long, lobes lanceolate, apically short-acuminate, lower lip 10-14 mm long, lanceolate, the three lobes abruptly acuminate. Corolla pale pink, sometimes nearly white, occasionally a darker pink, the standard with a prominent central dark maroon (to nearly black) central area (“eyespot’), 15 mm long, the blade 10-12 mm long and 7-9 mm wide, ovate, apex apiculate, the sides rolled or folded; wing petals 14 mm long, 5 mm wide, oblong, the apex rounded; keel petal upcurved, acuminate, 12 mm long. Mature fruits mostly 13-18 per inflorescence, the pods (20-) 24-30 (-35) mm long, (8-) 9-11 (-14) mm wide, ellipsoid, with an oblique acuminate beak and rounded base, wooly pubescent. Seeds few per fruit, orbicular, flattened, ca. 3.5 mm in diameter, pale gray with darker gray spots.

Figure 5 (A-C). *Lupinus aridorum*. A - Habit of typical erect suffrutescent plant in flower, Polk County, Florida, March 10, 2012. B - Branched vegetative plant, with leaves clustered near the branch tips, Orange County, Florida, April 5, 2012. C - Inflorescence, Polk County, Florida, March 10, 2012.

Figure 5 (D-F). *Lupinus aridorum.* D - Leaves (showing no free stipule tips, Polk County, Florida, March 20, 2012. E - Plant in early flower, Orange County, Florida, April 5, 2012. F - Inflorescence, Polk County, Florida, March 20, 2012.

Lupinus aridorum is a sand gap specialist (Richardson et al. 2014) of Florida scrub, known from the Winter Haven Ridge in Polk County and the Mount Dora Ridge in Orange County (Richardson et al. 2014, Christman and Judd 1990) (Fig. 18). It is now only known from white sand entisols of the St. Lucie series (Typic Quartzipsamments). Historically it occurred in both scrub and sandhill communities in peninsular Florida (Kane 1978), with 60% having white sand entisols, 30% gray sand entisols, and 10% yellow sand entisols.

McFarlin first named and gave a short account of this species on page 119 of his unpublished 'Flora of the Central Portion of the Lake Region of Florida' (1935). *Lupinus aridorum* was much later described by John Beckner (in 1982) after seeing McFarlin's manuscript and a small number of populations. The status of *L. aridorum* as a species distinct from *L. westianus* has since been doubted and the species was reduced to a variety of *L. westianus* by Isely (1986). We recognize both as deeply divergent reciprocally monophyletic sister species based on Nevado et al. 2024 (Fig. 3), a consistent set of morphological differences, and their strongly disjunct geographical ranges, with *L. aridorum* restricted to sand ridges in central peninsular Florida and *L. westianus* to the Florida panhandle.

Lupinus aridorum is typically less robust than *L. westianus*, with smaller leaves and a shorter average plant height. Although plant size is quite variable, no *L. aridorum* plants were seen which were more than 80 cm tall and 160 cm wide, whereas the largest *L. westianus* plants were 120 cm tall and 260 cm wide. Some of the main lateral branches of *L. aridorum* seem to diverge below ground level, perhaps indicating a longer life span and burial of branches by shifting sands, and are typically leafy only in the upper third to half of their length. The lateral branches of *L. westianus* all diverge well above ground level and are typically leafy for most of their length. There may be other characters differentiating these species, but the field measurements made in the limited number of populations studied (morphometrics from 4 populations of each) had enough overlap that there is insufficient confidence to describe other differences. Both *L. aridorum* and *L. westianus* can sometimes have evident free stipule tips, contrary to some references (e.g., Wunderlin and Hansen, 2008) which use the single key character "stipules obsolete" to distinguish these from other Florida unifoliolate *Lupinus* species. Plants with free stipule tips were seen most frequently in the inland populations of *L. westianus* in Washington County, Florida, but with variation both within and between individuals, some with no free stipule tips and others having one or rarely two per leaf up to 11 mm long.

NatureServe currently considers *Lupinus aridorum* as a variety of *L. westianus*, and so ranks it as G3T1 (Species Globally Vulnerable, variety Globally Critically Imperiled). With reinstatement as a full species, it should be ranked as G1 (Globally Critically Imperiled). Perhaps only one or two viable natural populations remain, although it has been artificially introduced at several additional sites. Although it is possible that these introduced populations may help preserve the species from extinction, this is still uncertain, given that insufficient time has passed to determine whether these introduced populations will become long-term stable viable populations. Several natural populations have been destroyed in the last two decades, long after it was listed as Federally Endangered in 1987, mostly to residential development.

3. LUPINUS WESTIANUS Small, Torreya 26: 91. 1926. *Lupinus villosus* subsp. *villosus* Phillips (pro parte), Res. Stud. St. Coll. Wash. 23: 201. 1955. **TYPE: Florida**. Bay Co.: St Andrews Bay, 4 May 1926, *Small, Mosier, & Matthous* (holotype: NY!; isotype: GA!). Figure 6.

Plants suffrutescent, typically biennial, or short-lived perennials (either semelparous or iteroparous) from a thick, deep taproot, typically with an erect main stem up to 1 cm in diameter, and few to numerous ascending lateral branches, (40-) 60-80 (-120) cm tall, (60-) 100-170 (-260) cm wide, generally (1.0-) 1.5-2.2 (-2.4) times as wide as tall, the main stem with 5-15 ascending lateral


A
B

Figure 6 (A-F). *Lupinus westianus.* A - Habitat, Washington County, Florida, May 4, 2012. B - Plant habit, Washington County, Florida, May 4, 2012. C - Leaf, with no free stipule tips, Walton County, Florida, May 3, 2012. D - Leaf, with free stipule tips, Washington County, Florida, May 4, 2012. E - Inflorescence in flower, Walton County, Florida, May 1, 2012. F - Inflorescence in fruit, Bay County, Florida, May 3, 2012.

branches, these mostly starting at the plant base in coastal plants, sometimes to 65 cm above the soil level in inland plants. Stems terete, stiff, gray to brown, densely tomentose, the lower leaves typically absent by the time of flowering. Leaves alternate, simple (unifoliolate), petioles (25-) 40-50 (-65) mm long, (1-) 2.5-3.0 (-3.5) mm wide, densely villous with mostly ascending tawny hairs 1-3 mm long. Stipules adnate to leaf petioles, the free stipule tips absent, represented by a tuft of hairs, or present and 1-3 (-8) mm long, curvate, densely villous with spreading hairs 1-3 mm long. Leaf blades (larger leaf blades on each plant) elliptic, rounded, and apiculate at tip, tapered to rounded at base, (55-) 75-85 (-93) mm long, (20-) 40-45 (-47) mm wide, mostly (1.7-) 1.9-2.1 (-2.5) times as long as wide, both leaf surfaces finely and densely appressed villous with hairs mostly 1-2 mm long, velvety to the touch. Inflorescence racemose, mostly 12-17 cm long, 2-5 cm wide, pedicels stout, mostly 2-4 mm long, the inflorescence axis, pedicels, and calyx all densely villous with tawny appressed-ascending hairs mostly 1-2 mm long. Calyx ca. 1 cm long, the tube broadly campanulate, 2-lipped, the upper lip low and broad, the lower lip longer than the tube, the lobes 5-9 mm long, linear-triangular. Corolla ca. 1 cm long, the standard slightly longer than the wings and keel, short-clawed, the blade broadly ovate to nearly orbicular, emarginate, the sides folded upwards, a bright pale blue with a broad dark blue to purplish lower central portion (the “eye spot”); wings short-clawed, pale blue, the blades narrowly obovate, falcate; keel pale blue, strongly curved upward to arrow, yellowish-orange firm tips. Stamens 10, filaments fused below the middle, with one set of 5 longer, rounded anthers and one set of 5 with shorter, elongate anthers. Ovary superior, narrow, densely silky-hairy, the long style curved upward. Fruit an oblong-elliptic nearly cylindric beaked legume 25-32 (-45) mm long, 10-11 mm wide, densely villous with white to tawny hairs up to 6 mm long.

Lupinus westianus is endemic to the Florida panhandle, where most populations are found in coastal sand pine scrub, often in association with other endemic or restricted species of these habitats (i.e., *Conradina canescens, Polygonella macrophylla).* A secondary area of distribution occurs inland on the Greenhead Slope, centered on the Bay - Washington County line (Fig. 18). This is an area of extensive deep sandy uplands pocketed with steep-sided sinkhole ponds known for the narrow endemics on their fluctuating margins (e.g. *Paronychia minima, Hypericum lissophloeus*, *Rhexia salicifolia, Xyris longisepala, X. isoetifolia*). The uplands of this hilly landscape were once covered by extensive *Pinus palustris/ Quercus laevis/ Aristida beyrichiana* xeric sandhill woodlands. *Lupinus diffusus* occurs in similar longleaf pine (*Pinus palustris)* sandhill habitats to the west of this karst region, and *L. villosus* to the east, but neither of these appear to occur in the Greenhead Slope region of inland *L. westianus* populations.

Lupinus westianus (as *Lupinus westianus* var. *westianus*) is currently ranked as G3T3 by NatureServe, the species and the variety both considered Vulnerable. However, based on the accelerated pace of residential and commercial development within its narrow range it should be considered as a G2 (Globally Imperiled) species. Many small populations that persisted in vacant lots within coastal sand pine scrub only a few decades ago are now extirpated, with little potential habitat remaining in some counties where it was once common in coastal xeric scrub. There are few protected populations in its coastal range. The inland population of the Greenhead Slope region, which is genetically distinct from the coastal population region, is under threat by rapidly increasing residential development. Some of its habitat was dissected by roads and divided into lots as part of land sale schemes decades ago, but these remained mostly in natural vegetation until rather recently. As an area of relatively high elevations and well drained soils only a short drive from the coast, these inland habitats will be increasingly subject to development pressure due to sea level rise and subsequent inland migration of the human population. Both the inland and coastal areas of its range should be independently evaluated for conservation of this species, with coastal populations facing the most imminent pressure from land clearing and sprawling coastal development. *Lupinus westianus* appears to be a sand gap specialist, from which it can spread into disturbed sites, often germinating from the seed bank following both natural and artificial disturbance. Plants with first year vegetative growth can precociously flower, but this species typically flowers during the second year of growth from the first year's stems (Isley 1990), often with mass flowering displays.

4. LUPINUS DIFFUSUS Nutt., Gen. N. Amer. Pl. 2: 93. 1818. *Lupinus villosus* var. *diffusus* (Nutt.) Torr. & Gray, Fl. N. Amer. 1(3): 382. 1840. *Lupinus villosus* subsp. *diffusus* (Nutt.) Phillips, Res. Stud. St. Coll. Wash. 23: 201. 1955. **TYPE: North Carolina**. Around Wilmington, *Nuttall s.n.* (holotype: PH 00026523). Figure 7.

The PH sheet has Nuttall's annotation as *Lupinus diffusus* and his asterisk (= new species). K 829313 has Nuttall's annotation of "Lupinus diffusus Alabama" and someone else has added "Nuttall 1829." BM 1046841 has a branch of *Lupinus* mounted with two other species, without indication that it was collected or seen by Nuttall.

Plants annual (probably semelparous), prostrate, mat-forming, generally about 15-50 cm tall, with ascending flowering branches at the ends of the branched stems, the mats mostly 25-80 cm in diameter. Stems densely sericeous to canescent, the hairs mostly appressed to ascending and 0.5-1.0 mm long. Leaf petioles 60-80 mm long, stipules connate to the petiole, their free linear to filiform tips mostly 10-16 mm long. Leaf blades 4-10 cm long, 15-35 mm wide, the leaf length:width ratio mostly over 3:1, often exceeding 4:1, the tips acute to obtuse, mostly rounded and mucronulate at the tip, oblanceolate to obovate, with appressed to ascending hairs on both surfaces, but the hairs not usually obscuring the green leaf surface. Inflorescence racemose, 8-20 cm long, 10-20 mm in diameter, the flowers verticillate to subverticillate, bracts 3-5 mm long, subulate, caducous. Pedicels 2-3 mm long at anthesis, 3-4 mm long in fruit.

Populations from peninsular Florida previously assigned to *Lupinus diffusus* are now included within the four species of the *L. cumulicola* complex (see below). *Lupinus diffusus*, as now recircumscribed, ranges from southern Mississippi east though southern Alabama, the Florida Panhandle, and eastern Georgia north to South Carolina and southern North Carolina (Fig. 17; Dunn, 1971). It occurs in clearings and on margins of woodlands of oak-palmetto and pine on deep sands. Although we have not sampled populations of *Lupinus* in northeast Florida, based on iNaturalist photographic records and herbarium specimens we believe these to represent the northernmost extent of the range of *L. ocalensis*. The range discontinuity between *L. diffusus* in south-central Georgia and the central Florida Panhandle, and *L. ocalensis* in northeast Florida is mostly correlated with the "Suwannee Straits", a lowland area where both species are absent. Although the Suwannee Straits developed during a much earlier geological period than the divergence times of the species of the Floridian unifoliolate *Lupinus* clade, it represents a discontinuity in dry sandy upland habitats, since it is generally of lower elevation than the sand ridges of the Florida Peninsula and the inland Florida Panhandle. During times of more recent high sea levels, it would have been inundated while the central ridge "islands" of peninsular Florida were above sea level. This could explain why the entities of the *L. cumulicola* complex are more related to each other than to *L. diffusus,* which is now restricted to areas north of the Suwannee Straits.

Lupinus diffusus has not been globally ranked by NatureServe as to its range-wide status but is ranked as S3 (State Vulnerable) in North Carolina and Mississippi, at the periphery of its range. Even with its new, narrower, definition and range, it is probably best ranked as G4 (Globally Apparently Secure). It does not seem to be of immediate conservation concern, despite destruction and fire-suppression of much of its sandhill habitat in the Western Highlands of the Florida Panhandle. It is a gap specialist and a seedbank re-seeder, often germinating from the seedbank following soil disturbance or burning of its habitat.


A
B
C

Figure 7 (A-E). *Lupinus diffusus*. A - Habit, Scotland County, North Carolina, May 17, 2021. B - Inflorescence, Okaloosa County, Florida, May 2, 2012. C - Inflorescence in fruit, Okaloosa County, Florida, May 2, 2012. D - Habit of typical sized plant, Okaloosa County, Florida, May 2, 2012. E - Habit of several individual plants in a small area, Okaloosa County, Florida, May 2, 2012.

5. LUPINUS CUMULICOLA Small, Man. S.E. Fl. 681. 1933. *Lupinus villosus* subsp. *diffusus* (Nutt.) Phillips (pro parte), Res. Stud. St. Coll. Washington 23: 201. 1955. **LECTOTYPE** (Dunn 1971): **Florida.** Highlands Co. (orig. as "DeSota" County): Sandhills E of Sebring, 1 May 1919, *J.K. Small & J.B. DeWinkeler 9081* (NY!; isolectotype- GH!). Figure 8.

Annual or winter biennial herb (probably iteroparous), or becoming a short lived suffrutescent perennial, the main stem usually stiffly erect, becoming suffrutescent or somewhat woody, forming plants mostly (42-) 70-110 (-210) cm in diameter, usually (40-) 60-90 (-200) cm tall at the tallest point (plant width/height ratio mostly from 1.1 to 1.5), the main stems usually with 7 to 16 primary branches, with the first branch mostly formed from 4 to 10 cm, sometimes more than 30 cm, above the soil surface, the plants mostly with an erect shrubby aspect except when collapsed or recumbent from their own weight. Stems and lateral branches mostly leafy only in the upper half, leafless for half or more of their length, the young stems and leaf petioles densely appressed-pubescent with 0.5-1.5 mm long tightly appressed-ascending hairs, mostly obscuring the surface of the stems and leaf petioles. Leaves narrowly to broadly elliptic, petiolate, the petiole (10-) 30-42 (-80) mm long, (1-) 2-3 (-5) mm wide, densely appressed-villous; free portion of stipules mostly 10-14 mm long, rarely longer; leaf blade narrowly to broadly elliptic, (45-) 65-82 (-103) mm long, (26-) 34-44 (-58) mm wide, the leaf length/width ratio mostly from 1.8 to 2.1, very densely appressed-ascending pubescent on both surfaces, the hairs tightly appressed to the leaf surface, slightly more evident along the leaf margin, the fresh leaves velvety with a reflective bluish-slivery cast due to the dense pubescence, the leaf surface not evident below the pubescence. Inflorescence indeterminate, mostly 10-20 cm long and 25-30 mm wide in flower when fully developed, the inflorescence rachis densely appressed-ascending pubescent with short, curled hairs. Bracts lanceolate, 4-5 mm long, densely spreading pubescent with hairs 1-2 mm long. Pedicels spreading, and 2-3 mm long at anthesis, becoming reflexed, stouter and 4-5 mm long in fruit. Calyx bilabiate, 9-11 mm long, densely ascending-appressed villous with light brown hairs obscuring the outer surface. Flowers ca. 1 cm long, the banner 9-10 mm long, light to vivid sky blue, or sometimes bluish-lavender, with a white to cream-colored „eyespot“, wings 9-11 mm long, slightly curvate, keel 9-10 mm long, strongly curvate. Legume (13-) 24-35 (-45) mm long, (5-) 8-11 (-19) mm wide, with a usually strongly recurved 4-6 mm long easily detached beak, densely villous with erect to ascending tawny to light brown hairs, the longest hairs 1-2 mm long, irregularly smooth to barbellate or sometimes pectinately branched, evenly pubescent, over a dense layer of shorter, dense, curled hairs on the legume surface. Ovules 4-6, the legume with short, scarcely evident incomplete cross-partitions, typically maturing 2-4 seeds per legume. Seeds suborbicular, 3.5-4 mm long, 3-3.5 mm wide, flattened at the hilum end and slightly indented at the hilum, laterally somewhat to strongly flattened, glabrous except for a tuft of translucent to whitish branched hairs at the hilum, the seed surface varying from pale gray to dark brown, the color comprised of irregular anastomizing light brown and dark brown patches and dots, with few light brown patches, the rim of the laterally compressed seed somewhat lighter colored than the seed faces.

A broader concept of *Lupinus cumulicola*, including some specimens now referred to as *L. floridanus*, was followed by Dunn (1971). In contrast, our concept of *L. cumulicola* is identical with *L. cumulicola* as described by Small (1933). *Lupinus cumulicola* is a Lake Wales Ridge near-endemic and is most common on the upper terraces of the ridge, at some of the highest elevations in peninsular Florida (Fig. 17). It is found exclusively on yellow sandy entisols of current or former longleaf pine (*Pinus palustris)* sandhills, from Lake County south to Highlands County, barely extending into far western Osceola and Orange counties, and with a single record from the highest terrace of the Lake Henry Ridge in Polk County. Only one location clearly referable to *L. cumulicola* based on both morphological and genetic evidence was found off the Lake Wales Ridge, this being at a high elevation on the Lake Henry Ridge about 17 km west of the Lake Wales Ridge. All other *Lupinus* populations on the Lake Henry Ridge showed evidence of introgression between *L. cumulicola* and *L. pilosior*.


A
B
C
D

Figure 8 (A-G). *Lupinus cumulicola*. A - Habit, Polk County, Florida, March 3, 2014. B - Inflorescence, Highlands County, Florida, March 6, 2012. C - Inflorescence, Highlands County, Florida, February 11, 2014. D - Inflorescence, Highlands County, Florida, April 8, 2021. E - Habit, Highlands County, Florida, March 29, 2023. F - Leaf with free stipule tips, Lake County, Florida, March 15, 2012. G - Inflorescence in fruit, Polk County, Florida, April 4, 2011.

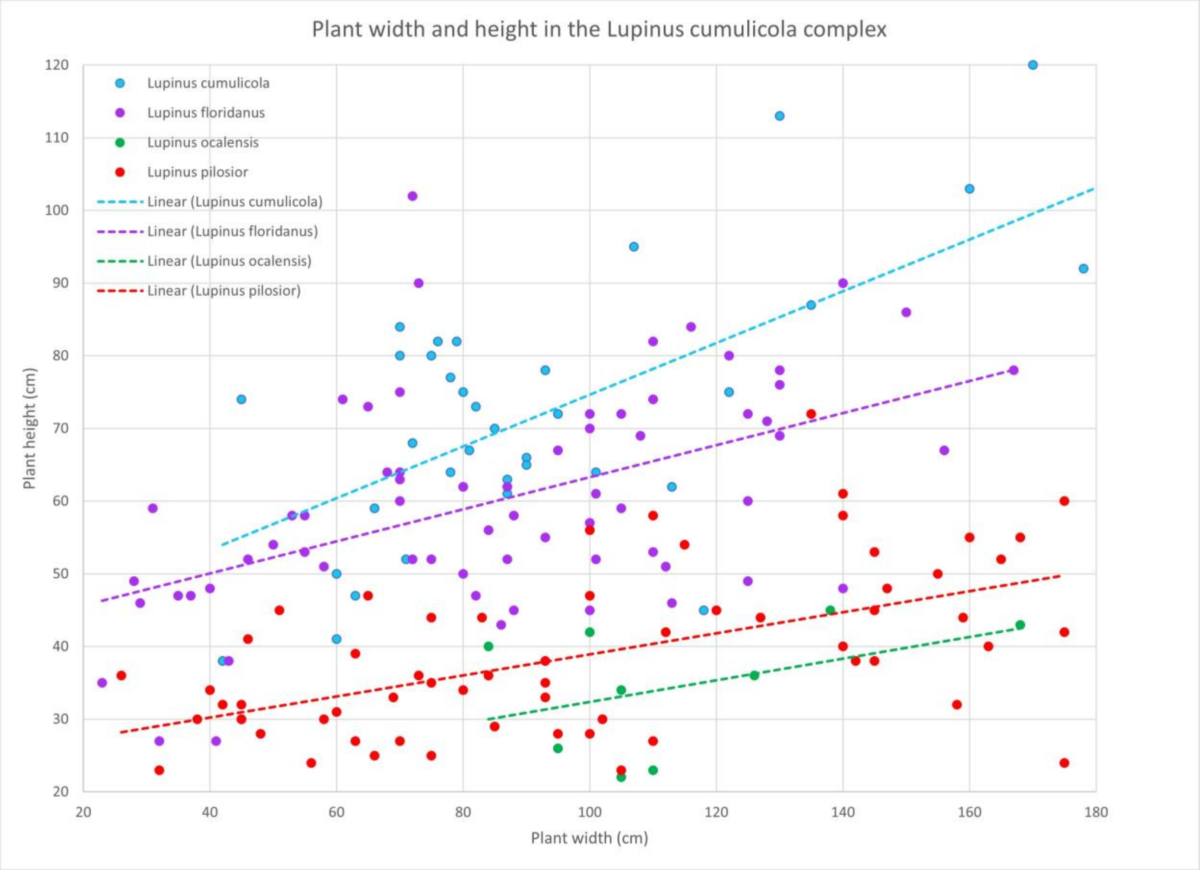


Figure 9. Plant width (cm) and Plant height (cm) in species of the *Lupinus cumulicola* complex, based on field measurements of individual plants from sampled populations. Dashed lines are linear regressions of all individual measurements for each species.

Lupinus cumulicola is listed as a species in NatureServe Explorer but is not given a global or state (Florida) conservation rank. We propose it be ranked as G2 (Globally Imperiled), and therefore also as S2 (State Imperiled) for Florida. It was once a common species of longleaf pine (*Pinus palustris*) sandhill savannas on the Lake Wales Ridge, and was considered to be a characteristic shrub (note – recognizing its woody stems and upright habit) of this natural community class by Harper (1921, 1927). There are a few preserves remaining where viable populations can reliably be found, including Tiger Creek Preserve (Polk County) and the Silver Lake and Carter Creek units of Lake Wales Ridge Wildlife and Environmental Area (Highlands County). As recently as a decade ago, it could be found in many areas adjacent to former or current citrus groves along the Lake Wales Ridge, but these are rapidly being converted to residential and commercial developments, particularly the northern section of the Lake Wales Ridge from Haines City north to Clermont, now part of the rapidly growing megalopolis sprawl of Orlando. Perhaps as many as half or more of the populations sampled in this study have been extirpated due to development or are now surrounded by commercial or residential development. Habitat fragmentation and alteration of the historic fire regime (reduced fire frequency and changes in fire seasonality) threaten the long-term viability of the remaining sites. Cogon grass and natal grass invasion is a serious threat to populations remaining in degraded habitats. *Lupinus cumulicola* is a sand gap specialist, and a re-seeder from the seedbank following disturbance to the soil, or post-fire. Episodic flowering of large silvery-leaved shrub size plants with masses of sky-blue flowers is a striking sight in intact south-central Florida sandhills.

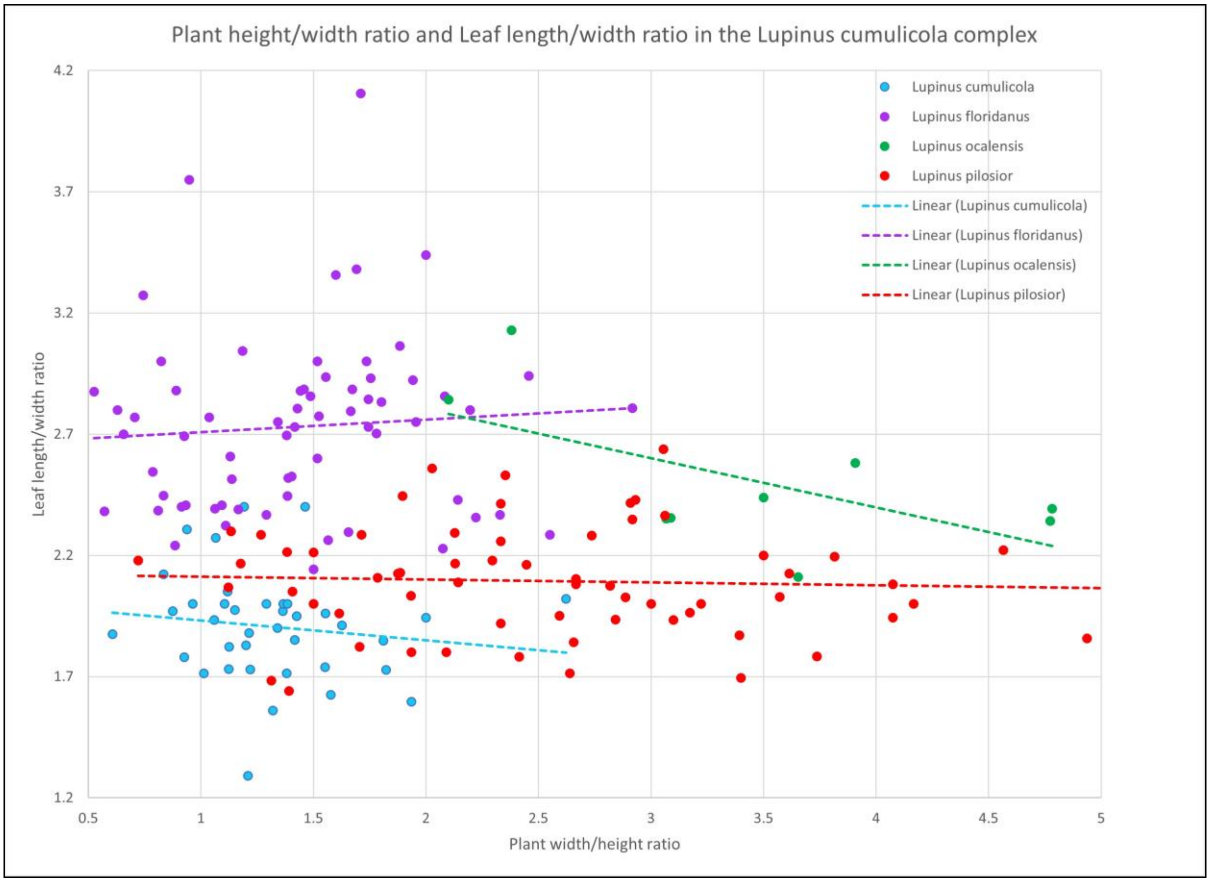


Figure 10. Plant width/height ratio and leaf blade length/width ratio in species of the *Lupinus cumulicola* complex, based on field measurements of individual plants from sampled populations. Dashed lines are linear regressions of all individual measurements for each species.

Under its original concept, *Lupinus cumulicola* was essentially restricted to sand ridges from the Lake Wales Ridge in Lake, Highlands and Polk counties of central peninsular Florida. Here we return the concept of *L. cumulicola* to the original definition by Small (1933). *Lupinus cumulicola* is part of a complex of four species which include all material from peninsular Florida previously assigned to *L. diffusus*, and consisting of four geographically and phylogenetically structured incipient species (Figs. 1, 2 & 3; Table 2) which correspond to the NFL (= *L. ocalensis*), SWFL (= *L. pilosior*) and EFL (= *L. floridanus*) populations discussed above. The four species in this complex are essentially allopatric, occupying a series of mostly isolated xeric deep sand ridges within a narrow geographic range. Other than the Lake Wales Ridge (*L. cumulicola*), the Atlantic Coastal Ridge, Tenmile Ridge and Bombing Range Ridge (*L. floridanus*), the southwest Florida coastal sand ridges (*L. pilosior*), and the Brooksville Ridge and Ocala Upland (*L. ocalensis*), there are no large and relatively contiguous areas of suitable habitat for these taxa, and as such they are found as isolated populations, often separated by several kilometers or more from the nearest populations by unsuitable habitats (Fig. 2).

Table 2: Morphometric field data for species of the *Lupinus cumulicola* complex.

Species	*L. cumulicola*	*L. floridanus*	*L. pilosior*	*L. ocalensis*
Plant height (cm)	(40-) 60-90 (-200)	(25-) 50-75 (-170)	(20-) 30-45 (-72)	(22-) 26-42 (-45)
Plant diameter (cm)	(42-) 70-110 (-210)	(25-) 62-110 (-170)	(25-) 65-140 (-175)	(85-) 100-125 (-168)
Plant height/ diameter ratio	1.1 to 1.5	1.0 to 1.7	1.9 to 3.1	2.9 to 4.1
Number of primary branches	(3-) 7-16 (-28)	(0-) 5-15 (-30)	(2-) 5-10 (-22)	(5-) 6-8 (-12)
Height of first branch (cm)	(0-) 4-10 (-30)	(0-) 5-15 (-34)	(0-) 0-8 (-14)	(0-) 0 (-10)
Length of stem and petiole hairs (mm)	0.5-1.5	0.5-1.5	2-3	0.2-0.5
Length of leaf blade surface hairs (mm)	0.5-1.0	0.5-1.0	2-3	0.2-0.5
Petiole length (mm)	(10-) 30-42 (-80)	(10-) 22-35 (-56)	(18-) 32-45 (-70)	(40-) 50-65 (-100)
Petiole width (mm)	(1-) 2-3 (-5)	(1-) 2-3 (-4)	(2-) 3-4 (-6)	(2-) 2-3 (-5)
Free stipule length (mm)	(6-) 10-14 (-23)	(0-) 8-12 (-23)	(13-) 19-25 (-39)	(14-) 17-21 (-30)
Leaf blade length (mm)	(45-) 65-82 (-103)	(40-) 65-85 (-113)	(55-) 70-90 (-118)	(67-) 82-100 (-118)
Leaf blade width (mm)	(26-) 34-44 (-58)	(15-) 25-32 (-44)	(25-) 35-43 (-60)	(28-) 32-38 (-45)
Leaf blade length/ width ratio	1.8 to 2.1	2.4 to 2.9	1.9 to 2.2	2.3 to 2.7
Legume length (mm)	(13-) 24-35 (-45)	(13-) 32-40 (-47)	(18-) 36-44 (-54)	(17-) 20-27 (-35)
Legume width (mm)	(5-) 8-11 (-19)	(5-) 9-11 (-15)	(8-) 10-12 (-17)	(6-) 6.7-7.0 (-8)

Measurements for quantitative characters based on field data are given in the following format: (minimum -) 25th percentile - 75th percentile (- maximum). For *L. cumulicola* measurements, leaf and petiole n=240, plant width and height n=49, and fruits n=171, from 16 populations in Highlands, Lake, Osceola, and Polk counties. For *L. floridanus* measurements, leaf and petiole n=363, plant width and height n=66, and fruits n=316, from 25 populations in Brevard, Highlands, Martin, Okeechobee, Osceola, Palm Beach, Polk, and St. Lucie counties. For *L. pilosior* measurements, leaf and petiole n=195, plant width and height n=63, and fruits n=137, from 15 populations in Collier, Hardee, Manatee, and Sarasota counties. For *L. ocalensis* measurements, leaf and petiole n= 27, plant width and height n=9, and fruits n=16, from 2 populations in Marion and Sumter counties.

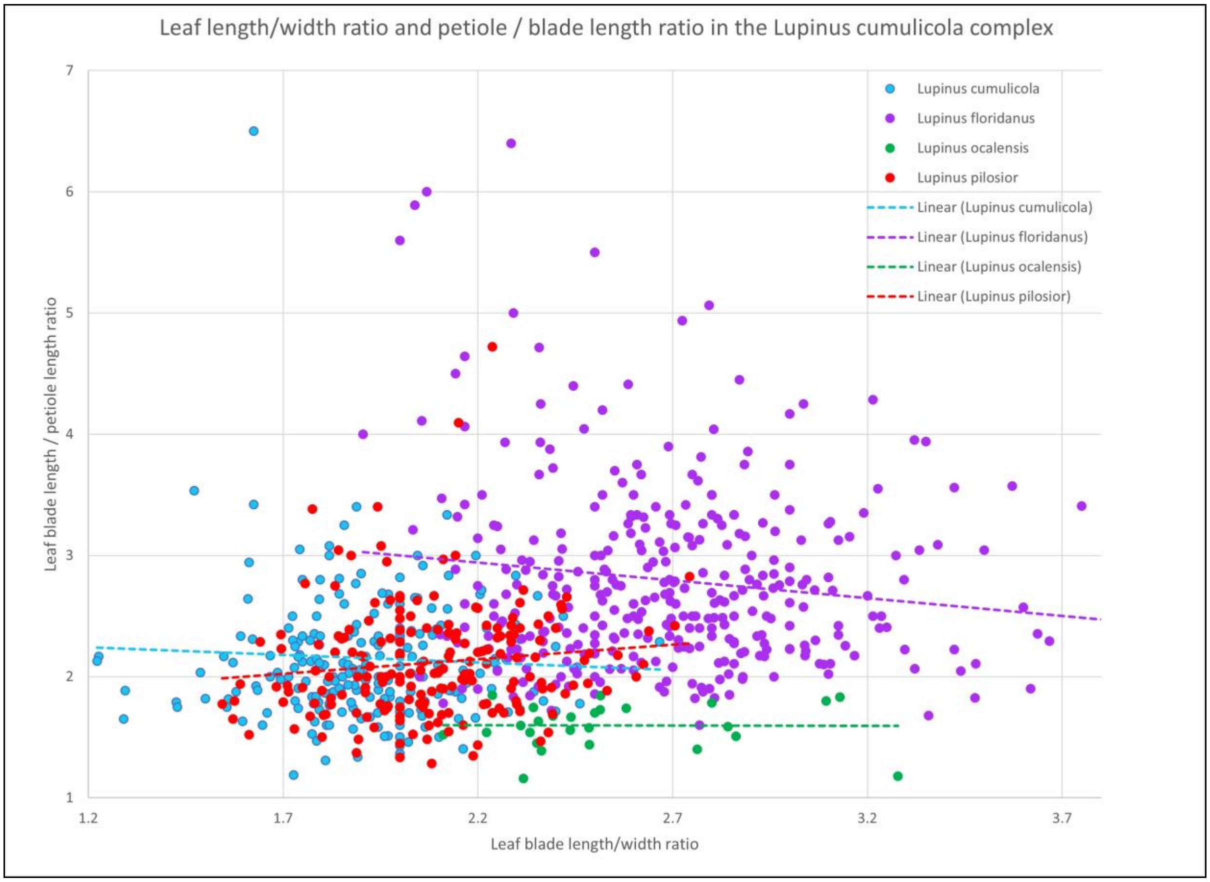


Figure 11. Leaf blade length/width ratio and leaf blade length / petiole length ratio in species of the *Lupinus cumulicola* complex, based on field measurements of individual plants from sampled populations. Dashed lines are linear regressions of all individual measurements for each species.

The morphology of each of these species is quite consistent within most of their ranges (Figs. 9-12), with intermediate forms only found at occasional sites where their ranges meet. We have attempted to find as many of these potential intermediates as possible, and many were included in the initial molecular analyses. While most of the putative hybrids were excluded from subsequent phylogenetic analyses, there are perhaps a few introgressed populations of uncertain placement still in these subsequent analyses, most notably the accessions from the extremely fragmented and extensively developed Orlando metropolitan area, where there are few remaining sites within what was already a naturally fragmented area. The majority of populations sampled for each species show coherence in terms of geographic ranges, habitats, morphology, and phylogeny. The three previously unrecognized species within the *Lupinus cumulicola* complex are described below.

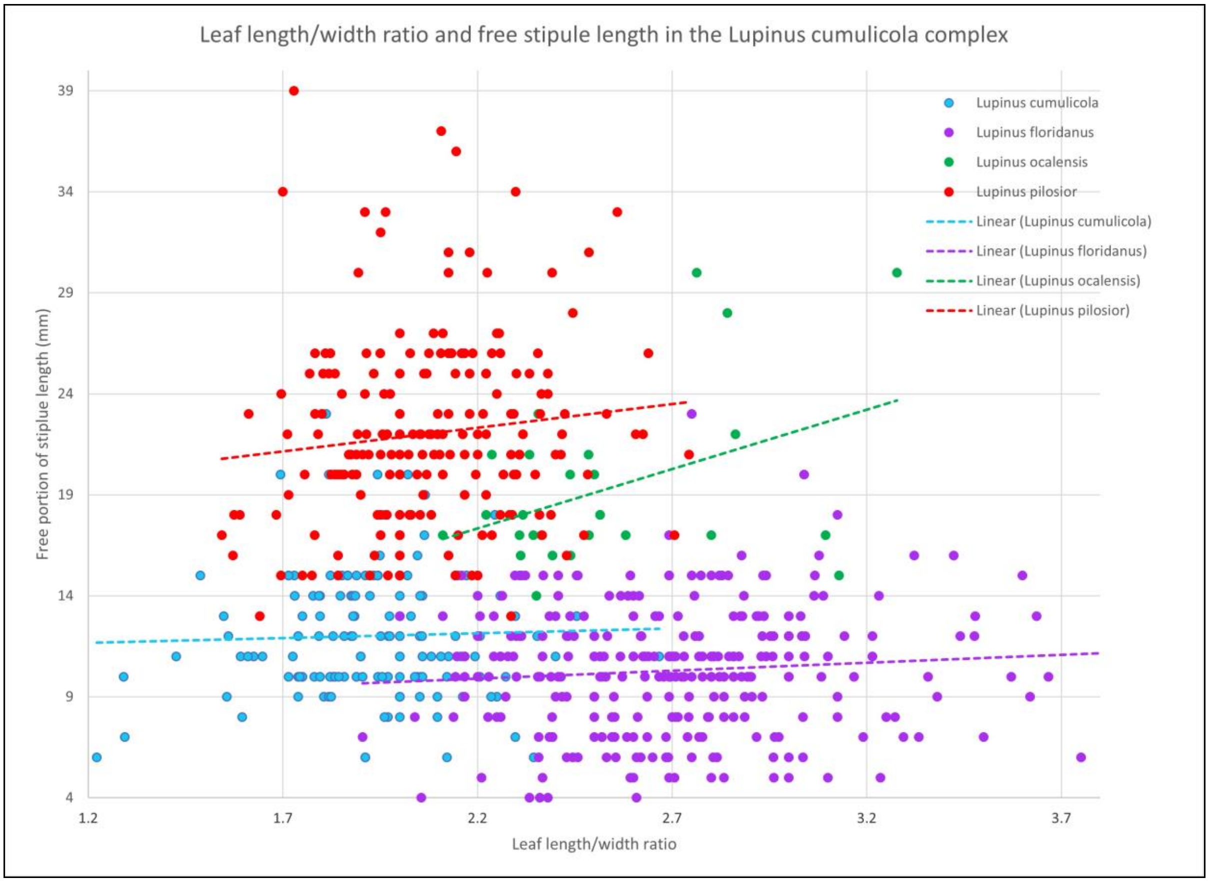


Figure 12. Leaf blade length/width ratio and free portion of stipule length (mm) in species of the *Lupinus cumulicola* complex, based on field measurements of individual plants from sampled populations. Dashed lines are linear regressions of all individual measurements for each species.

6. LUPINUS OCALENSIS E. Bridges & Orzell, **sp. nov. TYPE: Florida.** Sumter Co.: *Pinus palustris / Quercus laevis* sandhill woodland, long unburned and mostly overgrown, on SE side of County Road 243B, just W of jct Co Rd 243F, N of FL 44 at a point ca. 1.1 mi W of jct FL 475 and 2.9 mi W of I-75, ca. 6 air mi W of Wildwood; NEQ, SEQ, SEQ, Sec. 31, T18S, R22E; Lake Panasoffkee NW 7.5' Quad.; 28°52'33' N, 82°08'13" W, Soils - Candler sand (Typic Quartzipsamments), 12 Mar 2012, *Orzell & Bridges 26576* (holotype: FLAS; isotypes: USF, Z). DNA sample F35 in this study. Figure 13.

Like *Lupinus cumulicola*, differing in its prostrate growth habit, longer petioles (50-65 mm) and leaf blades (82-100 mm), and longer free stipules (17-21 mm).

Annual or winter biennial herb, the main stem branched from at or near the base to form a prostrate to slightly erect diffusely branched mat from (85-) 100-125 (-168) cm in diameter, usually only (22-) 26-42 (-45) cm tall at the tallest point (plant width/height ratio mostly from 2.9 to 4.1), the main stems usually with 6 to 8 primary branches, these then further branched and prostrate to decumbent, the ultimate branch tips curving upwards. Stems leafy throughout, the larger leaves often held erect, perpendicular to the ground surface, the young stems moderately to densely puberulent, the hairs mostly tightly appressed, the longest hairs 0.2-0.5 mm long, not obscuring the surface of the stems and leaf petioles, the leaf blade surface smooth to the touch. Leaves narrowly elliptic, petiolate, the petiole (40-) 50-65 (-100) mm long, 2-3 (-5) mm wide, finely and moderately densely appressed-ascending puberulent; free portion of stipules (14-) 17-21 (-30) mm long; leaf blade narrowly elliptic, (67-) 82-100 (-118) mm long, (28-) 32-38 (-45) mm wide, the leaf length/width ratio mostly from 2.3 to 2.7, moderately very finely appressed-puberulent on both surfaces, the hairs tightly appressed-ascending, 0.2-0.4 mm long, slightly longer (to 0.5-1.0 mm) along the main vein on the abaxial surface, barely evident to the naked eye along the leaf margin, the bright green leaf surface evident. Inflorescence indeterminate, mostly 15-25 cm long and 25-30 mm wide in flower when fully developed, the flowers bright sky blue, the inflorescence rachis finely and densely puberulent with appressed-ascending hairs. Bracts caducous, linear-subulate, 6-7 mm long, tightly and densely appressed-villous with hairs mostly less than 0.5 mm long. Pedicels spreading at anthesis, 1.5-2.5 mm long in flower, becoming recurved, stouter and 3-5 mm long in fruit. Calyx bilabiate, 8-9 mm long, densely ascending-appressed villous with tan to light brown hairs obscuring the outer surface. Flowers ca. 1 cm long, the banner 8-10 mm long, medium to dark blue with a white to cream-colored central eyespot, wings 8-10 mm long, somewhat curvate, the keel 8-10 mm long, strongly curvate. Legumes (17-) 20-27 (-35) mm long, (6-) 6.7-7.0 (-8) mm wide, with a usually strongly recurved 4-5 mm long easily detached beak, densely villous with erect to ascending whitish to tawny hairs, the longest hairs 1-2 mm long, mostly unbranched to occasionally slightly pectinately branched hairs, over a dense layer of shorter, dense, curled hairs on the legume surface. Ovules 3-5, the legume with short, incomplete cross-partitions, typically maturing 1-4 seeds per legume. Seeds suborbicular, 3-3.5 mm long, 3 mm wide, flattened at the hilum end and slightly indented at the hilum, laterally somewhat flattened, glabrous except for a tuft of translucent to whitish branched hairs at the hilum, the seed surface varying from light tan to dark brown, the color comprised of irregular anastomizing light brown and dark brown patches and lines, and irregularly spaced brown dots, occasionally with some light brown patches, the rim of the laterally compressed seed somewhat lighter colored than the seed faces.

The name "*ocalensis*" is derived from Ocala, the county seat of Marion County, which is close to the center of the range of *Lupinus ocalensis*. We have observed populations in Citrus, Hernando, Lake, Marion, Orange, Seminole, and Sumter counties in central Florida, and based on their connectivity, we suspect that its range extends northeastward to the Jacksonville area but may not extend into Georgia. The morphological characters which separate *L. ocalensis* and *L. diffusus* are relatively weak, and without genetic evidence we may not have recognized it as an evolutionary lineage separate from *L. diffusus* populations from the Florida Panhandle and *L. diffusus* populations sampled from other States (Mississippi, Georgia, South Carolina, and North Carolina). Further investigation is needed to determine the full extent of the range of this species within northeast Florida. The confirmed populations of *L. ocalensis* are separated by about 400 km from the nearest known site for *L. diffusus* in the Florida Panhandle, with the common unifoliolate *Lupinus* in the intervening region being *L. villosus*. Additional genetic sampling and morphometric study of *Lupinus* populations from northeast Florida and southeast Georgia would be beneficial in determining range limits of *L. diffusus* and whether *L. ocalensis* occurs in southeast Georgia.

With its range restricted to north-central and northeast Florida, and its occurrence in prime habitat for upland development, *Lupinus ocalensis* meets the criteria for NatureServe ranking as G3 (Globally Vulnerable) and S3 (State Vulnerable). There are several known populations on protected sites, including the Withlacoochee State Forest and the Marjorie Harris Carr Cross Florida Greenway. It is likely that populations exist in the Ocala National Forest and in other state and local parks and preserves in the region. *Lupinus ocalensis* is a sandhill gap specialist found on xeric yellow sands in fire-maintained sites and is a re-seeder following soil disturbance or post-fire, germinating from the seedbank. Maintenance of its fire-frequented habitat should increase population viability, but mechanical site preparation techniques (roller chopping, mowing, etc.) at sites in the Greenway or other locations should be strongly discouraged as a surrogate to prescribed fire.


A
B

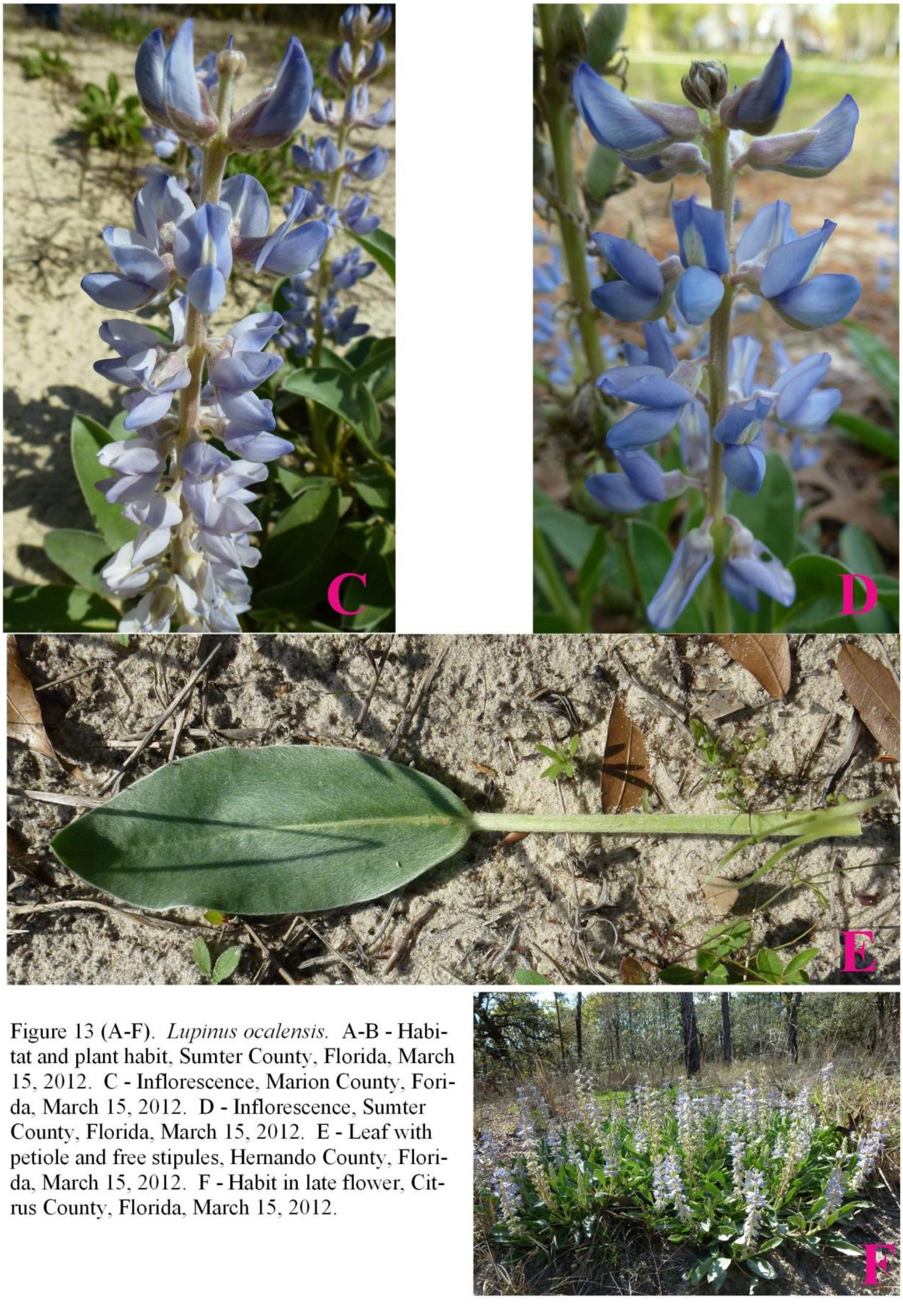


Figure 13 (A-F). *Lupinus ocalensis.* A-B - Habitat and plant habit, Sumter County, Florida, March 15, 2012. C - Inflorescence, Marion County, Forida, March 15, 2012. D - Inflorescence, Sumter County, Florida, March 15, 2012. E - Leaf with petiole and free stipules, Hernando County, Florida, March 15, 2012. F - Habit in late flower, Citrus County, Florida, March 15, 2012.

7. LUPINUS PILOSIOR E. Bridges & Orzell, **sp. nov.** **TYPE: Florida.** Charlotte Co.: *Pinus palustris / Quercus laevis / Aristida beyrichiana* sandhill savanna on yellow sand, on S side of Creek Trail, ca. 1.1 km W of jct Boxwood Road, ca. 300 m S of Fla 764 (Washington Loop Road), in the Ridge Harbor community, 26°59'30" N, 81°55'53" W, Soil type - Electra fine sand (Arenic Ultic Haplaquods), 6 Mar 2012, *Orzell & Bridges 26566* (holotype: FLAS; isotypes: USF, Z). DNA sample F08 in this study. Figure 14.

Like *Lupinus cumulicola*, differing in its prostrate growth habit, densely spreading arachnoid-villous leaf petioles and blades, and longer (19-25 mm) free stipules.

Annual or winter biennial herb (nearly always semelparous), the main stem branched from at or near the base to form a prostrate to slightly erect diffusely branched mat from (25-) 65-140 (-175) cm in diameter, usually only (20-) 30-45 (-72) cm tall at the tallest point (plant width/height ratio from 1.9 to 3.1), the main stems usually with 5 to 10 primary branches, these then further branched and prostrate to decumbent. Stems leafy throughout, the young stems and leaves densely white arachnoid-villous, the hairs mostly appressed-ascending to somewhat spreading, never tightly appressed, the longest hairs 2-3 mm long, mostly obscuring the surface of the stems and leaf petioles, the leaf blade surface softly velvety to the touch. Leaves narrowly elliptic, petiolate, the petiole (18-) 32-45 (-70) mm long, (2-) 3-4 (-6) mm wide, densely ascending-villous to spreading villous; free portion of stipules (13-) 19-25 (-39) mm long; leaf blade narrowly elliptic, (55-) 70-90 (-118) mm long, (25-) 35-43 (-60) mm wide, the leaf length/width ratio mostly from 1.9 to 2.2, densely arachnoid-villous on both surfaces, the hairs somewhat spreading, particularly near the leaf margin, grayish-green from the dense pubescence, but the green leaf surface usually visible. Inflorescence indeterminate, mostly 10-20 cm long and 25-30 mm wide in flower when fully developed, the flowers subverticillate, light blue to light lavender, the rachis densely pubescent, the hairs somewhat spreading. Bracts narrowly lanceolate, 4-5 mm long, densely ascending to spreading villous with hairs to 2 mm long. Pedicels spreading and 1.5-2.5 mm long in flower, becoming reflexed, stouter and 4-5 mm long in fruit. Calyx bilabiate, 7-10 mm long, densely ascending-appressed villous with light brown hairs obscuring the outer surface. Flowers ca. 1 cm long, the banner 9-10 mm long, light to medium blue or bluish-lavender with a white to cream-colored central eyespot, the wings 9-10 mm long, strongly curvate, keel 9-10 mm long, strongly curvate. Legumes (18-) 36-44 (-54) mm long, (8-) 10-12 (-17) mm wide, with a usually strongly recurved 4-5 mm long easily detached beak, densely villous with erect to ascending whitish hairs, the longest hairs 3-4 mm long, irregularly pectinately branched, often arranged in distinct lines with narrow undulating longitudinal furrows between the densely villous zones, over a dense layer of shorter, dense, curled hairs on the legume surface. Ovules 3-5, the legume with short, incomplete cross-partitions, typically maturing 1-3 seeds per legume. Seeds suborbicular, 4.5-5 mm long, 3.5-4.5 mm wide, flattened at the hilum end and slightly indented at the hilum, laterally somewhat flattened, glabrous except for a tuft of translucent to whitish branched hairs at the hilum, the seed surface varying from light tan to dark brown, the color comprised of irregular anastomizing dark brown patches and lines, and irregularly spaced brown dots, occasionally with some light brown patches, the rim of the laterally compressed seed somewhat lighter colored than the seed faces.

The name "*pilosior*" is derived from an unpublished manuscript by James B. McFarlin, which he had used on herbarium labels for this entity as early as 1931, as a form of *Lupinus diffusus*, but which was never published (McFarlin 1935). Since his use of this name was at the rank of *forma*, and was never published, it was not necessary for us to use his name for this species. However, it is an appropriate name for this species with its dense pilose indumentum, so much so that McFarlin sometimes confused it with *L. villosus* when not in flower. Examination of the McFarlin specimens at MICH with this name (all from Manatee and Sarasota counties) revealed that the name was being applied only to plants in this study with the dense, long spreading, pilose indumentum of this species, which corresponds to the variant SWFL (see above), occurring at scattered localities in Hillsborough,


A
B
C
D

Figure 14 (A-G). *Lupinus pilosior*. A - Habit, Charlotte County, Florida, March 6, 2012, the prostrate branches under fallen *Quercus laevis* leaves. B - Inflorescence, Charlotte County, Florida, March 6, 2012. C - Inflorescence, Hardee County, Florida, March 11, 2012. D - Inflorescence in fruit, Hardee County, Florida, March 11, 2012. E - Habit, Hardee County, Florida, March 11, 2012. F - Leaf with free stipule tips, Collier County, Florida, March 28, 2012. G - Inflorescence in early bud, Hardee County, Florida, March 11, 2012.

Manatee, Hardee, Sarasota, Desoto and Charlotte counties, west from near the Lake Wales Ridge to the Gulf of Mexico coast (Fig. 2). The densely long-pilose indumentum found on *L. pilosior* is matched only by *L. villosus* among the Florida unifoliolate species, but these do not overlap in geographic range and differ in flower color.

Lupinus pilosior is occasional on isolated xeric sandy uplands west of the Lake Wales Ridge in west-central and southwest Florida, from Pasco County south to Collier County, and inland to Hardee and Desoto counties. No other species of *Lupinus* is found within its range. There are some populations just west of the Lake Wales Ridge where *L. pilosior* seems to intergrade with *L. cumulicola*, mostly on the Lake Henry Ridge and isolated xeric sand ridges west of Lake Wales. It is possible that there could also be intergradation with *L. ocalensis* at its northern limit, but none were noted. This would be most likely in Pasco County, where there are older specimens of both species. *Lupinus pilosior* displays ballistic seed dispersal, where the seeds are forcefully expelled once the legume fruit reaches maturity and dries. This type of seed dispersal has been documented in other sandhill plants from peninsular Florida sandhills (Stamp & Lucas 1990).

Lupinus pilosior is a sandhill gap-specialist, and a likely reseeder from a persistent seedbank. It is becoming increasingly difficult to locate populations of *Lupinus pilosio*r in intact sandhill habitats. Several of our study sites were degraded xeric areas within pastures, as *Lupinus* is not palatable to cattle or horses, but grazing animals create enough soil disturbance for seed germination. Nonetheless, pasture maintenance is detrimental to long-term population viability, and it would not require much change in management to eliminate it from many pastures. Since the foliage is poisonous to livestock when ingested, there could be active efforts to eliminate it from pastures. Populations occurring on lands slated for future phosphate mining, a large extent of its current range, will eventually be destroyed by mining operations. Extant intact populations were only found at Lake Manatee State Park, Oscar Scherer State Park, and very few other locations. For these reasons we believe it qualifies for ranking as G2 (Globally Imperiled) and S2 (State Imperiled), using NatureServe conservation ranking criteria.

8. LUPINUS FLORIDANUS E. Bridges & Orzell, **sp. nov. TYPE. Florida:** Polk Co.: *Pinus palustris / Quercus laevis / Aristida beyrichiana* sandhill, E of Billig Rd and W of Bravo Range on Avon Park Air Force Range, NWQ, SWQ, Sec. 4, T32S, R30E, 27°43'28" N, 81°19'52" W, 21 Feb 2017, *Orzell & Bridges 27287* (holotype: NY; isotypes: FLAS, FSU, GH, NCU, USF, Z). Figure 15.

Like *Lupinus cumulicola*, differing in its narrower (length:width ratio 2.4 to 2.9), and yellowish-green conduplicate leaf blades, the leaf blade color visible through the appressed pubescence.

Annual or winter biennial herb (almost always semelparous), sometimes persisting into a third year if not flowering the second year, the main stem usually stiffly erect, becoming suffrutescent or somewhat woody, forming plants mostly (25-) 62-110 (-170) cm in diameter, usually (25-) 50-75 (-170) cm tall at the tallest point (plant width/height ratio mostly from 1.0 to 1.7), the main stems usually with 5 to 15 primary branches, with the first branch mostly formed from 5 to 15 cm, sometimes more than 30 cm, above the soil surface, the plants mostly with an erect shrubby aspect except when collapsed or recumbent from their own weight. Stems and lateral branches mostly leafy only in the upper half, leafless for half or more of their length, the young stems and leaf petioles densely appressed-pubescent with 0.5-1.5 mm long tightly appressed-ascending hairs, mostly obscuring the surface of the stems and leaf petioles, the leaf blade surface with sparse to moderately dense tightly appressed hairs 0.5-1.0 mm long, not obscuring the green leaf surface, the leaves like suede leather to the touch. Leaves narrowly elliptic, petiolate, the petiole (10-) 22-35 (-56) mm long, (1-) 2-3 (-4) mm wide, densely appressed-villous; free portion of stipules mostly 8-12 mm long, rarely longer; leaf blade narrowly elliptic, (40-) 65-85 (-113) mm long, (15-) 25-32 (-44) mm wide, the leaf length/width ratio mostly from 2.4 to 2.9, sparsely to moderately dense appressed-ascending

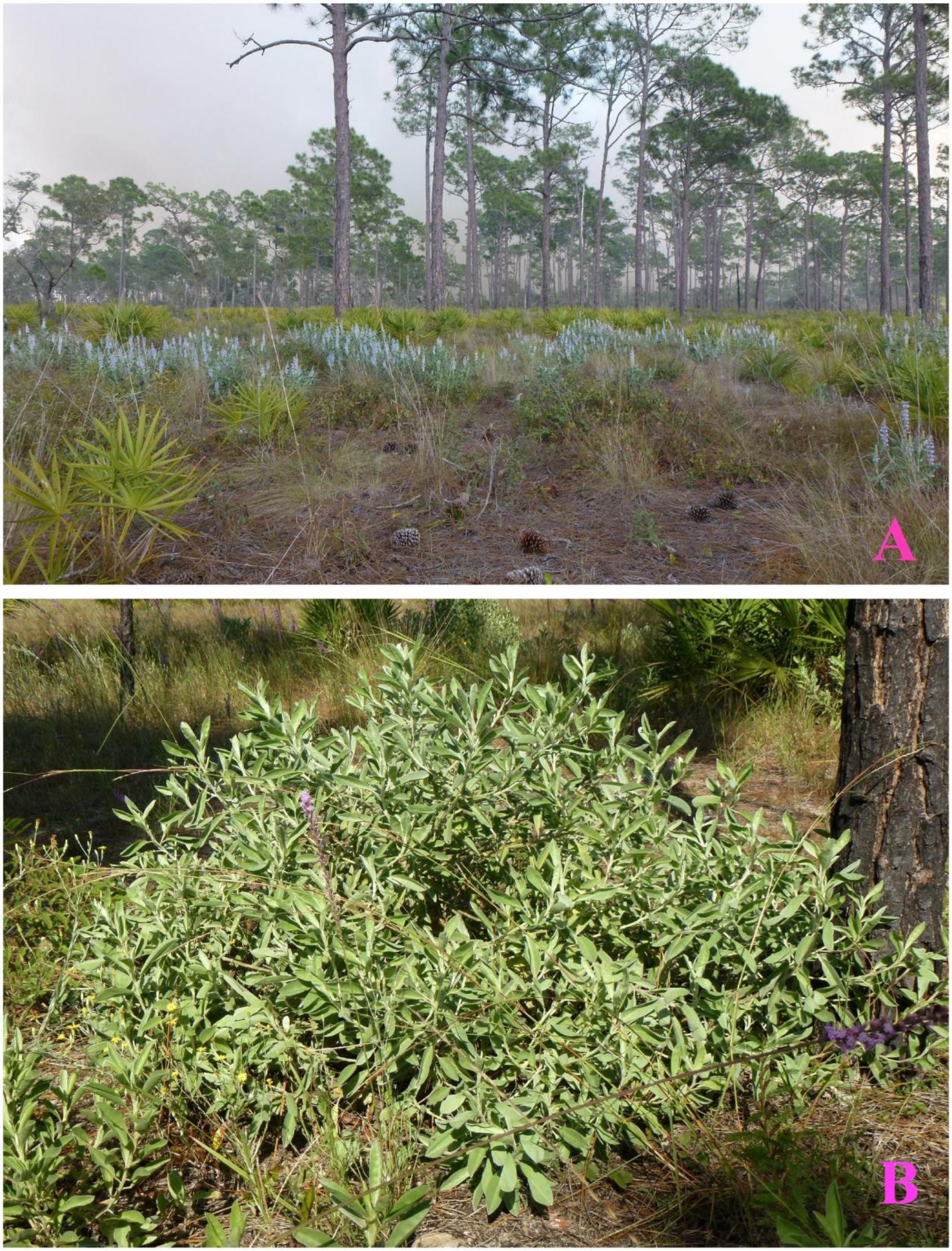
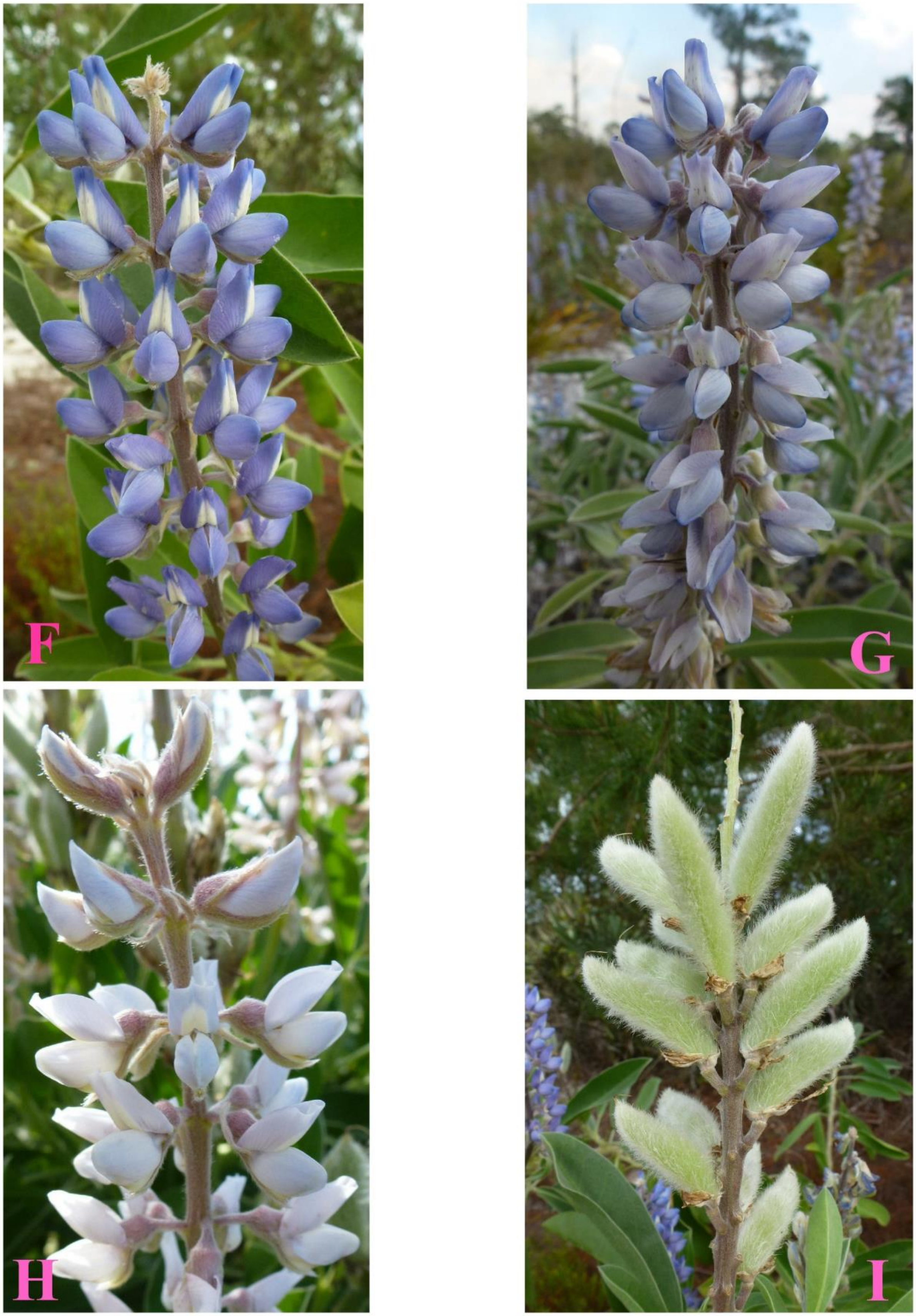

A
B

Figure 15 (A-I). *Lupinus floridanus*. A - Habitat, Polk County, Florida, March 4, 2015. B - Large plant, 1 m tall by 2 meters in diameter, Highlands County, Florida, October 16, 2011. C - Typical plant, Polk County, Florida, February 21, 2017. D - Lower stem branching, same plant as C. E - Habit, Polk County, Florida, January 21, 2022. F - Inflorescence, Martin County, Florida, March 8, 2012. G - Inflorescence, St. Lucie County, Florida, March 12, 2012. H - Inflorescence, Polk County, Florida, March 4, 2015. I - Inflorescence in fruit, Martin County, Florida, March 8, 2012.


F
G
H
I

pubescent on both surfaces, the hairs tightly appressed to the leaf surface, slightly more evident along the leaf margin, the fresh leaves yellowish-green, the green leaf surface evident below the pubescence. Inflorescence indeterminate, mostly 5-15 cm long and 25-30 mm wide in flower when fully developed, inflorescence rachis densely and finely appressed-ascending pubescent, the flowers very pale blue or pale lavender to almost white. Bracts caducous, narrowly lanceolate, 4-5 mm long, densely villous with hairs mostly 0.5-1 mm long. Pedicels ascending to spreading at anthesis, 2-3 mm long, becoming spreading to reflexed, stouter and 4-5 mm long in fruit. Calyx bilabiate, 9-10 mm long, densely ascending-appressed villous with light brown hairs obscuring the outer surface. Flowers ca. 1 cm long, the banner 9-10 mm long, wings 9-11 mm long, slightly curvate, keel 9-10 mm long, strongly curvate. Legumes (13-) 32-40 (-47) mm long, (5-) 9-11 (-15) mm wide, with a usually strongly recurved 4-6 mm long easily detached beak, densely villous with erect to ascending tawny to light brown hairs, the longest hairs 1-2 mm long, irregularly smooth to barbellate or sometimes pectinately branched, evenly pubescent, over a dense layer of shorter, dense, curled hairs on the legume surface. Ovules 4-6, the legume with short, incomplete cross-partitions, typically maturing 3-6 seeds per legume. Seeds suborbicular, 3.5-5 mm long, 3-4 mm wide, flattened at the hilum end and slightly indented at the hilum, laterally somewhat to strongly flattened, glabrous except for a tuft of translucent to whitish branched hairs at the hilum, the seed surface varying from ivory to light tan to dark brown, the color comprised of irregular anastomizing light brown and dark brown patches and lines, and irregularly spaced brown dots, occasionally with some light brown patches, the rim of the laterally compressed seed somewhat lighter colored than the seed faces.

The name "*floridanus*" refers to its restricted range limited to central peninsular Florida, along with the entire *Lupinus cumulicola* complex. In the past, some specimens of this species had been assigned to *L. cumulicola* (e.g., Dunn 1971) based on its shared shrubby erect habit. However, there are significant differences in leaf shape, color, and pubescence between *L. floridanus* and typical *L. cumulicola*. This species prompted our initial curiosity concerning unifoliate lupines, given its morphological distinctiveness from both *L. diffusus* and *L. cumulicola*, even in populations separated by only a few kilometers from *L. cumulicola*.

Lupinus floridanus is a sand gap specialist, and an obligate re-seeder, germinating following fire or soil disturbance. Within intact habitats it germinates between 12-18 months post-fire from a long-persistent seedbank. It mostly occurs in fire-maintained scrubby pinelands and sandhills, with coastal populations in scrubby pinelands and scrub. *Lupinus floridanus* occurs infrequently along the Atlantic Coastal Ridge and on isolated sand ridges east of the Lake Wales Ridge in central and south Florida (Fig. 2) and corresponds to populations assigned to EFL in Nevado et al. 2024. On the Atlantic Coastal Ridge it occurs from Brevard County south to Palm Beach County (with a historical record from as far south as Broward County). It is also found on most of the isolated sand ridges and xeric uplands in St. Lucie, Okeechobee, Osceola, and eastern Highlands and Polk counties. It is the only species of *Lupinus* east of the Lake Wales Ridge (south of the ranges of *L. aridorum* and *L. ocalensis* in Orange County). It is locally abundant on the Bombing Range Ridge in Polk and Highlands counties, where there is more remaining intact habitat at Avon Park Air Force Range than elsewhere within its range. Because of its abundance at Avon Park Air Force Range and presence at several protected sites on the Atlantic Coastal Ridge from Palm Beach to Brevard counties, it may be less threatened as the other peninsular Florida endemic species. The populations at Avon Park Air Force Range have been subject to life history and demographic studies by the authors. These have resulted in opportunities to implement land management strategies which could ensure the viability of several large populations on this military installation. Although management favoring this species may be more difficult in the suburban landscape of preserves on the Atlantic Coastal Ridge, it is still likely that some sites will be maintained. However, these preserved sites still could have changes in land use and management which could threaten the long-term viability of *L. floridanus*. Therefore, we propose it be ranked as G3 (Vulnerable), with possible downgrading to G4 (Apparently Secure) with additional conservation actions, using NatureServe ranking criteria.

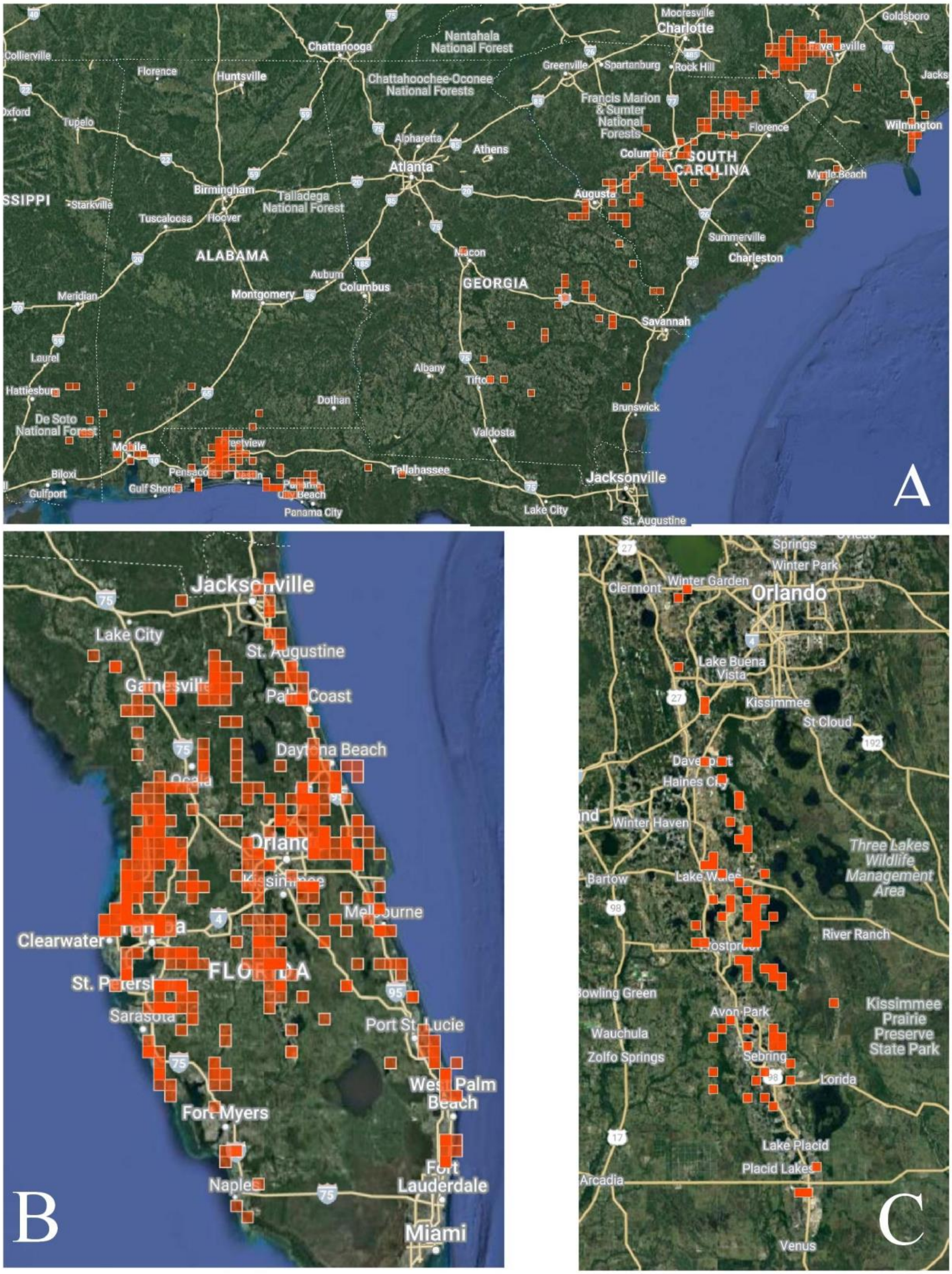


Figure 16. *Lupinus* range maps from iNaturalist as of November 8, 2023 - A - *Lupinus diffusus*. B - *Lupinus cumulicola* complex (including the new species named in this publication, not distinguished). C - *Lupinus cumulicola* (*sensu stricto*).

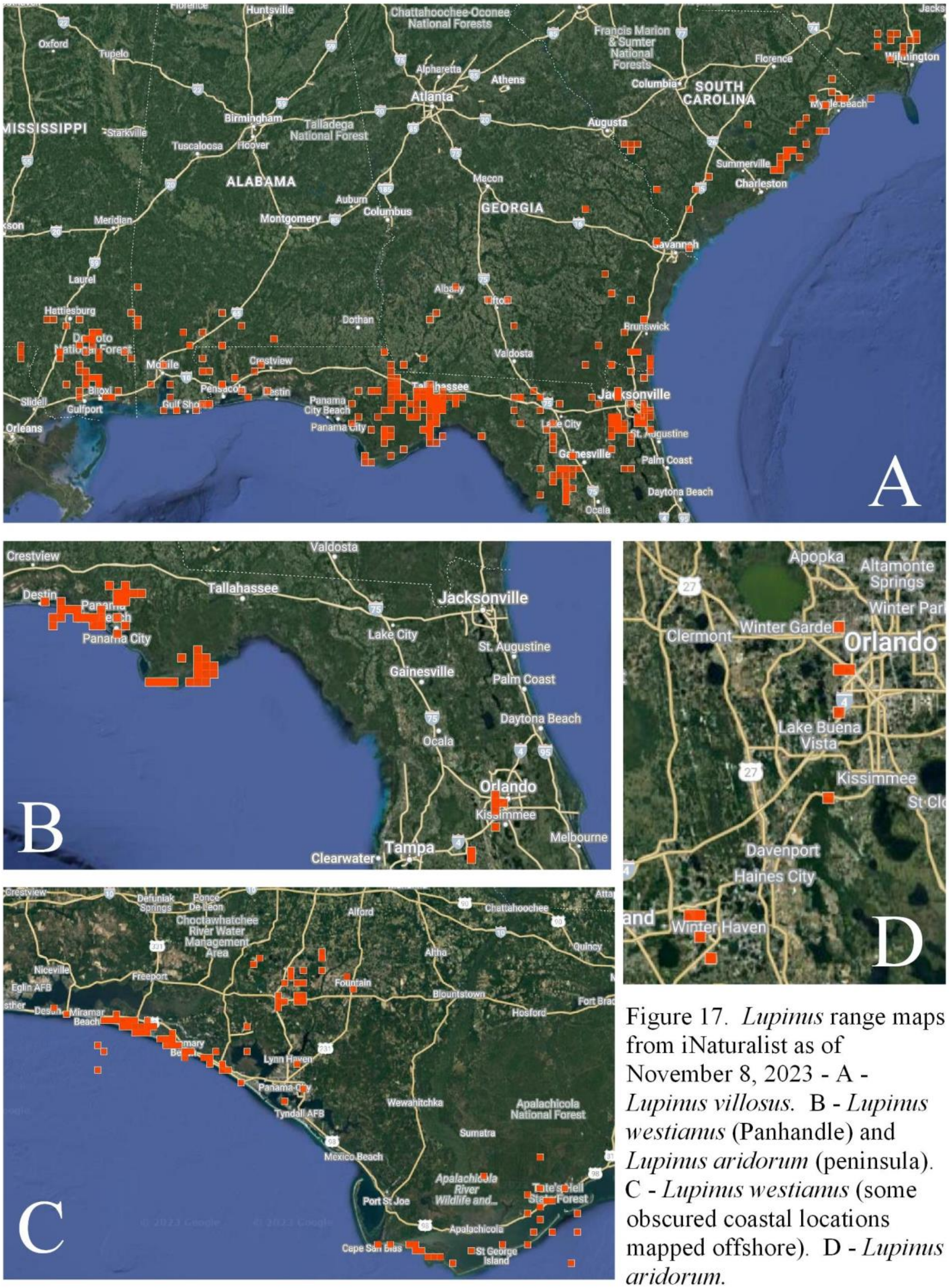


Figure 17. *Lupinus* range maps from iNaturalist as of November 8, 2023 - A - *Lupinus villosus*. B - *Lupinus westianus* (Panhandle) and *Lupinus aridorum* (peninsula). C - *Lupinus westianus* (some obscured coastal locations mapped offshore). D - *Lupinus aridorum*.

The status and disposition of the McFarlin *Lupinus* collections at MICH

During the week of July 9-13, 2012, the senior author examined the collections of *Lupinus* by James McFarlin that are housed in the herbarium of the University of Michigan at Ann Arbor (MICH). McFarlin had a strong interest in *Lupinus* in central Florida, and in the process of collecting his unfinished dissertation in 1931, made at least eleven collections of unifoliolate *Lupinus*, some of which are represented by multiple mounted sheets. In his dissertation manuscript, McFarlin references three unpublished names for new taxa, in addition to recognizing the existing species *L. cumulicola*, *L. diffusus*, and *L. villosus*. The senior author examined the specimens designated as the type material and other specimens referred by McFarlin to these taxa, in order to determine the relationship of his concepts to our current research on *Lupinus* in central Florida. Each McFarlin name will be discussed in the following sections.

Lupinus aridorum

McFarlin, in his manuscript, designated his collection number 4422 as the type of *Lupinus aridorum*. There are three sheets with this collection number at MICH, all with the location “in the scrub, Inwood, Winter Haven, Polk County”, collected on March 8, 1931. Inwood is located northwest of downtown Winter Haven, between Winter Haven and Auburndale. This area is now almost all urbanized. The three sheets of *McFarlin 4422* at MICH are all designated as the type for his name *Lupinus aridorum*, one marked “type for flower”, the second marked “type for fruit”, and the third as “part of type”. These sheets clearly match the concept of the currently recognized species *Lupinus aridorum* McFarlin ex Beckner. The leaves of these specimens are relatively small for a unifoliolate *Lupinus*, averaging 45-55 mm long and 15-21 mm wide, generally from 2-3 times as long as wide. The leaves are ellliptic to obovate, densely appressed pubescent, and with petioles mostly 20-30 mm long. The flowers are quite pale with a dark eye spot (actual color has been lost from the specimens, which are now mostly shades of tan to brown). The fruit is about 22-30 mm long, 7-9 mm wide, strongly villous, with the hairs somewhat spreading. These short fruits are also consistent with our current concept of *Lupinus aridorum*.

Although there are several other collection numbers for *Lupinus aridorum* cited in the McFarlin manuscript (1086, 7341, S.F. Poole s.n.), this is the only collection number for *L. aridorum* present in the MICH herbarium.

Lupinus cumulicola

There are two specimens of *Lupinus cumulicola* cited in the McFarlin manuscript, and both are present at MICH. *McFarlin 4294*, in early flower, was collected on Feb. 22, 1931, in “high pine land near Lake Marion, Polk Co.”. *McFarlin 5106*, in late fruit, was collected on May 6, 1931, in “high pine land east of Dundee, Polk Co.” Both are clearly within our current concept of *Lupinus cumulicola* Small. McFarlin stated in his manuscript that *L. cumulicola* “is the largest of our lupines, often forming upright shrubs.” The high pine land habitat cited for these specimens is also consistent with our observations that *L. cumulicola* prefers longleaf pine sandhill habitats.

There is one other collection of *Lupinus cumulicola* at MICH, *Stoutamire 1072,* collected in full flower on January 30, 1955 from “2 mi W of Avon Park, Highlands Co., Florida.” This collection location would be in the vicinity of the Avon Park airport property, which still supports large populations of *L. cumulicola*. The label mentions that these plants are “2 feet tall,” indicating an upright growth form.

Figure 18. McFarlin specimens of *Lupinus pilosior* from Manatee County, Florida at MICH - originally identified as *Lupinus villosus* and three *nomen nudum* forms of *L. diffusus* and *L. villosus.*

Lupinus diffusus* forma *pilosior

The name *Lupinus diffusus* Nutt. "forma *pilosior* McFarlin f. nov." does not appear in the McFarlin manuscript, but was present on a specimen in the collection at MICH. The fact that this name does not appear in the manuscript likely indicates that McFarlin had not seen this form in Polk County, and perhaps not in the entire “Lake Region” of central Florida. The collection designated as the type consists of two sheets of *McFarlin 4397,* collected in full flower on March 4, 1931, from “Sarasota, Sarasota Co.”. This collection has stems, petioles, and leaves with villous, somewhat spreading, pubescence. This unpublished entity is a clear match for what we are describing as *L. pilosior,* the “southwest Florida form” of the *L. cumulicola* complex (Figs. 10 and 18).

Curiously, McFarlin then collected this entity at least three more times in the same month in Manatee County, but identified all of these as *Lupinus villosus*, a species which has never been found this far south in peninsular Florida. It is apparent from these collections that McFarlin had probably never seen true *L. villosus,* and was emphasizing the villous pubescence of the leaves rather than the flower color in identifying his collections. The collections from Manatee County originally identified as *L. villosus* but referable to *L. pilosior* are these:

McFarlin 4520, March 20, 1931, “east of Manatee, Manatee Co., flrs purplish-blue”
McFarlin 4521, March 20, 1931, “east of Manatee, Manatee Co., flrs blue”
McFarlin 4536, March 22, 1931, “east of Manatee, Manatee Co., flrs purplish-blue”

There is also another McFarlin specimen of this form at MICH, *McFarlin 4374,* with a label giving no date, no location, and no determination. However, this number is cited in the McFarlin manuscript as from “Lake Alfred.” Lake Alfred is north of Winter Haven, perhaps near the north end of the Winter Haven Ridge. If this location is correct, this is a short-disjunct location of *Lupinus pilosior* or perhaps an introgressed intermediate form.

Another specimen at MICH referable to *Lupinus pilosior* is *Small, Mosier, and Small s.n.* from February 10, 1928, labelled as from “Myakka Prairie, southeast of Venice (on Pop-ash).” This would put this specimen in Sarasota County, but *L. pilosior* is definitely not an epiphyte on *Fraxinus cubensis,* so we cannot be certain that this label was placed with the correct specimen during distribution or mounting.

Lupinus villosus* forma *bartramianus

This unpublished name is cited in the McFarlin manuscript as the white flowered form of *Lupinus villosus*, but is said to not occur “within our range” (Polk County, or the Lake Region of central Florida). There is a specimen at MICH, *McFarlin and Poole 4535,* collected in March 1931 “in sand (scrub/) on Myakka City Rd, Manatee County” which is designated as the type (Fig. 18). This specimen consists of two sheets, both in flower, and notes as having “fls white.” Other than in flower color, this specimen appears to be identical with *L. pilosior*, and there is no obvious reason to refer it to *L. villosus* rather than to *L. pilosior*. In the absence of anthocyanin, the details of the flower color differences are obscured, and it appears that McFarlin referred this albino form to *L. villosus* solely on its leaf pubescence, shown in this study to be characteristic of southwest Florida populations of *L. pilosior*.

There is one additional specimen with this name at MICH, collected by S.F. Poole (s.n.) in March 1931 at the same location as the above and labelled in McFarlin’s handwriting. This implies that S.F. Poole perhaps was the first to find these albino plants, then led McFarlin to the site. “Myakka City Road” could refer to either FL 70 in southeastern Manatee County or to Wauchula Road, which connects FL 64 and FL 70.

Lupinus diffusus forma *albicoma*

This unpublished name is cited in the McFarlin manuscript from a single collection, *McFarlin 4865*, which is the only collection located of this entity at MICH (Fig. 18). The specimen is from "high pine land near Lake Garfield, Polk Co." and is stated as having "flrs pure white." We struggled at first with whether this specimen should be referred to *Lupinus cumulicola* or *Lupinus pilosior*, until we realized that Lake Garfield is just north of the northern end of the Lake Henry Ridge, east of Bartow. As such, a "high pine land near Lake Garfield" could be on the Lake Henry Ridge, where we have observed many *Lupinus* populations which are intermediate between *L. pilosior* and *L. cumulicola*. As such, we tentatively consider this collection as the albino form of the Lake Henry Ridge entity of *Lupinus*, an intermediate between those species.

Lupinus floridanus

There is one additional collection of *Lupinus* by McFarlin at MICH that is not clearly referrable to any of the above entities. This is *McFarlin 4728*, collected on April 8, 1931 from "Tiger Lake, Polk Co.," and cited as *L. diffusus* in the McFarlin manuscript. This specimen is stated on the label as "flrs light blue, plants decumbent." However, the specimen appears to be of a stiffly erect upper stem, and the leaves and stems have tightly appressed pubescence. The leaves are also narrowly elliptic, about three times as long as broad, which rules out the appressed-pubescent, but wider-leaved *L. cumulicola*. We are left with the meaning of "plants decumbent" as interpreted by McFarlin. McFarlin stated in his manuscript that *L. diffusus* is "usually decumbent or diffusely spreading, often forming large mats." In our current concept, *L. pilosior* is the entity in Polk County that is "diffusely spreading, often forming large mats", but the word "decumbent" can apply to either *L. pilosior* or *L. floridanus*, in which the lower branches are sometimes decumbent, or more often, technically, recumbent (arising from an erect stem but then bending downward, particularly with age and in fruit, towards the soil surface). Tiger Creek is not within the known range of our current concept of *L. pilosior*, but is a possible location for *L. floridanus*, particularly since it is east of the eastern edge of the Lake Wales Ridge, and at the same longitude and only about 10 km north of the nearest location of *L. floridanus* on Avon Park Air Force Range. Therefore, based on the combined evidence, we refer this specimen to *L. floridanus*. Any remaining accessible sandy uplands near Tiger Lake (north of FL 60, north of Indian Lake Estates and south of Lake Kissimmee State Park) should be searched for *Lupinus* to potentially confirm this identification.

If all of the above specimens are correctly placed within current concepts, McFarlin collected the following *Lupinus* entities:

Lupinus aridorum – only near Inwood in Polk County
Lupinus cumulicola – in sandhills east of Dundee and near Lake Marion, Polk County
Lupinus pilosior – in Manatee and Sarasota counties, and perhaps near Lake Alfred, north of Winter Haven, Polk County
Lupinus cumulicola x pilosior 'Lake Henry Ridge entity' – near Lake Garfield, perhaps near the north end of the Lake Henry Ridge
Lupinus floridanus– near Tiger Lake, about 10 km N of APAFR

McFarlin never collected typical *Lupinus diffusus*, since it does not occur in Polk County, and we suspect he never saw *L. villosus*, since it occurs in peninsular Florida south only to Levy and Alachua counties.

Biogeographical Patterns of Peninsular Florida Xeric Sandhill and Scrub Species

The xeric upland habitats of peninsular Florida are well known for their high levels of endemism, with recent descriptions of numerous endemic and previously unrecognized Lamiaceae and Asteraceae from this narrow region. Many of these recently described endemics are often in close proximity to species of the Floridian unifoliate *Lupinus* clade, in the same microhabitats or in

Ridge	*Lupinus* species	Other endemic species
Atlantic Coastal Ridge	*Lupinus floridanus*	*Chrysopsis delaneyi* *Conradina grandiflora* *Dicerandra immaculata* *Pityopsis latifolia* *Polygonella robusta* *Trichostema hobe*
Tenmile Ridge	*Lupinus floridanus*	*Conradina grandiflora* *Pityopsis latifolia*
Bombing Range Ridge	*Lupinus floridanus*	*Chrysopsis highlandsensis* *Pityopsis aequilifolia* *Polygonella basiramia* *Trichostema bridgesii-orzellii*
Lake Wales Ridge	*Lupinus cumulicola*	*Chrysopsis delaneyi* *Conradina brevifolia* *Dicerandra christmanii* *Dicerandra frutescens* *Dicerandra modesta* *Pityopsis aequilifolia* *Polygonella basiramia* *Trichostema bridgesii-orzellii*
Southwest Florida ridges	*Lupinus pilosior*	*Chrysopsis floridana* *Polygonella ciliata* *Polygonella brachystachya*
Brooksville Ridge	*Lupinus ocalensis*	*Calamintha coccinea* *Trichostema microphylla* *Chrysopsis linearifolia ssp. dressii*
Ocala upland	*Lupinus ocalensis*	*Conradina etonia* *Conradina cygniflora* *Dicerandra cornutissima* *Trichostema suffrutescens*
Panhandle Coastal Scrub	*Lupinus westianus*	*Chrysopsis cruiseana* *Chrysopsis godfreyi* *Conradina canescens* *Polygonella macrophylla* *Trichostema latens*
Greenhead slope	*Lupinus westianus*	*Chrysopsis lanuginosa* *Conradina sp. nov.* *Dicerandra fumella* *Trichostema dichotomum* *Polygonella polygama*

Table 3. Endemic species of selected genera in the range of *Lupinus* species

similar nearby xeric uplands. Although each species has a unique pattern of distribution, with some occupying a single sand ridge system and others present on multiple ridges, their ranges are often correlated. This implies that some of the same factors of geographic isolation and limited gene flow influencing the Floridian *Lupinus* may be impacting these other closely associated genera. Table 3 lists narrowly endemic species found in roughly the same ranges as some of the *Lupinus* in this study. The correlation of ranges of narrowly endemic species in mostly unrelated genera further supports the biographical distinction of these regions and of their endemic *Lupinus* species.

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