

# Conservation Status of the Plains Spotted Skunk, *Spilogale putorius interrupta*, in Texas, with an Assessment of Genetic Variability in the Species

A final report to the Texas Comptroller's Office

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Plains Spotted Skunk from Waller County, Texas  
Photo credit: Alexandra A. Shaffer

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## I. PROJECT OVERVIEW

In this report, we present results of research on the conservation status of the plains spotted skunk (*Spilogale putorius interrupta*) in Texas and an assessment of the genetic variability in populations throughout the range of the species. The conservation status portion of the study included (1) mapping the species' potential habitat in Texas using maximum entropy modeling (Maxent) with historic museum specimen records, (2) field-based surveying of locations in 10 counties to determine occurrence of the plains spotted skunk, (3) seeking additional occurrence records in Texas through crowd sourcing and citizen scientist approaches (4) using all current (2001 – 2017) occurrences to produce a model of probable geographic distribution in Texas and (5) assessing anthropogenic changes in land use, which may threaten the species' habitats, by mapping current and forecasted oil and gas development and urbanization within the species' modeled range. The species distribution model, combined with the land-change assessment, was used to select sites in 10 representative counties for field-based surveys in the hopes of revealing patterns of current distribution. Field surveys were carried out using live traps, enclosed track plates, and camera traps. These methods documented detections of plains spotted skunks ( $n = 12$ ) in 4 of the 10 sites sampled. All methods of detection were successful, but cameras and live traps out-performed track plates. Crowd-sourced approaches and citizen scientist camera trapping revealed an additional 82 occurrences in the state, 79 of which were since 2009. These recent records were used to produce a species distribution model that provides relative probability of occurrence for the plains spotted skunk in the state. Our land-change mapping revealed potential anthropogenic threats to habitats at 2 of the sites (Katy Prairie and Fort Hood), which also had robust populations of plains spotted skunks based on 25 and 51 detections, respectively).

For our genetic assessment, samples of tissue from three sources (i.e., field surveys, state agencies throughout the distribution of the eastern spotted skunk, and museum tissue collections) allowed a detailed assessment of the genetic variability in the species (*S. putorius*) using both microsatellite markers and cytochrome b gene sequence. Our analysis of 119 specimens was able to establish that genetic patterns were consistent with currently accepted taxonomy of the 3 recognized subspecies of *S. putorius* (*S. p. putorius*, *S. p. ambarvalis*, and *S. p. interrupta*). We also determined that there was no evidence for hybridization with the congener, *S. gracilis* (western spotted skunk), a species co-occurring with the eastern spotted skunk in parts of Texas. The differentiation between *S. p. putorius* and *S. p. ambarvalis* was less pronounced ( $F_{ST} = 0.178$ ; cytochrome b sequence divergence = 1.2%) than between these subspecies and the plains spotted skunk (average  $F_{ST} = 0.278$ ; cytochrome b sequence divergence = 2.9%). Overall, genetic variability (observed heterozygosity = 0.474) in the plains spotted skunk was lower than that seen in common carnivores (striped skunks, raccoons), but slightly higher than some endangered carnivores (black-footed ferret). The heterozygosity levels more closely resemble the levels found within the island spotted skunk (*S. gracilis amphiala*) from the Channel Islands of California and other vertebrates that have a "threatened" conservation status.

Key findings of the study include: 1) The current geographic distribution of the plains spotted skunk in Texas is reduced relative to historic records. 2) The species distribution model based on recorded occurrences since 2001 suggests areas of the state that are in need of further survey efforts. 3) genetic variability of plains spotted skunks is lower than more common carnivores, but higher than some recognized endangered species. 4) the subspecies, *S. p.*

*interrupta* is a distinct genetic subunit of the eastern spotted skunk; and 5) continued energy development and especially future urbanization in some parts of Texas may affect populations of the plains spotted skunk.

## II. BACKGROUND

The eastern spotted skunk, *Spilogale putorius*, is an uncommon mesocarnivore native to the central and eastern United States with a geographic range extending from Tamaulipas, Mexico to southern Pennsylvania, and an east-west distribution from the Continental Divide to southern Florida (Kinlaw 1995). Three subspecies of the eastern spotted skunk are currently recognized: the plains spotted skunk (*S. p. interrupta*), which is distributed largely throughout the Great Plains of the central and midwestern United States, the Appalachian spotted skunk (*S. p. putorius*), which occurs throughout the eastern United States and is generally associated with the Appalachian Mountain range, and the Florida spotted skunk (*S. p. ambarvalis*), which is restricted to peninsular Florida (Kinlaw 1995). Morphologically, all 3 subspecies retain the same striping pattern, yet differences in the width of these stripes, and therefore the relative ratio of black to white, serve to differentiate them (Van Gelder 1959). Specifically, *S. p. interrupta* exhibits the least amount of white overall, as noted by the thinner, white dorsal and shoulder stripes, a smaller, triangular nose patch, and the reduction or absence of white hairs present in the distal tip of the tail (Van Gelder 1959). In contrast, *S. p. ambarvalis* displays the greatest amount of white overall, as noted by the presence of thicker stripes, a larger nose patch, and the greater presence of white at the tip of the tail (Van Gelder 1959). In addition, adult males of the Florida subspecies attain the smallest average weight (400 g), in comparison to adult males of the Appalachian (600 g) and plains (660 g) subspecies (Van Gelder 1959). A sister species to the eastern spotted skunk, the western spotted skunk (*S. gracilis*) rarely occurs in sympatry with *S. putorius*, and can be differentiated from *S. putorius* by its occupation of the western United States, average smaller size, and presence of delayed implantation (Verts et al. 2001).

Although formerly considered a common carnivore in the midwestern United States, the eastern spotted skunk, and more specifically the plains subspecies, has experienced pronounced population declines throughout its range since the 1940s (Choate et al. 1974; Kaplan and Mead 1991; Gompper and Hackett 2005). Despite its past prevalence in the fur trade (annual multi-state harvests >100,000), overharvesting was not the only factor considered responsible for the decline of eastern spotted skunk populations (Gompper and Hackett 2005). Other possible contributors to the decline include large-scale changes in agricultural practices that occurred throughout the 20<sup>th</sup> century, disease, pesticide use, altered predator guilds, and population dynamics (Gompper 2017). Specifically, the modernization of farming methods is hypothesized to have been the primary impetus for the observed declines, as the destruction of dilapidated farm buildings, fence rows, creek bottoms, and wood piles (habitats historically abundant with spotted skunks) for industrial farming purposes served to reduce habitat and prey availability (Crabb 1948; Kaplan and Mead 1991; Gompper and Hackett 2005). Although it is agreed that anthropogenic activity instigated and hastened the decline of this in the 1940s, human-related activity is also thought to have facilitated the range expansion and local population size increases of the eastern spotted skunk during the late 19<sup>th</sup> century. For example, the then-marshy plains of the central United States were drained for farming efficiency, thereby enabling colonization of a previously uninhabitable area, and farm house and outbuilding construction provided shelter for

the skunks while affording them a steady food source in the form of crops, crop-eating insects, and commensal rodents (Van Gelder 1959; Choate et al. 1974).

The fluctuating nature of eastern spotted skunk populations over the past century has prompted concern over their conservation status, especially since this species is encountered very infrequently and is relatively understudied. Therefore, it is widely acknowledged that the eastern spotted skunk requires further population monitoring across its entire range. Currently, there is a paucity of studies aimed at assessing the status of local populations of eastern spotted skunks (Choate et al. 1974; Boppel and Long 1994; Reed and Kennedy 2000), with only a few focused on detecting (Hackett et al. 2007; Hardy 2013) or determining habitat requirements (McCullough and Fritzell 1984; Reed and Kennedy 2000; Lesmeister et al. 2008, 2009, 2013) for this elusive mesocarnivore. In response to the documented population declines and lack of *S. putorius* sightings, the International Union for Conservation of Nature (IUCN) now regards the eastern spotted skunk as vulnerable (Gompper and Jachowski 2016). Additionally, the U.S. Fish and Wildlife Service is currently considering the plains spotted skunk for listing as federally endangered (USFWS Federal Register 2012). Furthermore, on a state-by-state basis, the eastern spotted skunk is considered endangered, threatened, imperiled, or as a species of greatest conservation need in many states throughout its range (ESSCSG 2017).

The following report details the results of an assessment of the conservation status of the plains spotted skunk (*S. p. interrupta*) in Texas based on a field survey and modeling (Part III) and analysis of genetic variability across the species range (Part IV). Appendices 1 – 4 (Part V) are referenced in both of these sections.

## II. CONSERVATION ASSESSMENT AND MODELING FOR THE PLAINS SPOTTED SKUNK, *SPILOGALE PUTORIUS INTERRUPTA*, IN TEXAS

### INTRODUCTION

In Texas, historical trapping records for spotted skunks (often called civet cats) have been difficult to interpret, unlike those documented by Gompper and Hackett (2005) for the more northern parts of the range, because trapper surveys combined the eastern spotted skunk (*Spilogale putorius*) and western spotted skunk (*Spilogale gracilis*) into one species. This does not allow us to ascertain historical population numbers and trends in population for the plains subspecies following similar methods used by Gompper and Hackett (2005). Texas Parks and Wildlife Department classifies it as a species of greatest need ([https://tpwd.texas.gov/huntwild/wild/wildlife\\_diversity/nongame/tcap/sgcn.phtml](https://tpwd.texas.gov/huntwild/wild/wildlife_diversity/nongame/tcap/sgcn.phtml)).

Habitat associations of eastern spotted skunks across their range are varied and few studies have provided detailed assessments of habitat. Crabb (1948) found eastern spotted skunks to be closely associated with Iowa farms, many times with dens located in or under barns and other outbuildings. In Arkansas, eastern spotted skunks were found to inhabit both pine and hardwood forests, but showed a preference for early successional forests with dense understories (Lesmeister et al. 2009). Reed and Kennedy (2000) exclusively found spotted skunks in rhododendron (*Rhododendron* spp.) thickets along streams in the Appalachian Mountains of eastern Tennessee. In Texas, eastern spotted skunks are known to occur in wooded areas and prairies (Schmidly and Bradley 2016); however, this information is largely anecdotal because the habitat associations of the eastern spotted skunk have never been studied in Texas.

This report summarizes the results of an effort to determine the current status of populations of the plains spotted skunk in Texas based on field-based surveys, museum records, and data gathered from crowd sourced and citizen science approaches. We use these data to model high probability areas of the state expected to have populations of this uncommon species and examine the potential threats of energy development and urbanization to these populations.

### MATERIALS AND METHODS

As a preliminary assessment of the distribution of the plains spotted skunk in Texas, we gathered known museum records with georeferenceable locations from the late 1800s through the early part of the 21<sup>st</sup> century using VertNet (Constable et al. 2010) or information on museum specimen tags in research collections. These locations were used to produce a Maxent species distribution model (Version 3.3.3; Phillips et al. 2006; Phillips and Dudík 2008) to select field survey sites (Appendix 1). Additionally, we used a list of county economic importance (Comptroller 2015) to attempt to balance our survey between counties having higher or lower economic importance. Remaining core habitat for the species was also mapped (Appendix 2). By combining the species distribution model and the remnant core habitat, counties were ranked based upon the probability of occurrence of spotted skunks. From this ranking, 10 counties with a high probability of occurrence were chosen for sampling. The 10 counties surveyed were Wichita, Wise, Tarrant, Navarro, Coryell, Burleson, Waller, Harris, Calhoun, and Kleberg (Figure 1).

To increase the probability of detection and decrease the latency to detection for plains spotted skunks, we used multiple survey methods (Gompper et al. 2006; Hackett et al 2007). We

chose three such survey methods for the field component of this research and deployed the detection devices either as grids or transects depending on temporal or spatial constraints at a given study site. The maximum number of devices deployed per site was 120 arrayed as 40 live traps, 40 cameras, and 40 track plates. One device was deployed per station and stations were established 100 m from one another. Devices were deployed in such a manner that 2 devices of the same type were never at adjacent stations. Surveys were conducted over a 10-day period with devices being operational for 7 of the 10 days. All devices were checked daily during the operational period. All methods followed guidelines for use of mammals in research (Sikes et al. 2011). Research methods were approved by the Angelo State University Institution Animal Care and Use Committee (IACUC protocol 15-15).

Collapsible Tomahawk Live Traps (15 x 15 x 48 cm; Tomahawk Live Trap LLC, Hazelhurst, WI) were placed in the thickest available habitat, positioned so that the opening was oriented away from the thick cover. A 61 x 71 cm piece of burlap was placed over the trap to act as a cover. Traps were baited with a piece of chub mackerel (Chicken of the Sea International, Mt. Olive, NJ) placed in a shallow aluminum pan. Traps were checked as early as possible each morning and rebaited with fresh bait every other morning.

Two track plate designs were utilized to detect plains spotted skunks—one using a modified hair snare to collect hair samples (modified from Zielinski et al. 2006) and a control without a hair snare. Track plate devices were composed of an aluminum insert inside a corrugated plastic (Coroplast<sup>TM</sup>; Coroplast Inc, Dallas, TX) box (15 x 15 x 75 cm) and open on 1 end. The track plate insert was a 70 x 15 cm aluminum plate (modified from Zielinski et al. 2006; Hackett et al. 2007) with half of the insert (35 cm) covered with a tracking medium (recovered printer toner) and the other half covered with a 22-cm strip of contact paper, adhesive side up, and held in place by duct tape. Bait (chub mackerel) was placed in the closed end of the enclosure luring the animal over the tracking medium and registering its footprints on the contact paper.

Half of the track plates ( $n = 20$ ) were designed with a hair snare inserted into the track plate enclosure. The hair snare was placed halfway (35 cm) between the opening of the cover and the closed rear. The hair snare was comprised of a 15 x 2.5 cm strip cut from a commercially available glue board (Tomcat Glue Boards, Motomco, Madison, WI) stapled to the bottom side of a 30 cm long wooden stake. The hair snare was inserted through the sides of the enclosure, approximately 6 cm from the bottom and perpendicular to the long axis of the enclosure, with the glue strip positioned on the underside (Zielinski et al. 2006). The hair snare was designed to pivot upward on one end an additional 2 cm to allow for the passage of individual animals that vary in size (Zielinski et al. 2006). A second stake (without a glue strip) was placed horizontally above the hair snare to hinder individuals from climbing over rather than under the hair snare.

A positive detection was considered any footprint deposited on the contact paper. A negative detection was any footprint deposited solely in the carbon toner. Positive and negative detections of all species were recorded and the species identified using Elbroch (2003). Digital images of spotted skunk tracks were taken and the contact paper saved as a permanent record. Tracks of other species were not saved, except for those that needed additional consultation for identification of the species. Track plates were checked daily. After a visitation rendered the track plate non-operational, the tracking medium and contact paper were reapplied. Track plates were rebaited every other morning.

Bushnell Trophy Cameras (Bushnell Outdoor Products; Overland Park, KS) were set approximately 0.5 m above the ground and 5 to 6 m from the baited area. The cameras were attached to either a tree or to a t-post depending upon the availability of trees at the survey site. The cameras were angled slightly downward to maximize detection of the skunks and bait was deployed at a slightly lower height from the camera. Bait alternated between canned sardines (Beach Cliff Sardines; Bumble Bee Seafood; San Diego, CA) or commercial fish oil ([www.wildlifecontrolsupplies.com](http://www.wildlifecontrolsupplies.com)). All grass, limbs, and brush were removed between the camera and the bait to reduce false triggers. The cameras were set with the following settings: 3 picture burst, no delay between trigger events, and a normal trigger time. During part of the study a portion of the LED flash unit on cameras was covered with duct tape to decrease intensity of the flash and improve image quality.

In addition to field data collected on plains spotted skunks, we collected observations using crowd source methods. Initially, email blasts were sent out to the Texas Wildlife Rehab Coalition and to the Texas Society of Mammalogists. A “wanted poster” was later created and sent, via email blast, to these groups, the Texas Master Naturalist Program, and the Texas Chapter of the Wildlife Society (Figure 2). The wanted poster was shared on the Texas Society of Mammalogists, Texas Master Naturalist Program, the Texas Chapter of the Wildlife Society, Katy Prairie Conservancy, the Texas Trappers and Fur Harvesters, Texas Nature Trackers, and the Fort Worth Nature Center and Refuge’s home Facebook pages. The poster was further shared numerous (>300) times by members of these organizations.

Additionally, a project (Spotted Skunks of Texas) was created on the citizen scientist platform iNaturalist ([www.inaturalist.org](http://www.inaturalist.org)). This project along with the Mammals of Texas and Eastern Spotted Skunk projects was monitored for reported observations of *S. putorius interrupta* in Texas. An article featuring our research on skunks was published in Texas Parks and Wildlife Magazine (Roe 2016) and a dedicated project email address was published with a request for further information on spotted skunk observations.

Upon receipt of an observation, the species observed was first verified. The credibility of the observation and the temporal and spatial data associated with it were verified before the observation was added to the spotted skunk database. If temporal data were not provided, there was an attempt to associate the observation with either a month or a year. Observations were verified if they could at least be verified to the year level. If spatial data associated with the observation were lacking or not provided, the location was georeferenced with the assistance of the observer.

In addition to our primary field survey and crowd-sourced search for observations, we utilized citizen scientists to collect additional data at locations where a credible observation of *S. putorius interrupta* had previously been reported or at areas where the preliminary species distribution model showed a high probability of occurrence. Citizen scientist groups included multiple Texas Master Naturalist Program chapters, individual members of 4-H, the Texas Christian University chapter of The Wildlife Society in coordination with the Fort Worth Nature Center, spotted skunk research collaborators, and private citizens. Once a site was selected for survey, a group of 3 survey points were systematically selected. One of us (JCP) traveled to the site and assisted the citizen scientists with deploying the cameras using the methodology consistent with the full field surveys. The citizen scientists monitored the cameras for the following 3 weeks, checking and rebaiting the cameras every 7<sup>th</sup> day.

Throughout the project, we monitored natural history collections within the state for the accession of new skunks and sought additional records in museums that were not included in the

original database. The complete dataset of all observations underwent a quality check which included the removal of duplicate entries, georeferencing and confirmation of spatial data, and removal of entries that lacked sufficient spatial or temporal data.

After assembling the complete database of observations, it was important to filter observations to a current time frame, because some historical museum records were obviously collected in areas where land-use has drastically changed (i.e. collection locations from the early 20<sup>th</sup> century are in 21<sup>st</sup> century major metropolitan areas). While there is an approximately known year when population decline of *S. putorius interrupta* was first observed (1940), we felt that even this was too historic. To quantify a current time frame, we first filtered the dataset into quartiles. The 75%, or most recent, quartile was the year 2012 to present. Land cover is continually changing and datasets reflect this change; however, we felt that the rate of change in Texas would be minimal enough to use a 10-year buffer of records for the selected database. To wit, we selected a land cover database that was as close to 2012 as possible (2011) and all records within the 10 year buffer were selected for inclusion into the current dataset (2001 – 2017).

A species distribution model was created to predict the current probability of occurrence of *S. putorius interrupta* in Texas (Maxent version 3.4.0; Phillips et al. 2017). Environmental variable layers, such as topographical (slope and aspect), land cover, and climate were manipulated in ArcMap 10.2 (ESRI 2014). The model utilized 18 climate variables (Table 1) averaged between the years of 1970 to 2000 (Fick and Hijmans, 2017).

Current land-use data were downloaded from the 2011 National Land Cover Database (NLCD) (Homer et al. 2015). The raster was first cut to the study area based on the historic distribution of the plains spotted skunk in Texas (Dowler et al. 2008). Next, the NLCD raster was resampled to a 1 km resolution with land use being assessed as the majority category in a 4-km circular radius around each individual cell. The resample was performed to smooth the transition between habitats. Finally, the land-use categories were truncated into nine general categories with all similar habitats combined (Table 2).

With the assumption that spatial data for plains spotted skunks were biased towards areas with high human populations or areas with ongoing research, the spatial data were filtered. This bias was most evident at two locations, Katy Prairie and Fort Hood. Records at these locations accounted for 65% of all observations. First, the observations were buffered with a 1 km buffer which created a series of clumped observations at these locations. Next, the observations in each clump were randomly reduced to a single observation. Finally, the observations at Katy Prairie and Fort Hood locations were randomly filtered such that the number of observations at each location was equal and cumulatively they approximated 33% of the final observations.

Model validation was performed using a 2-fold cross validation (Fielding and Bell 1997; Franklin 2009). In this method, the presence data is split into 2 groups, a train group which calibrates the model and a test group which validates the model. The model fit was evaluated using area under curve (AUC) of the receiver operating characteristic (ROC) (Phillips et al. 2017, DeLong et al. 1988). When evaluating AUC, a value close to 1 is representative of a good model fit (Fielding and Bell 1997).

Landscape alteration from oil and gas development and urbanization, both current and projected for the future (2050), was assessed as having a potential impact on the plains spotted skunk. These factors were addressed by mapping drilling intensity as a proxy for landscape alteration. Similarly, we mapped current urbanization using NLCD plotting classes, following

the approach of Ryberg et al. (2017). Forecasted urbanization was mapped and quantified from 2010—2050 using the Theobald (2005) database, again following the approach of Ryberg et al. (2017). Details of this methodology are presented in Appendix 3.

## RESULTS

During the project, 233 fully verified, unique *S. putorius interrupta* observations in Texas were amassed (Figure 3). These records represent a time frame from 1891 to 2017. Records were gathered using 4 techniques (museum records, field surveys, crowd sourcing, and citizen scientist surveys). One hundred fifty-seven of these records were from museum specimens (verified and georeferenced), 58 were from crowd sourced observations, 12 were recorded during full field surveys, and 4 were recorded during citizen scientist camera surveys. One hundred and six fully verified records were recorded from the current (2001 to present) timeframe. Of these, 81 were deemed unique via temporal and spatial differences (Figure 4; Table 3).

Angelo State Natural History Collections also received 4 spotted skunks from the Texas Department of Health that had been submitted for rabies testing. All 4 were rabies negative. Due to privacy issues, the Department of Health could only release the county and year of submission for these specimens. For that reason, we were able to quantify the current occurrence of *S. putorius interrupta* in these counties, but the spatial data were not accurate enough to be utilized in the SDM. These skunks were submitted in 2011 ( $n = 2$ ) and 2017 ( $n = 2$ ). The counties of origin were Jack, Robertson, Gonzalez, and Caldwell. These 4 specimens are the only known records of occurrence for these counties within the current time frame.

### *Field Surveys*

Field surveys were initiated in September 2015 and concluded in January 2017. Sites in 10 counties were surveyed and *S. putorius interrupta* was detected in 4 of these counties. Survey devices were deployed 1179 times for a total of 8065 survey nights (Table 4). A minimum of nine skunks were detected 12 times (detection rate = 0.15%) and by all three survey devices (cameras, traps, and track plates). They were detected 6 times by traps (detection rate = 0.22%), five times by cameras (detection rate = 0.15%), and twice with track plates (detection rate = 0.07%). Skunks were detected in Coryell, Harris, Waller, and Wise counties with detection rates of 0.36%, 0.73%, 0.27%, and 0.12% respectively. In Coryell County, a minimum of 2 skunks were detected 3 times at Fort Hood Military Installation. In Harris County, a minimum of 4 skunks were detected 6 times at the Katy Prairie Conservancy's Warren Ranch property. In Waller County, 2 skunks were detected at a private landowner's ranch. In Wise County, 1 skunk was detected at the Sid Richardson Scout Ranch.

### *Crowd- sourced Data*

Eighty-two total observations were amassed of which at least 58 were unique and included in the dataset for analysis. These observations came from 18 counties including 3 (Austin, Shackelford, and Wilbarger) without prior county records (Schmidly and Bradley, 2016). Of these, the Austin County observation was a citizen-scientist report verified without pictorial evidence, the Shackelford County observation was an Abilene Christian University wildlife survey with trail camera photo documentation, and the Wilbarger County observation was a road-killed individual with picture and tissue voucher. The time frame for these

observations was from 1985 to 2017; however, 79 of the 82 observations were from 2009 to present with only 3 (1985, 1995, and 2003) prior to 2009.

#### *Citizen Scientist Surveys*

Field surveys by citizen scientists were initiated in September of 2016 and concluded in April of 2017. Nine locations in 6 counties were surveyed (Table 1). Survey devices were deployed 54 times for a total of 1206 survey nights. Spotted skunks were detected in 2 counties, Tarrant and Waller. A minimum of 2 skunks were detected 4 times (detection rate = 0.33%).

Plains spotted skunks were detected by cameras at a private location in Waller County twice. This location was also surveyed by Angelo State University (ASU) researchers during the fall of 2015 when 2 spotted skunks were detected in traps rather than by trail cameras. The landowner/collaborator also reported a visual observation on this property in early December 2016. Because of the spatial and temporal relatedness of the observations by the citizen scientist and the landowner, we considered all 3 observations to be of the same individual.

Skunks were detected at Fort Worth Nature Center and Refuge (FWNCR) in Tarrant County twice. Skunks were detected twice during a 24-hour period at the same camera, although during different nights. For this reason, we consider it to be multiple observations of the same individual. Although the FWNCR had verified observations in 2015 and 2016, researchers were not able to detect skunks during a fall 2015 survey. Historic flooding and mesocarnivore interference likely played a role in our inability to detect their occurrence with our methods.

#### *Species Distribution Model*

Only observations and records of *S. putorius interrupta* from the current time period (2001 – 2017) were used for the SDM. Of the 81 observations available for use in the model, only 72 were spatially distinct enough for use. After filtering observations at Fort Hood and Katy Prairie to account for redundancy, 45 (23 training, 22 testing) observations were used in the model.

The model showing probability of occurrence in Texas, predicted that the plains spotted skunk is presently found only in the central part of the state, west of the Piney Woods ecoregion and east of the Llano Estacado and Edwards Plateau (Figure 5). The model predicted high probability of occurrence in the Central Great Plains, Cross Timbers, Texas Blackland Prairies, East Central Texas Plains, Western Gulf Coast Plains, and the far eastern portion of the Edwards Plateau ecoregions (Griffith et al. 2004). Within these large ecoregions, mean probability of occurrence is relatively low, with the Cross Timbers having the highest probability at 40%. When examined at the county level, however, 25 counties have a mean probability of occurrence above 50% (Table 5).

The jack knife test of importance indicated that shrub and forests are important habitats in these areas (Figure 6). The jackknife test also indicated that annual precipitation (Bioclim 12), precipitation seasonality (Bioclim 15), and precipitation of wettest quarter (Bioclim 16) bioclimatic variables were the most important variables for predicting distribution. The receiver operator characteristics (ROC) area under curve (AUC) for the two models was 0.934 and 0.861 respectively (Figure 7).

Maps of both current and possible future landscape alteration due to oil and gas development and urbanization (Appendix 3, Figures 1 and 2) show potential threats to the known distribution of plains spotted skunks in Texas. Oil and gas development could affect small subsets of the range of the species; however, expanding urbanization patterns, in the Dallas-Fort

Worth metroplex and especially west of Houston appear to be distinct threats to current populations of plains spotted skunks in those areas. Details are provided in Appendix 3.

## DISCUSSION

Field surveys conducted for the plains spotted skunks were designed to allow us to evaluate the relative success of three methods of detection. Unfortunately, the low overall success rate of detections (12 detections for 8065 survey night, 0.15% detection rate) provided too few data to assess relative success of the 3 methods. We did establish that all methods detected plains spotted skunks; however, it became clear that few spotted skunks utilized the track plate enclosures. This is in contrast to previous data for eastern spotted skunks that showed enclosed track plates had greater efficiency, lower latency to detection, and higher probability of detection than cameras (Hackett et al. 2007). Only a single spotted skunk registered positive tracks or completely entered our enclosures. We believe that our reduction in the size of the track plate enclosures, relative to those used in previous studies (Hackett et al. 2007; Zielinski et al. 2006) inhibited the entry of spotted skunks. Our rationale for reducing the size to match our live trap dimensions was that this size might prevent larger mesocarnivores, such as raccoons and opossums, from entering the enclosures. This was not the case as both of those often entered or destroyed the enclosures in an effort to reach the bait. The color of our enclosures (white) may also have played a role, as those deployed in previous studies (e.g. Hackett et al. 2007) were a uniform dark color. In future studies, we recommend the use of cameras over track plates because of their efficacy in detecting spotted skunks and the reduction in time of both deploying and checking camera traps. If track plates are to be utilized, the enclosures should be enlarged to the size previously reported and dark in color. Although traps were efficient in detecting skunks, their use requires longer periods of time in the field than do cameras. Traps do provide the potential for collection of tissue, ectoparasites, and other biological sampling not possible with cameras.

Results of the project indicate that the distribution of the plains spotted skunk in Texas has been reduced to the central portion of the state. This conclusion is based on the fact that all but 1 of the 106 total recorded observations during the current time frame are from this region of the state. This block includes the cities of Dallas/Fort Worth, Houston, Waco, and Temple/Killeen. The lack of records of this species in the areas near San Antonio and Austin suggests that populations of the plains spotted skunk in these regions are either very uncommon or have been extirpated. An additional possibility is that *S. gracilis* is expanding its range eastward at the expense of *S. putorius interrupta*, though we have no evidence of this. Even within this remnant core range, the spotted skunk appears to be uncommon relative to other mesocarnivores. We were encouraged however that there are at least two areas of high local abundance, Fort Hood Military Installation and Katy Prairie.

Efforts to locate *S. putorius interrupta* were intensive, time consuming, and of limited success. While initially the success rate of the project looks bleak, our overall detection rate (0.15%) is comparable to the detection rates recently observed in Missouri, 0.37%, and Tennessee, 0.07% (Hackett et al. 2006; Reed and Kennedy, 2000). At the county level, the rates of 0.36% (Coryell) and 0.26% (Waller), compare favorably with those in Missouri and the rate for Harris (0.73%) is comparable with that seen in Arkansas, 0.81% (Hackett et al. 2006). This study confirms that live traps, track plates, and trail cameras will detect *S. putorius interrupta* if

the species is present. In fact, in areas of high local abundance, these methods, with the possible exception of track-plates, are excellent at detecting the species. This was seen at Fort Hood and Katy Prairie with the latency to detection period (the period from which the device was deployed to when it first detected a skunk). The period of 1 day, 1 day, and 5 days in 3 counties (Harris, Coryell, and Waller respectively) showed that when local abundance is high, devices readily detect presence.

With the lack of detections via field survey, crowd sourcing provided a low cost, low intensity alternative to locate additional records of occurrence. These methods were extremely effective and provided 82 additional *S. putorius interrupta* records. The distribution of our wanted poster, both by email blast and social media, provided the most verified records. Wanted posters also provided the most records in the recent past in Minnesota, albeit distribution of the posters was accomplished by alternate means (Wires and Baker, 1994). Although these methods were successful, we did not exhaust all options for locating crowd sourced records; notably farmers, who were regarded as experts as a result of the Minnesota survey (Wires and Baker, 1994), were not directly contacted. A concurrent *S. putorius* crowd sourcing project in Alabama has yielded verified reports by targeting agency-employed biologists and law enforcement officers as well as fur trappers, among other groups (Nick Sharp, [Alabama Wildlife and Freshwater Fisheries Division, Tanner, Alabama], personal communication, [September 2017]). These groups were not specifically targeted in Texas, although there was some partial overlap within these groups by targeting of other groups. In the future, we recommend that records be solicited from these groups via the crowd sourcing methodology

Dowler et al. (2008) used museum records to plot the range of *S. putorius interrupta* in Texas. The totality of current skunk reports, survey detections, and the species distribution model indicate that the distribution of the species has been reduced to the central portion of the state. Within this remnant core range, the skunk appears to be relatively uncommon, however, there are at least 2 areas of high local abundance, Fort Hood Military Installation and Katy Prairie.

In ecological modeling, care must be taken to work only within a species known range and with environmental variables germane to the species (Franklin 2009). Because of the unknown distribution and interaction with western spotted skunks (*Spilogale gracilis*), we ran the model on the entire state to potentially identify areas in and beyond the overlap zone where *S. p. interrupta* may occur. This could have potentially led to errors of commission (false positives) and the model does in fact show some predicted probability of occurrence west of San Antonio in an area where *S. putorius interrupta* has never been recorded. This area is part of the Edwards Plateau. The eastern and southern edge of the plateau shows up as a band with high probability of occurrence from north and west of Austin to west of San Antonio. This band includes the farthest extent of the plains spotted skunk's range in Texas, the overlap zone with *S. gracilis*, and some areas where *S. putorius interrupta* has never been reported. While a few historical records from this area exist, *S. putorius interrupta* was not recorded from this area during the project; however, multiple reports of *S. gracilis* were received. In the future, surveys capable of differentiating between the two species should be implemented in this area to help define the western extent of the range of *S. putorius interrupta* and whether the two species are truly sympatric in this area.

There are two other potential areas of commission (with false positive occurrences) deemed worthy of discussion—the Dallas/Fort Worth (DFW) metro area and a coastal band in the Western Gulf Coast Plains ecoregion. There were four records of *S. putorius interrupta* from

the DFW area (three at FWNCR and one road-killed individual near Cedar Hill State Park) included in the model. These four records were split evenly between the test dataset and the train dataset. The model shows low probability of occurrence for each city proper, but high probability for the more rural areas of Tarrant and Dallas county. While we can confirm that the skunk is still present in both counties, it is most likely extremely uncommon and probably isolated to pockets of suitable habitat such as at the refuge and state park. Although additional surveys for the skunk in this area should be initiated, we contend that issues encountered during our full survey of Fort Worth Nature Center and Refuge are still present and that survey methodology should be by trail cameras only.

The second area of potential commission is a narrow band in the western Gulf Coast Plains, from Matagorda Bay south to Brownsville. This area was surveyed twice; once in Calhoun County, on the southern shore of Matagorda Bay, and once in Kleberg County, slightly west of the predicted area of probable occurrence. *S. putorius interrupta* was not detected via survey; however, 1 crowd sourced observation was verified in Kleberg County. Eastern spotted skunks have been reported to utilize coastal wetlands, dunes, and adjacent thickets in Florida (Kinlaw et al. 1995). While it is possible that the skunk is still present in this band, it is also possible that this region is a false positive. We recommend further examination of the area to verify *S. putorius interrupta*'s presence or absence. If the skunk is present in this area, it is likely geographically isolated from other populations in Texas.

Current and future energy development and urbanization in Texas may represent a threat to populations of the plains spotted skunk in the coming decades. Our projected increase in urbanization west of Houston (Appendix 3-Figure 2) is of particular interest as the population of spotted skunks at remnants of the Katy Prairie in Harris and Waller counties, based on our field surveys, was among the most robust in the state. Efforts to reclaim additional areas of the Katy Prairie as protected areas or conservation easements, as is a current goal of the Katy Prairie Conservancy (<http://www.katyprairie.org/mission/>) should be pursued at local and state levels in Texas.

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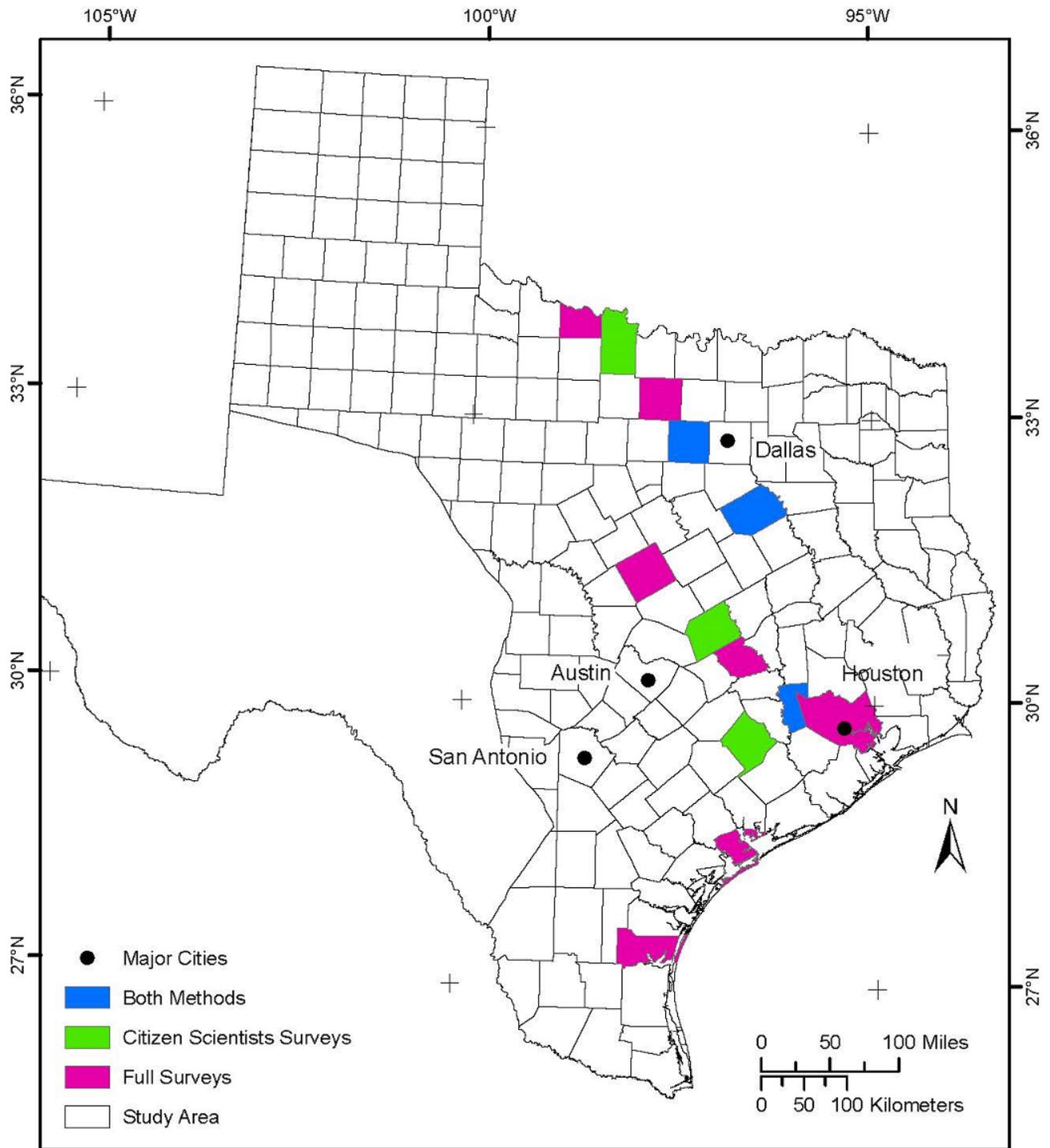
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**Figure 1.** Counties surveyed for plains spotted skunks (*Spilogale putorius interrupta*). Ten counties (purple and blue) were surveyed by Angelo State University researchers using full methods (traps, trail cameras, and track plates). Three of those counties (blue) had additional camera surveys conducted by citizen scientists. Counties surveyed using only cameras by citizen scientists are in green.

# Wanted: Spotted Skunks



**What:** All observations of Spotted Skunks (civet cats), statewide. Current, recent, and historical encounters sought.

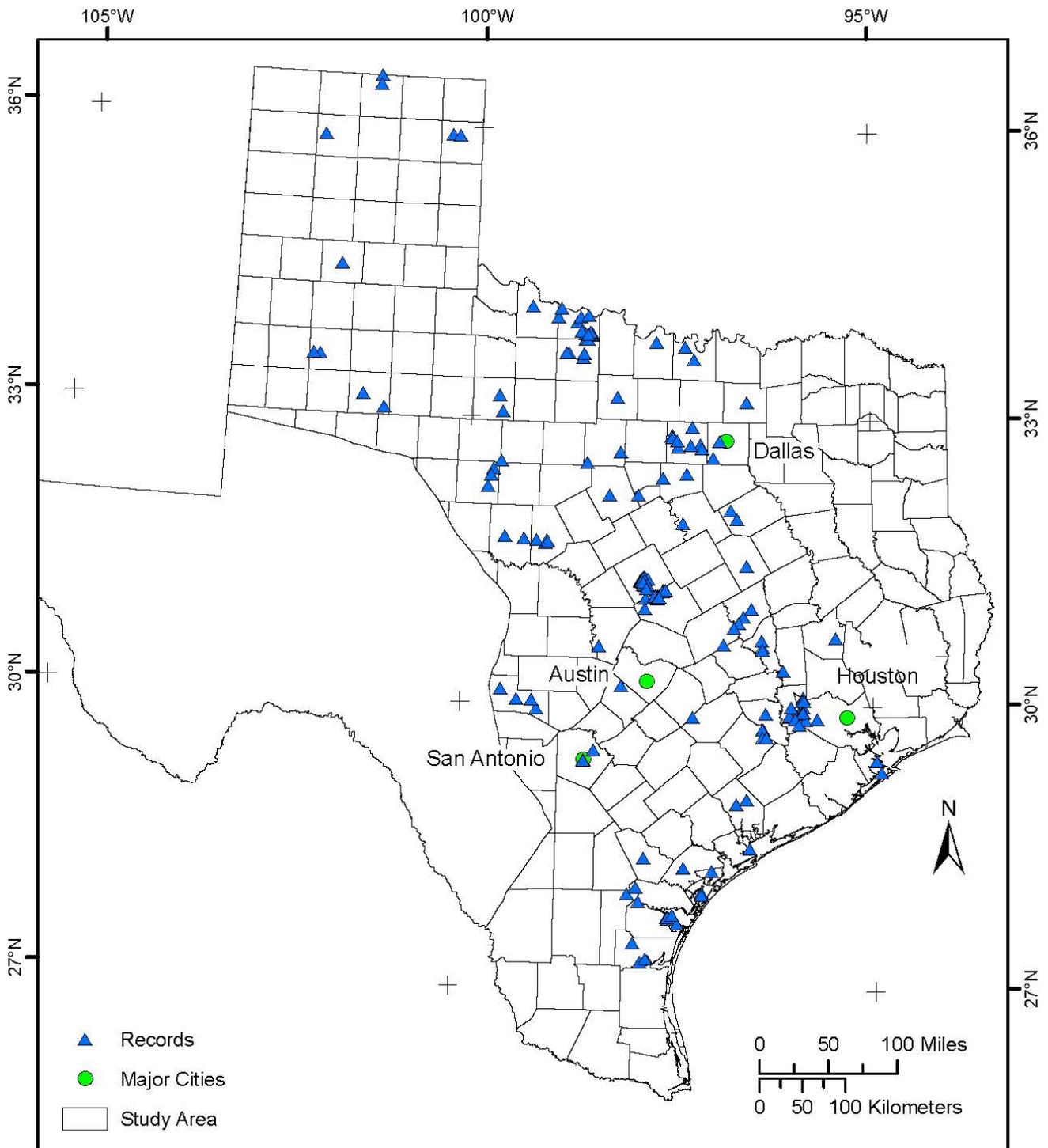
**Information Wanted:** Location, date, pictures (if available), and a short description of the encounter. If a road-killed animal, photograph and salvage any part possible. Call number below for further instructions.

**Contact:** Robert Dowler at [skunk.project@angelo.edu](mailto:skunk.project@angelo.edu) or (325) 486-6639. For immediate response, contact Clint Perkins at (318) 623-1678.

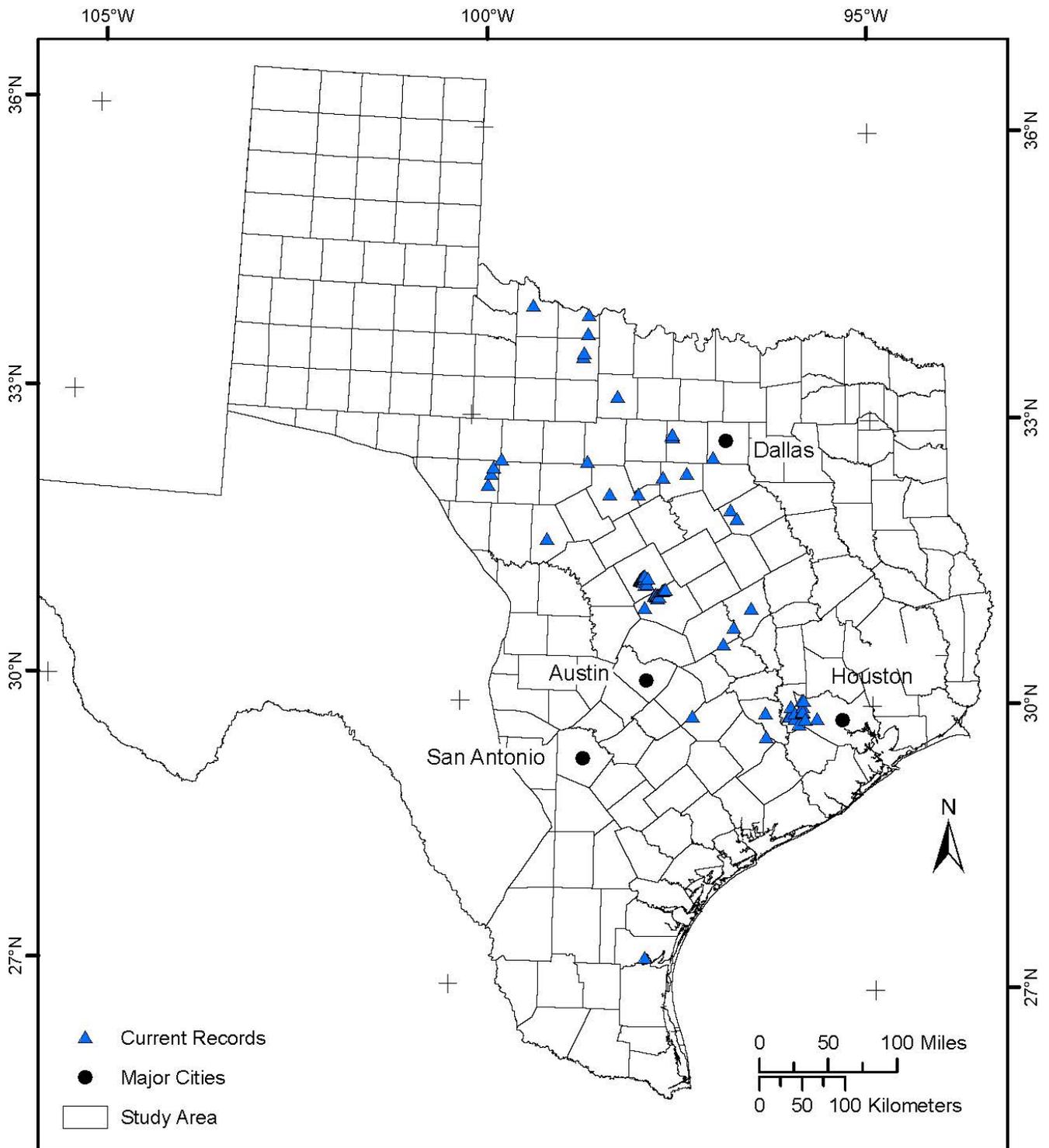


Department of Biology, Angelo State University, San Angelo, TX 76909

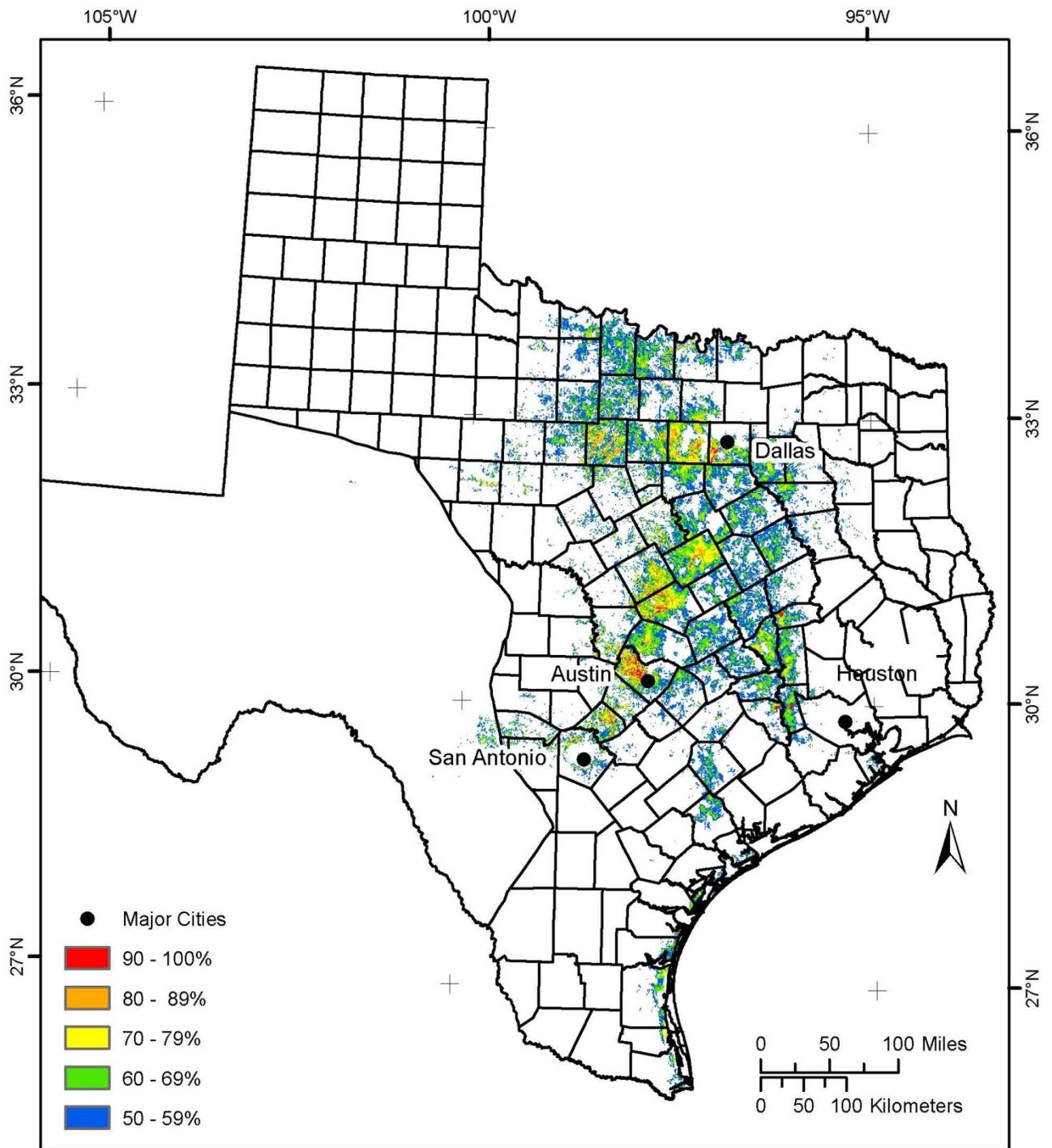
**Figure 2.** Wanted poster created to crowd source observations of both eastern (*Spilogale putorius*) and western (*Spilogale gracilis*) spotted skunks in Texas.



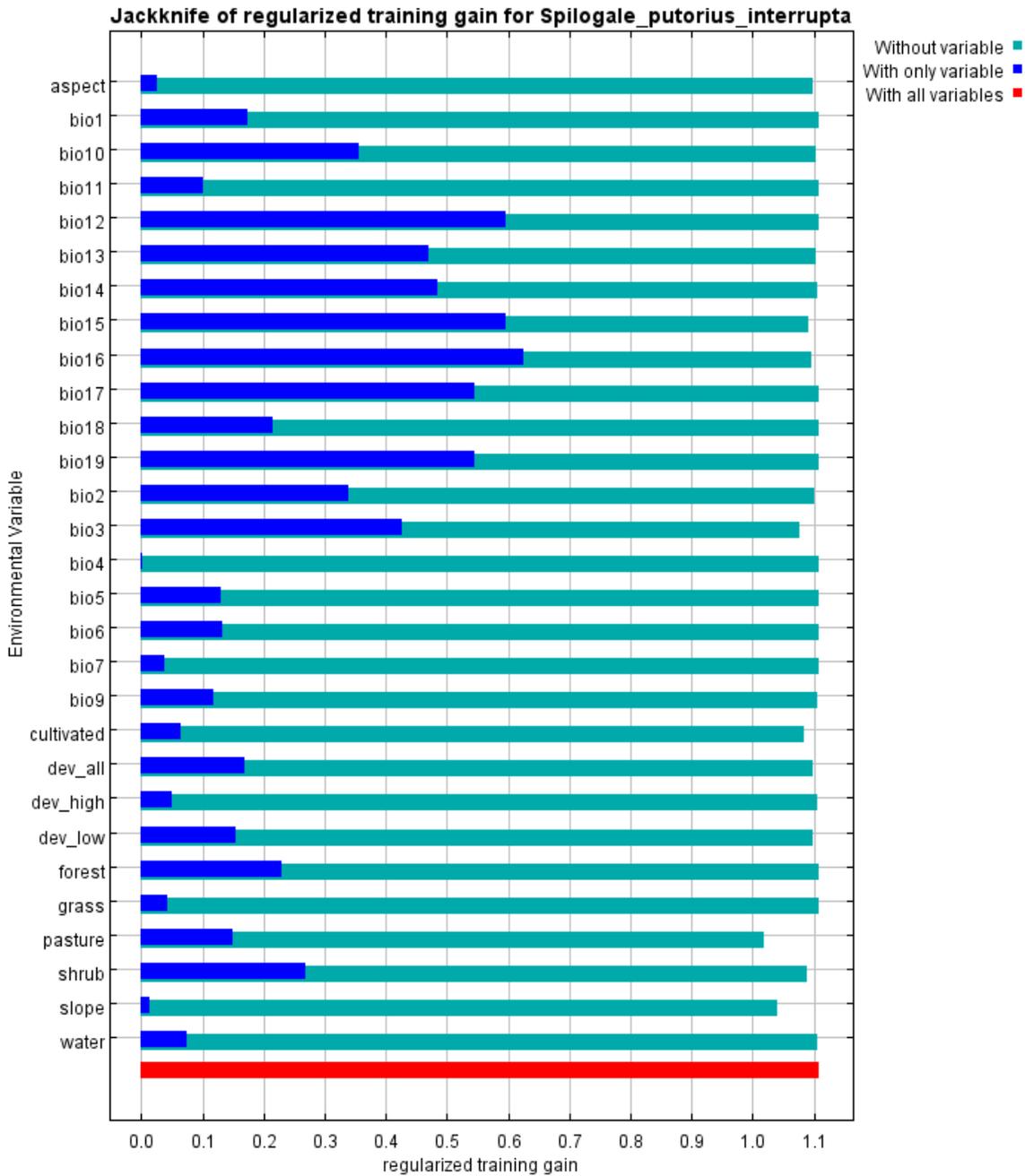
**Figure 3.** The location of all 233 known occurrences of plains spotted skunks (*Spilogale putorius interrupta*) in Texas including historical museum specimen records and those collected during the course of the project.



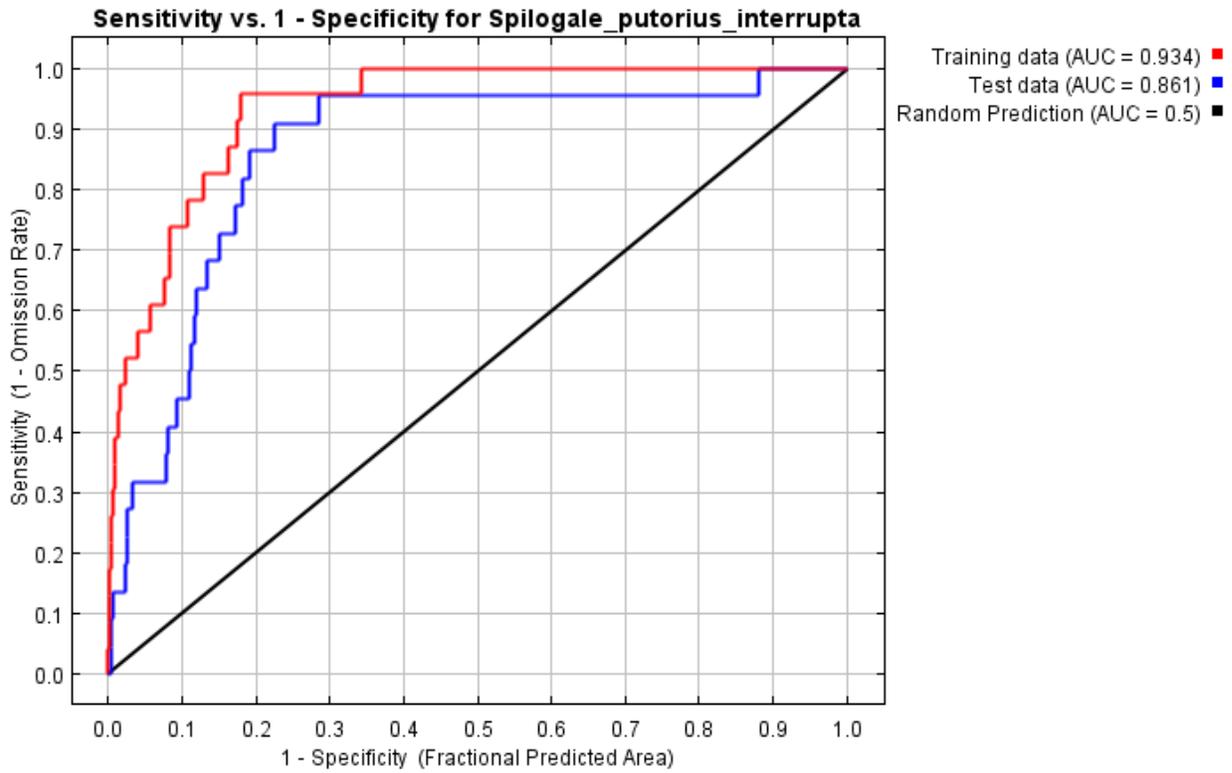
**Figure 4.** All 81 unique occurrences of plains spotted skunks (*Spilogale putorius interrupta*) amassed in Texas. These records represent the current time frame (2001 – 2017).



**Figure 5.** The current modeled distribution of plains spotted skunk (*Spilogale putorius interrupta*) in Texas using presence data from 2001 to 2017. This map shows > 50% probability of presence.



**Figure 6.** Jackknife test of input variable importance for the species distribution of plains spotted skunk (*Spilogale putorius interrupta*) (test gain). Bio 16 (precipitation of the wettest quarter) has the highest gain when used in isolation and therefore appears to have the most useful information in isolation. Pasture decreases the gain the most when removed and therefore appears to have the most information not found in other variables.



**Figure 7.** The area under the curve (AUC) for the receiver operating characteristic (ROC) curve. An AUC score close to 1 is considered excellent while 0.5 would be the hypothetical score of a random model. The AUC for test data (0.934) is considered excellent while the AUC on training data (0.8661) is considered above average.

**Table 1** – Variable, source, and covariates used in species distribution model for the plains spotted skunk (*Spilogale putorius interrupta*).

Variable	Description	Source
Aspect	Derived variable showing the rate of downhill change from a raster cell to neighbor cells	USGS (2014)
Climate	18 Bioclimate predictors representing monthly temperature and rainfall variables	Fick and Hijmans (2017)
Land-use	National Land-cover database (2011). Derived variables: Cultivated, Developed (high, all, low), Forest, Grass, Shrub, Pasture, and Water	Dewitz et al. (2011)
Slope	Derived variable showing the rate of maximum change in z-value from each cell of a raster surface	USGS (2014)

**Table 2** – Truncated land-use variables used in modeling the species distribution of the plains spotted skunk (*Spilogale putorius interrupta*) in Texas. All land-use variables originated from the 2011 National Land Cover Database (Homer et al. 2015).

Land-use variables	NLCD variables	Description
Cultivated	Cultivated Crops	Area used for annual production of crops and all land actively tilled.
Developed_all	Developed, Open Space	Impervious surface < 20% of total cover.
	Low Intensity	Impervious surface 20% - 49% of total cover.
	Medium Intensity	Impervious surface 50% - 79% of total cover.
	High Intensity	Impervious surface 80% -100% of total cover
Developed_high	Developed, Medium Intensity	Impervious surface 50% - 79% of total cover.
	High Intensity	Impervious surface 80% -100% of total cover
Developed_low	Developed, Open Space	Impervious surface < 20% of total cover.
	Low Intensity	Impervious surface 20% - 49% of total cover.
Forest	Deciduous Forest	Trees > 5 meters. Surface coverage >20%. >75% of trees shed foliage annually.
	Evergreen Forest	Trees > 5 meters. Surface coverage >20%. >75% of trees maintain foliage all year.
	Mixed Forest	Trees > 5 meters. Surface coverage >20%. Neither deciduous nor evergreen >75%.
Grass	Grassland/Herbaceous	Graminoid or herbaceous vegetation >80%.
Pasture	Pasture/Hay	Areas of grasses, legumes, or a mixture planted for grazing or hay production.
Shrub	Shrub/Scrub	Shrubs or young trees < 5 meters tall and >20% of the canopy.
Water	Open Water	Open water with < 25% cover of vegetation or soil
	Woody Wetlands	Forest or shrubland >20% of cover and the substrate periodically floods.
	Emergent Herbaceous	Perennial herbaceous vegetation >80% and the substrate periodically floods.

**Table 3** – All unique records of plains spotted skunk, *Spilogale putorius interrupta* from the current dataset (2001 to present). Table includes record type, year, county, general location, and coordinates. Coordinates for private landowners have been masked with X.

Record Type	Year	County	Location (General)	Latitude	Longitude
Crowd Sourced	2003	Wichita	Wichita Falls	X	X
Museum	2004	Brown	Brownwood	31.725902	-99.007089
Museum	2004	Harris	Hockley	29.999086	-95.84417
Museum	2005	Milam	Gause	30.8300617	-96.689062
Museum	2005	Waller	Brookshire	29.893333	-96.013056
Museum	2006	Archer	Archer City	33.641737	-98.619574
Museum	2007	Taylor	Tuscola	32.271839	-99.756913
Museum	2008	Waller	Pattison	29.81095	-95.889897
Museum	2008	Harris	Hockley	30.0656	-95.855
Crowd Sourced	2009	Navarro	Blooming Grove	X	X
Museum	2009	Harris	Hockley	30.060536	-95.841513
Crowd Sourced	2010	Waller	Waller	X	X
Crowd Sourced	2010	Harris	Katy	29.8608111	95.8152444
Crowd Sourced	2011	Coryell	Fort Hood	31.318902	-97.842629
Crowd Sourced	2011	Coryell	Fort Hood	31.307261	-97.838232
Crowd Sourced	2011	Coryell	Fort Hood	31.332948	-97.834532
Crowd Sourced	2011	Coryell	Fort Hood	31.336914	-97.834084
Crowd Sourced	2011	Coryell	Fort Hood	31.316997	-97.82754
Crowd Sourced	2011	Coryell	Fort Hood	31.319352	-97.822635
Crowd Sourced	2011	Coryell	Fort Hood	31.312193	-97.821169
Crowd Sourced	2011	Coryell	Fort Hood	31.313298	-97.814787
Crowd Sourced	2011	Coryell	Fort Hood	31.352818	-97.81037
Crowd Sourced	2011	Coryell	Fort Hood	31.306443	-97.806183
Crowd Sourced	2011	Coryell	Fort Hood	31.359323	-97.790134

**Table 3 (continued)** – Every unique *S. p. interrupta* record from the current dataset (2001 to present). Table includes record type, year, county, general location, and coordinates. Coordinates for private landowners have been masked with an X.

Record Type	Year	County	Location (General)	Latitude	Longitude
Crowd Sourced	2011	Bell	Fort Hood	31.202289	-97.566267
Crowd Sourced	2011	Bell	Fort Hood	31.211714	-97.54595
Crowd Sourced	2011	Bell	Fort Hood	31.223882	-97.534709
Crowd Sourced	2011	Bell	Fort Hood	31.222131	-97.526685
Crowd Sourced	2011	Harris	Katy Prairie Conservancy	29.948138	-95.84399
Crowd Sourced	2012	Bell	Fort Hood	31.029671	-97.777024
Crowd Sourced	2012	Bell	Fort Hood	31.151518	-97.665919
Crowd Sourced	2012	Bell	Fort Hood	31.17629	-97.645544
Crowd Sourced	2012	Bell	Fort Hood	31.145712	-97.640479
Crowd Sourced	2012	Bell	Fort Hood	31.16409	-97.631205
Crowd Sourced	2012	Bell	Fort Hood	31.15811	-97.630462
Crowd Sourced	2012	Bell	Fort Hood	31.128471	-97.628518
Crowd Sourced	2012	Bell	Fort Hood	31.144763	-97.607045
Crowd Sourced	2013	Kleberg	Roadway	27.333081	-97.700233
Crowd Sourced	2013	Johnson	Alvarado	32.43575	-97.289389
Crowd Sourced	2013	Fayette	Cistern	X	X
Crowd Sourced	2013	Colorado	Attwater Prairie Chicken NWR	29.68	-96.29
Crowd Sourced	2014	Coryell	Fort Hood	31.301126	-97.809665
Crowd Sourced	2014	Coryell	Fort Hood	31.326268	-97.791167
Crowd Sourced	2014	Coryell	Fort Hood	31.266467	-97.785985

**Table 3 (continued)** – Every unique *S. p. interrupta* record from the current dataset (2001 to present). Table includes record type, year, county, general location, and coordinates. Coordinates for private landowners have been masked with an X.

Record Type	Year	County	Location (General)	Latitude	Longitude
Crowd Sourced	2014	Coryell	Fort Hood	31.340457	-97.754994
Crowd Sourced	2014	Johnson	Godley	X	X
Crowd Sourced	2015	Palo Pinto	Palo Pinto Mountains SP	32.54356	-98.5343
Crowd Sourced	2015	Erath	Stephenville	32.205806	-98.240125
Crowd Sourced	2015	Tarrant	Fort Worth Nature Center	32.84488	-97.47169
Crowd Sourced	2015	Dallas	Cedar Hill	32.611039	-96.959206
Crowd Sourced	2015	Navarro	Silver City	31.973947	-96.657453
Crowd Sourced	2015	Austin	Bellville	29.926079	-96.301872
Survey	2015	Waller	Pattison	X	X
Survey	2015	Waller	Pattison	X	X
Crowd Sourced	2015	Austin	Bellville	29.926079	-96.301872
Museum	2016	Taylor	Abilene	32.45983	-99.70029
Crowd Sourced	2016	Shackelford	Abilene	32.544929	-99.600675
Crowd Sourced	2016	Wilbarger	Vernon	34.15931	-99.27255
Survey	2016	Wise	Sid Richardson Scout Ranch	32.216031	-97.888789
Survey	2016	Coryell	Fort Hood	31.317725	-97.826272
Survey	2016	Coryell	Fort Hood	31.296775	-97.806683
Survey	2016	Coryell	Fort Hood	31.296417	-97.8056
Crowd Sourced	2016	Coryell	Fort Hood	31.279227	-97.760779
Crowd Sourced	2016	Tarrant	Fort Worth Nature Center	32.83914879	-97.481016
Crowd Sourced	2016	Milam	Milano	X	X
CS Camera Survey	2016	Waller	Pattison	X	X

**Table 3 (continued)** – Every unique *S. p. interrupta* record from the current dataset (2001 to present). Table includes record type, year, county, general location, and coordinates. Coordinates for private landowners have been masked with an X.

Record Type	Year	County	Location (General)	Latitude	Longitude
Crowd Sourced	2016	Waller	Pattison	X	X
Survey	2016	Harris	Katy Prairie Conservancy	29.95334	-95.8559
Survey	2016	Harris	Katy Prairie Conservancy	29.94134	-95.84726
Survey	2016	Harris	Katy Prairie Conservancy	29.94134	-95.84726
Survey	2016	Harris	Katy Prairie Conservancy	29.947272	-95.843897
Museum	2017	Taylor	Abilene	32.391535	-99.723615
Crowd Sourced	2017	Archer	Archer City	33.6819	-98.609806
Museum	2017	Wichita	Burkburnett	34.078588	-98.557528
CS Camera Survey	2017	Tarrant	Fort Worth Nature Center	32.8264	-97.473106
Crowd Sourced	2017	Waller	Waller	29.993817	-95.993072
Crowd Sourced	2017	Waller	Pattison	29.873697	-95.944894
Crowd Sourced	2017	Waller	Pattison	29.81105	-95.88997
Crowd Sourced	2017	Harris	Houston	29.87303	-95.670761

**Table 4** – The location of all surveys for plains spotted skunks (*Spilogale putorius interrupta*) in Texas from 2015 to 2017. Listed are county, site, type of survey (full surveys completed by Angelo State personnel with 3 survey devices), survey dates, survey devices deployed, and survey nights.

County	Location	Survey Type	Survey Dates	Devices Deployed	Survey Nights
Waller	Private Property	Full	03 -11 Oct 2015	108	748
Tarrant	Fort Worth Nature Center	Full	21 - 29 Nov 2015	120	741
Burleson	Lake Somerville State Park	Full	07 - 15 Jan 2016	111	768
Wise	Sid Richardson Scout Ranch	Full	26 Feb - 05 Mar 2016	120	839
Kleberg	King Ranch	Full	13 -21 Mar 2016	120	840
Clay	Lake Arrowhead State Park	Citizen Scientist	07 - 28 Sep 2016	6	126
Wichita	Private Property	Full	01 - 09 Oct 2016	121	825
Wichita	Private Property	Full	01 - 09 Oct 2016	121	825
Waller	Private Property	Citizen Scientist	08 - 29 Nov 2016	3	63
Waller	Private Property	Citizen Scientist	08 - 29 Nov 2016	3	63
Navarro	Private Property	Full	19 - 27 Nov 2016	111	804
Coryell	Fort Hood	Full	14 - 22 Dec 2016	120	838
Calhoun	Powderhorn Ranch	Full	05 - 13 Jan 2017	120	837
Tarrant	Fort Worth Nature Center	Citizen Scientist	13 Feb - 15 Mar 2017	24	504
Milam	Private Property	Citizen Scientist	17 Mar - 18 Apr 2017	3	49
Milam	Private Property	Citizen Scientist	16 Mar - 08 Apr 2017	3	56
Milam	Private Property	Citizen Scientist	17 Mar - 18 Apr 2017	3	63
Colorado	Private Property	Citizen Scientist	18 Mar - 09 Apr 2017	3	63
Navarro	Private Property	Citizen Scientist	19 Mar - 09 Apr 2017	6	126

**Table 5** – Texas counties within the current range of plains spotted skunk, *Spilogale putorius interrupta* with a mean probability of occurrence > 50%. Table also notes whether a skunk was recorded for the county during the 2015 – 2017 survey period.

County	Area (Km <sup>2</sup> )	P <sub>mean</sub>	Record
McLennan	2750	63	
Palo Pinto	2550	62	Yes
Bell	2814	59	Yes
Tarrant	2327	58	Yes
Travis	2656	57	
Brazos	1529	57	
Comal	1489	56	
Coryell	2736	56	Yes
Kaufman	2091	56	
Parker	2350	55	
Montague	2424	54	
Jack	2383	54	Yes
Waller	1343	52	Yes
Clay	2866	52	
Johnson	1901	52	Yes
Robertson	2242	51	Yes
Ellis	2464	51	
Washington	1609	51	
Freestone	2314	51	
Milam	2648	51	Yes
Hill	2552	50	
Grimes	2080	50	
Navarro	2817	50	Yes
Young	2409	50	

### **III. GENETIC VARIATION IN SUBSPECIES OF THE EASTERN SPOTTED SKUNK (*SPILOGALE PUTORIUS*) WITH EMPHASIS ON THE PLAINS SPOTTED SKUNK (*S. P. INTERRUPTA*)**

#### **INTRODUCTION**

Despite the plethora of federal and state-level conservation status designations for *Spilogale putorius*, there remains an absence of genetic data for the entire species. Genetic markers, such as microsatellites, are especially useful when researching rare and understudied species, as they are capable of amplifying homologous sequences in closely related taxa, thus eliminating the need to develop *de novo* markers on a species-by-species basis. A multitude of studies have validated the use of these nuclear markers across species boundaries and have been successful in addressing topics relating to the genetic variability and differentiation of populations, conservation, and hybridization (Kyle et al. 2004; Grobler et al. 2005; Floyd et al. 2011; McManus et al. 2015). Specifically within *Spilogale*, cross-species microsatellites have been utilized by Floyd et al. (2011) to determine genetic differentiation within and among mainland western spotted skunks (*S. gracilis*) and island spotted skunks (*S. g. amphiala*) and by Jones et al. (2013) to determine the spatial and genetic organization of the island spotted skunk. However, microsatellites, nor any other molecular marker, have ever been used to analyze the genetic diversity of eastern spotted skunks, according to extensive literature searches.

Therefore, the four objectives of this study were: (1) determine the genetic variability of the plains spotted skunk using microsatellite markers, (2) compare the genetic variability of the plains spotted skunk to that of the Appalachian and Florida spotted skunks, (3) test the validity of the 3 eastern spotted skunk subspecies designations using molecular techniques, as morphological differences among them are the only metric currently supporting their distinction, and (4) determine the presence of hybridization between the western spotted skunk and the plains subspecies where they are sympatric in the state of Texas.

#### **MATERIALS AND METHODS**

*Sample collection (plains spotted skunk).* —From October 2015–May 2016 and October 2016–January 2017, we conducted field surveys for the plains spotted skunk throughout the state of Texas. Ten counties were surveyed (Burleson, Calhoun, Coryell, Harris, Kleberg, Navarro, Tarrant, Waller, Wichita, and Wise counties), with sampling lasting 7 days at each location. We anesthetized (with a 10 mg/kg dose of ketamine) live-trapped individuals (Tomahawk Live Trap, Hazelhurst, WI) in order to: (1) ascertain the overall condition, sex, and reproductive status, (2) obtain standard museum measurements, (3) collect ectoparasites, urine, and fecal samples when possible, (4) affix a unique, identifying ear tag (National Band and Tag Co., Newport, KY), (5) and acquire a 2 mm ear clip from the distal tip of the pinna for genetic analysis (Talbot et al. 2012; Jones et al. 2013). We stored ear clips in liquid nitrogen until they could be transferred to a -80°C freezer for permanent storage. All trapped individuals were handled following the American Society of Mammalogists' guidelines for the use of wild animals in research (Sikes et al. 2011), and all sampling protocols were approved by the Angelo State University Institutional Animal Care and Use Committee (IACUC Approval No: 15-15).

*Sample collection (all subspecies).*—To supplement the number of individuals obtained by field surveys, and to obtain tissue from the Appalachian and Florida spotted skunks, tissue samples representing all eastern spotted skunk subspecies were requested from museum collections, when available (Appendix 4, Fig. 1). Other sources of genetic material included the use of material from museum specimens at the National Museum of Natural History (USNM) prepared more than 100 years ago, salvaging of road-killed animals, and obtaining individuals from fur trapper harvests. In addition, samples from non-vouchered specimens were obtained via donations from researchers throughout the United States (Appendix 4). A majority of these donations were in the form of dried ear clips or hair samples. Ear clips were frozen at  $-80^{\circ}\text{C}$  once received, while hair samples remained stored at room temperature in an air-tight container containing silica desiccant. Additionally, tissues from western spotted skunks were analyzed in order to address the potential for hybridization between the western spotted skunk and the plains spotted skunk where they occur sympatrically in Texas. Eight western spotted skunks collected from areas of potential sympatry were included in the microsatellite analysis, all from the Angelo State Natural History Collections (ASK5307, ASK5719, ASK6259, ASK6778, ASK7359, ASK7910, ASK8487, and ASK9692).

*Laboratory methods.*—Genomic DNA from non-hair samples was extracted using the QIAGEN DNeasy Blood & Tissue kit (QIAGEN Inc., Valencia, CA) following the manufacturer's protocol. DNA from hair follicles was extracted using the QIAGEN kit following the modifications outlined in Iudica et al. (2001) or with InstaGene matrix (Bio-Rad Inc., Hercules, CA) following the Chelex protocols of Suenaga and Nakamura (2005), with the exception that 10 hairs, instead of 2, were utilized per extraction. All DNA extracts were quantified on a Qubit 1.0 fluorometer (Invitrogen Corp., Carlsbad, CA).

Claws, skull fragments, or turbinal bones were sampled from historic museum specimens (Appendix 1). Before removing claws from study skins, a 25% bleach solution was used to wipe the exterior of the claw followed by a rinse with sterile water. Material from the base of the claw was removed by drill bit or razor blade and placed in sterile centrifuge tubes containing buffer solution. Prior to extraction, bone fragments were exposed to UV light for 5 minutes to remove exogenous DNA from the surface and were incubated for 24 hours in EDTA to promote calcium breakdown. The claw and bone fragments were incubated at  $56^{\circ}\text{C}$  in a rotating heat block for 24-48 hours in buffer/digestion solution (80uL SDS, 35uL Proteinase K, and 20uL DTT). Once completely lysed, extractions were performed with a QIAGEN extraction kit. All DNA extractions were performed in an ancient DNA designated laboratory within the Center for Conservation Genomics, National Zoological Park, Smithsonian Institution, Washington DC.

A total of 16 cross-species microsatellite loci were amplified using primers originally developed for closely-related mephitids and mustelids (Table 1; Beheler et al. 2004; Bijlsma et al. 2000; Dragoo et al. 2009; Munguia-Vega et al. 2009; Floyd et al. 2011). Polymerase chain reaction (PCR) amplifications were performed in 10 or 25  $\mu\text{L}$  reactions, for non-hair and hair samples, respectively. PCR reaction and cycling conditions were modified from the original primer publications and were optimized for the analysis of eastern spotted skunks in this study (Table 2). Reactions contained 5–50 ng DNA, 0.25  $\mu\text{M}$  forward dye-labeled primer (Sigma-Aldrich Corp., St. Louis, MO), 0.25  $\mu\text{M}$  reverse primer (Alpha DNA, Montreal, Quebec), 1.5 mM  $\text{MgCl}_2$ , 0.80 mM dNTPs (0.20 mM each; Thermo Fisher Scientific Inc., Waltham, MA), 1X Standard *Taq* Reaction Buffer (New England BioLabs Inc., Ipswich,

MA), 0.4 U *Taq* DNA Polymerase (New England BioLabs Inc.), and de-ionized water as necessary to meet final reaction volumes. The only exception was for locus Meph42-25, whereby  $\text{MgCl}_2$  was increased to 3 mM. Dye-labeled PCR products were genotyped on a capillary electrophoretic genetic analysis system (CEQ<sup>TM</sup>8000, Beckman-Coulter Inc., Brea, CA) utilizing the 400 bp GenomeLab DNA Size Standard Kit (AB Sciex, Concord, Ontario) as the size standard. Genotypes were scored by eye. To mitigate the presence of scoring errors in the final dataset, and to reduce their negative effects in downstream analyses, approximately 25% of tissue samples and 35% of hair samples were reamplified and analyzed, in addition to approximately 20% of all samples analyzed being scored more than once to ensure consistent genotype calls (DeWoody et al. 2006).

The mitochondrial cytochrome *b* gene was amplified using universal primers LGL765 and LGL766 (Bickham et al., 1995). Total reaction volume was 12.5  $\mu\text{L}$  and contained 30–60 ng of DNA, 1 U of *Taq* DNA polymerase (New England BioLabs), 0.16 mM of each dinucleoside triphosphate, 1X Standard *Taq* Reaction Buffer (New England BioLabs), 1mM  $\text{MgCl}_2$ , and 0.16  $\mu\text{M}$  of each primer. *Cytb* was amplified with the following thermal profile: 1 cycle at 94°C for 2 min, 35 cycles at 95°C, 52°C, and 72°C each for 1 min, and a final extension step for 5 min at 72°C. PCR products were visualized on agarose gels, treated with ExoSAP-IT Express (Applied Biosystems, Foster City, CA) and Sanger sequencing was performed on both strands (Genomics Core Lab, Texas A&M Corpus Christi).

*Analysis of microsatellite variation.*—FreeNA was used to determine the frequency of null alleles for all loci and populations in the dataset (Chapuis and Estoup 2007). Scoring errors due to stutter and large-allele dropout were assessed with Micro-Checker v 2.2.3 (van Oosterhout et al. 2004). Tests for Hardy-Weinberg equilibrium and genotypic disequilibrium between loci were conducted using the Markov chain approximation (dememorization: 10,000; batches: 1,000; iterations per batch: 10,000) in GENEPOP v 4.5.1 (Raymond and Rousset 1995). *P*-values were adjusted for multiple pairwise comparisons using a Bonferroni correction in R (R Core Team 2016). GenAIEx v 6.5 (Peakall and Smouse 2006, 2012) was used to assess levels of genetic variation including the number of alleles per locus ( $N_A$ ), observed ( $H_O$ ) and expected heterozygosities ( $H_E$ ), and the number of private alleles ( $N_P$ ) within each population. Differences in genetic diversity among subspecies were determined using randomized t-tests in R (R Core Team 2016) and resulting *P*-values were adjusted using a Bonferroni correction.

*Microsatellite analysis of genetic structure.*—The program STRUCTURE v 2.3.4 (Pritchard et al. 2000) was used to determine the presence of genetic clusters within the eastern spotted skunk. Using the admixture model and correlated allele frequencies, 20 independent runs were performed at each assumed population number ( $K = 1-10$ ). No putative population origin information was provided *a priori*. The length of the burn-in period and number of Markov chain Monte Carlo iterations post-burn-in were set to 50,000 and 200,000, respectively. To determine the optimum number of population clusters present,  $\Delta K$  was calculated using STRUCTURE HARVESTER v 0.6.94 (Earl and vonHoldt 2012), following the recommendation by Evanno et al. (2005). CLUMPP v 1.1.2 was used to average individual membership coefficients from the 20 replicate STRUCTURE runs at the specified  $\Delta K$  using the FullSearch algorithm (Jakobsson and Rosenberg 2007). Finally, we used the program STRUCTURE PLOT v 2 (Ramamamy et al. 2014) to generate graphical displays of individual membership.

To further examine the presence of genetic structure, a principal coordinates analysis (PCoA) was conducted in GenAlEx v 6.5 (Peakall and Smouse 2006, 2012), with the input being a distance table (Smouse and Peakall 1999) generated from the final genotypic data. A permutational multivariate analysis of variance (PERMANOVA;  $n = 9,999$  permutations) was used to determine the significance of the PCoA clusters using the *adonis* function within the *vegan* package (Oksanen et al. 2017) in R (R Core Team 2016). The degree of genetic differentiation among subspecies was assessed by calculating pairwise  $F_{ST}$  values ( $n = 10,000$  replicates) using the *ENA* (excluding null alleles) correction method by Chapuis and Estoup (2007) in FreeNA. Null alleles, or the non-amplification of alleles due to sources such as mutation in the flanking region (primer sequence) or low-quality DNA templates, can positively bias  $F_{ST}$  values, as they generally function to reduce within-population diversity. The correction method implemented by FreeNA (Chapuis and Estoup 2007) has been shown to effectively correct for this positive bias that could result in the presence of null alleles. Rates of gene flow among subspecies were determined from  $Nm$ , the product of the effective population sizes ( $N$ ) and the rate of migration ( $m$ ) between them, using Wright's (1984) estimator:  $Nm = (1 / F_{ST} - 1) / 4$ . Additionally, 2-way Mantel tests were performed in R (R Core Team 2016) using the package *ade4* (Dray and Dufour 2007) to determine if genetic isolation by distance (IBD) was present within each subspecies.

*Phylogenetic analysis of mitochondrial data.*— In order to test the validity of the 3 eastern spotted skunk subspecies designations based on mitochondrial sequence data, we analyzed the phylogenetic patterns recovered from the analysis of the *cytb* gene. Forward and reverse sequences of the *cytb* gene were used to create a single consensus sequence for each individual in Sequencher (Gene Codes Corporation, Ann Arbor, Michigan) and were aligned in MEGA7 (Kumar et al. 2016). Maximum likelihood (ML) analyses were performed using the HKY+G model as selected by the Bayesian Information Criterion in MEGA7. The resulting topology was rooted with sequences of western spotted skunk (*S. gracilis*) downloaded from Genbank (AM711898.1, X94928, KY679913.1, KY679929.1, KY679935.1, KY679939.1, KY679940.1, KY679941.1, KY679942.1, KY679947.1, KY679949.1, KY679950.1, KY679952.1, KY679984.1, KY679988.1, KY679994.1, KY680001.1, KY680006.1). *S. gracilis* sequences were also evaluated for evidence of hybridization with *S. putorius*. Statistical nodal support was evaluated with 500 ML bootstrap replicates. Average sequence divergences (Kimura 2-parameter distances) were calculated in MEGA between species and subspecies of spotted skunks.

## RESULTS

*Sample collection.*—Field efforts led to the live capture of 6 plains spotted skunks from Coryell ( $n = 1$ ), Harris ( $n = 3$ ), and Waller ( $n = 2$ ) counties in Texas. All other acquired samples were obtained through tissue loans or donations ( $n = 106$ ), by salvaging road-killed animals ( $n = 8$ ), or from fur trapper harvests ( $n = 5$ ). In total, this study included 119 individuals representing all 3 subspecies: the plains spotted skunk ( $n = 64$ ), the Appalachian spotted skunk ( $n = 27$ ), and the Florida spotted skunk ( $n = 28$ ). Although hair samples from non-permanently marked individuals represented much of the Florida spotted skunk sample, all proved to be unique individuals.

*Microsatellite variation.*—Of the 16 cross-species microsatellite loci genotyped, 2 proved monomorphic for all 3 subspecies (Meme82 and Meme84) and were excluded from

further analyses. An additional 3 loci (Meme77, Meme88, and Meph22-89) were monomorphic within the plains and Appalachian subspecies. Due to the limited quantity of DNA from all Florida spotted skunks, only a few individuals representing a random subset were amplified across these 3 loci, in supposition that they would also prove monomorphic and uninformative for this study. This subset also proved to be monomorphic for the same allele, therefore no further individuals were analyzed at these loci. In all, with the exclusion of those 5 loci, the final genotypic dataset contained 97 individuals analyzed across 11 microsatellite loci (Appendix 1). The genotyping error rate for non-hair samples was approximately 1.50% and 2.30% for hair samples. All but 1 genotyping error was attributed to a single locus, Meph22-16, for hair samples. These error percentages are unlikely to affect conclusions relating to genetic diversity or structure, as it has been shown that estimates of  $H_E$ ,  $F_{ST}$ , and structure remain unbiased, even in the presence of high (>30%) genotyping error rates, when  $n > 10$  individuals per population (Smith and Wang 2014). The entire dataset contained 1.12% missing data, well below the maximum 20% suggested by Smith and Wang (2014) for the purposes of accurately examining population genetics.

Two microsatellite loci analyzed served to distinguish eastern spotted skunk subspecies. Locus Meme20 was perhaps the least informative marker with respect to its allelic richness ( $N_A = 1.33$ ) and heterozygosity levels (average  $H_O = 0.029$ ); however, it differentiated the plains subspecies from both the Appalachian and Florida subspecies due to its monomorphic nature in the latter and polymorphic nature in the former. Conversely, locus Meph22-14 was nearly monomorphic within the plains subspecies (one individual displayed an alternate genotype), yet was highly polymorphic within the Appalachian and Florida subspecies. Additionally, a unique pattern emerged within locus Meph42-25 (dinucleotide repeat), as all alleles ranging from 201–215 base pairs (bp) were odd-numbered fragment sizes, yet alleles from 218–236 bp were even-numbered sizes. As variation is known to occur both within and outside the repeat region of some microsatellite loci, it is possible that this source of error is due to an indel mutation outside the repeat motif. However, this pattern did not serve as a diagnostic character to differentiate subspecies, as all 3 contained bp fragments within the range of 201–236.

Null allele frequencies greater than 10% were present within *S. p. interrupta* at locus Meme20 (27.4%) and within all subspecies at locus Meph22-16 ( $11.3 < x < 13.0\%$ ). Across all loci and subspecies, the null allele frequency averaged  $4.61 \pm 0.010\%$  ( $\bar{X} \pm SE$ ). Scoring errors due to stutter might have affected genotyping of the plains subspecies at loci Meph22-16 and Meph22-26, while evidence of scoring errors due to large-allele dropout were not detected within any subspecies or at any locus. Because evidence of scoring errors due to stutter and high null allele frequencies were not consistently detected at specific loci across subspecies, these loci were retained in further analyses. Across loci,  $N_A$  for the 3 subspecies ranged from 4.73–6.64, while  $H_O$  and  $H_E$  ranged from 0.441–0.577 and 0.482–0.623, respectively (Table 3). For all loci and subspecies,  $N_A$  was  $5.61 \pm 0.550$  and  $H_O$  was  $0.498 \pm 0.053$  ( $\bar{X} \pm SE$ ; Table 3). Genetic diversity, with respect to  $N_A$ ,  $H_O$ , and  $H_E$ , was not significantly different among the 3 subspecies (randomized t-test;  $n = 10,000$  iterations;  $P_{adj} > 0.68$  for all comparisons). Private alleles, or alleles present only within a single population (in this case, subspecies) were 3.5 times more abundant within the plains spotted skunk in comparison to the Appalachian or Florida spotted skunks (Table 3).

Significant departure from Hardy-Weinberg equilibrium was present at locus Meph22-16 within the plains and Florida subspecies ( $P_{adj} < 0.01$ ), and within locus Meph22-

14 for the plains subspecies ( $P_{\text{adj}} = 0.046$ ), likely as a result of a single individual displaying an alternate genotype, whereas all others were monomorphic. When all 3 subspecies were pooled, a deviation from Hardy-Weinberg equilibrium occurred at locus Meph22-16 ( $P_{\text{adj}} < 0.001$ ; Table 1). These loci were included in all further analyses due to their deviations not being consistently encountered across subspecies, and because the individuals included in this analysis do not represent true populations and therefore are not expected to conform to Hardy-Weinberg equilibrium. Hardy-Weinberg equilibrium could not be determined for locus Meme20 when subspecies were pooled or for *S. p. putorius* and *S. p. ambarvalis* individually, as these 2 subspecies were monomorphic at this locus. Linkage disequilibrium was detected between loci Meme5 and Meph22-70 and Mel1 and Meph22-70 within the Florida subspecies ( $P_{\text{adj}} < 0.032$ ) and between Meme5 and Meph22-70 when subspecies were pooled ( $P_{\text{adj}} < 0.001$ ).

*Genetic structure.*—Tests of genetic structure including all 3 subspecies resulted in  $\Delta K = 2$  (Fig. 2A). All individuals belonging to *S. p. interrupta* formed a single cluster, while all individuals belonging to both *S. p. putorius* and *S. p. ambarvalis* comprised a second cluster (Fig. 2A). Because STRUCTURE identifies clusters corresponding to the uppermost hierarchical level of structure present (Evanno et al. 2005), a second STRUCTURE analysis was performed that excluded the plains subspecies to determine if a lower level of hierarchical structure was present between the Appalachian and Florida subspecies. The analysis excluding the plains subspecies resulted in  $\Delta K = 2$ , with *S. p. putorius* individuals and *S. p. ambarvalis* individuals forming separate clusters (Fig. 2B). STRUCTURE plots indicated a very low degree of admixture among subspecies (Fig. 2A, 2B). In addition, average membership coefficients were high for individuals within their respective subspecies and averaged within subspecies ( $\bar{X} \pm SE$ ): *S. p. interrupta* ( $99.45 \pm 0.001\%$ ), *S. p. putorius* ( $98.23 \pm 0.005\%$ ), and *S. p. ambarvalis* ( $97.90 \pm 0.006\%$ ).

The PCoA analysis further supported the presence of genetic structure among the 3 subspecies. The first axis, explaining 23.24% of the variation present, separated the plains subspecies from both the Appalachian and Florida subspecies (Fig. 3). The 2<sup>nd</sup> axis, explaining 10.61% of the variation present, separated the Appalachian and Florida subspecies and subdivided individuals within the plains subspecies, yet no geographical significance could be drawn from this subdivision (Fig. 3). Additionally, the PERMANOVA supported statistical significance of subspecies groupings ( $F = 45.19$ ,  $P < 0.0001$ ).

Corrected estimates of pairwise  $F_{ST}$  among subspecies ranged from 0.178 to 0.322 (Table 4), with the highest degree of genetic differentiation occurring between the plains and Appalachian subspecies, and the lowest degree occurring between the Appalachian and Florida subspecies. Uncorrected estimates of  $F_{ST}$  (range: 0.190–0.331) were similar to the *ENA* corrected estimates, but were inflated slightly, likely due to the presence of null alleles. Rates of gene flow among subspecies were low ( $Nm$  range: 0.526–1.155), most notably between the plains and Appalachian subspecies ( $Nm = 0.526$ ; Table 4). An association between geographic and genetic distance was detected within the plains spotted skunk ( $r = 0.27$ ;  $P < 0.001$ ) and less strongly in the Florida spotted skunk ( $r = 0.11$ ;  $P = 0.02$ ). No isolation by distance was detected in the Appalachian subspecies ( $r = 0.097$ ;  $P = 0.20$ ).

*Phylogenetic analysis of cytb.*—A total of 85 sequences of *S. putorius* were generated from the *cytb* gene and included in the analysis with 19 sequences from *S. gracilis*. The final alignment included 1072 base pairs from 7 *ambarvalis*, 20 *putorius*, and 58 *interrupta*. The ML tree was generated using the HKY+G model (alpha = 0.2382, log likelihood score = -

3409.8758). The plains subspecies formed a monophyletic lineage with high bootstrap support (91) (Fig. 4). The Appalachian and Florida subspecies formed a single lineage with bootstrap support (89), but these two subspecies were not distinct from each other. Two individuals from Florida clustered with South Carolina in the tree with the highest likelihood score, but there was no significant bootstrap support for this arrangement.

Average sequence divergence (K2P) based on the *cytb* gene was 9.8% between *S. putorius* and the outgroup *S. gracilis*. The *interrupta* subspecies was 2.9% divergent from the individuals in the *ambarvalis/putorius* clade. The *ambarvalis* and *putorius* subspecies were an average of 1.2% divergent.

Neither the microsatellite analysis (including 8 individuals of *S. gracilis*), nor the analysis of mitochondrial *cytb* sequence, detected any evidence of introgression between eastern and western spotted skunks. The degree of divergence between the two lineages was high (9.8%) which made the analysis of population structure using STRUCTURE invalid.

## DISCUSSION

Van Gelder (1959) initially identified *S. putorius* as a polytypic species composed of 15 subspecies, 3 of which are still recognized today (*S. p. interrupta*, *S. p. putorius*, *S. p. ambarvalis*). Results from our microsatellite structure analyses indicated the presence of 3 genetic clusters commensurate with the 3 subspecies designations. Although Van Gelder (1959) only utilized variation in external measurements (i.e. total, tail, and hind foot length), color pattern, and locality to designate *S. putorius* subspecies, we now add microsatellite variability to this list. Evidence of genetic structure and differentiation within the eastern spotted skunk was present in all analyses; however, the inability of STRUCTURE to separate *S. p. putorius* from *S. p. ambarvalis* in the first analysis was likely due to the less pronounced differentiation observed between these subspecies in comparison to the high degree of differentiation the plains spotted skunk shared with both the Appalachian and Florida subspecies. This high degree of differentiation likely resulted in  $\Delta K = 2$ , instead of  $\Delta K = 3$ , when all 3 subspecies were analyzed together. Mean  $\text{LnP}(K)$  for  $\Delta K = 3$  was less negative than  $\Delta K = 2$  (-2778.03 vs. -2966.27); however, the *SD* of this value was higher for  $\Delta K = 3$  (49.86 vs. 0.36). This pattern was consistent with the close relationship of *S. p. putorius* and *S. p. ambarvalis* in the phylogenetic analysis of *cytb* compared to the distinct mitochondrial lineage containing all members of *S. p. interrupta*.

Although IBD was present within the plains spotted skunk, the PCoA analysis and *cytb* tree did not reveal the same pattern, as individuals from Texas, South Dakota, Arkansas, and Nebraska clustered together with no discernable geographic pattern (Fig. 3, 4). Within the Florida subspecies, the presence of IBD was relatively unexpected, as all individuals were sampled from 1 contiguous population within a small geographic area. The greatest distance separating 2 trapped Florida spotted skunks was only 3.88 km, with an average distance of 1.51 km. As seasonal home ranges for the eastern spotted skunk (which are highly sex and season dependent) have been reported to range from 19–1,824 ha (Lesmeister et al. 2009), and the correlation coefficient for IBD was weak within this subspecies ( $r = 0.11$ ), it is possible that IBD does not play a key role in the structure of this subspecies at the scale we sampled. Interestingly, despite all Florida spotted skunk samples deriving from a single population, this subspecies displayed a pattern of genetic variation similar to that

observed in the plains and Appalachian subspecies, whose samples originated from as many as 5 states with a maximum distance of >1,500 km separating individuals.

A comparison of genetic diversity of the plains spotted skunk to other, and perhaps more common, North American mesocarnivores highlights the reduced diversity observed in this subspecies. Observed heterozygosity for the plains spotted skunk averaged 0.474, while  $H_O$  for subspecies of the North American badger (*Taxidea taxus*) averaged 0.757 (Kyle et al. 2004), studies on the striped skunk (*Mephitis mephitis*) reported  $H_O$  values of 0.764 and 0.683 (Barton and Wisely 2012; Brashear et al. 2015), and  $H_O$  for Florida populations of raccoons (*Procyon lotor*) ranged from 0.78 to 0.84. (Trujillo and Hoffman 2016). Allelic richness for the plains spotted skunk averaged 6.64, whereas  $N_A$  for *T. taxus* averaged 9.9 (Kyle et al. 2004), *M. mephitis* averaged 12.88 and 10.69 (Barton and Wisely 2012; Brashear et al. 2015), and *P. lotor* averaged 8.77 for mainland Florida populations (Trujillo and Hoffman 2016). Instead, average  $H_O$  and  $N_A$  of the plains subspecies more closely resembles the levels found within the island spotted skunk ( $H_O$ : 0.590,  $N_A$ : 4.5; Floyd et al. 2011), an insular subspecies of the western spotted skunk restricted to 2 islands within the Channel Island archipelago.

However, in contrast to the trend of lower genetic diversity observed in *S. p. interrupta* when compared to more common, North American carnivores, the plains subspecies exhibits levels of genetic diversity higher than those reported for endangered carnivores. For example, grassland and shrubland (sub)species such as the San Joaquin kit fox (*Vulpes macrotis mutica*) and the black-footed ferret (*Mustela nigripes*) contain low measures of genetic diversity due to reductions in population connectivity as a result of habitat alteration. Schwartz et al. (2005) reported ranges of  $N_A$  and  $H_O$  for the San Joaquin kit fox at 2.65–4.38 and 0.28–0.50, respectively. Cain et al. (2011) determined  $N_A = 2$  for 2 subpopulations of black-footed ferret with  $H_O$  ranging from 0.39–0.44. Other endangered carnivores such as the clouded leopard (*Neofelis nebulosa*) and Amur tiger (*Panthera tigris altaica*) exhibit the same trend of reduced genetic variability (Buckley-Beason et al. 2006; Henry et al. 2009). In a comparison across vertebrate taxa representing all 6 IUCN conservation ranks, Willoughby et al. (2015) determined that genetic diversity values ( $H_O$  and  $N_A$ ) were lower in threatened vertebrates, which exhibit some degree of extinction risk, in comparison to species of lesser conservation concern. Given the vulnerable status of the eastern spotted skunk by the IUCN, and that the conservation status of the plains subspecies is currently under review, the lower-than-average genetic diversity observed within each subspecies agrees with the pattern evidenced by Willoughby et al. (2015).

Levels of genetic diversity did not significantly differ among the 3 subspecies, therefore suggesting that the plains spotted skunk is no more depauperate genetically than the Appalachian or Florida spotted skunks. However, trends in sightings and capture rates for the 3 subspecies are not equal, suggesting relative abundances vary by subspecies. For example, past studies have reported that *S. p. ambarvalis* is abundant in southern (Kaplan and Mead 1991) and east-central (Kinlaw et al. 1995) Florida, and the recent trapping success rate by the Florida Fish and Wildlife Conservation Commission was substantially greater (approximately 42%; Tina Hannon, personal communication, June 2017) than those obtained in recent literature. A recent publication reporting on incidental captures of the Appalachian spotted skunk ( $n = 6$  over a month period; Diggins et al. 2015) and the number of *S. p. putorius* tissue donations received for this analysis ( $n = 17$ ) suggest that this subspecies is more locally abundant than the plains subspecies. For the plains spotted skunk, studies to

date have reported capture rates of 0.38% (Hackett et al. 2007) and 0.17% (this study), and game camera detections of 2 or 3 individuals over a period of 26 months (Hardy 2013), thus highlighting the rarity of this subspecies throughout its range in comparison to the other 2 subspecies.

Recent phylogeographic work by Ferguson et al. (2017) revealed that the genus *Spilogale* diverged from other mephitid lineages approximately 6.53 Ma, with eastern and western spotted skunks sharing a most recent common ancestor 2.71 Ma. Because it follows that differentiation achieved within *S. putorius* occurred after its divergence from *S. gracilis*, the intraspecies divergence observed likely occurred throughout the Quaternary, as opposed to occurring pre-Pleistocene. Biological communities in North America were affected continent-wide due to river system modifications, sea level changes, lake creation, and climate cooling that occurred as a result of alternating glacial and interglacial periods. Several geographic barriers to gene flow likely functioned to create the patterns of genetic differentiation and structure presently observed among eastern spotted skunk subspecies. The eastern spotted skunk is certainly not the only species that displays these patterns, as genetic signatures of isolation are abundant in the literature due to climatic and geological changes that occurred within the Quaternary (Hayes and Harrison 1992; Barton and Wisely 2012; Ferguson et al. 2017). Specifically, during the Wisconsinan glaciation of the Pleistocene, the southern United States and Mexico served as refugia for spotted skunks (Van Gelder 1959; Ferguson et al. 2017). Eventual retreat of the ice sheets enabled present day eastern spotted skunks to extend their range both northward and eastward, with individuals eventually branching east and west of the southern Mississippi River (Van Gelder 1959). Not only did this river serve as a strong isolating barrier, especially during periods of interglacial melt when river volume and width were substantial, but the floodplains and moist lowlands along the river also provided unsuitable habitat for eastern spotted skunks, further restricting gene flow across its banks (Van Gelder 1959). This divergence at the Mississippi River is congruent with the current subspecies boundary between *S. p. interrupta* and *S. p. putorius*, and is a well-documented barrier to gene flow in a variety of taxa (Burbrink et al. 2000; Brant and Ortí 2003; Soltis et al. 2006; Brandley et al. 2010; Near et al. 2001).

The divergence of the Florida spotted skunk from the plains and Appalachian subspecies is less clear, yet fossils indicate the earliest colonization of Florida by spotted skunks occurred in the early Pleistocene (Webb 1974). Climatic and glacial fluctuations that occurred throughout the Quaternary altered sea levels, with evidence for much of Florida being inundated over several periods from 188,000 to 72,000 BP (Cronin et al. 1981). This marine barrier likely served to isolate Florida populations of spotted skunks, much like it has in other species, such as the woodrat (*Neotoma* spp.; Hayes and Harrison 1992). With the recession of sea levels into the Holocene and the alleviation of the marine barrier, Florida populations could then achieve secondary contact with present day *S. p. putorius*. However, few to no specimens are documented or contained within museum collections along this subspecies contact zone, therefore making it difficult to interpret the degree of introgression that occurs. Moreover, much remains unknown with respect to the timing of genetic divergences among all 3 subspecies, thus future studies addressing the phylogeographic patterns of *S. putorius* are desperately needed.

Percent divergences among eastern spotted skunk subspecies ranged from 1.2–3.1%, yet the eastern spotted skunk exhibited divergence from the western spotted skunk at 9.8%. No degree of admixture was apparent in the ML *cytb* tree, as all *S. gracilis* clustered

separately from *S. putorius* with high bootstrap support. Therefore, from a mitochondrial perspective, there appears to be no hybridization occurring between these sister taxa. In fact, the divergence seen between these two species is comparable to that observed in many mammalian taxa (Bradley and Baker 2001; Baker and Bradley 2006). The lack of hybridization between these two skunks is not surprising, as several genetic, physiological, and temporal mechanisms likely prevent interspecies mating even where they occur sympatrically. Pre-zygotic isolating mechanisms include chromosomal differences ( $2n = 60$  for *S. gracilis* and 64 for *S. putorius*), the presence of delayed implantation in *S. gracilis*, and differences in mating season, with *S. putorius* mating in spring and *S. gracilis* in fall (Kinlaw 1995).

Fluctuating climatic conditions during the Quaternary could have dictated the structure and differentiation present within the eastern spotted skunk; however, modern-day anthropogenic activity has great capacity to exacerbate this differentiation by reducing population sizes and genetic variability. The negative effects of habitat fragmentation on the genetic variability of numerous carnivore species are well documented; however, these impacts have yet to be determined for the eastern spotted skunk. Prevalent anthropogenic sources that have the potential to act as barriers to gene flow for the plains spotted skunk include gas and oil drilling practices, urban sprawl, and agricultural modification of the landscape. Specifically, fragmentation has been shown to reduce gene flow and genetic variability within impacted carnivore populations (Riley et al. 2006; Haag et al. 2010; Schwalm et al. 2014; McManus et al. 2015) and often leads to the implementation of conservation and management strategies for the affected species. Although structure below the subspecies level was not found in this analysis, thus indicating impediments to gene flow within subspecies are not present, the intensification of anthropogenic activities throughout the central United States have the potential to restrict gene flow in this region. Therefore, any future management strategies for the plains spotted skunk should account for the dynamic nature of this habitat.

Although the 11 cross-species microsatellite markers utilized in this study enabled an in-depth analysis of the genetic structure and differentiation within this species, the development of *Spilogale*-specific primers would aid in future studies of the eastern spotted skunk. From an initial set of 26 molecular markers we tested on *Spilogale* that were originally developed for the striped skunk (Dragoo et al. 2009; Munguia-Vega et al. 2009), Eurasian badger (Bijlsma et al. 2000), and North American river otter (Beheler et al. 2004; Floyd et al. 2011), only 16 successfully amplified in *Spilogale*. Of these 16, 5 were monomorphic. As less than half of the loci tested were unsuccessfully amplified or proved uninformative for this study, the development of *Spilogale*-specific markers would not only enable the analysis of additional neutral sites, but would also ensure a higher prevalence of polymorphic loci for downstream analysis. Given that the conservation status of this species is insecure, markers specific for the eastern spotted skunk would be beneficial in addressing additional questions from individual to population level scales.

In conclusion, based on microsatellite and mitochondrial DNA sequences, the eastern spotted skunk displays strong patterns of genetic structuring and differentiation among subspecies, which are commensurate with previously reported morphological differences (Van Gelder 1959). The presence of private alleles found in all 3 subspecies, the degree of differentiation among them, the lack of gene flow, and high individual membership coefficients indicate the need to consider each subspecies as a unique evolutionarily

significant unit (Moritz 1994). A similar suggestion was provided by Floyd et al. (2011) for the island spotted skunk, as they determined that populations occupying 2 separate Channel Islands, Santa Cruz Island and Santa Barbara Island, were just as differentiated from each other as they were from mainland western spotted skunk subspecies. Future management strategies for the eastern spotted skunk should therefore consider the genetic dissimilarities present among subspecies, as it is possible that these genetic differences reflect behavioral, physiological, or habitat differences, as well. Although we were able to sample a wide geographic range representing all 3 subspecies, the inclusion of specimens representing additional states would help determine the amount of genetic introgression occurring. Furthermore, the inclusion of additional individuals that occupy subspecies contact zones would help refine our understanding of the geographic barriers that acted and are currently serving to divide the subspecies.

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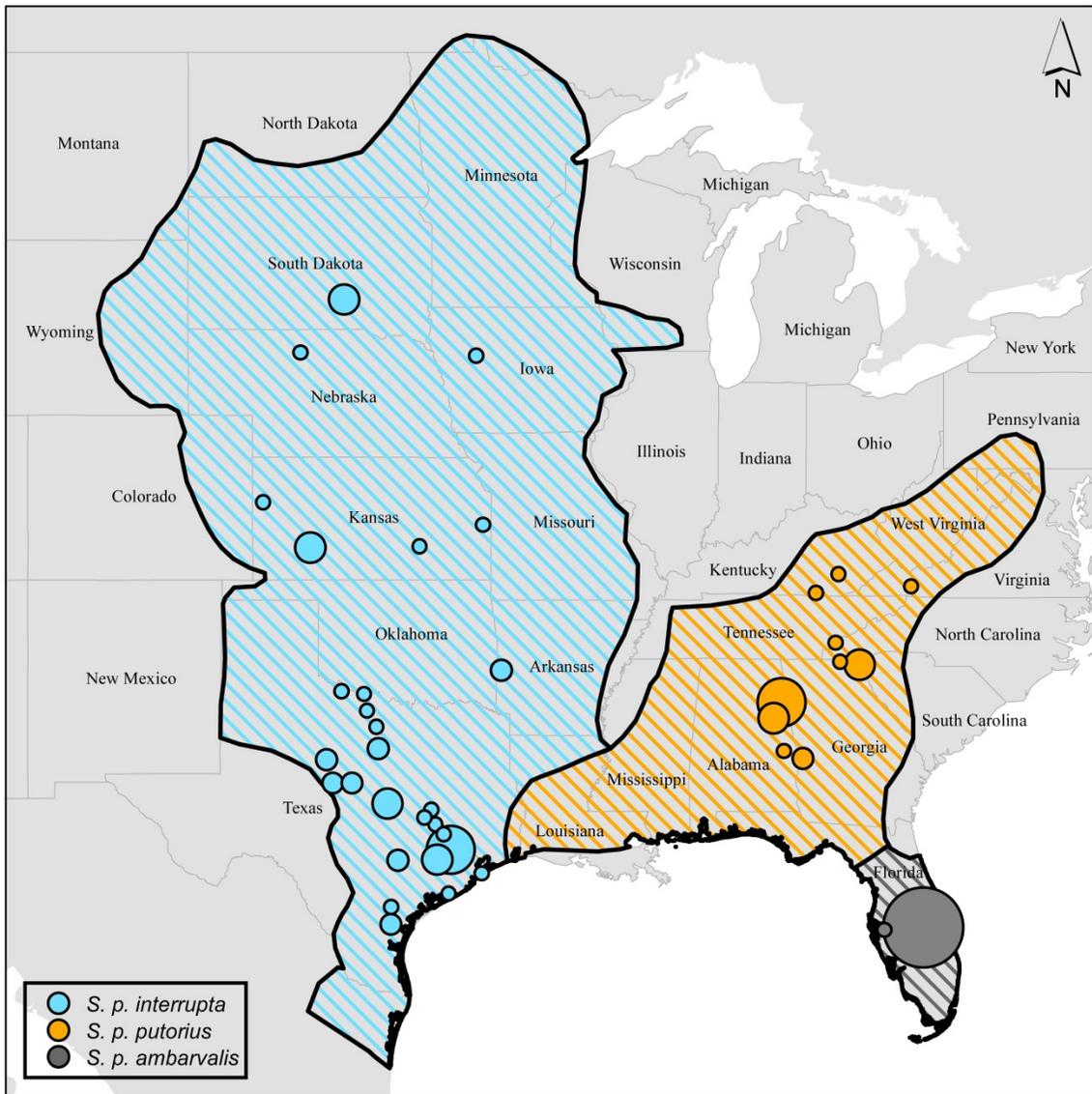
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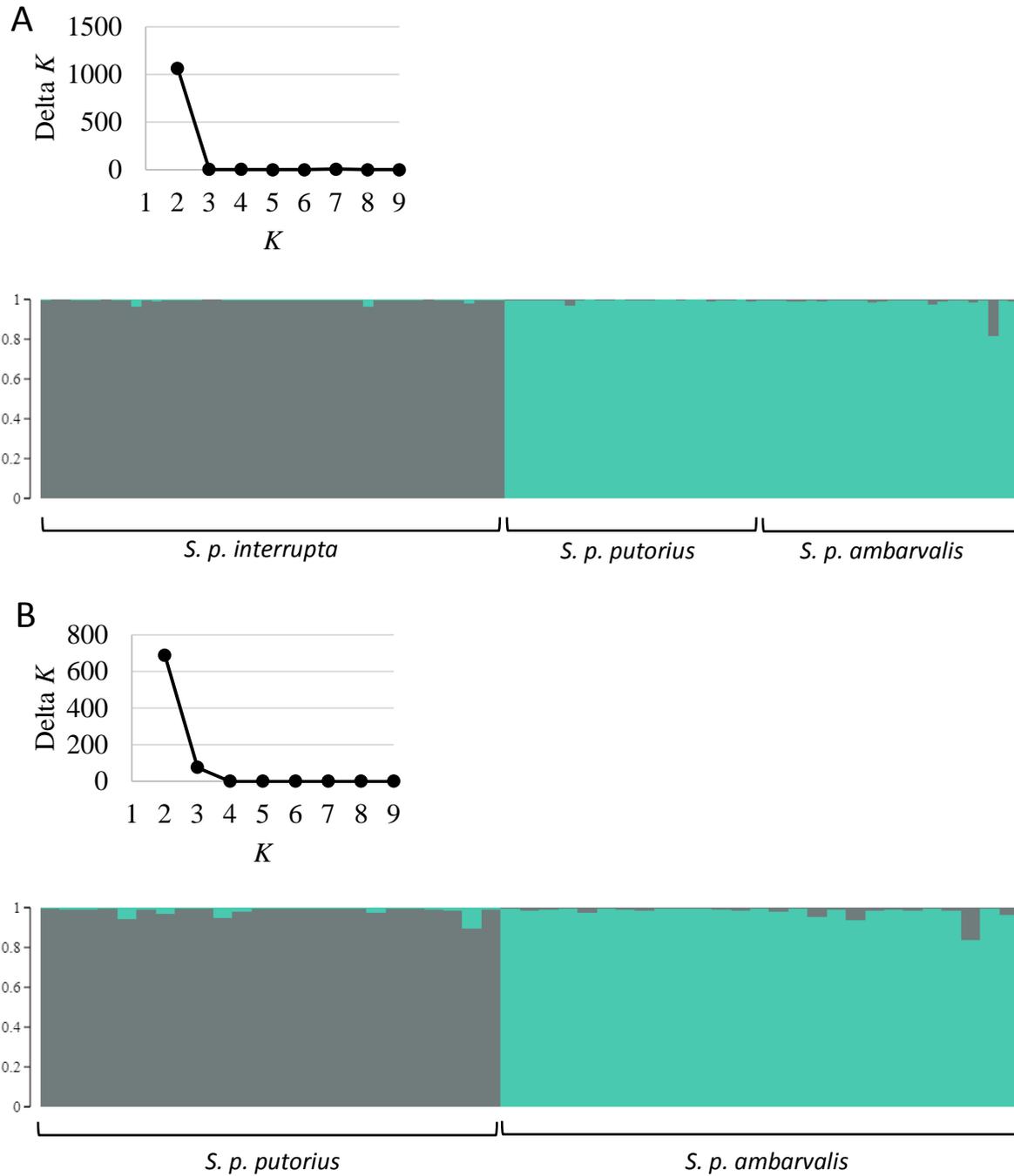
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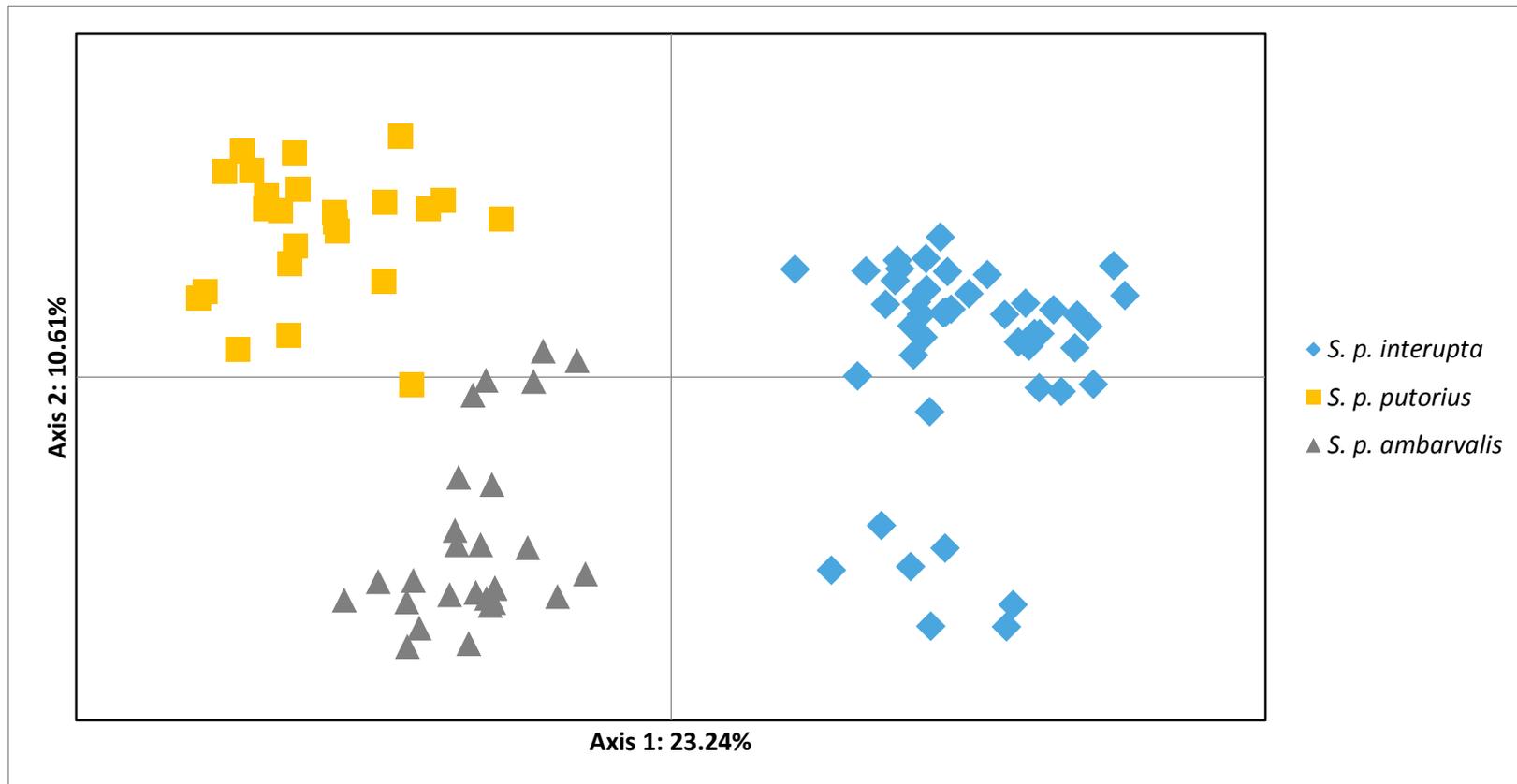
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**Fig. 1.**—Map indicating the collection locality of eastern spotted skunks ( $n = 97$ ) utilized in microsatellite analyses. From small to large, circles represent sample sizes of  $n = 1$ ,  $n = 2-3$ ,  $n = 4-5$ ,  $n = 9-12$ , and  $n = 27$ . Subspecies ranges are outlined in black and are color coded (cross-hatched) according to the respective subspecies (blue = *S. p. interrupta*, orange = *S. p. putorius*, gray = *S. p. ambarvalis*).



**Fig. 2.**—Plots of  $\Delta K$  for  $K = 1-10$  from STRUCTURE HARVESTER and the respective STRUCTURE PLOT bar graphs for *Spilogale putorius interrupta*, *S. p. putorius*, and *S. p. ambarvalis* (A) and *S. p. putorius* and *S. p. ambarvalis* (B), indicating  $\Delta K = 2$  for both analyses. For bar graphs, each bar represents 1 individual and its respective membership coefficient when  $K = 2$ .



**Fig. 3.**—Results from the principal coordinates analysis of genotypes of 11 microsatellite loci for subspecies of *Spilogale putorius*. The first axis explained 23.24% of the variation in the data and separated the plains subspecies from the Appalachian and Florida subspecies, while the second axis explained 10.61% of the variation in the data and separated the Appalachian from the Florida subspecies.

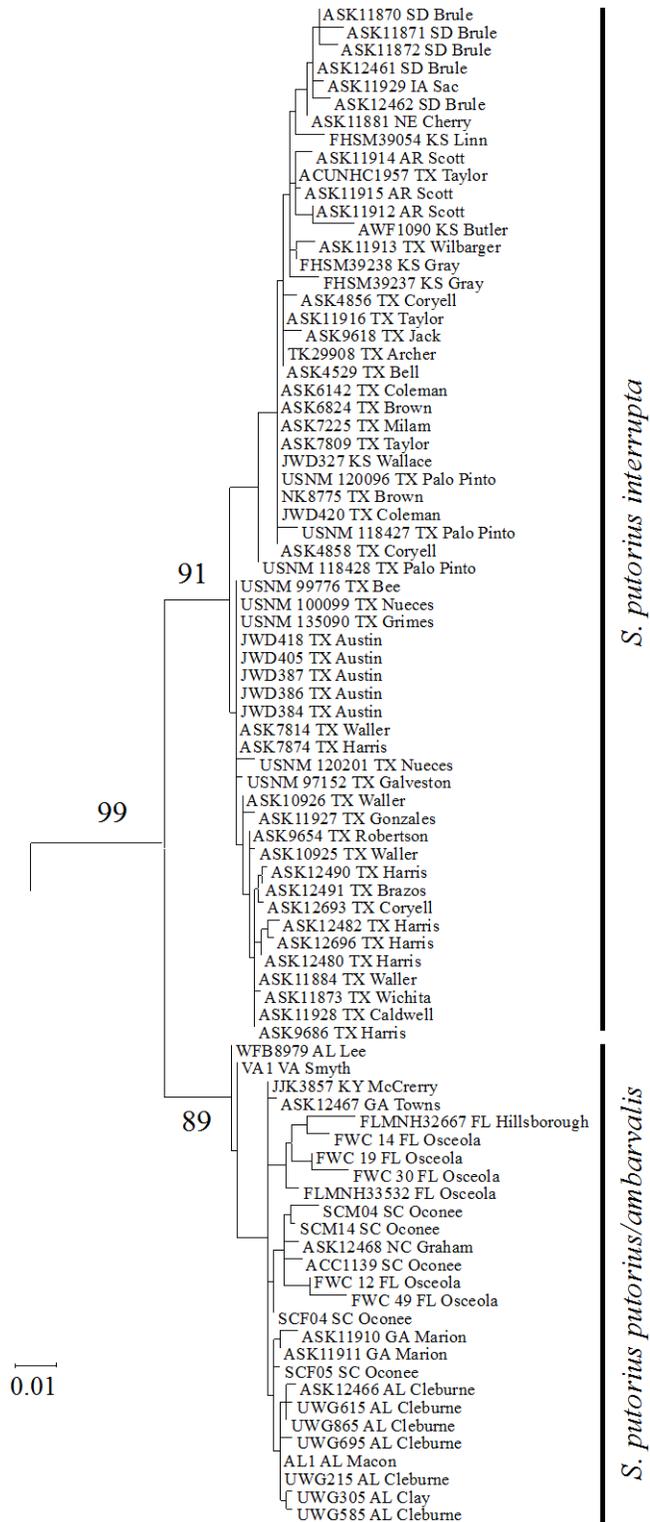


Fig. 4.—Maximum likelihood tree based on analysis of 1072 bases of cytochrome *b* gene for subspecies of *Spilogale putorius*. Numbers on branches are ML bootstrap values over 75%. Nineteen sequences from *S. gracilis* were used as the outgroup (removed for clarity).

**Table 1.**—Characterization of microsatellite loci optimized for genetic analysis of the eastern spotted skunk. Locus name, forward and reverse primer sequence, locus repeat motif, PCR annealing temperature ( $T_A$ , °C), the number of alleles per locus ( $N_A$ ), allelic size range (bp), observed ( $H_O$ ) and expected ( $H_E$ ) heterozygosities, and the original publication of each primer are noted. All forward primers were dye-labeled. “—” = Not determined.

Locus	Primer sequence 5'-3'	Repeat motif	$T_A$	$N_A$	Size range	$H_O$	$H_E$	Reference
Meme5	F: CCTGAATGCAGGAGATGGAT R: GATGACTGATTAAGCAGTCTGCC	(CA) <sub>26</sub>	55	8	176–198	0.548	0.597	Munguia-Vega et al. 2009
Meme20	F: CATGAGCCCTGACAGGTGTA R: TCTTGGAACTGCATCAAAA	(GT) <sub>29</sub>	55	2	120–135	0.029	0.165	Munguia-Vega et al. 2009
Meme75	F: GTGTAGCTCTTCAGAGATGGATAGG R: TTCCAGGATGAACCAGGATG	(GT) <sub>22</sub>	55	11	146–178	0.495	0.519	Munguia-Vega et al. 2009
Meme77 <sup>a</sup>	F: TCCACAATAGTCAAACAATGGAA R: GTTGCAAATGGCAGGATTTT	(CA) <sub>21</sub>	55	1	131–131	—	—	Munguia-Vega et al. 2009
Meme82 <sup>a</sup>	F: TACCCGCTAGTTCCATCCAC R: GAGCCTATATGCCCATCAACA	(CA) <sub>15</sub>	55	1	132–132	—	—	Munguia-Vega et al. 2009
Meme84 <sup>a</sup>	F: GCAAAGGATATATTTGATAAGGGATT R: AATGGCTTTGTTCCAGCAG	(CA) <sub>15</sub>	55	1	139–139	—	—	Munguia-Vega et al. 2009
Meme88 <sup>a</sup>	F: TAGCAGCAATGCCACAATA R: CATCTTTCTGATGGCTGCAT	(CA) <sub>24</sub>	55	1	122–122	—	—	Munguia-Vega et al. 2009
Meph22-14	F: CTTTTGGGTCATTAGTGCATTTATG R: GGAAAGAGGAAAAGAAAACCCATG	(GT) <sub>24</sub>	50	7	230–256	0.354	0.343	Dragoo et al. 2009
Meph22-16*	F: GATCCCCAAAACACAAAAACTATG R: GCTGGATAGCGCTGGCATG	(GT) <sub>17</sub>	52	4	318–328	0.000	0.348	Dragoo et al. 2009
Meph22-26	F: ATGCAGGCTTGATTTTCAACCTC R: GAAAATCATTTACCAGTGGTGTGG	(TG) <sub>7</sub> (CG) <sub>2</sub> (TG) <sub>12</sub>	52	11	220–240	0.788	0.810	Dragoo et al. 2009
Meph22-70	F: CAGATGCATCAGCAACGATTC R: GAGTGTTGCATTACAGCCTGTG	(CA) <sub>24</sub>	50	16	185–217	0.729	0.832	Dragoo et al. 2009

**Table 1.**—Continued

Locus	Primer sequence 5'-3'	Repeat motif	T <sub>A</sub>	N <sub>A</sub>	Size range	H <sub>O</sub>	H <sub>E</sub>	Reference
Meph22-89 <sup>a</sup>	F: GGCTCATATCCCCTGGGTAGG R: TGAAAGGGGGTGAAGAAGACTGG	(TG) <sub>6</sub> (AG)(TG) <sub>13</sub>	56	1	195–195	—	—	Dragoo et al. 2009
Meph42-25	F: ACCACTGTTGCACCAGTTCTATCAG R: CACAGTTAGAAGGCCCAGAACATTC	(TG) <sub>24</sub>	54	15	201–236	0.683	0.759	Dragoo et al. 2009
Meph42-73	F: AAAGGACAATCCCACAGGTCT R: TGGACATGGAATTCTGGTTG	(CA) <sub>14</sub>	50	7	152–168	0.716	0.704	Dragoo et al. 2009
Mel 1	F: CTGGGAAAATGGCTAAACC R: AATGCAGGCTTTGCAATTCC	(GT) <sub>20</sub>	60	9	258–278	0.724	0.746	Bijlsma et al. 2000
nRIO-08 <sup>b</sup>	F: TGAGGTGTTGGTGTGTTTGTCTAT R: TTGCCTGCTGACATTGAAGMT	(TG) <sub>15</sub>	58	10	139–159	0.408	0.448	Floyd et al. 2011

<sup>a</sup>Indicates monomorphic loci excluded from final genetic analysis

<sup>b</sup>Primer originally published by Beheler et al. (2004); annealing sequence modified for use in *Spilogale* by Floyd et al. (2011)

\*Deviates significantly from Hardy-Weinberg equilibrium ( $P_{\text{adj}} < 0.001$ )

**Table 2.**—Polymerase chain reaction thermal profiles utilized at each microsatellite locus for genetic analysis of the eastern spotted skunk. Locus specific annealing temperatures ( $T_A$ ) are provided in Table 1.

Thermal profile	Loci
95°C for 3min, followed by 35 cycles of 95°C for 30s, $T_A$ for 30s, and 72°C for 1min, with a final extension at 72°C for 10min	Meph22-14, Meph22-16, Meph22-26, Meph22-70, Meph22-89, Meph42-25, Meph42-73
94°C for 5min, followed by 40 cycles of 94°C for 30s, 55°C for 30s, and 72°C for 30s, with a final extension at 72°C for 5min	Meme5, Meme20, Meme75, Meme77, Meme82, Meme84, Meme88
94°C for 3min, followed by 30 cycles of 94°C for 1min, 60°C for 2min, and 72°C for 1.5min, with a final extension at 72°C for 10min	Mel1
94°C for 4min, followed by 40 cycles of 94°C for 40s, 58°C for 40s, and 72°C for 1min, with a final extension at 72°C for 5min	nRIO-08

**Table 3.**—Genetic diversity values for each eastern spotted skunk subspecies across 11 microsatellite loci.  $N_A$  is mean number of alleles per locus,  $H_O$  is observed heterozygosity,  $H_E$  is expected heterozygosity, and  $N_P$  is the number of private alleles. Values for  $N_A$ ,  $H_O$ , and  $H_E$  are mean  $\pm$  SE with ranges provided in parentheses.

Subspecies	$n$	$N_A$	$H_O$	$H_E$	$N_P$
<i>S. p. interrupta</i>	46	6.64 $\pm$ 1.208 (2–15)	0.474 $\pm$ 0.090 (0.000–0.761)	0.605 $\pm$ 0.081 (0.063–0.897)	28
<i>S. p. putorius</i>	24	4.73 $\pm$ 0.810 (1–10)	0.441 $\pm$ 0.086 (0.000–0.783)	0.482 $\pm$ 0.082 (0.000–0.849)	8
<i>S. p. ambarvalis</i>	27	5.46 $\pm$ 0.780 (1–9)	0.577 $\pm$ 0.101 (0.000–0.913)	0.623 $\pm$ 0.080 (0.000–0.865)	8
Average	32.3	5.61 $\pm$ 0.550	0.498 $\pm$ 0.053	0.570 $\pm$ 0.046	14.7

**Table 4.**—Degree of genetic differentiation ( $F_{ST}$ ; below diagonal) and rate of gene flow ( $N_m$ , above diagonal) among eastern spotted skunk subspecies.

Subspecies	<i>S. p. interrupta</i>	<i>S. p. putorius</i>	<i>S. p. ambarvalis</i>
<i>S. p. interrupta</i>	—	0.526	0.820
<i>S. p. putorius</i>	0.322	—	1.155
<i>S. p. ambarvalis</i>	0.234	0.178	—

## APPENDIX 1 – A SPECIES DISTRIBUTION MODEL TO DIRECT FIELD STUDIES

## EXECUTIVE SUMMARY

In light of possible declines in population of the Plains Spotted Skunk (*Spilogale putorius interrupta*) in Texas, we assessed habitat that may potentially support the species. We generated maps of modeled probability of occurrence using a Maxent species distribution model. This model used historic localities and a suite of physiogeographic features as inputs to evaluate potentially suitable habitat throughout the species' native range. This area included a large swath of Texas from south of San Antonio, along a zone east of Interstate Highway 35 to Dallas, and in a band to the northwest of Dallas to the Oklahoma border. We found that the model was most sensitive to mean summer temperature (i.e., temperature during the warmest and driest quarter), average percent sand in soil, and annual precipitation. It was least sensitive to compound topographic index, which generally indicates wetness or proximity to streams. Using this model, we provided a list of 23 counties with a mean modeled probability of occurrence ( $p_{\text{mean}} > 50\%$ ), which is an arbitrary cutoff we used to limit the counties considered for surveys. We also provide a ranking of top counties for economic activity, as estimated by the Texas Comptroller of Public Accounts (Comptroller). Based upon feedback from the Comptroller's office, we modified our project's scope of work to provide a ranking of the top 5 counties with and without economic importance — versus our original proposal of counties with and without oil and gas activities. Our ranking of counties based upon modeled probability of occurrence found that the five economically important counties in descending order of importance were:

- Tarrant: gross domestic product (GDP), construction value (Dallas-Fort Worth), gas production from the Barnett Shale Play,
- Gonzales: hydrocarbon production from the Eagle Ford Shale Play,
- Harris: GDP, agriculture production, construction value (Houston),
- Wise: hydrocarbon production from the Eagle Ford Shale Play, and
- Karnes: hydrocarbon production from the Eagle Ford Shale Play.

The  $p_{\text{mean}}$  for these counties are 54, 52, 44, 44, and 42, respectively. Other economically important counties with modeled habitat included Dallas, Johnson, Fort Bend, Travis, and Freestone ( $p_{\text{mean}}$ : 42, 41, 40, 40, and 39, respectively). The top five non-economically important counties were Wichita, Brazos, Burlason, Archer, and Washington ( $p_{\text{mean}}$ : 73, 73, 68, 65, and 63, respectively). The next highest non-economic counties were Waller, Lavaca, Victoria, Bee, and Robertson ( $p_{\text{mean}}$ : 63, 59, 55, 55, and 55, respectively). We do not intend these county rankings to be a definitive recommendation of counties to survey; however, the results of the model suggested that the greatest likelihood of finding the target species during future surveys may be when surveys are conducted within these 23 counties.

## INTRODUCTION

The native Texas habitats of the Plains Spotted Skunk (*Spilogale putorius interrupta*) have been altered by anthropogenic factors for over 100 years. These may include—but are not necessarily limited to—road construction, agricultural activities, urbanization, and oil and gas development (O&G). Despite range-wide alterations, few surveys have looked for the species in the past 30 years. However, a lack of specimens and sightings suggests a decline similar to that reported in other parts of the species' range. In light of the lack of current information for the species, we present an assessment of current habitat available to support the species. This assessment is comprised of three tasks:

1. Reviewing of all known historical records of the Plains Spotted Skunk and a compilation of landscape, vegetation, soils, and climate data within the known range;
2. Assessing availability of potential habitat within *S. p. interrupta*'s native Texas range using a Maxent species distribution model; and
3. Recommending survey locations, based on model results, in high-potential habitats.

### **Distribution Patterns, Preferred Habitat, and Population Trends**

A range-wide decline of *Spilogale putorius* has been documented by analysis of fur-trapping records that showed sharp decreases in numbers since the 1940s (Gompper and Hackett, 2005). As a result, *Spilogale putorius interrupta* (Plains Spotted Skunk) was named as a Texas priority species (TPWD, 2014) and the U.S. Fish and Wildlife Service (FWS) is considering listing the species as threatened or endangered (FWS, 2012).

The native range of the species originally included much of the eastern half of Texas, onto the Edwards Plateau, and into North-Central Texas (Schmidly, 2004). The species prefers wooded areas and tall-grass prairie and is reported to have historically been found near farms. They are omnivorous, eating mice, insects, birds, small mammals, and fruit.

Possible causes for the observed decline include shifts to large-scale farming, introduction of pesticides, overharvesting for the fur trade, and disease. These activities and others resulted in changes in land use throughout its native range which include conversion of native habitat to agriculture (i.e., crops and pasture), urban and exurban areas (i.e., developed areas of low to high density), and roads (Gompper 2017).

Anthropogenic land alterations may affect *S. putorius interrupta* through fragmentation and loss of habitat. We define “alteration” as the area of a landscape that was converted from native vegetation to agricultural production, oil and gas production, urban areas, and roads. We define “fragmentation” as a change in size of landscape classes such as “core”, “patch”, “edge”, etc. established by Vogt et al. (2007). Thus, this report provides recommendations for survey locations requiring an assessment of potential habitat.

## MATERIAL AND METHODS

We map habitat that may potentially support *Spilogale putorius interrupta* in its native Texas range (Dowler et al., 2008; Schmidly, 2004) using a species distribution model (SDM).

### Study Area

We identify potential habitat and recommend locations for directed surveys for the Plains Spotted Skunk within the five ecoregions of Texas (Griffith et al., 2004) where the species has been documented. Previous work by our research team established new locations extending the range of *S. putorius interrupta* (Dowler et al., 2008) west from its known distribution (**Figure 1**) in Texas (Schmidly, 2004).

### Review of Current and Historical Records

As no comprehensive review of all known historical records of the Plains Spotted Skunk had been conducted at the start of this study, we compiled records to establish both historic and current distribution in Texas. We started with historic localities of Dowler et al. (2008), which we augmented with additional museum records compiled recently by Dowler. We georeferenced some localities by converting text descriptions of a location to an approximate latitude-longitude coordinate nearby. Our protocol to georeference localities was the same as those of other similar projects such as HerpNet (<http://herpnet2.org/>) and MaNIS (<http://manisnet.org/>). All locations receive coordinates with an associated error radius calculated using an online calculator (<http://manisnet.org/gci2.html>).

### Modeling of Potentially Suitable Habitat with Maxent

We modeled skunk habitat using the Maxent species distribution model (Version 3.3.3; Phillips et al., 2006; Phillips and Dudík, 2008). This approach is recognized to generate robust species distribution models with presence-only records (Elith et al., 2006). It was also recently shown to be nearly mathematically equivalent to a Poisson regression model (Renner and Warton, 2013).

Probability of occurrence of *S. putorius interrupta* within its Texas range was modeled following the generalized approach of Labay et al. (2011) using skunk localities (Dowler et al., 2008). We used several physiographic variables for Maxent predictor variables (**Table 1**). These environmental data layers were divided into a grid of 30-arc-second cells (1 km<sup>2</sup> at equator). We implemented Maxent following default parameterization recommendations (Elith et al., 2011; Phillips and Dudík, 2008). These include cross-validating models with ten replicates to estimate errors around fitted functions. The performance of each model was evaluated using a receiver operating characteristic (ROC) analysis. The ROC analysis characterizes model performance at all possible thresholds using the area under the curve. An optimal model with perfect discrimination would have an AUC of one, whereas a model that predicted species occurrences at random would have an AUC of 0.5 (Hanley and McNeil, 1982).

The modeling software generated an ASCII grid of relative probability of occurrence. We mapped modeled distribution in ArcGIS using an average of the ten model results. We also calculated the mean probability of occurrence within Texas counties as well as state and federal land to assess areas with a high probability of occurrence. Additionally, because most land in Texas is privately held, it can be difficult to find enough land parcels to survey. Thus, we ranked habitat along major roads and highways (TxDOT, 2015) to direct road-side surveys.

We compiled 64 *S. putorius interrupta* specimens, which were used as inputs for the habitat modeling. We generated several maps of continuous probability of *S. putorius interrupta* occurrence using SDM results in ArcGIS. While the model results do not represent actual current skunk localities, they do indicate relative probability of occurrence of suitable habitat. We considered modeled probability of occurrence >50% to be potentially suitable habitat for the skunk. We suggest that areas with occurrence probabilities above this arbitrary threshold are prime suitable habitat.

### Recommended Survey Locations

In order to inform the best location for future surveys, we ranked counties based upon the modeled probability of *S. putorius interrupta* occurrence. We also recommend 10 counties with and without economically important activities where surveys could be directed. This differs somewhat from the original proposal, which suggested only evaluating counties with and without oil and gas development. We also evaluate what roads have the highest probability of occurrence.

## RESULTS

### Modeling of Potentially Suitable Habitat with Maxent

Our modeling revealed suitable habitat occurs across a large swath of Texas from ~200 miles south of San Antonio, along a zone ~100 miles east of Interstate Highway 35 to Dallas, and includes a ~100-mile wide band from northwest of Dallas to the Oklahoma border (**Figure 2**).

We discovered, based on a statistical analysis of each Maxent input variable, that the model is most sensitive to summer temperature (i.e., temperature during the warmest and driest quarter), average percent sand in soil, and annual precipitation (**Figure 3**). The model is least sensitive to the compound topographic index, which is a wetness index that is a function of slope and upstream contributing areas. The three other most important model inputs include—in descending order of importance—precipitation of the wettest quarter, annual precipitation, and maximum temperature in the warmest quarter. We also assess the relative importance of each input variable using a heuristic estimate (**Table 2**). Variables with >5% relative contribution are: mean temperature of the warmest quarter (19.2%), average percent sand in soil (11.3%), mean temperature of the driest quarter (7.6%), annual precipitation (7.4%), generalized soil order (6.5%), precipitation of the wettest quarter (5.7%), and relative wetland density (5.1%).

To highlight which counties had suitable habitat and which counties had the best potential habitat, we also generated a map of counties with a maximum probability of occurrence ( $P_{\max}$ ) >50% and mean probability of occurrence ( $P_{\text{mean}}$ ) >50% (**Figure 4; Table 3**). Counties with the highest modeled occurrence probability cluster east and southeast of Austin and San Antonio and northwest of Dallas. We also created a map of  $P_{\text{mean}}$  for watersheds classified using U.S. Geologic Survey 12-digit hydrologic unit codes (USGS, 2014b) (**Figure 5; Table 4**). This map highlights areas with better chances of finding the skunk using physical, rather than political boundaries. In addition, we mapped counties with economically important activities (**Figure 6**). We also created a map that shows state and federal land with  $P_{\text{mean}}$  >50% (**Figure 7; Table 5**). Finally, because publically-accessible land is limited in Texas, we generated a Google Earth KMZ file that shows where roads and highways cross potential habitat with modeled occurrence probability >70%

(TxDOT, 2015) (**Figure 8**). We also provide Google Earth KMZ map files of other derivative mapping products to help guide surveys.

### Recommended Survey Locations

We generated a list of the ten counties with and without economically important activities (20 counties total) with the highest modeled *S. putorius interrupta* occurrence probability. We suggest that future surveys be conducted in these 20 counties. The top ten economically important counties with the highest modeled probability of occurrence are, in descending order: Tarrant, Gonzales, Harris, Wise, Karnes, Dallas, Johnson, Fort Bend, Travis, and Freestone counties. The top 10 non-economically important counties with the highest modeled probability of occurrence are, in descending order: Wichita, Brazos, Burleson, Archer, and Washington, Waller, Lavaca, Victoria, Bee, and Robertson. Recognizing that Texas is a private property state, it may be important to survey state or federal lands, or public right-of-ways along roads and highways. To this end, the 5 most highly-ranked state lands are: Lake Somerville State Park, Lake Arrowhead State Park, Lake Somerville Wildlife Management Area, Mother Neff State Park, and Waco Lake. Lake Somerville is definitely a highly-ranked area worthy of surveying because it has the highest  $p_{\text{mean}}$  values. Additional road surveys could be directed to areas near Beeville, Victoria, College Station, Waco, Mineral Wells, and Wichita Falls.

## DISCUSSION

We assessed habitat that may potentially support *S. putorius interrupta* in Texas. These results suggest that the species may prefer parts of Texas with a certain combination of summer temperature, precipitation, and sand in soil. The target species does not appear to have an affinity for stream drainages or sloped terrain. The habitat analysis also reveals two possible groups based on geography: one east of the Balcones Escarpment and the second in North-Central Texas along the Oklahoma border. The results of genetic analyses (Task 4) should reveal if there are distinct populations.

### Recommended Survey Locations

The habitat analysis suggests that the best chance of finding the species is by surveying within the 23 counties shown on **Figure 4** and in **Table 3** with  $p_{\text{mean}} > 50\%$ . We originally proposed surveys in five counties with and without oil and gas activity (10 counties total). However, in light of conversations with the Comptroller's office, here we present recommended survey locations that include counties with and without important economic activity (**Figure 6** and **Table 4**). The economically important counties include oil and gas activity, in addition to construction, agriculture, and overall gross domestic product. Thus, these counties represent locations where future management actions may most strongly affect economic activity.

### Assumptions and Limitations of Approach

Our modeled probability of occurrence revealed a potential bias towards highly-sampled areas near urban areas. We partially accounted for this bias by including all skunk samples into the pseudo-absence dataset (Elith et al., 2011). Our habitat modeling may also have a temporal bias resulting from historic sampling in habitats where the species may no longer be found. Thus, the species'

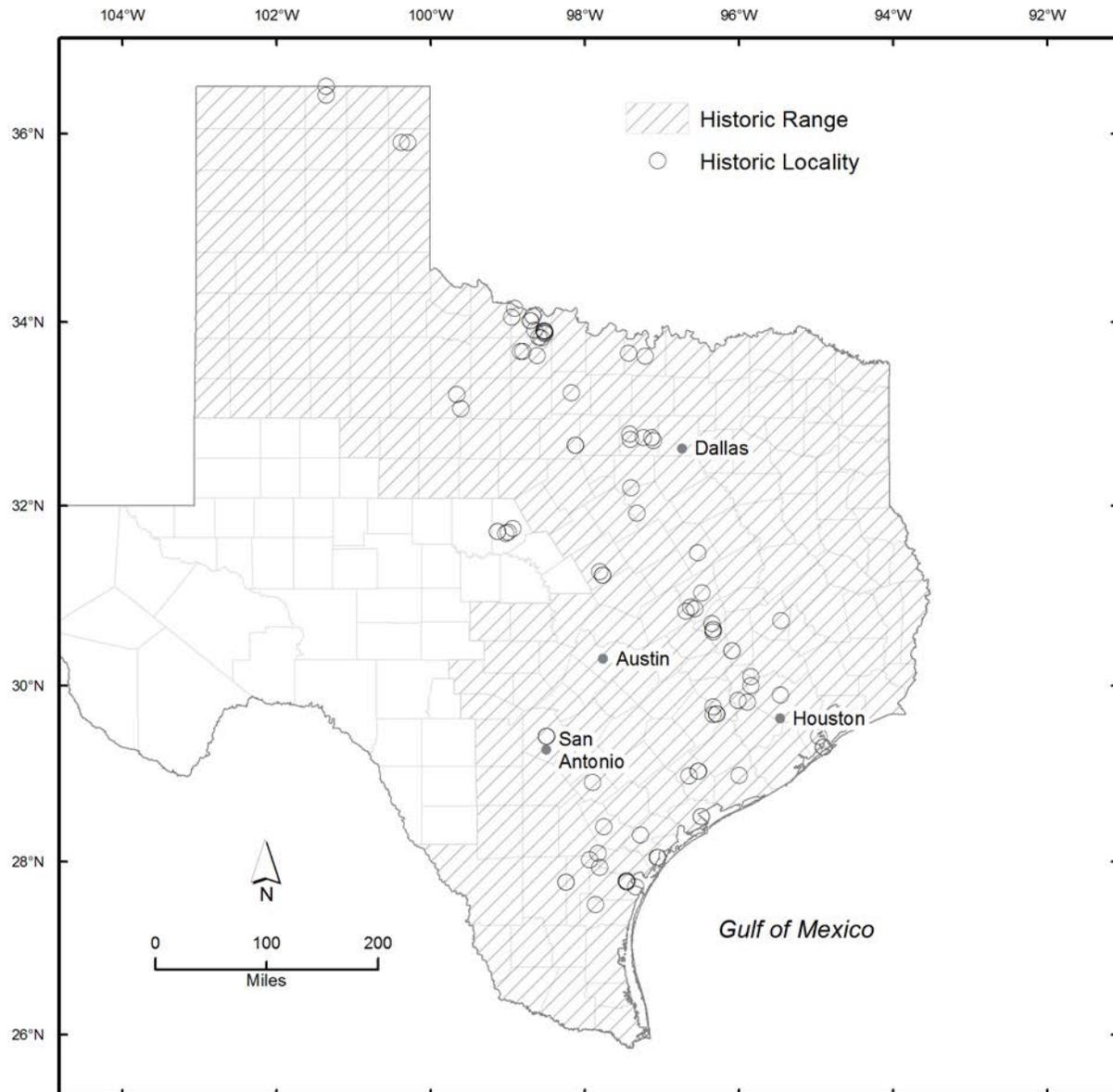
current actual distribution may not correspond with our habitat maps, which show where the species may potentially be found.

**CONCLUSIONS**

Key findings of this habitat suitability study include:

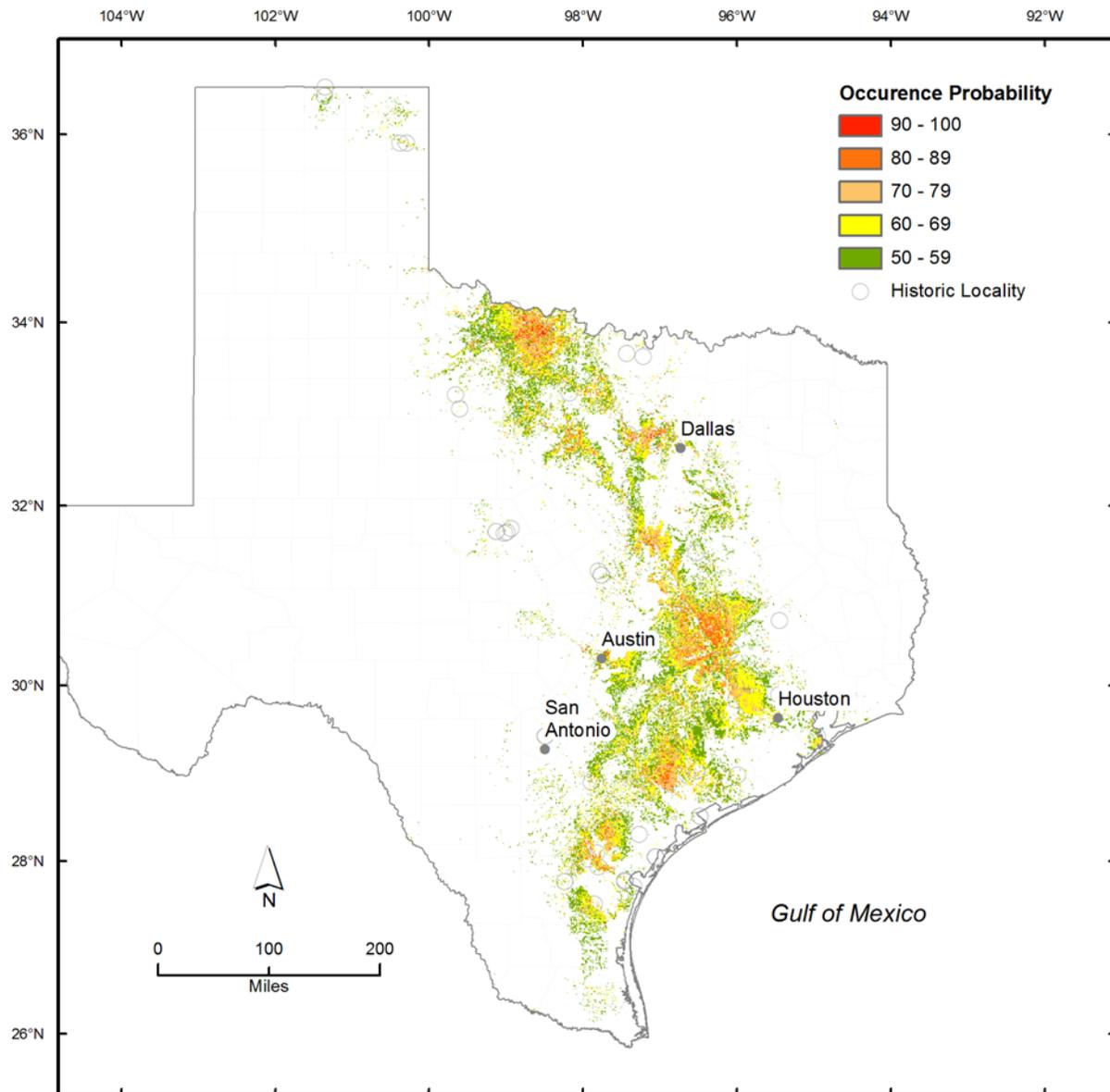
- Modeled potentially suitable habitat occurs across a large swath of Texas from south of San Antonio, along a zone east of Interstate Highway 35 to Dallas, and northwest of Dallas towards the Oklahoma border.
- The top ten economically important counties with the highest modeled probability of occurrence are, in descending order: Tarrant, Gonzales, Harris, Wise, Karnes, Dallas, Johnson, Fort Bend, Travis, and Freestone counties.
- The top ten non-economically important counties with the highest modeled probability of occurrence are, in descending order: Wichita, Brazos, Burleson, Archer, and Washington, Waller, Lavaca, Victoria, Bee, and Robertson.
- Surveys may have the best chance of finding the species if they are conducted in these counties.

**FIGURES—APPENDIX 1**



**Figure 1.** Map of historic *S. putorius interrupta* localities (Dowler et al., 2008; hollow circle) and generalized historic range in Texas (Dowler et al., 2008; cross-hatched polygons).

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**Figure 2.** Modeled probability of occurrence of *S. putorius interrupta* (occurrence records are hollow circles; Dowler et al., 2008).

Model results do not represent actual current skunk localities, but relative potentially suitable habitat—based on model inputs—that is likely to support the target species.

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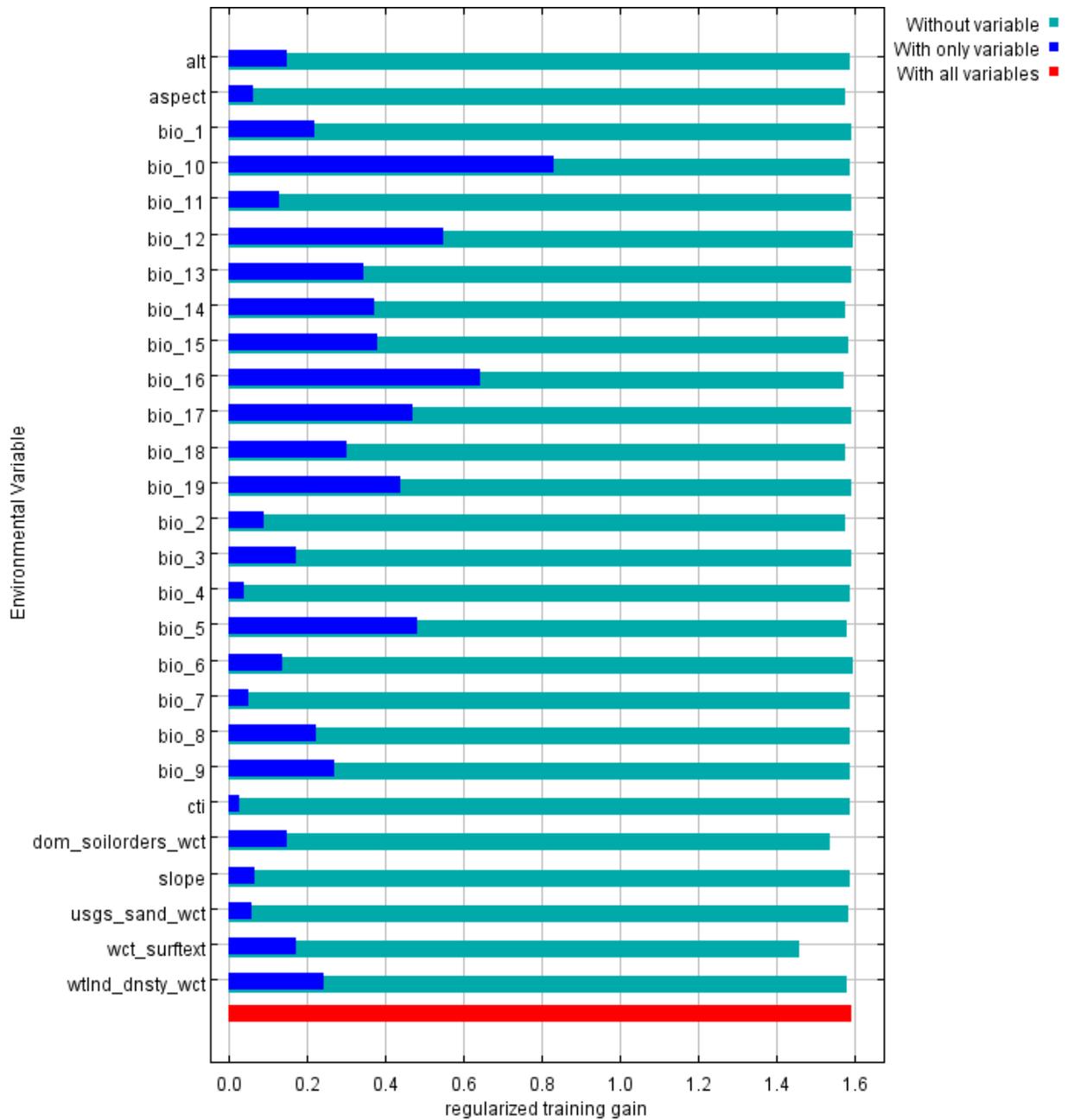
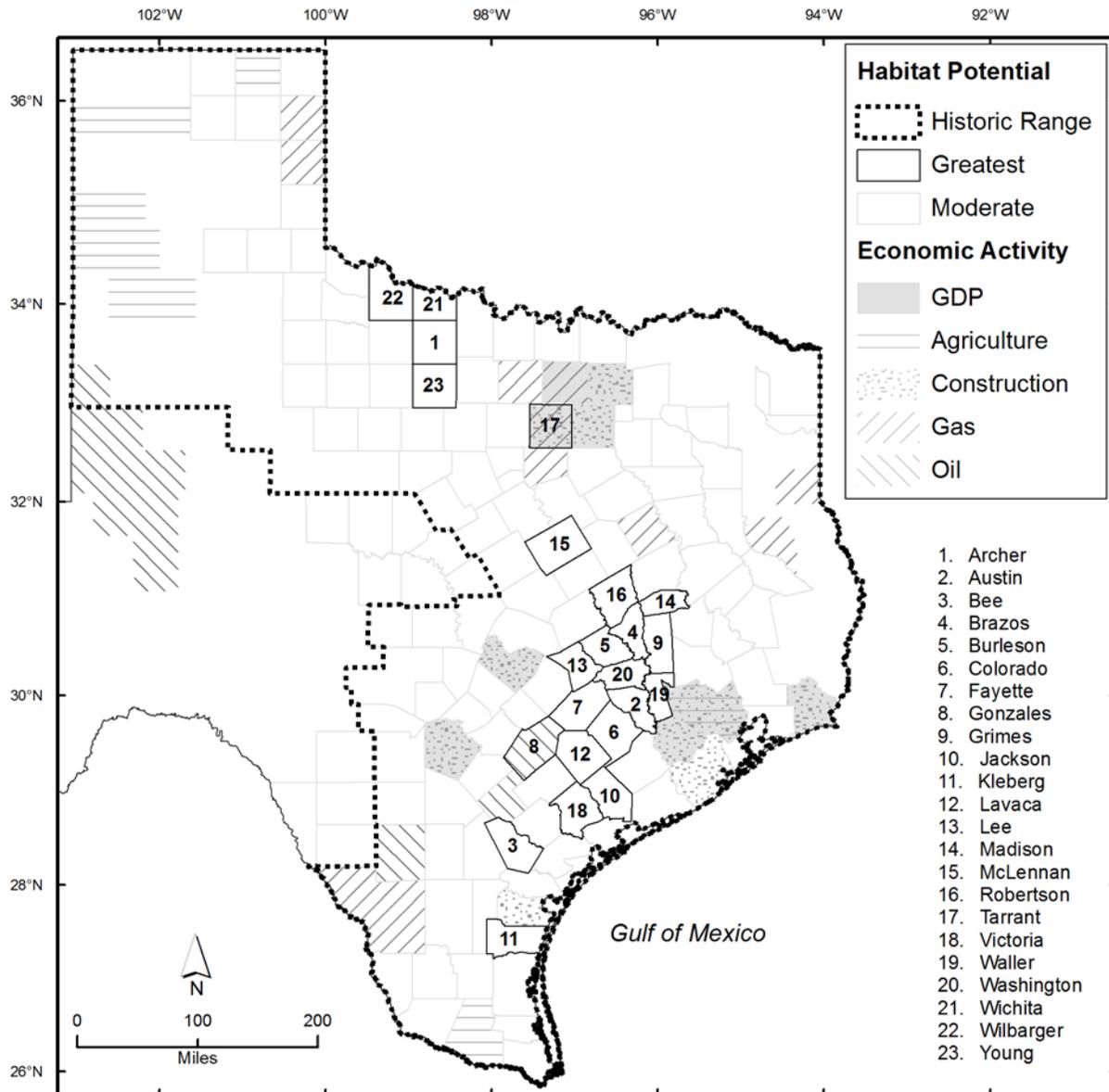


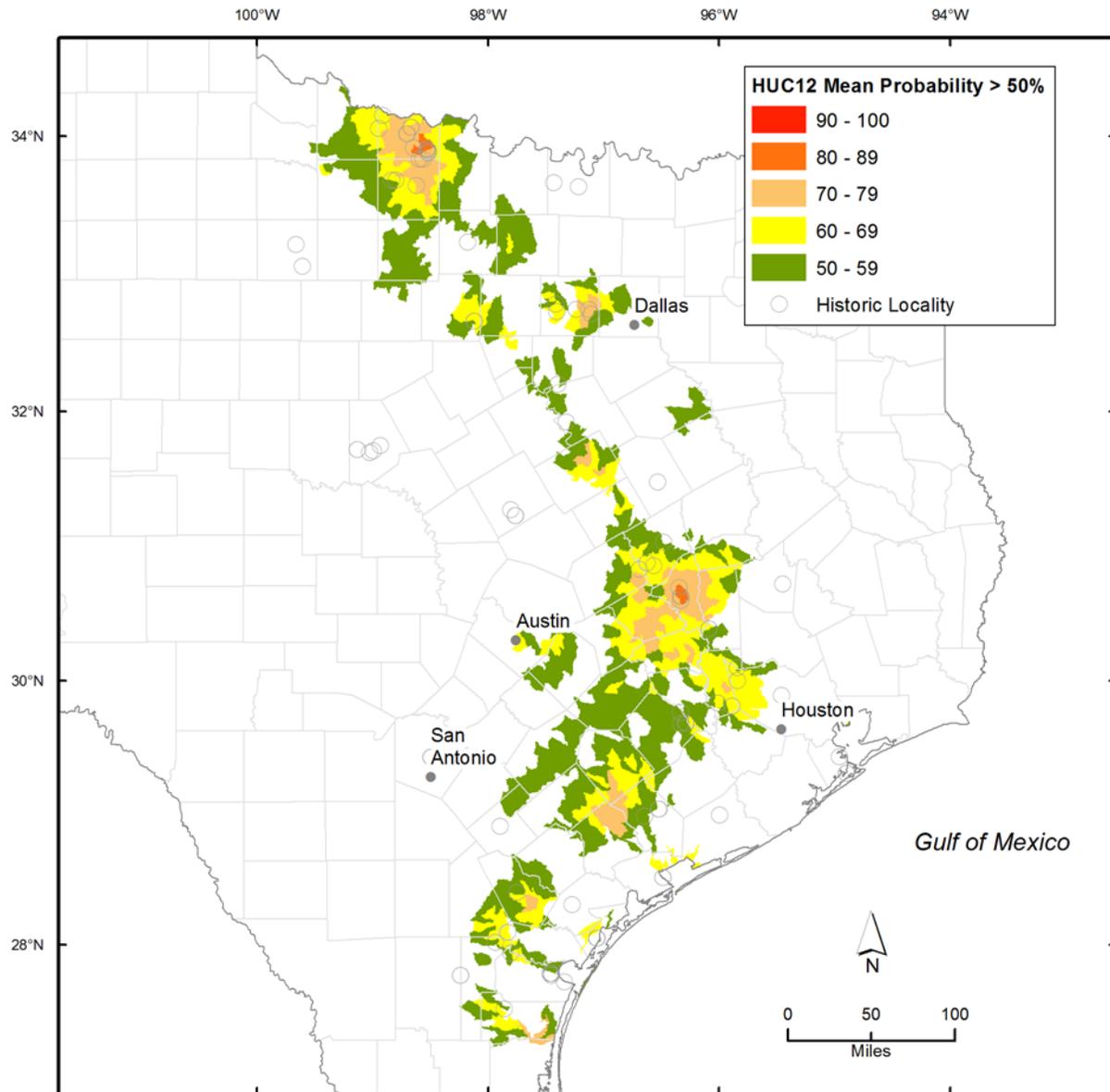
Figure 3. Relative importance of Maxent species distribution model inputs using jackknife test.

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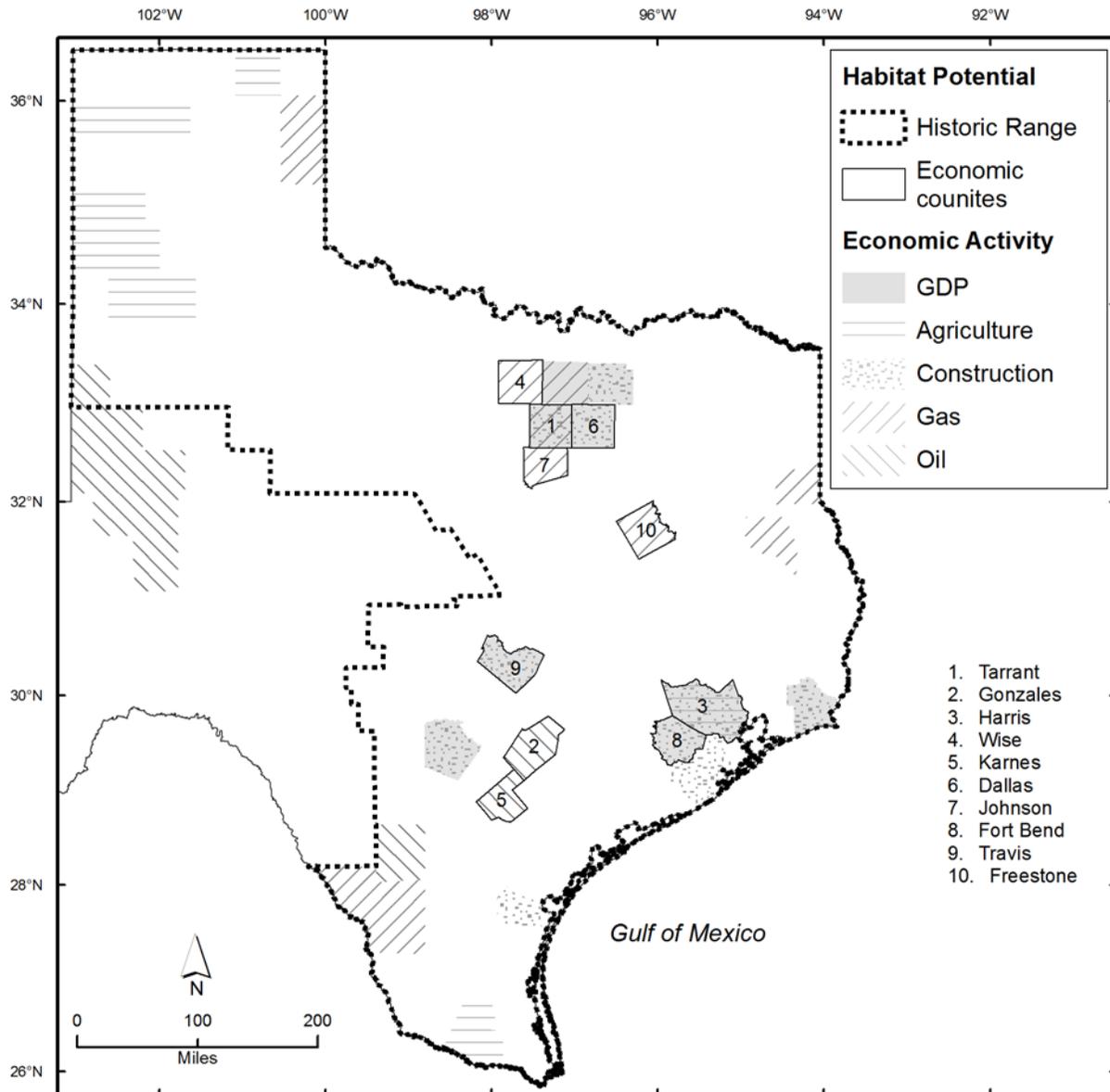
**Figure 4.** Counties with mean modeled probability of *S. putorius interrupta* occurrence >50% and top counties for economic activity (shaded, cross-hatched, and dappled counties; Comptroller, 2015). Refer to **Table 3** for numerical values.

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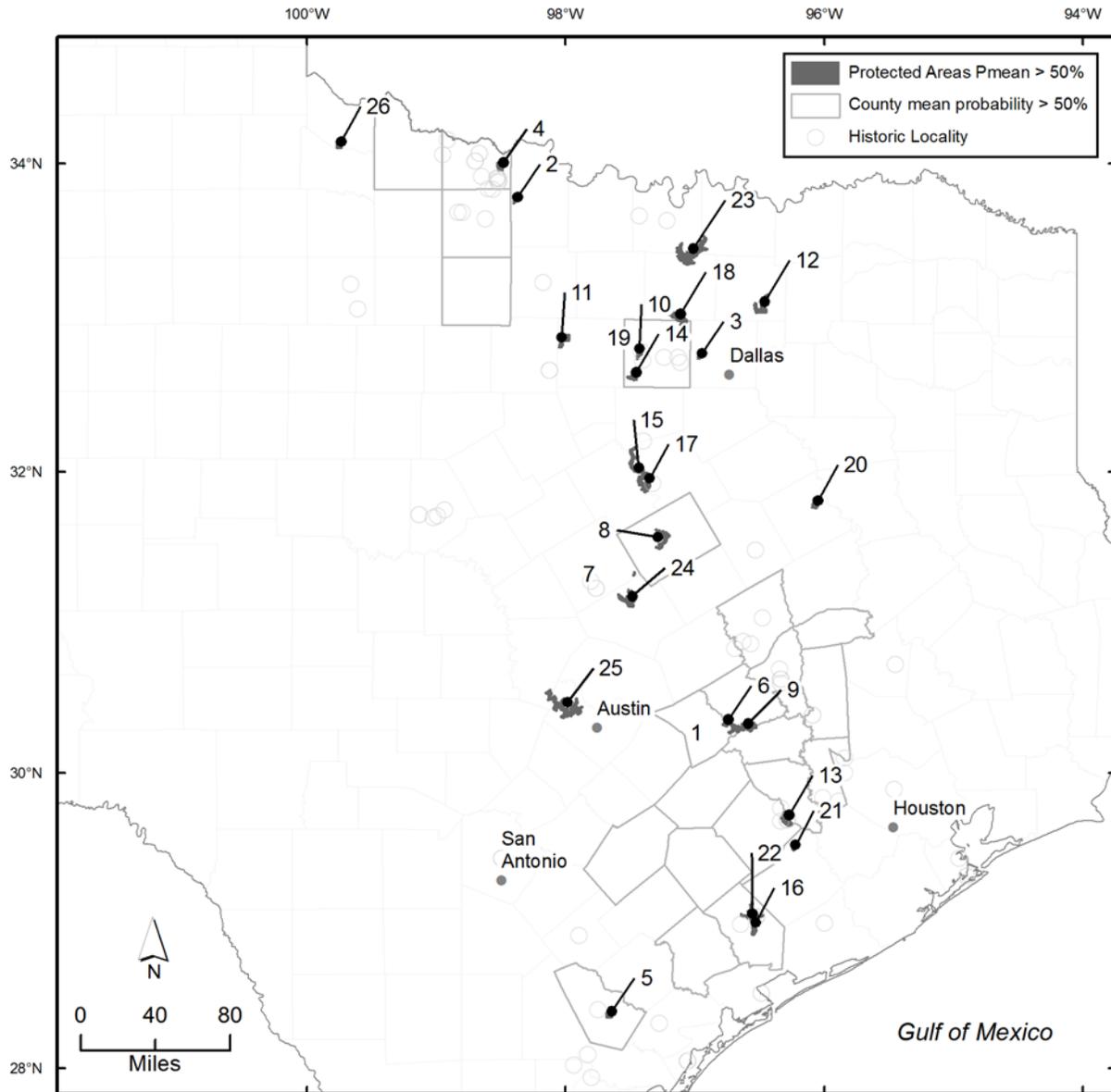
**Figure 5.** Watersheds with mean modeled probability of *S. putorius interrupta* occurrence >50%. Watersheds were classified using U.S. Geologic Survey 12-digit hydrologic unit codes (USGS, 2014b).

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**Figure 6.** Top counties for economic activity (shaded, cross-hatched, and dappled counties; Comptroller, 2015) with highest modeled probability of *S. putorius interrupta* occurrence, numbered in descending order of importance. Refer to **Table 4** for numerical values.

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**Figure 7.** State and federal properties with modeled *S. putorius interrupta* habitat >50% (calculated as zonal average within area of land parcel). Location numbers correspond to the “rank” in Table 5).

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**Figure 8.** Roads (TxDOT, 2015) near *S. putorius interrupta* habitat with probability of occurrence >50%.

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**TABLES—APPENDIX 1**

**Table 1.** Features used as predictor variables for Maxent species distribution modeling, following the approach of Labay et al. (2011).

Category	Description	Maxent Variable	Source
Topological	Altitude*	alt	1
	Aspect*	aspect	
	Slope*	slope	
	Compound topographic index* = (ln (accumulated flow/tan[slope] ))	cti	
Climate	Annual mean temperature	bio_1	2
	Mean diurnal range = (monthly mean (max temp-min temp))	bio_2	
	Isothermality (bio_2/bio_7)(*100)	bio_3	
	Temperature seasonality (sd *100)	bio_4	
	Maximum temp. of warmest month	bio_5	
	Minimum temperature of coldest month	bio_6	
	Temperature annual range (bio_5 – bio_6)	bio_7	
	Mean temperature of wettest quarter	bio_8	
	Mean temperature of driest quarter	bio_9	
	Mean temperature of warmest quarter	bio_10	
	Mean temperature of coldest quarter	bio_11	
	Annual precipitation	bio_12	
	Precipitation of wettest month	bio_13	
	Precipitation of driest month	bio_14	
	Precipitation seasonality (coefficient of variation)	bio_15	
	Precipitation of wettest quarter	bio_16	
Precipitation of driest quarter	bio_17		
Precipitation of warmest quarter	bio_18		
Precipitation of coldest quarter	bio_19		
Soils	Average percent sand in soil (from surface texture)*	surftext	3
	Generalized dominant soil order	dom_soilorders	3
	Area- and depth-weighted average soil	usgs_sand	3
Wetlands	Relative wetland density	wtlnd_dnsty	5

References: <sup>1</sup>30-arc second digital elevation model (USGS, 2014a), <sup>2</sup>WorldClim (2014), <sup>3</sup>SSURGO (USDA, 2014), <sup>4</sup>(Wieczorek, 2014), <sup>5</sup>(Dahl and Griffin, 2015). Note: \* indicates only these variables were used for a second Maxent model run.

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**Table 2.** Heuristic estimate of relative contributions environmental variables to Maxent model.

<b>Variable</b>	<b>Percent Contribution</b>
Mean Temperature of warmest quarter	19.2
Average percent sand in soil	11.3
Mean Temperature of driest quarter	7.6
Annual precipitation	7.4
Generalized soil order	6.5
Precipitation of wettest quarter	5.7
Relative wetland density	5.1
Precipitation of driest quarter	4.8
Maximum temperature of warmest month	4.5
Precipitation of warmest quarter	4.1
Precipitation seasonality	3.7
Mean Temperature of wettest quarter	3.5
Annual mean temperature	2.4
Altitude	2.0
Temperature annual range	2.0
Isothermality	1.9
Mean temperature of coldest quarter	1.6
Aspect	1.2
Precipitation of driest month	1.2
Temperature seasonality	1.1
Mean diurnal range	1.1
USGS sand percent	0.7
Precipitation of coldest quarter	0.5
Slope	0.4
Minimum temperature of coldest month	0.3
Compound topographic index	0.2

Note: All the parameters of Table 1 were used in this model run.

**Table 3.** Counties with mean probability of occurrence >50%, including those with economically important activities<sup>1</sup>.

<b>County</b>	<b>P<sub>mean</sub></b>	<b>Figure Label</b>	<b>Economically Important County<sup>1</sup></b>
Wichita	73	21	
Brazos	73	4	
Burleson	68	5	
Archer	65	1	
Washington	63	20	
Waller	63	19	
Lavaca	59	12	
Victoria	55	18	
Bee	55	3	
Robertson	55	16	
Fayette	54	7	
Tarrant	54	17	Yes
Colorado	53	6	
Grimes	53	9	
Madison	53	14	
Jackson	52	10	
Lee	52	13	
Gonzales	52	8	Yes
Wilbarger	52	22	
McLennan	51	15	
Kleberg	51	11	
Young	50	23	
Austin	50	2	

NOTE: <sup>1</sup>Comptroller (2015)

**Table 4.** Counties with economically important activities<sup>1</sup>.

<b>County</b>	<b>P<sub>mean</sub></b>	<b>Figure Label</b>
Tarrant	54	1
Gonzales	52	2
Harris	44	3
Wise	44	4
Karnes	42	5
Dallas	42	6
Johnson	41	7
Fort Bend	40	8
Travis	40	9
Freestone	39	10

NOTE: <sup>1</sup>Comptroller (2015)

**Table 5.** Ranking of state and federal lands based upon mean probability of occurrence for given county (location numbers on **Figure 5** correspond to “rank” listed here).

Rank	P <sub>mean</sub>	Location
1	93	Lake Somerville SP - Birch Creek
2	89	Lake Arrowhead SP
3	84	Dallas Naval Air Station (Closed)
4	76	Sheppard Air Force Base
5	76	Chase Field Naval Air Station (Closed)
6	73	Lake Somerville WMA
7	72	Mother Neff SP
8	72	Waco Lake
9	71	Somerville Lake
10	68	Carswell Air Force Base (Closed)
11	67	Lake Mineral Wells SP
12	64	Lavon Lake
13	63	Attwater Prairie Chicken National Wildlife Refuge
14	63	Benbrook Lake
15	62	Whitney Lake
16	61	Lake Texana SP
17	61	Lake Whitney SP
18	60	Grapevine Lake
19	59	Fort Wolters
20	57	Fairfield Lake SP
21	57	Wintermann WMA
22	56	Lake Texana
23	55	Lake Ray Roberts
24	51	Belton Lake
25	51	Lake Travis
26	51	Copper Breaks SP

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## APPENDIX 2 – LAND ALTERATION AND FRAGMENTATION OF POTENTIAL CORE

**EXECUTIVE SUMMARY**

In light of possible population declines of the Plains Spotted Skunk (*Spilogale putorius interrupta*) in Texas, we assessed landscape-scale factors modifying potential habitat from 2001 to 2012 for five economically important and five non-economically important counties in the species' Texas range (**Figure 1**). We quantified land alteration and fragmentation of expertly ranked potentially suitable core habitat. We selected these ten counties based on modeled probability of occurrence from the previously reported species distribution model (SDM) and anticipated land access. The five economically important counties are (in descending order of modeled probability of skunk occurrence,  $P_{\text{mean}}$ : 54, 52, 44, 44, and 39, respectively): **Tarrant** (gross domestic product; GDP, construction value, gas production); **Gonzales** (oil production); **Harris** (GDP, agriculture production, construction value); **Wise** (gas production); and **Freestone** (gas production). The five non-economically important counties selected are: **Wichita, Burleson, Waller, Jackson, and Kleberg** ( $P_{\text{mean}}$ : 73, 68, 63, 52, and 51, respectively). We assessed potential habitat alteration to ranked suitable habitat by overlaying land alteration from oil and gas development, urbanization, agricultural expansion, and road development. We found that Harris and Tarrant counties had the highest total landscape alteration (584 and 307 km<sup>2</sup>), which was primarily caused by urbanization and oil and gas development. The greatest loss of suitable habitat occurs in four of the five economically important counties for Harris, Tarrant, Freestone, Gonzales, and Kleberg counties (172, 80, 64, 54, 49, km<sup>2</sup>, respectively), which was caused by urbanization, oil and gas development, and agricultural expansion. The greatest loss of core habitat occurred in Freestone, Gonzales, and Burleson counties (438, 303, 293 km<sup>2</sup>, respectively), and was primarily attributable to perforating effect of oil and gas wells on land cover. We prioritize post-alteration core habitat using relative probability of occurrence from the SDM. These maps can be used to direct surveys to sites with the greatest probability of encountering the species. We found that Waller, Burleson, and Wichita counties had significant remaining core habitat area. While, Tarrant County had little remaining core habitat, because of urbanization and development of the Barnett Shale play. Western Harris County had remaining core habitat, but it was threatened by future urbanization. Integrating this study into the FWS's Species Status Assessment (SSA) framework was not originally proposed. However, future research mapping landscape alteration throughout the species' entire Texas range, forecasting future threats, and evaluating future habitat connectivity would improve an SSA for the species.

## INTRODUCTION

In the May 2015 Species Distribution Model Report, we provided an assessment of predicted probability of occurrence for the entire Texas range of *Spilogale putorius interrupta*. However, assessing landscape alteration for the entire Texas habitat of *S. putorius interrupta* was beyond the scope and resources of this study. Therefore, we used the predicted probability of occurrence of the previously reported SDM to choose ten counties on which to focus our analyses. Landscape-scale alteration processes occurring in these ten counties are representative of those across the entire *S. putorius interrupta* Texas range. Of these ten counties, five have substantial importance to the Texas economy and five are of lesser economic importance.

The native Texas habitats of the Plains Spotted Skunk (*Spilogale putorius interrupta*) have been altered by anthropogenic factors for over 100 years. These may include road construction, agricultural activities, urbanization, and oil and gas development (O&G). Despite range-wide alterations, few surveys have looked for the species in the past 30 years. However, a lack of specimens and sightings suggests a decline similar to that reported for the eastern spotted skunk (Hackett et al., 2007). In light of the lack of current information for the species, we present an assessment of current habitat available to support the species within our ten-county study area.

This assessment comprised three tasks:

**Task 1:** Reviewing of all known historical records of the Plains Spotted Skunk, compiling landscape, vegetation, soils, and climate data within the known range, and modeling probability of predicted occurrence using these data;

**Task 2:** Assessing remaining availability of *S. putorius interrupta* habitat within the newly defined 10-county study area using expertly ranked habitat types; and

**Task 3:** Recommending survey locations in remaining high-potential habitats.

## Distribution Patterns and Population Trends

A range-wide decline of *Spilogale putorius* has been documented by analysis of fur-trapping records that showed sharp decreases in numbers since the 1940s (Gompper and Hackett, 2005). As a result, *Spilogale putorius interrupta* (Plains Spotted Skunk) was named as a Texas priority species (TPWD, 2014a) and the U.S. Fish and Wildlife Service (FWS) is considering listing the species as threatened or endangered (FWS, 2012).

The native range of the species originally included much of the eastern half of Texas, onto the Edwards Plateau, and into North-Central Texas (Schmidly, 2004). The species prefers wooded areas and tall-grass prairie and is reported to have historically been found near farms. They are omnivorous, eating mice, insects, birds, small mammals, and fruit.

Possible causes for the observed decline include shifts to large-scale farming, introduction of pesticides, overharvesting for the fur trade, and disease. These activities and others resulted in changes in land use throughout its native range which include conversion of native habitat to agriculture (i.e., crops and pasture), urban and exurban areas (i.e., developed areas of low to high density), and roads.

Anthropogenic land alterations may affect *S. putorius interrupta* through fragmentation and loss of habitat. We define “alteration” as the area of a landscape that was converted from native vegetation to agricultural production, oil and gas production, urban areas, and roads. We define

“fragmentation” as a change in size of the “core” landscape class defined by Vogt et al. (2007). Core areas are buffered on the perimeter with ample suitable habitat and therefore are not degraded by edge effects to which many organisms are sensitive (Goodrich et al., 2004; Howell et al., 2006; Svobodová et al., 2010; Robson et al., 2011; McGarigal et al., 2005; Neel et al., 2004). Thus, providing recommendations for survey locations requires an assessment of remaining potential habitat, which is the primary goal of this study.

## METHODS

Mapping of habitat that may potentially support *Spilogale putorius interrupta* was done for a ten-county subset of its native Texas range (Dowler et al., 2008; Schmidly, 2004). We combined a landscape fragmentation model, which incorporated recent alteration of potential habitat with the predicted occurrence probability of a species distribution model (SDM) to establish a defined study area and to direct future surveying efforts.

### Review of Current and Historical Records

As no comprehensive review of all known historical records of the Plains Spotted Skunk had been conducted at the start of this study, we compiled museum specimen and trapper records, and confirmed sightings to establish past distribution in Texas. We started with historic localities of Dowler et al. (2008), which we augmented with additional museum records compiled recently by Dowler. We georeferenced some localities by converting text descriptions of a location to an approximate latitude-longitude coordinate nearby. Our protocol to georeference localities was the same as those of other similar projects such as HerpNet (<http://herpnet2.org/>) and MaNIS (<http://manisnet.org/>). All locations receive coordinates with an associated error radius calculated using an online calculator (<http://manisnet.org/gci2.html>).

### Modeling of Potentially Suitable Habitat with Maxent

Probability of occurrence of *S. putorius interrupta* within its Texas range was modeled using the Maxent species distribution model (Version 3.3.3; Phillips et al., 2006; Phillips and Dudík, 2008) following the generalized approach of Labay et al. (2011). We used the augmented skunk localities (mentioned above) of Dowler et al. (2008) and a suite of physiographic predictor variables (**Table 1**). A detailed methodology for the species distribution modelling is outlined in the Supplemental Information section.

### Selection of Ten-County Study Area

We select a ten-county subset of *S. putorius interrupta*'s range for our analyses using the highest county-level  $P_{\text{mean}}$  values from the SDM. The economic importance of each county was determined by the Texas Comptroller of Public Accounts (Comptroller, 2015). We further refine county selection based upon knowledge of anticipated permission to access survey sites and expert gestalt.

### Quantifying Land Disturbance and Remaining Core Potential Habitat

A substantial body of O&G infrastructure information exists for Texas, some of which is available from the Railroad Commission of Texas (RRC), the agency that regulates O&G operations, or commercial entities like Information Handling Service (IHS). Furthermore, datasets of land classification (topography, soil, vegetation, etc.) also exist from Federal (e.g., USDA National Agriculture Imagery Program (NAIP)), and State programs (e.g., Texas Parks and Wildlife

Ecological Mapping System (EMS; (TPWD, 2014b)). These data were organized into an integrated dataset that were used for assessing landscape status and planning field surveys. The EMS polygon data was converted to a 10m resolution raster format and used as a template for the preparation of all other spatial data to ensure all datasets had matching cell alignment and spatial extents.

### **Landscape Alteration from O&G Activities**

We mapped landscape alteration in the study area. We used a database containing O&G wells in the study area permitted between March 2001 and December 2012 from IHS (2015). Oil and gas transmission pipeline data was acquired from RRC (2014). We overlaid well and pipeline locations onto 2012 National Agriculture Imagery Program (NAIP) aerial photography for the ten-county study area. Using unsupervised image classification in ArcGIS 10.2, we specified ten classes to produce a 10m resolution thematic landscape map for the study area. The class which best captured areas where areas of bare ground existed in the imagery were used to create polygons representing alteration of the landscape within the study time period. We spatially joined wells that fell within 90 m of these polygons to extract the alteration that resulted from the development of drilling pad infrastructure. We identified outlier polygons in this joined layer using a spatial area standard deviation of three or more from the mean. We then visually inspected these outliers and either accepted or rejected these polygons from the drilling pad dataset. Additionally, any joined polygons that were less than 300 m<sup>2</sup> (1 or 2 pixel polygons; smaller than a typical drilling pad) were removed from the drilling pad dataset. Lastly, pixels representing land alteration were extracted from within a 30-m buffer of pipelines in the study area. The pad and pipeline alterations were then combined to represent total landscape alteration from O&G activities within the ten-county study area for the study time period.

### **Landscape Alteration from Urbanization and Agricultural Expansion**

We used the 30-m resolution National Land Cover Database (NLCD; Jin et al., 2013; USGS, 2014a) to assess recent urbanization and agricultural expansion in the 10-county study area. We resampled these datasets to 10-m resolution to match the O&G landscape alteration and the EMS. We used, in part, the approaches of Jantz et al. (2005) to identify where land cover changed between 2001 and 2011.

### **Landscape Alteration from Road Development**

We quantified recent road development by extracting the polygon datasets for roads, medians, and right-of-ways (ROWs) from an EMS dataset modified by the Texas Department of Transportation (TxDOT, 2014). TXDOT “burned” in the roads, medians, and ROWs into the EMS dataset because roadway infrastructure may not be adequately represented in the native EMS version. The extracted polygons were converted to a 10-m resolution raster format. It is important to note that datasets containing new road development for the time frame of the study were not available in a spatial format. For this reason, we used all roads, medians, and ROWs extracted from the TXDOT dataset to correct/adjust the native EMS. Therefore, any landscape alterations from road development presented in this study are from all TXDOT roads, medians, and ROWs and are not constrained to the twelve-year time frame as are the other landscape alteration regimes.

### **Isolation of Alteration Datasets**

We reconciled the alteration dataset so that we did not double-count for alteration factors. Due to

differing resolutions and methodologies for the creation of datasets from which we derived the alteration datasets for this study, we analyzed the alteration regimes for spatial overlaps. When any overlap occurred with road development (roads, medians, and ROWs), we assigned the alteration to road development because the road dataset was derived from infrastructure precisely located by TxDOT using survey-grade GPS. When overlaps occurred between the 30-m resolution NLCD urban and agricultural expansion datasets with the 12-year footprint of O&G alteration, we assigned the alteration to O&G because the O&G expansion was derived from the image classification of high resolution (1-m) NAIP imagery. Finally, no overlaps occurred between urban and agricultural expansion because they were derived from the same parent dataset.

### **Assessment of Habitat Fragmentation from Alteration Regimes**

Plains Spotted Skunk experts, Robert Dowler and Jerry Dragoo, ranked vegetation types of the EMS in the study area as to the suitability of habitat. The vegetation types were ranked from 0–5, with 5 being the most suited habitat and 0 being the least suited habitat for use by *S. putorius interrupta*. The ranked vegetation was then reclassified into a dichotomous layer with ranks 0, 1, and 2 considered to be unsuitable skunk habitat and 3, 4, and 5 considered to be suitable skunk habitat. A pre-alteration baseline fragmentation analysis was then conducted using morphological spatial pattern analysis (MSPA) in the GUIDOS toolbox (Soille and Vogt, 2009) which is based on methods established by Vogt et al. (2007). To assess the impact of each alteration type on skunk habitat, we intersected each alteration regime with the ranked dichotomous EMS layer and reclassified intersecting pixels of suitable habitat and landscape alterations to unsuitable habitat. Each resultant layer was reprocessed using MSPA in the GUIDOS toolbox. Lastly in order to assess an “all-inclusive” landscape alteration on skunk habitat, a combined alteration layer was created, used to reclassify the ranked dichotomous layer, and reprocessed with MSPA.

### **Directed surveys within Core Potential Habitat**

In order to optimize surveying efforts in the 10-county study area, we converted the available remaining core habitat pixels from the all-inclusive MSPA analysis to polygons and calculated the  $P_{\text{mean}}$  values from the SDM within each remaining core polygon. Skunk surveyors will be able to prioritize surveying efforts based on the size of remaining core areas and the associated  $P_{\text{mean}}$  values from the SDM results.

## **RESULTS**

### **Modeling of Potentially Suitable Habitat with Maxent**

We compiled 64 *Spilogale putorius interrupta* specimens, which we used as inputs for the SDM to map potential habitat. While the model results do not represent actual current skunk localities, they do indicate relative probability of occurrence of suitable habitat. We considered modeled probability of occurrence >50% to be potentially suitable habitat for the skunk. We suggest that areas with probabilities above this arbitrary threshold are prime suitable habitat. We found that modeled suitable habitat occurs across a large swath of Texas from ~200 miles south of San Antonio, along a zone ~100 miles east of Interstate Highway 35 to Dallas, and includes a ~100-mile wide band from northwest of Dallas to the Oklahoma border (**Figure 2**).

### **Total Land Alteration in 10-County Study Area**

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Total land alteration in the 10-county study area is ~1,322 km<sup>2</sup> (**Table 2**). It consists of ~520 km<sup>2</sup> of roadway development, ~500 km<sup>2</sup> of urban expansion, ~240 km<sup>2</sup> of O&G expansion, and ~70 km<sup>2</sup> of agricultural expansion (**Figure 3**). Harris County dominated the 10 counties in total land area alteration with ~585 km<sup>2</sup> of land conversion — primarily from urbanization and road construction (**Figure 5**). Tarrant County had the second highest amount of land conversion at ~310 km<sup>2</sup>. The remaining eight counties had total alterations of less than ~85 km<sup>2</sup> per county. When we examined where alterations occurred with respect to habitat, similar patterns emerged (**Table 3**). Harris County led with ~170 km<sup>2</sup> of suitable habitat loss. Tarrant had the second highest suitable habitat loss at ~80 km<sup>2</sup>. Wichita County had the lowest suitable habitat loss at ~15 km<sup>2</sup>.

We also assessed where alterations occurred in relation to core habitat. We found that Freestone County, where the dominant alteration regime is O&G expansion, had the highest amount of core habitat loss at ~440 km<sup>2</sup>. Gonzales, Burleson, and Jackson counties were also dominated by O&G alteration (~300 km<sup>2</sup>, ~290 km<sup>2</sup>, and ~145 km<sup>2</sup>, respectively). Kleberg County, whose dominant alteration source is agricultural expansion, had the fifth highest core loss nearly equal to Jackson County at ~145 km<sup>2</sup>. Interestingly, we found that alterations in Harris and Tarrant Counties, where land conversion is dominated by urban expansion and associated road building, suitable habitat losses were occurring on the edges of habitat and consequently these two counties had relatively low core habitat losses (**Table 4, Figure 6**).

We introduce the metric of suitable habitat to core habitat loss ratio, which we will refer to herein as the “S/C ratio”. When alterations occur in the middle of suitable habitats, MSPA immediately applies a habitat “edge” in the new landscape that did not exist previously. This results in a higher “core” habitat loss due to the newly assigned edge. Conversely, when alterations occur on the perimeters of suitable habitat a lower core habitat loss is the result. A low S/C ratio indicates that suitable habitat is being removed in the middle of core areas; whereas a high S/C ratio indicates that suitable habitat is being lost on the edges of core habitats or in areas of suitable habitat not large enough to be considered core and already degraded by edge effects. Harris and Tarrant Counties both had S/C ratios larger than one (**Table 4**), which indicates that more than one suitable habitat pixel is typically needed to be lost to remove one core habitat pixel. Kleberg County, where agriculture and O&G expansion dominate land conversion, had the lowest S/C ratio. Generally, counties with higher amounts of land conversion from O&G and agricultural expansion had lower S/C ratios.

#### **Land Alteration from O&G Expansion**

Oil and gas expansion had the highest impacts in Freestone and Gonzales Counties with suitable habitat losses at ~35 km<sup>2</sup> and ~20 km<sup>2</sup> and core habitat losses at ~375 km<sup>2</sup> and ~200 km<sup>2</sup>, respectively. The S/C ratio was overwhelmingly lower with O&G alteration (**Table 4**).

#### **Land alteration from Urban Expansion**

Urban expansion had the highest impacts in Harris and Tarrant Counties with ~145 km<sup>2</sup> and ~60 km<sup>2</sup> of suitable habitat loss and ~70 km<sup>2</sup> and ~30 km<sup>2</sup>. Surprisingly, urban expansion in Freestone County which amounted to ~10 km<sup>2</sup> of suitable habitat loss resulted in >70 km<sup>2</sup> of core habitat loss. This indicates that new urban development is occurring inside of suitable skunk habitat (**Table 3, Table 4**).

#### **Land alteration from agricultural expansion**

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Kleberg County experienced the largest loss of suitable habitat from agricultural expansion at ~35 km<sup>2</sup> of suitable habitat loss, which resulted in ~60 km<sup>2</sup> of core habitat loss. Gonzales County had the second highest amount of suitable habitat loss from agricultural expansion with ~15 km<sup>2</sup> of suitable habitat loss and ~40 km<sup>2</sup> of core habitat loss. Gonzales and Burleson counties had the lowest S/C ratios from agricultural expansion indicating the new agriculture is occurring inside of potential skunk habitat. Considering, *S. putorius interrupta*'s proclivity for occupying farmland, this land conversion regime may serve as a benefit to *S. putorius interrupta*.

### **Land Alteration from Roadway Development**

As previously stated, the roadway alteration layer was used to adjust and correct the EMS dataset because the EMS data do not accurately capture road networks. As development in the EMS spatial dataset progressed, roadway networks were eventually “burned” into the EMS dataset during the last few phases of development (7 phases total, last 3 had roads burned in; per comm. with Amie Truer-Kuehn, June 2015). Therefore, we refrain from discussing the effects roadway networks on skunk habitat. However, we have reported all values in **Tables 2, 3, and 4**. We caution the reader from drawing conclusions about the effects of roadways on skunk habitat from these data.

### **Directed Surveys within Core Potential Habitat**

We provide a study-wide map that displays the remaining core areas ranked by underlying SDM values (**Figure 8**) to demonstrate our surveying methodology. However, Plains Spotted Skunk surveyors will likely find the county level maps provided in the supplemental section to be most useful.

## **DISCUSSION**

We assessed habitat that may potentially support *Spilogale putorius interrupta* in Texas. These results suggest that the species may prefer parts of Texas with a certain combination of summer temperature, precipitation, and sand in soil. The species does not appear to have an affinity for stream drainages or sloped terrain. Based on geography, the habitat analysis indicates the possibility of two genetically isolated groups: one east of the Balcones Escarpment and the second in North-Central Texas along the Oklahoma border. The results of genetic analyses to be conducted should reveal if there are distinct populations, if sufficient samples are obtained.

We used this SDM habitat assessment to identify a ten-county study area including five economically important and five non-economically important counties. With a defined study area, we assessed the individual and cumulative habitat impacts from four different alteration regimes on expertly ranked skunk habitat. We combined the results from a species distribution and landscape fragmentation model to direct and optimize upcoming surveying efforts.

### **Assumptions and Limitations of Approach**

As with any model, it is only as good as its inputs. Modeled probability of occurrence reveals a potential bias towards highly-sampled areas near urban areas. We partially accounted for this bias by including all skunk samples into the pseudo-absence dataset (Elith et al., 2011). Our habitat modeling may also have a temporal bias resulting from historic sampling in habitats where the species may no longer be found. Thus, the species' current actual distribution may not correspond with our habitat maps, which show where the species may potentially be found.

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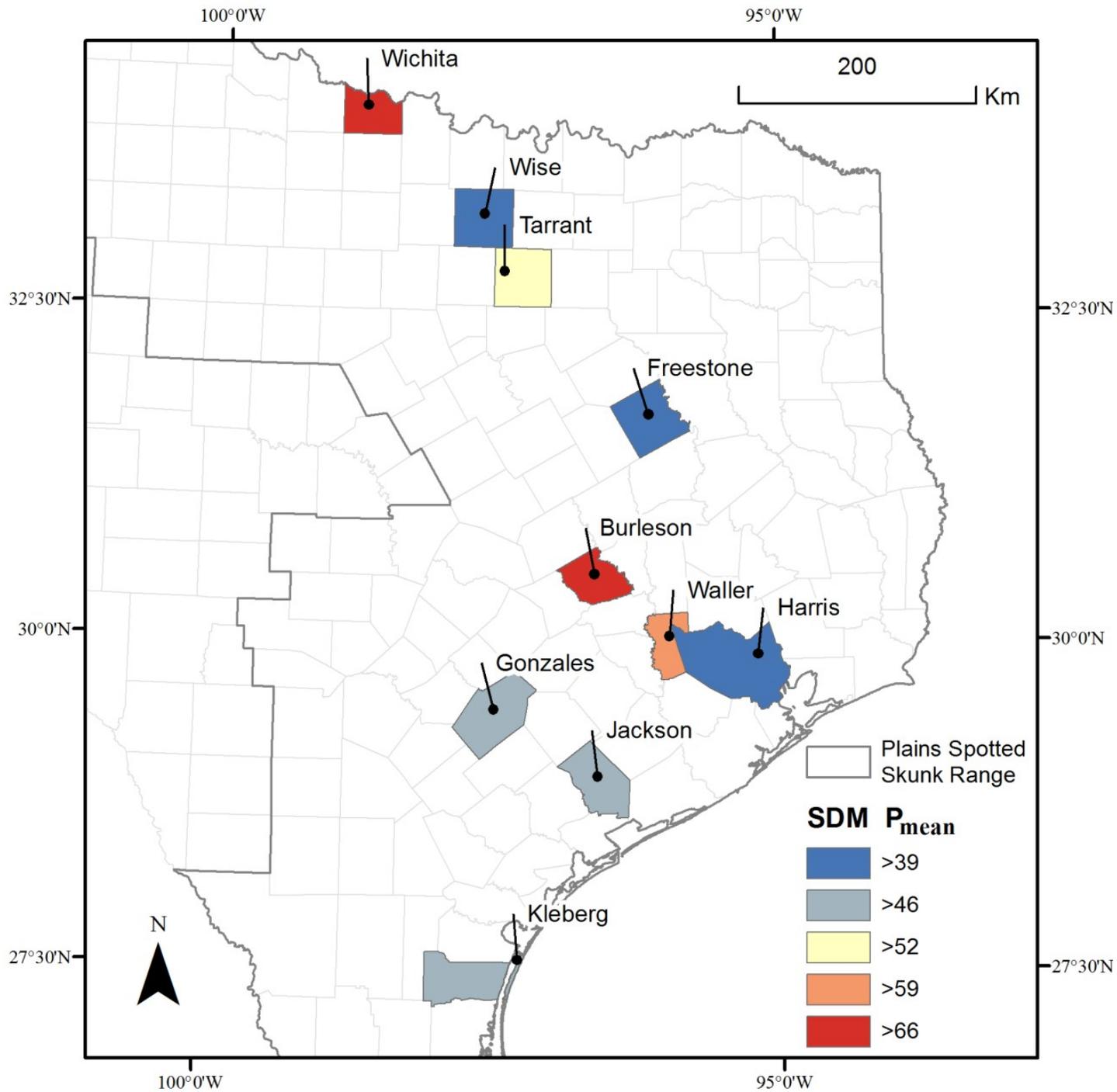
Additionally, we conducted our landscape fragmentation with datasets that were derived from parent datasets of differing resolutions. We were able to closely match the periods of the landscape alteration datasets with the exception of roadway networks. The roadway dataset used in this analysis includes all roads, medians, and ROWs built in Texas throughout time (at least in current TXDOT records). We were not able to constrain this dataset to the twelve-year timeframe of the study. However, our methodology provides the best scientific analysis possible as the roadway layer was created at a precise high resolution and we were able to use these data to adjust and correct the other datasets that were created at a lower native resolution.

**CONCLUSIONS**

The most important study results are:

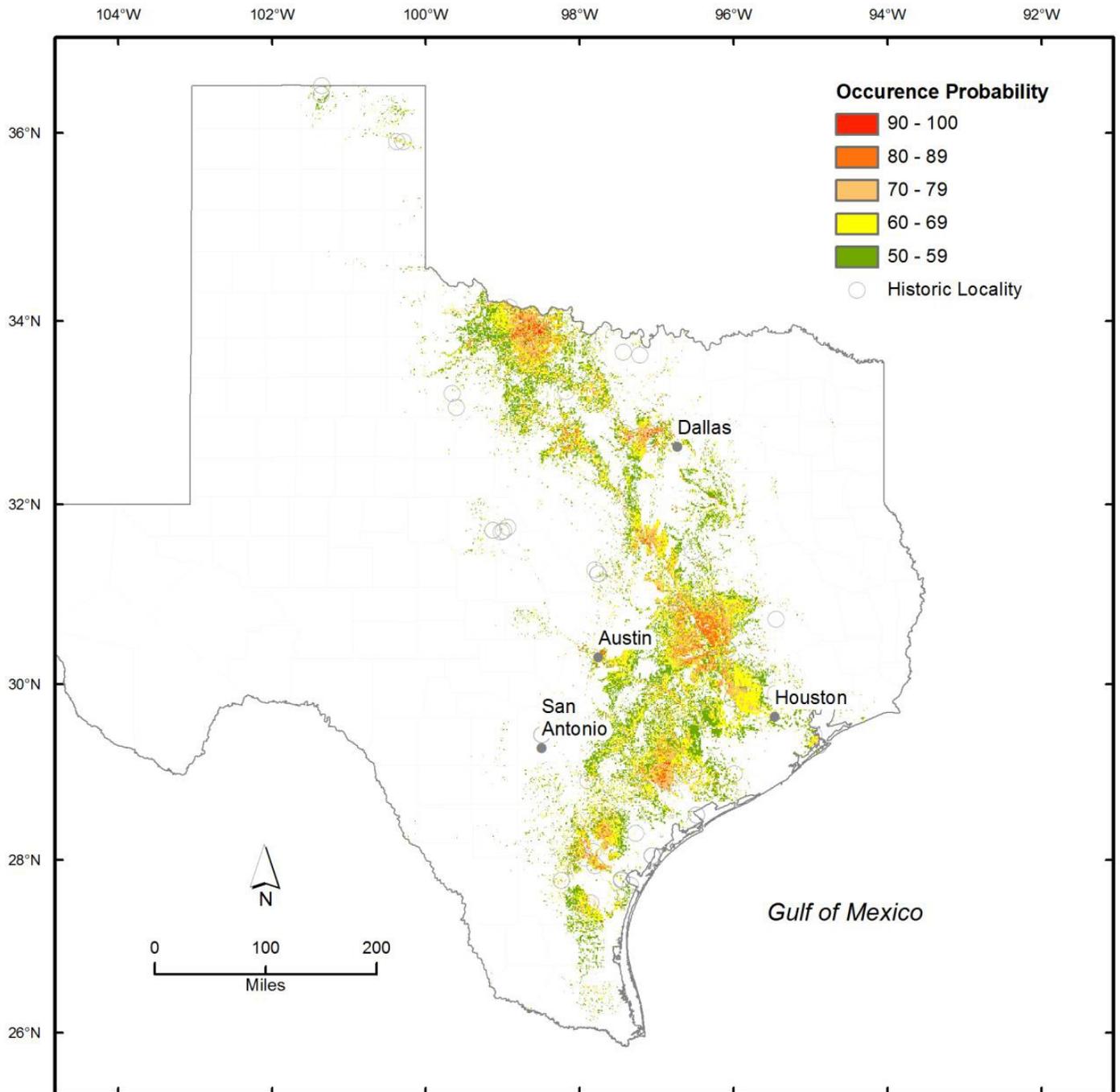
- We assessed alteration to suitable habitat from oil and gas development, urbanization, agricultural expansion, and road development and found Harris and Tarrant were the most altered counties (584 and 307 km<sup>2</sup>), mostly attributable to urbanization and gas development.
- Harris, Tarrant, Freestone, Gonzales, and Kleberg counties lost the most suitable habitat (172, 80, 64, 54, 49, km<sup>2</sup>, respectively), primarily from urbanization, oil and gas development, and agricultural expansion.
- Freestone, Gonzales, and Burleson counties had the greatest loss of core habitat (438, 303, 293 km<sup>2</sup>, respectively), primarily from the perforating effect of oil and gas wells and agriculture, which tend to occur inside habitat; however, Waller, Burleson, and Wichita counties still have significant remaining core habitat area.
- Western Harris County has remaining core habitat, but it is threatened by future urbanization, which (in addition to road construction) can cause progressive loss on the edges of the species' habitat.
- While integrating this study into the FWS's Species Status Assessment (SSA) framework was not originally part of the scope of work, future research mapping landscape alteration throughout the species' entire Texas range, forecasting future threats, and evaluating connectivity of future habitat could be integrated into a future SSA.

**FIGURES—APPENDIX 2**



**Figure 1.** Map of 10-county study area with county  $P_{mean}$  values from SDM. Tarrant, Gonzales, Harris, Wise, and Freestone counties are economically important (Comptroller, 2015).

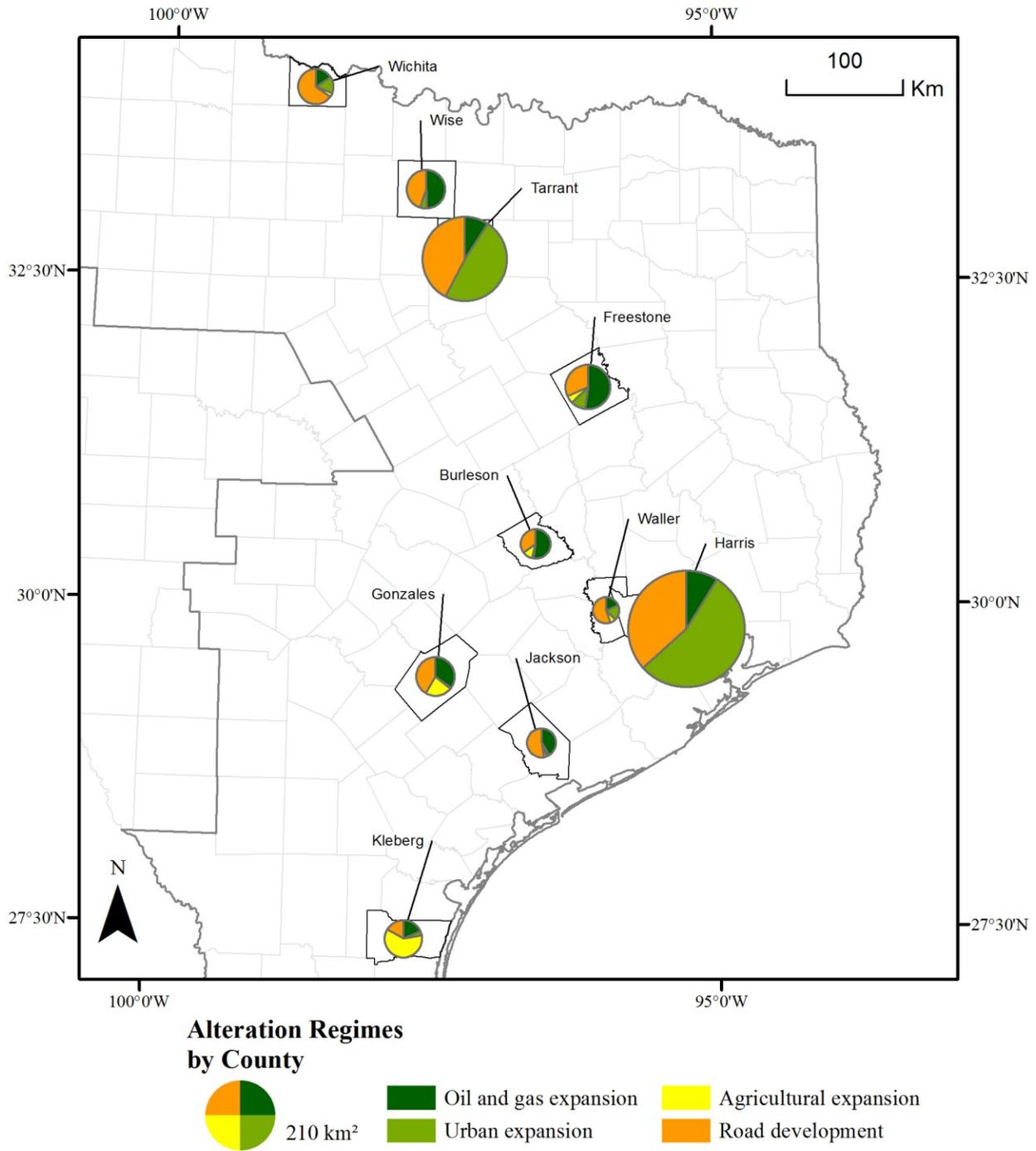
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**Figure 2.** Modeled probability of occurrence of *S. putorius interrupta* (occurrence records are hollow circles; Dowler et al., 2008).

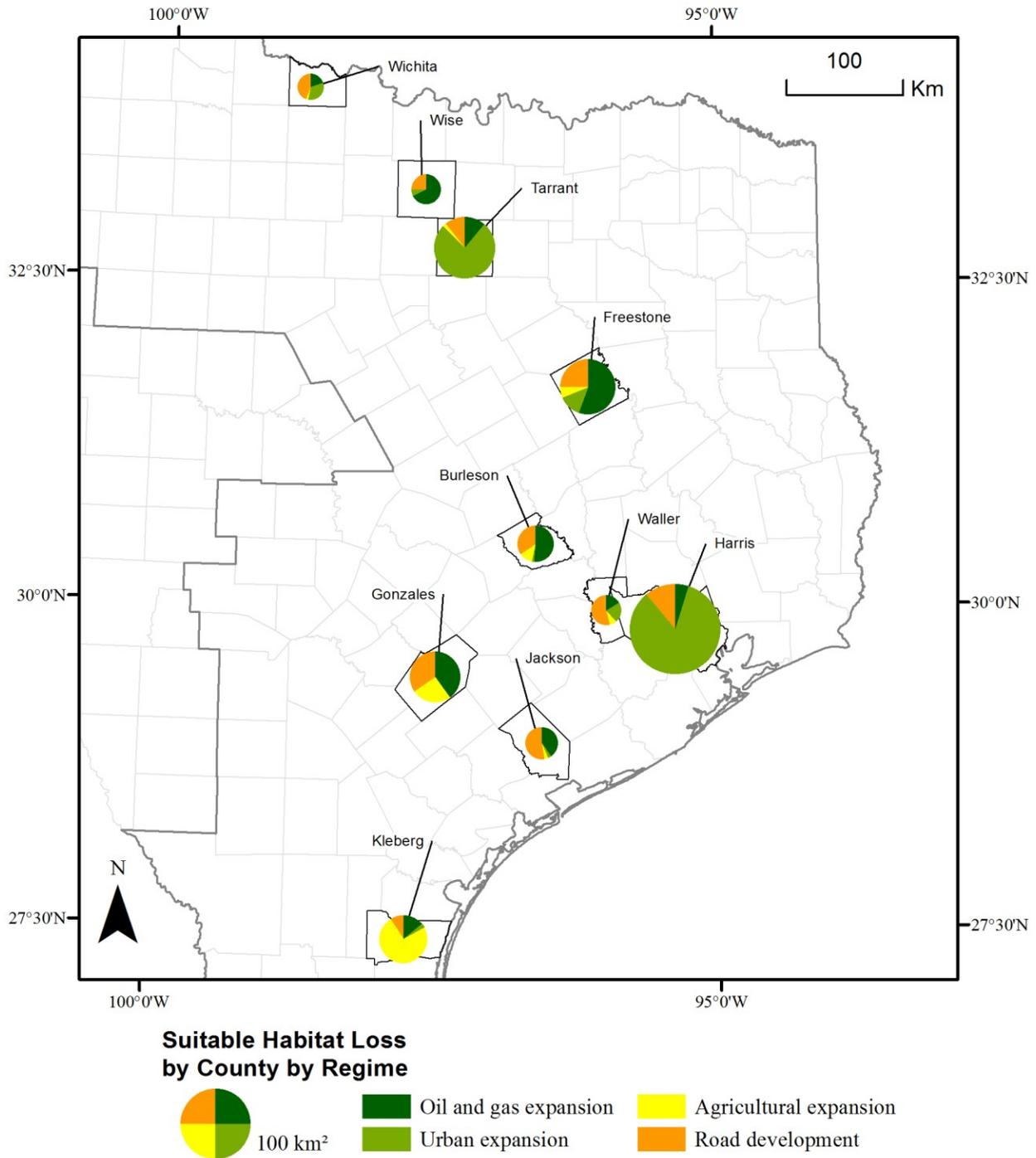
Model results do not represent actual current skunk localities, but relative potentially suitable habitat—based on model inputs—that is likely to support the target species.

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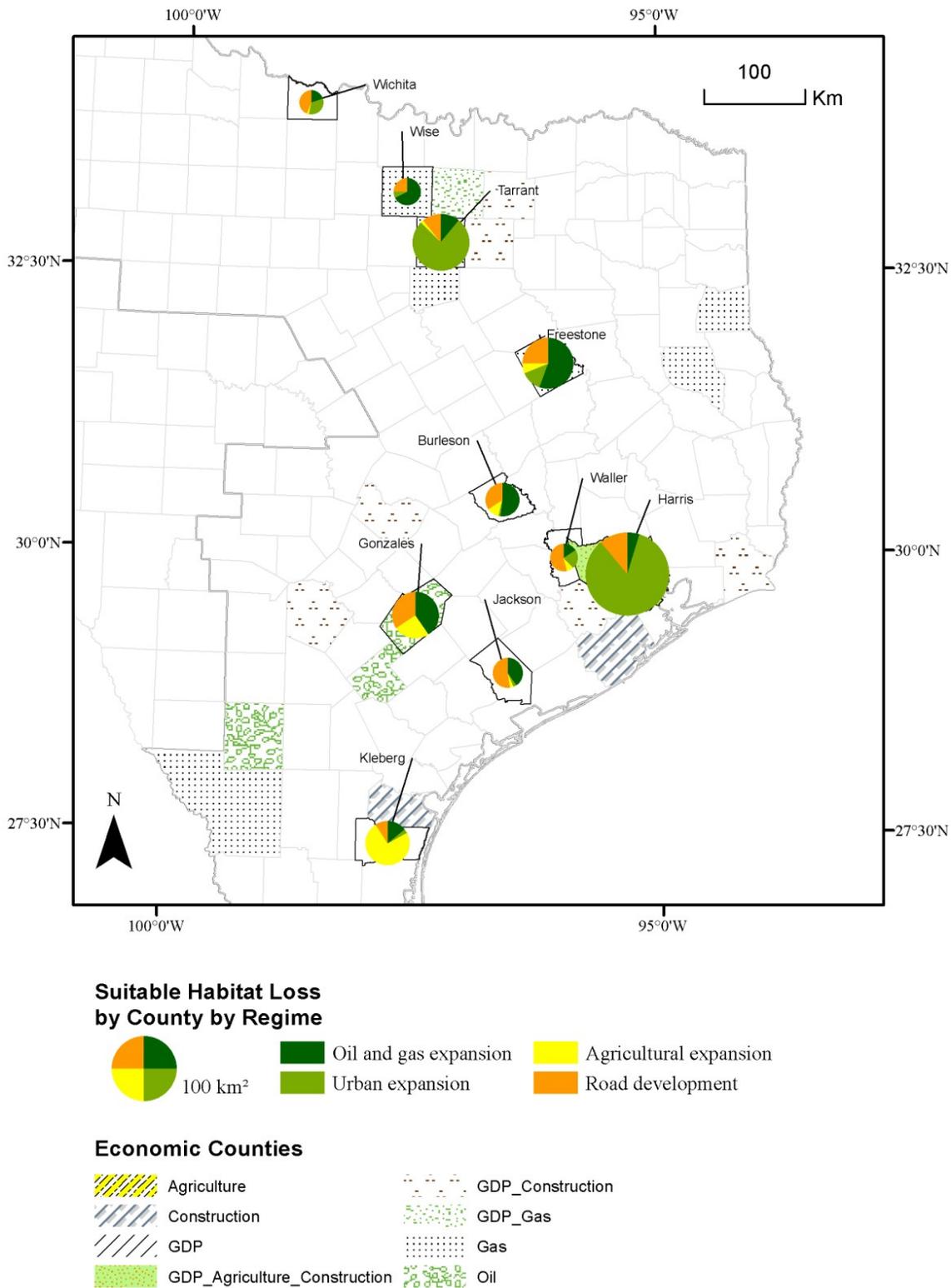
**Figure 3.** County breakdown of alteration regimes (i.e., total alteration within a given county). Pie chart size is proportional to total alteration amounts within the county.

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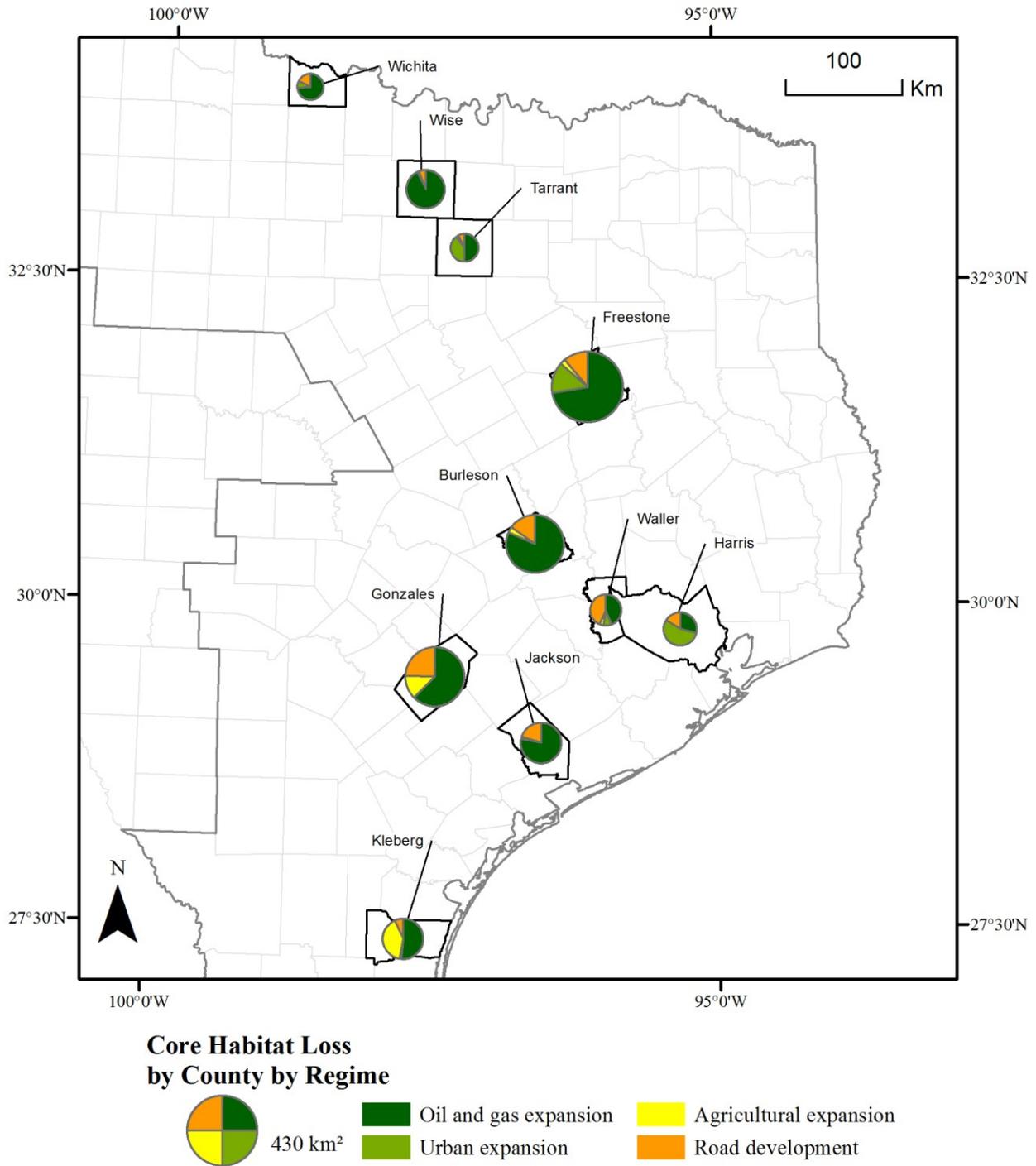
**Figure 4.** Suitable habitat loss by county by alteration regime.

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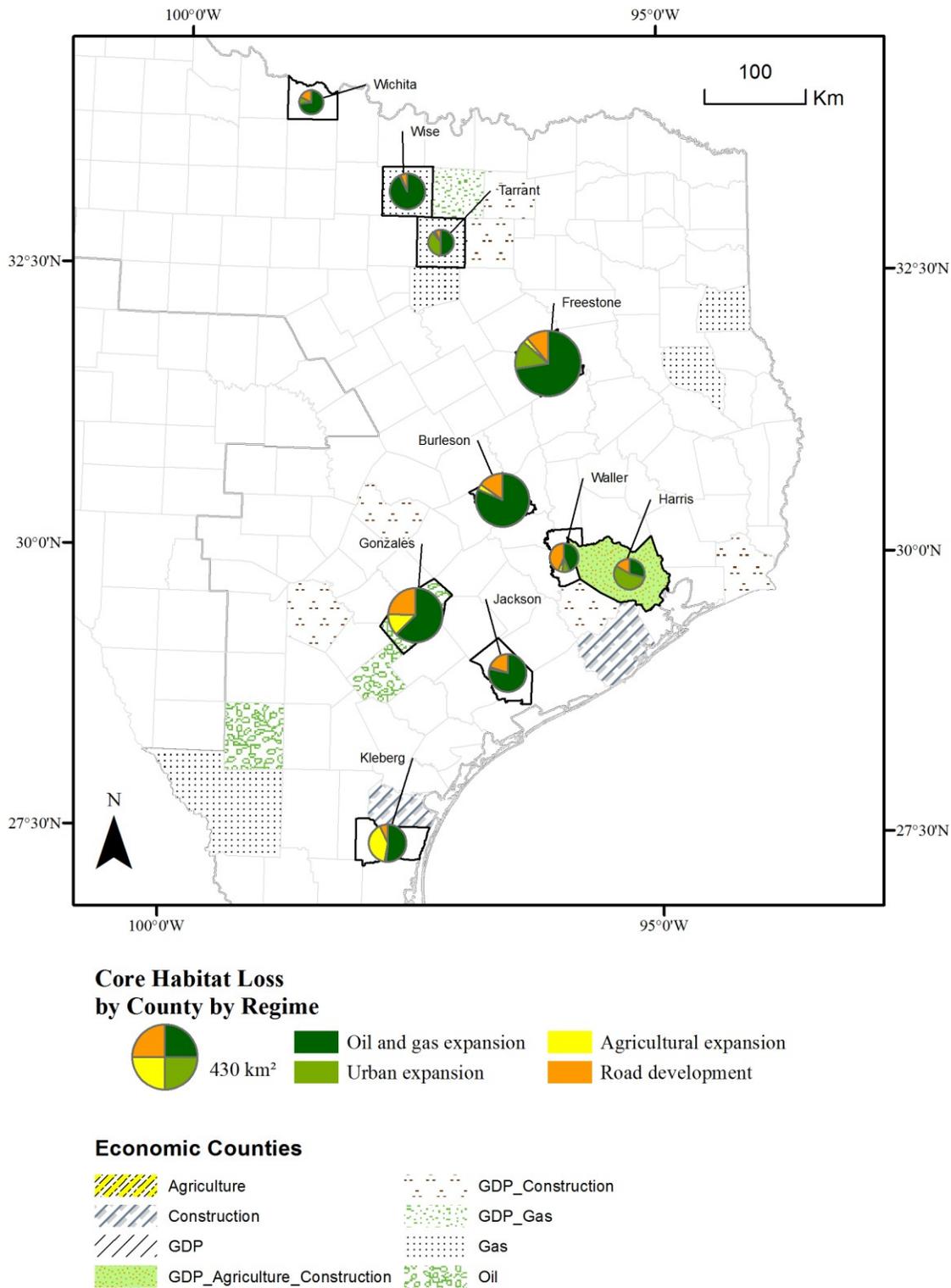
**Figure 5.** Suitable habitat loss by county by alteration regime overlaid onto important economic counties.

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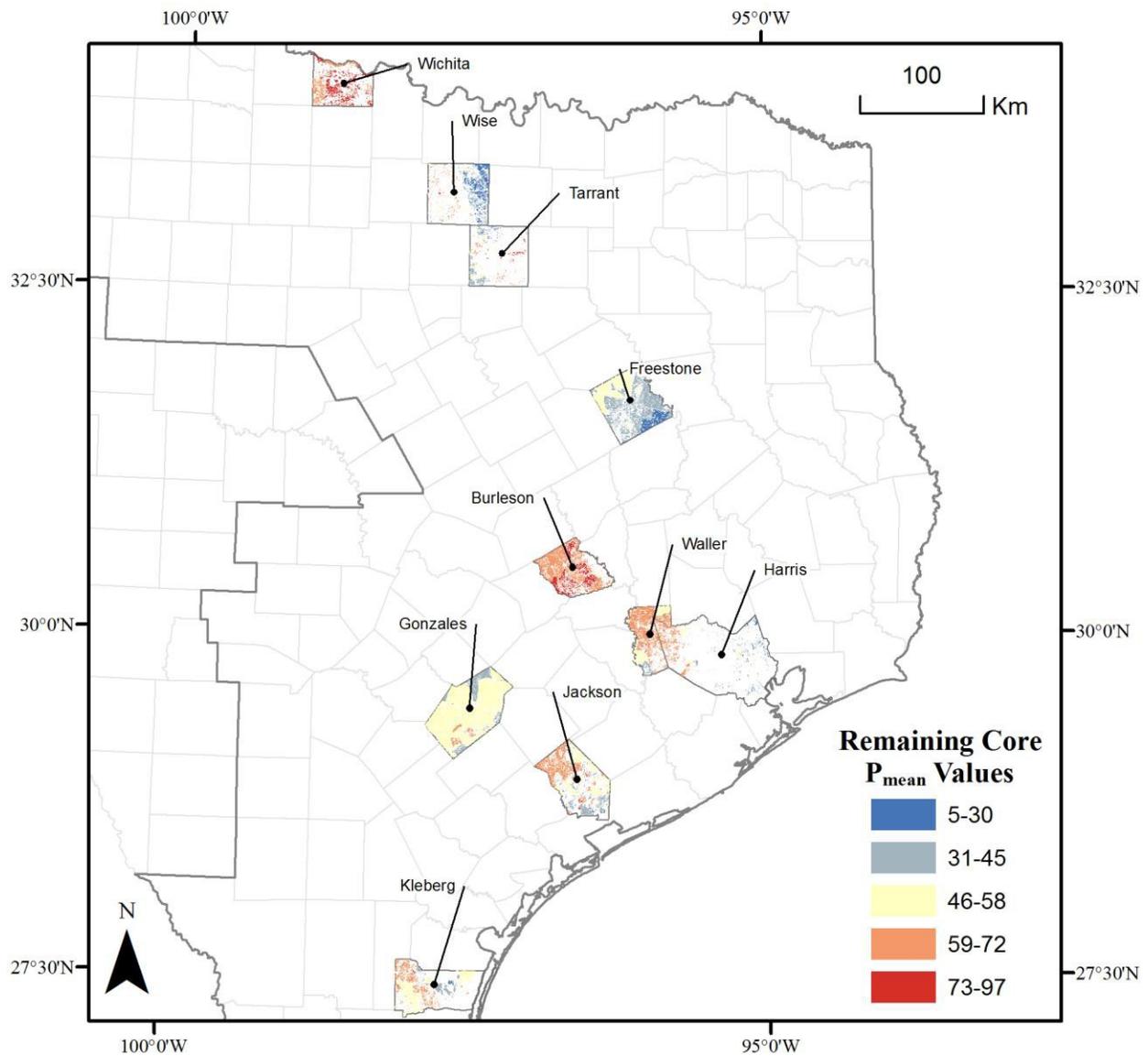
**Figure 6.** County core habitat loss by regime.

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**Figure 7.** Core habitat loss by county by alteration regime overlaid onto important economic counties.

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**Figure 8.** Core remaining after all landscape alteration, ranked by SDM  $P_{mean}$  values. Biologist will prioritize efforts by directing surveyors to large remaining core areas with high  $P_{mean}$  values.

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**TABLES—APPENDIX 2**

**Table 1.** Features used as predictor variables for Maxent Species Distribution Modeling, following the approach of Labay et al. (2011).

Category	Description	Maxent Variable	Source
Topological	Altitude*	alt	1
	Aspect*	aspect	
	Slope*	slope	
	Compound topographic index* = (ln (accumulated flow/tan[slope] ))	cti	
Climate	Annual mean temperature	bio_1	2
	Mean diurnal range = (monthly mean (max temp-min temp))	bio_2	
	Isothermality (bio_2/bio_7)(*100)	bio_3	
	Temperature seasonality (sd *100)	bio_4	
	Maximum temp. of warmest month	bio_5	
	Minimum temperature of coldest month	bio_6	
	Temperature annual range (bio_5 – bio_6)	bio_7	
	Mean temperature of wettest quarter	bio_8	
	Mean temperature of driest quarter	bio_9	
	Mean temperature of warmest quarter	bio_10	
	Mean temperature of coldest quarter	bio_11	
	Annual precipitation	bio_12	
	Precipitation of wettest month	bio_13	
	Precipitation of driest month	bio_14	
	Precipitation seasonality (coefficient of variation)	bio_15	
	Precipitation of wettest quarter	bio_16	
Precipitation of driest quarter	bio_17		
Precipitation of warmest quarter	bio_18		
Precipitation of coldest quarter	bio_19		
Soils	Average percent sand in soil (from surface texture) *	wct_surftext	3
	Generalized dominant soil order	dom_soilorder	3
	Area- and depth-weighted average soil	usgs_sand	3
Wetlands	Relative wetland density	wtlnd_dnsty	5

References: <sup>1</sup>30-arc second digital elevation model (USGS, 2014b), <sup>2</sup>WorldClim (2014), <sup>3</sup>SSURGO (USDA, 2014), <sup>4</sup>(Wieczorek, 2014), <sup>5</sup>(Dahl and Griffin, 2015). Note: \* indicates only these variables were used for a second Maxent model run.

**Table 2.** Total land alteration by county by regime.

COUNTY	Total alteration	O&G alteration	Urban alteration	Agriculture alteration	Road alteration
Wise County	63	30.9	3.8	0.6	28.0
Tarrant County	307	27.5	148.4	1.7	129.2
Freestone County	85	44.3	9.3	4.7	26.8
Harris County	584	51.0	317.5	1.6	213.5
Gonzales County	64	22.3	0.7	14.5	26.5
Wichita County	54	8.3	8.3	2.2	35.2
Burleson County	38	19.9	0.8	4.0	13.2
Waller County	30	5.6	5.9	2.1	16.5
Jackson County	36	14.9	1.1	1.2	19.0
Kleberg County	61	11.3	2.3	37.2	10.3
	1322	235.9	498.0	69.8	518.2

**Table 3.** Suitable habitat loss by county by regime.

COUNTY	Suitable habitat loss total alteration	Suitable habitat loss O&G	Suitable habitat loss Urban	Suitable habitat loss Agriculture	Suitable habitat loss Roads
Wise County	19	12.5	1.4	0.0	4.7
Tarrant County	80	8.6	61.1	1.4	8.5
Freestone County	64	35.6	8.5	4.3	15.8
Harris County	172	8.1	144.1	1.0	18.8
Gonzales County	54	21.5	0.5	13.5	18.4
Wichita County	15	2.9	4.9	0.4	6.5
Burleson County	28	14.5	0.5	3.3	9.7
Waller County	19	3.0	4.2	1.5	10.2
Jackson County	22	9.0	0.8	0.9	11.8
Kleberg County	49	6.9	1.4	36.2	4.4
	521	122.7	227.4	62.6	108.7

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**Table 4.** Core losses and suitable to core loss ratios by county by regime.

COUNTY	Core loss total alteration	Total alteration S/C ratio	Core loss O&G	O&G S/C ratio	Core loss Urban	Urban S/C ratio	Core loss Agriculture	Agriculture S/C ratio	Core loss Roads	Roads S/C ratio
Wise	129	0.1	123.1	0.1	1.5	0.9	0.0	5.7	8.1	0.6
Tarrant	67	1.2	39.3	0.2	30.6	2.0	2.1	0.7	6.2	1.4
Freestone	438	0.1	375.0	0.1	73.4	0.1	13.0	0.3	57.8	0.3
Harris	96	1.8	31.4	0.3	59.8	2.4	0.9	1.1	18.0	1.0
Gonzales	303	0.2	198.8	0.1	1.7	0.3	41.0	0.3	77.4	0.2
Wichita	60	0.2	46.3	0.1	5.6	0.9	0.4	1.1	11.1	0.6
Burleson	293	0.1	249.2	0.1	1.0	0.5	10.0	0.3	47.3	0.2
Waller	84	0.2	39.1	0.1	7.1	0.6	3.9	0.4	38.3	0.3
Jackson	145	0.2	119.9	0.1	1.3	0.6	2.3	0.4	31.1	0.4
Kleberg	145	0.3	76.6	0.1	2.1	0.7	59.9	0.6	10.3	0.4
	1760		1298.7		184.0		133.5		305.5	

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**SUPPLEMENTAL INFORMATION—APPENDIX 2****Modeling of Potentially Suitable Habitat with Maxent**

We discovered, based on a statistical analysis of each Maxent input variable, that the model is most sensitive to summer temperature (**Figure S1**). The model is least sensitive to the compound topographic index, which is a wetness index that is a function of slope and upstream contributing areas. The three other most important model inputs include—in descending order of importance—precipitation of the wettest quarter, annual precipitation, and maximum temperature in the warmest quarter. We also assess the relative importance of each input variable using a heuristic estimate (**Table S1**). Variables with >5% relative contribution are: mean temperature of the warmest quarter (19.2%), average percent sand in soil (11.3%), mean temperature of the driest quarter (7.6%), annual precipitation (7.4%), generalized soil order (6.5%), precipitation of the wettest quarter (5.7%), and relative wetland density (5.1%).

**SUPPLEMENTAL FIGURES—APPENDIX 2**

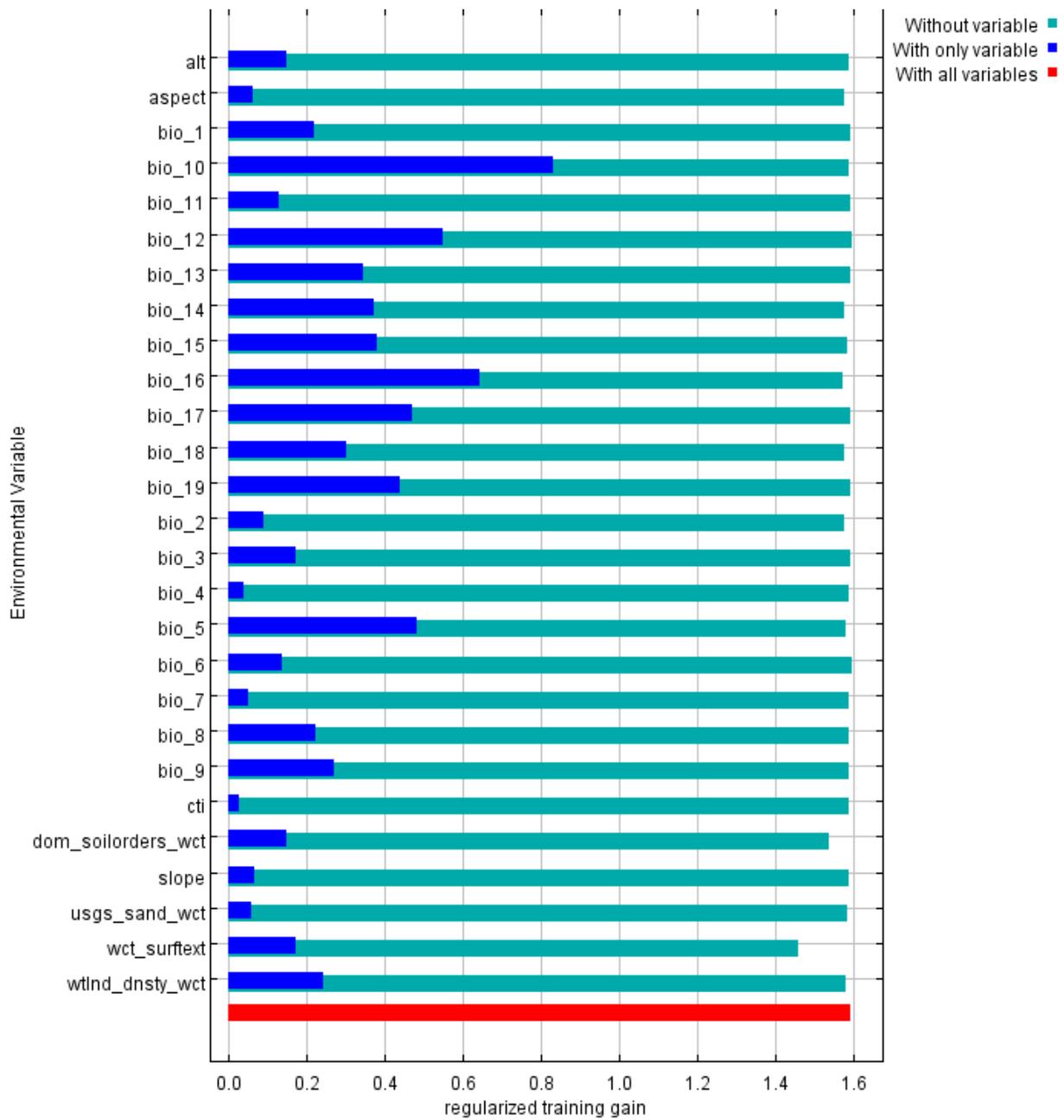


Figure S1. Relative importance of Maxent species distribution model inputs using jackknife test.

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**SUPPLEMENTAL TABLES—APPENDIX 2**

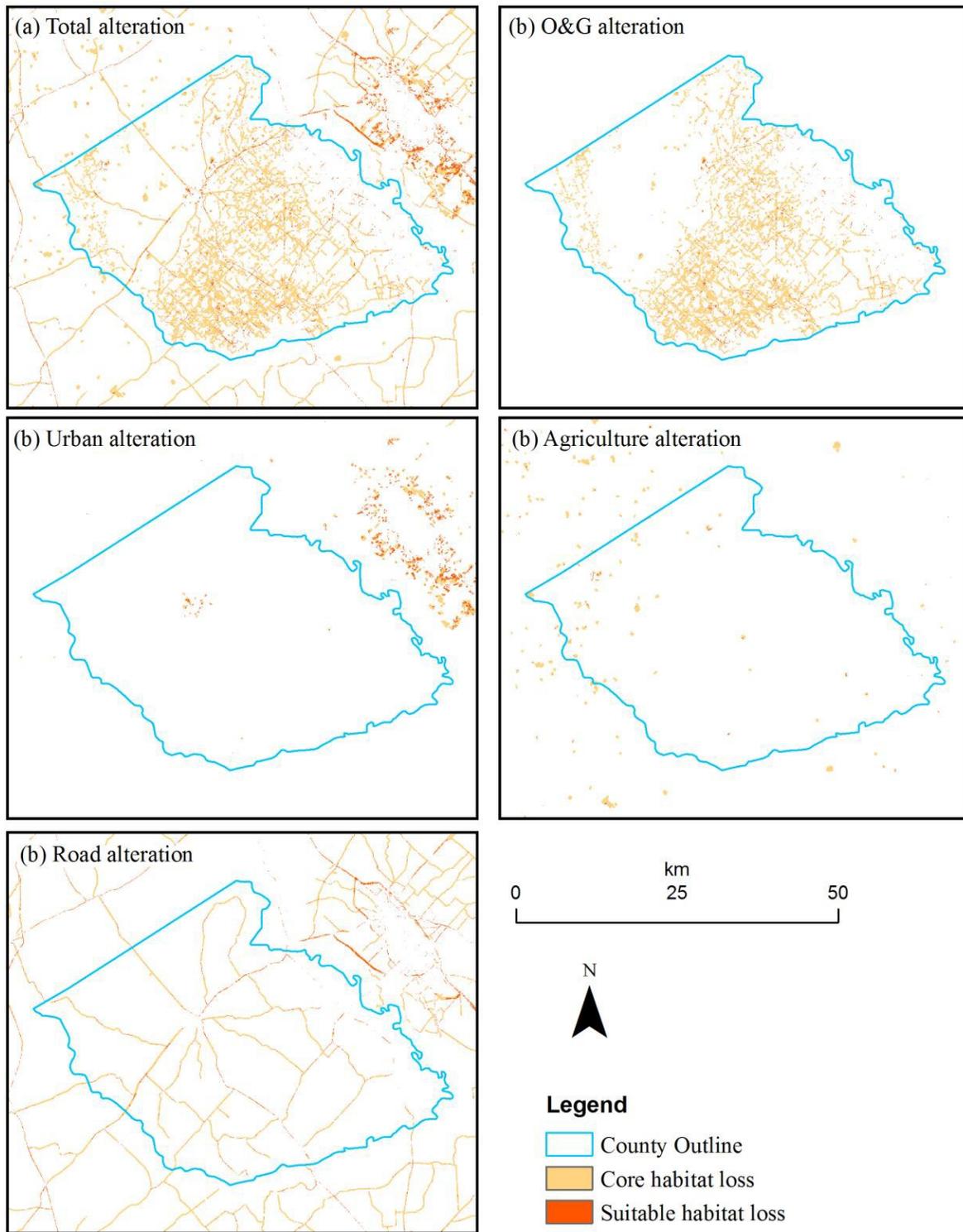
**Table S1.** Heuristic estimate of relative contributions environmental variables to Maxent model.

<b>Variable</b>	<b>Percent Contribution</b>
Mean Temperature of warmest quarter	19.2
Average percent sand in soil	11.3
Mean Temperature of driest quarter	7.6
Annual precipitation	7.4
Generalized soil order	6.5
Precipitation of wettest quarter	5.7
Relative wetland density	5.1
Precipitation of driest quarter	4.8
Maximum temperature of warmest month	4.5
Precipitation of warmest quarter	4.1
Precipitation seasonality	3.7
Mean Temperature of wettest quarter	3.5
Annual mean temperature	2.4
Altitude	2.0
Temperature annual range	2.0
Isothermality	1.9
Mean temperature of coldest quarter	1.6
Aspect	1.2
Precipitation of driest month	1.2
Temperature seasonality	1.1
Mean diurnal range	1.1
USGS sand percent	0.7
Precipitation of coldest quarter	0.5
Slope	0.4
Minimum temperature of coldest month	0.3
Compound topographic index	0.2

Note: All the parameters of Table 1 were used in this model run.

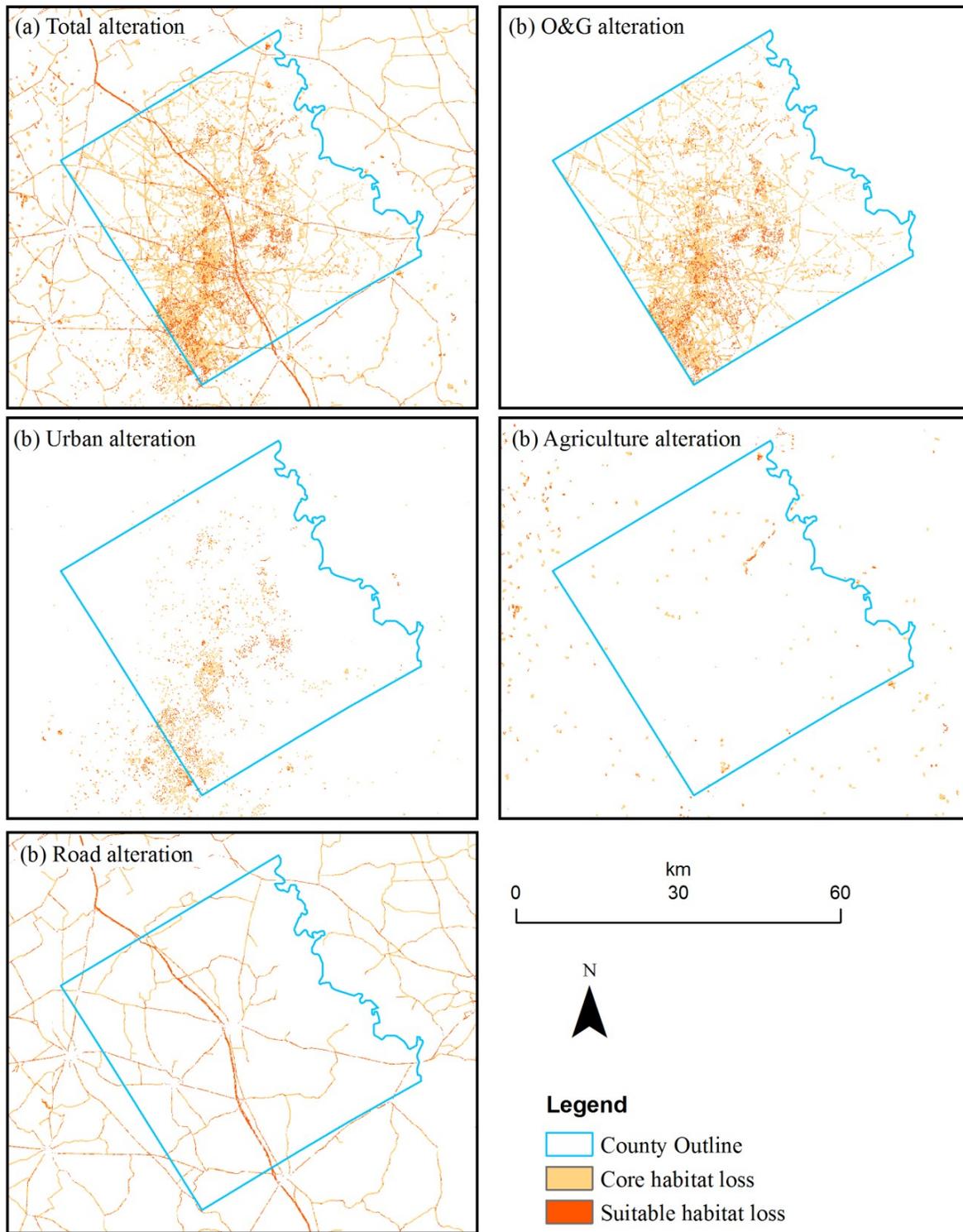
**SUPPLEMENTAL COUNTY FIGURES—APPENDIX 2**

**Skunk Habitat Alterations by County**



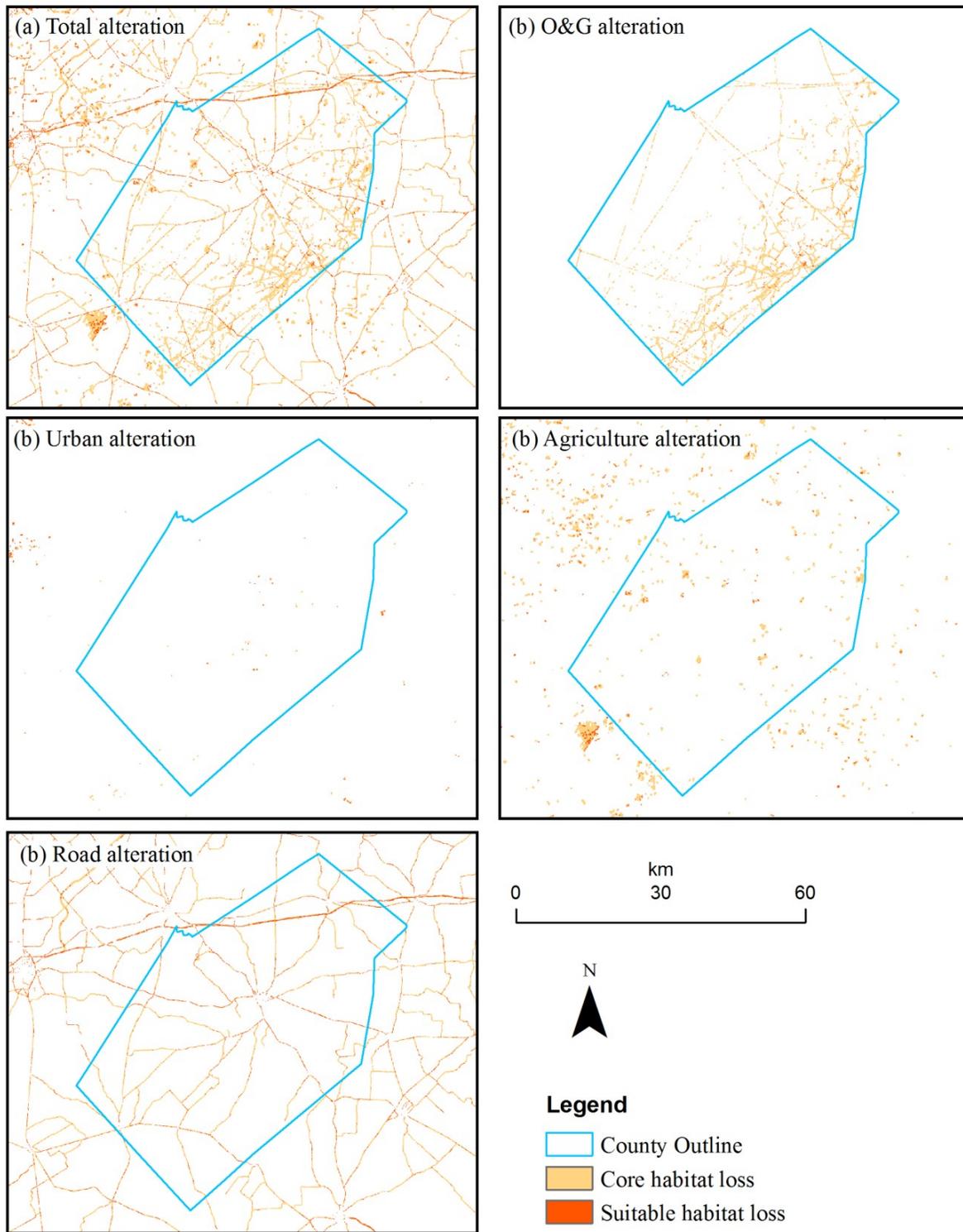
**Figure S2.** Burleson County suitable and core habitat losses by regime.

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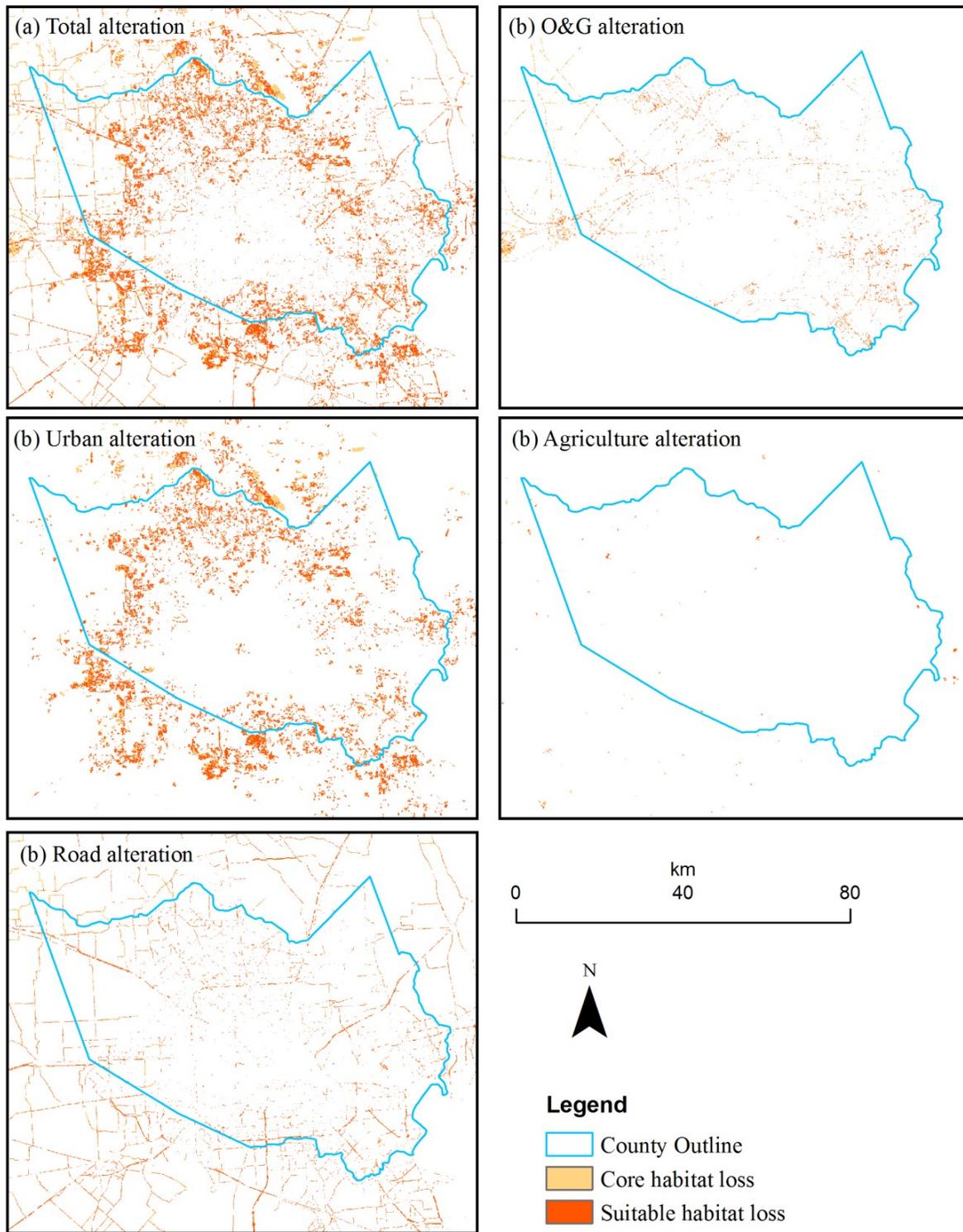
**Figure S3.** Freestone County suitable and core habitat losses by regime.

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**Figure S4.** Gonzales County suitable and core habitat losses by regime.

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**Figure S5.** Harris County suitable and core habitat losses by regime.

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**Figure S6.** Jackson County suitable and core habitat losses by regime.

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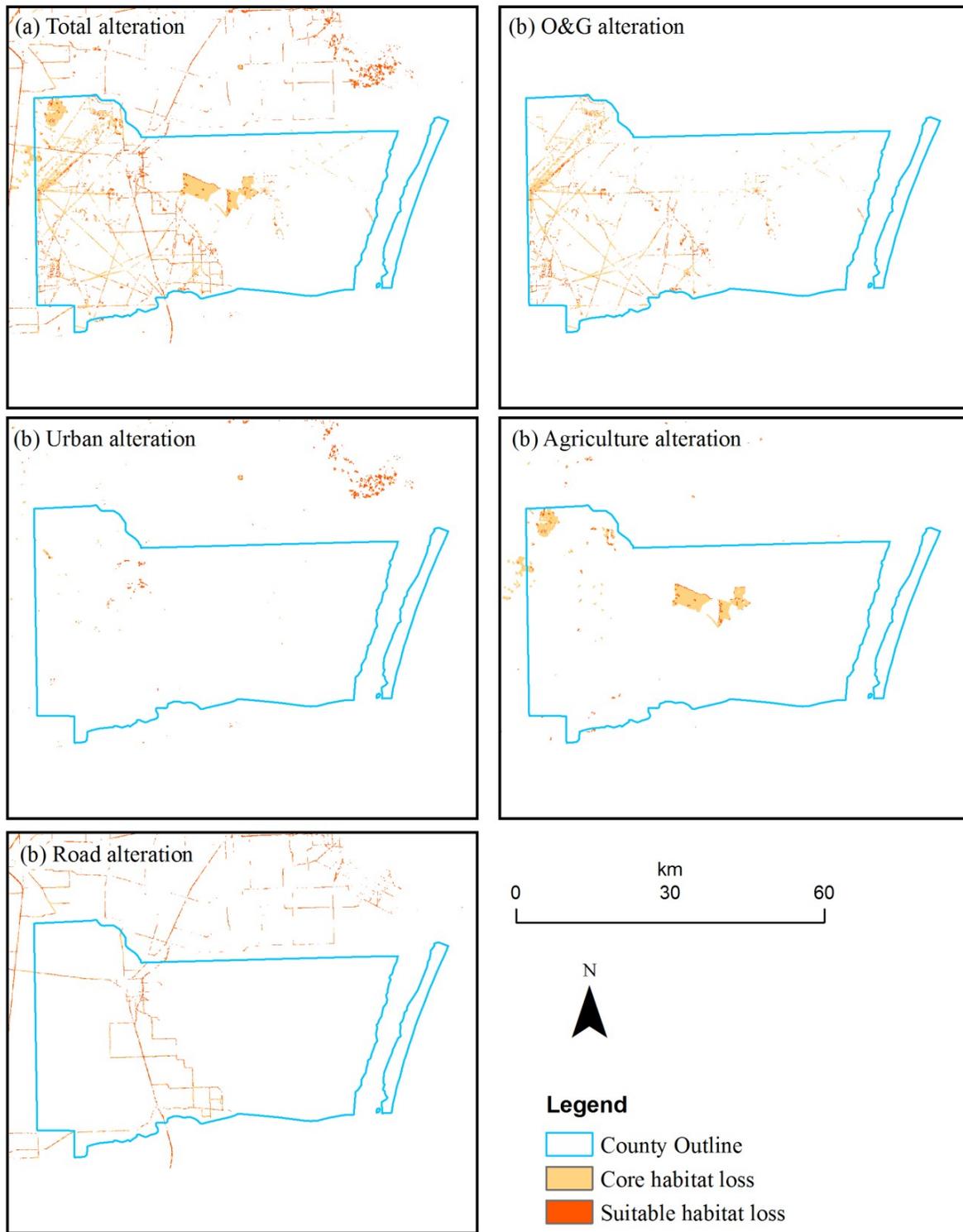
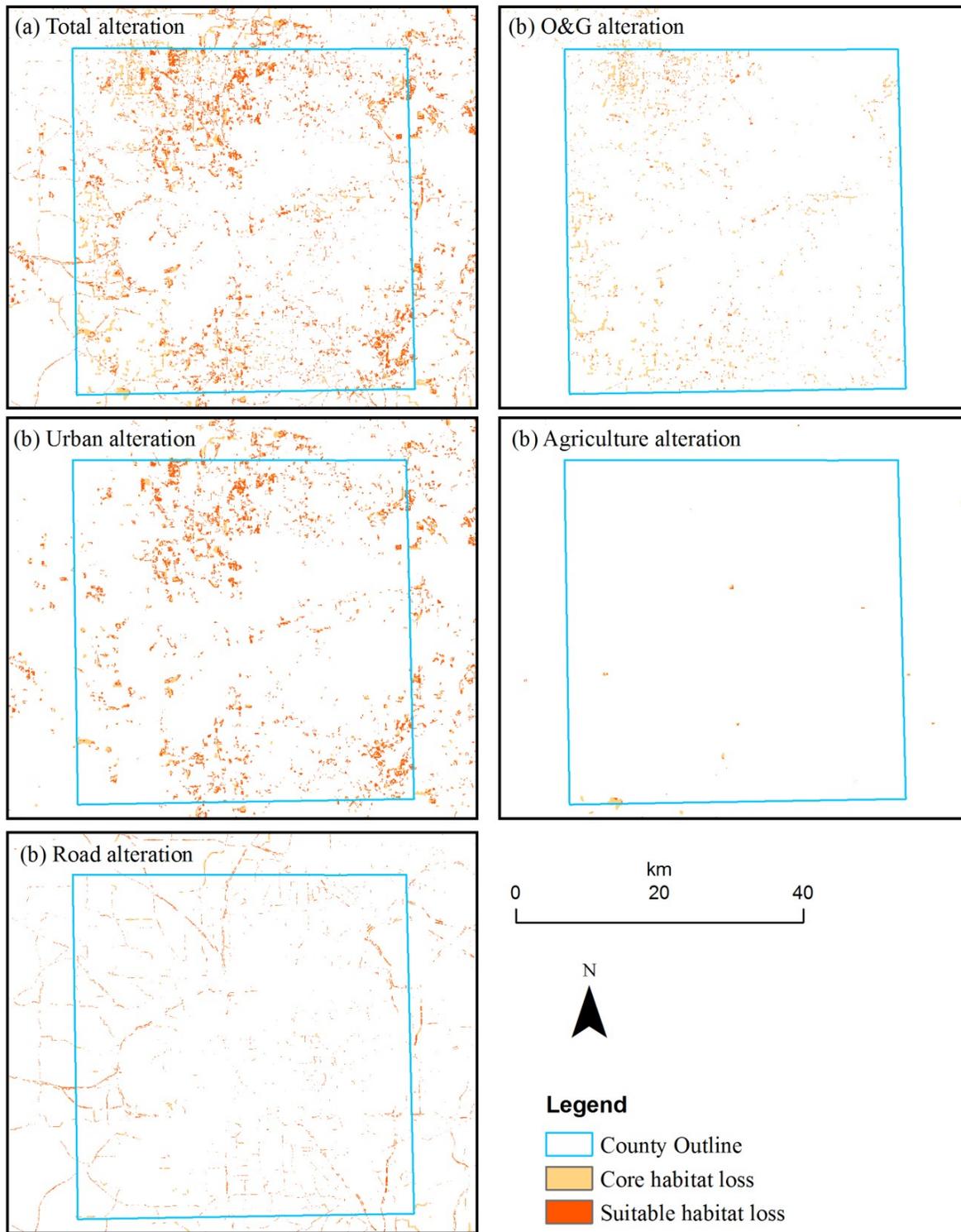


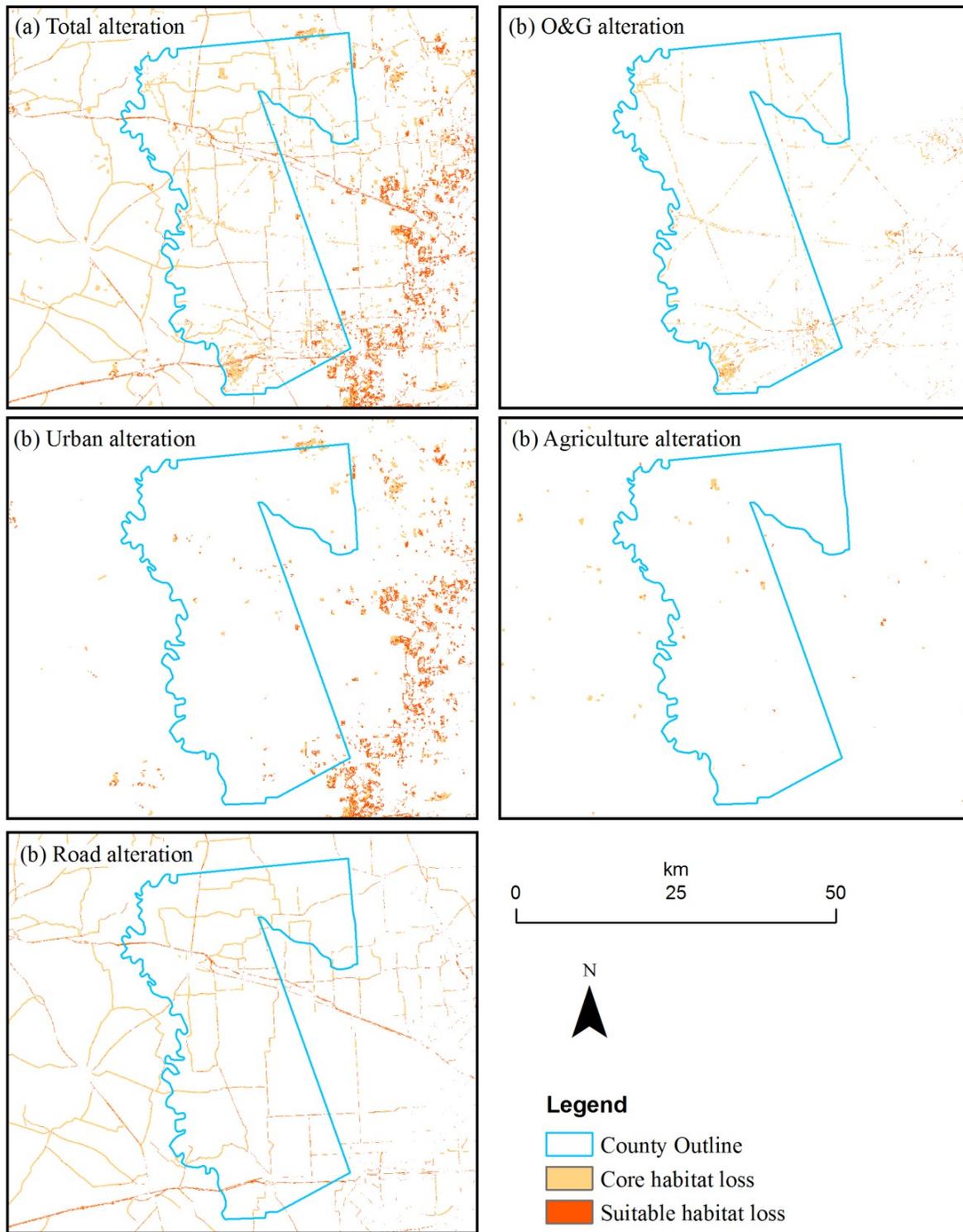
Figure S7. Kleberg County suitable and core habitat losses by regime.

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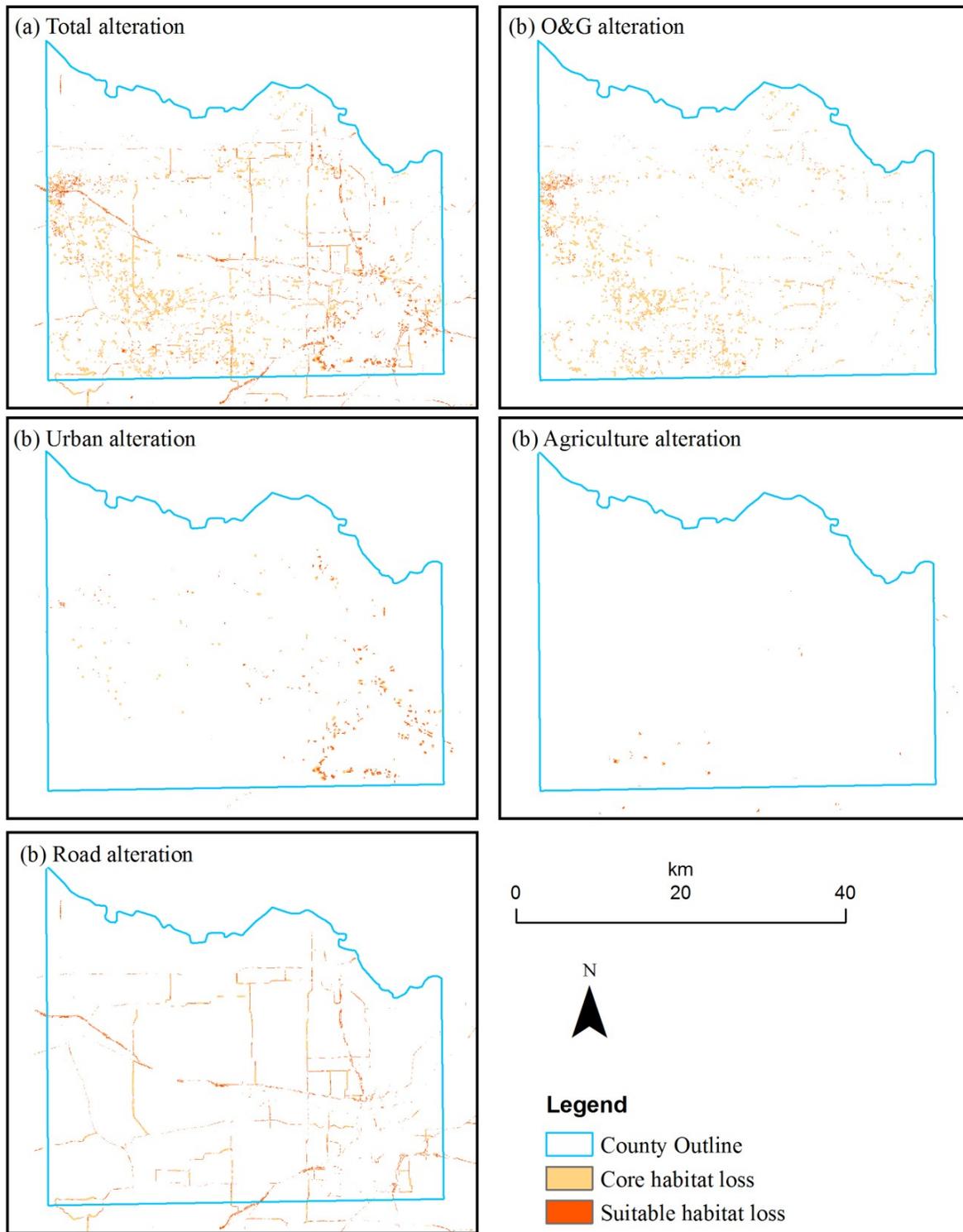
**Figure S8.** Tarrant County suitable and core habitat losses by regime.

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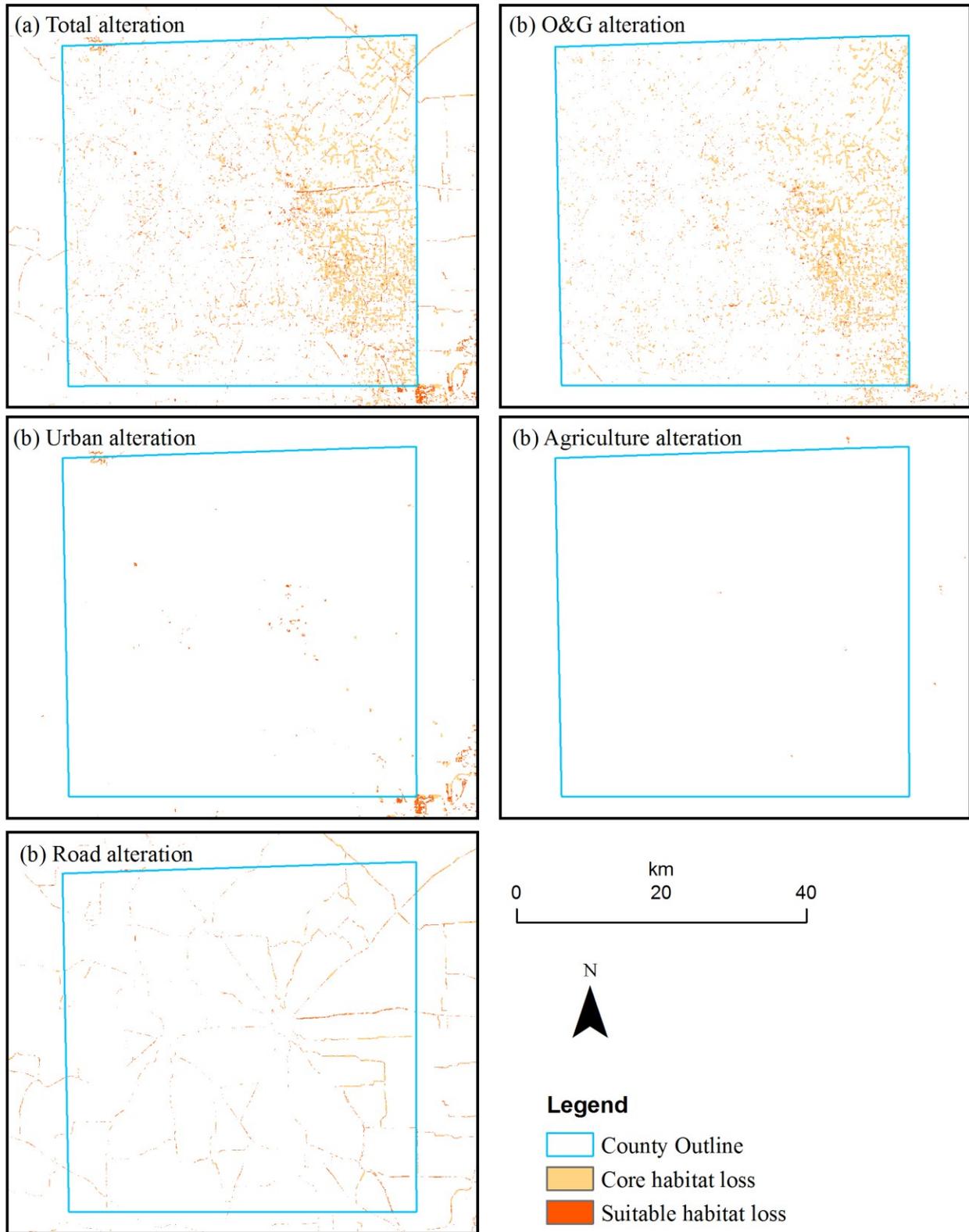
**Figure S9.** Waller County suitable and core habitat losses by regime.

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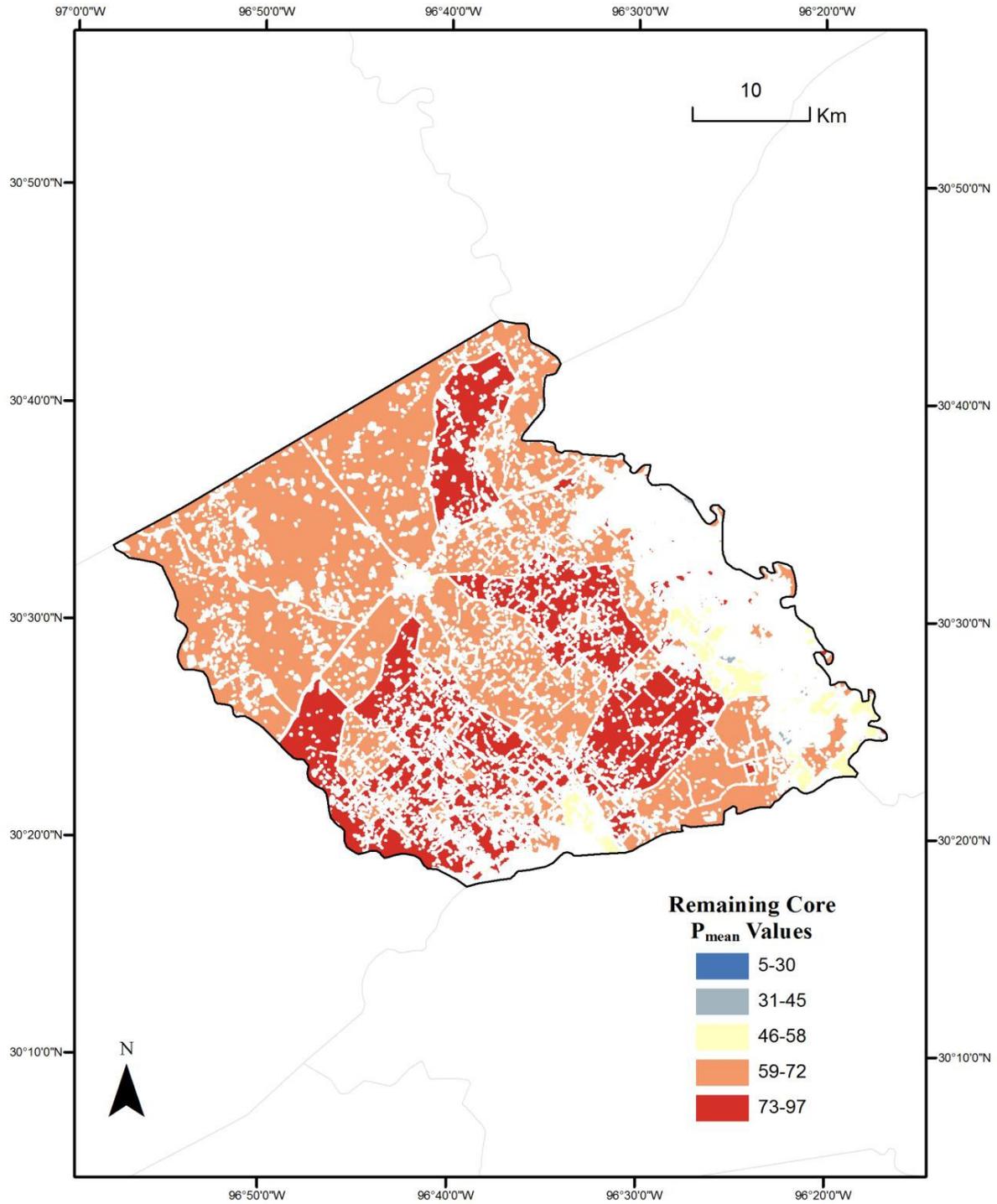
**Figure S10.** Wichita County suitable and core habitat losses by regime.

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**Figure S11.** Wise County suitable and core habitat losses by regime.

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**Figure S12.** Burlison County ranked core habitat for directed surveys.

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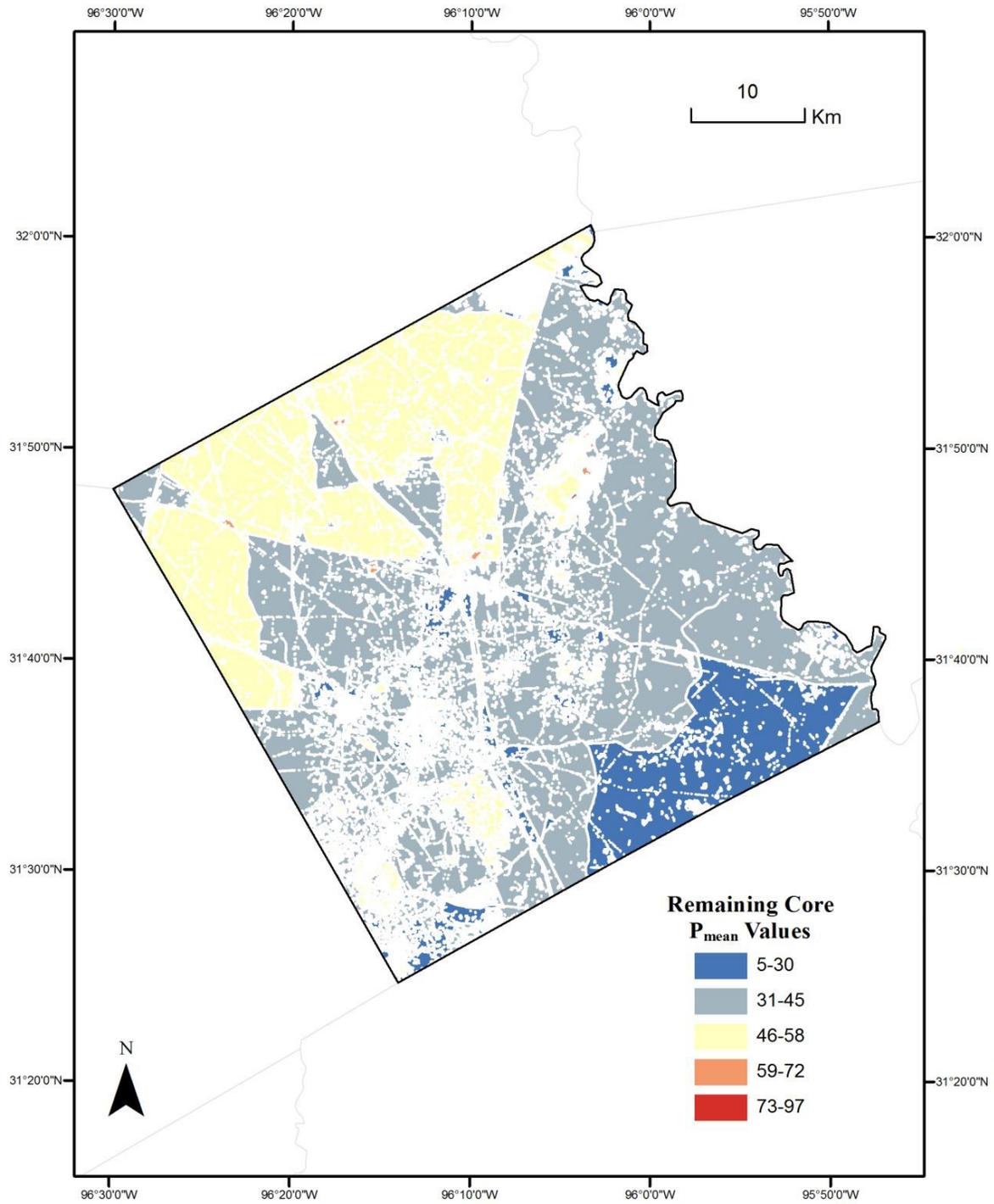
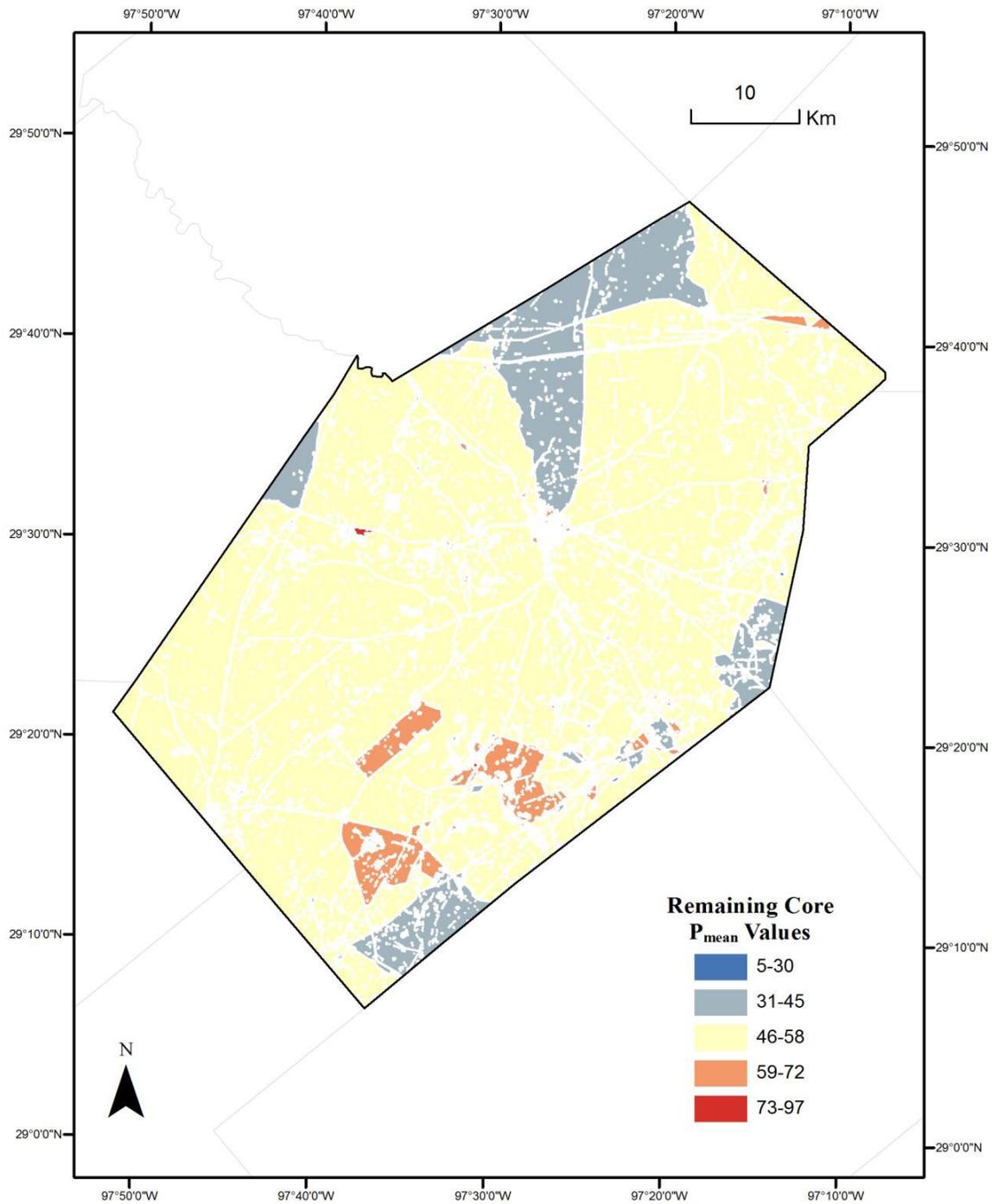


Figure S13. Freestone County ranked core habitat for directed surveys.

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**Figure S14.** Gonzales County ranked core habitat for directed surveys.

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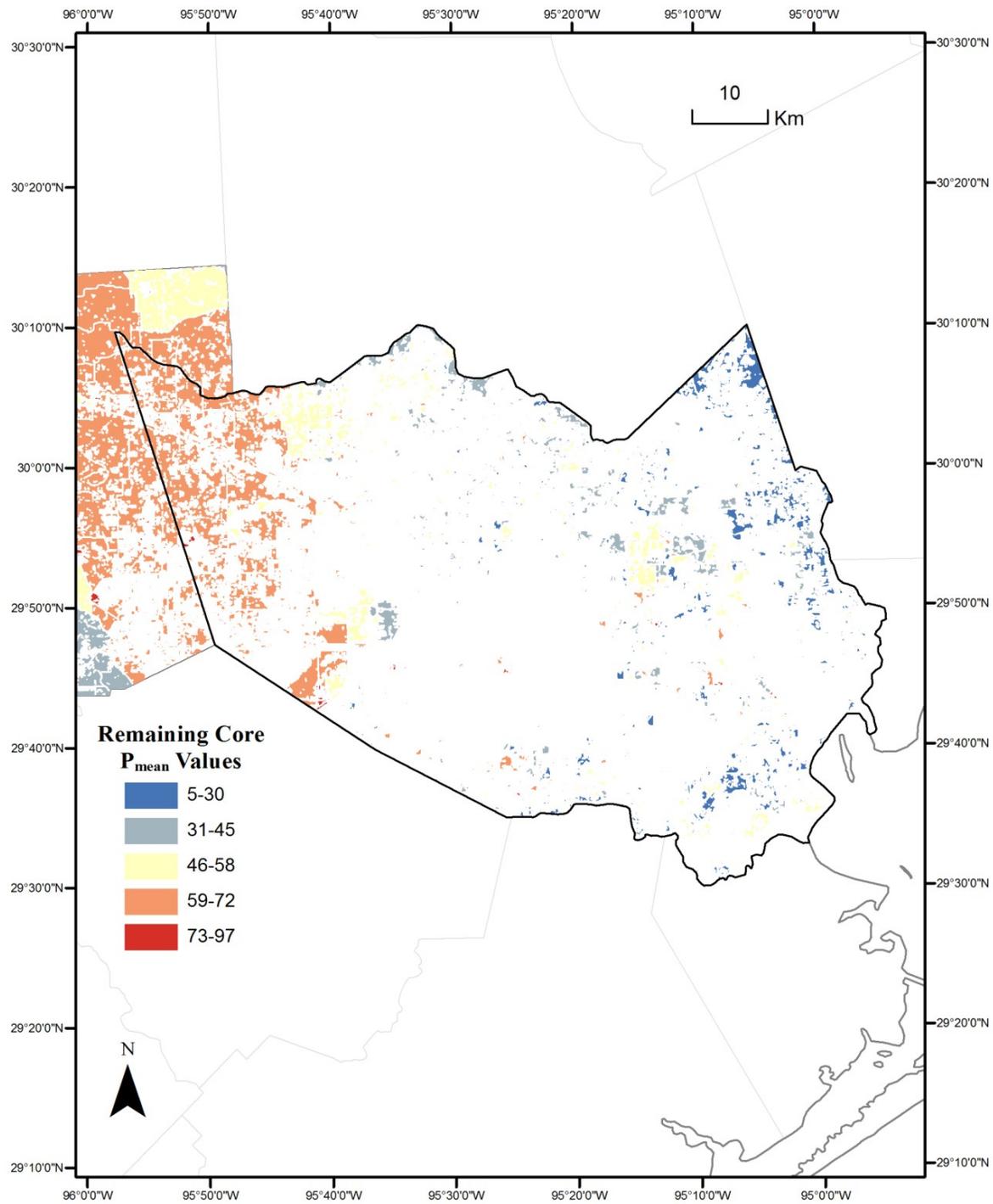


Figure S15. Harris County ranked core habitat for directed surveys.

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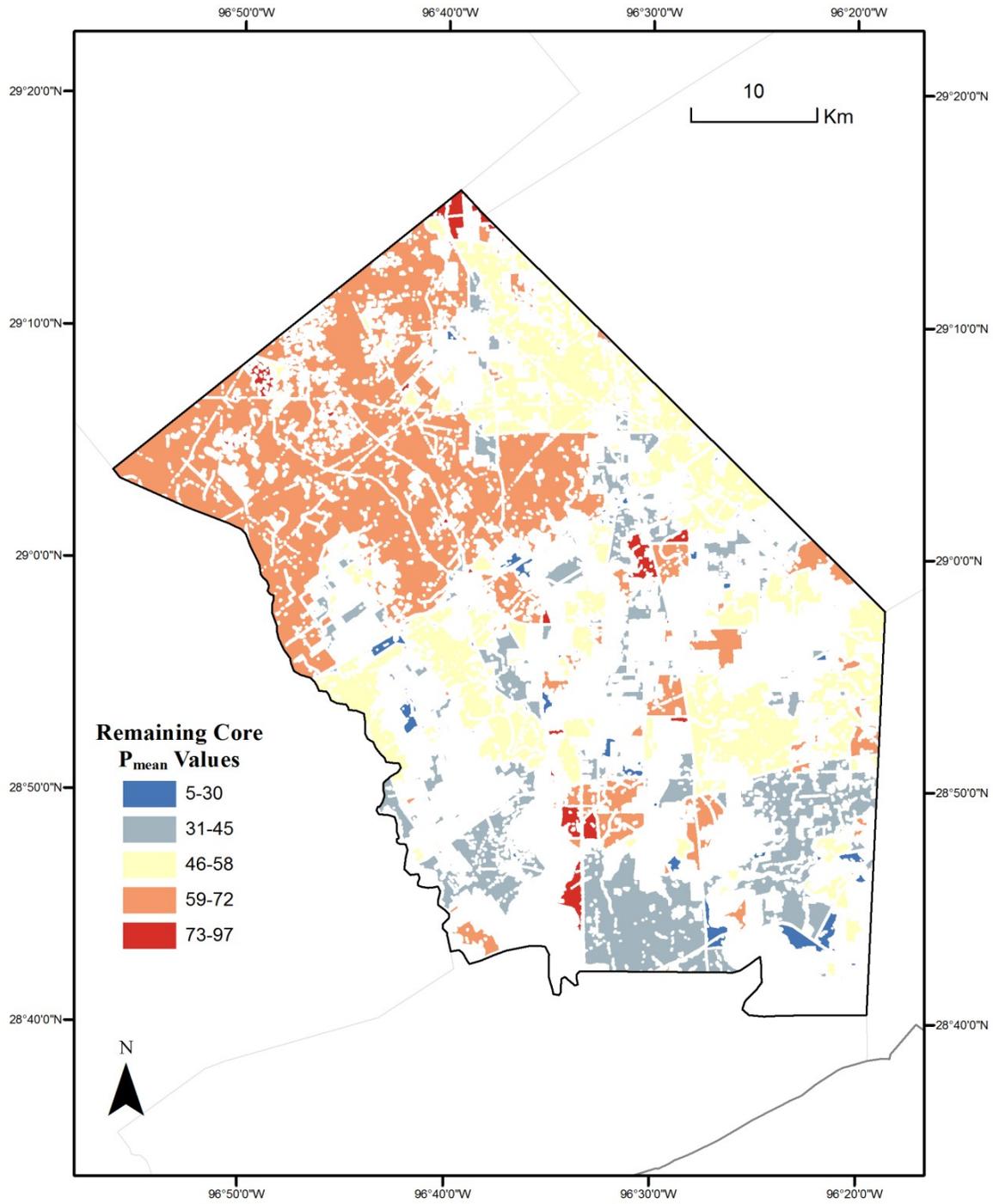


Figure S16. Jackson County ranked core habitat for directed surveys.

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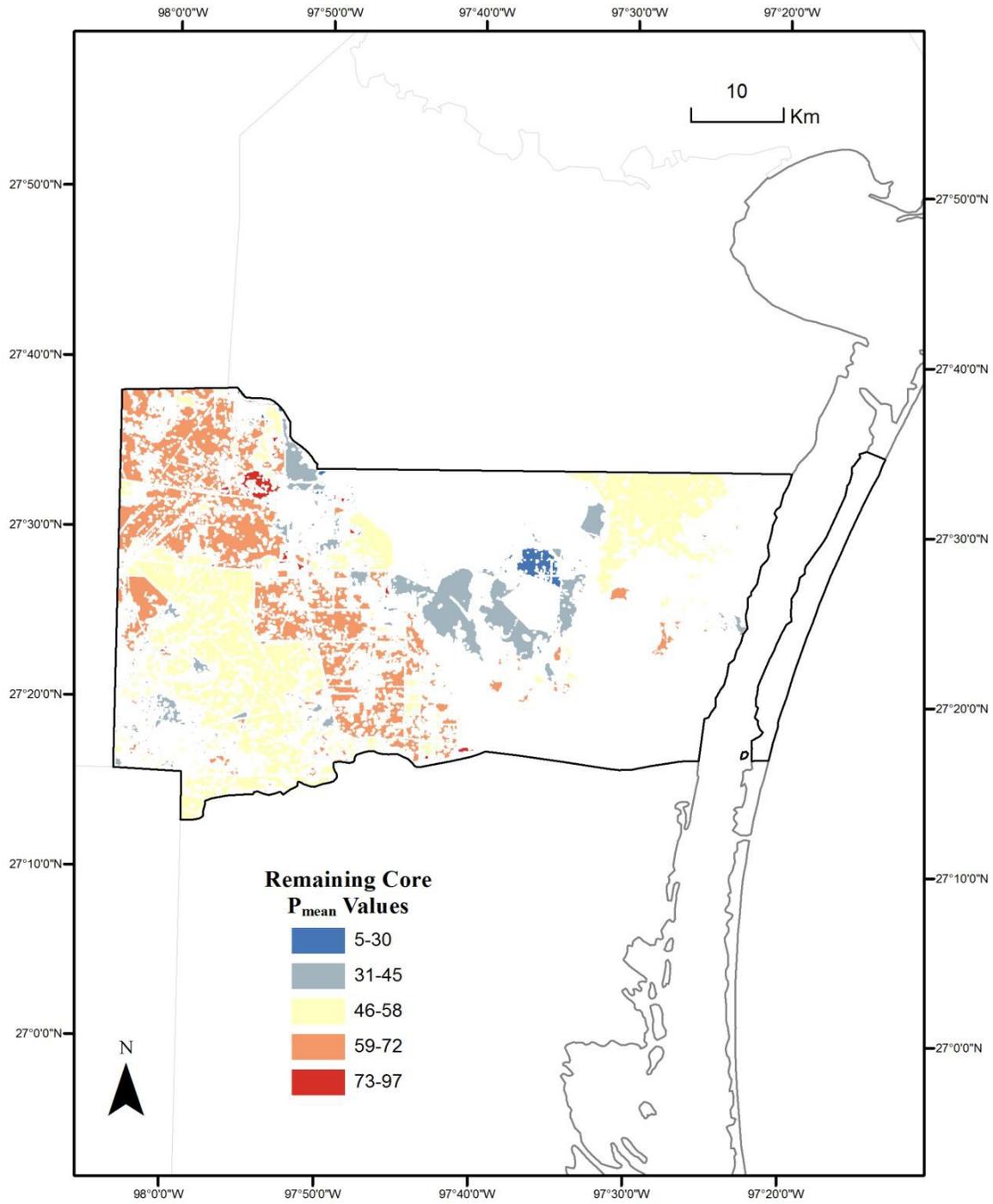


Figure S17. Kleberg County ranked core habitat for directed surveys.

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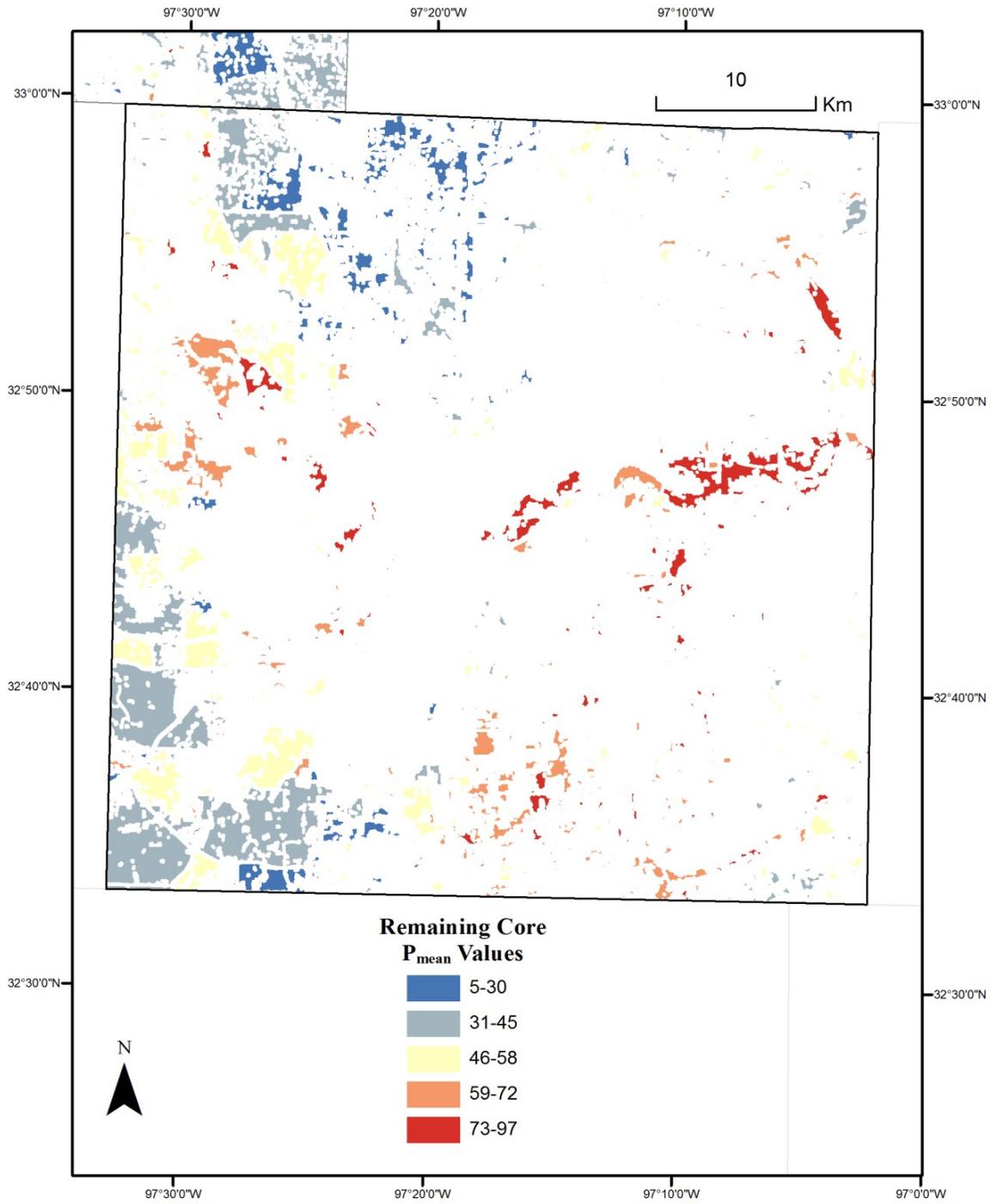
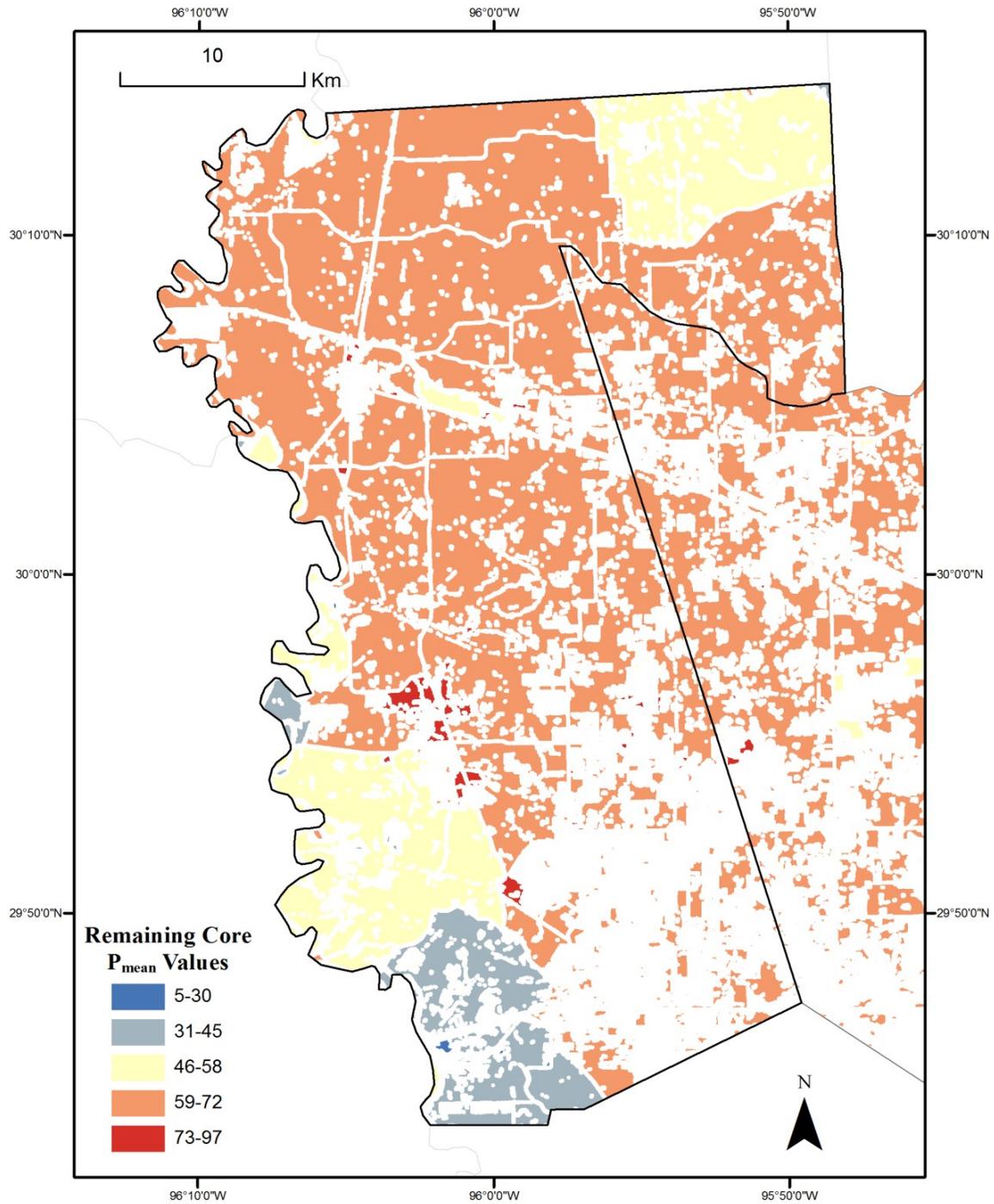


Figure S18. Tarrant County ranked core habitat for directed surveys.

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**Figure S19.** Waller County ranked core habitat for directed surveys.

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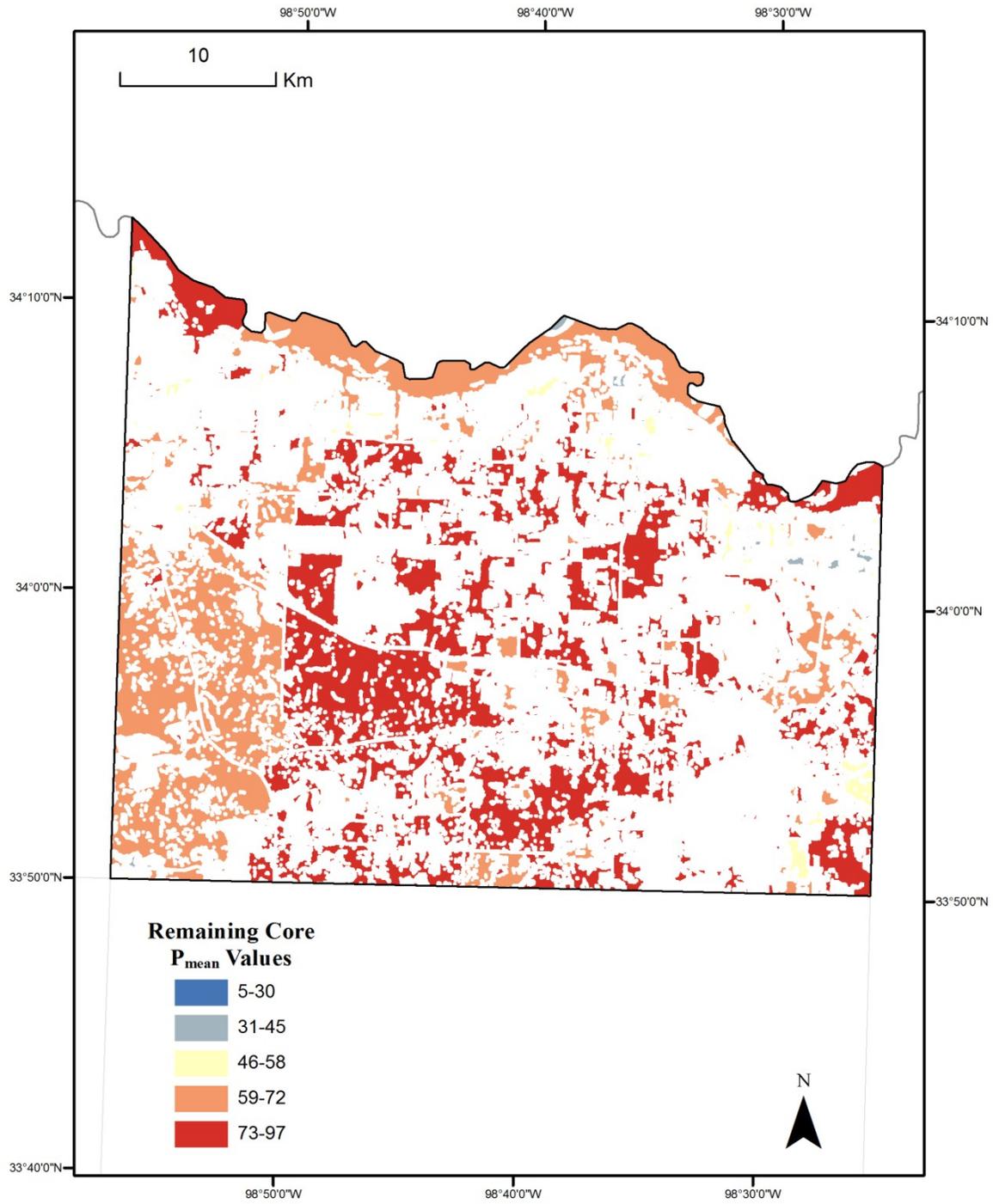


Figure S20. Wichita County ranked core habitat for directed surveys.

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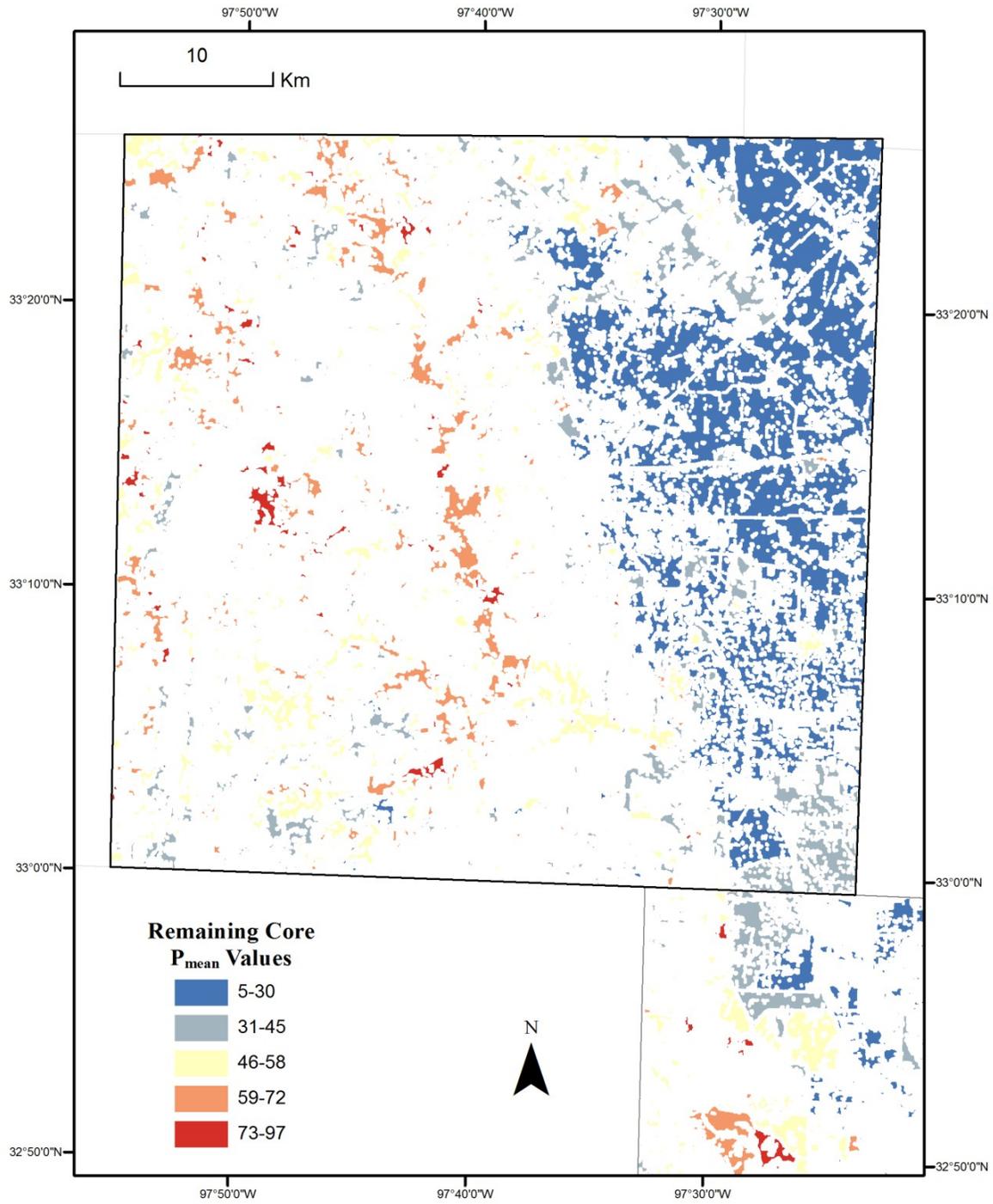


Figure S21. Wise County ranked core habitat for directed surveys.

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APPENDIX 3 – POTENTIAL LAND ALTERATION FROM FUTURE OIL AND  
GAS DEVELOPMENT AND URBANIZATION NEAR METROPOLITAN  
AREAS

Endangered Species Research Project on the Plains Spotted Skunk  
Final Report, UT Austin Additional Landscape Tasks

September 19, 2017

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## INTRODUCTION

This document presents additional landscape analyses to be included in the final report for the Plains Spotted Skunk (*Spilogale putorius interrupta*). To this end, here we present the spatial footprint of current oil and gas development and areas of possible future oil and gas activities. The study area includes numerous oil and gas resources, including the Barnett, Eagle Ford, and Haynesville shale plays and the Gulf Coast and Permian Basin (Fig. 1). We also present current and forecasted urbanization. The study area includes the cities of Austin, Dallas, Houston, and San Antonio (Fig. 2). While we had intended to conduct a vegetation analysis to assess if invasive plants were replacing native vegetation, state databases of vegetative cover do not specifically include invasive plants (Elliott et al., 2014); thus, the data do not currently exist to complete this portion of the project. Note that these analyses have been conducted in addition to those originally proposed. We completed these additional analyses so that the domain matches the area of modeled potential habitat by Angelo State University.

## METHODS

### 1. Landscape alteration from Eagle Ford Shale Play and Permian Basin oil and gas development within potential *Spilogale putorius interrupta* habitat

We assessed landscape alteration from oil and gas development by mapping drilling intensity, which we define as the number of permitted wells within a unit area. We consider drilling intensity to be a reasonable regional-scale proxy for landscape alteration caused by well pad and other infrastructure construction. We obtained well data from Information Handling Services, Inc. (IHS, 2017) on September 5, 2017; however, this database is continuously updated and a query made at a later date may result in a different suite of wells. We used the permit date, not the date drilling began (i.e., spud date) because landscape alteration occurs before a well is drilled when the well pad is constructed (e.g., Pierre et al., 2015). We chose wells starting in January 1, 2008 to coincide with the recent rapid expansion in drilling activity as a result of the commercialization of directional drilling with hydraulic fracturing (Pierre et al., 2017). The last permitted wells that show up in our database query are from Sept. 1, 2017. We then mapped drilling intensity by calculating the number of wells permitted on a one-square kilometer grid throughout the study area.

### 2. Areas of possible future oil and gas activities that may increase landscape alteration

We mapped areas of possible future oil and gas development within modeled potential habitat of *S. putorius interrupta*. We do not know exactly where drilling will occur in the future. Thus, we assume that areas of possible future oil and gas activity may be inferred from (1) current drilling intensity (i.e., future drilling is likely to occur in proximity of current drilling) and (2) the boundaries of conventional oil and gas plays (Dutton et al., 2005) and unconventional oil and gas plays (EIA, 2017). Thus, we mapped current play boundaries as proxies for future development.

### 3. Landscape alteration from current urbanization within potential *S. putorius interrupta* habitat

We investigated recent land use changes, which may represent threats to *S. putorius interrupta* habitat from urbanization, using the National Land Cover Database (NLCD; Jin et al., 2013; USGS, 2014). We mapped current urbanization using NLCD by plotting classes 21 (Developed, open space), 22 (Developed, low intensity), 23 (Developed, medium intensity), 24 (Developed, high intensity), following the approach of Ryberg et al. (2017).

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#### **4. Landscape alteration from forecasted urbanization within potential *S. putorius interrupta* habitat**

We assessed future threats to and alteration of *S. putorius interrupta* habitat caused by forecasted urbanization (2010–2050) using the same approach we used for current threats. We mapped and quantified future urban expansion beyond the urban fringe from 2010 to 2050 using the Theobald (2005) database, which presents forecasted increases in housing density in decadal increments. Following the approach of Ryberg et al. (2017), we considered urban areas in the Theobald dataset to include commercial and industrial institutions, >10 units/acre, 5–9.9 units/acre, 2–4.9 units/acre, 0.5–1.6 acre/unit, and 1.7–4.9 acre/unit. We selected these housing density classes because visual inspection of the 2010 Theobald dataset most closely agrees with patterns of urban development observed in current aerial photography (USDA, 2014) and developed land classes in the NLCD dataset. Forecasted housing development using these Theobald classes also resulted in the most qualitatively plausible pattern of 2050 housing development, given recent trends in urban expansion.

## **RESULTS**

### **1. Landscape alteration from Eagle Ford Shale Play and Permian Basin oil and gas development within potential *Spilogale putorius interrupta* habitat**

We mapped of current landscape alteration from oil and gas development within the modeled potential habitat of *S. putorius interrupta*, using drilling intensity as a proxy. This analysis revealed widespread drilling activity since 2008 resulting in drilling intensity of 1–5 wells/km<sup>2</sup> for a broad zone east of Dallas to southwest of San Antonio, which includes unconventional portions of the Eagle Ford and Haynesville Shale Plays and the Gulf Coast (Fig. 1). Much of the Eagle Ford Shale Play has drilling intensity of 1–5 wells/km<sup>2</sup>, though portions have drilling intensity of 6–25 wells/km<sup>2</sup>. Similar drilling densities were observed in the Barnett Shale Play; however, the highest drilling density (up to 145 wells/km<sup>2</sup>) is primarily focused within 50 km west of Dallas—where some current localities are found. The Permian Basin has a wide range of drilling densities; however, within the modeled range of *S. putorius interrupta*, these occur in more isolated clusters than the remainder of the study area.

### **2. Areas of possible future oil and gas activities that may increase landscape alteration**

Our assessment of possible future oil and gas within the modeled potential habitat of *S. putorius interrupta* includes essentially all areas that had recent drilling activity. We expect the trend of drilling in the Eagle Ford Shale Play observed by Pierre et al. (2017) to continue and that drilling density may increase into the future as infill drilling occurs. Similar trends may also be expected in the Barnett and Haynesville Shale Plays. Hydrocarbon resources along the Gulf Coast mapped by Dutton et al. (2005) may be expected to include reworking of existing oil and gas plays, with less drilling in previously undrilled areas. Conversely, over 20 billion barrels of oil and 16 trillion feet of gas are estimated to be yet undiscovered in the Wolfcamp Shale alone in the Permian Basin (Gaswirth et al., 2016). Thus, we expect new drilling, with possible increases in drilling intensity, to continue in the Permian Basin.

### **3. Landscape alteration from current urbanization within potential *S. putorius interrupta* habitat**

Our analysis revealed urbanization within much of the modeled potential habitat of *S. putorius interrupta* (Fig. 1). Urbanization near current localities was most intense to the west of Houston.

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Other areas of urbanization near current localities include to the southwest of Dallas and near Killeen, which is located ~70 miles north of Austin. However, much of the study areas is generally rural, suggesting habitat and migration pathways may remain.

#### **4. Landscape alteration from forecasted urbanization within potential *S. putorius interrupta* habitat**

Forecasted future urbanization through 2050 (Fig. 2) is expected to continue around areas of current urbanization. However, the most important future urbanization within modeled potential *S. putorius interrupta* habitat may occur near Houston, where several current localities area found. Urbanization around Dallas and Killeen may also cause habitat alteration, which may threaten the species.

### **DISCUSSION**

Potential habitat of *Spilogale putorius interrupta* is currently threatened, and will likely continue to be threatened, by urbanization in and around growing cities—particularly Houston and Dallas. Future urbanization may threaten the species near Killeen; however, the continued presence of the military base at Fort Hood (located in and around Killeen) may maintain habitat for the species into the future. Less clear, however, is the effect of oil and gas development on the species. As current localities are found throughout the Barnett Shale Play, perhaps widely dispersed drilling activity may not threaten the species. However, more research is needed to improve our understanding of the causal relationship between oil and gas development and the response of the species.

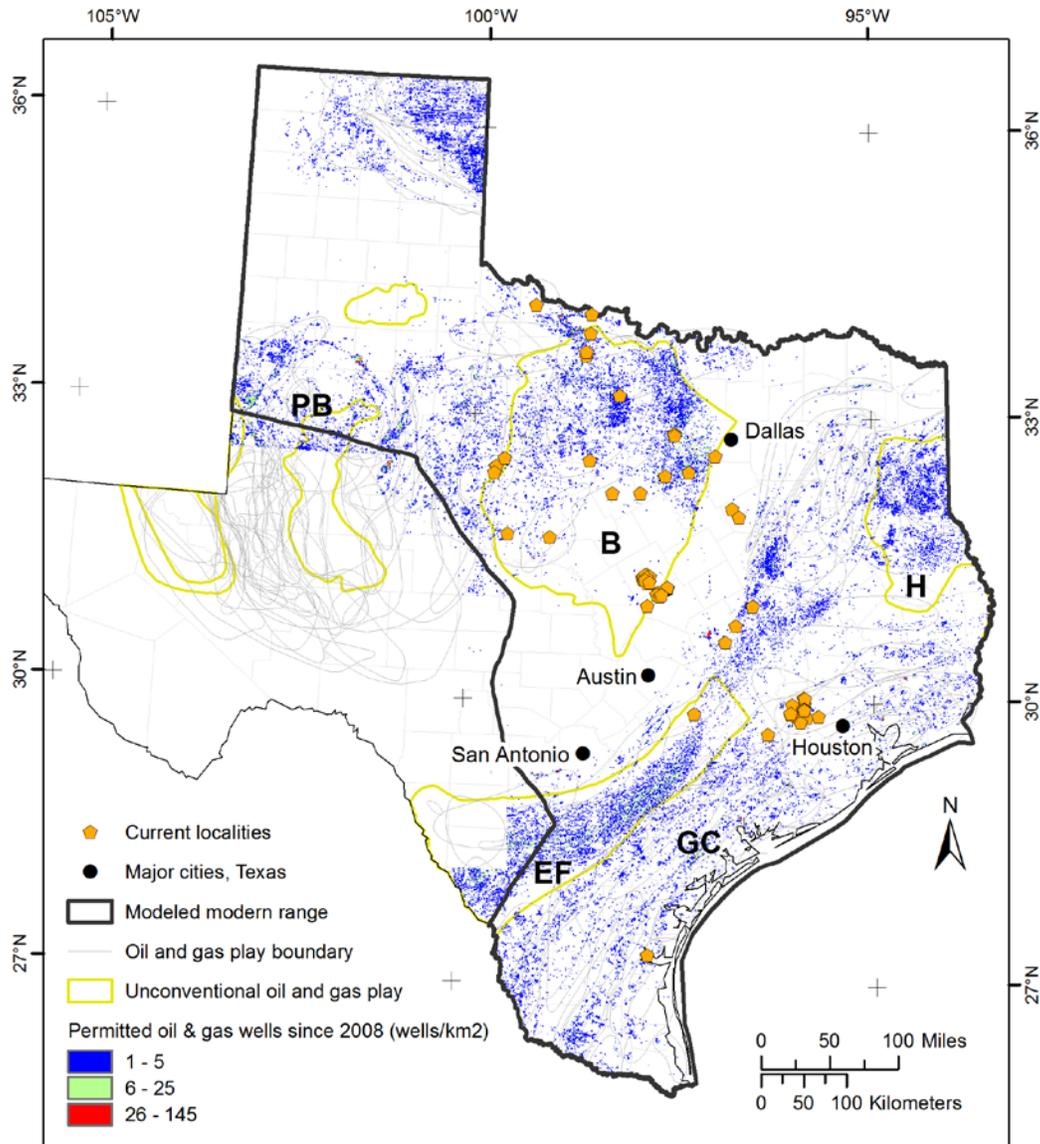
### **CONCLUSIONS**

- Forecasted trends in urbanization reveal that current localities near Houston, Dallas, and Killeen may be threatened by future urban growth; however, land use for Fort Hood near Killeen may maintain important habitats for the species there.
- Current localities in rural areas throughout the species' modeled potential habitat suggest that low-density urbanization may not adversely affect the species.
- The species is also currently found throughout the Barnett Shale Play; however, more research is needed to improve our understanding of the causal relationship between oil and gas development and the species' response.

**LITERATURE CITED—APPENDIX 3**

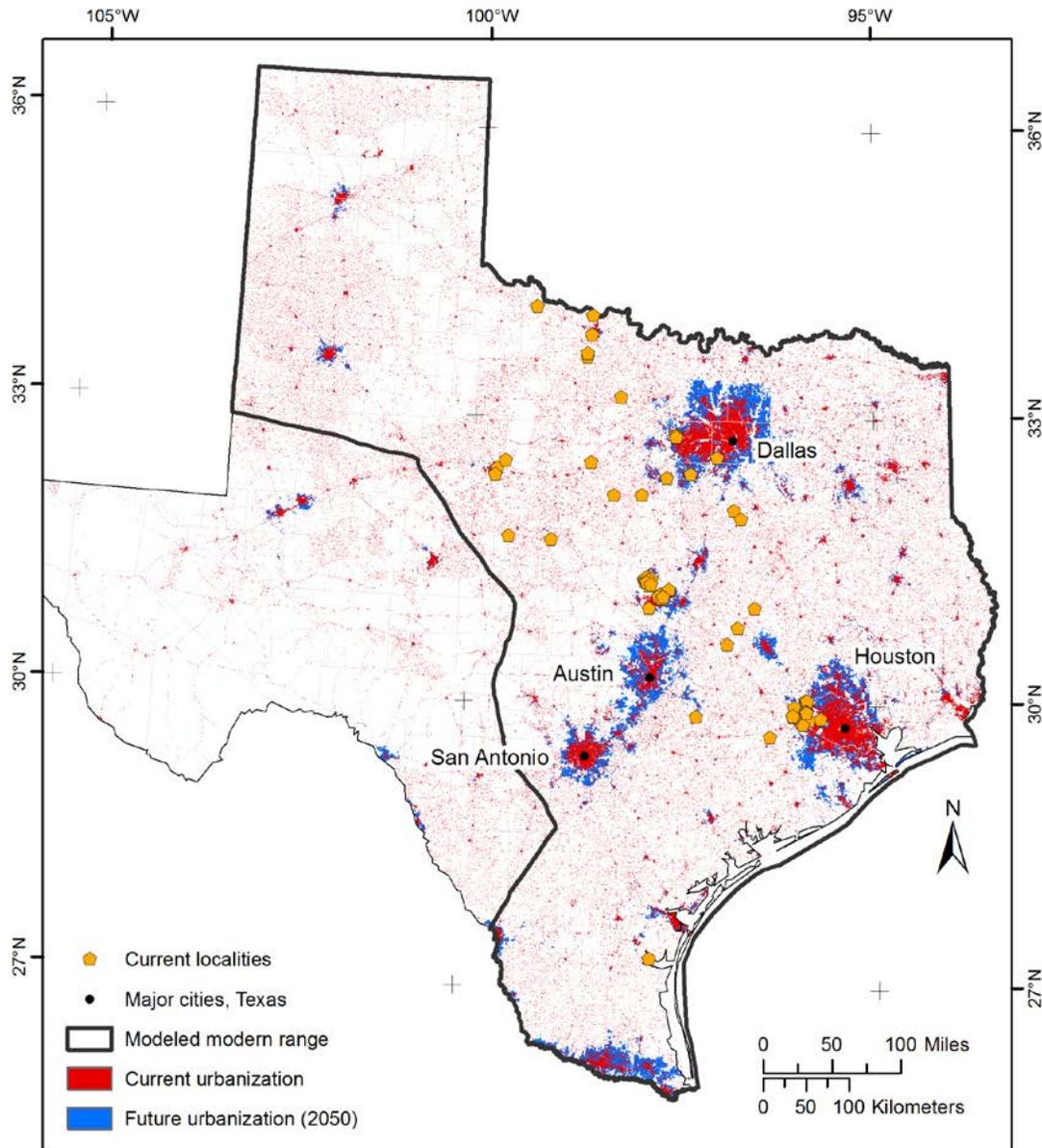
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FIGURES—APPENDIX 3



**Figure 1. Oil and gas development.** Current landscape alteration from oil and gas development is represented by drilling intensity, which is defined as the number of wells drilled since January 1, 2008 per km<sup>2</sup> (IHS, 2017). Areas of possible future oil and gas activities are inferred by current drilling intensity and also oil and gas play boundaries (gray line; Dutton et al., 2005) and unconventional oil and gas plays (gold line; EIA, 2017), which are favorable targets for future drilling. B=Barnett Shale Play, GC=Gulf Coast, EF=Eagle Ford Shale Play, H=Haynesville Shale Play, PB=Permian Basin.

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**Figure 2. Urbanization.** Current urbanization in red and forecasted future urbanization as of 2050 in blue. We assumed Theobald (2005) housing density classes of commercial and industrial institutions, > 10 units/ac, 5–9.9 units/ac, 2–4.9 units/ac, 0.5–1.6 ac/unit, and 1.7–4.9 ac/unit to represent urbanization.

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**Appendix 4: Specimens of *Spilogale putorius* and *S. gracilis* examined with microsatellite (M) and/or cytochrome b (C) sequence data.****Acronyms or abbreviations are as follows:**

ACUNHC (Abilene Christian University Natural History Collection), AGFC (Arkansas Game and Fish Commission), AMNH-MC (Anniston Museum of Natural History), ASNHC or ASK (Angelo State Natural History Collections), AWF (Adam Ferguson), CMNH (Campbell Museum of Natural History), DCNHTC (Dickinson College Natural History Teaching Collection), FWC (Florida Fish and Wildlife Conservation Commission), GMNH (Georgia Museum of Natural History), JJK (James Krupa), JWD (Jerry Drago), MWFB (Museum of Wildlife and Fish Biology, University of California-Davis), MWSU (Midwestern State University), NCSM (North Carolina Museum of Science), NDM (Nancy Moncrief), NK or MSB (University of New Mexico, Museum of Southwestern Biology), TCWC (Texas A&M Biodiversity Research and Teaching Collections), TTU or TK (Museum of Texas Tech University Genetic Resources Collection), UKVTC (University of Kentucky Vertebrate Teaching Collection), UMBMC (University of Missouri Bird and Mammal Collection), UNSM (University of Nebraska State Museum), USNM (National Museum of Natural History, Smithsonian Institution), and UWG (University of West Georgia). Tissue abbreviations: E (Ear clip), H (Heart), Hf (Hair follicle), HK (Heart or Kidney), K (Kidney), L (Liver), Mu (Muscle), T (Toe pad), Sk (Skin). NA = Not available, U = Unknown.

**Tasks 1, 2, and 3 Report: Plains Spotted Skunk Habitat Assessment**

**RFP No. 209b**

Subspecies	Tissue no.	Catalog no.	Institution	Tissue	State	County	Sex	Collection date	M	C
<i>ambarvalis</i>	FLMNH31053	FLMNH31053	FLMNH	L	FL	Brevard	M	24-Mar-2004	x	
<i>ambarvalis</i>	FLMNH32667	FLMNH32667	FLMNH	L	FL	Hillsborough	M	18-Mar-2009		x
<i>ambarvalis</i>	FLMNH33532	FLMNH33532	FLMNH	L	FL	Osceola	M	24-Feb-2016	x	x
<i>ambarvalis</i>	FLMNH33533	FLMNH33533	FLMNH	L	FL	Osceola	M	18-Feb-2016	x	
<i>ambarvalis</i>	FWC02	tissue only	FWC	Hf	FL	Osceola	U	7-Oct-2015	x	
<i>ambarvalis</i>	FWC06	tissue only	FWC	Hf	FL	Osceola	U	14-Oct-2015	x	
<i>ambarvalis</i>	FWC12	tissue only	FWC	Hf	FL	Osceola	U	10-Nov-2015	x	x
<i>ambarvalis</i>	FWC14	tissue only	FWC	Hf	FL	Osceola	U	10-Nov-2015	x	x
<i>ambarvalis</i>	FWC15	tissue only	FWC	Hf	FL	Osceola	U	12-Nov-2015	x	
<i>ambarvalis</i>	FWC16	tissue only	FWC	Hf	FL	Osceola	U	13-Nov-2015	x	
<i>ambarvalis</i>	FWC17	tissue only	FWC	Hf	FL	Osceola	U	13-Nov-2015	x	
<i>ambarvalis</i>	FWC18	tissue only	FWC	Hf	FL	Osceola	U	17-Nov-2015	x	
<i>ambarvalis</i>	FWC19	tissue only	FWC	Hf	FL	Osceola	U	17-Nov-2015	x	x
<i>ambarvalis</i>	FWC20	tissue only	FWC	Hf	FL	Osceola	U	20-Nov-2015	x	
<i>ambarvalis</i>	FWC22	tissue only	FWC	Hf	FL	Osceola	U	2-Feb-2016	x	
<i>ambarvalis</i>	FWC24	tissue only	FWC	Hf	FL	Osceola	U	3-Feb-2016	x	
<i>ambarvalis</i>	FWC26	tissue only	FWC	Hf	FL	Osceola	M	16-Mar-2016	x	
<i>ambarvalis</i>	FWC27	tissue only	FWC	Hf	FL	Osceola	F	16-Mar-2016	x	
<i>ambarvalis</i>	FWC28	tissue only	FWC	Hf	FL	Osceola	M	17-Mar-2016	x	
<i>ambarvalis</i>	FWC29	tissue only	FWC	Hf	FL	Osceola	U	1-May-2016	x	
<i>ambarvalis</i>	FWC30	tissue only	FWC	Hf	FL	Osceola	F	28-Mar-2016	x	x
<i>ambarvalis</i>	FWC32	tissue only	FWC	Hf	FL	Osceola	M	28-Mar-2016	x	
<i>ambarvalis</i>	FWC40	tissue only	FWC	Hf	FL	Osceola	F	1-Apr-2016	x	
<i>ambarvalis</i>	FWC41	tissue only	FWC	Hf	FL	Osceola	F	25-May-2016	x	
<i>ambarvalis</i>	FWC42	tissue only	FWC	Hf	FL	Osceola	F	25-May-2016	x	
<i>ambarvalis</i>	FWC49	tissue only	FWC	Hf	FL	Osceola	M	16-Aug-2016	x	x
<i>ambarvalis</i>	FWC50	tissue only	FWC	Hf	FL	Osceola	F	17-Aug-2016	x	
<i>ambarvalis</i>	FWC57	tissue only	FWC	Hf	FL	Osceola	F	19-Aug-2016	x	

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Subspecies	Tissue no.	Catalog no.	Institution	Tissue	State	County	Sex	Collection date	M	C
<i>interrupta</i>	ASK11912	tissue only	AGFC	Sk	AR	Scott	U	1-Jan-2016	x	x
<i>interrupta</i>	ASK11914	NA	UMBMC	Mu	AR	U	U	U	x	x
<i>interrupta</i>	ASK11915	NA	UMBMC	Mu	AR	Scott	U	19-Feb-2016	x	x
<i>interrupta</i>	ASK11929	not yet cataloged	ASNHC	L	IA	Sac	M	April 2016		x
<i>interrupta</i>	AWF1090	NA	University of Kansas	U	KS	Butler	M	12-Oct-2008	x	x
<i>interrupta</i>	FHSM37522	FHSM37522	Sternberg Museum	L	KS	Gray	M	11-Apr-2007	x	
<i>interrupta</i>	FHSM39054	FHSM39054	Sternberg Museum	L	KS	Linn	M	12-Jan-2010	x	x
<i>interrupta</i>	FHSM39235	FHSM39235	Sternberg Museum	L	KS	Ford	F	10-Mar-2009	x	
<i>interrupta</i>	FHSM39236	FHSM39236	Sternberg Museum	L	KS	Gray	M	20-Jan-2009	x	
<i>interrupta</i>	FHSM39237	FHSM39237	Sternberg Museum	L	KS	Gray	M	19-Apr-2010	x	x
<i>interrupta</i>	FHSM39238	FHSM39238	Sternberg Museum	L	KS	Gray	U	30-Oct-2008	x	x
<i>interrupta</i>	JWD327	NA	NA	DNA	KS	Wallace	U	U		x
<i>interrupta</i>	ASK11881	NA	UNSM	L	NE	Cherry	NA	20-Feb-2017	x	x
<i>interrupta</i>	ASK11870	not yet cataloged	ASNHC	L	SD	Brule	M	27-Mar-2017	x	x
<i>interrupta</i>	ASK11871	not yet cataloged	ASNHC	L	SD	Brule	M	2-Apr-2017	x	x
<i>interrupta</i>	ASK11872	not yet cataloged	ASNHC	L	SD	Brule	M	27-Mar-2017	x	x
<i>interrupta</i>	ASK12461	ASNHC18195	ASNHC	L	SD	Brule	M	Fall 2015	x	x
<i>interrupta</i>	ASK12462	ASNHC18196	ASNHC	L	SD	Brule	M	Fall 2015	x	x
<i>interrupta</i>	ACUNHC1957	ACUNHC1957	ACUNHC	L	TX	Taylor	M	10-Feb-2016	x	x
<i>interrupta</i>	ASK10925	tissue only	ASNHC	E	TX	Waller	M	8-Oct-2015	x	x
<i>interrupta</i>	ASK10926	tissue only	ASNHC	E	TX	Waller	M	10-Oct-2015	x	x
<i>interrupta</i>	ASK11873	not yet cataloged	ASNHC	L	TX	Wichita	M	16-Apr-2017	x	x
<i>interrupta</i>	ASK11884	not yet cataloged	ASNHC	K	TX	Waller	NA	3-Apr-2017	x	x
<i>interrupta</i>	ASK11913	tissue only	ASNHC	Sk	TX	Wilbarger	U	14-Mar-2016	x	x
<i>interrupta</i>	ASK11916	not yet cataloged	ACUNHC	L	TX	Taylor	M	9-Feb-2017	x	x
<i>interrupta</i>	ASK11927	not yet cataloged	ASNHC	Mu	TX	Caldwell	U	2017		x
<i>interrupta</i>	ASK11928	not yet cataloged	ASNHC	Mu	TX	Gonzales	U	2017		x

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Subspecies	Tissue no.	Catalog no.	Institution	Tissue	State	County	Sex	Collection date	M	C
<i>interrupta</i>	ASK12480	tissue only	ASNHC	E	TX	Harris	M	31-Oct-2016	x	x
<i>interrupta</i>	ASK12482	tissue only	ASNHC	E	TX	Harris	M	5-Nov-2016	x	x
<i>interrupta</i>	ASK12490	tissue only	ASNHC	E	TX	Harris	M	1-Nov-2016	x	x
<i>interrupta</i>	ASK12491	not yet cataloged	ASNHC	E	TX	Brazos	U	21-Oct-2015	x	x
<i>interrupta</i>	ASK12693	tissue only	ASNHC	E	TX	Coryell	M	16-Dec-2016	x	x
<i>interrupta</i>	ASK12696	not yet cataloged	ASNHC	L	TX	Harris		1-Jun-2017		x
<i>interrupta</i>	ASK4529	ASNHC10299	ASNHC	L	TX	Bell	F	6-Aug-1996	x	x
<i>interrupta</i>	ASK4856	ASNHC11774	ASNHC	L	TX	Coryell	F	20-May-1996	x	x
<i>interrupta</i>	ASK4858	ASNHC11773	ASNHC	K	TX	Coryell	F	24-Nov-1996	x	x
<i>interrupta</i>	ASK6142	ASNHC13370	ASNHC	L	TX	Coleman	M	21-Mar-2003	x	x
<i>interrupta</i>	ASK6824	ASNHC13369	ASNHC	HK	TX	Brown	M	22-Feb-2004	x	x
<i>interrupta</i>	ASK7225	ASNHC13371	ASNHC	L	TX	Milam	M	17-Apr-2005	x	x
<i>interrupta</i>	ASK7809	ASNHC13372	ASNHC	L	TX	Taylor	M	23-Apr-2007	x	x
<i>interrupta</i>	ASK7814	TCWC59601	TCWC	HK	TX	Waller	M	18-Mar-2005	x	x
<i>interrupta</i>	ASK7874	ASNHC13555	ASNHC	HK	TX	Harris	M	16-Apr-2008	x	x
<i>interrupta</i>	ASK7931	ASNHC13554	ASNHC	L	TX	Waller	M	16-Dec-2008	x	
<i>interrupta</i>	ASK9618	ASNHC14653	ASNHC	Mu	TX	Jack	U	29-Mar-2011	x	x
<i>interrupta</i>	ASK9654	ASNHC14878	ASNHC	Mu	TX	Robertson	U	15-Mar-2011	x	x
<i>interrupta</i>	ASK9686	ASNHC14891	ASNHC	L	TX	Harris	M	19-Mar-2004	x	x
<i>interrupta</i>	JWD384	NA	NA	DNA	TX	Austin	U	U		x
<i>interrupta</i>	JWD386	TCWC59551	TCWC	DNA	TX	Austin	U	U		x
<i>interrupta</i>	JWD387	TCWC59552	TCWC	DNA	TX	Austin	U	U		x
<i>interrupta</i>	JWD405	NA	NA	DNA	TX	Austin	U	U		x
<i>interrupta</i>	JWD418	TCWC59560	TCWC	DNA	TX	Austin	U	U		x
<i>interrupta</i>	JWD420	NA	NA	DNA	TX	Coleman	U	U		x
<i>interrupta</i>	NK8775	MSB135535	MSB	L	TX	Brown	M	26-Aug-1994		x
<i>interrupta</i>	TCWC60748	TCWC60748	TCWC	NA	TX	Harris	M	14-Apr-2009	x	
<i>interrupta</i>	TK29908	MWSU17840	TTU/MWSU	L	TX	Archer	F	18-Jan-91	x	x

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Subspecies	Tissue no.	Catalog no.	Institution	Tissue	State	County	Sex	Collection date	M	C
<i>interrupta</i>	USNM100099	USNM100099	USNM	claw	TX	Nueces	M	21-Apr-1900		x
<i>interrupta</i>	USNM118427	USNM118427	USNM	claw	TX	Palo Pinto	M	7-Oct-1902		x
<i>interrupta</i>	USNM118428	USNM118428	USNM	claw	TX	Palo Pinto	F	7-Oct-1902		x
<i>interrupta</i>	USNM120096	USNM120096	USNM	skull fragments	TX	Palo Pinto	F	7-Oct-1902		x
<i>interrupta</i>	USNM120201	USNM120201	USNM	claw	TX	Nueces	F	10-Apr-1902		x
<i>interrupta</i>	USNM135090	USNM135090	USNM	turbinals	TX	Grimes	M	15-Dec-1904		x
<i>interrupta</i>	USNM32422	USNM32422	USNM	claw	TX	Matagorda	M	15 Jan 1892		
<i>interrupta</i>	USNM97152	USNM97152	USNM	skull fragments	TX	Galveston	F	13 April 1899	x	
<i>interrupta</i>	USNM99776	USNM99776	USNM	claw	TX	Bee	M	12-Apr-1900		x
<i>putorius</i>	AL1		Univ of Auburn		AL	Macon		13-Dec-2013		x
<i>putorius</i>	ASK12466	2017.7.1	AMNH-MC	L	AL	Cleburne	M	7-Feb-2015	x	x
<i>putorius</i>	UWG215	tissue only	UWG	E	AL	Cleburne	M	25-Feb-2015	x	x
<i>putorius</i>	UWG305	tissue only	UWG	E	AL	Clay	M	8-Jul-2016	x	x
<i>putorius</i>	UWG308	tissue only	UWG	E	AL	Clay	M	23-Jul-2016	x	
<i>putorius</i>	UWG355	tissue only	UWG	E	AL	Cleburne	F	17-Jan-2015	x	
<i>putorius</i>	UWG389	tissue only	UWG	E	AL	Clay	M	31-Jul-2016	x	
<i>putorius</i>	UWG424	tissue only	UWG	E	AL	Clay	M	3-Sep-2016	x	
<i>putorius</i>	UWG525	tissue only	UWG	E	AL	Cleburne	M	4-Apr-2015	x	
<i>putorius</i>	UWG585	tissue only	UWG	E	AL	Cleburne	M	4-Apr-2015	x	x
<i>putorius</i>	UWG615	tissue only	UWG	E	AL	Cleburne	M	17-Jan-2015	x	x
<i>putorius</i>	UWG645	tissue only	UWG	E	AL	Cleburne	M	17-Jan-2015	x	
<i>putorius</i>	UWG695	tissue only	UWG	E	AL	Cleburne	M	4-Apr-2015	x	x
<i>putorius</i>	UWG865	tissue only	UWG	E	AL	Cleburne	M	30-Apr-2015	x	x
<i>putorius</i>	WFB8979	WFB8979	MWFB	Mu	AL	Lee	U	U	x	x
<i>putorius</i>	ASK11910	tissue only	UWG	Sk	GA	Marion	U	17-Mar-2017	x	x
<i>putorius</i>	ASK11911	DCNHTC329	DCNHTC	L	GA	Marion	U	31-Mar-2015	x	x
<i>putorius</i>	ASK12467	not yet cataloged	GMNH	H	GA	Towns	M	11-Apr-2015	x	x
<i>putorius</i>	JJK3648	JJK3648	UKVTC	T	KY	Clay	M	31-Mar-2016	x	
<i>putorius</i>	JJK3857	JJK3857	UKVTC	L	KY	McCrery	U	4-Mar-2017	x	x

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<b>Subspecies</b>	<b>Tissue no.</b>	<b>Catalog no.</b>	<b>Institution</b>	<b>Tissue</b>	<b>State</b>	<b>County</b>	<b>Sex</b>	<b>Collection date</b>	<b>M</b>	<b>C</b>
<i>putorius</i>	ASK12468	NC 2016-001	NCSM	H	NC	Graham	M	25-Feb-2016	x	x
<i>putorius</i>	ACC1139	ACC1139	CMNH	Mu	SC	Oconee	M	25-Dec-2006	x	x
<i>putorius</i>	SCF04	tissue only	NA	Hf	SC	Oconee	F	22-Feb-2017	x	x
<i>putorius</i>	SCF05	tissue only	NA	Hf	SC	Oconee	F	25-Feb-2017		x
<i>putorius</i>	SCM04	tissue only	NA	Hf	SC	Oconee	M	23-Feb-2017		x
<i>putorius</i>	SCM14	tissue only	NA	Hf	SC	Oconee	M	25-Feb-2016	x	x
<i>putorius</i>	VA1	NDM4385	VMNH		VA	Smyth	F	13-Dec-2013	x	x
<i>gracilis</i>	ASK5307	ASNHC12085	ASNHC	L	AZ	Pima	M	6-Oct-2001	x	x
<i>gracilis</i>	ASK6259	ASNHC13373	ASNHC	L	AZ	Pima	F	12-Mar-2003	x	x
<i>gracilis</i>	ASK6778	ASNHC13005	ASNHC	L	OR	Curry	M	16-Jun-2004	x	
<i>gracilis</i>	ASK5719	ASNHC12766	ASNHC	L	TX	Irion	M	10-Oct-2000	x	x
<i>gracilis</i>	ASK7359	ASNHC13300	ASNHC	L	TX	Brewster	M	28-Jan-2006	x	
<i>gracilis</i>	ASK7910	ASNHC13558	ASNHC	L	TX	Jeff Davis	M	19-Jul-2008	x	x
<i>gracilis</i>	ASK8487	ASNHC13833	ASNHC	L	TX	Bandera	M	25-Mar-2009	x	x
<i>gracilis</i>	ASK9692	ASNHC14890	ASNHC	L	TX	Tom Green	M	2-Jun-2011	x	