Distribution, Habitat Preferences, and Landscape Genetics of Appalachian Cottontail (*Sylvilagus obscurus*) in Western North Carolina

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Introduction

The Appalachian cottontail (*Sylvilagus obscurus*) is a medium-size rabbit native to the eastern United States and is distributed along the Appalachian Mountains south of the Hudson River in New York to northern Alabama (Chapman et al. 1992, Chapman 2007, Barry 2018, Edelman 2019). Appalachian cottontails were originally considered the same species as the New England cottontails (*S. transitionalis*) until they were determined to be genetically and morphologically separate species (Chapman et al. 1992). Appalachian cottontails are considered a Species of Concern by the U.S. Fish and Wildlife Service and are classified as vulnerable to critically imperiled throughout most of its range (Chapman 2007, NatureServe 2015). Additionally, the Appalachian cottontail is considered Near Threatened by the International Union for Conservation of Nature (IUCN; Barry and Lazell 2008). Within North Carolina, Appalachian cottontails are listed as a vulnerable species and are considered a Knowledge Gap Priority Species (NCWRC 2015).

Typically referred to as a cold-adapted, high-elevation specialist, Appalachian cottontails are usually associated with red spruce (*Picea rubens*) forests, northern hardwood forests, shrub balds, and ericaceous heath balds, although, at lower elevation sites, white pine-hemlock and oak hickory forests are also used by this species (Webster et al. 1985, Chapman et al. 1992, Chapman 2007). The majority of work on this species has been limited to western Maryland and West Virginia (Stevens and Barry 2002, Boyce and Barry 2007, Hartman and Barry 2010), while studies in the southern proportion of its range have been opportunistic or limited in scope (Blymyer 1976, Russell et al. 1999). Additionally, this species is found at lower elevations in Georgia, Alabama, and Kentucky (Sole 1999, Russell et al. 1999, Chapman 2007), indicating that this species is not restricted to high-elevation habitats within North Carolina. Habitat selection and home range estimates for this species in the southern proportion of its range are currently inferred from northern populations.

Appalachian cottontails are known to overlap with eastern cottontails (*S. floridanus*), although the geographic distribution of sympatry is unknown for the majority of the range of Appalachian cottontails. This may be due to the fact that the distribution of Appalachian cottontails is not well documented and is typically limited to county records (Campbell et al. 2010). While eastern cottontails are the most common species of rabbit east of the Rocky Mountains, their distribution within the southern Appalachian Mountains is also not well documented, limiting data on where these two species are sympatric or parapatric. Eastern cottontails are thought to compete with New England cottontails in habitats where they are sympatric (Probert and Litvaitis 1996, Fuller and Tur 2012). Currently there is no evidence of hybridization between eastern cottontails and New England or Appalachian cottontails (Litvaitis et al. 1997, Fuller and Tur 2012). Additionally, it is unknown if habitat competition occurs between eastern and Appalachian cottontails where they are sympatric.

Populations of the Appalachian cottontail are assumed to be declining in many parts of their range. However, the population status and trends in the majority of this species' range, including North Carolina, are unknown, making estimates of the rate at which the species is declining uncertain (Barry and Lazell 2008). Threats to the species are thought to include habitat fragmentation, indirect displacement by eastern cottontail, non-species specific hunting regulations, and lack of knowledge about the species (Barry and Lazell 2008). In order to better understand and better manage Appalachian cottontail populations, research on habitat preferences, distribution, and fragmentation effects on population genetics are needed. Studies from the northern part of the Appalachian cottontail's range and the much more complete body of literature on the New England cottontail have informed projections of the Appalachian cottontail's distribution in North Carolina (Southeast GAP Analysis Program 2011), but these models have not been verified. To the best of our knowledge, there have been no population genetic or habitat selection studies on this species in North Carolina.

Purpose

The purpose of this grant was to conduct a 2-year research project investigating the distribution, habitat preferences, and population genetic structure of the Appalachian cottontail in western North Carolina. Results from this study were aimed at improving the knowledge available to aid the North Carolina Wildlife Resource Commission's management of this species.

Objectives

Assessed habitat selection using scat samples from scat transect surveys and telemetry data from radio-collared individuals in western North Carolina. We estimated home range size from radio-collared individuals. From these data, we created a predictive occupancy map of Appalachian cottontail within the southern Appalachians. These data were used to 1) determine the distribution of Appalachian cottontail within western North Carolina, 2) highlight potential survey areas for monitoring, and 3) determine habitat preferences to guide in habitat management, especially in currently managed areas such as the Roan Mountain Highlands grassy balds.

1. Conducted a genetic analysis on Appalachian cottontail with ear punches from live captured individuals and scat collected from scat transects to determine 1) population genetic structure and migration patterns of Appalachian cottontails in western North Carolina, 2) potential hybridization with the sympatric eastern cottontail, and 3) estimates of parameters of identified populations (i.e. effective population size, genetic diversity, inbreeding levels, etc.). Understanding population size, gene flow between populations, and potential hybridization with eastern cottontails is important in determining management objectives for the species. Additionally, these data were used to determine impacts that habitat modification and climate change are having on eastern cottontail encroachment into Appalachian cottontail habitat.

Methods

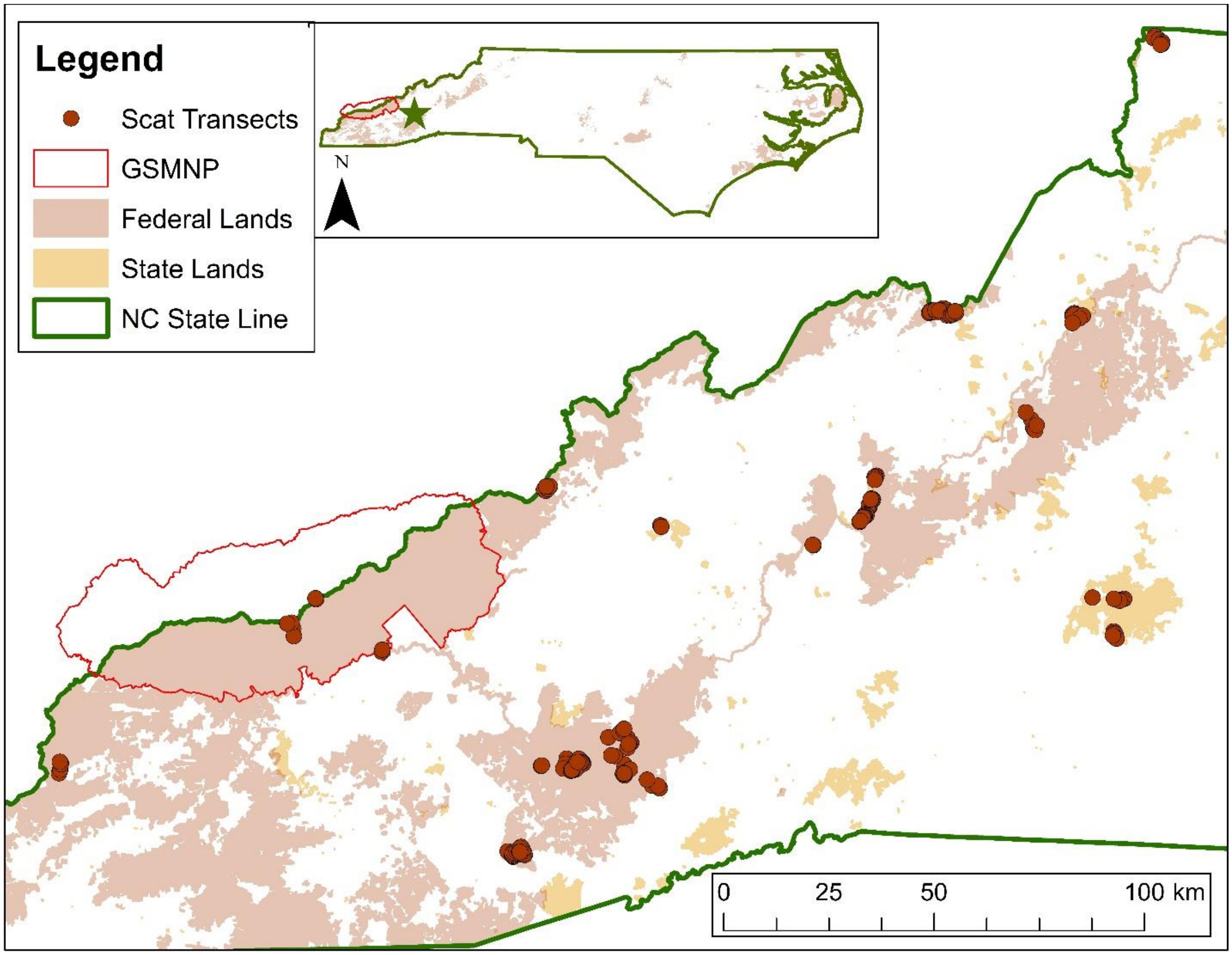
Study Area

Our study occurred in the Blue Ridge Mountain subregion of the southern Appalachian physiographic province in western North Carolina. This region is defined by deep valleys and high peaks and ridgelines. Habitats within the region range from high-elevation montane red spruce (*Picea rubens*) - Fraser fir (*Abies fraserii*) forests to low elevation oak (*Quercus* spp.) and white pine (*Pinus strobus*) - hemlock (*Tsgua* spp.) forests. The majority of sites in this study were second-growth and had been logged and/or burned during the industrial logging period at the turn of the 20th century. The only old-growth forest we surveyed was in Great Smoky Mountains National Park.

Scat Surveys

We employed scat surveys to assess large scale habitat use and preferences of Appalachian cottontail. Between May 2016 and March 2019, we conducted 227 scat surveys along 90 m transects spaced a minimum of 250 m apart at elevations ranging from 383 to 2006 m elevation. Focal study periods included May-June 2016, April 2017, March 2018, and Jan-Mar 2019. Our transects included surveys of xeric and montane oak forests, lowland cove forest, northern hardwood forest, spruce-fir forest and grass and shrub balds (Figure 1). Each transect included 10 plots of 2 m radius each, spaced 10 m apart. Transects were marked with a GPS and each 2 m radius plot was exhaustively searched for rabbit scat. We counted all scat appearing fresh (retaining a slight green or dark brown, rather than light tan color) and in clear clumps of pellets as a single scat detection, and all such clumps were tallied for each plot, with pellet clump counts serving as an index of rabbit use of each plot. We used all fresh samples for genetic analysis (see *Genetic Analysis* section below), and we only used those identified as Appalachian cottontail for spatial distribution modeling. In addition to transect-generated scat samples, fresh fecal samples were opportunistically collected as they were discovered during the other sampling efforts of this project. These samples were only used for population genetics and species distribution modeling efforts.

Figure 1. Location of scat transects for Appalachian cottontail (*Sylvilagus obscurus*) conducted in 2016, 2017, 2018, and 2019 in western North Carolina. GSMNP = Great Smoky Mountains National Park.



Opportunistic Roadkill Specimens

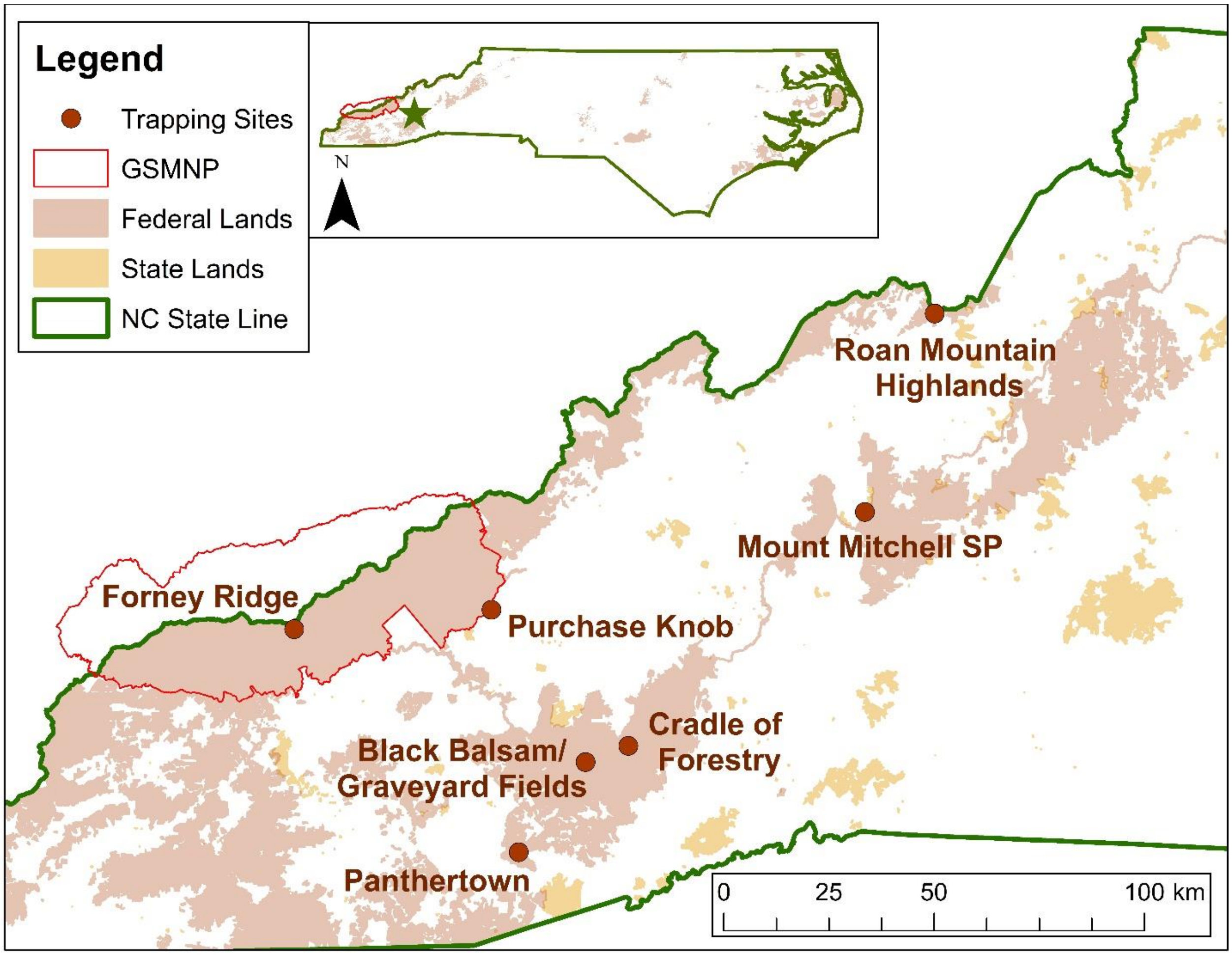
We obtained additional specimens by collecting roadkill samples and obtaining samples from state and federal partners (Table 1). We identified species by pelage characteristics and, if possible, measured ear length and hind foot length. We then collected 2 ear punch samples using a 2mm ear punch(Fine Science Tools, Inc., Foster City, CA; see *Genetic Analysis* section). For roadkill specimens, we collected genetic material, took measurements, and recorded the location of the roadkill but left the roadkill on the shoulder of the road by the location of death. For specimens collected by partners, we took genetic materials and measurements but returned specimens to the partners that originally collected them.

Live Trapping

To obtain live captures, we used Tomahawk live traps (Model 205; 26L x 9W x 9H cm; Tomahawk Live Trap Co., Hazelhurst, WI) to capture individual rabbits. During spring and fall of 2018 and fall 2019, we focused trapping effort at 4 high elevation sites and 3 low elevation sites (Figure 2). High-elevation sites were 1,500 - 2,000 m in elevation. Habitat at high-elevation sites included spruce-fir, northern hardwood, grassy balds, shrub balds, and heath balds. Spruce-fir forests were composed of red spruce, Fraser fir, yellow birch (*Betula alleghaniensis*), and mountain ash (*Sorbus americana*). Northern hardwood forests were composed of yellow birch, American beech (*Fagus grandifolia*), sugar maple (*Acer saccharum*), and yellow buckeye (*Aesculus flava*) in the overstory. Grassy balds were dominated by mountain oat grass (*Danthonia compressa*), as well as red sorrel (*Rumex acetosella*) and dwarf cinquefoil (*Potentilla candadensis*). Shrub balds were dominated by Allegheny blackberry (*Rubus allegheniensis*). Heath balds were mainly composed of Catawba rhododendron (*Rhododendron catawbiense*), mountain azalea (*Kalmia latifolia*), flame azalea, and blueberries (*Vaccinium* spp.). Low elevation sites ranged between 980-1,220 m in elevation. Habitat at low elevation sites included early successional habitat, oak, and pine-hemlock forests. Early successional habitat Oak forests are dominated by northern red oak (*Q. rubra*), white oak (*Q. alba*), chestnut oak (*Q. prinus*), scarlet oak (*Q. coccinea*), and red maple (*Acer rubrum*). Pine-hemlock forests are composed of white pine and eastern hemlock (*T. canadensis*) or Carolina helmock (*T. caroliniana*), typically with an understory of rhododendron. We opportunistically placed traps at sites, focusing on locations with understory cover to increase potential capture success. We covered traps with plastic wrap and duct tape and placed polyfil batting into each trap to reduce potential trap stress and hypothermia. We baited traps with apples. We did not set traps in inclement weather (i.e., heavy rain, snow storms), as these weather events might increase potential trap-induced mortality or restrict access to trapping sites at higher-elevations. We set traps 30-60 minutes before dusk and checked at dawn. We kept traps closed during the day due to low capture rates of Appalachian cottontails during daytime sessions as well as to to prevent potential heat stress of captures.

We removed individuals from traps and placed them in a cotton pillowcase, which kept the cottontail calm and prevented injury. We aged, sexed, measured (weight, ear length, right rear foot), and ear tagged each individual. We used self-piercing 1005-3 Monel ear tags (National Band and Tag Co., Newport, KY) for ear tagging. We differentiated Appalachian cottontails from eastern cottontails via pelage characteristics by using the field methods described by Livaitis et al. (1991). We took genetic samples using a 2 mm ear punch (see *Genetic Analysis* section). For individuals field identified as Appalachian cottontails, we radio-collared adult cottontails >700 g using MI-2 radio-collars (Holohil Systems Ltd., Carp, Ontario, Canada). The radio-collar weighted ~27g, consisting of <4% of the collared cottontail's total body weight. Our methods for capture and tagging were approved by the Virginia Tech Institutional Animal Care and Use Committee (permit #16-049-FIW). We released all captures at their capture site.

Figure 2. Trapping site locations for Appalachian cottontail (*Sylvilagus obscurus*) during 2018 and 2019 in western North Carolina.



Telemetry Surveys

We waited 48-72 hours after the initial capture of each radio-collared cottontail prior to tracking, allowing for individuals to adjust to the collar and resume normal movements (White and Garrott 1990). We tracked both diurnal and nocturnal movements of radio-collared cottontails to more accurately estimate home range and habitat use. We used simultaneous biangulation to obtain telemetry fixes on cottontails, while removing temporal bias (Schmutz and White 1990). This technique was appropriate for highly mobile small-bodied mammals that occupy relatively small homes ranges (<20 ha; Koprowski et al. 2008, Diggins and Ford 2017), such as Appalachian cottontails, which typically had home ranges <15 ha (Stevens and Barry 2002, Boyce and Barry 2007). Additionally, this technique reduced the potential impact of observer movement on activity of radio-collared cottontails since observers were stationary for the duration of the tracking period.

We placed telemetry stations >50 m apart and minimized bearings taken at <90° angles (White and Garrott 1990). We tracked cottontails during 4 hours sessions, where sessions were systematically blocked across a 24-hour time frame (i.e., 200-600, 600-1000, 1000-1400, etc.). We tracked individuals 1-3 times every 7-10 days and sessions were set a minimum of 12 hours apart, which allowed us to reduce travel to more remote sites. We rotated tracking sessions so each rabbit was tracked across the 24-hour period. We tracked individuals until their collars fell off, the individual died, or to the end of the study.

Data Analysis

Predictive Occupancy Map

Using live capture and scat-based occupancy data, we conducted species distribution modeling to determine which landscape factors Appalachian cottontail selected for across the study area. We used the program Maxent (version 3.4.1; Phillips et al. 2020), incorporating geospatial climatic (BioClim Version 2, Fick and Hijmans 2017; Appendix A) and habitat layers (SE-GAP), to conduct maximum entropy modeling of the geographic distribution of Appalachian cottontails in western North Carolina.

Only genetically-confirmed Appalachian cottontail points were used for this analysis. To adjust for spatial clustering due to focused sampling in certain areas (e.g. Black Balsam and Roan Highlands; Syfert et al. 2013), we randomly culled the 197 confirmed Appalachian cottontail records to 500 m minimum spacing in ArcGIS using the Create Random Points tool (ESRI 2019). This resulted in 46 input points for the model. We clipped BioClim and SE-GAP rasters to the same extent using the Extraction by Mask Layer tool in QGIS (QGIS 2019). The mask layer used was a boundary of North Carolina created by using a dissolve tool on a NC counties boundary shapefile downloaded from NC One Map (NCDOT 2020). We aligned our bioclimatic raster layers with the SE Gap raster (30 m pixels) using the “Clip” tool with a “Snap Raster Environment” in ArcGIS Pro. We then reprojected all rasters to WGS 84 CRS so the occurrence points would overlay using Maxent. The final pixel size was 0.00032, -0.00032 degrees. Our settings in Maxent included a 25% random test percentage with a maximum number of background points of 10,000; otherwise, all settings were the default options in this version of Maxent.

Home Range and Habitat Use Analysis

We entered locations of telemetry stations and biangulation bearings into the software program LOCATE II (Pacer Co., Truro, Nova Scotia, Canada) to obtain UTM coordinates of locations for radio-collared individuals. Using all locations for each cottontail, we estimated home ranges using convex polygon (MCP) and biased random bridge (BRB) estimators at the 50% (core habitat) and 95% isopleth in package *adehabitat* (Calenge 2006, 2020) in Program *R* version 3.1.2 (*R* Development Core Team 2020). We used MCP to compare home range estimates from our study with other studies on Appalachian and eastern cottontail, as this estimator is commonly used in older studies. We calculated home range using BRB, a method that used an advective-diffusive movement process to link sequential points to estimate local space use density, thereby incorporating abilities of animals to preferentially select for more attractive areas within home ranges while accounting for movement processes (Benhamou 2011). We compared home range size between the sexes and high- and low-elevation sites using Wilcoxon rank-sum tests in Program *R*.

We determined habitat use based on use vs. availability through a Euclidean distance-based analysis approach, which analyzes habitat use in a linear fashion, accounting for the use of ecotones and bias in radio-telemetry fixes (Conner et al. 2003). We evaluated habitat use with this method by comparing the distances of animal locations and random locations to the nearest edge of different habitat types (Conner and Plowman 2001). As this method was adaptable to multiple spatial scales, we assessed habitat selection at the 2nd order (home range selection within the landscape) and 3rd order (within home range selection) scale with the 95% BRB home range for each individual cottontail. At the 2nd order scale, we selected a 1000-m buffer around the home range of each radio-collared individual.

We generated random points equal to the number of telemetry fixes per cottontail on the 2nd order (i.e., within 1000-m buffer) and 3rd order scale (i.e., within 95% BRB home range) in ArcGIS 10.2 (Environmental Systems Research Institute, Inc., Redlands, CA). We grouped cottontails into two groups (high-elevation and low-elevation) for habitat selection analysis because vegetation communities are strongly correlated with elevation in the southern Appalachians. We reclassified habitat types using habitat shapefiles from Southeast Gap Analysis data (www.basic.ncsu.edu/segap/, verified October 2019) and aerial imagery (i.e., ArcMap imagery basemap) in ArcGIS using similar techniques to Diggins et al. (2017). We classified habitat for the Euclidean distance-based analysis depending on available habitat within the 1000m buffers and the home ranges of all cottontails in the high-elevation and low-elevation groups. For the 2nd order analysis, we classified high-elevation habitat as spruce-fir forests, northern hardwood, grassy balds, shrub balds, heath balds, and oak, whereas we classified low-elevation habitat as oak, conifer (i.e., white pine, hemlock), early successional, and heath bald. For the 3rd order analysis, we classified high-elevation habitat as spruce-fir, northern hardwood, grassy balds, shrub balds, and heath balds, whereas we classified low-elevation habitat as oak forests, conifer forests, and early successional.

We measured distances between random points and telemetry to each habitat type. We then created distance ratios of telemetry:random points using averaged distances for each individual cottontail to the closest representative habitat type for the 2nd and 3rd order scale. If habitat use of a particular habitat type was non-selective (i.e., occurred randomly), habitat ratios would equal 1.0. If habitat ratios are <1.0, the cottontail is using that habitat type more than expected given its availability on the landscape and the habitat was selectively used. However, if habitat ratios are >1.0, the cottontail was using that habitat type less than expected given its availability on the landscape and that habitat type was avoided. We followed the methodology outlined in Conner and Plowman (2001) for Euclidean distance-based analysis. First, we determined if habitat selection occurred between habitat types by running a multivariate analysis of variances (MANOVA) to determine if distance ratios were different from 1.0. If habitat selection occurred between habitat types, we tested to see if telemetry:random distance ratios for each habitat type differed from 1.0 using t-tests to determine cottontails' selection or avoidance of each habitat type based on the availability of that habitat type on the landscape at both spatial scales. Finally, we ranked the habitat preferences of all combinations of habitat type distance ratios at both spatial scales using a series of pairwise t-tests. We conducted all habitat selection analysis in Program *R* and considered statistical significance if $\alpha \geq 0.05$.

Genetic Analysis

Species Identification

Due to the cryptic nature of Appalachian and eastern cottontails, all scat and tissue samples were tested for positive identification as Appalachian cottontail prior to their use in habitat use modeling efforts. We extracted DNA from tissue using Qiagen DNEasy extraction kits. We extracted scat samples using a QIAamp DNA Stool Mini Kit (Qiagen #51504) following manufacturer instructions on all but centrifuging techniques, the time for which will be doubled to maximize supernatant yield. We initially conducted species identification using restriction enzyme-based methods adapted from those outlined in Litvaitis and Litvaitis (1996) and Kovach *et al.* (2003). We amplified extracted samples via polymerase chain reaction and ran them on an eGel to select for bands of approximately 600 base pairs, the length of the target region. Samples were digested using Bfa I, a restriction enzyme which produced two fragments for Appalachian cottontails and three fragments for eastern cottontails. These fragment patterns could be easily discerned via gel electrophoresis (Figure 3). However, after questionable preliminary results, we decided to sequence the target gene. Sequencing showed that the fragment approach of Litvaitis and Litvaitis (1996) did not identify all *S. obscurus* due to single nucleotide polymorphisms (SNPs) in the area of the gene targeted by the restriction enzymes. This is not surprising, as Litvaitis and Litvaitis (1996) designed their protocol for New England cottontails and not Appalachian cottontails. Thus, we decided to develop a quantitative PCR (qPCR) approach to species identification. Quantitative PCR is a much more reliable method for species identification than fragment analysis and has the advantage of less steps and thus less chance of contamination.

We designed primer probes from the Cytochrome b region of the mitochondrial genome using Geneious (V. 7.1.9). We designed primers and probes (Table 2) independently for Appalachian and eastern cottontails. Our general approach was to run each sample in triplicate with each set of probe/primers and to compare average CT values between the two sets. We tested this approach with 40 known (sequenced) samples to ensure that tissues and scat could be accurately and repeatedly identified and we did not find any inaccurate identifications. Both probe/primer sets were run for an initial 15 minute denaturing step at 95℃, followed by 50 cycles of a 94℃ denaturing step for 1 minute and a 62℃ annealing step. Data collection occurred during the annealing step. We recorded cycle threshold (CT) values for each sample (in triplicate).

Table 2. Probes and primers designed for *Sylvilagus floridanus* and *Syvilagus obscurus*. Primers are from the Cytochrome b region of the mitochondrial genome.

Primer Name	Primer
Sf_cytb_probe	/56-FAM/CTGCCTTTA/ZEN/TATACACGTCGGCC/3IABkFQ/
So_cytb_probe	/56-FAM/CTTCTTCGC/ZEN/GTTCCATTTTATCTTACCA/3IABkFQ/
Sf_cytb_F	CGTTATCTTCACGCTAATGGA
Sf_cytb_R	CCTATGAATGCTGTAGCTATCAC
So_cytb_F	ACATCGGAACGACTTTAGTC
So_cytb_R	CCGGTTTCGTGAAGAAAAAGT

Population Genetics

For this study, we employed a relatively new technique referred to as RADcap (Hoffberg et al. 2016). RADcap combined the best features of two commonly used "next generation sequencing" (NGS) techniques: sequence capture (Okou et al. 2007, Gnirke et al. 2009) and restriction-site associated DNA sequencing (RADseq; Miller et al. 2007, Baird et al. 2008, Davey and Blaxter 2010, Davey et al. 2011, Peterson et al. 2012). The melding of these approaches provided an ideal combination of sufficient genetic data (hundreds to thousands of data points) with extensive geographic representation (i.e. hundreds to thousands of individuals).

We developed molecular "baits" by first sequencing 12 individuals using a RADseq approach. Individual extractions were normalized and prepared using a 3RAD library procedure (Adapterama III; Bayona-Vásquez et al. 2019: bioRxiv: 205799). The three enzymes used during the digestion step were BAMHI, MSPI, and ClaI. Each sample was then quadruple-indexed, limited-cycled in PCR, and cleaned using speed beads (Rohland and Reich 2012) following the 3RAD procedure. Finally, we pooled samples together, size selected for 500 bp on a Pippin Prep (Sage Science), and sequenced on an Illumina Next-Seq 500 V.2 150 bp SR flow cell (Illumina Inc.) at the NC State University Genomic Sciences Laboratory with 5 million reads per sample. The 3RAD sequence data was demultiplexed, quality assessed, clustered, consensus called, and assembled de novo, using ipyrad v0.7.28 (Eaton and Overcast 2016).

The resultant 22,386 SNPs were then filtered in VCFtools v0.1.14 (Danecek et al. 2011). Resultant SNPs met the following requirements: minimum and maximum number of alleles per site of 2, minimum mean depth of coverage of 5, minor allele frequency of 0.2 (to remove singletons), and present in at least 50% of samples. We removed indels. Using these resultant SNPs, we produced a second SNP dataset by excluding heterozygous sites. We then selected informative SNPs across both species and sent them to Arbor BioSciences (Ann Arbor, MI) to develop 2,500 molecular baits. We then ran all 55 tissues samples according to the methods outlined in Hoffberg et al. (2016).

Population Genetic Structuring

We used two Bayesian methods to investigate the genetic structuring of populations. The first was implemented in TESS version 2.3 (Chen et al. 2007). TESS used hidden Markov random fields to model spatial dependence among individuals (Chen et al. 2007). This approach had the advantage that it incorporates the a priori assumption that individuals near one another are more likely to have similar allele frequencies than individuals from widely separated localities. We ran TESS for 100,000 simulations with a burn-in of 20,000. To estimate K (where K equals the number of populations), we ran 100 replicates each for K values ranging from 3 to 9. For each K, we averaged the 10 best deviance-information-criterion values and plotted them. Once we established the K value, the 10 runs with the lowest deviance-information-criterion values for that K were exported to CLUMPP version 1.1.2 (Jakobsson and Rosenberg 2007). We used the FullSearch algorithm in CLUMPP to match cluster membership over multiple runs. We repeated the above procedure using both the admixture and the no-admixture models implemented in TESS version 2.3 (Chen et al. 2007). Because the models did not differ significantly, we used the results from the no-admixture model, as recommended by the authors. We also studied the spatial genetic patterns by means of STRUCTURE 2.3.4 (Pritchard et al. 2000). STRUCTURE uses a Bayesian framework to assign individuals to populations by maximizing HWE within populations, and is one of the most commonly used structuring programs. The DK method of Evanno et al. (2005) was used to assess the best value of K. For each run of STRUCTURE, the program was run for 1,000,000 MCMC cycles, with a burn-in of 100,000 and default settings. We also assessed the number of populations using a K-means clustering approach (Meirmans 2012) in Genodive (Meirmans and Van Tienderen 2004) using a Bayesian Information Criterion.

Genetic Diversity, Gene Flow, and Hybrid Detection

Once populations were defined, we uploaded data into GenoDive v2.0b25 (Miermans and Tienderen 2004). Measurements of genetic diversity and differentiation were calculated at the individual, population, and species level. These include F'_{ST} and G'_{ST} for population differentiation, G_{IS} for a measure of inbreeding, and an Analysis of Molecular Variance (AMOVA). We identified potential hybrids using population assignment likelihood ratios in GenoDive v2.0b25 (Miermans and Tienderen 2004) and by Structure 2.3.4 (Pritchard et al. 2000).

RESULTS

Objective 1

Predictive Occupancy Model

After randomly culling our data set to reduce spatial bias, we used the resulting 46 genetically-confirmed Appalachian cottontail records along with climatic and land cover variables to predict this species' occupancy in the state of North Carolina (Figure 3). The resulting model demonstrated strong predictive performance, as indicated by AUC values of 0.985 and 0.948 for training and testing data, respectively (Figure 4).

Figure 3. Predictive occupancy map for Appalachian cottontail (*Sylvilagus obscurus*) in North Carolina. Models were run for the entire state; however, only the region with predicted occupancy is shown.

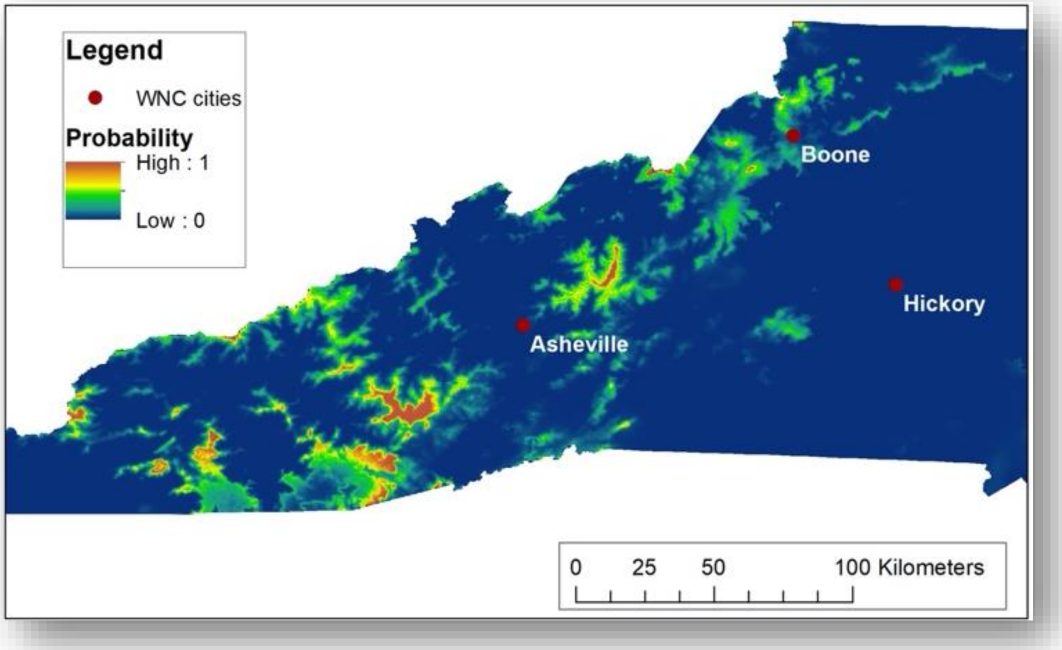
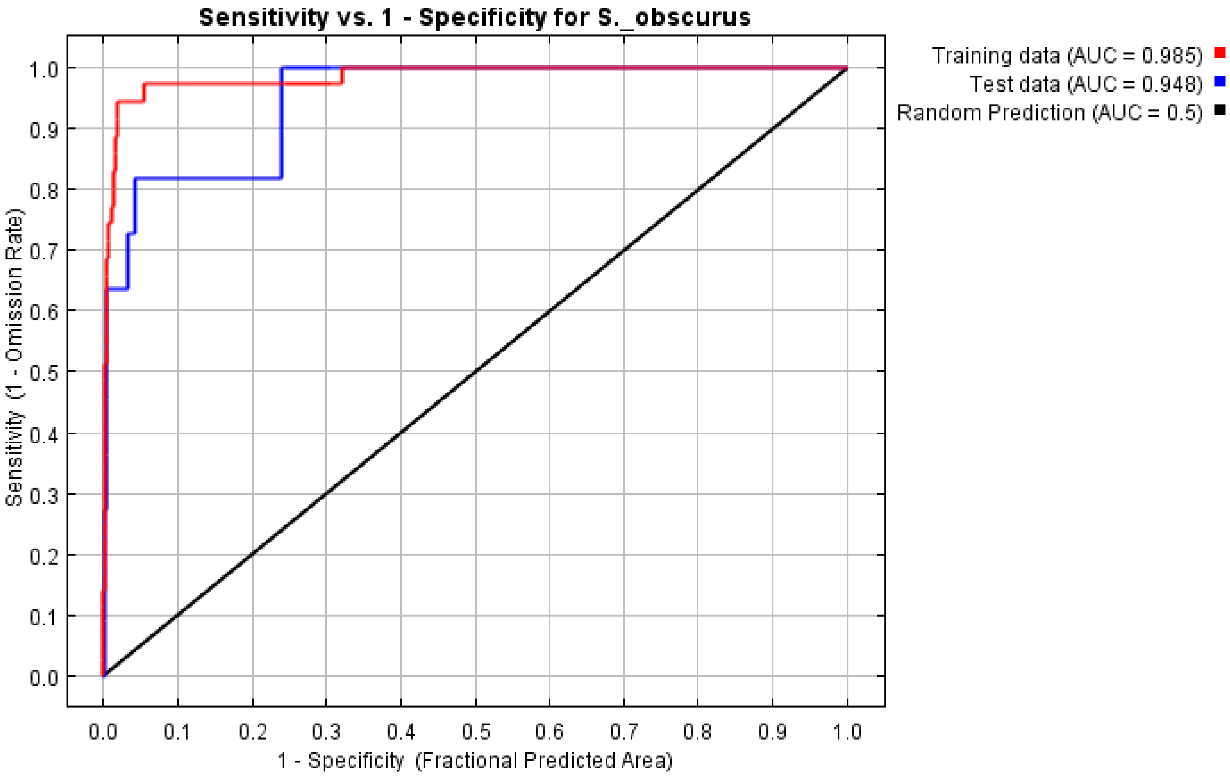


Figure 4. Receiver operating characteristic (ROC) curve for the *S. obscurus* Maxent model, which utilized 46 locations and a 25% random test percentage (i.e. n=35 for training data and n=11 for test data). AUC values above 0.9 are considered representative of strong model prediction performance.



Variables contributing most to model fit, as indicated by permutation importance in Maxent, were bio_2: mean diurnal temperature range, bio_8: mean temperature of wettest quarter, and bio_15: precipitation seasonality (Table 3). Plots of predicted occupancy's dependence on each of these variables as well as all other variables tested can be seen in Appendix B. Jacknife tests of variable importance indicated several additional variables as influential in predicting Appalachian cottontail occupancy, including bio_5: maximum temperature of warmest month, bio_10: mean temperature of warmest quarter, bio_1: mean annual temperature, and land cover 3).

To disentangle the complex predictive power of co-varying climate variables, we produced a Pearson's correlation coefficient matrix using the Band Collection Statistics tool in ArcGIS (ESRI 2019; Appendix C). Correlated variables of interest included a suite of temperature and seasonality variables, demonstrating statewide trends in temperature and precipitation seasonality: bio_1 (mean annual temperature) was correlated with bio_5 (maximum temperature of warmest month; r=0.90), bio_8 (mean temperature of wettest quarter; 0.71), bio_10 (mean temperature of warmest quarter; r=0.97), and bio_15 (precipitation seasonality; r=0.72). Bio_5 was also correlated with bio_10 (mean temperature of warmest quarter; r=0.96). Bio_8 and bio_10 were also correlated (r=0.75).

Table 3. Influential variables in species distribution modeling for Appalachian cottontail (*Sylvilagus obscurus)*. For specific information on the nature of the relationship between each variable and Appalachian cottontail predicted occupancy, please reference Appendix B. Permutation importance represents the degree to which the final Maxent model depended on a given variable, training gain represents the amount of explanatory gain acquired in a univariate model generated from the training data (the 75% of points selected randomly by Maxent), test gain represents similar gain for a model using the 25% test data, and AUC (area under curve) values are measures of fit for univariate models (Phillips 2017). Maximum value for a given metric is bolded.

Variable Code	**Variable Name/ Description**	**Relationship to *S. obscurus* occupancy**	**Permutation Importance (%)**	**Training Gain**	**Test Gain**	**AUC**
bio_15	Precipitation Seasonality	Peak occupancy at low seasonality (areas with consistent precipitation throughout the year)	23.2	1.35	1.5	0.92
bio_8	Mean Temperature of Wettest Quarter	Peak occupancy at low temperatures (2-12℃)	26.2	1.79	1.3	0.92
bio_2	Mean Diurnal Range (Mean of monthly (max temp - min temp))	Peak occupancy at mid-range: 6-9℃	**29**	2.0	0.8	0.83
bio_5	Max Temperature of Warmest Month	Peak occupancy in mid/upper range: ~20℃	0	**2.86**	1.94	**0.95**
bio_10	Mean Temperature of Warmest Quarter	Peak occupancy in mid-range: ~15℃	0	2.76	1.92	**0.95**
bio_1	Mean Annual Temperature	Peak occupancy in mid-range, ~6-8℃	0	2.67	1.90	0.94
SE-GAP	Land cover	Most predictive habitat types (all 100%): • Central and Southern Appalachian Northern Hardwood • Central and Southern Appalachian Spruce-fir Forest • Southern Appalachian Grass and Shrub Bald	0	1.9	**2.93**	0.92

Home Range and Habitat Use

Over the course of 5,488 trap nights, we captured 52 cottontails (40 Appalachian cottontails, 12 eastern cottontails; Appendix D) and collared 26 of those individuals (14 males, 12 females). Additional non-target captures include 20 Virginia opossums (*Didelphis virginiana*), 9 raccoons (*Procyon lotor*), 9 red squirrels (*Tamiasciurus hudsonicus*), 4 eastern gray squirrels (*Sciurus carolinensis*), 1 Carolina northern flying squirrel (*Glaucomys sabrinus coloratus*), 1 mouse (*Peromyscus* spp.), and 1 common rat (*Rattus rattus*; Appendix D). Post-genetic analysis showed 3 of our collared rabbits were eastern cottontails and 2 had inconclusive genetic confirmation; therefore, we removed these individuals from further analysis. Of the 22 genetically confirmed Appalachian cottontails we tracked, 20 had >30 telemetry locations (12 males, 8 females; Appendix E). We gathered 1,762 telemetry points (average of 88±10 points/cottontail; range: 34-200) on these 20 individuals. We tracked individuals for an average of 13±1.5 weeks (range: 3-24). Inter-locations of radio-collared cottontails were non-normally distributed with a strong positive skew and individuals moved an average of 31.5 m ±3.4 SE (range: 4.9-87.9) between locations.

Home Range

For Appalachian cottontails, average MCP home range estimates were non-normally distributed and had a strong positive skew at both the 50% and 95% levels. Average MCP at the 50% and 95% home range was 0.80 ha ± 0.13 SE (range: 0.21-2.16) and 3.4 ± 0.75 (0.55-13.78), respectively (Appendix E). We did not find any differences in MCP home range size between males and females at the 50% (*W*=44.5, *p*=0.82) or 95% level (*W*=49, *p*=0.97). There was no difference in MCP home range size between high- and low-elevation cottontails at the 50% (*W*=46, *p*=0.91) or 95% level (*W*=43, *p*=073).

The average BRB diffusion parameter was 67.9 $m^2 min^{-2}$ + 17.6 SE (range: 6.0-342.9). Home range estimates at both the 50% and 95% levels were non-normally distributed and displayed a strong positive skew. Average BRB at the 50% and 95% home range was 1.08 ha ± 0.18 SE (range: 0.11-2.58) and 5.72 ± 1.15 (0.83-19.44), respectively (Appendix E). There were no differences in BRB home range sizes between males and females at the 50% (*W*=46, *p*=0.91) or 95% level (*W*=33, *p*=0.27). Additionally, we found no differences in BRB home range sizes between high- and low-elevation sites at the 50% (*W*=52, *p*=0.79) or 95% (*W*=45, *p*=0.85). We showed figures of all BRB home range estimates in Appendix F.

Habitat Use

We tracked 13 Appalachian cottontails at high-elevation sites and 7 at low-elevation sites. At the high-elevation sites, the average distance between Appalachian cottontail telemetry points and the nearest spruce-fir was 90.1 m ± 35.8 SE, northern hardwood was 220.4 ± 64.5, grassy bald was 388.7 ± 136.8, shrub bald was 144.3 ± 111.6, heath bald was 151.3 ± 82.2, early successional was 977.2 ± 139.8, and oak was 1342.4 ± 79.4. On the 2nd order scale, the average distance between random points and the nearest spruce-fir was 86.1 m ± 26.2 SE, northern hardwood was 130.7 ± 28.9, grassy bald was 437.1 ± 84.94, shrub bald 280.4 ± 45.9, heath bald 317.2 ± 76.3, early successional 917.0 ± 126.6, and oak habitats 1165.7 ± 82.3. On the 3rd order scale, the average distance between random points and the nearest spruce-fir was 129.9 m ± 46.5 SE, northern hardwood was 240.7 ± 72.2, grassy bald was 309.0 ± 104.1, shrub bald was 78.2±39.9, and heath bald was 204.4 ± 86.9.

At low-elevation sites, the average distance between Appalachian cottontail telemetry points and the nearest oak was 61.3 m ± 16.1 SE, pine/hemlock was 15.6 ± 9.8, early successional was 36.4±15.9, and heath bald habitats was 1160.5±95.9. On the 2nd order scale, the average distance between random points and the nearest oak was 9.4±1.5, pine/hemlock was 294.3±32.4, early successional was 267.2±11.5, and heath balds was 1240.2±103.9. On the 3rd order scale, the average distance between random points and the nearest oak was 58.2±14.7, pine/hemlock was 22.9±11.7, and early successional was 41.7±14.7.

We found habitat selection was occurring on the 2nd and 3rd order scale at both high-elevation ($F_{7,6}$=248.37, P=0.000; $F_{5,8}$=34, P=0.000, respectively) and low-elevation sites ($F_{4,3}$ =4200, P=0.000; $F_{4,3}$=95.3, P=0.000, respectively). In high-elevation sites, cottontails selected for heath balds on the 2nd order scale more than expected given that habitat's availability on the landscape, and they avoided oak forests, using that habitat less than expected given oak availability on the landscape (Table 4). Cottontails did not select or avoid spruce-fir, northern hardwood, grassy bald, shrub bald, or early successional habitat and used these habitats proportionally to their availability on the landscape (Table 4). On the 3rd order scale, cottontails selected for heath balds more than expected, whereas other habitat types were neither avoided nor selected for given their availability on the landscape (Table 4). In low-elevation sites, cottontails at the 2nd scale selected for pine/hemlock, early successional, and heath balds, whereas they avoided oak forest more than expected based on their availability (Table 4). At the 3rd scale, cottontails selected for pine/hemlock more than expected and they did not select for or avoid early successional or oak habitat within their home ranges (Table 4). Ranked habitats showed preferential selection of certain habitat types over others in both high- and low-elevation habitats (Table 5). At high-elevation sites on the 2nd order scale, we found cottontails significantly closer to shrub bald < heath bald < spruce-fir < grassy bald < oak < northern hardwood < early successional. At low-elevation sites on the 2nd order scale, we found cottontails significantly closer to pine/hemlock < early successional < heath bald <oak. We found cottontails equally close to all habitat types on the 3rd order scale for both high and low elevation sites.

Table 4. T-tests of Appalachian cottontail (*Sylvilagus obscurus*) distance ratios for telemetry:random points indicating use versus availability on the landscape (2nd order scale) and home range (3rd order scale). Results are t-statistics (p-values).

	Habitat	**2nd order scale**	**3rd order scale**
High-elevation	Spruce-fir	-0.96 (0.357)	0.24 (0.817)
	Northern hardwood	1.92 (0.079)	-0.59 (0.566)
	Grassy bald	-0.50 (0.627)	0.98 (0.348)
	Shrub bald	-1.99 (0.070)	0.80 (0.440)
	Heath bald	-5.8 (0.000)	-2.4 (0.036)
	Early successional	0.94 (0.366)	---
	Oak	3.1 (0.009)	---
Low-elevation	Oak	2.7 (0.037)	0.38 (0.72)
	Pine/hemlock	-10.7 (0.000)	-2.5 (0.047)
	Early successional	-11.6 (0.000)	-1.25 (0.259)
	Heath Bald	-4.7 (0.003)	---

Table 5. Ranking matrix of Appalachian cottontails (*Sylvilagus obscurus*) habitat selection in western North Carolina during 2018-2020. Results are t-statistics (P-values) of pairwise comparisons of habitat type telemetry:random distance ratios. Values shown indicate t-statistic (p-value). Negative t-statistics indicate the column habitat was selected over the row habitat, whereas positive t-statistics indicate the row habitat was selected over the column habitat.

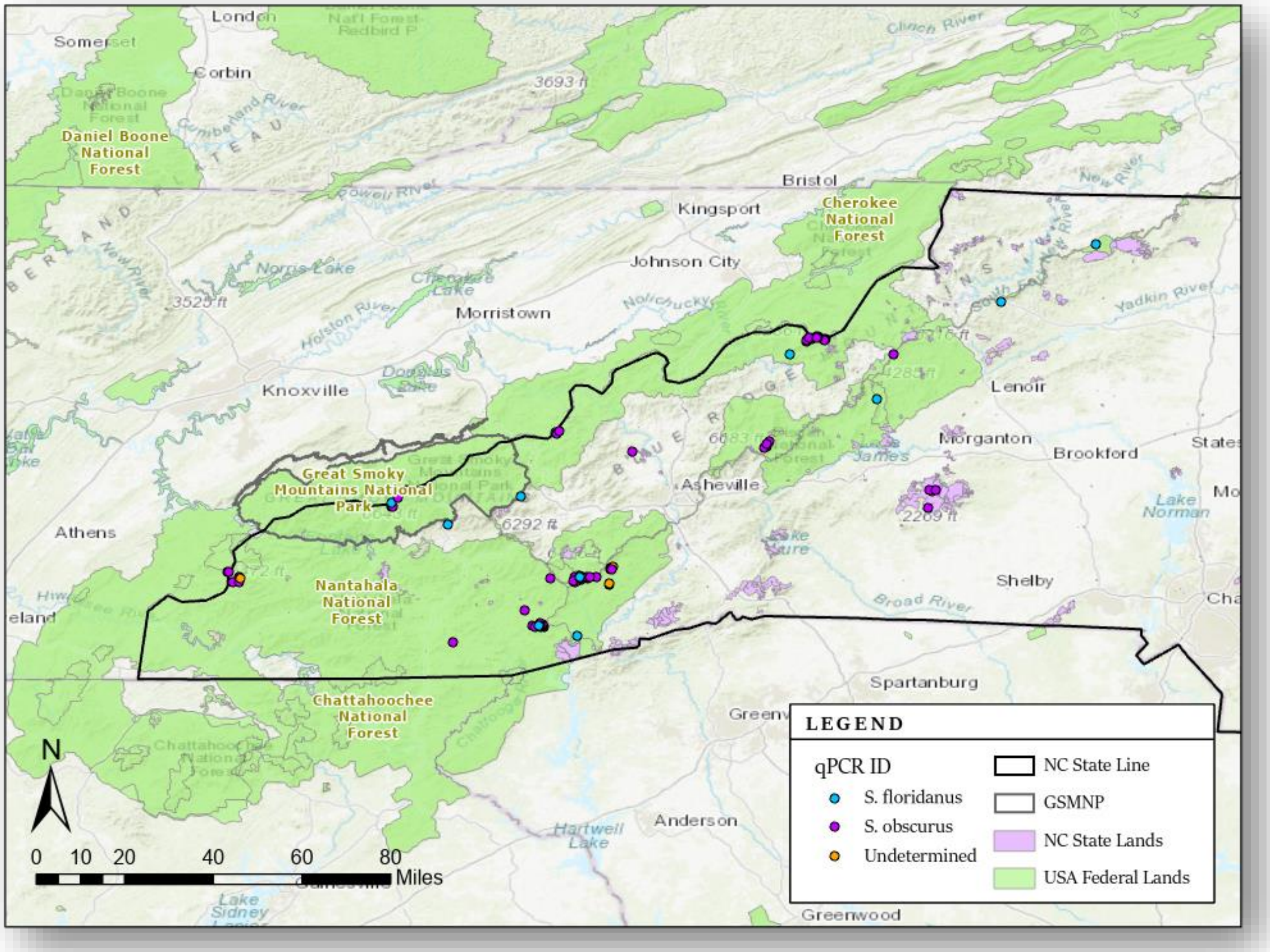
	Spruce-fir	Northern hardwood	Grassy bald	Shrub bald	Heath bald	Early successional	Oak
High-elevation sites							
2nd order scale							
Spruce-fir	---	1.92 (0.079)	0.19 (0.849)	-0.85 (0.408)	-1.46 (0.171)	1.27 (0.227)	1.47 (0.169)
Northern hardwood	-1.92 (0.079)	---	-1.58 (0.140)	-3.03 (0.011)	-3.38 (0.005)	0.16 (0.878)	-0.99 (0.339)
Grassy bald	-0.19 (0.849)	1.58 (0.140)	---	-4.06 (0.002)	-1.36 (0.198)	1.03 (0.321)	1.17 (0.266)
Shrub bald	0.85 (0.408)	3.03 (0.011)	4.06 (0.002)	---	0.08 (0.936)	1.74 (0.108)	2.71 (0.019)
Heath bald	1.46 (0.171)	3.38 (0.005)	1.36 (0.198)	-0.08 (0.936)	---	1.95 (0.075)	5.59 (0.000)
Early successional	-1.27 (0.227)	-0.16 (0.878)	-1.03 (0.321)	-1.74 (0.108)	-1.95 (0.075)	---	-0.55 (0.590)
Oak	-1.47 (0.169)	0.99 (0.339)	-1.17 (0.266)	-2.71 (0.019)	-5.59 (0.000)	0.55 (0.590)	---
3rd order scale							
Spruce-fir	---	-0.41 (0.691)	0.95 (0.363)	0.76 (0.461)	-1.18 (0.259)	---	---
Northern hardwood	0.41 (0.691)	---	0.99 (0.338)	0.83 (0.425)	-1.41 (0.185)	---	---
Grassy bald	-0.95 (0.363)	-0.99 (0.338)	---	-1.63 (0.128)	-1.07 (0.304)	---	---
Shrub bald	-0.76 (0.461)	-0.83 (0.425)	1.63 (0.128)	---	-0.92 (0.375)	---	---
Heath bald	1.18 (0.259)	1.41 (0.185)	1.07 (0.304)	0.92 (0.375)	---	---	---

	Oak	Pine/hemlock	Early successional	Heath bald
Low-elevation site				
2nd order scale				
Oak	---	-3.01 (0.024)	-2.99 (0.024)	-2.68 (0.03)
Pine/hemlock	3.01 (0.024)	---	3.04 (0.022)	9.34 (0.000)
Early successional	2.99 (0.024)	-3.04 (0.022)	---	10.04 (0.000)
Heath bald	2.68 (0.03)	-9.34 (0.000)	-10.04 (0.000)	---
3rd order scale				
Oak	---	-1.53 (0.176)	-1.02 (0.346)	---
Pine/hemlock	1.53 (0.176)	---	0.61 (0.566)	---
Early successional	1.02 (0.346)	-0.61 (0.566)	---	---

Species identification

We identified 272 unique samples from 270 unique localities (Fig. 5). In total, we tested 207 scat samples and 65 tissue samples (Appendix G). Overall, the qPCR approach worked very well, with only 3 tissue samples coming back as ambiguous. However, it was later revealed by sequencing that those 3 samples were likely hybrids (see below). The qPCR approach was also reliable and effective for the scat samples, with 207 samples positively identified and 16 samples undetermined, likely because of degraded DNA.

Figure 5. Sampling localities and qPCR identification for 272 *Sylvilagus sp.* samples.



Objective 2

Overall genetic results

Sequencing of the molecular baits resulted in 648 informative SNPs across all samples. While there were many more SNPs available that had some missing data or that were not as informative across all populations, we felt that 648 SNPs were more than adequate to address our objectives.

Population genetic structure

All approaches used to identify population genetic structure showed strong support for 4 populations when all samples from both species were included. Not surprisingly, these approaches identified eastern cottontail as one of the groupings and 3 populations of Appalachain cottontail (Fig 6). The 3 populations of *S. obscurus* were 1.) a Great Smoky National Park (GSMNP) grouping that included samples from Forney Ridge and Purchase Knob, 2.) a Pisgah grouping that included the Cradle of Forestry, Panthertown, Black Balsam, and Graveyard Fields, and 3.) a Roan mountain grouping that included all samples from the Roan area. When Appalachian cottontail samples were removed, additional eastern cottontail structure was identified for comparative purposes. These groupings were 1.) Mistletoe Meadows (near Stone Mountain State Park), 2.) a western group that was samples near GSMNP, 3.) a Black Balsam area grouping and 4.) a Roan Mountain area grouping.

Genetic diversity, gene flow, and hybrid detection

Population genetic analyses revealed a high amount of differentiation between genetic groupings (Table 6). Conversely, genetic groupings for eastern cottontails showed virtually no differentiation. However, Appalachian cottontail populations did not have excessively high F_{IS} values (Pisgah- 0.064, GSMNP-0.033, Roan- 0.032). An Analysis of Molecular VAriation (AMOVA) of Appalachian cottontail samples (Table 8) largely corroborated the F'_{ST} results. Likelihood ratio tests identified 3 probable hybrids between Appalachian cottontails and eastern cottontails, one from Mistletoe Meadows (unknown morphology), one from Roan (eastern morphology), and one from The Blue Ridge Parkway near Black Balsam (Appalachian morphology). All hybrids appear to be crosses between female eastern cottontails and Appalachian cottontail males.

Table 6. F'_{ST} values between Appalachian cottontail genetic groupings and all eastern cottontail samples grouped.

	Pisgah	**GSMNP**	**Roan**	**eastern cottontail**
Pisgah	0	0.233	0.175	0.639
GSMNP	0.233	0	0.395	0.577
Roan	0.175	0.395	0	0.668
Eastern Cottontail	0.639	0.577	0.668	0

Table 7. F'_{ST} values between eastern cottontail genetic groupings.

	Mistletoe Meadows	**Western**	**Black Balsam**	**Roan**
Mistletoe Meadows	0	0.029	0.008	0.032
Western	0.029	0	-0.075	-0.092
Black Balsam	0.008	-0.075	0	-0.078
Roan	0.032	-0.092	-0.078	0

Figure 6. Distribution of Appalachian cottontails (*Sylvilagus obscurus*) genetic groupings and eastern cottontail (*S. floridanus*) samples from genetic ear punch samples.

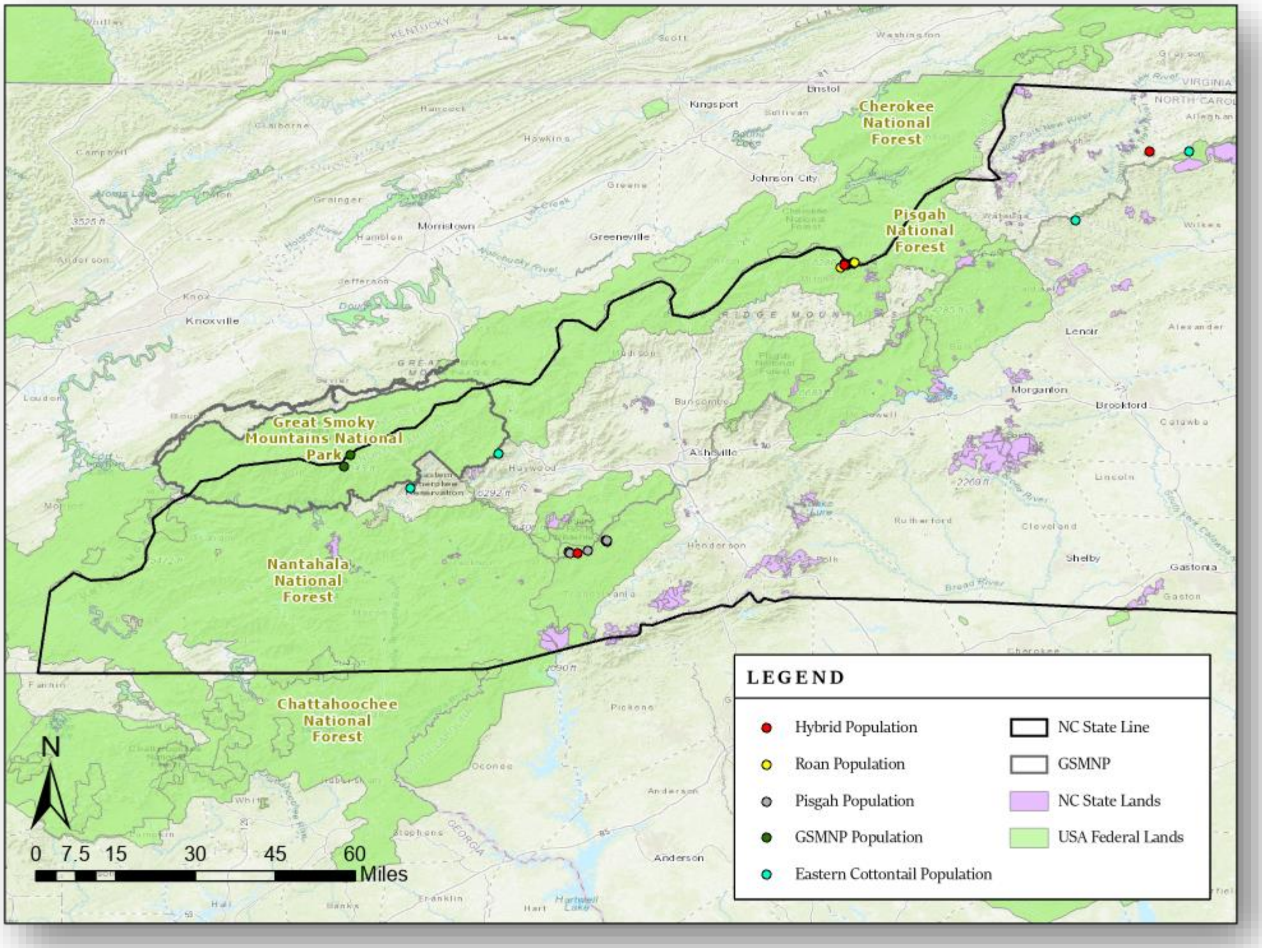


Table 8. Analysis of Molecular Variation (AMOVA) for Appalachian cottontail genetic groupings.

Source of Variation	**%var**	**F-stat**	**F-value**	**Std.Dev.**	**c.i.2.5%**	**c.i.97.5%**	**P-value**	**F'-value**
Within Individual	0.748	F_{IT}	0.252	0.065	0.127	0.377	--	--
Among Individual	0.04	F_{IS}	0.051	0.041	-0.029	0.132	--	--
Among Population	0.212	F_{ST}	0.212	0.061	0.098	0.332	0.001	0.223

DISCUSSION

Habitat Selection and Home Range

We found Appalachian cottontails preferentially selected for habitat within the surrounding landscape and within their home range, similar to eastern cottontails in the Southeast (Bond et al. 2002). At high-elevation sites, Appalachian cottontails used heath balds more than expected given their availability on the landscape on the 2nd and 3rd order scales. This finding was consistent with previous work in West Virginia and Maryland that observed Appalachian cottontails were associated with ericaceous cover, such as rhododendron, mountain laurel, or blueberries (Chapman and Morgan 1973, Chapman et al. 1992, Boyce and Barry 2007). While dominant vegetation in heath balds is typically not consumed in Appalachian cottontail diets (Hartman and Barry 2010), they provide cover for cottontails, which may help prevent predation (Boyce and Barry 2007, Cheeseman et al. 2019). Stevens and Barry (2002) found Appalachian cottontails in West Virginia avoided areas >10 m away from cover, indicating that proximity to cover is an important habitat feature for this species. Additionally, these habitat types possibly provided thermal cover during winter, since freezing temperatures were a contributing factor to higher mortality rates of cottontails during this time of year (Bond et al. 2002, Boyce and Barry 2007, Hartman and Barry 2010).

At low-elevation sites, pine/hemlock forests were an important habitat type Appalachian cottontails preferentially selected for at both spatial scales. However, telemetry work at lower elevations has not previously occurred for this species, so habitat associations at low elevation sites had been derived from live-trapping and hunting records. A previous study observed Appalachian cottontails in a pine-dominate forest in South Carolina (Russell et al. 1999). Radio-collared cottontails avoided oak forests on the landscape scale, but did not select for or avoid oak forests within their home ranges. Appalachian cottontails were previously observed in hardwood stands void of conifers (Blymyer 1976, Sole 1999). In Kentucky, Appalachian cottontails were collected with young hardwood stands that occasionally had mountain laurel, blueberries, or greenbrier (*Smilax spp*.) in the understory (Sole 1999). Blymer (1976) found Appalachian cottontails in a 6-7 year old clearcut with hardwood regeneration. Variation in the time of year these studies occurred and methods to determine habitat associations (i.e., live-trapping vs. telemetry) may be why we did not see cottontails selecting for hardwood dominant stands. Additionally, rhododendron and mountain laurel were common in the understory at both low elevation sites, which may provide important understory cover in forest types dominated by conifers or hardwoods.

The majority of our tracking took place during the cold season, when deciduous leaves were off. Therefore, the habitats Appalachian cottontails selected for potentially provided better thermal cover and concealment from predators during this time of year. Spruce-fir, pine/hemlock, and heath bald habitats provided cover throughout the year. While our study found these cover types to be preferred to hardwood dominated habitats, the time of year we tracked our cottontails may have resulted in higher selection of these habitat types. Since the highest capture rates of Appalachian cottontails occurred in the fall, tracking was limited during the spring and summer. Additionally, most cottontails radio-collared in the fall were initially tracked during leaf-on prior to leaf-off in October, however, this composed of only a few weeks of tracking and did not allow for a large enough sample size to compare habitat selection between seasons. Therefore, it is possible that these cover types may have more use during the colder months and this should be further explored. The leaf-on occurs between May through October. While we tracked several individuals during the leaf-on season, we did not have a large enough sample size to determine if habitat use or home range size varied between these times of year.

We found home range estimates of Appalachian cottontails in the southern Appalachians to be similar to this species in the central Appalachians. In Savage River State Forest in western Maryland, Stevens and Barry (2002) found 95% MCP home ranges of Appalachian cottontails ranged from 1.4-8.3 ha. In the Allegheny Mountains of West Virginia, 95% adaptive kernel home ranges of cottontails ranged from 0.9-9.0 ha (Boyce and Barry 2007). Similar to Stevens and Barry (2002), we did not find any differences between the home range size of males and females, but their study had a small sample size of 8 rabbits. Most studies on cottontails in the eastern United States find significant differences in home range size between the sexes depending on the time of year (Althoff and Storm 1989, Bond et al. 2001, Boyce and Berry 2007, Cheeseman et al. 2019). Male cottontails tend to have larger home ranges during the leaf-on season, potentially due to the availability of more cover and food resources (Boyce and Berry 2007). Additionally, because cottontails have polygamous mating systems, males may increase home range size during leaf-on season to increase fitness by attempting to find more mates (Bond et al. 2001, Cheeseman et al. 2019). Females typically maintain similar sized home ranges between the leaf-on and leaf-off seasons (Boyce and Barry 2007) because they have parental duties that may require them to remain closer to the den with their young (Bond et al. 2001). Our low sample sizes between leaf-on and leaf-off seasons did not allow us to determine if Appalachian cottontails in western North Carolina exhibited these trends in home range size between seasons, requiring further investigation.

Scat Surveys and Predictive Occupancy Modeling

Spatial clustering of our samples necessitated the culling of most points to reduce bias in our model (Syfert et al. 2013). Despite the resulting relatively small sample size and the challenges of covarying climatic factors, we achieved a model with good predictive capacity (AUCs >0.94) and clear trends for Appalachian cottontail preferences in North Carolina.

Our species distribution model, using Appalachian cottontail detections from scat surveys, live captures, and opportunistic scat collection, indicated that this species associates with areas that exhibit moderate to cool temperatures and consistent year-round precipitation. They also favored bald, spruce-fir, and northern hardwood habitat types. This species has often been described as restricted to high-elevation habitats (Webster et al. 1985, Chapman et al. 1992, Chapman 2007). While our research supports the claim that this species inhabits these areas, our documentation of Appalachian cottontails as low as 383 m and 590 m, in South Mountains State Park and Sandy Mush Gameland, respectively, also suggests this species can inhabit lower elevation sites, as has been documented in other southeastern states (Campbell 2010).

Our distribution modeling results cannot parse apart direct and indirect climate relationships. One possible direct climatic driver of the species' distribution is cold stress, which has been documented for other lagomorph species (Katzner et al. 1997, Beever et al. 2010). In our live trapping efforts, we documented higher mortality rates in freezing temperatures, consistent with the species' selection of moderate to cool, but not the coldest, regions of the state. Indirect drivers of these climatic relationships are likely to include the vegetation associations of this species, including cool, wet forest and bald habitats.

Population Genetics

Results from genomic sequencing identified well differentiated populations of Appalachian cottontails across the landscape. Though our sampling was somewhat limited, it is clear that there are genetically isolated populations associated with high mountain peaks in Western North Carolina (WNC). Namely, along the Tennessee border in the Great Smoky Mountains National Park, in the Pisgah/Black Balsams region, and in the Roan Highlands. This is supported by Bayesian population structuring, K-means clustering, an Analysis of Molecular Variance (AMOVA), and F statistics. The AMOVA indicated that roughly 21% of molecular variance can be explained by this isolated population structure. This is a fairly high amount of differentiation for a relatively vagile mammal. The New England cottontail, the sister species of the Appalachian, also displays a high amount of population differentiation, though the Appalachian cottontail populations sampled here appear to be much more isolated (Fenderson et al. 2011).

The relatively high amount of genetic differentiation is also supported strongly by F statistics (Table 6). In fact, WNC populations of Appalachian cottontails have much higher F_{ST} values than New England cottontail populations found at similar distances apart (Fenderson et al. 2011), and are orders of magnitude higher than eastern cottontails on the same landscape (Table 7). There are two possible explanations for the differences between Appalachian and New England cottontails, the first being that Appalachian cottontails have been isolated for much longer in their habitat patches than New England cottontails. The second is that there is much less current gene flow between patches. In all likelihood, the observed pattern is a result from a combination of these factors. A more detailed genetic sampling scheme and GIS analysis would be necessary to uncover the factors that drive the pattern.

Despite the high amount of genetic differentiation found between Appalachian cottontail populations, there does not seem to be evidence of genetic issues arising from isolation. All Appalachian cottontail genetic groupings had a relatively low inbreeding coefficient (F_{IS} value), indicative of fairly large and randomly breeding populations. Thus, even though populations appear to be highly fragmented with low gene flow, at this time, differentiation is likely due to genetic drift rather than inbreeding.

An alternative explanation for low inbreeding levels may come from our discovery of hybridization events between Appalachian and eastern cottontails. If there is a high amount of hybridization occurring, then heterozygosity levels could appear higher than they are in natural Appalachian cottontail populations. This is of course a threat to the genetic integrity of the species and needs to be investigated further. Of interest, Chapman and Morgan (1973) mentioned potential hybrids, so this is unlikely to be a new phenomenon.

Future Research Needs

While this study increased understanding of distribution of Appalachian cottontails in western North Carolina, there is a need to understand fine scale distribution of eastern cottontails in the Appalachian Mountains and highlight which areas the two species co-exist. There is also a larger need to understand what factors influence fine scale distribution between eastern and Appalachian cottontails and if hybridization events are linked to those factors or are more random in nature.

As mentioned earlier, the threat of hybrid events for Appalachian cottontails is troubling. As we have seen for many imperiled species, such as red wolves and California tiger salamanders, hybridization can cause rapid declines. There are two main mechanisms for how hybridization can affect rare species (Todesco et al. 2016). The first being outbreeding depression, which causes lowered fitness levels and therefore wasted reproduction effort. This is often referred to as demographic swamping (Wolf et al. 2001). The other potential outcome is referred to as genetic swamping, or the replacement by one lineage (almost always the less common lineage) by hybrids. Genetic swamping has been found to be more common (Todesco et al. 2016) and is likely the greater threat for Appalachian cottontails. Thus, there is a need to study Appalachian populations in greater detail rather than the broad approach we took in this study. A detailed population genetic study would also help to better understand the population genetic health of each population and identify areas where habitat restoration is needed to expand population sizes and/or gene flow.

One factor that is likely to play a large role in influencing hybridization is the configuration of habitat. This includes fragmentation by roads and human development. Understanding the effects of habitat configuration will also be important in identifying dispersal corridors, especially since cottontails are known to use habitat along roads to disperse (Litvaitis et al. 2003). This may help inform models of species co-occurrence in the region. Likewise, we do not understand eastern cottontail habitat selection and how sympatric eastern and Appalachian cottontails compete for space. Related, a diet study between the two species would be especially helpful in determining management recommendations.

There is also a need to understand the influence that predators have on Appalachian cottontail populations. Recent decades have seen an increasing number of mesopredators, including increasing populations of bobcats (Roberts and Crimmins 2010) and the expanded range of coyotes to the eastern U.S. (Hill et al. 1987, DeBow et al. 1998, Kays et al. 2008). Certainly, a higher number of predators on the landscape has a negative influence on any Sylvilagus species present. One approach could be to conduct a mortality study and determine how cover availability and seasonal movements of Appalachian cottontails influence population growth rates.

Finally, there is a need for Appalachian cottontail surveys and studies in other areas of WNC that weren't surveyed for this study. Our model predicted the species in several areas of interest, including parts of the Nantahala National Forest in Clay, Graham, Jackson, Macon, and Swain Counties and sites near Boone, including Elk Knob (Watauga County) and Three Top Mountain (Ashe County).

Conclusions and Management implications

Very little is known about the Appalachian cottontail range wide, much less in Western North Carolina. This study has provided a strong foundation of important information for the management of the species. At the broad level, we now have a much better understanding of the distribution and potential distribution of the species throughout Western North Carolina. We also have a better idea of how genetic diversity is distributed and fragmented across that distribution. At the local level, we now have an improved understanding of the climactic variables that influence presence/absence, habitat use, home range size, and genetic health of populations, and we have uncovered evidence for hybridization between Appalachian and eastern cottontails. However, there is clearly a lot left to learn about this elusive species and we highly recommend continued research to improve our understanding of it.

We suggest that the following research programs, listed in order of importance

1. Further investigation into the levels and threats of hybridization with Eastern cottontails.

2. Research into how habitat configuration influences Appalachian cottontails.
3. Fine scale population genetics and genetic health of the known populations.
4. Direct competition studies between Eastern and Appalachian cottontails, including a diet study and habitat selection.
5. Ground truthing and species distribution model validation. That is, searching for new populations.
6. A study on the effects of meso-predator abundance and Appalachian cottontail abundance/survival.

Another needed future research program that we do not know how to rank yet is on the threat, effects, and spread of RHDV2 in Appalachian cottontail populations. Given the seemingly small size of Appalachian cottontail populations, a disease such as RHDV2 could be devastating. It could also be another threat that is exacerbated by dense populations of Eastern cottontails. If it is found that RHDV2 is prevalent in the areas where Appalachian cottontails are found, then this research need would certainly be among the top needs.

Our data has revealed is that at higher elevations, heath balds play a disproportionately important role for the species and should be maintained. It is also likely that the continued restoration of high elevation red spruce would have an overall positive effect on the species. These two actions together represent the most important and direct management implications for the species. We recommend that the NCWRC work together with the USFS to develop a management plan for heath balds and red spruce that would benefit the Appalachian cottontail in areas where we found good populations through trapping, scat surveys, and genetic analysis. We also recommend a more concerted effort to survey for Appalachian cottontails in unknown areas and at potential edges of the known populations to determine species range limits.

DELIVERABLES

Conferences

Presented

C.A. Diggins, L.P. Erb, and J.J. Apodaca. Habitat use and home range of Appalachian cottontails in western North Carolina. Oral presentation. 30th Annual Colloquium on the Conservation of Mammals in the Southeastern U.S. Asheville, NC. February 14, 2020.

Accepted abstracts

C.A. Diggins, L.P. Erb, and J.J. Apodaca. Habitat selection and home range of Appalachian cottontails in the southern Appalachian Mountains. Oral presentation in the Endangered Species Conservation and Management Section to be presented at 8:40 pm on Wednesday, September 30, 2020. 27th Annual Conference of the Wildlife Society, Louisville, KY.

Publications

Diggins, C.A., L.P. Erb, and J.J. Apodaca. *In Preparation*. Multi-scale habitat selection and home range of a high-elevation lagomorph in the southern Appalachian Mountains. Journal of Wildlife Management.

L.P. Erb, J. Shields, C.A. Diggins, M. Olszack, and J.J. Apodaca. *In Preparation*. Refining species distribution models for a rare lagomorph using land cover and bioclimatic layers. Journal of Mammalogy.
J.J. Apodaca, M. Olszack, L.P. Erb, and C.A. Diggins. *In Preparation.* A new method for identifying Appalachian cottontails from congeners. Southeastern Naturalist
J.J. Apodaca, M. Olszack, L.P. Erb, and C.A. Diggins. *In Preparathion.* RADSeq reveals a complex genetic structure and hybridization in the Appalachian cottontail. Conservation Genetics

Workshops

- A planned workshop on the results of this study for partners scheduled for June 2020. Originally scheduled for March 17th, 2020 but rescheduled due to the Covid-19 pandemic.

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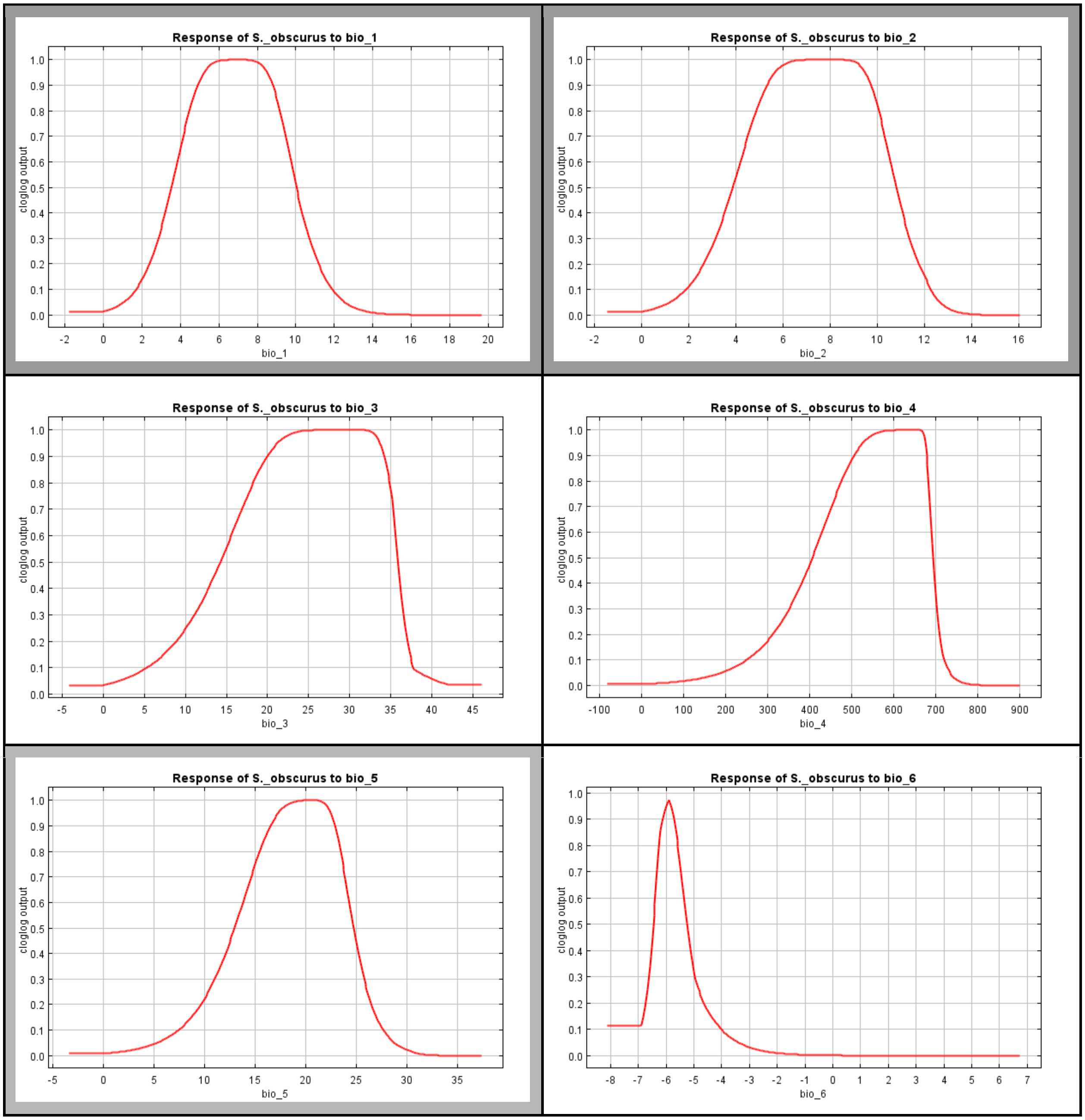
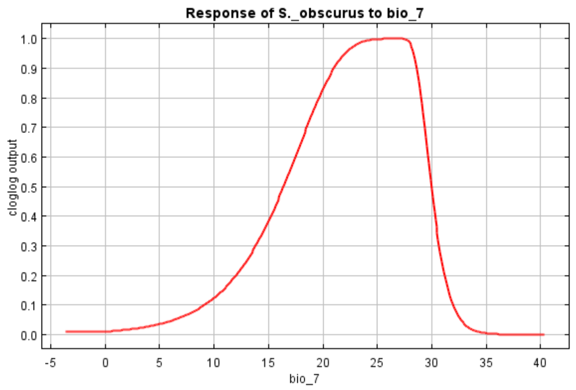
Appendix A. Bioclim variables used in species distributional modeling (courtesy of Fick and Hijmans 2017)

Code	**Variable Description**
BIO1	Annual Mean Temperature
BIO2	Mean Diurnal Range (Mean of monthly (max temp - min temp))
BIO3	Isothermality (BIO2/BIO7) (×100)
BIO4	Temperature Seasonality (standard deviation ×100)
BIO5	Max Temperature of Warmest Month
BIO6	Min Temperature of Coldest Month
BIO7	Temperature Annual Range (BIO5-BIO6)
BIO8	Mean Temperature of Wettest Quarter
BIO9	Mean Temperature of Driest Quarter
BIO10	Mean Temperature of Warmest Quarter
BIO11	Mean Temperature of Coldest Quarter
BIO12	Annual Precipitation
BIO13	Precipitation of Wettest Month
BIO14	Precipitation of Driest Month
BIO15	Precipitation Seasonality (Coefficient of Variation)
BIO16	Precipitation of Wettest Quarter
BIO17	Precipitation of Driest Quarter
BIO18	Precipitation of Warmest Quarter
BIO19	Precipitation of Coldest Quarter

Appendix B. The dependence of the predicted probability of *S. obscurus* presence on each variable included in our Maxent species distribution model. Relationships are depicted via univariate Maxent model. Trends for climatic variables may also reflect correlations with similar variables (see Appendix C). We have highlighted influential variables in gray.

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0.8
0.7
0.6
0.5
0.4
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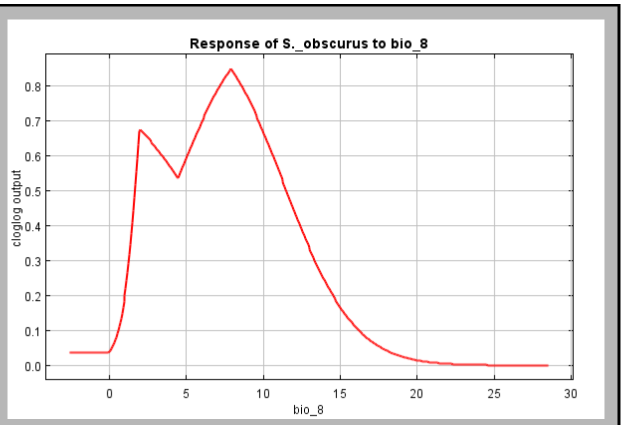
0.1
0.0
-5
0
5
10
15
20
25
30
35
40
bio_7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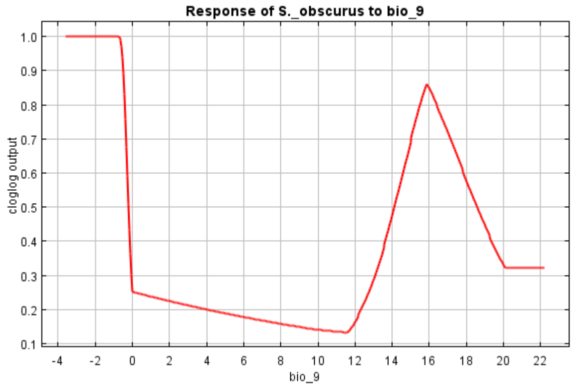
0
5
10
15
20
25
30
bio_8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cloglog output
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0.6
0.5
0.4
0.3
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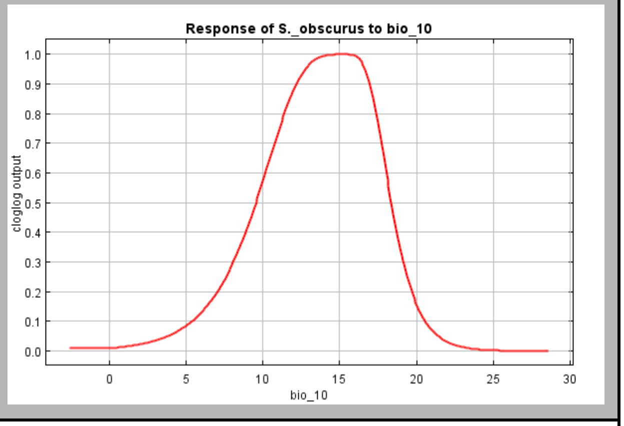
-2
0
2
4
6
8
10
12
14
16
18
20
22
bio_9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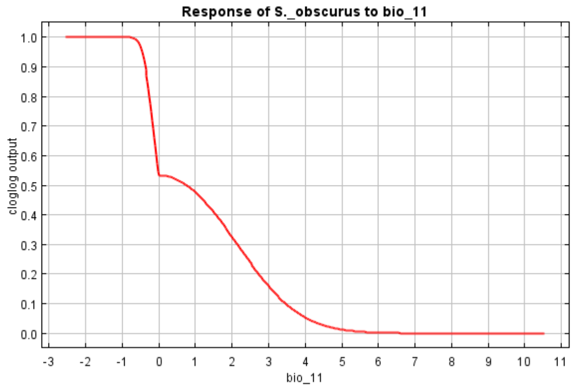
0
5
10
15
20
25
30
bio_10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6
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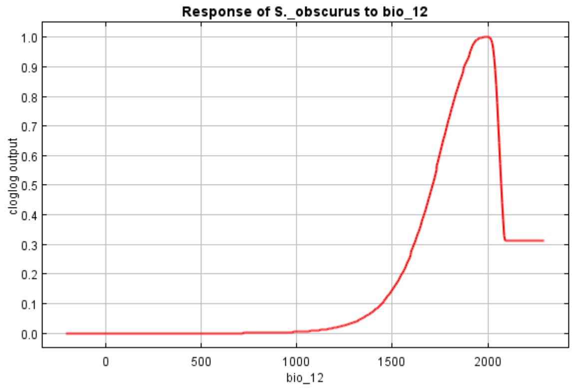
8
9
10
11
bio_11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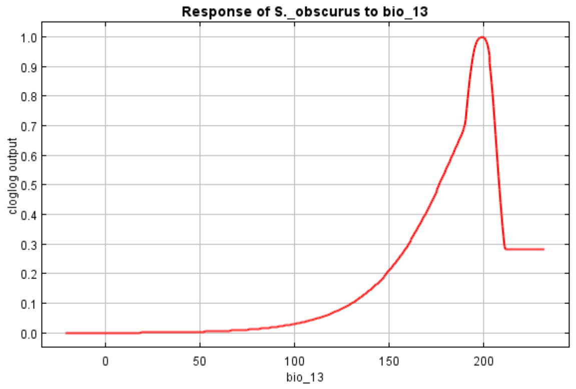
bio_12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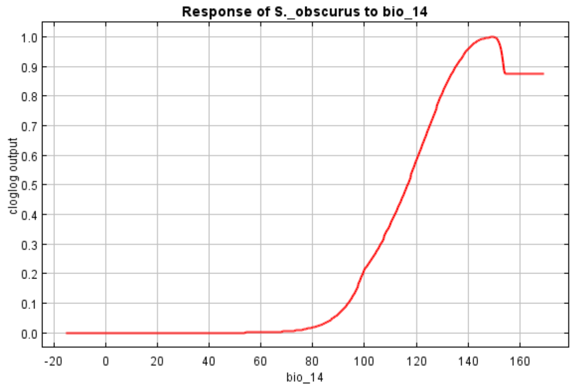
100
150
200
bio_13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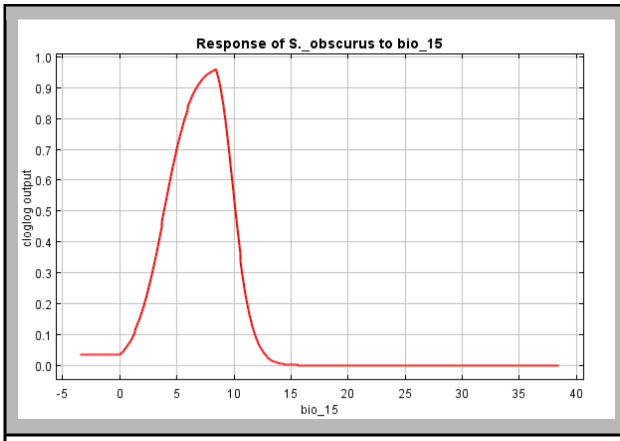
60
80
100
120
140
160
bio_14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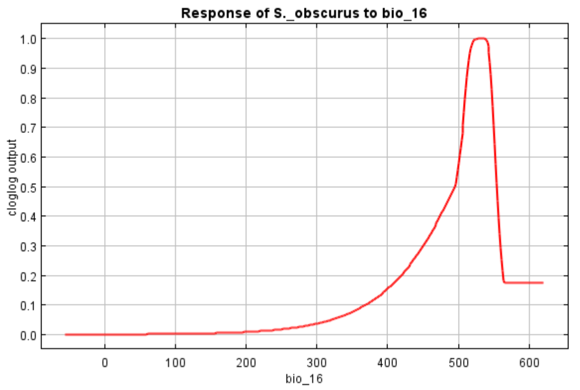
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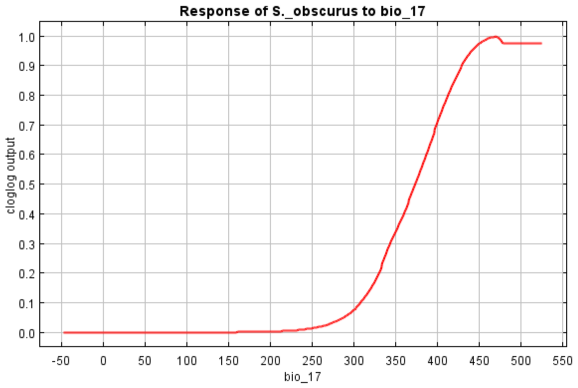
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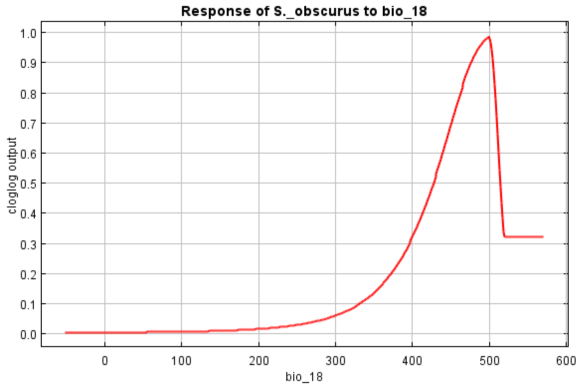
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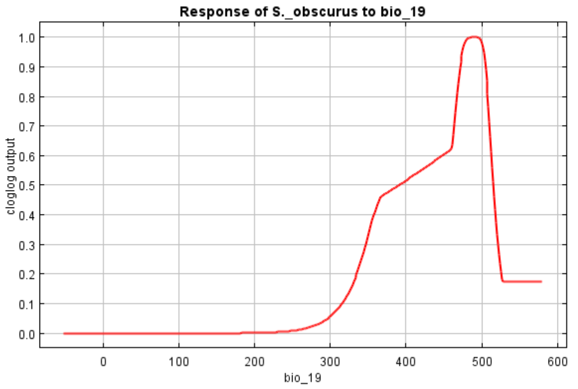
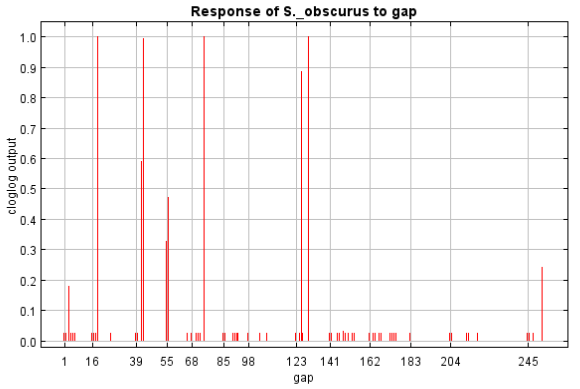

Response of S._obscurus to bio_19


Response of S._obscurus to gap

Appendix C. Correlation matrix

Appendix D. All captures obtained during trapping sessions for Appalachian cottontail (*Sylvilagus obscurus*) conducted in the western North Carolina in 2018 and 2019.

Date	Location	NAD 83	X	Y	Species	Sex	Mass (g)	Right Hindfoot (cm)	Right Ear Length (cm)	Left Ear Length (cm)	Ear Tag	Ear Sample (Y/N)	Fecal Sample (Y/N)	Collar Frequency
3/14/2018	Cradle of Forestry	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	F	800	9.1	5.9	.	.	Y	Y	.
3/30/2018	Cradle of Forestry	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	F	1225	8.8	5.5	5.6	.	Y	Y	.
4/20/2018	Smokies	17S	[redacted]	[redacted]	*Tamiasciurus hudsonicus*	.	.	.	.	.	.	.	.	.
4/20/2018	Smokies	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	F	1161	9.9	5.7	5.7	.	Y	Y	151.189
4/26/2018	Black Balsam	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	M	1040	9	5.5	5.4	7	Y	Y	151.110
5/1/2018	Black Balsam	17S	[redacted]	[redacted]	*Tamiasciurus hudsonicus*	.	.	.	.	.	.	.	.	.
5/1/2018	Black Balsam	17S	[redacted]	[redacted]	*Tamiasciurus hudsonicus*	.	.	.	.	.	.	.	.	.
5/1/2018	Black Balsam	17S	[redacted]	[redacted]	*Tamiasciurus hudsonicus*	.	.	.	.	.	.	.	.	.
5/1/2018	Black Balsam	17S	[redacted]	[redacted]	*Tamiasciurus hudsonicus*	.	.	.	.	.	.	.	.	.
5/1/2018	Black Balsam	17S	[redacted]	[redacted]	*Tamiasciurus hudsonicus*	.	.	.	.	.	.	.	.	.
5/1/2018	Black Balsam	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	.	.	.	.	.	.	.	Y	151.110
5/2/2018	Black Balsam	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	F	1360	9.8	5.5	5.4	5	Y	Y	.
5/2/2018	Black Balsam	17S	[redacted]	[redacted]	*Tamiasciurus hudsonicus*	.	.	.	.	.	.	.	.	.
5/12/2018	Black Balsam	17S	[redacted]	[redacted]	*Tamiasciurus hudsonicus*	.	.	.	.	.	.	.	.	.
5/12/2018	Cradle of Forestry	17S	[redacted]	[redacted]	*Didelphis virginiana*	.	.	.	.	.	.	.	.	.
5/13/2018	Cradle of Forestry	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	F	1190	9	5.3	5.3	4	Y	Y	151.070

10/24/2018	Roan Mountain	17S	[redacted]	[redacted]	*Didelphis virginiana*	.	.	.	.	.	.	.	.	.
10/25/2018	Roan Mountain	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	F	960	N	5.5	5.5	.	Y	Y	151.150
10/30/2018	Roan Mountain	17S	[redacted]	[redacted]	*Sylvilagus floridanus*	M	710	8.8	5	5.2	15	Y	?	.
10/30/2018	Roan Mountain	17S	[redacted]	[redacted]	*Sylvilagus floridanus?*	U	.	.	.	.	.	N	N	.
10/30/2018	Roan Mountain	17S	[redacted]	[redacted]	*Sylvilagus spp*	F	1130	9.9	5.9	6	13	Y	?	.
10/30/2018	Roan Mountain	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	M	620	8.6	.	.	14	Y	Y	.
10/30/2018	Roan Mountain	17S	[redacted]	[redacted]	*Didelphis virginiana*	.	.	.	.	.	.	.	.	.
10/30/2018	Roan Mountain	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	F	760	8.9	5.6	5.5	.	Y	Y	151.090
10/30/2018	Roan Mountain	17S	[redacted]	[redacted]	*Didelphis virginiana*	.	.	.	.	.	.	.	.	.
10/30/2018	Roan Mountain	17S	[redacted]	[redacted]	*Didelphis virginiana*	.	.	.	.	.	.	.	.	.
10/31/2018	Roan Mountain	17S	[redacted]	[redacted]	*Didelphis virginiana*	.	.	.	.	.	.	.	.	.
10/31/2018	Roan Mountain	17S	[redacted]	[redacted]	*Didelphis virginiana*	.	.	.	.	.	.	.	.	.
10/31/2018	Roan Mountain	17S	[redacted]	[redacted]	*Procyon lotor*	.	.	.	.	.	.	.	.	.
10/31/2018	Roan Mountain	17S	[redacted]	[redacted]	*Peromyscus spp*	.	.	.	.	.	.	.	.	.
10/31/2018	Roan Mountain	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	M	1130	9.7	5.6	5.6	.	Y	Y	151.229
10/31/2018	Roan Mountain	17S	[redacted]	[redacted]	*Sylvilagus obscurus*		.	.	.	.	.	.	.	151.090
10/31/2018	Roan Mountain	17S	[redacted]	[redacted]	*Tamiasciurus hudsonicus*	.	.	.	.	.	.	.	.	.
10/31/2018	Roan Mountain	17S	[redacted]	[redacted]	*Didelphis virginiana*	.	.	.	.	.	.	.	.	.
10/31/2018	Roan Mountain	17S	[redacted]	[redacted]	*Didelphis virginiana*	.	.	.	.	.	.	.	.	.

11/2/2018	Cradle of Forestry	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	M	980	9.5	5.5	5.6	.	Y	Y	151.209
11/2/2018	Cradle of Forestry	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	F	1200	8.9	5.6	5.6	.	Y	Y	150.809
11/2/2018	Cradle of Forestry	17S	[redacted]	[redacted]	*Procyon lotor*	.	.	.	.	.	.	.	.	.
11/2/2018	Cradle of Forestry	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	M	560	8	4.7	4.6	12	Y	Y	.
11/2/2018	Cradle of Forestry	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	F	1410	9.8	5.6	5.5	.	Y	Y	151.169
11/11/2018	Cradle of Forestry	17S	[redacted]	[redacted]	*Procyon lotor*	.	.	.	.	.	.	.	.	.
11/12/2018	Cradle of Forestry	17S	[redacted]	[redacted]	*Procyon lotor*	.	.	.	.	.	.	.	.	.
11/12/2018	Cradle of Forestry	17S	[redacted]	[redacted]	*Sciurus carolinensis*	.	.	.	.	.	.	.	.	.
11/19/2018	BRP/215	17S	[redacted]	[redacted]	*Procyon lotor*	.	.	.	.	.	.	.	.	.
11/20/2018	Cradle of Forestry	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	F	1190	9.5	5.8	5.8	.	Y	Y	151.009
11/20/2018	Cradle of Forestry	17S	[redacted]	[redacted]	*Procyon lotor*	.	.	.	.	.	.	.	.	.
11/20/2018	Cradle of Forestry	17S	[redacted]	[redacted]	*Sciurus carolinensis*	.	.	.	.	.	.	.	.	.
11/20/2018	Cradle of Forestry	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	M	690	8.2	4.9	4.9	12	.	Y	.
11/20/2018	Cradle of Forestry	17S	[redacted]	[redacted]	*Didelphis virginiana*	.	.	.	.	.	.	.	.	.
11/26/2018	Cradle of Forestry	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	M	700	8.5	4.9	4.9	17	Y	Y	.
11/29/2018	BRP/215	17S	[redacted]	[redacted]	*Glaucomys sabrinus coloratus*	.	.	.	.	.	.	.	.	.
12/4/2018	Hatchery	17S	[redacted]	[redacted]	*Didelphis virginiana*	.	.	.	.	.	.	.	.	.
1/8/2019	Cradle of Forestry	17S	[redacted]	[redacted]	*Didelphis virginiana*	.	.	.	.	.	.	Y	.	.
1/9/2019	Hatchery	17S	[redacted]	[redacted]	*Sciurus carolinensis*	.	.	.	.	.	.	.	.	.

1/9/2019	Hatchery	17S	[redacted]	[redacted]	*Sciurus carolinensis*	.	.	.	.	.	.	.	.	.
9/6/2019	Roan	17S	[redacted]	[redacted]	*Didelphis virginiana*	.	.	.	.	.	.	.	.	.
9/6/2019	Roan	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	F	1075	9.7	6.2	6.2	20	Y	N	150.670
9/8/2019	Roan	17S	[redacted]	[redacted]	*Didelphis virginiana*	.	.	.	.	.	.	.	.	.
9/8/2019	Roan	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	F	660	7.8	4.6	4.6	22	Y	Y	.
9/9/2019	Roan	17S	[redacted]	[redacted]	*Procyon lotor*	.	.	.	.	.	.	.	.	.
9/9/2019	Roan	17S	[redacted]	[redacted]	*Sylvilagus floridanus*	M	990	9.7	5.8	5.8	18	Y	Y	.
9/12/2019	Roan	17S	[redacted]	[redacted]	*Didelphis virginiana*	.	.	.	.	.	.	.	.	.
9/15/2019	Roan	17S	[redacted]	[redacted]	*Didelphis virginiana*	.	.	.	.	.	.	.	.	.
9/16/2019	Roan	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	M	540	7.7	4.7	4.7	.	Y	Y	.
9/16/2019	Roan	17S	[redacted]	[redacted]	*Sylvilagus floridanus?*	F	830	9.7	6	6	23	Y	Y	.
9/16/2019	Roan	17S	[redacted]	[redacted]	*Sylvilagus floridanus?*	M	820	9.8	5.5	5.5	19	Y	Y	.
9/16/2019	Roan	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	M	880	8.9	5.6	5.5	25	Y	Y	.
9/16/2019	Roan	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	F	1280	9.5	5.3	5.2	16	Y	Y	150.629
9/17/2019	Roan	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	M	820	9.3	5.4	5.3	27	Y	Y	151.129
9/17/2019	Roan	17S	[redacted]	[redacted]	*Sylvilagus floridanus*	F	880	9.5	5.7	5.7	24	Y	Y	.
9/17/2019	Roan	17S	[redacted]	[redacted]	*Sylvilagus floridanus*	F	1080	9.7	5.7	5.6	21	Y	N	.
9/17/2019	Roan	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	M	700	8.3	5.2	5.2	28	Y	Y	151.029
9/17/2019	Roan	17S	[redacted]	[redacted]	*Didelphis virginiana*	.	.	.	.	.	.	.	.	.

9/17/2019	Roan	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	F	1050	9.2	5.2	5.2	29	Y	Y	151.369
9/18/2019	Roan	17S	[redacted]	[redacted]	*Didelphis virginiana*	.	.	.	.	.	.	.	.	.
9/18/2019	Roan	17S	[redacted]	[redacted]	*Didelphis virginiana*	.	.	.	.	.	.	.	.	.
9/24/2019	Mount Mitchell	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	M	650	8.5	4.9	4.9	32	Y	Y	.
9/24/2019	Mount Mitchell	17S	[redacted]	[redacted]	*Sylvilagus floridanus?*	M	740	8.8	5	5	31	Y	Y	.
9/24/2019	Mount Mitchell	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	M	670	8.4	5.1	5.1	30	Y	Y	.
9/25/2019	Mount Mitchell	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	M	1010	9.6	5.1	5	34	Y	Y	151.474
9/25/2019	Mount Mitchell	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	M	1220	9.6	5.6	5.6	33	Y	Y	151.431
9/26/2019	Mount Mitchell	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	M	670	8.4	5.1	5.1	30	.	Y	.
10/10/2019	Black Balsam	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	F	460	7.8	4.8	4.7	35	Y	Y	.
10/10/2019	Black Balsam	17S	[redacted]	[redacted]	*Sylvilagus obscurus?*	M	1000	9.3	5.6	5.6	46	Y	Y	.
10/11/2019	Black Balsam	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	F	460	7.8	4.8	4.7	35	.	N	.
10/11/2019	Black Balsam	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	M	1060	9.3	5.3	5.4	36	Y	Y	151.454
10/12/2019	Black Balsam	17S	[redacted]	[redacted]	*Sylvilagus obscurus?*	F	1500	9.2	5.8	5.8	37	Y	Y	.
10/12/2019	Black Balsam	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	M	1175	10	6	5.9	39	Y	Y	151.590
10/17/2019	Black Balsam	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	M	1255	10.1	5.8	5.8	40	Y	Y	151.609
10/17/2019	Black Balsam	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	F	1170	9.8	5.9	5.8	47	Y	Y	150.549
10/18/2019	Black Balsam	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	M	1020	9.6	5.5	5.6	42	Y	Y	150.449
10/24/2019	Black Balsam	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	M	1210	9.7	5.9	5.9	41	Y	N	151.647

10/24/2019	Black Balsam	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	M	790	9.6	5.5	5.6	43	Yes	Yes	151.689
11/4/2019	Panthertown	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	M	1120	9.4	5.9	5.9	44	Yes	Yes	151.710
11/5/2019	Panthertown	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	M	1160	9.4	5.9	5.9	44	.	Yes	151.710
11/11/2019	Panthertown	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	M	1120	9.4	4.9	5.9	44	.	Yes	151.710
11/11/2019	Panthertown	17S	[redacted]	[redacted]	*Sylvilagus obscurus?*	M	1500	10.2	6.2	6.2	38	Yes	Yes	.
11/18/2019	Cradle of Forestry	17S	[redacted]	[redacted]	*Didelphis virginiana*	.	.	.	.	.	.	.	.	.
11/18/2019	Cradle of Forestry	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	F	1450	10.2	5.8	5.7	45	Yes	Yes	151.770 (151.009)
11/22/2019	Cradle of Forestry	17S	[redacted]	[redacted]	*Procyon lotor*	.	.	.	.	.	.	.	.	.
11/26/2019	Cradle of Forestry	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	F	1160	9.7	5.7	5.7	48	Yes	Yes	151.289
11/26/2019	Cradle of Forestry	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	F	1450	10.2	5.8	5.7	45	.	Yes	151.770 (151.009)
11/29/2019	Panthertown	17S	[redacted]	[redacted]	*Procyon lotor*	.	.	.	.	.	.	.	.	.
12/2/2019	Cradle of Forestry	17S	[redacted]	[redacted]	*Rattus rattus*	.	.	.	.	.	.	.	.	.
12/2/2019	Cradle of Forestry	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	M	790	9.2	5.2	5.1	50	Yes	Yes	151.249
12/2/2019	Cradle of Forestry	17S	[redacted]	[redacted]	*Sylvilagus obscurus*	F	1450	10.2	5.8	5.7	45	.	Yes	151.770

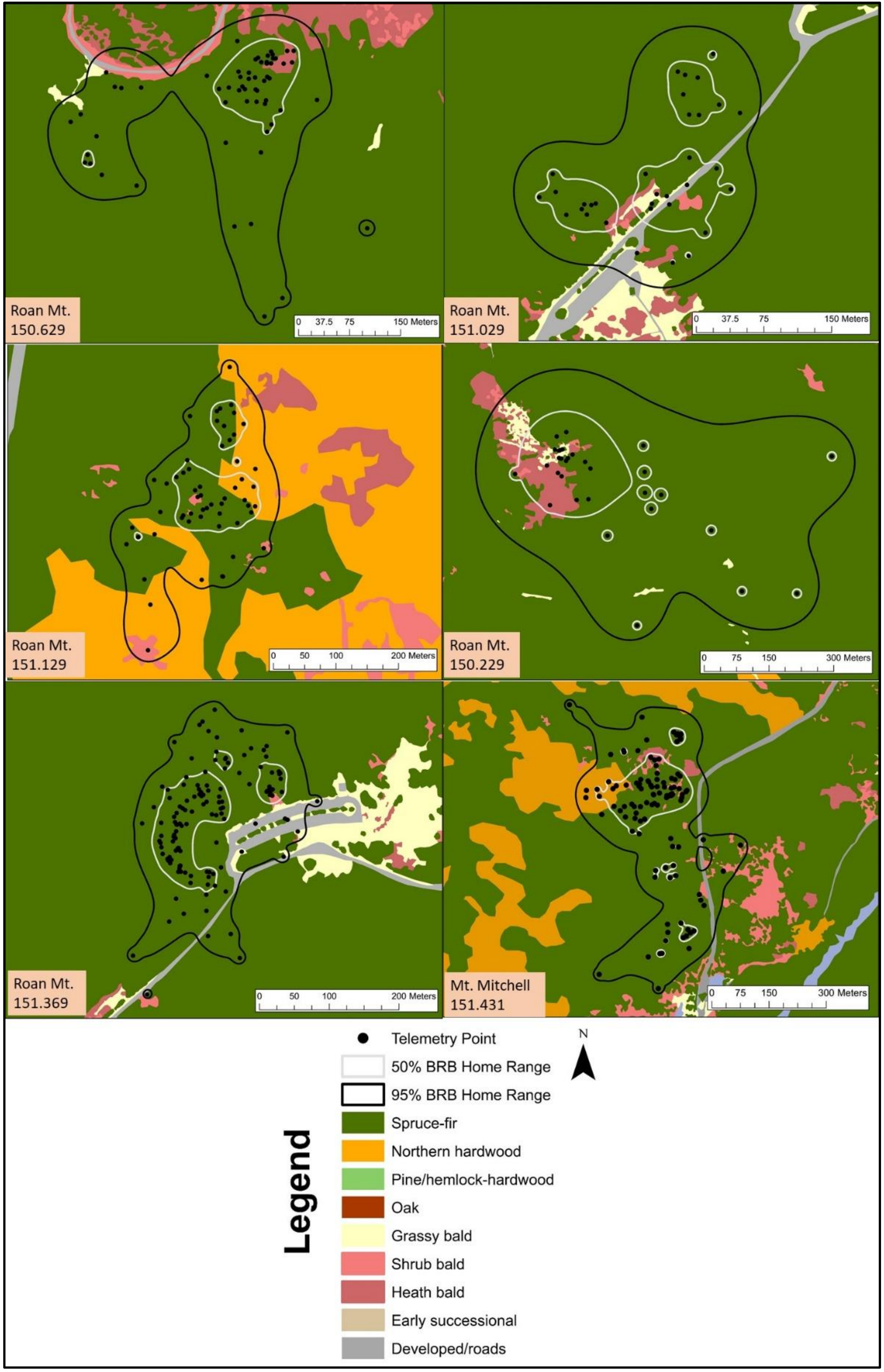
Appendix E. Individual information on radio-collared Appalachian cottontails (*Sylvilagus obscurus*) tracked the southern Appalachian Mountains in western North Carolina during 2018-2019 and 2019-2020. Sex is indicated as male (M) or female (F). Minimum convex polygon (MCP) and biased random bridges (BRB) home ranges are shown in hectares.

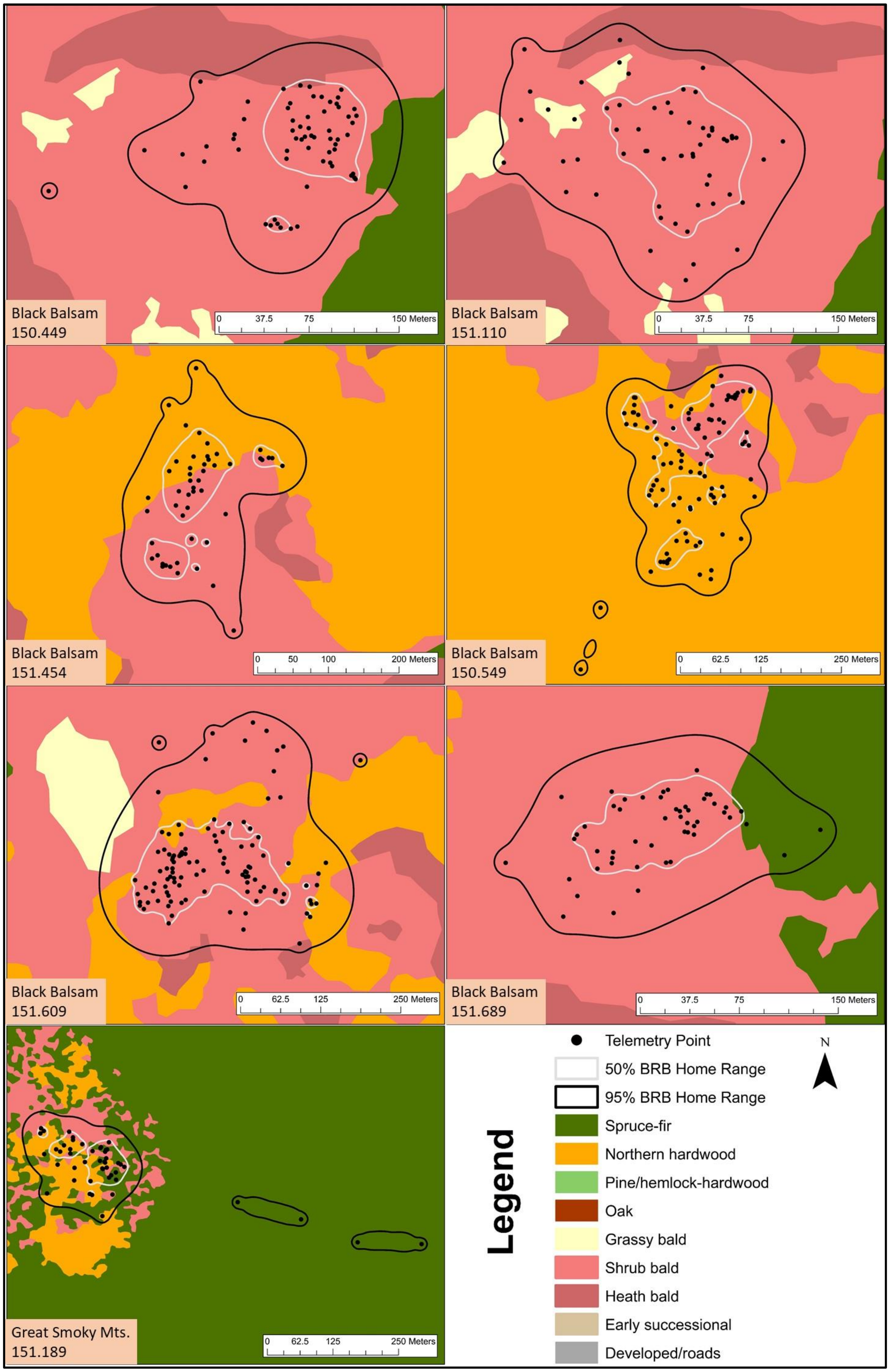
Location	Collar No.	Sex	Mass (g)	No. of Points	Weeks Tracked	50% MCP	95% MCP	50% BRB	95% BRB
Black Balsam	150.449	M	1020	64	12	0.27	0.83	0.38	1.90
Black Balsam	150.549	F	1170	101	19	1.28	3.09	1.17	4.41
Black Balsam	151.110	M	1040	60	6	0.45	1.72	0.65	2.60
Black Balsam	151.454	M	1060	50	6	0.52	1.80	0.82	3.65
Black Balsam	151.590	M	1175	23[+]	2	---	---	---	---
Black Balsam	151.609	M	1255	111	19	1.15	4.50	1.63	7.26
Black Balsam	151.689	M	790	52	12	0.19	0.67	0.41	1.73
Cradle of Forestry	150.809	F	1320	0*	---	---	---	---	---
Cradle of Forestry	151.070	F	1190	34	3	0.21	0.82	0.40	1.95
Cradle of Forestry	151.169	F	1560	149	20	2.16	13.78	2.5	19.44
Cradle of Forestry	151.209	M	1080	148	20	1.02	6.10	2.58	14.33
Cradle of Forestry	151.249	M	790	82	11	0.26	0.55	0.11	0.83
Cradle of Forestry	151.289	F	1160	105	14	0.28	1.07	0.27	1.17
Cradle of Forestry	151.770	F	1450	200	24	0.26	0.985	0.33	3.84
Great Smoky Mts.	151.189	F	1161	48	7	0.3	2.24	0.52	2.78
Mt. Mitchell	151.431	M	1220	110	22	1.65	9.05	2.23	11.92
Panthertown	151.710	M	1120	119	16	1.83	7.03	1.34	5.01
Roan Mts.	150.629	F	1280	61	7	0.66	4.32	0.74	5.40
Roan Mts.	151.029	M	700	33	4	0.74	1.81	0.93	3.36
Roan Mts.	151.129	M	820	58	7	0.72	3.0	0.97	4.47
Roan Mts.	151.229	M	1250	39	10	0.96	2.49	2.63	14.36
Roan Mts.	151.369	F	1050	138	23	1.07	2.96	0.91	4.04

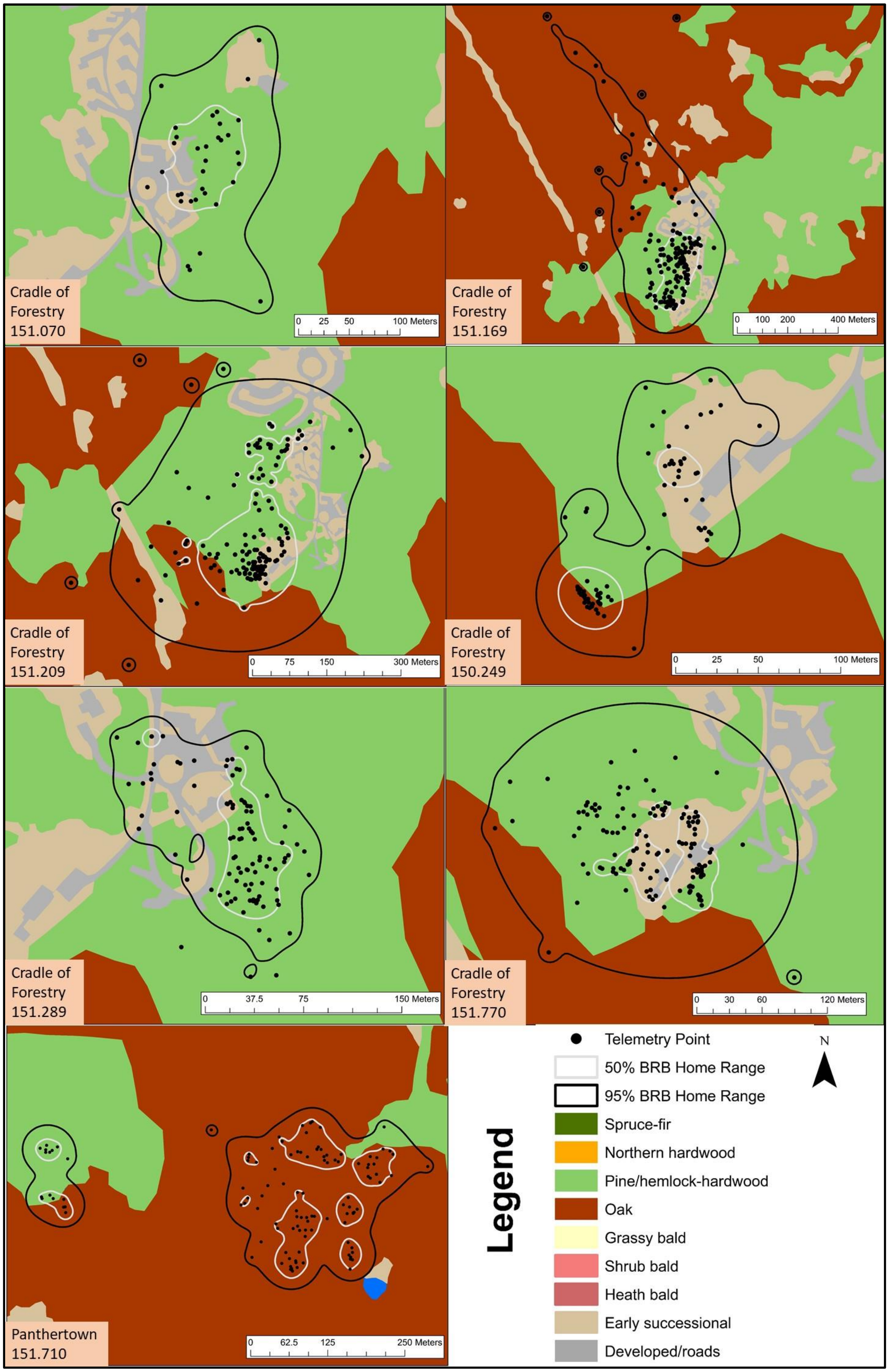
[+] Had under 30 telemetry points, so was excluded from home range and habitat analysis.

*Died 2 days after collaring, so no telemetry data was collected.

Appendix F. Individual biased random bridge home range estimates for radio-collared Appalachian cottontails (*Sylvilagus obscurus*) tracked in western North Carolina in 2018-2020.




Black Balsam
150.449
0 37.5 75 150 Meters
Black Balsam
151.110
0 37.5 75 150 Meters
Black Balsam
151.454
0 50 100 200 Meters
Black Balsam
150.549
0 62.5 125 250 Meters
Black Balsam
151.609
0 62.5 125 250 Meters
Black Balsam
151.689
0 37.5 75 150 Meters
Great Smoky Mts.
151.189
0 62.5 125 250 Meters
Legend
N
Telemetry Point
50% BRB Home Range
95% BRB Home Range
Spruce-fir
Northern hardwood
Pine/hemlock-hardwood
Oak
Grassy bald
Shrub bald
Heath bald
Early successional
Developed/roads


Cradle of Forestry 151.070
0 25 50 100 Meters
Cradle of Forestry 151.169
0 100 200 400 Meters
Cradle of Forestry 151.209
0 75 150 300 Meters
Cradle of Forestry 150.249
0 25 50 100 Meters
Cradle of Forestry 151.289
0 37.5 75 150 Meters
Cradle of Forestry 151.770
0 30 60 120 Meters
Panthertown 151.710
0 62.5 125 250 Meters
Legend
N
Telemetry Point
50% BRB Home Range
95% BRB Home Range
Spruce-fir
Northern hardwood
Pine/hemlock-hardwood
Oak
Grassy bald
Shrub bald
Heath bald
Early successional
Developed/roads

Appendix G. ALL SAMPLES qPCRed. A qPCR label of “SF” refers to *Sylvilagus floridanus*, “SO” refers to *Sylvilagus obscurus*, and “UND” refers to undetermined.

Field Label	Lab Label	qPCR ID	Date Collected	Collection Location	Region	Elevation (meters)	Sample Type	DD Lat	DD Long
1	SO 054	SF			Roan	1856	tissue	[redacted]	[redacted]
104010101	SO 103	SO			Black Balsam	1795	scat	[redacted]	[redacted]
104010201	SO 104	SO			Black Balsam	1795	scat	[redacted]	[redacted]
104010301	SO 105	SO			Black Balsam	1795	scat	[redacted]	[redacted]
104010302	SO 106	SO			Black Balsam	1795	scat	[redacted]	[redacted]
104010901	SO 107	SO			Black Balsam	1788	scat	[redacted]	[redacted]
104011001	SO 108	SO			Black Balsam	1788	scat	[redacted]	[redacted]
104011002	SO 109	SO			Black Balsam	1788	scat	[redacted]	[redacted]
104011003	SO 110	SO			Black Balsam	1788	scat	[redacted]	[redacted]
104020101	SO 111	SO			Black Balsam	1799	scat	[redacted]	[redacted]
104020102	SO 112	SO			Black Balsam	1799	scat	[redacted]	[redacted]
104020201	SO 114	SO			Black Balsam	1799	scat	[redacted]	[redacted]
104020301	SO 115	SO			Black Balsam	1799	scat	[redacted]	[redacted]
104020302	SO 116	SO			Black Balsam	1799	scat	[redacted]	[redacted]
104020304	SO 118	SO			Black Balsam	1799	scat	[redacted]	[redacted]
104020401	SO 119	SO			Black Balsam	1799	scat	[redacted]	[redacted]
104020402	SO 120	SO			Black Balsam	1799	scat	[redacted]	[redacted]
104020501	SO 121	UND			Black Balsam	1799	scat	[redacted]	[redacted]
104020601	SO 122	SO			Black Balsam	1780	scat	[redacted]	[redacted]
104020701	SO 123	SO			Black Balsam	1780	scat	[redacted]	[redacted]
104020702	SO 124	SO			Black Balsam	1780	scat	[redacted]	[redacted]
104020703	SO 125	SO			Black Balsam	1780	scat	[redacted]	[redacted]
104020802	SO 127	SO			Black Balsam	1780	scat	[redacted]	[redacted]
1504010101	SO129	SO			Black Balsam	1077	scat	[redacted]	[redacted]

1504010102	SO130	SO			Black Balsam	1077	scat	[redacted]	[redacted]
1504010103	SO131	SO			Black Balsam	1077	scat	[redacted]	[redacted]
1504010104	SO132	SO			Black Balsam	1077	scat	[redacted]	[redacted]
1504010301	SO133	SO			Black Balsam	1077	scat	[redacted]	[redacted]
1504010302	SO134	SO			Black Balsam	1077	scat	[redacted]	[redacted]
1902010301	SO 090	SO			Roan	1807	scat	[redacted]	[redacted]
1902010401	SO 091	SO			Roan	1807	scat	[redacted]	[redacted]
1902010402	SO 092	SO			Roan	1807	scat	[redacted]	[redacted]
1902010901	SO 093	SF			Roan	1807	scat	[redacted]	[redacted]
1902010902	SO 094	SF			Roan	1807	scat	[redacted]	[redacted]
1902010903	SO 095	SF			Roan	1807	scat	[redacted]	[redacted]
1902020401	SO 096	UND			Roan	1787	scat	[redacted]	[redacted]
1902020402	SO 097	SO			Roan	1787	scat	[redacted]	[redacted]
1902020403	SO 098	SF			Roan	1787	scat	[redacted]	[redacted]
1902020501	SO 099	SO			Roan	1787	scat	[redacted]	[redacted]
0104OPP1	SO 128	SO			Black Balsam	1780	scat	[redacted]	[redacted]
150401OPP01	SO135	SF			Black Balsam	1061	scat	[redacted]	[redacted]
1902010201 Old	SO 089	SF			Roan	1807	scat	[redacted]	[redacted]
190202OPP 2BB	SO 102	SO			Roan	1776	scat	[redacted]	[redacted]
190202OPP1	SO 100	SO			Roan	1787	scat	[redacted]	[redacted]
190202OPP2	SO 101	SO			Roan	1776	scat	[redacted]	[redacted]
4-14-18 Flat Laurel - W 4 175 0327382, 3910362	SO194	SO	4/14/2018	Flat Laurel W-4	Black Balsam	1679	scat	[redacted]	[redacted]
50-11 large	SO 061	SO			Roan	1832	scat	[redacted]	[redacted]
50-11 small	SO 062	SO			Roan	1832	scat	[redacted]	[redacted]
50-13 fresher large	SO 072	SO			Roan	1816	scat	[redacted]	[redacted]

50-13 weathered large	SO 071	SO			Roan	1816	scat	[redacted]	[redacted]
50-14 unknown	SO 068	SO			Roan	1811	scat	[redacted]	[redacted]
50-15 large	SO 069	SF			Roan	1803	scat	[redacted]	[redacted]
50-19 large	SO 063	SO			Roan	1771	scat	[redacted]	[redacted]
50-19 Small	SO 052	UND			Roan	1771	scat	[redacted]	[redacted]
50-19 small	SO 064	UND			Roan	1771	scat	[redacted]	[redacted]
50-20 large	SO 066	SO			Roan	1763	scat	[redacted]	[redacted]
50-20 small	SO 053	SO			Roan	1763	scat	[redacted]	[redacted]
50-20 small	SO 065	UND			Roan	1763	scat	[redacted]	[redacted]
50-rogue small	SO 051	SO			Roan	1763	scat	[redacted]	[redacted]
50-rogue small	SO 067	SO			Roan	1763	scat	[redacted]	[redacted]
BBOP01	SO136	SO			Black Balsam	1054	scat	[redacted]	[redacted]
BBOPP03	SO137	SO			Black Balsam	1096	scat	[redacted]	[redacted]
BBOPP201	SO138	SO			Black Balsam	1082	scat	[redacted]	[redacted]
BBOPP202	SO139	SO			Black Balsam	1082	scat	[redacted]	[redacted]
BBOPP203	SO140	SO			Black Balsam	1082	scat	[redacted]	[redacted]
BBOPP204	SO141	SO			Black Balsam	1082	scat	[redacted]	[redacted]
Big Knob 0-1	SO192	SO	3/23/2018	Big Knob 0-1	GRSM??	1320	scat	[redacted]	[redacted]
Black Balsam Flat Laurel-West-2A	SO195	SO	4/14/2018	Flat Laurel W-2A	Black Balsam	1673	scat	[redacted]	[redacted]
Black Balsam Opp. 1	SO206	SO	4/25/2018	Black Balsam - opportunistic	Black Balsam	1746	scat	[redacted]	[redacted]
Black Balsam Rd W-0-1	SO169	SO	3/17/2018	Black Balsam Road W pt #0	Black Balsam	1448	scat	[redacted]	[redacted]
Black Balsam Rd W-9-1	SO189	SO	3/17/2018	Black Balsam Road W pt #9	Black Balsam	1759	scat	[redacted]	[redacted]
Black Balsam Rd W-9-2	SO190	SO	3/17/2018	Black Balsam Road W pt #9	Black Balsam	1759	scat	[redacted]	[redacted]

Black Balsam Rd W-9-3	SO191	SO	3/17/2018	Black Balsam Road W pt #9	Black Balsam	1759	scat	[redacted]	[redacted]
BlackMountainMount Mitchell2-10-1	SO270	SO	14-Feb-19	Black Mountain, Mount Mitchell 2	Black Mountains	2006	scat	[redacted]	[redacted]
BlackMountainVisitor Center4-4-1	SO271	SO	14-Feb-19	Black Mountain Visitor Center 4	Black Mountains	1817	scat	[redacted]	[redacted]
BRP Roadkill	SO357	SO	25-Apr-19	Flat Rock	Blue Ridge Parkway	1208	tissue	[redacted]	[redacted]
Carver's Gap SYOB male Trap CG8 no tag, YOY	SO424	SO	9/16/2019	Carver's Gap	Roan	1644	tissue	[redacted]	[redacted]
Carvers Gap 151.129 Trap CG6 male	SO416	SO	9/17/2019	Carver's Gap	Roan	1629	tissue	[redacted]	[redacted]
CF2 Trap 012	SO223	SO	11/3/2018	Cradle of Forestry	Pisgah NF	1003	Tissue	[redacted]	[redacted]
CG1 015	SO217T	SF	10/30/2018	Carver's Gap - Roan Mtn.	Roan	1657	Tissue	[redacted]	[redacted]
CG15 013	SO218T	SF	10/30/2018	Carver's Gap	Roan	1675	Tissue	[redacted]	[redacted]
CG6 014	SO219	SO	10/30/2018	Carver's Gap	Roan	1653	Tissue	[redacted]	[redacted]
ChestnutBald1-1-1	SO247	SO	21-Jan-19	Chestnut Bald 1	276	1791	scat	[redacted]	[redacted]
Clingman's Dome 0-1	SO204	SF	4/20/2018	Clingman's Dome 0-1	GRSM	2000	scat	[redacted]	[redacted]
Clingman's Dome 0-2	SO205	SF	4/20/2018	Clingman's Dome 0-2	GRSM	2000	scat	[redacted]	[redacted]
Clingman's Dome 2-10-1	SO166	SO	3/16/2018	Clingman's Dome 2 pt #10	GRSM	1616	scat	[redacted]	[redacted]
Clingman's Dome 2-4-1	SO163	SO	3/16/2018	Clingman's Dome 2 pt #4	GRSM	1624	scat	[redacted]	[redacted]
Clingman's Dome 2-4-2	SO164	SO	3/16/2018	Clingman's Dome 2 pt #4	GRSM	1624	scat	[redacted]	[redacted]
Clingman's Dome 2-4-3	SO165	SO	3/16/2018	Clingman's Dome 2 pt #4	GRSM	1624	scat	[redacted]	[redacted]
Clingman's Dome Rd	SO167	SO	3/17/2018	Clingman's Dome Road	GRSM	1725	scat	[redacted]	[redacted]
Commissary Ridge	SO366	SO	9/24/2019	Commissary Ridge Mt Mitchell	Black Mtns	1809	tissue	[redacted]	[redacted]

CR11 - tissue									
Commissary Ridge CR6 - tissue	SO363	SO	9/24/2019	Commissary Ridge Mt Mitchell	Black Mtns	1842	tissue	[redacted]	[redacted]
Cradle of Forestry CF11 151.289 048 - tissue	SO391	SO	11/26/2019	Cradle of Forestry	Pisgah NF	1003	tissue	[redacted]	[redacted]
Cradle of Forestry CF2 151.249 050 - tissue	SO394	SO	12/2/2019	Cradle of Forestry	Pisgah NF	1005	tissue	[redacted]	[redacted]
Devil's Cthouse N-5	SO179	SO	3/13/2018	Devil's Cthouse N-5	Black Balsam	1673	scat	[redacted]	[redacted]
Devil's Cthouse SE-2	SO184	SO	3/13/2018	Devil's Cthouse SE-2	Black Balsam	1653	scat	[redacted]	[redacted]
Devil's Cthouse-7	SO180	SO	3/13/2018	Devil's Cthouse-7	Black Balsam	1672	scat	[redacted]	[redacted]
Devil's Cthouse-N-2A	SO174	SO	3/13/2018	Devil's Cthouse-N-2A	Black Balsam	1671	scat	[redacted]	[redacted]
Devil's Cthouse-N-2B	SO175	SO	3/13/2018	Devil's Cthouse-N-2B	Black Balsam	1671	scat	[redacted]	[redacted]
Devil's Cthouse-N-2C	SO176	SO	3/13/2018	Devil's Cthouse-N-2C	Black Balsam	1671	scat	[redacted]	[redacted]
Devil's Cthouse-N-3A	SO177	SO	3/13/2018	Devil's Cthouse-N-3A	Black Balsam	1672	scat	[redacted]	[redacted]
Devil's Cthouse-N-3B	SO178	SO	3/13/2018	Devil's Cthouse-N-3B	Black Balsam	1672	scat	[redacted]	[redacted]
Devil'sCourthouseN2-1-1	SO248	SO	21-Jan-19	Devil's Courthouse N 2	Black Balsam	1708	scat	[redacted]	[redacted]
Flat Laurel - West -2B	SO196	SO	4/14/2018	Flat Laurel W-2B	Black Balsam	1673	scat	[redacted]	[redacted]
Flat Laurel Branch 0-1	SO150	SO	3/18/2018	Flat Laurel Branch 0-1	Black Balsam	1691	scat	[redacted]	[redacted]
Flat Laurel Branch 0-2	SO151	SO	3/18/2018	Flat Laurel Branch 0-2	Black Balsam	1691	scat	[redacted]	[redacted]
Flat Laurel Branch 0-2 E	SO152	SO	3/17/2018	Flat Laurel Branch 0-2	Black Balsam	1725	scat	[redacted]	[redacted]
Flat Laurel Branch 151.110	SO207T	SO	4/26/2018	Flat Laurel Branch Trap FLBA1	Black Balsam	1729	tissue	[redacted]	[redacted]

original capture									
Flat Laurel Branch C-5	SO162	SO	3/18/2018	Flat Laurel Branch Central pt #5	Black Balsam	1670	scat	[redacted]	[redacted]
Flat Laurel Branch E-5-1	SO157	SO	3/18/2018	Flat Laurel Branch E pt #5	Black Balsam	1721	scat	[redacted]	[redacted]
Flat Laurel Branch E-5-2	SO158	SO	3/18/2018	Flat Laurel Branch E pt #5	Black Balsam	1721	scat	[redacted]	[redacted]
Flat Laurel Branch E-7	SO159	SO	3/18/2018	Flat Laurel Branch E pt #7	Black Balsam	1717	scat	[redacted]	[redacted]
Flat Laurel Branch E-9-1	SO160	SO	3/18/2018	Flat Laurel Branch E pt #9	Black Balsam	1714	scat	[redacted]	[redacted]
Flat Laurel Branch E-9-2	SO161	SO	3/18/2018	Flat Laurel Branch E pt #9	Black Balsam	1714	scat	[redacted]	[redacted]
Flat Laurel Branch NE-3-1	SO153	SF	3/18/2018	Flat Laurel Branch NE pt # 3	Black Balsam	1751	scat	[redacted]	[redacted]
Flat Laurel Branch NE-3-2	SO154	SF	3/18/2018	Flat Laurel Branch NE pt # 3	Black Balsam	1751	scat	[redacted]	[redacted]
Flat Laurel Branch NE-3-3	SO155	SF	3/18/2018	Flat Laurel Branch NE pt # 3	Black Balsam	1751	scat	[redacted]	[redacted]
Flat Laurel Branch NE-3-4	SO156	SF	3/18/2018	Flat Laurel Branch NE pt # 3	Black Balsam	1751	scat	[redacted]	[redacted]
Flat Laurel FL18 151.647 male	SO410	SF	10/24/2019	Flat Laurel	Black Balsam	1737	tissue	[redacted]	[redacted]
Flat Laurel FL2 150.449 - tissue	SO379	SO	10/18/2019	Flat Laurel	Black Balsam	1730	tissue	[redacted]	[redacted]
Flat Laurel FL2 151.590 - tissue	SO382	SO	10/12/2019	Flat Laurel	Black Balsam	1730	tissue	[redacted]	[redacted]
Flat Laurel FL23 151.689 - tissue	SO385	SO	10/24/2019	Flat Laurel	Black Balsam	1737	tissue	[redacted]	[redacted]
Flat Laurel FL8 035 - tissue	SO376	SO	10/10/2019	Flat Laurel	Black Balsam	1723	tissue	[redacted]	[redacted]
Flat Laurel W-10	SO197	SO	4/14/2018	Flat Laurel W-10	Black Balsam	1669	scat	[redacted]	[redacted]

Forney Ridge 0-1	SO202	SO	3-9-18?	Forney Ridge 0-1	GRSM	1852	scat	[redacted]	[redacted]
Graveyard Fields female FL18	SO408	SF	10/12/2019	Graveyard Fields	Black Balsam	1762	tissue	[redacted]	[redacted]
Graveyard Fields GF10 151.454 - tissue	SO402	SO	10/11/2019	Graveyard Fields	Black Balsam	1553	tissue	[redacted]	[redacted]
Graveyard Fields GF24 151.609 - tissue	SO397	SO	10/17/2019	Graveyard Fields	Black Balsam	1563	tissue	[redacted]	[redacted]
Graveyard Fields GF27 150.549 - tissue	SO399	SO	10/17/2019	Graveyard Fields	Black Balsam	1543	tissue	[redacted]	[redacted]
Graveyard Fields GF4 046 tissue	SO360	SO	10/10/2019	Graveyard Fields	Black Balsam	1538	tissue	[redacted]	[redacted]
Green Mountain male GM7 151.710	SO409	SO	11/4/2019	Green Mountain	Panthertown	1181	tissue	[redacted]	[redacted]
Green Mountain male tag038 GM14	SO411	SF	11-Nov-19	Green Mountain	Panthertown	1238	tissue	[redacted]	[redacted]
GSMNP	SO221	SF	10/18/2018	Purchase Knob	GRSM	1499	Tissue	[redacted]	[redacted]
Juv, F	SO 058	SO			Roan	1706	tissue	[redacted]	[redacted]
Juv, F	SO 059	SF			Roan	1877	tissue	[redacted]	[redacted]
Juv, F, Trap 2	SO 056	SO			Roan	1685	tissue	[redacted]	[redacted]
LinvilleGorge3-8-1	SO269	SF	11-Feb-19	Linville Gorge 3	Linville	1053	scat	[redacted]	[redacted]
Little Sam's Knob	SO198	SO	4/14/2018	Little Sam's Knob	Black Balsam	1690	scat	[redacted]	[redacted]
Little Sam's Knob	SO199	SO	4/14/2018	Little Sam's Knob	Black Balsam	1694	scat	[redacted]	[redacted]
Little Sam's Knob	SO200	SO	4/14/2018	Little Sam's Knob	Black Balsam	1696	scat	[redacted]	[redacted]
livetrap Black Balsam Trap	SO209T	SO	5/2/2018	Black Balsam Trap 15	Black Balsam	1793	tissue	[redacted]	[redacted]

livetrap Cradle	SO168T	SO	3/14/2018	Cradle of Forestry	Pisgah NF	1007	tissue	[redacted]	[redacted]
livetrap Cradle SYOB? female	SO193T	SO	3/30/2018	Cradle of Forestry	Pisgah NF	998	tissue	[redacted]	[redacted]
livetrap Forney Ridge, GSMNP	SO203T	SO	4/20/2018	Forney Ridge GSMNP	GRSM	1777	tissue	[redacted]	[redacted]
livetrap SYOB Cradle	SO212T	SO	5/13/2018	Cradle of Forestry	Pisgah NF	1003	tissue	[redacted]	[redacted]
LookingGlass1-10-1	SO349	UND	20-Mar-19	Looking Glass 1	Looking Glass Rock	1053	scat	[redacted]	[redacted]
LookingGlass1-5-1	SO350	UND	20-Mar-19	Looking Glass 1	Looking Glass Rock	1053	scat	[redacted]	[redacted]
LookingGlass2-3-1,	SO351	UND	20-Mar-19	Looking Glass 2	Looking Glass Rock	1103	scat	[redacted]	[redacted]
LookingGlass2-3-2	SO352	UND	20-Mar-19	Looking Glass 2	Looking Glass Rock	1103	scat	[redacted]	[redacted]
MaxPatch1-3-1	SO272	SF	20-Feb-19	Max Patch 1	Max Patch	1357	scat	[redacted]	[redacted]
MaxPatch2-1-1	SO273	SO	20-Feb-19	Max Patch 2	Max Patch	1320	scat	[redacted]	[redacted]
MaxPatch2-3-1	SO274	SO	20-Feb-19	Max Patch 2	Max Patch	1320	scat	[redacted]	[redacted]
MaxPatch4-4-1	SO275	SO	20-Feb-19	Max Patch 4	Max Patch	1273	scat	[redacted]	[redacted]
Mt Mitchell Campground CG15 032 - tissue	SO373	SO	9/24/2019	Mt Mitchell CG	Black Mtns	1883	tissue	[redacted]	[redacted]
NWOP2	SO143	SF	2/3/2018	Roan Mtn - NW bald	Roan	1873	scat	[redacted]	[redacted]
NWOP3	SO144	SF	2/3/2018	Roan Mtn - NW bald	Roan	1878	scat	[redacted]	[redacted]
NWOPP1	SO142	SF	2/3/2018	Roan Mtn - NW bald	Roan	1871	scat	[redacted]	[redacted]
OPP-2-A Devil's Cthouse	SO170	SO	3/13/2018	Devil's Courthouse - Opportunistic	Black Balsam	1671	scat	[redacted]	[redacted]
OPP2-B Devil's Cthouse	SO171	SO	3/13/2018	Devil's Courthouse - Opportunistic	Black Balsam	1671	scat	[redacted]	[redacted]
OPP2-C Devil's Cthouse	SO172	SO	3/13/2018	Devil's Courthouse - Opportunistic	Black Balsam	1671	scat	[redacted]	[redacted]
OPP2-D Devil's Cthouse	SO173	SO	3/13/2018	Devil's Courthouse -	Black Balsam	1671	scat	[redacted]	[redacted]

				Opportunistic					
OppOakKnob 1-1	SO355	UND	12-Mar-19	Unicoi Mountains 9	Unicois	1651	scat	[redacted]	[redacted]
Panthertown1-1-1	SO331	SO	18-Mar-19	Panthertown 1	Panthertown	1228	scat	[redacted]	[redacted]
Panthertown11-1-1	SO337	SO	19-Mar-19	Panthertown 11	Panthertown	1220	scat	[redacted]	[redacted]
Panthertown11-6-1	SO338	SO	19-Mar-19	Panthertown 11	Panthertown	1220	scat	[redacted]	[redacted]
Panthertown12-2-1	SO339	SO	19-Mar-19	Panthertown 12	Panthertown	1217	scat	[redacted]	[redacted]
Panthertown12-7-1	SO340	SO	19-Mar-19	Panthertown 12	Panthertown		scat	[redacted]	[redacted]
Panthertown13-2-1	SO332	SO	18-Mar-19	Panthertown 13	Panthertown		scat	[redacted]	[redacted]
Panthertown13-6-1	SO333	SO	18-Mar-19	Panthertown 13	Panthertown	1197	scat	[redacted]	[redacted]
Panthertown14-2-1	SO334	SO	18-Mar-19	Panthertown 14	Panthertown	1209	scat	[redacted]	[redacted]
Panthertown15-4-1	SO341	SO	19-Mar-19	Panthertown 15	Panthertown	1191	scat	[redacted]	[redacted]
Panthertown15-8-1	SO342	SO	19-Mar-19	Panthertown 15	Panthertown	1191	scat	[redacted]	[redacted]
Panthertown16-3-1	SO343	SO	19-Mar-19	Panthertown 16	Panthertown	1219	scat	[redacted]	[redacted]
Panthertown17-10-1	SO344	SO	19-Mar-19	Panthertown 17	Panthertown	1173	scat	[redacted]	[redacted]
Panthertown18-1-1	SO345	SO	19-Mar-19	Panthertown 18	Panthertown	1156	scat	[redacted]	[redacted]
Panthertown19-1-1	SO346	SO	19-Mar-19	Panthertown 19	Panthertown	1142	scat	[redacted]	[redacted]
Panthertown19-4-1	SO347	SO	19-Mar-19	Panthertown 19	Panthertown	1142	scat	[redacted]	[redacted]
Panthertown4-1-1	SO335	SO	18-Mar-19	Panthertown 4	Panthertown	1263	scat	[redacted]	[redacted]
Panthertown4-2-1	SO336	SO	18-Mar-19	Panthertown 4	Panthertown	1263	scat	[redacted]	[redacted]
Pathertown 12-4-1	SO348	SO	19-Mar-19	Panthertown 12	Panthertown	1217	scat	[redacted]	[redacted]
PinkBeds 3-10-1	SO353	UND	15-Jan-19	Pink Beds		995	scat	[redacted]	[redacted]
R49-1	SO 030	SF			Roan	1871	scat	[redacted]	[redacted]
R49-1	SO 088	SF			Roan	1871	scat	[redacted]	[redacted]
R4950-1	SO 031	SO			Roan	1855	scat	[redacted]	[redacted]
R4950-2	SO 032	SO			Roan	1850	scat	[redacted]	[redacted]
R4950-3	SO 033	UND			Roan	1845	scat	[redacted]	[redacted]
R4950-4	SO 083	SO			Roan	1840	scat	[redacted]	[redacted]
R4950-6	SO 035	SO			Roan	1853	scat	[redacted]	[redacted]
R4950-7	SO 082	SO			Roan	1846	scat	[redacted]	[redacted]
R4950-8	SO 081	SO			Roan	1840	scat	[redacted]	[redacted]
R4950-9	SO 036	SF			Roan	1836	scat	[redacted]	[redacted]
R5455-1	SO 075	SF			Roan	1885	scat	[redacted]	[redacted]
R5455-2	SO 037	SF			Roan	1883	scat	[redacted]	[redacted]

R5455-3	SO 073	SF			Roan	1882	scat	[redacted]	[redacted]
R5455-3	SO 086	SF			Roan	1882	scat	[redacted]	[redacted]
R5455-4	SO 074	SF			Roan	1881	scat	[redacted]	[redacted]
R5455-4	SO 087	SF			Roan	1881	scat	[redacted]	[redacted]
R5455-5	SO 038	SF			Roan	1881	scat	[redacted]	[redacted]
R5455-6	SO 039	SF			Roan	1887	scat	[redacted]	[redacted]
R5455-7	SO 040	SF			Roan	1888	scat	[redacted]	[redacted]
RB1-1	SO 084	SO			Roan	1687	scat	[redacted]	[redacted]
RB1-2	SO 076	SO			Roan	1682	scat	[redacted]	[redacted]
RB1-3	SO 041	SO			Roan	1678	scat	[redacted]	[redacted]
RB1-4	SO 042	SO			Roan	1670	scat	[redacted]	[redacted]
RB1-5	SO 043	SF			Roan	1666	scat	[redacted]	[redacted]
RB1-6	SO 044	SO			Roan	1690	scat	[redacted]	[redacted]
RB1-7	SO 077	SO			Roan	1691	scat	[redacted]	[redacted]
RB1-8	SO 078	SO			Roan	1694	scat	[redacted]	[redacted]
RB2-1	SO 079	SF			Roan	1748	scat	[redacted]	[redacted]
RB2-2	SO 080	SO			Roan	1747	scat	[redacted]	[redacted]
RB2-3	SO 045	SF			Roan	1745	scat	[redacted]	[redacted]
RB2-4	SO 046	SF			Roan	1745	scat	[redacted]	[redacted]
RB2-5	SO 047	SF			Roan	1746	scat	[redacted]	[redacted]
RB2-6	SO 048	SF			Roan	1749	scat	[redacted]	[redacted]
RB2-7	SO 049	SF			Roan	1749	scat	[redacted]	[redacted]
RHBT10 151.090	SO220T	UND	10/30/2018	Roan Mtn Bluff	Roan	1886	Tissue	[redacted]	[redacted]
Rhodo Garden Male 023 Trap RG15	SO415	SO	9/16/2019	Rhodo Garden	Roan	1741	tissue	[redacted]	[redacted]
Rhodo Garden SYOB male 151.029 trap RG19	SO421	SO	9/17/2019	Rhodo Garden	Roan	1893	tissue	[redacted]	[redacted]
Rhodo Gardens female TrapRG9 Tag022	SO413	SO	9/8/2019	Rhodo Gardens Trap RG9	Roan	1857	tissue	[redacted]	[redacted]
Road Kill Bunny (Fork Mt Road)	SO227	SF	3/4/2019	NW of Bakersville	Honeycutt, NC	858	Tissue	[redacted]	[redacted]
Roadkill BRP Bluff Mtn	SO211	SF	5/7/2018	BRP Bluff Mountain Overlook	Blue Ridge Parkway	1017	tissue	[redacted]	[redacted]
Roadkill BRP Johns Rock	SO208	SF	5/1/2018	BRP Johns Rock Overlook	Blue Ridge Parkway	1626	tissue	[redacted]	[redacted]
Roadkill BRP Looking Glass	SO215	SO	6/22/2018	BRP Looking Glass Overlook	Looking Glass	1372	tissue	[redacted]	[redacted]

Roadkill BRP Stoney Fork	SO210	SF	5/7/2018	BRP Stoney Fork Overlook (MP 278)	Blue Ridge Parkway	1047	tissue	[redacted]	[redacted]
Roadkill Cherokee	SO201	SF	4/19/2018	Cherokee	Cherokee	600	tissue	[redacted]	[redacted]
Roadkill Clingman's Road	SO213	SO	5/24/2018	Clingman's Dome Rd, GSMNP	GRSM	1783	tissue	[redacted]	[redacted]
Roadkill Cradle	SO214	SF	5/24/2018	Cradle of Forestry	Pisgah NF	998	tissue	[redacted]	[redacted]
Roan Loop SYOB female 150.629 trap RL20	SO420	SO	9/16/2019	Roan Loop	Roan	1867	tissue	[redacted]	[redacted]
Round Bald female SYFL 021 Trap RB2	SO419	SF	9/17/2019	Round Bald	Roan	1682	tissue	[redacted]	[redacted]
Round Bald female SYFL 024 Trap 14	SO417	SF	9/17/2019	Round Bald	Roan	1741	tissue	[redacted]	[redacted]
Round Bald female Tag023 Trap 14	SO412	SF	16-Sep-19	Round Bald Trap 14	Roan	1741	tissue	[redacted]	[redacted]
Round Bald male SYFL Trap RB14 Tag 018	SO418	SF	9/9/2019	Round Bald	Roan	1884	tissue	[redacted]	[redacted]
Round Bald SYFL male Tag 019 Trap 15	SO423	SF	9/16/2019	Round Bald	Roan	1739	tissue	[redacted]	[redacted]
Sandy Mush 1A-1	SO185	SO	3/15/2018	Sandy Mush 1A pt #9	Sandy Mush	600	scat	[redacted]	[redacted]
Sandy Mush 1A-2	SO186	SO	3/15/2018	Sandy Mush 1A pt #2	Sandy Mush	600	scat	[redacted]	[redacted]
Sandy Mush 1A-8	SO187	SO	3/15/2018	Sandy Mush 1A pt #8	Sandy Mush	590	scat	[redacted]	[redacted]
Sandy Mush 1A-8	SO188	SO	3/15/2018	Sandy Mush 1A pt #8	Sandy Mush	590	scat	[redacted]	[redacted]
SouthMountainGamelands10-6-1	SO277	SO	25-Feb-19	South Mountain Gamelands 10	South Mountains	762	scat	[redacted]	[redacted]

SouthMountainGamelands11-1-1	SO279	SO	25-Feb-19	South Mountain Gamelands 11	South Mountains	877	scat	[redacted]	[redacted]
SouthMountainGamelands11-10-1	SO278	SO	25-Feb-19	South Mountain Gamelands 11	South Mountains	877	scat	[redacted]	[redacted]
SouthMountainGamelands11-2-1	SO280	SO	25-Feb-19	South Mountain Gamelands 11	South Mountains	877	scat	[redacted]	[redacted]
SouthMountainGamelands4-4-1	SO276	SO	21-Feb-19	South Mountain Gamelands 4	South Mountains	383	scat	[redacted]	[redacted]
SouthMountainGamelands7-1-1	SO281	SO	25-Feb-19	South Mountain Gamelands 7	South Mountains	742	scat	[redacted]	[redacted]
SR-01	SO 050	SO			Black Balsam	1793	scat	[redacted]	[redacted]
SR-01	SO 085	SO			Roan	1793	scat	[redacted]	[redacted]
Stepp's Gap RS8 151.431 - tissue	SO370	SO	9/25/2019	Stepp's Gap Mt Mitchell	Black Mtns	1846	tissue	[redacted]	[redacted]
SYOB Road Kill (Rt 64, East of Rosman)	SO407	SF	Unknown	? Road Kill	Lake Toxaway	840	tissue	[redacted]	[redacted]
Tollhouse Gap Roan female 670	SO414	SF	9/6/2019	Tollhouse Gap Roan	Roan	1877	tissue	[redacted]	[redacted]
Tollhouse Gap SYOB female 151.369 trap TG4	SO422	SO	9/17/2019	Tollhouse Gap Roan	Roan	1871	tissue	[redacted]	[redacted]
Trap CF1 151.009	SO228T	SO	11/20/2018	Cradle of Forestry	Pisgah NF	1006	tissue	[redacted]	[redacted]
Trap CF1 151.209	SO225	SO	11/3/2018	Cradle of Forestry	Pisgah NF	1006	Tissue	[redacted]	[redacted]
Trap CF15 150.809	SO224	SO	11/3/2018	Cradle of Forestry	Pisgah NF	998	Tissue	[redacted]	[redacted]
Trap CF7 151.169	SO226	SO	11/3/2018	Cradle of Forestry	Pisgah NF	995	Tissue	[redacted]	[redacted]
Trap PBP3 017	SO231T	SO	11/26/2018	Pink Beds Picnic	Pisgah NF	998	tissue	[redacted]	[redacted]
Trap RG14	SO216T	SF	10/25/2018	Rhodo Garden - Roan Mtn.	Roan	1857	Tissue	[redacted]	[redacted]

Trap RG20 151.229	SO222	SO	10/31/2018	Rhodo Garden - Roan Mtn.	Roan	1885	Tissue	[redacted]	[redacted]
UnicoiMountains1-1-1	SO325	SO	12-Mar-19	Unicoi Mountains 1	Unicois		scat	[redacted]	[redacted]
UnicoiMountains10-8-1	SO324	SO	12-Mar-19	Unicoi Mountains 10	Unicois	1686	scat	[redacted]	[redacted]
UnicoiMountains2-5-1	SO326	SO	12-Mar-19	Unicoi Mountains 2	Unicois		scat	[redacted]	[redacted]
UnicoiMountains2-8-1	SO327	SO	12-Mar-19	Unicoi Mountains 2	Unicois		scat	[redacted]	[redacted]
UnicoiMountains6-1-1	SO328	SO	12-Mar-19	Unicoi Mountains 6	Unicois		scat	[redacted]	[redacted]
UnicoiMountains6-2-1	SO329	SO	12-Mar-19	Unicoi Mountains 6	Unicois		scat	[redacted]	[redacted]
UnicoiMountains8-5-1	SO330	SO	12-Mar-19	Unicoi Mountains 8	Unicois	1645	scat	[redacted]	[redacted]
WC-4	SO148	SO	2/3/2018	Roan Mtn - WC spruce-fir	Roan	1824	scat	[redacted]	[redacted]
WC8	SO149	SO	2/3/2018	Roan Mtn - WC spruce-fir	Roan	1815	scat	[redacted]	[redacted]
WCOP1	SO145	SO	2/3/2018	Roan Mtn - WC spruce-fir	Roan	1834	scat	[redacted]	[redacted]
WCOP2	SO146	SF	2/3/2018	Roan Mtn - WC spruce-fir	Roan	1833	scat	[redacted]	[redacted]
WCOP3	SO147	SO	2/3/2018	Roan Mtn - WC spruce-fir	Roan	1823	scat	[redacted]	[redacted]
Wet Devil's Cthouse-9A	SO181	SO	3/13/2018	Wet Devil's Cthouse-9A	Black Balsam	1668	scat	[redacted]	[redacted]
Wet Devil's Cthouse-9B	SO182	SO	3/13/2018	Wet Devil's Cthouse-9B	Black Balsam	1668	scat	[redacted]	[redacted]
Wet Devil's Cthouse-9C	SO183	SO	3/13/2018	Wet Devil's Cthouse-9C	Black Balsam	1668	scat	[redacted]	[redacted]