FINAL REPORT

As Required by

THE ENDANGERED SPECIES PROGRAM

TEXAS

Grant No. E - 57

Endangered and Threatened Species Conservation

Conservation Genetics of the Black-capped Vireo (*Vireo atricapilla*)

Prepared by:

Bob Zink and Andy Jones

TEXAS PARKS & WILDLIFE

Robert Cook
Executive Director

Matt Wagner
Program Director, Wildlife Diversity

Mike Berger
Division Director, Wildlife

December 20, 2006

FINAL REPORT

STATE: Texas **GRANT NUMBER:** E - 57

GRANT TITLE: Conservation Genetics of the Black-capped Vireo (*Vireo atricapilla*)

REPORTING PERIOD: 1 October 2004 to 30 September 2006

OBJECTIVE(S):

1) To determine from mitochondrial DNA analysis whether the Black-capped Vireo consists of more than one Distinct Population Segment (DPS),
2) To assess variability and gene flow among populations, or DPSs, and
3) To assess spatio-temporal patterns of colonization in Mexico, Texas, and Oklahoma

Significant Deviations:

Samples of Dwarf Vireo (*V. nelsoni*) were not obtained; no recent specimens exist in museums and none were captured for this study. This did not affect results (please see Attachment A).

Summary Of Progress:

Please see Attachment A.

Location: Several sites in Oklahoma, Texas, and Mexico.

Cost: _____

Prepared by: Craig Farquhar **Date:** December 20, 2006

Approved by: _____ **Date:** _____
Neil (Nick) E. Carter

ATTACHMENT A

FINAL REPORT FOR E-57

PI Robert M. Zink, University of Minnesota

19 December 2006

Significant Deviation

We were unable to locate and tissues of Dwarf Vireo (*V. nelsoni*) which has been proposed as the sister taxon of Black-capped Vireo ("probably conspecific" Phillips 1991, p. 195); no tissues are known to exist in any natural history museum (A. T. Peterson, pers. comm.). However, this does not affect the results of this study because there was no phylogenetic structure to the haplotype tree; hence, rooting could not change the outcome or interpretation.

CONSERVATION GENETICS OF THE BLACK-CAPPED VIREO

ABSTRACT

The Black-capped Vireo (*Vireo atricapilla*) breeds in habitat fragments in Oklahoma, Texas, Coahuila, Nuevo Leon, and Tamaulipas, and is endangered throughout its range due to habitat destruction and fragmentation; population declines are accelerated by cowbird parasitism. We used mitochondrial DNA (mtDNA) sequences from the ND2 gene to examine population structure. Feather samples from 108 individuals were taken, covering all five states where this bird occurs. Despite the geographic isolation of existing populations, no phylogeographic breaks were found. Sequence divergence was quite low (<1%) and few haplotypes were found, as expected for a species with a small

global distribution and no described subspecific variation. Mismatch distributions were unimodal, suggesting a recent population bottleneck, likely during the Pleistocene. This pattern of recent population growth is repeated when analyzing southern and northern samples separately, precluding inferences of Pleistocene movements. We suggest that recent isolation of populations is of insufficient duration and intensity to cause genetic differentiation in neutral markers in this species. Lack of geographic structure in an endangered bird in North America is a fairly common finding in the literature; we discuss the implications of this result to conservation and management priorities.

Key words: Endangered Species Act, mtDNA, phylogeography

INTRODUCTION

The Black-capped Vireo (*Vireo atricapillus*) is a small passerine that breeds in scrubby habitats in the states of Oklahoma and Texas in the United States, and Coahuila, Nuevo Leon, and Tamaulipas in Mexico. These birds migrate southwest to the lowlands along the Pacific slope of Mexico in winter. This species was formerly more widespread, occurring regularly in Nebraska and Kansas until the early 1900s. Black-capped Vireos have been widely studied (e.g. Grzybowski 1995). Attention has been focused on the Black-capped Vireo because it displays several interesting features. This species has an exceedingly large repertoire of song syllables, high even among the Vireonidae. Further, Black-capped Vireos show sexual dichromatism and delayed plumage maturation (second-year males are visibly distinct from after-second-year males); both of these characteristics are unusual among the Vireonidae (Grzybowski 1995).

Black-capped Vireo populations have declined recently due primarily to habitat loss and cowbird parasitism. As a result of this decline this species is listed as endangered by the United States Fish and Wildlife Service. Their specific habitat is dominated by oaks or junipers and is declining at a fairly high rate; this habitat is also home to the Federally endangered (in the United States) Golden-cheeked Warbler (*Dendroica chrysoparia*). Management efforts have focused on minimizing effects from the cowbirds that parasitize them, and establishment and maintenance of appropriate habitat (Grzybowski 1995).

Despite the attention paid to this species for various aspects of its natural history, its evolutionary history is not well known. Conservation efforts should be informed with genetic data whenever possible, and for a species that exists in fragmented populations

documentation of cryptic lineages or other population subdivision is important when prioritizing land conservation and management efforts. The Black-capped Vireo was the subject of an allozyme analysis (Fazio et al. 1992); from limited sampling (three Texas populations and one Oklahoma population) they found significant population differentiation. However, their data included two loci that were under selection, biasing their conclusions.

In this paper we include samples from all states where Black-capped Vireos are extant, present findings on population differentiation in this species, and discuss how these findings are relevant to management goals in endangered species.

METHODS

Sampling

Feathers were collected from live birds in the states of Oklahoma and Texas (United States of America) and Coahuila, Tamaulipas, and Nuevo Leon (Mexico) during the breeding seasons of 2002-2005. Exact locations are detailed in Appendix 1. Permits for feather sampling are detailed in the Acknowledgments section. Voucher specimens were not obtained due to conservation issues across the range of this taxon. The following outgroup sequences were taken from Genbank: *Vireo olivaceus* (AY136614), *V. solitarius* (AY030137), *V. gilvus* (AY030135), *V. latimeri* (AF281020), *V. leucophrys* (AY030134), and *V. plumbeus* (AY030136). We were unable to locate and tissues of Dwarf Vireo (*V. nelsoni*) which has been proposed as the sister taxon of Black-capped Vireo ("probably conspecific" Phillips 1991, p. 195); no tissues are known to exist in any natural history museum (A. T. Peterson, pers. comm.).

Laboratory Techniques

Feathers were sent to RMZ's laboratory at the University of Minnesota. Two to three contour feathers (or, occasionally only a single rectrix was available) were then extracted using a Qiagen DNeasy Tissue Extraction kit (Qiagen Inc., Valencia, California), following manufacturer's protocols except that the initial addition of Buffer ATL and Proteinase K was supplemented with 30 uL of DTT (Dithiothreitol) and incubated at 55° C for 24h. Extracts were amplified with polymerase chain reactions (PCR) with the following conditions: 15m at 94°, 35 cycles of [45s at 94°, 45s at 50°, and 90s at 72°], 5m at 72°, and then held at 4°. The mitochondrial ND2 (NADH dehydrogenase subunit 2) gene was amplified in all individuals in either two or three pieces, depending on how degraded the DNA was. Primers are listed in Table 1; three were designed by MCW for this study. These PCR products were then cleaned with a QIAquick PCR cleaning kit, and submitted in 6 uL sequencing reactions to the BioMedical Genomics Center at the University of Minnesota. Sequences were aligned with Sequencher 4.1.1 (Gene Codes Corp., Ann Arbor, Michigan). Mitochondrial origin was confirmed in Sequencher by inspecting for unexpected stop codons and insertions or deletions.

Population Genetic Analyses

Sequences were reduced to unique haplotypes, and a neighbor-joining tree produced to depict population subdivision, using the program DnaSP 4.10 (Rozas et al. 2003). Sequences from all individuals were analyzed for patterns of population size changes over time using mismatch distributions (Rogers and Harpending 1992). Samples were pooled for mismatch analyses into three groups: Oklahoma (10 individuals), Texas (80), and Mexico (16), with significance judged by Tajima's D (Tajima 1989) and Fu's Fs

(Fu 1997). Sequences with more than 5% missing data were deleted from mismatch analyses.

RESULTS

We obtained 1041 base pairs, representing the entire mitochondrial ND2 gene from 108 sampled Black-capped Vireos. The 39 variable sites (22 parsimony informative) resolved 27 unique haplotypes, with a haplotype diversity of 0.866. No stop codons or indels were observed.

A neighbor-joining phylogeny of haplotypes demonstrated no major phylogenetic breaks, and no visible geographic structuring within the topology (Figure 1). Further, no haplotypes are geographically restricted. The most common haplotype occurred at all sampled localities. Intraspecific variation was low, with an average of 3.41 differences between haplotypes (0.328% sequence divergence, uncorrected).

Two of the 108 sampled individuals were removed for population genetic analyses due to high levels of missing data. Nucleotide diversity (π) for all samples together was 0.0035; pooled values were: Mexico $\pi = 0.0046$, Texas $\pi = 0.0037$, and Oklahoma $\pi = 0.00256$. Mismatch distributions were unimodal when all samples were combined (Tajima's D: -0.08562, $p = 0.00700$; Fu's Fs: -0.28435, $p < 0.00001$) and ragged for the Oklahoma (Tajima's D: -0.04127, $p = 0.653$; Fu Fs = 0.28764, $p = 0.47879$) and Mexico (Tajima's D: -0.10823, $p = 0.11900$; Fu's Fs: 0.04543, $p = 0.69000$). Texas samples were significant for only Fu's Fs (Tajima's D: -0.0054, $p = 0.04600$; Fu's Fs: -0.22106, $p = 0.00100$).

DISCUSSION

The combination of an intraspecific haplotype phylogeny without major topological or geographic structure, and an overall unimodal mismatch distribution, describes the Black-capped Vireo as a monotypic species that has been expanding in recent history. This seemingly violates expectations for a patchily distributed species with well documented declines across its range. However, very recent effects might not leave a detectable signature on the mitochondrial genome, or any neutral molecular marker. Furthermore, the ragged mismatch distribution for the Mexican and Oklahoma samples suggests that there might be regions where the species has not been increasing. The possibility for a south to north expansion is consistent with this observation.

Fazio et al. (2004) reported a notable difference at the PNP locus, in which samples from Fort Hood (Texas) and Wichita Mtns (Oklahoma) shared a different common allele from their samples from Kickapoo Caverns (Texas) and Kerr WMA (Texas). This locus, as well as frequency differences at other loci, resulted in an unrooted UPGMA phenogram that separated these two pairs of samples. This is unusual for a passerine bird, especially given that the samples were relatively close geographically (Zink and Remsen 1986). No such division, however, was apparent in the rooted mtDNA haplotype phylogeny (Fig. 2). The disparity in the two results is problematic given that coalescence theory predicts that divergence in mtDNA will precede that in nuclear genes (e.g., allozymes) because of the former marker having ¼ the effective population size (Moore 1995). Because the allozyme results effectively hinge on a single locus (PNP), it would be prudent to investigate other nuclear markers. Importantly, sequences of nuclear genes, rather than frequency based approaches such as

149 microsatellites, are vastly preferable given that more sophisticated coalescence
150 approaches can be used to analyze the data.

151 Resolution of this apparent conflict between molecular markers could preclude
152 effective management. If there really are two groups of populations with different
153 histories, they would qualify potentially as distinct population segments (DPS) under the
154 Endangered Species Act (USFW/NMSF 1996). If, however, the mtDNA results prove
155 correct, there is no evidence for multiple DPSs, at least using genetic criteria. We instead
156 recovered the genetic signature of widespread historical gene flow despite low modern
157 gene flow, and our results demonstrate that maintaining large populations in several
158 locations could conserve genetic diversity.

159

160 ## ACKNOWLEDGMENTS

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164 Ballesteros Medrano (UANL), John P. Maresh, Victor W. Fazio III, Joseph A.
165 Grzybowski. This project was funded by Texas Parks and Wildlife Department and
166 University of Minnesota.

167 Feathers were collected under the following permits: SPR-1090-299 (Texas) and
168 USFWS 21999 to the University of Louisiana. Texas Endangered Species Permit to
169 Texas Parks and Wildlife Department #TE814933-2. Master Banding Permit #22365 to
170 CCF. Feathers imported from Mexico by CCF with Declaration for Importation or
171 Exportation of Fish or Wildlife permit #IE804570-1, the Import permit used for the

Declaration permit: issued to Rob Powell, #MA064189-1. Mexican feather collection permit Secretaria de Medio Ambiente y Recursos Naturales (SEMARNAT): #12160 issued to JIGR.

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TABLES

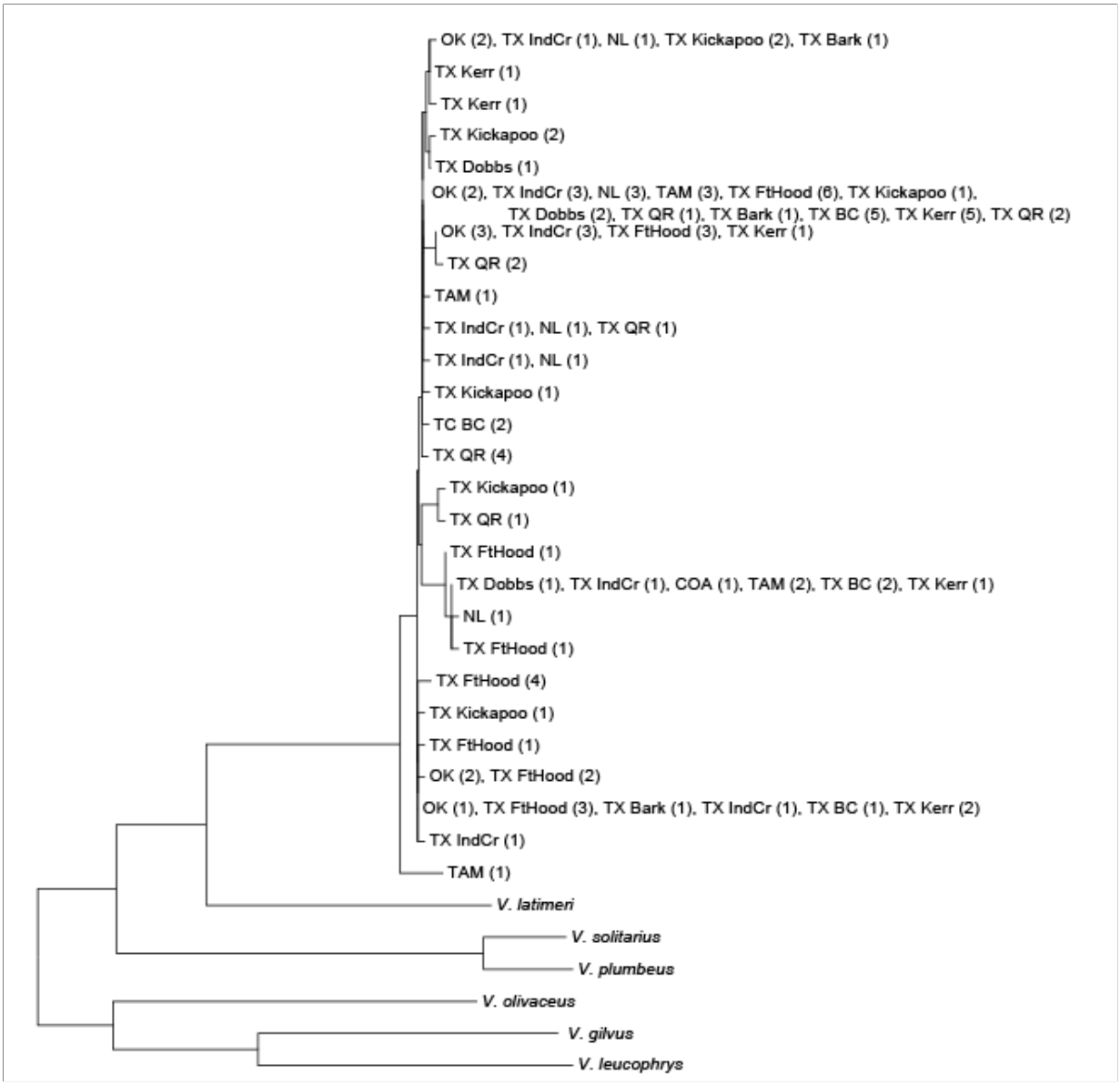
TABLE 1. Primers used in this study.

Primer Name	Sequence	Author
L5215	5’-TATCGGGCCCATACCCCGAATAT-3'	Hackett 1996
H1064	5’-CTTTGAAGGCCTTCGGTTTA-3'	Drovetski et al. 2004
L347	5’-CCATTCCACTTCTGATTCCC-3'	Drovetski et al. 2004
H5578	5’-CCTTGAAGCACTTCTGGGAATCAGA-3'	Hackett 1996
VA-ND2-L1	5’-CTAGTACCATTCCACTTCTGATTC-3'	MCW, this study
VA-ND2-L2	5’-CCTAACCTTCAACTCAATCAAAAC-3'	MCW, this study
VA-ND2-H1	5’-GTTTTGATTGAGTTGAAGGTTA-3'	MCW, this study

FIGURES

FIGURE 1. Neighbor-joining tree depicting relationships among sampled haplotypes. Haplotypes are designated with standardized state abbreviations, the number of individuals in parentheses, and, where known, an abbreviated locality name (see Appendix 1).

FIGURE 2. Graphs of all pairwise comparisons of sampled individuals (mismatch distributions). A: all samples (n=106), B: Oklahoma samples (n=10), C: Texas samples (n=80), D: Mexico samples (n=16). Values of Tajima's D are significantly negative for A and not significant for comparisons B, C, and D; Fu's Fs values are significantly negative for A and C but not B and D.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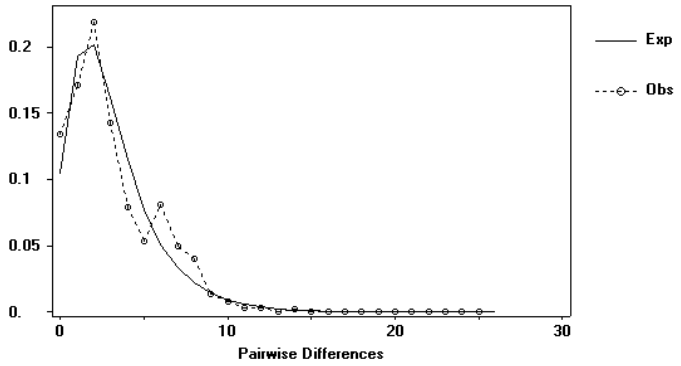
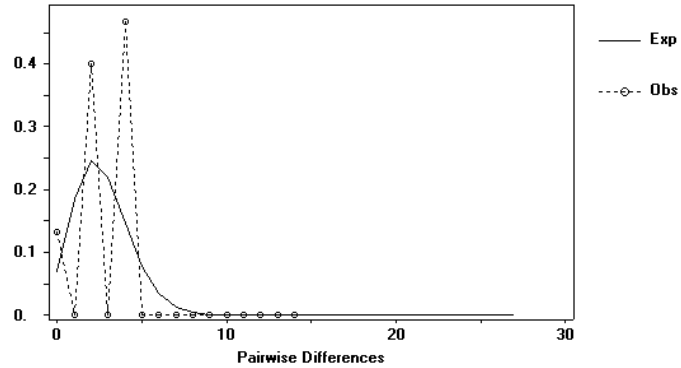
TX Kerr (1)
TX Kerr (1)
TX Kickapoo (2)
TX Dobbs (1)
OK (2), TX IndCr (3), NL (3), TAM (3), TX FtHood (6), TX Kickapoo (1),
TX Dobbs (2), TX QR (1), TX Bark (1), TX BC (5), TX Kerr (5), TX QR (2)
OK (3), TX IndCr (3), TX FtHood (3), TX Kerr (1)
TX QR (2)
TAM (1)
TX IndCr (1), NL (1), TX QR (1)
TX IndCr (1), NL (1)
TX Kickapoo (1)
TC BC (2)
TX QR (4)
TX Kickapoo (1)
TX QR (1)
TX FtHood (1)
TX Dobbs (1), TX IndCr (1), COA (1), TAM (2), TX BC (2), TX Kerr (1)
NL (1)
TX FtHood (1)
TX FtHood (4)
TX Kickapoo (1)
TX FtHood (1)
OK (2), TX FtHood (2)
OK (1), TX FtHood (3), TX Bark (1), TX IndCr (1), TX BC (1), TX Kerr (2)
TX IndCr (1)
TAM (1)
V. latimeri
V. solitarius
V. plumbeus
V. olivaceus
V. gilvus
V. leucophrys

A


0.2
0.15
0.1
0.05
0.
0
10
20
30
Pairwise Differences
Exp
Obs

B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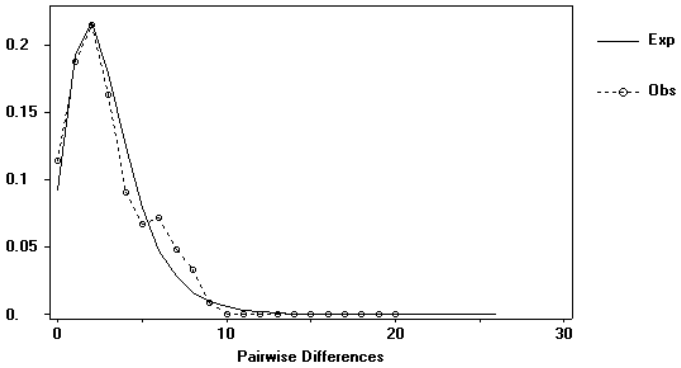
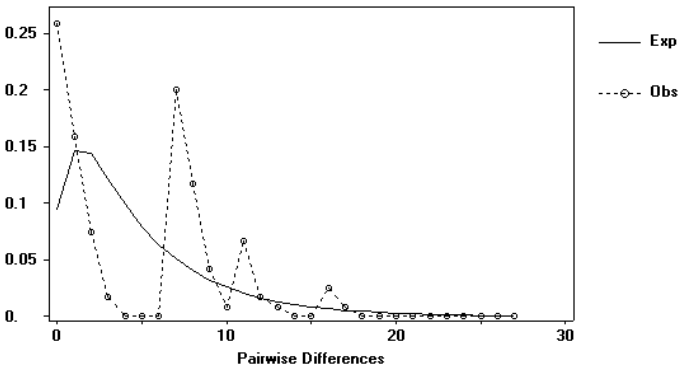
0.3
0.2
0.1
0.
0
10
20
30
Pairwise Differences
Exp
Obs

C


0.2
0.15
0.1
0.05
0.
0
10
20
30
Pairwise Differences
Exp
Obs

D


0.25
0.2
0.15
0.1
0.05
0.
0
10
20
30
Pairwise Differences
Exp
Obs

Appendix 1. Sampling locations for each individual.

Individual #	Band or Field #	Country	State	County	Locality
VA001	55791	USA	Oklahoma	Comanche	Fort Sill
VA002	55798	USA	Oklahoma	Comanche	Fort Sill
VA003	55799	USA	Oklahoma	Comanche	Fort Sill
VA004	55794	USA	Oklahoma	Comanche	Fort Sill
VA005	55793	USA	Oklahoma	Comanche	Fort Sill
VA006	55782	USA	Oklahoma	Comanche	Fort Sill
VA007	55779	USA	Oklahoma	Comanche	Fort Sill
VA008	55769	USA	Oklahoma	Comanche	Fort Sill
VA009	55775	USA	Oklahoma	Comanche	Fort Sill
VA010	55772	USA	Oklahoma	Comanche	Fort Sill
VA011	1770-34980	USA	Texas	Terrell	Chandler Independence Creek Preserve or TNC property nearby
VA012	1770-34981	USA	Texas	Terrell	Chandler Independence Creek Preserve or TNC property nearby
VA013	1770-34982	USA	Texas	Terrell	Chandler Independence Creek Preserve or TNC property nearby
VA014	1770-34991	USA	Texas	Terrell	Chandler Independence Creek Preserve or TNC property nearby
VA015	1760-75160	USA	Texas	Terrell	Chandler Independence Creek Preserve or TNC property nearby
VA017	no band	Mexico	Nuevo Leon	n/a	unknown
VA018	1350-74991	Mexico	Nuevo Leon	n/a	unknown
VA019	2070-98642	Mexico	Nuevo Leon	n/a	unknown
VA020	2070-98643	Mexico	Nuevo Leon	n/a	unknown
VA021	1350-49918	Mexico	Nuevo Leon	n/a	unknown
VA022	1350-49919	Mexico	Nuevo Leon	n/a	unknown
VA023	2400-45320	USA	Texas	Coryell	Ft. Hood
VA024	2400-45321	USA	Texas	Coryell	Ft. Hood
VA025	2400-45322	USA	Texas	Coryell	Ft. Hood
VA026	2320-60041	USA	Texas	Coryell	Ft. Hood
VA027	2400-45323	USA	Texas	Coryell	Ft. Hood
VA028	2400-45324	USA	Texas	Coryell	Ft. Hood
VA029	2400-45325	USA	Texas	Coryell	Ft. Hood
VA030	2400-45327	USA	Texas	Coryell	Ft. Hood
VA031	2360-63136	USA	Texas	Coryell	Ft. Hood
VA032	2360-63137	USA	Texas	Coryell	Ft. Hood
VA033	2360-63138	USA	Texas	Coryell	Ft. Hood
VA034	2360-63141	USA	Texas	Coryell	Ft. Hood
VA035	2360-63142	USA	Texas	Coryell	Ft. Hood
VA036	2360-63143	USA	Texas	Coryell	Ft. Hood
VA037	2360-63144	USA	Texas	Coryell	Ft. Hood
VA038	2360-63145	USA	Texas	Coryell	Ft. Hood
VA039	2340-14595	USA	Texas	Coryell	Ft. Hood
VA040	2360-63146	USA	Texas	Coryell	Ft. Hood
VA041	2400-45483	USA	Texas	Coryell	Ft. Hood
VA042	2360-63147	USA	Texas	Coryell	Ft. Hood

VA043	2070-98656	USA	Texas	Edwards	Kickapoo Cavern State Park
VA044	2070-98658	USA	Texas	Edwards	Kickapoo Cavern State Park
VA045	2070-98661	USA	Texas	Edwards	Kickapoo Cavern State Park
VA046	2070-98662	USA	Texas	Edwards	Kickapoo Cavern State Park
VA047	2070-98668	USA	Texas	Edwards	Kickapoo Cavern State Park
VA048	2070-98679	USA	Texas	Edwards	Kickapoo Cavern State Park
VA049	2070-98680	USA	Texas	Edwards	Kickapoo Cavern State Park
VA050	2070-98681	USA	Texas	Edwards	Kickapoo Cavern State Park
VA051	2070-98682	USA	Texas	Edwards	Kickapoo Cavern State Park
VA052	2070-98683	USA	Texas	Edwards	Kickapoo Cavern State Park
VA053	JPM1	USA	Texas	Edwards	Dobbs Mtn.
VA054	JPM2	USA	Texas	Edwards	Dobbs Mtn.
VA055	JPM3	USA	Texas	Edwards	Dobbs Mtn.
VA056	JPM4	USA	Texas	Edwards	Dobbs Mtn.
VA057	JPM5	USA	Texas	Somervell	Quail Ridge
VA058	JPM6	USA	Texas	Taylor	Camp Barkeley
VA060	JPM8	USA	Texas	Taylor	Camp Barkeley
VA061	JPM9	USA	Texas	Taylor	Camp Barkeley
VA062	JPM10	USA	Texas	Terrell	Independence Creek
VA063	JPM11	USA	Texas	Terrell	Independence Creek
VA065	JPM13	USA	Texas	Terrell	Independence Creek
VA066	JPM14	USA	Texas	Terrell	Independence Creek
VA068	JPM16	USA	Texas	Terrell	Independence Creek
VA069	JPM17	USA	Texas	Terrell	Independence Creek
VA071	JPM19	USA	Texas	Terrell	Independence Creek
VA090	2370-56103	Mexico	Nuevo Leon	n/a	Rcho Minas Viejas, Villaldama
VA091	2370-56104	Mexico	Nuevo Leon	n/a	Rcho Minas Viejas, Villaldama
VA092	2370-56105	Mexico	Nuevo Leon	n/a	Rcho Minas Viejas, Villaldama
VA093	2370-56106	Mexico	Tamaulipas	n/a	Ejid. Los Arrieros, Palmillas, Tamaulipas
VA094	2370-56107	Mexico	Coahuila	n/a	Pajaros Azules, Coahuila
VA095	2370-56112	Mexico	Tamaulipas	n/a	Carretera del Rcho.Capulin a Bustamante, Tamaulipas
VA096	2370-56113	Mexico	Tamaulipas	n/a	Carretera del Rcho.Capulin a Bustamante, Tamaulipas
VA097	2370-56114	Mexico	Tamaulipas	n/a	Carretera a Miquihuana km 9
VA098	2370-56115	Mexico	Tamaulipas	n/a	Carretera a Miquihuana km 10
VA099	2370-56116	Mexico	Tamaulipas	n/a	Carretera a Miquihuana km 11
VA100	2370-56200	Mexico	Tamaulipas	n/a	Carretera a Miquihuana km 9
VA105	2410-77101	USA	Texas	Burnet / Williamson	Balcones Canyonlands National Wildlife Refuge
VA106	2410-77102	USA	Texas	Burnet / Williamson	Balcones Canyonlands National Wildlife Refuge
VA107	2410-77103	USA	Texas	Burnet / Williamson	Balcones Canyonlands National Wildlife Refuge
VA108	2410-77104	USA	Texas	Burnet / Williamson	Balcones Canyonlands National Wildlife Refuge
VA109	2410-77105	USA	Texas	Burnet / Williamson	Balcones Canyonlands National Wildlife Refuge
VA110	2410-77106	USA	Texas	Burnet / Williamson	Balcones Canyonlands National Wildlife Refuge

VA111	2410-77107	USA	Texas	Burnet / Williamson	Balcones Canyonlands National Wildlife Refuge
VA112	2410-77108	USA	Texas	Burnet / Williamson	Balcones Canyonlands National Wildlife Refuge
VA113	2410-77109	USA	Texas	Burnet / Williamson	Balcones Canyonlands National Wildlife Refuge
VA114	2410-77110	USA	Texas	Burnet / Williamson	Balcones Canyonlands National Wildlife Refuge
VA115	2430-90819	USA	Texas	Kerr	Kerr Wildlife Management Area
VA116	2430-90820	USA	Texas	Kerr	Kerr Wildlife Management Area
VA117	2430-90821	USA	Texas	Kerr	Kerr Wildlife Management Area
VA118	2430-90822	USA	Texas	Kerr	Kerr Wildlife Management Area
VA119	2430-90823	USA	Texas	Kerr	Kerr Wildlife Management Area
VA120	2430-90824	USA	Texas	Kerr	Kerr Wildlife Management Area
VA121	2430-90825	USA	Texas	Kerr	Kerr Wildlife Management Area
VA122	2430-90826	USA	Texas	Kerr	Kerr Wildlife Management Area
VA123	2430-90827	USA	Texas	Kerr	Kerr Wildlife Management Area
VA124	2430-90828	USA	Texas	Kerr	Kerr Wildlife Management Area
VA125	2430-90837	USA	Texas	Somervell	Quail Ridge
VA126	2430-90838	USA	Texas	Somervell	Quail Ridge
VA127	2430-90839	USA	Texas	Somervell	Quail Ridge
VA128	2430-90840	USA	Texas	Somervell	Quail Ridge
VA129	2430-90841	USA	Texas	Somervell	Quail Ridge
VA130	2430-90842	USA	Texas	Somervell	Quail Ridge
VA131	2430-90843	USA	Texas	Somervell	Quail Ridge
VA132	2430-90844	USA	Texas	Somervell	Quail Ridge
VA133	2430-90845	USA	Texas	Somervell	Quail Ridge
VA134	2430-90846	USA	Texas	Somervell	Quail Ridge
VA135	2430-90847	USA	Texas	Somervell	Quail Ridge