PHYLOGEOGRAPHIC ANALYSIS OF NORTHERN YELLOW BATS, *DASYPTERUS INTERMEDIUS,* BY MOLECULAR ANALYSIS

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ABSTRACT

Northern yellow bats (*Dasypterus intermedius*) are tree-roosting bats in the family Vespertilionidae comprised of two subspecies: *D. intermedius intermedius* and *D. intermedius floridanus* distributed in North and Central America. The two subspecies lineages are thought to be geographically separated but this has never been tested with a molecular approach. In this study, mitochondrial sequence data from 38 *D. intermedius* and nuclear microsatellite data from 92 *D. intermedius* (across 8 loci) from across their range were used to test the hypothesis that genetically defined groups will correspond geographically with the two morphologically-defined subspecies. Though high levels of divergence of the mitochondrial sequence (11.6%) resulted in clusters that corresponded to geographic origin, no genetic structure in the population based on nuclear markers was recovered. This study suggests that *D. intermedius* has a single continuous population with gene flow between the two subspecies and relatively high genetic diversity levels (H_0=0.621).

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INTRODUCTION

Northern yellow bats (*Dasypterus intermedius*) are large, insectivorous vespertilionid bats found in the southeastern United States, the southeastern counties of Texas, and parts of Central America (Webster et al. 1980; Fig. 1). They are rare across their range and are not known to migrate during winter months (Ammerman et al. 2012). Along with *D. intermedius*, there are 3 other species of yellow bats (*D. ega, D. xanthinus, and D. insularis*) that were previously placed in the genus *Lasiurus* until it was recently determined that enough genetic diversity existed between yellow bats and the other members of *Lasiurus* for yellow bats to be elevated to the genus *Dasypterus* (Baird et al. 2015). This taxonomic shift is still controversial among mammalogists due to an apparent lack of substantial morphological divergence among the three monophyletic clades (colloquially deemed the hoary bats, the red bats, and the yellow bats) within *Lasiurus* (Novaes et al. 2018). *Dasypterus intermedius* is currently listed as a species of least concern on the IUCN Red List, but little is known about their estimated population size or structure (Miller and Rodriguez 2016). Northern yellow bats are solitary and roost in Spanish moss (*Tillandsia usneoides*), usually associated with oak trees, and among the dead fronds of palm trees in coastal regions (Jimenez 2016; Ammerman et al. 2012).

Two subspecies of Northern yellow bats have been recognized, *D. i. intermedius* and *D. i. floridanus*, which are largely differentiated by the color of their pelage and their size.

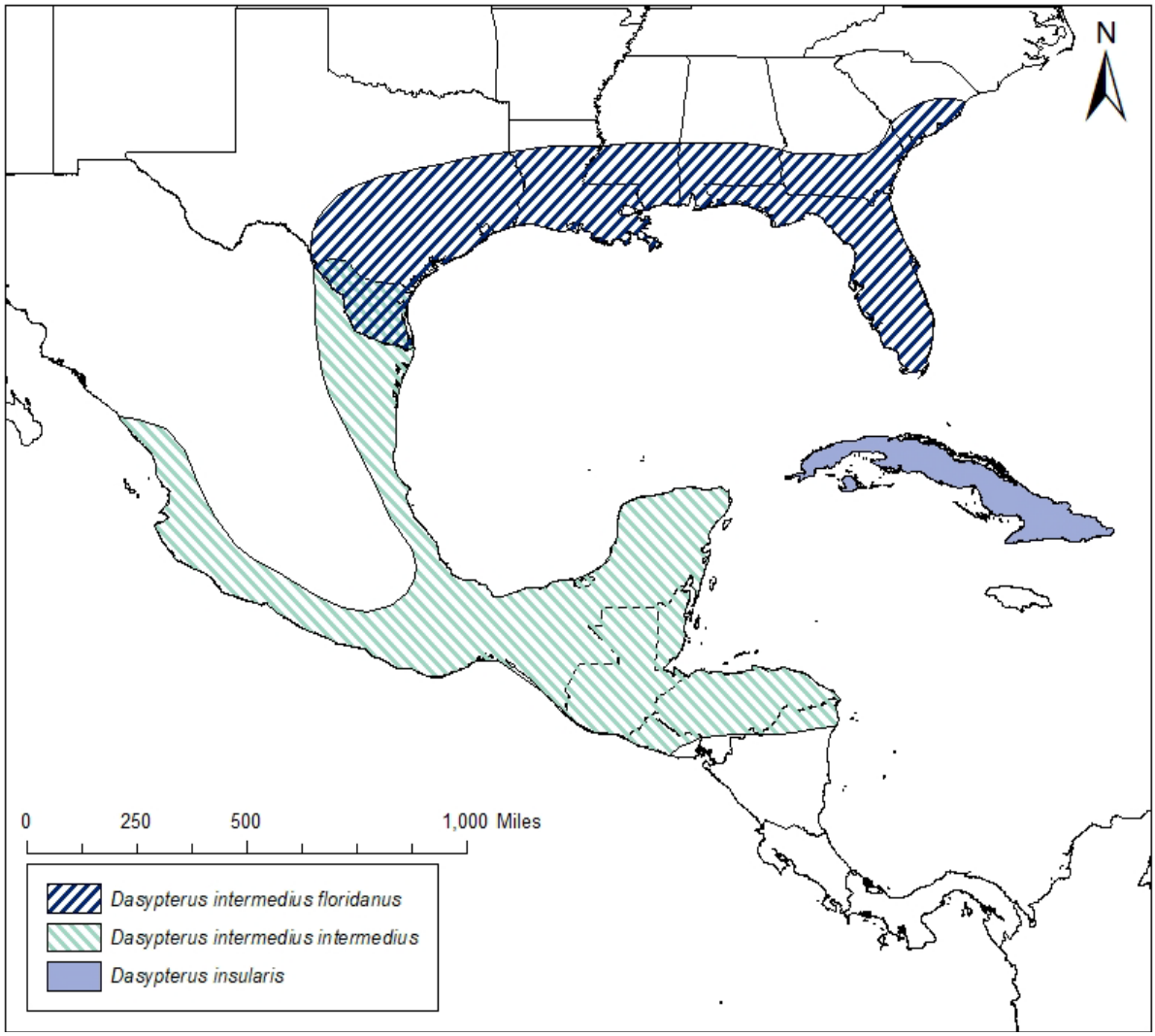

Fig. 1. Geographic distribution of *Dasypterus intermedius* subspecies: *D. i. floridanus* (from South Carolina to southern Texas) and *D. i. intermedius* (from southern Texas to Nicaragua). This distribution map was updated from the historical distribution to account for recent range expansion.

D. i. intermedius are typically larger and have more intensely yellow pelage compared to *D. i. floridanus*, which are typically less than 13.5 cm in total body length (Hall and Jones 1961). Hall and Jones (1961) showed that forearm length and certain cranial measurements such as condylocanine length could be used to distinguish the two subspecies, although intergradation has been observed across their range. Based on morphology, the two subspecies, originally described as separate species, are found in southeastern Texas (Hall and Jones 1961), but whether or not they are genetically distinct has not been tested.

A subspecies is defined as an aggregate of phenotypically similar populations of a species inhabiting a geographic subdivision of the range of that species and differing taxonomically from other populations of that species (Mayr and Ashlock 1991). Variation exists between subspecies due to the occupation of different environments across their range and differential reproduction, but controversy has surrounded the degree to which this variation should be reflected in taxonomy (Monroe Jr. 1982). Phylogeographic studies can contribute to the legitimacy of the recognition of subspecies or alternatively can recognize species-level divergences that were not evident based solely on morphological differences.

Phylogenetic studies in general help contribute to many disciplines, the most obvious of which is the understanding of evolution. Phylogeography is the study of the geographic distributions of species in light of genetics to reveal historical processes that explain genetic diversity across contemporary distributions (Avise 2000). Phylogeographic studies have uncovered major evolutionary processes, especially in light of climatic shifts

and extinction events. For example, a phylogeographic study of a species of frog, *Feirana taihangnica*, endemic to the Qinling Mountains of China used mitochondrial DNA data paired with geographic distribution to reveal deep genetic diversity likely due to the use of the mountains as glacial refugia during the late Pleistocene epoch (Wang et al. 2013). This species was hypothesized to have experienced secondary contact of genetically diverse clades due to range expansion after glaciers receded (Wang et al. 2013). Historical evidence of evolutionary change due to climatic shifts can be used to speculate on future conditions of biodiversity.

Phylogenetics and phylogeography have applications in regard to human health as well. Coevolution of viruses and their vectors has been implicated in multiple human health cases such as the hantaviruses (Nichol 1999). Prior to the 1993 hantavirus outbreak in the United States, pathogenic strains of the virus were thought to be restricted to Europe and Asia but new research has found dozens of previously unknown hantaviruses, most of which are highly specific to their rodent vector (Nichol 1999). Estimates based on current rodent phylogenies and the discovery of new members of the *Hantavirus* genus suggest that there are numerous pathogenic strains yet to be discovered that could potentially cause outbreaks (Yates et al. 2002). This application is especially notable in studies of bats, which have been implicated as important reservoirs for multiple types of viruses that can affect human health including, but not limited to, Lyssavirus, Henipavirus, and Ebolavirus (Calisher et al. 2006). Knowledge of the evolutionary lineages of virus reservoirs can help combat potential pathogenic outbreaks and understand viral evolution.

Animals submitted to the Texas Department of State Health Services (DSHS) for rabies testing can be an invaluable source of material for genetics studies. Between 2001 and 2010, 393 Northern yellow bats were submitted to the DSHS for rabies testing, 356 of which tested negative for the virus, with only 8.6% of Northern yellow bat submissions testing positive for rabies (Mayes et al. 2013). Data recovered from submissions and other species can provide insight on distribution trends, recently accounting for several new county records for multiple species (Demere et al. 2012). Additionally, these specimens provide a source of material for genetic studies. I used museum specimens, primarily ones originally acquired by the Texas DSHS, to test the hypothesis that clusters based on molecular data will correspond geographically with the morphologically-defined subspecies of *D. intermedius* using DNA sequencing data from mitochondrial cytochrome *b* and microsatellite markers. If there is no gene flow between the morphologically-described subspecies, then I expected to be able to recover two distinct lineages of Northern yellow bats.

MATERIALS AND METHODS

Sample design – A total of 100 samples of tissue from Northern yellow bats were obtained from Angelo State Natural History Collection (ASNHC), Natural Science Research Lab - Texas Tech University (NSRL-TTU), and the Florida Museum of Natural History (FLMNH) via tissue loan (Table 1). Samples were chosen based on the geographic location from which the specimen was found in order to include samples from across the range of *D. intermedius*. Specimens donated by the Texas Department of State Health Services (DSHS) were dissected, removing organ tissue (liver, heart, kidney, lung, spleen, and/or muscle depending on degradation of the tissues; stored in -80°C), and the voucher specimen was accessioned into the ASNHC as a fluid-preserved specimen in 70% ethanol after fixation in 10% formalin.

Loaned samples were shipped either frozen on dry ice or in 95% ethanol. Samples that were received on ice were kept at -80°C prior to DNA extraction. Five samples from FLMNH were shipped in ethanol and were rehydrated using 500 µL 1X PBS buffer for 10 minutes 3 times prior to extraction. Samples were derived from 27 counties in Texas, 4 counties in Florida, and the Yucatan region of Mexico. DNA was extracted from liver tissue (if liver was degraded or unavailable heart, kidney, or muscle tissue was used for DNA extraction) according to the standard protocols from the DNeasy Blood and Tissue Kit (Qiagen Inc., Valencia, CA), with the exception that two elutions of 50 µL each of AE buffer were recovered. Samples were quantified for DNA yield and purity using a NanoDrop Lite

Table 1. Vouchered specimens of *Dasypterus intermedius* used in this study including the tissue and catalog number from each institution, collection information for each individual, and whether they were analyzed using cytochrome *b* (*Cytb*) or microsatellites (MSats). If cytochrome *b* sequences were recovered, GenBank (GB) accession numbers are listed for those individuals. Abbreviations are as follows: DSHS (Texas Department of State Health Services), ASNHC or ASK (Angelo State Natural History Collections), TK or TTU (Natural Science Research Laboratory at Texas Tech University), and FLMNH (Florida Museum of Natural History). Specimens were male (M), female (F), or unknown (U). DNA was collected from some samples that failed to return mitochondrial or microsatellite data.

Tissue no.	Catalog no.	State	County	Sex	Collection date	*Cytb*	MSats	GB accession no.
ASK421	ASNHC1408	Yucatan	Tinum	F	31-Jul-1984	X	X	MK876254
ASK422	ASNHC1409	Yucatan	Tinum	F	31-Jul-1984	X	X	KP341748.1
ASK9499	ASNHC15829	TX	Cameron	F	15-Mar-2013		X	
ASK10517	ASNHC15830	TX	Brazoria	F	23-Oct-2012			
ASK10518	ASNHC15831	TX	Webb	F	5-Sep-2012	X	X	MK876250
ASK10519	ASNHC15832	TX	Hidalgo	M	15-Jun-2012		X	
ASK10520	ASNHC15833	TX	Webb	M	5-Sep-2012			
ASK10523	ASNHC15836	TX	Comal	M	11-Sep-2012		X	
ASK10525	ASNHC16292	TX	San Patricio	F	10-Jul-2012			
ASK10529	ASNHC15862	TX	San Patricio	F	21-Jul-2012		X	
ASK10530	ASNHC15863	TX	Montgomery	M	17-Jul-2012	X	X	MK876235
ASK10536	ASNHC15869	TX	Nueces	M	8-Jun-2012	X	X	MK876264
ASK10837	ASNHC19034	TX	Hidalgo	M	29-May-2014		X	
ASK10838	ASNHC19035	TX	Hidalgo	U	1-Mar-2014		X	
ASK10839	ASNHC19036	TX	Hidalgo	M	29-Mar-2014		X	
ASK10840	ASNHC19037	TX	Hidalgo	F	29-May-2014	X	X	MK876263
ASK10882	ANHC19045	TX	Hidalgo	F	6-Jul-2015		X	
ASK10891	ASNHC19046	TX	Hidalgo	F	6-Jul-2015	X	X	MK876239
ASK10892	ASNHC19047	TX	Fort Bend	F	21-Aug-2015	X	X	MK876233
ASK10893	ASNHC19038	TX	Nueces	M	7-Aug-2015		X	

Table 1. Continued

Tissue no.	Catalog no.	State	County	Sex	Collection date	*Cytb*	MSats	GB accession no.
ASK10894	ASNHC19039	TX	Nueces	M	24-Jun-2015		X	
ASK10895	ASNHC19040	TX	Nueces	F	4-Aug-2015		X	
ASK10896	ASNHC19048	TX	Nueces	F	25-Jun-2015		X	
ASK10897	ASNHC19041	TX	Nueces	M	18-Jun-2015		X	
ASK10898	ASNHC19042	TX	Nueces	F	24-Jun-2015		X	
ASK10899	ASNHC19049	TX	Cameron	F	17-Jul-2015		X	
ASK10900	ASNHC19055	TX	Hidalgo	M	10-Jul-2015		X	
ASK10901	Not Yet Cataloged	TX	Hidalgo	F	3-Jul-2015		X	
ASK10902	ASNHC19052	TX	Hidalgo	F	3-Jul-2015		X	
ASK10903	ASNHC19056	TX	Hidalgo	F	9-Jul-2015		X	
ASK10904	ASNHC19054	TX	Hidalgo	F	17-Sep-2015	X	X	MK876240
ASK10905	ASNHC19053	TX	Hidalgo	F	10-Jul-2015		X	
ASK10906	ASNHC19051	TX	Hidalgo	F	5-Jul-2015		X	
ASK10907	ASNHC19058	TX	Webb	M	12-Sep-2015	X	X	MK876260
ASK10908	ASNHC19057	TX	Webb	F	12-Sep-2015	X	X	MK876241
ASK10909	ASNHC19062	TX	Webb	M	1-Jul-2015		X	
ASK10910	ASNHC19059	TX	Wharton	M	23-Jun-2015	X	X	MK876253
ASK11624	ASNHC17401	TX	Washington	M	7-Mar-2014	X	X	MK876252
ASK12581	ASNHC17871	TX	Fort Bend	U	28-Apr-2016	X	X	MK876257
ASK12582	ASNHC17872	TX	Montgomery	M	15-Sep-2016			
ASK12583	ASNHC17873	TX	Lee	M	6-Oct-2016	X	X	MK876234
ASK12585	ASNHC17875	TX	San Patricio	F	30-Jun-2016		X	
ASK12586	ASNHC17876	TX	Bee	F	4-Oct-2016		X	
ASK12587	ASNHC17877	TX	Angelina	F	7-Sep-2016	X	X	MK876232
ASK12588	ASNHC17878	TX	Webb	M	27-Jun-2016		X	
ASK12589	ASNHC17879	TX	Willacy	M	9-Jul-2016		X	
ASK12590	ASNHC17880	TX	Fort Bend	M	6-Jun-2016		X	

Table 1. Continued

Tissue no.	Catalog no.	State	County	Sex	Collection date	*Cytb*	MSats	GB accession no.
ASK12591	ASNHC17881	TX	Fort Bend	M	16-Jun-2016	X	X	MK876248
DSHS2008-7103	ASNHC14892	TX	Nueces	F	2008	X	X	MK876262
DSHS2009-4378	ASNHC14896	TX	Hidalgo	F	Jun-2009		X	
DSHS2010-3432	ASNHC14894	TX	Nueces	U	May-2010		X	
DSHS2010-6432	ASNHC14898	TX	Hidalgo	F	Oct-2010		X	
DSHS2011-3585	ASNHC14900	TX	Cameron	M	Jun-2010	X	X	MK876237
DSHS2011-5464	ASNHC14897	TX	Hidalgo	F	Jul-2010	X	X	MK876238
DSHS2011-6374	ASNHC14899	TX	Hidalgo	F	Sep-2011	X	X	MK876259
FLMNH31318	FLMNH31318	FL	Broward	F	12-Jun-2006		X	
FLMNH31325	FLMNH31325	FL	Escambia	F	2-Oct-2006	X	X	MK876242
FLMNH31528	FLMNH31528	FL	Broward	M	26-Jun-2008		X	
FLMNH31997	FLMNH31997	FL	Volusia	F	28-Sep-2010	X	X	MK876243
FLMNH32472	FLMNH32472	FL	Brevard	M	23-Jun-2013	X	X	MK876244
TK51125	TTU-M69634	TX	Galveston	F	12-Mar-1996	X	X	MK876246
TK51126	TTU-M69635	TX	Harris	F	19-Mar-1996		X	
TK51159	TTU-M69668	TX	Harris	F	14-Sep-1994		X	
TK51160	TTU-M69669	TX	Harris	M	8-Nov-1994		X	
TK51161	TTU-M69670	TX	Harris	M	12-Dec-1994			
TK51162	TTU-M69671	TX	Galveston	M	20-Sep-1994		X	
TK51163	TTU-M69672	TX	Galveston	M	1-Nov-1994		X	
TK53777	TTU-M69781	TX	Guadalupe	M	17-Sep-1993		X	
TK53962	TTU-M69966	TX	San Patricio	F	28-Sep-1993			
TK53963	TTU-M69967	TX	San Patricio	F	26-Aug-1993			
TK53964	TTU-M69968	TX	Dallas	F	13-Oct-1993			
TK171005	TTU-M113393	TX	Travis	M	15-Sep-2008		X	
TK171015	TTU-M113403	TX	Webb	F	12-Jan-2010		X	
TK171017	TTU-M113405	TX	Frio	F	18-Mar-2010	X	X	MK876249

Table 1. Continued

Tissue no.	Catalog no.	State	County	Sex	Collection date	*Cytb*	MSats	GB accession no.
TK171021	TTU-M113409	TX	Uvalde	M	Unknown	X	X	MK876230
TK171022	TTU-M113410	TX	Webb	F	15-Jun-2010		X	
TK171070	TTU-M113458	TX	Kleberg	F	Unknown		X	
TK171071	TTU-M113459	TX	Webb	F	22-Apr-2009	X	X	MK876258
TK171073	TTU-M113461	TX	Willacy	F	13-Mar-2009		X	
TK171074	TTU-M113462	TX	Willacy	F	8-Jul-2009	X	X	MK876231
TK171079	TTU-M113467	TX	Kleberg	F	Unknown		X	
TK171080	TTU-M113468	TX	Kleberg	M	Unknown		X	
TK171086	TTU-M113474	TX	Kleberg	U	18-Jun-2010		X	
TK171127	TTU-M113515	TX	Hidalgo	M	22-Jan-2011		X	
TK171137	TTU-M113525	TX	Hidalgo	F	12-Jun-2009		X	
TK171148	TTU-M113536	TX	Hidalgo	M	22-Jan-2011	X	X	MK876261
TK171177	TTU-M113565	TX	Jim Wells	M	17-Jul-2008	X	X	MK876247
TK171205	TTU-M113593	TX	Nueces	F	27-Jun-2008		X	
TK171207	TTU-M113595	TX	Starr	M	26-Jul-2008		X	
TK171495	TTU-M114083	TX	Harris	U	22-Jun-2011	X	X	MK876256
TK173032	TTU-M114532	TX	Cameron	M	10-Sep-2011	X	X	MK876265
TK173064	TTU-M114564	TX	Cameron	M	15-Jun-2011		X	
TK173076	TTU-M114576	TX	Willacy	F	24-Jun-2011		X	
TK173079	TTU-M114579	TX	Nueces	M	24-Jun-2011	X	X	MK876236
TK173082	TTU-M114582	TX	Willacy	F	24-Jun-2011		X	
TK173113	TTU-M114613	TX	Cameron	F	11-Jun-2011		X	
TK173116	TTU-M114616	TX	Travis	F	12-Feb-2011	X	X	MK876255
TK173119	TTU-M114619	TX	Cameron	F	1-Jun-2011	X	X	MK876245
TK173123	TTU-M114623	TX	Travis	F	2-Nov-2011	X	X	MK876251
TK173128	TTU-M114628	TX	Willacy	M	24-Jun-2011		X	

(Thermo Fisher Scientific Inc., Waltham, MA) spectrophotometer or a Qubit 1.0 fluorometer (Invitrogen Corp., Carlsbad, CA).

Mitochondrial sequence amplification – For this study, a mitochondrial gene was amplified, sequenced, and analyzed to determine phylogenetic relationships. Cytochrome *b* (*Cytb*) was chosen for use in this study due to its sequence variability and because it is commonly used in phylogenetic studies of mammals, making data for it readily available on genetic databases for comparison. I chose the PCR primers LGL765 and LGL766 (Bickham et al. 1995) to amplify *Cytb* because although they were first developed based on *Cytb* in humans, they have been successful in amplifying *Cytb* for phylogenetic studies of vespertilionid bats (Bickham et al. 2004; Baird et al. 2015). The polymerase chain reactions (PCR) of *Cytb* contained 5-50 ng of template DNA, 1 U *Taq* polymerase (New England Biolabs, Ipswich, MA), 0.16 mM of each dNTP (Thermo Fisher Sci., Waltham, MA), 1X standard *Taq* reaction buffer (New England BioLabs, Ipswich, MA), 1.0 mM $MgCl_2$ (New England BioLabs, Ipswich, MA), 0.16 µM of each forward and reverse primers (AlphaDNA, Montreal, Quebec, Canada), and RNase free water as needed to meet a final volume of 12.5 µL.

Cytb reactions were amplified using the following thermalcycler profile: an initial denaturing step of 94°C for 2 minutes, followed by 35 cycles of 92°C for 1 minute, annealing at 52°C for 1 minute, 72°C for 1 minute and a final extension of 72°C for 5 minutes. Amplified cytochrome *b* samples were verified using gel electrophoresis with 1.0% agarose gels using sodium borate buffer and 0.05 mg ethidium bromide per 50 mL of gel to visualize

DNA. Verified samples were then purified with ExoSAP-IT Express PCR Product Cleaning Reagent (Thermo Fisher Sci., Waltham, MA) following the manufacturer's protocol and purified samples were shipped to the Texas A&M Corpus Christi Genomic Core Sequencing Lab on dry ice where they underwent Sanger sequencing of both DNA strands. All sequences were submitted to GenBank (accession MK876230 - MK876265).

Analysis of mitochondrial sequence – Forward and reverse sequences were aligned into contigs and edited with Sequencher v. 5.1. A consensus sequence for each individual sample was aligned using the MUSCLE method in MEGA 7 (Kumar et al. 2016). MEGA 7 also was used to perform maximum likelihood analysis using the Hasegawa-Kishino-Yano model of DNA substitution with Gamma distribution and 1000 bootstrap replicates. This analysis included two additional *D. intermedius* sequences from GenBank (ASK422: accession KP341748.1 and LSUMZ_M352: accession KC747687.1) as well as sequences for the Cuban yellow bat, *D. insularis* (TK32049: accession KP341747.1) and the Southern yellow bat, *D. ega* (NK15304: accession KP341743.1) to use as outgroups. A median-joining haplotype network analysis using PopART was also performed to determine mutational steps between haplotype groups (Leigh and Bryant 2015). For the haplotype network analysis, samples were grouped into 7 distinct geographic areas represented by a regional identifier: Cuba (n=1) (the *D. insularis* haplotype), Mexico (n=3), Florida (n=3), Austin (n=11; Texas counties Angelina, Fort Bend, Galveston, Harris, Lee, Montgomery, Travis, Washington, and Wharton), Brownsville (n=10; Texas counties Cameron, Hidalgo, and Willacy), Corpus Christi (n=4; Texas counties Nueces and San Patricio), and Laredo (n=6; Texas counties Frio, Uvalde,

and Webb). The group of counties represented by Austin in this analysis included samples from a wide radius around Austin while this regional identifier radius was narrowed for counties in the area of proposed sympatry (Brownsville, Corpus Christi, and Laredo). Sequence data for *D. ega* was omitted from this analysis, but *D. insularis* was included as an outgroup.

Microsatellite amplification – Microsatellite primers designed for *Corynorhinus rafinesquii* (Rafinesque's big-eared bat)*, Corynorhinus townsendii* (Townsend's big-eared bat), *Plecotus auritus* (brown long-eared bat), *Aeorestes cinereus* (hoary bat), and *Lasiurus borealis* (Eastern red bat) were used to amplify microsatellites in the Northern yellow bats (Table 2; Burland et al. 1998; Piaggio et al. 2008, 2009; Keller et al. 2014; Korstian et al. 2014; Vonhof and Russell 2015). These primers were selected for cross-species amplification because the loci had been shown to be polymorphic in *L. borealis*, suggesting that they might provide meaningful genetic data for other closely related species such as *D. intermedius* (Burland et al. 1998; Piaggio et al. 2008, 2009; Keller et al. 2014; Korstian et al. 2014; Vonhof and Russell 2015). The microsatellite amplification reactions contained 6.25 µL of master mix (Type-it Microsatellite PCR Kit; Qiagen Inc., Valencia, CA), 0.625 µL of 10 µM dye-labeled forward primer (Sigma-Aldrich Corp., St. Louis, MO), 0.625 µL of 10µM non-labeled reverse primer (AlphaDNA, Montreal, Quebec), 4 µL of sterile water, and 1 µL of template DNA.

Microsatellite loci were amplified using various thermalcycler profiles that were optimized for each locus (Table 3). All loci except for Paur03 and the loci with the LAS prefix

Table 2. Microsatellite loci amplified for the analysis of *Dasypterus intermedius*. Included are locus name (* indicates loci that were dropped because PCR conditions could not be optimized for *D. intermedius*), forward and reverse primer sequence, PCR annealing temperature (T_A; °C), fluorescent WellRed dye label, repeat motif ([a] indicates that the repeat motif recovered in this study differed from the published repeat motif), and the publication for each primer reported.

Locus	Primer Sequence - 5'-3'	T_A	Dye Label	Repeats	Reference
CoraF11	F: AAGCTCAGAGACTGCTCCTTC R: ATCCATTATGTTTGCTGATGTTC	60	D3	Dinucleotide	Piaggio et al. (2009)
CotoG12	F: TGCAAGTCTTAACTCACCTCATT R: CCACTCCCCTAGTTTTCATCTAC	60	D2	Dinucleotide	Piaggio et al. (2008)
LbG*	F: CTGGGATCACATGGGGAACT R: ATGTGGACTCAGCTCACACA	60	D4	Dinucleotide	Korstian et al. (2014)
LbK*	F: TCTCTCTCCTACCCCTTCCT R: ACTGGCCTCTGGAATTGTGA	60	D4	Dinucleotide	Korstian et al. (2014)
LbT	F: TCCTCTGTCTGGGCACATAC R: TCGCAGATTCCCAAGGATCC	60	D4	Dinucleotide	Korstian et al. (2014)
LcO*	F: GAGGTCCTGTTTGTGCCAAG R: CAGGTCCGCGGTTAATTACG	61	D2	Dinucleotide	Korstian et al. (2014)
PrLb02*	F: AGATGAAAAGCGCGTGTTGT R: GGCCCATGCTCATCATCTA	61	D3	Dinucleotide	Korstian et al. (2014)
D226*	F: ATCCCAGTTCAAGCAGAGTATG R: ATTCAGGGCTCTGCATTTTAG	54	D3	Tetranucleotide	Vonhof and Russell (2015)
D200	F: TGCACAGATGTTTTAAGGTTTG R: TCATGAAATTTGGTTGTCCAC	54	D2	Dinucleotide[a]	Vonhof and Russell (2015)
Paur03	F: CTGGAGTGTTGTTTTGCCCTTC R: GCTGATGGTGGAGTCTCCTTTTTC	55	D4	Dinucleotide	Burland et al. (1998)

Table 2. Continued

Locus	Primer Sequence - 5'-3'	T_A	Dye Label	Repeats	Reference
LAS4206	F: GRGAATTCTGCCTTGACTGGG R: GGACCCAGTGCCAATCCAAC	61	D2	Dinucleotide[a]	Keller et al. (2014)
LAS8539	F: CCAGTTCCAGCCTTGCACAG R: TGCTTTGGTGCCTCGAACAG	61	D3	Dinucleotide[a]	Keller et al. (2014)
LAS8830	F: GATGGGAATAAGGACTAGAGTG R: CCAATTTGGCACCATTCCCAG	61	D4	Dinucleotide[a]	Keller et al. (2014)
LAS8843*	F: TCAGACAAAGAAGGCCTGTC R: CTGAGGACGGGTGYGTTCCC	57	Not Labeled	Not Published	Keller et al. (2014)
LAS8953*	F: CCAGGGAGTAGGCCAGTGG R: CCAGGGAGTAGGCCAGTGG	57	Not Labeled	Not Published	Keller et al. (2014)
LAS9613*	F: TCCTAACACCTGTCCCTGCC R: AACACTCCCAATCCAAGCAC	64	Not Labeled	Not Published	Keller et al. (2014)

Table 3. Polymerase chain reaction thermal profiles utilized for each microsatellite locus in *Dasypterus intermedius*. T_A: annealing temperature.

Thermal profile	Loci (Locus)
95˚C for 5 min, followed by 35 cycles of 94˚C for 30s, T_A for 1.5 min, and 72˚C for 1.5 min, with a final extension at 60˚C for 30 min	CoraF11, CotoG12, LbT, D226, D200, LbG, LbK, LcO, PrLb02
95˚C for 5 min, followed by 35 cycles of 94˚C for 30s, 55˚C for 1.5 min, and 72˚C for 1.5 min, with a final extension at 72˚C for 10 min	Paur03
95˚C for 15 min, followed by 35 cycles of 94˚C for 30s, T_A for 30s, and 72˚C for 30s, with a final extension at 72˚C for 30 min	LAS4206, LAS8539, LAS8830, LAS8843, LAS8953, LAS9612

had profiles in accordance with the Type-it Microsatellite PCR Kit (Qiagen Inc., Valencia, CA). Paur03 had a higher final extension temperature for a shorter period of time to optimize amplification and loci with the LAS prefix used a thermalcycler profile published with the primers (Keller et al. 2014). To test primer utility, subsets of samples were visualized using gel electrophoresis. Each gel was 1.5% agarose in sodium borate with 0.05 g ethidium bromide per 50 mL gel to visualize DNA. Once PCR conditions were optimized for each locus, microsatellite PCR products were sequenced on a capillary electrophoresis genetic analysis system (CEQ™8000, Beckman-Coulter Inc., Brea, CA) with a 400 base-pair size standard (AB Sciex, Concord, Ontario). Genotypes were assigned by visualizing chromatograms and assigning size scores to peaks.

Analysis of microsatellite variation – The program Micro-Checker v 2.2.3 (van Oosterhout et al. 2004) was used to assess scoring error due to stutter and large-allele dropout, and the presence of null alleles was determined using the program FreeNA (Chapuis and Estoup 2007). Stutter during genotyping can occur due to slipping by *Taq* polymerase which may make it difficult to correctly interpret whether an individual is homozygous or heterozygous at a locus and can cause scoring error (van Oosterhout et al. 2004). Large-allele dropout can result from the preferential amplification of the smaller allele in a heterozygous individual, making the individual appear to be homozygous at that locus (DeWoody et al. 2006). Null alleles are alleles that consistently fail to amplify at detectable levels by polymerase chain reaction (Dakin and Avise 2004). To check for linkage disequilibrium between alleles and assess if loci were in Hardy-Weinberg equilibrium, I used

the program GENEPOP v 4.2 (Raymond and Rousset 1995). Linkage disequilibrium is the association of alleles at different loci and can cause an overestimation of genetic clusters (Kaeuffer et al. 2007). Hardy-Weinberg equilibrium was assessed in all loci to determine if the microsatellite markers were neutral and not under selective pressure. Scoring error due to stutter, large-allele dropout, presence of null alleles, linkage disequilibrium, and analysis of Hardy-Weinberg equilibrium are all ways to evaluate the suitability of the chosen microsatellite markers for use in evaluating population structure. To assess genetic diversity in the dataset, GenAlEx v 6.5 (Peakall and Smouse 2006, 2012) was used to calculate number of alleles per locus (N_a), expected (H_e) and observed heterozygosity (H_o).

Analysis of genetic structure – The program STRUCTURE v 2.3.4 (Pritchard et al. 2000) was used to determine population structure of my samples and assess the most likely number of populations. Ten iterations were performed at each assumed population number (K= 1-5) and the length of the burn-in period and number of Markov chain Monte Carlo iterations after burn-in were 30,000 and 100,000, respectively. This analysis utilized the admixture model and correlated allele frequencies. STRUCTURE HARVESTER v 0.6.94 (Earl and von Holdt 2012) was then used to determine the number of populations by calculating ΔK (Evanno et al. 2005). Lastly, STRUCTURE PLOT v 2 (Ramasamy et al. 2014) was used to create plot displays of STRUCTURE results. A subset of the samples including only those recovered by the mitochondrial phylogeny were used for a principal coordinates analysis in GenAlEx v 6.5 (Peakall and Smouse 2006, 2012) to further investigate genetic structure because these samples could be assigned to populations. Additionally, GenAlEx v 6.5

(Peakall and Smouse 2006, 2012) was used to calculate F_{ST} to determine the degree of genetic differentiation between populations recovered by the cytochrome *b* analysis.

RESULTS

Cytochrome b analysis – *Cytb* sequence data was obtained for 36 samples of Northern yellow bats. The maximum likelihood analysis used a fragment of 938 base pairs from the 40 sequences to recover three major lineages: the Cuban yellow bat, a cluster containing individuals primarily from the range of *D. i. floridanus*, and a cluster containing individuals primarily from the range of *D. i. intermedius* (Fig. 2). These groups roughly reflect the proposed subspecies distributions with an area of sympatry in extreme southern Texas. Samples from the counties of Webb, Cameron, Hidalgo, and Nueces could be found in both mitochondrial lineages. This analysis also demonstrated that *D. intermedius* is not a monophyletic group due to the highly supported (98% bootstrap value) grouping of the Cuban yellow bat with the *D. i. floridanus* samples (Fig. 2). Additionally, a pairwise distance analysis was performed to test divergence levels between the clades recovered from the maximum likelihood analysis and recovered 11.6% divergence between the two subspecies of Northern yellow bats (Table 4). The haplotype network analysis also recovered three clusters of haplotypes, corresponding to the three clades recovered by the maximum likelihood analysis, and revealed similar geographic patterns (Fig. 3). This analysis recovered 21 haplotypes and revealed 79 mutational steps between the two main haplotype groups.

Microsatellite primer optimization – CoraF11, CotoG12, LbT, D200, and Paur03 amplified successfully using their respective published thermal profiles. LAS4206, LAS8830, and LAS8593 amplified at an increased annealing temperature (61°C), but showed

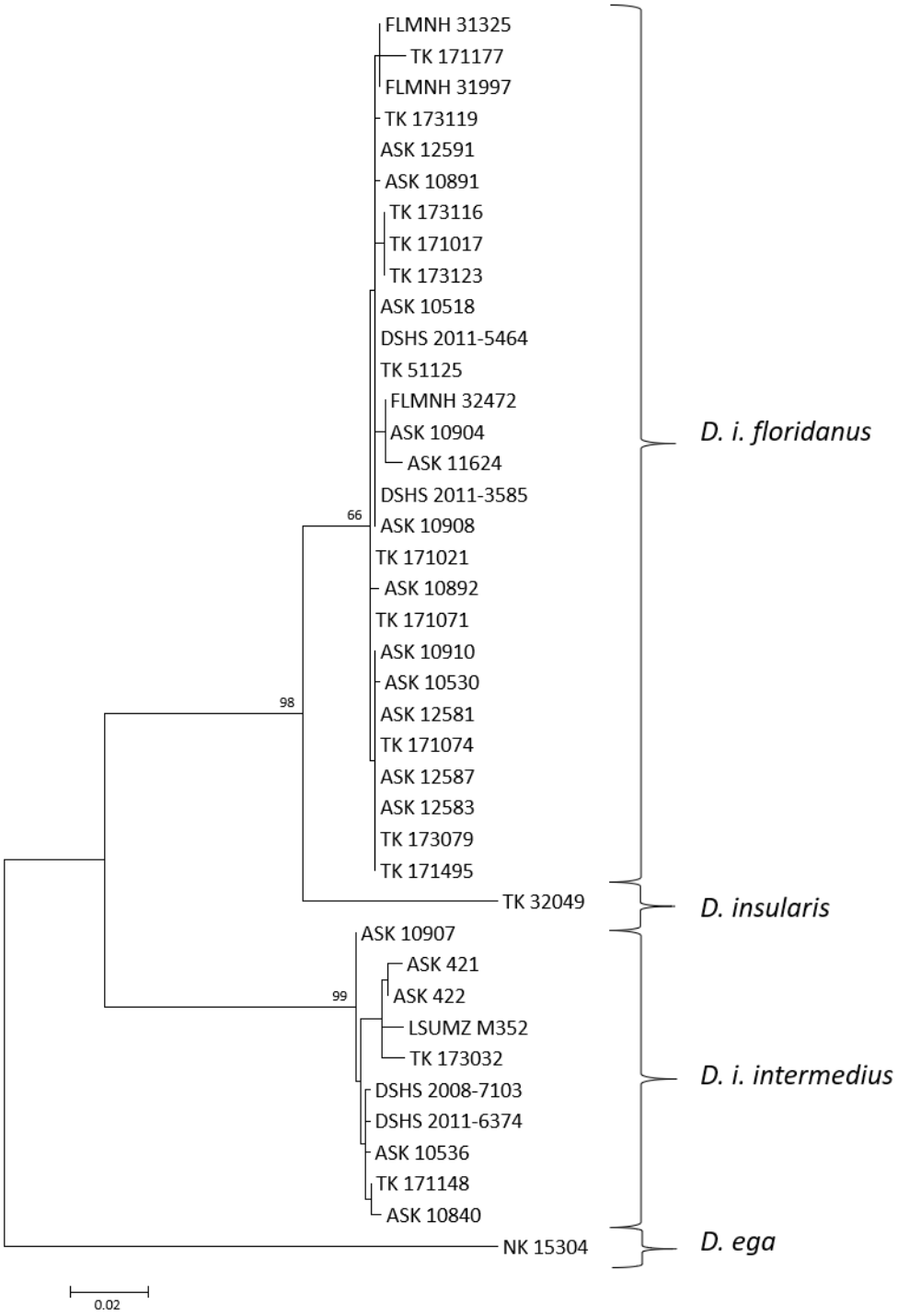

Fig. 2. Maximum likelihood phylogenetic tree of a 938 base pair fragment of the cytochrome *b* gene for 40 samples of *Dasypterus*. Maximum likelihood analysis was performed using the Hasegawa-Kishino-Yano model of DNA substitution with Gamma distribution (α= 0.3743). Bootstrap values based on 1000 replicates are shown at main branches.

Table 4. Pairwise distance analysis of average distances between clades recovered in the maximum likelihood phylogenetic analysis of cytochrome *b* sequence (Fig. 2).

	DIF	*DI*	*DII*
Dasypterus intermedius floridanus (DIF)	–	–	–
Dasypterus insularis (DI)	0.061	–	–
Dasypterus intermedius intermedius (DII)	0.116	0.129	–
Dasypterus ega	0.165	0.188	0.161

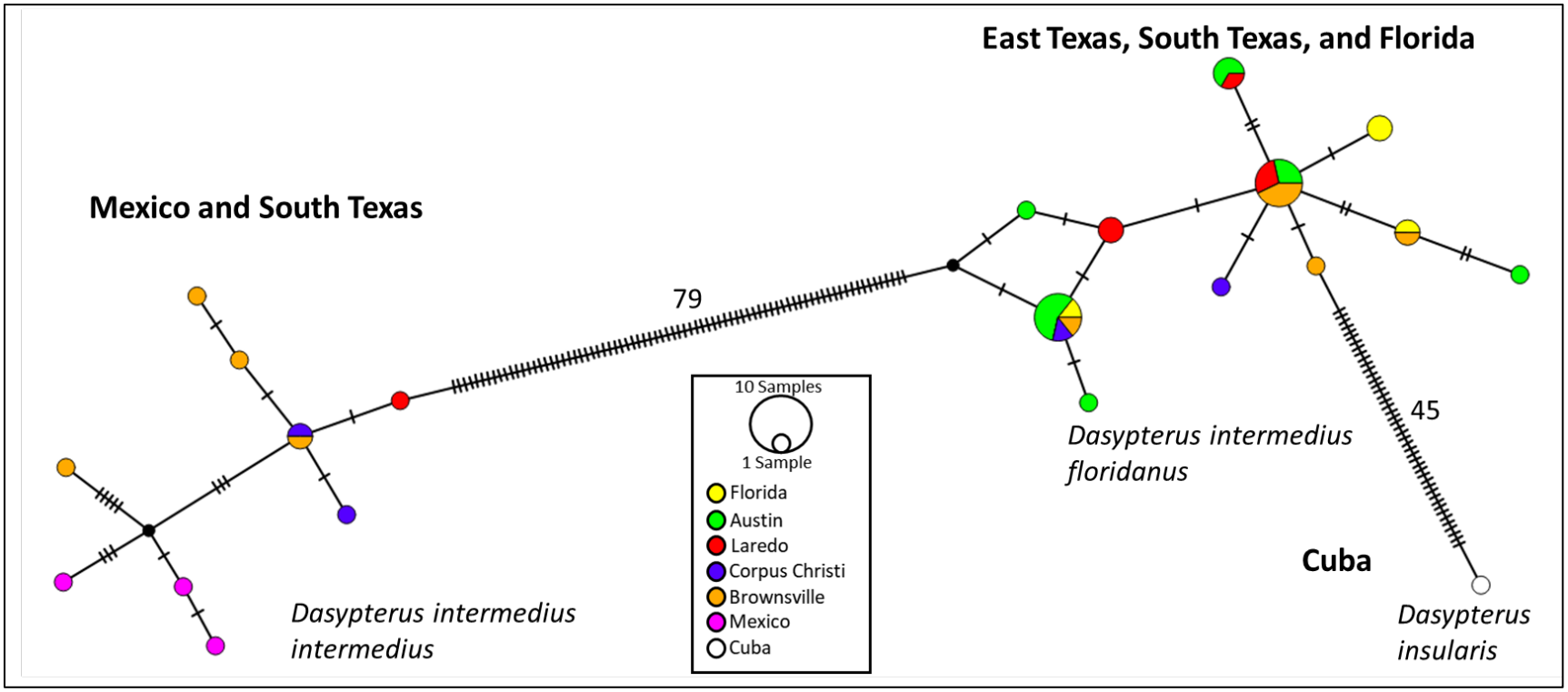

Fig. 3. Median joining haplotype network corresponding to the samples recovered by the phylogeny (Fig. 2), excluding *D. ega*. Tick marks represent the number of mutations between different haplotypes and small black nodes represent hypothetical haplotypes. Haplotypes are colored according to the area from which the sample originated.

non-specific banding patterns. Decreasing the annealing and extension times from the reported values (Keller et al. 2014: 90 seconds annealing and extension) to 30 seconds cleared up banding patterns and revealed a single band for each locus. Eight loci were excluded from the study as I was unable to obtain cross-species amplification products for the following loci: LbG, LbK, LcO, PrLb02, D226, LAS8843, LAS8953, LAS9613. D226 appeared to amplify at its published annealing temperature (54°C) and although bands were visualized on gel electrophoresis, peaks were not detected during sequencing. PrLb02 and LcO showed no visible bands after amplification, while LbK, LbG, LAS8843, and LAS8953 had non-specific banding patterns. To try to optimize LAS8843 and LAS8953, annealing temperatures of 59°C, 60°C, 61°C, 62°C, 63°C, and 64°C were tried as well as two different annealing and extension times of 60 and 30 seconds. These loci still did not amplify a specific region and were dropped from the study. LbK and LbG were not tested further for amplification due to budget restraints. LbK, LbG, LcO, and PrLb02 also did not amplify target loci in a close relative, Southern yellow bats, *Dasypterus ega* (Harrison 2018). LAS9613 showed a single band of amplified product after increasing the annealing temperature to 64°C and decreasing the annealing and extension times to 30 seconds, but due to budgetary constraints, this locus was not sequenced. Samples with over 50% missing data (data missing for four loci or more) after genotyping were dropped from the study. The overall missing data for 92 individuals across 8 loci was 2.04%.

Microsatellite variation – Analysis to determine null alleles found null allele frequency greater than 10% in LAS8539 (10.95%) and LAS4206 (11.65%). None of the loci in

this analysis exhibited large-allele dropout or scoring error due to stutter. Tests for Hardy-Weinberg equilibrium found that five out of eight loci (LbT, D200, Paur03, LAS4206, and LAS8539) deviated significantly from Hardy-Weinberg (HW) equilibrium (α=0.05; *P*=0.000 for all five loci). The following pairs of loci were determined to be linked (α=0.05): CoraF11/D200 (*P*=0.005), LbT/D200 (*P*=0.000), CoraF11/Paur03 (*P*=0.000), LbT/Paur03 (*P*=0.033), D200/Paur03 (*P*=0.000), LbT/LAS8539 (*P*=0.004), D200/LAS8539 (*P*=0.000), LbT/LAS4206 (*P*=0.000), and LbT/LAS8830 (*P*=0.016). Because Paur03 is an X-linked marker, HW equilibrium tests and linkage disequilibrium analyses were repeated after excluding males from the sample population. Without males in the analysis, Paur03 was in HW equilibrium but was still found to be in linkage disequilibrium with other loci: CoraF11/Paur03 (*P*=0.000) and D200/Paur03 (*P*=0.000).

Genetic variation and population structure – To assess genetic variation in *D. intermedius*, overall heterozygosity was calculated for the sample population as well as heterozygosity values for individual loci (Table 5). The overall heterozygosity was 0.621 with a standard error of 0.101 and heterozygosity values for individual loci ranged from 0.066–0.901. The number of alleles recovered for each locus ranged from 7–14, with an overall mean number of alleles of 9.0 ± 0.964 (Table 5). The STRUCTURE analysis resulted in a $\Delta K=2$ as the optimum number of populations based on the Evanno method; however, the membership coefficients suggest a single panmictic population because a gradual decrease in membership coefficient across the samples can be observed rather than distinct separation that corresponds to geographic location of the collected specimen (Fig. 4).

Table 5. Values of genetic diversity in *Dasypterus intermedius* across 8 microsatellite loci. N is number of genotyped individuals, N_a is number of alleles at each locus, H_o is observed heterozygosity, H_e is expected heterozygosity, and HWE is the p-value for the Hardy-Weinberg Equilibrium test.

Locus	N	N_a	H_o	H_e	HWE
CoraF11	92	7	0.582	0.486	*P*=0.552
CotoG12	92	14	0.879	0.861	*P*=0.997
LbT	92	7	0.066	0.126	*P*=0.000
D200	91	7	0.789	0.572	*P*=0.000
Paur03	92	12	0.901	0.665	*P*=0.000
LAS4206	88	10	0.581	0.768	*P*=0.000
LAS8539	87	7	0.379	0.502	*P*=0.000
LAS8830	87	8	0.791	0.796	*P*=0.434
Overall	92	9.0 ± 0.964	0.621 ± 0.101	0.595 ± 0.084	

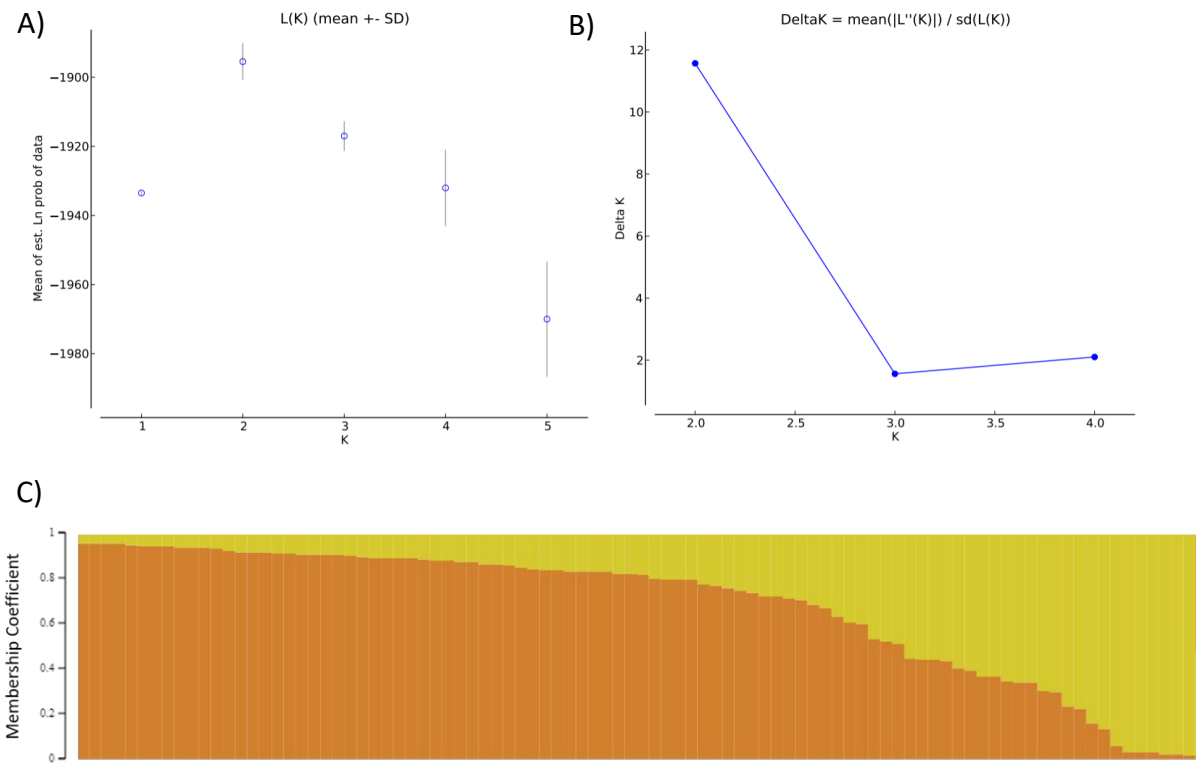

Fig. 4. A) Mean likelihood scores for each number of estimated populations (K) **B)** STRUCTURE HARVESTER plot of ΔK for 92 *Dasypterus intermedius* samples, indicating ΔK=2 for these individuals. **C)** The bar plot shows membership coefficients for each individual (1 individual = 1 bar) when ΔK=2.

No obvious pattern was revealed in the distribution of the samples into the two clusters defined by STRUCTURE analysis. Samples from the *D. i. floridanus cytb* lineage (Fig. 2) had membership coefficients for the orange cluster ranging from 0.12 to 0.95; similarly, samples from the *D. i. intermedius cytb* lineage (Fig. 2) had membership coefficients for the same orange cluster ranging from 0.23 to 0.96 (Fig. 4). A principal coordinates analysis showed no distinction between the two mitochondrially distinct clusters (Fig. 5) and an average F_{ST} value of 0.022 ± 0.007 indicates a high level of gene flow between the two lineages.

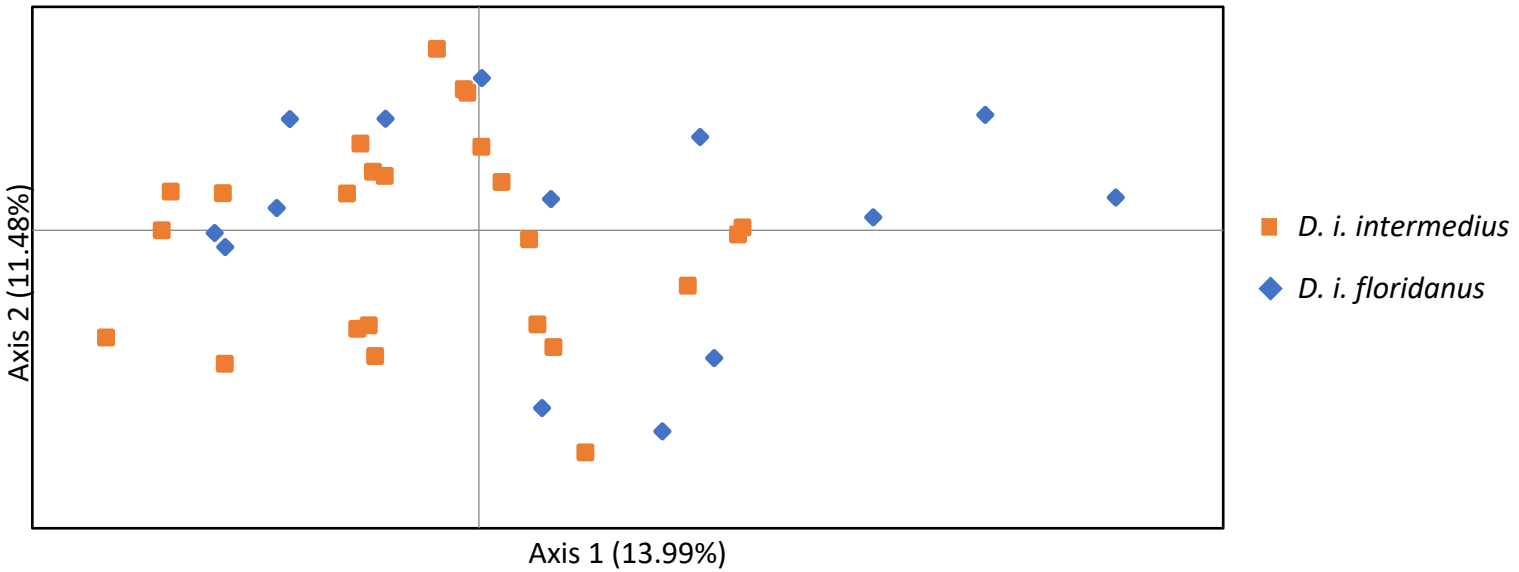

Fig. 5. Results of the principal coordinates analysis of the genotypes of 8 microsatellite loci for the two mitochondrial lineages of *Dasypterus intermedius*. The first axis explained 13.99% of the variation in the data, while the second axis explained 10.28% of the variation in the data. No separation of the two lineages was recovered by this analysis.

DISCUSSION

Northern yellow bats are relatively rare throughout their range and thus are a historically understudied species. Utilizing samples from natural history collections, especially those originally donated by the Texas DSHS, allowed me to assess the phylogeography, genetic diversity, and population structure of this elusive bat. The two main analyses performed contradict one another. The mitochondrial sequence data for cytochrome *b* returned two genetically distinct maternal lineages. A divergence level of 11.6% between the two clades is consistent with species-level recognition in accordance with the genetic species concept based on *Cytb* (Bradley and Baker 2001). This concept suggests that divergence in the *Cytb* sequence between sister species generally range from 4—11% as applied to bats and rodents, with an average divergence level of 6.83% for bat sister taxa (Bradley and Baker 2001). This analysis and the way that the phylogeny and haplotype analyses reflect proposed subspecies distribution suggest two highly divergent lineages of Northern yellow bats. Furthermore, the grouping of *D. insularis* with the *D. i. floridanus* clade to the exclusion of *D. i. intermedius* in the phylogeny make this species a paraphyletic group, violating the phylogenetic species concept (Cracraft 1983).

High levels of mitochondrial divergence within a species has also been reported in barred owls, *Strix varia*, which are difficult to differentiate at the subspecific level (Barrowclough et al. 2011). Divergence between the subspecies of barred owls was reported to follow similar geographic patterns as recovered in *D. intermedius* with the hypothesis that the divergences are a result of isolation of populations in geographically

isolated refugia during one of the glaciation events of the Pleistocene epoch (Barrowclough et al. 2011). Climate and associated changes in habitat could have created an unsuitable environment for the species and caused their distributions to shift southward. These refugia were proposed to occur in central Mexico, the Gulf Coast of Texas or Louisiana, and the southern Atlantic coast near Florida (Barrowclough et al. 2011). It is possible that *D. intermedius* populations also were isolated in two of these refugia for long enough time that their mitochondrial genomes diverged and later they experienced postglacial expansion of their range to form their current distributions. The same hypothesis was proposed to explain mitochondrial divergences in cottonmouth snakes, *Agkistrodon piscivorus*, explaining that secondary contact occurred as a result of postglacial range expansion and that gene flow between populations allowed for bi-parentally inherited marker exchange (Strickland et al. 2014).

A lack of genetic structure recovered from the analysis of nuclear microsatellite data suggests a single, continuous population in which gene flow is occurring. This directly contradicts the mitochondrial sequence data; however, this phenomenon has been reported before. Two distinct maternal lineages have also been reported in pallid bats, *Antrozous pallidus*, with over 12% divergence in the mitochondrial marker but no genetic structure was found when assessing bi-parentally inherited nuclear microsatellites (Arnold and Wilkinson 2015). This pattern was attributed to female pallid bats exhibiting philopatry to their natural roost sites whereas males are mating with females from different colonies (Arnold and Wilkinson 2015). Philopatry in female *Corynorhinus townsendii ingens* was also

hypothesized to play a role in divergent mitochondrial lineages that lacked population structure during microsatellite analysis (Weyandt et al. 2005). Although little is known about the ecology of *D. intermedius*, especially in regards to mating and reproduction, because of the geographic distribution of the mitochondrial lineages it is unlikely that philopatry can explain the pattern of high level of mitochondrial diversity and low nuclear diversity in Northern yellow bats.

The lack of evidence for genetic structure within *D. intermedius* based on microsatellites could indicate that there are two subspecies rather than two species as suggested by the cytochrome *b* analysis; however, the grouping of the Cuban yellow bat, *D. insularis* with the *D. i. floridanus* lineage makes the group paraphyletic. Further confirmation is needed, especially nuclear markers from the Cuban yellow bat, but based on the current evidence the taxon *D. insularis* might need to be absorbed into *D. intermedius* as its own insular subspecies to satisfy the phylogenetic species concept (Cracraft 1983). Obtaining samples from *D. insularis* has been difficult as political issues impede scientific collection in Cuba. Additionally, the Cuban yellow bat is listed as threatened on the IUCN Red List due to the rarity of the species and increasing habitat loss (Mancina 2012). To validate the results of this study, more information from the nuclear genome should be assessed such as additional microsatellite loci or a nuclear gene sequence. Though cross-species amplification of microsatellite loci for genetic analyses has been reported to be viable (Burland et al. 1998), this project demonstrates the need for development of species-specific microsatellite loci to provide more appropriate data.

The utility of cross-species amplification of microsatellite loci designed for several other species of vespertilionid bats in *D. intermedius* posed some challenges in this study. Of the eight loci successfully optimized, amplified, and genotyped, five loci deviated significantly from Hardy-Weinberg equilibrium, two loci had frequencies of null alleles greater than 10%, and 9 pairs of loci were determined to be linked. After eliminating males from the data set to correct for Y-chromosome linked loci, 3 loci were still out of HW equilibrium and there were four pairs of linked loci. Significant deviation from HW equilibrium may indicate that a locus is under selection or is being affected by gene flow, genetic drift, or mutation and is thus not a neutral marker. Neutral markers are generally preferred for population genetics analyses because they are not subject to selective forces that might bias interpretations of the data. Linkage disequilibrium can be problematic as it can result in overrepresentation of allele combinations and overestimation of the number of population clusters when admixture is present between populations (Falush et al. 2003). The presence of null alleles may result in a large number of homozygotes at a certain locus if the smaller allele is preferentially amplified or due to slippage of *Taq* polymerase during amplification (Gagneaux et al. 1997; Shinde et al. 2003). Increased homozygosity in the population reduces genetic diversity estimates. Null alleles are especially frequent in cross-species amplification studies in which the primer sets were not designed to be optimal to the study species (Chapuis and Estoup 2007). The difficulty that I found in using microsatellite loci designed for other verspertilionid bats, namely *Lasiurus borealis* and

Aeorestes cinereus, in *Dasypterus intermedius* may provide additional support for the splitting of the previous *Lasiurus* genus suggested by Baird et al. (2015).

Overall levels of heterozygosity at H_0=0.621 ± 0.101 are lower than that seen in Eastern red bats (H_0=0.805 ± 0.050) and hoary bats (H_0=0.859 ± 0.026) from Texas (Korstian et al. 2015). Though both of these species are closely related to *D. intermedius* (previously classified as the same genus, *Lasiurus*), both Eastern red bats and hoary bats are migratory whereas Northern yellow bats are not known to migrate (Ammerman et al. 2012). Migration could lead to more opportunities to increase genetic diversity within a species if individuals are not confined to mating within a specific population (Korstian et al. 2015). Overall observed heterozygosity in *D. intermedius* may be comparable to the average observed heterozygosity of the non-migratory Serotine bat, *Eptesicus serotinus*, (H_0=0.637) across much of their distribution in Poland (Bogdanowicz et al. 2013). This species has a wide distribution throughout western and central Europe as well as parts of Asia and is listed as a species of least conservation concern on the IUCN Red List (Hutson et al. 2008). On the other hand, some endangered bats such as the gray bat, *Myotis grisescens*, have similar heterozygosity levels as recovered in this study (H_0=0.639 ± 0.017; Lindsay et al. 2015). Reductions in heterozygosity levels could indicate a need for conservation assessment in Northern yellow bats.

Vertebrate species listed as threatened and exhibiting a degree of extinction risk also tend towards lower genetic diversity values, such as heterozygosity, than species of least concern (Willoughby et al. 2015). Low genetic diversity within a species affects its

ability to adapt to the ever-changing environment. Due to the rarity of this species and lack of ecological information on it, the relatively low level of heterozygosity within the Northern yellow bat samples could indicate a need for conservation efforts, although factors such as linkage disequilibrium and deviation from HW equilibrium in the tested loci might be contributing to this low value. Developing species-specific markers should provide a more reliable measure of genetic diversity in this species. Furthermore, the samples used in this study only cover a portion of this species' range and additional genetic diversity may exist in unsampled areas.

Such environment changes include global climate change. A recent boom in the wind energy production industry has led to the estimated annual death of over 500,000 bats in North America (Frick et al. 2017). Tree roosting species of bats, like *D. intermedius*, as well as migratory bats, are especially vulnerable (Frick et al. 2017). It is unknown to what extent these potential mortalities will have on populations of Northern yellow bats, but decreased genetic diversity could lead to the demise of the species. This may be an area in which regulation and policy can be applied, along with private enterprise if wind farm owners are required to adhere to guidelines to decrease bat mortalities. Populations of *D. intermedius* may also be threatened by increasing urban expansion as roost sites are subjected to landscaping practices such as the removal of dead fronds from palm trees (Jimenez 2016). It is notable that samples outside of the species' historical range were used in this analysis, with specimens from Frio county, Uvalde county, and Webb county included. Demere et al. (2012) reported this recent range expansion but did not speculate why that expansion

occurred. Northern yellow bats may be taking advantage of the increasing number of ornamental palm trees as roosting sites in urbanized areas, leading to wider distributions or might be expanding their range due to necessity while suitable roosting sites are destroyed for landscaping (Jimenez 2016).

With stigma still surrounding bats in many communities, public education on the benefits of bats may help decrease further habitat loss and aid in conservation efforts. Lack of aesthetic appeal and the perceived risk to humans contribute to the negative image of bats (Kingston 2015). Increased public outreach and education might be able to change the way the general public across cultures respond to bats in the environment. This study could also have implications in agricultural economics. Though the diet of Northern yellow bats has not been extensively studied, they likely consume crop pests such as those found in the previously recognized suborder Homoptera (Sherman 1939). Insectivorous bats are valuable to the North American agricultural industry (calculated at $22.9 billion per year) and likely contribute to lower levels of pesticide applications (Boyles et al. 2011). Bats contribute enormously to the ecosystem and *D. intermedius* are not an exception. Further studies on the ecology of these bats is needed to estimate their full impact on the ecosystem.

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VITA

Sydney Kay Decker was born in Heinsberg, Germany to Kimberly and Morris Decker, both veterans of the United States Air Force. After moving around every few years, she completed high school at Central High School in San Angelo, Texas, in May of 2015. Sydney has attended Angelo State University for all four years of her undergraduate career. She began her research in her first year after being awarded the First Year Research Experience Grant from ASU. She continued this research throughout her undergraduate years, presenting it at multiple conferences. She will be graduating from ASU with a Bachelor of Science in Biology and Highest University Honors in May of 2019 and in the fall will be attending The Ohio State University to pursue a Ph.D. in the Evolution, Ecology, and Organismal Biology program.

Appendix 1. Genotypes for *Dasypterus intermedius* (n=92) across 8 microsatellite loci. U = genotype unavailable.

Tissue No.	CoraF11	CotoG12	LbT	D200	Paur03	LAS8539	LAS4206	LAS8830
ASK421	154/154	226/228	256/256	U	222/234	164/170	280/280	252/256
ASK422	136/154	226/230	256/256	146/154	220/234	164/170	282/282	252/258
ASK9499	146/154	225/226	256/256	146/154	220/234	164/164	278/280	254/260
ASK10518	146/154	226/230	256/256	146/154	222/234	164/164	276/278	258/258
ASK10519	154/154	224/226	256/256	146/154	220/234	164/164	282/282	256/258
ASK10523	132/146	218/224	252/252	140/150	212/234	U	U	U
ASK10529	154/154	224/228	256/256	146/154	208/234	170/170	280/286	252/258
ASK10530	146/154	220/222	256/256	146/154	218/234	164/170	278/278	254/258
ASK10536	144/154	222/230	256/256	154/154	208/234	170/170	280/280	258/258
ASK10837	144/154	220/230	256/256	146/154	218/234	164/170	278/280	252/254
ASK10838	146/154	224/230	256/256	146/154	222/234	164/164	282/282	250/252
ASK10839	154/154	222/232	256/256	146/154	214/234	164/164	276/280	252/258
ASK10840	154/154	222/228	256/256	146/154	234/234	164/164	280/280	258/252
ASK10882	154/154	224/230	256/256	142/154	208/234	164/170	280/282	256/258
ASK10891	138/154	222/224	256/256	144/154	214/234	164/170	276/276	252/256
ASK10892	144/154	222/232	256/256	146/154	218/234	164/164	278/280	254/256
ASK10893	144/154	224/228	256/256	144/154	218/234	164/170	274/280	252/254
ASK10894	146/154	226/232	256/256	146/154	220/234	164/170	276/276	250/252
ASK10895	146/154	228/228	256/256	146/154	222/234	170/170	276/276	252/254
ASK10896	154/154	226/230	256/256	146/154	218/234	164/164	280/280	254/258
ASK10897	154/154	224/230	256/256	146/154	218/234	164/164	278/278	252/258
ASK10898	136/154	212/222	256/256	144/154	208/234	164/170	278/280	258/262
ASK10899	138/154	222/226	256/256	144/154	212/234	164/164	282/282	254/258
ASK10900	144/154	228/230	256/256	146/154	218/234	170/170	280/280	250/252
ASK10901	146/154	224/228	256/256	146/154	218/234	164/164	276/280	252/252
ASK10902	146/154	224/228	256/256	146/154	220/234	164/170	280/280	252/252

Appendix 1. Continued

Tissue No.	CoraF11	CotoG12	LbT	D200	Paur03	LAS8539	LAS4206	LAS8830
ASK10903	146/154	224/230	256/256	146/154	222/234	164/164	274/276	252/258
ASK10904	146/154	220/224	256/256	146/154	222/234	170/172	276/278	250/260
ASK10905	146/154	220/222	256/256	146/154	220/234	164/164	276/276	252/260
ASK10906	154/154	226/228	256/256	146/154	208/234	164/170	278/280	252/252
ASK10907	154/154	222/226	256/256	146/154	220/234	164/170	276/280	254/258
ASK10908	154/154	220/228	256/256	144/154	214/234	164/170	276/282	252/254
ASK10909	154/154	230/232	256/256	146/154	218/234	164/170	280/280	254/256
ASK10910	138/154	224/226	256/256	146/154	222/234	164/164	278/280	252/264
ASK11624	154/154	224/224	228/256	154/154	234/234	U	U	U
ASK12581	154/154	222/224	256/256	154/154	234/234	U	U	U
ASK12583	146/154	218/224	256/256	146/154	218/234	164/170	278/278	254/258
ASK12585	144/154	226/228	256/256	146/154	218/234	164/170	282/282	250/252
ASK12586	154/154	220/228	256/256	140/150	234/234	169/168	266/276	256/262
ASK12587	154/154	212/226	256/256	154/154	208/234	164/170	278/278	252/258
ASK12588	146/154	222/226	256/256	150/150	220/234	168/168	266/278	256/260
ASK12589	146/154	224/226	252/252	146/150	222/234	168/168	264/274	258/260
ASK12590	146/154	218/220	256/256	148/154	222/234	172/172	278/280	256/258
ASK12591	146/154	220/224	256/256	146/154	220/234	164/164	278/278	254/262
DSHS2008-7103	144/154	222/226	256/256	146/154	222/234	164/164	280/280	256/254
DSHS2009-4378	154/154	228/228	256/256	140/154	208/234	164/164	276/280	252/258
DSHS2010-3432	154/154	228/228	256/256	142/154	216/234	164/170	276/278	252/258
DSHS2010-6432	144/154	222/224	256/256	144/154	218/234	164/164	280/280	252/252
DSHS2011-3585	144/154	230/232	256/256	146/154	218/234	164/264	274/280	252/258
DHSH2011-5464	144/154	226/232	256/256	146/154	218/234	164/164	278/286	252/254
DSHS2011-6374	144/154	226/228	230/256	154/154	234/234	170/170	282/282	256/258
FLMNH31197	154/154	212/220	256/256	146/154	208/234	164/164	276/278	256/256

Appendix 1. Continued

Tissue No.	CoraF11	CotoG12	LbT	D200	Paur03	LAS8539	LAS4206	LAS8830
FLMNH31318	154/154	220/226	230/256	154/154	222/234	164/164	U	U
FLMNH31525	154/154	226/230	256/256	146/154	220/234	164/170	278/280	250/264
FLMNH31528	146/154	212/232	256/256	154/154	234/234	164/164	280/280	250/258
FLMNH32472	138/154	212/220	256/256	154/154	214/234	164/172	276/276	254/256
TK51125	154/154	224/224	256/256	154/154	214/234	164/172	278/280	252/260
TK51126	144/154	212/222	256/256	154/154	218/234	164/164	278/278	250/254
TK51159	154/154	212/224	256/256	146/154	218/234	164/172	278/282	254/254
TK51160	146/154	220/224	256/256	146/154	220/234	164/164	278/278	256/256
TK53777	154/154	212/224	256/256	146/154	218/234	164/170	276/278	252/258
TK53962	154/154	224/230	256/256	146/154	208/234	164/164	280/282	252/256
TK53963	154/154	224/230	256/256	154/154	214/234	164/170	280/280	250/264
TK171005	146/154	220/228	252/256	142/150	220/234	168/168	268/268	260/260
TK171015	146/154	206/226	256/256	146/154	222/234	164/164	280/280	254/258
TK171017	146/154	224/226	256/256	146/154	224/234	164/164	278/282	252/258
TK171021	154/154	212/222	256/256	146/154	220/234	164/170	276/278	252/252
TK171022	146/154	216/224	248/250	150/154	228/240	166/166	264/278	250/252
TK171070	154/154	208/226	256/256	146/154	234/234	164/170	280/282	252/258
TK171071	154/154	212/228	256/256	146/154	208/234	164/164	274/276	252/258
TK171073	154/154	222/224	256/256	154/154	208/234	164/164	276/276	252/258
TK171074	154/154	228/228	256/256	154/154	234/234	164/170	278/280	250/260
TK171079	144/154	224/226	256/256	146/154	218/234	164/164	278/282	254/260
TK171080	146/154	224/226	256/256	146/154	218/234	164/164	276/278	252/256
TK171086	146/154	224/224	256/256	146/154	220/234	164/164	276/282	252/260
TK171127	154/154	224/226	256/256	144/154	214/234	164/164	280/280	252/254
TK171137	144/154	224/232	256/256	146/154	218/234	164/164	276/276	254/254
TK171148	144/154	228/230	256/256	154/154	210/234	156/156	276/280	252/258

Appendix 1. Continued

Tissue No.	CoraF11	CotoG12	LbT	D200	Paur03	LAS8539	LAS4206	LAS8830
TK171177	146/154	228/232	256/256	146/154	222/234	164/164	276/280	252/252
TK171205	146/154	220/230	256/256	146/154	220/234	164/164	280/289	254/260
TK171207	146/154	222/238	256/256	146/154	222/234	164/170	274/276	252/258
TK171495	146/154	222/224	256/256	146/154	220/234	164/164	278/280	250/254
TK173032	154/154	228/228	224/230	154/154	234/234	U	U	U
TK173064	154/154	220/222	256/256	154/154	220/234	164/170	280/282	252/252
TK173076	154/154	226/226	256/256	154/154	208/234	164/170	280/280	258/258
TK173079	146/154	220/228	256/256	146/154	222/234	164/164	280/282	258/258
TK173082	154/154	230/232	256/256	154/154	214/234	170/170	280/280	258/258
TK173113	144/154	220/226	256/256	146/154	218/234	164/164	276/278	250/258
TK173116	154/154	226/226	256/256	146/154	220/234	164/164	280/282	250/258
TK173119	146/154	226/230	256/256	146/154	218/234	164/164	276/280	252/258
TK173123	154/154	224/226	256/256	154/154	208/234	164/170	274/282	254/258
TK173128	156/154	226/226	256/256	146/154	220/234	164/170	280/280	258/258