EVALUATION OF BLOOD GAS ANALYTES AND ECTOPARASITES FROM SOUTH TEXAS BIRDS

A Thesis

by

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ABSTRACT

The handheld point of care analyzer is a quick and feasible option to obtain hematology data from individuals. The iSTAT-1® was used to evaluate select venous blood analytes obtained via jugular venipuncture from 238 passerine birds from South Texas. These data were used to assess the health of birds in the area while taking into consideration life history (migratory or sedentary), locale, seasonality, sex, and age. Migratory birds had increased concentrations of pO_2, hematocrit, hemoglobin, and glucose as compared to sedentary birds. This can be attributed to the increased need of oxygen and carrying capacity involved with long duration flights. Increased glucose and lower ionized calcium concentrations were observed in migratory birds as a result of breakdown of fat deposits in the body to fuel the increased levels of muscular activity. During the hotter months of the year, birds' response to handling environmental stress was exhibited with relative respiratory acidosis. When sedentary birds sampled from South Texas were compared to a previous study from Central Texas, venous blood analytes differed by locale but were within the ranges of healthy populations. This leads to the conclusion that sedentary avian communities can be used as bioindicators of a healthy ecosystem.

Few assessments of louse-host associations in Texas involving multiple host families and genera have occurred. My assessment of 446 birds captured in South Texas revealed 64 host associations, of which 31 were previously unknown in the literature. In

addition to these new host associations, I also was able to identify 25 unique genetic lineages. There are 17 unique genetic lineages that are associated with new host associations. This leads to the possibility of having at minimum 17 and as many as 25 potential new species from this study. Morphologically I was unable to identify any lice to species, but sequences from GenBank assisted with some specimen identification to species. Using louse-host associations and the unique genetic lineages found, I was able to identify specimens that could represent new sequences to GenBank or new species to science.

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Part 1. Faculty Committee Recognition

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The Texas maps in Chapter II and Chapter III were possible with assistance of Aleyda Galán and Adrian Castellanos.

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CHAPTER I

INTRODUCTION

Approximately 95% of all Texas land is privately owned, which can make it difficult for researchers to be able to access these lands and address biological questions about the organisms that inhabit the area. This study was conducted on East Foundation lands as part of larger biodiversity assessment of amphibians/reptiles, birds, and small mammals in South Texas.

Hematological assessments are becoming a popular method within the veterinary community to assess health, due to the minimal impact on the bird and small amount of blood required (Deem et al. 2011; Fokidis, Greiner, and Deviche 2008; Sheldon et al. 2008). Point of care analyzers have allowed for assessment of respiratory and cardiovascular systems via measurement of avian acid-base status, biochemical fluid balance, electrolytes, and blood gases (Heatley et al. 2013). Blood gas analytes are defined as the biochemical composition of the blood. The results that the point of care analyzers provide give insight to individual health and possibly physiological changes brought on from the environment. Several studies have investigated effects of ecosystem health and the birds hematological response; these studies were able to show differences in hematology linked to negative ecosystem health (Llacuna et al. 1996, Ruiz et al. 2002)

Chewing lice (Insecta: Phthiraptera) are obligate ectoparasites that can be found all around the world on their avian hosts (Marshall 1981). Chewing lice belong to two suborders: Amblycera and Ischnocera with distinctive morphologies (Waterhouse 1953,

Johnson et al. 2012). These ectoparasites feed on feathers and skin debris and occasionally on blood (Waterhouse 1953). Although lice are obligate parasites, they utilize two methods of transmission to move to a new host. Vertical transmission happens by direct contact to another individual and horizontal transmission via phoresis (Keirans 1975, Johnson and Clayton 2003). Texas is an unstudied area, when it comes to louse-host associations. Although, a few studies have examined lice parasitizing doves in from Cameron and Hidalgo Counties (Johnson et al. 2002a, Moyer et al. 2002, Bush and Clayton 2006) which is close to sampling localities from this study in South Texas.

This thesis will focus on creating baseline information of blood gas analytes from birds in South Texas, comparing blood analyte values of migratory and sedentary birds, comparing bird analyte values from the South Texas to the Central Texas ecoregion, and determine is sedentary passeriforme communities are useful bioindicators of ecosystem health. In addition, this study will also assess avian chewing louse diversity in South Texas by investigating louse-host associations and determine relationships within groups of lice using phylogenetic analysis.

CHAPTER II

USING SELECT VENOUS BLOOD GAS ANALYTES TO ASSESS INTRINSIC FACTORS ON PASSERIFORME HEALTH IN SOUTH TEXAS

II.1 Introduction

Effects of habitat alteration or destruction are frequently investigated, but many studies only approach the issue from a habitat management perspective. To fully understand the effects that environmental changes have, especially on organisms such as birds, we need to address their physiological response to the change (Albano et al 2012). Environmental changes can cause stress to the inhabitants of the area and have been linked to nutritional deficiencies, hormonal imbalance, inflammation, and chronic infection (Briggs et al. 1996). Several studies have looked at the effects of an altered (polluted or degraded) ecosystem and the hematological response of the birds that inhabit the area (Llacuna et al. 1996, Ruiz et al. 2002, Elezaj et al. 2011). These studies were able to show differences of certain hematological parameters that impact bird health and can be associated with the conditions affecting health of the ecosystem.

Hematological assessment is an increasingly popular method to assess health with minimal negative impact on the individual bird (Fokidiset et al. 2008, Sheldon et al. 2008, Deem et al. 2011). Point of care analyzers have allowed for assessment of respiratory and cardiovascular systems via measurement of avian acid-base status, biochemical fluid balance, electrolytes, and blood gases (Heatley et al. 2013). The iSTAT-1® analyzer requires only .15 µl of blood and provides results within two

minutes for various blood analytes. The iSTAT-1® has been used to determine multiple analytes for avian species such as chickens, passerines, and parrots (Steinmetz et al. 2007, Paula et al. 2008, Martin et al. 2010, Harms and Harms 2012, Heatley et al. 2013). This study aims to assess the health of free living passerines in southern Texas via select venous blood analytes, and by including life history traits (migration), locale, seasonality, and other intrinsic variables as covariates. I also compare my results from southern Texas to similar data collected from birds occupying a distinctly different habitat in Central Texas.

I hypothesize that sedentary and migratory bird blood analytes will differ based on the physiological needs of migration. I further hypothesize that differences amongst analytes of birds from East Foundation ranches, and between South and Central Texas will be minimal and generally reflective of good health. Finding significant differences of these hematological parameters could be important to the larger scheme of understanding free-living passerine health and their interaction with environmental conditions. Should venous blood analytes of sedentary avian species be altered by local factors in healthy ecosystems, they could represent good local bioindicators of their ecosystem.

II.2 Materials and Methods

Field Sampling

Passerine birds (Table 1, Table 2) were sampled on East Foundation lands (Fig. 1) from March 2014 to November 2015. Specific East Foundation lands included San

Antonio Viejo Ranch (located inland, in Jim Hogg and Starr Counties) and, El Sauz Ranch (located coastally in Kenedy and Willacy Counties: Fig. 1). Birds were captured via mist net and placed in cloth bags for a short period, allowing them to calm before sampling occurred. Birds were restrained by hand for collection of 0.2-0.5 mL of blood via jugular venipuncture with needle and syringe. Blood samples were transferred to lithium heparin microtubes (Terumo America Inc, Elkton, MD, USA) to prevent clotting.

Table 1. Summary of the 238 passerines sampled for hematology on East Foundation ranches in South Texas.

Family	**Total**	**Males**	**Females**	**Adult**	**First Year**
Cardinalidae	72	37	34	38	12
Emberizidae	20	8	5	11	2
Fringillidae	3	2	1	1	2
Icteridae	36	10	18	17	5
Mimidae	25	3	4	7	6
Paridae	5	2	2	4	1
Parulidae	51	14	22	28	9
Troglodytidae	4	2	1	3	0
Turdidae	2	0	2	2	0
Tyrannidae	13	3	4	8	1
Vireonidae	7	1	2	3	1
El Sauz Ranch	194	71	72	99	24
San Antonio Viejo Ranch	44	11	23	23	12
Total	238	82	95	122	36

Table 2. Migratory and Sedentary birds from East Foundation ranches sampled for blood gas and electrolytes.

Migratory	**Sedentary**
***N*=128**	***N*=110**
Baltimore Oriole (Icterus galbula)	Audubon's oriole (Icterus graduacauda)
Bay Breasted Warbler (Setophaga castanea)	Bewick's wren (Thryomanes bewickii)
Black and white Warbler (Mniotilta varia)	Black crested titmouse (Baeolophus atricristatus)
Black throated green Warbler (Setophaga virens)	Bronzed cowbird (Molothrus aeneus)
Blue Grosbeak (Passerina caerulea)	Brown headed cowbird (Molothrus ater)
Blue headed vireo (Vireo solitarius)	Common yellowthroat (Geothlypis trichas)
Brown Crested flycatcher (Myiarchus tyrannulus)	Couch's kingbird (Tyrannus couchii)
Canada warbler (Cardellina canadensis)	Curve billed thrasher (Toxostoma curvirostre)
Clay colored sparrow (Spizella pallida)	Great kiskadee (Pitangus sulphuratus)
Dickcissel (Spiza americana)	Lark sparrow (Chondestes grammacus)
Eastern Phoebe (Sayornis phoebe)	Lesser goldfinch (Spinus psaltria)
Gray catbird (Dumetella carolinensis)	Long billed thrasher (Toxostoma longirostre)
Gray cheeked thrush (Catharus minimus)	Northern Cardinal (Cardinalis cardinalis)
Great crested flycatcher (Myiarchus crinitus)	Norther mockingbird (Mimus polyglottos)
Golden winged warbler (Vermivora chrysoptera)	Olive sparrow (Arremenops rufivirgatus)
Hooded oriole (Icterus cucullatus)	Pyrrhuloxia (Cardinalis sinuatus)

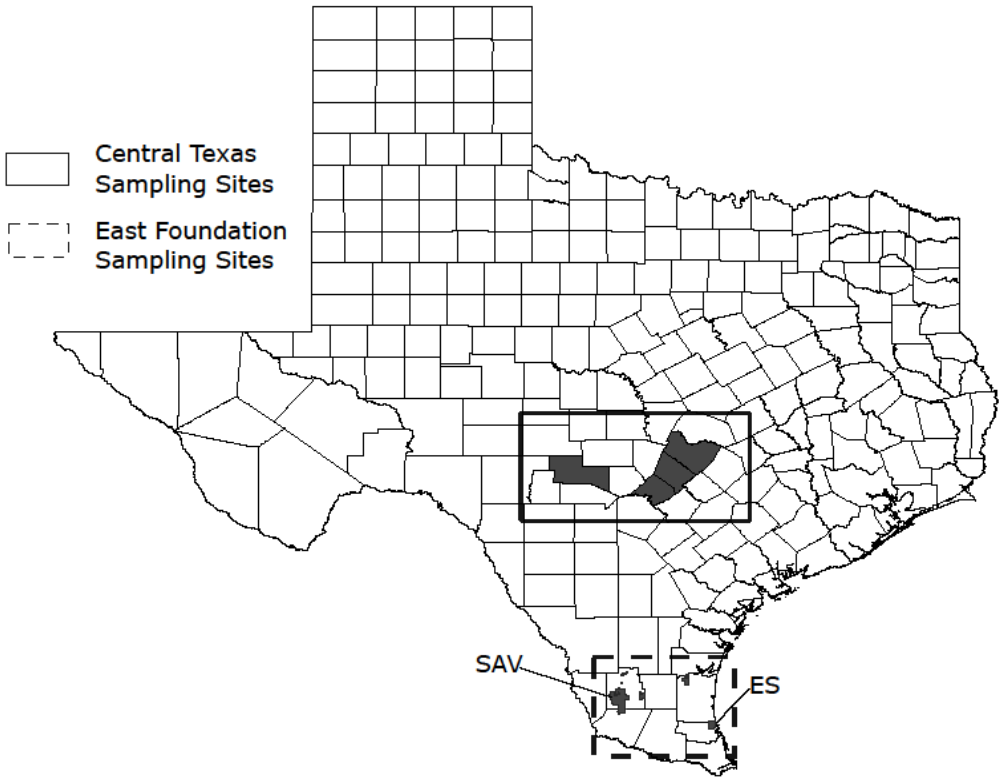
Table 2. Continued

Migratory	**Sedentary**
***N*=128**	***N*=110**
House Wren (Troglodytes aedon)	Red winged blackbird (Agelaius phoeniceus)
Indigo bunting (Passerina cyanea)	White eyed vireo (Vireo griseus)
Lincoln's sparrow (Melospiza lincolnii)	
Louisiana Waterthrush (Parkesia motacilla)	
Magnolia Warbler (Setophaga magnolia)	
Nashville warbler (Leiothlypis ruficapilla)	
Northern Waterthrush (Parkesia noveboracensis)	
Orange crowned warbler (Leiothypis celata)	
Painted bunting (Passerina ciris)	
Scarlet tanager (Piranga olivacea)	
Scissor tailed flycatcher (Tyrannus forficatus)	
Summer tanager (Piranga rubra)	
Swainson's thrush (Catharus ustulatus)	
Tennessee warbler (Oreothlypis peregrina)	
Warbling vireo (Vireo gilvus)	
Willow flycatcher (Empidonax traillii)	
Worm eating warbler (Helmitheros vermivorum)	

Table 2. Continued

Migratory	**Sedentary**
***N*=128**	***N*=110**
Yellow breasted chat (Icteria virens)	
Yellow warbler (Setophaga petechia)	

Figure 1. Sampling localities for birds included in this study. The red rectangle outlines the East Foundation properties with San Antonio Viejo (SAV) and El Sauz (ES) ranches noted. The blue rectangle outlines counties on the Central Texas, from which birds from a previous study (Heatley et al. 2013) are used for analyte comparisons to South Texas.



Sample Analysis

Blood sample analysis occurred within 5 minutes of sample collection using a handheld point of care analyzer, iSTAT-1® system (Abbott Laboratories. Abbott Park, IL, USA). Blood sample analysis was performed with the blood gas cartridge (CG4+ or CG8+) first, followed by the Chem 8 cartridge. Venous blood values (iSTAT-1® system manual 2012) were determined for the following analytes: pH, pCO_2 (carbon dioxide partial pressure), pO_2 (oxygen partial pressure), lactate, bicarbonate, total CO_2, base excess, sO_2 (dissolved oxygen), ionized calcium, glucose, blood urea nitrogen (BUN), hematocrit, hemoglobin, sodium, potassium, and chloride. The iSTAT-1® system measures most values directly, but total CO_2, base excess, hemoglobin, and sO_2 are calculated. Blood samples were loaded into ammonium heparin microhematocrit (Drummond Scientific Co, Broomall, PA, USA) capillary tubes and centrifuged (Clay-Adams, Inc. New York, USA) at 13,000 g for 5 minutes within 24 hours of collection to access packed cell volume. A physical examination and assignment of body condition score (BCS) was performed on each bird after blood collection (Manual of Exotic Pet Practice 2009). The body condition was scored on a scale of 1-4 by assessing the mass of the pectoral muscle and fat located on the chest, with BCS 1 being the lowest condition score and BCS 4 the highest. Scoring was performed by using the thumb and fore finger to palpate muscle along the keel, examining the contour of the breast muscle. In this technique, higher body condition scores are representative of better health. After sampling, some birds were humanely sacrificed via thoracic compression and prepared as voucher specimens for the Biodiversity Teaching and Research Collection at Texas

A&M University, while other birds released back to the ecosystem. Intrinsic variables such as species, sex, and age were recorded in the field, if possible, by external field markings and confirmed during specimen preparation. Migratory or sedentary status was assigned using life history information (Guide to Birds of North America 2011).

Statistical Methods

Analysis of the data was performed using Analyse-it for Microsoft Excel® statistical software (version 2.20 Microsoft Office 2010, Analyse-it® Software Ltd, http://www. analyse-it.com/, 2009). Normality for each analyte was assessed by histogram and Shapiro-Wilk test ($P>0.05$). The effects of migratory status, BCS, age, sex, season (fall, spring, or summer), and locality were evaluated using Student's t-test ($P<0.05$) for parametric data and by Kruskal-Wallis test ($P<0.05$) nonparametric data. Season and BCS were assessed using a one-way analysis of variance ($P<0.05$). The effect of species was also measured using a one-way analysis of variance for five sedentary species from South and Central Texas, that had a minimum of 10 individuals sampled. Five sedentary species, for a total of 108 birds (Table 3) were sampled from these two ecosystems. A Bland-Altman plot was constructed to assess the agreement between measurement of packed cell volume by centrifugation and hematocrit by the iSTAT-1®. For all statistical analysis except determination of normality ($P >0.05$) significance was accepted at $P<0.05$.

II.3 Results

East Foundation Passeriformes

A total of 238 passerines were sampled from East Foundation Lands, comprising 12 families, 52 species, and representing both migratory and sedentary life histories (Tables 1 and 2). Analyte data from birds captured on both ranches from South Texas had parametric distribution for nine analytes and, non-parametric distribution for eight analytes (Table 4). Bird sex was determined for only 177 individuals (82 males, 95 females) based on lack of sexually dimorphic field markings in combination with release of the bird post blood collection. Female birds had increased concentrations (all values to be read as mean ± standard deviation) of ionized calcium (0.964 ± 0.017 mg/dl) compared to males (0.918 ± 0.018 mg/dl: P=0.0515). Age classifications (adult and first year) were determined for 145 birds based on field markings and assessment of skull ossification during the museum preparation. Total carbon dioxide concentrations were significantly decreased in first year birds (22.5 ± 17.5 mmol/L) compared to adults (24.5 ± 20.9 mmol/L: P=0.0352).

Table 3. Numbers of specimens of five sedentary species selected from Central Texas and South Texas.

Species	Central Texas	South Texas	Total
Bewick's Wren	7	3	10
Black-crested Titmouse	14	5	19
Northern Cardinal	7	39	46
Northern Mockingbird	1	20	21
White-eyed Vireo	5	8	13

Table 4. Blood gas and electrolyte intervals from passeriformes sampled in South Texas.

Analyte	**Units**	**Birds sampled**	**Mean**	**95% CI**	***P* value**
pH*	pH	222	7.659	7.644-7.673	0.0187
pCO_2	mm Hg	222	20.7	20.0-21.5	0.5626
pO_2*	mm Hg	225	54.1	51.2-57.0	<0.0001
Base excess	mmol/L	226	2.6	1.9-3.2	0.1475
Bicarbonate	mmol/L	225	23.1	22.5-23.7	0.7881
TCO_2	mmol/L	202	23.7	23.1-24.3	0.3259
sO_2*	%	226	90.4	89.5-91.3	<0.0001
Lactate*	mmol/L	83	4.47	4.12-4.82	<0.0001
Glucose	mg/dl	200	330.2	319.6-340.8	0.0728
BUN*	mg/dl	9	3.9	2.8-4.9	0.0032
Sodium	mmol/L	203	155.7	155.1-156.3	0.0651
Potassium*	mmol/L	202	4.2	4.0-4.3	<0.0001
Chloride	mmol/L	80	122.6	121.6-123.6	0.3084
iCa	mg/dl	169	0.956	0.937-0.975	0.343
Hct*	%	203	39.9	39.3-40.7	0.0133
Hgb*	g/dl	203	13.6	13.3-13.8	<0.0001
PCV*	%	231	47.0	46.1-47.8	0.0002

With respect to life history differences migratory birds had increased concentrations of pO_2, hematocrit, and hemoglobin as compared to sedentary birds, whereas sodium and ionized calcium concentrations were decreased in migratory birds (Table 5). Samples collected in the fall had relatively increased values for pH, pO_2, sO_2, and lower concentrations of pCO_2 and lactate than those obtained in spring or summer

(Table 6). Additionally, chloride concentrations were higher (P=0.04) in fall than in spring. Ionized calcium concentrations were increased in summer compared to spring and potassium concentrations were relatively decreased during the summer (Table 6). All birds were issued a body condition score (BCS; range from 1-4), with most birds assigned a BCS of 2 or 3. For BCS 3 birds I found an increase in pCO_2 (P=0.0050) but a decrease in pO_2 (P=0.0534), percent sO_2 (P=0.0162), chloride (P=0.0202), and ionized calcium, as compared to BCS 2 birds.

Table 5. Venous blood analytes of passerine birds that differ based on life history strategy (migratory v sedentary) collected from East Foundation properties.

Analyte	**Units**	***N***	**Migratory**	**Sedentary**	***P* value**
pO_2*	mm Hg	225	56.8, (±2.2), 123	51.0, (±1.9), 102	0.0506
Sodium	mmol/L	203	155.2, (±0.4), 109	156.4, (±0.4), 94	0.0370
iCa	mg/dl	169	0.922, (±0.014), 79	0.986, (±0.013), 90	0.0008
Hct*	%	203	40.94, (±0.48), 109	38.84, (±0.53), 94	0.0011
Hgb*	g/dl	203	13.9, (±0.18), 109	13.2, (±0.18), 94	0.0011
Glucose	mg/dl	200	342.9, (±7.8), 107	315.8, (±7.0), 93	0.0119

All values given as mean, (standard error), individuals sampled.

p value represents Students t-test; those denoted * are non-normally distributed where *p* represents Kruskal-Wallis test.

Table 6. Effect of seasonality on venous blood analytes from passerine birds sampled on East Foundation properties.

Analyte	**Units**	***N***	**Fall**	**Spring**	**Summer**	***P* value**
pH*	pH	222	7.747, (7.708-7.788), 24	7.648, (7.631-7.665), 166	7.647, (7.618-7.678), 32	<.0001
pCO_2	mm Hg	225	15.14, (13.36-16.91), 26	21.47, (20.61-22.34), 167	21.34, (19.60-23.09), 32	<.0001
pO_2*	mm Hg	225	77.6, (66.2-89.0), 26	52.2, (49.0-55.3), 167	45.3, (42.2-48.4), 32	<.0001
sO_2*	%	226	97.0, (95.8-98.3), 27	89.6, (88.6-90.7), 167	88.8, (86.8-90.8), 32	<.0001
Lactate*	mmol/L	83	3.89, (3.47-4.30), 26	4.93, (4.24-5.63), 25	4.57, (3.92-5.23), 32	0.008
Potassium*	mmol/L	202	4.37, (4.05-4.68), 28	4.20, (4.07-4.33), 146	3.61, (3.34-3.88), 28	0.0055
iCa	mg/dl	169	-----	0.941, (0.920-0.963), 141	1.047, (1.008-1.086), 28	0.0007

All values given as mean (95% confidence interval) individuals sampled.

iCa *P* value represents Student t-test.

The Bland-Altman plot showed fair agreement between the measurement of packed cell volume by centrifugation and hematocrit by the iSTAT-1®, based on criteria from Rettenmund et al. (2014). Packed cell volume differed from the iSTAT-1® measurement by, on average, seven percent.

Sedentary Passeriformes

When migrants were excluded from the analysis few hematologic parameters differed in samples obtained from coastal versus inland properties in southern Texas. Blood samples obtained from birds on El Sauz Ranch (coastal) had lower decreased concentrations for TCO_2 (P=0.0025) and ionized calcium (P<0.0001), while blood samples of birds captured on the San Antonio Viejo Ranch (inland) had relatively higher glucose concentrations (P=0.0346).

South Texas versus Central Texas Comparisons

To assess whether habitat drives variation in analytes, the data from select sedentary birds sampled in South Texas (East Foundation properties) were analyzed in conjunction with data from a previous study in which birds were sampled from the ecologically and elevational different Edward's Plateau in Central Texas (Fig. 1: Heatley et al. 2013). Sex was determined for 74 individuals: 39 males and 35 females. Within the sedentary species' blood analytes collectively, sex effect was not found for any of the tested analytes or hematology values. Seasonality was evaluated for spring and summer, with fall being excluded based on lack of data (just 3 samples). Electrolytes, blood gases, lactate and hematology parameters were not affected by season. A decrease of pH

(P=0.0359) and base excess (P=0.0159) values occurred in summer compared to spring. Species affected base excess, sO_2, glucose, sodium, and ionized calcium. Notably, many analytes differed based on locality. As compared to Central Texas birds, samples collected in South Texas showed decreased pH values and decreased concentrations of HCO_3, pCO_2, TCO_2, glucose, ionized calcium, hematocrit, hemoglobin. South Texas birds also showed an increased pO_2 and sO_2 (Table 7)

Table 7. Venous blood analyte differences in passerine birds captured in Central Texas and in South Texas.

Analyte	**Units**	***N***	**Central Texas**	**South Texas**	***P*value**
pH	pH	94	7.593, (7.560-7.626), 28	7.672, (7.646-7.698), 66	<0.0001
pCO_2	mm Hg	94	25.11, (23.15-27.08), 28	19.54, (18.19-20.89), 66	<0.0001
pO_2*	mm Hg	93	38.1, (35.7-40.4), 28	51.3, (46.4-56.2), 65	<0.0001
Bicarbonate	mmol/L	94	23.95, (22.69-25.21), 28	21.79, (20.69-22.89), 66	0.024
TCO_2	mmol/L	97	24.7, (23.4-26.0), 28	22.7, (21.8-23.6), 69	0.0261
sO_2*	%	93	81.3, (78.5-84.0), 28	90.2, (88.6-91.8), 65	<0.0001
iCa	mg/dl	65	1.074, (0.977-1.172), 9	0.970, (0.937-1.003), 56	0.0237
Glucose	mg/dl	86	374.8, (350.0-399.6), 28	299.6, (282.9-316.2), 58	<0.0001
Hgb*	g/dl	90	14.2, (13.7-14.6), 31	12.8, (12.4-13.2), 59	<0.0001
Hct*	%	90	41.7, (40.4-42.9), 31	37.6, (36.5-38.8), 59	<0.0001

All values given as mean, (95% confidence interval), individuals sampled.

P value represents Students t-test; those denoted * are non-normally distributed where *P* represents Kruskal-Wallis test

II.4 Discussion

East Foundation Passeriformes

Several other studies have assessed venous blood gases in free living birds using a point of care analyzer. Reviewing Table 3, the analytes reported here are similar to previous literature and indicate healthy values (Paula et al. 2008, Harms and Harms 2012, Heatley et al. 2013, Heatley et al. 2015). Many similar studies have used a temperature correction formula to represent their data. The necessity of performing this temperature correction is controversial (Cowley et al. 2013); I did not obtain body temperature data and were not able to perform this correction.

The measurement method of hematocrit by iSTAT-1® or packed cell volume by centrifugation can be interchangeable because most points fall within the 95% limits of agreement. I attribute the differences that occur to human error when processing and measuring the microhematocrit tubes.

Sex and age had minimal impact on most analytes I assessed in this study. Female birds had increased blood concentrations of ionized calcium compared to males, which is in agreement with previous research (Howard et al. 2004). Ionized calcium plays an important role in many physiological processes, to include eggshell calcification. Therefore, the increased demands for ionized calcium expected during ovulation (de Matos 2008) could explain the relatively increased concentrations I observed in female birds. First year birds exhibited lower total CO_2 than adults possibly

based on a lack of endurance and increased effort to maintain flight in inexperienced juveniles (Heatley et al. 2013).

The increased hematocrit and hemoglobin concentrations found in migratory birds were expected based on reports in other passerine species (Barlein and Totzke 1992, Morton 1994, Piersma and Everaarts 1996). Migration is an energetically taxing activity for birds which increases the need for oxygen, subsequently increasing hematocrit and hemoglobin (Swanson 1990, Barlein and Totzke 1992, Hõrak et al. 1998). The need for increased blood oxygen during migration was also associated with increased oxygen saturation and partial pressures of oxygen (pO_2). Several factors can influence pO_2 values such as cardiac function, tissue metabolic rate, hematocrit, or cellular oxygen use (Swanson 1990, Barlein and Totzke 1992, Heatley et al. 2013). Decreased sodium concentrations might be explained by the birds' water consumption. While at stop over sites or reaching the final destination of their migration (i.e. when they were sampled), birds could be consuming extraordinary amounts of water which dilutes blood plasma and decreases sodium concentrations (Pierce and McWilliams 2004). Lower ionized calcium concentrations from migratory species may stem from the role calcium has in the muscular work of respiration and prolonged flight. Expected glucose concentrations for passerines via the iSTAT-1® have been recorded to be around 300 mg/dL (Fokidis et al. 2011, Heatley et al. 2013), which is similar to glucose concentrations determined in this study**.** During migration, birds must rely on fat reserves for energy. Increases of glucagon facilitate metabolism of fat deposits and increases the amount of blood glucose concentrations (Barlein and Totzke 1992). This

appears to be a seasonal change that occurs before migration and can still be observed even after prolonged flight. This suggest migratory birds captured at the South Texas sites are not exhausting all their nutrients and have adequate glucose reserves. This process might precisely explain the relatively increased glucose concentrations I observed in migratory birds.

BCS of 2 may have experienced greater physical exertion than BCS 3 birds. Decreased BCS score in birds has been associated with smaller pectoralis muscle and fat reserves (Gregory and Robins 1998). The blood oxygen and carbon dioxide interaction values could be indicating an acute tissue hypoxia that reduces pectoral muscle mass and justifies the original body score.

Both Central and South Texas commonly experience extreme weather condition variation during the year. One of these noticeable changes is seen during the spring and summer months**,** where there is a high increase in ambient temperatures as compared to fall months. In the spring and summer months, temperature in South and Central Texas routinely exceed 40°C and 35°C respectively. During this transition to higher temperatures, it takes approximately two weeks of heat exposure and conditioning for birds to regulate their bodies' acid-base homeostasis (Marder 1990). Increased PCO_2, blood lactate concentration, and a more acidic venous blood pH can be associated with higher ambient temperatures and the birds response environmental stress (Levine 1975). Handling stress in this study may have resulted in relative respiratory acidosis of birds sampled during the hotter summer months. Nutrient variability is likely to vary

seasonally. Nutrients from cacti and other woody plants from South Texas contain enough potassium to satisfy the dietary needs for avian activity during the harsh environmental conditions in the summer months (Everitt and Alaniz 1981). Since potassium appears available in the environment and accessible through dietary intake, the relatively lower concentrations of potassium, chloride, ionized calcium in birds from South Texas ranches might indicate relative euhydration/hyperhydration (Heatley et al. 2013). There are multiple methods to assess hydration status such as measuring total solids, pack cell volume, total protein, skin turgor, but these objectives were beyond the scope of this study. However, none of the analytes I recorded were clinically abnormal or lower than expected in a healthy bird.

Throughout the year, Passeriformes may exhibit differing needs of oxygen demands based on changing environmental conditions. During times of the year when ambient temperature is much cooler, increased oxygen need may be based on metabolic processes (shivering) to stay warm. Decreased concentrations of lactate from birds sampled in fall suggest decreased anaerobic metabolic processes (Swanson 1991). Blood samples of birds in fall exhibited no change in hematocrit or hemoglobin which suggests a functional change of oxygen affinity at the tissue level or lack of need in sea level non-migratory species in relatively warm ambient temperatures (Swanson 1990). A mechanism to satisfy the oxygen needs of hypoxic tissue might involve an accumulation of localized hemoglobin acting as a larger molecule and increasing oxygen saturation (Lapennas and Reeves 1983). Birds sampled in fall appear to handle changes in oxygen requirements similar to those previously recorded in other passerines (Swanson 1990).

The differences amongst East Foundation ranches was minimal and may be explained as birds adapting to their specific ecosystem. With one coastal ranch and the other more inland, the changes in electrolytes observed could indicate differing ecosystem acclimation at the same latitude. Overall, the blood gas data suggests the values observed within the sampling of the East Foundation properties, may be indicators of reasonable passerine health in the ecoregion.

Sedentary Passeriformes

Assessment of blood analytes from sedentary birds from the East Foundation and Central Texas failed to demonstrate expected changes in hematocrit and hemoglobin based on sex (Archawaranon 2005, Norte et al. 2009, Heatley et al. 2013). The decrease of pH and base excess in sedentary species sampled during summer months may represent metabolic acidosis, most likely based on the birds' response to handling stress during high ambient temperatures. The results that were seen when combining the Central Texas and South Texas sedentary bird species were similar to those seen previously on and off the Edward's Plateau. Glucose, ionized calcium, pH, hematocrit, and hemoglobin were values that were not changed and may exhibit normal levels for Central Texas (Heatley et al. 2015).

When select sedentary species were tested against each other based on geography (South versus Central Texas), most blood gas and hematology was not different. The significant parameters that were seen are most likely based on differences in dietary need or other physiological adaptations (Heatley et al. 2013). This suggests that sedentary

species from each region have adapted to their ecosystem. Each ecosystem may be host to many unique physiological adaptations by their sedentary bird species. Each locality, though different, harbors healthy bird communities. This data suggests that rather than a single bird species, there is potential to use a community of birds as bioindicators of ecosystem health.

This study was designed to assess the health of birds in South Texas using multiple venous blood analytes and compare them to other ecoregions. I also investigated the differences in blood physiology associated with migration. Migratory birds' venous blood analytes differed from sedentary species, particularly those associated with oxygen transport and capacity. Venous blood analytes of non-migratory passeriformes of most apparent use as bioindicators include lactate, electrolyte concentrations and blood gases. Sedentary species are advantageous as bioindicators as they directly reflect the year-round adaptations needed to survive in their ecosystem. As many analytes I studied appear similar in the sedentary species from Central Texas and South Texas, I suggest that sedentary birds, on a macro level, are similar and adapted to the local environmental stresses of their habitat. I find on a macro level of ecosystems; sedentary birds differ greatly in hematology from both locations.

Although the iSTAT-1® was a useful field tool to assess venous blood analytes from birds at the time of sampling, it does have limitations. Overfill, underfill and other reasons for cartridge failure were not uncommon, and has been documented in other studies. I observed 32 percent failure rate which is higher than previously recorded

(Rettenmund et al. 2014). The most common failure I observed dealt with poor contact between the analyzer and cartridge, dirty contact pads on cartridges or dirty connector in the analyzer, and temperature (Table 8). The poor contact and dirty components of the cartridge and analyzer are likely associated with sand and other debris in the air, which are common to South Texas habitats. Further, the analyzer is sensitive to ambient temperature and would fail if not maintained within 26-30°C, allowing for a limited working window for my studies in South Texas where temperatures over 40°C are common. Based on my findings I suggest that future research areas of passerine birds and ecosystem health would be best focused on sedentary avian communities to assess local ecosystem health and develop a more comprehensive baseline of hematology parameters.

CHAPTER III

ASSESSMENT OF AVIAN CHEWING LICE (INSECTA: PHTHIRAPTERA) FROM SOUTH TEXAS BIRDS

III.1 Introduction

Chewing lice (Insecta: Phthiraptera) are small, dorsoventrally flattened ectoparasites found on numerous avian species and some mammalian species (Johnson and Clayton 2003, Price et al. 2003). Chewing lice are placed into two suborders: Amblycera and Ischnocera, both of which feed on feathers and dead skin while some amblycerans also feed on blood or secretions (Waterhouse 1953). Each suborder is characterized by distinctive morphologies, and partition the body based on feeding strategy and host preening avoidance (Johnson et al. 2012). These are obligate parasites that spend their entire life cycle on their host (Marshal 1981, Catanach and Johnson 2015). However, chewing lice have two main methods of transport among hosts: vertical transmission, by direct contact with another individual, and horizontal transmission via phoresis, described as "hitchhiking" on hippoboscid flies (Keirans 1975, Johnson and Clayton 2003, Bartlow et al. 2016). Although phoresis is not common, it is more frequently used by ischnoceran lice than amblyceran lice (Johnson and Clayton 2003, Bartlow et al. 2016). This method of transmission allows lice to escape competition from other lice on the same host and potentially encounter a novel host (Harbison et al. 2009). One louse species or even an entire louse genus may be either host specific or a host generalist. Host generalist lice have the potential to parasitize multiple host species over

a large geographic area, which can lead to many interesting questions about the distributions and host associations of these lice.

Several studies have investigated the louse-host associations from select avian species North America. For example, ectoparasites from Wild turkey (*Meleagris gallopavo*) in California (Lane et al. 2006), community structure of lice on the Western scrub jay (*Aphelocoma californica*) in the Southwestern United States (Bush et al. 2009), phylogenetic relationships of lice on *Catharus* thrushes in Illinois (Bueter et al. 2009), and louse-host associations of select dove species in Manitoba, Canada (Galloway and Palma 2008) have been examined. With the exception of a few studies examining populations genetics, parasitism rates, and host switching of lice parasitizing doves (Johnson et al. 2002a, Moyer et al. 2002, Bush and Clayton 2006), there have been few studies that have examined louse relationships in Texas. Texas, not unlike Mexico and Central America, is an understudied area with respect to louse-host associations. Several studies have been examined co-evolutionary processes and speciation of lice parasitizing birds in Mexico, but limited to doves species (Johnson et al. 2002a, Clayton and Johnson 2003, Malenke et al. 2003) . For example, only a few studies from Costa Rica (Lindell et al. 2002, Sychra et al. 2010, Kounek et al. 2011) and Panama (Price and Johnson 2009) exist, that provide descriptions of new louse species. Many questions remain unanswered about biogeography, louse distributions and genetic relationships of lice across a significant portion of the New World. The objectives of this study were to assess louse diversity, louse-host associations, and to construct molecular phylogenies of lice to determine relationships of lineages of lice collected from birds surveyed in South Texas

to those described elsewhere. To accomplish this, I attempted collection of lice from approximately 450 birds during a biodiversity assessment of several private ranches in \South Texas. These birds represent a diverse subset of species from the area, and include both sedentary and migratory species. I hypothesize that I will find several novel louse-host associations and unique genetic lineages that could potentially represent new species of lice, based on lack of investigation in this area of Texas. Further, microclimate differences related to temperature and humidity are thought to affect louse parasitism rates (Moyer et al. 2002). Therefore, I hypothesize there will be different levels of parasitism between sampling localities, given the different microclimates associated with each.

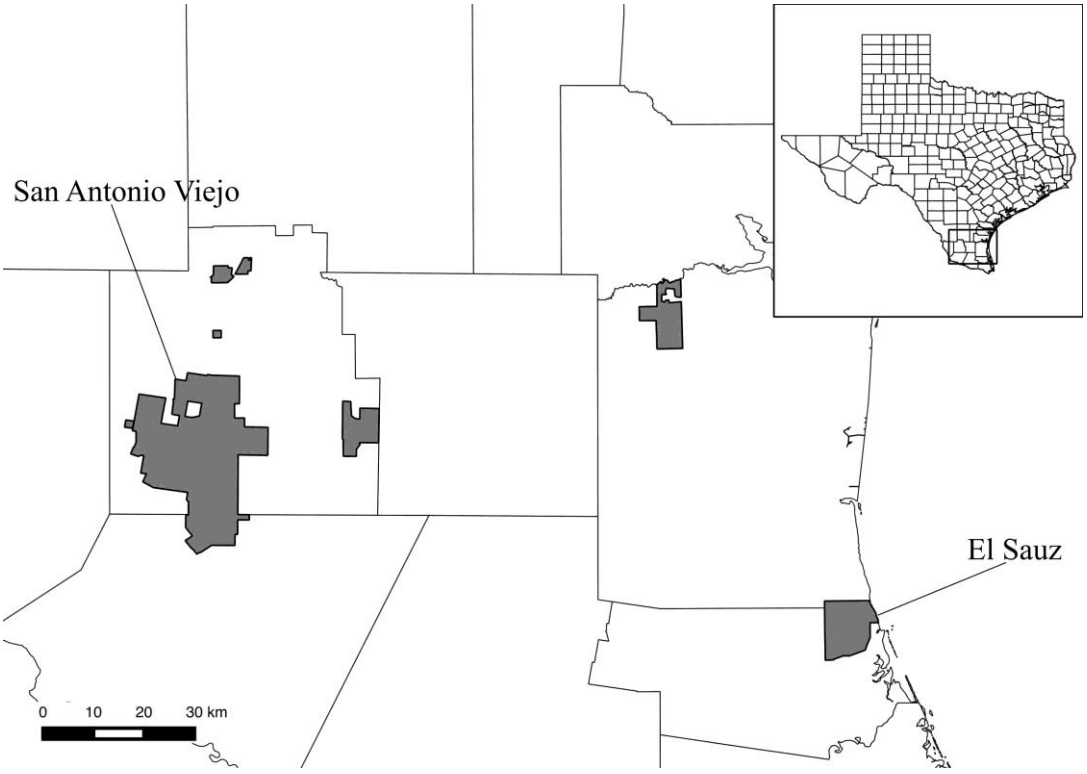
III.2 Materials and Methods

Louse Sampling and Examination

Lice were collected from birds intermittently between 2013-2015 on East Foundation ranches located in southern Texas (Figure 2). The East Foundation properties occupy approximately 88221 hectares of land in Jim Hogg, Starr, Willacy, and Kenedy Counties. For this study, samples were obtained from San Antonio Viejo Ranch (60179 hectares, located inland in Jim Hogg and Starr Counties) and El Sauz Ranch (11082 hectares, located coastally in Kenedy and Willacy Counties: Figure 2). Four biomes are represented among the two ranches: grassland, shrubland, woodland, and wetland. At San Antonio Viejo arrowfeather threeawn (*Aristida purpurescens*) dominates the grassland biome, hogplum (*Colubrina texensis*) and blackbrush (*Acacia*

rigidula) dominate shrubland, and mesquite (*Prosopis* sp.) dominates the woodland biome. At El Sauz gulf cordgrass (*Spartina spartinae*) dominates the grassland biome, lotebush (*Ziziphus obtusifolia*) dominates shrubland, mesquite (*Prosopis* sp.) dominates woodland, and marshhay cordgrass (*Spartina patens*) dominates the wetland biome. Birds were captured in the field via mist net. Some birds were sacrificed, and prepared as voucher specimens to be deposited at the Biodiversity Research and Teaching Collections located at Texas A&M University, while other birds were released. In both instances, birds were ruffled for lice. Ruffling is a method of louse collection in which a toothbrush is used to thoroughly brush the feathers over collecting paper (Clayton et al. 1992, Clayton and Drown 2001). For birds that were sacrificed, prior to ruffling, each was put into its' own individual bag with a cotton ball dipped in ethyl acetate, which acts as fumigation to release the lice from their host (Clayton and Drown 2001). Those birds not collected were dusted with flea powder (Zodiac®, Schaumburg, IL, USA) to aid in releasing ectoparasites from their plumage and ruffled prior to release (Walther and Clayton 1997). After lice were collected from a bird, they were stored either dry or in ethanol, at -80°C. Lice were identified to genus (when possible) using published dichotomous keys (Price et al., 2003). Digital vouchers were created before DNA extraction for each louse by using an Olympus SZX10 microscope, Intralux 6000 light source and SPOT v4.6 software (2009 Diagnostic Instruments).

Figure 2. Map of Texas with emphasis of South Texas and all East Foundation properties, sampling locations of San Antonio Viejo and El Sauz.



DNA Extraction, Amplification, and Sequencing

DNA extraction was performed using the E.Z.N.A® Tissue DNA Kit (Omega Bio-Tek Inc., Norcross, Georgia, USA) following louse specific protocols (Cruickshank et al. 2001). Before the extraction process began, the lice were washed in 1X phosphate-buffered saline solution to remove possible contaminants from the specimen. After the wash, the louse specimen's abdomen was sliced using a sterile surgical blade. The manufacturer's extraction protocol was followed throughout the process, except the total

DNA elution volume was lowered to 70 µL. Upon completion of DNA extraction, each louse exoskeleton was preserved in ethanol and stored at -80°C to be retained as a voucher specimen.

Polymerase chain reaction (PCR) was performed on all lice to amplify a portion of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene, using the primers L6625 and H7005 (Hafner et al. 1994). Each PCR reaction consisted of 25 µL solution containing 10 µL Emerald Master Mix (Takara Bio Inc.), 12 µL water, 1 µL forward primer (10 µM concentration), 1 µL reverse primer (10 µM concentration), and 1 µL DNA. The amplification protocol started with 5 minutes of denaturation at 94°C, followed by 40 cycles of denaturation at 94°C for 45 seconds, annealing at 44°C for 55 seconds, extension at 72°C for 1 minutes and final elongation at 72°C for 5 minutes (Light et al. 2016). The amplified PCR products were electrophoresed with 2 µL of 100bp Promega DNA ladder (Applied Biosystems) on an agarose gel to determine PCR success.

Successfully amplified PCR products were purified using ExoSAP-IT (United States Biochemical Corporation, Cleveland, Ohio, USA). The cleaned PCR products were sent to Beckman-Coulter Genomics (Danvers, Maryland, USA) or Yale University (New Haven, Connecticut, USA) for sequencing in both forward and reverse direction using the PCR primers. Forward and reverse strands were combined and sequences were edited using Sequencher v.4.2.2 (Gene Codes Corporation, Ann Arbor, Michigan, USA), aligned using MUSCLE (Edgar 2004), verified by eye.

Data Analysis

Each louse sequence was compared to published sequences located on GenBank by using the Basic Local Alignment Search Tool (BLAST). Closely related sequences resulting from the BLAST search were added to the data set for phylogenetic analyses (Table 8). Phylogenetic analysis occurred in two separate analyses, one for the suborder Amblycera and one for the suborder Ischnocera. Each analysis used two specimens from the louse suborder Anoplura as outgroups (*Haematopinus eurysternus* and *Fahrenholzia zacatecae*; GenBank numbers HM171422 and HM171445, respectively). ParitionFinder v1.1.1 (Lanfear et al. 2012) was used with the Bayesian information criterion to select the appropriate number of partitions and model of evolution at each partition. For analyses of both Amblycera and Ischnocera, 3 partitions were identified as HKY+G, GRT+I+G, and GTR+I+G for the first, second, and third codon positions respectively. Bayesian phylogenetic analyses were performed using MrBayes 3.2 (Ronquist et al. 2012). Bayesian analysis for each suborder consisted of 2 simultaneous runs for 10 million generations with four heated chains (Ronquist and Huelsenbeck 2003) and sampling occurred every 2000 generations with a 25% burn-in. Each independent run was assessed for convergence using Tracer v1.6 (Rambaut et al. 2014). A 50% majority rule consensus tree was constructed in FigTree v1.4.2 (Rambaut 2014) and the percentage of samples recovered in a particular clade was assumed to represent that clade's posterior probability (Huelsenbeck and Ronquist 2001). Average uncorrected *p*-distances were calculated in PAUP* v 4.0 (Swofford 2002) to examine genetic difference among taxa.

Table 8. Louse GenBank sequences that were included in the Bayesian analysis with South Texas sequences. Host species and collection locality data were recorded if known.

Louse species	Louse Host	Collection Locatility	GenBank Accesion No.
Amblycera Taxa			
Hohorstiella passerinae	*Columbina passerina*	USA	AF545716
Menacanthus camelinus	*Lanius collurio*	Costa Rica	KJ730544
Menacanthus eurysternus	*Tanagera dowii*	Costa Rica	KJ730661
Menacanthus eurysternus	*Turdus grayi*	Costa Rica	KJ730647
Menacanthus sp.	*Attila spadiceus*	Costa Rica	AF545726
Myrsidea cruickshanki	*Tanagera dowii*	Panama	GQ454449
Myrsidea fusca	*Ramphocelus passerinii*	Panama	FJ171267
Myrsidea incerta	*Catharus minimus*	USA (Illinois)	FJ171270
Myrsidea incerta	*Catharus ustulatus*	USA (Illinois)	FJ171268
Myrsidea nesomimi	*Mimus parvulus*	Ecuador	JF734299
Myrsidea sp.	*Hylocichla mustelina*	USA (Illinois)	FJ171284
Myrsidea sp.	*Psilorhinus morio*	Mexico	FJ171281
Myrsidea sp.	*Seiurus aurocapilla*	USA (Illinois)	FJ171289
Myrsidea sp.	*Troglodytes aedon*	Panama	KF614514
Myrsidea simplex	*Catharus fuscater*	Panama	FJ171276
Myrsidea textoris	*Ploceus velatus*	South Africa	KF768814
Ricinus mugimaki	*Cossypha dichroa*	South Africa	KF768816
Ricinus sp.	*Cyanocompsa parellina*		AF385014
Ischnocera Taxa			
Anaticola crassicornis	*Anas crecca*		KT587831

Table 8. Continued

Louse species	**Louse Host**	**Collection Locatility**	**GenBank Accesion No.**
Brueelia anamariae	*Troglodytes aedon*	USA (Illinois)	FJ171220
Brueelia antiqua	*Hylocichla mustelina*	USA (Illinois)	FJ171222
Brueelia brunneinucha	*Dumatella carolinensis*	USA (Illinois)	FJ171223
Brueelia cedrorum	*Bombycilla cedrorum*	USA	KT892073
Brueelia deficiens	*Aphelocoma califonica*	USA	KT892285
Brueelia dorsale	*Toxostoma rufum*	USA (Illinois)	FJ171224
Brueelia iliaci	*Turdus migratorius*	USA	KT892078
Brueelia ornatissima	*Agelaius phoeniceus*	USA	KT892090
Brueelia ornatissima	*Molothrus ater*	USA	KT892087
Brueelia pallidula	*Pheucticus ludovianus*	USA	KT892089
Brueelia picturata	*Sturnella sp.*	USA	KT892320
Brueelia sp.	*Cardellina canadensis*	USA	KT892275
Brueelia sp.	*Carduelis pinus*	USA	KT892116
Brueelia sp.	*Sialia currucoides*	USA	KT892319
Brueelia sp.	*Dolichonyx oryzivorus*	USA (Illinois)	FJ171230
Brueelia sp	*Melanerpes cruentatus*		KT892195
Brueelia sp.	*Melanerpes erythrocephalus*	USA	KT892330
Brueelia sp.	*Melospia lincolnii*	USA	KT892201
Brueelia sp.	*Melanerpes carolinus*	USA	KT892329
Brueelia vulgata	*Zonotrichia albicollis*	USA (Illinois)	FJ171234
Brueelia xanthocephali	*Xanthocephalus xanthocephalus*	USA	KT892325
Columbicola passerinae	*Columbina passerina*	Texas	AF414730
Columbicola passerinae	*Columbina passerina*	Mexico	AF414728
Columbicola macrourae	*Zenaida macroura*	USA	FJ656460

Table 8. continued

Louse species	**Louse Host**	**Collection Locatility**	**GenBank Accesion No.**
Cotingacola stotzi	*Querula purpurata*	Brazil	JN662444
Cuculoecus sp.	*Cerococcyx olivinus*	Africa	KU187329
Cummingsiella longirostricola	*Numernius americanus*	USA	JN900159
Degeeriella fulva	*Buteo regalis*		AF444861
Goinodes sp.	*Callipepla californica*	USA	AF545708
Lunaceps actophilus	*Calidris alba*	USA (Florida)	JN900147
Penenirmus auritus	*Mecopicos poertae*	Africa	AF356701
Penenirmus sp.	*Psaltiparus minimus*	USA (Utah)	AY149409
Philopterus sp.	*Motmotus momota*	Brazil	AF356716
Philopterus sp.	*Spizella pusilla*	USA	KF841436
Picicola faucetti	*Galbula cyanicollis*	Brazil	EF101571
Picicola galbulica	*Galbula tombacea*	Peru	EF101575
Picicola snodgrassi	*Melanerpes carolinus*	USA	AF444868
Picicola striata	*Monasa nigrifrons*	Bolivia	EF101577
Picicola oneilli	Notharchus macrorhynchos	Peru	EF101573
Trogoninirmus sp.	*Trogon melanocephalus*	Mexico	AF444876
Outgroup Taxa			
Fahrenholzia zacatecae	*Chaetodipus eremicus*		HM171445
Haematopinus eurysternus	*Bos sp.*		HM171422

III.3 Results

A total of 446 bird specimens from 2 sampling localities in South Texas were examined for ectoparasites (data available upon request). The host specimens examined were taxonomically diverse, representing a total of 156 species, 40 families, and 16 orders. Of these taxonomic categories, 28.8%, 50.0%, and 50.0%, were parasitized by ectoparasites, respectively. There were 164 birds collected from San Antonio Viejo ranch, of which 14.0% were parasitized, and 282 birds collected from El Sauz, of which 16.7% parasitized (Table 9). In total, 70 birds examined were parasitized by lice (15.5%) and 64 host associations were recorded, with 31 of these associations being new to science (Table 10). There were multiple instances (13) where a host species was parasitized by more than one species of louse (Table 10). Lice from both suborders were found to be parasitizing the same host specimen 5 times and parasitizing the same host species 8 times. It is possible there could be additional host associations but some louse specimens were excluded due to problems identifying them. These problems include poor reference material or poor condition of specimens.

Table 9. Species of birds that were parasitized by chewing lice in South Texas from the two sampling localities: El Sauz and San Antonio Viejo ranches during 2013-2015, where (n) is total number of host species examined.

El Sauz	**San Antonio Viejo**
Species (n)	**Species (n)**
Northern shoveler (*Anas clypeata*)	Red-winged blackbird (*Agelaius phoeniceus*) (4)
Black-crested titmouse (*Baeolophus atricristatus*)	Verdin (*Auriparus flaviceps*)
Sanderling (*Calidris alba*)	Cactus wren (*Campylorhynchus brunneicapillus*)
Northern cardinal (*Cardinalis cardinalis*) (6)	Northern cardinal (*Cardinalis cardinalis*)
Lark sparrow (*Chondestes grammacus*)	Yellow-billed cuckoo (*Coccyzus americanus*)
Northern bobwhite (*Colinus virginianus*)	American Kestrel (*Falco sparverius*)
Common ground dove (*Columbina passerina*)	Audubon's oriole (*Icterus graduacauda*)
Green jay (*Cyanocorax yncas*) (2)	Tennessee warbler (*Oreothlypis peregrina*)
Horned lark (*Eremophila alpestris*)	Golden-fronted woodpecker (*Melanerpes aurifrons*)
Hooded oriole (*Icterus cucullatus*)	Northern mockingbird (*Mimus polyglottos*)
Lincoln's sparrow (*Melospiza lincolnii*)	Bronzed cowbird (*Molothrus aeneaus*)

Table 9. Continued

El Sauz	**San Antonio Viejo**
Species (n)	**Species (n)**
Black and white warbler (*Mniotilta varia*)	Varied bunting (*Passerina versicolor*)
Bronzed cowbird (*Molothrus aeneaus*) (2)	Cassin's sparrow (*Pecaea cassini*)
Brown-headed cowbird (*Molothrus ater*)	Vermillion flycatcher (*Pyrocephalus rubinus*)
Brown-crested flycatcher (*Myiarchus tyrannulus*)	Eastern meadowlark (*Sturnella magna*)
Long-billed curlew (*Numenius americanus*)	Curve-billed thrasher (*Toxostoma curvirostre*) (2)
Harris's hawk (*Parabuteo unicinctus*)	Long-billed thrasher (*Toxostoma longirostre*)
Louisiana waterthrush (*Parkesia motacilla*) (2)	Bell's vireo (*Vireo bellii*)
Northern waterthrush (*Parkesia noeboracensis*)	
Summer tanager (*Piranga rubra*) (2)	
Northern parula (*Setophaga americana*)	
Black-throated green warbler (*Setophaga virens*)	
Lesser goldfinch (*Spinus psaltria*) (2)	

Table 9. Continued

El Sauz	**San Antonio Viejo**
Species (n)	**Species (n)**
Long-billed thrasher (*Toxostoma longirostre*) (2)	
House wren (*Troglodytes aedon*)	
Scissor-tailed flycatcher (*Tyrannus forficatus*)	
Golden-winged warbler (*Vermivora chrysoptera*)	
White-eyed vireo (*Vireo griseus*)	
White-tailed hawk (*Geranoaetus albicaudatus*)	
Mourning dove (*Zenaida macroura*)	

Table 10. Louse-host associations of birds from South Texas during 2013-2015 where (n) is number of host species examined. Daggers (†) indicate new host associations and asteriks (*) indicate species with no molecular data.

Host Family	Host Species (n)	Louse Suborder	Louse Family	Louse sample
Host Order: Accipitriformes				
Accipitridae	*Parabuteo unicinctus*	Ischnocera	Philopteridae	*Degeeriella fulva* †
	Geranoaetus albicaudatus	Ischnocera	Philopteridae	*Philopterus* sp. † *
Host Order: Anseriformes				
Anatidae	*Anas clypeata*	Ischnocera	Philopteridae	*Anaticola crassicornis*
		Ischnocera	Philopteridae	*Anatoecus* sp.
Host Order: Charadriformes				
Scolopacidae	*Calidris alba*	Ischnocera	Philopteridae	*Lunaceps actophilus*
	Numenius americanus	Ischnocera	Philopteridae	*Cummingsiella longirostricola*
Host Order: Columbiformes				
Columbidae	*Columbina passerina*	Ischnocera	Philopteridae	*Columbicola passerinae*
		Amblycera	Menopidae	*Hohorstiella passerinae*
	Zenaida asiatica	Ischnocera	Philopteridae	*Columbicola macrourae*

Table 10. Continued

Host Family	Host Species (n)	Louse Suborder	Louse Family	Louse Sample
Odontophoridae	*Colinus virginianus*	Ischnocera	Goniodidae	*Goniodes* sp. *
Host Order: Cuculiformes				
Cuculidae	*Coccyzus americanus*	Ischnocera	Philopteridae	*Cuculoecus* sp. *
Host Order: Falconiformes				
Falconidae	*Falco sparverius*	Ischnocera	Philopteridae	*Degeeriella fulva*
Host Order: Passeriformes				
Alaudidae	*Eremophila alprestris*	Amblycera	Menopidae	*Menacanthus* sp. †*
Cardinalidae	*Cardinalis cardinalis (7)*	Amblycera	Menopidae	*Menacanthus eurysternus*
		Amblycera	Menopidae	*Myrsidea* sp. †
		Ischnocera	Menopidae	*Brueelia pallidula* †
	Passerina ciris	Amblycera	Menopidae	*Myrsidea* sp. † *
		Ischnocera	Philopteridae	*Philopterus* sp. † *
	Passerina versicolor	Amblycera	Ricinidae	*Ricinus* sp.
	Piranga rubra (2)	Ischnocera	Philopteridae	*Philopterus* sp. † *
		Amblycera	Menopidae	*Myrsidea* sp. † *
Corvidae	*Cyanocorax yncas (2)*	Amblycera	Menopidae	*Myrsidea* sp. † *
		Amblycera	Menopidae	*Menacanthus* sp.
		Ischnocera	Philopteridae	*Philopterus* sp. † *

Table 10. Continued

Host Family	Host Species (n)	Louse Suborder	Louse Family	Louse Sample
Emberizidae	*Chondestes grammacus*	Amblycera	Menopidae	*Myrsidea* sp. † *
	Melospiza lincolnii	Ischnocera	Philopteridae	*Brueelia* sp.
	Pecaea cassinii	Ischnocera	Philopteridae	*Philopterus* sp. † *
Fringillidae	*Spinus psaltria (2)*	Ischnocera	Philopteridae	*Philopterus* sp. † *
		Amblycera	Ricinidae	*Ricinus* sp. *
Iceteridae	*Agelaius phoeniceus (4)*	Ischnocera	Philopteridae	*Brueelia xanthocephali*
		Amblycera	Menopidae	*Myrsidea* sp. † *
		Amblycera	Menopidae	*Myrsidea* sp. † *
		Ischnocera	Philopteridae	*Philopterus* sp. *
	Icterus cucullatus	Amblycera	Menopidae	*Menacanthus* sp. †
		Ischnocera	Philopteridae	*Philopterus* sp. † *
	Icterus graduacauda	Ischnocera	Philopteridae	*Brueelia vulgata*
	Molothrus aeneaus (3)	Ischnocera	Philopteridae	*Brueelia xanthocephali*
	Molothrus ater (2)	Ischnocera	Philopteridae	*Brueelia xanthocephali*
		Amblycera	Menopidae	*Myrsidea* sp.
	Sturnella magna	Ischnocera	Philopteridae	*Brueelia picturata*
Mimidae	*Mimus polyglottos (6)*	Ischnocera	Philopteridae	*Brueelia brunneinucha*
		Amblycera	Menopidae	*Myrsidea* sp. † *

Table 10. Continued

Host Family	Host Species (n)	Louse Suborder	Louse Family	Louse Sample
		Ischnocera	Philopteridae	*Philopterus* sp. *
	Toxostoma curvirostre	Ischnocera	Philopteridae	*Brueelia dorsale*
	Toxostoma longirostre (5)	Ischnocera	Philopteridae	*Brueelia dorsale* †
		Ischnocera	Philopteridae	*Brueelia brunneinucha* †
		Ischnocera	Philopteridae	*Philopterus* sp. † *
Paridae	*Baeolophus atricristatus*	Amblycera	Menopidae	*Myrsidea* sp. † *
Parulidae	*Mniotilta varia*	Ischnocera	Philopteridae	*Philopterus* sp. † *
	Oreothlypis peregrina	Amblycera	Ricinidae	*Ricinus* sp. *
	Parkesia noveboracensis	Amblycera	Ricinidae	*Ricinus* sp. *
	Parkesia motacilla (2)	Amblycera	Menopidae	*Myrsidea* sp. *
	Setophaga americana	Amblycera	Menopidae	*Menacanthus* sp. † *
	Setophaga virens	Amblycera	Menopidae	*Myrsidea* sp. †
	Vermivora chrysoptera	Amblycera	Ricinidae	*Ricinus* sp. † *
Remizidae	*Auriparus flaviceps*	Ischnocera	Philopteridae	*Brueelia* sp. *
Troglodytidae	*Campylorhynchus brunneicapillus*	Ischnocera	Philopteridae	*Brueelia dorsale* †
	Troglodytes aedon	Amblycera	Menopidae	*Myrsidea* sp.
Tyrannidae	*Myiarchus tyrannulus*	Amblycera	Menopidae	*Menacanthus* sp. † *

Table 10. Continued

Host Family	Host Species (n)	Louse Suborder	Louse Family	Louse Sample
	Pyrocephalus rubinus	Amblycera	Ricinidae	*Ricinus* sp.
	Tyrannus forficatus	Ischnocera	Philopteridae	*Picicola* sp. *
Vireonidae	*Vireo griseus*	Ischnocera	Philopteridae	*Brueelia dorsale* †
	Vireo bellii	Ischnocera	Philopteridae	*Brueelia* sp. † *
Host Order: Piciformes				
Picidae	*Melanerpes aurifrons*	Ischnocera	Philopteridae	*Penenirmus* sp. *
		Ischnocera	Philopteridae	*Picicola snodgrassi*

There were a total of 150 louse specimens included in the phylogenetic analysis, 81 specimens (34 Amblycera and 47 Ischnocera) from South Texas and 69 (20 Amblycera and 49 Ischnocera) from GenBank (Figures 2 and 3, Table 2). There were high average pairwise sequence divergences (uncorrected *p*-distances) within each suborder. Across amblyceran taxa the average uncorrected *p*-distance was 23.3% with a range of 0-35.7%. Across ischnoceran taxa the average uncorrected *p*-distance was 26.1% with a range of 0-37.3%. From this study, there were 27 unique genetic lineages (15 from Ischnocera and 12 from Amblycera) being approximately 15% genetically divergent (uncorrected *p*-distance) from their closest relative (Figures 3 and 4).

Phylogenetic analysis of the Amblyceran taxa resulted in 3 clades with strong Bayesian posterior probability (PP) support; each clade circumscribes a major louse genus (Figure 3). *Myrsidea* had the strongest support (PP=1), and the *Ricinus* and *Menacanthus* were strongly supported as well (PP=0.99). Support for relationships within each clade varied. In *Menacanthus* and *Ricinus*, more relationships were strongly supported. However, most relationships within *Myrsidea* were poorly supported effectively resulting in a major polytomy within this genus. The analysis also included the amblyceran genus *Hohorstiella*, which was placed as sister *Menacanthus*. However, this relationship was poorly supported (PP=0.73).

Figure 3. Phylogeny of amblyceran taxa from South Texas constructed using Bayesian analysis. Posterior probabilities ≥0.90 are shown and sequences grayed out are from GenBank. Unique genetic lineages are denoted with asteriks (*).

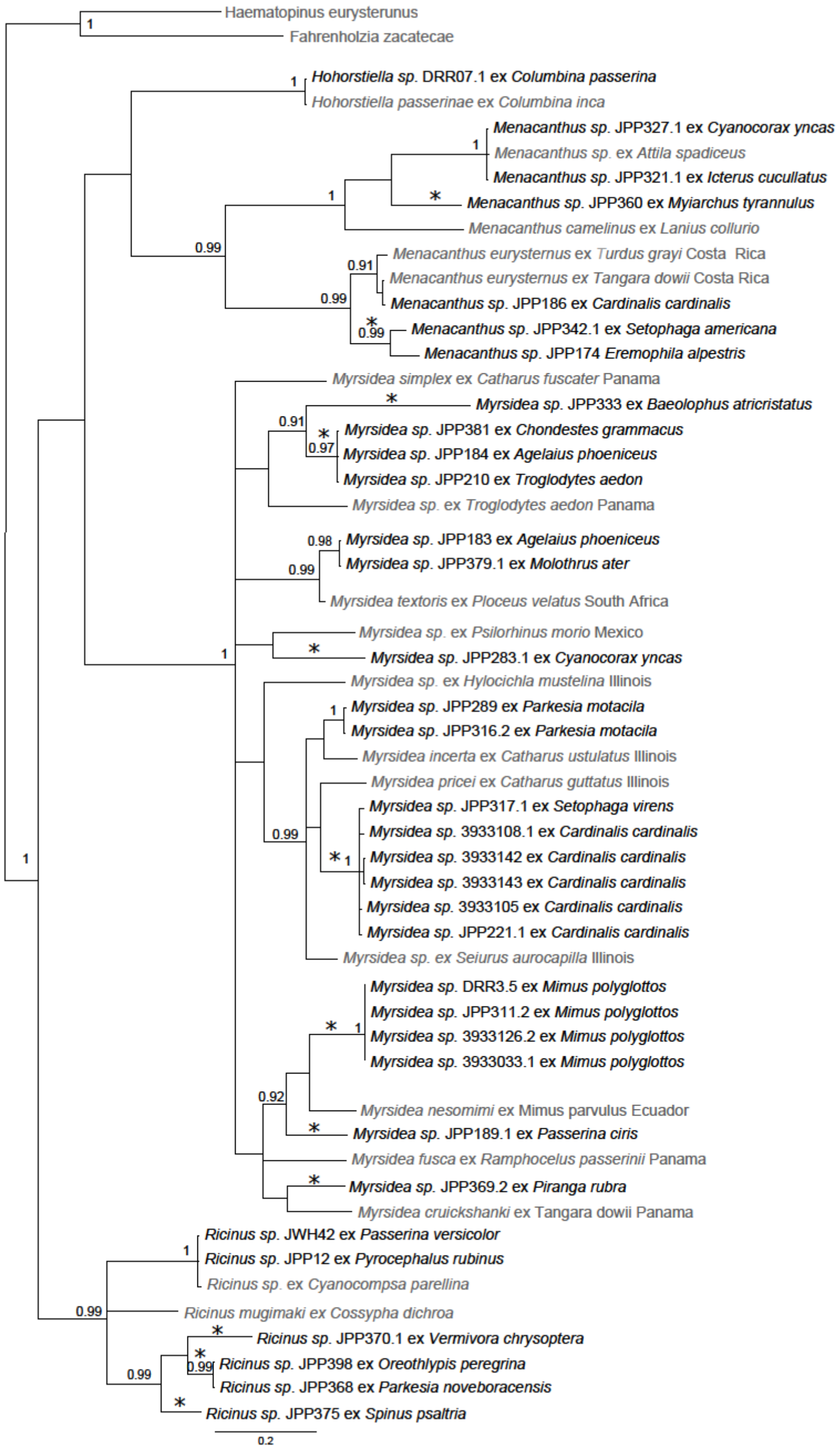
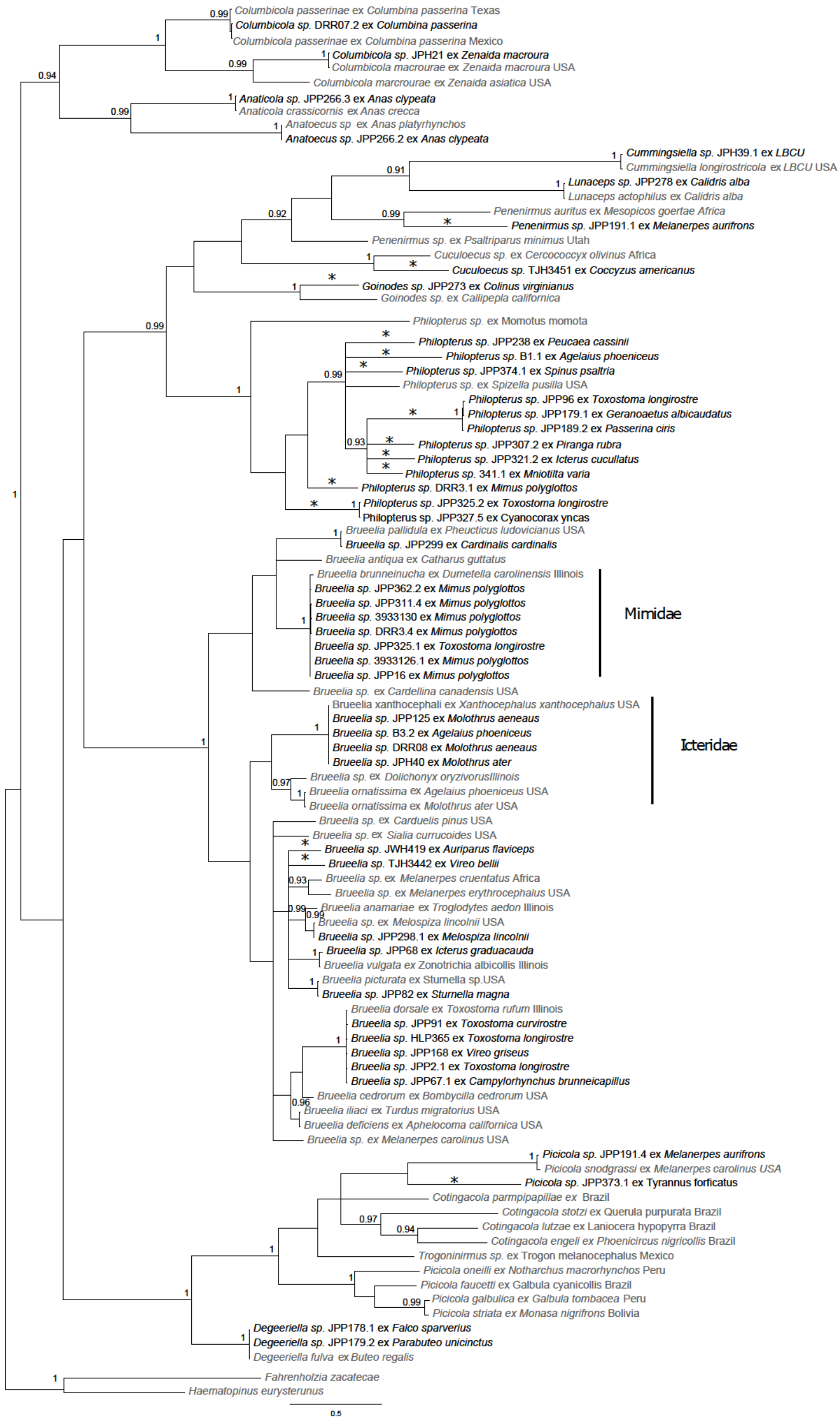

Haematopinus eurysternus
Fahrenholzia zacatecae
Hohorstiella sp. DRR07.1 ex Columbina passerina
Hohorstiella passerinae ex Columbina inca
Menacanthus sp. JPP327.1 ex Cyanocorax yncas
Menacanthus sp. ex Attila spadiceus
Menacanthus sp. JPP321.1 ex Icterus cucullatus
Menacanthus sp. JPP360 ex Myiarchus tyrannulus
Menacanthus camelinus ex Lanius collurio
Menacanthus eurysternus ex Turdus grayi Costa Rica
Menacanthus eurysternus ex Tangara dowii Costa Rica
Menacanthus sp. JPP186 ex Cardinalis cardinalis
Menacanthus sp. JPP342.1 ex Setophaga americana
Menacanthus sp. JPP174 Eremophila alpestris
Myrsidea simplex ex Catharus fuscater Panama
Myrsidea sp. JPP333 ex Baeolophus atricristatus
Myrsidea sp. JPP381 ex Chondestes grammacus
Myrsidea sp. JPP184 ex Agelaius phoeniceus
Myrsidea sp. JPP210 ex Troglodytes aedon
Myrsidea sp. ex Troglodytes aedon Panama
Myrsidea sp. JPP183 ex Agelaius phoeniceus
Myrsidea sp. JPP379.1 ex Molothrus ater
Myrsidea textoris ex Ploceus velatus South Africa
Myrsidea sp. ex Psilorhinus morio Mexico
Myrsidea sp. JPP283.1 ex Cyanocorax yncas
Myrsidea sp. ex Hylocichla mustelina Illinois
Myrsidea sp. JPP289 ex Parkesia motacila
Myrsidea sp. JPP316.2 ex Parkesia motacila
Myrsidea incerta ex Catharus ustulatus Illinois
Myrsidea pricei ex Catharus guttatus Illinois
Myrsidea sp. JPP317.1 ex Setophaga virens
Myrsidea sp. 3933108.1 ex Cardinalis cardinalis
Myrsidea sp. 3933142 ex Cardinalis cardinalis
Myrsidea sp. 3933143 ex Cardinalis cardinalis
Myrsidea sp. 3933105 ex Cardinalis cardinalis
Myrsidea sp. JPP221.1 ex Cardinalis cardinalis
Myrsidea sp. ex Seiurus aurocapilla Illinois
Myrsidea sp. DRR3.5 ex Mimus polyglottos
Myrsidea sp. JPP311.2 ex Mimus polyglottos
Myrsidea sp. 3933126.2 ex Mimus polyglottos
Myrsidea sp. 3933033.1 ex Mimus polyglottos
Myrsidea nesomimi ex Mimus parvulus Ecuador
Myrsidea sp. JPP189.1 ex Passerina ciris
Myrsidea fusca ex Ramphocelus passerinii Panama
Myrsidea sp. JPP369.2 ex Piranga rubra
Myrsidea cruickshanki ex Tangara dowii Panama
Ricinus sp. JWH42 ex Passerina versicolor
Ricinus sp. JPP12 ex Pyrocephalus rubinus
Ricinus sp. ex Cyanocompsa parellina
Ricinus mugimaki ex Cossypha dichroa
Ricinus sp. JPP370.1 ex Vermivora chrysoptera
Ricinus sp. JPP398 ex Oreothlypis peregrina
Ricinus sp. JPP368 ex Parkesia noveboracensis
Ricinus sp. JPP375 ex Spinus psaltria
0.2

Figure 4. Phylogeny of ischnoceran taxa from South Texas constructed using Bayesian analysis. Posterior probabilities ≥0.90 are shown and sequences grayed out are from GenBank. Unique genetic lineages are denoted with asteriks (*).


Columbicola passerinae ex Columbina passerina Texas
Columbicola sp. DRR07.2 ex Columbina passerina
Columbicola passerinae ex Columbina passerina Mexico
Columbicola sp. JPH21 ex Zenaida macroura
Columbicola macrourae ex Zenaida macroura USA
Columbicola marcrourae ex Zenaida asiatica USA
Anaticola sp. JPP266.3 ex Anas clypeata
Anaticola crassicornis ex Anas crecca
Anatoecus sp ex Anas platyrhynchos
Anatoecus sp. JPP266.2 ex Anas clypeata
Cummingsiella sp. JPH39.1 ex LBCU
Cummingsiella longirostricola ex LBCU USA
Lunaceps sp. JPP278 ex Calidris alba
Lunaceps actophilus ex Calidris alba
Penenirmus auritus ex Mesopicos goertae Africa
Penenirmus sp. JPP191.1 ex Melanerpes aurifrons
Penenirmus sp. ex Psaltriparus minimus Utah
Cuculoecus sp. ex Cercococcyx olivinus Africa
Cuculoecus sp. TJH3451 ex Coccyzus americanus
Goinodes sp. JPP273 ex Colinus virginianus
Goinodes sp. ex Callipepla californica
Philopterus sp. ex Momotus momota
Philopterus sp. JPP238 ex Peucaea cassinii
Philopterus sp. B1.1 ex Agelaius phoeniceus
Philopterus sp. JPP374.1 ex Spinus psaltria
Philopterus sp. ex Spizella pusilla USA
Philopterus sp. JPP96 ex Toxostoma longirostre
Philopterus sp. JPP179.1 ex Geranoaetus albicaudatus
Philopterus sp. JPP189.2 ex Passerina ciris
Philopterus sp. JPP307.2 ex Piranga rubra
Philopterus sp. JPP321.2 ex Icterus cucullatus
Philopterus sp. 341.1 ex Mniotilta varia
Philopterus sp. DRR3.1 ex Mimus polyglottos
Philopterus sp. JPP325.2 ex Toxostoma longirostre
Philopterus sp. JPP327.5 ex Cyanocorax yncas
Brueelia pallidula ex Pheucticus ludovicianus USA
Brueelia sp. JPP299 ex Cardinalis cardinalis
Brueelia antiqua ex Catharus guttatus
Brueelia brunneinucha ex Dumetella carolinensis Illinois
Brueelia sp. JPP362.2 ex Mimus polyglottos
Brueelia sp. JPP311.4 ex Mimus polyglottos
Brueelia sp. 3933130 ex Mimus polyglottos
Brueelia sp. DRR3.4 ex Mimus polyglottos
Brueelia sp. JPP325.1 ex Toxostoma longirostre
Brueelia sp. 3933126.1 ex Mimus polyglottos
Brueelia sp. JPP16 ex Mimus polyglottos
Mimidae
Brueelia sp. ex Cardellina canadensis USA
Brueelia xanthocephali ex Xanthocephalus xanthocephalus USA
Brueelia sp. JPP125 ex Molothrus aeneaus
Brueelia sp. B3.2 ex Agelaius phoeniceus
Brueelia sp. DRR08 ex Molothrus aeneaus
Brueelia sp. JPH40 ex Molothrus ater
Icteridae
Brueelia sp. ex Dolichonyx oryzivorusIllinois
Brueelia ornatissima ex Agelaius phoeniceus USA
Brueelia ornatissima ex Molothrus ater USA
Brueelia sp. ex Carduelis pinus USA
Brueelia sp. ex Sialia currucoides USA
Brueelia sp. JWH419 ex Auriparus flaviceps
Brueelia sp. TJH3442 ex Vireo bellii
Brueelia sp. ex Melanerpes cruentatus Africa
Brueelia sp. ex Melanerpes erythrocephalus USA
Brueelia anamariae ex Troglodytes aedon Illinois
Brueelia sp. ex Melospiza lincolnii USA
Brueelia sp. JPP298.1 ex Melospiza lincolnii
Brueelia sp. JPP68 ex Icterus graduacauda
Brueelia vulgata ex Zonotrichia albicollis Illinois
Brueelia picturata ex Sturnella sp.USA
Brueelia sp. JPP82 ex Sturnella magna
Brueelia dorsale ex Toxostoma rufum Illinois
Brueelia sp. JPP91 ex Toxostoma curvirostre
Brueelia sp. HLP365 ex Toxostoma longirostre
Brueelia sp. JPP168 ex Vireo griseus
Brueelia sp. JPP2.1 ex Toxostoma longirostre
Brueelia sp. JPP67.1 ex Campylorhynchus brunneicapillus
Brueelia cedrorum ex Bombycilla cedrorum USA
Brueelia iliaci ex Turdus migratorius USA
Brueelia deficiens ex Aphelocoma californica USA
Brueelia sp. ex Melanerpes carolinus USA
Picicola sp. JPP191.4 ex Melanerpes aurifrons
Picicola snodgrassi ex Melanerpes carolinus USA
Picicola sp. JPP373.1 ex Tyrannus forficatus
Cotingacola parmpipapillae ex Brazil
Cotingacola stotzi ex Querula purpurata Brazil
Cotingacola lutzae ex Laniocera hypopyrra Brazil
Cotingacola engeli ex Phoenicircus nigricollis Brazil
Trogoninirmus sp. ex Trogon melanocephalus Mexico
Picicola oneilli ex Notharchus macrorhynchos Peru
Picicola faucetti ex Galbula cyanicollis Brazil
Picicola galbulica ex Galbula tombacea Peru
Picicola striata ex Monasa nigrifrons Bolivia
Degeeriella sp. JPP178.1 ex Falco sparverius
Degeeriella sp. JPP179.2 ex Parabuteo unicinctus
Degeeriella fulva ex Buteo regalis
Fahrenholzia zacatecae
Haematopinus eurysternus
0.5

Analysis of the ischnoceran suborder revealed several well supported clades (Figure 4). The louse genera *Columbicola*, *Anaticola*, and *Anatoecus* were recovered together in a clade, with moderate support (PP=0.94). The *Degeeriella* complex, comprised the genera *Degeeriella*, *Picicola*, *Trogoninirmus*, and *Contingacola*, also strongly supported (PP=1). Within this clade, intergeneric relationships were not strongly supported and *Picicola* was not recovered as monophyletic. Members of the louse genera *Cummingsiella*, *Lunaceps*, *Penenirmus* (not recovered as monophyletic), *Cuculoecus* and *Goinodes* comprised a poorly supported sister clade to *Philopterus*. While *Philopterus* was strongly supported (PP=0.99), the relationships within the genus were not. The same is true for the largest ischnoceran clade comprised members of the hyper-diverse genus *Brueelia*; this clade was strongly supported (PP=1) however, support for relationships within the clade was lacking.

III.4 Discussion

The findings of this study were not consistent with the extraordinarily high parasitism rate of 60% or more observed in other studies (Lindell et al. 2002, Szczykutowicz et al. 2005); instead I found a parasitism rate of 15.5%. This could be attributed to abiotic factors, such as climate. There is strong evidence that shows that aridity could be a driving variable in reducing the amount of louse parasitism rates among birds inhabiting arid environments (Moyer et al. 2002). The area of South Texas where sampling occurred, has been described as semi-arid habitat that can be heavily influenced by long periods of drought (Hernandez and Uddameri 2013). Indeed, during

the first year of my work, South Texas was at the tail end of a two year drought event. The average precipitation during those years was approximately 334 millimeters per year as compared to the 536 millimeters average precipitation per year recorded in the area from 1981-2010; provided by PRISM Climate Group, Oregon State University, (http://prism.oregonstate.edu).

When individual sampling areas were compared (San Antonio Viejo, which is arid and inland versus El Sauz, which is humid and coastal), there was little difference in louse parasitism rates. This is likely the due to the South Texas climate heavily influencing overall parasitism rates rather than the microclimates between areas, although the aforementioned drought cycle may have obscured any potential differences related to microclimate. However, birds sampled from the El Sauz ranch were more heavily parasitized based on the number of lice obtained from each bird from San Antonio Viejo. Rather than finding 1-3 lice per host from San Antonio Viejo, birds from El Sauz were more than often parasitized with 5 or more, with as many as 40 lice per host individual.

Within the amblyceran tree, there are 7 unique genetic lineages of *Myrsidea* (Figure 3). The genus *Myrsidea* is the most speciose genus in Phthiraptera, with 350 described species and new species frequently being described (e.g. Price and Dalgleish 2007, Palma and Price 2010, Valim and Weckstein 2013). High divergence was recorded in the louse collected from the Green jay (*Cyanocorax yncas*, 19.3% uncorrected *p*-distance) compared to its closest genetic relative, which parasitized the Brown jay

(*Psilorhinus morio*) from Mexico, which has a similar distribution and belongs to the same family (Corvidae) as the Green jay. While this is a unique lineage, it is also a novel host association, which strengthens the potential of this being a species new to science. A similar divergence (18.7% uncorrected *p*-distance) was recorded from the louse parasitizing the Black-crested titmouse (*Baeolophus atricristatus*) as compared to the louse species parasitizing House wren (*Troglodytes aedon*), Red-winged blackbird (*Agelaius phoeniceus*), and Lark sparrow (*Chondestes grammacus*). Additionally, the lice on these three hosts (House wren, Red-winged blackbird, and Lark sparrow) were on average 16.1% genetically different (uncorrected *p*-distance) than their closest genetic relative from GenBank. All of these represent novel host associations, excluding the House wren. The lice parasitizing the 5 Northern cardinals (*Cardinalis* cardinalis) and Black-throated green warbler (*Setophaga* virens) were genetically similar (on average 1.8% uncorrected *p*-distance). Collectively, the lice species parasitizing this group of hosts, represent two novel host associations and were 11.9% divergent (uncorrected *p*-distance) from *Myrsidea pricei*. A unique genetic lineage (on average 15.4% uncorrected *p*-distance) from its closest relatives and novel host association was found from the *Myrsidea* louse parasitizing the Painted bunting (*Passerina ciris*). The *Myrsidea* sp. parasitizing the Summer tanager (*Piranga* rubra) is a novel host association and was 14.5% genetically different than *Myrsidea cruickshanki* that was found parasitizing Spangle-cheeked tanager (*Tangara* dowii). The lice collected from Northern mockingbirds (*Mimus* polyglottos) are on average 14.4% genetically different from the closest GenBank sequence (*Myrsidea nesomimi*) collected from the Galapagos

mockingbird (*Mimus parvulus*). With such a high amount of genetic divergence and being a novel host association, this louse is likely representative of a new species. Notably, a previous phylogenetic study concluded the Galapagos mockingbird's closest relative is a member of the North American mockingbird family, Mimidae (Arbogast et al. 2006). This could be an example where we see the host phylogeny and louse phylogeny co-evolving. Finally within the *Myrsidea*, there is an interesting relationship with the louse *Myrsidea textoris*. This louse has not been recorded anywhere except Africa, yet two separate hosts (*Agelaius phoeniceus* and *Molothrus ater*) from South Texas were likely parasitized by this louse species based on an average 4.9% genetic divergence (uncorrected *p*-distance) from the GenBank *Myrsidea textoris*. This suggests an intercontinental distribution for this louse species.

The *Ricinus* lice that parasitized the Golden-winged warbler (*Vermivora chrysoptera*), Tennessee warbler (*Oreothlypis peregrina*), Northern waterthrush (*Parkesia motacilla*), and Lesser goldfinch (*Spinus psaltria*) formed one clade that are on average 11.1-14.1% genetically different from each other. This same clade is on average 16.7% genetically different from the other species within the *Ricinus* clade. When looking at known louse-host associations, different species of *Ricinus* are known to parasitize each of those host species within this study. Therefore, we could be molecularly identifying three separate *Ricinus* species within this clade (based on genetic divergences, the Tennessee warbler and Northern waterthrush are likely parasitized by the same louse species). There is a known host association of *Ricinus picturatus* parasitizing members from the genus *Vermivora*. It is possible that the new host

association recorded for the Golden-winged warbler could be representative of this particular species of *Ricinus*.

In the *Menacanthus* clade lice that parasitized Green jay (*Cyanocorax yncas*) and Hooded oriole (*Icterus cucullatus*) were almost identical genetically (average uncorrected *p*-distance 0.2%) to the GenBank *Menacanthus* sp. from Bright-rumped attila (*Attila spadiceus*): these likely represent the same species. This *Menacanthus* subclade genetically differed on average 16.9% from the *Menacanthus* sp. parasitizing the Great-crested flycatcher (*Myiarchus crinitus*). Based on the amount of divergence and a novel host association, this flycatcher louse is likely a new species. The Clay-colored thrush (*Turdis grayi*) and Spangle-cheeked Tanager (*Tanager dowii*) are hosts to *Menacanthus eurysternus*. A louse specimen collected from a Northern cardinal (*Cardinalis cardinalis*) was genetically similar to *Menacanthus eurysternus* (1.4% uncorrected *p*-distance). The lice from Northern parula (*Setophaga americana*) and Horned Lark (*Eremophila alpestris*) are on average 11.2% divergent from *Menacanthus eurysternus*, and 6% divergent from each other; both of these represent novel host associations. Between these five host species, there is considerable species distribution overlap with Central America. Notably, *Menacanthus eurysternus* complex is a widely distributed louse species, parasitizing numerous host families and species (Price 1976), and is likely a complex of multiple species. Although there are two novel host associations with a unique genetic lineage within this complex, additional morphological and probable molecular work is needed to support the individual lice as possible new species.

Within the ischnoceran clade, I found 15 genetically unique lineages as compared to GenBank sequences (Figure 4). This is not particularly novel as numerous ischnoceran species and host associations are being discovered and described from around the world (Najer et al. 2014, Moodi et al 2013, Valim and Palma 2015, Valim and Kuabara 2015). However, these new descriptions often come from areas that are traditionally understudied in terms of host and louse diversity. The fact that there are 15 unique lineages from the United States is a bit surprising; nine of these come from one genus and four are known louse-host associations with a lack of genetic reference material. The *Penenirmus* collected from the Golden-fronted woodpecker (*Melanerpes aurifrons*) was highly supported as sister to a louse collected from another Picidae species, African gray woodpecker (*Mesopicos goertae*). The *Penenirmus* louse from the Golden-fronted woodpecker was significantly divergent (21.3% uncorrected *p*-distance) from the African gray woodpecker and 24.5% divergent from the *Penenirmus* louse from the passerine bird, Bush tit (*Psaltriparus minimus*). My results support previous literature finding a lack of monophyly for *Penenirmus* (Johnson et al. 2001), while showing two distinct clades based on host association, one from order Piciformes and one from order Passeriformes.

All *Brueelia* in this study were genetically identical or nearly identical to sequences obtained from GenBank, with the exception of two specimens (Figure 4). The two exceptions were collected from a Verdin (*Auriparus flaviceps*) and Bell's vireo (*Vireo bellii*), and they are 23.9% and 24.3% different from the nearest GenBank match, respectively. These unique genetic lineages coupled with novel host associations, reveal

a strong possibility of new species within *Brueelia*. There have been numerous studies over the past few years that have focused efforts on collecting *Brueelia* sp. and using molecular analysis to resolve the *Brueelia complex*, and most recently Bush et al. (2016) recognizing this group to as paraphyletic. My results show this genus is monophyletic, but this is likely due to a small sample size for the group. This genus is one of the largest groups within ischnocera containing over 260 described species of which roughly 90% are host specific (Johnson et al. 2002b) and my phylogeny reflects host specificity of certain species. For example, the bird family Mimidae was only parasitized by one species, likely *B. brunneinucha* based on genetic divergence from the GenBank specimen. This louse species targets other mimids as well, with previously described associations of *B. brunneinucha* with Tropical mockingbird (*Mimus gilvus*), Blue and white mockingbird (*Melanotis hypoleucus*), and Bahama mockingbird (*Mimus gundlachii*: Cicchino 1986). Another example of host specificity in *Brueelia* applies to *B. xanthocephali* and *B. ornatissima* exclusively parasitize members of family Icteridae (Figure 3). However, there is also a group of hosts that appear to be parasitized by a non-host specific louse, *B. dorsale* (species identification based on genetic similarity with the GenBank specimen). I identified this louse parasitizing four different host species from three different families (Figure 4). Interestingly, these four host species all inhabit southern Texas thorn-scrub habitat, suggesting that *B. dorsale* is an opportunistic parasite within that habitat. This study reiterates what Johnson et al. (2002b) suggests regarding *Brueelia* having members host specific and host generalist.

The genus *Philopterus* is effectively a large polytomy with a high amount of divergence (average uncorrected *p*-distance 20.7%) among lineages (Figure 4). As such, it is difficult to determine relationships. This difficulty is exacerbated by the general lack of genetic references on GenBank. One well supported group (PP=1) within *Philopterus* consisted of three individuals that average 23.8% divergence from the rest of the lineages in this clade. These three individuals parasitized three separate hosts (*Toxostoma longirostre, Geranoaetus albicaudatus,* and *Passerina ciris*). Unfortunately, as many of the samples in this clade were nymphs it was difficult to confidently identify them any further than to genus. Of the nine recorded unique lineages within this genus, seven are associated with novel host associations (Figure 4, Table 10). Most of the records detailing *Philopterus* and their host associations in North America are many decades old (Geist 1935, Peters 1936) and therefore based on morphology. In general, there is little recent information about the genus with the exception of Price et al. (2003) and Bush et al. (2009) who provided a distribution of a *Philopterus crassipes* parasitizing the Western scrub jay (*Aphelcoma californica*).

A well supported clade that is referred to as the *Degeeriella* complex Clay (1958) was recovered within the ischnoceran phylogeny. This clade is often defined as being strongly supported based on morphology and as having high Bayesian support among lineages, although many genera within this group are paraphyletic (Johnson et al. 2002c, Johnson et al. 2008). Similar results were seen in this study as the genus *Picicola* was paraphyletic (Figure 4). While *Picicola* is known to parasitize the Scissor-tailed flycatcher (*Tyrannus forficatus*), molecular data demonstrating this association is not

available. Therefore, I report here new molecular information about this host association and possibly a new species should the louse from this study be found to be morphologically different from the louse identified in the original louse-host association. Also within this complex, I discovered as one new louse-host association of *Degeeriella fulva* parasitizing the American Kestrel (*Falco sparverius*).

This study was designed to assess the diversity of lice from host associations and at the molecular level from a largely underexplored region in the United States. Although my sampling was relatively small (446 hosts), I still report 31 new louse-host associations, and 12 amblyceran and 15 ischnoceran genetically unique lineages that are new species to GenBank and possibly new to science. Future studies in Texas should increase sampling localities throughout the state and to other unrepresented regions (e.g. neighboring states or Mexico) to expand our knowledge of host associations, determine louse distributions, and host specificity (or lack thereof). This knowledge will allow broader studies of biogeography, and the ability to identify possible underlying causes of louse distributions, such as climate or ecological variation.

CHAPTER IV

SUMMARY

A portion of this study was to assess the health of birds in South Texas using multiple venous blood analytes and compare them to other ecoregions. Also, investigate the differences in blood physiology associated with migration. Migratory birds' venous blood analytes differed from sedentary species, particularly those associated with oxygen transport and capacity. Sedentary species are advantageous as bioindicators as they directly reflect the year-round adaptations needed to survive in their ecosystem. As many analytes we studied appear similar in the sedentary species from Central Texas and South Texas, we suggest that sedentary birds, on a macro level, are similar and adapted to the local environmental stresses of their habitat. We find on a macro level of ecosystems; sedentary birds differ greatly in hematology from both locations, which suggests that rather than a single bird species, there is potential to use a community of birds as bioindicators of ecosystem health.

The second portion of this study was assess the avian chewing louse diversity from birds in South Texas. This study recorded a large number of new louse-host associations (31) and unique genetic louse lineages (27). The amount of novel host association found in this area is not surprising given that the studies involving louse-host relationships in Texas are limited to dove species (Johnson et al. 2002a, Moyer et al. 2002, Bush and Clayton 2006). Finding so many unique lineages was very surprising since North America is thought to be so well studied, but if we look at genetic lineages

and known host associations there are only 10 unique genetic lineages that correspond with novel host associations. Collectively, this study expands the louse literature and has implications for future research in this area.

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