# University of New Mexico UNM Digital Repository

# **Biology ETDs**

**Electronic Theses and Dissertations** 

Spring 5-15-2017

# Natural history and evolution of an elevational generalist, the Cinereous Conebill (Conirostrum cinereum)

Andrea N. Chavez University of New Mexico

Chandresekhar Natarajan University of Nebraska - Lincoln

Jay F. Storz University of Nebraska - Lincoln

Angela Fago *Aarhus University* 

Christopher C. Witt University of New Mexico

Follow this and additional works at: https://digitalrepository.unm.edu/biol\_etds Part of the <u>Biology Commons</u>

## **Recommended** Citation

Chavez, Andrea N.; Chandresekhar Natarajan; Jay F. Storz; Angela Fago; and Christopher C. Witt. "Natural history and evolution of an elevational generalist, the Cinereous Conebill (Conirostrum cinereum)." (2017). https://digitalrepository.unm.edu/biol\_etds/199

This Thesis is brought to you for free and open access by the Electronic Theses and Dissertations at UNM Digital Repository. It has been accepted for inclusion in Biology ETDs by an authorized administrator of UNM Digital Repository. For more information, please contact disc@unm.edu.

Andrea N. Chavez

Biology Department

This thesis is approved, and it is acceptable in quality and form for publication:

Approved by the Thesis Committee:

Christopher C. Witt, Chairperson

Joseph Cook

Michael Andersen

# NATURAL HISTORY AND EVOLUTION OF AN ELEVATIONAL GENERALIST, THE CINEREOUS CONEBILL (CONIROSTRUM CINEREUM)

by

# ANDREA N. CHAVEZ

# **B.S., BIOLOGY, UNIVERSITY OF NEW MEXICO, 2008**

THESIS

Submitted in Partial Fulfillment of the Requirements for the Degree of

Master of Science Biology

The University of New Mexico Albuquerque, New Mexico

May 2017

#### ACKNOWLEDGMENTS

I would like to first thank my major advisor, Dr. Christopher C. Witt, for the invaluable guidance and support he has provided throughout my tenure as a graduate student at the University of New Mexico. I would also like to thank my committee members, Drs. Joseph Cook and Michael Andersen, for the scientific insight and research assistance they have provided in support of my research. Pursuing a graduate degree while working as a professional wildlife biologist for a federal agency has been difficult. I greatly appreciate my committee for their patience, understanding, and support for my career goals and aspirations throughout this time. For intellectual discourse, I would like to thank Elizabeth Beckman, Ariel Gaffney, Spencer C. Galen, Andrew Johnson, Chauncey Gadek, Matthew J. Baumann, C. Jonathan Schmitt and Ashley Smiley. I would also like to thank Fernando Angulo, Emil Bautista, Daniel F. Lane, Sheila Figeroa, L. Monica Flores, Willy Ñáñez, Jano Nuñez, Alessandra Quiñonez Z., Donna C. Schmitt, Dora Susanibar, Thomas Valqui, Walter Vargas, and many associates of Centro de Ornitología y Biodiversidad (CORBIDI) for assisting with fieldwork to obtain key specimens for this research. The American Museum of Natural History Department of Ornithology graciously loaned voucher specimens that greatly improved this work. Furthermore, this research would not have been possible without funding from NSF DEB- 1146491, NSF DEB-0543556, the UNM Center for Evolutionary and Theoretical Immunology (CETI), the Graduate and Professional Student Association, the University of New Mexico Latina/o Graduate and Professional Student Fellowship, the Donald Caughran Memorial Scholarship, the Molly Hayes Scholarship and the late Robert W. Dickerman. I thank my former supervisor at the Bureau of Land Management Lori Kimball for

iii

supporting my pursuit of a graduate degree while working as a permanent employee. I thank Marikay Ramsey for the academic advice and professional mentorship she has provided throughout my career as a wildlife biologist. I would like to especially thank Vincent Ortega for his unending support for me throughout all my educational and professional endeavors.

# NATURAL HISTORY AND EVOLUTION OF AN ELEVATIONAL GENERALIST, THE CINEREOUS CONEBILL (CONIROSTRUM CINEREUM)

by

Andrea N. Chavez

B.S., University of New Mexico, 2008

M.S., University of New Mexico, 2017

## ABSTRACT

Elevational generalism is relatively rare in the tropical Andes Mountains, likely owing to the inherent requirements of enduring a high degree of climatic zonation and coping with hypoxic stress. The Cinereous Conebill (*Conirostrum cinereum*) appears to be an exception, and inhabits a continuous elevational distribution that spans over 4,500 m. Two subspecies, *cinereum* and *fraseri*, are restricted to high elevations and may be isolated, whereas the third and most widespread, *littorale*, occurs continuously along the western slope of the Andes from 0 to over 4,500 m. First, we aim to characterize the morphology, genetics, and climatic niche of the three subspecies using a comparative biogeographic approach to explore patterns and timing of differentiation and to consider possible mechanisms of diversification. Second, we study whether hemoglobin adaptation plays a role in this elevational generalist's ability to thrive in high-elevation environments, and whether localized adaptation is possible despite altitudinal migration and gene flow. We used a comparative phylogeographic framework to examine whether lineage divergence within C. cinereum is associated with climatic, geographic, and/or physiological barriers leading to incipient speciation.

v

INTRODUCTION	1
CHAPTER 1	3
INTRODUCTION	3
Overview	5
Identification	8
Distribution	16
Life History	20
Conservation	23
FIGURES	26
TABLES	
References	
CHAPTER 2	
INTRODUCTION	35
Methods	41

# **TABLE OF CONTENTS**

INTRODUCTION	5
Methods	.1
Morphological traits4	-1
DNA extraction, PCR amplification, and sequencing of mtDNA4	.1
Environmental Data4	.3
Blood properties4	.4
Cloning and preliminary sequencing of globin genes4	.4
DNA extraction, PCR amplification, and sequencing of globins4	.5
Characterization of hemoglobin isoform composition4	.6

Measurement of hemoglobin functional properties	47
RESULTS	48
Morphological traits	48
Population Genetic Analysis	50
Molecular Dating and Population Delimitation	51
Environmental Data	54
Blood Properties	56
Altitudinal patterns of sequence variation	58
Hemoglobin isoform composition	59
Oxygenation properties of HbA and HbD isoforms	60
Comparison of high- and low-altitude HbA and HbD variants	61
DISCUSSION	61
FIGURES	70
TABLES	83
REFERENCES	86
APPENDICES	97
Appendix A	97
Appendix B	100
Appendix C	109
Appendix D	114
Appendix E	119
Appendix F	160

#### **INTRODUCTION**

In History of Animals, published circa 340 B.C.E., Aristotle wrote, "Animals differ from one another in their modes of subsistence, in their actions, in their habits, and in their parts." At that time, a reasonably complete catalog of all species on Earth would not be completed for more than two millenia. Aristotle described at great length the many distinctions and commonalities found among a wide variety of creatures, and he used these to infer taxonomic groups. Although he frequently used the term *species*, the evolutionary significance of taxonomic units would not be discovered until Charles Darwin published On the Origin of Species in 1859 C.E. Remarkably, modern understanding of molecular evolution was foreshadowed in some of Aristotle's writings, wherein he refers to "parts in which there appears to be an adaptation to an end...having been appropriately constituted by an internal spontaneity..." The concept that an organism's traits evolve adaptively to fit to the environment has been around since at least Aristotle, yet the evolutionary process of adaptation is difficult to study and aspects of it remain mysterious to modern evolutionary biologists. In particular, our understanding is still in its early stages with respect to the ways that the adaptive process manifests across different branches of the tree of life, across geographic space, and on different spatial and temporal scales. Though scientific and technological advancements continue to drastically boost our understanding of the evolutionary history of life on Earth, new questions continue to emerge due to the tremendous complexity of biological systems.

It has been predicted that Earth's eukaryotic biota consists of  $\sim 8.7$  million species,  $\sim 90\%$  of which still await description (Mora et al. 2011). Moreover, the various methods

available for estimating the number of global species is not without limitations, and depending on the method and current global taxonomic inventory, extrapolations based on existing data can be associated with very large margins of error (Bebber et al. 2007). Uncertainty about global biodiversity poses a challenge to the quantification of global change. Better understanding of species distributions and relationships allows for more robust comparative analyses that can solve unanswered ecological and evolutionary questions. Exploring the link between geography and speciation can illuminate how past evolutionary processes may have shaped contemporary biological distributions, and will inform predictions of how global climate change may affect biodiversity into the future. In this thesis, I study one lineage segment, or tip, of the great tree of life, using multiple approaches to describe geographic and molecular patterns of recent evolution in order to shed light on fundamental evolutionary processes. I selected this species because it has an unusually broad ecoclimatic niche for an Andean songbird; thus, it provides an excellent opportunity to examine the geographic and ecoclimatic causes of recent evolutionary diversification.

#### **CHAPTER 1**

## Natural History of the Cinereous Conebill (Conirostrum cinereum)

# **INTRODUCTION**

This first chapter of my thesis summarizes what is known about this focal species, the Cinereous Conebill, in a species account published in Neotropical Birds Online (Chavez 2016). The account presented here assembles the meager previously published information on this poorly known species along with novel findings that have emerged through my Master's research and through Museum of Southwestern Biology ornithological field expeditions to Peru over the last decade. Similar to the early catalogues of life on Earth, modern species accounts seek to summarize all knowledge about a species, including its taxonomic classification, geographic distribution, life history traits, reproduction, behavior, habitat requirements, conservation status, and economic importance. In particular, avian species accounts are an important resource for ornithologists, wildlife biologists, conservationists, teachers and students, and even recreational bird enthusiasts. Scientists often use this information to inform research. Furthermore, a large amount of species occurrence data around the world is known from the contribution of citizen science (i.e., bird watchers). It is therefore important that species identification, distribution and habitat information be easily accessible to the public.

The information contained in this detailed species account can be accessed via Neotropical Birds Online. It is formatted here in sections as they appear in the online species account (link below).

http://neotropical.birds.cornell.edu/portal/species/overview?p\_p\_spp=588236

#### **OVERVIEW**

This is a widespread species of conebill found from southernmost Colombia, south to northernmost Chile and central Bolivia. Two of the three putative subspecies of cinerous conebill are restricted to high elevations, whereas the third ranges from sea level to over 4500 m elevation, an unusually large elevational range for a songbird. Interestingly, there are two ecological zones it inhabits. The Andean population is found in more humid areas with shrubby habitats or open woodlands, up to treeline. Coastal birds are found in virtually any habitat that has some woody vegetation in the arid zone, including lomas formations, riparian thickets, gardens, and hedge rows. Coastal birds are buffier below than Andean birds, and the isolated population in Ecuador and Colombia is larger and browner than the southern birds. All have a pointed, cone-shaped bill, a distinct and bold pale supercilium, and a pale panel on the bases of the primaries that forms a white "speculum."

**OVERVIEW PAGE –** RIGHT SIDE PANEL INFO (UNDER DISTRIBUTION MAP):

Migration/Movement: Resident (nonmigratory).

<u>Primary Habitat</u>: Arid montane or coastal scrub (*littorale*); semi-humid to humid montane scrub (*cinereum*); *cinereum* and *fraseri* are both restricted to the Humid Temperate Zone. <u>Foraging Strata</u>: Canopy. Subspecies *littorale* and *cinereum* occur in the canopy of *Polylepis* (Lloyd 2008; Benham et al. 2011) or humid elfin forest or second growth. *Cinereum* forages at all heights in shrubs and small trees. Pairs glean in foliage near tips of dense bushes, shrubs, and stunted trees (J. Silliman). <u>Foraging Behavior</u>: Glean. Arboreal foliage gleaner; feeds principally on outermost edges of trees, such as *Polylepis*, in which they use hang-up and hang-down prey attack maneuvers (Lloyd 2008). Observations of secondary nectar robbing have been recorded in Quito (2850 m; ssp. *fraseri*); in these cases, cinereous conebills foraged for nectar using holes made by flowerpiercers at the bases of corollas (Vogt 2006).

<u>Diet</u>: Insectivorous. Mostly insects, some vegetation and seeds (Museum of Southwestern Biology specimen data). Burns et al. (2014) classified genus *Conirostrum* as arthropod feeders. Opportunistic nectar feeders, at least occasionally (Vogt 2006).

<u>Sociality</u>: Pairs/Family Groups. Can be solitary, but most often travels in groups of 2-6 individuals. May flock with tamarugo conebill, and may also associate with white-browed conebill (Schulenberg et al. 2007).

# Mating System: Monogamy.

<u>Nest Form</u>: Cup. Johnson (1967) described cup shape nests made of root fibers interwoven with cotton or vegetable wool and lined with black horse-hair. The average external diameter is 9 cm and 5 cm across the cup, with a depth of 6 cm and 3.5 cm inside the cup. The eggs, 3 in number, roundish in shape, and pale Etain's blue with numerous small spots of pale purplish gray. Average measurements are 16.6+/-0.34 by 13.7+/-0.04 mm. Johnson also reported the same clutch size and very similar egg colors and nest shape for the cinereous conebill (*C. cinereum*) in northern Chile as the tamarugo conebill (*C. tamarugense*). The nest is small and hemispheric in structure with a slightly longer vertical axis, being 6.5 to 9.0 cm in diameter and 7.0 to 10.0 cm in height. It is constructed using small twigs, feathers, sheep wool, and the rachis of tamarugo leaves.

The egg color is pale gray with irregular brown spots (Figure 1). Estades & Lopez-Calleja (1995) also reported the clutch to be comprised of three eggs.

**Figure 1**. Egg of *C. cinereum* (No. 5) displayed along side other members of the Diglossinae (Johnson 1967).

<u>Clutch</u>: Irregular brown spots – 3. Three eggs per clutch.

IUCN Status: Least Concern

#### **IDENTIFICATION**

#### SUMMARY

The three subspecies vary from light slate-gray to brownish-olive above with warm, buffy underparts. All subspecies have the pointed, cone-shaped bill that is typical of the genus, as well as a bold pale supercilium, and a whitish panel at the bases of the primaries that forms a conspicuous "speculum." Subspecies vary subtly in plumage color, size, and proportions.

#### SIMILAR SPECIES

Within Conebills, similar only to Tamarugo Conebill (*C. tamarugense*). However, Tamarugo Conebill is easily distinguishable by its rufous superciliary, throat, and vent. Cinereous Conebill could also be mistaken for other tanager species such as the Superciliaried Hemispingus (*Hemispingus superciliaris*) populations of central Peru (ssp. *insignis* and *leucogastrus*). The latter species is a small, warbler-like tanager roughly the same size as Cinereous Conebill; each species is gray above, dull white below, and with a white supercilium; the hemispingus has a slightly thicker bill and lacks the pale speculum at the base of the primaries. See also female Rusty Flowerpiercer (*Diglossa sittoides*).

#### VOCALIZATIONS

The song is a series of high-pitched notes of variable speed, somewhat reminiscent of a squeaky wheel, that can sound jumbled and fast, or as a rhythmic series of couplets consisting of two ("whee-chee") or three ("whee-chee-tee") repeating, up-and-down notes; individual songs typically have five to 25 of these couplets and last one to four

seconds. The call is a high, thin "tseep" (Schulenberg et al. 2007). Links to vocalization recordings are below. The common foraging call is a fine, 2-noted, "tsip tsip" (J. Silliman); song a rapid jumble of twittering phrases, virtually indistinguishable from song of the Carbonated Flower-piercer (Moynihan 1963).

C. cinereum littorale: http://avocet.zoology.msu.edu/recordings/12531 (Rasmussen

2011). C. cinereum littorale and cinereum: http://www.xeno-

<u>canto.org/species/Conirostrum-cinereum</u> (various recordings/citations).

# NON-VOCAL SOUND

(No information on non-vocal sound currently available.)

# **DETAILED DESCRIPTION** (appearance)

10.5-11.5 cm (*fraseri* largest). Ssp. *cinereum*: above light slate-gray, crown dusky; wingbar, patch at base of primaries, edge of tertials, narrow forehead, and supercilium white. Below pale gray, vent buff. Ssp. *littorale* similar, but supercilium shorter and supercilium and underparts tinged buff; ssp. *fraseri* much darker, back brownish-olive, fairly long and broad supercilium warm buff, underparts brownish clay to warm buff. Immature has buffy wash to wingbar and edges of tertials. Juvenile has feathers of back and breast faintly dark tipped, supercilium and underparts faintly tinged yellowish (Fjelsda & Krabbe 1990).

## **BARE PARTS**

For Sspp. *cinereum* and *littorale*, irides are brown to dark brown. Bill ranges from gray to black with the mandible typically being gray and the maxilla being dark gray or black. Tarsi are dark gray or black. Toes are gray or black with toe pads ranging from light to dark gray (Museum of Southwestern Biology specimen data). For Ssp. *fraseri*, tarsi and toes dark brown to black, with toe pads dark brown/gray, and dark brown bill (American Museum of Natural History specimen data).

#### **MEASUREMENTS**

Morphological measurements of museum specimens suggest that the three subspecies differ significantly in body size. No difference was found in culmen length. However, body mass, tarsus, tail, and wing chord are largest in the highland *cinereum* and smallest in the coastal *littorale* (no body mass data was available for *fraseri*). *Fraseri* is intermediate in size between *cinereum* and *littorale*, but shows no significant difference with *cinereum* for tarsus length. The larger individuals appear to occur in areas above 2500 m elevation where temperatures are substantially cooler than at lower elevations. These morphological characters do not vary within *littorale* across elevation. The results of statistical comparisons can be seen in Table 1.

<u>Mass</u>: Body mass varies by subspecies, with *cinereum* weighing over a gram more than *littorale*. Males and females in either subspecies do not differ [*cinereum*:  $\mu$ =9.337, SD=0.264, n=9; *littorale*:  $\mu$ =8.125, SD=0.612, n=42; t-test: t=9.377, p=2.495e-10]. No body mass data for *fraseri* were available.

<u>Culmen</u>: Culmen length does not vary by subspecies [*cinereum*:  $\mu$ =7.222 mm, SD=0.339, n=10; *littorale*:  $\mu$ =7.159, SD=0.328, n=54; *fraseri*:  $\mu$ =7.025, SD= 0.494, N=23].

<u>Tarsus</u>: Tarsus length is significantly longer in *cinereum* and *fraseri* than in *littorale* [*cinereum*:  $\mu = 18.241$ , SD=0.831, n=10; *littorale*:  $\mu = 17.0$ , SD=0.808, n=54; *fraseri*:  $\mu = 18.264$ , SD=0.667, N=23].

<u>Wing Chord</u>: In *littorale*, male wing chord length was found to average 3 mm longer than females, but no difference between sexes was observed in *cinereum*. Wing chord length is ~5 mm longer in *cinereum* than in *littorale* [*cinereum*:  $\mu$ =61.466, SD=2.088, n=10; *littorale*:  $\mu$ =56.265, SD=2.708, n=54; fraseri:  $\mu$ =58.713, SD=2.529, N=23]. <u>Tail</u>: Tail length is ~7 mm longer in *cinereum* than in *littorale* [*cinereum*:  $\mu$ =52.5, SD=1.779, n=10; *littorale*:  $\mu$ =45.528, SD=2.416, n=53; fraseri:  $\mu$ =50.282, SD=2.9612, N=23].

# MOLTS

Based on an assessment of museum specimen data, molt can occur throughout most months of the year in Peruvian populations of *C. cinereum* that occur between 5 and 12 degrees south latitude. Figure 2 shows the frequency in type of molt occurring throughout the year for subspecies *littorale* and *cinereum*. No molt data for subspecies *fraseri* was available. The vast majority of wing and tail molt was observed during July and August, for both subspecies (Figures 3 and 4). Other types of molt are more protracted, occurring during all months in which there are available specimen data. This sampling is preliminary, and more data are needed to understand the phenology of this species, including variation across latitudes and among subspecies. Data available to describe the

breeding season of *C. cinereum* are limited and primarily comprised of gonadal measurements of museum specimens; these suggest that breeding commences between September and December, but it also may occur at other times of the year (i.e., May). The phenology of the observed molting pattern is consistent with the hypothesized breeding period as adult molting typically occurs pre- or post-reproduction. However, a more robust and systematic sampling effort for all three subspecies is necessary to reduce the uncertainty apparent in the current data.

## **GEOGRAPHIC VARIATION**

The three subspecies of *C. cinereum* vary in geographic range, plumage, morphology, and genetics. The nominate (highland) subspecies *cinereum* is light slate-gray above with a dusky crown and distinct wingbar (patch at base of primaries) at the edge of the tertials. It has a narrow forehead and a white supercilium. It is distinctly pale gray below with a buffy vent. The coastal subspecies *littorale* is similar, but its supercilium is shorter and its underparts are tinged buff. The northern highland subspecies *fraseri* is much darker with a brownish-olive back. Its supercilium is very distinctly long, broad, and colored a warm buff. Its underparts are brownish clay to warm buff. These differences may be distinguishable by the common observer (Figure 5).

*Cinereum* is the largest of the three, *littorale* the smallest, and *fraseri* intermediate but closer in size to *cinereum*. Body size was assessed by length of the culmen, tarsus, wing chord, tail, and mass from museum specimens in the Museum of Southwestern Biology and the American Museum of Natural History (no mass data was available for *fraseri*).

The only character that did not differ between the three subspecies was culmen.

*Cinereum* and *fraseri* are both larger-bodied, which is likely related to their restriction to humid or semi-humid high-altitude environments, as neither of them occur below ~2500 meters. This pattern of variation is consistent with Bergman's Rule, which predicts that within a broadly distributed taxonomic clade, populations with larger size will be found in colder environments (Bergman 1847). The subspecies are not known to overlap geographically.

Size and plumage differ between the subspecies, even though culmen size and shape do not. It is therefore likely that these populations are subject to little or no inter-subspecies geneflow, but that they are geographic replacements of one another that inhabit similar, but slightly varying environmental niches. A phylogenetic analysis based on the mitochondrial DNA gene, ND2, supports the hypothesis of isolation, placing cinereum in its own monophyletic clade (Chavez 2016). First, Bayesian phylogenetic estimation in BEAST with substantial within-subspecies sampling (cinereum N=27, fraseri N=8, *littorale* N=83) revealed monophyly for *cinereum* and paraphyly for *littorale* and *fraseri* (posterior probabilities: cinereum=1, littorale/fraseri=1). Though littorale and fraseri appear to be paraphyletic, most *fraseri* individuals grouped together, with the exception of 3 that occur within the southern most part of the *fraseri* range and the northern most part of the *littorale* range where the populations may overlap. Second, tests of gene flow and genetic variation support significant differentiation and isolation of subspecies; Fst values between *littorale* and *cinereum* (0.738), *littorale* and *fraseri* (0.419), and *cinereum* and *fraseri* (0.738) were all significantly greater than zero.

#### **Systematics**

The Thraupidae (Tanagers) is the second largest family of birds in the world and the largest radiation of Neotropical songbirds (Howard & Moore 2013). The family consists of 371 species, and displays a remarkable range of plumage colors and patterns, behaviors, morphologies and ecotypes (Burns et al. 2014; Winkler et al. 2015). Interestingly, the trait diversity observed in the Thraupidae approaches that found within all passerines. Until recently, the evolutionary history of this genus and family was poorly understood, and taxonomic confusion was likely due to this immense diversity in life history traits and lack of a unifying set of defining characters. Burns et al. (2014) presented a comprehensive species-level phylogeny for the Thraupidae. The family now includes many species that were formerly spread across multiple avian families. Included among these are the conebills (genera *Conirostrum* and *Oreomanes*), which were previously placed with new world warblers in the Parulidae, likely owing to their warbler-like bills.

Along with 64 species from 14 genera, conebills fall within the subfamily Diglossinae, another particularly diverse group in terms of feeding morphologies and behaviors. Burns et al (2014) recommended the name "highland tanagers" to describe the Diglossinae because, despite some lowland species, over 80% of the members of this group have their center of abundance at or above 900 m elevation. Burns et al (2014) support the monophyly of *Conirostrum* + *Oreomanes* (giant conebill). They also suggest that, due to the nested placement of the clade of highland conebill taxa, highland conebills evolved from a lowland conebill ancestor.

Within the "highland tanagers," *Conirostrum cinereum* is a rather unique taxon because it inhabits both low and high altitude environments, both humid and arid ecosystems, and appears to be a habitat generalist. The subspecies are geographically isolated and genetically distinct (see *Geographic Variation*, above). The nominate subspecies appears to be more genetically differentiated from the other two subspecies than they are to each other. The habitats it has been found in are also in stark contrast to those of its hypothesized parapatric conspecific *littorale*. While *cinereum* occupies a more mesic region of the eastern Andean highlands characterized by high humidity and lower average temperatures, *littorale* is found in more arid regions of the western slope of the Andes where xeric habitats are dominant.

#### **DISTRIBUTION**

#### **THE AMERICAS**

The cinereous conebill is found from southernmost Colombia, south to northernmost Chile and central Bolivia. It has a broad elevational range, from sea level up to 4500 m (Fjeldsa & Krabbe 1990), and at least occasionally to 4750 m in Peru (Benham et al. 2011). In Colombia, it occurs from 2600 – 3600 m (Hilty & Brown 1986), in Ecuador from 2300-4000 m (Ridgely and Greenfield 2001), and in Bolivia probably mainly above 3000 m (del Hoyo et al. 2003).

There are three putative subspecies, *C.c. cinereum*, *C.c. littorale*, and *C.c. fraseri*. West slope and coastal birds (i.e., *littorale*) are buffier below than eastern Andean birds (i.e., *cinereum*), and the population in Ecuador to Colombia (i.e., *fraseri*) is larger and browner than the southern birds. All have a classic pointed cone-shaped bill, a distinct and bold pale supercilium, and a pale panel on the bases of the primaries.

The extent of each subspecies' range has yet to be determined with certainty. The nominate subspecies is purported to be restricted to high elevations in the central highlands and tree-line on the east slope of the Andes in Peru, south to northern Bolivia and northern Chile. There are a large number of eBird records of *C. cinereum* in northern Chile, but they do not discern between subspecies, likely owing to the difficulty of field identification. The photographic documentation accompanying many eBird observations in this region suggests the birds are buffier and possibly belonging to the *littorale* group. A photo posted to the Internet Bird Collection of a purported *littorale* sighted in the Arica

and Purinacota region of Chile appeared to be accurate. However, photographic identification can be subjective as the lighting, perspective, and quality of the images may obscure positive identification. According to Zimmer's 1942 Studies of Peruvian Birds XLIII, there are also records of *littorale* from Chacalluta and Pica, Tarapaca, both in the two northernmost provinces of Chile. Though it has been posited that *cinereum* occurs in northern Chile, this remains unclear given existing evidence.

Littorale occurs in western and northeastern Peru and south to northern Chile (del Hoyo et al. 2003). However, it is likely that del Hoyo et al. err in their extension of littorale to northeastern Peru. According to Fjelsda and Krabbe (1990), littorale occurs from the west slope of the western Andes and upper Utcubamba and Marañon valleys of northern Peru, south through the western Andes to Tarapaca of northern Chile. Fraseri possibly occurs separately from *littorale* and *cinereum*, in Colombia in the southern part of the central and east Andes, south to southern Ecuador (del Hoyo et al. 2003). A mapping effort of fraseri occurrences from Zimmer's Studies of Peruvian Birds XLIII suggested that the furthest south it occurs is Loja, Ecuador. The southernmost museum specimen from the American Museum of Natural History is from Loja as well. According to Fjelsda and Krabbe (1990), *fraseri* occurs from Cauca at the southern end of the central Andes and Narino, Colombia to Loja in southern Ecuador, which is consistent with Zimmer's specimens. The existing data place the likely boundary between *fraseri* and *littorale* in the region between Loja in southern Ecuador and Cajamarca in northern Peru, possibly corresponding the Maranon Valley and North Peruvian Low.

Interestingly there are two ecological zones *C. cinereum* inhabits. In the eastern Andean highlands (~2100-4000 m), it is found near tree-line in dry to moderately humid zones, especially bushy woodland borders, patches of dense stunted shrubbery, gardens and other lightly wooded drier habitats, and also in *Polylepis* woodland. The birds of the coastal desert and arid west slope of the Andes (sea level ~ 4500 m) occur in shrubby sites, riparian thickets, gardens, scrubby vegetation on slopes, and riparian vegetation along dry streambeds (del Hoyo et al. 2003).

#### **OUTSIDE THE AMERICAS**

Endemic to South America.

# HABITAT

Although *cinereum* and *fraseri* are restricted to the Humid Temperate Zone, *littorale* inhabits both Temperate and Tropical zones in their arid subdivisions (Zimmer 1942). Generally speaking, *C. cinereum* occupies arid to humid scrub and shrubbery, forest edge, riparian thickets, gardens, *Polylepis* woodland, hedgerows, etc. (Fjelsda & Krabbe 1990). There are two main ecological zones it inhabits. In the eastern Andean highlands (~2100-4000 m), it is found near tree-line in semi-arid to humid zones, especially bushy woodland borders, patches of dense stunted shrubbery, gardens and other lightly wooded drier habitats, and also in *Polylepis* woodland. The birds of the coastal desert and arid west slope of the Andes (sea level ~ 4500 m) occur in shrubby sites, riparian thickets, gardens, scrubby vegetation on slopes, and riparian vegetation along dry streambeds (del

Hoyo et al. 2003). Although *cinereum* and *fraseri* are restricted to the Humid Temperate Zone, *littorale* inhabits arid to semi-humid areas of both Temperate and Tropical zones.

# **HISTORICAL CHANGES**

*C. cinereum* occurs in such a wide variety of habitats, and it is therefore difficult to assess historical changes to its habitat. It is likely not especially sensitive to disturbance, as it occurs in heavily modified environments in many parts of its range. However, it does require woody vegetation, a habitat that has been steadily declining in the high Andes over the past several centuries due to human activities (Bush et al. 2005). Throughout the Northern and Central Andes, woody vegetation is cleared to make room for farmland and grazing pastures (Andersen et al. 1999). It is possible this widespread activity may have caused a reduction in suitable habitat for the species. See discussion on land use practices in the *Effects of Human Activity on Status* section.

## FOSSIL HISTORY

No known fossil records of this species.

#### LIFE HISTORY

# FOOD

*C. cinereum* primarily consumes insects, but sometimes vegetation and seeds, and opportunistically nectar (Museum of Southwestern Biology specimen label data). Lloyd (2008) classified *C. cinereum* as an arboreal foliage gleaner, and observed it feeding principally on the outermost edges of *Polylepis* trees using mostly hang-up and hang-down prey attack maneuvers. This species was also classified as forest-dependent in *Polylepis* habitat (Lloyd & Marsden 2008). These studies were conducted in the high-Andes of the Cordillera Vilcanota, Peru, and therefore represent the nominate subspecies (*C. c. cinereum*). Observations of secondary nectar robbing have also been recorded, in which *C. cinereum* forages for nectar using holes made by Flowerpiercers at the base of corollas. These observations likely represent subspecies *fraseri*, as they were made in Quito, Ecuador at 2850 m elevation (Vogt 2006).

#### **BEHAVIOR**

*C. cinereum* often occurs alone, in pairs or groups. It is very active, nervous, and often found in mixed flocks. Restlessly flitters about for insect and berries, often hanging, and spending most time along middle branches inside the vegetation (Fjelsda & Krabbe 1990). Pairs glean in foliage near tips of dense bushes, shrubs, or stunted trees (Hilty & Brown 1986). Males sing from perches in center of established territories (Moynihan 1963).

# TERRITORIALITY

Male sings from perches in center of established territories (Moynihan 1963).

# SEXUAL BEHAVIOR

(No information on sexual behavior currently available.)

# SOCIAL AND INTERSPECIFIC BEHAVIOR

Can be solitary, but most often travels in groups of 2-6 individuals. May flock with tamarugo conebill, and may also associate with white-browed conebill (Schulenberg et al. 2007).

# PREDATION

(No information on predation currently available.)

#### REPRODUCTION

Little is known about the mating system of *C. cinereum*. Two breeding condition females were recorded in February in Purace (CO) (Carriker), a song in March at Parque Nacional Purace (Brown), and a territorial dispute and display in May in Ecuador (Moynihan 1963; Hilty and Brown 1986). Based on an assessment of museum specimen data, molt can occur throughout the year in Peruvian populations of *C. cinereum* that occur between 5 and 12 degrees south latitude. The majority of molt (including both body and flight feathers) occurs in July and August, which likely marks the end of the annual breeding cycle. Museum of Southwestern Biology specimen data show enlarged gonads in *littorale* 

from May thru October, with a pulse in January. However the majority of enlarged gonads were observed in May (Figure 6). Museum samples of *cinereum* were more limited, but showed the majority of enlarged gonads in August through December with a pulse in March.

The geographic range of *C*. *cinereum* overlaps with several other closely related conebills. Since they share similar habitats and occur in the highlands, the breeding phenology of those species may be similar to that of *C. cinereum*. The tamarugo conebill (*C. tamarugense*), which is known to share habitat and even occur in mixed flocks with *C. cinereum*, breeds at mid-elevation from September to December (northern Chile) (Estades & Lopez-Calleja 1995). The giant conebill (*Oreomanes fraseri*) also occupies *Polylepis* forest between 2700-4850 m elevation, and breeding appears to coincide with the onset of the rainy season (October through December); no nests have been discovered between January and September (Bolivia) (Cahill et al. 2008). *C. cinereum*'s broad elevational range is important to consider when evaluating its reproductive phenology as variation in temperature and precipitation may lead to differences between high- and low-altitude populations.

# **POPULATIONS AND DEMOGRAPHY**

C. cinereum is relatively common where it occurs.

# **CONSERVATION**

# **CONSERVATION STATUS**

The International Union for Conservation of Nature has listed *C. cinereum* as Least Concern on the Red List of Threatened Species (IUCN 2014). BirdLife International acted as the assessor for this species, and for the following reasons evaluated it as Least Concern:

- The species has a very large range, and does not approach the thresholds for Vulnerable under the range size criterion.
- 2. The population trend appears to be stable, and hence the species does not approach the thresholds for Vulnerable under the population trend criterion.
- 3. The population size has not been quantified, but it is not believed to approach the thresholds for Vulnerable under the population size criterion.

Previously published Red List Assessments include:

2009: Least Concern (LC)
2008: Least Concern (LC)
2004: Least Concern (LC)
2000: Lower Risk/least concern (LRlc)
1994: Lower Risk/least concern (LRlc)
1988: Lower Risk/least concern (LRlc)

# **EFFECTS OF HUMAN ACTIVITY ON STATUS**

*C. cinereum* is likely not especially sensitive to disturbance, as it occurs in heavily modified environments in many parts of its range. However, it does require woody

vegetation, a habitat that is subject to long-term decline due to human activities in the high Andes (Bush et al. 2005). Humans have inhabited the high Andes for  $\sim 12,000$  years, and have drastically altered landscapes for fuel wood harvest and agricultural uses such as farming and livestock grazing. Throughout history and continued into the present (though with varying frequency and intensity), these practices have commonly involved the clearing of woodlands and native vegetation such that the upper tree line is thought to be substantially lower than the natural potential tree line (Ellenberg 1979; Andersen et al. 1999). More specifically, *Polylepis* forests have become increasingly fragmented, and are being rapidly depleted by rural communities, though some reforestation efforts are underway (various projects and partnerships by Antamina, ECOAN, American Bird Conservancy, and U.S. Fish & Wildlife Service, and others). The IUCN Red List currently identifies 14 of the c. 27 species of Polylepis as Vulnerable and one as nearthreatened. While C. cinereum doesn't depend on Polylepis exclusively, Polylepis forest patches provide habitat for C. cinereum where no other woody vegetation exists; thus, it is likely that habitat conversion due to widespread human activities over the past several centuries in the Andean highlands may be causing a reduction in suitable habitat for C. cinereum. Further research on the ecology of C. cinereum in human-dominated landscapes is recommended.

## FUTURE RESEARCH

Although the IUCN has listed *C. cinereum* as a species of Least Concern on the most recent Red List of Threatened Species based on its large range size, its true population size has not been quantified, and sufficient data to establish accurate species and

population trends are not available. Little is known of the breeding biology of the species or the effects of human activity on populations and habitat. Furthermore, it is unknown how high-elevation species such as *C. cinereum* will respond to temperature increases occurring due to climate change. Species or populations restricted to high elevation by physiological limitations related to temperature may experience contractions due to a reduced geographic range in which they can track optimal thermal zones. The cinereous conebill provides a unique opportunity to study physiological tolerances across a broad range of elevations (and therefore temperatures) because it has both elevational generalist and elevational specialist subspecies. There is some evidence that *C. c. littorale* individuals or populations are subject to seasonal elevational movements, but this requires further confirmation. Further comparative physiological and evolutionary studies of this widespread species would shed light on the potential impact of climate change on Andean birds.

# **Chapter 1**

# FIGURES

**Figure 1.** Egg of *C. cinereum* (No. 5) displayed along side other members of the Diglossinae (Johnson 1967).



**Figure 2.** Frequency of flight and body molt per month in *C. cinereum* museum specimens from the Museum of Southwestern Biology. Includes individuals from subspecies *littorale* and *cinereum*. Sample sizes per month are indicated at the top of each bar. "None" category refers to individuals for which molt was not found. No data were collected for the months of April and June.



Figure 3. Proportion of C. c. littorale museum specimens that exhibited molt, displayed by month of the year. Black bars represent specimens in which molt was detected, and white bars represent individuals in which it was not detected. Fields with no bars indicate no specimens of C. c. littorale were available from those months. Sample sizes per month are indicated on the top of each bar.



Figure 4. Proportion of C. c. cinereum museum specimens that exhibited molt, displayed by month of the year. Black bars represent specimens in which molt was detected, and white bars represent individuals in which it was not detected. Fields with no bars indicate no specimens of C. c. cinereum were collected in those months. Sample sizes per month are indicated on the top of each bar.


**Figure 5**. Geographic variation in plumage among the three subspecies of *C. cinereum*. Three male individuals of each plumage morph are displayed. *Cinereum* and *littorale* specimens are from the Museum of Southwestern Biology. *Fraseri* specimens are from the American Museum of Natural History.



**Figure 6**. Boxplots representing frequency distributions of gonad size in *C. cinereum* adults, broken down by subspecies and sex. Measurements taken from Museum of Southwestern Biology specimens. No gonad measurements were available for *fraseri*. Sample sizes are indicated below x-axis labels.



## Chapter 1

### TABLES

**Table 1.** Multivariate analysis of variance (MANOVA) results for morphological character comparisons between subspecies. Significance indicated by coded: '\*\*\*'0.001, '\*\*'0.01, '\*'0.05.

Comparison	Dependent	Df	Sum Sq	Mean Sq	F	р
	Variable					
cinereum	Culmen	1	0.0377	0.037658	0.3428	0.5604
VS.	Tarsus	1	12.944	12.944	19.319	4.497e-05 ***
littorale	Wing Chord	1	229.89	229.893	32.818	3.322e-07 ***
	Tail	1	408.83	408.83	75.073	3.19e-12 ***
cinereum	Culmen	1	0.2699	0.26989	1.305	0.262
VS.	Tarsus	1	0.0039	0.00394	0.0076	0.9309
fraseri	Wing Chord	1	52.788	52.788	9.0887	0.005095 **
	Tail	1	34.269	34.269	4.798	0.03614 *
fraseri	Culmen	1	0.2706	0.27055	1.8143	0.1821
VS.	Tarsus	1	25.635	25.6350	42.704	7.148e-09 ***
littorale	Wing Chord	1	98.29	98.285	13.754	0.0004003 ***
	Tail	1	362.46	362.46	54.011	2.183e-10 ***

#### Chapter 1

#### REFERENCES

- ANDERSEN, P.N., T. HJARSEN, AND N. MORAYWILLIAMS. 1999. Monitoring and management of high Andean biodiversity. DIVA, Technical Report No 6. Centre for Research on the Cultural and Biological Diversity of Andean Rainforests (DIVA).
- ARISTOTLE. 350 B.C.E. The History of Animals. Translated by D'Arcy Wentworth Thompson. Accessed online via

http://classics.mit.edu/Aristotle/history\_anim.html.

- BEBBER, D.P., F.H.C. MARRIOTT, K.J. GASTON, S.A. HARRIS, R.W. SCOTLAND. 2007.
   Predicting unknown species numbers using discovery curves. *Proceedings of the Royal Society B* 274:1651-1658.
- BENHAM, P.M., E.J. BECKMAN, S.G. DUBAY, M. FLORES, A. JOHNSON, M.J. LELEVIER,
  C.J. SCHMITT, N.A. WRIGHT, AND C.C. WITT. 2011. Satellite imagery reveals new critical habitat for endangered bird species in the high Andes of Peru. *Enangered Species Research* 13(2): 145-157.
- BERGMANN, C. 1847. "Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse". *Göttinger Studien*. 3 (1): 595–708.

BURNS, K.J., A.J. SHULTZ, P.O. TITLE, N.A. MASON, F.K BARKER, J. KLICKA, S.M.
LANYON, I.J. LOVETTE. 2014. Phylogenetics and diversification of tanagers
(Passeriformes: Thraupidae), the largest radiation of Neotropical songbirds. *Molecular Phylogenetics and Evolution* 75(41-77).

- CAHILL, J. R. A., E. MATTHYSEN, AND N. E. HUANCA. 2008. Nesting biology of the Giant Conebill (*Oreomanes fraseri*) in the high Andes of Bolivia. *Wilson Journal of Ornithology*. 120: 545-549.
- DEL HOYO, J., ELLIOT, A., & CHRISTIE, D.A. EDS. (2003). Handbook of the Birds of the World. Vol. 16. Tanagers to New World Blackbirds. Lynx Edicions, Barcelona.
- ELLENBERG, H. 1979. Man's influence on tropical mountain ecosystems in South America. *Journal of Ecology* 67:401-416.
- ESTADES, C.F. AND M.V. LOPEZ-CALLEJA. 1995. First nesting record of the Tamarugo Conebill. The Auk 112(3): 797-800.
- FJELDSA, J. & N. KRABBE. 1990. Birds of the High Andes. Jon Fjelsda & Niels Krabbe, and Zoological Museum, University of Copenhagen, and Apollo Books, Svendborg, Denmark.
- JOHNSON, A.W. 1967. The Birds of Chile and Adjacent Regions of Argentina, Bolivia and Peru.
- HILTY, S.L. AND W.L. BROWN. 1986. A Guide to the Birds of Colombia. Princeton University Press.
- IUCN. 2014. The International Union for Conservation of Nature Red List of Threatened Species. Version 2014.2. <<u>www.iucnredlist.org</u>>. Downloaded on 02 October 2014.
- LLOYD, HUW. 2008. Foraging ecology of High Andean insectivorous birds in remnant Polylepis Forest patches. *The Wilson Journal of Ornithology*, 120(3): 531-544.

- LLOYD, H. & S.J. MARSDEN. 2008. Bird community variation across Polylepis woodland fragments and matrix habitats: Implications for biodiversity conservation within a high Andean landscape. *Biodiversity Conservation*, 17:2645-2660.
- MORA, C. D.P. TITTENSOR, S. ADL, A.G.B SIMPSON, AND B. WORM. 2011. How many species are there on earth and in the ocean? *PLoS Biology* 9(8):1-8.
- RASMUSSEN, P.C. 2011. Avian Vocalizations Center: Cinereous Conebill, *Conirostrum cinereum littorale*. Recording AV#12531.
- RIDGLEY, R.S. AND P.J. GREENFIELD. 2001. The Birds of Ecuador. Cornell University Press.
- SCHULENBERG, T.S., D.F. STOTZ, D.F. LANE, J.P. O'NEILL & T.A. PARKER III. 2007. Birds of Peru. Princeton University Press.
- VOGT, C.A. 2006. Secondary nectar robbing, a previously unsubstantiated foraging behavior of the Cinereous Conebill (*Conirostrum cinereum*). *Ornitologia Neotropical*, 17: 613-617.
- ZIMMER, J.T. 1942. Studies of Peruvian Birds. No. XLIII: Notes on the genera Dacnis, Xenodacnis, Coereba, Conirostrum, and Oreomanes (Conirostrum cinereum cinereum and Conirostrum cinereum littorale, p14-15). Published by The American Museum of Natural History.

#### **CHAPTER 2**

# Incipient speciation in the Andes: relative roles of climate versus isolation in the adaptive diversification of the Cinereous Conebill

Andrea N. Chavez, Chandrasekhar Natarajan, Jay F. Storz, Angela Fago,

and Christopher C. Witt

#### **INTRODUCTION**

For Andean songbirds, the average elevational range breadth is ~1250 m, and relatively few species have elevational ranges broader than 3000 m (Parker et al. 1996). This pattern is surprising in light of the fact that the Andes mountains are characterized by steep topographic inclines across relatively short distances. In the Peruvian Andes, elevations of over 4000 m are as close as ~50 km to sea-level along the Pacific coast or near-sea-level in the Amazonian lowlands. Climatic variables such as temperature and precipitation vary greatly along elevational gradients and interact with a variety of topographic features to create a diverse distribution of ecosystems. This diversity is what characterizes mountains as "evolutionary engines" and consequent biodiversity hotspots, especially in the tropics.

Janzen (1967) hypothesized that mountain passes are 'higher in the tropics' where selection favors organisms with more narrow thermal tolerances, and consequently elevational ranges, as compared to organisms at higher latitudes (Janzen 1967). At very high elevations, organisms experience a much broader climatic range, with temperature fluctuations being much greater than they are in the lowlands. This increased climatic

variability, coupled with other limitations at high altitude such as decreased partial pressure of oxygen, may pose physiological challenges for life. As a possible result of these challenges, fewer species inhabit the extreme highlands, and those that do possess adaptive traits to cope with low oxygen and colder temperatures (Natarajan et al. 2016). Nonetheless, elevational generalists spanning ranges upward of 3,000 m occur in the Andes Mountains. These organisms provide opportunity for studying the evolutionary implications of broad versus narrow elevational ranges.

It is possible that elevational generalists are an exception to Janzen's hypothesis, and perhaps possess adaptive or physiological traits suited for coping with high environmental variability. An alternative hypothesis is that evolutionary instability is an inherent quality of widely distributed populations spanning geographically and climatically heterogeneous expanses, and therefore selection should ultimately favor specialization (Ricklefs & Cox 1972). Genetic examination of broadly distributed taxa can reveal population structure and gene flow, potentially illuminating the processes of incipient divergence and speciation. Furthermore, the physiological and genetic consequences of the elevational gradient are a critical component of any discussion of diversification in elevational generalist species as high-altitude hypoxia can be implicated in the restriction of species distributions.

Among the ~3,500 species of birds that inhabit the Andes, >200 occur above 4000 m elevation, where oxygen availability is reduced by ~40% relative to sea level (Fjelsda & Krabbe 1990). This remarkable degree of hypobaric hypoxia imposes severe challenges

for the oxygen uptake that is required to sustain aerobic metabolism (Storz 2016). Among the environmental conditions contributing to the difficulty of life at high altitude (e.g., increased solar radiation, decreased temperatures), the paucity of oxygen may be the most challenging for air-breathing organisms. Among high-altitude animals, birds are of extraordinary interest due to their use of metabolically demanding powered flight as their main form of locomotion. Despite its relative efficiency over long distances, flight is the most energetically costly mode of locomotion (Schmidt-Nielson 1972), and it becomes increasingly difficult to generate lift as barometric pressure decreases with altitude (Altschuler & Dudley 2006). Not only must birds cope with limited ambient oxygen availability for basic metabolic functioning, they must simultaneously cope with the intense metabolic demands of flapping flight (Chai & Dudley 1995).

Birds inherently possess oxygen transport characteristics that distinguish them from other vertebrates such as enhanced gas-exchange efficiency in the lungs, maintenance of oxygenation in the brain during hypoxia, augmented oxygen diffusion capacity in peripheral tissues, and high aerobic capacity (Scott 2011). Although these traits allow birds in general to cope with certain relatively low levels of hypoxia, most species cannot tolerate severe hypoxia (Black & Tenney 1980). An increasing body of literature seeks to understand the physiological attributes that facilitate the remarkable ability of some birds to overcome the physical limitations of hypoxic stress and ultimately thrive in high-altitude environments.

Several wild vertebrates restricted to high altitude environments have been found to possess adaptations for overcoming the physiological challenge of hypoxia (Storz et al. 2010). Many studies of high-altitude adaptation in the past have taken an interspecific comparative approach examining taxa with contrasting elevational distributions. While early studies often compared distantly related organisms (Tucker 1968; Bishop 1997; Piiper & Scheid 1972), more recent work has focused on comparisons of closely related or congeneric species (McCracken 2009; Projecto-Garcia et al. 2013) and intraspecific study of altitudinal generalists (Storz et al. 2009; Bulgarella et al. 2011; Galen et al. 2014; Cheviron et al. 2014).

Species that occur continuously across broad elevational distributions can improve the study of molecular adaptation through incorporation of biogeographic elements. It is important to note that ecotonal turnover is more rapid across elevational gradients than latitudinal gradients. This gradation in environmental variation across elevation is important when considering the relative roles of genotypic specialization and phenotypic plasticity in physiological adaptation (Storz et al 2010). Elevational generalists are therefore appropriate subjects on which to investigate adaptive mechanisms for high-altitude life.

Birds that fly at high altitudes must be capable of intense exercise in an oxygen-limited environment. Therefore, to understand the evolutionary implications of high-altitude colonization, we should pay attention to the various steps in the oxygen transport pathway. Many species possess high hemoglobin-oxygen (Hb- $O_2$ ) affinities, manifested

as a left-shifted oxygen dissociation curve (decrease in  $P_{50}$ , the partial pressure of  $O_2$  at which blood is 50% saturated with O<sub>2</sub>) (Chapell et al. 1988; Jurgens et al. 1988; Weber et al. 2002; Jessen et al. 1991; Scott & Milsom 2007; Storz et al. 2009; Galen et al. 2015). This adaptation in the circulatory oxygen delivery step of the pathway allows for improved oxygen delivery to the blood and circulation throughout the body. Max Perutz proposed that adaptive modifications of hemoglobin function would likely be due to a small number of amino acid replacements at key positions that affect the protein's physical structure (Perutz 1983). This is the case in Andean geese, in which a single substitution at the Leu-55 $\beta$ (D6) position changes a leucine to a serine, resulting in a 2-carbon gap at the  $\alpha 1\beta 1$  subunit contact, relaxing the deoxy structure, thus increasing O<sub>2</sub> affinity in high-altitude populations (Weber et al. 1993). Other types of mutations increase Hb-O<sub>2</sub> affinity by suppressing Hb sensitivity to binding of allosteric effectors such as Cl- and Inositol Pentaphosphate that otherwise preferentially bind and stabilize the low-affinity conformation of avian hemoglobin (Storz and Moriyama 2008). The role of these types of adaptations in facilitating the ability of elevational generalists to cope with life at both high and low elevations is still not fully understood.

Elevational generalism is relatively rare in the tropical Andes Mountains, likely owing to the inherent requirements of enduring a high degree of climatic zonation and coping with hypoxic stress. The Cinereous Conebill (*Conirostrum cinereum*) appears to be an exception, and inhabits a continuous elevational distribution that spans over 4500 m. Three putative subspecies have been described. Two subspecies, *cinereum* and *fraseri*, are restricted to high elevations and may be isolated, whereas the third and most widespread, *littorale*, occurs continuously along the western slope of the Andes from 0 to over 4500 m. They are purported to differ in plumage, body size, elevational distribution, and ecological zone of occurrence. However, no quantitative evaluation of these or other traits exists. First, we aim to characterize the morphology, genetics, and climatic niche of the three subspecies using a comparative biogeographic approach to explore patterns and timing of differentiation and to consider possible mechanisms of diversification. Second, we aim to discover whether hemoglobin adaptation plays a role in this elevational generalist's ability to thrive in high-elevation environments, and whether localized adaptation is possible despite altitudinal migration and gene flow.

The broad, continuous elevational distribution of *C. cinereum* on the west slope of the Andeas and its apparent isolation from high-altitude restricted populations on the eastern side of the Andean altiplano provide a natural experiment to test the effects of environmental variation relative to the effects of isolation as causes of functional divergence. Using a comparative phylogeographic framework, we examine whether lineage divergence within *C. cinereum* is associated with climatic, geographic, and/or physiological barriers leading to incipient speciation. Here, we use museum specimens of *C. cinereum* to quantify morphological differences, test for genetic differentiation, and compare blood properties among populations. Using bioclimatic data, we analyze patterns of lineage diversification and distribution as they relate to climate and geography. Characterizing the genetic structure of hemoglobin and testing protein functional properties between high- and low-elevation individuals will give us insight into mechanisms of high-altitude adaptation.

If we do not find evidence for diversification among populations, it would suggest that *C*. *cinereum* is in fact an exception to Janzen's hypothesis. Conversely, if we find morphological and genetic evidence of lineage differentiation, it would suggest the presence of isolating barriers which may be leading to consequent incipient speciation. If genetic structure is detected within the continuously distributed coastal subspecies, *littorale*, it would signal limited geneflow and therefore increased opportunity for population divergence. This result would suggest that C. cinereum is not an exception to Janzen's hypothesis, and is in fact diversifying possibly due to the exclusive or cumulative effects of climate, geography (e.g., elevation, latitude).

#### **METHODS**

#### Morphological traits

To assess differences in body size and proportions among populations of cinereous conebills, we used body mass and four morphological variables that are commonly used in avian studies: wing chord, tarsus, tail, and culmen. These variables were measured from 87 museum skins (64 from the Museum of Southwestern Biology, 23 from the American Museum of Natural History; Appendix A).

#### DNA extraction, PCR amplification, and sequencing of mtDNA

We extracted genomic DNA from frozen muscle or liver tissue from 125 cinereous conebill specimens from the Peruvian Andes and adjacent lowlands (elevational range: 39 to 4330 meters; Museum of Southwestern Biology (MSB) specimens; Appendix B). We also extracted DNA from the dry toe pads of 38 cinereous conebill specimens from Andean localities in Peru, Ecuador and Colombia (American Museum of Natural History (AMNH) specimens, Appendix B). All MSB tissue samples were collected by flashfreezing in liquid nitrogen within a few hours of death, and were subsequently stored at -80°C. DNA extraction from frozen tissues was conducted using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. Toe pad samples were obtained from museum skins that were greater than 50 years old. Therefore, we used a modified DNA extraction protocol, using reagents from the DNeasy Blood and Tissue Kit and spin columns from the QIA amp DNA Micro Kit (Qiagen, Valencia, CA, USA). This protocol was largely based on three protocols (protocol designed by Brian T. Smith at AMNH, Ancient DNA extraction protocol designed by Sabrina Taylor at Louisiana State University, and extraction protocol designed in John McCormack lab at the Moore Laboratory of Zoology) and modified by Andres Cuervo. Polymerase chain reaction (PCR) was used to amplify 1041 base pairs (bp) of the cinereous conebill mitochondrial gene ND2. For the MSB specimens we used the primers L5219 and H6313 (Sorenson et al. 1999). Each 15-µl PCR reaction contained 1 µl template DNA, 1.5 µl 1X Taq-GOLD PCR buffer, 2 µl MgCl<sub>2</sub>, 0.3 DNTP, 0.75 µl of each primer, 8.5  $\mu$ l DNA-free H<sub>2</sub>0, and 0.15  $\mu$ l of Taq-GOLD DNA polymerase. The thermal profile consisted of an initial 8 minute step at 94°C, followed by 35 cycles of 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 45 seconds with a final 10 minute extension at 72°C. Due to the much shorter DNA fragments extracted from AMNH toe pad samples (~200 bp), we used a combination of published primers and designed novel primers from known sequences to amplify the entire ND2 gene in 6 fragments (Table 1). These PCR reactions used the same protocol. Each 15-µl PCR reaction contained 2 µl template DNA,

1.5  $\mu$ l 1X Taq-GOLD PCR buffer (Life Technologies), 1.25 MgCl<sub>2</sub>, 0.3 DNTP, 0.75  $\mu$ l of each primer, 8.25  $\mu$ l DNA-free H<sub>2</sub>0, and 0.15  $\mu$ l Taq-GOLD DNA polymerase. The thermal profile consisted of an initial 8 minute step at 94°C, followed by 40 cycles of 94°C for 30 seconds, 49°C for 30 seconds, and 72°C for 1 minute and 15 seconds with a final 8 minute extension at 72°C.

Negative controls were included in each PCR to check for contamination. No contamination was detected during the course of study. All PCR products were stained with SYBR Safe (Invitrogen) and visualized on a 2% agarose gel to check for the presence of a PCR product of the expected length gauged by 1 kb DNA ladder. All successful amplifications were purified using Exo-Sap-IT (USB, Cleveland, OH) and sequenced in both directions using dye terminator cycle sequencing (Big Dye, ABI) on an ABI 3130 automated sequencer (Applied Biosystems, Foster City, CA). Sequences were aligned using Sequencher Version 5.4.6 (Gene Codes Corporation, Ann Arbor, MI, USA) and Geneious Version 8.1 (Kearse et al. 2012).

#### Environmental Data

To evaluate climatic variation across the distribution of *C. cinereum*, we used the WorldClim dataset (Hijmans et al. 2005), which is a set of 19 bioclimatic variables representing various averages and extremes of temperature and precipitation, as interpolated from weather stations worldwide (Appendix C). We downloaded global environmental grids at a 30 arc-second spatial resolution and used the ArcGIS data management toolbox to extract data on all variables for each individual collection

locality. The elevation dataset was compiled from museum specimen locality data. Samples representing the subspecies *fraseri* were obtained from the American Museum of Natural History, and, due to their age, did not have accompanying geographic coordinates. The written locality descriptors contained place names and approximate elevations, and were used to manually estimate the collection localities for those specimens.

#### **Blood** properties

Data on blood properties were collected from live birds in the field. Birds were netted for scientific collection. Whole arterial blood was drawn from the brachial vein, from which hemoglobin concentration and hematocrit were measured. Hemoglobin concentration was measured using 10  $\mu$ l samples of blood in a Hemocue Hb 201+ Analyzer in g/dL. Heparinized capillary tubes were filled ~ 2/3 to 3/4 full with whole blood, centrifuged at 2500 rpm for 4 minutes, immediately after which the ratio of volume of red blood cells to total volume of blood was measured with digital calipers (Appendix D).

#### Cloning and preliminary sequencing of globin genes

We initially screened sequence variation in a panel of 14 conebill specimens (7 highland and 7 lowland birds). We extracted RNA from whole blood using the RNeasy kit, and we amplified full-length cDNAs of the  $\alpha^A$ -,  $\alpha^D$ -, and  $\beta^A$ -globin genes using a OneStep RT-PCR kit (Qiagen, Valencia, CA, USA). We designed paralog-specific primers using 5' and 3' UTR sequences, as have been previously described (Grispo et al. 2012; Cheviron et al. 2014; Galen et al. 2015; Natarajan et al. 2015; Opazo et al. 2015). We

cloned reverse transcription (RT)-PCR products into pCR4-TOPO vector using the TOPO<sup>®</sup> TA Cloning<sup>®</sup> Kit (Invitrogen, Carlsbad, CA, USA), and we sequenced at least five clones per gene in each individual in order to recover both alleles. This enabled us to determine full diploid genotypes for each of the three adult-expressed globin genes in each individual specimen. As explained in the results section, we also conducted a survey of  $\alpha^{4}$ -globin sequence variation in a larger panel of conebill specimens. Methods are in the following section. All sequences were deposited in GenBank (Appendix B).

#### DNA extraction, PCR amplification, and Sanger sequencing of globin genes

We extracted genomic DNA from frozen muscle and liver tissue from 125 cinereous conebill specimens from the Peruvian Andes and adjacent lowlands, sampling putative subspecies *cinereum* and *littorale* (elevational range: 39 to 4330 meters; Museum of Southwestern Biology (MSB) specimens; Appendix B). All tissue samples were collected by flash-freezing in liquid nitrogen within a few hours of death, and were subsequently stored at -80°C. DNA extraction from frozen tissues was conducted using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol.

Polymerase chain reaction (PCR) was used to amplify 429 bp of Hb  $\alpha$ -A (full gene) and 315 bp of Hb  $\beta$ -A (exons 1-2). The primers used to amplify these loci can be found in Table 2. For Hb  $\alpha$ -A, each 15  $\mu$ l PCR reaction contained 2  $\mu$ l template DNA, 1.5  $\mu$ l 1X Taq-GOLD PCR buffer, 2  $\mu$ l MgCl<sub>2</sub>, 0.3 DNTP, 0.75  $\mu$ l of each primer, 7.5  $\mu$ l DNA-free H<sub>2</sub>0, and 0.15  $\mu$ l of Taq-GOLD DNA polymerase. The thermal profile consisted of an

initial 8 minute step at 94°C, followed by 35 cycles of 94°C for 30 seconds, 71°C for 30 seconds, and 72°C for 1 minute and 30 seconds with a final 10 minute extension at 72°C. For Hb  $\beta$ -A, each 15  $\mu$ l PCR reaction contained 1  $\mu$ l template DNA, 1.5  $\mu$ l 1X Taq-

GOLD PCR buffer, 2  $\mu$ l MgCl<sub>2</sub>, 0.3 DNTP, 0.75  $\mu$ l of each primer, 8.5  $\mu$ l DNA-free H<sub>2</sub>0, and 0.15  $\mu$ l of Taq-GOLD DNA polymerase. The thermal profile consisted of an initial 8 minute step at 94°C, followed by 35 cycles of 94°C for 30 seconds, 50°C for 30 seconds, and 72°C for 45 seconds with a final 10 minute extension at 72°C.

Negative controls were included in each PCR to check for contamination. No contamination was detected during the course of study. All PCR products were stained with SYBR Safe (Invitrogen) and visualized on a 2% Agarose gel to check for the presence of a PCR product of the expected length gauged by 1 kb DNA ladder. All successful amplifications were purified using Exo-Sap-IT (USB, Cleveland, OH) and sequenced in both directions using dye terminator cycle sequencing (Big Dye, ABI) on an ABI 3130 automated sequencer (Applied Biosystems, Foster City, CA). Alignments of forward and reverse primer sequences and alignments of mtDNA sequence for each individual were calculated in Sequencher Version 5.4.6 (Gene Codes Corporation, Ann Arbor, MI, USA) and Geneious Version 8.1 (Kearse et al. 2012).

#### Characterization of Hb isoform composition

We used a combination of tandem mass spectrometry (MS/MS) and isoelectric focusing (IEF) to characterize the Hb isoform composition of red blood cells from the same 14 conebill specimens used in the initial survey of globin sequence variation. The HbA and HbD isoforms were separated by means of IEF using precast Phast gels (pH 3–9)(GE

Healthcare Bio-Sciences, Pittsburgh, PA, USA; 17-0543-01). IEF gel bands were then excised and digested with trypsin, and MS/MS was used to identify the resultant peptides, as described previously (Storz et al. 2010, 2011; Revsbech et al. 2013; Opazo et al. 2015; Storz et al. 2015). Database searches of the resultant MS/MS spectra were performed using Mascot (Matrix Science, v1.9.0, London, UK); peptide mass fingerprints were queried against a custom database of avian globin sequences, including the full complement of embryonic and adult  $\alpha$ - and  $\beta$ -type globin genes annotated in avian genome assemblies (Hoffmann et al. 2010, 2011; Opazo et al. 2015). We identified all significant protein hits that matched more than one peptide with *P*<0.05. After electrophoretically separating the HbA and HbD isoforms in each individual hemolysate, we used densitometric measurements (Abramoff et al. 2004) to quantify the relative abundance of the two isoforms.

#### Measurement of Hb functional properties

For hemolysates of birds with known genotypes for each of the adult-expressed globin genes, the HbA and HbD isoforms were separated and were stripped of organic phosphates and other anions by ion-exchange fast-protein liquid chromatography (FPLC) using a HiTrap QHP column (GE Healthcare). O<sub>2</sub>-equilibrium curves were measured on 3  $\mu$ l thin-film samples (0.3 mM heme concentration) at 37°C, 0.1 M HEPES buffer (pH 7.4). Using standard experimental conditions, we measured oxygenation properties of purified Hb solutions under four treatments: (*i*) in the absence of allosteric effectors ('stripped'), (*ii*) in the presence of Cl<sup>-</sup> (in the form of KCl (0.1 M)), (*iii*) in the presence of IHP (IHP/Hb tetramer ratio = 2.0), and (*iv*) in the simultaneous presence of both

effectors. The curves were measured using a modified  $O_2$  diffusion chamber and absorption at 436 nm was monitored while subjecting thin-film samples to varying  $O_2$ tensions of gas mixtures (prepared using Wösthoff gas-mixing pumps that perfuse the chamber; Weber et al. 2004; Storz et al. 2012).  $P_{50}$  ( $O_2$  tension at half-saturation) and  $n_{50}$ (Hill's cooperativity coefficient at half-saturation) values were obtained by fitting the Hill equation to the experimental  $O_2$  saturation data using a nonlinear regression model. Free Cl<sup>-</sup> concentrations were controlled with a model 926S Mark II chloride analyzer (Sherwood Scientific Ltd, Cambridge, UK).

#### RESULTS

#### Morphological Differentiation

All statistical analyses of morphological data were conducted in the program R Version 3.3.2 (R Core Team 2016). We conducted a principal component analysis to visualize the variation among subspecies. We also conducted a series of pairwise comparisons of morphological traits between subspecies samples. A Welch's two-sample t-test was used to compare mass between *littorale* and *fraseri*. We used a multiple analysis of variance (MANOVA) to test for differences in all other morphological measures. We tested for normality and equal variance among sample means using Shapiro-Wilks and F-tests, respectively. These tests indicated the pairwise tests performed were appropriate. Results of the principal component analysis suggested that over 95% of the variation among subspecies is explained by wing chord and tail length, with *cinereum* and *littorale* grouping completely separate from one another (Figure 1). The results of pairwise comparisons revealed significant morphological differences among subspecies (Figure 2;

Table 3). Body mass varies by subspecies, with *cinereum* weighing over a gram more than *littorale* [*cinereum*: µ=9.337, SD=0.264, n=9; *littorale*: µ=8.125, SD=0.612, n=42; t-test: t=9.377, p=2.495e-10]. No body mass data for *fraseri* was available. Tarsus length was significantly longer in *cinereum* and *fraseri* than in *littorale*, but there was no difference between *cinereum* and *fraseri* [*cinereum*:  $\mu = 18.241$ , SD=0.831, n=10; littorale: µ=17.0, SD=0.808, n=54; fraseri: µ=18.264, SD=0.667, N=23]. In littorale, male wing chord length was found to average 3 mm longer than females, but no difference between sexes was observed in *cinereum*. Wing chord length was approximately 5 mm longer in *cinereum* than in *littorale* [*cinereum*:  $\mu$ =61.466, SD=2.088, n=10; *littorale*: µ=56.265, SD=2.708, n=54; *fraseri*: µ=58.713, SD=2.529, N=23]. Tail length was approximately 7 mm longer in *cinereum* than in *littorale* [*cinereum*:  $\mu$ =52.5, SD=1.779, n=10; *littorale*:  $\mu$ =45.528, SD=2.416, n=53; *fraseri*:  $\mu$ =50.282, SD=2.9612, N=23]. Culmen length did not vary by subspecies [*cinereum*:  $\mu$ =7.222 mm, SD=0.339, n=10; *littorale*:  $\mu$ =7.159, SD=0.328, n=54; *fraseri*:  $\mu$ =7.025, SD= 0.494, N=23].

These data suggest that the three subspecies differ in body size and proportions. No difference was found in culmen length. However, body mass, tarsus, tail, and wing chord were largest in the highland *cinereum* and smallest in the coastal *littorale* (Figure 2). *Fraseri* appears to be intermediate in size between *cinereum* and *littorale*, but shows no significant difference from *cinereum* for tarsus length.

Since *littorale* occurs throughout a broad elevational range, we compared these traits between the low- and high-altitude *littorale* populations. Individuals occurring below 1000 m were considered low-altitude, and individuals occurring above 2000 m were considered high-altitude. Remarkably, no morphological characters varied within *littorale* across elevation, even when individuals from the most extreme ends of the elevational range were compared (i.e., low=0-1000 m, high=3000-4500 m). The pattern of morphological uniformity within ssp. *littorale* is in contrast to the between-subspecies pattern in which larger bodies characterized both high-altitude subspecies, each of which generally occurs above 2500 m elevation.

#### **Population Genetic Differentiation**

We ran a series of statistical analyses on molecular data to characterize divergence within *C. cinereum*. Using the Gene Flow and Genetic Differentiation function in the program DnaSP Version 5 (Librado & Rozas 2009), we compared mitochondrial gene ND2 sequences between subspecies, which were assigned based on plumage morph and collection locality. Fst values were significant for all three pairwise comparisons (p<0.001) (Figure 3). As with the morphological data, we also compared low- and high-altitude populations of *littorale* to assess genetic variation along the western elevational cline of the Peruvian Andes. This comparison yielded an extremely low Fst value and no significant difference between low- and high-altitude individuals, suggesting there is likely substantial gene flow among *littorale* populations despite its broad elevational distribution (Figure 3).

<u>Haplotype Network</u>. To assess genetic structure within and among populations, we estimated haplotype diversity (Hd=0.8839), and generated a haplotype data file in DnaSP, which was then imported into the program Population Analysis with Reticulate Trees (PopART). We generated a median-joining haplotype network for our intraspecific phylogeny using the 30 haplotypes, calculated in DnaSP (Figure 4). Consistent with the genetic differentiation results, individuals purportedly belonging to *littorale* and *cinereum* populations grouped together. However, not all individuals from the *fraseri* population grouped together. Two similar haplotypes were much more closely related to *littorale* than to other *fraseri* haplotypes (Figure 4). The three individuals representing these haplotypes had plumage characteristics typical of *fraseri*, and were collected from southern Ecuador, which has been proposed as the southern edge of *fraseri*'s geographic range where it abuts or potentially overlaps with the northern edge of the range of *littorale*. These specimens were collected from  $\sim -5^{\circ}$  latitude, which raises the question of whether latitude plays a role in geographic division of these populations.

#### Molecular Dating and Population Delimitation

We first estimated tree topology using maximum likelihood methods in RAxML (Stamatakis 2014). To estimate the maximum likelihood tree, we executed a rapid bootstrap analysis and searched for the best scoring ML tree with 100 bootstrap replicates in one run, using a GTRGAMMA mode of nucleotide evolution. We included 87 individuals from *littorale*, 26 from *cinereum*, and 12 from *fraseri*, and *Conirostrum sitticolor* as the outgroup. We visualized the tree using FigTree Version 1.4.2 (Rambaut 2006-2014). The ML tree supports the clear monophyly of *cinereum* with a bootstrap

value of 84. The genetic structure between *littorale* and *fraseri* was more difficult to characterize, as 9 out of the 12 *fraseri* samples grouped together with a bootstrap value of 77, while the remaining 3 forming a separate clade that was nested within the *littorale* clade and whose monophyly was supported with a bootstrap value of 70. Both clades of *fraseri* were closer to *littorale* than to *cinereum* (Figure 5). These phylogenetic results indicate that *fraseri* mtDNA is not monophyletic, but they are not consistent with ongoing geneflow between subspecies. Subspecies *cinereum* is monophyletic, as is the clade containing *fraseri* plus *littorale*.

Using Bayesian inference, we estimated divergence time in BEAST v. 1.7.5 (Drummond et al. 2012; Drummond et al. 2002) implemented with BEAGLE (Ayers et al. 2012). We included 87 individuals from *littorale*, 26 from *cinereum*, and 12 from *fraseri*. We conducted the analysis with *Conirostrum sitticolor* as the outgroup, and again including ND2 sequences from other members of the conebill clade to place the evolutionary analysis of *C. cinereum* into a broader phylogenetic context. These taxa included *Conirostrum bicolor*, *Conirostrum, margaritae*, *Conirostrum speciosum*, *Conirostrum leucogenys*, *Conirostrum albifrons*, *Oreomanes fraseri*, *Conirostrum ferrugineiventre*, *Conirostrum sitticolor*, and *Conirostrum tamarugense*, all of which were obtained from Genbank with accession numbers listed in Appendix A. The only missing species from this clade is *Conirostrum rufum*, for which the published ND2 sequence was unavailable. We used a Yule speciation process for the tree prior with a strict clock and a clock.rate of 0.029 (equivalent to 2.9% Myr<sup>-1</sup>) derived from Lerner et al. (2011). We used a lognormal prior distribution for the *clock.rate* parameter with mean = 0.029 and standard deviation

= 0.25. Substitution rate priors derived from ND2 substitution rates for Hawaiian honeycreepers were used as upper and lower rate bounds (0.024 and 0.033 substitutions per site Myr<sup>-1</sup>) (Andersen et al. 2015; Lerner et al. 2011). We ran 2 independent MCMC chains for 10 million generations and sampled every 1000th generation. We examined burn-in and convergence diagnostics in TRACER Version 1.6.0; burn-in values were specific to each run with at least 20% of samples discarded. After reviewing the trace file in TRACER and verifying that the run converged on the posterior distributions and reached stationarity, we combined the sampled trees into a single tree file using Tree Annotator v2.4.0 (Rambaut & Drummond 2016) to produce a maximum-clade credibility tree, setting a burn-in percentage of 20, thus discarding the first 20% of the samples in the tree file. We visualized the tree using FigTree Version 1.4.2 (Rambaut 2006-2014). Lacking fossil calibration data for this group, we relied on published rates of mtDNA sequence evolution to calibrate our divergence dating analyses. Using a general substitution rate from distantly related species is not ideal (e.g. tanagers versus honeycreepers), but we note that mtDNA substitution rates across birds cluster around this value (Weir & Price 2011; Weir et al. 2008). Regardless, these date estimates can only be used as a rough guide to clade ages. We chose ND2 because it is one of the fastest-evolving mitochondrial gene regions in birds (Lerner et al. 2011) and it is used widely among avian systematists and phylogeographers and is appropriate for intraspecies comparisons (Andersen et al. 2015).

Tree topologies inferred from maximum-likelihood and Bayesian runs were highly concordant. The BA tree supports a monophyletic *cinereum* with a Bayesian posterior

probability of 1. As in the ML analysis, the relationship between *littorale* and *fraseri* is less clear (Figure 6). There is limited phylogenetic structure at the base of the clade containing *littorale* and *fraseri*. This may have occurred due to incomplete lineage sorting (ILS) during the diversification of this clade; the likelihood of ILS is high during rapid successive branching as occurred in this clade (Figure 6). Alternatively, lack of mtDNA monophyly in fraseri could indicate historic admixture between southern populations of *fraseri* and northern populations of *littorale*, a possibility that would be difficult to rule out even with high coverage genomic data. The same placement of *fraseri* samples observed in the ML tree was seen in the BA tree as well. The same 9 out of 12 samples grouped together with a posterior probability of 1, and the remaining 3 samples also grouped together with a posterior probability of 0.94. Figure 6 shows the most well supported Bayesian tree with posterior probabilities included for the major branches of interest. Estimated divergence time are exceptionally recent, entirely late Pleistocene to Holocene. This analysis approximates the split between its closest relative (included in this analysis), Conirostrum tamarugense, at 700,000 years before the present, and suggests that *cinereum* split from *fraseri* and *littorale* slightly ~150,000 years ago. The *fraseri-littorale* clade is ~100,000 years old, whereas the most recent common ancestor of *cinereum* is ~140,000 years old, and the age of the northern clade of *fraseri* is ~50,000 years.

#### **Environmental Data**

To shed light on the potential role of environmental factors in the distribution of *C*. *cinereum*, we analyzed a set of 19 bioclimatic variables representing various averages

and extremes of temperature and precipitation, as interpolated from weather stations worldwide. These values are listed in Table 4. We ran a principal component analysis using the program R Version 3.3.2 (R Core Team 2016), which included all 19 variables, and visualized the data to look for obvious patterns of variation among subspecies. We looked for variables that exhibited a near-zero variance to exclude those from analysis, but none existed. The results suggested that over 97% of the variance was explained by elevation and temperature seasonality (Figure 7). Annual precipitation and annual temperature range also appeared to be important. We therefore examined these three variables in greater detail. We expected to see a trend related to elevation because higher variability in temperature and, at least within the range of *littorale*, higher amounts of precipitation characterize high elevations. We plotted frequencies of temperature seasonality, annual temperature range, and annual precipitation by subspecies, splitting out *littorale* into high- and low-elevation groups (Figure 8). Individuals occurring below 1000 m were considered low-elevation, and individuals occurring above 2500 m were considered high-elevation.

As predicted, the distribution of precipitation values showed that higher elevation populations experience higher levels of annual precipitation (Figure 8c). However, temperature seasonality and annual temperature range did not show the elevational structure we expected. Annual temperature range was higher in *cinereum* and high*littorale* than it was for low-*littorale*, but the average range for *fraseri* was drastically lower than all other groups (Figure 8b). The latter result is almost certainly due to latitude, *fraseri* being closest to the equator (see below). Similarly, temperature

seasonality was drastically lower in *fraseri* than all other groups. It was also drastically higher in low-*littorale* than all other groups (Figure 8c). These results reflect complex daily and seasonal temperature variation across the complex topography and latitudinal gradient of the tropical Andes.

The northern boundary of *C. cinereum*'s geographic distribution centers on the equator, and is coincident with the described distribution of the *fraseri* subspecies. The sampled localities represent a substantial latitudinal gradient along which these variables may change. To determine the possible effect of latitude on these climatic variables, we ran linear regression analyses against latitude. Temperature seasonality and annual temperature range were significantly negatively correlated with latitude (Figure 9a, 9b), and precipitation was significantly positively correlated with latitude (Figure 9c). Note that these correlations are opposite of what is typical of North American studies because we treated latitudes south of the equator as negative values. Birds farther from the equator experience higher temperature variability and decreased rainfall (Figure 9). These data suggest that *fraseri* experiences much lower temperature variation than expected given its high elevation distribution, due to its proximity to the equator.

#### **Blood Properties**

Comparisons of hematocrit (Hct) and hemoglobin concentration ([Hb]) between subspecies indicate that these blood properties do not differ between *cinereum* and *littorale*. I performed Shapiro-Wilks tests for normality of data and tests for equal sample variances in the statistical program R Version 3.3.2 (R Core Team 2016). Based on these

results along with visual inspection of the data suggest that the data are normal and that sample variances do not differ (Shapiro-Wilk normality tests: *littorale* Hct, W=0.9801, p=0.195; *cinereum* Hct, W=0.92871, p=0.2326; *littorale* [Hb], W=0.98578, p=0.6816; *cinereum* [Hb], W=0.97856, p=0.9587). I also conducted F tests to compare variances, which indicated sample variances do not differ (Hct: F=1.7112, df=87, *p*=0.2409; [Hb]: F=1.0517, df=62, *p*=0.9758). I therefore conducted Welch two-sample t-tests to compare sample means of Hct and [Hb] between subspecies. Mean Hct did not differ between subspecies (t=1.3764, df=25.362, p-value=0.1807, 95 % CI= -0.007990957, 0.040259067,  $\mu_{cin}$ = 0.5242172,  $\mu_{lit}$ =0.5080832). Mean [Hb] also did not differ between subspecies (t=1, df=21.584, p-value=0.3284, 95 % CI= -0.6460591, 1.8466940,  $\mu_{cin}$ =17.52000,  $\mu_{lit}$ =16.91968).

Subspecies *littorale* occurs along a substantial elevational range from sea level to ~4500 m. We therefore compared high- and low-altitude populations of *littorale*. We calculated simple linear regressions to predict Hct and [Hb], respectively, based on elevation. We found a significant positive relationship between elevation and Hct (F=53.63; p < 0.001; R<sup>2</sup>=0.38; Figure 10), and a significant positive relationship between elevation and [Hb] (F=73.52, p<0.001, R<sup>2</sup><sub>adj</sub>=0.5391; Figure 10). Interestingly, the elevated Hct and [Hb] levels detected in the high-altitude populations of *littorale* were similar to the values observed in *cinereum* (highland population). Welch two-sample t-tests comparing means of Hct and [Hb] between *cinereum* and high-altitude *littorale* populations indicate that all high-altitude individuals of *C. cinereum* (>2500 m) have slight but significantly elevated Hct and [Hb] (t= -0.4819, df= 20.178, p-value = 0.6351, 95% CI= -1.5149978,

0.9461089,  $\mu_{cin}$ =17.52,  $\mu_{lit=high}$ =17.80) (Figure 10). Low elevation *littorale* have reduced values likely to optimize oxygen uptake and delivery in a normobaric environment.

#### Altitudinal patterns of sequence variation

In our initial panel of highland and lowland conebill specimens, we identified a single amino acid polymorphism in the  $\alpha^{A}$ -globin gene (134Thr/Ser) that exhibited a pronounced altitudinal difference in allele frequency. The Ser variant was present at a frequency of 0.64 in the sample of highland birds and it was altogether absent in the lowland birds (*n*=14 alleles in each population sample). By contrast, the  $\alpha^{D}$ - and  $\beta^{A}$ globin genes harbored a small number of low-frequency amino acid variants, but were otherwise invariant. On the basis of these findings, we then surveyed  $\alpha^{A}$ -globin sequence variation in a larger panel of conebill specimens to characterize the elevational patterns of variation. A test for gene flow and genetic differentiation was conducted between *littorale* and *cinereum* for Hb-  $\alpha^A$ , Hb- $\beta^A$ , and their associated introns. The resulting Fst values were significantly different from zero and suggest differentiation between subspecies (Figure 11). We conducted the same test between low- and high-altitude populations of *littorale*, and no difference was detected (Figure 12). Low altitude was considered anything below 1000 m, and high altitude was considered anything above 2500 m. These results suggest that adaptation is not likely to occur where there is gene flow.

Our phylogeographic analysis of *C. cinereum* allowed us to analyze the extended panel of globin gene sequences under a phylogenetic framework. We compared allele frequencies

of the Hb- $\alpha^4$  substitution at amino acid position 134 (Thr/Ser) between subspecies as determined by mtDNA clades. The larger panel of *C. cinereum* specimens produced results that were somewhat different from the preliminary analysis, but were improved by our new understanding of the mitochondrial genetic structure among subspecies. The nominate subspecies was fixed for Serine, while the *littorale* population exhibited allele frequencies of 0.76 and 0.24 for Threonine and Serine, respectively. Within the *littorale* population, we compared allele frequencies between low- and high-altitude individuals. Low altitude was considered anything below 1000 m, and high altitude was considered anything below 1000 m, and high altitude was considered anything above 2500 m. Allele frequencies were roughly the same for both low- and high-altitude groups (Low: Ser=0.21, Thr=0.79; High: Ser=0.26, Thr=0.74) (Figure 13).

#### Hb isoform composition

We used a combination of isoelectric focusing (IEF) and tandem mass spectrometry (MS/MS) to characterize Hb isoform composition in the red blood cells of the same 14 conebill specimens from our initial sequencing panel. These analyses revealed that adult conebills express two structurally distinct Hb isoforms, HbA (pI = 8.8-9.0) and HbD (pI = 7.2-7.3). The HbA isoform ( $\alpha^{A_2}\beta^{A_2}$ ) incorporates products of the  $\alpha^{A_2}$ -globin gene, whereas the minor HbD isoform ( $\alpha^{D_2}\beta^{A_2}$ ) incorporates products of the  $\alpha^{D_2}$ -globin gene; both isoforms share the same  $\beta$ -type subunit (Hoffmann and Storz 2007; Grispo et al. 2012). In the hemolysates of wild-caught conebills, HbA accounted for >60% of total Hb, consistent with data from the majority of other passerine taxa examined to date (Grispo et al. 2012; Cheviron et al. 2014; Galen et al. 2015; Natarajan et al. 2016). There was no statistically significant difference in the proportion of HbD in the highland and lowland

birds (mean  $\pm 1$  SE: 36.9  $\pm 1.0$  [*n*=7] and 37.3  $\pm 1.1$  [*n*=7]). MS/MS analysis confirmed that the subunit components of the two adult Hb isoforms represent products of the  $\alpha^A$ ,  $\alpha^D$ -, and  $\beta^A$ -globin genes.

#### Oxygenation properties of HbA and HbD isoforms

We purified HbA and HbD isoforms from red cell lysates of highland and lowland birds that were alternative homozygotes at  $\alpha^A 134$  (*n*=2 highland birds homozygous for Ser, and *n*=2 lowland birds homozygous for Thr). We selected these four particular specimens for the experimental tests because the sequence data indicated that there was no confounding amino acid variation at other sites in the  $\alpha^A$ - or  $\beta^A$ -globin genes. Consequently, the comparison between highland and lowland HbA variants cleanly isolated the functional effect of the  $\alpha^A 134$ Thr $\rightarrow$ Ser mutation. We also confirmed that there was no amino acid variation in the  $\alpha^D$ -globin genes of these four specimens, so the HbD isoforms of the highland and lowland birds were structurally identical.

We measured the oxygenation properties of purified HbA and HbD solutions in the presence and absence of two main allosteric effectors that regulate Hb-O<sub>2</sub> affinity: Cl<sup>-</sup> ions (added as 0.1 M KCl) and inositol hexaphosphate (IHP, a chemical analog of inositol pentaphosphate, at two-fold molar excess over tetrameric Hb). In samples of both highland and lowland birds, analysis of O<sub>2</sub>-equilibrium curves revealed that the HbD isoform exhibited a uniformly higher O<sub>2</sub>-affinity than the HbA isoform, both in the absence ('stripped') and presence of allosteric effectors (Table 5, Figure 14). This is indicated by the lower values of  $P_{50}$  (the partial pressure of O<sub>2</sub> at which heme is 50%)

saturated) for HbD relative to HbA (Table 5, Figure 14). Both isoforms exhibited a high degree of cooperative O<sub>2</sub>-binding, as estimated Hill coefficients ( $n_{50}$ 's) were consistently >2.4 in the presence of IHP (Table 5).

#### Comparison of high- and low-altitude HbA and HbD variants

The highland HbA variant exhibit a slightly higher intrinsic  $O_2$ -affinity than the lowland variant (the  $P_{50}$  of the 'stripped' Hb was slightly lower; Table 5, Figure 14). This slight difference persisted in the simultaneous presence of both allosteric effectors (the 'KCl+IHP' treatment; Table 5, Figure 14). The 'KCl+IHP' treatment is most relevant to *in vivo* conditions in avian red blood cells, but it is not clear that the observed ~1 torr difference in  $P_{50}$  between the highland and lowland HbA variants would translate into a physiologically meaningful difference in blood- $O_2$  transport. Similarly, comparisons between HbD isoforms from the highland and lowland birds revealed no appreciable differences in  $O_2$ -binding properties (Table 5, Figure 14).

#### DISCUSSION

A fundamental question in evolutionary biology concerns the roles of geography and climate as drivers of adaptive change. We looked for evidence of adaptive divergence between populations that were, in one case, in similar climates but separated by a physical barrier and, in a second case, continuously distributed along a major climatic gradient. Our phylogeographic study of mtDNA variation across the distribution of *C. cinereum* confirmed the effect of the physical barrier, the high Andean altiplano, on genetic isolation between subspecies *cinereum* in the east, and subspecies *littorale* in the west. The mtDNA data also revealed a complete genetic connectivity between *littorale* 

populations from the high Andes from those on the Pacific coast. Based on the complete species-level phylogeny estimated by Barker et al. (2015), we can infer that populations of C. cinereum spanning the Andean west slope elevational gradient evolved recently from ancestors that were high-altitude restricted; whereas the extant populations comprised of subspecies *fraseri* and *cinereum* are high-elevation specialists, unchanged from the ancestral condition for the species. Our phylogenetic analysis suggests that subspecies *cinereum* split from *littorale* and *fraseri* circa 150,000 years ago (Figure 6). Consistent with Barker et al. (2015), we estimated that the species split from its most recent common ancestor around 700,000 years ago. While our estimate is subject to substantial uncertainty (see *Methods*), it is reasonable to assume that the split occurred sometime in the late-Pleistocene, a period of cyclic glaciation. Substantial evidence now exists for major shifts in tropical temperature and precipitation during the late-Pleistocene (Bromley et al. 2009). C. cinereum likely expanded during this period, possibly due to episodic forest expansion, leading to geographic isolation across barriers that were already in place and remain as barriers to dispersal at present (e.g., low arid valleys and the high altiplano).

Our analyses reveal body size variation among populations of *C. cinereum* that is consistent with evolutionary divergence over a short time frame of a few tens of thousands of years. These patterns of body size evolution were mostly, but not entirely consistent with Bergmann's Rule. Populations at higher elevations were larger than those at lower elevations. The equatorial *fraseri* was slightly smaller overall than its counterpart in southern Peru, *cinereum*. In the western Andean *littorale*, we found no

difference between high- and low-altitude populations. The evolution of geneticallybased morphological changes is less likely to occur in a contiguous population that is subject to high rates of gene flow (Benham and Witt 2016). Our mtDNA results and analysis of specimen localities indicate that *cinereum* is geographically isolated and that, despite mitochondrial non-monophyly, *fraseri* is most likely essentially isolated from littorale as well. The complete lack of elevational or latitudinal mitochondrial DNA genetic structure within *littorale* suggests there is gene flow occurring across the western Andean elevational gradient and throughout the latitudinal range of that subspecies. Stable isotope analyses for a coastal population of C. cinereum in Lima, Peru also show no structure with elevation, further suggesting elevational movements of individual birds are occurring along this gradient (Gadek et al., in prep). Although it is possible that the body size variation in C. cinereum evolved by chance, the short evolutionary time frame of its diversification suggests that C. cinereum includes more than one ecotype (genetically distinct geographical population, which is adapted to specific environmental condition; Turesson 1922; Turrill 2006). The relatively large body size of subspecies *cinereum* may reflect adaptation to its colder and more mesic environment that is subject to higher temperature variability. The secondary reduction in body size that occurred in *littorale* was likely an evolutionary response to increased temperature that it experienced after secondary colonization of the lowlands. High elevation *littorale* populations were identical in size to low elevation ones, suggesting that selection on body size was not sufficiently strong to overcome the homogenizing effects of gene flow. The results of our analysis of bioclimatic factors are consistent with this hypothesis. While body sizes diverged, bill size was subject to stasis, suggesting conserved foraging niches.

Carl Bergmann observed that within broadly distributed endotherm taxa, populations of larger body size are found in colder environments, and those of smaller size are found in warmer regions (Bergmann 1847). By reducing the surface area to volume ratio of their own body size, he posited that organisms would radiate less body heat per unit mass and therefore maintain warmer body temperatures in cold climates. Although originally formulated in terms of species within a genus, it is most aptly applied to comparisons of intraspecific populations (Rensch 1938; Mayr 1956), and it has been recast in terms of latitude. Bergmann's Rule has received much criticism owed to its sensitivity to the evolutionary depth of comparison and its failure to address the many other factors that affect body size (Geist 1987; Paterson 1990). Nonetheless, empirical studies have shown that Bergmann's rule appears to be a valid generalization for both birds and mammals (Meiri & Dayan 2003; Millien et al. 2006; Rodriguez et al. 2008).

Bergmann's Rule, like all generalizations, has exceptions. A recent study by Freeman (2016) tested whether intra- and interspecific patterns in body mass along elevational gradients follow Bergmann's rule, and found little evidence that tropical montane passerines have larger body masses at higher elevations where temperatures are colder. Further research is necessary as his sample of mist-netted passerines along a lmited elevational gradient (from 0 - 2500 m) in southeastern Peru was not representative of all tropical passerines within the region he sampled, nor of other tropical regions. In contrast to Freeman's results, our analysis of morphological variation in *C. cinereum* spans a broader range of elevation, and a broader geographic scope of sampling. Gutierrez-Pinto et al. (2014) suggest that the likelihood of documenting ecogeographical 'rules' depends
on the environmental context in which the variation is examined, pointing out the importance of the relative influence of environmental stressors.

Annual temperature range explained a substantial portion of the variation in climate experienced by each subspecies. Lower values indicate lower variation in temperature and therefore a more stable climate, whereas higher values indicate a greater degree of temperature fluctuation throughout the year. We would expect this variable to be positively correlated with elevation and latitude. Not surprisingly, *cinereum* had the highest annual temperature range. It was followed by high-elevation *littorale*, low-elevation *littorale*, then *fraseri*. After comparing this variable across latitude, it became clear that *fraseri* likely has such a low annual temperature range due to its distribution being situated right on the equator. Interestingly, high-elevation *littorale* may be experiencing similar environmental conditions as *cinereum*, but does not show an increase in body size, likely as a result of ongoing gene flow inhibiting local adaptation.

Our comparisons between the HbA variants of the highland and lowland conebills suggest that the  $\alpha^4$ 134Thr $\rightarrow$ Ser mutation produces a slight increase in Hb-O<sub>2</sub> affinity (Table 2, Figure 5). The direction of this altitudinal difference is seemingly consistent with a documented positive relationship between Hb-O<sub>2</sub> affinity and native elevation in birds (Natarajan et al. 2016; Storz 2016). However, it is not clear that the subtle difference between the conebill HbA variants is sufficiently physiologically meaningful to be adaptive. The same  $\alpha$ 134Thr $\rightarrow$ Ser mutation that we documented in conebill HbA has also been documented in human Hb (Hb Kenton), and it is not associated with any

clinically relevant hematological effects (http://globin.bx.psu.edu/hbvar; HbVar ID 2650). In the absence of other independent lines of evidence, there is no compelling reason to invoke spatially varying selection to explain the altitudinal difference in  $\alpha^4$ 134Thr/Ser allele frequencies. There are other examples of broadly distributed Andean birds that do not exhibit clear differentiation in Hb O<sub>2</sub>-binding affinity between highland and lowland populations (Cheviron et al. 2014; Natarajan et al. 2015, 2016). In the case of Cheviron et al. (2014), there was clear population genetic evidence for spatially varying selection, comprised of an anomalous elevational pattern of Hb- $\alpha$  allele frequency variation. In this case, elevated genetic structure at Hb exons between *littorale* and *cinereum* is also consistent with local adaptation to elevation; however, the mtDNA is also structured between this pair of populations, and a genome-wide assessment of differentiation would be required to test whether Hb-gene structure is truly anomalous.

It is clear that *C. cinereum* is able to tolerate a broad range of elevations without any substantial evolutionary changes in the oxygenation properties of hemoglobin that could be locally adaptive. The  $\alpha^4$ 134 change from serine to threonine had an effect on oxygen affinity that was subtle, at best, according to our measurements. However, it is possible that our functional assays do not capture the full range of ways in which hemoglobin can optimize function relative to ambient oxygen pressure. The maintenance of adequate tissue oxygen supply in animals exposed to high-altitude hypoxia depends on an ensemble of adaptive responses, which include both evolutionary changes in molecular structures as well as non-genetic adjustments that modulate the flux capacity of oxygen transport (Storz 2010; Scott et al. 2010). Along the steep coastal slope of the Peruvian

Andes, ambient oxygen tensions vary dramatically over relatively short geographic distances. For example, in central Peru, elevation changes from sea level to 4000 m in just 50 km. This fine-scaled environmental variation may be expected to select for phenotypic plasticity over genotypic specialization in species with broad elevational ranges (Cheviron et al. 2014).

Plastic phenotypic responses to  $PO_2$  in blood properties or other aspects of the respiratory system may explain C. cinereum ecological success across a range of ambient elevations. Lowland conebills exhibit reduced hematocrit and [Hb] relative to highland conebills, regardless of whether they are connected by gene flow or not. This suggests that a plastic shift in the O2-transport system is at least one component of the evolutionary response to secondary colonization of the lowlands. To compensate for a genetic ancestry that was adapted to high altitude, *littorale* at low elevations appears to flexibly reduce its blood O<sub>2</sub> carrying capacity, an adjustment that likely has advantages for  $O_2$  delivery resulting from reduced blood viscocity. This plasticity may be essential for the maintenance of a >4000 m elevational range. Many vertebrates experience erythropoiesis under hypoxia due to the body's attempt to increase oxygen delivery to the cells by boosting production of red blood cells, which can be detected in measurements of hematocrit and hemoglobin concentration. In humans, Tibetan highlanders were found to have sea-level-like hemoglobin concentrations when compared with acclimatized Tibetan lowlanders and Andean highlanders, which leads to a blunted erythropoietic response (Beall et al. 1990). At low levels of increase, this erythropoietic response can be beneficial. However, drastic increases in hematocrit can lead to polycythemia and high blood viscosity, thus impairing

cardiac and circulatory functions. This occurs in Andean humans (Beall 2007), who are also known to reduce their hematocrits upon descent to sea-level (Heinicke et al. 2003). Species that are genetically adapted to high altitude through mechanisms that increase Hb-O<sub>2</sub> affinity do not generally show increased levels of hematocrit and hemoglobin concentration (Carpenter 1975), whereas species that have been transplanted to high elevations have exhibited increased hemoglobin concentrations (Monge & Leon-Velarde 1991; Beall et al. 1990). This difference in response is thought to be due to the history of residence at high altitude (Beall 2007).

An alternative explanation for the contrasting patterns of divergence between *cinereum* versus high-elevation *littorale* and high-elevation versus low-elevation littorale, may be that the former divergence occurred earlier. *C. cinereum* might have a relatively short history as an elevational generalist, although it shows no evidence of recent population expansion on the Andean west slope (Gadek et al. in prep). Our phylogenetic analysis of mtDNA estimates that *C. cinereum* split from its close highland relatives circa 700,000 years ago, and that the highland conebills split from their lowland sister species circa 150,000 years ago. They are one of the youngest species of conebill. Recent expansions and diversification across their broad elevational extent may reflect an evolutionary instability that is inherent to broad elevational ranges.

In summary, *C. cinereum* evolved from a montane ancestor and now includes three or four geographically isolated populations, including an elevational generalist population that secondarily colonized the lowlands. Lowland individuals within this population

responded to the increase in pressure by lowering hematocrit and [Hb]. A novel Hb allele arose in the elevational generalist population and spread to high frequency, causing significant population genetic structure between elevational generalist and montane taxa. However, the functional effects of that novel allele are subtle, at best. Local adaptation within the elevation generalist clade cannot be detected, likely because of rampant gene flow. These results show the importance of physical isolation to local adaptation on two levels: (1) the rapid, but subtle functional Hb divergence of *littorale* from *cinereum* during the short period of isolation of these species; and (2) the failure of *littorale* to locally adapt to specific parts of the elevational gradient along which it occurs abundantly.

# Chapter 2

### **FIGURES**

**Figure 1.** Plot of principal component analysis for morphological measurements of museum specimens (skins). Points are color coded by subspecies (as determined by plumage morph and collection locality). High and low elevation populations of *C*. *littorale* were combined because they were indistinguishable by measurements.



**Figure 2.** Frequency distribution of morphological measurements, colored by subspecies. Letters on x-axis indicate significance differences between subspecies for each trait. High and low elevation populations of *C. littorale* were combined because they were indistinguishable by measurements.



Figure 3. Fst values of ND2 comparisons. \*p<0.0001.



**Figure 4.** Median-joining network of ND2 haplotypes. Circle color represents putative subspecies category (based on plumage and collection locality), and circle size is proportional to the number of individuals (smallest circle is 1, largest is 28).



**Figure 5.** Maximum-likelihood tree estimated using RAxML with outgroup *Conirostrum sitticolor*. Bootstrap values indicated at nodes of interest. Individual identifiers are color coded to represent the subspecies population from which the individuals belong based on plumage morph and locality. These identifiers correspond to their collection localities in Colombia, Ecuador, and Peru shown on a map of South America.



**Figure 6.** Phylogenetic tree estimated with Bayesian inference using BEAST, with outgroup *Conirostrum sitticolor*. Posterior probabilities indicated at nodes of interest. Individual identifiers are color coded to represent the subspecies population from which the individuals belong based on plumage morph and locality. These identifiers correspond to their collection localities in Colombia, Ecuador, and Peru shown on a map of South America.



**Figure 7.** Biplot of principal component analysis of 19 bioclimatic variables from the WorldClim dataset. Points are color coded by subspecies (as determined by plumage morph and collection locality). *C.c. littorale* is split into high- and low elevational categories. Bioclimatic variable codes are described in Table 3.



**Figure 8.** Bioclimatic variable frequencies plotted by subspecies: a) temperature seasonality, b) annual temperature range, and c) annual precipitation. *C. c. littorale* is split into high- and low elevational categories.





**Figure 9.** a) Temperature seasonality, b) Annual temperature range, and c) Annual precipitation plotted against latitude.

**Figure 10.** Linear regression plots of a) hematocrit (Hct) as a function of elevation (adjusted  $R^2 = 0.377$ ; F=53.63, *p*<0.001), and b) hemoglobin concentration ([Hb]) as a function of elevation (adjusted  $R^2 = 0.539$ ; F=73.52, *p*<0.001).



**Figure 11.** Fst values of *littorale-cinereum* comparisons for 3 loci. Intron data are included. \*p<0.0001.



**Figure 12.** Fst values of low- and high-altitude population comparisons for subspecies *littorale*. Values for all comparisons are non-significant.



**Figure 13.** Allele frequencies for the non-synonymous substitution found at amino acid position 134 of the Hb- $\alpha^{A}$  protein sequence. Elevation is not to scale.



**Figure 14.** O2 affinities of HbA and HbD isoforms from high- and low-altitude populations of cinereous conebills. (A) P50 values (mean  $\pm$  SEM) for purified HbA variants of highland and lowland conebills measured in 0.1 M Hepes buffer at pH 7.4 and 37 °C in the absence (stripped) and presence of allosteric effectors ([Cl<sup>-</sup>], 0.1 M; IHP/Hb tetramer ratio, 2.0; [heme], 0.3 mM). (B) P50 values for HbD variants of highland and lowland conebills (experimental conditions as in A).



# Chapter 2

## TABLES

**Table 1.** Primer pairs used to amplify ND2 gene fragments from ancient DNA extracted from toe pads greater than 50 years old.

Fragment	Primer Name	Primer Sequence
Amplified		
0-200 bp	L5219 (forward)	(See Sorenson 1999)
	0_200REV2 (reverse)	5'-GAATAAGACAAGGGCAGAGGCAGCTG-3'
200-400 bp	201_400F1 (forward)	5'-CTGCCCTTGTCTTATTCTCCAGCATAAC-3'
	201_400R3 (reverse)	5'-GCTTCATGATGGTAGATAGGAGG-3'
400-600 bp	401_600F1 (forward)	5'-CTACCATCATGAAGCTCCCCCAAT-3'
	401_600R3 (reverse)	5'-GTTGTAGATGACGACGGCTGTTATTC-3'
600-800 bp	601_800F1 (forward)	5'-GCCGTCGTCATCTACAACCCCAAAC-3'
	601_800R1 (reverse)	5'-GTTAGCTCTTGGATGATGAGTCAC-3'
800-950 bp	801_950F1 (forward)	5'-CATCATCCAAGAGCTAACTAAACAGGAC-3'
	801_950R1 (reverse)	5'-GCCAGTACGTCATTGTTTCATATGGTTTG-3'
950-1041 bp	950_1041F2 (forward)	5'-CACACTACAAACCATATGAAACAATGACG-3'
	H6313 (reverse)	(See Sorenson 1999)

**Table 2.** Primer pairs used to amplify globin genes.

Gene Amplified	Primer Name	Primer Sequence
Hb α-A (exons	AlphaAF	(See Galen et al. 2015)
1-3)	(forward)	
	AlphaAR (reverse)	(See Galen et al. 2015)
	_	
Hb β-A (exons	LFlankFwd1	5'- CTCCAGCCGACGCCATGGTGCAGTGGACA-3'
1-2)	(forward	
	BetaAex2Rev3	5'-
	(reverse)	CTGAAGTTCTCGGKGTCCACGTGCAGCTTGTCG-
		3'

Comparison	Dependent	Df	Sum Sq	Mean Sq	F	р
	Variable					
cin vs. lit	Culmen	1	0.0377	0.037658	0.3428	0.5604
	Tarsus	1	12.944	12.944	19.319	4.497e-05 ***
	Wing Chord	1	229.89	229.893	32.818	3.322e-07 ***
	Tail	1	408.83	408.83	75.073	3.19e-12 ***
cin vs. fras	Culmen	1	0.2699	0.26989	1.305	0.262
	Tarsus	1	0.0039	0.00394	0.0076	0.9309
	Wing Chord	1	52.788	52.788	9.0887	0.005095 **
	Tail	1	34.269	34.269	4.798	0.03614 *
fras vs. lit	Culmen	1	0.2706	0.27055	1.8143	0.1821
	Tarsus	1	25.635	25.6350	42.704	7.148e-09 ***
	Wing Chord	1	98.29	98.285	13.754	0.0004003 ***
	Tail	1	362.46	362.46	54.011	2.183e-10 ***

**Table 3.** Multivariate effects from pairwise comparisons of morphological characters among subspecies. Significance indicated by codes: 0 '\*\*\*' 0.001 '\*\*' 0.01 '\*' 0.05.

**Table 4.** WorldClim bioclimatic variable descriptions. Bioclimatic variables are derived from the monthly temperature and rainfall values in order to generate more biologically meaningful variables. These are often used in species distribution modeling and related ecological modeling techniques. The bioclimatic variables represent annual trends (e.g., mean annual temperature, annual precipitation) seasonality (e.g., annual range in temperature and precipitation) and extreme or limiting environmental factors (e.g., temperature of the coldest and warmest month, and precipitation of the wet and dry quarters). A quarter is a period of three months (1/4 of the year).

Variable	Description
Bio_1	Annual Mean Temperature
Bio_2	Mean Diurnal Range (Mean of monthly (max temp – min temp))
Bio_3	Isothermality $(BIO_2/BIO_7)(*100)$
Bio_4	Temperature Seasonality
Bio_5	Max Temperature of Warmest Month
Bio_6	Min Temperature of Coldest Month
Bio_7	Temperature Annual Range (BIO <sub>5</sub> – BIO <sub>6</sub> )
Bio_8	Mean Temperature of Wettest Quarter
Bio_9	Mean Temperature of Driest Quarter
Bio_10	Mean Temperature of Warmest Quarter
Bio_11	Mean Temperature of Coldest Quarter
Bio_12	Annual Precipitation
Bio_13	Precipitation of Wettest Month
Bio_14	Precipitation of Driest Month
Bio_15	Precipitation Seasonality (Coefficient of Variation)
Bio_16	Precipitation of Wettest Quarter
Bio_17	Precipitation of Driest Quarter
Bio_18	Precipitation of Warmest Quarter
Bio_19	Precipitation of Coldest Quarter

**Table 5.** O<sub>2</sub> affinities ( $P_{50}$ , torr) and cooperativity coefficients ( $n_{50}$ ) of purified HbA and HbD isoforms from highland and lowland specimens of *Conirostrum cinereum* (denoted by a parenthetical 'H' or 'L', respectively). O<sub>2</sub> equilibria were measured in 0.1 mM HEPES buffer at pH 7.4 (± 0.01) and 37°C in the absence (stripped) and presence of Cl<sup>-</sup> ions (0.1 M KCl]) and IHP (at two-fold molar excess over tetrameric Hb).  $P_{50}$  and  $n_{50}$  values were derived from single O<sub>2</sub> equilibrium curves, where each value was interpolated from linear Hill plots based on 4 or more equilibrium steps between 25 and 75% saturation.

Taxon	IsoHb	Stripped		+KCl		+IHP		+KCl + IHP	
		$P_{50}$	<i>n</i> <sub>50</sub>	$P_{50}$	$n_{50}$	$P_{50}$	<i>n</i> <sub>50</sub>	$P_{50}$	<i>n</i> <sub>50</sub>
High	HbA	3.63±0.05	$1.94{\pm}0.06$	4.90±0.02	$2.07{\pm}0.02$	$48.48{\pm}0.89$	$2.41\pm0.09$	39.47±0.33	$2.72 \pm 0.07$
	HbD	$2.07 \pm 0.01$	$2.00{\pm}0.02$	$3.06 \pm 0.04$	$2.37{\pm}0.08$	$28.35 \pm 0.74$	$2.73 \pm 0.18$	$20.80 \pm 0.71$	2.57±0.22
Low	HbA	3.98±0.06	$1.98{\pm}0.06$	$5.67 \pm 0.17$	$2.06 \pm 0.13$	$47.80 \pm 0.52$	$2.42\pm0.08$	$40.50 \pm 1.17$	2.93±0.25
	HbD	2.17±0.02	$1.94{\pm}0.05$	$2.80{\pm}0.02$	$2.19{\pm}0.05$	$25.46 \pm 0.43$	2.86±0.13	19.06±0.06	$2.94{\pm}0.03$

#### Chapter 2

#### REFERENCES

- ABRAMOFF, M.D., P.J. MAGELHAES, AND S.J. RAM. 2004. Image processing with Image. *International Journal of Biophotonics* 11:36.
- ALTSHULER, D.L. AND R. DUDLEY. 2006. The physiology and biomechanics of avian flight at high altitude. *Integrative and Comparative Biology* 46:62-71.
- ANDERSEN ET AL. 2015. Rapid diversification and secondary sympatry in Australo-Pacific kingfishers (Aves: Alcedinidate: *Todiramphus*). *Royal Society of Science* http://dx.doi.org/10.1098/rsos.140375.
- AYRES, D.L. ET AL. 2012. BEAGLE: an application programming interface and highperformance computing library for statistical phylogenetics. *Syst. Biol.* 61, 170– 173.
- BANDELT, H., P. FORSTER, AND A. RÖHL. 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* 16(1):37–48.
- BARKER, K. ET AL. 2015. New insights into new-world biogeography: an integrated view from the phylogeny of blackbirds, cardinals, sparrows, tanagers, warblers, and allies. *The Auk* 132(2):333-348.
- BEALL, C.M. AND M.C. GOLDSTEIN. 1990. Hemoglobin concentration, percent oxygen saturation and arterial oxygen content of Tibetan nomads at 4,850 to 5,460 meters. In J.R. Sutton, G. Coates, and J.E. Remmers (eds.): *Hypoxia: The Adaptations*. Toronto, British Columbia: C. Decker, Inc., pp. 59–65.
- BEALL, C.M. 2007. Two routes to functional adaptation: Tibetan and Andean highaltitude natives. *Proceedings of the National Academy of Sciences* USA 104:

8655-8660.

BENHAM, P.M. AND C.C. WITT. 2016. The dual role of Andean topography in primary divergence: functional and neutral variation among populations of the hummingbird, Metallura tyrianthina. BMC Evolutionary Biology. DOI 10.1186/s12862-016-0595-2.

- BENHAM, P.M., E.J. BECKMAN, S.G. DUBAY, M. FLORES, A. JOHNSON, M.J. LELEVIER,
  C.J. SCHMITT, N.A. WRIGHT, AND C.C. WITT. 2011. Satellite imagery reveals new critical habitat for endangered bird species in the high Andes of Peru. *Enangered Species Research* 13(2): 145-157.
- BERGMANN, C. 1847. "Über die Verhältnisse der Wärmeökonomie der Thiere zu ihrer Grösse". *Göttinger Studien*. 3 (1): 595–708.
- BLACK, C.P. AND S.M. TENNEY. 1980. Oxygen transport during progressive hypoxia in high altitude and sea level waterfowl. *Respiratory Physiology* 39:217-239.

BURNS, K.J., A.J. SHULTZ, P.O. TITLE, N.A. MASON, F.K BARKER, J. KLICKA, S.M.
LANYON, I.J. LOVETTE. 2014. Phylogenetics and diversification of tanagers
(Passeriformes: Thraupidae), the largest radiation of Neotropical songbirds. *Molecular Phylogenetics and Evolution* 75(41-77).

- BROMLEY, G.R.M. ET AL. 2009. Relative timing of the last glacial maximum and late glacial events in the central tropical Andes. *Quaternary Science Reviews* doi:10.1016/j.quascirev.2009.05.012
- BULGARELLA, M., J.L. PETERS, C. KOPUCHIAN, T. VALQUI, R.E. WILSON, K.G. MCCRACKEN. 2011. Multilocus coalescent analysis of haemoglobin

differentiation between low- and high-altitude populations of crested ducks (*Lophonetta specularioides*). 2011. *Molecular Ecology* 21:350-368.

- CARPENTER, F.L. 1975. Bird hematocrits: effects of high altitude and strength of flight. Comparative Biochemistry and Physiology A: Physiology 50(2):415-417.
- CHAI, P. AND R. DUDLEY. 1995. Limits to vertebrate locomotor energetics suggested by hummingbirds hovering in heliox. *Nature* 377:722-725.
- CHAPPELL, M.A., J.P. HAYES, L.R.G. SNYDER. 1988. Hemoglobin polymorphisms in deer mice (Peromyscus maniculatus): physiology of beta-globin variants and alphaglobin recombinants. *Evolution* 42(4): 681-688.
- CHEVIRON, Z.A. C. NATARAJAN, J. PROJECTO-GARCIA, D.K. EDDY, J. JONES, M.D.
  CARLING, C.C. WITT, H. MORIYAMA, R.E. WEBER, A. FAGO, J.F. STORZ. 2014.
  Integrating evolutionary and functional tests of adaptive hypotheses: a case study of altitudinal differentiation in hemoglobin function in an Andean sparrow, *Zonotrichia capensis. Molecular Biology and Evolution* 31:2948.
- DEL HOYO, J., ELLIOT, A., & CHRISTIE, D.A. EDS. (2003). Handbook of the Birds of the World. Vol. 16. Tanagers to New World Blackbirds. Lynx Edicions, Barcelona.
- DRUMMOND, A.J., M.A. SUCHARD, D. XIE, AND A. RAMBAUT. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* 29:1969–1973.
- DRUMMOND, A.J., G.K. NICHOLLS, A.G. RODRIGO, W. SOLOMON. 2002 Estimating mutation parameters, population history and genealogy simultaneously from temporally spaced sequence data. *Genetics* 161, 1307–1320.

- FJELDSA, J. & N. KRABBE. 1990. Birds of the High Andes. Jon Fjelsda & Niels Krabbe, and Zoological Museum, University of Copenhagen, and Apollo Books, Svendborg, Denmark.
- FREEMAN, B.G. 2016. Little evidence for Bergmann's rule body size clines in passerines along tropical elevational gradients. *Journal of Biogeography*.
   DOI: 10.1111/jbi.12812.
- GADEK, C.R. *ET AL. IN PREP.* Why are tropical mountain passes 'low' for some species? A test of seasonal movements and genetic differentiation in four elevational generalist songbirds.
- GALEN, S.C. ET AL. 2015. Contribution of a mutational hot spot to hemoglobin adaptation in high-altitude Andean house wrens. *Proceedings of the National Academy of Sciences, USA* **112**:13958.
- GEIST, V. 1987. Bergmann's rule is invalid. Canadian Journal of Zoology 65:1035–1038.
- GRISPO, M.T., C. NATARAJAN, J. PROJECTO-GARCIA, H. MORIYAMA, R.E. WEBER, J.F. STORZ. 2012. Gene duplication and the evolution of hemoglobin isoform differentiation in birds. *Journal of Biological Chemistry* 287:12.

- GUTIERREZ-PINTO, N., K.G. MCCRACKEN, L. ALZA, P. TUBARO, C. KOPUCHIAN, A. ASTIE, AND C.D. CADENA. 2014. The validity of ecogeographical rules is context dependent: testing for Bergmann's and Allen's rules by latitude and elevation in a widespread Andean duck. Biological Journal of the Linnean Society 111(4):850-862.
- HEINICKE, K. ET AL. 2003. Long term exposure to intermittent hypoxia results in increased hemoglobin mass, reduced plasma volume, and elevated erythropoetic plasma levels in man. European Journal of Applied Physiology 88:535-543.
- HIJMANS, R.J., S.E. CAMERON, J.L. PARRA, P.G. JONES AND A. JARVIS. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25:1965-1978.
- HILTY, S.L. AND W.L. BROWN. 1986. A Guide to the Birds of Colombia. Princeton University Press.
- HOFFMANN, F.G., J.F. STORZ, T.A. GORR, AND J.C. OPAZO. 2010. Lineage-specific patterns of functional diversification in the α- and β-globin gene families of tetrapod vertebrates. *Molecular Biology and Evolution* 27:1126.
- HOFFMANN, F.G., J.C. OPAZO, AND J.F. STORZ. 2011. Differential loss and retention of cytoglobin, myoglobin, and globin-E during the radiation of vertebrates. *Genome Biology and Evolution* 3:588.
- HOWARD AND MOORE. 2013. Complete checklist of the birds of the world, 4<sup>th</sup> Edition, Volume 2 (Passerines).
- ISLER, M.L AND P.R. ISLER. 1987. The Tanagers: Natural history, distribution, and identification. Smithsonian Institution Press, Washington, D.C.

- JANZEN, D. 1967. Why mountain passes are higher in the tropics. American Naturalist 101(919):233-249.
- JESSEN, T.H., R.E. WEBER, & G. FERMI. 1991. Proceedings of the National Academy of Sciences of the United States of America 88(5): 6519-6522.
- JURGENS, K.D., M. PIETSCHMANN, & K. YAMAGUCHI. 1988. Oxygen binding properties, capillary densities and heart weights in high-altitude camelids. *Journal of Comparative Physiology B – Biochemical systemic and environmental physiology* 158(4): 469-477.
- KEARSE, M., R. MOIR, A. WILSON, S. STONES-HAVAS, M. CHEUNG, S. STURROCK, S.
  BUXTON, A. COOPER, S. MARKOWITZ, C. DURAN, T. THIERER, B. ASHTON, P.
  MENTJIES, AND A. DRUMMOND. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. *Bioinformatics* 28(12):1647-1649.
- LERNER, H.R., M. MEYER, H.F. JAMES, M. HOFREITER, R.C. FLEISCHER. 2011. Multilocus resolution of phylogeny and timescale in the extant adaptive radiation of Hawaiian honeycreepers. *Current Biology* 21, 1838–1844.
- LIBRADO, P. AND J. ROZAS. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 25: 1451-1452
- MAYR, E. 1956. Geographical character gradients and climatic adaptation. *Evolution* 20:105-108.
- McCracken, K.G., C.P. Barger, M. Bulgarella, K.P. Johnson, S.A. Sonsthagen, J. Trucco, T.H. Valqui, R.E. Wilson, K. Winkler, and M.D. Sorenson. 2009. Parallel evolution in the major haemoglobin genes of eight species of Andean

waterfowl. Molecular Ecology 18:3992-4005.

- MEIRI, S. AND T. DAYAN. 2003. On the validity of Bergmann's rule. *Journal of Biogeography* 30(3):331-351.
- MILLIEN, V., S.K. LYONS, L. OLSON, F.A. SMITH, A.B. WILSON, AND Y. YOM-TOV. 2006. Ecotypic variation in the context of global climate change: revisiting the rules. *Ecology Letters* 9(7):853-869.
- MONGE, C. AND F. LEON-VELARDE. 1991. Physiological adaptation to high altitude: oxygen transport in mammals and birds. Physiological Reviews 71(4):1135-1172.

NATARAJAN, C. J. PROJECTO-GARCIA, H. MORIYAMA, R.E. WEBER, V. MUNOZ-FUENTES,
A.J. GREEN, C. KOPUCHIAN, P.L. TUBARO, L. ALZA, M. BULGARELLA, M.M.
SMITH, R.E. WILSON, A. FAGO, K.G. MCCRACKEN, AND J.F. STORZ. 2015.
Convergent evolution of hemoglobin function in high-altitude Andean waterfowl involves limited parallelism at the molecular sequence level. *PloS Genetics* 11(12):e1005681.

- NATARAJAN, C., F. G. HOFFMANN, R. E. WEBER, A. FAGO, C. C. WITT, AND J. F. STORZ.
  2016. Predictable convergence in hemoglobin function has unpredictable molecular underpinnings. *Science* 354: 336-340.
- OPAZO, J.C., F.G. HOFFMAN, C. NATARAJAN, C.C. WITT, M. BERENBRINK, AND J.F. STORZ. 2015. Gene turnover in the avian globin gene families and evolutionary changes in hemoglobin isoform expression. *Molecular Biology and Evolution* 32:871.
- PARKER, T. A. ET AL. 1996. *Neotropical Bird Ecology and Conservation* (TA Parker and DK Moskovits, Eds.). University of Chicago Press.

- PATERSON, J.D. 1990. Comment Bergmann's rule is invalid: a reply to V. Geist. *Canadian Journal of Zoology* 68(7):1610-1612.
- PERUTZ, M.F. 1983. Species adaptation in a protein molecule. *Respir. Physiol. Neurobiol.* 144.
- PIETRUSKA, K. 2005. Geocontext topographic profiler. Center for Geographic Analysis. http://www.geocontext.org/publ/2010/04/profiler/en/

POPART. Population Analysis with Reticulate Trees. http://popart.otago.ac.nz

- PROJECTO-GARCIA, J., C. NATARAJAN, H. MORIYAMA, R.E. WEBER, A. FAGO, Z.A.
  CHEVIRON, R. DUDLEY, J.A. MCGUIRE, C.C. WITT, AND J.F. STORZ. 2013.
  Repeated elevational transitions in hemoglobin function during the evolution of Andean hummingbirds. *Proceedings of the National Academy of Sciences USA* 110:20669-20674.
- RAMBAUT AND DRUMMOND. 2006-2014. BEAST: Bayesian Evolutionary Analysis by Sampling Trees. BMC Evolutionary Biology 7(1):214.
- REVSBECH, I., D.M. TUFTS, J. PROJECTO-GARCIA, H. MORIYAMA, R.E. WEBER, J.F. STORZ, AND A. FAGO. 2013. Hemoglobin function and allosteric regulation in semi-fossorial rodents (family Sciuridae) with different altitudinal ranges. *Journal of Experimental Biology* 216: 4264-4271.
- RICKLEFS, R.E. AND G.C. COX. 1972. Taxon cycles in the West Indian avifauna. American Naturalist 106: 195–219.
- RIDGLEY, R.S. AND P.J. GREENFIELD. 2001. The Birds of Ecuador. Cornell University Press.

- R CORE TEAM 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <u>https://www.R-project.org/</u>.
- RENSCH, B. 1938. Some problems of geographical variation and species formation. *Proceedings of the Linnean Society of London* 150(4):275-285.
- RODRIGUEZ, M.A., M.A. OLALLA-TARRAGA, AND B.A. HAWKINS. 2008. Bergmann's rule and the geography of mammal body size in the Western Hemisphere. *Global Ecology and Biogeography* 17(2):274-283.
- SCHMIDT-NIELSON, K. 1972. Locomotion: Energy cost of swimming, flying and running. *Science*, New Series, 177(4045):222-228.
- SORENSON ET AL. 1999. Avian mtDNA primers.
- STAMATAKIS, A. 2014. RAxML Version 8: A tool for Phylogenetic Analysis and Post-Analysis of Large Phylogenies. *Bioinformatics* 22(21): 2688-2690.
- SCOTT, G. 2011. Elevated performance: the unique physiology of birds that fly at high altitudes. *Journal of Experimental Biology* 214:2455-2462.
- SCOTT, G.R. & W.K. MILSOM. 2007. American Journal of Physiology-Regulatory Integrative and Comparative Physiology 293(1): R379-R391.
- SORENSON, M., J.C. AST, D.E. DIMCHEFF, AND D. MINDELL. 1999. Primers for a PCRbased approach to mitochondrial genome sequencing in birds and other vertebrates. *Molecular Phylogenetics and Evolution* 12(2):105-114.
- STORZ, J.F. 2016. Hemoglobin-oxygen affinity in high-altitude vertebrates: Is there evidence for an adaptive trend? *Journal of Experimental Biology* 219:3190-3203.

- STORZ, J.F. AND H. MORIYAMA. 2008. Mechanisms of hemoglobin adaptation to high altitude hypoxia. *High Altitude Medicine and Biology* 9(2):148-157.
- STORZ, J.F., A.M. RUNK, H. MORIYAMA, R.E. WEBER, AND A. FAGO. 2010. Genetic differences in hemoglobin function between highland and lowland deer mice. *Journal of Experimental Biology* 213, 2565.
- STORZ, J.F., F.G. HOFFMANN, J.C. OPAZO, T.J. SANGER, AND H. MORIYAMA. 2011.
   Developmental regulation of hemoglobin synthesis in the green anole lizard
   *Anolis carolinensis. Journal of Experimental Biology* 214, 575.
- STORZ, J.F., R.E. WEBER, AND A. FAGO. 2012. Oxygenation properties and oxidation rates of mouse hemoglobins that differ in reactive cysteine content. *Comparative Biochemistry and Physiology A: Molecular and Integrative Physiology*, 161, 265.
- STORZ, J.F., A.M RUNK, S.J. SABATINO, J.K. KELLY, N. FERRAND, H. MORIYAMA, R.E.
  WEBER, A. FAGO & C.M. BELL. 2009. Evolutionary and Functional Insights into the Mechanism Underlying High-Altitude Adaptation of Deer Mouse Hemoglobin . *Proceedings of the National Academy of Sciences of the United States of America* 106(34): 14450-14455.
- STORZ, J.F. C. NATARAJAN, H. MORIYAMA, F.G. HOFFMAN, T. WANG, A. FAGO, H. MALTE, J. OVERGAARD, R.E. WEBER. 2015. Oxygenation properties and isoform diversity of snake hemoglobins. *American Journal of Physiology: Regulatory, Integrative, and Comparative Physiology* 309, R1178.
- TUCKER, V.A.1968. Respiratory physiology of house sparrows in relation to high-altitude flight. *Journal of Experimental Biology* 48:55-66.

TURISSON, G. 1922. The species and the variety as ecological units. Hereditas, Lund

3:100-113.

- TURRILL 2006. The ecotype concept: A consideration with appreciation and criticism, especially of recent trends. *New Phytologist* 45(1):34-43.
- WEBER, R.E., W. VOELTER, A. FAGO, H. ECHNER, E. CAMPANELLA, AND P.S. LOW. 2004.
  Modulation of red cell glycolysis: interactions between vertebrate hemoglobins and cytoplasmic domains of band 3 red cell membrane proteins. *American Journal of Physiology: Regulatory, Integrative, and Comparative Physiology* 287, R454.
- WEBER, RE., H. OSTOJIC, AND A. FAGO. 2002. Novel mechanism for high-altitude adaptation in hemoglobin of the Andean frog Telmatobius peruvianus. *American Journal of Physiology - Regulatory Integrative Comparative Physiology* 283(5):R1052-R1060.
- WEIR, J.T., E. BERMINGHAM, M.J. MILLER, J. KLICKA, M.A. GONZALEZ. 2008. Phylogeography of a morphologically diverse Neotropical montane species, the common bush-tanager (*Chlorospingus ophthalmicus*). *Molecular Phylogenetics and Evolution* 47, 650–664.
- WEIR, J.T., T.D. PRICE. 2011. Limits to speciation inferred from times to secondary sympatry and ages of hybridizing species along a latitudinal gradient. *American Naturalist* 17(7) 462–469.
- WINKER ET AL. 2015. Bird Families of the World. *Lynx Editions* and *Cornell Lab of Ornithology*.

**Appendix A.** Specimen data used in morphometric subspecies comparisons, listed by museum specimen number. Museum of Southwestern Biology specimens are labeled with 'NK' prefix, and American Museum of Natural History specimens are labeled with 'AM' prefix. Plumage class designated by specimen collectors and verified by museum curatorial staff.

Individual	Locality	Plumage	Culmen	Tarsus	Wing	Tail	Mass
NK172688	Apurimac, PE	cinereum	7.56	18.04	62.11	53	
NK172470	Apurimac, PE	cinereum	7.05	19.01	59.33	53	8.98
NK172650	Cusco, PE	cinereum	7.08	17.85	62.13	49.5	9.58
NK172649	Cusco, PE	cinereum	7.11	16.97	58.64	52	9.66
NK168308	Cusco, PE	cinereum	7.99	19.89	61.02	53	9.59
NK159760	Cusco, PE	cinereum	6.78	17.6	65.8	54	9.6
NK159712	Cusco, PE	cinereum	7.03	18.04	63.27	55	9.2
NK159819	Cusco, PE	cinereum	7.25	18.39	62.01	49.5	9.2
NK168319	Cusco, PE	cinereum	7.32	18.87	60.15	52.5	9.13
NK159832	Cusco, PE	cinereum			60.2	53.5	9.1
AM168304	Bestion, EC	fraseri	7.5	18.34	59.19	54	
AM810790	Bosques del Corazon, EC	fraseri	7.11	19.01	59.19	48.5	
AM810791	Bosques del Corazon, EC	fraseri	7.65	18.55	57.88	50	
AM810789	Bosques del Pichincha, EC	fraseri	7.34	18.17	56.82	51.5	
AM508415	Cayambe, EC	fraseri	6.73	17.88	60.64	52	
AM508405	Cupallacla, EC	fraseri	7.22	18.07	60.89	49.5	
AM168305	El Paso, EC	fraseri	7.24	18.63	55.25	44.5	
AM117205	Huila, CO	fraseri	5.26	19.27	59.27	51.5	
AM117204	Huila, CO	fraseri	7.04	17.33	56.76	51	
AM117203	Huila, CO	fraseri	7.23	18.45	56.99	55.5	
AM117206	Huila, CO	fraseri	7.09	17.08	59.51	49	
AM508417	Ibarra, EC	fraseri	7.27	18.01	54.61	45	
AM508419	Ibarra, EC	fraseri	6.27	17.31	59.13	53	

(Cont'd)							
Individual	Locality	Plumage	Culmen	Tarsus	Wing	Tail	Mass
AM508416	Ibarra, EC	fraseri	7.12	17.57	56.19	49.5	
AM508418	Ibarra, EC	fraseri	7.31	18.07	58.2	50	
AM447392	Llanganate, EC	fraseri	7.32	19.27	66.58	53	
AM130378	Loja, EC	fraseri	7.25	18.34	58.46	50.5	
AM508409	Pichincha, EC	fraseri	6.39	17.72	58.66	48	
AM40355	Quito, EC	fraseri	7.03	19.47	57.36	46.5	
AM508410	Quito, EC	fraseri	7.19	18.37	58.41	50.5	
AM508414	Riobamba, EC	fraseri	6.92	18.32	63	53	
AM168303	Salvias, EC	fraseri	7.14	19.19	57.72	46	
AM168302	Tarraguacocha, EC	fraseri	6.96	17.67	59.71	54.5	
NK169482	Ancash, PE	litttorale	6.97	15.93	57.15	45	8.24
NK173837	Ancash, PE	litttorale	7	16.49	60.55	46	7.68
NK169453	Ancash, PE	litttorale	7.07	16.52	54.45	41.5	6.14
NK169491	Ancash, PE	litttorale	7.08	16.51	58.38	42.5	7.5
NK171579	Ancash, PE	litttorale	7.09	16.9	56.14	47.5	8.01
NK169464	Ancash, PE	litttorale	7.25	17.23	58.81	48	8.29
NK169478	Ancash, PE	litttorale	7.26	17.36	58.12	41.5	8.15
NK169460	Ancash, PE	litttorale	7.79	16.66	53.07	44	8.54
NK172291	Arequipa, PE	litttorale	6.75	15.66	57.8	47	7.83
NK172249	Arequipa, PE	litttorale	6.78	16.95	53.97	43	7.97
NK172131	Cajamarca, PE	litttorale	7.06	17.84	54.91	42	
NK172142	Cajamarca, PE	litttorale	7.31	16.17	56.87	50	7.89
NK171965	Cajamarca, PE	litttorale	7.18	17.52	55.27	43.88	
NK171994	Cajamarca, PE	litttorale	7.25	18	59.09	49.5	
NK171996	Cajamarca, PE	litttorale	7.28	17.48	53.99	45	8.92

Appendix A

(Cont d)							
Individual	Locality	Plumage	Culmen	Tarsus	Wing	Tail	Mass
NK171928	Cajamarca, PE	litttorale	7.38	17.55	54.84	48.5	8.02
NK171997	Cajamarca, PE	litttorale	7.48	17.65	58.71	44.97	
NK171967	Cajamarca, PE	litttorale	7.63	17.87	59	49	8.93
NK172104	Cajamarca, PE	litttorale	7.79	16.82	56.17	46	8.01
NK168221	Huancavelica, PE	litttorale	7.41	17.02	57.2		8.1
NK168216	Huancavelica, PE	litttorale	7.42	18.01	60.11	45	8.1
NK168214	Huancavelica, PE	litttorale	7.5	17.32	58.25	46.5	8.1
NK220136	La Libertad, PE	litttorale	7.03	16.76	60.92	49.5	7.83
NK220142	La Libertad, PE	litttorale	7.22	16.62	57.46	49.84	9.02
NK220132	La Libertad, PE	litttorale	7.26	18.41	56.17	49	8.76
NK171457	La Libertad, PE	litttorale	6.66	16.52	49.77	41.5	7.8
NK171452	La Libertad, PE	litttorale	6.85	15.79	52.57	46	7.45
NK171495	La Libertad, PE	litttorale	7	15.7	57.24	45.5	7.74
NK171414	La Libertad, PE	litttorale	7.31	16.72	53.39	46	
NK162984	Lima, PE	litttorale	6.83	16.13	59.23	47	8.18
NK168664	Lima, PE	litttorale	6.25	17.54	53.53	48	7.3
NK168099	Lima, PE	litttorale	6.74	17.32	53.61	47.5	7.1
NK168114	Lima, PE	litttorale	6.86	16.5	60.64	42	8.9
NK175512	Lima, PE	litttorale	6.89	19.25	52.56	45	
NK172790	Lima, PE	litttorale	6.98	16.77	52.08	41.45	8
NK168569	Lima, PE	litttorale	7.02	15.66	58.6	47	8.7
NK168144	Lima, PE	litttorale	7.03	16.81	53.3	43.5	7.7
NK168666	Lima, PE	litttorale	7.03	16.95	57.87	45.5	8.1
NK168150	Lima, PE	litttorale	7.04	16.04	51.83	44	7.7
NK168697	Lima, PE	litttorale	7.07	16.95	56.71	46	8.4

Appendix A (Cont'd)

Appendix A (Cont'd)							
Individual	Locality	Plumage	Culmen	Tarsus	Wing	Tail	Mass
NK168165	Lima, PE	litttorale	7.1	16.11	54.1	46	7.7
NK175533	Lima, PE	litttorale	7.2	17.71	51.77	43.89	
NK168211	Lima, PE	litttorale	7.27	16.25	54.29	44.5	7.4
NK168129	Lima, PE	litttorale	7.28	17.18	57.97	47	9.5
NK168156	Lima, PE	litttorale	7.34	17.12	54.39	44	8.4
NK163451	Lima, PE	litttorale	7.46	18.14	53.44	44	8.9
NK163024	Lima, PE	litttorale	7.49	18.48	58.31	45.5	8.28
NK168685	Lima, PE	litttorale	7.52	17.46	55.88	45	8.6
NK168132	Lima, PE	litttorale	7.53	17.01	55.81	41.5	8.6
NK168076	Lima, PE	litttorale	7.63	18.6	57.44	45.5	8
NK172779	Lima, PE	litttorale	7.9	16.88	55.25	42.5	8
NK172220	Tacna, PE	litttorale	6.56	17.16	59.41	46.5	8.46
NK171762	Tacna, PE	litttorale	6.57	16.24	60.11	47.5	8.97
NK171808	Tacna, PE	litttorale	6.98	15.81	59.34	49	8.98
Gene	base	Genbank					
-----------	-------	-------------	-----------------				
Sequence	pairs	Accession #	Institution ID				
Hb-AlphaA	429	KY349295	MSB:Birds:27056				
Hb-AlphaA	429	KY349296	MSB:Birds:27057				
Hb-AlphaA	429	KY349297	MSB:Birds:27102				
Hb-AlphaA	429	KY349300	MSB:Birds:27159				
Hb-AlphaA	429	KY349299	MSB:Birds:28300				
Hb-AlphaA	429	KY349298	MSB:Birds:31424				
Hb-AlphaA	429	KY349294	MSB:Birds:31460				
Hb-AlphaA	429	KY349293	MSB:Birds:31779				
Hb-AlphaA	429	KY349292	MSB:Birds:31783				
Hb-AlphaA	429	KY349291	MSB:Birds:32905				
Hb-AlphaA	429	KY349290	MSB:Birds:32942				
Hb-AlphaA	429	KY349289	MSB:Birds:32960				
Hb-AlphaA	429	KY349288	MSB:Birds:32965				
Hb-AlphaA	429	KY349287	MSB:Birds:32972				
Hb-AlphaA	429	KY349309	MSB:Birds:32976				
Hb-AlphaA	429	KY349311	MSB:Birds:32984				
Hb-AlphaA	429	KY349312	MSB:Birds:32993				
Hb-AlphaA	429	KY349314	MSB:Birds:33100				
Hb-AlphaA	429	KY349313	MSB:Birds:33273				
Hb-AlphaA	429	KY349308	MSB:Birds:33308				
Hb-AlphaA	429	KY349307	MSB:Birds:33325				
Hb-AlphaA	429	KY349306	MSB:Birds:33344				
Hb-AlphaA	429	KY349305	MSB:Birds:33350				
Hb-AlphaA	429	KY349304	MSB:Birds:33401				
Hb-AlphaA	429	KY349303	MSB:Birds:33447				

**Appendix B**. Accession numbers for mitochondrial (ND2) and globin (Hb- $\alpha^{A}$  and Hb- $\beta^{A}$ ) sequence data used in this study and subsequently deposited on Genbank for archival purposes and for access by the public.

Appendix B	(cont'd)		
Gene	base	Genbank	
Sequence	pairs	Accession #	Institution ID
Hb-AlphaA	429	KY349302	MSB:Birds:33652
Hb-AlphaA	429	KY349310	MSB:Birds:33665
Hb-AlphaA	429	KY349301	MSB:Birds:34227
Hb-AlphaA	429	KY349266	MSB:Birds:34233
Hb-AlphaA	429	KY349267	MSB:Birds:34238
Hb-AlphaA	429	KY349268	MSB:Birds:34239
Hb-AlphaA	429	KY349269	MSB:Birds:34252
Hb-AlphaA	429	KY349272	MSB:Birds:34253
Hb-AlphaA	429	KY349271	MSB:Birds:34256
Hb-AlphaA	429	KY349270	MSB:Birds:34257
Hb-AlphaA	429	KY349265	MSB:Birds:34265
Hb-AlphaA	429	KY349264	MSB:Birds:34266
Hb-AlphaA	429	KY349263	MSB:Birds:34731
Hb-AlphaA	429	KY349262	MSB:Birds:34768
Hb-AlphaA	429	KY349261	MSB:Birds:34792
Hb-AlphaA	429	KY349260	MSB:Birds:34874
Hb-AlphaA	429	KY349259	MSB:Birds:34978
Hb-AlphaA	429	KY349258	MSB:Birds:35036
Hb-AlphaA	429	KY349257	MSB:Birds:35082
Hb-AlphaA	429	KY349281	MSB:Birds:35296
Hb-AlphaA	429	KY349282	MSB:Birds:35494
Hb-AlphaA	429	KY349283	MSB:Birds:35505
Hb-AlphaA	429	KY349284	MSB:Birds:35565
Hb-AlphaA	429	KY349286	MSB:Birds:35568
Hb-AlphaA	429	KY349285	MSB:Birds:35744
Hb-AlphaA	429	KY349280	MSB:Birds:35984

Appendix B	(cont'd)		
Gene	base	Genbank	
Sequence	pairs	Accession #	Institution ID
Hb-AlphaA	429	KY349279	MSB:Birds:35996
Hb-AlphaA	429	KY349278	MSB:Birds:36006
Hb-AlphaA	429	KY349277	MSB:Birds:36010
Hb-AlphaA	429	KY349276	MSB:Birds:36029
Hb-AlphaA	429	KY349275	MSB:Birds:36560
Hb-AlphaA	429	KY349274	MSB:Birds:31072
Hb-AlphaA	429	KY349273	MSB:Birds:31073
Hb-BetaA	312	KY349243	MSB:Birds:27057
Hb-BetaA	312	KY349245	MSB:Birds:27159
Hb-BetaA	312	KY349242	MSB:Birds:27172
Hb-BetaA	312	KY349239	MSB:Birds:31779
Hb-BetaA	312	KY349253	MSB:Birds:32942
Hb-BetaA	312	KY349252	MSB:Birds:32959
Hb-BetaA	312	KY349249	MSB:Birds:32960
Hb-BetaA	312	KY349246	MSB:Birds:32972
Hb-BetaA	312	KY349241	MSB:Birds:32989
Hb-BetaA	312	KY349244	MSB:Birds:33273
Hb-BetaA	312	KY349256	MSB:Birds:33350
Hb-BetaA	312	KY349255	MSB:Birds:33447
Hb-BetaA	312	KY349254	MSB:Birds:33487
Hb-BetaA	312	KY349250	MSB:Birds:33652
Hb-BetaA	312	KY349251	MSB:Birds:35568
Hb-BetaA	312	KY349237	MSB:Birds:35743
Hb-BetaA	312	KY349238	MSB:Birds:35744
Hb-BetaA	312	KY349247	MSB:Birds:36560
Hb-BetaA	312	KY349248	MSB:Birds:31063

Appendix B	(cont'd)		
Gene	base	Genbank	
Sequence	pairs	Accession #	Institution ID
Hb-BetaA	312	KY349240	MSB:Birds:31072
ND2	1041	KY349329	AMNH:Birds:SKIN117203
ND2	1041	KY349333	AMNH:Birds:SKIN117204
ND2	1041	KY349332	AMNH:Birds:SKIN129216
ND2	1041	KY349335	AMNH:Birds:SKIN129217
ND2	1041	KY349334	AMNH:Birds:SKIN129218
ND2	1041	KY349338	AMNH:Birds:SKIN129219
ND2	1041	KY349315	AMNH:Birds:SKIN130378
ND2	1041	KY349336	AMNH:Birds:SKIN145689
ND2	1041	KY349337	AMNH:Birds:SKIN145690
ND2	1041	KY349326	AMNH:Birds:SKIN145691
ND2	1041	KY349339	AMNH:Birds:SKIN150041
ND2	1041	KY349340	AMNH:Birds:SKIN150042
ND2	1041	KY349316	AMNH:Birds:SKIN150043
ND2	1041	KY349321	AMNH:Birds:SKIN150044
ND2	1041	KY349318	AMNH:Birds:SKIN168302
ND2	1041	KY349323	AMNH:Birds:SKIN168303
ND2	1041	KY349322	AMNH:Birds:SKIN68304
ND2	1041	KY349325	AMNH:Birds:SKIN168305
ND2	1041	KY349328	AMNH:Birds:SKIN408731
ND2	1041	KY349330	AMNH:Birds:SKIN408732
ND2	1041	KY349331	AMNH:Birds:SKIN508414
ND2	1041	KY349327	AMNH:Birds:SKIN508415
ND2	1041	KY349317	AMNH:Birds:SKIN508417
ND2	1041	KY349320	AMNH:Birds:SKIN508424
ND2	1041	KY349319	AMNH:Birds:SKIN810790

Appendix B	(cont'd)		
Gene	base	Genbank	
Sequence	pairs	Accession #	Institution ID
ND2	1041	KY349324	AMNH:Birds:SKIN810791
ND2	1029	KY349354	MSB:Birds:27056
ND2	1029	KY349364	MSB:Birds:27057
ND2	951	KY349435	MSB:Birds:27102
ND2	1029	KY349361	MSB:Birds:27159
ND2	1020	KY349410	MSB:Birds:27172
ND2	1029	KY349366	MSB:Birds:28300
ND2	1029	KY349377	MSB:Birds:31424
ND2	1029	KY349367	MSB:Birds:31460
ND2	1020	KY349424	MSB:Birds:31779
ND2	1029	KY349370	MSB:Birds:31783
ND2	1029	KY349350	MSB:Birds:32904
ND2	1020	KY349415	MSB:Birds:32905
ND2	1029	KY349379	MSB:Birds:32927
ND2	951	KY349437	MSB:Birds:32935
ND2	1011	KY349342	MSB:Birds:32942
ND2	969	KY349396	MSB:Birds:32957
ND2	972	KY349434	MSB:Birds:32959
ND2	903	KY349436	MSB:Birds:32960
ND2	1026	KY349390	MSB:Birds:32965
ND2	1011	KY349432	MSB:Birds:32972
ND2	1020	KY349431	MSB:Birds:32976
ND2	1017	KY349427	MSB:Birds:32978
ND2	1029	KY349378	MSB:Birds:32983
ND2	1029	KY349353	MSB:Birds:32984
ND2	1011	KY349393	MSB:Birds:32989

Appendix B	(cont'd)		
Gene	base	Genbank	
Sequence	pairs	Accession #	Institution ID
ND2	1029	KY349381	MSB:Birds:32993
ND2	1017	KY349385	MSB:Birds:27005
ND2	1029	KY349380	MSB:Birds:27041
ND2	1029	KY349352	MSB:Birds:33100
ND2	1020	KY349411	MSB:Birds:33273
ND2	1014	KY349403	MSB:Birds:33296
ND2	1020	KY349422	MSB:Birds:33308
ND2	1029	KY349363	MSB:Birds:33319
ND2	1011	KY349343	MSB:Birds:33325
ND2	993	KY349402	MSB:Birds:33344
ND2	1020	KY349430	MSB:Birds:33350
ND2	1023	KY349399	MSB:Birds:33417
ND2	1026	KY349389	MSB:Birds:33445
ND2	1029	KY349356	MSB:Birds:33447
ND2	1020	KY349428	MSB:Birds:33466
ND2	1029	KY349358	MSB:Birds:33478
ND2	1020	KY349429	MSB:Birds:33487
ND2	1020	KY349421	MSB:Birds:33652
ND2	1017	KY349404	MSB:Birds:33665
ND2	1020	KY349416	MSB:Birds:34227
ND2	1017	KY349383	MSB:Birds:34233
ND2	1023	KY349398	MSB:Birds:34234
ND2	1029	KY349376	MSB:Birds:34237
ND2	1020	KY349346	MSB:Birds:34238
ND2	1029	KY349375	MSB:Birds:34239
ND2	1026	KY349392	MSB:Birds:34252

Appendix B	(cont'd)		
Gene	base	Genbank	
Sequence	pairs	Accession #	Institution ID
ND2	1020	KY349418	MSB:Birds:34253
ND2	942	KY349438	MSB:Birds:34256
ND2	1020	KY349419	MSB:Birds:34257
ND2	1020	KY349420	MSB:Birds:34265
ND2	1011	KY349409	MSB:Birds:34266
ND2	1020	KY349417	MSB:Birds:34688
ND2	1020	KY349372	MSB:Birds:34712
ND2	1014	KY349384	MSB:Birds:34726
ND2	1020	KY349345	MSB:Birds:34729
ND2	1026	KY349387	MSB:Birds:34731
ND2	1029	KY349369	MSB:Birds:34768
ND2	1014	KY349397	MSB:Birds:34769
ND2	1020	KY349414	MSB:Birds:34792
ND2	966	KY349371	MSB:Birds:34853
ND2	1023	KY349405	MSB:Birds:34978
ND2	1020	KY349412	MSB:Birds:34986
ND2	1023	KY349400	MSB:Birds:35036
ND2	1029	KY349347	MSB:Birds:35052
ND2	1029	KY349374	MSB:Birds:35082
ND2	972	KY349341	MSB:Birds:35202
ND2	1029	KY349349	MSB:Birds:35239
ND2	1026	KY349388	MSB:Birds:35241
ND2	1029	KY349365	MSB:Birds:35268
ND2	1023	KY349401	MSB:Birds:35270
ND2	1026	KY349391	MSB:Birds:35271
ND2	1029	KY349359	MSB:Birds:35296

Appendix B	(cont'd)		
Gene	base	Genbank	
Sequence	pairs	Accession #	Institution ID
ND2	1023	KY349395	MSB:Birds:35378
ND2	1029	KY349355	MSB:Birds:35474
ND2	1029	KY349348	MSB:Birds:35494
ND2	1023	KY349407	MSB:Birds:35505
ND2	1029	KY349362	MSB:Birds:35523
ND2	1029	KY349368	MSB:Birds:35564
ND2	1026	KY349386	MSB:Birds:35565
ND2	1014	KY349394	MSB:Birds:35568
ND2	1029	KY349382	MSB:Birds:35743
ND2	1029	KY349360	MSB:Birds:35744
ND2	1029	KY349357	MSB:Birds:35941
ND2	1020	KY349423	MSB:Birds:35952
ND2	1011	KY349408	MSB:Birds:35984
ND2	1020	KY349413	MSB:Birds:35996
ND2	1014	KY349426	MSB:Birds:36006
ND2	1023	KY349406	MSB:Birds:36029
ND2	1014	KY349425	MSB:Birds:36029
ND2	1017	KY349433	MSB:Birds:36560
ND2	1029	KY349351	MSB:Birds:36581
ND2	1023	KY349373	MSB:Birds:31063
ND2	1020	KY349344	MSB:Birds:31072
ND2	819	KY349439	MSB:Birds:31073

Individual	Locality	Elev	Plumage	Latitude	Longitude	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
NK168878	Apurim., PE	3602	cinereum	-14.4067	-73.0882	89	168	75	1197	193	-29	222	98	74	99	70	954	193	11	84	560	42	215	49
NK168891	Apurim., PE	3602	cinereum	-14.4067	-73.0882	89	168	75	1197	193	-29	222	98	74	99	70	954	193	11	84	560	42	215	49
NK172469	Apurim., PE	2338	cinereum	-14.0521	-73.1892	159	165	79	914	264	57	207	162	146	170	146	633	132	7	85	371	26	159	26
NK172470	Apurim., PE	2338	cinereum	-14.0521	-73.1892	159	165	79	914	264	57	207	162	146	170	146	633	132	7	85	371	26	159	26
AM129216	Cusco, PE	3497	cinereum	-13.53195	-71.867463	101	158	73	1356	195	-20	215	111	82	113	80	696	151	3	86	387	14	226	16
AM129217	Cusco, PE	3497	cinereum	-13.53195	-71.867463	101	158	73	1356	195	-20	215	111	82	113	80	696	151	3	86	387	14	226	16
AM129218	Cusco, PE	3497	cinereum	-13.53195	-71.867463	101	158	73	1356	195	-20	215	111	82	113	80	696	151	3	86	387	14	226	16
AM129219	Cusco, PE	3497	cinereum	-13.53195	-71.867463	101	158	73	1356	195	-20	215	111	82	113	80	696	151	3	86	387	14	226	16
NK159711	Cusco, PE	3120	cinereum	-13.6253	-71.718	123	155	74	1264	214	7	207	132	104	134	104	582	119	4	86	325	14	178	14
NK159712	Cusco, PE	3120	cinereum	-13.6253	-71.718	123	155	74	1264	214	7	207	132	104	134	104	582	119	4	86	325	14	178	14
NK159760	Cusco, PE	4330	cinereum	-13.1993	-72.16	44	164	71	1372	145	-84	229	53	25	56	22	795	152	7	76	413	31	240	37
NK159819	Cusco, PE	3380	cinereum	-13.2493	-72.1692	99	157	74	1196	193	-19	212	106	82	110	80	711	149	4	81	373	15	244	24
NK159832	Cusco, PE	3380	cinereum	-13.2493	-72.1692	99	157	74	1196	193	-19	212	106	82	110	80	711	149	4	81	373	15	244	24
NK168319	Cusco, PE	3835	cinereum	-13.1881	-72.2314	62	163	73	1293	161	-62	223	70	44	74	41	772	150	6	76	398	26	241	34
NK168492	Cusco, PE	2953	cinereum	-13.3259	-71.9568	137	153	76	1035	229	30	199	142	122	148	122	491	97	4	82	266	18	147	18
AM145691	Huarac., PE	3349	cinereum	-13.413324	-72.208249	115	155	75	1174	207	1	206	122	98	126	97	689	154	2	85	367	7	253	16
NK168248	Junin, PE	3520	cinereum	-11.4888	-74.8961	105	147	78	815	189	1	188	106	93	115	93	1005	158	17	58	444	71	320	71
AM150041	Limbani, PE	3720	cinereum	-14.13785	-69.699913	99	151	70	1526	190	-24	214	111	76	112	76	882	145	10	66	418	50	279	51
AM150042	Limbani, PE	3720	cinereum	-14.13785	-69.699913	99	151	70	1526	190	-24	214	111	76	112	76	882	145	10	66	418	50	279	51
AM150043	Limbani, PE	3720	cinereum	-14.13785	-69.699913	99	151	70	1526	190	-24	214	111	76	112	76	882	145	10	66	418	50	279	51
AM150044	Limbani, PE	3720	cinereum	-14.13785	-69.699913	99	151	70	1526	190	-24	214	111	76	112	76	882	145	10	66	418	50	279	51
AM508424	Limbani, PE	3720	cinereum	-14.13785	-69.699913	99	151	70	1526	190	-24	214	111	76	112	76	882	145	10	66	418	50	279	51
AM145689	Ollanta., PE	2846	cinereum	-13.258369	-72.264268	144	155	77	944	239	40	199	147	131	155	131	694	130	5	81	368	25	229	25
AM145690	Ollanta., PE	2846	cinereum	-13.258369	-72.264268	144	155	77	944	239	40	199	147	131	155	131	694	130	5	81	368	25	229	25
AM408731	Tarma, PE	3074	cinereum	-11.419335	-75.688949	121	145	79	786	203	21	182	126	109	129	109	459	82	5	67	226	24	139	24
AM408732	Tarma, PE	3074	cinereum	-11.419335	-75.688949	121	145	79	786	203	21	182	126	109	129	109	459	82	5	67	226	24	139	24
AM168304	Bestion, EC	3064	fraseri	-3.409889	-79.025278	110	92	83	585	168	58	110	113	102	115	100	831	114	31	35	310	119	200	121
AM810790	BDC, EC	2861	fraseri	-0.137427	-78.475194	138	126	89	138	209	68	141	139	137	139	137	1124	166	19	46	432	91	372	91
AM810791	BDC, EC	2861	fraseri	-0.137427	-78.475194	138	126	89	138	209	68	141	139	137	139	137	1124	166	19	46	432	91	372	91

**Appendix C**. Bioclimatic data used in this study, accessed from the WorldClim dataset. Geographic coordinates and locality information are included.

Appendix C (contra)	Appe	ndix C	(cont'd)
---------------------	------	--------	----------

Individual	Locality	Elev	Plumage	Latitude	Longitude	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
AM508415	Cayambe, EC	2806	fraseri	0.027441	-78.150742	137	124	89	198	206	68	138	137	134	138	134	863	111	18	42	306	88	290	88
AM168305	El Paso, EC	2786	fraseri	-3.3622	-79.023659	113	93	84	587	171	61	110	117	105	117	103	793	109	29	35	296	113	193	115
AM117203	Huila, CO	3547	fraseri	2.54919	-75.52767	254	111	88	272	318	193	125	251	254	256	251	1452	223	43	46	548	148	396	229
AM117204	Huila, CO	3547	fraseri	2.54919	-75.52767	254	111	88	272	318	193	125	251	254	256	251	1452	223	43	46	548	148	396	229
AM508417	Ibarra, EC	2280	fraseri	0.339095	-78.122234	162	123	86	175	233	90	143	163	159	163	159	627	94	15	45	235	66	235	66
AM130378	Loja, EC	2187	fraseri	-3.990164	-79.211277	165	100	81	409	229	107	122	167	160	169	158	1007	139	52	29	360	177	229	195
AM508414	Riobam., EC	2770	fraseri	-1.663528	-78.654646	139	119	82	633	211	67	144	143	129	144	129	553	69	23	31	193	90	129	90
AM168303	Salvias, EC	1316	fraseri	-3.638174	-79.547851	223	113	88	342	292	164	128	219	223	227	218	1526	265	9	80	776	49	147	772
AM168302	Tarrag., EC	1329	fraseri	-3.68727	-79.610794	219	115	87	326	289	158	131	214	219	222	214	1583	285	8	83	820	42	91	820
NK169453	Ancash, PE	115	litttorale	-10.0214	-78.0498	193	88	61	1948	271	129	142	213	181	220	171	17	4	0	63	9	0	8	2
NK169459	Ancash, PE	39	litttorale	-10.0681	-78.136	193	84	60	1983	271	131	140	221	193	221	171	9	2	0	72	5	0	5	1
NK169460	Ancash, PE	39	litttorale	-10.0681	-78.136	193	84	60	1983	271	131	140	221	193	221	171	9	2	0	72	5	0	5	1
NK169463	Ancash, PE	39	litttorale	-10.0681	-78.136	193	84	60	1983	271	131	140	221	193	221	171	9	2	0	72	5	0	5	1
NK169464	Ancash, PE	39	litttorale	-10.0681	-78.136	193	84	60	1983	271	131	140	221	193	221	171	9	2	0	72	5	0	5	1
NK169465	Ancash, PE	39	litttorale	-10.0681	-78.136	193	84	60	1983	271	131	140	221	193	221	171	9	2	0	72	5	0	5	1
NK169478	Ancash, PE	39	litttorale	-10.0681	-78.136	193	84	60	1983	271	131	140	221	193	221	171	9	2	0	72	5	0	5	1
NK169479	Ancash, PE	39	litttorale	-10.0681	-78.136	193	84	60	1983	271	131	140	221	193	221	171	9	2	0	72	5	0	5	1
NK169482	Ancash, PE	39	litttorale	-10.0681	-78.136	193	84	60	1983	271	131	140	221	193	221	171	9	2	0	72	5	0	5	1
NK169483	Ancash, PE	39	litttorale	-10.0681	-78.136	193	84	60	1983	271	131	140	221	193	221	171	9	2	0	72	5	0	5	1
NK169491	Ancash, PE	39	litttorale	-10.0681	-78.136	193	84	60	1983	271	131	140	221	193	221	171	9	2	0	72	5	0	5	1
NK169492	Ancash, PE	39	litttorale	-10.0681	-78.136	193	84	60	1983	271	131	140	221	193	221	171	9	2	0	72	5	0	5	1
NK171579	Ancash, PE	2972	litttorale	-8.75478	-78.04845	120	155	81	780	204	14	190	128	109	128	109	217	51	1	81	125	6	125	6
NK171704	Ancash, PE	3439	litttorale	-9.5721	-77.8472	160	141	81	1070	236	64	172	173	145	173	145	143	43	0	132	115	0	115	0
NK171712	Ancash, PE	3439	litttorale	-9.5721	-77.8472	160	141	81	1070	236	64	172	173	145	173	145	143	43	0	132	115	0	115	0
NK173815	Ancash, PE	3931	litttorale	-8.75236	-78.03402	87	153	80	826	172	-19	191	97	75	97	75	433	94	3	75	228	13	228	13
NK173827	Ancash, PE	3931	litttorale	-8.75236	-78.03402	87	153	80	826	172	-19	191	97	75	97	75	433	94	3	75	228	13	228	13
NK173837	Ancash, PE	3740	litttorale	-8.74247	-78.0407	89	153	79	825	174	-18	192	99	77	99	77	416	90	3	76	219	12	219	12
NK173841	Ancash, PE	3740	litttorale	-8.74247	-78.0407	89	153	79	825	174	-18	192	99	77	99	77	416	90	3	76	219	12	219	12
NK173860	Ancash, PE	3740	litttorale	-8.74247	-78.0407	89	153	79	825	174	-18	192	99	77	99	77	416	90	3	76	219	12	219	12
NK172249	Arequipa, PE	3200	litttorale	-15.8143	-72.6671	93	172	76	1252	196	-28	224	106	79	106	74	273	85	0	137	229	2	229	2
NK172290	Arequipa, PE	3200	litttorale	-15.8143	-72.6671	93	172	76	1252	196	-28	224	106	79	106	74	273	85	0	137	229	2	229	2

Appendix C (cont'd)

Individual	Locality	Elev	Plumage	Latitude	Longitude	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
NK172291	Arequipa, PE	3200	litttorale	-15.8143	-72.6671	93	172	76	1252	196	-28	224	106	79	106	74	273	85	0	137	229	2	229	2
NK172294	Arequipa, PE	3200	litttorale	-15.8143	-72.6671	93	172	76	1252	196	-28	224	106	79	106	74	273	85	0	137	229	2	229	2
NK171928	Cajamarca, PE	2500	litttorale	-7.398	-78.7783	130	154	83	818	212	28	184	140	119	140	119	676	151	4	76	358	27	358	27
NK171965	Cajamarca, PE	2500	litttorale	-7.398	-78.7783	130	154	83	818	212	28	184	140	119	140	119	676	151	4	76	358	27	358	27
NK171967	Cajamarca, PE	2622	litttorale	-7.3982	-78.776	130	154	83	818	212	28	184	140	119	140	119	676	151	4	76	358	27	358	27
NK171994	Cajamarca, PE	2500	litttorale	-7.398	-78.7783	130	154	83	818	212	28	184	140	119	140	119	676	151	4	76	358	27	358	27
NK171996	Cajamarca, PE	2625	litttorale	-7.3982	-78.776	130	154	83	818	212	28	184	140	119	140	119	676	151	4	76	358	27	358	27
NK171997	Cajamarca, PE	2622	litttorale	-7.3982	-78.776	130	154	83	818	212	28	184	140	119	140	119	676	151	4	76	358	27	358	27
NK172022	Cajamarca, PE	2500	litttorale	-7.398	-78.7783	130	154	83	818	212	28	184	140	119	140	119	676	151	4	76	358	27	358	27
NK172104	Cajamarca, PE	2550	litttorale	-7.4038	-78.776	135	153	83	836	217	34	183	145	123	145	123	654	153	4	80	359	25	359	25
NK220285	Huancavelica, PE	3630	litttorale	-13.336333	-75.3248333	78	151	79	997	162	-29	191	88	62	88	62	691	171	1	108	480	8	480	8
NK220294	Huancavelica, PE	3630	litttorale	-13.336333	-75.3248333	78	151	79	997	162	-29	191	88	62	88	62	691	171	1	108	480	8	480	8
NK220295	Huancavelica, PE	3630	litttorale	-13.33633	-75.32483	78	151	79	997	162	-29	191	88	62	88	62	691	171	1	108	480	8	480	8
NK171414	La Libertad, PE	309	litttorale	-8.38685	-78.64518	187	91	64	1748	263	121	142	211	176	211	167	35	9	1	90	22	3	22	3
NK171438	La Libertad, PE	309	litttorale	-8.38685	-78.64518	187	91	64	1748	263	121	142	211	176	211	167	35	9	1	90	22	3	22	3
NK171452	La Libertad, PE	309	litttorale	-8.38685	-78.64518	187	91	64	1748	263	121	142	211	176	211	167	35	9	1	90	22	3	22	3
NK171455	La Libertad, PE	309	litttorale	-8.38685	-78.64518	187	91	64	1748	263	121	142	211	176	211	167	35	9	1	90	22	3	22	3
NK171457	La Libertad, PE	309	litttorale	-8.38685	-78.64518	187	91	64	1748	263	121	142	211	176	211	167	35	9	1	90	22	3	22	3
NK171494	La Libertad, PE	309	litttorale	-8.38685	-78.64518	187	91	64	1748	263	121	142	211	176	211	167	35	9	1	90	22	3	22	3
NK171495	La Libertad, PE	309	litttorale	-8.38685	-78.64518	187	91	64	1748	263	121	142	211	176	211	167	35	9	1	90	22	3	22	3
NK171518	La Libertad, PE	309	litttorale	-8.38685	-78.64518	187	91	64	1748	263	121	142	211	176	211	167	35	9	1	90	22	3	22	3

Individual	Locality	Elev	Plumage	Latitude	Longitude	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
NK162893	Lima, PE	3750	litttorale	-11.7619	-76.5489	47	138	77	1217	122	-56	178	62	30	62	30	606	149	1	98	383	8	383	8
NK162988	Lima, PE	372	litttorale	-12.0015	-76.9208	189	97	55	2742	291	116	175	158	183	227	157	15	2	0	41	6	1	5	6
NK163024	Lima, PE	352	litttorale	-12.0089	-76.9232	189	97	55	2754	292	116	176	158	184	227	157	15	2	0	41	6	1	5	6
NK163451	Lima, PE	2390	litttorale	-11.7442	-76.6088	144	134	75	1436	225	48	177	162	124	162	124	197	80	0	138	150	0	150	0
NK163455	Lima, PE	2390	litttorale	-11.7442	-76.6088	144	134	75	1436	225	48	177	162	124	162	124	197	80	0	138	150	0	150	0
NK168076	Lima, PE	935	litttorale	-12.0295	-76.6508	168	122	68	1980	258	80	178	194	149	194	143	80	30	0	147	69	0	69	0
NK168077	Lima, PE	935	litttorale	-12.0295	-76.6508	168	122	68	1980	258	80	178	194	149	194	143	80	30	0	147	69	0	69	0
NK168099	Lima, PE	935	litttorale	-12.0295	-76.6508	168	122	68	1980	258	80	178	194	149	194	143	80	30	0	147	69	0	69	0
NK168107	Lima, PE	935	litttorale	-12.0295	-76.6508	168	122	68	1980	258	80	178	194	149	194	143	80	30	0	147	69	0	69	0
NK168114	Lima, PE	352	litttorale	-12.0089	-76.9232	189	97	55	2754	292	116	176	158	184	227	157	15	2	0	41	6	1	5	6
NK168129	Lima, PE	352	litttorale	-12.0089	-76.9232	189	97	55	2754	292	116	176	158	184	227	157	15	2	0	41	6	1	5	6
NK168131	Lima, PE	352	litttorale	-12.0089	-76.9232	189	97	55	2754	292	116	176	158	184	227	157	15	2	0	41	6	1	5	6
NK168132	Lima, PE	352	litttorale	-12.0089	-76.9232	189	97	55	2754	292	116	176	158	184	227	157	15	2	0	41	6	1	5	6
NK168137	Lima, PE	352	litttorale	-12.0089	-76.9232	189	97	55	2754	292	116	176	158	184	227	157	15	2	0	41	6	1	5	6
NK168144	Lima, PE	352	litttorale	-12.0089	-76.9232	189	97	55	2754	292	116	176	158	184	227	157	15	2	0	41	6	1	5	6
NK168148	Lima, PE	352	litttorale	-12.0089	-76.9232	189	97	55	2754	292	116	176	158	184	227	157	15	2	0	41	6	1	5	6
NK168150	Lima, PE	352	litttorale	-12.0089	-76.9232	189	97	55	2754	292	116	176	158	184	227	157	15	2	0	41	6	1	5	6
NK168155	Lima, PE	352	litttorale	-12.0089	-76.9232	189	97	55	2754	292	116	176	158	184	227	157	15	2	0	41	6	1	5	6
NK168156	Lima, PE	352	litttorale	-12.0089	-76.9232	189	97	55	2754	292	116	176	158	184	227	157	15	2	0	41	6	1	5	6
NK168161	Lima, PE	352	litttorale	-12.0089	-76.9232	189	97	55	2754	292	116	176	158	184	227	157	15	2	0	41	6	1	5	6
NK168165	Lima, PE	352	litttorale	-12.0089	-76.9232	189	97	55	2754	292	116	176	158	184	227	157	15	2	0	41	6	1	5	6
NK168211	Lima, PE	3000	litttorale	-11.75	-76.5917	98	140	79	1188	174	-2	176	112	81	112	81	287	102	0	142	237	0	237	0
NK168515	Lima, PE	4062	litttorale	-11.7677	-76.5346	34	138	76	1286	110	-70	180	50	16	50	16	716	157	3	90	424	14	424	14
NK168527	Lima, PE	4093	litttorale	-11.7686	-76.5333	36	138	77	1279	111	-68	179	52	18	52	18	700	155	3	90	417	14	417	14
NK168538	Lima, PE	3970	litttorale	-11.7677	-76.5346	34	138	76	1286	110	-70	180	50	16	50	16	716	157	3	90	424	14	424	14
NK168544	Lima, PE	3910	litttorale	-11.7636	-76.5425	47	138	77	1217	122	-56	178	62	30	62	30	606	149	1	98	383	8	383	8
NK168563	Lima, PE	3907	litttorale	-11.7677	-76.5346	34	138	76	1286	110	-70	180	50	16	50	16	716	157	3	90	424	14	424	14
NK168569	Lima, PE	3905	litttorale	-11.7677	-76.5346	34	138	76	1286	110	-70	180	50	16	50	16	716	157	3	90	424	14	424	14
NK168636	Lima, PE	4056	litttorale	-11.7677	-76.5346	34	138	76	1286	110	-70	180	50	16	50	16	716	157	3	90	424	14	424	14
NK168664	Lima, PE	4131	litttorale	-11.7694	-76.5328	36	138	77	1279	111	-68	179	52	18	52	18	700	155	3	90	417	14	417	14
NK168666	Lima, PE	4082	litttorale	-11.7685	-76.5333	36	138	77	1279	111	-68	179	52	18	52	18	700	155	3	90	417	14	417	14

Appendix C (cont'd)

Appendix C	Appendix C (cont'd)																							
Individual	Locality	Elev	Plumage	Latitude	Longitude	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
NK168685	Lima, PE	4178	litttorale	-11.7697	-76.531	36	138	77	1279	111	-68	179	52	18	52	18	700	155	3	90	417	14	417	14
NK168697	Lima, PE	4140	litttorale	-11.7696	-76.5319	36	138	77	1279	111	-68	179	52	18	52	18	700	155	3	90	417	14	417	14
NK168706	Lima, PE	4088	litttorale	-11.7686	-76.5333	36	138	77	1279	111	-68	179	52	18	52	18	700	155	3	90	417	14	417	14
NK172779	Lima, PE	2086	litttorale	-11.757188	-76.597222	98	140	79	1188	174	-2	176	112	81	112	81	287	102	0	142	237	0	237	0
NK172790	Lima, PE	2086	litttorale	-11.757188	-76.597222	98	140	79	1188	174	-2	176	112	81	112	81	287	102	0	142	237	0	237	0
NK175512	Lima, PE	2086	litttorale	-11.757188	-76.597222	98	140	79	1188	174	-2	176	112	81	112	81	287	102	0	142	237	0	237	0
NK175533	Lima, PE	2086	litttorale	-11.757188	-76.597222	98	140	79	1188	174	-2	176	112	81	112	81	287	102	0	142	237	0	237	0
NK171762	Tacna, PE	740	litttorale	-17.5614	-70.6724	180	115	63	2208	272	92	180	154	187	209	152	11	2	0	76	5	0	3	4
NK171778	Tacna, PE	740	litttorale	-17.5614	-70.6724	180	115	63	2208	272	92	180	154	187	209	152	11	2	0	76	5	0	3	4
NK171808	Tacna, PE	3728	litttorale	-17.227	-70.2621	75	168	75	1831	176	-47	223	92	51	94	47	255	78	0	134	210	1	183	3
NK172200	Tacna, PE	2975	litttorale	-17.3209	-70.2477	117	160	75	1720	214	2	212	134	95	136	92	109	37	0	136	94	0	83	1
NK172220	Tacna, PE	2975	litttorale	-17.3209	-70.2477	117	160	75	1720	214	2	212	134	95	136	92	109	37	0	136	94	0	83	1
NK172231	Tacna, PE	2975	litttorale	-17.3209	-70.2477	117	160	75	1720	214	2	212	134	95	136	92	109	37	0	136	94	0	83	1

Individual	Elevation	Subspecies	Hct	[Hb]	Latitude	Longitude
NK172469	2338	cinereum	0.534598214	16.9	-14.0521	-73.1892
NK172470	2338	cinereum	0.467554389	15.2	-14.0521	-73.1892
NK168492	2953	cinereum	0.456539139	17.4	-13.3259	-71.9568
NK159711	3120	cinereum	0.53084	19.4	-13.6253	-71.718
NK159712	3120	cinereum	0.57803	18.8	-13.6253	-71.718
NK159819	3380	cinereum	0.52686	17.4	-13.2493	-72.1692
NK159832	3380	cinereum	0.56667	19.3	-13.2493	-72.1692
NK168248	3520	cinereum	0.47832	16.6	-11.4888	-74.8961
NK172666	3563	cinereum	0.476978826	13	-14.6787	-73.1478
NK172688	3563	cinereum	0.494543165	16.5	-14.6787	-73.1478
NK172692	3563	cinereum	0.502873078	15.2	-14.6787	-73.1478
NK168878	3602	cinereum	0.526513123	17.9	-14.4067	-73.0882
NK168891	3602	cinereum	0.565578123	18.2	-14.4067	-73.0882
NK168319	3835	cinereum	0.533649232	20.9	-13.1881	-72.2314
NK168308	4030	cinereum	0.567768704		-13.188	-72.2313
NK159760	4330	cinereum	0.58016	20.1	-13.1993	-72.16
NK169459	39	litttorale		14.1	-10.0681	-78.136
NK169460	39	litttorale	0.570979335	16.6	-10.0681	-78.136
NK169463	39	litttorale	0.530531845	16.9	-10.0681	-78.136
NK169464	39	litttorale	0.473989557	14.1	-10.0681	-78.136
NK169465	39	litttorale	0.50124805	16.8	-10.0681	-78.136
NK169478	39	litttorale	0.497915482		-10.0681	-78.136
NK169479	39	litttorale	0.479024819		-10.0681	-78.136

**Appendix D**. Field data used in analysis of blood properties; Museum of Southwestern Biology specimen numbers (NK), Elevation of occurrence, subspecies (as determined by plumage an genetic analysis), hematocrit (Hct), hemoglobin concentration ([Hb], and locality data (in decimal degrees, geodetic datum WGS1984).

4	Append. D	(cont d)					
	Individual	Elevation	Subspecies	Hct	[Hb]	Latitude	Longitude
	NK169482	39	litttorale	0.50123533		-10.0681	-78.136
	NK169483	39	litttorale	0.477741197		-10.0681	-78.136
	NK169491	39	litttorale	0.476022664		-10.0681	-78.136
	NK169492	39	litttorale	0.490948588		-10.0681	-78.136
	NK171414	309	litttorale	0.495520062	14.5	-8.38685	-78.64518
	NK171438	309	litttorale	0.476910828	13.4	-8.38685	-78.64518
	NK171452	309	litttorale	0.505690252	13.6	-8.38685	-78.64518
	NK171455	309	litttorale	0.52388535	14.6	-8.38685	-78.64518
	NK171457	309	litttorale	0.494238358	14.6	-8.38685	-78.64518
	NK171494	309	litttorale	0.485790072	13.5	-8.38685	-78.64518
	NK171495	309	litttorale		16.3	-8.38685	-78.64518
	NK171518	309	litttorale	0.443134391	11.5	-8.38685	-78.64518
	NK163024	352	litttorale	0.41041	14.54	-12.0089	-76.9232
	NK168114	352	litttorale	0.478648728		-12.0089	-76.9232
	NK168123	352	litttorale	0.453018539		-12.0089	-76.9232
	NK168129	352	litttorale	0.449869946		-12.0089	-76.9232
	NK168131	352	litttorale	0.434494196		-12.0089	-76.9232
	NK168132	352	litttorale	0.44661808		-12.0089	-76.9232
	NK168137	352	litttorale	0.433046248		-12.0089	-76.9232
	NK168144	352	litttorale	0.494361193		-12.0089	-76.9232
	NK168148	352	litttorale	0.487975592		-12.0089	-76.9232
	NK168150	352	litttorale	0.308694379		-12.0089	-76.9232
	NK168155	352	litttorale	0.405132603		-12.0089	-76.9232
	NK168156	352	litttorale	0.431220096		-12.0089	-76.9232
	NK168161	352	litttorale	0.427193302		-12.0089	-76.9232
	NK168165	352	litttorale	0.479635873		-12.0089	-76.9232

Append. D	(cont d)						
Individual	Elevation	Subspecies	Hct	[Hb]	Latitude	Longitude	
NK162984	372	litttorale	0.53177	17	-12.0014	-76.9208	
NK162988	372	litttorale	0.39442	12.9	-12.0015	-76.9208	
NK171762	740	litttorale	0.463717331	15	-17.5614	-70.6724	
NK171778	740	litttorale	0.455921053	14.8	-17.5614	-70.6724	
NK168076	935	litttorale	0.541843339		-12.0295	-76.6508	
NK168077	935	litttorale	0.480073174		-12.0295	-76.6508	
NK168099	935	litttorale	0.503940274		-12.0295	-76.6508	
NK168107	935	litttorale	0.522642293		-12.0295	-76.6508	
NK163451	2390	litttorale	0.525575113		-11.7442	-76.6088	
NK163455	2390	litttorale	0.524816278		-11.7442	-76.6088	
NK171928	2500	litttorale	0.536088475	17.1	-7.398	-78.7783	
NK171965	2500	litttorale	0.495110866	16.5	-7.398	-78.7783	
NK172131	2535	litttorale	0.516751519	16.8	-7.4038	-78.7797	
NK172104	2550	litttorale	0.521342576	16.9	-7.4038	-78.776	
NK172142	2550	litttorale	0.540705752	17	-7.4038	-78.7797	
NK171967	2622	litttorale	0.42352835	14.1	-7.3982	-78.776	
NK171997	2622	litttorale	0.499036052	16.6	-7.3982	-78.776	
NK171996	2625	litttorale	0.479806779	16.5	-7.3982	-78.776	
NK171579	2972	litttorale	0.555650393	17.2	-8.75478	-78.04845	
NK171600	2972	litttorale	0.595573146	16.3	-8.75478	-78.04845	
NK172200	2975	litttorale	0.490343348	17.1	-17.3209	-70.2477	
NK172220	2975	litttorale	0.465686275	15	-17.3209	-70.2477	
NK172231	2975	litttorale	0.496557659	16.2	-17.3209	-70.2477	
NK168211	3000	litttorale	0.5428	18.5	-11.75	-76.5917	
NK172249	3200	litttorale	0.561110055	18	-15.8143	-72.6671	
NK172290	3200	litttorale	0.512847078	16.2	-15.8143	-72.6671	

Append D (cont'd)

Append. D	(cont'd)					
Individual	Elevation	Subspecies	Het	[Hb]	Latitude	Longitude
NK172291	3200	litttorale	0.500303674	17.6	-15.8143	-72.6671
NK172294	3200	litttorale	0.487974255	16.1	-15.8143	-72.6671
NK171704	3439	litttorale	0.534651701	18.1	-9.5721	-77.8472
NK171712	3439	litttorale	0.476680384	15.5	-9.5721	-77.8472
NK168214	3630	litttorale		19.2	-13.3363	-75.3248
NK168216	3630	litttorale	0.52697	17.4	-13.3363	-75.3248
NK168221	3630	litttorale	0.55784	20.5	-13.3363	-75.3248
NK220285	3630	litttorale	0.51996	18	-13.3363333	-75.3248333
NK220293	3630	litttorale	0.63793	21.7	-13.3363333	-75.3248333
NK220294	3630	litttorale	0.62322	20.9	-13.3363333	-75.3248333
NK220295	3630	litttorale	0.56875	17.7	-13.33633	-75.32483
NK171808	3728	litttorale	0.564072693	16.6	-17.227	-70.2621
NK173837	3740	litttorale	0.574676724	17.6	-8.74247	-78.0407
NK173841	3740	litttorale	0.548362994	17.9	-8.74247	-78.0407
NK173860	3740	litttorale	0.549365522	16.3	-8.74247	-78.0407
NK162883	3750	litttorale	0.53747	17.9	-11.7618	-76.5488
NK162937	3750	litttorale	0.57545	20.2	-11.7618	-76.5488
NK162953	3750	litttorale	0.54591	17.8	-11.7618	-76.5488
NK168569	3905	litttorale	0.527654654		-11.7677	-76.5346
NK168563	3907	litttorale	0.494216867		-11.7677	-76.5346
NK168544	3910	litttorale	0.598566759		-11.7636	-76.5425
NK173827	3931	litttorale	0.594767442	16.8	-8.75236	-78.03402
NK168538	3970	litttorale	0.546803426	20.7	-11.7677	-76.5346
NK168620	4056	litttorale	0.540443071	18	-11.7677	-76.5346
NK168636	4056	litttorale	0.536689255	18.2	-11.7677	-76.5346
NK168515	4062	litttorale	0.521426558	19.3	-11.7677	-76.5346

Append. D	(cont'd)					
Individual	Elevation	Subspecies	Het	[Hb]	Latitude	Longitude
NK168666	4082	litttorale	0.56303016	19.6	-11.7685	-76.5333
NK168706	4088	litttorale	0.528304442	19	-11.7686	-76.5333
NK168527	4093	litttorale	0.510651951	19.5	-11.7686	-76.5333
NK168664	4131	litttorale	0.510613752	17.2	-11.7694	-76.5328
NK168697	4140	litttorale	0.588646967	20	-11.7696	-76.5319
NK168685	4178	litttorale	0.577135748	19.9	-11.7697	-76.531

**Appendix E.** *Conirostrum cinereum* specimen and observation data available on GBIF and VERNET databases with associated locality and individual specimen information (based on availability). Individuals are sorted by latitude, then subspecies designation (according to respective source).

Latitude	Longitude	Subspp.	Elev.	<b>Record Type</b>	Institution	Catalog. #	Preparation	Mass	Habitat
-18.50000	-70.41667	littorale		Specimen	SMF-Aves	77467			
-18.50000	-70.25000	littorale		Specimen	SMF-Aves	77466			
-18.50000	-70.41667	littorale		Specimen	SMF-Aves	77467			
-18.50000	-70.25000	littorale		Specimen	SMF-Aves	77466			
-18.48000	-70.33000	littorale		Specimen	LACM-Birds	26159			
-18.48000	-70.33000	littorale		Specimen	LACM-Birds	26159	Skin; skull		
-18.37300	-69.55000	unknown		Observation	CLO-Birds	167959			
-18.37300	-69.55000	unknown		Observation	CLO-Birds	167960			
-18.19641	-69.56603	unknown		Observation	CLO-Birds	167957			
-17.56136	-70.67236	unknown	740	Specimen	MSB-Birds	35036	skin; heart, liver, muscle; blood; partial skeleton; muscle; blood	8.97	dry scrub near agricultural fields and river
-17.56136	-70.67236	unknown	740	Specimen	MSB-Birds	35052	muscle (RNAlater); heart, liver, muscle (frozen); blood (slide smear); blood (flash- frozen)	9.15	dry scrub around agricultural fields
-17.56136	-70.67236	unknown	740	Specimen	MSB-Birds	35052			
-17.56136	-70.67236	unknown	740	Specimen	MSB-Birds	35036			
-17.32088	-70.24773	unknown	2975	Specimen	MSB-Birds	35474	blood (flash-frozen); muscle (RNAlater); lung (frozen); heart; blood (slide smear); partial skeleton; liver, muscle (frozen)	8.82	Arid riparian scrub

Ap	pendix	E	(cont'd)
1 I P	penann		come a,

Latitude	Longitude	Subspp.	Elev.	<b>Record Type</b>	Institution	Catalog. #	<sup>‡</sup> Preparation	Mass	Habitat
-17.32088	-70.24773	unknown	2975	Specimen	MSB-Birds	35505	blood (flash-frozen); muscle (RNAlater); lung (frozen); heart; blood (slide smear); partial skeleton; liver, muscle (frozen)	7.29	Arid riparian scrub
-17.32088	-70.24773	unknown	2975	Specimen	MSB-Birds	35494	skin; muscle (RNAlater); heart; liver, muscle (frozen); blood (slide smear); blood (flash-frozen); lung (frozen)	8.46	Arid riparian scrub
-17.32088	-70.24773	unknown	2975	Specimen	MSB-Birds	35494			
-17.32088	-70.24773	unknown	2975	Specimen	MSB-Birds	35505			
-17.32088	-70.24773	unknown	2975	Specimen	MSB-Birds	35474			
-17.22700	-70.26208	unknown	3728	Specimen	MSB-Birds	35082			
-17.22700	-70.26208	unknown	3728	Specimen	MSB-Birds	35082	skin; heart, liver, muscle (frozen); blood (slide smear); muscle (RNAlater); blood (flash-frozen)	8.98	dry montane scrub along agricultural fields
-17.00000	-72.10000	unknown		Observation	CLO-Birds	33926			
-16.42000	-71.42000	unknown		Observation	CLO-Birds	33846			
-16.42000	-71.42000	unknown		Observation	CLO-ML	33855			
-16.40000	-71.50000	unknown		Observation	CLO-Birds	33886			
-16.40000	-71.50000	unknown		Observation	CLO-Birds	33909			
-15.82500	-68.64583	unknown		Observation	CLO-Birds	168152			
-15.81433	-72.66712	unknown	3200	Specimen	MSB-Birds	35523	skin; muscle (RNAlater); heart; liver, muscle (frozen); blood (slide smear); partial skeleton; blood (flash- frozen); lung (frozen)	7.97	Arid riparian scrub

Appendix E (cont'd)

Latitude	Longitude	Subspp.	Elev.	Record Type	Institution	Catalog. #	<pre># Preparation</pre>	Mass	Habitat
-15.81433	-72.66712	unknown	3200	Specimen	MSB-Birds	35565	skin; heart, liver, muscle (frozen); blood (slide smear); muscle (RNAlater); blood (flash-frozen)	7.83	dry montane scrub
-15.81433	-72.66712	unknown	3200	Specimen	MSB-Birds	35564	muscle (RNAlater); blood (flash-frozen); heart, liver, muscle (frozen); partial skeleton; blood (slide smear)	8.3	dry montane scrub
-15.81433	-72.66712	unknown	3200	Specimen	MSB-Birds	35568	muscle (RNAlater); blood (flash-frozen); heart, liver, muscle (frozen); partial skeleton; blood (slide smear)	8.87	dry montane scrub
-15.81433	-72.66712	unknown	3200	Specimen	MSB-Birds	35568			
-15.81433	-72.66712	unknown	3200	Specimen	MSB-Birds	35523			
-15.81433	-72.66712	unknown	3200	Specimen	MSB-Birds	35565			
-15.81433	-72.66712	unknown	3200	Specimen	MSB-Birds	35564			
-14.67873	-73.14780	unknown	3563	Specimen	MSB-Birds	35851	blood (slide smear); blood (flash-frozen); muscle (RNAlater); heart, liver, muscle (frozen)	9.67	Polylepis forest
-14.67873	-73.14780	unknown	3563	Specimen	MSB-Birds	35877	blood (slide smear); blood (flash-frozen); muscle (RNAlater); heart, liver, muscle (frozen)		Polylepis forest
-14.67873	-73.14780	unknown	3563	Specimen	MSB-Birds	35873	skin; blood (flash-frozen); muscle (RNAlater); blood (slide smear); heart, liver, muscle (frozen)		Polylepis forest
-14.67873	-73.14780	unknown	3563	Specimen	MSB-Birds	35851			
-14.67873	-73.14780	unknown	3563	Specimen	MSB-Birds	35873			
-14.67873	-73.14780	unknown	3563	Specimen	MSB-Birds	35877			

Appendix E (cont'd)

Latitude	Longitude	Subspp.	Elev.	Record Type	Institution	Catalog. #	<sup>‡</sup> Preparation	Mass	Habitat
-14.40667	-73.08815	unknown	3602	Specimen	MSB-Birds	33665			
-14.40667	-73.08815	unknown	3602	Specimen	MSB-Birds	33652			
-14.40667	-73.08815	unknown	3602	Specimen	MSB-Birds	33652	skin; heart, liver, muscle (frozen); blood (slide smear); muscle (RNAlater); blood (flash-frozen)	8.35	semi-humid montane scrub
-14.40667	-73.08815	unknown	3602	Specimen	MSB-Birds	33665	muscle (RNAlater); heart, liver, muscle (frozen); blood (slide smear); blood (flash- frozen)	8.58	semi-humid montane scrub
-14.05210	-73.18917	unknown	2338	Specimen	MSB-Birds	35744			
-14.05210	-73.18917	unknown	2338	Specimen	MSB-Birds	35743			
-14.05210	-73.18917	unknown	2338	Specimen	MSB-Birds	35744	skin; muscle (RNAlater); heart; liver, muscle (frozen); blood (slide smear); partial skeleton; blood (flash- frozen); lung (frozen)	8.98	among trees along river in valley
-14.05210	-73.18917	unknown	2338	Specimen	MSB-Birds	35743	blood (flash-frozen); muscle (RNAlater); lung (frozen); heart; blood (slide smear); partial skeleton; liver, muscle (frozen)	9.246	among trees along river in valley
-13.68500	-76.20800	littorale		Specimen	KU-Birds	113462			
-13.68500	-76.20800	littorale		Specimen	KU-Birds	113462	Skin - 1; Tissue - 100	9	
-13.62533	-71.71800	unknown	3120	Specimen	MSB-Birds	27057	skin; blood (flash-frozen); heart, liver, muscle (frozen); muscle (RNAlater)	9.2	semi- arid montane scrub
-13.62533	-71.71800	unknown	3120	Specimen	MSB-Birds	27056			
-13.62533	-71.71800	unknown	3120	Specimen	MSB-Birds	27057			

Appendix E (cont'd)

Latitude	Longitude	Subspp.	Elev.	<b>Record Type</b>	Institution	Catalog. #	Preparation	Mass	Habitat
-13.62533	-71.71800	unknown	3120	Specimen	MSB-Birds	27056	muscle (RNAlater); heart, liver, muscle (frozen); blood (flash-frozen)	9.5	Ca. ruinas y frente a Laguna Huacarpay
-13.60860	-75.34860	unknown	2850	Specimen	USNM-Birds	637897			
-13.60860	-75.34860	unknown	2850	Specimen	USNM-Birds	637897.44 25	Skeleton: Whole		3 m up from tree near agricultural fields
-13.60000	-71.70000	unknown		Specimen	UAM-Birds	26258			
-13.60000	-71.70000	unknown		Specimen	UAM-Birds	25922			
-13.60000	-71.70000	unknown		Specimen	UAM-Birds	26050			
-13.60000	-71.70000	unknown		Specimen	UAM-Birds	25922	skin; tissue (frozen); stomach; skeleton	9.5	
-13.60000	-71.70000	unknown		Specimen	UAM-Birds	26050	tissue (frozen); skeleton; stomach	9.5	
-13.60000	-71.70000	unknown		Specimen	UAM-Birds	26258	tissue (frozen); skeleton; stomach	10	
-13.54933	-70.89113	unknown	2240	Specimen	MSB-Birds	33540	muscle (frozen)	7.8	montane second growth
-13.54933	-70.89113	unknown	2240	Specimen	MSB-Birds	33540			
-13.54773	-70.88902	unknown	2292	Specimen	MSB-Birds	33558	muscle (frozen)	7.51	montane second growth
-13.54773	-70.88902	unknown	2292	Specimen	MSB-Birds	33558			
-13.33633	-75.32483	littorale	3630	Specimen	MVZ-Birds	183362	skeleton; tissue (flash- frozen)	6.7	
-13.33633	-75.32483	littorale	3630	Specimen	MVZ-Birds	183361	skeleton; tissue (flash- frozen)	6.3	
-13.33633	-75.32483	unknown	3630	Specimen	MSB-Birds	27007	skin; blood (slide smear); heart, liver, muscle (frozen); muscle (RNAlater)	8.1	hillside scrub

Appendix	E (cont'd)								
Latitude	Longitude	Subspp.	Elev.	<b>Record Type</b>	Institution	Catalog. #	Preparation	Mass	Habitat
-13.33633	-75.32483	unknown	3630	Specimen	MSB-Birds	27009	skin; heart, liver, muscle (frozen); blood (slide smear); muscle (RNAlater); blood (flash-frozen)	8.1	hillside scrub
-13.33633	-75.32483	littorale	3630	Specimen	MVZ-Birds	183362			
-13.33633	-75.32483	littorale	3630	Specimen	MVZ-Birds	183361			
-13.33633	-75.32483	unknown	3630	Specimen	MSB-Birds	27009			
-13.33633	-75.32483	unknown	3630	Specimen	MSB-Birds	27014			
-13.33633	-75.32483	unknown	3630	Specimen	MSB-Birds	27007			
-13.33633	-75.32483	unknown	3630	Specimen	MSB-Birds	27014	skin; heart, liver, muscle (frozen); blood (slide smear); muscle (RNAlater); blood (flash-frozen)	8.1	hillside scrub
-13.32783	-74.19583	cinereum		Specimen	KU-Birds	113483	Skin - 1; Tissue - 100	9.5	
-13.32783	-74.19583	cinereum		Specimen	KU-Birds	113483			
-13.32590	-71.95682	unknown	2953	Specimen	MSB-Birds	33273			
-13.32590	-71.95682	unknown	2953	Specimen	MSB-Birds	33273	skin; blood (flash-frozen); muscle (RNAlater); blood (slide smear); heart, liver, muscle (frozen)	8.98	garden
-13.24933	-72.16917	unknown	3380	Specimen	MSB-Birds	27172			
-13.24933	-72.16917	unknown	3380	Specimen	MSB-Birds	27159			
-13.24933	-72.16917	unknown	3380	Specimen	MSB-Birds	27159	skin; blood (flash-frozen); heart, liver, muscle (frozen); muscle (RNAlater)	9.2	semi-humid montane scrub near stream
-13.24933	-72.16917	unknown	3380	Specimen	MSB-Birds	27172	skin; blood (flash-frozen); heart, liver, muscle (frozen); muscle (RNAlater)	9.1	semi-humid montane scrub near stream

Appendix E (cont'd)

Latitude	Longitude	Subspp.	Elev.	<b>Record Type</b>	Institution	Catalog. #	Preparation	Mass	Habitat
-13.19933	-72.16000	unknown	4330	Specimen	MSB-Birds	27102			
-13.19933	-72.16000	unknown	4330	Specimen	MSB-Birds	27102	skin; muscle (RNAlater); blood (flash-frozen); heart, liver, muscle (frozen)	9.6	Bosque de Polylepis.
-13.18808	-72.23137	unknown	4030	Specimen	MSB-Birds	33089	skin; heart, liver, muscle (frozen); blood (slide smear); muscle (RNAlater); blood (flash-frozen)	9.58	bosque de Polylepis
-13.18808	-72.23137	unknown	3835	Specimen	MSB-Birds	33100	skin; heart, liver, muscle (frozen); blood (slide smear); muscle (RNAlater); blood (flash-frozen)	9.13	bosque de Polylepis
-13.18808	-72.23137	unknown	3835	Specimen	MSB-Birds	33100			
-13.18808	-72.23137	unknown	4030	Specimen	MSB-Birds	33089			
-13.17758	-72.59600	unknown	3361	Specimen	MSB-Birds	35835	skin; partial skeleton; muscle (RNAlater)	9.58	Semihumid montane scrub
-13.17758	-72.59600	unknown	3361	Specimen	MSB-Birds	35834	skin; partial skeleton; muscle (RNAlater)	9.66	Semihumid montane scrub
-13.17758	-72.59600	unknown	3361	Specimen	MSB-Birds	35834			
-13.17758	-72.59600	unknown	3361	Specimen	MSB-Birds	35835			
-13.17000	-72.55000	unknown		Observation	CLO-ML	24095			
-13.15000	-72.30000	unknown		Observation	CLO-Birds	35460			
-12.78000	-73.82000	unknown	3520	Specimen	USNM-Birds	512016			
-12.78000	-73.82000	unknown	3520	Specimen	USNM-Birds	512016.42 64	Alcoholic: Whole		
-12.13000	-77.03000	unknown		Observation	CLO-Birds	13336			
-12.02950	-76.65078	unknown	935	Specimen	MSB-Birds	32935			
-12.02950	-76.65078	unknown	935	Specimen	MSB-Birds	32927			
-12.02950	-76.65078	unknown	935	Specimen	MSB-Birds	32905			

Appendix E (cont'd)

Latitude	Longitude	Subspp.	Elev.	<b>Record Type</b>	Institution	Catalog. #	<pre># Preparation</pre>	Mass	Habitat
-12.02950	-76.65078	unknown	935	Specimen	MSB-Birds	32904			
-12.02950	-76.65078	unknown	935	Specimen	MSB-Birds	32905	blood (slide smear); heart, liver, muscle (frozen); tissue (RNAlater); blood (frozen)	7.7	capturado en net, chacra con Membrillo y Manzana
-12.02950	-76.65078	unknown	935	Specimen	MSB-Birds	32904	skin; heart, liver, muscle (frozen); tissue (RNAlater); blood (slide smear); blood (frozen)	8	capturado en net, chacra con Membrillo y Manzana
-12.02950	-76.65078	unknown	935	Specimen	MSB-Birds	32927	skin; heart, liver, muscle (frozen); tissue (RNAlater); blood (slide smear); blood (frozen)	7.1	capturado en red en chacra con Membrillo y Manzana
-12.02950	-76.65078	unknown	935	Specimen	MSB-Birds	32935	blood (slide smear); heart, liver, muscle (frozen); tissue (RNAlater); blood (frozen)	8.1	capturado en net, chacra con Manzana y Membrillo, Sisicaya
-12.00890	-76.92323	unknown	352	Specimen	MSB-Birds	32942			
-12.00890	-76.92323	unknown	352	Specimen	MSB-Birds	32976			
-12.00890	-76.92323	unknown	352	Specimen	MSB-Birds	32993			
-12.00890	-76.92323	unknown	352	Specimen	MSB-Birds	32978			
-12.00890	-76.92323	unknown	352	Specimen	MSB-Birds	32983			
-12.00890	-76.92323	unknown	352	Specimen	MSB-Birds	32984			
-12.00890	-76.92323	unknown	352	Specimen	MSB-Birds	32951			
-12.00890	-76.92323	unknown	352	Specimen	MSB-Birds	32957			
-12.00890	-76.92323	unknown	352	Specimen	MSB-Birds	32960			
-12.00890	-76.92323	unknown	352	Specimen	MSB-Birds	32965			

Appendix E (cont'd)

Latitude	Longitude	Subspp.	Elev.	<b>Record Type</b>	Institution	Catalog. #	# Preparation	Mass	Habitat
-12.00890	-76.92323	unknown	352	Specimen	MSB-Birds	32972			
-12.00890	-76.92323	unknown	352	Specimen	MSB-Birds	32959			
-12.00890	-76.92323	unknown	352	Specimen	MSB-Birds	32989			
-12.00890	-76.92323	unknown	352	Specimen	MSB-Birds	31460			
-12.00890	-76.92323	unknown	352	Specimen	MSB-Birds	32942	skin; blood (flash-frozen); muscle (RNAlater); blood (slide smear); heart, liver, muscle (frozen)	8.9	riparian scrub in urban industrial zone
-12.00890	-76.92323	unknown	352	Specimen	MSB-Birds	32957	skin; blood (flash-frozen); muscle (RNAlater); blood (slide smear); heart, liver, muscle (frozen)	9.5	riparian scrub in urban industrial zone
-12.00890	-76.92323	unknown	352	Specimen	MSB-Birds	32978	skin; blood (flash-frozen); muscle (RNAlater); blood (slide smear); heart, liver, muscle (frozen)	7.7	riparian scrub in urban industrial zone
-12.00890	-76.92323	unknown	352	Specimen	MSB-Birds	32983	blood (slide smear); blood (flash-frozen); muscle (RNAlater); heart, liver, muscle (frozen)	8.4	riparian scrub in urban industrial zone
-12.00890	-76.92323	unknown	352	Specimen	MSB-Birds	32993	skin; blood (flash-frozen); muscle (RNAlater); blood (slide smear); heart, liver, muscle (frozen)	7.7	riparian scrub in urban industrial zone
-12.00890	-76.92323	unknown	352	Specimen	MSB-Birds	31460	skin; blood (flash-frozen); muscle (RNAlater); blood (slide smear); heart, liver, muscle (frozen)	8.28	riparian scrub in urban industrial zone
-12.00890	-76.92323	unknown	352	Specimen	MSB-Birds	32951	blood (slide smear); blood (flash-frozen); muscle (RNAlater); heart, liver, muscle (frozen)	7.7	riparian scrub in urban industrial zone

Appendix E (cont'd)

Latitude	Longitude	Subspp.	Elev.	<b>Record Type</b>	Institution	Catalog. #	# Preparation	Mass	Habitat
-12.00890	-76.92323	unknown	352	Specimen	MSB-Birds	32959	blood (slide smear); blood (flash-frozen); muscle (RNAlater); heart, liver, muscle (frozen)	9	riparian scrub in urban industrial zone
-12.00890	-76.92323	unknown	352	Specimen	MSB-Birds	32960	skin; blood (flash-frozen); muscle (RNAlater); blood (slide smear); heart, liver, muscle (frozen)	8.6	riparian scrub in urban industrial zone
-12.00890	-76.92323	unknown	352	Specimen	MSB-Birds	32965	blood (slide smear); blood (flash-frozen); muscle (RNAlater); heart, liver, muscle (frozen)	8.6	riparian scrub in urban industrial zone
-12.00890	-76.92323	unknown	352	Specimen	MSB-Birds	32972	skin; blood (flash-frozen); muscle (RNAlater); blood (slide smear); heart, liver, muscle (frozen)	7.7	riparian scrub in urban industrial zone
-12.00890	-76.92323	unknown	352	Specimen	MSB-Birds	32976	blood (slide smear); blood (flash-frozen); muscle (RNAlater); heart, liver, muscle (frozen)	8	riparian scrub in urban industrial zone
-12.00890	-76.92323	unknown	352	Specimen	MSB-Birds	32984	skin; blood (flash-frozen); muscle (RNAlater); blood (slide smear); heart, liver, muscle (frozen)	8.4	riparian scrub in urban industrial zone
-12.00890	-76.92323	unknown	352	Specimen	MSB-Birds	32989	blood (slide smear); blood (flash-frozen); muscle (RNAlater); heart, liver, muscle (frozen)	8.3	riparian scrub in urban industrial zone
-12.00148	-76.92082	unknown	372	Specimen	MSB-Birds	31420	skin; blood (flash-frozen); muscle (RNAlater); blood (slide smear); heart, liver, muscle (frozen)	8.18	corn field in urban industrial zone

Appendix E (cont'd)

Latitude	Longitude	Subspp.	Elev.	<b>Record Type</b>	Institution	Catalog. #	Preparation	Mass	Habitat
-12.00148	-76.92082	unknown	372	Specimen	MSB-Birds	31424	blood (slide smear); blood (flash-frozen); muscle (RNAlater); heart, liver, muscle (frozen)	7.96	corn field in urban industrial zone
-12.00148	-76.92082	unknown	372	Specimen	MSB-Birds	31424			
-12.00148	-76.92082	unknown	372	Specimen	MSB-Birds	31420			
-11.76970	-76.53102	unknown	4178	Specimen	MSB-Birds	33466			
-11.76970	-76.53102	unknown	4178	Specimen	MSB-Birds	33466	skin; heart, liver, muscle (frozen); blood (flash- frozen); muscle (RNAlater); lung (frozen)	8.6	bosque pequeno
-11.76958	-76.53193	unknown	4140	Specimen	MSB-Birds	33478	skin; heart, liver, muscle (frozen); blood (flash- frozen); muscle (RNAlater); lung (frozen)	8.4	bosque pequeno
-11.76958	-76.53193	unknown	4140	Specimen	MSB-Birds	33478			
-11.76942	-76.53278	unknown	4131	Specimen	MSB-Birds	33445			
-11.76942	-76.53278	unknown	4131	Specimen	MSB-Birds	33445	skin; heart, liver, muscle (frozen); blood (flash- frozen); muscle (RNAlater); lung (frozen)	7.3	bosque pequeno
-11.76857	-76.53327	unknown	4088	Specimen	MSB-Birds	33487	,		
-11.76857	-76.53327	unknown	4093	Specimen	MSB-Birds	33308			
-11.76857	-76.53327	unknown	4093	Specimen	MSB-Birds	33308	muscle (RNAlater); heart, liver, muscle (frozen); blood (flash-frozen); lung (frozen)	7.5	bosque pequeno
-11.76857	-76.53327	unknown	4088	Specimen	MSB-Birds	33487	muscle (RNAlater); heart, liver, muscle (frozen); blood (flash-frozen); lung (frozen)	8.1	bosque pequeno
-11.76847	-76.53325	unknown	4082	Specimen	MSB-Birds	33447			

Appendix E (cont'd)

Latitude	Longitude	Subspp.	Elev.	Record Type	Institution	Catalog. #	Preparation	Mass	Habitat
-11.76847	-76.53325	unknown	4082	Specimen	MSB-Birds	33447	skin; heart, liver, muscle (frozen); blood (flash- frozen); muscle (RNAlater); lung (frozen)	8.1	bosque pequeno
-11.76770	-76.53462	unknown	4056	Specimen	MSB-Birds	33417			
-11.76770	-76.53462	unknown	3907	Specimen	MSB-Birds	33344			
-11.76770	-76.53462	unknown	4056	Specimen	MSB-Birds	33401			
-11.76770	-76.53462	unknown	3970	Specimen	MSB-Birds	33319			
-11.76770	-76.53462	unknown	4062	Specimen	MSB-Birds	33296			
-11.76770	-76.53462	unknown	3905	Specimen	MSB-Birds	33350			
-11.76770	-76.53462	unknown	3970	Specimen	MSB-Birds	33319	muscle (RNAlater); heart, liver, muscle (frozen); blood (flash-frozen); lung (frozen)	10.2	bosque pequeno
-11.76770	-76.53462	unknown	3907	Specimen	MSB-Birds	33344	muscle (RNAlater); heart, liver, muscle (frozen); blood (flash-frozen); lung (frozen)	8.6	bosque pequeno
-11.76770	-76.53462	unknown	4056	Specimen	MSB-Birds	33401	heart, liver, muscle (frozen); blood (flash-frozen); lung (frozen); muscle (RNAlater)	9.4	bosque pequeno
-11.76770	-76.53462	unknown	4056	Specimen	MSB-Birds	33417	heart, liver, muscle (frozen); blood (flash-frozen); lung (frozen); muscle (RNAlater)	9	bosque pequeno
-11.76770	-76.53462	unknown	4062	Specimen	MSB-Birds	33296	muscle (RNAlater); heart, liver, muscle (frozen); blood (flash-frozen); lung (frozen)	9.02	bosque pequeno
-11.76770	-76.53462	unknown	3905	Specimen	MSB-Birds	33350	skin; blood (flash-frozen); lung (frozen); heart, liver, muscle (frozen); muscle (RNAlater)	8.1	bosque pequeno
-11.76730	-76.52477	unknown	4142	Specimen	MSB-Birds	36581			

Appendix E (cont'd)

Latitude	Longitude	Subspp.	Elev.	<b>Record Type</b>	Institution	Catalog. #	<pre># Preparation</pre>	Mass	Habitat
-11.76730	-76.52477	unknown	4142	Specimen	MSB-Birds	36581	muscle (RNAlater); heart, liver, muscle (frozen); blood (slide smear); blood (flash- frozen)	8	
-11.76360	-76.54250	unknown	3910	Specimen	MSB-Birds	33325			
-11.76360	-76.54250	unknown	3910	Specimen	MSB-Birds	33325	muscle (RNAlater); heart, liver, muscle (frozen); blood (flash-frozen); lung (frozen)	9.7	bosque pequeno
-11.76188	-76.54887	unknown	3750	Specimen	MSB-Birds	28336	muscle (RNAlater); blood (slide smear); blood (flash- frozen)	7.2	montane scrub
-11.76188	-76.54887	unknown	3750	Specimen	MSB-Birds	28291	muscle (RNAlater); blood (slide smear); blood (flash- frozen)	8.1	montane scrub
-11.76188	-76.54887	unknown	3750	Specimen	MSB-Birds	28300	muscle (RNAlater)		montane scrub
-11.76188	-76.54887	unknown	3750	Specimen	MSB-Birds	28350	blood (flash-frozen); muscle (RNAlater)	7.8	montane scrub
-11.76188	-76.54887	unknown	3750	Specimen	MSB-Birds	28336			
-11.76188	-76.54887	unknown	3750	Specimen	MSB-Birds	28291			
-11.76188	-76.54887	unknown	3750	Specimen	MSB-Birds	28350			
-11.76188	-76.54887	unknown	3750	Specimen	MSB-Birds	28300			
-11.76188	-76.54887	unknown	3750	Specimen	MSB-Birds	28258	skin; blood (flash-frozen); blood (slide smear); muscle (RNAlater)	21.5	montane scrub
-11.76077	-76.56767	unknown	3700	Specimen	MSB-Birds	31661	( )		
-11.76077	-76.56767	unknown	3620	Specimen	MSB-Birds	28442			
-11.76077	-76.56767	unknown	3620	Specimen	MSB-Birds	28442	tissue (95% ethanol)	8.2	montane scrub
-11.76077	-76.56767	unknown	3700	Specimen	MSB-Birds	31661	tissue (95% ethanol)		montane scrub
-11.76030	-76.59757	unknown	3177	Specimen	MSB-Birds	35952			
-11.76030	-76.59757	unknown	3177	Specimen	MSB-Birds	35941			

Appendix E (cont'd)

Latitude	Longitude	Subspp.	Elev.	Record Type	Institution	Catalog. #	<sup>‡</sup> Preparation	Mass	Habitat
-11.76030	-76.59757	unknown	3177	Specimen	MSB-Birds	35941	skin; heart, liver, muscle (frozen); muscle (RNAlater)	8	
-11.76030	-76.59757	unknown	3177	Specimen	MSB-Birds	35952	heart, liver, muscle (frozen); muscle (RNAlater)	8	bosque de Eucalipto
-11.75125	-76.52703	unknown	4166	Specimen	MSB-Birds	36560			-
-11.75125	-76.52703	unknown	4166	Specimen	MSB-Birds	36560	skin; heart, liver, muscle (frozen); blood (slide smear); muscle (RNAlater); blood (flash-frozen)	8.04	bosque peque�o de arbusto
-11.75000	-76.59167	unknown	3000	Specimen	MSB-Birds	27005			
-11.75000	-76.59167	unknown	3000	Specimen	MSB-Birds	27006			
-11.75000	-76.59167	unknown	3000	Specimen	MSB-Birds	27006	heart, liver, muscle (frozen); muscle (RNAlater)		hillside scrub
-11.75000	-76.59167	unknown	3000	Specimen	MSB-Birds	27005	skin; blood (flash-frozen); blood (slide smear); muscle (RNAlater)	7.4	hillside scrub
-11.74417	-76.60883	unknown	2390	Specimen	MSB-Birds	31783			
-11.74417	-76.60883	unknown	2390	Specimen	MSB-Birds	31779			
-11.74417	-76.60883	unknown	2390	Specimen	MSB-Birds	31779	skin; muscle (RNAlater); blood (flash-frozen); heart, liver, muscle (frozen)	8.9	montane scrub and agricultural matrix
-11.74417	-76.60883	unknown	2390	Specimen	MSB-Birds	31783	heart, liver, muscle (frozen); blood (flash-frozen); muscle (RNAlater)	9.2	montane scrub and agricultural matrix
-11.53100	-74.94300	unknown		Specimen	KU-Birds	114620			
-11.53100	-74.94300	unknown		Specimen	KU-Birds	114620	Skeleton - 1; Tissue - 100	8.8	
-11.48875	-74.89608	unknown	3520	Specimen	MSB-Birds	27041			

Appendix E (cont'd)

Latitude	Longitude	Subspp.	Elev.	<b>Record</b> Type	Institution	Catalog. #	# Preparation	Mass	Habitat
-11.48875	-74.89608	unknown	3520	Specimen	MSB-Birds	27041	blood (slide smear); blood (flash-frozen); muscle (RNAlater); heart, liver, muscle (frozen)	8.8	humid elfin forest edge
-11.48800	-74.89200	unknown		Specimen	KU-Birds	113946			
-11.48800	-74.89200	unknown		Specimen	KU-Birds	113946	Skin - 1; Tissue - 100	9	
-11.40000	-77.30000	littorale	396.24	Specimen	MVZ-Birds	157914			
-11.40000	-77.30000	unknown	396.24	Specimen	MVZ-Birds	174867			
-11.37323	-77.36222	littorale		Specimen	UMMZ-Birds	156852	Skel, Y	8.4	
-11.37322	-77.36222	littorale		Specimen	UMMZ-Birds	156852			
-10.06812	-78.13603	unknown	39	Specimen	MSB-Birds	34257			
-10.06812	-78.13603	unknown	39	Specimen	MSB-Birds	34252			
-10.06812	-78.13603	unknown	39	Specimen	MSB-Birds	34253			
-10.06812	-78.13603	unknown	39	Specimen	MSB-Birds	34239			
-10.06812	-78.13603	unknown	39	Specimen	MSB-Birds	34266			
-10.06812	-78.13603	unknown	39	Specimen	MSB-Birds	34233			
-10.06812	-78.13603	unknown	39	Specimen	MSB-Birds	34265			
-10.06812	-78.13603	unknown	39	Specimen	MSB-Birds	34234			
-10.06812	-78.13603	unknown	39	Specimen	MSB-Birds	34237			
-10.06812	-78.13603	unknown	39	Specimen	MSB-Birds	34256			
-10.06812	-78.13603	unknown	39	Specimen	MSB-Birds	34238			
-10.06812	-78.13603	unknown	39	Specimen	MSB-Birds	34233	muscle (RNAlater); heart, liver, muscle (frozen); blood (slide smear)	8.74	chacra de esparrago
-10.06812	-78.13603	unknown	39	Specimen	MSB-Birds	34238	skin; muscle (RNAlater); heart; liver, muscle (frozen); blood (slide smear); blood (flash-frozen); lung (frozen)	8.29	chacra de esparrago

Appendix	E (cont'd)								
Latitude	Longitude	Subspp.	Elev.	<b>Record Type</b>	Institution	Catalog. #	Preparation	Mass	Habitat
-10.06812	-78.13603	unknown	39	Specimen	MSB-Birds	34239	muscle (RNAlater); blood (flash-frozen); heart, liver, muscle (frozen); partial skeleton; blood (slide smear)	7.93	along edge of corn and Asparagus field
-10.06812	-78.13603	unknown	39	Specimen	MSB-Birds	34256	skin; heart, liver, muscle (frozen); blood (flash- frozen); blood (slide smear); muscle (RNAlater); lung (frozen)	8.24	chacra de esparrago
-10.06812	-78.13603	unknown	39	Specimen	MSB-Birds	34234	skin; muscle (RNAlater); heart; liver, muscle (frozen); blood (slide smear); partial skeleton; blood (flash- frozen); lung (frozen)	8.54	edge of asparagus & corn field
-10.06812	-78.13603	unknown	39	Specimen	MSB-Birds	34237	blood (flash-frozen); muscle (RNAlater); lung (frozen); heart; blood (slide smear); partial skeleton; liver, muscle (frozen)	8.66	along edge of corn and Asparagus field
-10.06812	-78.13603	unknown	39	Specimen	MSB-Birds	34252	skin; heart, liver, muscle (frozen); blood (flash- frozen); blood (slide smear); muscle (RNAlater); lung (frozen)	8.15	chacra de esparrago
-10.06812	-78.13603	unknown	39	Specimen	MSB-Birds	34253	muscle (RNAlater); heart, liver, muscle (frozen); blood (slide smear); blood (flash- frozen)	8.32	chacra de esparrago
-10.06812	-78.13603	unknown	39	Specimen	MSB-Birds	34257	muscle (RNAlater); lung (frozen); heart, liver, muscle (frozen); blood (slide smear); blood (flash-frozen)	7.85	chacra de esparrago

Appendix E (cont'd)

Latitude	Longitude	Subspp.	Elev.	Record Type	Institution	Catalog. #	<pre># Preparation</pre>	Mass	Habitat
-10.06812	-78.13603	unknown	39	Specimen	MSB-Birds	34265	skin; heart, liver, muscle (frozen); blood (flash- frozen); blood (slide smear); muscle (RNAlater); lung (frozen)	7.5	chacra de esparrago
-10.06812	-78.13603	unknown	39	Specimen	MSB-Birds	34266	muscle (RNAlater); heart, liver, muscle (frozen); blood (slide smear); blood (flash- frozen)	7.36	chacra de esparrago
-10.02162	-78.04977	unknown	115	Specimen	MSB-Birds	34227			
-10.02162	-78.04977	unknown	115	Specimen	MSB-Birds	34227	skin; blood (slide smear); lung (frozen); muscle (RNAlater); heart; partial skeleton; liver, muscle (frozen)	6.14	Acacia scrub along river
-9.57212	-77.84745	unknown	3439	Specimen	MSB-Birds	34978			
-9.57212	-77.84745	unknown	3439	Specimen	MSB-Birds	34986			
-9.57212	-77.84745	unknown	3439	Specimen	MSB-Birds	34978	muscle (RNAlater); heart, liver, muscle (frozen); blood (slide smear); blood (flash- frozen)	7.77	semi-humid elfin forest
-9.57212	-77.84745	unknown	3439	Specimen	MSB-Birds	34986	muscle (RNAlater); heart, liver, muscle (frozen); blood (slide smear); blood (flash- frozen)	9.75	semi-humid elfin forest
-8.75478	-78.04845	unknown	2972	Specimen	MSB-Birds	34853			
-8.75478	-78.04845	unknown	2972	Specimen	MSB-Birds	34874			
-8.75478	-78.04845	unknown	2972	Specimen	MSB-Birds	34853	skin; heart, liver, muscle (frozen); blood (slide smear); muscle (RNAlater); blood (flash-frozen)	8.01	semi-arid montane scrub

Appendix E (cont'd)

Latitude	Longitude	Subspp.	Elev.	<b>Record Type</b>	Institution	Catalog. #	Preparation	Mass	Habitat
-8.75478	-78.04845	unknown	2972	Specimen	MSB-Birds	34874	muscle (RNAlater); heart, liver, muscle (frozen); blood (slide smear); blood (flash- frozen)	7.54	semi-arid montane scrub
-8.75236	-78.03402	unknown	3931	Specimen	MSB-Birds	35996	,		
-8.75236	-78.03402	unknown	3931	Specimen	MSB-Birds	35984			
-8.75236	-78.03402	unknown	3931	Specimen	MSB-Birds	35984	muscle (RNAlater); heart, liver, muscle (frozen); partial skeleton	8.54	semi-arid montane scrub
-8.75236	-78.03402	unknown	3931	Specimen	MSB-Birds	35996	blood (flash-frozen); muscle (RNAlater); lung (frozen); heart; blood (slide smear); partial skeleton; liver, muscle (frozen)	8.45	semi-arid montane scrub
-8.74247	-78.04070	unknown	3740	Specimen	MSB-Birds	36010			
-8.74247	-78.04070	unknown	3740	Specimen	MSB-Birds	36029			
-8.74247	-78.04070	unknown	3740	Specimen	MSB-Birds	36006			
-8.74247	-78.04070	unknown	3740	Specimen	MSB-Birds	36010	blood (flash-frozen); muscle (RNAlater); lung (frozen); heart; blood (slide smear); partial skeleton; liver, muscle (frozen)	9.63	semi-arid montane scrub
-8.74247	-78.04070	unknown	3740	Specimen	MSB-Birds	36029	skin; muscle (RNAlater); heart; liver, muscle (frozen); blood (slide smear); partial skeleton; blood (flash- frozen); lung (frozen)	8.88	semi-arid montane scrub
-8.74247	-78.04070	unknown	3740	Specimen	MSB-Birds	36006	skin; heart, liver, muscle (frozen); blood (slide smear); partial skeleton; muscle (RNAlater); blood (flash-frozen)	7.68	semi-arid montane scrub
Appendix E (cont'd)

Latitude	Longitude	Subspp.	Elev.	Record Type	Institution	Catalog. #	<sup>‡</sup> Preparation	Mass	Habitat
-8.50000	-78.00000	unknown		Observation	CLO-Birds	10474			
-8.38685	-78.64518	unknown	309	Specimen	MSB-Birds	34792			
-8.38685	-78.64518	unknown	309	Specimen	MSB-Birds	34729			
-8.38685	-78.64518	unknown	309	Specimen	MSB-Birds	34712			
-8.38685	-78.64518	unknown	309	Specimen	MSB-Birds	34731			
-8.38685	-78.64518	unknown	309	Specimen	MSB-Birds	34768			
-8.38685	-78.64518	unknown	309	Specimen	MSB-Birds	34726			
-8.38685	-78.64518	unknown	309	Specimen	MSB-Birds	34769			
-8.38685	-78.64518	unknown	309	Specimen	MSB-Birds	34688			
-8.38685	-78.64518	unknown	309	Specimen	MSB-Birds	34731	skin; muscle (RNAlater); heart; liver, muscle (frozen); partial skeleton; blood (flash-frozen); lung (frozen)	7.8	mesquite scrub
-8.38685	-78.64518	unknown	309	Specimen	MSB-Birds	34768	blood (flash-frozen); muscle (RNAlater); lung (frozen); heart; blood (slide smear); partial skeleton; liver, muscle (frozen)	8.4	mesquite scrub
-8.38685	-78.64518	unknown	309	Specimen	MSB-Birds	34769	skin; blood (slide smear); heart, liver, muscle (frozen); muscle (RNAlater)	7.74	acacia scrub near agricultural fields or beans and corn
-8.38685	-78.64518	unknown	309	Specimen	MSB-Birds	34688	skin; muscle (RNAlater); heart; liver, muscle (frozen); blood (slide smear); partial skeleton; blood (flash- frozen); lung (frozen)	9	mesquite scrub

Appendix E (cont'd)

Latitude	Longitude	Subspp.	Elev.	<b>Record Type</b>	Institution	Catalog. #	Preparation	Mass	Habitat
-8.38685	-78.64518	unknown	309	Specimen	MSB-Birds	34712	muscle (RNAlater); blood (flash-frozen); heart, liver, muscle (frozen); partial skeleton; blood (slide smear)	7.57	dry acacia scrub near agricultural fields
-8.38685	-78.64518	unknown	309	Specimen	MSB-Birds	34726	skin; heart, liver, muscle (frozen); blood (slide smear); partial skeleton; muscle (RNAlater); blood (flash-frozen)	7.45	acacia scrub near agricultural fields
8.38685	-78.64518	unknown	309	Specimen	MSB-Birds	34729	blood (flash-frozen); muscle (RNAlater); lung (frozen); heart; blood (slide smear); partial skeleton; liver, muscle (frozen)	7.55	Acacia scrub near agricultural field
8.38685	-78.64518	unknown	309	Specimen	MSB-Birds	34792	muscle (RNAlater); heart, liver, muscle (frozen); partial skeleton; blood (flash-frozen)	8.63	dry acacia scrub near agricultural fields
-7.94858	-77.48775	littorale		Specimen	MVZ-Birds	163962			
7.94858	-77.48775	littorale		Specimen	MVZ-Birds	163962	study skin	9.9	
-7.75893	-77.76685	unknown	3549	Specimen	MSB-Birds	43052	muscle (RNAlater); heart, liver, muscle (frozen); partial skeleton	9.02	bosque alizo
-7.75893	-77.76685	unknown	3549	Specimen	MSB-Birds	43052	1		
-7.75882	-77.76467	unknown	3543	Specimen	MSB-Birds	43042			
-7.75882	-77.76467	unknown	3543	Specimen	MSB-Birds	43042	skin; partial skeleton; heart, liver, muscle (frozen); muscle (RNAlater)	8.76	bosque alizo
-7.75805	-77.76440	unknown	3551	Specimen	MSB-Birds	43046	× /		
.7.75805	-77.76440	unknown	3551	Specimen	MSB-Birds	43046	muscle (RNAlater); heart, liver, muscle (frozen); partial skeleton	7.83	bosque alizo

Appendix E (cont'd)

Latitude	Longitude	Subspp.	Elev.	<b>Record Type</b>	Institution	Catalog. #	# Preparation	Mass	Habitat
-7.40383	-78.77603	unknown	2550	Specimen	MSB-Birds	35378	skin; heart, liver, muscle (frozen); blood (slide smear); muscle (RNAlater); blood (flash-frozen)	8.01	semi-humid scrub
-7.40383	-78.77978	unknown	2550	Specimen	MSB-Birds	35416	skin; muscle (RNAlater); heart, liver, muscle (frozen); blood (slide smear); partial skeleton; blood (flash- frozen); lung (frozen)	7.89	Semi-humid montane scrub
-7.40383	-78.77978	unknown	2550	Specimen	MSB-Birds	35405	skin; heart, liver, muscle (frozen); blood (flash- frozen); blood (slide smear); muscle (RNAlater); lung (frozen)	9.14	Semi-humid montane scrub
-7.40383	-78.77978	unknown	2550	Specimen	MSB-Birds	35416			
-7.40383	-78.77603	unknown	2550	Specimen	MSB-Birds	35378			
-7.40383	-78.77978	unknown	2550	Specimen	MSB-Birds	35405			
-7.40315	-79.56333	littorale		Specimen	UMMZ-Birds	22844	Skin, Y		
-7.40315	-79.56333	unknown		Specimen	UMMZ-Birds	25856	Skin, Y		
-7.40315	-79.56333	littorale		Specimen	UMMZ-Birds	22844			
-7.40315	-79.56333	unknown		Specimen	UMMZ-Birds	25856			
-7.39817	-78.77603	unknown	2625	Specimen	MSB-Birds	35270			
-7.39817	-78.77603	unknown	2622	Specimen	MSB-Birds	35271			
-7.39817	-78.77603	unknown	2622	Specimen	MSB-Birds	35241			
-7.39817	-78.77603	unknown	2625	Specimen	MSB-Birds	35270	skin; heart, liver, muscle (frozen); blood (flash- frozen); blood (slide smear); muscle (RNAlater); lung (frozen)	8.92	Semi-humid montane scrub egde of forest

Appendix E (cont'd)

Latitude	Longitude	Subspp.	Elev.	Record Type	Institution	Catalog. #	Preparation	Mass	Habitat
-7.39817	-78.77603	unknown	2622	Specimen	MSB-Birds	35241	skin; heart, liver, muscle (frozen); blood (slide smear); muscle (RNAlater); blood (flash-frozen)	8.93	Semi-humid montane forest
-7.39817	-78.77603	unknown	2622	Specimen	MSB-Birds	35271	muscle (RNAlater); heart, liver, muscle (frozen); blood (slide smear); blood (flash- frozen)		Semi-humid montane forest
-7.39803	-78.77827	unknown	2500	Specimen	MSB-Birds	35239	blood (flash-frozen); muscle (RNAlater); lung (frozen); heart; blood (slide smear); partial skeleton; liver, muscle (frozen)	7.48	Semi-humid montane forest
-7.39803	-78.77827	unknown	2500	Specimen	MSB-Birds	35268	skin; heart, liver, muscle (frozen); partial skeleton; muscle (RNAlater); lung (frozen)	8.09	Semi-humid montane scrub egde of forest
-7.39803	-78.77827	unknown	2500	Specimen	MSB-Birds	35202	skin; muscle (RNAlater); heart; liver, muscle (frozen); blood (slide smear); partial skeleton; blood (flash- frozen): lung (frozen)	8.02	Semi-humid montane forest
-7.39803	-78.77827	unknown	2500	Specimen	MSB-Birds	35268			
-7.39803	-78.77827	unknown	2500	Specimen	MSB-Birds	35239			
-7.39803	-78.77827	unknown	2500	Specimen	MSB-Birds	35202			
-6.55252	-79.99749	littorale	30.48	Specimen	MVZ-Birds	157915			
-6.55252	-79.99749	littorale	30.48	Specimen	MVZ-Birds	157915	study skin	8	
-5.87558	-81.04113	littorale	30.48	Specimen	MVZ-Birds	157917			
-5.87558	-81.04113	littorale	30.48	Specimen	MVZ-Birds	157916			
-5.87558	-81.04113	littorale	30.48	Specimen	MVZ-Birds	157916	study skin	8	
-5.87558	-81.04113	littorale	30.48	Specimen	MVZ-Birds	157917	study skin	7	

Appendix E (cont'd)

Latitude	Longitude	Subspp.	Elev.	<b>Record Type</b>	Institution	Catalog. #	<b>Preparation</b>	Mass Habitat
-5.60000	-79.90000	unknown		Observation	CLO-Birds	13350		
-5.23000	-79.40000	unknown		Observation	CLO-Birds	21835		
-4.58395	-81.27068	cinereum		Specimen	<b>ROM-Birds</b>	511397	eggs	
-4.58395	-81.27068	cinereum		Specimen	<b>ROM-Birds</b>	511397		
-4.58361	-81.08528	cinereum		Specimen	<b>ROM-Birds</b>	28652	skin	
-4.58361	-81.08528	cinereum		Specimen	<b>ROM-Birds</b>	28652		
-4.53333	-81.15000	littorale	30.48	Specimen	MVZ-Birds	163963	study skin	6.5
-4.53333	-81.15000	littorale	30.48	Specimen	MVZ-Birds	163963		
-4.51012	-81.28542	cinereum		Specimen	<b>ROM-Birds</b>	28653		
-4.51012	-81.28542	cinereum		Specimen	ROM-Birds	28653	skin	
-4.48030	-80.10136	cinereum		Specimen	ROM-Birds	511398		
-4.48030	-80.10136	cinereum		Specimen	ROM-Birds	511398	eggs; nest	
-4.44006	-81.16618	littorale	30.48	Specimen	MVZ-Birds	161159		
-4.44006	-81.16618	littorale	30.48	Specimen	MVZ-Birds	161159	study skin	6.9
-3.10502	-79.10298	unknown		Specimen	MLZ-Birds	9850	skin	
-3.10502	-79.10298	unknown		Specimen	MLZ-Birds	9852	skin	
-3.10501	-79.10298	unknown		Specimen	MLZ-Birds	9852		
-3.10501	-79.10298	unknown		Specimen	MLZ-Birds	9850		
-1.96810	-78.39856	unknown		Specimen	MLZ-Birds	9851		
-1.96810	-78.39856	unknown		Specimen	MLZ-Birds	7517		
-1.96810	-78.39856	unknown		Specimen	MLZ-Birds	3353		
-1.96810	-78.39856	unknown		Specimen	MLZ-Birds	9851	skin	
-1.96810	-78.39856	unknown		Specimen	MLZ-Birds	3353	skin	
-1.96810	-78.39856	unknown		Specimen	MLZ-Birds	7517	skin	
-1.67098	-78.64712	unknown		Specimen	MLZ-Birds	66084	skin	
-1.67098	-78.64712	unknown		Specimen	MLZ-Birds	66085	skin	

Appendix E (cont'd)

Latitude	Longitude	Subspp.	Elev. Record Type	Institution	Catalog. #	# Preparation	Mass Habitat
-1.67098	-78.64712	unknown	Specimen	MLZ-Birds	66131	skin	
-1.67098	-78.64712	unknown	Specimen	MLZ-Birds	7518	skin	
-1.67098	-78.64712	unknown	Specimen	MLZ-Birds	7520	skin	
-1.67098	-78.64712	unknown	Specimen	MLZ-Birds	66132	skin	
-1.67098	-78.64712	unknown	Specimen	MLZ-Birds	66133	skin	
-1.67098	-78.64712	unknown	Specimen	MLZ-Birds	7515	skin	
-1.67098	-78.64712	unknown	Specimen	MLZ-Birds	7520		
-1.67098	-78.64712	unknown	Specimen	MLZ-Birds	7518		
-1.67098	-78.64712	unknown	Specimen	MLZ-Birds	7515		
-1.67098	-78.64712	unknown	Specimen	MLZ-Birds	66133		
-1.67098	-78.64712	unknown	Specimen	MLZ-Birds	66132		
-1.67098	-78.64712	unknown	Specimen	MLZ-Birds	66131		
-1.67098	-78.64712	unknown	Specimen	MLZ-Birds	66085		
-1.67098	-78.64712	unknown	Specimen	MLZ-Birds	66084		
-1.45275	-78.74119	unknown	Specimen	MLZ-Birds	66083		
-1.41939	-78.66207	unknown	Specimen	MLZ-Birds	66083	skin	
-1.40000	-78.42000	fraseri	Specimen	LACM-Birds	83544		
-1.40000	-78.42000	fraseri	Specimen	LACM-Birds	83544	skin   skull	
-0.41383	-78.36606	fraseri	Specimen	<b>ROM-Birds</b>	30497		
-0.41383	-78.36606	fraseri	Specimen	<b>ROM-Birds</b>	30497	skin	
-0.40941	-78.36591	fraseri	Specimen	<b>ROM-Birds</b>	30498		
-0.40941	-78.36591	fraseri	Specimen	<b>ROM-Birds</b>	30498	skin	
-0.36667	-78.13333	unknown	Specimen	MLZ-Birds	7514		
-0.36667	-78.13333	unknown	Specimen	MLZ-Birds	7514	skin	
-0.33985	-78.14717	unknown	Specimen	MLZ-Birds	7516		
-0.33985	-78.14717	unknown	Specimen	MLZ-Birds	7516	skin	

Appendix E (cont'd)

Latitude	Longitude	Subspp.	Elev.	<b>Record</b> Type	Institution	Catalog. #	Preparation	Mass Habitat
-0.23330	-78.25000	fraseri	3000	Specimen	YPM-VertZoo	YPM ORN		
0.00000	70.25000	<i>c</i> .	2000	G .		121582	100/ 6	
-0.23330	-78.25000	fraseri	3000	Specimen	YPM-Birds	121582	10% form>/0% alc.	
-0.22000	-78.40000	fraseri		Specimen	LACM-Birds	50748		
-0.22000	-78.40000	fraseri		Specimen	LACM-Birds	50748	skin   skull	
-0.21400	-78.40489	unknown		Specimen	MLZ-Birds	7513		
-0.21400	-78.40489	unknown		Specimen	MLZ-Birds	7513	skin	
-0.06670	-78.58330	fraseri		Specimen	YPM-VertZoo	YPM ORN		
						011592		
-0.06670	-78.58330	fraseri		Specimen	YPM-Birds	YPM ORN	skin, round	
-0 05600	-78 / 5869	frasari		Specimen	ROM-Birds	011592	skin	
-0.05077	-70.+3007	jruseri		Speemien	KOM-Dilds	4	5 Kill	
-0.05699	-78.45869	fraseri		Specimen	<b>ROM-Birds</b>	33.9.1.115		
				~ .		4		
0.36461	-78.11760	fraseri		Specimen	ROM-Birds	62626	skin	
0.36461	-78.11760	fraseri		Specimen	<b>ROM-Birds</b>	62626		
0.98189	-77.13919	unknown	2740	Specimen	IAvH	IAvH-A-		
1 011(7	77 27021	<i>c</i> ·		с ·	DOM D. 1	15420	-1-i	
1.2116/	-//.2/921	fraseri		Specimen	ROM-Birds	94806	SKIN	
1.21167	-77.27921	fraseri		Specimen	ROM-Birds	94806		
2.36639	-76.40667	fraseri	3380	Specimen	IAvH-A	IAvH-A-		
2 40000	-76 45000	fraseri		Specimen	VPM-VertZoo	2525 VPM ORN		
2.70000	10.12000	jruseri		speemen		027204		
2.40000	-76.45000	fraseri		Specimen	YPM-VertZoo	YPM ORN		
				-		027203		
2.40000	-76.45000	fraseri	2750	Specimen	LACM-Birds	36739		

Appendix E (cont'd)

Latitude	Longitude	Subspp.	Elev.	<b>Record</b> Type	Institution	Catalog. #	<sup>4</sup> Preparation	Mass Habitat
2.40000	-76.45000	fraseri		Specimen	YPM-Birds	YPM ORN	l skin, round	
						027203		
2.40000	-76.45000	fraseri	2750	Specimen	LACM-Birds	36739	skin   skull	
2.40000	-76.45000	fraseri		Specimen	YPM-VZ	YPM ORN	skin, round	
		cinereum		Specimen	AMNH-Birds	027204 Skin- 145691	skin	
		cinereum		Specimen	AMNH-Birds	Skin-	skin	
						408732		
		cinereum		Occurrence	ANSP-ORN	102423		
		cinereum	3352.8	Occurrence	ANSP-ORN	133803		
		cinereum	3352.8	Occurrence	ANSP-ORN	91585		
		cinereum	3352.8	Occurrence	ANSP-ORN	91586		
		cinereum	3352.8	Occurrence	ANSP-ORN	91587		
		cinereum	3000	Specimen	CM-Birds	P120072	study skin	
		cinereum	3500	Specimen	FMNH-Birds	217815	skin(r)	
		cinereum	3353	Specimen	FMNH-Birds	299793	skin(r)	
		cinereum	3353	Specimen	FMNH-Birds	299947	skin(r)	
		cinereum	3500	Specimen	FMNH-Birds	217816	skin(r)	
		cinereum	2647	Specimen	FMNH-Birds	183920	skin(r)	
		cinereum	3200	Specimen	FMNH-Birds	299406	skin(r)	
		cinereum	3500	Specimen	FMNH-Birds	217817	skin(r)	
		cinereum	2743	Specimen	FMNH-Birds	287842	skin(r)	
		cinereum		Specimen	MCZ-Orn	179359	skin (dry)	
		cinereum		Specimen	MCZ-Orn	179360	skin (dry)	
		cinereum		Specimen	MCZ-Orn	179361	skin (dry)	
		cinereum		Specimen	UMMZ-Birds	113292	Skin, Y	

Latitude	Longitude	Subspp.	Elev.	<b>Record Type</b>	Institution	Catalog. #	Preparation	Mass Habitat
		cinereum		Specimen	UMZC-Verts	27/Paru/5/c	Skin (un-mounted)	
			0774	a :		/1	<b>C1 W</b> 711.	
		cinereum	2774	Specimen	USNM-Birds	273396.43	Skin: whole	
		cinereum	2774	Specimen	USNM-Birds	273398.43	Skin: Whole	
		cinereum		Specimen	USNM-Birds	529286.43	Skin: Whole	
		cinereum	2743	Specimen	USNM-Birds	273397.43	Skin: Whole	
		cinereum		Specimen	AMNH-Birds	Skin- 145689	skin	
		cinereum		Specimen	AMNH-Birds	Skin- 145690	skin	
		cinereum		Specimen	AMNH-Birds	Skin- 408731	skin	
		cinereum		Occurrence	ANSP-ORN	102421		
		cinereum		Occurrence	ANSP-ORN	102422		
		cinereum		Occurrence	ANSP-ORN	102425		
		cinereum		Occurrence	ANSP-ORN	109323		
		cinereum	3261.3 6	Occurrence	ANSP-ORN	119555		
		cinereum	3352.8	Occurrence	ANSP-ORN	133804		
		cinereum	3230.8 8	Occurrence	ANSP-ORN	133805		
		cinereum	3230.8 8	Occurrence	ANSP-ORN	133806		
		cinereum	2970	Occurrence	ANSP-ORN	91589		
		cinereum	2970	Occurrence	ANSP-ORN	91590		
		cinereum		Specimen	CM-Birds	P117836	study skin	
		cinereum		Specimen	CM-Birds	P117837	study skin	
		cinereum	3000	Specimen	CM-Birds	P120071	study skin	

Latitude	Longitude	Subspp.	Elev.	<b>Record</b> Type	Institution	Catalog. # P	reparation	Mass Habitat
		cinereum	3000	Specimen	FMNH-Birds	181917 sk	kin(r)	
		cinereum		Specimen	FMNH-Birds	118753 sk	kin(r)	
		cinereum	2743	Specimen	FMNH-Birds	287841 sk	kin(r)	
		cinereum	2896	Specimen	FMNH-Birds	283730 sk	kin(r)	
		cinereum	2743	Specimen	FMNH-Birds	287840 sk	kin(r)	
		cinereum		Specimen	MCZ-Orn	123013 sk	kin (dry)	
		cinereum		Specimen	USNM-Birds	273662.43 S	kin: Whole	
		cinereum		Occurrence	ANSP-ORN	102418		
		cinereum		Occurrence	ANSP-ORN	102420		
		cinereum		Specimen	USNM-Birds	32671.429 SI 96	kin: Whole	
		fraseri		Specimen	AMNH-Birds	SKIN- sk 810789	kin	
		fraseri		Specimen	AMNH-Birds	SKIN- sk 810791	kin	
		fraseri	3474.7	Occurrence	ANSP-ORN	149814		
		fraseri	3200.4	Occurrence	ANSP-ORN	149816		
		fraseri	3474.7 2	Occurrence	ANSP-ORN	149818		
		fraseri	3383.2 8	Occurrence	ANSP-ORN	149823		
		fraseri	1188.7 2	Occurrence	ANSP-ORN	150523		
		fraseri	3300	Occurrence	ANSP-ORN	162159		
		fraseri		Occurrence	ANSP-ORN	164238		
		fraseri	2740	Occurrence	ANSP-ORN	59893		
		fraseri	2740	Occurrence	ANSP-ORN	59894		

Appendix E (cont'd)

Latitude	Longitude	Subspp.	Elev.	<b>Record</b> Type	Institution	Catalog. #	Preparation	Mass Habitat
		fraseri	3048	Occurrence	ANSP-ORN	59896		
		fraseri	3200.4	Occurrence	ANSP-ORN	59898		
		fraseri	3200.4	Occurrence	ANSP-ORN	59901		
		fraseri	3200.4	Occurrence	ANSP-ORN	59902		
		fraseri		Specimen	FMNH-Birds	374934	skin(r)	
		fraseri		Specimen	MCZ-Orn	139426	skin (dry)	
		fraseri		Specimen	MCZ-Orn	139427	skin (dry)	
		fraseri		Specimen	MCZ-Orn	199331	skin (dry)	
		fraseri	3353	Specimen	USNM-Birds	447397.43	Skin: Whole	
		fraseri	2591	Specimen	USNM-Birds	447396.43	Skin: Whole	
		fraseri	2200	Specimen	YPM-VZ	YPM ORN	skin, round	9.5
		fraseri		Specimen	AMNH-Birds	088969 SKIN- 810790	skin	
		fraseri	3657.6	Occurrence	ANSP-ORN	149815		
		fraseri	3474.7 2	Occurrence	ANSP-ORN	149819		
		fraseri	3200.4	Occurrence	ANSP-ORN	149820		
		fraseri	3657.6	Occurrence	ANSP-ORN	149821		
		fraseri	3474.7 2	Occurrence	ANSP-ORN	149822		
		fraseri	3383.2 8	Occurrence	ANSP-ORN	150524		
		fraseri	3383.2 8	Occurrence	ANSP-ORN	150525		
		fraseri		Occurrence	ANSP-ORN	164237		
		fraseri		Occurrence	ANSP-ORN	164239		
		fraseri		Occurrence	ANSP-ORN	164241		

Appendix E (cont'd)

Latitude	Longitude	Subspp.	Elev.	<b>Record</b> Type	Institution	Catalog. #	Preparation	Mass Habitat
		fraseri		Occurrence	ANSP-ORN	164242		
		fraseri		Occurrence	ANSP-ORN	164243		
		fraseri		Occurrence	ANSP-ORN	165438		
		fraseri	35000	Occurrence	ANSP-ORN	183533		
		fraseri	3200.4	Occurrence	ANSP-ORN	59897		
		fraseri	3200.4	Occurrence	ANSP-ORN	59899		
		fraseri	3200.4	Occurrence	ANSP-ORN	59900		
		fraseri	2134	Specimen	FMNH-Birds	53537	skin(r)	
		fraseri		Specimen	MCZ-Orn	113954	skin (dry)	
		fraseri		Specimen	MCZ-Orn	139425	skin (dry)	
		fraseri		Specimen	MCZ-Orn	151661	skin (dry)	
		fraseri		Specimen	MCZ-Orn	199329	skin (dry)	
		fraseri		Specimen	MCZ-Orn	199330	skin (dry)	
		fraseri		Specimen	MCZ-Orn	199332	skin (dry)	
		fraseri		Specimen	MCZ-Orn	199333	skin (dry)	
		fraseri		Specimen	UMMZ-Birds	98190	Skin, Y	
		fraseri	3200	Specimen	USNM-Birds	447398.43	Skin: Whole	
		fraseri	2743	Specimen	USNM-Birds	447399.43	Skin: Whole	
		fraseri		Occurrence	ANSP-ORN	164236		
		fraseri		Specimen	USNM-Birds	375725.43	Skin: Whole	
		fraseri		Specimen	USNM-Birds	101246.43	Skin: Whole	
		fraseri		Occurrence	ANSP-ORN	3937		
		fraseri		Occurrence	ANSP-ORN	59895		
		fraseri		Specimen	MCZ-Orn	34514	skin (dry)	
		fraseri		Specimen	NHMO-Birds	63582/1-P	Skin	
		fraseri		Specimen	USNM-Birds	55307.429	Skin: Whole	

Appendix E (cont'd)

Latitude	Longitude	Subspp.	Elev. Record Type	Institution	Catalog. #	Preparation	Mass Habitat
		fraseri	Specimen	USNM-Birds	55339.429	Skin: Whole	
					96		
		fraseri	Specimen	USNM-Birds	236487.43	Skin: Whole	
		fraseri	Occurrence	ANSP-ORN	164240		
		fraseri	Occurrence	ANSP-ORN	3936		
		fraseri	Specimen	USNM-Birds	236490.43	Skin: Whole	
		fraseri	Specimen	USNM-Birds	236489.43	Skin: Whole	
		fraseri	Specimen	USNM-Birds	236488.43	Skin: Whole	
		littorale	Specimen	AMNH-Birds	Skin-	skin	
					229153		
		littorale	Specimen	AMNH-Birds	Skin-	skin	
		littorala	Spaaiman	AMNU Dirda	229154 Skin	skin	
		intorate	specifien	Alvinni-Dilus	508427	SKIII	
		littorale	Specimen	AMNH-Birds	Skin-	skin	
			1		508431		
		littorale	Occurrence	ANSP-ORN	102430		
		littorale	Occurrence	ANSP-ORN	102434		
		littorale	Occurrence	ANSP-ORN	109320		
		littorale	Occurrence	ANSP-ORN	109322		
		littorale	Occurrence	ANSP-ORN	109324		
		littorale	Occurrence	ANSP-ORN	109327		
		littorale	Occurrence	ANSP-ORN	109329		
		littorale	Occurrence	ANSP-ORN	53968		
		littorale	Occurrence	ANSP-ORN	53969		
		littorale	Occurrence	ANSP-ORN	53970		
		littorale	2743.2 Specimen	CM-Birds	P117760	study skin	

Latitude	Longitude	Subspp.	Elev.	<b>Record Type</b>	Institution	Catalog. #	Preparation	Mass Habitat
		littorale		Specimen	CM-Birds	P117877	study skin	
		littorale		Specimen	FMNH-Birds	287843	skin(r)	
		littorale	2438	Specimen	FMNH-Birds	120745	skin(r)	
		littorale	1219	Specimen	FMNH-Birds	61791	skin(r)	
		littorale		Specimen	FMNH-Birds	60366	skin(r)	
		littorale	2743	Specimen	FMNH-Birds	53370	skin(r)	
		littorale		Specimen	FMNH-Birds	53373	skin(r)	
		littorale	610	Specimen	FMNH-Birds	286716	skin(r)	
		littorale	3170	Specimen	FMNH-Birds	60370	skin(r)	
		littorale	3719	Specimen	FMNH-Birds	60368	skin(r)	
		littorale		Specimen	FMNH-Birds	60367	skin(r)	
		littorale		Specimen	FMNH-Birds	53372	skin(r)	
		littorale	1219	Specimen	FMNH-Birds	61794	skin(r)	
		littorale	1219	Specimen	FMNH-Birds	61797	skin(r)	
		littorale		Specimen	MCZ-Orn	179358	skin (dry)	
		littorale	396.24	Specimen	MVZ-Birds	157914	study skin	
		littorale		Specimen	MVZ-Birds	157918	study skin	
		littorale	3000	Specimen	MVZ-Birds	164617	study skin	
		littorale	1180	Specimen	UF-Audio	35661	skeleton	
		littorale		Specimen	WFVZ-Birds	55733	eggs	
		littorale		Specimen	AMNH-Birds	Skin-	skin	
		1		<b>C</b>		508426	alrin	
		iittorale		Specimen	AMINH-BIRds	508428	5K111	
		littorale		Specimen	AMNH-Birds	Skin-	skin	
				1		508429		
		littorale		Specimen	AMNH-Birds	508430	skin	

Appendix E (cont'd)

Latitude	Longitude	Subspp.	Elev.	<b>Record Type</b>	Institution	Catalog. #	<b>Preparation</b>	Mass Habitat
		littorale		Occurrence	ANSP-ORN	102426		
		littorale		Occurrence	ANSP-ORN	102429		
		littorale		Occurrence	ANSP-ORN	102431		
		littorale		Occurrence	ANSP-ORN	102432		
		littorale		Occurrence	ANSP-ORN	102433		
		littorale		Occurrence	ANSP-ORN	102435		
		littorale		Occurrence	ANSP-ORN	102436		
		littorale		Occurrence	ANSP-ORN	102437		
		littorale		Occurrence	ANSP-ORN	102438		
		littorale		Occurrence	ANSP-ORN	102439		
		littorale		Occurrence	ANSP-ORN	109325		
		littorale		Occurrence	ANSP-ORN	109326		
		littorale	2740	Occurrence	ANSP-ORN	91581		
		littorale	2740	Occurrence	ANSP-ORN	91582		
		littorale		Specimen	FMNH-Birds	53371	skin(r)	
		littorale	1219	Specimen	FMNH-Birds	61792	skin(r)	
		littorale		Specimen	FMNH-Birds	61798	skin(r)	
		littorale	1219	Specimen	FMNH-Birds	61789	skin(r)	
		littorale	1219	Specimen	FMNH-Birds	61796	skin(r)	
		littorale	3170	Specimen	FMNH-Birds	60371	skin(r)	
		littorale		Specimen	FMNH-Birds	275672	skin(r)	
		littorale	1219	Specimen	FMNH-Birds	61795	skin(r)	
		littorale	1219	Specimen	FMNH-Birds	61790	skin(r)	
		littorale	3170	Specimen	FMNH-Birds	60369	skin(r)	
		littorale	1219	Specimen	FMNH-Birds	61793	skin(r)	
		littorale		Specimen	MCZ-Orn	179356	skin (dry)	

Latitude	Longitude	Subspp.	Elev. Record Ty	pe Institution	Catalog. #	Preparation	Mass Habitat
		littorale	Specimen	MCZ-Orn	179357	skin (dry)	
		littorale	1180 Specimen	UF-Birds	38215	skeleton	
		littorale	Specimen	AMNH-Birds	Skin-	skin	
		littorale	Occurrenc	e ANSP-ORN	508425 3935		
		littorale	Specimen	MCZ-Orn	76710	skin (dry)	
		littorale	Specimen	AMNH-Birds	Skin- 508432	skin	
		littorale	Occurrence	e ANSP-ORN	3939		
		unknown	Specimen	UMZC-Verts	27/Paru/5/c	e Skin (un-mounted)	
		unknown	Specimen	UMZC-Verts	27/Paru/5/c	c Skin (un-mounted)	
		unknown	Specimen	AMNH-Birds	FLUID- 1739	fluid	
		unknown	Specimen	AMNH-Birds	Skin- 129216	skin	
		unknown	Specimen	AMNH-Birds	Skin- 129217	skin	
		unknown	Specimen	AMNH-Birds	Skin- 129218	skin	
		unknown	Specimen	AMNH-Birds	Skin- 150041	skin	
		unknown	Specimen	AMNH-Birds	Skin-	skin	
		unknown	Specimen	AMNH-Birds	Skin- 461817	skin	
		unknown	Specimen	AMNH-Birds	Skin- 508423	skin	
		unknown	Observatio	n CLO-ML	171059		

Appendix E (cont'd)

Latitude	Longitude	Subspp.	Elev. Record Type	Institution	Catalog. #	<pre># Preparation</pre>	Mass Habitat
		unknown	Specimen	DMNH-Birds	58978	skin	
		unknown	Specimen	DMNH-Birds	58979	skin	
		unknown	Specimen	DMNH-Birds	58982	skin	
		unknown	Specimen	MCZ-Orn	287253		
		unknown	Specimen	MCZ-Orn	287254		
		unknown	Specimen	MCZ-Orn	299790		
		unknown	396.24 Specimen	MVZ-Birds	174867	whole organism (ethanol)	
		unknown	Observation	UF-Audio	1709		Polylepis- Gynoxis Association Woodland
		unknown	Observation	UF-Audio	19437		
		unknown	Specimen	UF-Audio	36135	study skin	
		unknown	Specimen	WFVZ-Birds	18464	study skin	
		unknown	Specimen	WFVZ-Birds	34027	study skin	7
		unknown	Specimen	WFVZ-Birds	39592	study skin	8
		unknown	Specimen	WFVZ-Birds	39594	study skin	9
		unknown	Specimen	WFVZ-Birds	39595	study skin	9
		unknown	Specimen	WFVZ-Birds	39596	study skin	9
		unknown	Specimen	WFVZ-Birds	39598	study skin	9
		unknown	Specimen	WFVZ-Birds	39599	study skin	9
		unknown	Specimen	WFVZ-Birds	39600	study skin	9
		unknown	Specimen	WFVZ-Birds	39601	study skin	9
		unknown	Specimen	WFVZ-Birds	39606	study skin	9
		unknown	Specimen	WFVZ-Birds	39610	study skin	9
		unknown	Specimen	WFVZ-Birds	42946	study skin	10

Latitude	Longitude	Subspp.	Elev.	<b>Record Type</b>	Institution	Catalog. #	<sup>‡</sup> Preparation	Mass Habitat
		unknown		Specimen	WFVZ-Birds	48243	study skin	10.1
		unknown		Specimen	WFVZ-Birds	167971	round skin	
		unknown		Specimen	WFVZ-Birds	168808	eggs   nest	
		unknown		Specimen	WFVZ-Birds	178654	round skin	
		unknown		Specimen	AMNH-Birds	FLUID- 1740	fluid	
		unknown		Specimen	AMNH-Birds	Skin- 129219	skin	
		unknown		Specimen	AMNH-Birds	Skin- 150042	skin	
		unknown		Specimen	AMNH-Birds	Skin- 150043	skin	
		unknown		Specimen	AMNH-Birds	Skin- 150044	skin	
		unknown		Specimen	AMNH-Birds	Skin- 468583	skin	
		unknown		Specimen	AMNH-Birds	Skin- 468584	skin	
		unknown		Specimen	AMNH-Birds	Skin- 508424	skin	
		unknown		Specimen	AMNH-Birds	SKIN- 820794	skin	
		unknown		Specimen	DMNH-Birds	58980	skin	
		unknown		Specimen	DMNH-Birds	58981	skin	
		unknown		Specimen	DMNH-Birds	58983	skin	
		unknown		Specimen	DMNH-Birds	58984	skin	
		unknown	3000	Specimen	MCNB-Chord	MZB 82- 3008	pell d'estudi (sec)	
		unknown		Specimen	MCZ-Orn	299791		

Latitude	Longitude	Subspp.	Elev. Record Type	Institution	Catalog. #	<sup>‡</sup> Preparation	Mass Habitat
		unknown	Specimen	MCZ-Orn	299792		
		unknown	Specimen	WFVZ-Birds	35069	study skin	
		unknown	Specimen	WFVZ-Birds	39593	study skin	10
		unknown	Specimen	WFVZ-Birds	39597	study skin	9
		unknown	Specimen	WFVZ-Birds	39602	study skin	9
		unknown	Specimen	WFVZ-Birds	39603	study skin	8
		unknown	Specimen	WFVZ-Birds	39604	study skin	9
		unknown	Specimen	WFVZ-Birds	39605	study skin	9
		unknown	Specimen	WFVZ-Birds	39607	study skin	9
		unknown	Specimen	WFVZ-Birds	39608	study skin	9
		unknown	Specimen	WFVZ-Birds	39609	study skin	9
		unknown	Specimen	WFVZ-Birds	42947	study skin	9.8
		unknown	Specimen	WFVZ-Birds	46344	study skin	14
		unknown	Specimen	WFVZ-Birds	47360	study skin	11.5
		unknown	Specimen	WFVZ-Birds	49369	study skin	
		unknown	Specimen	WFVZ-Birds	49370	study skin	
		unknown	Specimen	WFVZ-Birds	168813	eggs   nest	
		unknown	Specimen	KU-Birds	125289	Tissue - 100	
		unknown	Specimen	LSUMZ-Birds	100881	study skin	
		unknown	Specimen	LSUMZ-Birds	100882	study skin	
		unknown	Specimen	LSUMZ-Birds	100884	study skin	
		unknown	Specimen	LSUMZ-Birds	112792	study skin	
		unknown	Specimen	LSUMZ-Birds	114226	study skin	
		unknown	Specimen	LSUMZ-Birds	114228	study skin	
		unknown	Specimen	LSUMZ-Birds	120839	skeleton	
		unknown	Specimen	LSUMZ-Birds	128096	study skin	

Latitude	Longitude	Subspp.	Elev. Record Type	Institution	Catalog. #	Preparation	Mass Habitat
		unknown	Specimen	LSUMZ-Birds	162506	study skin	
		unknown	Specimen	LSUMZ-Birds	162509	study skin	
		unknown	Specimen	LSUMZ-Birds	162510	study skin	
		unknown	Specimen	LSUMZ-Birds	162514	study skin	
		unknown	Specimen	LSUMZ-Birds	162515	study skin	
		unknown	Specimen	LSUMZ-Birds	179620	study skin	
		unknown	Specimen	LSUMZ-Birds	181891	study skin	
		unknown	Specimen	LSUMZ-Birds	34710	study skin	
		unknown	Specimen	LSUMZ-Birds	64523	study skin	
		unknown	Specimen	LSUMZ-Birds	65332	skeleton	
		unknown	Specimen	LSUMZ-Birds	68935	study skin	
		unknown	Specimen	LSUMZ-Birds	70270	skeleton	
		unknown	Specimen	LSUMZ-Birds	71380	study skin	
		unknown	Specimen	LSUMZ-Birds	72773	study skin	
		unknown	Specimen	LSUMZ-Birds	73087	skeleton	
		unknown	Specimen	LSUMZ-Birds	73088	skeleton	
		unknown	Specimen	LSUMZ-Birds	74557	study skin	
		unknown	Specimen	LSUMZ-Birds	75362	study skin	
		unknown	Specimen	LSUMZ-Birds	79110	study skin	
		unknown	Specimen	LSUMZ-Birds	79111	study skin	
		unknown	Specimen	LSUMZ-Birds	79112	study skin	
		unknown	Specimen	LSUMZ-Birds	79113	study skin	
		unknown	Specimen	LSUMZ-Birds	79114	study skin	
		unknown	Specimen	LSUMZ-Birds	79621	alcoholic	
		unknown	Specimen	LSUMZ-Birds	79622	alcoholic	
		unknown	Specimen	LSUMZ-Birds	79887	skeleton	

Latitude	Longitude	Subspp.	Elev. Record Type	Institution	Catalog. #	<sup>£</sup> Preparation	Mass Habitat
		unknown	Specimen	LSUMZ-Birds	79888	skeleton	
		unknown	Specimen	LSUMZ-Birds	79889	skeleton	
		unknown	Specimen	LSUMZ-Birds	82293	study skin	
		unknown	Specimen	LSUMZ-Birds	83911	alcoholic	
		unknown	Specimen	LSUMZ-Birds	86145	study skin	
		unknown	Specimen	LSUMZ-Birds	86146	study skin	
		unknown	Specimen	LSUMZ-Birds	86147	study skin	
		unknown	Specimen	LSUMZ-Birds	93774	study skin	
		unknown	Specimen	LSUMZ-Birds	93775	study skin	
		unknown	Specimen	LSUMZ-Birds	94202	skeleton	
		unknown	Specimen	LSUMZ-Birds	98856	study skin	
		unknown	Specimen	LSUMZ-Birds-	103840	Liquid Nitrogen	
		_		Tiss			
		unknown	Specimen	LSUMZ-Birds-	49632	Liquid Nitrogen	
		unknown	Specimen	l ISS I SUMZ_Birds_	61265	Liquid Nitrogen	
		unknown	Speemen	Tiss	01205	Elquid Milogen	
		unknown	Specimen	LSUMZ-Birds-	62856	Liquid Nitrogen	
				Tiss			
		unknown	Specimen	WFVZ-Birds	34029	study skin	8
		unknown	Specimen	WFVZ-Birds	34030	study skin	9
		unknown	Specimen	WFVZ-Birds	34034	study skin	8
		unknown	Specimen	AMNH-Birds	FLUID-	fluid	
			~ ·		3956	T. 100	
		unknown	Specimen	KU-Birds	125596	Tissue - 100	
		unknown	Specimen	KU-Birds	125688	Tissue - 100	
		unknown	Specimen	KU-Birds	126079	Tissue - 100	

Latitude	Longitude	Subspp.	Elev. Record Type	Institution	Catalog. #	Preparation	Mass Habitat
		unknown	Specimen	KU-Birds	127881	Tissue - 100	
		unknown	Specimen	LSUMZ-Birds	100883	study skin	
		unknown	Specimen	LSUMZ-Birds	100885	study skin	
		unknown	Specimen	LSUMZ-Birds	114227	study skin	
		unknown	Specimen	LSUMZ-Birds	120815	alcoholic	
		unknown	Specimen	LSUMZ-Birds	128095	study skin	
		unknown	Specimen	LSUMZ-Birds	128097	study skin	
		unknown	Specimen	LSUMZ-Birds	128098	study skin	
		unknown	Specimen	LSUMZ-Birds	128099	study skin	
		unknown	Specimen	LSUMZ-Birds	129403	study skin	
		unknown	Specimen	LSUMZ-Birds	129404	study skin	
		unknown	Specimen	LSUMZ-Birds	162505	study skin	
		unknown	Specimen	LSUMZ-Birds	162507	study skin	
		unknown	Specimen	LSUMZ-Birds	162508	study skin	
		unknown	Specimen	LSUMZ-Birds	162511	study skin	
		unknown	Specimen	LSUMZ-Birds	162512	study skin	
		unknown	Specimen	LSUMZ-Birds	162513	study skin	
		unknown	Specimen	LSUMZ-Birds	182801	study skin	
		unknown	Specimen	LSUMZ-Birds	35291	study skin	
		unknown	Specimen	LSUMZ-Birds	35292	study skin	
		unknown	Specimen	LSUMZ-Birds	38119	study skin	
		unknown	Specimen	LSUMZ-Birds	64522	study skin	
		unknown	Specimen	LSUMZ-Birds	73089	skeleton	
		unknown	Specimen	LSUMZ-Birds	79115	study skin	
		unknown	Specimen	LSUMZ-Birds	79116	study skin	
		unknown	Specimen	LSUMZ-Birds	80819	study skin	

Latitude	Longitude	Subspp.	Elev. Record Type	Institution	Catalog. #	Preparation	Mass Habitat
		unknown	Specimen	LSUMZ-Birds	85370	study skin	
		unknown	Specimen	LSUMZ-Birds	86144	study skin	
		unknown	Specimen	LSUMZ-Birds	89299	study skin	
		unknown	Specimen	LSUMZ-Birds	90350	skeleton	
		unknown	Specimen	LSUMZ-Birds	93772	study skin	
		unknown	Specimen	LSUMZ-Birds	93773	study skin	
		unknown	Specimen	LSUMZ-Birds	94201	skeleton	
		unknown	Specimen	LSUMZ-Birds	97540	skeleton	
		unknown	Specimen	LSUMZ-Birds	98857	study skin	
		unknown	Specimen	LSUMZ-Birds-	103863	Liquid Nitrogen	
			_	Tiss			
		unknown	Specimen	LSUMZ-Birds-	103871	Liquid Nitrogen	
		1	<b>C</b>	Tiss	40047	Timid Nites and	
		unknown	Specimen	LSUMZ-BIRdS-	48247	Liquid Nillogen	
		unknown	Specimen	LSUMZ-Birds-	48250	Liquid Nitrogen	
			Speeinen	Tiss	10200	1	
		unknown	Specimen	LSUMZ-Birds-	61266	Liquid Nitrogen	
				Tiss			
		unknown	Specimen	LSUMZ-Birds-	66643	Liquid Nitrogen	
			<u>Caracian en</u>	TISS	0200	Liquid Nitrogon	
		unknown	Specimen	LSUMZ-BIRdS-	8300	Liquid Millogen	
		unknown	Specimen	WFVZ-Birds	34028	study skin	8
		unknown	Specimen	WFVZ-Birds	34031	study skin	8
		unknown	Specimen	WFVZ-Birds	34032	study skin	8
		unknown	Specimen	WFVZ-Birds	34033	study skin	8

**Appendix F**. Map showing all georeferenced museum specimens and eBird records for *Conirostrum cinereum*. Specimens for which subspecies was determined are color coded. Specimens for which subspecies was not determined and eBird records are shown in white.

