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WHY ARE THERE SO MANY BIRDS IN THE TROPICS: USING COMPARATIVE APPROACHES TO INVESTIGATE THE CONFLUENCE OF LANDSCAPE, ECOLOGY, AND GENOME IN THE GENERATION OF NEOTROPICAL AVIAN DIVERSITY

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BY THE COMMITTEE CONSISTING OF

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Abstract

The Neotropics are home to an astonishing level of avian diversity, and thus naturally a focus for the study of the generation of biodiversity. Studies of tropical speciation often focus on large-scale landscape changes, such as the impacts of Pleistocene climate change, the formation of the isthmus of Panama, or the uplift of the Andes. However, the full scope of Neotropical avian diversity is not yet wholly understood, and increasing evidence suggests that other processes beyond these geographical factors contribute to speciation in tropical birds. Here, I examine genetic data, from mitochondrial markers to whole genomes, in a diverse array of Neotropical birds, to better understand the interplay between landscape, genome, and ecology in the development of reproductive isolation. In my first chapter, I use mitochondrial barcodes from over 2,000 birds to detect potential cryptic species across Panama. I find that species-level splits occur in 19% of sampled species, suggesting avian diversity in Panama is substantially underestimated. These disproportionately occurred in species with ecological characteristics associated with low dispersal ability. This is reinforced by the next chapter, in which I use reduced-representation genomic data in ten taxa of lowland Panamanian birds to test whether time is the most important predictor of the outcomes of secondary contact. I find no evidence that time plays a role in determining hybrid zone width in these taxa, and only a partial role in the generation of genomic variation. Instead, diet, which is again linked to dispersal ability as well as demographic changes, is a much better predictor. Finally, I move my focus in Chapter 3 to the Andes, using low-coverage whole genomes to examine the speciation history of the hummingbird genus *Aglaeactis*. This rapid radiation has significant mitonuclear discord, with completely different phylogenies reconstructed from mitochondrial and nuclear markers. In the two clades defined in the nuclear data, each made of three taxa, I then examined how divergence was distributed across the genome. The three southern taxa, all of which are in allopatry, have overall higher genomic divergence, but it is spread evenly across the genome. In the northern clade, though, I find much lower divergence punctuated by outliers of elevated differentiation. These northern taxa do have contact zones between them, and it is likely that gene flow in that geographic scenario has had a hand in shaping the genomic landscape for the development of reproductive isolation. Taken together, each of these chapters explores how

reproductive isolation is the outcome of multiple facets of an organism interacting, and sheds further light on how avian biodiversity is generated.

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Completing a PhD is a daunting endeavor in the best of times. Completing one between 2017 and 2022 has been downright Herculean. I've spent these past four years dodging a seemingly never-ending parade of obstacles, and you can't do that sort of thing alone. The completion of this dissertation is in large part a testament to the support and community I am blessed to be a part of. It may be cliched to say I couldn't have done this without you all, but nevertheless: I couldn't have done this without you all.

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General Introduction

"...whilst this planet has gone cycling on according to the fixed law of gravity, from so simple a beginning endless forms most beautiful and most wonderful have been, and are being, evolved." (Darwin 1859)

From the beginning of the study of evolution, one of the questions at the very heart of biology has been how the myriad of species that exist and have existed have come to be (Darwin 1859; Mayr 1963; Otte and Endler 1989; Coyne and Orr 2004). Such an enormous question must be tackled from a variety of angles, from the role of ecological interactions in supporting diverse communities in organisms (Hutchinson 1959; Brown 1981) to the mechanisms by which genetic variation arises (Dobzhansky 1937). The increasing availability of high-throughput (HTS) or next-generation sequencing (NGS) has allowed for further investigation into even more questions: what exactly must happen in the genome for divergent lineages to become species? What factors of variation across the genome are associated with the development of reproductive isolation? What rules, if any, are there in determining why certain taxa speciate more frequently, and how? With the ability to cost-effectively sequence ever-larger portions of the genome in an array of species, comparative genomic studies can allow us to directly explore these questions more effectively (Wolf, Lindell, and Backström 2010; Seehausen *et al.* 2014; Termignoni-Garcia *et al.* 2022).

A major conceptual framework for studying the speciation process is the speciation continuum (Drès and Mallet 2002; Hendry *et al.* 2009; Merot *et al.* 2017; Stankowski and Ravinet 2021), particularly in comparative studies (Stankowski and Ravinet 2021). In these studies, each taxon pair represents one discrete point in the process of speciation, from the earliest stages of differentiation to complete reproductive isolation (Hendry *et al.* 2009; Stankowski and Ravinet 2021), and examination of common patterns amongst them or striking differences between them may reveal more general rules in how reproductive isolation develops in incipient species (Tollis *et al.* 2018; Gagnaire 2020). This is

particularly useful for testing hypotheses that are not easily amenable to direct controlled experiments given the time scale of evolutionary processes, such as how long it takes for reproductive isolation to develop (Coyne and Orr 1989; Edmands 2002; Price and Bouvier 2002; Coyne and Orr 2004; Matute and Cooper 2021), the relative rates of development of pre- and post-zygotic isolation (Mendelson 2003; Charistianson, Swallow, and Wilkinson 2005; Moyle, Olson, and Tiffin 2004; Turissini *et al.* 2018; Rosser *et al.* 2019), and the relationship between introgression rates and genetic divergence (Wiens, Engstrom, and Chippindale 2006; Kronforst *et al.* 2013; Hamlin, Hibbins, and Moyle 2020; McLaughlin *et al.* 2020). However, as more and more studies have been conducted, it has become ever clearer that much variation exists in the answers to these questions (Matute and Cooper 2021; Stankowski and Ravinet 2021). This leads to the inevitable question: what causes such variation in the process exist, and what factors lead to particular outcomes in a given system?

Testing mechanisms of speciation in a species-rich area

While comparative approaches can be applied to other geographic regions, tropical regions, with their massive biodiversity, are of particular interest. An estimated 62% of global terrestrial vertebrate biodiversity occurs within the tropics (Pillay *et al.* 2021). The question of tropical biodiversity can be approached from multiple angles, but can be broadly broken into two main categories of mechanisms by which species diversity increases- either by accumulation of species, or as a generator of them (Stenseth 1984; Gaston and Blackburn 1996; McKenna and Farrell 2006). These are reflected in the classic framing of the tropics as either a cradle or museum of species (Stebbins 1974; Stenseth 1984); i.e., as a hotspot for creating new species, or as an area with lower extinction rates where older lineages are more likely to persist than at other latitudes (Stebbins 1974). While it is likely that this framing simplifies a more complex set of processes (Chown and Gaston 2000; McKenna and Farrell 2006; Arita and Vázquez-Domínguez 2008; Vasconcelos, O'Meara, and Beaulieu 2022), the evidence that the tropics likely have at least some elevated rates of speciation (McKenna and Farrell 2006; Moreau and Bell 2013) makes them of particular interest for the study of speciation.

With this overall biodiversity comes an especially notable level of avian diversity, with over 3000 estimated species in the Neotropics alone (Stotz *et al.* 1996). Many studies of avian speciation have focused on temperate regions, but the Neotropics, with their overwhelming diversity of species, are an obvious laboratory for understanding how species form. Studies of temperate zone birds suggest some general patterns – such as an average time to speciation between 1-2 million years (Weir and Schluter 2007; Price 2008) and that prezygotic barriers play a more important role than postzygotic isolation in forming species (Martin, Montgomerie, and Lougheed 2010; Weir and Wheatcroft 2011; Weir, Wheatcroft, and Price 2012). This may not be the case, however, in tropical systems. Many Neotropical bird species are older, between 2-4 million years old (Bates, Hackett, and Goerck 1999; Marks, Hackett, and Capparella 2002; González *et al.* 2003; Weir and Schluter 2007; Price 2008; Miller *et al.* 2008, 2011; Weir and Price 2011; Weir and Lawson 2015) and include notable levels of cryptic diversity (Hackett 1995; Hackett 1996; Bates, Hackett, and Goerck 1999; Aleixo 2002; Marks, Hackett, and Capparella 2002; González *et al.* 2003; Miller *et al.* 2011; Lopez *et al.* 2016; Pulido-Santacruz, Aleixo, and Weir 2018).

Gene flow has been found between sister taxa across splits far older than in comparable temperate cases (Weir and Price 2011; Weir *et al.* 2015; Pulido-Santacruz, Aleixo, and Weir 2018, 2020; Miranda, Prestes, and Aleixo 2021). These observations lead to a fundamental question of whether the balance of the many mechanisms that lead to reproductive isolation in birds is shifted in tropical systems--whether key processes in the diversification of tropical bird species are those outweighed by other factors in temperate systems.

Models of Neotropical speciation center the landscape

Historically, the primary spotlight of the investigation of avian speciation has focused on the landscape. Ornithology has been particularly influenced by the work of Ernst Mayr (Cracraft 1983;

McKitrick and Zink 1988; Haffer 1992; Zink and McKitrick 1995; Price 2008), and thus consideration of the speciation process has been firmly rooted in the biological species concept (Cracraft 1983; Price 2008). With this paradigm comes the primary role of allopatry in creating the perquisite isolation in at least some point in the process (Jordan 1905; Dobzhansky 1937, 1940; Mayr 1942, 1963; Rosenzweig 1995). This has been particularly true in studies of Neotropical avian diversity, which have tended to center specific landscape-based models in their investigations.

Initially, most studies tended to focus on how long-term changes in the landscape created allopatric splits. Examples of this are found in discussion of the uplift of the Andes (Burns and Naoki 2004); the formation of the isthmus of Panama (DaCosta and Klicka 2008; Cortés-Ortiz, Rylands, and Mittermeier 2015); and the competing "rocks, rivers, or refugia" hypotheses for diversification in the Amazon (Wallace 1853; Cracraft 1985; Nelson *et al.* 1990; Bush 1994; Haffer 1997; Hayes and Sewlal 2004; Hoorn *et al.* 2010; Cortés-Ortiz, Rylands, and Mittermeier 2015). In brief, these each emphasize the barriers created by large-scale geologic change (Burns and Naoki 2004; Naka *et al.* 2012), large rivers (Wallace 1853; Sick 1967; Capparella 1988; Gascon *et al.* 2000; Hayes and Sewlal 2004; Naka and Brumfield 2018), and encroaching grasslands which fragmented forest habitats during the periodic increased aridity during Pleistocene glaciation (Haffer 1969, 1985). These models remain highly influential, and in more recent work shifted to exploration of how such large-scale changes create the matrix shaping dispersal patterns and gene flow (Smith *et al.* 2014; Harvey and Brumfield 2015; Oswald *et al.* 2017). In most, though, the main focus remains on geography, even though the influence of landscape on organisms is only one facet shaping how species diversify. While geography can explain some patterns, there are often species in which these explanations fail (Smith *et al.* 2014) and focusing only on those taxa which fit these patterns ignores the key question of *why* specific taxa do not fit into these paradigms.

Speciation is driven by more than just landscape

Geographic isolation and its consequence, differentiation through drift and adaptation, do not act alone– it acts on the pre-existing landscape of variation and diversity across the genome of diverging populations. As diverging populations move along the speciation continuum (Hendry *et al.* 2009), the genome does not diverge uniformly (Harrison 1991; Turelli, Barton, and Coyne 2001; Nosil, Funk, and Ortiz-Barrientos 2009; Vijay *et al.* 2016, 2017). If geographic changes bring populations that have diverged in allopatry back together, the outcomes of secondary contact are likely to be different in a population pair that, for example, have relatively undifferentiated genomes with a few key loci with heightened divergence, compared to a similar pair that have overall higher divergence, but more evenly spread across the genome (Feder *et al.* 2013, 2014; Nosil *et al.* 2017). This has important implications for the formation and maintenance of reproductive barriers, and whether populations diverge into full-fledged species. Some of this genomic background will be shaped by intrinsic genomic factors (such as variation in recombination rate; Burri *et al.* 2015), but often neglected is how life history, demography, and ecology interact to generate and maintain genomic variation.

In this dissertation, I investigate the genomic mechanisms of speciation in two systems (Figure I.1). In Chapters 1 and 2, I focus on speciation in lowland Panama, where multiple species of birds show evidence of rapid turnover despite the lack of obvious geographic barriers, pointing to a stronger role for ecological and demographic factors in driving the development of reproductive isolation. In Chapter 3, I shift my focus to how geographic patterns of allopatry and parapatry can shape the landscape of divergence across the genome, using whole genomes to characterize the genomic landscape of speciation in a rapidly diverging group of Andean hummingbirds. These two systems, though they stand as mirrors of each other in respect to the pace of speciation, each create excellent natural laboratories to investigate how non-geographic factors drive speciation in the Neotropics.

Figure I.1: Map of the Neotropics, using bioregion classifications from Olson *et al.* (2001; modified by Antonelli *et al.* 2018). Highlighted are my two areas of study: Panama (Chapters 1 and 2), shown in the inset with bioregions defined by Smith and Bermingham (2005); and the Andes (Chapter 3).

Chapter 1: Comparative phylogeography reveals widespread cryptic diversity driven by ecology in Panamanian birds

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Author Contributions

JFM and MJM conceived the study, based on the barcoding efforts initiated by MJM, KS, LDL, and OLC and with input from SL, RD, and JRL. JFM wrote the initial draft with input from MJM and drew all figures. All authors then contributed to later drafts. Sequencing was carried out by CA, WGN-G, Data processing and analysis was conducted by JFM, LCA, AA, BA, RC, AT, SJV, JB, KMB and MJM. Original illustrations created by JFM.

Abstract

Identifying cryptic species is important for conservation, and can also provide insights into the speciation process as they often represent phenotypically similar sister taxa that are of particular interest in studying the early stages of speciation. Here we identify potential cryptic species of Panamanian birds based on a comprehensive database of COI DNA barcodes from 2,333 birds from 429 species across Panama, representing 391 (59%) of the 659 resident landbird population of the country, as well as opportunistically sampled waterbirds. We then expanded this dataset using whole mitochondrial genomes from 20 taxa (species or species-complexes), allowing us to then supplement these data with publicly available mitochondrial sequences from other loci such as ND2 and cytochrome *b*, increasing our genetic sampling across Panama. Using BINS, which is a numerical taxonomic system that provides an unbiased estimate of potential species-level diversity, we find multiple mitochondrial barcode identification numbers (BINs), likely representing cryptic species, in 19% of landbird species with widespread geographic sampling, highlighting the hidden diversity in even the relatively well-described avifauna of Panama. While some of these splits corresponded with recognized geographic features, such as the Cordillera Central, that have served to isolate populations, the majority (74%) of lowland splits were between eastern and western populations. Timing of these splits did not coincide, making historical landscape change associated with the formation of the Isthmus of Panama or with potential habitat contraction during Pleistocene climatic cycles unlikely as the primary driver of diversification. However, strong trends in ecological characteristics were observed in species with mitochondrial splits, with forest species, understory species, insectivores, and strongly territorial species all more likely to have multiple BINs in Panama. Additionally, hand-wing

index, a proxy for dispersal capability, was significantly lower in species with multiple BINs. Together these suggest that dispersal ability plays an important role in generating diversity in the Neotropical avifauna, and underscores the need for studies of the evolution of tropical bird communities to take ecological factors into account along with geographic explanations, as dispersal ability alone may drive differentiation even in the absence of obvious landscape barriers.

Introduction

A key part in understanding the development of biodiversity in a given region is simply knowing how many species occur there (Bickford *et al.* 2007; Allendorf and Luikart 2009; Pérez-Ponce de León and Nadler 2010). However, this is not always an easy task. Cryptic species, reproductively isolated and distinct taxa that are nevertheless not recognized as distinct species, are a well-known issue in both evolutionary (Bickford *et al.* 2007; Struck *et al.* 2018) and conservation biology (Fouquet *et al.* 2007; Crawford *et al.* 2013; Yan *et al.* 2018; Chenuil *et al.* 2019; Levy and Cox 2020). From a conservation perspective, it is rather intuitive that it is difficult to make decisions that protect an area's biodiversity without accurately defining it (Bickford *et al.* 2007; Fouquet *et al.* 2007; Trontelj and Fišer 2009; Funk, Caminer, and Ron 2012). Also important, however, is how the occurrence of cryptic species may illuminate the evolutionary processes that generate this biodiversity (Campbell, Braile, and Winker 2016; Bickford *et al.* 2007; Struck *et al.* 2018). While cryptic species are often framed as an issue of not describing and cataloging variation (Pérez-Ponce de León and Nadler 2010; Korshunova *et al.* 2017), it is also worth considering them from the perspective of why some species diversify without being easily identifiable as distinct entities (Roux *et al.* 2016; Pulido-Santacruz, Aleixo, and Weir 2018). From this frame of reference, the question becomes less one of lack of thorough biodiversity survey effort, but one of what factors, such as geographical, historical, ecological, or any of the above in combination, are actually driving the generation of such variation (Bickford *et al.* 2007; Struck *et al.* 2018). This can then provide valuable insights into the mechanisms of speciation and diversification, informing not just conservation decision-making but also the study of evolutionary processes (Bickford *et al.* 2007; Saitoh *et al.* 2015; Campbell, Braile, and Winker 2016; Struck *et al.* 2018).

The Neotropics are home to incredible avian species diversity, with around 1 in 4 global bird species found in the region (Haffer 1985; Orme *et al.* 2005). However, despite that stunning richness, it is likely that even this is an underestimate (Tavares *et al.* 2011; Milá *et al.* 2012; Rheindt, Cuervo, and Brumfield 2013; Mendoza *et al.* 2016), as studies frequently find unrecognized species-level diversity within recognized species. This is particularly the case in "widespread" species, where closer examination often indicates that taxa reveal widespread variation characteristic not of single species but species complexes, such as *Cantorchilus* wrens (González *et al.* 2003), trogons (DaCosta and Klicka 2008), *Cyanocompsa* grosbeaks (Bryson *et al.* 2014), *Mionectes* (Miller *et al.* 2008) and *Zimmerius* flycatchers (Rheindt, Cuervo, and Brumfield 2013), *Lepidothrix* (Cheviron, Hackett, and Capparella 2005) and *Manacus* (Brumfield *et al.* 2008) manakins, *Habia* ant-tanagers (Lavinia *et al.* 2015; Ramírez-Barrera *et al.* 2018, 2019), *Pachyramphus* becards (Musher and Cracraft 2018), *Malacoptila* puffbirds (Ferreira *et al.* 2017), *Arremon* brush-finches (Cadena, Klicka, and Ricklefs 2007; Navarro-Sigüenza *et al.* 2008; Cadena and Cuervo 2010) and *Phaeothlypis* wood-warblers (Lovette 2004). Not only does this widespread underestimation of avian biodiversity across the Neotropics have practical implications for conservation (Bickford *et al.* 2007; Allendorf and Luikart 2009; Valentini, Pompanon, and Taberlet 2009; Lohman *et al.* 2010; Funk, Caminer, and Ron 2012; Crawford *et al.* 2013; Gonçalves *et al.* 2015; Mendoza *et al.* 2016), it also hampers our understanding of the evolutionary processes and biogeographic factors that have made the Neotropics such an engine of species diversification (Bickford *et al.* 2007). While there is some risk that the search for cryptic species may lead to oversplitting and taxonomic inflation which hamper our understanding of the evolutionary processes at play (Chaitra, Vasudevan, and Shanker 2004; Isaac, Mallet, and Mace 2004; Hundsdoerfer *et al.* 2019; Chan *et al.* 2020), the evidence in birds specifically suggests that the error is far more likely in the direction of underestimation (Sangster 2009; Barrowclough *et al.* 2016), and even if such taxa may not represent fully-fledged species in their own right, they still provide insight into the processes at play by providing examples of other potential outcomes.

Mitochondrial barcoding provides us with a powerful tool to further detect potential cryptic species (Arnot, Roper, and Bayoumi 1993; Floyd *et al.* 2002; Hebert, Ratnasingham, and deWaard 2003; Hebert and Gregory 2005; Kerr *et al.* 2009; Valentini, Pompanon, and Taberlet 2009; Milá *et al.* 2012). Previous efforts using mitochondrial markers have documented cryptic variation in multiple Panamanian birds (González *et al.* 2003; Miller *et al.* 2011; Bryson *et al.* 2014; Loaiza *et al.* 2016; Lopez *et al.* 2016). However, as useful as single-taxon studies are, they provide only snapshots into patterns of phylogeographic diversity of Panamanian birds. Mitochondrial barcoding, with its relative ease and low cost, provides a simple but powerful tool to build large scale comparative datasets (Kerr *et al.* 2007, 2009; Mendoza *et al.* 2016), using a single locus present in a wide range of taxa (Bronstein, Kroh, and Haring 2018). With such datasets, we can better estimate the occurrence of cryptic species in Panama, which in turn allows us to better document the avian diversity of the region.

Large-scale barcoding allows us to do more than simply catalog previous undescribed diversity. It allows us to investigate whether there are patterns in where such turnovers occur geographically (e.g., such as corresponding with known suture zones in other taxa) and what patterns of ecological factors are more common in taxa with cryptic variation (Bickford *et al.* 2007; Struck *et al.* 2018). Mitochondrial barcodes, while only a portion of the genome and thus only a window into a narrow portion of the evolutionary history, can be a powerful tool for this scale of study, allowing for the assembly of datasets with far more individuals than with multi-locus studies and by their relatively well understood rates of molecular change compared with other potential loci (Mendoza *et al.* 2016; Barreira, Lijtmaer, and Tubaro 2016). Again, the vast biodiversity of the Neotropics raises multiple questions that we can empirically test with large comparative phylogeographic datasets (Kerr *et al.* 2009; Tavares *et al.* 2011; Miller *et al.* 2021). Hypotheses on the origin of Neotropical diversity tend to fall into a few broad groups. First, there are biogeographic explanations that emphasize how the geologic and environmental history of the landscape itself have repeatedly created isolated populations that lead to diversification (Sick 1967; Haffer 1969, 1985, 1997; Bush 1994; Sedano and Burns 2010; Smith *et al.* 2014; Ferreira *et al.* 2017). In Panama, these are further grouped into those emphasizing

the process of the formation of the Isthmus of Panama (DaCosta and Klicka 2008; Smith and Klicka 2010; Leigh, O'Dea, and Vermeij 2014), and those emphasizing paleoclimatic fluctuations driving a changing mosaic of forest and savannah (Smith, Amei, and Klicka 2012). Secondly, there are explanations which focus on the role of ecological assemblages driving competition and diversification, with the profusion of niches driving the diversification of species to fill them (Klopfer and MacArthur 1960, 1961; Emerson and Kolm 2005; Brown 2014; Moles and Ollerton 2016). While overall, both likely play roles in generating tropical biodiversity (Bush 1994; Smith *et al.* 2014), the question of which prevail in a given region and at finer scales remains unclear.

With widespread DNA barcoding, we can test hypotheses which fall under both of these categories. The Panamanian region is a topographically and ecologically diverse region, especially considering its small size (Ridgely and Gwynne 1992; Siegel and Olson 2008; Angehr and Dean 2010). With multiple islands and disjunct highlands (Figure 1.1A), we may expect that these will be home to a disproportionate number of cryptic species, as has been found elsewhere (Saitoh *et al.* 2015; Campbell, Braile, and Winker 2016). Both these populations and the more continuously distributed lowland taxa may also have been subject to historic isolation, especially by dispersal and then isolation prior to the final closure of the Isthmus of Panama approximately 2.7 to 4.2 million years ago (Leigh, O'Dea, and Vermeij 2014; O'Dea *et al.* 2016; Jaramillo *et al.* 2017), or by possible expansion of savannah habitats and formation of forest refugia during the Pleistocene (Smith, Amei, and Klicka 2012). In particular, lowland Panama has been recognized as a hotspot for species turnover- replacement of a given taxa with a sister taxa- in birds (Miller, Bermingham, and Ricklefs 2007; Miller *et al.* 2011; Loaiza *et al.* 2016; Lopez *et al.* 2016; McLaughlin, Garzón, *et al.* 2020), as well as in freshwater fish (Bermingham and Martin 1998; Martin and Bermingham 2000; Perdices *et al.* 2002; Smith and Bermingham 2005; Bagley and Johnson 2014), mammals (Cortés-Ortiz *et al.* 2003), herpetofauna (Crawford 2003; Crawford and Smith 2005; Bagley and Johnson 2014), insects (Bagley and Johnson 2014; Eskildsen *et al.* 2018), and plants (Dick, Abdul-Salim, and Bermingham 2003).We can investigate if this is an important factor in generating avian diversity by testing if breaks are broadly coincident in time (Naka

and Brumfield 2018). Beyond biogeographic explanations, our diverse sampling (Figure 1.2) allows us to investigate whether specific ecological traits, such as habitat preference (Zhang *et al.* 2012; Berner and Thibert-Plante 2015; Harvey *et al.* 2017), dispersal ability (Claramunt *et al.* 2012; Weeks and Claramunt 2014; Crouch *et al.* 2019), territoriality (Tobias *et al.* 2016), and diet (Sheard *et al.* 2020; Miller *et al.* 2021) are overrepresented among taxa with mitochondrial splits.

We set out to investigate the connections between geography, ecology, and the occurrence of cryptic phylogeographic variation with a multifaceted mitochondrial dataset. First, we barcoded 429 species of birds, including 391 landbird species, which represents 59% of the documented 659 resident landbirds in the country. This allowed us to estimate the occurrence of mitochondrial breaks in the Panamanian avifauna, and to identify if certain ecological or phylogenetic patterns predicted the likelihood of such breaks. We found that specific ecological traits, including dispersal ability, territoriality, diet, and habitat, were significantly over-represented in taxa with mitochondrial breaks, suggesting these may strongly contribute to the diversification of Neotropical birds.

Materials and Methods

COI barcode survey of Panamanian birds

We developed a COI barcode dataset of 2333 birds from across Panama in 429 (as defined in the original taxon dataset from BOLD; Angehr and Dean 2010 define 484 by splitting several taxa that are not in the taxonomy used in BOLD) species as part of sequencing for the Barcode of Life Database (BOLD; Ratnasingham and Hebert 2007, 2013). The majority of these birds were sequenced through the Smithsonian Institution (SI)'s Barcode of Life initiative which provided funds for barcoding sequencing at the National Museum of Natural History (Schindel *et al.* 2011) and the Smithsonian Tropical Research Institute (original data presented here). With a few exceptions, every sequence in the SI barcoding datasets is represented by a museum voucher specimen (Table A1.1).

We determined whether a species had mitochondrial splits by using the barcode index number (BIN; (Ratnasingham and Hebert 2013) as implemented in BOLD (Ratnasingham and Hebert 2007). In brief, this numerical taxonomy method uses the mitochondrial barcode gap to define distinct units of evolutionary diversity, which are frequently used as indication of potential cryptic speciation (Ratnasingham and Hebert 2013). Mitochondrial COI sequences are aligned and then clustered, with individuals that are more than twice the distance of divergence within a cluster being taken as the start of a new cluster, followed by use of a Markovian analysis to refine clusters (Ratnasingham and Hebert 2013). Key benefits of this method are in the ease and low-cost of the method (Tavares *et al.* 2011; Milá *et al.* 2012), and the general relatively high reliability in assigning individuals to species in past studies (Yoo *et al.* 2006; Kerr *et al.* 2007). It does carry the standard limitations of any single-marker method of evaluating diversity, namely that a single locus may not be reflective of the total evolutionary history of a taxon. However, mitochondrial studies are still valuable where large-scale nuclear sequencing is not feasible, and are useful in determining where to focus with more in-depth sequencing efforts (see Chapter 2).

Improving geographic coverage through mitogenomic haplotyping

While covering over 2000 birds, our COI database does not fully capture available data on the distribution of mitochondrial diversity and structure in Panamanian birds that is available either as part of previously published studies, (e.g., Miller *et al.* 2010; Smith *et al.* 2014; Miller *et al.* 2021), or from our group's unpublished non-COI mtDNA datasets. Because mitochondrial DNA is non-recombining, whole mitochondrial genomes can "connect" disparate mtDNA datasets into congruent haplogroups, functioning as a "Rosetta Stone" to leverage multiple mitochondrial loci into a large common dataset. For 20 bird taxa identified with distinct COI BINs in Panama that had whole genome data available, we mined whole mitogenomes from genomic datasets (e.g., do Amaral *et al.* 2015). We then harvested 215 additional mitochondrial sequences, including ND2, cytB, ND3,

ATPase 8, and ATPase6, from NCBI (Table 1.1; details by individual in Table A1.1), increasing sampling density across Panama.

Mitogenome assembly, annotation, and analysis

As part of several long-term projects on the comparative genomics of Panamanian lowlands, we filtered mtDNA reads from whole genome shotgun sequencing for the above 20 taxa of resident lowland birds sampled in western (Bocas del Toro) and eastern (Darién) Panama. We sequenced two individuals from each of those populations, preparing genomic libraries with the NEB Ultra II protocol and sequencing them on an Illumina NovaSeq. We then used bbduk, a utility within the bbmap program (Bushnell 2014), to trim and perform initial quality control on reads.

Assembly of one individual per population was performed using NovoPlasty v. 3.4 (Dierckxsens, Mardulyn, and Smits 2017), using either COI or ND2 as the seed, depending on availability. In some individuals with a high number of reads, we subsampled the initial reads with BBSplit, a part of the BBMap package (Bushnell 2014), to increase computational efficiency by only including putative mitochondrial reads in our assembly inputs. Mitogenomes were then aligned and annotated with MitoAnnotator (Iwasaki *et al.* 2013), from which we calculated the pairwise K2P genetic distance for each of the protein-coding genes between eastern and western populations.

Generating trees

In order to determine as best as possible where mitochondrial breaks occur, we generated locus-specific sequence alignments of available mitochondrial sequences for the 34 taxa which had mitochondrial breaks. Sequences were downloaded from the BOLD and NCBI databases (Table A1.1). We then generated MUSCLE alignments (Edgar 2004) in MEGA7 (Kumar, Stecher, and Tamura 2016), using these to build neighbor-joining trees in PAUP* (Swofford 2001) or MEGA (Kumar, Stecher, and

Tamura 2016) and ML trees in RAxML (Stamatakis 2014) using with the GTR substitution model with Lewis ascertainment bias correction for 100 bootstrap replicates. For the 21 taxa with multiple mitochondrial loci available (Table 1.1), the BOLD barcodes were used to define groups, and then additional individual sequences were aligned to the whole mitochondrial genomes for that taxon, which had already been assigned to BINs, and haplotyped accordingly.

Testing predictors of mitochondrial divergence

In order to test how ecological factors such as habitat openness, forest stratum, diet, and elevational distribution influenced the likelihood of mitochondrial splits, we compiled and scored these data for all 659 resident, breeding landbirds of Panama, using species accounts from The Handbook of the Birds of the World Online (Billerman *et al.* 2020) supplemented as needed from individual species accounts in Angehr and Dean (2010), Ridgely and Gwynne (1992), Stotz *et al.* (1996), and Wetmore (1965, 1968, 1972; 1984). Habitat was scored as forest, edge, or open (Figure 1.5C). Stratum was scored as ground (primarily terrestrial foraging, and/or prefers walking to flying), understory (forages primarily in undergrowth or directly above ground), midstory (primarily found in middle strata of forest, up to subcanopy), canopy (primarily found in the subcanopy and above), and aerial (forages above the forest canopy; almost exclusively swifts and swallows). We also included data on territoriality, hand-wing index (HWI), body size, and annual precipitation in range sourced from Sheard *et al* (2020). Finally, we identified whether each species had been sampled across multiple geographic regions of Panama (Figure 1.1A), to determine if sampling was geographically sufficient to actually identify variation across the region.

First, we tested for sampling biases in these three categories, comparing the total list and sampled subset by *chi*-squared tests to check if our 429 sampled taxa reflected the distribution of the above traits within the total Panamanian avifauna. Then we tested for whether those taxa which had been sampled across multiple geographic regions of Panama (Figure 1.1A) were likewise representative of the total Panamanian avifauna. Taxa were considered widespread enough for inclusion in these tests if

they occurred in two or more of the defined geographic regions of Panama (Figure 1.1A), which yielded a total of 181 species.

Within the 181 species in our overall sample which had been widely sampled across Panama based on the above criteria, we then tested whether certain geographic, ecological, and morphological traits were over- or under-represented in species with two or more mitochondrial BINs. For each of the above traits (stratum, territoriality, diet, habitat, HWI, body size, and annual precipitation), we tested using either a *chi*-squared test or student's *t*-test whether there were significant differences in the representation of traits between split and non-split taxa.

Testing timing of splits

To see whether splits were coincident in time, and thus likely to have been driven by the same biogeographic events, we estimated divergence time in BEAST v. 2.6.2 (Bouckaert *et al.* 2014) as implemented on CIPRES (Miller, Pfeiffer, and Schwartz 2011), for all COI barcodes, trimmed to no missing data. We used a strict clock rate of 1.8 % divergence per million years (Lavinia *et al.* 2016), gamma site model with JC69 substitution model, and five fossil calibration points (Table A1.3). We ran this model for 4 billion generations, sampling every 10,000 generations, and visualized results in Tracer v. 1.7.1 (Rambaut *et al.* 2018). We calculated the mean divergence time for each taxon using the rate of 1.8% divergence per million years previously found for avian COI (Lavinia *et al.* 2016), and compared this with the BEAST estimated means, and along with the 95% confidence intervals constructed in the latter, used them to establish whether divergence times were broadly coincident.

Results

Barcoding and data collection

We successfully barcoded 2,333 individuals from 429 species across Panama, 391 of which were resident landbird species. This sample was fairly representative of the general Panamanian avifauna, as similar proportions of highland and lowland birds were present in the whole as in our sample ($\chi^2 = 0$, $df = 1$, $p = 1$) as well as similar proportions by diet ($\chi^2=13.343$, $df = 9$, $p=0.148$) and habitat ($\chi^2=6.18$, $df = 3$, $p=0.103$; Table 2). Of these 429 species, 181 were sampled across two or more geographic regions (Figure 1.1A) and were likewise representative of the whole population of resident landbirds (Table 2).

Thirty-four of these 181 taxa had more than 1 mitochondrial BIN, represented by a total of 419 individuals barcoded in BOLD. We then increased this to a total of 634 individuals by adding 215 additional sequences from NCBI (Table A1.1). Twenty-one species were able to be supplemented by this method, but the remainder did not have the required samples of both whole mitochondrial genomes to allow the building of a multi-locus data transect.

Mitochondrial splits

We characterized the geography of the thirty-four taxa with multiple BINs, plus two waterbirds (*Laterallus albigularis* and *Jacana spinosa*) not included in the prediction testing due to overall low sampling of waterbirds (Figure 1.3). Among landbirds, splits, as defined by BOLD's barcode gap method (Ratnasingham and Hebert 2007) were observed in 20 families of the 37 widely sampled families (Figure 1.2), or 33% of the 61 resident landbird families documented in Panama. Seven taxa, all lowland, had three BINs in Panama (Figure 1.3). Overall, we found 41 splits across 34 landbird species, out of the 181 taxa with sufficient sampling across multiple geographic regions.

Geographically, we observed two primary patterns in the distribution of splits (Figure 1.3). The first, observed in seven species, was a break between southwest Panama, in particular the Burica peninsula (Chiriquí province) (Figure 1.1A), and the rest of Panama. This pattern was restricted to lowland taxa.

The second primary pattern was one of splits between eastern and western Panama, observed in 35 splits (Figure 1.3). This included both highland taxa (4 splits) and lowland (31 splits). In highland taxa, these splits were between the Cordillera Central and the highland areas of the east. However, in lowland taxa, there were two general clusters of regions of rapid geographic replacement of BINs across multiple taxa. The first, involving 15 splits (48% of lowland taxa with east-west splits), was along the Caribbean versant in Veraguas province, extending into Colón province in some cases (Figure 1.1B). The second, involving seven splits, was roughly located along the border of Darién and Panama provinces (Figure 1.1B). Three lowland splits were in central Panama (Figure 1.4), and an additional three, while representing distinct BINs in extreme eastern and western Panama, lacked samples from between these that would allow us to locate the precise area of turnover (Figure 1.4). One predicted geographic pattern that was not observed was differentiation of island and mainland taxa, despite island samples being included in most species and preferentially including those taxa thought to include such distinct island groups (Table A1.1)

Prediction testing

While our overall dataset of 181 species may have been ecologically and geographically representative of resident landbird taxa (Table 1.2), there were marked differences for many of these in species where splits were observed. While insectivores made up 47% of non-split species, they accounted for significantly more (68%) of species with splits (χ^2=17.27, *df*= 7, *p*=0.016; Figure 1.4B). Forest birds, while comprising the majority of non-split species, at 62%, had even greater representation among the split species, at 85% (χ^2=6.488, *df*= 2, *p*=0.039; Figure 1.4C). When considering habitat stratum, we found that while on non-split species were evenly distributed throughout strata, with only 40% being classed as understory residents, in split taxa understory birds were the overwhelming majority, at 74% of species (χ^2=14.04, *df*= 4, *p*=0.0072; Figure 1.4C). Hand-wing index (HWI) was significantly (*t*= -5.52, *df*- 154.29, *p*=1.43 × 10^{-7}) lower in split species (Figure 1.4A). Finally, split taxa were far more

likely to be strongly territorial, with 62% of split taxa versus 35% of non-split (χ^2=12.038, *df*= 2, *p*=0.0024; Figure 1.4D).

Some traits, however, were represented at similar proportions in both split and non-split species (Table A1.2). Highland taxa were in both cases a minority of species, at 11% of non-split and 12% of split species (χ^2=4.10 × 10^{-29}, *df*= 1, *p*=1). Annual precipitation was similar for both, at 2209 mm/yr in split and 2179 mm/yr in non-split (*t*= 0.28, *df*= 48.22, *p*=0.78). Finally, scaled body size was largely similar, with non-split species being very slightly larger, but not significantly so (*t*= 0, *df*=292, *p*=1).

Timing of splits

Depths of splits varied considerably. Pairwise differences in COI ranged from 1.24% to 8.49% for those defined as having multiple BINs by BOLD. Median pairwise divergence was 3.31%. These are equivalent to between 689 kya and 4.71 mya (Figure 1.5), with a median of 1.84 mya, using the rate of 1.8% divergence every million years observed for avian COI (Lavinia *et al.* 2016). The majority of the splits post-date the typical estimate of the formation of the Isthmus of Panama approximately 3 mya (Leigh, O'Dea, and Vermeij 2014). However, many of the dates are concentrated between one and three mya (Figure 1.5), which while contrary to assertions of a fully formed isthmus during the Pliocene, are congruent with more recent descriptions of this process being a gradual emergence of land (O'Dea *et al.* 2016). However, as our confidence intervals were frequently very wide (Figure 1.5), we cannot draw definitive conclusions about the role of the formation of the Isthmus in the broader patterns we see, instead focusing on the ecological factors above.

Discussion

Despite its small size, Panama is nevertheless home to remarkable avian diversity, with 1,000 currently recognized species occurring in the region (Wetmore 1965, 1968, 1972; Wetmore, Pasquier, and Olson 1984; Ridgely and Gwynne 1992; Angehr and Dean 2010). While some of this is likely due to its

position as a literal bridge between North and South America (DaCosta and Klicka 2008; Smith and Klicka 2010; Leigh, O'Dea, and Vermeij 2014), the region itself plays an important role in generating biodiversity. The diverse elevational and climatic range of habitats across the isthmus provide opportunity for endemism (Stiles 1983; Barrantes 2009; Chavarría-Pizarro *et al.* 2010; Batista *et al.* 2020), but this alone may not be the only driving force behind avian diversity. Consistent with other recent work in the region (Miller *et al.* 2021), we find support for ecological factors strongly associated with cryptic diversity in lowland Panama in particular, emphasizing the need to look beyond biogeographic drivers of Neotropical biodiversity.

In this study, we find that the frequency of cryptic diversity in Panamanian resident landbirds is 18.8% (Table 1.2). This is higher than estimates from similar barcoding efforts, including the 2.7% of North American birds (Kerr *et al.* 2007), 11% of Korean birds (Yoo *et al.* 2006), 7.5% of Palearctic birds (Kerr *et al.* 2009), 3.3% of Argentinian birds (Kerr *et al.* 2009), and 3.6% of South American birds more generally (Tavares *et al.* 2011), although though due to variations in study designs, some caution is needed in directly comparing these without controlling for differences in sample size, taxonomic biases, and similar potential sources of variation between studies. The studies which found higher incidence of potential cryptic diversity tended to focus on narrow subsets of birds that may be particularly predisposed towards splits. For example, Milá *et al.* (2012) found evidence of interspecific-level variation within 33 of 40 forest understory birds in the Amazon, a habitat profile that we find to be significantly overrepresented in lineage with potential cryptic species in our study. However, many barcoding studies have tended to focus on large-scale questions of overall divergence, rather than explicitly examining whether specific ecological traits were over- or under-represented in taxa with potential splits (Yoo *et al.* 2006; Kerr *et al.* 2007, 2009; Kerr *et al.* 2009; Tavares *et al.* 2011).

Geographical patterns of cryptic diversity

We sampled widely across Panama and were able to test for the existence of several biogeographic patterns in cryptic diversity that have been previously proposed (Wetmore 1959; Summers *et al.* 1997; Anderson and Handley 2001; Miller *et al.* 2011; Kaviar, Shockey, and Sundberg 2012; Miller *et al.* 2015). Many of the taxa included samples from the various islands of Panama, including Isla Coiba in the Pacific and the Caribbean islands of San Cristobal, Bastimentos, Cayo Agua, and Escudo de Veraguas. Islands are a natural focus of investigation for undescribed biodiversity, and previous studies have found evidence for island endemics in both birds and other taxa in Panama (Summers *et al.* 1997; Anderson and Handley 2001; Kaviar, Shockey, and Sundberg 2012). Escudo, for example, has had four of its eight to ten resident breeding birds described as endemic subspecies (Wetmore 1959). The Escudo hummingbird (*Amazilia (tzacatl) handleyi*) is both phenotypically and genetically distinct from mainland populations (Miller *et al.* 2011) and is treated as a separate species by some (Wetmore 1968; Angehr and Dean 2010). However, it has also been observed that the islands of Panama, particularly in Bocas del Toro, are relatively close to the mainland (approximately 15 km for the furthest islands of Bocas del Toro, 20 km to Isla Escudo, and 25 km to Isla Coiba), and are likely to have been connected to it repeatedly due to Pleistocene sea level fluctuations (Miller *et al.* 2011). Escudo itself, as the furthest of the Caribbean islands, is estimated to have become isolated only around 9000 years ago (Summers *et al.* 1997; Miller *et al.* 2011). This lowers the probability of the development of distinctive island endemics (MacArthur and Wilson 2001; Mayr 1965). The majority of the island avifauna are also found on the mainland (Wetmore 1965, 1968, 1972; Wetmore, Pasquier, and Olson 1984; Ridgely and Gwynne 1989; Angehr and Dean 2010), suggesting that there has been a high degree of connectivity between them over time. Our findings are consistent with this, as only one taxon, *Setophaga petechia*, had a distinctive BIN on Isla Coiba, but this was likely an individual of the migratory subspecies rather than the resident, as both occur in Panama. While islands may be the source of haplotype diversity in some taxa (González *et al.* 2003), they are not sufficiently diverged to be split under the BIN system.

Another area with likely unrecognized diversity is the Pacific coast of Chiriquí, particularly the Burica Peninsula (Figure 1.1A). This region, separated from most of the rest of Panama by the Cordillera Central, receives markedly less precipitation than the rest of the country (Wetmore 1965), and is thus home to much more xeric ecosystems than the parts of the country subject to heavier rains from the Caribbean side (Blanco *et al.* 2013). In this case, we did find support for this region being a hotspot of unrecognized avian diversity, with six splits of the 41 being located in this relatively small area (Figure 1.3).

The final geographic pattern we observed, and by far the most common, was repeated differentiation between eastern and western Panama. These splits could be further subdivided into three general patterns: disjunct highland populations from the Cordillera Central and the highlands of eastern Panama, a suture zone in the Caribbean versant of Veraguas and Colón, and a second suture zone in Darién (Figure 1.1B).

The four highland splits are all between the two main highland regions of Panama (Figure 1.4). The predominant east-west split is consistent with the numerous species-pairs previously observed to follow this pattern (Wetmore 1965, 1968, 1972; Wetmore, Pasquier, and Olson 1984; Ridgely and Gwynne 1992; Angehr and Dean 2010). Likewise, the Azuero Peninsula has been previously noted for several potential endemic species (Miller *et al.* 2015). However, across our overall dataset, highland splits are very much in the minority of those observed, making up only 14.7% of splits.

This disproportionate representation of the lowlands in the taxa with cryptic species-level variation bears further examination. It is possible that this is an artifact of efforts to locate potential cryptic species tending to focus on taxa with disjunct ranges, so that most of the highland splits of equivalent depth to those we find in this study are more likely to already have been found and classified as separate species. Overall, few highland taxa met our geographic sampling criteria, representing only 15.1% of widely distributed species and 11.5% of those barcoded across multiple geographic regions

when they make up more than 30% of the total avifauna (Table 1.2), lending some support to the hypothesis that this is potential due to bias in prior taxonomic description of variation. But while this may go some way towards explaining the discrepancy in the rates of splits in lowland and highland taxa, it does not explain what may generate that lowland variation in the first place.

Many of Panama's lowland bird species are continuously distributed (Wetmore 1965, 1968, 1972; Wetmore, Pasquier, and Olson 1984; Ridgely and Gwynne 1992; Angehr and Dean 2010), unlike the largely disjunct ranges of highland species. This may not have historically been the case, though, as Pleistocene climate variation may have caused ranges to contract (Haffer 1969; Moritz *et al.* 2000; Weir, Bermingham, and Schluter 2009; Smith, Amei, and Klicka 2012; Lavinia *et al.* 2015). Most of the split bird species (85%) are forest birds (Table 1.2), and the increased aridity of the Pleistocene is thought to have caused widespread advancement of savannah into formerly forested areas throughout the tropics (Haffer 1985; Webb 1991) Thus, currently continuously distributed species may have not always been connected, and this climatic history may have driven the diversification of taxa across Panama. But it is unclear the extent to which forests actually contracted in Panama throughout the Pleistocene (Bush and Colinvaux 1990; Colinvaux, De Oliveira, and Bush 2000), and we find little support for the observed splits dating to even roughly similar time periods (Table 1.1). Clearly, while Panama's diverse landscape has played a role in generating its rich avian diversity, simple biogeographic and historic factors alone cannot explain this high rate of cryptic variation and mitochondrial turnover in lowland Panamanian birds.

The lack of strongly convergent timing in the split species (Figure 1.5) casts further doubt on simple biogeographic models. Previous work in Panama and lower Central America (LCA) more broadly has focused on the timing of the emergence of the isthmus (Smith and Klicka 2010), and in the resulting biotic interchange between North and South America (DaCosta and Klicka 2008; Weir, Bermingham, and Schluter 2009). However, it is unlikely that dispersal following the formation of the isthmus is responsible for the patterns of turnovers we see in our taxa. Our oldest splits (~4 mya) predate the 3

mya date generally accepted for the formation of the isthmus (Leigh, O'Dea, and Vermeij 2014), while the youngest (< 1 mya) are well after this time. However, a more gradual emergence of the isthmus would possibly better explain some of the splits (O'Dea *et al.* 2016). In particular, we find a group of splits in the Veraguas/Colón suture zone around the Pleistocene-Pliocene boundary (Figure 1.5), which line up with a potential episode of seawater breaching the newly formed isthmus approximately 2.45 mya (Groeneveld *et al.* 2014; O'Dea *et al.* 2016). While overall our dates do not support a scenario of dispersal prior to the formation of the isthmus, followed in most lowland cases by secondary contact, a more gradual process in which a changing landscape of newly emerged land acted as a tenuous bridge for dispersal over a longer period may be likely.

Ecological predictors of mitochondrial turnover

If biogeographic explanations alone cannot explain the generation of diversity in lowland Panamanian birds, what does? Our results highlight that ecological traits alone have the potential to drive divergence, even in a landscape that has historically lacked obvious barriers to dispersal (Bush and Colinvaux 1990). The landscape alone is not the only potential source of dispersal barriers, and we find evidence that limitations to dispersal from traits such as habitat use, diet, and morphology can be effective in generating diversity. We found that the majority of taxa with mitochondrial splits in Panama disproportionately shared specific ecological traits. Insectivores (68% of sampled taxa with splits vs. 47% non-split taxa), forest birds (85% vs 62%), understory foragers (74% vs 40%), and strongly territorial species (62% vs. 35%) were all overrepresented in lineages with mitochondrial turnover (Figure 1.4).

A potential explanation for at least some of these factors, particularly habitat and stratum use, is that they are more likely to have undescribed avian diversity not because they are more likely to create diversity, but simply that denser habitats would make it more difficult for human observers to observe and document any differences. Therefore, we must consider the possibility that the apparent

overabundance of cryptic types in understory forest birds is in fact a relative scarcity of human observations. Detectability has real ramifications for studying some groups, as has been covered particularly in the context of conservation (Clark and May 2002; Ducatez and Lefebvre 2014; McKenzie and Robertson 2015; Smith *et al.* 2020). This can also extend to taxonomic considerations, as more easily observed and "charismatic" organisms have been shown to be more likely to be oversplit (Pillon and Chase 2007). However, this does not explain all of the observed relationships, and the overall well-described nature of the Panamanian avifauna make it less likely than elsewhere in the Neotropics (Angehr and Dean 2010; Ridgely and Gwynne 1992; Wetmore 1965, 1968, 1972; Wetmore, Pasquier, and Olson 1984), and many splits within inconspicuous understory birds have been previously described (e.g., Saucier, Sánchez, and Carling 2015).

The traits observed to have a strong link to mitochondrial turnover share a common characteristic: they are all associated with low dispersal capability. This is most obvious in the much lower HWI of birds with mitochondrial splits. HWI is a well-recognized proxy of dispersal ability (Claramunt *et al.* 2012; Claramunt and Wright 2017; Sheard *et al.* 2020), as it describes wing shape and thus the ability for sustained flight (Kipp 1959; Lockwood, Swaddle, and Rayner 1998; White 2016; Claramunt and Wright 2017; Sheard *et al.* 2020), and the lower HWI of split species shows that lower dispersal ability is significantly associated with mitochondrial turnover.

This association of ecological factors linked to lower dispersal ability holds through other tested traits. Forest birds have much lower dispersal abilities than edge or open area species (Moore *et al.* 2008; Burney and Brumfield 2009; Weir, Bermingham, and Schluter 2009), especially those which primarily use the understory (Burney and Brumfield 2009; Woltmann and Sherry 2011). These species were disproportionately represented in the split taxa, demonstrating how these ecological factors can drive divergence. Likewise, strongly territorial species were overrepresented in the split taxa. As these species are less likely to disperse once they have established a territory (Greenwood 1980), this provides further weight to the role of dispersal.

Diet may at first glance seem less potentially linked with dispersal, but previous studies have found strong evidence that diet type, and especially the extent to which a given species relies on plant-based food sources, can shape dispersal and demography (Westcott and Graham 2000; Moore *et al.* 2008; Burney and Brumfield 2009; Miller *et al.* 2021). While both plant and animal food sources are typically available year-round in the tropics, seed, nectar, and fruit tend to be spatially and temporally clustered (Morton 1973; Levey and Stiles 1994). While insectivores in particular may reliably find arthropods in a given home range (Levey and Stiles 1992; Burney and Brumfield 2009), those feeding primarily on fruit, seeds, and nectar will by necessity need to travel more widely to seek out food sources throughout and between years (Westcott and Graham 2000). Furthermore, the relative availability of these resources varies between years to different extents. While arthropods are certainly subject to population cycles, they are usually less extreme (Jahn *et al.* 2010) than the fluctuations between mast years and lean years in fruit and seed-bearing species typically relied on for food by frugivorous and granivorous birds (Faaborg, Arendt, and Kaiser 1984; Wheelwright 1986; Levey, Moermond, and Denslow 1994; Brawn, Karr, and Nichols 1995; Ryder and Sillett 2016; Macario *et al.* 2017). As a result, birds which primarily feed on plants are more subject to boom-and-bust population dynamics (Faaborg, Arendt, and Kaiser 1984; Greenberg and Gradwohl 1986; Şekercioğlu *et al.* 2002; Woltmann and Sherry 2011; Sherry *et al.* 2020), and during boom years will experience increased dispersal, potentially connecting populations more regularly and slowing the accumulation of divergence between them.

The importance of traits directly or indirectly tied to dispersal ability may be considered at first glance to be driving a simple isolation-by-distance (IBD) effect. Poor dispersers will develop greater divergence across a given space than better dispersers, so that further populations will be increasingly genetically differentiated (Wright 1943, 1946; Slatkin 1993). However, while that may play a part for some of the taxa in our study, it is unlikely for all the observed splits. While some taxa, such as *Mionectes oleagineus* and *Baryphthengus martii*, have repeated mitochondrial breaks with increasing

divergence across Panama (Figure 1.4), others have sharp turnovers within Panama with equal or greater divergence estimates, yet these haplotypes are still found hundreds of kilometers away in Nicaragua (*Arremon auraniirostris*, *Cyanocompsa cyanoides*), Honduras (*Arremon aurantiirostris*), Belize (*Cyanocompsa cyanoides*), and Ecuador (*Cantorchilus nigricapillus*). Thus, it is likely that dispersal is the driver of divergence in concert with other ecological factors in many cases.

Conclusions

Panama is widely recognized as an area of high biodiversity; however, we find that avian diversity is significantly underestimated. Potential cryptic species are in some cases associated with landscape and geography, such as highland taxa and those in southwestern Chiriquí, but the bulk of the observed splits are in lowland taxa in the absence of geographic barriers. The varying ages of the observed splits, from approximately 0.75 - 4.2 mya, makes it unlikely that all the observed variation is driven by a single historical factor. We find instead strong correlations between dispersal ability, both directly (HWI) and indirectly (through ecological traits such as habitat, diet, and territoriality), and the occurrence of mitochondrial turnover. This sheds light on how intrinsic ecological and life history traits can be a major factor in driving species turnover and the accumulation of biodiversity in the tropics, and illustrates how examining cryptic species can provide insights into evolutionary processes that may be missed otherwise. The potential cryptic species we identify are good candidates for further sequencing across the nuclear genome, allowing us to explore the evolutionary processes more deeply in play. Overall, we demonstrate that barcode data is useful both for identifying drivers of divergence and in directing the focus of future genomic studies.

Acknowledgements

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Figures

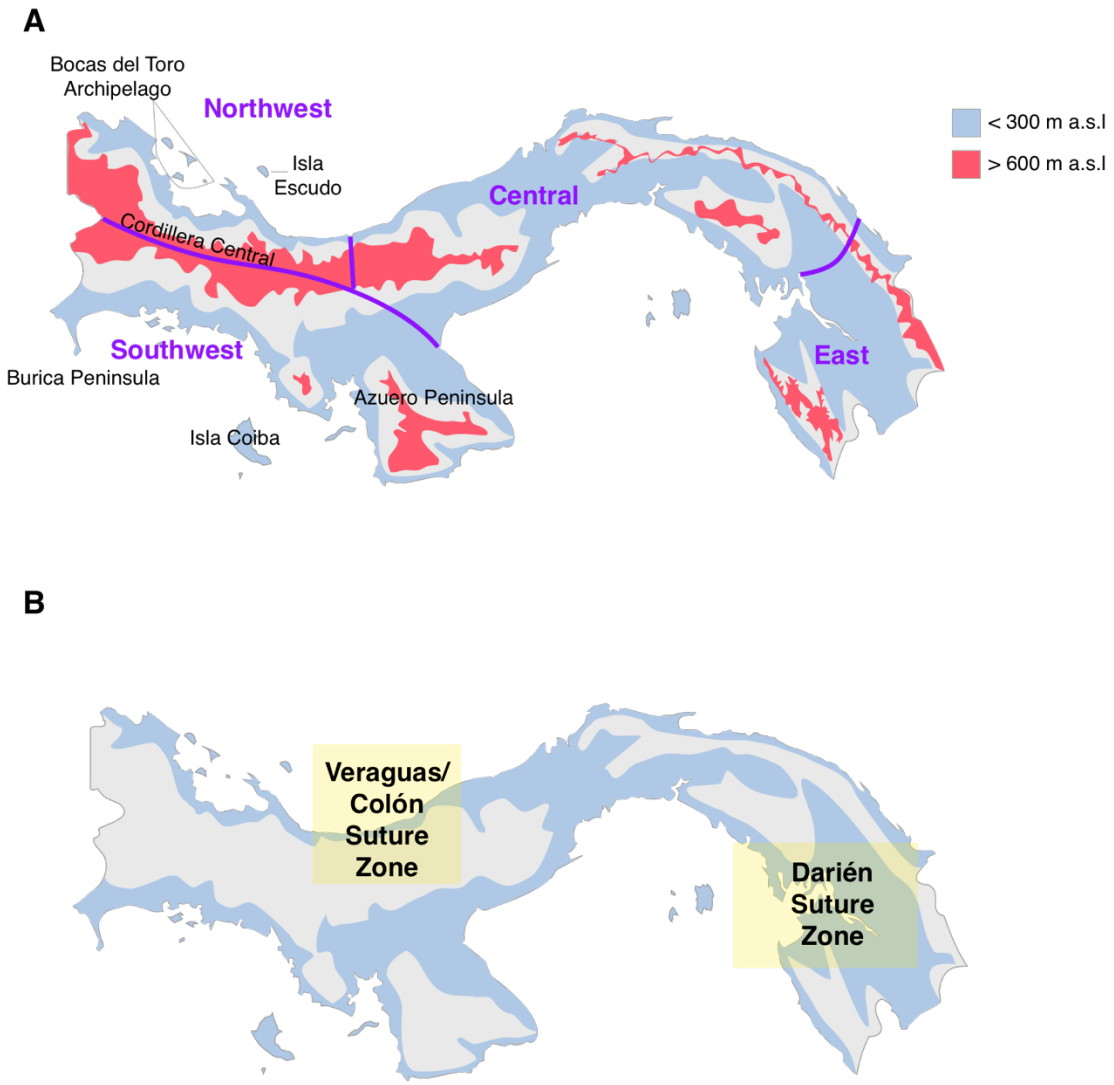


Figure 1.1: Map of Panama indicating major features considered in this study. A) Pink areas are highland regions with greater than 600 m.a.s.l elevation, while light blue show those with less than 300 m.a.s.l. Purple lines indicate the broad geographic areas used to define sampling, with names in purple.

B) Lowland suture zones. Light yellow shading indicates general locations of suture zones described in this study.

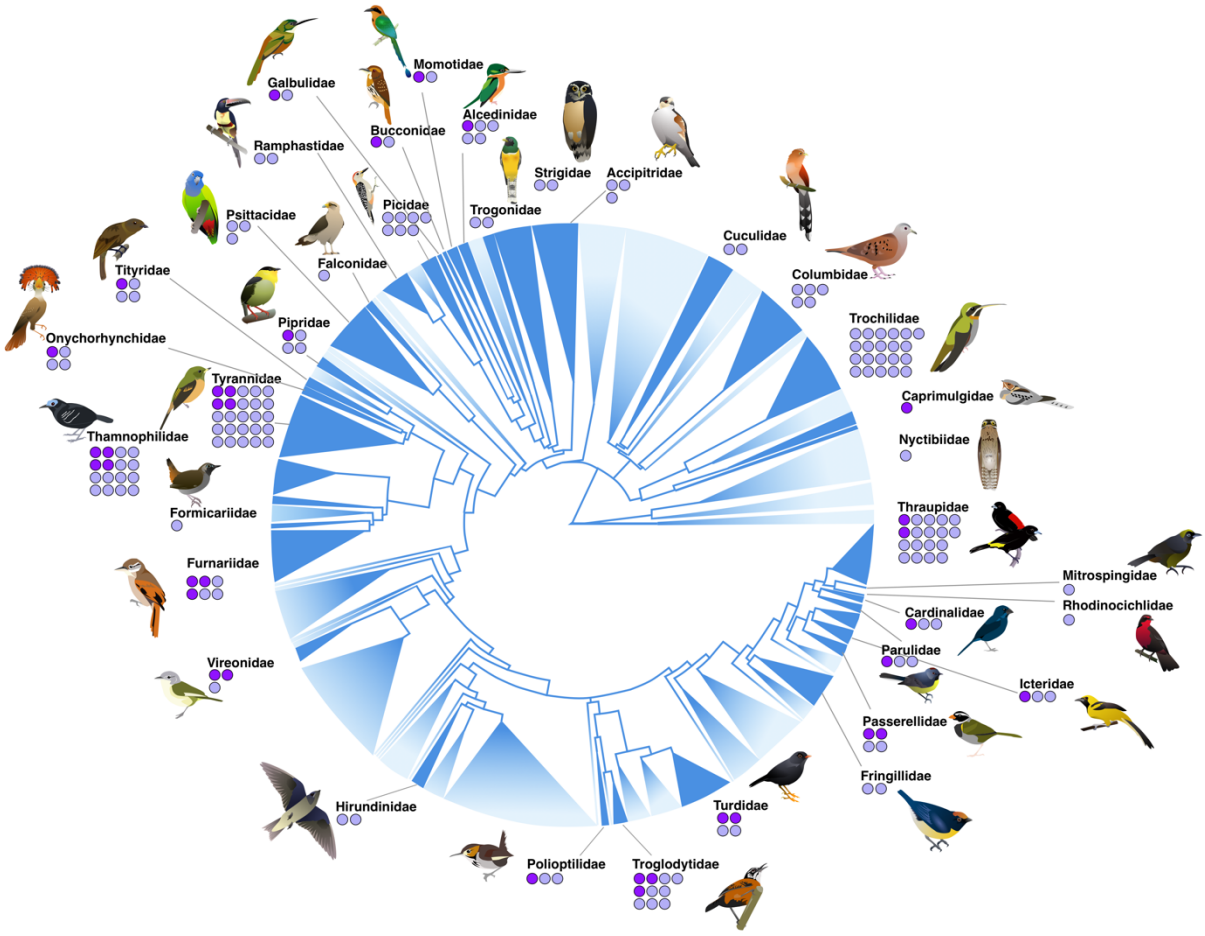


Figure 1.2: Phylogenetic sampling, with overall avian tree of life modified from Jetz *et al* (2012). Sampled families are shown in dark blue, with unsampled lineages shown with topology collapsed and faded out, but all maintain scaling by number of species. For each sampled family, dots indicate the number of taxa sampled across multiple regions of Panama. Light purple dots indicate lineages with a single mitochondrial BIN; dark purple indicate those with two or more.

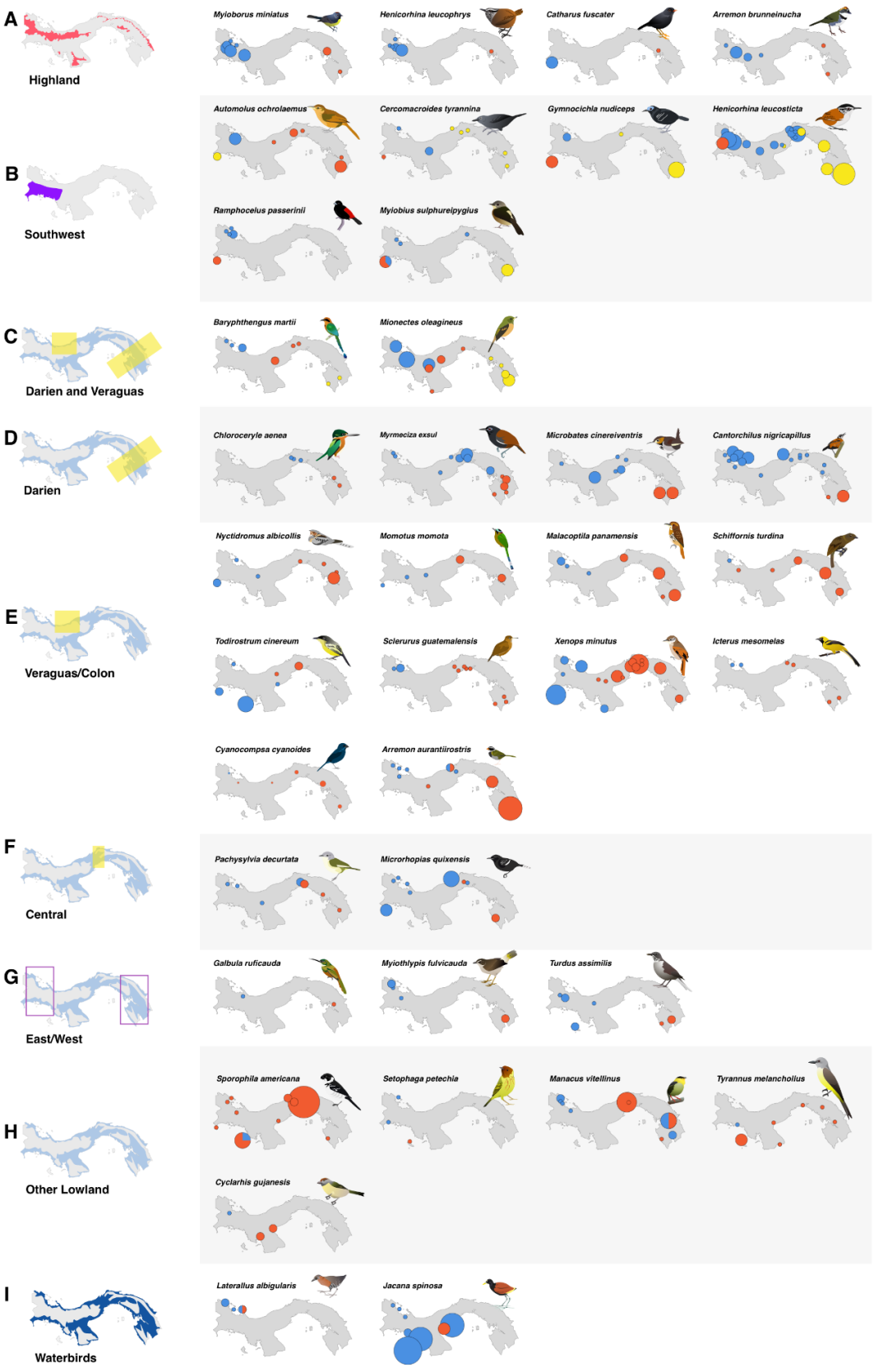

A
Highland
Myioborus miniatus
Henicorhina leucophrys
Catharus fuscater
Arremon brunneinucha
B
Southwest
Automolus ochrolaemus
Cercomacroides tyrannina
Gymnocichla nudiceps
Henicorhina leucosticta
Ramphocelus passerinii
Myiobius sulphureipygius
C
Darien and Veraguas
Baryphthengus martii
Mionectes oleagineus
D
Darien
Chloroceryle aenea
Myrmeciza exsul
Microbates cinereiventris
Cantorchilus nigricapillus
E
Veraguas/Colon
Nyctidromus albicollis
Momotus momota
Malacoptila panamensis
Schiffornis turdina
Todirostrum cinereum
Sclerurus guatemalensis
Xenops minutus
Icterus mesomelas
Cyanocompsa cyanoides
Arremon aurantiirostris
F
Central
Pachysylvia decurtata
Microrhopias quixensis
G
East/West
Galbula ruficauda
Myiothlypis fulvicauda
Turdus assimilis
H
Other Lowland
Sporophila americana
Setophaga petechia
Manacus vitellinus
Tyrannus melancholius
Cyclarhis gujanesis
I
Waterbirds
Laterallus albigularis
Jacana spinosa

Figure 1.3: Maps of the distribution of haplotypes (defined initially by BIN for COI data, and then by sequence for additional markers) for all taxa with observed mitochondrial breaks, grouped by geographic region of splits. Highland species are separated from lowland birds (A), and lowland species are displayed by (B) southwest vs rest of Panama, with or without additional splits; (C) splits in both the Veraguas and Darién suture zones; (D) Darién suture zone splits; (E) Veraguas/Colón splits; (F) splits in central Panama, typically around Cerro Azul; (G) lowland taxa which have distinctive haplotypes in east and west, but lack sufficient sampling across the transect to determine the precise locality of the turnover; (H) taxa with idiosyncratic patterns that fit none of the above; and (I) waterbirds, which were generally excluded from analyses due to less consistent sampling. Dot colors indicate distinct BINs, size scaled by the number of samples from a given locality.

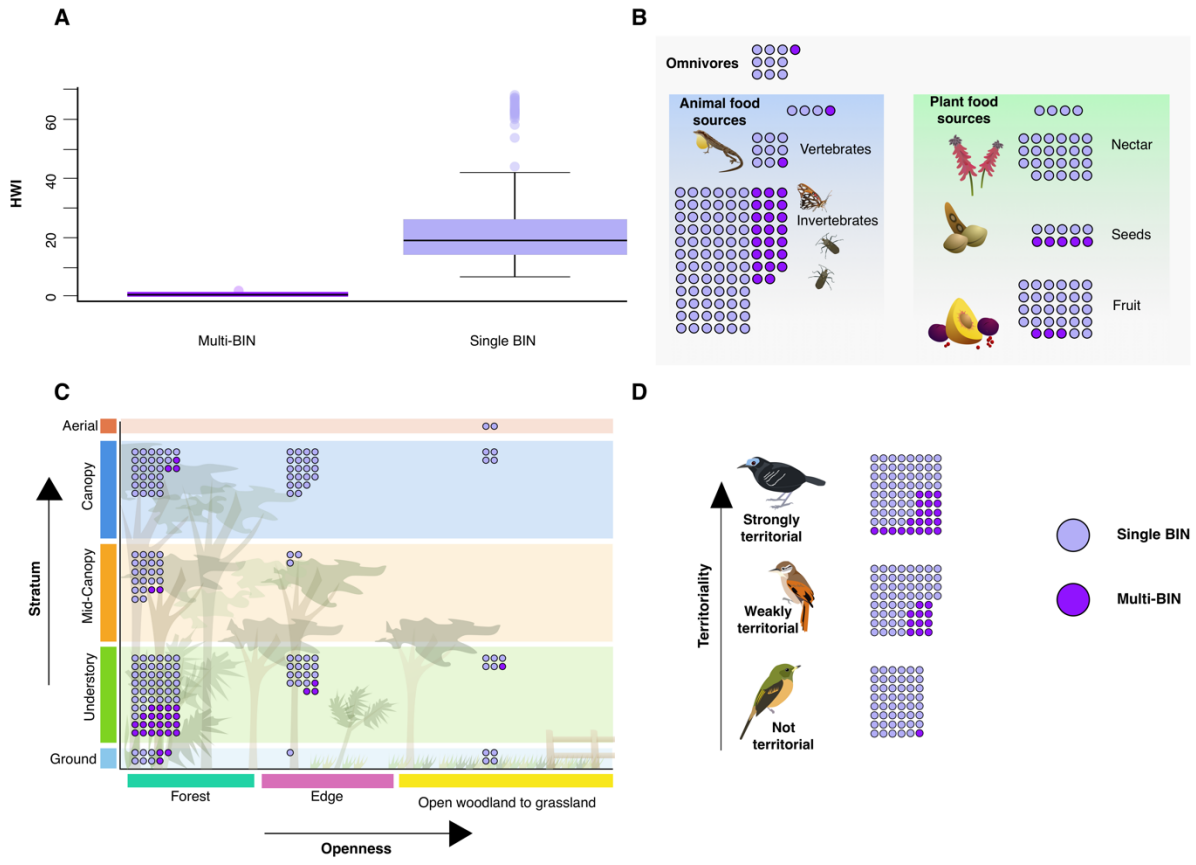
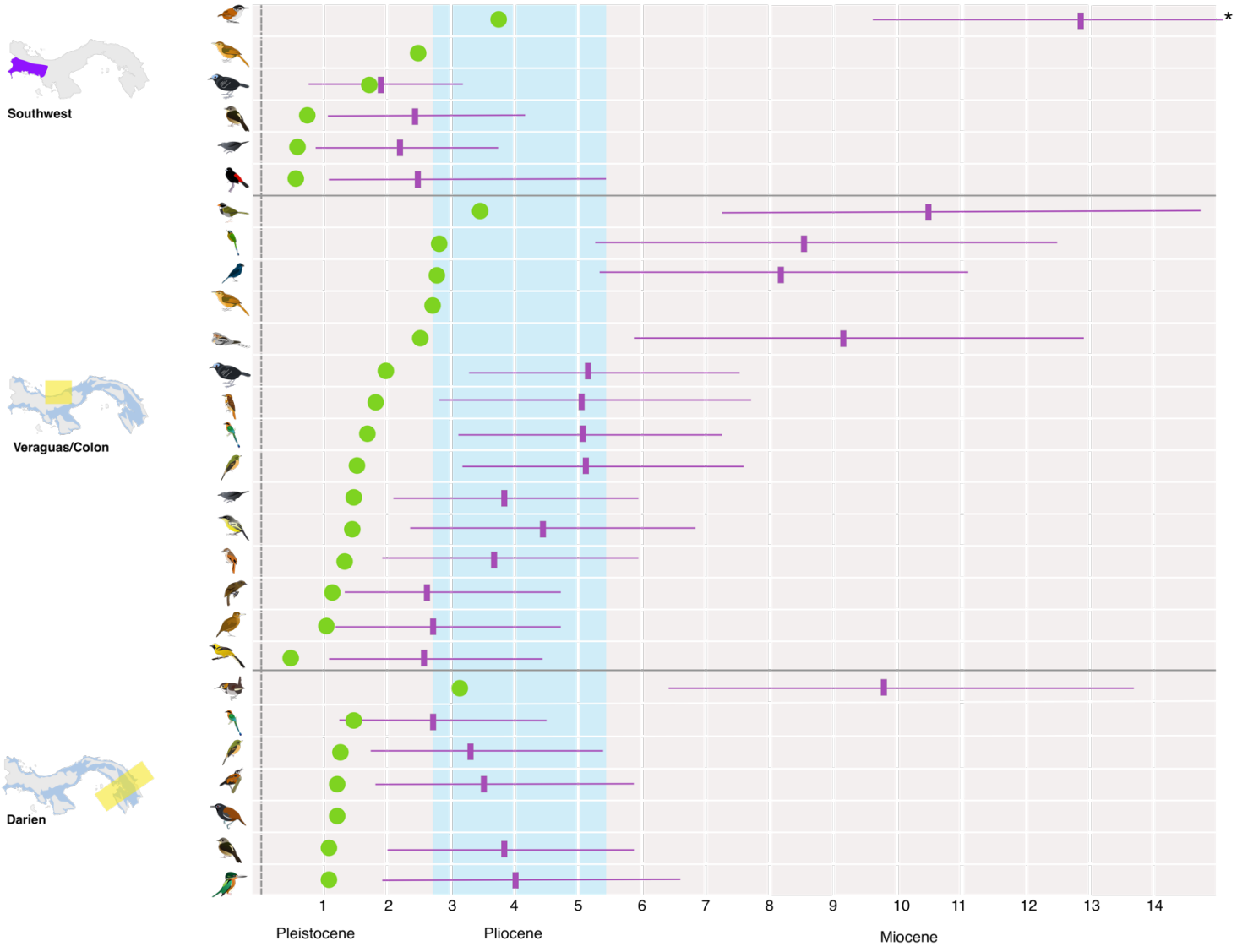


Figure 1.4: Associations of ecological traits with mitochondrial splits. Throughout the figure, dark purple circles represent taxa with two or more BINs, while light purple indicate those with only one. A) Hand wing index (HWI) is significantly lower in taxa with splits (t= -5.52, df- 154.29, p=1.43 × 10^{-7}), indicating lower physical dispersal capability is associated with mitochondrial turnover. B) Primary diet, showing that insectivores are significantly overrepresented (χ^2=17.27, df= 7, p=0.016) in taxa with splits. C) A visual representation of habitat use by split and non-split taxa, showing that habitat type as measured by openness (χ^2=6.488, df= 2, p=0.039) and stratum (χ^2=14.04, df= 4, p=0.0072) are associated with mitochondrial turnover, with it becoming increasingly likely in the closed forest understory. D) Despite the relatively even distribution of territoriality across our sample, strongly territorial taxa were overrepresented among those with mitochondrial turnover (χ^2=12.038, df= 2, p=0.0024).


Southwest
Veraguas/Colon
Darien
*
1
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4
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7
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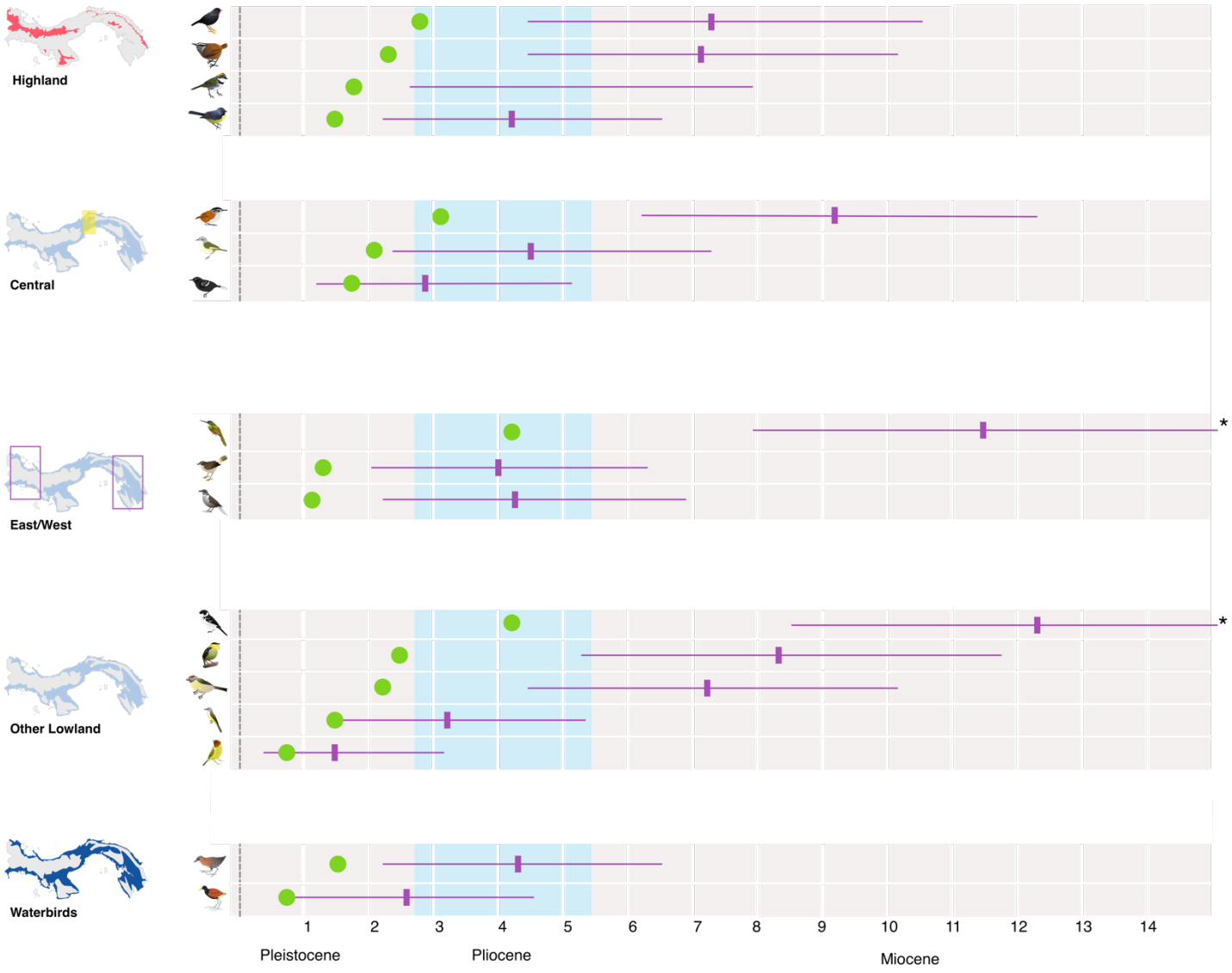
13
14
Pleistocene
Pliocene
Miocene


Highland
Central
East/West
Other Lowland
Waterbirds
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*
1
2
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4
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7
8
9
10
11
12
13
14
Pleistocene
Pliocene
Miocene

Figure 1.5: Timing of mitochondrial splits, with time calculated from pairwise COI divergence (green) and in BEAST2 (purple, shown with 95% confidence intervals). Splits are grouped by region as in Figure 1.3, with those taxa with multiple splits being shown for each. Some CIs are truncated due to space (indicated with asterisk); see Table 1.3 for full details.

Tables

Table 1.1: Species with COI splits defined as multiple BINs, with additional mitochondrial markers indicated. Distances calculated on BOLD-aligned sequences with K2P method. Species with more than two unique BIN assignments have pairwise COI divergence for all inter-BIN comparisons listed.

Species	Family	Pairwise COI divergence	Other included markers
Nyctidromus albicollis	Caprimulgidae	5.16%	cytB, ND2, whole mitogenome
Laterallus albigularis	Rallidae	3.20%	
Jacana spinosa	Jacanidae	1.72%	
Chloroceryle aenea	Alcedinidae	2.19%	ND2, whole mitogenome
Baryphthengus martii	Momotidae	3.48% 3.31% 3.35%	Whole mitogenome
Momotus momota	Momotidae	5.64%	Whole mitogenome

Malacoptila panamensis	Bucconidae	3.84%	ND3, whole mitogenome
Galbula ruficauda	Galbulidae	8.25%	cytB, whole mitogenome
Manacus vitellinus	Pipridae	5.08%	
Schiffornis turdina	Tityridae	2.20%	Whole mitogenome
Myiobius sulphureipygius	Onychorhynchidae	2.19%	
Tyrannus melancholicus	Tyrannidae	3.00%	
Cercomacroides tyrannina	Thamnophilidae	3.00%	
Todirostrum cinereum	Tyrannidae	2.87%	
Microrhopias quixensis	Thamnophilidae	2.51%	ND2, cytB, whole mitogenome
Gymnocichla nudiceps	Thamnophilidae	3.99%	ND2, cytB, whole mitogenome
Myrmeciza exsul	Thamnophilidae		ND2, cytB, whole mitogenome
Automolus ochrolaemus	Furnariidae	5.38%	cytB, whole mitogenome
Sclerurus guatemalensis	Furnariidae	2.08%	ND2, whole mitogenome

Xenops minutus	Furnariidae	2.77%	ND2, cytB, whole mitogenomes
Cyclarhis gujanensis	Vireonidae	4.66%	
Pachysylvia decurtata	Vireonidae	4.20%	ND2, whole mitogenome
Microbates cinereiventris	Polioptilidae	6.12%	ND2, whole mitogenome
Cantorchilus nigricapillus	Troglodytidae	2.51%	cytB, ATPase 8 and 6, whole mitogenome
Henicorhina leucosticta	Troglodytidae	6.13% 6.89% 7.61%	cytB, ATPase 8 and 6, ND2, whole mitogenome
Henicorhina leucophrys	Troglodytidae	4.63%	
Turdus assimilis	Turdidae	2.52%	
Catharus fuscater	Turdidae	5.67%	
Arremon aurantiirostris	Passerellidae	6.92%	ND2, whole mitogenome
Arremon brunneinucha	Passerellidae	3.49%	
Myiothlypis fulvicauda	Parulidae	2.86%	

Setophaga petechia	Parulidae	1.24%	
Myioborus miniatus	Parulidae	3.18%	
Icterus mesomelas	Icteridae	1.24%	
Cyanocompsa cyanoides	Cardinalidae	5.60%	ND2, cytB, whole mitogenome
Ramphocelus passerini	Thraupidae	1.65%	Whole mitogenomes
Sporophila americana	Thraupidae	8.49%	Whole mitogenomes

Table 1.2: Distribution of traits across Panamanian birds, showing across all 658 resident landbirds, those distributed across multiple regions, those barcoded (including both widespread and regional species), those resident landbirds with widespread distributions that were barcoded, and those within that last group found to have more than one BIN.

	All species	**Widely distributed**	**Barcoded (landbirds)**	**Sampled**	**Split**
Total	658	338	388	181	34
Highland	199 (30.2%)	55 (15.1%)	105 (27.1%)	20 (11.5%)	4 (11.8%)

	All species	**Widely distributed**	**Barcoded (landbirds)**	**Sampled**	**Split**
Habitat: Forest Edge Open	 446 (67.8%) 132 (20.1%) 76 (11.5%)	 224 (66.3%) 70 (20.7%) 44 (13.0%)	 275 (70.9%) 74 (19.1%) 38 (9.80%)	 121 (66.8%) 44 (24.3%) 16 (8.84%)	 29 (85.3%) 4 (11.8%) 1 (2.94%)
Stratum: Aerial Canopy Mid-canopy Undergrowth Ground	 25 (3.80%) 274 (41.6%) 82 (12.5%) 207 (31.5%) 69 (10.5%)	 17 (5.03%) 134 (39.6%) 47 (13.9%) 102 (30.2%) 38 (11.2%)	 6 (1.55%) 146 (37.6%) 47 (12.1%) 150 (38.6%) 38 (9.80%)	 2 (1.10%) 56 (30.9%) 25 (13.8%) 84 (46.4%) 14 (7.73%)	 0 4 (11.8%) 2 (5.89%) 25 (73.5%) 3 (8.82%)
Diet: Omnivore	 49 (7.45%)	 18 (5.32%)	 36 (9.28%)	 10 (5.52%)	 1 (2.94%)
Plant-based:	240 (36.5%)	106 (31.4%)	141 (36.3%)	66 (36.5%)	8 (23.5%)

	All species	**Widely distributed**	**Barcoded (landbirds)**	**Sampled**	**Split**
General plant-based	26 (3.95%)	11 (3.25%)	15 (3.87%)	4 (2.21%)	0
Nectarivore	60 (9.12%)	22 (6.51%)	42 (10.3%)	23 (12.7%)	0
Granivore	41 (6.23%)	18 (5.32%)	20 (5.15%)	10 (5.52%)	5 (14.7%)
Frugivore	113 (17.2%)	55 (16.3%)	64 (16.5%)	29 (16.0%)	3 (8.82%)
Animal-based:	357 (54.3%)	207 (61.2%)	207 (53.3%)	105 (58.0%)	25 (73.5%)
General animal-based	32 (4.86%)	25 (7.40%)	13 (3.35%)		1 (2.94%)
Insectivore	296 (45.0%)	154 (45.6%)	181 (46.6%)	4 (2.21%)	23 (67.6%)
Vertebrates	29 (4.41%)	28 (8.28%)	13 (3.35%)	92 (50.8%)	1 (2.94%)
				9 (4.97%)	

Table 1.3: Estimated divergence time as calculated in BEAST2 for the above 34 taxa.

Taxon	**Median divergence time (My), fossil calibrated**	**95% confidence interval (My), fossil calibrated**
Nyctidromus albicollis	9.10	5.72 – 12.97
Laterallus albigularis	4.27	2.25 – 6.59
Jacana spinosa	2.48	0.91 – 4.35
Chloroceryle aenea	3.99	1.93 – 6.55
Baryphthengus martii	2.76 5.05	1.36 – 4.44 3.03 – 7.39
Momotus momota	9.44	6.01 – 13.40
Malacoptila panamensis	5.07	2.77 – 7.82
Galbula ruficauda	11.66	7.95 – 15.89
Manacus vitellinus	8.28	5.20 – 11.71
Schiffornis turdina	2.76	1.26 – 4.73
Myiobius sulphureipygius	2.43 3.87	1.02 – 4.12 2.06 – 5.96
Mionectes oleagineus	3.42	1.84 – 5.39

Taxon	**Median divergence time (My), fossil calibrated**	**95% confidence interval (My), fossil calibrated**
	5.16	3.13 – 7.58
Tyrannus melancholicus	3.22	1.50 – 5.37
Cercomacroides tyrannina	2.15 3.88	0.86 – 3.72 2.07 – 6.11
Todirostrum cinereum	4.44	2.37 – 6.93
Microrhopias quixensis	2.92	1.25 – 5.11
Gymnocichla nudiceps	1.97 5.14	0.82 – 3.37 3.15 – 7.59
Automolus ochrolaemus	8.58	5.44 – 12.24
Sclerurus guatemalensis	2.78	1.14 – 4.80
Xenops minutus	3.72	1.89 – 5.99
Cyclarhis gujanensis	7.14	4.44 – 10.33

Taxon	**Median divergence time (My), fossil calibrated**	**95% confidence interval (My), fossil calibrated**
Pachysylvia decurtata	4.66	2.48 – 7.25
Microbates cinereiventris	9.82	6.40 – 13.52
Cantorchilus nigricapillus	3.58	1.79 – 5.93
Henicorhina leucosticta	9.06 12.93	6.22 – 12.38 9.66 – 16.64
Henicorhina leucophrys	7.09	4.42 – 10.13
Turdus assimilis	4.32	2.14 – 6.90
Catharus fuscater	7.27	4.43 – 10.46
Arremon aurantiirostris	10.49	7.31 – 13.94
Arremon brunneinucha	5.09	2.64 – 7.96

Taxon	**Median divergence time (My), fossil calibrated**	**95% confidence interval (My), fossil calibrated**
Myiothlypis fulvicauda	3.99	2.01 – 6.33
Setophaga petechia	1.64	0.53 – 3.15
Myioborus miniatus	4.19	2.20 – 6.52
Icterus mesomelas	2.61	1.10 – 4.49
Cyanocompsa cyanoides	8.23	5.31 – 11.52
Ramphocelus passerini	2.52	1.10 – 4.39
Sporophila americana	12.21	8.52 – 16.20

Chapter 2: Time in allopatry does not predict the outcome of secondary contact in lowland Panamanian birds

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Author Contributions

JFM conceived the study in consultation with MJM. JFM generated genomic libraries to supplement the original dataset, conducted all analyses, created original illustrations, and wrote the initial draft, with feedback from MJM on subsequent drafts.

Data Accessibility

Scripts, input files, and other associated files for analysis are available at https://github.com/jfmclaughlin92/panama_uce. Sequence data has been uploaded to the NCBI Short read archive (SRA).

Abstract

Geographic speciation models assume that time in allopatry should predict the degree of reproductive isolation (RI) between populations, but this has rarely been tested. Here we test the prediction that greater time in allopatry results in greater RI using comparative ultraconserved element (UCE) data from ten bird lineages in secondary contact in Panama. The best-fit models for the proportion of fixed Z-linked and autosomal loci to our data includes a combination of both time (as measured by

mtDNA divergence) and hand-wing index (HWI), emphasizing that the role of time is tempered by dispersal capability. Furthermore, time does not predict the extent of genome-wide introgression as measured by the median width of diagnostic loci clines or the degree of variation in cline centers or widths. These metrics of the outcome of secondary contact were best predicted by ecological and genomic factors, including diet, HWI, and genome-wide F_{ST} respectively, that are understood to serve as proxies for dispersal, the variability of population size, and overall genomic divergence. We find a primary role for ecological factors instead of isolation time in determining secondary contact outcomes for a lineage, highlighting how ecology shapes the development of RI.

Introduction

Historically, animal speciation has been understood to be driven in its early stages by population isolation in allopatry (Mayr 1942, 1963; Bush 1975; Lynch 1989; Otte and Endler 1989; Rosenzweig 1995; Barraclough and Vogler 2000; Coyne and Orr 2004). Mayr (1963) outlined the basic model of allopatric speciation that has remained largely unchanged for nearly 60 years, in which an ancestral species is divided into two allopatric populations by vicariance or dispersal, leading to isolation without gene flow. During their time in isolation, differences between the populations, phenotypic and genetic, accumulate. Subsequently, one or both populations experience range expansion, and the populations come into secondary contact, where speciation is completed. Later authors would expand on this, describing the outcome of secondary contact as a continuum (Wu 2001; Hey *et al.* 2003; Mallet *et al.* 2007; Nosil *et al.* 2009; Seehausen *et al.* 2014), from population fusion – also called reticulation or reverse speciation (Webb *et al.* 2011; Grant and Grant 2016; Kearns *et al.* 2018; Slager *et al.* 2020) – to complete reproductive isolation (RI) without any introgression (Cowles and Uy 2019; Merot *et al.* 2017). Intermediate along this continuum is the formation of a hybrid zone, with partial RI (Hendry *et al.* 2009). This current paradigm for animal speciation is the result of research demonstrating the pervasiveness of gene flow during the speciation process (Mallet 2005; Nosil 2008;

Mallet and Dasmahapatra 2012; Martin *et al.* 2013; Seehausen *et al.* 2014; Ottenburghs *et al.* 2017; Palacios *et al.* 2019; McLaughlin *et al.* 2020a).

Implicit in both Mayr's model and more modern interpretations that allow for gene flow is a central role for time in allopatry for driving differentiation and, eventually, speciation. As time increases, the likelihood of evolutionary changes occurring increases in parallel. Time allows for selection for local environmental conditions and stochastic changes through drift to take place (Orr 1995; Singhal and Moritz 2013). Orr and Turelli (2001) described the 'speciation clock' as the outcome of molecular evolution coupled with the accumulation of Dobzhansky-Muller incompatibilities. Genomic variations that arise and become fixed, such as chromosomal inversions (Bush *et al.* 1977), the mutations within them (Berdan *et al.* 2020), or mitonuclear incompatibilities (Hill 2016, 2017), occur at a largely consistent rate in similarly sized populations (Wright 1931), but as time in isolation passes, it becomes more likely that any one or more of them occur and accumulate. Over time, this accumulation would increase the likelihood of such changes leading to the development of RI. Experimental evidence demonstrates hybrid viability decreasing over time (Sasa *et al.* 1998; Tubaro and Lijtmaer 2002; Lijtmaer *et al.* 2003; Bolnick and Near 2005; Singhal and Moritz 2013; Dufresnes *et al.* 2015).

Time, therefore, makes intuitive sense as a predictor of where a lineage is in the process of speciation, and the expected extent of introgression in secondary contact. Indeed, there is some evidence from birds that supports this hypothesis. Studies of hybridization in birds have shown a relationship between time (measured by mitochondrial divergence) and the development of RI (Price and Bouvier 2002; Price 2008), with hybrid infertility and inviability becoming more likely with increased divergence time (Tubaro and Lijtmaer 2002; Lijtmaer *et al.*2003). Likewise, traits linked with prezygotic isolation also show this tendency (Winger and Bates 2015; Winger 2017), which is relevant given the general pattern in birds of prezygotic isolation arising before postzygotic (Edwards *et al.* 2005; Price 2008). From these observations, it is reasonable to predict that as time in isolation

increases, so will RI from both pre- and postzygotic mechanisms. Thus, in the context of secondary contact, the extent of introgression will decrease in tandem.

However, many factors could decouple time from the outcome of secondary contact and the development of RI. Birds, which tend to have higher rates of hybridization than many vertebrates (Fitzpatrick 2004; Price 2008), have been a useful group to study these factors. However, in many cases it has proved difficult to detect a reduction in fitness of hybrids in wild populations, and introgression even appears to be selected for in some cases (Gee 2004; Lipshutz *et al.* 2019). Some of this propensity for hybridization possibly arises from the nature of bird genomes themselves (Ellegren 2013). Bird genomes are small compared to other tetrapods (Bachmann *et al.* 1972; Tiersch and Wachtel 1991), likely driven by the metabolic demands of flight (Wright *et al.* 2014; Kapusta *et al.* 2017; Gregory 2002). This is then compounded by the relatively conserved synteny of avian chromosomes (Shetty *et al.* 1999; Ellegren 2010). As a result of these attributes, there are fewer structural variants which lead to postzygotic barriers seen in other taxa (Ellegren 2010). This likely provides an explanation for the tendency of RI in birds to be maintained by prezygotic barriers (Edwards *et al.* 2005), but it simultaneously informs why these barriers may be less effective in perpetuating that isolation. Postzygotic barriers are far more effective in limiting hybridization than assortative mating (Irwin 2020), and prezygotic barriers can be eroded by environmental changes (Nemeth *et al.* 2013) or even by sexual selection in systems such as manakins (Parsons *et al.* 1993; Brumfield and Braun 2001; Stein and Uy 2006; Parchman *et al.* 2013), fairywrens (Baldassarre and Webster 2013; Baldassarre *et al.* 2014), and jacanas (Lipshutz *et al.* 2019), where the phenotype of the courtship-dominant sex in one taxon is actually preferred by the other. These factors all mean that time may not be a predictor of the outcome of secondary contact.

We investigated this question using loci linked to ultraconserved elements (UCEs) in ten lineages of birds in secondary contact spanning a range of divergence dates across Panama. We tested several predictions that arise from the time-in-allopatry hypothesis. Firstly, we predict that the varying depths

of mitochondrial breaks, reflective of differing lengths of time in isolation, are correlated with the development of differentiation across the nuclear genome (Figure 1A). We expected that if this is the case, the proportion of loci fixed between the eastern and western Panama populations would be higher in taxa with a greater mitochondrial divergence. Lastly, in older taxa, the geographic extent of admixture will be reduced (Figure 1B), and that geographic clines should be narrower and more consistently located along the transect (Figure 1C).

Methods

Taxonomic and genetic sampling

The lowlands of Panama are an excellent system to test hypotheses about how time shapes the outcomes of secondary contact. Connected by largely continuous forest prior to the arrival of settler-colonial agricultural practices (Piperno *et al.* 1991; Bush *et al.* 1992) and lacking obvious geographic barriers, this region is nevertheless a notable suture zone where many sister taxa rapidly replace each other, with varying degrees of apparent hybridization (see Chapter 1). We focused on taxa that are found widely across this region and for which genetic resources from vouchered museum specimens were available (Table S1). We also considered preliminary data from mitochondrial barcoding (see Chapter 1) to include as wide a range of split depths as possible, along with diet, habitat, and family, to include an ecologically diverse sample of species. This resulted in a final dataset of ten lineages with mtDNA divergence between 2.75% and 9.83% (pairwise distance of all mt coding regions).

We examined loci associated with UCEs to measure clinal variation in secondary contact in hundreds or thousands of homologous loci, and to estimate the degree of genome-wide fixed variation between populations at terminal populations across transects. UCEs are useful for comparative studies, as they allow for robust sampling of directly comparable orthologous loci across large taxonomic divides (Faircloth *et al.* 2012; Smith *et al.* 2014; Harvey *et al.* 2016; McLaughlin *et al.* 2020a). For each of taxa,

we attempted to sample 3-4 individuals per population from populations along an east-west transect across lowland Panama, but in several cases were only able to include one or two individuals from a locality, resulting in a dataset of 180 individuals (Table S1). Preliminary analyses suggested that two species— *Cyanocompsa cyanoides* and *Arremon aurantiirostris*—had signals of admixture in the westernmost population sampled in northwest Panama. Therefore, for these species we sampled added populations from Nicaragua and Honduras for both species, and Belize for *Cy. cyanoides*. Similarly, we added samples from Ecuador in *Cantorchilus nigricapillus*, ensuring that all 10 species had purely parental populations in the westernmost and easternmost terminal populations.

We extracted genomic DNA from muscle tissue samples from vouchered museum specimens (Table S1) and prepared UCE-enriched libraries following Faircloth *et al.* (2012) and Glenn *et al.* (2019). For a few individuals, UCEs were harvested from whole-genome shotgun sequenced libraries. In both cases, libraries were prepared following the NEB Ultra II protocol. For the UCE-enriched libraries, we then followed the protocol from Glenn *et al* (2019) using the 5k Tetrapod set v.1 of 5,060 probes (Faircloth *et al.* 2012), while non-enriched samples proceeded directly to sequencing (Table S1).

Bioinformatics

We used either Trimmomatic v. 0.32 (Bolger *et al.* 2014; implemented in Illumiprocessor, Faircloth 2013) or BBDuk (part of BBMap v. 37.93; Bushnell 2014) for quality checking and adapter-trimming in raw reads, with the former being used for UCE-only reads as part of the original UCE pipeline and the latter for our whole genome reads. We generated a UCE loci-only reference for each taxon with *de novo* assemblies using Trinity v. 2.5.1 (Grabherr *et al.* 2011), filtering the assembly contigs to ensure that they contained only single-copy UCE loci without capture bycatch with phyluce (Faircloth 2016). Read number is not necessarily predictive of enrichment quality, so we generated a UCE assembly for all individuals, and selected the assembly with the most recovered UCE loci as the reference for that taxon. However, for three taxa, we had no UCE-enriched libraries. Assuming that

the number of total reads was proportional to UCE-derived reads in these samples, we selected the single individual with the greatest number of reads to generate a reference for that taxon. For that sample's trimmed reads, we filtered the UCE-derived reads using BBSplit (part of BBMap) and a concatenated reference derived from a set of closely related taxa as the BBSplit mapping target. For *Myrmeciza exsul* and *Schiffornis*, the three closely related taxa were *Xenops minutus, Henicorhina leucosticta*, and *Mionectes oleagineus* (developed for separate project); for *Ramphocelus*, we used *Pachysylvia decurtata*, *Cy. cyanoides*, and *A. aurantiirostris.* Using the BBSplit reads, we generated a final reference pseudo-genome for each species, as above.

To recover diploid genotypes for all UCE loci for all individuals per species, we followed the Genome Analysis Toolkit (GATK; McKenna *et al.* 2010) best practices for resequencing, making an alignment using all reads mapped to the reference using BWA. The alignment was indexed and cleaned using Samtools v. 1.7 (Li *et al.* 2009) and Picard v. 2.18.0 (Broad Institute 2019). Genotypes were generated in GATK v. 4.0.12 using HaplotypeCaller and GenotypeGVCF, generating a variant file per taxon. Using VCFTools v. 0.1.13 (Danecek *et al.* 2011), the variants were filtered to include only variants with biallelic SNPs present in all individuals with a minimum GQ of 10 and mean coverage depth of 10. This filtered dataset was used for window-based analyses of nucleotide diversity and genome-wide F_{ST}. We further thinned this dataset to include one SNP per locus by using the thin function of VCFTools, retaining the first 5' SNP for each locus. This became our canonical SNP dataset for each taxon for non-windowed analyses.

We also identified Z-linked loci to test how fixation rates differed between autosomal and sex-linked chromosomes. Z chromosome variants should be considered separately for three primary reasons. Firstly, its effective population size is only ¾ that of the autosomes, and thus under neutral conditions it will accumulate fixed differences at twice the rate of autosomes (Irwin 2018). Secondly, the Z chromosome has been indicated to disproportionately be the site of loci responsible for the development of reproductive barriers in many studies of avian speciation (Backström *et al.* 2010;

Storchová *et al.* 2010; Ellegren 2013; Lavretsky *et al.* 2015, 2019), making separate consideration of them important. Finally, Z chromosomes are likely to be the sites of incompatibilities as predicted by Haldane's rule -- if hybrid fitness is lower in one sex than the other, it will be in the heterogametic sex (Haldane 1922; Laurie 1997; Coyne and Orr 2004; Price 2008), possibly as incompatibilities arise between the different sex chromosomes (Coyne 1985), between the Z chromosome and autosomes (Johnson and Lachance 2012), or between the Z chromosome and mitochondrial genomes (Trier *et al.* 2014). The master UCE probe sequence list (Faircloth *et al.* 2012) was matched to the *Taeniopygia guttata* genome (GCA_008822105.2) with BLAST (Zhang *et al.* 2000) to generate a list of UCE loci located on the Z chromosome, as chromosomal synteny in birds is relatively conserved (Ellegren 2010; Shetty *et al.*1999; Ellegren 2013). This list was then used to reference against per-site F_{ST} to determine autosomal versus Z-linked fixation.

Determining the mitochondrial haplotype

We assembled mitochondrial genomes for both the eastern and western parental populations in each with NOVOplasty v. 3.4 (Dierckxsens *et al.* 2017). We then determined haplotype per individual as detailed in Chapter 1.

Population structure analyses

To verify that each species included just two parental genotypes across the study area, to ensure that each parental genotype was adequately sampled, and to provide an initial assessment of population structure, for each species we conducted PCA and DAPC in the R package adegenet v. 2.1.3 (Jombart and Ahmed 2011). We determined the number of clusters by minimizing the value of the Bayesian Information Criterion (BIC), and individuals were assigned to these clusters. We used STRUCTURE v2.3.4 (Pritchard *et al.* 2000) to provide an additional assessment of population structure, and to assign individuals as either the western or eastern parental genotypes or as admixed between the two

We ran STRUCTURE for 30 replicates for each of K = 2–4. We combined all replicates for each K using CLUMPP v. 1.12 (Jakobsson and Rosenberg 2007). We confirmed the best value of K as 2 in STRUCTURE HARVESTER v. 0.6.94 (Earl and vonHoldt 2012) using the Evanno method (Evanno *et al.* 2005), and plotted results with distruct (Rosenberg *et al.* 2002).

Geographic cline width analysis

Variation in geographic cline width and center provides information about the permeability of genomes to introgression. More permeable genomes are expected to show greater cline width and have a wider span of cline centers; narrow cline width and tightly coincident cline centers are a signature of RI (Barton 1979; Szymura and Barton 1986; Richard G. Harrison 1990; Szymura and Barton 1991; Derryberry *et al.* 2014; Rieseberg *et al.* 1999). We generated geographic clines as follows: we filtered our SNP datasets to include only diagnostic loci (Lipshutz *et al.* 2019), defined as loci where the westernmost populations had a combined allele frequency for genotype p of at least 0.75, while the combined allele frequency in the easternmost populations was no more than 0.25 for p. We generated geographic clines for these diagnostic loci using the R package hzar (Derryberry *et al.* 2014), testing 3 cline models per locus using a custom script (https://github.com/jfmclaughlin92/panama_uce). Using the best-fit model for each locus, we calculated median cline center and width for each taxon, as well as the set of cline width and center values that represent 95% of the observed variation. We then plotted all best-fit clines together for each taxon, calculating the median width and the variance of the width and center across all loci.

Statistics and prediction testing

We obtained counts of fixed SNPs using VCFTOOLS, including across all loci, autosomal sites only, and Z-linked loci only, as determined by alignment of the UCE probe sequences to the zebra finch genome. We then constructed a general linear model for each response parameter. In addition to

mitochondrial distance, we also included variables to test for how other factors contribute to shaping the outcomes of secondary contact. We included hand-wing index (HWI), a quantification of the aspect ratio of a bird's wing that is an indicator of dispersal ability, a known driver of divergence in Neotropical birds (Claramunt *et al.* 2012). We additionally included diet, coded as plant-based (frugivores and granivores) or insectivorous. These diet types are linked to differing dispersal capabilities (Miller *et al.* 2021) and characteristic demographic patterns (Morton 1973; Karr 1976; Levey and Stiles 1994; Westcott and Graham 2000). Finally, weighted genome-wide F_{ST} was included as a generalized measurement of genomic differentiation, and π for overall nucleotide diversity. These were calculated from only the parental populations, and clines were not calculated from them in such a way to make them autocorrelated. For cline parameters (median width, variance in width, and variance in center), these were F_{ST}, mitochondrial distance, HWI (Sheard *et al.* 2020), and diet. For autosomal and Z-linked fixation rates, we excluded F_{ST}, as that would be auto-correlated with the response variables. We tested which error structure best described each dataset, and from there constructed generalized linear models (GLMs) using all combinations of the explanatory variables. From these models produced for each response variable, we then used AICc to select the best model.

Results

Sequencing results

We sequenced between 0.22 and 6.5 million reads for each UCE-enriched sample and between 40.4 and 229.6 million reads for WGS samples (Table S1). From this, we recovered between 1855 and 4292 loci per taxon, with an average coverage of 39.8× (Table S1). Overall, coverage from non-enriched and enriched libraries was similar, but slightly higher in non-enriched samples (48.2x vs 37.1x). However, several unenriched samples were dropped due to low numbers of recovered UCE loci (between 500-1000 loci), demonstrating that while UCEs can be recovered with ease from WGS reads when circumstances call for such an approach, it is less reliable.

Mitochondrial divergence and fixation rates

Pairwise divergence of all mitochondrial protein-coding regions ranged from 2.75% (*X. minutus*) to 9.83% (*Schiffornis*) (Table 1). F_{ST} of UCE-linked loci ranged from 0.265 (*Cy. cyanoides*) and 0.688 (*Schiffornis*), and nucleotide diversity (π) between 0.00195 (*P. decurtata*) and 0.00298 (*H. leucosticta*; Table 1). The total proportion of fixed SNPs in these loci was between 0.0126 (*Ca. nigricapillus*) and 0.226 (*Schiffornis*), with autosomal fixation ranging from 0.0105 (*Ca. nigricapillus*) to 0.220 (*Schiffornis*) and Z-linked fixation ranging from 0.0221 (*P. decurtata*) to 0.480 (*Malacoptila panamensis*; Table 1). While average divergence in insectivores was lower than in birds reliant on plant foods, 4.07% vs 5.92%, the difference was not significant (t = -1.50, df = 6.57, p = 0.180).

Population structure and admixture detection

In all taxa, the Evanno method indicated that K=2 provided the best fit for our STRUCTURE results. Individuals with admixture proportions greater than 1% were detected in seven of the ten taxa, including all taxa considered conspecific across Panama except *Ma. panamensis* (Figure 2). When observed, the geographic extent of admixture varied widely (Figure 2) from admixed individuals occurring across our sampling transect in Panama, such as in *A. aurantiirostris*, occurring across nearly 350 km, to being confined to a roughly 5 km span between Cerro Azul and Cerro Jefe in central Panama, as seen in *H. leucosticta*.

Mitochondrial and nuclear mismatch

Mismatch between mitochondrial haplotype and nuclear genotype –defined as having the mitochondrial haplotype of one parental population with a nuclear genotype above 50% derived from the other parental population – was limited to three lineages (Figure 2). In *P. decurtata*, two

individuals near the median cline center for that lineage had haplotypes characteristic of the parental population that was less than 50% of the nuclear genome. In *Ca. nigricapillus*, samples that had nuclear genotypes almost completely assignable to the Darién group nevertheless had the western mitochondrial haplotype. Again, this occurred very close to the median clinal center, and at one locality included only two of the three birds from the location. Finally, the central Panama localities for *A. aurantiirostris* were admixed with the greater assignment probability to the eastern Darién population, but with one exception, had the western haplotype.

Cline analysis

For both geographic and genomic cline analyses, our reduced diagnostic-only SNP datasets-- SNPs with a frequency of one genotype of 0.75 or more in one parental population and of 0.25 or less in the other-- included between 72 (*A. aurantiirostris*) and 717 (*Ma. panamensis*) SNPs (Table 2). Median width varied from 2.4 km (*Ma. panamensis*) to 373.6 km (*A. aurantiirostris*; Table 2; Figure 2). Within each taxon, the widths best fit a Poisson distribution (Figure S1), with most cline widths very narrow and with a long tail of wider clines and the overall proportion of the loci in this tail varying across taxa. The median cline centers were likewise wide ranging (Figure 2C), but normally distributed (Figure S2). When distance in kilometers from the beginning of a taxon's transect was converted back into degrees longitude, this estimate ranged between -78.22 and -81.63 degrees, corresponding approximately with the western edge of the Valiente Peninsula and the western edge of Darién province, respectively (Figure 2C). Within this area, two clusters of cline centers were observed. The first, including *Schiffornis*, *My. exsul,* and *Ca. nigricapillus*, occur near the border of Darién, with the three occurring very closely together. The other, less tightly clustered, group, occurs along the Caribbean coast of Veraguas, and includes *Ramphocelus*, *Ma. panamensis, X. minutus*, and *Cy. cyanoides.*

Prediction testing

The best-fit models for our five response variables (median cline width, variance in single-locus clinal width, clinal center variance, autosomal fixation rate, and Z-chromosome fixation rate) varied. When we fit models for Z-chromosome and autosomal fixation rates, we found that mitochondrial distance and HWI were the best predictors in both cases (Table 3). For the primary cline variable-- the median width of clines across all loci-- diet alone was the best predictor, with weaker support for other models (Table 3). This was a weakly statistically supported relationship (t = -2.2662, df = 5.3811, p = 0.06904; Figure 3C), likely due to the overall small number of taxa included, but it is enough to say with reasonable certainty that insectivores have smaller median cline widths (mean = 40.887 km) than birds reliant on plant diets (mean = 178.35 km). For variance in clinal center (i.e., spatial coincidence of the centers of hybrid zones), HWI was the best predictor, although moderate support was also found for the following predictors, in order of increasing delta AICc: F_{ST} alone, mitochondrial distance + HWI, mitochondrial distance alone, and π + HWI (Table 3). This relationship was moderately statistically supported (Adj R^2 = 0.2995, p = 0.05884; Figure 3A). For variance in clinal width (i.e., how much the spatial extent of hybridization varied), F_{ST} alone was the best predictor, with moderate support also found for $\pi + F_{ST}$. However, this was not significant, with weak support found for the linear relationship between the two (Adj R^2 = 0.0719, p = 0.3323; Figure 3B). While these results were weakly significantly significant, much of this is likely due to the small number of taxa included relative to the variation, and though not definitive, is still indicative of a biologically significant relationship between these variables that bears further investigation with more taxa.

Discussion

Time plays a role in predicting the accumulation of differentiation between populations. Yet time alone does not, in lowland Panama, tell the full story of how populations in secondary contact respond. Despite time often being implicitly assumed to play a key role in driving the development of

RI in incipient species, our results show that time alone is not a predictor of these outcome of secondary contact in lowland Panama. It certainly plays a role in predicting the accumulation of fixed loci on both the autosomes and Z chromosome, supporting the mechanism of time allowing for the development of variation within isolated populations. Yet genomic differentiation alone does not necessarily indicate the development of RI, and for our most direct measure of the development of RI-- median cline width-- time played no role as a predictor, indicating a disconnect between the mere accumulation of genomic variation and the development of differences that could lead to speciation.

Predictors of geographic cline width and stability

Our most striking result was the robust recovery of diet alone as the best predictor of median cline width, placing ecological factors as key in determining the outcomes of secondary contact. Taxa that relied on plant-based foods, primarily seeds and/or fruit, had significantly wider clines than insectivores. Diet differences have been shown to be significantly linked with differences in dispersal ability in Neotropical birds (Westcott and Graham 2000; Moore *et al.* 2008). Seeds and fruit are available year-round, but not necessarily in the same place simultaneously (Morton 1973; Levey *et al.* 1994). This results in birds reliant on such resources needing to disperse more widely (Westcott and Graham 2000). Meanwhile, insectivores, especially the understory insectivores examined here, can generally rely on a steady supply of food items year-round within a small area, and consequently are much more sedentary (Levey and Stiles 1992; Burney and Brumfield 2009) and have smaller home ranges and are often very weak fliers (Moore *et al.* 2008).

However, this link to dispersal ability is unlikely to be the sole reason for the strong relationship observed between diet and cline width. HWI, which reflects the morphological constraints on flight ability (Lockwood *et al.* 1998; Kipp 1959) and more directly measures dispersal ability (Burney and Brumfield 2009; Claramunt *et al.* 2012; Weeks and Claramunt 2014; Kennedy *et al.* 2016; Chua *et al.* 2017; Claramunt and Wright 2017; Pigot *et al.* 2018; Sheard *et al.* 2020), was not a predictor, so we

must consider other demographic traits associated with diet that may be more influential in determining cline width. Notably, birds reliant on fruits and seeds have much wider variation in population sizes. Availability of these resources varies not just across a single year, but between years, with individual trees varying in fruit and seed production across multiple years (Wheelwright 1986), driving more pronounced cycles in population highs and lows in birds dependent on them (Faaborg *et al.* 1984; Levey *et al.* 1994; Brawn *et al.* 1995; Ryder and Sillett 2016; Macario *et al.* 2017). While arthropods are also subject to declines, such cycles are generally relatively less drastic and usually specific to certain prey species (Jahn *et al.* 2010). Because of this, Neotropical understory insectivores are observed to have far less year-to-year demographic fluctuation (Greenberg and Gradwohl 1986; Faaborg *et al.* 1984; Şekercioğlu *et al.* 2002; Woltmann and Sherry 2011; Sherry *et al.* 2020). Thus, possibly the differences in demography among birds dependent on plant-based foods and those that primarily subsist on arthropods drive these patterns, although the limitations with UCE-linked loci make it difficult to test directly for historical demographic fluctuations.

Cline width alone is not the only indicator of the outcomes of secondary contact examined here. The coincidence of clines, as measured by the variation in cline center and width, is a measure of how spatially consistent the geographic center and extent of turnover will be across loci. Again, this was not predicted by time. The variance in cline center was best predicted by HWI alone. This is of interest because while dispersal ability as measured via the most accepted proxy was not important in determining the width of the hybrid zone, it is the best predictor behind how geographically concentrated introgression is. However, variance in width-- or how much variation in how far a locus will introgress across the transect-- was predicted by neither time nor ecological factors, but by F_{ST}. This suggests that as overall genomic divergence between parental types increases, differential introgression of loci decreases, an observation corroborated by observations of decreased hybridization with greater genetic divergence in other systems (Montanari *et al.* 2014).

The development of RI is a key step in the process of speciation, but it can be difficult to disentangle outside of controlled model systems, making hybrid zones an important opportunity to gain insight into the strength of RI in non-model organisms (Barton and Hewitt 1985; Hewitt 1988; Mallet and Dasmahapatra 2012). Cline analyses can be powerful tools in gaining insight into how RI develops in the early stages of speciation (Szymura and Barton 1986, 1991; Derryberry *et al.* 2014). The geographic range of hybridization can be informative of the extent of RI in a pair of incipient species, as it reflects the extent to which introgressed loci are selected against (Szymura and Barton 1986; Derryberry *et al.* 2014). Thus, median cline width across loci is a key measurement of the extent of RI.

Geography of hybridization in lowland Panama

Of our ten taxa, seven of them had median cline centers in one of two narrow regions (Figure 2C). Three of the taxa-- *Schiffornis, My. exsul,* and *Ca. nigricapillus--* had median cline centers estimated in western Darién, which also corresponded with where they experienced mitochondrial turnover. This corresponds to previous findings that this region represents a major suture zone between Central and South American taxa (Chapman 1917). The second cluster, consisting of the *Ramphocelus* tanagers, *Ma. panamensis, X. minutus,* and *Cy. cyanoides*, is located along the Caribbean coast of Veraguas. This region, whose avifauna has been much less well-documented than much of Panama (Wetmore 1965, 1968, 1972; Wetmore *et al.* 1984; Ridgely and Gwynne 1992; Siegel and Olson 2008; Angehr and Dean 2010), appears to be an important suture zone (McLaughlin *et al.* 2020b). In both cases, though, it is notable what they each lack: major biogeographic barriers that would easily explain the occurrence of such rapid turnover, a feature observed in other Neotropical lowland contact zones (Lovette 2004; Cheviron *et al.* 2005; Vázquez-Miranda *et al.* 2009; Miller *et al.* 2011; Milá *et al.* 2012; Miller *et al.* 2021). Because of the apparent absence of barriers, these suture zones provide promising windows into how Neotropical diversity is shaped not by the landscape alone, but by traits inherent to ecological characteristics of the taxa in contact.

Predictors of genomic differentiation

We found that time plays a role in generating variation across the genome, particularly the autosomes. This is consistent with predictions of neutral theory (Kimura 1968; Kimura 1983; Gillespie 2004), as most of the genome accumulates variation at a predictable rate (Kimura and Ota 1971; Bromham and Penny 2003). But just as there are fluctuations in the molecular clock that erode any simple link between the accumulation of mutations and time (Ohta 1992; Bromham and Penny 2003; Weir and Schluter 2008), the relationship between time and fixation rate is also less straightforward. While time was a significant predictor of autosomal fixation in our best-fit model, HWI was as well. The impact of time is clearly important-- in addition to being a predictor in our best-fit model, time alone was the second best-fit model for autosomal fixation rate (Table 3) -- but in this system, the relationship between the time and fixation is modulated by the dispersal capability. This is consistent with previous findings that high dispersal ability can hamper divergence in tropical birds (Claramunt *et al.* 2012; Weeks and Claramunt 2014) but shows that this also extends to the accumulation of neutral variation.

The Z-chromosome is often the focus of searches for loci driving the development of RI (Qvarnström and Bailey 2009; Backström *et al.* 2010; Storchová *et al.* 2010; Ellegren 2013; Irwin 2018). The accumulation of fixed differences on the sex chromosomes has implications for the development of RI (Wright 1933; Charlesworth *et al.* 1987; Ellegren 2013; Irwin 2018), as they can lead to incompatibilities (Ellegren 2013; Hooper *et al.* 2019) or be linked to traits involved in male-biased (in ZW systems) sexual selection or conflict (Rice 1987; Kaiser and Ellegren 2006; Storchová and Divina 2006). We found that while time was a predictor of Z-chromosome differentiation, it was not significant, while the other included predictor, HWI, was. Dispersal ability, of which HWI is a well-established proxy (Burney and Brumfield 2009; Claramunt *et al.* 2012; Sheard *et al.* 2020; Weeks and Claramunt 2014), then, appears to play a role in driving divergence in the specific portion of the genome most frequently implicated in speciation in birds (Irwin 2018; Lavretsky *et al.* 2015; Ellegren 2013). As dispersal ability has been previously shown to play a major role in shaping hybrid zone

dynamics (McEntee *et al.* 2020), this is not unexpected. However, it is notable that this was the only response variable for which dispersal ability in itself, as measured with HWI, is a predictor.

Time drives the generation of variation, but not of RI

Our results confirm that time does play a role in the development of genomic differentiation, those differences do not necessarily indicate the development of RI. Disentangling how genomic variation, phenotypic differences, and reproductive barriers are intertwined can prove challenging. Previous work in birds, including wood-warblers (Toews *et al.* 2016), hummingbirds (Palacios *et al.* 2019), and seedeaters (Campagna *et al.* 2017) show that remarkably little genomic variation can nevertheless result in striking phenotypic variation. Yet most such examples recover evidence of substantial gene flow that indicate that such differences should not in and of themselves be taken as evidence for RI, which has been backed up in other systems where overall genetic differentiation has not necessarily been indicative of the extent of reproductive barriers (Edmands 2002; Hogner *et al.* 2012; but see Dufresnes *et al.* 2021 for counterexample).

Lessons from secondary contact on the formation of species

Hybrid zones have long been acknowledged as a powerful window into the evolutionary mechanisms driving speciation (Barton and Hewitt 1985; Hewitt 1988; Hewitt 2001; Barton and Hewitt 1989; Gompert *et al.* 2017). Regions like lowland Panama, where hybrid zones in multiple taxa co-occur, have even greater potential for investigation of broader questions about the speciation process. However, many such studies tend to focus on questions of how landscape factors across time have impacted divergence in large suites of organisms (McLaughlin *et al.* 2020a). Lowland Panama provides an important setting for a different set of questions. As the range of split times suggests no single historic factor and the region lacks the strong geographic barriers characteristic of other classic suture

zones, the spotlight can instead be shifted onto the inherent traits of the organisms themselves. Our results drive home the necessity of considering ecological factors such as demographic stability and dispersal capability as key drivers of outcomes of contact between diverging populations.

Conclusions

Our findings provide strong evidence against time being the primary driver of the outcomes of secondary contact in lowland Panama, and indicate that instead ecological and genomic factors are better predictors of the development of reproductive isolation. We also find evidence of a high degree of cryptic variation in lowland Panama, which has implications for conservation. Lowland Panama is an excellent laboratory for understanding the early stages of speciation, and future work in this area will likely provide further insights into avian diversification.

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Figures

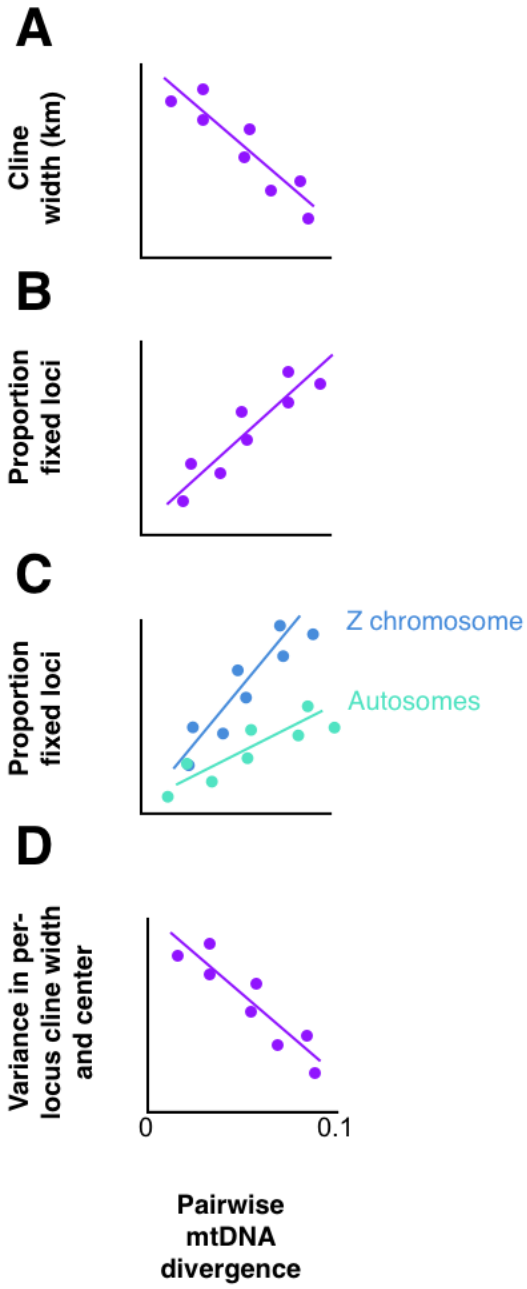

Figure 1: Predictions given time as the main driving factor in determining secondary contact outcomes. According to this hypothesis, A) median cline width should decrease with increasing mitochondrial distance, as hybrid zones narrow; B) the proportion of fixed loci will increase as isolated

populations accumulate fixed differences independently of each over, either by drift or by adaptive pressures; C) the Z chromosome, with ¾ the effective population size, will accumulate these differences at approximately twice the rate in autosomes, and D) variance in the location of the cline center and the width across loci will decrease, as the hybrid zone stabilizes and narrows under selection pressures against hybrid offspring.

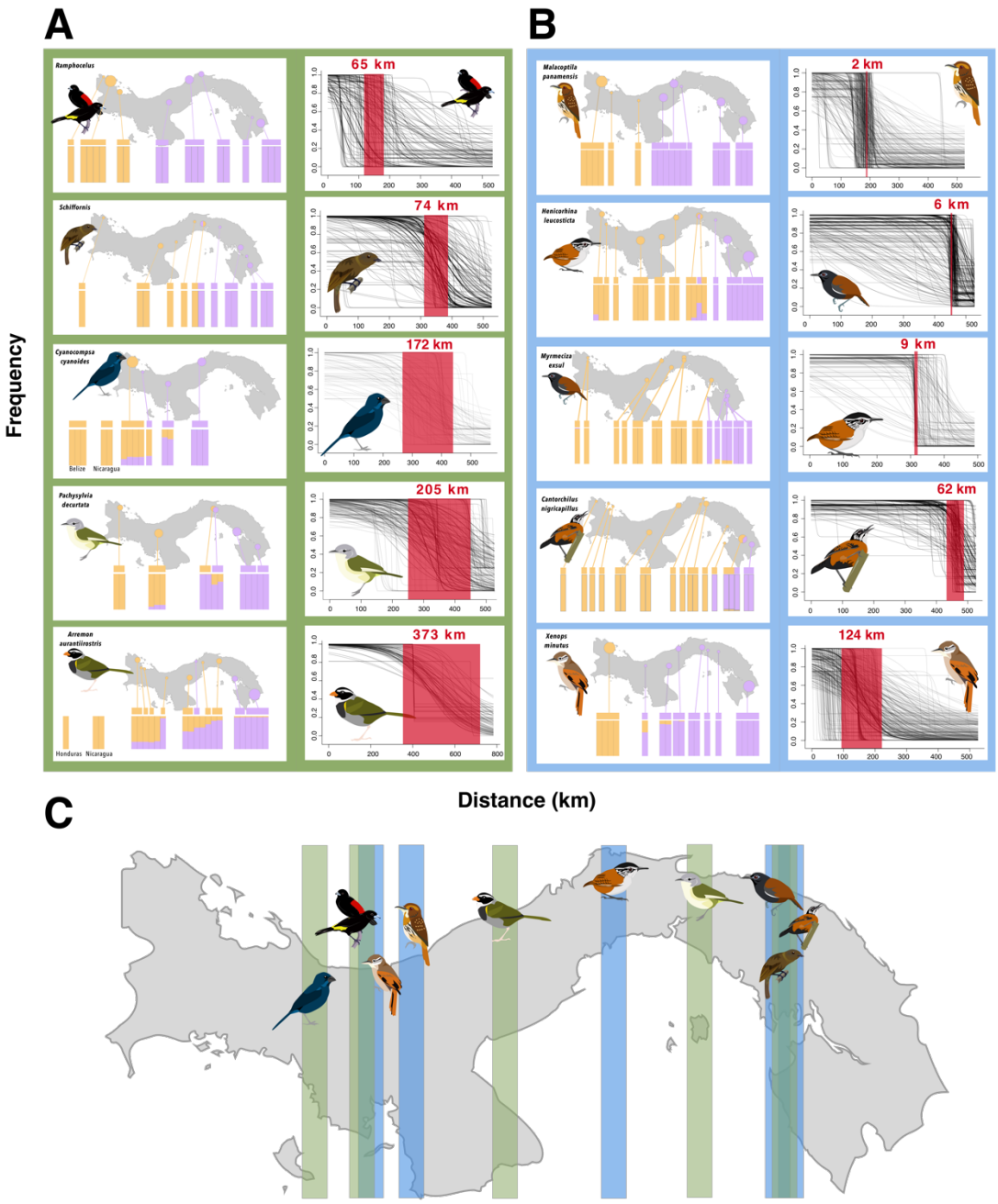

Figure 2: Genotypes of all birds, with points indicating locality, and cline results for the ten study taxa, group by diet guild with blue (A) indicating insectivores, and green (B) for granivores and frugivores. Structure plots in each taxon are grouped by locality, and bars on top indicate the mitochondrial haplotype of each individual. Genotypes for individuals from localities outside of Panama are labeled below each plot. Clines for each taxon are show per locus, with median clinal

width highlighted in red. In C), approximate longitude of median cline centers for each taxon are mapped, with diet guild again indicated.

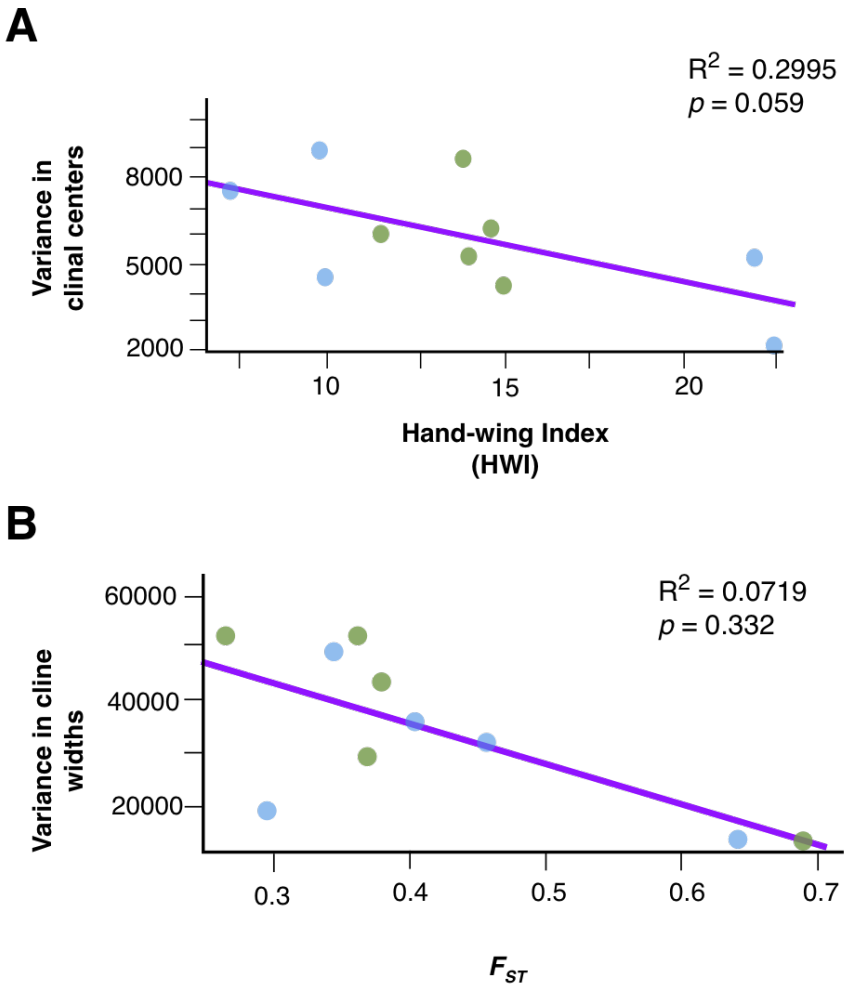
A
R^2 = 0.2995
p = 0.059
Variance in clinal centers
8000
5000
2000
10
15
20
Hand-wing Index (HWI)
B
R^2 = 0.0719
p = 0.332
Variance in cline widths
60000
40000
20000
0.3
0.4
0.5
0.6
0.7
F_{ST}
C
p = 0.069
Median Cline Width (km)
300
200
100
0
Insectivores
Frugivores/Granivores
Diet

Figure 3: Best fit models for cline parameters. Plant-based diets indicated in green, insectivores in blue. A) For variance in cline center, HWI was the best fit, with a higher HWI corresponding with greater coincidence of the geographic center of clines (Adj R^2 = 0.2995, p = 0.05884). B) Variance in cline width was predicted instead by F_{ST}, with cline widths becoming more consistent as overall genome differentiation increased, although this relationship was weak and not statistically supported (Adj R^2 = 0.0719, p = 0.3323). C) Median cline width was best predicted by diet type, with frugivores and granivores having wider clines than insectivores (t = -2.2662, df = 5.3811, p = 0.06904).

Tables:

Table 2.1: Proportion of fixed SNPs by taxon, broken down by autosomal and sex chromosomes. mtDNA distance calculated as pairwise difference of all coding regions.

Taxon		**mtDNA distance**	**Total proportion fixed SNPs**	**Proportion fixed autosomal SNPs**	**Proportion fixed Z-linked SNPs**	**Proportion Z vs autosomal fixed SNPs**
Arremon aurantiirostris	Orange-billed Sparrow	6.36%	0.0202 (40/1979)	0.0156 (29/1836)	0.0887 (11/124)	5.68
Cantorchilus nigricapillus	Bay Wren	3.30%	0.0126 (37/2935)	0.0105 (29/2757)	0.0449 (8/178)	4.28
Cyanocompsa cyanoides	Blue-black Grosbeak	5.01%	0.0214 (85 / 3975)	0.0172 (64 / 3718)	0.0817 (21 / 257)	4.75
Henicorhina leucosticta	White-breasted Wood-wren	5.94%	0.0151 (28 / 1855)	0.0119 (21 / 1763)	0.0761 (7 / 92)	6.39
Malacoptila panamensis	White-whiskered Puffbird	5.24%	0.1224 (523 / 4271)	0.0932 (368 / 3948)	0.4799 (155 / 323)	5.15

Taxon		mtDNA distance	Total proportion fixed SNPs	Proportion fixed autosomal SNPs	Proportion fixed Z-linked SNPs	Proportion Z vs autosomal fixed SNPs
Myrmeciza exsul	Chestnut-backed Antbird	3.14%	0.0553 (202/3649)	0.0553 (192/3475)	0.0588 (10/170)	1.06
Pachysylvia decurtata	Lesser Greenlet	4.11%	0.0310 (132 / 4257)	0.0221 (87 / 3939)	0.1415 (45 / 318)	6.40
Ramphocelus passerinii/flammigeris	Scarlet-rumped/ Flame-rumped Tanager	4.30%	0.0270 (66/2444)	0.0245 (57/2325)	0.0756 (9/119)	3.08
Schiffornis veraepacis/turdina	Northern/ Brown-winged Schiffornis	9.83%	0.2258 (499/2209)	0.2205 (470/2132)	0.3766 (29/77)	1.72
Xenops minutus	Plain Xenops	2.75%	0.0510 (219 / 4292)	0.0373 (148 / 3971)	0.2212 (71 / 321)	5.93

Table 2.2: Clinal characters of all taxa, including number of diagnostic loci for each set of analyses.

Taxon	Diagnostic loci	Median Cline Width (km)	Variance in Cline Width	Median cline center (degrees longitude)	Variance in Cline Center
Arremon aurantiirostris	72	373	43502	-80.27414	6009
Cantorchilus nigricapillus	225	62.2	48930	-78.22381	8955
Cyanocompsa cyanoides	81	172	52274	-81.63481	5207
Henicorhina leucosticta	163	9.3	19116	-79.53446	4566
Malacoptila panamensis	717	2.4	14048	-80.86800	2225
Myrmeciza exsul	389	6.1	36187	-78.30600	7573
Pachysylvia decurtata	333	205	52330	-78.98317	6285
Ramphocelus passerinii/flammigeris	162	65.3	28828	-81.27781	8638
Schiffornis veraepacis/turdina	505	74.6	13165	-78.30600	4254
Xenops minutus	509	124	32521	-81.15118	5287

Table 2.3: Delta AICc values for all tested GLMs. Model family for each response variable indicated. mt= pairwise mitochondrial divergence, diet= diet type, coded as insectivore or plant-based, HWI= hand-wing index, π = nucleotide diversity as measured between parental populations, and F_{ST} = F_{ST} as measured between parental populations.

Model	**Width (km)**	**Variance in median cline center**	**Variance in median cline width**	**Proportion of fixed SNPs, Z chromosome**	**Proportion of fixed SNPs, autosomes**
Family	Exponential	Normal	Normal	Lognormal	Lognormal
mt	4.214	1.9394	3.8661	28.5584	4.2268
diet	0	4.6538	6.1298	31.1308	18.8783
HWI	4.3411	0	5.6552	22.5728	18.4172
mt+diet	3.8852	5.9979	3.7498	33.106	9.3678
mt+HWI	10.2074	1.1929	8.1912	0	0
mt+HWI+diet	12.4882	7.0191	10.2497	8.211	8.9972
π	4.1762	3.1347	3.5422	31.00977	16.8023
π+mt	8.8498	7.9326	9.1347	27.5523	6.7076
π+diet	5.4149	8.9702	8.0879	37.0029	58.2586
π+HWI	10.1603	1.1956	6.5121	25.597	12.121
π+HWI+mt	17.4404	9.3129	15.3711	7.3951	8.9506
π+HWI+diet	13.6657	10.0002	13.7598	22.7699	11.3519
π+mt+diet	11.5466	14.4985	12.6647	30.76086	10.1848

Model	**Width (km)**	**Variance in median cline center**	**Variance in median cline width**	**Proportion of fixed SNPs, Z chromosome**	**Proportion of fixed SNPs, autosomes**
π+mt+diet+HWI	26.4634	22.0105	25.1983	21.2965	23.9419
π+mt+diet+HWI+F_{ST}	56.4637	47.9162	53.0133		
F_{ST}	3.4974	1.0662	0		
π+F_{ST}	9.3288	5.7325	1.6746		
mt+F_{ST}	8.4155	6.1728	5.3842		
diet+F_{ST}	5.2962	7.0185	4.8775		
HWI+F_{ST}	9.4568	4.8904	5.9269		
π+mt+F_{ST}	17.0978	14.7248	8.5747		
π+diet+F_{ST}	14.1625	14.6222	8.2292		
π+HWI+F_{ST}	18.1157	9.8913	10.5113		
mt+diet+F_{ST}	12.8778	14.1682	10.0004		
mt+HWI+F_{ST}	15.0016	10.1161	14.3831		
diet+HWI+F_{ST}	12.137	13.8501	13.7774		
mt+diet+HWI+F_{ST}	27.0654	19.9168	24.5132		

Model	**Width (km)**	**Variance in median cline center**	**Variance in median cline width**	**Proportion of fixed SNPs, Z chromosome**	**Proportion of fixed SNPs, autosomes**
π+diet+HWI+F_{ST}	27.132	24.7173	23.0242		
π+mt+HWI+F_{ST}	28.9677	24.3128	23.5694		
π+mt+diet+F_{ST}	26.4895	29.1582	23.1425		

Chapter 3: Genomic insights into rapid speciation in a clade of Andean hummingbirds (*Aglaeactis*)

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Author Contributions

JFM, CCW, and MJM conceived the study. CCW provided the samples. JFM generated the genomic libraries and modified the protocols in consultation with MJM. JFM performed the computational analyses and wrote the initial draft, with all authors contributing to the final draft. DA provided key feedback on analyses and the initial draft. Original illustrations created by JFM.

Data Accessibility

Scripts, input files, and other associated files for analysis are available at https://github.com/jfmclaughlin92/aglaeactis. Sequence data has been uploaded to the NCBI Short read archive (SRA).

Abstract

The high Andes are a geologically recent and challenging environment, with dynamic communities that have shifted, diversified, and adapted extensively since the Pleistocene. As such, they have

provided a stage for multiple rapid radiations of organisms and serve as an excellent study system for how the physical and genomic landscapes interact to drive speciation. We used whole-genome re-sequencing and thorough geographic sampling to describe the speciation history and genomic landscape of divergence in a high-altitude hummingbird radiation, the sunbeams (genus *Agleaeactis*), sequencing 46 individuals of all six named taxa in the genus at low coverage. Mitochondrial and nuclear phylogenies indicate rapid, recent speciation with notable mitonuclear discordance, pointing to either lineages still in the process of sorting or a central role for gene flow in the diversification of the genus. Two broad groups were recovered in our nuclear phylogeny: *A. cupripennis* and the extremely range-restricted *A. aliciae* in one, and *A. castelnaudii* and *A. pamela* in the other. Overall, differentiation across the genome was extremely low, with a very small proportion of sites being fixed between taxa. However, while overall genome-wide differentiation was higher in the southern clade ($F_{ST} > 0.20$), where all taxa are in allopatry, this was evenly distributed across the genome, with no regions significantly elevated above the mean. Meanwhile, the northern clade, all of which come into some degree of contact with each other, had much lower overall differentiation ($F_{ST} < 0.05$), but in all pairwise comparisons between the three taxa in the clade we found regions with significantly elevated differentiation, despite filtering which accounted for this difference in the overall divergence levels in the comparisons. Demographic modeling recovered estimates of gene flow one to two orders of magnitude higher in the north than the south and a history of continuous recent gene flow that contrasts with the strong signature of isolation in the southern populations. We propose that this difference in gene flow, likely driven by the differing geographic patterns observed in northern and southern *Aglaeactis*, is responsible for the observed variation in divergence across the genome, and that future higher-coverage genome scans should focus on these regions in the search for loci likely under selection for incipient reproductive isolation. Our results illustrate that the differing ranges resulting from the diverse topography and climate of the Andes have had a key role in shaping the genomic landscape of divergence in *Aglaeactis*.

Introduction

One of the key questions in the study of speciation is what genomic mechanisms drive the development of reproductive isolation (Via 2012; Seehausen *et al.* 2014; Gante *et al.* 2016; Wolf and Ellegren 2017; Campbell, Poelstra, and Yoder 2018; Dong *et al.* 2019; Salisbury *et al.* 2020; Greenway *et al.* 2021). This is often combined with the search for loci that can be linked to traits implicated in either prezygotic (Saetre *et al.* 2003; Hermann *et al.* 2013; Brelsford, Toews, and Irwin 2017; Van Belleghem *et al.* 2017; Rosser *et al.* 2019) or postzygotic (Orr and Presgraves 2000; Sætre *et al.* 2003; Pulido-Santacruz *et al.* 2018) barriers. However, the landscape of variation in the genomes of incipient species can be shaped by multiple factors (Turner and Hahn 2010; Cruickshank and Hahn 2014), with selection for local adaptation (Rundle and Nosil 2005; Losos *et al.* 2006; Papadopulos *et al.* 2014; Soria-Carrasco *et al.* 2014; Malinsky *et al.* 2015; Han *et al.* 2017; Malinsky *et al.* 2018; Marchán *et al.* 2020), demographic variation (Quilodrán *et al.* 2020), and linked selection (Burri *et al.* 2015; Wang *et al.* 2016; Duranton *et al.* 2018) all having been supported as potential alternative drivers of "islands of speciation" (Nosil, Funk, and Ortiz-Barrientos 2009; Ellegren *et al.* 2012; Nadeau *et al.* 2012), or more generally, heterogeneous genomic divergence (Harrison 1991; Avise 2000; Via 2001; Mallet 2005; Noor and Feder 2006; Nosil, Funk, and Ortiz-Barrientos 2009; Mallet *et al.* 2007), as regions of heightened divergence are often interpreted as loci responsible for driving reproductive isolation (Turner *et al.* 2005; Malinsky *et al.* 2015; Wolf and Ellegren 2017; Han *et al.* 2017; Ma *et al.* 2018). One common prediction is that these genomic patterns are the outcome of speciation with gene flow that may be more likely in sympatry or parapatry than allopatry, as the former two are more likely to host the requisite gene flow for selection to act counter to (Turelli, Barton, and Coyne 2001), but evidence for geography predicting the occurrence of such islands is mixed at best (Michel *et al.* 2010; Renaut *et al.* 2013).

Mountain ranges, with their complex topography and climatic heterogeneity, are systems particularly suited to studies of the geography of speciation (Ruggiero and Hawkins 2008), as their topographic and climatic complexity create more opportunities for a wide array of allopatric, parapatric, and sympatric distributions among diverging lineages (Rahbek *et al.* 2019). Along the latitudinal axis of

the Andes, habitats are less contiguous at higher elevations, a pattern thought to make the high Andean fauna more susceptible to range fragmentation due to physical dispersal barriers, with genetic drift and isolation then leading to speciation (Graves 1985). There is ample evidence of rapid speciation in high Andean taxa, including in birds (Benham and Witt 2016), herpetofauna (Hutter, Guayasamin, and Wiens 2013; Hutter, Lambert, and Wiens 2017), and plants (Lagomarsino *et al.* 2016; Pérez-Escobar *et al.* 2017; Nevado *et al.* 2018). The rich biodiversity of the Andes is often attributed to two main historical factors. First, the uplift of the range in the Miocene and Pliocene (Picard, Sempere, and Plantard 2008) contributed to vicariance events (Hughes and Eastwood 2006; Chaves *et al.* 2007; Chaves, Weir, and Smith 2011; Weir and Price 2011a). Second, more recent Pleistocene glacial cycles and associated climate change (Haselton, Hilley, and Strecker 2002; Martini *et al.* 2017; Zech *et al.* 2017) created opportunities for range shifts (Gentry 1982; Koscinski *et al.* 2008; Kolář, Dušková, and Sklenář 2016; Paz, González, and Crawford 2019) and refugia (Gentry 1982; Muellner *et al.* 2005; Zemlak *et al.* 2008; Vera-Escalona *et al.* 2012; Marín *et al.* 2013; Baker *et al.* 2020), while the often steep elevational gradients lead to strong selection for local adaptation (Lim *et al.* 2021).

However, an alternative framework for explaining the repeated occurrence of rapid, recent radiations in the high Andes is that its dynamic environment creates many opportunities for reproductive barriers to arise as a consequence of the interplay of genomic architecture and the environment. In this view, while environmental factors create the opportunity for speciation, whether speciation in fact occurs is determined by genomic and ecological factors intrinsic to the organisms themselves (Schluter 2001; Coyne and Orr 2004; Price 2008). Instead of the particulars of the landscape, such as the specifics of a bout of glaciation or a certain refugium, the focus of this hypothesis is on how the broad-scale patterns of change in the landscape interact with these ecological and genomic constraints to produce biodiversity.

A particularly promising group to test this interplay of landscape and genome are hummingbirds. Hummingbirds (Trochilidae) are an extremely species-rich avian family, its 349 currently recognized species surpassed only by the tyrant-flycatchers (Tyrannidae; 422) and tanagers (Thraupidae; 377) (Winkler, Billerman, and Lovette 2020). One of the notable features of their genomes are their small size, which at an average 1.03 Gbp are the smallest of any birds (Gregory *et al.* 2009). The role of genome size in speciation rate is still a topic of debate (Kraaijeveld 2010). On one hand, small genomes are usually proportionately denser in coding regions, so that a mutation would more likely result in phenotypic change that could be subject to selection (Gregory 2005; Kraaijeveld 2010). On the other hand, large genomes can contain many more transposable elements and gene duplications increasing the probability of genomic incompatibilities in hybridization (Gregory 2005; Kraaijeveld 2010). Overall, in most taxa it appears that smaller genomes are correlated with faster speciation (Kraaijeveld 2010), but this varies between taxa. This relationship suggests that hummingbirds would be a particularly useful group for studying how genome, landscape, and ecology interact in the early stages of speciation (Figure I.1); they may be already more likely to speciate, and the changes in question will be easier to spot on a smaller genome. Hummingbird diversity is particularly high in the Andes (Bleiweiss 1998; McGuire *et al.* 2007), containing approximately 40% of the species of the family (McGuire *et al.* 2014), providing ample opportunity for studying how these factors may influence speciation outcomes in a system that is particularly prone to landscape changes that lead to population separation and diversification.

Four species of Andean hummingbirds are currently recognized in the genus *Aglaeactis* (Figure 3.1). The most widespread, *A. cupripennis*, ranges from North Santander, Colombia, south to Cuzco, Peru (Schuchmann 1999). It is polytypic, with the nominate subspecies distributed in the northern portion of the range down through Huánuco, Peru, and *A. c. caumatonota* to the south (Schuchmann 1999). *Aglaeactis castelnaudii* has a disjunct distribution, with subspecies *A. c. regalis* in Junín, Huánuco, and Pasco, and the nominate in the higher elevations around Cuzco and Apurímac. *Aglaeactis pamela* is further restricted in range, distributed in the Cordillera Real in Bolivia, with no recorded range overlap

with any of its congeners. Finally, the endangered (BirdLife International 2016) *A. aliciae* is restricted to a small area in the upper Marañón valley of La Libertad, Peru (Lambert and Angulo-Pratolongo 2007). As *A. cupripennis caumatonota* and *A. castelnaudii* have areas of range overlap, the potential for hybridization has been previously noted and likely hybrids described (Zimmer 1951), but questions remain as to whether these truly represent hybrids (Schuchmann 1999) and hybrids have not been reported between the other taxa (Schuchmann 1999). *Aglaeactis* provides a particularly intriguing group for studies of how the geographic context of divergence interacts with genomic architecture, because of both the general traits of hummingbird genomes and for the occurrence of both allopatric and parapatric distributions within the genus (Figure 3.1). These factors provide a fertile ground for testing hypotheses on the early speciation process.

Methods

We sampled frozen tissues from vouchered museum specimens that were collected as part of integrative site inventory work in the high Andes of Ecuador, Peru, and Bolivia. We extracted DNA from pectoral muscle in forty-six individuals in the genus *Aglaeactis* (Table 3.1), and one individual each from *Ensifera ensifera* and *Coeligena coeligena* as outgroups, using the Qiagen DNeasy protocol for blood and tissue.

We performed two rounds of library preparations, constructing two duplicate libraries using the SeqWell plexWell Library Preparation Kit which were then sequenced separately. Each DNA sample was diluted to 10 ng/μL and loaded into two wells of the 96-well plate, which contained well-specific barcodes, resulting in four independent sequencing events per individual between our two plates. After barcoding, samples were then pooled, purified with a 1:1 ratio of MAGwise beads, and barcoded with a plate-specific index. This was purified as above with MAGwise beads.

For each round of library preparation, we used different amplification and size selection protocols to determine the optimal method. In the first, we amplified the library using the following program:

72°C for 10 min, 95°C for 3 min; 12 cycles of 95°C for 30 sec, 64°C for 15 sec, and 72°C for 30 sec; and finally 72°C for 2 min. To size select, we then used a multi-stage bead cleanup, with sequential clean-up of 0.4X, 0.2X, and finally 1.0X AmpPure beads. For the second round, we adjusted this by amplifying as above, but splitting the library in half and amplifying each before repooling, to minimize the rate of PCR duplicates. We then used a simpler clean-up with 1.0X AmpPure, but first diluted the library to 205 μL in ddH_2O, using half of this diluted library for clean-up. The first library was sequenced on an Illumina HiSeq 3000, and the second on a NextSeq. Based on an expected sequencing yield of ~ 100 GB of sequence data, and an expected *Aglaeactis* genome size of 1.1 GB, we expected to recover on average 1.9X coverage across the genome per run.

Raw read processing

For each of the four pairs of read files generated above, we inspected raw reads using FastQC (Andrews 2010) for coverage, duplication rate, and base call quality. We trimmed low quality bases and removed adaptors using bbduk, which is part of the bbMAP package (Bushnell 2014). Next, we separated reads mapping to the mitochondrial genome of *Heliodoxa aurescens* (Genbank: KP853094) using the bbMap bbsplit package, creating a single mtDNA read file for each sample and a separate file of only nuclear reads.

Assembling mitochondrial genomes

We used NOVOplasty (Dierckxsens, Mardulyn, and Smits 2017) to assemble a whole mitochondrial genome using ND2 from *Aglaeactis castelnaudii* as an initial seed and a single high-coverage *A. ca. castelnaudii* individual (MSB:Birds:34095) as reads. We annotated the mito-assemblies using DOGMA (Wyman, Jansen, and Boore 2004). We then aligned for quality-filtered reads for all 48 samples to this reference with BWA-MEM (Li 2013) and created consensus sequences with samtools *mpileup* (Li *et al.* 2009; Li and Durbin 2009) followed by bcftools *call* (Li 2011) to identify variants.

Creating a reference

Because of the lack of a closely related hummingbird genome to use as a reference, we prepared a pseudo-reference of remapped *Aglaeactis* reads. We mapped high-coverage reads from *Aglaeactis aliciae* generated as part of another sequencing effort to a chromosome-level *Calypte anna* genome (Genbank GCA_003957555.2; used as it is the only chromosome-level hummingbird assembly available at the time of writing) with BWA-MEM, creating an alignment with an average 114X coverage (calculated in Qualimap v. 2.2.1; García-Alcalde *et al.* 2012). We then cleaned these bam files with picard (Broad Institute 2019) to remove duplicates and called SNPs with samtools *mpileup* and bcftools *call*, then output a FASTA of the consensus sequence with bcftools *consensus*. This created a chromosome-level assembly that was modified with *Aglaeactis* base calls to increase mapping rates and call variants with higher confidence. As chromosomal synteny is high in birds (Shetty, Griffin, and Graves 1999; Edwards *et al.* 2005; Ellegren 2010, 2013), we also used this to examine the chromosomal distribution of variation with reasonable confidence.

Remapping nuclear genomes

For each read file, we mapped non-mtDNA reads to our reference using BWA-MEM. We cleaned, marked read groups, and removed duplicates with picard (Broad Institute 2019), before merging the individual well alignments for each sampled bird in samtools (Li *et al.* 2009).

After merging, bam files were sorted with samtools, and variants were called with samtools *mpileup* function with default parameters, followed by bcftools *call* (Li 2011), using the multiallelic alternative caller and outputting only variants. Output files were filtered in bcftools to select only high-quality SNPs, using multiple filtering criteria based on GQ scores (10, 12, 15, and 20), Q scores (15, 20, and 30), and minimum coverage (1 and 2X), and then further filtered in vcftools (Danecek *et al.* 2011) to

include only biallelic SNPs, and then were filtered at minimum mean DP thresholds of 2, 5, and 10 to select the best quality filter for analyses. Multiple datasets were constructed for downstream analyses based on the requirements, with either complete or 90% complete SNP tables and either all SNPs or one SNP per 2,000 bp.

Working with low-coverage data presents challenges for accuracy and confidence in variant calling (Lou *et al.* 2021; Zhang *et al.* 2019; Rustagi *et al.* 2017). Our primary concern was the false calling of variants, and particularly of heterozygotes (Yu and Sun 2013), as we found that the popular HaplotypeCaller tool in GATK created erroneous heterozygous SNPs when a sample was homozygous for the alternate allele relative to the reference (also observed by Lefouili and Nam 2022; see Appendix 3 for details). To minimize these, we evaluated our filtering schema (see above), and only selected positions with enough depth that this was less likely. Furthermore, we attempted to validate our analyses conducted with the samtools/bcftools pipeline by re-calling all data based on genotype likelihood scores obtained with the program ANGSD (Korneliussen, Albrechtsen, and Nielsen 2014). We called SNPs based on genotype likelihood scores using the samtools model, outputting to the BEAGLE file format for later conversion to VCF, and on allele frequencies calculated from with a fixed major, unknown minor frequency, and a uniform posterior prior inferred from the genotype likelihood. These were then restricted to sites with a *p*-value of less than or equal to 0.05, with at least 2X coverage and less than 50X coverage (to minimize impact of paralogs and repetitive regions), and only calling genotypes with at least 0.90 posterior probability. However, high proportions of missing data reduced both the number of variants available for analysis and the number of individuals able to be included, rendering the datasets of little value for validation through replication.

In general, error in low coverage datasets is introduced in two ways. First, very low coverage sites will not have the read depth to detect base-call errors, introducing false calls into the dataset. Several potential outcomes can result from this. First, a site that is truly homozygous for the ancestral allele (A/A) may be called with a variant, such as A/T, creating a false heterozygote for a derived alleles present in the population. Secondly, a truly variant allele may be called as the ancestral, replacing an

A/T call with a A/A and reducing variation in the dataset. Similarly, a miscalled base to the ancestral allele on a derived homozygote T/T will inflate the heterozygosity of the sample as in the first example. Finally, any of these can happen with an allele not already present in the population, such as an A/T locus miscalled as C/T, falsely creating a variant site altogether (i.e., a truly monomorphic locus becomes polymorphic) or turning a biallelic site into a multiallelic one that is then removed from analyses that can assume biallelic sites. Errors can likewise be introduced on the high end of coverage, as reads that are associated with paralogs are mistakenly called as the same locus, likely creating false heterozygotes when two paralogs with slight differences are assumed to be the same site. For all the analyses described below, a brief description of which of these errors has the greatest impact on inferences is provided.

Generating a mitochondrial tree

We generated a phylip file with vcf2phylip v2.0 (Ortiz 2019) of complete mitochondrial sequences from all individuals. We then used this to build a maximum likelihood tree in MEGAX (Kumar, Stecher, and Tamura 2016), with *Coeligena coeligena* and *Ensifera ensifera* serving as outgroups (aligned to the same reference). We bootstrapped the analyses 500 times, and then viewed the results in FigTree (Rambaut 2009). As mitochondrial DNA is typically oversequenced relative to the nuclear genome (due to multiple copies being present per cell), corrections for low coverage were not relevant.

Generating nuclear phylogenies

We constructed maximum likelihood trees for all nuclear SNPs in IQ-TREE v. 1.3.11.1 (Nguyen *et al.* 2015), using 186,011 biallelic SNPs with minimum DP of 2 with 90% complete data under the GTR+I model with 1,000 bootstrap replicates, with *Coeligena coeligena* and *Ensifera ensifera* as the outgroups. Additionally, we ran the same analysis on 74,924 SNPs excluding *A. aliciae* to confirm the overall topology without potential rooting issues from the highly ambiguous signal from *A. aliciae.* The full analysis was also replicated in RAxML (Stamatakis 2014), using the GTR+GAMMA model

with the rapid hill-climb algorithm and 100 bootstrap replicates on 68,541 unlinked nuclear SNPs with the *Coeligena* and *Ensifera* outgroups. Additionally, Splitstree v. 4.14.8 (Huson and Bryant 2006) was run on 71,515 biallelic unlinked SNPs, thinned to one SNP per 2,000 bp from the above dataset, from the 46 *Aglaeactis* individuals to generate an unrooted phylogeny, to provide an independent analysis to confirm the IQ-TREE and RAxML results. All tree methods are potentially sensitive to false heterozygotes, but as each of these methods is likely to be impacted to somewhat varying extents, use of all three together can help mitigate these impacts.

Population genomics

STRUCTURE v. 2.3.4 (Pritchard, Stephens, and Donnelly 2000) was run the dataset of 71,515 SNPs for the 46 *Aglaeactis* individuals, thinned to 1 SNP per 2 kbp to avoid the effects of linkage on inferences. Possible impacts of low coverage on this analysis are 1) base-call errors being creating nonexistent variants or obscuring a true variant, leading to either artificially inflated signals of divergence or obscured true signal of divergence and 2) increased likelihood and influence of paralogs in the dataset due to the strict missing data requirements. We ran a 100,000 iteration burn in, followed by 500,000 k iterations, bootstrapping each value of *K* from two to six 30 times. We determined the best value of *K* in STRUCTURE HARVESTER v. 0.6.94 (Earl and vonHoldt 2012) with the Evanno method (Evanno, Regnaut, and Goudet 2005). This was then repeated with both the *cupripennis* + *aliciae* and the *castelnaudii* + *pamela* groups.

For each taxon pair of interest, we measured per-site and windowed F_{ST} using vcftools. This analysis is potentially particularly sensitive to errors introduced by false base calls, especially in *A. aliciae* and *A. pamela*, with their small sample sizes making the frequency of an error appear to be relatively high. We used a non-overlapping sliding window of 50 kbp to calculate F_{ST} between each group of interest: first, a comparison between the northern and southern groups, followed by pairwise comparisons with each group's three taxa (Table 2). This was done to determine at what scale of divergence outlier windows

were most frequent, and if the two major groups of *Aglaeactis* were distinguished by any differentiated loci that were not strongly differentiated with these groups. We used a significance-based threshold to identify outliers rather than a single cut-off value to control for variation in the overall levels of differentiation in different comparisons (i.e., so that we would not miss outlier windows just because overall differentiation in a given comparison was lower or higher than other comparisons). D_{xy} was calculated with the same window sizes.

Detecting gene flow

To test the role of gene flow in the evolutionary history of *Aglaeactis,* we fit models of divergence for each pairwise comparison in δaδi v. 1.7.0 (Gutenkunst *et al.* 2009), following methods for pairwise demographic modeling from Everson *et al.* (2019). As low-frequency SNPs are less likely to be polymorphic in low-coverage data, the estimated site frequency spectrum (SFS) may be biased towards intermediate allele frequencies, losing many of the rare variants (Lou *et al.* 2022). We generated a vcf file with only the variable sites for each pair of taxa under investigation with vcftools and jvarkit v. 20200713 (Lindenbaum 2015) and then thinned these to one SNP per 1000 bp to minimize linkage effects, converting this to the specific δaδi input format with easySFS (Overcast 2017). We tested a variety of models both with and without gene flow (Figure A3.1). In brief, these included: a) "neutral", in which populations were panmictic, b) "island", a split between populations with no subsequent gene flow, accompanied by population growth, c) "IM", a split between populations with population growth that includes gene flow, d) "split_nomig", where populations split without growth, and do not experience gene flow, e) "split_1m", a split with no growth and equal gene flow between populations, f) "split_2m", a split with no growth and separate migration parameters that allow for the detection of asymmetric gene flow, g) "SC_1m", a split with a period of no gene flow, followed by symmetric gene flow between populations to model secondary contact, and h) "SC_2m", secondary contact as above but with asymmetric gene flow. These models were modified from those used in McLaughlin *et al.* (2020), with the secondary contact models originally based on those used in

Rougemont *et al* (2017). Model optimization followed the procedure used in Mclaughlin *et al.* 2020, running all models and adjusting parameter bounds until estimates no longer approached the upper or lower bounds, and then the appropriate set of bounds was used to run that model fifteen times to ensure estimation was reliable. From there, the lowest maximum log composite likelihood score (MLCL) was recorded and used to calculate AICc. While two of our populations, *aliciae* and *pamela*, were only represented by two individuals each, ðaði parameter estimates are consistently calculated at that sample size (McLaughlin and Winker 2020), so our results are likely to be reliable for all comparisons. We interpreted these values using a mutation rate of $1.24x10^{-9}$ sites/year, calculated by comparing the reference *A. aliciae* genome against that of *Calypte anna* (GCA_003957555.2) and using a divergence date of 16.4 mya (McGuire *et al.* 2014), and a generation time of 4.2 years (BirdLife International 2021).

We also conducted ABBA-BABA tests for an independent method of testing for gene flow. The SNP dataset used for STRUCTURE analyses was converted to a SNP frequency table using the conStruct package (Bradburd, Coop, and Ralph 2018) and then ABBA-BABA tests were performed with an R script (available at https://github.com/jfmclaughlin92/aglaeactis). Because there was potential gene flow between all members of a given clade, the tests were performed with each potential arrangement of taxa, mirroring the pairwise set-up of the ðaði modeling.

Results

We generated over 1.2 billion reads in total. Read number per individual was between 10.0 and 37.8 million, with an average of 26.4 million (Table 3.1). PCR duplicates were higher than anticipated, with around 30% of reads being flagged as duplicates. This is likely due to use of PlexWell protocols that were still being refined for tetrapods at the time of sequencing, and there was both more even coverage and lower PCR duplicate rates (approximately 20%) in the second round of library preparation than the first after updating to the newer versions of the kits. Once initial quality control was completed, coverage was calculated as 0.98X to 3.28X, with an average of 2.35X (Table 3.1).

Phylogenetic estimation

Overall, our nuclear phylogenies reflect the current taxonomy of *Aglaeactis*, with the exception of *A. aliciae* (Figure 3.2B, C). Both *A. aliciae* individuals are outgroups relative to *A. cupripennis sensu lato* (100% and 99% bootstrap support), but do not form a clade themselves. The two *A. cupripennis* subspecies form a monophyletic group (100% bootstrap support), as do the two *A. castelnaudii* (100% support; Figure 3.2B, C). Overall, we recovered two clades: the "northern" clade of *A. cupripennis sensu lato* and *A. aliciae*, and the "southern" clade of *A. castelnaudii sensu lato* and *A. pamela*, named for their overall relative geographies. This result was robust and was still obtained when *A. aliciae* was excluded from the IQ-TREE analysis and when the tree was constructed with all taxa in Splitstree (Figure 3.3).

Our mitochondrial and nuclear trees varied substantially (Figure 3.2A). Most notably, we found that *cupripennis sensu lato* is non-monophyletic with 100% bootstrap support on the mitochondrial tree, contrasting with its monophyly on the nuclear tree. Meanwhile, the relationships of the clade of *castelnaudii sensu lato* + *pamela* remained largely consistent across the nuclear and mitochondrial trees (Figure 3.2). We found strong support (96% bootstrap support) that *A. cu. caumatonota* alone was the sister group of *A. castelnaudii sensu lato* + *A. pamela*. *A. aliciae* again did not form a monophyletic clade, with one individual resolved as part of the nominate *cupripennis* group and the other in *caumatonota*. Other individuals which had been placed within clades concordant with their taxonomic assignment in the nuclear phylogeny were recovered elsewhere in the mitochondrial, including a *caumatonota* from Huánuco as sister to nominate *A. castelnaudii* and one of each *A. castelnaudii* subspecies with *A. cu. caumatonota* (Figure 3.2A).

Population genomics

We found support for an overall value of $K = 2$ across all *Aglaeactis*, with little evidence for introgression between the two (Figure 3.4). These clusters consisted of on one hand the two *A. cupripennis* taxa and *aliciae*, and both *A. castelnaudii* taxa and *pamela* on the other, which matches the finding of a northern and southern clade from the nuclear phylogenies. When each of these groups was separately analyzed, we again found that $K = 2$ was the best supported value in each (Figure 3.4B, 3.4C). However, the distribution of admixed individuals differed substantially in these two groups. In the southern clade, we found that the two *A. castelnaudii* subspecies corresponded to the two clusters found by Structure. However, *A. pamela* was not found to be a distinct cluster, and instead both individuals had approximately 75% assignment probability to *regalis* and 25% to *A. castelnaudii* nominate (Figure 3.4C). This is particularly of note given the geographic distribution of the three taxa (Figure 3.1), as *A. ca. regalis* is the more distant of the two *A. castelnaudii* subspecies from the range of *pamela.*

In the northern clade, meanwhile, while *caumatonota* was, with a few individual exceptions, mostly entirely assigned to one of the clusters, the other group had no individuals assigned solely to it (Figure 3.4B), despite multiple attempted runs. It is possible this represents widespread admixture from *A. cu. caumatonota* into *A. cupripennis* nominate, as the assignment probabilities to the *A. cu. caumatonota* group are highest in the southern parts of the nominate range, particularly Cajamarca, where the two ranges come into contact (Figure 3.1). We found that, similarly to the ambiguous placement in our other analyses, *A. aliciae* once again was unclear in its assignment, with one individual almost entirely assigned to the same group as *caumatonota* and the other a having a 12% assignment probability to nominate *A. cupripennis* (Figure 3.4B).

Distribution of genomic differentiation

Overall weighted Weir and Cockerham F_{ST} between the northern (*A. cu. cupripennis* + *A. cu. caumatonota* + *A. aliciae*) and southern groups (*A. ca. castelnaudii* + *A. ca. regalis* + *A. pamela*) was

0.2197, calculated with 200,975 SNPs with sliding, non-overlapping 50 kbp windows. We found that there were no significant F_{ST} outlier windows between these two main clades (Figure 3.5A), and overall low differentiation across all chromosomes. This pattern of low differentiation was consistent across all comparisons (Figure 3.5; Table 3.2). In the three comparisons in the northern clade (Figure 3.5 B, C, D), only 0.11–0.20% of windows were elevated above genome-wide significance, spread widely across the genome. Only one of these windows in *A. aliciae*/*A. cu. caumatonota* (Figure 3.5B) and three in *A. cupripennis* nominate/*A. cu. caumatonota* were located on the Z chromosome, with the rest located across the autosomes. Meanwhile, in contrast to this even limited number of outlier windows, in comparisons between the three southern taxa, no significant outlier windows were found between *A. pamela*, nominate *A. castelnaudii*, or *A. regalis* (Figure 3.5 E, F, G). This is despite overall higher genomic divergence ($F_{ST} > 0.20$) in these comparisons, as opposed to the far lower overall F_{ST} in the northern comparisons ($F_{ST} < 0.05$).

Demographic modeling and tests of gene flow

Our demographic modeling with δaδi further supported the finding of differing modes of divergence in the northern and southern clades (Table 3.3; see Figures S4-9 for site frequency spectrum (SFS) plots used for model-fitting process), with the signal of gene flow being far more pervasive between the three northern taxa. Best fit models varied among comparisons, with all recovering a single unambiguous best fit (Table 3.3). Split migration, in which gene flow occurs pervasively after the split but no population expansion occurs, was the best fit for two comparisons, *caumatonota/cupripennis* nominate and *A. aliciae/A. cu. cupripennis*, with the equal migration model being the best in both. In the comparisons between *A. pamela* and each of the *A. castelnaudii* subspecies, the secondary contact models provided a better fit, with gene flow only occurring after a discrete period of isolation. Effective population sizes were small and similar between all populations (Table 3.4).

Gene flow estimates were extremely different between the northern and southern clades. Best fit parameters were one to two orders of magnitude higher in the north (1.41–7.67 vs. 0.04--0.71). In the north, this was equivalent to 0.5 - 2 birds per generation-- or an estimated 1% of the effective population size in the most extreme case, which, as migration is calculated with the effective population sizes, is a proportion that holds even if the true effective population size is different than that we calculate (see above). This high level of gene flow is only observed in our three pairwise comparisons that occur in parapatry and have outliers of heightened genomic divergence. Time estimates, however, for *A. aliciae/A. cu. cupripennis* and *A. cu. cupripennis/A. cu. caumatonota* were extremely low (191 and 210 years, respectively), possibly as a result of multiple bouts of high gene flow obscuring the signal of the original split (McLaughlin *et al* 2020a). Meanwhile, while gene flow was detected in the south, it was far lower, equivalent to around 0.10 birds per generation, and occurred after a period of isolation approximately 85-170 kya (Table 3.4). These were broadly supported by the ABBA-BABA results, which found strong evidence for gene flow between *A. aliciae* and each of the *A. cupripennis* subspecies (D= 0.4568 and 0.465; Figure 3.6) and only weak support for gene flow in the southern clade (*D*=-0.147 – 0.120). The largest point of disagreement is in the presence of gene flow between the two *A. cupripennis* subspecies (*D*=0.002), which our demographic models indicated was subject to significant ongoing gene flow, but the ABBA-BABA test did not support.

Discussion

The dynamic geologic and climatic history of the Andes has been noted as driver of rapid speciation in multiple taxonomic groups (e.g., Hughes and Eastwood 2006; Hutter, Guayasamin, and Wiens 2013; Benham and Witt 2016; Lagomarsino *et al.* 2016; Hutter, Lambert, and Wiens 2017), due to both the relatively recent uplift of the range in the Miocene and Pliocene (Picard, Sempere, and Plantard 2008; Antonelli *et al.* 2009; Smith *et al.* 2014), and by the subsequent repeated cycles of habitat contraction and expansion during the Pleistocene (Ferreira *et al.* 2017). This complex topography and history have impacted the evolutionary history of *Aglaeactis*, as we find evidence of rapid diversification in the

genus, discordant phylogenies likely caused by past gene flow, and a genomic landscape shaped by the geographic context of allopatry or parapatry and the prevalence of gene flow.

Phylogenetics of Aglaeactis

Our phylogeny of *Aglaeactis* reinforces the importance of several key biogeographic barriers in Andean speciation. Most notably, our phylogenies recovered clades that correspond to major valleys in Peru and Bolivia. The two *A. cupripennis* subspecies are found north and south of the Marañón Valley, and *A. aliciae* is endemic to the valley (Figure 1), which is in line with the Marañón's known role as both a suture zone between taxa and as a hotspot of endemism (Särkinen *et al.* 2011; Guzman *et al.* 2021). Moving further south, the Apurímac Valley, previously noted for its role in shaping species distributions (Cracraft 1985; Hosner *et al.* 2015) separates the two *castelnaudii* clades, but interestingly does not correspond with either phylogenetic splits (Figure 2.A) or population structure (Figure 3) within *A. cu. caumatonota*. Finally, the dry inter-Andean valleys between southern Peru and Bolivia correspond with the break between *A. castelnaudii* and *A. pamela*, consistent with previous work (Guzman *et al.* 2021).

We recovered strongly discordant phylogenies for the *Aglaeactis* complex from mitochondrial and nuclear data. The most striking difference was in the placement of *A. cu. caumatonota* (Figure 3.2), as nuclear data recovered a topology in which it was in a monophyletic clade sister to nominate *A. cupripennis*, while mitochondrial markers consistently recovered nominate *A. cupripennis* as sister to all other *Aglaeactis*. While individuals other than *aliciae* formed clades with the rest of their taxa in the nuclear phylogeny, several individuals had discordant mitochondrial placements (Figure 3.2B). Such mitonuclear discord is relatively frequently observed (Linnen and Farrell 2007; Humphries and Winker 2011; Toews and Brelsford 2012; Pavlova *et al.* 2013; Kutschera *et al.* 2014; Peters *et al.* 2014; Toews *et al.* 2014; McLean *et al*. 2016; Sloan, Havird, and Sharbrough 2017; Després 2019;

McLaughlin, Faircloth, *et al.* 2020; Marshall *et al.* 2021), but the mechanisms by which it might arise vary.

The two most prominent potential explanations at play for this mitonuclear discordance are incomplete lineage sorting and gene flow (Funk and Omland 2003; Kutschera *et al.* 2014; Mallet, Besansky, and Hahn 2016; Lavretsky *et al.* 2019), both of which are strong explanatory candidates in this rapidly diversifying group. Our demographic models recover a strong signal of gene flow in all comparisons investigated, particularly among the northern taxa. In northern *Aglaeactis,* the signal of gene flow was on the order of 1% of the effective population size in a given taxon, an amount which is highly likely to result in discordance as introgression repeatedly "shuffles" the genome. This is likely the reason for the strikingly low divergence dates observed in two of the comparisons, as multiple bouts of such high gene flow may erode the signal of earlier periods of isolation (McLaughlin *et al.* 2020a). Even in southern *Aglaeactis*, where gene flow is much less prominent in the demographic histories of each taxon, we find a consistent signal of introgression as a result of secondary contact. Although for the reasons discussed above the exact date of this secondary contact that we recovered may not be fully reliable, our tentative placement of this contact around 110 kya coincides with the beginning of the last Pleistocene glacial period (Martinson *et al.* 1987), suggesting that the current allopatry of the southern clade has been periodically disrupted by climate-driven range shifts.

Advantages and limitations of low-coverage data

Low coverage whole genomes, such as we use in this study (e.g., under 10X coverage), have become a relatively accessible option for studies of variant identification, population genetics, and evolutionary history (Rustagi *et al.* 2017, Homburger *et al*. 2019, Zhang et al. 2019, Lou *et al.* 2021). Our work here demonstrates some of the potential pitfalls of the technique, such as issues with bias from repetitive regions and issues with software not optimized for such datasets, as well as how meaningful evolutionary inference may still be gained despite these. While higher coverage will remain the gold-standard, practical realities of limited resources mean that for the vast majority of researchers, trade-

offs between sampling scope (i.e., number of individuals) and number (McLaughlin *et al.* 2020b) and/or depth (Buerkle and Gompert 2013) of sequenced variants will remain key considerations for the foreseeable future.

Most importantly, extra care is needed when calling variants from low-coverage data (Yu and Sun 2013), as many SNP-calling algorithms are not optimized for these use cases and may produce specific major errors (Lefouili and Nam 2022). The most pressing of these errors are 1) bias introduced by a high proportion of orthologs and replicated regions in retained higher-coverage sites and 2) incorrect SNP calls from low-coverage regions. The former is likely to result in inferences skewed by the relationships in these parts of the genome, which may not reflect the true predominant evolutionary relationships, while the latter introduce false data that are completely unrelated to the relationships within taxa. The specific technical errors introduced by particular programs are discussed in more detail in Appendix 3, but overall mitigation factors will be summarized here. First, low coverage data will likely require more careful handling of all stages of bioinformatics processing, as the "black box" approach unfortunately common in many studies is usually optimized for higher coverage data and, if run as-is, will introduce errors that will likely go undetected. Secondly, rigorous filtering for depth of coverage (excluding both very low and very high coverage sites) alongside other standard filtering parameters (e.g., Q scores, GQ scores, and percent missing data is key to minimize the above biases. Finally, using a variety of analyses and drawing conclusions from consideration of all of them together becomes especially important, as the differing underlying methods (e.g., tree-based models of relationships vs. demographic models based on the site frequency spectrum vs. Bayesian clustering of allele frequencies) are not uniformly susceptible to these primary biases. While the challenges imposed by low coverage data require great care, with these general principles in mind such datasets can still provide a rich and detailed picture of a variety of evolutionary scenarios.

Patterns of genomic differentiation and the geography of speciation

Overall genomic differentiation was low in all comparisons, which is consistent with other speciation events associated with Pleistocene climate change across multiple ecoregions (Knowles 2000; Peterson and Nyári 2008; Hawlitschek *et al.* 2012; Winker, Glenn, and Faircloth 2018; Nevado *et al.* 2018), and with other hummingbirds (Judy 2018; Sornoza-Molina *et al.* 2018; Palacios *et al.* 2019; Battey 2020; Henderson and Brelsford 2020). However, while in the southern clade this was distributed evenly across the genome (Figure 3.5), in the northern clade, the genomic landscape was far more heterogeneous, with multiple outlier peaks across the genome.

The propensity of genomes of hybridizing taxa to develop islands of divergence has been widely observed (Turner, Hahn, and Nuzhdin 2005; Ellegren *et al.* 2012; Nadeau *et al.* 2012; Poelstra *et al.* 2014; Irwin *et al.* 2018) as whole genome sequencing has become more widely available in the past two decades. While many studies have found processes other than selection for reproductive barriers driving the development of such islands (Burri *et al.* 2015; Wolf and Ellegren 2017; Quilodrán *et al.* 2020), in some bird species these islands have been strongly implicated in leading to phenotypic differences that may function as reproductive barriers (Ruegg *et al.* 2014; Toews *et al.* 2016). This explanation is particularly compelling in *Aglaeactis*, as the cases for which gene flow rather than other mechanisms lead to these islands have strong phenotypic differences with very little genomic variation (Toews *et al.* 2016; Turbek *et al.* 2021). In these cases, premating isolation from assortative mating by phenotype is usually implicated as the causal mechanism (Turbek *et al.* 2021).

Overall relative genomic differentiation was higher in the southern clade, in which all taxa are allopatric. This is in line with classic thinking of the geography of speciation, which emphasizes that such divergence must necessarily occur in isolation, lest gene flow erode differences before they can lead to reproductive barriers (Mayr 1942, 1963; Coyne and Orr 2004; Price 2008). The striking difference in the distribution of outlier windows in the northern and southern clades may be related to their different range distributions, in line with these models of the geography of speciation. In the

southern group, each of the three taxa (nominate *A. castelnaudii*, *A. ca. regalis*, and *A. pamela*) are distributed in relatively small areas not in direct contact with one another (Figure 3.1), with the only other *Aglaeactis* in direct range overlap being *A. cu. caumatonota.* Meanwhile, two of the northern taxa, *A cu. cupripennis* and *A. cu. caumatonota*, are far more widespread, and come into contact with both each other and *A. aliciae* (Figure 3.1). This difference in distributions and the potential for gene flow makes it possible that the predominant modes of speciation may differ radically in each clade, with a strictly allopatric model best describing the southern taxa while speciation with gene flow prevails in the parapatric north.

The overall lack of outlier windows on the Z chromosome is particularly striking. Sex chromosomes have repeatedly been implicated as the location of loci associated with the development of reproductive isolation (e.g., Carling and Brumfield 2009; Sætre and Sæther 2010; Storchová, Reif, and Nachman 2010; Ruegg *et al.* 2014; Lavretsky *et al.* 2015; Oyler-McCance *et al.* 2015; Battey 2020; Sendell-Price *et al.* 2020). It is possible that the lack of Z chromosome outliers is due to the low coverage of our genomes, which is more likely to impact the chromosome that is heterozygous in approximately half of our samples. This makes drawing conclusions from this result difficult, and reinforces that while low-coverage whole genomes can be a useful tool for addressing many issues in speciation genomics, it does come with its own limitations that need to be taken into account during study design. The lack of Z-linked outliers in rapidly diverging taxa is an intriguing possibility, given how it differs from typical observations in other avian systems, but with these data we cannot draw any specific conclusions.

Pervasive gene flow and a potential hybrid species

The finding of a far more heterogeneous pattern of genomic divergence in the north than the south suggests differing histories of gene flow. This hypothesis is supported by more detailed demographic modeling. In northern *Aglaeactis*, gene flow is far greater, and would create stronger selection for loci

involved in reproductive isolation, either due to reducing the chance of mating, as in the case of plumage, or in reducing the fitness of hybrids in the specific local environment, as seen in ecological speciation.

Effective population sizes were small and similar between all populations (Table 3.4). While this was unsurprising in some of the taxa, particularly *aliciae*, which at last survey had an estimated census population size of 1,000–2,500 individuals (BirdLife International 2021), it is surprising in many of the others, which, while no population estimates are available, have larger ranges than the extremely range-restricted *A. aliciae* (Schuchmann 1985, 1999; BirdLife International 2021). However, this estimate is sensitive to error in the generation time, and the available estimate used in IUCN assessments of 4.2 years (BirdLife International 2021) may not be accurate due to the difficulties of determining survivorship and reproductive life history in hummingbirds that live in the high Andes. Overall, our effective population sizes are likely overly conservative due to this uncertainty in life history, but as the error in this estimation is the same across all six taxa (as all have similar ecological niches and likely have similar annual survival and generation time), they are still useful in examining whether there are disparities in population sizes that would be an alternative driver of the patterns of genomic divergence we observe. All of the estimated population sizes are quite similar, and as the error in estimating them comes from the uncertainty in factors that apply equally to all, this remains true even if the estimates change by revising the mutation rate or generation time.

When viewed in light of this nearly ubiquitous gene flow, the ambiguity of the placement of *A. aliciae* in both phylogenomic (Figure 3.2) and SNP-based clustering analyses (Figure 3.4) raises the intriguing possibility that the taxon itself may be the result of hybridization between the two *A. cupripennis* subspecies. Although at first blush the taxa in question would not appear to be particularly similar, the white coloration in the face and breast that is characteristic of *A. aliciae* is similar to the patterns of orange seen in particularly *A. cu. caumatonota*, and thus a simple change in one of the genes implicated in coloration in birds (e.g., *ASIP*, (Toews *et al.* 2016; Campagna *et al.* 2017; Stryjewski and Sorenson

2017; Abolins-Abols *et al.* 2018); MC1R, (Stryjewski and Sorenson 2017; Funk and Taylor 2019; Palacios *et al.* 2019); FST, (Toews *et al.* 2016; Toomey *et al.* 2018; Kim *et al.* 2019)) could be driving the observed phenotypic variation. We believe that a likely scenario of the history of *aliciae* would run as follows: both *A. cu. cupripennis* taxa, having diverged previously with or without gene flow, have a contact zone similar to today's, but including the Marañón Valley. Climatic shifts then lead to the contraction of the two parent lineages' ranges, isolating a mix of both types in the Marañón. In this small population, existing reproductive barriers would be more likely to break down due to drift (Hubbs 1955; Rhymer and Simberloff 1996; Grant and Grant 1997; McCracken and Wilson 2011; Klein, Lagache-Navarro, and Petit 2017), and once the plumage change arose, it could quickly become fixed due to either drift or selection. Finally, post-glacial range expansions to the current distributions of *A. cu. cupripennis* and *A. cu. caumatonota* occur, and the gene flow we detect between all three taxa resumes, potentially increasing sexual selection on the novel *A. aliciae* phenotype. This scenario would fit with the observed patterns of genomic and phenotypic divergence we observe, but much more in-depth sampling, likely augmented with spectrophotometric evaluation of the plumage phenotypes of all three northern clade taxa, would be needed to further test this hypothesized history.

Dynamic landscapes and the genomics of rapid speciation

The speciation process is a continuum of divergence states, but the continuum is not necessarily a smooth one. The accumulation of divergence and the development of reproductive isolation is shaped by factors such as the pace of landscape-level changes and genomic organization (Flaxman, Feder, and Nosil 2013; Flaxman *et al.* 2014; Nosil *et al.* 2017; Riesch *et al.* 2017; McLaughlin, Faircloth, *et al.* 2020), and in *Aglaeactis* we see how both of these factors are at play in the radiation of the genus. Dynamic landscapes such as the Andes promote diversification not only by creating opportunities for geographic isolation and ecological selection, but additionally by favoring the rapid development of reproductive barriers that preserve incipient diversification once the landscape changes again in ways that would otherwise erode those differences. Furthermore, we see how the landscape of genomic

divergence is shaped by these differences in gene flow and geography, providing a road map for further investigation into which parts of the genome are responsible for creating and maintaining reproductive isolation against a backdrop of constant geographic change.

Speciation is a process shaped by the confluence of factors derived from the landscape on which an organism occurs, its ecological characteristics, and the intrinsic traits arising from the architecture of the genome. In *Aglaeactis,* these first and last factors are of particular importance, as the geographic situation of the northern versus the southern clades has played a key role in the development of the genomic landscape. Future work should examine whether genes potentially linked to phenotypic differences are located in these islands, and more comprehensively examine the patterns of discordant phylogenies across the genome. With our current low-coverage dataset, caution should be applied to our results and interpretation; however, we believe that the findings presented here illustrate the complexity of the evolutionary history of *Aglaeactis* and provide a starting point for further investigation.

Acknowledgments

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Figures

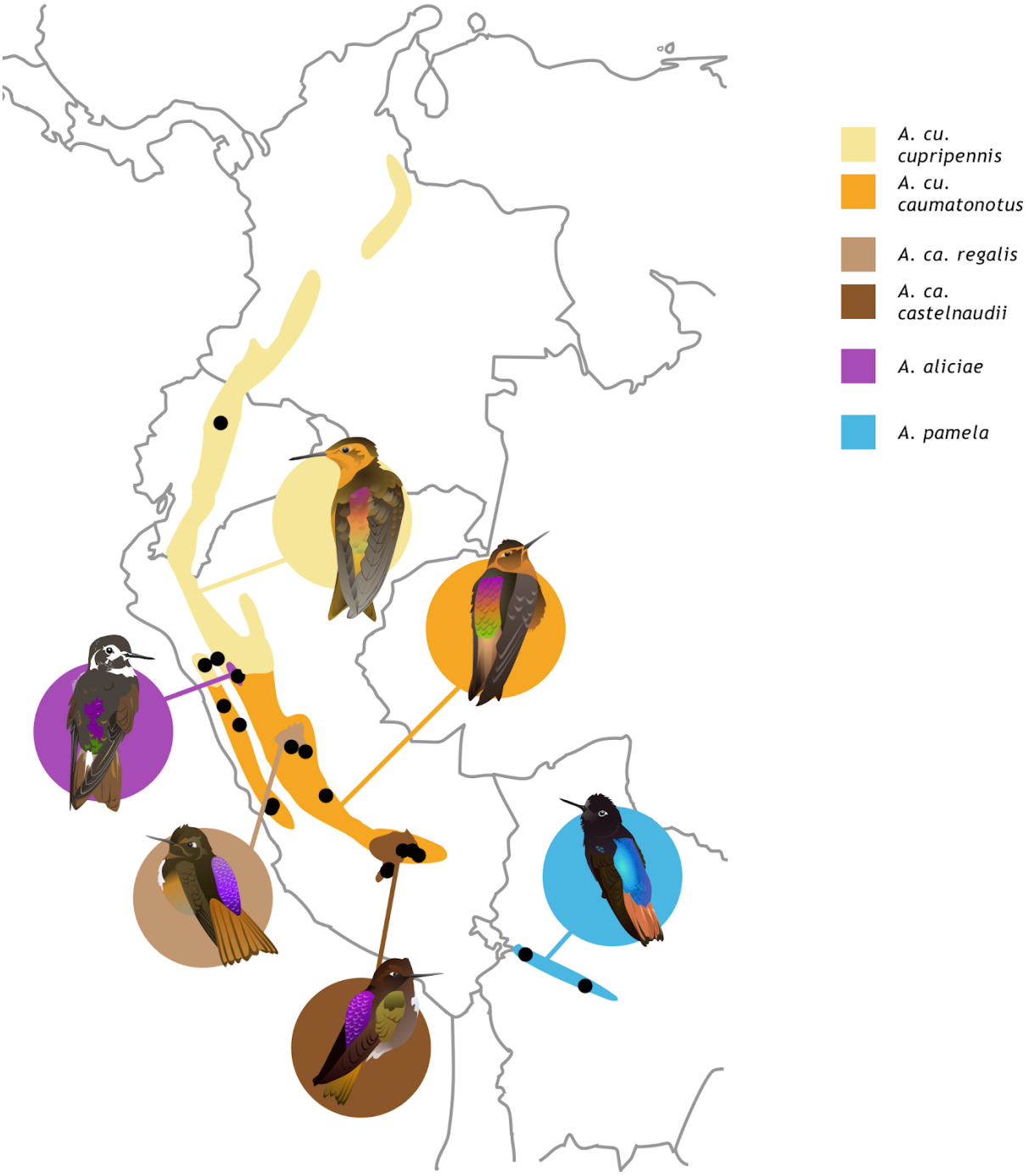

Figure 3.1: Ranges and collection localities of the six taxa in the genus *Aglaeactis*. Ranges sourced from Handbook of the Birds of the World (Schuchmann 1999).

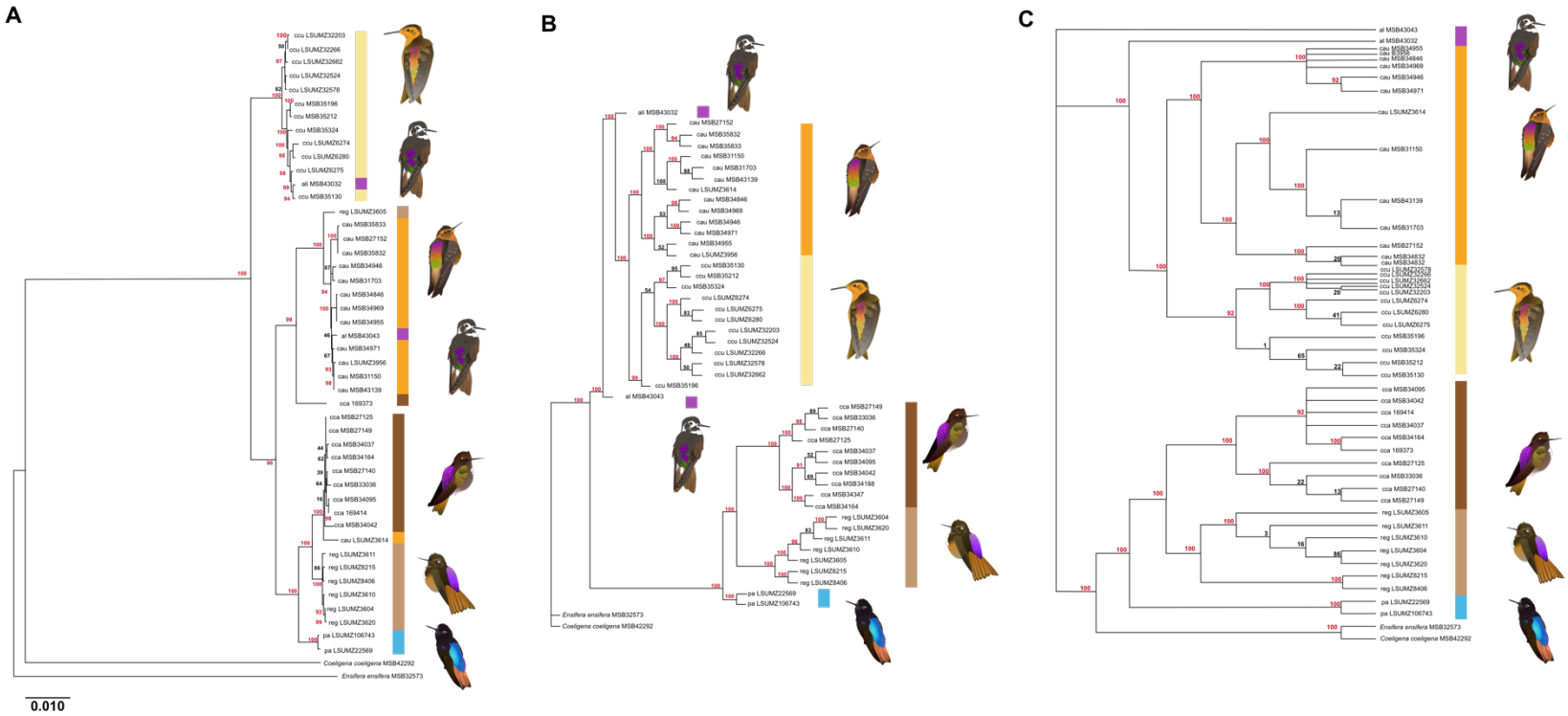

Figure 3.2: (A) Maximum-likelihood tree from whole mitogenomes, (B) ML tree generated in IQ-TREE with 186,011 nuclear SNPs, and (C) ML tree generated in RAxML with 68,541 nuclear SNPs, with taxa indicated by color-codes. Bootstrap values indicated on nodes, with values >90 in red. Colors and species as in Figure 3.1.

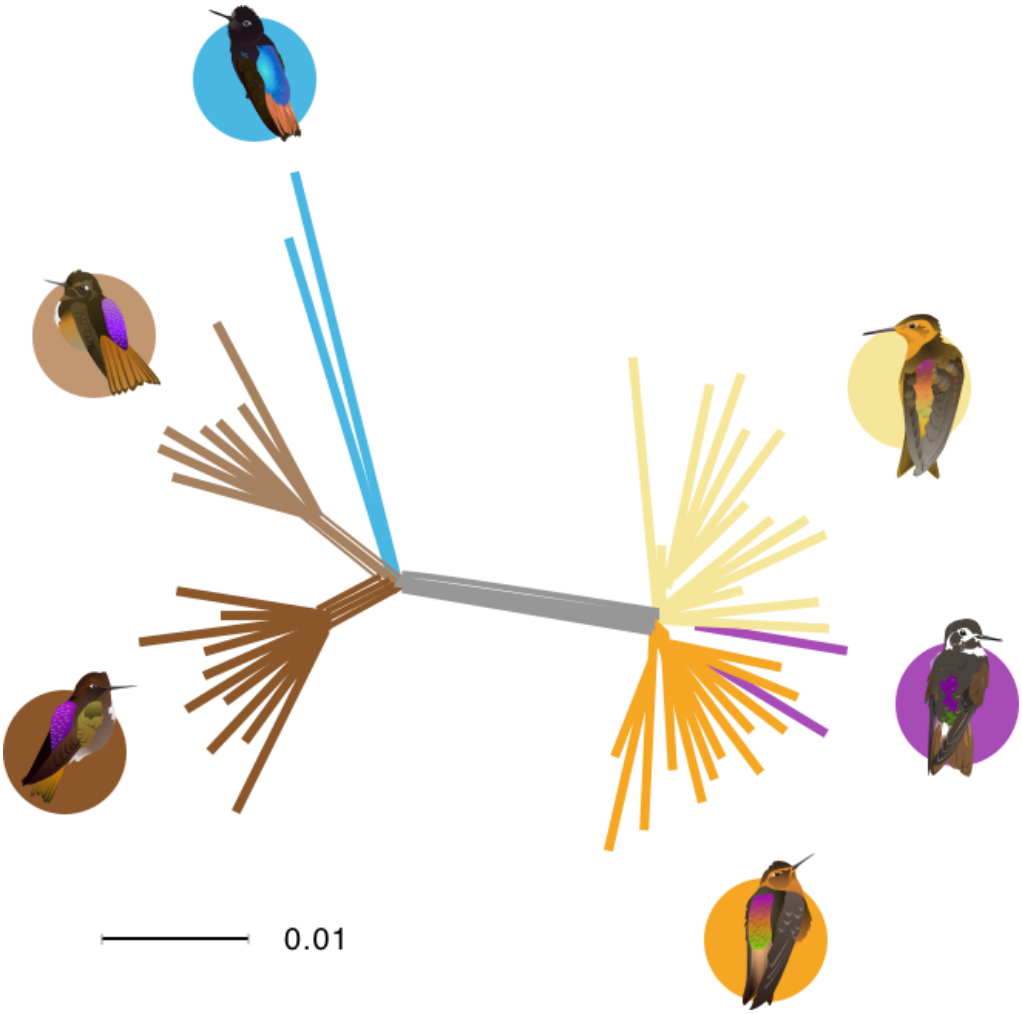

Figure 3.3: Splitstree phylogeny created from 71,505 SNPs. We find that in this unrooted tree, we recover the same clades as with our nuclear ML tree constructed in IQ-TREE, with the same northern and southern clades as seen above. Colors and species as in Figure 3.1.

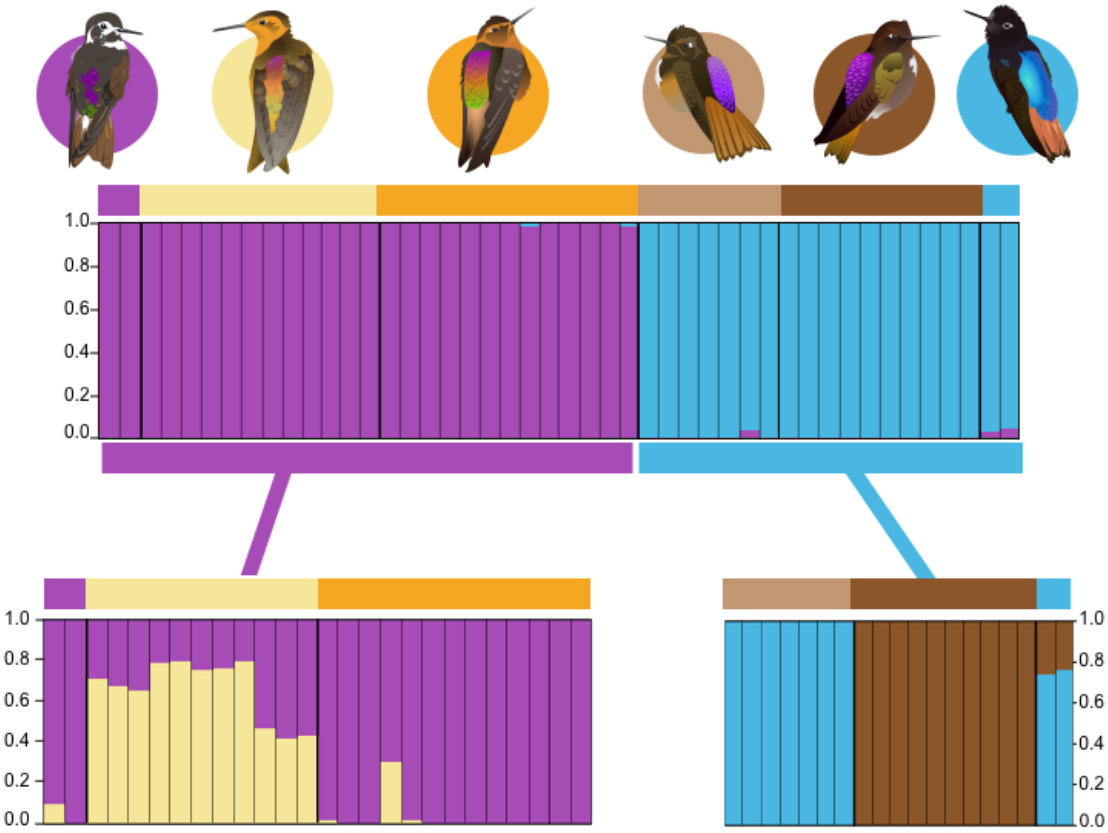

Figure 3.4: STRUCTURE analysis of 71,505 SNPs in 46 *Aglaeactis* individuals. Overall, the best fit value was $K = 2$, with clustering corresponding to northern (both *cupripennis* subspecies, oranges, and *aliciae*, purple) and southern (*castelnaudii*, browns, subspecies and *pamela*, blue) groups. When these two groups were analyzed separately, $K = 2$ was again found as the best fit, with *aliciae* and *pamela* not found to be distinctive clusters.

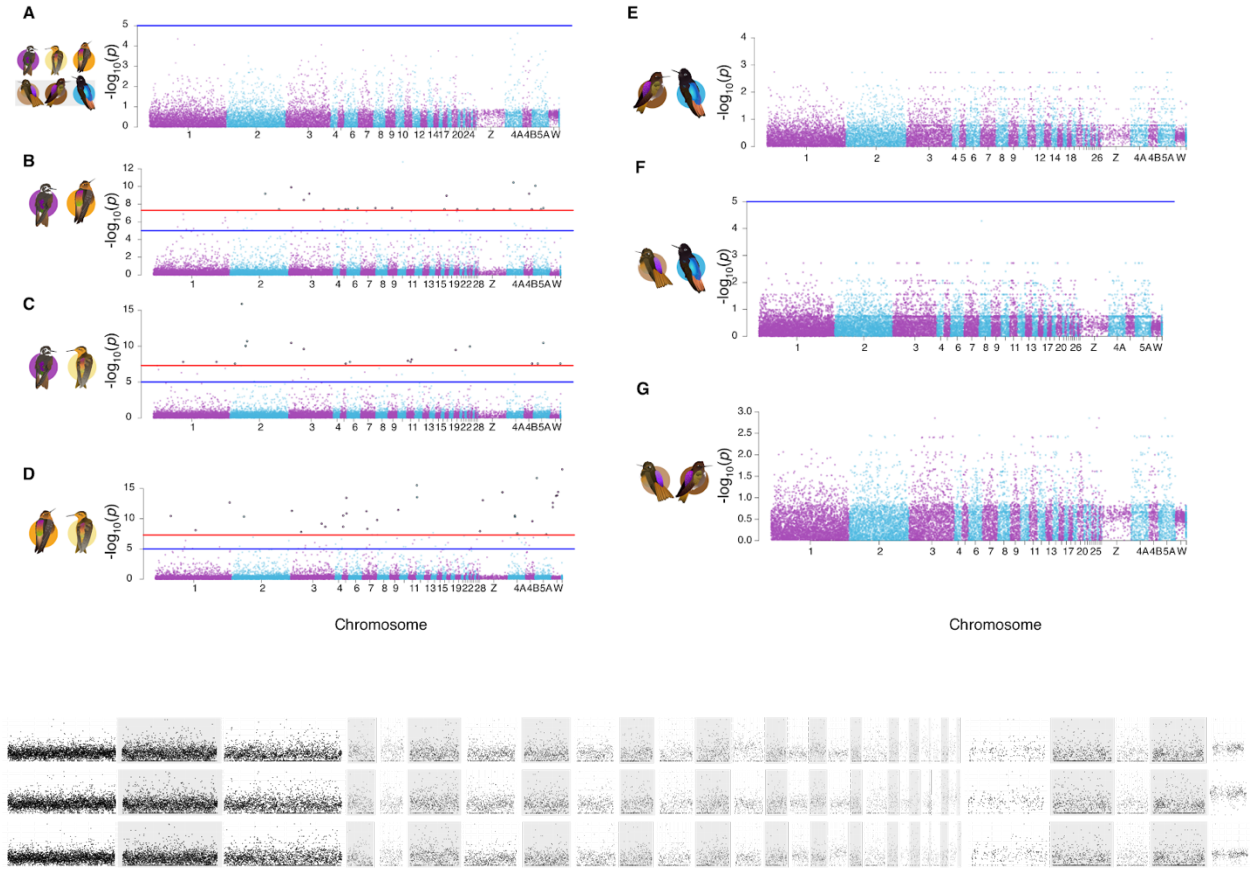

Figure 3.5: Distribution of F_{ST} by windows between (A) northern and southern *Aglaeactis*, (B) *aliciae* and *caumatonota*, (C) *aliciae* and nominate *cupripennis*, (D) nominate *cupripennis* and *caumatonota*, (E) nominate *castelnaudii* and *pamela*, (F) *regalis* and *pamela*, and (G) nominate *castelnaudii* and *regalis.* Colors and species as in Figure 1. Horizontal blue lines (shown on A, B, C, D, and F) indicate $-\log_{10}p(1 \times 10^{-5})$, with windows in excess of that being potentially elevated, though not significantly so. Horizontal red lines (only shown on B, C, and D) indicate $-\log_{10}(5 \times 10^{-8})$, with windows above that being significantly elevated above genome-wide F_{ST}. Panels lacking one or both lines did not have any windows in excess of either value. All comparisons are characterized by overall relative differentiation, with few outlier windows. In (H), *Dxy* across the genome is shown.

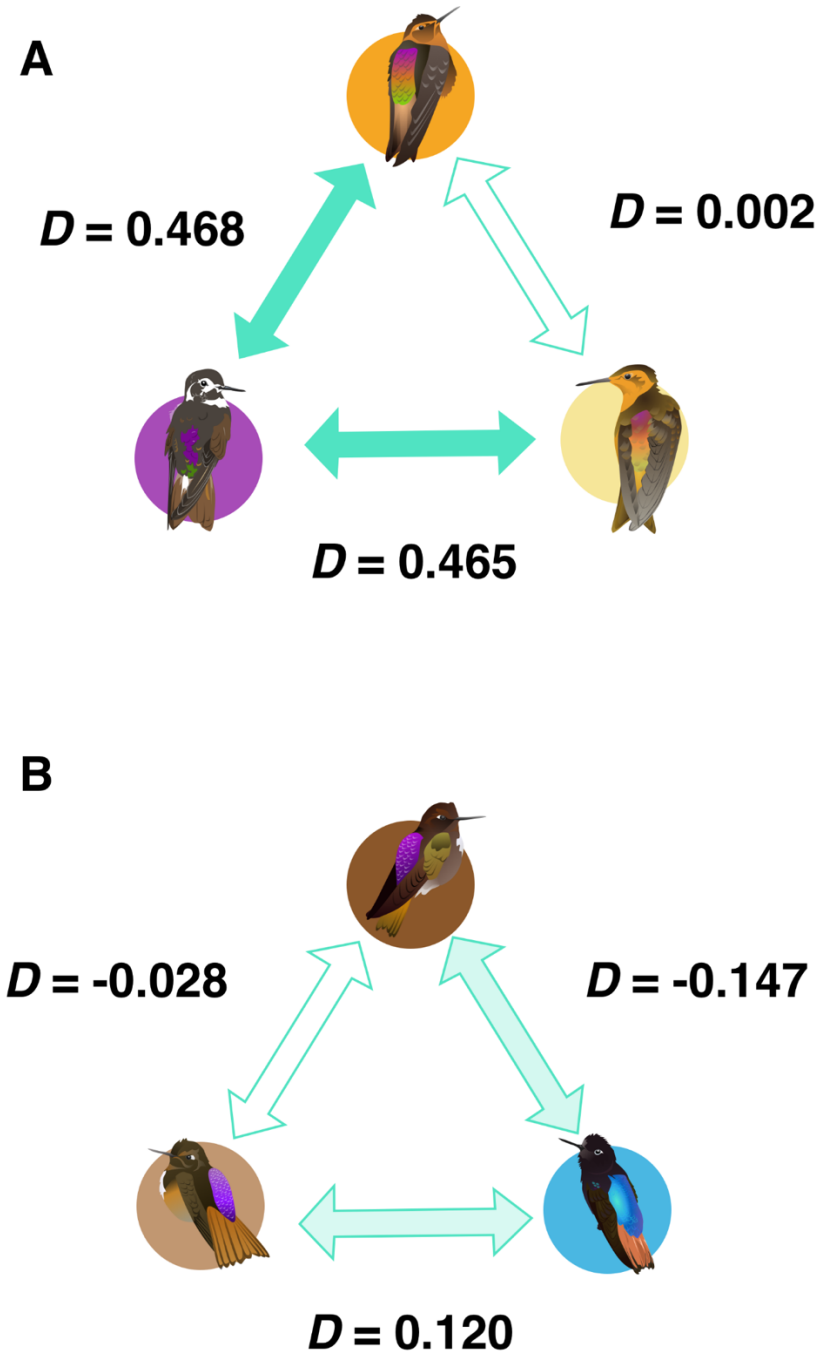

Figure 3.6: ABBA-BABA results for pairwise comparisons in the (A) northern and (B) southern clades. *D* values are shown for each pairwise comparison. Strong evidence of gene flow is indicated with fully colored-in arrows, weak evidence with lightly colored arrows, and no evidence with outlines only.

Tables

Table 3.1: Localities of specimens used in this study, with sequencing results and NCBI SRA numbers. Institution codes: Museum of Southwestern Biology (MSB), Louisiana State University Museum of Zoology (LSUMZ). Note that some individuals could not be assigned geographic coordinates. Reads are counted post-quality control, with coverage from aligned reads following duplicate removal.

Taxon	Locality	Catalog number	Reads (millions)	Coverage (average from aligned reads after duplicate removal)	SRA accession number
Aglaeactis aliciae	Peru: La Libertad, El Molino Viejo (-7.759, -77.765, 3543 m)	MSB:Birds:43032	21.3	1.88	SAMN28788481
	Peru: La Libertad, El Molino Viejo (-7.758, -77.764, 3551 m)	MSB:Birds:43043	32.9	2.89	SAMN28788482
Aglaeactis ca. castelnaudii	Peru: Cuzco, Urubamba (-13.199, -72.16, 4330 m)	MSB:Birds:27125	33.9	2.90	SAMN28788509
	Peru: Cuzco, Urubamba (-13.199, -72.16, 4400 m)	MSB:Birds:27140	21.3	1.89	SAMN28788510
	Peru: Cuzco, Urubamba (-13.199, -72.16, 4470 m)	MSB:Birds:27149	25.6	2.21	SAMN28788508
	Peru: Cuzco, Urubamba (-13.188, -72.231, 4030 m)	MSB:Birds:33036	32.4	2.91	SAMN28788511

	Peru: Apurímac, Abancay (-14.059, -73.003, 4411 m)	MSB:Birds:34037	25.1	2.27	SAMN28788512
	Peru: Apurímac, Abancay (-14.060, -73.008, 4369 m)	MSB:Birds:34042	27.5	2.48	SAMN28788513
	Peru: Apurímac, Abancay (-14.060, -73.008, 4375 m)	MSB:Birds:34095	24.1	2.13	SAMN28788514
	Peru: Apurímac, Abancay (-14.069, -73.017, 4578 m)	MSB:Birds:34147	13.5	1.24	SAMN28788515
	Peru: Apurímac, Abancay (-14.059, -73.002, 4512 m)	MSB:Birds:34164	18.8	1.67	SAMN28788516
	Peru: Apurímac, Abancay (-14.058, -73.001, 4485 m)	MSB:Birds:34188	29.5	2.61	SAMN28788517
Aglaeactis ca. regalis	Peru: Huánuco, Quebrada Shugush (-9.934, -76.453, 3100 m)	LSUMZ3604	24.6	2.91	SAMN28788518
	Peru: Huánuco, Quebrada Shugush (-9.934, -76.453, 3100 m)	LSUMZ3605	24.8	2.09	SAMN28788519
	Peru: Huánuco, Quebrada Shugush (-9.934, -76.453, 3100 m)	LSUMZ3610	21.1	1.75	SAMN28788520
	Peru: Huánuco, Quebrada Shugush (-9.934, -76.453, 3100 m)	LSUMZ3611	29.9	2.60	SAMN28788521
	Peru: Huánuco, Quebrada Shugush (-9.934, -76.453, 3100 m)	LSUMZ3620	37.7	3.23	SAMN28788522

	Peru: Pasco, Millpo (-9.9, -75.73, 3700 m)	LSUMZ8 215	13.6	1.19	SAMN28788523
	Peru: Huánuco, Hurao (-9.867, -75.801, 3500 m)	LSUMZ8 406	33.8	2.99	SAMN28788524
Aglaeactis cu. caumatonota	Peru: Cuzco, Urubamba (-13.199, -72.16, 4300)	MSB:Birds :27152	33.4	2.99	SAMN28788483
	Peru: Junín, Puente Carrizales (-11.489, -74.896, 3520 m)	MSB:Birds :31150	37.8	3.28	SAMN28788484
	Peru: Lima, Carhuayumac (-11.762, -76.549, 3750 m)	MSB:Birds :31703	21.1	1.87	SAMN28788485
	Peru: Ancash, Macate (-8.755, -78.048, 2972 m)	MSB:Birds :34846	31.4	2.82	SAMN28788486
	Peru: Ancash, Carhuaz (-9.572, -77.847, 3439 m)	MSB:Birds :34946	18.3	1.65	SAMN28788491
	Peru: Ancash, Carhuaz (-9.572, -77.847, 3439 m)	MSB:Birds :34955	29.8	2.71	SAMN28788487
	Peru: Ancash, Carhuaz (-9.572, -77.847, 3439 m)	MSB:Birds :34971	27.8	2.53	SAMN28788492
	Peru: Ancash, Carhuaz (-9.572, -77.847, 3439 m)	MSB:Birds :34969	34.5	2.99	SAMN28788488
	Peru: Cuzco, Paucartambo (-13.178, -72.596, 3361 m)	MSB:Birds :35832	20.0	1.80	SAMN28788496

	Peru: Cuzco, Paucartambo (-13.178, -72.596, 3361 m)	MSB:Birds:35833	20.3	1.82	SAMN28788497
	Peru: Lima, Huarochiri (-11.925, -76.652, 937 m)	MSB:Birds:43139	25.5	2.27	SAMN28788490
	Peru: Huánuco, Quebrada Shugush (-9.934, -76.453, 3100 m)	LSUMZ3614	27.2	2.44	SAMN28788507
	Peru: Huánuco	LSUMZ3956	33.4	2.93	SAMN28788498
Aglaeactis cu. cupripennis	Peru: Cajamarca, Contumaza (-7.398, -78.778, 2500 m)	MSB:Birds:35130	29.7	2.63	SAMN28788489
	Peru: Cajamarca, Contumaza (-7.398, -78.778, 2500 m)	MSB:Birds:35196	10.0	0.98	SAMN28788493
	Peru: Cajamarca, Contumaza (-7.398, -78.778, 2500 m)	MSB:Birds:35212	32.1	2.88	SAMN28788494
	Peru: Cajamarca, Contumaza (-7.398, -78.778, 2500 m)	MSB:Birds:35324	32.6	2.94	SAMN28788495
	Ecuador: Pichincha	LSUMZ6274	26.0	2.33	SAMN28788499
	Ecuador: Pichincha	LSUMZ6275	27.2	2.44	SAMN28788500
	Ecuador: Pichincha	LSUMZ6280	21.7	1.97	SAMN28788501

	Peru: Cajamarca	LSUMZ32203	21.4	1.84	SAMN28788502
	Peru: Cajamarca	LSUMZ32266	23.6	2.11	SAMN28788503
	Peru: Cajamarca	LSUMZ32524	27.3	2.45	SAMN28788504
	Peru: Cajamarca	LSUMZ32578	21.7	1.95	SAMN28788505
	Peru: Cajamarca	LSUMZ32662	21.1	1.89	SAMN28788506
Aglaeactis pamela	Bolivia: La Paz	LSUMZ22569	32.0	2.82	SAMN28788525
	Bolivia: Cochabamba	LSUMZ106743	23.2	1.99	SAMN28788526
Coeligena coeligena	Peru: Amazonas, Las Pinas (-6.049, -78.227, 2150 m)	MSB:Birds:42292	28.3	2.45	SAMN28788527
Ensifera ensifera	Peru: Amazonas, Tullanya (-6.080, -78.325, 2937 m)	MSB:Birds:32573	37.4	3.26	SAMN28788528

Table 3.2: Measures of genomic differentiation between A) northern and southern clades of *Aglaeactis* and B) pairwise comparisons of individual *Aglaeactis* taxa

Comparison		F_{ST}	Outlier windows
Northern clade (*cupripennis* nominate, *caumatonota*, *aliciae*)	Southern clade (*castelnaudii* nominate, *regalis, pamela*)	0.2197	0% (0/17,287)
aliciae	*cupripennis*	0.0297	0.11% (18/16,563)
aliciae	*caumatonota*	0.0274	0.15% (24/16,414)
cupripennis	*caumatonota*	0.0484	0.20% (33/16,869)
pamela	*castelnaudii*	0.2085	0% (0/16,086)
pamela	*regalis*	0.2031	0% (0/15,944)
castelnaudii	*regalis*	0.2486	0% (0/16,391)

Table 3.3: ðaði model fitting for each comparison. Maximum log composite likelihood (MLCL) for each is shown, followed by AICc weights in parentheses. Numbers in bold indicate best-fit models, with cases of partial support being italicized. Sample sizes correspond with the order of the names in the first column.

Comparison	Sample size	Neutral	IM	Island	Split (no migration)	Splitmig (equal migration)	Splitmig_2m	SC_1m	SC_2m
aliciae/caumatonota	2:	-1176.13	-3099.24	-8207.71	-8838.96	-1075.74	-1643.25	-1109.	**-722.38**
aliciae/cupripennis nominate	2:	-1779.25 (2.77×10^{-85})	-2398.46 (0)	-6312.76 (0)	-11287.46 (0)	**-1582.55** (1)	-2202.59 (1.9×10^{-270})	-1924.19 (1.6×10^{-149})	-1645.84 (4.41×10^{-29})
caumatonota/cupripennis nominate		-8419.19 (0)	-5983.23 (0)	-20405.42 (0)	-34029.45 (0)	**-3563.93** (1)	-4509.98 (0)	-4749.79 (0)	-3762.66 (6.7×10^{-88})
pamela/castelnaudii nominate	2:	-7567.60	-744.90	-4818.24	-5512.78	-683.32	-4064.88		**-176.65**
pamela/ regalis	2:7	-6313.56 (0)	-696.52 (1.5×10^{-261})	-5024.86 (0)	-3422.68 (0)	-521.62 (3.8×10^{-185})	-669.41 (9.1×10^{-250})	-521.21 (7.7×10^{-186})	**-95.97** (1)
regalis/castelnaudii nominate	7:	-39034.75 (0)	-4444.20 (0)	-9965.02 (0)	-15328.13 (0)	-2247.07 (2.5×10^{-207})	-5758.96 (0)	-1919.00 (7.9×10^{-65})	**-1770.40** (1)

Table 3.4: Estimated demographic variables from δaδi modeling, with raw parameters in parentheses. Included are estimates of effective population sizes (N_e) of each population, effective population size of the ancestral population (N_{ref}), time since divergence in years (T_S) and, for applicable models, time of secondary contact (T_{SC}), and migration rates, either total (M) or in each direction from population 1 to 2 (M_{12}) or vice versa (M_{21}).

Comparison	N_e first taxon (individuals)	N_e second taxon (individuals)	T_S	T_{SC}	M	M_{12}	M_{21}	N_{ref} (individuals)
aliciae/caumatonota	491.69 (1.0221)	828.51 (1.7222)	43,549 (10.7769)	3,709 (0.9180)		2.27 (4.4565)	0.088 (0.1725)	481.07 (4079.51)
aliciae/ cupripennis nominate	201.85 (0.1211)	873.12 (0.5238)	191.24 (0.0137)		2.01 (7.67)			1,666.91 (13,033.70)
caumatonota/cupripennis nominate	196.08 (0.1355)	244.17 (0.1687)	210.79 (0.0173)		0.4934 (5.8491)			1,447.47 (12,717.91)
pamela/castelnaudii nominate	601.38 (0.9384)	70.51 (0.1100)	25,110.57 (4.6645)	973.92 (0.1809)		0.9280 (1.9779)	0.3239 (5.8895)	640.87 (5212.37
pamela/ regalis	350.16 (0.1629)	941.66 (0.4377)	178,878.15 (9.896)	101,203.52 (5.600)		0.0098 (0.0449)	0.1031 (0.4710)	2,151.24 (14,555.62)
regalis/castelnaudii nominate	589.95 (0.2432)	486.30 (0.2007)	85,266.92 (4.1888)	55,886.21 (2.7454)		0.0703 (0.7012)	0.04198 (0.4184)	2,423.38 (20,539.62)

General Conclusions

In studying the process of speciation, it is natural to be drawn to regions particularly rich in biodiversity to test hypotheses on the mechanisms that lead to the development and maintenance of reproductive isolation. Using genetic data from single COI barcodes to full genomes, I have tested several specific hypotheses on how intrinsic organismal traits shape the speciation process, and how they interact with the more commonly studied landscape history to generate the rich avifaunal diversity of the Neotropics.

Time does not predict reproductive isolation

One of my most striking findings is that time in allopatry does not predict the development of reproductive isolation, as observed in the context of secondary contact (Chapter 2). This is counter to both theoretical predictions and previous experimental evidence. In their foundational 1989 paper, Coyne and Orr state that "[t]he divergence time of taxa must obviously be correlated with the amount of reproductive isolation between them, because all species begin as populations that are not reproductively isolated." While this has been supported by evidence of both increasing prezygotic (Coyne and Orr 1989, 1997; Turissini *et al.* 2018; Mendelson 2003; Tilley, Verrell, and Arnold 1990; Arnegard *et al.* 2010; P. A. Moran *et al.* 2020) and postzygotic isolation (Mendelson 2003; Charistianson, Swallow, and Wilkinson 2005; Scopece *et al.* 2007; Edmands 2002; Gourbière and Mallet 2010; Coughlan and Matute 2020), for other reproductive barriers, this prediction has not held up (Gleason and Ritchie 1998; Campagna *et al.* 2012; R. L. Moran *et al.* 2017). The probability of hybridization with increasing divergence has been examined less often (Matute and Cooper 2021), but it has generally been found to decrease as the age of the taxa split grows (Wiens, Engstrom, and Chippindale 2006; Kronforst *et al.* 2013; Hamlin, Hibbins, and Moyle 2020; Mallet 2007; Pereira, Monahan, and Wake 2011; Sánchez-Guillén *et al.* 2014).

I do not find evidence that time increases reproductive isolation in Panamanian birds, contrary to these predictions.

Ecology predicts diversification and reproductive isolation

I find strong evidence that in Panamanian birds, ecological traits linked to dispersal capability are linked to both the occurrence of species-level mitochondrial variation and to the outcomes of secondary contact. The role of ecology in driving the generation of species is of course foundational to the very study of biology, but often focuses on the particulars of a population's adaptation to the environment and community in which it finds itself. By instead asking what ecological traits are associated with specific outcomes in the tempo and mode of speciation, we can build models of speciation that are less about the particulars of a landscape, and more about how the characteristics of an organism can predict its evolutionary trajectory.

The role of dispersal and gene flow in speciation

In all my chapters, I find that dispersal and gene flow are heavily intertwined in shaping the speciation process. In Panama, traits associated with lower dispersal ability (namely smaller HWI, strong territoriality, forest undergrowth habitats, and insectivory) were overrepresented in taxa with two or more mitochondrial BINs. This is in line with previous work that ties greater diversity to lower dispersal (Belliure *et al.* 2000; Miller *et al.* 2021).

Low dispersal capability is not only tied to the development of differentiation, but to the maintenance of it during secondary contact. Diet was the best predictor of median cline width, and HWI was, in combination with time, part of the best fit model for fixation rates on both the autosomes and Z chromosome. We see this key role in dispersal shaping the genomic landscape in early speciation again in *Aglaeactis*, where the distribution of heightened divergence is associated with higher levels of gene

flow. This drives home how gene flow does not act as merely a destroyer of genetic differentiation, but can instead lead to a variety of outcomes as populations come into contact.

Future directions

A key piece that warrants further exploration in both study systems is the role of demography. In Panama, the best predictor of median cline width is diet. While diet is associated with dispersal ability in birds (Sheard *et al.* 2020), HWI is a much more direct proxy for this (Claramunt and Wright 2017; Sheard *et al.* 2020), and this was not recovered as a predictor of cline width. As there are striking differences in the demographic dynamics associated with diet type, disentangling their role in determining the outcomes of secondary contact is an important next step. Our ability to test this directly is limited by our current data, however, as loci linked to ultraconserved elements are, by nature of their linkage to highly conserved regions, likely to be biased conservatively in their ability to detect demographic changes (McLaughlin *et al.* 2020). Population demographic models with loci less likely to be under purifying selection are needed to properly address this question.

A more in-depth investigation of demographics is also warranted in the *Aglaeactis* system. Our low-coverage data, while adequate for the analyses performed in Chapter 3, is not robust enough for confident modeling of population changes over time beyond our relatively simple demographic models. As one travels back into the evolutionary history of a population pair, distinguishing between discrete periods of gene flow or multiple bouts of population expansion and contraction become increasingly difficult (Gutenkunst *et al.* 2009), and require more sites to be confidently called to come to a reliable answer. Greater sequencing coverage of at least some of the individuals in each population would provide the necessary data quality to make these inferences.

Final thoughts

The process by which a population becomes a species is one driven by the interactions of an untold myriad of factors, working in concert to generate the outcomes we can see in the rich array of life on Earth. Some of the threads in this dense web of forces will be thick and obvious; others will be the faintest thought of a fiber. It is easy to unpick the most obvious threads, but these subtler threads may reward investigation with a deeper understanding of the web as a whole. My dissertation is an exploration of some of these less prominent threads. Species arise not merely from passive organisms being acted upon by a landscape, but the combination of landscape, ecology, and the nature of the genome itself. By stepping back and considering all of these threads, we come closer to fully taking in, to quote Darwin, "the grandeur in this view of life."

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Appendix 1: Supplementary Information for Chapter 1

Table A1.1: Samples used in this study, including the individual museum catalog number, field catalog number(s) used to refer to the sample in BOLD, NCBI, or other public repositories, locality, sequence type, and accession information on NCBI or BOLD. Some samples, listed with no museum number, are available only as tissues in the STRI Bird Tissue collection under the provided field catalog number, unless otherwise noted. ANSP: Academy of Natural Sciences of Philadelphia, CUMV: Cornell University Museum of Vertebrates, FMNH: Field Museum of Natural History, KU:O: University of Kansas Ornithology Collection, LSUMZ: Louisiana State University Museum of Zoology, STRIBC: Smithsonian Tropical Research Institute Bird Collection, UAM: University of Alaska Museum, USNM: US National Museum, Smithsonian Institute, UWBM: University of Washington Burke Museum. In some cases, museum numbers were not available, and so the sample is only referred to by its label from either BOLD or NCBI.

Species	**Museum Number**	**Other Associated Numbers**	**Locality**	**Type**	**Accession Numbers**
Arremon aurantiirostris	USNM607183	B00383	Panama: Bocas del Toro, Isla San Cristobal	COI	BOLD:608287
Arremon aurantiirostris	USNM608001	B01223	Panama: Comarca Ngäbe-Buglé, Valiente Peninsula, Punta Alegre	COI	BOLD:1608286

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Arremon aurantiirostris	USNM608246	B01982	Panama: Panama: Bocas del Toro, Chiriquí Grande	COI	BOLD:1608285
Arremon aurantiirostris	FMNH470778	JMD671	Panama: Panama, Chiman, Cerro Chucantí	COI	BOLD: 3741539
Arremon aurantiirostris	FMNH470779	JMD675	Panama: Panama, Chiman, Cerro Chucantí	COI	BOLD: 3741541
Arremon aurantiirostris	UWBM108617	GMS996	Panama: Veraguas, Santa Fé	COI	BOLD: 6288801
Arremon aurantiirostris	UAM22804	JMM937	Panama: Darién, Cana	COI	BOLD: 2616640
Arremon aurantiirostris	UAM22808	JMM933	Panama: Darién, Cana	COI	BOLD: 2616639
Arremon aurantiirostris	UAM28805	JMM917	Panama: Darién, Cana	COI	BOLD: 2616637

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Arremon aurantiirostris		PA-AAR-PC5	Panama: Panama: Colón, Achiote	COI	BOLD: 4567590
Arremon aurantiirostris	UAM22807	PA-AAU2005 MJM2005	Panama: Darién, Cana	COI ND2	BOLD: 603996 KR781507.1
Arremon aurantiirostris	CUMV51277	PA-AAU67 IJL04-067	Panama: Bocas del Toro, Chiriquí Grande, Rio La Gloria	COI	BOLD: 603990
Arremon aurantiirostris	FMNH470775	PA-AAU672 JMD672	Panama: Panama, Chiman, Cerro Chucantí	COI	BOLD: 3741540
Arremon aurantiirostris	UWBM106601	GMS1179	Panama: Colón, Achiote	Whole mitogenome	KR780063.1
Arremon aurantiirostris	UWBM111383	JK04-303	Panama: Colón, Achiote	ND2	KR781504.1

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Arremon aurantiirostris	UAM25730	MJM1443	Panama: Panama: Colón, 20 km west of Gatún	ND2	KR781505.1
Arremon aurantiirostris	UWBM106577	GMS1154	Panama: Colón, Achiote	ND2	KR781506.1
Arremon aurantiirostris	UAM25716	MJM1931	Panama: Darién, Cana	ND2	KR781508.1
Arremon aurantiirostris	UAM22806	MJM1948	Panama: Darién, Cana	ND2	KR781509.1
Arremon aurantiirostris	FMNH470780	GMS2011	Panama: Bocas del Toro, Bosque Protector de Palo Seco	ND2	KR781510.1
Arremon aurantiirostris	UWBM123617	JK06-229	Panama: Bocas del Toro, Bosque Protector de Palo Seco	ND2	KR781511.1

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Arremon aurantiirostris	UWBM123533	JK06-148	Panama: Panama: Bocas del Toro, Bosque Protector de Palo Seco	ND2	KR781512.1
Arremon brunneinucha	KU:O:87123	B05474	Panama: Chiriquí, Los Planes	COI	BOLD: 1608498
Arremon brunneinucha	KU:O:87102	B05329	Panama: Chiriquí, Los Planes	COI	BOLD: 1608281
Arremon brunneinucha	KU:O:87101	B05407	Panama: Chiriquí, Los Planes	COI	BOLD: 1608280
Arremon brunneinucha	UWBM108337	JMD126	Panama: Veraguas, Santa Fé	COI	BOLD: 6288803
Arremon brunneinucha	STRIBC3164	MJM7675	Panama: Comarca Ngäbe-Buglé, Nole Duima, Altos Las Nubes	COI	BOLD: 3741605

Species	**Museum Number**	**Other Associated Numbers**	**Locality**	**Type**	**Accession Numbers**
Arremon brunneinucha	STRIBC3165	MJM7676	Panama: Comarca Ngäbe-Buglé, Nole Duima, Altos Las Nubes	COI	BOLD: 3741606
Arremon brunneinucha	STRIBC2304	MJM5982	Panama: Chiriquí, Boquete	COI	BOLD: 3741562
Arremon brunneinucha	UWBM108891	PA-BBR702	Panama: Panama, Chiman, Cerro Chucantí	COI	BOLD: 603997
Arremon brunneinucha	UAM25737	MJM1011	Panama: Darién, Tropic Star Lodge	COI	BOLD: 2616651
Automolus ochrolaemus	UAM36650	MJM2047	Panama: Darién, Cana	COI	BOLD: 2616660
Automolus ochrolaemus	STRIBC3611	MJM8066	Panama: Coclé, La Pintada, El Copé	COI	BOLD: 3740856

Species	**Museum Number**	**Other Associated Numbers**	**Locality**	**Type**	**Accession Numbers**
Automolus ochrolaemus	UAM25368	JMM957	Panama: Darién, Cana	COI	BOLD: 3740858
Automolus ochrolaemus		PA-OCR155 JTW155	Panama: Bocas del Toro, Chiriquí Grande	COI	BOLD: 4431395
Automolus ochrolaemus	USNM614009	B1990	Panama: Bocas del Toro, Chiriquí Grande	cytB	KM079758.1
Automolus ochrolaemus	LSU51424		Panama: Chiriquí	cytB	KM079757.1
Automolus ochrolaemus		BAR15332	Panama: Panamá, Cerro Jefe	cytB	KM079762.1
Automolus ochrolaemus	LSU2241		Panama: Darién, Cana	cytB ND3	KM079763.1
Automolus ochrolaemus	LSU26528		Panama: Colón, Gamboa	cytB ND3	KM079764.1

Species	**Museum Number**	**Other Associated Numbers**	**Locality**	**Type**	**Accession Numbers**
Automolus ochrolaemus	LSU26537		Panama: Colón, Gamboa	cytB	KM079765.1
Automolus ochrolaemus	FMNH470676	JMD874	Panama: Chiriquí, Burica Peninsula	cytB	KM079760.1
Automolus ochrolaemus	FMNH470677	JMD867	Panama: Chiriquí, Burica Peninsula	cytB	KM079759.1
Baryphthengus martii	USNM606819	B00285	Panama: Bocas del Toro, Isla San Cristobal	COI	BOLD: 1641196
Baryphthengus martii	USNM613419	B01306	Panama: Comarca Ngäbe-Buglé, Valiente Peninsula, Punta Alegre	COI	BOLD: 1608120
Baryphthengus martii	USNM613420	B01341	Panama: Comarca Ngäbe-Buglé, Valiente Peninsula, Punta Alegre	COI	BOLD: 1608119

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Baryphthengus martii	UAM20530	MJM922	Panama: Darién, Tropic Star Lodge	COI	BOLD: 2616795
Baryphthengus martii	STRIBC0477	MJM3485	Panama: Coclé, La Pintada, El Copé	COI	BOLD: 3740933
Baryphthengus martii	STRIBC0476	MJM3511	Panama: Coclé, La Pintada, El Copé	COI	BOLD: 3740934
Baryphthengus martii	CUMV50835	IJL04-075 PA-BMR75	Panama: Bocas del Toro, Chiriquí Grande, Rio La Gloria	COI	BOLD: 604041
Baryphthengus martii		MJM455	Panama: Panamá, Cerro Azul	COI	BOLD: 604044
Baryphthengus martii	UAM24957	JMM1056	Panama: Darién, Cana	COI	BOLD: 604043

Species	**Museum Number**	**Other Associated Numbers**	**Locality**	**Type**	**Accession Numbers**
Baryphthengus martii	ANSP189174	PA-BMR3781	Panama: Colón, Gamboa	COI	BOLD: 604042
Cantorchilus leucotis	UAM34575	JMM889 PA-TLU889	Panama: Colón, Gamboa	COI	BOLD: 604773
Cantorchilus leucotis	STRIBC3442	MJM7832	Panama: Darién, Chepigana, Aruza Abajo	COI	BOLD: 2616752
Cantorchilus leucotis	STRIBC3520	MJM7898	Panama: Darién, Chepigana, Aruza Abajo	COI	BOLD: 2616776
Cantorchilus modestus	USNM613332	B0103	Panama: Chiriquí, Los Planes	COI	BOLD: 1608396
Cantorchilus modestus	KU:O:87012	B05397	Panama: Chiriquí, Los Planes	COI	BOLD: 1608395
Cantorchilus modestus	STRIBC1504	MJM6741	Panama: Colón, Achiote	COI	BOLD: 3741146

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Cantorchilus modestus		GMS2174 PA-TMO2174	Panama: Chiriquí, Burica Peninsula	COI	BOLD: 604776
Cantorchilus modestus		PA-TMO-PC95	Panama: Colón, French Canal	COI	BOLD: 604777
Cantorchilus modestus		PA-TMO46466	Panama: Bocas del Toro, Changuinola	COI	BOLD: 604775
Cantorchilus modestus	UAM26274	MJM631 PA-TLU631	Panama: Panamá, Cerro Azul	COI	BOLD: 604774
Cantorchilus nigricapillus	USNM607864	B01007	Panama: Bocas del Toro, Isla Escudo de Veraguas	COI	BOLD: 1608397
Cantorchilus nigricapillus	USNM607869	B01102	Panama: Bocas del Toro, Cayo Agua	COI	BOLD: 1608398
Cantorchilus nigricapillus	USNM605408	B01745	Panama: Bocas del Toro, Bastimentos Island	COI	BOLD: 853609

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Cantorchilus nigricapillus	CUMV44260	IJL04-038	Panama: Bocas del Toro, Chiriquí Grande, Rio La Gloria	COI	BOLD: 604781
Cantorchilus nigricapillus	UWBM111237	JK04-146	Panama: Veraguas, Santa Fé	COI	BOLD: 604794
Cantorchilus nigricapillus	UWBM77020	PA-TNI181 VGR181	Panama: Panama, Panama City, confluence of Rios Chagres and Chagrecito	COI	BOLD: 604765
Cantorchilus nigricapillus		PA-TNI302	Panama: Bocas del Toro, Isla San Cristobal	COI	BOLD: 604789
Cantorchilus nigricapillus	UWBM111384	JK04-304	Panama: Colón, Achiote	COI	BOLD: 604784
Cantorchilus nigricapillus	UAM25353	MJM607	Panama: Colón, Gamboa	COI	BOLD: 604785

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Cantorchilus nigricapillus	UAM25350	MJM647	Panama: Panamá, Cerro Azul	COI	BOLD: 604790
Cantorchilus nigricapillus	UWBM101013	JTW720	Panama: Darién, Puerto Piña	COI	BOLD: 604787
Cantorchilus nigricapillus		PA-TNI1029	Panama: Bocas del Toro, Isla Escudo de Veraguas	COI	BOLD: 604788
Cantorchilus nigricapillus		PA-TNI1249	Panama: Comarca Ngäbe-Buglé, Valiente Peninsula, Punta Alegre	COI	BOLD: 604793
Cantorchilus nigricapillus	UWBM123299	GMS1906	Panama: Panama, Chiman, Cerro Chucantí	COI	BOLD: 604791
Cantorchilus nigricapillus		PA-TNI1986	Panama: Bocas del Toro, Chiriquí Grande	COI	BOLD: 604779

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Cantorchilus nigricapillus		PA-TNI2269	Panama: Darién, Cana	COI	BOLD: 604791
Cantorchilus nigricapillus		PA-TNI2272	Panama: Darién, Cana	COI	BOLD: 605554
Cantorchilus nigricapillus		PA-TNI26392	Panama: Panamá, Serranía de San Blas, west end	COI	BOLD: 604795
Cantorchilus nigricapillus	N.A. (blood only)		Panama: Darién, Cana	cytB	DQ415701.1
Cantorchilus nigricapillus		PA-TNI1028	Panama: Bocas del Toro, Isla Escudo de Veraguas	ATP8/6	AY103277.1
Cantorchilus nigricapillus		PA-TNI1103	Panama: Bocas del Toro, Cayo Agua		AY103279.1

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Cantorchilus nigricapillus		PA-TNI1250	Panama: Comarca Ngäbe-Buglé, Valiente Peninsula, Punta Alegre		AY103281.1
Cantorchilus nigricapillus		PA-TNI1762	Panama: Bocas del Toro, Bastimentos Island		AY103283.1
Cantorchilus nigricapillus		PA-TNI28552	Panama: Colón, Achiote		AY103285.1
Cantorchilus nigricapillus		PA-TNI28559	Panama: Colón, Achiote		AY103286.1
Cantorchilus nigricapillus		PA-TNI305	Panama: Bocas del Toro, Isla San Cristobal		AY103288.1
Cantorchilus nigricapillus		PA-TNI494	Panama: Bocas del Toro, Tierra Oscura, Isla San Cristóbal		AY103289.1

Species	**Museum Number**	**Other Associated Numbers**	**Locality**	**Type**	**Accession Numbers**
Cantorchilus nigricapillus		PA-TNI26393	Panama: Panamá, Chepo		AY103291.1
Catharus fuscater	USNM607615	B01433	Panama: Chiriquí, Gualaca	COI	BOLD: 1641287
Catharus fuscater	USNM607617	B01537	Panama: Chiriquí, Gualaca	COI	BOLD: 1608408
Catharus fuscater	KU:O:87033	B05427	Panama: Chiriquí, Los Planes	COI	BOLD: 1608409
Catharus fuscater	UWBM112249	PA-CT62 JK06-062	Panama: Panama, Chiman, Cerro Chucantí	COI	BOLD: 605109
Cercomacroides tyrannina	STRIBC2963	MJM6937	Panama: Veraguas, Santa Fé	COI	BOLD: 3741716
Cercomacroides tyrannina	STRIBC3901	MJM7908	Panama: Darién, Chepigana, Chucanaque, El Salto	COI	BOLD: 2616779
Cercomacroides tyrannina		PA-CTYPC10	Panama: Colón, Achiote	COI	BOLD: 604269

Species	**Museum Number**	**Other Associated Numbers**	**Locality**	**Type**	**Accession Numbers**
Cercomacroides tyrannina	UWBM112249	JMD143 PA-CTY143	Panama: Veraguas, Santa Fé	COI	BOLD: 604269
Cercomacroides tyrannina	UWBM12372	JK06-383 PA-CTY383	Panama: Chiriquí, Burica Peninsula	COI	BOLD: 604268
Cercomacroides tyrannina	UAM36986	JMM581 PA-CTY581	Panama: Colón, Gamboa	COI	BOLD: 604270
Cercomacroides tyrannina	UAM39468	MJM1905 PA-CTY1905	Panama: Bocas del Toro, Isla Colón	COI	BOLD: 604267
Cercomacroides tyrannina	UAM31147	KSW4805 PA-CTY4805	Panama: Darién, Cana	COI	BOLD: 604267
Cercomacroides tyrannina		JMM222 PA-MSC222	Panama: Panama, Panama City, confluence of Rios Chagres and Chagrecito	COI	BOLD: 604336

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Chloroceryle aenea	STRIBC2909	MJM6639	Panama: Coclé, San Juan, Aguas Claras	COI	BOLD: 3726997
Chloroceryle aenea	STRIBC3197	MJM7404	Panama: Darién, Chepigana, Aruza Abajo	COI	BOLD: 2616729
Chloroceryle aenea		PA-CAE46523	Panama: Panamá, Pacora	COI	BOLD: 603916
Chloroceryle aenea		PA-CAE46550	Panama: Darién, Rancho Frío	COI	BOLD: 603919
Chloroceryle aenea		PA-CHI-PA115	Panama: Colón, Gamboa	COI	BOLD: 603918
Chloroceryle aenea	UAM24686	MJM1464	Panama: Colón, 20 km west of Gatún	ND2	FJ175782.1
Chloroceryle aenea		JMM572	Panama: Colón, Gamboa	ND2	FJ175783.1
Chloroceryle aenea	UAM24685	JMM825	Panama: Colón, Gamboa	ND2	FJ175784.1
Cyanocompsa cyanoides		GMS1923	Panama: Panama, Chiman, Cerro Chucantí	COI	BOLD: 3740657

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Cyanocompsa cyanoides	UAM20470	MJM377	Panama: Coclé, Cerro Moreno	COI	BOLD: 3585518
Cyanocompsa cyanoides	UAM20491	MJM406	Panama: Panamá, Cerro Azul	COI	BOLD: 3585519
Cyanocompsa cyanoides	UAM20553	MJM438	Panama: Panamá, Cerro Azul	COI	BOLD: 3585517
Cyanocompsa cyanoides	UAM24022	MJM2070	Panama: Darién, Cana	COI	BOLD: 2616663
Cyanocompsa cyanoides	UWBM112219	PA-CCY24 JK06-024	Panama: Panama, Chiman, Cerro Chucantí	COI	BOLD: 3740681
Cyanocompsa cyanoides		PA-CCY263 JTW263	Panama: Bocas del Toro, Chiriquí Grande	COI	BOLD: 4481331
Cyanocompsa cyanoides		PA-CCY304 JTW304	Panama: Comarca Ngäbe-Buglé, Cerro Chalite	COI	BOLD: 603937

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Cyanocompsa cyanoides	UAM25743	PA-CCY927 JMM927	Panama: Darién, Cana	COI	BOLD: 603940
Cyanocompsa cyanoides	UWBM107312	PA-CCY1922 GMS1922	Panama: Panama, Chiman, Cerro Chucantí	COI	BOLD: 603942
Cyanocompsa cyanoides	UAM20622	MJM574	Panama: Panamá, Cerro Azul	ND2	FJ176191.1
Cyanocompsa cyanoides	UAM20594	MJM515	Panama: Panamá, Cerro Azul	ND2	FJ176193.1
Cyanocompsa cyanoides	UAM20251	MJM157	Panama: Coclé, La Pintada, Coclesito	ND2	FJ176194.1
Cyanocompsa cyanoides		MJM707	Panama: Panamá, Cerro Azul	ND2	FJ176195.1
Cyanocompsa cyanoides	UAM20309	MJM215	Panama: Coclé, La Pintada, Coclesito	ND2	FJ176196.1

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Cyanocompsa cyanoides	UAM19200	MJM538	Panama: Panamá, Cerro Azul	ND2	FJ176197.1
Cyanocompsa cyanoides	UAM20602	MJM531	Panama: Panamá, Cerro Azul	ND2	FJ176198.1
Cyanocompsa cyanoides	UAM20374	MJM280	Panama: Coclé, La Pintada, Coclesito	ND2	FJ176199.1
Cyanocompsa cyanoides	UWBM76875	RCF2010	Panama: Panama, Panama City, confluence of Rios Chagres and Chagrecito	cytB	KU923785.1
Cyanocompsa cyanoides	UWBM76959	SMB210	Panama: Panama, Panama City, confluence of Rios Chagres and Chagrecito	cytB, COI	KU923785.1
Cyanocompsa cyanoides	UWBM76861	GKD262	Panama: Panamá, Panamá, Rio Esperanza Playa Grande	cytB, COI	KU923783.1

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Cyanocompsa cyanoides	UWBM76869	GKD270	Panama: Panamá, Panamá, Rio Esperanza Playa Grande	cytB, COI	
Cyclarhis gujanensis	STRIBC1426	MJM5137	Panama: Herrera, Parita, Herrera	COI	BOLD: 3741421
Cyclarhis gujanensis	STRIBC1425	MJM5150	Panama: Herrera, Parita, Herrera	COI	BOLD: 3741422
Cyclarhis gujanensis	STRIBC6689	MJM6045	Panama: Chiriquí, Boquete	COI	BOLD: 3741442
Cyclarhis gujanensis	UWBM123573	JK06-421 PA-CGU421	Panama: Veraguas, Golfo de Montijo	COI	BOLD: 605409
Cyclarhis gujanensis		JMD921 PA-CGU921	Panama: Veraguas, Golfo de Montijo	COI	BOLD: 605410
Galbula ruficauda	STRIBC3624	MJM7875	Panama: Darién, Chepigana, Aruza Abajo	COI	BOLD: 2616767

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Galbula ruficauda	PA-GRU283	JTW283	Panama: Comarca Ngäbe-Buglé, Cerro Chalite	COI	BOLD: 604024
Gymnocichla nudiceps	USNM612378	B00448	Panama: Bocas del Toro, Tierra Oscura, Isla San Cristóbal	COI	BOLD: 1608211
Gymnocichla nudiceps	USNM612379	B00468	Panama: Bocas del Toro, Tierra Oscura, Isla San Cristóbal	COI	BOLD: 1853520
Gymnocichla nudiceps	UAM31470	MJM2002	Panama: Darién, Cana	COI	BOLD: 2616658
Gymnocichla nudiceps		PA-GNU34	Panama: Colón, Achiote	COI	BOLD: 604285
Gymnocichla nudiceps		JMD891 PA-GNU891	Panama: Chiriquí, Burica Peninsula	COI	BOLD: 604284
Gymnocichla nudiceps	UAM25364	JMM1013 PA-GNU1013	Panama: Darién, Cana	COI	BOLD: 604287

Species	**Museum Number**	**Other Associated Numbers**	**Locality**	**Type**	**Accession Numbers**
Gymnocichla nudiceps	UAM20241	MJM1029 PA-GNU1029	Panama: Chiriquí, Burica Peninsula	COI	BOLD: 291383
Gymnocichla nudiceps	UAM31893	MJM2075 PA-GNU2075	Panama: Darién, Cana	COI	BOLD: 604286
Gymnocichla nudiceps		B2228	Panama: Darién, Cana	cytB, ND2, ND3	EF639948.1
Gymnocichla nudiceps	UWBM111372	MBM14845 JK04-291	Panama: Colón, Achiote	ND2	FJ175911.1
Gymnocichla nudiceps		JTW448	Panama: Chiriquí, Burica Peninsula	cytB	
Henicorhina leucophrys	KU:O:87013	B05419	Panama: Chiriquí, Los Planes	COI	BOLD: 1608391
Henicorhina leucophrys	KU:O:87015	B05298	Panama: Chiriquí, Los Planes	COI	BOLD: 1608390

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Henicorhina leucophrys	USNM607610	B01480	Panama: Chiriquí, Los Planes	COI	BOLD: 1608389
Henicorhina leucophrys		GMS2114 PA-HLE2114GS	Panama: Comarca Ngäbe-Buglé, Nole Duima, Altos Las Nubes	COI	BOLD: 604745
Henicorhina leucophrys		GMS2024 PA-HLE2024	Panama: Comarca Ngäbe-Buglé, Nole Duima, Altos Las Nubes	COI	BOLD: 4567597
Henicorhina leucophrys		JTW482 PA-HLC482	Panama: Chiriquí, Bugaba, Volcán	COI	BOLD: 604746
Henicorhina leucophrys		JK06-058 PA-HLE58	Panama: Panama, Chiman, Cerro Chucantí	COI	BOLD: 604748
Henicorhina leucophrys		JK06-284 PA-HLE284	Panama: Bocas del Toro, Bosque Protector de Palo Seco	COI	BOLD: 604743

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Henicorhina leucophrys		KSW4457 PA-HLC4457	Panama: Chiriquí, Boquete	COI	BOLD: 4567595
Henicorhina leucosticta	UWBM120904	GMS1913	Panama: Panama, Chiman, Cerro Chucantí	COI	BOLD: 3741114
Henicorhina leucosticta	UWBM120908	JMD657	Panama: Panama, Chiman, Cerro Chucantí	COI	BOLD: 3741116
Henicorhina leucosticta	FMNH470759	JMD664	Panama: Panama, Chiman, Cerro Chucantí	COI	BOLD: 3741117
Henicorhina leucosticta	STRIBC1534	MJM3373	Panama: Coclé, La Pintada, El Copé	COI	BOLD: 3741122
Henicorhina leucosticta	STRIBC1536	MJM4504	Panama: Colón, Achiote	COI	BOLD: 3741125
Henicorhina leucosticta		GMS1107 PA-HLC1107	Panama: Coclé, El Valle	COI	BOLD: 604747

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Henicorhina leucosticta	UWBM120907	GMS2165 PA-HLC2165	Panama: Chiriquí, Bugaba, Volcán	COI	BOLD: 604754
Henicorhina leucosticta		JTW089 PA-HLE89	Panama: Bocas del Toro, Chiriquí Grande	COI	BOLD: 4567593
Henicorhina leucosticta		JTW090 PA-HLE90	Panama: Bocas del Toro, Chiriquí Grande	COI	BOLD: 604750
Henicorhina leucosticta	UWBM108349	JMD138 PA-HLE138	Panama: Veraguas, Santa Fé	COI	BOLD: 604759
Henicorhina leucosticta	CUMV50230	JTW203 PA-HLE203	Panama: Bocas del Toro, Chiriquí Grande	COI	BOLD: 4567594
Henicorhina leucosticta		JTW280 PA-HLE280	Panama: Comarca Ngäbe-Buglé, Cerro Chalite	COI	BOLD: 604749
Henicorhina leucosticta		JTW332 PA-HLE332	Panama: Panamá, Cerro Campana	COI	BOLD: 604752

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Henicorhina leucosticta		JTW641 PA-HLE641	Panama: Darién, Puerto Piña	COI	BOLD: 4567596
Henicorhina leucosticta	UAM22765	MJM684 PA-HLE684	Panama: Panamá, Cerro Azul	COI	BOLD: 604757
Henicorhina leucosticta	UAM22726	MJM696 PA-HLE696	Panama: Panamá, Cerro Jefe	COI	BOLD: 604758
Henicorhina leucosticta		JTW728 PA-HLE728	Panama: Darién, Puerto Piña	COI	BOLD: 604751
Henicorhina leucosticta	UAM22762	MJM2112 PA-HLE2112	Panama: Darién, Cerro Pirre	COI	BOLD: 604756
Henicorhina leucosticta	UAM22769	KSW4893 PA-HLE4893	Panama: Darién, Cana	COI	BOLD: 604755
Henicorhina leucosticta		B28632	Panama: Panamá, Paraiso	cytB	KM080565.1

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Henicorhina leucosticta		B41623	Panama: Bocas del Toro, Chiriquí Grande	cytB	KM080566.1
Henicorhina leucosticta		B28784	Panama: Colón, Achiote	cytB	KM080567.1
Henicorhina leucosticta		B2236	Panama: Darién, Cana	cytB	KM080573.1
Henicorhina leucosticta		B1357	Panama: Darién, Cana	cytB	KM080574.1
Henicorhina leucosticta		B2097	Panama: Darién, Cana	cytB	KM080575.1
Henicorhina leucosticta		PA-HLE319 JTW319	Panama: Comarca Ngäbe-Buglé, Cerro Chalite	ND2	EU983495.1
Henicorhina leucosticta		PA-HLC2007 GMS2007	Panama: Bocas del Toro, Bosque Protector de Palo Seco	ND2	EU983498.1

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Henicorhina leucosticta	UWBM123642	JK06-125	Panama: Bocas del Toro, Bosque Protector de Palo Seco	ND2	EU983499.1
Henicorhina leucosticta	UWBM123523	JK06-130	Panama: Bocas del Toro, Bosque Protector de Palo Seco	ND2	EU983500.1
Henicorhina leucosticta	UWBM123380	JMD754	Panama: Bocas del Toro, Bosque Protector de Palo Seco	ND2	EU983501.1
Henicorhina leucosticta	UWBM123646	JK06-124	Panama: Bocas del Toro, Bosque Protector de Palo Seco	ND2	EU983503.1
Henicorhina leucosticta	STRIBC1525	MJM4821	Panama: Chiriquí, Bugaba, Volcán	ND2	EU983504.1
Henicorhina leucosticta	STRIBC1524	MJM4863	Panama: Chiriquí, Bugaba, Volcán	ND2	EU983505.1
Henicorhina leucosticta	UAM22765	MJM684	Panama: Panamá, Cerro Azul	ND2	EU983508.1

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Henicorhina leucosticta	UAM24661	MJM1044	Panama: Panamá, Lago Bayano	ND2	EU983509.1
Henicorhina leucosticta	UAM22727	MJM303	Panama: Panamá, Lago Alajuela	ND2	EU983510.1
Henicorhina leucosticta	UAM22728	MJM1057	Panama: Panamá, Cerro Jefe	ND2	EU983511.1
Henicorhina leucosticta	UAM22726	MJM696	Panama: Panamá, Cerro Azul	ND2	EU983512.1
Henicorhina leucosticta	UAM24580	JMM907	Panama: Panamá, Cerro Azul	ND2	EU983513.1
Henicorhina leucosticta	UAM24660	MJM1420	Panama: Colón, 20 km west of Gatún	ND2	EU983515.1
Henicorhina leucosticta	UAM24476	MJL055	Panama: Darién, Puerto Piña	ND2	EU983518.1

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Henicorhina leucosticta	UAM22768	KSW4880	Panama: Darién, Cana	ND2	EU983519.1
Henicorhina leucosticta	UAM22770	KSW4902	Panama: Darién, Cana	ND2	EU983520.1
Henicorhina leucosticta	UAM22762	MJM2112	Panama: Darién, Cana	ND2	EU983521.1
Henicorhina leucosticta	UAM22767	MJM2114	Panama: Darién, Cana	ND2	EU983522.1
Henicorhina leucosticta	UAM22766	MJM2113	Panama: Darién, Cana	ND2	EU983523.1
Henicorhina leucosticta	UAM22761	MJM1987	Panama: Darién, Cana	ND2	EU983524.1
Henicorhina leucosticta	UAM22769	KSW4893	Panama: Darién, Cana	ND2	EU983525.1

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Henicorhina leucosticta	UAM24008	MJM2089	Panama: Darién, Cana	ND2	FJ231670.1
Henicorhina leucosticta		PA-HLE1 JTW001	Panama: Coclé, La Pintada, El Copé	ATP8, ATP6	AY304322.1
Henicorhina leucosticta		TR50111	Panama: Panamá, Barro Colorado Island	ATP8, ATP6	AY304326.1
Henicorhina leucosticta		TR50122	Panama: Panamá, Barro Colorado Island	ATP8, ATP6	AY304327.1
Henicorhina leucosticta		PA-HLE273 JTW273	Panama: Bocas del Toro, Valle de Risco	ATP8, ATP6	AY304328.1
Henicorhina leucosticta		PA-HLE222 JTW222	Panama: Bocas del Toro, Valle de Risco	ATP8, ATP6	AY304335.1
Henicorhina leucosticta	UWBM111260	JK04-169	Panama: Veraguas, Santa Fé	ATP8, ATP6	MK014722.1

Species	**Museum Number**	**Other Associated Numbers**	**Locality**	**Type**	**Accession Numbers**
Pachysylvia decurtata	USNM612431	B00429	Panama: Bocas del Toro, Tierra Oscura, Isla San Cristóbal	COI	BOLD: 1608478
Pachysylvia decurtata		B17498	Panama: Bocas del Toro, Changuinola	COI	BOLD: 3741413
Pachysylvia decurtata	UWBM111193	JK04-100	Panama: Panamá, Cerro Jefe	COI	BOLD: 3741418
Pachysylvia decurtata		JMM910	Panama: Panamá, Cerro Azul	COI	BOLD: 3741420
Pachysylvia decurtata	STRIBC1422	MJM6350	Panama: Bocas del Toro, Changuinola	COI	BOLD: 3741443
Pachysylvia decurtata	UWBM111263	JK04-172 PA-HDE172	Panama: Veraguas, Santa Fé	COI	BOLD: 605417
Pachysylvia decurtata	UWBM108897	JMD719 PA-HDE719	Panama: Panama, Chiman, Cerro Chucantí	COI	BOLD: 605413

Species	**Museum Number**	**Other Associated Numbers**	**Locality**	**Type**	**Accession Numbers**
Pachysylvia decurtata	UWBM108154	GMS983 PA-HDE983	Panama: Panamá, Cerro Jefe	COI	BOLD: 605415
Pachysylvia decurtata		B46600 PA-HDE46600	Panama: Darién, Rancho Frío	COI	BOLD: 605414
Pachysylvia decurtata	UWBM112234	JK06-044 PA-HYD44	Panama: Panama, Chiman, Cerro Chucantí	COI	BOLD: 605416
Pachysylvia decurtata	UWBM123479	JK06-093	Panama: Panamá, Cerro Azul	ND2	FJ176019.1
Icterus mesomelas	USNM607085	B00377	Panama: Bocas del Toro, Isla San Cristobal	COI	BOLD: 1608127
Icterus mesomelas	USNM608015	B01235	Panama: Comarca Ngäbe-Buglé, Valiente Peninsula, Punta Alegre	COI	BOLD: 1641207
Icterus mesomelas		JX16068	Panama: Darién	COI	BOLD: 3632767

Species	**Museum Number**	**Other Associated Numbers**	**Locality**	**Type**	**Accession Numbers**
Icterus mesomelas	UAM25193	MJM995	Panama: Darién, Tropic Star Lodge	COI	BOLD: 2616800
Icterus mesomelas	UWBM106614	GMS1192 PA-IME1192	Panama: Colón, Achiote	COI	BOLD: 604031
Icterus mesomelas		PA-IME-PC98	Panama: Colón, French Canal	COI	BOLD: 265548
Icterus mesomelas	LSUMZ109279		Panama: Darién	partial mt genome	JX516068.1
Jacana spinosa	USNM606725	B00286	Panama: Bocas del Toro, Isla San Cristobal	COI	BOLD: 1641205
Jacana spinosa	USNM613969	B02002	Panama: Bocas del Toro, Chiriquí Grande	COI	BOLD: 1608124
Jacana spinosa		B46451	Panama: Bocas del Toro, Changuinola	COI	BOLD: 3022348

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Jacana spinosa		JTW587	Panama: Coclé, Rosario	COI	BOLD: 3022406
Jacana spinosa		JTW588	Panama: Coclé, Rosario	COI	BOLD: 3022407
Jacana spinosa		JTW589	Panama: Coclé, Rosario	COI	BOLD: 3022408
Jacana spinosa	STRIBC3121	MJM7659	Panama: Veraguas, Las Palmas	COI	BOLD: 3022350
Jacana spinosa	STRIBC3266	MJM7661	Panama: Veraguas, Las Palmas	COI	BOLD: 3022352
Jacana spinosa	STRIBC3170	MJM7662	Panama: Veraguas, Las Palmas	COI	BOLD: 3022353
Jacana spinosa	STRIBC3368	MJM7666	Panama: Veraguas, Las Palmas	COI	BOLD: 3022357
Jacana spinosa	STRIBC3316	MJM7744	Panama: Chiriquí, Río Tabasará	COI	BOLD: 3022359
Jacana spinosa	STRIBC3338	MJM7758	Panama: Veraguas, Las Palmas	COI	BOLD: 3022365
Jacana spinosa	STRIBC3335	MJM7761	Panama: Chiriquí, Río Tabasará	COI	BOLD: 3022367
Jacana spinosa	STRIBC3334	MJM8237	Panama: Chiriquí, Boca Chica, Playa Hermosa	COI	BOLD: 3022377

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Jacana spinosa	STRIBC3325	MJM8238	Panama: Chiriquí, Boca Chica, Playa Hermosa	COI	BOLD: 3022378
Jacana spinosa	STRIBC3326	MJM8245	Panama: Chiriquí, Boca Chica, Playa Hermosa	COI	BOLD: 3022379
Jacana spinosa	STRIBC3333	MJM8246	Panama: Chiriquí, Boca Chica, Playa Hermosa	COI	BOLD: 3022380
Jacana spinosa	STRIBC3331	MJM8247	Panama: Chiriquí, Boca Chica, Playa Hermosa	COI	BOLD: 3022381
Jacana spinosa	STRIBC3332	MJM8248	Panama: Chiriquí, Boca Chica, Playa Hermosa	COI	BOLD: 3022382
Jacana spinosa	STRIBC3314	MJM8249	Panama: Chiriquí, Boca Chica, Playa Hermosa	COI	BOLD: 3022383
Jacana spinosa	STRIBC3315	MJM8287	Panama: Chiriquí, San Félix, Playa Las Lajas	COI	BOLD: 3022384

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Jacana spinosa	STRIBC3317	MJM8288	Panama: Chiriquí, San Félix, Playa Las Lajas	COI	BOLD: 3022385
Jacana spinosa	STRIBC3319	MJM8289	Panama: Chiriquí, San Félix, Playa Las Lajas	COI	BOLD: 3022386
Jacana spinosa	STRIBC3328	MJM8290	Panama: Chiriquí, San Félix, Playa Las Lajas	COI	BOLD: 3022387
Jacana spinosa	STRIBC3366	MJM8292	Panama: Chiriquí, San Félix, Playa Las Lajas	COI	BOLD: 3022389
Jacana spinosa	STRIBC3318	MJM8293	Panama: Chiriquí, San Félix, Playa Las Lajas	COI	BOLD: 3022390
Jacana spinosa	STRIBC3370	MJM8430	Panama: Veraguas, Las Palmas	COI	BOLD: 3022394
Laterallus albigularis	USNM612271	B00403	Panama: Bocas del Toro, Isla San Cristobal	COI	BOLD: 1608030

Species	**Museum Number**	**Other Associated Numbers**	**Locality**	**Type**	**Accession Numbers**
Laterallus albigularis	USNM607646	B01270	Panama: Comarca Ngäbe-Buglé, Valiente Peninsula, Punta Alegre	COI	BOLD: 1608029
Laterallus albigularis	USNM607647	B01389	Panama: Comarca Ngäbe-Buglé, Valiente Peninsula, Punta Alegre	COI	BOLD: 1608028
Laterallus albigularis		MJM6328	Panama: Bocas del Toro, Changuinola	COI	BOLD: 3732463
Laterallus albigularis		PA-LAL17501 B17501	Panama: Bocas del Toro, Changuinola	COI	BOLD: 604248
Malacoptila panamensis	USNM612334	B00441	Panama: Bocas del Toro, Tierra Oscura, Isla San Cristóbal	COI	BOLD: 1608343

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Malacoptila panamensis	USNM607732	B01381	Panama: Comarca Ngäbe-Buglé, Valiente Peninsula, Punta Alegre	COI	BOLD: 1608342
Malacoptila panamensis	STRIBC7888	JFM074	Panama: Veraguas, Río Luis	COI	BOLD: 10637102
Malacoptila panamensis	FMNH470657	JMD695	Panama: Panama, Chiman, Cerro Chucantí	COI	BOLD: 3740551
Malacoptila panamensis	FMNH470656	JMD696	Panama: Panama, Chiman, Cerro Chucantí	COI	BOLD: 3740552
Malacoptila panamensis	FMNH470655	JMD697	Panama: Panama, Chiman, Cerro Chucantí	COI	BOLD: 3740553
Malacoptila panamensis	UAM27609	JMM1017	Panama: Darién, Cana	COI	BOLD: 2616630

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Malacoptila panamensis		JMM1057	Panama: Darién, Cana	COI	BOLD: 2616632
Malacoptila panamensis	UAM28307	MJM2098	Panama: Darién, Cerro Pirre	COI	BOLD: 3740554
Malacoptila panamensis	STRIBC0500	MJM3097	Panama: Bocas del Toro, Changuinola	COI	BOLD: 3740555
Malacoptila panamensis	STRIBC2786	MJM4298	Panama: Bocas del Toro, Changuinola	COI	BOLD: 3740556
Malacoptila panamensis	STRIBC0501	MJM4484	Panama: Colón, Achiote	COI	BOLD: 3740557
Malacoptila panamensis	STRIBC0503	MJM4485	Panama: Colón, Achiote	COI	BOLD: 3740558
Malacoptila panamensis		MJM6314	Panama: Darién, Rancho Frío	COI	BOLD: 2616674

Species	**Museum Number**	**Other Associated Numbers**	**Locality**	**Type**	**Accession Numbers**
Malacoptila panamensis		PA-MPN5	Panama: Bocas del Toro, Chiriquí Grande, Rio La Gloria	COI	BOLD: 603927
Malacoptila panamensis		JTW671 PA-MPN671	Panama: Darién, Puerto Piña	COI	BOLD: 603930
Malacoptila panamensis	LSU52944		Panama: Darién, Puerto Piña	cytB, ND3	KX769336.1
Malacoptila panamensis	LSU46564		Panama: Darién, Cana	ND3	KX773223.1
Manacus vitellinus	USNM608158	B01896	Panama: Bocas del Toro, Changuinola	COI	
Manacus vitellinus	USNM608166	B01907	Panama: Bocas del Toro, Changuinola	COI	
Manacus vitellinus	USNM608138	B01966	Panama: Bocas del Toro, Chiriquí Grande	COI	

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
′*Manacus vitellinus*	UAM31178	JMM976 PA-MVI976	Panama: Darién, Cana	COI	
Manacus vitellinus	STRIBC4367	MJM7356	Panama: Darién, Chepigana, Aruza Abajo	COI	
Manacus vitellinus	STRIBC4184	MJM7439	Panama: Darién, Chepigana, Aruza Abajo	COI	
Manacus vitellinus		JTW244 PA-MVI244	Panama: Bocas del Toro, Valle de Risco	COI	
Manacus vitellinus		IJL04-098 PA-MVI98	Panama: Bocas del Toro, Chiriquí Grande, Rio La Gloria	COI	
Manacus vitellinus	UAM27506	MJM1938 PA-MVI1938	Panama: Darién, Cana	COI	
Manacus vitellinus		CDC078	Panama: Coclé, San Juan, Aguas Claras	COI	

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Manacus vitellinus	STRIBC3306	MJM7317	Panama: Darién, Chepigana, Aruza Abajo	COI	
Manacus vitellinus	UAM38584	JMM776 PA-MVI776	Panama: Colón, Gamboa	COI	
Manacus vitellinus		JTW629 PA-MVI629	Panama: Darién, Puerto Piña	COI	
Manacus vitellinus		CDC058	Panama: Coclé, San Juan, Aguas Claras	COI	
Manacus vitellinus		CDC062	Panama: Coclé, San Juan, Aguas Claras	COI	
Manacus vitellinus	STRIBC3021	MJM7318	Panama: Darién, Chepigana, Aruza Abajo	COI	
Manacus vitellinus		CDC075	Panama: Coclé, San Juan, Aguas Claras	COI	

Species	**Museum Number**	**Other Associated Numbers**	**Locality**	**Type**	**Accession Numbers**
Manacus vitellinus		CDC077	Panama: Coclé, San Juan, Aguas Claras	COI	
Microbates cinereiventris	UAM34036	MJM2116	Panama: Darién, Cana	COI	BOLD: 2616672
Microbates cinereiventris	STRIBC1588	MJM6430	Panama: Colón, Sherman Station	COI	BOLD: 3741102
Microbates cinereiventris	STRIBC4459	MJM6619	Panama: Colón, Río Mendoza	COI	BOLD: 3741102
Microbates cinereiventris	UWBM123649	JK06-208 PA-MCI208	Panama: Bocas del Toro, Bosque Protector de Palo Seco	COI	BOLD: 604252
Microbates cinereiventris		JTW339 PA-MCI339	Panama: Panamá, Cerro Campana	COI	BOLD: 604201
Microbates cinereiventris		JTW340 PA-MCI340	Panama: Panamá, Cerro Campana	COI	BOLD: 604202

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Microbates cinereiventris		JTW636 PA-MCI636	Panama: Darién, Puerto Piña	COI	BOLD: 604199
Microbates cinereiventris		JTW647 PA-MCI3647	Panama: Darién, Puerto Piña	COI	BOLD: 604200
Microbates cinereiventris		B2175	Panama: Darién, Cana	ND2	MG902966.1
Microbates cinereiventris		B2282	Panama: Darién, Cana	ND2	MG902967.1
Microbates cinereiventris	UWBM100984	BTS08-041	Panama: Darién, Puerto Piña	ND2	MG902968.1
Microbates cinereiventris	UWBM106546	GMS1122	Panama: Coclé, El Valle	ND2	MG902978.1
Microbates cinereiventris	UWBM108334	JMD123	Panama: Veraguas, Santa Fé	ND2	MG902981.1

Species	**Museum Number**	**Other Associated Numbers**	**Locality**	**Type**	**Accession Numbers**
Microbates cinereiventris	UWBM108333	JMD122	Panama: Veraguas, Santa Fé	ND2	MG902980.1
Microbates cinereiventris	UWBM108157	GMS986	Panama: Veraguas, Santa Fé	ND2	MG902979.1
Microrhopias quixensis	USNM607754	B01232B	Panama: Comarca Ngäbe-Buglé, Valiente Peninsula, Punta Alegre	COI	BOLD: 1608198
Microrhopias quixensis	USNM606233	B01813	Panama: Bocas del Toro, Isla Colón	COI	BOLD: 1641221
Microrhopias quixensis		IJL04-083	Panama: Bocas del Toro, Chiriquí Grande, Rio La Gloria	COI	BOLD: 3741667
Microrhopias quixensis		JTW733	Panama: Darién, Puerto Piña	COI	BOLD: 2616645
Microrhopias quixensis	STRIBC0778	MJM4502	Panama: Colón, Achiote	COI	BOLD: 3741690

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Microrhopias quixensis		JTW078 PA-MQU78	Panama: Chiriquí, Burica Peninsula	COI	BOLD: 604303
Microrhopias quixensis		JTW301 PA-MQU301	Panama: Comarca Ngäbe-Buglé, Cerro Chalite	COI	BOLD: 604302
Microrhopias quixensis	UWBM106569	GMS1146 PA-MQU1146	Panama: Colón, Achiote	COI	BOLD: 604307
Microrhopias quixensis		MJM1172 PA-MQU1172	Panama: Panamá, Cerro Azul	COI	BOLD: 604308
Microrhopias quixensis		PA-MQU3773	Panama: Colón, Gamboa	COI	BOLD: 604306
Microrhopias quixensis	UAM20400	MJM307	Panama: Coclé, Molejon, Finca Moreno	ND2	FJ175883.1
Microrhopias quixensis	UWBM106592	GMS1169	Panama: Colón, Achiote	ND2	FJ175886.1

Species	**Museum Number**	**Other Associated Numbers**	**Locality**	**Type**	**Accession Numbers**
Microrhopias quixensis	UWBM106591	MBM14842 GMS1168	Panama: Colón, Achiote	ND2	FJ175887.1
Microrhopias quixensis		JTW422	Panama: Chiriquí, Burica Peninsula	cytB	GU215241.1
Microrhopias quixensis		JTW724	Panama: Darién, Puerto Piña	cytB	GU215243.1
Microrhopias quixensis		JTW423	Panama: Chiriquí, Burica Peninsula	cytB	GU215242.1
Momotus momota	STRIBC3068	MJM7364	Panama: Darién, Chepigana, Aruza Abajo	COI	BOLD: 2616716
Momotus momota	STRIBC3069	MJM7406	Panama: Darién, Chepigana, Aruza Abajo	COI	BOLD: 2616730
Momotus momota	STRIBC3142	MJM7684	Panama: Comarca Ngäbe-Buglé, Nole Duima, Altos Las Nubes	COI	BOLD: 3740938

Species	**Museum Number**	**Other Associated Numbers**	**Locality**	**Type**	**Accession Numbers**
Momotus momota	UAM24928	JMM660 PA-EPL660	Panama: Colón, Gamboa	COI	BOLD: 604050
Momotus momota		JMM862 PA-EPL862	Panama: Colón, Gamboa	COI	BOLD: 604051
Momotus momota		JTW386 PA-MMO386	Panama: Chiriquí, Burica Peninsula	COI	BOLD: 604049
Momotus momota	UWBM108166	GMS995 PA-MMO995	Panama: Veraguas, Santa Fé	COI	BOLD: 604052
Myiobius sulphureipygius	USNM542846	B01553	Panama: Chiriquí, Burica Peninsula	COI	BOLD: 1608450
Myiobius sulphureipygius	USNM607592	B01510	Panama: Chiriquí, Burica Peninsula	COI	BOLD: 1608449
Myiobius sulphureipygius		JTW164 PA-MYB164	Panama: Bocas del Toro, Chiriquí Grande	COI	BOLD: 605288

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Myiobius sulphureipygius	UWBM123639	JK06-133 PA-MYB133	Panama: Bocas del Toro, Bosque Protector de Palo Seco	COI	BOLD: 605287
Myiobius sulphureipygius	UWBM108287	JMD089 PA-MYB89	Panama: Panamá, Cerro Jefe	COI	BOLD: 605292
Myiobius sulphureipygius	UWBM123390	JMD807 PA-MYB807	Panama: Chiriquí, Burica Peninsula	COI	BOLD: 605289
Myiobius sulphureipygius	UAM38488	JMM1049 PA-MYB1049	Panama: Darién, Cana	COI	BOLD: 605291
Myiobius sulphureipygius	UAM38740	MJM2041 PA-MYB2041	Panama: Darién, Cana	COI	BOLD: 605290
Myiobius sulphureipygius	UAM34054	MJM2082	Panama: Darién, Cana	COI	BOLD: 2616665
Myioborus miniatus	KU:O:87052	B05337	Panama: Chiriquí, Los Planes	COI	BOLD: 1608097
Myioborus miniatus	KU:O:87054	B05386	Panama: Chiriquí, Los Planes	COI	BOLD: 1608096

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Myioborus miniatus	KU:O:87124	B05475	Panama: Chiriquí, Los Planes	COI	BOLD: 1608095
Myioborus miniatus		GU932120	Panama: Chiriquí	COI	BOLD: 3590190
Myioborus miniatus	STRIBC4438	MJM6105	Panama: Chiriquí, Boquete	COI	BOLD: 3740973
Myioborus miniatus	STRIBC6742	MJM6126	Panama: Chiriquí, Boquete	COI	BOLD: 3740974
Myioborus miniatus	STRIBC1773	MJM6165	Panama: Chiriquí, Boquete	COI	BOLD: 3740975
Myioborus miniatus	STRIBC3408	MJM7719	Panama: Comarca Ngäbe-Buglé, Nole Duima, Altos Las Nubes	COI	BOLD: 3740988
Myioborus miniatus	STRIBC3698	MJM7774	Panama: Comarca Ngäbe-Buglé, Nole Duima, Altos Las Nubes	COI	BOLD: 3740990
Myioborus miniatus	STRIBC3701	MJM7780	Panama: Comarca Ngäbe-Buglé, Nole Duima, Altos Las Nubes	COI	BOLD: 3740991

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Myioborus miniatus		JTW185 PA-MMN185	Panama: Bocas del Toro, Chiriquí Grande	COI	BOLD: 604094
Myioborus miniatus	UWBM112334	JK06-324 PA-MMN324	Panama: Bocas del Toro, Bosque Protector de Palo Seco	COI	BOLD: 604086
Myioborus miniatus		JTW454 PA-MMN454	Panama: Chiriquí, Volcan, Alto Chiquero	COI	BOLD: 604095
Myioborus miniatus	UWBM108895	JMD709 PA-MMN709	Panama: Panama, Chiman, Cerro Chucantí	COI	BOLD: 604089
Myioborus miniatus		PA-MMN1423 MJB606	Panama: Darién, Cerro Pirre	COI	BOLD: 604088
Myioborus miniatus	UAM36354	KSW4463 PA-MMN4463	Panama: Chiriquí, Volcan, Alto Chiquero	COI	BOLD: 604087
Myioborus miniatus	UWBM116714	GMS2149 PA-MYM2149	Panama: Panama, Chiman, Cerro Chucantí	COI	BOLD: 605123

Species	**Museum Number**	**Other Associated Numbers**	**Locality**	**Type**	**Accession Numbers**
Myiothlypis fulvicauda	KU:O:87066	B05341	Panama: Chiriquí, Hornito, Fortuna Dam	COI	BOLD: 1608091
Myiothlypis fulvicauda		GU932065	Panama: Darién, Cana	COI	BOLD: 3590270
Myiothlypis fulvicauda	STRIBC1819	MJM2837	Panama: Bocas del Toro, Changuinola	COI	BOLD: 3740958
Myiothlypis fulvicauda	STRIBC5543	MJM2881	Panama: Bocas del Toro, Changuinola	COI	BOLD: 3740959
Myiothlypis fulvicauda		IJL04-100 PA-BFU100	Panama: Bocas del Toro, Chiriquí Grande, Rio La Gloria	COI	BOLD: 604058
Myiothlypis fulvicauda		PA-BFU2233 SML696	Panama: Darién, Cerro Pirre	COI	BOLD: 604059
Myrmeciza exsul		B01106	Panama: Bocas del Toro	COI	BOLD: 1608197
Myrmeciza exsul		B01405	Panama: Bocas del Toro	COI	BOLD: 1608196
Myrmeciza exsul		B01789	Panama: Bocas del Toro	COI	BOLD: 1641220

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Myrmeciza exsul	STRIBC2962	MJM7360	Panama: Darién, Chepigana, Aruza Abajo	COI	BOLD: 2616714
Myrmeciza exsul		JK04-103 PA-MYE103	Panama: Veraguas, Santa Fé	COI	BOLD: 604311
Myrmeciza exsul	UAM23995	MJM1900 PA-MYE1900	Panama: Bocas del Toro, Isla Colón	COI	BOLD: 604309
Myrmeciza exsul	UAM20240	MJM167	Panama: Coclé, Molejon, Finca Moreno	cytB, ND2	EF639963.1
Myrmeciza exsul	UWBM112356	JK06-366	Panama: Chiriquí, Burica Peninsula	ND2	FJ229379.1
Myrmeciza exsul		PA-MYE55 JTW055	Panama: Chiriquí, Burica Peninsula	ND2	FJ229380.1
Myrmeciza exsul	UWBM123891	JMD890	Panama: Chiriquí, Burica Peninsula	ND2	FJ229381.1

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Myrmeciza exsul	UWBM108956	JMD877	Panama: Chiriquí, Burica Peninsula	ND2	FJ229382.1
Myrmeciza exsul		PA-MYE63 JTW063	Panama: Chiriquí, Burica Peninsula	ND2	FJ229383.1
Myrmeciza exsul		PA-MYE2179 GMS2179	Panama: Chiriquí, Burica Peninsula	ND2	FJ229384.1
Myrmeciza exsul		PA-MYE2191 GMS2191	Panama: Chiriquí, Burica Peninsula	ND2	FJ229385.1
Myrmeciza exsul	UWBM108952	JMD872	Panama: Chiriquí, Burica Peninsula	ND2	FJ229386.1
Myrmeciza exsul		GMS2185	Panama: Chiriquí, Burica Peninsula	ND2	FJ229387.1
Myrmeciza exsul	UWBM107533	GMS2184	Panama: Chiriquí, Burica Peninsula	ND2	FJ229388.1

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Myrmeciza exsul	UWBM106472	GMS1047	Panama: Veraguas, Restingue, southwest tip of Azuero Peninsula	ND2	FJ229389.1
Myrmeciza exsul	UWBM108363	JMD152	Panama: Veraguas, Restingue, southwest tip of Azuero Peninsula	ND2	FJ229390.1
Myrmeciza exsul		PA-MYE10 IJL04-010	Panama: Bocas del Toro, Chiriquí Grande, Rio La Gloria	ND2	FJ229391.1
Myrmeciza exsul	CU44211	IJL04-027	Panama: Bocas del Toro, Chiriquí Grande, Rio La Gloria	ND2	FJ229393.1
Myrmeciza exsul	CU50916	IJL04-036	Panama: Bocas del Toro, Chiriquí Grande, Rio La Gloria	ND2	FJ229392.1
Myrmeciza exsul		IJL04-033	Panama: Bocas del Toro, Chiriquí Grande, Rio La Gloria	ND2	FJ229394.1
Myrmeciza exsul	CU50834	IJL04-051	Panama: Bocas del Toro, Chiriquí Grande, Rio La Gloria	ND2	FJ229395.1

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Myrmeciza exsul		IJL04-012	Panama: Bocas del Toro, Chiriquí Grande, Rio La Gloria	ND2	FJ229396.1
Myrmeciza exsul	UAM23991	JTW323	Panama: Comarca Ngäbe-Buglé, Cerro Chalite	ND2, cytB	FJ229397.1
Myrmeciza exsul	UAM23993	JTW324	Panama: Comarca Ngäbe-Buglé, Cerro Chalite	ND2, cytB	FJ229398.1
Myrmeciza exsul		JTW309	Panama: Comarca Ngäbe-Buglé, Cerro Chalite	ND2, cytB	FJ229399.1
Myrmeciza exsul		JTW258	Panama: Bocas del Toro, Valle de Risco	ND2, cytB	FJ229400.1
Myrmeciza exsul		JTW289	Panama: Comarca Ngäbe-Buglé, Cerro Chalite	ND2, cytB	FJ229401.1
Myrmeciza exsul		JTW287	Panama: Comarca Ngäbe-Buglé, Cerro Chalite	ND2	FJ229402.1
Myrmeciza exsul		AWK3231		ND2	FJ229403.1

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Myrmeciza exsul		AWK3301		ND2	FJ229404.1
Myrmeciza exsul	UWBM108351	JMD140	Panama: Veraguas, Santa Fé	ND2	FJ229405.1
Myrmeciza exsul	UWBM108314	JMD103	Panama: Veraguas, Santa Fé	ND2	FJ229406.1
Myrmeciza exsul	UWBM108315	JMD104	Panama: Veraguas, Santa Fé	ND2	FJ229407.1
Myrmeciza exsul	UWBM106459	GMS1032	Panama: Veraguas, Santa Fé	ND2	FJ229408.1
Myrmeciza exsul	UAM20446	MJM353	Panama: Coclé, Cerro Moreno	ND2	FJ229409.1
Myrmeciza exsul	UAM20273	MJM177	Panama: Coclé, Molejon, Finca Moreno	ND2	FJ229411.1
Myrmeciza exsul	UAM20404	MJM311	Panama: Coclé, Molejon, Finca Moreno	ND2	FJ229412.1
Myrmeciza exsul	UAM20271	MJM179	Panama: Coclé, Molejon, Finca Moreno	ND2	FJ229413.1
Myrmeciza exsul	UAM20276	MJM182	Panama: Coclé, Molejon, Finca Moreno	ND2	FJ229414.1

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Myrmeciza exsul		JTW596	Panama: Coclé, La Pintada, El Copé	ND2	FJ229415.1
Myrmeciza exsul		JTW595	Panama: Coclé, La Pintada, El Copé	ND2	FJ229416.1
Myrmeciza exsul		JK04-311		ND2	FJ229417.1
Myrmeciza exsul		PA-MYE17		ND2	FJ229418.1
Myrmeciza exsul	UWBM106567	GMS1144	Panama: Colón, Achiote	ND2	FJ229419.1
Myrmeciza exsul	UWBM106566	GMS1143	Panama: Colón, Achiote	ND2	FJ229420.1
Myrmeciza exsul	UWBM111350	JK04-266	Panama: Colón, Achiote	ND2	FJ229421.1
Myrmeciza exsul	UWBM106568	GMS1145	Panama: Colón, Achiote	ND2	FJ229422.1
Myrmeciza exsul	UWBM111360	JK04-276	Panama: Colón, Achiote	ND2	FJ229423.1
Myrmeciza exsul	UWBM111396	JK04-318	Panama: Colón, Achiote	ND2	FJ229424.1
Myrmeciza exsul	MJM1418	MJM1418	Panama: Colón, 20 km west of Gatún	ND2	FJ229425.1

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Myrmeciza exsul	MJM1446	MJM1446	Panama: Colón, 20 km west of Gatún	ND2	FJ229426.1
Myrmeciza exsul	PA-MYE44	PA-MYE44	?	ND2	FJ229427.1
Myrmeciza exsul	UAM36677	JMM666	Panama: Colón, Gamboa	ND2	FJ229428.1
Myrmeciza exsul	UAM20591	MJM503	Panama: Panamá, Cerro Azul	ND2	FJ229429.1
Myrmeciza exsul	UWBM76896	RCF2031	Panama: Panama, Panama City, confluence of Rios Chagres and Chagrecito	ND2	FJ229430.1
Myrmeciza exsul	UWBM76855	GKD256	Panama: Panamá, Panamá, Rio Esperanza Playa Grande	ND2	FJ229431.1
Myrmeciza exsul	UWBM76972	SMB223	Panama: Panamá, Panamá, Rio Esperanza Playa Grande	ND2	FJ229432.1
Myrmeciza exsul	UAM24573	KSW4791	Panama: Darién, Cana	ND2	FJ229433.1
Myrmeciza exsul	UAM24683	KSW4790	Panama: Darién, Cana	ND2	FJ229434.1
Myrmeciza exsul	UAM24689	JMM1018	Panama: Darién, Cana	ND2	FJ229435.1

Species	**Museum Number**	**Other Associated Numbers**	**Locality**	**Type**	**Accession Numbers**
Myrmeciza exsul	UAM24574	MJM2023	Panama: Darién, Cana	ND2	FJ229436.1
Myrmeciza exsul	UAM24473	JMM946	Panama: Darién, Cana	ND2	FJ229437.1
Myrmeciza exsul		B46542		ND2	FJ229438.1
Myrmeciza exsul		B46551		ND2	FJ229439.1
Myrmeciza exsul		B46593		ND2	FJ229440.1
Myrmeciza exsul	UAM23992	MJM985	Panama: Darién, Tropic Star Lodge	ND2	FJ229441.1
Myrmeciza exsul	UAM23994	MJM937	Panama: Darién, Tropic Star Lodge	ND2	FJ229442.1
Myrmeciza exsul	UAM23994	MJM938	Panama: Darién, Tropic Star Lodge	ND2	FJ229443.1
Myrmeciza exsul		JTW086		cytB	GU215249.1
Nyctidromus albicollis	STRIBC4370	MJM7945	Chucanaque	COI	BOLD: 2616780

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Nyctidromus albicollis	STRIBC3348	MJM7982	Panama: Darién, Chepigana, Aruza Abajo	COI	BOLD: 2616789
Nyctidromus albicollis	STRIBC3347	MJM7983	Panama: Darién, Chepigana, Aruza Abajo	COI	BOLD: 2616790
Nyctidromus albicollis	STRIBC3349	MJM7984	Panama: Darién, Chepigana, Aruza Abajo	COI	BOLD: 2616791
Nyctidromus albicollis		JTW412 PA-NAL412	Panama: Chiriquí, Burica Peninsula	COI	BOLD: 603934
Nyctidromus albicollis	UWBM123402	JMD851 PA-NAL851	Panama: Chiriquí, Burica Peninsula	COI	BOLD: 603933
Nyctidromus albicollis	UWBM108165	GMS994 PA-NAL994	Panama: Veraguas, Santa Fé	COI	BOLD: 603936
Nyctidromus albicollis	UWBM123839	GMS1869 PA-NAL1869	Panama: Panamá, Higueronal	COI	BOLD: 603935

Species	**Museum Number**	**Other Associated Numbers**	**Locality**	**Type**	**Accession Numbers**
Nyctidromus albicollis	UWBM123431	JMD734	Felipillo Marsh	ND2	FJ175723.1
Nyctidromus albicollis	USNM612300		Panama: Bocas del Toro, Tierra Oscura, Isla San Cristóbal	CO1	DQ433846.1
Ramphocelus flammigerus		B01238	Panama: Bocas del Toro	COI	BOLD: 1608243
Ramphocelus flammigerus		B01267	Panama: Bocas del Toro	COI	BOLD: 1608242
Ramphocelus flammigerus		B01275	Panama: Bocas del Toro	COI	BOLD: 1608241
Ramphocelus flammigerus	UWBM106616	GMS1194 PA-RFL1194	Panama: Colón, Achiote	COI	BOLD: 604469
Ramphocelus flammigerus	STRIBC3822	MJM6728	Panama: Colón, Achiote	COI	BOLD: 3741821

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Ramphocelus flammigerus	UAM34480	KSW4857PA-RFL4857	Panama: Darién, Cana	COI	BOLD: 604470
Ramphocelus flammigerus	STRIBC4385	MJM6825	Panama: Colón, Achiote	COI	BOLD: 3741831
Ramphocelus flammigerus		JTW711 PA-RIC711	Panama: Darién, Puerto Piña	COI	BOLD: 604471
Ramphocelus passerinii		B01152	Panama: Bocas del Toro, Cayo Agua	COI	BOLD: 1853532
Ramphocelus passerinii		B01183	Panama: Bocas del Toro, Cayo Agua	COI	BOLD: 1853531
Ramphocelus passerinii		B02321	Panama: Chiriquí, Burica Peninsula	COI	BOLD: 1608240
Ramphocelus passerinii		IJL04-015 PA-RCS15	Panama: Bocas del Toro, Panama: Bocas del Toro, Chiriquí Grande, Rio La Gloria	COI	BOLD: 604462

Species	**Museum Number**	**Other Associated Numbers**	**Locality**	**Type**	**Accession Numbers**
Ramphocelus passerinii	UAM25722	MJM1906 PA-RPA1906	Panama: Bocas del Toro, Isla Colón	COI	BOLD: 604473
Ramphocelus passerinii	UWBM123507	JK06-214 PA-RPA214	Panama: Bocas del Toro, Chiriquí Grande, Punta Robalo	COI	BOLD: 604472
Ramphocelus passerinii	UWBM108975	JMD912 PA-RPA912	Panama: Chiriquí, Burica Peninsula	COI	BOLD: 604474
Schiffornis		B05457		COI	BOLD: 1608066
Schiffornis		B05498		COI	BOLD: 1608065
Schiffornis		B01421		COI	BOLD: 1608064
Schiffornis	UWBM76978	SMB229 PA-STU229	Panama: Panamá, Panamá, Rio Esperanza Playa Grande	COI	BOLD: 605362
Schiffornis	UWBM108283	JMD085 PA-STU85	Panama: Panamá, Cerro Jefe	COI	BOLD: 605361
Schiffornis		JTW099	Panama: Bocas del Toro, Chiriquí Grande	COI	BOLD: 3741171

Species	**Museum Number**	**Other Associated Numbers**	**Locality**	**Type**	**Accession Numbers**
Schiffornis	STRIBC3600	MJM7878	Panama: Darién, Chepigana, Aruza Abajo	COI	BOLD: 2616768
Schiffornis	UWBM123426	JMD798 PA-SCT798	Panama: Chiriquí, Gualaca	COI	BOLD: 605359
Sclerurus guatemalensis	USNM606856	B00300	Panama: Bocas del Toro, Isla San Cristobal	COI	BOLD: 1641212
Sclerurus guatemalensis	USNM606857	B00309	Panama: Bocas del Toro, Isla San Cristobal	COI	BOLD: 1608157
Sclerurus guatemalensis	UAM21890	MJM946	Panama: Darién, Tropic Star Lodge	COI	BOLD: 2616797
Sclerurus guatemalensis	STRIBC0548	MJM3964	Panama: Bocas del Toro, Changuinola	COI	BOLD: 3740824
Sclerurus guatemalensis	STRIBC0549	MJM6433	Panama: Colón, Sherman Station	COI	BOLD: 3740832

Species	**Museum Number**	**Other Associated Numbers**	**Locality**	**Type**	**Accession Numbers**
Sclerurus guatemalensis	UWBM108285	MBM15326 JMD087	Panama: Panamá, Cerro Jefe	ND2	FJ175819.1
Sclerurus guatemalensis	UAM20498	MJM418	Panama: Panamá, Cerro Azul	ND2	FJ175820.1
Sclerurus guatemalensis	PA-SGU23	PA-SGU23	Panama: Colón, Achiote	ND2	FJ175821.1
Sclerurus guatemalensis		B26538	Panama: Colón, Gamboa	ND2, cytB, ND3	JF975131.1
Sclerurus guatemalensis		B46563	Panama: Darién, Rancho Frío	ND2, cytB, ND3	JQ903761.1
Sclerurus guatemalensis		B1393	Panama: Darién, Cerro Pirre	ND2, cytB, ND3	JQ903762.1
Setophaga petechia		MJM2296	Panama: Veraguas, Isla Coiba	COI	BOLD: 3740957
Setophaga petechia		JK06-409 PA-DPE409	Panama: Chiriquí, Alanje, Divalá	COI	BOLD: 604075

Species	**Museum Number**	**Other Associated Numbers**	**Locality**	**Type**	**Accession Numbers**
Setophaga petechia	UAM35673	JMM652 PA-DPE652	Panama: Bocas del Toro, Isla Colón	COI	BOLD: 604074
Todirostrum cinereum	USNM606985	B00332	Panama: Bocas del Toro, Isla San Cristobal	COI	BOLD: 1608474
Todirostrum cinereum		PA-TCI46723	Panama: Veraguas, Isla Coiba	COI	BOLD: 3742059
Todirostrum cinereum	STRIBC1077	MJM6542	Panama: Panamá, Panama City, Albrook	COI	BOLD: 3741978
Todirostrum cinereum		B46771	Panama: Veraguas, Isla Coiba	COI	BOLD: 3741880
Todirostrum cinereum		PA-TCI46760 B46760	Panama: Veraguas, Isla Coiba	COI	BOLD: 3741876
Todirostrum cinereum		PA-TCI46747 B46747	Panama: Veraguas, Isla Coiba	COI	BOLD: 3741873

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Todirostrum cinereum	UAM20346	MJM252 PA-TCI252	Panama: Coclé, Molejon, Finca Moreno/Molejon	COI	BOLD: 605377
Todirostrum cinereum		JMD898 PA-TDC898	Panama: Chiriquí, Burica Peninsula	COI	BOLD: 605377
Todirostrum cinereum		JTW453 PA-TOG453	Panama: Chiriquí, Burica Peninsula	COI	BOLD: 605380
Todirostrum cinereum	UWBM123599	JK06-078 PA-TDC78	Felipillo Marsh	COI	BOLD: 605378
Todirostrum cinereum	STRIBC1070	MJM5241	Panama: Coclé, Penenomé	COI	BOLD: 3741939
Turdus assimilis		B46660	Panama: Veraguas, Isla Coiba	COI	BOLD: 2616627
Turdus assimilis		B46669	Panama: Veraguas, Isla Coiba	COI	BOLD: 2616628
Turdus assimilis	STRIBC6747	MJM6134	Panama: Chiriquí, Boquete	COI	BOLD: 3741385
Turdus assimilis	STRIBC1672	MJM6251	Panama: Chiriquí, Boquete	COI	BOLD: 3741390

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Turdus assimilis		JTW672 PA-TAB672	Panama: Darién, Puerto Piña	COI	BOLD: 605128
Turdus assimilis	UAM25334	KSW4515 PA-TAB4515	Panama: Chiriquí, Boquete	COI	BOLD: 605127
Turdus assimilis	UWBM111256	JK04-165 PA-TAS165	Panama: Veraguas, Santa Fé	COI	BOLD: 605131
Turdus assimilis	UAM30578	JMM1002 PA-TAS1002	Panama: Darién, Cana	COI	BOLD: 605130
Turdus assimilis	UAM36675	KSW4826 PA-TAS4826	Panama: Darién, Cana	COI	BOLD: 605129
Tyrannus melancholicus		PA-TML46705 B46705	Panama: Veraguas, Isla Coiba	COI	BOLD: 3741861
Tyrannus melancholicus		B46765	Panama: Veraguas, Isla Coiba	COI	BOLD: 3741877

Species	**Museum Number**	**Other Associated Numbers**	**Locality**	**Type**	**Accession Numbers**
Tyrannus melancholicus		PA-TML46774 B46774	Panama: Veraguas, Isla Coiba	COI	BOLD: 4568008
Tyrannus melancholicus	STRIBC1215	MJM5895	Panama: Los Santos	COI	BOLD: 3741948
Tyrannus melancholicus	STRIBC3044	MJM7386	Panama: Darién, Chepigana, Aruza Abajo	COI	BOLD: 2616724
Tyrannus melancholicus	UWBM111181	PA-TML88 JK04-088	Felippio	COI	BOLD: 605402
Tyrannus melancholicus		GMS1872PA-TML1872	Panama: Panamá, Chepo	COI	BOLD: 4568013
Tyrannus melancholicus	STRIBC1213	MJM5221	Panama: Coclé, Penenomé	COI	BOLD: 4568014
Tyrannus melancholicus	UAM36836	MJM1883 TR-TML1883	Panama: Chiriquí, Remedios	COI	BOLD: 605397
Xenops minutus		JFM012	Panama: Veraguas, Río Luis	COI	BOLD: 10636702

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Xenops minutus	STRIBC0576	MJM2904	Panama: Bocas del Toro, Changuinola	COI	BOLD: 3740816
Xenops minutus	STRIBC0577	MJM3110	Panama: Bocas del Toro, Changuinola	COI	BOLD: 3740820
Xenops minutus	STRIBC6928	MJM6650	Panama: Coclé, San Juan, Aguas Claras	COI	BOLD: 3740844
Xenops minutus	UAM19458	MJM693 PA-XMI693	Panama: Panamá, Cerro Azul	COI	BOLD: 604022
Xenops minutus	UAM22110	MJM700 PA-XMI700	Panama: Panamá, Cerro Jefe	COI	BOLD: 604023
Xenops minutus		JMD879 PA-XMI879	Panama: Chiriquí, Burica Peninsula	COI	BOLD: 604018
Xenops minutus	UAM24576	MJM2051 PA-XMI2051	Panama: Darién, Cana	COI	BOLD: 604020
Xenops minutus		PA-XMI-PA21	Panama: Colón, Gamboa	COI	BOLD: 604019

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Xenops minutus	UAM20338	MJM244	Panama: Coclé, Molejon, Finca Moreno	ND2	FJ175808.1
Xenops minutus	UAM20495	MJM410	Panama: Panamá, Cerro Azul	ND2	FJ175809.1
Xenops minutus	UAM24684	KSW4392	Panama: Panamá, Cerro Azul	ND2	FJ175810.1
Xenops minutus	UAM20326	MJM232	Panama: Coclé, Molejon, Finca Moreno	ND2	FJ175811.1
Xenops minutus	UAM22111	MJM407	Panama: Panamá, Cerro Azul	ND2	FJ175812.1
Xenops minutus	UAM20350	MJM256	Panama: Coclé, Molejon, Finca Moreno	ND2	FJ175813.1
Xenops minutus	UAM24577	MJM1462	Panama: Colón, 20 km west of Gatún	ND2	FJ175814.1
Xenops minutus	UAM22105	MJM675	Panama: Panamá, Cerro Azul	ND2	FJ175815.1
Xenops minutus	UAM24578	MJM1461	Panama: Panamá, Lago Bayano	ND2	FJ175817.1
Xenops minutus	UWBM107239	GMS1842	Panama: Panamá, Lago Bayano	cytB	KM081460.1

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Xenops minutus	USNM1283		Panama: Comarca Ngäbe-Buglé, Valiente Peninsula, Punta Alegre	cytB	KM081438.1
Xenops minutus	USNM1302		Panama: Comarca Ngäbe-Buglé, Valiente Peninsula, Punta Alegre	cytB	KM081439.1
Xenops minutus	USNM1400		Panama: Comarca Ngäbe-Buglé, Valiente Peninsula, Punta Alegre	cytB	
Xenops minutus	LSUMZ28628		Panama: Panamá, Paraiso	cytB	KM081443.1
Xenops minutus	LSUMZ28753		Panama: Colón, 20 km west of Gatún	cytB	KM081455.1
Xenops minutus	LSUMZ26932		Panama: Panamá, Paraiso	cytB	KM081456.1
Xenops minutus	UWBM108478	JMD270	Panama: Panama, Panama City, confluence of Rios Chagres and Chagrecito	cytB	KM081457.1

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Xenops minutus	LSUMZ2209		Panama: Darién, Cana	cytB	KM081461.1
Xenops minutus	LSUMZ26497		Panama: Colón, Gamboa	cytB	KM081462.1
Xenops minutus		GMS2186	Panama: Chiriquí, Burica Peninsula	cytB	KM081448.1
Xenops minutus	UWBM107485	GMS2125	Panama: Chiriquí, Bugaba, Volcán	cytB	KM081437.1
Xenops minutus	UWBM107189	GMS1758	Panama: Panamá, Lago Bayano	cytB	KM081458.1
Xenops minutus	CU50191		Panama: Chiriquí, Burica Peninsula	cytB	KM081451.1
Xenops minutus	CU50738		Panama: Coclé, La Pintada, El Copé	cytB	KM081444.1
Xenops minutus		GMS2187	Panama: Chiriquí, Burica Peninsula	cytB	KM081449.1
Xenops minutus		GMS2192	Panama: Chiriquí, Burica Peninsula	cytB	KM081450.1

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Xenops minutus	CU51309		Panama: Panamá, Cerro Campana	cytB	KM081445.1
Xenops minutus	UWBM111291	JK04-203	Panama: Veraguas, Restingue, southwest tip of Azuero Peninsula	cytB	KM081453.1
Xenops minutus	UWBM107199	GMS1778	Panama: Panamá, Lago Bayano	cytB	KM081459.1
Xenops minutus	ANSP7207		Panama: Veraguas, Restingue, southwest tip of Azuero Peninsula	cytB	?term=xenops+minutus+ANSP7207
Sporophila americana		B46772	Panama: Veraguas, Isla Coiba	COI	BOLD: 3741517
Sporophila americana	UAM35704	JMM635	Panama: Bocas del Toro, Isla Colón	COI	BOLD: 603991
Sporophila americana	UWBM108980	JMD917	Panama: Chiriquí, Burica Peninsula	COI	BOLD: 603992

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Sporophila americana	STRIBC6952	MJM6736	Panama: Colón, Achiote	COI	BOLD: 3741592
Sporophila americana	STRIBC2120	MJM6574	Panama: Panamá, Panama City, Albrook	COI	BOLD: 3741583
Sporophila americana		JTW265 PA-SAM265	Panama: Bocas del Toro, Valle de Risco	COI	BOLD: 604482
Sporophila americana	STRIBC2117	MJM6513	Panama: Panamá, Panama City, Albrook	COI	BOLD: 3741576
Sporophila americana	STRIBC2119	MJM6511	Panama: Panamá, Panama City, Albrook	COI	BOLD: 3741575
Sporophila americana	STRIBC6855	MJM6463	Panama: Panamá, Panama City, Albrook	COI	BOLD: 3741570
Sporophila americana		CDC101	Panama: Colón, Achiote	COI	BOLD: 3741531

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Sporophila americana		CDC079	Panama: Coclé, San Juan, Aguas Claras	COI	BOLD: 3741529
Sporophila americana		CDC063	Panama: Coclé, San Juan, Aguas Claras	COI	
Sporophila americana		CDC050	Panama: Panamá, Panama City, Albrook	COI	
Sporophila americana		CDC040	Panama: Panamá, Panama City, Albrook	COI	
Sporophila americana		CDC026	Panama: Panamá, Panama City, Albrook	COI	
Sporophila americana		B46750	Panama: Veraguas, Isla Coiba	COI	
Sporophila americana		B46749	Panama: Veraguas, Isla Coiba	COI	

Species	Museum Number	Other Associated Numbers	Locality	Type	Accession Numbers
Sporophila americana		B46735	Panama: Veraguas, Isla Coiba	COI	
Sporophila americana		JTW124 PA-SAM124	Panama: Bocas del Toro, Chiriquí Grande	COI	
Sporophila americana		JTW586 PA-SPO586	Panama: Coclé, Rosario	COI	
Sporophila americana		JTW688 PA-SAM688JW	Panama: Darién, Puerto Piña	COI	
Sporophila americana	STRIBC2114	MJM6515	Panama: Panamá, Panama City, Albrook	COI	

Table A1.2: Table of all barcoded taxa with ecological data for diet, stratum, habitat, and distribution. Diet: O, omnivore; P, plant generalist; N, nectarivore; G, granivore; F, frugivore; A, animal generalist; I, invertebrate diet; V, sourced compiled from Stotz *et al.* 1996, supplemented as needed from Angehr and Dean 2010.

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Little Tinamou	*Crypturellus soui*	Tinamidae	P	G	forest	L	N	Y	0
Gray-headed Chachalaca	*Ortalis cinereiceps*	Cracidae	P	G	forest	L	N	Y	0
Crested Guan	*Penelope purpurascens*	Cracidae	P	G	forest	L	N	Y	0
Black Guan	*Chamaepetes unicolor*	Cracidae	P	G	forest	H	N	N	0
Tawny-faced Quail	*Rhynchortyx cinctus*	Odontiphoridae	GI	G	forest	L	N	Y	0
Black-breasted Wood-Quail	*Odontophorus leucolaemus*	Odontiphoridae	GI	G	forest	H	N	N	0
Spotted Wood-Quail	*Odontophorus guttatus*	Odontiphoridae	GI	G	forest	H	N	N	0

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
White-crowned Pigeon	*Patagioenas leucocephala*	Columbidae	F	C	forest	L	N	Y	0
Band-tailed Pigeon	*Patagioenas fasciata*	Columbidae	F	C	forest	H	N	N	0
Short-billed Pigeon	*Patagioenas nigrirostris*	Columbidae	F	C	forest	L	N	Y	0
Ruddy Ground-Dove	*Columbina talpacoti*	Columbidae	G	G	open	L	N	Y	1
Blue Ground-Dove	*Claravis pretiosa*	Columbidae	G	G	open	L	N	Y	0
Maroon-chested Ground-Dove	*Claravis mondetoura*	Columbidae	G	U	forest	H	N	N	0
Ruddy Quail-Dove	*Geotrygon montana*	Columbidae	F	G	forest	L	N	Y	1
Violaceous Quail-Dove	*Geotrygon violacea*	Columbidae	F	U	forest	L	N	Y	0

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
White-tipped Dove	*Leptotila verreauxi*	Columbidae	P	G	open	L	N	Y	1
Gray-headed Dove	*Leptotila plumbeiceps*	Columbidae	G	U	forest	L	N	N	0
Brown-backed Dove	*Leptotila battyi*	Columbidae	G	U	forest	L	N	N	0
Gray-chested Dove	*Leptotila cassinii*	Columbidae	G	G	secondary	L	N	N	1
Buff-fronted Quail-Dove	*Zentrygon costaricensis*	Columbidae	G	G	forest	H	N	N	0
Chiriquí Quail-Dove	*Zentrygon chiriquensis*	Columbidae	P	G	forest	H	N	N	0
Russet-crowned Quail-Dove	*Zentrygon goldmani*	Columbidae	G	G	forest	H	N	N	0
Little Cuckoo	*Coccycua minuta*	Cuculidae	I	U	edge	L	N	N	0

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Squirrel Cuckoo	*Piaya cayana*	Cuculidae	I	M	forest	L	N	Y	1
Mangrove Cuckoo	*Coccyzus minor*	Cuculidae	I		forest	L	N	N	0
Greater Ani	*Crotophaga major*	Cuculidae	I	U	forest	L	N	N	0
Smooth-billed Ani	*Crotophaga ani*	Cuculidae	I	C	open	L	N	Y	1
Groove-billed Ani	*Crotophaga sulcirostris*	Cuculidae	I	G	open	L	N	Y	0
Short-tailed Nighthawk	*Lurocalis semitorquatus*	Caprimulgidae	I	C	forest	L	N	Y	0
Common Pauraque	*Nyctidromus albicollis*	Caprimulgidae	I	U	forest	L	N	Y	1
Great Potoo	*Nyctibius grandis*	Nyctibiidae	I	C	edge	L	N	Y	0
Common Potoo	*Nyctibius griseus*	Nyctibiidae	I	C	edge	L	N	Y	1

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
White-necked Jacobin	*Florisuga mellivora*	Trochilidae	N	C	forest	L	N	Y	1
White-tipped Sicklebill	*Eutoxeres aquila*	Trochilidae	N	U	forest	H	N	Y	1
Bronzy Hermit	*Glaucis aeneus*	Trochilidae	N	U	edge	L	N	N	1
Rufous-breasted Hermit	*Glaucis hirsutus*	Trochilidae	N	U	forest	L	N	N	1
Band-tailed Barbthroat	*Threnetes ruckeri*	Trochilidae	N	U	forest	L	N	Y	1
Green Hermit	*Phaethornis guy*	Trochilidae	N	U	forest	H	N	Y	1
Long-billed Hermit	*Phaethornis longirostris*	Trochilidae	N	U	forest	L	N	Y	1
Pale-bellied Hermit	*Phaethornis anthophilus*	Trochilidae	N	U	forest	L	N	N	0
Stripe-throated Hermit	*Phaethornis striigularis*	Trochilidae	N	U	forest	L	N	Y	1

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Green-fronted Lancebill	*Doryfera ludovicae*	Trochilidae	N	M	forest	H	N	Y	0
Purple-crowned Fairy	*Heliothryx barroti*	Trochilidae	N	C	edge	L	N	Y	1
Green-breasted Mango	*Anthracothorax prevostii*	Trochilidae	N	U	open	L	N	N	0
Black-throated Mango	*Anthracothorax nigricollis*	Trochilidae	N	U	open	L	N	Y	0
Veraguan Mango	*Anthracothorax veraguensis*	Trochilidae	N	U	open	L	N	N	0
Rufous-crested Coquette	*Lophornis delattrei*	Trochilidae	N	U	edge	L	N	Y	0
Green-crowned Brilliant	*Heliodoxa jacula*	Trochilidae	N	U	forest	H	N	Y	0
Fiery-throated Hummingbird	*Panterpe insignis*	Trochilidae	N	C	forest	H	N	N	0

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Long-billed Starthroat	*Heliomaster longirostris*	Trochilidae	N	U	edge	L	N	Y	1
White-bellied Mountain-gem	*Lampornis hemileucus*	Trochilidae	N	C	forest	H	N	N	0
Purple-throated Mountain-gem	*Lampornis calolaemus*	Trochilidae	N	C	forest	H	N	N	0
White-throated Mountain-gem	*Lampornis castaneoventris*	Trochilidae	N	C	forest	H	N	N	0
Magenta-throated Woodstar	*Calliphlox bryantae*	Trochilidae	N	M	edge	H	N	N	0
Scintillant Hummingbird	*Selasphorus scintilla*	Trochilidae	N	U	edge	H	N	N	0
Garden Emerald	*Chlorostilbon assimilis*	Trochilidae	N	U	edge	L	N	Y	1
Scaly-breasted Hummingbird	*Phaeochroa cuvierii*	Trochilidae	N	C	edge	L	N	Y	1

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Violet Sabrewing	*Campylopterus hemileucurus*	Trochilidae	N	U	forest	H	N	N	0
Stripe-tailed Hummingbird	*Eupherusa eximia*	Trochilidae	N	C	forest	H	N	N	0
Black-bellied Hummingbird	*Eupherusa nigriventris*	Trochilidae	N	C	forest	H	N	N	0
White-tailed Emerald	*Elvira chionura*	Trochilidae	N	C	forest	H	N	N	1
Snowcap	*Microchera albocoronata*	Trochilidae	N	C	forest	L	N	N	0
White-vented Plumeleteer	*Chalybura buffonii*	Trochilidae	N	C	forest	L	N	N	1
Bronze-tailed Plumeleteer	*Chalybura urochrysia*	Trochilidae	N	U	forest	L	N	Y	1
Violet-crowned Woodnymph	*Thalurania colombica*	Trochilidae	N	U	forest	L	N	N	1

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Blue-chested Hummingbird	*Amazilia amabilis*	Trochilidae	N	U	edge	L	N	Y	1
Charming Hummingbird	*Amazilia decora*	Trochilidae	N	U	edge	L	N	N	0
Snowy-bellied Hummingbird	*Amazilia edward*	Trochilidae	N	U	open	L	N	Y	1
Rufous-tailed Hummingbird	*Amazilia tzacatl*	Trochilidae	N	U	edge	L	N	Y	1
Violet-capped Hummingbird	*Goldmania violiceps*	Trochilidae	N	U	forest	H	N	N	0
Sapphire-throated Hummingbird	*Lepidopyga coeruleogularis*	Trochilidae	N	U	edge	L	N	Y	1
Violet-bellied Hummingbird	*Juliamyia julie*	Trochilidae	N	U	forest	L	N	N	1
Blue-throated Goldentail	*Hylocharis eliciae*	Trochilidae	N	C	forest	L	N	N	1

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Black Vulture	*Coragyps atratus*	Cathartidae	V	A	open	L	N	Y	0
Pearl Kite	*Gampsonyx swainsonii*	Accipitridae	V	C	open	L	N	Y	1
White-tailed Kite	*Elanus leucurus*	Accipitridae	V	A	open	L	N	Y	0
Hook-billed Kite	*Chondrohierax uncinatus*	Accipitridae	I	M	forest	L	N	Y	0
Gray-headed Kite	*Leptodon cayanensis*	Accipitridae	A	C	forest	L	N	Y	0
Double-toothed Kite	*Harpagus bidentatus*	Accipitridae	A	C	forest	L	N	Y	0
Tiny Hawk	*Accipiter superciliosus*	Accipitridae	V	C	forest	L	N	Y	0
Bicolored Hawk	*Accipiter bicolor*	Accipitridae	V	C	forest	L	N	Y	0
Plumbeous Kite	*Ictinia plumbea*	Accipitridae	I	C	edge	L	N	Y	0

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Common Black Hawk	*Buteogallus anthracinus*	Accipitridae	A	C	open	L	N	Y	1
Barred Hawk	*Morphnarchus princeps*	Accipitridae	A	C	forest	H	N	Y	0
Roadside Hawk	*Rupornis magnirostris*	Accipitridae	A	U	edge	L	N	Y	1
White Hawk	*Pseudastur albicollis*	Accipitridae	V	C	forest	L	N	Y	0
Semiplumbeous Hawk	*Leucopternis semiplumbeus*	Accipitridae	A	M	forest	L	N	Y	0
Zone-tailed Hawk	*Buteo albonotatus*	Accipitridae	V	A	open	L	N	Y	0
Vermiculated Screech-Owl	*Megascops guatemalae*	Strigidae	A	M	forest	L	N	Y	1
Bare-shanked Screech-Owl	*Megascops clarkii*	Strigidae	A	C	forest	H	N	N	0

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Spectacled Owl	*Pulsatrix perspicillata*	Strigidae	V	M	forest	L	N	Y	1
Lattice-tailed Trogon	*Trogon clathratus*	Trogonidae	F	M	forest	L	N	N	0
Slaty-tailed Trogon	*Trogon massena*	Trogonidae	F	C	forest	L	N	Y	1
Black-throated Trogon	*Trogon rufus*	Trogonidae	F	M	forest	L	N	Y	1
Tody Motmot	*Hylomanes momotula*	Momotidae	A	U	forest	L	N	N	0
Rufous Motmot	*Baryphthengus martii*	Momotidae	A	U	forest	L	N	Y	1
Broad-billed Motmot	*Electron platyrhynchum*	Momotidae	A	M	forest	L	N	Y	1
Ringed Kingfisher	*Megaceryle torquata*	Alcedinidae	V	M	forest	H	N	Y	1

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Amazon Kingfisher	*Chloroceryle amazona*	Alcedinidae	V	M	forest	H	N	Y	1
Green Kingfisher	*Chloroceryle americana*	Alcedinidae	V	M	forest	H	N	Y	1
Green-and-rufous Kingfisher	*Chloroceryle inda*	Alcedinidae	V	M	forest	L	N	Y	1
American Pygmy Kingfisher	*Chloroceryle aenea*	Alcedinidae	V	U	forest	L	N	Y	1
Barred Puffbird	*Nystalus radiatus*	Bucconidae	I	M	forest	L	N	N	0
Pied Puffbird	*Notharchus tectus*	Bucconidae	I	C	forest	L	N	Y	1
White-whiskered Puffbird	*Malacoptila panamensis*	Bucconidae	I	U	forest	L	N	Y	1
Gray-cheeked Nunlet	*Nonnula frontalis*	Bucconidae	I	U	forest	L	N	N	0

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
White-fronted Nunbird	*Monasa morphoeus*	Bucconidae	I	C	forest	L	N	Y	0
Rufous-tailed Jacamar	*Galbula ruficauda*	Galbulidae	I	U	forest	L	N	Y	1
Spot-crowned Barbet	*Capito maculicoronatus*	Capitonidae	O	C	forest	L	N	N	0
Red-headed Barbet	*Eubucco bourcierii*	Capitonidae	O	C	forest	H	N	N	0
Prong-billed Barbet	*Semnornis frantzii*	Semnornithidae	F	C	forest	H	N	N	0
Blue-throated Toucanet	*Aulacorhynchus caeruleogularis*	Ramphastidae	O	C	forest	H	N	N	0
Collared Aracari	*Pteroglossus torquatus*	Ramphastidae	F	C	forest	L	N	Y	1
Yellow-eared Toucanet	*Selenidera spectabilis*	Ramphastidae	F	C	forest	L	N	Y	0

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Keel-billed Toucan	*Ramphastos sulfuratus*	Ramphastidae	F	C	forest	H	N	Y	1
Yellow-throated Toucan	*Ramphastos ambiguus*	Ramphastidae	F	C	forest	H	N	Y	0
Olivaceous Piculet	*Picumnus olivaceus*	Picidae	I	U	edge	L	N	Y	0
Golden-naped Woodpecker	*Melanerpes chrysauchen*	Picidae	I	C	forest	L	N	N	0
Black-cheeked Woodpecker	*Melanerpes pucherani*	Picidae	I	C	forest	L	N	Y	1
Red-crowned Woodpecker	*Melanerpes rubricapillus*	Picidae	I	C	forest	L	N	Y	1
Stripe-cheeked Woodpecker	*Piculus callopterus*	Picidae	I	U	forest	L	N	N	0
Cinnamon Woodpecker	*Celeus loricatus*	Picidae	I	C	forest	L	N	Y	1

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Chestnut-colored Woodpecker	*Celeus castaneus*	Picidae	I	M	forest	L	N	N	0
Lineated Woodpecker	*Dryocopus lineatus*	Picidae	I	C	edge	L	N	Y	1
Crimson-bellied Woodpecker	*Campephilus haematogaster*	Picidae	I	U	forest	L	N	N	1
Crimson-crested Woodpecker	*Campephilus melanoleucos*	Picidae	I	U	forest	L	N	Y	1
Pale-billed Woodpecker	*Campephilus guatemalensis*	Picidae	I	U	forest	L	N	N	1
Collared Forest-Falcon	*Micrastur semitorquatus*	Falconidae	V	M	forest	L	N	Y	0
Crested Caracara	*Caracara cheriway*	Falconidae	A	G	open	L	N	N	0

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Yellow-headed Caracara	*Milvago chimachima*	Falconidae	A	G	open	L	N	Y	1
Sulphur-winged Parakeet	*Pyrrhura hoffmanni*	Psittacidae	P	C	edge	H	N	N	0
Olive-throated Parakeet	*Eupsittula nana*	Psittacidae	P	C	forest	L	N	N	0
Orange-chinned Parakeet	*Brotogeris jugularis*	Psittacidae	P	C	edge	L	N	N	1
Brown-hooded Parrot	*Pyrilia haematotis*	Psittacidae	P	C	forest	L	N	Y	0
Blue-headed Parrot	*Pionus menstruus*	Psittacidae	P	C	forest	L	N	Y	1
White-crowned Parrot	*Pionus senilis*	Psittacidae	P	C	forest	H	N	N	0
Red-lored Parrot	*Amazona autumnalis*	Psittacidae	F	C	edge	L	N	Y	1

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Mealy Parrot	*Amazona farinosa*	Psittacidae	F	C	forest	L	N	Y	0
Yellow-crowned Parrot	*Amazona ochrocephala*	Psittacidae	F	C	edge	L	N	Y	0
Fasciated Antshrike	*Cymbilaimus lineatus*	Thamnophilidae	I	M	forest	L	N	Y	1
Great Antshrike	*Taraba major*	Thamnophilidae	I	U	forest	L	N	Y	0
Barred Antshrike	*Thamnophilus doliatus*	Thamnophilidae	I	U	forest	L	N	Y	1
Black Antshrike	*Thamnophilus nigriceps*	Thamnophilidae	I	U	forest	L	N	N	1
Black-hooded Antshrike	*Thamnophilus bridgesi*	Thamnophilidae	I	U	forest	L	N	N	1
Black-crowned Antshrike	*Thamnophilus atrinucha*	Thamnophilidae	I	U	forest	L	N	Y	1
Speckled Antshrike	*Xenornis setifrons*	Thamnophilidae	I	U	forest	L	N	N	0

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Russet Antshrike	*Thamnistes anabatinus*	Thamnophilidae	I	C	forest	L	N	Y	0
Plain Antvireo	*Dysithamnus mentalis*	Thamnophilidae	I	U	forest	H	N	Y	0
Spot-crowned Antvireo	*Dysithamnus puncticeps*	Thamnophilidae	I	U	forest	L	N	Y	1
White-flanked Antwren	*Myrmotherula axillaris*	Thamnophilidae	I	U	forest	L	N	Y	1
Slaty Antwren	*Myrmotherula schisticolor*	Thamnophilidae	I	U	forest	H	N	Y	1
Checker-throated Antwren	*Epinecrophylla fulviventris*	Thamnophilidae	I	U	forest	L	N	Y	1
Dot-winged Antwren	*Microrhopias quixensis*	Thamnophilidae	I	M	forest	L	N	Y	1
White-fringed Antwren	*Formicivora grisea*	Thamnophilidae	I	U	forest	L	N	N	0

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Rufous-rumped Antwren	*Euchrepomis callinota*	Thamnophilidae	I	C	forest	H	N	Y	0
Dusky Antbird	*Cercomacroides tyrannina*	Thamnophilidae	I	U	forest	L	N	Y	1
Jet Antbird	*Cercomacra nigricans*	Thamnophilidae	I	U	forest	L	N	N	0
Bare-crowned Antbird	*Gymnocichla nudiceps*	Thamnophilidae	I	U	forest	L	N	Y	1
White-bellied Antbird	*Myrmeciza longipes*	Thamnophilidae	I	G	forest	L	N	Y	1
Chestnut-backed Antbird	*Myrmeciza exsul*	Thamnophilidae	I	G	forest	L	N	Y	1
Spotted Antbird	*Hylophylax naevioides*	Thamnophilidae	I	G	forest	L	N	Y	1
Bicolored Antbird	*Gymnopithys bicolor*	Thamnophilidae	I	U	forest	L	N	Y	1

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Ocellated Antbird	*Phaenostictus mcleannani*	Thamnophilidae	I	U	forest	L	N	Y	0
Streak-chested Antpitta	*Hylopezus perspicillatus*	Grallariidae	I	G	forest	L	N	Y	0
Thicket Antpitta	*Hylopezus dives*	Grallariidae	I	G	forest	L	N	Y	0
Silvery-fronted Tapaculo	*Scytalopus argentifrons*	Rhinocryptidae	I	G	forest	H	N	N	0
Black-faced Antthrush	*Formicarius analis*	Formicariidae	I	G	forest	L	N	Y	1
Rufous-breasted Antthrush	*Formicarius rufipectus*	Formicariidae	I	G	forest	H	N	Y	0
Scaly-throated Leaftosser	*Sclerurus guatemalensis*	Furnariidae	I	G	forest	L	N	Y	1
Long-tailed Woodcreeper	*Deconychura longicauda*	Furnariidae	I	U	forest	L	N	Y	0

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Ruddy Woodcreeper	*Dendrocincla homochroa*	Furnariidae	I	U	forest	L	N	Y	0
Plain-brown Woodcreeper	*Dendrocincla fuliginosa*	Furnariidae	I	U	forest	L	N	Y	1
Wedge-billed Woodcreeper	*Glyphorynchus spirurus*	Furnariidae	I	U	forest	L	N	Y	1
Black-banded Woodcreeper	*Dendrocolaptes picumnus*	Furnariidae	I	U	forest	H	N	N	0
Cocoa Woodcreeper	*Xiphorhynchus susurrans*	Furnariidae	I	M	forest	L	N	Y	1
Black-striped Woodcreeper	*Xiphorhynchus lachrymosus*	Furnariidae	I	C	forest	L	N	Y	0
Spotted Woodcreeper	*Xiphorhynchus erythropygius*	Furnariidae	I	C	forest	L	N	Y	0
Red-billed Scythebill	*Campylorhamphus trochilirostris*	Furnariidae	I	U	forest	L	N	N	0

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Brown-billed Scythebill	*Campylorhamphus pusillus*	Furnariidae	I	U	forest	H	N	Y	0
Streak-headed Woodcreeper	*Lepidocolaptes souleyetii*	Furnariidae	I	C	open	L	N	Y	0
Spot-crowned Woodcreeper	*Lepidocolaptes affinis*	Furnariidae	I	M	forest	H	N	N	0
Plain Xenops	*Xenops minutus*	Furnariidae	I	M	forest	L	N	Y	1
Slaty-winged Foliage-gleaner	*Philydor fuscipenne*	Furnariidae	I	U	forest	L	N	N	0
Lineated Foliage-gleaner	*Syndactyla subalaris*	Furnariidae	I	U	forest	H	N	Y	0
Streak-breasted Treehunter	*Thripadectes rufobrunneus*	Furnariidae	I	U	forest	H	N	N	0
Buff-throated Foliage-gleaner	*Automolus ochrolaemus*	Furnariidae	I	U	forest	L	N	Y	1
Chiriquí Foliage-gleaner	*Automolus exsertus*	Furnariidae	I	U	forest	L	N	N	0

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Spotted Barbtail	*Premnoplex brunnescens*	Furnariidae	I	U	forest	H	N	N	0
Ruddy Treerunner	*Margarornis rubiginosus*	Furnariidae	I	C	forest	H	N	N	0
Pale-breasted Spinetail	*Synallaxis albescens*	Furnariidae	I	U	open	L	N	Y	0
Slaty Spinetail	*Synallaxis brachyura*	Furnariidae	I	U	edge	L	N	Y	0
Lance-tailed Manakin	*Chiroxiphia lanceolata*	Pipridae	F	U	forest	L	N	Y	1
White-ruffed Manakin	*Corapipo altera*	Pipridae	F	U	forest	L	N	Y	1
Blue-crowned Manakin	*Lepidothrix coronata*	Pipridae	F	U	forest	L	N	Y	1
White-collared Manakin	*Manacus candei*	Pipridae	F	U	forest	L	N	N	0

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Orange-collared Manakin	*Manacus aurantiacus*	Pipridae	F	U	forest	L	N	N	0
Golden-collared Manakin	*Manacus vitellinus*	Pipridae	F	U	forest	L	N	Y	1
White-crowned Manakin	*Dixiphia pipra*	Pipridae	F	U	forest	H	N	N	0
Purple-throated Fruitcrow	*Querula purpurata*	Cotingidae	I	C	forest	L	N	Y	0
Bare-necked Umbrellabird	*Cephalopterus glabricollis*	Cotingidae	F	M	forest	H	N	N	0
Three-wattled Bellbird	*Procnias tricarunculatus*	Cotingidae	F	C	forest	H	N	N	0
Northern Schiffornis	*Schiffornis veraepacis*	Tityridae	F	U	forest	L	N	N	1
Russet-winged Schiffornis	*Schiffornis stenorhyncha*	Tityridae	F	U	forest	L	N	N	0

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Speckled Mourner	*Laniocera rufescens*	Tityridae	O	U	forest	L	N	Y	0
Masked Tityra	*Tityra semifasciata*	Tityridae	F	C	forest	L	N	Y	1
Black-crowned Tityra	*Tityra inquisitor*	Tityridae	F	C	forest	L	N	Y	0
Cinnamon Becard	*Pachyramphus cinnamomeus*	Tityridae	I	C	edge	L	N	Y	1
White-winged Becard	*Pachyramphus polychopterus*	Tityridae	I	C	forest	L	N	Y	1
Black-and-white Becard	*Pachyramphus albogriseus*	Tityridae	I	C	forest	H	N	N	0
Rose-throated Becard	*Pachyramphus aglaiae*	Tityridae	I	C	forest	H	N	N	0
Royal Flycatcher	*Onychorhynchus coronatus*	Onychorhynchidae	I	M	forest	L	N	Y	1

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Ruddy-tailed Flycatcher	*Terenotriccus erythrurus*	Onychorhynchidae	I	M	forest	L	N	Y	1
Sulphur-rumped Flycatcher	*Myiobius sulphureipygius*	Onychorhynchidae	I	U	forest	L	N	Y	1
Black-tailed Flycatcher	*Myiobius atricaudus*	Onychorhynchidae	I	U	forest	L	N	Y	1
Stub-tailed Spadebill	*Platyrinchus cancrominus*	Tyrannidae	I	U	forest	L	N	N	0
White-throated Spadebill	*Platyrinchus mystaceus*	Tyrannidae	I	U	forest	H	N	Y	0
Golden-crowned Spadebill	*Platyrinchus coronatus*	Tyrannidae	I	U	forest	L	N	Y	1
Olive-striped Flycatcher	*Mionectes olivaceus*	Tyrannidae	F	U	forest	L	N	Y	1

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Ochre-bellied Flycatcher	*Mionectes oleagineus*	Tyrannidae	F	U	forest	L	N	Y	1
Slaty-capped Flycatcher	*Leptopogon superciliaris*	Tyrannidae	I	M	forest	H	N	Y	1
Rufous-browed Tyrannulet	*Phylloscartes superciliaris*	Tyrannidae	I	C	forest	H	N	Y	0
Black-capped Pygmy-Tyrant	*Myiornis atricapillus*	Tyrannidae	I	U	forest	L	N	Y	0
Scale-crested Pygmy-Tyrant	*Lophotriccus pileatus*	Tyrannidae	I	M	forest	H	N	Y	1
Northern Bentbill	*Oncostoma cinereigulare*	Tyrannidae	I	U	forest	L	N	N	0
Slate-headed Tody-Flycatcher	*Poecilotriccus sylvia*	Tyrannidae	I	U	edge	L	N	N	1

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Common Tody-Flycatcher	*Todirostrum cinereum*	Tyrannidae	I	U	forest	L	N	Y	1
Black-headed Tody-Flycatcher	*Todirostrum nigriceps*	Tyrannidae	I	C	forest	L	N	Y	1
Brownish Twistwing	*Cnipodectes subbrunneus*	Tyrannidae	I	U	forest	L	N	N	1
Eye-ringed Flatbill	*Rhynchocyclus brevirostris*	Tyrannidae	I	C	forest	L	N	Y	1
Olivaceous Flatbill	*Rhynchocyclus olivaceus*	Tyrannidae	I	M	forest	L	N	N	1
Yellow-olive Flycatcher	*Tolmomyias sulphurescens*	Tyrannidae	I	M	forest	L	N	Y	1
Yellow-margined Flycatcher	*Tolmomyias assimilis*	Tyrannidae	I	C	forest	L	N	Y	1

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Yellow-breasted Flycatcher	*Tolmomyias flaviventris*	Tyrannidae	I	C	forest	L	N	N	0
Brown-capped Tyrannulet	*Ornithion brunneicapillus*	Tyrannidae	I	C	forest	L	N	Y	1
Yellow Tyrannulet	*Capsiempis flaveola*	Tyrannidae	I	U	edge	L	N	Y	0
Yellow-crowned Tyrannulet	*Tyrannulus elatus*	Tyrannidae	I	C	forest	L	N	Y	0
Greenish Elaenia	*Myiopagis viridicata*	Tyrannidae	I	C	edge	L	N	Y	1
Yellow-bellied Elaenia	*Elaenia flavogaster*	Tyrannidae	F	C	edge	L	N	Y	1
Lesser Elaenia	*Elaenia chiriquensis*	Tyrannidae	F	C	open	L	N	Y	1
Mountain Elaenia	*Elaenia frantzii*	Tyrannidae	F	C	open	H	N	N	0

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Paltry Tyrannulet	*Zimmerius vilissimus*	Tyrannidae	F	C	edge	L	N	Y	1
Bright-rumped Attila	*Attila spadiceus*	Tyrannidae	I	C	edge	L	N	Y	1
Rufous Mourner	*Rhytipterna holerythra*	Tyrannidae	I	C	forest	L	N	Y	1
Dusky-capped Flycatcher	*Myiarchus tuberculifer*	Tyrannidae	I	C	forest	L	N	Y	1
Panama Flycatcher	*Myiarchus panamensis*	Tyrannidae	I	C	edge	L	N	Y	0
Great Kiskadee	*Pitangus sulphuratus*	Tyrannidae	I	C	open	L	N	Y	0
Rusty-margined Flycatcher	*Myiozetetes cayanensis*	Tyrannidae	I	C	edge	L	N	N	0
Social Flycatcher	*Myiozetetes similis*	Tyrannidae	I	C	edge	L	N	Y	1

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Gray-capped Flycatcher	*Myiozetetes granadensis*	Tyrannidae	I	M	edge	L	N	Y	0
Golden-bellied Flycatcher	*Myiodynastes hemichrysus*	Tyrannidae	I	C	forest	H	N	N	0
Streaked Flycatcher	*Myiodynastes maculatus*	Tyrannidae	I	C	forest	L	N	Y	0
Tropical Kingbird	*Tyrannus melancholicus*	Tyrannidae	I	C	edge	L	N	Y	1
Bran-colored Flycatcher	*Myiophobus fasciatus*	Tyrannidae	I	M	forest	L	N	N	0
Tufted Flycatcher	*Mitrephanes phaeocercus*	Tyrannidae	I	M	edge	H	N	Y	0
Pied Water-Tyrant	*Fluvicola pica*	Tyrannidae	I	U	open	L	N	N	1
Northern Scrub-Flycatcher	*Sublegatus arenarum*	Tyrannidae	I	U	edge	L	N	Y	0

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Long-tailed Tyrant	*Colonia colonus*	Tyrannidae	I	C	edge	L	N	Y	1
Rufous-browed Peppershrike	*Cyclarhis gujanensis*	Vireonidae	I	C	forest	L	N	Y	1
Scrub Greenlet	*Hylophilus flavipes*	Vireonidae	I	U	edge	L	N	N	0
Tawny-crowned Greenlet	*Tunchiornis ochraceiceps*	Vireonidae	I	U	forest	L	N	Y	1
Lesser Greenlet	*Pachysylvia decurtata*	Vireonidae	G	C	forest	L	N	Y	1
Yellow-winged Vireo	*Vireo carmioli*	Vireonidae	I	C	forest	H	N	N	0
Brown-capped Vireo	*Vireo leucophrys*	Vireonidae	I	C	forest	H	N	N	0
Azure-hooded Jay	*Cyanolyca cucullata*	Corvidae	O	C	forest	H	N	N	0
Brown Jay	*Psilorhinus morio*	Corvidae	O	C	edge	L	N	N	0

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Black-chested Jay	*Cyanocorax affinis*	Corvidae	O	C	forest	L	N	N	0
Gray-breasted Martin	*Progne chalybea*	Hirundinidae	I	A	open	L	N	Y	1
Mangrove Swallow	*Tachycineta albilinea*	Hirundinidae	I	A	open	L	N	Y	0
Southern Rough-winged Swallow	*Stelgidopteryx ruficollis*	Hirundinidae	I	A	open	L	N	Y	1
Southern Nightingale-Wren	*Microcerculus marginatus*	Troglodytidae	I	G	forest	L	N	Y	1
House Wren	*Troglodytes aedon*	Troglodytidae	I	U	open	L	N	Y	1
Ochraceous Wren	*Troglodytes ochraceus*	Troglodytidae	I	M	forest	H	N	Y	0
White-headed Wren	*Campylorhynchus albobrunneus*	Troglodytidae	I	M	forest	L	N	N	1

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Band-backed Wren	*Campylorhynchus zonatus*	Troglodytidae	I	C	edge	L	N	N	0
Black-throated Wren	*Pheugopedius atrogularis*	Troglodytidae	I	U	forest	L	N	N	0
Rufous-breasted Wren	*Pheugopedius rutilus*	Troglodytidae	I	M	edge	L	N	Y	1
Black-bellied Wren	*Pheugopedius fasciatoventris*	Troglodytidae	I	C	edge	L	N	N	1
Rufous-and-white Wren	*Thryophilus rufalbus*	Troglodytidae	I	U	edge	L	N	N	1
Stripe-breasted Wren	*Cantorchilus thoracicus*	Troglodytidae	I	U	edge	L	N	N	0
Bay Wren	*Cantorchilus nigricapillus*	Troglodytidae	I	U	forest	L	N	Y	1
Riverside Wren	*Cantorchilus semibadius*	Troglodytidae	I	U	forest	L	N	N	0

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Buff-breasted Wren	*Cantorchilus leucotis*	Troglodytidae	I	U	edge	L	N	N	1
White-breasted Wood-Wren	*Henicorhina leucosticta*	Troglodytidae	I	U	forest	L	N	Y	1
Gray-breasted Wood-Wren	*Henicorhina leucophrys*	Troglodytidae	I	U	forest	H	N	Y	1
Song Wren	*Cyphorhinus phaeocephalus*	Troglodytidae	I	U	forest	L	N	Y	0
Tawny-faced Gnatwren	*Microbates cinereiventris*	Polioptilidae	I	U	forest	L	N	Y	1
Long-billed Gnatwren	*Ramphocaenus melanurus*	Polioptilidae	I	M	forest	L	N	Y	1
Tropical Gnatcatcher	*Polioptila plumbea*	Polioptilidae	I	C	edge	L	N	Y	1
Slate-throated Gnatcatcher	*Polioptila schistaceigula*	Polioptilidae	I	C	forest	L	N	N	0

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Black-faced Solitaire	*Myadestes melanops*	Turdidae	F	C	forest	H	N	N	0
Varied Solitaire	*Myadestes coloratus*	Turdidae	F	C	forest	H	N	N	0
Black-billed Nightingale-Thrush	*Catharus gracilirostris*	Turdidae	I	G	forest	H	N	N	0
Orange-billed Nightingale-Thrush	*Catharus aurantiirostris*	Turdidae	I	G	forest	H	N	N	0
Slaty-backed Nightingale-Thrush	*Catharus fuscater*	Turdidae	I	U	forest	H	N	Y	1
Ruddy-capped Nightingale-Thrush	*Catharus frantzii*	Turdidae	I	U	forest	H	N	N	0

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Mountain Thrush	*Turdus plebejus*	Turdidae	O	C	forest	H	N	N	0
Pale-vented Thrush	*Turdus obsoletus*	Turdidae	O	M	forest	H	N	Y	1
Clay-colored Thrush	*Turdus grayi*	Turdidae	O	G	forest	L	N	Y	1
White-throated Thrush	*Turdus assimilis*	Turdidae	O	U	forest	L	N	Y	1
Tropical Mockingbird	*Mimus gilvus*	Mimidae	O	U	open	L	I	N	0
Black-and-yellow Silky-flycatcher	*Phainoptila melanoxantha*	Ptiliogonatidae	F	M	forest	H	N	N	0
Golden-browed Chlorophonia	*Chlorophonia callophrys*	Fringillidae	F	C	forest	H	N	N	0

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Yellow-crowned Euphonia	*Euphonia luteicapilla*	Fringillidae	F	C	edge	L	N	Y	0
Thick-billed Euphonia	*Euphonia laniirostris*	Fringillidae	F	C	edge	L	N	Y	1
Fulvous-vented Euphonia	*Euphonia fulvicrissa*	Fringillidae	F	C	forest	L	N	N	0
Olive-backed Euphonia	*Euphonia gouldi*	Fringillidae	F	M	forest	L	N	N	0
White-vented Euphonia	*Euphonia minuta*	Fringillidae	F	C	forest	L	N	Y	0
Tawny-capped Euphonia	*Euphonia anneae*	Fringillidae	F	U	forest	H	N	Y	1
Yellow-bellied Siskin	*Spinus xanthogastrus*	Fringillidae	G	C	edge	H	N	N	0
Rosy Thrush-Tanager	*Rhodinocichla rosea*	Rhodinocichlidae	I	U	forest	L	N	N	1

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Yellow-green Finch	*Pselliophorus luteoviridis*	Passerellidae	O	U	forest	H	N	N	0
Orange-billed Sparrow	*Arremon aurantiirostris*	Passerellidae	G	U	forest	L	N	Y	1
Sooty-faced Finch	*Arremon crassirostris*	Passerellidae	O	U	forest	H	N	Y	0
Chestnut-capped Brushfinch	*Arremon brunneinucha*	Passerellidae	G	U	forest	H	N	Y	1
Black-striped Sparrow	*Arremonops conirostris*	Passerellidae	O	U	edge	L	N	Y	1
White-naped Brushfinch	*Atlapetes albinucha*	Passerellidae	O	U	forest	H	N	N	0
Rufous-collared Sparrow	*Zonotrichia capensis*	Passerellidae	G	G	open	H	N	N	0
Common Chlorospingus	*Chlorospingus flavopectus*	Passerellidae	O	M	forest	H	N	N	0

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Tacarcuna Chlorospingus	*Chlorospingus tacarcunae*	Passerellidae	O	M	forest	H	N	N	0
Pirre Chlorospingus	*Chlorospingus inornatus*	Passerellidae	O	C	forest	H	N	N	0
Sooty-capped Chlorospingus	*Chlorospingus pileatus*	Passerellidae	O	M	forest	H	N	N	0
Yellow-throated Chlorospingus	*Chlorospingus flavigularis*	Passerellidae	O	U	forest	H	N	N	0
Wrenthrush	*Zeledonia coronata*	Zeledoniidae	I	G	forest	H	N	N	0
Eastern Meadowlark	*Sturnella magna*	Icteridae	I	G	open	L	N	N	0
Red-breasted Meadowlark	*Leistes militaris*	Icteridae	I	G	open	L	N	Y	0
Yellow-billed Cacique	*Amblycercus holosericeus*	Icteridae	I	U	edge	L	N	N	1
Montezuma Oropendola	*Psarocolius montezuma*	Icteridae	O	C	edge	L	N	N	0

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Scarlet-rumped Cacique	*Cacicus uropygialis*	Icteridae	O	C	forest	L	N	Y	1
Black-cowled Oriole	*Icterus prosthemelas*	Icteridae	O	C	edge	L	N	N	0
Yellow-backed Oriole	*Icterus chrysater*	Icteridae	O	C	edge	L	N	Y	0
Orange-crowned Oriole	*Icterus auricapillus*	Icteridae	O	C	edge	L	N	N	0
Yellow-tailed Oriole	*Icterus mesomelas*	Icteridae	I	U	edge	L	N	Y	1
Bronzed Cowbird	*Molothrus aeneus*	Icteridae	O	G	open	L	N	N	0
Giant Cowbird	*Molothrus oryzivorus*	Icteridae	P	C	open	L	N	Y	0
Great-tailed Grackle	*Quiscalus mexicanus*	Icteridae	O	G	open	L	N	Y	1

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Flame-throated Warbler	*Oreothlypis gutturalis*	Parulidae	I	C	edge	H	N	N	0
Olive-crowned Yellowthroat	*Geothlypis semiflava*	Parulidae	I	U	open	L	N	N	0
Tropical Parula	*Setophaga pitiayumi*	Parulidae	I	C	edge	H	N	Y	1
Yellow Warbler	*Setophaga petechia*	Parulidae	I	U	edge	L	N	Y	1
Buff-rumped Warbler	*Myiothlypis fulvicauda*	Parulidae	I	G	forest	L	N	Y	1
Rufous-capped Warbler	*Basileuterus rufifrons*	Parulidae	I	M	edge	L	N	N	1
Black-cheeked Warbler	*Basileuterus melanogenys*	Parulidae	I	U	forest	H	N	N	0
Golden-crowned Warbler	*Basileuterus culicivorus*	Parulidae	I	U	forest	H	N	N	1

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Costa Rican Warbler	*Basileuterus melanotis*	Parulidae	I	U	forest	H	N	N	
Slate-throated Redstart	*Myioborus miniatus*	Parulidae	I	C	forest	H	N	Y	1
Collared Redstart	*Myioborus torquatus*	Parulidae	I	C	forest	H	N	N	0
Dusky-faced Tanager	*Mitrospingus cassinii*	Mitrospingidae	O	U	edge	L	N	Y	1
Flame-colored Tanager	*Piranga bidentata*	Cardinalidae	F	C	forest	H	N	N	0
White-winged Tanager	*Piranga leucoptera*	Cardinalidae	F	C	forest	H	N	N	0
Red-crowned Ant-Tanager	*Habia rubica*	Cardinalidae	I	U	forest	L	N	Y	1
Red-throated Ant-Tanager	*Habia fuscicauda*	Cardinalidae	I	U	forest	L	N	Y	1

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Lemon-spectacled Tanager	*Chlorothraupis olivacea*	Cardinalidae	I	U	forest	H	N	N	0
Black-faced Grosbeak	*Caryothraustes poliogaster*	Cardinalidae	O	C	forest	L	N	N	0
Blue-black Grosbeak	*Cyanocompsa cyanoides*	Cardinalidae	G	U	forest	L	N	Y	1
Blue-and-gold Tanager	*Bangsia arcaei*	Thraupidae	O	C	forest	H	N	N	0
Blue-gray Tanager	*Thraupis episcopus*	Thraupidae	O	C	edge	L	N	Y	1
Palm Tanager	*Thraupis palmarum*	Thraupidae	O	C	edge	L	N	Y	1
Golden-hooded Tanager	*Tangara larvata*	Thraupidae	F	C	forest	L	N	Y	1
Speckled Tanager	*Tangara guttata*	Thraupidae	F	C	forest	H	N	Y	0

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Green-naped Tanager	*Tangara fucosa*	Thraupidae	F	C	forest	H	N	N	0
Spangle-cheeked Tanager	*Tangara dowii*	Thraupidae	F	M	forest	H	N	N	0
Plain-colored Tanager	*Tangara inornata*	Thraupidae	F	C	forest	L	N	Y	1
Bay-headed Tanager	*Tangara gyrola*	Thraupidae	F	C	forest	L	N	Y	1
Silver-throated Tanager	*Tangara icterocephala*	Thraupidae	F	C	forest	H	N	Y	1
White-eared Conebill	*Conirostrum leucogenys*	Thraupidae	O	C	edge	L	N	N	0
Green Honeycreeper	*Chlorophanes spiza*	Thraupidae	F	C	forest	L	N	Y	0
Black-and-yellow Tanager	*Chrysothlypis chrysomelas*	Thraupidae	F	C	forest	H	N	Y	1

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Sulphur-rumped Tanager	*Heterospingus rubrifrons*	Thraupidae	F	C	forest	L	N	Y	0
Scarlet-browed Tanager	*Heterospingus xanthopygius*	Thraupidae	I	C	forest	L	N	N	0
Blue-black Grassquit	*Volatinia jacarina*	Thraupidae	G	U	open	L	N	Y	1
Gray-headed Tanager	*Eucometis penicillata*	Thraupidae	F	U	forest	L	N	Y	1
White-shouldered Tanager	*Tachyphonus luctuosus*	Thraupidae	F	M	forest	L	N	Y	0
Tawny-crested Tanager	*Tachyphonus delatrii*	Thraupidae	O	U	edge	L	N	Y	1
White-throated Shrike-Tanager	*Lanio leucothorax*	Thraupidae	I	M	forest	H	N	N	0

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Scarlet-rumped Tanager	*Ramphocelus passerinii*	Thraupidae	F	U	edge	L	N	N	1
Crimson-backed Tanager	*Ramphocelus dimidiatus*	Thraupidae	F	U	edge	L	N	N	1
Shining Honeycreeper	*Cyanerpes lucidus*	Thraupidae	F	C	forest	L	N	Y	0
Red-legged Honeycreeper	*Cyanerpes cyaneus*	Thraupidae	F	C	forest	L	N	Y	1
Blue Dacnis	*Dacnis cayana*	Thraupidae	P	C	edge	L	N	Y	1
Bananaquit	*Coereba flaveola*	Thraupidae	N	C	edge	L	N	N	1
Yellow-faced Grassquit	*Tiaris olivaceus*	Thraupidae	G	U	open	L	N	Y	1
Slate-colored Seedeater	*Sporophila schistacea*	Thraupidae	G	U	edge	L	N	Y	0
Ruddy-breasted Seedeater	*Sporophila minuta*	Thraupidae	G	U	open	L	N	Y	0

English Name	Scientific Name	Family	Diet	Stratum	Habitat	Lowland/ Highland	Vagrant/ Introduced	Widespread?	Sampled E and W?
Variable Seedeater	*Sporophila americana*	Thraupidae	G	U	open	L	N	Y	1
Buff-throated Saltator	*Saltator maximus*	Thraupidae	F	M	edge	L	N	Y	1
Slate-colored Grosbeak	*Saltator grossus*	Thraupidae	G	C	forest	L	N	Y	0
Streaked Saltator	*Saltator striatipectus*	Thraupidae	O	U	edge	L	N	Y	0

Table A1.3: Fossil calibration used for BEAST analysis.

Node	Date (Mya)	Priors (offset, mean, sd)	Source
Parulidae + Icteridae	18-23	18, 1.0, 1.25	(Päckert *et al.* 2016)
Cyanocompsa	4.5-10	4.5, 1.0, 1.25	(Päckert *et al.* 2016)
Oscines	27.25-56.0	43.9, 1.0, 1.25	(Oliveros *et al.* 2019)
Wrens	17.2-56.0	25.5, 1.0, 1.25	(Oliveros *et al.* 2019)
Passerines	51.81-66.5	56.26, 1.0, 1.25	(Oliveros *et al.* 2019)

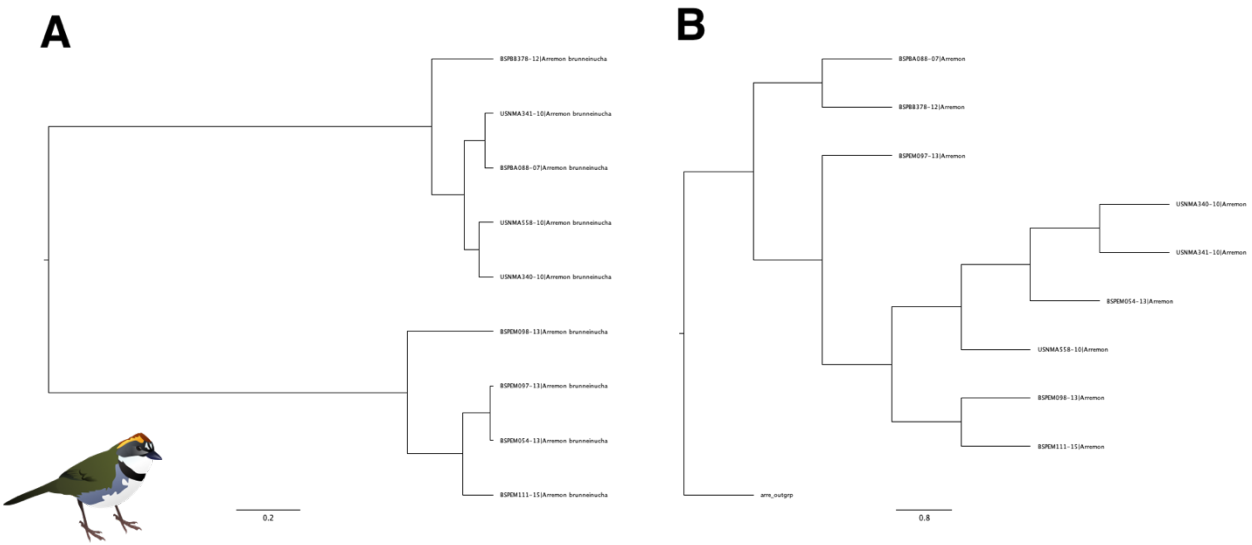

Figure A1.1: COI trees of *Arremon brunneinucha*, with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

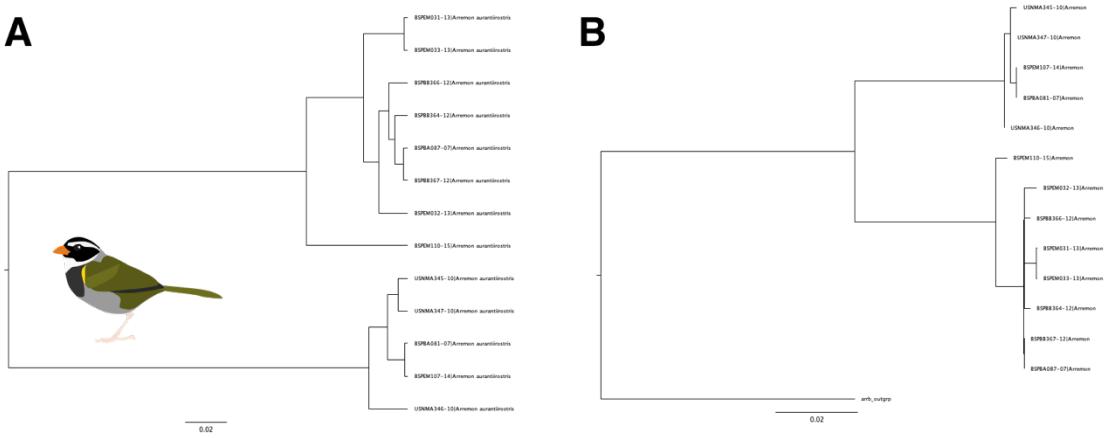

Figure A1.2: COI trees of *Arremon aurantiirostris* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

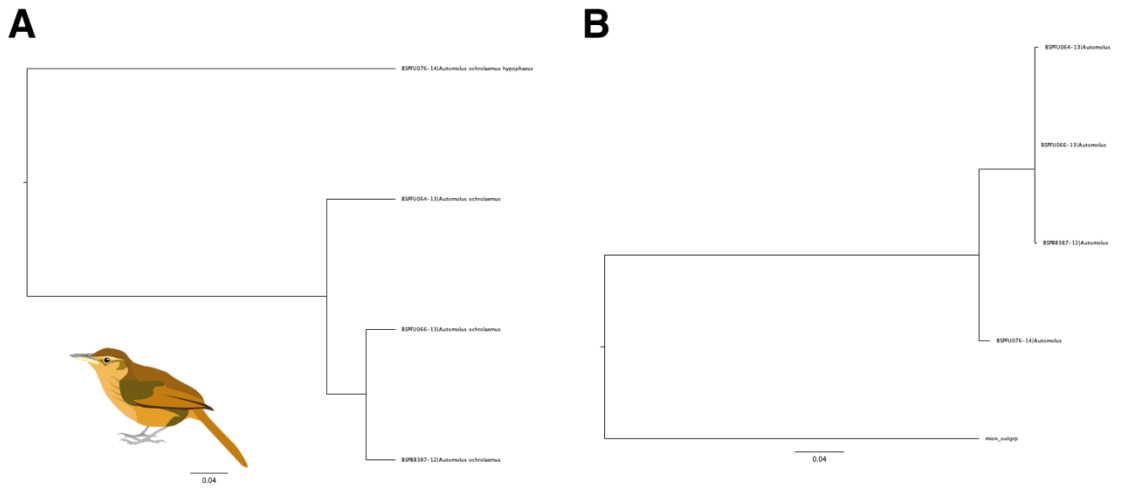

Figure A1.3: C COI trees of *Automolus ochrolaemus* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

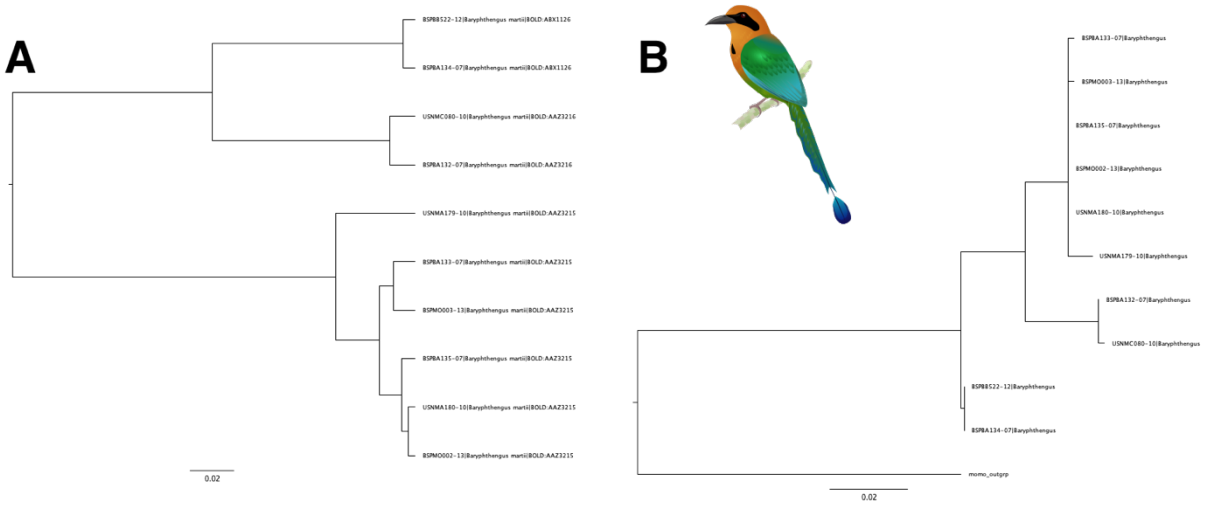

Figure A1.4: COI trees of *Baryphthengus martii* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

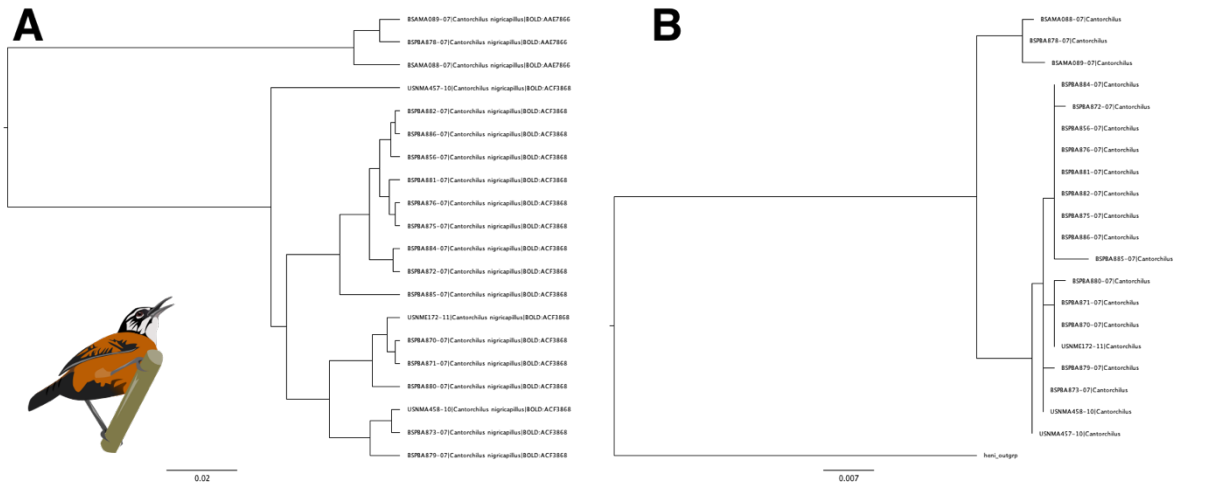

Figure A1.5: COI trees of *Cantorchilus nigricapillus* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

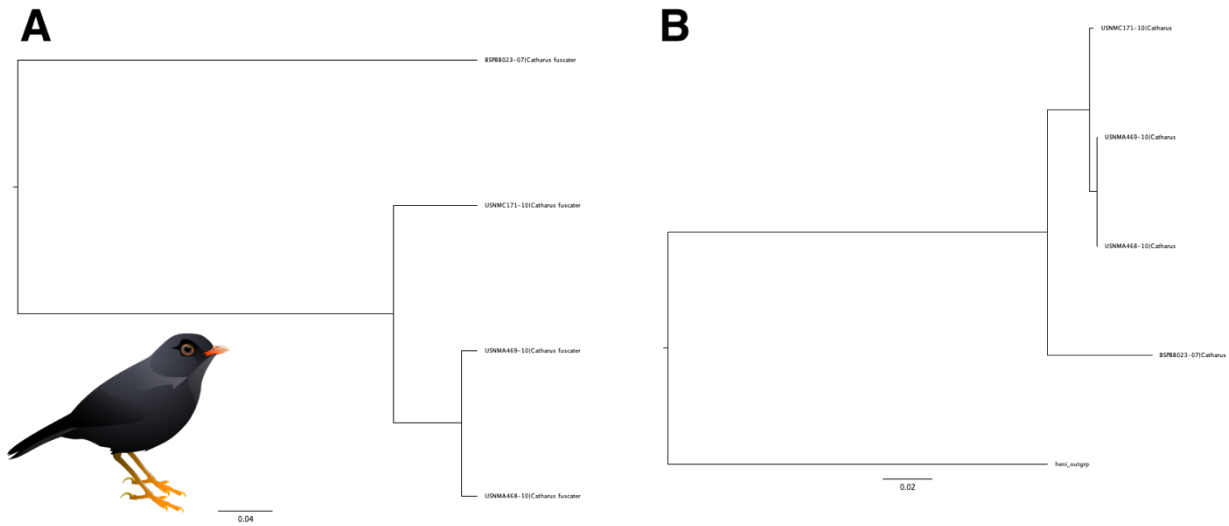

Figure A1.6: COI trees of *Catharus fuscater* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

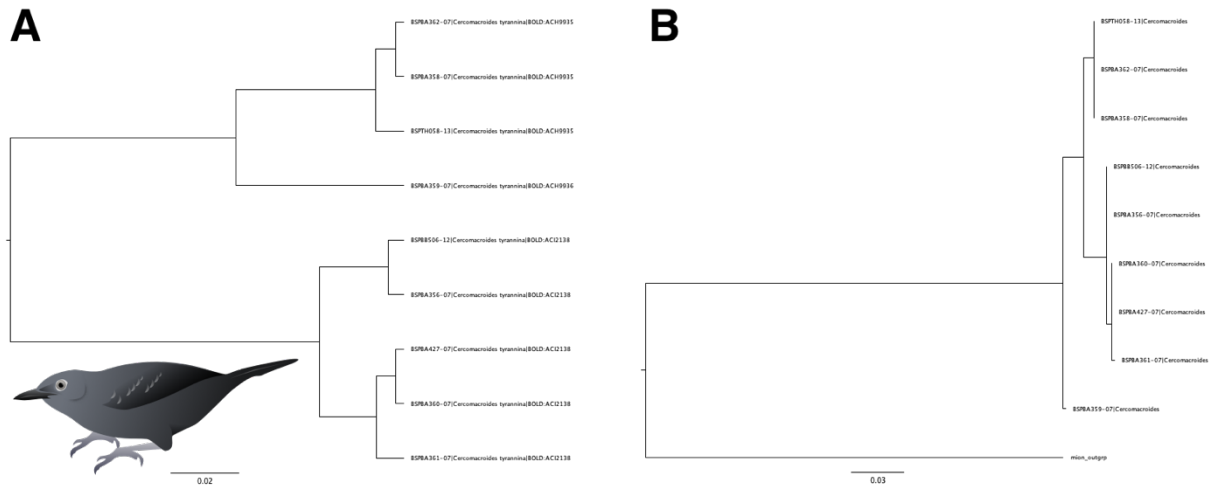

Figure A1.7: COI trees of *Cercomacra tyrannina* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

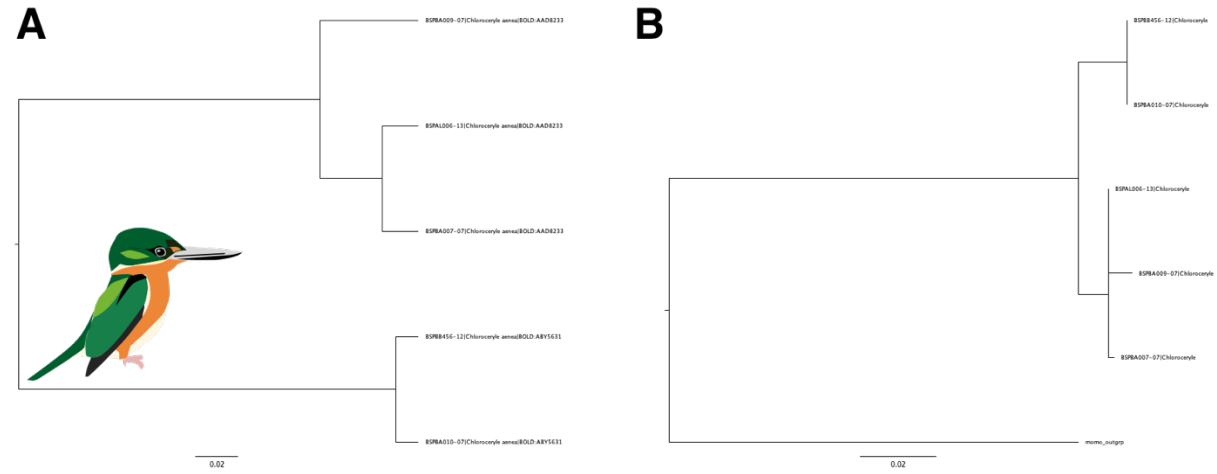

Figure A1.8: COI trees of *Chloroceryle aenea* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

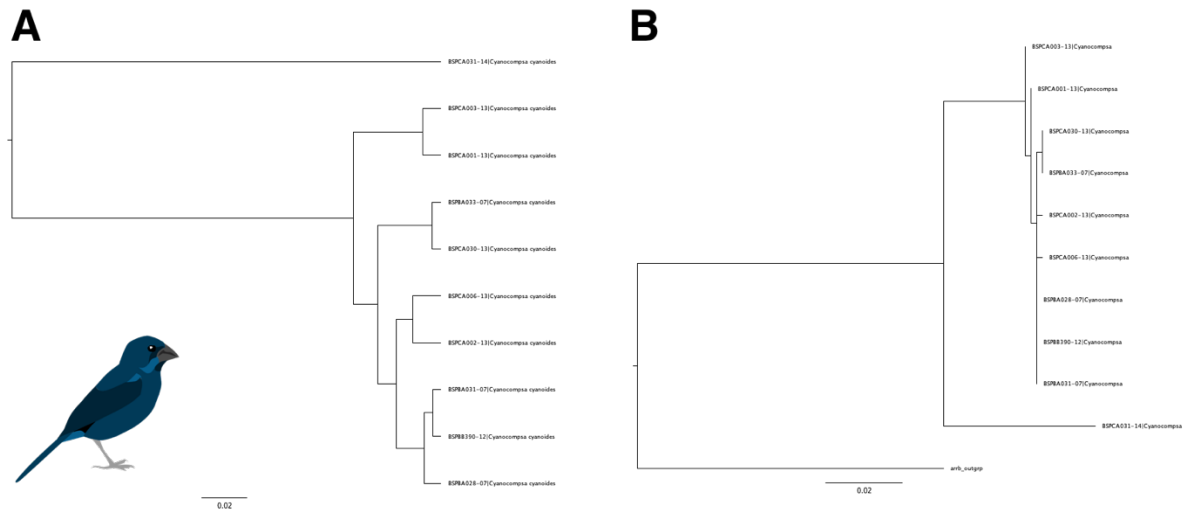

Figure A1.9: COI trees of *Cyanocompsa cyanoides* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

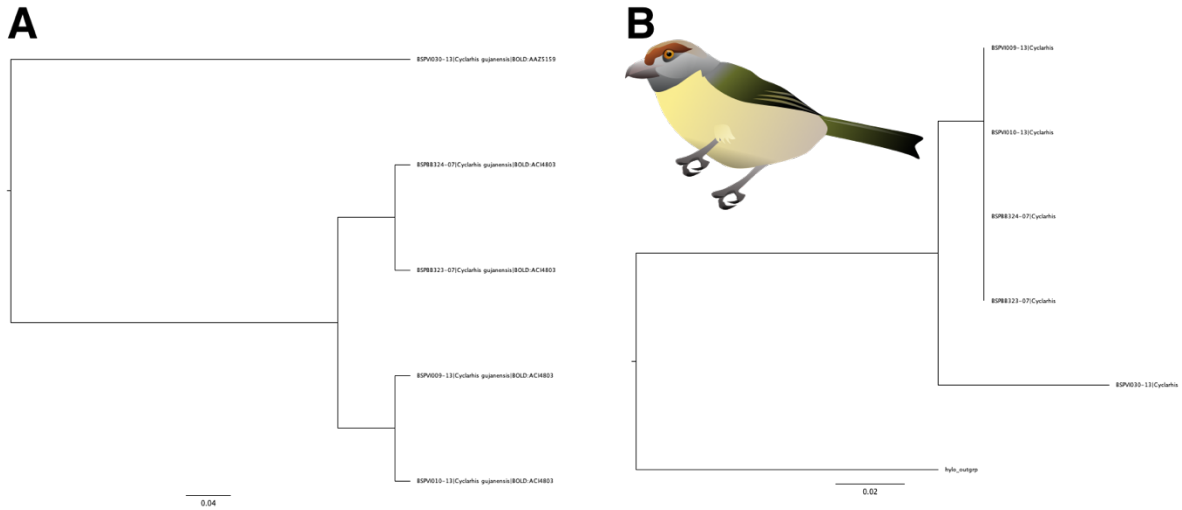

Figure A1.10: COI trees of *Cyclarhis gujanensis* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

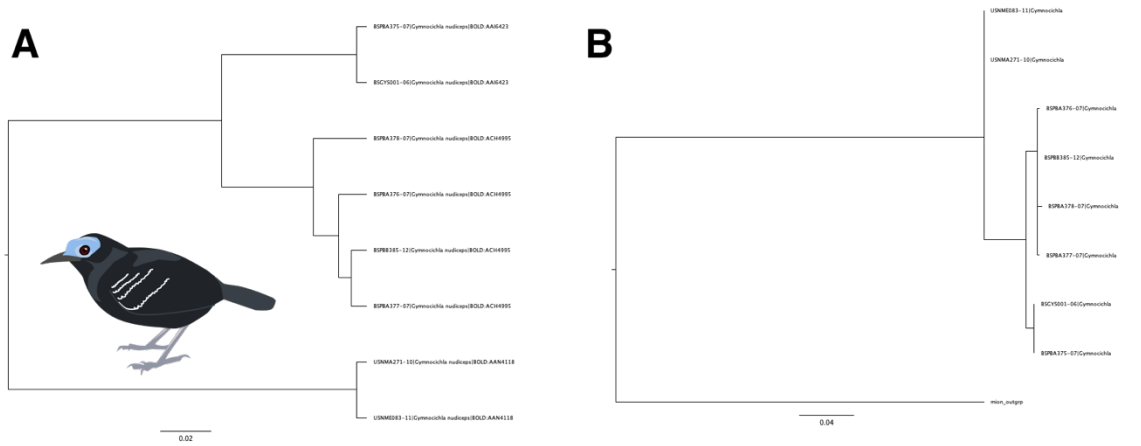

Figure A1.11: COI trees of *Gymnocichla nudiceps* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

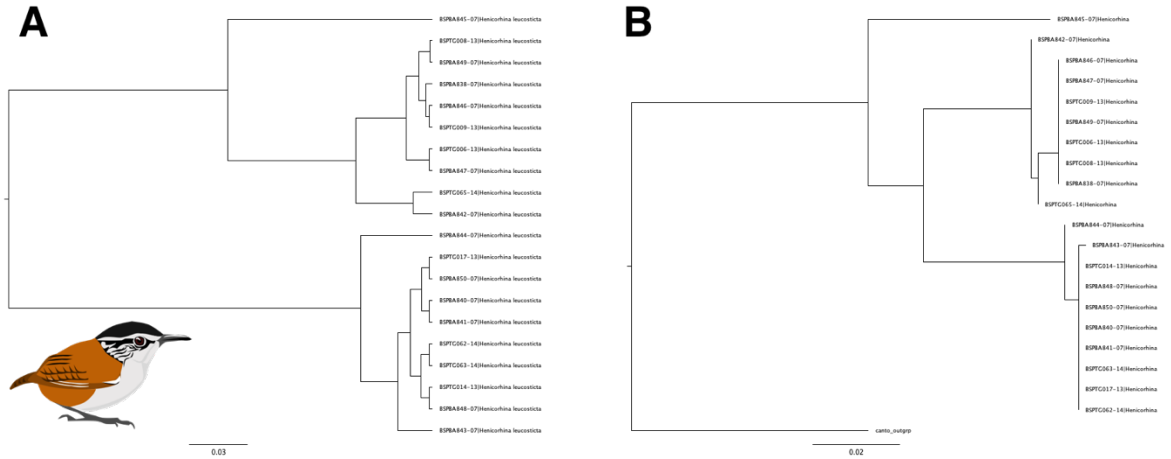

Figure A1.12: COI trees of *Henicorhina leucosticte* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

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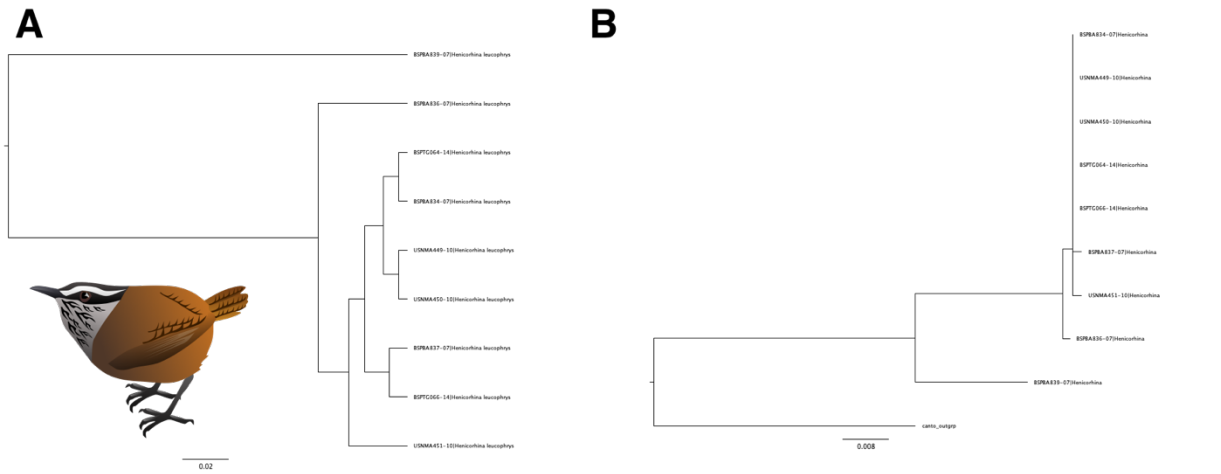


Figure A1.13: COI trees of *Henicorhina leucophrys* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

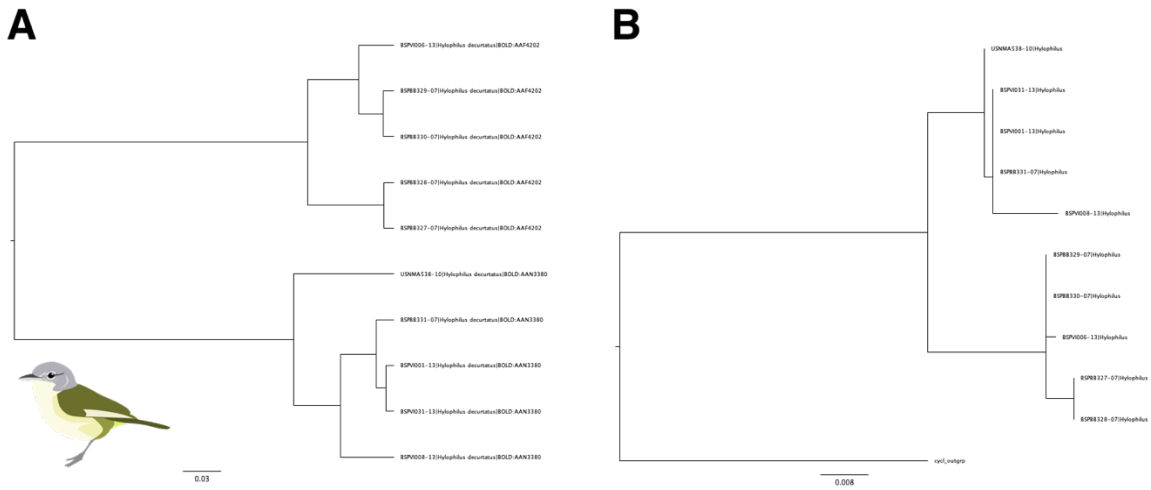


Figure A1.14: COI trees of *Pachysylvia decurtata* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

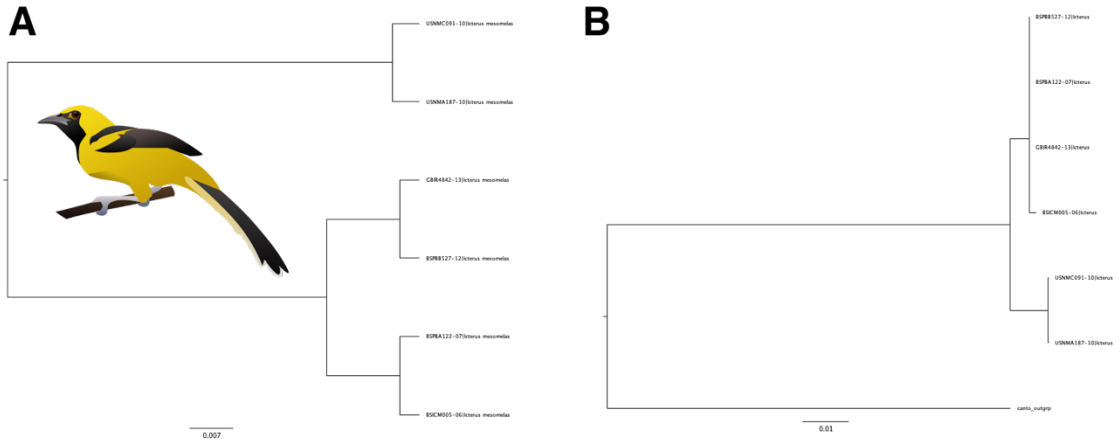


Figure A1.15: COI trees of *Icterus mesomelas* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

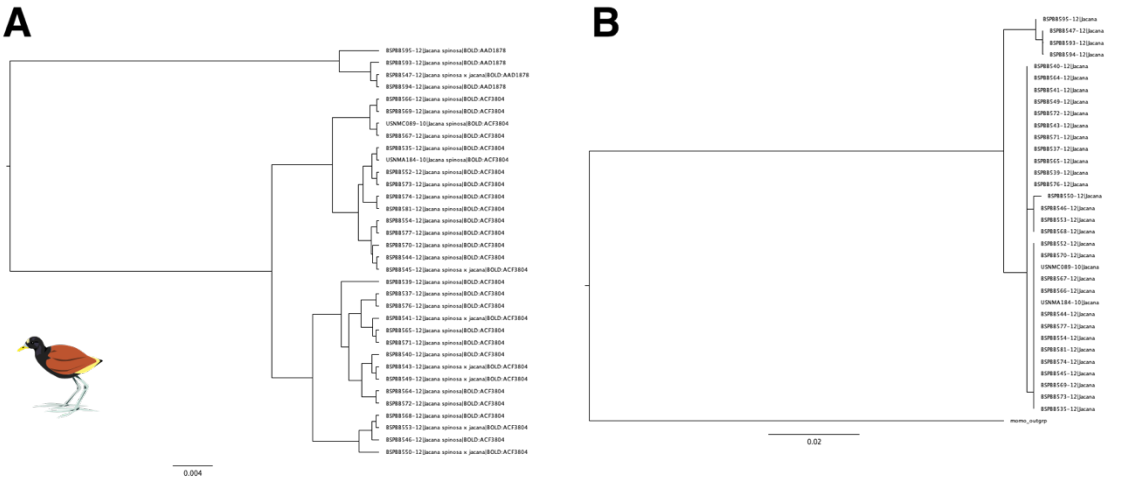


Figure A1.16: COI trees of *Jacana spinosa* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

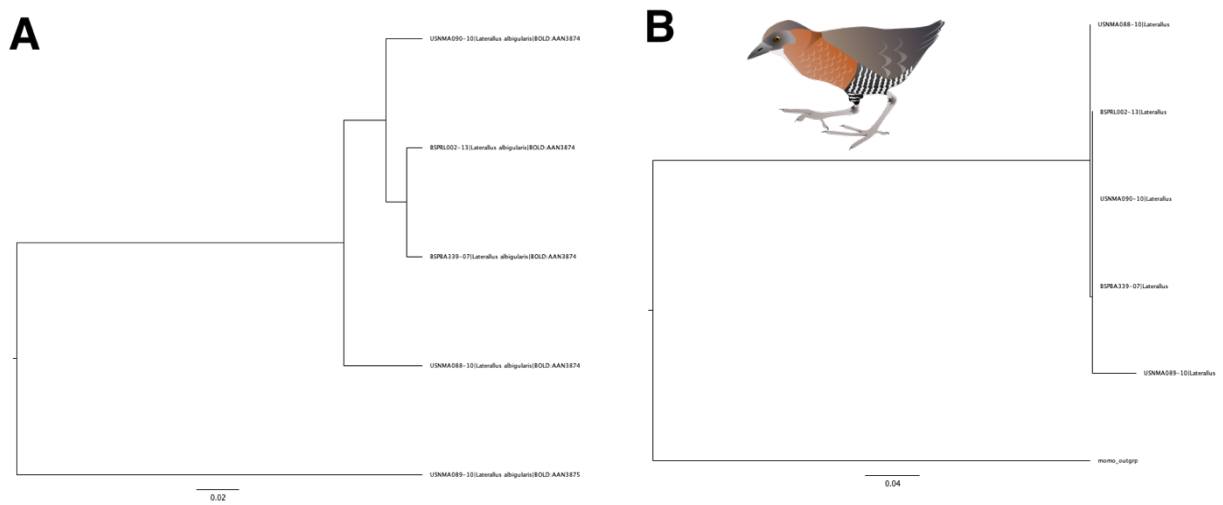


Figure A1.17: COI trees of *Laterallus albigularis* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

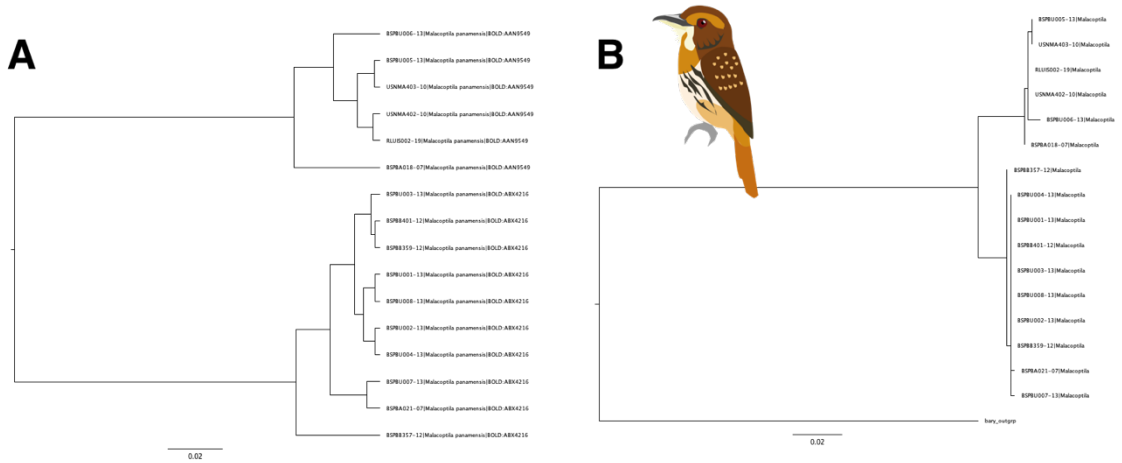


Figure A1.18: COI trees of *Malacoptila panamensis* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

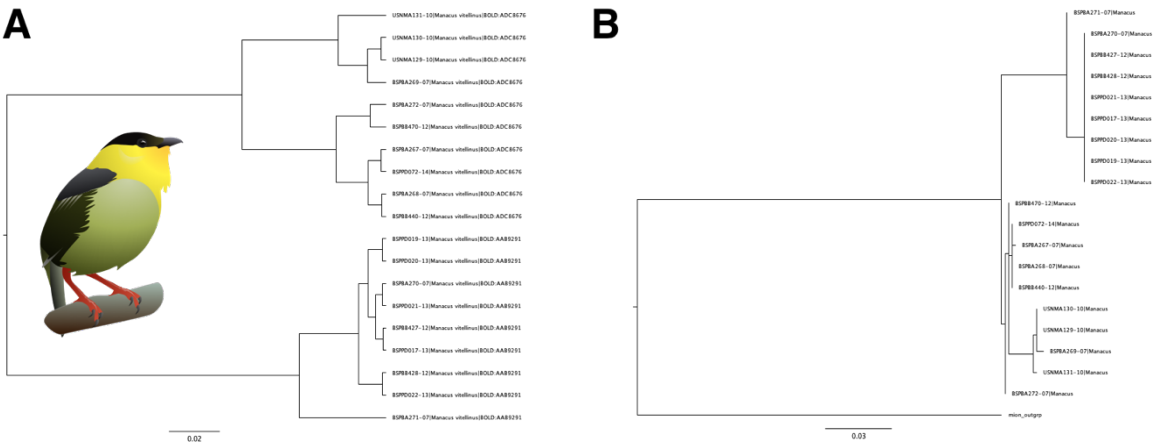


Figure A1.19: COI trees of *Manacus vitellinus* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

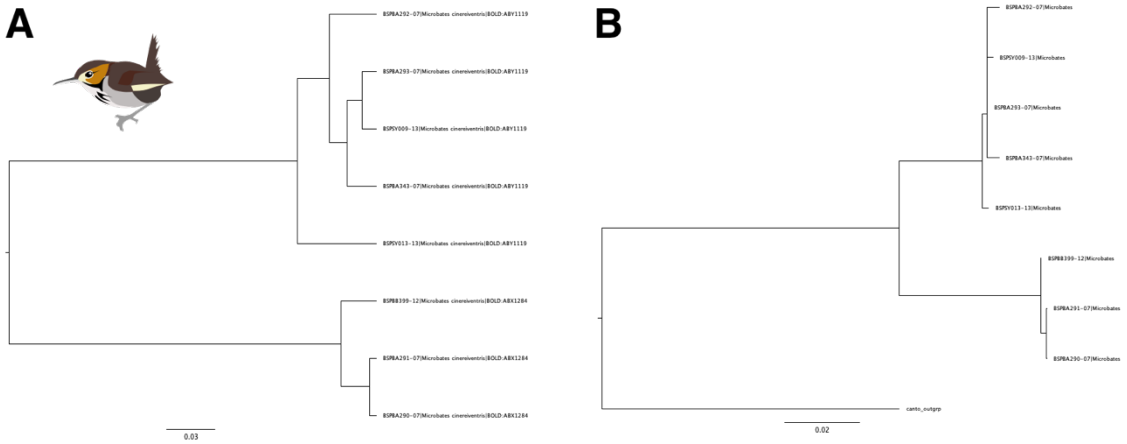


Figure A1.20: COI trees of *Microbates cinereiventris* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

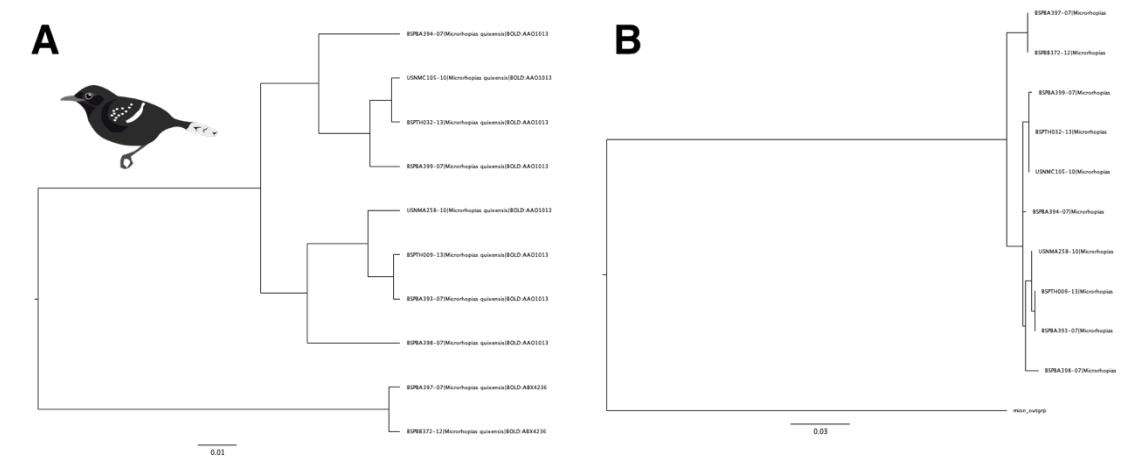


Figure A1.21: COI trees of *Microrhopias quixensis* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

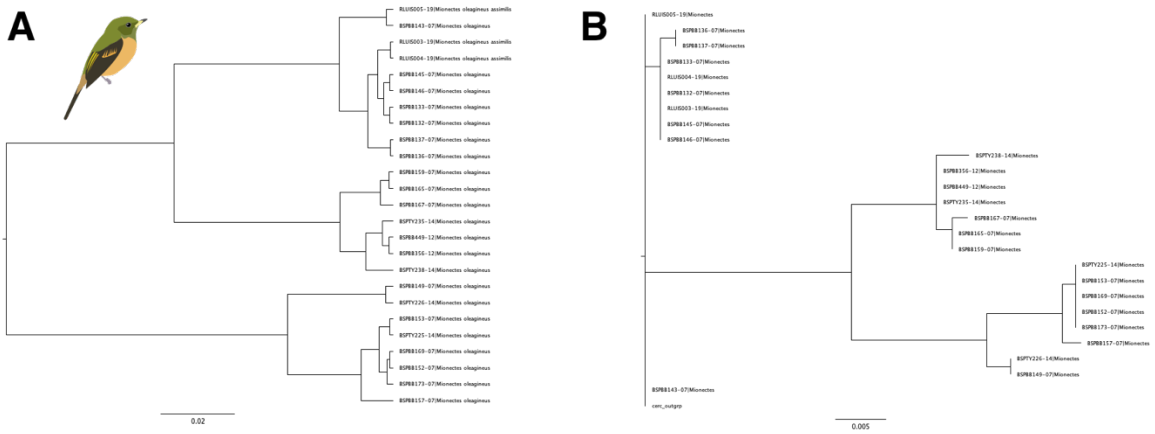


Figure A1.22: COI trees of *Mionectes oleagineus* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

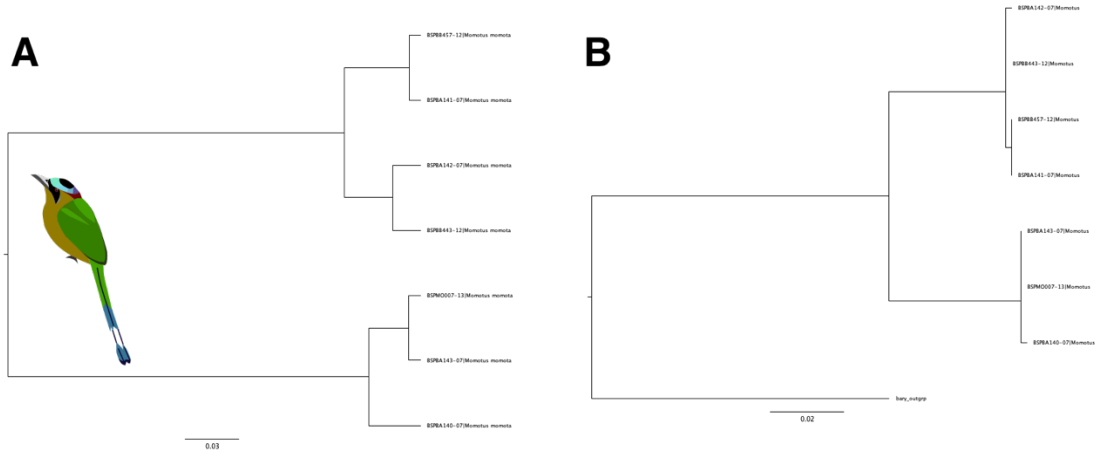


Figure A1.23: COI trees of *Momotus momota* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

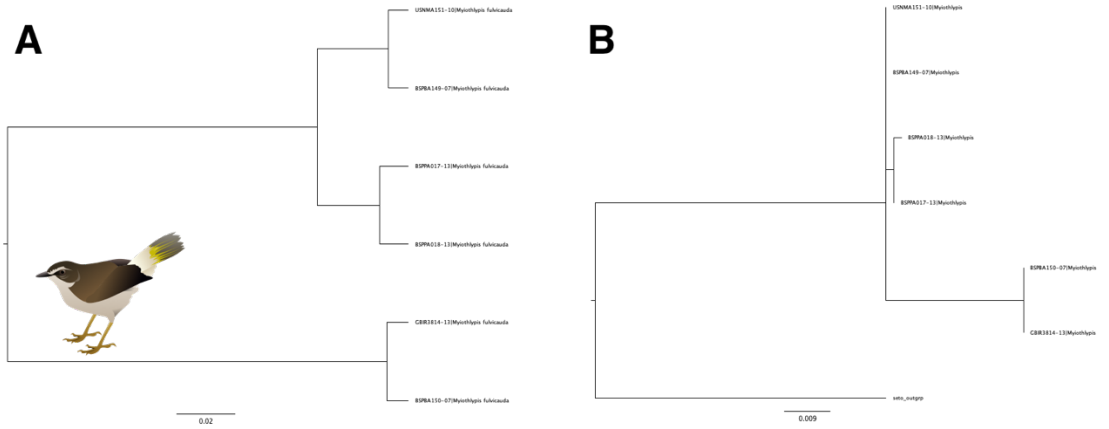


Figure A1.24: COI trees of *Myiothlypis fulvicauda* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

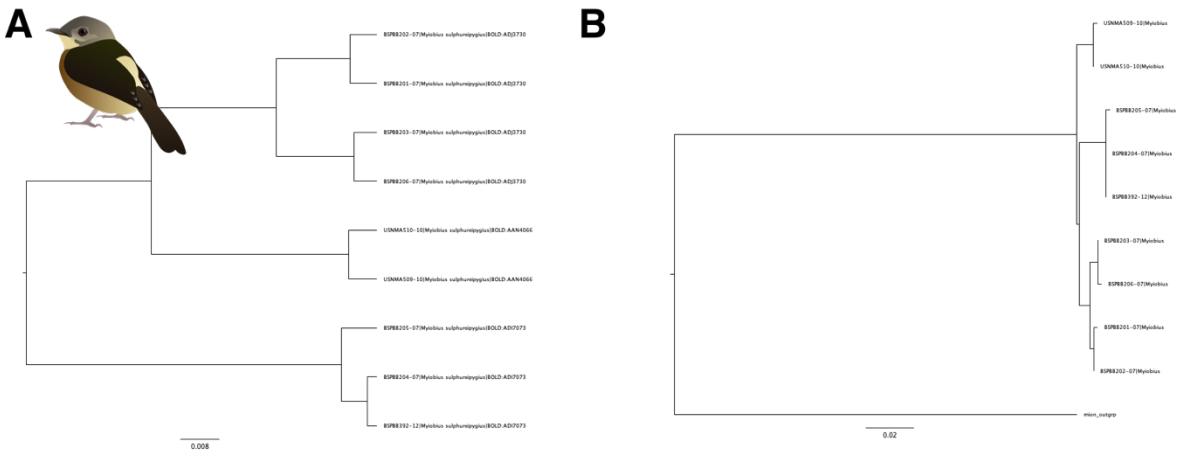


Figure A1.25: COI trees of *Myiobius sulphureipygius* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

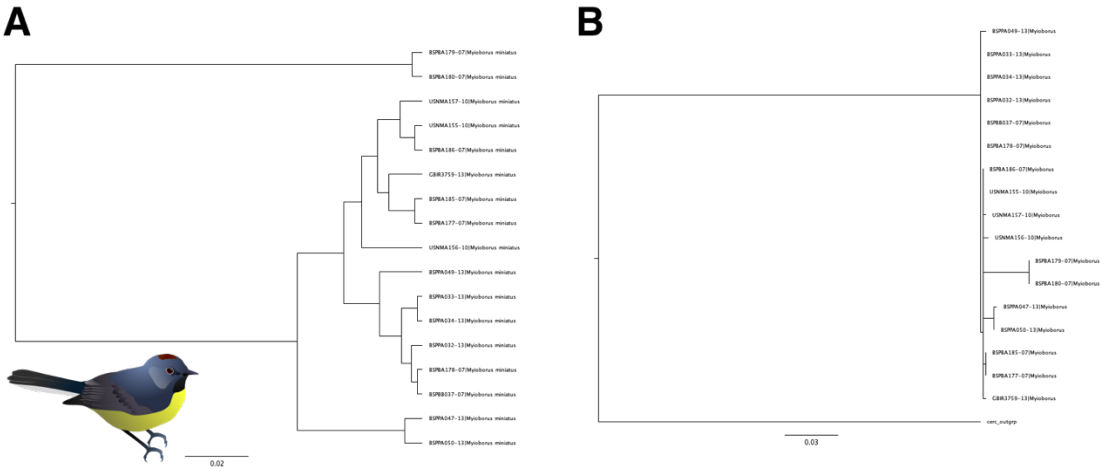


Figure A1.26: COI trees of *Myioborus miniatus* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

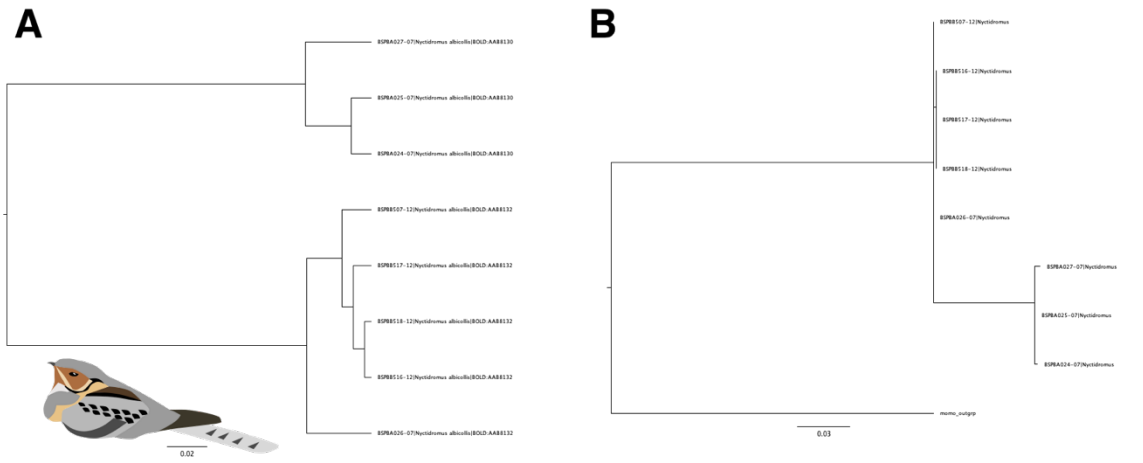


Figure A1.27: COI trees of *Nyctidromus albicollis* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

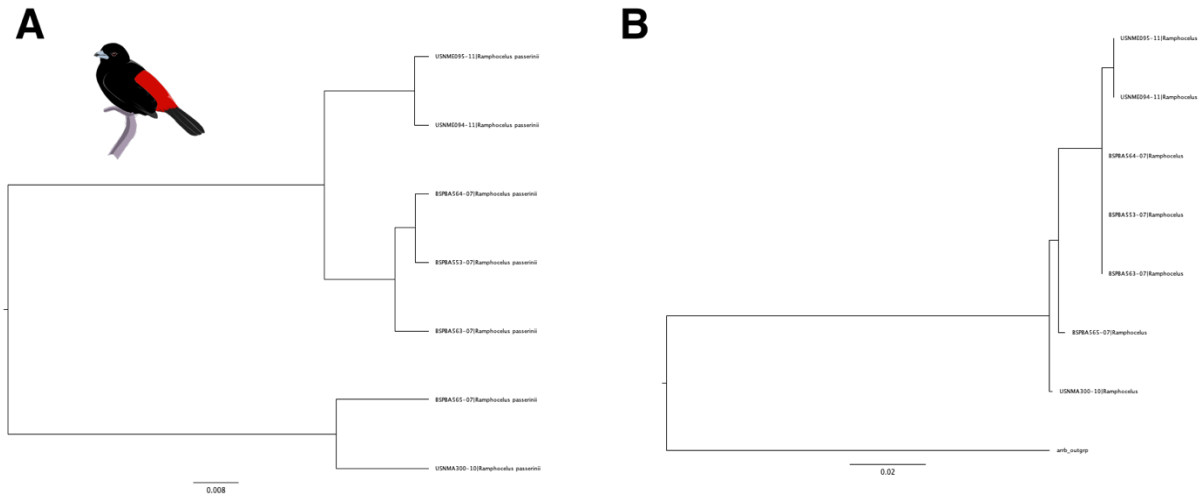


Figure A1.28: COI trees of *Ramphocelus flammigerus* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

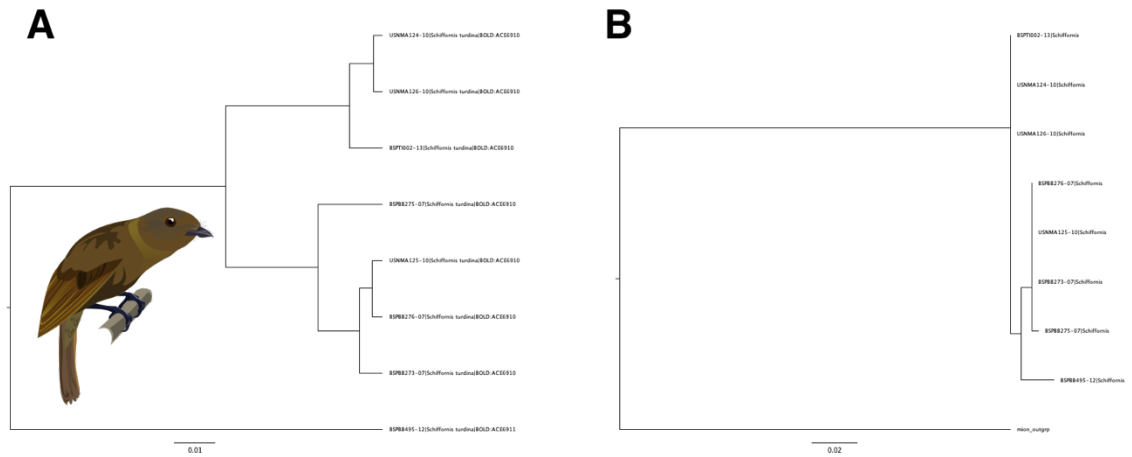


Figure A1.29: COI trees of *Schiffornis "turdina"* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

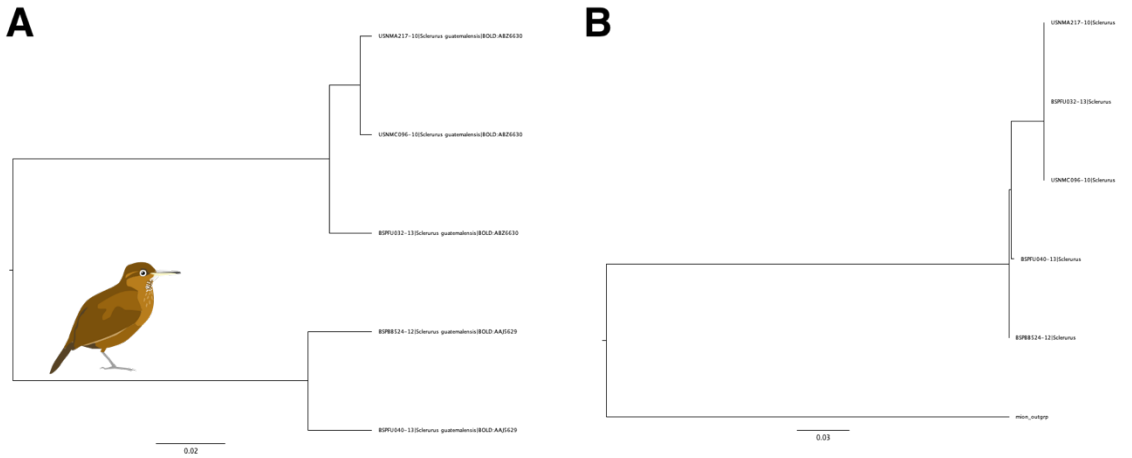


Figure A1.30: COI trees of *Sclerurus guatemalensis* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

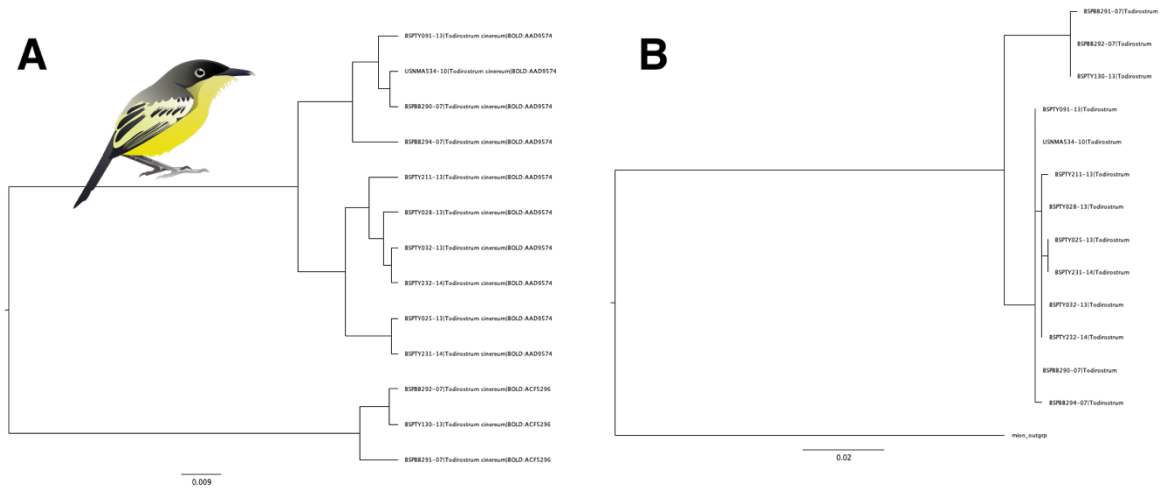


Figure A1.31: COI trees of *Todirostrum cinereum* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

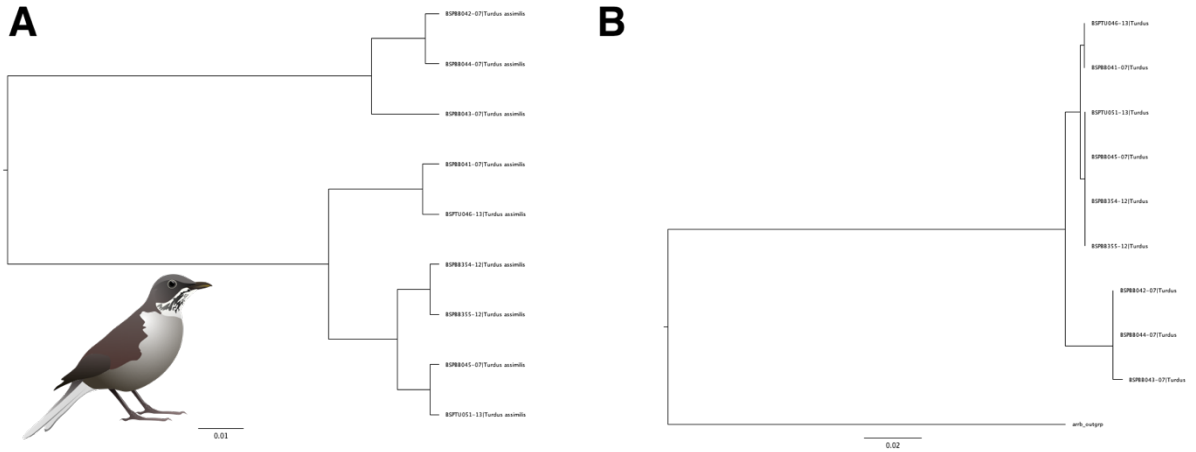


Figure A1.32: COI trees of *Turdus assimilis* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

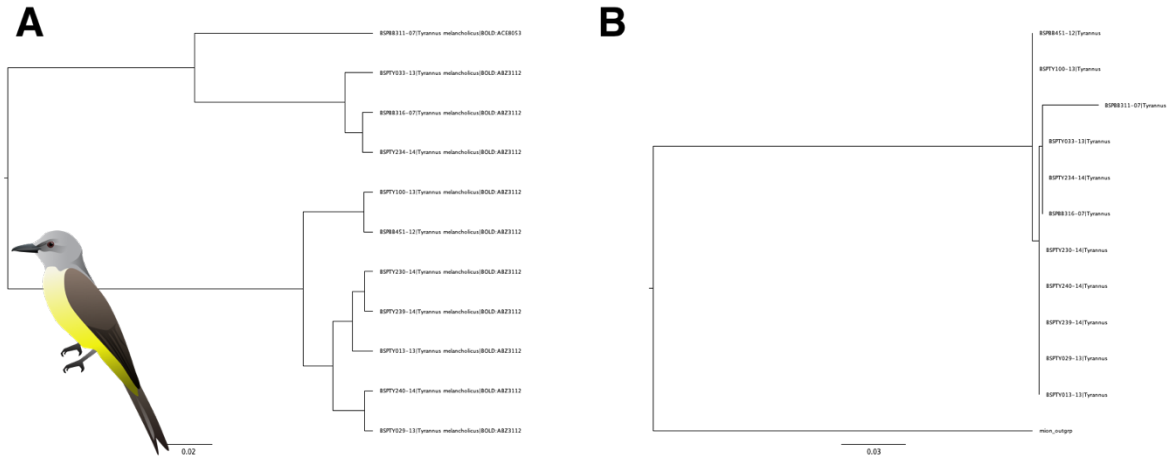


Figure A1.33: COI trees of *Tyrannus melancholicus* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

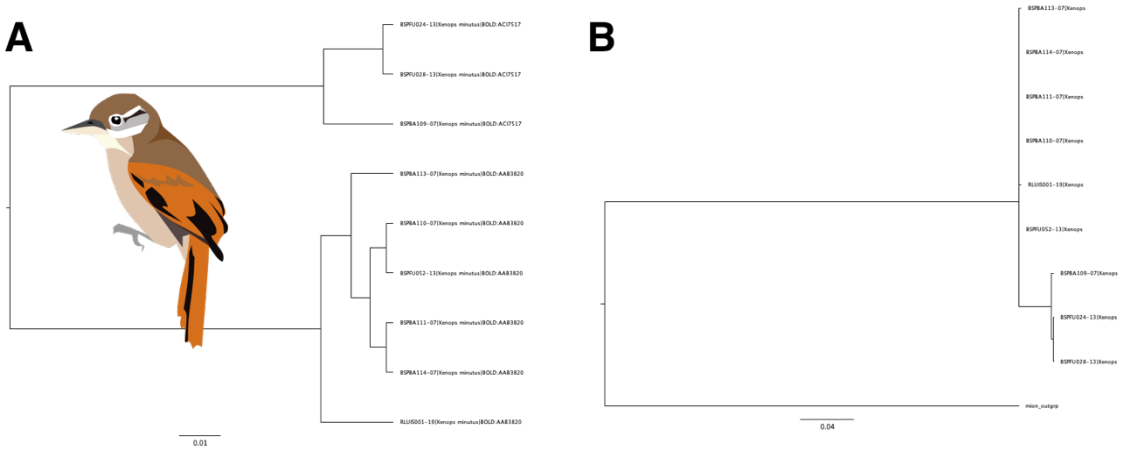


Figure A1.34 COI trees of *Xenops minutus* with both A) a NJ tree constructed in MEGA and B) a ML tree constructed in RaxML.

Appendix to Chapter 2: Supplementary Tables and Figures

Table A2.1: Sequencing and UCE recovery results. UCE= UCE enriched sample, WGS= unenriched whole genome shotgun library. Museum numbers provided where specimens have been fully cataloged, with field catalog numbers provided for samples which have not been added to museum databases. CUMV: Cornell University Museum of Vertebrates, FMNH: Field Museum of Natural History, STRIBC: Smithsonian Tropical Research Institute Bird Collection, UAM: University of Alaska Museum, UWBM: University of Washington Burke Museum.

Taxon	Museum Number	Catalog Number	Population	Type	Reads (millions)	SRA Accession Number
Arremon aurantiirostris	UWBM93635		Honduras: Copán	WGS	82.8	SAMN28920643
Arremon aurantiirostris	UWBM56060		Nicaragua: La Luz	WGS	120.6	SAMN28920644
Arremon aurantiirostris	UWBM70031		Nicaragua: La Luz	WGS	104.4	SAMN28920645
Arremon aurantiirostris		MJM2298	Panama: Bocas del Toro, Bosque Protector de Palo Seco	WGS	47.4	SAMN28920646
Arremon aurantiirostris	STRIBC5367	MJM2299	Panama: Bocas del Toro, Bosque Protector de Palo Seco	WGS	42.8	SAMN28920647
Arremon aurantiirostris	STRIBC2287	MJM2930	Panama: Bocas del Toro, Changuinola	UCE		SAMN28920648

Taxon	Museum Number	Catalog Number	Population	Type	Reads (millions)	SRA Accession Number
Arremon aurantiirostris	STRIBC2266	MJM4225	Panama: Bocas del Toro, Changuinola	UCE		SAMN28920649
Arremon aurantiirostris		JTW288	Panama: Comarca Ngäbe-Buglé, Cerro Chalite	UCE		SAMN28920650
Arremon aurantiirostris	FMNH470780	GMS2011	Panama: Bocas del Toro, Bosque Protector de Palo Seco	UCE		SAMN28920651
Arremon aurantiirostris		JFM022	Panama: Veraguas, Río Luis	WGS	72.8	SAMN28920652
Arremon aurantiirostris		JFM037	Panama: Veraguas, Río Luis	WGS	203.8	SAMN28920653
Arremon aurantiirostris		JFM044	Panama: Veraguas, Río Luis	WGS	40.8	SAMN28920654
Arremon aurantiirostris		JFM047	Panama: Veraguas, Río Luis	WGS	126.4	SAMN28920655
Arremon aurantiirostris	UWBM108325	JMD114	Panama: Veraguas, Santa Fé	UCE	4.68	SAMN28920656
Arremon aurantiirostris	STRIBC2762	MJM6862	Panama: Veraguas, Santa Fé	UCE	0.42	SAMN28920657

Taxon	Museum Number	Catalog Number	Population	Type	Reads (millions)	SRA Accession Number
Arremon aurantiirostris	STRIBC2577	MJM6871	Panama: Veraguas, Santa Fé	UCE	1.12	SAMN28920658
Arremon aurantiirostris	STRIBC2811	MJM6932	Panama: Veraguas, Santa Fé	UCE	3.35	SAMN28920659
Arremon aurantiirostris	STRIBC4312	MJM8129	Panama: Coclé, La Pintada, Coclesito	UCE	0.85	SAMN28920660
Arremon aurantiirostris	STRIBC3568	MJM8186	Panama: Coclé, La Pintada, Coclesito	UCE	4.87	SAMN28920661
Arremon aurantiirostris	UWBM106601	GMS1179	Panama: Colón, Achiote	UCE	0.60	SAMN28920662
Arremon aurantiirostris	UWBM106602	GMS1180	Panama: Colón, Achiote	UCE	0.67	SAMN28920663
Arremon aurantiirostris	STRIBC2810	MJM6628	Panama: Coclé, San Juan, Aguas Claras	UCE	0.73	SAMN28920664
Arremon aurantiirostris	STRIBC2268	MJM7004	Panama: Coclé, San Juan, Aguas Claras	UCE	3.33	SAMN28920665
Arremon aurantiirostris	UWBM76885	RCF2020	Panama: Panamá, Panama City, confluence of Rios Chagres and Chagrecito	UCE	0.66	SAMN28920666

Taxon	Museum Number	Catalog Number	Population	Type	Reads (millions)	SRA Accession Number
Arremon aurantiirostris	UWBM76954	SMB205	Panama: Panamá, Panama City, confluence of Rios Chagres and Chagrecito	UCE	3.96	SAMN28920667
Arremon aurantiirostris	STRIBC3944	MJM8562	Panama: Panama, Chiman, Cerro Chucantí	UCE	0.96	SAMN28920668
Arremon aurantiirostris	STRIBC3713	MJM8563	Panama: Panama, Chiman, Cerro Chucantí	UCE	3.20	SAMN28920669
Arremon aurantiirostris	UAM22809	MJM1933	Panama: Darién, Cana	WGS	64.3	SAMN28920670
Arremon aurantiirostris	UAM25715	MJM1949	Panama: Darién, Cana	UCE	0.65	SAMN28920671
Arremon aurantiirostris	UAM22803	MJM1979	Panama: Darién, Cana	UCE	0.75	SAMN28920672
Arremon aurantiirostris	UAM22807	MJM2005	Panama: Darién, Cana	UCE	0.61	SAMN28920673
Arremon aurantiirostris	UAM25716	MJM1931	Panama: Darién, Cana	UCE	0.68	SAMN28920674
Cantorchilus nigricapillus	STRIBC1447	MJM2656	Panama: Bocas del Toro, Isla Escudo de Veraguas	WGS	229.4	SAMN28920675

Taxon	Museum Number	Catalog Number	Population	Type	Reads (millions)	SRA Accession Number
Cantorchilus nigricapillus		JFM052	Panama: Veraguas, Río Luis	WGS	226.8	SAMN28920676
Cantorchilus nigricapillus		JFM053	Panama: Veraguas, Río Luis	WGS	40.4	SAMN28920677
Cantorchilus nigricapillus	STRIBC3992		Panama: Bocas del Toro, Isla Escudo de Veraguas	WGS	123.6	SAMN28920678
Cantorchilus nigricapillus	STRIBC3982		Panama: Bocas del Toro, Isla Escudo de Veraguas	WGS	34.3	SAMN28920679
Cantorchilus nigricapillus	STRIBC1474	MJM2759	Panama: Colón, Achiote	UCE	3.8	SAMN28920680
Cantorchilus nigricapillus	STRIBC1476	MJM4664	Panama: Colón, Achiote	UCE	4.0	SAMN28920681
Cantorchilus nigricapillus		PA-TNI472	Panama: Panamá, Cerro Azul	UCE	0.50	SAMN28920682
Cantorchilus nigricapillus	STRIBC4216		Panama: Colón, Santa Isabel, Palenque	WGS	127.4	SAMN28920683
Cantorchilus nigricapillus		PA-TNI26392	Panama: Panamá, Serranía de San Blas, west end	UCE	0.71	SAMN28920684

Taxon	Museum Number	Catalog Number	Population	Type	Reads (millions)	SRA Accession Number
Cantorchilus nigricapillus		PA-TNI26393	Panama: Panamá, Serranía de San Blas, west end	UCE	0.68	SAMN28920685
Cantorchilus nigricapillus		PA-TNI1906	Panama: Panama, Chiman, Cerro Chucantí	UCE	0.66	SAMN28920686
Cantorchilus nigricapillus	PA-TNI666		Panama: Darién, Puerto Piña	WGS	211.7	SAMN28920687
Cantorchilus nigricapillus	PA-TNI46569		Panama: Darién, Rancho Frío	WGS	78.7	SAMN28920688
Cantorchilus nigricapillus	STRIBC4840		Panama: Darién, Rancho Frío	WGS	51.9	SAMN28920689
Cantorchilus nigricapillus	STRIBC4841		Panama: Darién, Rancho Frío	WGS	96.9	SAMN28920690
Cantorchilus nigricapillus	UAM25132	JMM1041	Panama: Darién, Cana	UCE	1.00	SAMN28920691
Cantorchilus nigricapillus	UAM31187	JMM1042	Panama: Darién, Cana	WGS	60.1	SAMN28920692
Cantorchilus nigricapillus	UAM31480	JMM1043	Panama: Darién, Cana	WGS	82.9	SAMN28920693

Taxon	Museum Number	Catalog Number	Population	Type	Reads (millions)	SRA Accession Number
Cantorchilus nigricapillus	UAM27613	KSW4884	Panama: Darién, Cana	UCE	0.90	SAMN28920694
Cantorchilus nigricapillus		MJM2125	Panama: Darién, Cana	UCE	0.53	SAMN28920695
Cantorchilus nigricapillus		EC-TNI2047	Ecuador: Pichincha, Mindo	WGS	189.4	SAMN28920696
Cantorchilus nigricapillus		EC-TNI12053	Ecuador: Pichincha, Mindo	WGS	111.0	SAMN28920697
Cyanocompsa cyanoides	UAM24530	ABJ481	Belize: Toledo, Big Falls	UCE	3.7	SAMN28920698
Cyanocompsa cyanoides	UAM18351	ABJ865	Belize: Toledo, Big Falls	UCE	0.7	SAMN28920699
Cyanocompsa cyanoides	UAM15286	KSW3847	Belize: Toledo, Big Falls	UCE	5.0	SAMN28920700
Cyanocompsa cyanoides	UWBM70035	DAB1176	Nicaragua: La Luz	WGS	100.6	SAMN28920701
Cyanocompsa cyanoides	UWBM56337	DAB1256	Nicaragua: La Luz	WGS	93.6	SAMN28920702

Taxon	Museum Number	Catalog Number	Population	Type	Reads (millions)	SRA Accession Number
Cyanocompsa cyanoides	STRIBC2421	MJM3014	Panama: Bocas del Toro, Changuinola	UCE	5.5	SAMN28920703
Cyanocompsa cyanoides	STRIBC2420	MJM4149	Panama: Bocas del Toro, Changuinola	UCE	5.6	SAMN28920704
Cyanocompsa cyanoides	STRIBC2422	MJM3016	Panama: Bocas del Toro, Changuinola	UCE	5.0	SAMN28920705
Cyanocompsa cyanoides	CUMV51040	IJL04191	Panama: Bocas del Toro, Chiriquí Grande, Rio La Gloria	UCE	4.5	SAMN28920706
Cyanocompsa cyanoides	STRIBC2435	MJM2337	Panama: Comarca Ngäbe-Buglé, Cerro Chalite	UCE	6.0	SAMN28920707
Cyanocompsa cyanoides	STRIBC2423	MJM6963	Panama: Veraguas, Santa Fé	UCE	5.0	SAMN28920708
Cyanocompsa cyanoides	UWBM111233	JK04142	Panama: Veraguas, Santa Fé	UCE	5.4	SAMN28920709
Cyanocompsa cyanoides	STRIBC2428	MJM6699	Panama: Colón, Achiote	UCE	4.1	SAMN28920710
Cyanocompsa cyanoides	STRIBC2438	MJM6683	Panama: Colón, Achiote	UCE	6.1	SAMN28920711

Taxon	Museum Number	Catalog Number	Population	Type	Reads (millions)	SRA Accession Number
Cyanocompsa cyanoides	STRIBC3390	MJM7996	Panama: Colón, Achiote	UCE	5.1	SAMN28920712
Henicorhina leucosticta	UWBM123523	JK06130	Panama: Bocas del Toro, Changuinola	UCE	0.37	SAMN28920713
Henicorhina leucosticta	UWBM123380	JMD754	Panama: Bocas del Toro, Changuinola	UCE	0.44	SAMN28920714
Henicorhina leucosticta	UWBM123642	JK06125	Panama: Bocas del Toro, Changuinola	UCE	0.58	SAMN28920715
Henicorhina leucosticta		JTW280	Panama: Comarca Ngäbe-Buglé, Cerro Chalite	UCE	1.34	SAMN28920716
Henicorhina leucosticta	UWBM106448	GMS1021	Panama: Veraguas, Santa Fé	UCE	0.88	SAMN28920717
Henicorhina leucosticta	STRIBC1528	MJM6908	Panama: Veraguas, Santa Fé	UCE	0.54	SAMN28920718
Henicorhina leucosticta	UWBM111225	JK04134	Panama: Veraguas, Santa Fé	UCE	1.12	SAMN28920719
Henicorhina leucosticta	STRIBC1534	MJM3373	Panama: Coclé, La Pintada, El Copé	UCE	0.35	SAMN28920720

Taxon	Museum Number	Catalog Number	Population	Type	Reads (millions)	SRA Accession Number
Henicorhina leucosticta	STRIBC1531	MJM3463	Panama: Coclé, La Pintada, El Copé	UCE	0.57	SAMN28920721
Henicorhina leucosticta	UAM24660	MJM1420	Panama: Colón, Achiote	UCE	1.11	SAMN28920722
Henicorhina leucosticta	STRIBC1536	MJM4504	Panama: Colón, Achiote	UCE	0.94	SAMN28920723
Henicorhina leucosticta	UAM24661	MJM1044	Panama: Panamá, Cerro Azul	UCE	0.32	SAMN28920724
Henicorhina leucosticta	UAM24580	JMM907	Panama: Panamá, Cerro Azul	UCE	0.61	SAMN28920725
Henicorhina leucosticta	UAM22726	MJM696	Panama: Panamá, Cerro Jefe	UCE	0.23	SAMN28920726
Henicorhina leucosticta	UAM22728	MJM1057	Panama: Panamá, Cerro Jefe	UCE	0.92	SAMN28920727
Henicorhina leucosticta	UWBM107228	GMS1830	Panama: Panamá, Lago Bayano	UCE	0.22	SAMN28920728
Henicorhina leucosticta	UWBM120908	JMD657	Panama: Panama, Chiman, Cerro Chucantí	UCE	1.04	SAMN28920729

Taxon	Museum Number	Catalog Number	Population	Type	Reads (millions)	SRA Accession Number
Henicorhina leucosticta	UWBM120904	GMS1913	Panama: Panama, Chiman, Cerro Chucantí	UCE	0.83	SAMN28920730
Henicorhina leucosticta	FMNH470759	JMD664	Panama: Panama, Chiman, Cerro Chucantí	UCE	0.75	SAMN28920731
Henicorhina leucosticta	UAM22767	MJM2114	Panama: Darién, Cana	UCE	0.62	SAMN28920732
Henicorhina leucosticta	UAM22766	MJM2113	Panama: Darién, Cana	UCE	0.53	SAMN28920733
Henicorhina leucosticta	UAM24008	MJM2089	Panama: Darién, Cana	UCE	0.22	SAMN28920734
Henicorhina leucosticta	UAM22761	MJM1987	Panama: Darién, Cana	UCE	0.16	SAMN28920735
Malacoptila panamensis	STRIBC2788	MJM4404	Panama: Bocas del Toro, Changuinola	UCE	5.0	SAMN28920736
Malacoptila panamensis	STRIBC2786	MJM4298	Panama: Bocas del Toro, Changuinola	UCE	4.4	SAMN28920737
Malacoptila panamensis	STRIBC0500	MJM3097	Panama: Bocas del Toro, Changuinola	UCE	5.2	SAMN28920738

Taxon	Museum Number	Catalog Number	Population	Type	Reads (millions)	SRA Accession Number
Malacoptila panamensis	STRIBC2787	MJM4308	Panama: Bocas del Toro, Changuinola	UCE	4.0	SAMN28920739
Malacoptila panamensis		JTW318	Panama: Comarca Ngäbe-Buglé, Cerro Chalite	UCE	2.5	SAMN28920740
Malacoptila panamensis	STRIBC7888	JFM074	Panama: Veraguas, Río Luis	WGS	81.6	SAMN28920741
Malacoptila panamensis	UAM20359	MJM265	Panama: Coclé, La Pintada, Coclesito	UCE	4.3	SAMN28920742
Malacoptila panamensis	STRIBC3597	MJM8074	Panama: Coclé, La Pintada, Coclesito	UCE	5.0	SAMN28920743
Malacoptila panamensis	UAM20417	MJM324	Panama: Coclé, La Pintada, Coclesito	UCE	5.3	SAMN28920744
Malacoptila panamensis	STRIBC2564	MJM2819	Panama: Colón, Achiote	UCE	6.4	SAMN28920745
Malacoptila panamensis	STRIBC2563	MJM2822	Panama: Colón, Achiote	UCE	4.3	SAMN28920746
Malacoptila panamensis	STRIBC0503	MJM4485	Panama: Colón, Achiote	UCE	5.1	SAMN28920747

Taxon	Museum Number	Catalog Number	Population	Type	Reads (millions)	SRA Accession Number
Malacoptila panamensis	STRIBC2908	MJM7208	Panama: Colón, Gamboa	UCE	4.3	SAMN28920748
Malacoptila panamensis	FMNH470655	JMD697	Panama: Panama, Chiman, Cerro Chucantí	UCE	3.7	SAMN28920749
Malacoptila panamensis	FMNH470657	JMD695	Panama: Panama, Chiman, Cerro Chucantí	UCE	4.2	SAMN28920750
Malacoptila panamensis	FMNH470656	JMD696	Panama: Panama, Chiman, Cerro Chucantí	UCE	4.8	SAMN28920751
Malacoptila panamensis	STRIBC4814	MJM9375	Panama: Darién, Rancho Frío	UCE	4.1	SAMN28920752
Malacoptila panamensis		B17539	Panama: Darién, Rancho Frío	UCE	4.4	SAMN28920753
Malacoptila panamensis	STRIBC4836	MJM9397	Panama: Darién, Rancho Frío	UCE	4.3	SAMN28920754
Myrmeciza exsul		B58101	Almirante	WGS	79.3	SAMN28920755
Myrmeciza exsul	STRIBC 0825	MJM4263	Panama: Bocas del Toro, Changuinola	WGS	105.6	SAMN28920756
Myrmeciza exsul	STRIBC4141		Panama: Coclé, La Pintada, Coclesito	WGS	118.3	SAMN28920757

Taxon	Museum Number	Catalog Number	Population	Type	Reads (millions)	SRA Accession Number
Myrmeciza exsul	STRIBC0796		Panama: Colón, Achiote	WGS	55.3	SAMN28920758
Myrmeciza exsul	STRIBC 2961	MJM7205	Panama: Colón, Gamboa	WGS	59.2	SAMN28920759
Myrmeciza exsul	STRIBC 3856	MJM8033	Panama: Colón, Gamboa	WGS	49.9	SAMN28920760
Myrmeciza exsul		GKD256	Panama: Panamá, Panama City, confluence of Rios Chagres and Chagrecito	WGS	99.4	SAMN28920761
Myrmeciza exsul		RCF2031	Panama: Panamá, Panama City, confluence of Rios Chagres and Chagrecito	WGS	145.5	SAMN28920762
Myrmeciza exsul		SMB223	Panama: Panamá, Panama City, confluence of Rios Chagres and Chagrecito	WGS	61.0	SAMN28920763
Myrmeciza exsul		MJM0503	Panama: Panamá, Cerro Azul	WGS	88.2	SAMN28920764
Myrmeciza exsul	STRIBC 0799	MJM5636	Panama: Panamá, Cerro Azul	WGS	90.1	SAMN28920765
Myrmeciza exsul	STRIBC 3921	MJM8564	Panama: Panama, Chiman, Cerro Chucantí	WGS	74.6	SAMN28920766
Myrmeciza exsul	STRIBC 3742	MJM8565	Panama: Panama, Chiman, Cerro Chucantí	WGS	89.9	SAMN28920767

Taxon	Museum Number	Catalog Number	Population	Type	Reads (millions)	SRA Accession Number
Myrmeciza exsul	STRIBC3538		Panama: Darién, Chepigana, Chucanaque, El Salto	WGS	140.5	SAMN28920768
Myrmeciza exsul	STRIBC 4140	MJM9020	Panama: Comarca Emberá-Wounann, Cémaco, Peña Bijagual	WGS	96.8	SAMN28920769
Myrmeciza exsul	STRIBC 4389	MJM9148	Panama: Comarca Emberá-Wounann, Cémaco, Peña Bijagual	WGS	153.8	SAMN28920770
Myrmeciza exsul		MJM985	Tropic Star	WGS	77.2	SAMN28920771
Myrmeciza exsul	STRIBC4835		Panama: Darién, Rancho Frío	WGS	111.8	SAMN28920772
Myrmeciza exsul	STRIBC4837		Panama: Darién, Rancho Frío	WGS	102.7	SAMN28920773
Myrmeciza exsul	MJM2023		Panama: Darién, Cana	WGS	46.5	SAMN28920774
Pachysylvia decurtata	STRIBC1422	MJM6350	Panama: Bocas del Toro, Bosque Protector de Palo Seco	UCE	4.5	SAMN28920775
Pachysylvia decurtata	STRIBC1423	B17498	Panama: Bocas del Toro, Bosque Protector de Palo Seco	UCE	4.4	SAMN28920776
Pachysylvia decurtata	UWBM111263	JK04172	Panama: Veraguas, Santa Fé	UCE	4.1	SAMN28920777
Pachysylvia decurtata	UWBM111255	JK04164	Panama: Veraguas, Santa Fé	UCE	5.2	SAMN28920778

Taxon	Museum Number	Catalog Number	Population	Type	Reads (millions)	SRA Accession Number
Pachysylvia decurtata	UWBM106463	GMS1036	Panama: Veraguas, Santa Fé	UCE	4.1	SAMN28920779
Pachysylvia decurtata	STRIBC1423	MJM2660	Panama: Panamá, Cerro Azul	UCE	5.1	SAMN28920780
Pachysylvia decurtata	UWBM111193	JK04100	Panama: Panamá, Cerro Jefe	UCE	4.0	SAMN28920781
Pachysylvia decurtata		JMM910	Panama: Panamá, Cerro Azul	UCE	4.7	SAMN28920782
Pachysylvia decurtata	UWBM108154	GMS983	Panama: Panamá, Cerro Jefe	UCE	4.0	SAMN28920783
Pachysylvia decurtata	STRIBC3764	MJM8573	Panama: Panama, Chiman, Cerro Chucantí	UCE	3.5	SAMN28920784
Pachysylvia decurtata	UWBM112234	JK06044	Panama: Panama, Chiman, Cerro Chucantí	UCE	3.5	SAMN28920785
Pachysylvia decurtata	UWBM108897	JMD719	Panama: Panama, Chiman, Cerro Chucantí	UCE	4.6	SAMN28920786
Pachysylvia decurtata		B46582	Panama: Darién, Rancho Frío	UCE	4.2	SAMN28920787
Pachysylvia decurtata		B46600	Panama: Darién, Rancho Frío			SAMN28920788
Ramphocelus passerinii		PA-RPA46461	Panama: Bocas del Toro, Guabito	WGS	90.0	SAMN28920789
Ramphocelus passerinii	STRIBC4562	MJM4054	Panama: Bocas del Toro, Changuinola	WGS	70.8	SAMN28920790

Taxon	Museum Number	Catalog Number	Population	Type	Reads (millions)	SRA Accession Number
Ramphocelus passerinii	STRIBC1898	MJM4007	Panama: Bocas del Toro, Changuinola	WGS	71.7	SAMN28920791
Ramphocelus passerinii	STRIBC1901	MJM4146	Panama: Bocas del Toro, Changuinola	WGS	97.0	SAMN28920792
Ramphocelus passerinii	STRIBC4629	MJM4170	Panama: Bocas del Toro, Changuinola	WGS	63.9	SAMN28920793
Ramphocelus passerinii		PA-RPA46444	Panama: Bocas del Toro, Chiriquí Grande	WGS	98.5	SAMN28920794
Ramphocelus passerinii		PA-RPA46475	Panama: Bocas del Toro, Chiriquí Grande	WGS	70.1	SAMN28920795
Ramphocelus flammigerus	STRIBC3623	MJM8197	Panama: Coclé, La Pintada, Coclesito	WGS	53.6	SAMN28920796
Ramphocelus flammigerus	STRIBC7345	MJM8132	Panama: Coclé, La Pintada, Coclesito	WGS	134.0	SAMN28920797
Ramphocelus flammigerus	STRIBC1915	MJM2437	Panama: Colón, Achiote	WGS	89.6	SAMN28920798
Ramphocelus flammigerus	UWBM106617	GMS1195	Panama: Colón, Achiote	WGS	100.3	SAMN28920799

Taxon	**Museum Number**	**Catalog Number**	**Population**	**Type**	**Reads (millions)**	**SRA Accession Number**
Ramphocelus flammigerus	STRIBC1918	MJM2406	Panama: Colón, Achiote	WGS	76.6	SAMN28920800
Ramphocelus flammigerus	STRIBC4280	MJM8966	Panama: Colón, Santa Isabel, Palenque	WGS	70.2	SAMN28920801
Ramphocelus flammigerus	STRIBC7584	MJM8967	Panama: Colón, Santa Isabel, Palenque	WGS	77.1	SAMN28920802
Ramphocelus flammigerus	STRIBC4278	MJM9030	Panama: Comarca Emberá-Wounann, Cémaco, Peña Bijagual	WGS	75.4	SAMN28920803
Ramphocelus flammigerus	UAM34480	KSW4857	Panama: Darién, Cana	WGS		SAMN28920804
Ramphocelus flammigerus	UAM31175	JMM1079	Panama: Darién, Cana	WGS	90.5	SAMN28920805
Ramphocelus flammigerus	UAM25724	JMM1102	Panama: Darién, Cana	WGS	118.5	SAMN28920806
Schiffornis veraepacis		RCF3	Panama: Bocas del Toro, Changuinola		105.6	SAMN28920807
Schiffornis veraepacis	STRIBC1223	MJM3356	Panama: Coclé, La Pintada, El Copé	WGS	45.5	SAMN28920808

Taxon	Museum Number	Catalog Number	Population	Type	Reads (millions)	SRA Accession Number
Schiffornis veraepacis	STRIBC1224	MJM3495	Panama: Coclé, La Pintada, El Copé	WGS	78.1	SAMN28920809
Schiffornis veraepacis	UWBM106535	GMS1112	Panama: Coclé, El Valle	WGS	70.8	SAMN28920810
Schiffornis veraepacis	UWBM76978	SMB229	Panama: Panamá, Panama City, confluence of Rios Chagres and Chagrecito	WGS	72.5	SAMN28920811
Schiffornis veraepacis	UWBM108416	JMD208	Panama: Panamá, Cerro Azul	WGS	99.4	SAMN28920812
Schiffornis veraepacis	UWBM108283	JMD085	Panama: Panamá, Cerro Azul	WGS	78.7	SAMN28920813
Schiffornis stenorhyncha	STRIBC1228	MJM5753	Panama: Panamá, Lago Bayano	WGS	126.8	SAMN28920814
Schiffornis stenorhyncha	STRIBC4338		Panama: Comarca Emberá-Wounann, Cémaco, Peña Bijagual	WGS	112.8	SAMN28920815
Schiffornis stenorhyncha	STRIBC3075	MJM7345	Panama: Darién, Chepigana, Aruza Abajo	WGS	76.2	SAMN28920816
Schiffornis stenorhyncha	STRIBC3600	MJM7878	Panama: Darién, Chepigana, Aruza Abajo	WGS	105.1	SAMN28920817
Xenops minutus	STRIBC0576	MJM2904	Panama: Bocas del Toro, Changuinola	UCE	4.5	SAMN28920818

Taxon	Museum Number	Catalog Number	Population	Type	Reads (millions)	SRA Accession Number
Xenops minutus	STRIBC0577	MJM3110	Panama: Bocas del Toro, Changuinola	UCE	4.5	SAMN28920819
Xenops minutus	STRIBC0575	MJM4121	Panama: Bocas del Toro, Changuinola	UCE	7.4	SAMN28920820
Xenops minutus	STRIB0578	MJM4374	Panama: Bocas del Toro, Changuinola	UCE	4.6	SAMN28920821
Xenops minutus	STRIBC7889	JFM012	Panama: Veraguas, Río Luis	WGS	77.8	SAMN28920822
Xenops minutus	STRIBC0579	MJM3443	Panama: Coclé, La Pintada, El Copé	UCE	3.9	SAMN28920823
Xenops minutus	STRIBC0580	MJM3525	Panama: Coclé, La Pintada, El Copé	UCE	3.6	SAMN28920824
Xenops minutus	STRIBC0586	MJM4675	Panama: Colón, Achiote	UCE	3.8	SAMN28920825
Xenops minutus	STRIBC0583	MJM5323	Panama: Colón, Achiote	UCE	4.9	SAMN28920826
Xenops minutus	STRIBC0584	MJM5612	Panama: Panamá, Cerro Azul	UCE	4.5	SAMN28920827
Xenops minutus	STRIBC0589	MJM5646	Panama: Panamá, Cerro Azul	UCE	4.5	SAMN28920828
Xenops minutus	STRIBC0581	MJM5735	Panama: Panamá, Lago Bayano	UCE	5.4	SAMN28920829
Xenops minutus	UWBM107189	GMS1758	Panama: Panamá, Lago Bayano	UCE	5.3	SAMN28920830

Taxon	Museum Number	Catalog Number	Population	Type	Reads (millions)	SRA Accession Number
Xenops minutus	UAM36707	JMM1012	Panama: Darién, Cana	UCE	3.9	SAMN28920831
Xenops minutus	UAM36654	KSW4789	Panama: Darién, Cana	UCE	4.1	SAMN28920832
Xenops minutus	UAM36653	MJM2045	Panama: Darién, Cana	UCE	4.2	SAMN28920643
Xenops minutus	UAM24576	MJM2051	Panama: Darién, Cana	UCE	4.2	SAMN28920644

Table A2.2: Distances used for clinal analyses. Localities blank if taxon not sampled at that site. For each, the start of the transect is indicated with a bold "0", with subsequent distances measured from there. All distances in straight-line kilometers.

Location	Coordinates	*Arremon*	*Cantorchilus*	*Cyanocompsa*	*Henicorhina*	*Malacoptila*	*Myrmeciza*	*Pachysylvia*	*Ramphocelus*	*Schiffornis*	*Xenops*
La Luz, Nicaragua	13.702, -84.854	**0**		**0**							
Guabito	9.4733, -82.565								**0**		
Rio Changuinola	9.133, -82.501	258.59	**0**	258.59	**0**	**0**	**0**		7.04	**0**	**0**

Location	**Coordinates**	***Arremon***	***Cantorchilus***	***Cyanocompsa***	***Henicorhina***	***Malacoptila***	***Myrmeciza***	***Pachysylvia***	***Ramphocelus***	***Schiffornis***	***Xenops***
Almirante	9.307, -82.423						8.65				
Rio La Gloria	8.9844, -82.233			288.09							
Palo Seco	8.7934, -82.189	292.925						**0**			
Chiriquí Grande	8.7934, -82.18885								41.37		
Cerro Chalite	8.858611, 82.06055	307.04		307.04	48.44	48.44					
Cayo Agua	9.155, -82.037		51.15								
Rio Luis	8.598, -81.206	401.03	142.54			142.54					142.54
Santa Fe	8.56645, -81.191	402.69	144.41	402.69	144.41			109.76			
Coclesito	8.77532, -80.54823	473.38				214.93	214.93		221.83		214.93

Location	**Coordinates**	***Arremon***	***Cantorchilus***	***Cyanocompsa***	***Henicorhina***	***Malacoptila***	***Myrmeciza***	***Pachysylvia***	***Ramphocelus***	***Schiffornis***	***Xenops***
El Copé	8.6697, -80.593				210.33					209.87	
El Valle	8.633, -80.155									263.49	
Achiote	9.18352, -79.98356	535.49		535.49	276.65	276.65	276.65		283.68		276.65
Agua Claras	9.187, -79.69	567.63									
Gamboa	9.16933, -79.7529					302.01	302.01				
Cerro Azul	9.1611, -79.416		340.04		340.04		340.04	304.96		339.30	340.04
Cerro Jefe	9.2333, -79.35				347.34			312.25			
Palenque	9.5734, -79.352		347.10						353.07		
Upper Rio Chagres	9.3875, -79.34317	605.93					348.09			347.34	

Location	**Coordinates**	***Arremon***	***Cantorchilus***	***Cyanocompsa***	***Henicorhina***	***Malacoptila***	***Myrmeciza***	***Pachysylvia***	***Ramphocelus***	***Schiffornis***	***Xenops***
San Blas	9.3558, -78.97929		388.21								
Lago Bayano	9.1564, -78.698				419.17					418.26	419.17
Cerro Chucantí	8.78932, -78.45137	704.02	445.52		445.52	445.52	445.52	411.09			
Tropic Star Lodge	7.57, -78.19						475.21				
Aruza Abajo	8.3613, -77.									500.64	
Puerto Piña	7.6333, -78.183		475.95								
El Salto	8.3135, -77.7884						519.48				
Rancho Frío	8.02, -77.732		525.73			525.73	525.73	490.25			
Cana	8.02, -77.733	783.18	525.73		525.73		525.73		531.15	524.58	525.73

Location	**Coordinates**	***Arremon***	***Cantorchilus***	***Cyanocompsa***	***Henicorhina***	***Malacoptila***	***Myrmeciza***	***Pachysylvia***	***Ramphocelus***	***Schiffornis***	***Xenops***
Cémaco	8.25343, -77.72						527.51		532.92	526.58	

Table A2.3: Regression models of mtDNA pairwise divergence vs other parameters. Models where the regression is significant in bold.

Variable	**Slope**	**Adjusted R^2**	**p-value**
Cline width	6.593	-0.1093	0.7451
Cline width variance	-3739	0.1698	0.1304
Cline center variance	-492.9	0.1495	0.1467
Proportion total SNPs fixed	**0.02261**	**0.4231**	**0.02479**
Proportion autosomal SNPs fixed	**0.2266**	**0.4679**	**0.01746**
Proportion Z SNPs fixed	0.03267	0.1058	0.1887
Enrichment of fixed Z loci	-0.1811	-0.08119	0.5847

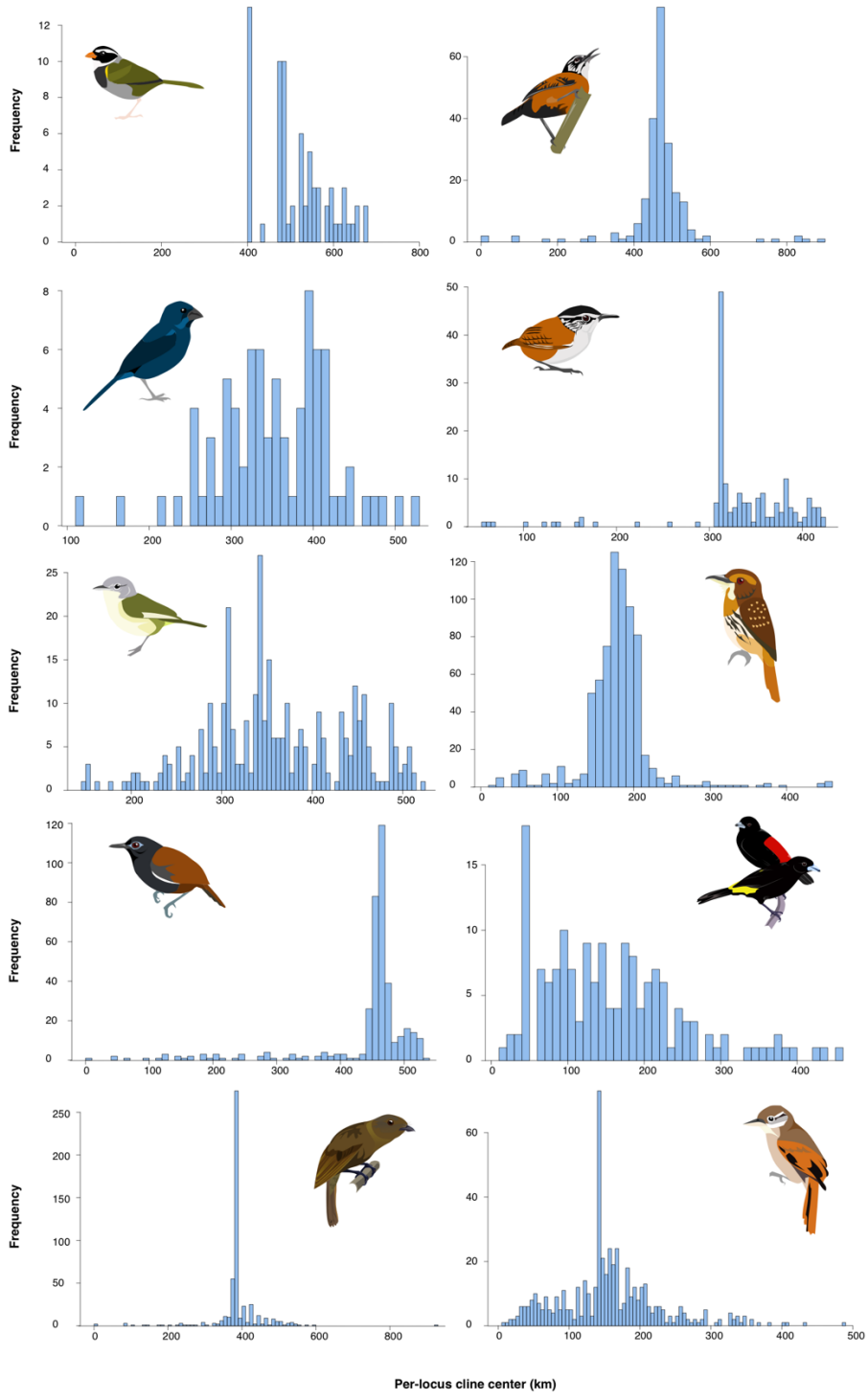


Figure A2.1: Distribution of per-locus geographic cline center estimates fit in HZAR, shown for each study taxon.

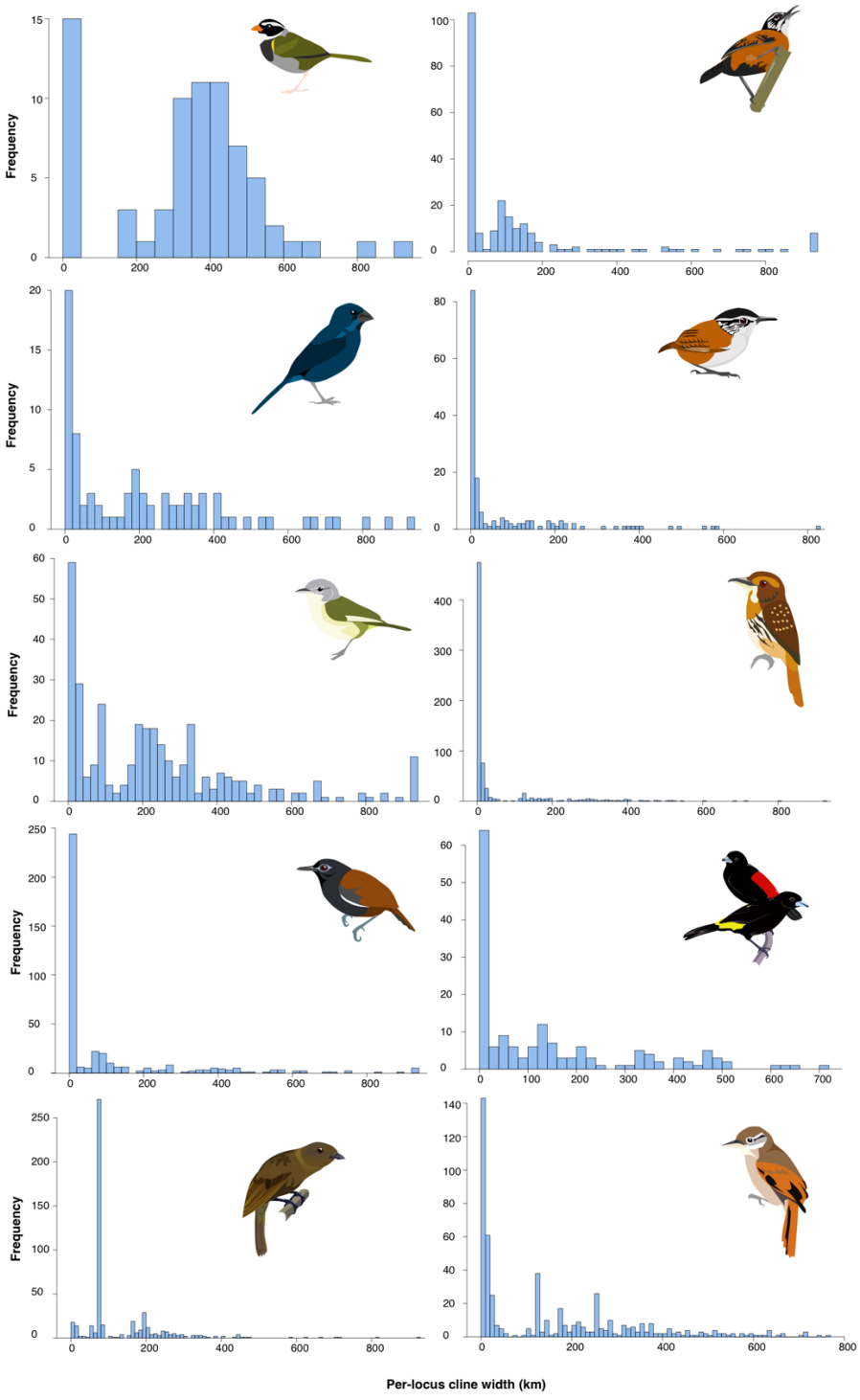


Figure A2.2: Distribution of per-locus geographic cline width estimates fit in HZAR, shown for each study taxon.

Appendix to Chapter 3: Supplemental Methods

Additional variant-calling approaches

Low-coverage data presents additional challenges for accurate identification of variants (Yu and Sun 2013; Lou *et al.* 2021). Initially, we followed a similar protocol to that used in Chapter 2, using the Genome Analysis ToolKit HaplotypeCaller tool (McKenna *et al.* 2010). However, on examination, we discovered a major issue with specifically the heterozygotes called by this method. In brief, GATK miscalls many sites that are homozygous for the alternative allele (1/1) as heterozygotes (0/1) when coverage is low. This error is the result of the assumption within the calling method that it is more likely that a site where all reads are the derived allele are the result of missing data rather than evolutionary change. It is difficult to identify these sites outside of the Z chromosome of female birds, which can be identified as errors since heterozygous sites here are impossible. As such, we cannot regard sites in low-coverage data identified with GATK as reliable. An abundance of false heterozygotes is a major issue with any attempts at population genetic analyses, and the use of GATK in such datasets thus should be avoided. This has since also been supported by published findings from other researchers who recently independently observed this and other phenomena, and recommend the use of mpileup in non-model systems by other researchers (Lefouili and Nam 2022).

For this same reason, ANGSD also requires caution when used with data of this nature. Many pipelines which use ANGSD for calling variants based on genotype likelihood (GL) scores use an option within ANGSD to calculate GL with the GATK method. This can result in similar errors to those described above.

Testing filtering strategies

Proper filtering and quality control of NGS data is an important but often under-considered part of bioinformatics pipeline development that can have substantial impacts on results (Shafer *et al.* 2017;

O'Leary *et al.* 2018; Linck & Battey 2019). I conducted extensive trials of different filtering schema (Table A3.1), which were evaluated on the following criteria:

1) Did the filter leave enough data (both in number of SNPs and individuals retained) to allow for robust inference? Many analyses perform best with more sites included (Nazareno *et al.* 2017), one of the main drivers of using genome-wide data to begin with. Datasets failed this criterion if 50% or more of the individuals from one of the six taxa were removed (which for *A. aliciae* and *A. pamela*, would be a single individual), as this would lead to questionable results for any inferences on those populations. There was no set boundary for the number of variants, but datasets of under 10,000 SNPs were removed.
2) Does the filter minimize the biases inherent in low-coverage data? These include particularly the overrepresentation of repetitive regions and higher likelihood of base-calling errors going undetected in low-coverage sites (Lou *et al.* 2021). Filtering by depth and by missing data may mitigate the influence of both very low and very high coverage sites. Datasets were evaluated as not performing well in this criterion if they had a high proportion of missing data (with 50% missingness leading to discarding the dataset and greater than 30% missingness being deprioritized over more complete datasets). Sites with high coverage (i.e., possible paralogs) were removed and, if the resulting dataset had too few variants to analyze (see criterion 1), the dataset was not considered. Differing levels of filtering for maximum depth were applied to evaluate this metric (Table A3.1).
3) Does the analysis to be performed with these data require specific thresholds for missingness, linkage, or minor allele frequency? Among the analyses presented here, missing data and linkage are particular issues to be taken into consideration. Specifically, δaδi and STRUCTURE require unlinked data, and perform best when missing data is minimized (i.e., as close to no missing data as possible, as model fitting becomes increasingly challenging with more missing data). However, other analyses, such as window-based analyses, required all SNPs, without MAF or linkage filters. This criterion was therefore evaluated for each specific analysis.

The datasets created in ANGSD did not perform well on points 1 and 2, as they left few variants for analyses and those remaining variants had a high proportion of missing data that did not perform well with the attempted analyses (Table A3.1). Most concerningly, they did not meet the inclusion criteria on a per-individual basis, as they did not include (at minimum) one of the *A. aliciae* individuals. The samtools datasets performed better on all three of the criteria, even if they were somewhat less rigorous in the second area than ANGSD.

The main choice was then between different levels of missing data in the samtools dataset. In addition to being required by certain analyses, it also can help further mitigate the biases introduced by low-coverage data by removing sites prone to very low coverage *and* minimize the influence of exceptionally high coverage sites (as these are likely to occur in only a small subset of individuals at any given site and be absent in most). The dataset with no missing data was somewhat small, however, and so the 90% complete set was selected instead as balancing the considerations of criteria 1 and 2.

One point of potential confusion in how filtering by missing data operates is that when applying stricter filters (which can minimize the influence of coverage outlier sites and is necessary for some analyses that handle missing data less well) is that at a certain point, a penalty is incurred for including *more* individuals, even if the initial number of variants called is higher (see Table A3.1). When these data are filtered to include minimal missing data, eventually the inclusion of more *individuals increases the likelihood of any given SNP not meeting the criteria* (Figure A3.2). This pattern applies whenever the filtering is stricter than the average missing data rate. You can see that again on Table A3.1, as the outgroup dataset is larger up through a missing data rate of 25%, and only reverses for the 10% and 0% missing datasets. This is a widely observed phenomenon, mostly in low-coverage or reduced-representation data where the missingness rate is high enough to reach the inflection point in normal data-range cut-offs.

Additional tree analyses

The combination of low coverage with low genomic variation further limits which analyses can be performed. In particular, it heightens the difficulty in handling phylogenies when there are high levels of both gene flow and shared ancestral variation. Thus, it is likely unsurprising that our data did not meet the assumptions of some programs.

TreeMix (Pickrell and Pritchard 2012) assumes the data have an underlying tree-like structure, and then infers migration on that tree. This creates issues in cases where significant hybridization has led to violation of this assumption- i.e., the number of admixed populations exceeds the number of unadmixed population, and so both false positives and false negatives will be generated because the background variation within populations is too great to differentiate migration events. As all of our *Aglaeactis* populations show at least some evidence of admixture (Figure 3.4, Figure 3.6), this assumption is violated. Second, Treemix assumes that migration events are short and singular occurrences (Pickrell and Pritchard 2012), and its developers explicitly warn against its use for modeling continuous migration. Again, our data likely violate this assumption, based on the recovery of continuous migration scenarios as best-fit in two of our pairwise demographic models (Table 3.4). Unsurprisingly given these violations of the method assumptions, we were unable to recover any consistently converging models with this method, in regards to either the underlying topology or the inferred migration events (Figure A3.3).

Questions remained as to how variant calling and filtering would impact downstream analyses. I performed several of these analyses with multiple datasets. In particular, concerns were raised on whether our nuclear tree topology, with its discordance from the mitochondrial tree, could be a result of data processing. IQ-TREE was run with multiple input datasets, with SNPs called with both mpileup and ANGSD. Mpileup datasets recovered consistent topologies (Figure A3.4), regardless of filtering schema, that were practically identical to the one presented in the main paper (Figure 3.2B). This includes data where aggressive two-sided depth filtering (using mean DP minimum of 2 and maximum of 5) was applied to test whether the signal was resulting from repetitive regions not properly excluded. Meanwhile, all ANGSD datasets tested, regardless of filtering, recovered a massive

polytomy. This is likely due to the level of information loss incurred in the ANGSD variant calling pipeline versus the mpileup one, and indicates that while filtering is important for minimizing potential biases, overall SNP detection method plays a much stronger role in shaping variation in topology.

Testing for statistical difference in F_{ST} outliers

One question of interest with the observed F_{ST} outliers is if there is a statistically significant difference in the distribution of outliers between comparisons- i.e., if the same regions show elevated divergence in different sets of taxa. Conceptually, one way to do this is to ask if the distribution of values in the dataset are statistically similar. This is commonly attempted with a Mann-Whitney U Test, which is also used to establish statistical significance in distribution of variation between genomic regions. However, while this latter use may not necessarily violate assumptions, such a comparison between two scans of divergence using the same reference genome and, in some cases, the same individuals in the populations under consideration, is likely to due to nonindependence of observations. As a result, I am skeptical of this approach, and there appears to be no consensus from the literature on a statistically sound way to do this precise test.

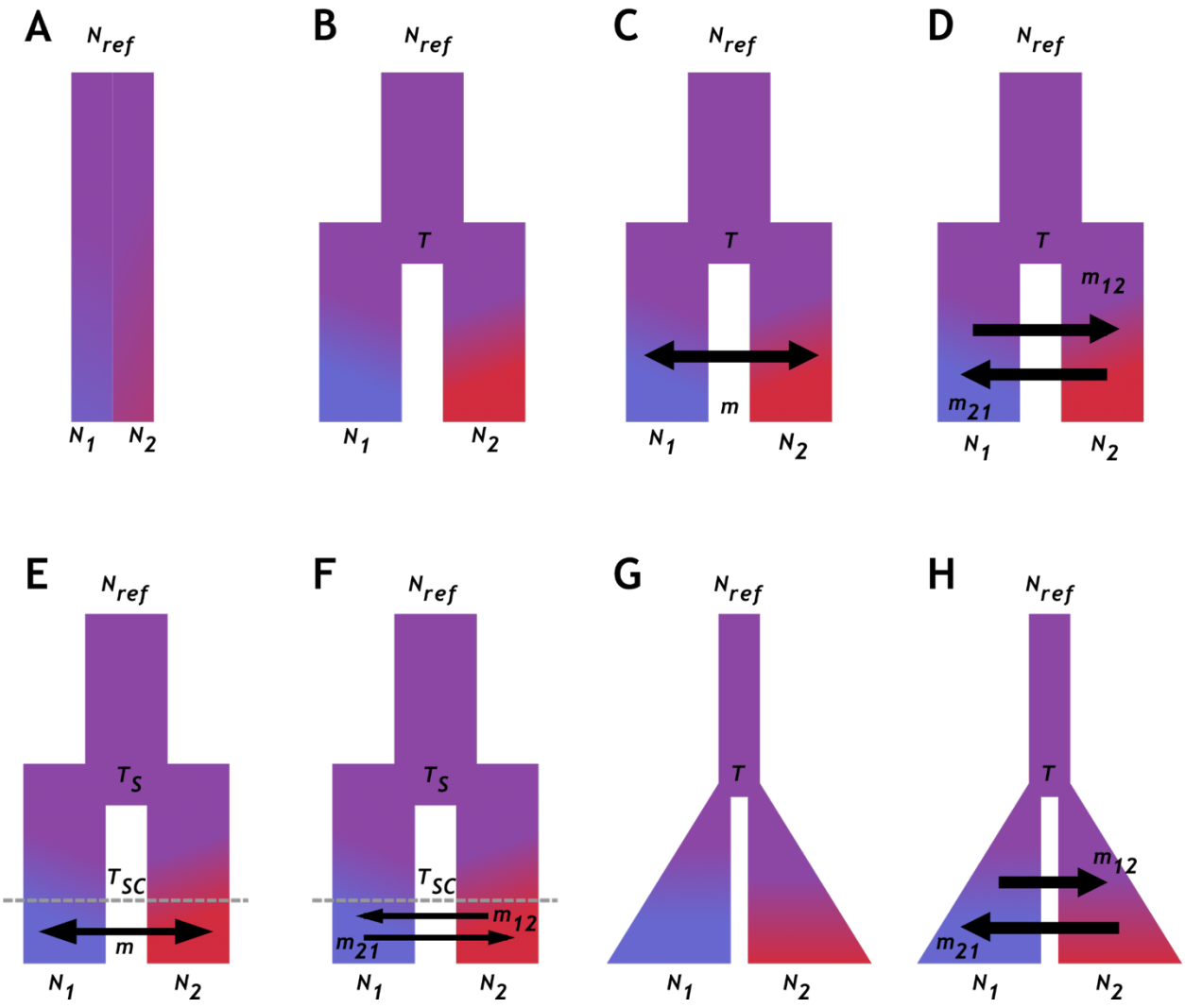


Figure A3.1: Demographic models tested in δaδi. Figure reproduced from (McLaughlin, Faircloth *et al.* 2020). Models are: (A) neutral model (no divergence), (B) split (divergence) followed by isolation and no migration (gene flow), (C) split-migration ("splitmig") with unidirectional migration parameter (i.e., roughly similar levels of gene flow in both directions), (D) split-migration with two migration estimates (i.e., asymmetrical gene flow), (E) secondary contact ("SC") with single migration parameter (i.e., divergence with intermittent gene flow), (F) secondary contact with two migration parameters (i.e., divergence with asymmetric intermittent gene flow), (G) split and isolation with population growth and no gene flow ("island"), and (H) split with migration (gene flow) and

population growth ("IM"). Model sequence reflects underlying architecture and then increasing complexity within model family. Single arrows indicate models with gene flow in both directions being roughly equal; double arrows are models with different levels of gene flow in each direction. Models B and G are no-gene-flow models. Rectangles indicate unchanging population sizes; triangles indicate population growth. Colors suggest increasing population differentiation.

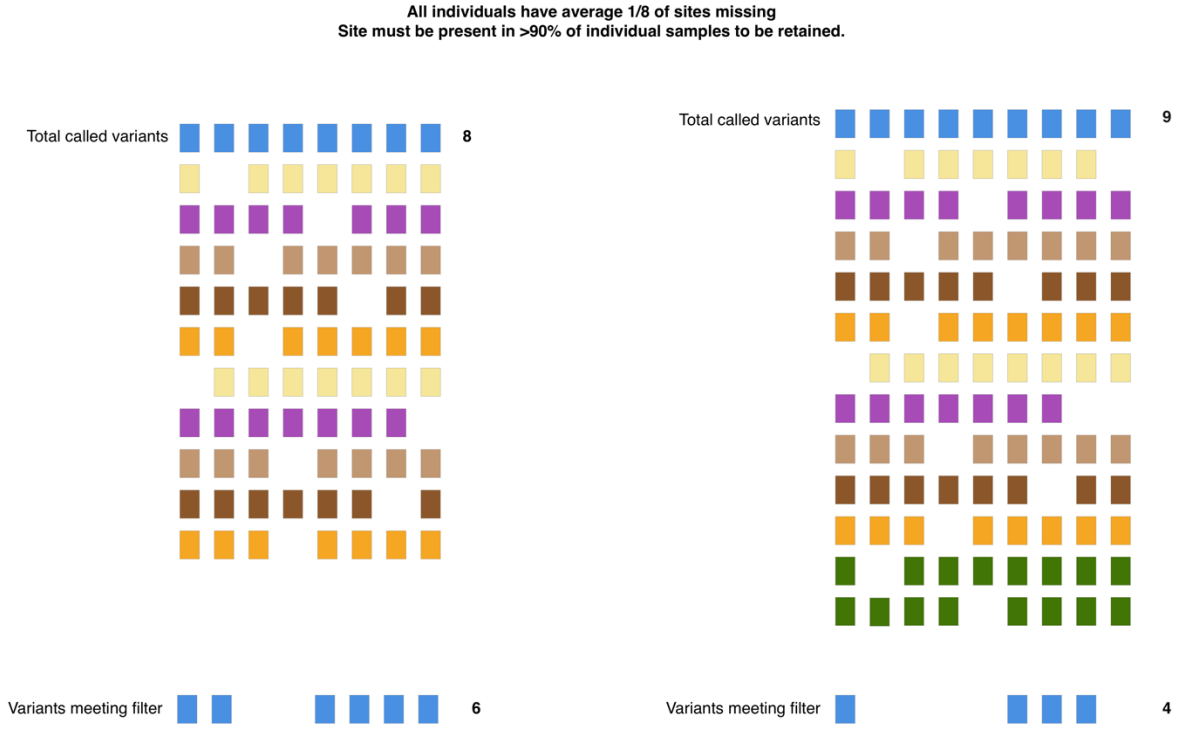
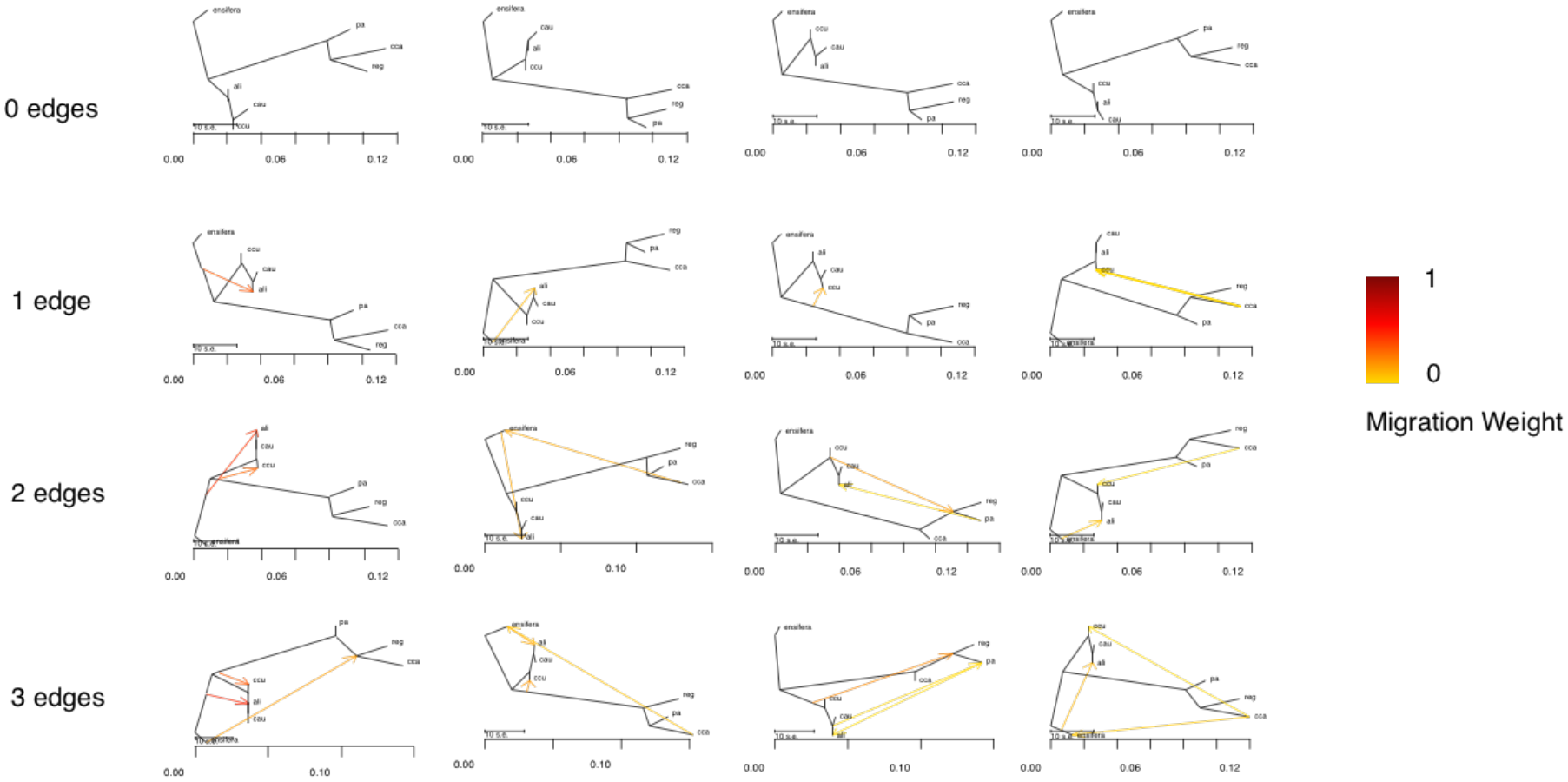
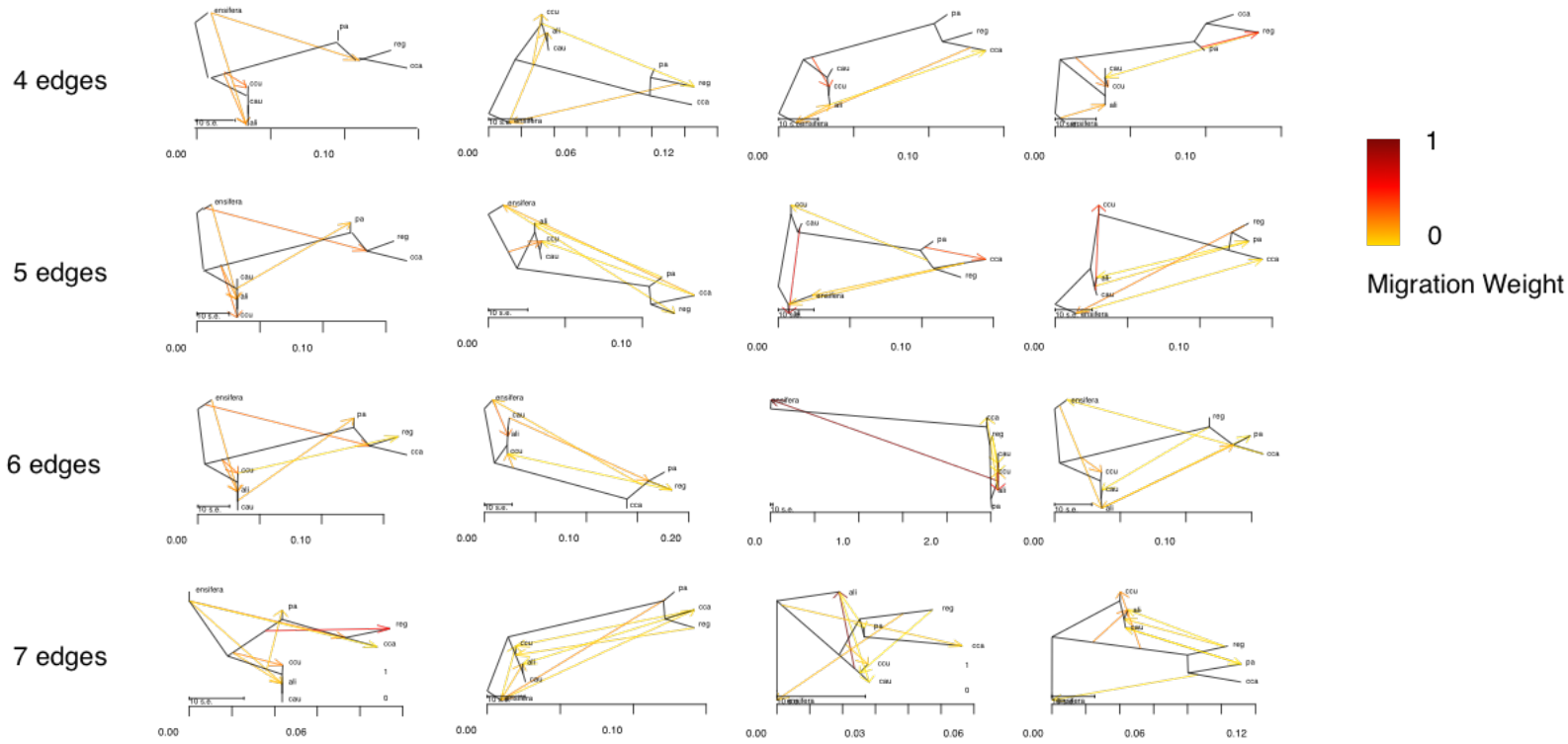


Figure A3.2: Impacts of including more individuals when filtering by missing data. On the left, we see a scenario in which a dataset of ten individuals leads to a total of 8 called SNPs. On average, each individual is missing 1/8 of the sites, and the data are filtered so that a site must be present in 90% or more of individuals to be retained (the level used for most of my datasets). In practice, this means that

while one individual can be missing for a site, if two or more are, the site is removed. In this first case, 6 of the initial 8 sites are retained. On the right, two outgroup individuals (green) are added, resulting in 9 total SNPs being called. However, the same error rate and filtering strategy are still applied, and with the additional individuals, fewer sites meet the criteria. Thus, even though the initial pool of variants is larger, only 4 are retained after filtering.


0 edges
1 edge
2 edges
3 edges
1
0
Migration Weight


4 edges
5 edges
6 edges
7 edges
1
0
Migration Weight

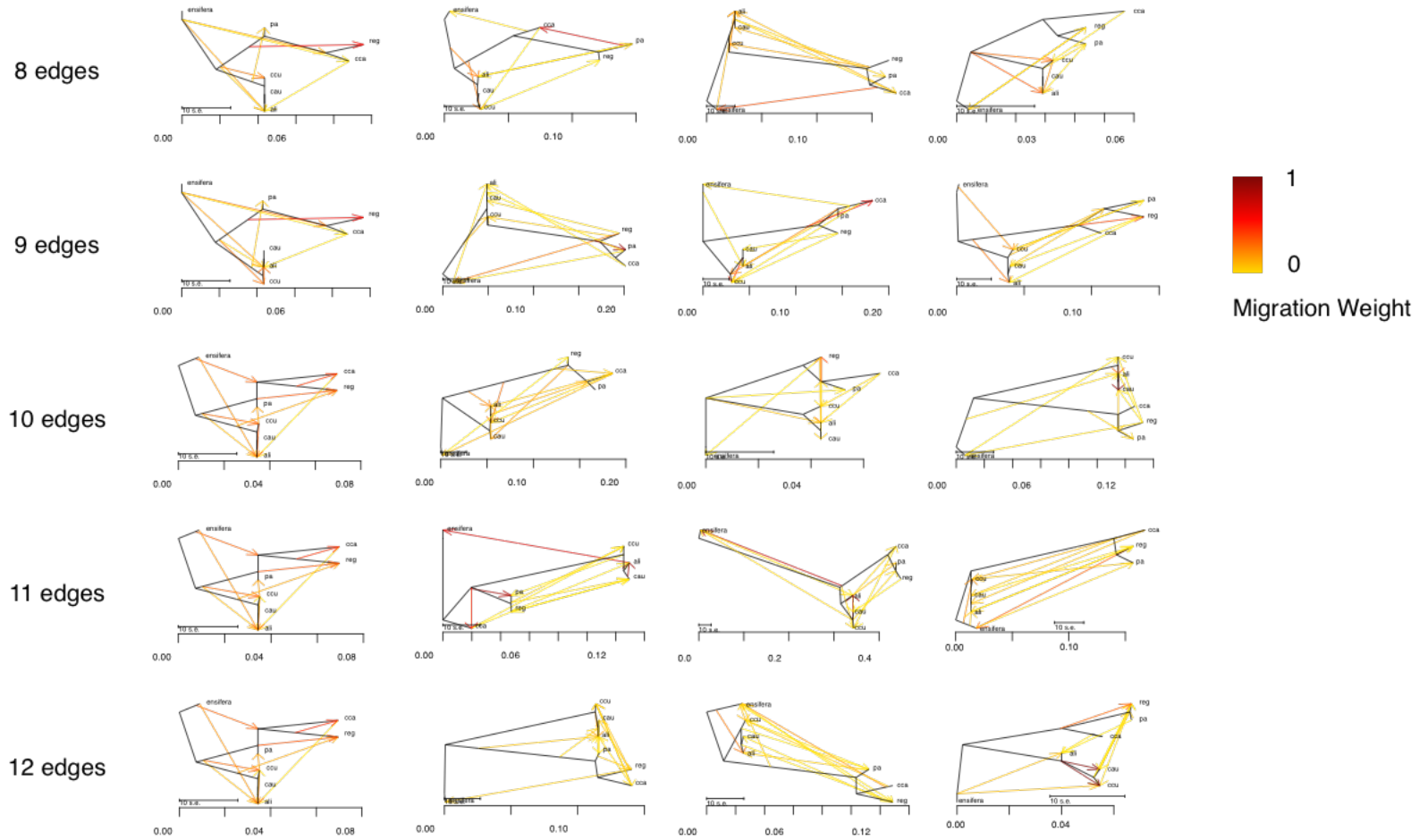
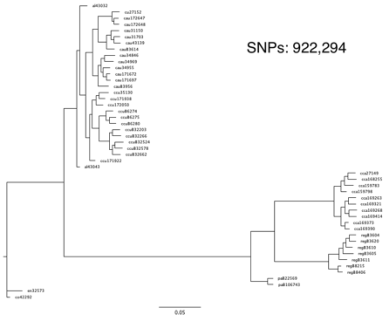
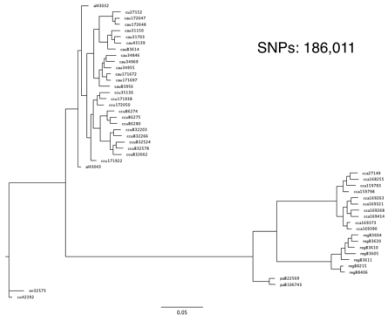

8 edges
9 edges
10 edges
11 edges
12 edges
1
0
Migration Weight

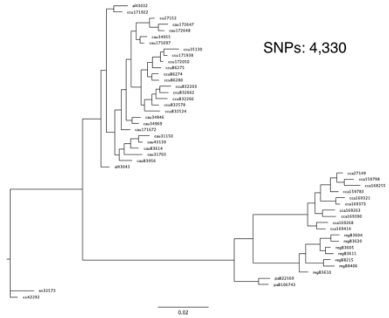
Figure A3.3: A selection of TreeMix results with the large samtools/bcftools dataset (68,451 SNPs), with four different runs to test for 0 to 12 migration events (arranged in columns). Each run was conducted with exactly the same dataset and input parameters, yet produced widely variable results. X-axis shows drift parameter, migration weight indicated by color scale. "al" = *A. aliciae*, "cau" = *A. cu. caumatonota*, "cca" = *A. ca. castelnaudii*, "ccu" = *A. cu. cupripennis*, "ensifera" = *Ensifera ensifera* (outgroup), "pa" = *A. pamela*, "reg" = *A. ca. regalis.*



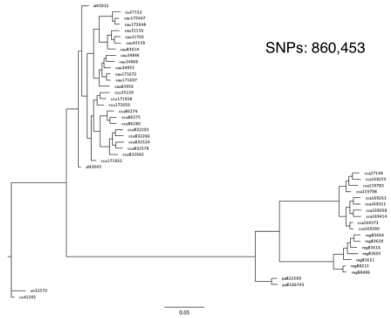
mpileup
min mean DP: 2



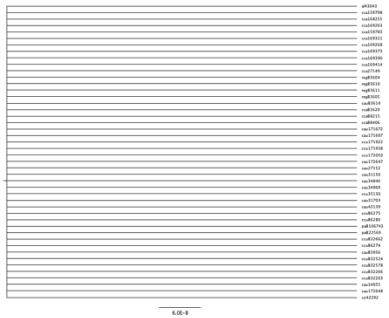
mpileup
min mean DP: 2
biallelic
25% missing data



mpileup
min mean DP: 2
biallelic
no missing data

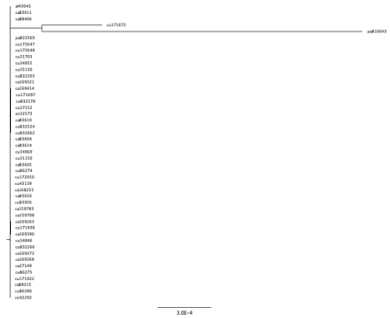


mpileup
min mean DP: 2
max mean DP: 5
biallelic
all data



ANGSD
min mean DP: 1
max mean DP: 50
p-value: 0.05
30% missing data
N= 46

ANGSD
min mean DP: 2
max mean DP: 50
p-value: 0.05
50% missing data
Thinning: 2000
N= 44



ANGSD
min mean DP: 2
max mean DP: 50
p-value: 0.05
50% missing data
N= 47

ANGSD
min mean DP: 2
max mean DP: 50
30% missing data
N= 44

Figure A3.4: Variant IQ-TREE topologies under multiple filtering schema with the same parameters (detailed in the Methods section). The top row shows the almost identical topologies generated by four SNP datasets created with the mpileup/bcftools call pipeline. From left to right, these were: all SNPs with a minimum coverage of 2X; biallelic SNPs with a minimum coverage of 2X present in at least 75% of individuals; biallelic SNPs with at least 2X coverage present in all 48 individuals; and all biallelic SNPs with at least 2X but no more than 5X coverage. The bottom row shows four representative ANGSD SNP datasets, which all recover massive polytomies. From left to right, these datasets are: SNPs with a genotype likelihood meeting a p-value cutoff of 0.05 with between 1X and 50X coverage present in 70% of 46 individuals (two dropped for lack of data); SNPs meeting the p=0.05 cutoff with 2X-50X coverage present in 50% of 44 individuals, thinned to 1 SNP per 2,000 bp; SNPs meeting the p=0.05 cutoff with 2X-50X coverage present in 50% of 47 individuals; and all SNPs (regardless of genotype likelihood) between 2X and 50X in 70% of 44 individuals.

Table A3.1: Number of variants called and retained with various filtering protocols. Please note that the filtering schema differ between programs based on variations in how they do or do not retain quality information in the VCF file output. In the final column, the number of individuals in the dataset is given, and "n=46" in that category indicates the two individuals excluded were not the two outgroup individuals. Not all filtering schema applied for all sets of individuals; indicated with "- ".

Filter	**All individuals (n=48)**	**All *Aglaeactis* (n=46)**	**Excluding low-coverage individuals**
Samtools/bcftools:			
Total called variants	22,264,196	20,337,188	
Without indels	18,131,968	17,170,101	
Without indels, biallelic only	18,070,732	17,118,339	
Without indels, biallelic only, min mean DP = 2	893,717	803,815	
Without indels, biallelic only, min mean DP = 2, no missing data	4,330	5,208	
Without indels, biallelic only, min	186,011	200,975	

Filter	**All individuals (n=48)**	**All *Aglaeactis* (n=46)**	**Excluding low-coverage individuals**
mean DP = 2, max missing data = 10%			
Without indels, biallelic only, min mean DP = 2, max missing data = 25%	855,654	764,622	
Without indels, biallelic only, min mean DP = 2, thinned to 1 SNP per 2000 bp (10% missing)	68,451	71,505	
ANGSD:			
Unfiltered variants	41,195,989		
GATK, Minimum Q score = 13, min depth= 2, max depth = 50, GL $p = 1.0x10^{-6}$	3,181,172		
GATK, Minimum Q score = 13, min depth= 2, max depth =	466,317		

Filter	**All individuals (n=48)**	**All *Aglaeactis* (n=46)**	**Excluding low-coverage individuals**
50, GL $p = 1.0x10^{-6}$, 50% missing data			
GATK, Minimum Q score = 13, min depth= 2, max depth = 50, GL $p = 1.0x10^{-6}$, 50% missing data, 1 SNP per 2000 bp	148,849	-	
GATK, Minimum Q score = 13, min depth= 2, max depth = 50, GL $p = 1.0x10^{-6}$, 40% missing data	-	-	97,185 (n=45) 71,153 (n=46) 56,983 (n =47)
GATK, Minimum Q score = 13, min depth= 2, max depth = 50, GL $p = 1.0x10^{-6}$, 30% missing data	-	-	995 (n = 45) 1,495 (n=47)
GATK, Minimum Q score = 13, min depth= 2, max depth = 50, GL $p = 0.5$	-	-	6,996,985 (n=47)

Filter	**All individuals (n=48)**	**All *Aglaeactis* (n=46)**	**Excluding low-coverage individuals**
GATK, Minimum Q score = 13, min depth= 2, max depth = 50, GL *p* = 0.5, 25% missing data	-	-	239 (n =43, no outgroup) 119 (n = 45)
GATK, Minimum Q score = 13, min depth= 1, max depth = 50, GL *p* = 0.5, 50% missing data	1,675,969		
GATK, Minimum Q score = 13, min depth= 1, max depth = 50, GL *p* = 0.5, 30% missing data	2,476		
GATK, Minimum Q score = 13, min depth= 1, max depth = 50, GL *p* = 0.5, 25% missing data	119		
Samtools, Minimum Q score = 13, min			

Filter	**All individuals (n=48)**	**All *Aglaeactis* (n=46)**	**Excluding low-coverage individuals**
depth= 1, max depth = 50, GL *p* = 0.5			
GATK, Minimum Q score = 13, min depth= 1, max depth = 50, GL *p* = 0.5, 50% missing data	1,125,609		
GATK, Minimum Q score = 13, min depth= 1, max depth = 50, GL *p* = 0.5, 30% missing data	1,513		