Biogeography and Genetic Population Structure of the Buckeye Butterflies (Genus *Junonia*) in the Western Hemisphere: Patterns of Hybridization, Dispersal, and Speciation

by

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Abstract

The New World buckeye butterflies (genus *Junonia*) are a valuable experimental model organisms, but the taxonomy of this group has been problematic and contentious. I have clarified the taxonomy of the *Junonia* species in North America using molecular and morphological data from contemporary and museum collections, focusing on Florida, the American Southwest, and Mexico. *Junonia* populations in Florida have been assigned to different species and *J. coenia grisea* in the American Southwest has been elevated to full species status. Using this framework, I reconstructed the invasion history of the tropical buckeye (*J. zonalis*) into South Florida. For the species that occur in the American Southwest and Mexico, I have plotted the contemporary distributions of the five species that occur in this region. Evidence of hybridization was documented and a cryptic species pair was identified (*J. coenia* and *J. grisea*). An improved taxonomy will encourage and support further comparative biology research.

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Dedication

For my son Landen and my great grandmother Juanita who no matter what told me I could do this. Landen is and will continue to be my biggest motivation no matter what I do in life.

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Chapter 1: Introduction

Mitochondrial DNA: Function and Uses

Mitochondrial deoxyribonucleic acid (mtDNA) is found within the mitochondrion, which is the primary energy producing organelle in almost all eukaryotic cells (Avise 2000). Mitochondria are originally derived from bacterial endosymbionts, and thus have a genome that is distinct from the eukaryotic nuclear genome (Lane 2005; Godfrey-Smith 2015). Mitochondrial DNA is haploid and in most animals is passed almost exclusively from female to offspring with no genetic input from the male (Vawter & Brown 1986; Avise 2000; Hebert *et al.* 2003; Zink & Barrowclough 2008). These sequences are considered to be highly conserved between all animal species due to its biological importance in maintaining cell function (Brown *et al.* 1979; Vawter & Brown 1986).

What makes mtDNA useful as a molecular tool is that it occurs in high copy number on a per cell basis, it has a rapid mutation rate, and lacks recombination (Brown *et al.* 1979; Avise 2000; Hebert *et al.* 2003; Zink & Barrowclough 2008). Mitochondrial DNA has been used for a vast array of studies including delimitation of cryptic species (De Barro & Ahmed 2011), observing historical patterns of genetic diversity (Leonard 2008) , tracking patterns of migration (Wilkinson & Fleming 1996) and species invasion (De Barro & Ahmed 2011), biogeography (Wahlberg *et al.* 2005), reconstructing phylogeographic ranges (Pons *et al.* 2006; Keyghobadi *et al.* 2013), defining matrillineages (Avise 2000; Zink & Barrowclough 2008), and determining the geographical and taxonomic limits of recently diverged groups (Zink & Barrowclough 2008). One advantage to using mtDNA is that its high copy number on a per cell basis gives researchers a more usable template when performing molecular analysis. In contrast, nuclear DNA (nDNA) occurs in low copy number and when using small or degraded tissue samples, extracting usable nuclear DNA can be difficult (Vawter & Brown 1986; Watts *et al.* 2007; Keyghobadi *et al.* 2013).

The mitochondrial *cytochrome oxidase subunit 1* (*COI*) has been one of the most widely used gene sequences in molecular analysis (Wahlberg *et al.* 2005; Kerr *et al.* 2007; Hubert *et al.* 2008; Pfeiler *et al.* 2012; Gemmell *et al.* 2014; Gemmell & Marcus 2015; Jose & Harikrishanan 2016). Its gene product is part of the enzyme complex IV subunit in the electron transport chain, which catalyzes the last enzymatic reaction during oxidative phosphorylation (Li *et al.* 2006; Balsa *et al.* 2012; Aras *et al.* 2013). Oxidative phosphorylation is the process by which cells convert energy into stores of adenosine triphosphate (ATP), which is used for many cellular processes (Wilson *et al.* 2012). Enzyme complex IV, also called cytochrome c oxidase, aids in the final steps in transferring electrons to the final electron acceptor (oxygen) producing water and ATP (Li *et al.* 2006). This process is essential in the energy production of all eukaryotic cells that conduct aerobic respiration, and therefore the gene sequences associated with respiratory metabolism are considered to be highly conserved (Brown *et al.* 1979; Vawter & Brown 1986).

The barcode region of the mitochondrial *cytochrome oxidase subunit I* gene is a 658 base pair (bp) region near the 5' end of the gene, where robust universal primers exist that allow for isolation of this gene from nearly any animal species (Folmer *et al.* 1994; Hebert *et al.* 2003; Hajibabaei *et al.* 2006). The *COI* gene sequence differs somewhat in length, depending on which group of organisms is being examined, so a highly conserved segment of this gene is utilized (Marshall 2005). The amino acids within this gene sequence change more slowly than in any other mitochondrial gene (Lynch & Jarrell 1993), and DNA sequences from the barcode region can often be used as a diagnostic tool for species identification (Folmer *et al.* 1994; Hebert *et al.* 2003; Hajibabaei *et al.* 2006). The phylogenetic signal in the barcode region of *COI* has allowed assignment of organisms into higher taxonomic classes with little difficulty (Hebert *et al.* 2003; Ratnasingham & Hebert 2007). Databases for DNA barcodes have been created (eg. BOLD (Ratnasingham & Hebert 2007)) and there are examples where amplification of this gene sequence from type specimens has allowed for the correct identification of insect species (Meusnier *et al.* 2008; Price *et al.* 2015). However, some issues with species delimitation do still exist (Janzen *et al.* 2005). Species that have diverged from one another very recently are sometimes problematic as their gene sequences have not had enough time to develop distinguishable variation (Pfeiler *et al.* 2012; Borchers & Marcus 2014; Gemmell *et al.* 2014; Gemmell & Marcus 2015). About 3% of the Lepidoptera species defined by morphological criteria, cannot be distinguished from closely related species by their *COI* barcodes (Janzen *et al.* 2005). Similar problems occur in organisms which have undergone hybridization and experience transfer of mitochondria between species (Halbert & Derr 2007; Good 2008).

Even given these considerations, mtDNA is a powerful tool for phylogeographic studies (Chapters 2 and 4), and examining historical patterns of migration and species distributions (Goldstein & Desalle 2003; Keyghobadi *et al.* 2013; Heintzman *et al.* 2014; Hernandez-Triana *et al.* 2014)(Chapter 3). Having well-developed molecular tools already available allows for the possibility of determining mitochondrial genotypes from specimens in museum collections (Watts *et al.* 2007; Winston 2007). Museum collections hold immense potential for scientific study (Leonard 2008; Saarinen & Daniels 2012). They are compilations of specimens collected by the museum itself and from donated private collections that may span entire species ranges over hundreds of years (Goldstein & Desalle 2003; Habel *et al.* 2009; Heintzman *et al.* 2014). Specimens within these collections have collection data associated with them and include such information as, where and when the specimens were collected, as well as who collected them (Winston 2007).

Having large spatial and time series data sets available allows for the observation of changes in allele frequencies in a population over time, migration patterns of organisms over time, biogeographic changes in species distributions, and biological invasions of non-native species (Harper *et al.* 2006; Estoup *et al.* 2010; Ugelvig *et al.* 2011; Keyghobadi *et al.* 2013). Museum collections have been used to explore various questions relating to population genetics and evolution in both vertebrates (Iudica *et al.* 2001; Estoup *et al.* 2010; Smith *et al.* 2011) and invertebrates (Goldstein & Desalle 2003; Harper *et al.* 2006; Habel *et al.* 2009; Saarinen & Daniels 2012; Keyghobadi *et al.* 2013; Heintzman *et al.* 2014). These questions focused on looking at specific time points and comparing them to contemporary populations in order to observe changes in allele frequencies, Metagenomic analysis, and bottleneck effects (Harper *et al.* 2003; Harper *et al.* 2006; Habel *et al.* 2009; Ugelvig *et al.* 2011; Saarinen & Daniels 2012).

One particular interest in the scientific community over the last 200 years has been the topic of invasion biology (Reichard & White 2003; Falk-Petersen *et al.* 2006; Davis 2009; Cristescu 2015). Invasion biology includes many topics of interest that include but are not limited to adaptive radiation, the creation of secondary contact zones, invasion events by non-native species, long-range dispersal events, speciation, and hybridization events (Mooney & Cleland 2001; Didham *et al.* 2005; Durand *et al.* 2009; Stigall 2010; Flohr *et al.* 2013; Cristescu 2015). Non-native species in some cases are referred to as invasive species, as they are organisms that have potential to establish populations and habituate to new habitats (Reichard & White 2003; Didham *et al.* 2005; Falk-Petersen *et al.* 2006). When such occurrences take place it is of great concern, as these species may have no natural predators, outcompete native species for habitat and resources, and/or hybridize with native species if reproductive isolation mechanisms do not exist (Mooney & Cleland 2001; Reichard & White 2003; Didham *et al.* 2005). This thesis will explore many of these issues as they pertain to the butterfly genus *Junonia* (Lepidoptera: Nymphalidae).

***Junonia* Butterfly Taxonomy**

The buckeye butterflies of the genus *Junonia* originated in Africa, and this group contains both Old and New World representatives (Kodandaramaiah & Wahlberg 2007; Kodandaramaiah 2009). The New World *Junonia* have long been thought to be monophyletic (Forbes 1928; Forbes 1947; Kodandaramaiah & Wahlberg 2007), but it has recently been suggested that patterns of mitochondrial variation in the genus may be consistent with multiple colonization events from the Old World (Gemmell & Marcus 2015).

In the New World there are currently 9 or 10 described species of buckeye butterflies from the genus *Junonia*: *J. coenia, J. divaricata, J. evarete, J. genoveva, J. litoralis, J. neildi, J. vestina, J. wahlbergi, and J. zonalis.* (Gemmell et al. 2014). For example, some authors also include *J. nigrosuffusa* as a full species (Brown *et al.* 1992) while others refer to it as a subspecies of *J. evarete* (Hafernik 1982). Because of its phenotypic distinctiveness and its unique larval host plants (not used by other *Junonia*), I will follow Brown et al. (1992) in treating it as a full species.

The taxonomy of the New World *Junonia* butterflies has not always been clear and still under revision today. Clear species definitions and proper identification of some populations have not been established in some cases, and have caused the taxonomic history in this group to be very complicated (Schwartz 1989; Neild 2008; Calhoun 2010; Brévignon & Brévignon 2011; Brévignon & Brévignon 2012; Gemmell *et al.* 2014). For example, the grey buckeye, *J. coenia grisea* (Austin & Emmel 1998), is rejected as a distinctive subspecies by some authours (Brock & Kaufman 2003; Knerl & Bowers 2013), but based on my on research, I will argue deserves full species status (Chapter 4), is an example of a *Junonia* taxon with a murky taxonomic past (Gemmell & Marcus 2015). Other factors that have further complicated the taxonomy within the genus *Junonia* include: hybridization (Forbes 1928; Rutkowski 1971; Hafernik 1982; Minno & Emmel 1993); phenotypic variation occurring both geographically and seasonally within species (Forbes 1928; Clark 1932; Mather 1967; Remington 1985; Smith 1991; Rountree & Nijhout 1995); the close phenotypic resemblance of some forms (DeVries 1987; Glassberg 2007); vague species descriptions (Cramer 1775; Cramer 1780; Turner & Parnell 1985); the loss or absence of type specimens (Munroe 1951; Neild 2008); and the interchangeability of the genus names *Junonia* and *Precis* by many authors (De Lesse 1952; Kimball 1965; Wahlberg *et al.* 2005).

Because of their importance as an experimental model organism in many fields of scientific study, the taxonomy of the genus *Junonia* is important. Some work has been done to aid in the taxonomic ambiguities that exist. Wahlberg et al. (2005) determined using molecular phylogenetics that *Junonia* and *Precis* (restricted to Africa) were not synonymous genera, and in fact were not even sister clades. Additional progress has been made as new type specimens have been established, better species definitions published, and cryptic species identified (Brévignon 2004; Brévignon 2008; Neild 2008; Brévignon 2009; Brévignon & Brévignon 2011; Brévignon & Brévignon 2012). *Junonia* from different geographic locations in the New World have been studied in an attempt to associate geographic variation within species with appropriate taxonomic designations. A key problem with theses associations is that naming authorities have not been specified when submitting barcodes for reference specimens (Gemmell & Marcus 2015).

Mitochondrial Haplotypes in New World *Junonia*

The buckeye butterflies in the New World can be divided into two major mitochondrial haplotype groups using DNA barcodes; haplotype group A and haplotype group B (Pfeiler *et al.* 2012). Within these two major groups there are populations where these haplotypes exhibit enough variation to be considered unique (Pfeiler *et al.* 2012). With this added variation, four distinct haplotype groups can be found within the Western Hemisphere *Junonia*. Haplotype group A_1 is found in populations at high elevations in Peru restricted to a sole species (*Junonia vestina*), Haplotype group A_2 is predominant throughout South America, Haplotype group B is predominant in North and Central America, and B^{CA} is found in populations in the South Western United States (Pfeiler *et* *al.* 2012; Gemmell *et al.* 2014; Gemmell & Marcus 2015). The Caribbean seems to be a zone of genetic admixture where only the A_2 and B haplotypes are present (Gemmell *et al.* 2014; Gemmell & Marcus 2015). Within the genus *Junonia* it has also been found that all of the mitochondrial haplotypes can be found within all species (Brévignon & Brévignon 2012; Borchers & Marcus 2014; Gemmell *et al.* 2014). Using these DNA barcodes to distinguish species is not useful but they can be used to distinguish specific geographic locations as the signals present in these sequences differ (Gemmell *et al.* 2014; Gemmell & Marcus 2015). The distribution of haplotype groups A and B will be used to explore current (Chapter 2) and historical (Chapter 3) biogeographic patterns of *Junonia* in Florida, while the distribution of haplotype groups B and B^{CA} will be used to explore biogeographic patterns of *Junonia* in western North America (Chapter 4).

Florida *Junonia*

In Florida, there are three species of *Junonia* butterflies that occur: the common buckeye (*J. coenia*), the mangrove buckeye (*J. genoveva)*, and the tropical buckeye (*J. evarete*). The identity of the common buckeye, *J. coenia,* has not been disputed but the identity of the other two species has been the subject of much discussion (Calhoun 2010). I will note that all of the species designations that will be discussed here are based solely on morphological characteristics and geography with the exception of the taxonomic designations proposed based on work done in this thesis. Prior to 1928 it was thought that only *J. coenia* occurred in Florida and that there was seasonal and individual variation within this species (Forbes 1928). Then, it was recognized based on morphology that not only the common buckeye occurred in Florida but an additional form as well, the mangrove buckeye (Davis 1928; Forbes 1928). It was not until 1951 when it was observed that there were potentially three forms of buckeye butterflies in Florida; the common buckeye, the tropical buckeye and the mangrove buckeye (Klots 1951; Munroe 1951). It should also be noted that while the identity of the common buckeye (*J. coenia*) was considered to be distinct and was largely undisputed, both the tropical and mangrove buckeyes were considered to be seasonal forms of the same species based on morphology; the wet seasonal form and the dry seasonal form respectively (Munroe 1951). Based on morphology and geography this was still the main view in 1977 (Clench 1977).

In 1980 it was recognized that the tropical (*Junonia evarete*) and mangrove (*Junonia genoveva*) buckeyes were actually distinct species bringing the number of *Junonia* species in Florida and the Bahamas to three based on morphology (Clench & Bjorndal 1980). In 1985 Turner & Parnell (also using morphology) also agreed that the tropical and mangrove buckeyes were separate species but suggested that the taxonomic names be switched (the tropical buckeye switched to *J. genoveva* and the mangrove buckeye switched to *J. evarete* (Turner & Parnell 1985)). In 2008 Neild suggested based on morphology that the correct taxonomic designations were those proposed by Clench and Bjorndal in 1980 (Clench & Bjorndal 1980; Neild 2008).

Subsequent work done in 2011 and 2012 found that *J. evarete* and *J. genoveva* were actually restricted to South America and thus did not occur in the Caribbean (Brévignon & Brévignon 2011; Brévignon & Brévignon 2012). The mangrove buckeye in the region that includes the Caribbean and Florida was therefore properly referred to as *J. neildi* and the tropical buckeye as *J. zonalis* (Brévignon & Brévignon 2011; Brévignon

& Brévignon 2012; Gemmell *et al.* 2014). It was also determined that neither *J. evarete* nor *J. genoveva* use black mangrove (*Avicennia germinans*) as a larval host plant, and that the mangrove-feeding buckeyes of Central and South America belong to a third species, *J. litoralis*, which is also distinct from the mangrove-feeding buckeye species, *J. neildi,* in the Caribbean (Brévignon & Brévignon 2011; Brévignon & Brévignon 2012). *Junonia zonalis,* the tropical buckeye occurs in Florida, the Caribbean, and in Central America (Gemmell *et al.* 2014). The work described in this thesis (Chapters 2 & 3) will clarify the distributions, colonization history, and patterns of hybridization in Florida *Junonia.*

***Junonia* of Western North America**

Within the southwestern United States of America (California, Nevada, New Mexico, Arizona, Texas, New Mexico, Oklahoma, Colorado, southern Oregon, and southern Wyoming) and Mexico there are at least 5 different forms of *Junonia* in this region with overlapping ranges. These forms include *J. coenia, J. grisea, J. litoralis, J. nigrosuffusa,* and *J. zonalis* (Barnes & McDunnough 1916; Forbes 1928; Tilden 1970; Rutkowski 1971; Schwartz 1987; Minno & Emmel 1993; Paulsen 1996; Elster *et al.* 1999; Walker 2001; Neild 2008; Calhoun 2010; Gemmell & Marcus 2015). The recent diversification (within the last 3 million years) of this genus in the New World is the result of the *Junonia* ancestors invading from the Old World (McCullagh 2016). The Old World sister clade to almost all of the New World *Junonia* appears to be *J. villida* from the Indo-Pacific region (Gemmell & Marcus 2015; McCullagh 2016). Hybridization between species of the New World *Junonia* occurs at some frequency, also suggesting recent divergence (Hafernik 1982; Borchers & Marcus 2014; Gemmell *et al.* 2014). The degree to which hybridization occurs between species in the wild is still unknown, but putative hybrids have been found in the wild in some regions where multiple forms of *Junonia* occur (Forbes 1928; Rutkowski 1971; Hafernik 1982; Minno & Emmel 1993).

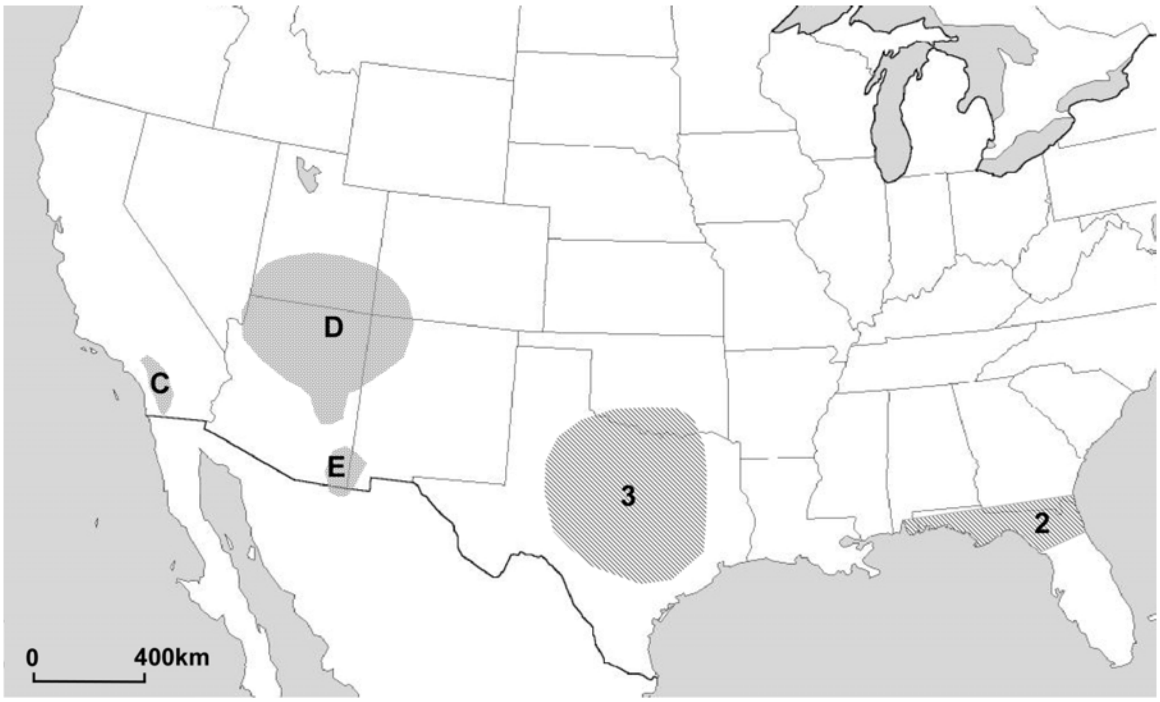
The distributions of some *Junonia* species in this region are known in part, but have not yet been fully documented. *Junonia grisea* (northern or gray buckeye) was described from southern California, and was thought to occur in California and adjacent parts of Arizona, USA and Baja California, Mexico (Austin & Emmel 1998). Hafernik (1982) made range maps of the *Junonia* in this region, but did not distinguish between *J. coenia* and *J. grisea*. *Junonia nigrosuffusa* can be found in southern Arizona, and southern Texas in the USA, and throughout north and central Mexico (Hafernik 1982). *Junonia zonalis* can be found in south Texas USA, along the eastern coast of Mexico and throughout southern Mexico (Hafernik 1982). It should be noted that Hafernik (1982) also did not distinguish between *J. zonalis* and *J. litoralis* and he considered both to be *J. zonalis* in his distribution maps. *Junonia litoralis* uses black mangrove (*Avicennia germinans*) as a larval host plant, and its distribution is closely associated with the presence of the larval host in marine coastal regions (Brévignon & Brévignon 2011; Brévignon & Brévignon 2012). Previous attempts to clarify the distribution of the various *Junonia* forms in this region suffered from limited sampling and difficulties interpreting the observed phenotypic variation (Hafernik 1982; Gemmell & Marcus 2015). Using mitochondrial genotyping to supplement analysis of phenotypic variation will clarify species distributions, patterns of hybridization, and produce a stable taxonomy for the forms that occur in the region (Chapter 4).

Historical Biogeography of *Junonia*

The complex pattern of overlapping *Junonia* species, with possible hybridization between them, in western North America mirrors geographically similar patterns in many other western species (Remington 1968; Remington 1985). Remington (1968) proposed a series of suture zones (or hybrid belts) within North America: 6 major suture zones and 6 minor or little-known zones based on observations of multiple hybrids between many species pairs. It was speculated that these suture zones represented boundaries where organisms met after sheltering in different glacial refuges during the late Pleistocene (Remington 1968; Remington 1985). The removal of the glacial barriers post-Pleistocene (8,000-11,000 years ago) would have allowed for species to move into these areas and possibly for closely related species to hybridize (Remington 1968). The suture zones he presented that are of particular interest include two of the major suture zones (zones 2 (Northern Florida) and 3 (Central Texas)) and three of the minor zones (zones C (California Desert-Pacific slope), D (Rocky Mountain-Sonoran), and E (Southwestern New Mexico)) (Figure 1-1; (Remington 1968)) because these zones are within the region of multiple *Junonia* species overlap.

At the same time, our understanding of the glacial history of North America has changed enormously since Remington (1968) proposed these suture zones. For many decades, it was thought that there were 4 major glaciation events that took place during the Pleistocene (from oldest to most recent): the Nebraskan (2.5-0.55 mya (million years ago), Kansan (0.55-0.2 mya; furthest southward expansion of the Laurentide ice sheet), Illinoian (0.2-0.16 mya), and Wisconsin (0.16-0.011 mya = 160,000 – 11,000 years ago

Figure 1-1. Suture zones proposed by Remington (1968). Numbers represent the major suture zones (2=Northern Florida; 3=Central Texas) and letters represent the minor suture zones (C=California Desert-Pacific Slope; D=Rocky Mountain-Sonoran; E=Southwestern New Mexico Arizona). Map modified from the Cartographic Research Lab, University of Alabama (http://alabamamaps.ua.edu/contemporarymaps/usa/basemaps/usa1.jpg).



(ya)) glacial stages (Boellstorff 1978). More recent reinterpretation of the deposits left by the various glacial advances suggests that the Nebraskan and Kansan stages were actually composites of multiple glacial advances, rather than singular events (Boellstorff 1978; Rovey & McLouth 2015). It is now understood that there were many glaciation events during the Pleistocene that are now classified into 3 major periods: Pre-Illinoian (2.4-0.2 mya; corresponds to the time periods formerly making up the Nebraskan and Kansan stages), Illinoian (0.2-0.16 mya), and Wisconsin (0.16-0.011 mya) (Lane 1994; Zeiller 2005; Rovey & Balco 2011). During each of these glacial advances in North America, continental populations were subdivided into eastern and western glacial refuges, in many cases contributing to speciation (Lovette 2005). The maximal glacial advance during the entire Pleistocene (associated with glacial tills formerly assigned to the Kansan glacial stage) appears to have taken place about 1.2 mya (Wanner *et al.* 2008; Rovey & Balco 2011; Roberts & Hamann 2015).

The glacial advances that occurred in North America during the Pleistocene coincide with some patterns of *Junonia* haplotype divergence and diversification in the New World. During the Pre-Illinoian period, approximately 2.31±0.42 mya (Table 1-1) haplotype B diverged from the lineage that gave rise to *J. villida* and New World haplotype group A, but it is not known whether this vicariance event took place in the New World or in the Pacific (McCullagh 2016). The split of haplotype group B^{CA} from haplotype group B coincides with the maximum glacial advance in North America during the Pleistocene at 1.18±0.29 mya (Table 1-1; Rovey & Balco 2011; McCullagh 2016). This suggests that *Junonia* carrying haplotype group B were in North America by 1.2 million years ago, and the subdivision of haplotype group B may be related to subdivision of North America *Junonia* populations being split into eastern and western glacial refuges.

The split of haplotype A from *J. villida* also falls within the Pre-Illinoian period (1.58±0.32 mya; Table 1-1) but again, it cannot yet be determined whether the split occurred in the Old or New World (McCullagh 2016). The diversification of haplotype A within the New World dates to 0.96±0.29 mya (Table 1-1) and unlike haplotype group B shows no East-West split, possibly because this haplotype may not have been present in the New World at the 1.2 mya Pleistocene glacial maximum (McCullagh 2016).

Table 1-1. Haplotype divergence times for the buckeye butterflies (genus *Junonia*) in the New World. Adapted from McCullagh (2016).

Divergence Event	Divergence Time (in millions of years)
Haplotype B from Haplotype A and *J. villida*	2.31±0.42
Haplotype A from *J. villida*	1.58±0.32
Haplotype B^{CA} from Haplotype B (Haplotype B diversification in the New World)	1.18±0.29
Haplotype A diversification in the New World	0.96±0.29

Junonia Biogeography: Florida

The current interglacial period is called the Holocene (11,000 ya to present (Wanner *et al.* 2008; Roberts & Hamann 2015)). From 11,000-6,000 ya there was an overall warming trend in global climate (Kaufman *et al.* 2004). Increases in air temperature resulted in glacial retreat and extensive melting, forming enormous mid-continental lakes filled with glacial meltwater (e.g. glacial Lake Aggasiz, glacial Lake Ojibway) (Teller 1987). As temperatures continued to increase and the lakes grew larger, new lake outlets were created, resulting in several catastrophic drainages (Condron & Winsor 2011; Teller 2013). Each time this took place, massive amounts of cold, fresh water spilled into the Gulf of Mexico or the Atlantic Ocean, but each time from a different outlet (Joyce *et al.* 1993; Clark *et al.* 2001; Teller 2013; Hill & Condron 2014). The massive amounts of cold water being poured into the Atlantic Ocean, caused lower ocean temperatures, changes in ocean currents, and rapid increases in sea level (Wanless 1989; Ganopolski *et al.* 1998). Coastal ecosystems were disrupted during this period (Wanless 1989), with tropical and subtropical species probably being disproportionately affected. During this period, the Florida peninsula was predominantly desert scrub habitat (Delcourt 2002). While this habitat may have been suitable and served as a possible refuge for *Junonia coenia,* it was likely too cold for the survival of larval host plants of the other 2 *Junonia* species that currently occur in Florida.

Approximately 8,000 ya the last of the glacial lakes drained and the ocean currents changed causing an overall warming trend in North America and the surrounding bodies of water (Li *et al.* 2012; Hill & Condron 2014). Particularly, the circulation patterns in the Atlantic Ocean reversed bringing the warm waters from the tropics north, instead of cold waters flowing southwards, as had occurred during the drainage of the glacial lakes (Clark *et al.* 2001; Hill & Condron 2014). The climate became warmer and wetter (Beck *et al.* 1997), and the vegetation found in the Florida interior came to resemble the modern tropical/subtropical flora about 5000 years ago (Watts 1975; Delcourt 2002), as tropical and subtropical species moved north from glacial refuges.

Finally, by 3,000 ya the sea level stabilized (Wanless 1982) and this stabilization would have allowed for coastal species such as mangroves to establish (Wanless 1989). Thus, in Florida, *Junonia neildi,* which depends on black mangrove (*Avicennia germinans*) as a larval host plant (Turner & Parnell 1985; Paulsen 1996; Elster *et al.* 1999), probably arrived from more southerly refuges in the last 3000 years. The final species, *Junonia zonalis,* which feeds on frost-sensitive larval host plants, appears to have arrived in Florida during the mid-20th Century (Minno & Emmel 1993; Glassberg *et al.* 2000; Calhoun 2010).

Junonia Biogeography: Western North America

During the late Pleistocene and early to middle Holocene in the South Western USA and Mexico the climatic conditions were reversed when compared to the North Atlantic (Benson *et al.* 1997). This same trend is apparent when comparing both the Northern Hemisphere and Southern Hemisphere climate data (eg. Northern Hemisphere warming coinciding with Southern Hemisphere cooling (Shakun *et al.* 2012)). Vegetation reconstructions in Mexico during the Pleistocene shed light into where the *Junonia* species in Western North America may have found refuge (Ceballos *et al.* 2010). At this time the climate was cooler and wetter (Benson *et al.* 1997) than the present day warm and drier conditions, which would have allowed for more temperate plant species to be present during this time (Delcourt & Delcourt 1979).

During the Pleistocene, in Baja California and the region presently known as the Sonora Desert, the main vegetation type was xeric scrub (Ceballos *et al.* 2010). This is the habitat that *J. grisea* currently prefers (Scott 1975; Stout 2016), and it is possible to speculate that this species may have taken refuge here during times of glacial advancement. During glacial retreat, *J. grisea* may have migrated from these refuges into suitable habitat west of the Rocky Mountains in modern-day California. The unsuitable high elevation habitats of the Rocky Mountains may have permitted *J. grisea,* which carries the distinctive B^{CA} mitochondrial haplotype, to maintain its distinctiveness by limiting east-west migration.

The predominant vegetation in Northeastern Mexico (presently known as the Chihuahua Desert) during this time was also primarily composed of xeric scrub (Ceballos *et al.* 2010). At the time of the Wisconsin glaciation (160,000 – 11,000 years ago (ya)) there was a split in the continental populations of distinct species into eastern and western glacial refuges (Lovette 2005). It is possible to speculate during this split that *J. coenia* was divided into two separate populations, one taking refuge in Florida and the Caribbean in the East, and the other in South Texas and Northeastern Mexico in the West, both of which continue to carry mitochondrial haplotype group B. In this scenario, the populations in Northeastern Mexico evolved a phenotypic darkening of the wings (Hafernik 1982), underwent a larval host plant switch, which resulted in host plant specialization (Tilden 1970; Glassberg 2001), and gave rise to the dark buckeye, *J. nigrosuffusa.*

Tropical rainforest and tropical dry forest habitats in Mexico during the Pleistocene were restricted to latitudes below 20° N latitude (Ceballos *et al.* 2010). The 20° N latitude is also believed to be the most northern extent of black mangrove (*Avicennia germinans*) populations on the Gulf and Pacific Coasts of Mexico during the Wisconsin glacial advance (Sandoval-Castro *et al.* 2014). Black mangrove is the sole larval host plant of *J. litoralis* (Brévignon 2009; Brévignon & Brévignon 2012) and it can be assumed based on the close association of this butterfly species with black mangrove, that it also was also restricted to habitats below 20° N latitude. *J. zonalis* would may likewise been restricted to same northern latitudinal maximum limits as its host plants occur in coastal regions, tropical grasslands, and gaps in forested areas, as they are extremely frost sensitive (Turner & Parnell 1985; Glassberg *et al.* 2000).

During the early to middle Holocene, western North America became warmer, arid, and desert-like shrub became the predominant vegetation in this region (Benson *et al.* 1997). This shift in climate would have allowed for *J. coenia* to expand Westward from Eastern glacial refuges into much of Western North America. This change in climate would also have allowed for the other species of *Junonia* and their respective host plants from glacial refuges in Mexico to also expand their ranges northward. A product of the expansion north would have brought these 5 species into close proximity and in some cases allowed for habitats to overlap to create the current species distributions.

Objectives and rationale of the thesis:

1. To determine the relative distributions of the three *Junonia* species which occur in Florida USA based on morphological characteristics, as well as determine the relative species and haplotype distributions of these species within Florida using molecular markers (Chapter 2).
2. To investigate whether the *Junonia* species that occur in Florida are the same as those in Central America, Southern America and the Caribbean using morphological and molecular markers to help clarify to the taxonomy in this region (Chapter 2).
3. To determine the relative distribution of haplotypes in Florida using historical data to observe the creation of a secondary contact zone and observe the pattern of gene flow over both space and time (Chapter 3).
4. To reconstruct the historical timeline of the invasion of the tropical buckeye into Florida, USA, using museum specimens and attempt to determine whether it was a single invasion event or multiple invasion events (Chapter 3).

5. To determine the number of *Junonia* species that occur in southwestern America and Mexico and determine species ranges using morphological characteristics and collection data from museum collections (Chapter 4).
6. To determine the distribution of haplotypes in the southwestern America's and Mexico and observe the frequency of haplotype group B (standard *Junonia coenia*) vs. B^{CA} (private haplotype group associated with *J. grisea*) (Chapter 4).
7. To investigate whether *Junonia coenia* and *Junonia grisea* are discrete species or if *J. grisea* is a subspecies of *J. coenia* using morphological characteristics, species distributions and molecular markers (Chapter 4).

Conclusion

Junonia butterflies have been used for studies as diverse as the evolution and development of colour patterns, (Nijhout 1991; Carroll *et al.* 1994; Kodandaramaiah 2009), larval host plant specialization (Bowers 1984; Camara 1997; Knerl & Bowers 2013; Gemmell *et al.* 2014), and insect physiology (Nijhout 2010), and the development of seasonal polymorphisms (Daniels *et al.* 2012; Daniels *et al.* 2014). However, the vast majority of this work has focused on a single species: *J. coenia.* Exploiting natural diversity within the genus will provide important resources for the study of these and other subjects. By better defining each species, their phenotypes and natural geographic ranges, and the nature of the species boundaries, this thesis will attempt to fill in the gaps in knowledge for this model system for the study of the generation and the maintenance of biodiversity.

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Chapter 2: The taxonomy and population structure of the buckeye butterflies (genus *Junonia,* Nymphalidae: Nymphalini) of Florida, USA

Abstract

The buckeye butterflies (genus *Junonia*) that occur in Florida, USA have been an ongoing taxonomic challenge for over 100 years. Schwartz (1987) described the situation succinctly: "No other lepidopteran on the Florida Keys nor in south Florida, presents the taxonomic problems as *Junonia*." The current consensus recognizes 3 different forms of *Junonia* in Florida based on morphological characters, but there is a general lack of consensus regarding the appropriate scientific names for each form. Similarities between the species that occur in Florida, intraspecific variation, and possible hybridization between species have often made it challenging to identify specimens, define the population structure of *Junonia* butterflies, or to understand the relationships of these butterfly populations to those elsewhere in the New World. I use a combination of morphological characters, mitochondrial *cytochrome oxidase I* barcodes, and nuclear *wingless* DNA sequences, from *Junonia* from Florida, the Caribbean, and North and South America to resolve issues of taxonomy and population structure in this genus. I conclude that the common buckeye (*J. coenia*), the mangrove buckeye (*J. neildi*), and the tropical buckeye (*J. zonalis*) occur in Florida and that hybridization between these species takes place in this region.

Introduction

The New World buckeye butterflies (genus *Junonia*, Lepidoptera: Nymphalidae) have had a complicated taxonomic history (Schwartz 1989; Neild 2008; Calhoun 2010; Brévignon & Brévignon 2011; Brévignon & Brévignon 2012; Gemmell *et al.* 2014). Establishing clear species definitions and proper identification of many species in this genus has been very difficult because of geographical variation (Forbes 1928; Remington 1985) and seasonal variability in colour patterns (Clark 1932; Mather 1967; Smith 1991; Rountree & Nijhout 1995) within some *Junonia* species, the close resemblance between some *Junonia* forms (DeVries 1987; Glassberg 2007), and the possible presence of naturally occurring hybrids between them (Forbes 1928; Hafernik 1982; Minno & Emmel 1993; Rutkowski 1971). This has been further complicated by vague early species descriptions (Cramer 1779; Cramer 1780; Turner & Parnell 1985), the complete absence or loss of type specimens for some species (Munroe 1951; Neild 2008), the interchangeable use of the generic names *Precis* and *Junonia* (De Lesse 1952), and the inadvertent use of invalid taxonomic homonyms (*Precis/Junonia lavinia*) (Cramer 1775; Comstock 1944; Munroe 1951). To paraphrase very liberally (with apologies) from Winston Churchill's (1939) wartime description of Russia, the butterfly genus *Junonia* has been a taxonomic puzzle (Riley 1975; DeVries 1987) , wrapped in a muddle , inside an enigma (Neild 2008).

As a consequence of these factors, researchers studying this genus have used an astounding variety of scientific names for New World *Junonia* species. The three *Junonia*, species found in Florida (the common buckeye, the mangrove buckeye, and the tropical or West Indian buckeye) are a case in point: they have been known by so many different names (Table 2-1) that the scientific literature associated with this genus has become very difficult to decipher. Kimball (1965) wrote "This [genus] has been bandied about so in the past few years as regards name that it would not be surprising were its oldest friends to fail to recognize it….Under how many and what aliases it has paraded recently I leave to the historian of curiosities."

Table 2-1. Taxonomic names used by various authorities for the three *Junonia* species found in Florida, USA**.**

Authority	Common Buckeye	Mangrove Buckeye	Tropical Buckeye
Maynard 1891	*Junonia coenia*		*J. genoveva*
Holland 1898	*J. coenia*	*J. lavinia*[1]	*J. genoveva*[1]
Swainson 1901			*J. genoveva*
Fruhstorfer 1907	*J. lavinia coenia*	*J. lavinia lavinia*	*J. lavinia zonalis*[2]
Longstaff 1908, 1912	*Precis lavinia coenia*	*P. lavinia lavinia*	*P. lavinia genoveva*
Barnes & McDunnough 1916	*J. coenia*	*J. genoveva*[*]	*J. genoveva*[*]
Grossbeck 1917	*J. coenia*	*J. lavinia*	
Walker 1917	*J. coenia*	*J. genoveva*	
Seitz 1924	*P. lavinia coenia*	*P. lavinia genoveva*[3]	*P. lavinia lavinia*[3]
Davis 1928	*P. lavinia coenia*[4]		*P. lavinia genoveva*[4]
Forbes 1928	*J. lavinia coenia*	*J. lavinia zonalis*[5] (dry season form)	*J. lavinia zonalis*[5]
Bates 1935	*P. coenia*	*P. zonalis*[*]	*P. zonalis*[*]
Wolcott 1936	*J. coenia*	*J. lavinia*	*J. genoveva & P. zonalis*
Dethier 1941			*P. zonalis*
Carpenter & Lewis 1943	*P. coenia*	*P. lavinia zonalis*[5] (dry season form)	*P. lavinia zonalis*[5]
Comstock 1944	*J. evarete coenia*	*J. evarete genoveva*	*J. evarete zonalis*
Avinoff & Shoumatoff 1946		*J. genoveva*	*J. zonalis*
Corbet 1948	*P. orithya lavinia*		
Wolcott 1948	*J. evarete coenia*	*J. evarete genoveva*	*J. evarete zonalis*
Eliot 1949	*P. lavinia coenia*	*P. lavinia lavinia*[*]	*P. lavinia lavinia*[*]
Klots 1951	*P. lavinia coenia*	*P. lavinia zonalis*	*P. lavinia genoveva*
Rindge 1952	*J. coenia*	*J. evarete zonalis*[*]	*J. evarete zonalis*[*]
Munroe 1951	*J. coenia*	*J. evarete zonalis*[5]	*J. evarete zonalis*[5]

		(dry season form)	
Young 1955	*P. coenia*		*P. lavinia zonalis*
Ehrlich & Ehrlich 1961	*P. lavinia*	*P. genoveva*[*]	*P. genoveva*[*]
Kimball 1965	*P. orithya evarete*	*P. orithya zonalis*	
Remington 1968	*J. coenia*	*J. evarete*	
Barcant 1970		*P. lavinia zonatis*[*]	*P. lavinia zonatis*[*]
Tilden 1970	*P. coenia*	*P. evarete evarete*	*P. evarete zonalis*
Rutkowski 1971	*J. coenia*	*J. evarete zonalis*[*]	*J. evarete zonalis*[*]
Brown & Heineman 1972	*P. evarete coenia*	*P. evarete zonalis*[5] (dry season form)	*P. evarete zonalis*[5]
Percival 1974		*Junonia* species "B"	*Junonia* species "A"
Gorelick 1975	*P. coenia*	*P. evarete zonalis*[*]	*P. evarete zonalis*[*]
Riley 1975	*J. coenia*	*J. evarete zonalis*[5] (dry season form)	*J. evarete zonalis*[5]
Clench 1977	*J. coenia*	*J. evarete zonalis* form *genoveva*	*J. evarete zonalis*
Clench & Bjorndal 1980		*J. genoveva*	*J. evarete zonalis*
Lenczewski 1980	*J. coenia*	*J. evarete*	
Pyle *et al.* 1981	*J. coenia*	*J. evarete*[*]	*J. evarete*[*]
Baggett 1982a	*J. coenia*	*Junonia* species "B"	*Junonia* species "A"
Baggett 1982b	*J. coenia*	*J. evarete* species "B"	*J. evarete*, nr. ssp. *michaelesi* "A"
Hafernik 1982	*J. coenia*		*J. evarete zonalis*
Schwartz 1983			*J. evarete zonalis*
Harvey 1984[6]	*J. coenia*	*Junonia* species "B"	*Junonia* species "A"
Remington 1985	*J. coenia*	*J. zonalis*[*]	*J. zonalis*[*]
Turner & Parnell 1985	*J. coenia*	*J. evarete*	*J. genoveva*
Scott 1986	*P. coenia*	*P. genoveva*	*P. evarete*
Alayo & Hernandez 1987	*J. coenia*	*J. evarete zonalis* (dry season form *genoveva*)	*J. evarete zonalis*
de la Maza 1987	*P. coenia*	*P. evarete zonalis*[*]	*P. evarete zonalis*[*]
Schwartz 1987	*J. evarete*	*J. coenia*	*J. coenia*
Schwartz *et al.* 1987		*J. evarete*	*J. genoveva zonalis*
Schwartz 1989	*J. coenia*	*J. genoveva zonalis*[7]	*J. genoveva zonalis*[7]
Miller *et al.* 1992		*J. genoveva*	
Opler & Malikul 1992	*J. coenia*	*J. evarete*	*J. genoveva*
Minno & Emmel 1993	*J. coenia*	*J. evarete*	*J. genoveva*
Smith *et al.* 1994	*J. coenia*	*J. evarete*	*J. genoveva*
Austin *et al.* 1996		*J. evarete*	*J. genoveva*
Paulsen 1996	*P. coenia*	*P. evarete*	

Meerman 1999		*J. evarete zonalis*	*J. genoveva*
Glassberg *et al.* 2000	*J. coenia*	*J. evarete*	*J. genoveva*
Opler & Warren 2002	*J. coenia*	*J. genoveva*	*J. evarete*
Brock & Kaufman 2003	*J. coenia*	*J. evarete*	*J. genoveva*
Brévignon 2003		*J. genoveva* n. ssp.	*J. evarete michaelesi*
Brévignon 2004		*J. genoveva neildi*	*J. evarete swifti*
Hernandez 2004	*J. coenia*	*J. evarete zonalis*	*J. genoveva*
Lamas 2004	*J. coenia*	*J. genoveva*	*J. evarete*
Cech & Tudor 2005	*J. coenia*	*J. evarete*	*J. genoveva*
Lazell 2005		*J. evarete*	*J. genoveva*
Marcus 2005	*J. coenia*	*J. evarete*	
Kodandaramaiah & Wahlberg 2007	*J. coenia*	*J. genoveva*	*J. evarete*
Askew & Stafford 2008	*J. coenia*	*J. evarete*	*J. genoveva*
Beccaloni *et al.* 2008	*J. coenia*	*J. genoveva*	*J. evarete*
Brévignon 2008	*J. coenia*	*J. genoveva neildi*	*J. evarete*
Neild 2008	*J. coenia*	*J. genoveva*	*J. evarete*
Brévignon 2009	*J. coenia*	*J. neildi*	*J. evarete*
Kodandaramaiah 2009	*J. coenia*	*J. genoveva*	*J. evarete*
Perez-Asso *et al.* 2009		*J. evarete michaelisi*	*J. genoveva neildi*
Calhoun 2010	*J. coenia*	*J. genoveva*	*J. evarete zonalis*
Nijhout 2010	*P. coenia*	*P. evarete*	
Brévignon & Brévignon 2011, 2012	*J. coenia*	*J. neildi*	*J. zonalis*
Gemmell & Marcus 2015	*J. coenia*	*J. "evarete"*	*J. "genoveva"*
This study	*J. coenia*	*J. neildi*	*J. zonalis*

[*]Does not distinguish between these forms.
[1]Holland (1898) was not aware that these species occurred in Florida, and illustrates these species based on material from Texas and Caribbean.
[2]Fruhstorfer (1907) also establishes subspecies *J. lavinia michaelisi* form Puerto Rico and the lesser Antilles.
[3]Seitz (1924) considered *P. lavinia lavinia* and *P. lavinia genoveva* to be intraspecific variants that co-occurred in various parts of Latin America. He viewed P. *lavinia zonalis* as an aberrant form.
[4]Davis (1928) considers these to be alternate forms of the same species.

[5]Considers phenotypes associated with mangrove buckeye to be seasonal forms of *J. zonalis*. Dry season form is *genoveva.*
[6]Harvey (1984) is a manuscript that was never published, but which was circulated in draft form, was cited several times (e.g. (Bowers 1984; DeVries 1987)), and had an important influence on taxonomic usage.
[7]Schwartz (1989) identifies the phenotype associated with the mangrove buckeye, but considers it to be an extreme form of the tropical buckeye.

The taxonomic history of *Junonia* can be understood as having undergone four major phases. The first phase began with the first observations and early descriptions of *Junonia* in the New World (Sloane 1725; Cramer 1775; Cramer 1779; Cramer 1780; Abbot & Smith 1797), and is characterized by very limited information available to natural historians about the number of species that occur and their geographic ranges. This period continued until about 1900, when many of the North American forms were recognized, though in many cases their respective ranges still remained ambiguous (Holland 1898; Swainson 1901). The second phase of *Junonia* taxonomy began in the early 20th century and interpreted many phenotypic differences between the species, as intraspecific geographic (Fruhstorfer 1907; Longstaff 1908; Seitz 1924) or seasonal variability (also known as polyphenism) (Forbes 1928). Drawing from observations of seasonal polyphenism in the supposed Old World congeners *Precis octavia* (Marshall 1898; Munroe 1951) and *P. antilope* (Rogers 1911), and reinforced by observations of seasonal polyphenism in the New World *J. coenia* (Clark 1932), seasonal variability became the primary lens through which variation in *Junonia* was interpreted for much of the 20th century (Forbes 1928; Munroe 1951; Brown & Heineman 1972; Clench 1977; Alayo & Hernandez 1987). Even when multiple forms of *Junonia* were observed simultaneously in the same habitat, this was often interpreted as the effects of microclimate on the development of larvae (Carpenter & Lewis 1943; Munroe 1951; Brown & Heineman 1972).

In the third phase of this taxonomic history, beginning in the early 1980s, (Clench & Bjorndal 1980; Baggett 1982a; Baggett 1982b) a general consensus developed that three distinct *Junonia* species occur in Florida (Figure 2-1): the common buckeye, the mangrove buckeye, and the tropical (or West Indian) buckeye. While there was general agreement that the common buckeye should be called *J. coenia,* opinions differed with respect to the correct taxonomy for the mangrove and tropical buckeyes (Table 2-1). For clarity, this phase can be split into three segments based on the time in which these views came into the literature, and they will be designated as phase 3.1, phase 3.2, and phase 3.3 respectively. Phase 3.1 assigned the mangrove buckeye with the scientific name *J. genoveva* and the tropical buckeye with *J. evarete,* (Clench & Bjorndal 1980; Scott 1986) maintaining the traditional associations of names with the same phenotypes observed by many prior authors, but now recognizing them as distinct species rather than seasonal forms. Phase 3.2 began when Turner and Parnell (1985) inspected the hand-coloured published plates of the original species descriptions of *J. evarete* and *J. genoveva* from Cramer (1779, 1780) and compared them to *Junonia* material from Jamaica and Florida. Discrepancies in wing patterns between the illustrations (depicting material collected in Suriname) and their own material prompted Turner and Parnell (1985) to switch the species names (the mangrove buckeye became *J. evarete* and the tropical buckeye became *J. genoveva*), compared to prior common usage. The taxonomic viewpoint associated with phase 3.2 predominated in the literature until 2002, and is still in use by authors who focus primarily on the butterfly fauna of the Greater Antilles and eastern

North America, including Florida (Opler & Malikul 1992; Minno & Emmel 1993; Smith *et al.* 1994; Glassberg *et al.* 2000; Cech & Tudor 2005; Askew & Stafford 2008). In Phase 3.3, the names of the mangrove and tropical buckeyes were switched back to the original designations in phase 3.1. This switch was originally justified based on unpublished work by Andrew Neild (Lamas 2004; Opler & Warren 2002). The full justification of this change was published several years later (Neild 2008) based on the inspection of the original water colors that were used as the basis for the published plates of Cramer (1779, 1780), and the consideration of additional diagnostic morphological characters, especially associated with the antennae (Calhoun 2010). This view was adopted by a group of *Junonia* researchers who tended to be more focused on the taxa in South American and the Lesser Antilles (Brévignon 2003; Kodandaramaiah & Wahlberg 2007; Brévignon 2008; Kodandaramaiah 2009; Calhoun 2010). However, while both the antennal characters emphasized by Neild (2008) and the color pattern characters used by many prior authors generally support each other to produce similar conclusions when distinguishing between *J. evarete* and *J. genoveva* in Venezuela, the antennal and wing characters do not correspond very well to the interspecific variation seen in Florida and can yield opposite species determinations (Table 2-2).

The fourth phase in *Junonia* taxonomy is based on the recent recognition that the South American species are distinct from the Caribbean species. First, Brévignon (2004) named new subspecies for each of the two *Junonia* species that occurred in Guadeloupe:

Figure. 2-1. Adult photos of *Junonia* species found in Florida, USA. (**a**) Dorsal view common buckeye (*J. coenia*), Old Ingram Highway, Everglades National Park, Dade County, Florida, USA, 10 January 2007. (**b**) Dorsal view mangrove buckeye (*J. neildi*), Jack Island Preserve State Park, St. Lucie County, Florida, USA, 22 May 2006. (**c**) Dorsal view tropical buckeye (*J. zonalis*), North Trailhead, Everglades Greenway, Homestead, Dade County, Florida, USA, 12 January 2007. (**d**) Ventral view common buckeye (*J. coenia*), Zip Track Site, Paducah, Kentucky, McCracken County, Kentucky, USA, 6 September 2003. (**e**) Ventral view mangrove buckeye (*J. neildi*), Lower Sugarloaf Key, Monroe County, Florida, USA, 16 March 2007. (**f**) Ventral view tropical buckeye (*J. zonalis*), North Trailhead, Everglades Greenway, Homestead Dade County, Florida, USA, 12 January 2007.

Table 2-2. Defining characteristics for the three *Junonia* species found in Florida, USA.

Attributes	***Junonia coenia* (Common Buckeye)**	***Junonia neildi* (Mangrove Buckeye)**	***Junonia zonalis* (Tropical Buckeye)**
Size of forewing	18-27.5 mm (Minno & Emmel 1993)	26-31 mm (Minno & Emmel 1993)	23-28 mm (Minno & Emmel 1993)
Subapical patches on dorsal forewings	White or cream coloured (Glassberg *et al.* 2000)	Suffused with orange of pink pigment (Turner & Parnell 1985; Glassberg *et al.* 2000)	Suffused with orange or pink pigment (Turner & Parnell 1985)
Forewing band	White; surrounds larger eyespot (Minno & Emmel 1993)	Pale orange (Minno & Emmel 1993)	Pinkish-white (Minno & Emmel 1993)
Colouration on ventral hindwing	Light colouration, variably prominent submarginal reddish band (Glassberg *et al.* 2000)	Dark colouration, dull grey to dark brown (Turner & Parnell 1985)	Light colouration, prominent white postmedian band (Turner & Parnell 1985; Minno & Emmel 1993)
Eyespots on ventral hindwing	Prominent (Forbes 1928)	Not very prominent (Turner & Parnell 1985)	Prominent (Turner & Parnell 1985)
Eyespots on dorsal hindwings	Anterior eyespot is larger than posterior (Forbes 1928)	Eyespots are nearly identical in size (Paulsen 1996)	Anterior eyespot is larger than posterior (Turner & Parnell 1985)
Antennae	Dark antennal tips, dark undersides of antennal tips (Minno, pers comm. 2015)	Dark antennal tips, underside of tip is dark brown or brownish-black contrasting the colour of the shaft (Calhoun 2010)	Dark antennal tips, underside of tip is pale and similar in colour to the ventral shaft (Calhoun 2010)
Habitat preferences	Salt marsh, sand dune and grassland habitats (Paulsen 1996; Glassberg *et al.* 2000)	Coastal mangrove swamps (Turner & Parnell 1985; Paulsen 1996; Glassberg *et al.* 2000)	Salt marsh, sand dune and grassland habitats. In Florida, restricted to the Florida Keys and the frost-free portions of mainland Florida (in the extreme South) (Turner & Parnell 1985; Glassberg *et al.* 2000).

Larval host plants in Florida	Several species of false foxglove, (*Agalinis fasciculata, A. maritima,* and *A. purpurea*), American blueheart (*Buchnera americana*) black-senna (*Seymeria* sp.), toadflax (*Linaria sp.*), and plantain (*Plantago sp.*) (Tilden 1970; Glassberg *et al.* 2000)	Black Mangrove (*Avicennia germinans*) (Turner & Parnell 1985; Paulsen 1996; Glassberg *et al.* 2000)	Blue Porterweed (*Stachytarpheta jamaicensis*) (Glassberg *et al.* 2000), possibly also frog fruit (*Lippia nodiflora,* documented from some Caribbean populations, but may be an inferior host) (Brown & Heineman 1972). Recently, this species and it hybrids have also been reared from wild-collected larvae found on *Agalinis* and *Buchnera* (M. Minno, *pers. comm.*)
Larval phenotype	Larvae nearly black with two lateral rows of cream spots. Bluish-black spines dorsally on each segment, with orange spots at the base of each lateral spine. Prolegs orange. (Glassberg *et al.* 2000; Wagner 2010)	Larvae almost completely black without lateral markings. Bluish-black spines dorsally on each segment. Prolegs black. (Glassberg *et al.* 2000)	Larvae nearly black dorsally with broad gray-brown lateral bands. Lateral rows of cream spots greatly reduced. Bluish-black spines dorsally on each segment. Prolegs gray-brown. (Glassberg *et al.* 2000)
Pupal mass (all reared on *Plantago laneolata* and *P. major* in captivity)	mean = 0.346 g st. dev. = 0.053 g N = 118 (this study)	mean = 0.550 g st. dev. = 0.083 g N = 134 (this study)	mean = 0.327 g st. dev. = 0.050 g N = 82 (this study)

J. genoveva neildi whose larval host plant is black mangrove (*Avicennia germinans*) and *J. evarete swifti,* whose larvae feed on blue porterweed (*Stachytarpheta jamaicensis*) and frog fruit (*Phyla nodiflora*). Brévignon (2009) later realized that *J. genoveva* in French

Guiana does not feed on black mangrove, and differs phenotypically from the form in Guadeloupe, prompting him to elevate *J. neildi* to a full species. More recently, a similar disparity between larval host plant use and various features of larval and adult morphology between South American and Caribbean forms of *J. evarete*, suggests that these are distinct species as well (Brévignon & Brévignon 2011; Brévignon & Brévignon 2012). Thus, *J. evarete* (type locality Suriname) was no longer considered to be synonymous with *J. zonalis* (type locality Cuba), and the form that occurs in Guadeloupe would be called *J. zonalis swifti* in this taxonomy. Genetic evidence supports the separation of both Caribbean forms (*J. neildi* and *J. zonalis*) from *Junonia* species that occur on the South American mainland (Gemmell *et al.* 2014). The many competing taxonomic hypotheses for the New World *Junonia*, and the long duration of taxonomic ambiguity has given rise to repeated calls to revise the taxonomy of *Junonia* over the last 100 years (Longstaff 1908; Bates 1935; Klots 1951; Schwartz 1989).

The problems caused by uncertain nomenclature in *Junonia* are of concern beyond the domain of taxonomists. This is because the genus *Junonia* is a valuable experimental model in several research areas such as the evolution of wing color patterns (Kodandaramaiah 2009; Kodandaramaiah *et al.* 2013), insect physiology and development (Martin & Reed 2010; Nijhout 2010; Dhungel & Otaki 2013; Martin & Reed 2014), the mechanisms of larval host plant preference (Camara 1997; Knerl & Bowers 2013; Gemmell *et al.* 2014), quantitative genetics (Paulsen 1996; Marcus 2005), phylogenetics (Kodandaramaiah & Wahlberg 2007; Pfeiler *et al.* 2012a), and ring species evolution (Gemmell & Marcus 2015). Tools for manipulating gene expression (Lewis *et al.* 1999; Lewis & Brunetti 2006; Dhungel *et al.* 2013) and for making transgenics

(Beaudette *et al.* 2014) in *Junonia* have been developed, suggesting that its importance as a model system will continue as these tools for genome manipulation are deployed.

The uncertainty of the taxonomy has made conducting research on *Junonia* butterflies challenging and has impeded progress in some instances. Ambiguity in taxon names has caused some authors to transpose biological details between species (e.g. (Opler & Malikul 1992)). Records of larval host plant use by each species have been particularly prone to misattribution (Tietz 1972; Robinson *et al.* 2002; Beccaloni *et al.* 2008). Since identification by DNA barcode is predicated on properly and unambiguously identified reference specimens (Hebert *et al.* 2003), the effectiveness of DNA barcoding efforts in New World *Junonia* has been compromised because researchers doing faunal surveys that include DNA barcoding have often failed to provide the taxonomic authority they followed when associating *Junonia* species names with barcodes (Janzen *et al.* 2005; Hajibabaei *et al.* 2006; Janzen & Hajibabaei 2009; Hebert *et al.* 2010; Escobedo 2011; Janzen 2012; Mitter 2013). In a group like *Junonia*, in which so many different taxonomic hypotheses are being used simultaneously (Table 2-1), this compromises the value of DNA barcodes for species identification, and has greatly complicated analyses that attempted to use New World *Junonia* barcodes from the faunal surveys for phylogenetic inference (Pfeiler *et al.* 2012a; Pfeiler *et al.* 2012b; Gemmell *et al.* 2014). In some cases in the past, it has been possible to use photo or specimen vouchers to resolve ambiguous or suspect taxonomic identifications, but in other cases these resources have not been readily available and the barcodes cannot be assigned to particular species (Pfeiler *et al.* 2012a; Borchers & Marcus 2014; Gemmell *et al.* 2014; Gemmell & Marcus 2015).

Fortunately, the community of researchers interested in the genus has finally resolved some of the enduring taxonomic issues that have plagued the New World *Junonia.* Molecular phylogenetics has determined that *Junonia* and *Precis* are distinct genera and not even sister clades (Wahlberg *et al.* 2005). The genus *Precis* is restricted to Africa (Wahlberg *et al.* 2005) while *Junonia* occurs throughout the world (except Europe and Antarctica), with the greatest species diversity in the tropics (Forbes 1928; Kodandaramaiah & Wahlberg 2007). New types have been established for some existing taxa (Neild 2008). Better species definitions have been published, including the description of previously unidentified cryptic species (Brévignon 2004; Brévignon 2008; Brévignon 2009; Brévignon & Brévignon 2011; Brévignon & Brévignon 2012). While there are probably still at least a few unnamed species within the New World *Junonia* (especially in South America), much of the remaining taxonomic work among the New World members of the genus will be to associate the forms in each geographic region with the appropriate species names. Because there is evidence from a variety of sources that hybridization can and does occur between at least some *Junonia* species (reviewed in (Gemmell *et al.* 2014; Gemmell & Marcus 2015), operationally the Marcus laboratory uses the isolation species concept that defines species as systems of populations such that genetic exchange between these systems is limited or prevented by one or more reproductive isolating mechanisms (Dobzhansky 1970; Templeton 1989)

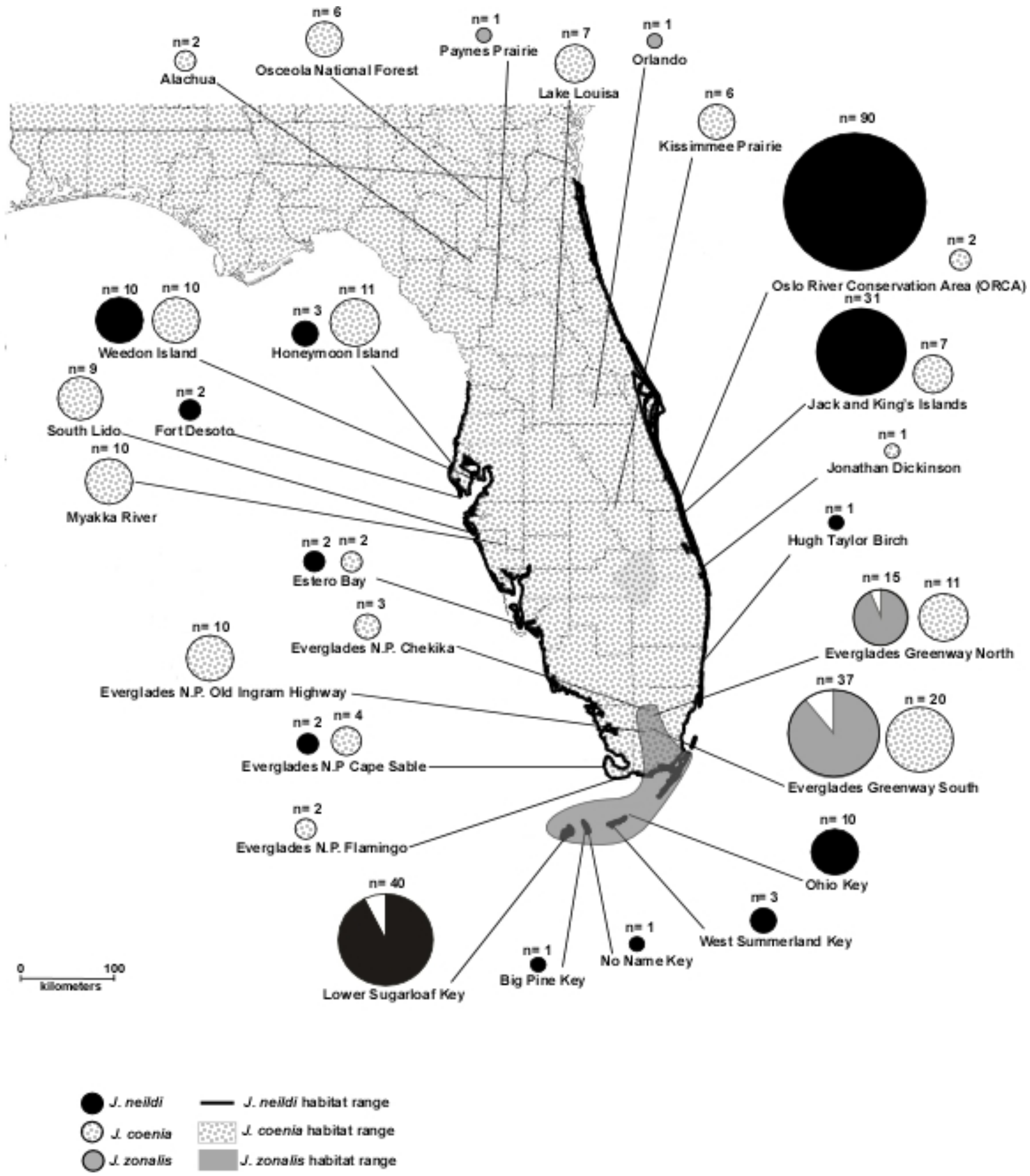
In this paper, I evaluate the 3 *Junonia* species that occur in Florida, USA (Figure 2-1) on the basis of morphology and with mitochondrial *cytochrome oxidase I* (*COI*) haplotypes, as well as nuclear *wingless* (*wg*) sequences, molecular markers with a track record for clarifying the taxonomy and population structure of *Junonia* from South

America and the Caribbean (Borchers & Marcus 2014; Gemmell *et al.* 2014). Prior work has identified two principle *COI* haplotype groups (A and B) in the *Junonia* of the Western Hemisphere (Pfeiler *et al.* 2012a) that differ from each other by 4% sequence divergence (Borchers & Marcus 2014). Haplotype group A is most prevalent in South America (Borchers & Marcus 2014; Gemmell *et al.* 2014), while *Junonia* in North and Central America carry almost exclusively haplotype group B (Gemmell & Marcus 2015). The Caribbean seems to be a zone of genetic admixture and both haplotype groups appear to be common in all Caribbean *Junonia* populations (Gemmell *et al.* 2014; Gemmell & Marcus 2015). Florida is the only region of North America where haplotype group A has been documented (except for 1 specimen from Veracruz, Mexico), but it occurs there at low frequency (Gemmell & Marcus 2015), and is likely the result of gene flow from the Caribbean. In spite of these geographic trends, both haplotype groups can be found in individuals from almost all New World *Junonia* species (Gemmell & Marcus 2015). Since mitochondiral *COI* haplotypes are not species-diagnostic in *Junonia,* data from the variation in the nuclear *wingless* locus is very useful as an additional molecular tool for defining genetically distinct *Junonia* populations and species (Borchers & Marcus 2014; Gemmell *et al.* 2014).

The common buckeye, *J. coenia*, is widespread throughout Florida (Figure 2-2), where it feeds on several species of larval host plants (Table 2-2). In recent years, the other two *Junonia* species found in Florida have generally been referred to as the mangrove buckeye (*J. evarete*) and the tropical buckeye (*J. genoveva*) after Turner and Parnell (1985), but the recent changes in the taxonomy of the South American and Caribbean *Junonia* species introduced uncertainty into the nomenclature for these species

(Gemmell & Marcus 2015). The mangrove buckeye is almost always found in close association with its larval host plant, the black mangrove, *Avicennia germinans* (Glassberg *et al.* 2000), which is largely restricted to coastal areas (Figure 2-2), though occasional strays have been reported from the interior of peninsular Florida (Lotts & Naberhaus 2014). The tropical buckeye is similarly closely associated with its primary larval host plant in Florida, blue porterweed, *Stachytarpheta jamaicensis*, which is not frost-tolerant and is most abundant in Miami-Dade and Monroe Counties in south Florida. Like the mangrove buckeye, occasional strays of the tropical buckeye are found outside of its primary range (Figure 2-2). The common buckeye and mangrove buckeye are resident in Florida, where they have been recognized as distinct from each other by at least some authors since the early 20^{th} Century (Walker 1917), while the tropical buckeye appears to have colonized Florida from the Caribbean (possibly from Cuba or the Bahamas) in recent times (Minno & Emmel 1993; Cech & Tudor 2005; Calhoun 2010). The earliest documented occurrence of the tropical buckeye in Florida is a specimen collected on Key Largo in 1961 (Calhoun 2010), but this species was apparently not abundant or widespread until about 1978 (Scott 1986; Minno & Emmel 1993; Cech & Tudor 2005). It was first recognized in Florida in 1981 when it was very abundant in multiple locations in both the upper and lower Keys, as well as on the mainland in the vicinity of Homestead (Baggett 1982a; Baggett 1982b). Occurrences prior to 1981 were determined from previously captured specimens present in collections. Since 1981, the abundance of tropical buckeyes has varied considerably in Florida (Cech & Tudor 2005), and during the period when the collections for this study were made (2004-2011), this species was most abundant in the vicinity of Homestead in Miami-Dade County and was relatively rare in the Florida Keys.

Figure. 2-2. Map of Florida, USA showing the known distributions of *Junonia* species, collection localities, and *cytochrome oxidase subunit I* (*COI*) haplotype group assignments for the specimens included in this study. The area of each pie graph is proportional to the sample size from each collection locality. Group A *COI* haplotypes are indicated by white areas of each pie graph. Group B *COI* haplotypes are indicated by filled areas of each pie graph. The mangrove buckeye (*J. neildi*) is indicated by black shading, the common buckeye (*J. coenia*) is indicated by light gray shading, and the tropical buckeye (*J. zonalis*) in indicated by dark grey shading.



By comparing the phenotypes and molecular markers of *Junonia* present in Florida with those from elsewhere in North America, South America, and the Caribbean (Gemmell *et al.* 2014), I have been able to determine that the most appropriate nomenclature is to refer to the common buckeye as *J. coenia,* the mangrove buckeye as *J. neildi,* and the tropical buckeye as *J. zonalis* after Brévignon and Brévignon (2012). Clarifying the taxonomy of this fascinating group of butterflies will facilitate further research in all of the disciplines that use *Junonia* as an experimental model.

Materials and Methods

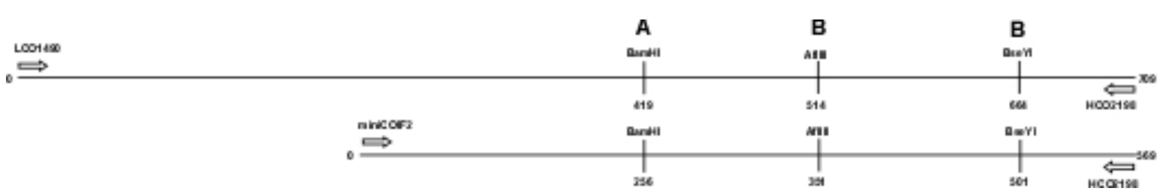
Specimen Collection and Preparation. *Junonia* specimens were collected from the wild using hand-held butterfly nets and stored at 4°C until they were frozen at -20°C. Before being frozen, some individuals of each species were released into plexiglass flight cages and allowed to oviposit on *Plantago lanceolata* or *P. major* before the adults were placed in the freezer. Larvae were reared on *Plantago* at 25°C and a 12h light/12h dark photoperiod until they pupated. Live pupae were weighed using an electronic balance. A few additional wild-caught adult specimens were obtained from other collectors and were shipped to the Marcus laboratory at room temperature before they were frozen at -20°C. Data from a total of 370 wild-caught specimens from Florida (USA) were compared to specimens from Kentucky and Missouri (USA) representing typical North American continental populations; to specimens from Cuba, the Dominican Republic, and Jamaica representing Greater Antilles populations; to specimens from Martinique and Guadeloupe representing Lesser Antilles populations; and to specimens from French Guiana representing mainland South American populations (Brévignon & Brévignon 2012; Gemmell *et al.* 2014). Specimens were identified on the basis of morphological characters (Table 2-2) (Turner & Parnell 1985; Neild 2008; Calhoun 2010; Brévignon & Brévignon 2012).

DNA isolation was accomplished using a single leg from each specimen and the Qiagen DNEasy Blood and Tissue kit either manually or with the assistance of a Qiagen QIAcube extraction robot (Qiagen, Düsseldorf, Germany) using the Animal tissue DNA program, following the manufacturers protocol with modifications as previously described (Gemmell & Marcus 2015). DNA concentration of each sample was evaluated using a Nanodrop 2000 spectrophotometer (Nanodrop, Wilmington, Delaware, USA) and then stored at -20˚C.

Mitochondrial *Cytochrome Oxidase I* (*COI*). *Cytochrome oxidase subunit I* gene products were obtained using polymerase chain reaction (PCR). The earliest amplifications were performed using the *COI* primers Ron (GGA TCA CCT GAT ATA GCA TTC CC) and Nancy (CCC GGT AAA ATT AAA ATA TAA ACT TC) which produce a 479 bp product (including primer sequences, 430 bp without primers) (Monteiro & Pierce 2001). However, in order to maximize the compatibility of the *Junonia COI* data set with those of other researchers, the bulk of the *COI* PCR experiments were performed using the gene specific primers LCO1490 (GGT CAA CAA ATC ATA AAG ATA TTG G) and HCO2198 (TAA ACT TCA GGG TGA CCA AAA AAT CA) (Folmer *et al.* 1994),which yield a 709 base pair product (including primer sequences, 658 bp without primers). PCR reaction conditions were: 95˚C for 5 minutes; 35 cycles of 94˚C for 1 minute, 46˚C for 1 minute, 74˚C for 1 minute, 94˚C for 1 minute; and a final extension for 5 minutes at 72˚C, then a 4˚C hold. Samples were run on a QIAxcel Advanced capillary electrophoresis instrument (Qiagen) fitted with a DNA Screening Cartridge with QX Size Markers (250 bp–4 kb v. 2.0) and QX Alignment

Markers (50 bp-5 kb) using the AL320 electrophoresis method as reported previously (Gemmell & Marcus 2015). If satisfactory bands were detected, they were either sequenced as previously described (Borchers and Marcus 2014) or a diagnostic triple restriction enzyme digest was performed using AflIII, BseYI and BamHI restriction endonucleases (New England Biolabs (NEB), Ipswich, MA, USA) to determine the haplotype of each specimen (Figure 2-3).

Figure. 2-3. Restriction digest map used for the determination of haplotype groups. The top line represents the *cytochrome oxidase subunit I* (COI) amplification product generated with primers LCO1490 and HCO2198. The bottom line represents the smaller amplification product created using primers HCO2198 and miniCOIF2. The specific enzyme cut sites for BamHI, AflIII, and BseYI, are shown using a vertical bar. BamHI restriction sites are found only in haplotype group A alleles whereas AflIII and BseYI restriction sites are found only in haplotype group B alleles. The base position of each cut site in each PCR product is indicated below and the haplotype associated with each cut is indicated above the line.



For the diagnostic restriction enzyme digest 10µL of the PCR product was mixed with 2µL NEB Buffer3, 2µL BSA (10X, 1mg/mL), 4µL deionized distilled water, 0.5µL AflIII, 0.5µL BseYI, and 1 µL BamHI, in a 1.5mL microcentrifuge tube and incubated at 37˚C for 1 hour. Enzyme deactivation was done in a 70˚C water bath for 10 minutes. The digested products were then separated with a QIAxcel Advanced instrument as described above. Haplotypes were assigned by the size of the bands obtained: Haplotype Group A genotypes have a single BamHI cut site that produces 2 bands in this triple digest (419 bp and 290 bp). Haplotype Group B cuts once each with AflIII and BseYI and produces 3 bands in this triple digest (514 bp, 150 bp, 45 bp).

If no PCR products were obtained from the first amplification, they were reamplified using miniCOIF2 (ATA CTA TTG TTA CAG CCT CAT GC) (Gemmell *et al.* 2014) and HCO2198 , yielding a shorter 569 base pair product (including primer sequences, 520 bp without primers). The PCR program and visualization was conducted as described above. These PCR products were then assigned to haplotype groups using the same diagnostic triple restriction enzyme digest as described above, as all enzyme cut sites were also present within this smaller fragment (Figure 2-3). The digested products were then visualized as before. Haplotypes were assigned by the size of the bands obtained: Haplotype Group A produces 2 bands (313 bp and 256 bp) due to the BamHI restriction site, and Haplotype Group B produces 3 bands (351 bp, 218 bp, 68 bp) due to the AflII and BseYI cut sites.

Mitochondrial haplotype assignments based on both DNA sequencing and restriction digest genotyping methods for all of the *Junonia* specimens collected from each collection locality were pooled. Haplotype frequencies for each species and each locality were calculated, and plotted on a map of Florida, USA.

Estimating Gene Flow with the Island Model. Wright (1931) proposed the island model that relates the amount of population differentiation between an island population and a mainland population, as measured by the fixation index (F_{ST}), to the number of migrants per generation, which is equal to the product of the effective population size (N) and the migration rate (m). The relationship between these variables is described by the equation $F_{ST} \simeq 1/(4Nm + 1)$ which is based on the interaction between genetic drift and migration and assumes that gene flow between the 2 populations is at equilibrium (Hutchison & Templeton 1999). F_{ST} can also be calculated from allele frequencies, F_{ST} = variance(p)/[mean(p) (1-mean(p))], where p is the allele frequency of 1 allele in a 2-allele system, in a pair of populations(Holsinger & Weir 2009). Once F_{ST} has been calculated from allele frequencies, the number of migrants per generation Nm can be estimated by algebraic manipulation (King & Lawson 1997). To estimate F_{ST} and Nm for *J. zonalis,* I used the reported *COI* haplotype group A allele frequency of *J. zonalis* of p=0.35 from Cuba (Gemmell & Marcus 2015) and the frequency of the same allele p=0.096 across all of the South Florida *J. zonalis* populations that I sampled (Appendix I).

Nuclear *wingless.* DNA for the nuclear *wingless* (*wg*) locus was isolated using the gene specific primers lepwg1 (GAR TGY AAR TGY CAY GGY ATG TCT GG) and lepwg2 (ACT NCG CRC ACC ATG GAA TGT RCA) (Brower & DeSalle 1998). The reaction volumes were 25 µL with the following reaction conditions: 94°C for 5 min; 40 cycles of 94°C for 1 min, 46°C for 1 min, 72°C for 2 min; a final extension at 72°C for 10 min and a final hold at 4°C. The PCR product obtained was 460 base pairs and was evaluated, sequenced, trimmed, and aligned in the same manner as *COI*. The final products were trimmed to a size of 402 base pairs after primers removal (Borchers & Marcus 2014). The coding sequence of the *Junonia wingless* locus contains a great deal of allelic variation and most individual specimens are heterozygous (Borchers & Marcus 2014; Gemmell *et al.* 2014). Heterozygotes for single nucleotide polymorphisms (SNPs) were identified from the Sanger sequence traces as previously described (Borchers and Marcus 2014) and the genotypes for each variable position for each individual were entered into PHASE v2.1.1 (Stephens & Scheet 2005) and run using the recombination model (MR).

The most likely alleles identified in PHASE v2.1.1 were assigned to each individual and the data entered into GENEPOP v4.0.10 (Rousset 2008), which tests pairs of populations for genic differentiation (Exact *G* test) by determining if alleles of each population were drawn from the same distribution (Raymond & Rousset 1995). GENEPOP settings used for testing all populations were a dememorisation of 1,000, 100 batches, and 1,000 iterations per batch as previously described (Borchers & Marcus 2014). To correct for multiple comparisons, the sequential Bonferroni method (Holm 1979) was used to adjust significance thresholds.

Results

Mitochondrial *Cytochrome Oxidase I* (*COI*). Using the *Cytochrome Oxidase subunit I* gene from 370 Florida *Junonia* samples, haplotype groups were assigned. A total of 3 samples were determined by sequencing PCR products amplified with the gene specific primers Ron and Nancy (Genbank Accessions KR094173 - KR094175), 171 were determined by sequencing LCO1490 and HCO2198 PCR amplifications (KF4191814, KJ469115-KJ469116, and KM288076-KM288247) (previously published in (Brévignon & Brévignon 2012; Mitter 2013; Gemmell & Marcus 2015)), and 196 LCO1490/HCO2198 and miniCOIF2/HCO2198 PCR amplifications were genotyped by restriction enzyme digests. The specimen haplotypes were plotted on a map of Florida (Figure 2-2) according to their collection locality. The mitochondrial haplotype A was found to be restricted to the most southern parts of Florida, occurring only in 2 South Florida populations of *J. zonalis* (frequency 6% and 12%, respectively, 9.6% across both *J. zonalis* populations combined) and in 1 population of *J. neildi* in the Florida Keys (frequency 8%). Except for these populations, haplotype group B was found at 100% frequency throughout the state of Florida in all 3 *Junonia* species (but note that *J. zonalis* was only found as an occasional stray individual except for the 2 populations described above).

Estimating Gene Flow with the Island Model. The fixation index (F_{ST}) between Cuba and South Florida populations of *J. zonalis* was determined to be 0.185. Assuming that gene flow is at equilibrium and no other evolutionary forces are acting on the allele frequences, I calculated an expected 1.09 migrants per generation (Nm) between Cuba and South Florida.

Nuclear *wingless* (*wg*). Full length (402 bp) *wingless* sequences were obtained from 262 *Junonia* specimens (Genbank Accessions KR094177-KR094437). To facilitate population genetic comparisons with a prior study of poorly preserved DNA from Caribbean and South American *Junonia* (Gemmell *et al.* 2014), the *wingless* sequences were also trimmed to a shorter length of 137 bp and then analyzed in combination with with previously published shorter or "mini" *wingless* fragments. The full-length *wingless* sequences contained a total of 69 single nucleotide polymorphic (SNP) sites, whereas the mini *wingless* sequence had 38 SNP sites. PHASE was used to determine the allele combinations present in each individual based on this SNP variation and a total of 158 alleles were found across all of the *Junonia* populations. Assigned alleles for each individual were used for further analysis in GENEPOP (Rousset 2008).

Statistical results for full length *wingless* fragments and "mini" *wingless* fragments were virtually identical, so only the more complete set of comparisons based on and "mini" *wingless* are presented here (Table 2-3). First, populations of each of the 3 *Junonia* species found in Florida are significantly distinct from one other on the basis of allelic variation in *wingless,* reinforcing the interpretation that they are in fact different species.

Second, when compared to populations of suspected conspecifics, *J. coenia* from Florida did not show significant genic differentiation from *J. coenia* elsewhere in North America. Similarly, Florida *J. zonalis* was not genetically distinct from *J. zonalis* in the Caribbean. However, Florida *J. neildi* were significantly genically differentiated from Caribbean *J. neildi*, but this was based on comparison with sequences from a small number of specimens (n=6), all of which were from the Lesser Antilles.

Finally, populations of *J. zonalis* and *J. neildi* were both significantly genetically differentiated from *J. evarete, J. genoveva,* and (in the case of *J. neildi*) *J. litoralis*, suggesting that these South America populations, which had historically been considered as conspecifics with the Florida taxa by some authorities, are actually different species.

Table 2-3. Results of the population genetic analysis test for genic differentiation between *Junonia* populations carried out using nuclear *wingless* (*wg*) alleles. Tests were corrected for multiple comparisons with the sequential Bonferroni method (Holm 1979).

Species	Chi-Squared Value	p-Value[a]	Sequential Bonferroni Threshold Value	Significant Differentiation
Comparisons among Florida *Junonia*				
J. coenia Florida (n=69) X *J. neildi* Florida (n=127)	∞	0	0.00833	yes
J. coenia Florida (n=69) X *J. zonalis* (n=39)	17.56083	0.00015	0.0125	yes
J. neildi Florida (n=127) X *J. zonalis* (n=39)	∞	0	0.00833	yes
Comparisons between Florida *Junonia* and suspected conspecifics				
J. coenia Florida (n=69) X *J. coenia* KY & MO[b] (n=9)	4.89189	0.08664	0.05	no
J. zonalis (n=39) X *J. zonalis* Caribbean (n=34)	6.46205	0.03952	0.025	no
J. neildi Florida (n=127) X *J. neildi* Caribbean[c] (n=6)	∞	0	0.00833	yes
Comparisons between Florida *Junonia* and suspected heteropecifics				
J. zonalis Florida (n=39) X *J. genoveva* FG[d] (n=30)	∞	0	0.00833	yes
J. zonalis Florida (n=39) X *J. evarete* FG[d] (n=3)	12.61721	0.00182	0.01667	yes
J. neildi Florida (n=127) X *J. genoveva* FG[d] (n=30)	17.73148	0.00014	0.01	yes
J. neildi Florida (n=127) X *J. evarete* FG[d] (n=3)	∞	0	0.00833	yes
J. neildi Florida (n=127) X *J. litoralis* FG[d] (n=3)	∞	0	0.00833	yes

[a]Degrees of freedom = 2
[b]Kentucky and Missouri, USA
[c]All Caribbean samples of *J. neildi* used in this comparison are from the lesser Antilles
[d]French Guiana

Discussion

Morphology and Taxonomy. *Junonia evarete* and *J. genoveva* were originally described from the mainland of South America (Cramer 1775; Cramer 1780), and based on morphological characteristics alone, for a long time, it was not certain whether the forms in Florida actually corresponded to the taxa known from South America (Calhoun, 2010; Gemmell *et al.*2014; Gemmell & Marcus 2015; Hafernik 1982; Schwartz 1987; Tilden 1970). Morphologically all of these buckeye butterflies share some similarities. All have one small and one large eyespot on the dorsal surface of the forewing, two large eyespots on the dorsal surface of the hindwing, as well as changes in ground color and the degree of transverse stripe development on the ventral hindwings that vary dependent on season (Brakefield & French 1993; Forbes 1928; Minno & Emmel 1993; Paulson 1996; Tilden 1970). The distinguishing morphological features for each of the three *Junonia* species can be found in Table 2-1, and careful examination of these features, in combination with insights from molecular analysis have allowed me to conclude that the taxa in Florida are *J. coenia* (common buckeye), *J. neildi* (mangrove buckeye), and *J. zonalis* (tropical buckeye). This is in agreement with the taxonomic hypotheses established previously based on morphology and host plant use for the *Junonia* of the Lesser Antilles (Brévignon & Brévignon 2012).

Nuclear Genetic Variation. Allelic variation in nuclear *wingless* sequences has proven to be of considerable value as an aid in resolving uncertainties of species designations (Table 2-3). All three species of *Junonia* in Florida were found to have significantly different gene sequences from one another, confirming the hypothesis that three separate species do exist here. Comparison between Florida *J. coenia* and other North American *J. coenia* populations were found to be conspecific, which was expected as the identity of this particular species has not been disputed (Table 2-3).

The mangrove buckeye nuclear *w*ingless sequences from Florida, USA were compared to proposed conspecifics *J. evarete* (Turner & Parnell 1985), *J. genoveva* (Neild 2008), and the mangrove feeders *J. litoralis* from French Guiana and *J. neildi* from the Lesser Antilles (Brévignon & Brévignon 2012). The Florida mangrove buckeyes were found to be significantly different from all 4 species (Table 2-3). However, based on morphology and colour pattern, the Florida mangrove buckeye is very similar to *J. neildi* from the Lesser Antilles and the *wingless* comparison was based on a very small number of Caribbean samples (n=6). *Wingless* sequences from additional *J. neildi* samples, especially from the Greater Antilles (including Hispaniola, Cuba, Puerto Rico and the Bahamas) may be able to further reinforce my tentative conclusion based on morphology and colour pattern that the Florida mangrove buckeye is conspecific with *J. neildi.*

Similarly, the Florida tropical buckeye was compared with proposed conspecifics *J. evarete* (Turner & Parnell 1985) and *J. genoveva* (Neild 2008) from French Guiana, and with *J. zonalis* (Brévignon & Brévignon 2012) from Cuba and Jamaica (Table 2-3). It was determined that the Florida tropical buckeye is distinct from *J. evarete* and *J. genoveva*, but is not genetically distinct from *J. zonalis,* suggesting that these populations are conspecific. By clarifying the correct species identifications for Florida *Junonia* species, I hope to establish a stable basis on which future research progress can be built. Previous work, which either had too limited taxonomic sampling (Kodandaramaiah & Wahlberg 2007; Kodandaramaiah 2009) or placed too much emphasis on mitochondrial genotyping (Brévignon & Brévignon 2012; Pfeiler *et al.* 2012a; Gemmell *et al.* 2014; Gemmell & Marcus 2015) was insufficient for this purpose.

Mitochondrial Variation. In Florida, the vast majority of *Junonia* butterflies (≥ 88%), regardless of species, carry alleles from haplotype group B (Gemmell & Marcus 2015). This makes the Florida forms very unlike *Junonia* forms from South America that putatively belonged to the same species, but which carried haplotype group B at a maximum frequency of 15% (Pfeiler *et al.* 2012a; Gemmell & Marcus 2015). However, the Caribbean *Junonia* populations had intermediate haplotype group frequencies, apparently allowing invading *J. zonalis* to transport group A haplotypes to south Florida (Gemmell & Marcus 2015). The appearance of haplotype A alleles occurs in the contemporary Florida samples analyzed appear in only two (*J. neildi* and *J. zonalis*) of the three species in south Florida (Figure 2-2) and at a low frequency. Haplotype group A is only found in *J. neildi* populations on Lower Sugarloaf Key, although samples for this species were only available for few populations in Florida Keys (Lower Sugarloaf Key, Big Pine Key, No Name Key, West Summerland Key, and Ohio Key; Figure 2-2).

Junonia zonalis appears in the data set from two populations found near Homestead, Florida (Everglades Greenway North and Everglades Greenway South; Figure 2-2). The Marcus lab was not able to locate populations of *J. zonalis* in the Florida Keys in spite of considerable time in the field, suggesting that this species was relatively rare in the Florida Keys during the sampling period. Based on mitochondrial haplotype allele frequencies in *J. zonalis* in Florida (9.6% haplotype group A; this study) and in Cuba (35% haplotype group A; Gemmell & Marcus 2015), and assuming equilibrium, the number of migrants per generation (Nm) between Cuba and South Florida was calculated to be 1.09 individuals. *Junonia zonalis* is active year-round and has 5 or more generations per year (Turner & Parnell 1985), suggesting that a similar number of migrants between Cuba and South Florida would be necessary to maintain haplotype group A in Florida to counteract the tendency of genetic drift to remove this rare allele from the population. Since mitochondrial haplotypes are maternally inherited (McCullagh & Marcus 2015), only migrant females make a contribution to allele frequencies in future generations, so the actual migration rate (including both males and females) is likely greater than what has been calculated here. Other butterfly species native to Cuba, but not native to Florida, periodically disperse to South Florida in substantial numbers (tens to many hundreds) (Minno & Emmel 1993), suggesting that this may be a realistic scenario.

Future directions. This study provides much needed clarity to the taxonomy of the *Junonia* from South Florida. It also provides details about the genotypes present in Florida *J. zonalis,* suggesting that this species is qualitatively different from the other two Florida *Junonia* species, consistent with its recent arrival from the Caribbean. It would be fascinating to make a more detailed examination of Florida *J. zonalis* throughout its invasion history in order to more fully reconstruct the temporal and spatial dynamics of this biological invasion.

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Chapter 3: Entomological Time Travel: Reconstructing the Invasion History of the Buckeye Butterflies (genus *Junonia*) from Florida, USA

Abstract

Invasion biology focuses on the process by which non-native species integrate into new habitats. Three species of buckeye butterflies (genus *Junonia*) currently reside in Florida, USA, including *Junonia coenia* (polyphagous and widely distributed), *J. neildi* (monophagous and restricted to coastal areas), and *J. zonalis* (polyphagous tropical species restricted to frost-free south Florida). Two species have long been resident in Florida, whereas *J. zonalis* was first detected in Florida in 1981. Diagnostic morphological and molecular markers exist for determining *Junonia* with Caribbean ancestry, allowing observation of the invasion and creation of a secondary contact zone over space and time. I developed techniques to quickly, inexpensively, and unambiguously determine mitochondrial genotypes from museum specimens collected in Florida and the Caribbean over the last 150 years. I reconstructed the invasion of *J. zonalis* as it colonized Florida using 798 museum specimens from 1865 to 2015, including the oldest insect museum specimens genotyped to date. Significant correlations were found between the presence of Caribbean genotypes and latitude, longitude, and time. *Junonia zonalis* colonized Florida from Cuba by the 1930s, followed by hybridization with resident species, with ongoing gene flow between Cuba and the Florida Keys. Episodic gene flow between the Keys and mainland Florida populations of *J. zonalis* may also be occurring. Mainland mitochondrial genotypes appear to be more resilient than Caribbean genotypes at extreme high and low temperatures. Although *Junonia* is not an agricultural pest, this time series can be used as a model for understanding the behavior of other insect invasion events.

Introduction

Invasion biology has been a topic of scientific interest for at least the last 200 years (Reichard & White 2003; Falk-Petersen *et al.* 2006; Davis 2009). It encompasses phenomena such as invasion events by non-native species, naturally occurring long-range dispersal events, human-mediated dispersal events, adaptive radiation, speciation, the creation of secondary contact zones, and hybridization events (Mooney & Cleland 2001; Didham *et al.* 2005; Stigall 2010; Flohr *et al.* 2013). Invasive species have been of great concern as they have the potential to outcompete native species for resources and habitat (Mooney & Cleland 2001; Didham *et al.* 2005; Falk-Petersen *et al.* 2006). If sizeable populations are established, the invasive species may expand their range, overtake other habitats, and colonize new ecological niches; this is known as adaptive radiation (Reichard & White 2003; Falk-Petersen *et al.* 2006; Flohr *et al.* 2013).

There are also occurrences where invasive species do not outcompete the native populations of related species for resources or habitat, but may potentially hybridize with them if the species are genetically similar enough for this to occur, and no effective reproductive isolation mechanisms exist (Mayr 1963; Mooney & Cleland 2001; Didham *et al.* 2005; Falk-Petersen *et al.* 2006). Hybridization events may introduce genes into the gene pool of the native species that may be beneficial and increase the fitness of hybrids, or detrimental and decrease fitness (Anderson & Hubricht 1938; Mooney & Cleland 2001; Aliabadian *et al.* 2005; Durand *et al.* 2009; Stigall 2010). Gene flow can also occur in the opposite direction, with the invader incorporating genetic material from the native species by hybridization and introgression, and in some cases, this contributes to the success of the invader (Arnold 2004). This zone of hybridization is referred to as a secondary contact zone (Aliabadian *et al.* 2005; Durand *et al.* 2009). Hybridization events between species may also initiate speciation events under some circumstances (Mayr 1963; Mooney & Cleland 2001; Stigall 2010).

Molecular techniques are important tools in any field of study where one would like to assess the genetic structure of an organism or group of organisms. For fresh samples or ones that are suitably preserved using recommended storage methods, obtaining nuclear DNA to do analysis is a relatively easy task (Mandrioli *et al.* 2006). For older samples, such as those found in many museum collections, which have been preserved using various methods, possibly with compounds unfavourable to DNA preservation, the task of isolating useable nuclear DNA is sometimes not as easily accomplished (Dillon *et al.* 1996; Mandrioli *et al.* 2006; Watts *et al.* 2007). As specimens age, the quality of the DNA in general degrades and becomes fragmented (Mandrioli *et al.* 2006; Watts *et al.* 2007; Strange *et al.* 2009). This problem is particularly acute for studies of nuclear DNA, as copy numbers are low to begin with (Watts *et al.* 2007), and when the amount of preserved tissue is small as is the case in small-bodied organisms like insects. It has been found that some fumigants commonly used in the past in museum collections can prevent reliable PCR amplification from nuclear DNA after less than a year of exposure (Espeland *et al.* 2010). As a practical matter, nuclear DNA from pinned insect specimens that have been stored at room temperature in museum collections can be PCR amplified and sequenced using the conventional Sanger method with some hope of success for about 50 years, but the likelihood of successful PCR and sequencing declines over time and becomes very uncertain when specimens have been in storage for 20 years or longer (Marcus *et al.* In prep.). Beyond 20 years in storage, obtaining usable nuclear

DNA (usually short fragments or microsatellites) is dependent on the way in which specimens have been preserved (Watts *et al.* 2007).

The mitochondrion, the primary energy processing organelle of the eukaryotic cell, contains numerous copies of its own DNA and many mitochondria are found in each cell (Avise 2000; Zink & Barrowclough 2008). Mitochondrial DNA (mtDNA) is passed solely from female to offspring with no contribution from the males in most animal species, allowing easy identification of matrilineages (Avise 2000; Zink & Barrowclough 2008). It has been used routinely in many areas of study including the DNA barcode project, which employs a portion of the mitochondrial *cytochrome oxidase subunit I* gene to distinguish different species (Hebert *et al.* 2003; Janzen *et al.* 2005; Hajibabaei *et al.* 2006; Ratnasingham & Hebert 2007). Mitochondrial DNA (mtDNA) occurs in higher copy number than nuclear DNA in most cells (Watts *et al.* 2007), which is useful when trying to recover DNA from older specimens. Obtaining usable mtDNA from museum specimens has been achieved in many population genetics based studies and satisfactory recovery of mtDNA has been documented from insect specimens collected as long ago as the 1870s (Goldstein & Desalle 2003; Strange *et al.* 2009; Saarinen & Daniels 2012; Heintzman *et al.* 2014; Wells *et al.* 2015).

Historical museum specimens have been used to explore questions relating to various population genetics and evolutionary questions in both vertebrates and insects (Goldstein & Desalle 2003; Harper *et al.* 2006; Winston 2007; Habel *et al.* 2009; Saarinen & Daniels 2012; Keyghobadi *et al.* 2013; Heintzman *et al.* 2014). Vertebrate specimens are often relatively large, so tissues are abundant and getting sufficient tissue for analysis has not been an issue, but the quality of the DNA can be dependent on preservation technique (Bouzat *et al.* 1998; Iudica *et al.* 2001). For many insects, specimens are small and little tissue is available for analysis; a partial leg or other small body part is often the sole tissue available for genetic analysis as preservation of the remainder of the specimen may be required for proper identification by morphological characteristics (Watts *et al.* 2007). DNA quality recovered from preserved insect specimens is also dependent on preservation method and storage conditions (Dillon *et al.* 1996; Watts *et al.* 2007) and on the amount of time specimens have been in storage (Watts *et al.* 2007; Heintzman *et al.* 2014). To date, much of the work using historical insect museum specimens has focused on comparing genetic variation from historical populations over time and with comparing genetic variation in historical and extant populations (Goldstein & Desalle 2003; Harper *et al.* 2006; Habel *et al.* 2009).

Here historical mitochondrial DNA recovered from museum specimens will be employed to study biological processes related to invasion biology in the butterfly genus *Junonia* (buckeye butterflies). *Junonia* is already a valuable model in many biological fields of research including evolutionary developmental biology (especially of wing colour patterns such as eyespots), plant-insect interactions, hybrid zones, ring species, and other ecological and evolutionary processes (Bowers 1984; Camara 1997; Wahlberg *et al.* 2005; Kodandaramaiah & Wahlberg 2007; Wahlberg & Wheat 2008; Knerl & Bowers 2013; Gemmell *et al.* 2014). *Junonia* are attractive, conspicuous, and abundant group of butterflies, that are well-represented in many insect collections, including specimens dating back to the 18th century (Linnaeus 1758). For the New World species of *Junonia,* abundant specimens are available in museum collections for at least the last 100 years with few gaps.

Within Florida, USA, there are currently three species of buckeye butterflies; the common buckeye (*Junonia coenia*), the mangrove buckeye (*J. neildi*), and the tropical buckeye (*J. zonalis*). *Junonia zonalis* was absent from Florida until the mid-20th century when specimens began appearing in Florida (presumably migrants from Caribbean populations of this species), and has since established breeding populations in the frost-free regions of South Florida where its preferred larval host plant blue porterweed (*Stachytarpheta jamaicensis*) can survive (Minno & Emmel 1993; Glassberg *et al.* 2000). There are several locations in the Florida Keys where *J. zonalis* and one or both other species co-exist at the same localities (Minno & Emmel 1993; Glassberg *et al.* 2000). This invasion event has created a secondary contact zone between *J. zonalis* and each of the two resident *Junonia* species. The creation of this secondary contact zone is recent and specimens from the entire period of colonization are available from museum collections (Gemmell *et al.* 2014; Gemmell & Marcus 2015). The source population for *J. zonalis* now found in Florida has been widely suspected to be Cuba (Minno & Emmel 1993; Cech & Tudor 2005; Calhoun 2010), but this species has been collected frequently and repeatedly in Key Largo, suggesting that the nearby Bahamas is another possible source of migrants.

Two working hypothesis have been proposed to explain the arrival of *J. zonalis* in Florida. The first is that *J. zonalis* arrived in South Florida in a single invasion event from the Caribbean (Minno & Emmel 1993), carrying with it mitochondrial genotypes common in the Caribbean, but rare in the North American mainland (haplotype group A (Pfeiler *et al.* 2012)). Based on limited prior sampling, haplotype A occurs in Cuba at a frequency of about 35% (Gemmell & Marcus 2015), while haplotype A frequency in the

Bahamas had not been estimated prior to this study. Hybridization with congeners in South Florida combined with the process of genetic drift is expected to cause the frequency of Haplotype A to decrease over time in Florida populations of *J. zonalis* (Chapter 2; Gemmell & Marcus 2015). The second hypothesis is that the invasions are episodic (Cech & Tudor 2005). In an episodic invasion one would expect to see multiple and periodic influxes of A haplotypes over time, perhaps followed by dilution by hybridization with resident *Junonia* and gradual loss of the rarer allele due to genetic drift, leading to what would look like an oscillating pattern of allele frequencies over time.

The genus *Junonia* is a good system for studying invasion biology and its effects on native species because museum collections of the invading species and of its congeners cover the South Florida region over an extended period of time, allowing us to reconstruct the recent biogeographic history of this group; before, during and after invasion. I have adapted molecular based approaches to the available museum material to generate species distribution maps and biogeographic population genetics data over time and space. I will be focusing specifically on the frequency of Caribbean mitochondrial haplotype A alleles in each of the 3 *Junonia* species, to provide insights into patterns of invasion and gene flow between the three species of *Junonia* in Florida (which otherwise carry mitochondrial alleles belonging to North American *Junonia* haplotype group B; Chapter 2; Gemmell & Marcus 2015). Diagnostic morphological (Chapter 2: Table 2-1) characteristics exist for determination of species and molecular markers exist for determination of the mitochondrial haplotypes for this genus. Having this biogeographic history allows for the study of the different stages of the process of colonization, dispersal and interactions with closely related native species (including hybridization within a taxonomic) group that are integral to the successful establishment of an invading species.

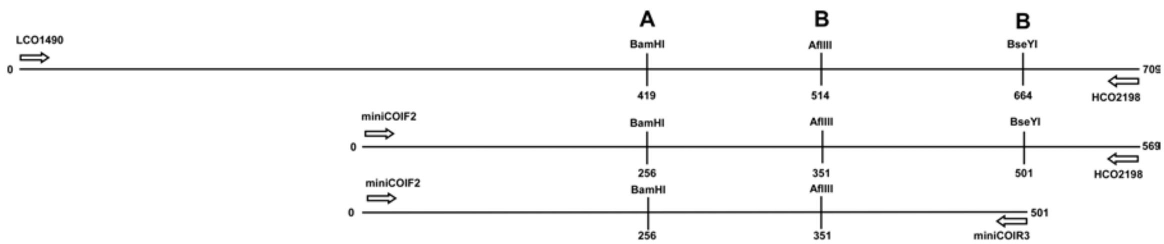
Materials & Methods

Specimen Collection and Preparation. Specimens were chosen based on specific geographic location (South Florida, Cuba, Bahamas), species identification (*J. coenia*, *J. neildi*, and *J. zonalis*), and date of collection. In addition to specimens collected by members of the Marcus laboratory for previous studies, (Chapter 2; (Gemmell & Marcus 2015)), additional specimens were obtained from both museum collections and private collectors (Appendix II). Each specimen was identified to species on the basis of morphological characters (Chapter2; Table 2-2). DNA was isolated from a single leg from each specimen with the Qiagen DNEasy Blood and Tissue kit, either manually or with the assistance of a Qiagen QIAcube extraction robot (Qiagen, Düsseldorf, Germany) using the Animal tissue DNA program, following the manufacturers protocol with modifications as previously described (Chapter 2; (Gemmell & Marcus 2015)), plus the replacement of the Qiagen DNEasy lysis buffer with “mouse tail-tip” lysis buffer (1% SDS, 0.1M NaCl, 0.1M EDTA, 0.05M Tris and deionized distilled water) prepared in the laboratory in order to increase the recovery of extracted DNA. Sample DNA concentrations were evaluated using a Nanodrop 2000 spectrophotometer (Nanodrop, Wilmington, Delaware, USA) and then stored at -20˚C.

Mitochondrial *Cytochrome Oxidase I* (*COI*). The barcode region of the *Cytochrome oxidase subunit I* (*COI*) gene was amplified by polymerase chain reaction (PCR). The amplification of the *COI* gene products used the gene specific primers LCO1490 (GGT CAA CAA ATC ATA AAG ATA TTG G) and HCO2198 (TAA ACT TCA GGG TGA CCA AAA AAT CA) (Folmer *et al.* 1994) which yield a 709 base pair (bp) product (including primer, 658 bp with primers sequences removed). PCR reaction conditions were: 95˚C for 5 minutes; 35 cycles of 94˚C for 1 minute, 46˚C for 1 minute, 74˚C for 1 minute, 94˚C for 1 minute; and a final extension for 5 minutes at 72˚C, then a 4˚C hold. Visualization of amplification products from samples were run on a QIAxcel Advanced capillary electrophoresis instrument (Qiagen) fitted with a DNA Screening Cartridge with QX Size Markers (250 bp–4 kb v. 2.0) and QX Alignment Markers (50 bp-5 kb) using the AL320 electrophoresis method as reported previously (Gemmell & Marcus 2015). If satisfactory bands were detected, a diagnostic triple restriction enzyme digest was performed using AflIII, BseYI and BamHI restriction endonucleases (New England Biolabs (NEB), Ipswich, MA, USA) to unambiguously determine the haplotype group of each specimen as previously described (Gemmell & Marcus 2015)(Figure 3-1). These protocols for PCR and visualization of PCR products and restriction digests were used for all experiments described here, unless otherwise specified.

The diagnostic restriction enzyme digest was performed using 10 µL of the PCR product mixed with 2 µL NEB Buffer 3, 2 µL BSA (10X, 1 mg/mL), 4 µL deionized distilled water, 0.5 µL AflIII, 0.5 µL BseYI, and 1 µL BamHI, in a 1.5 mL microcentrifuge tube, and was then incubated at 37˚C for 1 hour. Enzymes were deactivated for 10 minutes in a 70˚C water bath. The digested products were then

Figure 3-1. Restriction digest map used for the determination of haplotype groups from *cytochrome oxidase subunit I* (*COI*) amplification products generated from PCR using the primers LCO1490 and HCO2198, miniCOIF2 and HCO2198, and miniCOIF2 and miniCOIR3. To determine haplotype group, the specific enzyme cut sites for BamHI, AflIII, and BseYI are shown using a vertical bar. Haplotype group A alleles only contain the cut site for BamHI, while Haplotype group B alleles contain cut sites for both AflIII and BseYI. The haplotype associated with each cut is shown above the vertical line and the position of the cut site is shown below it.



resolved using a QIAxcel Advanced instrument as described above for evaluating PCR products. Haplotypes were assigned based on the size of the DNA fragments obtained: Haplotype Group A specimens have a single BamHI cut site, which produces 2 bands in this triple digest (419 bp and 290bp). Haplotype Group B cuts once each with AflIII and BseYI and produces 3 distinct bands (514 bp, 150 bp, 45 bp).

If no PCR products were obtained from the LCO1490/ HCO2198 amplification, they were reamplified using miniCOIF2 (ATA CTA TTG TTA CAG CCT CAT GC) and HCO2198, yielding a shorter 569 bp product (with primers and 520 bp with primers excluded) (Gemmell & Marcus 2015). The individual PCR products were then assigned to haplotype groups utilizing the same diagnostic triple restriction enzyme digest as described above, as all enzyme cut sites are present within the smaller PCR fragment (Fig 3-1). The digested products were then visualized as described above. Haplotypes were assigned on the basis of the size of the bands obtained: Haplotype Group A with 2 bands (313 bp and 256 bp) due to the BamHI restriction site, and Haplotype Group B with 3 bands (351 bp, 218 bp, 68 bp) due to the AflII and BseYI cut sites (Fig. 3-1).

If no PCR products were obtained from the miniCOIF2/ HCO2198 amplification they were reamplified using miniCOIF2 and miniCOIR3 (TAT TTC GAT CTG TTA AAA GTA TAG) (Gemmell & Marcus 2015) using the DNA from the miniCOIF2/LCO1490 amplification as the template, which yields a 501 base pair product (including primers, 454 bp without primers). The shorter amplification product produced in these experiments does not include the BseYI restriction site so digests of the PCR products did not include BseYI (and 0.5 µL ddH2O was added to the digest instead). The digested products were visualized as above, and haplotypes were assigned based on band sizes obtained: Haplotype Group A produces 2 bands (256 bp and 245 bp) due to the BamHI restriction site, and Haplotype Group B produces 2 bands (351 bp and 150 bp) due to the AflIII cut site.

Haplotype Frequency Changes in Space and Time. Samples were sorted into collection localities by species. A small number of specimens that lacked collection dates from the Gemmel & Marcus (2015) data set were excluded from analyses using date of collection. For each locality, the total numbers of haplotype A and haplotype group B were tallied for each species. Pie charts were created using Illustrator CS6 (Adobe, San Jose, CA, USA), for the proportion of A and B haplotypes. The area of each pie graph was standardized and made proportional to the total sample size from each collection locality. Pie charts were added to a template map of South Florida (Pilsbry 1946) and were positioned according to locality using Canvas 14 software (ACD Systems, Seattle, WA, USA). Species ranges were added to the map based on specimens collected by the Marcus lab (Chapter 2), data from specimens from museum collections, and published reports.

Haplotype frequency graphs were generated using Microsoft Excel (Redmond, WA, USA) using the proportion of haplotype A for each species. Data sets where sorted by place (mainland Florida, the Florida Keys, Cuba, and Bahamas), by species, and then by year. The proportion of haplotype A for each decade (eg. 1950-1959) were then calculated and plotted. Standard error for each percentage was calculated using standard methods by taking the square root of ((proportion A*(1-proportion A))/total sample size) for each decade (Stuart 1963).

Yearly mean, maximum, and minimum temperatures from the Florida Mainland were compiled using data compiled for Homestead, Florida from Homestead Air Force Base (Weather Source 2009a), Homestead General Aviation (Weather Source 2009c), and Homestead Experimental Station (Weather Source 2009b) weather stations. Yearly temperature data from the Florida Keys was compiled from Tavernier, Florida (Weather Source 2009d) and Key West International Airport (Schmidt 2016) weather stations. Temperature graphs were generated using Microsoft Excel.

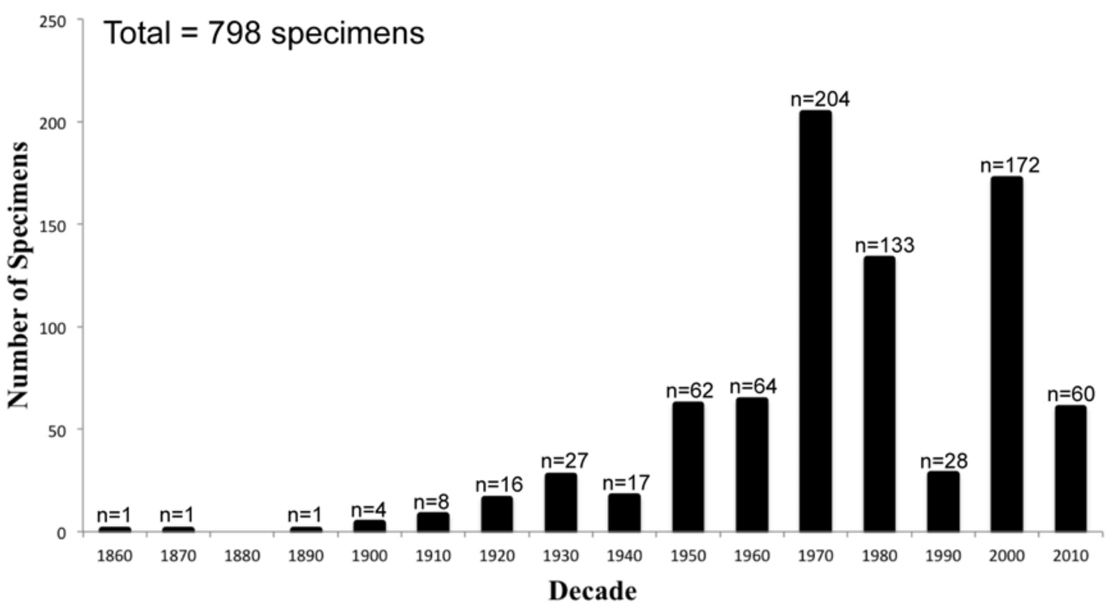
Statistical Analysis. To test whether there are significant differences in geographic variation per species in the abundance of haplotype A, point biserial correlations

((Kornbrot 2014); used to test for correlation between a binary and continuous variable) were performed using both latitude and longitude (GPS coordinates from each collection locality were converted to decimal degree format) as continuous variables and haplotype A as the binary variable (0=absent, 1=present), with each specimen analyzed as an independent data point. A single sample from Alachua County Florida was considered a stray and was not included in this analysis. Three early specimens (1875, 1894 and 1919) were also excluded due to uncertain locality data. The significant geographic trends detected in this analysis suggested that the data for each species should be subdivided by whether they were caught on the Florida mainland or in the Florida Keys for further statistical analyses. To test whether there were significant changes in the frequency of haplotype A over time, separate point biserial correlation analyses were conducted using year as a continuous variable and using the presence of absence of haplotype A as a binary variable for each species in the Florida Keys and in mainland South Florida. In addition, to test the hypothesis that the much lower air temperatures that sometimes occur on mainland Florida are responsible for limiting the frequency of haplotype A there, point biserial correlations were performed with annual maximum, minimum and mean temperatures (from the sources described above) as continuous variables and haplotype A as the binary variable.

Results

A total of 798 specimens were evaluated including 310 *J. coenia,* 265 *J. neildi*, 181 *J. zonalis,* and 42 hybrids. Specimens from South Florida (635), Cuba (111), and the Bahamas (52), spanning the years 1866-2015 were genotyped. The temporal distribution of the collection dates is displayed in Figure 3-2. The major decline in the frequency of *Junonia* collections in South Florida in the 1990s is a consequence of a well-publicized butterfly poaching case in 1992 that included a few specimens caught illegally in South Florida (Kral 1996; Laufer 2010). After that case became public, it became very difficult to obtain collecting permits and there was aggressive deterrence of collectors without permits for any public lands in South Florida and especially in the Florida Keys for a period of about 10 years (Laufer 2010). By the mid-2000s regulatory authorities became willing to authorize collecting permits for specific taxa and the Marcus laboratory was able to obtain permits to collect *Junonia* in many South Florida jurisdictions.

Figure 3-2. Histogram showing the sampling of *Junonia* from South Florida, Cuba, and the Bahamas over time analyzed for this study including museum specimens and specimens from contemporary collections.



Geographic Patterns of *Junonia* Haplotypes. The distribution of *Junonia coenia* in South Florida is shown in Figure 3-3 This species is found throughout South Florida, but with reduced abundance in the Florida Keys. The frequency of haplotype A increases in southern populations of this species, as revealed by a significant negative point biserial correlation between latitude and the presence of haplotype A (Table 3-1). With the exception of rare strays (Chapter 2), the distribution of *Junonia neildi* (Figure 3-4) is restricted by the distribution of its larval host plant, black mangrove (*Avicennia germinans*), which occurs primarily in coastal habitats. There is no correlation between latitude and the presence of haplotype A in South Florida (Table 3-1), but extensive contemporary sampling in Central and North Florida (n=214; Chapter 2; not included in the current statistical analysis to make the treatment of this species consistent with the other *Junonia* species) shows that haplotype A is completely absent from *J. neildi* populations in those regions. Therefore in *J. neildi,* haplotype A alleles are far more common in South Florida than in the rest of the Florida distribution of this species. A significant correlation between longitude and haplotype A was detected in J. *neildi* populations (Table 3-1), showing that the abundance of haplotype A increases from west to east in South Florida.

The distribution of *J. zonalis* (Figure 3-5) is almost exclusively restricted to frost-free regions of Florida where its preferred larval host plants persist, with the exception of a small number of recent strays (Chapter 2, Figure 2-2) and a single specimen

Figure 3-3. Map of South Florida, USA showing the distributions of *Junonia coenia* using collection localities, and *cytochrome oxidase subunit I* (*COI*) haplotype group assignments from this study. For this and all subsequent map figures, the area of each circle is proportional to the number of samples from each locality. Haplotype group A is represented by the grey areas in each pie graph, while haplotype group B is represented by the black areas.

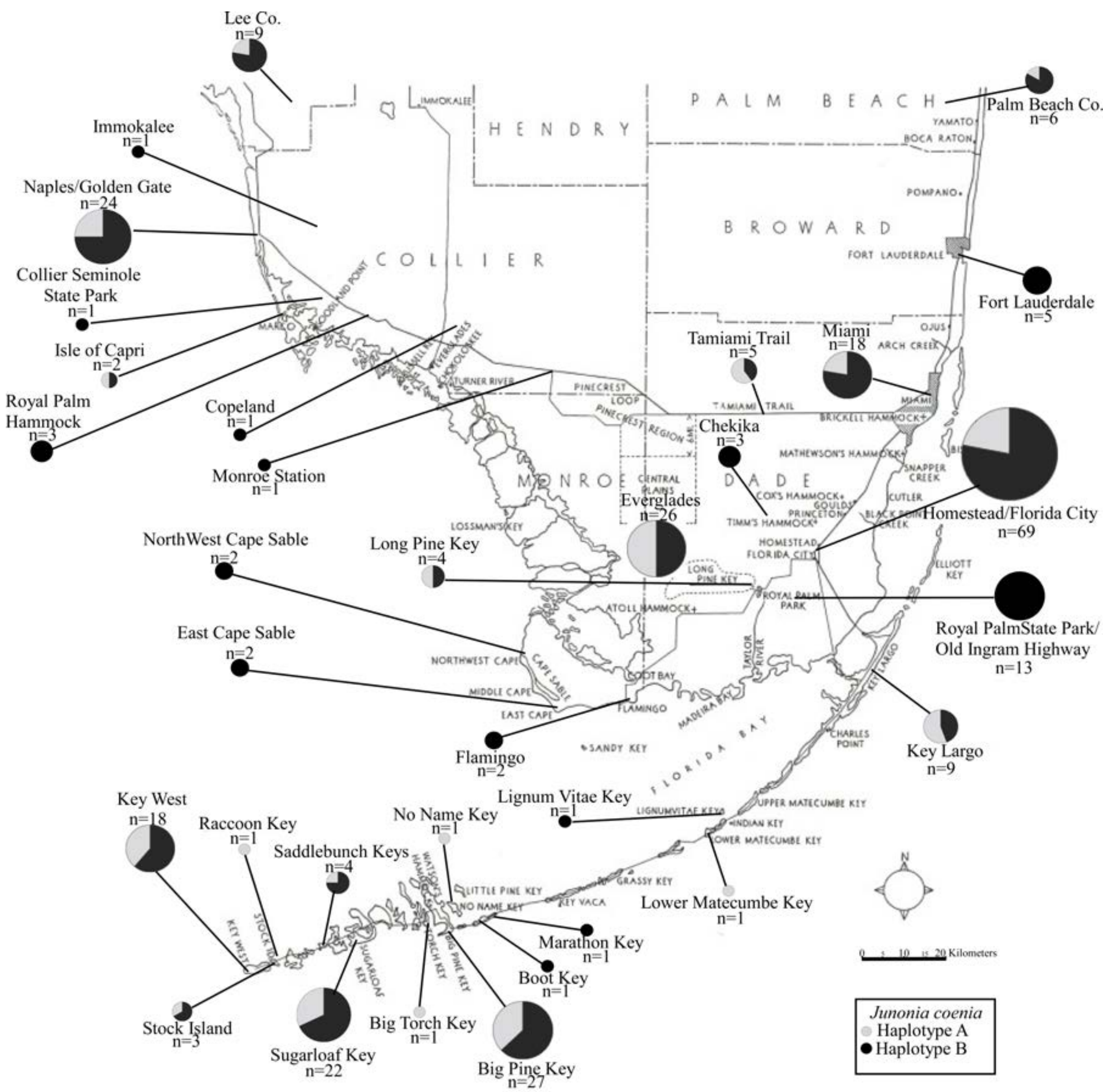
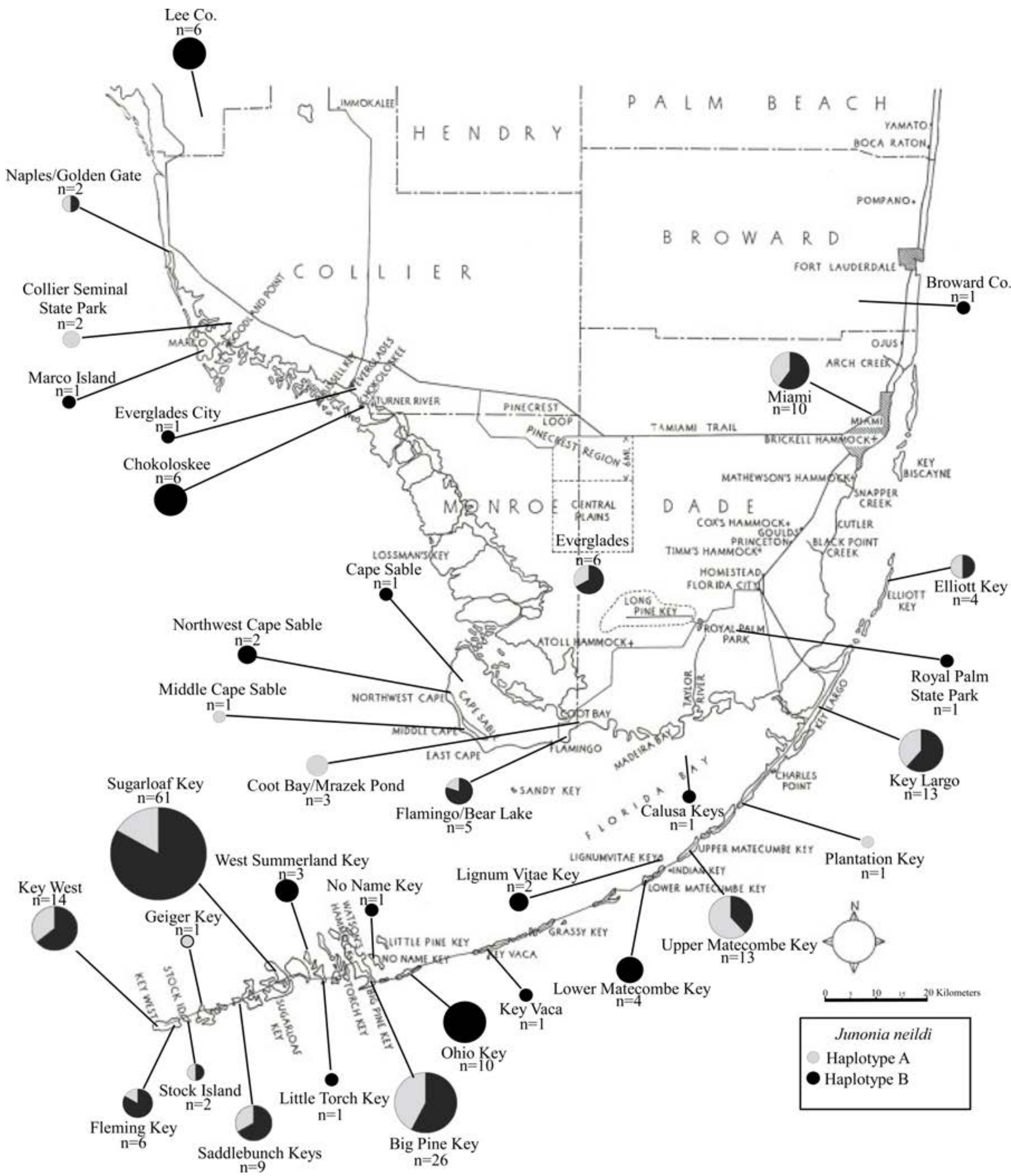


Table 3-1. Point biserial correlations between latitude or longitude and the presence of mitochondrial haplotype A for each of the 3 *Junonia* species in South Florida, USA, including samples from both the South Florida mainland and the Florida Keys.

	r	d.f.	p
J. coenia latitude	-0.136	261	0.027
J. coenia longitude	-0.095	261	0.126
J. neildi latitude	0.042	216	0.534
J. neildi longitude	0.177	216	0.009
J. zonalis latitude	-0.284	69	0.016
J. zonalis longitude	-0.131	69	0.276
J. zonalis with hybrids latitude	-0.279	106	0.003
J. zonalis with hybrids longitude	-0.300	106	0.002

phenotypically possibly a hybrid between *J. zonalis* and *J. coenia*) captured in 1966 in Alachua County, Florida (Appendix II). After excluding these strays, *Junonia zonalis* exhibits the same trend as *J. coenia* with the proportion of haplotype A increasing in more Southerly populations and a negative correlation between latitude and the presence of haplotype A (Table 3-1) is observed. There is no significant trend between haplotype A and longitude for *J. coenia* in South Florida, but for *J. zonalis* (with hybrids; Table 3-1) there is a significant correlation with a decrease in the abundance of haplotype A from west to east in South Florida.

Figure 3-4. Map of South Florida, USA showing the distributions of *Junonia neildi* using collection localities, and *cytochrome oxidase subunit I* (*COI*) haplotype group assignments from this study.



Temporal Patterns of *Junonia* Haplotypes. Few *J. coenia* were collected in the Florida Keys prior to the 1960s (Figure 3-6 A). Starting in the 1960s, *J. coenia* shows a consistent proportion of haplotype A in the Keys over time (Figure 3-6 A), with a

haplotype frequency of ~40%. Point biserial correlation analysis shows no significant trend in the abundance of haplotype A over time (Table 3-2). The earliest collections of *J. coenia* from the mainland Florida populations that were preserved in museum collections were from the 1870s, but the number of available samples increased in the 1920s and continued steadily to the present decade (Figure 3-6 B). Starting in the 1920s, when sampling becomes sufficient to calculate allele frequencies, the abundance of haplotype A appears to oscillate dramatically with time. From the 1920s to the 1950s there is a relatively consistent frequency of haplotype A of ~30%. In the 1960s the frequency decreases to 0% followed by an increase in the frequency of haplotype A in the 1970s to ~40% which remained consistent through the 1990s. In the 2000s there is another decrease in the frequency of haplotype A to 0%, perhaps followed by a recovery (documented by few samples) in the current decade (2010s). As a result of this temporal variation in haplotype A alleles, there is an overall statistically significant negative point biserial correlation between year and the abundance of haplotype A in mainland South Florida.

Few *J. neildi* specimens from the Florida Keys prior to the 1960s were found in museum collections (Figure 3-6 C). From the 1960s onward, there is some fluctuation in the frequency of haplotype A in this region. Before 1990 haplotype A was relatively abundant with frequencies between 40-60%. There were no *J. neildi* specimens from the Keys were available for genotyping. In the 2000s the haplotype frequency dropped to less

Figure 3-5. Map of South Florida, USA showing the distributions of *Junonia zonalis* using collection localities, and *cytochrome oxidase subunit I* (*COI*) haplotype group assignments from this study.

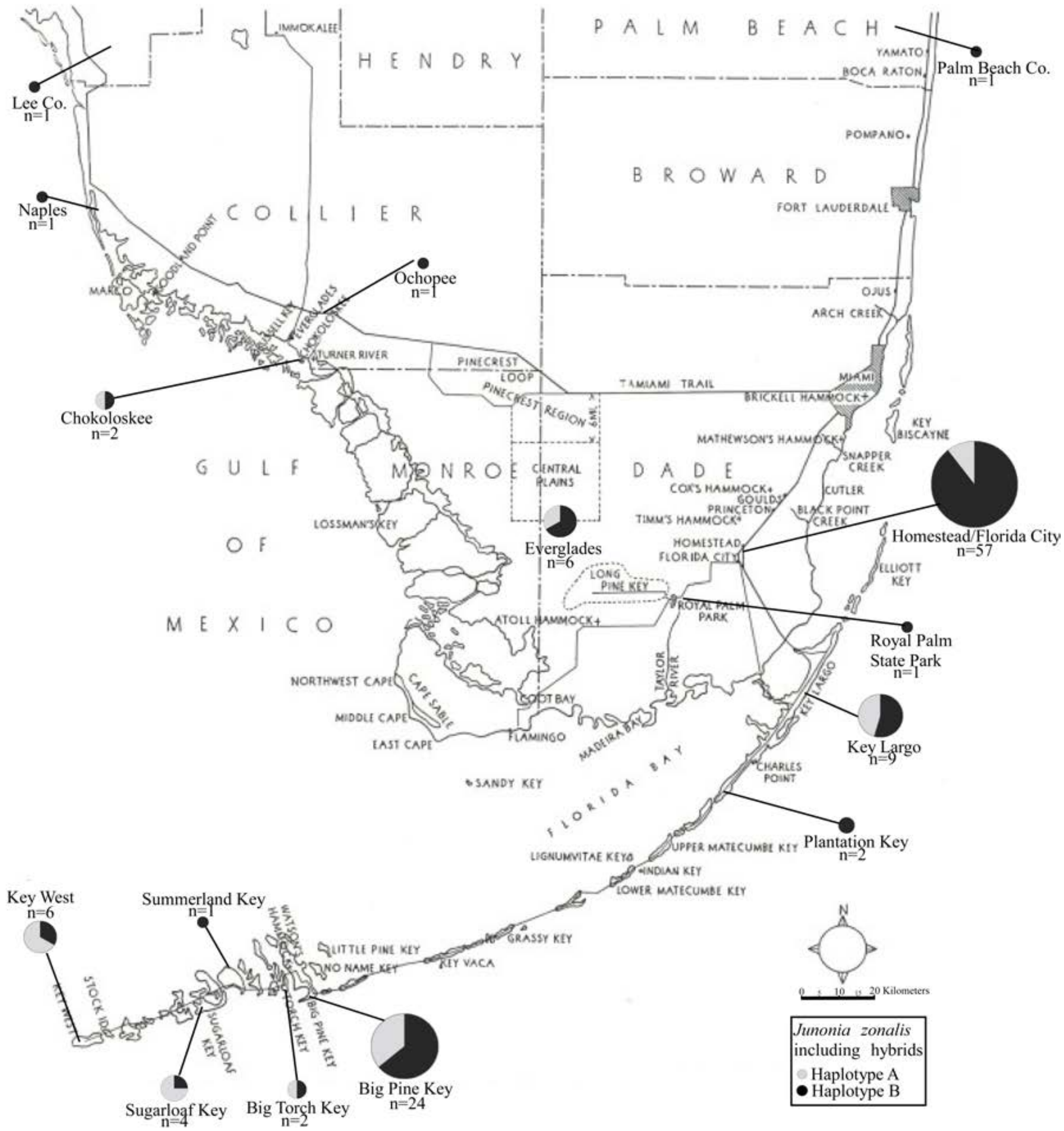


Figure 3-6. Proportion of Haplotype A (+/- 1 standard error) over time in Florida, USA, comparing the Florida Keys to Mainland Florida separated by species. (A) *Junonia coenia* Florida Keys, (B) *J. coenia* Mainland Florida, (C) *J. neildi* Florida Keys (D) *J. neildi* Mainland Florida (E) *J. zonalis* Florida Keys (including *J. zonalis* Hybrids) (F) *J. zonalis* Mainland Florida (including *J. zonalis* Hybrids) (G) Maximum, Mean and Minimum annual temperatures in the Florida Keys. (H) Maximum, Mean and Minimum annual temperatures for Homestead on the South Florida Mainland.

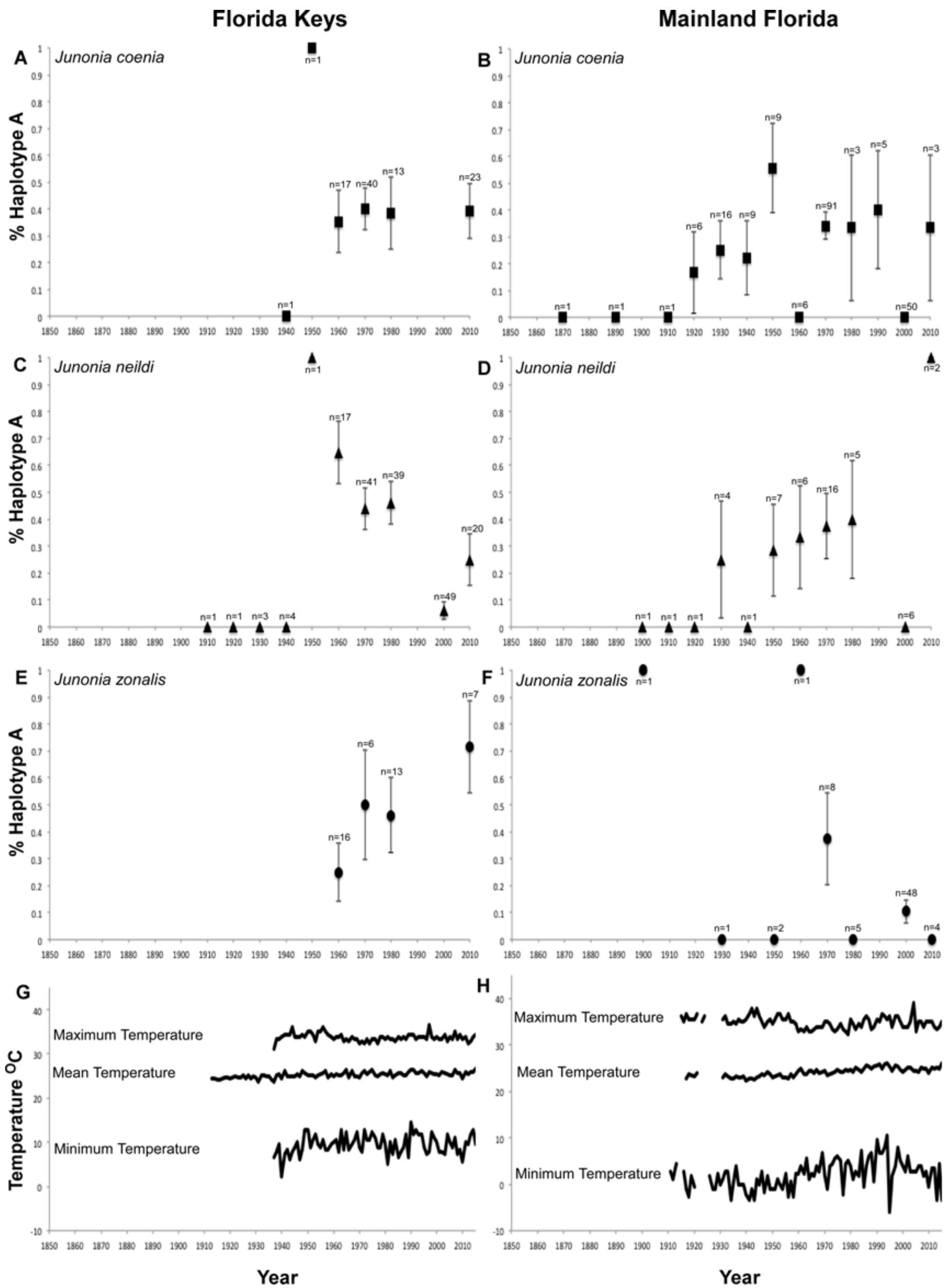

Florida Keys
Mainland Florida
A
Junonia coenia
B
Junonia coenia
C
Junonia neildi
D
Junonia neildi
E
Junonia zonalis
F
Junonia zonalis
% Haplotype A
G
H
Maximum Temperature
Mean Temperature
Minimum Temperature
Temperature °C
Year

Table 3-2. Point biserial correlations between time (year of collection) and the presence of mitochondrial haplotype A for each of the 3 *Junonia* species in the Florida Keys and the South Florida Mainland.

	r	d.f.	p
J. coenia Keys	0.008	93	0.941
J. coenia Mainland	-0.163	199	0.021
J. neildi Keys	-0.151	174	0.046
J. neildi Mainland	0.140	48	0.333
J. zonalis Keys	0.449	12	0.107
J. zonalis Mainland	-0.082	57	0.535
J. zonalis with hybrids Keys	0.321	41	0.036
J. zonalis with hybrids Mainland	-0.293	68	0.014

than 10% followed by what may be a recovery in the 2010s. Point biserial correlation analysis shows a significant negative correlation between haplotype A and time in the Florida Keys (Table 3-2).

The earliest surviving specimens for *J. neildi* from mainland populations that were found in museum collections were collected in the early 1900s (Figure 3-6 D), but sampling increased starting in the 1930s and remained robust until present, with one gap in the 1990s. Haplotype A frequencies have remained relatively constant on the mainland for *J. neildi*, between 30-40% since at least the 1930s. In the 2000s the frequency of haplotype A dropped to 0%, although the sample size is small with only 6 samples. Point biserial correlation analysis shows no significant trend in the abundance of haplotype A over time in mainland populations (Table 3-2).

Junonia zonalis was not detected in the Florida Keys until 1981 (Baggett 1982b; Baggett 1982a), but after it was identified, a review of previously collected material identified specimens of this species collected as early as 1961 (Calhoun 2010). In the current study, I was able to find additional (previously unidentified) *J. zonalis* specimens, as well as probable hybrids between *J. zonalis* and *J. coenia* and (more rarely) between *J. zonalis* and *J. neildi* among material labeled in museum collections as other *Junonia* species (Fig 3-6 E, F). This incudes apparent hybrid *J. zonalis* from the South Florida mainland from the 1900s (n=1), 1930s (n=1), and 1950s (n=2) (Fig 3-6 F), suggesting that this species occurred in Florida prior to 1961. However, it should be noted that the 1900s specimen is labeled as coming from Chokoloskee; many specimens from elsewhere in the Neotropics were incorrectly labeled with this locality during this time period (Heppner 1993), so the collection data for this particular specimen is somewhat suspect.

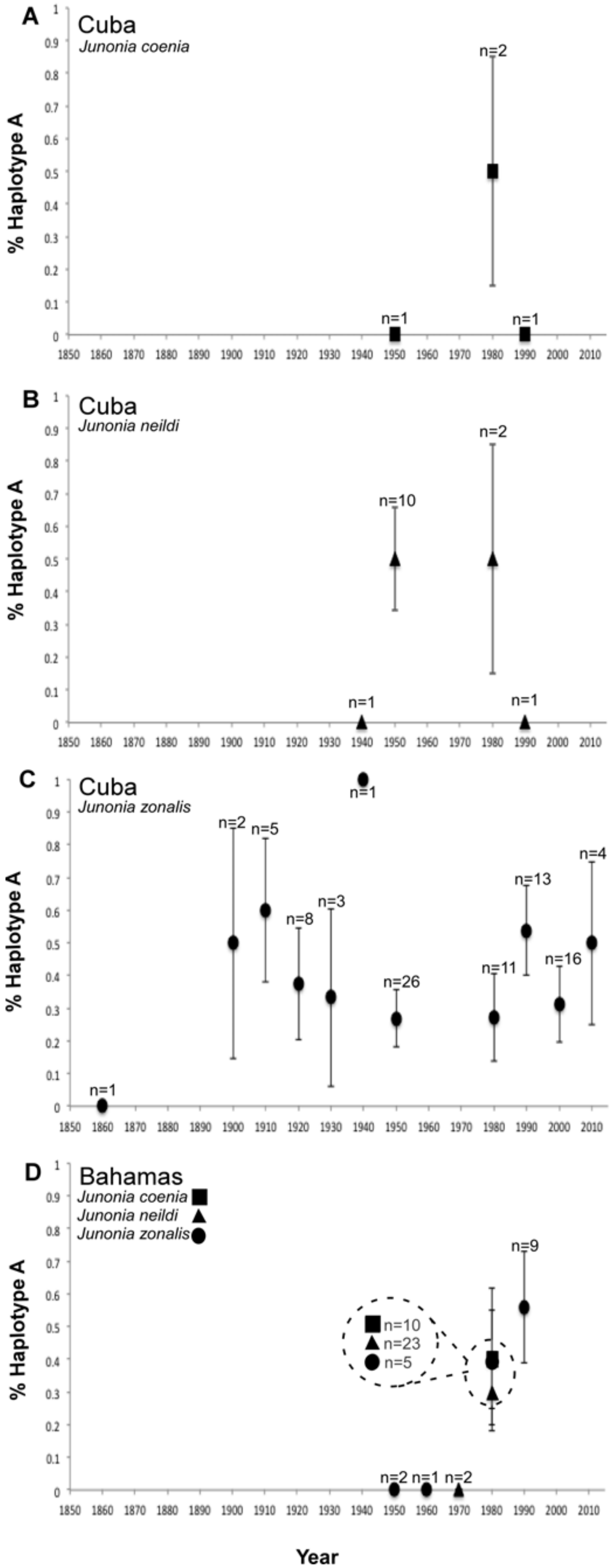
In the Florida Keys, the earliest identified specimens of *J. zonalis* found in museum collections remain from the 1960s (Fig 3-6 E) as reported previously (Calhoun 2010). In the Keys, the haplotype A frequency starts at 20% in the 1960s and increases to 45% in the 1980s. For the 1990s and 2000s there are no samples available for the same reasons mentioned above for other *Junonia* species. By the 2010s haplotype A frequency of nearly 70% was observed, although the sample size is very small. The point biserial correlation analysis shows non-significant results when only *J. zonalis* specimens are taken into account, probably due to insufficient sample size. However, when *J. zonalis* hybrids (with both *J. coenia* and *J. neildi*) are taken into account a statistically significant positive correlation is observed, indicating an overall increase of haplotype A over time (Table 3-2).

Mainland populations of *J. zonalis* were not sampled extensively until the 1970s with a haplotype A frequency of 40% (Figure 3-6 F). In the 1980s the haplotype frequency dropped to 0% and appears to have recovered somewhat by the 2000s with a frequency of 20%. For the 1990s again there are no samples, and in the 2010s the haplotype A frequency dropped to 0% (only 4 samples). Point biserial correlation analysis for the mainland populations yielded a significant negative correlation indicating a decrease in haplotype A over time (Table 3-2).

Junonia coenia specimens from Cuba (Fig 3-7 A) were rare in the collections that were consulted, yielding a total of only 4 samples. This makes speculation about the frequencies of haplotype A over time inappropriate. *Junonia neildi* populations from Cuba (Fig 3-7 B) where better sampled with the largest number of samples from the 1950s. The frequency of haplotype A in the 1950s was close to 40%. *Junonia zonalis* from Cuba (Fig 3-7 C) were the most abundant *Junonia* species in the museum collections. Cuban specimens of *J. zonalis* became well represented in museum collections beginning in the 1910s, although there are two samples that do appear prior to this in the data set. The frequency of haplotype A in Cuba has remained relatively constant with a frequency of ~40% throughout the sampled time interval.

All *Junonia* species from the Bahamas were all plotted on the same graph (Fig 3-7 D) as the total number of samples obtained was small. Reasonable sampling for all 3 species was obtained from the 1980s and all species had a frequency of haplotype A of ~40%.

Figure 3-7. Proportion of Haplotype A (+/- 1 standard error) over time for (A) *J. coenia* from Cuba, (B) *J. neildi* from Cuba, (C) *J. zonalis* from Cuba (3 *J. zonalis* X *J. neildi* hybrids from the 1950s were not included), (D) all 3 species from the Bahamas, *J. coenia* is represented by a black square, *J. neildi* is represented by a black circle and *J. zonalis* is represented by a black triangle.


A
Cuba
Junonia coenia
B
Cuba
Junonia neildi
C
Cuba
Junonia zonalis
D
Bahamas
Junonia coenia
Junonia neildi
Junonia zonalis
% Haplotype A
Year

Relationship between Climate and Haplotype. Historical records for annual maximum, minimum, and average temperatures for the Florida Keys (Figure 3-6 G) and for mainland South Florida (Figure 3-6 H) show several interesting patterns. The mean annual temperatures in Key West and Homestead, Florida are very similar, but the annual maximum and minimum temperatures in Homestead are more extreme (annual maximum is typically higher, annual minimum is typically lower) than in Key West. There is also much more year-to-year variability in the annual maximum and minimum temperatures on the mainland as represented by the Homestead temperature record than in the Keys. The more limited temperature fluctuations in the Keys are due to their more tropical latitude and the temperature-buffering effects of ocean waters on small islands, making the climate of the Florida Keys more similar to that of Caribbean islands.

Point biserial correlation analysis between historical temperature records and the presence of Haplotype A for mainland Florida yielded statistically significant results for two of the three species (Table 3-3). There is a significant negative correlation between mean annual temperature and the abundance of haplotype A and a marginally significant negative correlation between minimum annual temperature and the abundance of haplotype A in mainland populations of *J. coenia*. *Junonia zonalis* yielded a significant negative correlation (Table 3-3) only when hybrids where included in the data set and only with annual maximum temperatures, suggesting that high temperature may be the limiting factor for the persistence of haplotype A in this species on the Florida mainland. For the Florida Keys the only species that showed significant correlations between temperature and haplotype A abundance was *J. neildi* (Table 3-3). All three analyses (annual maximum, annual minimum and annual mean temperatures) yielded significant negative correlations with the presence of haplotype A, suggesting that temperature plays a major role in the abundance of haplotype A in this species in this region.

Table 3-3. Point biserial correlations between maximum (Max), minimum (min), and mean annual temperatures and the presence of haplotype A for each of the 3 *Junonia* species in the Florida Keys and the South Florida mainland.

	r	**d.f.**	**p**
J. coenia Keys Max	0.041	93	0.696
J. coenia Keys Min	0.030	93	0.772
J. coenia Keys Mean	0.090	93	0.387
J. coenia Mainland Max	0.106	193	0.142
J. coenia Mainland Min	-0.131	196	0.067
J. coenia Mainland Mean	-0.185	193	0.010
J. neildi Keys Max	-0.294	165	0.00011
J. neildi Keys Min	-0.167	165	0.031
J. neildi Keys Mean	-0.172	165	0.026
J. neildi Mainland Max	-0.067	44	0.658
J. neildi Mainland Min	0.034	46	0.819
J. neildi Mainland Mean	0.132	44	0.383
J. zonalis Keys Max	-0.180	12	0.539
J. zonalis Keys Min	0.153	12	0.602
J. zonalis Keys Mean	0.098	12	0.739
J. zonalis Mainland Max	-0.193	57	0.143
J. zonalis Mainland Min	0.137	57	0.301
J. zonalis Mainland Mean	0.003	57	0.980
J. zonalis with hybrids Keys Max	-0.077	41	0.622
J. zonalis with hybrids Keys Min	-0.148	41	0.343
J. zonalis with hybrids Keys Mean	-0.177	41	0.256
J. zonalis with hybrids Mainland Max	-0.284	65	0.020
J. zonalis with hybrids Mainland Min	0.146	65	0.240
J. zonalis with hybrids Mainland Mean	-0.085	65	0.495

Discussion

Species Distributions. This study confirms the previously reported distributions of all 3 *Junonia* species in South Florida (Glassberg *et al.* 2000). *Junonia coenia* is commonly found throughout mainland Florida but is less common in the Florida Keys because the open habitats favored by this species are not abundant, and are often lost to human development (Figure 3-3)(Minno & Emmel 1993). *Junonia coenia* is the most common species in this genus in mainland South Florida and is represented by specimens in museum collections starting in 1875 on the South Florida mainland, and starting in 1948 in the Florida Keys. It seems to be more common in the lower Florida Keys than in the Upper Florida Keys, with the largest extant populations on Key West and Big Pine Key.

The other resident species, *J. neildi* is common in mangrove swamp habitats in coastal areas in both mainland South Florida and in the Florida Keys, where it is among the most abundant butterfly species in these habitats year-round (Figure 3-4)(Schwartz 1987; Glassberg *et al.* 2000). Historical specimens of *J. neildi* are well represented in museum collections, with specimens dating back to the early 1900s. *Junonia neildi* has been classified as a species in decline by some organizations, largely due to destruction of mangrove habitats by human development (Minno 2016), but recent fieldwork (Chapter 2) suggests that it is present in sizeable populations where appropriate habitats remain in both mainland Florida and in the Florida Keys.

The invading species, *J. zonalis* remains relatively rare in Florida. It is currently present in greatest abundance in two of the same localities where it was first observed in Florida in 1981: Homestead and Big Pine Key (Baggett 1982b; Baggett 1982a). It has become less common on Key Largo due to development of its preferred open habitat by humans, but this species has also been found in Key West in recent years (Appendix II). Stray individuals of this species are also occasionally observed far to the north of its core range in eastern South Florida and the Florida Keys (Figure 3-5; Figure 2-2; Chapter 2)(Mitter 2013). Specimens of *J. zonalis* and its hybrids with *J. coenia* have been observed in western mainland South Florida, but it appears to be rarer in the west than it is in eastern South Florida or in the Florida Keys. The relative rarity of *J. zonalis,* and the human development of its habitat on Key Largo have led some to describe this species as being imperiled in Florida (Minno 2016). Of the extant populations of *J. zonalis* in South Florida, the Homestead population, which the Marcus laboratory has been monitoring since 2006, appears to be the largest and most stable.

Invasion History of *Junonia zonalis.* Retrospective reviews of specimens from museum collections by prior authors seeking additional *J. zonalis* material from Florida located several specimens from the 1970s and 1980s as well as a Key Largo specimen collected in 1961 (Calhoun 2010). Prior to this study, the 1961 Key Largo *J. zonalis* specimen was the "index case" or first reported instance of this species occurring in Florida. Previously, these early collections of *J. zonalis* had been interpreted as either variation within *J. coenia* or *J. neildi* (Chapter 2) (Turner & Parnell 1985), or in some cases as hybrids between them (Remington 1968; Rutkowski 1971; Remington 1985; Scott 1986). As I consulted museum collections, while I found no earlier "pure" *J. zonalis,* I found several earlier specimens that appear to be hybrids between *J. zonalis* and other *Junonia* species, with the earliest hybrid specimen with firm collection data captured in 1930 in Royal Palm State Park (now part of Everglades National Park). This suggests that the process of invasion by *J. zonalis* into Florida began much earlier than was previously recognized (Minno & Emmel 1993; Cech & Tudor 2005; Calhoun 2010) and that hybridization was an important feature of the early stages of colonization by this species. This is consistent with predictions from theory that suggest that the ability to hybridize and produce fertile offspring with a resident species may allow early colonists of an invasive species to overcome the challenge of gamete limitation (caused by low availability of conspecific mates) during the earliest stages of an invasion (Hall 2016).

Contemporary specimens of *J. zonalis* captured in Florida are phenotypically very similar to *J. zonalis* from the Caribbean with respect to wing color patterns (Calhoun 2010) and flight behaviour (Turner & Parnell 1985). However, at least in the Florida Keys populations, *J. zonalis* host plant preferences appear to have shifted from its primary larval host in the Caribbean, blue porterweed (*Stachytarpheta jamaicensis*), to saltmarsh false foxglove (*Agalinis maritima*) and American blueheart (*Buchnera americana*), preferred larval host plants for *J. coenia,* and in at lease one case to black mangrove (*Avicennia germinans*), the preferred larval host plant for *J. neildi* (M.C. Minno, pers. comm.). Larvae from recent collections of *J. zonalis* from the Florida Keys grew slowly with high mortality when fed *Stachytarpheta* (M.C. Minno, pers. comm.), while in the 1980s, larval *J. zonalis* from the Keys performed well on this host plant (Baggett 1982a). This suggests that invading *J. zonalis* may have acquired genetic variation associated with larval host plant choice and performance from its Florida native congeners by hybridization and introgression, and is similar to what has been observed in some other systems (Hall 2016). Based on the phenotypes and frequency of the putative *J. zonalis* hybrids, hybridization between *J. zonalis* and *J. coenia* appears to be much more common than hybridization between *J. zonalis* and *J. neildi.* This is consistent with observations that hybridization between *J. zonalis* and *J. neildi* is also extremely rare or absent in most of the Caribbean (T. W. Turner, pers. comm.).

Temporospatial Dynamics of Mitochondrial haplotype frequencies. Prior work looking into the distributions of the mitochondrial *Cytochrome oxidase subunit I* (*COI*) haplotypes, had found that haplotype A was almost completely absent from mainland Florida and was relatively rare in the Florida Keys (Chapter 2; Gemmell & Marcus 2015). Prior to this study, *Junonia neildi* and *J. zonalis* were the only species known to carry the A haplotype in contemporary populations in North America, each represented by a few individuals with this genotype in South Florida (Chapter 2). Based on the current work, the first specimen of *J. neildi* with a haplotype A genotype in mainland Florida was collected in 1934, while the first from the Florida Keys was collected in 1964. The first specimens of *J. zonalis* carrying haplotype A on the Florida mainland were collected in 1973, although there is a *J. zonalis* X *J. coenia* hybrid with a haplotype A genotype from the 1930s. For the Florida Keys, the first haplotype A found in *J. zonalis* was collected in 1981 while there are both *J. zonalis* X *J. coenia* and *J. zonalis* X *J. neildi* hybrids from 1967 carrying haplotype A in the Florida Keys. Surprisingly, while haplotype A was absent in contemporary collections of *J. coenia* in mainland Florida (Chapter 2), expanding the dataset to include contemporary specimens from the Florida Keys and historical *J. coenia* specimens shows that this species often carries haplotype group A alleles, with the earliest alleles appearing in the 1920s.

In contemporary populations, of *Junonia* haplotype B occurs at nearly 100% frequency on the Florida mainland and at a high frequency in the Florida Keys (Chapter 2). This prompted questions regarding trends in latitudinal and longitudinal trends in historical populations. *Junonia coenia* demonstrates a statistically significant negative point biserial correlation between latitude and the abundance of haplotype A (Table 3-1). This decrease in haplotype A abundance with increasing latitude corresponds to what we do know with contemporary populations as Central and Northern Florida have a 100% frequency of haplotype B (Chapter 2; Gemmell & Marcus 2015), while haplotype A becomes more common in South Florida (Figure 3-3).

When *J. zonalis* is considered (Table 3-1), it showed a similar a statistically significant negative point biserial correlation between latitude and the abundance of haplotype A as *J. coenia*. Since the sample size of *J. zonalis* specimens was relatively small when compared to the other species, *J. zonalis* hybrids were added into the data set and the biserial correlation analysis was repeated. When hybrids were included, statistically significant correlations were observed for both latitude and longitude (Table 3-1), indicating a decrease in the frequency of haplotype A from South to North in South Florida as well as from West to East, with the highest frequencies of haplotype A in the lower Florida Keys (Figure 3-5). The proximity of Cuba to the lower Florida Keys and the consistent high frequency of haplotype A in Cuban *J. zonalis* suggest possible gene flow between these populations and is consistent with earlier suspicions that Cuba may be the source of *J. zonalis* colonists invading Florida (Cech & Tudor 2005; Calhoun 2010).

Populations of *J. neildi* show a general increase in haplotype A with longitude (Table 3-1) suggesting an increase from western to eastern Florida. The high abundance of haplotype A alleles in southeastern Florida (Key Largo, the opposite of the trend of that found in *J. zonalis,* Figure 3-5), may be an indication of gene flow between these populations and populations of *J. neildi* in the nearby Bahamas, which also have a high frequency of haplotype A (Figure 3-7D), especially when compared to haplotype frequencies in more Northern populations of *J. neildi* in Florida (Chapter 2).

Significant biserial correlations between the presence of haplotype A and both longitude and latitude in some *Junonia* species (Table 3-1) prompted me to take mainland Florida and the Florida Keys populations and analyze them separately (Table 3-2). Consistent with what is known about contemporary populations on the mainland for *J. coenia*, a significant negative biserial correlation was observed: haplotype A was found to decrease in frequency over time. In the Florida Keys *J. neildi* populations also showed a significant negative biserial correlation and a decrease in the frequency of haplotype A over time. For *J. zonalis* without hybrids, no significant results were obtained on either the South Florida mainland or in the Florida Keys. Once hybrid specimens were included in the analysis, a significant negative biserial correlation and a decrease in the frequency of haplotype A was found on the Florida mainland, while significant positive biserial correlation and an increase in haplotype A in the Florida Keys over time was observed.

Originally there was speculation that *J. zonalis* invaded South Florida in a single event (Baggett 1982b; Baggett 1982a; Minno & Emmel 1993) bringing with it the haplotype A allele from the Caribbean (Gemmell & Marcus 2015). Initial observations suggested that in Florida mainland populations of *J. zonalis,* haplotype A frequencies (< 5%) were also much lower than in Caribbean *Junonia* populations (Gemmell & Marcus 2015) (Chapter 2). Cuba, the closest source population of *J. zonalis* where allele frequencies had been measured, had a haplotype A frequency of approximately 35% (Gemmell & Marcus 2015). This suggested that an interaction between the evolutionary forces of migration (which would tend to increase the frequency of A) and genetic drift (which would cause a decrease in the frequency of A), perhaps in combination with the dilution of A alleles by hybridization with other *Junonia* species, may be responsible for determining haplotype frequencies in Florida (Chapter 2). Under these circumstances, in the case of a single migration event, one would predict a single peak, followed by an exponential decay in the frequency of haplotype A in *J. zonalis* populations.

An alternative to the single invasion event hypothesis was proposed by Cech and Tudor (2005), who suggested that episodic invasions of *J. zonalis* from the Caribbean might be responsible for the dramatic swings in the abundance of *J. zonalis* in Florida. Episodic invasion in combination with drift and hybridization would be expected to produce an oscillating pattern of haplotype A allele frequencies, with peaks of high haplotype A abundance associated with each invasion event, followed by exponential decay.

The behavior of haplotype A frequencies in Florida Keys populations of *J. zonalis* (Figure 3-6E) is not consistent with either of these invasion-haplotype frequency decay scenarios and were observed to increase significantly over time (Table 3-2). This could be consistent with a single invasion event where haplotype A is subsequently maintained in populations by selection or with ongoing immigration of *J. zonalis* migrants to Florida from elsewhere in the Caribbean, or with some combination of both. In mainland South Florida overall, there is a statistically significant decrease in haplotype A frequency (Table 3-2), but there are peaks of relative abundance of haplotype A in *J. zonalis* in the

1970s and the 2000s, each followed by a subsequent disappearance of the allele (Figure 3-6F). This pattern is consistent with the oscillations in allele frequency that are predicted by the episodic invasion hypothesis, though mainland populations might be receiving immigrants from the Florida Keys as well as from the Caribbean. Upon reflection, ongoing gene flow between the Florida Keys and Cuba (distance 170 km), and episodic gene flow between the Florida Keys and the Florida mainland (distance 165 km) populations of *J. zonalis* is not terribly surprising, giving that these distances are well within the large dispersal abilities of species in this genus (Harris 1988; Shapiro 1991).

Given the importance of hybridization in the early stages of many successful invasion events (Hall 2016), scanning additional museum collections for more specimens of *J. zonalis* and its hybrids from the early stages (pre-1960s) of the invasion of South Florida, would yield better temporospatial resolution and reveal further details of the course of this invasion that will improve our understanding of the mode and tempo of invasion biology more generally.

Temperature and haplotype frequencies. Recognizing that haplotype frequencies are changing over time, and that haplotype frequencies are behaving differently in Florida Keys versus mainland populations leads to the additional question of what factors may be contributing to these fluctuations. Climate is an important factor influencing species distributions, by its direct effects on organisms (Rank & Dahlhoff 2002), and by its indirect effects on predator-prey relationships (Grigaltchick *et al.* 2012), species competition (Alexander *et al.* 2015), the availability of suitable habitat, and the distribution of food resources (Miller-Struttmann *et al.* 2015). There are many important components that contribute to climate, but comparative data was not available for most of them to match the large temporal and spatial scale of my *Junonia* data set. However, annual records for minimum, maximum, and mean temperature, a major component of climate, are available for both the Keys and the mainland with few interruptions for the last 100 years (Weather Source 2009a; Weather Source 2009c; Weather Source 2009b; Weather Source 2009d; Schmidt 2016). This provides a starting point for understanding how climate may be influencing *Junonia* populations in Florida.

The mean annual temperatures of the Florida Keys and Homestead, on the Florida mainland, are nearly identical, but on the mainland the annual maximum temperatures are higher and the minimum annual temperatures are lower (Figure 3-6 G, H). In addition, the Florida Keys experience less temperature fluctuation over time (Figure 3-6 G). This is largely due to their more southerly position and the moderating effect of warm ocean waters on the small islands that make up the Florida Keys, both of which will act to keep island temperatures relatively stable.

The significant negative biserial correlation results for mean annual temperatures and the marginally significant negative correlation for minimum annual temperatures for *J. coenia* on the mainland (Table 3-3) suggests that there is a decrease in the presence of haplotype A on the mainland when temperatures are low. There are similar significant negative correlations between the presence of haplotype A alleles and maximum, minimum, and mean annual temperatures for *J. neildi* in the Florida Keys. Finally, there is a negative correlation between the presence of haplotype A in *J. zonalis* (when hybrids are included) and maximum annual temperatures. While not every *Junonia* species yields a statistically significant result, the consistency of these findings suggest that haplotype A may decreases in frequency at times and in places that experience high temperature conditions (Table 3-3). This may account for the low frequencies of haplotype A in all *Junonia* species on the Florida mainland in the 2000s (Figure 3-6). There are a number of other loci that have shown similar patterns related to geography and temperature in other insect species including the nuclear genes *phosphoglucose isomerase* (*PGI*)(Rank & Dahlhoff 2002; Karl *et al.* 2009a; Wheat 2010) and *heatshock protein 70* (*HSP70*)(Rank & Dahlhoff 2002; Karl *et al.* 2009b) and the mitochondrial gene *cytochrome oxidase II* (*COII*)(Roberts *et al.* 2014).

At the same time, I recognize that the climate data that I have used for these analyses is extremely coarse. It represents temperature data summarized on an annual basis, and in some cases (especially on the Florida mainland), specimens were collected at sites far from the temperature recording stations. Also, at least in some cases, it is short-term weather events, rather than climate, that may be exerting selective forces with powerful effects on the evolution of populations (Brown & Bomberger Brown 2000). Ideally, it would be desirable to reinforce the findings here with laboratory experiments that measure aspects of physiological performance in *Junonia* carrying mitochondrial haplotypes A or B at various biologically relevant temperatures, as has been done in some other insect systems (Rank & Dahlhoff 2002; Karl *et al.* 2009b).

Historical biogeography and invasion biology of *J. coenia* and *J. neildi*. Given this new understanding of the time course of the *J. zonalis* invasion of Florida, it is tempting to examine the other two species in Florida and to try to reconstruct their biogeographic history as well. It has been estimated that mitochondrial haplotype groups A and B carried by *J. coenia* and *J. neildi* invaded the Western Hemisphere from the Asia–Pacific region at least 0.96 $\pm$ 0.29 mya to 1.18 $\pm$ 0.29 mya (McCullagh 2016), respectively. Both of these species evolved within the New World, but predate the most recent glacial maximum, approximately 11,000 years ago at the end of the Pleistocene Epoch.

During the course of the Wisconsin glaciation in the late Pleistocene (85,000 years ago to 11,000 years ago) (Pielou 1991), the Laurentide Ice Sheet grew to cover most of North America, and as this glacial ice sheet advanced, temperate deciduous forest was pushed southward, before finally reaching its maximum just north of Florida (Delcourt & Delcourt 1979; Delcourt 2002; Hill & Condron 2014). The climate in Florida during this time would have been dry and the habitat in much of peninsular Florida would have been sandy and composed mostly of desert scrub (Delcourt 2002). This is a preferred habitat type for *J. coenia* (Opler & Malikul 1992) and its larval host plants would have been present on the Florida peninsula (Brown & Heineman 1972; Lane 1994), suggesting that *J. coenia* was likely resident in Florida throughout the Wisconsin glaciation. During this glacial episode, coastal temperatures were too low to support mangrove growth (Cavanaugh *et al.* 2014), so the black mangrove (*Avicennia germinans*), the larval host of *J. neildi,* was excluded from Florida (Turner & Parnell 1985; Paulsen 1996; Elster *et al.* 1999).

At the end of the Wisconsin glaciation, rising air temperatures began to allow tropical species to migrate North from glacial refuges (Lane 1994; Zeiller 2005). Between 11,000 and 6,000 years ago, periodic catastrophic drainages of glacial lakes overflowing with melt water repeatedly spilled massive amounts of cold water and icebergs first into the Gulf of Mexico, and then into the Atlantic Ocean (Hill & Condron

2014). These massive spillages caused rapid increases in sea level and sent flows of cold water and icebergs down along the North American Atlantic continental shelf as far south as Key Largo, Florida (Bard *et al.* 2000; Hill & Condron 2014). These conditions would have continued to prevent the formation of mangrove swamps long after inland air temperatures might have otherwise permitted their growth (Cavanaugh *et al.* 2014).

Approximately 6,000 years ago, the last of the glacial lakes drained (Pielou 1991). The warm waters from the tropics began to move northward along the Atlantic coast as the Gulf Stream current and the ocean circulation became similar to modern circulation patterns. By 3,000 years ago, sea level rise slowed and stabilized, the climate had warmed in Florida to produce modern subtropical conditions, and mangrove habitats, including *A. germinans,* were re-established on both the Atlantic and Gulf coasts of Florida (Lane 1994; Zeiller 2005) from glacial refuges in the Caribbean and on the Atlantic coast of Mexico (Cavanaugh *et al.* 2014; Sandoval-Castro *et al.* 2014). This suggests that *J. neildi* probably arrived in Florida within the last 6,000 years, perhaps from refuges in the Caribbean, creating a secondary contact zone with resident *J. coenia*.

Under laboratory conditions, hybridization between *J. neildi* and *J. coenia* occurs readily (Paulsen 1994; Paulsen 1996; Marcus 2005), but in most of Florida, phenotypically intermediate specimens that would be expected from hybrids between these 2 species are extremely rare in the wild. This is the case even though *J. coenia* frequently co-occurs in mangrove swamps with *J. neildi* (Chapter 2, Appendix I, Appendix II). Apparent hybrids appear to be most common at sites where *J. neildi* is periodically extirpated by cold environmental conditions at the northern extremes of its range (Glassberg *et al.* 2000), and it reinvades from populations located farther South.

Repeated seasonal reinvasion of habitat in the northern parts of species ranges is a common feature of how many *Junonia* species behave in North America (Shapiro 1991). Clusters of phenotypic intermediates between *J. neildi* and *J. coenia* are known from New Port Richey on the Florida Gulf Coast (J. R. Slotten, pers. comm.) and from New Smyrna on the Atlantic Coast (T. W. Turner, pers. comm.). During the reinvasion of these habitats by *J. neildi*, the invading species is initially very rare while *J. coenia* is common, resembling the circumstances described for the *J. zonalis* invasion of South Florida which produced plentiful *J. zonalis* X *J. coenia* hybrids, especially during the early stages of the invasion, and what is expected from invasion biology models (Hall 2016). Due to the rarity of conspecific mates, early colonists in a biological invasion may be forced to mate with heterospecifics, overcoming longstanding reproductive isolating mechanisms that may still be in effect in other localities where there is sympatry between *Junonia* species.

Implications for future work obtaining genotypes from museum specimens. Previous work using historical DNA from insect museum specimens has been mainly focused on nuclear genotyping based on polymorphic microsatellite amplification product sizes (e.g. (Strange *et al.* 2009; Saarinen & Daniels 2012)), while mitochondrial DNA genotyping has been studied by Sanger-sequencing based approaches (e.g. (Goldstein & Desalle 2003; Heintzman *et al.* 2014)). Fragment-based techniques like microsatellites are less sensitive to poor DNA quality, but mitochondrial DNA occurs in much higher copy number (Watts *et al.* 2007), making DNA-sequencing based techniques possible for much older specimens. However, for both microsatellites and mitochondrial-sequencing based approaches, the likelihood of successful genotyping decreases as the age of the specimens increases (Mandrioli *et al.* 2006; Watts *et al.* 2007; Strange *et al.* 2009). The mitochondrial DNA restriction digest genotyping strategy that I have used takes advantage of both the high copy number of mitochondrial DNA and the relative robustness of fragment-based genotyping methods.

To date, the oldest specimens successful yielding microsatellite genotypes have been from Hymenoptera (*Bombus*) collected in 1893 (Strange *et al.* 2009), Lepidoptera (*Parnassius* and *Polyommatus*) collected in 1895 and 1896 (Habel *et al.* 2009) (Harper *et al.* 2006), and Ondonata (*Coenagrion*) collected in 1954 (Watts *et al.* 2007). The oldest specimens successfully yielding mitochondrial DNA sequences have been from Lepidoptera (*Speyeria*) collected in 1945 (Keyghobadi *et al.* 2013), Diptera (*Gigantodax* and *Simulium*) collected in 1953 (Hernandez-Triana *et al.* 2014), and Coleoptera (*Cicindela* and *Amara*) collected in the early 1870s (Goldstein & Desalle 2003; Heintzman *et al.* 2014), but it should be noted that DNA degradation in preserved beetle specimens seems to take place more slowly than in most other insects (Heintzman *et al.* 2014).

Success rates from previous historical DNA experiments for insects vary enormously (Goldstein & Desalle 2003; Watts *et al.* 2007; Strange *et al.* 2009; Ugelvig *et al.* 2011; Keyghobadi *et al.* 2013; Heintzman *et al.* 2014; Hernandez-Triana *et al.* 2014). One of the problems encountered when estimating the proportion of successful DNA amplifications from museum specimens from the literature, is that some authors do not report how many specimens out of the data set were successful, state that only successful amplifications were used in analysis, or simply state how many sites were amplified successfully (Harper *et al.* 2006; Habel *et al.* 2009; Saarinen & Daniels 2012). When taking into account only published historical data sets that do report enough detail to calculate success rates, the rates vary between 0% and 97%, but two trends were observed. First, across many studies, it was consistently observed that museum specimens showed a declining success rate in obtaining suitable DNA for analysis as the age of the specimens increased (Goldstein & Desalle 2003; Watts *et al.* 2007; Strange *et al.* 2009; Ugelvig *et al.* 2011; Keyghobadi *et al.* 2013; Heintzman *et al.* 2014; Hernandez-Triana *et al.* 2014). Second, the success rate for genotyping museum specimens was dependent on the size of the fragment of mt DNA with smaller amplified fragments resulting in higher success rates (Meusnier *et al.* 2008; Keyghobadi *et al.* 2013).

By using high copy number mitochondrial templates, a fragment-detection based genotyping assay, an effective DNA extraction protocol, and an extremely sensitive capillary electrophoresis instrument (Qiagen QiAxcel) for detecting amplification products and restriction digest fragments, I was able to successfully assign mitochondrial haplotypes to a total of 798 specimens with a 100% success rate. This total includes genotypes from butterflies collected as early as 1866 (Figure 3-2), making them the oldest insect museum specimens genotyped to date and 30 years older than the next-oldest Lepidoptera that have been successfully genotyped (Habel *et al.* 2009) (Harper *et al.* 2006). This method also has the additional advantages of being fast (going from intact specimen to genotype in a single day is possible in our laboratory), with reasonably high throughput (hundreds of specimens can be genotyped in a week), and produces genotypes at approximately 1/10th the cost of Sanger sequencing on a per-individual basis. The museum specimens analyzed for the current study includes only a small number of specimens from the 19th century for this group (Appendix II), so the maximum age of specimens from which it can reliably produce genotypes is still not well defined. Sampling from 1900-present is much more robust, and the method can be considered reliable for specimens up to 100-120 years old. An effort to genotype additional New World specimens of *Junonia* from the 19th century (and from the 18th century, if they can be located) would be very helpful in defining the maximum temporal limits of the method. Electronic searches of the better-catalogued North American entomology collections suggest that appropriate *Junonia* specimens exist for at least the period of 1865-1900 (Cobb 2016). Additional searches of the larger museum collections in North America and Europe with large Lepidoptera holdings from the 19th century may reveal additional appropriate specimens collected from earlier periods in order to validate the method for use with older specimens more comprehensively.

Conclusion

The genotyping method that I have developed was essential for reconstructing the invasion history of *Junonia zonalis* into South Florida, as well as the possible interactions of the invading species with the 2 resident *Junonia* species through hybridization as well as potential assimilation of traits (such as larval host plant preference from native *Junonia* populations). Careful reviews of museum collections dates the initial invasion of *J. zonalis* to no later than 1930, some 30 years prior to what had been previously believed.

These innovations in restriction fragment-based mitochondrial genotyping of museum specimens permitted the genotyping of preserved butterflies from 1866 to present. Because of its robustness when working with old samples, its speed, and low cost, this method will have many applications in studies of invasion biology, conservation biology, and in documenting the responses of organisms to climate change.

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Chapter 4: Getting Western: Biogeographical analysis of morphological variation and mitochondrial haplotypes reveals cryptic species and hybrid zones in the *Junonia* butterflies of the American Southwest and Mexico

Abstract

The American Southwest and Northern Mexico have a great degree of endemic diversity compared to the rest of North America. The Pleistocene glaciations and the dispersal of species from glacial refuges in this region have been important engines for the production of biodiversity in the region. The New World *Junonia* are a recently diverged group of butterflies that are thought to have spent time in these refuges in periods of glacial advancement. I have reconstructed the plausible movements, and the contemporary geographic distributions of the five species (*J. coenia*, *J. grisea*, *J. litoralis*, *J. nigrosuffusa*, and *J. zonalis*) that occur in the American Southwest and Northern Mexico using phenotypic and genotypic information from specimens preserved in museum collections. Evidence of cryptic species and hybridization events were observed using both mitochondrial haplotype data and morphological characteristics. *Junonia grisea* and *J. coenia* are morphologically very similar, but differences in morphology, life history traits, and a discrete mitochondrial haplotypes suggest that they are a cryptic species pair, thus elevating *J. grisea* to a full species from a subspecies of *J. coenia*.

Introduction

The topography and glacial history of the American Southwest and Northern Mexico has caused a pronounced increase in the biological diversity of this region, when compared to other parts of the North American continent (Mengel 1964; Mengel 1970). Groups as diverse as birds (Bermingham *et al.* 1992), mammals (Stein *et al.* 2000), fish (Olden & Poff 2005), plants (e.g. *Helianthus* (Ode et al. 2011)), and butterflies (e.g. *Apodemia* (Proshek et al. 2015), *Limenitis* (Platt 1983), and *Speyeria* (Williams 2001)) have greater endemic biodiversity in this region than in eastern North America. Remington (1968, 1986) proposed a series "suture zones" where populations of many different pairs of related organisms expanded their ranges after emerging from glacial refugia, came into secondary contact, and began to interact with one another, in some cases hybridizing and creating hybrid zones (Porter 1989; Porter 1990). Remington (1968, 1986) saw suture zones as a way of reconstructing the possible geographical locations for Pleistocene glacial refuges.

Recently, a number of studies have employed molecular markers to develop a better understanding of the population structure of organisms in this region and to reconstruct the glacial history as well as the geographical locations of glacial refuges (e.g. (Peñaloza-Ramírez *et al.* 2010; Roberts & Hamann 2015). At the same time, such studies have provided important information about contemporary species ranges, patterns of hybridization, and the evolutionary forces acting on organisms in hybrid zones (Ross & Harrison 2002; Rieseberg *et al.* 2007). Since hybridization among clusters of related species can actually accelerate evolutionary innovation and speciation (Genner & Turner

2012), the interactions between geography, climate history, and hybridization may be an important driver of biological diversification in western North America.

The American Southwest (encompassing the US states of California, Nevada, Arizona, Utah, New Mexico, Texas, Oklahoma, Colorado, southern Wyoming, and southern Oregon, USA) and northern Mexico is a region where multiple forms of *Junonia* butterflies (Lepidoptera: Nymphalidae) have overlapping ranges. There are 5 different forms in the region: *J. coenia, J. grisea, J. litoralis, J. nigrosuffusa,* and *J. zonalis* (Barnes & McDunnough 1916; Forbes 1928; Tilden 1970; Rutkowski 1971; Schwartz 1987; Minno & Emmel 1993; Paulsen 1996; Elster *et al.* 1999; Walker 2001; Neild 2008; Calhoun 2010; Gemmell & Marcus 2015). The New World members of this genus are the result of recent diversification (within the last 3 million years) from Old World ancestors (McCullagh 2016), and the New World species appear to hybridize with some frequency (Borchers & Marcus 2014; Gemmell *et al.* 2014).

There have been only 4 described subspecies of *J. coenia,* 2 of which occur in this region: the *nominate* subspecies *J. coenia coenia,* the common buckeye, and what I will refer to as *J. grisea,* the Northern or gray buckeye (Austin & Emmel 1998). The third subspecies, *J. coenia bergi* is found only in Bermuda (Avinoff 1926; Peters & Marcus In Press) and will not be considered further here. The fourth subspecies, *J. coenia nigrosuffusa* will be discussed below. When it was first described, *J. grisea* was documented to occur in California, Oregon, Nevada, and Arizona, and was thought likely to occur in at least the northern part of Baja California (Austin & Emmel 1998). Those who have sampled *J. coenia* in areas outside this region (including all of Mexico) have generally assumed that specimens belong to the nominate subspecies (Brown *et al.* 1992;

Beutelspacher 1996; Warren & Llorente 1999; Glassberg 2007), but the actual ranges of the two subspecies and the degree to which they hybridize in the wild is unknown (Gemmell & Marcus 2015). However, it is known that *J. grisea* can hybridize with congeners in the lab (Hafernik 1982).

Unlike almost all other New World *Junonia* subspecies and species, *J. grisea* is characterized by distinct mitochondrial haplotypes (Pfeiler *et al.* 2012a; Gemmell *et al.* 2014; Gemmell & Marcus 2015), which I will refer to as B^{CA} alleles. Based on these mitochondrial DNA sequences, it is more genetically distinct from the common buckeye, *J. coenia* (which is widespread in eastern North America and typically carries haplotype B alleles), than most full species of *Junonia* are from each other (Gemmell & Marcus 2015; McCullagh 2016). The divergence time between haplotypes B and B^{CA} has been estimated to be 1.18 ± 0.29 million years (McCullagh 2016). The subtle morphological differences in combination with the molecular distinctiveness suggest that *J. grisea* and *J.coenia* may be an example of a cryptic species pair. Cryptic species are often difficult to identify as separate species based on morphology alone, but can be differentiated using molecular tools (Smith *et al.* 2011). The characteristic mitochondrial haplotypes in *Junonia* can be used as a genetic marker to supplement identification by morphology (Table 4-1) to determine the range of *J. grisea*, and the extent to which there is genetic admixture between this form and other *Junonia.*

The dark buckeye, *J. nigrosuffusa,* has been alternately understood as a subspecies of *J. coenia* (Barnes & McDunnough 1916) or of *J. evarete* (Hafernik 1982; Wauer 2006), or as a full species (Tilden 1970; Brown *et al.* 1992). *Junonia nigrosuffusa* can be found throughout northern Mexico, south to the Mexican states of Veracruz and Oaxaca, and north to the US states of Arizona, New Mexico, and Texas. It occurs as a rare stray in many other Western US states. The primary larval host plant is *Stemmodia*, which is apparently avoided by other *Junonia* species (Tilden 1970). While *J. nigrosuffusa* mitochondrial genotypes have been sampled in Sonora, Mexico and Texas, USA, they exhibit typical haplotype B alleles and there are not yet any diagnostic genetic markers for this form (Pfeiler *et al.* 2012a; Gemmell & Marcus 2015), but its distinctive morphology, habitat, larval host plant preference, and geographic range suggest that it is an independent evolutionary lineage.

Populations of western mangrove buckeye, *J. litoralis,* were previously classified as *J. genoveva* (Opler & Warren 2002; Lamas *et al.* 2004; Neild 2008; Pfeiler 2011; Pfeiler *et al.* 2012a; Pfeiler *et al.* 2012b), while populations of the tropical or West Indian buckeye, *J. zonalis,* were previously assigned to *J. evarete* (Opler & Warren 2002; Lamas *et al.* 2004; Neild 2008; Pfeiler 2011; Pfeiler *et al.* 2012a; Pfeiler *et al.* 2012b). However, recent scholarship based on morphology (Brévignon & Brévignon 2011; Brévignon & Brévignon 2012) and molecular markers (Gemmell & Marcus 2015)(Chapter 2) have shown that *J. genoveva* and *J. evarete* are restricted to South America and are distinct from the forms considered here. I have made taxonomic assignments for the *Junonia* species of the American Southwest and Mexico on the basis of morphological characteristics (Table 4-1), larval host plant use (Table 4-2), (Brévignon & Brévignon 2011; Brévignon & Brévignon 2012) and available molecular characters (Gemmell *et al.* 2014; Gemmell & Marcus 2015). These characters suggest that the western mangrove buckeye, *J. litoralis* is conspecific with the mangrove-feeding *Junonia* taxon from South America (Gemmell *et al.* 2014), while the tropical or

Table 4-1. Morphological characteristics of the *Junonia* of the American Southwest and Mexico.

Attributes	***Junonia coenia***	***Junonia grisea***	***Junonia nigrosuffusa***	***Junonia litoralis***	***Junonia zonalis***
Forewing length	18-27.5 mm (Minno & Emmel 1993)	22-26.2 mm (Austin & Emmel 1998)	21-29 mm (This study)	31-32 mm (Brévignon 2009)	23-28 mm (Minno & Emmel 1993)
Subapical patches on dorsal forewings	Cream coloured or white (Glassberg *et al.* 2000)	Cream coloured (Austin & Emmel 1998)	Suffused with orange pigment or filled with grey or black pigment to match ground colour (Hafernik 1982)	Cream coloured or suffused with brown pigment to match ground colour (Brévignon 2009; Brévignon & Brévignon 2012)	Suffused with pink or orange pigment (Turner & Parnell 1985)
Forewing band	Surrounds larger eyespots; white in colour (Minno & Emmel 1993)	Surrounds larger eyespots; white in colour (Austin & Emmel 1998)	Orange band (Glassberg 2007)	White but brown towards the apex (Brévignon 2009)	Whitish-pink (Minno & Emmel 1993)
Dorsal Forewing anterior eyespot	Visible (This study)	Small, often Missing (This study)	Visible (This study)	Visible (This study)	Visible (This study)
Dorsal ground colour	Chocolate brown (Austin & Emmel 1998)	Greyish-brown (Austin & Emmel 1998)	Dark grey or black (Hafernik 1982)	Chocolate brown (Brévignon & Brévignon 2012)	Light to Chocolate brown Minno & Emmel 1993)
Orange Submarginal Bands on Dorsal Forewing and Hindwing	Typically wide and bright (this study)	Typically narrow or absent (this study)	Narrow or often absent (Hafernik 1982)	Very narrow (Brévignon & Brévignon 2012; Pfeiler et al. 2012)	Typically wide and bright (Turner & Parnell 1985)

Attributes	***Junonia coenia***	***Junonia grisea***	***Junonia nigrosuffusa***	***Junonia litoralis***	***Junonia zonalis***
Colouration on ventral hindwing	Prominent reddish submarginal band. Postmedian band is absent. Light tan to reddish ground colouration (Glassberg *et al.* 2000)	Variably prominent submarginal reddish band. Postmedian band is absent. Light to reddish ground colouration, red ground colour not as intense as in *J. coenia* (Austin & Emmel 1998; Daniels *et al.* 2012)	Highly variable reddish submarginal band. Postmedian band is prominent and white. Light tan to deep reddish ground colour, sometimes with iridescence (Hafernik 1982; Glassberg 2007)	Postmedian band is prominent but disjoint from the thin white submarginal band. Ground colouration typically light to medium brown. Ventral hindwings weakly marked (but more strongly marked when compared with *J. neildi* from Florida and the Caribbean (Brévignon 2009; Brévignon & Brévignon 2012)	Highly variable reddish submarginal band. Postmedian band is prominent and white. Light tan to deep reddish ground colouration (Turner & Parnell 1985; Minno & Emmel 1993)
Eyespots on ventral hindwing	Prominent (Forbes 1928)	Usually prominent (Austin & Emmel 1998)	Varies among individuals from prominent to obscure (Hafernik 1982)	Barely visible but of equal size (Brévignon 2009)	Prominent (Turner & Parnell 1985)
Eyespots on dorsal hindwings	Anterior eyespot larger than posterior (Forbes 1928)	Anterior eyespot larger than posterior (Austin & Emmel 1998)	Anterior eyespot larger than posterior (Glassberg 2007)	Anterior eyespot is nearly identical in size to posterior (Brévignon 2009)	Anterior eyespot larger than posterior (Turner & Parnell 1985)

Attributes	***Junonia coenia***	***Junonia grisea***	***Junonia nigrosuffusa***	***Junonia litoralis***	***Junonia zonalis***
Antennae	Dark undersides of antennal tips, dark antennal tips (This study)	Dark undersides of antennal tips, dark antennal tips (This study)	White undersides of antennal tips, typically white antennal tips (rarely dark antennal tips; This study)	Black dorsal surface and brown ventral surface of shaft, brown to black antennal tips (Brévignon 2009)	Pale underside of tip that is similar in colour to that of the ventral shaft, dark antennal tip (Calhoun 2010)
Habitat preferences	Grassland, salt marsh and sand dune habitats (Paulsen 1996; Glassberg *et al.* 2000)	Grassland and other open areas, agricultural areas, disturbed areas, dry ravines, and near water courses (Scott 1975; Stout 2016)	Arid open areas (Glassberg 2001), sand dunes, and along water courses (Hafernik 1982)	Mangrove swamps (Brévignon 2009)	Grassland, salt marsh and sand dune habitats (Turner & Parnell 1985; Glassberg *et al.* 2000)

Attributes	***Junonia coenia***	***Junonia grisea***	***Junonia nigrosuffusa***	***Junonia litoralis***	***Junonia zonalis***
Larval Host Plants	Plantain (*Plantago sp.*), black-senna (*Seymeria sp.*), false foxglove (*Agalinis sp.*), and toadflax (*Linaria sp.*) (Tilden 1970; Glassberg *et al.* 2000)	Plantain (*Plantago sp.*) (Shapiro 1974; Scott 1975), cutleaf indian paintbrush (*Castilleja lacera*) (Shields 1966; Bowers 1984; Knerl & Bowers 2013), purple owls clover (*Castilleja exserta exserta*) (Orsak 1977; Bowers 1984; Knerl & Bowers 2013), orange bush monkey flower (*Diplacus aurantiacus*)(Shapiro 1974), azure penstemon (*Penstemon azureus*) (Shapiro 1978; Bowers 1984), lanceleaf frogfruit (*Lippia lanceolata*) (Shapiro 1974)	Frogfruits, ruellias (Glassberg 2001), *Stemodia tomentosa* (Tilden 1970), false foxglove (*Agalinis sp.*), *Mimulus sp., Veronica sp*. (Hafernik 1982)	Black mangrove (*Avicennia germinans*) (Brévignon 2009; Brévignon & Brévignon 2012)	Blue Porterweed (*Stachytarpheta jamaicensis*) (Glassberg *et al.* 2000), also possibly frog fruit (*Lippia nodiflora*) (Brown & Heineman 1972)

Table 4-2. Life cycle data for the 5 *Junonia* species found within the American Southwest and Mexico.

	Junonia coenia	***Junonia grisea***	***Junonia litoralis***	***Junonia nigrosuffusa***	***Junonia zonalis***	***Junonia neildi***
Days to Hatching	4-6	3	-	3-4	3-4	6-9
Days as Larvae	30-35	14-18	~21	26-28	25-27	24-28
Days as pupae	5	9	7-10	5-8	5-8	7-10
Entire life cycle	40-45	26-30	-	34-40	33-39	37-47
Pupal mass	mean = 0.346 g st. dev. = 0.053 g N = 118	mean = 0.392 g st. dev. = 0.049 g N = 155	Not available	mean = 0.341 g st. dev. = 0.048 g N = 32	mean = 0.327 g st. dev. = 0.050 g N = 82	mean = 0.550 g st. dev. = 0.083 g N = 134
Population Source	Everglades Greenway, Miami-Dade Co. FL	Point Richmond, Contra Costa Co., CA	Sonora Mexico (Pfeiler 2011)	South Padre Island, Cameron Co. TX	Everglades Greenway, Miami-Dade Co. FL	ORCA, Indian River Co. FL
Larval diet data	*Plantago lanceolata, P. major*	*Plantago lanceolata, P. major*	*Avicennia germinans*	*Plantago lanceolata, P. major*	*Plantago lanceolata, P. major*	*Plantago lanceolata, P. major*

West Indian buckeye, *J. zonalis* is conspecific with a polyphagous *Junonia* taxon that occurs in Central America, the Caribbean and in south Florida (Chapter 2).

This impressive diversity, in combination with the powerful experimental tools available for the study of the developmental biology of morphology and color patterns in *Junonia* (Carroll *et al.* 1994; Marcus 2005; Nijhout 2010; Dhungel *et al.* 2013; Beaudette *et al.* 2014; Daniels *et al.* 2014), provides a remarkable opportunity to study phenotypic change in a crucible of evolutionary novelty and innovation. As a step towards exploring this promising system for understanding the evolution of phenotype in the natural environment, I have conducted an extensive survey of morphology and mitochondrial genotypes from hundreds of *Junonia* museum specimens. This has been supplemented by rearing experiments, and reviews of published larval host plant records and life history data in order to distinguish among *Junonia* forms, map their distributions, and observe patterns of gene flow, hybridization, and the identification of a cryptic species.

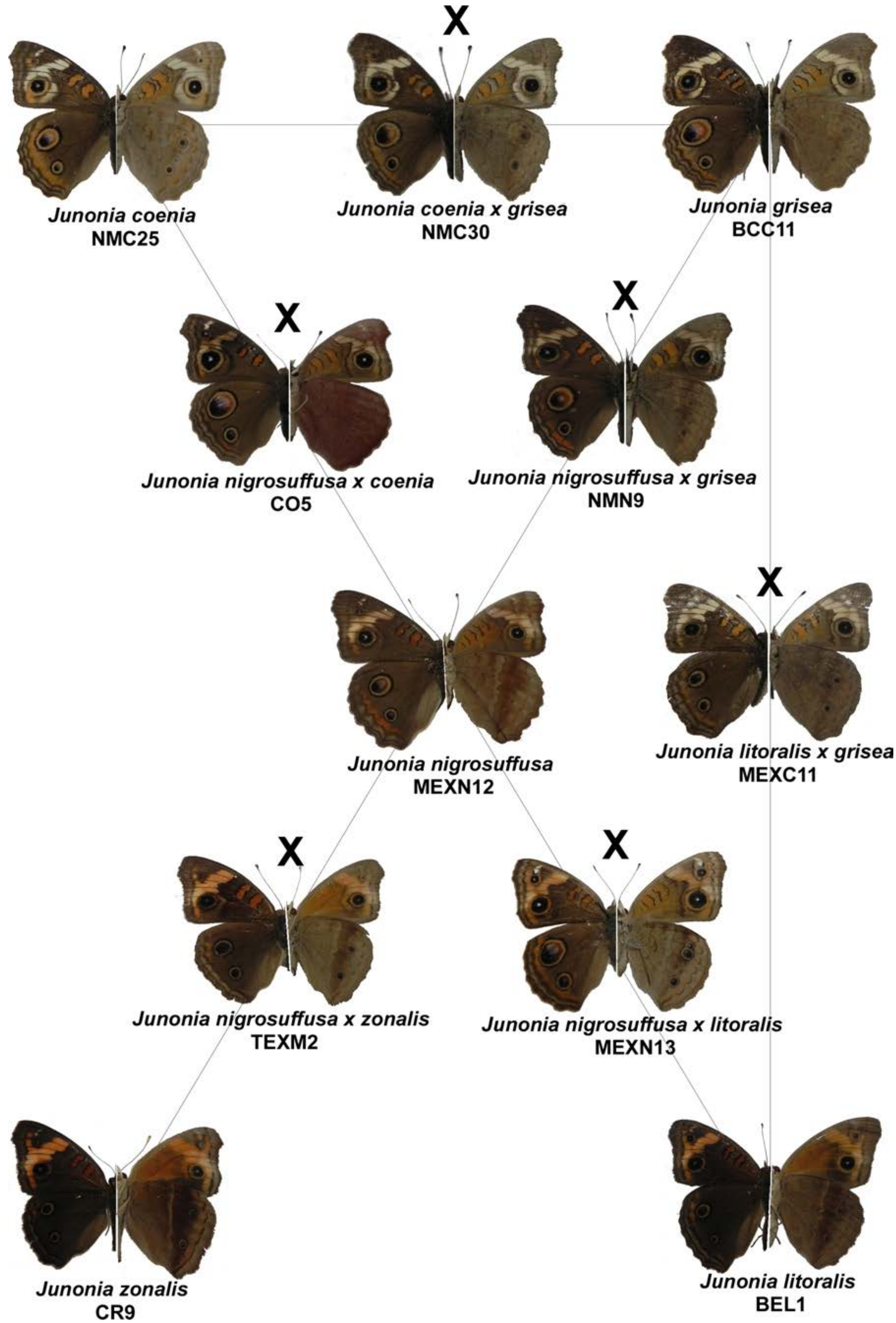
Material & Methods

***Junonia grisea* Life History.** Live specimens of *Junonia grisea* were collected from the Miller-Knox Regional Shoreline, (Port Richmond, Contra Costa County, California, USA) with hand-held butterfly nets and stored at 4°C until arrival at the laboratory. The individuals were released into a plexiglass flight cage and allowed to oviposit on *Plantago major*. Larvae were reared on *Plantago* (*P. major* and *P. lanceolata*) at 25°C and a 12h light/12h dark photoperiod until they pupated. Live pupae were weighed using an electronic balance. Life history data was available for *J. neildi*, *J. zonalis*, *J.*

nigrosuffusa and, *J. coenia* from previous unpublished experiments from the Marcus laboratory. Data for *J. litoralis* is from (Pfeiler 2011). Larval host plant data was collected from diverse sources from the literature (Table 4-1).

Specimen Collection and Preparation. Specimens were obtained from both museum collections and private collectors (Appendix 3). Specimens were chosen based on specific geographic location (California, New Mexico, Arizona, Texas, Oregon, Nevada, Oklahoma, Colorado, Wyoming, and Mexico) and identified on the basis of morphological characteristics (Table 4-1; (Turner & Parnell 1985; Neild 2008; Calhoun 2010; Brévignon & Brévignon 2012)). Most specimens were easily assigned to species on the basis of the phenotypic characteristics, but a subset had features that were intermediate between two *Junonia* species, which I interpreted as being possible hybrids (Figure 4-1).

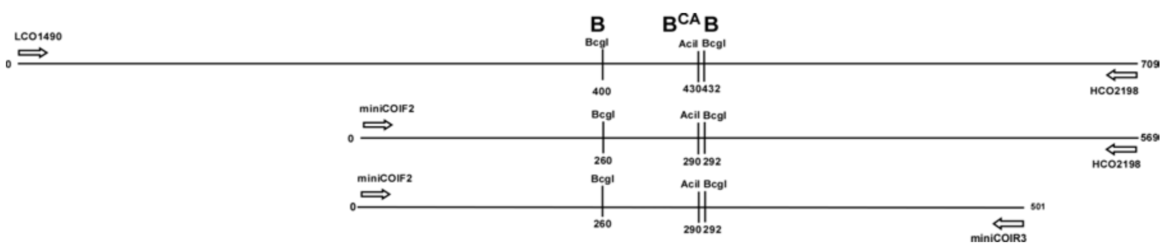
Figure 4-1. Dorsal (left) and ventral (right) wing surfaces of the 5 *Junonia* species that occur in the American Southwest and Mexico, with the putative hybrid forms that have been observed between them (indicated by X above the image). Lines have been drawn between species that appear to have produced hybrid offspring included among the specimens sampled here. Several of these hybrid types have been observed previously in the wild in this study region (Hafernik 1982). *Junonia coenia* X *J. zonalis* putative hybrids were not observed in the specimens sampled, but have been observed by others in south Texas (Hafernik 1982) and in Florida (Chapter 3). *Junonia zonalis* X *J. litoralis* hybrids have not yet been observed, but *J. zonalis* does appear to hybridize rarely with *J. neildi* in Florida (Chapter 3). *Junonia litoralis* and *J. neildi* feed on the same larval host plant, *Avicennia germinans,* and may be sister taxa.


X
Junonia coenia
NMC25
Junonia coenia x grisea
NMC30
Junonia grisea
BCC11
X
Junonia nigrosuffusa x coenia
CO5
X
Junonia nigrosuffusa x grisea
NMN9
X
Junonia nigrosuffusa
MEXN12
Junonia litoralis x grisea
MEXC11
X
Junonia nigrosuffusa x zonalis
TEXM2
X
Junonia nigrosuffusa x litoralis
MEXN13
Junonia zonalis
CR9
Junonia litoralis
BEL1

DNA was isolated from a single leg with the Qiagen DNEasy Blood and Tissue kit, either manually or with the assistance of a Qiagen QIAcube extraction robot (Qiagen, Düsseldorf, Germany) using the animal tissue DNA program, following the manufacturers protocol with modifications as previously described (Gemmell & Marcus 2015). In addition, in order to improve mitochondrial DNA recovery from museum specimens, the lysis buffer from the DNEasy kit was replaced with a lysis buffer commonly used for DNA extractions from mouse tail tips (1% SDS, 0.1M NaCl, 0.1M EDTA, 0.05M Tris, and deionized distilled water) DNA concentration of each sample was evaluated using a Nanodrop 2000 spectrophotometer (Nanodrop, Wilmington, Delaware, USA) and then stored at -20˚C.

Mitochondrial *Cytochrome Oxidase I* (*COI*). The *Cytochrome oxidase subunit I* (*COI*) gene was amplified by polymerase chain reaction (PCR) and the gene specific primers LCO1490 (GGT CAA CAA ATC ATA AAG ATA TTG G) and HCO2198 (TAA ACT TCA GGG TGA CCA AAA AAT CA) (Folmer *et al.* 1994),which yield a 709 base pair product (including primer, 658 bp without). PCR reaction conditions were: 95˚C for 5 minutes; 35 cycles of 94˚C for 1 minute, 46˚C for 1 minute, 74˚C for 1 minute, 94˚C for 1 minute; and a final extension for 5 minutes at 72˚C, then a 4˚C hold. Visualization of amplification products from samples were run on a Qiagen QIAxcel Advanced capillary electrophoresis instrument fitted with a DNA Screening Cartridge with QX Size Markers (250 bp–4 kb v. 2.0) and QX Alignment Markers (50 bp-5 kb) using the AL320 electrophoresis method as reported previously (Gemmell & Marcus 2015). If satisfactory bands were detected, a diagnostic restriction enzyme digest was performed using AciI and BcgI restriction endonucleases (New England Biolabs (NEB), Ipswich, MA, USA) to determine the haplotype group of each specimen (Figure 4-2).

Figure 4-2. Restriction digest map used for the determination of mitochondrial haplotype groups. The top line represents the *cytochrome oxidase subunit I* (COI) amplification product obtained using the primers LCO1490 and HCO2198. The middle line represents a smaller amplification product created using the primers miniCOIF2 and HCO2198. The bottom line represents the smallest amplification product obtained using the primers miniCOIF2 and miniCOIR3. The specific enzyme cut sites for BcgI and AciI are shown using a vertical bar. BcgI restriction sites are found solely in haplotype group B alleles whereas AciI restriction sites are found exclusively in haplotype group B^{CA} alleles. The base position of each cut site in each PCR product is indicated below the vertical line and the haplotype associated with each cut is indicated above the line.



AciI and BcgI have cut sites within 2 base pairs of each other (Figure 4-2) therefore similar sized restriction digest products are produced. To determine haplotype, the diagnostic restriction enzyme digest was then performed using two separate digests (one for each enzyme, per sample), run in parallel. For AciI, 10µL of the PCR product mixed with 2µL Cutsmart Buffer, 7.5 µL deionized distilled water, 0.5µL AciI in a 1.5mL microcentrifuge tube. For BcgI, 10 µL of the PCR product was mixed with 2 µL

NEB Buffer 3.1, 7 µL deionized distilled water, 0.5 µL SAM, and 0.5 µL BcgI in a 1.5 mL microcentrifuge tube. For each reaction the incubation and enzyme deactivation were identical (incubated at 37˚C for 1 hour in a water bath; deactivation at 70˚C for 10 minutes in a water bath. The digested products were then separated with a QIAxcel Advanced instrument as described above, with each sample pair. Haplotypes were assigned based on the presence of which enzyme cut the PCR product and specific band sizes obtained: Haplotype Group B with 3 bands (400 bp, 277 bp and 32 bp) due to the BcgI restriction site (note that unlike most restriction endonuclease enzymes, BcgI makes 2 cuts per target site, exactly 32 bp apart), and Haplotype Group B^{CA} with 2 bands (430 bp and 279 bp) due to the AciI cut site (Figure 4-2).

If no detectable PCR products were obtained from the first amplification, they were reamplified using miniCOIF2 (ATA CTA TTG TTA CAG CCT CAT GC) (Gemmell *et al.* 2014) and HCO2198 , yielding a shorter 569 base pair product (520bp without primers). The same PCR program and visualization method was used as described above. The individual PCR products were then assigned to haplotype groups utilizing the same diagnostic restriction enzyme digest as described above, as all enzyme cut sites are present within the smaller PCR fragment (Figure 4-1). The digested products were then visualized as described above. Haplotypes were assigned based on which enzyme cut which PCR product, and the specific band sizes obtained: Haplotype Group B yields 3 bands (277 bp, 260 bp and 32 bp) due to the BcgI restriction site, and Haplotype Group B^{CA} yields 2 bands (290 bp and 279 bp) due to the AciI cut site (Figure 4-2). If no PCR products were obtained from the second amplification they were once again reamplified using miniCOIF2 and miniCOIR3 (TAT TTC GAT CTG TTA AAA GTA

TAG)(Gemmell & Marcus 2015), which yields a 501 base pair product (454 bp without primers). The same protocols described above were used for amplification and visualization. Haplotypes were assigned based on the presence of which enzyme cut the PCR product and specific band sizes obtained: Haplotype Group B with 3 bands (241 bp, 209 bp and 32 bp) due to the BcgI restriction site, and Haplotype Group B^{CA} with 2 bands (290 bp and 211 bp) due to the AciI cut site (Figure 4-2). A very small number of PCR products (N=3) cut with neither BcgI nor AciI. These were further evaluated for the presence of haplotype group A by restriction digest by BamH1 as previously described (Gemmell & Marcus 2015) (Chapters 2 and 3). *Junonia* haplotype group A is very rare in North America, but is common in South America and the Caribbean. In all cases, when these products were digested with BamH1, they could be assigned unambiguously to Haplotype A.

Haplotype map generation. Samples were sorted by collection locality and species and grouped together by county in the United States and by state in Mexico. For each grouping, the total number of specimens with haplotype groups B, B^{CA}, and A were tallied for each species. Pie charts for individual species were created using Illustrator CS6 (Adobe, San Jose, CA, USA), for the proportion of haplotypes. The area of each pie graph was standardized and made proportional to the total sample size from each collection locality. Using Canvas 14 (ACD Systems, Seattle, WA, USA), pie charts were added to a map of the American Southwest and Mexico and were grouped and positioned according to locality. Core species ranges (determined from observations of collectors in the Marcus lab, the distribution of museum specimens and consultation with published sources such as (Hafernik 1982)) were added to the map to distinguish between samples collected within the normal range of each *Junonia* species and specimens that were collected as rare strays. Finally, rare specimens that appear to be intermediate between 2 *Junonia* species based on colour pattern phenotypes and other morphological characters were identified and mapped separately in order to delimit possible hybrid zones.

Heat map generation. Specimen haplotypes for all species were grouped together by county in the United States and by state in Mexico. Heat maps were generated for both the percent haplotype B^{CA} and B, as well as the total number of B and B^{CA} haplotypes for each place using OpenHeatMap (Warden 2010).

Results

***Junonia* Life Histories and larval host plant use.** The life history traits of each of the five *Junonia* species that occur in the study region are quite distinct. Life cycle data was collected within the Marcus Lab under uniform conditions over a period of several years, except for *J. litoralis* where data was obtained from the literature (Pfeiler 2011). *Junonia grisea* develops more quickly (26-30 days) than any other species in the American Southwest and Mexico, followed by *J. zonalis, J. nigrosuffusa, J. coenia,* and *J. neildi* had the longest life cycle (37-47 days; Table 4-2). Complete life cycle data are not yet available for *J. litoralis,* and it has not yet been reared on *Plantago* to facilitate comparisons, but the available data suggest that its life cycle is very similar to *J. neildi* (to which it is also morphologically similar and probably closely related). There is no clear relationship between length of the life cycle and body size: *J. grisea* had the shortest life cycle, but the mean pupal mass for this species was one of the largest at 0.392±0.049 g; *J. neildi* had the longest life cycle and had the largest body size of 0.550±0.083 g; *J. zonalis* have the smallest pupal mass at 0.327±0.050 g and a life cycle of intermediate length (33-39 days) (Table 4-2).

Larval host plant use was compiled separately for native and introduced plant species for all five *Junonia* species that occur in the American Southwest and Mexico (Table 4-3; Table 4-4). There is very little overlap between *Junonia* species with respect to native host plant use (the overlap that exists is due to isolated use of alternate native host plants by *J. nigrosuffusa* when its primary hosts in the genus *Stemodia* are unavailable) (Table 4-3). While their native host plants are distinct, all five species of *Junonia* can be reared on the same two introduced larval host plants (*Plantago lanceolata* and *P. major*) in the laboratory (Table 4-4). Two species, *J. coenia* and *J. grisea,* have been able to switch to using many non-native plant species as larval hosts in the wild, and have substantial overlap in introduced host plant use (Table 4-4).

Biogeographic Species Distributions. Core ranges of each species were inferred based on the density of specimens in museum collections reviewed for this study in combination with previous reports from the literature. In the study region, *Junonia coenia* is distributed throughout Texas and Oklahama, as well as much of New Mexico and Arizona, and the southeastern corner of Colorado. It is also found throughout much of Mexico, reaching the Mexican states of Guerrero and Oxaca on the Pacific coast (Figure 4-3). Outside of the study region, it occurs throughout the eastern United States, on the Niagara Peninsula of Ontario (Canada) as well as in Cuba, the Bahamas, and Bermuda

(Gemmell & Marcus 2015) (Chapter 2). Sampling in this study is less dense in Mexico than in the United States for all *Junonia* species, so consequently the definition of this portion of the core ranges is less certain in all cases. The predominant haplotype for all *J. coenia* genotyped is haplotype group B. Haplotype B^{CA} does occur in this species with most examples in the most western parts of the *J. coenia* core range (Arizona and New Mexico). Stray individuals of *J. coenia* have been collected from the western Great Plains (E.g. northern Colorado, Wyoming), and in California, with a large cluster of them (N=11) in the region surrounding San Francisco Bay.

The range of *J. grisea* includes southern Oregon, California, Arizona, New Mexico, and the westernmost part of Texas in the United States, and at least the northern parts of the Mexican states of Baja California du Notre, Sonora, Chihuahua, and Coahuila (Figure 4-4). The predominant mitochondrial haplotype in this species is haplotype group B^{CA}, although haplotype B does occur in some specimens, especially in the San Francisco Bay area, and in Arizona and New Mexico where the range of *J. grisea* overlaps with the core ranges of *J. coenia* and *J. nigrosuffusa.*

The distribution of *J. nigrosuffusa* includes Arizona, southeastern and central New Mexico, Baja California del Sud, west and south Texas and much of northern and central Mexico (Figure 4-5). The principle haplotype group for *J. nigrosuffusa* is haplotype group B, although haplotype group B^{CA} occurs in this species in the parts of its range that overlap with or are close to the core range of *J. grisea*. One specimen of *J. nigrosuffusa* from Veracruz, Mexico carries an allele from haplotype group A, which is more common in the Caribbean and in South America.

Table 4-3. Native larval host plant preferences for the *Junonia* of the American Southwest and Mexico.

Order	**Family**	**Species**	***Junonia coenia***	***Junonia grisea***	***Junonia nigrosuffusa***	***Junonia neildi***	***Junonia zonalis***
Lamiales							
	Acanthaceae	*Avicennia germinans*				X	
	Orobanchaecae	*Agalinis* (*Gerardia*) *purpurea* (and other *Agalinis* species)	X		X		
		Castilleja exserta exserta (=*Orthocarpus pupurascens*)		X			
		Castilleja lacera (=*Orthocarpus lacerus*)		X			
	Plantaginaceae	*Plantago coronopus*		X			
		Plantago hookeriana		X			
		Nattallanthus (=*Linaria*) *Canadensis*	X				
		Veronica sp.			X		
	Phyrmacceae	*Diplacus aurantiacus*		X			
		Mimulus sp.			X		
	Scrophulariaceae	*Buchnera floridana*	X				
		Penstemon azureus		X			
		Stemodia tomentosa (=*Stemodia lanata*)			X		
		Stemodia durantifolia			X		
	Verbenaceae	*Lippia* (=*Phyla*) *lanceolata*		X	X		
		Stachytarpheta jamaicensis					X

Table 4-4. Introduced larval host plant preferences for the *Junonia* of the American Southwest and Mexico.

Order	Family	Species	***Junonia coenia***	***Junonia grisea***	***Junonia nigrosuffusa***	***Junonia neildi***	***Junonia zonalis***
Cornales	Cornaceae	*Acuba japonica*	X				
Lamiales	Plantaginaceae	*Plantago lanceolata*	X*	X*	X*	X*	X*
		Plantago major	X*	X*	X*	X*	X*
		Cymbalaria muralis		X			
		Kickxia elatine		X			
		Kickxia spuria		X			
		Linaria maroccana	X	X			
		Linaria vulgaris	X	X			
Lamiales	Scrophulariaceae	*Antirrinum majus*	X	X			
		Diascia vigilis	X				
		Rusella equisetiformis	X				
Lamiales	Verbenaceae	*Verbena bornariensis*		X			

* indicates that the larval host plant has been used successfully for rearing the species in a laboratory setting.

Figure 4-3. Sketch of the American Southwest and Mexico showing the distributions of *Junonia coenia* with collection localities, and *cytochrome oxidase subunit I* (*COI*) haplotype group assignments. For this and subsequent figures, the area of each circle is proportional to the number of samples from each locality. Haplotype group B is represented by the black areas in each pie graph and, haplotype group B^{CA} is represented by the white areas. When present, haplotype group A is represented by grey sectors of the pie graph. The shaded grey portion of the map represents the core range of the species distribution.

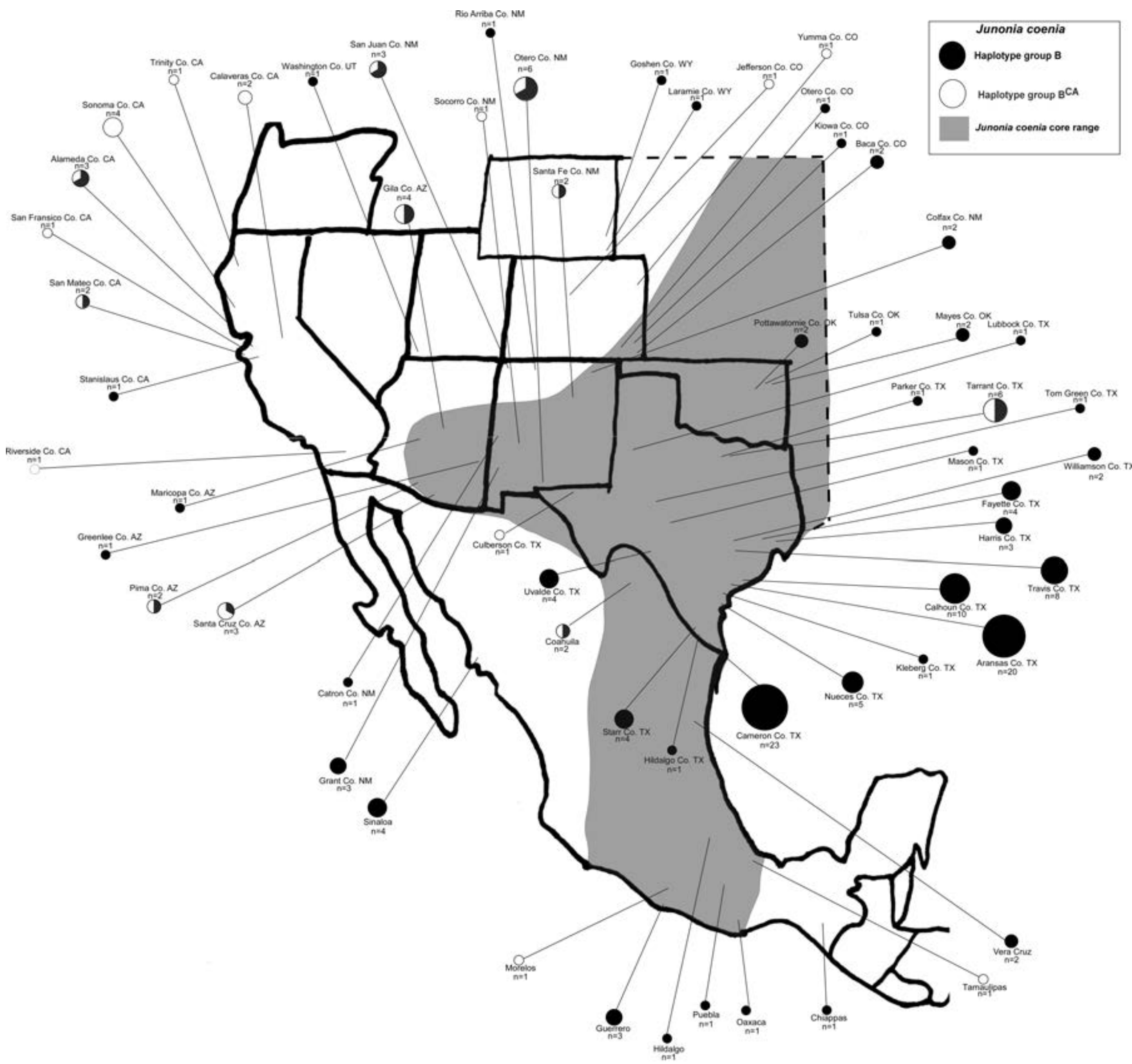


Figure 4-4. Sketch of the American Southwest and Mexico showing the distributions of *Junonia grisea* with collection localities, and *cytochrome oxidase subunit I* (*COI*) haplotype group assignments.

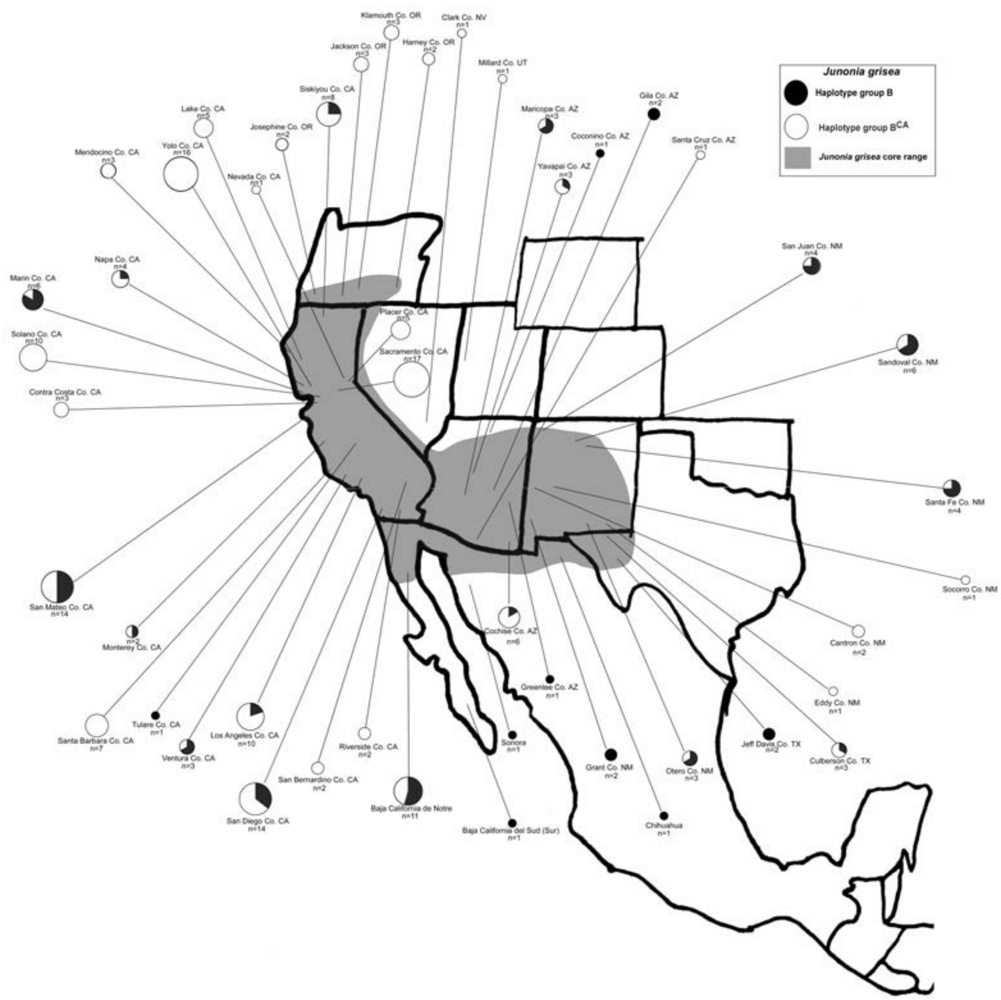
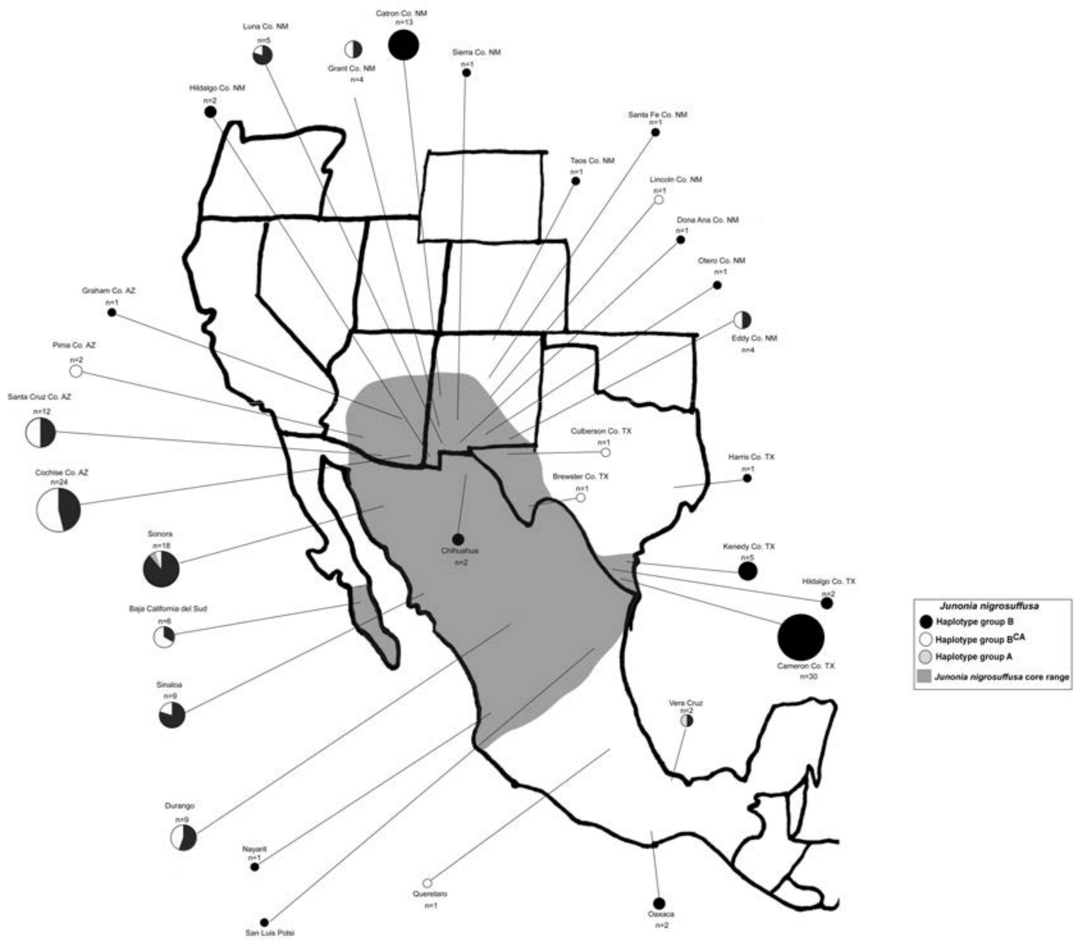
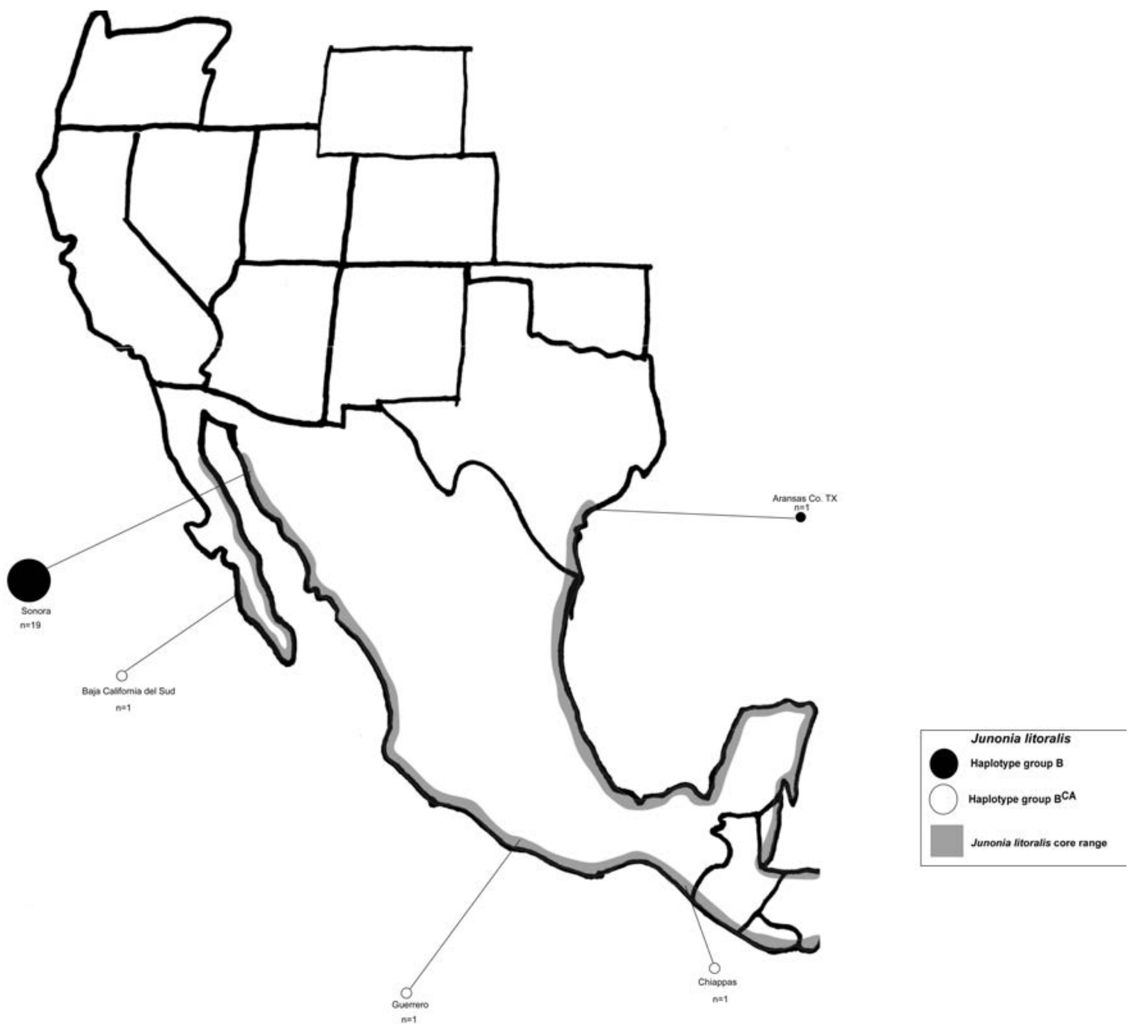


Figure 4-5. Sketch of the American Southwest and Mexico showing the distributions of *Junonia nigrosuffusa* with collection localities, and *cytochrome oxidase subunit I* (*COI*) haplotype group assignments.



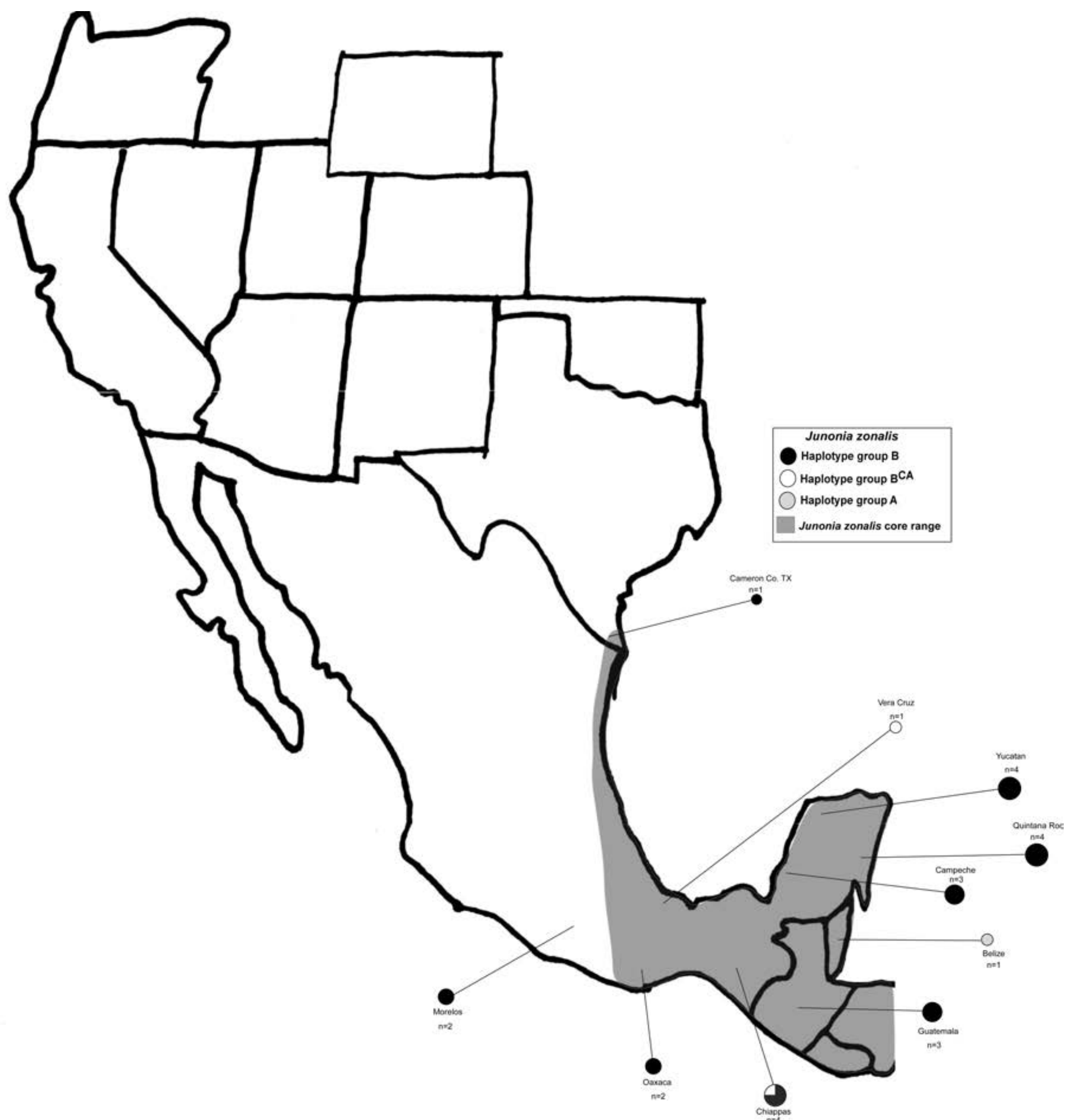
Junonia litoralis uses black mangrove (*Avicennia germinans*) almost exclusively as its larval host plant in the wild, so its distribution is restricted to the coastal regions of Mexico (including Baja California) and south Texas (Figure 4-6). This species is not well-represented in the samples examined for this study, but based on the available data, the most northern populations of *J. litoralis* appear to be exclusively haplotype group B while specimens with haplotype group B^{CA} do occur further south in Mexico (N=3).

Figure 4-6. Sketch of the American Southwest and Mexico showing the distributions of *Junonia litoralis* with collection localities, and *cytochrome oxidase subunit I* (*COI*) haplotype group assignments.



In the study region, *J. zonalis* is restricted to the most southern regions of Mexico and along the Gulf Coast (Figure 4-7). Beyond the study region, *J. zonalis* occurs

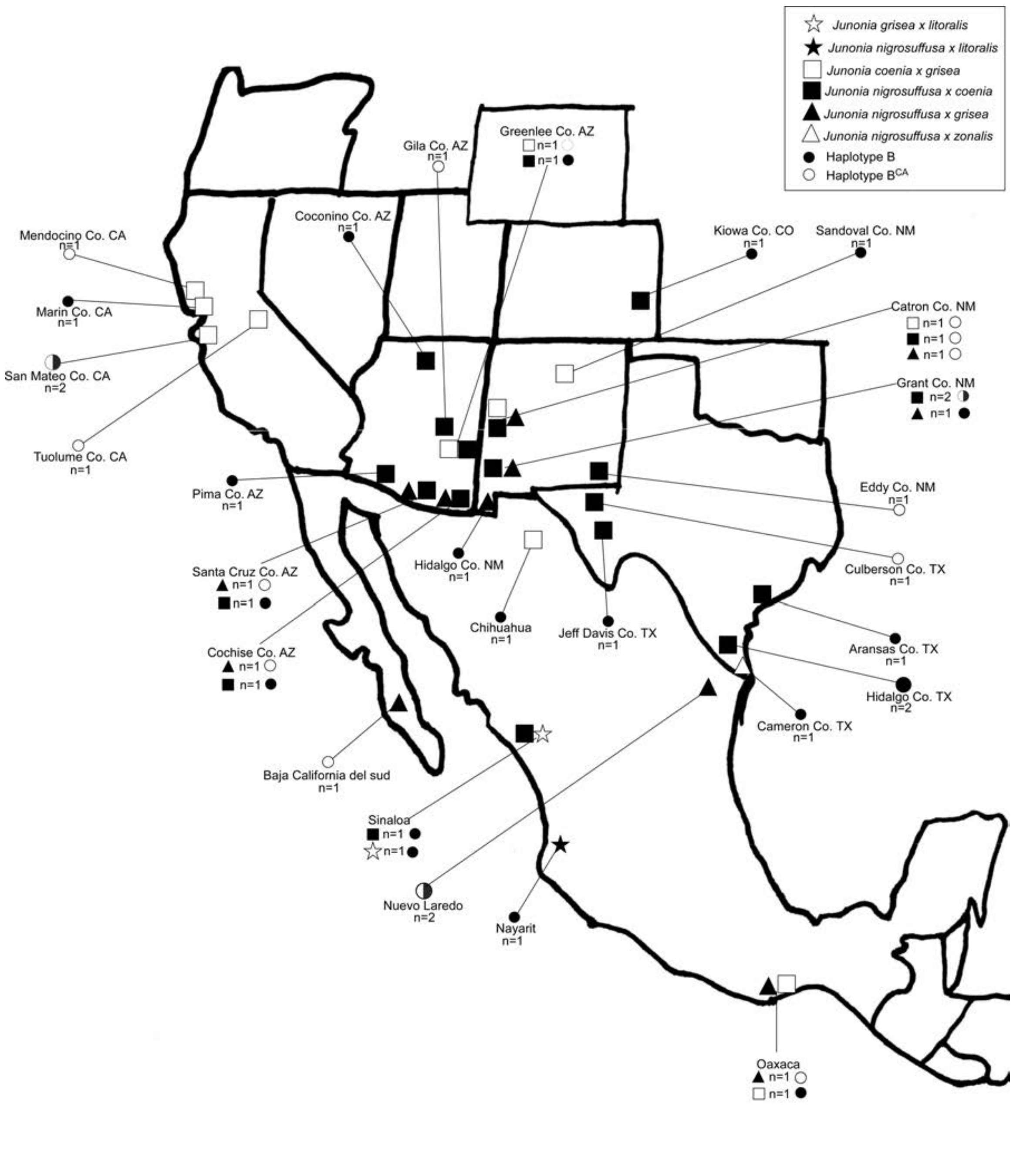
Figure 4-7. Sketch of the American Southwest and Mexico showing the distributions of *Junonia zonalis* with localities, and *cytochrome oxidase subunit I* (*COI*) haplotype group assignments.



throughout Central America, Colombia, the Caribbean, and in South Florida (Gemmell & Marcus 2015) (Chapter 2). The principle haplotype group for the samples that I studied is haplotype group B, which is consistent with prior findings that in Central America, this species primarily carries alleles from this group (Gemmell & Marcus 2015). One haplotype group B^{CA} allele was found in *J. zonalis* from each of Chiappas and Veracruz, Mexico. One haplotype group A allele was obtained from specimens from Belize, and there are rare haplotype group A alleles known from *J. zonalis* in both Costa Rica and Panama (Gemmell & Marcus 2015).

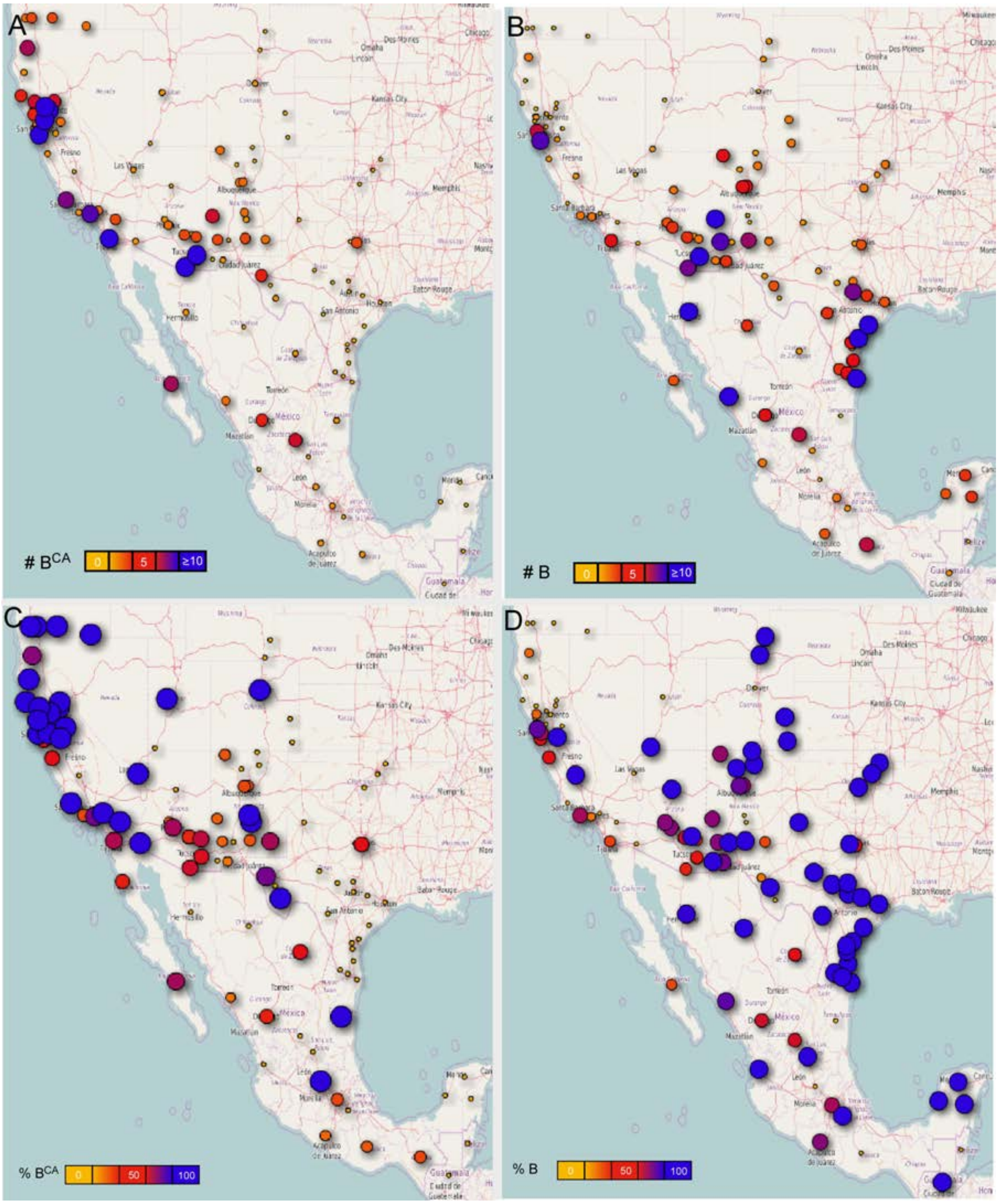
***Junonia* hybrids.** Using morphological characteristics I detected the presence of six different classes of *Junonia* with intermediate morphological and colour pattern phenotypes in the study region, which I am interpreting as likely hybrids (Figure 4-1). The highest frequency of hybrids was found in localities in the south and eastern portions of Arizona and from western New Mexico (Figure 4-8) where the range of *J. nigrosuffusa* (Figure 4-5) overlaps with the ranges of *J. coenia* (Figure 4-3) and *J. grisea* (Figure 4-4). The most common hybrids encountered in collections was *J. nigrosuffusa* X *J. coenia* (17 out of 39 total hybrids). *Junonia coenia* X *J. grisea* (10/39) and *J. nigrosuffusa* X *J. grisea* (9/39) specimens were found at almost an equal frequency. The three remaining hybrid types (*J. grisea* X *J. litoralis*, *J. nigrosuffusa* X *J. litoralis*, and *J. nigrosuffusa* X *J. zonalis*) are represented by a single specimen each.

Figure 4-8. Sketch of the American Southwest and Mexico showing the distributions of *Junonia species* hybrids using collection localities, and *cytochrome oxidase subunit I* (*COI*) haplotype group assignments from this study. The specific hybrid combinations are shown in the figure legend.



Biogeographic Haplotype distributions. Heat maps were generated to observe the total number (Figure 4-9 A,B) and percentage (Figure 4-9 C,D) of haplotypes B^{CA} and B irrespective of species based on collection locality. Haplotype B^{CA} predominates along the Pacific coast (Figure 4-9 A,C), while haplotype B is the predominant allele east of the Rocky Mountains and in most of Mexico (Figure 4-9 B,D). Zones of admixture appear to be in the region around San Francisco Bay, and in Baja California, Arizona and New Mexico.

Figure 4-9. The distributions of *cytochrome oxidase subunit I* (*COI*) haplotype groups in the American Southwest and Mexico pooled across all 5 *Junonia* species. (A) the number of haplotype group B^{CA} alleles sampled from each locality, (B) the number of haplotype group B alleles sampled from each locality, (C) the percent frequency of haplotype group B^{CA} alleles from each locality, and (D) the percent frequency of haplotype group B alleles from each locality.


A
B
C
D
B^CA
0
5
≥10
B
0
5
≥10
% B^CA
0
50
100
% B
0
50
100

Discussion

Mitochondrial Haplotypes. In the Western hemisphere there are two principle haplotype groups that are shared by all *Junonia* species: Haplotype group A and Haplotype group B (Pfeiler *et al.* 2012a; Gemmell *et al.* 2014; Gemmell & Marcus 2015). There is a variant of haplotype group A (A_1) that occurs in *J. vestina* at high elevation in the South American Andes, but this haplotype does not occur in the study region and will not be considered further for the purposes of this project (Gemmell & Marcus 2015; McCullagh 2016). Haplotype group B is most predominant in North America and Central America while Haplotype group A is most predominant in South America (Gemmell & Marcus 2015). The Caribbean seems to be a zone of genetic admixture having varying frequencies of each haplotype, with haplotype group A frequencies decreasing in the northern parts of the Caribbean (Gemmell & Marcus 2015). A distinctive variant of haplotype group B occurs in California populations of *Junonia* (Pfeiler *et al.* 2012a; Gemmell & Marcus 2015) and which I refer to as haplotype group B^{CA}.

Haplotype B^{CA} was originally believed to occur solely in California populations of *Junonia grisea* (Pfeiler *et al.* 2012a; Gemmell & Marcus 2015) and that *J. grisea* was the only species of *Junonia* in California. It appears that this is not the case: in the current study haplotype B^{CA} has been documented in five *Junonia* species in the study region. Further, a population of *J. coenia* appears to occur in the San Francisco Bay area of California. Haplotype group B is also found in scattered locations and at low frequency throughout California. All haplotype group B alleles are very similar in sequence composition, throughout its distribution, including eastern and western North America, the Caribbean, and the northern parts of South America (Gemmell *et al.* 2014; Gemmell

& Marcus 2015; McCullagh 2016). Understanding the sequence divergence of haplotypes and the distributions in the Western Hemisphere are useful for understanding which species were together in which glacial refuges, when species came back into contact (suture zones), and patterns of hybridization.

Diversification of haplotype B^{CA} from haplotype B in the new world is quite recent, 1.18±0.29 million years ago (mya) (McCullagh 2016; Chapter 1; Table 1-1). This time point coincides with the maximum glacial advance of the Pre-Illinoian glaciation period (and of the entire Pleistocene) 1.2 mya which at times of glacial advance would have pushed North American species into southern glacial refuges (Wanner *et al.* 2008; Rovey & Balco 2011; Roberts & Hamann 2015). At the glacial maximum, North American species groups would have been split into eastern and western glacial refuges allowing for enough time for the diversification of haplotypes to take place. *Junonia grisea* is thought to have taken refuge in Baja California (a western glacial refuge), as based on habitat reconstructions, its preferred open grassland/desert scrub habitat and its larval host plants would have been present at this time (Scott 1975; Ceballos *et al.* 2010; Stout 2016). Eastern populations of *J. coenia* may have been restricted to refuges in Florida or the Caribbean at this stage of glacial advance. This east-west split is likely the origin of both the *J. coenia*-*J. grisea* divergence and the haplotype B-haplotype B^{CA} divergence.

More recent glacial events (e.g. the Illinoisan and Wisconsin glaciations), probably also forced North American *Junonia* into glacial refuges, but as these glacial advances were not as extensive as the 1.2 mya event, the number and placement of the refuges may have differed somewhat. During the Wisconsin glaciation (85,000-11,000 ya), it appears that there may have been 3 or more glacial refuges. In addition to Florida and Baja California, Northeastern Mexico and South Texas featured temperate grassland habitat suitable for *Junonia* (Ceballos *et al.* 2010). The western *J. grisea* population may have retreated to Baja California as it did in previous glacial events, but the eastern *Junonia* populations may have found refuge in both the Florida/Caribbean refuge and in Northeastern Mexico/South Texas, each carrying haplotype group B. The populations in Northeastern Mexico and South Texas (western refuge) are speculated to have evolved distinct characteristics from the eastern populations giving rise to *J. nigrosuffusa* (Chapter 1), while the eastern populations (Florida and the Caribbean) retained phenotypic characteristics that are now recognized as *J. coenia*. *Junonia litoralis* and *J. zonalis* utilize tropical species as larval host plants (Turner & Parnell 1985; Glassberg *et al.* 2000; Brévignon 2009; Brévignon & Brévignon 2012) which are not frost tolerant so glacial refuges for these species would have been restricted to even more southern regions, perhaps in Central America. The most northern limit of the frost-free zone during this time would have been at approximately 20° N latitude (Ceballos *et al.* 2010). Modern populations of *J. litoralis* and *J. zonalis* in Central America carry primarily haplotype group B, (Pfeiler *et al.* 2012a; Gemmell *et al.* 2014; Gemmell & Marcus 2015), so this genotype was either present in the refuges, or it was transferred to these populations by hybridization after the refuge populations emerged during an interglacial period.

As North America became warmer after that last of the glaciers had receded (early to middle Holocene), species from glacial refuges would have been allowed to expand into new habitats. Since *J. coenia* is hypothesized to have taken refuge in Florida and the Caribbean, this would have allowed for this species to expand westward into the

American Southwest and Mexico and bringing with it haplotype group B. Similarly, populations of *J. grisea* carrying haplotype B^{CA} would have spread to the north and east. This warming event would have allowed for the five species of *Junonia* in the Southwestern Americas and Mexico to come into close proximity to each other and made hybridization between these species possible.

Species and haplotype distributions. Species distributions for all five of the *Junonia* species in the American Southwest and Mexico have been documented previously (Hafernik 1982; Glassberg 2001; Gemmell & Marcus 2015), but failure to identify species correctly made these earlier maps very difficult to use. Using a list of morphological characteristics that I compiled (Table 4-1), I was able to unambiguously identify specimens from museum collections (Appendix III) and, after sampling leg tissue, assign mitochondrial haplotypes to each. Using locality data species ranges and haplotype distributions were generated. *Junonia coenia* has the broadest range spanning much of the study area (Figure 4-3). The core range of this species extends from the Eastern United States, outside of the current study area, to Southeastern Colorado, most of New Mexico, South-central Arizona, and the most of Mexico. The principle haplotype group in this core range is haplotype group B, although B^{CA} becomes more common in the western parts of the range where hybridization between *J. coenia* and *J. grisea* has taken place (Figure 4-3). *Junonia grisea* follows the opposite trend of *J. coenia* with the highest proportion of specimens from the western United States with the principle haplotype group being B^{CA} (Figure 4-4). Pooling haplotypes across all species, it is possible to observe the transition between haplotypes B and B^{CA} across the study region

(Figure 4-9). The core range of *J. nigrosuffusa* is in between *J. coenia* and *J. grisea* (Figure 4-5) and shows both B and B^{CA} haplotypes, especially in Arizona and New Mexico where these 3 species have the most extensive overlap, and where there appears to be a large hybrid zone (Figure 4-8). *Junonia litoralis* is restricted to coastal habitats and unfortunately is represented by only a few samples in this study (Figure 4-6). In the study region, *J. zonalis* occurs at the highest frequency in southeastern Mexico (Figure 4-7), and although the number of samples that I obtained was low, haplotype B is the most common haplotype for this species.

Evidence of Hybridization. A total of 36 probable hybrids were found based on intermediate morphology and colour pattern phenotypes found among 630 museum specimens (5.7%; Figure 4-1). The localities of these rare hybrid specimens were usually located in areas of overlap between the ranges of *Junonia* species (Figure 4-8). *J. nigrosuffusa* was the only species to have been found to hybridize with all of the other species in the study area, which does make sense as its core range overlaps with all of the other *Junonia* species found in this geographic region (Figure 4-5; Figure 4-8).

Hybrids between *J. coenia* and *J. grisea* showed an unexpected geographic pattern as a cluster of 3 suspected hybrids were found with locality data from the San Francisco Bay area in California (Figure 4-8), which is made up of Alameda, Contra Costa, Marin, Napa, San Francisco, San Mateo, Santa Clara, Solano, and Sonoma Counties (Appendix III). An unusually large concentration of stray *J. coenia* outside of the core range were also documented from the San Francisco Bay area (Figure 4-3), suggesting that a disjunct population of *J. coenia* may have existed in the region. This is further reinforced by a high proportion of haplotype B (otherwise rare in *J. grisea*) found in *Junonia* from the San Francisco Bay area (Figure 4-9). The first *J. coenia* in the data set is from San Francisco County collected in 1913 although the haplotype of this specimen was B^{CA}. The first haplotype B found within this region appears in a specimen from Marin County *J. grisea* collected in 1925. The first *J. coenia* haplotype B from the San Francisco Bay area was collected in 1959 from Alameda County. It is not clear if the San Francisco Bay area population of *J. coenia* is still extant, or whether it has been extirpated. The most recent *J. coenia* from the San Francisco Bay area was collected in 1965 and the most recent Bay area B haplotype in any *Junonia* species was collected in 1971 from Napa County. Recent collections (2015) of *Junonia* from Northern California (including Contra Costa County in the Bay area) included in this study did not find any *J. coenia, J. coenia* X *J. grisea* hybrids, or specimens carrying mitochondrial haplotype B.

Evidence for the elevation of *Junonia grisea* to a full species. Many authorities have included *Junonia grisea* within the nominate subspecies *J. coenia coenia* (Brown *et al.* 1992; Beutelspacher 1996; Warren & Llorente 1999; Glassberg 2007; Knerl & Bowers 2013), even after Austin & Emmel (1998) described *J. grisea* as a subspecies of *J. coenia* from California based primarily on differences in wing pigmentation. I have examined specimens from a much larger geographic region than Austin & Emmel (1998), and can identify 4 morphological traits that can be used to distinguish *J. grisea* consistently: a very small or often absent absent dorsal forewing anterior eyespot, a grey-brown ground colour for the dorsal wing surfaces, reduced or absent orange submarginal bands on the dorsal wing surfaces, and finally, a variably prominent submarginal band on the ventral hindwing that is usually less distinct and more orange in colour (rather than red) than what is seen in *J. coenia* (Table 4-1). Previous authors have also reported that *J. grisea* has a reduced degree of seasonal polyphenism, compared with *J. coenia* (Daniels et al. 2012), a difference that was initially mistaken for variation in polyphenims expressivity between populations.

When originally described *J. grisea* was documented to occur in California, Oregon, Nevada, Arizona and speculated to occur in the northern part of Baja California (Austin & Emmel 1998). I have been able to confirm that these original occurrences of *J. grisea* are correct but have observed that the core range of this species also includes southern Oregon, northern Mexico and western Texas (Figure 4-4). The distinctive mitochondrial haplotype B^{CA} is associated primarily with *J. grisea* (Figure 4-4), hybrids involving *J. grisea* as a parent (Figure 4-8), and in geographic regions where B^{CA} may have been transferred to other *Junonia* species by hybridization with *J. grisea* (Figure 4-9). This makes *J. grisea* very unusual among the North American *Junonia* in that most other forms do not have diagnostic mitochondrial genotypes (Gemmell & Marcus 2015).

Life cycle data was compiled for all five species in the study area (Table 4-2), but I will focus on *J. coenia* and *J. grisea* here. *Junonia grisea* has a substantially shorter generation time than *J. coenia* (Table 4-2). This is especially apparent when looking at the total number of days spent as larvae as *J. coenia* spends nearly twice as long at this stage compared to *J. grisea*, but *J. grisea* have a longer pupal stage (Table 4-2). While *J. coenia* and *J. grisea* can be reared successfully on the same introduced larval host plants in the laboratory, and utilize many of the same introduced larval host plants in the wild (Table 4-4), there is no native host plant overlap between these 2 species (Table 4-3).

Many populations of *J. grisea* have switched almost entirely to consuming introduced larval host plants (Scott 1975; Bowers 1984; Camara 1997), which has made their dissimilarity in host range with *J. coenia* less apparent.

While there is some apparent hybridization between *J. grisea* and *J. coenia* in the wild (Figure 4-8), this occurs primarily where the species ranges overlap, and hybridization among species in the wild appears to be a common feature of the New World *Junonia* (Hafernik 1982; Borchers & Marcus 2014; Gemmell *et al.* 2014). Collectively, the distinctive morphological differences, mitochondrial haplotype, life history traits, pattern of larval host plant use, and geographic range suggest that *J. grisea* is clearly distinct from *J. coenia coenia* and that it is appropriate to elevate *Junonia grisea* to full species status.

Future Directions

The identification of *Junonia grisea* as a cryptic species, as well as clearer maps of the hybrid zones among *Junonia* in the American Southwest and Mexico, reveals a number of interesting avenues to pursue. For example, understanding when the population of *J. coenia* in the San Francisco Bay area first appeared, and whether it is still extant would be an interesting invasion biology case study, especially in comparison with the history of invasion for *J. zonalis* in Florida (Chapter 3).

It is not clear whether the Bay area population of *J. coenia* is the result of natural dispersal, or whether it has somehow been augmented by human activity. Among butterflies, *Junonia* is particularly adept at long-range dispersal, and is often one of the only butterfly species present on remote oceanic islands (Harris 1988; Vane-Wright &

Tennent 2011; Peters & Marcus In Press). In California, some northern populations of *Junonia* have been extirpated by extreme cold weather events, and then replaced by migrants from protected refuges at low elevation or in the south (Shapiro 1991). The concentration of *J. coenia* and haplotype B in the San Francisco Bay area may very well be the product of ongoing natural long-range dispersal of *J. coenia* into all parts of California, as the first haplotype B that I have identified from California was from a specimen captured in Los Angeles County in 1879, and the most recent was from a specimen collected in Ventura County in 1989 (Appendix III). It is also possible that road construction, shipping, or other human activities may have facilitated the dispersal and establishment of the species in the San Francisco Bay area. A careful examination of the *Junonia* material from the San Francisco Bay area from the entomology collections at the California Academy of Science, the University of California at Berkley, the University of California at Davis, and the Los Angeles County Museum (each of these collections has large holdings of appropriate material that have not been used in the current study), in combination with contemporary sampling of *Junonia* should provide a much more detailed history of this *J. coenia* population, reveal whether it is still extant, and determine if it has left any genetic trace in the current populations of *J. grisea*. The robust genotyping techniques that I have developed should allow for determination of mitochondrial haplotypes of all of this material, regardless of its age or state of preservation.

It would also be desirable to deploy techniques for nuclear genotyping of Western *Junonia* specimens, as mitochondrial DNA can sometimes produce a distorted view of evolutionary history because of its uniparental inheritance. Population genetic analysis of nuclear *wingless* alleles, and of random amplified DNA fingerprints (RAF) has been effective for illuminating population structure of *Junonia* in other geographic regions, but these techniques have not yet been adapted for use with museum material (Chapter 2; Gemmell *et al.* 2014; Borchers & Marcus 2014), which describes the bulk of the samples considered here. The optimization of these techniques for use with museum specimens and/or the accumulation of suitable newly caught and well-preserved *Junonia* material would permit the more rigorous evaluation of additional forms, such as *J. nigrosuffusa* where there is a lack of consensus about taxonomic status (Barnes & McDunnough 1916; Tilden 1970; Gorelick 1975; Hafernik 1982; Brown *et al.* 1992; Wauer 2006), but which unlike *J. grisea* do not possess a distinctive mitochondrial haplotype.

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Chapter 5: Discussion & Future Directions

The use of appropriate and consistent biological taxonomy is extremely important. Especially for groups of organisms that are used as model systems in scientific research, it is crucial to have clear species designations so that other researchers can easily access pertinent information about the species that they are doing work on, and distinguish that information from data collected in other species. The genus *Junonia* is an example of a genus that is used in a vast array of studies, but due to inconsistent use of taxonomic identifiers and a lack of clarity in the nomenclature over time, there has been much confusion regarding which species have actually been studied (Chapter 2; Table 2-1). The taxonomic mess that this genus had become can be attributed to many factors such as the interchangeability of genus names (*Precis*/*Junonia*)(Cramer 1775; Comstock 1944; Munroe 1951; De Lesse 1952), vague early descriptions of species (Cramer 1779; Cramer 1780; Turner & Parnell 1985), variation of species geographically (Forbes 1928; Remington 1985) as well as seasonally (Clark 1932; Mather 1967; Smith 1991; Rountree & Nijhout 1995), morphologically similar species, and natural hybridization (Chapter 2; Chapter 4)(Forbes 1928; Rutkowski 1971; Hafernik 1982; DeVries 1987; Minno & Emmel 1993; Glassberg 2007).

Bad Taxonomy leads to bad Science. Problems arise when biological taxonomy for a group of organisms is unclear and *Junonia* is a prime example. In this group many taxa have been referred to by the same name. *Junonia evarete* is a species that is endemic to South America (Brévignon & Brévignon 2011; Brévignon & Brévignon 2012) but authors have used this species designation for *J. neildi* from Florida and the Caribbean(Turner & Parnell 1985; Gemmell & Marcus 2015); *J. zonalis* from Texas, Mexico, and Central America; *J. nigrosuffusa* from Texas (Wauer 2006; Pfeiler *et al.* 2012), and *J. wahlbergi* from Venezuela (Neild 2008). At the same time, the name *Junonia genoveva* was used for *J. zonalis* from Florida (Turner & Parnell 1985; Gemmell & Marcus 2015) and the Caribbean and *J. litoralis* from Texas and Mexico (Pfeiler *et al.* 2012; Gemmell & Marcus 2015). Similarly, many *J. grisea* have been classified as *J. coenia* in the American Southwest and Mexico due to similarities in morphological characteristics (Brown *et al.* 1992; Knerl & Bowers 2013). Based on work in Chapter 4 it is now clear that *J. grisea* is a cryptic species based on subtle differences in morphology, a distinct larval host plant range, differences in life history characteristics, and a discrete mitochondrial genotype.

In the New World *Junonia* the same taxon has also been referred to by different names in different geographic regions. For example, *J. zonalis* in Florida, USA and the Caribbean have been referred to as *J. genoveva* (Turner & Parnell 1985; Gemmell & Marcus 2015), while populations in Texas, USA, Mexico and Central America have been referred to as *J. evarete* (Wauer 2006). In a second example, *J. neildi* in Florida, USA and the Greater Antilles has been called *J. evarete* (Turner & Parnell 1985; Gemmell & Marcus 2015) while populations in the Lesser Antilles have been called *J. genoveva neildi* (Brévignon 2009). As discussed in Chapter 2, *J. genoveva* and *J. evarete* are endemic to South America (Brévignon & Brévignon 2012), but the history of the usage of these names means that virtually all publications employing the names *J. genoveva* and *J. evarete*, regardless of the geographic origin of the source material, becomes very difficult to decipher or to assign biological data to the correct species.

An example of how faulty taxonomy compromises scientific research conducted using a group of organisms can be seen in *Junonia* larval host plant associations. Many different (and incorrect) larval host plants have been associated with *J. evarete* (Beccaloni et al. 2008). The larval host plants for *J. zonalis* and *J. neildi* have been transposed in publications (Opler & Malikul 1992; Calhoun 2010). There have also been cases where larval host plant data for multiple lineages combined that has contributed to obscuring cryptic species (Knerl & Bowers 2013), as has been the case for *J. grisea* (Chapter 4).

Benefits of good taxonomy. Establishment of morphological characteristics (Table 2-2; Table 4-1) that allows for the delimitation of species can aid in the proper identification and provide a sound basis for scientific communication (Paterlini 2007). By associating populations of the same species from different geographical regions with each other it allows for the assignment of appropriate taxonomic names. I have established species nomenclature based on morphological characteristics from descriptions of species found in the literature (Table 2-2; Table 4-1) and also by use of molecular markers. This establishment of a common language has allowed for the association of larval host plants with particular species, life cycle data being associated with specific species, the establishment of species ranges, observation of possible hybrid zones, a cryptic species that was hidden in this genus, and have reconstructed of the invasion history of *J. zonalis* into South Florida (Chapter 3). Having these associations with specific species will allow for more sophisticated comparative biology research to occur and have a larger impact in the scientific community.

Future Directions. The genus *Junonia* is a valuable model for many fields of research and many molecular techniques exist to conduct this work. There is ongoing work that will produce within the next year the full genome sequence of *J. coenia* (Marcus Pers. Com.). The availability of this reference genome sequence will greatly facilitate comparative biology experiments with other *Junonia* species, but having the correct species identities when doing such work is important. If incorrect taxonomic designations are assigned and full genome sequences are generated and released, it will not only become an enormous waste of resources but also magnify the taxonomic ambiguities that already exist in this genus. With proper morphological identifications of species being associated with gene sequences using a common language, it will further aid in the work that is currently being done and revolutionize the field allowing for more comparative biological research to occur in using this invaluable model system.

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Appendix I. *Junonia* specimens for the contemporary Florida data set

Code	*Junonia* Species	Location	Date	GPS Northing	GPS Westing	Genotyping Method	Haplotype	Primers
FL1	neildi	USA: Florida, St. Lucie County, USA: Florida, St. Lucie County, Jack Island Pre	13 v 2004	27 29.970'	080 18.905'	Restriction Digest	B	Ron/Nancy
FL2	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, Jack Island	14 v 2004	28 29.970'	81 18.905'	Restriction Digest	B	Ron/Nancy
FL3	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, Jack Island	15 v 2004	29 29.970'	82 18.905'	Restriction Digest	B	Ron/Nancy
FLC1	coenia	USA: Florida, Kissimmee Prairie Preserve State Park	10 v 2005	27 34.875	081 01.354'	KM288092	B	HCO/LCO
FLC10	coenia	USA: Florida, Lake County, Lake Louisa State Park	9 v 2005	28 27.308	081 43.727	KM288093	B	HCO/LCO
FLC11	coenia	USA: Florida, Lake County, Lake Louisa State Park	9 v 2005	28 27.308	081 43.727	KM288094	B	HCO/LCO
FLC12	coenia	USA: Florida, Lake County, Lake Louisa State Park	9 v 2005	28 27.135	081 43.829	KM288095	B	HCO/LCO
FLC13	coenia	USA: Florida,Pinellas County,Dunedin, Honeymoon Island State Park	12 v 2005	28 04.404	082 49.880	KM288096	B	HCO/LCO
FLC14	coenia	USA: Florida,Pinellas County,Dunedin, Honeymoon Island State Park	12 v 2005	28 04.723	082 49.953	KM288097	B	HCO/LCO
FLC15	coenia	USA: Florida,Pinellas County,Dunedin, Honeymoon Island State Park	12 v 2005	28 04.723	082 49.953	KM288098	B	HCO/LCO
FLC16	coenia	USA: Florida, Kissimmee Prairie Preserve State Park	11 V 2005	27 34.719	081 01.356	KM288099	B	HCO/LCO
FLC17	coenia	USA: Florida, Kissimmee Prairie Preserve State Park	11 V 2005	27 34.703	081 01.356	KM288100	B	HCO/LCO
FLC18	coenia	USA: Florida, Kissimmee Prairie Preserve State Park	11 V 2005	27 34.867	081 01.355	KM288101	B	HCO/LCO
FLC19	coenia	USA: Florida, Sarasota County, South Lido Nature Park	5 xi 2005	27 18.4526'	082 34.2907'	Restriction Digest	B	HCO/LCO
FLC2	coenia	USA: Florida, Kissimmee Prairie Preserve State Park	11 V 2005	27 34.874	081 01.357	KM288102	B	HCO/LCO
FLC20	coenia	USA: Florida, Sarasota County, South Lido Nature Park	5 xi 2005	27 18.4526'	082 34.2907'	Restriction Digest	B	HCO/LCO
FLC2004.16	coenia	USA: Florida, St. Lucie County, Jack Island Preserve State Park, USA: Florida, S	24 xi 2004	27 34.875	080 19.213	Restriction Digest	B	HCO/LCO
FLC2004.17	coenia	USA: Florida, St. Lucie County, Jack Island Preserve State Park, King's Island	24 xi 2004	27 34.875	080 19.213	Restriction Digest	B	HCO/LCO
FLC2004.18	coenia	USA: Florida, St. Lucie County, Jack Island Preserve State Park, King's Island	24 xi 2004	27 34.875	080 19.213	Restriction Digest	B	HCO/LCO
FLC2004.5	coenia	USA: Florida, St. Lucie County, Jack Island Preserve State Park, King's Island	13 v 2004	27 34.875	080 19.213	Restriction Digest	B	HCO/LCO
FLC2004.6	coenia	USA: Florida, St. Lucie County, Jack Island Preserve State Park, King's Island	13 v 2004	27 34.875	080 19.213	Restriction Digest	B	HCO/LCO
FLC2010.1	coenia	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLC21	coenia	USA: Florida, Sarasota County, South Lido Nature Park	5 xi 2005	27 18.4526'	082 34.2907'	Restriction Digest	B	HCO/LCO
FLC22	coenia	USA: Florida, Sarasota County, South Lido Nature Park	5 xi 2005	27 18.4526'	082 34.2907'	Restriction Digest	B	HCO/LCO
FLC3	coenia	USA: Florida, St. Lucie County, Jack Island Preserve State Park, King's Island	10 v 2005	27 34.875	080 19.213	KM288103	B	HCO/LCO
FLC4	coenia	USA: Florida, St. Lucie County, Jack Island Preserve State Park, King's Island	10 v 2005	27 35.318	080 22.066	KM288104	B	HCO/LCO
FLC5	coenia	USA: Florida, Lake County, Lake Louisa State Park	9 v 2005	28 25.806	081 43.506	KM288105	B	HCO/LCO
FLC6	coenia	USA: Florida, Lake County, Lake Louisa State Park	9 v 2005	28 27.208	081 43.873	KM288106	B	HCO/LCO
FLC7	coenia	USA: Florida, Kissimmee Prairie Preserve State Park	11 V 2005	27 34.908	081 01.352	KM288107	B	HCO/LCO
FLC8	coenia	USA: Florida, Lake County, Lake Louisa State Park	9 v 2005	28 27.197	081 43.828	KM288108	B	HCO/LCO
FLC9	coenia	USA: Florida, Lake County, Lake Louisa State Park	9 v 2005	28 27.097	081 43.829	KM288109	B	HCO/LCO
FLCD1	coenia	USA: Florida, Everglades National Park, Flamingo, Trialhead Coastal Prairie Tra	16 xi 2007	25° 08.173'	080° 56.894'	KM288076	B	HCO/LCO
FLCD2	coenia	USA: Florida, Everglades National Park, Flamingo, Trialhead Coastal Prairie Tra	16 xi 2007	25° 08.173'	080° 56.893'	KM288077	B	HCO/LCO
FLCD3	coenia	USA: Florida, Everglades National Park, North-West Cape Sable	18 xi 2007	25° 13.899'	081° 10.251'	KM288078	B	HCO/LCO
FLCD4	coenia	USA: Florida, Everglades National Park, North-West Cape Sable	18 xi 2007	25° 12.652'	081° 09.428'	KM288079	B	HCO/LCO
FLCD5	coenia	USA: Florida, Everglades National Park, East Cape Sable	18 xi 2007	25° 08.673'	081° 06.585'	KM288080	B	HCO/LCO
FLCD6	coenia	USA: Florida, Everglades National Park, East Cape Sable	18 xi 2007	25° 08.673'	081° 06.585'	KM288081	B	HCO/LCO
FLCJ1	coenia	USA: Florida, Osceola National Forest	19 vi 2006	30° 19.045'	082° 22.313'	KM288110	B	HCO/LCO
FLCJ2	coenia	USA: Florida, Osceola National Forest	19 vi 2006	30° 19.045'	082° 22.313'	KM288111	B	HCO/LCO
FLCJ3	coenia	USA: Florida, Osceola National Forest	19 vi 2006	30° 19.045'	082° 22.313'	KM288112	B	HCO/LCO
FLCJ4	coenia	USA: Florida, Osceola National Forest	19 vi 2006	30° 19.045'	082° 22.313'	KM288113	B	HCO/LCO
FLCJ5	coenia	USA: Florida, Osceola National Forest	19 vi 2006	30° 19.045'	082° 22.313'	KM288114	B	HCO/LCO
FLCJ6	coenia	USA: Florida, Osceola National Forest	19 vi 2006	30° 19.045'	082° 22.313'	KM288115	B	HCO/LCO
FLCJa1	coenia, worn small male	USA: Florida, Dade County, Everglades Greenway	11 i 2007	25° 24.392'	080° 33.554'	KM288219	B	HCO/LCO
FLCJa10	coenia	USA: Florida, Dade County, Everglades Greenway--North End	12 i 2007	25° 38.144'	080° 27.849'	KM288220	B	HCO/LCO
FLCJa11	coenia , faint white ventr	USA: Florida, Dade County, Everglades Greenway--North End	12 i 2007	25° 38.144'	080° 27.849'	KM288221	B	HCO/LCO
FLCJa12	coenia	USA: Florida, Dade County, Everglades Greenway--North End	12 i 2007	25° 38.144'	080° 27.849'	KM288222	B	HCO/LCO
FLCJa2	coenia, very worn	USA: Florida, Dade County, Everglades Greenway--North End	11 i 2007	25° 38.178'	080° 29.851'	KM288223	B	HCO/LCO
FLCJa3	coenia	USA: Florida, Dade County, Everglades Greenway--North End	12 i 2007	25° 38.144'	080° 27.849'	KM288224	B	HCO/LCO
FLCJa4	coenia	USA: Florida, Dade County, Everglades Greenway--North End	12 i 2007	25° 38.144'	080° 27.849'	KM288225	B	HCO/LCO
FLCJa5	coenia, worn faint ventra	USA: Florida, Dade County, Everglades Greenway--North End	12 i 2007	25° 38.144'	080° 27.849'	KM288226	B	HCO/LCO
FLCJa6	coenia, faint line below	USA: Florida, Dade County, Everglades Greenway--North End	12 i 2007	25° 38.144'	080° 27.849'	KM288227	B	HCO/LCO
FLCJa7	coenia	USA: Florida, Dade County, Everglades Greenway--North End	12 i 2007	25° 38.144'	080° 27.849'	KM288228	B	HCO/LCO
FLCJa8	coenia, very worn	USA: Florida, Dade County, Everglades Greenway--North End	12 i 2007	25° 38.144'	080° 27.849'	KM288229	B	HCO/LCO
FLCJa9	coenia	USA: Florida, Dade County, Everglades Greenway--North End	12 i 2007	25° 38.144'	080° 27.849'	KM288230	B	HCO/LCO
FLCM1	coenia	USA: Florida, Dade County, Everglades Greenway	17 v 2006	25° 24.511'	080° 33.554'	KM288116	B	HCO/LCO
FLCM10	coenia	USA: Florida, Dade County, Everglades Greenway	18 v 2006	25° 25.448'	080° 33.556'	Restriction Digest	B	HCO/LCO
FLCM11	coenia	USA: Florida, Dade County, Everglades Greenway	18 v 2006	25° 25.183'	080° 33.557'	KM288117	B	HCO/LCO
FLCM12	coenia	USA: Florida, Dade County, Everglades Greenway	18 v 2006	25° 25.186'	080° 33.557'	KM288118	B	HCO/LCO

Appendix I. *Junonia* specimens for the contemporary Florida data set

Code	*Junonia* Species	Location	Date	GPS Northing	GPS Westing	Genotyping Method	Haplotype	Primers
FLCM13	coenia	USA: Florida, Dade County, Everglades Greenway	18 v 2006	25° 25.081'	080° 33.557'	KM288119	B	HCO/LCO
FLCM14	coenia	USA: Florida, Dade County, Everglades Greenway	18 v 2006	25° 25.081'	080° 33.557'	Restriction Digest	B	HCO/LCO
FLCM15	coenia	USA: Florida, Dade County, Everglades Greenway	18 v 2006	25° 24.528'	080° 33.553'	KM288120	B	HCO/LCO
FLCM16	coenia	USA: Florida, Dade County, Everglades Greenway	18 v 2006	25° 24.574'	080° 33.551'	KM288121	B	HCO/LCO
FLCM17	coenia	USA: Florida, Dade County, Everglades Greenway	18 v 2006	25° 24.652'	080° 33.553'	Restriction Digest	B	HCO/LCO
FLCM18	coenia	USA: Florida, Dade County, Everglades Greenway	18 v 2006	25° 25.020'	080° 33.557'	KM288122	B	HCO/LCO
FLCM19	coenia	USA: Florida, Dade County, Everglades Greenway	18 v 2006	25° 25.020'	080° 33.557'	KM288123	B	HCO/LCO
FLCM2	coenia	USA: Florida, Dade County, Everglades Greenway	17 v 2006	25° 24.926'	080° 33.555'	KM288124	B	HCO/LCO
FLCM20	coenia	USA: Florida, USA: Florida,Lee County, Estero Bay Preserve State Park Preserv	19 v 2006	26° 28.328'	081° 54.020'	KM288125	B	HCO/LCO
FLCM21	coenia	USA: Florida, USA: Florida,Lee County, Estero Bay Preserve State Park Preserv	19 v 2006	26° 28.354'	081° 53.974'	KM288126	B	HCO/LCO
FLCM22	coenia	USA: Florida, Jonathan Dickinson State Park	21 v 2006	27° 00.727'	080° 06.674'	KM288127	B	HCO/LCO
FLCM23	coenia	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	22 v 2006	27° 35.532'	080° 21.987'	Restriction Digest	B	HCO/LCO
FLCM3	coenia	USA: Florida, Dade County, Everglades Greenway	17 v 2006	25° 24.970'	080° 33.554'	KM288125	B	HCO/LCO
FLCM4	coenia	USA: Florida, Dade County, Everglades Greenway	17 v 2006	25° 25.090'	080° 33.555'	KM288126	B	HCO/LCO
FLCM5	coenia	USA: Florida, Dade County, Everglades Greenway	17 v 2006	25° 25.192'	080° 33.554'	KM288127	B	HCO/LCO
FLCM6	coenia	USA: Florida, Dade County, Everglades Greenway	17 v 2006	25° 25.452'	080° 33.557'	KM288128	B	HCO/LCO
FLCM7	coenia	USA: Florida, Dade County, Everglades Greenway	18 v 2006	25° 24.908'	080° 33.553'	KM288129	B	HCO/LCO
FLCM8	coenia	USA: Florida, Dade County, Everglades Greenway	18 v 2006	25° 24.582'	080° 33.553'	KM288130	B	HCO/LCO
FLCM9	coenia	USA: Florida, Dade County, Everglades Greenway	18 v 2006	25° 25.040'	080° 33.554'	KM288131	B	HCO/LCO
FLCMar1	coenia	USA: Florida, Everglades National Park, Intersection W. Rd. with Old Ingram H	12 iii 2007	25° 22.493'	080° 37.481'	Restriction Digest	B	HCO/LCO
FLCMar10	coenia	USA: Florida, Everglades National Park, Intersection W. Rd. with Old Ingram H	12 iii 2007	25° 22.491'	080° 37.555'	Restriction Digest	B	HCO/LCO
FLCMar11	coenia	USA: Florida, Everglades National Park, Dade County, Chekika	13 iii 2007	25° 37.039'	080° 34.966'	Restriction Digest	B	HCO/LCO
FLCMar12	coenia	USA: Florida, Everglades National Park, Dade County, Chekika	13 iii 2007	25° 36.971'	080° 34.902'	Restriction Digest	B	HCO/LCO
FLCMar13	coenia	USA: Florida, Everglades National Park, Dade County, Chekika	13 iii 2007	25° 36.871'	080° 34.520'	Restriction Digest	B	HCO/LCO
FLCMar2	coenia	USA: Florida, Everglades National Park, Intersection W. Rd. with Old Ingram H	12 iii 2007	25° 22.491'	080° 37.485'	Restriction Digest	B	HCO/LCO
FLCMar3	coenia	USA: Florida, Everglades National Park, Intersection W. Rd. with Old Ingram H	12 iii 2007	25° 22.491'	080° 37.555'	Restriction Digest	B	HCO/LCO
FLCMar4	coenia	USA: Florida, Everglades National Park, Intersection W. Rd. with Old Ingram H	12 iii 2007	25° 22.491'	080° 37.555'	Restriction Digest	B	HCO/LCO
FLCMar5	coenia	USA: Florida, Everglades National Park, Intersection W. Rd. with Old Ingram H	12 iii 2007	25° 22.491'	080° 37.555'	Restriction Digest	B	HCO/LCO
FLCMar6	coenia	USA: Florida, Everglades National Park, Intersection W. Rd. with Old Ingram H	12 iii 2007	25° 22.491'	080° 37.555'	Restriction Digest	B	HCO/LCO
FLCMar7	coenia	USA: Florida, Everglades National Park, Intersection W. Rd. with Old Ingram H	12 iii 2007	25° 22.491'	080° 37.555'	Restriction Digest	B	HCO/LCO
FLCMar8	coenia	USA: Florida, Everglades National Park, Intersection W. Rd. with Old Ingram H	12 iii 2007	25° 22.491'	080° 37.555'	Restriction Digest	B	HCO/LCO
FLCMar9	coenia	USA: Florida, Everglades National Park, Intersection W. Rd. with Old Ingram H	12 iii 2007	25° 22.491'	080° 37.555'	Restriction Digest	B	HCO/LCO
FLCN1	coenia	USA: Florida, Pinellas County, Weedon Island Preserve	4 xi 2005	27° 51.465'	082° 37.089'	KM288136	B	HCO/LCO
FLCN10	coenia	USA: Florida, Pinellas County, Weedon Island Preserve	4 xi 2005	27° 51.465'	082° 37.089'	KM288137	B	HCO/LCO
FLCN11	coenia	USA: Florida, Sarasota County, South Lido Park	5 xi 2005	27° 18.563'	082° 34.208'	KM288138	B	HCO/LCO
FLCN12	coenia	USA: Florida, Sarasota County, South Lido Park	5 xi 2005	27° 18.563'	082° 34.208'	KM288139	B	HCO/LCO
FLCN13	coenia	USA: Florida, Sarasota County, South Lido Park	5 xi 2005	27° 18.563'	082° 34.208'	KM288140	B	HCO/LCO
FLCN14	coenia	USA: Florida, Sarasota County, South Lido Park	5 xi 2005	27° 18.563'	082° 34.208'	KM288141	B	HCO/LCO
FLCN15	coenia	USA: Florida, Sarasota County, Myakka River State Park	5 xi 2005	27° 14.903'	082°17.029'	KM288142	B	HCO/LCO
FLCN16	coenia	USA: Florida, Sarasota County, Myakka River State Park	5 xi 2005	27° 14.903'	082°17.029'	KM288143	B	HCO/LCO
FLCN17	coenia	USA: Florida, Sarasota County, Myakka River State Park	6 xi 2005	27° 14.903'	082°17.029'	KM288144	B	HCO/LCO
FLCN18	coenia	USA: Florida, Sarasota County, Myakka River State Park	6 xi 2005	27° 14.903'	082°17.029'	KM288145	B	HCO/LCO
FLCN19	coenia	USA: Florida, Sarasota County, Myakka River State Park	6 xi 2005	27° 14.903'	082°17.029'	KM288146	B	HCO/LCO
FLCN2	coenia	USA: Florida, Pinellas County, Weedon Island Preserve	4 xi 2005	27° 51.465'	082° 37.089'	KM288147	B	HCO/LCO
FLCN20	coenia	USA: Florida, Sarasota County, Myakka River State Park	6 xi 2005	27° 14.903'	082°17.029'	KM288148	B	HCO/LCO
FLCN21	coenia	USA: Florida, Sarasota County, Myakka River State Park	6 xi 2005	27° 14.903'	082°17.029'	KM288149	B	HCO/LCO
FLCN22	coenia	USA: Florida, Sarasota County, Myakka River State Park	6 xi 2005	27° 14.903'	082°17.029'	KM288150	B	HCO/LCO
FLCN23	coenia	USA: Florida, Sarasota County, Myakka River Wilderness Preserve	6 xi 2005	27°14.449'	082°19.327'	KM288151	B	HCO/LCO
FLCN24	coenia	USA: Florida, Sarasota County, Myakka River Wilderness Preserve	6 xi 2005	27°14.449'	082°19.327'	KM288152	B	HCO/LCO
FLCN25	coenia	USA: Florida, Sarasota County, South Lido Park	7 xi 2005	27° 18.563'	082° 34.208'	KM288153	B	HCO/LCO
FLCN26	coenia	USA: Florida,Pinellas County,Dunedin, Honeymoon Island State Park	7 xi 2005	28 04.404	082 49.880	KM288154	B	HCO/LCO
FLCN27	coenia	USA: Florida,Pinellas County,Dunedin, Honeymoon Island State Park	7 xi 2005	28 04.404	082 49.880	KM288155	B	HCO/LCO
FLCN28	coenia	USA: Florida,Pinellas County,Dunedin, Honeymoon Island State Park	7 xi 2005	28 04.404	082 49.880	KM288156	B	HCO/LCO
FLCN29	coenia	USA: Florida,Pinellas County,Dunedin, Honeymoon Island State Park	7 xi 2005	28 04.404	082 49.880	KM288157	B	HCO/LCO
FLCN3	coenia	USA: Florida, Pinellas County, Weedon Island Preserve	4 xi 2005	27° 51.465'	082° 37.089'	KM288158	B	HCO/LCO
FLCN4	coenia	USA: Florida, Pinellas County, Weedon Island Preserve	4 xi 2005	27° 51.465'	082° 37.089'	KM288159	B	HCO/LCO
FLCN5	coenia	USA: Florida, Pinellas County, Weedon Island Preserve	4 xi 2005	27° 51.465'	082° 37.089'	KM288160	B	HCO/LCO
FLCN6	coenia	USA: Florida, Pinellas County, Weedon Island Preserve	4 xi 2005	27° 51.465'	082° 37.089'	KM288161	B	HCO/LCO

Appendix I. *Junonia* specimens for the contemporary Florida data set

Code	*Junonia* Species	Location	Date	GPS Northing	GPS Westing	Genotyping Method	Haplotype	Primers
FLCN7	coenia	USA: Florida, Pinellas County, Weedon Island Preserve	4 xi 2005	27° 51.465’	082° 37.089’	KM288162	B	HCO/LCO
FLCN8	coenia	USA: Florida, Pinellas County, Weedon Island Preserve	4 xi 2005	27° 51.465’	082° 37.089’	KM288163	B	HCO/LCO
FLCN9	coenia	USA: Florida, Pinellas County, Weedon Island Preserve	4 xi 2005	27° 51.465’	082° 37.089’	KM288164	B	HCO/LCO
FLCO3	coenia	USA: Florida,Pinellas County,Dunedin, Honeymoon Island State Park	29 x 2006	28° 04.701’	082° 49.945’	Restriction Digest	B	HCO/miniCOIF2
FLCO5	coenia	USA: Florida,Pinellas County,Dunedin, Honeymoon Island State Park	29 x 2006	28° 04.610’	082° 49.929’	Restriction Digest	B	HCO/LCO
FLCO6	coenia	USA: Florida,Pinellas County,Dunedin, Honeymoon Island State Park	29 x 2006	28° 04.067’	082° 49.874’	Restriction Digest	B	HCO/LCO
FLCO7	coenia	USA: Florida,Pinellas County,Dunedin, Honeymoon Island State Park	29 x 2006	28° 04.189’	082° 49.956’	Restriction Digest	B	HCO/LCO
FLE 2009.1	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	17 xi 2009	24° 35.875’	081° 34.996’	Restriction Digest	B	HCO/LCO
FLE 2009.10	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	17 xi 2009	24° 35.875’	081° 34.996’	Restriction Digest	B	HCO/LCO
FLE 2009.2	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	17 xi 2009	24° 35.875’	081° 34.996’	Restriction Digest	B	HCO/LCO
FLE 2009.3	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	17 xi 2009	24° 35.875’	081° 34.996’	Restriction Digest	B	HCO/LCO
FLE 2009.4	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	17 xi 2009	24° 35.875’	081° 34.996’	Restriction Digest	B	HCO/LCO
FLE 2009.5	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	17 xi 2009	24° 35.875’	081° 34.996’	Restriction Digest	B	HCO/LCO
FLE 2009.6	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	17 xi 2009	24° 35.875’	081° 34.996’	Restriction Digest	B	HCO/LCO
FLE 2009.7	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	17 xi 2009	24° 35.875’	081° 34.996’	Restriction Digest	B	HCO/LCO
FLE 2009.8	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	17 xi 2009	24° 35.875’	081° 34.996’	Restriction Digest	A	HCO/LCO
FLE 2009.9	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	17 xi 2009	24° 35.875’	081° 34.996’	Restriction Digest	B	HCO/miniCOIF2
FLE1	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	10 v 2005	27 35.446'	080 21.909'	KM288165	B	HCO/LCO
FLE10	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, Jack Island	10 v 2005	27 30.089	080 18.693	KM288166	B	HCO/LCO
FLE11	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, Jack Island	10 v 2005	27 30.089	080 18.693	KM288167	B	HCO/LCO
FLE12	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, King's Island	10 v 2005	27 30.403	080 19.053	KM288168	B	HCO/LCO
FLE13	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	10 v 2005	27 35.320	080 22.086	Restriction Digest	B	HCO/LCO
FLE14	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	10 v 2005	27 35.474	080 22.140	KM288169	B	HCO/LCO
FLE15	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	10 v 2005	27 35.385	080 21.894	KM288170	B	HCO/LCO
FLE16	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	10 v 2005	27 35.318	080 22.038	KM288171	B	HCO/LCO
FLE2	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	10 v 2005	27 35.322'	080 22.030'	KM288173	B	HCO/LCO
FLE2004.1	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, King's Island	13 v 2004	27 34.875	080 19.213	Restriction Digest	B	HCO/LCO
FLE2010.1	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	20 iv 2010	24° 35.875’	081° 34.996’	Restriction Digest	B	HCO/LCO
FLE2010.10	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	080 21.894	Restriction Digest	B	HCO/LCO
FLE2010.11	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	080 21.894	Restriction Digest	B	HCO/LCO
FLE2010.12	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.13	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.14	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.15	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.16	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.17	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.18	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.19	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.2	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	20 iv 2010	24° 35.875’	081° 34.996’	Restriction Digest	B	HCO/LCO
FLE2010.20	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	080 21.894	Restriction Digest	B	HCO/LCO
FLE2010.21	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	080 21.894	Restriction Digest	B	HCO/LCO
FLE2010.22	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.23	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.24	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.25	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.26	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.27	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.28	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.29	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.3	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.30	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/miniCOIF2
FLE2010.31	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/miniCOIF2
FLE2010.32	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.33	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.34	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.35	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.36	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.37	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO

Appendix I. *Junonia* specimens for the contemporary Florida data set

Code	*Junonia* Species	Location	Date	GPS Northing	GPS Westing	Genotyping Method	Haplotype	Primers
FLE2010.38	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.39	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.4	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.40	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.41	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.42	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.43	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.44	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.45	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.46	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.47	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.48	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.49	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.5	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.50	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.51	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.52	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.53	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.54	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/miniCOIF2
FLE2010.55	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.56	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/miniCOIF2
FLE2010.57	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.58	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.59	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/miniCOIF2
FLE2010.6	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.60	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/miniCOIF2
FLE2010.61	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.62	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/miniCOIF2
FLE2010.63	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/miniCOIF2
FLE2010.64	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.65	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/miniCOIF2
FLE2010.66	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.67	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/miniCOIF2
FLE2010.68	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.69	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.7	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.70	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/miniCOIF2
FLE2010.71	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/miniCOIF2
FLE2010.72	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	3 ii 2010	27 35.385	80 21.894	Restriction Digest	B	HCO/LCO
FLE2010.73	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	2 ii 2010	24° 35.875'	081° 34.996'	Restriction Digest	B	HCO/miniCOIF2
FLE2010.74	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	2 ii 2010	24° 35.875'	081° 34.996'	Restriction Digest	B	HCO/LCO
FLE2010.75	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	2 ii 2010	24° 35.875'	081° 34.996'	Restriction Digest	B	HCO/miniCOIF2
FLE2010.76	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	2 ii 2010	24° 35.875'	081° 34.996'	Restriction Digest	B	HCO/miniCOIF2
FLE2010.77	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	2 ii 2010	24° 35.875'	081° 34.996'	Restriction Digest	B	HCO/miniCOIF2
FLE2010.8	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	080 21.894	Restriction Digest	B	HCO/LCO
FLE2010.9	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	21 iv 2010	27 35.385	080 21.894	Restriction Digest	B	HCO/LCO
FLE2011.1	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	23 i 2011	27 35.446'	080 21.909'	Restriction Digest	B	HCO/LCO
FLE2011.2	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	23 i 2011	27 35.446'	080 21.909'	Restriction Digest	B	HCO/LCO
FLE2011.3	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	23 i 2011	27 35.446'	080 21.909'	Restriction Digest	B	HCO/LCO
FLE2011.4	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	23 i 2011	27 35.446'	080 21.909'	Restriction Digest	B	HCO/LCO
FLE2011.5	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	23 i 2011	27 35.446'	080 21.909'	Restriction Digest	B	HCO/miniCOIF2
FLE2011.6	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	23 i 2011	27 35.446'	080 21.909'	Restriction Digest	B	HCO/LCO
FLE3	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	10 v 2005	27 35.536	080 22.029	KM288174	B	HCO/LCO
FLE4	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, Jack Island	10 v 2005	27 30.137	080 18.879	KM288175	B	HCO/LCO
FLE5	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, Jack Island	10 v 2005	27 30.087	080 18.488	KM288176	B	HCO/LCO
FLE6	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	10 v 2005	27 35.446	080 21.911	KM288177	B	HCO/LCO
FLE7	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	10 v 2005	27 35.423	080 21.903	KM288178	B	HCO/LCO
FLE8	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, King's Island (2	10 v 2005	27 30.471'	080 18.895'	KM288179	B	HCO/LCO

Appendix I. *Junonia* specimens for the contemporary Florida data set

Code	*Junonia* Species	Location	Date	GPS Northin	GPS Westing	Genotyping Method	Haplotype	Primers
FLE9	neildi = FLE19	USA: Florida, St. Lucie County, Jack Island Preserve State Park, Jack Island	10 v 2005	27 29.973	080 18.899	KM288172	B	HCO/LCO
FLED1	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	17 xi 2007	24° 35.875'	081° 34.996'	KM288082	B	HCO/LCO
FLED10	neildi	USA: Florida, Everglades National Park, E of North-West Cape Sable	18 xi 2007	25° 12.652'	081° 09.428'	KM288083	B	HCO/LCO
FLED2	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	17 xi 2007	24° 35.875'	081° 34.996'	KM288084	B	HCO/LCO
FLED3	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	17 xi 2007	24° 35.875'	081° 34.996'	KM288085	B	HCO/LCO
FLED4	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	17 xi 2007	24° 35.876'	081° 34.995'	KM288086	B	HCO/LCO
FLED5	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	17 xi 2007	24° 35.876'	081° 34.995'	KM288087	B	HCO/LCO
FLED6	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	17 xi 2007	24° 35.876'	081° 34.995'	KM288088	A	HCO/LCO
FLED7	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	17 xi 2007	24° 35.876'	081° 34.995'	KM288089	B	HCO/LCO
FLED8	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	17 xi 2007	24° 35.971'	081° 34.784'	KM288090	A	HCO/LCO
FLED9	neildi	USA: Florida, Everglades National Park, E of North-West Cape Sable	18 xi 2007	25° 12.629'	081° 09.411'	KM288091	B	HCO/LCO
FLEJa1	neildi	USA: Florida, Monroe County, Ohio Key	13 i 2007	24° 40.365'	081° 14.595'	Restriction Digest	B	HCO/miniCOIF2
FLEJa10	neildi	USA: Florida, Monroe County, Ohio Key	14 i 2007	24° 40.298'	081° 14.628'	Restriction Digest	B	HCO/miniCOIF2
FLEJa11	neildi	USA: Florida, Monroe County, West Summerland Key, S. of Bahia Honda Chan	14 i 2007	24° 39.338'	081° 17.884'	Restriction Digest	B	HCO/LCO
FLEJa12	neildi	USA: Florida, Monroe County, West Summerland Key, S. of Bahia Honda Chan	14 i 2007	24° 39.342'	081° 17.841'	Restriction Digest	B	HCO/miniCOIF2
FLEJa13	neildi	USA: Florida, Monroe County, West Summerland Key, S. of Bahia Honda Chan	14 i 2007	24° 39.342'	081° 17.833'	Restriction Digest	B	HCO/miniCOIF2
FLEJa14	neildi	USA: Florida, Monroe County, Big Pine Key	14 i 2007	24° 43.687'	081° 23.331'	Restriction Digest	B	HCO/LCO
FLEJa15	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	14 i 2007	24° 35.910'	081° 35.014'	Restriction Digest	B	HCO/LCO
FLEJa16	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	14 i 2007	24° 35.997'	081° 35.228'	Restriction Digest	B	HCO/LCO
FLEJa17	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	14 i 2007	24° 35.963'	081° 35.225'	Restriction Digest	B	HCO/miniCOIF2
FLEJa18	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	15 i 2007	24° 35.915'	081° 35.018'	Restriction Digest	B	HCO/LCO
FLEJa19	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	15 i 2007	24° 35.996'	081° 35.214'	Restriction Digest	B	HCO/LCO
FLEJa2	neildi	USA: Florida, Monroe County, Ohio Key	13 i 2007	24° 40.365'	081° 14.595'	Restriction Digest	B	HCO/LCO
FLEJa20	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	15 i 2007	24° 35.975'	081° 35.249'	Restriction Digest	B	HCO/miniCOIF2
FLEJa21	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	15 i 2007	24° 35.970'	081° 35.226'	Restriction Digest	B	HCO/LCO
FLEJa22	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	15 i 2007	24° 35.971'	081° 35.318'	Restriction Digest	B	HCO/LCO
FLEJa23	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	15 i 2007	24° 35.984'	081° 35.214'	Restriction Digest	B	HCO/miniCOIF2
FLEJa24	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	15 i 2007	24° 35.970'	081° 35.226'	Restriction Digest	B	HCO/miniCOIF2
FLEJa25	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key	15 i 2007	24° 35.987'	081° 35.191'	Restriction Digest	B	HCO/miniCOIF2
FLEJa3	neildi	USA: Florida, Monroe County, Ohio Key	13 i 2007	24° 40.300'	081° 14.620'	Restriction Digest	B	HCO/miniCOIF2
FLEJa4	neildi	USA: Florida, Monroe County, Ohio Key	13 i 2007	24° 40.227'	081° 14.663'	Restriction Digest	B	HCO/miniCOIF2
FLEJa5	neildi	USA: Florida, Monroe County, Ohio Key	13 i 2007	24° 40.401'	081° 14.609'	Restriction Digest	B	HCO/LCO
FLEJa6	neildi	USA: Florida, Monroe County, Ohio Key	14 i 2007	24° 40.227'	081° 14.665'	Restriction Digest	B	HCO/LCO
FLEJa7	neildi	USA: Florida, Monroe County, Ohio Key	14 i 2007	24° 40.303'	081° 14.622'	Restriction Digest	B	HCO/LCO
FLEJa8	neildi	USA: Florida, Monroe County, Ohio Key	14 i 2007	24° 40.387'	081° 14.614'	Restriction Digest	B	HCO/LCO
FLEJa9	neildi	USA: Florida, Monroe County, Ohio Key	14 i 2007	24° 40.338'	081° 14.607'	Restriction Digest	B	HCO/miniCOIF2
FLEM1	neildi	USA: Florida,Lee County, Estero Bay Preserve State Park	19 v 2006	26° 28.328'	081° 54.020'	KM288181	B	HCO/LCO
FLEM10	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	22 v 2006	27° 35.537'	080° 22.149'	KM288180	B	HCO/LCO
FLEM11	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, Jack Island	22 v 2006	27° 30.092'	080° 18.656'	KM288181	B	HCO/LCO
FLEM12	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, Jack Island	22 v 2006	27° 30.088'	080° 18.675'	KM288182	B	HCO/LCO
FLEM13	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, Jack Island	22 v 2006	27° 30.081'	080° 18.708'	KM288183	B	HCO/LCO
FLEM14	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, Jack Island	22 v 2006	27° 30.068'	080° 18.737'	KM288184	B	HCO/LCO
FLEM15	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, Jack Island	22 v 2006	27° 30.047'	080° 18.782'	KM288185	B	HCO/LCO
FLEM16	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, Jack Island	22 v 2006	27° 30.036'	080° 18.802'	KM288186	B	HCO/LCO
FLEM17	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, Jack Island	22 v 2006	27° 30.180'	080° 18.871'	Restriction Digest	B	HCO/miniCOIF2
FLEM18	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, Jack Island	22 v 2006	27° 30.198'	080° 18.686'	KM288187	B	HCO/LCO
FLEM19	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, Jack Island	22 v 2006	27° 30.342'	080° 18.846'	KM288188	B	HCO/LCO
FLEM2	neildi	USA: Florida,Lee County, Estero Bay Preserve State Park	19 v 2006	26° 28.354'	081° 53.974'	KM288190	B	HCO/LCO
FLEM20	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, Jack Island	22 v 2006	27° 30.464'	080° 18.901'	KM288189	B	HCO/LCO
FLEM21	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, King's Island	22 v 2006	27° 30.426'	080° 18.997'	KM288192	B	HCO/LCO
FLEM22	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, King's Island	22 v 2006	27° 30.328'	080° 19.277'	KM288193	B	HCO/LCO
FLEM23	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, Jack Island	22 v 2006	27° 30.151'	080° 18.877'	KM288194	B	HCO/LCO
FLEM24	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, Jack Island	22 v 2006	27° 30.024'	080° 18.899'	KM288195	B	HCO/LCO
FLEM25	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, Jack Island	22 v 2006	27° 29.972'	080° 18.905'	KM288196	B	HCO/LCO
FLEM26	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, Jack Island	22 v 2006	27° 30.021'	080° 18.834'	KM288197	B	HCO/LCO
FLEM3	neildi	USA: Florida,Broward County, Hugh Taylor Birch State Park	19 v 2006	26° 08.802'	080° 06.396'	KM288198	B	HCO/LCO
FLEM30	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, Jack Island	23 v 2006	27° 30.023'	080° 18.694'	KM288199	B	HCO/LCO
FLEM31	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, Jack Island	23 v 2006	27° 30.067'	080° 18.737'	Restriction Digest	B	HCO/LCO

Appendix I. *Junonia* specimens for the contemporary Florida data set

Code	*Junonia* Species	Location	Date	GPS Northin	GPS Westing	Genotyping Method	Haplotype	Primers
FLEM32	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, Jack Island	23 v 2006	27° 29.981'	080° 18.909'	Restriction Digest	B	HCO/LCO
FLEM5	neildi	USA: Florida, St. Lucie County, Jack Island Preserve State Park, Jack Island	21 v 2006	27° 30.031'	080° 18.896'	KM288200	B	HCO/LCO
FLEM6	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	22 v 2006	27° 35.537'	080° 22.031'	KM288201	B	HCO/LCO
FLEM7	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	22 v 2006	27° 35.549'	080° 21.960'	KM288202	B	HCO/LCO
FLEM8	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	22 v 2006	27° 35.537'	080° 22.149'	KM288203	B	HCO/LCO
FLEM9	neildi	USA: Florida, Indian River County, Vero Beach, Oslo Riverfront Conservation A	22 v 2006	27° 35.571'	080° 22.074'	KM288204	B	HCO/LCO
FLEMar1	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key--Entry Rd	16 iii 2007	24° 35.862'	081° 34.969'	Restriction Digest	B	HCO/LCO
FLEMar2	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key--Entry Rd	16 iii 2007	24° 35.862'	081° 34.969'	Restriction Digest	B	HCO/LCO
FLEMar3	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key--Entry Rd	16 iii 2007	24° 35.862'	081° 34.969'	Restriction Digest	B	HCO/miniCOIF2
FLEMar4	neildi	USA: Florida, Monroe County, Lower Sugarloaf Key--Entry Rd	16 iii 2007	24° 35.862'	081° 34.969'	Restriction Digest	B	HCO/LCO
FLEMar5	neildi	USA: Florida, Monroe County, No Name Key	17 iii 2007	24° 41.855'	081° 19.107'	Restriction Digest	B	HCO/LCO
FLEN1	neildi=FLEN11	USA: Florida, Pinellas County, Weedon Island Preserve	4 xi 2005	27° 51.465'	082° 37.089'	Restriction Digest	B	HCO/miniCOIF2
FLEN10	neildi	USA: Florida, Pinellas County, Weedon Island Preserve	4 xi 2005	27° 51.465'	082° 37.089'	KM288205	B	HCO/LCO
FLEN11	neildi = FLEN1	USA: Florida,Pinellas County,Dunedin, Honeymoon Island State Park	7 xi 2005	28 04.404	082 49.880	KM288206	B	HCO/LCO
FLEN2	neildi	USA: Florida, Pinellas County, Weedon Island Preserve	4 xi 2005	27° 51.465'	082° 37.089'	KM288207	B	HCO/LCO
FLEN3	neildi	USA: Florida, Pinellas County, Weedon Island Preserve	4 xi 2005	27° 51.465'	082° 37.089'	KM288208	B	HCO/LCO
FLEN4	neildi	USA: Florida, Pinellas County, Weedon Island Preserve	4 xi 2005	27° 51.465'	082° 37.089'	KM288209	B	HCO/LCO
FLEN5	neildi	USA: Florida, Pinellas County, Weedon Island Preserve	4 xi 2005	27° 51.465'	082° 37.089'	KM288210	B	HCO/LCO
FLEN6	neildi	USA: Florida, Pinellas County, Weedon Island Preserve	4 xi 2005	27° 51.465'	082° 37.089'	KM288211	B	HCO/LCO
FLEN7	neildi	USA: Florida, Pinellas County, Weedon Island Preserve	4 xi 2005	27° 51.465'	082° 37.089'	KM288212	B	HCO/LCO
FLEN8	neildi	USA: Florida, Pinellas County, Weedon Island Preserve	4 xi 2005	27° 51.465'	082° 37.089'	KM288213	B	HCO/LCO
FLEN9	neildi	USA: Florida, Pinellas County, Weedon Island Preserve	4 xi 2005	27° 51.465'	082° 37.089'	KM288214	B	HCO/LCO
FLEO1	neildi	USA: Florida, Pinellas County, Fort De Soto	27 x 2006	27° 38.347'	082° 44.085'	Restriction Digest	B	HCO/miniCOIF2
FLEO2	neildi	USA: Florida, Pinellas County, Fort De Soto	27 x 2006	27° 38.584'	082° 44.849'	Restriction Digest	B	HCO/miniCOIF2
FLEO4	neildi	USA: Florida,Pinellas County,Dunedin, Honeymoon Island State Park	29 x 2006	28° 04.807'	082° 49.944'	Restriction Digest	B	HCO/miniCOIF2
FLEO8	neildi	USA: Florida,Pinellas County,Dunedin, Honeymoon Island State Park	29 x 2006	28° 04.190'	082° 49.913'	Restriction Digest	B	HCO/miniCOIF2
FLG2009.1	zonalis	USA: Florida, Dade County, Everglades Greenway	18 xi 2009	25° 24.997'	080° 33.555'	Restriction Digest	B	HCO/LCO
FLG2009.10	zonalis	USA: Florida, Dade County, Everglades Greenway	18 xi 2009	25° 24.997'	080° 33.555'	Restriction Digest	B	HCO/LCO
FLG2009.11	zonalis	USA: Florida, Dade County, Everglades Greenway	18 xi 2009	25° 24.997'	080° 33.555'	Restriction Digest	B	HCO/LCO
FLG2009.12	zonalis	USA: Florida, Dade County, Everglades Greenway	18 xi 2009	25° 24.997'	080° 33.555'	Restriction Digest	B	HCO/LCO
FLG2009.13	zonalis	USA: Florida, Dade County, Everglades Greenway	18 xi 2009	25° 24.997'	080° 33.555'	Restriction Digest	B	HCO/LCO
FLG2009.14	zonalis	USA: Florida, Dade County, Everglades Greenway	18 xi 2009	25° 24.997'	080° 33.555'	Restriction Digest	B	HCO/LCO
FLG2009.15	zonalis	USA: Florida, Dade County, Everglades Greenway	18 xi 2009	25° 24.997'	080° 33.555'	Restriction Digest	B	HCO/LCO
FLG2009.16	zonalis	USA: Florida, Dade County, Everglades Greenway	18 xi 2009	25° 24.997'	080° 33.555'	Restriction Digest	B	HCO/LCO
FLG2009.17	zonalis	USA: Florida, Dade County, Everglades Greenway	18 xi 2009	25° 24.997'	080° 33.555'	Restriction Digest	B	HCO/LCO
FLG2009.18	zonalis	USA: Florida, Dade County, Everglades Greenway	18 xi 2009	25° 24.997'	080° 33.555'	Restriction Digest	B	HCO/LCO
FLG2009.19	zonalis	USA: Florida, Dade County, Everglades Greenway	18 xi 2009	25° 24.997'	080° 33.555'	Restriction Digest	B	HCO/LCO
FLG2009.2	zonalis	USA: Florida, Dade County, Everglades Greenway	18 xi 2009	25° 24.997'	080° 33.555'	Restriction Digest	B	HCO/LCO
FLG2009.20	zonalis	USA: Florida, Dade County, Everglades Greenway	18 xi 2009	25° 24.997'	080° 33.555'	Restriction Digest	B	HCO/LCO
FLG2009.21	zonalis	USA: Florida, Dade County, Everglades Greenway	18 xi 2009	25° 24.997'	080° 33.555'	Restriction Digest	B	HCO/LCO
FLG2009.22	zonalis	USA: Florida, Dade County, Everglades Greenway	18 xi 2009	25° 24.997'	080° 33.555'	Restriction Digest	B	HCO/LCO
FLG2009.23	zonalis	USA: Florida, Dade County, Everglades Greenway	18 xi 2009	25° 24.997'	080° 33.555'	Restriction Digest	A	HCO/miniCOIF2
FLG2009.24	zonalis	USA: Florida, Dade County, Everglades Greenway	18 xi 2009	25° 24.997'	080° 33.555'	Restriction Digest	A	HCO/miniCOIF2
FLG2009.25	zonalis	USA: Florida, Dade County, Everglades Greenway	18 xi 2009	25° 24.997'	080° 33.555'	Restriction Digest	B	HCO/LCO
FLG2009.26	zonalis	USA: Florida, Dade County, Everglades Greenway	18 xi 2009	25° 24.997'	080° 33.555'	Restriction Digest	A	HCO/miniCOIF2
FLG2009.3	zonalis	USA: Florida, Dade County, Everglades Greenway	18 xi 2009	25° 24.997'	080° 33.555'	Restriction Digest	B	HCO/LCO
FLG2009.4	zonalis	USA: Florida, Dade County, Everglades Greenway	18 xi 2009	25° 24.997'	080° 33.555'	Restriction Digest	B	HCO/LCO
FLG2009.5	zonalis	USA: Florida, Dade County, Everglades Greenway	18 xi 2009	25° 24.997'	080° 33.555'	Restriction Digest	B	HCO/LCO
FLG2009.6	zonalis	USA: Florida, Dade County, Everglades Greenway	18 xi 2009	25° 24.997'	080° 33.555'	Restriction Digest	B	HCO/LCO
FLG2009.7	zonalis	USA: Florida, Dade County, Everglades Greenway	18 xi 2009	25° 24.997'	080° 33.555'	Restriction Digest	B	HCO/LCO
FLG2009.8	zonalis	USA: Florida, Dade County, Everglades Greenway	18 xi 2009	25° 24.997'	080° 33.555'	Restriction Digest	B	HCO/LCO
FLG2009.9	zonalis	USA: Florida, Dade County, Everglades Greenway	18 xi 2009	25° 24.997'	080° 33.555'	Restriction Digest	B	HCO/LCO
FLG2010.1	zonalis	USA: Florida, Dade County, Everglades Greenway	21 iv 2010	25° 24.997'	080° 33.555'	Restriction Digest	B	HCO/LCO
FLGJa1	zonalis	USA: Florida, Dade County, Everglades Greenway	11 i 2007	25° 25.381'	080° 33.558'	KM288231	B	HCO/LCO
FLGJa10	zonalis	USA: Florida, Dade County, Everglades Greenway--North End	12 i 2007	25° 38.144'	080° 27.849'	KM288232	B	HCO/LCO
FLGJa11	zonalis	USA: Florida, Dade County, Everglades Greenway--North End	12 i 2007	25° 38.144'	080° 27.849'	KM288233	B	HCO/LCO
FLGJa12	zonalis	USA: Florida, Dade County, Everglades Greenway--North End	12 i 2007	25° 38.144'	080° 27.849'	KM288234	B	HCO/LCO
FLGJa13	zonalis--no white line be	USA: Florida, Dade County, Everglades Greenway--North End	12 i 2007	25° 38.144'	080° 27.849'	KM288235	B	HCO/LCO

Appendix I. *Junonia* specimens for the contemporary Florida data set

Code	*Junonia* Species	Location	Date	GPS Northing	GPS Westing	Genotyping Method	Haplotype	Primers
FLGJa14	zonalis	USA: Florida, Dade County, Everglades Greenway--North End	12 i 2007	25° 38.144’	080° 27.849’	KM288218	A	HCO/LCO
FLGJa15	zonalis--faint markings b	USA: Florida, Dade County, Everglades Greenway--North End	12 i 2007	25° 38.144’	080° 27.849’	KM288236	B	HCO/LCO
FLGJa16	zonalis	USA: Florida, Dade County, Everglades Greenway--North End	12 i 2007	25° 38.144’	080° 27.849’	KM288237	B	HCO/LCO
FLGJa17	zonalis	USA: Florida, Dade County, Everglades Greenway--North End	12 i 2007	25° 38.144’	080° 27.849’	KM288238	B	HCO/LCO
FLGJa18	zonalis	USA: Florida, Dade County, Everglades Greenway--North End	12 i 2007	25° 38.144’	080° 27.849’	Restriction Digest	B	HCO/LCO
FLGJa19	zonalis	USA: Florida, Dade County, Everglades Greenway--North End	12 i 2007	25° 38.144’	080° 27.849’	Restriction Digest	B	HCO/LCO
FLGJa2	zonalis	USA: Florida, Dade County, Everglades Greenway	11 i 2007	25° 25.394’	080° 33.558’	KM288239	B	HCO/LCO
FLGJa20	zonalis	USA: Florida, Dade County, Everglades Greenway--North End	12 i 2007	25° 38.144’	080° 27.849’	KM288240	B	HCO/LCO
FLGJa21	zonalis	USA: Florida, Dade County, Everglades Greenway--North End	12 i 2007	25° 38.144’	080° 27.849’	KM288241	B	HCO/LCO
FLGJa22	zonalis	USA: Florida, Dade County, Everglades Greenway--North End	12 i 2007	25° 38.144’	080° 27.849’	KM288242	B	HCO/LCO
FLGJa3	zonalis	USA: Florida, Dade County, Everglades Greenway	11 i 2007	25° 25.330’	080° 33.557’	KM288243	B	HCO/LCO
FLGJa4	zonalis	USA: Florida, Dade County, Everglades Greenway	11 i 2007	25° 25.392’	080° 33.556’	Restriction Digest	B	HCO/LCO
FLGJa5	zonalis	USA: Florida, Dade County, Everglades Greenway	11 i 2007	25° 24.973’	080° 33.556’	KM288244	B	HCO/LCO
FLGJa6	zonalis	USA: Florida, Dade County, Everglades Greenway	11 i 2007	25° 24.499’	080° 33.554’	KM288245	B	HCO/LCO
FLGJa7	zonalis	USA: Florida, Dade County, Everglades Greenway	11 i 2007	25° 24.966’	080° 33.554’	KM288246	A	HCO/LCO
FLGJa8	zonalis	USA: Florida, Dade County, Everglades Greenway--North End	11 i 2007	25° 38.178’	080° 29.851’	KM288247	B	HCO/LCO
FLGJa9	zonalis	USA: Florida, Dade County, Everglades Greenway--North End	12 i 2007	25° 38.144’	080° 27.849’	Restriction Digest	B	HCO/miniCOIF2
FLGM1	zonalis	USA: Florida, Dade County, Everglades Greenway	17 v 2006	25° 24.997’	080° 33.555’	KM288215	B	HCO/LCO
FLGM2	zonalis	USA: Florida, Dade County, Everglades Greenway	18 v 2006	25° 25.273’	080° 33.557’	KM288216	B	HCO/LCO
FLGM3	zonalis	USA: Florida, Dade County, Everglades Greenway	18 v 2006	25° 25.012’	080° 33.556’	KM288217	B	HCO/LCO
FLO1	zonalis	USA: Florida, Orange County, Orlando, Florida (Male)	24 v 1998	28° 32.300'	081° 22.754'	Restriction Digest	B	HCO/miniCOIF2

Appendix II. *Junonia* specimens for the historical Florida data set

Specimen Identifier	Collection	Museum Accession Number	Species	Locality	GPS Coordinates	Collection Date	Collector	Genotyping Method	Haplotype Group
BAHZ1991.1	Milwaukee Public Museum	1, IZ1993-35B.1, AS 27914	*Junonia zonalis*	Bahamas Is.: Exuma Cays, Great Exuma I.		13-Jul-91	D.W. Buden	Restriction Digest	B
BAHZ1991.2	Milwaukee Public Museum	2, IZ1993-35B.2, AS 27915	*Junonia zonalis*	Bahamas Is.: Exuma Cays, Great Exuma I.		13-Jul-91	D.W. Buden	Restriction Digest	B
BAHZ1994.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	Bahamas: Andros Island, 1-2 km SE Andros Town Airport, less than 5m		23 May 1994	L.D. Miller & M.Junonia Simon	Restriction Digest	A
BAHZ1994.2	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	Bahamas: Andros Island, 1-2 km SE Andros Town Airport, less than 5m		23 May 1994	L.D. Miller & M.Junonia Simon	Restriction Digest	B
BAHZ1994.3	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	Bahamas: Andros Island, 1-2 km SE Andros Town Airport, less than 5m		23 May 1994	L.D. Miller & M.Junonia Simon	Restriction Digest	A
BAHZ1994.5	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	Bahamas: Andros Island, 1-2 km SE Andros Town Airport, less than 5m		23 May 1994	L.D. Miller & M.Junonia Simon	Restriction Digest	A
BAHZ1994.6	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	Bahamas: Andros Island, 1-2 km SE Andros Town Airport, less than 5m		23 May 1994	L.D. Miller & M.Junonia Simon	Restriction Digest	A
BAHZ1994.4	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	Bahamas: Andros, 1km S. Fresh Creek, less than 5 m		23 May 1994	L.D. Miller & M.Junonia Simon	Restriction Digest	B
BAHN1982.1	Yale Peabody	YPM ENT 729938	*Junonia neildi*	Bahamas: Crooked Island, Colonel Hill		29-Oct-82	D.S. Dodge	Restriction Digest	B
BAHN1982.2	Yale Peabody	YPM ENT 729937	*Junonia neildi*	Bahamas: Crooked Island, Colonel Hill		29-Oct-82	D.S. Dodge	Restriction Digest	B
BAHZ1991.3	Milwaukee Public Museum	3, IZ1993-35B.3, AS 28008	*Junonia zonalis*	Bahamas: Exuma Cays, Great Exuma		09-Jul-91	D.W. Buden	Restriction Digest	A
BAHC1988.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	Bahamas: Grand Bahamas, Id. Freeport		2 October 1988	D.L. Bauer	Restriction Digest	A
BAHC1988.2	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	Bahamas: Grand Bahamas, Id. Freeport		2 October 1988	D.L. Bauer	Restriction Digest	B
BAHC1989.3	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	Bahamas: Grand Bahamas, Id. Lucayan		24 October 1989	D.L. Bauer	Restriction Digest	A
BAHC1989.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	Bahamas: Grand Bahamas, Id. Lucayan		26 october 1989	D.L. Bauer	Restriction Digest	A
BAHC1989.2	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	Bahamas: Grand Bahamas, Id. Lucayan		26 october 1989	D.L. Bauer	Restriction Digest	B
BAHC1989.4	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	Bahamas: Grand Bahamas, Id. Lucayan		26 october 1989	D.L. Bauer	Restriction Digest	B
BAHC1989.5	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	Bahamas: Grand Bahamas, Id. Lucayan		26 october 1989	D.L. Bauer	Restriction Digest	B
BAHC1989.6	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	Bahamas: Grand Bahamas, Id. Lucayan		26 october 1989	D.L. Bauer	Restriction Digest	B
BAHC1989.7	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	Bahamas: Grand Bahamas, Id. Lucayan		26 october 1989	D.L. Bauer	Restriction Digest	A
BAHC1989.8	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	Bahamas: Grand Bahamas, Id. Lucayan		27 october 1989	D.L. Bauer	Restriction Digest	B
BAHZ1980.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	Bahamas: Great Abaco, 1 mi N. Andy's Auto NW. Treasure Cayk		18 October 1980	C. Redfern	Restriction Digest	A
BAHN1979.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	Bahamas: Great Abaco, 1 mi. SE Andy's Auto NW Treasure Cayk		26 October 1979	C. Redfern	Restriction Digest	B
BAHN1979.2	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	Bahamas: Great Abaco, 1 mi. SE Andy's Auto NW Treasure Cayk		11 November 197	C. Redfern	Restriction Digest	B
BAHZ1985.2	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	Bahamas: Great Inagua Island, Horse Pond, circa 2mi NE Matthewtown		11 October 1985	L.D. Miller & M.Junonia Simon	Restriction Digest	B
BAHZ1985.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	Bahamas: Great Inagua Island, Man-O-War Bay nr. Calf Pond		9 October 1985	L.D. Miller & M.Junonia Simon	Restriction Digest	B
BAHN1986.3	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	Bahamas: Great Inuaga, Salt Works		1 October 1986	M.Junonia Simon & R. Miller	Restriction Digest	B
BAHN1986.4	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	Bahamas: Great Inuaga, Salt Works		3 October 1986	M.Junonia Simon & R. Miller	Restriction Digest	B
BAHN1986.5	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	Bahamas: Great Inuaga, Salt Works		3 October 1986	M.Junonia Simon & R. Miller	Restriction Digest	B
BAHN1987.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	Bahamas: Great Inuaga, Salt Works		22 September 198	M.Junonia Simon & R. Miller	Restriction Digest	A
BAHN1987.2	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	Bahamas: Great Inuaga, Salt Works		22 September 198	M.Junonia Simon & R. Miller	Restriction Digest	B
BAHN1987.5	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	Bahamas: Great Inuaga, Salt Works		22 September 198	M.Junonia Simon & R. Miller	Restriction Digest	A
BAHN1987.6	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	Bahamas: Great Inuaga, Salt Works		22 September 198	M.Junonia Simon & R. Miller	Restriction Digest	B
BAHN1987.7	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	Bahamas: Great Inuaga, Salt Works		22 September 198	M.Junonia Simon & R. Miller	Restriction Digest	B
BAHN1987.4	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	Bahamas: Great Inuaga, Salt Works		25 September 198	M.Junonia Simon & R. Miller	Restriction Digest	B
BAHN1987.3	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	Bahamas: Great Inuaga, Salt Works		26 September 198	M.Junonia Simon & R. Miller	Restriction Digest	A
BAHN1987.8	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	Bahamas: Great Inuaga, Salt Works		26 September 198	M.Junonia Simon & R. Miller	Restriction Digest	B
BAHN1987.9	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	Bahamas: Great Inuaga, Salt Works		26 September 198	M.Junonia Simon & R. Miller	Restriction Digest	B
BAHN1986.2	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	Bahamas: Great Inuaga, Salt Works		1 October 1986	M.Junonia Simon & R. Miller	Restriction Digest	A
BAHN1986.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi* (male)	Bahamas: Great Inuaga, Salt Works		1 October 1986	M.Junonia Simon & R. Miller	Restriction Digest	A
BAHN1988.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	Bahamas: Long Island, Glintons		27 September 198	L.D. Miller & M.Junonia Simon	Restriction Digest	B
BAHN1988.2	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	Bahamas: Long Island, Glintons		27 September 198	L.D. Miller & M.Junonia Simon	Restriction Digest	B
BAHN1988.3	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	Bahamas: Long Island, Glintons		27 September 198	L.D. Miller & M.Junonia Simon	Restriction Digest	A
BAHN1988.4	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	Bahamas: Long Island, Glintons		27 September 198	L.D. Miller & M.Junonia Simon	Restriction Digest	B
BAHN1988.5	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	Bahamas: Long Island, Glintons		27 September 198	L.D. Miller & M.Junonia Simon	Restriction Digest	A
BAHN1988.6	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	Bahamas: Long Island, Glintons		27 September 198	L.D. Miller & M.Junonia Simon	Restriction Digest	B
BAHN1988.7	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	Bahamas: Long Island, Glintons		27 September 198	L.D. Miller & M.Junonia Simon	Restriction Digest	B
BAHZ1988.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	Bahamas: Long Island, Glintons		27 September 198	L.D. Miller & M.Junonia Simon	Restriction Digest	A
BAHZ1960.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	Bahamas: New Providence		13 June 1960	L. Brush	Restriction Digest	B
BAHZ1957.1	Yale Peabody	YPM ENT 839737	*Junonia zonalis*	Bahamas: New Providence, Long Point		02-Aug-57	JunoniaG. Coutsis	Restriction Digest	B
BAHZ1957.2	Yale Peabody	YPM ENT 414496	*Junonia zonalis*	Bahamas: New Providence, Long Point		02-Aug-57	JunoniaG. Coutsis	Restriction Digest	B
BAHZ1987.1	Illinois Natural History Survey	0990	*Junonia zonalis*	Bahamas: San Andros		May 31-June 12, 1	Charles A. Bridges	Restriction Digest	B
CUBZ1866.1	Yale Peabody	YPM ENT 414455	*Junonia zonalis*	Cuba		roughly 1866	Suffert	Restriction Digest	B
CUBN1994.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	Cuba: Baracoa Boca de Yumuri		27 June 1994	L.C. & JunoniaY. Miller & L.R. Hernandez	Restriction Digest	B
CUBZ1994.2	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	Cuba: Baracoda, Camarones Minas Amores Rd., 6.7 km, 20m		28 June 1994	L.D. & JunoniaY. Miller & L.R. Hernandez	Restriction Digest	A
CUBZ1994.3	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	Cuba: Baracoda, Camarones Minas Amores Rd., 6.7 km, 20m		28 June 1994	L.D. & JunoniaY. Miller & L.R. Hernandez	Restriction Digest	A
CUBZ1994.4	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	Cuba: Baracoda, Camarones Minas Amores Rd., 6.7 km, 20m		28 June 1994	L.D. & JunoniaY. Miller & L.R. Hernandez	Restriction Digest	A
Cub 10			*Junonia zonalis*	Cuba: Gran Piedra, Sierra Maestre, Santiago		Aug-2009		KM288255	A
Cub 11			*Junonia zonalis*	Cuba: Gran Piedra, Sierra Maestre, Santiago		Aug-2009		KM288256	A
Cub 12			*Junonia zonalis*	Cuba: Gran Piedra, Sierra Maestre, Santiago		Aug-2009		Restriction Digest	B
Cub 13			*Junonia zonalis*	Cuba: Gran Piedra, Sierra Maestre, Santiago		Aug-2009		Restriction Digest	A
Cub 14			*Junonia zonalis*	Cuba: Gran Piedra, Sierra Maestre, Santiago		Aug-2009		Restriction Digest	B
Cub 15			*Junonia zonalis*	Cuba: Gran Piedra, Sierra Maestre, Santiago		Aug-2009		KM288257	B
Cub 4			*Junonia zonalis*	Cuba: Gran Piedra, Sierra Maestre, Santiago		Aug-2009		KM288250	B
Cub 5			*Junonia zonalis*	Cuba: Gran Piedra, Sierra Maestre, Santiago		Aug-2009		KM288251	B
Cub 6			*Junonia zonalis*	Cuba: Gran Piedra, Sierra Maestre, Santiago		Aug-2009		KM288252	A
Cub 7			*Junonia zonalis*	Cuba: Gran Piedra, Sierra Maestre, Santiago		Aug-2009		KM288253	A
Cub 9			*Junonia zonalis*	Cuba: Gran Piedra, Sierra Maestre, Santiago		Aug-2009		KM288254	B
Cub 17			*Junonia zonalis*	Cuba: Gran Piedra, Sierra Maestre, Santiago		5-Aug-2009		Restriction Digest	B
Cub 18			*Junonia zonalis*	Cuba: Gran Piedra, Sierra Maestre, Santiago		5-Aug-2009		Restriction Digest	B
Cub 19			*Junonia zonalis*	Cuba: Gran Piedra, Sierra Maestre, Santiago		5-Aug-2009		Restriction Digest	B
Cub 16			*Junonia zonalis*	Cuba: Gran Piedra, Sierra Maestre, Santiago (500-1000 m)		5-Aug-2009		KM288258	B
Cub 20			*Junonia zonalis*	Cuba: Gran Piedra, Sierra Maestre, Santiago (500-1000 m)		5-Aug-2009		KM288259	B
CUBZ1994.5	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	Cuba: Granma Jot. Palma De Parro & Victorino Rds., 300m		7 July 1994	L.D. & JunoniaY. Miller & M.Junonia Simon & L.R. Hernandez	Restriction Digest	B
CUBZ1912.2	Harvard University (MCZ-Entomology 170324)	170324	*Junonia zonalis (marked as Juno*	Cuba: Greater Antilles, Vicinity of Havana		1912	Thomas Barbour	Restriction Digest	B
CUBZ1912.3	Harvard University (MCZ-Entomology 170291)	170291	*Junonia zonalis (marked as Juno*	Cuba: Greater Antilles, Vicinity of Havana		1912	Thomas Barbour	Restriction Digest	A
CUBZ1912.1	Harvard University (MCZ-Entomology 170328)	170328	*Junonia zonalis (marked as Juno*	Cuba: Greater Antilles, Vicinity of Havana		6 xii 1912	Thomas Barbour	Restriction Digest	A
CUBZ1919.1	Yale Peabody	YPM ENT 414451	*Junonia zonalis*	Cuba: Guantanamo Bay		21-Jun-19	P.S. Remington	Restriction Digest	B
CUBZ1919.2	Yale Peabody	YPM ENT 414453	*Junonia zonalis*	Cuba: Guantanamo Bay		26-Jun-19	P.S. Remington	Restriction Digest	A
CUBZ1989.1	Milwaukee Public Museum	5, IZ1993-35B.4, AS 23773	*Junonia zonalis*	Cuba: Guantanamo, 15.8 km NW Baracoa (on coast road), 10m		27-Feb-89	A. Estrada, S. B. Hedges	Restriction Digest	B
CUBZ1990.1	Milwaukee Public Museum	6, IZ1993-35B.5, AS 26139	*Junonia zonalis*	Cuba: Guantanamo, 3.9 km N Hatibonico, in the Montongas coast, 85m		14-Jun-90	S.B. Hedges	Restriction Digest	B
CUBZ1995.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	Cuba: Guantanamo, Los Camarones Minas Amores Rd., 60m		12 July 1995	L.D. & JunoniaY Miller & M.Junonia Simon, & L.R. Hernandez	Restriction Digest	B
CUBZ1995.2	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	Cuba: Guantanamo, Los Camarones Minas Amores Rd., 60m		12 July 1995	L.D. & JunoniaY Miller & M.Junonia Simon, & L.R. Hernandez	Restriction Digest	B
CUBZ1995.3	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	Cuba: Guantanamo, Los Camarones Minas Amores Rd., 60m		12 July 1995	L.D. & JunoniaY Miller & M.Junonia Simon, & L.R. Hernandez	Restriction Digest	A
CUBZ1995.4	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	Cuba: Guantanamo, Los Camarones Minas Amores Rd., 60m		12 July 1995	L.D. & JunoniaY Miller & M.Junonia Simon, & L.R. Hernandez	Restriction Digest	A
CUBZ1985.1	Milwaukee Public Museum	4, IZ1993-35B.6, AS 15252	*Junonia zonalis*	Cuba: Guantanamo, U.S. Naval Base, east side of bay		09-Oct-85	B.I. Crother	Restriction Digest	B
CUBZ1955.1	Yale Peabody	YPM ENT 414448	*Junonia zonalis*	Cuba: Harvard Gardens, Soleclad, L.U.		26-Aug-55	G.E. Watson	Restriction Digest	A
CUBX1928.1	Yale Peabody	YPM ENT 414449	*Junonia zonalis*	Cuba: Havana		27-Dec-28	S.A. Hessel	Restriction Digest	A
CUBX1929.1	Yale Peabody	YPM ENT 839757	*Junonia zonalis*	Cuba: Havana		Dec-29	S.A. Hessel	Restriction Digest	B
CUBX1929.2	Yale Peabody	YPM ENT 839756	*Junonia zonalis*	Cuba: Havana		Dec-29	S.A. Hessel	Restriction Digest	B
CUBZ1929.1	Yale Peabody	YPM ENT 414431	*Junonia zonalis*	Cuba: Havana		Dec-29	S.A. Hessel	Restriction Digest	A
CUBZ1929.2	Yale Peabody	YPM ENT 839750	*Junonia zonalis*	Cuba: Havana		Dec-29	S.A. Hessel	Restriction Digest	A
CUBZ1929.3	Yale Peabody	YPM ENT 839745	*Junonia zonalis*	Cuba: Havana		Dec-29	S.A. Hessel	Restriction Digest	B
CUBZ1929.4	Yale Peabody	YPM ENT 839741	*Junonia zonalis*	Cuba: Havana		Dec-29	S.A. Hessel	Restriction Digest	B
CUBZ1929.5	Yale Peabody	YPM ENT 839779	*Junonia zonalis*	Cuba: Havana		Dec-29	S.A. Hessel	Restriction Digest	B
CUBN1955.1	Yale Peabody	YPM ENT 414314	*Junonia neildi*	Cuba: Havana, Havana		09-Oct-55	G.E. Watson	Restriction Digest	B
CUBN1955.3	Yale Peabody	YPM ENT 843934	*Junonia neildi*	Cuba: Havana, Havana		09-Oct-55	G.E. Watson	Restriction Digest	B
CUBN1955.4	Yale Peabody	YPM ENT 843933	*Junonia neildi*	Cuba: Havana, Havana		09-Oct-55	G.E. Watson	Restriction Digest	A
CUBN1955.6	Yale Peabody	YPM ENT 843924	*Junonia neildi*	Cuba: Havana, Havana		09-Oct-55	G.E. Watson	Restriction Digest	A
CUBN1955.2	Yale Peabody	YPM ENT 414307	*Junonia neildi*	Cuba: Havana, Havana		14-Oct-55	G.E. Watson	Restriction Digest	B
CUBN1955.5	Yale Peabody	YPM ENT 843932	*Junonia neildi*	Cuba: Havana, Havana		14-Oct-55	G.E. Watson	Restriction Digest	A

Appendix II. *Junonia* specimens for the historical Florida data set

Specimen Identifier	Collection	Museum Accession Number	Species	Locality	GPS Coordinates	Collection Date	Collector	Genotyping Method	Haplotype Group
CUBX1955.2	Yale Peabody	YPM ENT 843929	*Junonia neildi (ZxN)*	Cuba: Havana, Havana		09-Oct-55	G.E. Watson	Restriction Digest	B
CUBZ1955.3	Yale Peabody	YPM ENT 414438	*Junonia zonalis*	Cuba: Havana, Havana		09-Oct-55	G.E. Watson	Restriction Digest	B
CUBZ1955.2	Yale Peabody	YPM ENT 414440	*Junonia zonalis*	Cuba: Havana, Lake Arguanabo		05-Sep-55	G.E. Watson	Restriction Digest	B
CUBZ1994.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	Cuba: Jauco Mpio Maiai, 60-100m		26 June 1994	L.D. & JunoniaY. Miller & L.R. Hernandez	Restriction Digest	A
CUBZ1940.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	Cuba: Madruga		12 September 1940		Restriction Digest	A
CUBN1950.2	Yale Peabody	YPM ENT 414352	*Junonia neildi*	Cuba: Mantanzas Province, Playa		21-Jul-50	S.L. de la Torre y Callejas	Restriction Digest	B
CUBN1950.3	Yale Peabody	YPM ENT 414353	*Junonia neildi*	Cuba: Mantanzas Province, Playa		21-Aug-50	S.L. de la Torre y Callejas	Restriction Digest	B
CUBX1950.1	Yale Peabody	YPM ENT 414351	*Junonia neildi (ZxN)*	Cuba: Mantanzas Province, Playa		16-Aug-51	S.L. de la Torre y Callejas	Restriction Digest	B
CUBC1950.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	Cuba: Matanzas Playa		21 August 1950	Salvador L. de la Torro	Restriction Digest	B
CUBZ1950.3	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	Cuba: Matanzas Playa		14 July 1950	Salvador L. de la Torro	Restriction Digest	A
CUBN1950.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	Cuba: Matanzas Playa		27 July 1950	Salvador L. de la Torro	Restriction Digest	A
CUBZ1950.4	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	Cuba: Matanzas Playa		25 July 1950	Salvador L. de la Torro	Restriction Digest	A
CUBZ1950.6	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	Cuba: Matanzas Playa		8 August 1950	Salvador L. de la Torro	Restriction Digest	A
CUBZ1950.2	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	Cuba: Matanzas Playa		8 October 1950	Salvador L. de la Torro	Restriction Digest	A
CUBZ1951.2	Yale Peabody	YPM ENT 414446	*Junonia zonalis*	Cuba: Matanzas Province, Playa		10-Aug-51	S.L. Torre y Dallejas	Restriction Digest	B
CUBZ1951.3	Yale Peabody	YPM ENT 414445	*Junonia zonalis*	Cuba: Matanzas Province, Playa		16-Aug-51	S.L. Torre y Dallejas	Restriction Digest	B
CUBZ1951.4	Yale Peabody	YPM ENT 414442	*Junonia zonalis*	Cuba: Matanzas Province, Playa		17-Sep-51	S.L. Torre y Dallejas	Restriction Digest	B
CUBZ1950.5	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	Cuba: Matanzas Yumuri		30 August 1950	Salvador L. de la Torro	Restriction Digest	B
CUBZ1950.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis?*	Cuba: Matanzas Yumuri		2 October 1950	Salvador L. de la Torro	Restriction Digest	B
CUBZ1951.5	Yale Peabody	YPM ENT 414435	*Junonia zonalis*	Cuba: Matanzas, Los Practicos		15-Sep-51	S.L. Torre y Dallejas	Restriction Digest	B
CUBZ1951.6	Yale Peabody	YPM ENT 414433	*Junonia zonalis*	Cuba: Matanzas, Los Practicos		16-Sep-51	S.L. Torre y Dallejas	Restriction Digest	B
CUBZ1951.8	Yale Peabody	YPM ENT 839755	*Junonia zonalis*	Cuba: Matanzas, Los Practicos		16-Sep-51	S.L. Torre y Dallejas	Restriction Digest	B
CUBC1988.1	Milwaukee Public Museum	7, IZ1993-35B.7, AS 23414	*Junonia coenia*	Cuba: Matanzas, soplillar, ca. 5m (E Playa Larga)		June 18, 1988	Hass, S.B. Hedges, R. Thomas	Restriction Digest	B
CUBC1988.2	Milwaukee Public Museum	8, IZ1993-35B.8,AS 23415	*Junonia coenia*	Cuba: Matanzas, soplillar, ca. 5m (E Playa Larga)		June 18, 1988	Hass, S.B. Hedges, R. Thomas	Restriction Digest	A
CUBZ1988.1	Milwaukee Public Museum	9, IZ1993-35B.9, AS 23418	*Junonia zonalis*	Cuba: Matanzas, Soplillar, ca. 5m (E Playa Larga)		18-Jun-88	Hass, S. B. Hedges, R. Thomas	Restriction Digest	B
CUBZ1988.2	Milwaukee Public Museum	10, IZ1993-35B.10, AS 23419	*Junonia zonalis*	Cuba: Matanzas, Soplillar, ca. 5m (E Playa Larga)		18-Jun-88	Hass, S. B. Hedges, R. Thomas	Restriction Digest	B
CUBZ1988.3	Milwaukee Public Museum	11, IZ1993-35B.11, AS 23425	*Junonia zonalis*	Cuba: Matanzas, Soplillar, ca. 5m (E Playa Larga)		18-Jun-88	Hass, S. B. Hedges, R. Thomas	Restriction Digest	A
CUBZ1951.10	Yale Peabody	YPM ENT 839748	*Junonia zonalis*	Cuba: Matanzas, Versalles		15-Sep-51	S.L. Torre y Dallejas	Restriction Digest	A
CUBZ1951.11	Yale Peabody	YPM ENT 839736	*Junonia zonalis*	Cuba: Matanzas, Versalles		15-Sep-51	S.L. Torre y Dallejas	Restriction Digest	B
CUBZ1951.12	Yale Peabody	YPM ENT 839778	*Junonia zonalis*	Cuba: Matanzas, Versalles		15-Sep-51	S.L. Torre y Dallejas	Restriction Digest	B
CUBZ1951.13	Yale Peabody	YPM ENT 839777	*Junonia zonalis*	Cuba: Matanzas, Versalles		15-Sep-51	S.L. Torre y Dallejas	Restriction Digest	B
CUBZ1951.14	Yale Peabody	YPM ENT 839761	*Junonia zonalis*	Cuba: Matanzas, Versalles		15-Sep-51	S.L. Torre y Dallejas	Restriction Digest	B
CUBZ1951.7	Yale Peabody	YPM ENT 414427	*Junonia zonalis*	Cuba: Matanzas, Versalles		15-Sep-51	S.L. Torre y Dallejas	Restriction Digest	B
CUBZ1951.9	Yale Peabody	YPM ENT 839751	*Junonia zonalis*	Cuba: Matanzas, Versalles		15-Sep-51	S.L. Torre y Dallejas	Restriction Digest	B
CUBZ1951.1	Yale Peabody	YPM ENT 414450	*Junonia zonalis*	Cuba: Matanzas, Yumuri		15-Sep-51	S.L. Torre y Dallejas	Restriction Digest	A
CUBC1991.1	Milwaukee Public Museum	14, IZ1993-35B.14, AS 27669	*Junonia coenia*	Cuba: Pinar del Rio, 4.4 km NW Guane, 15 m		July 20, 1991	Hedges, A. Estrada	Restriction Digest	B
CUBZ1991.1	Milwaukee Public Museum	12, IZ1993-35B.12, AS 27666	*Junonia zonalis*	Cuba: Pinar del Rio, 4.4 km NW Guane, 15 m		20-Jul-91	Hedges, A. Estrada	Restriction Digest	A
CUBZ1991.2	Milwaukee Public Museum	13, IZ1993-35B.13, AS 27668	*Junonia zonalis*	Cuba: Pinar del Rio, 4.4 km NW Guane, 15 m		20-Jul-91	Hedges, A. Estrada	Restriction Digest	B
CUBZ1990.2	Milwaukee Public Museum	15, IZ1993-35B.15, AS 26509	*Junonia zonalis*	Cuba: Pinar del Rio, 7.5 km S. Soroa, 10 m		13-14 jul 1990	S.B. Hedges	Restriction Digest	B
CUBZ2015.1	Carlos A. Cruz		*Junonia zonalis*	Cuba: Pinar del Río, Pinar del Río		January 3, 2015	Carlos A. Cruz	Restriction Digest	A
CUBZ2015.2	Carlos A. Cruz		*Junonia zonalis*	Cuba: Pinar del Río, Pinar del Río		January 3, 2015	Carlos A. Cruz	Restriction Digest	B
CUBZ2015.3	Carlos A. Cruz		*Junonia zonalis*	Cuba: Pinar del Río, Pinar del Río		January 3, 2015	Carlos A. Cruz	Restriction Digest	B
CUBZ2015.4	Carlos A. Cruz		*Junonia zonalis*	Cuba: Pinar del Río, Pinar del Río		January 3, 2015	Carlos A. Cruz	Restriction Digest	A
CUBN1948.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	Cuba: Playa Habana		15 July 1948	S.L. de la Torrec	Restriction Digest	B
CUBX1955.1	Yale Peabody	YPM ENT 414305	*Junonia neildi (ZxN)*	Cuba: Playa Havana		14-Oct-55	G.E. Watson	Restriction Digest	B
OLD 22.1	Marcus Lab		*Junonia zonalis*	Cuba: Santiago de Cuba		1933	H. Frère Clémente, F.E. Church	Restriction Digest	B
CUBZ1932.2	Milwaukee Public Museum	126, IZ117458	*Junonia zonalis*	Cuba: Santiago de Cuba		15-Aug-32	R.E. Griffin	Restriction Digest	A
CUBZ1989.2	Milwaukee Public Museum	16, IZ1993-35B.16, AS 23887	*Junonia zonalis*	Cuba: Santiago de Cuba, Las Cuevas, SW base Pico Turquino, 5 m		06-Aug-89	S.B. Hedges, R. Thomas	Restriction Digest	A
CUBZ1989.3	Milwaukee Public Museum	17, IZ1993-35B.17, AS 23889	*Junonia zonalis*	Cuba: Santiago de Cuba, Las Cuevas, SW base Pico Turquino, 5 m		06-Aug-89	Hedges, R. Thomas	Restriction Digest	B
CUBZ1989.4	Milwaukee Public Museum	18, IZ1993-35B18, AS 23891	*Junonia zonalis*	Cuba: Santiago de Cuba, Las Cuevas, SW base Pico Turquino, 5 m		06-Aug-89	Hedges, R. Thomas	Restriction Digest	B
CUBZ1989.5	Milwaukee Public Museum	19, IZ1993-35B.19, AS 23893	*Junonia zonalis*	Cuba: Santiago de Cuba, Las Cuevas, SW base Pico Turquino, 5 m		06-Aug-89	Hedges, R. Thomas	Restriction Digest	B
CUBZ1989.6	Milwaukee Public Museum	20, IZ1993-35B.20, AS 23900	*Junonia zonalis*	Cuba: Santiago de Cuba, Las Cuevas, SW base Pico Turquino, 5 m		06-Aug-89	Hedges, R. Thomas	Restriction Digest	B
CUBZ1989.7	Milwaukee Public Museum	21, IZ1993-35B.21, AS 23901	*Junonia zonalis*	Cuba: Santiago de Cuba, Las Cuevas, SW base Pico Turquino, 5 m		06-Aug-89	Hedges, R. Thomas	Restriction Digest	A
CUBN1989.1	Milwaukee Public Museum	22, IZ1993-35B.23, AS 24175	*Junonia neildi*	Cuba: Santiago de Cuba, South Side, Laguna de Baconao, 0m		16 August 1989	R.Thomas	Restriction Digest	B
CUBN1989.2	Milwaukee Public Museum	23, IZ1993-35B.22, AS 24171	*Junonia neildi*	Cuba: Santiago de Cuba, South Side, Laguna de Baconao, 0m		16 August 1989	R.Thomas	Restriction Digest	A
OLD24.1	Marcus Lab		*Junonia zonalis*	Cuba: Sierra Maestra, Cuba		1953	H. Fre're Cle'mente, F.E. Church	Restriction Digest	B
OLD23.1	Marcus Lab		*Junonia zonalis*	Cuba: Tanamo		approx. 1903	W. Schaus	Restriction Digest	B
OLD21.1	Marcus Lab		*Junonia zonalis*	Cuba: Tanamo		approx. 1903	W. Schaus	Restriction Digest	A
CUBZ1932.1	Harvard University (MCZ-Entomology 170306)	170306	*Junonia zonalis (marked as Juno*	Cuba: Villa Clara Province, Greater Antilles, Santa Clara, Soledad		29 xii 1932	D. Marston Bates, Alexander G.B. Fairchild	Restriction Digest	B
CUBZ1953.1	Milwaukee Public Museum	119, IZ1980-01.1	*Junonia zonalis*	Cuba: West Indies		1953	D. Glanz	Restriction Digest	A
CUBZ1953.2	Milwaukee Public Museum	120, IZ1980-01.2	*Junonia zonalis*	Cuba: West Indies		1953	D. Glanz	Restriction Digest	B
CUBZ1953.3	Milwaukee Public Museum	121, IZ1980-01.3	*Junonia zonalis*	Cuba: West Indies		1953	D. Glanz	Restriction Digest	B
FLC1875.1	Yale Peabody	YPM ENT 415390	*Junonia coenia*	USA: Florida mainland		1875	C.P. Whitney	Restriction Digest	B
FLC1894.1	Yale Peabody	YPM ENT 415282	*Junonia coenia*	USA: Florida mainland		1894	S.Stone	Restriction Digest	B
FLE1919.1	Harvard University	110879	Junonia neildi (marked as Junoni	USA: Florida mainland		ii 1919	John B. Paine Jr.	Restriction Digest	B
FLE1967.4	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida Monroe Co., Big Pine Key, Long Beach		09-Aug-67	F. Rutkowski	Restriction Digest	A
FLE1967.5	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida Monroe Co., Big Pine Key, Long Beach		09-Aug-67	F. Rutkowski	Restriction Digest	A
FLC1978.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida Monroe Co., Key Largo		22-Oct-78	Bob Hollister	Restriction Digest	A
FLX1966.1	Yale Peabody	YPM ENT 415261	*Junonia coenia (CxG)*	USA: Florida, Alachua County, 10 mi. N. Gainesville		27-Sep-66	Mays, D. L.	Restriction Digest	A
FLC1977.3	Michigan State University		*Junonia coenia*	USA: Florida, Alligator Alley		12-Feb-77	from the Bruce Wilson Collection	Restriction Digest	B
FLC1925.1	University of Michigan	UMMZI-00204379	*Junonia coenia*	USA: Florida, Broward Co., Fort Lauderdale		26 vii 1925	D. Marston Bates	Restriction Digest	B
FLC1923.1	University of Michigan	UMMZI-00204385	*Junonia coenia*	USA: Florida, Broward Co., Fort Lauderdale		28 i 1923	D. Marston Bates	Restriction Digest	B
FLC1935.2	University of Michigan	UMMZI-00204392	*Junonia coenia*	USA: Florida, Broward Co., Fort Lauderdale		30 vi 1935	I.Junonia Cantrall	Restriction Digest	B
FLC1935.1	University of Michigan	UMMZI-00204390	*Junonia coenia*	USA: Florida, Broward Co., Fort Lauderdale		30 vii 1935	I.Junonia Cantrall	Restriction Digest	B
FLC1920.1	University of Michigan	UMMZI-00204381	*Junonia coenia*	USA: Florida, Broward Co., Fort Lauderdale		xii 1920	D. Marston Bates	Restriction Digest	B
FLC1982.2	Mississippi		*Junonia coenia*	USA: Florida, Collier Co., 12 mi. SW of Immok on hwy S46 (In flight, road ditach on Everglades Blvd.)		October 10, 1982	JunoniaJunonia Williams	Restriction Digest	B
FLE1979.5	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Collier Co., Chokoloskee		10-Nov-79	D. Leston	Restriction Digest	B
FLE1981.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Collier Co., Chokoloskee		19-Nov-81	Bob Hollister	Restriction Digest	B
FLE1981.2	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Collier Co., Chokoloskee		19-Nov-81	Bob Hollister	Restriction Digest	B
FLE1981.3	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Collier Co., Chokoloskee		19-Nov-81	Bob Hollister	Restriction Digest	B
FLE1900.1	Milwaukee Public Museum	123, IZ2015-02.2	*Junonia neildi*	USA: Florida, Collier Co., Chokoloskee		Dec 3 early 1900's		Restriction Digest	B
FLE1979.12	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi* (dark top and orar	USA: Florida, Collier Co., Chokoloskee		10-Nov-79	D. Rivera	Restriction Digest	B
FLX1900.1	Milwaukee Public Museum	122, IZ2015-02.1	*Junonia X (EXG)*	USA: Florida, Collier Co., Chokoloskee		Decmeber 3 early 1900's		Restriction Digest	A
FLG1980.1	McGuire Center for Lepidoptera and Biodiversity		Junonia zonalis (but marked as J	USA: Florida, Collier Co., Chokoloskee		pre-1980	Accessioned Allyn Museum 1980	Restriction Digest	B
FLE1978.3	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Collier Co., Collier Seminal State Park		22-Nov-78	Bob Hollister	Restriction Digest	A
FLE1985.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Collier Co., Collier Seminal State Park		##############	L.C.D	Restriction Digest	A
FLC1967.2	Yale Peabody	YPM ENT 415236	*Junonia coenia*	USA: Florida, Collier Co., Collier-Seminole Park, 1 mile SE of Royal Palm		10-May-67	D.A. Wright	Restriction Digest	B
FLC1957.1	Michigan State University		*Junonia coenia*	USA: Florida, Collier Co., Copeland		01-Feb-57	R.R. Carryl	Restriction Digest	B
FLC1923.2	California Academy of Science		*Junonia coenia*	USA: Florida, Collier Co., Everglades		17 June 1923		Restriction Digest	A
FLG1973.1	California Academy of Sciences		*Junonia zonalis*	USA: Florida, Collier Co., Everglades		27-Jun-73	JunoniaW. Tilden	Restriction Digest	B
FLE1956.1	Colorado State University		*Junonia neildi*	USA: Florida, Collier Co., Everglades City		August 10, 1956	RWH	Restriction Digest	B
FLC1965.3	Yale Peabody	YPM ENT 839681	*Junonia coenia*	USA: Florida, Collier Co., Everglades National Park, Royal Palm Hammock		28-Mar-65	D.S. Chambers	Restriction Digest	B
FLC1965.5	Yale Peabody	YPM ENT 839696	*Junonia coenia*	USA: Florida, Collier Co., Everglades National Park, Royal Palm Hammock		28-Mar-65	D.S. Chambers	Restriction Digest	B
FLC1965.1	Yale Peabody	YPM ENT 415223	*Junonia coenia (rosa)*	USA: Florida, Collier Co., Everglades National Park, Royal Palm Hammock		28-Mar-65	D.S. Chambers	Restriction Digest	B
FLE1976.2	McGuire Center for Lepidoptera and Biodiversity		*Junonai neildi* (Female)	USA: Florida, Collier Co., Golden Gate, 10 ft.		17-Nov-76		Restriction Digest	B
FLC1976.13	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Collier Co., Golden Gate, 10ft.		17-Nov-76		Restriction Digest	B
FLC1976.7	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia (female)*	USA: Florida, Collier Co., Golden Gate, 10ft.		17-Nov-76		Restriction Digest	B
FLC1976.12	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia (female, rosa)*	USA: Florida, Collier Co., Golden Gate, 10ft.		17-Nov-76		Restriction Digest	B
FLC1976.8	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia* (female, rosa)	USA: Florida, Collier Co., Golden Gate, 10ft.		17-Nov-76		Restriction Digest	B
FLC1976.11	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia (male)*	USA: Florida, Collier Co., Golden Gate, 10ft.		17-Nov-76		Restriction Digest	A
FLC1976.6	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia (male)*	USA: Florida, Collier Co., Golden Gate, 10ft.		17-Nov-76		Restriction Digest	A
FLC1976.10	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia (male, rosa)*	USA: Florida, Collier Co., Golden Gate, 10ft.		17-Nov-76		Restriction Digest	A

Appendix II. *Junonia* specimens for the historical Florida data set

Specimen Identifier	Collection	Museum Accession Number	Species	Locality	GPS Coordinates	Collection Date	Collector	Genotyping Method	Haplotype Group
FLC1976.5	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia* (male, rosa)	USA: Florida, Collier Co., Golden Gate, 10ft.		17-Nov-76		Restriction Digest	A
FLC1976.9	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia (male, rosa)*	USA: Florida, Collier Co., Golden Gate, 10ft.		17-Nov-76		Restriction Digest	B
FLC1976.4	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia* (male; rosa)	USA: Florida, Collier Co., Golden Gate, 10ft.		17-Nov-76		Restriction Digest	B
FLC1993.1	Mississippi		*Junonia coenia*	USA: Florida, Collier Co., Isle of Capri		July 18, 1993	Ricky Patterson	Restriction Digest	A
FLC1993.2	Mississippi		*Junonia coenia*	USA: Florida, Collier Co., Isle of Capri		July 18, 1993	Ricky Patterson	Restriction Digest	B
FLC1963.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Collier Co., Monroe Station		01-Sep-63	Harold L. King	Restriction Digest	B
FLC1970.36	Yale Peabody	YPM ENT 839821	*Junonia coenia*	USA: Florida, Collier Co., Naples		17-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.4	Yale Peabody	YPM ENT 415203	*Junonia coenia*	USA: Florida, Collier Co., Naples		17-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.57	Yale Peabody	YPM ENT 839837	*Junonia coenia*	USA: Florida, Collier Co., Naples		17-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.58	Yale Peabody	YPM ENT 839836	*Junonia coenia*	USA: Florida, Collier Co., Naples		17-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.64	Yale Peabody	YPM ENT 839830	*Junonia coenia*	USA: Florida, Collier Co., Naples		17-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.65	Yale Peabody	YPM ENT 839828	*Junonia coenia*	USA: Florida, Collier Co., Naples		17-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.66	Yale Peabody	YPM ENT 839827	*Junonia coenia*	USA: Florida, Collier Co., Naples		17-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.67	Yale Peabody	YPM ENT 839826	*Junonia coenia*	USA: Florida, Collier Co., Naples		17-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.68	Yale Peabody	YPM ENT 839825	*Junonia coenia*	USA: Florida, Collier Co., Naples		17-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.70	Yale Peabody	YPM ENT 839823	*Junonia coenia*	USA: Florida, Collier Co., Naples		17-Jun-70	T.R. Manley	Restriction Digest	B
FLC1996.1	University of Manitoba	306293	*Junonia coenia*	USA: Florida, Collier Co., Naples		15-Sep-96	S. Lenberger	Restriction Digest	B
FLC1996.2	University of Manitoba	328967	*Junonia coenia*	USA: Florida, Collier Co., Naples		September 15, 19(	S. Lenberger	Restriction Digest	A
FLC1996.3	University of Manitoba	328966	*Junonia coenia*	USA: Florida, Collier Co., Naples		September 15, 19(	S. Lenberger	Restriction Digest	B
FLX1970.6	Yale Peabody	YPM ENT 843434	*Junonia coenia (CxG)*	USA: Florida, Collier Co., Naples		17-Jun-70	T.R. Manley	Restriction Digest	B
FLE1969.6	Yale Peabody	YPM ENT 792474	*Junonia neildi*	USA: Florida, Collier Co., Naples		16-Aug-69		Restriction Digest	A
FLX1959.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia X Junonia zonali*	USA: Florida, Collier Co., Ochopee, Monroe Station		07-May-59	John Junonia Bowe	Restriction Digest	B
FLC1986.1	C.V. Covell Collection		*Junonia coenia (female?)*	USA: Florida, Collier Co., Rt. 17		May 17, 1986	C.V. Covell III	Restriction Digest	A
FLE1979.8	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Dade Co., Card Sound Rd.		02-Mar-79	B. Lenczewski	Restriction Digest	B
FLE1969.4	Yale Peabody	YPM ENT 793065	*Junonia neildi*	USA: Florida, Dade Co., Deering Estate, Cutler Ridge		15-Aug-69	P.B. Mason	Restriction Digest	B
FLE1979.9	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Dade Co., Elliot Key		02-May-79	B. Rivera	Restriction Digest	A
FLE1979.6	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Dade Co., Elliot Key		02-Aug-79	D. Leston	Restriction Digest	B
FLE1972.2	C.V. Covell Collection		*Junonia neildi*	USA: Florida, Dade Co., end of Bear Lake Trail Flamingo, Everglades National Park		10-May-72	C.V. Covell	Restriction Digest	B
FLE1955.1	Yale Peabody	YPM ENT 414330	*Junonia neildi*	USA: Florida, Dade Co., Everglades National Park		18-Jan-55	C.L. Remington	Restriction Digest	B
FLE1978.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Dade Co., Everglades National Park		23-Oct-78	Bob Hollister	Restriction Digest	B
FLE1978.2	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Dade Co., Everglades National Park		23-Oct-78	Bob Hollister	Restriction Digest	A
FLE1978.5	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Dade Co., Everglades National Park		23-Oct-78	Bob Hollister	Restriction Digest	B
FLE1978.6	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Dade Co., Everglades National Park		23-Oct-78	Bob Hollister	Restriction Digest	A
FLE1978.4	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Dade Co., Everglades National Park		23-Nov-78	Bob Hollister	Restriction Digest	B
FLC1970.10	Yale Peabody	YPM ENT 415309	*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park		18-Jun-70	T.R. Manley	Restriction Digest	A
FLC1970.47	Yale Peabody	YPM ENT 843288	*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park		18-Jun-70	T.R. Manley	Restriction Digest	A
FLC1970.71	Yale Peabody	YPM ENT 843333	*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park		18-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.72	Yale Peabody	YPM ENT 843328	*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park		18-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.73	Yale Peabody	YPM ENT 843326	*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park		18-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.74	Yale Peabody	YPM ENT 843325	*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park		18-Jun-70	T.R. Manley	Restriction Digest	A
FLC1970.75	Yale Peabody	YPM ENT 843324	*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park		18-Jun-70	T.R. Manley	Restriction Digest	A
FLC1970.76	Yale Peabody	YPM ENT 843322	*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park		18-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.77	Yale Peabody	YPM ENT 843320	*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park		18-Jun-70	T.R. Manley	Restriction Digest	A
FLC1970.78	Yale Peabody	YPM ENT 843319	*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park		18-Jun-70	T.R. Manley	Restriction Digest	A
FLC1970.79	Yale Peabody	YPM ENT 843313	*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park		18-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.80	Yale Peabody	YPM ENT 843312	*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park		18-Jun-70	T.R. Manley	Restriction Digest	A
FLC1970.81	Yale Peabody	YPM ENT 843311	*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park		18-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.82	Yale Peabody	YPM ENT 843307	*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park		18-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.83	Yale Peabody	YPM ENT 843306	*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park		18-Jun-70	T.R. Manley	Restriction Digest	A
FLC1970.84	Yale Peabody	YPM ENT 843302	*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park		18-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.85	Yale Peabody	YPM ENT 843301	*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park		18-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.86	Yale Peabody	YPM ENT 843292	*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park		18-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.87	Yale Peabody	YPM ENT 843291	*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park		18-Jun-70	T.R. Manley	Restriction Digest	A
FLC1970.88	Yale Peabody	YPM ENT 843290	*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park		18-Jun-70	T.R. Manley	Restriction Digest	B
FLG1970.2	Yale Peabody	YPM ENT 843300	*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park		18-Jun-70	T.R. Manley	Restriction Digest	B
FLX1970.5	Yale Peabody	YPM ENT 843874	*Junonia coenia (CxG)*	USA: Florida, Dade Co., Everglades National Park		18-Jun-70	T.R. Manley	Restriction Digest	B
FLX1970.7	Yale Peabody	YPM ENT 843327	*Junonia coenia (CxG)*	USA: Florida, Dade Co., Everglades National Park		18-Jun-70	T.R. Manley	Restriction Digest	A
FLX1970.8	Yale Peabody	YPM ENT 843321	*Junonia coenia (CxG)*	USA: Florida, Dade Co., Everglades National Park		18-Jun-70	T.R. Manley	Restriction Digest	B
FLC1956.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park, 20ft.		30-Jun-56		Restriction Digest	A
FLE1955.5	Yale Peabody	YPM ENT 414362	*Junonia neildi*	USA: Florida, Dade Co., Everglades National Park, Coot Bay		18-Jan-55	JunoniaE. Remington, C.L. Remington, E.E. Remington	Restriction Digest	A
FLE1955.6	Yale Peabody	YPM ENT 843916	*Junonia neildi*	USA: Florida, Dade Co., Everglades National Park, Coot Bay		18-Jan-55	JunoniaE. Remington, C.L. Remington, E.E. Remington	Restriction Digest	A
FLCMar11	Marcus Lab		*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park, Dade County, Chekika	25° 37.039' 080° 34.966'	13 iii 2007	Jeffrey M. Marcus	Restriction Digest	B
FLCMar12	Marcus Lab		*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park, Dade County, Chekika	25° 36.971' 080° 34.902'	13 iii 2007	Jeffrey M. Marcus	Restriction Digest	B
FLCMar13	Marcus Lab		*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park, Dade County, Chekika	25° 36.871' 080° 34.520'	13 iii 2007	Jeffrey M. Marcus	Restriction Digest	B
FLCMar2	Marcus Lab		*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park, Intersection W. Rd. with Old Ingram Highway	25° 22.491' 080° 37.485'	12 iii 2007	Jeffrey M. Marcus	Restriction Digest	B
FLCMar3	Marcus Lab		*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park, Intersection W. Rd. with Old Ingram Highway	25° 22.491' 080° 37.555'	12 iii 2007	Jeffrey M. Marcus	Restriction Digest	B
FLCMar5	Marcus Lab		*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park, Intersection W. Rd. with Old Ingram Highway	25° 22.491' 080° 37.555'	12 iii 2007	Jeffrey M. Marcus	Restriction Digest	B
FLCMar7	Marcus Lab		*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park, Intersection W. Rd. with Old Ingram Highway	25° 22.491' 080° 37.555'	12 iii 2007	Jeffrey M. Marcus	Restriction Digest	B
FLCMar8	Marcus Lab		*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park, Intersection W. Rd. with Old Ingram Highway	25° 22.491' 080° 37.555'	12 iii 2007	Jeffrey M. Marcus	Restriction Digest	B
FLCMar9	Marcus Lab		*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park, Intersection W. Rd. with Old Ingram Highway	25° 22.491' 080° 37.555'	12 iii 2007	Jeffrey M. Marcus	Restriction Digest	B
FLCMar10	Marcus Lab		*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park, Intersection W. Rd. with Old Ingram Highway	25° 22.491' 080° 37.555'	12 iii 2007	Jeffrey M. Marcus	Restriction Digest	B
FLCMar6	Marcus Lab		*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park, Intersection W. Rd. with Old Ingram Highway	25° 22.491' 080° 37.555'	12 iii 2007	Jeffrey M. Marcus	Restriction Digest	B
FLC1932.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park, Long Pine Key		31-Jul-32	Florence M. Grimshawe	Restriction Digest	A
FLC1955.4	Yale Peabody	YPM ENT 839790	*Junonia coenia*	USA: Florida, Dade Co., Everglades National Park, near Long Pine Key		18-Jan-55	JunoniaE. Remington, C.L. Remington, E.E. Remington	Restriction Digest	A
FLC1955.3	Yale Peabody	YPM ENT 415246	*Junonia coenia (rosa)*	USA: Florida, Dade Co., Everglades National Park, near Long Pine Key		18-Jan-55	JunoniaE. Remington, C.L. Remington, E.E. Remington	Restriction Digest	B
FLCMar1	Marcus Lab		*Junonia coenia*	USA: Florida, Dade Co., Everglades NP--Intersection W. Rd. with Old Ingram Highway	25° 22.493' 080° 37.481'	12 iii 2007	Jeffrey M. Marcus	Restriction Digest	B
FLE1955.2	Yale Peabody	YPM ENT 414302	*Junonia neildi*	USA: Florida, Dade Co., Flamingo		18-Jan-55	E.E. Remington, C.L. Remington, P.S. Remington	Restriction Digest	B
FLE1955.3	Yale Peabody	YPM ENT 843679	*Junonia neildi*	USA: Florida, Dade Co., Flamingo		18-Jan-55	E.E. Remington, C.L. Remington, P.S. Remington	Restriction Digest	B
FLE1955.4	Yale Peabody	YPM ENT 843674	*Junonia neildi*	USA: Florida, Dade Co., Flamingo		18-Jan-55	E.E. Remington, C.L. Remington, P.S. Remington	Restriction Digest	B
FLC1948.1	Yale Peabody	YPM ENT 415240	*Junonia coenia*	USA: Florida, Dade Co., Florida City		12-Aug-48	L.P. Brower	Restriction Digest	B
FLC1970.11	Yale Peabody	YPM ENT 415306	*Junonia coenia*	USA: Florida, Dade Co., Florida City		18-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.13	Yale Peabody	YPM ENT 839693	*Junonia coenia*	USA: Florida, Dade Co., Florida City		18-Jun-70	T.R. Manley	Restriction Digest	A
FLC1970.14	Yale Peabody	YPM ENT 839686	*Junonia coenia*	USA: Florida, Dade Co., Florida City		18-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.15	Yale Peabody	YPM ENT 839684	*Junonia coenia*	USA: Florida, Dade Co., Florida City		18-Jun-70	T.R. Manley	Restriction Digest	A
FLC1970.16	Yale Peabody	YPM ENT 839675	*Junonia coenia*	USA: Florida, Dade Co., Florida City		18-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.17	Yale Peabody	YPM ENT 839673	*Junonia coenia*	USA: Florida, Dade Co., Florida City		18-Jun-70	T.R. Manley	Restriction Digest	A
FLC1970.18	Yale Peabody	YPM ENT 839672	*Junonia coenia*	USA: Florida, Dade Co., Florida City		18-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.40	Yale Peabody	YPM ENT 839727	*Junonia coenia*	USA: Florida, Dade Co., Florida City		18-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.41	Yale Peabody	YPM ENT 839726	*Junonia coenia*	USA: Florida, Dade Co., Florida City		18-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.42	Yale Peabody	YPM ENT 839706	*Junonia coenia*	USA: Florida, Dade Co., Florida City		18-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.43	Yale Peabody	YPM ENT 839705	*Junonia coenia*	USA: Florida, Dade Co., Florida City		18-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.44	Yale Peabody	YPM ENT 839704	*Junonia coenia*	USA: Florida, Dade Co., Florida City		18-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.45	Yale Peabody	YPM ENT 839702	*Junonia coenia*	USA: Florida, Dade Co., Florida City		18-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.46	Yale Peabody	YPM ENT 839700	*Junonia coenia*	USA: Florida, Dade Co., Florida City		18-Jun-70	T.R. Manley	Restriction Digest	A
FLX1970.2	Yale Peabody	YPM ENT 839697	*Junonia coenia (CxG)*	USA: Florida, Dade Co., Florida City		18-Jun-70	T.R. Manley	Restriction Digest	A
FLC1982.1	Milwaukee Public Museum	24, IZ1993-35B.24, AS 7711	*Junonia coenia*	USA: Florida, Dade Co., Haven School		05-Jun-82	Thomas	Restriction Digest	B
FLC1970.1	California Academy of Sciences		*Junonia coenia*	USA: Florida, Dade Co., Homestead		26-Aug-70	B.S.Smith	Restriction Digest	A
FLG1981.5	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	USA: Florida, Dade Co., Homestead, IFAS Station		04-Nov-81	H.D. Bagett	Restriction Digest	B
FLG1981.6	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	USA: Florida, Dade Co., Homestead, IFAS Station		04-Nov-81	H.D. Bagett	Restriction Digest	B
FLG1981.7	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	USA: Florida, Dade Co., Homestead, IFAS Station		10-Nov-81	R.C. Godfroi	Restriction Digest	B
FLG1981.13	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis (in James Scott l*	USA: Florida, Dade Co., Homestead, IFAS Station		21-Nov-81	H.D. Bagett	Restriction Digest	B
FLC1978.3	Milwaukee Public Museum	25, IZ1993-35B.25	*Junonia coenia*	USA: Florida, Dade Co., Krome Ave, 0.5 mi. S. Tamiami Trail		20-May-78	Schaus Coll.	Restriction Digest	A

Appendix II. *Junonia* specimens for the historical Florida data set

Specimen Identifier	Collection	Museum Accession Number	Species	Locality	GPS Coordinates	Collection Date	Collector	Genotyping Method	Haplotype Group
FLC1943.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Dade Co., Matheson Hammock, Coral Gables		27-Jul-43		Restriction Digest	A
FLC1918.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Dade Co., Miami		11-Oct-18	Mrs. C.N. Grimshawe	Restriction Digest	B
FLC1935.3	California Academy of Science		*Junonia coenia*	USA: Florida, Dade Co., Miami		12 December 1935		Restriction Digest	B
FLC1935.4	California Academy of Science		*Junonia coenia*	USA: Florida, Dade Co., Miami		12 December 1935		Restriction Digest	A
FLC1935.5	California Academy of Science		*Junonia coenia*	USA: Florida, Dade Co., Miami		12 December 1935		Restriction Digest	B
FLC1936.2	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Dade Co., Miami		04-Jul-36	F.M. Grimshawe	Restriction Digest	B
FLC1936.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Dade Co., Miami		12-Jul-36	F.M. Grimshawe	Restriction Digest	A
FLC1936.3	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Dade Co., Miami		12-Jul-36	F.M. Grimshawe	Restriction Digest	B
FLC1936.4	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Dade Co., Miami		12-Jul-36	F.M. Grimshawe	Restriction Digest	B
FLC1946.6	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Dade Co., Miami		10-May-46		Restriction Digest	B
FLC1946.2	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia* (male)	USA: Florida, Dade Co., Miami		06-May-46		Restriction Digest	B
FLC1946.3	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia* (male)	USA: Florida, Dade Co., Miami		06-May-46		Restriction Digest	B
FLC1946.4	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia* (male)	USA: Florida, Dade Co., Miami		06-May-46		Restriction Digest	B
FLC1946.5	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia* (male)	USA: Florida, Dade Co., Miami		06-May-46		Restriction Digest	B
FLE1934.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Dade Co., Miami		16-Sep-34	C.N. Grimshawe	Restriction Digest	A
FLE1935.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Dade Co., Miami		28-Oct-35	C.N. Grimshawe	Restriction Digest	B
FLE1940.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Dade Co., Miami		15-Oct-40	C.N. Grimshawe	Restriction Digest	B
FLE1930.2	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi* (little orange on t	USA: Florida, Dade Co., Miami		Aug-30	Mrs. C.N. Grimshawe	Restriction Digest	B
FLE1979.7	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Dade Co., Mrazek Pond, Everglades National Park		09-Sep-79	D. Leston	Restriction Digest	A
FLC1970.19	Yale Peabody	YPM ENT 839665	*Junonia coenia*	USA: Florida, Dade Co., Redland		22-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.21	Yale Peabody	YPM ENT 839659	*Junonia coenia*	USA: Florida, Dade Co., Redland		22-Jun-70	T.R. Manley	Restriction Digest	A
FLC1970.23	Yale Peabody	YPM ENT 839652	*Junonia coenia*	USA: Florida, Dade Co., Redland		22-Jun-70	T.R. Manley	Restriction Digest	A
FLC1970.24	Yale Peabody	YPM ENT 839651	*Junonia coenia*	USA: Florida, Dade Co., Redland		22-Jun-70	T.R. Manley	Restriction Digest	A
FLC1970.25	Yale Peabody	YPM ENT 839644	*Junonia coenia*	USA: Florida, Dade Co., Redland		22-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.26	Yale Peabody	YPM ENT 839629	*Junonia coenia*	USA: Florida, Dade Co., Redland		22-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.27	Yale Peabody	YPM ENT 839628	*Junonia coenia*	USA: Florida, Dade Co., Redland		22-Jun-70	T.R. Manley	Restriction Digest	A
FLC1970.28	Yale Peabody	YPM ENT 839627	*Junonia coenia*	USA: Florida, Dade Co., Redland		22-Jun-70	T.R. Manley	Restriction Digest	A
FLC1970.29	Yale Peabody	YPM ENT 839626	*Junonia coenia*	USA: Florida, Dade Co., Redland		22-Jun-70	T.R. Manley	Restriction Digest	A
FLC1970.3	Yale Peabody	YPM ENT 415231	*Junonia coenia*	USA: Florida, Dade Co., Redland		22-Jun-70	T.R. Manley	Restriction Digest	A
FLC1970.30	Yale Peabody	YPM ENT 839618	*Junonia coenia*	USA: Florida, Dade Co., Redland		22-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.31	Yale Peabody	YPM ENT 839617	*Junonia coenia*	USA: Florida, Dade Co., Redland		22-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.33	Yale Peabody	YPM ENT 839612	*Junonia coenia*	USA: Florida, Dade Co., Redland		22-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.34	Yale Peabody	YPM ENT 839662	*Junonia coenia*	USA: Florida, Dade Co., Redland		22-Jun-70	T.R. Manley	Restriction Digest	A
FLC1970.5	Yale Peabody	YPM ENT 839611	*Junonia coenia*	USA: Florida, Dade Co., Redland		22-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.6	Yale Peabody	YPM ENT 839605	*Junonia coenia*	USA: Florida, Dade Co., Redland		22-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.7	Yale Peabody	YPM ENT 839602	*Junonia coenia*	USA: Florida, Dade Co., Redland		22-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.8	Yale Peabody	YPM ENT 839600	*Junonia coenia*	USA: Florida, Dade Co., Redland		22-Jun-70	T.R. Manley	Restriction Digest	A
FLC1930.2	Yale Peabody	YPM ENT 415259	*Junonia coenia*	USA: Florida, Dade Co., Royal Palm State Park		28-Mar-roughly19	F.M. Jones	Restriction Digest	B
FLX1930.1	Yale Peabody	YPM ENT 415220	*Junonia coenia (CxG)*	USA: Florida, Dade Co., Royal Palm State Park		roughly 1930	F.M. Jones	Restriction Digest	B
FLC1930.1	Yale Peabody	YPM ENT 415216	*Junonia coenia (rosa)*	USA: Florida, Dade Co., Royal Palm State Park		16-Mar-roughly19	F.M. Jones	Restriction Digest	B
FLC1930.3	Yale Peabody	YPM ENT 415258	*Junonia coenia (rosa)*	USA: Florida, Dade Co., Royal Palm State Park		28-Feb-roughly19	F.M. Jones	Restriction Digest	B
FLE1930.3	Yale Peabody	YPM ENT 414315	*Junonia neildi*	USA: Florida, Dade co., Royal Palm State Park		01-Mar-roughly19	F.M. Jones	Restriction Digest	B
FLE1981.7	Milwaukee Public Museum	26, IZ1993-35B.26, AS 7384	*Junonia neildi*	USA: Florida, Dade Co., south end Ludlow Road		29-Dec-81	S. (Schwartz)	Restriction Digest	A
FLC1979.3	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Dade Co., Tamiami Trail		April 29, 1979	B. Rivera	Restriction Digest	B
FLC1937.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Dade Co., Tamiami Trail, Everglades		08-Aug-37		Restriction Digest	A
FLC1940.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Dade Co., Tamiami Trail, Everglades		08-Aug-40	F.H. Chermock	Restriction Digest	A
FLCMar4	Marcus Lab		*Junonia coenia*	USA: Florida, Dade Co.,Everglades National Park, Intersection W. Rd. with Old Ingram Highway	25° 22.491' 080° 37.555'	12 iii 2007	Jeffrey M. Marcus	Restriction Digest	B
FLCM1	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway		17-May-2006	Jeffrey M. Marcus	KM288116	B
FLCM2	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway		17-May-2006	Jeffrey M. Marcus	KM288124	B
FLCM3	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway		17-May-2006	Jeffrey M. Marcus	KM288129	B
FLCM4	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway		17-May-2006	Jeffrey M. Marcus	KM288130	B
FLCM5	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway		17-May-2006	Jeffrey M. Marcus	KM288131	B
FLCM6	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway		17-May-2006	Jeffrey M. Marcus	KM288132	B
FLCM11	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway		18-May-2006	Jeffrey M. Marcus	KM288117	B
FLCM12	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway		18-May-2006	Jeffrey M. Marcus	KM288118	B
FLCM13	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway		18-May-2006	Jeffrey M. Marcus	KM288119	B
FLCM15	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway		18-May-2006	Jeffrey M. Marcus	KM288120	B
FLCM16	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway		18-May-2006	Jeffrey M. Marcus	KM288121	B
FLCM18	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway		18-May-2006	Jeffrey M. Marcus	KM288122	B
FLCM19	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway		18-May-2006	Jeffrey M. Marcus	KM288123	B
FLCM7	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway		18-May-2006	Jeffrey M. Marcus	KM288133	B
FLCM8	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway		18-May-2006	Jeffrey M. Marcus	KM288134	B
FLCM9	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway		18-May-2006	Jeffrey M. Marcus	KM288135	B
FLCJa1	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway		11-Jan-2007	Jeffrey M. Marcus	KM288219	B
FLCM10	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway	25° 25.448' 080° 33.556'	18 v 2006	Jeffrey M. Marcus	Restriction Digest	B
FLGM1	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		17-May-2006	Jeffrey M. Marcus	KM288215	B
FLGM2	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		18-May-2006	Jeffrey M. Marcus	KM288216	B
FLGM3	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		18-May-2006	Jeffrey M. Marcus	KM288217	B
FLGJa1	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		11-Jan-2007	Jeffrey M. Marcus	KM288231	B
FLGJa2	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		11-Jan-2007	Jeffrey M. Marcus	KM288239	B
FLGJa3	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		11-Jan-2007	Jeffrey M. Marcus	KM288243	B
FLGJa5	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		11-Jan-2007	Jeffrey M. Marcus	KM288244	B
FLGJa6	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		11-Jan-2007	Jeffrey M. Marcus	KM288245	B
FLGJa7	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		11-Jan-2007	Jeffrey M. Marcus	KM288246	A
FLGJa8	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		11-Jan-2007	Jeffrey M. Marcus	KM288247	B
FLGJa10	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		12-Jan-2007	Jeffrey M. Marcus	KM288232	B
FLGJa11	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		12-Jan-2007	Jeffrey M. Marcus	KM288233	B
FLGJa12	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		12-Jan-2007	Jeffrey M. Marcus	KM288234	B
FLGJa13	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		12-Jan-2007	Jeffrey M. Marcus	KM288235	B
FLGJa14	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		12-Jan-2007	Jeffrey M. Marcus	KM288218	A
FLGJa15	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		12-Jan-2007	Jeffrey M. Marcus	KM288236	B
FLGJa16	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		12-Jan-2007	Jeffrey M. Marcus	KM288237	B
FLGJa17	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		12-Jan-2007	Jeffrey M. Marcus	KM288238	B
FLGJa20	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		12-Jan-2007	Jeffrey M. Marcus	KM288240	B
FLGJa21	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		12-Jan-2007	Jeffrey M. Marcus	KM288241	B
FLGJa22	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		12-Jan-2007	Jeffrey M. Marcus	KM288242	B
FLG2009.1	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		18 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLG2009.10	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		18 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLG2009.11	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		18 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLG2009.12	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		18 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLG2009.13	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		18 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLG2009.14	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		18 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLG2009.15	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		18 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLG2009.16	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		18 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLG2009.17	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		18 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLG2009.18	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		18 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLG2009.19	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		18 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLG2009.2	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		18 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLG2009.20	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		18 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLG2009.21	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		18 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLG2009.22	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		18 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLG2009.23	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		18 xi 2009	Jeffrey M. Marcus	Restriction Digest	A

Appendix II. *Junonia* specimens for the historical Florida data set

Specimen Identifier	Collection	Museum Accession Number	Species	Locality	GPS Coordinates	Collection Date	Collector	Genotyping Method	Haplotype Group
FLG2009.24	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		18 xi 2009	Jeffrey M. Marcus	Restriction Digest	A
FLG2009.25	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		18 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLG2009.26	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		18 xi 2009	Jeffrey M. Marcus	Restriction Digest	A
FLG2009.3	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		18 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLG2009.4	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		18 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLG2009.5	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		18 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLG2009.6	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		18 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLG2009.7	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		18 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLG2009.8	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		18 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLG2009.9	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		18 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLG2010.1	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway		21 iv 2010	Jeffrey M. Marcus	Restriction Digest	B
FLGJa4	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway	25° 25.392' 080° 33.556'	11 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLCJa2	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway--North End		11-Jan-2007	Jeffrey M. Marcus	KM288223	B
FLCJa10	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway--North End		12-Jan-2007	Jeffrey M. Marcus	KM288220	B
FLCJa11	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway--North End		12-Jan-2007	Jeffrey M. Marcus	KM288221	B
FLCJa12	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway--North End		12-Jan-2007	Jeffrey M. Marcus	KM288222	B
FLCJa3	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway--North End		12-Jan-2007	Jeffrey M. Marcus	KM288224	B
FLCJa4	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway--North End		12-Jan-2007	Jeffrey M. Marcus	KM288225	B
FLCJa5	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway--North End		12-Jan-2007	Jeffrey M. Marcus	KM288226	B
FLCJa6	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway--North End		12-Jan-2007	Jeffrey M. Marcus	KM288227	B
FLCJa7	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway--North End		12-Jan-2007	Jeffrey M. Marcus	KM288228	B
FLCJa8	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway--North End		12-Jan-2007	Jeffrey M. Marcus	KM288229	B
FLCJa9	Marcus Lab		*Junonia coenia*	USA: Florida, Dade County, Everglades Greenway--North End		12-Jan-2007	Jeffrey M. Marcus	KM288230	B
FLGJa18	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway--North End	25° 38.144' 080° 27.849'	12 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLGJa19	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway--North End	25° 38.144' 080° 27.849'	12 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLGJa9	Marcus Lab		*Junonia zonalis*	USA: Florida, Dade County, Everglades Greenway--North End	25° 38.144' 080° 27.849'	12 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLC1955.1	Yale Peabody	YPM ENT 415232	*Junonia coenia (rosa)*	USA: Florida, Everglades National Park, Lone Pine Key		20-Jan-55	JunoniaE. Remington, C.L. Remington, E.E. Remington	Restriction Digest	B
FLC1965.2	Yale Peabody	YPM ENT 415221	*Junonia coenia*	USA: Florida, Everglades National Park, N. of Shark River Tower, S. of Tamiami Trail		29-Mar-65	D.S. Chambers	Restriction Digest	B
FLC1959.2	California Academy of Science		*Junonia coenia*	USA: Florida, Everglades National Park, Route 94		25 March 1959	R. Smith	Restriction Digest	A
FLC1955.2	Yale Peabody	YPM ENT 415249	*Junonia coenia*	USA: Florida, Lee Co., Boca Grande, on Gasparilla Island		22-Jan-55	C.L. Remington	Restriction Digest	A
FLX1954.1	Yale Peabody	YPM ENT 415247	*Junonia coenia (CxG)*	USA: Florida, Lee Co., Boca Grande, on Gasparilla Island		30-Mar-54	E.E. Remington	Restriction Digest	B
FLC1958.1	Yale Peabody	YPM ENT 415242	*Junonia coenia(rosa)*	USA: Florida, Lee Co., Boca Grande, on Gasparilla Island		12-Jan-58	E.E. Remington	Restriction Digest	B
FLC1978.2	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Lee Co., Cape Coral		20-Nov-78	Bob Hollister	Restriction Digest	B
FLCM20	Marcus Lab		*Junonia coenia*	USA: Florida, Lee Co., Estero Bay Preserve State Park		19-May-2006	Jeffrey M. Marcus	KM288125	B
FLCM21	Marcus Lab		*Junonia coenia*	USA: Florida, Lee Co., Estero Bay Preserve State Park		19-May-2006	Jeffrey M. Marcus	KM288126	B
FLC1972.2	Yale Peabody	YPM ENT 725967	*Junonia coenia*	USA: Florida, Lee Co., Sanibel, Sanibel Island		23-Mar-72	JunoniaS. Ingraham	Restriction Digest	A
FLC1972.3	Yale Peabody	YPM ENT 725964	*Junonia coenia*	USA: Florida, Lee Co., Sanibel, Sanibel Island		25-Mar-72	JunoniaS. Ingraham	Restriction Digest	B
FLX1955.1	Yale Peabody	YPM ENT 414316	*Junonia coenia (CxE)*	USA: Florida, Lee Co., Sanibel, Sanibel Island		01-Dec-55		Restriction Digest	A
FLC1977.2	Harvard University	153021	*Junonia coenia*	USA: Florida, Lee Co., Sanibel-Captiva Islands		18 iv 1977	William D. Winter, Jo B. Winter	Restriction Digest	B
FLC1977.1	Harvard University	153020	*Junonia coenia*	USA: Florida, Lee Co., Sanibel-Captiva Islands		27 ix 1977	William D. Winter, Jo B. Winter	Restriction Digest	B
FLE1977.1	Harvard University	153011	Junonia neildi (marked as Junoni	USA: Florida, Lee Co., Sanibel-Captiva Islands		16 iv 1977	William D. Winter, Jo B. Winter	Restriction Digest	B
FLE1965.1	Yale Peabody	YPM ENT 414361	*Junonia neildi*	USA: Florida, Lee Co., Useppa Island		21-Mar-65	S.A. Hessel	Restriction Digest	B
FLE1965.2	Yale Peabody	YPM ENT 843914	*Junonia neildi*	USA: Florida, Lee Co., Useppa Island		21-Mar-65	S.A. Hessel	Restriction Digest	B
FLE1978.8	C.V. Covell Collection		*Junonia neildi*	USA: Florida, Miami- Dade Co., Elliott Key, Biscayne Nationa Park		16-May-78	C.V. Covell Jr.	Restriction Digest	A
FLC1978.4	C.V. Covell Collection		*Junonia coenia*	USA: Florida, Miamia-Dade Co., Elliott Key, Biscayne National Park		May 16, 1978	C.V. Covell Jr.	Restriction Digest	A
FLC1946.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia (male)*	USA: Florida, Miami-Dade Co., 10 mi. S. Homestead		27-Apr-46		Restriction Digest	B
FLE2011.97	Marc C. Minno		*Junonia neildi (female)*	USA: Florida, Miami-Dade Co., Chapman field Park		September 22, 20	Marc C. Minno	Restriction Digest	A
FLE2011.98	Marc C. Minno		*Junonia neildi (male)*	USA: Florida, Miami-Dade Co., Chapman field Park		September 22, 20	Marc C. Minno	Restriction Digest	A
FLE2001.1	University of Michigan	UMMZI-00204391	Junonia neildi	USA: Florida, Miami-Dade Co., Coral Gables, Matheson Co. Park		09 vii 2001	Carol Landry	Restriction Digest	B
FLE2010.93	Marc C. Minno		*Junonia neildi (male)*	USA: Florida, Miami-Dade Co., Elliott Key		August26, 2010	Marc C. Minno	Restriction Digest	A
FLC1973.3	C.V. Covell Collection		*Junonia coenia*	USA: Florida, Miami-Dade Co., Homestead		11-May-73	C.V. Covell Jr.	Restriction Digest	B
FLC2010.163	Marc C. Minno		*Junonia coenia (female)*	USA: Florida, Miami-Dade Co., W. of Florida City		August 26, 2010	Marc C. Minno	Restriction Digest	B
FLC2010.68	Marc C. Minno		*Junonia coenia (female)*	USA: Florida, Miami-Dade Co., W. of Florida City		August 26, 2010	Marc C. Minno	Restriction Digest	B
FLC2010.42	Marc C. Minno		*Junonia coenia (female)*	USA: Florida, Miami-Dade Co., W. of Florida City		October 31, 2010	Marc C. Minno	Restriction Digest	A
FLC1975.1	C.V. Covell Collection		*Junonia coenia*	USA: Florida, Monroe Co. Big Pine Key		10-May-75	C.V. Covell Jr.	Restriction Digest	A
FLG1981.3	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	USA: Florida, Monroe Co. North Key Largo		01-Nov-81	H.D. Bagett	Restriction Digest	A
FLG1981.4	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	USA: Florida, Monroe Co. North Key Largo		01-Nov-81	H.D. Bagett	Restriction Digest	A
FLC1979.6	Milwaukee Public Museum	27, IZ1993-35B.27	*Junonia coenia*	USA: Florida, Monroe Co., 5.2 mi W Pinecrest		14-Apr-79	unknown	Restriction Digest	B
FLC1979.7	Milwaukee Public Museum	28, IZ1993-35B.28	*Junonia coenia*	USA: Florida, Monroe Co., 5.2 mi W Pinecrest		14-Apr-79	Wisor	Restriction Digest	B
FLC1979.8	Milwaukee Public Museum	29, IZ1993-35B.29	*Junonia coenia*	USA: Florida, Monroe Co., 5.2 mi W Pinecrest		14-Apr-79	S. (Schwartz)	Restriction Digest	A
FLE1979.4	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Bear Lake Rd., Everglades National Park		01-Nov-79	B. Rivera	Restriction Digest	A
FLG1967.1	Yale Peabody	YPM ENT 415233	*Junonia coenia*	USA: Florida, Monroe Co., Big Pine Key		05-Aug-67	F.E. Rutkowski	Restriction Digest	A
FLC1967.4	Yale Peabody	YPM ENT 415195	*Junonia coenia*	USA: Florida, Monroe Co., Big Pine Key		09-Aug-67	F.E. Rutkowski	Restriction Digest	B
FLC1968.1	Yale Peabody	YPM ENT 839814	*Junonia coenia*	USA: Florida, Monroe Co., Big Pine Key		20-Oct-68	F.E. Rutkowski	Restriction Digest	B
FLC1969.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Monroe Co., Big Pine Key		16-Oct-69	Larry Brown	Restriction Digest	B
FLC1973.4	Marcus Lab		*Junonia coenia*	USA: Florida, Monroe Co., Big Pine Key		12-May-73		Restriction Digest	B
FLC1973.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Monroe Co., Big Pine Key		24-Oct-73	Larry Brown	Restriction Digest	B
FLC1975.2	Yale Peabody	YPM ENT 792650	*Junonia coenia*	USA: Florida, Monroe Co., Big Pine Key		17-Jul-75	V.Junonia Mason	Restriction Digest	B
FLC1983.1	Milwaukee Public Museum	30, IZ1993-35B.30, AS 9421	*Junonia coenia*	USA: Florida, Monroe Co., Big Pine Key		02-May-83	S. (Schwartz)	Restriction Digest	A
FLC1986.2	Milwaukee Public Museum	33, IZ1993-35B.34, AS 15815	*Junonia coenia*	USA: Florida, Monroe Co., Big Pine Key		02-Jan-86	Junonia Escobio	Restriction Digest	A
FLC1986.3	Milwaukee Public Museum	35, IZ1993-35B.35, AS 17039	*Junonia coenia*	USA: Florida, Monroe Co., Big Pine Key		26-Apr-86	Junonia Escobio	Restriction Digest	B
FLC1986.4	Milwaukee Public Museum	36,IZ1993-35B.36, AS 17045	*Junonia coenia*	USA: Florida, Monroe Co., Big Pine Key		26-Apr-86	Junonia Escobio	Restriction Digest	B
FLC1986.5	Milwaukee Public Museum	38, IZ1993-35B.38, AS 20184	*Junonia coenia*	USA: Florida, Monroe Co., Big Pine Key		11-Oct-86	A. Schwartz	Restriction Digest	B
FLC1986.6	Milwaukee Public Museum	39, IZ1993-35B.39, AS 20186	*Junonia coenia*	USA: Florida, Monroe Co., Big Pine Key		11-Oct-86	Junonia Escobio	Restriction Digest	B
FLC1986.7	Milwaukee Public Museum	40, IZ1993-35B.40, AS 20187	*Junonia coenia*	USA: Florida, Monroe Co., Big Pine Key		11-Oct-86	Junonia Escobio	Restriction Digest	B
FLC1986.8	Milwaukee Public Museum	41, IZ1993-35B.41, 20189	*Junonia coenia*	USA: Florida, Monroe Co., Big Pine Key		11-Oct-86	A. Schwartz	Restriction Digest	A
FLC1986.10	Milwaukee Public Museum	43, IZ1993-35B.43, AS 21081	*Junonia coenia*	USA: Florida, Monroe Co., Big Pine Key		27-Nov-86	Junonia Escobio	Restriction Digest	B
FLC1986.9	Milwaukee Public Museum	42, IZ1993-35B.42, AS 21078	*Junonia coenia*	USA: Florida, Monroe Co., Big Pine Key		27-Nov-86	Junonia Escobio	Restriction Digest	B
FLX1967.1	Yale Peabody	YPM ENT 415238	*Junonia coenia (CxG)*	USA: Florida, Monroe Co., Big Pine Key		04-Aug-67	F.E. Rutkowski	Restriction Digest	A
FLC1965.4	Yale Peabody	YPM ENT 839711	*Junonia coenia (rosa)*	USA: Florida, Monroe Co., Big Pine Key		23-Mar-65	D.S. Chambers	Restriction Digest	A
FLE1973.4	California Academy of Science		*Junonia neildi*	USA: Florida, Monroe Co., Big Pine Key		27-Jan-73	R.A. Anderson	Restriction Digest	B
FLE1973.16	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Big Pine Key		24-Oct-73	Larry Brown	Restriction Digest	B
FLE1973.12	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Big Pine Key		30-Dec-73	JunoniaF. Williams	Restriction Digest	B
FLE1973.13	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Big Pine Key		30-Dec-73	JunoniaF. Williams	Restriction Digest	A
FLE1973.14	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Big Pine Key		30-Dec-73	JunoniaF. Williams	Restriction Digest	A
FLE1981.4	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Big Pine Key		01-Nov-81	Charles M. Stevens	Restriction Digest	B
FLE1981.6	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Big Pine Key		01-Nov-81	Charles F. Zeiger	Restriction Digest	B
FLE1983.1	Milwaukee Public Museum	31, IZ1993-35B.31, AS 9476	*Junonia neildi*	USA: Florida, Monroe Co., Big Pine Key		04-May-83	Correa	Restriction Digest	A
FLE1985.2	Milwaukee Public Museum	32, IZ1993-35B.32, AS 15498	*Junonia neildi*	USA: Florida, Monroe Co., Big Pine Key		24-Dec-85	Junonia Escobio	Restriction Digest	B
FLE1985.3	Milwaukee Public Museum	34, IZ1993-35B.33, AS 15653	*Junonia neildi*	USA: Florida, Monroe Co., Big Pine Key		31-Dec-85	Junonia Escobio	Restriction Digest	A
FLE1986.1	Milwaukee Public Museum	44, IZ1993-35B.44, AS 21091	*Junonia neildi*	USA: Florida, Monroe Co., Big Pine Key		27-Nov-86	A. Schwartz	Restriction Digest	B
FLE1964.3	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Big Pine Key		21-26 December 1	F. Rutkowski	Restriction Digest	B
FLE1964.4	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Big Pine Key		21-26 December 1	F. Rutkowski	Restriction Digest	A
FLE1964.5	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Big Pine Key		21-26 December 1	F. Rutkowski	Restriction Digest	B
FLE1973.17	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi* (male)	USA: Florida, Monroe Co., Big Pine Key		26-Jul-73	I.L. Finkelstein	Restriction Digest	A
FLE2010.92	Marc C. Minno		*Junonia neildi (male)*	USA: Florida, Monroe Co., Big Pine Key		August 29, 2010	Marc C. Minno	Restriction Digest	B
FLE1976.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi* (very small)	USA: Florida, Monroe Co., Big Pine Key		27-May-76		Restriction Digest	A
FLX1981.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi X Junonia zonalis*	USA: Florida, Monroe Co., Big Pine Key		01-Nov-81	Charles F. Zeiger	Restriction Digest	A
FLX1972.2	Milwaukee Public Museum	125, IZ1994-11.2	*Junonia X (CXG)*	USA: Florida, Monroe Co., Big Pine Key		15-Oct-72	R.A. Anderson	Restriction Digest	B
FLX1986.1	Milwaukee Public Museum	37, IZ1993-35B.37, AS 17052	*Junonia X (CXG)*	USA: Florida, Monroe Co., Big Pine Key		26 April, 1986	A. Schwartz	Restriction Digest	A
FLG2011.138	Marc C. Minno		*Junonia zonalis (female)*	USA: Florida, Monroe Co., Big Pine Key		30-Nov-11	Marc C. Minno	Restriction Digest	A

Appendix II. *Junonia* specimens for the historical Florida data set

Specimen Identifier	Collection	Museum Accession Number	Species	Locality	GPS Coordinates	Collection Date	Collector	Genotyping Method	Haplotype Group
FLG1967.3	Yale Peabody	YPM ENT 843920	*Junonia neildi*	USA: Florida, Monroe Co., Big Pine Key		09-Aug-67	F.E. Rutkowski	Restriction Digest	B
FLE1975.1	Yale Peabody	YPM ENT 414359	*Junonia neildi*	USA: Florida, Monroe Co., Big Pine Key		29-Nov-75	W.B. Wright	Restriction Digest	B
FLE1979.13	Yale Peabody	YPM ENT 414360	*Junonia neildi*	USA: Florida, Monroe Co., Big Pine Key		28-Dec-79	H.D. Baggett	Restriction Digest	B
FLX1967.9	Yale Peabody	YPM ENT 843433	*Junonia neildi (CxE)*	USA: Florida, Monroe Co., Big Pine Key		09-Aug-67	F.E. Rutkowski	Restriction Digest	A
FLX1967.10	Yale Peabody	YPM ENT 843431	*Junonia neildi (GxE)*	USA: Florida, Monroe Co., Big Pine Key		09-Aug-67	F.E. Rutkowski	Restriction Digest	B
FLG1982.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	USA: Florida, Monroe Co., Big Pine Key (on ground)		11-Oct-82		Restriction Digest	B
FLX1966.2	Yale Peabody	YPM ENT 839782	*Junonia coenia (ExG)*	USA: Florida, Monroe Co., Big Pine Key, Bogie Channel		19-May-66	F.E. Rutkowski	Restriction Digest	B
FLE1986.2	Milwaukee Public Museum	45, IZ1993-35B.45, AS 16284	*Junonia neildi*	USA: Florida, Monroe Co., Big Pine Key, East End Road		15-Feb-86	P.A. Arana	Restriction Digest	A
FLE1986.3	Milwaukee Public Museum	46, IZ1993-35B.46, AS 17115	*Junonia neildi*	USA: Florida, Monroe Co., Big Pine Key, East End Road		27-Apr-86	Junonia Escobio	Restriction Digest	B
FLX1968.1	Yale Peabody	YPM ENT 415237	*Junonia coenia (CxG)*	USA: Florida, Monroe Co., Big Pine Key, Long Beach		22-Oct-68	F.E. Rutkowski	Restriction Digest	A
FLE1967.3	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Big Pine Key, Long Beach		09-Aug-67	F. Rutkowski	Restriction Digest	B
FLG1967.2	Yale Peabody	YPM ENT 414355	*Junonia neildi*	USA: Florida, Monroe Co., Big Pine Key, Long Beach		09-Aug-67	F.E. Rutkowski	Restriction Digest	A
FLX1967.4	Yale Peabody	YPM ENT 843928	*Junonia neildi (CXG)*	USA: Florida, Monroe Co., Big Pine Key, Long Beach		09-Aug-67	F.E. Rutkowski	Restriction Digest	B
FLX1967.11	Yale Peabody	YPM ENT 843430	*Junonia neildi (GxE)*	USA: Florida, Monroe Co., Big Pine Key, Long Beach		09-Aug-67	F.E. Rutkowski	Restriction Digest	B
FLX1967.12	Yale Peabody	YPM ENT 843439	*Junonia neildi (GxE)*	USA: Florida, Monroe Co., Big Pine Key, Long Beach		09-Aug-67	F.E. Rutkowski	Restriction Digest	A
FLX1967.5	Yale Peabody	YPM ENT 839695	*Junonia neildi (GxE)*	USA: Florida, Monroe Co., Big Pine Key, Long Beach		09-Aug-67	F.E. Rutkowski	Restriction Digest	B
FLX1967.6	Yale Peabody	YPM ENT 415215	*Junonia neildi (GxE)*	USA: Florida, Monroe Co., Big Pine Key, Long Beach		09-Aug-67	F.E. Rutkowski	Restriction Digest	B
FLX1967.7	Yale Peabody	YPM ENT 415266	*Junonia neildi (GxE)*	USA: Florida, Monroe Co., Big Pine Key, Long Beach		09-Aug-67	F.E. Rutkowski	Restriction Digest	A
FLX1967.8	Yale Peabody	YPM ENT 843437	*Junonia neildi (GxE)*	USA: Florida, Monroe Co., Big Pine Key, Long Beach		09-Aug-67	F.E. Rutkowski	Restriction Digest	B
FLE1967.2	McGuire Center for Lepidoptera and Biodiversity		Junonia neildi (marked as Junoni	USA: Florida, Monroe Co., Big Pine Key, Long Beach		09-Aug-67	F. Rutkowski	Restriction Digest	A
FLC1967.8	Yale Peabody	YPM ENT 839688	*Junonia coenia*	USA: Florida, Monroe Co., Big Pine Key, near Bogie Channel		07-Aug-67	F.E. Rutkowski	Restriction Digest	B
FLC1967.3	Yale Peabody	YPM ENT 415204	*Junonia coenia*	USA: Florida, Monroe Co., Big Pine Key, near Bogie Channel		09-Aug-67	F.E. Rutkowski	Restriction Digest	A
FLC1967.5	Yale Peabody	YPM ENT 839683	*Junonia coenia*	USA: Florida, Monroe Co., Big Pine Key, near Bogie Channel		09-Aug-67	F.E. Rutkowski	Restriction Digest	A
FLC1967.6	Yale Peabody	YPM ENT 839671	*Junonia coenia*	USA: Florida, Monroe Co., Big Pine Key, near Bogie Channel		09-Aug-67	F.E. Rutkowski	Restriction Digest	B
FLC1967.7	Yale Peabody	YPM ENT 839682	*Junonia coenia*	USA: Florida, Monroe Co., Big Pine Key, near Bogie Channel		09-Aug-67	F.E. Rutkowski	Restriction Digest	B
FLG1967.4	Yale Peabody	YPM ENT 839732	*Junonia coenia*	USA: Florida, Monroe Co., Big Pine Key, near Bogie Channel		09-Aug-67	F.E. Rutkowski	Restriction Digest	B
FLX1967.2	Yale Peabody	YPM ENT 843917	*Junonia coenia (CxE)*	USA: Florida, Monroe Co., Big Pine Key, near Bogie Channel		07-Aug-67	F.E. Rutkowski	Restriction Digest	B
FLX1967.3	Yale Peabody	YPM ENT 843922	*Junonia coenia (CxG)*	USA: Florida, Monroe Co., Big Pine Key, near Bogie Channel		07-Aug-67	F.E. Rutkowski	Restriction Digest	B
FLX1967.13	Yale Peabody	YPM ENT 843918	*Junonia coenia (CxG)*	USA: Florida, Monroe Co., Big Pine Key, near Bogie Channel		09-Aug-67	F.E. Rutkowski	Restriction Digest	B
FLG1968.2	Yale Peabody	YPM ENT 839754	*Junonia coenia*	USA: Florida, Monroe Co., Big Pine Key, U.S. 1		25-Oct-68	F.E. Rutkowski	Restriction Digest	B
FLX1968.2	Yale Peabody	YPM ENT 415193	*Junonia coenia (CxG)*	USA: Florida, Monroe Co., Big Pine Key, U.S. 1		20-Oct-68	F.E. Rutkowski	Restriction Digest	B
FLC1972.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Monroe Co., Big Torch Key		04-Sep-72	JunoniaB. Heppner	Restriction Digest	A
FLX1972.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia X Junonia zonali*	USA: Florida, Monroe Co., Big Torch Key		04-Sep-72	JunoniaB. Heppner	Restriction Digest	A
FLC1967.1	Harvard University	110864	*Junonia coenia*	USA: Florida, Monroe Co., Boot Key		21 vii 1967	Robert E. Silberglied	Restriction Digest	B
FLE1967.1	Harvard University	110881	Junonia neildi (marked as Junoni	USA: Florida, Monroe Co., Calusa Keys, near E9		2-4 viii 1967	Robert E. Silberglied	Restriction Digest	B
FLE1925.1	University of Michigan	UMMZI-00204398	Junonia neildi	USA: Florida, Monroe Co., Cape Sable		1 ix 1925	D. Marston Bates	Restriction Digest	B
FLCD5	Marcus Lab		*Junonia coenia*	USA: Florida, Monroe Co., Everglades National Park, East Cape Sable		18-Nov-2007	Jeffrey M. Marcus	KM288080	B
FLCD6	Marcus Lab		*Junonia coenia*	USA: Florida, Monroe Co., Everglades National Park, East Cape Sable		18-Nov-2007	Jeffrey M. Marcus	KM288081	B
FLCD4	Marcus Lab		*Junonia coenia*	USA: Florida, Monroe Co., Everglades National Park, East of North-West Cape Sable		18-Nov-2007	Jeffrey M. Marcus	KM288079	B
FLED10	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe Co., Everglades National Park, East of North-West Cape Sable		18-Nov-2007	Jeffrey M. Marcus	KM288083	B
FLED9	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe Co., Everglades National Park, East of North-West Cape Sable		18-Nov-2007	Jeffrey M. Marcus	KM288091	B
FLCD1	Marcus Lab		*Junonia coenia*	USA: Florida, Monroe Co., Everglades National Park, Flamingo, Trialhead Coastal Prairie Trail		16-Nov-2007	Jeffrey M. Marcus	KM288076	B
FLCD2	Marcus Lab		*Junonia coenia*	USA: Florida, Monroe Co., Everglades National Park, Flamingo, Trialhead Coastal Prairie Trail		16-Nov-2007	Jeffrey M. Marcus	KM288077	B
FLCD3	Marcus Lab		*Junonia coenia*	USA: Florida, Monroe Co., Everglades National Park, North-West Cape Sable		18-Nov-2007	Jeffrey M. Marcus	KM288078	B
FLE1979.2	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Fleming Key		10-Jul-79	Acree & Weems	Restriction Digest	B
FLE1979.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Fleming Key		26-Dec-79	Acree & Weems	Restriction Digest	B
FLE1980.2	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Fleming Key		21-Feb-80	H.E. Williams & H.V. Weems Jr. (insect flight trap)	Restriction Digest	B
FLE1980.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Fleming Key		02-Mar-80	H.E. Williams & H.V. Weems Jr. (insect flight trap)	Restriction Digest	A
FLE1980.3	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Fleming Key		14-Mar-80	H.E. Williams & H.V. Weems Jr. (insect flight trap)	Restriction Digest	B
FLE1980.4	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Fleming Key		25-Mar-80	H.E. Williams & H.V. Weems Jr. (insect flight trap)	Restriction Digest	B
FLE1916.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi* (female)	USA: Florida, Monroe Co., Florida Keys (prob. Key West)		Apr-16		Restriction Digest	B
FLE1972.3	Milwaukee Public Museum	124, IZ1994-11.1	*Junonia neildi*	USA: Florida, Monroe Co., Geiger Key		08-Oct-72	R.A. Anderson	Restriction Digest	A
FLE1970.2	Yale Peabody	YPM ENT 414312	*Junonia neildi*	USA: Florida, Monroe Co., Higher Matecumbe Key		01-Mar-70	D.B. Wright	Restriction Digest	A
FLE1961.2	McGuire Center for Lepidoptera and Biodiversity		*Junonai neildi*	USA: Florida, Monroe Co., Key Largo		28-Aug-61	John Plomley	Restriction Digest	B
FLC1950.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Monroe Co., Key Largo		11-Aug-50		Restriction Digest	A
FLC1973.5	Yale Peabody	YPM ENT 793289	*Junonia coenia*	USA: Florida, Monroe Co., Key Largo		03-Jul-73	JunoniaW. Mason	Restriction Digest	B
FLC1974.2	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Monroe Co., Key Largo		11-May-74	I.L. Finkelstein	Restriction Digest	A
FLC1976.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Monroe Co., Key Largo		14-Aug-76	L.C. Dow	Restriction Digest	B
FLC1976.2	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Monroe Co., Key Largo		14-Aug-76	L.C. Dow	Restriction Digest	B
FLC1976.3	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia (male)*	USA: Florida, Monroe Co., Key Largo		14-Aug-76	L.C. Dow	Restriction Digest	B
FLE1944.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Key Largo		22-Apr-44	Florence M. Grimshawe	Restriction Digest	B
FLE1969.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Key Largo		27-Jul-69	John Plomley	Restriction Digest	B
FLE1969.2	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Key Largo		27-Jul-69	John Plomley	Restriction Digest	B
FLE1969.3	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Key Largo		27-Jul-69	John Plomley	Restriction Digest	B
FLE1969.5	Yale Peabody	YPM ENT 792631	*Junonia neildi*	USA: Florida, Monroe Co., Key Largo		14-Aug-69	JunoniaM. Mason	Restriction Digest	B
FLE1973.19	Yale Peabody	YPM ENT 793064	*Junonia neildi*	USA: Florida, Monroe Co., Key Largo		03-Jul-73	P.B. Mason	Restriction Digest	B
FLE1979.10	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Key Largo		21-Mar-79	I.L. Finkelstein	Restriction Digest	A
FLE1979.11	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Key Largo		14-Dec-79	I.L. Finkelstein	Restriction Digest	A
FLE1980.6	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Key Largo		09-Mar-80	F.D. Fee	Restriction Digest	A
FLE1977.2	McGuire Center for Lepidoptera and Biodiversity		Junonia neildi (marked as Junoni	USA: Florida, Monroe Co., Key Largo		26-Aug-77	H.L. King	Restriction Digest	B
FLE1977.3	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi* (very large)	USA: Florida, Monroe Co., Key Largo		26-Aug-77	H.L. King	Restriction Digest	A
FLG1981.8	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	USA: Florida, Monroe Co., Key Largo		01-Nov-81	H.D. Bagett	Restriction Digest	B
FLG1981.10	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	USA: Florida, Monroe Co., Key Largo		03-Nov-81	R.C. Godfroi	Restriction Digest	B
FLG1979.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis* (female)	USA: Florida, Monroe Co., Key Largo		19-Dec-79	I.L. Finkelstein	Restriction Digest	A
FLG1981.9	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis* (in James Scott I	USA: Florida, Monroe Co., Key Largo		01-Nov-81	H.D. Bagett	Restriction Digest	B
FLX1979.1	Yale Peabody	YPM ENT 414328	*Junonia neildi (GxE)*	USA: Florida, Monroe Co., Key Largo		27-Dec-79	H.D. Baggett	Restriction Digest	B
FLG1981.11	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	USA: Florida, Monroe Co., Key Largo		29-Dec-81	R.W. Boscoe	Restriction Digest	A
FLG1981.12	McGuire Center for Lepidoptera and Biodiversity		*Junonia zonalis*	USA: Florida, Monroe Co., Key Largo		29-Dec-81	R.W. Boscoe	Restriction Digest	A
FLX1965.1	Yale Peabody	YPM ENT 414318	*Junonia neildi (GxE)*	USA: Florida, Monroe Co., Key Largo, 1 mi. N of Tavernier		26-Mar-65	D.S. Chambers	Restriction Digest	B
FLG1961.1	University of Michigan	UMMZI-00204399	*Junonia zonalis*	USA: Florida, Monroe Co., Key Largo, S-905		16 viii 1961	Tom Pliske	Restriction Digest	B
FLE1964.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Key Vaca		30-Jul-64	Malcolm G. Douglas	Restriction Digest	B
FLC2011.62	Marc C. Minno		*Junonia coenia (male)*	USA: Florida, Monroe Co., Key West		September 21, 20	Marc C. Minno	Restriction Digest	A
FLC2011.65	Marc C. Minno		*Junonia coenia (male)*	USA: Florida, Monroe Co., Key West		September 21, 20	Marc C. Minno	Restriction Digest	B
FLC2010.38	Marc C. Minno		*Junonia coenia (female)*	USA: Florida, Monroe Co., Key West		August 28, 2010	Marc C. Minno	Restriction Digest	B
FLC2010.43	Marc C. Minno		*Junonia coenia (female)*	USA: Florida, Monroe Co., Key West		August 28, 2010	Marc C. Minno	Restriction Digest	A
FLC2010.45	Marc C. Minno		*Junonia coenia (female)*	USA: Florida, Monroe Co., Key West		August 28, 2010	Marc C. Minno	Restriction Digest	A
FLC2010.47	Marc C. Minno		*Junonia coenia (female)*	USA: Florida, Monroe Co., Key West		August 28, 2010	Marc C. Minno	Restriction Digest	B
FLC2010.48	Marc C. Minno		*Junonia coenia (female)*	USA: Florida, Monroe Co., Key West		August 28, 2010	Marc C. Minno	Restriction Digest	A
FLC2011.61	Marc C. Minno		*Junonia coenia (female)*	USA: Florida, Monroe Co., Key West		September 21, 20	Marc C. Minno	Restriction Digest	B
FLC2011.63	Marc C. Minno		*Junonia coenia (female)*	USA: Florida, Monroe Co., Key West		September 21, 20	Marc C. Minno	Restriction Digest	B
FLC2011.66	Marc C. Minno		*Junonia coenia (female)*	USA: Florida, Monroe Co., Key West		September 21, 20	Marc C. Minno	Restriction Digest	A
FLC2011.67	Marc C. Minno		*Junonia coenia (female)*	USA: Florida, Monroe Co., Key West		September 21, 20	Marc C. Minno	Restriction Digest	A
FLC2010.36	Marc C. Minno		*Junonia coenia (female?)*	USA: Florida, Monroe Co., Key West		August 28, 2010	Marc C. Minno	Restriction Digest	B
FLC2010.37	Marc C. Minno		*Junonia coenia (female?)*	USA: Florida, Monroe Co., Key West		August 28, 2010	Marc C. Minno	Restriction Digest	B
FLC2010.39	Marc C. Minno		*Junonia coenia (male)*	USA: Florida, Monroe Co., Key West		July 17, 2010	Marc C. Minno	Restriction Digest	B
FLC2010.44	Marc C. Minno		*Junonia coenia (male)*	USA: Florida, Monroe Co., Key West		August 28, 2010	Marc C. Minno	Restriction Digest	B
FLC2010.46	Marc C. Minno		*Junonia coenia (male)*	USA: Florida, Monroe Co., Key West		August 28, 2010	Marc C. Minno	Restriction Digest	A
FLC2010.49	Marc C. Minno		*Junonia coenia (male)*	USA: Florida, Monroe Co., Key West		August 28, 2010	Marc C. Minno	Restriction Digest	B
FLE1970.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Key West		08-Mar-70		Restriction Digest	B
FLE1972.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Key West		25-Dec-72	R.A. Anderson	Restriction Digest	B
FLE1973.3	California Academy of Science		*Junonia neildi*	USA: Florida, Monroe Co., Key West		07-Jan-73	R.A. Anderson	Restriction Digest	A
FLE1973.11	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Key West		14-Jan-73	R.A. Anderson	Restriction Digest	B

Appendix II. *Junonia* specimens for the historical Florida data set

Specimen Identifier	Collection	Museum Accession Number	Species	Locality	GPS Coordinates	Collection Date	Collector	Genotyping Method	Haplotype Group
FLE1973.2	California Academy of Science		*Junonia neildi*	USA: Florida, Monroe Co., Key West		14-Jan-73	R.A. Anderson	Restriction Digest	B
FLE1973.5	California Academy of Science		*Junonia neildi*	USA: Florida, Monroe Co., Key West		14-Jan-73	R.A. Anderson	Restriction Digest	A
FLE1973.6	California Academy of Science		*Junonia neildi*	USA: Florida, Monroe Co., Key West		16-Mar-73	R.A. Anderson	Restriction Digest	A
FLE1973.7	California Academy of Science		*Junonia neildi*	USA: Florida, Monroe Co., Key West		03-Jun-73	R.A. Anderson	Restriction Digest	B
FLE1973.8	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Key West		22-Aug-73	R.A. Anderson	Restriction Digest	B
FLE1973.10	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Key West		13-Oct-73	R.A. Anderson	Restriction Digest	B
FLE1974.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Key West		02-Jan-74	JunoniaF. Williams	Restriction Digest	A
FLE1973.1	Marcus Lab		Junonia neildi	USA: Florida, Monroe Co., Key West		20 xi 1973	B. Houtz	Restriction Digest	B
FLE1957.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi* (small: coenia x?)	USA: Florida, Monroe Co., Key West		27-Apr-57		Restriction Digest	A
FLX2010.57	Marc C. Minno		*Junonia zonalis X Junonia coenia? (*	USA: Florida, Monroe Co., Key West		July 17, 2010	Marc C. Minno	Restriction Digest	B
FLX2010.59	Marc C. Minno		*Junonia zonalis X Junonia coenia? (*	USA: Florida, Monroe Co., Key West		August 28, 2010	Marc C. Minno	Restriction Digest	B
FLG2012.137	Marc C. Minno		*Junonia zonalis (female)*	USA: Florida, Monroe Co., Key West		September 12, 20	Marc C. Minno	Restriction Digest	A
FLX2010.58	Marc C. Minno		*Junonia zonalis X Junonia coenia? (f*	USA: Florida, Monroe Co., Key West		August 28, 2010	Marc C. Minno	Restriction Digest	A
FLX2011.64	Marc C. Minno		*Junonia zonalis X Junonia coenia? (f*	USA: Florida, Monroe Co., Key West		September 21, 20	Marc C. Minno	Restriction Digest	A
FLX2010.1	Marc C. Minno		*Junonia zonalis? X Junonia neildi (fe*	USA: Florida, Monroe Co., Key West		December 11, 201	Marc C. Minno	Restriction Digest	A
FLC1975.3	Yale Peabody	YPM ENT 414321	*Junonia neildi*	USA: Florida, Monroe Co., Key West Golf Course		25-Mar-75	P.Junonia Russell, S. Russell	Restriction Digest	B
FLC1979.4	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Monroe Co., Lignum Vitae Key		May 17, 1979	B. Rivera	Restriction Digest	B
FLE1979.3	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Lignum vitae Key		16-Mar-79	B. Rivera	Restriction Digest	B
FLE1980.5	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Lignum vitae Key		18-Jan-80	Denis Leston	Restriction Digest	B
FLE1967.6	Yale Peabody	YPM ENT 414325	*Junonia neildi*	USA: Florida, Monroe Co., Little Torch Key, South Road.		roughly 1967	F.E. Rutkowski	Restriction Digest	B
FLE1927.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Lower Matacombe Key		Apr-27		Restriction Digest	B
FLE1930.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Lower Matacombe Key		Jan-80		Restriction Digest	B
FLC1985.1	California Academy of Science		*Junonia coenia*	USA: Florida, Monroe Co., Lower Matecumbe Key		January 3, 1985	Dana E. Shafer	Restriction Digest	A
FLE1930.5	Yale Peabody	YPM ENT 414308	*Junonia neildi*	USA: Florida, Monroe Co., Lower Matecumbe Key		10-Apr-roughly19	F.M. Jones	Restriction Digest	B
FLE1930.4	Yale Peabody	YPM ENT 414311	*Junonia neildi*	USA: Florida, Monroe Co., Lower Matecumbe Key		17-Mar-roughly19	F.M. Jones	Restriction Digest	B
FLC1986.11	Milwaukee Public Museum	83, IZ1993-35B.83, AS 21133	*Junonia coenia*	USA: Florida, Monroe Co., Lower Sugarloaf Key		27-Nov-86	Junonia Escobio	Restriction Digest	A
FLE1982.1	Milwaukee Public Museum	68, IZ1993-35B.68, AS 9058	*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key		18-Dec-82	A. Schwartz	Restriction Digest	B
FLE1982.2	Milwaukee Public Museum	69, IZ1993-35B.69, AS 9079	*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key		18-Dec-82	R.W. Wisor	Restriction Digest	B
FLE1982.3	Milwaukee Public Museum	70, IZ1993-35B.70, AS 9098	*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key		19-Dec-82	A. Schwartz	Restriction Digest	A
FLE1982.4	Milwaukee Public Museum	71, IZ1993-35B.71, AS 9109	*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key		19-Dec-82	A. Schwartz	Restriction Digest	B
FLE1982.5	Milwaukee Public Museum	72, IZ1993-35B.72, AS 9127	*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key		19-Dec-82	R.W. Wisor	Restriction Digest	B
FLE1983.2	Milwaukee Public Museum	73, IZ1993-35B.73, AS 9255	*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key		29-Apr-83	JunoniaC. Correa	Restriction Digest	B
FLE1986.14	Milwaukee Public Museum	74, IZ1993-35B.74, AS 16372	*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key		16-Feb-86	A. Schwartz	Restriction Digest	A
FLE1986.15	Milwaukee Public Museum	75, IZ1993-35B.75, AS 16373	*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key		16-Feb-86	A. Schwartz	Restriction Digest	B
FLE1986.16	Milwaukee Public Museum	76, IZ1993-35B.76, AS 16440	*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key		15-Mar-86	A. Schwartz	Restriction Digest	A
FLC1986.12	Milwaukee Public Museum	84, IZ1993-35B.84, AS 21134	*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key		27-Nov-86	Junonia Escobio	Restriction Digest	B
FLC1986.13	Milwaukee Public Museum	85, IZ1993-35B.85, AS 21140	*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key		27-Nov-86	A. Schwartz	Restriction Digest	A
FLE1986.17	Milwaukee Public Museum	77, IZ1993-35B.77, AS 21117	*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key		27-Nov-86	Junonia Escobio	Restriction Digest	A
FLED1	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key		17-Nov-2007	Jeffrey M. Marcus	KM288082	B
FLED2	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key		17-Nov-2007	Jeffrey M. Marcus	KM288084	B
FLED3	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key		17-Nov-2007	Jeffrey M. Marcus	KM288085	B
FLED4	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key		17-Nov-2007	Jeffrey M. Marcus	KM288086	B
FLED5	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key		17-Nov-2007	Jeffrey M. Marcus	KM288087	B
FLED6	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key		17-Nov-2007	Jeffrey M. Marcus	KM288088	A
FLED7	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key		17-Nov-2007	Jeffrey M. Marcus	KM288089	B
FLED8	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key		17-Nov-2007	Jeffrey M. Marcus	KM288090	A
FLEJa15	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key	24° 35.910’ 081° 35.014’	14 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLEJa17	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key	24° 35.963’ 081° 35.225’	14 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLEJa19	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key	24° 35.996’ 081° 35.214’	15 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLEJa21	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key	24° 35.970’ 081° 35.226’	15 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLEJa22	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key	24° 35.971’ 081° 35.318’	15 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLEJa23	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key	24° 35.984’ 081° 35.214’	15 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLEJa24	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key	24° 35.970’ 081° 35.226’	15 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLEJa25	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key	24° 35.987’ 081° 35.191’	15 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLEJa26	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key		16 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLEJa16	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key	24° 35.997’ 081° 35.228’	14 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLEJa18	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key	24° 35.915’ 081° 35.018’	15 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLEJa20	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe Co., Lower Sugarloaf Key	24° 35.975’ 081° 35.249’	15 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLC1948.2	Yale Peabody	YPM ENT 415252	*Junonia coenia*	USA: Florida, Monroe Co., Marathon Key		17-Aug-48	L.P. Brower	Restriction Digest	B
FLE1978.7	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Marco Island		28-Aug-78		Restriction Digest	B
FLE1966.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Middle Cape Sable, Everglades National Park		08-Apr-66	F.W. Mead	Restriction Digest	A
FLC1973.2	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Monroe Co., No Name Key (line ventral hindwing)		24-Oct-73	Larry Brown	Restriction Digest	A
FLE1981.5	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi* (female)	USA: Florida, Monroe Co., North Key Largo		23-Dec-81	R.W. Boscoe	Restriction Digest	A
FLC1974.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Monroe Co., Northern half Big Pine Key		08-Aug-74	Larry Brown	Restriction Digest	A
FLE1964.2	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Plantation Key		30-Jul-64	Malcolm G. Douglas	Restriction Digest	A
FLG1981.2	Calhoun		Junonia zonalis (but marked as J	USA: Florida, Monroe Co., Plantation Key (female)		16 xii 1981	John V. Calhoun	Restriction Digest	B
FLG1981.1	Calhoun		Junonia zonalis (but marked as J	USA: Florida, Monroe Co., Plantation Key (male)		15 xii 1981	John V. Calhoun	Restriction Digest	B
FLC2010.60	Marc C. Minno		*Junonia coenia (female)*	USA: Florida, Monroe Co., Raccoon Key		August 28, 2010	Marc C. Minno	Restriction Digest	A
FLC2011.51	Marc C. Minno		*Junonia coenia (male)*	USA: Florida, Monroe Co., Saddlebunch Keys		June 4, 2011	Marc C. Minno	Restriction Digest	B
FLC2011.52	Marc C. Minno		*Junonia coenia (male)*	USA: Florida, Monroe Co., Saddlebunch Keys		June 4, 2011	Marc C. Minno	Restriction Digest	B
FLC2011.53	Marc C. Minno		*Junonia coenia (female)*	USA: Florida, Monroe Co., Saddlebunch Keys		June 4, 2011	Marc C. Minno	Restriction Digest	B
FLC2011.50	Marc C. Minno		*Junonia coenia (male)*	USA: Florida, Monroe Co., Saddlebunch Keys		June 4, 2011	Marc C. Minno	Restriction Digest	A
FLE1947.1	Yale Peabody	YPM ENT 414299	*Junonia neildi*	USA: Florida, Monroe Co., Saddlebunch Keys		26-Dec-47		Restriction Digest	B
FLE1947.2	Yale Peabody	YPM ENT 843667	*Junonia neildi*	USA: Florida, Monroe Co., Saddlebunch Keys		26-Dec-47		Restriction Digest	B
FLE1947.3	Yale Peabody	YPM ENT 843985	*Junonia neildi*	USA: Florida, Monroe Co., Saddlebunch Keys		26-Dec-47		Restriction Digest	B
FLX2010.55	Marc C. Minno		*Junonia neildi X Junonia coenia? (f*	USA: Florida, Monroe Co., Saddlebunch Keys		June 18, 2010	Marc C. Minno	Restriction Digest	B
FLX2010.56	Marc C. Minno		*Junonia neildi X Junonia coenia? (f*	USA: Florida, Monroe Co., Saddlebunch Keys		June 18, 2010	Marc C. Minno	Restriction Digest	A
FLX2010.54	Marc C. Minno		*Junonia neildi X Junonia coenia? (f*	USA: Florida, Monroe Co., Saddlebunch Keys		July 17, 2010	Marc C. Minno	Restriction Digest	A
FLX1947.1	Yale Peabody	YPM ENT 843925	*Junonia neildi (CxE)*	USA: Florida, Monroe Co., Saddlebunch Keys		26-Dec-47		Restriction Digest	A
FLE2010.95	Marc C. Minno		*Junonia neildi (female)*	USA: Florida, Monroe Co., Saddlebunch Keys		November 14, 20	Marc C. Minno	Restriction Digest	A
FLE2010.94	Marc C. Minno		*Junonia neildi (male)*	USA: Florida, Monroe Co., Saddlebunch Keys		November 14, 20	Marc C. Minno	Restriction Digest	A
FLE2010.96	Marc C. Minno		*Junonia neildi (male)*	USA: Florida, Monroe Co., Saddlebunch Keys		November 14, 20	Marc C. Minno	Restriction Digest	B
FLE2012.103	Marc C. Minno		*Junonia neildi (male)*	USA: Florida, Monroe Co., Saddlebunch Keys		February 27, 2012	Marc C. Minno	Restriction Digest	B
FLE2012.100	Marc C. Minno		*Junonia neildi (male)*	USA: Florida, Monroe Co., Saddlebunch Keys		March, 2012	Marc C. Minno	Restriction Digest	A
FLE2012.99	Marc C. Minno		*Junonia neildi (male)*	USA: Florida, Monroe Co., Saddlebunch Keys		March, 2012	Marc C. Minno	Restriction Digest	B
FLE1986.18	Milwaukee Public Museum	94, IZ1993-35B.94, AS 16245	*Junonia neildi*	USA: Florida, Monroe Co., Stock Island		14-Feb-86	A. Schwartz	Restriction Digest	A
FLE1986.19	Milwaukee Public Museum	95, IZ1993-35B.95, AS 16246	*Junonia neildi*	USA: Florida, Monroe Co., Stock Island		14-Feb-86	A. Schwartz	Restriction Digest	B
FLC1981.3	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Monroe Co., Stock Island		August 25, 1981	R. Godefroi	Restriction Digest	B
FLC1979.1	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Monroe Co., Stock Island Botanical Garden (next to Key West)		July 12, 1979	Larry Brown	Restriction Digest	B
FLC1979.2	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Monroe Co., Stock Island Botanical Garden (next to Key West)		July 12, 1979	Larry Brown	Restriction Digest	A
FLC1970.12	Yale Peabody	YPM ENT 415265	*Junonia coenia*	USA: Florida, Monroe Co., Sugarloaf Key		21-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.35	Yale Peabody	YPM ENT 839822	*Junonia coenia*	USA: Florida, Monroe Co., Sugarloaf Key		21-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.37	Yale Peabody	YPM ENT 839820	*Junonia coenia*	USA: Florida, Monroe Co., Sugarloaf Key		21-Jun-70	T.R. Manley	Restriction Digest	A
FLC1970.38	Yale Peabody	YPM ENT 839796	*Junonia coenia*	USA: Florida, Monroe Co., Sugarloaf Key		21-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.39	Yale Peabody	YPM ENT 839766	*Junonia coenia*	USA: Florida, Monroe Co., Sugarloaf Key		21-Jun-70	T.R. Manley	Restriction Digest	A
FLC1970.49	Yale Peabody	YPM ENT 839851	*Junonia coenia*	USA: Florida, Monroe Co., Sugarloaf Key		21-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.50	Yale Peabody	YPM ENT 839850	*Junonia coenia*	USA: Florida, Monroe Co., Sugarloaf Key		21-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.51	Yale Peabody	YPM ENT 839849	*Junonia coenia*	USA: Florida, Monroe Co., Sugarloaf Key		21-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.52	Yale Peabody	YPM ENT 839848	*Junonia coenia*	USA: Florida, Monroe Co., Sugarloaf Key		21-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.53	Yale Peabody	YPM ENT 839846	*Junonia coenia*	USA: Florida, Monroe Co., Sugarloaf Key		21-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.54	Yale Peabody	YPM ENT 839845	*Junonia coenia*	USA: Florida, Monroe Co., Sugarloaf Key		21-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.55	Yale Peabody	YPM ENT 839842	*Junonia coenia*	USA: Florida, Monroe Co., Sugarloaf Key		21-Jun-70	T.R. Manley	Restriction Digest	A

Appendix II. *Junonia* specimens for the historical Florida data set

Specimen Identifier	Collection	Museum Accession Number	Species	Locality	GPS Coordinates	Collection Date	Collector	Genotyping Method	Haplotype Group
FLC1970.56	Yale Peabody	YPM ENT 839838	*Junonia coenia*	USA: Florida, Monroe Co., Sugarloaf Key		21-Jun-70	T.R. Manley	Restriction Digest	A
FLC1970.59	Yale Peabody	YPM ENT 839835	*Junonia coenia*	USA: Florida, Monroe Co., Sugarloaf Key		21-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.60	Yale Peabody	YPM ENT 839834	*Junonia coenia*	USA: Florida, Monroe Co., Sugarloaf Key		21-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.61	Yale Peabody	YPM ENT 839833	*Junonia coenia*	USA: Florida, Monroe Co., Sugarloaf Key		21-Jun-70	T.R. Manley	Restriction Digest	A
FLC1970.62	Yale Peabody	YPM ENT 839832	*Junonia coenia*	USA: Florida, Monroe Co., Sugarloaf Key		21-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.63	Yale Peabody	YPM ENT 839831	*Junonia coenia*	USA: Florida, Monroe Co., Sugarloaf Key		21-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.69	Yale Peabody	YPM ENT 839824	*Junonia coenia*	USA: Florida, Monroe Co., Sugarloaf Key		21-Jun-70	T.R. Manley	Restriction Digest	A
FLC1970.9	Yale Peabody	YPM ENT 415337	*Junonia coenia*	USA: Florida, Monroe Co., Sugarloaf Key		21-Jun-70	T.R. Manley	Restriction Digest	B
FLG1970.1	Yale Peabody	YPM ENT 839857	*Junonia coenia*	USA: Florida, Monroe Co., Sugarloaf Key		21-Jun-70	T.R. Manley	Restriction Digest	A
FLE1973.18	Mississippi		*Junonia coenia*	USA: Florida, Monroe Co., Sugarloaf Key		##############	Gaulet	Restriction Digest	B
FLX1970.4	Yale Peabody	YPM ENT 843221	*Junonia coenia (CxG)*	USA: Florida, Monroe Co., Sugarloaf Key		21-Jun-70	T.R. Manley	Restriction Digest	B
FLX1970.3	Yale Peabody	YPM ENT 843223	*Junonia coenia (ExG)*	USA: Florida, Monroe Co., Sugarloaf Key		21-Jun-70	T.R. Manley	Restriction Digest	A
FLC2010.40	Marc C. Minno		*Junonia coenia (male)*	USA: Florida, Monroe Co., Sugarloaf Key		June 19, 2010	Marc C. Minno	Restriction Digest	B
FLE1970.4	Yale Peabody	YPM ENT 843919	*Junonia neildi*	USA: Florida, Monroe Co., Sugarloaf Key		21-Jun-70	T.R. Manley	Restriction Digest	A
FLE1970.5	Yale Peabody	YPM ENT 843915	*Junonia neildi*	USA: Florida, Monroe Co., Sugarloaf Key		21-Jun-70	T.R. Manley	Restriction Digest	B
FLE1970.6	Yale Peabody	YPM ENT 843913	*Junonia neildi*	USA: Florida, Monroe Co., Sugarloaf Key		21-Jun-70	T.R. Manley	Restriction Digest	B
FLE2012.105	Marc C. Minno		*Junonia neildi (female)*	USA: Florida, Monroe Co., Sugarloaf Key		January 21, 2012	Marc C. Minno	Restriction Digest	A
FLE2012.107	Marc C. Minno		*Junonia neildi (female)*	USA: Florida, Monroe Co., Sugarloaf Key		January 21, 2012	Marc C. Minno	Restriction Digest	B
FLE2012.104	Marc C. Minno		*Junonia neildi (male)*	USA: Florida, Monroe Co., Sugarloaf Key		January 21, 2012	Marc C. Minno	Restriction Digest	B
FLE2012.106	Marc C. Minno		*Junonia neildi (male)*	USA: Florida, Monroe Co., Sugarloaf Key		January 21, 2012	Marc C. Minno	Restriction Digest	B
FLX1985.1	Milwaukee Public Museum		*Junonia X (EXG)*	USA: Florida, Monroe Co., Sugarloaf Key		29 December, 198	A. Schwartz	Restriction Digest	A
FLE1970.3	Yale Peabody	YPM ENT 414357	*Junonia neildi*	USA: Florida, Monroe Co., Sugarloaf Key mislabeled as California		21-Jun-70	T.R. Manley	Restriction Digest	A
FLX1986.2	Milwaukee Public Museum	116, IZ1993-35B.116, AS 21017	*Junonia X (CXG)*	USA: Florida, Monroe Co., Summerland Key		12 October, 1986	A. Schwartz	Restriction Digest	B
FLC1979.5	McGuire Center for Lepidoptera and Biodiversity		*Junonia coenia*	USA: Florida, Monroe Co., Upper Key Largo		October 27, 1979	D. Leston	Restriction Digest	A
FLE1973.9	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Upper Matecombe Key		28-Dec-73	JunoniaF. Williams	Restriction Digest	A
FLE1973.15	McGuire Center for Lepidoptera and Biodiversity		*Junonia neildi*	USA: Florida, Monroe Co., Upper Matecombe Key		29-Dec-73	D. & Junonia Jenkins	Restriction Digest	B
FLE1986.10	Milwaukee Public Museum	63, IZ1993-35B.63, AS 16186	*Junonia neildi*	USA: Florida, Monroe Co., Upper Matecumbe Key, NE Islamorada		08-Feb-86	Junonia Escobio	Restriction Digest	B
FLE1986.11	Milwaukee Public Museum	64, IZ1993-35B.64, AS 16187	*Junonia neildi*	USA: Florida, Monroe Co., Upper Matecumbe Key, NE Islamorada		08-Feb-86	A. Schwartz	Restriction Digest	B
FLE1986.12	Milwaukee Public Museum	65, IZ1993-35B.65, AS 16188	*Junonia neildi*	USA: Florida, Monroe Co., Upper Matecumbe Key, NE Islamorada		08-Feb-86	A. Schwartz	Restriction Digest	A
FLE1986.13	Milwaukee Public Museum	66, IZ1993-35B.66, AS 16190	*Junonia neildi*	USA: Florida, Monroe Co., Upper Matecumbe Key, NE Islamorada		08-Feb-86	A. Schwartz	Restriction Digest	A
FLE1986.4	Milwaukee Public Museum	57, IZ1993-35B.57, AS 16172	*Junonia neildi*	USA: Florida, Monroe Co., Upper Matecumbe Key, NE Islamorada		08-Feb-86	Junonia Escobio	Restriction Digest	A
FLE1986.5	Milwaukee Public Museum	58, IZ1993-35B.58, AS 16173	*Junonia neildi*	USA: Florida, Monroe Co., Upper Matecumbe Key, NE Islamorada		08-Feb-86	Junonia Escobio	Restriction Digest	A
FLE1986.6	Milwaukee Public Museum	59, IZ1993-35B.59, AS 16174	*Junonia neildi*	USA: Florida, Monroe Co., Upper Matecumbe Key, NE Islamorada		08-Feb-86	Junonia Escobio	Restriction Digest	B
FLE1986.7	Milwaukee Public Museum	60, IZ1993-35B.60, AS 16179	*Junonia neildi*	USA: Florida, Monroe Co., Upper Matecumbe Key, NE Islamorada		08-Feb-86	Junonia Escobio	Restriction Digest	A
FLE1986.8	Milwaukee Public Museum	61, IZ1993-35B.61, AS 16180	*Junonia neildi*	USA: Florida, Monroe Co., Upper Matecumbe Key, NE Islamorada		08-Feb-86	Junonia Escobio	Restriction Digest	B
FLE1986.9	Milwaukee Public Museum	62, IZ1993-35B.62, AS 16181	*Junonia neildi*	USA: Florida, Monroe Co., Upper Matecumbe Key, NE Islamorada		08-Feb-86	Junonia Escobio	Restriction Digest	A
FLEJa14	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe County, Big Pine Key	24° 43.687' 081° 23.331'	14 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLC1967.12	Yale Peabody	YPM ENT 839707	*Junonia coenia*	USA: Florida, Monroe County, Big Pine Key, near Bogie Channel		07-Aug-67	F.E. Rutowski	Restriction Digest	A
FLC1967.10	Yale Peabody	YPM ENT 839738	*Junonia coenia*	USA: Florida, Monroe County, Big Pine Key, near Bogie Channel		09-Aug-67	F.E. Rutowski	Restriction Digest	B
FLC1967.11	Yale Peabody	YPM ENT 839733	*Junonia coenia*	USA: Florida, Monroe County, Big Pine Key, near Bogie Channel		09-Aug-67	F.E. Rutowski	Restriction Digest	B
FLC1967.9	Yale Peabody	YPM ENT 839740	*Junonia coenia*	USA: Florida, Monroe County, Big Pine Key, near Bogie Channel		09-Aug-67	F.E. Rutowski	Restriction Digest	A
FLE 2009.1	Marcus Lab		Junonia neildi	USA: Florida, Monroe County, Lower Sugarloaf Key		17 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLE 2009.10	Marcus Lab		Junonia neildi	USA: Florida, Monroe County, Lower Sugarloaf Key		17 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLE 2009.2	Marcus Lab		Junonia neildi	USA: Florida, Monroe County, Lower Sugarloaf Key		17 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLE 2009.3	Marcus Lab		Junonia neildi	USA: Florida, Monroe County, Lower Sugarloaf Key		17 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLE 2009.4	Marcus Lab		Junonia neildi	USA: Florida, Monroe County, Lower Sugarloaf Key		17 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLE 2009.5	Marcus Lab		Junonia neildi	USA: Florida, Monroe County, Lower Sugarloaf Key		17 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLE 2009.6	Marcus Lab		Junonia neildi	USA: Florida, Monroe County, Lower Sugarloaf Key		17 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLE 2009.7	Marcus Lab		Junonia neildi	USA: Florida, Monroe County, Lower Sugarloaf Key		17 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLE 2009.8	Marcus Lab		Junonia neildi	USA: Florida, Monroe County, Lower Sugarloaf Key		17 xi 2009	Jeffrey M. Marcus	Restriction Digest	A
FLE 2009.9	Marcus Lab		Junonia neildi	USA: Florida, Monroe County, Lower Sugarloaf Key		17 xi 2009	Jeffrey M. Marcus	Restriction Digest	B
FLE2010.73	Marcus Lab		Junonia neildi	USA: Florida, Monroe County, Lower Sugarloaf Key		2 ii 2010	Jeffrey M. Marcus	Restriction Digest	B
FLE2010.74	Marcus Lab		Junonia neildi	USA: Florida, Monroe County, Lower Sugarloaf Key		2 ii 2010	Jeffrey M. Marcus	Restriction Digest	B
FLE2010.75	Marcus Lab		Junonia neildi	USA: Florida, Monroe County, Lower Sugarloaf Key		2 ii 2010	Jeffrey M. Marcus	Restriction Digest	B
FLE2010.76	Marcus Lab		Junonia neildi	USA: Florida, Monroe County, Lower Sugarloaf Key		2 ii 2010	Jeffrey M. Marcus	Restriction Digest	B
FLE2010.77	Marcus Lab		Junonia neildi	USA: Florida, Monroe County, Lower Sugarloaf Key		2 ii 2010	Jeffrey M. Marcus	Restriction Digest	B
FLE2010.1	Marcus Lab		Junonia neildi	USA: Florida, Monroe County, Lower Sugarloaf Key		20 iv 2010	Jeffrey M. Marcus	Restriction Digest	B
FLE2010.2	Marcus Lab		Junonia neildi	USA: Florida, Monroe County, Lower Sugarloaf Key		20 iv 2010	Jeffrey M. Marcus	Restriction Digest	B
FLEMar1	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe County, Lower Sugarloaf Key--Entry Rd	24° 35.862' 081° 34.969'	16 iii 2007	Jeffrey M. Marcus	Restriction Digest	B
FLEMar2	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe County, Lower Sugarloaf Key--Entry Rd	24° 35.862' 081° 34.969'	16 iii 2007	Jeffrey M. Marcus	Restriction Digest	B
FLEMar3	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe County, Lower Sugarloaf Key--Entry Rd	24° 35.862' 081° 34.969'	16 iii 2007	Jeffrey M. Marcus	Restriction Digest	B
FLEMar4	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe County, Lower Sugarloaf Key--Entry Rd	24° 35.862' 081° 34.969'	16 iii 2007	Jeffrey M. Marcus	Restriction Digest	B
FLEMar5	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe County, No Name Key	24° 41.855' 081° 19.107'	17 iii 2007	Jeffrey M. Marcus	Restriction Digest	B
FLEJa3	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe County, Ohio Key	24° 40.300' 081° 14.620'	13 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLEJa5	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe County, Ohio Key	24° 40.401' 081° 14.609'	13 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLEJa10	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe County, Ohio Key	24° 40.298' 081° 14.628'	14 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLEJa7	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe County, Ohio Key	24° 40.303' 081° 14.622'	14 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLEJa1	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe County, Ohio Key	24° 40.365' 081° 14.595'	13 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLEJa2	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe County, Ohio Key	24° 40.365' 081° 14.595'	13 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLEJa4	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe County, Ohio Key	24° 40.227' 081° 14.663'	13 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLEJa6	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe County, Ohio Key	24° 40.227' 081° 14.665'	14 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLEJa8	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe County, Ohio Key	24° 40.387' 081° 14.614'	14 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLEJa9	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe County, Ohio Key	24° 40.338' 081° 14.607'	14 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLEJa11	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe County, West Summerland Key, S. of Bahia Honda Channel	24° 39.338' 081° 17.884'	14 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLEJa12	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe County, West Summerland Key, S. of Bahia Honda Channel	24° 39.342' 081° 17.841'	14 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLEJa13	Marcus Lab		*Junonia neildi*	USA: Florida, Monroe County, West Summerland Key, S. of Bahia Honda Channel	24° 39.342' 081° 17.833'	14 i 2007	Jeffrey M. Marcus	Restriction Digest	B
FLC1928.1	University of Michigan	UMMZI-00204380	*Junonia coenia*	USA: Florida, Monroe/Miami-Dade Co., Everglades		9 iv 1928	D. Marston Bates	Restriction Digest	B
FLC1928.2	University of Michigan	UMMZI-00204382	*Junonia coenia*	USA: Florida, Monroe/Miami-Dade Co., Everglades		9 iv 1928	D. Marston Bates	Restriction Digest	B
FLE2011.7	Michigan State University		*Junonia neildi*	USA: Florida, Munroe Co. Sugarloaf Key (Hwy 1, milepost 17)		23-25-Dec-2011	F.W. Stehr	Restriction Digest	B
FLC1937.3	Yale Peabody	YPM ENT 415244	*Junonia coenia*	USA: Florida, Palm Beach Co., Lake Worth		29-Jan-37	M.B. Bishop	Restriction Digest	B
FLC1937.2	Yale Peabody	YPM ENT 839610	*Junonia coenia (rosa)*	USA: Florida, Palm Beach Co., Lake Worth		29-Jan-37	M.B. Bishop	Restriction Digest	B
FLC1970.2	Yale Peabody	YPM ENT 415239	*Junonia coenia*	USA: Florida, Palm Beach Co., South Bay		23-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.20	Yale Peabody	YPM ENT 839663	*Junonia coenia*	USA: Florida, Palm Beach Co., South Bay		23-Jun-70	T.R. Manley	Restriction Digest	B
FLC1970.22	Yale Peabody	YPM ENT 839654	*Junonia coenia*	USA: Florida, Palm Beach Co., South Bay		23-Jun-70	T.R. Manley	Restriction Digest	A
FLC1970.32	Yale Peabody	YPM ENT 839614	*Junonia coenia*	USA: Florida, Palm Beach Co., South Bay		23-Jun-70	T.R. Manley	Restriction Digest	B
FLX1970.1	Yale Peabody	YPM ENT 839653	*Junonia coenia (CxG)*	USA: Florida, Palm Beach Co., South Bay		23-Jun-70	T.R. Manley	Restriction Digest	B
FLC1959.1	Oregon State		*Junonia coenia*	USA: Florida, Route 94, Everglades National Park		25-Mar-59	R. Smith	Restriction Digest	A
FLG1973.2	California Academy of Sciences		*Junonia zonalis*	USA: Florida, Route 94, Monroe Co. (Probably Collier Co.)		27-Jun-73	JunoniaW. Tilden	Restriction Digest	A
FLEM3	Marcus Lab		*Junonia neildi*	USA: Florida,Broward County, Hugh Taylor Birch State Park		19-May-2006	Jeffrey M. Marcus	KM288198	B
FLEM1	Marcus Lab		*Junonia neildi*	USA: Florida,Lee County, Estero Bay Preserve State Park		19-May-2006	Jeffrey M. Marcus	KM288189	B
FLEM2	Marcus Lab		*Junonia neildi*	USA: Florida,Lee County, Estero Bay Preserve State Park		19-May-2006	Jeffrey M. Marcus	KM288190	B
FLC1970.48	Yale Peabody	YPM ENT 839868	*Junonia coenia*	USA: Florida. Dade County. Florida City.		18-Jun-70	T.R. Manley	Restriction Digest	B

Appendix III. *Junonia* specimens for the American Southwest and Mexico data set

Specimen Identifier	Collection	Museum Accession Number	Species	Locality	GPS Coordinates	Collection Date	Collector	Genotyping Method	Haplotype Group
OLD 6	Marcus Lab		Junonia zonalis	Belize				Restriction Digest	A
GUA1	Michigan State University		Junonia zonalis	Guatemala: San Cristobal Verapaiz, Alta Verapaz, Baleu (1350 meters elev.)		June 19, 1966	Eduardo C. Welling	Restriction Digest	B
GUA2	Oregon State	875890	Junonia zonalis (female)	Guatemala: SaydaxchŽ, el Peten		September 3, 1963		Restriction Digest	B
GUA3	McGuire Center for Lepidoptera and Biodiversity		Junonia zonalis (male)	Guatemala: Tenedores Isabel		27-Jun-65	Edward Welling	Restriction Digest	B
BM41	Marcus Lab		Junonia nigrosuffusa	Mexico				Restriction Digest	B
BCC11	Colorado State University		Junonia grisea	Mexico: Baja California (2000')		October 19, 1969	RWH	Restriction Digest	B
BCC13	Colorado State University		Junonia grisea	Mexico: Baja California de Notre, Meling Ranch (2000')		September 12, 1970	RWH	Restriction Digest	B
BCC12	Colorado State University		Junonia grisea	Mexico: Baja California de Notre, San Pedro Martir (2000')		May 20, 1970	RWH	Restriction Digest	B
BCN3	California Academy of Science		Junonia nigrosuffusa	Mexico: Baja California del Sud, Rancho Cayucos, 7 miles up Canyon San Pedro from Caduano		May 7, 1959		Restriction Digest	B
BCN6	California Academy of Science		Junonia nigrosuffusa	Mexico: Baja California del Sud, Rancho Potrero, 14 miles up Canyon San Pedro from Caduano		May 10, 1959	Don Patterson	Restriction Digest	B
BCN4	California Academy of Science		Junonia nigrosuffusa	Mexico: Baja California del Sud, Rancho Potrero, 14 miles up Canyon San Pedro from Caduano		May 9, 1959	Don Patterson	Restriction Digest	B/CA
BCN2	California Academy of Science		Junonia nigrosuffusa	Mexico: Baja California del Sud, Rancho Potrero, 14 miles up Canyon San Pedro from Caduano		May 10, 1959	Don Patterson	Restriction Digest	B/CA
BCN5	California Academy of Science		Junonia nigrosuffusa	Mexico: Baja California del Sud, Rancho Potrero, 14 miles up Canyon San Pedro from Caduano		May 10, 1959	Don Patterson	Restriction Digest	B/CA
BCN1	California Academy of Science		Junonia nigrosuffusa	Mexico: Baja California del sude, Rancho Potrero, 14 miles up Canyon San Pedro		October 5, 1959	Don Patterson	Restriction Digest	B/CA
BCM1	California Academy of Science		Junonia litoralis	Mexico: Baja California sur, Santa Maria Bay		23-Feb-28	T. Craig	Restriction Digest	B/CA
BCC1	California Academy of Science		Junonia grisea	Mexico: Baja California sur., 8 mi. east of La Paz (in mangrove swamp)		September 17, 1972	Don Patterson	Restriction Digest	B
BCC9	California Academy of Science		Junonia grisea	Mexico: Baja California, de Norte Sierra Norte Sierra San Pedro Maitre Trail, La Grulla to La Encantada, E. Meling Ranch		May 3, 1958		Restriction Digest	B/CA
BCC7	California Academy of Science		Junonia grisea	Mexico: Baja California, de Norte 10 mi. N. or San Vicente,		March 25, 1973	J.A. Powell	Restriction Digest	B
BCC4	California Academy of Science		Junonia grisea	Mexico: Baja California, de Norte 10 mi. N. or San Vicente,		March 25, 1973	J.A. Powell	Restriction Digest	B
BCC8	California Academy of Science		Junonia grisea (female)	Mexico: Baja California, de Norte Sierra San Pedro Martir		June 4, 1958		Restriction Digest	B/CA
BCC6	California Academy of Science		Junonia grisea	Mexico: Baja California, de Norte Sierra San Pedro Martir, La Grulla, E. Meling Ranch, 7000ft		May 29, 1958	Don Patterson	Restriction Digest	B
BCC3	California Academy of Science		Junonia grisea (female)	Mexico: Baja California, de Norte Sierra San Pedro Martir, La Grulla, E. Meling Ranch, 7000ft		May 29, 1958	Don Patterson	Restriction Digest	B/CA
BCC5	California Academy of Science		Junonia grisea	Mexico: Baja California, de Norte, 19 mi. SE of Maneadero		March 25, 1973	J. Donohoe & Don Patterson	Restriction Digest	B/CA
BCC2	California Academy of Science		Junonia grisea	Mexico: Baja California, de Notre Sierra San Pedro Martir Trail, Las Encimas to La Sanjo, E. Meling Ranch, 5000-7000ft.		May 27, 1958	Don Patterson	Restriction Digest	B/CA
BCC10	California Academy of Science		Junonia grisea x nigrosuffu	Mexico: Baja California, El Requeson		February 20, 1978		Restriction Digest	B/CA
MAL 02876	International Barcode of Life		Junonia zonalis	Mexico: Campeche, Calakmul, Reserva de la Biosfera de Calakmul, Ejido Cristobal Colon, Zona C		25-Sep-99	Santiago Uc	GU659436	B
MAL 02881	International Barcode of Life		Junonia zonalis	Mexico: Campeche, Calakmul, Reserva de la Biosfera de Calakmul: Zona Arqueologica de Calakmul `B`		29-Sep-04	David Berdugo	GU659432	B
MAL 02884	International Barcode of Life		Junonia zonalis	Mexico: Campeche, El Chorro, Ejido Nuevo Becal		12-Aug-03	Pablo Beutelspacher	GU659427	B
NW162 7	Nicholas Wahlberg		Junonia zonalis	Mexico: Cerro Frio, Morelos		13-Jul-07	Luc Legal	JQ430731	B
MEX1	Marcus Lab		Junonia zonalis	Mexico: Chiapas		July 1, 2013		Restriction Digest	B
MEX2	Marcus Lab		Junonia zonalis	Mexico: Chiapas		July 1, 2013		Restriction Digest	B
MEX3	Marcus Lab		Junonia zonalis	Mexico: Chiapas		July 1, 2013		Restriction Digest	B
MEXZ3	Colorado State University		Junonia zonalis	Mexico: Chiapas, K-140, Mex 200		August 18, 1971	RWH	Restriction Digest	B/CA
MEXC1	McGuire Center for Lepidoptera and Biodiversity		Junonia coenia	Mexico: Chiapas, Palenque			D & J. Jenkins	Restriction Digest	B
MEXM2	Illinois Natural History Survey	590	Junonia litoralis	Mexico: Chiappas, Lago De Montebellio		late Feb 1974	Bill Bier	Restriction Digest	B/CA
MEXN31	Colorado State University		Junonia nigrosuffusa	Mexico: Chihuahua, 5 mi up Rio Piedras Verdez from Colonia Juarez (5500')		June 27, 1979	RWH	Restriction Digest	B
MEXC16	Colorado State University		Junonia grisea	Mexico: Chihuahua, 5 mi. NW of Colonia Juarez, on Rio Piedras Verdes (5500')		June 26, 1979	RWH	Restriction Digest	B
MEXC9	Colorado State University		Junonia coenia x grisea	Mexico: Chihuahua, Mex. Hwy. 2, Puerto San Luis Can. On side Chih-Son. State line (5500')		May 4, 1994	RWH & ESC	Restriction Digest	B
MEXN34	Colorado State University		Junonia nigrosuffusa	Mexico: Chihuahua, Mex. Hwy. 45, Chih. K-1431 nr. Cd. Jiminez (4500')		August 27, 1967	RWH	Restriction Digest	B
MEX7	Michigan State University		Junonia zonalis	Mexico: Chiltepec, Oaxaca		March 2, 1970	Peter Hubbey	Restriction Digest	B
MEX6	Michigan State University		Junonia zonalis	Mexico: Chiltepec, Oaxaca		October 1, 1970	Peter Hubbey	Restriction Digest	B
MEXC13	Colorado State University		Junonia coenia	Mexico: Coahuila, Saltillo		October 9, 2003	E. & P. Opler	Restriction Digest	B
MEXC14	Colorado State University		Junonia coenia	Mexico: Coahuila, Saltillo		October 9, 2003	E. & P. Opler	Restriction Digest	B/CA
MEX8	Michigan State University		Junonia coenia	Mexico: Dos Amates, Vera Cruz		November 1, 1970	Peter Hubbell	Restriction Digest	B
MEXN11	Colorado State University		Junonia nigrosuffusa	Mexico: Durango, 10 km W of Mex. 39 on rd. Of Topia (7000')		August 6, 1981	RWH	Restriction Digest	B/CA
MEXN22	Colorado State University		Junonia nigrosuffusa	Mexico: Durango, 10 km W of Mex. 39 on rd. Of Topia (7000')		August 6, 1981	RWH	Restriction Digest	B/CA
MEXN32	Colorado State University		Junonia nigrosuffusa	Mexico: Durango, 28 mi E of El Salto (8300')		August 5, 1981	RWH	Restriction Digest	B
MEXN17	Colorado State University		Junonia nigrosuffusa	Mexico: Durango, 3 mi E Rio Mimbres, Hwy. 45		July 27, 1981	RES	Restriction Digest	B/CA
MEXN14	Colorado State University		Junonia nigrosuffusa	Mexico: Durango, 3 mi. NW Las Nieves, Hwy 45 Rio Florida		July 26, 1981	RES	Restriction Digest	B
MEXN15	Colorado State University		Junonia nigrosuffusa	Mexico: Durango, 3 mi. NW Las Nieves, Hwy 45 Rio Florida		July 26, 1981	RES	Restriction Digest	B
MEXN16	Colorado State University		Junonia nigrosuffusa	Mexico: Durango, 3 mi. NW Las Nieves, Hwy 45 Rio Florida		July 26, 1981	RES	Restriction Digest	B
MEXN18	Colorado State University		Junonia nigrosuffusa	Mexico: Durango, 8 mi W. Durango Hwy. 45 nr. Dump		July 27, 1981	RES	Restriction Digest	B/CA
MEXN3	Oregon State	875914	Junonia nigrosuffusa (rosa	Mexico: Durango, Rio Chico Gorge, Hwy 40 C. 20 miles W. of Cd. Durango		August 28, 1985	D. McCorkle & D. Mulins	Restriction Digest	B
JM6 10	Pfeiler Lab		Junonia zonalis	Mexico: El Lim—n, Morelos		29-Jun-07	Luc Legal	JQ430733	B
MEX4	Michigan State University		Junonia coenia	Mexico: Guerrero, 20 m S Taxco		February 6, 1964	R.R. Dreisbach	Restriction Digest	B
MEXC2	McGuire Center for Lepidoptera and Biodiversity		Junonia coenia	Mexico: Guerrero, Acanuizotla		09-Jun-59	T. Escalante	Restriction Digest	B
MEXC1977.2	McGuire Center for Lepidoptera and Biodiversity		Junonia coenia	Mexico: Guerrero, Acanuizotla		14-Aug-77	D & J. Jenkins	Restriction Digest	B
MEXM1	Oregon State	875901	Junonia litoralis	Mexico: Guerrero, Ixtapa		October 5, 1986		Restriction Digest	B/CA
MEXC12	Colorado State University		Junonia coenia	Mexico: Hildalgo, nr. Zimapan, Hwy 85		August 4, 1981	RES	Restriction Digest	B
MEXC3	McGuire Center for Lepidoptera and Biodiversity		Junonia coenia	Mexico: Morelos, Rancho viejo		09-Nov-82	D.W. Jenkins	Restriction Digest	B/CA
MEX5	Michigan State University		Junonia nigrosuffusa	Mexico: Nayarit-Jalisco Border on Mex-15 (3900 feet elev.)		July 18, 1963	Julian P. Donahue	Restriction Digest	B
MEXN13	Colorado State University		Junonia nigrosuffusa x litoı	Mexico: Nayarit, Languan Santa Maria (2200')		July 31, 1971	RWH	Restriction Digest	B
MEXN9	California Academy of Science		Junonia nigrosuffusa x gris	Mexico: Nuevo Laredo, 10 mi. S		December 22, 1940	G.E. Bohart	Restriction Digest	B/CA
MEXX2	California Academy of Science		Junonia grisea x.nigrosuffu	Mexico: Nuevo Leon, Monterrey		November 26, 1929		Restriction Digest	B
MEXX4	Colorado State University		Junonia nigrosuffusa	Mexico: Oaxaca, 20 km S. of Oaxaca on Rd. to Puerto Angel		December 23, 1978	RWH	Restriction Digest	B
MEXN23	Colorado State University		Junonia nigrosuffusa x gris	Mexico: Oaxaca, 20 km S. of Oaxaca on Rd. to Puerto Angel (5000')		July 23, 1978	RWH	Restriction Digest	B/CA
MEXX3	Colorado State University		Junonia coenia x grisea	Mexico: Oaxaca, 20 km SE of Oaxaca Macuilxochitl (5000')		July 22, 1978	RWH	Restriction Digest	B
MEXC1977.1	McGuire Center for Lepidoptera and Biodiversity		Junonia coenia	Mexico: Oaxaca, Las Animas Tr. 190 S.		12-Jun-77		Restriction Digest	B
MEXN12	Colorado State University		Junonia nigrosuffusa	Mexico: Oaxaca, R-115, Mex. 190 E. of Oaxaca (2200')		August 6, 1971	RWH	Restriction Digest	B
OLD 9	Marcus Lab		Junonia nigrosuffusa	Mexico: Presidio, Vera Cruz			T.Escalante	Restriction Digest	A
MEXC8	Colorado State University		Junonia coenia	Mexico: Puebla, 5 mi. S. of Izucar de Matamoros, Mex 190 (5000')		July 4, 1970	RWH	Restriction Digest	B
MEXN4	Andrew Warren Collection		Junonia nigrosuffusa	Mexico: Queretaro, Mpio El Salto, Hwy 40, 1 km SW Cerro el Mad rono, 2450m		29-May-98	Andrew D. Warren	Restriction Digest	B/CA
MAL 02874	International Barcode of Life		Junonia zonalis	Mexico: Quintana Roo, 2.5 km camino al Ejido San Carlos		01-Nov-90	Enrique Escobedo	HQ990188	B
MAL 02875	International Barcode of Life		Junonia zonalis	Mexico: Quintana Roo, Chetumal, Universidad de Quintana Roo		03-May-92	Nancy Crisostomo	GU659435	B
MAL 02883	International Barcode of Life		Junonia zonalis	Mexico: Quintana Roo, Reserva de la Biosfera Sian ka`an: Estacion Santa Teresa		09-Mar-04	Emigdio May	GU659426	B
MAL 02882	International Barcode of Life		Junonia zonalis	Mexico: Quintana Roo, Reserva de la Biosfera Sian ka`an: Pulticub		04-Nov-04	Alejandro Franco	GU659425	B
CIAD10 B01	Pfeiler Lab		Junonia litoralis	Mexico: San Carlos, Sonora		08-Jan-10	E. Pfeiler	JQ430692	B
CIAD10 B10	Pfeiler Lab		Junonia litoralis	Mexico: San Carlos, Sonora		21-Jul-10	E. Pfeiler	JQ430693	B
CIAD10 B11	Pfeiler Lab		Junonia litoralis	Mexico: San Carlos, Sonora		16-Aug-10	E. Pfeiler	JQ430694	B
CIAD10 B12	Pfeiler Lab		Junonia litoralis	Mexico: San Carlos, Sonora		16-Aug-10	E. Pfeiler	JQ430695	B
CIAD10 B13	Pfeiler Lab		Junonia litoralis	Mexico: San Carlos, Sonora		20-Aug-10	E. Pfeiler	JQ430696	B
CIAD10 B14	Pfeiler Lab		Junonia litoralis	Mexico: San Carlos, Sonora		20-Aug-10	E. Pfeiler	JQ430697	B
CIAD10 B15	Pfeiler Lab		Junonia litoralis	Mexico: San Carlos, Sonora		20-Aug-10	E. Pfeiler	JQ430698	B
CIAD10 B16	Pfeiler Lab		Junonia litoralis	Mexico: San Carlos, Sonora		25-Sep-10	E. Pfeiler	JQ430699	B
CIAD10 B17	Pfeiler Lab		Junonia litoralis	Mexico: San Carlos, Sonora		25-Sep-10	E. Pfeiler	JQ430700	B
CIAD10 B18	Pfeiler Lab		Junonia litoralis	Mexico: San Carlos, Sonora		25-Sep-10	E. Pfeiler	JQ430701	B
CIAD10 B19	Pfeiler Lab		Junonia litoralis	Mexico: San Carlos, Sonora		25-Sep-10	E. Pfeiler	JQ430702	B

Appendix III. *Junonia* specimens for the American Southwest and Mexico data set

Specimen Identifier	Collection	Museum Accession Number	Species	Locality	GPS Coordinates	Collection Date	Collector	Genotyping Method	Haplotype Group
CIAD10 B20	Pfeiler Lab		Junonia litoralis	Mexico: San Carlos, Sonora		25-Sep-10	E. Pfeiler	JQ430703	B
CIAD10 B21	Pfeiler Lab		Junonia litoralis	Mexico: San Carlos, Sonora		25-Sep-10	E. Pfeiler	JQ430704	B
CIAD10 B22	Pfeiler Lab		Junonia litoralis	Mexico: San Carlos, Sonora		25-Sep-10	E. Pfeiler	JQ430705	B
CIAD10 B23	Pfeiler Lab		Junonia litoralis	Mexico: San Carlos, Sonora		25-Sep-10	E. Pfeiler	JQ430706	B
CIAD10 B24	Pfeiler Lab		Junonia nigrosuffusa	Mexico: San Carlos, Sonora		01-Oct-10	E. Pfeiler	JQ430711	B
CIAD10 B26	Pfeiler Lab		Junonia nigrosuffusa	Mexico: San Carlos, Sonora		01-Oct-10	E. Pfeiler	JQ430713	B
CIAD10 B25	Pfeiler Lab		Junonia nigrosuffusa	Mexico: San Carlos, Sonora		08-Oct-10	E. Pfeiler	JQ430712	B
CIAD10 B27	Pfeiler Lab		Junonia litoralis	Mexico: San Carlos, Sonora		11-Oct-10	E. Pfeiler	JQ430707	B
CIAD10 B28	Pfeiler Lab		Junonia litoralis	Mexico: San Carlos, Sonora		11-Oct-10	E. Pfeiler	JQ430708	B
CIAD10 B29	Pfeiler Lab		Junonia litoralis	Mexico: San Carlos, Sonora		01-Nov-10	E. Pfeiler	JQ430709	B
CIAD10 B30	Pfeiler Lab		Junonia nigrosuffusa	Mexico: San Carlos, Sonora		01-Nov-10	E. Pfeiler	JQ430714	B
CIAD10 B31	Pfeiler Lab		Junonia nigrosuffusa	Mexico: San Carlos, Sonora		01-Nov-10	E. Pfeiler	JQ430715	B
CIAD10 B32	Pfeiler Lab		Junonia nigrosuffusa	Mexico: San Carlos, Sonora		01-Nov-10	E. Pfeiler	JQ430716	B
CIAD10 B33	Pfeiler Lab		Junonia nigrosuffusa	Mexico: San Carlos, Sonora		01-Nov-10	E. Pfeiler	JQ430717	B
CIAD10 B34	Pfeiler Lab		Junonia nigrosuffusa	Mexico: San Carlos, Sonora		02-Nov-10	E. Pfeiler	JQ430718	B
CIAD10 B35	Pfeiler Lab		Junonia nigrosuffusa	Mexico: San Carlos, Sonora		05-Nov-10	E. Pfeiler	JQ430719	B
CIAD10 B36	Pfeiler Lab		Junonia nigrosuffusa	Mexico: San Carlos, Sonora		05-Nov-10	E. Pfeiler	JQ430720	B
CIAD10 B37	Pfeiler Lab		Junonia litoralis	Mexico: San Carlos, Sonora		09-Nov-10	E. Pfeiler	JQ430710	B
MEXN2	California Academy of Science		Junonia nigrosuffusa	Mexico: San Luis Potosi, 15 mile N Vallies		January 6, 1941	G.E. Bohart	Restriction Digest	B
MEXN1	California Academy of Science		Junonia nigrosuffusa	Mexico: Sinaloa twixt, Caliacan & Mazatlan		July 2, 1963	E.A. Evans	Restriction Digest	B
MEXN21	Colorado State University		Junonia nigrosuffusa	Mexico: Sinaloa, Can. Gelo El. Palmito (5500')		October 7, 1970	RWH	Restriction Digest	B
MEXN24	Colorado State University		Junonia nigrosuffusa	Mexico: Sinaloa, Chirimollos, Hwy 40		23 Nov to 2 Dec 2003	P.A & E.M. Opler	Restriction Digest	B
MEXN25	Colorado State University		Junonia nigrosuffusa	Mexico: Sinaloa, Chirimollos, Hwy 40		23 Nov to 2 Dec 2003	P.A & E.M. Opler	Restriction Digest	B
MEXN26	Colorado State University		Junonia nigrosuffusa	Mexico: Sinaloa, Chirimollos, Hwy 40		23 Nov to 2 Dec 2003	P.A & E.M. Opler	Restriction Digest	B
MEXC11	Colorado State University		Junonia litoralis x grisea	Mexico: Sinaloa, Isla de Piedra		November 24, 2003	P.A. Opler	Restriction Digest	B
MEXN5	Andrew Warren Collection		Junonia nigrosuffusa	Mexico: Sinaloa, Loberas (on Hwy. 40), 1900-1940m		30-Nov-96	Andrew D. Warren	Restriction Digest	B
MEXN6	Andrew Warren Collection		Junonia nigrosuffusa	Mexico: Sinaloa, Loberas (on Hwy. 40), 1900-1940m		30-Nov-96	Andrew D. Warren	Restriction Digest	B
MEXN7	Andrew Warren Collection		Junonia nigrosuffusa	Mexico: Sinaloa, Loberas (on Hwy. 40), 1900-1940m		30-Nov-96	Andrew D. Warren	Restriction Digest	B/CA
MEXC10	Colorado State University		Junonia coenia	Mexico: Sinaloa, Mex Hwy. 15, K-1187 nr. Mazatlan		September 1, 1967	RWH	Restriction Digest	B
MEXX1	Andrew Warren Collection		Junonai coenia x nigrosuffı	Mexico: Sinaloa, Mpio Mazatlan, Mazatlan		01-Dec-96	Andrew D. Warren	Restriction Digest	B
MEXC4	Andrew Warren Collection		Junonia coenia	Mexico: Sinaloa, Mpio Mazatlan, Mazatlan		01-Dec-96	Andrew D. Warren	Restriction Digest	B
MEXC5	Andrew Warren Collection		Junonia coenia	Mexico: Sinaloa, Mpio Mazatlan, Mazatlan		01-Dec-96	Andrew D. Warren	Restriction Digest	B
MEXC6	McGuire Center for Lepidoptera and Biodiversity		Junonia coenia	Mexico: Sinaloa, Mpio Mazatlan, Mazatlan		01-Dec-96	Andrew D. Warren	Restriction Digest	B
MEXN8	McGuire Center for Lepidoptera and Biodiversity		Junonia nigrosuffusa (male	Mexico: Sinaloa, nr. Mazatian Mex. 40, 5000ft.		08-Sep-77		Restriction Digest	B/CA
MEXN27	Colorado State University		Junonia nigrosuffusa	Mexico: Sonora, 5 mi N. of Mesa Tres Rios at Rio de Los Lobos (5100')		July 1, 1979	RWH	Restriction Digest	B
MEXN29	Colorado State University		Junonia nigrosuffusa	Mexico: Sonora, 5 mi N. of Mesa Tres Rios at Rio de Los Lobos (5100')		July 1, 1979	RWH	Restriction Digest	B
MEXN28	Colorado State University		Junonia nigrosuffusa	Mexico: Sonora, 5 mi N. of Mesa Tres Rios at Rio de Los Lobos (5100')		July 1, 1979	RWH	Restriction Digest	B/CA
MEXC15	Colorado State University		Junonia grisea	Mexico: Sonora, c. 8 mi. NE of Bavispe, on road to Janos, Chih. Rio Bavispe (Sonora) side of ridge E of Rio Bavispe (5000')		5-6 September, 1994	RWH & ESC	Restriction Digest	B
OLD10.1	Marcus Lab		Junonia nigrosuffusa	Mexico: Sonora, Gurocoba (about 1400ft, 425 m)		Aug. 9-16, 1954	R. Zweifel	Restriction Digest	A
MEXN19	Colorado State University		Junonia nigrosuffusa	Mexico: Sonora, Imuris (2800')		September 2, 1966	RWH	Restriction Digest	B
MEXN20	Colorado State University		Junonia nigrosuffusa	Mexico: Sonora, Imuris (2800')		September 2, 1966	RWH	Restriction Digest	B
MEXN33	Colorado State University		Junonia nigrosuffusa	Mexico: Sonora, Imuris (2800')		September 2, 1966	RWH	Restriction Digest	B
MEXN30	Colorado State University		Junonia nigrosuffusa	Mexico: Sonora, Tres Rios, Rio Bavispe (4800')		April 12, 1978	RWH	Restriction Digest	B
MEXC7	California Academy of Sciences		Junonia coenia	Mexico: Tamaulipas, Monterry		November 16, 1929	L.I. Hewes Collection	Restriction Digest	B/CA
MEX9	Michigan State University		Junonia coenia	Mexico: Vera Cruz		November 1, 1970	Peter Hubbell	Restriction Digest	B
MEXZ2	McGuire Center for Lepidoptera and Biodiversity		Junonia zonalis	Mexico: Vera Cruz, dos Amateo		Oct-69		Restriction Digest	B/CA
MEXN10	California Academy of Science		Junonia nigrosuffusa	Mexico: Vera Cruz, Fontivin de Las Flores		July 30, 1955	J.E. Opler	Restriction Digest	B
MEXZ1	McGuire Center for Lepidoptera and Biodiversity		Junonia zonalis (male)	Mexico: Yucatan, Piste		10-Jul-67		Restriction Digest	B
MAL 02880	International Barcode of Life		Junonia zonalis	Mexico: Yucatan, Ria Lagartos, Peten Tucha		21-Jan-07	David Berdugo	GU659431	B
MAL 02879	International Barcode of Life		Junonia zonalis	Mexico: Yucatan, Tizimin, La Florida		20-Jan-07	Aixchel Maya	GU659430	B
MAL 02878	International Barcode of Life		Junonia zonalis	Mexico: Yucatan, Uman, Hacienda Poxila		03-Nov-04		GU659429	B
AZN7	Arizona State University	ASUHIC0003805	Junonia nigrosuffusa	USA, Arizona, Cochise County, 5131 S Bannock Street, Sierra Vista, Huachuca Mountains (found in backyard)	31.475908 -110.269907 WGS84	22-May-84	Roman S. Wielgus	Restriction Digest	B
AZN4	Arizona State University	ASUHIC0003801	Junonia nigrosuffusa	USA, Arizona, Cochise County, Carr Canyon, Huachuca Mountains	31.438463 -110.291882 WGS84	28-May-78	Roman S. Wielgus	Restriction Digest	B
AZN5	Arizona State University	ASUHIC0003803	Junonia nigrosuffusa	USA, Arizona, Cochise County, Miller Canyon, Huachuca Mountains	31.414168 -110.278539 WGS84	18-Aug-78	Roman S. Wielgus	Restriction Digest	B
AZN6	Arizona State University	ASUHIC0003804	Junonia nigrosuffusa	USA, Arizona, Cochise County, Miller Canyon, Huachuca Mountains	31.414168 -110.278539 WGS84	12-Jun-84	Roman S. Wielgus	Restriction Digest	B/CA
AZC19	Arizona State University	ASUHIC0003798	Junonia coenia	USA, Arizona, Gila County, 3 mi. NE of Gisela (from larva; emerged 1977-07-03)	34.132928 -111.241569 WGS84	25-Jun-77	William B. Warner	Restriction Digest	B
AZC22	Arizona State University	ASUHIC0003797	Junonia coenia	USA, Arizona, Gila County, 3 mi. NE of Gisela (from larva; emerged 1977-07-03)	34.132928 -111.241569 WGS84	25-Jun-77	William B. Warner	Restriction Digest	B/CA
AZC18	Arizona State University	ASUHIC0003790	Junonia coenia	USA, Arizona, Gila County, Rose Creek, Sierra Ancha	33.8548 -110.983996 WGS84	10-May-69	Roman S. Wielgus & Dale Wielgus	Restriction Digest	B
AZC16	Arizona State University	ASUHIC0003792	Junonia coenia	USA, Arizona, Gila County, Rose Creek, Sierra Ancha	33.8548 -110.983996 WGS84	28 June 1969	Roman S. Wielgus & Dale Wielgus	Restriction Digest	B/CA
Jn2015.3	Marcus Lab		Junonia nigrosuffusa	USA: Arizona, Cochise Co., Sycamore Cyn.		September 5-7, 2015	Victoria Bic	Restriction Digest	B
Jn2015.7	Marcus Lab		Junonia nigrosuffusa	USA: Arizona, Cochise Co., Sycamore Cyn.		September 5-7, 2015	Victoria Bic	Restriction Digest	B
Jn2015.8	Marcus Lab		Junonia nigrosuffusa	USA: Arizona, Cochise Co., Sycamore Cyn.		September 5-7, 2015	Victoria Bic	Restriction Digest	B
Jn2015.1	Marcus Lab		Junonia nigrosuffusa	USA: Arizona, Cochise Co., Sycamore Cyn.		September 5-7, 2015	Victoria Bic	Restriction Digest	B/CA
Jn2015.10	Marcus Lab		Junonia nigrosuffusa	USA: Arizona, Cochise Co., Sycamore Cyn.		September 5-7, 2015	Victoria Bic	Restriction Digest	B/CA
Jn2015.2	Marcus Lab		Junonia nigrosuffusa	USA: Arizona, Cochise Co., Sycamore Cyn.		September 5-7, 2015	Victoria Bic	Restriction Digest	B/CA
Jn2015.4	Marcus Lab		Junonia nigrosuffusa	USA: Arizona, Cochise Co., Sycamore Cyn.		September 5-7, 2015	Victoria Bic	Restriction Digest	B/CA
Jn2015.5	Marcus Lab		Junonia nigrosuffusa	USA: Arizona, Cochise Co., Sycamore Cyn.		September 5-7, 2015	Victoria Bic	Restriction Digest	B/CA
Jn2015.6	Marcus Lab		Junonia nigrosuffusa	USA: Arizona, Cochise Co., Sycamore Cyn.		September 5-7, 2015	Victoria Bic	Restriction Digest	B/CA
Jn2015.9	Marcus Lab		Junonia nigrosuffusa	USA: Arizona, Cochise Co., Sycamore Cyn.		September 5-7, 2015	Victoria Bic	Restriction Digest	B/CA
AZN9	California Academy of Science		Junonia nigrosuffusa	USA: Arizona, Cochise County Benson (46 mi E of Tucson)		11-Aug-46	James K. Lochead	Restriction Digest	B/CA
USJunGen1	Marcus Lab		Junonia nigrosuffusa	USA: Arizona, Cochise County, Palmerlee		04-08-08	Igor Udod	Restriction Digest	B (used to be weird A)
AZN10	California Academy of Science		Junonia nigrosuffusa	USA: Arizona, Cochise County, 5 mi w Portal		21-Aug-58	P.A. Opler	Restriction Digest	B
AZN3	Arizona State University	ASUHIC0003806	Junonia nigrosuffusa	USA: Arizona, Cochise County, 5131 S Bannock Street, Sierra Vista, Huachuca Mountains (found in backyard)	31.475908 -110.269907 WGS84	23-May-84	Roman S. Wielgus	Restriction Digest	B
AZN11	California Academy of Science		Junonia nigrosuffusa	USA: Arizona, Cochise County, Apache, 5 mi SE		11-Aug-58	P.A. Opler	Restriction Digest	B/CA
AZC12	Arizona State University	ASUHIC0003777	Junonia grisea	USA: Arizona, Cochise County, Carr Canyon, Huachuca Mountains	31.438463 -110.291882 WGS84	29-Apr-78	Roman S. Wielgus	Restriction Digest	B
AZN1	Arizona State University	ASUHIC0003802	Junonia nigrosuffusa	USA: Arizona, Cochise County, Carr Canyon, Huachuca Mountains	31.438463 -110.291882 WGS84	29-Apr-78	Roman S. Wielgus	Restriction Digest	B
AZC1	Arizona State University	ASUHIC0003774	Junonia coenia x grisea	USA: Arizona, Cochise County, Carr Canyon, Huachuca Mountains	31.438463 -110.291882 WGS84	29 April 1978	Roman S. Wielgus	Restriction Digest	B
AZN2	Arizona State University	ASUHIC0003800	Junonia nigrosuffusa	USA: Arizona, Cochise County, Carr Canyon, Huachuca Mountains	31.438463 -110.291882 WGS84	02-Apr-78	Roman S. Wielgus	Restriction Digest	B/CA
AZC3	Arizona State University	ASUHIC0003780	Junonia grisea	USA: Arizona, Cochise County, Carr Canyon, Huachuca Mountains	31.438463 -110.291882 WGS84	28-Apr-78	Roman S. Wielgus	Restriction Digest	B/CA
AZC13	Arizona State University	ASUHIC0003775	Junonia grisea	USA: Arizona, Cochise County, Carr Canyon, Huachuca Mountains	31.438463 -110.291882 WGS84	29-Apr-78	Roman S. Wielgus	Restriction Digest	B/CA
AZC14	Arizona State University	ASUHIC0003776	Junonia grisea	USA: Arizona, Cochise County, Carr Canyon, Huachuca Mountains	31.438463 -110.291882 WGS84	29-Apr-78	Roman S. Wielgus	Restriction Digest	B/CA
AZC20	Arizona State University	ASUHIC0003778	Junonia grisea	USA: Arizona, Cochise County, Carr Canyon, Huachuca Mountains	31.438463 -110.291882 WGS84	29-Apr-78	Roman S. Wielgus	Restriction Digest	B/CA
AZC9	Arizona State University	ASUHIC0003779	Junonia grisea	USA: Arizona, Cochise County, Carr Canyon, Huachuca Mountains	31.438463 -110.291882 WGS84	29-Apr-78	Roman S. Wielgus	Restriction Digest	B/CA
AZX2	Oregon State	875900	Junonia coenia x nigrosuff	USA: Arizona, Cochise County, Portal SW. Research Station (1650 m)	30û53 min N, 109û13 min W	26-Aug-98	A. Brower & A. Sohns	Restriction Digest	B/CA
AZN16	Oregon State	875917	Junonia nigrosuffusa	USA: Arizona, Cochise County, San Raefel Valley		September 2, 1981	H.E. Rice	Restriction Digest	B
AZN18	Oregon State	875918	Junonia nigrosuffusa	USA: Arizona, Cochise County, San Raphael Valley		September 2, 1981	H.E. Rice	Restriction Digest	B/CA
AZC7	Arizona State University	ASUHIC0003787	Junonia nigrosuffusa	USA: Arizona, Cochise County, Saw Mill Canyon, Huachuca Mountains	31.453735 -110.376851 WGS84	28-Jun-84	Roman S. Wielgus	Restriction Digest	B

Appendix III. *Junonia* specimens for the American Southwest and Mexico data set

Specimen Identifier	Collection	Museum Accession Number	Species	Locality	GPS Coordinates	Collection Date	Collector	Genotyping Method	Haplotype Group
AZC10	Arizona State University	ASUHIC0003786	Junonia grisea	USA: Arizona, Cochise County, Saw Mill Canyon, Huachuca Mountains	31.453735 -110.376851 WGS84	28 June 1984	Roman S. Wielgus	Restriction Digest	B
AZN8	University of Manitoba	306295	Junonia nigrosuffusa	USA: Arizona, Cochise County, Sierra Vista				Restriction Digest	B/CA
AZN15	Oregon State	875895	Junonia nigrosuffusa	USA: Arizona, Cochise County, vicinity SW Research Station near Portal		June 3, 1978	S.G. Jewette Jr.	Restriction Digest	B/CA
AZC2	Arizona State University	ASUHIC0003781	Junonia grisea	USA: Arizona, Coconino County, Clover Springs	34.506575 -111.362496 WGS84	25-Aug-78	Roman S. Wielgus	Restriction Digest	B
AZC21	Arizona State University	ASUHIC0003796	Junonia coenia x nigrosuffu	USA: Arizona, Coconino County, Oak Creek Canyon, Sedona	34.912719 -111.72606 WGS84	26-Jul-64	Roman S. Wielgus	Restriction Digest	B
AZC11	Arizona State University	ASUHIC0003789	Junonia grisea	USA: Arizona, Gila County, Rose Creek, Sierra Ancha	33.8548 -110.983996 WGS84	28-Jun-69	Dale Wielgus	Restriction Digest	B
AZC15	Arizona State University	ASUHIC0003791	Junonia grisea	USA: Arizona, Gila County, Rose Creek, Sierra Ancha	33.8548 -110.983996 WGS84	28-Jun-69	Roman S. Wielgus	Restriction Digest	B
AZC17	Arizona State University	ASUHIC0003793	Junonia coenia x nigrosuffu	USA: Arizona, Gila County, Rose Creek, Sierra Ancha	33.8548 -110.983996 WGS84	11 May 1969	Roman S. Wielgus & Dale Wielgus	Restriction Digest	B/CA
AZN23	McGuire Center for Lepidoptera and Biodiversity		Junonia nigrosuffusa	USA: Arizona, Graham County, B.S. Gap, San Carlos I.R.		15-Jul-83	R.A. Bailowitz	Restriction Digest	B
AZ11	University of New Mexico		Junonia grisea (was NMC1	USA: Arizona, Greenlee Co, Big Lue Mountains, 7mi W 4.5mi SW, Mule Creek		18-May-95	J.P. Hubbard	Restriction Digest	B
AZ12	University of New Mexico		Junonia coenia (was NMC1	USA: Arizona, Greenlee Co, Big Lue Mountains, 7mi W 4.5mi SW, Mule Creek		18-May-95	J.P. Hubbard	Restriction Digest	B
AZ4	Marcus Lab		Junonia coenia x nigrosuffu	USA: Arizona, Greenlee County, 191 @ Cambell Blue River		21-Jul-05	Mark Deering	Restriction Digest	B
AZ3	Marcus Lab		Junonia coenia x grisea	USA: Arizona, Greenlee County, 191 @ Cambell Blue River		21-Jul-05	Mark Deering	Restriction Digest	B/CA
AZC6	Arizona State University	ASUHIC0003783	Junonia coenia	USA: Arizona, Maricopa County, Phoenix	33.44833 -112.07333 WGS84	03-Jun-74	Roman S. Wielgus	Restriction Digest	B
AZ6	Rutowski Lab		Junonia grisea	USA: Arizona, Maricopa County, Tempe		1980	Ron Rutowski	Restriction Digest	B
AZ7	Rutowski Lab		Junonia grisea	USA: Arizona, Maricopa County, Tempe		May-83	Ron Rutowski	Restriction Digest	B
AZ8	Rutowski Lab		Junonia grisea	USA: Arizona, Maricopa County, Tempe		May-83	Ron Rutowski	Restriction Digest	B/CA
AZN17	Oregon State	875896	Junonia nigrosuffusa	USA: Arizona, Pima County, Arivaca		August 31, 1981	H.E. Rice	Restriction Digest	B/CA
AZX1	Oregon State	875908	Junonia coenia x nigrosuff	USA: Arizona, Pima County, Box Canyon near NH. Forest Border (R15E T93510)		18-Oct-98		Restriction Digest	B
AZC27	McGuire Center for Lepidoptera and Biodiversity		Junonia coenia	USA: Arizona, Pima County, Elkhorn Canyon, Baboquivari mts.		04-Jul-79	R.A. Bailowitz	Restriction Digest	B
AZN24	McGuire Center for Lepidoptera and Biodiversity		Junonia nigrosuffusa	USA: Arizona, Pima County, Fionda Cyn Sta. Rita mts.		05-Sep-77	R.A. Bailowitz	Restriction Digest	B/CA
AZC28	McGuire Center for Lepidoptera and Biodiversity		Junonia coenia	USA: Arizona, Pima County, Tuscon, Tanqueverd Ranch		31-Mar-87	John J. Bowe	Restriction Digest	B/CA
AZC26	McGuire Center for Lepidoptera and Biodiversity		Junonia coenia	USA: Arizona, Santa Cruz County San Rafael Valley, 10.5 mi SE Patagonia		3-4 September 1983	B. O'Hara	Restriction Digest	B
AZX4	McGuire Center for Lepidoptera and Biodiversity		Junonia coenia x nigrosuff	USA: Arizona, Santa Cruz County San Rafael Valley, 10.5 mi SE Patagonia		3-4 September 1983	B. O'Hara	Restriction Digest	B
AZN20	McGuire Center for Lepidoptera and Biodiversity		Junonia nigrosuffusa	USA: Arizona, Santa Cruz County San Rafael Valley, 10.5 mi SE Patagonia		3-4 September 1983	B. O'Hara	Restriction Digest	B
AZX3	McGuire Center for Lepidoptera and Biodiversity		Junonia nigrosuffusa x gris	USA: Arizona, Santa Cruz County San Rafael Valley, 10.5 mi SE Patagonia		04-Nov-86	B. O'Hara	Restriction Digest	B/CA
AZC24	McGuire Center for Lepidoptera and Biodiversity		Junonia coenia	USA: Arizona, Santa Cruz County San Rafael Valley, 10.5 mi SE Patagonia		3-4 September 1983	B. O'Hara	Restriction Digest	B/CA
AZC25	McGuire Center for Lepidoptera and Biodiversity		Junonia coenia	USA: Arizona, Santa Cruz County San Rafael Valley, 10.5 mi SE Patagonia		3-4 September 1983	B. O'Hara	Restriction Digest	B/CA
AZ9	Marcus Lab		Junonia nigrosuffusa	USA: Arizona, Santa Cruz County, Atacosa Mountains west of Pena Blanca Lake (female)			B. Houtz	Restriction Digest	B
AZ2	Marcus Lab		Junonia nigrosuffusa	USA: Arizona, Santa Cruz County, Atacosa Mountains west of Pena Blanca Lake (female)				Restriction Digest	B/CA
AZ1	Marcus Lab		Junonia nigrosuffusa	USA: Arizona, Santa Cruz County, Atacosa Mountains west of Pena Blanca Lake (male)				Restriction Digest	B/CA
AZ10	Marcus Lab		Junonia nigrosuffusa	USA: Arizona, Santa Cruz County, Atacosa Mountains west of Pena Blanca Lake (male)			B. Houtz	Restriction Digest	B/CA
AZ5	Marcus Lab		Junonia nigrosuffusa	USA: Arizona, Santa Cruz County, Harshaw Creek Road		11-Oct-97	Mark Deering	Restriction Digest	B
AZC23	Oregon State	875905	Junonia grisea	USA: Arizona, Santa Cruz County, Nogalis		29-Apr-79	H.E. Rice	Restriction Digest	B/CA
AZN12	Oregon State	875911	Junonia nigrosuffusa	USA: Arizona, Santa Cruz County, Patagonia		May 3, 1979	H.E. Rice	Restriction Digest	B
AZN14	Oregon State	875892	Junonia nigrosuffusa (rosa	USA: Arizona, Santa Cruz County, Patagonia		September 1, 1981	H.E. Rice	Restriction Digest	B
AZN19	McGuire Center for Lepidoptera and Biodiversity		Junonia nigrosuffusa	USA: Arizona, Santa Cruz County, San Rafael Valley at Santa Cruz River		03-Sep-92	B. O'Hara	Restriction Digest	B
AZN21	McGuire Center for Lepidoptera and Biodiversity		Junonia nigrosuffusa	USA: Arizona, Santa Cruz County, San Rafael Valley at Santa Cruz River		03-Sep-92	B. O'Hara	Restriction Digest	B/CA
AZN22	McGuire Center for Lepidoptera and Biodiversity		Junonia nigrosuffusa	USA: Arizona, Santa Cruz County, San Rafael Valley at Santa Cruz River		03-Sep-92	B. O'Hara	Restriction Digest	B/CA
AZN13	Oregon State	875887	Junonia nigrosuffusa	USA: Arizona, Santa Cruz County, Sonoita Creek at Patagonia Lake (R15E T226S30)		October 21, 1998		Restriction Digest	B/CA
AZC5	Arizona State University	ASUHIC0003782	Junonia grisea	USA: Arizona, Yavapai County, 2 mi. S of Jerome	34.719876 -112.11306 WGS84	14-May-78	Roman S. Wielgus	Restriction Digest	B/CA
AZC8	Arizona State University	ASUHIC0003784	Junonia grisea	USA: Arizona, Yavapai County, 9 mi. NW of Kirkland, D.C. Ranch, found at creek	34.519932 -112.814013 WGS84	19-May-73	Roman S. Wielgus	Restriction Digest	B
AZC4	Arizona State University	ASUHIC0003785	Junonia grisea	USA: Arizona, Yavapai County, NW of Mayer, Big Bug Creek (found along Big Bug Creek)	34.323789 -112.126769 WGS84	26-May-73	J. Wielgus	Restriction Digest	B/CA
Ca92	California Academy of Science		Junonia coenia	USA: California, Alameda County, Alameda (collected dead on bluegrass lawn Nr. Brackish Lagoon, Residential District)		October 6, 1969	Jay B. Osborne	Restriction Digest	B/CA
Ca93	California Academy of Science		Junonia coenia	USA: California, Alameda County, Albany		June 29, 1959	P.A. Opler Collection	Restriction Digest	B
Ca91	California Academy of Science		Junonia coenia	USA: California, Alameda County, Oakland Canyon N. of Oakland Knoll Naval Hospital		April 27, 1969	T. W. Davies	Restriction Digest	B
Ca88	California Academy of Science		Junonia coenia	USA: California, Calaveras County, 1 mi. N. of Railroad Flat near south Fork Mokelumne River		July 25, 1969	J. R. Carr	Restriction Digest	B/CA
Ca89	California Academy of Science		Junonia coenia	USA: California, Calaveras County, 1 mi. N. of Railroad Flat near south Fork Mokelumne River		July 25, 1969	J. R. Carr	Restriction Digest	B/CA
JMM2015.1	Marcus Lab		Junonia grisea	USA: California, Contra Costra CO.,Port Richmont;Miller-knox Regional Shoreline		08-Aug-15	Jeffrey Marcus	Restriction Digest	BCA
JMM2015.2	Marcus Lab		Junonia grisea	USA: California, Contra Costra CO.,Port Richmont;Miller-knox Regional Shoreline		08-Aug-15	Jeffrey Marcus	Restriction Digest	BCA
JMM2015.3	Marcus Lab		Junonia grisea	USA: California, Contra Costra CO.,Port Richmont;Miller-knox Regional Shoreline		08-Aug-15	Jeffrey Marcus	Restriction Digest	BCA
Ca11	Marcus Lab		Junonia grisea	USA: California, Lake County, above Clear Lake		16-Sep-10	Robert Dowell	KM287939	B/CA
Ca12	Marcus Lab		Junonia grisea	USA: California, Lake County, above Clear Lake		16-Sep-10	Robert Dowell	KM287940	B/CA
Ca13	Marcus Lab		Junonia grisea	USA: California, Lake County, above Clear Lake		16-Sep-10	Robert Dowell	KM287941	B/CA
Ca14	Marcus Lab		Junonia grisea	USA: California, Lake County, above Clear Lake		16-Sep-10	Robert Dowell	KM287942	B/CA
Ca15	Marcus Lab		Junonia grisea	USA: California, Lake County, above Clear Lake		16-Sep-10	Robert Dowell	KM287943	B/CA
Ca54	McGuire Center for Lepidoptera and Biodiversity		Junonia grisea (male)	USA: California, Los Angeles County, Big Rock Creek nr. Valyermo, 4200 ft.		24-Sep-74		Restriction Digest	B/CA
Ca49	McGuire Center for Lepidoptera and Biodiversity		Junonia grisea (male)	USA: California, Los Angeles County, Burbank, 800ft		Jan-41		Restriction Digest	B/CA
Ca50	McGuire Center for Lepidoptera and Biodiversity		Junonia grisea (male)	USA: California, Los Angeles County, Burbank, 800ft		Jan-41		Restriction Digest	B/CA
Ca46	McGuire Center for Lepidoptera and Biodiversity		Junonia grisea	USA: California, Los Angeles County, Orcas Park		06-Oct-77	B O'Hara	Restriction Digest	B
Ca51	McGuire Center for Lepidoptera and Biodiversity		Junonia grisea (female)	USA: California, Los Angeles County, Ranger Station, Big Pines, 6200ft.		06-Jul-75		Restriction Digest	B/CA
Ca52	McGuire Center for Lepidoptera and Biodiversity		Junonia grisea (male)	USA: California, Los Angeles County, Ranger Station, Big Pines, 6200ft.		06-Jul-75		Restriction Digest	B/CA
Ca104	Illinois Natural History Survey	640,071	Junonia grisea	USA: California, Los Angeles County, Santa Monica		Mar 20, 1879		Restriction Digest	B
Ca53	McGuire Center for Lepidoptera and Biodiversity		Junonia grisea (male)	USA: California, Los Angeles County, Sierra Pelona Rd., 3000 ft.		24-Jun-67		Restriction Digest	B/CA
Ca47	McGuire Center for Lepidoptera and Biodiversity		Junonia grisea	USA: California, Los Angeles County, Woodland hills		10-Feb-70	Joseph Cicero	Restriction Digest	B/CA
Ca48	McGuire Center for Lepidoptera and Biodiversity		Junonia grisea	USA: California, Los Angeles County, Woodland hills		10-Feb-70	Joseph Cicero	Restriction Digest	B/CA
Ca81	California Academy of Science		Junonia grisea	USA: California, Marin County, Hwy.1 19 mi. NE Muir Beach		April 3, 1963	P.H. Arnaud, Jr.	Restriction Digest	B
Ca77	California Academy of Science		Junonia grisea	USA: California, Marin County, Mill Valley		August 30, 1925		Restriction Digest	B
Ca80	California Academy of Science		Junonia coenia x grisea	USA: California, Marin County, Mill Valley		September 20, 1965	P.H. Arnaud, Jr.	Restriction Digest	B
Ca82	California Academy of Science		Junonia grisea	USA: California, Marin County, Mill Valley		September 23, 1965	P.H. Arnaud, Jr.	Restriction Digest	B
Ca83	California Academy of Science		Junonia grisea	USA: California, Marin County, Mill Valley		September 23, 1965	P.H. Arnaud, Jr.	Restriction Digest	B
Ca84	California Academy of Science		Junonia grisea	USA: California, Marin County, Mill Valley		September 23, 1965	P.H. Arnaud, Jr.	Restriction Digest	B
Ca85	California Academy of Science		Junonia grisea	USA: California, Marin County, Mill Valley		September 16, 1965	P.H. Arnaud, Jr.	Restriction Digest	B/CA
CA35	Michigan State University		Junonia grisea x coenia	USA: California, Mendocino County, Calpella		December 24, 1962	R. W. Mathews	Restriction Digest	B/CA
CA38	Michigan State University		Junonia grisea	USA: California, Mendocino County, Covelo		September 9, 1980	R.L. Fischer	Restriction Digest	B/CA
CA43	Michigan State University		Junonia grisea	USA: California, Mendocino County, Covelo		September 9, 1980	R.L. Fischer	Restriction Digest	B/CA
CA44	Michigan State University		Junonia grisea	USA: California, Mendocino County, Covelo		September 9, 1980	R.L. Fischer	Restriction Digest	B/CA
CA39	Michigan State University		Junonia grisea	USA: California, Monterey County, nr. Tassajara Hot springs		June 12, 1957	M.J. McKenney	Restriction Digest	B
Ca87	California Academy of Science		Junonia grisea	USA: California, Monterey County, Partington Canyon at Highway 1		July 8, 1963	P.H. Arnaud, Jr.	Restriction Digest	B/CA
CA41	Michigan State University		Junonia grisea	USA: California, Napa County, Calistoga, Franz Valley		September 28, 1971	L.C. Clarence	Restriction Digest	B
CA40	Michigan State University		Junonia grisea	USA: California, Napa County, Oakville		May 18, 1968	G. Belyea	Restriction Digest	B/CA
CA37	Michigan State University		Junonia grisea (rosa)	USA: California, Napa County, Riffts Canyon nr. Pope Valley		March 10, 1983	A.H. Porter	Restriction Digest	B/CA
CA42	Michigan State University		Junonia grisea (rosa)	USA: California, Napa County, Riffts Canyon nr. Pope Valley		March 10, 1983	A.H. Porter	Restriction Digest	B/CA
AMS2015.39	Shapiro Collection		Junonia grisea	USA: California, Nevada CO.,Long Crossing;South Yuba River;Solera west Slope; 5000 ¡elevation		24-May-15	Arthur M. Shapiro	Restriction Digest	BCA
Ca1	Marcus Lab		Junonia grisea	USA: California, Placer County, West of Nyack exit I-80		25-Aug-10	Robert Dowell	KM287929	B/CA
Ca2	Marcus Lab		Junonia grisea	USA: California, Placer County, West of Nyack exit I-81		25-Aug-10	Robert Dowell	KM287930	B/CA
Ca3	Marcus Lab		Junonia grisea	USA: California, Placer County, West of Nyack exit I-82		25-Aug-10	Robert Dowell	KM287931	B/CA

Appendix III. *Junonia* specimens for the American Southwest and Mexico data set

Specimen Identifier	Collection	Museum Accession Number	Species	Locality	GPS Coordinates	Collection Date	Collector	Genotyping Method	Haplotype Group
Ca4	Marcus Lab		Junonia grisea	USA: California, Placer County, West of Nyack exit I-83		25-Aug-10	Robert Dowell	KM287932	B/CA
Ca5	Marcus Lab		Junonia grisea	USA: California, Placer County, West of Nyack exit I-84		25-Aug-10	Robert Dowell	KM287933	B/CA
Ca76	California Academy of Science		Junonia grisea	USA: California, Riverside County, Corona		March 22, 1931	Robert T. Reeves Collection	Restriction Digest	B/CA
Ca28	Arizona State University	ASUHIC0003794	Junonia grisea	USA: California, Riverside County, Riverside (found on flowers of Heteromeles arbutifolia (Lindl.) M. Roem. (toyon) [USDA])	33.95333 -117.39528 WGS84	25-Jun-60	Carol Chaney	Restriction Digest	B/CA
Ca24	Arizona State University	ASUHIC0003795	Junonia coenia	USA: California, Riverside County, Riverside, found on flowers of Heteromeles arbutifolia (Lindl.) M. Roem. (toyon) [USDA]	33.95333 -117.39528 WGS84	25-Jun-60	Carol Chaney	Restriction Digest	B/CA
Ca10	Marcus Lab		Junonia grisea	USA: California, Sacramento County, San Juan Rd. west of junction El Centro Rd.		26-Aug-10	Robert Dowell	KM287938	B/CA
Ca6	Marcus Lab		Junonia grisea	USA: California, Sacramento County, San Juan Rd. west of junction El Centro Rd.		26-Aug-10	Robert Dowell	KM287934	B/CA
Ca7	Marcus Lab		Junonia grisea	USA: California, Sacramento County, San Juan Rd. west of junction El Centro Rd.		26-Aug-10	Robert Dowell	KM287935	B/CA
Ca8	Marcus Lab		Junonia grisea	USA: California, Sacramento County, San Juan Rd. west of junction El Centro Rd.		26-Aug-10	Robert Dowell	KM287936	B/CA
Ca9	Marcus Lab		Junonia grisea	USA: California, Sacramento County, San Juan Rd. west of junction El Centro Rd.		26-Aug-10	Robert Dowell	KM287937	B/CA
CA36	Michigan State University		Junonia grisea	USA: California, San Bernardina County, Forhia Big Bear		November 30, 1958	Newman??	Restriction Digest	B/CA
Ca79	California Academy of Science		Junonia grisea	USA: California, San Bernardino County, San Antonia Canyon, 3000 ft		October 1, 1931	T. Craig collection	Restriction Digest	B/CA
Ca95	California Academy of Science		Junonia coenia	USA: California, San Francisco County, San Francisco		June 10, 1913		Restriction Digest	B/CA
Ca21	Arizona State University	ASUHIC0003763	Junonia coenia	USA: California, San Mateo County, Alameda Road and Crystal Springs Road, San Mateo	37.555508 -122.336313 WGS84	10-Aug-65	Roman S. Wielgus	Restriction Digest	B
Ca19	Arizona State University	ASUHIC0003770	Junonia grisea	USA: California, San Mateo County, Parrott Drive, S of Borel, San Mateo	37.528009 -122.335624 WGS84	03-Oct-65	Roman S. Wielgus	Restriction Digest	B
Ca32	Arizona State University	ASUHIC0003788	Junonia grisea	USA: California, San Mateo County, Parrott Drive, S of Borel, San Mateo	37.528009 -122.335624 WGS84	03-Oct-65	Roman S. Wielgus	Restriction Digest	B/CA
Ca27	Arizona State University	ASUHIC0003758	Junonia grisea	USA: California, San Mateo County, Parrott Drive, S of Borel, San Mateo (found in vacant lot)	37.528009 -122.335624 WGS84	18-Sep-65	Roman S. Wielgus	Restriction Digest	B/CA
Ca22	Arizona State University	ASUHIC0003765	Junonia grisea	USA: California, San Mateo County, Parrott Drive, S of Borel, San Mateo (found in vacant lot)	37.528009 -122.335624 WGS84	03-Oct-65	Roman S. Wielgus	Restriction Digest	B/CA
Ca31	Arizona State University	ASUHIC0003759	Junonia grisea	USA: California, San Mateo County, Parrott Drive, S of Borel, San Mateo (found in vacant lot)	37.528009 -122.335624 WGS84	03-Oct-65	Roman S. Wielgus	Restriction Digest	B/CA
Ca18	Arizona State University	ASUHIC0003757	Junonia grisea	USA: California, San Mateo County, San Mateo	37.56444 -122.35056 WGS84	02-Oct-65	Roman S. Wielgus	Restriction Digest	B
Ca25	Arizona State University	ASUHIC0003772	Junonia coenia x grisea	USA: California, San Mateo County, San Mateo	37.56444 -122.35056 WGS84	16-Oct-65	Roman S. Wielgus	Restriction Digest	B
Ca20	Arizona State University	ASUHIC0003769	Junonia grisea	USA: California, San Mateo County, San Mateo	37.56444 -122.35056 WGS84	16-Oct-65	Dale Wielgus	Restriction Digest	B
Ca30	Arizona State University	ASUHIC0003761	Junonia grisea	USA: California, San Mateo County, San Mateo	37.56444 -122.35056 WGS84	16-Oct-65	Roman S. Wielgus	Restriction Digest	B
Ca33	Arizona State University	ASUHIC0003767	Junonia coenia x grisea	USA: California, San Mateo County, San Mateo	37.56444 -122.35056 WGS84	18-Sep-65	Roman S. Wielgus	Restriction Digest	B/CA
Ca23	Arizona State University	ASUHIC0003768	Junonia grisea	USA: California, San Mateo County, San Mateo	37.56444 -122.35056 WGS84	18-Sep-65	Roman S. Wielgus	Restriction Digest	B/CA
Ca26	Arizona State University	ASUHIC0003764	Junonia coenia	USA: California, San Mateo County, San Mateo	37.56444 -122.35056 WGS84	16-Oct-65	Roman S. Wielgus	Restriction Digest	B/CA
Ca29	Arizona State University	ASUHIC0003766	Junonia grisea	USA: California, San Mateo County, San Mateo	37.56444 -122.35056 WGS84	12-Nov-65	Dale Wielgus	Restriction Digest	B/CA
Ca100	Illinois Natural History Survey	640, 065	Junonia grisea	USA: California, San Mateo County, San Mateo Hills		July 8, 1952	D.L. Baber	Restriction Digest	B/CA
Ca103	Illinois Natural History Survey	640,067	Junonia grisea	USA: California, San Mateo County, Spring Valley Lakes		February 4, 1954	D.L. Baber	Restriction Digest	B
Ca101	Illinois Natural History Survey	640,066	Junonia grisea	USA: California, San Mateo County, Spring Valley Lakes		February 9, 1954	D.L. Baber	Restriction Digest	B
Ca102	Illinois Natural History Survey	640,068	Junonia grisea	USA: California, San Mateo County, Spring Valley Lakes		February 9, 1954	D.L. Baber	Restriction Digest	B
CA65	California Academy of Science		Junonia grisea	USA: California, Sandiego County		01-Jun-54	F.X. Williams	Restriction Digest	B/CA
CA67	California Academy of Science		Junonia grisea	USA: California, Sandiego County, Me. La Mesa		01-Jun-54	F.X. Williams	Restriction Digest	B/CA
CA66	California Academy of Science		Junonia grisea (male)	USA: California, Sandiego County, Reservoir SE of Chula Vista		18-Mar-51		Restriction Digest	B/CA
CA56	California Academy of Science		Junonia grisea	USA: California, Sandiego County, Sandiego		30-Aug-46	L.D. Coy	Restriction Digest	B
CA57	California Academy of Science		Junonia grisea	USA: California, Sandiego County, Sandiego		30-Aug-46	L.D. Coy	Restriction Digest	B
CA61	California Academy of Science		Junonia grisea	USA: California, Sandiego County, Sandiego		30-Aug-46	L.D. Coy	Restriction Digest	B
CA62	California Academy of Science		Junonia grisea	USA: California, Sandiego County, Sandiego		30-Aug-46	L.D. Coy	Restriction Digest	B
CA64	California Academy of Science		Junonia grisea (female)	USA: California, Sandiego County, Sandiego		20-Oct-29	L.D. Coy	Restriction Digest	B/CA
CA55	California Academy of Science		Junonia grisea	USA: California, Sandiego County, Sandiego		30-Aug-46	L.D. Coy	Restriction Digest	B/CA
CA58	California Academy of Science		Junonia grisea	USA: California, Sandiego County, Sandiego		30-Aug-46	L.D. Coy	Restriction Digest	B/CA
CA59	California Academy of Science		Junonia grisea	USA: California, Sandiego County, Sandiego		30-Aug-46	L.D. Coy	Restriction Digest	B/CA
CA60	California Academy of Science		Junonia grisea	USA: California, Sandiego County, Sandiego		30-Aug-46	L.D. Coy	Restriction Digest	B/CA
CA63	California Academy of Science		Junonia grisea	USA: California, Sandiego County, Sandiego		30-Aug-46	L.D. Coy	Restriction Digest	B/CA
CA34	Michigan State University		Junonia grisea	USA: California, Sandiego County, Sandiego, Anza Borrego State Park		November 3, 1984	M. Arduser	Restriction Digest	B
CIAD10 B03	Pfeiler Lab		Junonia grisea	USA: California, Santa Barbara Co,, Santa Barbara (130 m)		19-Jun-10	E. Pfeiler	JQ430685	B/CA
CIAD10 B04	Pfeiler Lab		Junonia grisea	USA: California, Santa Barbara Co,, Santa Barbara (130 m)		19-Jun-10	E. Pfeiler	JQ430686	B/CA
CIAD10 B05	Pfeiler Lab		Junonia grisea	USA: California, Santa Barbara Co,, Santa Barbara (130 m)		19-Jun-10	E. Pfeiler	JQ430687	B/CA
CIAD10 B06	Pfeiler Lab		Junonia grisea	USA: California, Santa Barbara Co,, Santa Barbara (130 m)		19-Jun-10	E. Pfeiler	JQ430688	B/CA
CIAD10 B07	Pfeiler Lab		Junonia grisea	USA: California, Santa Barbara Co,, Santa Barbara (130 m)		19-Jun-10	E. Pfeiler	JQ430689	B/CA
CIAD10 B08	Pfeiler Lab		Junonia grisea	USA: California, Santa Barbara Co,, Santa Barbara (130 m)		19-Jun-10	E. Pfeiler	JQ430690	B/CA
CIAD10 B09	Pfeiler Lab		Junonia grisea	USA: California, Santa Barbara Co,, Santa Barbara (130 m)		19-Jun-10	E. Pfeiler	JQ430691	B/CA
Ca69	California Academy of Science		Junonia grisea	USA: California, Siskiyou County, Lower McCloud River		August 29, 1925	T. Craig collection	Restriction Digest	B
Ca73	California Academy of Science		Junonia grisea	USA: California, Siskiyou County, Lower McCloud River		August 29, 1925	T. Craig collection	Restriction Digest	B
Ca68	California Academy of Science		Junonia grisea	USA: California, Siskiyou County, Lower McCloud River		August 29, 1925	T. Craig collection	Restriction Digest	B/CA
Ca70	California Academy of Science		Junonia grisea	USA: California, Siskiyou County, Lower McCloud River		August 29, 1925	T. Craig collection	Restriction Digest	B/CA
Ca71	California Academy of Science		Junonia grisea	USA: California, Siskiyou County, Lower McCloud River		August 29, 1925	T. Craig collection	Restriction Digest	B/CA
Ca72	California Academy of Science		Junonia grisea	USA: California, Siskiyou County, Lower McCloud River		August 29, 1925	T. Craig collection	Restriction Digest	B/CA
Ca74	California Academy of Science		Junonia grisea	USA: California, Siskiyou County, Lower McCloud River		August 29, 1925	T. Craig collection	Restriction Digest	B/CA
Ca75	California Academy of Science		Junonia grisea	USA: California, Siskiyou County, Lower McCloud River		August 29, 1925	T. Craig collection	Restriction Digest	B/CA
AMS2015.1	Shapiro Collection		Junonia grisea	USA: California, Solano Co., Gates Canyon;1.6 miles NW of Vocaville;350 ¡ elevation.		23-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.10	Shapiro Collection		Junonia grisea	USA: California, Solano Co., Gates Canyon;1.6 miles NW of Vocaville;350 ¡ elevation.		23-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.2	Shapiro Collection		Junonia grisea	USA: California, Solano Co., Gates Canyon;1.6 miles NW of Vocaville;350 ¡ elevation.		23-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.3	Shapiro Collection		Junonia grisea	USA: California, Solano Co., Gates Canyon;1.6 miles NW of Vocaville;350 ¡ elevation.		23-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.4	Shapiro Collection		Junonia grisea	USA: California, Solano Co., Gates Canyon;1.6 miles NW of Vocaville;350 ¡ elevation.		23-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.5	Shapiro Collection		Junonia grisea	USA: California, Solano Co., Gates Canyon;1.6 miles NW of Vocaville;350 ¡ elevation.		23-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.6	Shapiro Collection		Junonia grisea	USA: California, Solano Co., Gates Canyon;1.6 miles NW of Vocaville;350 ¡ elevation.		23-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.7	Shapiro Collection		Junonia grisea	USA: California, Solano Co., Gates Canyon;1.6 miles NW of Vocaville;350 ¡ elevation.		23-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.8	Shapiro Collection		Junonia grisea	USA: California, Solano Co., Gates Canyon;1.6 miles NW of Vocaville;350 ¡ elevation.		23-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.9	Shapiro Collection		Junonia grisea	USA: California, Solano Co., Gates Canyon;1.6 miles NW of Vocaville;350 ¡ elevation.		23-May-15	Arthur M. Shapiro	Restriction Digest	BCA
Ca96	California Academy of Science		Junonia coenia	USA: California, Sonoma County, Forestville		October 18, 1961	R.M. Brown Collection	Restriction Digest	B/CA
Ca97	California Academy of Science		Junonia coenia	USA: California, Sonoma County, Forestville		October 18, 1961	R.M. Brown Collection	Restriction Digest	B/CA
Ca98	California Academy of Science		Junonia coenia	USA: California, Sonoma County, Forestville		October 18, 1961	R.M. Brown Collection	Restriction Digest	B/CA
Ca99	California Academy of Science		Junonia coenia	USA: California, Sonoma County, Forestville		October 18, 1961	R.M. Brown Collection	Restriction Digest	B/CA
Ca94	California Academy of Science		Junonia coenia	USA: California, Stanislaus County, Del Puerto Canyon Frank Raines Par, 1100'		July 5, 1971	P.H. and M. Aranaud	Restriction Digest	B
Ca90	California Academy of Science		Junonia coenia	USA: California, Trinity County, Weaverville		May 30, 1931	L.I. Hewes Collection	Restriction Digest	B/CA
Ca78	California Academy of Science		Junonia grisea	USA: California, Tulare County, Mineral King, 10,500'		July 30, 1935	G. Heid	Restriction Digest	B
Ca86	California Academy of Science		Junonia coenia x grisea	USA: California, Tuolume County, Basin Cr. Camp Ground		May 31, 1963	P.H. Arnaud, Jr.	Restriction Digest	B/CA
CA45	Michigan State University		Junonia grisea	USA: California, Ventura County, Foster Park		December 1, 1959	J. E. Bath	Restriction Digest	B
Ca16	University of New Mexico		Junonia grisea	USA: California, Ventura County, Foster Park, Vetum River bed between Hwy 33 and Santa Ann Rd		19-Aug-89	M.M. Fuller	Restriction Digest	B
Ca17	University of New Mexico		Junonia grisea	USA: California, Ventura County, Foster Park, Vetum River bed between Hwy 33 and Santa Ann Rd		19-Aug-89	M.M. Fuller	Restriction Digest	B/CA
AMS2015.27	Shapiro Collection		Junonia grisea	USA: California,Sacramento CO.,North Sacramento;American River Floodplain; 35 ¡elevation		26-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.28	Shapiro Collection		Junonia grisea	USA: California,Sacramento CO.,North Sacramento;American River Floodplain; 35 ¡elevation		26-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.29	Shapiro Collection		Junonia grisea	USA: California,Sacramento CO.,North Sacramento;American River Floodplain; 35 ¡elevation		26-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.30	Shapiro Collection		Junonia grisea	USA: California,Sacramento CO.,North Sacramento;American River Floodplain; 35 ¡elevation		26-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.31	Shapiro Collection		Junonia grisea	USA: California,Sacramento CO.,North Sacramento;American River Floodplain; 35 ¡elevation		26-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.32	Shapiro Collection		Junonia grisea	USA: California,Sacramento CO.,North Sacramento;American River Floodplain; 35 ¡elevation		26-May-15	Arthur M. Shapiro	Restriction Digest	BCA

Appendix III. *Junonia* specimens for the American Southwest and Mexico data set

Specimen Identifier	Collection	Museum Accession Number	Species	Locality	GPS Coordinates	Collection Date	Collector	Genotyping Method	Haplotype Group
AMS2015.33	Shapiro Collection		Junonia grisea	USA: California,Sacramento CO.,North Sacramento;American River Floodplain; 35 ¡elevation		26-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.34	Shapiro Collection		Junonia grisea	USA: California,Sacramento CO.,North Sacramento;American River Floodplain; 35 ¡elevation		26-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.35	Shapiro Collection		Junonia grisea	USA: California,Sacramento CO.,North Sacramento;American River Floodplain; 35 ¡elevation		26-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.36	Shapiro Collection		Junonia grisea	USA: California,Sacramento CO.,North Sacramento;American River Floodplain; 35 ¡elevation		26-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.37	Shapiro Collection		Junonia grisea	USA: California,Sacramento CO.,North Sacramento;American River Floodplain; 35 ¡elevation		26-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.38	Shapiro Collection		Junonia grisea	USA: California,Sacramento CO.,North Sacramento;American River Floodplain; 35 ¡elevation		26-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.11	Shapiro Collection		Junonia grisea	USA: California,Yolo CO., West Sacramento; Midway between Davis and Sacranento;35 ¡ elevation		25-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.12	Shapiro Collection		Junonia grisea	USA: California,Yolo CO., West Sacramento; Midway between Davis and Sacranento;35 ¡ elevation		25-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.13	Shapiro Collection		Junonia grisea	USA: California,Yolo CO., West Sacramento; Midway between Davis and Sacranento;35 ¡ elevation		25-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.14	Shapiro Collection		Junonia grisea	USA: California,Yolo CO., West Sacramento; Midway between Davis and Sacranento;35 ¡ elevation		25-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.15	Shapiro Collection		Junonia grisea	USA: California,Yolo CO., West Sacramento; Midway between Davis and Sacranento;35 ¡ elevation		25-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.16	Shapiro Collection		Junonia grisea	USA: California,Yolo CO., West Sacramento; Midway between Davis and Sacranento;35 ¡ elevation		25-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.17	Shapiro Collection		Junonia grisea	USA: California,Yolo CO., West Sacramento; Midway between Davis and Sacranento;35 ¡ elevation		25-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.18	Shapiro Collection		Junonia grisea	USA: California,Yolo CO., West Sacramento; Midway between Davis and Sacranento;35 ¡ elevation		25-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.19	Shapiro Collection		Junonia grisea	USA: California,Yolo CO., West Sacramento; Midway between Davis and Sacranento;35 ¡ elevation		25-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.20	Shapiro Collection		Junonia grisea	USA: California,Yolo CO., West Sacramento; Midway between Davis and Sacranento;35 ¡ elevation		25-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.21	Shapiro Collection		Junonia grisea	USA: California,Yolo CO., West Sacramento; Midway between Davis and Sacranento;35 ¡ elevation		25-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.22	Shapiro Collection		Junonia grisea	USA: California,Yolo CO., West Sacramento; Midway between Davis and Sacranento;35 ¡ elevation		25-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.23	Shapiro Collection		Junonia grisea	USA: California,Yolo CO., West Sacramento; Midway between Davis and Sacranento;35 ¡ elevation		25-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.24	Shapiro Collection		Junonia grisea	USA: California,Yolo CO., West Sacramento; Midway between Davis and Sacranento;35 ¡ elevation		25-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.25	Shapiro Collection		Junonia grisea	USA: California,Yolo CO., West Sacramento; Midway between Davis and Sacranento;35 ¡ elevation		25-May-15	Arthur M. Shapiro	Restriction Digest	BCA
AMS2015.26	Shapiro Collection		Junonia grisea	USA: California,Yolo CO., West Sacramento; Midway between Davis and Sacranento;35 ¡ elevation		25-May-15	Arthur M. Shapiro	Restriction Digest	BCA
CO3	Colorado State University		Junonia coenia	USA: Colorado, Baca County, Two Butes Dam		September 9, 1990		Restriction Digest	B
CO4	Colorado State University		Junonia coenia	USA: Colorado, Baca County, Two Butes Dam		September 9, 1990		Restriction Digest	B
CO7	Colorado State University		Junonia coenia	USA: Colorado, Jefferson County, Platte Cu. (5650')		October 4, 1992	RES	Restriction Digest	B/CA
CO5	Colorado State University		Junonia coenia x nigrosuffu	USA: Colorado, Kiowa County, Nee Sopah Rsvr.		September 22, 1984	P.A. Opler	Restriction Digest	B
CO1	Colorado State University		Junonia coenia	USA: Colorado, Kiowa County, Queens Lake, SWA		October 4, 1987	P.A. Opler	Restriction Digest	B
CO2	Colorado State University		Junonia coenia	USA: Colorado, Otero County, Rockey Road, SWA		October 3, 1987	P.A. Opler	Restriction Digest	B
CO6	Colorado State University		Junonia coenia	USA: Colorado, Yuma County, Bonny Recr. Area		July 30, 1972	R.E. Stanford	Restriction Digest	B/CA
NEV1	Yale Peabody	YPM ENT 415364	Junonia grisea	USA: Nevada, Clark Co.,Kyle Canyon, Mt. Charleston. Eleve2286	(Lat-Lon) 36.29472 -115.44444	14-Jun-65	T.W. Davies & W.T. Davies	Restriction Digest	B/CA
NMN18	Colorado State University		Junonia nigrosuffusa	USA: New Mexico, Catron C., 6.4 mi. N of NM12 on USFS 11 (Alamocito Can.) Sec. 31, T.3S.R13W, SE slope Mangas Mts.		April 25, 1995	RWH	Restriction Digest	B
NMN26	Colorado State University		Junonia nigrosuffusa	USA: New Mexico, Catron Co., 3 mi. N. of Apache cr., SW slope, Gallo Mts.		June 27, 1995	RWH	Restriction Digest	B
NMX8	Colorado State University		Junonia nigrosuffusa x coe	USA: New Mexico, Catron Co., Apache Cr., NM 12 at NM 32, SW slope Gallo Mts.		July 23, 1994	RWH & ESC	Restriction Digest	B/CA
NMN30	Colorado State University		Junonia nigrosuffusa	USA: New Mexico, Catron Co., Hardcastle Creek at USFS 23 nr. Fitzgerald Cienega, S. slope Gallo Mts.		July 27, 1994	RWH & ESC	Restriction Digest	B
NMN2	University of New Mexico		Junonia nigrosuffusa	USA: New Mexico, Catron Co., Pleasanton		30-Apr-95	J.P. Hubbard	Restriction Digest	B
NMN4	University of New Mexico		Junonia nigrosuffusa	USA: New Mexico, Catron Co., Pleasanton		30-Apr-95	J.P. Hubbard	Restriction Digest	B
NMN1	University of New Mexico		Junonia nigrosuffusa	USA: New Mexico, Catron Co., Pleasanton		23-May-95	J.P. Hubbard	Restriction Digest	B
NMC6	University of New Mexico		Junonia coenia	USA: New Mexico, Catron Co., Pleasanton		25-May-95	J.P. Hubbard	Restriction Digest	B
NMN6	University of New Mexico		Junonia nigrosuffusa	USA: New Mexico, Catron Co., Pleasanton		25-May-95	J.P. Hubbard	Restriction Digest	B
NMN7	University of New Mexico		Junonia nigrosuffusa	USA: New Mexico, Catron Co., Pleasanton		25-May-95	J.P. Hubbard	Restriction Digest	B
NMN8	University of New Mexico		Junonia nigrosuffusa	USA: New Mexico, Catron Co., Pleasanton		26-May-95	J.P. Hubbard	Restriction Digest	B
NMN9	University of New Mexico		Junonia nigrosuffusa	USA: New Mexico, Catron Co., Pleasanton		28-May-95	J.P. Hubbard	Restriction Digest	B
NMC2	University of New Mexico		Junonia grisea	USA: New Mexico, Catron Co., Pleasanton		22-Jun-95	J.P. Hubbard	Restriction Digest	B/CA
NMC4	University of New Mexico		Junonia coenia x grisea	USA: New Mexico, Catron Co., Pleasanton		03-Nov-95	J.P. Hubbard	Restriction Digest	B/CA
NMC5	University of New Mexico		Junonia grisea	USA: New Mexico, Catron Co., Pleasanton		14-Nov-95	J.P. Hubbard	Restriction Digest	B/CA
NMN17	Colorado State University		Junonia nigrosuffusa	USA: New Mexico, Catron Co., Porcupine Spr. Nr. Quenado Lake, Mangas/Gallo Mts.		May 13, 1995	RWH & SJC	Restriction Digest	B
NMN33	Colorado State University		Junonia nigrosuffusa	USA: New Mexico, Catron Co., S. fork of Wall Lake, nr. Beaverhead		August 9, 1993	RWH	Restriction Digest	B
NMN28	Colorado State University		Junonia nigrosuffusa	USA: New Mexico, Catron Co., Wall Lake, S of Beaverhead W. slope, Black Range		August 7, 1993	RWH & ESC	Restriction Digest	B
NMN29	Colorado State University		Junonia nigrosuffusa x gris	USA: New Mexico, Catron Co., Wall Lake, S of Beaverhead W. slope, Black Range		August 7, 1993	RWH & ESC	Restriction Digest	B/CA
NMC22	Colorado State University		Junonia coenia	USA: New Mexico, Colfax Co., 14 mi W of T-25, SW of Raton		October 4, 1997	RWH	Restriction Digest	B
NMC21	Colorado State University		Junonia coenia	USA: New Mexico, Colfax Co., 2 mi. E Trinchera Pass, N. slope Johnson Mesa		August 3, 1997	RWH	Restriction Digest	B
NMN12	Colorado State University		Junonia nigrosuffusa	USA: New Mexico, Dona Ana Co., Finley Can., S. Slope Organ Mts.		May 5, 1979	RWH	Restriction Digest	B
NMX5	Colorado State University		Junonia grisea	USA: New Mexico, Eddy Co., Black River nr. Rattlesnake Spr., E. Slope Guadalupe Mts.		July 14, 1986	RWH	Restriction Digest	B/CA
NMN23	Colorado State University		Junonia nigrosuffusa	USA: New Mexico, Eddy Co., Rattlesnake Spr., Carlsbad Caverns N.P NM, E. slope Guadalupe Mts.		July 26, 1986	RWH & SJC	Restriction Digest	B/CA
NMN21	Colorado State University		Junonia nigrosuffusa	USA: New Mexico, Eddy Co., Rattlesnake Spr., Carlsbad Caverns NP		August 31, 1986	RWH	Restriction Digest	B
NMN35	Colorado State University		Junonia nigrosuffusa	USA: New Mexico, Eddy Co., Rattlesnake Spr., Carlsbad Caverns NP		August 31, 1986	RWH	Restriction Digest	B
NMN22	Colorado State University		Junonia nigrosuffusa	USA: New Mexico, Eddy Co., Rattlesnake Spr., Carlsbad Caverns NP		August 9, 1986	RWH	Restriction Digest	B/CA
NMX7	Colorado State University		Junonia nigrosuffusa x coe	USA: New Mexico, Eddy Co., Rattlesnake Spr., Carlsbad Caverns NP		August 31, 1986	RWH	Restriction Digest	B/CA
NMC1	University of New Mexico		Junonia coenia	USA: New Mexico, Grant Co. Big Burro Mountains, Thompson Canyon		19-Oct-96	J.P. Hubbard	Restriction Digest	B
NMX3	University of New Mexico		Junonia coenia x nigrosuff	USA: New Mexico, Grant Co. Big Burro Mountains, Thompson Canyon		19-Oct-96	J.P. Hubbard and E.C. Espinoza	Restriction Digest	B
NMX2	University of New Mexico		Junonia nigrosuffusa	USA: New Mexico, Grant Co. Big Burro Mountains, Thompson Canyon		19-Oct-96	J.P. Hubbard and E.C. Espinoza	Restriction Digest	B/CA
NMN3	University of New Mexico		Junonia nigrosuffusa	USA: New Mexico, Grant Co., Big Burro Mountains, Gold Gulch, T20SR16W Sec 17		24-Apr-95	J.P. Hubbard	Restriction Digest	B/CA
NMC7	University of New Mexico		Junonia coenia	USA: New Mexico, Grant Co., Big Lue Mountains, Pine Cienaga area		27-Apr-95	J.P. Hubbard	Restriction Digest	B
NMC9	University of New Mexico		Junonia coenia	USA: New Mexico, Grant Co., Big Lue Mountains, Pine Cienaga area		27-Apr-95	J.P. Hubbard	Restriction Digest	B
NMC8	University of New Mexico		Junonia grisea	USA: New Mexico, Grant Co., Big Lue Mountains, Pine Cienaga area		27-Apr-95	J.P. Hubbard	Restriction Digest	B
NMC3	University of New Mexico		Junonia grisea x nigrosuffu	USA: New Mexico, Grant Co., Big Lue Mountains, Pine Cienaga area		27-Apr-95	J.P. Hubbard	Restriction Digest	B
NMN13	Colorado State University		Junonia nigrosuffusa	USA: New Mexico, Grant Co., Mimbree River at San Lorenza		July 24, 1993	RWH	Restriction Digest	B
NMX6	Colorado State University		Junonia nigrosuffusa x coe	USA: New Mexico, Grant Co., NM 61 and Rio Mimbres at Royal John Mine Rd. nr. Sherman		June 4, 1994	RWH	Restriction Digest	B/CA
NMX4	Colorado State University		Junonia grisea	USA: New Mexico, Grant Co., Rio Mimbres at San Lorenzo		July 24, 1993	RWH	Restriction Digest	B
NMN20	Colorado State University		Junonia nigrosuffusa	USA: New Mexico, Grant Co., Upper Black Canyon CG NM 61 (Otter Loop Dr.)		June 13, 1986	RWH	Restriction Digest	B
NMN32	Colorado State University		Junonia nigrosuffusa	USA: New Mexico, Hidalgo Co., Grey Rch. La Cienega		July 20, 1991	RWH	Restriction Digest	B
NMX1	University of New Mexico		Junonia grisea x.nigrosuffu	USA: New Mexico, Hidalgo Co., Guadealope Canyon		21-Nov-95	J.P. Hubbard	Restriction Digest	B
NMN34	Colorado State University		Junonia nigrosuffusa	USA: New Mexico, Hidalgo Co., Skeleton Can. W. slope Peloncillo Mts.		August 1, 1986	SJC	Restriction Digest	B
NMN27	Colorado State University		Junonia nigrosuffusa	USA: New Mexico, Lincoln Co., 6 mi N. of Tinnie, SE slope CapitanMts.		June 4, 1995	RWH	Restriction Digest	B/CA
NMN10	Colorado State University		Junonia nigrosuffusa	USA: New Mexico, Luna Co., 1 Mi. S. Lobo Cyn., Florida Mts.		October 6, 1985	SJC	Restriction Digest	B/CA
NMN24	Colorado State University		Junonia nigrosuffusa	USA: New Mexico, Luna Co., Montezuma Can. At windmill NW slope, Cookes Peak		June 25, 1995	RWH, ESC & SJC	Restriction Digest	B
NMN25	Colorado State University		Junonia nigrosuffusa	USA: New Mexico, Luna Co., Montezuma Can. At windmill NW slope, Cookes Peak		June 25, 1995	RWH, ESC & SJC	Restriction Digest	B
NMN15	Colorado State University		Junonia nigrosuffusa	USA: New Mexico, Luna Co., N. slope Hadley Draw, Cooke Peak		April 15, 1995	RWH & SJC	Restriction Digest	B
NMN31	Colorado State University		Junonia nigrosuffusa	USA: New Mexico, Luna Co., Slate Spr., Hadley Draw NE slope, Cooke Peak		May 7, 1989	RWH	Restriction Digest	B
NMC18	Illinois Natural History Survey	584	Junonia coenia	USA: New Mexico, Otero Co., Alamogondo, T16S, RIDE (4500')		June 24 1975	Charles A. Bridges	Restriction Digest	B/CA
NMC11	Illinois Natural History Survey	579	Junonia grisea	USA: New Mexico, Otero Co., Alamogondo, Washington St. ditch (4500')		August 11, 1975	Charles A. Bridges	Restriction Digest	B
NMC17	Illinois Natural History Survey	580	Junonia coenia	USA: New Mexico, Otero Co., Alamogondo, Washington St. ditch (4500')		August 26, 1975	Charles A. Bridges	Restriction Digest	B
NMC14	Illinois Natural History Survey	576	Junonia grisea	USA: New Mexico, Otero Co., Alamogondo, Washington St. ditch (4500')		August 26, 1975	Charles A. Bridges	Restriction Digest	B
NMC13	Illinois Natural History Survey	575	Junonia coenia	USA: New Mexico, Otero Co., Alamogondo, Washington St. ditch (4500')		August 30, 1975	Charles A. Bridges	Restriction Digest	B
NMN11	Illinois Natural History Survey	582	Junonia coenia	USA: New Mexico, Otero Co., Alamogondo, Washington St. ditch (4500')		aug 30, 1975	Charles A. Bridges	Restriction Digest	B
NMC15	Illinois Natural History Survey	583	Junonia grisea	USA: New Mexico, Otero Co., Alamogondo, Washington St. ditch (4500')		August 11, 1975	Charles A. Bridges	Restriction Digest	B/CA
NMC12	Illinois Natural History Survey	581	Junonia coenia	USA: New Mexico, Otero Co., Alamogondo, Washington St. ditch (4500')		August 26, 1975	Charles A. Bridges	Restriction Digest	B/CA

Appendix III. *Junonia* specimens for the American Southwest and Mexico data set

Specimen Identifier	Collection	Museum Accession Number	Species	Locality	GPS Coordinates	Collection Date	Collector	Genotyping Method	Haplotype Group
NMN19	Colorado State University		Junonia nigrosuffusa	USA: New Mexico, Otero Co., Head Spr., Mescalero Apache Res., W. slope, Sacramento Mts.		April 15, 1995	RWH & SJC	Restriction Digest	B
NMC16	Illinois Natural History Survey	578	Junonia coenia	USA: New Mexico, Otero Co., High Rolls-Mnt-Park (92û58.0'N 105û50.0'W; 8500')		September 25, 1977	Charles A. Bridges	Restriction Digest	B
NMC28	Colorado State University		Junonia coenia	USA: New Mexico, Rio Arriba Co., San Lorenzo, Rio Del Oso NE slope Jemez Mts.		June 28, 1984	RWH	Restriction Digest	B
NMC31	Colorado State University		Junonia coenia	USA: New Mexico, San Juan Co., Palisades Cr., 1.8 mi up Whiskey Cr. From Nav12		August 19, 1995	RWH	Restriction Digest	B
NMC37	Colorado State University		Junonia grisea	USA: New Mexico, San Juan Co., Red Lake nr. Navajo, NM, Chuska Mts.		July 30, 1978	RWH	Restriction Digest	B
NMC36	Colorado State University		Junonia grisea	USA: New Mexico, San Juan Co., Red Lake nr. Navajo, NM, Chuska Mts.		August 6, 1978	RWH	Restriction Digest	B
NMC39	Colorado State University		Junonia grisea	USA: New Mexico, San Juan Co., Toadlena, Chuska Mts.		July 3, 1978	RWH	Restriction Digest	B
NMC38	Colorado State University		Junonia coenia	USA: New Mexico, San Juan Co., Toadlena, Chuska Mts.		July 22, 1978	RWH	Restriction Digest	B/CA
NMC19	Colorado State University		Junonia coenia	USA: New Mexico, San Juan Co., Whickey Cr., 1.8 mi. E. of N-12, Chuska Mts.		August 5, 1978	RWH	Restriction Digest	B
NMC20	Colorado State University		Junonia grisea	USA: New Mexico, San Juan Co., Whickey Cr., 1.8 mi. E. of N-12, Chuska Mts.		August 5, 1978	RWH	Restriction Digest	B/CA
NMC30	Colorado State University		Junonia coenia x grisea	USA: New Mexico, Sandoval Co., 3 mi. N of San Felipe Pueblo on Rio Grande		September 3, 1984	RWH	Restriction Digest	B
NMC32	Colorado State University		Junonia grisea	USA: New Mexico, Sandoval Co., Jemez Spr. S slope Jemez Mts.		August 27, 1983	RWH	Restriction Digest	B/CA
NMC33	Colorado State University		Junonia grisea	USA: New Mexico, Sandoval Co., San Pablo Can., 5 mi. W of Red Top, W slope Jemez Mts.		June 27, 1983	RWH	Restriction Digest	B
NMC35	Colorado State University		Junonia grisea	USA: New Mexico, Sandoval Co., Sec. 4, San Juan Can., S slope Jemez Mts.		May 21, 1984	RWH	Restriction Digest	B/CA
NMC40	Colorado State University		Junonia grisea	USA: New Mexico, Sandoval Co., Summit Joaquin Can., W Slope, Jemez Mts		July 11, 1984	RWH	Restriction Digest	B
NMC41	Colorado State University		Junonia grisea	USA: New Mexico, Sandoval Co., Tent Rocks, SE slope nr Cochiti Pueblo Jemez Mts.		September 17, 1983	RWH	Restriction Digest	B
NMC42	Colorado State University		Junonia grisea	USA: New Mexico, Sandoval Co., Tent Rocks, SE slope nr Cochiti Pueblo Jemez Mts.		September 17, 1983	RWH	Restriction Digest	B
NMC25	Colorado State University		Junonia coenia	USA: New Mexico, Santa Fe Co., 1 mi. W. of La Cienega in Santa Fe Can.		August 19, 1984	RWH	Restriction Digest	B
NMC26	Colorado State University		Junonia nigrosuffusa	USA: New Mexico, Santa Fe Co., 1 mi. W. of La Cienega in Santa Fe Can.		August 19, 1984	RWH	Restriction Digest	B
NMC27	Colorado State University		Junonia grisea	USA: New Mexico, Santa Fe Co., 1 mi. W. of La Cienega in Santa Fe Can.		August 19, 1984	RWH	Restriction Digest	B/CA
NMC23	Colorado State University		Junonia grisea	USA: New Mexico, Santa Fe Co., Golondrinas Museum nr. Santa Fe Downs		August 18, 1984	RWH	Restriction Digest	B
NMC29	Colorado State University		Junonia grisea	USA: New Mexico, Santa Fe Co., Golondrinas Museum nr. Santa Fe Downs		August 18, 1984	RWH	Restriction Digest	B
NMC34	Colorado State University		Junonia grisea	USA: New Mexico, Santa Fe Co., Golondrinas Museum nr. Santa Fe Downs		August 18, 1984	RWH	Restriction Digest	B
NMC24	Colorado State University		Junonia coenia	USA: New Mexico, Santa Fe Co., Golondrinas Museum nr. Santa Fe Downs		August 18, 1984	RWH	Restriction Digest	B/CA
NMN14	Colorado State University		Junonia nigrosuffusa	USA: New Mexico, Sierra Co., Percha Dam		July 31, 1986	SJC	Restriction Digest	B
NMN5	University of New Mexico		Junonia grisea	USA: New Mexico, Socorro Co. Sevilleta National Wildlife Refuge (desert grasland), N34.36447 W106.68485		28-Sep-14	D.C. Lightfoot	Restriction Digest	B/CA
NMC10	Oregon State	875902	Junonia coenia	USA: New Mexico, Socorro Co., 1.5 miles S. San Antonio State Hwy1		July 31, 1989	J. Hinchliff	Restriction Digest	B/CA
NMN16	Colorado State University		Junonia nigrosuffusa	USA: New Mexico, Taos Co., 2 mi. N Taos Plaza		August 16, 1995	RWH	Restriction Digest	B
OK2	Illinois Natural History Survey	640,070	Junonia coenia	USA: Oklahoma, Mayes County, Grand Pt. Resort		August 10, 1964	Mike Toliver	Restriction Digest	B
OK3	Illinois Natural History Survey	640,072	Junonia coenia	USA: Oklahoma, Mayes County, Grand Pt. Resort		August 11, 1964	Mike Toliver	Restriction Digest	B
OK4	Univeristy of Manitoba	356784	Junonia coenia	USA: Oklahoma, Pottawatomie Co., Wanette		27-Sep-68	W.B. Preston	Restriction Digest	B
OK5	Univeristy of Manitoba	356785	Junonia coenia	USA: Oklahoma, Pottawatomie Co., Wanette		27-Sep-68	W.B. Preston	Restriction Digest	B
OK1	Illinois Natural History Survey	640,069	Junonia coenia	USA: Oklahoma, Tulsa County, Tulsa		June 13, 1965	Mike Toliver	Restriction Digest	B
ORC1	Oregon State	875915	Junonia grisea	USA: Oregon, Harney County, Alvord Dunes (T36S R35ES9)		October 14, 1979	M.J. Smith	Restriction Digest	B/CA
ORC8	Oregon State	875910	Junonia grisea	USA: Oregon, Harney County, McCoy Creek, Steens Mt. (8000 ft.)		July 30, 1963	E.J. Dornfeld	Restriction Digest	B/CA
ORC2	Oregon State	875907	Junonia grisea	USA: Oregon, Jackson County, South side Siskyou Summit (2700ft)		May 30, 1978	J. Hinchliff	Restriction Digest	B/CA
ORC3	Oregon State	875884	Junonia grisea	USA: Oregon, Jackson County, South side Siskyou Summit (2700ft)		May 30, 1978	J. Hinchliff	Restriction Digest	B/CA
ORC5	Oregon State	875904	Junonia grisea	USA: Oregon, Jackson County, South side Siskyou Summit (2700ft)		May 30, 1978	J. Hinchliff	Restriction Digest	B/CA
ORC10	Oregon State	875893	Junonia grisea	USA: Oregon, Josephine County, vicinity O'Brien		July 2, 1977	S.G. Jewette Jr.	Restriction Digest	B/CA
ORC9	Oregon State	875899	Junonia grisea	USA: Oregon, Josephine County, Wilderville, Slate Creek		July 17, 1977		Restriction Digest	B/CA
ORC4	Oregon State	875906	Junonia grisea	USA: Oregon, Klamath County, Bly Mt., Hwy 66 (5000ft)		June 17, 1963	E.J. Dornfeld	Restriction Digest	B/CA
ORC7	Oregon State	875885	Junonia grisea	USA: Oregon, Klamath County, Bly Mt., Hwy 66 (5000ft)		June 17, 1963	E.J. Dornfeld	Restriction Digest	B/CA
ORC6	Oregon State	875913	Junonia grisea	USA: Oregon, Klamath County, Crescent Creek (4470 ft)		August 14, 1963	E.J. Dornfeld	Restriction Digest	B/CA
TXC24	Marcus Lab		Junonia coenia	USA: Texas, Aransas County, Aransas National Wildlife Refuge, Birding Trail #2		03-Dec-07	Jeffrey M. Marcus	KM287986	B
TXC25	Marcus Lab		Junonia coenia	USA: Texas, Aransas County, Aransas National Wildlife Refuge, Birding Trail #2		03-Dec-07	Jeffrey M. Marcus	KM287987	B
TXC26	Marcus Lab		Junonia coenia	USA: Texas, Aransas County, Aransas National Wildlife Refuge, Birding Trail #2		03-Dec-07	Jeffrey M. Marcus	KM287988	B
TXC27	Marcus Lab		Junonia coenia	USA: Texas, Aransas County, Aransas National Wildlife Refuge, Birding Trail #2		03-Dec-07	Jeffrey M. Marcus	KM287989	B
TXC28	Marcus Lab		Junonia coenia	USA: Texas, Aransas County, Aransas National Wildlife Refuge, Birding Trail #2		03-Dec-07	Jeffrey M. Marcus	KM287990	B
TXC29	Marcus Lab		Junonia coenia	USA: Texas, Aransas County, Aransas National Wildlife Refuge, Birding Trail #2		03-Dec-07	Jeffrey M. Marcus	KM287991	B
TXC23	Marcus Lab		Junonia coenia	USA: Texas, Aransas County, Aransas National Wildlife Refuge, Heron Flats Trail		03-Dec-07	Jeffrey M. Marcus	KM287985	B
TXC1	Marcus Lab		Junonia coenia	USA: Texas, Aransas County, Aransas National Wildlife Refuge, N. Boundary Road		14-Oct-07	Chad Stinson	KM287970	B
TXC10	Marcus Lab		Junonia coenia	USA: Texas, Aransas County, Aransas National Wildlife Refuge, N. Boundary Road		14-Oct-07	Chad Stinson	KM287971	B
TXC11	Marcus Lab		Junonia coenia	USA: Texas, Aransas County, Aransas National Wildlife Refuge, N. Boundary Road		14-Oct-07	Chad Stinson	KM287972	B
TXC12	Marcus Lab		Junonia coenia	USA: Texas, Aransas County, Aransas National Wildlife Refuge, N. Boundary Road		14-Oct-07	Chad Stinson	KM287973	B
TXC2	Marcus Lab		Junonia coenia	USA: Texas, Aransas County, Aransas National Wildlife Refuge, N. Boundary Road		14-Oct-07	Chad Stinson	KM287981	B
TXC3	Marcus Lab		Junonia coenia	USA: Texas, Aransas County, Aransas National Wildlife Refuge, N. Boundary Road		14-Oct-07	Chad Stinson	KM287992	B
TXC4	Marcus Lab		Junonia coenia	USA: Texas, Aransas County, Aransas National Wildlife Refuge, N. Boundary Road		14-Oct-07	Chad Stinson	KM288003	B
TXC5	Marcus Lab		Junonia coenia	USA: Texas, Aransas County, Aransas National Wildlife Refuge, N. Boundary Road		14-Oct-07	Chad Stinson	KM288006	B
TXC6	Marcus Lab		Junonia coenia	USA: Texas, Aransas County, Aransas National Wildlife Refuge, N. Boundary Road		14-Oct-07	Chad Stinson	KM288007	B
TXC7	Marcus Lab		Junonia coenia	USA: Texas, Aransas County, Aransas National Wildlife Refuge, N. Boundary Road		14-Oct-07	Chad Stinson	KM288008	B
TXC8	Marcus Lab		Junonia coenia	USA: Texas, Aransas County, Aransas National Wildlife Refuge, N. Boundary Road		14-Oct-07	Chad Stinson	KM288009	B
TXC9	Marcus Lab		Junonia coenia	USA: Texas, Aransas County, Aransas National Wildlife Refuge, N. Boundary Road		14-Oct-07	Chad Stinson	KM288010	B
TXCS2	Marcus Lab		Junonia coenia	USA: Texas, Aransas County, Stedman Island		25-Sep-08	Charles J. Sassine	KM288012	B
TXCS1	Marcus Lab		Junonia litoralis	USA: Texas, Aransas County, Stedman Island		25-Sep-08	Charles J. Sassine	KM288011	B
TEX3	California Academy of Science		Junonia nigrosuffusa x coe	USA: Texas, AransasCounty, Goose Island		June 15, 1968	J.W. Tilden	Restriction Digest	B
TEXN2	McGuire Center for Lepidoptera and Biodiversity		Junonia nigrosuffusa	USA: Texas, Brewster County, Trap Spring nr. Mule Ear Overlook, 16 mi. S Hwy 118 Big Bend National Park		September 9, 1993	R.F. Denno & E.E. Grissell	Restriction Digest	B/CA
TEXM2	California Academy of Science		Junonia nigrosuffusa x zon	USA: Texas, Brownsville, Cameron County		October 21, 1963	J.W. Tilden	Restriction Digest	B
TEXM3	California Academy of Science		Junonia zonalis	USA: Texas, Brownsville, Cameron County		October 24, 1973	J.W. Tilden	Restriction Digest	B
TXC13	Marcus Lab		Junonia coenia	USA: Texas, Calhoun County, Aransas National Wildlife Refuge, Whitmire Division		09-Nov-07	Chad Stinson	KM287974	B
TXC14	Marcus Lab		Junonia coenia	USA: Texas, Calhoun County, Aransas National Wildlife Refuge, Whitmire Division		09-Nov-07	Chad Stinson	KM287975	B
TXC15	Marcus Lab		Junonia coenia	USA: Texas, Calhoun County, Aransas National Wildlife Refuge, Whitmire Division		09-Nov-07	Chad Stinson	KM287976	B
TXC16	Marcus Lab		Junonia coenia	USA: Texas, Calhoun County, Aransas National Wildlife Refuge, Whitmire Division		09-Nov-07	Chad Stinson	KM287977	B
TXC17	Marcus Lab		Junonia coenia	USA: Texas, Calhoun County, Aransas National Wildlife Refuge, Whitmire Division		09-Nov-07	Chad Stinson	KM287978	B
TXC18	Marcus Lab		Junonia coenia	USA: Texas, Calhoun County, Aransas National Wildlife Refuge, Whitmire Division		09-Nov-07	Chad Stinson	KM287979	B
TXC19	Marcus Lab		Junonia coenia	USA: Texas, Calhoun County, Aransas National Wildlife Refuge, Whitmire Division		09-Nov-07	Chad Stinson	KM287980	B
TXC20	Marcus Lab		Junonia coenia	USA: Texas, Calhoun County, Aransas National Wildlife Refuge, Whitmire Division		09-Nov-07	Chad Stinson	KM287982	B
TXC21	Marcus Lab		Junonia coenia	USA: Texas, Calhoun County, Aransas National Wildlife Refuge, Whitmire Division		09-Nov-07	Chad Stinson	KM287983	B
TXC22	Marcus Lab		Junonia coenia	USA: Texas, Calhoun County, Aransas National Wildlife Refuge, Whitmire Division		09-Nov-07	Chad Stinson	KM287984	B
TXG	Marcus Lab		Junonia coenia	USA: Texas, Cameron County, Highway 2480		28-Oct-07	Bill Dempwolf	KM288026	B
TXF1	Marcus Lab		Junonia coenia	USA: Texas, Cameron County, Ted Hunt & Highway 510 Near Bayview		27-Oct-07	Bill Dempwolf	KM288023	B
TXF2	Marcus Lab		Junonia coenia	USA: Texas, Cameron County, Ted Hunt & Highway 510 Near Bayview		27-Oct-07	Bill Dempwolf	KM288024	B
TXF3	Marcus Lab		Junonia coenia	USA: Texas, Cameron County, Ted Hunt & Highway 510 Near Bayview		27-Oct-07	Bill Dempwolf	KM288025	B
TXC0	Marcus Lab		Junonia coenia	USA: Texas, Cameron County, Loma Alta Skeet and Trap Club		27-Oct-07	Bill Dempwolf	KM287969	B
TXB1	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		27-Oct-07	Bill Dempwolf	KM287961	B
TXB2	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		27-Oct-07	Bill Dempwolf	KM287962	B
TXB3	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		27-Oct-07	Bill Dempwolf	KM287963	B
TXB4	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		27-Oct-07	Bill Dempwolf	KM287964	B

Appendix III. *Junonia* specimens for the American Southwest and Mexico data set

Specimen Identifier	Collection	Museum Accession Number	Species	Locality	GPS Coordinates	Collection Date	Collector	Genotyping Method	Haplotype Group
TXB5	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		27-Oct-07	Bill Dempwolf	KM287965	B
TXB6	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		27-Oct-07	Bill Dempwolf	KM287966	B
TXB7	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		27-Oct-07	Bill Dempwolf	KM287967	B
TXB8	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		27-Oct-07	Bill Dempwolf	KM287968	B
TXC30	Marcus Lab		Junonia coenia	USA: Texas, Cameron County, South Padre Island Convention Center		01-Dec-07	Jeffrey M. Marcus	KM287993	B
TXC31	Marcus Lab		Junonia coenia	USA: Texas, Cameron County, South Padre Island Convention Center		01-Dec-07	Jeffrey M. Marcus	KM287994	B
TXC32	Marcus Lab		Junonia coenia	USA: Texas, Cameron County, South Padre Island Convention Center		01-Dec-07	Jeffrey M. Marcus	KM287995	B
TXC33	Marcus Lab		Junonia coenia	USA: Texas, Cameron County, South Padre Island Convention Center		01-Dec-07	Jeffrey M. Marcus	KM287996	B
TXC34	Marcus Lab		Junonia coenia	USA: Texas, Cameron County, South Padre Island Convention Center		01-Dec-07	Jeffrey M. Marcus	KM287997	B
TXC35	Marcus Lab		Junonia coenia	USA: Texas, Cameron County, South Padre Island Convention Center		01-Dec-07	Jeffrey M. Marcus	KM287998	B
TXC36	Marcus Lab		Junonia coenia	USA: Texas, Cameron County, South Padre Island Convention Center		01-Dec-07	Jeffrey M. Marcus	KM287999	B
TXC37	Marcus Lab		Junonia coenia	USA: Texas, Cameron County, South Padre Island Convention Center		01-Dec-07	Jeffrey M. Marcus	KM288000	B
TXC38	Marcus Lab		Junonia coenia	USA: Texas, Cameron County, South Padre Island Convention Center		01-Dec-07	Jeffrey M. Marcus	KM288001	B
TXC39	Marcus Lab		Junonia coenia	USA: Texas, Cameron County, South Padre Island Convention Center		01-Dec-07	Jeffrey M. Marcus	KM288002	B
TXC40	Marcus Lab		Junonia coenia	USA: Texas, Cameron County, South Padre Island Convention Center		01-Dec-07	Jeffrey M. Marcus	KM288004	B
TXC41	Marcus Lab		Junonia coenia	USA: Texas, Cameron County, South Padre Island Convention Center		01-Dec-07	Jeffrey M. Marcus	KM288005	B
TXT1	Marcus Lab		Junonia coenia	USA: Texas, Cameron County, South Padre Island Convention Center		01-Dec-07	Jeffrey M. Marcus	KM288074	B
TXT2	Marcus Lab		Junonia coenia	USA: Texas, Cameron County, South Padre Island Convention Center		01-Dec-07	Jeffrey M. Marcus	KM288075	B
TXN1	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		01-Dec-07	Jeffrey M. Marcus	KM288061	B
TXN3	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		01-Dec-07	Jeffrey M. Marcus	KM288062	B
TXN5	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		01-Dec-07	Jeffrey M. Marcus	KM288063	B
TXN6	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		01-Dec-07	Jeffrey M. Marcus	KM288064	B
TXMC4	Marcus Lab		Junonia coenia	USA: Texas, Cameron County, South Padre Island Convention Center		26-May-08	Jeffrey M. Marcus	KM288038	B
TXMC5	Marcus Lab		Junonia coenia	USA: Texas, Cameron County, South Padre Island Convention Center		26-May-08	Jeffrey M. Marcus	KM288039	B
TXMN1	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		26-May-08	Jeffrey M. Marcus	KM288042	B
TXMN10	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		26-May-08	Jeffrey M. Marcus	KM288043	B
TXMN11	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		26-May-08	Jeffrey M. Marcus	KM288044	B
TXMN2	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		26-May-08	Jeffrey M. Marcus	KM288048	B
TXMN3	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		26-May-08	Jeffrey M. Marcus	KM288055	B
TXMN4	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		26-May-08	Jeffrey M. Marcus	KM288056	B
TXMN5	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		26-May-08	Jeffrey M. Marcus	KM288057	B
TXMN8	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		26-May-08	Jeffrey M. Marcus	KM288058	B
TXMN9	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		26-May-08	Jeffrey M. Marcus	KM288059	B
TXMC7	Marcus Lab		Junonia coenia	USA: Texas, Cameron County, South Padre Island Convention Center		28-May-08	Jeffrey M. Marcus	KM288040	B
TXMC8	Marcus Lab		Junonia coenia	USA: Texas, Cameron County, South Padre Island Convention Center		28-May-08	Jeffrey M. Marcus	KM288041	B
TXMN12	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		28-May-08	Jeffrey M. Marcus	KM288045	B
TXMN14	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		28-May-08	Jeffrey M. Marcus	KM288046	B
TXMN18	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		28-May-08	Jeffrey M. Marcus	KM288047	B
TXMN20	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		28-May-08	Jeffrey M. Marcus	KM288049	B
TXMN21	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		28-May-08	Jeffrey M. Marcus	KM288050	B
TXMN22	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		28-May-08	Jeffrey M. Marcus	KM288051	B
TXMN23	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		28-May-08	Jeffrey M. Marcus	KM288052	B
TXMN24	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		28-May-08	Jeffrey M. Marcus	KM288053	B
TXMN25	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Cameron County, South Padre Island Convention Center		28-May-08	Jeffrey M. Marcus	KM288054	B
TEXN3	Colorado State University		Junonia nigrosuffusa	USA: Texas, Culberson County, Choza Spr. E. slope Guadalupe Mts. (5000')		September 14, 1986	RWH	Restriction Digest	B/CA
TEX6	Colorado State University		Junonia nigrosuffusa x coe	USA: Texas, Culberson County, Delaware Ridge, 2 mi SE US 62 nr. GMNP (5400')		July 18, 1987	RWH	Restriction Digest	B/CA
TEXC1	Colorado State University		Junonia grisea	USA: Texas, Culberson County, Dog Can. CG, W. slope Guadalupe Mts (6200')		July 27, 1986	RWH	Restriction Digest	B
TEXC5	Colorado State University		Junonia grisea	USA: Texas, Culberson County, Guadalupe Mts. N.P., Pine Springs Cyn., SE slope Guadalupe Mts. (5700')		June 9, 1986	RWH & SJC	Restriction Digest	B/CA
TEXC4	Colorado State University		Junonia grisea	USA: Texas, Culberson County, Guadalupe Mts. N.P., Pine Springs Cyn., SE slope Guadalupe Mts. (5700')		June 19, 1986	RWH & SJC	Restriction Digest	B/CA
TEXC2	Colorado State University		Junonia coenia	USA: Texas, Culberson County, S. McKittrick Can. GMNP, SE slope Guadalupe Mts. (5300')		18-May-86	RWH	Restriction Digest	B/CA
TXQ1	Marcus Lab		Junonia coenia	USA: Texas, Fayette County		17-Nov-07	Bill Dempwolf	KM288068	B
TXQ2	Marcus Lab		Junonia coenia	USA: Texas, Fayette County		17-Nov-07	Bill Dempwolf	KM288069	B
TXQ3	Marcus Lab		Junonia coenia	USA: Texas, Fayette County		17-Nov-07	Bill Dempwolf	KM288070	B
TXQ4	Marcus Lab		Junonia coenia	USA: Texas, Fayette County		18-Nov-07	Bill Dempwolf	KM288071	B
TXR	Marcus Lab		Junonia coenia	USA: Texas, Harris County, Houston, North Braes Bayou		20-Oct-07	Victor Hitchings	KM288072	B
TXS	Marcus Lab		Junonia coenia	USA: Texas, Harris County, Houston, North Braes Bayou		20-Oct-07	Victor Hitchings	KM288073	B
TEX1	California Academy of Science		Junonia coenia	USA: Texas, Harris County, Kirry Stn.		October 7, 1967	Roy Alan JamesonCollection	Restriction Digest	B
TEXM1	California Academy of Science		Junonia nigrosuffusa	USA: Texas, Hidalgo County, Santa Ana Refuge		October 15, 1970	J.W. Tilden	Restriction Digest	B
TEXN1	California Academy of Science		Junonia coenia	USA: Texas, Hidalgo County, Santa Ana Refuge		November 8, 1972	J.W. Tilden	Restriction Digest	B
TEXM4	California Academy of Science		Junonia nigrosuffusa x coe	USA: Texas, Hidalgo County, Santa Ana Refuge		October 6, 1973	J.W. Tilden	Restriction Digest	B
TEX4	California Academy of Science		Junonia nigrosuffusa	USA: Texas, Hidalgo County, Santa Ana Refuge		November 16, 1974	J.W. Tilden	Restriction Digest	B
TEX2	California Academy of Science		Junonia nigrosuffusa x coe	USA: Texas, Hidalgo County, Sullivan City		October 11, 1974	J.W. Tilden	Restriction Digest	B
TEX5	Colorado State University		Junonia nigrosuffusa x coe	USA: Texas, Jeff Davis County, Limia cn. 9 mi NE of Ft. Davis		October 18, 1984	RES	Restriction Digest	B
TEXC10	Colorado State University		Junonia grisea	USA: Texas, Jeff Davis County, Mt. Locke		October 18, 1984	RES	Restriction Digest	B
TEXC14	Colorado State University		Junonia grisea	USA: Texas, Jeff Davis County, Mt. Locke		October 18, 1984	RES	Restriction Digest	B
TXE1	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Kenedy County, I77 at Armstrong		26-Oct-07	Bill Dempwolf	KM288018	B
TXE2	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Kenedy County, I77 at Armstrong		26-Oct-07	Bill Dempwolf	KM288019	B
TXE3	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Kenedy County, I77 at Armstrong		26-Oct-07	Bill Dempwolf	KM288020	B
TXE4	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Kenedy County, I77 at Armstrong		26-Oct-07	Bill Dempwolf	KM288021	B
TXE5	Marcus Lab		Junonia nigrosuffusa	USA: Texas, Kenedy County, I77 at Armstrong		26-Oct-07	Bill Dempwolf	KM288022	B
TXI	Marcus Lab		Junonia coenia	USA: Texas, Kleberg County, Kingsville, Kleberg Park		26-Oct-07	Bill Dempwolf	KM288027	B
TEXC3	Colorado State University		Junonia coenia	USA: Texas, Lubbock County, Lubbock		October 18, 1987	P. Gordy	Restriction Digest	B
TEXC16	Colorado State University		Junonia coenia	USA: Texas, Mason County, 12 mi. S of Mason		April 15, 1986	RES	Restriction Digest	B
TXD1	Marcus Lab		Junonia coenia	USA: Texas, Nueces County, Bishop Cemetary		26-Oct-07	Bill Dempwolf	KM288013	B
TXD2	Marcus Lab		Junonia coenia	USA: Texas, Nueces County, Bishop Cemetary		26-Oct-07	Bill Dempwolf	KM288014	B
TXD3	Marcus Lab		Junonia coenia	USA: Texas, Nueces County, Bishop Cemetary		26-Oct-07	Bill Dempwolf	KM288015	B
TXD4	Marcus Lab		Junonia coenia	USA: Texas, Nueces County, Bishop Cemetary		26-Oct-07	Bill Dempwolf	KM288016	B
TXD5	Marcus Lab		Junonia coenia	USA: Texas, Nueces County, Bishop Cemetary		26-Oct-07	Bill Dempwolf	KM288017	B
TEXC9	Colorado State University		Junonia coenia	USA: Texas, Parker County, Aledo		August 21, 1971	D.E. Allen	Restriction Digest	B
TXA1	Marcus Lab		Junonia coenia	USA: Texas, Starr County, Highway 650 near Highway 83		30-Oct-07	Bill Dempwolf	KM287957	B
TXA2	Marcus Lab		Junonia coenia	USA: Texas, Starr County, Highway 650 near Highway 83		30-Oct-07	Bill Dempwolf	KM287958	B
TXA3	Marcus Lab		Junonia coenia	USA: Texas, Starr County, Highway 650 near Highway 83		30-Oct-07	Bill Dempwolf	KM287959	B
TXA4	Marcus Lab		Junonia coenia	USA: Texas, Starr County, Highway 650 near Highway 83		30-Oct-07	Bill Dempwolf	KM287960	B
TEXC11	Colorado State University		Junonia coenia	USA: Texas, Tarrant County, Benbrook Lake		October 22, 1972	D.E. Allen	Restriction Digest	B
TEXC12	Colorado State University		Junonia coenia	USA: Texas, Tarrant County, Benbrook Lake		October 22, 1972	D.E. Allen	Restriction Digest	B
TEXC8	Colorado State University		Junonia coenia	USA: Texas, Tarrant County, Benbrook Lake		October 22, 1972	D.E. Allen	Restriction Digest	B
TEXC13	Colorado State University		Junonia coenia	USA: Texas, Tarrant County, Benbrook Lake		October 22, 1972	D.E. Allen	Restriction Digest	B/CA

Appendix III. *Junonia* specimens for the American Southwest and Mexico data set

Specimen Identifier	Collection	Museum Accession Number	Species	Locality	GPS Coordinates	Collection Date	Collector	Genotyping Method	Haplotype Group
TEXC6	Colorado State University		Junonia coenia	USA: Texas, Tarrant County, Benbrook Lake		October 22, 1972	D.E. Allen	Restriction Digest	B/CA
TEXC7	Colorado State University		Junonia coenia	USA: Texas, Tarrant County, Benbrook Lake		October 22, 1972	D.E. Allen	Restriction Digest	B/CA
TEXC15	Colorado State University		Junonia coenia	USA: Texas, Tom Green County, 11 mi NE San Angelo		April 12, 1986	RES	Restriction Digest	B
TXM	Marcus Lab		Junonia coenia	USA: Texas, Travis County		16-Aug-06	Bill Dempwolf	KM288037	B
TXL1	Marcus Lab		Junonia coenia	USA: Texas, Travis County		26-Aug-06	Bill Dempwolf	KM288035	B
TXL2	Marcus Lab		Junonia coenia	USA: Texas, Travis County		26-Aug-06	Bill Dempwolf	KM288036	B
TXK1	Marcus Lab		Junonia coenia	USA: Texas, Travis County, Dorsett Road		29-Apr-05	Bill Dempwolf	KM288030	B
TXK2	Marcus Lab		Junonia coenia	USA: Texas, Travis County, Dorsett Road		21-May-05	Bill Dempwolf	KM288031	B
TXK3	Marcus Lab		Junonia coenia	USA: Texas, Travis County, Dorsett Road		21-May-05	Bill Dempwolf	KM288032	B
TXK4	Marcus Lab		Junonia coenia	USA: Texas, Travis County, Dorsett Road		21-May-05	Bill Dempwolf	KM288033	B
TXK6	Marcus Lab		Junonia coenia	USA: Texas, Travis County, Dorsett Road		10-Jul-05	Bill Dempwolf	KM288034	B
TXO0	Marcus Lab		Junonia coenia	USA: Texas, Uvalde County		17-Oct-07	Bill Dempwolf	KM288065	B
TXO1	Marcus Lab		Junonia coenia	USA: Texas, Uvalde County		17-Oct-07	Bill Dempwolf	KM288066	B
TXO2	Marcus Lab		Junonia coenia	USA: Texas, Uvalde County		17-Oct-07	Bill Dempwolf	KM288067	B
TXN0	Marcus Lab		Junonia coenia	USA: Texas, Uvalde County, Concan, Neal's Cabins		12-Oct-07	Bill Dempwolf	KM288060	B
TXJ1	Marcus Lab		Junonia coenia	USA: Texas, Williamson County, West of Liberty Hill		20-Oct-07	Bill Dempwolf	KM288028	B
TXJ2	Marcus Lab		Junonia coenia	USA: Texas, Williamson County, West of Liberty Hill		20-Oct-07	Bill Dempwolf	KM288029	B
UT1	Colorado State University		Junonia grisea	USA: Utah, Millard County, 1.4 mi W. Scipio		August 25, 1983	RES	Restriction Digest	B/CA
NW38 18	Nicholas Wahlberg		Junonia coenia	USA: Utah, Washington County		28-May-98		AY248777	B
WY1	Colorado State University		Junonia coenia	USA: Wyoming, Goshen County, Lone Tree Cyn.		August 27, 2001	Opler & Buckner	Restriction Digest	B
WY2	Colorado State University		Junonia coenia	USA: Wyoming, Laramie County, Cheyenne		September 15, 1992	RES	Restriction Digest	B