Ecological genomics of *Myotis* bats and their ectoparasites in the Baja California peninsula.

Laura Alejandra Nájera Cortazar

Submitted in accordance with the requirements for the degree of

Doctor of Philosophy

The University of Leeds

School of Biology

September 2020

The candidate confirms that the work submitted is her own, and that appropriate credit has been given within the thesis where reference has been made to the work of others.

Material for fieldwork and information of some sites was proportioned by Dr. S. Ticul Álvarez Castañeda, from Centro de Investigaciones Biologicas del Noroeste. Dr. Winifred Frick, from University of California, Santa Cruz, also proportionated assistance and contacts for sampling sites. Drew Stokes, from the San Diego Natural History Museum donated bat bugs, *Myotis californicus* and *M. yumanensis* tissues for this study. Fieldwork was carried out with the assistance of Eduardo F. Aguilera Miller, Alejandro Perez Najera, Paul Heady, Quintin Frick, Alan Harper, Uriel H. Sarabia, and Simon Goodman, as well as the master students Thomas Kitching, Alex Keen, Haley O'Connor and Natasha Kebala, all across three years of sampling. Sequencing of ddRAD libraries and quality control was done by Ian Carr, from St. James's University Hospital, also assisting with analysis and performance in the "arc3" High Performance Computing system of the University of Leeds. Bioinformatics pipelines and python scripts for SNP calling were designed by Kathryn Maher, who also provided assistance with running scripts on the University of Sheffield High Performance Computing facility "iceberg". Simon Goodman contributed with R scripts for visualisation of *Q* analysis, and example scripts for parasite diversity analysis and visualization.

This thesis is constructed according to the "standard" thesis format, with a general introduction, data chapters and general discussion. To facilitate the dissemination of conducted work in future publications, data chapters are written using "we" instead of "I", with exception of the Abstract and General Discussion. References are collected at the end of the thesis and followed a 10th edition Harvard style referencing.

Acknowledgements

In the first place, I would like to thank the University of Leeds for giving me the opportunity to study my PhD, for funding part of it and for the travel grants provided during this time. It has been amazing to be part of this institution, as student and as a volleyball player (Go Leeds!). Equally, I would like to thank CONACYT, which gave me the opportunity to study abroad, funded most of my PhD studies and kept me fed for 4 years. Many thanks to the Rufford Foundation and Idea Wild for the grants provided for my fieldwork. My gratitude goes also to the NERC Biomolecular Analysis Facility-Sheffield for providing the huge grant that allowed me to conduct such challenging and amazing lab/bioinformatics work. I would also like to thank the Next Generation Sequencing Facility at St. James's Hospital, University of Leeds, for their help with sequencing the ddRAD libraries and bioinformatics help.

Massive thanks to my supervisor, Simon Goodman and my co-supervisor Hannah Dugdale, for supporting me and teaching how is life and work in Academia. I learnt a lot from you both in many different levels. Simon, thank you very much for your sincere enthusiasm for working with me since the very first day. Your trust for following my crazy ideas to build up this project was remarkable, but also your crazier ideas kept me pushing for more accomplishments and future projects. Thank you as well for your great patience in those many meetings and written documents, your scrutiny has indeed made me grow tons in research. This PhD adventure has been difficult but also very fun and valuable, thank you for all those times when I felt I was lost and you provided support. It has been a pleasure to be your student/lab technician/fieldwork General. Hannah, I thank you for sharing your knowledge in such an altruistic manner, not only for technical writing but also for guidance on how to develop other skills that are so important in academia. The ways you have supported me during difficult times and showing a sincere care about my wellbeing are priceless. I would also like to thank John Altringham for your advice and help with documents.

This work would not have been possible without the great help I got during fieldwork. Eduardo, you taught me so much during two fieldwork seasons and five amazing years. I would be forever grateful for this and more. Thanks to Uriel for volunteering in the first year. Thank you Alejandro, you were the one that brought me to biology, my bodyguard, my teacher. It was truly amazing to go into the wild with you one last time.

During fieldwork, I got outstanding help from “my” Master students, you guys were the soldiers that made it possible. Thanks Tom and Alex, you were the first ones to accept the challenge. I had an amazing time teaching and learning from you, and also laughing with you (and at you… sorry, but you know why). I am so deeply proud that I am the one to blame for your love and careers with bats. Haley and Natasha, thanks as well for joining to this adventure and to go through so many challenges in the last fieldwork season, I also learned a lot from you both. Haley, your amazing personality, work efficiency and friendly manner were invaluable.

Especially grateful with my former supervisor, Sergio Ticul Álvarez who thought me so much and gave me his steady support since my masters and up to date. I discovered my amazing Baja bats thanks to him. Thank you Winifred Frick for been so approachable and for giving me vital information and contacts for conducting a successful fieldwork. Paul Heady and Quintin Frick, I am truly grateful for your help since my first day of sampling: it was perfect, a free course on how to sample bats, and not to fail in the process. Especial thanks to Alan Harper, who has been a great friend and supporter, I have learned from you a lot (triple back up!); I am truly happy I had the pleasure to get your help during my fieldwork and also for my PhD studies. It would be great I could thank all the local people that allowed me to do my amazing surveys. Especial thanks goes to Thomas Wood for allowing me to do the best surveys in all Baja and his labour for promoting ecology studies in such a biodiverse place, thank you to everyone in San Basilio. I will definitely try to come back. Big thanks to Evelin Ríos for her help and accessibility for supporting my grant references. And finally, thanks to Enrique Alfaro, who was very excited by the project and help me over field.

Huge thanks to Drew Stokes and the people from the San Diego History Natural History Museum for providing me with bugs samples and *Myotis* tissues for my project. Many thanks to all the people in the CIBNOR who has helped me to defeat bureaucracy, Pedro Peña, Alfredo Ortega, Cintya Segura, Pedro Cruz and especially to Izmene Gutierrez, because she has been always amazing and committed to help. Thanks to Horacio de la Cueva from CICESE and Sergio Guerrero from UdeG for your help and collaboration with this project. Staff from the NBAF in the University of Sheffield, Deborah Dawson, Gavin Horsburgh, Katy Maher, Terry Burke and Helen Hipperson for their massive help during lab work over Sheffield, insightful lab meetings and complicated coding and programming. It was great to learn all that from you. Thanks to Vikram Chattre for his huge support and teaching on the structure analysis. Thanks to Michael Siva-Jothy for providing bed bug samples to be used in the laboratory analysis. Very ultra-special thanks to

Ian Carr, who help me so much by teaching me a lot of stuff, helping me with much other stuff, and saying very cool and funny stuff about real academia. Very important "stuff". I see light for real this time.

My friends and colleagues at Uni, my accomplices. I simply don't have the space in here to thank you all for how wonderful, supportive and brilliant you all were to me. You were there for good and for bad times, we shared laughs and tears, parties and courses, hugs and adventures. I would have not survive this PhD without you guys, you all know. I am truly sorry I am not properly writing messages, that would take another chapter-length in here, and this section is already getting too long! So… in order of appearance: Giovanna, Leandro, Laurin, Jens, Tom, Tom, Katy, Nick, Sonja, Sil, Michaela, Myrna, Jude, Liz, Marianne, Peter, Ali, David, Elisa, Ioannis, Katerina, George, Isabel, Tayah, Harri, Maarten and the great people in my and sister office. Thank you all! Special thanks to Liz for the many "back to reality" chats, great advices and support, always with a lot of laughs, and sometime silly tears. You are great. Many thanks to the great FBS staff, Lucy, Martha and Caroline, as well as Paul, Sarah and Chris, you were super helpful, making my life (and of many other students) easier.

Lastly, to my family in Leeds and in Mexico. Mohammed, Cecilia and Yoshi, when I arrived to Leeds, you kept me sane, thank you for that, I will treasure you guys forever. Katarina, Kat, Zsolti, Dimi, Bea and Robertito, you are my family in las Europas, you have been there, we have done so much, you are… I have no words. Thank you for loving me that much. I love you all loads. Gracias Yoselin, por darme ese calor y apoyo cubano, y los consejos tan importantes de ciencia y vida. Muchas gracias de nuevo Gio, Myrni y Elisa por ser la onda, mis paisas, mi española. Gracias Manuel por absolutamente todo con lo que me apoyaste, nunca lo olvidaré. Gracias a Gio, Ilse, Fermín, Tania, Nayelli y Jesús por la ayuda con análisis y enseñanzas de vida que me han dado, espero algún día devolverles tanto. Lalo, gracias por todo, por absolutamente todo. Y finalmente, mis heroínas: Gracias a mi madre y a mi tía Alicia, son ustedes las razones primordiales por las que llegué a hacer esto, las que he extrañado tanto y las que se han sacrificado inmensamente porque yo me supere de esta manera. Amá eres a quien más admiro y te agradezco la vida. Con eso trato de englobar lo que has hecho por mí, ya que no hay cantidad de palabras que puedan hacer justicia a lo que tú me has dado durante XX años. Y sé que lo que nos ha costado, ha valido la pena. Gracias.

Abstract

In this thesis I investigated diversity, population structure and species boundaries in *Myotis* bats and their ectoparasite community structure, from the Baja California peninsula and continental sites in Mexico. Using mitochondrial cyt*b* haplotypes and ddRAD derived SNP markers, traces of introgression were found in four sympatric *Myotis* species, particularly over the mid and north peninsula, and one continental site. Recent hybrids were detected among *Myotis yumanensis* and *M. californicus* individuals from mid and north peninsula. Population structure for *M. californicus* was detected, but also potential migrants, suggesting some long-distance dispersal. Cluster analysis suggested *M. californicus* to be split in two groups. *M. yumanensis* showed weak nuclear population structure, but strong mitochondrial differentiation, suggesting long-distance male dispersal and female philopatry. Admixture analyses with ddRAD SNP data showed that bats with ancestry similar to *M. velifer* clustered along with the putatively Baja endemic *M. peninsularis*. However, mitochondrial haplotypes and nuclear F_{ST} results indicated *M. peninsularis* population as demographically isolated from continental *M. velifer*. I also identified two potential new cryptic *Myotis* lineages with strongly divergent mitochondrial haplotypes: a population showing morphological features not matching currently recorded species, designated *M. sv*, and specimens from Baja that were morphologically assigned to *M. volans*. Bat ectoparasite phylogenetic analysis revealed multiple novel lineages of bat bugs, flies and ticks, five new records of known bat flies and one tick species previously unrecorded for the peninsula. Host-specific and generalist ectoparasites were found, as well as locally restricted and widely dispersed species, with the latter potentially reflecting bat dispersal across the peninsula. The main driver of ectoparasite diversity and community structure was host diversity and composition, with a strong pattern of phylogenetic association at the host family level. This research discovered novel cryptic diversity of bats and ectoparasites in north-western Mexico, showing porous species boundaries and potential incipient species. Both bat and ectoparasite research are highly relevant to disease ecology as potential vectors of zoonotic diseases.

Table of Contents

List of Figures

List of Tables

List of Appendices

Commonly used abbreviations

18S: 18s ribosomal Ribonucleic Acid.

Baja: Baja California península.

COI: Cytochrome c oxidase subunit I.

Cyt*b*: Cytochrome *b*.

IBD: Isolation by Distance.

MRM: Multiple Regression with distance-based Matrices.

mtDNA: mitochondrial Deoxyribonucleic Acid.

nDNA: nuclear Deoxyribonucleic Acid.

rDNA: ribosomal Deoxyribonucleic Acid.

Chapter 1: General introduction.

1.1. Background

1.1.1. Genetic diversity

Darwin's "On the origin of species by means of natural selection, or the preservation of favored races in the struggle of life" (Darwin, 1859), was one of the first concrete attempts to describe how species arise, vary and adapt to their environment as result of evolution through natural selection. Although it was more about changing species than the origin of them, it recognized that species not only evolve, but can also divide into separate lineages which give rise to new reproductively isolated species with differing adaptations (Darwin, 1859; Coyne and Orr, 2004). However, genetic analyses have shown that reproductive isolation is not always the main determinant to define a species. According to the Genetic Species Concept, a species is a group of genetically compatible interbreeding populations that is genetically isolated from other such groups (Baker and Bradley, 2006). This definition differs from the Biological Species Concept because it focusses on genetic isolation instead of reproductive isolation (Mayr, 1947), therefore allowing differences in levels of species interbreeding, implying the possibility of introgression and hybridisation (Baker and Bradley, 2006). If hybridisation exists, there will be species differentiation as long as the integrity of the gene pool of the two species is protected. In similar way, the Phylogenetic Species Concept defines species as the smallest population or groups of populations that holds a parental pattern of ancestry and descent, and unique combinations of character states (Cracraft, 1997). However, for this definition, subspecies are not relevant and allopatric populations interbreeding is not important for defining a species state (Baker and Bradley, 2006). Population genetics has been central to our understanding of how genetic barriers evolve between populations, and how the process of

differentiation of isolated populations will depend on the interaction of mutation, drift and migration, as well as the pressure exerted by selection (Feder *et al.*, 2013; Ellegren and Galtier, 2016).

Speciation is the evolutionary process by which members of a species diverge and eventually become genetically isolated, no longer sharing an evolutionary fate (Baker and Bradley, 2006). Three main scenarios of speciation and some of the evolutionary process and mechanisms that delimit them are shown in Fig. 1.1, describing different ways in which speciation may occur with and without contact (Smadja and Butlin, 2011). In ecological speciation, reproductive isolation results as a consequence of disruptive natural selection, either between populations that inhabit different environments or exploit different resources (Schluter, 2001; Seehausen *et al.*, 2014). In the mustard plant *Boechera stricta,* latitudinal genetic variation and isolation by distance was detected, where different local genotypes in allopatric and sympatric regions were predicted by specific environmental variables, like water availability (Lee and Mitchell-Olds, 2011). To evaluate populations under divergence like the previous example, it is necessary to quantify the amount of genetic variation associated with traits under selection, and to test these traits in different environmental conditions (Charmantier and Garant, 2005).This would provide evidence of trait fitness under different environmental scenarios.

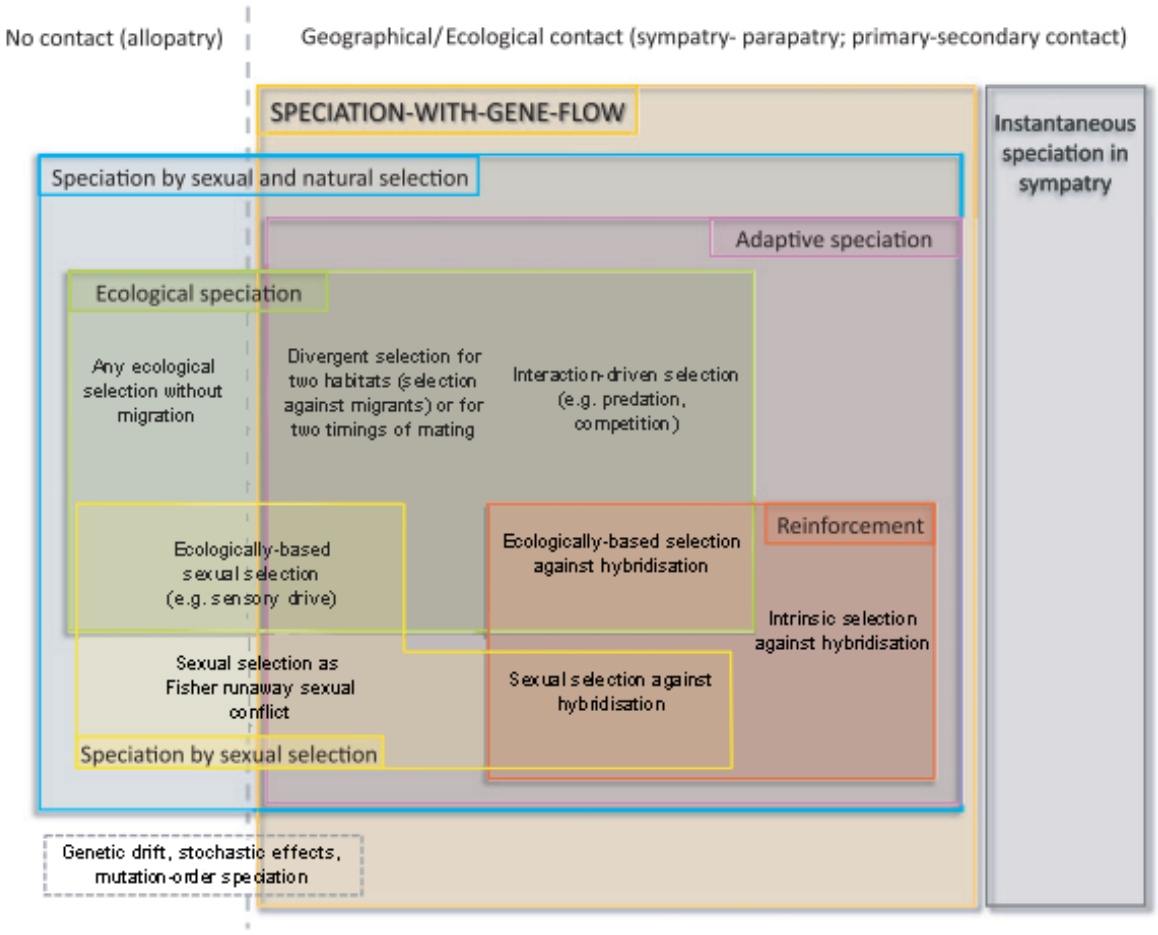


Figure 1.1. Scenarios of speciation. Diverging populations without contact (left of dashed line) from those with some geographical or ecological contact (right of dashed line). The different types of evolutionary and selective forces potentially involved (text) define and delimit the different modes and mechanisms of speciation (coloured frames). Modified from Smadja and Butlin (2011).

Assessing population structure can help to determine patterns of ecological and genetic variation correlated with geographic structure and environmental elements (Rieseberg, Church and Morjan, 2004). Population structure occurs when there is a deviation from random mating within a subgroup, with respect to the total population, where allele frequencies in sub-populations would be expected to diverge over time through genetic drift (Hartl and Clark, 2007). If gene flow tends to decrease between subpopulations, the isolated subpopulation may eventually experience inbreeding (Hartl and Clark, 2007). The rate at which population genetic differentiation happens will depend on population size, migration, selection and the gene flow acting to supress the potential for divergence (Bohonak, 1999; Holsinger and Weir, 2009; Seehausen *et al.*, 2014). How gene flow limits ecological differentiation (Hey, 2006; Abbott *et al.*, 2013; Seehausen *et al.*, 2014; Marques *et al.*, 2016), and the adaptation of populations to differing environmental conditions can be some of the most important factors influencing the development of divergence between species (Templeton, 1981; Coyne and Orr, 2004; Arnegard *et al.*, 2014; Hoffmann *et al.*, 2015).

1.1.2. Speciation with gene flow

Speciation is an ongoing, continuous process that can rarely be studied in sexually reproducing natural populations (Seehausen *et al.*, 2014). A classic example is the one involving fruit flies from the *Rhagoletis pomonella* sibling-species complex as parasites of various plants with differing fruiting times, where in the absence of geographic isolation, each host-specific parasite has radiated sympatrically by specialising on unique plants species which appeared to be infertile in crossings, forming an incipient sympatric speciation complex (Walsh, 1864; Berlocher and Feder, 2002; Linn *et al.*, 2004). Under this speciation continuum (i.e. continuous genetic variation between incipient species diverging on similar paths), genetic

changes can accumulate and/or combine gradually until they get fixed and are not available for recombination. (Seehausen *et al.*, 2014; Shaw and Mullen, 2014). Therefore, the homogenizing effect of gene flow functions as a major obstacle for ecological differentiation (i.e. recombination tends to break up gene associations involved in adaptive divergence). However, its effects can be mitigated if the relevant genes are sheltered against interspecific recombination through their location in the genome (Rice *et al.*, 2011; Seehausen *et al.*, 2014).

According to the "genomic island" perspective, the localization of early stages of differentiation can be limited to a small number of loci ("islands") of differentiation, where divergent selection is strong (Seehausen *et al.*, 2014). If these regions cause a reduction of the gene flow by increasing their size around selected sites (divergence hitchhiking), these will in turn spread across the entire genome (genome hitchhiking) facilitating divergence under gene flow (Feder *et al.*, 2013; Seehausen *et al.*, 2014). These levels of differentiation and isolating traits in distinctive temporal and spatial scales can allow periods of overlap with gene flow against periods of separation (Smadja and Butlin, 2011; Abbott *et al.*, 2013).

Hybrid zones provide ideal conditions for the study of the consequences of introgression in early stages of divergence (Poelstra *et al.*, 2014). As an example, a study of hybridisation between two European crow subspecies (*Corvus corone corone* and *C. c. cornix*) identified genome-wide introgression while maintaining phenotypic divergence under gene flow (Poelstra *et al.*, 2014), showing a successful landscape of hybrid adaptive differentiation. Patterns of introgression detected in some loci across hybrid offspring could potentially allow the identification of genes important for local adaptation and species differentiation (Payseur, 2010; Harrison and Larson, 2014). However, if hybrid offspring from genetically distinct

populations are successfully adapted, this might allow gene flow and recombination between populations to promote speciation by adaptive introgression (Barton and Hewitt, 1985; Abbott *et al.*, 2013; Harrison and Larson, 2014).

1.1.3. The genomics of ecological differentiation

High-throughput sequencing has proved its great potential as a revolutionary large-scale genome sequencing technique (Davey *et al.*, 2011; Rice *et al.*, 2011), making it possible to discover, validate and apply genetic markers in a lower cost, faster and more efficient way across several model and non-model genomes (Davey *et al.*, 2011; Rice *et al.*, 2011; Narum *et al.*, 2013; Benestan *et al.*, 2016). It has also enhanced the discovery and genotyping of hundreds of single nucleotide polymorphisms (SNPs) in *de novo* sequencing, producing tens to hundreds of gigabases of sequence data per run (Narum *et al.*, 2013; Ellegren, 2014). Genomic approaches have allowed us to decode and study complex and continuous systems, assess their evolutionary potential, and trace the adaptive patterns and responses of the species to environmental challenges (Zhou *et al.*, 2011; Ellegren, 2014). Genomics has huge potential in ecological speciation and conservation studies (Table 1.1), for example, revealing parallel patterns of nucleotide diversity and population differentiation across the genome (Hohenlohe *et al.*, 2010), to identify loci under divergence and/or selection (Ungerer, Johnson and Herman, 2008; Rice *et al.*, 2011; Jones *et al.*, 2012), and mapping the environment-dependent effects of phenotypic traits on hybrids (Arnegard *et al.*, 2014).

Table 1.1. Ecological speciation questions that can be addressed using genomic methods. Modified from (Rice *et al.*, 2011) and references therein.

	Ecological Question	Corresponding Genomic Question
Population divergence through ecological selection	To what extent does divergence result from divergent selection and adaptation vs. neutral processes?	• Are loci that are more divergent than expected under neutral evolution present? • Do these loci also show an association with phenotypic traits under divergent selection? • How many genes show signatures of divergent selection?
	Does exposure to similar environments lead to the evolution of similar adaptations?	• Do the same genes show signatures of selection in individuals sampled from different populations but exposed to similar eco logical environments? • Are the same genes responsible for parallel phenotypic changes?
Adaptation and reproductive isolation	What is the relative importance of different adaptations for reproductive isolation?	• How does the amount of introgression between populations (e.g. in populations forming ring species) correlate with the amount of divergence in different traits?
	Are the same adaptations important for reproductive isolation over the time course of speciation?	• Are the same genes coding for traits underlying ecological adaptations and sexual isolation eventually also causing incompatibilities at later stages of divergence?
	Do parallel adaptations achieved through different genetic pathways have a similar impact on the build-up of reproductive isolation?	• What is the relationship between variation in the genetic architecture underlying ecological adaptation and the degree of reproductive isolation?
	How does ecological divergence lead to genetic incompatibilities?	• What are the identity and genomic locations of genes causing genetic incompatibility?
Divergence despite gene exchange	What is the breadth and location of natural hybrid zones?	• Where does the transition between species-specific alleles occur? • How steep is the cline for different marker loci? • Which populations show the greatest degree of admixture?
	How much realized gene flow occurs between populations across the genome?	• What is the frequency of heterospecific genes in natural populations? • What is the cline breadth for multiple loci at secondary contact zones? • Do some loci show asymmetric clines or geographically displaced cline centres?
	How does the relationship between genes that have undergone adaptive divergence and genes associated with reproductive isolation allow the build-up of linkage disequilibrium?	• Do outlier loci map to regions associated with reproductive isolation? • Where are genes for reproductive isolation located in the genome relative to genes for adaptive divergence? • Do such genes tend to be located in chromosomal inversions, on sex chromosomes, or in other regions of reduced recombination?

Since most genome research involves comparing homologous sequences between individuals, the importance of choosing the best method based on each particular case, will dictate optimal marker resolution (Peterson *et al.*, 2012). Genotyping methods that target a larger fraction of the genome can optimize the number of genetic markers retrieved, adding flexibility to investigate different biological questions (Fig. 1.2). According to Davey *et al*. (2011), the most common methods are: 1) reduced-representation sequencing (reduced-representation libraries and complexity reduction of polymorphic sequences); 2) restriction-site associated DNA sequencing (RADseq); and 3) low coverage genotyping (multiplexed shotgun genotyping, and genotyping by sequencing). For the study of wild populations with no available reference genome, RADseq (Baird *et al.*, 2008) and double digest restriction-site associated DNA (ddRAD) sequencing (Peterson *et al.*, 2012) are appropriate, cost-effective methods that will generate a larger number of markers to ensure that the population parameters are well estimated (Davey *et al.*, 2011). However, without a reference genome, variant discovery will be restricted to those regions containing one or two polymorphic sites (Peterson *et al.*, 2012). Another important disadvantage of ddRAD and RAD sequencing versus whole-genome sequencing will be that only a proportion of the genome will be sequenced, limiting researchers to genotype a specific number of markers depending on the experiment planned (Peterson *et al.*, 2012).

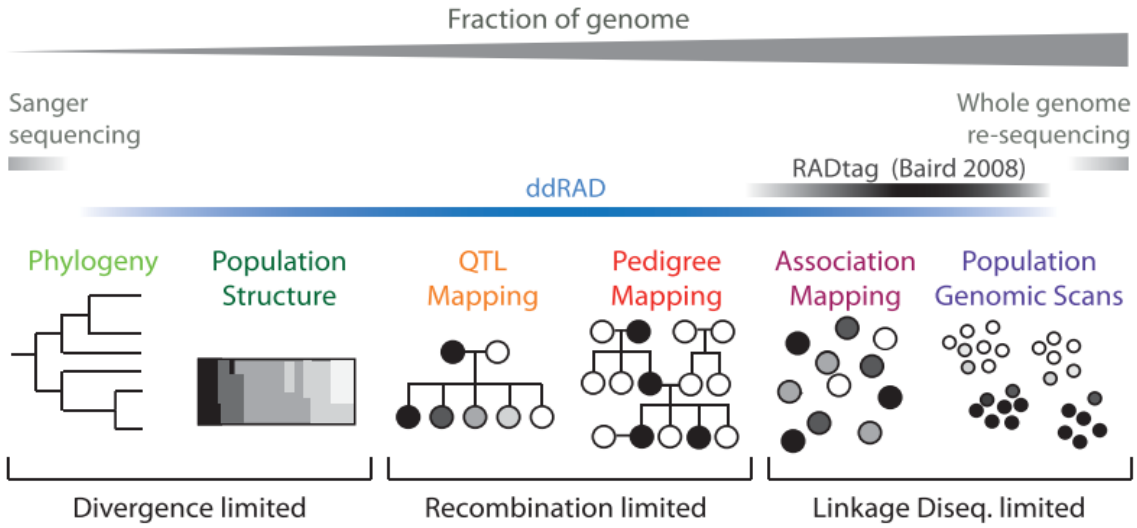


Figure 1.2. Scheme showing recent methods and techniques combining reduced representation library construction and next generation sequencing. Double digest RAD sequencing can provide marker sets targeting an intermediate number of regions with no requirement of prior genomic data. Modified from Peterson *et al.* (2012).

To generate RAD markers, genomic DNA is digested by an enzyme or a set of enzymes. After this, a P1 adapter (containing a forward amplification primer, an Illumina sequencing primer and a barcode) gets attached to random fragments of the genome, which are defined by cutting sites preselected by sequence barcodes (Baird *et al.*, 2008). These fragments are then combined, sheared and ligated to a second adapter (P2), which contains the reverse complement of the amplification primer (Baird *et al.*, 2008). These chunks of DNA then are aligned to other fragments or a reference genome to detect SNPs. In the case of ddRAD, a double restriction enzyme digestion is implemented, eliminating DNA-loss steps, also introducing a precise and repeatable selection of genomic fragments by size, adding more control on the regions represented in the libraries (Peterson *et al.*, 2012). Including a custom combinatorial indexing method, ddRAD libraries can be sequenced in combination of single or pair-end reads, using adapter barcodes depending on the chosen method, allowing to include

more samples per lane of sequencing making it also more cost effective (Davey *et al.*, 2011; Peterson *et al.*, 2012).

High-resolution provided by RADseq and ddRAD methods has improved the detection of admixture and hybridisation between cryptic and closely related species (Chattopadhyay *et al.*, 2016; Lavretsky, Janzen and McCracken, 2019; Sonsthagen *et al.*, 2019). An example is the landscape genomics study performed of the critical endangered Dahl´s Toad-headed turtle (*Mesoclemmys dahli*) and its evolutionary response to severe habitat modification (Gallego-García *et al.*, 2019). Using RAD sequencing, results of this study showed that landscape fragmentation was restricting gene flow, causing adaptation to the novel conditions. There were also negative consequences of habitat loss such as small effective population sizes, inbreeding, etc. Based on these results Gallego-García *et al*. (Gallego-García *et al.*, 2019) recommended monitoring the population and restoring gene flow to encourage genetic rescue to counteract these threats. Implementation of RAD techniques have had great implications for understanding species boundaries and their vulnerability in the face of many current threats to biodiversity (Gallego-García *et al.*, 2019; Lavretsky, Janzen and McCracken, 2019; Sonsthagen *et al.*, 2019), promoting their use in the continued evaluation of potential risks, and in developing strategies for management and conservation.

1.2. Bats

Bats belong to the order Chiroptera, the second largest order of mammals (Simmons, 2005), with approximately 1,400 species known to date that account for approximately 20% of mammals (Teeling *et al.*, 2018; Jebb *et al.*, 2020). Estimating bat species diversity and resolving taxonomic relationships is challenging due to cryptic morphological differences

among some species. Thus, the incidence of potential overlooked taxa is hypothesised to be high (Clare *et al*., 2011). The discovery of cryptic species has important implications for biodiversity estimates, conservation, wildlife management and pathogen research (Centeno-Cuadros *et al*., 2019). Recently, there has been frequent discovery of novel bat species around the world, as survey coverage improves, particularly in locations already known for high species diversity. Clare *et al*. (2013) gathered evidence for cryptic speciation within the *Pteronotus parnellii* species complex from Central and South America, which included four different species, showing genetic differentiation and divergent morphological and acoustic characters. Another example of recent cryptic species discovery includes two new species within the *Myotis nattereri* group in the Western Paleartic (Juste *et al*., 2019). Underexplored regions can also potentially contain high proportions of endemic and cryptic bat species, like the horseshoe bat recently found in the Andaman Islands, *Rhinolophus andamanensis*, which was previously described as a subspecies of *R. affinis* (Srinivasulu *et al.,* 2019). Expectation of novel species discovery among bats is increasing, and the integration of multiple morphological, acoustic and molecular approaches, especially genome-wide data, promises to significantly enhance prospects for the discovery of cryptic bat diversity worldwide (Chattopadhyay *et al*., 2016; Juste *et al*., 2018; Srinivasulu *et al*., 2019).

Previously, bats were grouped in two suborders: Microchiroptera, which included laryngeal echolocating bats from across the world; and Megachiroptera, non-laryngeal echolocating fruit bats from the old world (Koopman, 1993). Incorporating molecular data, this classification was proved to be wrong, which led to a revision of the taxonomy of bats, resulting in two suborders: Yinpterochiroptera and Yangochiroptera. In Yinpterochiroptera was included the Old-World fruit bat Pteropodidae family, and the echolocating bat families

Craseonycteridae, Hipposideridae, Megadermatidae, Rhinolophidae, Rhinonycteridae and Rhinopomatidae; and in Yangochiroptera, were included the rest of the bat families, like Vespertilionidae and Phyllostomidae (Teeling *et al.*, 2005, 2018; Tsagkogeorga *et al.*, 2013; Teeling, Jones and Rossiter, 2016). This phylogenomic reconstruction recovered a reciprocal monophyly from both suborders, hypothesizing that the evolution of laryngeal echolocation emerged multiple times, or it occurred as an evolutionary loss in Old World bats (Tsagkogeorga *et al.*, 2013).

There are still many gaps in knowledge regarding the lower level taxonomy of bats and their diversity around the world, especially in regions with difficult access for researchers. As an example, *Myotis planiceps* is an endemic and potentially small population in the north of Mexico, which in 1970 was thought to be extinct until a re-capture in 2004 (Haynie *et al.*, 2016). As with higher order taxonomic relationships, molecular phylogenetics is now largely replacing more traditional morphological approaches to bat systematics (Jones and Teeling, 2004; Bogdanowicz, Juste and Owen, 2005; Tschapka *et al.*, 2008; Monteiro and Nogueira, 2010; Evin, Horáček and Hulva, 2011). Mitochondrial DNA (mtDNA) has been one of the most frequently used source of markers for this purpose (Simmons and Conway, 2001; Stadelmann, Herrera, *et al.*, 2004; Stadelmann, Jacobs, *et al.*, 2004; Stadelmann *et al.*, 2007; Nájera-Cortazar, Álvarez-Castañeda and De Luna, 2015), but now the use of multiple markers is increasing resolution and is an important tool for understanding cryptic species relationships for bats.

Distributed in most continents and ecosystems of the world (except the Antarctic), bats possess unique adaptations. Body size varies from the smallest bumblebee bat *Craseonycteris*

thonglongyai with a weight of ~2 g, to the largest golden-capped fruit bat *Acerodon jubatu*, with a weight of ~1 kg and a wing span of ~1.5 m (Teeling *et al.*, 2018). The range and diversity of unique ecological and physiological adaptations found in bats make them important focuses of basic scientific and medical research, especially in fields of zoonotic disease, immunology or aging (Altringham, 2011; Brook and Dobson, 2015; Foley *et al.*, 2018; Kasso and Balakrishnan, 2013). Bats also have high economic significance for human activities, mainly because of their implications as reservoirs and vectors of zoonotic disease, where misinformation usually impacts bat conservation in a negative way (Turmelle and Olival, 2009); and positively through the ecosystem services they provide such as seed dispersal and pollination (Kunz *et al*., 2011). They are also important for enterprises such as ecotourism, and for agriculture through pest control and as a source of guano, which remains a commercially important source of fertiliser. Thus, bats are a crucial component in the equilibrium of most terrestrial systems.

Bats provide important study systems for topics in ecology and evolution, including phylogenetics and taxonomy (Rodriguez and Ammerman, 2004; Evin, Horáček and Hulva, 2011; Walters *et al.*, 2012; Nájera-Cortazar, Álvarez-Castañeda and De Luna, 2015); phylogeography and geographic structure (Ortega and Arita, 1998; Jones *et al.*, 2002; Teeling *et al.*, 2005; Dool *et al.*, 2013), migration and dispersal studies (Herrera Montalvo, 1997; Moussy *et al.*, 2012; Sovic, Carstens and Gibbs, 2016); aging and survival-reproductive trade-offs (Foley *et al.*, 2018; Culina *et al.*, 2019); pathogens (Atterby *et al.*, 2010; Drexler, Corman and Drosten, 2014; Anthony *et al.*, 2017; Joffrin *et al.*, 2018), and of course, lots of research related to conservation of this special group (Floyd *et al.*, 2010; McMahon, Teeling and Höglund, 2014; Barlow *et al.*, 2015; Aguiar *et al.*, 2016; Langwig *et al.*, 2016; Russo *et al.*,

2017). Recently, metagenomics and metabarcoding have focused on studying bat diet and microbiome (Razgour *et al.*, 2011; Ingala *et al.*, 2018; Zepeda Mendoza *et al.*, 2018), as well as studies related to bat viruses (Donaldson *et al.*, 2010; Li *et al.*, 2010; Ge *et al.*, 2012; Geldenhuys *et al.*, 2018). Genomics has been used to detect patterns of dispersal (Sovic, Carstens and Gibbs, 2016), evolutionary relationships (Tsagkogeorga *et al.*, 2013) and adaptation to climate change (Razgour, Taggart, *et al.*, 2018). The number of studies covering the features of these mammals are as diverse as the group itself.

1.2.1. Ecology

Bats are long-lived small mammals (Altringham, 2011; Kasso and Balakrishnan, 2013; Seim *et al.*, 2013), they usually have only one young per litter, with highest mortality during the first year (Altringham, 2011). There are both diurnal and nocturnal bats, utilising a wide variety of food and foraging strategies (carnivorous, frugivorous, nectarivorous, hematophagous) resources (Altringham, 2011). They are well represented at high latitudes (Altringham, 2011), but have especially high diversity in tropical areas in both the new world (Kalko, 1998; Rex *et al.*, 2008; Altringham, 2011) and the old world (Altringham, 2011; Ruedi *et al.*, 2012; Wordley *et al.*, 2015). Bats generally have long-distance aerial dispersal potential, that can go from nightly feeding flights of 80 km, to long-distance dispersal of more than 1000 km, as suggested in a migration modelling study of the silver-haired bat, *Lasionycteris noctivagans* (McGuire *et al.*, 2012), predicting migration distances per day of 250–275 km and up to 1,500 km total distance. For temperate bats, migration occurs over winter as a strategy for coping with the harsh conditions and lack of food. Bats migrate to hibernate or to feed in warmer zones (Cryan and Diehl, 2009; Piksa *et al.*, 2013). For tropical bats migration occurs mainly following food availability and they can remain residents when resources are available

in all seasons (Popa-Lisseanu, Voigt and Ecology, 2009; Moussy *et al.*, 2012). Sex-biased movements are also common among bats, were females are more likely to migrate than males, potentially due to the need of more food and suitable roosting sites for pregnancy and lactation. Males can cope with lower resources, granting more choices for roosting and foraging sites (Rivers, Butlin and Altringham, 2005; Moussy *et al.*, 2012; Angell, Butlin and Altringham, 2013; McGuire and Boyle, 2013).

Most bat species present different social aggregations during their annual life history, involving clustering for regular sleeping, foraging, hibernating and mating (Willis and Brigham, 2004; Senior, Butlin and Altringham, 2005; Altringham, 2011; Cvikel *et al.*, 2015). These aggregations are defined by seasonal movements that will facilitate gene flow, community structure, and influence the transmission of bat pathogens and parasites (Langwig *et al.*, 2012; Presley, 2012). For example, in a study of bat communities hibernating in caves in Poland, which were distributed along a temperate elevation gradient, Piksa *et al* . (2013) showed how the community composition was governed by altitude, and local geomorphological conditions, which determine the microclimate of the caves (Fig. 1.3). Their findings also suggested that the type of vegetation had impacts on the structure of hibernating bat assemblages and species richness between zones (Piksa *et al.*, 2013). This shows how environmental factors in different levels of micro ecosystems can have strong influences on bat dispersal, species richness and community structure of bats and their adjacent micro fauna.

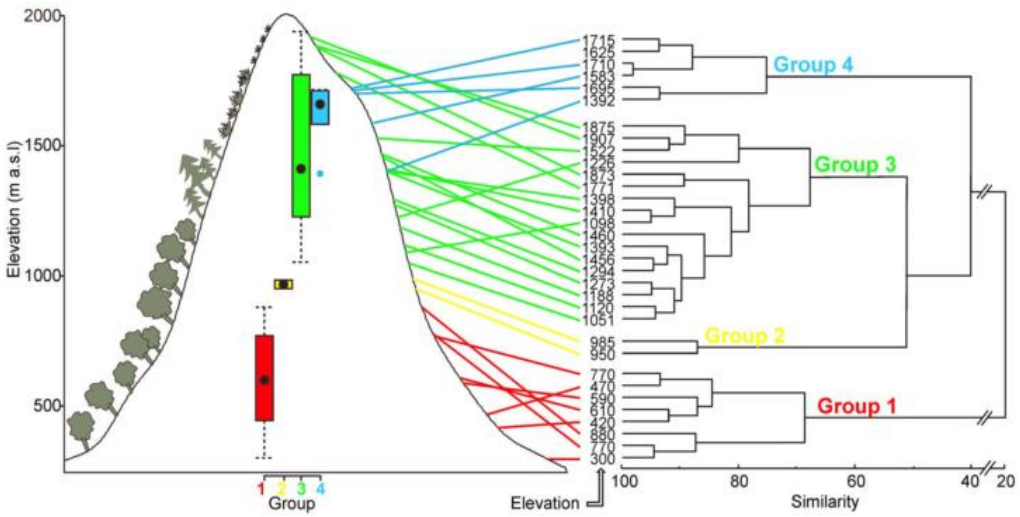


Figure 1.3. Cluster analysis of bat assemblages from the study in 33 caves in the Polish Carpathians. Four groups were distinguished and marked in different colours; the altitudinal position of openings of caves is projected on the slope and expressed with a boxplot. Four main vegetation zones are drawn on the left slope. Modified from Piksa *et al.* (2013).

1.2.2. Genetic diversity and population structure in bats

Molecular approaches for quantifying genetic population structure in bats have been applied in many species with varied ecologies to evaluate population boundaries and connectivity (Smith *et al.*, 2008; Speer *et al*., 2017), consequences of habitat fragmentation (Rossiter *et al.*, 2000), phylogeography and population history (Carstens, Lundrigan and Myers, 2002; Guevara-Chumacero *et al.*, 2010; Hulva *et al.*, 2010; Evin, Horáček and Hulva, 2011), landscape features and its effect on population composition (Razgour *et al*., 2014; Talbot *et al*., 2017), association of morphological variation with population structure and species identity (Marchán-Rivadeneira *et al.*, 2012), etc. Population structure analyses have been particularly focused on understanding mating systems and dispersal behaviour (Rossiter *et al.*, 2000; Carstens *et al.*, 2004; Rivers, Butlin and Altringham, 2005; Dixon, 2011; Johnson *et al.*, 2015), where maternity colonies and swarming sites act as hotspots for gene flow, allowing

interbreeding among different colonies (Erlangen *et al.*, 2000; Castella, Ruedi and Excoffier, 2001; Veith *et al.*, 2004; Angell, Butlin and Altringham, 2013; Miller-Butterworth *et al.*, 2014). It has been found that swarming areas of *Myotis nattereri* in the north of England support high genetic diversity from different populations (Rivers, Butlin and Altringham, 2005). Johnson *et al*. (2015) found similar results for the genetic structure in *M. lucifugus* in North America, where haplotype diversity was significantly higher at swarming sites than both distant and proximal summering sites.

Species presenting disjointed patterns of distribution generally have genetically structured populations. Over large geographical scales, migratory species tend to show less population structure due to long distance movements, mating outside breeding areas, and weak migratory connectivity (Moussy *et al.*, 2012). In contrast, populations of sedentary species tend to be more differentiated at smaller geographical scales (Moussy *et al.*, 2012). However, both sedentary and migratory behaviours have been observed in bats from different populations from the same species. An example of this are populations of *Tadarida brasiliensis,* a long-distance migrating bat that has both migratory and non-migratory populations, distributed over different scales of dispersal through North America (Fig. 1.4) (Russell, Medellin and Mccracken, 2005). Using mtDNA plus existing information from allozyme, banding, and natural history data, they found that there was not significant genetic structuring of behaviourally distinct migratory groups, explained by both gene flow by movement of bats from different groups (at least corroborated for C and D groups, figure1.4), and by coalescent stochasticity (Russell, Medellin and Mccracken, 2005). This lack of genetic structure may be explained by knowing how migratory behaviour might be driven by environmental cues, or even by previous learning. A more recent study using microsatellites, mitochondrial and morphological data, showed that *T.*

brasiliensis distributed across Florida and islands in The Bahamas showed high population structure between two islands, potentially as a result of ancient divergence and subsequent contact between both populations (Speer *et al*., 2017).

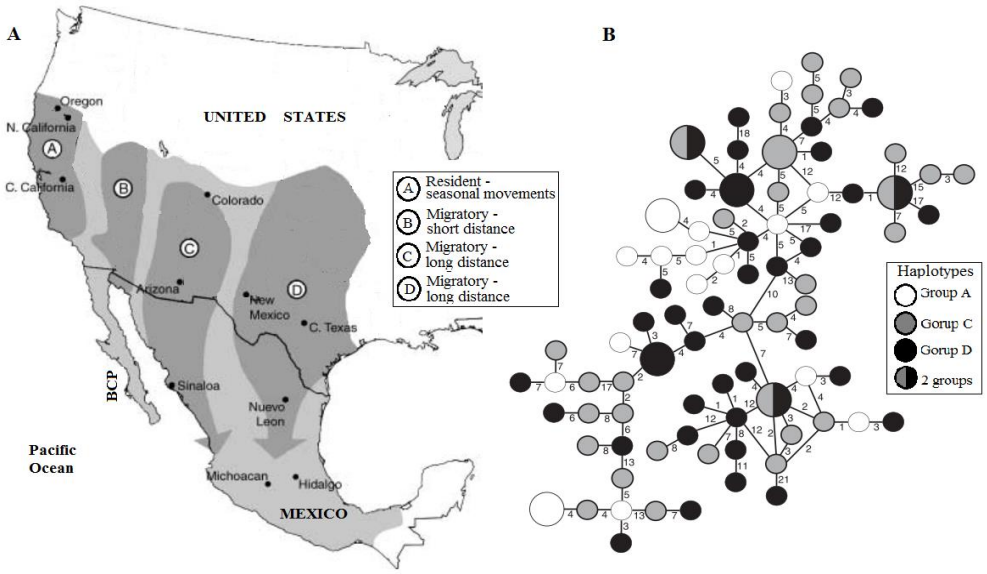


Figure 1.4. A, distribution of *Tadarida brasielinsis mexicana* (light grey) and range of putative migratory groups (shaded) from different colonies. B, minimum-spanning network of haplotypes (mtD-loop) from distinct migratory groups in A. Smaller circles represent unique haplotypes, and larger circles represent haplotypes found in two individuals. Number of mutations indicated beside each link line. Modified from Russell, Medellin and Mccracken (2005).

Large SNP datasets obtained by high-throughput sequencing approaches have increased power for quantifying weak and fine scale population structure, allowing the detection of admixture and introgression among genetic isolated populations (Sovic, Carstens and Gibbs, 2016; Loureiro, Engstrom and Lim, 2020), which in turn can help to detect vulnerable populations across different geographic systems. Using Genotyping-by-Sequencing, Loureiro, Engstrom and Lim (2020) studied phylogeographic patterns in mainland and island populations of *Molossus* bats, finding different ecological and historical constrains promoting population structure and population isolation. For *M. molossus*, the Amazon River, rainforest and savanna

habitats affected bat population structure, whereas neither the Andes Mountains nor oceanic barriers had an effect; and for *M. milleri,* oceanic barriers were isolating populations in the Greater Antilles, increasing their vulnerability to climate change (Loureiro, Engstrom and Lim, 2020).

A clear example of cryptic biodiversity discovery, using an integrative approach including high-throughput sequencing, is shown in the assessment of morphological and genetic variability of the Oriental fruit bats *Cynopterus sphinx* and *C. brachyotis* (Chattopadhyay *et al.*, 2016). Combining microsatellites, cyt*b* gene (1140 bp) and ddRAD sequencing (~700,000 bp), Chattopadhyay *et al.* (2016) evaluated the population structure and differentiation patterns of these two species, in search of potential hybridisation over the contact zone in southern India. They found substantial morphological overlap at higher altitudes where both species converged, but no hybrids were identified between the initial species comparison. However, they discovered a new cryptic Cynopterinae lineage coexisting with *C. sphinx*, and confirmed introgression in these two lineages (Chattopadhyay *et al.*, 2016). Combining novel sequencing techniques and traditional methods for discovering cryptic diversity, it is a step forward to understand the evolutionary processes of species radiations (Chattopadhyay *et al.*, 2016), also demonstrating that gene flow between species happens more often than what has been traditionally documented.

1.3. Bat ectoparasites

Ectoparasites are organisms that cling to the skin or fur/feathers of their host to feed, copulate or reproduce, while potentially causing a certain level of harm to the host (Hopla,

Durden and Keirans, 1994; Talbot, 2017). Ectoparasites can permanently accompany the host, or live from it only for certain periods to fulfil certain biological needs (e.g. feeding and/or completing a stage on their life cycle) (Morand, Krasnov and Poulin, 2006). This is generally related to host specificity and it varies according to the ectoparasite group biology and life cycles (Seneviratne, Fernando and Udagama-Randeniya, 2009). Ectoparasites can be generalists (i.e. capable of using two or more hosts during any stage of their life cycle), or specialists (i.e. associated to a single host at any stage or their whole life cycle) (Hopla, Durden and Keirans, 1994; Morand, Krasnov and Poulin, 2006). Parasites can be indicators of different trophic interactions in ecosystem functioning, pathogen transmission and/or hosts movements patterns at a population and community level (Hudson, Dobson and Lafferty, 2006; Miller-Butterworth *et al.*, 2014). This make them an accessible option to study ecological interactions of elusive hosts that are not easy to sample across their full range of distribution, and/or season.

Ectoparasites of bats are mainly composed by arthropods from the orders Mesostigmata (acari), Diptera (flies), Hemiptera (bugs), Ixodida (ticks), Siphonaptera (fleas) and Trombidiformes (chiggers) (Morand, Krasnov and Poulin, 2006; Seneviratne, Fernando and Udagama-Randeniya, 2009). In figure 1.5 there are represented some of the most common families of ectoparasites related to bats of each order. There are generalists ectoparasites, like bugs from the family *Cimicidae* that can parasitize humans and domestic animals (Booth *et al.*, 2015; Talbot, 2017); and specialist cases like bat flies from the families *Nycteribiidae* and *Streblidae*, bugs from the family *Polyctenidae*, and fleas from the family *Ischnopsyllidae* (Seneviratne, Fernando and Udagama-Randeniya, 2009; Dick and Miller, 2010). Host specificity is a consequence of co-existence between host and parasite lineage, including their phylogenetic relatedness and whether a parasite occupies the same or different hosts across a

geographic range (Seneviratne, Fernando and Udagama-Randeniya, 2009; Poulin, Krasnov and Mouillot, 2011). Bat parasites are considered mostly to be host specific due to their associated life histories strategies and bats' ecological specialization and dispersal (Seneviratne, Fernando and Udagama-Randeniya, 2009). Esser *et al*. (2016) investigated the dynamics of tick communities in Panama, finding strong phylogenetic relatedness from adult stage ticks. This study pointed out how high host-specificity may have implications on both hosts and parasite susceptibilities to changes on their environment, movement patterns and disease tolerance (Esser *et al.*, 2016), which is useful to determine dispersal routes of parasites and to evaluate spread risks

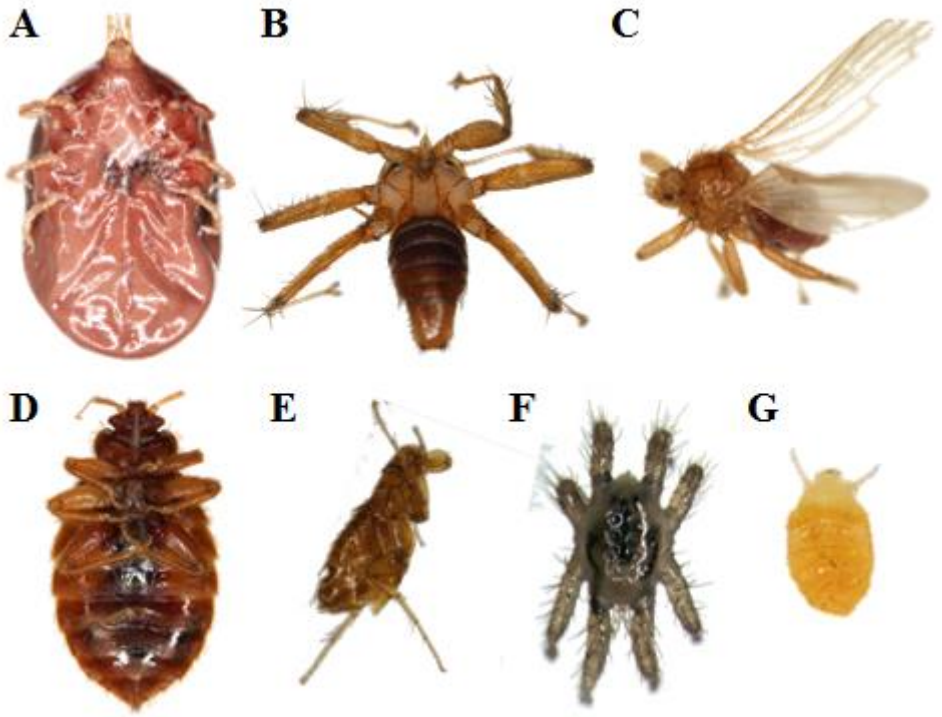


Figure 1.5. Different bat ectoparasite families. A, ticks *Argasidae;* B, wingless flies *Nycteribiidae;* C, winged flies *Streblidae;* D, bugs *Cimicidae;* E, fleas *Ischnosyllidae;* F, mites *Spinturnicidae;* and G, chiggers *Trombiculidae.*

1.3.1. Bat population structure and host-pathogen/parasite relationships

Bats are considered to be very important virus reservoirs, which is possibly due to their physiological, immunological and behavioural adaptations (e.g. ability to fly), that have impacted the way their genomes have coevolved and developed resistance to virulence (Brook and Dobson, 2015). Among the most studied viral associations in bats are members of the family *Rhabdoviridae* (e.g. rabies *Lyssavirus*), and the second most common family *Coronaviridae* (Anthony *et al.*, 2013; Mollentze, Biek and Streicker, 2014; Hayman, 2016; Albery *et al.*, 2019). For example, the discovery of bats as ubiquitous hosts for Hendra and Nipah viruses, after emergence into domestic animals with devastating effects, focused resources on the study of viruses from the family *Paramyxoviridae* (Drexler *et al.*, 2012; Hayman, 2016). Host-pathogen phylogenetic similarity (i.e. pathogens tend to infect or parasitize hosts that are phylogenetically related) and geographic structure are strong, non-linear predictors of viral sharing among mammal species (Albery *et al.*, 2019), therefore research on these topics is crucial for better prevention and management of potential zoonoses.

Landscape complexity influences patterns of bat dispersal, therefore affecting both gene flow and spread of pathogens among bats (Wilder, Kunz and Sorenson, 2015). This is particularly important when it concerns the dispersal and spread of lethal diseases, like the white-nose syndrome (WNS), the second major cause of mortality for bats during the last decade (Frick *et al.*, 2010; Johnson *et al.*, 2015; Vonhof, Russell and Miller-Butterworth, 2015; Wilder, Kunz and Sorenson, 2015; O'Shea *et al.*, 2016). Emerging in North America in 2006, WNS is associated with the fungus *Geomyces destructans*, affecting hibernating bats, causing skin lesions, aberrant behaviour and premature loss of critical fat reserves (Frick *et al.*, 2010). A study testing the relationship between population genetic structure and the dispersal of

Myotis lucifugus related to the WNS, found that the fungus was infrequent and/or locally restricted to the dispersal of the bats, suggesting limited opportunities for pathogen introduction from eastern to western North America (Wilder, Kunz and Sorenson, 2015). In Europe, presence of *G. destructans* was evaluated testing bats distributed over Germany, Switzerland, and Hungary using rRNA gene internal transcribed spacer region of DNA, where 21 of 23 bats from five species had this fungus Wibbelt *et al*. (2010). These authors hypothesized that bats over Europe were more immunologically or behaviourally resistant (Wibbelt *et al.*, 2010). The study of bat population structure and its relation with bat pathogens highlights the importance of investigating potential routes of dispersal across different scales, where collaborating to evaluate information gathered in long distance places would help to understand development and dispersal of potential zoonotic vectors.

Parasites may function as direct or intermediary vectors transmitting bacteria, fungi and viruses to other organisms that interact with their host (Hopla, Durden and Keirans, 1994; Olival *et al.*, 2013; Lučan *et al.*, 2016). The composition of ectoparasites and the amount of gene flow between them will also be driven by environmental features and behavioural aggregations from both hosts and parasites (Presley, 2012; Wu *et al.*, 2019), where the parasite's ability to adapt to a given host, is strongly linked to its own life history and the life history of its host (van Schaik *et al.*, 2014). An example including two bat ectoparasite mites *Spinturnix myoti* and *S. bechsteini*, and their respective hosts *Myotis myotis and M. bechsteini* showed that despite the mites' similar life histories, they had different population structure, driven by the variation in genetic drift and dispersal opportunities caused by the different social systems of their bat hosts (van Schaik *et al.*, 2014). This exemplifies how the host social system

affects the population structure of their parasites, as well as the evolutionary pressure effected by the ectoparasites over the hosts (van Schaik *et al.*, 2014).

Population and community-level structure studies of parasites can help us to understand cross-species interactions and to elucidate patterns of host movement that might not be detected by host studies alone (Olival *et al.*, 2013; van Schaik *et al.*, 2014). Olival *et al*. (2013) performed a population genetic structure assessment of the bat fly *Cyclopodia horsfieldi* from the bats *Pteropus vampyrus, P. hypomelanus,* and *P. lylei*, distributed in Malaysia, Cambodia, and Vietnam, and found a lack of genetic population subdivision and morphological variation. This was attributed to the frequent contact between the *Pteropus* species and subsequent high levels of parasite gene flow, suggesting *P. vampyrus* could be facilitating the movement of bat flies between the three *Pteropus* species in the region (Olival *et al.*, 2013). As shown in this study, how congruent the parasite population structure is with that of the host will be determined by how specialist or generalist the parasite is, if it has free-living lifecycle stages and the host-parasite degree of dispersal (Olival *et al.*, 2013; Wessels *et al.*, 2018). In addition, single hosts tend to be infected by different types of ectoparasites at the same time, with their own repertoire of pathogens, increasing the interactions occurring in each system (Wu *et al.*, 2019). Multilevel host-pathogens interactions can provide insights into why some organisms are more affected than others, and how pathogens are transferred among different hosts, offering multiple tools for dealing with the positive and negative effects on their fitness.

1.4. The *Myotis* bats and bat ectoparasites across the Baja California peninsula

Bats from the genus *Myotis* (Vespertillionidae) had an origin in the early Miocene, approximately 21 million years ago (Ruedi *et al.*, 2013). *Myotis* are distributed worldwide

(except the Antarctic), comprising around 100 species, although this number has potentially increased to date (Ruedi *et al.*, 2013; Platt *et al.*, 2018). The New World *Myotis* radiated from the Old World *Myotis* between 10-15 million years ago, and forming a monophyletic clade (Stadelmann *et al*., 2007; Clare *et al*., 2011; Larsen *et al*., 2012; Ruedi *et al.*, 2013). Members of this genus exhibit little morphological differentiation across multiple species complexes, also presenting frequent convergent evolution, resulting in one of the most cryptic genera among bats (Ruedi *et al*., 2001; Stadelmann *et al.*, 2007; Platt *et al.*, 2018).

In the Baja California peninsula, *Myotis* is the most abundant and understudied genus of bats, where their mostly cryptic morphology and elusive behaviour has been one of the main determinants. This thesis focuses on the evaluation of the taxonomic relationships of three sympatric putative *Myotis* species in the Baja California peninsula: *Myotis californicus, M. peninsularis* and *M. yumanensis.* Two sites on the Mexican continent were also included with the purpose of comparing the continental *M. velifer*, which exhibits cryptic morphology and is currently under ambiguous taxonomic relationships among *M. peninsularis* populations.

1.4.1. Baja California peninsula, Mexico.

The Baja California peninsula (Baja), located in the north west of Mexico, is limited by the Pacific Ocean and the Sea of Cortés (Fig. 1.6) to the west and east respectively, breaking its isolation by a connection to the state of Sonora in the north east, and to California, U. S. A, to the north. It is approximately 1,315 km in length, spanning nine degrees of latitude (Case, Cody and Ezcurra, 2002). It has a mountainous interior, mostly distributed along the eastern side of the peninsula. It has an altitudinal range from sea level up to 3150m in the Sierra de San Pedro Martir in the north, and to 2,080m in the Sierra la Laguna in the south (Riemann and Ezcurra,

2005; Ramirez-Acosta *et al.*, 2012). It is surrounded by 32 islands that vary in age, distance to peninsula or continent, and vegetation type (Frick, Hayes and Heady, 2008; Álvarez-Castañeda and Murphy, 2014), and which have been colonized by organisms from both the peninsula and the continent (Case, Cody and Ezcurra, 2002).

This region has a large number of species with restricted or local distribution, isolated not only by the geography of the peninsula, but also due to climatic ranges, ecological gradients and vegetation types. There are high levels of plant (Riemann and Ezcurra, 2005; González-Abraham, Garcillán and Ezcurra, 2010; Vanderplank, Rebman and Ezcurra, 2017) and animal endemism (Álvarez-Castañeda and Ríos, 2011; Nájera-Cortazar, Álvarez-Castañeda and De Luna, 2015; Herrera, Flores-Martínez and Sánchez-Cordero, 2017; Álvarez-Castañeda and Nájera-Cortazar, 2020). The approximate number of terrestrial vertebrate species is 754, of which 42 (6%) are endemic (reptiles 15%, mammals 6%, birds 2%) (Ramirez-Acosta *et al.*, 2012). The peninsula has seven natural protected areas along its length (Table 1.2, Fig. 1.6), six of which cover most the territory (Riemann and Ezcurra, 2005). There are also abundant marine resources, especially in the Upper Gulf of California, where there are several commercially important and endangered species distributed, like the totoaba (*Totoaba macdonaldi*) and the vaquita marina (*Phocoena sinus*) (Aragón-Noriega *et al.*, 2010). All this together makes the Baja California peninsula a region with a vast reservoir of natural resources holding opportunities to discover its biodiversity and evolutionary potential.

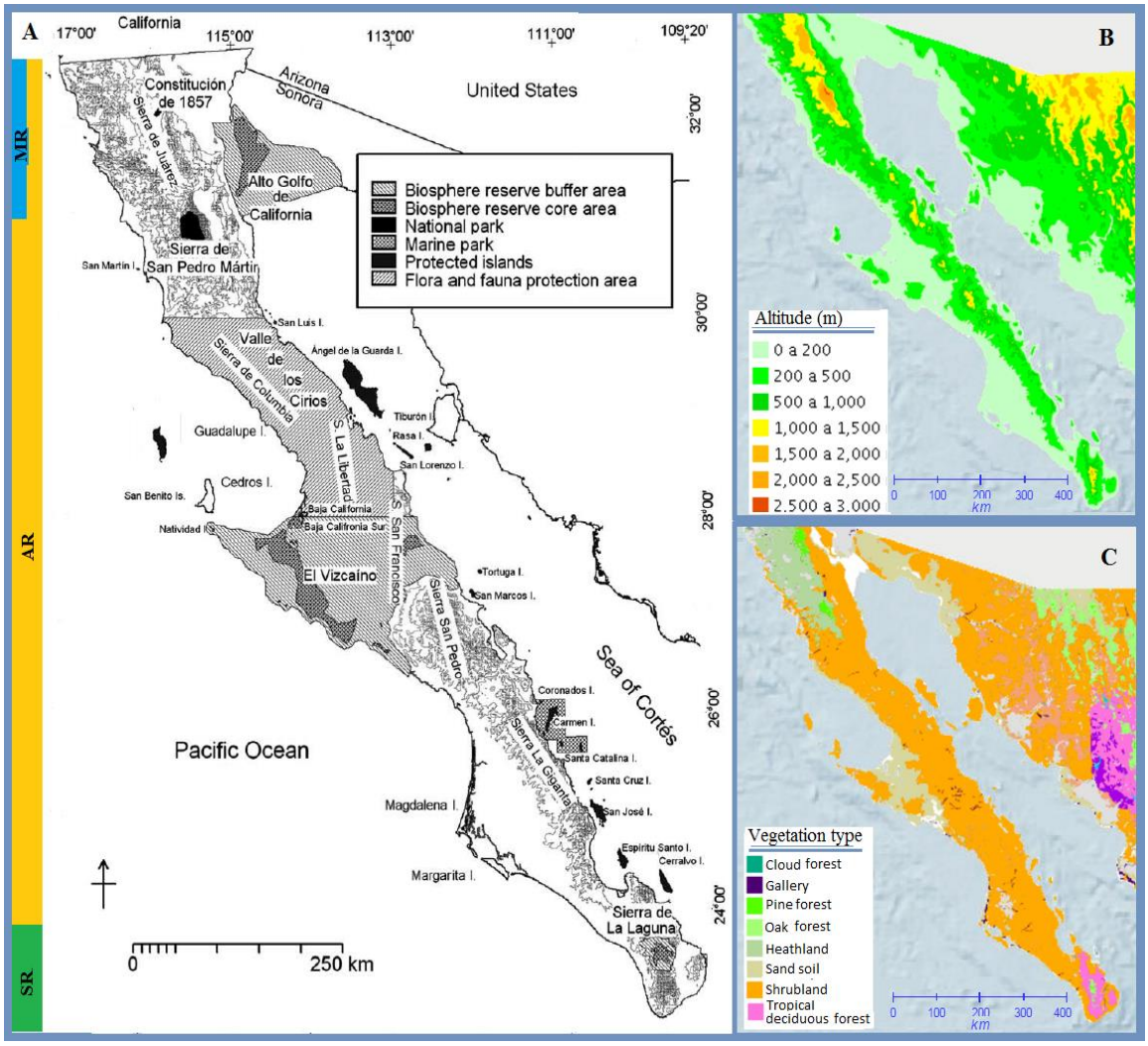


Figure 1.6. A, Natural protected areas in the Baja California peninsula and adjacent islands. The colour bar in the left indicates its three simplified vegetation regions: SR, Subtropical (green); AR, Arid (yellow); and MR, Mediterranean Region (blue). B, Hypsometry, and C, vegetation types in Baja. Modified from Riemann and Ezcurra (2005), and CONABIO (2012), respectively.

Table 1.2. Protected areas in the Baja California Peninsula. Modified from Ramirez-Acosta *et al.* (2012).

Protected area	Category	Area (ha)	Communities	Date of decree
Constitucion de 1857	National Park	4,950	Pine forest	SEMARNAT April/27/1962
Sierra de San Pedro Martir	National Park	72,910	Chaparral and pine forest	SEMARNAT April/26/1947
Valle de los Cirios	Flora and Fauna Protection Area	2,524,930	Desert scrub	SEMARNAT June/07/2000
Alto Golfo de California y Delta del Río Colorado	Biosphere Reserve	934,756 769,976[a]	Sand dunes, halophilic scrub[a]	SEMARNAT June/10/1993
El Vizcaino	Biosphere Reserve	2,546,790	Desert scrub, coastal dunes, halophilic scrub, mangroves	SEMARNAT November/30/ 1988
Sierra La Laguna	Biosphere Reserve	112,437	Pine oak forest, tropical dry forest, palm oases, columnar cacti and desert scrub	SEMARNAT June/06/1994
Cabo Pulmo	National Park	7,111 3,402[b]	Coral reef[b]	SEMARNAT Jun/07/2000
Cabo San Lucas	Flora and Fauna Protection Area	3,996 554[b]	Submarine sand fall[b]	SEMARNAT June/07/2000

[a] This area represents only the land and peninsular section of the reserve buffer area.
[b] This area represents the portion land of the protected area.

The unique characteristics of Baja peninsula have stimulated many studies of the processes that formed this region (Salinas-Zavala *et al.*, 1990; Riddle *et al.*, 2000; González-Abraham, Garcillán and Ezcurra, 2010). Within the geological history of the peninsula (Fig. 1.6), four vicariant events are postulated to have taken place during the Late Miocene to middle Pleistocene (Riddle *et al.*, 2000; Riemann and Ezcurra, 2005). These are the development of divisions between subtropical thornscrub (green) or desert (orange) biotas (Fig. 1.7), which gave rise to a middle Pleistocene mid-peninsula seaway; a late Pliocene northward transgression of the Sea of Cortés; and a Pliocene seaway across the southern peninsular Isthmus of La Paz. Riddle *et al* (2000) examined 12 mammalian, avian, amphibian, and reptilian species distributed in Baja, to assess the role of these vicariant events in the evolution and distribution of the taxa, finding strong correspondence with these events and their

phylogeographic structure (Riddle *et al.*, 2000). They also found a deep level of divergence between continental and peninsular taxa, supporting a Peninsular Desert differentiated from the more widespread Sonoran Desert, holding biota with cryptic evolutionary history and ecological patterns (Riddle *et al.*, 2000). This biological diversity that has evolved in step with the formation of Baja has the potential to provide natural model systems for understanding speciation processes.

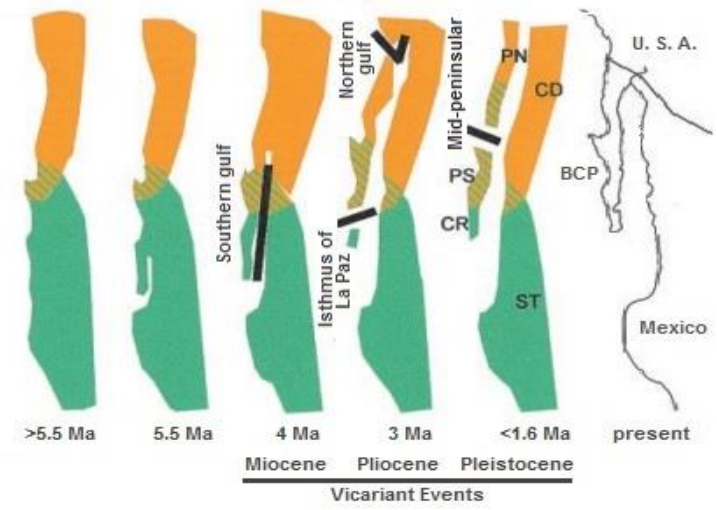


Figure 1.7. Simplified Late Miocene to middle Pleistocene geological history of the Baja California peninsula, and its areas of endemism resulting from vicariant events: PN, Peninsular north; PS, Peninsular south; CR, Peninsular Cape Region; CD, Continental Deserts; and ST, Continental Subtropical. Modified from Riddle *et al.* (2000).

Biogeographic patterns of populations over Baja are shaped by the many different ecological gradients that it holds. The current distribution of habitat types is essentially a continuous arid region covering the middle section of the peninsula (Fig. 1.8), between a subtropical portion in the south and a Mediterranean section in the north (Fig. 1.6) (CONABIO, 1997; Case, Cody and Ezcurra, 2002; Riemann and Ezcurra, 2005). This habitat distribution could be limiting population dispersal to some extent (Huber *et al.*, 2007; Traba *et al.*, 2010;

Marques *et al.*, 2016), forcing populations that are less tolerant of arid environments to be restricted to either extreme, and those more tolerant occupying larger territories, including transition zones dominated by arid vegetation. Using the cytochrome oxidase sub. III mtDNA marker, Riddle *et al*. (2000) found phylogeographic structure in six taxa distributed along Baja, where five mammal groups presented the same pattern of northern and southern peninsular phylogroups (Riddle *et al.*, 2000). Ecological differentiation along Baja appears as a consequence of all the historical and current geographical and ecological characteristics of the peninsula (Riddle *et al.*, 2000; Case, Cody and Ezcurra, 2002; De Queiroz, 2007), holding an important source of ecological differentiation that can be studied both historically and as an ongoing process.

Figure 1.8. Peninsular desert landscape, showing some of the common plants in "The Valley of the Cirios", including the rare Boojum tree or cirio, *Fouquieria columnaris*, endemic to north western Mexico (extreme right).

1.4.2. *Myotis* bats in Baja

There are 25 species of bat across the Baja California peninsula, from seven families (Hall, 1981). The vespertilionid family is the most abundant in this region with 14 species, and some of the best represented include *Antrozous pallidus, Eptesicus fuscus* and *Parastrellus hesperus* (Bogan, 1999). There are nine listed species from the *Myotis* genus distributed to some extent in Baja, and potentially two more species that could hypothetically be present at the northern tip of Baja (*M. lucifugus* and *M. velifer*, represented by "?" in Table 1.3), because their range edge overlaps within the peninsula (Fenton and Barclay, 1980; Fitch, Shump and Shump, 1981). The *Myotis* species most commonly found in Baja surveys are *M. californicus* and *M. yumanensis*, and including *M. volans*, they are distributed all along the peninsula. The other members of this complex are mainly distributed over the northern part of the peninsula, with the exceptions of the endemic population of *M. peninsularis* restricted to the south, and *M. vivesi*, restricted the coastal zones of the Sea of Cortés (Álvarez-Castañeda and Bogan, 1998; Bogan, 1999; Braun *et al.*, 2015). All *Myotis* are insectivorous, with the exception of *M. vivesi* which feeds on marine vertebrates and invertebrates. For Baja, data from *Myotis* bats are mostly focused on species richness around sampling points, general distribution range and taxonomy (Álvarez-Castañeda and Bogan, 1998; Álvarez-Castañeda and Patton, 1999; Rios and Álvarez-Castañeda, 2002), morphological assessments (Ospina Garcés, 2010; Nájera-Cortazar, Álvarez-Castañeda and De Luna, 2015), with biogeographical (Frick, Hayes and Heady, 2008) and limited molecular analyses for some species (Stadelmann, Herrera, *et al.*, 2004; Ortega and Maldonado, 2007; Nájera-Cortazar, Álvarez-Castañeda and De Luna, 2015).

Table 1.3. Distribution of *Myotis* species in the Baja California peninsula (BCP), range and conservation status from IUCN. Abbreviations are: MX, Mexico; US, United States; CAN, Canada; GT, Guatemala; HON, Honduras. The "?" represents a possibility for that species to be present at northern BCP. *Myotis milleri* is considered a synonym of *M. evotis* (Bogan 1999; IUCN 2020).

Myotis species	Vegetation/region in BCP			Range	IUCN
	Subtropical/ South	Arid/ Middle	Mediterranean/ North		
californicus	*	*	*	Western MX-US-CAN	LC
ciliolabrum				BCP, western MX-US-CAN	LC
evotis/milleri		*	*	BCP, western MX-US-CAN	LC
lucifugus?			*?	US-CAN	LC
melanorhinus			*	Central MX and north BCP western US-southern CAN	LC
peninsularis	*			Southern tip BCP, MX	EN
thysanodes			*	Central MX-western US-southern CAN	LC
velifer?			*?	MX-Mid and southern US-GT HON	LC
vivesi		*		BCP coast, MX	VU
volans	*	*	*	BCP, western MX-US-CAN	LC
yumanensis	*	*	*	BCP, western MX-US-CAN	LC

As a general issue for the *Myotis* genus, cryptic morphology is present around the peninsular species. In Fig. 1.9 are shown some of the *Myotis* captured along this project, exemplifying the poor external morphological variation present among them, with the exception of the highly specialised fishing bat *M. vivesi* (Blood and Clark, 1998), shown in picture H rom Fig. 1.9. In general, *M. peninsularis* (A, Fig. 1.9), has been distinguished from other *Myotis* in Baja by its larger size and restricted distribution (Álvarez-Castañeda and Bogan, 1998; Nájera-Cortazar, Álvarez-Castañeda and De Luna, 2015). From *M. velifer* (B, Fig. 1.9), its closest related species (Nájera-Cortazar, Álvarez-Castañeda and De Luna, 2015), lacks external morphological variation but is smaller in size and lighter fur colour. However, neither size or fur colour are determinant factors to discriminate among bat species with wider distribution nor large fur colour variation, as *M. velifer* is (Fitch, Shump and Shump, 1981). Other similar characteristics between *M. peninsularis* and *M. velifer* would be the presence of a furless spot in the dorsal base of the neck and absence of a keeled calcar (Álvarez-Castañeda

and Bogan, 1998). *M. yumanensis* (D and E, Fig. 1.9) mainly differs from other *Myotis* from Baja also by the absence of a keeled calcar (Braun *et al.*, 2015); and compared to *M. peninsularis*, the determinant characteristics would be the attachment of the plagiopatagium to side of foot in *M. peninsularis*, while in *M. yumanensis* plagiopatagium would be attach at the level of the fingers (Álvarez-Castañeda and Bogan, 1998). Only size and fur colour are considered for make a distinction for *M. yumanensis* when compared with *M. thysanodes* (Braun *et al.*, 2015), and bigger feet when compared with *M. californicus* (F and G, Fig. 1.9). There are mainly skull differences between *M. californicus* and *M. ciliolabrum*, where externally, they may be distinguished by the pattern of face fur colour and length of the snout (Simpson, 1993). However, these characteristics are not practical for field identification.

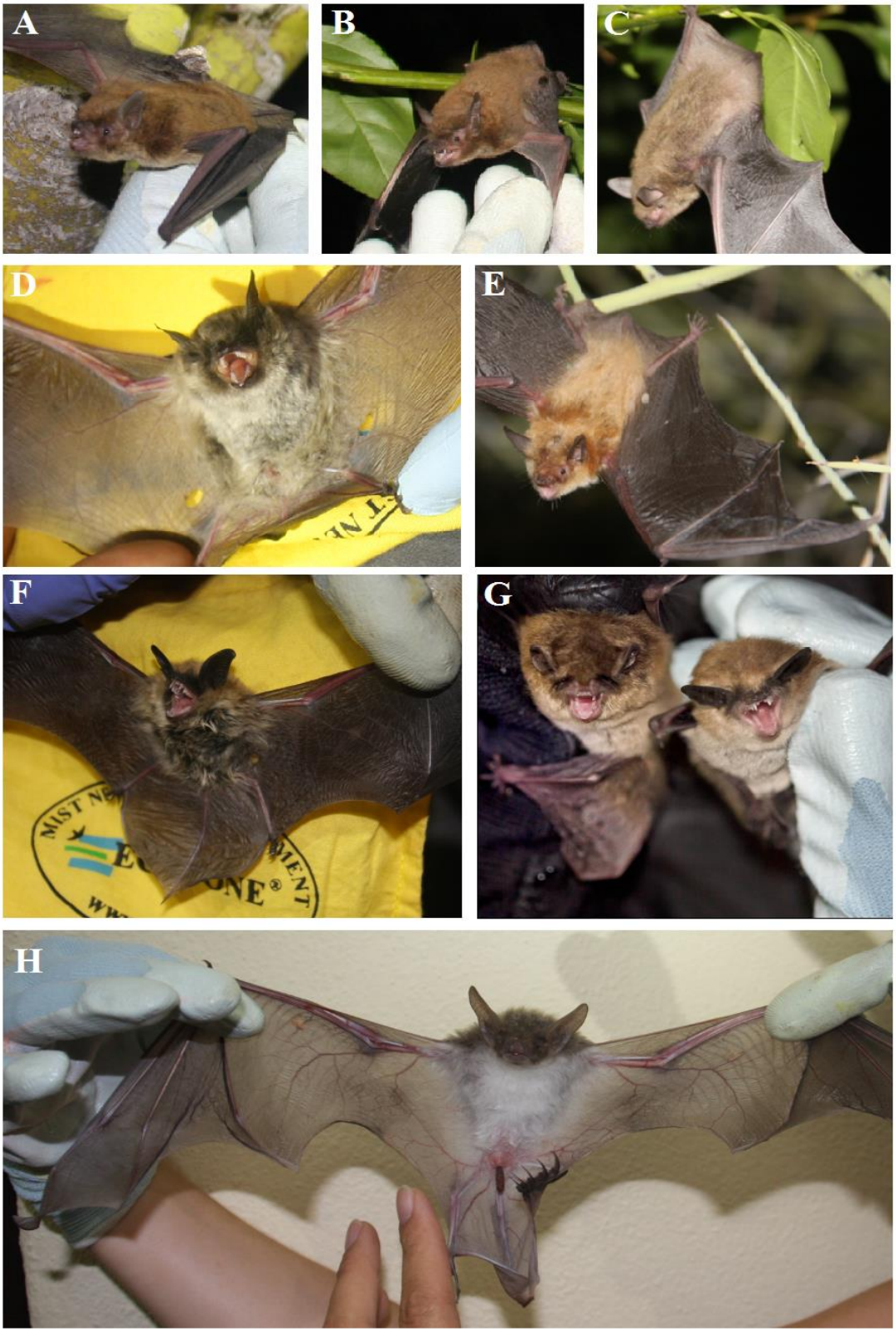


Figure 1.9. Pictures of some of the *Myotis* bats sampled in this study. A, *peninsularis*; B, putative *velifer*; C, *sv*; D, *yumanensis* 1; E, *yumanensis* 2; F, *californicus*; G, *volans* (left) and *californicus* (right); and H, *vivesi*.

Taxonomic relationships among the *Myotis* in Baja are poorly explored, and currently based only on a few molecular studies that do not include all the *Myotis* in the same analysis (Stadelmann *et al.*, 2007; Larsen*et al.*, 2012). The first molecular phylogenetic analysis including *M. peninsularis* was performed using mitochondrial DNA (COI and Cyt*b* markers) and geometric morphometric analysis (Nájera-Cortazar, Álvarez-Castañeda and De Luna, 2015), clustering along the *M. velifer* clade. They showed <2% genetic divergence between them, which falls in the intraspecific divergence range among bats (Bradley and Baker, 2001). Morphometric results from the same study demonstrated that there was also significant differentiation for each population analysed, but this variation fell within the expected intraspecific level of variation among *M. velifer* populations (Nájera-Cortazar, Álvarez-Castañeda and De Luna, 2015), concluding *M. peninsularis* and *M. velifer* could potentially be considered the same species. However, only few mitochondrial sequences from both species were used, and more resolution in combination with nuclear markers is needed to fully understand their taxonomic relationships and ecological complexity. As with *M. peninsularis*, there is little information for other *Myotis* species on detailed local distributions, ecology, mating systems and seasonal movements; and even less information about the pathogens and parasites they may host. It would be expected to discover more cases of cryptic lineages involving other *Myotis* within the outcomes of this study.

1.4.3. Potential bat-ectoparasite community structure in different environmental gradients

The host-parasite relationship is generally thought to result from a complex interaction between congruent phylogenetic, behavioural and ecological associations from both host and

parasite (Bruyndonckx *et al.*, 2009; van Schaik *et al.*, 2014). Parasites will hold and adapt to diverse strategies to colonize and survive into their host, which will be influenced by specificity on the host and seasonality (Sándor *et al.*, 2019; Wu *et al.*, 2019). Therefore, the host is the main environment of the parasite community, which will be also directly driven by environmental factors affecting the host (Bruyndonckx *et al.*, 2009). Bat ectoparasites have evolved to be able to cope with bat morphology and biology, like flying, thermal tolerance, behavioural aggregation etc. (Sándor *et al.*, 2019), conferring host-specific interaction over most of the ectoparasite species (van Schaik *et al.*, 2014; Estrada-Villegas *et al.*, 2018; Ossa *et al.*, 2019; Sándor *et al.*, 2019).

Environmental gradients may have a strong effect on infestation probabilities, and community structure of ectoparasites (Wu *et al.*, 2019). A study of prevalence and co-occurrence of lizard mites and ticks along environmental gradients found that mite infestation increased with vegetation cover and altitude, whereas tick infestations were higher, with more human disturbance and presence of livestock (Wu *et al.*, 2019). The peninsular landscape is a temperate-subtropical transitional zone, where variation in vegetation and habitat aridity seems to be associated with richness of bat communities in this desert ecosystem (Frick, Hayes and Heady, 2008). Along elevational gradients, patterns of bat richness in transitional zones can be related to a combined relationship between temperature and water availability (McCain, 2007). Assessing variation in richness and diversity of bats and their ectoparasites would help to understand distributional patterns and how bats and ectoparasites are affected by environmental factors.

Diversity of vegetation types and geographical barriers over Baja represent an interesting system to study bats and their ectoparasites composition, where their distributions seem to be related to ecological and environmental gradients and limited by differences in habitat types and resources regardless of their vagility (Frick, Hayes and Heady, 2008). There are only a few studies that describe the parasite repertoire of the peninsula, where works are focused on single host-parasite systems, usually of agricultural/economic importance. Existing works include parasites and ectoparasites in fish (Méndez *et al.*, 2010; Rodríguez-Santiago and Rosales-Casián, 2011), in lizards (Veiga *et al.*, 2000), owls (Bolaños-García, Rodríguez-Estrella and Guzmán-Cornejo, 2018) and rodents (Light, Durden and OConnor, 2020); usually related to list of records found on each species, prevalence and disease on livestock from small areas of Baja. Usinger (1966) described bugs from the family *Cimicidae* with distribution over North America, including Baja, but other ectoparasite occurrence is not listed or published. Given their zoonotic potential and their role in the ecology of diseases in wildlife, humans, agricultural and human companion animals, the importance of studying ectoparasites and their pathogens is becoming a matter of significant relevance.

1.5. Thesis aims and outline

In this thesis, I aim to: 1) evaluate the genetic diversity, taxonomic relationships and species boundaries of sympatric *Myotis* bat species distributed in the Baja California peninsula and northwestern Mexico, focussing on *Myotis californicus*, *M. peninsularis*, *M. velifer* and *M. yumanensis*. For this, the specific objectives are to: i) examine the taxonomic species identity of the *Myotis* bats sampled for this study using phylogenetic analyses with the mitochondrial cytochrome *b* marker; ii) to evaluate population genetic structure and species boundaries assessed through SNP variation using ddRAD sequencing; and, iii) obtain evidence for introgression and hybridisation within the *Myotis* bats. In this thesis, I also aim to: 2) evaluate the taxonomic identity of bat ectoparasites distributed along the same region, using phylogenetic analysis using the mitochondrial cytochrome oxidase subunit I marker, and the nuclear ribosomal 18S marker; and, 3) examine how bat-ectoparasite community structure is influenced by environmental variables, and host distribution, and/or host diversity. I briefly describe these aims in the following Chapters descriptions:

In Chapter 2, I describe the genetic diversity, evolutionary potential and species boundaries of a complex of sympatric *Myotis* bats, by performing a phylogenetic analysis using the mtDNA cytochrome *b*, plus population structure analysis using SNP nuclear data with ddRAD techniques. I aim to investigate genetic differentiation and population structure among the *Myotis* studied, particularly to gather evidence of hybridisation and introgression. I also aim to evaluate what levels of genetic differentiation are between the putative *M. peninsularis* and *M. velifer*. This work contributes with information regarding species boundaries in the worldwide cryptic *Myotis* genus, and discovers the complexity of the *Myotis* diversity in Baja.

Chapter 3 provides the first description of the bat ectoparasite diversity in the peninsula and an analysis of the phylogenetic relationships of bat bugs, bat flies and bat ticks. I aim to understand the ectoparasite diversity associated with bats along the peninsula. I describe multiple novel lineages for Baja, including the possibility of the discovery of new diversity for some of the ectoparasite groups. I also suggest how ectoparasite haplotypes can be an important tool for describing patterns of bat dispersal through North America.

In Chapter 4, I aim to evaluate how bat population structure is related to ectoparasite diversity found over Baja, and if there is any environmental pattern along their distribution. For this, I integrate the molecular diversity information of bats generated from this study to evaluate their community structure. I also analyse how the environmental gradients in Baja influence their diversity and distribution, and their implications for the discovery and dispersal of bat pathogens.

Finally, in Chapter 5, I discuss the results of the whole thesis as I integrate them to understand the main drivers of genetic differentiation in the *Myotis* system. I discuss the opportunities that the use of high-resolution genomic techniques can provide for a wider view of the evolutionary processes shaping bat diversity in the Baja California peninsula, and in any other cryptic ecosystem. I also highlight the need for increasing research regarding bat ectoparasites, fauna that have been neglected despite their importance as vectors of other pathogens of medical importance. I finish by prospecting the future work within this system, the still hidden diversity of bats and pathogens that need to be studied, and the future implications for the conservation of bats in North America.

Chapter 2: Ecological genomic structure of a complex of *Myotis* bats in the Baja California peninsula, Mexico.

2.1. Abstract

A fundamental question in evolutionary biology is to what extent gene flow limits ecological differentiation and speciation. High-throughput sequencing technologies can now generate high resolution, genome scale perspectives on how introgression and hybridisation occurs in face of gene flow. We investigated the evolutionary interactions and species boundaries of a complex of *Myotis* bats in the Baja California peninsula, Mexico and two continental sites. The taxonomic status of the putatively peninsular endemic *Myotis peninsularis*, is currently ambiguous. Our results demonstrate mitochondrial haplotypes from individuals phenotypically identified as *M. peninsularis*, mostly form a distinct haplogroup closely related to *M. velifer*. However, phylogenetic data clustered *M. peninsularis* along with *M. velifer* individuals. ddRAD SNP data results also clustered both species together, and showed evidence of introgression from these populations among bats sampled in mid-peninsula and a continental population. We identified this continental population as a potentially new phenotypically cryptic *Myotis* lineage, which is morphologically similar to *M. velifer*, but which has a mean sequence divergence at cytochrome *b* of more than 7% with the closest reference sequences in GenBank, also forming a distinct cluster with the ddRAD SNP data. Using mitochondrial phylogenetic analysis, we identified other potential cryptic *Myotis* lineage that was first identified as *M. volans*; which will need further investigation. Bayesian cluster analysis identified admixture among *Myotis yumanensis* individuals at several sites, reflecting gene flow with each of the other three *Myotis* species in the study. PCA and DAPC cluster analyses confirmed the Bayesian structure of the *Myotis* species complex, but detecting two different clusters for *M. californicus* individuals. With the SNP structure analysis, we detected

high levels of genetic differentiation and population structure within *M. californicus* populations, suggesting both local and long-distance dispersal. In contrast, there was poor structure among *M. yumanensis* individuals, which also showed conflictive phylogenetic clades, suggesting female philopatry and long distance male mediated dispersal. Our study represents an important step forward for the discovery of novel genetic variation and understanding species boundaries in New World *Myotis* bats, contributing also to inform the development of conservation strategies for *Myotis* bats in North America.

2.2. Introduction

The mechanisms by which genetic differentiation develops within and among species, and their relationship with the environment is an important topic for conservation and evolutionary biology (Jones *et al.*, 2012; Arnegard *et al.*, 2014). Distribution and dispersal of populations are limited by both physical and ecological barriers, plus the inherent characteristics of each organism. By quantifying genetic population structure, patterns of gene flow and demography within species can be characterised, and help to identify hybridisation and potential introgression among species (Lindtke *et al.*, 2013; DaCosta and Sorenson, 2014, 2016). High-throughput sequencing approaches now allow high-resolution analyses of population structure and patterns of gene flow in species with complex patterns of differentiation and introgression. In this Chapter, the population structure and species boundaries in a complex of sympatric *Myotis* bats distributed along the Baja California peninsula will be characterised using mitochondrial cytochrome *b* (Cyt*b*) haplotypes and double digest Restriction-Site Associated DNA (ddRAD) derived Single Nucleotide Polymorphism (SNP) markers.

High performance methods like ddRAD sequencing can recover thousands of loci and SNP data, allowing assessment of genetic variation at the genome scale. ddRAD sequencing therefore enables a wide range of population genetics studies and improves power and resolution for population parameter estimations (Davey *et al.*, 2011; Narum *et al.*, 2013). Research implementing ddRAD techniques has increased in the last years for population genetic (Peterson *et al.*, 2012; DaCosta and Sorenson, 2014; Alter, Munshi-South and Stiassny, 2017; Lavretsky, Janzen and McCracken, 2019), phylogenetics and phylogeographic analyses (DaCosta and Sorenson, 2016; Kobayashi *et al.*, 2018). ddRAD techniques can be particularly efficient for recovering weak patterns of population structure when dealing with species which disperse over large distances and/or have cryptic morphology, which as commonly seen in bats (Chattopadhyay *et al.*, 2016).

Species discovery and evaluating genetic boundaries among cryptic bat species are on-going tasks with conservation priority (Teeling *et al.*, 2018), highlighting the importance of efforts to understand worldwide bat diversity. Further, quantifying how genetic diversity is distributed in bat populations, provides tools for understanding how bats have adapted to varying ecological gradients throughout their ranges, and may respond to habitat fragmentation and changes in future environmental conditions (Razgour *et al*., 2014; Pylant *et al.*, 2016). Bats can be sensitive to finer-scale levels of geographic or ecological isolation (e.g. differences in vegetation types and level of aridity associated with richness of bat communities in desert ecosystems), reducing dispersal and promoting population structure (Frick, Hayes and Heady, 2008; Moussy *et al.*, 2012; Hua *et al.*, 2013; Morales *et al.*, 2017).

Investigating bat genetic diversity is also very important for evaluating their interaction with pathogens, and their potential to act as disease vectors for other bats, wildlife and humans. One example is the high mortality caused in bats by the fungus *Pseudogymnoascus destructans*, which causes a disease called White Nose Syndrome (Frick *et al.*, 2015). Using RADseq analyses, the population genetic structure of the bat *Myotis lucifugus* was examined to identify its relationships with the spread of this fungal disease in Eastern North America, and the fungus's potential expansion to western North America (Wilder, Kunz and Sorenson, 2015). The authors concluded that the high levels of genetic structure in western populations would act as a barrier of spread, reducing the risk of *P. destructans* spreading westwards carried by *M. lucifugus* (Wilder, Kunz and Sorenson, 2015). Such examples show that characterising both host and pathogen genetic diversity and population structure (Brook and Dobson, 2015; Teeling *et al.*, 2018), as well as modelling future scenarios of spread (Frick *et al.*, 2015; Plowright *et al.*, 2017; Hoyt *et al.*, 2018; Albery *et al.*, 2019), should be a priority for preventing future spill-overs and developing management strategies.

Detection of introgression and hybridisation in bats has been reported in several studies, suggesting that it may be a common process among related bat species (Carstens *et al.*, 2004; Miller-Butterworth *et al.*, 2014; Moussy *et al.*, 2015; Morales *et al.*, 2017). Using a ddRAD approach, Chattopadhyay *et al*. (2016) evaluated the morphological and genetic disparity in *Cynopterus sphinx* and *C. brachyotis* fruit bats from two natural contact zones and 17 allopatric sites in India. They did not detect hybridisation occurring among these two species in the contact zones. However, they found evidence for a new Cynopterine lineage, confirmed by the SNP data and mtDNA analysis, detecting signs of introgression and potential hybridisation between this new lineage and *C. sphinx* (Chattopadhyay *et al.*, 2016). Multi-species swarming

sites can be a common phenomenon for sympatric bat species, increasing the probabilities for introgression and hybridisation (Berthier, Excoffier and Ruedi, 2006; Bogdanowicz, Piksa and Tereba, 2012b; Chattopadhyay *et al.*, 2016; Centeno-Cuadros *et al.*, 2020). However, exactly how common introgression is among bats, and what the evolutionary implications influencing species boundaries are, remain to be fully explored.

The *Myotis* genus has a global distribution and is one of the most speciose among bats, with many cases of cryptic morphology (Simmons, 2005; Stadelmann *et al.*, 2007; Larsen *et al.*, 2012; Morales *et al.*, 2017). There are potentially 10 *Myotis* species distributed sympatrically along the Baja California peninsula (ranges described in Chapter 1, Table 1.3), including species which have wide continental distributions in North America, as well as putatively endemic species. The Baja California peninsula (hereafter referred to as Baja; Fig. 1.6), has a unique combination of habitats which vary over latitudinal and altitudinal gradients which are important in shaping its biodiversity (McCain, 2007; Piksa *et al.*, 2013). *Myotis* species' ecological requirements over peninsular habitats are not very well studied, but in general, they seem to follow those describe for each species in other regions (Fitch, Shump and Shump, 1981; Simpson, 1993; Álvarez-Castañeda and Bogan, 1998; Braun *et al.*, 2015). *Myotis californicus*, *M. velifer* and *M. yumanensis* have one of the largest sympatric distributions for bats in western North America, while *M. peninsularis* is putatively endemic to the southern tip of the peninsula (Fig. 2.1). Investigating the processes driving the different *Myotis* species' distributions over Baja will help to understand each species ecological constraints with respect to environmental variation.

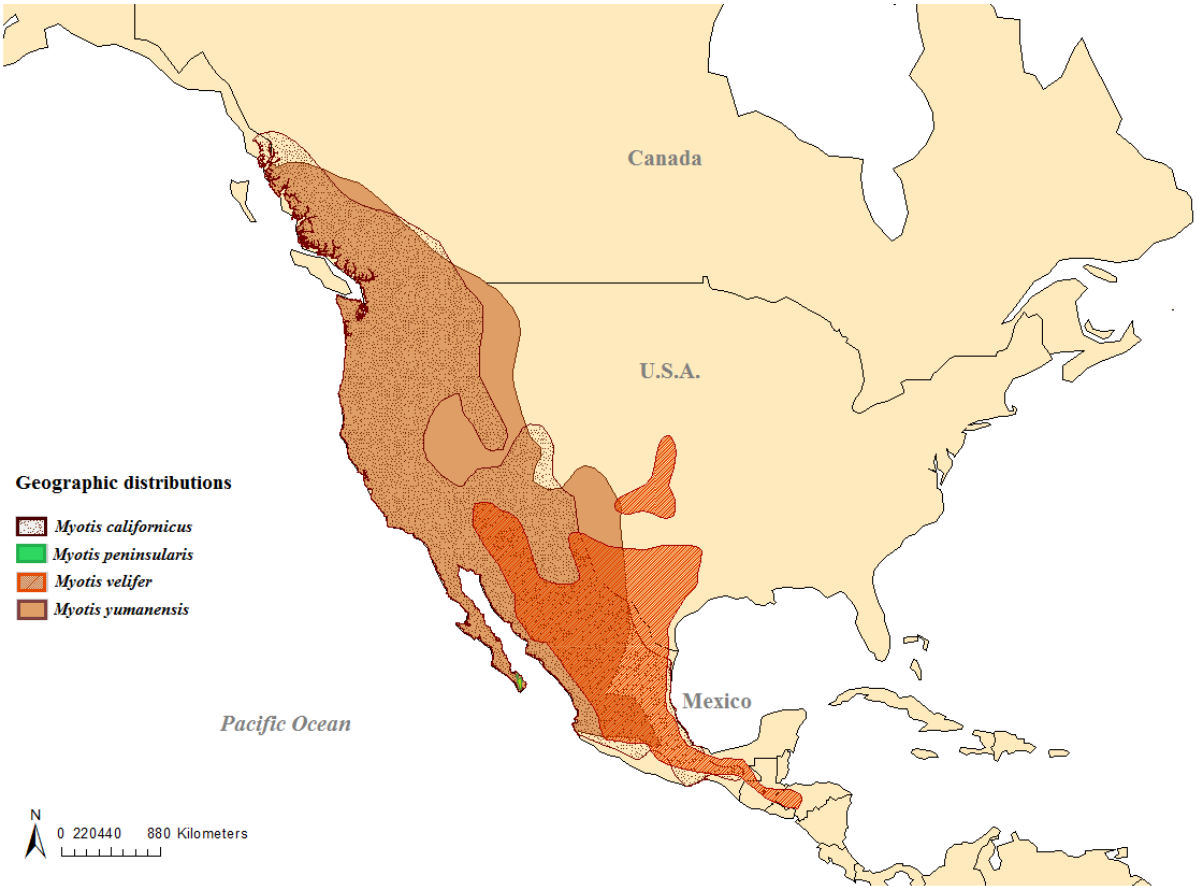


Figure 2.1. Distribution of the *Myotis* bat complex targeted for this study: *Myotis californicus, M. peninsularis, M. velifer* and *M. yumanensis.*

The taxonomic status of the putative endemic *Myotis peninsularis* (Álvarez-Castañeda and Bogan, 1998; Nájera-Cortazar, Álvarez-Castañeda and De Luna, 2015), which is restricted to the tropical deciduous forest area (see Chapter 1 for a detailed relationship description with *M. velifer*), is currently ambiguous given its genetic proximity to *M. velifer* and *M. yumanensis* (Nájera-Cortazar, Álvarez-Castañeda and De Luna, 2015). The distribution of *M. velifer* is so far documented to barely reach south-eastern California (Fitch, Shump and Shump, 1981; Parlos *et al.*, 2008; Krutzsch, 2009; Solari, 2019), where it is sympatric with *M. yumanensis* and *M. californicus* (Fig. 2.1). In the south of the peninsula, both *M. peninsularis* and *M. yumanensis* have sympatric distributions, and potentially share roosting sites with *M. californicus* (Simpson 1993). The latter species can be difficult to differentiate morphologically

from *M. ciliolabrum, M. leibii* and even *M. melanorhinus* (Simpson, 1993; Holloway and Barclay, 2001; Rodriguez and Ammerman, 2004), where so far, these bats are distinguishable only by skull characters. Based on field observations during this project, a continuum of morphological features were observed among this complex of *Myotis* bats, making their taxonomical identification challenging by using simple morphological characters only.

This study aims to re-evaluate the taxonomic relationships of *M. californicus*, *M. peninsularis, M. velifer* and *M. yumanensis*, using mitochondrial Cytochrome *b* and ddRAD SNP markers. We conducted population genetic and phylogenetic analyses to quantify patterns of population structure across the Baja California peninsula and two continental sites. We hypothesize that some level of admixture is occurring among sympatric bats in the south of Baja, which may account for the intermediate phenotypic features observed in bats of this region.

2.3. Methods

2.3.1. Ethics and permits.

All bat handling was carried out under the approval of the ethics committee of the Faculty of Biological Sciences, University of Leeds (AWCNRW170615); and following the Guidelines of the American Society of Mammalogists (Sikes *et al.*, 2016). Sampling was carried out under the permits SEMARNAT-DGVS-008972-16 and SEMARNAT-DGVS-001642-18 issued by Secretaría del Medio Ambiente y Recursos Naturales (SEMARNAT) in Mexico. The latter included two *Myotis* bat species listed in the Mexican Official Norm for the protection of native species of flora and fauna in Mexico (NOM-059-SEMARNAT-2010,

(Secretaría de Medio Ambiente y Recursos Naturales, 2010)), under the Pr (under special protection) and P (in danger of extinction) categories (*M. evotis* and *M. vivesi*, respectively); and sampling on protected reserves. When required, permission was also solicited and granted from private land owners. All samples were imported into the UK under permission of the Department for Environment, Food and Rural Affairs (DEFRA) from the Animal and Plant Health Agency (permit ITIMP19.0036).

2.3.2. Sampling.

Field work was carried out each summer between 2016 and 2018 covering 26 different sites around the Baja California peninsula, and including three localities in continental Mexico (Fig. 1.1, Appendix 2.1). Sites were chosen to represent the diversity of environmental and ecological gradients across the peninsula. Abbreviations for the complete sites and species labels used for this thesis are listed in Table 2.1. An initial set of sites was identified based on past records of *Myotis* occurrence from the Mammalogy Lab in the Centro de Investigaciones Biológicas del Noroeste, SC, and the mammals database accessed by GBIF (National Museum of Natural History, 2016), and then refined by identifying accessible locations near water bodies. Previously unsurveyed areas were also evaluated in Google Earth (2016-2018, Maxar Technologies, Google), to identify new sites with potential bat habitat. We targeted places with water bodies (natural or anthropogenic), to facilitate efficient bat capture, as bats congregate in such locations to feed and drink. Logistical considerations for safe access within the time and resource constraints of the project were also taken into account.

Table 2.1. List of sites sampled showing number, name, abbreviation (Site label) and geographic coordinates shown at the left of the table. List of bat species sampled and their short labels (Species label) shown at the right of the table. *Myotis sp* refers to any *Myotis* that were not morphologically identified during field, and *Myotis sv* refers to any unidentified *Myotis* morphologically resembling *M. velifer*, found at Ures site.

Site number	Site name	Site Label	Latitude	Longitude	Bat species	Species Label
0	San Diego	SanDi	32.927	-117.176	*Antrozous pallidus*	ANPA
1	Chabacanos	Chaba	32.566	-116.493	*Artibeus hirsutus*	ARHI
2	Mosqueda	Mosq	32.156	-115.279	*Artibeus intermedius*	ARIN
3	Ensenasda	Ense	31.770	-116.520	*Chiroderma salvini*	CHSA
4	Meling	Meli	30.972	-115.744	*Choeronycteris mexicana*	CHME
5	Jolla	Jolla	30.920	-115.601	*Corynorhinus townsendii*	COTO
6	Punta Matzo	Matz	30.436	-116.029	*Eptesicus fuscus*	EPFU
7	San Fernando	SanFe	29.971	-115.237	*Glossophaga soricina*	GLSO
8	Rosarito	Rosa	28.613	-114.047	*Lasiurus xanthinus*	LAXA
9	San Ignacio	SanIg	27.297	-112.898	*Leptonycteris yerbabuenae*	LEYE
10	Cueva Guano	Guano	26.879	-111.987	*Macrotus californicus*	MACA
11	Requeson	Reque	26.638	-111.833	*Mormoops megalophylla*	MOME
12	San Basilio	SanBa	26.371	-111.429	*Myotis californicus*	MYCA
13	Loreto	Loreto	26.012	-111.349	*Myotis evotis*	MYEV
14	San Javier	SanJa	25.862	-111.543	*Myotis peninsularis*	MYPE
15	Pocitas	Poza	24.403	-111.104	*Myotis sp*	MYsp
16	La Paz	LaPaz	24.103	-110.306	*Myotis sv*	MYsv
17	Testera	Teste	23.764	-110.055	*Myotis velifer*	MYVE
18	Parral	Parra	23.748	-110.058	*Myotis vivesi*	MYVI
19	Faro	Faro	23.427	-110.233	*Myotis volans*	MYVO
20	San Pedro	SanPe	23.390	-110.212	*Myotis yumanensis*	MYYU
21	Boca Sierra	Boca	23.385	-109.819	*Natalus mexicanus*	NAME
22	Tesos	Teso	23.175	-109.611	*Nyctinomops femorosaccus*	NYFE
23	Ures	Ures	29.433	-110.376	*Parastrellus hesperus*	PAHE
24	Tucson	Tucs	20.705	-103.336	*Sturnira parvidens*	STPA
25	Primavera	Prima	20.679	-103.602	*Tadarida brasiliensis*	TABR

Mist nets (NHBS) were used for capturing bats, from sunset to midnight. All captured bats were put into individual cotton bags to reduce stress and then released immediately after processing. For each bat, the sex age class, and reproductive status were recorded, along with the following morphometric measurements: Size (mm) of the right forearm, ear, tragus and foot, and mass (gr.). Wing biopsies were taken with a 3mm disposable biopsy punch (Integra, Miltex), one from each wing, in the plagiopatagium as close as possible to the body and avoiding blood vessels, ensuring a natural healing time of 2-4 weeks (Corthals *et al.*, 2015),

stored in 96% ethanol. Wing biopsies were then stored on ice in cool boxes for the duration of fieldwork (up to 30 days). On return to the laboratory samples were stored at -20°C prior to DNA extraction.

Scale-standardised photographs of ventral, dorsal and lateral profiles were taken to support species identifications if needed. Bats were initially identified on site using mammal field guides (Arita and Ceballos, 1997; Medellín, Arita and Sánchez, 2007; Álvarez-Castañeda, Álvarez and González-Ruíz, 2015). In general, bats were successfully identified to species in the field (n = 313), with the exception of 59 *Myotis* bats, that presented ambiguous characters. For the initial designations where individuals could not be identified as definitive species, they were recorded as 'MYsp' for those sampled in La Paz (site number 16), Baja California Sur; and 'MYsv' for those sampled in Ures (site number 23), Sonora (names and short names are listed in Table 2.1). *M. californicus* and its sister species *M. ciliolabrum*, have previously been noted to be challenging to distinguish by morphological methods in field (Rodriguez and Ammerman, 2004). Therefore, all the specimens sampled with a *M. californicus* like phenotype were initially assigned as this species in the field (Appendix 2.1). Additionally, archived tissue samples for seven *Myotis californicus* (n=7) and *M. yumanensis* (n=10) from the San Diego area (records CA1-CA11, Appendix 2.1), were donated by The San Diego Natural History Museum (SDNHM), United States of America and imported to the UK under the same DEFRA permit (ITIMP19.0036).

2.3.3. DNA extraction and mtDNA sequencing.

Laboratory procedures for genetic profiling of *Myotis* bats were performed at the NERC Bioanalysis Facility at The University of Sheffield, U.K. (NBAF Sheffield), under grant

NBAF1151 to S. Goodman and L. Najera Cortazar. DNA from wing tissue was extracted following the ammonium acetate precipitation method described by (Nicholls *et al.*, 2000), with an overnight digestion at 55°C using a rotating oven. DNA concentrations for each sample were quantified using a fluorimeter (FLUOstar OPTIMA; BMG LabTechnologies), and DNA quality was assessed by agarose gel electrophoresis. Samples with low DNA yields were concentrated (Concentrator plus, Eppendorf), and then re-quantified.

A fragment of the Cytochrome *b* gene (Cyt*b*) was amplified using the primers FL15162 (5'-GCAAGCTTCTACCATGAGGACAAATATC-3') and RH15915 (5'-AACTGCAGTCATCTCCGGTTTACAAGA-3') (Irwin, Kocher and Wilson, 1991), with an expected product size of approximately 750bp. PCR amplification for each sample was carried out in a total volume of a 10µl comprising: 4µl QIAGEN master mix (QIAGEN, Germany); 1µl of forward and 1µl of reverse primers (5mM) each; 3µl of sterile ddH2O; and 1µl of DNA template. PCR amplification conditions consisted of three minutes of initial denaturation at 95°C, followed by 35 cycles, each with 20 seconds of denaturation at 95°C and 20 seconds annealing at 50°C, finishing with 90 seconds of extension at 72°C. PCR products were purified using ExoSAP-IT (Thermo Fisher Scientific). Sequencing reactions for both strands were performed using BigDye Terminator 3.1 Cycle Sequencing Kit (Thermo Fisher Scientific). Products were sequenced using an Applied Biosystems (ABI3730) automated sequencer at the NBAF Sheffield node. Both sequenced strands for each sample were then cleaned and aligned by custom Bio-Linux and Perl scripts from the NBAF, generating a consensus sequence. After that, each sequence was inspected individually, quality controlled using BioEdit (Hall, 2005), and aligned along with references sequences.

2.3.4. Phylogenetic and phylogeographic analyses of Cytochrome *b* sequence data

A Nucleotide BLAST search via the NCBI Blast server was performed for each of the final sequences to identify the closest match to each sample. To identify all relevant sequences for comparative phylogenetic analyses, a systematic search was performed using the *AnnotationBurst* 1.2 package (Borstein, 2018) in Rstudio 1.1.456 (RStudio Team, 2015) with the terms "*Myotis californicus*", "*Myotis ciliolabrum*", "*Myotis velifer*", "*Myotis volans*" and "*Myotis yumanensis*", and the arguments "cytochrome *b*" and "Cyt*b"* to retrieve sequences that were available in GenBank. Additionally, sequences of other *Myotis* species listed from previous publications phylogenetically close to the species in this study were incorporated, when not included in the sequences retrieved by *AnnotationBurst* 1.2. A search for metadata was conducted to include the geographical location of the reference sequences when possible, for later use in the phylogeographic analyses. The complete set of sequences were aligned to check for errors in sequence direction, and to remove short (> 500pb) and/or poor-quality sequences (e.g. containing ambiguous or unresolved nucleotide calls).

Summary genetic statistics and haplotype diversity was generated in DNAsp5 (Librado and Rozas, 2009). Initial phylogenetic analysis was carried out in MEGA 10.1.7 (Kumar, Stecher and Tamura, 2016) applying the HKY G+I sequence evolution model obtained as the best fit model for this data in MEGA, with Maximum Likelihood (ML) tree construction, and 1000 bootstrap iterations. Additionally, a Bayesian phylogenetic (BA) analysis was performed in BEAST 1.10.4 (Suchard *et al.*, 2018) under the same model of evolution, with a MCMC chain length of 10,000,000, strict molecular clock under the tree prior Speciation Yule Process (Yule, 1925; Gernhard, 2008) and default priors, previously obtained with BEAUti 1.10.4 (Suchard *et al.*, 2018). Two separate runs were conducted in BEAST of 10,000,000 each with

10% burn-in. After, stationarity of BEAST results were assessed in Tracer 1.7.1 (Rambaut *et al.*, 2018). Both files were combined to obtain the final estimates of divergence using LogCombiner 1.10.4 (Suchard *et al.*, 2018), generating a single .log file and a single .tree file. A majority-rule consensus tree was inferred using TreeAnnotator 1.10.4 (Suchard *et al.*, 2018) with the combined .tree file generated and using a posterior probability limit of 0.6 and median nodes heights were summarised. Tree annotation was performed in iTOL 5.6.2 (Letunic and Bork, 2016).

The software PopArt (Leigh and Bryant, 2005) was used to construct a median-joining network (Bandelt, Forster and Röhl, 1999) of cytochrome *b* haplotypes obtained with DNAsp 5 (Librado and Rozas, 2009), for the whole set with references sequences to evaluate phenotypic assignment vs haplotypes position. A second median-joining network was constructed evaluating haplotypes from the present study only, annotated by geographic region of origin. Finally, an analysis of molecular variance (AMOVA), was used to test the partitioning of mitochondrial genetic variation within *Myotis* species overall, and across sampling localities for species. For this analysis, individuals were grouped based on phylogenetic identification. AMOVA was conducted in Arlequin 3.5.2.2 (Excoffier and Lischer, 2010) using the standard haplotype format with statistical significance assessed by 1000 permutations.

2.3.5. Generation of ddRAD libraries

Laboratory work for generating the ddRAD libraries was carried out in the NBAF Sheffield lab (see section 2.3.3 for more details). The protocol of DaCosta and Sorenson (DaCosta and Sorenson, 2014), with modifications was followed to generate three ddRAD libraries. In total 283 samples were processed, from which 63 were later excluded for quality

control reasons, for example, those samples with low DNA concentration (< 50ng) or with fewer than 100,000 reads after bioinformatic processing. For each sample, a standard 21µl volume of template DNA was digested for more than 18 hours at 37°C with the enzymes *SbfI* and *EcoRI* with the 10X NEBuffer 4 (New England Biolabs Inc.) reaction buffer, for a total volume of 25µl. Depending on extract concentration the starting quantity of DNA for each library varied between 50ng to more than 1000ng. Adapters were ligated to the digested DNA, comprising "P1" Illumina adapters with a unique barcode for each sample; and a "P2" paired-end compatible adapter. Samples were then quantified using a FLUOstar OPTIMA fluorimeter. Size selection was done using low melting point agarose gel electrophoresis (Cambridge Reagents), adding a 300 and 450 bp internal standards to each sample for identifying the selected size range, which was excised from the gel. Each gel fragment was transferred to an individual Eppendorf tube, and then purified using the MinElute PCR Purification kit (QIAGEN, Germany). Size-selected fragments where amplified using Phusion High-Fidelity PCR Master Mix and DNA Polymerase (New England Biolabs, Beverly, MA) using the primers RAD1.F (5′-AATGATACGGCGACCACCGAG-3′), and RAD2.R (5′-CAAGCAGAAGACGGCATACGAG-3′). PCR products were then purified using AMPure XP magnetic beads, transferring the product into a new plate. After this, products were quantified by qPCR using a library quantification kit (KAPA Library Quant Kit, Illumina), and then pooled in equimolar amounts into three libraries. Finally, each library was quantified using a Qubit Fluorometer 3.0 (Invitrogen), and then assessed for quality fragment size distribution and concentration using a TapeStation 4200 (Agilent Technologies). Bioinformatic analyses were carried using the University of Sheffield high performance computing (HPC) cluster, 'Iceberg', and the University of Leeds HPC platform, 'Arc3'.

Libraries were sent to the Next Generation Sequencing Facility at St. James's Hospital, University of Leeds, U.K., where each library was sequenced in an individual lane on an Illumina Hiseq3000, with 150bp paired-end reads. The data was base called using the bcl2fastq 2.17.1.17 (Illumina Inc.) application as documented by Illumina. Quality control procedures included to detect the presence of adaptor sequences and the prevalence of low-quality base calls in each read file was determined using FastQC (Andrews, 2014). Extended runs of low-quality base calls at the 3 and 5' ends of the reads as well as adaptor sequences were removed using Cutadapt 2.10 (Martin, 2011). The success of the data trimming was assessed using FastQC as before. The data was de-multiplexed using the P1 sample specific index, which was removed along with the restriction enzyme site sequences used to make the libraries using a bespoke application developed by the Next Generation Sequencing Facility of the University of Leeds. As qStacks requires all reads to be the same length, reads shorter than 140 bps were discarded, while those longer were trimmed to 140 bp in length. The reference genome assembly approach *ref_map.pl* algorithm was used in the STACKS 2.41 pipeline (Catchen *et al.*, 2013) to identify and call SNPs. Clean reads were aligned to the *Myotis lucifugus* reference genome (Kent *et al.*, 2002; Yu *et al.*, 2016), being the closest available assembled genome for the species from this study, using BWA (Heng and Richard, 2009), prior to SNP calling with the STACKS pipeline using the *mem* algorithm. Only uniquely mapped reads were retained, with the aligned data saved as unordered SAM (Sequence Alignment/Map) files. Once aligned, the reads were ordered by position in the reference sequence and then saved as a BAM (binary SAM version) file using SAMtools (Li *et al.*, 2009). We performed SNP calling for two different sets of species together in order to compare structure and the number of SNPs retrieved, based on phylogenetic taxonomic proximity: A) a global set including *M. californicus, M. peninsularis, M. sv, M. velifer* and *M. yumanensis*; and B) a separate set

including the closest related species, excluding *M. californicus*. We also SNP called three separate different sets of species that were sampled along different geographic locations, to tests population structure including a larger dataset of SNPs per species, based in posterior classifications of individuals from the Global test: C) *M. californicus;* D) *M. peninsularis*; and E) *M. yumanensis*.

The *population.pl* script from STACKS was used to generate unlinked SNPs, creating files including one SNP per locus, eliminating loci with observed heterozygosity of > 0.7 with the function --*max-obs-het*; applying a minor allele frequency cut-off of 0.05 with the function --*min_maf*, and with 80% of missing data per individuals (-*r* parameter). We used different number of populations in which a locus must be present according to each set tested (-*p* parameter), across different sets of individuals (see datasets above): A, $p = 4$; B, $p = 4$; C, $p = 4$; D, $p = 2$; and E, $p = 4$. Each set created a VCF file that were later used for population structure analysis.

2.3.6. Population genetic analyses

SNP data in VCF files were re-sorted using PLINK2 software (Purcell *et al.*, 2007; Chang *et al.*, 2015) to create BED files. Loci obtained were then filtered for Linkage Disequilibrium also in PLINK2 software, using -- *indep-pairwise* with a window size in variant count of 50, to shift in 10 and a variance inflation factor of 0.1. Departures from Hardy-Weinberg were calculated per population using --*hwe*, with a threshold of 0.001, and then outliers removed by VCFTOOLS, using the --*keep* function. BCFTOOLS was then used to index and merge the filtered VCF files. Using the filtered output files, we removed any relatives present in the datasets (leaving one of each pair), using --*king-cutoff* in PLINK2, with

a proportion of 0.354. Subsequently, *population.pl* was run again to obtain summary statistics from the filtered data, plus pairwise AMOVA corrected F_{ST} values, tested for significance with a Fisher's exact test and with a Bonferroni correction applied. Overall and pairwise F_{ST} values were estimated within each species to assess their population structure.

Using the global (A) set of retrieved loci common to all species, we assessed the population structure of *Myotis californicus, M. peninsularis, M. sv, M. velifer and M. yumanensis* (n = 218), and the four species set (B) excluding *M. californicus* (n = 172) to evaluate the relationship between phenotype and the mitochondrial/nuclear assignment. The software fastSTRUCTURE 1.0 (Raj, Stephens and Pritchard, 2014) was used to evaluate the number of populations contributing to pools of individuals, from K = 2 up to K = 6 for all the groups, and to identify cases of potential hybridisation and introgression. The optimal K was inferred by computing a range of values obtained by a model complexity that maximizes marginal likelihood, and a model of least components selecting the fewest clusters necessary, used to explain structure in the data (Raj, Stephens and Pritchard, 2014). Biological relevance was also taken into account for choosing K. Bar plots showing cluster membership were visualized using a modified version of the DISTRUCT 2.3 Python script (Raj, Stephens and Pritchard, 2014; Chhatre, 2018) and *ggplot* package (Wickham, 2016) in RStudio (RStudio Team, 2015). Admixed individuals were assessed based on the Q scores proportions from results of the optimal K test in fastSTRUCTURE and shown as percentage. Maps showing the proportion of admixed individuals per site were also performed in the *ggplot* package.

Individual based cluster analyses of genotype variation were performed for the global set using Principal Component Analysis (PCA) in the R packages *adegent* 2.1.0. (Jombart,

2008) and *ade4* (Dray and Dufour, 2007). Using the PCA reduced data, differences between groups were inferred using discriminant functions with a Discriminant Analysis (DAPC) with the same packages. The function *find.clusters* was used for identifying the optimal number of genetic clusters in function of the *k*-means algorithm, using the Bayesian Information Criterion (BIC); and to test cluster membership comparing *a priori* "original" groups versus inferred groups (Jombart and Collins, 2017). For these analyses, each individual was labelled by their phenotypic assignations.

Population structure across geographic locations was also assessed within the individual *Myotis* species runs, using the same methodology than for the global assessment. This comprised three separate analyses, one including specimens phenotypically assigned to *M. peninsularis* (*n* = 90) and *M. velifer* individuals (*n* = 2); one with specimens of *M. californicus* (*n* = 48), and one with *M. yumanensis* (*n* = 53). *M. sv* population was excluded since this was sampled at only one site. Isolation by distance (IBD) was evaluated using a Mantel test (Mantel, 1967) among sites between each species, and within species (*M. peninsularis* and *M. velifer* as one group), using the program ISOLDE in GENEPOP 4.7 (Raymond and Rousset, 1995; Rousset, 2008). The corrected F_{ST} values among population pairs from each of the filtered datasets, were transformed into $F_{ST}/(1-F_{ST})$ and a Log_{10} transformation was applied to linear geographic distances (Raymond and Rousset, 1995; Rousset, 2008). The correlation between genetic differentiation and geographic distance was quantified using a Pearson's correlation in R, and plotted with the function *ggscatter* from the package *ggpubr* 0.4.0.

2.4. Results

2.4.1. Bat sampling

During the 2016-2018 field work seasons 620 bats were sampled at 25 sites, with 22 sites on the Baja California peninsula, and three in continental Mexico (Fig 2.2). From these, 295 were assigned *Myotis* species classifications according to field phenotype identifications (Appendix 2.1). In total, 312 *Myotis* bats were analysed for this study, including the seven *Myotis californicus* and 10 *M. yumanensis* samples donated from the San Diego Natural History Museum. Numbers and species names of the *Myotis* used for this study and their location are shown in Fig. 2.3.

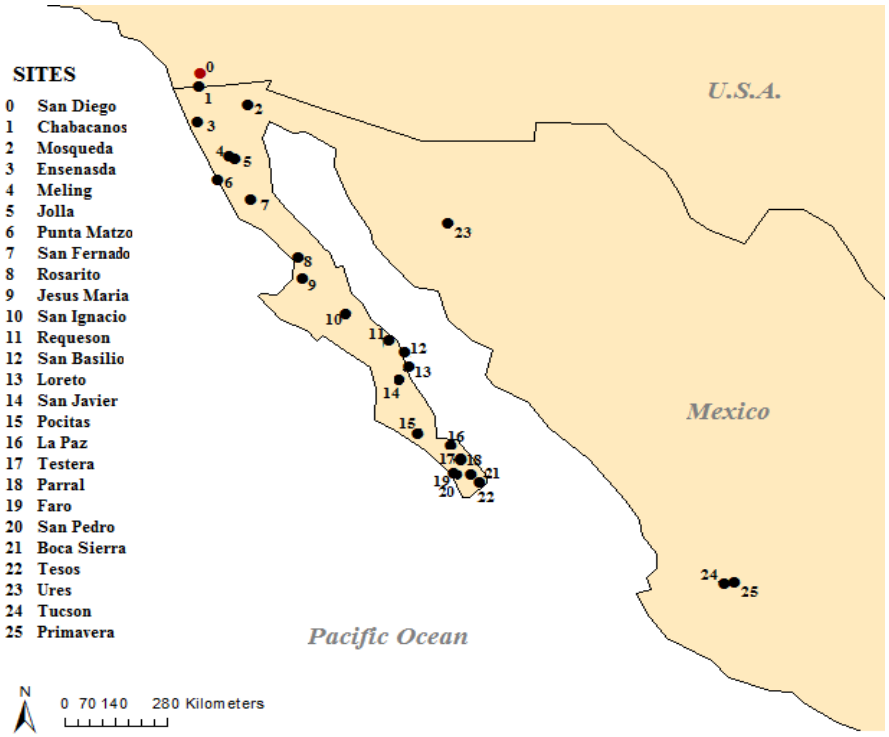


Figure 2.2. Sites sampled during 2016-2018 in the Baja California peninsula and continental Mexico. Site "0" (red dot) indicates a complex of sites in San Diego, California, from which the *Myotis* tissues donated were obtained from (Appendix 2.1, CA1 to CA17 records). Tesos, Testera and La Paz sites are maternity roosts, and the others were mainly at water-bodies areas.

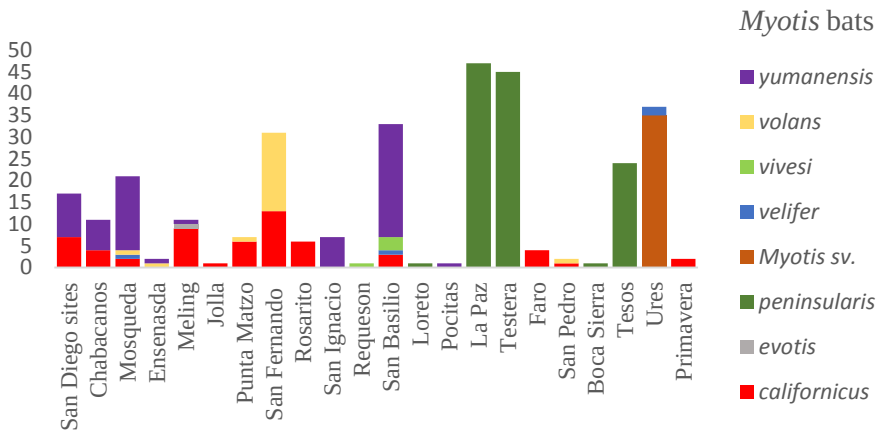


Figure 2.3. Number of *Myotis* species and their locations used for this study. "San Diego sites" refers to the 17 samples donated from San Diego Natural History Museum. Their respective locations are listed in Appendix 2.1.

Two groups of bat specimens were not immediately identified in the field because their morphological features were not consistent with either identification guides (Medellín, Arita and Sánchez, 2007; Álvarez-Castañeda, Álvarez and González-Ruíz, 2015), or my previous experience. Firstly, some specimens, named here as *M. sp*, were sampled from La Paz, along with *M. peninsularis* individuals. Secondly 31 individuals sampled in Ures site that exhibited ambiguous morphology similitudes with *M. velifer*. From these, 14 individuals with undefined morphology were named here as *M. sv*, and 17 individuals identified as *M. velifer*. Additionally, observations during sampling also revealed the presence of some *Myotis* individuals with intermediate morphological characteristics at different locations: intermediate between *M. peninsularis* and *M. yumanensis*, in La Paz and San Basilio (Fig. 2.1), and for *M. californicus* specimens all along its distribution, also occasionally for *M. yumanensis* individuals. Some of these intermediate characters included the presence of an undeveloped keel, area of attachment of the plagiopatagium to foot or finger, and body size (Álvarez-Castañeda and Bogan, 1998). Fur colour patterns were also difficult to assess, which is expected to vary among specimens of *M. californicus* and *M. yumanensis* given their wide

distribution, but not for the restricted *M. peninsularis*. However, more systematic data are needed to evaluate these morphological characters, which will not be addressed by this study.

2.4.2. Mitochondrial DNA phylogenetic analysis of the *Myotis* complex

DNA extraction and sequencing of the mtDNA cyt*b* marker yielded 284 sequences from the *Myotis* bats sampled, from which 2 did not amplify for the cyt*b* marker (sequences CA8_MYCA and CA11_MYCA, but these were included in nuclear analysis), and 15 sequences were discarded after checking for quality and sequencing errors, leaving a total of 267 for the mitochondrial analysis (Appendix 2.1). An additional 179 reference sequences were retrieved from GenBank (Appendix 2.2). Given the complexity of the phylogenetic classification of the cyt*b* haplotypes, species level haplotype summary statistics and phylogeographic results, will be described based on phylogenetic species classification results. For this study, determination of putative species/lineages was based on criteria for mammalian intra and interspecific cyt*b* sequence divergence (Ditchfield, 2000; Bradley and Baker, 2001; Baker and Bradley, 2006; Stadelmann *et al.*, 2007; Clare *et al.*, 2011), where maximum intraspecific divergence ranges are set from 2% to 5%; interspecific/sister species values from 2.5% to 8%; and interspecific/intrageneric values from 8% to 19%. Genetic divergence estimates are show in Table 2.2.

In total, 448 sequences (inclusive of GenBank references) from *Myotis* and two members of the Vespertilionid family as outgroups (*Eptesicus fuscus* and *Antrozous pallidus*) were analysed. The species obtained in this study, *Myotis californicus, M. evotis, M. peninsularis, M. sv, M. velifer, M. volans* and *M. yumanensis*, were concentrated in two main clades (> 0.90 of posterior probabilities; Fig 2.4.): clade 1 (C1) containing *M. californicus, M.*

evotis and *M. volans;* and clade 2 (C2) containing the rest of *Myotis* listed previously. For clearer visualization, trees for clades C1 and C2 are also presented separately (Fig. 2.5 and 2.6, respectively).

Table 2.2. Estimates of mean evolutionary divergence over sequence pairs between (B) groups (%Dist. B, left matrix) and within (W) groups (%Dist. W, right extreme column) shown in percentage. Groups are defined as in the clades resulted in the phylogenetic analysis. Abbreviation of clades and their *Myotis* species contents as in shown in the phylogenetic trees are as follows: CA1, CA2, and CA3 *californicus*; EV, *evotis*; PE, *peninsularis*; SV, *Myotis sv*; VO, *volans*; YU1, YU2, and YU3, *yumanensis*; VEL, *M. velifer* reference sequences; EVO, *M. evotis* reference sequences; VOL, *M. volans* reference sequences; YUM, *M. yumanensis* reference sequences; CA-CI, *M. californicus* and *M. ciliolabrum* reference sequences; THY, *M. thysanodes* reference sequences; FOR, *M. fortidens* reference sequences; and OUT, outgroup.

% dist B	VO	CA1	CA2	PE	yU3	YU2	YU1	CA3	EV	SV	vel	evo	vol	YUM	cA-ci	thY	for	% dist W
VO																		0.0
CA1	11.78																	1.45
CA2	12.59	30.29																2.38
PE	16.32	18.18	16.73															0.19
YU3	17.47	17.67	16.29	30.33														1.34
YU2	16.43	17.87	16.55	10.34	30.36													0.19
YU1	15.09	13.90	12.63	60.43	60.98	50.90												0.0
CA3	12.50	30.12	4.06	17.40	16.92	17.29	13.22											2.87
EV	13.86	12.38	11.73	16.57	17.04	16.07	14.78	12.41										NA
SV	16.70	16.31	15.75	11.91	11.77	10.85	10.43	16.70	15.31									0.73
VEL	16.27	17.66	16.37	10.51	30.57	10.96	50.97	16.88	15.98	11.47								1.6
evo	12.99	10.00	10.25	16.66	17.26	16.26	14.65	10.49	3.94	15.50	16.16							6.06
vol	11.77	12.37	12.43	14.79	14.85	14.14	14.06	12.74	11.99	16.01	14.68	12.44						8.9
yum	15.78	15.74	14.46	5.12	6.02	4.76	3.42	15.02	15.61	10.53	5.03	15.53	14.67					4.90
ca-ci	12.37	4.22	4.21	16.54	16.33	16.07	12.56	4.93	12.27	15.89	16.09	10.16	12.57	14.27				3.32
thy	14.22	12.75	12.96	13.77	13.78	13.50	13.61	12.92	5.48	14.90	13.37	7.23	12.86	13.95	13.35			8.27
for	15.06	15.93	14.84	10.87	10.73	10.07	11.21	15.59	16.46	6.95	10.64	16.19	15.45	10.44	15.09	15.54		3.07
out	20.93	17.59	17.42	20.00	19.32	19.83	18.46	17.69	18.15	18.21	19.77	18.04	19.26	19.04	17.66	18.49	18.62	20.0

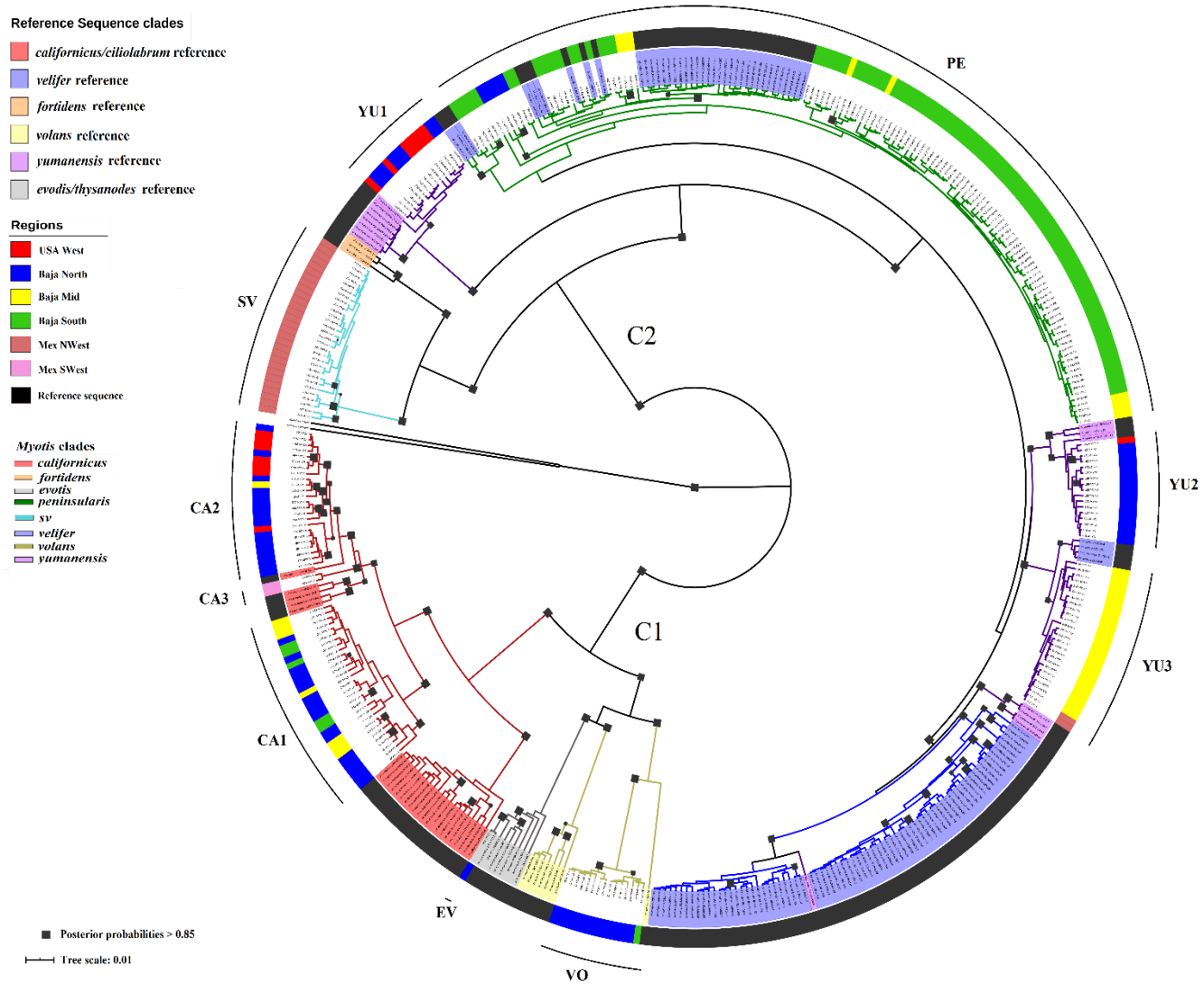


Figure 2.4. Full Bayesian phylogenetic analysis of *Myotis cytb* sequences. Clades branches and labels are coloured by species. Clade C1 includes *Myotis californicus, M. ciliolabrum, M. evotis and M. volans*. Subclades formed by *M. californicus* (CA1, CA2, CA3), and *M. volans* (VO) are delimited by a black curved line next to each position of each species from this study in the circle tree. Reference sequence subclades have no exterior curved line, and are coloured according to species. Posterior probabilities > 0.85 are shown by a dark square, size proportional to value obtained. Colour stripes relate to the region of origin of the sequences generated in this study only (Regions key); reference sequences have a black stripe with no region assignment.

The C1 clade comprises two well supported (posterior probability >0.90) subclades, representing *M. volans*, *M. evotis* and *M. californicus* haplotypes respectively (Fig. 2.5). Individuals sampled from the peninsula initially assigned to *M. volans*, are clustered with the *M. volans* reference sequences retrieved from GenBank (golden *Myotis* subclade branches legends, Fig. 2.4). However, peninsular *M. volans* presented between 8% to 16% divergence from *M. volans* reference sequences (Table 2.2, genetic distances within and among groups), which is considered to be genetic divergence at the interspecies level within genera (Bradley and Baker, 2001; Stadelmann *et al.*, 2007; Clare *et al.*, 2011). This suggests that peninsular *M. volans* from Baja (VO, Fig. 2.4) could potentially represent a currently unrecognised species. The *M. evotis/thysanodes* reference sequences formed a subcluster with the reference *M. volans* sequences, but with posterior support < 0.85.

BLAST searches within *M. californicus* sequences from this study retrieved both *M. californicus* and *M. ciliolabrum* reference records with percent similarity typically falling in a range of 96% to 98% ($E < 10^{-6}$ to E^{-10}, Pearson 2013). The phylogenetic analysis recovered three main clades for *M. californicus* individuals sampled from this study: *CA*1, is distributed throughout Baja; *CA*2, including mid and northern peninsular individuals, plus specimens from San Diego, USA; and *CA3*, comprising the two samples from south western Mexico (Fig. 2.2). The GenBank reference sequences (red clades collapsed, Fig. 2.5) formed 3 clades, 2 of which were nested within Baja haplotypes. These clades were composed of a mixture of sequences annotated as originating from *M. californicus* and *M. ciliolabrum*, plus one sequence each annotated as *M. melanorhinus* and *M. leibii* (Fig. 2.4). Genetic divergence ranged between 4.0-4.9% for the *M. californicus-ciliolabrum* reference sequences clades, and between 3.12 - 4.06% for sequences derived in this study (Table 2.2, CA-CI, and CA1-CA3, respectively). The

maximum divergence among the three Baja clades (4.06%, for CA2-CA3), was comparable to the comparisons among the reference sequences groups (CA-CI, Table 2.1).

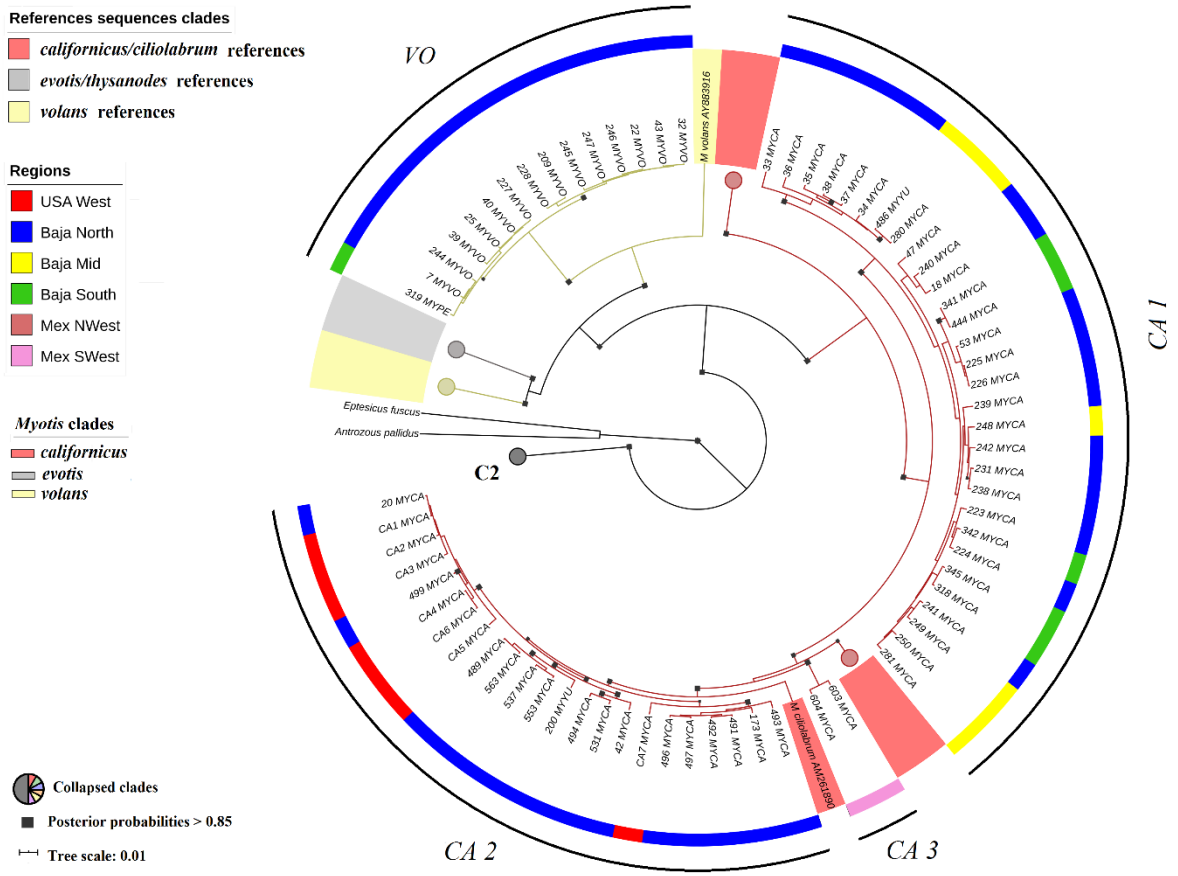


Figure 2.5. Section of the Bayesian phylogenetic tree under the HKY G+I model of evolution (Cyt*b* marker) showing only clade 1 (C1) that includes *Myotis californicus, M. ciliolabrum, M. evotis and M. volans*. Subclades formed by *M. californicus* (CA1, CA2, CA3), and *M. volans* (VO) are delimited by a black curved line next to each position of each species from this study. Clade 2 is collapsed, shown as a grey circle tip. Nodal support values (black squares) represents posterior probabilities greater than >0.85, with a proportional size up to 100%. The colour of clade tips refer to reference sequences only (reference sequences clades key). This tree is colour-coded, where the colour of clade lines represents each *Myotis* species (*Myotis* clades key). Colour stripes relate to the region of origin of the sequences generated in this study only (Regions key); there is no stripe for reference sequences.

Sequences in the phylogenetic analyses from individuals classed morphologically as *M. peninsularis, M. velifer*, and *M. yumanensis* formed a complex arrangement, which are not reciprocally monophyletic with respect to phenotype (Fig. 2.4 and 2.6). Sequence divergence within and among clades (typically <3% and up to 6% respectively; Table 2.2) is less than for the *californicus-volans* sub-tree, with fewer highly supported nodes. Sequences of *M. yumanensis* from this study divide into three main clades (YU1, YU2, YU3, Fig. 2.4 and 2.6). YU2 and YU3 are proximate in the tree, but group with other clades derived from reference sequences assigned to *velifer* (YU3), and *velifer* and *yumanensis* (YU2). YU1 is most divergent from the other clades and groups with a separate set of reference sequences assigned to *yumanensis*. The clades reflect geographic structuring with YU1 composed of individuals from western USA and northern Baja, YU2 primarily northern Baja, and YU3 primarily mid Baja.

Individuals assigned as *M. peninsularis* formed the biggest subclade (Clade 2, subclade PE, Fig. 2.4), but the subclade overall has posterior probability support of <0.85. The subclade also includes a number of *velifer* reference sequences, and based on BLAST comparisons, sequence similarity between *M. peninsularis* and *M. velife*r haplotypes ranged from 98.49% to 100%, and for *M. thysanodes* and *M. yumanensis* was ≤ 98.49%. Average divergence among all *M. peninsularis* and *M. velife*r sequences was 1.4%, which falls into the range for intraspecific genetic divergence (Bradley and Baker, 2001; Stadelmann *et al.*, 2007; Clare *et al.*, 2011). *M. peninsularis* individuals are distributed principally in southern Baja, the recognised restricted distribution of this bat. The PE subclade also includes individuals phenotypically assigned as *M. yumanensis* from mid-peninsula (Fig. 2.6, yellow stripes), and four individuals from the north, which could not be assigned to species based on morphology in the field. It is worth noting that largest subcluster of *M. peninsularis* haplotypes is not mixed with *M. velfier*

sequences, although the grouping does lack a high level of posterior support (≤60%), due to low divergence levels.

Lastly, from the 31 bats sampled in Ures, phenotypically all similar to *M. velifer*, there were 17 sequences identified by morphology as *M. velifer*, and 14 as *M. sv.* From these, 12 *M. sv* individuals had unique haplotypes and belong to a separate phylogenetic clade (Fig. 2.4, light blue branches). The two *M. sv* remaining individuals (bats 572 and 602, Appendix 2.1), were identified as *M. velifer* by BLAST with 99.84% (highest value among hits) similarity, but with *M. thysanodes* (99.68%, potentially misidentified), and *M. yumanensis* (98.05%) as lower ranked matches. However, in the phylogenetic analysis, this two *M. sv* individuals were included in YU3 clade (Fig. 2.4 and 2.6) along with putative *M. yumanensis* individuals sampled in mid-peninsula.

Sequences from the *M. sv* individuals sampled in Ures, and three *M. fortidens* reference sequences, formed a well-supported lineage which diverges at the base of the C2 clade. The *M. sv* and *M. fortidens* sequences form two separate, highly supported sub-clades, with a divergence of more than 6.5% (light blue clade lines, and orange clade, respectively, Fig. 2.4). Consistent with their phylogenetic position, *M. sv* sequences returned highest similarity with *M. fortidens* references (average 93.44%) in BLAST searches, with matches to *M. yumanensis* and *M. velifer* sequences returning hits at around 90% similarity. Given its phenotype which could not be classified in the field, and the level of sequence divergence from *M. fortidens*, which falls at the interspecies level within genera, *M. sv* may represent another currently unrecognised species.

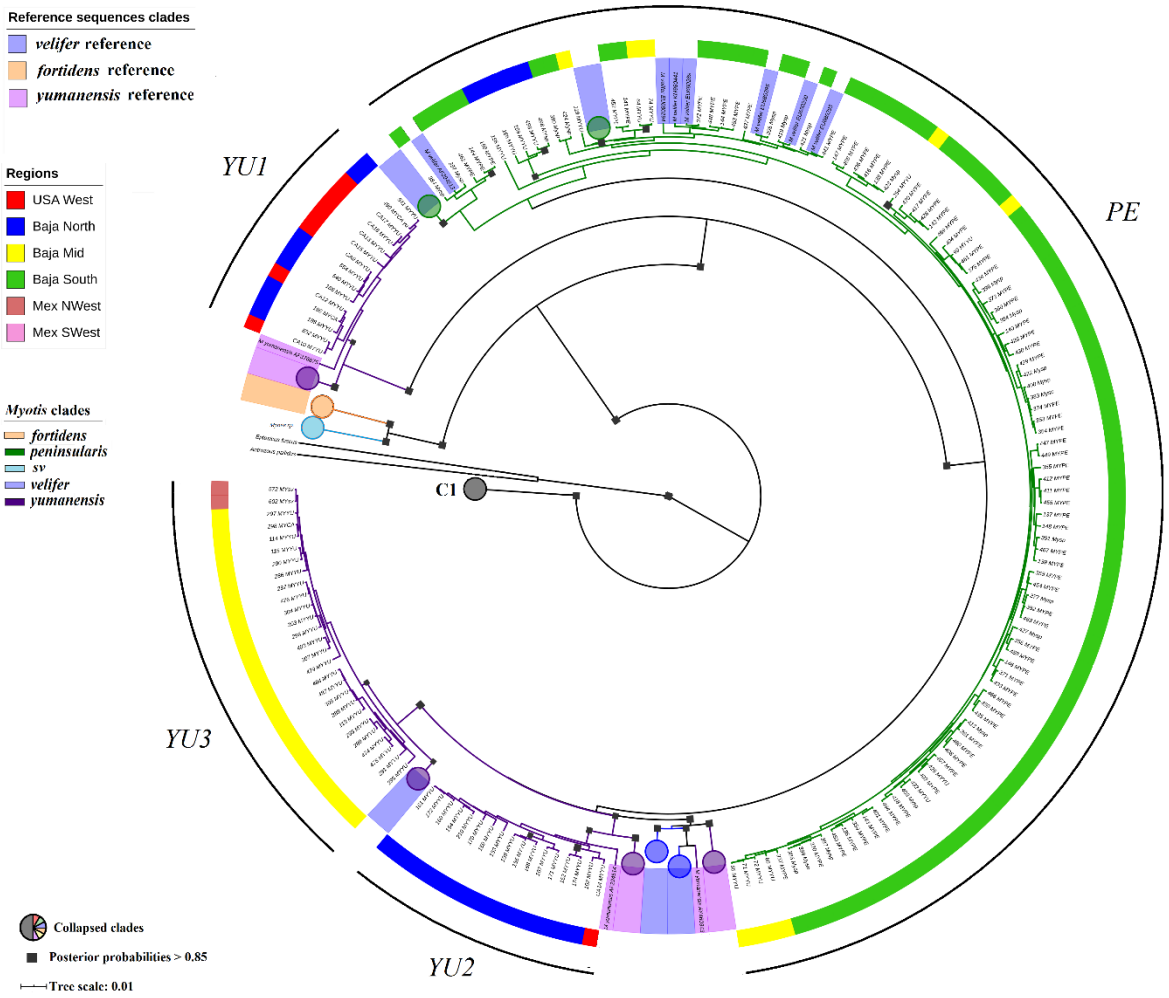


Figure 2.6. Section of the Bayesian phylogenetic tree under the HKY G+I model of evolution (Cyt*b* marker) showing only clade 2 (C2) that includes *Myotis peninsularis, M. yumanensis and M. velifer*. Subclade formed by *M. peninsularis* (PE) and those formed by *M. yumanensis* (YU1, YU2 and YU3) are delimited by a black curved line next to each position of each species from this study. Clade 2 is collapsed, shown as a grey circle tip. Nodal support values (black squares) represents posterior probabilities greater than >0.85, with a proportional size up to 100%. The colour of clade tips refer to reference sequences only (reference sequences clades key). This tree is colour-coded, where the colour of clade lines represents each *Myotis* species (*Myotis* clades key). Colour stripes relate to the region of origin of the sequences generated in this study only (Regions key); there is no stripe for reference sequences.

2.4.2.1. Haplotype diversity

Overall, there were analysed a total of 448 sequences (Appendix 2.3) with 96 haplotypes. From these, there were 50 haplotypes of 267 for samples sequenced in this study. Analysis of Baja *Myotis* at the clade level, the highest haplotype and nucleotide diversities was presented by *M. californicus* clades (range 0.908 - 1 and 0.005 - 0.284 respectively). The lowest values for both haplotype and nucleotide diversity were presented by *yumanensis* clades (Table 2.3). The largest clade, PE, included 108 *peninsularis* individuals but had low haplotype and nucleotide diversity despite having the largest sample size of all the groups. The VO clade presented few haplotypes as did YU clades, which generally had similar sample sizes. Excluding CA1, CA2 and CA3 subclades, subclade SV presented slightly higher nucleotide diversity compared to the other clades, despite coming from a single site sampled.

Table 2.3. Summary statistics of the *Myotis* species lineages from this study. Abbreviations of statistics are: *N*, number of sequences within each group; *S*, number of variable sites; *H*, number of haplotypes; *Hd*, haplotype diversity; SD, standard deviation; and, *Nd*, nucleotide diversity. Abbreviation of clades and their *Myotis* species contents as in shown in the phylogenetic trees are as follows: CA1, CA2, and CA3 *californicus*; EV, *evotis*; PE, *peninsularis*; SV, *Myotis sv,* VO, *volans*; YU1, YU2, and YU3, *yumanensis*.

Myotis clades	N	S	Number of mutations	H	Hd	Hd SD	Nd	Nd SD
CA1	30	21	24	14	0.874	0.05	0.0072	0.001
CA2	25	17	17	10	0.0877	0.04	0.011	0.0009
CA3	2	3	3	2	1	0.5	0.005	0.0024
PE	106	22	22	13	0.412	0.06	0.002	0.0005
SV	29	18	18	12	0.781	0.072	0.005	0.0097
VO	15	3	3	2	0.533	0.052	0.0027	0.0002
YU1	15	56	56	2	0.133	0.0112	0.018	0.0156
YU2	17	3	3	4	0.566	0.123	0.001	0.0003
YU3	27	0	0	1	0	0	0	0

2.4.2.2. Mitochondrial DNA haplogroups

The overall median-joining haplotype network recovered the same main divisions of sequences identified in the phylogenetic analysis, resulting in nine main haplogroups (Figure 2.7). As with the phylogenetic analysis, the placement of several *M. californicus, M. velifer*, *M. volans* and *M. yumanensis* haplotypes do not match their initial morphological attribution, or species annotation for GenBank reference sequences. This suggests cases of potential hybridisation/introgression among individuals from this study or individuals from reference sequences, and/or species misidentification/misannotation uploaded in GenBank.

Each haplogroup was defined for having at least 8-10 mutations away from the closest haplogroup. HG1 contains *M. californicus* and *M. ciliolabrum* individuals, HG2 includes *M. volans* from the present study only, whereas HG3 and HG4 include *M. volans* from reference sequences. HG5 comprises the single *M. evotis* individual from this study (EV1). Haplogroups HG6-HG9 were separated from the HG1-HG5 cluster by 28 substitutions (Figure 2.8). Clade HG6 includes *M. peninsularis*, *M. velifer* and *M. yumanensis* haplotypes; HG7 comprises *M. fortidens* reference sequences, while HG8 comprises *M. sv* bats (Fig. 2.8). HG9 includes one single haplotype with a putative *M. velifer* reference sequence, separated from the other *M. velifer* sequences by 19 substitutions, and therefore most likely represents a misidentification by the original authors.

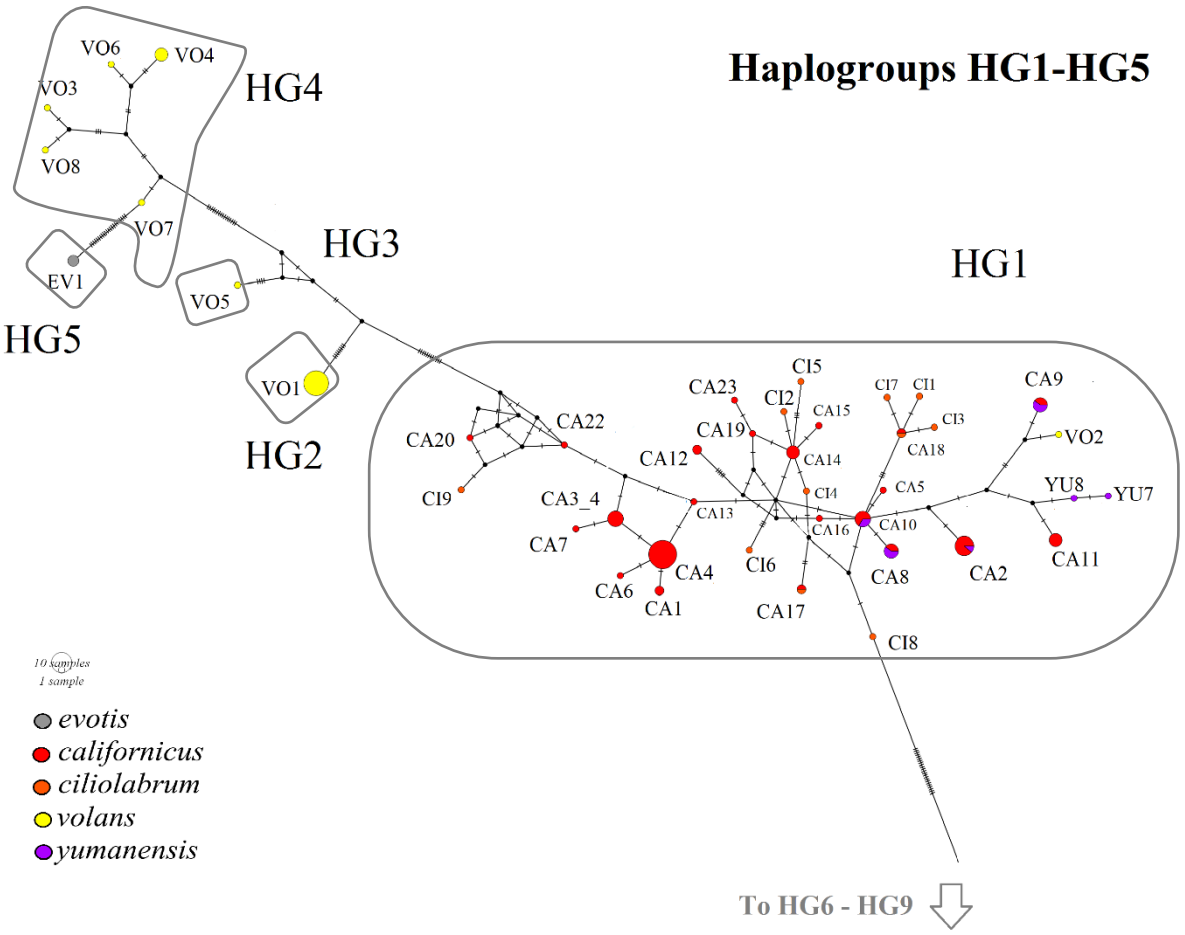


Figure 2.7. Median-joining network section for *Myotis* mitochondrial cytochrome *b* haplogroups. Each haplogroups includes the following individuals: HG1, haplotypes of *Myotis californicus* and *M. ciliolabrum*; HG2, *M. volans* from this study, HG3 and HG4; *M. volans*; and HG5, *M. evotis*. Haplogroups are indicated by a continue-line shape.

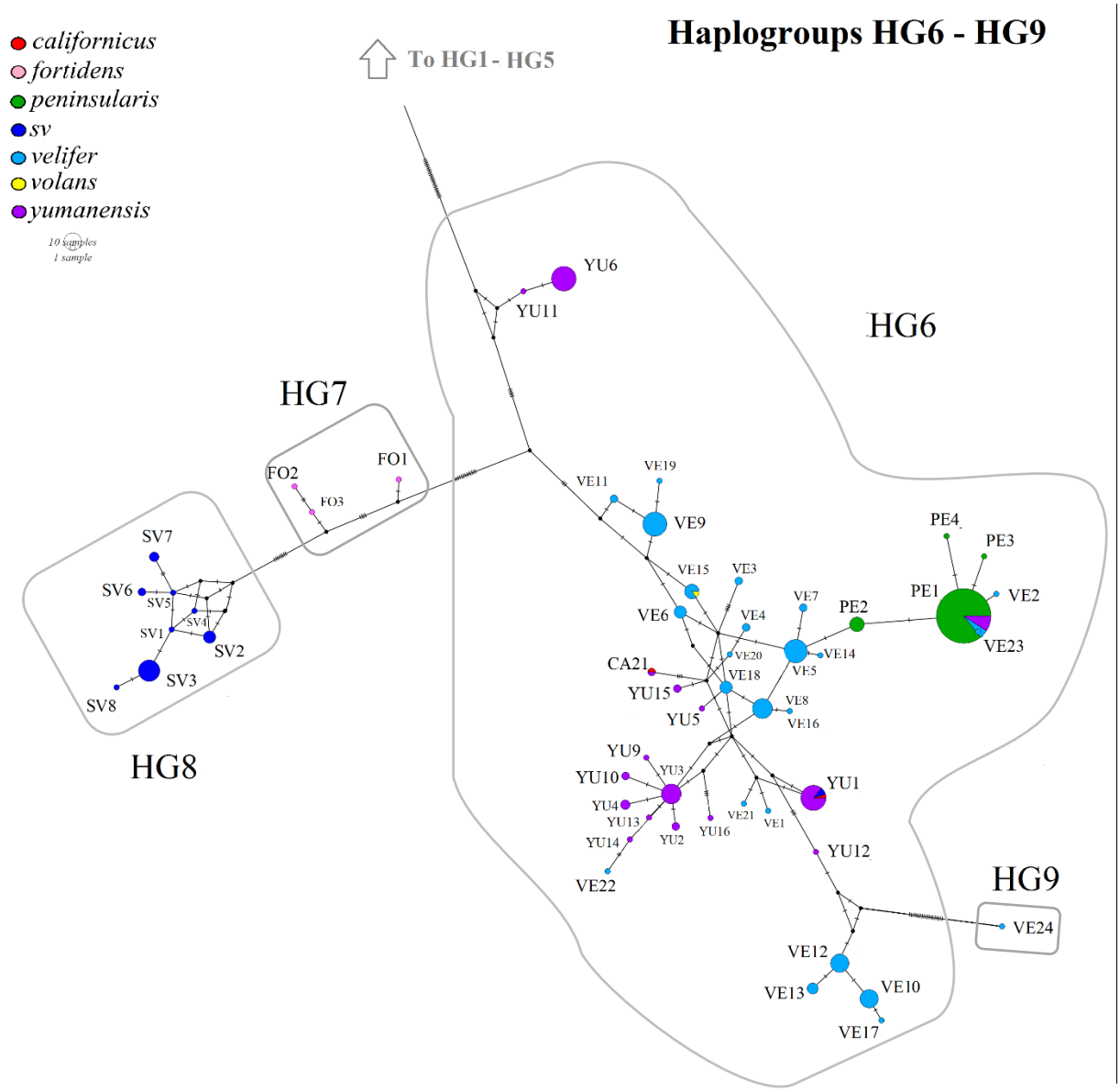


Figure 2.8. Median-joining network section for *Myotis* mitochondrial cytochrome *b* haplogroups. Each haplogroups includes the following individuals: HG6; haplotypes of *M. peninsularis, M. velifer,* and *M. yumanensis*; HG7, *M. fortidens;* HG8 haplotypes of *M. sv;* and HG9, a potentially misclassified *M. velifer* specimen from references sequences. Haplogroups are indicated by a continue-line shape.

Within HG6, there is a central cluster composed by members of *M. velifer* and *M. yumanensis*, mostly linked by one or two substitutions (Fig. 2.8). Individuals from *M.*

peninsularis are located at the right extreme, showing four haplotypes separated by single substitutions between each other, and from the closest *M. velifer* reference sequence haplotype (VE5). Most of the *M. peninsularis* individuals are represented within a single haplotype (PE1, 85 of 108 sequences), in which eight individuals were classified as *M. yumanensis* (codes are based on morphological assessments from this study, but this placement also agrees with phylogenetic results), plus six reference sequences, including one annotated as *M. levis* and five annotated as *M. velifer* (Appendix 2.3). There were two haplotypes from reference sequences annotated as *M. velifer*, separated by one mutation from PE1: Haplotype VE23 comes from an individual phenotypically classified as *M. peninsularis*, from Baja, but submitted to GenBank as *M. velifer* by the authors (see Nájera-Cortazar, Álvarez-Castañeda and De Luna, 2015). Haplotype VE2 derives from a bat sampled in New Mexico, U.S. (velifer_AF294513, Appendix 2.2).

Haplotypes YU11 and YU6, and VE10, VE12, VE13 and VE17 represent paraphyletic clusters of *M. velifer* and *M. yumanensis* haplotypes respectively, but also include several cases of individuals with *M. velifer* phenotype/reference annotation clustering among *M. yumanensis* haplotypes and vice versa. The *M. yumanensis* haplotypes (YU11 and YU6) were separated from the closest *M. velifer* haplotype from eight substitutions, suggesting demographic isolation. There was also a phenotypically misclassified *Myotis* contained in haplotype CA21, initially attributed to an individual with *M. californicus*, but later classified as *M. yumanensis* with the phylogenetic results.

2.4.2.3. Phylogeographic analysis of Cytochrome *b* haplotypes

A separate median joining network containing only haplotypes generated by the present study was constructed and annotated by region of origin (Fig. 2.9). Haplogroups retrieved are organized as follows: GHG1 including *M. californicus* individuals, GHG2 including *M. volans*, GHG3 including the single individual sampled of *M. evotis*, GHG4 including *M. sv*, GHG5 including *M. peninsularis*, GHG6 including most of *M. yumanensis* from northern peninsular sites, GHG7 including *M. yumanensis* from Chabacanos and San Diego sites, and GHG8 including most of *M. yumanensis* from San Basilio and the only two *M. velifer* obtained in Ures (sites in Fig. 2.2, Appendix 2.4).

The overall distribution of haplotypes among the regional haplogroups largely reflects the geographic species distributions and sample origins. For each of the species distributed across multiple regions (*M. californicus*, *M. volans* and *M. yumanensis*) rare haplotypes were typically found in only single sites in the same regions, but most of the more common haplotypes were distributed across multiple sites and regions (Figure 2.9. haplotype circles with mixed colours). For example, in *M. californicus*, there were 5 haplotypes (CA2b, CA4b, CA8, CA9 and CA10) that contained mixes of individuals sampled from multiple sites in different regions, with CA4b being distributed from the north to the south of the peninsula.

Interestingly, *M. peninsularis/velifer* haplotypes included individuals located in mid-peninsula, expanding their range of distribution known in the literature so far (Fitch, Shump and Shump, 1981; Álvarez-Castañeda and Bogan, 1998). *M. yumanensis* presented evidence for potentially 3 differentiated groups. One associated with haplotype YU6 haplotype (separated by least 24 substitutions the closest median) restricted to the Western USA (San Diego) and north

peninsula. A second associated with YU1 comprised of mid-peninsula *M. yumanensis* and the two specimens with *M. velifer* phenotypes sampled at the mainland site Ures (Fig. 2.2). GHG6 shows a final group of closely related haplotypes located around YU3 is mostly found in the north peninsula.

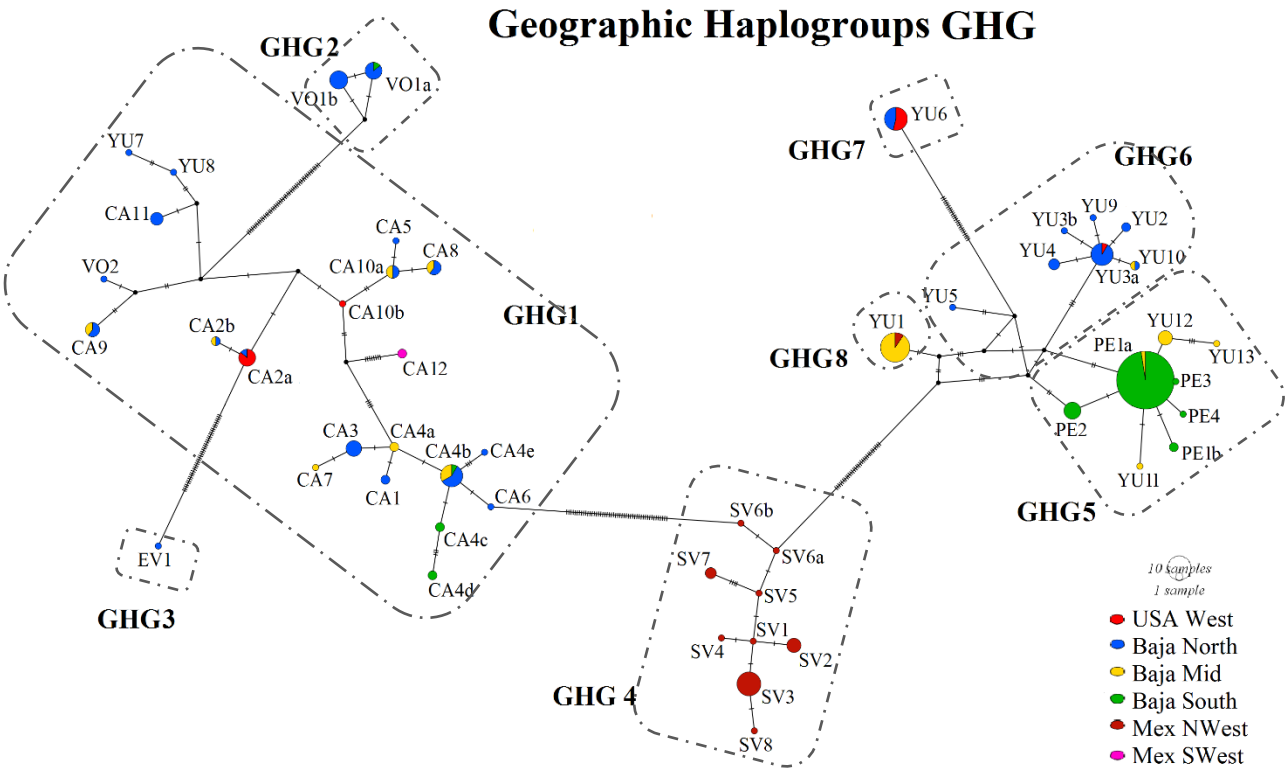


Figure 2.9. Median joining network showing mitochondrial cytochrome *b* haplotypes of *Myotis* obtained only in the present study and their geographic regions. The geographic haplogroups (GHG) from 1 to 8 included haplotypes of *Myotis californicus* (CA), *M. ciliolabrum* (CI), *M. evotis* (EV), *M. peninsularis* (PE), *M. sv* (SV), *M. velifer* (within YU1), *M. volans* (VO), and *M. yumanensis* (YU). Each haplogroup is numbered and indicated by discontinued dashed shapes.

2.4.2.4. AMOVA for populations and regions within species

For each species, grouped based on mitochondrial results, genetic differentiation based on sequence variation across sites and regions was estimated via an AMOVA. None of the species returned significant differentiation among regions, but *M. californicus*, *M. yumanensis*, and grouped *M. yumanensis/M. velifer* comparisons showed high levels of differentiation among sites, and for sites within regions (Table 2.4). Phenotypic *M. peninsularis* from the south peninsula showed no significant differentiation across sites, however, south *M. peninsularis* compared against individuals from mid-peninsula with 'peninsularis' haplotypes showed significant differentiation of haplotype frequencies across sites (F_{ST} = 0.3478, P < 0.001).

Table 2.4. AMOVA results for the mtDNA haplotypes analysis dataset grouped by haplotypes of *Myotis* species and their sites of distribution. *M. sv* was excluded for having only one site of sampling. Abbreviations: Among regions (A.R.), among populations within regions (A.P.W.R.), among sites (A.S.), degrees of freedom (D.F), sum of squares (S.S.), percentage (%), Fixation (F).

Variation	D.F.	S.S.	Variance	% Variation	F. index	F. index value	*P* value
M. californicus							
A.R.	3	2.753	-0.0315	-6.84	F_{CT}	-0.0684	0.715
A.P.W.R.	7	10.153	0.2399	52.02	F_{SC}	0.4868	<0.001
A.S.	42	10.63	0.2592	54.82	F_{ST}	0.4517	<0.001
M. peninsularis							
A.R.	3	0.989	0.0145	10.4	F_{CT}	0.1040	0.38
A.P.W.R.	2	0.2	-0.0017	-1.26	F_{SC}	-0.0141	0.39
A.S.	90	11.456	0.1272	90.86	F_{ST}	0.0914	0.14
M. peninsularis according to haplotypes							
A.R.	3	4.37	0.0747	35.9	F_{CT}	0.35899	0.29814
A.P.W.R.	2	0.2	-0.00232	-1.12	F_{SC}	-0.0174	0.42033
A.S.	96	13.028	0.13571	65.22	F_{ST}	0.3478	<0.001
M. volans							
A.R.	1	0.41	0.1	30	F_{CT}	0.3	0.4076
A.P.W.R.	3	0.667	-0.0166	-5	F_{SC}	-0.0714	0.7233
A.S.	8	2	0.25	75	F_{ST}	0.25	0.4858
M. yumanensis, M. velifer							
A.R.	3	10.268	0.12547	25.57	F_{CT}	0.25569	0.1662
A.P.W.R.	3	5.667	0.25234	51.42	F_{SC}	0.69086	<0.001
A.S.	54	6.097	0.11291	23.01	F_{ST}	0.76991	<0.001
M. yumanensis							
A.R.	2	8.044	0.0756	15.11	F_{CT}	0.1510	0.2551
A.P.W.R.	4	10.015	0.2949	58.93	F_{SC}	0.6941	<0.001
A.S.	59	7.669	0.1299	25.97	F_{ST}	0.7403	<0.001

Regions are based in sites from north, mid and south peninsula, and north west and south west continent, described in Methods 2.3. The *M. yumanensis* analysis included haplotypes of the two individuals assigned as *M. velifer* by BLAST and phylogenetic results.

2.4.3. Species boundaries and population structure assessed through ddRAD SNP variation

2.4.3.1. SNP calling and marker summary statistics

A range of parameter settings were evaluated in the *gstacks* pipeline to optimise SNP recovery among the five species included in the libraries (Table 2.5 A), changing the parameter *–p* (minimum number of populations a locus must be present in to process a locus) and *–r* (minimum percentage of individuals in a population required to process a locus for that population), to minimise missing data within species, while maximising the number of loci shared among species pairs (Paris, Stevens and Catchen, 2017; Rochette and Catchen, 2017). The parameters chosen for the global dataset analysis were *–p* = 4 and *–r* = 0.80, recovering a total of 249,913 genotyped loci, comprising 256,049,796 forward reads and 237,332,298 matching pair-end reads, with a mean insert length of 223.1bp (SD = 55.8). Effective per-sample coverage had a mean of 272.8x (SD = 189.5x; min = 5.3, max = 1,061.8x). Additionally, the same SNP pipeline was run separately for the global test excluding *Myotis californicus* individuals (Table 2.5 B), and each species (coverage data not shown), resulting in 44,789 to 197,631 loci and 35,056 to 46,642 variant SNPs (C, D and E, Table 2.5). Two relatives were removed from the global and the global excluding *Myotis californicus* datasets (specimen number 355 classified as *M. peninsularis*, and specimen number 586 classified as *M. sv*, Appendix 2.1), leaving a total of 218 individuals; and one from the *M. peninsularis* data set, leaving a total of 92 individuals for population structure analysis.

Table 2.5. Summary of ddRAD libraries settings per analysis made: A) All *Myotis* included (global analysis) divided into four species according to their mtDNA assignments; and populations of B) *M. californicus*, C) *M. peninsularis* and *M. velifer*, and D) *M. yumanensis*, clustered according to mtDNA and nuclear assignments. Number of populations considered (*p* parameter) in STACKS were set according to each set analysed. Proportion of missing data allowed (*r* parameter) was set to 0.80 for all the analysis.

ID	*Myotis* species/analysis	Number of individuals	Populations/species considered – p parameter	Loci total	Loci filtered	Loci kept after filtering	Number of sites in kept loci	Number of sites in kept loci filtered	Number of sites kept	Total variant sites (SNPs)
A*	All - Global	220	4	249,913	247,507	2,406	549,275	71,416	477,859	2,076
B*	Global-excluding *M. californicus*	170	3	230,969	224,001	6,968	1,601,496	20,075	1,581,421	57,409
C	*californicus*	48	4	44,789	40,111	4,678	1,073,751	18,039	1,055,712	46,642
D*	*peninsularis/velifer*	93	2	206,446	197,631	8,815	2,199,265	6,462	2,192,803	35,056
E	*yu*manensis	53	4	55,177	50,196	4,981	1,179,335	18,755	1,160,580	41,947

***For posterior analyses, two relatives were removed from sets A and B, and from those, one individual removed from set D.**

2.4.3.2. Genetic variation within species and *F*-statistics among species

Overall, levels of heterozygosity were low for both variant and fixed positions (Table 2.6), potentially because of the combination of different species over the same dataset (Chhatre *et al.*, 2018). *Myotis peninsularis* had the closest observed heterozygosity to the expected one, whereas the rest of the species had lower observed values than their respective expected, especially *M. californicus* (Table 2.6). This might be due to the high population structure this species presents.

Consistent with mtDNA results, *M. californicus* was the species with the highest genetic variation in the whole group, with 999 private alleles, and the highest nucleotide diversity (*Pi*, Table 2.6). *M. californicus* also had the highest levels of F_{IS}, corresponding with its heterogeneous geographic distribution, followed by *M. yumanensis*, where both species had the

widest sampled distributions from this study. Individuals of *M. sv* presented a high F_{IS} value and highest *Pi* compared to the rest of the species (except for *M. californicus*), considering its small group size and that it was sampled in only the Ures site (Fig. 2.3). Mean F_{ST} across paired species was 0.07231, with inter-species pair values ranging from 0.022 for *M. yumanensis* and *M. velifer*, to 0.146 between *M. peninsularis and M. sv* (Table 2.9).

Table 2.6. Summary statistics for the ddRAD global SNP calling, which included *Myotis californicus, M. peninsularis, M. sv, M. velifer* and *M. yumanensis* species, performed with *populations.pl*. Abbreviations are as follows: HWE, Hardy-Weinberg equilibrium; Obs. Het., proportion of heterozygote individuals; Exp. Het., expected heterozygosity under HWE; Obs. Hom., proportion of homozygote individuals; Exp. Hom., homozygosity expected under HWE; *Pi*, nucleotide diversity; F_{IS}, inbreeding coefficient of an individual relative to the subpopulation.

Myotis	Private alleles	Variant Sites	Variant sites out of HWE <0.05	Obs. Het.	Exp. Het.	Obs. Hom.	Exp. Hom.	*Pi*	F_{IS}
californicus	999	2058	632	0.02694	0.06967	0.97306	0.93033	0.07046	0.27236
yumanensis	297	2065	248	0.0139	0.02348	0.9861	0.97652	0.0238	0.11337
peninsularis	134	2076	48	0.01539	0.01802	0.98461	0.98198	0.01813	0.01605
velifer	62	1998	0	0.01802	0.02553	0.98198	0.97447	0.02917	0.02377
sv	21	191	19	0.01397	0.03568	0.98603	0.96432	0.03652	0.09737

Table 2.7. Population pair F_{ST} divergence statistics.

Myotis population pairs	F_{ST}
californicus-yumanensis	0.020782
californicus-peninsularis	0.03778
californicus-velifer	0.032297
californicus-sv	0.055175
yumanensis-peninsularis	0.026311
yumanensis-velifer	0.039016
yumanensis-sv	0.090553
peninsularis-velifer	0.060076
peninsularis-sv	0.084744
velifer-sv	0.22972

2.4.3.3. Population structure and admixture among species from Bayesian cluster analysis

In total, 218 individuals were analysed (excluding the two relatives found, set A, Table 2.5), including four putative *Myotis* species: *Myotis californicus, M. peninsularis/velifer, M. sv* and *M. yumanensis*. FastStructure analysis of individual SNP genotypes with $K = 2$ to $K = 6$ runs (full K cluster exploration is shown in Appendix 2.5) identified $K = 4$ as the optimal number of clusters, obtained by the maximal marginal likelihood and the least clusters essential to explain patterns showed by the data (Raj, Stephens and Pritchard, 2014). This K value also had greatest biological relevance by identifying clusters of individuals which approximated the number of putative species sampled. Hereafter, observations will be made based on the admixture proportions from the $K = 4$ clustering (bar plot, Fig. 2.10 A). The separate population structure analysis excluding *M. californicus* showed similar population structure than the global assessment. Bar plot showing population structure results for this set are visualised in Appendix 2.6.)

Most individuals phenotypically assigned as *M. californicus*, mapped to Cluster 1 (red, Fig. 2.10 A), and also carried M. *californicus* mtDNA (Appendix 2.7). Two individuals with *M. californicus* morphology were assigned to the *peninsularis* (green) and *yumanensis* (purple) clusters, and 12 showed varying admixture with other clusters. Two individuals carried *yumanensis* mtDNA in *californicus* nuclear background.

Most of the individuals with *M. peninsularis* morphology, which carried *peninsularis* mtDNA, were assigned to a single cluster (Cluster 2, green), with the exception of two specimens with *sp* morphology, appearing to have 100% (387-sp) and ~75% (150-PE)

yumanensis nuclear ancestry (Fig. 2.10 A, Appendix 2.5). The latter, showing admixture consistent with being a F2 backcross with *yumanensis* ancestry. There was at least one member of each other *Myotis* species included in the nuclear analysis, assigned as a member of *M. peninsularis*/*velifer* green cluster, although they were classified as other species by the mtDNA results.

From the 31 bats that were morphologically assigned to *M. velifer* or to *M. sv* sampled in Ures, 22 were included in this analysis. Of those, seven had majority Cluster 2 ancestry, but the remainder were assigned to a third cluster (Cluster 3, yellow), including 13 bats with *velifer* assigned morphology. There were five bats with majority ancestry to Cluster 3, but presented admixture with Cluster 2. The two individuals with mtDNA haplotypes classed as *M. velifer* by BLAST (Arrows, Fig. 2.10 A), were both morphologically classified as *M. sv*, but assigned to Cluster 2 in the SNP data.

Individuals with *yumanensis* morphology were genetically heterogeneous. Out of 56 bats, 26 showed 100% ancestry with a forth species cluster (Cluster 4, purple), with 23 carrying *yumanensis* mtDNA, three *peninsularis* mtDNA and one *californicus* mtDNA. Nine showed majority nuclear ancestry with Cluster 2, and one with Cluster 1, with the remainder showing more complex admixture among Clusters 1, 2 and 4. Three were potential misclassifications in the field, one having *californicus* mtDNA and 100% ancestry with Cluster 1, and 2 having *peninsularis* mtDNA and 100% ancestry with Cluster 2 (Fig. 2.10 A).

Admixed individuals were assessed based on the *Q* score proportions (*Q*) from the optimal *K*, and were considered admixed when the *Q* score percentage for the Cluster

contributing the majority ancestry < 98% (modified from descriptions in Melville *et al*. 2017). From the 218 *Myotis* analysed, 173 individuals were considered pure (*Q* > 98%), including 160 bats where the phenotypic species assignment matched with both mitochondrial and nuclear analysis, 8 bats matching mitochondrial assignment with phenotype (but not nuclear), and six bats matching nuclear assignment with phenotype, but not mitochondrial (Appendix 2.7). In total, there were 45 individuals with admixture proportions consistent with being F1, F2 backcrosses and further backcrosses (Table 2.10). From these, there were 17 individuals including ancestry components from the minor cluster of *Q* > 20%, 23 with *Q* > 10% and < 19.9%, and eleven specimens with *Q* between 9.9% and 2%, including along them three individuals showing admixed proportions with more than two clusters, and two F1 hybrid individuals. Most of the admixed cases were observed within individuals initially assigned as *M. yumanensis* (Cluster 4), followed by *M. sv* individuals (Cluster 3), and *M. californicus* (Cluster 1). There were only two individuals showing values between ~ 40%-60% admixture, which may represent F1 hybrids: 99_YU with 50.28% *yumanensis* and 49.69% *peninsularis*, and 115_YU with 59.56% *yumanensis*, 40.42% *peninsularis*, both from mid-peninsula (black circles in Fig. 2.10 A). There were four individuals detected sharing ancestry contributions from the three species, three from *M. yumanensis* (CA8, CA11 and CA16) from San Diego sites; and one *M. californicus* (CA_40) from San Fernando site (black circles in Fig. 2.10 A, see proportions in Table 2.10).

Introgression was detected across all the sampling area, but some regions were acting as hotspots of admixture (Fig. 2.10 B, left map). Northern sites hold more cases of introgression across species, concentrated in Mosqueda, Ures and San Diego sites (Fig. 2.2). Mid-peninsula also hold important cases of introgression, highlighting San Basilio and San Ignacio sites, (Fig.

2.10 B, mid-peninsula section chart pies in both maps), which included the two F1 hybrids found in this study (99_YU and 115_YU), and another four complex multispecies hybrids described below, indicated with a black circle in the top of each individual bar, structure plot of $K = 4$, Fig. 2.10 A). There was only one admixed individual from south peninsula, 150_PE from Tesos, but there were multiple initial misclassifications from *M. sp* to *M. peninsularis* (Fig. 2.10 B, right map). Three *M. yumanensis* bats that presented three-species admixture sampled in San Diego (CA8_YU, CA11_YU, CA16_YU), whereas the *M. californicus* one (42_CA) was sampled in San Fernando (Table 2.10). Cases of individuals classified as pure (no evidence of introgression in SNPs analysis), but that there was a mismatch with the morphological assignment by either mitochondrial or nuclear results, were classified at mtDNA introgressed and nDNA introgressed, respectively ($n = 14$, Appendix 2.7).

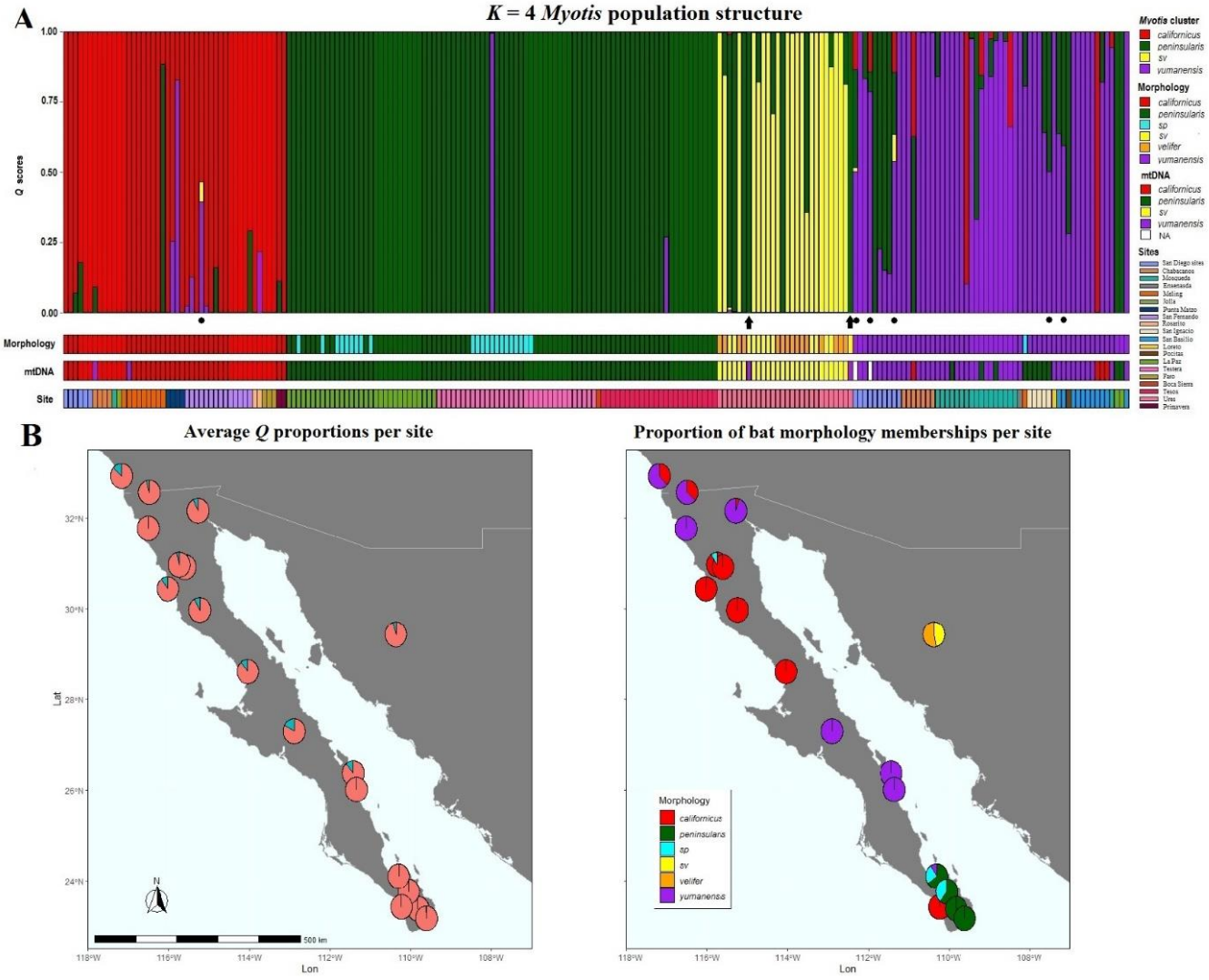


Figure 2.10. A, Admixture analysis for *K* = 4 of 218 *Myotis* individuals, ordered by their phenotypic identification. *Q*-score bar plot colours indicate ancestry of individuals assigned to different clusters. The correspondent morphological ("Morphology" bar plot), and molecular mitochondrial ("mtDNA" bar plot) are shown below. Sites of sampling are indicated in the bottom bar plot. Arrows indicate the only two individuals assigned to *M. velifer* by BLAST. Black circles indicate F1 hybrids and three-species admixed individuals. B, Average *Q* proportions per site, showing major cluster ancestry on orange, and the introgressed ancestry detected on blue (left map); and the proportion of *Myotis* morphologies per site (right map).

Table 2.8. Summary of *Q* scores proportions for admixed individuals obtained in the *K* = 4 structure test, plus their phenotypic, nuclear and mitochondrial assignments. Abbreviations: mtDNA, introgressed from mtDNA; nDNA, introgressed from nDNA; BC, Backcrossed. *Myotis* records are ordered by species phenotypic classifications (*californicus, peninsularis, sv* and *yumanensis*), and then from north to south.

Myotis Phenotype	*Myotis* mtDNA	*Myotis* nDNA	Sites	Cluster 1 *californicus* Red	Cluster 2 *peninsularis* /*velifer* Green	Cluster 3 *sv* Yellow	Cluster 4 *yumanensis* Purple	Classes of admixture
CA3_CA	*californicus*	*peninsularis*	SanDi	0.9296	0.0703	0.0001	0.0001	BC
CA4_CA	*californicus*	*californicus*	SanDi	0.8215	0.1784	0.0001	0.0001	BC
196_CA	*yumanensis*	*californicus*	Chaba	0.9068	0.0930	0.0001	0.0001	BC
499_CA	*californicus*	*peninsularis*	Meli	0.1136	0.8857	0.0003	0.0003	BC
35_CA	*californicus*	*californicus*	Matzo	0.7451	0.0001	0.0001	0.2547	BC
37_CA	*californicus*	*yumanensis*	Matzo	0.1711	0.0001	0.0001	0.8287	BC
18_CA	*californicus*	*californicus*	SanFe	0.9758	0.0001	0.0001	0.0240	BC
19_CA	*californicus*	*californicus*	SanFe	0.8728	0.0001	0.0001	0.1271	BC
42_CA	*californicus*	*californicus*	SanFe	0.5336	0.0001	0.0718	0.3945	BC
223_CA	*californicus*	*californicus*	SanFe	0.9765	0.0001	0.0001	0.0233	BC
225_CA	*californicus*	*californicus*	SanFe	0.8389	0.1610	0.0001	0.0001	BC
242_CA	*californicus*	*californicus*	SanFe	0.7091	0.2907	0.0001	0.0001	BC
53_CA	*californicus*	*californicus*	Rosa	0.7828	0.0001	0.0001	0.2171	BC
604_CA	*californicus*	*californicus*	Prima	0.8885	0.1113	0.0001	0.0001	BC
150_PE	*peninsularis*	*peninsularis*	Tesos	**0.0001**	**0.7305**	**0.0001**	**0.2694**	BC
567_VE	*sv*	*sv*	Ures	0.0009	0.1517	0.8466	0.0009	BC
574_sv	*sv*	*sv*	Ures	0.0013	0.1776	0.8202	0.0010	BC
577_sv	*sv*	*peninsularis*	Ures	0.0010	0.2960	0.7012	0.0018	BC
593_sv	*sv*	*sv*	Ures	0.0010	0.1248	0.8734	0.0008	BC
601_VE	*sv*	*sv*	Ures	0.0009	0.1857	0.8126	0.0008	BC
CA8_YU	-	*yumanensis*	SanDi	0.1200	0.3497	0.0135	0.5168	BC
CA10_YU	*yumanensis*	*yumanensis*	SanDi	0.0001	0.1662	0.0001	0.8336	BC
CA11_YU	-	*yumanensis*	SanDi	0.1470	0.1881	0.0001	0.6648	BC
CA13_YU	*yumanensis*	*peninsularis*	SanDi	0.0001	0.7489	0.0001	0.2509	BC
CA14_YU	*yumanensis*	*peninsularis*	SanDi	0.0001	0.8363	0.0001	0.1636	BC
CA15_YU	*yumanensis*	*peninsularis*	SanDi	0.0001	0.8577	0.0001	0.1422	BC
CA16_YU	*yumanensis*	*yumanensis*	SanDi	0.1435	0.2216	0.0858	0.5492	BC
200_YU	*californicus*	*peninsularis*	Chaba	0.3715	0.6284	0.0001	0.0001	BC
152_YU	*yumanensis*	*yumanensis*	Mosq	0.0553	0.0001	0.0001	0.9446	BC
153_YU	*yumanensis*	*yumanensis*	Mosq	0.0001	0.1581	0.0001	0.8418	BC
160_YU	*yumanensis*	*californicus*	Mosq	0.8976	0.0001	0.0001	0.1023	BC
161_YU	*yumanensis*	*yumanensis*	Mosq	0.0001	0.0227	0.0001	0.9772	BC
162_YU	*yumanensis*	*peninsularis*	Mosq	0.0001	0.6675	0.0001	0.3324	BC
166_YU	*peninsularis*	*yumanensis*	Mosq	0.1531	0.0489	0.0001	0.7979	BC
168_YU	*yumanensis*	*yumanensis*	Mosq	0.0240	0.1355	0.0001	0.8404	BC
169_YU	*peninsularis*	*yumanensis*	Mosq	0.0001	0.0324	0.0001	0.9674	BC
171_YU	*yumanensis*	*yumanensis*	Mosq	0.0001	0.0341	0.0001	0.9657	BC
172_YU	*yumanensis*	*yumanensis*	Mosq	0.3377	0.0001	0.0001	0.6622	BC
498_sp	*peninsularis*	*yumanensis*	Meli	0.0001	0.1939	0.0001	0.8058	BC
74_YU	*peninsularis*	*yumanensis*	SanIg	0.0001	0.3577	0.0001	0.6422	BC
99_YU	*peninsularis*	*yumanensis*	SanIg	0.0001	0.4970	0.0001	0.5029	F1
114_YU	*yumanensis*	*yumanensis*	SanBa	0.0001	0.3620	0.0001	0.6379	BC
115_YU	*yumanensis*	*yumanensis*	SanBa	0.0001	0.4042	0.0001	0.5956	F1
119_YU	*yumanensis*	*peninsularis*	SanBa	0.0001	0.7192	0.0001	0.2806	BC
487_YU	*californicus*	*yumanensis*	SanBa	0.0001	0.1780	0.0001	0.8218	BC

2.4.3.4. Cluster analysis of ddRAD derived SNPs

Principal component analysis (PCA) of the global *Myotis* set showed a clear separation between the *Myotis californicus* (red circles) complex and the rest of the *Myotis* for the first two Principal Components (PC)s (Fig. 2.11), along with some potential misclassification from *M. yumanensis* (purple circles) individuals. In the PC1 vs PC2, PC2 splits *M. californicus* into two clusters, and the rest of points are conglomerated near the centre of the multivariate space, denoting poor differentiation from the average. PCA2 vs PCA3 plot shows the same spatial distribution where *M. californicus* is well clustered opposite to the rest of the individuals, with a few points far from the cluster. PC1 explains only 6.8% of the variation, while PC2 and PC3 explains 3.6% and 3.3% each, decreasing percentage along the rest of 215 PCs explaining the data.

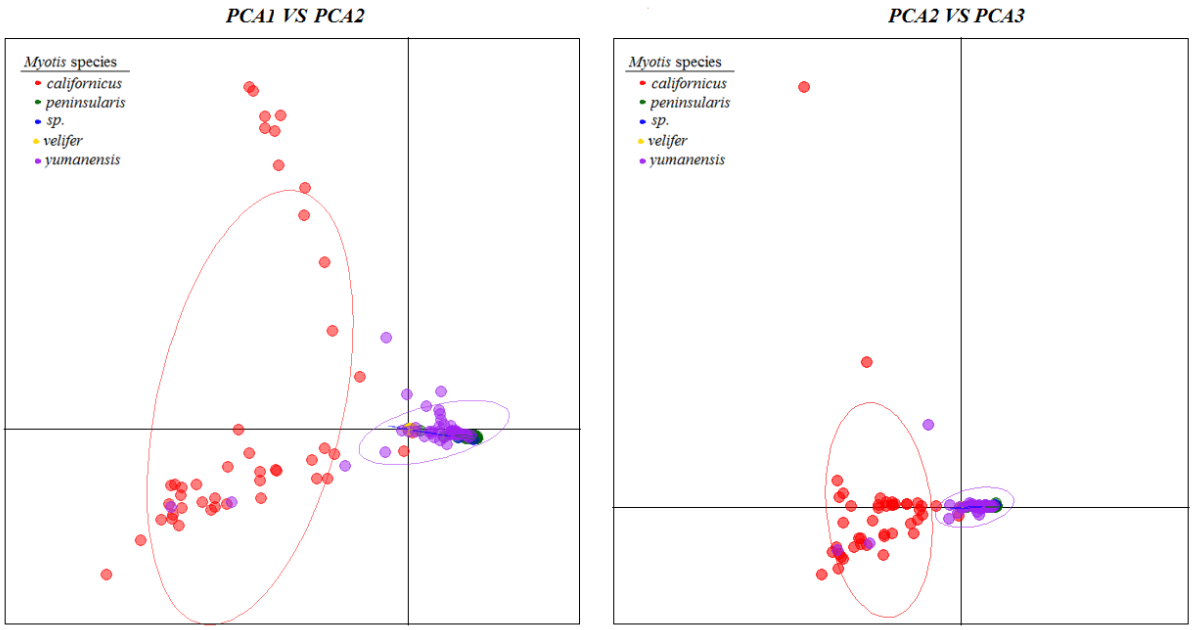


Figure 2.11. Principal component analysis of *Myotis* SNPs analysis, plotting PC1 against PC2 (left), and PC2 against PC3 (right).

Discriminant analysis based on PCA data reduction explained approximately 98% of the cumulative variance by at least 160 PCs, which were retained from the 218 total (Fig. 2.12). The BIC inference estimated from five to six clusters to best explain the data, based in the lowest value of BIC before a direction change (Fig. 2.12 A). The DAPC analysis inferred five clusters (Fig. 2.12 B), mostly located at the centre of the multivariate space over the discriminant analysis plane. This suggest that individuals within Clusters 1, 2 and 4 were sharing the most common genetic attributes along the whole data set, and Clusters 3 and 5 were more distinct among and between individuals. Membership of clusters were confirmed by the correlation made by the original vs inferred clustering groups (Fig. 2.12 C), where Cluster 1 (*n* = 43) included mostly *a priori* identified *Myotis yumanensis* individuals (*n* = 41). Cluster 2 (*n* = 40) included individuals from all the species, in majority composed by the phenotypically designated *M. sv* (*n* = 11) and *M. velifer* (*n* = 14) individuals from Ures population, (site 23 in Fig. 2.1, Appendix 2.1). Cluster 3 (*n* = 30) mainly included *M. californicus* individuals (*n* = 28); and Cluster 4 (*n* = 93) included mainly *M. peninsularis* individuals (*n* = 86). Cluster 5 was composed exclusively by *M. californicus* individuals (*n* = 11). This results provided more evidence for *a posteriori* classifications of *Myotis* individuals made by mitochondrial and nuclear analysis, particularly for *M. yumanensis*, *M. velifer/peninsularis* and *M. sv* individuals. In the case of *M. californicus* individuals, the PCA and DAPC analysis detected two clusters instead of one, suggesting genetic differentiation.

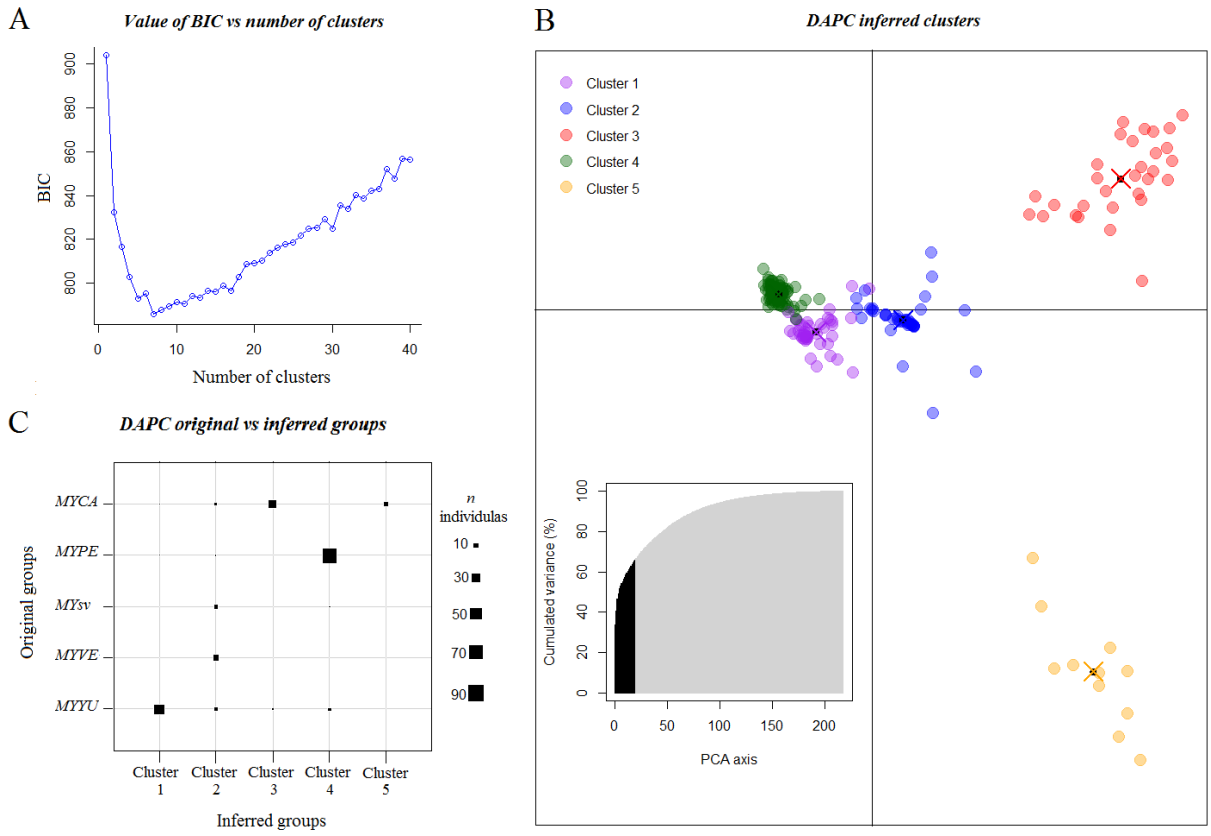


Figure 2.12. Cluster analysis. A) Discriminant analysis based on the PCA dataset, retrieved five clusters. Cumulative variance and number of PCA axis are shown in the graph located at the left bottom. B) Number of clusters inferred by Bayesian inference estimates; and C) Correlation and membership assignment of original clusters against inferred groups.

2.4.3.5. Population structure within *Myotis californicus* SNP dataset

For this analysis, a separate SNPs structure analysis was performed, including only *M. californicus* individuals that were assigned to Cluster 2 in the global *Myotis* analysis (Green, Fig. 2.10 A), and had also a mtDNA classification (Fig. 2.4). In total, 48 individuals (Table 2.5 C) sampled in 11 sites showed an overall F_{ST} of 0.17605. Pairwise values ranged from 0.03306, from San Fernando-San Basilio sites, to 0.77538 from San Basilio-La Jolla, sites (Fig. 2.2 and Table 2.9, *M. californicus*). San Fernando (SF) was the least genetically differentiated site, perhaps due to bats converging in this site to hunt and drink, which functions as an oasis in the

middle of the peninsular desert (González-Abraham, Garcillán and Ezcurra, 2010). Jolla (JO) had the highest F_{ST} values among these populations, followed by Primavera site (Fig. 2.2, Table 2.9, *M. californicus* section), both sites remotely located. Isolation by distance (IBD) was not detected among *M. californicus* individuals (r = 0.19, P = 0.17, Fig. 2.13).

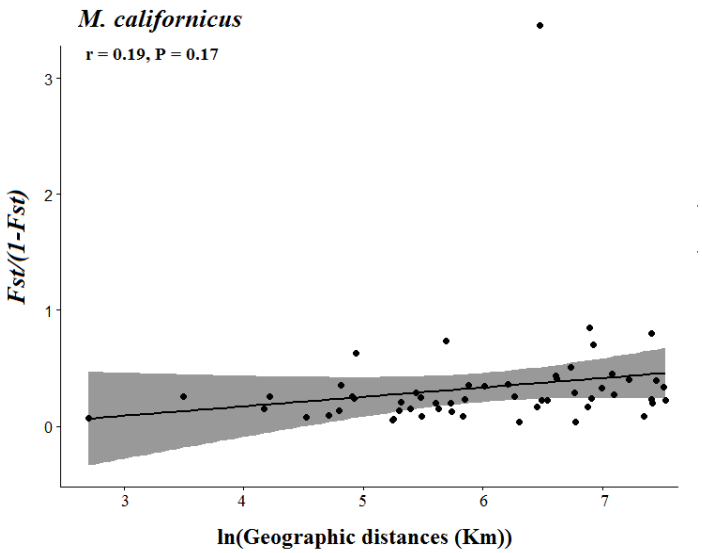


Figure 2.13. Isolation by distance analysis of *M. californicus* specimens.

Admixture analysis from K = 2 to K = 5 for *M. californicus* showed a clear population structure regarding capture site. Suggested K values resulted in between K = 3 and K = 4 (Fig. 2.14). K = 2 showed populations from Matzo to south peninsula to belong to one cluster, and the other one from Meling to the norther sites and including Primavera site from south continent. However, from K = 3 to 5, this structure changed, where populations followed the same well-defined cluster that included *M. californicus* from San Diego, California, to basically all the peninsular and the continental sites as one cluster, and a second one including Chabacanos and Meling/Jolla together. There is a third sign of admixture from another population that is detected in K = 3, present only one individual with full ancestry to a third

cluster (green cluster, 499-CA, K = 3), and two more individuals admixed with cluster yellow (green cluster, 35-CA and 37-CA, K = 3), which specimens were sampled in the north (Fig. 2.14). From K = 4 to K =5, there is a cluster with Q proportions of < 0.00009 within each plot, which are not considered as population clusters (visualised as a thin line from a different colour on each K plot, Fig. 2.14). Therefore, K = 3 was chosen as indicative of the real number of populations within the *M. californicus* dataset.

Table 2.9. F_{ST} values between sites for each species. *Myotis velifer* is included along *M. peninsularis*. Abbreviations listed in the table, from north to south, are: SD, San Diego; CH, Chabacanos; MO, Mosqueda; MA, Matzo; ME, Meling; JO, Jolla; SF, San Fernando; RO, Rosarito; SI, San Ignacio; SB, San Basilio; LO, Loreto; PO, Pocitas; LP, La Paz; MT, Testera; FA, Faro; BC, Boca de la Sierra; TE, Tesos; UR, Ures; and PR, Primavera.

M. californicus											
	SF	**MA**	**MO**	**RO**	**CH**	**FA**	**SB**	**ME**	**JO**	**PR**	**SD**
SF											
MA	0.0722										
MO	0.0814	0.1730									
RO	0.0525	0.1304	0.2566								
CH	0.1109	0.1985	0.2611	0.2648							
FA	0.0331	0.1450	0.2489	0.1837	0.3111						
SB	0.0330	0.1408	0.3040	0.2628	0.3362	0.1888					
ME	0.1162	0.1332	0.1957	0.1696	0.0582	0.1952	0.1826				
JO	0.0898	0.2036	0.3874	0.4243	0.1177	0.4605	0.7753	0.0659			
PR	0.0830	0.1901	0.2834	0.2891	0.2546	0.2922	0.4128	0.1660	0.4459		
SD	0.0779	0.1646	0.2043	0.2042	0.2037	0.2124	0.2260	0.1340	0.2247	0.1844	
M. peninsularis and M. velifer											
	MT	**TE**	**LP**	**BO**	**UR**						
MT											
TE	0.0118										
LP	0.0109	0.0098									
BO	0.0201	0.0169	0.0149								
UR	0.1037	0.1180	0.0964	0.2716							
M. yumanensis											
	SI	LO	SB	PO	MO	CH	LP	SD			
SI											
LO	0.1352										
SB	0.0584	0.0845									
PO	0.1478	0.5414	0.1075								
MO	0.0465	0.0485	0.0376	0.0330							
CH	0.0996	0.1391	0.0822	0.0884	0.0445						
LP	0.1937	0.5441	0.1499	0.5186	0.0690	0.1530					
SD	0.0817	0.0803	0.0770	0.0742	0.0416	0.0599	0.0489				

The continental individuals (Primavera, PR) were only detected as from a different cluster until *K* = 5. Despite been the furthest site, individuals from Primavera showed to be grouped with individuals from San Diego, the furthest site in the north, therefore suggesting a potential dispersal route. Admixture proportions for *K* = 4 detected a fourth population including *Q* scores of 0.0003 to 0.01%, and showing the same trend than for the analysis on *K* = 3 (*Q* scores not shown), therefore suggesting an external population influencing genetic population structure, but not as another population within this species.

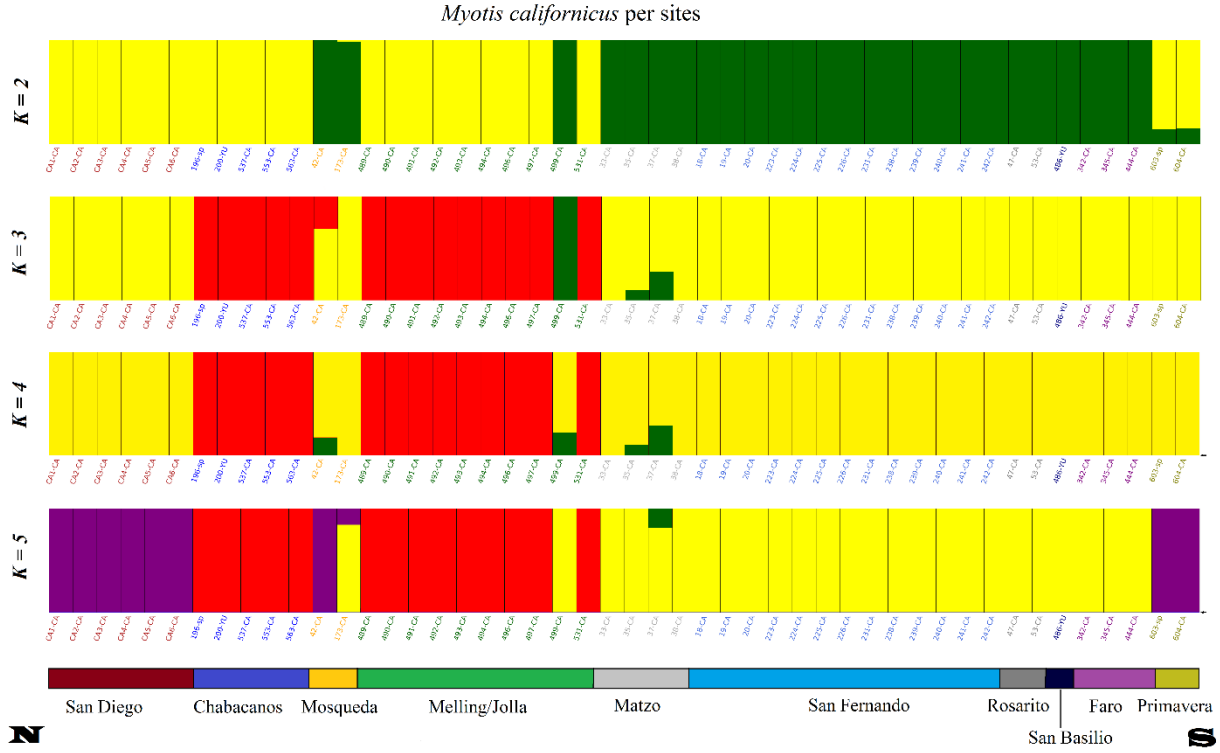


Figure 2.14. Admixture analysis from *K* = 2 to *K* = 5 of 48 *Myotis* individuals assigned to *M. californicus* with the mitochondrial analysis, but labelled as their initial phenotypic identification. Abbreviations: CA, *californicus*; YU, *yumanensis*; and *M. sp*, non-identified. Bar plot colours indicate different populations detected according to their HW clustering, progressing from *K* = 2 to *K* = 5 for evaluation. Text colours indicate site of sampling, correlated with the colour bar located at the bottom of the figure. Sites are ordered from north to south peninsula, and the north to south western Mexico. For the *K* = 4 to *K* = 5 plots, the 4th and 5th clusters are visualised by a thin coloured line (purple and blue, respectively), and do not represent a real population (black arrow at the end of each plot).

2.4.3.6. Population structure within *Myotis peninsularis/velifer* SNP dataset

For this analysis, a separate SNPs structure analysis was performed, including only *M. peninsularis/velifer* individuals that were assigned to Cluster 2 in the global *Myotis* analysis (Green, Fig. 2.10 A). In this case, the two individuals assigned to *M .velifer* by BLAST were included, despite having a *M. yumanensis* mtDNA classification (Fig. 2.6 YU3 class). In total, 92 individuals (Table 2.5 D*) sampled in six sites showed an overall F_{ST} of 0.0848. Pairwise values ranged from 0.0098, from Tesos-La Paz sites, to 0.2716 from Boca de la Sierra-Ures site (Fig. 2.2, Table 2.9 in *M. peninsularis/velifer* section). There is low genetic differentiation among most of the sites, where the populations of Ures was the most differentiated compared with the rest of the sites. Ures is located in in northwest continent, therefore this was expected (Fig. 2.2, Table 2.9 in *M. peninsularis and M. velifer* section). IBD was not detected (P = 0.21) (Fig. 2.15).

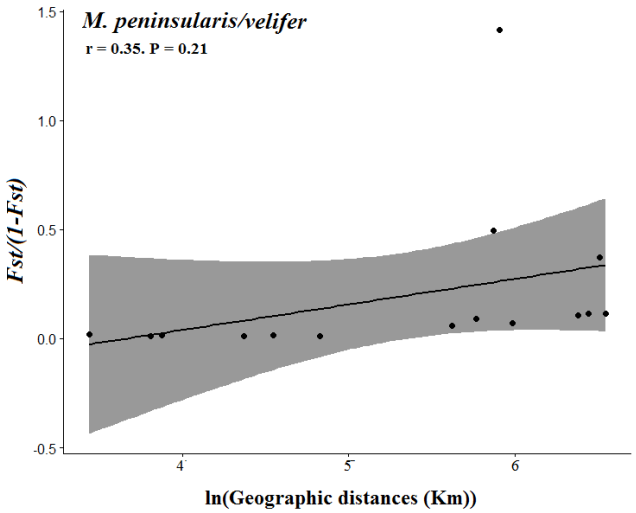


Figure 2.15. Isolation by distance analysis of *M. peninsularis/velifer* specimens.

Exploration of admixture from K = 2 to K = 5 for *M. peninsularis/velifer* was conducted, including the individuals assigned as *M. peninsularis/velifer* from the nuclear SNP

analysis of the putative *M. velifer* (572-sv and 602-sv, Fig. 2.10 A). Optimal *K* statistics for explaining the data was suggested as *K* = 2 (Fig. 2.16), where the two putative *M. velifer* individuals plus the individual 150-PE showed membership to a different cluster. From *K* = 3 to *K* =5 there was not major cluster present ($Q < 0.000002$), until *K* = 5, presenting a forced clustering with no biological or molecular meaning for each classified individual. Global *Q* scores for the admixed individuals (150_PE, 572_sv and 602_sv) showed a recent backcross of 14.31% with Cluster 3 (scores not shown).

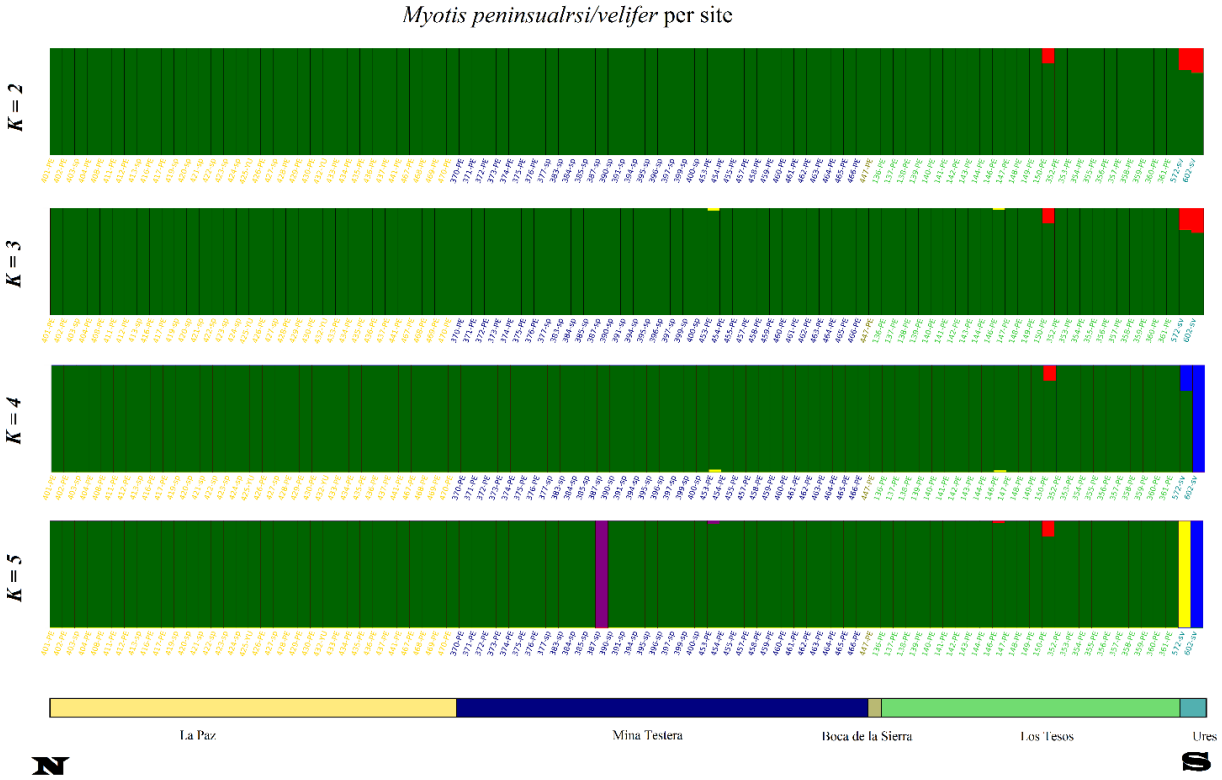


Figure 2.16. Admixture analysis from *K* = 2 to *K* = 5 of 93 *Myotis* individuals assigned to *M. peninsularis* with the mitochondrial analysis, but labelled as their initial phenotypic identification, plus two putative *M. velifer* individuals. Abbreviations: PE, *peninsularis*; sv, *M. sv*; sp, and *M. sp* (initially non-identified species). Bar plot colours indicate different populations detected according to their HW clustering, progressing from *K* = 2 to *K* = 5 for evaluation. Text colours indicate site of sampling, correlated with the colour bar located at the bottom of the figure. Sites are ordered from north to south peninsula, and the north to south western Mexico.

2.4.3.7. Population structure within *Myotis yumanensis* SNP dataset

For this analysis, a separate SNPs structure analysis was performed, including only *M. yumanensis* individuals that were assigned to Cluster 2 in the global *Myotis* analysis (Green, Fig. 2.10 A) and had mtDNA classification also (Fig. 2.4). In total, 53 individuals (Table 2.5 E) sampled in six sites showed an overall F_{ST} of 0.10519, presenting low to moderate genetic differentiation among sites in the north compared to the rest of sites sampled. Pairwise values ranged from 0.03306 from Mosqueda-Pocitas sites, to 0.54412 between Loreto-La Paz sites (Fig. 2.1, Table 2.9 in *M. yumanensis*). Loreto from mid-peninsula and La Paz and Pocitas sites from the south, are the most differentiated from the rest and between each other. In contrast, individuals from San Diego, Chabacanos and Mosqueda sites are the least differentiated among north and mid-peninsula sites (Table 2.11). No IBD was detected among *M. yumanensis* individuals (P = 0.12, Fig. 2.17).

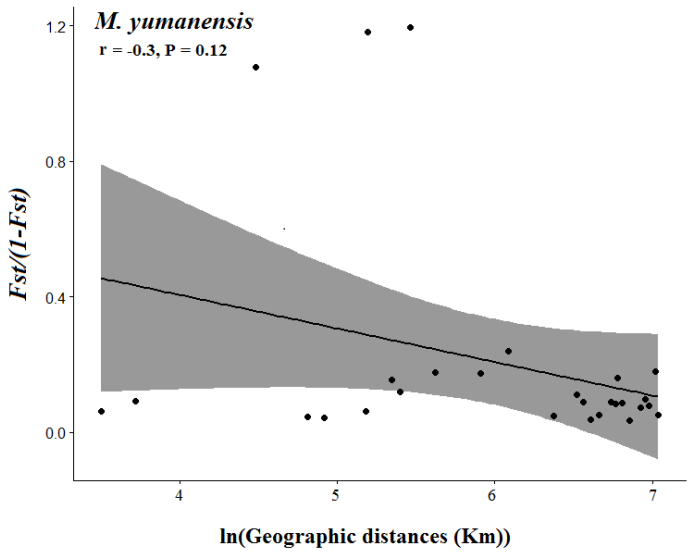


Figure 2.17. Isolation by distance analysis of *M. yumanensis* specimens.

Admixture analysis from $K = 2$ to $K = 5$ of putative *M. yumanensis* classified by nDNA results was performed for 8 sites. The optimal K for this dataset was defined as $K = 2$ to $K = 3$ (Fig. 2.18), where $K = 3$ showed one cluster involving most of the samples (green cluster) suggesting long distance dispersal, and the other two showing few individuals (red and yellow). Poor population structure was shown, detecting a subtle pattern of admixture between proximal sites in the case of individuals from the red cluster, but none for green and yellow in $K = 3$ (Fig. 2.18). Northern sites San Diego and Mosqueda, San Basilio in mid-peninsula, and La Paz in the south, were the places with more cases of admixture. This suggest that *M. yumanensis* individuals are potentially showing recurrent dispersal from the same population, with other populations occasionally overlapping sites.

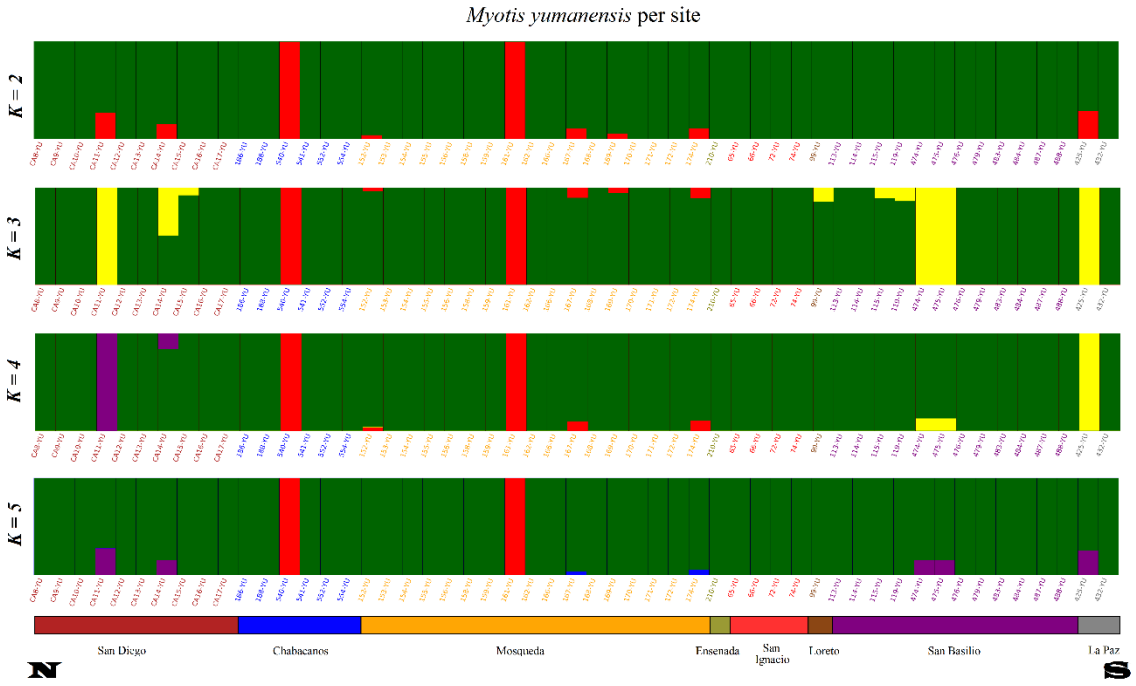


Figure 2.18. Admixture analysis from $K = 2$ to $K = 5$ of 53 *Myotis* individuals assigned to *M. yumanensis* with the mitochondrial analysis, but labelled as their initial phenotypic identification. Abbreviation: YU, *yumanensis*. Bar plot colours indicate different populations detected according to their HW clustering, progressing from $K = 2$ to $K = 5$ for evaluation. Text colours indicate site of sampling, correlated with the colour bar located at the bottom of the figure. Sites are ordered from north to south peninsula.

2.5. Discussion

We investigated the genetic diversity and species boundaries of sympatric *Myotis* bats in the Baja California peninsula, Mexico, using mitochondrial and nuclear data. We also evaluated genetic structure within species. The results reinforced the view that some *Myotis* species are extremely difficult to identify by morphological characteristics alone, highlighting the importance of using molecular markers to understand species boundaries. This study found two potential new cryptic species based on mtDNA divergence (*M. sv* and *M. volans* from Baja), one also confirmed by the Bayesian SNP clustering, plus PCA and DAPC analysis (*M. sv* lineage). These potential lineages need to be further studied, including more samples and detailed morphological analysis. We also revealed new records of *M. peninsularis/velifer* in new localities over mid-peninsula, which is out of the range previously stipulated for either species. We also found extensive hybridisation and introgression across four *Myotis* species, including individuals with apparent ancestry from more than two species. This suggest that *Myotis* bats species in Baja are prone to interbreeding, with porous species borders. There was evidence of population structure for some individuals, both regionally and through the west section of North America. This project provides the first insight in this region about genetic differentiation in a complex of *Myotis* bats by combining mitochondrial barcoding and ddRAD genome-wide data approaches, which significantly increases the knowledge of patterns of genetic differentiation and introgression among bats.

2.5.1. Bat sampling

Sampling *Myotis* bats in the Baja was a challenging task, as there are not many roosting sites known. Roosting sites are normally prioritised since they allow efficient sampling of large

numbers of individuals. However, there were only three maternity roost sites sampled for this study, in the south peninsula (Tesos, Testera and La Paz sites, Fig. 2.2), leading to some disparity in sample sizes with other sites. Elsewhere, targeting water bodies proved an effective sampling strategy, using both natural reservoirs and human-built water repositories. In bats, recognition of water bodies are mediated by cues that detect mirror-like reflection using echolocation (Greif and Siemers, 2010). Even targeting urban water bodies, which are subject to multiple disruptive elements, proved effective for random sampling of bat diversity over the mostly arid territory of the Baja California peninsula.

2.5.2. Phenotypic, mitochondrial and nuclear assessment of species boundaries

Overall, the level of complexity to characterise the *Myotis* bats in this study was very high. Morphology-based subdivision of *Myotis* species is poorly correlated with phylogenetic relationships (Ruedi and Mayer, 2001; Van Den Bussche and Hoofer, 2004) as is often seen when dealing with cryptic species. As an example, a pattern of field misidentifications occurred for bats sampled as part of this study, with individuals initially assigned as *M. yumanensis* and *M. volans* based on morphology, having mtDNA classification (haplotypes,YU7, YU8 and VO2) falling within *M. californicus* haplotypes (HG1, extreme right, Fig. 2.7). This suggests a link between the phenotypic assessments made on those particular sites, including specific morphological characteristics that lead to exclude them from the rest of *M. californicus* assessments in field. This would imply that those individuals shared the same ambiguous morphological characters, potentially suggesting them as a population with specific cryptic morphology, and/or potential plasticity of supposedly diagnostic features, or the influence of hybridisation and introgression. This seems to be a common issue among *Myotis* taxonomy, given the amount of incorrectly annotated reference sequences from GenBank (Leray *et al.*,

2019). This is evidence for the need for re-evaluations *Myotis* taxonomy, which may include higher species diversity than is currently thought. Phenotypically, bats from mid-peninsula were assigned to *M. yumanensis* because of the lack of keel in the calcar. However, "rudimentary" keel was detected in some of the bigger individuals (resembling members of *M. californicus*), and some other also had the plagiopatagium attached to a side of the foot (resembling *M. peninsularis*). This was also the case between *M. yumanensis* and *M. peninsularis* individuals in the south and mid-peninsula, where the misclassified *M. yumanensis* were classified as *M. peninsularis* given that they were smaller than most of the *M. peninsularis* found, a finer rostrum and paler colour. They were still showing traces of a keeled calcar, where neither bat species should have it (Álvarez-Castañeda and Bogan, 1998; Braun *et al.*, 2015). Between *M. yumanensis*, *M. californicus* and *M. sv* over the northern sites, complications to classify species were caused by differences in sizes and particular colour patterns from *M. californicus*, where rostrum mask was ambiguous, and calcar was sometimes presenting a barely visible keel (*M. californicus* should have a defined keeled calcar, Simpson 1993).

Recent signs of genetic admixture and F1 hybrids found in mid-peninsula and northern sites showed to be in congruence with phenotypic ambiguity and molecular results. This was not the case in the south, where the two of the *Myotis* that were classified as *yumanensis* by morphology, were assigned as *peninsularis* by mtDNA and nDNA results, and with no signs of admixture. The same happened with the morphologically unclassified *M. sp* individuals from the south, all assigned to *M. peninsularis* as well (Appendix 2.7, Fig. 2.4).

Morphological classification was particularly difficult at the continental site Ures, where most of the bats were not able to be fully classified to *M. velifer,* or any other currently recorded species (and were therefore given designation *M. sv*) by morphology only. *M. sv* phenotype individuals were genetically heterogeneous, being assigned to Cluster 2 and 3 (Fig. 2.10 A), but the only perceptible morphological differences were gradients of fur colour and size. This study reiterates that the use of morphological features to identify cryptic species is not enough to form any conclusion regarding taxonomic and ecological interactions, as it has been seen in other studies dealing with the same constrains in contact zones (Chattopadhyay *et al.*, 2016).

Mitochondrial Cyt*b* sequences did not always result in conclusive species assignments when they were compared with GenBank reference sequences, mainly because of the lack of existing references for Baja. Species identification in this genus is quite complex, which can lead to mistakes in the specimen designation and cause further problems after GenBank submission (Leray *et al.*, 2019). For example, haplogroup HG7 represented a single haplotype of *M velifer* retrieved from GenBank, which was at least 21 mutations away from other *M. velifer* and *M. yumanensis* (Fig. 2.8), representing a clear example of cryptic morphology influencing potentially erroneous species annotation for some GenBank submissions (Leray *et al.*, 2019). This may result in downstream errors in barcoding and phylogenetic studies which rely on such data.

In general, the phylogenetic clades and major haplogroups were consistent with previous phylogenies published for the *Myotis* evaluated in this study (Ruedi and Mayer, 2001; Stadelmann *et al.*, 2007; Larsen*et al.*, 2012; Nájera-Cortazar, Álvarez-Castañeda and De Luna,

2015; Platt *et al.*, 2018), including the same problems regarding the lack of clarity about their taxonomic designations. Genetic divergence among *Myotis* of this study did not always reflect their phenotypic variation, nor their ecological differentiations. This has been attributed to the *Myotis* rapid radiation (~5 and 10 MYA) and incomplete lineage sorting (Platt *et al.*, 2018).

2.5.3. The case of *Myotis peninsularis/velifer* populations

The status of the putative endemic *M. peninsularis* has been ambiguous due to its resemblance to *M. velifer* (Hayward, 1970; Fitch, Shump and Shump, 1981; Álvarez-Castañeda and Bogan, 1998), where the most recent study, which used analysis of skull morphology and a small Cyt*b* dataset, suggested to consider *M. peninsularis* as a population of *M. velifer* (Nájera-Cortazar, Álvarez-Castañeda and De Luna, 2015), but recommended the use of more data to further explore this idea. Phylogenetic analysis in the present study supported the previous mitochondrial conclusion, that *peninsularis* and *velifer* haplotypes are not reciprocally monophyletic and differed by only a small number of substitutions. However, all *peninsularis* individuals carried haplotypes which are rare or not found in the reference *velifer* samples. Additionally, the median joining network placed *M. velifer*/*yumanensis* and *M. peninsularis* haplotypes in separate sub-haplogroups (Fig. 2.8). These results might be consistent with a recent founder event for *M. peninsularis*, suggesting potential contributions of gene flow by dispersal between continental and peninsular populations of *M. velifer*. The only individuals sampled by this study with *velifer* morphology, in the continental site of Ures, carried *sv* type mtDNA, making their status ambiguous. Within this site, two other individuals (with *sv* morphology) had mtDNA haplotypes assigned by BLAST to *M. velifer* (572 and 602). However, in the phylogenetic analysis, these individuals were placed in *M. yumanensis*

subclade (YU3), and not the *M. peninsularis* subclade (Fig. 2.4). However, they were assigned to Cluster 2 in the from the SNP analysis (Fig. 2.10 A, arrows).

With the nuclear SNP data some individuals with *californicus*, *sv*, *velifer* and *yumanensis* phenotypes were assigned to Cluster 2 along with all *peninsularis* phenotype individuals. However, these all originate from sites outside the *peninsularis* range which implies they may derive ancestry from continental *velifer* populations, and a close association between peninsularis and velifer nuclear genotypes (Fig. 2.10 A). Evaluation of F_{ST} values and structure analyses by sites also showed evidence of genetic differentiation between *M. velifer* and *M. peninsularis* individuals. PCA and DAPC cluster analysis clustered the two putative *M. velifer* individuals along with *M. peninsularis,* and the rest of *M. sv* individuals as a separate cluster (Fig. 2.11. and 2.12), despite that population structure analysis classified more *M.sv* individuals (Fig. 2.10 A. yellow bars), to be part of *M. peninsularis* cluster (Fig. 2.10 A, green bars), agreeing with the phylogenetic analysis. FastStructure may lack of power to resolve contributions from unsampled populations (Raj, Stephens and Pritchard, 2014), and therefore the cluster attribution of the individuals from the northern sites could change if more confirmed *velifer* samples were available.

Overall, mitochondrial and nuclear results suggest that *M. peninsularis* and *M. velifer* share recent ancestry, but show incipient genetic differentiation from each other. More studies need to be done including more *M. velifer* individuals from across the species range, to account ecological variation, and for continent, continent-peninsula, and potential continent-sea/island-peninsula dispersal routes. This study gathered evidence for describing *M. peninsularis* as a separate entity from *M. velifer* into some extent, where from both mitochondrial and nuclear

points of view, there is a degree of demographic isolation and differentiation for the population of the south of Baja, but the magnitude of divergence is likely to lie in the intraspecies range.

2.5.4. *Myotis* bats population structure and species boundaries

Phylogenetic relationships described *M. yumanensis* as sister species of *M. velifer* and *M. peninsularis,* which belong to the Neotropical subclade; whereas *M. californicus,* which is less closely related, is located in the Nearctic subclade (Stadelmann *et al.*, 2007). A population structure study of European *Myotis* (Bogdanowicz, Piksa and Tereba, 2012b) showed that phylogenetically closer species, in this case *M. mystacinus* and *M. alcathoe* (Palearctic clade), produced more hybrids between each other than with *M. brandtii* (New World clade, but Palearctic according to Stadelmann *et al* . 2007). This SNP structure analysis showed that *M. yumanensis* was prone to admix with all the populations present in this study, supporting the hypothesis of potential hybridisation among its sister species *M. peninsularis/velifer*, as well as with *M. californicus* individuals (Fig. 2.10, Appendix 2.7). The mid-peninsula region functioned as one of the "hot-spots" for introgression in Baja, where F1 hybrids were detected in this study, along with other admixed individuals, phenotypically assigned *M. yumanensis* (Table 2.10). However, the analysis of *M. yumanensis* populations between sites (Fig. 2.18) did not show any strong admixture with the few individuals/sites included in this study. In this region, traces of introgression between *M. peninsularis*/*velifer* and *M. yumanensis* was evident (Fig. 2.10 green Cluster), where individuals assigned by the phylogenetic data as *M. yumanensis*, were clustered as *M. peninsularis* in the nuclear data. However, PCA and DAPC cluster analysis separated *M. yumanensis* as a unique cluster, and the same for *M. peninsularis* unique cluster (Fig. 2.11 and 2.12). In this analysis, the Cluster 2 included most of the *M. sv* individuals, as well as come *M. yumanensis* and *M. californicus* individuals, pointing out the

influence of the admixed individuals. A hypothesis for explaining this would be that these putative *M. yumanensis* bats are hybridising with *M. velifer*, therefore explaining the conflicting results between the mitochondrial and nuclear data over the north, and explaining their new detected distribution over mid-peninsula (see previous section, "The case of *Myotis peninsularis/velifer* populations").

The distribution of *M. yumanensis* covers from southern Alaska to Central Mexico (Braun *et al.*, 2015), and down to the southern part of Baja. Besides confirming records of *M. yumanensis* in the southern region, this study found evidence of the great capability of this species to travel long distances. The haplotype network including GenBank references (Fig. 2.7-2.8) showed individuals from this study sharing a haplotype with one sequence potentially from a bat captured in Alaska (Accession number KM370991 to 96). This might partially explain why there was no structure subdivision for most of the individuals in the analysis within sites, and no IBD detected, as is commonly find among volant species like birds and bats (Guevara-Chumacero *et al.*, 2010; Dixon, 2011; Miller *et al.*, 2014). It has been documented that bats may fail to present IBD even at large scales (Furmankiewicz and Altringham, 2007; Dixon, 2011), and they can present IBD when population dispersal is infrequent (e.g. geographic or ecological barriers, fragmentation), and/or there is natal philopatry among colonies (Guevara-Chumacero *et al.*, 2010; Dixon, 2011; Nagy *et al.*, 2013). Despite the relatively small sample sizes and few sites which may limit power to detect structure, SNPs and haplotypes results from this study supports large scale dispersal within *Myotis* populations of this study.

Mitochondrial results showed three well defined clades in the *M. peninsularis-velifer-yumanensis* phylogenetic tree (Fig. 2.4 and 2.6), and its own cluster of haplotypes with a transitional network of single substitutions (Fig. 2.8 and 2.9). However, all the analysis converged on finding a group of *M. yumanensis* that did not fit under either mtDNA (phylogenetic subclade YU3, phenotypic and geographic haplotype YU6, Fig. 2.4, and 2.8-2.9, respectively), nor nDNA *M. yumanensis* main cluster, (admixed *yumanensis* individuals in global assessment Fig. 2.10 A, Table 2.10). Included in these are the putative *M. yumanensis* individuals from San Diego and Chabacanos sites, plus other reference sequences from Alaska (Olson *et al.*, 2014). These sites proven to be another "hotspot" for bat introgression, located in the limits between peninsula and mainland (Fig. 2.2). For example, Chabacanos has a small lagoon in the middle of the desert, perfect for hunting/water drinking, where many bat species converge, and as a potential stop over for migrating bats over the western North America (Braun *et al.*, 2015). Consistent separation of these individuals from other *M. yumanensis,* from both this study and reference sequences, suggest a population which is potentially divergent at mtDNA but with high levels of nuclear admixture with other populations and species.

Myotis californicus was more closely related to *M. volans* than to the rest of species (Nearctic clade in Stadelmann *et al*. 2007). Phylogenetic and haplotype results showed that samples of this study were mixed with reference sequences from *M. californicus* and *M. ciliolabrum*, suggesting potential misclassifications based in phenotype from both this study and references sequences (Fig. 2.5 and 2.7). This has already been pointed out as an important issue by a morphological and molecular analysis of *M. californicus* and *M. ciliolabrum* (including the morphologically similar *M. melanorhinus* and *M. leibii*), where it was concluded that sequence divergence and their phylogenetic results did not support different lineages for

both species (Rodriguez and Ammerman, 2004). However, reference sequence clades were positioned separately from those generated by this study (Fig. 2.4), in agreement with the haplotype analysis results (Fig. 2.7), suggesting the populations of Baja are distinct from those sampled for other studies. Additionally, PCA and DAPC analysis split *M. californicus* individuals into two different clusters (Fig. 2.11 red spots, Fig. 2.12 Cluster 3 in red and Cluster 5 in yellow). This is suggesting that there can be potentially two differentiated populations, or even two different lineages that have a *M. californicus* cryptic morphology, which also agrees with phylogenetic results. Haplotype network analysis also showed a degree of isolation between groups of haplotypes included in the haplogroup HG1 (Fig. 2.7). *M. californicus* sampled for this study (CA1-CA12), were mainly spread in three clusters of haplotypes, split by *M. californicus* and *M. ciliolabrum* reference sequences, accumulating more than eight mutations between haplotypes (e.g. CA12 separated by eight mutations from CA10, its closest haplotype from this study). There is a possibility that included among this analysis there are in fact, one or more different cryptic lineages, which could be any of the other species suggested previously by the literature, including *M. ciliolabrum* and *M. leibii* (Simpson, 1993; Holloway and Barclay, 2001; Rodriguez and Ammerman, 2004). However, more samples are needed for understanding the degree of differentiation between this cryptic complex, as well as morphological and demographic studies, to further test this hypothesis.

Overall, there were high levels of genetic differentiation among *californicus* populations, and often more than the one that presented among species (overall F_{ST} and F_{SC} results, Table 2.4). *M. californicus* showed population structure related to sites, where the best differentiated SNP clusters showed a continuous population including members of San Diego, most of the sites in Baja and including continental specimens (K = 3, yellow cluster, Fig. 2.14).

This contrasted with the geographic network (Fig. 2.9), where haplotype CA2a and b and the CA15, are 16 mutations away from each other. Discrepancies among markers might be suggesting that philopatric females are contributing to a sub-structure of certain populations of this species. No IBD was detected, supporting long distance dispersal, similarly than with *M. yumanensis*.

Levels of admixture among *M. californicus* were also high, showing sites of the north acting as zones for genetic differentiation, also in agreement with *M. yumanensis* results. This added evidence of introgression and hybridisation among the other species (Table 2.10), despite not being sister species nor positioned in the *Myotis* Nearctic clade (Stadelmann *et al.*, 2007). Admixed individuals that were detected in the global assessment were also admixed in the population analysis (red and green clusters, Fig. 2.14). Red cluster referred to northern sites with higher altitudes (Meling 646, Chabacanos 758 and Jolla 1459 meter above sea, Appendix 2.1), implying a difference in climate and vegetation types (see Fig. 1.1, Chapter 1), which might be indicating ecological barriers for the distribution of this population. The San Fernando site yielded some of the admixed individuals, and it is located in the middle of a continuous arid extension, with an altitude of 469 meter above sea. Potentially, this area might be functioning not only as a stopover place for hunting and drinking, but also as a link in between the two main populations of *M. californicus*; where bats could be using it as a temporal place in between migration routes, where mating may be occurring between *Myotis* that are distributed along.

Despite ambiguity over some haplotype-species associations, this study identified two new highly divergent Cyt*b* lineages within the bats sampled for this study. First, the potentially

cryptic lineage *M. sv*, is not phylogenetically related to *M. velifer* as much as initially thought (~10% genetic divergence, see Fig. 2.1 and 2.4). Haplotype analysis showed 10 haplotypes including 29 sequences, suggesting these bats potentially came from a maternity roost with members of the same species but from different distribution. Admixture analysis corroborates that *M. sv* population presents admixture with *M. peninsularis/velifer* populations (Fig. 2.10 A, Cluster 3 yellow and Cluster 2 green, respectively). The most plausible explanation would be that there is gene flow among *M. velifer* populations near by the roosting place of *M. sv*. During the BLAST searches, only three sequences *M. fortidens* were obtained as the most similar species, then with an average genetic distance of 6.95% (Table 2.2). However, BLAST searches did not show another existing record from a partial mitochondrial genome sequenced from a museum specimen of *M. fortidens* (Platt *et al.*, 2018). Genetic divergence pilot analysis (not shown) resulted on a range of 0.2% to 1.2% between *M. sv* and the *M. fortidens* unverified sequence obtained from Platt *et al.* (accession number MF143483, Platt *et al*. 2018). Despite this, more studies need to be done to relate molecular with morphological data, since convergent ectomorphs are common with *Myotis* bats (Stadelmann *et al.*, 2007; Platt *et al.*, 2018).

Second, individuals of *M. volans* were mostly captured in the north of Baja, with an unexpected record in the south (green stripe, Fig. 2.2), where it was phenotypically assigned as *M. peninsularis*. Genetic divergence from *M. volans* of this study versus reference sequences was in average 11.78% (Table 2.2), which should be considered as an interspecific value (Bradley and Baker 2001; Ruedi and Mayer 2001), implying that the putative *M. volans* from Baja is a different species, and potentially a new lineage using the mitochondrial cyt*b* marker information available to date. The phylogenetic results showed three different clades of *M.*

volans sequences, with two subclades obtained by this study individuals (Fig. 2.4 and 2.5, golden branches). In the phylogeny performed by Stadelmann *et al*. (2007), two separate clades were obtained (*M. volans* A and B). In other study, using molecular markers, *M. volans* had a different placement among the other *Myotis* species, as a sister species of the New World *Myotis* clade along with *M. brandtii* (Platt *et al.*, 2018). In contrast, the mitochondrial phylogeny placed *M. volans* within the Nearctic clade (Stadelmann *et al.*, 2007; Platt *et al.*, 2018). All the outcomes together show a more complex taxonomy and evolutionary history of peninsular *M. volans* worth further studying.

This study has increased substantially the molecular and distribution information of North American bats, updating phylogenetic analysis of New World *Myotis*, and discovering the complexity of Baja *Myotis* inter-specific gene flow, dispersal and migration. Sampling efforts for Mexican bats are biased towards tropical regions, where there is high species richness, high accessibility and security (Zamora-Gutierrez, Amano and Jones, 2019), neglecting arid regions like the Baja California peninsula, where there is plenty unexplored territory and relatively little is known about bat diversity, biology and ecological interactions. Transitional regions (Nearctic-Neotropical) are usually environmentally under-sampled, with few knowledge about patterns of temporal, latitudinal and altitudinal dispersal of bats and their potential as pathogens vectors, therefore the necessity of greater sampling effort is urgent.

2.5.5. Synthesis

Overall, this work has discovered a high amount of cryptic diversity, which highlights the relevance for increasing resources to explore and study non-model organisms, especially over isolated regions and neglected wildlife worldwide. In general, cryptic morphology is

frequently present in bats worldwide (Clare *et al*., 2011, 2013; Razgour *et al*., 2011; Srinivasulu *et al*., 2019). The genus *Myotis* is one of the most diverse groups of bats in the world (Stadelmann *et al.*, 2007; Larsen *et al.*, 2012; Morales *et al.*, 2017; Platt *et al.*, 2018), and phylogenetic relationships are often controversial due to their cryptic morphology and poor sampling (Morales *et al.*, 2017). In the last years, several detections of new *Myotis* species have been published, suggesting a general increment in the discovery of species for this cryptic genus (Morales *et al*., 2017; Juste *et al.,* 2018; Moratelli *et al*., 2019; Simmons *et al*., 2021). It is expected that the potential for discovering more species may be greater in regions that are less explored or of more challenging sampling (Srinivasulu *et al.*, 2019).

Previously available sequences from public databases do not reflect the true diversity and genetic relationships among organisms that have no vouchers available, mitochondrial and nuclear markers data, or that are difficult to identify (Platt *et al.*, 2018; Leray *et al.*, 2019). *Myotis* systematics should reflect their ecological and behaviour constraints, plus their biogeographic and phylogeographic histories to better represent their taxonomic status and associations (Ruedi and Mayer, 2001; Hoofer and Van Den Bussche, 2003; Stadelmann *et al.*, 2007). We have provided high-resolution genetic data to further investigate the taxonomic identity of at least three *Myotis* lineages, and for the mechanisms promoting cryptic morphology despite genetic differentiation (Baker and Bradly, 2006; Chattopadhyay *et al*., 2016; Lavretsky, Janzen and McCracken, 2019). Gathering multiple independent sources of evidence for understanding cryptic speciation, will contribute to increase research of similar study cases, building an important database of ecologic, taxonomic and genetic information. To have an up-to-date biodiversity inventory is also highly relevant to prevent population decline and even extinction of endemic taxa.

The importance of interspecific gene flow, hybridisation and introgression in sympatric species has been an important topic for understanding the evolution and diversification of species (Jones *et al*., 2012; Abbott *et al*., 2013; Poelstra *et al.*, 2014; Supple *et al.*, 2015). According to Bogdanowicz, Piksa and Tereba (2012), there are few cases of hybridisation reported in bats, compared to plant and animal species. However, evidence about hybridisation in bats is increasing, because their complex behaviour, social interactions and long-distance dispersal provides a suitable scenario to study interspecific gene flow in sympatry and between cryptic species (Altringham, 2011; Chattopadhyay *et al*., 2016; Centeno-Cuadros *et al.*, 2019). In our study, the species assignments for the majority of *Myotis* individuals were consistent between mitochondrial and nuclear markers. However, a significant proportion of individuals showed some evidence of recent hybridisation, or older introgression events with SNP data, or had conflicts between mitochondrial and nuclear species assignments. Evidence suggests that hybridisation and introgression events in bats, mostly involves morphologically cryptic species, independently of if they are sister species or have recently diverged (Centeno-Cuadros *et al.*, 2020), as the *Myotis* complex from the present study. Cryptic speciation and hybridisation has been shown in two cryptic bat species *Eptesicus serotinus* and *E. isabellinus*, were male-mediated asymmetric hybridisation was found in sympatric colonies (Centeno-Cuadros *et al*., 2020). In this study, having evidence of interspecific hybridisation between these two *Eptesicus* species, had important zoonotic implications for interspecific transmission of European bat *Lyssavirus* type 1 (Centeno-Cuadros *et al*., 2020), showing the importance for understanding bat interspecific relationships and the mechanisms of genetic interchange.

Swarming behaviour in *Myotis* bats increases gene flow and connectivity between sites (Burns, Frasier and Broders, 2014; Johnson *et al.*, 2015), including distant populations and

different species. Evidence of gene flow along key sites in Baja, gathers important evidence of the complexity in a multispecies system, where introgression and hybridisation under gene flow is relatively frequent between high proportions of *Myotis* bats. Yet, *Myotis* bats in this system seems to maintain their species identity. In evolutionary terms, the presence of hybrids indicates that interspecific mate selection during swarming events may be a way to increase their reproductive success (Bogdanowicz, Piksa and Tereba, 2012), which can lead to either increase fitness, or to speciation. Selection may or may not favour hybrids, depending on the pressure of ecological and genetic constrains on the species. According to Morales *et al*. (2017), a sympatric vs allopatric mode of speciation has prevailed during the evolution of the *Myotis* bats. An example is another *Myotis* complex distributed in North America, comprised by *Myotis evotis*, *M. keenii* and *M. thysanodes*, which form a group of sister species (*M. evotis*, *M. keenii*) *M. thysanodes*), with paraphyletic relationships due to mitochondrial introgression, inferred by phylogenetic analysis using Cytochrome *b* (Dewey 2006). Incorporating the presence of gene flow and the use of a genomic data of ultraconserved elements, Morales *et al*. (2017) evaluated the evolutionary patterns of this *Myotis* complex, demonstrating that there was evidence of gene flow at the initial stages of divergence, and that may not occur in the present. For *Myotis* bats in Baja, the mechanisms for which different degrees of interspecific gene flow may occur in the present, or have occurred early during they divergence, need to be further studied. By the generation of high-resolution genetic data, we have provided with the material to further study how selection could affect levels of gene flow between the *Myotis* complex in Baja, providing an excellent opportunity to study different stages of the speciation continuum in a vertebrate system, which will help to understand how genomes diverge during speciation and in face of gene flow (Seehausen *et al*., 2014; Supple *et al*., 2015; Marques *et al*., 2016). As other studies have already tested in different taxa (Jones *et al*., 2012; Abbott *et al*.,

2013; Poelstra *et al.*, 2014; Supple *et al.*, 2015; Morales *et al*., 2017), our results contributes to change the traditional view of speciation under gene flow to be a rare event, showing that the nature of species boundaries in bat systems are not fixed, but is a dynamic and porous process that is shaped by each species evolutionary processes.

Among vertebrates, birds and bats are able to disperse over long distances, overcoming different environmental conditions and even oceanic barriers (Castella *et al*., 2000; Gómez-Díaz, Navarro and González-Solís, 2008; Sonsthagen *et al.*, 2019). Volant animals represent an important diver of genetic differentiation, which highlights the importance of understanding the mechanisms that allows them to disperse and cope with different environmental, physiological and pathogen challenges. Wide-range genomic information can provide signatures of selection and gene functions to investigate the genomic basis of adaptive evolution (Jones *et al*., 2012; Sonsthagen *et al.*, 2019), and ecological and physiological adaptations to different conditions through their range (Arnegard *et al.*, 2014). This has also important implications for understanding current and future adaptations to climate change and anthropogenic disturbance, which can be extrapolated to other organism's studies and conservation.

Chapter 3: Phylogenetic analyses show bat communities on the Baja California peninsula harbour a high diversity of novel cryptic ectoparasite species.

3.1. Abstract

Parasites are integral parts of ecosystems and fundamental drivers of evolutionary processes. Here, we used a phylogenetic approach to characterise ectoparasite taxon identity and diversity for 16 species of bats captured along the Baja California peninsula. This was done by sequencing mitochondrial Cytochrome Oxidase C subunit I and nuclear 18S ribosomal gene fragments using Bayesian and Maximum Likelihood methods. We revealed multiple potential novel lineages of bat bugs (Cimicidae), flies (Nycteribiidae and Streblidae) and soft ticks (Argasidae). Within families, the new linages showed more than 10% sequence divergence, which is consistent with separation at the species level. Both families of bat flies showed host specificity, particularly on *Myotis* species. We also identified new records in the peninsula of one tick (*Carios kelleyi*), and of five Streblid bat flies. One Nycteribiid bat fly haplotype from Pallid bat (*Antrozous pallidus*) hosts was found throughout the peninsula, suggesting potential host migration. Different bat bug and tick communities were found in the north and south of the peninsula, suggesting roosting sites and environmental factors may play a role in their range boundaries. This study is the first systematic survey of bat ectoparasites in the Baja California peninsula, discovering highly genetically differentiated lineages compared to other parts of North America. We also found patterns of bat migration through the ectoparasites haplotypes. This work is a first step for discovering ectoparasite diversity over the Baja peninsula, and understanding how ecological and evolutionary interactions shape bat ectoparasite repertoire along different host species and distribution.

3.2. Introduction

Characterising ectoparasite diversity is fundamental to studies of host-parasite interactions, evolution and conservation, as well as being key to understanding emerging disease threats for some vector borne pathogens (Morand, Krasnov and Poulin, 2006; Poulin, 2014; Spencer and Zuk, 2016). Bat ectoparasites are of high interest because of their influence on the hosts immune system and resultant coevolution, where infection can be an important evolutionary driver of diversity and ecosystem organization (Gómez and Nichols, 2013; Spencer and Zuk, 2016). Bat-ectoparasite relationships are also important as pathways for understanding bat dispersal, pathogen transmission and zoonotic disease risks (Klimpel and Mehlhorn, 2014; Wilder, Kunz and Sorenson, 2015; Joffrin *et al.*, 2018; Speer *et al.*, 2019). Despite the widely recognised need to increase sampling effort for pathogen/parasite discovery in bats, bat ectoparasites are still understudied in most parts of the world (Reinhardt and Siva-Jothy, 2007; Gay *et al.*, 2014; Brook and Dobson, 2015; de la Harpe *et al.*, 2017; Haelewaters *et al.*, 2017; Hornok *et al.*, 2019). Here we use a phylogenetic approach to characterise ectoparasite (bat bugs, flies and ticks) identity and diversity for 27 species of bat resident along the Baja California peninsula, and north western Mexico, based on mitochondrial Cytochrome Oxidase C subunit I (COI) and nuclear 18S ribosomal (18S) DNA amplicon sequences.

3.2.1. Ectoparasite diversity in bats

Among the arthropod groups that are bat-associated ectoparasites, we commonly find members of the Insecta class, including bat bugs (Hemiptera), fleas (Siphonaptera) and flies (Diptera); and members of the Arachnida class such as ticks (Ixodida) and mites (orders Mesostigmata and Trombidiformes) (Ter Hofstede and Fenton, 2005; Seneviratne, Fernando

and Udagama-Randeniya, 2009). All these ectoparasites are hematophagous (blood feeding) organisms, creating potential mechanisms for interactions with humans and domestic animals (Poulin, 2014; Hornok *et al.*, 2017).

Bat bugs are included in the family Cimicidae, which has a worldwide distribution and comprises approximately 110 known species within 24 genera (Balvín, Vilímová and Kratochvíl, 2013; Hornok *et al.*, 2017; Ossa *et al.*, 2019). The family also includes the common Bed Bug (*Cimex lectularius*), which is one of the most studied ectoparasite that interacts with bats, birds and humans (Reinhardt and Siva-Jothy, 2007), and is the potential vector of at least 65 pathogens (Zorrilla-Vaca, Silva-Medina and Escandón-Vargas, 2015; Hornok *et al.*, 2017). The majority of cimicid bugs are ecologically and biologically associated with bats, which are considered to be their ancestral host (Usinger, 1966; Balvín *et al.*, 2012; Balvín, Vilímová and Kratochvíl, 2013; Hornok *et al.*, 2017; Ossa *et al.*, 2019); therefore, their biology and ecology appears to be strongly influenced by bats in most cases (Ossa *et al.*, 2019).

Bat flies comprise two main families, Nycteribiidae and Streblidae, which have a common origin from a single lineage coevolving with bats (Wenzel and Tipton, 1966; Dittmar *et al.*, 2006). As of 2006, there were approximately 520 species described (Dittmar *et al.*, 2006). This number has increased substantially following many recent publications reporting new taxa and/or new records around the world (Scheffler *et al.*, 2015; de Vasconcelos *et al.*, 2016; Ramírez-Martínez *et al.*, 2016; Ramasindrazana *et al.*, 2017; Saldaña-Vázquez *et al.*, 2019; Szentiványi, Christe and Glaizot, 2019), but an updated review and synthesis of these families is yet to be published. Additional fly species associated with bats include the Hippoboscidae and Glossinidae which also parasitize other mammals and birds (Petersen *et al.*,

2007). These two families are monophyletic along with Nycteribiidae, while family Streblidae seems to be paraphyletic in respect to the other fly families. Recent work has been focused on describing diversity of bat flies in North America (Dick *et al.*, 2003; Cuxim-Koyoc *et al.*, 2015; Ramírez-Martínez *et al.*, 2016; Trujillo-Pahua and Ibáñez-Bernal, 2018), South America (Dick, 2006; Dick and Miller, 2010; Reeves *et al.*, 2016) and Western Europe and South West Asia (Tortosa *et al.*, 2013; Ramasindrazana *et al.*, 2017). Bat flies exhibit both host specificity and host-roosting site sharing (Dittmar *et al.*, 2006; Bennett, Turmelle and O'Grady, 2014; Ramasindrazana *et al.*, 2017; Estrada-Villegas *et al.*, 2018), although it has been shown that this varies accordingly to habitat type, roosting ecology and richness of hosts (Saldaña-Vázquez *et al.*, 2019). The family Nycteribiidae is considered to occur over subtropical, tropical and temperate regions; whereas Streblidae has been recorded for tropical and subtropical climates only (Dittmar *et al.*, 2006). Both families present reduced compound eyes, lack of ocelli and a spider-like shape; where nycteribiids present a backwardly folded head and a complete loss of wings (Dittmar *et al.*, 2006), and most of streblids present fully-developed wings (Bennett, Turmelle and O'Grady, 2014) and a membranous abdomen (Dittmar *et al.*, 2006).

Ticks are grouped in three families: Argasidae (soft ticks), Ixodidae (hard ticks) and *Nuttalliellidae*. The latter family comprises a single known species, *Nuttalliella namaqua* (Mans *et al.*, 2012; Burger *et al.*, 2014), which it has been suggested as the basal group among all the tick lineages (Mans *et al.*, 2012). There are approximately 894 known species of ticks worldwide, with 32 species of Argasidae and 68 species of Ixodidae in Mexico (Guglielmone *et al.*, 2010; Pérez *et al.*, 2014). Bat tick phylogenetic studies have been mainly conducted for old world species (Hornok *et al.*, 2014; Hornok, Kontschán, *et al.*, 2015; Sándor Hornok, Szőke,

Görföl, *et al.*, 2017), with a limited number for the Americas and some of them have also included ticks from distinct parts of the world (Black IV, Klompen and Keirans, 1997; Burger *et al.*, 2014; Hornok, Estrada-Peña, *et al.*, 2015; Venzal *et al.*, 2015; Sándor Hornok, Szőke, Tu, *et al.*, 2017). Rather focusing on tick systematics, many tick studies in bats list incidental records of ticks and hosts, or are focused on single host–tick systems as vectors of other pathogens (Hornok *et al.*, 2014, 2019; Tahir *et al.*, 2016; Sándor Hornok, Sándor, *et al.*, 2017; Sándor Hornok, Szőke, Tu, *et al.*, 2017; Sándor *et al.*, 2019), especially those of medical importance that have been found in bats, like *Anaplasma, Borrelia, Bartonella*, and *Rickettsia* (de la Fuente *et al.*, 2008; Reeves *et al.*, 2016; Burazerović *et al.*, 2018). Ticks parasitizing bats have evolved to adapt their unique morphology and life-style, therefore it is common to find host-specificity (Sándor *et al.*, 2019). Most of the work has been conducted over Europe, therefore host-specificity and life-cycle studies from bat ticks in other parts of the world are still scarce (Sándor *et al.*, 2019). Classification of taxonomic relationships among soft ticks are controversial (Burger *et al.*, 2014), and more studies are needed to accurately describe their taxonomic status (Estrada-Pena *et al.*, 2010; Burger *et al.*, 2014).

3.2.2. Phylogenetic studies of bat ectoparasites

Early studies of bat ectoparasite taxonomy and diversity have previously been published as lists of records of ectoparasites identified by morphology and descriptive host associations (Usinger, 1966; Graciolli, 2001; Steinlein, Durden and Cannon, 2001; Horak, Camicas and Keirans, 2002; Dick *et al.*, 2003; Estrada-Pena *et al.*, 2010). Molecular phylogenetic analyses have substantially improved the resolution of taxonomic studies for bat bugs (Balvín, Vilímová and Kratochvíl, 2013; Hornok *et al.*, 2017; Ossa *et al.*, 2019), bat ticks (Mans *et al.*, 2012; Hornok *et al.*, 2014; Sándor Hornok, Sándor, *et al.*, 2017; Sándor Hornok, Szőke, Görföl, *et al.*,

2017), and bat flies (Dittmar *et al.*, 2006; Olival *et al.*, 2013; Tortosa *et al.*, 2013; Trujillo-Pahua and Ibáñez-Bernal, 2018), which are often challenging to identify on the basis of morphology. Using molecular techniques, Hornok *et al*. (2017a) found two different lineages of *Cimex* bugs associated to Pipistrelle bats within *C. lectularius* bugs, implying potential associations of the human-related bed bugs with bat bugs. This was later corroborated by Roth *et al*. (2019) showing evidence of other bat related bugs parasitizing both bats and humans (e.g. *Leptoxcimex sp.*), or completely generalists parasitizing bats, humans and birds (e.g. *C. hemipterus*). Phylogenetic relationships of bat ectoparasites have often revealed unexpected host associations and new lineages of ectoparasites (Powell *et al.*, 2013; Tortosa *et al.*, 2013; Hornok *et al.*, 2014; Ramírez-Martínez *et al.*, 2016; Ramasindrazana *et al.*, 2017; Hornok *et al.*, 2017; Sándor Hornok, Sándor, *et al.*, 2017), suggesting that there is potential for high rates of misidentification based on morphology and many cryptic ectoparasite species across all groups.

Previous bat ectoparasite studies in North America have mostly focused on describing distribution and taxonomic status of bat flies, bugs and ticks (Jobling, 1938; Bradshaw and Ross, 1961; Usinger, 1966; Graciolli, 2001; Graciolli, Autino and Claps, 2007), and their medical importance (Steinlein, Durden and Cannon, 2001; Dick *et al.*, 2003). In Mexico, publications have focused on diversity studies of flies, ticks and mites (Pérez *et al.*, 2014; Guzmán-Cornejo *et al.*, 2017; Bolaños-García, Rodríguez-Estrella and Guzmán-Cornejo, 2018), with special attention on new records of bat flies (Cuxim-Koyoc *et al.*, 2015; Ramírez-Martínez *et al.*, 2016; Trujillo-Pahua and Ibáñez-Bernal, 2018), and studies including ecological relationships with bats and bat flies (Salinas-Ramos *et al.*, 2018; Saldaña-Vázquez *et al.*, 2019; Zamora-Mejías *et al.*, 2020), and *Rickettsia* presence in soft ticks in Mexico

(Sánchez-Montes *et al.*, 2016). To my knowledge, there has not been any studies specific to Mexico related to bat bugs.

The Baja California peninsula (Fig. 2.2, Chapter 2), holds a mosaic of habitats and high levels of biodiversity and endemism (Riddle *et al.*, 2000; González-Abraham, Garcillán and Ezcurra, 2010). There are approximately 25 species of bats distributed along this region (Álvarez-Castañeda and Patton, 1999; Medellín, Arita and Sánchez, 2007; Álvarez-Castañeda, Álvarez and González-Ruíz, 2015). While there are several studies describing Baja bat diversity, distributions and ecology (Álvarez-Castañeda and Patton, 1999; Frick, Hayes and Heady, 2008; Álvarez-Castañeda, Álvarez and González-Ruíz, 2015; Nájera-Cortazar, Álvarez-Castañeda and De Luna, 2015), there are no studies describing the bat ectoparasite fauna. This study is intended as a first step in describing the ectoparasite diversity of the bats in this region by performing phylogenetic analyses using mitochondrial Cytochrome oxidase sub 1 (COI) and the nuclear ribosomal 18S (18S) amplicons. The aims of this study are to characterise the species identity of sampled ectoparasites, to relate them to known or novel lineages within Baja and the rest of the Americas, and evaluate the extent to which these can be attributed to specific regions in Baja, or more widely distributed along their host's ranges. This research is also relevant for increasing knowledge of both bat and ectoparasite distribution for western North America, and to understand how ecological and evolutionary interactions shape parasite community structure along environmental gradients, which will be addressed in Chapter 4.

3.3. Methods

3.3.1. Sample collection

Bat sampling was conducted at 21 sites along the Baja California peninsula, three sites in continental Mexico during 2016-2018 as described in Chapter 2 (Appendix 2.1, Fig. 2.1, in Chapter 2). Bats were identified to species level in the field following published identification guides (Medellín, Arita and Sánchez, 2007; Álvarez-Castañeda, Álvarez and González-Ruíz, 2015), and subsequently with molecular assays (see Chapter 2). Ectoparasites were collected manually from bats using forceps, and transferred to labelled Eppendorf tubes with 96% ethanol for storage. Ectoparasites collected from the same bat but from different taxonomic groups were stored in separate tubes for each group, with corresponding labels and appropriate specimen source identifiers. All samples were kept on ice during fieldwork, until their arrival to the laboratory where they were stored at -20° C. Ectoparasites were photographed during field work using a portable Maozua 5MP 20-300X USB microscope, taking ventral and dorsal views. Preliminary classification of ectoparasites was done according to morphological characters specified in keys by McDaniel (1979), Usinger (1966), Knee and Proctor (2006) and Dick and Miller (2010), and adapted for North American ectoparasites by this study. In addition to ectoparasites collected in the field, 10 specimens of bat bugs were provided by Drew Stokes from San Diego Natural History Museum, from *Parastrellus hesperus* hosts, captured at Ramona, California, U.S.A.

3.3.2. DNA extraction, primer selection and PCR amplification

Individual ectoparasite bodies were first sectioned and crushed using sterile pestles, followed by digestion with Proteinase K at 56° C for 18 hours. DNA was extracted using either

a Thermo-Fisher DNA extraction and purification kit (Thermo Fisher Scientific Inc.) or QuickExtract kit (Epicentre, Illumina), following the manufacturers' protocols. PCR conditions were optimized separately for each ectoparasite group, and according to each primer set. Primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3) and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al.*, 1994) were used to amplify an approximately 700bp fragment of the mitochondrial COI gene in a 25 µl reaction. The reaction mix contained: 1 U of Flexi GoTaq Taq Polymerase, 5X GoTaq reaction buffer, 50 mM $MgCl_2$ (1.5 – 2.5 mM final concentration), 0.5 µl PCR nucleotide Mix (0.2 mM each), 0.5 µl of each set of primers (1 µM final concentration), 15.8 µl ddH_2O and 8 µl template DNA (extractions diluted at 1:10 with sterile distilled water). Thermal cycling parameters were as follows on a TECHNE thermocycler model TC-512: initial denaturation step at 95°C for 5 min, followed by 40 cycles of denaturation at 94°C for 40 seconds, annealing at 53°C - 56°C for 1 minute and extension at 72°C for 1 minute. Final extension was performed at 72°C for 10 minutes.

For the 18S rDNA gene, approximately 800bp amplicons were amplified using the primers 18S-1F (5' CTGGTTGATCCTGCCAGTAGT 3') and 18S-3R (5' GGTTAGAACTAGGGCGGTATCT 3') for bat bugs (Campbell *et al.*, 1995); a0.7-F (5' ATTAAAGTTGTTGCGGTT 3') and 7R (5' GCATCACAGACCTGTTATTGC 3') for flies (Whiting, 2002); and D-F (5' GGCCCCGTAATTGGAATGAGTA 3') and C-R (5' CTGAGATCCAACTACGAGCTT 3') for ticks (Mangold, Bargues and Mas-Coma, 1997). The same reaction mix quantities were used as for the COI gene, with $MgCl_2$ at a final concentration of 1.5 mM for all primer sets. Thermal cycling parameters were set as follows: initial denaturation step at 95°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 40s, annealing at 53°C for 1 min and extension at 72°C for 1 minute. Final extension was

performed at 72°C for 10 minutes. PCR products were visualized by 1% agarose gel electrophoresis and then sent for PCR product purification and Sanger sequencing to GENEWIZ (GENEWIZ Europe, 2018), with each amplicon sequenced in both forward and reverse directions.

3.3.3. Quality control and references sequences

Sequence quality for both the forward and reverse strands of each amplicon was evaluated in BioEdit 7.2.5 (Hall, 2005). Trimmed forward and reverse sequences were combined to generate a consensus sequence for each amplicon, and then analysed in BLAST (The National Library of Medicine, 2018) to generate initial taxon identities and identify reference sequences. Additionally, COI sequences were analysed in the BOLD system platform (Ratnasingham and Hebert, 2007), in search for other potential taxonomic matches. Reference sequences were also compiled from previous studies on each ectoparasite group (Dittmar *et al.*, 2006; Mans *et al.*, 2012; Tortosa *et al.*, 2013; Burger *et al.*, 2014), and by performing a systematic search using *AnnotationBustR* 1.2 package (Borstein, 2018) in Rstudio 1.1.456 (RStudio Team, 2015), searching for the closest genus for the sequences generated in this study and for those obtained by BLAST (see methods Chapter 2). Alignments combining reference sequences and those from this study were generated using CLUSTAL W (Thompson, Higgins and Gibson, 1994), implemented in BioEdit 7.2 (Hall, 2005), and were reviewed by eye, with manual correction of potential errors as required.

3.3.4. Sequence summary statistics and phylogenetic analyses

Genetic distance estimates among haplotypes and best fit sequence evolution models for the COI and 18S datasets were evaluated using MEGA 10.1.7 (Sudhir *et al.*, 2018). The Barcode Index Number system was followed to delimit a lineage, where intraspecific variation at COI is generally considered as groups of sequences with less than 2% divergence, and exhibiting more than 4% divergence from neighbouring lineages (Hebert, Ratnasingham and DeWaard, 2003; Ratnasingham and Hebert, 2013; Salinas-Ramos *et al.*, 2018). Phylogenetic analyses were also performed using the best fit evolution model identified for each group and each marker, assessing phylogenetic relationships based on the Maximum Likelihood approach in MEGA (Sudhir *et al.*, 2018), and under a Bayesian approach using BEAST 1.10.4 (Suchard *et al.*, 2018). For the latter, a MCMC chain length of 10,000,000 was used and priors specific to each parasite group and sequence evolution model selected using the program BEAUti 1.10.4 (Suchard *et al.*, 2018). Two separate runs using the same Bayesian settings file generated by BEAUti were run in BEAST (see methods Chapter 2), with a burn in of the 10% of the total number of iterations. Posterior probabilities results files were then formatted as described in Chapter 2. Phylogenetic trees were edited and visualized using iTOL 5.6.2 (Letunic and Bork, 2016).

Following phylogenetic analysis, haplotypes from this study were grouped by lineage, and haplotype diversity and genetic summary statistics were calculated in DNAsp ver. 5.10.01 (Librado and Rozas, 2009) as appropriate. Outgroups were chosen based on previous phylogenetic studies of each group, using *Bucimex chilensis, Primicimex cavernis* and *Anthocoris flavipes* for bat bugs (Ossa *et al.*, 2019); *Drosophila melanogaster*, *Chrysops niger*,

Musca domestica, *Sarcophaga bullata*, *Ornithomya avicularia* and *Spelobia bifrons* for bat flies (Dittmar *et al.*, 2006); and *Nuttalliella Namaqua* (Mans *et al.*, 2012) for ticks.

3.4. Results

3.4.1. Sampling and field identifications

A total of 1,988 ectoparasites were collected within the orders Diptera (flies), Hemiptera (bugs), Ixodida (ticks), Mesostigmata (mites), Siphonaptera (fleas) and Trombidiformes (chiggers). However, fleas, chiggers, mites and any non-identified specimens were excluded for the present study. Fleas, chiggers and mites will be briefly examined further in Chapter 4, along with spatial distributions and community composition across sampling sites. Bats captured parasitized by at least one group is listed in Appendix 3.1. The samples for the three ectoparasite groups considered here comprised 90 bat bugs, 213 bat flies and 126 ticks, collected from 138 individual bat hosts (Fig. 3.1) of 16 species.

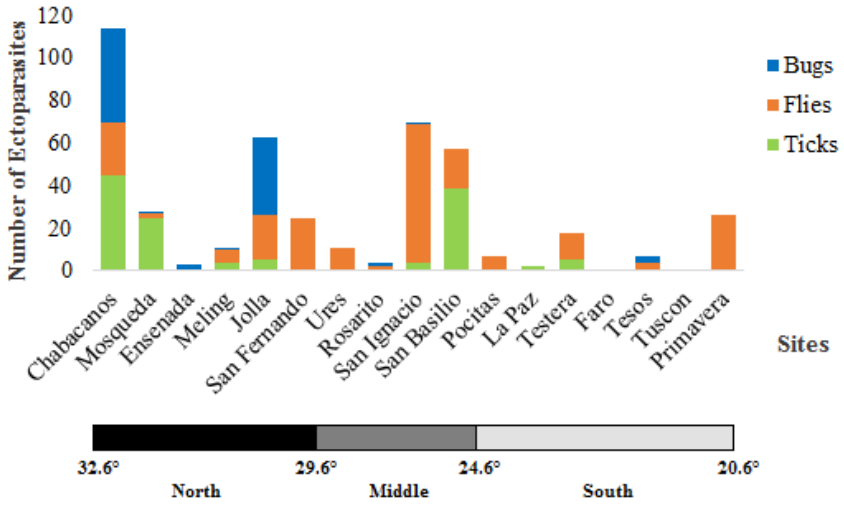


Figure 3.1. Number of bat bugs, flies and ticks captured per site in 2016-2018 fieldwork seasons. A latitudinal gradient bar at the bottom shows the limit established in this study to considered north, mid or south peninsula.

There was a notable majority of flies captured in most of the sites (13 sites), followed by ticks and bugs (eight sites). However, captures were more abundant in specific sites, were the majority of bugs were sampled in Chabacanos and Jolla; ticks in Chabacanos, Mosqueda and San Basilio; and San Ignacio for flies (Fig. 3.1). Field morphological evaluations suggested most specimens were related to *Cimex pilosellus* (Usinger, 1966). Most bats flies could be identified to the genus level, which were later corroborated with molecular data. On the basis of morphology, all bat ticks were identified as family Argasidae (soft ticks), with at least six different morphotypes present. Most of these were tentatively attributed to the genus *Ornithodoros*, but further classification was not possible at the time of sampling.

3.4.2. Molecular analyses

A total of 150 COI mitochondrial and 147 18S rDNA sequences were generated among the three groups: bugs n = 30/30 (COI/18S, respectively); flies n = 81/73; and ticks n = 39/44. When there was more than one ectoparasite specimen available per bat, one specimen for sequencing was selected based on morphological similarity, sequencing one individual from each morphological cluster found. Neither BLAST nor BOLD analysis suggested that any of the haplotypes from bugs and most of the tick specimens in this study matched existing sequences deposited in GenBank at the species level (all divergence > 4%), with the exception of a single match from the tick species *Carios kelleyi*, corresponding to one of the tick lineages (96.95% in GenBank, 97.07% in BOLD databases). For bat flies, seven of 39 COI sequences matched with five known species (see Table 3.1). Where comparable reference sequences were available, the results of 18S BLAST analyses were consistent with those from COI. For clarity and to provide comparability with larger numbers of reference sequences, further reporting of diversity, divergence statistics and taxonomic identity will be focused on COI results.

Overall, both Maximum Likelihood and Bayesian phylogenetic analyses using COI recovered similar tree topologies, and in each case they produced identical clade clustering for sequences generated by this study. For each parasite type, COI and 18S markers returned similar interspecies relationships allowing for differences in reference sequence availability. For brevity, here we present only Bayesian topologies for both COI and 18S analyses, with the exception of 18S sequences in streblid flies, where the alignment quality was poor and therefore the 18S phylogeny is not presented.

3.4.2.1. Genetic diversity

COI genetic diversity statistics for each phylogenetically defined ectoparasite lineage obtained in this study are summarised in Table 3.1. Excluding four lineages represented by single individuals (e.g. *Cimex* 1), and three lineages including only individuals with the same haplotype (e.g. *Basilia* 1), nucleotide diversity (Nd) ranging from 0.001-0.006, with the highest value 0.071, presented by Tick 4 lineage, with three haplotypes (H) in three sequences (Table 3.1). In general, number of haplotypes were close to or the same as the number of sequences tested per lineage, with some exceptions including *Cimex* 4 bugs; flies Nycteribiid 2, *Basilia* 2b and *Nycterophilia coxata*, and from ticks Tick 3 and Tick 5 having two less haplotypes than sequences, but still presenting high Hd (Table 3.1), suggesting potential structuring along most of the groups. *Basilia* 2b showed five haplotypes from 26 sequences all along the peninsula, implying a potential dispersal path by its only host, *A. palllidus* (Fig. 3.2).

Table 3.1. Summary statistics of the ectoparasites lineages from this study. Abbreviations are: *N*, number of sequences within each group; *S*, number of segregating sites; *H*, number of haplotypes; *Hd*, haplotype diversity; *Nd*, nucleotide diversity, and % Div., percentage of divergence.

	Lineage/putative genus	N	S	H	Hd	Nd	Host(s)	Closest reference species	% Div. from closest reference	Known congeneric ectoparasite species for host(s) when no molecular match
Bat bugs: Family Cimicidae	*Cimex* 1	1	0	1	0	0	*Antrozous pallidus*	*Cimex latipennis*	5.1	*C. incrassatus, C. pilosellus* [1]
	Cimex 2	2	4	2	1	0.006	*Myotis californicus*	*Cimex latippenis*	3.2	*Cimex adjunctus, C. antennatus, C. brevis, C. pilosellus, C. latippenis* [1]
	Cimex 3	5	2	2	0.4	0.001	*Antrozous pallidus, Myotis californicus*	*Cimex adjuntus*	5.9	*C. incrassatus, Paracimex cavernis* [1]
	Cimex 4	22	14	17	0.97	0.005	*Parastrellus hesperus, Antrozous pallidus*	*Cimex antennatus*	4.4	*C. antennatus, C. incrassatus, C. pilosellus* [1,8]
Bat flies: Family Nycteribiidae	Nycteribiid 1	5	6	5	1	0.003	*Myotis sv Myotis californicus Parastrellus hesperus*	*Nycteribia pygmaea*	9.7	*Basilia antrozoi, B. corynborhini, B. forcipata, B. rondani* [6, 8]
	Nycteribiid *2*	12	22	8	0.89	0.007	*Myotis volans Antrozous pallidus Myotis californicus Myotis yumanensis*	*Nycteribia pygmaea*	9.9	*Basilia antrozoi, B. corynborhini, B. forcipata, B. jellisoni, B. rondani* [5, 6, 8]
	Basilia 1	3	0	1	0	0	*Myotis yumanensis*	*Basilia boardmani*	4.9	*Basilia forcipata, B. jellisoni, B. rondani* [5, 6]
	Basilia 2a	3	1	2	0.67	0.0009	*Myotis vivesi*	*Basilia ortizi*	9.9	*Basilia plaumanni, B. pynzonix, B. producto* [6]
	Basilia 2b	26	4	5	0.58	0.001	*Antrozous pallidus*	*Basilia ortizi.*	8.9	*Basilia antrozoi, B. rondani* [6]
Bat flies: Family Streblidae	*Aspidoptera phyllostomatis*	2	0	1	0	0	*Sturnira parvidens*	*Aspidoptera phyllostomatis*	2.3	-
	Megistopoda aranea	3	29	3	1	0.029	*Sturnira parvidens Artibeus hirsutus*	*Megistopoda aranea*	0.76	-
	Nycterophilia coxata	9	9	4	0.75	0.006	*Leptonycteris yerbabuenae Macrotus californicus*	*Nycterophilia coxata*	0.0	-
	Paratrichobius 1	1	0	1	0	0	*Artibeus hirsutus*	*Paratrichobius longicrus*	4.7	*Aspidoptera phyllostomatis, Paratrichobius longicrus* [3]
	Streblid 1	4	15	3	0.83	0.011	*Macrotus californicus*	*Strebla sp.*	11.7	*Trichobius adamsi, Nycterophilia coxata* [8]
	Trichobius 1	2	6	2	1	0.008	*Mormoops megalophylla*	*Trichobius sphaeronotus*	6.3	*Trichobius sphaeronotus, T. galei, T. leionotus, Nycterophilia mormoopsis* [7]
	Trichobius 2	2	0	1	0	0	*Choeronycteris mexicana*	*Trichobius dugesii*	5.8	*Trichobius mixtus, Paratrichobius longicrus* [8]

	Trichobius 3	1	0	1	0	0	*Artibeus hirsutus*	*Trichobius joblingi*	5.3	*Aspidoptera phyllostomatis, Paratrichobius longicrus*[8]
	Trichobius dugesii	1	0	1	0	0	*Glossophaga soricina*	*Trichobius dugesii*	0.1	-
	Trichobius sphaeronotus	2	0	1	0	0	*Leptonycteris yerbabuenae*	*Trichobius sphaeronotus*	0.0	-
Bat Ticks. Family Argasidae	*Antricola* 1	1	0	1	0	0	*Macrotus californicus* *Myotis vivesi*	*Antricola marginatus*	10.7	*Ornithodoros sp.* [8]
	Carios kelleyi	10	33	9	0.98	0.011	*Antrozous pallidus*	*Carios kelleyi*	1.9	-
	Tick 1	3	2	3	1	0.002	*Antrozous pallidus* *Myotis peninsularis*	*Ornithodoros turicata*	12.3	*Ornithodoros sp., O. kelleyi,, O. rossi* [8, 9]
	Tick 2	2	1	2	1	0.001	*Antrozous pallidus* *Myotis yumanensis*	*Ornithodoro faccinii*	13.2	*Ornithodoros kelleyi, O. yumatensis, O. rossi* [5, 8, 9]
	Tick 3	6	15	4	0.8	0.008	*Myotis yumanensis*	*Carios vespertilionis*	13.1	*Ornithodoros kelleyi, O. yumatensis* [5, 8]
	Tick 4	3	72	3	1	0.071	*Antrozous pallidus* *Eptesicus fuscus*	*Ornithodoro faccinii*	11.6	*Ornithodoros sp., O. kelleyi,, O. rossi* [8, 9]
	Tick 5	10	16	8	0.93	0.005	*Myotis yumanensis* *Antrozous pallidus* *Parastrellus hesperus* *Eptesicus fuscus*	*Antricola marginatus*	11.8	*Ornithodoros sp., O. kelleyi,, O. rossi, O. yumatensis* [8, 9]
	Tick 6	3	1	2	0.67	0.001	*Myotis peninsularis*	*Ornithodoros yumatensis*	9.9	No previous record to our knowledge

1) (Usinger, 1966); 2) (Cuxim-Koyoc *et al.*, 2015); 3) (Trujillo-Pahua and Ibáñez-Bernal, 2018); 4) (Zamora-Mejías *et al.*, 2020); 5) (Braun *et al.*, 2015); 6) (Graciolli, Autino and Claps, 2007); 7) (Ramírez-Martínez *et al.*, 2016); 8) (Bradshaw and Ross, 1961); 9) (Steinlein, Durden and Cannon, 2001).

3.4.2.2. Phylogenetic assessment of Baja peninsula bat bug sequences

The best fit evolution model for the COI gene set was GTR+G+I, and K2+G+I for 18S. For each marker 30 bugs sequences were generated, which formed four novel lineages with respect to reference sequences in each case (Fig. 3.2). Genetic divergence between the four peninsular lineages ranged from 9.9% to 17.1% (Table 3.2), and between 7.2% and 20.9% against the reference sequences, where *C. latipennis, C. antennatus* and *C. adjuntus* presented the lowest divergence values from the novel lineages (Table 3.2). All the bugs collected appeared belong to the *Pilosellus* complex (Usinger, 1966; Balvín, Roth and Vilímová, 2015; Talbot *et al.*, 2016), which includes only North American members of the Cimicidae family, genus *Cimex: C. adjunctus, C. antennatus, C. brevis, C. incrassatus, C. latipennis* and *C. pilosellus* (Usinger, 1966). A representative photograph of a specimen from each lineage is shown in Appendix 3.2.

Table 3.2. Estimates of percentage divergence (COI) over sequence-pairs between groups (diagonal left matrix), and within groups (most left column) of bat bugs, showing the lineages obtained in this study versus closely related reference sequences from GenBank.

	Cimex 1	***Cimex 2***	***Cimex 3***	***Cimex 4***	***C. adjuntus***	***C. antennatus***	***C. brevis***	***C. hemipterus***	***C. latipennis***	***C. lectularius***	***C. pipistrelli***	***Cimex* Asia**	***Oeciacus***	**Within groups**
Cimex 1														n/c
Cimex 2	9.9													0.59
Cimex 3	16.3	16.0												0.12
Cimex 4	17.1	16.1	13.5											0.50
C. adjuntus	19.9	17.1	12.6	10.5										0.70
C. antennatus	16.9	15.2	12.1	9.3	9.7									n/c
C. brevis	14.2	13.1	13.5	14.6	15.8	14.3								n/c
C. hemipterus	19.3	17.9	20.9	18.1	19.6	19.7	17.7							0.10
C. latipennis	10.6	7.2	13.9	16.2	17.1	15.2	13.7	19.1						2.01
C. lectularius	18.7	17.3	19.8	18.0	19.1	17.9	17.4	18.5	18.6					3.51
C. pipistrelli	19.5	16.8	20.0	17.5	18.1	17.3	16.6	15.3	17.8	15.0				1.68
***Cimex* Asia**	19.1	16.4	20.1	17.8	17.8	17.4	16.6	15.3	17.5	15.2	2.3			n/c
Oeciacus	20.1	17.7	20.7	18.2	18.1	17.8	17.0	15.2	18.7	15.7	6.9	7.2		6.26

The *Cimex* 1 lineage is represented by a single specimen (EBCO155) obtained from a *Myotis yumanensis* host at Mosqueda (site 2), and forms a sister lineage to *Cimex* 2 represented by two haplotypes from two bugs parasitizing *M. californicus* hosts, which were also sampled from northern sites (Fig. 2.1 and 3.2). Both markers placed *Cimex latipenis*, which is distributed from Canada to the north western USA (Usinger, 1966), as the closest named molecular reference species to these new lineages. The *Cimex* 3 lineage is represented by a separate clade consisting of 2 haplotypes, sampled from specimens collected from four *Antrozous pallidus* and one *M. californicus* hosts, all distributed in the southern half of the peninsula (sites 8, 10 and 23). Reference sequences for *C. pilosellus* and *C. brevis* formed a clade which appeared to be ancestral to *Cimex* 3, with 13% divergence (Table 3.2). There is no molecular reference for *C. brevis* in the 18S analysis, where *C. antennatus* was positioned as the sister clade of *Cimex* 3, placing *C. adjuntus* among other species.

The *Cimex* 4 lineage includes 17 haplotypes derived from 22 specimens, where 16 of the bugs were found parasitizing *Parastrellus hesperus* individuals, and one from an *A. pallidus* (EBCO201), all from northern sites (Fig. 2.1, Chapter 2), with *C. antennatus* as the closet sequence match (9.3%; Fig. 3.1). In the sequence alignment, *C. antennatus* (KF018718) sequence is shorter than the rest of the samples, potentially adding a bias as to whether this species is truly the closest one to *Cimex* 4 lineage. Sequences of *C. adjunctus* in the 18S topology clustered in a clade with other *Cimex* species with mixed origins, including some none Palearctic species. This may represent a misidentification of those specimens, or a mistake in the annotation of the sequences submitted to GenBank. *Cimex incrassatus* is reported as occurring on *Antrozous pallidus* hosts (Usinger, 1966), but no reference sequences are available. Therefore, it is possible that one of the novel lineages may represent this species, although morphological assessments need to be done.

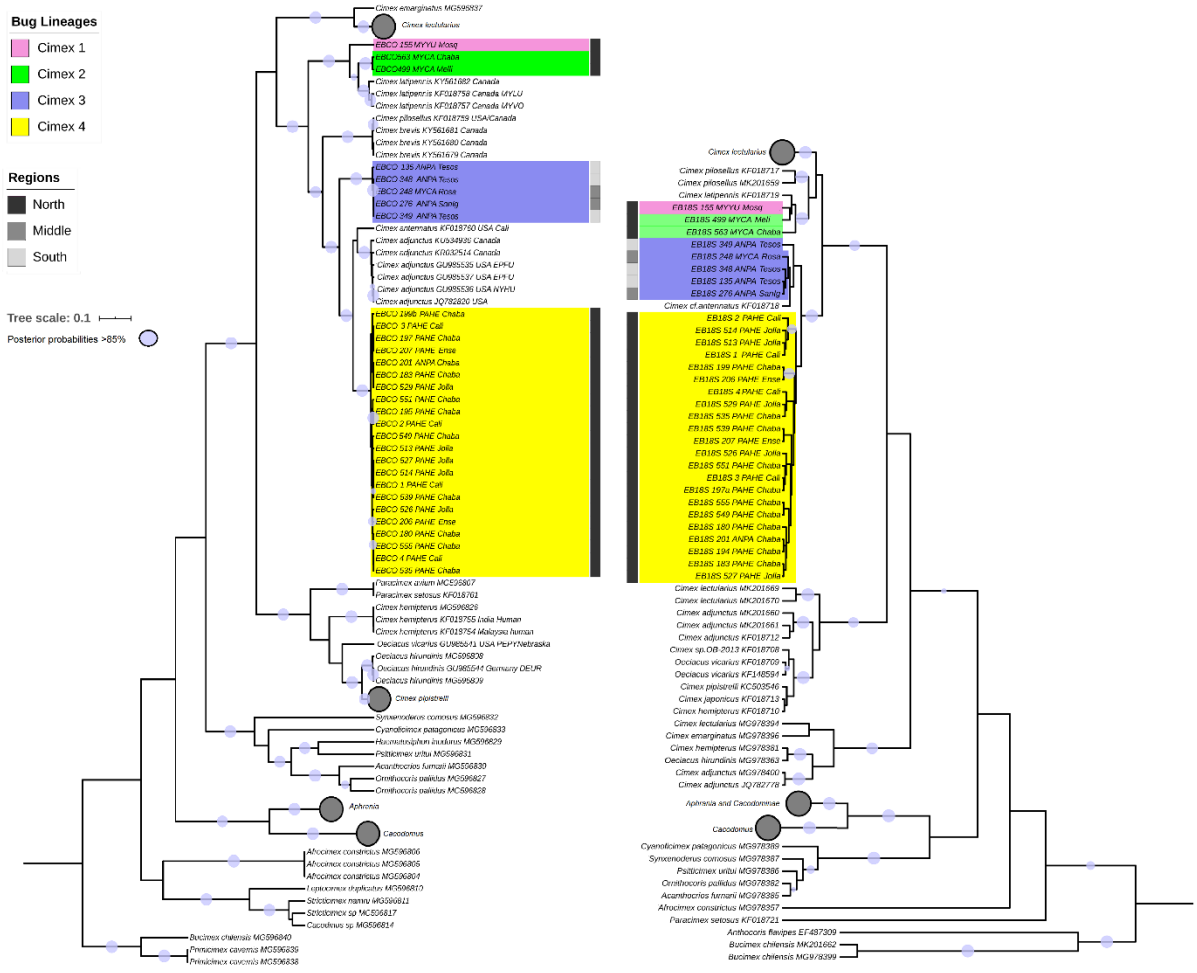


Figure 3.2. Bat bug phylogenetic tree obtained under Bayesian analysis using the mitochondrial COI (left) and the ribosomal 18S markers (right). Posterior probability support values (>0.85) are shown as light purple circles on the tree branches. When available, information of location and host is written next to each reference sequences label. To improve clarity of the tree, collapsed clades of references sequences are shown as grey circles.

3.4.2.3. Phylogenetic assessment of Baja peninsula bat fly sequences

The GTR+G model of evolution was obtained for the COI gene set, and T92+G+I for the 18S gene. In total, 77 bat flies were sequenced, yielding 76 sequences for COI and 73 for 18S amplicons respectively. Individual sequences for two specimens for COI, and three for 18S did not pass sequence quality thresholds and were discarded. In the final sequence set there were 49 sequences from the Nycteribiidae family (wingless bat flies) and 27 for the Streblidae family (winged bat flies), representing ten novel lineages and five new species records for Mexico. A photograph for fly specimens representing each of the lineages obtained in this study is presented in Appendix 3.3. The COI and 18S tree topologies for Nycteribiidae fly families showed a fairly consistent configuration of clades among analyses. Both trees showed *Eucampsipoda* (Family Hippoboscidae) as the furthest species from the family Nycteribiidae, and the link between the family Streblidae. However, the 18S Streblidae tree will not be considered because of the low quality and lack of overlapping against reference sequences, failing to provide any reliable tree, as previously mentioned in methods.

In the COI phylogenetic analysis, the 49 Nycteribiidae sequences formed five lineages, all of which appeared to be novel with respect to GenBank references. Genetic divergence among Baja lineages ranged from 2.9 to 14.5%, and up to 16% against their reference sequences (Table 3.3). Two peninsular lineages, Nycteribiid 1 and 2, formed sister clades with haplotypes from Asian *Nycteribia*, species, with 4.4% divergence between them, and more than 10% with their closest references (Table 3.3). Nycteribiid 1 and 2 lineages were primarily associated with *Myotis* bat hosts, but one specimen with a Nycteribiid 1 haplotype was recovered from *Parastrellus hesperus* host (specimen 514 from the 18S tree, Fig. 3.3).

The three other Baja lineages formed clades associated with *Basilia* sequences from species recorded in Madagascar, USA and Panama, from GenBank. *Basilia* 1 presented a divergence of 4.9% from *Basilia boardmani*, a bat fly distributed throughout the United States that has been found parasitizing *Myotis* bats (Graciolli, 2001; Graciolli, Autino and Claps, 2007). Specimens with *Basilia* 1 haplotypes were sampled at mid-peninsula, parasitizing *Myotis yumanensis*, which is widely distributed throughout North America (Braun *et al.*, 2015). With this exception, none of the other *Basilia* species reported as parasitizing the bat species sampled in our study have reference sequences deposited in GenBank (Table 3.1).

Genetic divergence among lineages *Basilia* 2a and 2b was 2.9% (Table 3.1), representing the threshold for intra/inter interspecific values (Hebert, Ratnasingham and DeWaard, 2003; Ratnasingham and Hebert, 2013; Salinas-Ramos *et al.*, 2018). We assigned them as different lineages due to their difference in host species, where *Basilia* 2a parasitize *M. vivesi*, *Basilia* 2b appears to be restricted to *Antrozous pallidus* in our sample (Fig. 3.3, blue and lilac clades, respectively). Based on *Basilia* 2 haplotypes distributions and samples from different years, *A. pallidus* dispersal over the peninsula was detected (Fig. 3.3), providing evidence of bat movement along Baja. Here *Basilia* 2b may represent *B. antrozoi*, as it has been previously reported to parasitize *A. pallidus* (Graciolli, Autino and Claps, 2007). However, a more detailed morphological study is required to be certain of taxonomic identity of all the *Basilia* specimens reported here.

Table 3.3. Estimates of evolutionary divergence (COI) in percentage over sequence-pairs between groups (diagonal left matrix), and within groups (most left columns) of bat flies from the family Nycteribiidae, versus closely related reference sequences from GenBank.

	Nycteribiid 1	**Nycteribiid 2**	***Nycteribia***	***Penicillidia***	***Basilia 1***	***Basilia 2a***	***Basilia 2b***	***Basilia boardmani***	**Basilia America**	**Basilia Africa**	**No Basilia**	**Within groups**
Nycteribiid 1												0.350
Nycteribiid 2	4.4											0.691
Nycteribia	10.7	10.5										5.910
Penicillidia	11.7	11.4	9.8									5.753
Basilia 1	14.1	13.0	13.3	13.6								0.000
Basilia 2a	13.5	11.4	12.9	14.6	9.8							0.109
Basilia 2b	12.9	12.7	13.6	15.1	9.2	2.9						0.098
Basilia boardmani	14.5	13.9	13.0	13.2	4.9	10.3	9.9					0.000
Basilia America	13.4	12.7	11.9	12.6	8.5	10.5	10.6	8.8				6.540
Basilia Africa	15.8	14.9	13.3	14.0	13.3	12.9	13.5	14.3	13.7			11.348
No Basilia	10.9	10.9	8.4	9.4	13.4	13.2	14.2	12.8	12.4	13.2		6.079

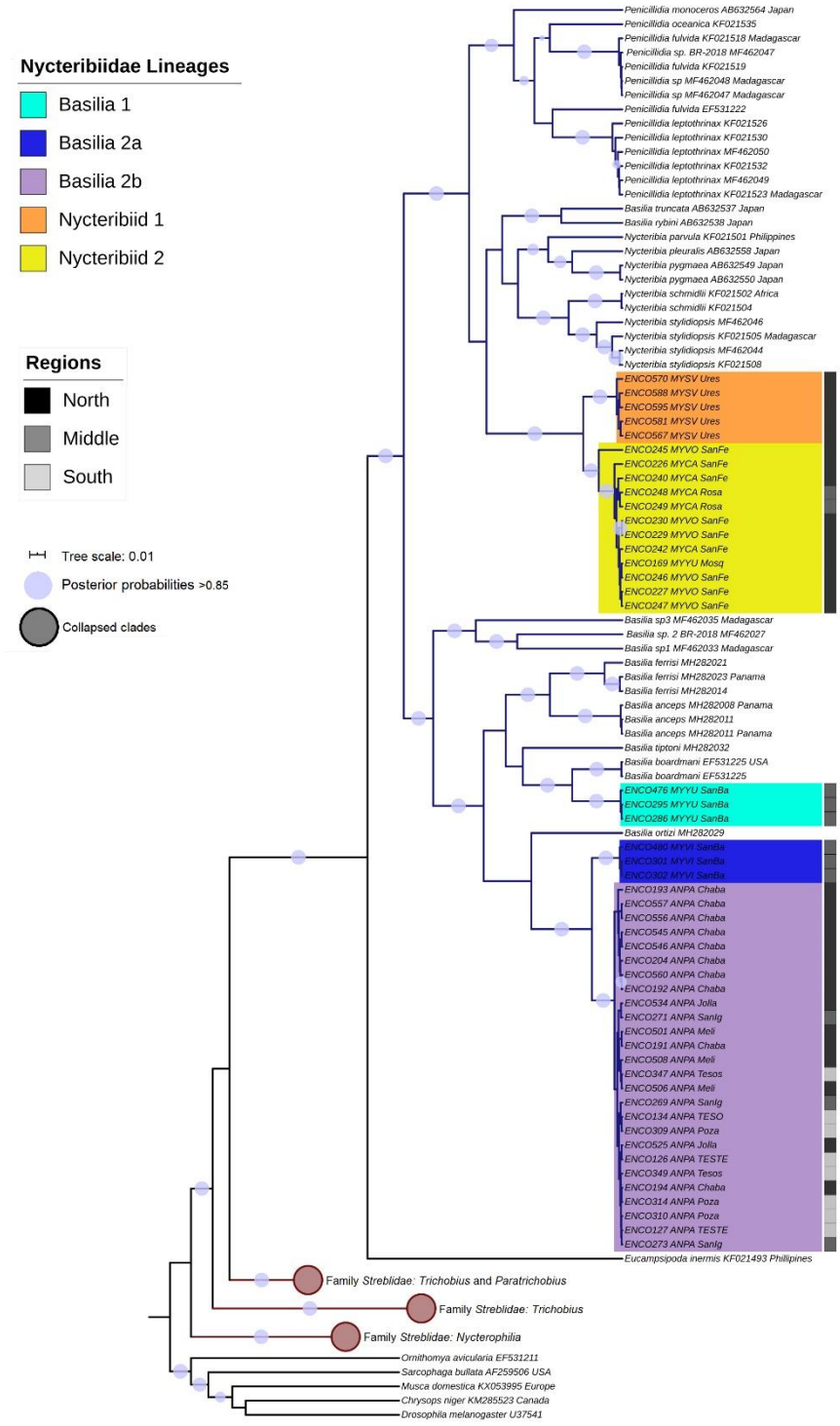


Figure 3.3. Bat flies of the family Nycteribiidae phylogenetic tree obtained under Bayesian analysis using the mitochondrial COI (left) and the ribosomal 18S markers (right). Posterior probability support values (>0.85) are shown as light purple circles on the tree branches. When available, information of location and host is written next to each reference sequences label. For clearer visualization of the tree, the family Streblidae sequences are collapsed (dark red circles), and references sequences that are not included in the Nycteribiidae family are shown as grey collapsed circles.

The ten lineages of the family Streblidae presented genetic divergence of 0.01% to 9.9% for sequences sampled within this study, and up to 18.2% against their reference sequences (Table 3.4). From the five novel lineages, as a putative classification four of these were attributed to the closest genus in the phylogenetic analysis, having an average genetic divergence of 5.6% with their closest reference: *Trichobius* 1, *Trichobius* 2, *Trichobius* 3 and *Paratrichobius* 1 (Table 3.4). Streblid 1 lineage presented a 9.9% divergence against its closest reference, *T. sphaeronotus* (Table 3.4, Fig. 3.4).

The other streblid clades matched sequences from five known species: *Aspidoptera phyllostomatis, Megistopoda aranea, Nycterophilia coxata, Trichobius dugesii* and *T. sphaeronotus* (Fig. 3.4), representing new records for these species in Baja and western Mexico. Fly species *A. phyllostomatis 1* and *M. aranea 1* lineages from this study had 2.3% and 2.9% divergence against their reference sequences respectively, and *N. coxata 1*, *T. dugesii 1* and *T. sphaeronotus 1* had less than 1.5% against the GenBank references (records obtained in this study are in bold and with a number at the end of the name in table 3.4). Most streblid lineages were parasitizing mainly fruit-nectar feeding bats (*Phyllostomidae*) over the mid and northern peninsula, with the exception of *Trichobius* 1 found on *Mormoops megalophylla* hosts, (family *Mormoopidae*), which feeds on insects. *Trichobius* 1 was the only peninsular streblid fly lineage found in the south of Baja as well. The other flies marked as to be distributed in the south belong to continental sites (sites 23 and 24, figure 2.1). Outside of the closest sequence matches none of the other species reported as parasitizing hosts in Baja (Table 3.1) appear to have reference sequences in GenBank.

Table 3.4. Estimates of evolutionary divergence in percentage over sequence-pairs between groups (diagonal left matrix), and within groups (most left columns) of bat flies from the family Streblidae, showing the lineages obtained in this study versus closely related reference sequences from GenBank. Names over the first column have the genus abbreviated.

	Aspidoptera phyllostomatis 1	*Megistopoda aranea 1*	*Nycterophillia coxata 1*	**Streblid 1**	*Trichobius 1*	*Trichobius 2*	*Trichobius sphaeronotus 1*	*Trichobius 3*	*Paratrichobius 1*	*Aspidoptera phyllostomatis*	*Megistopoda aranea*	*Nycterophilia coxata*	*Nycterophilia fairchildi*	*Nycterophilia parnelli*	*Paratrichobius dunni*	*Paratrichobius longricus*	*Trichobius dugesii*	*Trichobius joblingi*	*Trichobius major*
A. phyllostomatis 1																			
M. aranea 1	12.1																		
N. coxata 1	14.7	16.3																	
Streblid 1	12.8	13.2	14.1																
Trichobius 1	11.6	15.1	14.3	11.0															
Trichobius 2	8.1	12.9	15.6	13.5	11.9														
T. sphaeronotus 1	11.4	14.4	15.4	9.9	5.9	11.7													
Trichobius 3	6.4	11.2	14.5	12.3	10.7	7.0	11.7												
Paratrichobius 1	11.2	11.5	16.2	10.7	12.1	11.3	11.9	10.7											
A. phyllostomatis	2.3	13.5	15.6	13.7	11.8	8.4	12.3	6.8	11.9										
M. aranea	12.5	2.9	16.6	12.8	15.4	12.7	14.0	11.1	10.8	13.1									
N. coxata	15.4	15.9	1.2	13.8	14.5	15.7	15.5	14.5	16.5	16.0	16.0								
N. fairchildi	15.6	15.0	7.4	13.2	13.5	14.4	13.0	14.0	16.8	15.3	14.5	6.6							
N. parnelli	15.8	15.4	8.6	13.4	14.1	15.8	14.3	13.5	14.6	16.1	15.6	7.6	8.5						
P. dunni	12.1	13.6	17.5	13.1	12.2	11.2	12.4	12.1	7.9	11.8	12.9	17.4	16.3	16.1					
P. longricus	11.5	12.5	18.2	13.1	12.4	12.9	12.6	12.1	4.5	12.0	11.9	18.1	17.1	16.1	8.4				
T. dugesii	7.9	12.3	14.9	12.7	11.8	5.4	13.3	5.3	11.3	7.6	12.0	15.2	14.5	14.3	12.2	12.6			
T. joblingi	8.4	12.7	16.1	12.9	12.3	7.7	13.0	5.8	11.9	8.4	12.2	16.3	15.9	15.8	12.8	13.1	6.9		
T. major	11.6	17.0	12.1	11.8	9.9	11.9	10.7	11.6	13.6	12.3	17.0	12.3	12.9	15.8	14.2	14.0	14.7	14.0	
T. sphaeronotus	11.6	14.6	15.7	10.0	5.9	11.6	0.01	11.9	12.1	12.4	13.9	15.5	13.0	14.3	12.3	12.8	13.5	13.1	10.9
T. uniformis	13.4	12.8	16.2	12.5	9.0	11.5	8.6	12.3	11.2	14.1	12.7	16.6	13.8	14.5	10.2	12.2	11.0	12.7	14.4
T. urodermae	6.3	13.1	17.1	13.6	12.5	8.2	13.0	5.9	12.5	6.6	12.4	17.1	16.0	15.6	12.4	12.7	7.2	7.3	13.5

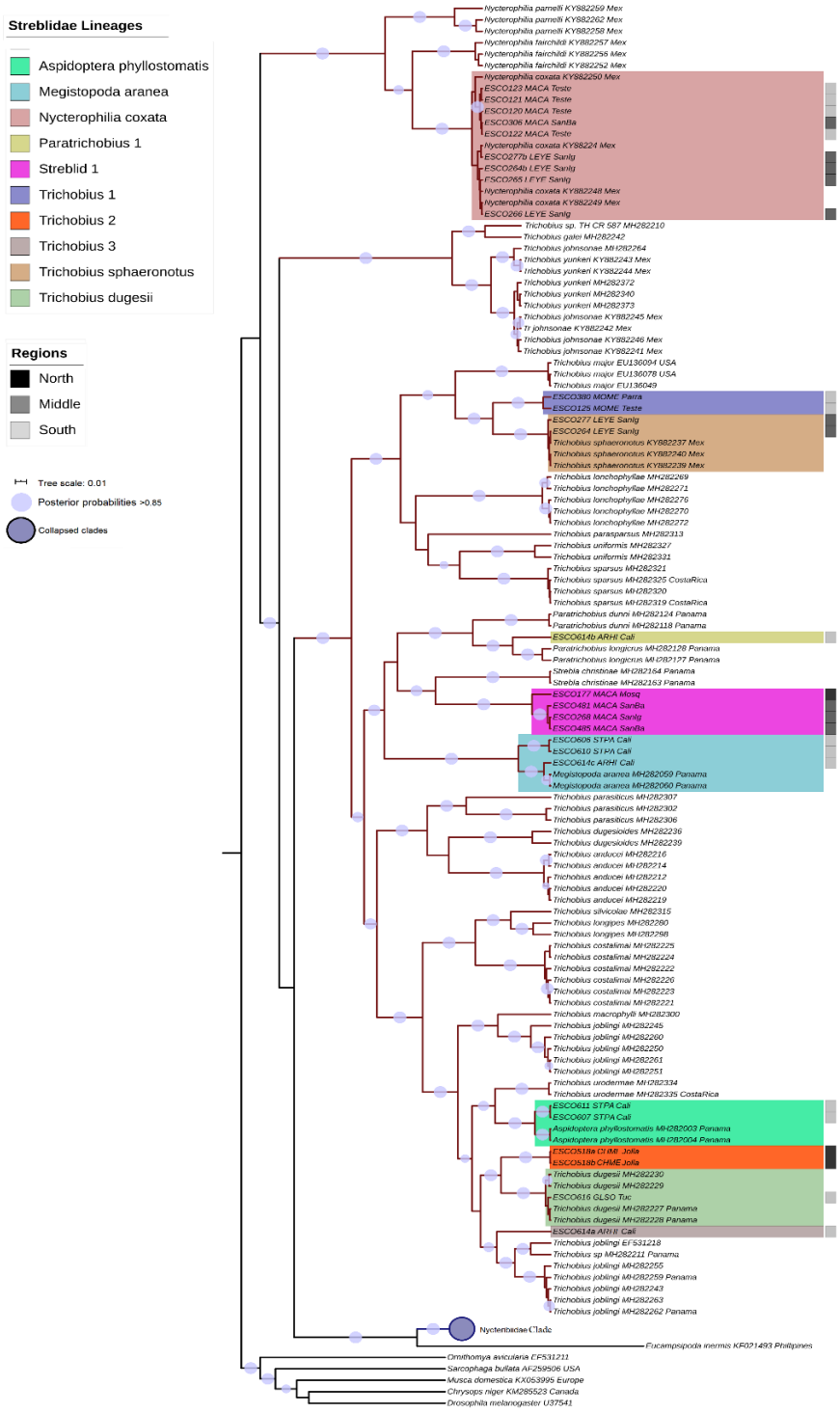


Figure 3.4. Bat flies of the family Streblidae phylogenetic tree obtained under Bayesian analysis using the mitochondrial COI marker. Posterior probability support values (>0.85) are shown as light purple circles on the tree branches. When available, information of location and host is written next to each reference sequences label. For terms of better visualization, the family Nycteribiidae clade is collapsed (dark blue).

3.4.2.4. Phylogenetic assessment of Baja peninsula bat tick sequences

The best fit sequence evolution models were GTR+G+I for the COI gene set, and K2+G+I for the 18S gene set. There were 45 ticks sequenced from the Argasidae family (soft ticks), with 39 sequences for the COI gene, and 44 sequences for the 18S gene, where six specimens failed to amplify for COI (specimen numbers 157, 306, 338, 480b, 480c and 485), and one specimen failed to amplify for 18S (specimen number 457). One new record and seven potential novel lineages were obtained (Fig. 3.5). A specimen photograph from each tick lineage is shown in Appendix 3.4 (with the exception of lineage Tick 2, which had no specimen left after sequencing). The only species that matched over GenBank and BOLD system databases (95.95% and 97.07%, respectively) was the soft tick *Carios kelleyi*, presenting a genetic divergence of between 1.94% and 2.47% with 11 individuals from this study, therefore identified as *C. kelleyi* lineage (Table 3.5). The lowest genetic divergence among sequences from this study was of 6.5%, showed by Tick 2 and *C. kelleyi* lineages, and between 11.4% and 19.3% for the rest of the lineages (Table 3.5). Aside from *C. kelleyi*, comparisons to the closest reference sequences ranged from 9.9% to 22.8% divergence (Table 3.1).

There were 32 haplotypes in 39 sequences from this study. Haplotype diversity was high, with all lineages represented by more than one samples having values greater than 0.67, while Argasidae nucleotide diversity was relatively low (Nd < 0.009) for five of the lineages, with the exception of *C. kelleyi* and Tick 3 (Hd = 0.011 and 0.071, respectively). However, these values are influenced by the small sample size for each of the lineages in this group.

Among the other closest reference sequences for COI data, *Antricola marginatus* had 10.6% divergence from *Antricola* 1 lineage, and 14.6 from *A. mexicanus* (Fig. 3.5). *Ornithodoros faccini* had a divergence of around 11% for clades *Carios kelleyi*, Tick 1 to 4, and *Ornithodoros yumatensis* showed divergence of around 9.9% with respect to Tick lineage 6 (Table 3.5). Ticks of lineage Tick 6 were recovered from *M. peninsularis* from which there are no previous records of bat ticks to our knowledge.

Table 3.5. Estimates of evolutionary divergence (COI) in percentage over sequence-pairs between groups (diagonal left matrix), and within groups (most left columns) of bat ticks.

	***Antricola* 1**	***Carios kelleyi***	**Tick 1**	**Tick 2**	**Tick 3**	**Tick 4**	**Tick 5**	**Tick 6**	***Antricola***	***Carios***	***Nothoaspis***	***Ornithodoros* Asia**	***Ornithodoros* America**	***Ornithodoros* Africa**	**Within groups**
***Antricola* 1**															0.99
Carios kelleyi	13.9														0.10
Tick 1	13.1	11.8													0.13
Tick 2	13.9	6.1	11.4												0.56
Tick 3	13.3	12.5	12.9	12.7											4.97
Tick 4	13.7	11.9	11.9	11.5	12.1										0.33
Tick 5	12.7	12.7	12.5	11.3	12.6	11.4									0.07
Tick 6	13.7	14.5	13.4	14.2	15.0	14.3	15.4								11.83
Antricola sp.	13.7	14.6	15.2	14.4	15.0	14.8	14.2	14.3							11.12
Carios	14.0	14.0	14.3	13.9	14.4	14.7	14.1	15.2	15.1						n/c
Nothoaspis	14.4	13.5	14.8	13.7	15.8	14.6	14.6	16.8	14.2	15.0					18.70
***Ornithodoros* Asia**	17.6	18.3	18.5	17.1	17.9	17.2	17.9	19.3	17.8	19.1	17.7				14.74
***Ornithodoros* America**	15.3	14.6	15.1	14.2	15.5	14.7	15.7	13.9	15.0	15.2	15.9	17.7			14.67
***Ornithodoros* Africa**	16.2	16.2	16.4	15.2	16.3	16.4	16.3	16.5	16.6	15.9	16.3	18.1	16.4		0.99

The sequences generated grouped in the same lineages for both COI and 18S genes (Fig. 3.5). Tick lineages *C. kelleyi* and Tick 1 to 5 also formed a monophyletic group with respect to the reference sequences; and the *Antricola* 1 and Tick 6 were positioned in separate clades. However, the topology of sister clade relationships varied slightly between markers, particularly around deeper nodes which had posterior probability support less than 0.85. This

suggests more data is required to resolve deeper taxonomic relationships among species. *Antricola* 1 and Tick 6 were separated around shallow deep nodes in both analyses, where the absence or presence of reference sequences influenced their topological proximity (Fig. 3.5). The closest reference sequences related to the new sequences presented here primarily derive from a mixture *Antricola*, *Carios*, and *Ornithodoros* genera, but none of them formed monophyletic groupings with respect to the nomenclature of the genus. There is current controversy regarding the taxonomic status of *Ornithodoros*, *Antricola* and *Carios* genus, sometimes used as synonyms of each other (Burger *et al.*, 2014). For this reason, aside from *Antricola* 1 and *C. kelleyi*, lineages for this group are called "Tick" plus its correspondent number.

Tick lineages 2 and 6 (orange and pale red, Fig. 3.5) were only observed in the south of the peninsula, on *A. pallidus* and *M. peninsularis* hosts for Tick 2, and only on *M. peninsularis* hosts for Tick 6. These two species of bats share at least one confirmed roosting site within the study region, at Tesos (Fig. 2.1 site 21), suggesting a potential interchange of host species for Tick lineage 2. Evidence of singular host specificity was observed for the lineages *C. kelleyi* (*A. pallidus*), Tick 3 (*M. yumanensis*), and Tick 6 (*M. peninsularis*), all observed exclusively on the same hosts across sites and field seasons. The *Antricola* 1 lineage (Fig. 3.5, lilac clade, ET301_MYVI,) was recovered from one specimen using the COI marker, and grouped with *Antricola marginatus* (found in the South East of Mexico), followed by *A. mexicanus*. In the 18S topology, five additional sequences were obtained, grouping them with *A. mexicanus* only, as no *A. marginatus* reference was available (Fig. 3.5 right tree). Two host species for ticks of this lineage were identified, *M. vivesi* and *Macrotus californicus*, sampled mostly at mid-peninsula (Fig. 2.1). Tick 5 lineage had the most diverse

host and spatial distribution, being found on four different bat species, and at sites from the north to the south of the peninsula (Fig. 3.5, light blue shading).

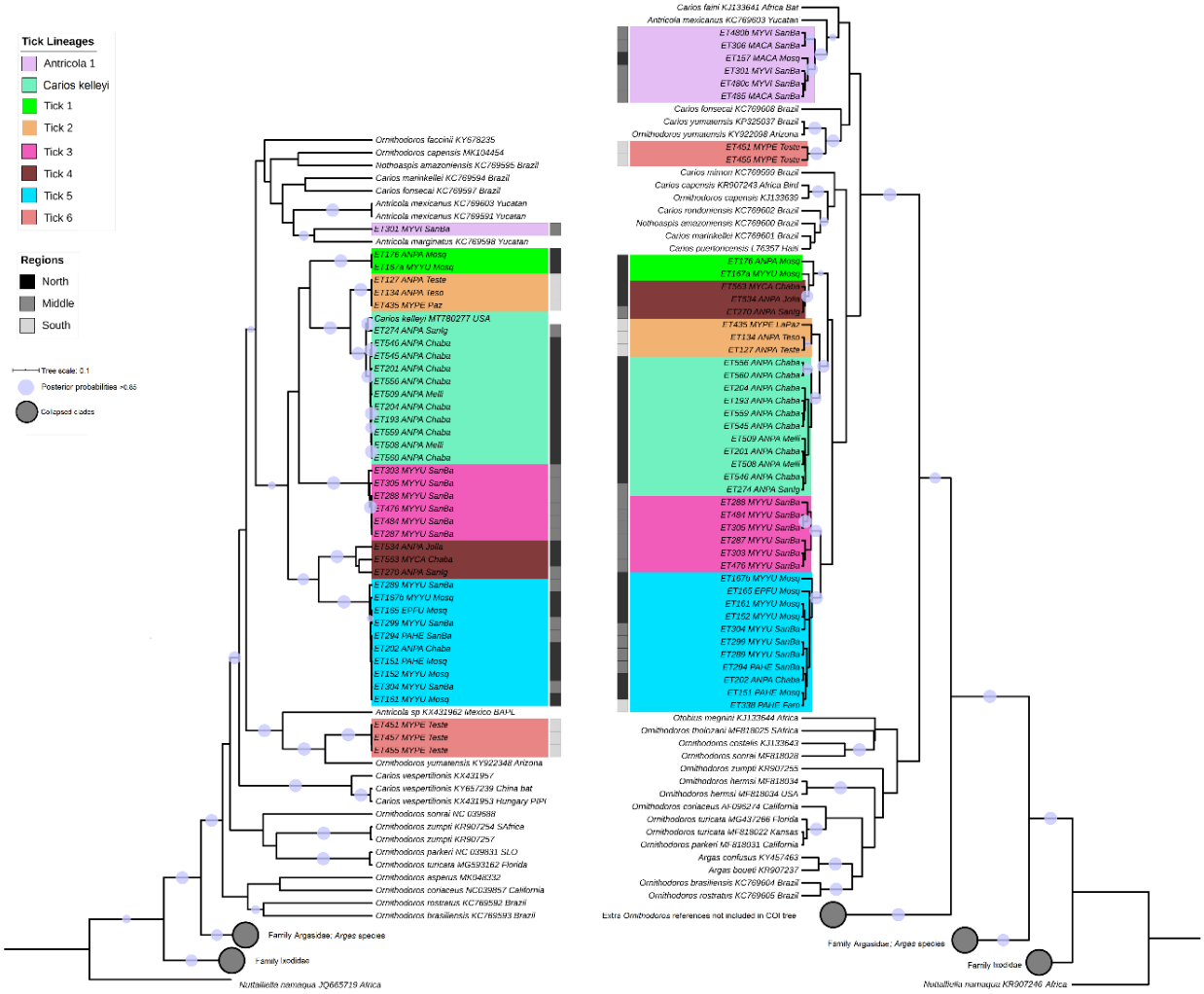


Figure 3.5. Bat ticks of the family Argasidae phylogenetic tree obtained under Bayesian analysis using the mitochondrial COI (left) and the ribosomal 18S markers (right). Posterior probability support values (>0.85) are shown as light purple circles on the tree branches. When available, information of location and host is written next to each reference sequences label. For clarity, reference sequences of other species not closely related to those from this study are collapsed in grey circles.

3.5 Discussion

3.5.1. Molecular analyses

From a total of 297 sequences identified from two markers, 21 novel genetic lineages plus six new species records for the Baja California peninsula were found among the three groups of ectoparasites sampled in this study. This highlights the high diversity of the ectoparasite fauna of bats from the North West region of Mexico. Some of the novel lineages may derive from previously recorded species with no reference sequences available, while others are likely to represent new species. Mitochondrial genetic divergence among the potential novel lineages from this study range from 3.2% (*Cimex* 2) to 13.2% (Tick 1) to its closes reference sequence (Table 3.1).

In general, molecular references were more abundant for the bat flies than bugs and ticks, especially for Mexico, were bat fly research has been lately increasing in central and southern areas of the country (Cuxim-Koyoc *et al.*, 2015; Ramírez-Martínez *et al.*, 2016; Salinas-Ramos *et al.*, 2018; Trujillo-Pahua and Ibáñez-Bernal, 2018; Saldaña-Vázquez *et al.*, 2019; Zamora-Mejías *et al.*, 2020). As shown in Table 3.1, there are potential records of ectoparasites that have been described before using morphological tools, associated to each species of bat host within this study. However, for the north-western part of Mexico there is little information from molecular studies of bat parasites, hence the need to address their taxonomic relationships using molecular tools.

This study is the first molecular work that has been done to understand the ectoparasite diversity of bats along the Baja California peninsula and north-west Mexico, highlighting the importance of prioritise conducting more ectoparasite surveys, especially

over relatively underexplored territories. However, it is important to highlight the need to include and combine morphological analyses with molecular tools (Burger *et al.*, 2014). The lack of background information and taxonomic references pose a great challenge for cross-validating species identification; therefore, it is imperative to design and increment surveys using multiple identification resources, helping to elucidate the diversity of cryptic lineages among ectoparasites.

3.4.1.1. Bat bugs.

Four novel lineages of *Cimex* bugs found in this study were distributed mostly along north of the peninsula, with lineages *Cimex* 3 and *Cimex* 24 suggesting host specificity (Fig. 3.1). Genetic divergence from *Cimex* 2 compared with *C. latippenis* was 3.2% (Table 3.2), which is above the threshold of 2% of genetic differentiation, but below the 4% suggested as the minimum percent to be classified as a sister species using the COI marker (Hebert, Ratnasingham and DeWaard, 2003). *C. latippenis* has not been recorded parasitizing *M. yumanensis* before (Braun *et al.*, 2015), which might suggest *Cimex* 2 as a cryptic bat bug for the family Cimicidae. However, more sampling effort needs to be done to obtain and analyse morphological differentiation paired up with genetic population structure across a bigger sample size, to better evaluate diversity and distribution of Mexican bat bugs.

Cimicid bugs have low inherent dispersal capacity, generally feeding for a few days, before dropping from the bat host to digest the blood in the roost, where they can survive without feeding for approximately 1.5 years (Ossa *et al.*, 2019). However, it has been documented that population structure of bat bugs is mainly influenced by bat movements (Usinger, 1966; Talbot *et al.*, 2016, 2017; Ossa *et al.*, 2019). Results showed that bat hosts

movements have allowed bat bugs to disperse across sites in the north, reaching the south of the peninsula (*Cimex* 4, Fig. 3.1 and 3.2, but samples sizes are too small to infer more specific patterns.

Interestingly, results from the haplotype analysis of *Myotis* bats from Chapter 2, showed that some *M. yumanensis* bats captured in northern localities shared haplotype with reference sequences from Alaska (see results YU6_14, Fig. 2.5, Chapter 2). Given that the *M. yumanensis* specimen 155 was also captured at a northern site (Mosqueda site, Fig. 2.1), it could be potentially sharing roosts with other migratory bats at that site or in previous roosts along its trajectory. This would increase the likelihood of bringing along bat bugs from other latitudes, and the pathogens that travel with them. There is evidence that bat bugs can parasitise other taxa including humans (Roth *et al.*, 2019), therefore, generating more studies of bat bugs in Mexico should be granted more attention.

3.4.1.2. Bat flies.

Ten potentially novel lineages and five new records of bat flies were found in this study. Nycteribiid flies were more abundant in the northern temperate sites, and less abundant over subtropical southern sites; while streblids were more abundant in the southern and more subtropical sites (Fig. 3.3 and 3.4, regions stripe colours), supporting the trends noted by Dittmar *et al*. (2006). In particular, all hosts of lineages Nycteribiid 1 and Nycteribiid 2 hold sympatric distributions over the northern half of the peninsula (Simpson, 1993; Navarro Frías, González Ruíz and Álvarez Castañeda, 2007; Braun *et al.*, 2015), and were sampled in a few common sites, suggesting potential roost sharing among these *Myotis* range. To our knowledge *Nycteribia* species have not previously been reported for bats with ranges in Baja

(Table 3.1). Streblids flies were present in bats from the Phyllostomidae family, which are in general fruit and nectar feeders, with the exception of *Macrotus californicus* that feeds on insects; and also *Mormoops megallophyla*, from the family Mormoopidae. Nycteribiids were parasitizing only bats from the family Vespertilionidae that includes insectivorous and omnivorous bats (Appendix 3.1).

Among lineages, host specificity appeared to be the general pattern as previously documented (Dittmar *et al.*, 2006; de Vasconcelos *et al.*, 2016; Saldaña-Vázquez *et al.*, 2019), with a few exceptions over the two families: Nycteribiid 2 (Nycteribiidae) and *Nycterophillia coxata* (Streblidae) lineages. Lack of host specificity has been documented for Nycteribiid flies (Olival *et al.*, 2013; Wilkinson *et al.*, 2016). Streblid winged flies have been described as mostly not host-specific due to conspecific roost sites sharing and high transfer potential (Dittmar *et al.*, 2006), also with noted exceptions (Wenzel and Tipton, 1966; Patterson, Ballard and Wenzel, 1999; Dittmar *et al.*, 2006). The streblid *N. coxata* was parasitizing *Macrotus californicus* and *Leptonycteris yerbabuenae* (Fig. 3.4), therefore showing at least two different hosts, included in the same family (Phyllostomidae). In Baja, we found a roosting site shared by both species at the same time (Mulegé, but there was no ectoparasite sampling over this site), implying horizontal transmission of *N. coxata* between the two species when roosting, as a potential explanation.

There were, in general, few haplotypes among lineages, suggesting a poor structure along Baja populations, reflected by their total dependence on the host. Lineages *Basilia* 2a (host: *Myotis vivesi*) and *Basilia* 2b (host: *Antrozous pallidus*) are another example of potential cryptic diversity and undergoing genetic differentiation, driven by host-specificity.

M. vivesi is endemic to the Gulf of Cortes, restricted to coastal habitats because of its marine diet (Blood and Clark, 1998; Herrera, Flores-Martínez and Sánchez-Cordero, 2017). The bat *A. pallidus* has a wider distribution along western North America and it is primarily an insectivorous feeder, which also includes scorpions and nectar in its diet (Frick, Hayes and Heady, 2009). *Basilia* fly species previously reported for *M. vivesi*, but without reference sequences include *B. plaumanni, B. pynzonix,* and *B. producto* (Graciolli, Autino and Claps, 2007), while flies parasitizing *A. pallidus* have been described *B. antrozoi* (Table 3.1). As mentioned before, bat flies tend to present host-specificity (Dittmar *et al.*, 2006; de Vasconcelos *et al.*, 2016; Saldaña-Vázquez *et al.*, 2019), especially in fruit bats; whereas host-specificity seems to be more relaxed for insectivorous bats, mostly influenced by the roosting ecology of the host (Ramasindrazana *et al.*, 2017; Saldaña-Vázquez *et al.*, 2019). Despite that *M. vivesi* and *A. pallidus* have differences in hunting strategies (sea and land, respectively), both species co-occur along the peninsula, increasing the probabilities for these bats to have shared the common ancestor of *Basilia* 2a and *Basilia* 2b at some point of time. As a result of this study and the tendency of bat flies to be host-specific (Olival *et al.*, 2013), we hypothesize that *Basilia* 2a and *Basilia* 2b are undergoing a process of divergence by host specialization, where host associations and specificity may lead to genetic differentiation even for parasites with high mobility potential (Bennett, Turmelle and O'Grady, 2014).

The geographic distribution of *Basilia* 2b sequences, reflect dispersal of its host *A. pallidus* from north to south peninsula (Fig. 3.3), showing for the first time in Baja, evidence of dispersal. In the study conducted by Speer *et al*. (2019), the streblid fly *Trichobius frequens,* have helped to elucidate patterns of the bat *Erophylla sezekorni* dispersal, across an oceanic channel. In agreement with this, *Basilia* 2b showed five haplotypes included in 26

sequences, low haplotype diversity and nucleotide diversity (Hd = 0.58 and Nd = 0.001, Table 3.5), where the same haplotype included sequences from widely separated locations. These results highlight the potential of ectoparasites for elucidating patterns of bat dispersal, which in turn will provide important information for the management and conservation of bats and their pathogens in cryptic systems.

3.4.1.3. Bat ticks.

A new record of *Carios kelleyi* tick and seven novel lineages belonging to the Argasidae family were found from north to south peninsula (Fig. 3.5), with more than 9.9% of genetic divergence against the closest reference sequence from GenBank (Tick 6, Table 3.1). Similar values were shown in between lineages (e.g. Tick 2 vs *C. kelleyi*, 6.1% divergence, Table 3.5), indicating high genetic divergence even among peninsular lineages. Compared to the family Ixodidae, the family Argasidae is in general poorly studied and with few molecular studies, with classifications at genus level still controversial (Burger *et al.*, 2014). Because of this, reference sequences are limited (Porter and Hajibabaei, 2018) and also difficult to interpret (i.e. for both markers, different genus were grouped together, and similar ones were sometimes in different clades, Fig. 3.5).

In general, there are not many studies regarding the members of this family over North America (Pérez *et al.*, 2014; Sánchez-Montes *et al.*, 2016; Guzmán-Cornejo *et al.*, 2017). Argasidae in Mexico is poorly studied, mostly due to the difficulties for sampling and identification of this group (Pérez *et al.*, 2014), and especially those inhabiting caves (Sánchez-Montes *et al.*, 2016). The vague lineage annotation in this study is directly linked to the lack of information for the family, and to the controversial taxonomic relationships

among the potential genus in this study. The genus *Antricola* and *Nothoaspis* are associated with bats and their roosting sites in Mexico. The genus *Ornithodoros,* associated to bats and birds, is under taxonomic uncertainty with the genus *Carios* (Gill *et al.*, 2004; Burger *et al.*, 2014). Despite that there are species of soft ticks already documented for the hosts of this study, none of them are conclusive and are included into the controversial genus mentioned before (see *Ornithodoros* sp., Table 3.1). Genetic genus-level divergence is estimated around 9%, whereas within family can be considered around 10% or more (Hebert *et al.*, 2003; Hebert, Ratnasingham and DeWaard, 2003). Genetic divergence among lineages of this study (from 6.1% up to 19.3%, Table 3.5), and among reference sequences (from 9.9% to 22.8%, Table 3.1), suggests that Baja lineages could be potentially representing novel species and even novel genus for Mexico.

Both host-specificity and generalist lineages were found within ticks, where lineages that were host-specific for this study, were distributed among proximate sites, while most generalists not. For example, *Carios kelleyi* parasitizing *A. pallidus,* was sampled in Chabacanos, Meling and San Ignacio (but *A. pallidus* dispersal has proven to be within all the peninsula); and Tick 3 parasitizing *M. yumanensis* was found only in San Basilio. Other lineages are constituted by low sample sizes, therefore no pattern can be truly inferred. It has been documented that host-tick systems in South Africa Argasidae may show a continuum of hosts-specificity, especially at the species level (Cumming, 1998; Esser *et al.*, 2016). However, tick stage-cycle must be considered to account for all possible sets of host-specificity. Immature ticks are considered to be more generalist than their adult conspecifics (Nava and Guglielmone, 2013; Esser *et al.*, 2016). In this study, no life cycle was assessed while collecting ticks, therefore it is highly possible that there are gaps in the information

regarding unidentified larvae that was not sequenced. However, tick lineages in this study could also show adult stage patterns host-specificity among their bat hosts. For this group, there is a relevant need to integrate morphological and molecular studies to better understand patterns of host-specificity.

Distribution of specimens showed a slight geographic pattern according to their resulting lineage, were the bat *A. pallidus* was the host species carrying five of the eight tick lineages (ANPA label, Fig. 3.5). As shown for the bat flies, patterns of dispersal of *A. pallidus* can be elucidated by the ticks found in the present study (Speer *et al.*, 2019). As mentioned before, individuals of the lineage *C. kelleyi* distributed in Baja were parasitized only by *A. pallidus*. However, the published molecular record from which this lineage was identified (*C. kelleyi*), belonged to a specimen sampled from an *Eptesicus fuscus* bat, from eastern U.S. (GenBank accession code: MT780277, Fig. 3.5). Both *A. pallidus* and *E. fuscus* bat species present an overlapping distribution in western North America, but not in the east of U.S. (Kurta and Baker, 1990; Lack, Wilkinson and Van Den Bussche, 2010). This could be suggesting another potential pattern of dispersal from *C. kelleyi* through either species of bat, or a regional host-specificity, but more studies needs to be done to provide with a plausible explanation.

The only Tick 5 lineage specimen recorded for south peninsula was found on *Parastrellus hesperus* (specimen 338, Faro site, visualized in the 18S tree only, Fig. 3.5). This bat was very rare in the south, found only in the last season and at one site (Appendix 3.1). Haplotypes for Tick 5 were shared among sites from north, mid and south peninsula, where *P. hesperus* was recorded as host in the three regions (Fig. 3.5, Tick 5, blue clade).

This comprises strong evidence of potential dispersal from *P. hesperus* from north to south peninsula, as it has been shown for other bats within this study.

3.5.2. Ectoparasite sampling: limitations and outcomes

Ectoparasite sampling is limited by the amount of effort for studying certain host's species, where ectoparasites are often sampled opportunistically and not as the main objective (Schad *et al.*, 2012; Holz *et al.*, 2018; Raya *et al.*, 2018). There is also a bias on the amount of studies done related to ectoparasites of agricultural and medical importance, leaving aside the study of ectoparasite of wildlife, disease ecology and conservation (Gómez and Nichols, 2013; Poulin, 2014; Spencer and Zuk, 2016). Additionally, ectoparasite sampling and morphological identification requires of more time of activity sampling on field, material preparation, specimen images taken with high-resolution equipment and the participation of expert taxonomists (Pérez *et al.*, 2014). In general, more field-based research needs to be done, designing a specific methodology for ectoparasite sampling, which in turn will help to increase efficiency of time and amount of data processed. This study is contributing through field-based research to building up a molecular diversity database for north-western Mexico, which is the first step for increasing the scope of ectoparasite research in this region.

For bat ectoparasites, the design of the survey must take into consideration the host ecology and its dispersal patterns. It would also have to include an assessment of ectoparasite fauna found in roosting sites against those found feeding directly from their hosts, targeting the ecology and host specificity of ectoparasite groups like bat bugs, that will spend just a portion of their life cycle tagged to the host (Talbot, 2017). Seasonality will change both the diversity of bats and ectoparasites present at the same area, as shown in smaller regions of

Mexico (Salinas-Ramos *et al.*, 2018). Therefore, we can expect to find more or less abundance and diversity of ectoparasite fauna depending on different sampling times through the year. Another very important factor would be the amount of people taking part in the survey. Ectoparasite sampling requires enough time and skill to not harm each one of the captured bats while retrieving a complete ectoparasite specimen. Proper training and enough personal to carefully process bats as fast as possible, will decrease the amount of stress and manipulation over bats (Sikes *et al.*, 2016), and efficiently collect ectoparasites data that will be reliable for long-term studies.

3.5.3. Novel ectoparasite genetic diversity

There has been an increased number of publications describing new molecular records of bat ectoparasites across different parts of the world (Martins *et al.*, 2014; Burazerović *et al.*, 2015, 2018; Potiwat *et al.*, 2016; Ramasindrazana *et al.*, 2017), including several Mexican regions, but mostly focused on bat flies and mites (Cuxim-Koyoc *et al.*, 2015; Ramírez-Martínez *et al.*, 2016; Trujillo-Pahua and Ibáñez-Bernal, 2018). In general, estimations of parasite diversity accounts for a large fraction of the world's biodiversity (Poulin, 2014). Molecular tools and more focused efforts have improved the discovery of new species of parasites, therefore it is expected that most surveys of new host species and regions are likely to reveal more new species. However, there is also a reasonable amount of literature describing ectoparasite diversity based on previous identifications with morphological characters (see Bradshaw and Ross 1961; Usinger 1966; Dick *et al*. 2003; Graciolli *et al*. 2007; Pérez *et al* . 2014). The insufficiency of molecular resources inherited by the understudied ectoparasite fauna limits their proper identification, how they affect host' fitness (Pérez *et al.*, 2014; Spencer and Zuk, 2016), their pathogen repertoire and their

function as potential vectors of zoonotic spill over, and associated estimation and monitoring of zoonotic disease risks (Morse *et al.*, 2012; Veikkolainen *et al.*, 2014; Zorrilla-Vaca, Silva-Medina and Escandón-Vargas, 2015; Reeves *et al.*, 2016; Hornok *et al.*, 2019).

Although there are studies reporting limited data on bat ectoparasites from North Western Mexico and South-Western USA (Bradshaw and Ross, 1961; Usinger, 1966; Pérez *et al.*, 2014; Braun *et al.*, 2015), these studies contain either morphological or molecular information, not including both resources on the same study. For the Baja California peninsula, information about bat distributions contains gaps about some species biology and ecology, roosting sites, patterns of dispersal/migration, and bat-bat associations across roosting sites and seasons. The nature of the peninsular terrain has also prevented performing biodiversity surveys compared to other regions, prioritising resources to evaluate other taxa targeted for specific projects (Álvarez-Castañeda, 1998; Ezcurra *et al.*, 2002; Ramirez-Acosta *et al.*, 2012; Vanderplank, Rebman and Ezcurra, 2017). For these reasons, bat ectoparasite surveys are so far not present over this region.

For this study, it was difficult to match up sequences with previously described species, either because there was no reference sequence available from voucher specimens, or because of poor species id/records and incorrect annotation of reference sequences. This issue is paired with the lack of studies in this groups in the region, and potentially, from incorrect or non-existent morphological assessments before submission. Bat tick lineages from Baja might be representing previously recorded species, but the input of an expert morphologist to account for different life stages and specific morphological characters is needed.

3.5.4. Ectoparasite and hosts spatial distributions

Association between hosts and ectoparasites may depend on several biotic (host abundance and distribution, behaviour, host specificity, host-host transferences of ectoparasite and pathogens, etc.) and abiotic (ecological requirements of host and ectoparasites, vegetation type, etc.) factors (Bruyndonckx *et al.*, 2009; van Schaik *et al.*, 2014; Lučan *et al.*, 2016). Distribution of ectoparasite haplotypes along the peninsula seemed to suggest that their composition is more associated to the hosts evolution and population structure (Krasnov *et al.*, 2012; Pulgarín-R *et al.*, 2018), and not due to phylogeographic events. This was reflected by the presence of ectoparasites following bat hosts distribution in this study. Patterns of geological isolation are potentially important for historical bat distributions (Talbot *et al.*, 2016), therefore also influencing current ectoparasite structure, but patterns of biogeographical isolation were not tested in the present study. In Baja, vicariant events have shaped the distribution of small mammals (Riddle *et al.*, 2000; Morrone, Espinosa-Organista and Llorente-Bousquets, 2002; Álvarez-Castañeda and Murphy, 2014; Álvarez-Castañeda and Nájera-Cortazar, 2019), potentially influencing the isolation of bat populations like *Myotis peninsularis/velifer* to the southern tip of the peninsula (See Chapter 2). For example, lineage Tick 6 was found exclusively on this species, suggesting this system as a potential case of study to test patterns of bat and ectoparasites isolation and ecological differentiation.

Bat ectoparasites have been considered to be mostly host-specific due to the ecological associations and life history strategies that are so particular of bats (Seneviratne, Fernando and Udagama-Randeniya, 2009). However, this will be dependent on the ectoparasite behaviour and life strategy as well (e.g. bat bugs stay most of their life cycle on

the bat´s roost, which can be shared by other bats species, wildlife and humans). Therefore, the degree of host-specificity is important to understand how successfully an ectoparasite can colonise new hosts, its survival or co-extinction, geographical distribution, and the different routes in which pathogens can be transmitted across other taxa (Esser *et al.*, 2016). Host-specific and generalist ectoparasites were found in all the groups from this study, where host-specificity was the case most times. Many of the bats captured were associated to water bodies or buildings that were in direct relation with humans. This highlights the importance for a better understanding of which associations are formed, their location across peninsula and in their distribution extent (Zamora-Mejías *et al.*, 2020), to evaluate and prevent any potential spread of pathogens.

Ectoparasite systems have the potential to describe different patterns of movement and population structure of elusive hosts, providing independent information about their hosts that can be used to test hypotheses that may be difficult to assess using host genetics alone. (Speer *et al.*, 2019). For example, latitudinal (Zamora-Mejías *et al.*, 2020) and seasonal variation (Salinas-Ramos *et al.*, 2018) in bats parasitized by *Nycterophilia coxata* flies, have been reported in the bat *Leptonycteris yerbabuenae* in Mexico. Bat migration patterns over Baja are unknown, therefore there is no information about when and where do they move. Results from this study present strong evidence of bat migration by the haplotypes obtained within the ectoparasites groups (Fig. 3.2-3.5), confirming for the first time that bats movements happen across the peninsula. As example, the detection of *Antrozous pallidus* dispersal in the peninsula due to the haplotypes distribution of the fly lineage *Basilia* 2b (Fig. 3.3), represents the usefulness of ectoparasites studies to discover patterns of dispersal in

understudied systems. However, more biological and abiotic factors need to be taken into account to evaluate patterns of dispersal at small and large scales.

Results of this research are producing basal data that is increasing ectoparasite information for North America, generating also a repository of ectoparasite samples and their interactions for future research in multiple areas (e.g. on pathogens). Investigating ectoparasite diversity has implications not only over species discovery, but also for evaluating risks on bat disease ecology, targeting areas of concern for potential vectors of zoonotic importance and in conservation (Poulin, 2014). With this study, it is also intended to highlight the importance of ectoparasites as integral part of their hosts and their ecosystem (Nichols and Gómez, 2011), where it is necessary to study their population status and evaluate their risk of extinction, directly linked with conservation of their hosts and the host's habitat. This study also highlight the importance of conducting medium-scale surveys, where patterns of spatial distribution and habitat components are important for understanding the bat-ectoparasite community diversity, which will be briefly examined in the following Chapter.

Chapter 4: Bat ectoparasite community structure in the Baja California peninsula.

4.1. Abstract

Evaluating the genetic and ecological mechanisms shaping host-parasite community structure can provide a better understanding of host movements and how this influence the dispersal of parasites and potential pathogens. We investigated the bat-ectoparasite community structure of 16 bat species distributed along the Baja California peninsula and three sites of continental Mexico, to understand how bat-ectoparasite community is shaped by hosts constrains, climate, and environmental structure. We analysed prevalence and intensity of two different community sets, one composed by total bat bugs, fleas, flies, mites and ticks sampled; and another one including only bat bugs, flies and ticks that were identified to the species level. Overall, mites were the most abundant and prevalent group, followed by bat flies. For the second set, we also conducted analysis of alfa and beta diversity, host and spatial associations. Ectoparasite associations were mainly composed by one or two hosts, and shared up to four hosts. Bat phylogenetic similarity showed to be a better predictor of ectoparasite diversity, where phyllostomid bats were exclusively parasitized by streblid flies. Ectoparasite richness was highly correlated with bat richness and diversity, not showing other climatic or environmental predictor. The three peninsular regions showed similar levels of bat richness and sampling effort, but differences in bat-ectoparasite diversity composition. Mid and north peninsula were the places with the highest values of bat and ectoparasite diversity. Finally, bat diversity was highly correlated with high temperature and precipitation rates, all converging in summer and late-summer. This work is a first step for discovering factors shaping bat-ectoparasite community structure in Baja, showing the effect of ecology and distribution of the bat hosts as main drivers of ectoparasite diversity. Our study provides

insights for the study of patterns of distribution of bat and ectoparasites as potential zoonotic vectors, highlighting the importance of increasing efforts in bat and ectoparasite research.

4.2. Introduction

The composition of parasite communities is shaped by a combination of ecological, biogeographical and evolutionary processes influencing host population structure, and by the interactions among hosts and other parasites (Krasnov *et al.*, 2012). In general, related host species may present similar values for parasite community richness or ecological characteristics because of shared evolutionary history, more than due to convergent ecology (Poulin, 1995). Patterns of species richness and abundance associated with latitudinal gradients are a common biogeographic feature of diversity distributions (Guégan, Morand and Poulin, 2004; Poulin, 2014; Chhatre *et al.*, 2019), where there is strong covariance between host species richness and parasite species richness (Poulin, 2014). Studying these and other temporal factors affecting hosts, like ecological and climatic conditions, seasonal processes, time and degree of dispersal, life-cycles and senescence, sex-biased assemblages, etc. (Poulin, 1995; Poulin and Morand, 2004; Saldaña-Vázquez *et al.*, 2019), would help to understand patterns parasite distribution and dispersal at different ecological scales.

Parasite-host studies in wild animal populations are increasingly gaining attention because of their relevance to diseases of zoonotic potential and as study systems for basic wildlife disease ecology (Morse *et al.*, 2012; Wilder, Kunz and Sorenson, 2015; Reeves *et al.*, 2016; Sánchez-Montes *et al.*, 2016; Hornok *et al.*, 2017; Hornok *et al.*, 2019). While most attention is focused on the negative impacts of parasites , parasite exposure may also have beneficial influences on some aspects of host fitness, such as being necessary for the

development of a host's functional immune system (Spencer and Zuk, 2016), and as regulators of host populations and host interaction with other pathogens (Hudson, Dobson and Lafferty, 2006). To understand the degree of beneficial or detrimental effects on different host systems, it is necessary to identify the epidemiological and ecological interactions over different hierarchical levels of diversity organisation.

For the study of parasite community composition, spatial scale will be defined by the host's level of assemblage and connectivity (Gómez-Díaz, Navarro and González-Solís, 2008). Community structure of parasites will be affected by the interaction of intrinsic and extrinsic characteristics of both parasite and host, along with environmental factors and host populations' connectivity (Morand, Krasnov and Poulin, 2006; Gómez-Díaz, Navarro and González-Solís, 2008; Krasnov *et al.*, 2012). These factors can be studied at different scales of organisation: infra-, component, compound and regional community levels (Table 4.1). A study evaluating the parasite community structure in multilevel community hierarchies of three shearwater hosts of the genus *Calonectris* showed that patterns of parasite aggregations changed across different spatial scales (Gómez-Díaz, Navarro and González-Solís, 2008). These communities were composed of overlapping mixes of ectoparasite species, including three lice species, *Halipeurus abnormis, Austromenopon echinatum* and *Saemundssonia peusi*, and one flea *Xenopsylla gratiosa*, but were separated along the host body (infracommunity level). In the next level, they presented aggregated abundances within colonies and types of ectoparasites (component community level), reflecting ecological differences between lice and fleas (Gómez-Díaz, Navarro and González-Solís, 2008). Lastly, these ectoparasite communities correlated with geographic distances among host colonies (regional level), but not with genetic distances (Gómez-Díaz, Navarro and González-Solís,

2008). In systems with high dispersal and aggregation breeding behaviour, the key determinants of ectoparasite community structure are: phylogenetic component of ectoparasites and hosts, spatial distribution in the host's body, host phylogeographic structure and seasonal host-host relationships (Guégan, Morand and Poulin, 2004; Poulin and Morand, 2004; Saldaña-Vázquez *et al.*, 2019).

Table 4.1. Parasite assemblage hierarchies in which different spatial and temporal factors may act. From Guégan et al., (2004) and Gómez-Díaz et al., (2008) and references therein.

Level	Definition	Description
Infracommunity	All parasite populations within an individual host	Temporally (e.g. host's breeding cycle) and spatially (e.g. resource heterogeneity within the host body) structured.
Component communities	All infracommunities within the same host population	Structured by host susceptibility to infestation (e.g. host's body size and condition), or from differences in parasite exposure among individual hosts (e.g. sex-based clustering).
Compound communities	All the parasite communities within an ecosystem	Larger scale processes may have a strong influence on local community structure and dynamics of parasites and pathogens (e.g. host long-distance seasonal migration from different colonies exchanging parasites at different assemblage points over temporal frames).
Regional community	All component communities within a host species	Structure is mainly determined by habitat characteristics (e.g. vegetation type defining hosts distributions), host population size and density (e.g. small or large colonies of hosts), and host connectivity (e.g. host's capacity of dispersal.

Bats represent an ideal system to understand parasite community dynamics, since this order harbours a rich diversity of arthropod ectoparasites (see Chapter 1, Ectoparasites of bats), that are considered to be mostly host-specific as a result of ancient lineages coevolving, and adapting to the bats particular ecological constraints, behaviour and life history strategies (Seneviratne, Fernando and Udagama-Randeniya, 2009; McKee *et al.*, 2019). The roosting

behaviour of bats means many bat species form aggregations that promote reproduction and horizontal transfer among certain groups of ectoparasites, such as flies and mites that are obligately dependent on the host for survival (Reckardt and Kerth, 2009; Lučan *et al.*, 2016; Saldaña-Vázquez *et al.*, 2019; Speer *et al.*, 2019). Additionally, roosting sites are perfect for the interchange of other ectoparasites like bat bugs, which after feeding from their host, can remain in the roosting site for long periods, therefore are potentially able to infect other bats species occupying the same roost in different seasons (Balvín, Vilímová and Kratochvíl, 2013; Hornok *et al.*, 2017). Social systems will also influence parasite population structure and coadaptation dynamics of different communities of ectoparasites in bats. As an example, two mite species with similar life-history traits, *Spinturnix myoti* and *S. bechesteini*, were shown to have a different genetic structure, most likely due to differences in patterns of dispersal and social structures of their hosts, *Myotis myotis* and *M. bechesteii*, respectively (van Schaik *et al.*, 2014). Accounting for multiple ecological and evolutionary processes influencing bat biology, distribution and genetic structure will help to understand how these multifactor scenarios can shape parasites and pathogens sharing among bat populations.

Figure 4.1. Maternity colony of *Myotis peninsularis* in the south of the Baja California peninsula. Juveniles can be detected in this picture by their finer and grey coloured fur.

Bat ectoparasites are vectors of other pathogens of relevant to the health of their hosts, and which are also of zoonotic importance (Morse *et al.*, 2012; Gay *et al.*, 2014; Lučan *et al.*, 2016; Haelewaters *et al.*, 2017; McKee *et al.*, 2019). The famous bed bug, *Cimex lectularius*, is a re-emerging human commensal parasite, derived from a bat ectoparasite ancestor (Roth *et al.*, 2019), and which can opportunistically feed from both bats and humans under appropriate conditions (e.g. humans and bats sharing shelter in buildings or mines). *C. lectularius* can be infected by *Rickettsia* and *Bartonella* (Zorrilla-Vaca, Silva-Medina and Escandón-Vargas, 2015), a bacteria of zoonotic importance that can be transmitted by bats ectoparasites (Veikkolainen *et al.*, 2014; Reeves *et al.*, 2016; Burazerović *et al.*, 2018). *Rickettsia* has been also shown to be transmitted by ticks and fleas. In Mexico, there are seven species of

Rickettsia bacteria, where *R. lusitaniae* has been detected in soft ticks from bat caves in Yucatan, Mexico (Sánchez-Montes *et al.*, 2016). *Bartonella*, a bacteria found in vertebrates that can be pathogenic to humans, has been detected globally in studies of bat flies, including Mexico, Guatemala and the U.S, highlighting their zoonotic importance (Morse *et al.*, 2012). Examining the mechanisms that shape bat ectoparasite diversity is fundamental for assessing these potential threats, and for understanding how vectors vary in their host-specificity, which taxa posse the greatest risks, and how to detect potential transmission routes of pathogens to humans and vulnerable wildlife (Olival *et al.*, 2017; Albery *et al.*, 2019; McKee *et al.*, 2019).

Bats can commonly be hosts of different groups of ectoparasites at the same time, where abundance and diversity will vary according to different factors. In general, studies of bat ectoparasite are focused on characterising the community composition of specific groups of ectoparasites from a particular region of the world (Dick *et al.*, 2003; Gay *et al.*, 2014; Booth *et al.*, 2015; Frank *et al.*, 2015; Bezerra, de Vasconcelos and Bocchiglieri, 2016; Potiwat *et al.*, 2016; Ramasindrazana *et al.*, 2017), with bat flies in South America being particular well represented (Graciolli, Autino and Claps, 2007; Venzal *et al.*, 2015; Muñoz-Leal *et al.*, 2016; Guzmán-Cornejo *et al.*, 2017). However, these studies are usually evaluating single host-ectoparasite associations, or including a single type of ectoparasite among different bat hosts and not as a community entity, where multilevel associations have effects on the composition of the complete repertoire on each bat population. Burazerović *et al.* (2018) performed a community analysis in terms of abundance and host specificity, including all bats and their ectoparasites captured in a three-year survey in the central Balkans. They found a total of 80 bat-ectoparasite associations, in which the largest number of ectoparasites parasitized primarily only one host species, *Miniopterus schreibersii*. The

most abundant ectoparasites in different hosts were the tick *Ixodes vespertilionis*, the fly *Nycteribia schmidlii*, and the mite *Spinturnix myoti*; which was also the most abundant, and along with the fly *Penicilidia dufouri*, the most distributed over the sites in the study (Burazerović *et al.*, 2018). Their results gathered the first set of patterns of abundance for hosts and ectoparasites for that region. This highlights the necessity for research of these associations in unexplored places, gathering useful information for future ecological, biogeographical and epidemiological questions.

In the Baja California peninsula (Fig. 2.2), there have only been a few studies targeting bat pathogens or parasites, and have focused on those of medical or agricultural importance, such as the potential for bats to act as reservoirs for lyssaviruses (rabies) (Velasco-Villa *et al.*, 2002). In Baja, *Lyssavirus* has been detected only in domestic animals (cattle, horses), bobcats (*Lynx rufus*) and skunks (*Spilogale putorius*). However, it is present in bats and other animals in continental Mexico (Velasco-Villa *et al.*, 2002). Apart from a monograph on cimicid bugs of North America (Usinger, 1966), there are no parasite studies covering bats from Baja. For the rest of Mexico, there is an increasing number of studies describing bat fly ectoparasite diversity (Cuxim-Koyoc *et al.*, 2015; Saldaña-Vázquez *et al.*, 2019), and a few including other groups such as mites (Pérez *et al.*, 2014; Ramírez-Martínez *et al.*, 2016; Bolaños-García, Rodríguez-Estrella and Guzmán-Cornejo, 2018), ticks (Steinlein, Durden and Cannon, 2001; Guzmán-Cornejo *et al.*, 2017), and ticks and associated pathogens (Sánchez-Montes *et al.*, 2016; Hornok *et al.*, 2019).

The first phylogenetic study of ectoparasites of Baja was presented in Chapter 3, showing that there are potentially 21 novel lineages of bat bugs, flies and ticks (Fig. 4.2).

This represents the first step for identifying and describing associations between the parasites and their bat hosts, and how they are influenced by the multiple variables including bat population structure in the peninsula. A range of patterns was observed for the geographic distribution of ectoparasite species on the peninsular, with some showing restricted host and spatial ranges, while others were widely distributed. This suggests that a diversity of processes influencing bat systems might be the main contributions of ectoparasite community organisation.

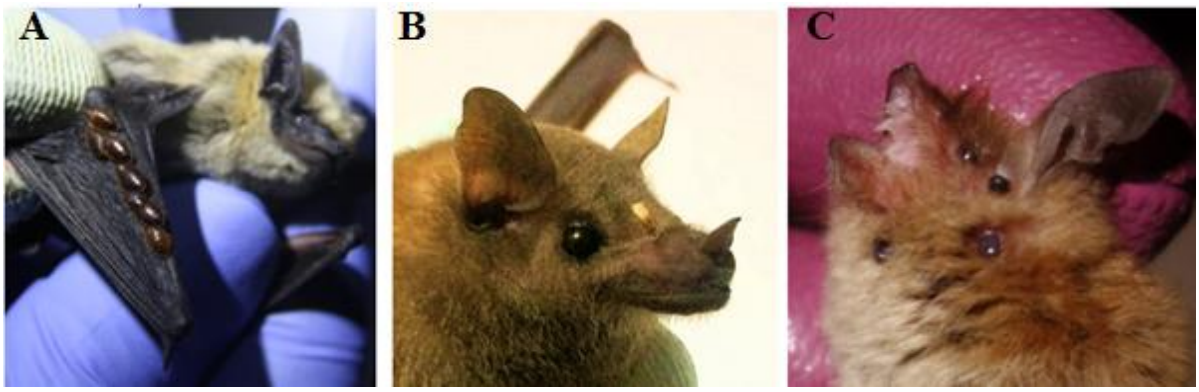


Figure 4.2. Bat bugs of the putative lineage *Cimex 4* in forearm of a *Parastrellus hesperus* bat; B, winged fly *Trichobius sphaeronotus* wondering on the face of a *Leptonycteris yerbabuenae* bat; and C, bat ticks of the putative lineage Tick *6* infesting a *Myotis yumanensis* bat (see Chapter 3).

This Chapter will investigate ectoparasite community structure on the Baja California peninsula; to understand how bat-ectoparasite community is influenced by hosts constrains and geographic structure, and if environmental and ecological variables will have a separate effect on the bat-ectoparasite associations. Predictions are that the highest parasite diversity will be present over sites with great abundance of hosts, also affected by bats geographic diversity.

4.3. Methods

4.3.1. Sampling and parasite identification

Study area, fieldwork assessment, bat capture and *Myotis* bats molecular identification were described in the methods section of Chapter 2. Ectoparasite collection methodology and other bat species sampled for this study were reported in Chapter 3. For this Chapter, bats and all the ectoparasites captured along the three fieldwork seasons were considered for an overall summary of collected ectoparasites, diversity of bat species and sampled sites. This overview included the abundance of bugs, flies, ticks, mites, fleas and any other non-identified ectoparasite, which included larvae and nymph states of mites and ticks that were too small to be identified in field (grouped as "Other"). This Chapter is based on the previously generated molecular identifications and phylogenetic results from both *Myotis* bats and parasites, therefore their lineage assignments are going to be used for the following analyses.

4.3.2. Bat and ectoparasite diversity

For each ectoparasite-bat association, summary statistics were calculated for two different data sets: firstly, one including all the ectoparasite classes captured over bats from this study (*n* hosts = 501); and a second data set using only bat bugs, flies and ticks, from hosts that included at least one of those ectoparasites (*n* hosts = 148). The first dataset was used to generate summary statistics at the level of parasite class, including abundance, mean abundance, median abundance, mean intensity of infestation and prevalence, which were calculated using Excel (Microsoft, 2013) and the Quantitative Parasitology software (QPweb) ver. 1.0.14 (Reiczigel *et al.*, 2019). To test for the level of uncertainty of the sample values,

95% confidence intervals were calculated for prevalence using Blaker's method (Blaker, 2000); and for the mean abundance, mean and median intensity by applying a bias-corrected and accelerated bootstrap of 2000 replicates (BCa, Efron and Tibshirani, 1994) performed in QPweb (Reiczigel *et al.*, 2019).

The second data set was used to estimate measures of species diversity, which were calculated only for ectoparasite lineages previously identified using the molecular methods described in Chapter 3, therefore excluding mites, fleas and any non-identified parasite, retaining identified bat bugs, flies and ticks. Bat species diversity indexes were also calculated for those included in this dataset to compare results. The software R studio (RStudio Team, 2015) was used to perform all the following analysis. The packages *vegan* (Oksanen *et al.*, 2019) and *BiodiversityR* (Kindt and Coe, 2005), were used for obtaining richness (S), diversity with Shannon-Wiener index (H) and Simpson's diversity index (1-D), which results were merged with the package *kableExtra* (Zhu, 2019). Beta diversity was calculated with the "Sørensen index of dissimilarity" (Oksanen, 2019) using the function *vegdist* from the same package.

Sample-based accumulation curves are commonly used for studying parasites, using individual hosts as measure of sampling effort (Dove and Cribb, 2006). Individual-based curves records the cumulative increase in richness against number of individual organisms exanimated, and they are not treated as equivalent samples units as they use abundance data, detecting better potential rare species to discover (Dove and Cribb, 2006). Ectoparasite and bat site sample-based species accumulation curves were calculated using Kindt's "exact" method (Oksanen, 2019), and for ectoparasites against number of bats (bats hosts instead of

sites). Individual-based curves using the "rarefaction" method, in *vegan.* This package was also used to perform a rarefaction analysis to find the expected species richness of ectoparasites and bats in the community, and for beta diversity analysis per group of ectoparasites and bats. Plot generation and visualization was done using the package *ggplot2* (Wickham, 2016).

4.3.3. Host associations with bat bugs, flies and ticks

A matrix representing each ectoparasite lineage abundance for each bat species was generated. With this, a co-occurrence network analysis was performed to understand bat-ectoparasite associations, and visualized using the package *Circlize* 0.4.9 (Gu *et al.*, 2014).

4.3.4. Spatial associations of bats and ectoparasites richness and diversity

Historic climate data for 1970-2000 was downloaded from WorldClim version 2.1 (Fick and Hijmans, 2017), including the 19 standard "bioclimatic" variables of 30 seconds resolution plus a monthly historical weather data at 30 seconds resolution (also 1970-2000), including average of maximum and minimum temperature (°C), precipitation (mm), wind speed (ms^{-1}), and water vapour pressure (kPa). From the monthly data, the annual average was obtained as well as the average from June to September, covering the months in which fieldwork was done. For this, the *raster* (Hijmans, 2020), *sp* (Bivand, Pebesma and Gomez-Rubio, 2013), and *mapview* (Appelhans *et al.*, 2020) packages were used to extract the data information from the coordinates of the sites included in this study. Each variable was tested for normality using a Shapiro-Wilk Normality test with the package *stats* (Royston, 1995; RStudio Team, 2015). After this, a Spearman non-parametric correlation was performed to

test for collinearity along the climatic variables using the package *Hmisc* (Harrell, 2020). Variables were then transformed using a Tukey's ladder of powers transformation from the package *rcompanion* (Mangiafico, 2020), for running a principal component analysis (PCA) with the function *prcomp* to account for spatial correlation and visualize direction of the variables against sites (RStudio Team, 2015).Variables with high collinearity ($r < 0.8$) were discarded, retaining only those that best explained the data based on the predominant variables affecting the summer season and direction of the variables: Annual mean maximum temperature, annual mean minimum temperature, mean temperature driest quarter, annual precipitation, precipitation wettest quarter, precipitation driest quarter, precipitation warmest quarter, precipitation coldest quarter, annual mean vapour pressure and annual mean wind speed. A dataset was then compiled including the climatic variables that were retained after testing for collinearity, plus species richness, Shannon-Wiener and Simpson's diversity indexes (for both bats and ectoparasites), latitude and elevation for each site, peninsular region for each site (north, middle, south), continental regions (mid and south, including one and two sites, respectively), and the dominant vegetation type at each site (obtained from the Comision Nacional para el Conocimiento y Uso de la Biodiversidad (Rzedowski, 2006)), were also included.

To test for spatial, environmental and ecological factors influencing ectoparasite and bat diversity, linear models were fitted in R, using the function *lm* from the package *stats* (RStudio Team, 2015). Diversity measures (species richness and Shannon-Wiener diversity index) were used as response variables. Explanatory variables were first fitted individually, and then included in a maximal model, followed by a model reduction procedure to evaluate models with multiple terms. Individual variables and model significance was tested using the

Akaike Information Criterion where appropriate, using the *AIC* function from *stats*. For the categorical variables (Region and Vegetation type), differences in pairwise comparisons of means for Shannon-Wiener index values were evaluated using Tukey's range test (Tukey Honest Significant Difference test), with a 95% family-wise confidence level.

Euclidean distances were calculated for pairwise comparisons between sites using the *dist* function in R (RStudio Team, 2015), for each of the retained climate variables, plus site altitude. Pairwise geographic distances (metres) between sites based on longitude and latitude coordinates were calculated using the *distm* function from the *geosphere* package 1.5-10 (Hijmans, 2019). Multiple regression with distance matrices (MRM) analyses were carried out for ectoparasite beta diversity (Sørensen index of dissimilarity) and for bat beta diversity across sites, and across environment and ecological variables. For this the *MRM* function was used from the *ecodist* package (Hijmans, 2019), with 100,000 permutations for each analysis. The maximal MRM models for each bat and ectoparasites beta diversity included the 10 WorldClim variables described above, plus bat beta diversity, ectoparasite beta diversity, geographic distances, and altitude. The minimal MRM analysis were carried out only with the variables that showed to be significantly correlated with each bat and ectoparasite beta diversity set.

4.4. Results

4.4.1. Sampling and overall parasite descriptive statistics

In total, 1,846 ectoparasites were collected from 313 bats (n = 501), where 188 bats had no visible ectoparasites recorded. From this, 63% were mites (including mites from the orders Mesostigmata and Trombidiformes), and 9% were non-identified larvae or nymphs

from either mites or ticks, grouped together since they were too small to be classified during fieldwork (purple group, Fig. 4.3 A). Bat flies were the second most common group of ectoparasites, followed by ticks and then bugs. Only six fleas were captured during all fieldwork seasons from five individuals, constituting 0.003% of the ectoparasites collected (pink legend, Fig. 4.3). The bat species *Antrozous pallidus* and *Parastrellus hesperus* were the most parasitized bat species in this study.

Ectoparasites were found in a total of 18 from 25 sites along the Baja California peninsula, and two sites in the continental Mexico (Fig. 2.2 and 4.4). The site with most parasite records was Chabacanos from the north, followed by Testera in the south peninsula. Most of the sites had at least one acari (mites, trombiculids, other and ticks groups) record, with the exception of Tucson, whereas three sites included only mite records (Requeson, San Pedro and Boca de la Sierra). The rest of the sites included at least one member of bug, fly or tick (see Fig. 3.1).

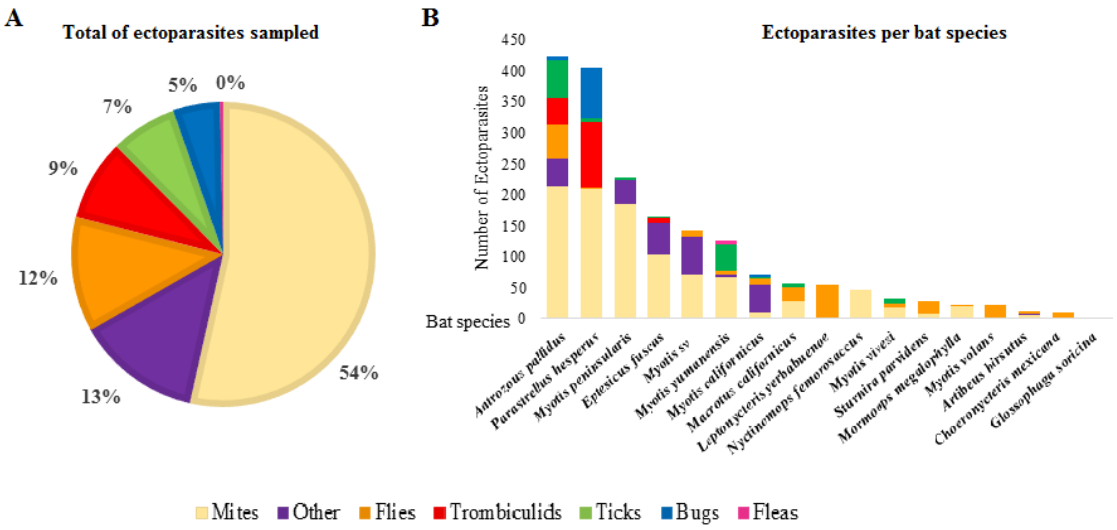


Figure 4.3. Total of ectoparasites collected along 2016-2018 fieldwork seasons, showing A, the proportion of ectoparasites collected per group; and B, ectoparasites collected per bat species. The group "Other" refers to any non-identified ectoparasite.

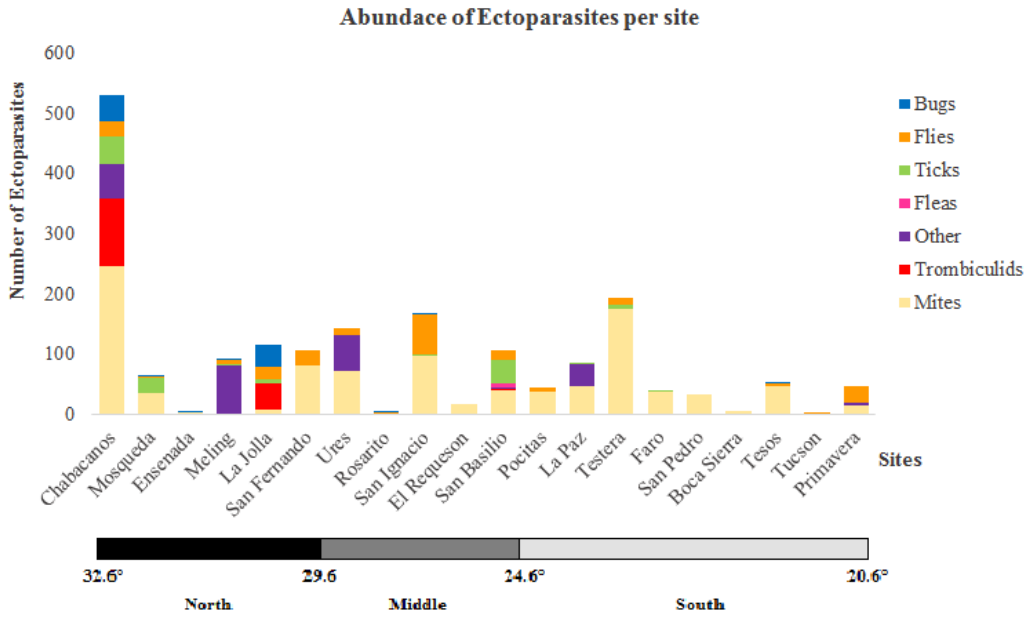


Figure 4.4. Total of ectoparasites found per sampling site. Sites in this graph are ordered from the northernmost (Chabacanos) in the left, to the southermost (Primavera) site over the peninsula in the right. (Sites "Ures", "Tucson" and "Primavera" are in continental land).

The overall prevalence varied extensively among different ectoparasite groups (Pr, Table 4.2), where the minimum was presented by fleas with 1.0%, found only in one location in five *Myotis yumanensis* hosts, and a maximum of 42.9% presented by the groups including all the families of mites and non-identified ectoparasites (potentially mite or tick larvae/nymphs). The per host intensity abundance of non-identified mites and larvae was appreciably higher than the rest of the ectoparasite groups, ranging between 1 to 15 mites (intensity and median intensity, rounded up from Table 4.2). Bat flies represented the group with the highest values after mites, except for median intensity. Bat ticks showed higher values than bugs, except for intensity.

Table 4.2. Overall summary of descriptive stats of all the ectoparasites found in this study. In the Table from left to right, ectoparasite group, number of infected hosts analysed (IH), percentage of prevalence (Pr), 95% Prevalence confidence intervals (CI) under the Blaker's method (P-CI), intensity (I), 95% confidence intervals under 2000 Bootstrap BCa replications for intensity (I-CI), mean abundance (MA), 95% confidence intervals under 2000 Bootstrap BCa replications for mean abundance (MA-CI), median intensity (MI), 95% Exact (nominal) confidence intervals for median intensity (MI-CI), and total abundance (A). NA refers to a sample size too small for calculations.

Group	IH	Pr (%)	Pr-CI (lower-upper)	I	I-CI (lower-upper)	MA	MA-CI (lower-upper)	MI	MI-CI (lower-upper)	A
Bugs	35	6.99	0.050-0.095	2.63	2.03-3.73	0.18	0.122-0.297	2.0	1-2	92
Flies	82	16.37	0.133-0.199	2.74	2.19-3.74	0.45	0.325-0.635	2.0	1-2	225
Ticks	57	11.4	0.088-0.144	2.25	1.86-2.74	0.25	0.186-0.351	2.0	1-2	129
Mites + Other*	215	42.91	0.386-0.473	6.74	5.84-7.82	2.89	2.41-3.47	4.0	1-3	1392
Fleas	5	1.0	0.004-0.023	1.20	1-1.4	0.01	0.004-0.026	1.0	NA	6

*** Mites + Other group includes all mites (Mesostigmata and Trombidiformes orders), and all non-identified larvae/nymphs from either mites or ticks.**

The number of ectoparasites species (lineages) analysed in the dataset including only bugs, flies and ticks was 27. Of these, five were found on single host individuals with a single record (S=1 and IH=1, Table 4.3), whereas the rest of ectoparasite lineages caught were represented by at least two hosts (from the same bat species or from different species. Estimated overall parasite richness was 29.5 (SD+-3.0; 95% CI of 27.4 to 42.6). The lineage with most infected hosts was NBasilia 2b (IH=29), which belonged to the family Nycteribiidae and parasitizing the bat *Antrozous pallidus* exclusively (potentially representing *Basilia antrozoi*), also with the highest prevalence among all the groups (Pr=19.6%), and followed by *Cimex* 4 bug lineage (Pr=18.9%), which was also the most abundant (A=84, Table 4.3). The tick lineage with the highest prevalence was Tick 5 (Pr=10.1%), parasitizing four different hosts species; and the most abundant *Carios kelleyi* lineage (Pr = 8.8%, A=42, Table 4.3), parasitizing only one species of bat, *A. pallidus*.

Table 4.3. Diversity descriptive stats of bat bugs, flies and ticks ectoparasites. In the Table from left to right, ectoparasite lineage, host richness (S), number of infected hosts analysed (IH), percentage of prevalence (Pr), 95% Prevalence confidence intervals (CI) under the Blaker's method (P-CI), intensity (I), 95% confidence intervals under 2000 Bootstrap BCa replications for intensity (I-CI), mean abundance (MA), 95% confidence intervals under 2000 Bootstrap BCa replications for mean abundance (MA-CI), median intensity (MI), 95% Exact (nominal) confidence intervals for median intensity (MI-CI), total abundance (A), and hosts species. Hosts species are listed in order of the most parasitized in relation to their respective ectoparasite lineage. NA refers to a sample size too small for calculations.

Lineage	S	IH	Pr (%)	Pr-CI (lower-upper)	I	I-CI (lower-upper)	MA	MA-CI (lower-upper)	MI	MI-CI (lower-upper)	A	Hosts
Cimex 1	1	1	0.7	0.000-0.035	1	NA	0.006	NA	1	NA	1	*Antrozous pallidus*
Cimex 2	1	2	1.4	0.002-0.048	1	NA	0.013	0-0.033	1	NA	2	*Myotis californicus*
Cimex 3	2	4	2.7	0.009-0.065	1.25	1-1.5	0.033	0.006-0.074	1	NA	5	*Antrozous pallidus*, *Myotis californicus*
Cimex 4	2	28	18.9	0.131-0.259	3	2.29-4.32	0.56	0.358-0.919	2	1to3	84	*Parastrellus hesperus*, *Antrozous pallidus*
T*Antricola*1	2	5	3.4	0.013-0.075	2.8	1-5.6	0.093	0.02-0.299	1	NA	14	*Macrotus californicus*, *Myotis vivesi*
Carios kelleyi	1	13	8.8	0.048-0.143	3.23	2.23-4.31	0.28	0.149-0.506	3	1to5	42	*Antrozous pallidus*
T1	2	2	1.4	0.002-0.048	2	NA	0.026	0-0.067	2	NA	4	*Antrozous pallidus*, *Myotis yumanensis*
T2	2	3	2	0.005-0.058	1	NA	0.02	0-0.047	1	NA	3	*Antrozous pallidus*, *Myotis peninsularis*
T3	1	8	5.4	0.024-0.102	2.12	1.38-3.12	0.115	0.047-0.241	2	1to3	17	*Myotis yumanensis*
T4	2	4	2.7	0.009-0.065	2.5	1.25-3.25	0.067	0.013-0.172	2.5	NA	10	*Antrozous pallidus*, *Eptesicus fuscus*
T5	4	15	10.1	0.058-0.160	1.73	1.2-2.47	0.176	0.094-0.311	1	1to3	32	*Myotis yumanensis*, *Antrozous pallidus*,

												Parastrellus hesperus, *Eptesicus fuscus*
T6	1	4	2.7	0.009-0.065	1	NA	0.026	0.006-0.054	1	NA	4	*Myotis peninsularis*
NNycte1	3	10	6.8	0.035-0.119	1.4	1.1-1.6	0.094	0.040-0.162	1	1to2	14	*Myotis sp*, *Myotis californicus*, *Parastrellus hesperus*
NNycte2	4	13	8.8	0.048-0.143	2.15	1.38-3.31	0.189	0.094-0.351	1	1to3	37	*Myotis volans*, *Antrozous pallidus*, *Myotis californicus*, *Myotis yumanensis*
N*Basilia*1	1	3	2	0.005-0.058	2	1-2.67	0.04	0.006-0.115	2	NA	6	*Myotis yumanensis*
N*Basilia*2a	1	3	2	0.005-0.058	2	1-2.67	0.04	0-0.113	2	NA	6	*Myotis vivesi*
N*Basilia*2b	1	29	19.6	0.136-0.269	1.9	1.45-2.43	0.372	0.236-0.554	1	1to2	61	*Antrozous pallidus*
S*A.phyllostomatis*	1	3	2	0.005-0.058-	1.67	1-2.67	0.033	0.006-0.087	2	NA	5	*Sturnira parvidens*
S*M.aranea*	2	6	4.1	0.018-0.085	3.17	1.83-4	0.128	0.04-0.264	3.5	1to5	19	*Sturnira parvidens*, *Artibeus hirsutus*
S*N.coxata*	2	10	6.8	0.035-0.119	3.8	2.3-5.4	0.257	0.115-0.507	2	2to7	38	*Leptonycteris yerbabuenae*, *Macrotus californicus*
S*Paratrichobius*1	1	1	0.7	-0.000-0.035	1	NA	0.006	0-0.027	1	NA	1	*Artibeus hirsutus*
SStreblid1	1	4	2.7	0.009-0.065	2.75	1-5.75	0.074	0.013-0.277	1.5	NA	11	*Macrotus californicus*
S*Trichobius*1	1	2	1.4	0.002-0.048	1	NA	0.013	0-0.033	1	NA	2	*Mormoops megalophylla*
S*Trichobius*2	1	1	0.7	0.000-0.035	10	NA	0.067	0-0.203	10	NA	10	*Choeronycteris mexicana*
S*Trichobius*3	1	1	0.7	0.000-0.035	1	NA	0.067	0-0.02	1	NA	1	*Artibeus hirsutus*
S*T.dugesii*	1	1	0.7	0.000-0.035	1	NA	0.067	0-0.02	1	NA	1	*Glossophaga soricina*
S*T.sphaeronotus*	1	3	2	0.005-0.058	9.33	2-13.3	0.189	0.013-0.58	12	NA	28	*Leptonycteris yerbabuenae*

Median intensity and its confidence intervals (MI and MI-CI, Table 4.3) showed a closer value to the common number of parasites load on each bat, where usually there were not more than two ectoparasites of one group per host, and with only rare cases of individuals with high loads, fitting a negative binomial distribution, common distribution shown over host-parasite systems (Reiczigel *et al.*, 2019). One extreme example was a *Parastrellus hesperus* individual sampled at La Jolla, with 11 bat bugs from *Cimex* 4 lineage (A, Fig. 4.2).

4.4.2. Bat and ectoparasite diversity

A summary of richness and diversity stats for ectoparasites per site, and bats per site is shown in Table 4.4. There were seven sites with only one ectoparasite species captured (e.g. Ensenada, Faro sites, Fig. 2.2), whereas the most diverse sites included seven different species of ectoparasites (Table 4.4). From these, the most diverse and with highest rarefaction value was San Basilio site, located at mid-peninsula. The same site had the highest diversity values for bat species, with eight species and also the highest rarefaction value. High values of richness and diversity index can be explained by two factors: San Basilio site had eight sampled nights in three years (NN and Y, Table 4.4), which are the highest values among sites; and San Basilio is located in the middle of the desert, tight at the edge of the sea, therefore is one of the few sources of drinking water over that area.

Table 4.4. Summary richness and diversity stats for ectoparasites lineages and bats species by site. Abbreviations: Number of nights (NN), number of seasons/years sampled (Y), richness (S), diversity indexes (H, Shannon-Wiener, and D, Simpson's diversity 1-D), and rarefaction analysis (Rar).

			Ectoparasite species				Bats species			
Sites	**NN**	**Y**	**S**	**H**	**D**	**Rar**	**S**	**H**	**D**	**Rar**
Chabacanos	5	2	7	1.522	0.746	4.116	5	1.472	0.745	4.161
Ensenada	1	1	1	0	0	1	4	1.277	0.694	4
Faro	3	2	1	0	0	1	4	1.264	0.694	3.909
Jolla	2	1	5	1.190	0.595	3.561	6	1.422	0.699	4.246
La Paz	4	2	2	0.693	0.500	2	2	0.176	0.081	1.384
Meling	1	1	3	0.916	0.562	2.909	5	1.334	0.703	3.823
Mosqueda	4	2	6	1.438	0.698	4.460	8	1.520	0.667	4.611
Parral	1	1	1	0	0	1	2	0.693	0.500	2
Pocitas	2	2	1	0	0	1	3	0.900	0.531	3
Primavera	1	1	4	0.797	0.426	2.703	5	1.304	0.651	4.462
Rosarito	2	2	2	0.693	0.500	2	5	1.330	0.693	3.963
San Basilio	8	3	7	1.781	0.809	5.106	8	1.749	0.790	4.923
San Fernando	4	2	1	0	0	1	7	1.688	0.788	4.740
San Ignacio	6	2	7	1.363	0.678	3.721	7	1.636	0.754	4.754
Tesos	2	2	3	1.004	0.612	3	3	0.701	0.431	2.269
Testera	3	3	5	1.335	0.685	4.110	6	0.971	0.449	3.104
Tucson	1	1	1	0	0	1	2	0.673	0.480	2
Ures	2	1	1	0	0	1	2	0.210	0.102	1.473

As expected, accumulation curves calculated for all the analysis did not reach the asymptote, showing that more sampling is needed, increasing both the number of sites and number of bat species to capture the full species diversity which is potentially present (Fig. 4.5). For both ectoparasites and bats, the individual-based analyses (right graphs, Fig. 4.5), appeared to be approaching asymptote, and approximate an exponential decay. This suggests that to increase the number of species detected would require approximately doubling sample sizes of individuals. Accumulation curves for sampling over sites appeared to be still increasing approximately linearly, suggesting that sampling new sites should be prioritised to increase species discovery.

Beta diversity analysis showed more values of dissimilarity (more similar=0, more dissimilar=1) for pairs of sites among the ectoparasites matrix (lower diagonal, Table 4.5), than the one for bats (upper diagonal, Table 4.5). For both bats and ectoparasites, Chabacanos was the site with most species in common with all other sites. Bat beta diversity values (Upper diagonal, Table 4.5), showed few pairs of sites that were completely dissimilar, where only Tucson had no species overlap with other sites at all. Site pairs sharing bat species only with each other included Parral – Testera, and Ures – San Basilio. Bat beta diversity for sites with at least one shared species ranged between 0.08 for the most similar (Jolla – San Fernando), to 0.85 for the least similar (San Basilio – Primavera). For ectoparasites beta diversity, the value of the most similar site pair was 0.33 (San Fernando – Rosarito, Lower diagonal, Table 4.5), and for the least similar pair was 0.86 (San Basilio – Chabacanos, Table 4.5). Primavera and Tucson, the southern sites sampled on the Mexican continent, failed to present any similarity on ectoparasite diversity with other sites from this study.

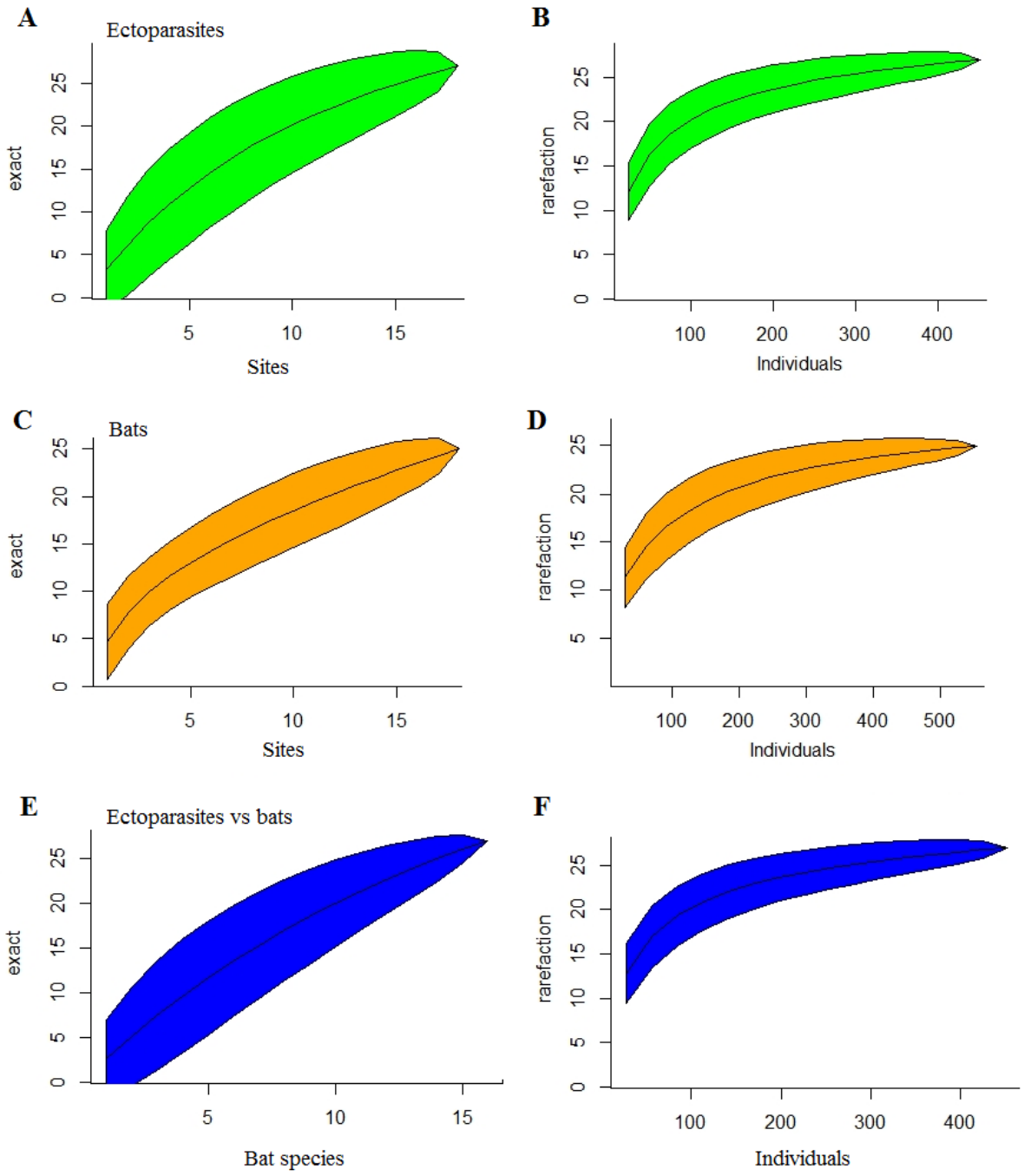


Figure 4.5. Species accumulation curves of ectoparasites (green) and bats (orange) per sites, and ectoparasites per bat species (blue). Analysis per sites based on the exact method (A, C) and bat species (E); and by individuals using the rarefaction method (B, D and F).

Table 4.5. Pairwise beta diversity (Sørensen index of dissimilarity) showing the values calculated for bats (upper diagonal) and for ectoparasites (lower diagonal) against sites of sampling. Values different from 1 are highlighted on green for a better appreciation. The minimum and maximum value of each matrix are in bold. Names of sites are abbreviated in top row.

SITES	Chaba	Ense	Faro	Jolla	LaPaz	Meli	Mosq	Parra	Poza	Prima	Rosa	SanBa	SanFe	SanIg	Teso	Teste	Tucs	Ures
Chabacanos		0.56	0.33	0.27	0.71	0.20	0.23	1.00	0.25	0.60	0.40	0.38	0.33	0.50	0.50	0.45	1.00	1.00
Ensenada	0.75		0.50	0.60	0.67	0.78	0.33	1.00	0.71	1.00	0.56	0.67	0.45	0.64	0.71	0.80	1.00	1.00
Faro	0.75	1.00		0.20	1.00	0.56	0.33	1.00	0.71	0.56	0.11	0.67	0.27	0.64	0.71	0.80	1.00	1.00
Jolla	0.50	0.67	1.00		1.00	0.45	0.29	1.00	0.56	0.64	0.27	0.57	**0.08**	0.54	0.56	0.67	1.00	1.00
La Paz	1.00	1.00	1.00	1.00		0.71	0.80	1.00	0.60	1.00	1.00	0.80	1.00	0.78	0.60	0.50	1.00	1.00
Meling	0.40	1.00	1.00	0.75	1.00		0.38	1.00	0.25	0.60	0.60	0.54	0.50	0.50	0.75	0.45	1.00	1.00
Mosqueda	0.69	1.00	0.71	1.00	1.00	1.00		1.00	0.45	0.69	0.38	0.38	0.20	0.33	0.64	0.43	1.00	1.00
Parral	1.00	1.00	1.00	1.00	1.00	1.00	1.00		1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.75	1.00	1.00
Pocitas	0.75	1.00	1.00	0.67	1.00	0.50	1.00	1.00		1.00	1.00	0.64	0.60	0.40	0.67	0.33	1.00	1.00
Primavera	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		1.00	**0.85**	0.67	0.83	1.00	0.82	1.00	1.00
Rosarito	0.78	1.00	1.00	1.00	1.00	1.00	0.75	1.00	1.00	1.00		0.69	0.33	0.67	0.75	0.82	1.00	1.00
San Basilio	**0.86**	1.00	0.75	1.00	1.00	1.00	0.54	1.00	1.00	1.00	1.00		0.60	0.60	0.64	0.57	1.00	0.80
San Fernando	0.75	1.00	1.00	1.00	1.00	1.00	0.71	1.00	1.00	1.00	**0.33**	1.00		0.57	0.60	0.69	1.00	1.00
San Ignacio	0.43	0.75	1.00	0.50	1.00	0.60	0.85	1.00	0.75	1.00	1.00	0.71	1.00		0.80	0.38	1.00	1.00
Tesos	0.80	1.00	1.00	0.75	0.60	0.67	1.00	1.00	0.50	1.00	0.60	1.00	1.00	0.80		0.56	1.00	1.00
Testera	0.83	1.00	1.00	0.80	0.43	0.75	1.00	0.67	0.67	1.00	1.00	0.83	1.00	0.67	0.50		1.00	1.00
Tucson	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00		1.00
Ures	1.00	1.00	1.00	0.67	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	

Summary statistics of ectoparasite and bat richness and diversity tested by region are shown in Table 4.6. Overall, total numbers of nights sampled and number of bat and ectoparasite species sampled across three fieldwork seasons were relatively even for mid and south peninsula. The northern region hold the highest number of nights (*n* = 19) and bats species (*n* = 9) sampled. In contrast, mid region had fewer nights (*n* = 14) and bat species sampled (*n* = 8), but highest number of ectoparasites species (*n* = 14), confirmed by the highest diversity (H and S) and rarefaction value (6.012) for ectoparasites (Table 4.6). Bat richness and diversity were even across the peninsular landscape, showing the lowest rarefaction values in the south. Despite having only two nights sampled in the southern continental sites (Table 4.4.), this region showed to be highly diverse for both bat and ectoparasites richness, diversity and rarefaction values (Table 4.6).

Table 4.6. Summary richness and diversity stats for ectoparasites lineages and bats species by region. Abbreviations: Number of nights (NN), richness (S), diversity indexes (H, Shannon-Wiener, and D, Simpson's diversity 1-D), and rarefaction analysis (Rar).

Regions		Ectoparasites species				Bats species			
	NN	S	H	D	Rar	S	H	D	Rar
North	19	13	1.902	0.804	5.086	8	1.829	0.823	5.098
Mid	14	14	2.204	0.858	6.012	8	1.83	0.814	5.101
South	15	7	1.669	0.771	4.866	8	1.202	0.515	3.533
Continent Mid	2	1	0.000	0.000	1.000	2	0.211	0.102	1.473
Continent South	2	5	0.926	0.466	3.034	5	1.369	0.683	4.516

Ectoparasites from sites in mid-peninsula (Lower diagonal, Table 4.5) had the lowest beta diversity values among each other, occasionally also among sites in the north. Overall bat beta diversity showed a wider range of diversity values compared to the ectoparasite ranges (A, Fig. 4.6), with bats presenting a mean of 0.71, and ectoparasites a mean of 0.91, reflecting the lack of similarity over pairs of sites among the ectoparasites ranges. Ectoparasites beta diversity analysis included only the peninsular regions, given that there

were only one and two sites per continental region, therefore not suitable for a beta diversity analysis. A higher range of variation of beta diversity was shown among sites in the north and south, compared to mid-peninsula, which also presented the highest mean value among peninsular regions (B, Mid peninsular region, Fig. 4.6), showing poor similarity among sites.

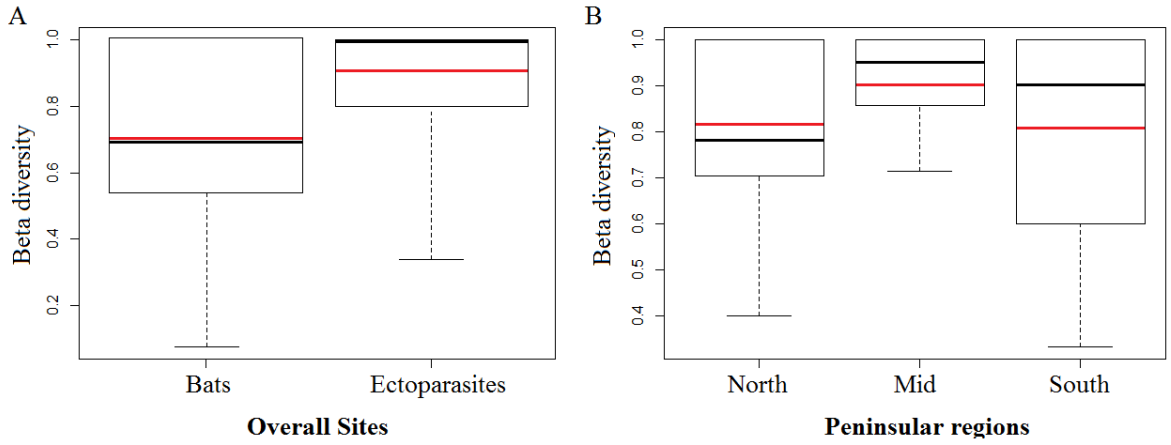


Figure 4.6. Boxplot showing summary stats for beta diversity of bats and ectoparasites among sites (A), and for ectoparasites among northern, middle and southern regions of Baja (B). The mid and south continental sites were not included. Mean value is shown with a red line. Minimum and maximum values are shown with whiskers. Quantiles are shown with a box, and median value denoted with a black ticker line.

4.4.3. Host associations among bat bugs, flies and ticks

In general, most of the host-parasites associations occurred between one parasite and one host, or one parasite and two hosts (Fig. 4.7). There were only three ectoparasite lineages that shared more hosts species: NNycte1 with three, and NNycte2 and Tick 5 lineages with four bat species hosts (Table 4.3). From the host's point of view, the bat *Antrozous pallidus* was the species with more interspecific associations among ectoparasite lineages (n = 9), followed by *Myotis californicus* (n = 7) and *M. yumanensis* (n = 6). *A. pallidus* was the most frequently sampled bat (n = 135 records), followed by *Parastrellus hesperus* (n = 87) (Fig.

4.7). *Cimex* 4 (almost restricted to *P. hesperus*) was the most frequently sampled ectoparasite (n = 84), followed by the nycteribiid flies from the lineage NBasilia2b (n = 61), and then for the soft tick lineage *Carios kelleyi* (n = 42).

Analysis among families showed a strong association among fruit bats (family Phyllostomidae), and winged flies (family Streblidae), where these flies were parasitizing only phyllostomid bats, showing a high co-specificity. The only bat belonging to a different family that presented the only other record of a streblid fly lineage (SParatrichobius1), was *Mormoops megallophylla*, (Mormoopidae), which is an insectivore as with other vespertilionids (Fig. 4.7). The family Vespertillionidae, host the rest of the lineages identified in this study, ranging from the *Eptesicus fuscus* presenting only a single record of the cosmopolitan Tick 5 lineage, to *A. pallidus*, which was parasitized by most of the bug, tick and wingless fly lineages. *Myotis* bats were also parasitized by most of the ectoparasite lineages, where Tick 6 was exclusive to *M. peninsularis*.

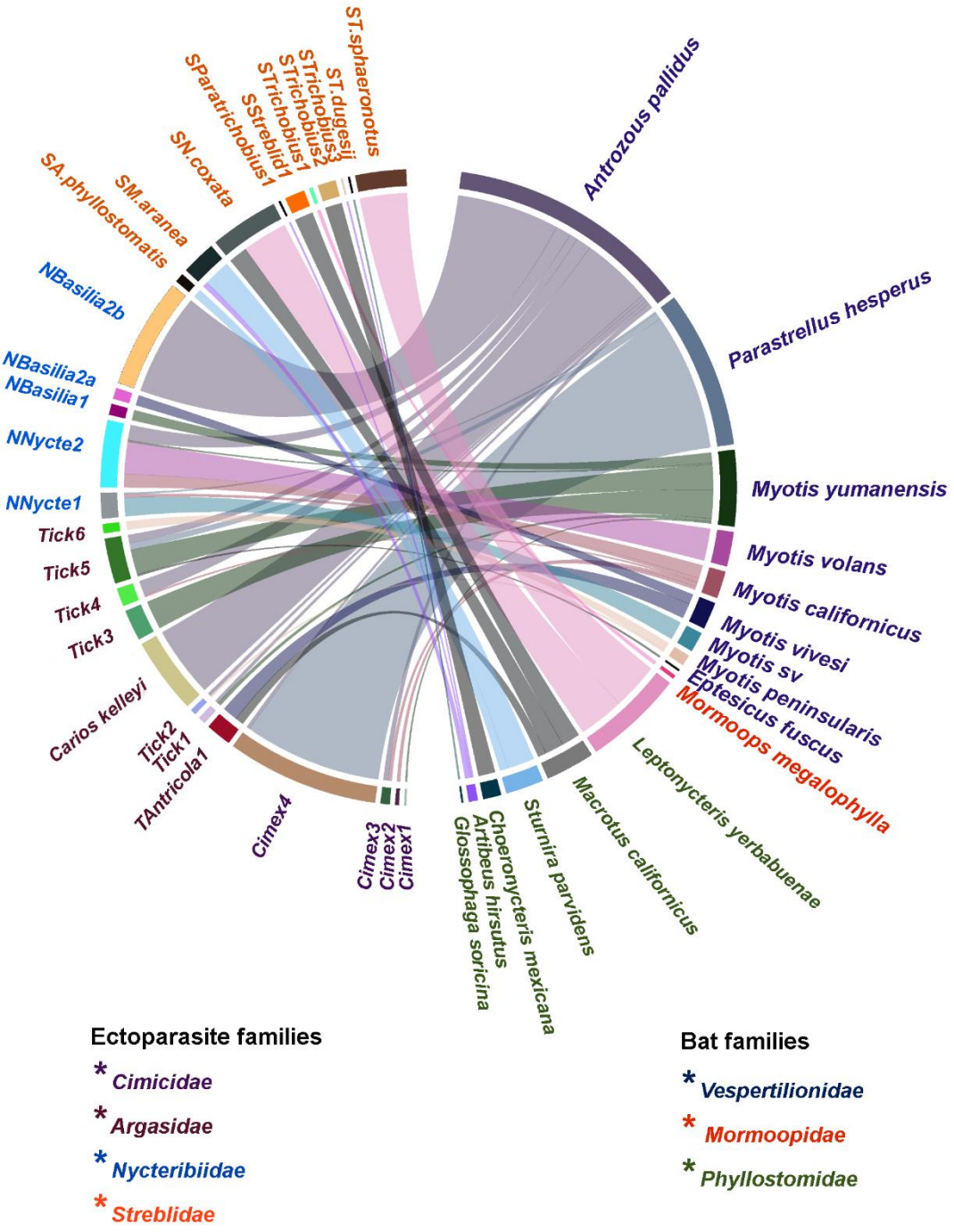


Figure 4.7. Circle plot describing ectoparasite-host relationships and families of each lineage. Bats (right half) and ectoparasites (left half) families are listed clockwise, denoted by each label coloured according to its family. Links to ectoparasite lineages are coloured as each bat species stripes. Length of each stripe is proportional to abundance.

4.4.4. Spatial associations of bats and ectoparasites richness and diversity

For model evaluations, only a small subset of explanatory variables fitted singularly returned significant results. These included association between ectoparasite and bat species richness, bat species richness and bat diversity with region, and bat diversity with mean maximum annual temperature (Table 4.6). Ectoparasite richness was significantly associated with bat richness (Coeff. = 0.82, $P < 0.001$). In the Tukey tests for comparison of richness and diversity between regions, there was only a significant difference in bat diversity (H) among south and north regions (Diff = -0.62, 95%CL = -1.19-0.04, $P = 0.03$).

Table 4.7. Linear model (LM) tests showing significant effects from environmental and spatial variables over Bat Richness and Bat Diversity (Shannon-Wiener index, H). Abbreviations: S.E., standard error; Pr (> |*t*|), probability value according to *t*; AIC, Akaike Information Criterion.

LM model call: Ectoparasite richness ~ Bat richness					
	Coeff.	**S. E.**	***t* value**	**Pr (> |*t*|)**	**Model significance**
Intercept	-0.6235	1.0169	-0.613	0.5484	Residual Std. Error = 1.7 on 16 D.F., Multiple R^2 =0.51, adjusted R^2 = 0.48, F-stats=16.93 on 1 and 16 D.F., P = 0.0008*. AIC = 74.05.
Bat Richness	0.8241	0.2003	4.114	0.0008*	
LM model call: Bat richness ~ Region					
	Coeff.	**S. E.**	***t* value**	**Pr (> |*t*|)**	**Model significance**
Intercept	5.5	0.8917	6.168	1.8E-05*	Residual Std. Error = 1.78 on 15 D.F., Multiple R^2 =0.34, adjusted R^2 = 0.25, F-stats=3.82 on 2 and 15 D.F., P = 0.04*. AIC = 76.63.
North	0.3333	1.1512	0.29	0.7761	
South	-2.125	1.0921	-1.946	0.0707	
LM model call: Bat diversity (H) ~ Region					
	Coeff.	**S. E.**	***t* value**	**Pr (> |*t*|)**	**Model significance**
Intercept	1.2313	0.2047	6.014	2.3E-05*	Residual Std. Error = 0.41 on 15 D.F., Multiple R^2 =0.35, adjusted R^2 = 0.26, F-stats = 4.05, 2 and 15 D.F., P = 0.039*. AIC = 23.66.
North	0.2209	0.2643	0.836	0.416	
South	-0.396	0.2508	-1.579	0.135	
LM model call: Bat diversity (H) ~ Precipitation warmest qrt. + Precipitation driest qrt.					
	Coeff.	**S. E.**	***t* value**	**Pr (> |*t*|)**	**Model significance**
Intercept	1.4117	0.1602	8.810	2.5E-07*	Residual Std. Error = 0.39 on 15 D.F., Multiple R^2 =0.38, Adjusted R^2 = 0.30, F-stats = 4.67, 2 and 15 D.F., P = 0.026*. AIC = 22.71 (Precip_warmest_qrt P = 0.007*, AIC = 20.98; Precip_driest_qrt P = 0.69, AIC = 29.24).
Precip_warmest_qrt	-0.0022	0.0007	-3.016	0.0086*	
Precip_driest_qrt	0.0082	0.0172	0.480	0.6381	

*** Significant $P < 0.05$ values.**

Multiple regression with distance matrices (MRM) of bat and ectoparasite beta diversity retrieved significant results correlated to ecological and environmental variables. For the bat beta diversity Maximum MRM model, only three variables were significantly correlated ($P < 0.05$, $R^2 = 0.35$, $P = 0.0002$; $F = 5.73$, $P = 0.0002$): Mean maximum temperature was positively correlated to bats beta diversity (Coeff. = 4.558734×10^{-2}). Mean temperature of the driest quarter (Coeff. = -3.866962×10^{-2}), and precipitation of the coldest quarter (Coeff. = -2.625548×10^{-3}) were negatively correlated. For the ectoparasite beta diversity Maximum MRM model, three variables were significantly correlated ($P < 0.05$, $R^2 = 0.34$, $P = 0.0003$; $F = 5.731$, $P = 0.0002$): a positive correlation was detected for bat beta diversity (Coeff. = 3.636171×10^{-1}), and annual precipitation (Coeff. = 1.031965×10^{-3}); and a negative correlation for precipitation of the wettest quarter (Coeff. = -1.946453×10^{-3}). Precipitation of the warmest quarter (Coeff. = 6.543056×10^{-4}, $P = 0.058$) was also chosen to be included, given that it had the lowest *P*-value among the rest of non-significant variables.

Minimal MRM model for bat beta diversity similarity correlation are shown in Table 4.8. For bat beta diversity it was shown that there was high similarity of bat communities correlated with the mean maximum temperature and the precipitation of the wettest quarter. These patterns are presented in most sites of the peninsula over late summer. There were two variables presenting a weak negative correlation: mean temperature of the driest quarter and mean temperature of the coldest quarter (Table 4.8). These two variables were related mostly to winter season, when bats are less abundant.

For the ectoparasite beta diversity, there was a high positive correlation to beta bat diversity, suggesting that bat-host effects were the main determinants of ectoparasite beta

diversity. Following the hosts-effects, there were only weak effects by the significant correlated variables (Table 4.8), giving more evidence of relatedness of ectoparasite communities depending mostly from host distribution. Annual precipitation and precipitation of the warmest quarter were also positive correlated with ectoparasites beta diversity.

Table 4.8. Minimal models results from the multiple regression with distance matrices (MRM), of bats beta diversity and ectoparasite beta diversity across environmental and ecological variables.

Minimal MRM Bat Beta Diversity			**Minimal MRM Ectoparasite Beta Diversity**		
Model: Bat.beta ~ Mean_temp_ driest_qrt+Precip_wetest_qrt.dist+Precip_coldest_qrt+ Mean_temp_Max			Model: Ectoparasite.beta ~ beta.bat+Annual_precip+Precip_warmest_qrtt+Pre cip_wettest_qrt		
Variables	**Coeff.**	***P*-value**	**Variables**	**Coeff.**	***P*-value**
Intercept	0.647417	0.0812	Intercept	0.6449	1
Mean_temp_driest_qrt	-0.040218	0.0137	Bat_Beta_diversity	0.319617	0.00003
Precip_wettest_qrt	0.000784	0.0024	Annual_precip	0.000916	0.0067
Precip_coldest_qrt	-0.002826	0.0187	Precip_warmest_qrt	0.000689	0.0126
Mean_temp_Max	0.053160	0.0041	Precip_wettest_qrt	-0.00166	0.0064
Model statistics of significance			**Model statistics of significance**		
$R^2 = 0.44$, $P = 0.0009$; F = 28.84, $P = 0.0009$			$R^2 = 0.29$, $P = 0.00001$; F = 15.04, $P = 0.00001$		

4.5. Discussion

The majority of ectoparasites found were mites, the group that infested the most hosts, and hold the highest values of abundance, intensity and prevalence from the overall ectoparasite captures (Table 4.2). However, mites and non-identified ectoparasites collected in this study have not been analysed yet given the size of the data set (approx. 1600 mites), and difficulty of phenotypic identification. In the case of ticks, only potential adults were analysed that were large enough to be safely extracted from the bats since larva and nymphs posed an infection risk if pieces of the body were left attached to the bat. Host prevalence and

specificity can differ among life stages, where immature ticks tend to be more generalists than ticks from the adult stage (Nava and Guglielmone, 2013; Esser *et al.*, 2016). Therefore, values of diversity and hosts-ectoparasites associations have to be analysed including different life stages, which in the case of ticks, it is relevant to better detect patterns of infection and potential risks of spread of pathogens. This highlights the importance of doing more studies specifically targeting bat ectoparasites, ensuring efficiency considering biological and ecological aspects of each specific ectoparasite group.

This study was designed to enhance capture of bats on a region with mostly unknown roosting sites. Ectoparasite captures were mostly opportunistic and targeting places with water, with varied effort in sampling nights. Groups like bat bugs, which are theoretically more associated with roosting sites (Reinhardt and Siva-Jothy, 2007), are usually biased when sampling only from mist-netting captured bats. A basic exploration of diversity and patterns of ectoparasites in the peninsula have been discovered through this study, pointing out the potential hidden ectoparasite richness awaiting further surveys. A more systematic sampling needs to be done now that the first stage has been successfully conducted, in order to retrieve a better view of the ectoparasite landscape.

Overall, ectoparasite richness in this study was correlated with bat richness. Mid-peninsula was the region that showed the highest level of richness, diversity and beta diversity for both bats and ectoparasites, followed closely by the north region. As a general pattern, there is strong covariance among hosts species richness and parasite species richness, following the species-area relationship (Lomolino, 2000; Poulin, 2014), where larger areas will contain more species. If there is high richness of hosts, there will be high richness of

ectoparasites, as shown in the present study. In the peninsula, the most differentiated regions in relation with bat diversity were north and south, converging with differences in vegetation and patterns of temperature and precipitation. There was an almost significant comparison between bat richness and south-north regions, suggesting that a latitudinal pattern might be influencing bat richness, but more power in sampling is needed to detect any correlation. The southern region is constituted mainly for Tropical Deciduous Forest with rains over late summer-autumn, while the northern region is composed by Heathland and Pine and Oak Forest, which are characterized by lower temperatures and precipitation over winter, and dry and warm summers, with casual storms caused by cyclones (González-Abraham, Garcillán and Ezcurra, 2010). Bat-ectoparasite community similarities presented a weak but significant influence from environmental factors, explained by seasonality, where maximum temperatures and rainy seasons in Baja both converge in late-summer (González-Abraham, Garcillán and Ezcurra, 2010).

Both LM and MRM models described the significant correlation between precipitation and alpha/beta diversity of bats and ectoparasites over the peninsula. This was corroborated by the presence of bat communities over mid and south peninsula overlapping with the highest temperature of the year, as well as the highest rate of precipitation (González-Abraham, Garcillán and Ezcurra, 2010). Arthropods will be more abundant when raining, therefore summer is when peninsular bats are most abundant, corresponding to increased food availability and increased feeding opportunities. This in turn overlaps with birth and development of pups, which occurs until late-summer and early autumn, when juveniles start flying (Simpson, 1993; Álvarez-Castañeda and Bogan, 1998; Braun *et al.*, 2015). Massive gatherings of pregnant females, mothers and pups, as well as high rates of

humidity, are important factors contributing to ectoparasites infestation, especially for those individuals spending most of the time in roosting sites (Salinas-Ramos *et al.*, 2018). In this study, a negative correlation was found with precipitation during the wettest quarter and higher rates of bat and ectoparasite diversity, corroborating the previous results as it overlaps with the warmest season, and shows more similarities among bat-ectoparasite communities that are distributed in Baja during summer and late-summer (González-Abraham, Garcillán and Ezcurra, 2010), when all these factors occurs at overlapping times.

The mid region of the peninsula was characterised by sites that had important water sources, surrounded by arid ecosystems, and isolated from other water resources in near proximity. High levels of bat activity and diversity have been associated with the presence of water resources in desert systems (Razgour, Persey, *et al.*, 2018), which are used by bats for both drinking and hunting. The mid-peninsula region presented few available water bodies in between large arid areas, it was expected for bats to converge in those water resources, depending on whichever was closer to their regular foraging ranges. Most North American desert bats are insectivorous (Patrick and Stevens, 2014), presenting different diet preferences depending on each bat species biology (e.g. *Myotis* bat size will define the hardness of the consumed arthropod, Segura-Trujillo *et al*., 2016, 2018). This might be reflected in resource partitioning, fulfilled by the high diversity of arthropods over North American deserts (Bang *et al.*, 2012), not excluding intra and interspecific competition for prey resources. However, there is no information of visitation patterns and how are water resources been used by bat communities over Baja. Specific characteristics and isolation degrees of sites sampled were out of the scope of this study. San Basilio and San Ignacio sites in mid-peninsula were important hotspots for bat diversity and gene flow over Baja (see results Chapter 2),

highlighting the importance of further studies evaluating resource use, composition and connectivity. Given the implications for bat's diversity and population structure within this region, more sampling effort should be focused in mid-peninsula, contributing to understand patterns of bat-ectoparasite dispersal and potential gene flow among ectoparasites from nearby roosting sites.

Attempts of estimating parasite biodiversity are currently limited by insufficient knowledge (Poulin, 2014), where wildlife ectoparasites have been neglected. The most important limiting factor for species classification in this study was the lack of morphological and molecular background for wildlife ectoparasites, especially for the north-western part of Mexico. Studies of parasites in Mexico have faced the same problem, where undescribed species from previous works and lack of fully revised and inclusive descriptions, have left many other records without any certainty classification (Ramírez-Martínez *et al.*, 2016). This has resulted in a complex phylogenies where multiple species are spread among different paraphyletic clades. In addition, taxonomic revisions of current species and genera needs to be done, as some have been published as duplicates of existence records (Ramírez-Martínez *et al.*, 2016), or are mixed up between species synonymies (Estrada-Pena *et al.*, 2010). In order to build up a reliable molecular database of ectoparasite diversity in this region, input of experts in morphology needs to be taken into account to couple it with molecular identification, biology of ectoparasites and hosts, plus ecological constrains from hosts.

This study works as evidence of the need to increase ectoparasite research over Mexico. Currently, there is an uprising interest for ectoparasite research, however, mostly focused in bat flies. This is reflected on the amount of recent publications related to this

group in Mexico (Cuxim-Koyoc *et al.*, 2015; Trujillo-Pahua and Ibáñez-Bernal, 2018; Saldaña-Vázquez *et al.*, 2019), that includes several records of undescribed fly species, confirming that it is expected a large rate of species discovery, in accordance with our results. Rarefaction analysis indicated that numbers of bats and ectoparasite species will increase with further sampling (Fig. 4.5), therefore, expanding sampling over more sites should be prioritise to get a better approach of Mexican bat and ectoparasite diversity.

In Baja, most of the host-parasites associations occurred between one parasite and one host, or one parasite and two hosts (MI values, Table 4.2). Streblid flies showed the highest values of MI (e.g. *Trichobius sp*. and *T. sphaeronotus*, MI = 10 and 12, respectively), but those were the only extreme cases of infestation in this study. These results are similar to those found in other ectoparasite community structure studies including combinations of bat bugs, flies and ticks (Dick *et al.*, 2003; Czenze and Broders, 2011; Bezerra, de Vasconcelos and Bocchiglieri, 2016; Burazerović *et al.*, 2018). However, mean intensity will vary accordingly to bat sex, reproductive stage, season, and geographical variation (Morand, Krasnov and Poulin, 2006; Poulin, Krasnov and Mouillot, 2011; Krasnov *et al.*, 2012; Poulin, 2014), which are out of the scope of this analysis. Overall, there were only three ectoparasite lineages that shared more hosts species: flies of NNycte1 with three, and the fly NNycte2 and Tick 5 lineages with four bat species hosts (Table 4.3).

From the host's point of view, the bat *Antrozous pallidus* was the species with more interspecific associations among ectoparasite lineages (n = 9), followed by *Myotis californicus* (n = 7) and *M. yumanensis* (n = 6, Fig. 4.7). In terms of ectoparasite abundance within hosts, *A. pallidus* also presented the highest abundance overall (n = 135) followed by

Parastrellus hesperus (*n* = 87), which was composed mainly *Cimex* bat bugs (Fig. 4.7). Results from Chapter 3 have indicated that hosts-specific and generalist ectoparasites lineages were potentially found across the whole study, where haplotype analysis showed patterns of peninsular dispersal among *A. pallidus.* The reason of the detection of multiple associations with *A. pallidus* is mainly the cosmopolitan presence and large sample size across the peninsula. This suggest that a better understanding of the ectoparasite systems in Baja will be achieved when more territory and more bats specimens are sampled, providing stronger evidence of other patterns detected in this study.

Overall, bats from the family Vespertilionidae were parasitized by at least one member of each one of the ectoparasite families, with the exception of flies from the family Streblidae (Fig. 4.7). Interestingly, all bats from family Phyllostomidae were exclusively parasitized by streblid flies, with the exception of *Macrotus californicus*, that shared cave roosts with other *Myotis* species and showed interaction with Antricola 1 tick lineage. Ectoparasite-host patterns of associations at family level agrees with patterns found at species level (see Chapter 3), where ectoparasite diversity seemed to be shaped by bat phylogenetic proximity and not driven by environmental or climatic influence (Krasnov *et al.*, 2012). Ectoparasite community structure is mostly influenced by host's relationships in this study, however, different scales of analysis may show different patterns of community structure.

Model testing of environmental variables showed evidence for weak spatial effects in bats, where latitudinal patterns were detected for mid-peninsula, and seasonal patterns where detected by the climatic analysis. This suggest a power issue with sampling and number of sites, probably affecting the ability to detect finer scale latitudinal effects. It has been shown

that in bats communities distributed in North American deserts, community assemblages showed to be phylogenetically clustered, suggesting a specific response to desert conditions in similar ways (Patrick and Stevens, 2014). Most of the bats within this study fall into the same type of biome, the Sonoran desert (González-Abraham, Garcillán and Ezcurra, 2010; Patrick and Stevens, 2014), suggesting that environmental and geographic variables may be shaping both bat and ectoparasites distributions. This highlights the importance of focusing more efforts in understanding the factors that shape bat distribution and connectivity inside Baja and between continental lands, which will elucidate different levels of bat-ectoparasite community structure.

Investigating bat ectoparasite community structure has shown to be very important for understanding each other's patterns of distribution, especially relevant when discovering patterns of dispersal of bats with cryptic distributions and different scales of dispersal (Speer *et al.*, 2019). Intra and inter-specific interactions between wildlife ectoparasites (Hellard *et al.*, 2015), and potential interactions between ectoparasites sharing multiple hosts have high implications on the study of host's health, disease ecology, epidemiology and ectoparasite conservation (Spencer and Zuk, 2016). Bats and bat ectoparasites can potentially be vectors of zoonotic importance, where spatial and temporal distribution of bats may strongly affect how pathogens are disperse and transmitted to other organisms (e.g.: potential introduction of new ectoparasites carried by drifts on migration routes from bats due to climate change, land use, roosting sites, etc.). Therefore, identifying the interaction mechanisms shaping bat and ectoparasites diversity is vital for disease management (Hellard *et al.*, 2015).

Balanced communities of bats and pathogens should maintain a healthy environment, contributing to a stronger bat immune system (Spencer and Zuk, 2016), and preventing from zoonotic spill overs due to anthropogenic causes. Bat-ectoparasite systems should be studied in conjunction, and not treat them as separate entities, which is important for ectoparasite conservation. In this study, ectoparasites restricted to single bat population hosts were identified (i.e. putative endemic *Myotis peninsularis* and Tick 6 lineage, populations isolated in south peninsula), which may represent an important source of information regarding this cryptic bat population, that not only lacks information regarding its biology and movement patterns, but also its health status. At the first stage, this study represents an important contribution in the identification of ectoparasite diversity, patterns of bat dispersal and connectivity, and how environmental and latitudinal gradients may influence bat diversity over North America.

Chapter 5: General Discussion

The mechanisms by which genetic differentiation develops within and among species, and their relationship with the environment is an important topic for conservation and evolutionary biology. One of the fundamental questions is to what extent gene flow limits ecological differentiation and speciation. New sequencing technologies are now being successfully applied in ecological speciation research, to evaluate how patterns of gene flow, environmental factors and phenotypic traits contribute to ecological differentiation, and ultimately speciation. However, exactly how rates of gene flow, selection and vicariance interact with the environment to influence ecological differentiation remains to be fully understood. This thesis investigated the genetic population structure and species boundaries of *Myotis* bats in the Baja California peninsula, comparing phenotype assignments with mitochondrial cytochrome *b* and ddRAD SNP derived markers. The genetic diversity and community structure of bat ectoparasites in Baja peninsula and continental sites was also evaluated, using a mitochondrial cytochrome oxidase sub-unit I marker, and the ribosomal 18S nuclear marker.

In Chapter 2, I found signs of hybridisation and introgression among the four *Myotis* species included in this study. There was evidence for population structure in *Myotis californicus,* suggesting a local and a long-distance route of dispersal. In contrast, *M. yumanensis* showed poor nuclear structure, but strong mitochondrial differentiation, suggesting long male dispersal and female philopatry. Using phylogenetic analysis, *M. peninsularis* cytochrome *b* sequences were clustered among *M. velifer* references. However, *M. peninsularis* had exclusive haplotypes not shared by *M. velifer*. Population structure analyses of ddRAD SNP data, showed that some *Myotis* individuals from the northern

peninsula and continent, outside *M. peninsularis'* know range, had nuclear ancestry which potentially derived from continental *M. velifer* and these formed a cluster with *M. peninsularis*. However, not having a larger sample of unambiguous continental *M. velifer* is a limitation for confirming genotypic clustering. With the evidence gathered so far, it is likely that *M. peninsularis* represents a potentially recently established subpopulation of *M. velifer*, which is at least partially demographically isolated from continental *M. velifer*.

We identified two potentially new cryptic *Myotis* lineages: a mainland population of bats with morphological features which did not match currently recorded species, designated here as *M. sv*; and a population of bats from Baja which morphology has been traditionally assigned to *M. volans*. In both cases, each formed a monophyletic clade with phylogenetic and haplotype data, plus genetic divergence of 6% and 11% with their respective closest reference in GenBank (Bradley and Baker, 2001; Stadelmann *et al.*, 2007). Consistent with this and the ddRAD SNP dataset, *M. sv* is confirmed as a distinct cluster, supporting its status as different species. Further genetic sampling and morphological studies should be prioritised to full evaluate its taxonomic status relative to other species.

In Chapter 3, using a phylogenetic approach, six new records of known species and 21 novel genetic lineages of bat ectoparasite were found in Baja. Of the new lineages some may be ectoparasites that have been sampled before but without any genetic assessments, but mostly are potentially representing new species. The ectoparasite lineages in this study lack detailed morphological assessments, which has been an important limiting factor. Collaboration with ectoparasite taxonomists will be important to fully evaluate their species status. Studies of ectoparasites communities in poorly explored areas have discovered new

lineages of ectoparasites (Czenze and Broders, 2011; Muñoz-Leal *et al*., 2016; Burazerović *et al.*, 2018), therefore, it was expected to find multiple novel lineages within this study.

In Chapter 4, I found evidence for strong host-ectoparasite associations, where ectoparasite community structure was mainly determined by host phylogeny, host community composition, host species richness and other factors influencing host distribution. With linear models, I found evidence of a potential effects of latitudinal, environmental and climatic variables influencing bat host diversity. Ectoparasite beta diversity was influenced by host community, with some weak effects from environmental variables and geographic distances. Lack of sampling power might be limiting the detection of other covarying factors influencing the diversity and distribution of bats in the peninsula. Understanding bat ectoparasite diversity and disease ecology has important implications for the management and prevention of potential future spill-overs of some diseases of zoonotic importance (Anthony *et al.*, 2013; Wilder, Kunz and Sorenson, 2015; Hayman, 2016).

5.1. Genetic diversity, species boundaries and the potential of gene flow

Myotis bats exhibit convergent morphological differentiation, posing diverse nuclear, mitochondrial and biogeographic histories (Stadelmann *et al.*, 2007; Larsen *et al.*, 2012; Platt *et al.*, 2018). This, along with its rapid diversification and their high rate of cryptic speciation has represented a challenge for understanding their taxonomic relationships (Stadelmann *et al.*, 2007; Platt *et al.*, 2018). The majority of current *Myotis* phylogenies are based on traditional mitochondrial and nuclear markers, usually limited by previous morphological misidentifications. Stadelmann *et al.*, (2007) has found evidence of potential misidentifications/mislabelling of voucher specimens (e.g. *Myotis latirostris* in Stadelmann *et*

al., 2007), suggesting that review of basal morphological identifications of *Myotis* should be done. In the present study, the use of high-throughput sequencing produced finer scale results than previous methods, which in combination, can achieve a better understanding of *Myotis* population structure and radiation. However, the lack of molecular references of *Myotis* from the north-western region of Mexico, and the accuracy of the annotation of existing reference comprises a limiting factor, where this study has contributed for generating more information for this and further investigations.

The biological species concept, the widest used definition, define "species" as a group of natural populations that interbreed and are reproductively isolated from other populations (Mayr, 1947; de Queiroz, 2005; Butlin *et al.*, 2012). This vision has been increasingly changed by evidence from many studies showing introgression and hybridisation between populations of different species (Seehausen *et al.*, 2008, 2014; Harrison and Larson, 2014; Poelstra *et al.*, 2014; Supple *et al.*, 2015; Chattopadhyay *et al.*, 2016; Marques *et al.*, 2016; Chhatre *et al.*, 2018; Lavretsky, Janzen and McCracken, 2019; Arteaga, Bello-Bedoy and Gasca-Pineda, 2020; McGee *et al.*, 2020). From recent studies at least 25% of plant species and 10% of vertebrates show evidence of hybridisation and introgression with other species (Mallet, 2008; Gourbière and Mallet, 2010; Bogdanowicz, Piksa and Tereba, 2012). Increasingly, the idea of a species as a fixed discrete entity is changing towards a more dynamic scenario, where species boundaries are more permeable than it was thought in the past (Mallet, 2007; Gourbière and Mallet, 2010; Seehausen *et al.*, 2014; McGee *et al.*, 2020).

According to Bogdanowicz, Piksa and Tereba (2012b), introgression and hybridisations in bats seems to be present in approximately 1.1% of 1260 bats species

evaluated in 2012, a number that has increased by using higher resolution methods, along with the number of bats species discovered so far (more than 1,400, Jebb *et al.*, 2020). For example, Chattopadhyay *et al.* (2016), found signs of hybridisation in a new cryptic lineage of *Cynopterus* bats using ddRAD techniques. Hybridisation and mtDNA introgression has been reported across different bat families worldwide (Berthier, Excoffier and Ruedi, 2006; Mao *et al.*, 2010; Centeno-Cuadros *et al.*, 2020). In *Myotis* bats, introgressive hybridisation, incomplete lineage sorting and phylogenetic error are factors that have driven conflicting estimations of taxonomic relationships (Berthier, Excoffier and Ruedi, 2006; Platt *et al.*, 2018). Results from this study are showing that introgression and hybridisation occurs among *Myotis* bats in Baja, occasionally involving more than two bat species, illustrating how complex this system is. Similar results have been found in sympatric colonies of vespertilionid bats from the Iberian peninsula, where no mtDNA introgression was detected, but male-mediated nuclear asymmetric hybridisation was found over the contact zone (Centeno-Cuadros *et al.*, 2020). The evolutionary processes that drive this level of admixture, might be a combination of ecological (e.g. level of sympatry), spatial (e.g. swarming sites/roosting sites used for resting during long-distance migration), and/or behavioural (lack of pre- zygotic barriers derived from perhaps indistinct mating ques, and/or post-zygotic barriers due to absence of genetic incompatibilities in co-adapted interacting genes) factors (Bogdanowicz, Piksa and Tereba, 2012; Seehausen *et al.*, 2014). These process that have promoted such porous species boundaries need to be studied in more depth, as well as the mechanisms that may promote either reproductive isolation, or maintaining some level of genetic and ecological differentiation.

This study detected at least two 'hotspots' of individuals with mixed species ancestry in strategic points in Baja, gathering evidence of continental-peninsular genetic interchange. High availability of water resources in these places represent the perfect habitat for hunting and mating, therefore suggesting that there is potentially swarming sites nearby these places. As mentioned in Chapter 2, swarming sites are crucial places for maintaining a healthy population, not only by promoting gene flow but also by sharing pathogens that have immunological and zoonotic importance (Rivers, Butlin and Altringham, 2005; Dixon, 2011). If long-distance migrating bats are converging over this hotspots/swarming sites, as suggested with population structure of *Myotis* from this study, efforts for increasing research for understanding patterns of dispersal should be implemented.

Hotspots of hybridisation detected here might be part of other sites with equal opportunities in different areas inside their range of migration. Results found here represent a "summer" view of these bats interactions, which might be influenced by admixture with non-sampled species in different areas, at the different times of the year. *Myotis* species over west North America mate in Autumn, with some species potentially over spring too (Warner and Czaplewski, 1984; Simpson, 1993; Braun *et al.*, 2015). Aside from this, there is no other information updating if this is a consistent pattern over different regions/species, nor what happens with resident populations versus migrants. More efforts to update and looking for patterns of reproduction and dispersal are crucial, not only in terms of connectivity and conservation of bats in North America, but also in how pathogens are dispersed within this bats.

5.2. Bat-ectoparasites systems

Parasites are integral parts of ecosystems and fundamental drivers of evolutionary processes and environmental structure (Rigaud, Perrot-Minnot and Brown, 2010; Poulin, 2014; Hellard *et al.*, 2015; Spencer and Zuk, 2016). It has been suggested that relative parasite biodiversity accounts for at least one-third of worldwide species (Guégan, Morand and Poulin, 2004; Poulin and Morand, 2004; Poulin, 2014). Despite not having a real estimate, these organisms represent a very understudied group, hence containing high discovery rates and unknown numbers of cryptic species (Poulin, 2014).

Studies understanding parasites and pathogens of zoonotic importance for agriculture and livestock have a high economic and medical impact, therefore they have been studied with more emphasis (Xu *et al.*, 2003; Gómez and Nichols, 2013; Fiset *et al.*, 2015; Morand, 2015). However, wildlife parasites have been neglected in the past, even though they represent the majority of emergent zoonotic pathogens for humans and other taxa (Gómez and Nichols, 2013). There are several reasons of this lack of ectoparasite wildlife attention, including the difficulty of sampling, the challenging task of quantifying their effects over wildlife hosts, and the lack of taxonomic expertise (Gómez and Nichols, 2013; Poulin, 2014). The present study faced those challenges, especially regarding lack of morphological guides and molecular references resources for bats in the Baja region. To our knowledge, this is the first bat ectoparasite survey conducted in Baja, where there is still undiscovered ectoparasite diversity and also unexplored challenges regarding their hosts-parasite-pathogen associations.

Another vital aspect of studying ectoparasite diversity is the zoonotic, and disease ecology risks. Publications regarding zoonotic disease and anthropogenic land-use change

have almost doubled up in the course of five years (2012 > 18 papers, 2017 > 33 papers, White and Razgour, 2020). In accordance to this, studies of pathogens carried by bat ectoparasites appear to have increased during the last years (Davis *et al.*, 2005; Donaldson *et al.*, 2010; Veikkolainen *et al.*, 2014; Sánchez-Montes *et al.*, 2016; Wilkinson *et al.*, 2016; Raya *et al.*, 2018; Hornok *et al.*, 2019; White and Razgour, 2020). Although that there has been evidence of bat ectoparasites interacting with humans (e.g. human blood has been reported in bat ticks of the genus *Carios* in Iowa, U.S., Gill *et al.*, 2004), the lack of basic ectoparasite and pathogens studies in western Mexico, decreases the opportunity of preventing and managing any potential risk.

Changes in land-use and other anthropogenic disturbances over the arid habitats in Mexico are still low in comparison to more easily accessed areas (Gonzalez-Abraham *et al.*, 2015). However, urbanization and massive developments are increasing over the peninsular coast (A. Ortega, CIBNOR, Pers. Comm.), implying strong impact over the natural landscape. It is highly important to understand the current bat ectoparasite and pathogens diversity, as well as their hosts patterns of dispersal, in order to better understand and modelling potential routes for spill-over, in current and future circumstances.

Bat ectoparasites studies are increasing, however, they are focused on richness and species discovery studies (Dick, 2006; Cuxim-Koyoc *et al.*, 2015; Frank *et al.*, 2015; Bezerra, de Vasconcelos and Bocchiglieri, 2016; Ramírez-Martínez *et al.*, 2016; Trujillo-Pahua and Ibáñez-Bernal, 2018). This is of course of high importance, given the high necessity of increasing ectoparasite biodiversity, particularly the one including multiple tools (i.e. multiple molecular and morphological techniques), for a proper identification and

classification. However, studying patterns of richness and diversity, and how these varies among hosts species and across geographical areas and different ecosystems should be also of high importance (Poulin, 2014).

Studies of bat-ectoparasites community structure involving several groups at the same time are scarce in comparison to single host-ectoparasite studies (Czenze and Broders, 2011; Krasnov *et al.*, 2012; Burazerović *et al.*, 2018; McKee *et al.*, 2019). Bat-ectoparasite community in Baja showed to be a complex system including both host-specific and generalist ectoparasites, as well as single and multiple groups of ectoparasite being carried at the same time by their hosts. The main driver of the ectoparasite composition was linked to each hosts phylogenetic relatedness and distribution, in agreement with other bat ectoparasite studies (Krasnov *et al.*, 2012; McKee *et al.*, 2019). However, weak environmental effects were detected over ectoparasite richness and diversity, implying that ecological and environmental factors can also influence the ectoparasite distribution and competition independently of their hosts. To be able to detect patterns at this level, enough sample size power is needed (Gannon and Willig, 1995). This highlights the importance of increasing studies targeting ectoparasite sampling, including a systematic approach for the accurate discovery of the mechanisms shaping ectoparasite community structure.

Overall, this thesis integrates multiple discoveries of North American biodiversity, which includes potentially two new lineages of *Myotis* bats and 21 lineages of ectoparasites. This investigation also highlights the lack of molecular references, particularly for ectoparasites, and the need for revisiting and updating current mis-annotated sequences uploaded in GenBank. Our results also showed that for both bat and ectoparasite diversity,

more sampling needs to be done to be able to recover more accurate diversity numbers, host-parasite interactions and different scales of dispersal. Ongoing search of taxonomic identities and their ecological interactions are important tasks for understanding how species are currently distributed and how they are dealing with environmental and anthropogenic related challenges.

By generating both bat and ectoparasite genetic data in the same study, we provided important insights about bat dispersal within and outside the peninsula (e.g. haplotype evidence of *Myotis yumanensis* bats moving from Alaska to the north of the peninsula), using different molecular markers. Ectoparasite phylogenetic data confirmed patterns of bat movements in the peninsula (e.g. the case of *Antrozous pallidus* bat and *Basilia* 2b fly lineage). Reciprocally, bat taxonomic identity supported multiple host-specific associations between peninsular bats and their respective ectoparasite lineage, suggesting that host phylogenetic proximity (e.g. bats of the family Phyllostomidae been parasitized by flies from the family Streblidae only), and host distribution were the most important factors determining ectoparasite community structure in Baja. Additionally, this study highlighted that investigating routes of bat-ectoparasite movement are important for understanding the potential for pathogen dispersal. We obtained samples from bats roosting in multiple human-related buildings, which is highly relevant for both conservation of bats and their ectoparasites, as well as to identify vulnerable populations and species of zoonotic importance, providing opportunities to prevent future spill-overs. While each chapter provides self-contained information, they come together to give an integrated view of this hosts-parasite system for Baja.

Locally and globally, there is still misinformation about the importance of bats, not only for a healthy ecosystem but also for human economy and wellbeing. As mentioned before, parasites in general are mostly considered as negative organisms affecting the health of their hosts, and not as an important co-evolutive organism, that has contributed to the host's immunology system and fitness adaptation. By increasing research in hots and their parasites individually, and in the hosts-parasite communities, all as one system, we are contributing to discover and evaluate the mechanisms that shape and maintain ecological interactions. Ultimately, understanding these mechanisms is relevant for many topics worldwide, including medical research, detection and prevention of spill-overs, adaptation to climate change and biodiversity conservation.

5.3. Future work

This study was the first exploration of the genomic basis of patterns of introgression over a complex of *Myotis* in Baja. The next step is to map regions of fixed genomic differentiation among species, which might contain loci contributing to maintain post-zygotic barriers due to genetic incompatibilities. Equally, given the important amount of introgression detected in this study, it would be really interesting to investigate what are the main drivers of preserving "species identities" in some of the pure *Myotis* individuals sampled. It has been shown that small numbers of loci can influence hybrid speciation (Zahn, 2018), or maintain species differentiation despite strong and common introgression (Poelstra *et al.*, 2014).

The case of *Myotis peninsularis/velifer* requires obtaining more individuals and more strategic sites for sampling. The possibility for this system to be under incipient speciation is

outstanding, where more attention need to be put on improving sample size from strategic sites across their ranges. This would provide the opportunity to investigate if there are regions of genomic differentiation acting to maintain or differentiate these continental and peninsular populations from each other (Michel *et al.*, 2010; Poelstra *et al.*, 2014; Malinsky *et al.*, 2015).

Additionally, it will be important to investigate signs of environmental adaptation through F_{ST} analysis (Poelstra *et al.*, 2014; Benestan *et al.*, 2016; Chattopadhyay *et al.*, 2016). Different loci may be diffusing across species boundaries at different rates, where it will depend on their effect on organisms fitness, and their genomic response to different habitats/ecosystems (Seehausen *et al.*, 2014; McGee *et al.*, 2020). Bats like *Myotis californicus* and *M. yumanensis*, that pose long-distance latitudinal dispersal, may potentially hold genes that are designed to cope with the climatic and environmental gradients that this imply. An opportunity for investigating gene functions adapted to cope with different environmental challenges represents an important future topic research, especially under a climate changing future.

Discovering patterns of bat dispersal and distribution are of high importance for the ecology and conservation of bats and ectoparasites. This study gathered evidence of latitudinal dispersal, and peninsular-continental gene flow. However, direction, route and duration of dispersal is still unknown. Ectoparasites turned to be very helpful for discovering these patterns. However, more sampling and different seasons need to be covered to start making inferences with sufficient power.

Bat and ectoparasite sampling in Mexican deserts are still understudied in comparison with temperate and tropical places (Zamora-Gutierrez, Amano and Jones, 2019). Baja hold different ecoregions which are composed by different levels of aridity and coastal effect (González-Abraham, Garcillán and Ezcurra, 2010). This ecosystem diversity has also been influenced by Baja's complex geological history, one of the main drivers of allopatric speciation events over the peninsula (Riddle *et al.*, 2000). To relate patterns of distribution to historical biogeographic processes represents another research topic that would reveal important information for Baja biodiversity.

5.4. Conservation implications

This study has set the grounds for future research in Baja peninsula, where the data generated will answer questions related to diversity and taxonomic status of bat and ectoparasite diversity, environmental modelling, pathogens from ectoparasites, and bat microbiome and virome diversity. Information generated here will be useful to formulate conservation and management strategies for bats in Baja, as well as to highlight the importance of pathogens research to prevent potential zoonotic spill-overs and to contribute to the investigation of pathogens diversity over North America.

My work has a large dataset compilation of bat and ectoparasite genetic and ecological information, and also resulted in Mexico-U.S.A trans-border collaborations that will increase the opportunities for a better understanding of diversity research without borders. Maintaining research in plant and animals that are distributed across Mexico and the U.S. is of high importance as well, particularly because closing borders would impact negatively in the biodiversity that does not care about anthropogenic issues (Peters *et al.*,

2018). Although bats are not directly affected by anthropogenic barriers, they represent an important example of how important is to maintain connectivity at a continental scale.

Myotis peninsularis is considered endemic to the south of Baja (Álvarez-Castañeda and Arroyo-Cabrales, 2008). The few known maternity colonies are in proximity to cities, and have already suffered from anthropogenic disturbance. Little is known about its movement patterns over the region and over the year. It has been recorded in winter (W. Frick, UCSC, pers. comms.), which implies that *M. peninsularis* is a resident bat population. Changing its taxonomic status may represent a challenge and an opportunity to promoting its conservation. Therefore, it is utterly important to generate more evidence of its evolutionary history, highlighting its position as incipient species.

Parasites, especially wildlife ectoparasites, have been ignored and neglected due to the negative effects that parasites pose over their hosts, which are in general more well-studied than the evolutionary and positive immunological implications they provide (Nichols and Gómez, 2011; Gómez and Nichols, 2013; Poulin, 2014; Spencer and Zuk, 2016). There is evidence that many parasite species are endangered, where their risk of extinction would disturb the normal functioning of ecosystems and the evolutionary potential of their hosts (Gómez and Nichols, 2013). Research on parasite richness and diversity is crucial for understanding these systems and building up taxonomic databases, the same than with their hosts. Parasite extinctions may have passed unaware from research due to the lack of previous records of their existence. Baja bat-ectoparasite community showed to be highly relevant for understanding dispersal patterns of their hosts, and that host-specificity was mostly present over this system. If something happens to hosts that hold cryptic host-specific

ectoparasite species (e.g. anthropogenic disturbance of an endemic bat population), the risk of that cryptic ectoparasite loss is high. This study has contributed in discovering Baja ectoparasite diversity and the different degrees of interactions with their hosts and environment, providing the first stage towards the inclusion of conservation for parasites in unexplored areas and cryptic bat populations in Baja. Parasites are part of Earth's biodiversity, and should be conserved not only by the critical roles they play but also by their own right (Spencer and Zuk, 2016).

5.5. Conclusion

The studies of bat and ectoparasites as a complex system are increasing, due to its importance for conservation, evolutionary and medical research. Overall, this thesis increased bat and ectoparasite diversity known over the north-western deserts of Mexico, where new potential lineages have been discovered. This thesis represents an important step forward for evolutionary research on poor studied taxa in unexplored regions, highlighting the vast diversity that still remains to be studied. My work updates phylogenetic analysis of New World *Myotis*, providing with this a more complex scenario, which involves taking into account multiple evolutionary processes that can be targeted with high-throughput sequencing techniques. *Myotis* bats in western North America have shown that species boundaries are not fixed in this system, with evidence of frequent, recurrent hybridisation and introgression.

In a global context, this work contributed to increase the number of molecular reference sequences and high-resolution genomic information than can be used in prospective research of how wildlife adapts to different habitats. This is especially important to track how

environmental change affects wildlife in a recent temporary scale, but also to model future implications. It has also contributed to add more evidence for the study of different stages of speciation. This work shows that defining a species has become a very complex matter, which involves multidisciplinary work and a deeper understanding of the mechanisms that allows pre and postzygotic reproductive isolation, providing the opportunity to investigate how genomes diverge under gene flow, introgression and hybridisation in a wildlife system.

This work has also emphasised on the importance of doing research over neglected organisms like ectoparasites, which are vital for a healthy ecosystem and evolutionary processes. Increasing efforts to study bats, ectoparasites and their pathogens are especially relevant during this times of change, where the implications of anthropogenic disturbance over wildlife systems and their pathogens have had strong worldwide consequences.

Supplementary Appendix

Chapter 2.

Appendix 2.1. List of individuals used for this study. Codes of *Myotis* bats analysed based on mitochondrial assignations: CA, *californicus*; EV, *evotis*; PE, *peninsularis*; *sv*, unknown lineage morphologically close to *velifer*; VE, *velifer*; VO, *volans*; YU, *yumanensis*. Abbreviations for states in Mexico are: BC, Baja California; BCS, Baja California Sur; Son, Sonora; and Jal, Jalisco. For the United Staes: Cali, California. Abbreviations for San Diego sites are: StTsa, Santa Ysabel Open Space Preserve; Otay, Otay Mountain; Cotton, Cottonwood Creek; Hollen, Hollenbeck Canyon Wildlife Area; CNF, Cleveland National Forest; Fairban, Fairbanks Ranch; Penas, Los Penasquitos Canyon Preserve; and SDNWR, San Diego National Wildlife Refuge. Altitude is measured as in meters above sea. Outgroups (Ot).

ID	*Myotis*	Site Label	Site	State	Altitude	Latitude	Longitude
7_VO	*volans*	SanFe	San Fernando	BC	469	29.971	-115.237
18_CA	*californicus*	SanFe	San Fernando	BC	469	29.971	-115.237
20_CA	*californicus*	SanFe	San Fernando	BC	469	29.971	-115.237
22_VO	*volans*	SanFe	San Fernando	BC	469	29.971	-115.237
25_VO	*volans*	SanFe	San Fernando	BC	469	29.971	-115.237
32_VO	*volans*	SanFe	San Fernando	BC	469	29.971	-115.237
33_CA	*californicus*	Matz	Matzo	BC	11	30.436	-116.029
34_CA	*californicus*	Matz	Matzo	BC	11	30.436	-116.029
35_CA	*californicus*	Matz	Matzo	BC	11	30.436	-116.029
36_CA	*californicus*	Matz	Matzo	BC	11	30.436	-116.029
37_CA	*californicus*	Matz	Matzo	BC	11	30.436	-116.029
38_CA	*californicus*	Matz	Matzo	BC	11	30.436	-116.029
39_VO	*volans*	Matz	Matzo	BC	11	30.436	-116.029
40_VO	*volans*	SanFe	San Fernando	BC	469	29.971	115.237
42_CA	*californicus*	Mosq	Mosqueda	BC	6	32.156	-115.279
43_VO	*volans*	Mosq	Mosqueda	BC	6	32.156	-115.279
47_CA	*californicus*	Rosa	Rosarito	BC	95	28.613	-114.047
53_CA	*californicus*	Rosa	Rosarito	BC	95	28.613	-114.047
64_YU	*yumanensis*	SanIg	San Ignacio	BCS	122	27.297	-112.898
65_YU	*yumanensis*	SanIg	San Ignacio	BCS	122	27.297	-112.898
66_YU	*yumanensis*	SanIg	San Ignacio	BCS	122	27.297	-112.898
71_YU	*yumanensis*	SanIg	San Ignacio	BCS	122	27.297	-112.898
72_YU	*yumanensis*	SanIg	San Ignacio	BCS	122	27.297	-112.898
74_YU	*yumanensis*	SanIg	San Ignacio	BCS	122	27.297	-112.898
99_PE	*peninsularis*	Lore	Loreto	BCS	7	26.012	-111.349
113_YU	*yumanensis*	SanBa	San Basilio	BCS	19	26.371	-111.429
114_YU	*yumanensis*	SanBa	San Basilio	BCS	19	26.371	-111.429
115_YU	*yumanensis*	SanBa	San Basilio	BCS	19	26.371	-111.429
119_YU	*yumanensis*	Poza	Pocitas	BCS	64	24.403	-111.104
136_PE	*peninsularis*	Teso	Tesos	BCS	179	23.175	-109.611
137_PE	*peninsularis*	Teso	Tesos	BCS	179	23.175	-109.611
138_PE	*peninsularis*	Teso	Tesos	BCS	179	23.175	-109.611
139_PE	*peninsularis*	Teso	Tesos	BCS	179	23.175	-109.611

140_PE	*peninsularis*	Teso	Tesos	BCS	179	23.175	-109.611
141_PE	*peninsularis*	Teso	Tesos	BCS	179	23.175	-109.611
142_PE	*peninsularis*	Teso	Tesos	BCS	179	23.175	-109.611
143_PE	*peninsularis*	Teso	Tesos	BCS	179	23.175	-109.611
144_PE	*peninsularis*	Teso	Tesos	BCS	179	23.175	-109.611
146_PE	*peninsularis*	Teso	Tesos	BCS	179	23.175	-109.611
147_PE	*peninsularis*	Teso	Tesos	BCS	179	23.175	-109.611
148_PE	*peninsularis*	Teso	Tesos	BCS	179	23.175	-109.611
149_PE	*peninsularis*	Teso	Tesos	BCS	179	23.175	-109.611
150_PE	*peninsularis*	Teso	Tesos	BCS	179	23.175	-109.611
152_YU	*yumanensis*	Mosq	Mosqueda	BC	6	32.156	-115.279
153_YU	*yumanensis*	Mosq	Mosqueda	BC	6	32.156	-115.279
154_YU	*yumanensis*	Mosq	Mosqueda	BC	6	32.156	-115.279
155_YU	*yumanensis*	Mosq	Mosqueda	BC	6	32.156	-115.279
156_VE	*yumanensis*	Mosq	Mosqueda	BC	6	32.156	-115.279
158_YU	*yumanensis*	Mosq	Mosqueda	BC	6	32.156	-115.279
159_YU	*yumanensis*	Mosq	Mosqueda	BC	6	32.156	-115.279
160_YU	*yumanensis*	Mosq	Mosqueda	BC	6	32.156	-115.279
161_YU	*yumanensis*	Mosq	Mosqueda	BC	6	32.156	-115.279
162_YU	*yumanensis*	Mosq	Mosqueda	BC	6	32.156	-115.279
166_YU	*yumanensis*	Mosq	Mosqueda	BC	6	32.156	-115.279
167_YU	*yumanensis*	Mosq	Mosqueda	BC	6	32.156	-115.279
168_YU	*yumanensis*	Mosq	Mosqueda	BC	6	32.156	-115.279
169_YU	*yumanensis*	Mosq	Mosqueda	BC	6	32.156	-115.279
170_YU	*yumanensis*	Mosq	Mosqueda	BC	6	32.156	-115.279
171_YU	*yumanensis*	Mosq	Mosqueda	BC	6	32.156	-115.279
172_YU	*yumanensis*	Mosq	Mosqueda	BC	6	32.156	-115.279
173_CA	*californicus*	Mosq	Mosqueda	BC	6	32.156	-115.279
174_YU	*yumanensis*	Mosq	Mosqueda	BC	6	32.156	-115.279
186_YU	*yumanensis*	Chaba	Chabacanos	BC	758	32.567	-116.493
188_YU	*yumanensis*	Chaba	Chabacanos	BC	758	32.567	-116.493
196_YU	*yumanensis*	Chaba	Chabacanos	BC	758	32.567	-116.493
200_CA	*californicus*	Chaba	Chabacanos	BC	758	32.567	-116.493
209_VO	*volans*	Ense	Ensenada	BC	53	31.770	-116.520
210_YU	*yumanensis*	Ense	Ensenada	BC	53	31.770	-116.520
223_CA	*californicus*	SanFe	San Fernando	BC	469	29.971	-115.237
224_CA	*californicus*	SanFe	San Fernando	BC	469	29.971	-115.237
225_CA	*californicus*	SanFe	San Fernando	BC	469	29.971	-115.237
226_CA	*californicus*	SanFe	San Fernando	BC	469	29.971	-115.237
227_VO	*volans*	SanFe	San Fernando	BC	469	29.971	-115.237
228_VO	*volans*	SanFe	San Fernando	BC	469	29.971	-115.237
231_CA	*californicus*	SanFe	San Fernando	BC	469	29.971	-115.237
238_CA	*californicus*	SanFe	San Fernando	BC	469	29.971	-115.237
239_CA	*californicus*	SanFe	San Fernando	BC	469	29.971	-115.237
240_CA	*californicus*	SanFe	San Fernando	BC	469	29.971	-115.237
241_CA	*californicus*	SanFe	San Fernando	BC	469	29.971	-115.237
242_CA	*californicus*	SanFe	San Fernando	BC	469	29.971	-115.237
244_VO	*volans*	SanFe	San Fernando	BC	469	29.971	-115.237
245_VO	*volans*	SanFe	San Fernando	BC	469	29.971	-115.237
246_VO	*volans*	SanFe	San Fernando	BC	469	29.971	-115.237
247_VO	*volans*	SanFe	San Fernando	BC	469	29.971	-115.237
248_CA	*californicus*	Rosa	Rosarito	BC	95	28.613	-114.047

249_CA	*californicus*	Rosa	Rosarito	BC	95	28.613	-114.047
250_CA	*californicus*	Rosa	Rosarito	BC	95	28.613	-114.047
254_YU	*yumanensis*	SanIg	San Ignacio	BCS	122	27.297	-112.898
280_CA	*californicus*	SanBa	San Basilio	BCS	19	26.371	-111.429
281_CA	*californicus*	SanBa	San Basilio	BCS	19	26.371	-111.429
286_YU	*yumanensis*	SanBa	San Basilio	BCS	19	26.371	-111.429
287_YU	*yumanensis*	SanBa	San Basilio	BCS	19	26.371	-111.429
288_YU	*yumanensis*	SanBa	San Basilio	BCS	19	26.371	-111.429
289_YU	*yumanensis*	SanBa	San Basilio	BCS	19	26.371	-111.429
290_YU	*yumanensis*	SanBa	San Basilio	BCS	19	26.371	-111.429
291_YU	*yumanensis*	SanBa	San Basilio	BCS	19	26.371	-111.429
295_YU	*yumanensis*	SanBa	San Basilio	BCS	19	26.371	-111.429
296_YU	*yumanensis*	SanBa	San Basilio	BCS	19	26.371	-111.429
297_YU	*yumanensis*	SanBa	San Basilio	BCS	19	26.371	-111.429
298_YU	*yumanensis*	SanBa	San Basilio	BCS	19	26.371	-111.429
299_YU	*yumanensis*	SanBa	San Basilio	BCS	19	26.371	-111.429
303_YU	*yumanensis*	SanBa	San Basilio	BCS	19	26.371	-111.429
304_YU	*yumanensis*	SanBa	San Basilio	BCS	19	26.371	-111.429
305_YU	*yumanensis*	SanBa	San Basilio	BCS	19	26.371	-111.429
307_YU	*yumanensis*	SanBa	San Basilio	BCS	19	26.371	-111.429
318_CA	*californicus*	SanPe	San Pedro	BCS	8	23.390	-110.212
319_VO	*volans*	SanPe	San Pedro	BCS	8	23.390	-110.212
341_CA	*californicus*	Faro	Faro	BCS	6	23.427	-110.233
342_CA	*californicus*	Faro	Faro	BCS	6	23.427	-110.233
345_CA	*californicus*	Faro	Faro	BCS	6	23.427	-110.233
352_PE	*peninsularis*	Teso	Tesos	BCS	179	23.175	-109.611
353_PE	*peninsularis*	Teso	Tesos	BCS	179	23.175	-109.611
354_PE	*peninsularis*	Teso	Tesos	BCS	179	23.175	-109.611
355_PE**	*peninsularis*	Teso	Tesos	BCS	179	23.175	-109.611
356_PE	*peninsularis*	Teso	Tesos	BCS	179	23.175	-109.611
357_PE	*peninsularis*	Teso	Tesos	BCS	179	23.175	-109.611
358_PE	*peninsularis*	Teso	Tesos	BCS	179	23.175	-109.611
359_PE	*peninsularis*	Teso	Tesos	BCS	179	23.175	-109.611
360_PE	*peninsularis*	Teso	Tesos	BCS	179	23.175	-109.611
361_PE	*peninsularis*	Teso	Tesos	BCS	179	23.175	-109.611
370_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
371_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
372_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
373_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
374_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
375_PE	*yumanensis*	Teste	Testera	BCS	598	23.764	-110.055
377_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
383_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
384_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
385_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
387_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
390_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
391_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
394_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
395_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
396_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
397_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055

399_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
400_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
401_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
402_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
403_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
404_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
408_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
411_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
412_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
413_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
416_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
417_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
418_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
419_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
421_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
422_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
423_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
424_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
425_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
426_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
427_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
428_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
429_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
430_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
432_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
433_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
434_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
435_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
436_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
437_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
441_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
444_CA	*californicus*	Faro	Faro	BCS	6	23.427	-110.233
447_PE	*peninsularis*	Boca	Boca de la Sierra	BCS	294	23.385	-109.819
448_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
449_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
450_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
452_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
453_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
454_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
455_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
457_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
458_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
459_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
460_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
461_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
462_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
463_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
464_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
465_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
466_PE	*peninsularis*	Teste	Testera	BCS	598	23.764	-110.055
467_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
468_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306

469_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
470_PE	*peninsularis*	LaPaz	La Paz	BCS	31	24.103	-110.306
474_VE	*velifer*	SanBa	San Basilio	BCS	34	26.371	-111.429
475_YU	*yumanensis*	SanBa	San Basilio	BCS	34	26.371	-111.429
476_YU	*yumanensis*	SanBa	San Basilio	BCS	34	26.371	-111.429
479_YU	*yumanensis*	SanBa	San Basilio	BCS	34	26.371	-111.429
483_YU	*yumanensis*	SanBa	San Basilio	BCS	34	26.371	-111.429
484_YU	*yumanensis*	SanBa	San Basilio	BCS	34	26.371	-111.429
486_CA	*californicus*	SanBa	San Basilio	BCS	34	26.371	-111.429
487_YU	*yumanensis*	SanBa	San Basilio	BCS	34	26.371	-111.429
488_YU	*yumanensis*	SanBa	San Basilio	BCS	34	26.371	-111.429
489_CA	*californicus*	Meli	Meling	BC	646	30.972	-115.744
490_CA	*californicus*	Meli	Meling	BC	646	30.972	-115.744
491_CA	*californicus*	Meli	Meling	BC	646	30.972	-115.744
492_CA	*californicus*	Meli	Meling	BC	646	30.972	-115.744
493_CA	*californicus*	Meli	Meling	BC	646	30.972	-115.744
494_CA	*californicus*	Meli	Meling	BC	646	30.972	-115.744
495_EV	*evotis*	Meli	Meling	BC	646	30.972	-115.744
496_CA	*californicus*	Meli	Meling	BC	646	30.972	-115.744
497_CA	*californicus*	Meli	Meling	BC	646	30.972	-115.744
498_YU	*yumanensis*	Meli	Meling	BC	646	30.972	-115.744
499_CA	*californicus*	Meli	Meling	BC	646	30.972	-115.744
531_CA	*californicus*	Jolla	Jolla	BC	1459	30.920	-115.601
537_CA	*californicus*	Chaba	Chabacanos	BC	763	32.566	-116.493
540_YU	*yumanensis*	Chaba	Chabacanos	BC	763	32.566	-116.493
541_YU	*yumanensis*	Chaba	Chabacanos	BC	763	32.566	-116.493
552_YU	*yumanensis*	Chaba	Chabacanos	BC	763	32.566	-116.493
553_CA	*californicus*	Chaba	Chabacanos	BC	763	32.566	-116.493
554_YU	*yumanensis*	Chaba	Chabacanos	BC	763	32.566	-116.493
563_CA	*californicus*	Chaba	Chabacanos	BC	763	32.566	-116.493
566_sv	*Sv*	Ures	Ures	Son	396	29.433	-110.376
567_sv	*Sv*	Ures	Ures	Son	396	29.433	-110.376
568_sv	*Sv*	Ures	Ures	Son	396	29.433	-110.376
569_sv	*Sv*	Ures	Ures	Son	396	29.433	-110.376
570_sv	*Sv*	Ures	Ures	Son	396	29.433	-110.376
571_sv	*Sv*	Ures	Ures	Son	396	29.433	-110.376
572_VE	*velifer*	Ures	Ures	Son	396	29.433	-110.376
573_sv	*Sv*	Ures	Ures	Son	396	29.433	-110.376
574_sv	*Sv*	Ures	Ures	Son	396	29.433	-110.376
575_sv	*Sv*	Ures	Ures	Son	396	29.433	-110.376
576_sv	*Sv*	Ures	Ures	Son	396	29.433	-110.376
577_sv	*Sv*	Ures	Ures	Son	396	29.433	-110.376
578_sv	*Sv*	Ures	Ures	Son	396	29.433	-110.376
579_sv	*Sv*	Ures	Ures	Son	396	29.433	-110.376
580_sv	*Sv*	Ures	Ures	Son	396	29.433	-110.376
581_sv	*Sv*	Ures	Ures	Son	396	29.433	-110.376
582_sv	*Sv*	Ures	Ures	Son	396	29.433	-110.376
583_sv	*sv*	Ures	Ures	Son	396	29.433	-110.376
584_sv	*sv*	Ures	Ures	Son	396	29.433	-110.376
586_sv**	*sv*	Ures	Ures	Son	396	29.433	-110.376
587_sv	*sv*	Ures	Ures	Son	396	29.433	-110.376
588_sv	*sv*	Ures	Ures	Son	396	29.433	-110.376

589_sv	*sv*	Ures	Ures	Son	396	29.433	-110.376
590_sv	*sv*	Ures	Ures	Son	396	29.433	-110.376
591_sv	*sv*	Ures	Ures	Son	396	29.433	-110.376
592_sv	*sv*	Ures	Ures	Son	396	29.433	-110.376
593_sv	*sv*	Ures	Ures	Son	396	29.433	-110.376
599_sv	*sv*	Ures	Ures	Son	396	29.433	-110.376
600_sv	*sv*	Ures	Ures	Son	396	29.433	-110.376
601_sv	*sv*	Ures	Ures	Son	396	29.433	-110.376
602_VE	*velifer*	Ures	Ures	Son	396	29.433	-110.376
603_CA	*californicus*	Prima	Primavera	Jal	1463	20.679	-103.602
604_CA	*californicus*	Prima	Primavera	Jal	1463	20.679	-103.602
CA1_CA	*californicus*	SanDi	StYsa-San Diego	Cali	NA	33.131	-116.648
CA2_CA	*californicus*	SanDi	Otay-San Diego	Cali	NA	32.7778	-116.4478
CA3_CA	*californicus*	SanDi	Cotton-San Diego	Cali	NA	32.8754	-116.4665
CA4_CA	*californicus*	SanDi	Cotton-San Diego	Cali	NA	32.8648	-116.4589
CA5_CA	*californicus*	SanDi	Hollen-San Diego	Cali	NA	32.8648	-116.4589
CA6_CA	*californicus*	SanDi	Boden-San Diego	Cali	NA	33.1189	-116.6657
CA7_CA	*californicus*	SanDi	CNF-San Diego	Cali	NA	32.9273	-117.1764
CA8_YU*	*yumanensis*	SanDi	Otay-San Diego	Cali	NA	33.1314	-116.6476
CA9_YU	*yumanensis*	SanDi	Fairban-San Diego	Cali	NA	32.7198	-116.9503
CA10_YU	*yumanensis*	SanDi	CNF-San Diego	Cali	NA	32.587	-116.9117
CA11_YU*	*yumanensis*	SanDi	CNF-San Diego	Cali	NA	32.5688	-116.7633
CA12_YU	*yumanensis*	SanDi	CNF-San Diego	Cali	NA	32.5688	-116.7633
CA13_YU	*yumanensis*	SanDi	CNF-San Diego	Cali	NA	32.679	-116.822
CA14_YU	*yumanensis*	SanDi	StYsa-San Diego	Cali	NA	33.09168	-116.896
CA15_YU	*yumanensis*	SanDi	Penas-San Diego	Cali	NA	32.799	-116.642
CA16_YU	*yumanensis*	SanDi	StYsa-San Diego	Cali	NA	32.606	-116.930
CA17_YU	*yumanensis*	SanDi	SDNWR-San Diego	Cali	NA	32.999	-117.2054
Ot205_ANPA	*Antrozous pallidus*	Prima	Chabacanos	BC	758	32.5673	-116.492
Ot368_EPFU	*Eptesicus fuscus*	Teste	Testera	BCS	606	23.7482	-110.0576

***Non- amplified sample for the cytochrome *b* marker, included only in the nuclear analysis. ** Removed relative for population structure analysis.**

Appendix 2.2. List of reference sequences (two sections) used in the phylogenetic and haplotype analysis of *Myotis* species from this study.

ID	*Myotis species*	Accession code	Country	Potential site	ID	*Myotis species*	Accession code	Country	Potential site
1	*californicus*	AY460353	U.S.	Arizona	91	*velifer*	EU680229	U.S.	Texas
2	*californicus*	AY460355	U.S.	Arizona	92	*velifer*	EU680230	U.S.	Texas
3	*californicus*	AY460350	U.S.	California	93	*velifer*	EU680231	U.S.	Texas
4	*californicus*	AY460351	U.S.	California	94	*velifer*	EU680232	U.S.	Texas
5	*californicus*	AM261887	Mexico	Durango	95	*velifer*	EU680233	U.S.	Texas
6	*californicus*	JX130442	Mexico	Jalisco	96	*velifer*	EU680234	U.S.	Texas
7	*californicus*	JX130524	Mexico	Michoacan	97	*velifer*	EU680235	U.S.	Texas
8	*californicus*	AY460347	U.S.	New Mexico	98	*velifer*	EU680236	U.S.	Texas
9	*californicus*	AY460348	U.S.	New Mexico	99	*velifer*	EU680237	U.S.	Texas
10	*californicus*	AY460346	U.S.	Texas	100	*velifer*	EU680238	U.S.	Texas
11	*californicus*	AY460352	U.S.	Texas	101	*velifer*	EU680239	U.S.	Texas
12	*californicus*	AY460354	U.S.	Texas	102	*velifer*	EU680240	U.S.	Texas
13	*californicus*	AY460356	U.S.	Texas	103	*velifer*	EU680241	U.S.	Texas
14	*californicus*	MF143469	U.S.	Oregon	104	*velifer*	EU680242	U.S.	Texas
15	*californicus*	AY460349	U.S.	Utah	105	*velifer*	EU680243	U.S.	Texas
16	*ciliolabrum*	AY460357	U.S.	Arizona	106	*velifer*	EU680244	U.S.	Texas
17	*ciliolabrum*	AY460362	U.S.	Arizona	107	*velifer*	EU680245	U.S.	Texas
18	*ciliolabrum*	AY460365	U.S.	Arizona	108	*velifer*	EU680246	U.S.	Texas
19	*ciliolabrum*	AM261889	Canada	Canada	109	*velifer*	EU680247	U.S.	Texas
20	*ciliolabrum*	AM261890	Mexico	Durango	110	*velifer*	EU680248	U.S.	Texas
21	*ciliolabrum*	AY460358	U.S.	New Mexico	111	*velifer*	EU680249	U.S.	Texas
22	*ciliolabrum*	AY460360	U.S.	New Mexico	112	*velifer*	EU680250	U.S.	Texas
23	*ciliolabrum*	AY460361	U.S.	New Mexico	113	*velifer*	EU680251	U.S.	Texas
24	*ciliolabrum*	AY460359	U.S.	Oklahoma	114	*velifer*	EU680252	U.S.	Texas
25	*ciliolabrum*	AY460364	U.S.	Texas	115	*velifer*	EU680253	U.S.	Texas
26	*ciliolabrum*	AY460363	U.S.	Utah	116	*velifer*	EU680254	U.S.	Texas
27	*evotis*	KC747695	Mexico	Baja California	117	*velifer*	EU680255	U.S.	Texas
28	*evotis*	AJ841949	Canada	Canada	118	*velifer*	EU680256	U.S.	Texas
29	*evotis*	AY883914	Canada	Canada	119	*velifer*	EU680257	U.S.	Texas

30	*evotis*	AY883917	Canada	Canada	120	*velifer*	EU680258	U.S.	Texas
31	*evotis*	MF143468	Mexico	Baja California	121	*velifer*	EU680259	U.S.	Texas
32	*evotis*	AY460344	U.S.	New Mexico	122	*velifer*	EU680260	U.S.	Texas
33	*fortidens*	KC747690	Mexico	Guerrero	123	*velifer*	EU680261	U.S.	Texas
34	*fortidens*	JX130439	Paraguay	Paraguay	124	*velifer*	EU680262	U.S.	Texas
35	*fortidens*	JX130437	Peru	Peru	125	*velifer*	EU680263	U.S.	Texas
36	*leibii*	MF143488	U.S.	Oklahoma	126	*velifer*	EU680264	U.S.	Texas
37	*melanorhinus*	KC747694	U.S.	Texas	127	*velifer*	EU680265	U.S.	Texas
38	*thysanodes*	JX130458	Ecuador	Ecuador	128	*velifer*	EU680266	U.S.	Texas
39	*thysanodes*	JX130461	Ecuador	Ecuador	129	*velifer*	EU680267	U.S.	Texas
40	*velifer*	EU680280	U.S.	Arizona/California	130	*velifer*	EU680268	U.S.	Texas
41	*velifer*	EU680282	U.S.	Arizona/California	131	*velifer*	EU680269	U.S.	Texas
42	*velifer*	EU680289	U.S.	Arizona/California	132	*velifer*	EU680270	U.S.	Texas
43	*velifer*	EU680293	U.S.	Arizona/California	133	*velifer*	EU680271	U.S.	Texas
44	*velifer*	EU680294	U.S.	Arizona/California	134	*velifer*	EU680272	U.S.	Texas
45	*velifer*	JX130462	Ecuador	Ecuador	135	*velifer*	EU680273	U.S.	Texas
46	*velifer*	JX130468	Ecuador	Ecuador	136	*velifer*	EU680274	U.S.	Texas
47	*velifer*	JX130477	Ecuador	Ecuador	137	*velifer*	EU680275	U.S.	Texas
48	*velifer*	JX130478	Salvador	El Salvador	138	*velifer*	EU680276	U.S.	Texas
49	*velifer*	KM980441	Mexico	La Paz	139	*velifer*	EU680277	U.S.	Texas
50	*velifer*	MF143499	Mexico	Baja California	140	*velifer*	EU680278	U.S.	Texas
51	*velifer*	JX130589	Mexico	Michoacan	141	*velifer*	EU680279	U.S.	Texas
52	*velifer*	EU680295	U.S.	Mohave	142	*velifer*	EU680281	U.S.	Texas
53	*velifer*	AF294513	U.S.	New Mexico	143	*velifer*	EU680283	U.S.	Texas
54	*velifer*	KM980442	Mexico	Nuevo Leon	144	*velifer*	EU680284	U.S.	Texas
55	*velifer*	KM980443	Mexico	Nuevo Leon	145	*velifer*	EU680285	U.S.	Texas
56	*velifer*	JX130438	Peru	Peru	146	*velifer*	EU680286	U.S.	Texas
57	*velifer*	AF376870	Mexico	Sonora	147	*velifer*	EU680287	U.S.	Texas
58	*velifer*	EU680196	U.S.	Texas	148	*velifer*	EU680288	U.S.	Texas
59	*velifer*	EU680197	U.S.	Texas	149	*velifer*	EU680290	U.S.	Texas
60	*velifer*	EU680198	U.S.	Texas	150	*velifer*	EU680291	U.S.	Texas
61	*velifer*	EU680199	U.S.	Texas	151	*velifer*	EU680292	U.S.	Texas
62	*velifer*	EU680200	U.S.	Texas	152	*velifer*	EU680296	U.S.	Texas
63	*velifer*	EU680201	U.S.	Texas	153	*velifer*	EU680297	U.S.	Texas
64	*velifer*	EU680202	U.S.	Texas	154	*velifer*	EU680298	U.S.	Texas
65	*velifer*	EU680203	U.S.	Texas	155	*velifer*	EU680299	U.S.	Texas

66	*velifer*	EU680204	U.S.	Texas	156	*velifer*	EU680300	U.S.	Texas
67	*velifer*	EU680205	U.S.	Texas	157	*volans*	JN628247	Mexico	Durango
68	*velifer*	EU680206	U.S.	Texas	158	*volans*	JN628248	Mexico	Durango
69	*velifer*	EU680207	U.S.	Texas	159	*volans*	AF376871	U.S.	Texas
70	*velifer*	EU680208	U.S.	Texas	160	*volans*	AF376872	U.S.	Texas
71	*velifer*	EU680209	U.S.	Texas	161	*volans*	JN628244	U.S.	Texas
72	*velifer*	EU680210	U.S.	Texas	162	*volans*	JN628245	U.S.	Texas
73	*velifer*	EU680211	U.S.	Texas	163	*volans*	JN628246	U.S.	Texas
74	*velifer*	EU680212	U.S.	Texas	164	*volans*	JX130590	U.S.	Texas
75	*velifer*	EU680213	U.S.	Texas	165	*volans*	AY883916	U.S.	Washington
76	*velifer*	EU680214	U.S.	Texas	166	*yumanensis*	KM370991	U.S.	Alaska
77	*velifer*	EU680215	U.S.	Texas	167	*yumanensis*	KM370992	U.S.	Alaska
78	*velifer*	EU680216	U.S.	Texas	168	*yumanensis*	KM370993	U.S.	Alaska
79	*velifer*	EU680217	U.S.	Texas	169	*yumanensis*	KM370994	U.S.	Alaska
80	*velifer*	EU680218	U.S.	Texas	170	*yumanensis*	KM370995	U.S.	Alaska
81	*velifer*	EU680219	U.S.	Texas	171	*yumanensis*	KM370996	U.S.	Alaska
82	*velifer*	EU680220	U.S.	Texas	172	*yumanensis*	AF376875	U.S.	California
83	*velifer*	EU680221	U.S.	Texas	173	*yumanensis*	JX130592	Mexico	Michoacan
84	*velifer*	EU680222	U.S.	Texas	174	*yumanensis*	AY460343	U.S.	Oklahoma
85	*velifer*	EU680223	U.S.	Texas	175	*yumanensis*	EF222340	Mexico	South Mexico
86	*velifer*	EU680224	U.S.	Texas	176	*yumanensis*	EF222343	Mexico	South Mexico
87	*velifer*	EU680225	U.S.	Texas	177	*yumanensis*	EF222376	Mexico	South Mexico
88	*velifer*	EU680226	U.S.	Texas	178	*yumanensis*	MF143485	U.S.	Texas
89	*velifer*	EU680227	U.S.	Texas	179	*yumanensis*	AF294514	U.S.	Utah
90	*velifer*	EU680228	U.S.	Texas	180	*yumanensis*	AY883907	U.S.	Washington

Appendix 2.3. List haplotypes used for the phenotype-based *Myotis* species from the mitochondrial DNA median joining network analysis. Haplotypes names (Hap. Name), frequency (FQ) and code of sequence (specimen number_field assignation_site), or GenBank accession number (*Myotis* species name_accession number). Relates to figure 2.7 and 2.8.

Hap. name	Fq	Code of sequence/Accession number
CA1	2	18_MYCA_SanFe,240_MYCA_SanFe
CA2	9	20_MYCA_SanFe,487_MYYU_SanBa,499_MYCA_Melli,CA1_MYCA_Cali,CA2_MYCA_Cali,CA3_MYCA_Cali,CA4_MYCA_Cali,CA5_MYCA_Cali,CA6_MYCA_Cali
CA3	6	33_MYCA_Matz,34_MYCA_Matz,35_MYCA_Matz,36_MYCA_Matz,37_MYCA_Matz,38_MYCA_Matz
CA4	19	47_MYCA_Rosa,53_MYCA_Rosa,223_MYCA_SanFe,224_MYCA_SanFe,225_MYCA_SanFe,226_MYCA_SanFe,231_MYCA_SanFe,239_MYCA_SanFe,241_MYCA_SanFe,242_MYCA_SanFe,248_MYCA_Rosa,249_MYCA_Rosa,250_MYCA_Rosa,281_MYCA_SanBa,318_MYCA_SanPe,341_MYCA_Faro,342_MYCA_Faro,345_MYCA_Faro,444_MYCA_Faro
CA5	1	173_MYCA_Mosq
CA6	1	238_MYCA_SanFe
CA7	1	280_MYCA_SanBa
CA8	5	475_MYYU_SanBa,476_MYYU_SanBa,491_MYCA_Melli,492_MYCA_Melli,493_MYCA_Melli
CA9	5	479_MYYU_SanBa,488_MYYU_SanBa,490_MYCA_Melli,494_MYCA_Melli,531_MYCA_Jolla
CA10	6	483_MYYU_SanBa,484_MYYU_SanBa,496_MYCA_Melli,497_MYCA_Melli,CA7_MYCA_Cali,califor_AY460350
CA11	4	489_MYCA_Melli,537_MYCA_Chaba,553_MYCA_Chaba,563_MYCA_Chaba
CA12	2	603_MYCA_Prima,604_MYCA_Prima
CA13	1	califor_AY460356
CA14	7	califor_AY460355,califor_AY460354,califor_AY460353,califor_AY460348,leibii_MF143488,melanorhi_MF143489,melanorhi_KC747694
CA15	1	califor_AY460352
CA16	1	califor_AY460351
CA17	2	califor_AY460349,ciliola_AY460360
CA18	2	califor_AY460347,ciliola_AY460363
CA19	1	califor_JX130442
CA20	1	califor_MF143469
CA21	2	califor_JX130524,yuma_JX130592
CA22	1	califor_AM261887
CA23	1	califor_AY460346
CI1	1	ciliola_AY460365
CI2	1	ciliola_AY460364
CI3	1	ciliola_AY460362
CI4	1	ciliola_AY460361
CI5	1	ciliola_AY460359
CI6	1	ciliola_AY460358
CI7	1	ciliola_AY460357

CI8	1	ciliola_AM261890
CI9	1	ciliola_AM261889
EV1	3	495_MYEV_Melli,evotis_MF143468,evotis_KC747695
FO1	1	fortidens_JX130439
FO2	1	fortidens_JX130437
FO3	1	fortidens_KC747690
PE1	99	64_MYYU_SanIg,65_MYYU_SanIg,66_MYYU_SanIg,71_MYYU_SanIg,72_MYYU_SanIg,74_MYYU_SanIg,99_MYYU_Palm,119_MYYU_SanBa,136_MYPE_Teso,137_MYPE_Teso,138_MYPE_Teso,139_MYPE_Teso,140_MYPE_Teso,141_MYPE_Teso,142_MYPE_Teso,143_MYPE_Teso,144_MYPE_Teso,146_MYPE_Teso,147_MYPE_Teso,148_MYPE_Teso,149_MYPE_Teso,150_MYPE_Teso,254_MYYU_SanIg,352_MYPE_Teso,355_MYPE_Teso,356_MYPE_Teso,357_MYPE_Teso,358_MYPE_Teso,359_MYPE_Teso,360_MYPE_Teso,361_MYPE_Teso,370_MYPE_Teste,371_MYPE_Teste,372_MYPE_Teste,373_MYPE_Teste,375_MYPE_Teste,377_MYPE_Teste,384_MYPE_Parra,385_MYPE_Parra,387_MYPE_Parra,390_MYPE_Parra,391_MYPE_Parra,394_MYPE_Parra,395_MYPE_Parra,396_MYPE_Parra,397_MYPE_Parra,399_MYPE_Parra,401_MYPE_LaPaz,402_MYPE_LaPaz,403_MYPE_LaPaz,404_MYPE_LaPaz,408_MYPE_LaPaz,411_MYPE_LaPaz,412_MYPE_LaPaz,413_MYPE_LaPaz,416_MYPE_LaPaz,417_MYPE_LaPaz,418_MYPE_LaPaz,419_MYPE_LaPaz,421_MYPE_LaPaz,423_MYPE_LaPaz,424_MYPE_LaPaz,425_MYPE_LaPaz,426_MYPE_LaPaz,427_MYPE_LaPaz,428_MYPE_LaPaz,430_MYPE_LaPaz,432_MYPE_LaPaz,433_MYPE_LaPaz,435_MYPE_LaPaz,436_MYPE_LaPaz,437_MYPE_LaPaz,441_MYPE_LaPaz,447_MYPE_Boca,448_MYPE_Teste,449_MYPE_Teste,450_MYPE_Teste,452_MYPE_Teste,453_MYPE_Teste,455_MYPE_Teste,457_MYPE_Teste,458_MYPE_Teste,459_MYPE_Teste,460_MYPE_Teste,461_MYPE_Teste,462_MYPE_Teste,463_MYPE_Teste,464_MYPE_Teste,466_MYPE_Teste,467_MYPE_LaPaz,468_MYPE_LaPaz,469_MYPE_LaPaz,470_MYPE_LaPaz,levis_MF143482,velifer_EU680295,velifer_EU680294,velifer_EU680293,velifer_EU680290,velifer_EU680289
PE2	7	353_MYPE_Teso,354_MYPE_Teso,374_MYPE_Teste,383_MYPE_Parra,400_MYPE_Parra,422_MYPE_LaPaz,429_MYPE_LaPaz
PE3	1	434_MYPE_LaPaz
PE4	1	454_MYPE_Teste
SV1	1	566_MYVE_Ures
SV2	5	567_MYVE_Ures,569_MYVE_Ures,583_MYVE_Ures,584_MYVE_Ures,588_MYVE_Ures
SV3	15	568_MYVE_Ures,570_MYVE_Ures,571_MYVE_Ures,574_MYVE_Ures,576_MYVE_Ures,577_MYVE_Ures,578_MYVE_Ures,579_MYVE_Ures,580_MYVE_Ures,581_MYVE_Ures,582_MYVE_Ures,591_MYVE_Ures,592_MYVE_Ures,600_MYVE_Ures,601_MYVE_Ures
SV4	1	573_MYVE_Ures
SV5	1	575_MYVE_Ures
SV6	2	586_MYVE_Ures,587_MYVE_Ures
SV7	3	589_MYVE_Ures,590_MYVE_Ures,593_MYVE_Ures
SV8	1	599_MYVE_Ures
VE1	1	velifer_AF376870
VE2	1	velifer_AF294513
VE3	2	velifer_EU680300,velifer_JX130477
VE4	2	velifer_EU680299,velifer_KM980442
VE5	18	velifer_EU680298,velifer_EU680281,velifer_EU680273,velifer_EU680272,velifer_EU680270,velifer_EU680269,velifer_EU680268,velifer_EU680267,velifer_EU680266,velifer_EU680265,velifer_EU680263,velifer_EU680262,velifer_EU680260,velifer_EU680258,velifer_EU680257,velifer_EU680224,velifer_EU680213,velifer_JX130478
VE6	5	velifer_EU680297,velifer_EU680291,velifer_EU680288,velifer_EU680287,velifer_EU680286
VE7	2	velifer_EU680296,velifer_EU680292

VE8	13	velifer_EU680285,velifer_EU680284,velifer_EU680236,velifer_EU680234,velifer_EU680232,velifer_EU680229,velifer_EU680228,velifer_EU680225,velifer_EU680214,velifer_EU680207,velifer_EU680202,velifer_EU680199,velifer_EU680197
VE9	19	velifer_EU680283,velifer_EU680279,velifer_EU680278,velifer_EU680255,velifer_EU680254,velifer_EU680247,velifer_EU680244,velifer_EU680238,velifer_EU680237,velifer_EU680230,velifer_EU680227,velifer_EU680226,velifer_EU680223,velifer_EU680222,velifer_EU680221,velifer_EU680219,velifer_EU680218,velifer_EU680216,velifer_EU680215
VE10	11	velifer_EU680282,velifer_EU680277,velifer_EU680250,velifer_EU680248,velifer_EU680241,velifer_EU680239,velifer_EU680211,velifer_EU680210,velifer_EU680209,velifer_EU680198,velifer_EU680196
VE11	2	velifer_EU680280,velifer_EU680201
VE12	11	velifer_EU680276,velifer_EU680264,velifer_EU680259,velifer_EU680252,velifer_EU680249,velifer_EU680246,velifer_EU680243,velifer_EU680217,velifer_EU680212,velifer_EU680206,velifer_EU680200
VE13	4	velifer_EU680275,velifer_EU680274,velifer_EU680261,velifer_EU680245
VE14	1	velifer_EU680271
VE15	7	velifer_EU680256,velifer_EU680251,velifer_EU680235,velifer_EU680231,velifer_EU680220,velifer_JX130468,volans_JX130590
VE16	1	velifer_EU680253
VE17	1	velifer_EU680242
VE18	5	velifer_EU680240,velifer_EU680233,velifer_EU680208,velifer_EU680205,velifer_EU680203
VE19	1	velifer_EU680204
VE20	1	velifer_JX130462
VE21	1	velifer_JX130438
VE22	1	velifer_KM980443
VE23	1	velifer_KM980441
VE24	1	velifer_MF143499
VO1	15	7_MYVO_SanFe,22_MYVO_SanFe,25_MYVO_SanFe,32_MYVO_SanFe,39_MYVO_Matz,40_MYVO_SanFe,43_MYVO_Mosq,209_MYVO_Ense,227_MYVO_SanFe,228_MYVO_SanFe,244_MYVO_SanFe,245_MYVO_SanFe,246_MYVO_SanFe,247_MYVO_SanFe,319_MYVO_SanPe
VO2	1	42_MYVO_Mosq
VO3	1	volans_AF376872
VO4	4	volans_AF376871,volans_JN628246,volans_JN628245,volans_JN628244
VO5	1	volans_AY883916
VO6	1	volans_MF143496
VO7	1	volans_JN628248
VO8	1	volans_JN628247
YU1	21	113_MYYU_SanBa,114_MYYU_SanBa,115_MYYU_SanBa,286_MYYU_SanBa,287_MYYU_SanBa,288_MYYU_SanBa,289_MYYU_SanBa,290_MYYU_SanBa,291_MYYU_SanBa,295_MYYU_SanBa,296_MYYU_SanBa,297_MYYU_SanBa,298_MYCA_SanBa,299_MYYU_SanBa,303_MYYU_SanBa,304_MYYU_SanBa,305_MYYU_SanBa,307_MYYU_SanBa,474_MYYU_SanBa,572_MYVE_Ures,602_MYVE_Ures
YU2	2	152_MYYU_Mosq,174_MYYU_Mosq
YU3	13	153_MYYU_Mosq,154_MYYU_Mosq,159_MYYU_Mosq,160_MYYU_Mosq,161_MYYU_Mosq,162_MYYU_Mosq,166_MYYU_Mosq,167_MYYU_Mosq,169_MYYU_Mosq,170_MYYU_Mosq,171_MYYU_Mosq,172_MYYU_Mosq,CA14_MYYU_Cali
YU4	3	155_MYYU_Mosq,158_MYYU_Mosq,168_MYYU_Mosq
YU5	1	156_MYYU_Mosq

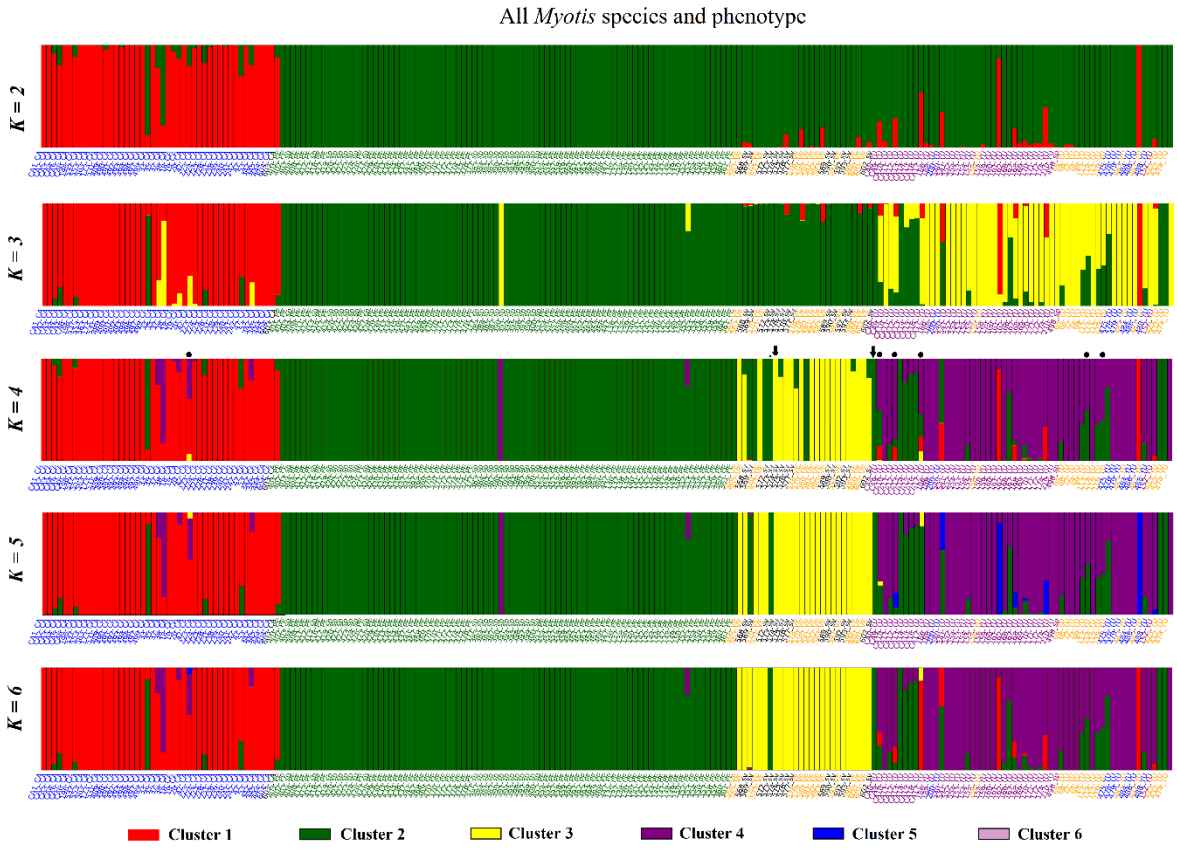
YU6	20	186_MYYU_Chaba,188_MYYU_Chaba,540_MYYU_Chaba,541_MYYU_Chaba,552_MYYU_Chaba,554_MYYU_Chaba,CA9_MYYU_Cali,CA10_MYYU_Cali,CA12_MYYU_Cali,CA13_MYYU_Cali,CA15_MYYU_Cali,CA16_MYYU_Cali,CA17_MYYU_Cali,yuma_KM370995,yuma_KM370996,yuma_KM370994,yuma_KM370993,yuma_KM370992,yuma_KM370991,yuma_AF376875
YU7	1	196_Mysp_Chaba
YU8	1	200_MYYU_Chaba
YU9	1	210_MYYU_Ense
YU10	2	486_MYYU_SanBa,498_MYYU_Melli
YU11	1	yuma_AY883907
YU12	1	yuma_AY460343
YU13	1	yuma_AF294514
YU14	1	yuma_EF222376
YU15	2	yuma_EF222343,yuma_EF222340
YU16	1	yuma_MF143485

Appendix 2.4. List of haplotypes used for the geographic region network median-joining analysis of *Myotis* species from this study. Haplotypes names (Hap. Name), frequency (FQ) and code of sequence (specimen number_field assignation_site). Relates to figure 2.9.

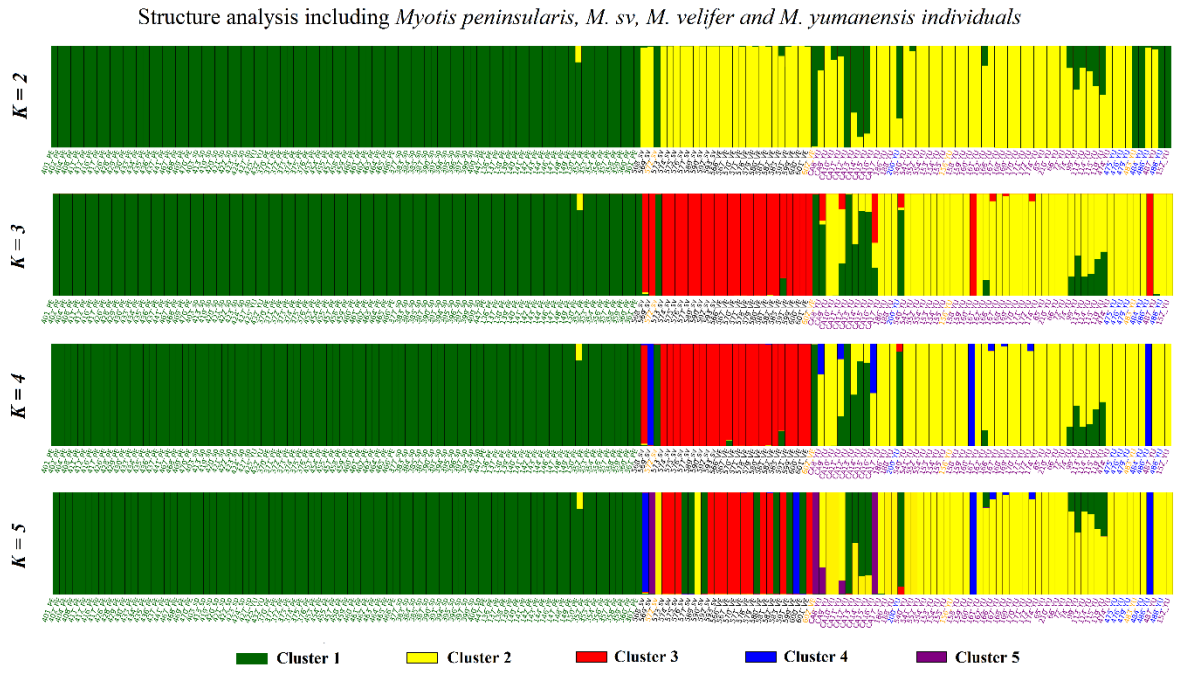
HAP. NAME *	FQ	Code of sequence
CA1	2	18_MYCA_SanFe,240_MYCA_SanFe
CA2	7	20_MYCA_SanFe,CA1_MYCA_Cali,CA2_MYCA_Cali,CA3_MYCA_Cali,CA4_MYCA_Cali, CA5_MYCA_Cali,CA6_MYCA_Cali
CA2b	2	487_MYYU_SanBa,499_MYCA_Melli
CA3	6	33_MYCA_Matz,34_MYCA_Matz,35_MYCA_Matz,36_MYCA_Matz,37_MYCA_Matz,38_M YCA_Matz
CA4a	2	47_MYCA_Rosa,250_MYCA_Rosa
CA4b	12	53_MYCA_Rosa,223_MYCA_SanFe,224_MYCA_SanFe,225_MYCA_SanFe,226_MYCA_San Fe,231_MYCA_SanFe,241_MYCA_SanFe,242_MYCA_SanFe,248_MYCA_Rosa,249_MYCA_ Rosa,281_MYCA_SanBa,342_MYCA_Faro
CA4c	2	318_MYCA_SanPe,345_MYCA_Faro
CA4d	2	341_MYCA_Faro,444_MYCA_Faro
CA4e	1	239_MYCA_SanFe
CA5	1	173_MYCA_Mosq
CA6	1	238_MYCA_SanFe
CA7	1	280_MYCA_SanBa
CA8	5	475_MYYU_SanBa,476_MYYU_SanBa,491_MYCA_Melli,492_MYCA_Melli,493_MYCA_M elli
CA9	5	479_MYYU_SanBa,488_MYYU_SanBa,490_MYCA_Melli,494_MYCA_Melli,531_MYCA_Jol la
CA10a	4	483_MYYU_SanBa,484_MYYU_SanBa,496_MYCA_Melli,497_MYCA_Melli
CA10b	1	CA7_MYCA_Cali
CA11	4	489_MYCA_Melli,537_MYCA_Chaba,553_MYCA_Chaba,563_MYCA_Chaba
CA12	2	603_MYCA_Prima,604_MYCA_Prima
EV1	1	495_MYEV_Melli
PE1	84	99_MYYU_Palm,119_MYYU_SanBa,136_MYPE_Teso,137_MYPE_Teso,138_MYPE_Teso,13 9_MYPE_Teso,140_MYPE_Teso,141_MYPE_Teso,142_MYPE_Teso,143_MYPE_Teso,144_M YPE_Teso,146_MYPE_Teso,147_MYPE_Teso,149_MYPE_Teso,150_MYPE_Teso,352_MYPE _Teso,355_MYPE_Teso,357_MYPE_Teso,358_MYPE_Teso,359_MYPE_Teso,360_MYPE_Tes o,361_MYPE_Teso,370_MYPE_Teste,371_MYPE_Teste,372_MYPE_Teste,373_MYPE_Teste, 375_MYPE_Teste,377_MYPE_Teste,384_MYPE_Parra,385_MYPE_Parra,387_MYPE_Parra,39 0_MYPE_Parra,391_MYPE_Parra,394_MYPE_Parra,395_MYPE_Parra,396_MYPE_Parra,397_ MYPE_Parra,399_MYPE_Parra,401_MYPE_LaPaz,402_MYPE_LaPaz,403_MYPE_LaPaz,404_ MYPE_LaPaz,408_MYPE_LaPaz,411_MYPE_LaPaz,412_MYPE_LaPaz,413_MYPE_LaPaz,41 6_MYPE_LaPaz,417_MYPE_LaPaz,418_MYPE_LaPaz,419_MYPE_LaPaz,421_MYPE_LaPaz, 423_MYPE_LaPaz,424_MYPE_LaPaz,425_MYPE_LaPaz,426_MYPE_LaPaz,427_MYPE_LaPa z,428_MYPE_LaPaz,430_MYPE_LaPaz,432_MYPE_LaPaz,433_MYPE_LaPaz,435_MYPE_La Paz,436_MYPE_LaPaz,437_MYPE_LaPaz,441_MYPE_LaPaz,447_MYPE_Boca,448_MYPE_T este,449_MYPE_Teste,450_MYPE_Teste,452_MYPE_Teste,453_MYPE_Teste,455_MYPE_Tes te,457_MYPE_Teste,458_MYPE_Teste,459_MYPE_Teste,460_MYPE_Teste,461_MYPE_Teste, 462_MYPE_Teste,463_MYPE_Teste,464_MYPE_Teste,466_MYPE_Teste,467_MYPE_LaPaz,4 68_MYPE_LaPaz,469_MYPE_LaPaz,470_MYPE_LaPaz
PE1b	2	148_MYPE_Teso,356_MYPE_Teso
PE2	7	353_MYPE_Teso,354_MYPE_Teso,374_MYPE_Teste,383_MYPE_Parra,400_MYPE_Parra,422 _MYPE_LaPaz,429_MYPE_LaPaz
PE3	1	434_MYPE_LaPaz
PE4	1	454_MYPE_Teste
SV1	1	566_MYVE_Ures
SV2	5	567_MYVE_Ures,569_MYVE_Ures,583_MYVE_Ures,584_MYVE_Ures,588_MYVE_Ures
SV3	15	568_MYVE_Ures,570_MYVE_Ures,571_MYVE_Ures,574_MYVE_Ures,576_MYVE_Ures,577 _MYVE_Ures,578_MYVE_Ures,579_MYVE_Ures,580_MYVE_Ures,581_MYVE_Ures, 582_MYVE_Ures,591_MYVE_Ures,592_MYVE_Ures,600_MYVE_Ures,601_MYVE_Ures
SV4	1	573_MYVE_Ures
SV5	1	575_MYVE_Ures
SV6	1	586_MYVE_Ures
SV6b	1	587_MYVE_Ures

SV7	3	589_MYVE_Ures,590_MYVE_Ures,593_MYVE_Ures
SV8	1	599_MYVE_Ures
VO1a	7	7_MYVO_SanFe,25_MYVO_SanFe,39_MYVO_Matz,40_MYVO_SanFe,227_MYVO_SanFe,244_MYVO_SanFe,319_MYPE_SanPe
VO1b	8	22_MYVO_SanFe,32_MYVO_SanFe,43_MYVO_Mosq,209_MYVO_Ense,228_MYVO_SanFe,245_MYVO_SanFe,246_MYVO_SanFe,247_MYVO_SanFe
VO2	1	42_MYVO_Mosq
YU1	21	113_MYYU_SanBa,114_MYYU_SanBa,115_MYYU_SanBa,286_MYYU_SanBa,287_MYYU_SanBa,288_MYYU_SanBa,289_MYYU_SanBa,290_MYYU_SanBa,291_MYYU_SanBa,295_MYYU_SanBa,296_MYYU_SanBa,297_MYYU_SanBa,298_MYCA_SanBa,299_MYYU_SanBa,303_MYYU_SanBa,304_MYYU_SanBa,305_MYYU_SanBa,307_MYYU_SanBa,474_MYYU_SanBa,572_MYVE_Ures,602_MYVE_Ures
YU2	2	152_MYYU_Mosq,174_MYYU_Mosq
YU3	12	153_MYYU_Mosq,154_MYYU_Mosq,159_MYYU_Mosq,160_MYYU_Mosq,161_MYYU_Mosq,162_MYYU_Mosq,166_MYYU_Mosq,167_MYYU_Mosq,170_MYYU_Mosq,171_MYYU_Mosq,172_MYYU_Mosq,CA14_MYYU_Cali
YU3b	1	169_MYYU_Mosq
YU4	3	155_MYYU_Mosq,158_MYYU_Mosq,168_MYYU_Mosq
YU5	1	156_MYYU_Mosq
YU6	13	186_MYYU_Chaba,188_MYYU_Chaba,540_MYYU_Chaba,541_MYYU_Chaba,552_MYYU_Chaba,554_MYYU_Chaba,CA9_MYYU_Cali,CA10_MYYU_Cali,CA12_MYYU_Cali,CA13_MYYU_Cali,CA15_MYYU_Cali,CA16_MYYU_Cali,CA17_MYYU_Cali
YU7	1	196_Mysp_Chaba
YU8	1	200_MYYU_Chaba
YU9	1	210_MYYU_Ense
YU10	2	486_MYYU_SanBa,498_MYYU_Melli
YU11	1	254_MYYU_SanIg
YU12	5	64_MYYU_SanIg,65_MYYU_SanIg,71_MYYU_SanIg,72_MYYU_SanIg,74_MYYU_SanIg
YU13	1	66_MYYU_SanIg

Appendix 2.5. Admixture analysis from *K*=2 to *K*=6 of 218 *Myotis* individuals ordered by their phylogenetic classification and labelled as their phenotypic identification. Abbreviations: MY, *Myotis*; CA, *californicus*; PE, *peninsularis*; YU, *yumanensis*; VE, *velifer*; *sp*, non-identified in the southern region of Baja California peninsula; and *SV*, non-identified from the north-west continental region. Bar plot colours indicate different populations detected according to their HW clustering. Text colours indicate mitochondrial assignation of each *Myotis* sequence: blue, *californicus*; dark green, *peninsularis*; black, *sv*; purple, *yumanensis*; and orange, *velifer*. Cluster 6 in *K* = 6 bar plot appear as a thin line on its respective individual bar.



Appendix 2.6. Admixture analysis from *K* = 2 to *K* = 5 of 171 *Myotis* individuals excluding *Myotis californicus*, ordered by their phylogenetic classification and labelled as their phenotypic identification. Abbreviations: MY, *Myotis*; PE, *peninsularis*; YU, *yumanensis*; VE, *velifer*; *sp*, non-identified in the southern region of Baja California peninsula; and *SV*, non-identified from the north-west continental region. Bar plot colours indicate different populations detected according to their HW clustering, progressing from *K* = 2 to *K* = 5 for evaluation. Text colours indicate mitochondrial assignation of each *Myotis* sequence: blue, *californicus*; dark green, *peninsularis*; black, *sv*; purple, *yumanensis*; and orange, *velifer*.



Appendix 2.7. List of individuals used in the global *Myotis* SNP calling analysis in *K* = 4, their phenotypic, mtDNA and nDNA assignments. *Q* scores per cluster are shown described by its colour and species membership over the structure cluster plot. Abbreviations: ID, identification number based on the order used in the global structure analysis cluster plot; CCP, clade code assigned in the phylogenetic analysis; and species that showed the admix value within the specified *Q* score range. Class relates to the *Q* scores obtained, describing each individual according to the amount of introgression hold: Pure, individuals ≥ 98.99% membership to one cluster; mtDNA, individuals that nDNA match morphology, but not mtDNA assignments and no evidence of introgression in SNPs; nDNA individuals that mtDNA match morphology, but not nDNA assignments and no evidence of introgression in SNPs; and backcrossed (BC), individuals with 98.97 ≤ *Q* ≥ 1% membership to more than one cluster. Relates to Figure 2.10.

ID	CCP	Phenotype assignment	mtDNA assignment	nDNA assignment	Sites	Cluster 1 Red *californicus*	Cluster 2 Green *peninsularis*	Cluster 3 Yellow *sv*	Cluster 4 Purple *yumanensis*	Class
1	CA2	CA1_CA	*californicus*	*californicus*	San Diego	99.98%	0.01%	0.01%	0.01%	Pure
2	CA2	CA2_CA	*californicus*	*californicus*	San Diego	99.98%	0.01%	0.01%	0.01%	Pure
3	CA2	CA3_CA	*californicus*	*californicus*	San Diego	92.96%	7.03%	0.01%	0.01%	BC
4	CA2	CA4_CA	*californicus*	*californicus*	San Diego	82.15%	17.84%	0.01%	0.01%	BC
5	CA2	CA5_CA	*californicus*	*californicus*	San Diego	99.98%	0.01%	0.01%	0.01%	Pure
6	CA2	CA6_CA	*californicus*	*californicus*	San Diego	99.98%	0.01%	0.01%	0.01%	Pure
7	YU1	196_CA	*yumanensis*	*californicus*	Chabacanos	90.68%	9.30%	0.01%	0.01%	BC
8	CA2	537_CA	*californicus*	*californicus*	Chabacanos	99.97%	0.01%	0.01%	0.01%	Pure
9	CA2	553_CA	*californicus*	*californicus*	Chabacanos	99.97%	0.01%	0.01%	0.01%	Pure
10	CA2	563_CA	*californicus*	*californicus*	Chabacanos	99.98%	0.01%	0.01%	0.01%	Pure
11	CA2	173_CA	*californicus*	*californicus*	Mosqueda	99.98%	0.01%	0.01%	0.01%	Pure
12	CA2	531_CA	*californicus*	*californicus*	Jolla	99.98%	0.01%	0.01%	0.01%	Pure
13	CA2	489_CA	*californicus*	*californicus*	Meling	99.98%	0.01%	0.01%	0.01%	Pure
14	YU1	490_CA	*yumanensis*	*californicus*	Meling	99.98%	0.01%	0.01%	0.01%	nDNA
15	CA2	491_CA	*californicus*	*californicus*	Meling	99.98%	0.01%	0.01%	0.01%	Pure

16	CA2	492_CA	*californicus*	*californicus*	Meling	99.98%	0.01%	0.01%	0.01%	Pure
17	CA2	493_CA	*californicus*	*californicus*	Meling	99.98%	0.01%	0.01%	0.01%	Pure
18	CA2	494_CA	*californicus*	*californicus*	Meling	99.97%	0.01%	0.01%	0.01%	Pure
19	CA2	496_CA	*californicus*	*californicus*	Meling	99.96%	0.01%	0.01%	0.01%	Pure
20	CA2	497_CA	*californicus*	*californicus*	Meling	99.92%	0.03%	0.03%	0.03%	Pure
21	CA2	499_CA	*californicus*	*peninsularis*	Meling	11.36%	88.57%	0.03%	0.03%	BC
22	CA1	33_CA	*californicus*	*californicus*	Matzo	99.98%	0.01%	0.01%	0.01%	Pure
23	CA1	35_CA	*californicus*	*californicus*	Matzo	74.51%	0.01%	0.01%	25.47%	BC
24	CA1	37_CA	*californicus*	*yumanensis*	Matzo	17.11%	0.01%	0.01%	82.87%	BC
25	CA1	38_CA	*californicus*	*californicus*	Matzo	99.97%	0.01%	0.01%	0.01%	Pure
26	CA1	18_CA	*californicus*	*californicus*	San Fernando	97.58%	0.01%	0.01%	2.40%	BC
27	-	19_CA	*californicus*	*californicus*	San Fernando	87.28%	0.01%	0.01%	12.71%	BC
28	CA2	20_CA	*californicus*	*californicus*	San Fernando	99.98%	0.01%	0.01%	0.01%	Pure
29	CA2	42_CA	*californicus*	*californicus*	San Fernando	53.36%	0.01%	7.18%	39.45%	BC
30	CA1	223_CA	*californicus*	*californicus*	San Fernando	97.65%	0.01%	0.01%	2.33%	BC
31	CA1	224_CA	*californicus*	*californicus*	San Fernando	99.98%	0.01%	0.01%	0.01%	Pure
32	CA1	225_CA	*californicus*	*californicus*	San Fernando	83.89%	16.10%	0.01%	0.01%	BC
33	CA1	226_CA	*californicus*	*californicus*	San Fernando	99.98%	0.01%	0.01%	0.01%	Pure
34	CA1	231_CA	*californicus*	*californicus*	San Fernando	99.98%	0.01%	0.01%	0.01%	Pure
35	CA1	238_CA	*californicus*	*californicus*	San Fernando	99.98%	0.01%	0.01%	0.01%	Pure
36	CA1	239_CA	*californicus*	*californicus*	San Fernando	99.98%	0.01%	0.01%	0.01%	Pure
37	CA1	240_CA	*californicus*	*californicus*	San Fernando	99.98%	0.01%	0.01%	0.01%	Pure
38	CA1	241_CA	*californicus*	*californicus*	San Fernando	99.98%	0.01%	0.01%	0.01%	Pure
39	CA1	242_CA	*californicus*	*californicus*	San Fernando	70.91%	29.07%	0.01%	0.01%	BC
40	CA1	47_CA	*californicus*	*californicus*	Rosarito	99.97%	0.01%	0.01%	0.01%	Pure
41	CA1	53_CA	*californicus*	*californicus*	Rosarito	78.28%	0.01%	0.01%	21.71%	BC
42	CA1	342_CA	*californicus*	*californicus*	Faro	99.98%	0.01%	0.01%	0.01%	Pure
43	CA1	345_CA	*californicus*	*californicus*	Faro	99.98%	0.01%	0.01%	0.01%	Pure

44	CA1	444_CA	*californicus*	*californicus*	Faro	99.98%	0.01%	0.01%	0.01%	Pure
45	CA3	604_CA	*californicus*	*californicus*	Primavera	88.85%	11.13%	0.01%	0.01%	BC
46	CA3	603_CA	*californicus*	*californicus*	Primavera	99.98%	0.01%	0.01%	0.01%	Pure
47	PE	401_PE	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
48	PE	402_PE	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
49	PE	403_sp	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
50	PE	404_PE	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
51	PE	408_PE	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
52	PE	411_PE	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
53	PE	412_PE	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
54	PE	413_sp	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
55	PE	416_PE	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
56	PE	417_PE	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
57	PE	419_sp	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
58	PE	420_sp	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
59	PE	421_sp	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
60	PE	422_sp	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
61	PE	423_sp	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
62	PE	424_sp	*peninsularis*	*peninsularis*	La Paz	0.02%	99.94%	0.02%	0.02%	Pure
63	PE	426_PE	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
64	PE	427_sp	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
65	PE	428_PE	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
66	PE	429_PE	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
67	PE	430_PE	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
68	PE	433_PE	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
69	PE	434_PE	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
70	PE	435_PE	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
71	PE	436_PE	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure

72	PE	437_PE	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
73	PE	441_PE	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
74	PE	467_PE	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
75	PE	468_PE	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
76	PE	469_PE	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
77	PE	470_PE	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
78	PE	370_PE	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
79	PE	371_PE	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
80	PE	372_PE	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
81	PE	373_PE	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
82	PE	374_PE	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
83	PE	375_PE	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
84	PE	376_PE	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
85	PE	377_sp	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
86	PE	383_sp	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
87	PE	384_sp	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
88	PE	385_sp	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
89	PE	387_sp	*peninsularis*	*yumanensis*	Testera	0.12%	0.12%	0.11%	99.65%	nDNA
90	PE	390_sp	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
91	PE	391_sp	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
92	PE	394_sp	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
93	PE	395_sp	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
94	PE	396_sp	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
95	PE	397_sp	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
96	PE	399_sp	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
97	PE	400_sp	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
98	PE	453_PE	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
99	PE	454_PE	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure

100	PE	455_PE	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
101	PE	457_PE	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
102	PE	458_PE	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
103	PE	459_PE	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
104	PE	460_PE	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
105	PE	461_PE	*peninsularis*	*peninsularis*	Testera	0.01%	99.97%	0.01%	0.01%	Pure
106	PE	462_PE	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
107	PE	463_PE	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
108	PE	464_PE	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
109	PE	465_PE	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
110	PE	466_PE	*peninsularis*	*peninsularis*	Testera	0.01%	99.98%	0.01%	0.01%	Pure
111	PE	447_PE	*peninsularis*	*peninsularis*	Boca	0.01%	99.98%	0.01%	0.01%	Pure
112	PE	136_PE	*peninsularis*	*peninsularis*	Tesos	0.01%	99.98%	0.01%	0.01%	Pure
113	PE	137_PE	*peninsularis*	*peninsularis*	Tesos	0.01%	99.97%	0.01%	0.01%	Pure
114	PE	138_PE	*peninsularis*	*peninsularis*	Tesos	0.01%	99.98%	0.01%	0.01%	Pure
115	PE	139_PE	*peninsularis*	*peninsularis*	Tesos	0.01%	99.98%	0.01%	0.01%	Pure
116	PE	140_PE	*peninsularis*	*peninsularis*	Tesos	0.01%	99.98%	0.01%	0.01%	Pure
117	PE	141_PE	*peninsularis*	*peninsularis*	Tesos	0.01%	99.98%	0.01%	0.01%	Pure
118	PE	142_PE	*peninsularis*	*peninsularis*	Tesos	0.01%	99.98%	0.01%	0.01%	Pure
119	PE	143_PE	*peninsularis*	*peninsularis*	Tesos	0.01%	99.98%	0.01%	0.01%	Pure
120	PE	144_PE	*peninsularis*	*peninsularis*	Tesos	0.01%	99.98%	0.01%	0.01%	Pure
121	PE	146_PE	*peninsularis*	*peninsularis*	Tesos	0.01%	99.98%	0.01%	0.01%	Pure
122	PE	147_PE	*peninsularis*	*peninsularis*	Tesos	0.01%	99.98%	0.01%	0.01%	Pure
123	PE	148_PE	*peninsularis*	*peninsularis*	Tesos	0.01%	99.98%	0.01%	0.01%	Pure
124	PE	149_PE	*peninsularis*	*peninsularis*	Tesos	0.01%	99.98%	0.01%	0.01%	Pure
125	PE	150_PE	*peninsularis*	*peninsularis*	Tesos	0.01%	73.05%	0.01%	26.94%	BC
126	PE	352_PE	*peninsularis*	*peninsularis*	Tesos	0.01%	99.98%	0.01%	0.01%	Pure
127	PE	353_PE	*peninsularis*	*peninsularis*	Tesos	0.01%	99.98%	0.01%	0.01%	Pure

128	PE	354_PE	*peninsularis*	*peninsularis*	Tesos	0.01%	99.98%	0.01%	0.01%	Pure
129	PE	356_PE	*peninsularis*	*peninsularis*	Tesos	0.01%	99.98%	0.01%	0.01%	Pure
130	PE	357_PE	*peninsularis*	*peninsularis*	Tesos	0.01%	99.98%	0.01%	0.01%	Pure
131	PE	358_PE	*peninsularis*	*peninsularis*	Tesos	0.01%	99.98%	0.01%	0.01%	Pure
132	PE	359_PE	*peninsularis*	*peninsularis*	Tesos	0.01%	99.98%	0.01%	0.01%	Pure
133	PE	360_PE	*peninsularis*	*peninsularis*	Tesos	0.01%	99.98%	0.01%	0.01%	Pure
134	PE	361_PE	*peninsularis*	*peninsularis*	Tesos	0.01%	99.98%	0.01%	0.01%	Pure
135	SV	566_VE	*sv*	*sv*	Ures	0.09%	0.08%	99.74%	0.08%	Pure
136	SV	567_VE	*sv*	*sv*	Ures	0.09%	15.17%	84.66%	0.09%	BC
137	SV	568_sv	*sv*	*peninsularis*	Ures	0.93%	97.04%	1.10%	0.93%	nDNA
138	SV	569_sv	*sv*	*peninsularis*	Ures	0.15%	99.55%	0.15%	0.15%	nDNA
139	SV	570_VE	*sv*	*sv*	Ures	0.11%	0.10%	99.69%	0.10%	Pure
140	SV	571_VE	*sv*	*peninsularis*	Ures	0.11%	99.67%	0.12%	0.10%	nDNA
141	YU3	572_sv	*yumanensis*	*peninsularis*	Ures	0.01%	99.98%	0.01%	0.01%	nDNA
142	SV	573_sv	*sv*	*sv*	Ures	0.11%	0.81%	99.00%	0.09%	Pure
143	SV	574_sv	*sv*	*sv*	Ures	0.13%	17.76%	82.02%	0.10%	BC
144	SV	575_sv	*sv*	*sv*	Ures	0.07%	0.08%	99.78%	0.08%	Pure
145	SV	576_sv	*sv*	*sv*	Ures	0.11%	0.07%	99.73%	0.09%	Pure
146	SV	577_sv	*sv*	*peninsularis*	Ures	0.10%	29.60%	70.12%	0.18%	BC
147	SV	578_VE	*sv*	*sv*	Ures	0.09%	0.09%	99.73%	0.09%	Pure
148	SV	579_VE	*sv*	*peninsularis*	Ures	0.11%	99.66%	0.13%	0.10%	nDNA
149	SV	580_VE	*sv*	*sv*	Ures	0.08%	0.08%	99.77%	0.08%	Pure
150	SV	581_VE	*sv*	*sv*	Ures	0.19%	0.19%	99.42%	0.19%	Pure
151	SV	582_VE	*sv*	*sv*	Ures	0.07%	0.09%	99.76%	0.08%	Pure
152	SV	583_VE	*sv*	*sv*	Ures	0.11%	0.10%	99.69%	0.10%	Pure
153	SV	589_sv	*sv*	*sv*	Ures	0.08%	0.07%	99.77%	0.07%	Pure
154	SV	590_sv	*sv*	*sv*	Ures	0.08%	0.08%	99.77%	0.08%	Pure
155	SV	591_VE	*sv*	*sv*	Ures	0.10%	0.07%	99.76%	0.08%	Pure

156	SV	592_sv	*sv*	*sv*	Ures	0.10%	0.07%	99.75%	0.08%	Pure
157	SV	593_sv	*sv*	*sv*	Ures	0.10%	12.48%	87.34%	0.08%	BC
158	SV	599_VE	*sv*	*sv*	Ures	0.11%	0.08%	99.71%	0.10%	Pure
159	SV	600_VE	*sv*	*sv*	Ures	0.06%	0.08%	99.79%	0.08%	Pure
160	SV	601_VE	*sv*	*sv*	Ures	0.09%	18.57%	81.26%	0.08%	BC
161	YU3	602_sv	*yumanensis*	*peninsularis*	Ures	0.01%	99.98%	0.01%	0.01%	mtDNA
162	-	CA8_YU	-	*yumanensis*	San Diego	12.00%	34.97%	1.35%	51.68%	BC
163	YU1	CA9_YU	*yumanensis*	*yumanensis*	San Diego	0.01%	0.01%	0.01%	99.98%	Pure
164	YU1	CA10_YU	*yumanensis*	*yumanensis*	San Diego	0.01%	16.62%	0.01%	83.36%	BC
165	-	CA11_YU	-	*yumanensis*	San Diego	14.70%	18.81%	0.01%	66.48%	BC
166	YU1	CA12_YU	*yumanensis*	*peninsularis*	San Diego	0.01%	99.98%	0.01%	0.01%	nDNA
167	YU1	CA13_YU	*yumanensis*	*peninsularis*	San Diego	0.01%	74.89%	0.01%	25.09%	BC
168	YU2	CA14_YU	*yumanensis*	*peninsularis*	San Diego	0.01%	83.63%	0.01%	16.36%	BC
169	YU1	CA15_YU	*yumanensis*	*peninsularis*	San Diego	0.01%	85.77%	0.01%	14.22%	BC
170	YU1	CA16_YU	*yumanensis*	*yumanensis*	San Diego	14.35%	22.16%	8.58%	54.92%	BC
171	YU1	CA17_YU	*yumanensis*	*yumanensis*	San Diego	0.01%	0.01%	0.01%	99.98%	Pure
172	YU1	186_YU	*yumanensis*	*yumanensis*	Chabacanos	0.01%	0.01%	0.01%	99.98%	Pure
173	YU1	188_YU	*yumanensis*	*yumanensis*	Chabacanos	0.01%	0.01%	0.01%	99.98%	Pure
174	CA2	200_YU	*californicus*	*peninsularis*	Chabacanos	37.15%	62.84%	0.01%	0.01%	BC
175	YU1	540_YU	*yumanensis*	*yumanensis*	Chabacanos	0.01%	0.01%	0.01%	99.96%	Pure
176	YU1	541_YU	*yumanensis*	*yumanensis*	Chabacanos	0.04%	0.04%	0.04%	99.87%	Pure
177	YU1	552_YU	*yumanensis*	*yumanensis*	Chabacanos	0.01%	0.01%	0.01%	99.96%	Pure
178	YU1	554_YU	*yumanensis*	*yumanensis*	Chabacanos	0.02%	0.02%	0.02%	99.93%	Pure
179	YU2	153_YU	*yumanensis*	*yumanensis*	Mosqueda	0.01%	15.81%	0.01%	84.18%	BC
180	YU2	154_YU	*yumanensis*	*yumanensis*	Mosqueda	0.01%	0.01%	0.01%	99.98%	Pure
181	YU2	155_YU	*yumanensis*	*yumanensis*	Mosqueda	0.01%	0.01%	0.01%	99.98%	Pure
182	PE	156_YU	*peninsularis*	*yumanensis*	Mosqueda	0.01%	0.01%	0.01%	99.98%	mtDNA
183	YU2	158_YU	*yumanensis*	*yumanensis*	Mosqueda	0.01%	0.01%	0.01%	99.98%	Pure

184	YU2	159_YU	*yumanensis*	*yumanensis*	Mosqueda	0.01%	0.01%	0.01%	99.98%	Pure
185	YU2	160_YU	*yumanensis*	*californicus*	Mosqueda	89.76%	0.01%	0.01%	10.23%	BC
186	YU2	161_YU	*yumanensis*	*yumanensis*	Mosqueda	0.01%	2.27%	0.01%	97.72%	BC
187	YU2	162_YU	*yumanensis*	*peninsularis*	Mosqueda	0.01%	66.75%	0.01%	33.24%	BC
188	PE	166_YU	*peninsularis*	*yumanensis*	Mosqueda	15.31%	4.89%	0.01%	79.79%	BC
189	YU2	167_YU	*yumanensis*	*yumanensis*	Mosqueda	0.01%	0.01%	0.01%	99.98%	Pure
190	YU2	168_YU	*yumanensis*	*yumanensis*	Mosqueda	2.40%	13.55%	0.01%	84.04%	BC
191	PE	169_YU	*peninsularis*	*yumanensis*	Mosqueda	0.01%	3.24%	0.01%	96.74%	BC
192	YU2	170_YU	*yumanensis*	*yumanensis*	Mosqueda	0.01%	0.01%	0.01%	99.98%	Pure
193	YU2	171_YU	*yumanensis*	*yumanensis*	Mosqueda	0.01%	3.41%	0.01%	96.57%	BC
194	YU2	172_YU	*yumanensis*	*yumanensis*	Mosqueda	33.77%	0.01%	0.01%	66.22%	BC
195	YU2	174_YU	*yumanensis*	*yumanensis*	Mosqueda	0.01%	0.01%	0.01%	99.98%	Pure
196	YU2	210_YU	*yumanensis*	*yumanensis*	Ensenada	0.01%	0.01%	0.01%	99.98%	Pure
197	PE	498_sp	*peninsularis*	*yumanensis*	Meling	0.01%	19.39%	0.01%	80.58%	BC
198	PE	65_YU	*peninsularis*	*yumanensis*	San Ignacio	0.01%	0.01%	0.01%	99.97%	mtDNA
199	PE	66_YU	*peninsularis*	*yumanensis*	San Ignacio	0.01%	0.01%	0.01%	99.98%	mtDNA
200	PE	72_YU	*peninsularis*	*yumanensis*	San Ignacio	0.01%	0.01%	0.01%	99.98%	mtDNA
201	PE	74_YU	*peninsularis*	*yumanensis*	San Ignacio	0.01%	35.77%	0.01%	64.22%	BC
202	PE	99_YU	*peninsularis*	*yumanensis*	San Ignacio	0.01%	49.70%	0.01%	50.29%	F1
203	YU3	113_YU	*yumanensis*	*yumanensis*	Loreto	0.01%	0.01%	0.01%	99.98%	Pure
204	YU3	114_YU	*yumanensis*	*yumanensis*	San Basilio	0.01%	36.20%	0.01%	63.79%	BC
205	YU3	115_YU	*yumanensis*	*yumanensis*	San Basilio	0.01%	40.42%	0.01%	59.56%	F1
206	PE	119_YU	*yumanensis*	*peninsularis*	San Basilio	0.01%	71.92%	0.01%	28.06%	BC
207	YU3	475_YU	*yumanensis*	*yumanensis*	San Basilio	0.01%	0.01%	0.01%	99.98%	Pure
208	YU3	476_YU	*yumanensis*	*yumanensis*	San Basilio	0.01%	0.01%	0.01%	99.98%	Pure
209	YU3	479_YU	*yumanensis*	*yumanensis*	San Basilio	0.01%	0.01%	0.01%	99.98%	Pure
210	YU3	483_YU	*yumanensis*	*yumanensis*	San Basilio	0.01%	0.01%	0.01%	99.98%	Pure
211	YU3	484_YU	*yumanensis*	*yumanensis*	San Basilio	0.01%	0.01%	0.01%	99.98%	Pure

212	YU3	486_YU	*californicus*	*californicus*	San Basilio	99.98%	0.01%	0.01%	0.01%	Pure
213	YU3	487_YU	*californicus*	*yumanensis*	San Basilio	0.01%	17.80%	0.01%	82.18%	BC
214	CA2	488_YU	*californicus*	*yumanensis*	San Basilio	0.01%	0.01%	0.01%	99.98%	mtDNA
215	YU2	152_YU	*yumanensis*	*yumanensis*	Mosqueda	5.53%	0.01%	0.01%	94.46%	BC
216	PE	425_YU	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
217	PE	432_YU	*peninsularis*	*peninsularis*	La Paz	0.01%	99.98%	0.01%	0.01%	Pure
218	YU3	474_YU	*yumanensis*	*yumanensis*	San Basilio	0.01%	0.01%	0.01%	99.98%	Pure

Chapter 3.

Appendix 3.1. List describing the type and amount of ectoparasites collected per bat individual that hold at least one type of ectoparasite.

ID	*Bat species*	Label	Site Label	Site	State	Bugs	Flies	Ticks	Mites	Fleas	Altitude	Latitude	Longitude
1	*Macrotus californicus*	MACA_120	Teste	Testera	BCS	0	2	0	0	0	598	23.764	-110.055
2	*Macrotus californicus*	MACA_121	Teste	Testera	BCS	0	2	0	1	0	598	23.764	-110.055
3	*Macrotus californicus*	MACA_122	Teste	Testera	BCS	0	2	0	0	0	598	23.764	-110.055
4	*Macrotus californicus*	MACA_123	Teste	Testera	BCS	0	2	0	2	0	598	23.764	-110.055
5	*Macrotus californicus*	MACA_124	Teste	Testera	BCS	0	0	0	2	0	598	23.764	-110.055
6	*Mormoops megalophylla*	MOME_125	Teste	Testera	BCS	0	1	0	0	0	598	23.764	-110.055
7	*Antrozous pallidus*	ANPA_126	Teste	Testera	BCS	0	0	0	0	0	598	23.764	-110.055
8	*Antrozous pallidus*	ANPA_127	Teste	Testera	BCS	0	1	1	0	0	598	23.764	-110.055
9	*Eptesicus fuscus*	EPFU_130	Teste	Testera	BCS	0	0	0	5	0	598	23.764	-110.055
10	*Eptesicus fuscus*	EPFU_131	Teste	Testera	BCS	0	0	0	2	0	598	23.764	-110.055
11	*Antrozous pallidus*	ANPA_134	Teso	Tesos	BCS	0	1	1	0	0	179	23.175	-109.611
12	*Antrozous pallidus*	ANPA_135	Teso	Tesos	BCS	1	0	0	1	0	179	23.175	-109.611
13	*Myotis peninsularis*	MYPE_141	Teso	Tesos	BCS	0	0	0	6	0	179	23.175	-109.611
14	*Myotis peninsularis*	MYPE_148	Teso	Tesos	BCS	0	0	0	2	0	179	23.175	-109.611
15	*Parastrellus hesperus*	PAHE_151	Mosq	Mosqueda	BC	0	0	2	0	0	6	32.156	-115.279
16	*Myotis yumanensis*	MYYU_152	Mosq	Mosqueda	BC	0	0	1	0	0	6	32.156	-115.279
17	*Myotis yumanensis*	MYYU_153	Mosq	Mosqueda	BC	0	0	4	0	0	6	32.156	-115.279
18	*Myotis yumanensis*	MYYU_155	Mosq	Mosqueda	BC	1	0	0	1	0	6	32.156	-115.279
19	*Myotis yumanensis*	MYVE_156	Mosq	Mosqueda	BC	0	0	0	6	0	6	32.156	-115.279
20	*Macrotus californicus*	MACA_157	Mosq	Mosqueda	BC	0	0	3	14	0	6	32.156	-115.279
21	*Myotis yumanensis*	MYYU_160	Mosq	Mosqueda	BC	0	0	1	0	0	6	32.156	-115.279
22	*Myotis yumanensis*	MYYU_161	Mosq	Mosqueda	BC	0	0	3	0	0	6	32.156	-115.279
23	*Myotis yumanensis*	MYYU_162	Mosq	Mosqueda	BC	0	0	1	0	0	6	32.156	-115.279
24	*Eptesicus fuscus*	EPFU_165	Mosq	Mosqueda	BC	0	0	1	0	0	6	32.156	-115.279
25	*Myotis yumanensis*	MYYU_167	Mosq	Mosqueda	BC	0	0	3	5	0	6	32.156	-115.279
26	*Myotis yumanensis*	MYYU_168	Mosq	Mosqueda	BC	0	0	0	3	0	6	32.156	-115.279
27	*Myotis yumanensis*	MYYU_169	Mosq	Mosqueda	BC	0	1	2	0	0	6	32.156	-115.279

28	*Myotis yumanensis*	MYYU_170	Mosq	Mosqueda	BC	0	0	0	3	0	6	32.156	-115.279
29	*Myotis yumanensis*	MYYU_172	Mosq	Mosqueda	BC	0	0	0	2	0	6	32.156	-115.279
30	*Myotis californicus*	MYCA_173	Mosq	Mosqueda	BC	0	0	0	1	0	6	32.156	-115.279
31	*Myotis yumanensis*	MYYU_174	Mosq	Mosqueda	BC	0	0	0	1	0	6	32.156	-115.279
32	*Eptesicus fuscus*	EPFU_175	Mosq	Mosqueda	BC	0	0	1	0	0	6	32.156	-115.279
33	*Antrozous pallidus*	ANPA_176	Mosq	Mosqueda	BC	0	0	2	0	0	6	32.156	-115.279
34	*Macrotus californicus*	MACA_177	Mosq	Mosqueda	BC	0	1	0	0	0	6	32.156	-115.279
35	*Macrotus californicus*	MACA_179	Mosq	Mosqueda	BC	0	1	0	0	0	6	32.156	-115.279
36	*Parastrellus hesperus*	PAHE_180	Chaba	Chabacanos	BC	2	0	0	19	0	758	32.567	-116.493
37	*Eptesicus fuscus*	EPFU_181	Chaba	Chabacanos	BC	0	0	0	0	0	758	32.567	-116.493
38	*Parastrellus hesperus*	PAHE_183	Chaba	Chabacanos	BC	2	0	0	12	0	758	32.567	-116.493
39	*Parastrellus hesperus*	PAHE_184	Chaba	Chabacanos	BC	2	0	0	6	0	758	32.567	-116.493
40	*Parastrellus hesperus*	PAHE_185	Chaba	Chabacanos	BC	3	0	0	4	0	758	32.567	-116.493
41	*Eptesicus fuscus*	EPFU_187	Chaba	Chabacanos	BC	0	0	0	40	0	758	32.567	-116.493
42	*Myotis yumanensis*	MYYU_188	Chaba	Chabacanos	BC	0	0	0	1	0	758	32.567	-116.493
43	*Antrozous pallidus*	ANPA_191	Chaba	Chabacanos	BC	0	5	0	8	0	758	32.567	-116.493
44	*Antrozous pallidus*	ANPA_192	Chaba	Chabacanos	BC	0	1	0	2	0	758	32.567	-116.493
45	*Antrozous pallidus*	ANPA_193	Chaba	Chabacanos	BC	0	2	1	21	0	758	32.567	-116.493
46	*Antrozous pallidus*	ANPA_194	Chaba	Chabacanos	BC	0	1	0	7	0	758	32.567	-116.493
47	*Parastrellus hesperus*	PAHE_195	Chaba	Chabacanos	BC	2	0	0	3	0	758	32.567	-116.493
48	*Parastrellus hesperus*	PAHE_197	Chaba	Chabacanos	BC	6	0	0	14	0	758	32.567	-116.493
49	*Parastrellus hesperus*	PAHE_198	Chaba	Chabacanos	BC	5	0	0	21	0	758	32.567	-116.493
50	*Parastrellus hesperus*	PAHE_199	Chaba	Chabacanos	BC	3	0	0	13	0	758	32.567	-116.493
51	*Antrozous pallidus*	ANPA_201	Chaba	Chabacanos	BC	1	0	1	6	0	758	32.567	-116.493
52	*Antrozous pallidus*	ANPA_202	Chaba	Chabacanos	BC	0	0	5	30	0	758	32.567	-116.493
53	*Antrozous pallidus*	ANPA_204	Chaba	Chabacanos	BC	0	2	3	0	0	758	32.567	-116.493
54	*Parastrellus hesperus*	PAHE_206	Ense	Ensenada	BC	2	0	0	0	0	53	31.770	-116.520
55	*Parastrellus hesperus*	PAHE_207	Ense	Ensenada	BC	1	0	0	0	0	53	31.770	-116.520
56	*Parastrellus hesperus*	PAHE_208	Ense	Ensenada	BC	0	0	0	2	0	53	31.770	-116.520
57	*Parastrellus hesperus*	PAHE_214	SanFe	San Fernando	BC	0	0	0	2	0	469	29.971	-115.237
58	*Parastrellus hesperus*	PAHE_215	SanFe	San Fernando	BC	0	0	0	22	0	469	29.971	-115.237
59	*Parastrellus hesperus*	PAHE_217	SanFe	San Fernando	BC	0	0	0	1	0	469	29.971	-115.237
60	*Parastrellus hesperus*	PAHE_221	SanFe	San Fernando	BC	0	0	0	5	0	469	29.971	-115.237
61	*Parastrellus hesperus*	PAHE_222	SanFe	San Fernando	BC	0	0	0	3	0	469	29.971	-115.237
62	*Myotis californicus*	MYCA_223	SanFe	San Fernando	BC	0	0	0	3	0	469	29.971	-115.237

63	*Myotis californicus*	MYCA_224	SanFe	San Fernando	BC	0	0	0	1	0	469	29.971	-115.237
64	*Myotis californicus*	MYCA_226	SanFe	San Fernando	BC	0	3	0	0	0	469	29.971	-115.237
65	*Myotis volans*	MYVO_227	SanFe	San Fernando	BC	0	6	0	1	0	469	29.971	-115.237
66	*Myotis volans*	MYVO_228	SanFe	San Fernando	BC	0	1	0	0	0	469	29.971	-115.237
67	*Myotis volans*	MYVO_229	SanFe	San Fernando	BC	0	1	0	0	0	469	29.971	-115.237
68	*Myotis volans*	MYVO_230	SanFe	San Fernando	BC	0	5	0	0	0	469	29.971	-115.237
69	*Myotis californicus*	MYCA_231	SanFe	San Fernando	BC	0	0	0	2	0	469	29.971	-115.237
70	*Parastrellus hesperus*	PAHE_232	SanFe	San Fernando	BC	0	0	0	4	0	469	29.971	-115.237
71	*Parastrellus hesperus*	PAHE_233	SanFe	San Fernando	BC	0	0	0	15	0	469	29.971	-115.237
72	*Parastrellus hesperus*	PAHE_234	SanFe	San Fernando	BC	0	0	0	4	0	469	29.971	-115.237
73	*Parastrellus hesperus*	PAHE_235	SanFe	San Fernando	BC	0	0	0	16	0	469	29.971	-115.237
74	*Parastrellus hesperus*	PAHE_237	SanFe	San Fernando	BC	0	0	0	2	0	469	29.971	-115.237
75	*Myotis californicus*	MYCA_240	SanFe	San Fernando	BC	0	2	0	0	0	469	29.971	-115.237
76	*Myotis californicus*	MYCA_242	SanFe	San Fernando	BC	0	1	0	0	0	469	29.971	-115.237
77	*Myotis volans*	MYVO_244	SanFe	San Fernando	BC	0	0	0	1	0	469	29.971	-115.237
78	*Myotis volans*	MYVO_245	SanFe	San Fernando	BC	0	4	0	0	0	469	29.971	-115.237
79	*Myotis volans*	MYVO_246	SanFe	San Fernando	BC	0	1	0	0	0	469	29.971	-115.237
80	*Myotis volans*	MYVO_247	SanFe	San Fernando	BC	0	1	0	0	0	469	29.971	-115.237
81	*Myotis californicus*	MYCA_248	Rosa	Rosarito	BC	2	1	0	0	0	95	28.613	-114.047
82	*Myotis californicus*	MYCA_249	Rosa	Rosarito	BC	0	1	0	0	0	95	28.613	-114.047
83	*Nyctinomops femorosaccus*	NYFE_251	SanIg	San Ignacio	BCS	0	0	0	2	0	122	27.297	-112.898
84	*Nyctinomops femorosaccus*	NYFE_252	SanIg	San Ignacio	BCS	0	0	0	4	0	122	27.297	-112.898
85	*Eptesicus fuscus*	EPFU_257	SanIg	San Ignacio	BCS	0	0	0	1	0	122	27.297	-112.898
86	*Eptesicus fuscus*	EPFU_259	SanIg	San Ignacio	BCS	0	0	0	2	0	122	27.297	-112.898
87	*Eptesicus fuscus*	EPFU_261	SanIg	San Ignacio	BCS	0	0	0	25	0	122	27.297	-112.898
88	*Nyctinomops femorosaccus*	NYFE_262	SanIg	San Ignacio	BCS	0	0	0	20	0	122	27.297	-112.898
89	*Nyctinomops femorosaccus*	NYFE_263	SanIg	San Ignacio	BCS	0	0	0	22	0	122	27.297	-112.898
90	*Leptonycteris yerbabuenae*	LEYE_264	SanIg	San Ignacio	BCS	0	18	0	0	0	122	27.297	-112.898
91	*Leptonycteris yerbabuenae*	LEYE_265	SanIg	San Ignacio	BCS	0	9	0	0	0	122	27.297	-112.898

92	*Leptonycteris yerbabuenae*	LEYE_266	SanIg	San Ignacio	BCS	0	8	0	0	0	122	27.297	-112.898
93	*Nyctinomops femorosaccus*	NYFE_267	SanIg	San Ignacio	BCS	0	0	0	0	0	122	27.297	-112.898
94	*Macrotus californicus*	MACA_268	SanIg	San Ignacio	BCS	0	7	0	5	0	122	27.297	-112.898
95	*Antrozous pallidus*	ANPA_269	SanIg	San Ignacio	BCS	0	1	0	3	0	122	27.297	-112.898
96	*Antrozous pallidus*	ANPA_270	SanIg	San Ignacio	BCS	0	0	2	5	0	122	27.297	-112.898
97	*Antrozous pallidus*	ANPA_271	SanIg	San Ignacio	BCS	0	1	0	0	0	122	27.297	-112.898
98	*Antrozous pallidus*	ANPA_272	SanIg	San Ignacio	BCS	0	0	0	13	0	122	27.297	-112.898
99	*Antrozous pallidus*	ANPA_273	SanIg	San Ignacio	BCS	0	1	0	0	0	122	27.297	-112.898
100	*Antrozous pallidus*	ANPA_274	SanIg	San Ignacio	BCS	0	0	2	0	0	122	27.297	-112.898
101	*Antrozous pallidus*	ANPA_276	SanIg	San Ignacio	BCS	1	0	0	0	0	122	27.297	-112.898
102	*Leptonycteris yerbabuenae*	LEYE_277	SanIg	San Ignacio	BCS	0	20	0	0	0	123	27.298	-112.905
103	*Antrozous pallidus*	ANPA_278	Reque	Requeson	BCS	0	0	0	11	0	12	26.638	-111.833
104	*Myotis vivesi*	MYVI_279	Reque	Requeson	BCS	0	0	0	5	0	12	26.638	-111.833
105	*Myotis californicus*	MYCA_280	SanBa	San Basilio	BCS	0	0	0	0	0	19	26.371	-111.429
106	*Parastrellus hesperus*	PAHE_283	SanBa	San Basilio	BCS	0	0	1	0	0	19	26.371	-111.429
107	*Myotis yumanensis*	MYYU_286	SanBa	San Basilio	BCS	0	2	0	0	0	19	26.371	-111.429
108	*Myotis yumanensis*	MYYU_287	SanBa	San Basilio	BCS	0	0	2	3	0	19	26.371	-111.429
109	*Myotis yumanensis*	MYYU_288	SanBa	San Basilio	BCS	0	0	1	0	0	19	26.371	-111.429
110	*Myotis yumanensis*	MYYU_289	SanBa	San Basilio	BCS	0	0	3	0	0	19	26.371	-111.429
111	*Myotis yumanensis*	MYYU_290	SanBa	San Basilio	BCS	0	0	1	2	0	19	26.371	-111.429
112	*Parastrellus hesperus*	PAHE_293	SanBa	San Basilio	BCS	0	0	0	10	0	19	26.371	-111.429
113	*Parastrellus hesperus*	PAHE_294	SanBa	San Basilio	BCS	0	0	1	6	0	19	26.371	-111.429
114	*Myotis yumanensis*	MYYU_295	SanBa	San Basilio	BCS	0	3	0	0	0	19	26.371	-111.429
115	*Myotis yumanensis*	MYYU_297	SanBa	San Basilio	BCS	0	0	0	1	0	19	26.371	-111.429
116	*Myotis yumanensis*	MYYU_299	SanBa	San Basilio	BCS	0	0	1	0	0	19	26.371	-111.429
117	*Myotis vivesi*	MYVI_301	SanBa	San Basilio	BCS	0	1	1	2	0	19	26.371	-111.429
118	*Myotis vivesi*	MYVI_302	SanBa	San Basilio	BCS	0	3	0	2	0	19	26.371	-111.429
119	*Myotis yumanensis*	MYYU_303	SanBa	San Basilio	BCS	0	0	3	5	0	19	26.371	-111.429
120	*Myotis yumanensis*	MYYU_304	SanBa	San Basilio	BCS	0	0	3	2	0	19	26.371	-111.429
121	*Myotis yumanensis*	MYYU_305	SanBa	San Basilio	BCS	0	0	1	0	0	19	26.371	-111.429
122	*Macrotus californicus*	MACA_306	SanBa	San Basilio	BCS	0	2	1	7	0	19	26.371	-111.429
123	*Myotis yumanensis*	MYYU_307	SanBa	San Basilio	BCS	0	0	0	1	0	19	26.371	-111.429

124	*Eptesicus fuscus*	EPFU_308	Poza	Pocitas	BCS	0	0	0	8	0	64	24.403	-111.104
125	*Antrozous pallidus*	ANPA_309	Poza	Pocitas	BCS	0	1	0	0	0	64	24.403	-111.104
126	*Antrozous pallidus*	ANPA_310	Poza	Pocitas	BCS	0	5	0	9	0	64	24.403	-111.104
127	*Eptesicus fuscus*	EPFU_311	Poza	Pocitas	BCS	0	0	0	10	0	64	24.403	-111.104
128	*Antrozous pallidus*	ANPA_312	Poza	Pocitas	BCS	0	0	0	2	0	64	24.403	-111.104
129	*Antrozous pallidus*	ANPA_313	Poza	Pocitas	BCS	0	0	0	6	0	64	24.403	-111.104
130	*Antrozous pallidus*	ANPA_314	Poza	Pocitas	BCS	0	1	0	4	0	64	24.403	-111.104
131	*Macrotus californicus*	MACA_315	SanPe	San Pedro	BCS	0	0	0	5	0	8	23.390	-110.212
132	*Parastrellus hesperus*	PAHE_316	SanPe	San Pedro	BCS	0	0	0	5	0	8	23.390	-110.212
133	*Parastrellus hesperus*	PAHE_317	SanPe	San Pedro	BCS	0	0	0	8	0	8	23.390	-110.212
134	*Myotis californicus*	MYCA_318	SanPe	San Pedro	BCS	0	0	0	2	0	8	23.390	-110.212
135	*Myotis volans*	MYVO_319	SanPe	San Pedro	BCS	0	0	0	4	0	8	23.390	-110.212
136	*Parastrellus hesperus*	PAHE_320	SanPe	San Pedro	BCS	0	0	0	1	0	8	23.390	-110.212
137	*Eptesicus fuscus*	EPFU_325	SanPe	San Pedro	BCS	0	0	0	4	0	8	23.390	-110.212
138	*Antrozous pallidus*	ANPA_326	SanPe	San Pedro	BCS	0	0	0	3	0	8	23.390	-110.212
139	*Parastrellus hesperus*	PAHE_338	Faro	Faro	BCS	0	0	1	5	0	6	23.427	-110.233
140	*Parastrellus hesperus*	PAHE_340	Faro	Faro	BCS	0	0	0	5	0	6	23.427	-110.233
141	*Eptesicus fuscus*	EPFU_343	Faro	Faro	BCS	0	0	0	30	0	6	23.427	-110.233
142	*Eptesicus fuscus*	EPFU_344	Faro	Faro	BCS	0	0	0	5	0	6	23.427	-110.233
143	*Antrozous pallidus*	ANPA_347	Teso	Tesos	BCS	0	1	0	1	0	179	23.175	-109.611
144	*Antrozous pallidus*	ANPA_348	Teso	Tesos	BCS	1	0	0	2	0	179	23.175	-109.611
145	*Antrozous pallidus*	ANPA_349	Teso	Tesos	BCS	1	1	0	3	0	179	23.175	-109.611
146	*Antrozous pallidus*	ANPA_350	Teso	Tesos	BCS	0	0	0	18	0	179	23.175	-109.611
147	*Antrozous pallidus*	ANPA_351	Teso	Tesos	BCS	0	0	0	4	0	179	23.175	-109.611
148	*Myotis peninsularis*	MYPE_353	Teso	Tesos	BCS	0	0	0	5	0	179	23.175	-109.611
149	*Myotis peninsularis*	MYPE_357	Teso	Tesos	BCS	0	0	0	1	0	179	23.175	-109.611
150	*Myotis peninsularis*	MYPE_358	Teso	Tesos	BCS	0	0	0	2	0	179	23.175	-109.611
151	*Myotis peninsularis*	MYPE_359	Teso	Tesos	BCS	0	0	0	1	0	179	23.175	-109.611
152	*Myotis peninsularis*	MYPE_370	Teste	Testera	BCS	0	0	0	10	0	598	23.764	-110.055
153	*Myotis peninsularis*	MYPE_371	Teste	Testera	BCS	0	0	0	6	0	598	23.764	-110.055
154	*Myotis peninsularis*	MYPE_373	Teste	Testera	BCS	0	0	0	4	0	598	23.764	-110.055
155	*Myotis peninsularis*	MYPE_374	Teste	Testera	BCS	0	0	0	4	0	598	23.764	-110.055
156	*Myotis yumanensis*	MYPE_375	Teste	Testera	BCS	0	0	0	6	0	598	23.764	-110.055
157	*Myotis peninsularis*	MYPE_377	Teste	Testera	BCS	0	0	0	6	0	598	23.764	-110.055
158	*Mormoops megalophylla*	MOME_378	Parra	Parra	BCS	0	0	0	6	0	606	23.748	-110.058

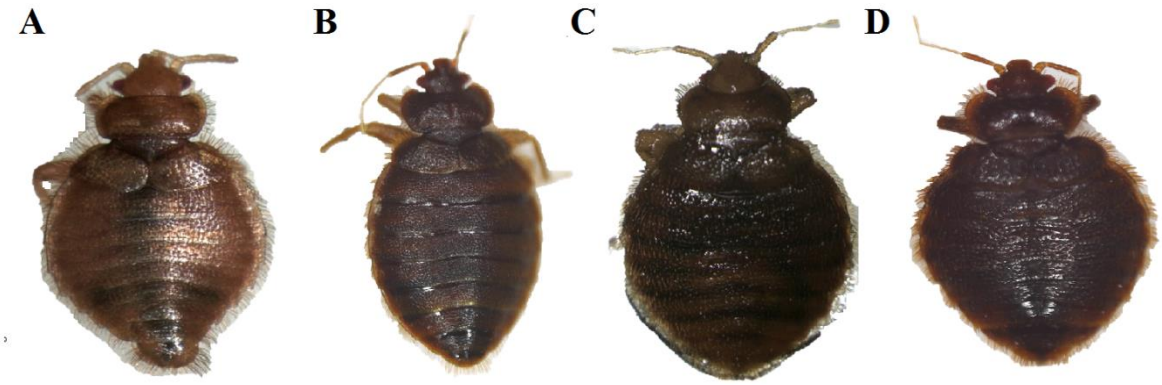
159	*Mormoops megalophylla*	MOME_380	Parra	Parra	BCS	0	1	0	0	0	606	23.748	-110.058
160	*Myotis peninsularis*	MYPE_383	Teste	Testera	BCS	0	0	0	8	0	598	23.764	-110.055
161	*Myotis peninsularis*	MYPE_387	Teste	Testera	BCS	0	0	0	0	0	598	23.764	-110.055
162	*Myotis peninsularis*	MYPE_388	Teste	Testera	BCS	0	0	0	1	0	598	23.764	-110.055
163	*Myotis peninsularis*	MYPE_389	Teste	Testera	BCS	0	0	0	2	0	598	23.764	-110.055
164	*Myotis peninsularis*	MYPE_398	Teste	Testera	BCS	0	0	0	17	0	598	23.764	-110.055
165	*Myotis peninsularis*	MYPE_399	Teste	Testera	BCS	0	0	0	15	0	598	23.764	-110.055
166	*Myotis peninsularis*	MYPE_400	Teste	Testera	BCS	0	0	0	12	0	598	23.764	-110.055
167	*Myotis peninsularis*	MYPE_402	LaPaz	La Paz	BCS	0	0	0	1	0	31	24.103	-110.306
168	*Myotis peninsularis*	MYPE_407	LaPaz	La Paz	BCS	0	0	0	11	0	31	24.103	-110.306
169	*Myotis peninsularis*	MYPE_408	LaPaz	La Paz	BCS	0	0	0	1	0	31	24.103	-110.306
170	*Myotis peninsularis*	MYPE_409	LaPaz	La Paz	BCS	0	0	0	2	0	31	24.103	-110.306
171	*Myotis peninsularis*	MYPE_410	LaPaz	La Paz	BCS	0	0	0	4	0	31	24.103	-110.306
172	*Myotis peninsularis*	MYPE_412	LaPaz	La Paz	BCS	0	0	0	2	0	31	24.103	-110.306
173	*Myotis peninsularis*	MYPE_422	LaPaz	La Paz	BCS	0	0	0	2	0	31	24.103	-110.306
174	*Myotis peninsularis*	MYPE_428	LaPaz	La Paz	BCS	0	0	0	1	0	31	24.103	-110.306
175	*Myotis yumanensis*	MYYU_432	LaPaz	La Paz	BCS	0	0	0	5	0	31	24.103	-110.306
176	*Myotis peninsularis*	MYPE_435	LaPaz	La Paz	BCS	0	0	1	0	0	31	24.103	-110.306
177	*Myotis peninsularis*	MYPE_436	LaPaz	La Paz	BCS	0	0	0	2	0	31	24.103	-110.306
178	*Parastrellus hesperus*	PAHE_445	Faro	Faro	BCS	0	0	0	3	0	6	23.427	-110.233
179	*Myotis peninsularis*	MYPE_447	Boca	Boca de Sierra	BCS	0	0	0	4	0	294	23.385	-109.819
180	*Myotis peninsularis*	MYPE_449	Teste	Testera	BCS	0	0	0	3	0	598	23.764	-110.055
181	*Myotis peninsularis*	MYPE_450	Teste	Testera	BCS	0	0	1	2	0	598	23.764	-110.055
182	*Myotis peninsularis*	MYPE_451	Teste	Testera	BCS	0	0	1	0	0	598	23.764	-110.055
183	*Myotis peninsularis*	MYPE_454	Teste	Testera	BCS	0	0	0	2	0	598	23.764	-110.055
184	*Myotis peninsularis*	MYPE_455	Teste	Testera	BCS	0	0	1	0	0	598	23.764	-110.055
185	*Myotis peninsularis*	MYPE_457	Teste	Testera	BCS	0	0	1	0	0	598	23.764	-110.055
186	*Myotis peninsularis*	MYPE_458	Teste	Testera	BCS	0	0	0	1	0	598	23.764	-110.055
187	*Myotis peninsularis*	MYPE_459	Teste	Testera	BCS	0	0	0	0	0	598	23.764	-110.055
188	*Myotis peninsularis*	MYPE_465	Teste	Testera	BCS	0	0	0	1	0	598	23.764	-110.055
189	*Myotis peninsularis*	MYPE_469	LaPaz	La Paz	BCS	0	0	0	9	0	31	24.104	-110.306
190	*Myotis peninsularis*	MYPE_470	LaPaz	La Paz	BCS	0	0	0	21	0	31	24.104	-110.306
191	*Parastrellus hesperus*	PAHE_473	SanBa	San Basilio	BCS	0	0	0	1	0	34	26.371	-111.429
192	*Myotis velifer*	MYVE_474	SanBa	San Basilio	BCS	0	0	0	1	0	34	26.371	-111.429
193	*Myotis californicus*	MYCA_475	SanBa	San Basilio	BCS	0	0	2	2	0	34	26.371	-111.429

194	*Myotis californicus*	MYCA_476	SanBa	San Basilio	BCS	0	2	2	0	0	34	26.371	-111.429
195	*Myotis californicus*	MYCA_479	SanBa	San Basilio	BCS	0	0	1	0	1	34	26.371	-111.429
196	*Myotis vivesi*	MYVI_480	SanBa	San Basilio	BCS	0	2	8	8	0	34	26.371	-111.429
197	*Macrotus californicus*	MACA_481	SanBa	San Basilio	BCS	0	2	0	0	0	34	26.371	-111.429
198	*Myotis velifer*	MYVE_483	SanBa	San Basilio	BCS	0	0	0	0	1	34	26.371	-111.429
199	*Myotis californicus*	MYCA_484	SanBa	San Basilio	BCS	0	0	5	0	1	34	26.371	-111.429
200	*Macrotus californicus*	MACA_485	SanBa	San Basilio	BCS	0	2	1	0	0	34	26.371	-111.429
201	*Myotis californicus*	MYCA_487	SanBa	San Basilio	BCS	0	0	0	0	2	34	26.371	-111.429
202	*Myotis californicus*	MYCA_488	SanBa	San Basilio	BCS	0	0	1	0	0	34	26.371	-111.429
203	*Myotis californicus*	MYCA_494	Meli	Meling	BC	0	0	0	20	0	646	30.972	-115.744
204	*Myotis californicus*	MYCA_496	Meli	Meling	BC	0	0	0	20	0	646	30.972	-115.744
205	*Myotis californicus*	MYCA_499	Meli	Meling	BC	1	0	0	0	0	646	30.972	-115.744
206	*Eptesicus fuscus*	EPFU_500	Meli	Meling	BC	0	0	0	20	0	646	30.972	-115.744
207	*Antrozous pallidus*	ANPA_501	Meli	Meling	BC	0	1	0	0	0	646	30.972	-115.744
208	*Eptesicus fuscus*	EPFU_502	Meli	Meling	BC	0	0	0	20	0	646	30.972	-115.744
209	*Antrozous pallidus*	ANPA_505	Meli	Meling	BC	0	1	0	0	0	646	30.972	-115.744
210	*Antrozous pallidus*	ANPA_506	Meli	Meling	BC	0	2	0	0	0	646	30.972	-115.744
211	*Antrozous pallidus*	ANPA_507	Meli	Meling	BC	0	1	0	0	0	646	30.972	-115.744
212	*Antrozous pallidus*	ANPA_508	Meli	Meling	BC	0	1	1	0	0	646	30.972	-115.744
213	*Antrozous pallidus*	ANPA_509	Meli	Meling	BC	0	0	3	0	0	646	30.972	-115.744
214	*Parastrellus hesperus*	PAHE_512	Jolla	Jolla	BC	5	0	0	0	0	1459	30.920	-115.601
215	*Parastrellus hesperus*	PAHE_513	Jolla	Jolla	BC	5	0	0	3	0	1459	30.920	-115.601
216	*Parastrellus hesperus*	PAHE_514	Jolla	Jolla	BC	1	1	0	0	0	1459	30.920	-115.601
217	*Eptesicus fuscus*	EPFU_516	Jolla	Jolla	BC	0	0	0	6	0	1459	30.920	-115.601
218	*Eptesicus fuscus*	EPFU_517	Jolla	Jolla	BC	0	0	0	1	0	1459	30.920	-115.601
219	*Choeronycteris mexicana*	CHME_518	Jolla	Jolla	BC	0	10	0	0	0	1459	30.920	-115.601
220	*Eptesicus fuscus*	EPFU_519	Jolla	Jolla	BC	0	0	0	7	0	1459	30.920	-115.601
221	*Eptesicus fuscus*	EPFU_520	Jolla	Jolla	BC	0	0	0	0	0	1459	30.920	-115.601
222	*Eptesicus fuscus*	EPFU_522	Jolla	Jolla	BC	0	0	0	1	0	1459	30.920	-115.601
223	*Antrozous pallidus*	ANPA_525	Jolla	Jolla	BC	0	5	1	7	0	1459	30.920	-115.601
224	*Parastrellus hesperus*	PAHE_526	Jolla	Jolla	BC	13	0	0	0	0	1459	30.920	-115.601
225	*Parastrellus hesperus*	PAHE_527	Jolla	Jolla	BC	5	0	0	7	0	1459	30.920	-115.601
226	*Parastrellus hesperus*	PAHE_528	Jolla	Jolla	BC	1	0	0	5	0	1459	30.920	-115.601
227	*Parastrellus hesperus*	PAHE_529	Jolla	Jolla	BC	5	0	0	2	0	1459	30.920	-115.601
228	*Parastrellus hesperus*	PAHE_530	Jolla	Jolla	BC	0	0	0	2	0	1459	30.920	-115.601

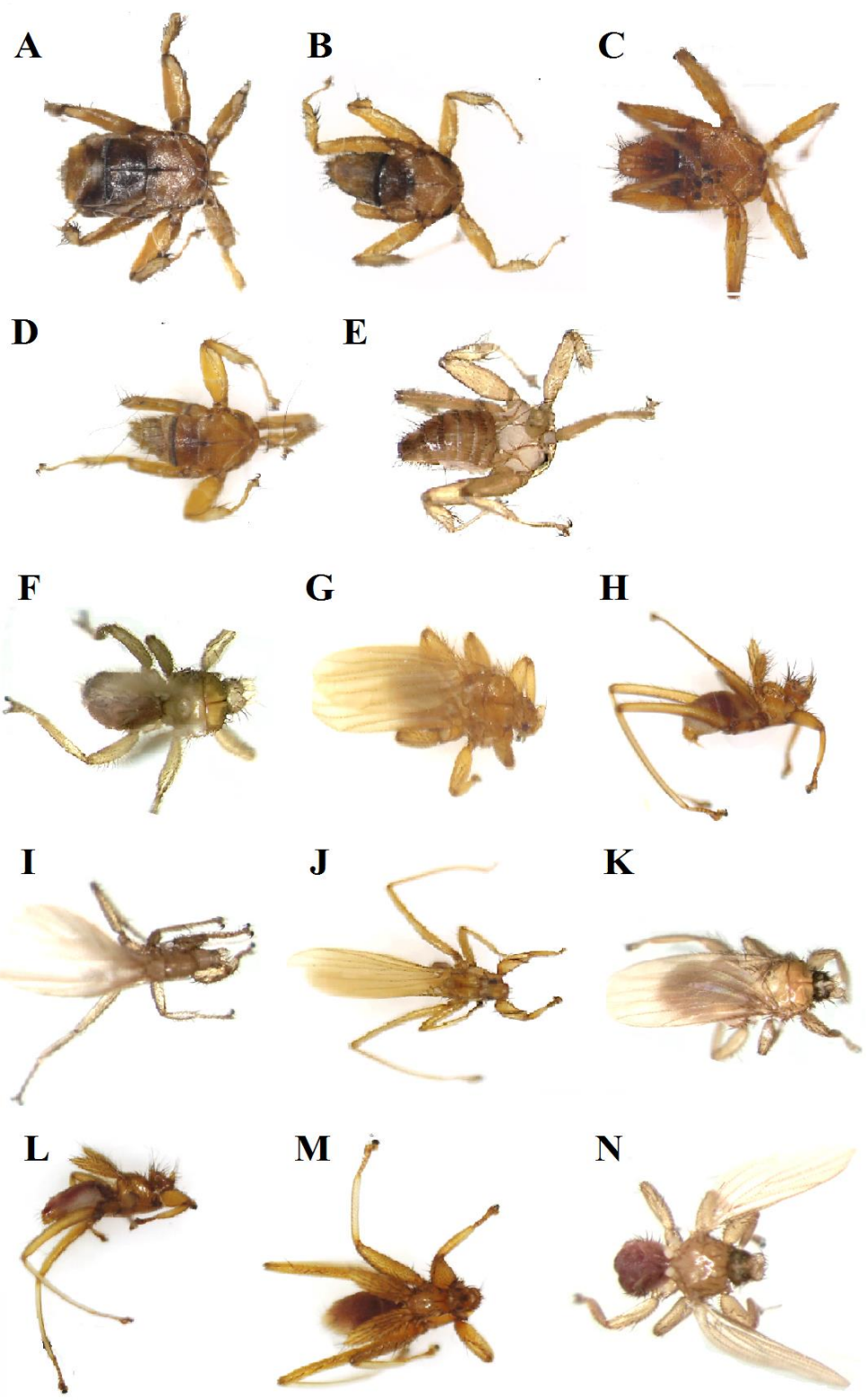
229	*Myotis californicus*	MYCA_531	Jolla	Jolla	BC	0	2	0	0	0	1459	30.920	-115.601
230	*Parastrellus hesperus*	PAHE_532	Jolla	Jolla	BC	0	0	0	5	0	1459	30.920	-115.601
231	*Parastrellus hesperus*	PAHE_533	Jolla	Jolla	BC	2	0	0	0	0	1459	30.920	-115.601
232	*Antrozous pallidus*	ANPA_534	Jolla	Jolla	BC	0	3	4	6	0	1459	30.920	-115.601
233	*Parastrellus hesperus*	PAHE_535	Chaba	Chabacanos	BC	3	0	0	0	0	763	32.566	-116.493
234	*Parastrellus hesperus*	PAHE_536	Chaba	Chabacanos	BC	1	0	0	1	0	763	32.566	-116.493
235	*Parastrellus hesperus*	PAHE_538	Chaba	Chabacanos	BC	0	0	0	20	0	763	32.566	-116.493
236	*Parastrellus hesperus*	PAHE_539	Chaba	Chabacanos	BC	4	0	0	20	0	763	32.566	-116.493
237	*Myotis yumanensis*	MYYU_540	Chaba	Chabacanos	BC	0	0	0	3	0	763	32.566	-116.493
238	*Myotis yumanensis*	MYYU_541	Chaba	Chabacanos	BC	0	0	0	1	0	763	32.566	-116.493
239	*Antrozous pallidus*	ANPA_544	Chaba	Chabacanos	BC	0	1	4	1	0	763	32.566	-116.493
240	*Antrozous pallidus*	ANPA_545	Chaba	Chabacanos	BC	0	4	5	0	0	763	32.566	-116.493
241	*Antrozous pallidus*	ANPA_546	Chaba	Chabacanos	BC	0	4	5	39	0	763	32.566	-116.493
242	*Antrozous pallidus*	ANPA_547	Chaba	Chabacanos	BC	0	0	0	32	0	763	32.566	-116.493
243	*Parastrellus hesperus*	PAHE_548	Chaba	Chabacanos	BC	1	0	0	8	0	763	32.566	-116.493
244	*Parastrellus hesperus*	PAHE_549	Chaba	Chabacanos	BC	4	0	0	11	0	763	32.566	-116.493
245	*Parastrellus hesperus*	PAHE_550	Chaba	Chabacanos	BC	0	0	0	8	0	763	32.566	-116.493
246	*Parastrellus hesperus*	PAHE_551	Chaba	Chabacanos	BC	2	0	0	7	0	763	32.566	-116.493
247	*Parastrellus hesperus*	PAHE_555	Chaba	Chabacanos	BC	1	0	0	8	0	763	32.566	-116.493
248	*Antrozous pallidus*	ANPA_556	Chaba	Chabacanos	BC	0	2	2	11	0	763	32.566	-116.493
249	*Antrozous pallidus*	ANPA_557	Chaba	Chabacanos	BC	0	1	2	19	0	763	32.566	-116.493
250	*Antrozous pallidus*	ANPA_558	Chaba	Chabacanos	BC	0	0	3	5	0	763	32.566	-116.493
251	*Antrozous pallidus*	ANPA_559	Chaba	Chabacanos	BC	0	0	6	21	0	763	32.566	-116.493
252	*Antrozous pallidus*	ANPA_560	Chaba	Chabacanos	BC	0	2	7	6	0	763	32.566	-116.493
253	*Parastrellus hesperus*	PAHE_562	Chaba	Chabacanos	BC	1	0	0	1	0	763	32.566	-116.493
254	*Myotis californicus*	MYCA_563	Chaba	Chabacanos	BC	1	0	1	0	0	763	32.566	-116.493
255	*Eptesicus fuscus*	EPFU_564	Chaba	Chabacanos	BC	0	0	0	12	0	763	32.566	-116.493
256	*Myotis sv*	MYsp_566	Ures	Ures	Sonora	0	1	0	2	0	396	29.433	-110.376
257	*Myotis sv*	MYsp_567	Ures	Ures	Sonora	0	2	0	2	0	396	29.433	-110.376
258	*Myotis sv*	MYsp_568	Ures	Ures	Sonora	0	0	0	1	0	396	29.433	-110.376
259	*Myotis sv*	MYsp_569	Ures	Ures	Sonora	0	0	0	26	0	396	29.433	-110.376
260	*Myotis sv*	MYsp_570	Ures	Ures	Sonora	0	2	0	20	0	396	29.433	-110.376
261	*Myotis sv*	MYsp_573	Ures	Ures	Sonora	0	0	0	7	0	396	29.433	-110.376
262	*Myotis sv*	MYsp_574	Ures	Ures	Sonora	0	0	0	4	0	396	29.433	-110.376
263	*Myotis sv*	MYsp_575	Ures	Ures	Sonora	0	1	0	6	0	396	29.433	-110.376

264	*Myotis sv*	MYsp_579	Ures	Ures	Sonora	0	0	0	1	0	396	29.433	-110.376
265	*Myotis sv*	MYsp_581	Ures	Ures	Sonora	0	1	0	0	0	396	29.433	-110.376
266	*Myotis sv*	MYsp_587	Ures	Ures	Sonora	0	0	0	1	0	396	29.433	-110.376
267	*Myotis sv*	MYsp_588	Ures	Ures	Sonora	0	1	0	3	0	396	29.433	-110.376
268	*Myotis sv*	MYsp_589	Ures	Ures	Sonora	0	1	0	0	0	396	29.433	-110.376
269	*Myotis sv*	MYsp_590	Ures	Ures	Sonora	0	0	0	1	0	396	29.433	-110.376
270	*Myotis sv*	MYsp_591	Ures	Ures	Sonora	0	0	0	9	0	396	29.433	-110.376
271	*Myotis sv*	MYsp_592	Ures	Ures	Sonora	0	0	0	1	0	396	29.433	-110.376
272	*Myotis sv*	MYsp_593	Ures	Ures	Sonora	0	0	0	6	0	396	29.433	-110.376
273	*Myotis sv*	MYsp_595	Ures	Ures	Sonora	0	2	0	26	0	396	29.433	-110.376
274	*Myotis sv*	MYsp_596	Ures	Ures	Sonora	0	0	0	2	0	396	29.433	-110.376
275	*Myotis sv*	MYsp_598	Ures	Ures	Sonora	0	0	0	2	0	396	29.433	-110.376
276	*Myotis sv*	MYsp_599	Ures	Ures	Sonora	0	0	0	4	0	396	29.433	-110.376
277	*Myotis sv*	MYsp_600	Ures	Ures	Sonora	0	0	0	4	0	396	29.433	-110.376
278	*Myotis sv*	MYsp_601	Ures	Ures	Sonora	0	0	0	4	0	396	29.433	-110.376
279	*Myotis californicus*	MYCA_603	Prima	Primavera	Jalisco	0	0	0	1	0	1463	20.679	-103.602
280	*Myotis californicus*	MYCA_604	Prima	Primavera	Jalisco	0	0	0	4	0	1463	20.679	-103.602
281	*Sturnira parvidens*	STPA_605	Prima	Primavera	Jalisco	0	0	0	0	0	1463	20.679	-103.602
282	*Sturnira parvidens*	STPA_606	Prima	Primavera	Jalisco	0	4	0	1	0	1463	20.679	-103.602
283	*Sturnira parvidens*	STPA_607	Prima	Primavera	Jalisco	0	6	0	4	0	1463	20.679	-103.602
284	*Sturnira parvidens*	STPA_608	Prima	Primavera	Jalisco	0	1	0	2	0	1463	20.679	-103.602
285	*Sturnira parvidens*	STPA_609	Prima	Primavera	Jalisco	0	1	0	0	0	1463	20.679	-103.602
286	*Sturnira parvidens*	STPA_610	Prima	Primavera	Jalisco	0	2	0	0	0	1463	20.679	-103.602
287	*Sturnira parvidens*	STPA_611	Prima	Primavera	Jalisco	0	7	0	1	0	1463	20.679	-103.602
288	*Artibeus hirsutus*	ARHI_614	Prima	Primavera	Jalisco	0	5	0	7	0	1463	20.679	-103.602
289	*Chiroderma salvini*	CHSA_615	Prima	Primavera	Jalisco	0	0	0	0	0	1463	20.679	-103.602
290	*Glossophaga soricina*	GLSO_616	Tucs	Tucson	Jalisco	0	1	0	0	0	1506	20.705	-103.336

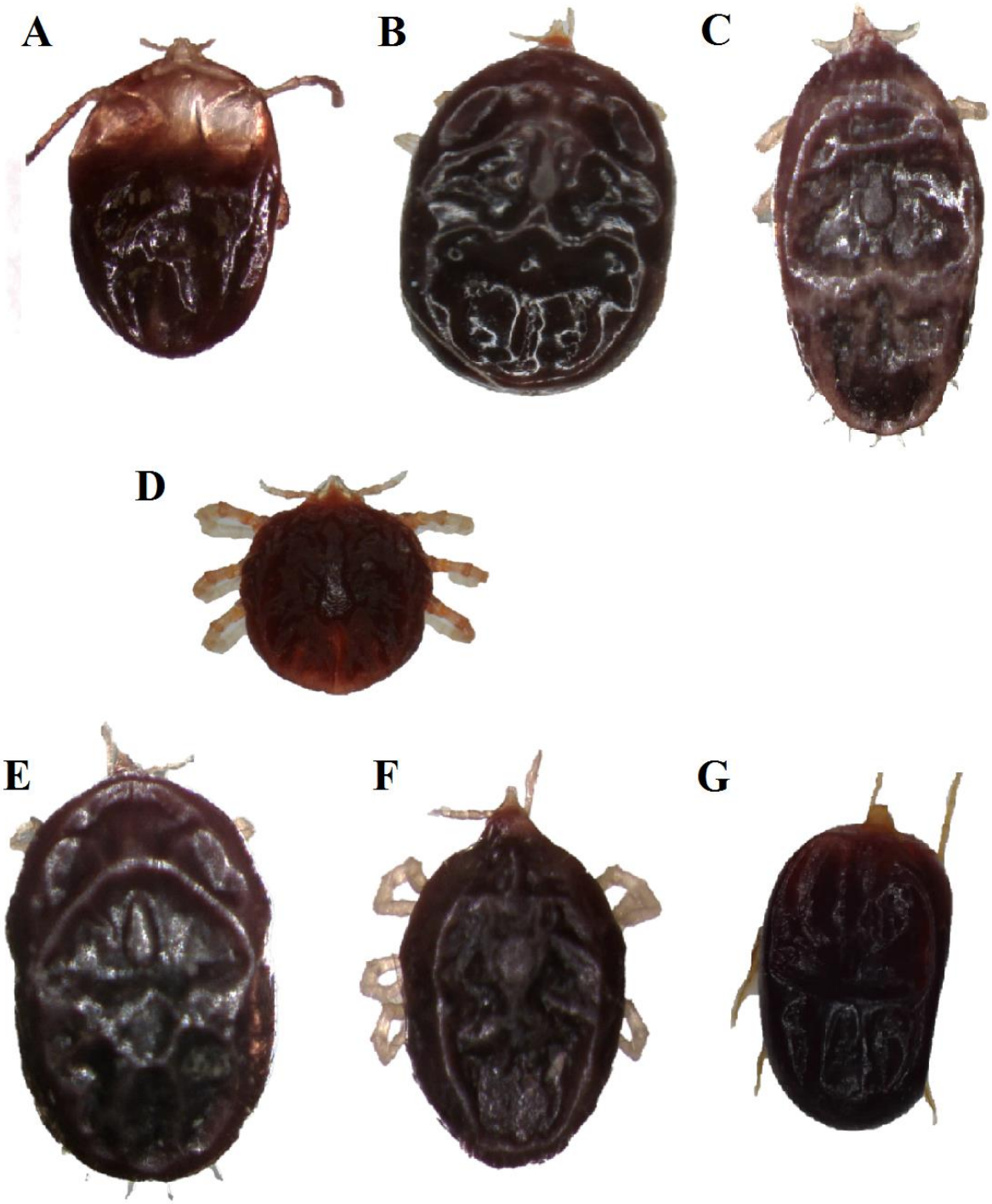
Appendix 3.2. Bat bug specimen of each lineage obtained: A, *Cimex* 1; B, *Cimex* 2; C, *Cimex* 3; and D, *Cimex* 4.



Appendix 3.3. Bat fly specimen of each lineage obtained: A, *Basilia* 1; B, *Basilia* 2a; C, *Basilia* 2b; D, Nycteribiid 1; E, Nycteribiid 2; F, *Aspidoptera phyllostomatis*; G, *Megistopoda aranea*; H, *Nycterophilia coxata*; I, *Paratrichobius* 1; J, *Trichobius* 1; K, *Trichobius* 2; L, *Trichobius* 3; M, *Trichobius sphaeronotus* 1; and N, *Trichobius dugesii*.



Appendix 3.4. Bat tick specimen of each lineage obtained: A, *Antricola* 1; B, *Carios kelleyi;* C, Tick 1; D, Tick 3; E, Tick 4; F, Tick 5 and G, Tick 6. There was not specimen available of Tick 2 lineage after sequencing.



References

Abbott, R. *et al.* (2013) 'Hybridization and speciation', *Journal of Evolutionary Biology*, 26(2), pp. 229–246. Doi: 10.1111/j.1420-9101.2012.02599.x.

Aguiar, L. M. S. *et al.* (2016) 'Should I stay or should I go? Climate change effects on the future of Neotropical savannah bats', *Global Ecology and Conservation*. Elsevier B.V., 5, pp. 22–33. Doi: 10.1016/j.gecco.2015.11.011.

Albery, G. F. *et al.* (2019) 'Predicting the global mammalian viral sharing network using phylogeography', *in Review*, (August), pp. 1–30. Doi: 10.1101/732255.

Alter, S. E., Munshi-South, J. and Stiassny, M. L. J. (2017) 'Genomewide SNP data reveal cryptic phylogeographic structure and microallopatric divergence in a rapids-adapted clade of cichlids from the Congo River', *Molecular Ecology*, 26(5), pp. 1401–1419. Doi: 10.1111/mec.13973.

Altringham, J. D. (2011) *Bats. From evolution to conservation*. 2nd edn. New York, U.S.A.: Oxford University Press.

Álvarez-Castañeda, S. T. (1998) 'Analysis of the Nomenclature and Taxonomy of the Species of Land Mammals of the Baja California Peninsula in the Last 30 Years', *Journal of the Arizona-Nevada Academy of Science*, 31(1), pp. 57–70.

Álvarez-Castañeda, S. T., Álvarez, T. and González-Ruíz, N. (2015) *Guía para la identificación de los mamíferos de México en campo y laboratorio*. La Paz, México: Centro de Investigaciones Biológicas del Noroeste, S. C. y Asociacion Mexicana de Mastozoología, A. C.

Álvarez-Castañeda, S. T. and Arroyo-Cabrales, J. (2008) *Myotis peninsularis, The IUCN Red List of Threatened Species*. Available at: http://dx.Doi.org/10.2305/iucn.uk.2008.rlts.t14189a4418135.en.

Álvarez-Castañeda, S. T. and Bogan, M. A. (1998) '*Myotis peninsularis*', *Mammalian Species*, 573, pp. 1–2.

Álvarez-Castañeda, S. T. and Murphy, R. W. (2014) 'The endemic insular and peninsular species *Chaetodipus spinatus* (Mammalia, Heteromyidae) breaks patterns for Baja California.', *PloS one*, 9(12), p. e116146. Doi: 10.1371/journal.pone.0116146.

Álvarez-Castañeda, S. T. and Nájera-Cortazar, L. A. (2020) 'Do island populations differ

in size and shape compared to mainland counterparts?', *Journal of Mammalogy*. 101(2), pp. 373-385. Doi: 10.1093/jmammal/gyz193.

Álvarez-Castañeda, S. T. and Patton, J. L. (eds) (1999) *Mamíferos del Noroeste de México*. México: Centro de Investigaciones Biológicas del Noroeste, S. C.

Álvarez-Castañeda, S. T. and Ríos, E. (2011) 'Revision of *Chaetodipus arenarius* (Rodentia: Heteromyidae)', *Zoological Journal of the Linnean Society*, 161(1), pp. 213–228.

Andrews, S. (2014) 'FastQC: A quality control tool for high throughput sequence data'. Babraham Bioinformatics. Available at: https://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

Angell, R. L., Butlin, R. K. and Altringham, J. D. (2013) 'Sexual segregation and flexible mating patterns in temperate bats', *PLoS ONE*, 8(1). Doi: 10.1371/journal.pone.0054194.

Anthony, S. J. *et al.* (2013) 'Coronaviruses in bats from Mexico', *Journal of General Virology*, 94, pp. 1028–1038. Doi: 10.1099/vir.0.049759-0.

Anthony, S. J. *et al.* (2017) 'Further evidence for bats as the evolutionary source of Middle East respiratory syndrome coronavirus', *mBio*, 8, pp. e00373-17.

Appelhans, T. *et al.* (2020) 'mapview: Interactive viewing of spatial data in R. R package version 2.7.8'. Available at: https://cran.r-project.org/package=mapview.

Aragón-Noriega, E. A. *et al.* (2010) 'Managing a protected marine area for the conservation of critically endangered vaquita (*Phocoena sinus* Norris, 1958) in the Upper Gulf of California', *International Journal of Sustainable Development and World Ecology*, 17(5), pp. 410–416. Doi: 10.1080/13504509.2010.500823.

Arita, H. T. and Ceballos, G. (1997) 'The mammals of mexico: Distribution and conservation status', *Revista Mexicana de Mastozoología*, 2, pp. 33–71.

Arnegard, M. E. *et al.* (2014) 'Genetics of ecological divergence during speciation', *Nature*. Nature Publishing Group, 511(7509), pp. 307–311. Doi: 10.1038/nature13301.

Arteaga, M. C., Bello-Bedoy, R. and Gasca-Pineda, J. (2020) 'Hybridization between Yuccas from Baja California : Genomic and environmental patterns', 11(May), pp. 1–

11. Doi: 10.3389/fpls.2020.00685.

Atterby, H. *et al.* (2010) 'Population genetic structure of the Daubenton's bat (*Myotis daubentonii*) in western Europe and the associated occurrence of rabies', *European Journal of Wildlife Research*, 56(1), pp. 67–81. Available at: http://dx.Doi.org/10.1007/s10344-009-0292-1.

Baird, N. A. *et al.* (2008) 'Rapid SNP discovery and genetic mapping using sequenced RAD markers', *PLoS ONE*, 3(10), pp. 1–7. Doi: 10.1371/journal.pone.0003376.

Baker, R. J. and Bradley, R. D. (2006) 'Speciation in mammals and the genetic species concept', *Journal of mammalogy*, 87(4), pp. 643–662.

Balvín, O. *et al.* (2012) 'Mitochondrial DNA and morphology show independent evolutionary histories of bedbug *Cimex lectularius* (Heteroptera: Cimicidae) on bats and humans', *Parasitology Research*, 111(1), pp. 457–469. Doi: 10.1007/s00436-012-2862-5.

Balvín, O., Roth, S. and Vilímová, J. (2015) 'Molecular evidence places the swallow bug genus *Oeciacus Stål* within the bat and bed bug genus *Cimex Linnaeus* (Heteroptera: Cimicidae)', *Systematic Entomology*, 40(3), pp. 652–665. Doi: 10.1111/syen.12127.

Balvín, O., Vilímová, J. and Kratochvíl, L. (2013) 'Batbugs (*Cimex pipistrelli* group, Heteroptera: Cimicidae) are morphologically, but not genetically differentiated among bat hosts', *Journal of Zoological Systematics and Evolutionary Research*, 51(4), pp. 287–295. Doi: 10.1111/jzs.12031.

Bandelt, H., Forster, P. and Röhl, A. (1999) 'Median-joining networks for inferring intraspecific phylogenies', *Molecular Biology and Evolution*, 16(1), pp. 37–48.

Bang, C. *et al.* (2012) 'Control of arthropod abundance, richness, and composition in a heterogeneous desert city', *Wiley on behalf of the Ecological Society of America*, 82(1), pp. 85–100.

Barlow, K. E. *et al.* (2015) 'Citizen science reveals trends in bat populations: The National Bat Monitoring Programme in Great Britain', *Biological Conservation*. Elsevier Ltd, 182, pp. 14–26. Doi: 10.1016/j.biocon.2014.11.022.

Barton, N. H. and Hewitt, G. M. (1985) 'Analysis of Hybrid Zones', *Annual Review of Ecology, Evolution, and Systematics*, 16(1985), pp. 113–148. Doi: 10.1146/annurev.es.16.110185.000553.

Benestan, L. *et al.* (2016) ‘Conservation genomics of natural and managed populations: building a conceptual and practical framework’, *Molecular Ecology*, pp. 1–11. Doi: 10.1111/mec.13647.

Bennett, G., Turmelle, A. and O’Grady, P. (2014) *Evolutionary insights into cospeciation and host switching in Neotropical Streblidae (Diptera), Figshare*. Doi: https://Doi.org/10.6084/m9.figshare.1221856.v1.

Berlocher, S. H. and Feder, J. L. (2002) ‘Sympatric speciation in phytophagous insects: Moving beyond controversy?’, *Annual Review of Entomology*, 47, pp. 773–815.

Berthier, P., Excoffier, L. and Ruedi, M. (2006) ‘Recurrent replacement of mtDNA and cryptic hybridization between two sibling bat species *Myotis myotis* and *Myotis blythii*’, *Proceedings of the Royal Society B: Biological Sciences*, 273(1605), pp. 3101–3109. Doi: 10.1098/rspb.2006.3680.

Bezerra, R. H. S., de Vasconcelos, P. F. and Bocchiglieri, A. (2016) ‘Ectoparasites of bats (Mammalia: Chiroptera) in Atlantic forest fragments in north-eastern Brazil’, *Parasitology Research*. Parasitology Research, 115(10), pp. 3759–3765. Doi: 10.1007/s00436-016-5137-8.

Bivand, R. S., Pebesma, E. and Gomez-Rubio, V. (2013) *Applied spatial data analysis with R*. 2nd edn. New York: Springer, NY.

Black IV, W. C., Klompen, J. S. H. and Keirans, J. E. (1997) ‘Phylogenetic relationships among tick subfamilies (Ixodida: Ixodidae: Argasidae) based on the 18S nuclear rDNA gene’, *Molecular Phylogenetics and Evolution*, 7(1), pp. 129–144.

Blaker, H. (2000) ‘Confidence curves and improved exact confidence intervals for discrete distributions’, *Canadian Journal of Statistics*, 28(4), pp. 783–798.

Blood, B. R. and Clark, M. K. (1998) ‘*Myotis vivesi’*, *Mammalian Species*, 588, pp. 1–5.

Bogan, M. A. (1999) ‘Family Vespertilionidae’, in Alvarez-Castañeda, S. T. and Patton, J. L. (eds) *Mamíferos del Noroeste de Mexico*. 1st edn. Baja California Sur, Mexico: Centro de Investigaciones Biológicas del Noroeste S. C., p. 237.

Bogdanowicz, W., Juste, J. and Owen, R. D. (2005) ‘Geometric morphometrics and cladistics: testing evolutionary relationships in mega- and microbats relationships in mega- and microbats’, *Acta Chiropterologica*, 7(1), pp. 39–49.

Bogdanowicz, W., Piksa, K. and Tereba, A. (2012a) ‘Genetic structure in three species of whiskered bats (genus *Myotis*) during swarming’, *Journal of Mammalogy*, 93(3), pp. 799–807.

Bogdanowicz, W., Piksa, K. and Tereba, A. (2012b) ‘Hybridization hotspots at bat swarming Sites’, *PLoS ONE*, 7(12), pp. e53334. Doi: 10.1371/journal.pone.0053334.

Bohonak, A. J. (1999) ‘Dispersal, gene flow, and population structure’, *The Quarterly Review of Biology*, 74(1), pp. 21–45.

Bolaños-García, R., Rodríguez-Estrella, R. and Guzmán-Cornejo, C. (2018) ‘Ectoparásitos asociados a polluelos del Búho Cornudo (Aves: Strigidae) en un paisaje fragmentado de la península de Baja California, México’, *Acta Zoológica Mexicana (N.S.)*, 34(1), pp. 1–15. Doi: 10.21829/azm.2018.3412142.

Booth, W. *et al.* (2015) ‘Host association drives genetic divergence in the bed bug, *Cimex lectularius*’, *Molecular Ecology*, 24(5), pp. 980–992. Doi: 10.1111/mec.13086.

Borstein, S. (2018) ‘AnnotationBustR: An R package to extract sub-sequences from GenBank annotations.’ Available at: https://cran.r-project.org/web/packages/AnnotationBustR/vignettes/AnnotationBustR-vignette.html.

Bradley, R. D. and Baker, R. J. (2001) ‘A test of the genetic species concept : cytochrome-b sequences and mammals’, *Journal of Mammalogy*, 82(4), pp. 960–973.

Bradshaw, G. V. R. and Ross, A. (1961) ‘Ectoparasites of Arizona Bats’, *Journal of the Arizona Academy of Science*, 1(4), p. 109. Doi: 10.2307/40026556.

Braun, J. K. *et al.* (2015) ‘*Myotis yumanensis* (Chiroptera: Vespertilionidae)’, *Mammalian Species*, 47(918), pp. 1–14. Doi: 10.1093/mspecies/sev001.

Brook, C. E. and Dobson, A. P. (2015) ‘Bats as “special” reservoirs for emerging zoonotic pathogens’, *Trends in Microbiology*, 23(3), pp. 172–180. Doi: 10.1016/j.tim.2014.12.004.

Bruyndonckx, N. *et al.* (2009) ‘Molecular cophylogenetic relationships between European bats and their ectoparasitic mites (Acari, Spinturnicidae)’, *Molecular Phylogenetics and Evolution*. Elsevier Inc., 51(2), pp. 227–237. Doi: 10.1016/j.ympev.2009.02.005.

Burazerović, J. *et al.* (2015) ‘Ticks (Acari: Argasidae, Ixodidae) parasitizing bats in the

central Balkans', *Experimental and Applied Acarology*, 66(2), pp. 281–291. Doi: 10.1007/s10493-015-9891-6.

Burazerović, J. *et al.* (2018) 'Patterns of Central Balkans', *Journal of Medical Entomology*, 55(1), pp. 20–28. Doi: 10.1093/jme/tjx189.

Burger, T. D. *et al.* (2014) 'Molecular phylogeny of soft ticks (Ixodida: Argasidae) inferred from mitochondrial genome and nuclear rRNA sequences', *Ticks and Tick-borne Diseases*, 5(2), pp. 195–207. Doi: 10.1016/j.ttbdis.2013.10.009.

Burns, L. E., Frasier, T. R. and Broders, H. G. (2014) 'Genetic connectivity among swarming sites in the wide ranging and recently declining little brown bat (*Myotis lucifugus*)', *Ecology and Evolution*, 4(21), pp. 4130–4149. Doi: 10.1002/ece3.1266.

Butlin, R. *et al.* (2012) 'What do we need to know about speciation?', *Trends in Ecology and Evolution*, 27(1), pp. 27–39. Doi: 10.1016/j.tree.2011.09.002.

Campbell, B. C. *et al.* (1995) 'Paraphyly of Homoptera and Auchenorrhyncha inferred from 18S rDNA nucleotide sequences', *Systematic Entomology*, 20(3), pp. 175–194. Doi: 10.1111/j.1365-3113.1995.tb00090.x.

Carstens, B. C. *et al.* (2004) 'Exploring population genetic structure in three species of Lesser Antillean bats', *Molecular Ecology*, 13(9), pp. 2557–2566. Doi: 10.1111/j.1365-294X.2004.02250.x.

Carstens, B. C., Lundrigan, B. L. and Myers, P. (2002) 'A phylogeny of the Neotropical nectar-feeding bats (Chiroptera: Phyllostomidae) based on morphological and molecular data', *Journal of Mammalian Evolution*, 9(1), pp. 23–53.

Case, T. J., Cody, M. L. and Ezcurra, E. (eds) (2002) *A new island biogeography of the Sea of Cortés*. New York, U.S.A.: Oxford University Press.

Castella, V., Ruedi, M. and Excoffier, L. (2001) 'Contrasted patterns of mitochondrial and nuclear structure among nursery colonies of the bat *Myotis myotis'*, *Journal of Evolutionary Biology*, 14(5), pp. 708–720. Doi: 10.1046/j.1420-9101.2001.00331.x.

Catchen, J. *et al.* (2013) 'Stacks: An analysis tool set for population genomics', *Molecular Ecology*, 22(11), pp. 3124–3140. Doi: 10.1111/mec.12354.

Centeno-Cuadros, A. *et al.* (2019) 'Comparative phylogeography and asymmetric hybridization between cryptic bat species', *Journal of Zoological Systematics and*

Evolutionary Research, 57(4), pp. 1004–1018. Doi: 10.1111/jzs.1231838.

Chang, C. C. *et al.* (2015) 'Second-generation PLINK: rising to the challenge of larger and richer datasets', *GigaScience*. BioMed Central, 4, p. 7. Doi: 10.1186/s13742-015-0047-8.

Charmantier, A. and Garant, D. (2005) 'Environmental quality and evolutionary potential: lessons from wild populations', *Proceedings. Biological sciences / The Royal Society*, 272(1571), pp. 1415–25. Doi: 10.1098/rspb.2005.3117.

Chattopadhyay, B. *et al.* (2016) 'Genome-wide data reveal cryptic diversity and genetic introgression in an Oriental cynopterine fruit bat radiation', *BMC Evolutionary Biology*. BMC Evolutionary Biology, 16(1), p. 41. Doi: 10.1186/s12862-016-0599-y.

Chhatre, V. E. *et al.* (2018) 'Adaptive introgression and maintenance of a trispecies hybrid complex in range-edge populations of *Populus'*, *Molecular Ecology*, 27(23), pp. 4820–4838. Doi: 10.1111/mec.14820.

Chhatre, V. E. (2018) 'DISTRUCT 2.3: A modified admixture plotting script'. http://distruct2.popgen.org. Available at: http://distruct2.popgen.org.

Chhatre, V. E. *et al.* (2019) 'Climatic niche predicts the landscape structure of locally adaptive standing genetic variation', *bioRxiv*, pp. 1–38. Doi: 10.1101/817411.

Clare, E. L. *et al.* (2011) 'Neotropical bats: estimating species diversity with DNA barcodes', *PloS one*, 6(7), p. e22648. Doi: 10.1371/journal.pone.0022648.

Clare, E. L. *et al*. (2013) 'Diversification and reproductive isolation: cryptic species in the only New World high-duty cycle bat, *Pteronotus parnellii*', *BMC evolutionary biology*, 13, p. 26. Doi: 10.1186/1471-2148-13-26.

CONABIO (1997) *Provincias Biogeográficas de México, Comisión Nacinal para el Uso y conservación Biológicas*. Available at: http://www.conabio.gob.mx/informacion/gis/layouts/rbiog4mgw.png.

CONABIO (2012) *Portal de geoinformación. Sistema nacional de información sobre biodiversidad, Comisión Nacional para el Conocimiento y Uso de la Biodiversidad*. Available at: http://www.conabio.gob.mx/informacion/gis/.

Corthals, A. *et al.* (2015) 'From the field to the lab: Best practices for field preservation of bat specimens for molecular analyses', *PLoS ONE*, 10(3), pp. 1–12. Doi:

10.1371/journal.pone.0118994.

Coyne, J. A. and Orr, H. A. (2004) *Speciation*. USA: Sinauer Associates, Inc.

Cracraft, J. (1997) 'Species concepts in systematics and conservation biology—an ornithological viewpoint', in Claridge, M. F., Dawah, H. A., and Wilson, M. R. (eds) Species: the units of biodiversity. London, United Kingdom: Chapman & Hal, pp. 325–340.

Cryan, P. M. and Diehl, R. H. (2009) 'Analyzing bat migration', in Kunz, T. H. and Parsons, S. (eds) *Ecological and behavioral methods for the study of bats*. 2nd edn. John Hopkins University Press, pp. 476–488.

Culina, A. *et al.* (2019) 'Live fast, don't die young: survival reproduction trade-offs in long-lived income breeders', *Journal of Animal Ecology*, (March 2018), pp. 1–11. Doi: 10.1111/1365-2656.12957.

Cumming, G. S. (1998) 'Host preference in African ticks (Acari: Ixodida): a quantitative data set', *Bulletin of the Entomological Research*, 88(4), pp. 379–406.

Cuxim-Koyoc, A. *et al.* (2015) 'Streblidae (Diptera: Hippoboscoidea) from Yucatan and Updated Species List for Mexico', *Journal of Medical Entomology*, 52(5), pp. 947–961. Doi: 10.1093/jme/tjv117.

Cvikel, N. *et al.* (2015) 'Bats aggregate to improve prey search but might be impaired when their density becomes too high', *Current Biology*. Elsevier Ltd, 25(2), pp. 206–211. Doi: 10.1016/j.cub.2014.11.010.

Czenze, Z. J. and Broders, H. G. (2011) 'Ectoparasite community structure of two bats (*Myotis lucifugus* and *M. septentrionalis*) from the maritimes of Canada', *Journal of Parasitology Research*, 2011, pp. 1–9. Doi: 10.1155/2011/341535.

DaCosta, J. M. and Sorenson, M. D. (2014) 'Amplification biases and consistent recovery of loci in a double-digest RAD-seq protocol', *PLoS ONE*, 9(9). Doi: 10.1371/journal.pone.0106713.

DaCosta, J. M. and Sorenson, M. D. (2016) 'ddRAD-seq phylogenetics based on nucleotide, indel, and presence-absence polymorphisms: Analyses of two avian genera with contrasting histories', *Molecular Phylogenetics and Evolution*. Elsevier Inc., 94, pp. 122–135. Doi: 10.1016/j.ympev.2015.07.026.

Darwin, C. (1859) *On the origin of species by means of natural selection or the preservation of favored races in the struggle of life*. London: John Murray.

Davey, J. W. *et al.* (2011) 'Genome-wide genetic marker discovery and genotyping using next-generation sequencing', *Nature Rev Genet*. Nature Publishing Group, 12(7), pp. 499–510. Doi: 10.1038/nrg3012.

Davis, A. *et al.* (2005) 'Experimental and natural infection of North American bats with West Nile virus', *American Journal of Tropical Medicine and Hygiene*, 73(2), pp. 467–469. PMID:16103624.

Degnan, J. H. and Rosenberg, N. A. (2009) 'Gene tree discordance, phyloge- netic inference and the multispecies coalescent', *Trends in Ecology & Evolution*, (24), pp. 332–340.

Dick, C. W. *et al.* (2003) 'Ectoparasite associations of bats from Central Pennsylvania', *Journal of Medical Entomology*, 40(6), pp. 813–819. Doi: 10.1603/0022-2585-40.6.813.

Dick, C. W. (2006) 'The streblid bat flies (Diptera: Streblidae) of Guatemala', *Biodiversidad de Guatemala*, 1(May), pp. 441–452.

Dick, C. W. and Miller, J. A. (2010) 'Streblidae (Bat Flies)', in Brown, B. V. *et al*. (eds) *Manual of Central American Diptera. Volume 2*. Canada: NRC Research Press, pp. 1249–1260.

Ditchfield, A. D. (2000) 'The comparative phylogeography of neotropical mammals: patterns of intraspecific mitochondrial DNA variation among bats contrasted to nonvolant small mammals', *Molecular ecology*, 9(9), pp. 1307–18.

Dittmar, K. *et al.* (2006) 'Molecular phylogenetic analysis of nycteribiid and streblid bat flies (Diptera: Brachycera, Calyptratae): Implications for host associations and phylogeographic origins', *Molecular Phylogenetics and Evolution*, 38(1), pp. 155–170. Doi: 10.1016/j.ympev.2005.06.008.

Dixon, M. D. (2011) 'Population genetic structure and natal philopatry in the widespread North American bat *Myotis lucifugus', Journal of Mammalogy*, 92(6), pp. 1343–1351. Doi: 10.1644/10-MAMM-A-426.1.

Donaldson, E. F. *et al.* (2010) 'Metagenomic analysis of the viromes of three North American bat species: Viral diversity among different bat species that share a common habitat', *Journal of Virology*, 84(24), pp. 13004–13018. Doi:

10.1128/JVI.01255-10.

Dool, S. E. *et al.* (2013) 'Phylogeography and postglacial recolonization of Europe by *Rhinolophus hipposideros*: Evidence from multiple genetic markers', *Molecular Ecology*, 22(15), pp. 4055–4070. Doi: 10.1111/mec.12373.

Dove, A. D. M. and Cribb, T. H. (2006) 'Species accumulation curves and their applications in parasite ecology', *Trends in Parasitology*, 22(12), pp. 568–574. Doi: 10.1016/j.pt.2006.09.008.

Dray, S. and Dufour, A. (2007) 'The ade4 Package: Implementing the duality diagram for ecologists', *Journal of Statistical Software*, 22(4), pp. 1–20. Doi: 10.18637/jss.v022.i04.

Drexler, J. F. *et al.* (2012) 'Bats host major mammalian paramyxoviruses', *Nature Communications*, 3. Doi: 10.1038/ncomms1796.

Drexler, J. F., Corman, V. M. and Drosten, C. (2014) 'Ecology, evolution and classification of bat coronaviruses in the aftermath of SARS', *Antiviral Research*. Elsevier B.V., 101(1), pp. 45–56. Doi: 10.1016/j.antiviral.2013.10.013.

Efron, B. and Tibshirani, R. J. (1993) *An introduction to the bootstrap*. Chapman & Hall, London, UK.

Ellegren, H. (2014) 'Genome sequencing and population genomics in non-model organisms', *Trends in Ecology and Evolution*. Elsevier Ltd, 29(1), pp. 51–63. Doi: 10.1016/j.tree.2013.09.008.

Ellegren, H. and Galtier, N. (2016) 'Determinants of genetic diversity', *Nat Rev Genet*. Nature Publishing Group, (7), pp. 422–433. Doi: 10.1038/nrg.2016.58.

Erlangen, D. U. *et al.* (2000) 'Mitochondrial DNA (mtDNA) reveals that female Bechstein's bats live in closed societies', *Molecular Ecology*, 9, pp. 793–800.

Esser, H. J. *et al.* (2016) 'Host specificity in a diverse Neotropical tick community: An assessment using quantitative network analysis and host phylogeny', *Parasites and Vectors*. Parasites & Vectors, 9(1), pp. 1–14. Doi: 10.1186/s13071-016-1655-6.

Estrada-Pena, A. *et al.* (2010) 'A review of the systematics of the tick family argasidae (Ixodida)', *Acarologia*, 50(3), pp. 317–333. Doi: 10.1051/acarologia/20101975.

Estrada-Villegas, S. *et al.* (2018) 'Bats and their bat flies: community composition and host specificity on a Pacific island archipelago', *Acta Chiropterologica*, 20(1), pp. 161–176. Doi: 10.3161/15081109ACC2018.20.1.012.

Evin, A., Horáček, I. and Hulva, P. (2011) 'Phenotypic diversification and island evolution of pipistrelle bats (*Pipistrellus pipistrellus* group) in the Mediterranean region inferred from geometric morphometrics and molecular phylogenetics', *Journal of Biogeography*, 38(11), pp. 2091–2105. Doi: 10.1111/j.1365-2699.2011.02556.x.

Excoffier, L. and Lischer, H. E. L. (2010) 'Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows', *Molecular Ecology Resources*, 10, pp. 564–567.

Ezcurra, E. *et al.* (2002) 'Los desiertos de Sonora y Baja California', in Mittermeier, R. A. *et al.* (eds) *Áreas silvestres: Las últimas regiones virgenes del planeta*. Conservation International y Agrupación Sierra Madre, pp. 315–332.

Feder, J. L. *et al.* (2013) 'Geographic mode of speciation and genomic divergence', *Annual Review of Ecology, Evolution, and Systematics*, 44(1), pp. 73–97. Doi: 10.1146/annurev-ecolsys-110512-135825.

Fenton, B. and Barclay, R. M. R. (1980) '*Myotis lucifugus*. Mammalian species', *Journal of Mammalogy*, (142), pp. 1–8Fick, S. E. and Hijmans, R. J. (2017) 'WorldClim 2: new 1km spatial resolution climate surfaces for global land areas', *International Journal of Climatology*, 37(12), pp. 4302–4315.

Fiset, J. *et al.* (2015) 'Phylogeographic structure of the white-footed mouse and the deer mouse, two lyme disease reservoir hosts in Québec', *PLoS ONE*, 10(12), pp. 1–18. Doi: 10.1371/journal.pone.0144112.

Fitch, J. H., Shump, K. A. J. and Shump, A. U. (1981) '*Myotis velifer*. Mammalian Species', *Journal of Mammalogy*, 149, pp. 1–5.

Floyd, C. H. *et al.* (2010) 'Conserving the endangered Mexican fishing bat (*Myotis vivesi*): Genetic variation indicates extensive gene flow among islands in the Gulf of California', *Conservation Genetics*, 11(3), pp. 813–822. Doi: 10.1007/s10592-009-9902-4.

Foley, N. M. *et al.* (2018) 'Growing old, yet staying young: The role of telomeres in bats' exceptional longevity', *Science Advances*, 4(2). Doi: 10.1126/sciadv.aao0926.

Folmer, O. *et al.* (1994) 'DNA primers for amplification of mitochondrial cytochrome c

oxidase subunit I from diverse metazoan invertebrates', *Molecular Marine Biology and Biotechnology*, 3(5), pp. 294–299. Doi: 10.1371/journal.pone.0013102.

Frank, R. *et al.* (2015) 'Parasite diversity of European *Myotis* species with special emphasis on *Myotis myotis* (Microchiroptera, Vespertilionidae) from a typical nursery roost', *Parasites & vectors*, 8(1), p. 101. Doi: 10.1186/s13071-015-0707-7.

Frick, W. F. *et al.* (2010) 'An emerging disease causes regional population collapse of a common North American bat species', *Science (New York, N.Y.)*, 329(5992), pp. 679–82. Doi: 10.1126/science.1188594.

Frick, W. F. *et al.* (2015) 'Disease alters macroecological patterns of North American bats', *Global Ecology and Biogeography*, 24(7), pp. 741–749. Doi: 10.1111/geb.12290.

Frick, W. F., Hayes, J. P. and Heady, P. A. (2008) 'Island biogeography of bats in Baja California, Mexico: Patterns of bat species richness in a near-shore archipelago', *Journal of Biogeography*, 35(2), pp. 353–364. Doi: 10.1111/j.1365-2699.2007.01798.x.

Frick, W. F., Hayes, J. P. and Heady, P. A. (2009) 'Nestedness of desert bat assemblages: Species composition patterns in insular and terrestrial landscapes', *Oecologia*, 158(4), pp. 687–697. Doi: 10.1007/s00442-008-1168-x.

de la Fuente, J. *et al.* (2008) 'Overview: Ticks as vectors of pathogens that cause disease in humans and animals', *Frontiers in Bioscience*, 13, pp. 6938–6946.

Furmankiewicz, J. and Altringham, J. D. (2007) 'Genetic structure in a swarming brown long-eared bat (*Plecotus auritus*) population: evidence for mating at swarming sites', *Conservation Genetics*, 8, pp. 913–923.

Gallego-García, N. *et al.* (2019) 'Landscape genomic signatures indicate reduced gene flow and forest-associated adaptive divergence in an endangered Neotropical turtle', *Molecular Ecology*, (28), pp. 2757–2771. Doi: 10.1111/mec.15112.

Gannon, M. R. and Willig, M. R. (1995) 'Ecology of ectoparasites from tropical bats', *Environmental Entomology*, pp. 1495–1503.

Gay, N. *et al.* (2014) 'Parasite and viral species richness of Southeast Asian bats: Fragmentation of area distribution matters', *International Journal for Parasitology: Parasites and Wildlife*. Australian Society for Parasitology, 3(2), pp. 161–170. Doi: 10.1016/j.ijppaw.2014.06.003.

Ge, X. *et al.* (2012) 'Metagenomic analysis of viruses from bat fecal samples reveals many novel viruses in insectivorous bats in China', *Journal of Virology*, 86(8), pp. 4620–4630. Doi: 10.1128/JVI.06671-11.

Geldenhuys, M. *et al.* (2018) 'A metagenomic viral discovery approach identifies potential zoonotic and novel mammalian viruses in Neoromicia bats within South Africa', *Plos One*, 13(3), p. e0194527. Doi: 10.1371/journal.pone.0194527.

Gernhard, T. (2008) 'Yule Process', *Journal of Theoretical Biology2*, (253), pp. 769–778.

Gill, J. *et al.* (2004) 'Detection of human blood in the bat tick *Carios* (*Ornithodoros*) *kelleyi* (Acari: Argasidae) in Iowa', *Journal of Medical Entomology*, 41(6), pp. 1179–1181. Doi: 10.1603/0022-2585-41.6.1179.

Gómez-Díaz, E., Navarro, J. and González-Solís, J. (2008) 'Ectoparasite community structure on three closely related seabird hosts: A multiscale approach combining ecological and genetic data', *Ecography*, 31(4), pp. 477–489. Doi: 10.1111/j.0906-7590.2008.05330.x.

Gómez, A. and Nichols, E. (2013) 'Neglected wild life: Parasitic biodiversity as a conservation target', *International Journal for Parasitology: Parasites and Wildlife*. Australian Society for Parasitology, 2(1), pp. 222–227. Doi: 10.1016/j.ijppaw.2013.07.002.

Gonzalez-Abraham, C. *et al.* (2015) 'The human footprint in Mexico: Physical geography and historical legacies', *PLoS ONE*, 10(3). Doi: 10.1371/journal.pone.0121203.

González-Abraham, C. E., Garcillán, P. and Ezcurra, E. (2010) 'Ecorregiones de la península de Baja California: una síntesis', *Boletín de la Sociedad Botánica de México*, (87), pp. 69–82.

Gourbière, S. and Mallet, J. (2010) 'Are species real? The shape of the species boundary with exponential failure, reinforcement, and the "missing snowball"', *Evolution*, 64(1), pp. 1–24. Doi: 10.1111/j.1558-5646.2009.00844.x.

Graciolli, G. (2001) 'Distribuição geográfica e hospedeiros quirópteros (Mammalia, Chiroptera) de moscas nicteribidas americanas (Diptea, Nycteribiidae)', *Revista Brasileira de Zoologia*, 18(1), pp. 307–322.

Graciolli, G., Autino, A. G. and Claps, G. L. (2007) 'Catalogue of American Nycteribiidae (Diptera, Hippoboscoidea)', *Revista Brasileira de Entomologia*, 51(2), pp. 142–159. Doi: 10.1590/S0085-56262007000200004.

Greif, S. and Siemers, B. M. (2010) ‘Innate recognition of water bodies in echolocating bats’, *Nature Communications*. Nature Publishing Group, 1(107), pp. 1–6. Doi: 10.1038/ncomms1110.

Gu, Z. *et al.* (2014) ‘circlize Implements and enhances circular visualization in R.’, *Bioinformatics (Oxford, England)*. England, 30(19), pp. 2811–2812. Doi: 10.1093/bioinformatics/btu393.

Guégan, J. F., Morand, S. and Poulin, R. (2004) ‘Are there general laws in parasite community ecology? The emergence of spatial parasitology and epidemiology’, in Thomas, F. (ed.) *Parasitism and Ecosystems*. Cambridge University Press, pp. 22–42. Doi: 10.1093/acprof:oso/9780198529873.003.0003.

Guevara-Chumacero, L. M. *et al.* (2010) ‘Molecular phylogeography of *Pteronotus davyi* (Chiroptera : Mormoopidae) in Mexico’, *Journal of Mammalogy*, 91(1), pp. 220–232. Doi: 10.1644/08-mamm-a-212r3.1.key.

Guglielmone, A. A. *et al.* (2010) ‘The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida) of the world: a list of valid species names’, *Zootaxa*, 2528(November 2019), pp. 1–28. Doi: 10.1023/A:1025381712339.

Guzmán-Cornejo, C. *et al.* (2017) ‘Molecular evidence and additional morphological characters to distinguish *Ornithodoros brodyi* and *Ornithodoros yumatensis* (Ixodida: Argasidae) in their different developmental stages’, *Acta Parasitologica*, 62(2), pp. 432–448. Doi: 10.1515/ap-2017-0051.

Haelewaters, D. *et al.* (2017) ‘Parasites of parasites of bats: Laboulbeniales (Fungi: Ascomycota) on bat flies (Diptera: Nycteribiidae) in central Europe’, *Parasites and Vectors*. Parasites & Vectors, 10(1), pp. 1–14. Doi: 10.1186/s13071-017-2022-y.

Hall, E. R. (1981) *The mammals of North Amercia*. 2nd edn. Edited by John Wiley & Sons. New York, USA: New York: Ronald Press.

Hall, T. (2005) ‘BioEdit. Biological sequence editor’. California, U.S.A. Available at: http://www.mbio.ncsu.edu/BioEdit/bioedit.html.

de la Harpe, M. *et al.* (2017) ‘Molecular ecology studies of species radiations: Current research gaps, opportunities and challenges’, *Molecular Ecology*, pp. 2608–2622. Doi: 10.1111/mec.14110.

Harrell, F. E. J. (2020) ‘Hmisc: Harrell Miscellaneous. R package version 4.4-0’. Available at: https://cran.r-project.org/package=Hmisc.

Harrison, R. G. and Larson, E. L. (2014) ‘Hybridization, introgression, and the nature of species boundaries’, *Journal of Heredity*, 105(S1), pp. 795–809. Doi: 10.1093/jhered/esu033.

Hartl, D. L. and Clark, A. G. (2007) *Principles of population genetics*. 4th edn. Massachusetts, U. S. A.: Sinauer Associates, Inc.

Hayman, D. T. S. (2016) ‘Bats as viral reservoirs’, *Annual Review of Virology*, 3(1), pp. 77–99. Doi: 10.1146/annurev-virology-110615-042203.

Haynie, M. L. *et al.* (2016) ‘Placement of the rediscovered *Myotis planiceps* (Chiroptera: Vespertilionidae) within the Myotis phylogeny’, *Journal of Mammalogy*, 97(3), pp. 701–712. Doi: 10.1093/jmammal/gyv216.

Hayward, B. J. (1970) ‘The natural history of the cave bat*, Myotis velifer’, Western New Mexico Univ. Res. Sci*, (1), pp. 1–74.

Hebert, P. D. N. *et al.* (2003) ‘Biological identifications through DNA barcodes’, *Proceedings. Biological sciences / The Royal Society*, 270(1512), pp. 313–321. Doi: 10.1098/rspb.2002.2218.

Hebert, P. D. N., Ratnasingham, S. and DeWaard, J. R. (2003) ‘Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species’, *Proceedings of the Royal Society B: Biological Sciences*, 270(SUPPL. 1), pp. 96–99. Doi: 10.1098/rsbl.2003.0025.

Hellard, E. *et al.* (2015) ‘Parasite-parasite interactions in the wild: How to detect them?’, *Trends in Parasitology*. Elsevier Ltd, 31(12), pp. 640–652. Doi: 10.1016/j.pt.2015.07.005.

Heng, L. and Richard, D. (2009) ‘BWA: Fast and accurate short read alignment with Burrows–Wheeler transform’, *Bioinformatics*, 25(14), pp. 1754–1760.

Herrera M., L. G., Flores-Martínez, J. J. and Sánchez-Cordero, V. (2017) ‘Geographical distribution and conservation status of an endemic insular mammal: the Vulnerable fish-eating bat Myotis vivesi’, *Oryx*, (September), pp. 1–6. Doi: 10.1017/S0030605317000874.

Herrera Montalvo, L. G. (1997) ‘Evidence of altitudinal movements of *Leptonycterys curasoae* (Chiroptera: Phyllostomidae) in Central Mexico’, *Revista Mexicana de Mastozoologia*, 2, pp. 116–118.

Hey, J. (2006) ‘Recent advances in assessing gene flow between diverging populations and species’, *Current Opinion in Genetics and Development*, 16(6), pp. 592–596. Doi: 10.1016/j.gde.2006.10.005.

Hijmans, R. J. (2019) ‘geosphere: Spherical Trigonometry. R package version 1.5-10’. Available at: https://cran.r-project.org/package=geosphere.

Hijmans, R. J. (2020) ‘raster: Geographic Data Analysis and Modeling. R package version 3.1-5’. Available at: https://cran.r-project.org/package=raster.

Hoffmann, A. *et al.* (2015) ‘A framework for incorporating evolutionary genomics into biodiversity conservation and management’, *Climate Change Responses*, 2(1), p. 1. Doi: 10.1186/s40665-014-0009-x.

Ter Hofstede, H. M. and Fenton, M. B. (2005) ‘Relationships between roost preferences, ectoparasite density, and grooming behaviour of Neotropical bats’, *Journal of Zoology*, 266(4), pp. 333–340. Doi: 10.1017/S095283690500693X.

Hohenlohe, P. A. *et al.* (2010) ‘Population genomics of parallel adaptation in threespine stickleback using sequenced RAD tags’, *PLoS Genetics*, 6(2). Doi: 10.1371/journal.pgen.1000862.

Holloway, B. G. L. and Barclay, R. M. R. (2001) ‘*Myotis ciliolabrum*’, *Mammalian Species*, 4(670), pp. 1–5.

Holsinger, K. E. and Weir, B. S. (2009) ‘Genetics in geographically structured populations: defining, estimating and interpreting F(ST)’, *Nature reviews. Genetics*, 10(September), pp. 639–650. Doi: 10.1038/nrg2611.

Holz, P. H. *et al.* (2018) ‘Virus survey in populations of two subspecies of bent-winged bats (*Miniopterus orianae bassanii* and *oceanensis*) in south-eastern Australia reveals a high prevalence of diverse herpesviruses’, *PLoS ONE*, 13(5), pp. 1–17. Doi: 10.1371/journal.pone.0197625.

Hoofer, S. R. and Van Den Bussche, R. A. (2003) ‘Molecular phylogenetics of the chiropteran family Vespertilionidae’, *Acta Chiropterologica*, 5(Suppl.), pp. 1–63.

Hopla, C. E., Durden, L. A. and Keirans, J. E. (1994) ‘Ectoparasites and classification’, *Revue Scientifique et Technique de l’OIE*, 13(4), pp. 985–1017. Doi: 10.20506/rst.13.4.815.

Horak, I. G., Camicas, J. and Keirans, J. E. (2002) 'The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida): a world list of valid tick names', *Experimental and Applied Acarology*, 28, pp. 27–54. Doi: 10.1023/A:1025381712339.

Hornok, S. *et al.* (2014) 'Bat ticks revisited: *Ixodes ariadnae sp*. nov. and allopatric genotypes of *I. vespertilionis* in caves of Hungary', *Parasites & Vectors*, 7(1), p. 202. Doi: 10.1186/1756-3305-7-202.

Hornok, S., Kontschán, J., *et al.* (2015) 'Contributions to the morphology and phylogeny of the newly discovered bat tick species, *Ixodes ariadnae* in comparison with *I. vespertilionis and I. simplex*', *Parasites and Vectors*, 8(1), pp. 1–7. Doi: 10.1186/s13071-015-0665-0.

Hornok, S., Estrada-Peña, A., *et al.* (2015) 'High degree of mitochondrial gene heterogeneity in the bat tick species *Ixodes vespertilionis, I. ariadnae* and *I. simplex* from Eurasia', *Parasites & Vectors*, 8(1), p. 457. Doi: 10.1186/s13071-015-1056-2.

Hornok, Sándor, Sándor, A. D., *et al.* (2017) 'Contributions to the phylogeny of *Ixodes (Pholeoixodes) canisuga, I. (Ph.) kaiseri, I. (Ph.) hexagonus* and a simple pictorial key for the identification of their females', *Parasites and Vectors*, 10(1). Doi: 10.1186/s13071-017-2424-x.

Hornok, Sándor, Szőke, K., Tu, V. T., *et al.* (2017) 'Mitochondrial gene heterogeneity of the bat soft tick *Argas vespertilionis* (Ixodida: Argasidae) in the Palaearctic', *Parasites & vectors*, 10(1), p. 109. Doi: 10.1186/s13071-017-2037-4.

Hornok, Sándor, Szőke, K., Görföl, T., *et al.* (2017) 'Molecular investigations of the bat tick *Argas vespertilionis* (Ixodida: Argasidae) and *Babesia vesperuginis* (Apicomplexa: Piroplasmida) reflect "bat connection" between Central Europe and Central Asia', *Experimental and Applied Acarology*, 72(1), pp. 69–77. Doi: 10.1007/s10493-017-0140-z.

Hornok, S. *et al.* (2017) 'Phylogenetic analyses of bat-associated bugs (Hemiptera: Cimicidae: Cimicinae and Cacodminae) indicate two new species close to *Cimex lectularius', Parasites and Vectors*, 10(1), pp. 1–10. Doi: 10.1186/s13071-017-2376-1.

Hornok, S. *et al.* (2019) 'Molecular detection of vector-borne bacteria in bat ticks (Acari: Ixodidae, Argasidae) from eight countries of the Old and New Worlds', *Parasites and Vectors*, 12(1), pp. 1–7. Doi: 10.1186/s13071-019-3303-4.

Hoyt, J. R. *et al.* (2018) 'Cryptic connections illuminate pathogen transmission within

community networks', *Nature*. Springer US. Doi: 10.1038/s41586-018-0720-z.

Hua, P. *et al.* (2013) 'Dispersal, mating events and fine-scale genetic structure in the Lesser Flat-Headed bats', *PLoS ONE*, 8(1). Doi: 10.1371/journal.pone.0054428.

Huber, S. K. *et al.* (2007) 'Reproductive isolation of sympatric morphs in a population of Darwin's finches', *Proceedings. Biological sciences/The Royal Society*, 274(1619), pp. 1709–1714. Doi: 10.1098/rspb.2007.0224.

Hudson, P. J., Dobson, A. P. and Lafferty, K. D. (2006) 'Is a healthy ecosystem one that is rich in parasites?', *Trends in Ecology and Evolution*, 21(7), pp. 381–385. Doi: 10.1016/j.tree.2006.04.007.

Hulva, P. *et al.* (2010) 'Mechanisms of radiation in a bat group from the genus *Pipistrellus* inferred by phylogeography, demography and population genetics', *Molecular Ecology*, 19(24), pp. 5417–5431. Doi: 10.1111/j.1365-294X.2010.04899.x.

Ingala, M. R. *et al.* (2018) 'Comparing microbiome sampling methods in a wild mammal: Fecal and intestinal samples record different signals of host ecology, evolution', *Frontiers in Microbiology*, 9(803), pp. 1–13. Doi: 10.3389/fmicb.2018.00803.

Irwin, D. M., Kocher, T. D. and Wilson, A. C. (1991) 'Evolution of the cytochrome b gene of mammals', *Journal of Molecular Evolution*, 32(2), pp. 128–144. Doi: 10.1007/BF02515385.

IUCN (2020) 'The IUCN Red List of Threatened Species', *The IUCN Red List of Threatened Species.* Version 2016-1. Available at: www.iucnredlist.org.

Jebb, D. *et al.* (2020) 'Six reference-quality genomes reveal evolution of bat adaptations', *Nature*. Springer US, 583(7817), pp. 578–584. Doi: 10.1038/s41586-020-2486-3.

Jobling, B. (1938) 'A revision of the species of the genus *Trichobius*: (Diptera Acalypterae, Streblidae)', *Parasitology*, 30(3), pp. 358–387. Doi: Doi:10.1017/S0031182000025932.

Joffrin, L. *et al.* (2018) 'Bat pathogens hit the road: But which one?', *PLOS Pathogens*, 14(8), p. e1007134. Doi: 10.1371/journal.ppat.1007134.

Johnson, L. N. L. *et al.* (2015) 'Population genetic structure within and among seasonal site types in the Little Brown Bat (*Myotis lucifugus*) and the Northern Long-Eared Bat (*M. septentrionalis*)', *PLoS ONE*, 10(5), pp. 1–18. Doi:

10.5061/dryad.47nm0.During.

Jombart, T. (2008) ‘adegenet: a R package for the multivariate analysis of genetic markers’, *Bioinformatics*, 24, pp. 1403–1405.

Jombart, T. and Collins, C. (2017) ‘A tutorial for Discriminant Analysis of Principal Components (DAPC) using adegenet 2.1.0’, pp. 1–43.

Jones, F. C. *et al.* (2012) ‘The genomic basis of adaptive evolution in threespine sticklebacks’, *Nature*, 484(7392), pp. 55–61. Doi: 10.1038/nature10944.

Jones, K. E. *et al.* (2002) ‘A phylogenetic supertree of the bats (Mammalia: Chiroptera).’, *Biological reviews of the Cambridge Philosophical Society*, 77(2), pp. 223–59. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12056748.

Jones, K. E. and Teeling, E. C. (2004) ‘Phylogenetic tools for examining character and clade evolution in bats’.

Juste, J. *et al*. (2018) ‘Two new cryptic bat species within the *Myotis nattereri* species complex (Vespertilionidae, Chiroptera) from the Western Palaearctic’, *Acta Chiropterologica*, 20(2), pp. 285–300. Doi: 10.3161/15081109ACC2018.20.2.001.

Kalko, E. K. V (1998) ‘Organisation and diversity of tropical bat communities through space and time’, *Zoology*, (101), pp. 281–297.

Kasso, M. and Balakrishnan, M. (2013) ‘Ecological and economic importance of bats (Order Chiroptera)’, *ISRN Biodiversity*, 2013(187415), pp. 1–9. Doi: 10.1155/2013/187415.

Kent, W. J. *et al.* (2002) ‘The human genome browser at UCSC’, *Genome research*, 12(6), pp. 996–1006.

Kindt, R. and Coe, R. (2005) ‘*Tree diversity analysis. A manual and software for common statistical methods for ecological and biodiversity studies*’, Nairobi: World Agroforestry Centre (ICRAF).

Klimpel, S. and Mehlhorn, H. (2014) ‘*Bats (Chiroptera) as vectors of diseases and parasites*’, Parasitology Research Monographs, Doi: 10.1007/978-3-642-39333-4.

Knee, W. and Proctor, H. (2006) ‘Keys to the Families and Genera of Blood and Tissue Feeding Mites Associated with Albertan Birds’, *Canadian Journal of Arthropod*

Identification, 2(2), pp. 1–18. Doi: 10.3752/cjai.2006.02.

Kobayashi, H. *et al.* (2018) 'ddRAD-seq based phylogeographic study of *Sargassum thunbergii* (Phaeophyceae, Heterokonta) around Japanese coast', *Marine Environmental Research*. Elsevier, 140(May), pp. 104–113. Doi: 10.1016/j.marenvres.2018.05.021.

Koopman, K. F. (1993) 'Chiroptera', in Wilson, D. E. and Reeder, D. M. (eds) *Mammalian Species of the World: A Taxonomic and Geographic Reference*. Second. Washington, D.C.: Smithsonian Institution Press, pp. 137–241.

Krasnov, B. R. *et al.* (2012) 'Compositional and phylogenetic dissimilarity of host communities drives dissimilarity of ectoparasite assemblages: Geographical variation and scale-dependence', *Parasitology*, 139(3), pp. 338–347. Doi: 10.1017/S0031182011002058.

Krutzsch, P. H. (2009) 'The reproductive biology of the Cave Myotis (*Myotis velifer*)', *Acta Chiropterologica*, 11(1), pp. 89–104. Doi: 10.3161/150811009X465712.

Kumar, S., Stecher, G. and Tamura, K. (2016) 'MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets', *Molecular biology and evolution*, 33(7), pp. 1870–1874. Doi: 10.1093/molbev/msw054.

Kurta, B. A. and Baker, R. H. (1990) '*Eptesicus fuscus*', *Mammalian Species*, 356, pp. 1–10.

Lack, J. B., Wilkinson, J. E. and Van Den Bussche, R. A. (2010) 'Range-wide population genetic structure of the Pallid Bat (Antrozous pallidus) — Incongruent results from nuclear and mitochondrial DNA', *Acta Chiropterologica*, 12(2), pp. 401–413. Doi: 10.3161/150811010X537981.

Langwig, K. E. *et al.* (2012) 'Sociality, density-dependence and microclimates determine the persistence of populations suffering from a novel fungal disease, white-nose syndrome', *Ecology Letters*, 15(9), pp. 1050–1057. Doi: 10.1111/j.1461-0248.2012.01829.x.

Langwig, K. E. *et al.* (2016) 'Drivers of variation in species impacts for a multi-host fungal disease of bats', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 371(1709), p. 20150456. Doi: 10.1098/rstb.2015.0456.

Larsen, Roxanne J. *et al.* (2012) 'Evolutionary history of Caribbean species of *Myotis*, with evidence of a third Lesser Antillean endemic', *Mammalian Biology*, 77(2), pp.

124–134. Doi: 10.1016/j.mambio.2011.11.003.

Larsen, Roxanne J *et al.* (2012) 'Genetic diversity of Neotropical *Myotis* (Chiroptera: Vespertilionidae) with an emphasis on South American species', *PloS one*, 7(10), p. e46578. Doi: 10.1371/journal.pone.0046578.

Lavretsky, P., Janzen, T. and McCracken, K. G. (2019) 'Identifying hybrids & the genomics of hybridization: Mallards & American black ducks of Eastern North America', *Ecology and Evolution*, 9(6), pp. 3470–3490. Doi: 10.1002/ece3.4981.

Lee, C.-R. and Mitchell-Olds, T. (2011) 'Quantifying effects of environmental and geographical factors on patterns of genetic differentiation.', *Molecular ecology*, 20(22), pp. 4631–42. Doi: 10.1111/j.1365-294X.2011.05310.x.

Leigh, J. W. and Bryant, D. (2005) 'POPART: Full-feature software for haplotype network construction', *Methods in Ecology and Evolution*, 6(9), pp. 1110–1116. Doi: https ://Doi.org/10.1111/2041-210X.12410.

Leray, M. *et al.* (2019) 'GenBank is a reliable resource for 21st century biodiversity research', *Proceedings of the National Academy of Sciences of the United States of America*, 116(45), pp. 22651–22656. Doi: 10.1073/pnas.1911714116.

Letunic, I. and Bork, P. (2016) 'Interactive tree of life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees', *Nucleic Acids Research*, 28(3), pp. 1–4. Doi: 10.1093/nar/gkw290.

Li, H. *et al.* (2009) 'The Sequence Alignment/Map format and SAMtools', *Bioinformatics*, 25(16), pp. 2078–9. Doi: 10.1093/bioinformatics/btp352.

Li, L. *et al.* (2010) 'Bat guano virome: predominance of dietary viruses from insects and plants plus novel mammalian viruses', *Journal of Virology*, 84(14), pp. 6955–6965. Doi: 10.1128/JVI.00501-10.

Librado, P. and Rozas, J. (2009) 'DnaSP v5: a software for comprehensive analysis of DNA polymorphism data', *Bioinformatics (Oxford, England)*, 25(11), pp. 1451–1452.

Light, J. E., Durden, L. A. and OConnor, B. M. (2020) 'Checklist of ectoparasites of cricetid and heteromyid rodents in México', *Therya*, 11(1), pp. 79–136. Doi: 10.12933/therya-20-785.

Lindtke, D. *et al.* (2013) 'Admixture mapping of quantitative traits in *Populus* hybrid

zones: power and limitations', *Heredity*, 111(6), pp. 474–85. Doi: 10.1038/hdy.2013.69.

Linn, C. E. *et al.* (2004) 'Postzygotic isolating factor in sympatric speciation in *Rhagoletis* flies: Reduced response of hybrids to parental host-fruit odors', *Proceedings of the National Academy of Sciences of the United States of America*, 101(51), pp. 17753–17758. Doi: 10.1073/pnas.0408255101.

Lomolino, M. V. (2000) 'Ecology's most general, yet protean pattern: The species-area relationship', *Journal of Biogeography*, 27(1), pp. 17–26. Doi: 10.1046/j.1365-2699.2000.00377.x.

Loureiro, L. O., Engstrom, M. D. and Lim, B. K. (2020) 'Comparative phylogeography of mainland and insular species of Neotropical molossid bats (*Molossus*)', *Ecology and Evolution*, 10(1), pp. 389–409. Doi: 10.1002/ece3.5903.

Lučan, R. K. *et al.* (2016) 'Ectoparasites may serve as vectors for the white-nose syndrome fungus', *Parasites & Vectors*, 9(1), p. 16. Doi: 10.1186/s13071-016-1302-2.

Malinsky, M. *et al.* (2015) 'Genomic islands of speciation separate cichlid ecomorphs in an East African crater lake', *Science*, 350(6267), pp. 1493–1498. Doi: DOI: 10.1126/science.aac9927.

Mallet, J. (2007) 'Concepts of Species', *Encyclopedia of Life*, pp. 1–15. Doi: 10.1016/b0122-2686(52)00481-8.

Mallet, J. (2008) 'Hybridization, ecological races, and the nature of species: empirical evidence for the ease of speciation', *Philosophical Transactions of the Royal Society Series B*, 363, pp. 2971–2986.

Mangiafico, S. (2020) 'rcompanion: Functions to support extension education program evaluation. R package version 2.3.25'. Available at: https://cran.r-project.org/package=rcompanion.

Mangold, A. J., Bargues, M. D. and Mas-Coma, S. (1997) '18S rRNA gene sequences and phylogenetic relationships of European hard-tick species (Acari: Ixodidae)', *Parasitology Research*, 84(1), pp. 31–37. Doi: 10.1007/s004360050352.

Mans, B. J. *et al.* (2012) 'The mitochondrial genomes of *Nuttalliella namaqua* (IxoDoidea: Nuttalliellidae) and *Argas africolumbae* (IxoDoidae: Argasidae): estimation of divergence dates for the major tick lineages and reconstruction of

ancestral blood-feeding characters', *PLoS ONE*, 7(11). Doi: 10.1371/journal.pone.0049461.

Mantel, N. (1967) 'The detection of disease clustering and a generalized regression approach', *Cancer Research*, 27, pp. 209–220.

Mao, X. *et al.* (2010) 'Historical male-mediated introgression in horseshoe bats revealed by multilocus DNA sequence data', *Molecular Ecology*, 19(7), pp. 1352–1366. Doi: 10.1111/j.1365-294X.2010.04560.x.

Marchán-Rivadeneira, M. R. *et al.* (2012) 'On the association between environmental gradients and skull size variation in the great fruit-eating bat, *Artibeus lituratus* (Chiroptera : Phyllostomidae)', *Biological Journal of the Linnean Society*, 105, pp. 623–634.

Marques, D. A. *et al.* (2016) 'Genomics of rapid incipient speciation in sympatric threespine stickleback', *PLOS Genetics*, 12(2), p. e1005887. Doi: 10.1371/journal.pgen.1005887.

Martin, M. (2011) 'Cutadapt removes adapter sequences from high-throughput sequencing reads', *EMBnet.journal*, 17(1), pp. 10–12. Doi: https://Doi.org/10.14806/ej.17.1.200.

Martins, T. F. *et al.* (2014) 'New tick records from the state of Rondônia, western Amazon, Brazil', *Experimental and Applied Acarology*, 62(1), pp. 121–128. Doi: 10.1007/s10493-013-9724-4.

Mayr, E. (1947) 'Ecological Factors in Speciation', *Society for the Study of Evolution*, 1(4), pp. 263–288.

McCain, C. M. (2007) 'Could temperature and water availability drive elevational species richness patterns? A global case study for bats', *Global Ecology and Biogeography*, 16(1), pp. 1–13. Doi: 10.1111/j.1466-8238.2006.00263.x.

McDaniel, B. (1979) *How to know the mites and ticks*. Dubuque, Iowa: W.C. Brown Co.

McGee, M. D. *et al.* (2020) 'The ecological and genomic basis of explosive adaptive radiation', *Nature*, (February). Doi: 10.1038/s41586-020-2652-7.

McGuire, L. P. *et al.* (2012) 'Migratory stopover in the long-distance migrant silver-haired bat, *Lasionycteris noctivagans'*, *Journal of Animal Ecology*, 81(2), pp. 377–

385. Doi: 10.1111/j.1365-2656.2011.01912.x.

McGuire, L. P. and Boyle, W. A. (2013) ‘Altitudinal migration in bats: Evidence, patterns, and drivers’, *Biological Reviews*, 88(4), pp. 767–786. Doi: 10.1111/brv.12024.

McKee, C. D. *et al.* (2019) ‘Host phylogeny, geographic overlap, and roost sharing shape parasite communities in European bats’, *Frontiers in Ecology and Evolution*, 7(MAR), pp. 1–21. Doi: 10.3389/fevo.2019.00069.

McMahon, B. J., Teeling, E. C. and Höglund, J. (2014) ‘How and why should we implement genomics into conservation?’, *Evolutionary Applications*, 7(9), pp. 999–1007. Doi: 10.1111/eva.12193.

Medellín, R. A., Arita, H. T. and Sánchez, O. (2007) *Identificación de los murciélagos de México. Claves de Campo*. 2nd edn. México: Universidad Nacional Autonóma de México, Instituto Nacional de Ecología.

Melville, J. *et al.* (2017) ‘Identifying hybridization and admixture using SNPs: Application of the DArTseq platformin phylogeographic research on vertebrates’, *Royal Society Open Science*, 4(7). Doi: 10.1098/rsos.161061.

Méndez, O. *et al.* (2010) ‘Helminth parasites of some freshwater fishes from Baja California Sur, Mexico’, *Zootaxa*, 2327(1), pp. 44–50.

Michel, A. P. *et al.* (2010) ‘Widespread genomic divergence during sympatric speciation’, *Proceedings of the National Academy of Sciences of the United States of America*, 107(21), pp. 9724–9729. Doi: 10.1073/pnas.1000939107.

Microsoft, C. (2013) ‘Microsoft Excel’. Available at: https://office.microsoft.com/excel.

Miller-Butterworth, C. M. *et al.* (2014) ‘Genetic structure of Little Brown Bats (*Myotis lucifugus*) corresponds with spread of White-Nose Syndrome among hibernacula’, *Journal of Heredity*, 105(3), pp. 354–364. Doi: Doi:10.1093/jhered/esu012.

Miller, M. J. *et al.* (2014) ‘Genetic and phenotypic characterization of a hybrid zone between polyandrous northern and wattled jacanas in Western Panama’, *BMC Evolutionary Biology*, 14, p. 227. Doi: 10.1186/s12862-014-0227-7.

Mollentze, N., Biek, R. and Streicker, D. G. (2014) ‘The role of viral evolution in rabies host shifts and emergence’, *Current Opinion in Virology*. Elsevier B.V., 8, pp. 68–72.

Doi: 10.1016/j.coviro.2014.07.004.

Monteiro, L. R. and Nogueira, M. R. (2010) ‘Adaptive radiations, ecological specialization, and the evolutionary integration of complex morphological structures’, *Evolution; International Journal of Organic Evolution*, 64(3), pp. 724–44. Doi: 10.1111/j.1558-5646.2009.00857.x.

Morales, A. E. *et al.* (2017) ‘Speciation with gene flow in North American *Myotis* bats’, *Systematic Biology*, 66(3), pp. 440–452. Doi: 10.1093/sysbio/syw100.

Morand, S. (2015) ‘(macro-) Evolutionary ecology of parasite diversity: From determinants of parasite species richness to host diversification’, *International Journal for Parasitology: Parasites and Wildlife*. The Author, 4(1), pp. 80–87. Doi: 10.1016/j.ijppaw.2015.01.001.

Morand, S., Krasnov, B. R. and Poulin, R. (2006) *Micromammals and macroparasites: from evolutionary ecology to management*. New York, USA: Springer Publishing.

Moratelli, R. and Carrion, C. (2019) ‘A new species of *Myotis* (Chiroptera, Vespertilionidae) from Peru’, *Special Publications, Museum of Texas Tech University*, (October).

Morrone, J. J., Espinosa-Organista, D. and Llorente-Bousquets, J. (2002) ‘Mexican biogeographic provinces: Preliminary scheme, general characterizations, and synonymies’, *Acta Zoológica Mexicana (nueva serie)*, (85), pp. 83–108.

Morse, S. F. *et al.* (2012) ‘Global distribution and genetic diversity of *Bartonella* in bat flies (Hippoboscoidea, Streblidae, Nycteribiidae)’, *Infection, Genetics and Evolution*. Elsevier B.V., 12(8), pp. 1717–1723. Doi: 10.1016/j.meegid.2012.06.009.

Moussy, C. *et al.* (2012) ‘Migration and dispersal patterns of bats and their influence on genetic structure’, *Mammal Review*, 43(3), pp. 183–195. Doi: 10.1111/j.1365-2907.2012.00218.x.

Moussy, C. *et al.* (2015) ‘Population genetic structure of serotine bats (*Eptesicus serotinus*) across Europe and implications for the potential spread of bat rabies (European bat lyssavirus EBLV-1)’, *Heredity*, 115(1), pp. 83–92. Doi: 10.1038/hdy.2015.20.

Muñoz-Leal, S. *et al.* (2016) ‘Ticks infesting bats (Mammalia: Chiroptera) in the Brazilian Pantanal’, *Experimental and Applied Acarology*, 69(1), pp. 73–85. Doi: 10.1007/s10493-016-0026-5.

Nagy, M. *et al.* (2013) 'Female-biased dispersal in a bat with a female-defence mating strategy', *Molecular Ecology*, 22(6), pp. 1733–1745. Doi: 10.1111/mec.12202.

Nájera-Cortazar, L. A., Álvarez-Castañeda, S. T. and De Luna, E. (2015) 'An analysis of *Myotis peninsularis* (Vespertilionidae) blending morphometric and genetic datasets', *Acta Chiropterologica*, 17(1), pp. 37–47. Doi: 10.3161/15081109ACC2015.17.1.003.

Narum, S. R. *et al.* (2013) 'Genotyping-by-sequencing in ecological and conservation genomics', *Molecular Ecology*, 22(11), pp. 2841–2847. Doi: 10.1016/j.surg.2006.10.010.Use.

National Museum of Natural History, S. I. (2016) *Mammal Species of the World. Checklist dataset, GBIF.org*. Doi: 10.15468.

Nava, S. and Guglielmone, A. A. (2013) 'A meta-analysis of host specificity in Neotropical hard ticks (Acari: Ixodidae)', *Bulletin of the Entomological Research*, 103(2), pp. 216–224.

Navarro Frías, J., González Ruíz, N. and Álvarez Castañeda, S. T. (2007) 'Los mamíferos silvestres de Milpa Alta, Distrito Federal: lista actualizada y consideraciones para su conservación', *Acta Zoologica Mexicana*, 23(003), pp. 103–124.

Nicholls, J. A. *et al.* (2000) 'The evolution of cooperative and pair breeding in thornbills *Acanthiza* (Pardalotidae)', *Journal of Avian Biology*, 31(2), pp. 165–176. Doi: 10.1034/j.1600-048X.2000.310208.x.

Nichols, E. and Gómez, A. (2011) 'Conservation education needs more parasites', *Biological Conservation*. Elsevier Ltd, 144(2), pp. 937–941. Doi: 10.1016/j.biocon.2010.10.025.

O'Shea, T. J. *et al.* (2016) 'Multiple mortality events in bats: a global review', *Mammal Review*, (2016), pp. 1–16. Doi: 10.1111/mam.12064.

Oksanen, J. *et al.* (2019) 'Package "vegan". A Community Ecology Package', *Community ecology package*, 2(9), pp. 1–297. Available at: https://cran.r-project.org/web/packages/vegan/vegan.pdf.

Oksanen, J. (2019) 'Vegan: ecological diversity', *R Package Version 2.4-4*, 1, p. 11. Available at: https://cran.r-project.org/package=vegan.

Olival, K. J. *et al.* (2013) 'Lack of population genetic structure and host specificity in the

bat fly, *Cyclopodia horsfieldi*, across species of *Pteropus* bats in Southeast Asia', *Parasites and Vectors*, 6(1). Doi: 10.1186/1756-3305-6-231.

Olival, K. J. *et al.* (2017) 'Host and viral traits predict zoonotic spillover from mammals', *Nature*. Nature Publishing Group, 546(7660), pp. 646–650. Doi: 10.1038/nature22975.

Olson, L. E. *et al.* (2014) 'First Records of Yuma *Myotis* (*Myotis yumanensis*) in Alaska', *Northwestern Naturalist*, 95(3), pp. 228–235. Doi: 10.1898/13-29.1.

Ortega, J. and Arita, H. T. (1998) 'Neotropical-Nearctic limits in middle America as determined by distributions of bats', *American Society of Mammalogists*, 79(3), pp. 772–783.

Ortega, J. and Maldonado, J. E. (2007) 'Caracterización y evolución de microsatélites en murciélagos (Orden: Chiroptera)', in Sánchez-Rojas, G. and Rojas-Martínez, A. (eds) *Tópicos en sistemática, biogeografía, ecología y conservación de mamíferos*. Universidad Autónoma del Estado de Hidalgo, pp. 71–79.

Ospina Garcés, S. M. (2010) *Análisis ecomorfológico del aparato masticatorio de Myotis vivesi (Chiroptera: Vespertilionidae)*. Universidad Nacional Autónoma de México.

Ossa, G. *et al.* (2019) 'The Klingon batbugs: Morphological adaptations in the primitive bat bugs, *Bucimex chilensis* and *Primicimex cavernis*, including updated phylogeny of Cimicidae', *Ecology and Evolution*, 9(4), pp. 1736–1749. Doi: 10.1002/ece3.4846.

Paris, J. R., Stevens, J. R. and Catchen, J. M. (2017) 'Lost in parameter space: a road map for stacks', *Methods in Ecology and Evolution*, 8(10), pp. 1360–1373. Doi: 10.1111/2041-210X.12775.

Parlos, J. A. *et al.* (2008) 'Population genetics of *Myotis velifer'*, *Direct submission. Biology, Texas State University-San Marcos.*

Patrick, L. E. and Stevens, R. D. (2014) 'Investigating sensitivity of phylogenetic community structure metrics using North American desert bats', *Journal of Mammalogy*, 95(6), pp. 1240–1253. Doi: 10.1644/14-mamm-a-007.

Patterson, B. D., Ballard, J. O. W. and Wenzel, R. (1999) 'Distributional evidence for cospeciation between Neotropical bats and their bat Xy ectoparasites', *Studies on Neotropical Fauna and Environment*, 33, pp. 76–84.

Payseur, B. A. (2010) ‘Using differential introgression in hybrid zones to identify genomic regions involved in speciation’, *Molecular Ecology Resources*, 10(5), pp. 806–820. Doi: 10.1111/j.1755-0998.2010.02883.x.

Pearson, W. R. (2013) ‘An introduction to sequence similarity (“homology”) searching’, *Current protocols in bioinformatics*, Chapter 3, p. Unit3.1-Unit3.1. Doi: 10.1002/0471250953.bi0301s42.

Pérez, T. M. *et al.* (2014) ‘Biodiversidad de ácaros en México’, *Revista Mexicana de Biodiversidad*, 85(SUPPL.), pp. 399–407. Doi: 10.7550/rmb.36160.

Peters, R. *et al.* (2018) ‘Nature Divide, Scientists United: U.S.-Mexico Border Wall Threatens Biodiversity and Binational Conservation’, *Bioscience*, pp. 1–4. Doi: 10.1093/biosci/biy063.

Petersen, F. T. *et al.* (2007) ‘The phylogeny and evolution of host choice in the Hippoboscoidea (Diptera) as reconstructed using four molecular markers’, *Molecular Phylogenetics and Evolution*, 45(1), pp. 111–122. Doi: 10.1016/j.ympev.2007.04.023.

Peterson, B. K. *et al.* (2012) ‘Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species’, *PLoS ONE*, 7(5). Doi: 10.1371/journal.pone.0037135.

Piksa, K. *et al.* (2013) ‘Nonlinear distribution pattern of hibernating bats in caves along an elevational gradient in mountain (Carpathians, Southern Poland)’, *PLoS ONE*, 8(7), pp. 1–10. Doi: 10.1371/journal.pone.0068066.

Platt, R. N. *et al.* (2018) ‘Conflicting evolutionary histories of the mitochondrial and nuclear genomes in New World *Myotis* bats’, *Systematic Biology*, 67(2), pp. 236–249. Doi: 10.1093/sysbio/syx070.

Plowright, R. K. *et al.* (2017) ‘Pathways to zoonotic spillover’, *Nature Reviews Microbiology*. Nature Publishing Group, 15(8), pp. 502–510. Doi: 10.1038/nrmicro.2017.45.

Poelstra, J. W. *et al.* (2014) ‘The genomic landscape underlying phenotypic integrity in the face of gene flow in crows’, *Science (New York, N.Y.)*, 344(6190), pp. 1410–4. Doi: 10.1126/science.1253226.

Popa-Lisseanu, A. G., Voigt, C. C. and Ecology, E. (2009) ‘Bats on the Move’, *Journal of Mammalogy*, 90(6), pp. 1283–1289. Doi: 10.1644/09-MAMM-S-130R2.1.

Porter, T. M. and Hajibabaei, M. (2018) ‘Over 2.5 million COI sequences in GenBank and growing’, *bioRxiv*, p. 353904. Doi: 10.1101/353904.

Potiwat, R. *et al.* (2016) ‘Identification of bat ectoparasite *Leptocimex inordinatus* from bat-dwelling cave, Kanchanaburi Province, Thailand’, *Southeast Asian Journal of Tropical Medicine and Public Health*, 47(1), pp. 16–22.

Poulin, R. (1995) ‘Phylogeny, ecology, and the richness of parasite communities in vertebrates’, *Ecological Monographs*, 65(3), pp. 283–302.

Poulin, R. (2014) ‘Parasite biodiversity revisited: Frontiers and constraints’, *International Journal for Parasitology*. Australian Society for Parasitology Inc., 44(9), pp. 581–589. Doi: 10.1016/j.ijpara.2014.02.003.

Poulin, R., Krasnov, B. R. and Mouillot, D. (2011) ‘Host specificity in phylogenetic and geographic space’, *Trends in Parasitology*. Elsevier Ltd, 27(8), pp. 355–361. Doi: 10.1016/j.pt.2011.05.003.

Poulin, R. and Morand, S. (2004) *Parasite biodiversity: book review, Smithsonian Institution Books*. Doi: 10.1111/1539-6975.00065.

Powell, T. H. Q. *et al.* (2013) ‘Genetic divergence along the speciation continuum: The transition from host race to species in *Rhagoletis* (Diptera: Tephritidae)’, *Evolution*, 67(9), pp. 2561–2576. Doi: 10.1111/evo.12209.

Presley, S. J. (2012) ‘Sex-based population structure of ectoparasites from Neotropical bats’, *Biological Journal of the Linnean Society*, 107(1), pp. 56–66. Doi: 10.1111/j.1095-8312.2012.01924.x.

Pulgarín-R, P. C. *et al.* (2018) ‘Host species, and not environment, predicts variation in blood parasite prevalence, distribution, and diversity along a humidity gradient in northern South America’, *Ecology and Evolution*, 8(8), pp. 3800–3814. Doi: 10.1002/ece3.3785.

Purcell, S. *et al.* (2007) ‘PLINK: A tool set for whole-genome association and population-based linkage analyses’, *American Journal of Human Genetics*, 81(3), pp. 559–575. Doi: 10.1086/519795.

Pylant, C. L. *et al.* (2016) ‘Geographic origins and population genetics of bats killed at -wind-energy facilities’, *Ecological Applications*, 26(5), pp. 1381–1395.

de Queiroz, K. (2005) ‘Ernst Mayr and the modern concept of species.’, *Proceedings of the National Academy of Sciences of the United States of America*, 102 Suppl, pp. 6600–6607. Doi: 10.1073/pnas.0502030102.

de Queiroz, K. (2007) ‘Species concepts and species delimitation.’, *Systematic biology*, 56(6), pp. 879–886. Doi: 10.1080/10635150701701083.

Raj, A., Stephens, M. and Pritchard, J. K. (2014) ‘FastSTRUCTURE: Variational inference of population structure in large SNP data sets’, *Genetics*, 197(2), pp. 573–589. Doi: 10.1534/genetics.114.164350.

Ramasindrazana, B. *et al.* (2017) ‘Hidden diversity of Nycteribiidae (Diptera) bat flies from the Malagasy region and insights on host-parasite interactions’, *Parasites and Vectors*. Parasites & Vectors, 10(1), pp. 1–8. Doi: 10.1186/s13071-017-2582-x.

Rambaut, A. *et al.* (2018) ‘Posterior summarization in Bayesian phylogenetics using Tracer 1.7’, *Systematic Biology*, 67(5), pp. 901–904. Doi: https ://Doi.org/10.1093/sysbi o/ syy032.

Ramirez-Acosta, J. *et al.* (2012) ‘Conservation of endemic terrestrial vertebrates in the protected areas of the Baja California Peninsula, Mexico’, *Natural Areas Journal*, 32(1), pp. 15–30. Doi: 10.3375/043.032.0104.

Ramírez-Martínez, M. *et al.* (2016) ‘New records of ectoparasitic Acari (Arachnida) and Streblidae (Diptera) from bats in Jalisco, Mexico’, *Journal of Vector Ecology*, 41(2), pp. 309–313. Doi: 10.1111/jvec.12228.

Ratnasingham, S. and Hebert, P. D. N. (2007) ‘BOLD: The Barcode of Life Data System (www.barcodinglife.org)’, *Molecular Ecology Notes*, 7, pp. 355–364. Doi: 10.1111/j.1471-8286.2006.01678.x.

Ratnasingham, S. and Hebert, P. D. N. (2013) ‘A DNA-Based Registry for All Animal Species: The Barcode Index Number (BIN) System’, *PLoS ONE*, 8(8), p. e66213. Doi: 10.1371/journal.pone.0066213.

Raya, A. P. *et al.* (2018) ‘Detection of *Bartonella* species, including *Candidatus Bartonella ovis* sp. nov, in ruminants from Mexico and lack of evidence of *Bartonella* DNA in saliva of common vampire bats (*Desmodus rotundus*) predating on them’, *Veterinary Microbiology*, 222(June), pp. 69–74. Doi: 10.1016/j.vetmic.2018.06.018.

Raymond, M. and Rousset, F. (1995) ‘GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism’, *Journal of Heredity*, pp. 248–249.

Razgour, O. *et al.* (2011) ‘High-throughput sequencing offers insight into mechanisms of resource partitioning in cryptic bat species’, *Ecology and Evolution*, 1(4), pp. 556–570. Doi: 10.1002/ece3.49.

Razgour, O. *et al*. (2014) ‘Scale-dependent effects of landscape variables on gene flow and population structure in bats’, *Diversity and Distribution*s, 20(10), pp. 1173–1185. Doi: 10.1111/ddi.12200.

Razgour, O. *et al.* (2018) ‘An integrated framework to identify wildlife populations under threat from climate change’, *Molecular Ecology Resources*, 18(1), pp. 18–31. Doi: 10.1111/1755-0998.12694.

Razgour, O. *et al.* (2018) ‘The role of climate, water and biotic interactions in shaping biodiversity patterns in arid environments across spatial scales’, *Diversity and Distributions*, 24(10), pp. 1440–1452. Doi: 10.1111/ddi.12773.

Razgour, O., Hanmer, J. and Jones, G. (2011) ‘Using multi-scale modelling to predict habitat suitability for species of conservation concern: The grey long-eared bat as a case study’, *Biological Conservation*. Elsevier Ltd, 144(12), pp. 2922–2930. Doi: 10.1016/j.biocon.2011.08.010.

Reckardt, K. and Kerth, G. (2009) ‘Does the mode of transmission between hosts affect the host choice strategies of parasites? Implications from a field study on bat fly and wing mite infestation of Bechstein’s bats’, *Oikos*, 118(2), pp. 183–190.

Reeves, W. K. *et al.* (2016) ‘Ecology of Bats, Their Ectoparasites, and Associated Pathogens on Saint Kitts Island’, *Journal of Medical Entomology*, 53(5), pp. 1218–1225. Doi: 10.1093/jme/tjw078.

Reiczigel, J. *et al.* (2019) ‘Biostatistics for Parasitologists – A Primer to Quantitative Parasitology’, *Trends in Parasitology*. Elsevier Ltd, 35(4), pp. 277–281. Doi: 10.1016/j.pt.2019.01.003.

Reinhardt, K. and Siva-Jothy, M. T. (2007) ‘Biology of the Bed Bugs (Cimicidae)’, *Annual Review of Entomology*, 52(1), pp. 351–374. Doi: 10.1146/annurev.ento.52.040306.133913.

Rex, K. *et al.* (2008) ‘Species richness and structure of three Neotropical bat assemblages’, *Biological Journal of the Linnean Society*, 94(3), pp. 617–629. Doi: 10.1111/j.1095-8312.2008.01014.x.

Rice, A. M. *et al.* (2011) ‘A guide to the genomics of ecological speciation in natural

animal populations', *Ecology Letters*, 14(1), pp. 9–18. Doi: 10.1111/j.1461-0248.2010.01546.x.

Riddle, B. R. *et al.* (2000) 'Cryptic vicariance in the historical assembly of a Baja California peninsular desert biota', *Proceedings of the National Academy of Sciences of the United States of America*, 97(26), pp. 14438–43. Doi: 10.1073/pnas.250413397.

Riemann, H. and Ezcurra, E. (2005) 'Plant endemism and natural protected areas in the peninsula of Baja California, Mexico', *Biological Conservation*, 122(1), pp. 141–150. Doi: 10.1016/j.biocon.2004.07.008.

Rieseberg, L. H., Church, S. A. and Morjan, C. L. (2004) 'Integration of populations and differentiation of species', *New Phytologist*, 161(1), pp. 59–69. Doi: 10.1046/j.1469-8137.2003.00933.x.

Rigaud, T., Perrot-Minnot, M. J. and Brown, M. J. F. (2010) 'Parasite and host assemblages: Embracing the reality will improve our knowledge of parasite transmission and virulence', *Proceedings of the Royal Society B: Biological Sciences*, 277(1701), pp. 3693–3702. Doi: 10.1098/rspb.2010.1163.

Rios, E. and Álvarez-Castañeda, S. T. (2002) 'Mamíferos de la Reserva del Valle de los Cirios, Baja California, México', *Acta Zoológica Mexicana*, 86, pp. 51–85.

Rivers, N. M., Butlin, R. K. and Altringham, J. D. (2005) 'Genetic population structure of Natterer's bats explained by mating at swarming sites and philopatry', *Molecular Ecology*, 14(14), pp. 4299–4312. Doi: 10.1111/j.1365-294X.2005.02748.x.

Rochette, N. C. and Catchen, J. M. (2017) 'Deriving genotypes from RAD-seq short-read data using Stacks', *Nature Protocols*. Nature Publishing Group, 12(12), pp. 2640–2659. Doi: 10.1038/nprot.2017.123.

Rodríguez-Santiago, M. A. and Rosales-Casián, J. A. (2011) 'Parasite structure of the Ocean Whitefish *Caulolatilus princeps* from Baja California, México (East Pacific)', *Helgoland Marine Research*, 65(2), pp. 197–202. Doi: 10.1007/s10152-010-0215-2.

Rodriguez, R. M. and Ammerman, L. K. (2004) 'Mitochondrial DNA divergence does not reflect morphological difference between *Myotis californicus* and *Myotis ciliolabrum*', *Journal of Mammalogy*, 85(5), pp. 842–851. Doi: 10.1644/1545-1542(2004)085<0842.

Rossiter, S. J. *et al.* (2000) 'Genetic variation and population structure in the endangered

greater horseshoe bat *Rhinolophus ferrumequinum*', *Molecular Ecology*, 9(8), pp. 1131–1135. Doi: 10.1046/j.1365-294x.2000.00982.x.

Roth, S. *et al.* (2019) 'Bedbugs evolved before their bat hosts and did not co-speciate with ancient humans', *Current Biology*, pp. 1–7. Doi: 10.1016/j.cub.2019.04.048.

Rousset, F. (2008) 'Genepop'007: a complete reimplementation of the Genepop software for Windows and Linux', *Molecular Ecology Resources*, (8), pp. 103–106.

Royston, P. (1995) 'Remark AS R94: A remark on Algorithm AS 181: The W test for normality', *Applied Statistics*, 44, pp. 547–551. Doi: 10.2307/2986146.

RStudio Team, C. (2015) 'RStudio: Integrated Development for R'. Boston, MA: RStudio, Inc. Available at: http://www.rstudio.com/.

Ruedi, M. *et al.* (2012) 'Biogeography of Old World emballonurine bats (Chiroptera: Emballonuridae) inferred with mitochondrial and nuclear DNA', *Molecular phylogenetics and evolution*. Elsevier Inc., 64(1), pp. 204–11. Doi: 10.1016/j.ympev.2012.03.019.

Ruedi, M. *et al*. (2013) 'Molecular phylogenetic reconstructions identify East Asia as the cradle for the evolution of the cosmopolitan genus *Myotis* (Mammalia, Chiroptera)', *Molecular Phylogenetics and Evolution*. Elsevier Inc., 69(3), pp. 437–449. Doi: 10.1016/j.ympev.2013.08.011.

Ruedi, M. and Mayer, F. (2001) 'Molecular systematics of bats of the genus Myotis (Vespertilionidae) suggests deterministic ecomorphological convergences', *Molecular phylogenetics and evolution*, 21(3), pp. 436–48. Doi: 10.1006/mpev.2001.1017.

Russell, A. L., Medellin, R. A. and Mccracken, G. F. (2005) 'Genetic variation and migration in the Mexican free-tailed bat (*Tadarida brasiliensis mexicana*)', *Molecular Ecology*, 14(7), pp. 2207–2222. Doi: 10.1111/j.1365-294X.2005.02552.x.

Russo, D. *et al.* (2017) 'Collection of voucher specimens for bat research: conservation, ethical implications, reduction, and alternatives', *Mammal Review*. Doi: 10.1111/mam.12095.

Rzedowski, J. (2006) *Vegetación de México*. 1st edn. Comisión Nacional para el Conocimiento y Uso de la Biodiversidad (CONABIO).

Saldaña-Vázquez, R. A. *et al.* (2019) 'Host ecology moderates the specialization of Neotropical bat-fly interaction networks', *Parasitology Research*. Parasitology Research, 118(10), pp. 2919–2924. Doi: 10.1007/s00436-019-06452-1.

Salinas-Ramos, V. B. *et al.* (2018) 'Seasonal variation of bat-flies (Diptera: Streblidae) in four bat species from a tropical dry forest', *Mammalia*, 82(2), pp. 133–143. Doi: 10.1515/mammalia-2016-0176.

Salinas-Zavala, C. A. *et al.* (1990) 'Distribución geográfica y variabilidad climática de los regímenes pluviométricos en Baja California Sur, México', *Atmósfera*, (3), pp. 217–237.

Sánchez-Montes, S. *et al.* (2016) '*Rickettsia lusitaniae* associated with *Ornithodoros yumatensis* (Acari: Argasidae) from two caves in Yucatan, Mexico', *Ticks and Tick-borne Diseases*, 7(6), pp. 1097–1101. Doi: 10.1016/j.ttbdis.2016.09.003.

Sándor, A. D. *et al.* (2019) 'Bats and ticks: host selection and seasonality of bat-specialist ticks in Eastern Europe', *Parasites & vectors*. BioMed Central, 12(1), p. 605. Doi: 10.1186/s13071-019-3861-5.

Schad, J. *et al.* (2012) 'Evidence for the "good genes" model: Association of MHC Class II DRB alleles with ectoparasitism and reproductive state in the Neotropical Lesser Bulldog bat, *Noctilio albiventris'*, *PLoS ONE*, 7(5). Doi: 10.1371/journal.pone.0037101.

van Schaik, J. *et al.* (2014) 'The effect of host social system on parasite population genetic structure: comparative population genetics of two ectoparasitic mites and their bat hosts', *BMC Evolutionary Biology*. BMC Evolutionary Biology, 14(1), p. 18. Doi: 10.1186/1471-2148-14-18.

Scheffler, I. *et al.* (2010) 'Ectoparasites of bats in Mongolia (Ischnopsyllidae, Nycteribiidae, Cimicidae and Spinturnicidae)', *Erforsch. Biol. Ress. Mongolei*. 11:367-381.

Schluter, D. (2001) 'Ecology and the origin of species', *Trends in Ecology & Evolution*, 16(7), pp. 372–380.

Secretaría de Medio Ambiente y Recursos Naturales (2010) *Norma Oficial Mexicana NOM-059-SEMARNAT-2010. Protección ambiental-especies nativas de México de flora y fauna silvestres-Categorías de riesgo y especificaciones para su inclusión, exclusión o cambio-lista de especies en riesgo*. Secretaría de Medio Ambiente y Recursos Naturales, Mexico. Available at:

https://dof.gob.mx/nota_detalle_popup.php?codigo=5173091

Seehausen, O. *et al.* (2008) 'Speciation reversal and biodiversity dynamics with hybridization in changing environments', *Molecular Ecology*, 17(1), pp. 30–44. Doi: 10.1111/j.1365-294X.2007.03529.x.

Seehausen, O. *et al.* (2014) 'Genomics and the origin of species', *Nature reviews. Genetics*. Nature Publishing Group, 15(3), pp. 176–92. Doi: 10.1038/nrg3644.

Segura-Trujillo, C. A., Lidicker, W. Z. and Álvarez-Castañeda, S. T. (2016) 'New perspectives on trophic guilds of arthro-podivorous bats in North and Central America', *Journal of Mammalogy*, 92, pp. 644–654.

Segura-Trujillo, C. A., Willig, M. R. and Álvarez-Castañeda, S. T. (2018) 'Correspondence between ecomorphotype and use of arthropod resources by bats of the genus *Myotis'*, *Journal of Mammalogy*, (May), pp. 1–9. Doi: 10.1093/jmammal/gyy049.

Seim, I. *et al.* (2013) 'Genome analysis reveals insights into physiology and longevity of the Brandt's bat *Myotis brandtii*', *Nature communications*. Nature Publishing Group, 4, p. 2212. Doi: 10.1038/ncomms3212.

Seneviratne, S. S., Fernando H, C. and Udagama-Randeniya, P. V (2009) 'Host specificity in bat ectoparasites: A natural experiment', *International Journal for Parasitology*. Australian Society for Parasitology Inc., 39(9), pp. 995–1002. Doi: 10.1016/j.ijpara.2008.12.009.

Senior, P., Butlin, R. K. and Altringham, J. D. (2005) 'Sex and segregation in temperate bats', *Proceedings of the Royal Society B: Biological Sciences*, 272(1580), pp. 2467–2473. Doi: 10.1098/rspb.2005.3237.

Shaw, K. L. and Mullen, S. P. (2014) 'Speciation continuum', *Journal of Heredity*, 105(S1), pp. 741–742. Doi: 10.1093/jhered/esu060.

Sikes, R. S. *et al.* (2016) '2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education', *Journal of Mammalogy*, 97(3), pp. 663–668. Doi: 10.1093/jmammal/gyw078.

Simmons, N. B. (2005) 'Order Chiroptera', in Wilson, D. E. and Reeder, D. M. (eds) *Mammals of the World: a taxonomic and geographic reference*. 3rd edn. USA: Johns Hopkings University Press, p. 2142. Available at: http://www.press.jhu.edu.

Simmons, N. B. and Conway, T. M. (2001) ‘Phylogenetic relationships of Mormoopid bats (Chiroptera: Mormoopidae) based on morphological data’, *Bulletin of the American Museum of Natural History*, 258(1), p. 1. Doi: 10.1206/0003-0090(2001)258<0001:PROMBC>2.0.CO;2.

Simmons, N. B. *et al.* (2021) ‘A new dichromatic species of *Myotis* (Chiroptera: Vespertilionidae) from the Nimba Mountains, Guinea’, *American Museum Novitates*, (3963), pp. 1–37

Simpson, M. R. (1993) ‘*Myotis californicus*’, *Mammalian Species*, 428, pp. 1-4. Doi: 10.2307/3504100.

Smadja, C. M. and Butlin, R. K. (2011) ‘A framework for comparing processes of speciation in the presence of gene flow’, *Molecular Ecology*, 20(24), pp. 5123–5140. Doi: 10.1111/j.1365-294X.2011.05350.x.

Smith, S. J. *et al.* (2008) ‘Subspecific affinities and conservation genetics of Western Big-eared bats (*Corynorhinus townsendii pallescens*) at the edge of their distributional range’, *Journal of Mammalogy*, 89(4), pp. 799–814. Doi: 10.1644/06-MAMM-A-279.1.

Solari, S. (2019) *Myotis velifer. The IUCN Red List of Threatened Species 2019: e.T14208A22063586, IUCN Red List of Threatened Species*. Doi: https://dx.Doi.org/10.2305/IUCN.UK.2019-1.RLTS.T14208A22063586.en.

Sonsthagen, S. A. *et al.* (2019) ‘Coast to coast: High genomic connectivity in North American scoters’, *Ecology and Evolution*, 9(12), pp. 7246–7261. Doi: 10.1002/ece3.5297.

Sovic, M. G., Carstens, B. C. and Gibbs, H. L. (2016) ‘Genetic diversity in migratory bats: Results from RADseq data for three tree bat species at an Ohio windfarm’, *PeerJ*, 4, p. e1647. Doi: 10.7717/peerj.1647.

Speer, K. A. *et al*. (2017) ‘Population structure of a widespread bat (*Tadarida brasiliensis*) in an island system’, *Ecology and Evolution*, 7(19), pp. 7585–7598. Doi: 10.1002/ece3.3233.

Speer, K. A. *et al.* (2019) ‘A fly on the cave wall: parasite genetics reveal fine-scale dispersal patterns of bats’, *Journal of Parasitology*, 105(4), p. 555. Doi: 10.1645/19-20.

Spencer, H. G. and Zuk, M. (2016) ‘For host’s sake: The pluses of parasite preservation’,

Trends in Ecology and Evolution. Elsevier Ltd, 31(5), pp. 341–343. Doi: 10.1016/j.tree.2016.02.021.

Srinivasulu, C. *et al.* (2019) 'Integrated approaches to identifying cryptic bat species in areas of high endemism: The case of *Rhinolophus andamanensis* in the Andaman Islands', *PLoS ONE*, 14(10), pp. 1–15. Doi: 10.1371/journal.pone.0213562.

Stadelmann, B. *et al.* (2004) 'Molecular systematics of the fishing bat *Myotis* (*Pizonix*) *vivesi', Journal of Mammalogy*, 85(1), pp. 133–139.

Stadelmann, B. *et al.* (2004) 'Phylogeny of African *Myotis* bats (Chiroptera , Vespertilionidae) inferred from cytochrome b sequences', *Acta Chiropterologica*, 6(2), pp. 177–192.

Stadelmann, B. *et al.* (2007) 'Molecular phylogeny of New World *Myotis* (Chiroptera, Vespertilionidae) inferred from mitochondrial and nuclear DNA genes', *Molecular Phylogenetics and Evolution*, 43(1), pp. 32–48. Doi: 10.1016/j.ympev.2006.06.019.

Steinlein, D. B., Durden, L. and Cannon, W. L. (2001) 'Tick (Acari) infestations of bats in New Mexico.', *Journal of medical entomology*, 38(4), pp. 609–611. Doi: 10.1603/0022-2585-38.4.609.

Suchard, M. A. *et al.* (2018) 'Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10', *Virus Evolution*, 4(1), p. vey016.

Sudhir, K. *et al.* (2018) 'MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms', *Molecular Biology and Evolution*, 35, pp. 1547–1549.

Supple, M. A. *et al.* (2015) 'Divergence with gene flow across a speciation continuum of *Heliconius* butterflies', *BMC evolutionary biology*. BMC Evolutionary Biology, 15(1), p. 204. Doi: 10.1186/s12862-015-0486-y.

Szentiványi, T., Christe, P. and Glaizot, O. (2019) 'Bat flies and their microparasites: Current knowledge and distribution', *Frontiers in Veterinary Science*, 6(April), pp. 1–12. Doi: 10.3389/fvets.2019.00115.

Tahir, D. *et al.* (2016) 'New *Rickettsia* species in soft ticks *Ornithodoros hasei* collected from bats in French Guiana', *Ticks and Tick-borne Diseases*, 7(6), pp. 1089–1096. Doi: 10.1016/j.ttbdis.2016.09.004.

Talbot, B. *et al.* (2016) 'Range-wide genetic structure and demographic history in the bat

ectoparasite *Cimex adjunctus'*, *BMC Evolutionary Biology*. BMC Evolutionary Biology, 16(1), pp. 1–13. Doi: 10.1186/s12862-016-0839-1.

Talbot, B. (2017) *Evolutionary genetic aspects of host association in generalist ectoparasites*. The University of Western Ontario.

Talbot, B. *et al.* (2017) 'Population structure in two geographically sympatric and congeneric ectoparasites: *Cimex adjuntus* and *Cimex lectularius*, in the North American Great Lakes region', *Canadian Journal of Zoology*, (95), pp. 901–907.

Talbot, B. *et al.* (2017) 'Comparative analysis of landscape effects on spatial genetic structure of the big brown bat and one of its cimicid ectoparasites', *Ecology and Evolution*, 7(20), pp. 8210–8219. Doi: 10.1002/ece3.3329.

Teeling, E. *et al.* (2018) 'Bat Biology, Genomes, and the Bat1K Project: To Generate Chromosome-Level Genomes for all Living Bat Species', *Annual Review of Animal Biosciences*, 6(1), p. annurev-animal-022516-022811. Doi: 10.1146/annurev-animal-022516-022811.

Teeling, E. C. *et al.* (2005) 'A molecular phylogeny for bats illuminates biogeography and the fossil record', *Science (New York, N.Y.)*, 307, pp. 580–584. Doi: 10.1126/science.1105113.

Teeling, E. C., Jones, G. and Rossiter, S. J. (2016) 'Phylogeny, genes, and hearing: implications for the evolution ofecholocation in bats', in Fenton, M. B. *et al*. (eds) *Bat Bioacoustics*. New York, USA: Springer, pp. 24–54.

Templeton, A. R. (1981) 'Mechanisms of speciation - A population genetic approach', *Annual Review of Ecology and Systematics*, 12(1), pp. 23–48. Doi: 10.1146/annurev.es.12.110181.000323.

The National Library of Medicine (2018) *The Basic Local Alignment Search Tool (BLAST), The National Center for Biotechnology Information*. Available at: http://blast.ncbi.nlm.nih.gov/Blast.cgi.

Thompson, J. D., Higgins, D. G. and Gibson, T. J. (1994) 'CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice', *Nucleic Acids Research*, (22), pp. 4673–4680.

Tortosa, P. *et al.* (2013) 'Evolutionary history of indian ocean nycteribiid bat flies mirroring the ecology of their hosts', *PLoS ONE*, 8(9). Doi:

10.1371/journal.pone.0075215.

Traba, J. *et al.* (2010) 'Habitat selection by two sympatric rodent species in the Monte desert, Argentina. First data for Eligmodontia moreni and *Octomys mimax*', *Journal of Arid Environments*, 74(2), pp. 179–185. Doi: 10.1016/j.jaridenv.2009.06.017.

Trujillo-Pahua, L. and Ibáñez-Bernal, S. (2018) 'New geographical records of bat flies (Diptera: Streblidae) associated with Phyllostomid bats (Chiroptera: Phyllostomidae) in the West Highlands of Mexico', *Journal of Medical Entomology*, (September), pp. 0–11. Doi: 10.1093/jme/tjy166.

Tsagkogeorga, G. *et al.* (2013) 'Phylogenomic analyses elucidate the evolutionary relationships of bats', *Current Biology*. Elsevier Ltd, 23(22), pp. 2262–2267. Doi: 10.1016/j.cub.2013.09.014.

Tschapka, M. *et al.* (2008) 'Diet and cranial morphology of *Musonycteris harrisoni*, a highly specialized nectar-feeding bat in western Mexico', *Journal of Mammalogy*, 89(4), pp. 924–932.

Turmelle, A. S. and Olival, K. J. (2009) 'Correlates of viral richness in bats (Order Chiroptera)', *EcoHealth*, 6(4), pp. 522–539. Doi: 10.1007/s10393-009-0263-8.

Ungerer, M. C., Johnson, L. C. and Herman, M. A. (2008) 'Ecological genomics: understanding gene and genome function in the natural environment', *Heredity*, 100(2), pp. 178–183. Doi: 10.1038/sj.hdy.6800992.

Usinger, R. L. (1966) 'Monograph of Cimicidae (Hemiptera-Heteroptera)', *Entomological Society of America*, VII, p. 572. Available at: http://apps.webofknowledge.com/full_record.do?product=UA&search_mode=GeneralSearch&qid=32&SID=Y2m6OyGYg7txy2Oc2bE&page=1&doc=3.

Van Den Bussche, R. and Hoofer, S. R. (2004) 'Phylogenetic relationships among recent chiropteran families and the importance of choosing appropriate out- group taxa', *Journal of Mammalogy*, 85(2), pp. 321–330.

Vanderplank, S. E., Rebman, J. P. and Ezcurra, E. (2017) 'Where to conserve? Plant biodiversity and endemism in mediterranean Mexico', *Biodiversity and Conservation*. Springer Netherlands. Doi: 10.1007/s10531-017-1424-7.

de Vasconcelos, P. F. *et al.* (2016) 'Parasite-host interactions of bat flies (Diptera: Hippoboscoidea) in Brazilian tropical dry forests', *Parasitology Research*, 115(1), pp. 367–377. Doi: 10.1007/s00436-015-4757-8.

Veiga, J. P. *et al.* (2000) ‘Skin pockets and the location of ectoparasitic mites on lizards of Baja California’, *Herpetological Journal*, 10, pp. 73–74.

Veikkolainen, V. *et al.* (2014) ‘Bats as reservoir hosts of human bacterial pathogen, *Bartonella mayotimonensis’*, *Emerging Infectious Diseases*, 20(6), pp. 960–967. Doi: 10.3201/eid2006.130956.

Veith, M. *et al.* (2004) ‘The role of swarming sites for maintaining gene flow in the brown long-eared bat (*Plecotus auritus*)’, *Heredity*, 93(4), pp. 342–349. Doi: 10.1038/sj.hdy.6800509.

Velasco-Villa, A. *et al.* (2002) ‘Antigenic diversity and distribution of rabies virus in Mexico’, *Journal of Clinical Microbiology*, 40(3), pp. 951–958. Doi: 10.1128/JCM.40.3.951.

Venzal, J. M. *et al.* (2015) ‘Two new species of *Ornithodoros* (Ixodida; Argasidae) from the Southern Cone of South America’, *Experimental and Applied Acarology*, 66(1), pp. 127–139. Doi: 10.1007/s10493-015-9883-6.

Vonhof, M. J., Russell, A. L. and Miller-Butterworth, C. M. (2015) ‘Range-wide genetic analysis of little brown bat (*Myotis lucifugus*) populations: Estimating the risk of spread of white-nose syndrome’, *PLoS ONE*, 10(7), pp. 1–23. Doi: 10.1371/journal.pone.0128713.

Walsh, B. D. (1864) ‘On phytophagic varieties and phytophagic species’, *Proceedings of the Entomological Society of Philadelphia*, (3), pp. 403–430.

Walters, C. L. *et al.* (2012) ‘A continental-scale tool for acoustic identification of European bats’, *Journal of Applied Ecology*, 49(5), pp. 1064–1074. Doi: 10.1111/j.1365-2664.2012.02182.x.

Warner, B. R. M. and Czaplewski, N. J. (1984) ‘*Myotis volans*’, *Mammalian Species*, 07(224), pp. 2–5.

Wenzel, R. and Tipton, V. J. (1966) ‘Some relationships between mammal hosts and their ectoparasites’, in Wenzel, R. and Tipton, V. J. (eds) *Ectoparasites of Panama*. Chicago: Field Museum of Natural History, pp. 677–723.

Wessels, C. *et al.* (2018) ‘Comparative mtDNA phylogeographic patterns reveal marked differences in population genetic structure between generalist and specialist ectoparasites of the African penguin (Spheniscus demersus)’, *Parasitology Research*. Parasitology Research.

White, R. J. and Razgour, O. (2020) ‘Emerging zoonotic diseases originating in mammals: a systematic review of effects of anthropogenic land-use change’, *Mammal Review*. Doi: 10.1111/mam.12201.

Whiting, M. F. (2002) ‘Mecoptera is paraphyletic: Multiple genes and phylogeny of Mecoptera and Siphonaptera’, *Zoologica Scripta*, 31(1), pp. 93–104. Doi: 10.1046/j.0300-3256.2001.00095.x.

Wibbelt, G. *et al.* (2010) ‘White-nose syndrome fungus (*Geomyces destructans*) in bats, Europe’, *Emerging Infectious Diseases*, 16(8), pp. 1237–1242. Doi: 10.3201/eid1608.100002.

Wickham, H. (2016) *ggplot2: Elegant Graphics for Data Analysis*. New York, USA: Springer-Verlag.

Wilder, A. P., Kunz, T. H. and Sorenson, M. D. (2015) ‘Population genetic structure of a common host predicts the spread of white-nose syndrome, an emerging infectious disease in bats’, *Molecular Ecology*, 24, pp. 5495–5506. Doi: 10.1111/mec.13396.

Wilkinson, D. *et al.* (2016) ‘The bacteriome of bat flies (Nycteribiidae) from the Malagasy region: a community shaped by host ecology, bacterial transmission mode, and host-vector specificity’, *Applied and Environmental Microbiology*, 82(6), pp. 1778–1788. Doi: 10.1128/AEM.03505-15.Editor.

Willis, C. K. R. and Brigham, R. M. (2004) ‘Roost switching, roost sharing and social cohesion: forest-dwelling big brown bats, *Eptesicus fuscus*, conform to the fission-fusion model’, *Animal Behaviour*, 68(3), pp. 495–505. Doi: 10.1016/j.anbehav.2003.08.028.

Wordley, C. F. R. *et al.* (2015) ‘Landscape scale habitat suitability modelling of bats in the Western Ghats of India: Bats like something in their tea’, *Biological Conservation*. Elsevier B.V., 191, pp. 529–536. Doi: 10.1016/j.biocon.2015.08.005.

Wu, Q. *et al.* (2019) ‘Environmental variation mediates the prevalence and co-occurrence of parasites in the common lizard, *Zootoca vivipara’*, *BMC Ecology*. BioMed Central, 19(1), pp. 1–11. Doi: 10.1186/s12898-019-0259-3.

Xu, G. *et al.* (2003) ‘Molecular phylogenetic analyses indicate that the *Ixodes ricinus* complex is a paraphyletic group.’, *Journal of Parasitology*, 89(3), pp. 452–457. Doi: 10.1645/0022-3395(2003)089[0452:MPAITT]2.0.CO;2.

Yu, D. *et al.* (2016) ‘The complete mitochondrial genome of *Myotis lucifugus*

(Chiroptera: Vespertilionidae)', *Mitochondrial DNA Part A*. Taylor & Francis, 27(4), pp. 2423–2424. Doi: 10.3109/19401736.2015.1030625.

Yule, G. U. (1925) 'Yule Process', *Philosophical Transactions of the Royal Society B: Biological Sciences1*, (213), pp. 21–87.

Zahn, L. M. (2018) 'Rapid hybrid speciation in Darwin's finches', *Science*, 359(6372), p. 172. Doi: 10.1126/science.359.6372.172-d.

Zamora-Gutierrez, V., Amano, T. and Jones, K. E. (2019) 'Spatial and taxonomic biases in bat records: Drivers and conservation implications in a megadiverse country', *Ecology and Evolution*, 00(October), pp. 1–12. Doi: 10.1002/ece3.5848.

Zamora-Mejías, D. *et al.* (2020) 'Does latitudinal migration represent an advantage in the decrease of ectoparasite load in *Leptonycteris yerbabuenae* (Chiroptera)?', *Journal of Mammalogy*, (X), pp. 1–11. Doi: 10.1093/jmammal/gyaa075.

Zepeda Mendoza, M. L. *et al.* (2018) 'Hologenomic adaptations underlying the evolution of sanguivory in the common vampire bat', *Nature Ecology and Evolution*, 2(4), pp. 659–668. Doi: 10.1038/s41559-018-0476-8.

Zhou, R. *et al.* (2011) 'Population genetics in nonmodel organisms: II. Natural selection in marginal habitats revealed by deep sequencing on dual platforms', *Molecular Biology and Evolution*, 28(10), pp. 2833–2842. Doi: 10.1093/molbev/msr102.

Zhu, H. (2019) 'kableExtra: Construct Complex Table with "kable" and Pipe Syntax'. Available at: https://cran.r-project.org/package=kableExtra.

Zorrilla-Vaca, A., Silva-Medina, M. M. and Escandón-Vargas, K. (2015) 'Bedbugs, *Cimex* spp.: their current world resurgence and healthcare impact', *Asian Pacific Journal of Tropical Disease*, 5(5), pp. 342–352. Doi: 10.1016/S2222-1808(14)60795-7.