

**Description of the cophylogenetic relationship between the *Myrsidea*  
chewing louse and its Neotropical *Mionectes* flycatcher host.**

by

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B.SC., The University of British Columbia, 2013

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE

DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

(Zoology)

UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

December 2016

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## **Abstract**

Cospeciation is when two species share similar or identical phylogenies as a result of their interactions and is often predicted with ectosymbionts, organisms that complete the majority of their lifecycle on a host. When an organism is dependent on another to complete its reproductive cycle, there is potential for cospeciation. Due to limited dispersal capabilities beyond contact between a current and potential host species, many ectosymbionts have phylogenies that mirror their hosts'. In this thesis, I test this prediction using *Myrsidea* feather lice found on Neotropical *Mionectes* flycatchers from Manu National Park, Peru. My results show strong evidence of cospeciation by comparing trees built using sequences from the *Myrsidea*'s mitochondrial (CO1) and nuclear (EF-1 $\alpha$ ) genes to previously described host trees from Miller *et al.* (2008) and Jetz *et al.* (2012). These findings agree with previous morphological descriptions of two *Myrsidea* lice species co-occurring with *Mionectes* hosts and provides evidence that there are currently two previously undescribed species of *Myrsidea*. This adds to our knowledge and understanding of how ectosymbionts and hosts interact through evolutionary time, and provides the basis for future studies on the description of *Myrsidea* which is an under-described group of widespread organisms.

## **Preface**

This thesis is original, unpublished work by the author, A. T. Cook.

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## List of Abbreviations

### Models of Evolution:

- JC: Jukes-Cantor model of evolution. All substitutions are equally likely, equal frequencies of nucleotide base pairs (Jukes & Cantor 1969).
- HKY: Hasegawa-Kishino-Yano model of evolution. Separate transition and transversion rates, variable frequencies of nucleotide base pairs possible (Hasegawa *et al.* 1985).
- GTR: General time reversal model of evolution. Symmetrical substitution matrix, variable frequencies of nucleotide base pairs possible (Rodriguez *et al.* 1990).
- GTR + G: General time reversal model of evolution with a gamma distribution of rates. Variable transition and transversion rates between nucleotide base pairs.
- GTR + G + I: A general time reversal model of evolution with gamma distribution of rates that incorporates unchanging base pairs in sequences.

## **Glossary**

Station: Area including base camp and several netting sites

Site: Area or trail where groups of 5 – 10 nets were set up

## **Acknowledgements**

Thank you to my advisor, Jill Jankowski, for giving me the opportunity to explore a discovered passion for ectoparasites and for providing me with experiences and an environment that have made me a better scientist. Thank you also to my supervisory committee members (current and past), Diane Srivastava, Darren Irwin, and Kathy Martin for your guidance in navigating my thesis as well as always providing (prompt) feedback. I would also not have been able to complete my work without a supportive faculty and staff in the Zoology department and UBC at large, with a special thank you to Doug Altshuler, Alice Liou, Katie Beall, and Gerard Cowan for your invaluable assistance with the logistics of graduate school.

This thesis would not have been possible if not for the guidance with all things genetic from Julie Allen. Thank you as well to Therese Catanach for answering my late night Skypes and troubleshooting with me and Kevin Johnson for providing the lab space and materials for my sequencing. For their intellectual and emotional support, I thank my lab mates Jenny Munoz, Santiago David, and especially Micah Scholer. For making the endless hours in the field and in the lab looking at vials memorably enjoyable, thank you Keenan Yakola and Dani Valgardson. For helping me stay motivated and making my social life not completely absent, thank you to Kathryn Anderson, Natalie Caulk, and Meagan Grabowski. My ability to complete this thesis is in no small part because of all of you.

To my family and friends that are family, thank you for putting up with me and my choice to pursue a degree that demands so much time. Especially thank you to Antonia Musso, who walked with me through it all.

And also Morris.

For every science educator that has helped me learn about this wonderful world around us.

## **Chapter 1: Introduction to ectosymbionts and their impact on hosts.**

### **1.1 Ectosymbionts**

Understanding the evolution and diversification of species has been a central focus of modern biology since the time of Darwin. Describing these evolutionary histories has become much easier with the advent of the powerful tool of genetics, inferring lineages through molecular phylogenies in combination with life histories. These tools have recently led to the identification of several new cryptic species or the collapse of species previously considered distinct (Malenke *et al.* 2009; Toews & Irwin 2008; Bush *et al.* 2016). Although these tools are powerful, there are still several instances where they are yet to be fully utilized. Particularly, using genetics in conjunction with life histories can be used to resolve relationships between species which have intimate and prolonged interactions (such as symbiotic relationships) and to differentiate congeneric species which typically appear morphologically similar.

Symbiosis is the ecological phenomenon where two organisms rely heavily on each other for survival, including providing food resources, modes of reproduction, or a physical environment to live on or in. A symbiont is an organism that has a persistent and intimate interaction with a host organism of another species and can either occur on the external surface (ectosymbiont) or within a host (endosymbiont). These relationships are typically studied in the context of human impact, focusing on those that benefit humans or are considered pests. Well studied examples of symbiotic relationships are arthropod pollinators and flowering plants (Weiblen & Bush 2002), endosymbiotic mitochondria and chloroplasts in eukaryotes (McFadden 2001), or nitrogen fixing fungi and plant root associations (Doyle 1998).

In all cases of symbiosis, there are costs and benefits associated with the interaction to both

participants. If the both the symbiont and host derive a benefit from the interaction, it is considered a mutual symbiosis. If the outcome for the host is neutral and there is a benefit to the symbiont, it is a commensal interaction. Finally, if there is a cost to the host and a benefit to the symbiont, it is a parasitic relationship. Parasites typically derive their benefit at a cost to their hosts. This cost can be through reducing available energy by directly consuming nutrients via blood and body fluids or indirectly by consuming structures that can be regrown, increasing the cost of maintenance to the host (Clayton *et al.* 2003). Parasites can also have physical impacts on their hosts, such as disrupting airflow over wing surfaces increasing the energetic costs of flight (Clayton *et al.* 2003; Moller & Rozsa 2005; Holmstad *et al.* 2008). Ectoparasites, such as lice, complete the majority of their lifecycles on the surface of their hosts, consuming host fluids and tissues for nutrients. Due to this obligatory association, ectoparasites are largely reliant on host movements and host conspecific interactions for dispersal (Marshall 1981). In the context of their evolutionary history, this reliance of parasites on their host for dispersal one would predict that ectoparasite phylogenies will mirror their hosts. That is, that ectoparasites will cospeciate with their hosts.

Although this is an appealing framework when approaching host-parasite associations, there are several examples where ectoparasite species occur on multiple host species or where cospeciation fails to occur (Banks *et al.* 2006). This can happen through a varying number of mechanisms (Johnson *et al.* 2003; Weckstein 2004; Banks *et al.* 2006). For example, an extinction of an ectoparasite on host A's lineage may be replaced secondarily by a second ectoparasite occurring on host B, resulting in a single parasite occurring on multiple host species (Johnson *et al.* 2003). Alternatively, ectoparasites can fail to speciate with their hosts due to higher rates of gene flow between parasites than between hosts (Johnson *et al.* 2003). This can be

accomplished via ectoparasites dispersing through phoresis (temporarily attaching to an intermediate organism for dispersal) or by recurring close contact either spatially or temporally by the hosts. Because of these potentially complex relationships between ectoparasites and hosts, there is a need to examine and study these relationships when attempting to describe ectoparasite species.

## 1.2 Study System

*Mionectes* flycatchers are Tyrannidae flycatchers found throughout South America (Schulenberg *et al.* 2007; Miller *et al.* 2008). Although their name may imply that they sustain themselves only on catching insects, *Mionectes* flycatchers supplement their diet with fruits (Aguilar *et al.* 2000; Westcott & Smith 1994; Greeney *et al.* 2006; Westcott 1991; Price *et al.* 2005). In Peru, there are four species of *Mionectes*, which occur along an elevational gradient (Schulenberg *et al.* 2007). These species can overlap geographically as well as at a local scale, and therefore have the potential to have recurring or historic contact among congeners. This contact between species could be in the form of male leks during mating displays or in mixed species flocks where multiple *Mionectes* species may forage simultaneously (Willis *et al.* 1978; Snow & Snow 1979; Westcott & Smith 1994; Pizo & Aleixo 1998). This ongoing close contact can lead to a higher potential for transfer of ectoparasites between hosts as ectoparasites can be dispersal limited and therefore higher rates of host contact increase transfer potential (Weckstein 2004; McCoy *et al.* 2005). *Mionectes* are parasitized by *Myrsidea* lice, which feed on their hosts' feathers (Price *et al.* 2005). *Myrsidea* are chewing lice in the Amblyceran suborder of lice (Marshall 1981; Price *et al.* 2003). Like other Amblyceran lice, *Myrsidea* lice feed by consuming feather materials and incidentally on blood and fluids from growing feather tips (Marshall 1981; Price *et al.* 2003; Moller & Rozsa 2005). Feather maintenance may be important for thermoregulatory function as

well as potential influences on female choice driven mate selection and birds will invest significant time in several behaviors to reduce parasite load (Clayton et al. 2010). Due to this intimate relationship between *Myrsidea* lice and their *Mionectes* hosts, they make an ideal system to examine cospeciation in an ectosymbiont-host association.

### **1.3 Research Objectives**

The objective of my thesis is to describe the evolutionary relationship between *Myrsidea* chewing lice and their *Mionectes* hosts by comparing phylogenies between host and symbiont. To do this I used samples collected from Parque Nacional del Manu (Manu National Park), Peru from 2011 to 2013. Four species of *Mionectes* flycatchers occur in Peru: *Mionectes macconnelli*, *Mionectes oleagineus*, *Mionectes olivaceus*, and *Mionectes striaticollis*. There are four potential louse species, each occurring on a single host. Previously, only two have been described, *M. oleaginei* and *M. olivacei* occurring on *M. oleagineus* and *M. olivaceus* respectively. I will incorporate previous host phylogenies from Miller *et al.* (2008) and Jetz *et al.* (2012) to compare with the ones that I construct using both mitochondrial and nuclear sequences extracted from *Myrsidea* lice.

## Chapter 2: Exploring the cophylogenetic relationship between hosts and their ectoparasites using *Mionectes* flycatchers and *Myrsidea* feather lice.

### 2.1 Introduction

#### 2.1.1 The Feather Louse *Myrsidea*

A symbiont is an organism that has a persistent and intimate interaction with a host organism of another species and can either occur on the external surface (ectosymbiont) or within a host (endosymbiont). These can be broken down into three relationship types, those that benefit only the symbiont with no effect on the host (commensalistic); benefit both symbiont and host (mutualistic); or benefit the symbiont at a cost to the host (parasitic). Lice are a group of parasitic insects that feed on the blood and external keratinized structures (skin, hair, feathers, etc.) of their hosts (Marshall 1981; Johnson & Clayton 2003). Historically, parasitic lice were classified into either Mallophaga (chewing lice) or Anoplura (sucking lice); however, these groups were paraphyletic (Johnson & Clayton 2003). More recently, they have been reclassified as sucking lice (Anoplura) and chewing lice (Amblycera, Ischnocera, and Rhynchophthirina). Of interest for this study is the genus *Myrsidea*, a chewing louse in the suborder Amblycera. It is a common parasite on several avian species (Price et al. 2003; Valim et al. 2011). *Myrsidea* are hemimetabolous and complete their entire lifecycle on their hosts (Marshall 1981). Unlike Ischnocera, Amblycera lice like *Myrsidea* retain many ancestral characteristics (Johnson & Clayton 2003). They generally lack structures and behaviours to specialize on a specific body area of their host (e.g. wing, body, head) and are more free living being able to abandon a dead or dying host (Johnson & Clayton 2003; Johnson et al. 2009).

Although *Myrsidea* have generalized feeding behaviours on a host, this does not necessarily translate to generalizing on multiple host species. For example, a study of *Myrsidea* (Amblycera) and *Brueelia* (Ischnocera) lice on *Catharus* thrushes found higher rates of host specificity and a

strong signal of biogeography in *Myrsidea* when comparing phylogenies built from mitochondrial and nuclear DNA markers (Bueter et al. 2009). This increased host specificity can be widely seen in *Myrsidea*, and specifically within *Myrsidea* parasitizing Tyrannid avian hosts, where there is an average of 1.07 *Myrsidea* species per host species (Valim & Weckstein 2013). This high host fidelity is likely due to reduced means of dispersing between host species (Marshall 1981). One potential method of dispersal common in lice taxa is phoresy, where the louse attaches to a hippoboscid fly (Diptera: Hippoboscidae) and travels between individuals (Marshall 1981). However, this trait is uncommon in Amblycera and has not been observed in *Myrsidea*, where transmission only occurs: vertically through intimate contact from parent to offspring; horizontally between mating pairs; or through proximity of a suitable conspecific to a dying host (Marshall 1981; Johnson & Clayton 2003). These restrictions to gene flow have likely lead to a large number of speciation events and likely contribute to the large number of theorized undescribed Amblyceran lice species (Valim & Weckstein 2013). Given that Amblyceran lice complete their entire lifecycle on a host and have reduced means of transmission, they have likely coevolved with their hosts and are thus excellent candidates for exploring cophylogenetic relationships between avian hosts and ectoparasites.

In summary, lice in the genus *Myrsidea* are widespread taxa found on passerine birds and exhibit high host specificity (Bueter *et al.* 2009). *Myrsidea*, like most Amblyceran lice, are body generalists and consume feather material, skin, and body fluids such as blood from emerging feathers (Marshall 1981; Johnson & Clayton 2003). Feather louse feeding can reduce host fitness, lowers feather weight and bird mass, and leads to lower survival (Clayton et al. 1999; Clayton et al. 2003; Holmstad et al. 2008). Due to their limited dispersal capabilities, high rates of host specificity, and the likely hundreds of undescribed species, *Myrsidea* are an appealing

system to study cophylogenetic relationships.

### **2.1.2 The Neotropical Tyrant Flycatcher: *Mionectes***

*Mionectes* flycatchers are a genus of small passerines from the family Tyrannidae occurring in Central and South America, found in habitats ranging from humid primary and secondary lowland evergreen and flooded forest to lower montane forest (Janzen 1983; Stotz *et al.* 1996). In Peru, they are common in lowland Amazonia and along the slopes of the Andes (Schulenberg *et al.* 2007). There are five species of *Mionectes*. Occurring at low elevation in the lowlands and foothills of the Andes (below 1200 m) are *M. macconnelli* (McConnell's), *M. oleaginous* (Ochre-bellied), and *M. rufiventris* (Grey-hooded). At higher elevations, found in cloud forest and elfin forest (above 1200 m), are *M. olivaceus* (Olive Striped) and *M. striaticollis* (Streak-necked) (Miller *et al.* 2008). *Mionectes* are drab birds with limited sexual dimorphism (Skutch 1960; Schulenberg *et al.* 2007). Birds are olive-green in colour on their wings and back, with ochre to yellow breast feathers and underparts. *Mionectes rufiventris* and *M. striaticollis* have pronounced grey hoods (Schulenberg *et al.* 2007). The genus is widespread throughout Central and northern South America (Miller *et al.* 2008), and all but *M. rufiventris* occur on the east slope of the Andes in Peru (Schulenberg *et al.* 2007).

Species that co-occur in Peru specialize in habitat usage: *M. oleaginous* lives in second growth forest, clearings, and along forest edge, whereas *M. macconnelli* uses older growth forests and plantations (Willis *et al.* 1978). *Mionectes olivaceus* has a wider range, occurring from low-elevation *terra firme* to cloud forest. There it is replaced by *M. striaticollis*, which occurs from mid to higher elevations, from cloud forest to shrub or elfin forest (Schulenberg *et al.* 2007; Merkord 2010). Some *Mionectes* species are also documented elevational migrants moving upward for the wet season including *M. striaticollis*, *M. olivaceus*, and *M. oleaginous*, possibly

in response to seasonal changes in local resource availability (Blake & Loiselle 2000; Merkord 2010). This has the potential to increase contact of individuals both within a host species as well as between host species.

Tyrant flycatchers (Tyrannidae) are generally insectivorous, as their name implies. However, *Mionectes* are unique within the group in that a major portion of their diet comes from berries and fruit (Skutch 1960; Willis *et al.* 1978; Stouffer & Borges 2001; Greeney *et al.* 2006). For example, in *M. striaticollis* fruit consumption accounts for roughly 25% of their diet (Greeney *et al.* 2006). While foraging in the understory, *Mionectes* species frequently participate in mixed species flocks (Willis *et al.* 1978; Greeney *et al.* 2006; Blake & Loiselle 2000; Merkord 2010). Although they have been observed in mixed species flocks, *Mionectes* individuals are also regularly found foraging alone, implying that their association with mixed species flocks may be facultative or possibly incidental.

During the breeding season, *Mionectes* are territorial and partially lekking. Males in all species have been observed occupying and displaying in territories either solitarily or in groups of two to six individuals (Aguilar *et al.* 2000; Willis *et al.* 1978; Westcott & Smith 1994; Snow & Snow 1979; Pizo & Aleixo 1998; Greeney *et al.* 2006). Male *Mionectes* display by perching on low branches, singing, and raising alternate wings. Breeding occurs at the beginning of the rainy season, likely corresponding with increases in food resources (Greeney *et al.* 2006). Female *Mionectes* build pyriform (pear-shaped) nests suspended from epiphyte roots or flexible branches above flowing water (Skutch 1960; Snow & Snow 1979; Willis *et al.* 1978; Greeney *et al.* 2006). Only females provision the nestlings and typically lay between 2-3 eggs with 1-2 offspring fledging. This is not surprising as high nest mortality rates are typical for tropical birds (Skutch

1985).

The phylogenetic relationships among *Mionectes* species are not clearly understood. There is agreement that ‘high elevation’ species, *M. striaticollis* and *M. olivaceus*, form a clade, and ‘low elevation’ *M. rufiventris*, *M. oleaginous*, and *M. macconnelli* form a second clade (Jetz *et al.* 2012; Miller *et al.* 2008). However, Miller *et al.* (2008) found molecular evidence suggesting that *M. macconnelli* may be paraphyletic, being composed of two distinct species: *Mionectes macconnelli* from south west Amazonia being basal to all other ‘low elevation’ species; and *M. macconnelli* from the Guiana Shield forming a sister group with *M. rufiventris* and *M. oleagineus*. Miller *et al.* (2008) constructed these phylogenies using the cytochrome *b* mitochondrial sequence (mtDNA) and partial sequences from RAG-1 and *c-myc* nuclear sequences (nDNA) collected from birds across South America. This complexity certainly raises some questions about the relationships, however it is useful for a comparative studies of *Mionectes* in Peru to use a more simplified phylogeny as *M. rufiventris* and the second *M. macconnelli* species do not occur in Peru (Figure 2.1). In Peru, ‘high elevation’ *M. striaticollis* and *M. olivaceus* are a sister group to the ‘low elevation’ *M. oleagineus* and *M. macconnelli* (Figure 2.2).

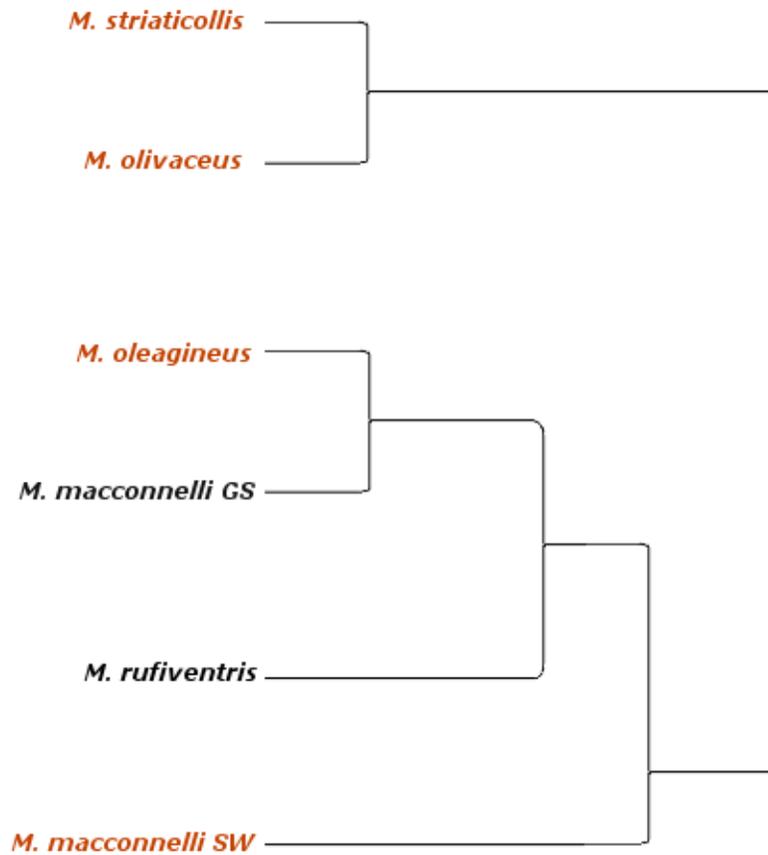


Figure 2.1: *Mionectes* phylogeny adapted from Miller *et al.* (2008) and Jetz *et al.* (2012). For *M. macconnelli*, GS represents *Mionectes* sampled from the Guiana Shield in the eastern Amazon by Miller *et al.* (2008). SW represents *M. macconnelli* sampled from SW Amazon. Species highlighted in red represent *Mionectes* species which occur in Manu National Park, Peru.

### 2.1.3 Research Goals

The presence of *Myrsidea* louse species on Neotropical birds is well documented, however their diversity is poorly described (Valim & Weckstein 2013). Currently, there are two documented louse species that occur on Neotropical tyrant flycatchers: *Myrsidea oleaginei* on *Mionectes*

*oleagineus* and *Myrsidea olivacei* on *Mionectes olivaceus*. This leaves two hosts (*M. striaticollis* and *M. macconnelli*) and the potential co-phylogenetic relationships between *Myrsidea* and *Mionectes* un-described. One of the first steps required to catalogue the diversity within *Myrsidea* is to compare host and louse phylogenies to determine if co-speciation has occurred between *Myrsidea* and their *Mionectes* hosts. Previous research would suggest that unique host-parasite pairs exist due to *Myrsidea*'s poor dispersal capabilities (Valim & Weckstein 2013). However, due to the presence of multiple sister taxa of *Mionectes* within the same local region it is still possible for a single *Myrsidea* species to occur on multiple hosts. This could occur either through *Myrsidea*'s failure to speciate with their hosts or through host switching events where a parasite is introduced to a new host species secondarily. Secondary host switching also has the potential to cause *Myrsidea* and *Mionectes* phylogenies to disagree. My research goal is to begin the steps of description of *Myrsidea* in the widespread and abundant *Mionectes* genus by building phylogenies from genetic markers in *Myrsidea* from sampled netting efforts in Manu National Park, Peru and comparing them to host phylogenies adapted from Miller *et al.* (2008) and Jetz *et al.* (2012) (Figure 2.2).

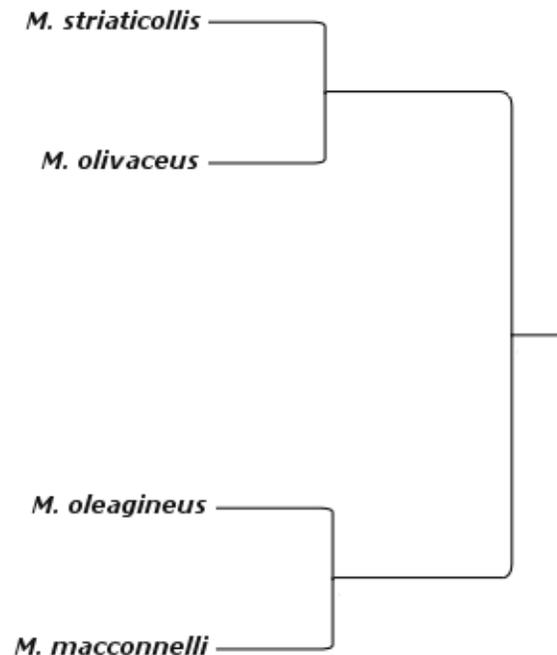


Figure 2.2 Phylogeny of *Mionectes* flycatchers occurring in Manu National Park, Peru. Adapted from phylogeny constructed by Miller *et al.* (2008) and Jetz *et al.* (2012) using mitochondrial and nuclear sequences.

## 2.2 Methods

### 2.2.1 Study Area

All field sites were located either in Parque Nacional del Manu (Manu National Park) or in a buffer zone surrounding the park, which encompasses the World Heritage site on the Eastern slope of the Andes in Peru ( $11^{\circ}51'23''$  S,  $71^{\circ}43'17''$  W) (World Heritage Committee 2009). Field teams were based out of stations Pantiacolla, San Pedro, and Wayqecha, with limited sampling at Villa Carmen and two trails (Trochas) originating from the San Pedro station and traversing ridges to higher elevation. The low elevation field station at Pantiacolla was located in the forest surrounding Pantiacolla Lodge (a tourist lodge) at roughly 400 m. a.s.l., with habitats consisting

of *terra firme* and floodplain forest, with large patches of *Guadua* spp. bamboo. San Pedro station was based out of the Cock of the Rock Lodge at 'mid' elevation (~1400 m) and consisted of lower montane and cloud forest with patches of both *Chusquea* spp. and *Guadua* spp. bamboo. Wayqecha Biological Station, maintained by the Asociación para la Conservación de la Cuenca Amazónica (ACCA), is located at 'high' elevation (~2800 m) and harbours a mixture of cloud forest and drier, low canopy shrub forest, with *Schefflera* spp. and *Oreocallis* spp. as some of the dominant species. One additional field station was located at Villa Carmen Biological Station (also operated by ACCA), in foothill forest, generally dominated by *Guadua* spp. bamboo patches at 500-700m, but extending to lower montane forest along a ridge up to 1200 m. Two long transects at higher elevation include Trocha Lucho, located in cloud forest along a ridge running from ~1400 to 2000 m, and Trocha Union, a long ridge transect from ~1600 to 3600 m, which traverses habitats from the lower limits of cloud forest at 1700 m to higher cloud forest dominated by *Weinmannia* spp. (Cunoniaceae) and *Clusia* spp. (Clusiaceae) and several genera from Melastomataceae and Rubiaceae just below treeline at ~3600 m (Lutz et al. 2013). Villa Carmen and the two Trochas were not sampled regularly.

### **2.2.2 Sample Collection**

Collection occurred inclusively between 2011 and 2013 and sites were visited from late July to mid December each year. This is generally in the transition between the dry and rainy season, which begins in late December. In 2011 only San Pedro was visited, and Wayqecha was only visited in 2013 (Table 2.1). Field teams would visit sites simultaneously along the gradient, reducing temporal variation in sampling. Field teams consisted of three members, and contained a minimum of one experienced bander. Field teams consisted of roughly 40 international volunteers participating in the Manu Park Bird Project over the three year sampling period.

Volunteers gained experience in Neotropical bird identification and bird banding protocols in exchange for sample and data collection. Between years, consistency was attempted by distributing returning experienced field team members evenly between sampling locations. The majority of my work with the samples was cataloguing and organizing the previously collected samples (approximately 14000 blood, ectoparasite, and feather samples). All information was recorded in the field and then transcribed to digital records during and after the field season. All records are stored at the Jankowski lab at the University of British Columbia (Vancouver, BC, Canada).

Table 2.1: Years that major sites were visited and ecto-symbiont samples were collected.

Station	Years Visited	Ecto-Symbionts Collected
Pantiacolla	2012, 2013	2012, 2013
San Pedro	2011, 2012, 2013	2011, 2012, 2013
Wayqecha	2011, 2012, 2013	2013

Avian hosts were captured using passive mist netting. Teams set up approximately 10 sites per station, which were selected to maximize coverage of habitat types. Each site contained between 5 and 10 nets placed roughly 5 meters apart (where terrain allowed this kind of placement) and oriented perpendicular to the slope or parallel to natural and man-made breaks in the forest to maximize capture rates. Nets were 38 mm mesh, 12 x 2.6 m (Avinet, Freeville, NY, USA), and were opened as early as possible, just after dawn (average 06:30) and closed at sundown for the

safety of both teams and birds. Nets were not opened or temporarily closed if weather conditions were poor (moderate rain or strong winds). Captured birds were identified to species using Birds of Peru (Schulenberg *et al.* 2007) and measured for wing chord, tail and tarsus length, exposed culmen, bill width, and mass for use in morphometric studies. Age was determined by one or a combination of: broader worn feather tips on the 8<sup>th</sup>, 9<sup>th</sup>, and 10<sup>th</sup> primaries; skull ossification; and presence of a previous year's band (Wolfe *et al.* 2009)□. Sex was determined by the visible presence of an egg in the oviduct, brood patch, and/or the presence of an emarginated 8<sup>th</sup> (*M. macconnelli* and *M. oleagineus*) or 9<sup>th</sup> (*M. olivaceus* and *M. striaticollis*) primary in males (Zimmer 1941; Wolfe *et al.* 2009). Birds were also checked for the presence and development of brood patch and cloacal protuberances as possible indicators for sex or timing of mating. Images of identifying characteristics were taken in cases of ambiguous identification species or sex for future consultation. Each capture event received a unique event number to keep track of all metrics and samples collected.

From birds deemed in good condition, with no obvious signs of distress (e.g. panting or a prolonged net extraction), ecto-community samples were collected by massaging approximately 1 mL of a 1.00% pyretherin insecticide powder (EverGreen® Pyrethrum Dust, McLaughlin Gormley King Company®, Minneapolis, MN, USA) into the body feathers of the bird over a piece of paper. The resulting powder and ecto-symbionts were collected on the paper and funneled in to a 2 mL screw cap tube (Fisherbrand™ Sterile Microcentrifuge Tubes with Screw Caps, Waltham, MA, USA), topped up with a 96% ethanol solution, and stored in the field until transport to the lab for processing. Ethanol was purchased in the field from a variety of reputable pharmacies. During storage, prior to identification, samples were checked yearly to refill evaporated ethanol as needed.

### **2.2.3 Sorting and Initial Identification**

To determine louse presence, quantity, and identification to genus, ecto-symbiont samples were examined under magnification in the lab using Stemi 508 Stereomicroscopes (Carl Zeiss Canada Ltd., Toronto ON, Canada). *Myrsidea* were identified using descriptions found in Price *et al.* (2005) and Clay (1966) as well as through visual confirmation from Dr. Kevin Johnson at the University of Illinois (*pers. comm.*). To ensure all ecto-symbionts were removed from the vial, samples were emptied into plastic petri dishes and sample vials were rinsed a minimum of three times with a 95% ethanol solution or until there was no visible residue remaining. Petri dishes were checked systematically, scanning the perimeter and then in sequential horizontal bands. Dishes were reused but flushed thoroughly between uses first with water, then with 95% ethanol, then allowed to air dry with residual ethanol being removed by Kimwipe (Kimberly-Clark Professional Canada, Mississauga, ON, Canada). Before dishes were flushed, all samples were checked over by a second observer to ensure no loss of ecto-symbionts during sorting. The number and genera of lice and any other ecto-parasites per sample were recorded. Lice were separated from the other ecto-parasites into separate 2 mL vials to reduce the risk of contamination during extraction and for ease of manipulation in future steps.

### **2.2.4 DNA Extraction and Sequencing**

In preparation for DNA extraction, lice were sliced mid abdomen and just posterior to the head using a modified 30 gauge Precisionglide© syringe (Sigma-Aldrich, St. Louis, MO, USA) bent at a 30 degree angle to turn the beveled end into a cutting edge. This allowed for extraction of DNA without destruction of the sample.

For extraction, I used the DNeasy Blood and Tissue Kit (Qiagen N.V., Hilden, Germany). Dried

specimens were placed in 180  $\mu\text{L}$  of ATL buffer with 25  $\mu\text{L}$  of proteinase K. The solution was allowed to incubate for ~32-48 hours at 55  $^{\circ}\text{C}$  and agitated with a vortex mixer every 2 hours (excluding overnight). After incubation, 200  $\mu\text{L}$  of the AL buffer was mixed into each tube and then the solution was incubated again for 10 minutes at 70  $^{\circ}\text{C}$ . Next, 200  $\mu\text{L}$  of 95% ethanol was mixed in to each tube. Liquid contents were moved to a spin column. Once extraction was complete, specimen vouchers were stored in 70% ethanol in a -80  $^{\circ}\text{C}$  freezer in Dr. Kevin Johnson's lab at the Illinois Natural History Survey, University of Illinois at Urbana-Champaign.

The extracted DNA was separated from solution via centrifuge (1 minute at 8000 rpm). The DNA was passed through two rinse cycles to remove any remaining reagents: 500  $\mu\text{L}$  AW1 followed by 8000 rpm for 1 minute and 500  $\mu\text{L}$  of AW2 followed by 14000 rpm for 3 minutes. All flow-through was discarded. Two final washes of the filter with 50  $\mu\text{L}$  of AE buffer extracted the remaining DNA. After each wash, the filter and buffer were incubated for 5 minutes at 70  $^{\circ}\text{C}$  in a heating block and centrifuged in to the final collection vial using 8000 rpm for 1 minute. The final extractions were kept at -20  $^{\circ}\text{C}$  unless immediately processed.

Two genes were amplified, on mitochondrial gene (mtDNA) cytochrome oxidase 1 (CO1) and one nuclear gene (nDNA) elongation factor-1 $\alpha$  (EF-1 $\alpha$ ), these genes are commonly sequenced barcode genes that are used to identify species level differences between lice (Johnson *et al.* 2002; Bush *et al.* 2016; Johnson *et al.* 2007). The CO1 gene is a mitochondrial sequence which produces a subunit of the enzyme Cytochrome oxidase 1 (Garcia-Horsmant *et al.* 1994) while EF-1 $\alpha$  is a nuclear autosomal sequence (nDNA) responsible for ribosome function (Andersen & Nyborg 2001). I ran all samples through a polymerase chain reaction (PCR) using the New England Biolabs 5x master mix and protocols (New England Biolabs, Ipswich, MA, USA). For

each sample, 5  $\mu\text{L}$  of master mix was combined with 0.5  $\mu\text{L}$  of primer, 16  $\mu\text{L}$  of  $\text{dH}_2\text{O}$ , and 3  $\mu\text{L}$  of the cleaned DNA. Primers for CO1 were H7005F and L6625R, and primers for EF-1 $\alpha$  were FOB3F and CHO10R (Johnson *et al.* 2004). The PCR reaction was run in a thermocycler at 94  $^\circ\text{C}$  for 2.5 minutes, annealing for 0.5 minutes, then at 72  $^\circ\text{C}$  for a 0.5 minute cycle. This was repeated for 30 cycles before finishing with 72  $^\circ\text{C}$  for 7 minutes and holding at 4  $^\circ\text{C}$ .

The resulting DNA was sequenced using the Sanger approach. Sequencing was done through the University of Illinois Urbana-Champaign Biotechnology Centre's Core Facility.

### **2.2.5 Tree Building**

Contiguous sequences were assembled using *de novo* sequence assembling implemented in Geneious (9.0.2, Biomatters Ltd.) and then aligned utilizing the MUSCLE plugin (Edgar 2004)□. Sequence editing was done in Geneious and potential base pair misreads were identified by disagreement between forward and backward reads of a single sample. They were further checked after alignments and indels identified and the chromatograms again checked for errors. To test for contamination, all sequences were blasted against the NCBI nucleotide data base to ensure that the top hit was a *Myrsidea*. To remove primer ends, sequences were aligned to sequences with primers already removed by the Johnson lab (Catanach, *pers. comm.*). Once sequences were satisfactorily aligned and edited, reading frame was determined using the translate feature in Geneious, utilizing the Invertebrate Mitochondrial translation table for CO1 and the standard translation table for EF-1 $\alpha$ . Reading frame was classified as the frame that has no stop codons within the sequence. The number of potentially informative sites was calculated using the 'pis' function in the 'ips' package in R (R Core Team 2016).

To generate the best partition schemes and models of evolution to inform tree building software,

I used PartitionFinder (1.1.1; Lanfear *et al.* 2012) on the extracted sequences. This program selects the best partitioning schemes and model of evolution using the Akaike information criterion (AIC). The inputted sequences were separated by gene and codon position. The program searched for all schemes and was run with linked branch lengths and models were filtered by those used in RAxML (Stamatakis 2014) and MrBayes (Ronquist *et al.* 2011)□. All tree building software used the partitions generated in this step.

The maximum likelihood (ML) and Bayesian (BP) trees were built using RAxML and MrBayes plugins in Geneious and rooted using CO1 and EF-1 $\alpha$  sequences from *Myrsidea* from a *Rhynchocyclus olivaceus* host (GenBank Accession numbers: FJ171317 and FJ171288). The *Myrsidea* from *R. olivaceus* was chosen because the host is also a member of Tyrannidae and because both sequences were available from a single louse. To determine support for each node in the ML tree, 1000 bootstrap replicates were calculated. The Bayesian Inference tree was run for  $1.1 \times 10^5$  generations and 10% was removed as burn-in. Two chains were run as the default in MrBayes and they were checked for stationarity by examining likelihood scores for stability.

To test if trees built with concatenated sequences in place of multiple sequences would have produced different topologies, individual gene trees were also built using RAxML and MrBayes. Trees were visually compared for changes in clade topology. To concatenate sequences, an individual's CO1 and EF-1 $\alpha$  were concatenated when both were available in SeaView (4.6) for input to partition finder and tree-building software (Gouy *et al.* 2010).

## **2.3 Results**

### **2.3.1 Samples collected**

A total of 304 ectoparasite samples were collected in the field, and after sorting, 119 of these

samples had at least one louse present (Table 2.2). DNA was successfully extracted from 61 of the 119 samples, all confirmed to be *Myrsidea* before extraction. From this pool of successful extractions, only 41 CO1 and 20 EF-1 $\alpha$  extractions produced useable reads to construct contiguous sequences. These rates of extraction were expected as lice have very little soft tissue in relation to recommended tissue for DNA extraction (Allen, *pers. comm.*), decreasing the volume of useable DNA and increasing the risk of extraction failure. There were 13 individual lice, which had both CO1 and EF-1 $\alpha$  sequences, and the remaining 48 individuals had only one of either (Table 2.3). Once sequences were aligned and primer ends removed, CO1 had 409 base pairs (bp) and EF-1 $\alpha$  had 344 bp.

### **2.3.2 Partitions and Models of Evolution**

Maximum likelihood and Bayesian individual gene trees produced similar trees to those produced through concatenated sequences (Figure 2.3, Figure 2.4, Figure A.1, Figure A.2, Figure A.3, Figure A.4). Therefore, CO1 and EF-1 $\alpha$  sequences were concatenated for all future steps. Partition finder suggested two partitions for RAxML tree building. The model of evolution which best described positions 1-2 and 1-3 of CO1 and EF-1 $\alpha$  respectively was a GTR+I+G model of evolution. Only CO1 position 3 used a GTR+G model of evolution. There were three partitions suitable for MrBayes. Position 1 for both CO1 and EF-1 $\alpha$  followed a GTR model of evolution, positions 2 for CO1 and 2-3 for EF-1 $\alpha$  are best described with the JC model of evolution. Finally, position 3 of CO1 used a HKY model of evolution. There were 138 parsimony informative sites.

Table 2.2: Summary count of Myrsidea lice collected, extracted, and sequenced by location and species.

Collection Location	Host	Count of <i>Myrsidea</i>			Count of Successful Sequences	
		Samples Collected	Individuals Present	Successfully Extracted	C01	EF-1 $\alpha$
Pantiacolla	<i>M. macconnelli</i>	19	8	5	3	5
	<i>M. oleagineus</i>	38	11	11	4	7
	<i>M. olivaceus</i>	38	17	10	8	3
San Pedro	<i>M. olivaceus</i>	43	26	10	8	1
	<i>M. striaticollis</i>	100	40	14	13	3
Wayqecha	<i>M. striaticollis</i>	64	15	8	5	1
Villa Carmen	<i>M. olivaceus</i>	2	2	2	0	0
Total		304	119	60	41	20

Table 2.3: List of samples and associated sequenced DNA

Event Number	Host Species	Collection Location	Year	C01	EF-1 $\alpha$
Mysp.Mimac.P121314	<i>M. macconnelli</i>	Pantiacolla	2012	X	X
Mysp.Mimac.P121315	<i>M. macconnelli</i>	Pantiacolla	2012		X
Mysp.Mimac.P121316	<i>M. macconnelli</i>	Pantiacolla	2012		X
Mysp.Mimac.P13279	<i>M. macconnelli</i>	Pantiacolla	2013	X	X

Event Number	Host Species	Collection Location	Year	C01	EF-1 $\alpha$
Mysp.Mimac.P13396	<i>M. macconnelli</i>	Pantiacolla	2013	X	X
Mysp.Miole.P121493	<i>M. oleagineus</i>	Pantiacolla	2012	X	X
Mysp.Miole.P12958	<i>M. oleagineus</i>	Pantiacolla	2012	X	
Mysp.Miole.p13245	<i>M. oleagineus</i>	Pantiacolla	2013	X	X
Mysp.Miole.p13267	<i>M. oleagineus</i>	Pantiacolla	2013	X	X
Mysp.Miole.P13329	<i>M. oleagineus</i>	Pantiacolla	2013		X
Mysp.Miole.P13331	<i>M. oleagineus</i>	Pantiacolla	2013		X
Mysp.Miole.P13348	<i>M. oleagineus</i>	Pantiacolla	2013		X
Mysp.Miole.p13382	<i>M. oleagineus</i>	Pantiacolla	2013		X
Mysp.Mioli.P121244	<i>M. olivaceus</i>	Pantiacolla	2012	X	
Mysp.Mioli.P121254	<i>M. olivaceus</i>	Pantiacolla	2012	X	
Mysp.Mioli.P121256	<i>M. olivaceus</i>	Pantiacolla	2012	X	X
Mysp.Mioli.P12848	<i>M. olivaceus</i>	Pantiacolla	2012	X	X
Mysp.Mioli.P12942	<i>M. olivaceus</i>	Pantiacolla	2012	X	X

Event Number	Host Species	Collection Location	Year	C01	EF-1 $\alpha$
Mysp.Mioli.P13179	<i>M. olivaceus</i>	Pantiacolla	2012	X	
Mysp.Mioli.P13204	<i>M. olivaceus</i>	Pantiacolla	2013	X	
Mysp.Mioli.P13291	<i>M. olivaceus</i>	Pantiacolla	2013	X	
Mysp.Mioli.SP110053	<i>M. olivaceus</i>	San Pedro	2011	X	
Mysp.Mioli.SP110142	<i>M. olivaceus</i>	San Pedro	2011	X	X
Mysp.Mioli.SP110229	<i>M. olivaceus</i>	San Pedro	2011	X	
Mysp.Mioli.SP121168	<i>M. olivaceus</i>	San Pedro	2012	X	
Mysp.Mioli.SP121181	<i>M. olivaceus</i>	San Pedro	2012	X	
Mysp.Mioli.SP121203	<i>M. olivaceus</i>	San Pedro	2012	X	
Mysp.Mioli.SP121241	<i>M. olivaceus</i>	San Pedro	2012	X	
Mysp.Mioli.SP121279	<i>M. olivaceus</i>	San Pedro	2012	X	
Mysp.Mistr.SP110062	<i>M. striaticollis</i>	San Pedro	2011	X	X
Mysp.Mistr.SP110106	<i>M. striaticollis</i>	San Pedro	2011	X	
Mysp.Mistr.SP120034	<i>M. striaticollis</i>	San Pedro	2012	X	X

Event Number	Host Species	Collection Location	Year	C01	EF-1 $\alpha$
Mysp.Mistr.SP121154	<i>M. striaticollis</i>	San Pedro	2012	X	X
Mysp.Mistr.SP121167	<i>M. striaticollis</i>	San Pedro	2012	X	
Mysp.Mistr.SP121182	<i>M. striaticollis</i>	San Pedro	2012	X	
Mysp.Mistr.SP121186	<i>M. striaticollis</i>	San Pedro	2012	X	
Mysp.Mistr.SP121187	<i>M. striaticollis</i>	San Pedro	2012	X	
Mysp.Mistr.SP121373	<i>M. striaticollis</i>	San Pedro	2012	X	
Mysp.Mistr.SP121428	<i>M. striaticollis</i>	San Pedro	2012	X	
Mysp.Mistr.TL120085	<i>M. striaticollis</i>	San Pedro	2012	X	
Mysp.Mistr.TL120099	<i>M. striaticollis</i>	San Pedro	2012	X	
Mysp.Mistr.TU120030	<i>M. striaticollis</i>	San Pedro	2012	X	
Mysp.Mistr.W130196	<i>M. striaticollis</i>	Wayqecha	2013	X	X
Mysp.Mistr.W130329	<i>M. striaticollis</i>	Wayqecha	2013	X	
Mysp.Mistr.W130364	<i>M. striaticollis</i>	Wayqecha	2013	X	
Mysp.Mistr.W130554	<i>M. striaticollis</i>	Wayqecha	2013	X	

Event Number	Host Species	Collection Location	Year	C01	EF-1 $\alpha$
Mysp.Mistr.W130773	<i>M. striaticollis</i>	Wayqecha	2013	X	

### 2.3.3 Maximum Likelihood Tree

Using a multi-loci approach, *Myrsidea* broadly fell in to four major clades, representative of their host's phylogenies (Figure 2.3). Lice occurring on higher elevation host species (*M. striaticollis* and *M. olivaceus*) form a clade while lice on 'low' elevation species (*M. oleagineus* and *M. macconnelli*) form a sister group (ML = 99%). Within the low and high elevation clades, *Myrsidea* is further split between their respective host species. At high elevation there is strong support for dividing the lice between hosts (ML=95%), whereas at low elevation there is lower but still substantial support for *Myrsidea* splitting along host species lineages (ML=82%). Within the cluster of *M. macconnelli*, there is a single *Myrsidea* sample that is labelled as having been found on a *M. oleagineus* host (Figure 2.3). In spite of this single case of disagreement, there is strong support for distinct louse clades split by host species.

There was no effect of elevation or year on relationships between *Myrsidea*. With the exception of the single *M. oleagineus* louse, lice were split exclusively by host species.

### 2.3.4 Bayesian Tree

The Bayesian tree had similar findings to the ML tree (Figure 2.4). Division of lice grouped according to host species with the exception of a single *M. oleagineus* within the *M. macconnelli* clade. There were four distinct clades of *Myrsidea*, all grouped by host species. The Bayesian posterior probabilities (BP) for the *M. striaticollis*, *olivaceus*, and *macconnelli* clades were 100% and the *M. oleagineus* clade was supported with a 97% posterior probability. The Bayesian

phylogeny also broadly split the groups into high and low elevation groups, with *Mionectes striaticollis* and *olivaceus* forming one group (BP = 100%) and *M. oleagineus* and *macconnelli* forming a sister group (BP = 100%). Similar to the maximum likelihood trees, elevation or year did not appear to influence topology and a single *M. oleagineus* was clustered with the *M. macconnelli* clade.

### **2.3.5 Consensus Tree**

The consensus tree was constructed using trees assembled from both the ML and BP approaches (Figure 2.5). This tree includes the previously described *Myrsidea* species *M. olivacei* and *M. oleaginei* occurring on *M. olivaceus* and *M. oleagineus* hosts respectively. Tree was rooted using *Myrsidea* sequences from a *R. olivaceus* host.

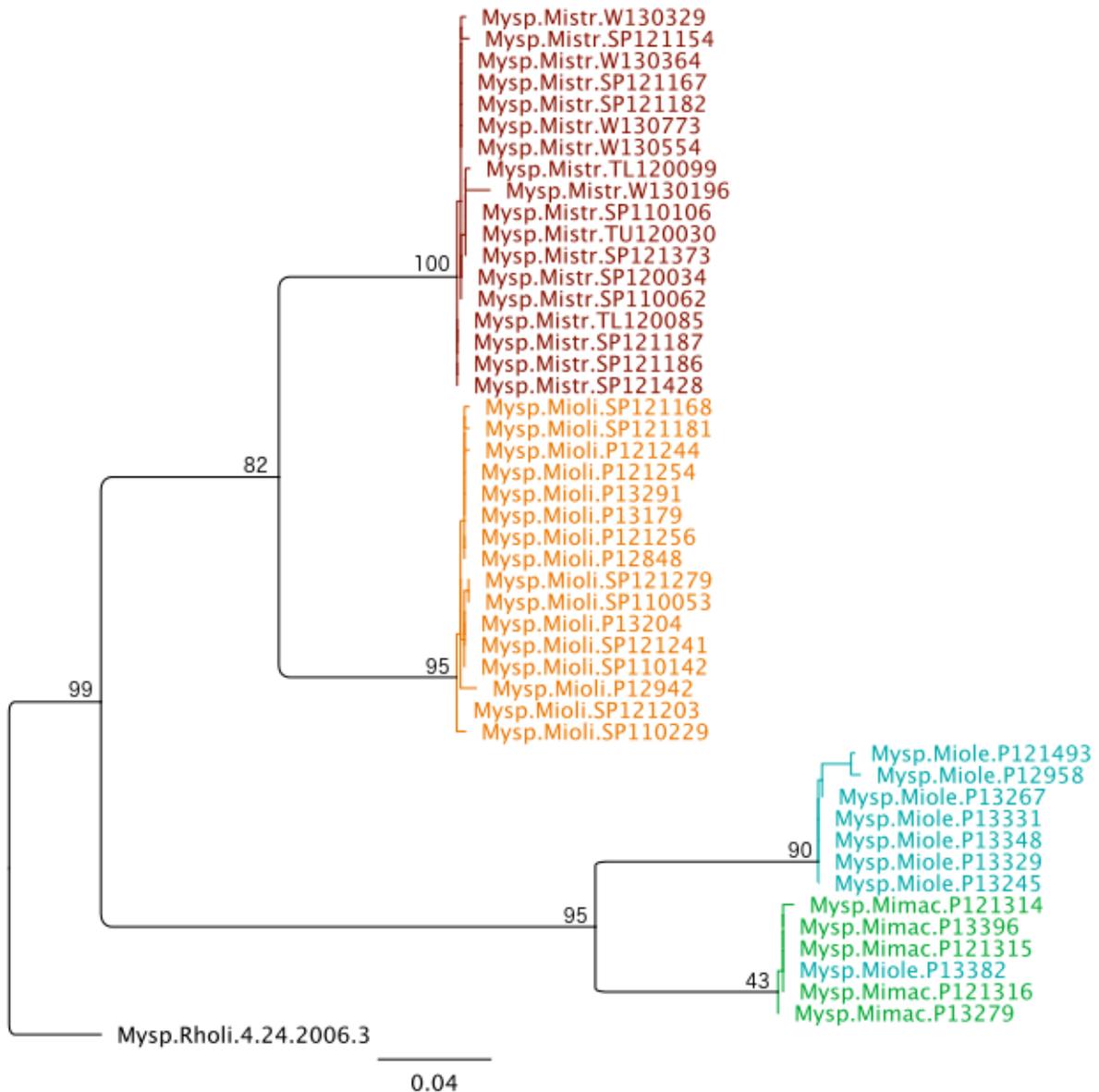


Figure 2.3: Phylogeny of *Myrsidea* lice occurring on *Mionectes* hosts from Manu Park, Peru using maximum likelihood tree builder RAxML. Node support values are above the branches and were calculated using 1000 bootstraps. Phylogenies were constructed using CO1 and EF-1 $\alpha$  sequences. Colour indicates host species: red, *M. striaticollis*; orange, *M. olivaceus*; blue, *M. oleaginosa*; green, *M. macconnelli*. Outgroup is *Myrsidea* occurring on *Rhynchocycclus olivaceus*. Only support values of major clades are displayed for clarity. Scale bar is the number of substitutions per site.

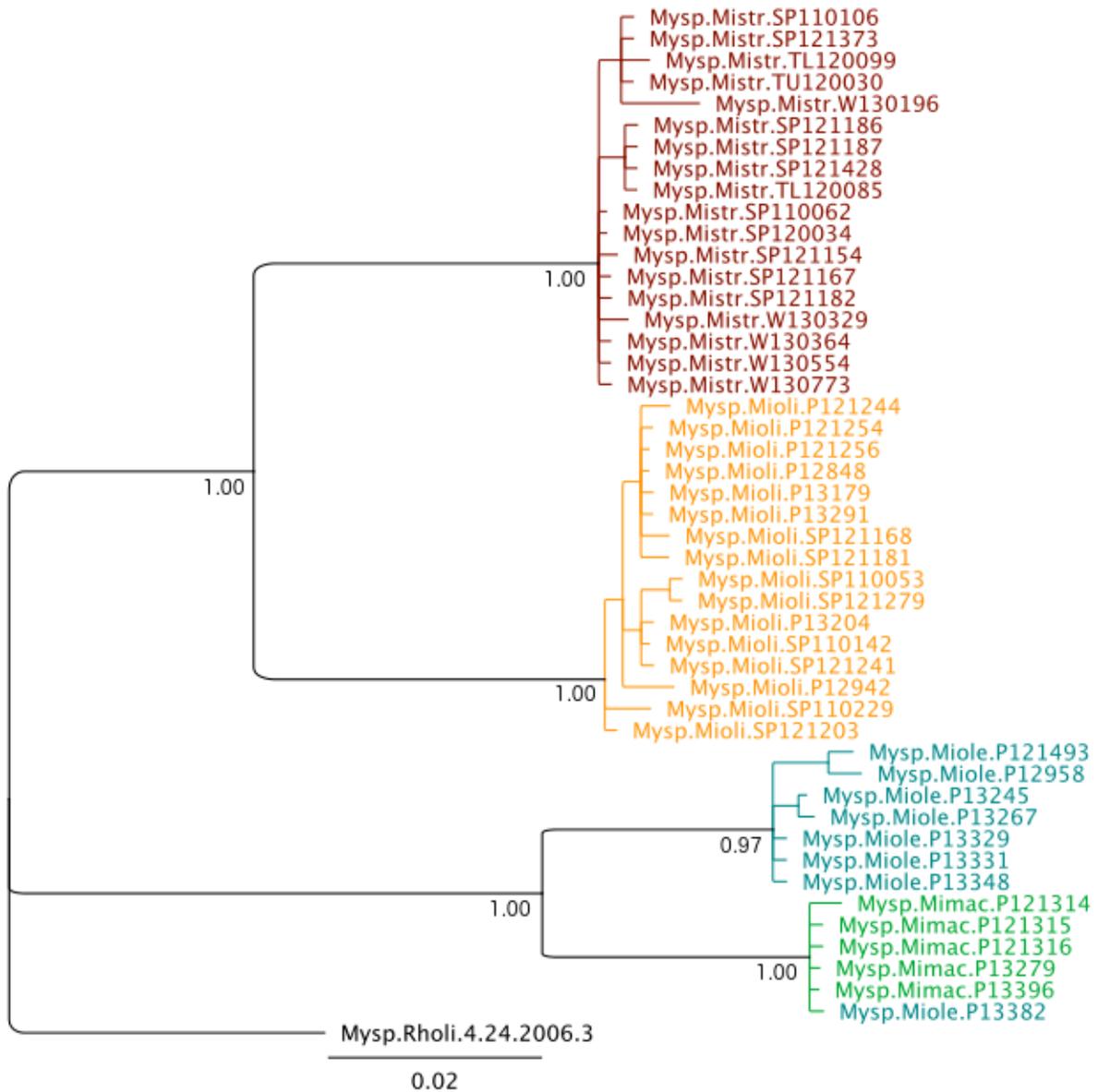


Figure 2.4: Phylogeny of *Myrsidea* lice occurring on *Mionectes* hosts from Manu Park, Peru using Bayesian tree builder MrBayes. Node support values are below the branches and were calculated using 1,000,000 chain length with a 100,000 burn-in. Phylogenies were constructed using CO1 and EF-1 $\alpha$  sequences. Colour indicates host species: red, *M. striaticollis*; orange, *M. olivaceus*; blue, *M. oleaginous*; green, *M. macconnelli*. Outgroup is *Myrsidea* occurring on *Rhynchocyclus olivaceus*. Only support values of major clades are displayed for clarity. Scale bar is the number of substitutions per site.

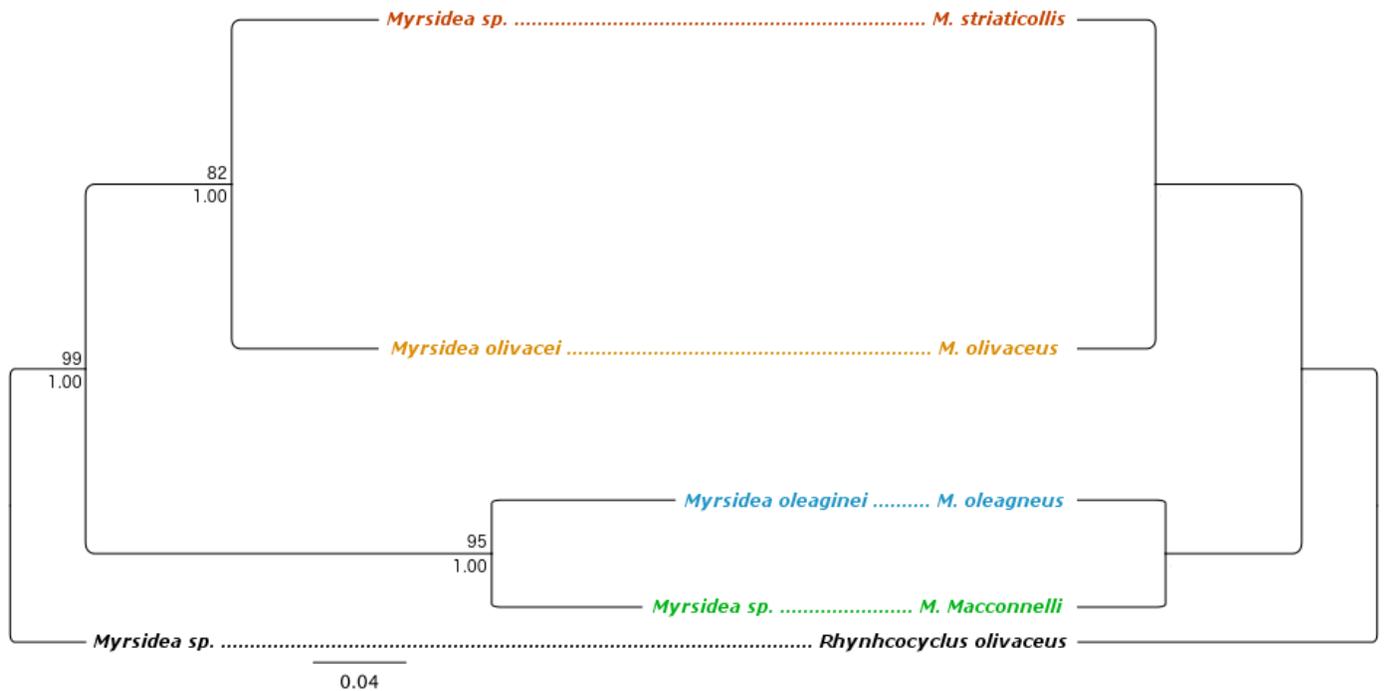


Figure 2.5: Consensus *Myrsidea* phylogeny (left) constructed using maximum likelihood and Bayesian trees of CO1 and EF-1 $\alpha$  sequences in comparison to *Mionectes* host phylogeny (right) adapted from Miller *et al.* (2008) and Jetz *et al.* (2012). Maximum likelihood node supports are above the branches, and were constructed using 1,000 bootstraps. Bayesian node support values are below the branches and were constructed using 1,000,000 chain lengths with a 1,000 burn-in. Colour indicates host species *Myrsidea* were collected from: red, *M. striaticollis*; orange, *M. olivaceus*; blue, *M. oleaginous*; green, *M. macconnelli*. Phylogeny is rooted using *Myrsidea* from *R. olivaceus*. Dotted lines are highlighting similar topologies between parasite and host phylogenies. Scale bar is the number of substitutions per site and applies only to left hand *Myrsidea* tree, right hand *Mionectes* tree is scaled for clarity.

## 2.4 Discussion

Overall, there is strong support that *Myrsidea* lineages have a cophylogenetic relationship with their *Mionectes* hosts (Figure 2.5). Both ML and Bayesian trees constructed from concatenated

sequences provide strong support for the division of *Myrsidea* into four distinct clades, which reflect speciation in parallel with their hosts. Branch supports for the ML tree have the majority of bootstrap values above 90% with a single branch (*M. striaticollis* and *M. oleagineus*) having 82% (Figure 2.3). Bayesian branches are all supported with > 95% posterior probabilities (Figure 2.4). Concatenation was rationalized due to Maximum likelihood and Bayesian trees for individual gene sequences produced similar trees and therefore not having conflicting evolutionary trajectories (Figure A.1, Figure A.2, Figure A.3, Figure A.4); however, the EF-1 $\alpha$  trees did not detect a difference between *Myrsidea* sampled from *M. striaticollis* and *M. olivaceus* (Figure A.2, Figure A.4). Sequences were still concatenated as this difference was not likely to be a true indication of gene flow or differences in gene trajectories between CO1 and EF-1 $\alpha$ . The gene EF-1 $\alpha$  has shown lower rates of sequence divergence than mtDNA sequences such as CO1 in previous studies of *Myrsidea* lice across multiple host species (Bueter et al. 2009; Johnson et al. 2007). High rates of sequence conservation in EF-1 $\alpha$  is supported within this data as there are 129 parsimony informative sites (an estimate of the number of bp mutations differing between samples) in CO1 and 14 in EF-1 $\alpha$ . Further, the differences between *M. macconnelli* and *M. oleagineus* consists of mutations at only three positions. Therefore, concatenation was a reliable means of building phylogenies for *Myrsidea*.

Evidence of cospeciation between *Myrsidea* and their hosts has been demonstrated across multiple host taxa (Bueter et al. 2009; Valim & Weckstein 2013), however it should be noted that cospeciate is not common in the majority of studies between avian hosts and other symbionts (Paterson & Gray 1997; Paterson *et al.* 1999). There are several mechanisms through which ectosymbionts can fail to speciate with their hosts (Johnson *et al.* 2003; Banks *et al.* 2006). Host switching (introduction of a louse to a new host) can occur when two host species have intimate

contact either through predator-prey interactions (Whiteman *et al.* 2004) or inter-specific mating between closely related species. Exchange between host species can also occur when ectosymbionts are able to move phoretically by attaching themselves to more mobile hippoboscids (Johnson *et al.* 2011; Sychra *et al.* 2014; Bueter *et al.* 2009). These events can also occur when several hosts reuse the same nesting cavities, such as tree hollows (Weckstein 2004). These exchanges can either result in gene flow between symbionts on different host species or symbiont phylogenies that do not mirror their hosts. However, in *Myrsidea* we do not see evidence of recent gene flow or transfer between hosts in their molecular data.

If host switching were prevalent in *Myrsidea* the most likely candidate for host-host transfer would be through hybridization events. However the limited molecular work done on *Mionectes* and field observations do not suggest that hybridization occurs at any detectable rate (Miller *et al.* 2008; Jetz *et al.* 2012). There are also likely strong selective barriers to inter-specific mating between hosts. All *Mionectes* have been described as at least partially lekking, meaning that several males will display in a territory simultaneously. Females choose males from this available pool, presumably by selecting males with desirable traits (Snow & Snow 1979; Westcott & Smith 1994; Pizo & Aleixo 1998; Willis *et al.* 1978). Mechanisms of female choice often select for strong species-specific traits, driving divergent characteristics between males or choosiness in females to increase species recognition (Eberhard 2004; Verzijden *et al.* 2005). Even in situations where hosts have significant geographic overlap, such as *M. macconnelli* and *M. oleaginous*, these species tend to inhabit different areas or habitats within lowland forests: *M. macconnelli* occupying *terra firme* and forest interior; and *M. oleagineus* more common in floodplain forests, second growth, and forest edges (Willis *et al.* 1978). This reduces the chances of congeneric host interactions, maintaining distinct lineages of *Myrsidea*. Finally, the

ectosymbiont host switching can also happen between hosts that share nests (Weckstein 2004). Lice are left in nesting material when the host leaves the nest and can persist on remaining skin and feather debris. When a potential host species reuses the nesting cavity, the louse may colonize and persist on a new host. This can result in taxonomies that are more reflective of biogeography than phylogeny (Johnson et al. 2011; Weckstein 2004). Although CO1 and EF-1 $\alpha$  sequencing is not intended to detect fine scale geographic resolution, there is no evidence of geographic structuring in the generated trees. The lack of structuring in conjunction with the nesting behavior of the host species makes it unlikely that *Myrsidea* are being transferred via nesting materials. *Mionectes* build pendular nests over water and are not known to reuse nests from congeners either within or between seasons (Willis *et al.* 1978; Aguilar *et al.* 2000; Westcott & Smith 1994; Greeney *et al.* 2006). Nesting in all *Mionectes* species also corresponds with the peak of the rainy season, meaning that females are foraging for nesting material simultaneously. This would reduce the chances of female *Mionectes* recycling nesting material from abandoned nests, which could also increase the dispersal of *Myrsidea*.

It is unlikely that host switching occurs often enough to create species level trends; the majority of sequences are grouped by host species. However, a single *M. oleagineus* sequence appears in the *M. macconnelli* clade (Figure 2.3 and Figure 2.4). Although this may be evidence that host switching occurred, the high support for the *M. oleagineus* and *M. macconnelli* branches and the lack of observational evidence supporting inter-species leks would mean this is a very recent transfer, that the *Myrsidea* was transferred to this individual host. Although the potential of a ‘snapshot’ capturing a host switching event cannot be eliminated as an explanation, this claim cannot be made with confidence. Low elevation species are similar in plumage colouration and morphology, making them difficult to distinguish to untrained observers. At the beginning of the

field season, there is a greater potential for field teams to misidentify the host. Typically, confirmation of host identification is accomplished by secondarily checking species against detailed photos taken in the field. Unfortunately, there is no image of the specific individual host for ID verification. Overall, host identification is robust. This is because as field collection continued and trained field teams returned from previous years, knowledge was retained between years and incoming groups were more prepared to identify hosts. It is likely that the only case of misidentification was a *M. macconnelli* as all cases determined to be misidentified were between *M. oleagineus* and *M. macconnelli* and they are the most morphologically similar (Schulenberg *et al.* 2007).

Although these results indicate strong support for distinct clades, there is little structure of *Myrsidea* occurring within a species. One possibility is that *M. striaticollis* have greater rates of contact between geographically distant hosts because they are elevational migrants, moving upslope from the dry to wet season (Greeney *et al.* 2006; Merkord 2010). This increased mobility along the gradient may mean that they have a higher rate of mating between individuals which occur more distantly, resulting in a panmixis within a larger geographical range. Low elevation host species, being territorial and moving little between locations, would have higher rates of divergence over shorter distances. An alternative possibility is that higher elevation species face stronger selection for metabolic-associated traits. The lower oxygen environment at higher elevations may be acting as a strong selective pressure for metabolic processes, including those at a cellular level. Selection of mitochondrial DNA has been implied when examining mitochondrial sequences in migratory Yellow-rumped Warblers (Toews *et al.* 2013). This may mean mitochondrial genes such as CO1 may actually be selected for in the high elevation *M. striaticollis* because of their potential effect on metabolic efficiency. This could explain the lack

of structure within the montane *M. striaticollis* and *M. olivaceus* clade. At this time, it is unclear if this has an impact on *Myrsidea*. Closer investigation may be necessary to understand if low oxygen environments at high elevation are driving selection for specific mitochondrial DNA.

Deep divergence between co-occurring species implies that louse speciation occurred relatively early in the evolutionary history of the host (Miller *et al.* 2008). This is likely due to reduced inter-specific host interactions which are supported by the elevational and habitat partitioning between *Mionectes* species. Host clades were likely established in their current pattern for the last 1.5 million years (Jetz *et al.* 2012; Miller *et al.* 2008). This long standing reduction in gene flow between *Mionectes* species likely corresponds with a lack of physical contact between hosts. As *Myrsidea*'s main means for host transfer is through mating or nesting, it is likely that *Myrsidea* species have been isolated from each other for as long as their hosts. Alternatively, *Myrsidea* could be too well adapted to their host species. Lice which can successfully disperse to a new host species could be selected against and have lower fitness than species already occurring on the new host resulting in a failure to establish. For example, in a cross species study, increased nestling T-cell immune response was correlated with increases in Amblyceran species richness (Moller & Rozsa 2005). The authors argued that immune responses could affect parasite feeding success and fitness. Further, Clayton *et al.* (2003) showed differences in host size impacted the ability of *Columbicola* feather lice to avoid preening. Lice grown on one size of host had lower fitness when transferred to hosts of a different size in spite of their ability to attach and feed. Broader sampling and establishment of a genetic clock could help to place the timing of ectoparasitic *Myrsidea* relative to their hosts' divergence. As well, direct studies of fitness impacts on both the parasite and host may also help establish mechanisms which maintain strong patterns of co-speciation.

Through this work I have established that *Myrsidea* occurring on *Mionectes* hosts along the eastern slope of the Andes, Peru have distinct lineages that follow a cophylogenetic pattern. Previous distinctions between *Myrsidea* species have relied solely on morphological data for descriptions (Clay 1966; Price *et al.* 2005). This inclusion of molecular data on the relationship between *Myrsidea* and their *Mionectes* hosts strengthens support for the classification of lice occurring on separate hosts as distinct. It also illustrates that there are two species *Myrsidea* occurring on *M. striaticollis* and *M. macconnelli* that appear to be distinct species from *M. oleaginei* and *M. olivacei*. For every sequenced *Myrsidea* specimen, vouchers are available for morphological comparison as the next step is to describing the biodiversity of *Myrsidea* on *Mionectes* flycatchers. Combining both genetic and morphological data when describing these species is also important to prevent over splitting due to possible biases when researchers assume different ectosymbionts occur on different hosts. In previous work by Valim & Weckstein (2013) genetic information was used in combination with morphologies to estimate the potential undescribed number of *Myrsidea* in Brazil which is hypothesized as being an order of magnitude great than those already described. It is therefore imperative that genetic sequencing be used to confirm distinct host-symbiont relationships.

Although this is the first step in describing *Myrsidea* occurring on *Mionectes* hosts, there are still several future directions that these results could inform. Our sampling sites were relatively close geographically. Banding data collected from the project indicates that individual *Mionectes* do not travel between stations, however for robust species level conclusions it would be prudent to expand sampling efforts to birds from across South America. Also, as highlighted in Miller *et al.* (2008), *M. macconnelli* at low elevations across the Amazon appear to be paraphyletic, being composed of two species; one species occurring in Western Amazonia and a second found east

on the Guiana shield. Researchers have proposed that symbionts, such as *Myrsidea*, could be valuable potential tools in discerning recent relationships in hosts when host molecular data are inconclusive (Whiteman & Parker 2005). Due to shorter life cycles, ecto-symbionts often have increased rates of mitochondrial mutation than their hosts. Combined with their limited dispersal capabilities, ecto-symbionts could be indicators of recent population movements and may shed some light on the complex relationship across *Mionectes* species across South America. Finally, pyrietherin is a general purpose insecticide which is also a miticide, effective against the ectosymbiotic feather mites which co-occur with *Myrsidea* on *Mionectes* hosts. These mites were also collected from the field and are available for future genetic analysis. If the strong pattern of cospeciation found in *Myrsidea* is the result of *Mionectes* life history traits such as limited contact of congeners and one time use pyriform nests, then other ecto-symbionts groups such as the mites should also have high host fidelity. Two ecto-symbiont species with similar patterns of co-speciation would strengthen the co-speciation argument.

## **Chapter 3: Conclusion**

### **3.1 Summary and Implications**

Phylogenies built from *Myrsidea* sequence data show strong evidence of cospeciation with *Mionectes* hosts. This supports previous findings based on morphological differences between lice that some *Myrsidea* have speciated in concert with their hosts. This support is from both Bayesian and maximum likelihood approaches for tree building and incorporates mitochondrial and nuclear sequences. In the greater context of ectosymbionts on passerine hosts, this contributes to our growing understanding and description of *Myrsidea*. Previous work has highlighted the lack of *Myrsidea* louse descriptions, particularly in the tropics (Valim & Weckstein 2013).

### **3.2 Future research**

Future research should use these findings as the first step in pursuing more robust species descriptions in chewing lice as well as *Myrsidea*'s potential use as a tool for inferring host phylogenies. Work by Miller *et al.* (2008) has indicated that there may be paraphyletic groups within *Mionectes*, specifically within *M. macconnelli*. Because *Myrsidea* have a faster reproductive rate, they can be helpful indicators of recent host lineages when host DNA is either inconclusive or would benefit from additional data to increase resolution (Whiteman & Parker 2005). Applying this approach may be able to explain or confirm the proposed evolutionary history of *Mionectes* that *M. macconnelli* occurring in the southwest Amazon are basal to the low elevation clade. Further, there is a fifth species of *Mionectes* which does not occur within our study area. *Mionectes rufiventris* share many life history traits with other *Mionectes* species, such as lekking behavior and pyriform nests. *Myrsidea* is a widespread genus and likely occurs on *M. rufiventris*. Broadening the geographical sampling to include more locations as well as *M.*

*rufiventris* would be the next step in fully describing the *Myrsidea* genus occurring on *Mionectes*. As well, this study would determine if a similar pattern of co-speciation occurs across all *Mionectes* and their *Myrsidea* lice. Given the similarities in life histories including nest habitat and construction it is likely that they exhibit similar co-speciation patterns to other *Mionectes* species. As well, *Myrsidea*'s lack of phoretic dispersal would reinforce this pattern.

Whole genome Illumina sequencing was also conducted on several *Myrsidea* samples collected from the same sites. The sequences used to generate trees in this study (CO1 and EF-1 $\alpha$ ) resulted in phylogenies that are too coarse to identify within population level movements along the gradient with reliability. However, the Illumina data could provide other valuable markers that could be looked at for describing louse movement at the population level. Being able to determine host movements from a single sampling regime has potential for reducing the need for long term studies. The most widely used method for documenting host movement is through mark recapture programs, generally referred to as 'banding' or 'ringing' in birds. Using lice to infer host movements through the environment in evolutionary time could replace, in some circumstances, time and logistically expensive projects. Monitoring efforts which utilize this potential tool have already been successful in the field (Whiteman & Parker 2005).

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## Appendix: Phylogenies built using individual gene sequences

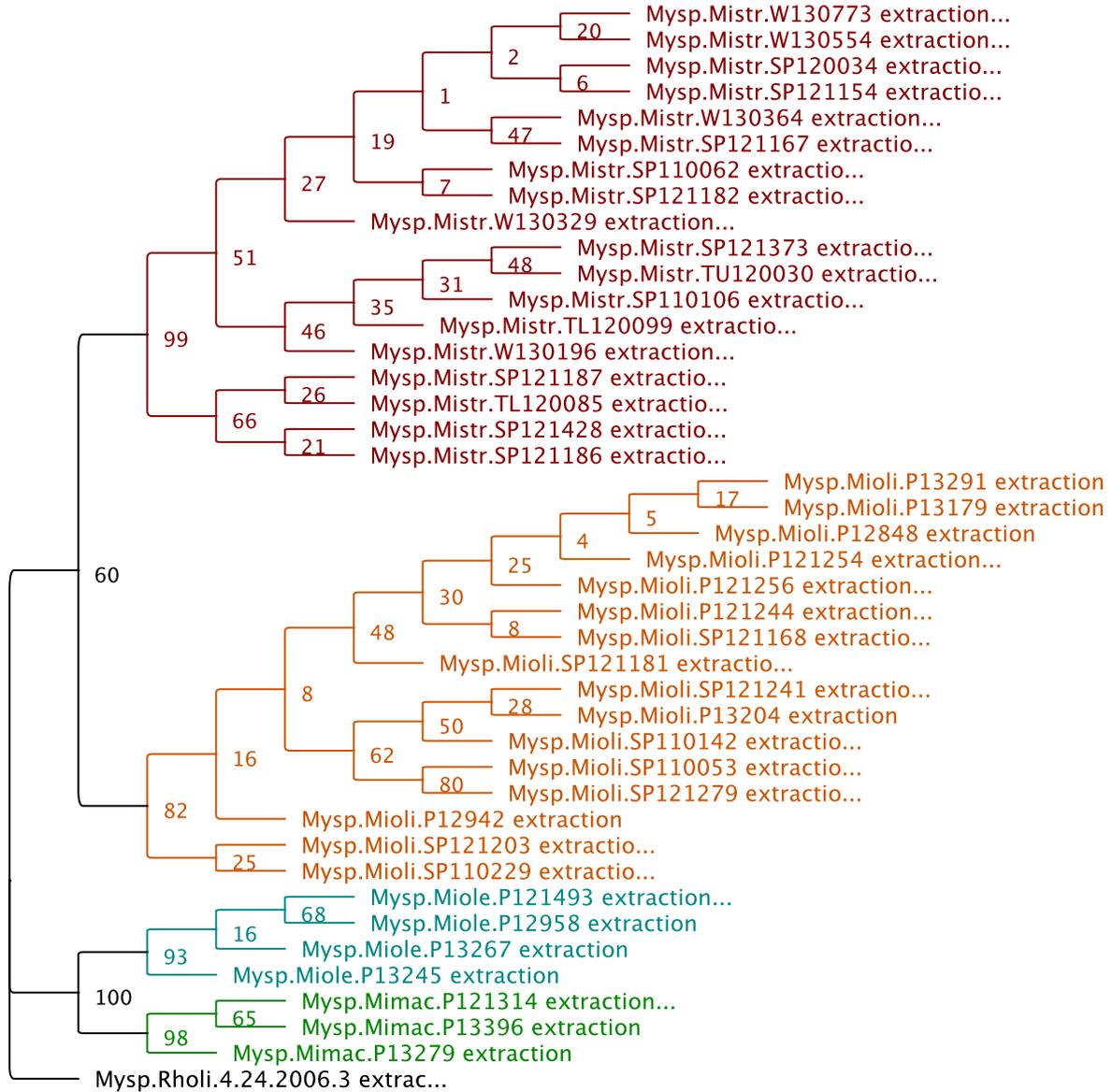


Figure A.1: Phylogeny of *Myrsidea* lice occurring on *Mionectes* hosts from Manu Park, Peru using maximum likelihood tree builder RAxML. Node support values are to the right of the nodes and were calculated using 1000 bootstraps. Phylogenies were constructed using CO1 sequences. Colour indicates host species: red, *M. striaticollis*; orange, *M. olivaceus*; blue, *M. oleaginosa*; green, *M. macconnelli*. Outgroup is *Myrsidea* occurring on *Rhynchocycclus olivaceus*.

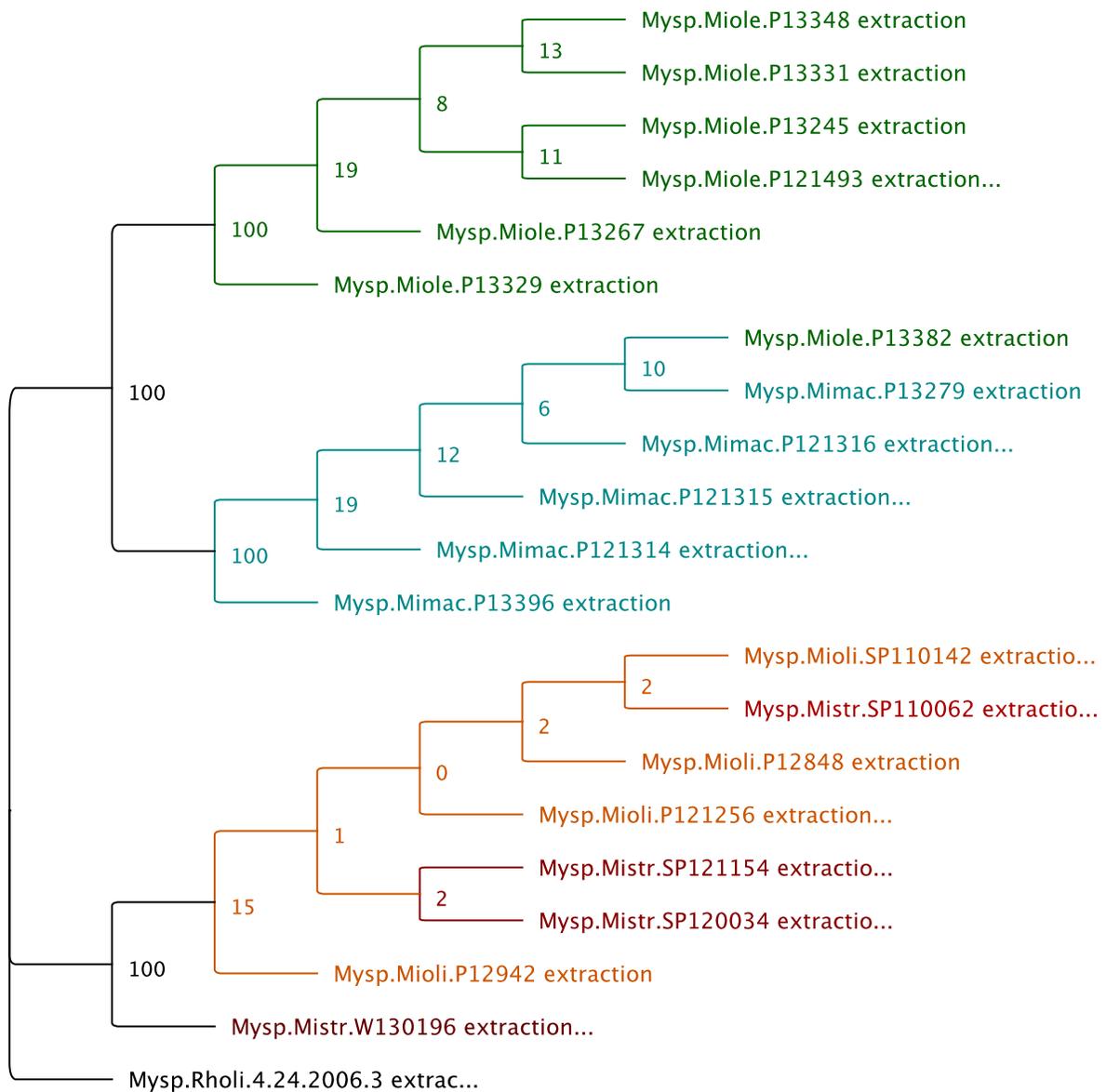


Figure A.2: Phylogeny of *Myrsidea* lice occurring on *Mionectes* hosts from Manu Park, Peru using maximum likelihood tree builder RAXML. Node support values are to the right of the nodes and were calculated using 1000 bootstraps. Phylogenies were constructed using EF-1 $\alpha$  sequences. Colour indicates host species: red, *M. striaticollis*; orange, *M. olivaceus*; blue, *M. oleaginous*; green, *M. macconnelli*. Outgroup is *Myrsidea* occurring on *Rhynchocyclus olivaceus*.

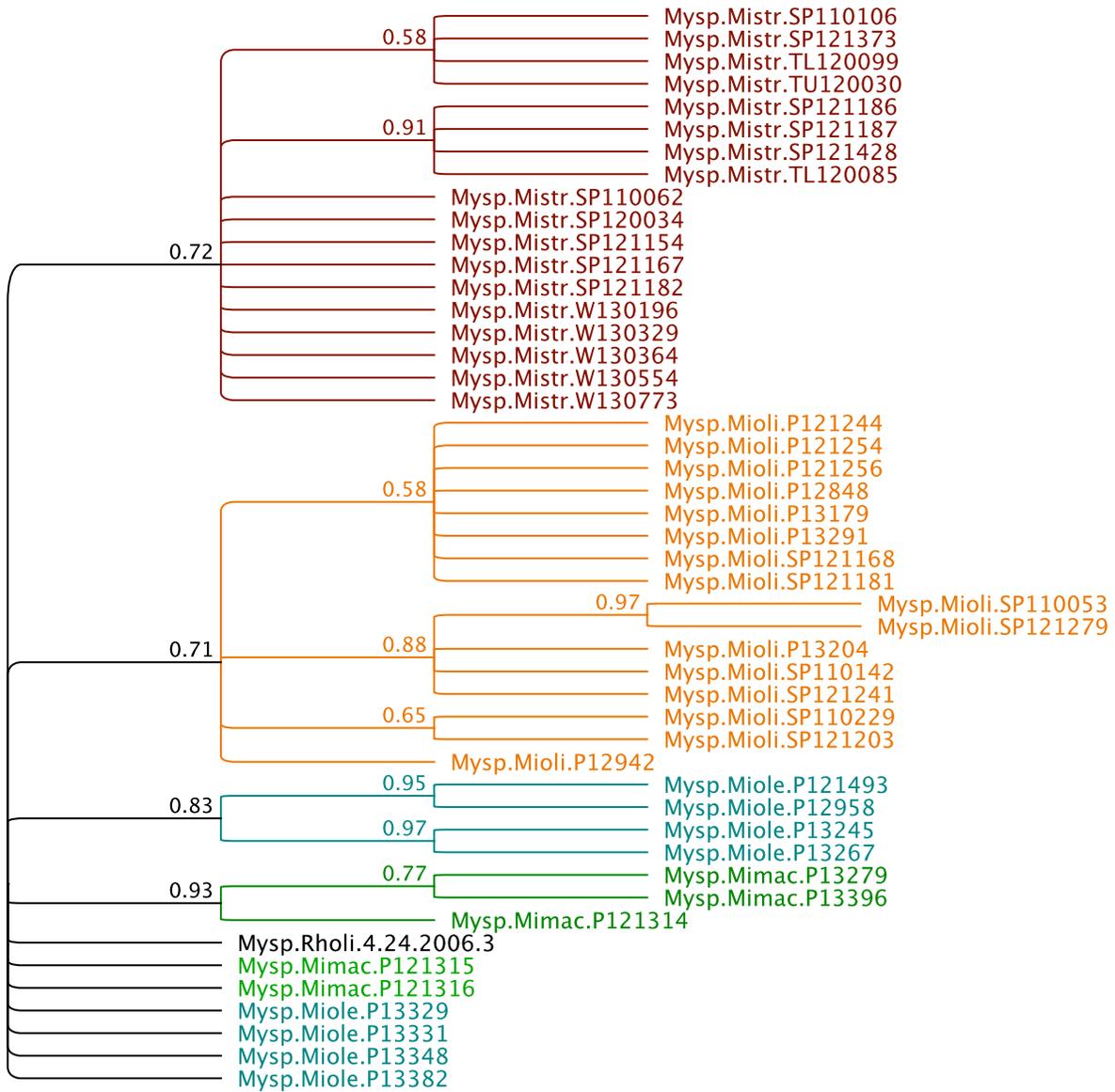


Figure A.3: Phylogeny of *Myrsidea* lice occurring on *Mionectes* hosts from Manu Park, Peru using Bayesian tree builder MrBayes. Node support values are above the branches and were calculated using 1,000,000 chain length with a 100,000 burn-in. Phylogenies were constructed using CO1 sequences. Colour indicates host species: red, *M. striaticollis*; orange, *M. olivaceus*; blue, *M. oleaginous*; green, *M. macconnelli*. Outgroup is *Myrsidea* occurring on *Rhynchocyclus olivaceus*.

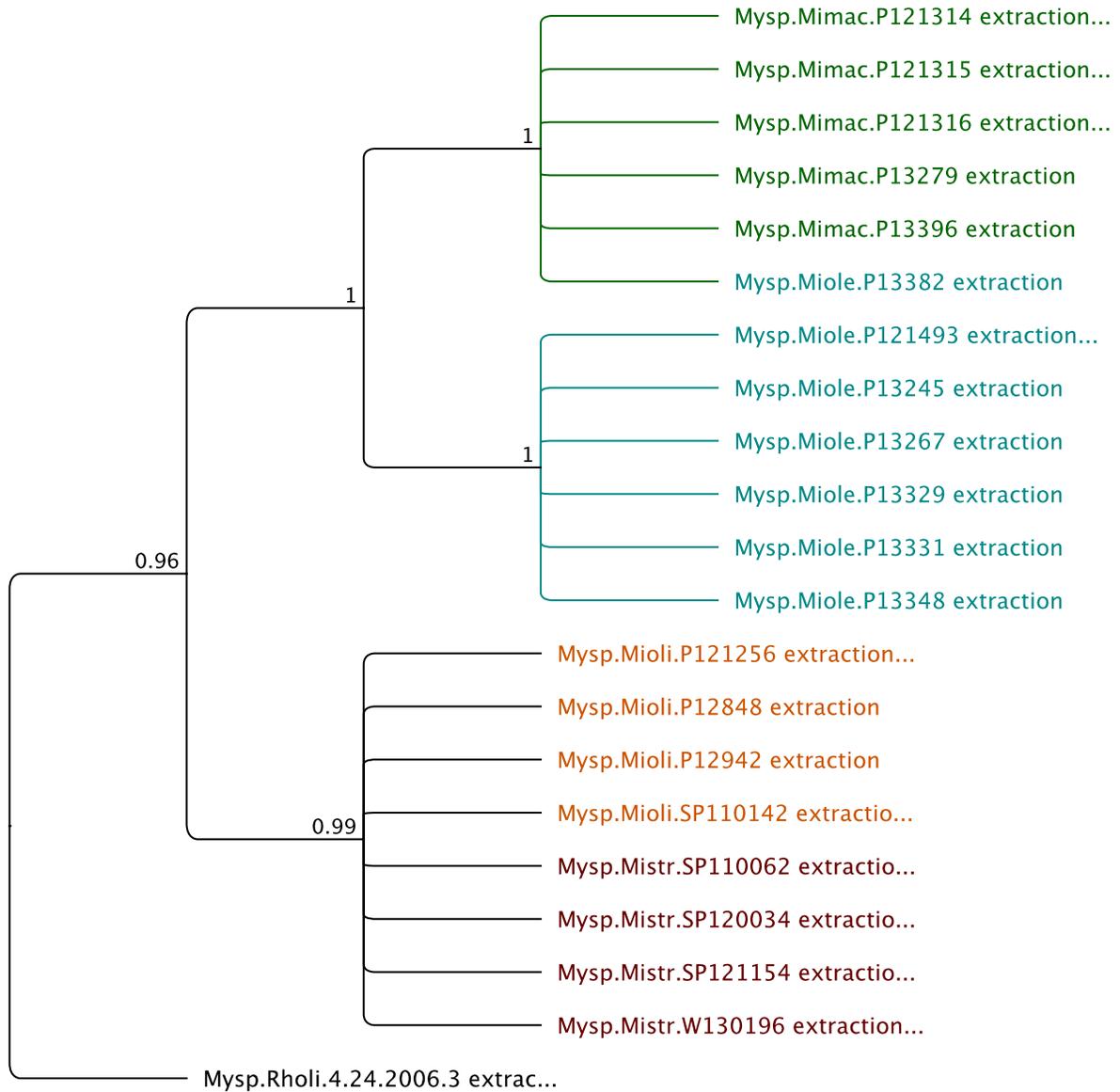


Figure A.4: Phylogeny of *Myrsidea* lice occurring on *Mionectes* hosts from Manu Park, Peru using Bayesian tree builder MrBayes. Node support values are above the branches and were calculated using 1,000,000 chain length with a 100,000 burn-in. Phylogenies were constructed using EF-1 $\alpha$  sequences. Colour indicates host species: red, *M. striaticollis*; orange, *M. olivaceus*; blue, *M. oleaginous*; green, *M. macconnelli*. Outgroup is *Myrsidea* occurring on *Rhynchocyclus olivaceus*.