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DNA barcoding expands dietary identification and reveals dietary similarity in Jamaican frugivorous bats

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A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Biology

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DNA BARCODING EXPANDS DIETARY IDENTIFICATION AND REVEALS
DIETARY SIMILARITY IN JAMAICAN FRUGIVOROUS BATS

(Thesis format: Monograph)

by

Colin E. Hayward

Graduate Program in Biology

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

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Abstract

Detailed identification of diet is imperative for investigations of community structure, pollination and seed dispersal. Using DNA barcoding, I studied the diets of Jamaican fruitbats and how they compared. I identified dietary constituents of three morphologically distinct bat species, *Artibeus jamaicensis*, *Ariteus flavescens* and *Glossophaga soricina* from 135 fecal samples collected in Cockpit Country, Jamaica. DNA barcoding identified 11 fruit taxa in the fruitbats' diets, seven more taxa than detected by traditional methods. Dietary overlap among fruitbat species was significantly high ($O = 0.66$, $p < 0.05$) despite distinct morphologies but *A. jamaicensis* and *G. soricina* consumed some fruit taxa exclusively. *A. jamaicensis* (largest) had the broadest diet. Morphology alone did not partition the bats' diets. A canonical correspondence analysis also indicated that age, sex and reproductive status influence diet. I show that DNA barcoding is a high resolution tool for diet investigations of frugivores that enables effective dietary studies.

Keywords

DNA barcoding, *rbcL*, diet, faecal analysis, frugivory, dietary overlap, niche breadth, *Ariteus*, *Artibeus jamaicensis*, *Glossophaga soricina*

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Chapter 1 - Introduction

1.1 Dietary investigation

Detailed dietary investigations are fundamental to the understanding of community structure and function. The acquisition and transfer of energy and nutrients are central to trophic theory (e.g. Elton 1927), which provides an ecological framework that describes community organization. As a first step, dietary investigations allow insight into potential sources of competition within trophic levels and predatory relationships across trophic levels. Furthermore, dietary investigations are integral to understanding major ecological processes, such as plant pollination and seed dispersal, that emerge from these trophic interactions. Because of this, a wide range of efforts to identify the diets of herbivores have emerged (e.g., Beeston et al. 2005, Donadio & Buskirk 2006, Lopez & Vaughan 2007, Nagelkerken et al. 2009, Razgour et al. 2011) to place species within food webs and to identify their ecological roles. The purpose of my research was to use molecular methods to investigate the diet of frugivorous bats (Mammalia: Chiroptera) as a first step to identifying their trophic relationships and ecological roles.

Investigations of predation or, in the case of plant eating animals, herbivory, are a means to identify food resources. Herbivory is a vertical interaction (across trophic levels) that transfers energy and nutrients up trophic levels from primary producers to primary consumers. Although often described as exploitative, herbivory frequently results in mutualistic relationships between plants and consumers through dispersal of gametes and seeds. Animal-mediated seed and pollen dispersal, or zoochory, may be more effective than abiotic dispersal vectors such as wind and gravity for many plant species under the escape, colonization and direct dispersal hypotheses (reviewed by Howe & Smallwood 1982, Connell 1971, Janzen 1970). Plants offer a reward to entice vertebrates into acquiring and moving propagules away from the parent (Herrera 1981, Howe & Smallwood 1982). Several syndromes such as ornithichory/ornithophily (fruits or flowers that target birds; e.g., Albuquerque et al. 2006, Armesto & Rozzi 1989, Debussche et al. 1982) and chiropterochory/chiropterophily (fruits or flowers that target bats; e.g., Albuquerque et al. 2006, Sazima et al. 2003, Schlumpberger et al. 2006) emerged from

this relationship where plants attract specific consumers that are effective seed and pollen dispersers. Dietary investigations provide the first step to identifying potential dispersers and pollinators.

Although some plant species attract specific dispersers (e.g., Ware et al. 1993) many plants attract a wide range of dispersers (e.g., Waser et al. 1996). Competition among consumers is often a result of this. Interspecific competition occurs between consumers within similar trophic levels that use the same resources. It is thought to be a major mechanism that structures communities (Pianka 1976, Schoener 1983). Dietary overlap provides a means of identifying shared resources that are potentially under competition (Pianka 1976). Clode and Macdonald (1995) established the diets of mink (*Mustela vison*) and otters (*Lutra lutra*) to assess if they were competing for food and found that there was strong dietary overlap which is often indicative of competition (Schoener 1983). When shared resources are limiting (e.g. low abundance or low fecundity fruits) one consumer species of a competing pair will emerge as a greater competitor and exclude the other (Hardin 1960). As a means of avoiding competition, alternate food sources can be exploited. Recent dietary investigations found that alternate food sources that are rarely exploited are very important for community structure, as a means of reducing the effects of competition (Nagelkerken et al. 2009, Razgour et al. 2011). Dietary breadth (Levins 1968) indicates the potential for alternative food sources. In the absence of alternative resources, coexisting species using limiting resources are thus in the midst of exclusion or have differentiated their ecological niches sufficiently to persist (McNab 1971). However, where resources are non-limiting, ecologically similar species are able to coexist.

Selection of food is influenced by a range of factors intrinsic to the consumer. Morphological differences, such as body size and skull structure, are a means of niche separation (Brown 1981, Hutchinson 1959, Wilson 1975, Woodward et al. 2005) observed in a range of ecologically similar taxa that coexist in communities (e.g., mammals: Andreas et al. 2012, Birks & Dunstone 1985, Pratt & Stiles 1985, Tamsitt 1967, and fish: Mittelbach, 1984). Larger consumers are able to exploit larger food items. However, there are always exceptions creating communities where morphology does not

appear to provide a mechanism for the coexistence of ecologically similar consumers (e.g., mammals: Razgour et al. 2011, birds: Rotenberry 1980, and reptiles: Sutherland 2011) or where resources are not limiting.

Consumer sex and age are also factors that affect food selection. Dietary differences between males and females are not uncommon in vertebrates as observed in mammals (e.g., Birks & Dunstone 1985, Fritts & Sealander 1978) and birds (e.g., Beeston et al. 2005, Durell et al. 1993). Many consumers are sexually dimorphic leading to size differences between males and females (e.g., Myers 1978, Shine 1991, Székely et al. 2000). Furthermore, dietary adjustments, such as proportional increases, decreases or substitutions, during female reproductive preparation are documented in birds (e.g., Morrissey et al. 2010) and in bats (Zortéa 2003, Lopez & Vaughan, 2007). Ontogenetic niche shifts are also common in vertebrate consumers and influence food selection (reviewed by Werner & Gilliam 1984, Field et al. 2006). Dietary differences between consumers are influenced by age and sex.

1.2 Dietary identification

To date most dietary reconstructions for herbivores have involved low resolution methods. Direct observation and food removal experiments were among the first techniques used to identify the diets of species and continue to be used (eg. Fleming et al. 1985, Margalida et al. 2005, Palmeirim et al. 1989). However, these methods are less feasible where the consumer species of interest are cryptic, nocturnal/crepuscular, rare or otherwise difficult to observe. As taxonomists collected and identified reference material, the list of available methods expanded to include analyses of stomach contents and fecal remains (Bumrungsri et al. 2007, Collopy 1983, Fleming et al. 1985, Lopez & Vaughan 2007, Strüssmann et al. 1984). In many herbivorous diets, seeds often remain intact after passing through the alimentary tract and can be used for identification (e.g. Fleming 1988, Fleming & Heithaus 1981, Olson & Blum 1968). However, these methods are limited by the level of mastication and digestion of the consumer taxa and may render many dietary elements unidentifiable. Additionally, species may maintain highly diverse diets (e.g. Aragona & Setz 2001, Clare et al. 2009, Newmaster et al. 2013) further confounding the

structures or seeds available for morphological distinction. Stable isotope analyses are effective for identifying generalized trophic levels, but these methods provide low resolution dietary determinations (Herrera et al. 2001). High resolution methods of diet identification are imperative for detailed dietary investigations. Ideally, the best means of identification has high resolution, is accurate to the lowest taxonomic level possible, non-invasive and inexpensive.

DNA barcoding (Hebert et al. 2003) is a good candidate for herbivorous dietary identification. Molecular taxonomists have generated large sequence databases, such as the Barcode of Life Database (BOLD) and GenBank, for loci that are suitable for discriminating organisms at low taxonomic levels. When coupled with sequencing technologies and proper analyses, DNA sequence databases facilitate the identification of dietary constituents even in degraded tissues such as stomach contents and faeces. To date, DNA barcoding and subsequent molecular methods have been used to investigate a wide range of consumer species including but not limited to fish (e.g. Corse et al. 2010), reptiles (e.g. Brown et al. 2012), birds (e.g. Joo & Park 2012, Jarman et al. 2002, Jedlicka et al. 2013) and mammals (e.g. Clare et al. 2009, Deagle et al. 2005). Although DNA barcoding was initially developed to investigate animal diversity (using the mitochondrial gene cytochrome *c* oxidase I or COI), the method can equally be applied to plant identification using different gene loci such as *rbcL* and *trnH* (Fazekas et al. 2008, Kress & Erickson 2007, Newmaster et al. 2006, Newmaster et al. 2007). DNA barcoding and subsequent molecular methods provide feasible methods for dietary identification in a broad range of consumers.

Faeces are a readily available and minimally invasive source of data for molecular diet identification. In cases where food is thoroughly digested and identifiable structures are absent (precluding morphological analyses) prey DNA is often present in sufficient quantities to be detected via molecular sequencing (e.g., Clare et al. 2009, Deagle et al. 2005, Jedlicka et al. 2013, Razgour et al. 2011). However, fecal samples are particularly troublesome for DNA extraction. Even when fresh, the prey DNA in fecal samples may be highly degraded due to endogenous endonucleases, depurination, strand breakage,

oxidative damage, bacterial digestion, molecular crosslinking, and many other digestive processes (Höss et al. 1996, Lindahl 1993, Mitchell et al. 2005, Pääbo 1989). Digestive processes add a number of substances such as mucopolysaccharides, polysaccharides, blood, bile, and bilirubin that are inhibitory to restriction enzymes and DNA polymerases (Monteiro 1997). Additionally, degradation continues after deposition of fecal matter due to environmental conditions and decomposition (Brinkman et al. 2009).

Molecular analysis of herbivore fecal matter is particularly problematic because plants contain many secondary metabolites that often co-purify during DNA isolation (Ivanova et al. 2008). Compounds such as polysaccharides, phenols, tannins, lignans, alkaloids, proteins and RNA are common in plant material and may inhibit downstream molecular reactions (Pirttilä et al. 2001). However, there are methods to deal with these issues (e.g. Xu et al. 2004). These problems are also somewhat alleviated as DNA barcoding uses short sequences (ca. 600 bp) to identify species (Hebert et al. 2003, Newmaster et al. 2006). Although faeces provide inherent difficulties for molecular identification, the development of robust methodologies has generated high resolution and accurate dietary data (Deagle et al. 2005, Razgour et al. 2011, Jedlicka et al. 2013).

1.3 Investigating frugivores

As primary consumers, fruitbats (Mammalia: Chiroptera) provide a model system for investigating molecular dietary determination methods. Bats are the second most speciose mammalian order and represent nearly one fifth of all mammals (Buckley et al. 2010). Bats of the new world family Phyllostomidae have the most diverse diets in mammals ranging from carnivory (e.g., insects, fish, and amphibians) to herbivory (e.g., fruits, pollen and nectar) to sanguivory or in some species a combination of carnivory and herbivory (Freeman 2000, Giannini & Kalko 2004, Wetterer et al. 2000). Recent technological advances have enabled effective molecular dietary analyses of insectivorous bats (Clare et al. 2009, Razgour et al. 2011, Zeale et al. 2011) and provide opportunities to extend these methods in fruit eating species. Dietary analyses are important because bats are widely regarded as integral components to ecosystem health due to their consumer-resource interactions which lead to ecosystem services (reviewed

by Kunz et al. 2011), seed dispersal (Galindo-González et al. 2008, Lobova & Mori 2003, Medellín & Gaona 1999, Muscarella & Fleming 2007, Silveira et al. 2011) and pollination (Fleming et al. 2009, Fujita & Tuttle 1991, Muscarella & Fleming 2007). Bats are also suggested to be good indicators of disturbance and ecosystem health (Fenton et al. 1992, Medellín et al. 2000). As bats demonstrate their involvement in key ecological processes, detailed investigation of their consumer-resource relationships are important to identify for effective management and conservation applications.

MacArthur (1965) suggested that island communities maintain reduced species diversity, which is advantageous when conducting studies of consumer-resource interactions. As the number of consumer and resource species increase, networks become increasingly complex. Therefore, simpler communities, such as those found on islands, are desirable. For example, in Jamaica the community of bats that rely on fruit comprises only 6 species (Genoways et al. 2005), compared to ca. 15 species in adjacent continental locations such as Costa Rica and Panama (Fleming et al. 1972, Lopez & Vaughan 2007). This feeding guild is composed of two frugivores, *Artibeus jamaicensis* and *Ariteus flavescens*, and four nectarivores that periodically eat fruit, *Glossophaga soricina*, *Monophyllus redmani*, *Erophylla sezekorni*, and *Phylonycteris aphylla* (Genoways et al. 2005). Similarly, the vascular flora of Jamaica is relatively depauperate, with only ca. 3300 spp. (28% of which are endemic) compared to ca. 10,000 spp. in similar areas on the mainland (Davis et al. 1997). Using the Jamaican fruit-feeding bat guild and DNA barcoding, it is possible to investigate consumer-resource interactions at a high resolution with relatively few complicating effects from high diversity.

Ariteus flavescens (Grey) is a relatively small fruitbat (11.9 ± 0.9 g mass and 38.7 ± 0.3 mm forearm length; Genoways et al. 2005, Howe 1974) that is endemic to Jamaica and remains largely unstudied. It is a member of the Short-faced bat clade (Subtribe Stenodermatina) that also includes the genera *Centurio*, *Pygoderma*, *Ametrida*, *Sphaeronycteris*, *Ardops*, *Stenoderma*, and *Phyllops* (Wetterer et al. 2000). The common ancestor of the Short-faced bats colonized the Antilles before the Pleistocene (10.8-20.7 Ma) and subsequently diversified (Dávalos 2007). The Short-faced bats then appear to

have diversified in the Caribbean and later one lineage reinvaded the mainland (Dávalos 2007, Genoways et al. 2005). This resulted in the emergence of *A. flavescens* as a species on the island of Jamaica. *Ariteus flavescens* has often been overlooked due to sampling bias towards caves (Dávalos & Eriksson 2003, Howe 1974) and what little is known about the diet of *A. flavescens* is largely based on anecdotal evidence provided only by direct observation of the species around fruiting trees such as Fustic (*Malcura tinctoria*; syn. *Chlorophora tinctoria*) and Naseberry (*Manilkara zapota*) trees (Genoways et al. 2005, Howe 1974). *Ariteus flavescens* have shortened rostrums, similar to most new world frugivorous bat species, which is indicative of relatively increased bite force (Dumont 2004, Nogueira et al. 2009). There have been no studies on the roosting ecology of *A. flavescens* but it was not found in caves and is assumed to roost in foliage (Genoways et al. 2005). Overall, *A. flavescens* has been little studied.

Artibeus jamaicensis (Leach) are relatively large fruit-eating phyllostomids (43.4 ± 1.1 g mean \pm standard error and 60.4 ± 0.4 mm forearm length; ter Hofstede & Fenton 2005). They occur from Mexico and the Caribbean to Peru. The species initially invaded Jamaica in the late-Pleistocene and has likely dispersed back to the continent since (Genoways et al. 2005, Larsen et al. 2007, Phillips et al. 1991, Williams 1952). The diet of *A. jamaicensis* has been well established using traditional methods and shows relatively high flexibility. It eats fruit including but not limited to *Ficus* spp., *Cecropia* spp. (Giannini & Kalko 2004, Teixeira et al. 2009) and *Piper* spp. (Lopez & Vaughan 2007), foliage (Kunz & Diaz 1995), pollen (Giannini & Kalko 2004), and insects (Giannini & Kalko 2004) found across its distribution. As a relatively large bat, *A. jamaicensis* is able to exploit a wide range of fruits due to greater bite force potential (Herrel et al. 2008, Nogueira et al. 2009) and the ability to commute greater distances (Fenton 1997). *Artibeus jamaicensis* roost in caves, foliage and hollow trees (Genoways et al. 2005, Kunz & McCracken 1996, McFarlane 1986). *Artibeus jamaicensis* also shows relatively high divergence between the Jamaican and continental populations (Phillips et al. 1991). Much of the data for *A. jamaicensis* is from the continental Neotropics where the community of fruit bats is vastly different from that of Jamaica. Very few comparisons have been made between this island community and that of the mainland.

Of the nectarivorous species, *Glossophaga soricina* (Pallas) is relatively common, widely known to eat fruit (e.g. Giannini & Kalko 2004, Nogueira et al. 2009, Willig et al. 1993, Zortéa 2003) and maintains continental populations where the other three nectarivores are endemic to the Caribbean (Genoways et al. 2005). This allows comparisons for continental and island *G. soricina* and it is a good candidate to represent all of the nectarivorous species. *Glossophaga soricina* is a relatively small (10.1 ± 0.1 g and 35.7 ± 0.1 mm forearm length; ter Hofstede & Fenton 2005) nectarivorous bat that has a wide distribution from Mexico to the north of Argentina. Other than Trinidad and Tobago, Jamaica is the only Caribbean island inhabited by this species. It is unclear when *G. soricina* invaded Jamaica. Williams (1952) found *Glossophaga* remains and fossils only in the surface and subsurface layers of Jamaican caves. *Artibeus jamaicensis* fossils were also found only in these layers, suggesting a similar time frame for invasion; *G. soricina* is also likely a recent invader. *Glossophaga soricina* populations in Jamaica are genetically similar to continental populations (Hoffmann and Baker 2001). *Glossophaga soricina* is traditionally known as a nectarivorous species and maintains the specialized morphology for nectarivory, such as an elongated rostrum that leads to less bite force (Aguirre et al. 2002, Harper et al. 2013, Nogueira et al. 2009, Winter & von Helversen 2003). However, recent dietary investigations have identified greater dietary flexibility and the inclusion of fruit such as *Ficus* spp., *Cecropia* spp., and *Vismia* sp. (Giannini and Kalko 2004) and insects (Clare et al. In Press, Herrera et al. 2001, Zortéa, 2003). *Glossophaga soricina* are cave roosting species that may also roost in buildings (Fenton et al. 2001, Genoways et al. 2005). As with *A. jamaicensis*, the majority of dietary studies are focused on continental populations.

1.4 Objectives

The objectives of my research were **(1)** to investigate the applicability of DNA barcoding as a tool for plant identification from frugivorous bat faeces and **(2)** to investigate the dietary intake of a community of fruit eating bats using these molecular identification tools, and then to compare diets within and across species.

(1) DNA barcoding should provide a greater resolution of the diet of frugivorous bats

than traditional morphological identification methods because of its universal applicability and ability to identify taxa from amorphous fecal remains. I predicted that I would be able to identify more fruit taxa in the diet with DNA barcoding than with traditional methods.

(2) Morphological factors of *Ariteus flavescens*, *Artibeus jamaicensis*, and *Glossophaga soricina* lead to dietary differentiation because greater body size allows frugivorous vertebrates to exploit a greater diversity of fruit including larger bodied and harder fruits.

(a) I predicted that the fruit species consumed by each bat species would be largely exclusive to their respective bat species.

(b) I predicted that *A. jamaicensis*, as the largest species, would have the greatest dietary breadth of the three species.

(c) I predicted that *A. flavescens* would have a narrow diet relative to *A. jamaicensis*

(d) I predicted that *G. soricina*, as a nectarivore, would have a narrow fruit diet breadth.

Chapter 2 - Materials and Methods

2.1 Study area

My study was conducted in September-October 2011 and May-June 2012 in the Windsor (18°21'22.50" N, 77°38'48.72" W) and Coxheath (18°22'59.21"N, 77°37'45.33"W) areas in the north of Cockpit Country, Jamaica (elevation 100-500 m). Cockpit Country is a karst landscape (ca. 600 km²) of predominately "wet limestone forest" habitat covering hillsides and valleys (historically described as cockpits by the British). The canopy can reach 30 m but is more frequently 15 to 20 m in height. The wet seasons occur in May and September to October when overall rainfall exceeds 100 mm. The driest months (less than 100 mm of rain) are December through March (Windsor Research Centre, unpublished data). Annual rainfall ranges from 1500 to 2000 mm, increasing towards the centre of the area (Koenig 2001). The average temperature of the region varies annually but remains from low 20s to mid 30s °C (Koenig 2001).

The Cockpit Country has a legacy and continued effect of disturbance from resource exploitation (e.g., lumber and bauxite) and agriculture (e.g., yams, bananas, mangos, coffee and cattle pastures). The Windsor area is typified by early successional or primary wet limestone forest with a sparse understory (Dávalos & Eriksson 2003, Koenig 2001). Agricultural operations are typically pastures and cultivation (e.g., yams, bananas, and coffee). The Coxheath area has a greater incidence of human disturbance than Windsor, both development and agriculture, leading to relatively less forest cover. Coxheath and Windsor Cave are about 3.5 km apart and have similar habitat but less human disturbance closer to Windsor.

I chose this area because of its proximity to the Windsor Great Cave which provides roost sites for several bat species but for *A. jamaicensis* and *G. soricina* more specifically. This area is also inhabited by *A. flavescens* (Genoways et al. 2005, Dávalos and Eriksson 2003, Dávalos 2007) which allowed the potential capture of all three species at similar sites. Using the Windsor and Coxheath areas also allowed an inspection of the effects of differing human activity on the diet of Jamaican fruitbats.

2.2 Sample collection

I captured bats using mist nets (2.5 m x 10 m, 32 mm mesh size; Ecotone, Gdynia) in forested and open areas and harp traps (Forest Strainer, Bat Conservation and Management Inc., Carlisle, PA, USA or custom built 1.5 m x 1.5 m harp trap) at the Windsor Cave upper and lower entrances. I deployed two to six nets prior to dusk (approximately 18:00 hrs) and closed them shortly before dawn (approximately 5:00 hrs) in both field seasons.

I recorded 14 factors relevant to the morphology, location and time of each captured individual. To assess morphology I recorded species, sex, reproductive condition (visibility of testes in males, pregnancy, lactation and nipple status in females) and age (sub-adult or adult) of each bat. To assess size specifically, I measured mass using a digital scale (± 0.1 g), and forearm length using a ruler or digital calipers (± 0.001 mm). I estimated the distension of the abdomen, an approximation of stomach contents, by visualizing the abdomen of bats that recently defecated and were kept for an extended time period (0%) and full bats (100%) and assigning a value to approximate the distension of captured bats based on those reference points. I recorded the time of capture both on a nightly basis (hour) and the season of capture (early, mid or late wet season).

To obtain faecal samples, I placed the bats in small cloth bags and kept them for a period of approximately one hour before releasing them. In all instances, I placed bats in a new, clean cloth bag to avoid cross contamination. I placed the faeces in 1.5 mL microcentrifuge tubes. I placed fecal samples collected in the 2011 field season (September - October) into a freezer at approximately -18°C for a period of one year. I placed samples collected in the 2012 field season (May - June) and those from the 2011 field season (after one year of freezing) on silica gel to desiccate the samples and kept them at ambient temperature for up to two months. Once in Canada, the samples were frozen at approximately -20°C .

Table 1. Mist net locations for Windsor and Coxheath areas, Trelawny, Jamaica.

Site name	Site code	Latitude	Longitude	Site description
Windsor Research Centre	WRC	18°21'22.50"N	77°38'48.72"W	Driveway, forested near pasture
WRC- Road by House	RBH	18°21'22.15"N	77°38'45.10"W	Road near a Fig tree and Coffee glade
Windsor Little Bridge	LB	18°21'15.52"N	77°38'48.48"W	Road with small river, fig tree
Windsor Big Bridge	BB	18°21'18.49"N	77°38'50.72"W	Open area near river
Windsor Cave Upper Entrance	UE	18°21'00.78"N	77°38'46.68"W	Large cave entrance
Windsor Cave Lower Entrance	LE	18°21'07.98"N	77°38'50.64"W	Small cave entrance
Trail Head	TH	18°21'06.23"N	77°38'46.83"W	Trail intersection near road end and pasture
Mike's Five Acres	M5A	18°21'27.30"N	77°38'34.10"W	Forested near road, farm and Coffee glade
Coxheath Hill	CH	18°22'59.30"N	77°37'52.20"W	Edge of a farm
Coxheath Hill Naseberry	CHN	18°23'02.90"N	77°37'51.60"W	Near a Naseberry tree (Manilkara zapota)
Miss Lilly's	ML	18°22'59.21"N	77°37'45.33"W	Backyard garden near a nightclub and road
Across from Miss Lilly's	A-ML	18°23'01.07"N	77°37'47.53"W	Backyard garden near road
Miss Lilly's Pasture	MLP	18°23'00.83"N	77°37'42.19"W	Forest edge near pasture and road
Coxheath Road	CR	18°22'53.50"N	77°37'40.40"W	On a road near pasture

In the 2011 field season many bats did not produce faeces, even over extended periods of time. As a solution, during the 2012 field season I placed some bats in inverted and perforated 2 L plastic jugs and performed feeding trials to attempt to collect faeces from previous meals. Following Delorme and Thomas (1996), I placed a wire mesh composed of vinyl hardware cloth in each chamber to allow the bats to climb and hang while preventing them from flying. I added small cups of homogeneous mashed banana with blue food colouring (Blue No. 2) to each chamber. I monitored bats for ingestion and excretion for a maximum of 3 hours and collected faeces dropped in the neck of the bottle.

Mist net sampling effort was not standardized. Instead, bats were netted at accessible sites where *A. flavescens* has been reported or in areas where the landscape features appeared to provide favourable conditions for capturing frugivorous bats such as corridors (roads and edges) or near fruiting trees (Table 1). Some sites were also located on farms and in backyard gardens. Because bats tend to avoid areas where they have been caught in mist nets (Kunz and Brock 1975), I netted each site for a maximum of three consecutive nights with at least three nights before returning to that capture location. As little is known about *A. flavescens*, I assessed the general rarity of the species in Windsor and Coxheath by calculating the number of bats captured per mist net per hour relative to *A. jamaicensis* and *G. soricina* captures.

2.3 Morphological identification

To address the resolution of identification using DNA barcoding relative to traditional methods and the diet of each species I examined bat droppings under a dissecting microscope to identify plant species present as accurately as possible with reference to a field guide (Cornejo and Janovec 2010) and reference seeds I had collected in the field. Target plants were those that researchers had observed bats approaching, teeth marks, and attributes believed to be of interest to frugivorous bats (Susan Koenig, Pers. Comm.) I collected these reference seeds by locating and indentifying the target plants with a trained local field assistant and a reference manual (Adams 1972). I treated the seeds using the same preservation methods as for faeces. When seeds of more than one species

were present in a fecal sample, I divided them into two separate extractions. I recombined these samples after identification for all subsequent analyses. I captured images of almost all fecal samples using a Hirox digital light microscope (50-400x magnification) and associated software (Hirox-USA, Hackensack, NJ) for reference and accession into the BOLD database.

2.4 Molecular identification

2.4.1 DNA extraction

With help from Dr. Royce Steeves at the Biodiversity Institute, University of Guelph, ON, I extracted whole genomic DNA from the silica gel preserved bat faeces using a custom DNA extraction method. In total I extracted DNA from 130 fecal samples, and one seed found attached to a bat's fur using a custom protocol employing a CTAB based lysis buffer, chloroform:isoamyl alcohol, and DNA precipitation on magnetized silica beads with chaotropic salts. I placed all samples in 1 mL strip cap tubes with a stainless steel bead, froze them in liquid nitrogen 1-2 minutes, and lysed using a TissueLyser II (Qiagen). Full details of the custom DNA extraction protocol are described (Appendix 2).

2.4.2 PCR amplification

I performed PCR amplification of *rbcL* in 20 μ L reaction volumes containing 2 μ g of BSA (New England Biolabs cat# B9001S), 1x Phire hotstart PCR buffer, 0.4 mM Gene Amp dNTPs (Applied Biosystems, Foster City, CA, USA), 0.2 μ M of each primer (*rbcL*-AF: 5'-ATGTCACCACAAACAGAGACTAAAGC-3', Kress & Erickson 2007 and *rbcL* 634R: GAAACGGTCTCTCCAACGCAT-3' Fazekas et al. 2008), 0.3 μ L of Phire hotstart DNA polymerase (Finnzymes, Espoo, Finland), and 1 μ L of genomic DNA as template. I used a Viriti® thermal cycler (Applied Biosystems) to perform PCR with an initial denaturation phase at 98°C for 1 min, followed by 35 cycles of 98°C for 5 s, 57°C for 5 s, and 72°C for 12 s, and a final elongation at 72°C for 1 min. The PCR products were held at 10°C until removed from the apparatus. To confirm successful amplification, I combined 4 μ L of PCR products with 1 μ L of Promega Blue/Orange loading dye and ran on a 1% agarose gel for 30 min at 5 V/cm. I imaged the gels using an Alpha imager.

2.4.3 DNA sequencing

I prepared the amplification products using the same primers used in PCR and the following reaction mixture: a 10.5 μL reaction volume containing 0.5 μL of BigDye terminator mix v3.1, 1.88 μL of 5x sequencing buffer (Applied Biosystems), 1 μM primer and 0.5 μL of band re-amplification product. Thermal cycling parameters were: 96°C for 2 min; 30 cycles of 96°C for 30 s, 56°C for 30 s, and 60°C for 4 min; and a 10°C hold. I used Sephadex columns (Cat. no. S5897, Sigma-Aldrich, St. Louis, MO, USA) to clean the products from each reaction and ran the clean samples on an ABI 3730 sequencer (Applied Biosystems) at the University of Guelph Genomics Facility. For a small subset of samples (n=18) I only sequenced the forward direction using the *rbcL*-AF primer.

2.4.4 DNA analysis

I assembled contigs and visually inspected them using Sequencher 4.8 (Gene Codes Corporation, Ann Arbor, MI, USA). Initially, I performed a Nucleotide BLAST (NCBI) with all sequences to tentatively identify the genus and/or species associated with the DNA sequences. This was done to select outgroup taxa for comparison in cladistic analyses. I mined sequences from putative related taxa from GenBank and aligned with the fecal sequences using the default settings of the ClustalW algorithm (Thompson et al. 1994) in Bioedit (Hall 1999) and adjusted manually.

I identified fecal and GenBank-derived fruit sequences to the lowest taxonomic level possible by performing a cladistic analysis using Mr. Bayes v3.1.2 (Ronquist and Huelsenbeck 2003) implemented using CIPRES (Miller et al. 2012). Mrmodeltest (Nylander 2004) selected HKY+I+G as the most suitable nucleotide substitution model under the Akaike Information Criterion (AIC). I performed ten million generations using 4 chains and 2 runs with trees sampled every 1000 generations. I estimated posterior probabilities using a burn-in of 25,000 trees as log-likelihood values stabilized after 2.5 million generations. I used Treegraph2 (Stover & Muller 2010) to display and edit consensus trees. I identified faeces-derived sequences if they formed a monophyletic clade, at least with respect to the species known from Jamaica. All sequences generated in

this study have been deposited in the Barcode of Life Database (BOLD) as well as GenBank (Appendix 3). Images of fecal samples are available for most samples on the BOLD.

2.5 Statistical analyses

2.5.1 Niche breadth, overlap, and richness

I assessed the dietary specialization of each fruitbat species by using standardized Levins' (1968) index (equation 1) of niche breadth,

$$B_A = \frac{\frac{1}{\sum P_i^2} - 1}{n-1}, \quad (1)$$

where B_A is the standardized measure of Levins' index, P_i is the proportion of records for each plant taxon in each bat species diet and n is the number of possible plant taxa in the diet.

To identify the exclusivity or lack thereof for each fruitbat species' diet, I used Pianka's (1973) measure of niche overlap (equation 2) to quantify dietary resource overlap between the three bat species.

$$O_{jk} = \frac{\sum_i^n P_{ij} P_{ik}}{\sqrt{\sum_i^n P_{ij}^2 \sum_i^n P_{ik}^2}}, \quad (2)$$

where P_{ij} is the proportion of plant taxa i of the total plant taxa consumed by bat species j ; P_{ik} is the proportion that plant taxa i is of the total plant taxa consumed by bat species k ; and n is the number of plant taxa. To test if the extent of overlap was greater than expected by chance I used null models. I generated 10,000 simulated matrices of randomized diet composition using EcoSim software (v7; <http://grayentsminger.com/ecosim.htm>) with Randomization algorithm 3 and compared observed and randomly simulated extents of niche overlap.

I constructed a rarefaction curve for each species using PAST software version 2.17c (Hammer et al., 2001). Following this curve, I further assessed the sample sizes by conducting a resampling test on the dietary data by randomly selecting seven samples (the

maximum number of *A. flavescens* samples) from the original pools of samples without replacement and identified the plant taxa found within the faeces. This process was repeated 100 times. I used this test to calculate the mean number of plant species captured for each bat species. These means were then compared using Kruskal-Wallis and a multiple comparison post hoc test.

2.5.2 Influence of factors on diet

To determine the influence of the 14 factors, I used multivariate analyses to explore variation in Jamaican fruit bat diets and to identify considerable axes of variation in the data, which were related to bat factors; these include species, age (sub-adult/adult), sex (m/f), mass (g), abdominal distension (%), forearm length (mm), male reproductive status (testes descended and visible), female reproductive status (hair or hairless nipples, lactation, and pregnancy), year of capture, season of capture, time of capture, and location of capture. I used CANOCO 4.5 (ter Braak & Smilauer 2002) to explore variation in the diet of *A. jamaicensis*, *A. flavescens* and *G. soricina* constrained by the aforementioned 14 factors. A detrended correspondence analysis (DCA; ter Braak 1986) allowed me first to identify the length of the ordination axis (i.e., the extent of variation in the axis scores for the 14 factors) and determine the need for either a linear or unimodal ordination model. The length of the gradient (2.42 SD) justified the use of a canonical correspondence analysis (CCA) to characterize variation in diet among the three bat species. I constructed a matrix of 14 bat factors and used it to constrain the variation in the bats' diet. I used multivariate statistics to identify important factors used in canonical correspondence analysis, of which I used absolute t -value > 2.1 to indicate important canonical coefficients (ter Braak 1998) and significant ($P < 0.01$) inter-set correlations.

To determine if there was a significant difference among the diets of the three bat species based on the 14 factors, I conducted Kruskal-Wallis rank sum tests (Kruskal & Wallis 1952) on the scores obtained from the CCA. Each test was conducted independently and followed by Siegel and Castellan's (1988) post hoc multiple comparison test. I made these comparisons between the sexes within and across each species. I also made comparisons between age and sex groups for only *A. jamaicensis*.

2.5.3 Inter- and intraspecific size differences

To identify significant differences in size between males and females and age groups within and across each species I compared mass and forearm length using Kruskal-Wallis rank sum tests (Kruskal & Wallis 1952). I used R 3.0.1 statistical package (R Development Core Team 2008) to accomplish these comparisons. I used a non-parametric test because the groups were non-normal in distribution. Each test was conducted independently and followed by Siegel and Castellan's (1988) post hoc multiple comparison test. Where samples sizes were small, <3 samples, I made no statistical comparisons. I made comparisons between each species, and males and females within and across, for all three variables. I also conducted this analysis on groups delineated by age and sex simultaneously for *A. jamaicensis* (intraspecific comparison) because sample sizes were sufficiently large ($16 \leq n \leq 55$).

Chapter 3 - Results

3.1 Sample collection

I captured a total of 408 bats in the Coxheath and Windsor areas of Jamaica in 2011 and 2012 and when I include 37 other bats caught during concurrent studies, I sampled 445 bats (Table 2). My capture effort was a total of 1552.5 mist net hours (one hour open per one mist net) and the capture success varied by species and by site (Table 2). I caught all three species throughout the night. Of the three species, I caught *G. soricina* the least frequently in forested areas. However, this species was common at the cave entrances and was more commonly caught in harp traps. I caught *A. flavescens* infrequently and as this species is unavailable to capture at the cave, is the rarest of the three species. I caught *A. jamaicensis* the most frequently and it is the most common of the fruitbats. From the 445 bats, I collected 11 fecal samples from *A. flavescens*, 90 from *A. jamaicensis*, and 34 from *G. soricina* totaling 135 samples. These include samples collected from clean cloth bags, in nets, and during feeding trials.

3.2 Morphological identification

Ninety of the samples I examined contained discernible seeds identifiable at some taxonomic level. Of these 90 seed-containing faecal samples, three were identifiable to only family (Solanaceae), 12 to genus (*Piper*), and 72 to species (only *Cecropia peltata*; Table 4). I excluded 14 samples identified as *C. peltata* from molecular sequencing methods as the seeds of this species were easily identifiable. A total of 24 samples did not contain any visible seeds and were not identifiable morphologically. Morphological identification revealed four identifiable plant taxa in the guano of the three species of bats.

Table 2. Capture effort for *A. flavescens* (AF) *A. jamaicensis* (AJ) and *G. soricina* (GS) at a range of sites in the Windsor and Coxheath areas of Trelawny, Jamaica. Mist nets were open from dusk until dawn. Capture efforts are expressed as the number of bats per mist net per hour of operation.

Location	2011			2012			Bats captured				Net-hours	Capture rate (bats/net-hour)			
	Nights	Nets	Time (h)	Nights	Nets	Time (h)	AF	AJ	GS	Total		AF	AJ	GS	ALL
WRC	10	3	91.5				2	49	2	53	274.5	0.01	0.18	0.01	0.19
UE	2	1*		1	1*			4	4	8					
LE	2	1*		2	1*				27	27					
M5A	11	6	87				7	12	1	20	522	0.01	0.02	0.002	0.04
LB	8	2	79.5	1	3	10	6	80	1	87	159	0.04	0.50	0.006	0.55
BB				1	2	1.5		3		3	3		1.00		1.00
RBH	3	2	28.5				5	8		13	57	0.09	0.14		0.23
TH	3	2	20.5				1	30	1	32	41	0.02	0.73	0.02	0.78
ML	3	2	20	7	1-3	67.5	1	52	1	54	137	0.01	0.38	0.01	0.39
A-ML	2	2	19	2	2-3	22.5	3	29		32	84.5	0.04	0.34		0.38
MLP				3	2	20.5		5		5	41		0.12		0.12
CH				11	1-3	112.5	7	68		75	152	0.05	0.45		0.49
CHN				2	2	21	2	7		9	42	0.05	0.17		0.21
CR				2	2-3	16.5		1		1	39.5		0.03		0.03
TOTAL							34	348	37	419	1552.5	0.022	0.22	0.004	0.2699

*Harp trap at the cave entrances. These values are not included in capture rate.

Table 3. Molecularly or morphologically identified plant taxa recovered from the faeces of bats belonging to *Ariteus flavescens* (AF), *Artibeus jamaicensis* (AJ), and *Glossophaga soricina* (GS). Samples that were unidentifiable by either means were excluded.

Family	Genera and species	Consumer	Number of samples	
			Molecular ID	Morphological ID
Boraginaceae	<i>Cordia spp.</i>	AJ	1	
Combretaceae	<i>Terminalia sp.</i>	AJ	1	
Moraceae	<i>Castilla elastica</i>	AJ	9	
	<i>Brosimum alicastrum</i>	AF, AJ	4 ^a	
	<i>Ficus spp.</i>	AJ	10	
Melastomataceae	Unknown sp.	GS	2	
Myrtaceae	<i>Psidium spp.</i>	AJ	2	
Piperaceae	<i>Piper spp.</i>	GS	12	12
Simaroubaceae	<i>Simarouba glauca</i>	AJ	2	
Solanaceae	<i>Solanum spp.</i>	AJ	3	
	Unknown 1	AJ		3 ^d
Cecropiaceae	<i>Cecropia peltata</i>	AF, AJ, GS	58 ^f	72
	<i>Musa</i>	AF	2 ^b	
	<i>Desmodium</i>	GS	1 ^c	
Unknown	Unknown 2	AF, AJ		3 ^e
		TOTAL	107	90

^a One sample of dry *B. alicastrum* tissue, identified by molecular means, found in the net with the bat

^b Provided to bats during feeding trials

^c Found on the exterior of the bat

^d Identified by the molecular methods as *Solanum spp*

^e Identified by the molecular methods as *B. alicastrum*

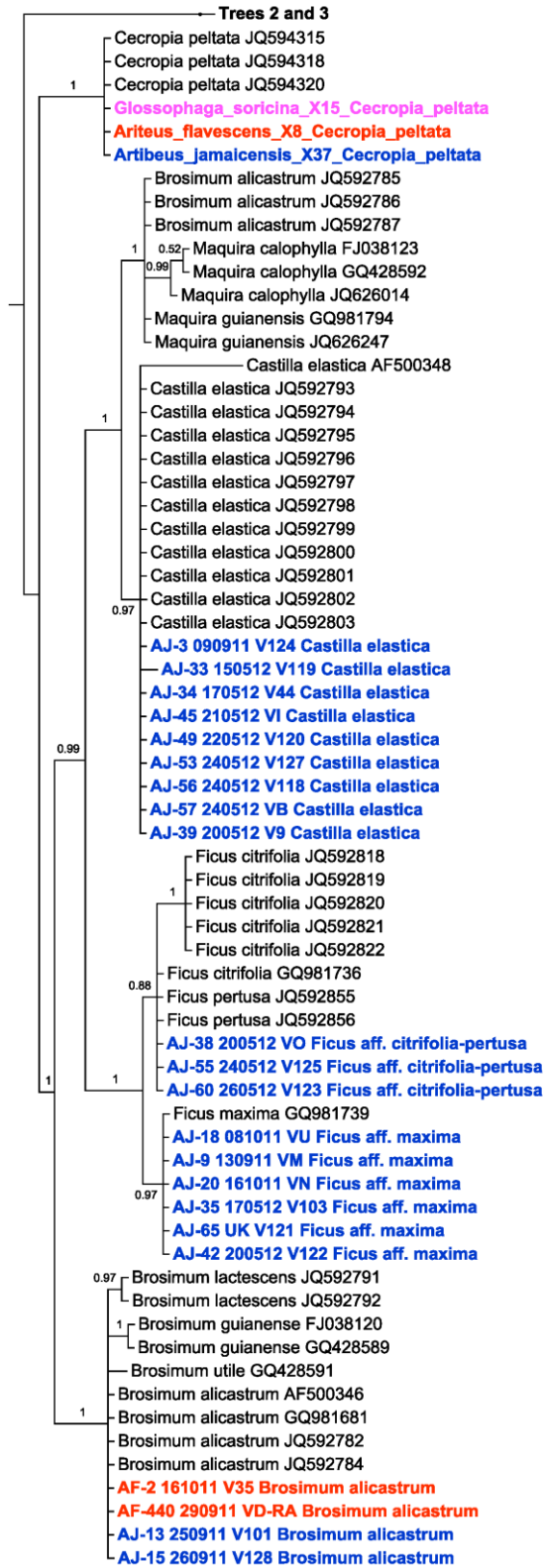
^f 24 *C. peltata* samples excluded from molecular identification

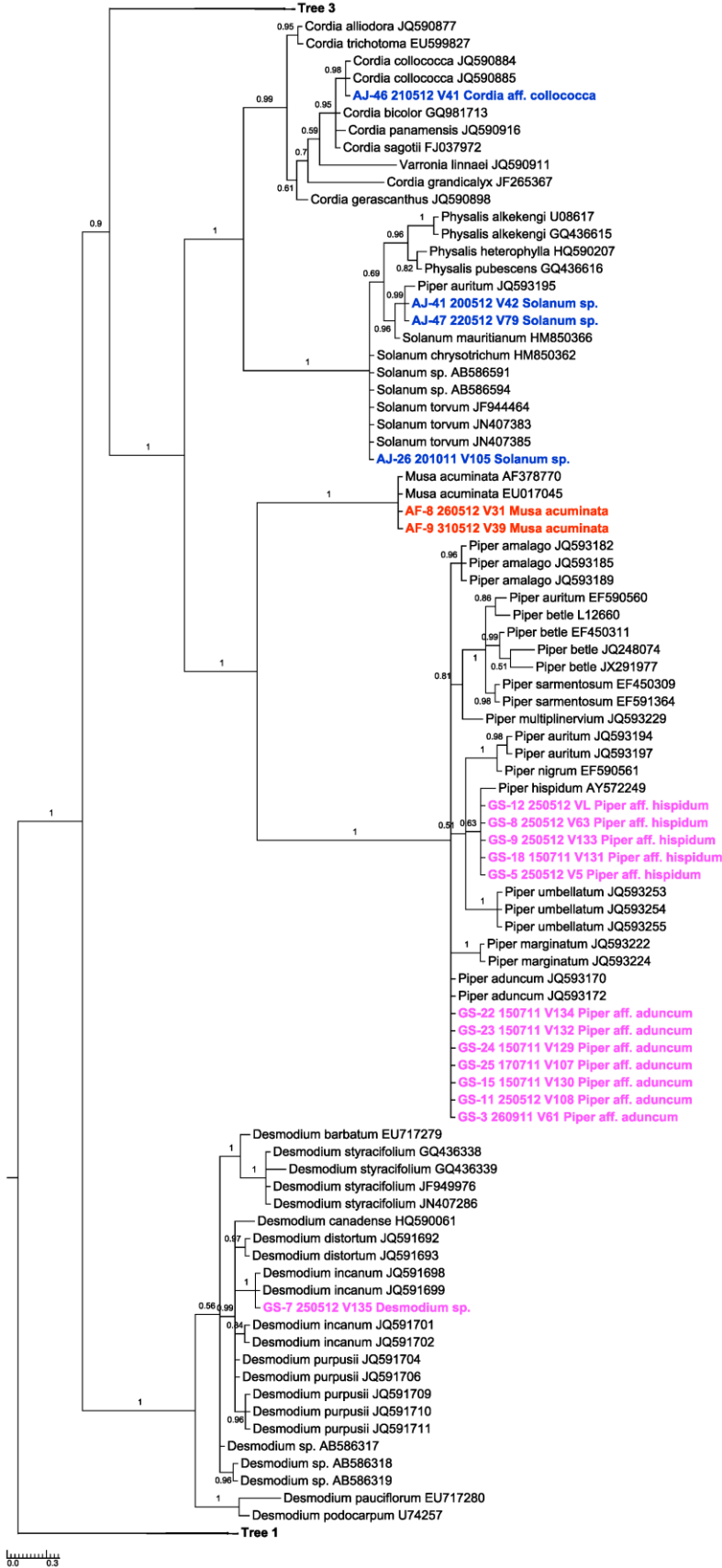
3.3 Molecular identification

I obtained high quality *rbcL* sequences for 107 of 116 faecal samples subjected to sequencing reactions (92% successful). To minimize computation time, I included only three guano-derived *C. peltata* sequences in the cladistic analysis (Fig. 1) as I observed no sequence polymorphisms among the samples. The final alignment of 51 guano-derived sequences, six fruit plant sequences and 123 plant sequences from GenBank contained 622 characters, 205 of which were variable. I found one stop codon in the alignment of

180 sequences but I included the sequence (*Clidemia petiolaris*: GenBank accession AJ235777) in the analysis nevertheless. I constructed a Bayesian consensus tree with posterior probabilities and proportional branch lengths (Fig. 1). Of the 108 sequences subjected to molecular identification, I was able to identify two to family, 29 to genus, and 72 to species (Table 4). In total, I detected 11 plant taxa present in the scat of the three species of bats.

I found that *Ariteus flavescens* ate two Moraceae species (Fig. 2). *Artibeus jamaicensis* maintained a more diverse diet composed of nine species from six families, including four Moraceae, one species of Myrtaceae, one species of Solanaceae, one species of Simaroubaceae, one species of Combretaceae, and one species of Boraginaceae (Fig. 2). *Glossophaga soricina*, traditionally known as a nectarivore, ate three species from three families including one species of Moraceae, at least one species of Piperaceae and one species of Melastomataceae (Fig 2). *Cecropia peltata* represented greater than 50% of the diet for each species. I found that *A. jamaicensis* had differing dietary proportions for groups delineated by age and sex. *Cecropia peltata* composed the main proportion for each group at over half (Fig. 3). The remaining fruit taxa were relatively rare with the highest representation of 14% (Fig. 3).





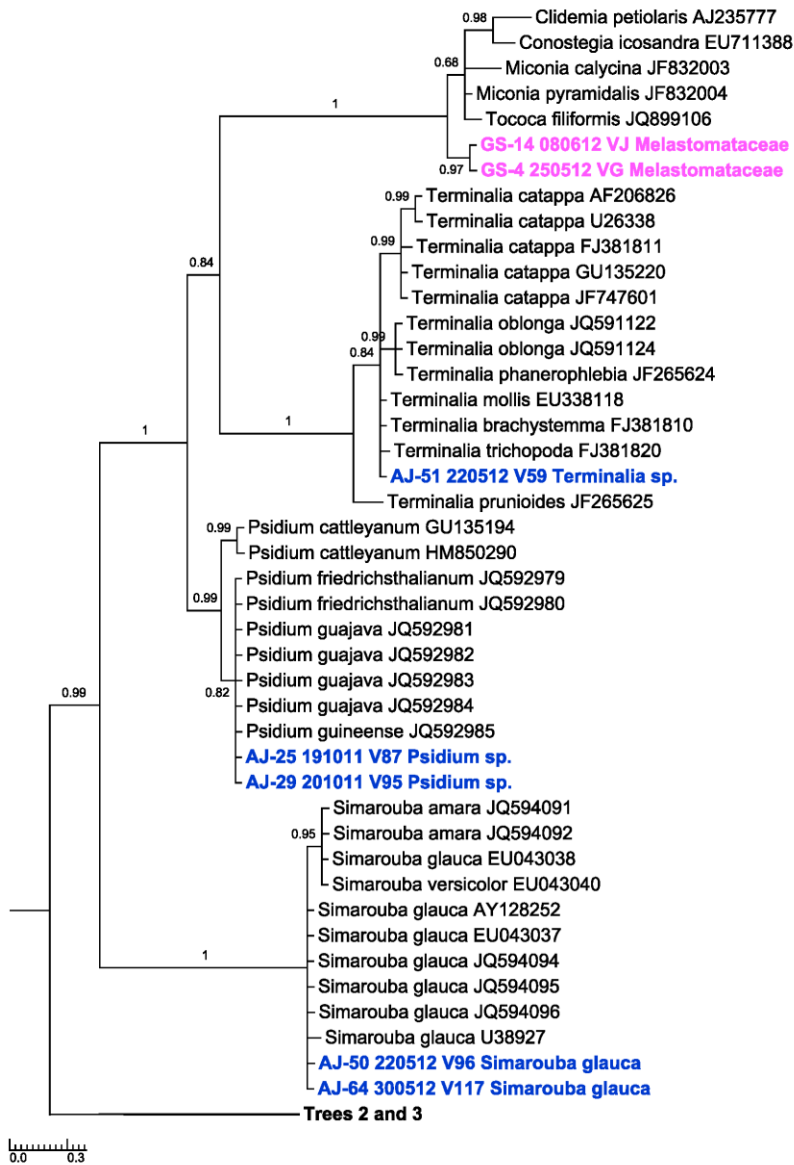


Figure 1. Bayesian consensus tree indicating the flora consumed by *Ariteus flavescens* (AF), *Artibeus jamacensis* (AJ), and *Glossophaga soricina* (GS) compared to voucher sequences acquired from BOLD and GenBank. Branch lengths are proportionate and bayesian posterior probabilities are indicated above their respective branch.

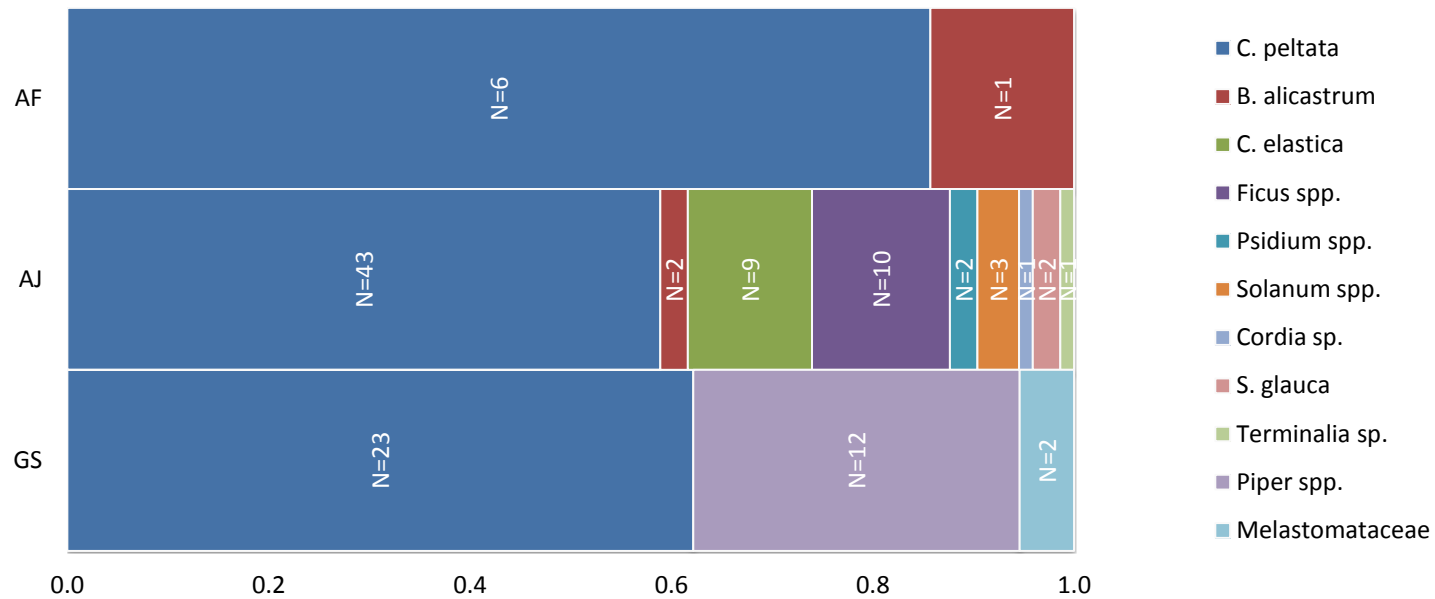


Figure 2. Proportions of dietary constituents in *Ariteus flavescens* (AF; N=7), *Artibeus jamaicensis* (AJ; N=73), and *Glossophaga soricina* (GS; N=37). Bats consumed: *Cecropia peltata*, *Brosimum alicastrum*, *Castilla elastica*, *Ficus* spp., *Psidium* spp., *Solanum* spp., *Cordia* sp., *Simarouba glauca*, *Terminalia* sp., *Piper* spp., and an unknown from the family Melastomataceae. The number of bats that consumed each taxa are indicated inside each bar.

*From left to right:

AF: *C. peltata*, *B. alicastrum*

AJ: *C. peltata*, *B. alicastrum*, *C. elastica*, *Ficus* spp., *Psidium* spp., *Solanum* spp., *Cordia* sp., *S. glauca*, *Terminalia* sp.

GS: *C. peltata*, *Piper* spp., Melastomataceae

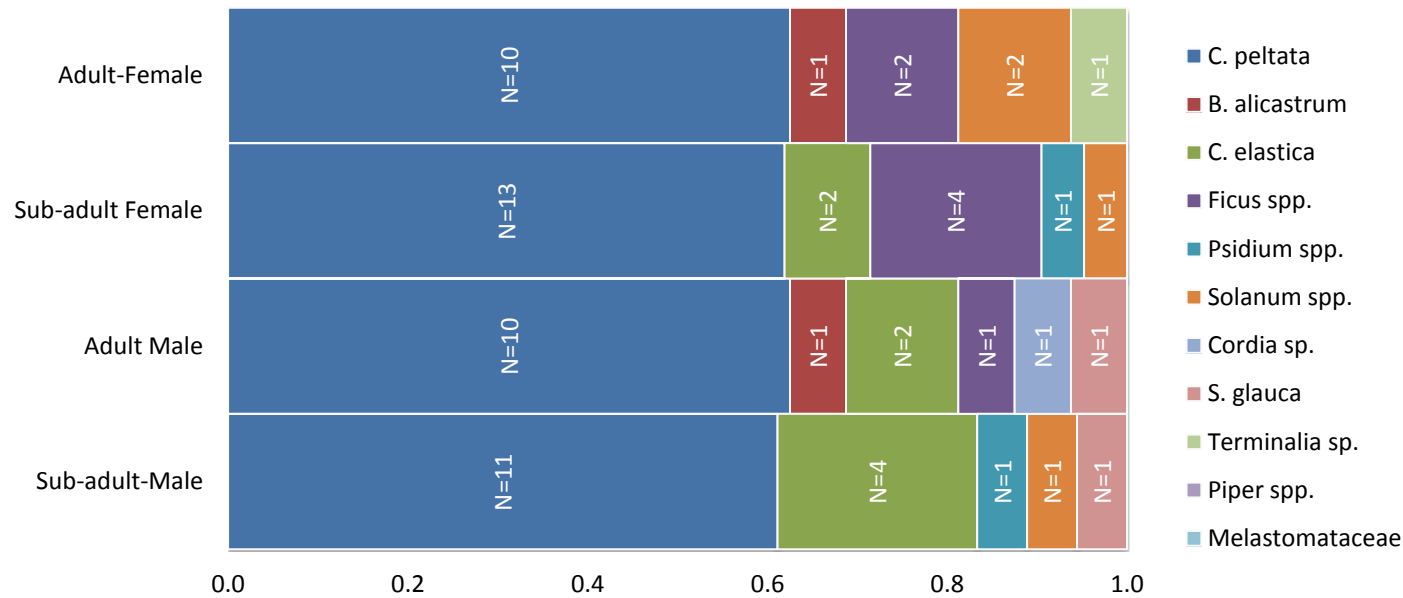


Figure 3. Proportions of dietary constituents for *Artibeus jamaicensis* adult females (N = 16), sub-adult females (N=21), adult males (N = 16) and sub-adult males (N = 18). Bats consumed: *Cecropia peltata*, *Brosimum alicastrum*, *Castilla elastica*, *Ficus spp.*, *Psidium spp.*, *Solanum spp.*, *Cordia sp.*, *Simarouba glauca*, *Terminalia sp.*, *Piper spp.*, and an unknown from the family Melastomataceae. The number of bats that consumed each taxa are indicated inside each bar.

* From left to right:

Adult female: *C. peltata*, *B. alicastrum*, *Ficus spp.*, *Solanum spp.*, *Terminalia sp.*

Sub-adult female: *C. peltata*, *C. elastica*, *Ficus spp.*, *Psidium sp.*, *Solanum spp.*

Adult male: *C. peltata*, *B. alicastrum*, *C. elastica*, *Ficus spp.*, *Cordia sp.*, *S. glauca*

Sub-adult male: *C. peltata*, *C. elastica*, *Psidium spp.*, *Solanum spp.*, *S. glauca*

3.4 Dietary comparisons between and within fruitbat species

3.4.1 Niche breadth, overlap, and richness

Ariteus flavescens was more specialized ($B_A=0.03$) in the plants they ate than either *A. jamaicensis* or *G. soricina*. *G. soricina* followed closely ($B_A=0.09$) and also occupied a relatively narrow niche. *A. jamaicensis* had the greatest niche breadth ($B_A=0.15$). Congruently, rarefaction indicated greater richness in *A. jamaicensis* followed by *G. soricina* and *A. flavescens*. At seven samples (the full extent that *A. flavescens* is represented) *A. jamaicensis* consumed 3.3 ± 2.5 (± 1 SD) fruit taxa, *G. soricina* consumed 2.3 ± 1.3 fruit taxa, and *A. flavescens* consumed 2 fruit taxa (Fig. 5). The resampling test output means had a similar pattern. *Artibeus jamaicensis* consumed 3.31 ± 1.01 fruit taxa, *G. soricina* consumed 2.34 ± 0.49 fruit taxa and *A. flavescens* consumed two fruit taxa. The Kruskal-Wallis test of these means indicated a significant difference between them ($H=131.86$, $p<0.001$) and post hoc analysis indicated that dietary plant taxa richness in each species is significantly different from each other (Fig. 6).

Although *A. jamaicensis* demonstrated greater dietary species richness, there was little evidence for resource partitioning in the three bat species. Niche overlap was significantly higher than expected by chance ($O_{jk}=0.66$, $p<0.05$).

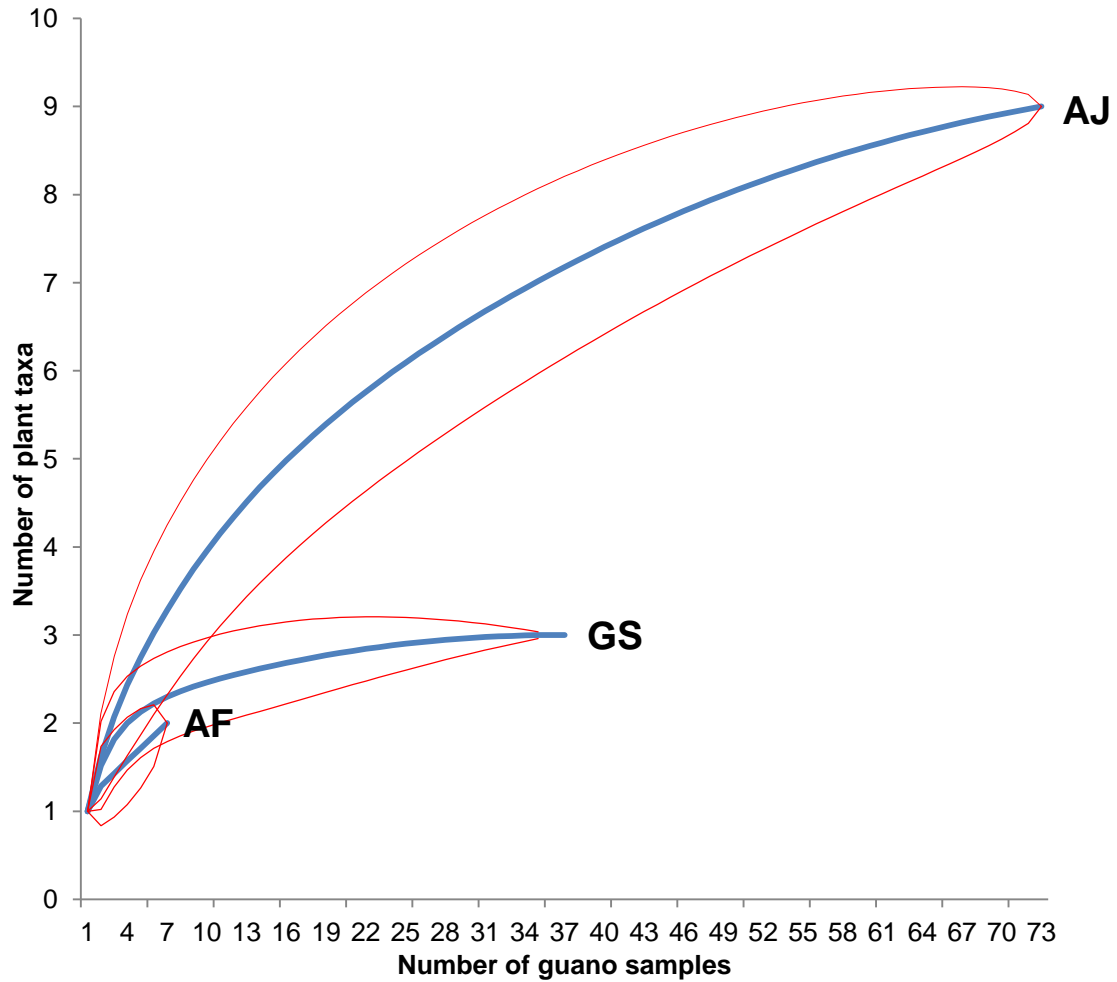


Figure 4. Rarefaction analysis for the number of plant taxa detected in the diets of *Ariteus flavescens* (AF), *Artibeus jamaicensis* (AJ) and *Glossophaga soricina* (GS). The rarefaction curve, plotting the number of fruit taxa found in randomly and sequentially added guano samples, was computed using PAST. Red lines indicate the 95% confidence intervals.

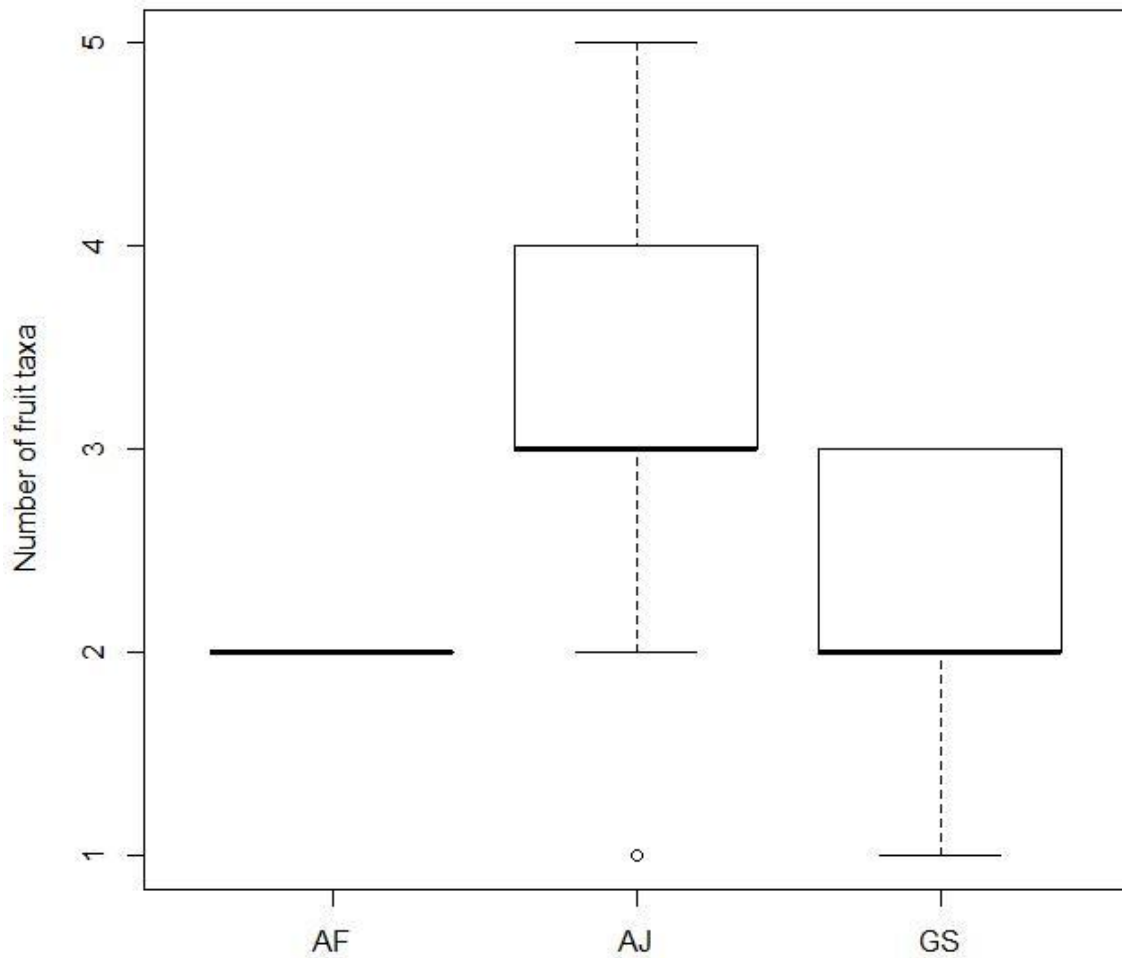


Figure 5. Reduced sample size comparison of the number of fruit taxa found within the diet of *Ariteus flavescens* (AF), *Artibeus jamaicensis* (AJ) and *Glossophaga soricina* (GS). Each group is represented by 100 replications of seven randomly sampled, without replacement, fecal samples to discern the number of fruit taxa contained within a reduced sample. A Kruskal-Wallis test followed by a multiple comparison post hoc test determined that all groups were significantly different from each other ($p < 0.005$).

3.4.2 Factors associated with dietary diversity

There is considerable variation in the diets of Jamaican fruitbats. The canonical correspondence analysis (CCA) revealed relationships between the species of bats and the diversity of fruits they consumed (Fig 7; Table 8). The overall inertia was 9.8 indicating considerable dispersion among the dietary diversity data. Eigenvalues indicated that the first 2 axes explain a considerable amount of the variation in the data; CCA 1 explains 30.1% and CCA 2 explains an additional 20.6% of the variation. Each of the axes are highly correlated (>60%; Table 4). The CCA ordination displayed a clear relationship between the bats as consumers and their diversity of fruit prey (Fig 7). Within the ordination, all bat species converge on a single common fruit species, *C. peltata*, that is central to the ordination. *Ariteus flavescens* and *A. jamaicensis* also converge near centrally on *B. alicastrum*. *Ariteus flavescens* remain central whereas *A. jamaicensis* and *G. soricina* individuals are dispersed outwards and apart from each other consuming exclusive plant species. *Artibeus jamaicensis* is found predominantly on the left where *G. soricina* is found on the right of the ordination. Further separation of *A. jamaicensis* by age and sex is also indicated by the ordination (Fig 8). Males and females converge centrally overlapping on several of the fruit taxa but males disperse upwards and females disperse downwards on the ordination demonstrating some exclusively consumed fruit taxa.

Table 4. Summary of Canonical Correspondence Analysis (CCA) of variation in DNA barcodes among 11 plant taxa recovered from the scat of 113 bats belonging to *Ariteus flavescens* (AF), *Artibeus jamaicensis* (AJ) and *Glossophaga soricina* (GS) individuals. The majority of the variation is explained by the 1st and 2nd axes.

Summary variables	Axes				Total inertia
	CCA 1	CCA 2	CCA 3	CCA 4	
Eigenvalues	0.459	0.308	0.238	0.142	9.806
Species-Factor correlations	0.713	0.617	0.491	0.378	
Cumulative percentage variance	30.1	50.7	66.6	76.1	

Dietary diversity could be partially explained by the factors of the bats. Fourteen factors explained a considerable amount of the variance in the dietary diversity of the three bat species. High species-factor correlations indicated a close correspondence between the species and factors that constrained each Monte Carlo permutation test. This confirmed that the first two axes were statistically significant ($P < 0.01$) in explaining 50.7% of the variation in the dietary data (Table 8). The respective eigenvalues for each axis confirmed that the first two axes are the most important in explaining variation in the dietary diversity of the bat species. There was considerable variation in the species scores of which the bi-plot indicates the direction and relative influence of several factors intrinsic to the bats. Significant interspecies correlations ($P < 0.01$) and t -values were used to identify eight important factors of which three are associated with the first axis and five with the second axis (Table 9). The CCA identified species, female nipple condition, and pregnancy as the most important variables that explained variation along the first CCA axis. Visibility of male testes, abdominal distension, year, sex, female lactation and age correlated strongly with the second CCA axis in the ordination.

The scores obtained from the CCA1 axis differed significantly for the species and sex comparison ($H = 35.11$, $p < 0.01$; Fig. 9 C). The post hoc analysis revealed that *A. jamaicensis* males and females are significantly different and that female *A. jamaicensis* were significantly different from female *G. soricina*. The scores from CCA2 axis also differed significantly ($H = 25.27$, $p < 0.001$; Fig. 9 D). Post hoc analysis indicated that female and male *A. jamaicensis* differ and that female *A. jamaicensis* differ from female *G. soricina*.

The CCA1 scores for *A. jamaicensis* alone were not significantly different ($H = 3.37$, $p > 0.1$; Fig. 10 C). When comparing CCA2 scores for *A. jamaicensis* groups, I observed a significant difference ($H = 11.59$, $p < 0.05$; Fig 10 D). However, the post hoc test indicated no differences between the groups. I then applied a Mann-Whitney U test with Bonferroni corrections to assess the potential for a type II error made by the previous test. This resulted in a significant difference ($p < 0.05$) between adult females (AJAF) and sub-adult males (AJSAM). In light of this difference, I applied this post hoc test to all other comparisons and found congruency between the methods.

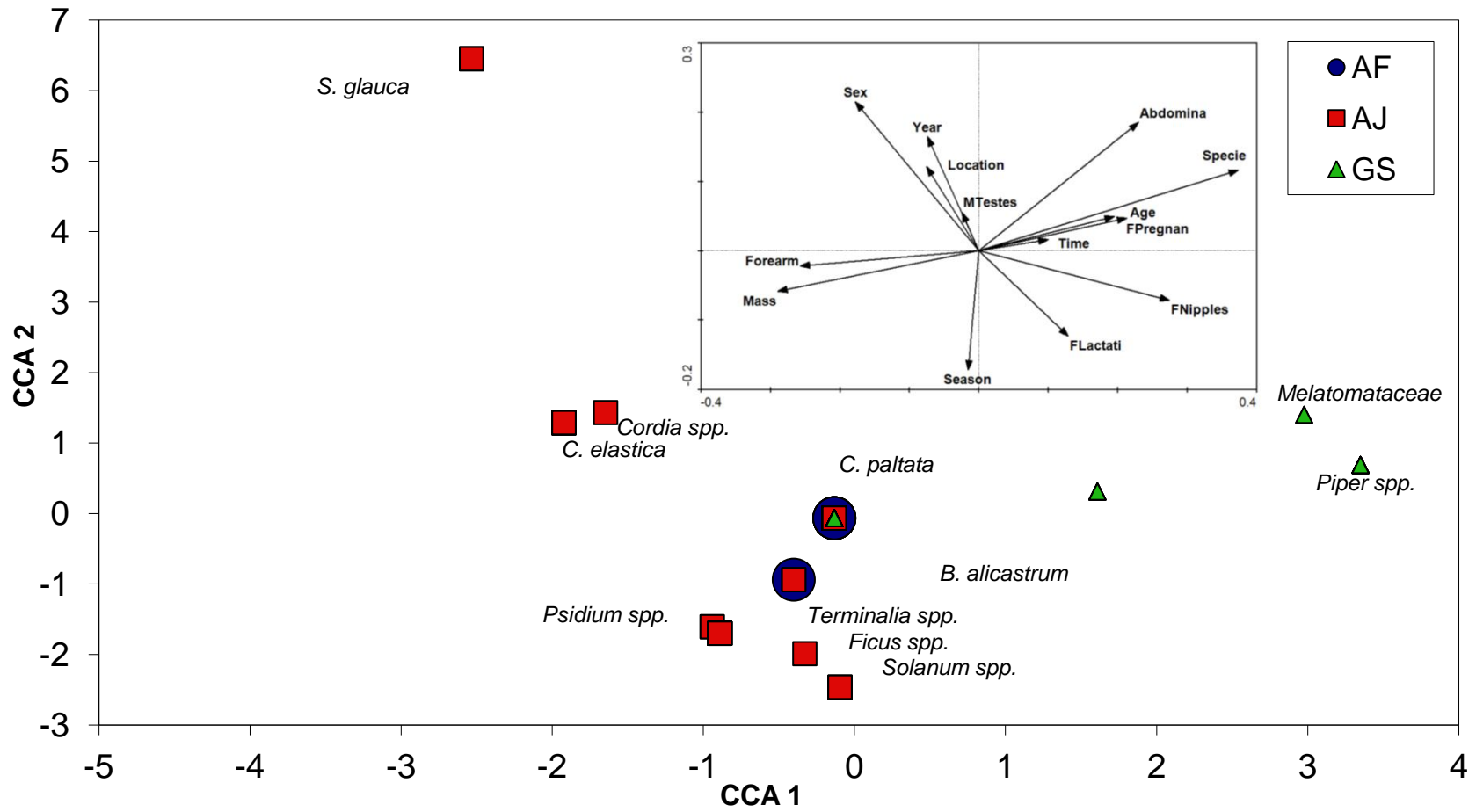


Figure 6. Dietary diversity ordination from a Canonical Correspondence Analysis (CCA) of variation in DNA barcodes among 11 plant taxa recovered from the scat of 113 bats belonging to the three species *Ariteus flavescens* (AF), *Artibeus jamaicensis* (AJ), and *Glossophaga soricina* (GS). Biplot represents important factors in explaining the variation along the respective CCA axes.

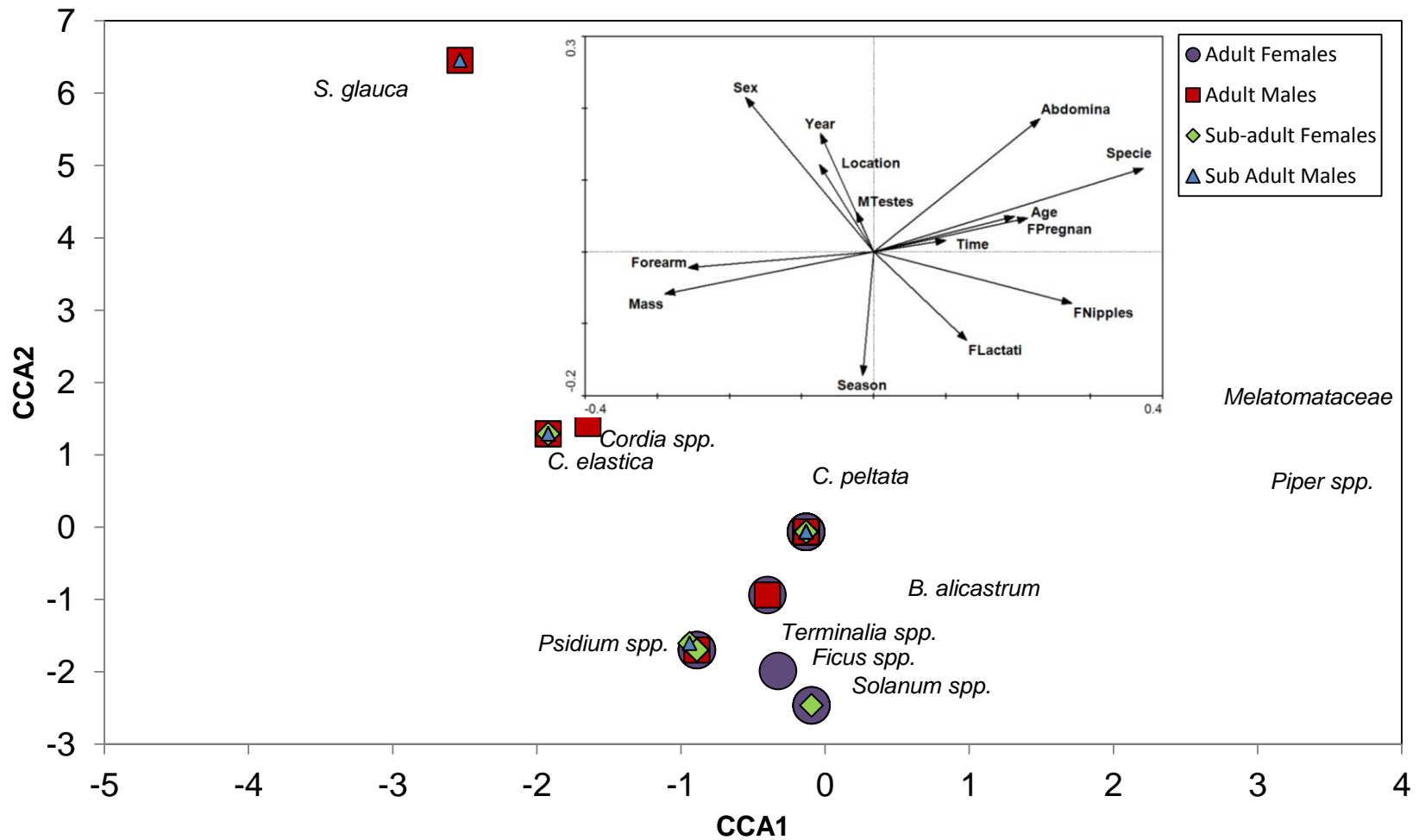


Figure 7. Dietary diversity ordination from a Canonical Correspondence Analysis (CCA) of variation in DNA barcodes among 11 plant taxa recovered from the scat of 113 bats belonging to the three species indicating only *A. jamaicensis* individuals demonstrating age and sex of each individual.

Table 5. Statistics for explanatory variables used in Canonical Correspondence Analysis (CCA) of 113 bats and 11 plant taxa constrained by 14 factors. Bolded values indicate variables with significant correlation and canonical coefficients.

	Factors	Inter set correlations		Regression/canonical coefficients		t-values of regression coefficients	
		CCA1	CCA2	CCA1	CCA2	CCA1	CCA2
Morphology	Species	0.5355	0.14	0.4655	0.1571	3.8986	1.1773
	Mass	-0.4146	-0.0706	-0.3166	-0.1428	-1.834	-0.7402
	Abdominal distension	0.3297	0.2231	0.0656	0.412	0.5157	2.8976
	Forearm length	-0.3684	-0.0266	0.059	0.071	0.3053	0.3292
	Age	0.3054	0.0566	0.0726	0.3759	0.4408	2.1431
	Sex	-0.2544	0.259	-0.1792	0.3368	-1.4324	2.4089
Reproductive status	M Testes	-0.0341	0.0664	0.2019	-0.5123	1.2955	-2.942
	F Nipples	0.3933	-0.0863	0.3874	-0.2228	2.182	-1.0715
	F Lactation	0.1847	-0.1489	-0.0315	-0.4196	-0.201	-2.3968
	F Pregnancy	0.2798	0.0595	0.201	-0.1306	2.1291	-1.1218
Temporal	Year	-0.1057	0.1985	0.2726	0.7295	1.1448	2.7409
	Season	-0.0217	-0.208	0.3972	0.2559	1.9677	1.1342
	Time	0.143	0.0193	0.1182	-0.0527	1.2312	-0.4911
Spatial	Location	-0.1074	0.1465	-0.1957	0.0717	-1.8297	0.5998

3.4.3 Inter- and intraspecific comparisons of size

Forearm lengths differed significantly ($H = 159$, $p < 0.001$) across the species (Fig. 9 A). The post hoc analysis indicated that male and female *A. flavescence* and *G. soricina* did not differ from each other. Male and female *A. jamaicensis* were significantly different from both *A. flavescens* and *G. soricina*. The analysis of mass showed the same pattern (Fig. 9 B). Mass differed significantly ($H = 222$, $p < 0.001$) across species. Male and female *A. flavescens* and *G. soricina* did not differ within and across species but collectively differed from male and female *A. jamaicensis*. In both forearm length and mass, male and female *A. jamaicensis* did not differ significantly.

Forearm lengths of *A. jamaicensis* did not differ significantly when delineated by age and sex ($H = 6.7$, $p > 0.05$; Fig. 10 A) . However, mass was significantly different across groups ($H = 94$, $p < 0.001$ Fig. 10 B). Post hoc analysis indicated significant differences between adults and sub-adults but no differences between the sexes.

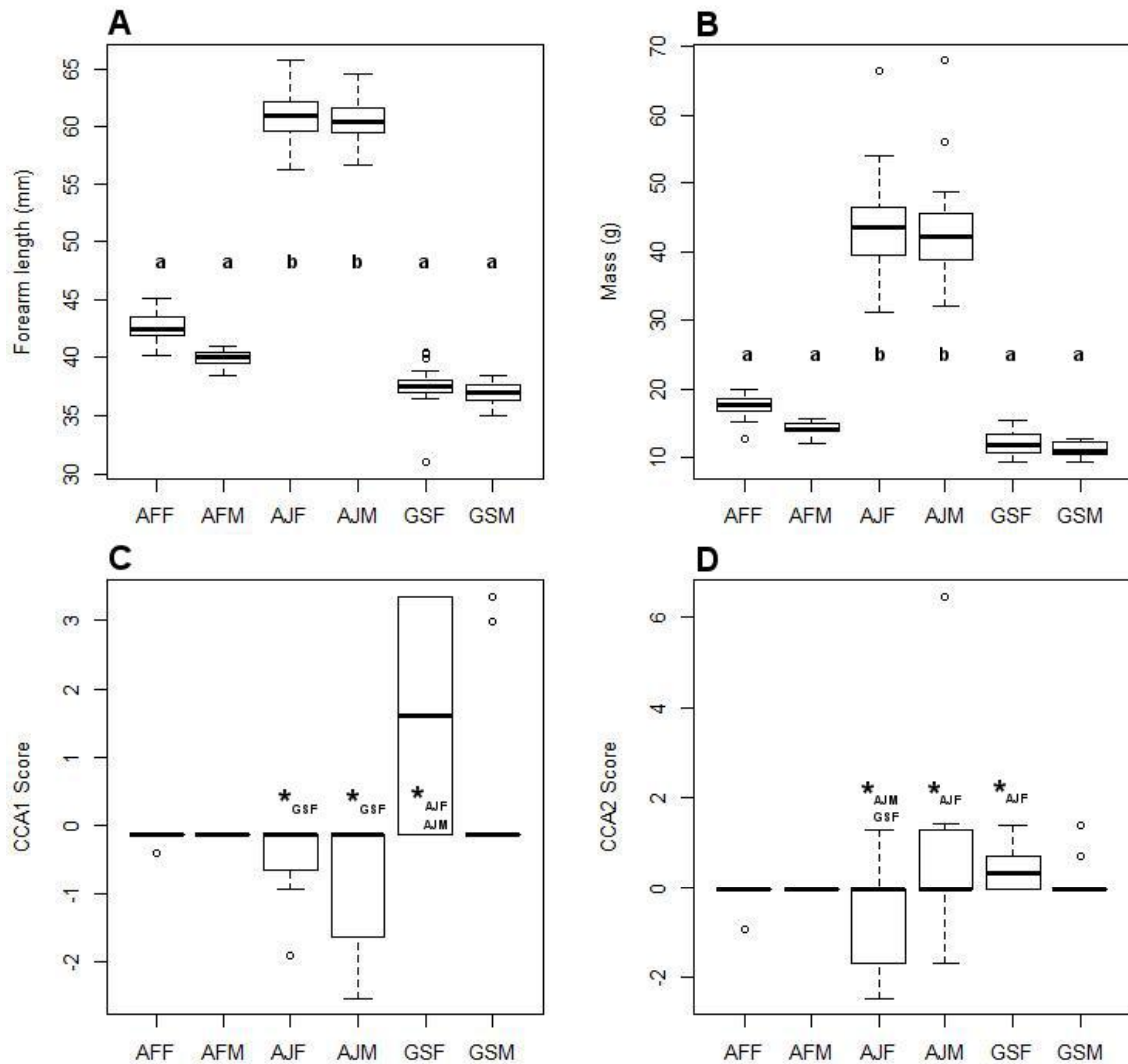


Figure 8. Comparisons across species and sex groups for forearm length (**A**; $N_{AFF}=23$, $N_{AFM}=11$, $N_{AJF}=98$, $N_{AJM}=105$, $N_{GSF}=21$, $N_{GSM}=13$), mass (**B**; $N_{AFF}=23$, $N_{AFM}=11$, $N_{AJF}=100$, $N_{AJM}=107$, $N_{GSF}=26$, $N_{GSM}=17$), and scores obtained from the first of the canonical correspondence analysis (**C** and **D**; $N_{AFF}=5$, $N_{AFM}=2$, $N_{AJF}=36$, $N_{AJM}=33$, $N_{GSF}=13$, $N_{GSM}=6$). Different letters indicate significant differences ($p < 0.05$) obtained by Kruskal-Wallis and multiple comparison post hoc tests. * indicates a significant pair-wise difference for the corresponding group.

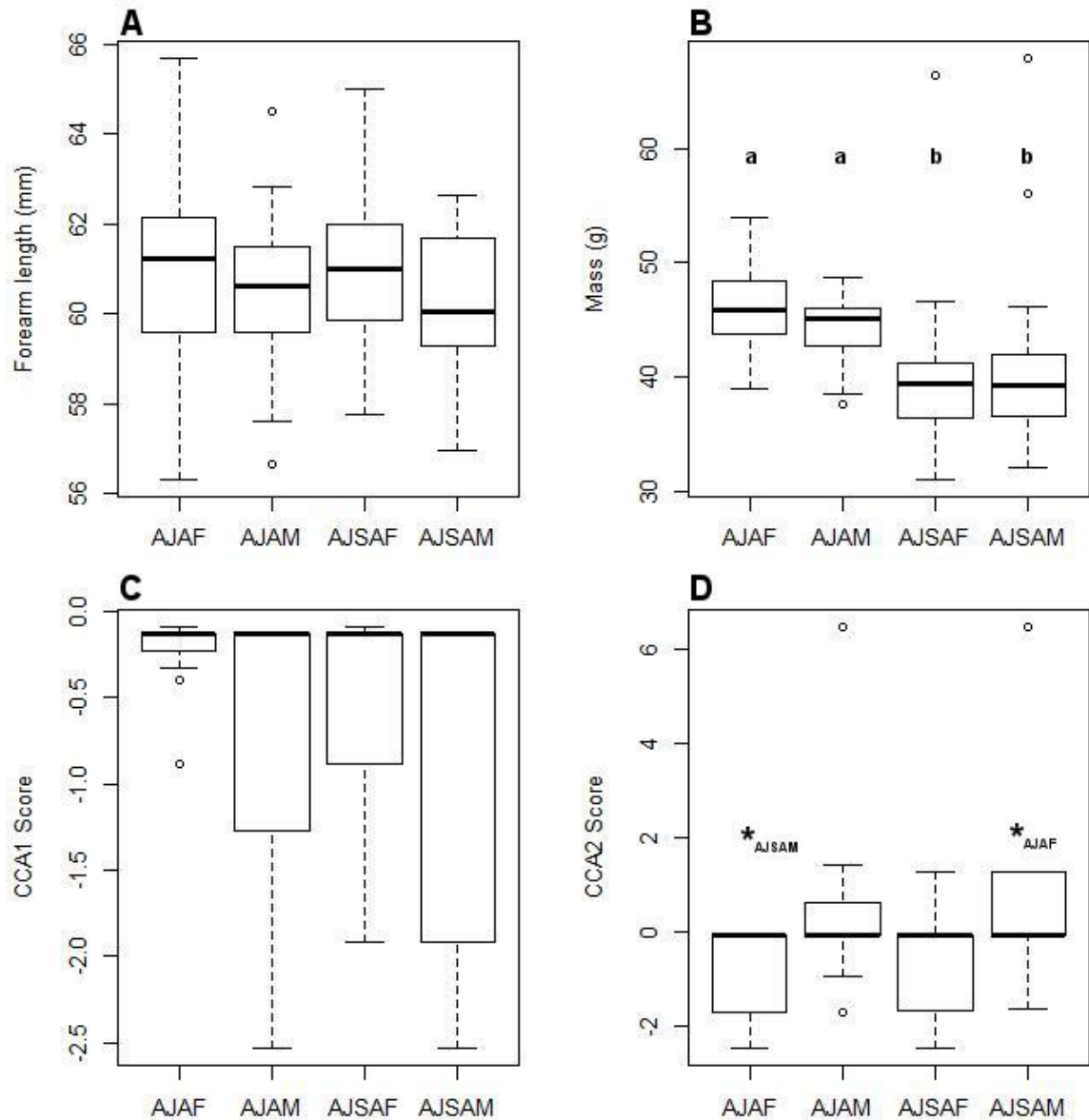


Figure 9. Comparisons across age and sex groups of *Artibeus jamaicensis* (AJAF - adult females, AJAM - adult males, AJSAF - sub-adult females, and AJSAM - sub-adult males). Forearm length (**A**; $N_{AF}=51$, $N_{AM}=55$, $N_{SAF}=47$, $N_{SAM}=50$), mass (**B**; $N_{AF}=52$, $N_{AM}=55$, $N_{SAF}=48$, $N_{SAM}=52$), and scores obtained from first two axes of the canonical correspondence analysis (**C** and **D**; $N_{AF}=16$, $N_{AM}=16$, $N_{SAF}=19$, $N_{SAM}=17$). Different letters indicate significant differences ($p<0.05$) obtained by Kruskal-Wallis and multiple comparison post hoc tests.* indicates a significant pair-wise differences for the corresponding group.

Chapter 4 - Discussion

4.1 Dietary resource identification

DNA barcoding increased the resolution of dietary plant identification. Using only morphological identification methods would have resulted in a low resolution dietary reconstruction. Pulp samples (those without seeds or identifiable structures) are frequently overlooked or ignored in dietary studies of vertebrates (e.g., Alves-Costa & Eterovick 2007, Corbett 1989) and samples of this type are rarely reported. Exclusion of pulp samples is common in dietary studies of fruitbats (e.g., Charles-Dominique 1991, García-Morales et al. 2012, Marinho-Filho 1991, Mello et al. 2008, Palmeirim et al. 1989, Teixeira et al. 2009). In some cases it is possible to identify pulp samples because they often resemble the fruit consumed but the sample must be fresh and certainty is low (Fleming 1988). DNA barcoding provides a solution to the loss of data due to the limitations of traditional methods.

In the present study, the exclusion of pulp samples would represent a reduction of 18% in sample identification. Frequently, focus is given to small seeds retained in the faeces because seed dispersal is easily studied. However, identification of pulp samples is imperative because bats also consume large seeded fruits which they transport away from parent plants (Howe 1986, Melo et al. 2009). These relatively large seeds are too large to pass through the alimentary tract of the bats, and may not be collected at mist nets, but their pulp may be available in faeces. Because bats are important dispersers (e.g., Albuquerque et al. 2006, Lobo & Mori 2003) the identification of all dietary fruit taxa is integral to investigations of dispersal. The use of DNA barcoding increased the resolution of identification which has a great effect on dietary reconstructions.

Recently, alternative methods have emerged to address the lower resolution of traditional methods. Bumrungsri et al. (2007) and Long and Racey (2007) used methods such as odour detection and chemical analyses to identify fruit pulp in fruitbat faeces successfully. However, these methods have a limited breadth and are not widely applicable. For example, Bumrungsri et al. (2007) identified *Acronychia peduncula* using its minty odour but could not distinguish other plant species based on odour. Similarly,

observational colour changes in faecal pulp when subjected to NaOH allowed Bumrungsri et al. (2007) to identify a *Diospyros* sp. Without extensive sampling of the available fruit taxa it is impossible to conclude that only the *Diospyros* sp. reacts in that manner. Newmaster et al. (2013) compared traditional methods to DNA barcoding of faeces from woodland caribou (*Rangifer tarandus caribou*) and found molecular identification to be superior; traditional methods provided no resolution at the species level, video recordings identified 42 species and DNA barcoding identified 67 species. Although DNA barcoding is a recently developed methodology, Yoccoz et al. (2012) found that molecular methods used on soil samples were accurate to 85% of the species determined by traditional methods in boreal and tropical vascular plant communities. Molecular identification methods provide a robust means of dietary identification which overcome the shortcomings of alternative methods.

Although effective, DNA barcoding has limitations. Of primary concern, the specific gene region selected for barcoding is controversial because different gene regions provide different taxonomic distinctions (Moritz & Cicero 2004, Newmaster et al. 2007, Cräutlein et al. 2011). Newmaster et al. (2007) found that a combination of gene regions provided the best resolution in taxonomic identification as individual gene regions could not consistently discern plant species. Cräutlein et al. (2011) also demonstrated various successes and failures between the most common plant barcoding gene regions: *rbcL*, *matK*, *trnH-psbA* and ITS when used individually, indicating that a single standardized barcode region is unrealistic. In the present study, *rbcL* was sufficient for identification to the level of species where taxa were monotypic genera in Jamaica, but otherwise identification was made to level of genus and not beyond. This is likely compounded by a major caveat of molecular identification; it is contingent upon a library of relevant sequences acquired from a wide range of species (Frézal & Leblois 2008, Hajibabaei et al. 2005, Hajibabaei et al. 2007, Razgour et al. 2011). Moritz and Cicero (2004) indicated that any species being barcoded requires sister species to be present in the analysis; full exploration of genera is imperative to properly assign individuals to species. This is evident in dietary constituents of the fruitbats where six of the fruits could only be identified to genus and two samples only to family (Melastomataceae). Without proper construction of a comprehensive sequence library, species identification is difficult

regardless of the number of gene regions included. A combination of traditional methods and DNA barcoding can alleviate impediments of either method used alone.

DNA barcoding and successive molecular analyses show promise for future studies. As a precursor, DNA barcoding can indicate that further taxonomic inspection is required for plant identification but remains inexpensive and rapid (Hajibabaei et al. 2007, Newmaster et al. 2006, Newmaster et al. 2007). When paired with emerging technologies such as next-generation sequencing, DNA barcoding can allow for increased scale of analyses and more detailed inspection of consumer-resource interactions in food webs (Valentini 2008, Razgour et al. 2011). Beyond identification, molecular methods provide numerous applications for a more detailed understanding of bat ecology. Godoy and Jordano (2001) demonstrated the value of paternity analysis to identify seed sources. An expansion of this application to seeds found in bat faeces could provide insight for dispersal, competition, landscape and resource use, among many other areas of study. In the present study, DNA barcoding allowed greater resolution into the identification of diet and subsequent dietary comparisons while also providing new avenues for future analyses.

4.2 Dietary comparison and the influence of factors

The bats exhibited high dietary overlap despite morphological differences. *Cecropia peltata* was a common resource for all three species of fruitbats (>58% of total diet) and *B. alicastrum* was shared by *A. jamaicensis* and *A. flavescens*. Contrary to my second prediction, the diets maintained by each species were not largely exclusive. Genoways et al. (2005) suggested that *A. flavescens* and *A. jamaicensis* maintain completely exclusive diets due to distinct alimentary tract differences. Differences in body size can also lead to dietary resource partitioning (Birks & Dunstone 1985, Pratt & Stiles 1985, Muller & Reis 1993, Andreas et al. 2012). Tamsitt (1967) observed a comparable size comparison between *Phyllostomus discolor* and *Phyllostomus hastatus* (24.19 mm difference in forearm length) and found little dietary overlap. Fleming (1991) found that even small differences of 8.2 g in mass and 6.9 mm in forearm length are sufficient to separate dietary proportions in Costa Rican *Carollia* spp. I observed a considerable difference in size (25 g in mass and 20 mm in forearm length) between *A. jamaicensis* and the smaller bats, *A. flavescens* and *G. soricina* but little overall dietary separation. High levels of

dietary overlap are more frequently observed in morphologically similar and closely related species (Palmeirim et al. 1989, Barclay & Brigham 1991, Razgour et al. 2011). Despite the differences observed in many fruitbat communities, morphological differences appear not to differentiate the Jamaican bats' diets.

Morphology did not completely distinguish the diets of each fruitbat species. However, *A. jamaicensis* consumed exclusive fruit taxa that may be explained by its morphology relative to the other two bat species. *Artibeus jamaicensis*, the largest species, showed a broad diet (nine fruit taxa) relative to *A. flavescens* (two fruit taxa) and *G. soricina* (three fruit taxa) supporting my third prediction that the larger species would have greater dietary species richness. Greater bite forces (Aguirre et al. 2002, Dumont 1999, 2004, Herrel 2008), commuting distances (Fenton 1997), and the ability to carry more weight (Bonaccorso 1979) are consequences of greater body size and may contribute to the relatively higher species richness observed in the diet of *A. jamaicensis*. Unripe *Terminalia catappa* (one of three potential *Terminalia* spp. consumed by *A. jamaicensis*) and *Ficus* spp. fruit are relatively hard (pers. obs.; August 1981) and may have been only exploitable by high bite force species, or in this case the larger fruit bat *A. jamaicensis*. *Artibeus jamaicensis* can also travel 1-4 km (Handley et al. 1991) or upwards of ca. 8 km (Morrison 1978a) between day roosts and foraging sites allowing greater access to more widely spaced food patches. *Artibeus flavescens* appears not to travel great distances, at least between day roosts (Appendix 4). Fruitbats also demonstrate feeding behaviours that involve transportation of the fruit to a feeding roost away from the fruit tree to a feeding roost believed to be safe from predators (Morrison 1978b). Larger fruit bats can move heavier fruit species to new locations (Howe 1986, Melo et al. 2009) allowing exploitation of both small fruit and large fruit. Smaller fruit bats are unable to carry such a wide range of fruit and are limited to carrying only small fruit. Large body size allows frugivores to exploit more resources as demonstrated by *A. jamaicensis* in Jamaica.

Glossophaga soricina also consumed fruit taxa that the other bat species did not but had a narrower fruit niche breadth. This supports my third prediction, that nectarivorous species will maintain a narrow fruit diet. It is surprising that *G. soricina* was the only species to consume *Piper* spp. Fleming (1988, 1991) suggested that *Piper* spp. represent highly

nutritional resources that are ideal for bats. *Piper* spp. are typically shrubs and occur in the undergrowth or cluttered areas (Adams 1972, Gartner 1989). Fleming (1991) found that the proportion of *Piper* spp. in the diets of *Carollia* spp. was negatively correlated with forearm length. Large body size lowers maneuverability (Bonaccorso et al. 2007), and inversely, small body size leads to increased maneuverability (Norberg & Rayner 1987). *Artibeus jamaicensis* are known to forage in the canopy (Clarke et al. 2005) which is also evident by the large proportion of tree fruit I observed in their diet. This may be why I did not observe *Piper* spp. in the diet of *A. jamaicensis* and observed it in the diet of the smaller, *G. soricina*.

Morphological specializations also allow nectarivores, and more specifically *G. soricina*, to exploit nectar and pollen as food resources while simultaneously precluding hard fruit from their diet (Forman et al. 1979, Harper et al. 2013, Winter & von Helversen 2003). Competition with the remaining three nectarivorous bats of Jamaica may have led to increased exploitation of fruit resources such as *Piper* spp.. However, *G. soricina* appear to be more omnivorous, consuming insects as well (Clare et al. In Press, Herrera et al. 2001). As small bats, *G. soricina* also maintain relatively short commutes, employing resource defense and trap-line foraging along routinely travelled routes in smaller areas (Lemke 1984). Maintaining the ability to exploit several resources may reduce competition between similar species but each resource will have narrow breadths as specialization for nectarivory precludes a wide fruit and insect diet in terms of size and morphology.

Ariteus flavescens did not consume any fruit taxa that the other bat species did not. The diet of *A. flavescens* was completely overlapped by the diet of *A. jamaicensis*. *Ariteus flavescens* maintained a narrow niche breadth. This supports my third prediction, that larger bats will have greater dietary species richness. However, the small sample size for *A. flavescens* precludes detailed conclusions. After the rarefaction analysis (i.e. reduction of sample size), I continued to observe this pattern suggesting that larger bats indeed have broader diets. By consuming *C. peltata* and *B. alicastrum*, both locally native plant species (Adams 1972), *A. flavescens* consumed locally native fruit species. Consumption of locally native fruit is widespread in bat species (e.g., Fleming & Williams 1990, Lopez

& Vaughan 2007, Muscarella & Fleming 2007). I observed instances where *A. jamaicensis* consumed *T. catappa* and *C. elastica*, both introduced tree species (Adams 1972). This is not surprising as fruitbats also adjust their diet to consume introduced fruit species (e.g., Bumrungsri et al. 2007, Long & Racey 2007). I did not detect *Glossophaga soricina* consuming any introduced species but *Piper* spp. and the Melastomataceae are likely locally native. Aside from the presence of exotics in the diet of *A. jamaicensis*, small samples size in *A. flavescens* makes it difficult to assess the absence of introduced species. Although *A. flavescens* consumed locally native fruit species, I was unable observe higher proportions than that of the other two species.

Although some fruitbat communities demonstrate dietary separation, dietary similarity between morphologically distinct species occurs. Lopez and Vaughan (2007) observed a similar level of overlap between *Artibeus jamaicensis* and *Vampyressa nymphae*, both of which consumed *Cecropia* spp. This species pair comparison is similar to the comparison between *A. jamaicensis* and *A. flavescens* (ca. 35 g and 20 mm forearm length difference; Giannini & Kalko 2004, Kalko et al. 1996). Willig et al. (1993) also observed dietary differences between neotropical fruitbats but they found no statistical support for size as a mechanism for the separation. Recent analyses indicate that dietary overlap is also high for animalivorous (i.e. a diet of animals such as fish, amphibians, insects, etc.) bat feeding-guilds (Schoeman & Jacobs 2011, Razgour et al. 2011) Similar levels of overlap exist in other taxa despite body size (e.g., reptiles: Sutherland, 2011). Dietary similarities are possible for morphologically distinct species and body size does not preclude resource similarities between consumer species.

The identification of exclusive and rare dietary constituents is important for an evaluation of community structure. Heinrich (1979) discovered that bumble bees visiting plants select "major" flowers but when abundances are reduced they shift their selection to "minor" flowers. Although major plant species represent an important dietary portion, "minor" or rare species are integral in the face of competition or reduced abundances. Nagelkerken et al. (2009) suggested that dietary partitioning of minor prey items is important in reducing competition and facilitating coexistence of coral reef fish. Razgour et al. (2011) and Schoeman and Jacobs (2011) also indicated the importance of

partitioning minor prey items in the community structure of insectivorous bats. Fleming (1986) suggested that bat-plant interactions are key to the structure of bat communities. Exploitation of alternative or minor fruit resources in the face of competition or reduced abundance may be an important factor in the structure and stability of the Jamaican fruitbat guild as well as other frugivorous communities.

The CCA confirmed the dietary overlap and distinction observed for each species and revealed that age, sex, and reproductive status are important factors that influence diet. Fleming (1988) observed different foraging behavior between males and females of *Carollia perspicillata* and subsequently a difference in diet. Ontogenetic shifts occur in bats (Adams 1996) and appear to influence dietary selection in Jamaican fruit bats. Reproductive status was also significant. Most lactating *A. jamaicensis* females deviated from consumption of *C. peltata* to fruit species including *Ficus* spp., *Solanum* spp., and *Terminalia* spp. Nelson et al. (2005) observed that lactating females appear to seek calcium and readily exploited calcium blocks more frequently than non-lactating females and males. *Ficus* spp. are known to have high concentrations of calcium (Bravo et al. 2012) which may account for the dietary shift. It appears that Jamaican fruitbat diets are partially determined by sex and age.

The CCA also indicated abdominal distension, visibility of male testes, and year of capture as significant but these "factors" are likely artifacts of the analysis. Abdominal distension was an approximation of stomach contents to adjust mass values to reflect water and meals. Male testes migrate based on ambient and body temperature (Jolly & Blackshaw 1988). Although often used (e.g., Zortéa 2003), visibility of male testes without detailed inspection does not provide an accurate estimation of reproductive status. Because netting was not standardized, it was difficult to detect differences between years. Netting was focused in Windsor in 2011 and Coxheath in 2012 this likely reflects a small difference in location. However, the CCA did not indicate capture location as significant.

Sample sizes were a limitation for this analysis. Not all sample sizes were equal and *A. flavescens* had low representation ($N = 7$). I observed that *A. flavescens* is a relatively uncommon species (0.02 bats per mist net hour, Table 3). Bats are difficult to catch and often demonstrate learned avoidance of capture techniques (Kunz & Brock 1975). The

number of *A. flavescens* I caught might be the most realistic outcome in terms of species rarity and capture success. Further investigation is required to assess the status of the population. Recent dietary studies of insectivorous bats used sample sizes of ca. 30 to determine diet (Razgour et al. 2011). Other studies had samples sizes for species that range from eight to 130 (Willig et al. 1993). Dietary studies of frugivorous bats also had limited sample sizes ranging from three to 267 samples (Lopez & Vaughan, 2007). Low sample sizes are a common problem for dietary investigations of bats.

Despite the low *A. flavescens* sample size, I was able to address my predictions with these analyses. The diets of all three species overlapped indicating that the bats do not partition resources based only on morphology. I detected that *A. jamaicensis* had the greatest dietary richness and through rarefaction, can confidently suggest that *A. jamaicensis* still has the broadest diet even at increased sample sizes. I observed that *G. soricina* has a relatively narrow fruit diet. What I was unable to investigate was whether *A. flavescens* consumes locally native species in greater proportions than the other two bat species. Although I observed the bats consuming native species, I detected the same species in the diets of the other bat species. Only extensive sampling can provide further insights into this question.

4.3 Island community vs. continental community

When compared to adjacent mainland areas such as Costa Rica and Panama, the fruit bat community of Jamaica exhibits differences. Most notably, Jamaica lacks the fruitbat community (Fleming 1993, Genoways et al. 2005, Lopez & Vaughan 2007) and vascular plant diversity (Davis et al. 1997) seen in continental areas. This reduction provides room for differing trophic interactions. Continental *A. jamaicensis* consumed *Piper* spp. (Lopez & Vaughan 2007, García-Morales et al. 2012), albeit rarely, whereas on Jamaica there is no evidence of *Piper* spp. in their diet. *Glossophaga soricina* exploited *Piper* spp. on Jamaica where on the mainland *Piper* spp. specialists such as *Carollia* spp. appear to dominate that resource (Giannini & Kalko 2004). Lopez and Vaughan (2007) also found that *A. jamaicensis* maintain broader niches on the mainland than I observed in Jamaica. In the absence of a complex community it appears that Jamaican fruit bats had the opportunity to expand their niches into otherwise occupied niches on the continent.

Ariteus flavescens is an endemic species and may augment the organization of the Jamaican community through competition for resources. However, it is more likely that fruit resources on Jamaica are abundant and non-limiting given the high levels of dietary overlap. Reduced bat consumer diversity appears to structure Jamaican fruitbat diets differently from continental conspecifics.

4.4 *Cecropia peltata*, a major resource

All three bat species ate *C. peltata* despite differences in morphology. It composed the majority of each species diet. *Cecropia* spp. are often consumed by bats and birds and dispersal appears to be highly influenced by these taxa (Fleming & Williams 1990, Lobova & Mori 2003, Staudacher et al. 2011). Bats frequently disperse pioneer species' seeds into forest gaps and have an important role in regeneration of degraded forest (Galindo-González et al. 2008, Medellín & Gaona 1999, Muscarella & Fleming 2007, Silveira et al. 2011). The proportion of seeds that successfully germinate is also increased when consumed and excreted by bats (Fleming & Heithaus 1981, Fleming 1988, Olson & Blum 1968). The Windsor and Coxheath areas have degraded forest and open patches ideal for pioneer species. *Cecropia peltata* appears to benefit from both ease of dispersal into suitable habitat and increased rates of germination. Overall, *C. peltata* benefits immensely from this consumer-resource relationship. However, *C. peltata* fruits are nutritionally poor compared to other available fruits (Fleming & Williams 1990, Herbst 1986). This raises the question, why do Jamaican bats prefer *C. peltata* over alternative fruits?

The relatively reduced vascular floral diversity of Jamaica may provide an answer in that there may not be as many suitable fruit options. However this is unlikely as *A. jamaicensis* consumed a similar number of fruit taxa in Costa Rica as observed in Jamaica (Lopez & Vaughan 2007). Despite *C. peltata* having relatively less nutritional value, Fleming (1988) noted that ca. eight *C. peltata* fruits are sufficient for a night and that if short foraging times are favorable, *C. peltata* may in fact be the best fruit resource option. Furthermore, *C. peltata* is also abundant as is it a common pioneer species found in recently disturbed habitats and fruits sporadically all year providing a predictable food source (Adams 1972). Bats prefer predictable food sources (Fleming 1988), which further

supports selection for *C. peltata*. Despite limited fruit diversity it appears that the high abundance and predictability of *C. peltata* makes it a dietary staple for Jamaican fruitbats.

Coupled with its apparent dietary importance, *C. peltata* also provides medicinal effects. *Cecropia peltata* extracts have hypoglycemic effects (Andrade-Cetto & Vázquez 2010, Andrade-cetto 2007). This is observed in another species *Terminalia catappa*, also found in the Windsor and Coxheath areas (Nagappa et al. 2003). Fruit diets are typically low in nitrogen and high in energy. Often in the attempt to acquire sufficient amounts of protein high excesses of sugars are acquired (Thomas 1984). In the case of *G. soricina*, high metabolisms and flight quickly use sugars (Welch et al. 2008). However, Delorme and Thomas (1996, 1999) found that fruitbats require very little dietary nitrogen. The high levels of *C. peltata* consumed by the fruitbats suggest that the bats may be affected by the hypoglycemic effect. The potential medicinal effects of fruits consumed by bats warrant more detailed investigations.

Chapter 5 - Summary

I approached this research with two major questions. First, can DNA barcoding increase the resolution of dietary studies and second, how do the diets of three frugivorous bats compare given distinct morphological differences?

5.1 Molecular identification

I found that DNA barcoding increased the resolution of the present dietary investigation and prevented the loss of seven otherwise undetectable fruit taxa. My work provides support for the wider application of DNA barcoding to frugivorous diet studies and beyond to herbivorous studies. Although there are limitations, molecular methods expanded my ability to identify pulp and provide an avenue for future research. Furthermore, DNA barcoding allows insight into previously unknown minor resources that have great effects on community structure. With the global effort to sequence all known taxa and create a comprehensive library, DNA barcoding and subsequent molecular methods will improve the scope of dietary investigations.

5.2 Dietary comparison

I observed both dietary differences and high levels of overlap between the three bat species. Despite differences in morphology each species centralized on *Cecropia peltata* in the greatest proportion of their diet. This is contrary to my prediction that the morphologically distinct Jamaican fruitbats would maintain largely exclusive diets. Beyond this overlap, *A. jamaicensis* maintained the broadest diet and *A. flavescens* and *G. soricina* maintained relatively narrow fruit diets supporting my predictions that morphology influences dietary breadth. I identified minor fruit resources for *A. jamaicensis* which may provide alternative food sources and reduce competition during low resource abundances. Reproductive status and age also appear to influence the diets of these fruit bats and warrant further research into potential ontogenetic diet shifts and reproductive preparation.

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Appendices

Appendix 1 - Study location maps



Figure 1. Approximate area of Cockpit country, Jamaica. Windsor and Coxheath are contained within.

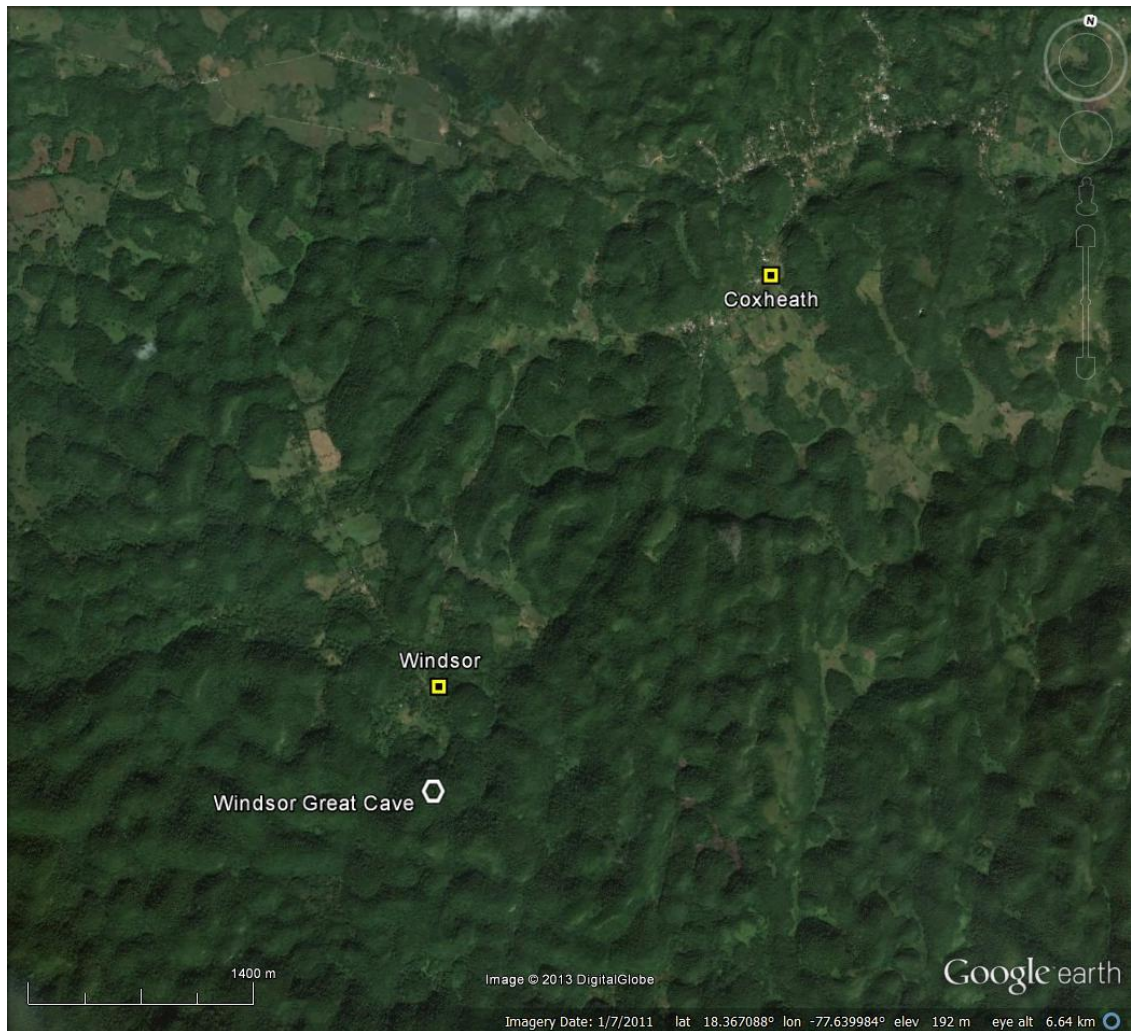


Figure 2. Locations of Coxheath and Windsor relative to each other and the upper entrance of the Windsor Great Cave.

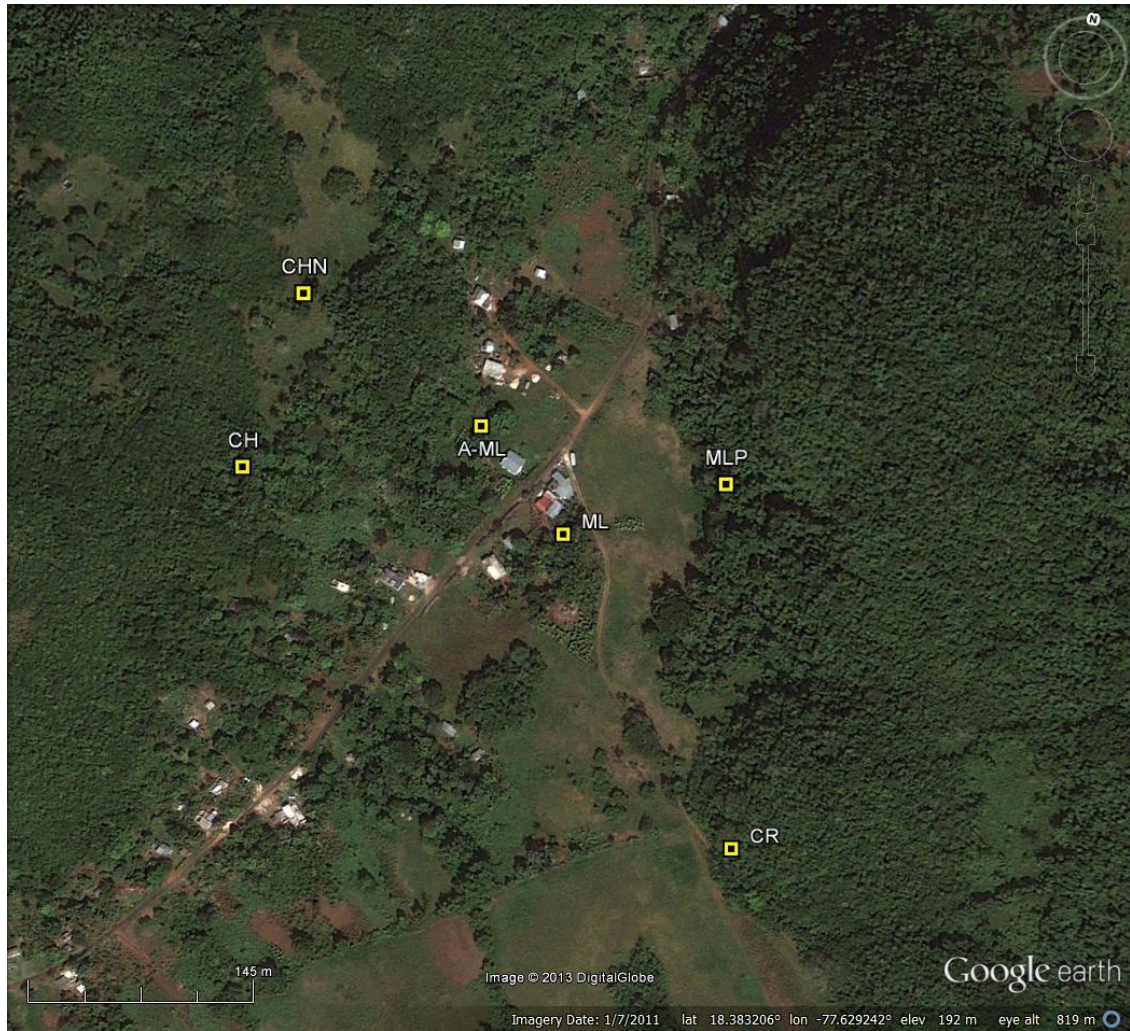


Figure 3. Mist net locations in the Coxheath area. Netting sites include Coxheath hill (CH), Coxheath hill naseberry (CHN), across from Miss Lillie's (A-ML), Miss Lillie's (ML), Miss Lillie's pasture (MLP) and Coxheath road (CR).

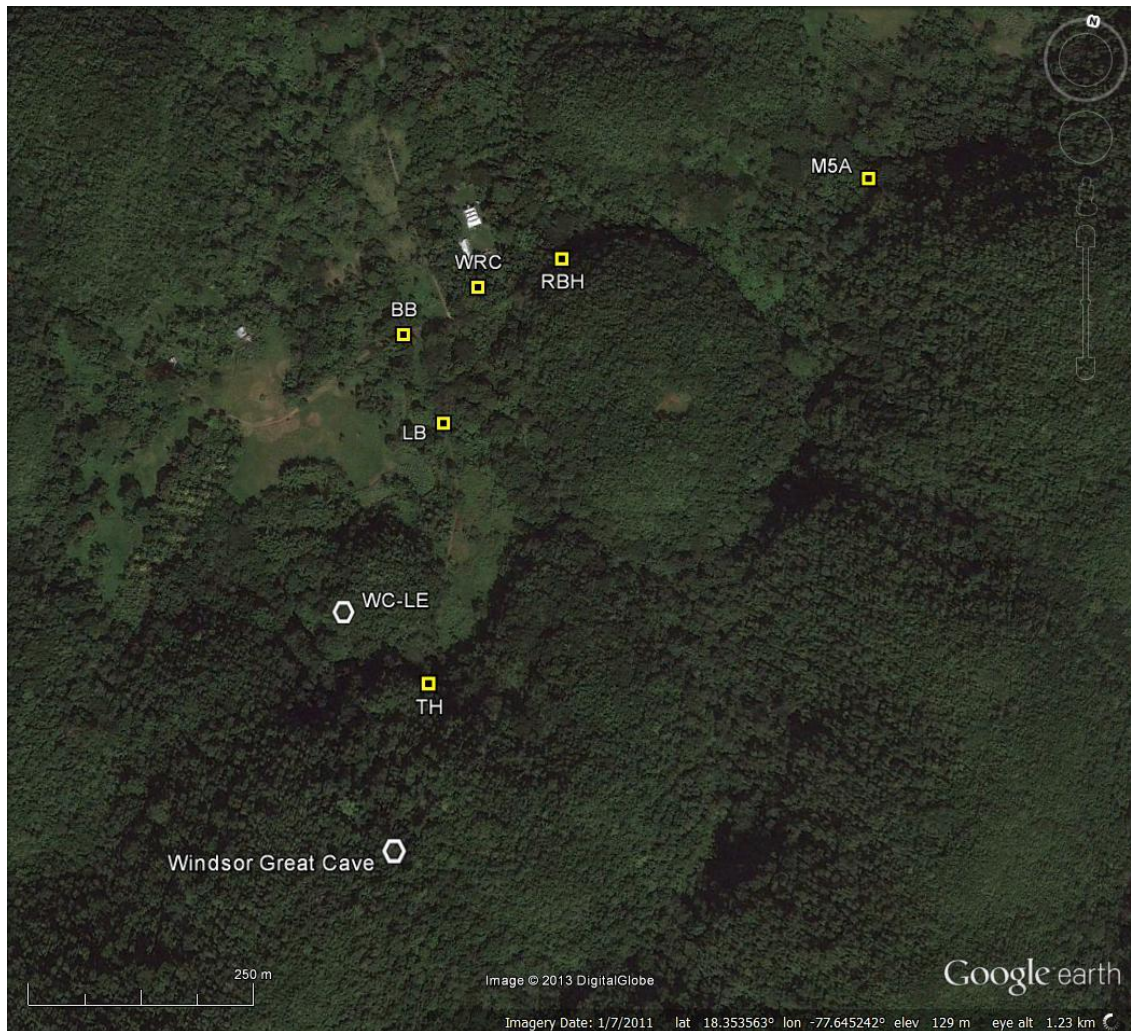


Figure 4. Mist net locations in the Windsor area relative to the upper entrance of the Windsor Great Cave. Netting sites include Mike's five acres (M5A), road by house (RBH), Windsor Research Centre (WRC), big bridge (BB), little bridge (LB) and the trailhead (TH).

Appendix 2 - Detailed molecular methods

Specimen handling and contamination control

Forceps were flame-sterilized between the handling of individual samples and plastic weigh-boats were used to contain samples and were discarded after each use to minimize the risk of cross-sample contamination. Additionally, blanks were included during each extraction, amplification, and sequencing procedure to monitor for contamination.

Custom DNA extraction protocol

Solutions and materials in order of use:

Lysis: 2% CTAB w/v, 20 mM EDTA pH 8.0, 100 mM Tris-HCl pH 8.0, 1.5 M NaCl, 2% w/v PVP-40 (mw=40,000), 1% beta-mercaptoethanol, and 0.5 mg of RNase/sample. pH of buffer should be 8-8.4. (Adapted from Doyle and Doyle 1987)

Chloroform: Isoamyl alcohol: 24:1, Sigma-Aldrich cat#: C0549-1PT

Binding Buffer: 5 M Guanidine thiocyanate (Sigma-Aldrich, cat#: G9277), 3% triton-x v/v, 300 mM sodium acetate (Sigma Aldrich cat#: S7899). pH of buffer should be 5-5.2. (Adapted from Rohland et al. 2010).

Magnetic Silica Suspension: MagAttract Suspension G (Qiagen, City)

Wash buffer I: 2.5 M Guanidine thiocyanate (Sigma-Aldrich, cat#: G9277), 50% v/v ethanol, 25 mM Tris-HCl pH 8.0, 12.5 mM NaCl, 1% Triton-x v/v. (Adapted from Ivanova et al. 2008)

Wash buffer II: 80% ethanol, 1 mM EDTA pH 8.0, 10 mM Tris-HCl pH 8.0. (Kuch and Poinar 2012).

Elution Buffer: 0.1 mM EDTA pH 8.0, 1 mM Tris-HCl pH 8.0, 0.05% tween-20 v/v. pH of this solution should be around 8. (Kuch and Poinar 2012)

Caution: Steps 1-4 of the protocol should be performed in a fume hood to minimize exposure to chloroform-isoamylalcohol and beta-mercaptoethanol. Additional care should be taken in the handling and disposal of solutions containing guanidine thiocyanate, beta-mercaptoethanol, and chloroform-isoamyl alcohol.

Stepwise custom DNA extraction protocol:

1. Place 10-25 mg of scat sample in a 1.0 mL strip tube containing a stainless steel bead (Product#-Montreal Biotech), place in a Styrofoam cooler containing liquid Nitrogen and incubate for 1-2 minutes.
2. Remove from liquid nitrogen and mechanically disrupt samples with a TissueLyser II or another appropriate instrument.
3. Allow tubes to warm to near room temperature and add **900 μ L of lysis buffer** and 0.5 mg of RNase per sample.
4. Incubate for 1-2 hours at 56-60°C with moderate magnetic agitation (if scat is resistant to homogenization you can use a powerful magnet, such as a small neodymium magnet, to agitate the steel bead within the microcentrifuge tube to further break up the sample).
5. Add the lysate to a new 2.0 mL microcentrifuge tube containing **900 μ L of chloroform-isoamyl alcohol** (24:1), re-cap, mix several times (10-20) by inversion and spin for 5 min at 17,000 x g to separate the phases.
6. Carefully remove about 800 μ L of the aqueous phase (top phase) by pipetting while being careful not to disturb the protein-rich interphase and place into a new tube containing **400 μ L of binding buffer** and **12 μ L of magnetic silica suspension**.
7. Gently vortex mixture to suspend silica particles and incubate for 1 hour with gentle agitation at room temperature protected from strong light.
8. Use a neodymium magnet to pellet the silica on the side of the tube and remove as much lysate-binding buffer mixture as possible by pipetting being careful to not disturb the silica pellet. Note: the pelleting of the magnetic silica particles generally happens in 2-5 seconds but may take longer with weaker magnets and more viscous lysates.
9. Add **500 μ L of wash buffer I**, re-suspend the silica pellet by pipetting and pellet on the side of the tube by magnetization.
10. Remove as much buffer 1 as possible by pipeting and then add **500 μ L of wash buffer II and re-suspend**.
11. Pellet by magnetization, remove buffer II and add another **500 μ L of wash buffer**

II.

12. Remove as much of the buffer as possible by pipetting and place tube in a 56°C dry bath for about 5-10 minutes to evaporate residual ethanol.
13. Add **50-120 µL of elution buffer** to the dry silica pellet and incubate at 56°C with agitation for 20 minutes.
14. Pellet the silica particles with a magnet and transfer the eluate to a sterile microcentrifuge tube by pipetting, try not to take any silica particles.
15. Allow DNA eluate to completely freeze and thaw once as this may precipitate some PCR inhibiting substances (Kuch and Poinar 2012).

Notes: The above protocol may be scaled up or down to accommodate smaller or larger fecal samples. We do not recommend exceeding 25mg of fecal material/~900 µL of lysis buffer as this may overwhelm the buffering capacity of the lysis solution. The lysis solution may need to be pre-warmed to 60°C to fully dissolve CTAB. We found that a 1-2 small neodymium magnets (disks 3 mm thick, 8 mm diameter) to be sufficiently strong for pelleting silica particles in microcentrifuge tubes.

Appendix 3 - BOLD and GenBank accession numbers

Table 1. Barcode of Life Database (BOLD) sample ID's, Genbank accession numbers, bat and plant species of 108 fecal samples collected by Colin Hayward at the Windsor Research Centre and Coxheath, Jamaica in the years 2011 and 2012. Additional collection information is available on the Barcode of Life Database (www.boldsystems.org). Bat species are *Ariteus flavescens* (*A. flav*), *Artibeus jamaicensis* (*A. jam*) and *Glossophaga soricina* (*G. sor*).

Bat Species	Sample ID	Field ID	rbcl seq ref	rbcl genbank #	Collection Date	rbcl Identification	rbcl Seq. Length
<i>A. flav</i>	AF-2_161011_V35	AF_161011_06-01	BSCAT001-13.rbcl	KF270096	16-Oct-2011	<i>Brosimum alicastrum</i>	625[0n]
<i>A. jam</i>	AJ-13_250911_V101	AJ_250911_03-03	BSCAT002-13.rbcl	KF270097	25-Sep-2011	<i>Brosimum alicastrum</i>	626[0n]
<i>A. jam</i>	AJ-15_260911_V128	AJ_260911_01-01	BSCAT003-13.rbcl	KF270098	26-Sep-2011	<i>Brosimum alicastrum</i>	598[0n]
<i>A. flav</i>	AF-440_290911_VD-RA	AF_290911_0440	BSCAT004-13.rbcl	KF270099	29-Sep-2011	<i>Brosimum alicastrum</i>	611[0n]
<i>A. jam</i>	AJ-3_090911_V124	AJ_090911_01-03	BSCAT005-13.rbcl	KF270106	09-Sep-2011	<i>Castilla elastica</i>	604[0n]
<i>A. jam</i>	AJ-33_150512_V119	AJ_150512_ML001	BSCAT006-13.rbcl	KF270107	15-May-2012	<i>Castilla elastica</i>	601[0n]
<i>A. jam</i>	AJ-34_170512_V44	AJ_170512_MLR001	BSCAT007-13.rbcl	KF270108	17-May-2012	<i>Castilla elastica</i>	628[0n]
<i>A. jam</i>	AJ-45_210512_VI	AJ_210512_CH005	BSCAT008-13.rbcl	KF270100	21-May-2012	<i>Castilla elastica</i>	611[0n]
<i>A. jam</i>	AJ-49_220512_V120	AJ_220512_CH003	BSCAT009-13.rbcl	KF270101	22-May-2012	<i>Castilla elastica</i>	602[0n]
<i>A. jam</i>	AJ-53_240512_V127	AJ_240512_LB002	BSCAT010-13.rbcl	KF270102	24-May-2012	<i>Castilla elastica</i>	599[0n]
<i>A. jam</i>	AJ-56_240512_V118	AJ_240512_LB006	BSCAT011-13.rbcl	KF270103	24-May-2012	<i>Castilla elastica</i>	597[0n]
<i>A. jam</i>	AJ-57_240512_VB	AJ_240512_LB008	BSCAT012-13.rbcl	KF270105	24-May-2012	<i>Castilla elastica</i>	611[0n]
<i>A. jam</i>	AJ-39_200512_V9	AJ_200512_ML008	BSCAT013-13.rbcl	KF270104	20-May-2012	<i>Castilla elastica</i>	623[0n]
<i>A. flav</i>	AF-3_181011_V37	AF_181011_01-04	BSCAT014-13.rbcl	KF270109	18-Oct-2011	<i>Cecropia peltata</i>	624[1n]

Bat Species	Sample ID	Field ID	rbcl seq ref	rbcl genbank #	Collection Date	rbcl Identification	rbcl Seq. Length
<i>A. flav</i>	AF-4_210512_V34	AF_210512_CH004	BSCAT015-13.rbcl	KF270144	21-May-2012	<i>Cecropia peltata</i>	626[0n]
<i>A. flav</i>	AF-1_240911_V33	AF_240911_01-01	BSCAT016-13.rbcl	KF270143	24-Sep-2011	<i>Cecropia peltata</i>	625[0n]
<i>A. flav</i>	AF-7_290512_V32	AF_290512_CHN001	BSCAT017-13.rbcl	KF270142	29-May-2012	<i>Cecropia peltata</i>	625[0n]
<i>A. flav</i>	AF-5_220512_VQ	AF_220512_FT-7-2	BSCAT018-13.rbcl	KF270141	22-May-2012	<i>Cecropia peltata</i>	609[2n]
<i>A. flav</i>	AF-5_220512_VV	AF_220512_FT7-2	BSCAT019-13.rbcl	KF270140	22-May-2012	<i>Cecropia peltata</i>	610[1n]
<i>A. flav</i>	AF-6_270512_VW	AF_270512_FT8-1	BSCAT020-13.rbcl	KF270139	27-May-2012	<i>Cecropia peltata</i>	592[0n]
<i>A. flav</i>	AF-7_300512_V38	AF_300512_FT9-2	BSCAT021-13.rbcl	KF270138	30-May-2012	<i>Cecropia peltata</i>	627[0n]
<i>A. jam</i>	AJ-1_90911_V1	AJ_90911_01-01	BSCAT022-13.rbcl	KF270137	09-Sep-2011	<i>Cecropia peltata</i>	623[0n]
<i>A. jam</i>	AJ-2_90911_V2	AJ_90911_01-02	BSCAT023-13.rbcl	KF270136	09-Sep-2011	<i>Cecropia peltata</i>	629[0n]
<i>A. jam</i>	AJ-8_130911_V3	AJ_130911_03-06	BSCAT024-13.rbcl	KF270135	13-Sep-2011	<i>Cecropia peltata</i>	630[0n]
<i>A. jam</i>	AJ-4_090911_V85	AJ_090911_01-04	BSCAT025-13.rbcl	KF270134	09-Sep-2011	<i>Cecropia peltata</i>	624[0n]
<i>A. jam</i>	AJ-6_130911_V93	AJ_130911_03-04	BSCAT026-13.rbcl	KF270133	13-Sep-2011	<i>Cecropia peltata</i>	629[0n]
<i>A. jam</i>	AJ-7_130911_V69	AJ_130911_03-05	BSCAT027-13.rbcl	KF270132	13-Sep-2011	<i>Cecropia peltata</i>	626[0n]
<i>A. jam</i>	AJ-10_150911_V102	AJ_150911_02-02	BSCAT028-13.rbcl	KF270131	15-Sep-2011	<i>Cecropia peltata</i>	626[0n]
<i>A. jam</i>	AJ-19_161011_V90	AJ_161011_06-02	BSCAT029-13.rbcl	KF270130	16-Oct-2011	<i>Cecropia peltata</i>	625[0n]
<i>A. jam</i>	AJ-22_181011_V81	AJ_181011_05-03	BSCAT030-13.rbcl	KF270129	18-Oct-2011	<i>Cecropia peltata</i>	626[0n]
<i>A. jam</i>	AJ-24_191011_V75	AJ_191011_01-03	BSCAT031-13.rbcl	KF270128	19-Oct-2011	<i>Cecropia peltata</i>	625[0n]
<i>A. jam</i>	AJ-23_191011_V82	AJ_191011_05-01	BSCAT032-13.rbcl	KF270127	19-Oct-2011	<i>Cecropia peltata</i>	626[0n]
<i>A. jam</i>	AJ-36_200512_V97	AJ_200512_ch002	BSCAT033-13.rbcl	KF270126	20-May-2012	<i>Cecropia peltata</i>	626[0n]
<i>A. jam</i>	AJ-27_201011_V100	AJ_201011_01-02	BSCAT034-13.rbcl	KF270125	20-Oct-2011	<i>Cecropia peltata</i>	614[0n]
<i>A. jam</i>	AJ-28_201011_V83	AJ_201011_01-04	BSCAT035-13.rbcl	KF270124	20-Oct-2011	<i>Cecropia peltata</i>	624[0n]
<i>A. jam</i>	AJ-44_210512_V70	AJ_210512_ch003	BSCAT036-13.rbcl	KF270123	21-May-2012	<i>Cecropia peltata</i>	626[0n]
<i>A. jam</i>	AJ-48_220512_V84	AJ_220512_ch002	BSCAT037-13.rbcl	KF270122	22-May-2012	<i>Cecropia peltata</i>	625[0n]
<i>A. jam</i>	AJ-52_230512_V92	AJ_230512_ch003	BSCAT038-13.rbcl	KF270121	23-May-2012	<i>Cecropia peltata</i>	624[0n]
<i>A. jam</i>	AJ-54_240512_V86	AJ_240512_lb003	BSCAT039-13.rbcl	KF270120	24-May-2012	<i>Cecropia peltata</i>	626[0n]

Bat Species	Sample ID	Field ID	rbcl seq ref	rbcl genbank #	Collection Date	rbcl Identification	rbcl Seq. Length
<i>A. jam</i>	AJ-58_240512_V72	AJ_240512_lb009	BSCAT040-13.rbcl	KF270119	24-May-2012	<i>Cecropia peltata</i>	625[0n]
<i>A. jam</i>	AJ-14_240911_V77	AJ_240911_04-19	BSCAT041-13.rbcl	KF270118	24-Sep-2011	<i>Cecropia peltata</i>	625[0n]
<i>A. jam</i>	AJ-59_250512_V89	AJ_250512_bb001	BSCAT042-13.rbcl	KF270117	25-May-2012	<i>Cecropia peltata</i>	624[0n]
<i>A. jam</i>	AJ-11_250911_V98	AJ_250911_03-01	BSCAT043-13.rbcl	KF270116	25-Sep-2011	<i>Cecropia peltata</i>	625[0n]
<i>A. jam</i>	AJ-12_250911_V55	AJ_250911_03-02	BSCAT044-13.rbcl	KF270115	25-Sep-2011	<i>Cecropia peltata</i>	625[0n]
<i>A. jam</i>	AJ-16_260911_V78	AJ_260911_01-03	BSCAT045-13.rbcl	KF270114	26-Sep-2011	<i>Cecropia peltata</i>	626[0n]
<i>A. jam</i>	AJ-17_260911_V80	AJ_260911_01-04	BSCAT046-13.rbcl	KF270113	26-Sep-2011	<i>Cecropia peltata</i>	627[0n]
<i>A. jam</i>	AJ-61_270512_V94	AJ_270512_ch001	BSCAT047-13.rbcl	KF270112	27-May-2012	<i>Cecropia peltata</i>	625[0n]
<i>A. jam</i>	AJ-30_271011_VT	AJ_271011_08-01	BSCAT048-13.rbcl	KF270111	27-Oct-2011	<i>Cecropia peltata</i>	611[0n]
<i>A. jam</i>	AJ-32_281011_V88	AJ_281011_09-01	BSCAT049-13.rbcl	KF270110	28-Oct-2011	<i>Cecropia peltata</i>	624[0n]
<i>A. jam</i>	AJ-31_281011_V76	AJ_281011_09-03	BSCAT050-13.rbcl	KF270168	28-Oct-2011	<i>Cecropia peltata</i>	626[0n]
<i>A. jam</i>	AJ-66_220611_V53	AJ_220611_2	BSCAT051-13.rbcl	KF270167	22-Jun-2011	<i>Cecropia peltata</i>	627[0n]
<i>A. jam</i>	AJ-62_300512_V73	AJ_300512_chn001	BSCAT052-13.rbcl	KF270166	30-May-2012	<i>Cecropia peltata</i>	625[0n]
<i>A. jam</i>	AJ-63_300512_V56	AJ_300512_CHN002	BSCAT053-13.rbcl	KF270165	30-May-2012	<i>Cecropia peltata</i>	625[0n]
<i>A. jam</i>	AJ-67_170711_V74	AJ_170711_4	BSCAT054-13.rbcl	KF270164	17-Jul-2011	<i>Cecropia peltata</i>	626[0n]
<i>A. jam</i>	AJ-43_210512_V10	AJ_210512_CH002	BSCAT055-13.rbcl	KF270163	21-May-2012	<i>Cecropia peltata</i>	630[0n]
<i>A. jam</i>	AJ-37_200512_V7	AJ_200512_CH004	BSCAT056-13.rbcl	KF270162	20-May-2012	<i>Cecropia peltata</i>	627[1n]
<i>A. jam</i>	AJ-40_200512_V8	AJ_200512_CH008	BSCAT057-13.rbcl	KF270161	20-May-2012	<i>Cecropia peltata</i>	627[0n]
<i>G. sor</i>	GS-20_150711_V65	GS_150711_10	BSCAT058-13.rbcl	KF270160	15-Jul-2011	<i>Cecropia peltata</i>	625[0n]
<i>G. sor</i>	GS-21_150711_V66	GS_150711_11	BSCAT059-13.rbcl	KF270159	15-Jul-2011	<i>Cecropia peltata</i>	626[0n]
<i>G. sor</i>	GS-1_130911_V110	GS_130911_03-02	BSCAT060-13.rbcl	KF270158	13-Sep-2011	<i>Cecropia peltata</i>	626[0n]
<i>G. sor</i>	GS-22_150711_V67	GS_150711_14	BSCAT061-13.rbcl	KF270157	15-Jul-2011	<i>Cecropia peltata</i>	623[0n]
<i>G. sor</i>	GS-23_150711_V68	GS_150711_15	BSCAT062-13.rbcl	KF270156	15-Jul-2011	<i>Cecropia peltata</i>	627[0n]
<i>G. sor</i>	GS-6_250512_V115	GS_250512_le003	BSCAT063-13.rbcl	KF270155	25-May-2012	<i>Cecropia peltata</i>	625[0n]
<i>G. sor</i>	GS-7_250512_V114	GS_250512_le004	BSCAT064-13.rbcl	KF270154	25-May-2012	<i>Cecropia peltata</i>	626[0n]

Bat Species	Sample ID	Field ID	rbcl seq ref	rbcl genbank #	Collection Date	rbcl Identification	rbcl Seq. Length
<i>G. sor</i>	GS-9_250512_V62	GS_250512_LE006	BSCAT065-13.rbcl	KF270153	25-May-2012	<i>Cecropia peltata</i>	624[0n]
<i>G. sor</i>	GS-10_250512_V111	GS_250512_le007	BSCAT066-13.rbcl	KF270152	25-May-2012	<i>Cecropia peltata</i>	625[0n]
<i>G. sor</i>	GS-11_250512_V116	GS_250512_le008-seed2	BSCAT067-13.rbcl	KF270151	25-May-2012	<i>Cecropia peltata</i>	612[0n]
<i>G. sor</i>	GS-13_250512_VX	GS_250512_LE010	BSCAT068-13.rbcl	KF270150	25-May-2012	<i>Cecropia peltata</i>	608[1n]
<i>G. sor</i>	GS-2_250911_V106	GS_250911_05-10	BSCAT069-13.rbcl	KF270149	25-Sep-2011	<i>Cecropia peltata</i>	625[0n]
<i>G. sor</i>	GS-2_250911_V109	GS_250911_05-10	BSCAT070-13.rbcl	KF270148	25-Sep-2011	<i>Cecropia peltata</i>	630[0n]
<i>G. sor</i>	GS-16_150711_V64	GS_150711_3	BSCAT071-13.rbcl	KF270147	15-Jul-2011	<i>Cecropia peltata</i>	625[0n]
<i>G. sor</i>	GS-17_150711_V113	GS_150711_5	BSCAT072-13.rbcl	KF270146	15-Jul-2011	<i>Cecropia peltata</i>	627[0n]
<i>G. sor</i>	GS-5_150711_VS	GS_150711_9	BSCAT073-13.rbcl	KF270145	15-Jul-2011	<i>Cecropia peltata</i>	611[0n]
<i>A. jam</i>	AJ-46_210512_V41	AJ_210512_CH007	BSCAT074-13.rbcl	KF270169	21-May-2012	<i>Cordia aff. collococca</i>	590[2n]
<i>G. sor</i>	GS-7_250512_V135	GS_250512_LE004-Stick-tight	BSCAT075-13.rbcl	KF270170	25-May-2012	<i>Desmodium</i> sp.	598[0n]
<i>A. jam</i>	AJ-38_200512_VO	AJ_200512_CH007	BSCAT076-13.rbcl	KF270173	20-May-2012	<i>Ficus aff. citrifolia-pertusa</i>	611[0n]
<i>A. jam</i>	AJ-55_240512_V125	AJ_240512_LB005	BSCAT077-13.rbcl	KF270172	24-May-2012	<i>Ficus aff. citrifolia-pertusa</i>	598[0n]
<i>A. jam</i>	AJ-60_260512_V123	AJ_260512_CH001	BSCAT078-13.rbcl	KF270171	26-May-2012	<i>Ficus aff. citrifolia-pertusa</i>	598[0n]
<i>A. jam</i>	AJ-18_081011_VU	AJ_081011_02-01	BSCAT079-13.rbcl	KF270174	08-Oct-2011	<i>Ficus aff. maxima</i>	617[0n]
<i>A. jam</i>	AJ-9_130911_VM	AJ_130911_03-07	BSCAT080-13.rbcl	KF270178	13-Sep-2011	<i>Ficus aff. maxima</i>	615[0n]
<i>A. jam</i>	AJ-20_161011_VN	AJ_161011_06-03	BSCAT081-13.rbcl	KF270177	16-Oct-2011	<i>Ficus aff. maxima</i>	629[0n]
<i>A. jam</i>	AJ-35_170512_V103	AJ_170512_mlp001	BSCAT082-13.rbcl	KF270176	17-May-2012	<i>Ficus aff. maxima</i>	626[0n]
<i>A. jam</i>	AJ-65_UK_V121	AJ_UK_1	BSCAT083-13.rbcl	KF270175		<i>Ficus aff. maxima</i>	599[0n]
<i>A. jam</i>	AJ-42_200512_V122	AJ_200512_CH010	BSCAT084-13.rbcl	KF270179	20-May-2012	<i>Ficus aff. maxima</i>	598[0n]

Bat Species	Sample ID	Field ID	rbcl seq ref	rbcl genbank #	Collection Date	rbcl Identification	rbcl Seq. Length
<i>G. sor</i>	GS-14_080612_VJ	GS_080612_UE001	BSCAT085-13.rbcl	KF270181	08-Jun-2012	Melastomataceae	632[0n]
<i>G. sor</i>	GS-4_250512_VG	GS_250512_LE001	BSCAT086-13.rbcl	KF270180	25-May-2012	Melastomataceae	615[0n]
<i>A. flav</i>	AF-8_260512_V31	AF_260512_CH003	BSCAT087-13.rbcl	KF270183	26-May-2012	<i>Musa acuminata</i>	624[0n]
<i>A. flav</i>	AF-9_310512_V39	AF_310512_FT10-1435	BSCAT088-13.rbcl	KF270182	31-May-2012	<i>Musa acuminata</i>	626[0n]
<i>G. sor</i>	GS-22_150711_V134	GS_150711_14	BSCAT089-13.rbcl	KF270189	15-Jul-2011	<i>Piper aff. aduncum</i>	594[4n]
<i>G. sor</i>	GS-23_150711_V132	GS_150711_15	BSCAT090-13.rbcl	KF270188	15-Jul-2011	<i>Piper aff. aduncum</i>	597[0n]
<i>G. sor</i>	GS-24_150711_V129	GS_150711_16	BSCAT091-13.rbcl	KF270187	15-Jul-2011	<i>Piper aff. aduncum</i>	598[0n]
<i>G. sor</i>	GS-25_170711_V107	GS_170711_18	BSCAT092-13.rbcl	KF270186	17-Jul-2011	<i>Piper aff. aduncum</i>	628[0n]
<i>G. sor</i>	GS-15_150711_V130	GS_150711_1	BSCAT093-13.rbcl	KF270185	15-Jul-2011	<i>Piper aff. aduncum</i>	597[0n]
<i>G. sor</i>	GS-11_250512_V108	GS_250512_le008-Seed1	BSCAT094-13.rbcl	KF270184	25-May-2012	<i>Piper aff. aduncum</i>	622[0n]
<i>G. sor</i>	GS-3_260911_V61	GS_260911_06-03	BSCAT095-13.rbcl	KF270190	26-Sep-2011	<i>Piper aff. aduncum</i>	628[0n]
<i>G. sor</i>	GS-12_250512_VL	GS_250512_LE004	BSCAT096-13.rbcl	KF270192	25-May-2012	<i>Piper aff. hispidum</i>	611[0n]
<i>G. sor</i>	GS-8_250512_V63	GS_250512_LE005	BSCAT097-13.rbcl	KF270193	25-May-2012	<i>Piper aff. hispidum</i>	625[0n]
<i>G. sor</i>	GS-9_250512_V133	GS_250512_LE006	BSCAT098-13.rbcl	KF270194	25-May-2012	<i>Piper aff. hispidum</i>	597[0n]
<i>G. sor</i>	GS-18_150711_V131	GS_150711_8	BSCAT099-13.rbcl	KF270195	15-Jul-2011	<i>Piper aff. hispidum</i>	599[0n]

Bat Species	Sample ID	Field ID	rbcl seq ref	rbcl genbank #	Collection Date	rbcl Identification	rbcl Seq. Length
<i>G. sor</i>	GS-5_250512_V5	GS_250512_BE002	BSCAT100-13.rbcl	KF270191	25-May-2012	<i>Piper aff. hispidum</i>	597[1n]
<i>A. jam</i>	AJ-25_191011_V87	AJ_191011_01-05	BSCAT101-13.rbcl	KF270197	19-Oct-2011	<i>Psidium</i> sp.	628[0n]
<i>A. jam</i>	AJ-29_201011_V95	AJ_201011_01-05	BSCAT102-13.rbcl	KF270196	20-Oct-2011	<i>Psidium</i> sp.	627[0n]
<i>A. jam</i>	AJ-50_220512_V96	AJ_220512_ch004	BSCAT103-13.rbcl	KF270198	22-May-2012	<i>Simarouba glauca</i>	627[0n]
<i>A. jam</i>	AJ-64_300512_V117	AJ_300512_CHN003	BSCAT104-13.rbcl	KF270199	30-May-2012	<i>Simarouba glauca</i>	596[0n]
<i>A. jam</i>	AJ-41_200512_V42	AJ_200512_CH009	BSCAT105-13.rbcl	KF270200	20-May-2012	<i>Solanum</i> sp.	631[0n]
<i>A. jam</i>	AJ-26_201011_V105	AJ_201011_01-01	BSCAT106-13.rbcl	KF270202	20-Oct-2011	<i>Solanum</i> sp.	625[0n]
<i>A. jam</i>	AJ-47_220512_V79	AJ_220512_ch001	BSCAT107-13.rbcl	KF270201	22-May-2012	<i>Solanum</i> sp.	627[0n]
<i>A. jam</i>	AJ-51_220512_V59	AJ_220512_CH005	BSCAT108-13.rbcl	KF270203	22-May-2012	<i>Terminalia</i> sp.	626[0n]

Appendix 4 - Roost ecology of *Ariteus flavescens*

Methods

To identify and locate roosts used by *A. flavescens* opportunistically, I fitted individual *Ariteus flavescens* with LB-2 radio transmitters (Holohil Systems Ltd, Carp, Ontario, Canada) attached to interscapular dorsal region using ostomy liquid bonding cement (Torbot Group Inc., Cranston, Rhode Island, USA). Following Aldridge and Brigham (1988), the mass of the transmitters and glue was less than 5% of the bats total body mass. I released bats with transmitters at their sites of capture and began tracking them the next day using two 4 element yagi antennae and receivers (Telonics Inc., Mesa, AZ, USA). I used a homing method to approach the roost location where direction was no longer discernible at a gain of near zero (White & Garrott 1990, Russo et al. 2002, Ralista et al. 2010). I recorded the latitude and longitude and elevation of each day roost using Garmin eTrex Vista H handheld GPS units (Garmin International, Inc., Olathe, KS, USA) and measured distances using the fossil package (Vavrek 2011) in R v3.0.1 (R Development Core Team 2008). I assessed the approximate location of the roost in the tree (foliage, small/large branch, bole cavity, near/away from bole). I then recorded the tree species and characteristics of the roost tree and its immediate surroundings. These characteristics include the approximate height of the tree, the diameter at breast height (DBH), the presence or absence of fruit and its ripeness, and an approximation of crown density (mean of two independent observers).

I assessed roost fidelity using an index (equation 1) proposed by Chaverri and Kunz (2006),

$$F = \frac{2(stay)+1(return)-1(move)}{stay+return+move}, \quad (1)$$

where F is the fidelity index, $(stay)$ is the number of consecutive uses of a roost, $(return)$ is the number of instances a bat returned to a previous roost and $(move)$ is the number of times bats moved to a new roost. Bats that remain in the same roost will express higher fidelities than bats that return to a small subset of roosts. Values range from -1 (complete

infidelity) to 2 (complete fidelity). I calculated all values using the first located roost as a baseline.

Results

I attached radio transmitters to 16 *A. flavescens* but did not subsequently locate 6 of them (Table 1). One individual used the same area as a Jamaican Boa (*Epicrates subflavus*) concurrently tracked by an ongoing study (Brent Newman, unpublished data). The tagged bat and tagged boa were in the same tree, and the snake appeared to have eaten the bat. I could clearly see the boa and a bat-sized swelling in its abdomen. Both radio signals indicated that the bat and boa were in the same location, at approximately the same height and moving in unison. I tracked the remaining nine bats for a total of 59 roost days (One day per roost per bat; Table 2).

I located a total of 23 roosts and identified four unknown roosts ($\bar{X} = 3$ roosts/bat, range 1-6 roosts). The bats were inconspicuous in their roosts but I located the approximate location of each bat (Fig. 1; Table 2). I identified unknown roosts by tracking the bats to areas that did not contain previous roosts and I was unable to locate the specific roost tree. The 23 roosts I was able to locate were in ten tree species (Table 2). Of these species, *Ficus spp.*, *Terminalia catappa*, and *Castilla elastica*, were the only species used by multiple bats. The mean tracking time per bat was relatively low when compared to the nominal battery life of 14 days ($\bar{X} = 6.5 \pm 5$ days) but many signals were lost during tracking for periods of several days. Three bats spent one day in their respective roosts and moved on the next day one of which was found again at a roost nearby the previous. I tracked the remaining six bats for at least six days ($\bar{X} = 9.2 \pm 3.9$ days, range 6 to 15 days) and used these bats to assess roost fidelity.

Roost fidelity varied across the six bats and they switched roosts readily ($\bar{X} = 3.8 \pm 2$ roosts/bat). The duration each bat spent in each roost also varied ($\bar{X} = 2.5 \pm 3.2$ days, range 1-14 days). The Chaverri-Kunz index also varied among the bats ($\bar{X}_F = 0.7 \pm 1.1$, range -1 to 2) but indicated that *A. flavescens* remain in preferred roosts. When considering roost areas (50m radius) and adjusting a roost moves within a central 50 m

radius to reflect returns, I observed an increase in roost fidelity ($\bar{X}_F = 1.0 \pm 1.1$, range -1 to 2). *A. flavescens* appear to be faithful to their roosts and more so to roost areas over a relatively short period of time.

Roost trees preferred by some bats appeared to be preferred by other bats. I observed two instances of roost tree overlap between four bats. One instance involved 0360 and 0480 which roosted in a fig tree (*Ficus sp.*) with six days of concurrent overlap. The second instance involved 0237 and 0379 which asynchronously shared a rubber tree (*C. elastica*) and an almond tree (*T. catappa*) roost.

I located roosts in the valleys (cockpits) and on the hills ($\bar{X}_{\text{Elevation}} = 140 \pm 56$ m, range 99 to 261 m). When changing roosts, the bats maintained a near consistent elevation ($\bar{X}_{\Delta\text{Elevation}} = 7.6 \pm 32$ m, range -13 to 116 m) relative to their previous roost excluding one (0160) which roosted as high as 261 m and as low as 133 m with one roost change that dropped 116 m in elevation. After removing 0160, the change in roost elevation of the remaining eight bats was near zero ($\bar{X}_{\Delta\text{Elevation}} = 0.7 \pm 8$ m, range -13 to 14 m).

Although elevation remained similar, the distances bats travelled varied greatly from capture point to roost ($\bar{X}=232\pm315$ m, min=5 m, max=1075 m) and roost to roost ($\bar{X}=173\pm253$ m, range 5 to 880 m). I caught four of the bats (0237, 0339, 0360, and 0480) within ca. 100 m of all of their roosts ($\bar{X}=66\pm23$ m, range 28 to 106 m). Of these individuals, 0360 and 0480, used the same roost for the entire tracking period and did not move from their initial location. Conversely, other individuals used roosts at greater distances ($\bar{X}=673\pm216$ m, range 286 to 1075 m) from their capture location. On one occasion, I observed a bat (0440) travelling greater than one kilometer from the point of capture to its initial roost. The bats exhibited a range of motility between roost locations.

Table 1. *Arietues flavescens* fitted with LB-2 radio transmitters to locate roosts using radio telemetry.

Transmitter frequency (kHz)	Start Date	End date	Days tracked	Roosts	Forearm Length (mm)	Mass (g)	%	Age	Sex	Reproductive status	Capture Location
150.160	09/10/2011	21/10/2011	6	6	45.00	17.2	2.7	A	F	NP, NL	M5A
150.237	19/10/2011	29/10/2011	6	4	40.50	15.5	3.0	A	M	Desc.	WRC
150.280	19/10/2011	26/10/2011	2	2	42.00	17.5	2.7	A	F	NP, NL	TH
150.299*	13/09/2011				42.00	15.1	3.1	SA	F	NP, NL	LB
150.339	16/09/2011	01/10/2011	13	4	38.70	13.7	3.4	A	M	Desc.	M5A
150.360	24/09/2011	09/10/2011	6	1	40.25	17.7	2.7	A	F	NP, NL	LB
150.379	25/09/2011	09/10/2011	9	6	41.00	13.1	3.6	A	M	Not desc.	WRC
150.440	29/09/2011	03/10/2011	1	1	43.00	18.9	2.5	A	F	NP, NL	M5A
150.480	03/10/2011	17/10/2011	15	1	39.50	14.0	3.4	A	M	Desc.	LB
150.499	08/10/2011	19/10/2011	1	1	43.00	16.4	2.9	A	F	NP, NL	M5A

% Mass of the radio transmitter as a percentage of the bats mass.

^{NP} Not pregnant.

^{NL} Not lactating.

^{Desc.} Testes are exteriorly visible.

* Bat consumed by a Jamaican Boa, *Epicrates subflavus*



Figure 1. Locations of *Ariteus flavescens* roosts relative to point of capture. Unknown roosts are excluded. Groups represent bats captured in the same location located within the indicated group. The upper entrance of the Windsor Great Cave provides a point of reference.

Table 2 a. Roosts used by nine radio-tagged *Ariteus flavescens*. For each roost (numbered by individual) the elevation (AMSL), Roost days, scientific name of the roost tree species, roost tree characteristics and approximate location of the roost are provided.

	Roost	AMSL (m)	Roost days	Roost tree species	Fruit presence	Roost tree height (m)	Crown density (%)	DBH (cm)	Roost location	Roost height (m)
0160	1	243	1	<i>Oxandra lanceolata</i>		15	70		F	8
	2	250	1	<i>Ficus spp.</i>	unripe	20	25	72	LB, away	19
	3	261	1	<i>Ficus spp.</i>	unripe	20	25	75	SB, away	19
	4		1	Unknown						
	5	249	1	<i>Ficus spp.</i>		20	85	148	SB, away	19
	6	133	1	<i>Ficus spp.</i>		20	85	72	LB, away	19
0237	1*		1	<i>Mammea americana</i>	ripe	20			LB, away	15
	2	101	1	<i>Cordia gerascanthus</i>		20	40	162	F	15
	3 ^a	107	2	<i>Terminalia catappa</i>	ripe	20	30	65	SB, away	10
	4 ^b	111	2	<i>Castilla elastica</i>		25	40	59	F	22
0280	1	169	1	<i>Syzygium malaccense</i>	ripe	15	30	127	SB, away	12
	2	155	1	<i>Syzygium malaccense</i>		17	30	123	SB, away	15
0339	1	116	8	<i>Guazuma ulmifolia</i>		17	45	40	F	17
	2	116	1	<i>Guazuma ulmifolia</i>		15	45	43	F	10
	3	116	3	<i>Guazuma ulmifolia</i>		20	35	102	F	19
	4		1	Unknown						
0360	1 ^c	107	6	<i>Ficus spp.</i>	ripe	20	70	153	SB, near	8

* Roost located on a hillside and could be sighted but not approached. Characteristics are approximated.

^{abc} Roosts with the same letter are the same individual tree.

Roost location note: F= Foliage, LB=Large branch, SB=Small branch, Away/Near = away or near bole.

Table 2 b. Roosts used by nine radio-tagged *Ariteus flavescens*. For each roost (numbered by individual) the elevation (AMSL), number of days in roost (Roost days), scientific name of the roost tree species, roost tree characteristics and approximate location of the roost are provided.

	Roost	AMSL (m)	Roost days	Roost tree species	Fruit presence	Roost tree height (m)	Crown density (%)	DBH (cm)	Roost location	Roost height (m)
0379	1	100	1	<i>Castilla elastica</i>		20	55	25	F	20
	2	100	1	<i>Castilla elastica</i>		17	45	14	F	15
	3	99	1	<i>Swietenia mahagoni</i>		20	40	44	F	15
	4	99	1	Unknown		20	18	37	F	17
	5 ^b	112	4	<i>Castilla elastica</i>		25	40	59	F	22
	6 ^a	107	1	<i>Terminalia catappa</i>	ripe	20	30	65	SB, away	12
0440	1	99	1	<i>Ficus spp.</i>	unripe	20	70		SB, away	10
0480	1		1	Unknown						
	2 ^c	107	14	<i>Ficus spp.</i>	ripe	20	70	153	SB, near	8
0499	1	165	1	<i>Cola acuminata</i>	unripe	20	75		LB, away	15

* Roost located on a hillside and could be sighted but not approached. Characteristics are approximated.

^{abc} Roosts with the same letter are the same individual tree.

Roost location note: F= Foliage, LB=Large branch, SB=Small branch, Away/Near = away or near bole.

Discussion

My results indicate that *Ariteus flavescens* roosted predominantly in foliage but also roosted on branches near to and away from the tree bole. Foliage roosts are common in a range of bat species (Brooke et al. 2000, Hutchinson & Lacki 2000). Where other bat species, such as *Artibeus* spp., create tents (folded leaf enclosures; (e.g., Campbell et al. 2006, Charles-Dominique 1993, Chaverri & Kunz, 2006) I did not observe this in *A. flavescens*. Foliage roosts can provide protection from terrestrial predators because small branches cannot support the predator and there is a high likelihood of jarring the roost bat (Timm & Mortimer 1976). Cryptic colouration can provide camouflage in foliage (Hutchinson & Lacki 2000). Hendricks (2000) suggested that birds roosting in foliage do so to increase predator detection. Most predators of bats appear to be nocturnal as is observed for the barn owl (*Tyto alba*; McFarlane & Garrett, 1989) and *Epicrates* spp. (Rodriguez & Reagan 1984, Rodríguez-Durán 1996) that hunt at cave entrances (Pers. obs.) and in trees as is evident by the predation event I observed. Foliage roosts do not provide protection from disturbances. Despite this, I observed roosts located immediately adjacent to roads and agricultural clearings frequently visited by humans. Hurricanes and storms may also influence the roost preferences of *A. flavescens* over acute to extended periods of time. Gannon and Willig (1994) investigated the effects of hurricane Hugo and determined that bat densities dropped and species recovery took ca. two years where populations recovered at all. Tree roosting species may experience the effects of these disturbances more so than cave roosting species as loss of roost habitat is additional to the other effects. The vast majority of *A. jamaicensis* that I captured had obvious ectoparasites suggesting that they were roosting in the cave. The presence of ectoparasite and lack of observed tents suggests that *A. jamaicensis* and *A. flavescens* partition their roosts.

The bats used a range of tree species that include locally native and introduced species. The most common roost species across the tracked bats were *Ficus* spp., *C. elastica*, and *T. catappa*. The bats that I observed roosting in *Ficus* spp. often returned to the same tree or to other *Ficus* spp. in the near area. Where other bat species make commutes to food

patches (e.g., Fleming & Heithaus 1986, Morrison 1980) *A. flavescens* appear to roost in the fruiting trees they may be exploiting for food. This could also be true for the introduced species as I observed *A. flavescens* roosting in *C. elastica* and *T. catappa*, both species consumed by Jamaican fruitbats. Long and Racey (2007) found that bats may become highly reliant on introduced tree species for food resources and my data indicate that *A. flavescens* may also rely on introduced tree species for roosts. In the present study, I documented that *A. flavescens* roost both in native and exotic tree species.

Although I found most roosts in the valleys, I also found bats roosting on hilltops and hillsides. Elevation is known to be a limiting factor for roost selection in other species (Cryan et al. 2000). I did not observe any limitation of elevation in *A. flavescens* roosts but I did observe that their roost preferences skew towards the valleys. The small difference in elevation, *ca.* 150 m, between hilltops and valleys is unlikely to limit *A. flavescens*. Genoways et al. (2005) report *A. flavescens* captures from a range of elevation in Jamaica. Also, bats often move across greater elevations (Neubaum et al. 2006). Flora proportions differ between the hills and valleys (Kelly et al. 1988) which may present more favorable tree species in the valleys. Despite differences observed between hilltops and valleys, *A. flavescens* uses roost resources at both altitudes.

The *A. flavescens* individuals that I tracked were generally roost faithful over the relatively short tracking period but most of the bats used multiple roosts. My observations indicate that *A. flavescens* maintains relatively low roost fidelity when compared to other tree roosting species (Brooke et al. 2000, Heithaus & Fleming 1978, Vehrencamp et al. 1977) who can be detected in similar roosts over periods of weeks to months. *Ariteus flavescens* is more comparable to low fidelity tree roosting species that change roosts frequently but remain faithful to small areas (Vonhof & Barclay 1996). Roost switching is a strategy to avoid ectoparasites (Reckardt & Kerth 2007). I observed no ectoparasite on *A. flavescens*, suggesting that roost switching may indeed be an effective means of parasite avoidance. More likely, foliage offers abundant roost space and is ephemeral (Lewis 1995) so it is not surprising that the bats changed roosts somewhat frequently. Many species of bats return to previously used roosts (e.g., Cryan et al., 2001). In this way the bats are faithful to their roosts but maintain several roosts that they use

interchangeably. This could be true of *A. flavescens* as I observed the bats travelling relatively short distances between successive roosts and I observed one individual returning to a roost it had previously used.

On the other end of the spectrum, some individuals were highly faithful to their roosts. Over a period of 14 days I observed a bat remain in the same roost. Brigham and Fenton (1986) noted that reproductive success can be reduced if bats move involuntarily or too frequently. Although group size factors into many roost selection aspects (Lewis 1995) I am unable to comment on the effect of group size. Numerous phyllostomids roost in groups (e.g., Kunz & McCracken 1996, Olson & Barclay 2013) but I did not reliably detect this during radio-tracking. I tracked two individuals simultaneously to the same roost in approximately the same location. This is the only suggestion that *A. flavescens* roost in groups. More detailed research is required to further investigate the roost ecology of this endemic species

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Appendix 5 - Permissions

AUSPC



2008-003-04::3:

AUP Number: 2008-003-04
AUP Title: Behavioural Ecology of Bats

The YEARLY RENEWAL to Animal Use Protocol (AUP) 2008-003-04 has been approved.

1. This AUP number must be indicated when ordering animals for this project.
2. Animals for other projects may not be ordered under this AUP number.
3. Purchases of animals other than through this system must be cleared through the ACVS office.
Health certificates will be required.

REQUIREMENTS/COMMENTS

Please ensure that individual(s) performing procedures on live animals, as described in this protocol, are familiar with the contents of this document.

The holder of this Animal Use Protocol is responsible to ensure that all associated safety components (biosafety, radiation safety, general laboratory safety) comply with institutional safety standards and have received all necessary approvals. Please consult directly with your institutional safety officers.

Submitted by: Thompson, Sharla H
on behalf of the Animal Use Subcommittee



NATIONAL ENVIRONMENT & PLANNING AGENCY

Ref. No. 18/27

15 July 2011

Mr. Colin Hayward
Department of Biology
University of Western Ontario

Dear Mr. Hayward

Re: Application to capture Jamaican bats

The National Environment and Planning Agency (NEPA) is in receipt of your Wildlife Research Application dated 24 February 2011 to capture bats to determine the ecology of the endemic *Arctus flavescens*.

The Agency wishes to advise that permission has been granted for you and your associates, Dr. Brock Fenton, Dr. Susan Koenig, Dr. Steve Newmaster, Mr. Matthew Enrich and Ms. Carole Ann Lacroix, to capture bats for the research project entitled "Ecosystems services and conservation of the endemic Jamaican fig-eating bat *Arctus flavescens*" from 1 September – 15 December 2011, subject to the following conditions:

1. The capture of bats for the project shall be conducted over the period 1 September – 15 December 2011 from Windsor Great Cave and Coxheath area, Trelawny.
2. The project shall only involve the capture of the bat species *Arctus flavescens* and *Arctus jamaicensis* and the collection of wing punches and fecal samples from the animals. A maximum of twenty (20) *Arctus flavescens* shall be fitted with radio transmitters.
3. The project shall only involve the collection of the plant species listed in Annex 1 from Windsor and Coxheath, Trelawny.
4. No specimens of plants or any other animal species shall be collected during the implementation of the project without prior permission from NEPA.
5. A Material Transfer Agreement for the non-commercial use for the endemic species *Piper amalago* var. *nigrinodum*, *Ocotea staminea*, *Ocotea robertsoniae*, *Ocotea jamaicensis*, *Orphanesia triandra*, *Terminalia latifolia*, *Psidium montanum*, *Bumelia nigra*, *Bumelia rotundifolia*, *Bumelia octosepala*, *Bumelia montana*, *Bumelia bullata*, *Bumelia* sp. A and *Bumelia* sp. B shall be signed prior to the collection of the specimens. The cost is one thousand Jamaican dollars (J\$1000).
6. An export permit is required under the Endangered Species (Protection, Conservation and Regulation of Trade) Act, 2000 to export specimens of *Ocotea harrisii*, *Ocotea stamineoides*, *Bumelia bullata*, *Bumelia* sp. A and *Bumelia* sp. B from Jamaica. The cost is Jamaican One Thousand Dollars (\$1,000.00) for the application fee and Two Thousand Dollars (\$2,000.00) for two export permits. A copy of an application form is enclosed.

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Any reply or subsequent reference to this communication should be addressed to the Chief Executive Officer, to the attention of the officer dealing with the matter, and the reference quoted where applicable.

*Managing and protecting Jamaica's land, wood and water
A Government of Jamaica Agency*



NATIONAL ENVIRONMENT & PLANNING AGENCY

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Ref. #: 18/27

2 March 2012

Mr. Colin Hayward
Department of Biology
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Canada

Dear Mr. Hayward:

Re: Application to Capture Jamaican Bats

The National Environment and Planning Agency (NEPA) is in receipt of your email dated 23 January 2012 requesting an extension of the research for the project entitled "Ecosystems services and conservation of the endemic Jamaican fig-eating bat (*Artibeus flavescens*)".

The Agency has reviewed your request and wishes to advise that the project end date as specified in the permission letter dated 13 July 2011 has been extended to 15 December 2012.

Please note that all the other conditions outlined in the letter dated 15 July 2011 are still applicable except for Condition 10 which has been amended to read as follows:

- A project report shall be sent to the Chief Executive Office of NEPA by 31 March 2013 at [redacted]. The report should include but not limited to the following: a list of species collected and observed, total number of each species collected and the location of collection sites (latitude and longitude coordinates). A final report on the findings of the project shall be submitted to NEPA by 30 June 2013.

You are also reminded that NEPA reserves the right to revoke the permit if it is found that the researcher(s) or their associates acted in violation of the terms outlined and if the species to be collected are threatened for any reason.

Yours sincerely
National Environment and Planning Agency

Peter Knight, JP
Chief Executive Officer

/mc

c. Ms. Marilyn Headley, Chief Executive Officer & Conservator of Forests, Forestry Department
Dr. M. B. Fentosa, Emeritus Professor of Biology, University of Western Ontario
Dr. Susan Koenig, Director of Research, Windsor Research Centre Ltd.

Any reply or subsequent reference to this communication should be addressed to the Chief Executive Officer, to the attention of the officer dealing with the matter, and the reference quoted where applicable.

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A Government of Jamaica Agency*

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