The Ecology, Evolution and Natural History of the Endangered Carnivores of Cozumel Island, Mexico

Katherine Walton McFadden

Submitted in partial fulfillment of the
Requirements for the degree
of Doctor of Philosophy
in the Graduate School of Arts and Sciences

COLUMBIA UNIVERSITY

2004

ABSTRACT

The Ecology, Evolution and Natural History of the Endangered Carnivores of Cozumel Island, Mexico

Katherine W. McFadden

Understanding the basic ecology and evolutionary history of a species is important for the conservation and management plans of endangered species. This dissertation examined the phylogenetic uniqueness, feeding ecology, population size and morphological variation found in two endemic carnivores, they pygmy raccoon (*Procyon pygmaeus*) and dwarf coati (*Nasua nelsoni*), on Cozumel Island, Mexico. Data was collected by trapping 78 individual pygmy raccoons (38 males and 40 females) for three field seasons from 2001-2003**.** Results from the mtDNA analyses suggest that island coatis are distinct from their mainland sister taxa, while the mainland and island raccoons seem more closely related. Using a molecular clock and the average sequence divergence between island and Yucatan coatis and raccoons these species are estimated to have diverged from their mainland conspecifics in the last 46-51,000 years. Using data derived from stable carbon and nitrogen isotope analyses combined with scat data, feeding ecology analyses indicate that the pygmy raccoon utilizes an omnivorous diet in which the three most prevalent food items were crab, which constitutes > 50% of the diet, followed by fruits and insects. Trapping efforts identified the most northwestern section of the island, despite what appears to be suitable habitat elsewhere, as the location of the main populations of the pygmy raccoon on Cozumel Island. Using mark-recapture models, the current population of pygmy raccoons on Cozumel Island is estimated to be

fewer than 250 mature individuals at this time. Results from this study indicate that both the pygmy raccoon and dwarf coati should be listed as critically endangered by the World Conservation Union (IUCN). Data from morphological measures indicated that like most species of *Procyon*, the pygmy raccoon exhibits significant male biased sexual dimorphism in the form of increased mass and canine length. The average mass for adult male and female pygmy raccoons was 3.68 and 3.28 kg, respectively, and male canines were approximately 1 cm longer than females. Adult and subadult morphometric values, including mass, did not fluctuate seasonally. In comparing the island and mainland raccoons, a 15% size reduction was estimated for the pygmy raccoon species.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES:

ACKNOWLEDGMENTS

I thank Matt Gompper for his untiring guidance and direction of my dissertation work, for his immediate support and confidence in me, and for the opportunity to work with him at the Center for Environmental Research and Conservation, and elsewhere. I acknowledge Matt for laying the intellectual foundation for my studies, for his consistent support and encouragement of both field and lab work, and for generously providing the necessary funding for both field and laboratory research. Similarly, I am deeply grateful to my committee members, Drs. Juan Carlos Morales, Rob DeSalle, Rodrigo Medellín and Ray Sambrotto for many helpful suggestions on project design, data analysis and manuscript review. I want to thank J.C Morales for his help in learning the ropes of both laboratory techniques and various phylogenetic analyses. I thank Rob DeSalle for allowing me to work in his laboratory and for providing guidance in the ancient DNA analyses and for his help in developing my phylogeographic hypotheses. I thank Rodrigo Medellín for his helpful advice on both the design of the feeding ecology portion of this study and extremely useful help in the writing process. I thank Ray Sambrotto for his patience and assistance in my extensive stable isotope analyses and for his genuine enthusiasm for my project.

I would also like to acknowledge the other research scientists who made significant contributions to my intellectual growth. I am deeply grateful to Pruthu Fernando for taking me under his wing in the laboratory and for assisting with my molecular laboratory techniques and analyses at every step of the way. His patience, assistance, and encouragement in the laboratory were invaluable. I acknowledge Ben

Evans for his assistance in learning various phylogenetic programs. I also thank Goncalo Ferraz for his assistance in learning MARK and for Sarah Greene who patiently let me bend her ear many times and helped me through many difficulties in lab work.

I thank my family for their support during my seemingly never-ending trip towards obtaining my PhD. I thank the many friends at Columbia who provided a thought-provoking, stimulating, and thoroughly entertaining environment in which to work and play and who helped me keep graduate school in perspective to "the big picture". I acknowledge my litany of office mates, beginning with Hugh Cross and Chris Fiorello, and through Anders De Silva and Angelica Cibrian for their friendship and companionship in the long road towards completing my PhD.

I would also like to thank all the people who have been there for me throughout my time in NY and who made it a truly fun and memorable experience: Okan Demirmen, Amanda Mark, Mark Bingham, Chris Fiorello, Julian Faivovich, Sushma Reddy, Jessica Satkoski, Danielle Whittaker, Chanda Bennett and Hugh Cross. I thank them all for their encouragement, friendship and being incredible friends and support systems. I thank Ashley Meeks, Carolyn Kurle and my partner in crime, Chris Fiorello, for having continually been there for me through everything, their round the clock therapy sessions, and being such wonderful lifetime friends. I thank those who constantly provided encouragement every step of the way: Jessie Bushell, Lee Johnson, and Martha Cantwell

There are many people and institutions in both Mexico and the United States who made this study possible. Logistical support on Cozumel Island was provided by FideCaribe, PROFEPA, Jesus Benavides (Punta Sur Park), Raul Lopez Osorio (Coparmex), Fundacíon de Parques y Museos, Martin Delgadillo and Benny Campos

(Cozumel Country Club), and Ernesto Vivas (CAPA). The carnivore research was made possible by collaboration with numerous researchers at the Universidad de Nacional de Mexico (UNAM), Carmen Pozo from EcoSur, and the hospitality and efforts of many wonderful people in Mexico including Rita Sheese, Carlos Naredo, Rafael Chacón, Héctor González, and Ilan Zaviv (Wild Tours Inc.). Rita Sheese was instrumental in introducing me to many contacts on Cozumel, for taking an interest in my research, for allowing me to trap on her land, for never laughing at my Spanish, and her genuine friendship. I thank Alfredo Cuarón and David Valenzuela for their assistance and support in completing this project (and of course the wonderful, yet temperamental Lazy Lizard). Both their logistical and scientific advice is greatly appreciated. I especially thank David Valenzuela for his patience with my pathetic Spanish and cultural missteps, and for his genuine helpfulness in getting my feet wet on Cozumel. I am extremely grateful for logistical assistance provided by Ana Laborde, Christopher Rodriguez, Denise Garcia-Vasco, Irene Romero, and Lupita Navarro. Each one of them made my fieldwork truly pleasurable and fun. Many thanks also to Dr. Nee from the NY Botanical Garden for identification of fruit samples.

I also acknowledge and thank the Department of Ecology, Evolution and Environmental Biology (E3B), the Center for Environmental Research and Conservation at Columbia University, and the University of Missouri, the Sigma Xi Grants-in-Aid, Roosevelt Grant from the American Museum of Natural History, the Explorer's Club, and Sigma Delta Epsilon for supporting this research through research grants.

This dissertation is dedicated to my hero and friend, Mark K. Bingham (1970-2001)

PREFACE

This dissertation is divided into six chapters. The first chapter provides a brief review of the current ecology, evolutionary history, population ecology and current conservation status of the endangered dwarf carnivores of Cozumel Island. Chapter two is a paper to be submitted to *Animal Conservation*; this chapter examines the genetic uniqueness of the dwarf carnivores and dates their appearance on the island of Cozumel. Chapter three is a paper to be submitted to *Oecologia;* this chapter examines the feeding habits of the pygmy raccoons and examines spatial and temporal variation in their diet. Chapter four is a paper to be submitted to *Journal of Zoology*; this chapter estimates population size and capture probabilities of various age and sex classes as well as the temporal and spatial variability of the pygmy raccoons. Chapter five is a paper to be submitted to *Journal of Mammalogy;* this chapter examines the morphological variation of various subpopulations of pygmy raccoon found on Cozumel Island. Chapter six is a brief summary of major conclusions of this dissertation and future directions for related research.

Chapter 1: INTRODUCTION

Cozumel Island, located 15 km off the northeast coast of Quintana Roo, is the largest inhabited island off the Yucatán Peninsula and spans an area of approximately 490 km^2. Human expansion on Cozumel has increased dramatically since the 1960s, concurrent with the increased popularity of the island as a tourist destination. The 1970 population of 10,000 has today grown to more than 65,000 people. This human population growth has accelerated the loss of wildlife resources due to habitat loss, fragmentation and alteration.

Cozumel Island is unique because of its high level of mammalian endemism and contains a distinct biota within the Yucatán Peninsula (Goldman & Moore 1945). As is the trend for island fauna, Cozumel is home to a number of mammals that are smaller, or dwarfed, compared to their mainland ancestors. These island taxa present several conservation dilemmas. As Cozumel's environment is rapidly altered, a conflict exists between animal conservation objectives and the tremendous pressures of economic development, which underlie the human population increases. This dissertation examines the biology and current knowledge of the status of two procyonids endemic to Cozumel Island: the dwarf coati (*Nasua nelsoni*) and the pygmy raccoon (*Procyon pygmaeus*). Both taxa are listed by IUCN as endangered; the raccoon is EN C2a and the coati is EN D1.

Natural History:

Procyonids are comparatively small carnivores (0.8 – 12.0 kg) confined to the New World, and all are semiarboreal, have a plantigrade gait, and tend to prefer

temperate and tropical vegetational zones (Bekoff & Daniels 1984; Kaufmann 1982b). Four species of procyonids are thought to have historically existed (Martinez Meyer et al. 1998) on Cozumel Island: the kinkajou (*Potos flavus*), fox (*Urocyon cinereoargenteus*), dwarf coati (*Nasua nelsoni*) and pygmy raccoon (*Procyon pygmaeus*). The only known kinkajou skeleton in recent times was found in 1995 (Martinez Meyer et al. 1998) and both this and the fox are thought to be currently rare.

Both the coati and raccoon are adapted to a variety of habitats, but they generally occur only where there is tree cover. These species are able to tolerate different habitat types ranging from dry deciduous forests, mangrove stands, sandy palm areas to multistratal tropical evergreen forests. Most species in this family are solitary, although raccoons are sometimes seen in extended family groups (Lotze & Anderson 1979) and coatis live in female-banded groups with as many as 10-40 adults (Fritzell 1978; Gompper 1995; Gompper & Decker 1998; Kaufmann 1962; Russell 1982; Russell 1983).

The order Carnivora can be divided into two groups: 1) omnivores, species in which meat constitutes less than 60% of the diet and 2) carnivores, species in which mean accounts for over 60% of the diet (Gittleman 1986). The coati and raccoon fall into the omnivorous category of feeding strategies and thus have relatively heavier offspring, longer gestation periods, and lengthier periods of dependence prior to weaning, as compared to more exclusively carnivorous species (Bekoff & Daniels 1984).

Procyon pygmaeus:

Two subgenera and seven species are currently recognized under the genus *Procyon*. Merriam (1901) first described the pygmy raccoon (*P. pygmaeus*) as a distinct

species from its mainland congener, *Procyon lotor shufeldti*, based on a number of morphological traits. These include being markedly smaller, both externally and cranially (Merriam 1901). Merriam (1901) described it as very easy to distinguish from *P. lotor* because of its "broad black throat band and golden yellow tail, short posteriorly expanded and rounded nasals and peculiarities of the teeth". Goldman (1950) agreed with Merriam's assessment on the distinctiveness of *P. pygmaeus*, and noted that the teeth are remarkably reduced, and that these and other characteristics point to a long period of isolation. Other authors have also examined additional specimens of *P. pygmaeus* and have concurred that the taxa is deserving of specific recognition (Genoways & Jones 1975; Jones & Lawlor 1965). An archaeological study of animal use by Cozumel Maya (Hamblin 1984) also identified raccoon bones of reduced stature dating to 1300-1500 years bp. Thus, it is unlikely that the size reduction is a recent (colonial) phenomenon. Recent studies using morphometric data have confirmed this species as a true example of dwarfism (Helgen & Wilson 2004).

Nasua nelsoni:

Coatis are easily distinguishable from related members of the family Procyonidae including the raccoons, ringtails, olingos, and kinkajous. They are notably long, slender, and have a non-prehensile tail equal in length to the head and body and often held vertically erect. The canines are sharper and narrower than in raccoons, and the premolars and molars have comparatively high crowns with sharp cusps.

The Cozumel coati (*N. nelsoni; N. thersites* are synonyms- Thomas 1901) has historically been considered a distinct species because it is strikingly smaller morphologically and cranially than *N. narica* (Merriam 1901; Thomas 1901). Jones and

Lawlor (1965) also retained *N. nelsoni* as a separate species based on its reduced size relative to *N. narica yucatanica* of the adjacent mainland. Glatson (1994) accepted *N. nelsoni* as a separate species but indicated that some researchers believe the Mayans may have introduced the population to Cozumel. Decker (1991) measured males and females of this species and found total length (cm) = 744, 785; length of tail = 348, 328; and length of hind food 79, 84.

In contrast, Decker (1991) contends that the taxonomic status of the Cozumel coati is controversial because original descriptions were based on a small number of highly variable features. Decker compared island and mainland coatis and concluded that based on morphological features *N. nelsoni* should be considered conspecific with the mainland species of *N. narica*. Thus, Decker would designate the island taxa as a subspecies, *N. narica nelsoni* (Gompper 1995). As Decker notes, however, five of the six specimens of *N. nelsoni* used in her study were collected from the same locality at the same time and may derive from a single family unit. A larger, more representative sample of individuals across the island should be collected before conclusions can be made based on the differences in morphology between the two species.

As with Cozumel raccoons, Hamblin (1984) reported the excavation of *N. nelsoni* bones dating to the Classic Period (ca. 1300-1700 years bp) on Cozumel. During Mayan times, the Cozumel coati was widespread; archaeological excavations have found them to be located at several sites on the island with a high number of coati remains being found at each site (Hamblin 1984). Most Cozumel local residents agree that the coati is currently very rare, but was much more abundant only a decade ago (Cuarón et al. 2004).

Conservation Threats:

Little published information exists on the status and abundance of these endangered island endemics beyond a brief raccoon survey carried out in the 1980s (Cuarón et al. 2004; Navarro & Suarez 1989). This survey was carried out before Hurricane Gilbert (1988), which caused severe damage to Cozumel, and the authors emphasized their concern over the hurricane and its influence on the survival of both raccoons and coatis. Since Navarro and Suarez's survey, however, no broad-based field study of these taxa has been carried out and little formal conservation action has been implemented to ensure their protection (Cuarón et al. 2004). Little is known about the ecology of the Cozumel raccoon and virtually nothing is known about the ecology of the Cozumel coati.

There is an urgent need to undertake 'base-line' studies on the small carnivores in biodiversity rich areas like Cozumel (Cuarón et al. 2004). Cozumel Island has been considered as a priority region for conservation by the Mexican government and an 'Important Area for the Conservation of Birds' (AICA) (Cabrera et al. 1998; Escalante et al. 1999). Examples of protected areas on Cozumel include Punta Sur Park, Chankanaab Park, the Mayan ruins areas of San Gervasio and El Cedral; each of these areas is protecting significant habitats of the small carnivores of Cozumel. However, protection remains inadequate for these severely declining populations and further conservation management is needed. Further research is also needed in order to clarify the taxonomic status of dwarf procyonids before appropriate conservation management strategies can be implemented.

In a recent review, Cuarón et al. (2004) list six suspected threats to the Cozumel procyonids: habitat loss and fragmentation, hunting and poisoning, threats from introduced predators, disease spill-over from domestic animals, collection as pets, and genetic introgression.

Habitat loss and fragmentation remain critical issues in Cozumel as elsewhere. Recent touristic development has focused on the coastline of western Cozumel. Perhaps more worrisome is the recent increased development along the cross-island highway which bisects the island along a Northwest-Southeast axis. In 1999-2000, small-scale family farms and home building began along the highway, with a larger complex of home parcels delineated by signage and survey markers at the Atlantic tip of the highway. Development along this highway effectively splits the island into a northeastern region and a southwestern region with reduced opportunities for movement between the two areas.

Other factors which may negatively impact the populations of procyonids on the island including collection of these animals as pets and the practice of hunting; though to date the extent of these activities on Cozumel procyonids is unclear. Navarro and Suarez (1989) reported that because of the potential damage inflicted on agricultural crops the raccoon is often hunted or poisoned in some places such as El Cedral. Signs of hunting have also been observed on Isla de Pasión (Navarro & Suarez 1989) and the northwestern mangrove areas on Cozumel Island. These species are important animals for subsistence hunters (Aranda 1991; Gompper 1995; Hamblin 1984) and increased human hunting pressure has caused a noticeable decline in the population in the past (Valenzuela, personnel communication). Coatis skins have also been known to be sold in Mexico

(Aranda 1991; Gompper 1995) and these animals are an important food source for subsistence hunters (Hamblin 1984; Redford & Robinson 1991) on the mainland. Although no quantitative data exists regarding the prevalence of hunting on Cozumel, a study by Jorgenson (1993) found that the mainland coati (*Nasua nasua*) was one of the mostly frequently harvested taxa. This study also identified the coati as a troublesome crop predator and found large groups of up to 30-40 females and their young causing extensive damage to corn crops (Jorgenson 1993). Other studies (Kaufmann 1987) have found widespread hunting of *N. narica* for food in northern Mexico and has reportedly greatly reduced population sizes.

Carnivores on islands can be especially susceptible to impacts of predators and disease as these species often lack natural enemies, thus they may be relatively defenseless when exotic predators or parasites are introduced (Bowen & Van Vuren 1997; Case et al. 1992; Primack 1998; Van Riper et al. 1986). One such predator, the *Boa constrictor*, is thought to have been introduced on Cozumel in 1971, and now is a concern for the conservation of several endemic and other native terrestrial vertebrates of the island (Cuarón et al. 2004). This species is also of concern for the conservation of procyonids, as large boas are certainly capable of killing adult procyonids (Janzen 1970). Domestic or feral dogs could also be a problem for the procyonid populations; Punta Sur Park managers have observed coatis being chased or killed by dogs within the park (Cuarón et al. 2004).

The movement of diseases from domestic animals into populations of wild animals has recently been identified as an important conservation issue (Daszak et al. 2000) and this appears especially true for carnivores (Funk et al. 2001). Diseases and

parasites pose serious threats to rare species and have likely caused extinctions (MacDonald 1996; McCallum & Dobson 1995). Serious infectious disease outbreaks, such as mange (Valenzuela et al. 2000), rabies, and canine, distemper in the domestic dog population has the potential to spillover into the procyonid populations (McFadden et al., in prep). Epizootic outbreaks of exotic diseases have been identified as a primary threat to many island populations including the island fox populations (*Urocyon littoralis*) on the Channel Islands, California (Garcelon et al. 1992).

The size of feral cat populations on Cozumel is unclear, but a recent localized coati population decline in western Mexico due to a *Notedres cati* (notoendric mange) epizootic (Valenzuela et al. 2000) indicates that domestic cats should also be of concern. The large population of feral dogs on the island also is of concern as disease spillover of viruses is also likely (McFadden et al., in prep).

There is no evidence that hybridization with animals transported to Cozumel from the mainland has had any influence on the Cozumel procyonids. However, pet coatis have been observed on Cozumel (to date there are at least 10 "pet" continental coatis living on Cozumel) and it is likely that all of these were originally captured on the mainland. Should mainland animals escape, or be released, the potential exists for introgression of mainland alleles into the Cozumel population (Glatston 1994). Since the habitat and environment of Cozumel is somewhat different than that found elsewhere in the mainland range of *N. narica* and *P. lotor*, it is likely that strongly coadapted gene complexes exist and thus enhance survival of the island animals. Genetic introgression risks the loss of these coadapted gene complexes.

Specifically, before conservation efforts can be implemented or considered for such a species, scientists must evaluate if the island taxa are indeed a different species than their mainland relatives. Since indigenous peoples are known to have traveled from the mainland to islands, faunal exchange between these two areas are likely common and it is possible that the individuals released onto islands are either founder individuals or are individuals which have simply interbred with animals already existing on the island and thereby hybridizing the species. If the latter is true, such a species may not be significantly different from their mainland counterparts and the management implications for this scenario will consequently be radically different in their scope.

Cozumel Island:

Cozumel Island is unique because of its high level of vertebrate endemism, and contains a distinct biota within the Yucatán Peninsula. Cozumel contains a total of 26 endemic taxa: 3 species and 3 subspecies of mammals, 4 species and 15 subspecies of birds, and 1 lizard species.

Cozumel Island is approximately 486 km^2- with dimensions of 36 km long and 15km wide. It is a relatively flat island, which averages 5 m in elevation, although some areas are twice that. The island is surrounded on the east, south and west by 400 m of water and was clearly not a land bridge to the mainland. The geologic history of the island is complex due to its location on a faulted continental margin and because of the wide variety of geophysical characteristics to this island (Ward 1985). A reconstruction of the geological history of the island was conducted by Ward and Weidie (1985) and is thought to have resulted from faulting off the eastern Yucatan continental margin during the late Jurassic. Cozumel Island was submerged at least two times during the late

Pleistocene (121,000 ± 6,000). During the Wisconsin glaciation, sea level dropped to greater than –100 m between 15,000 and 20,000 ypb (Milliman & Emery 1968; Morner 1971). Sea levels rose through the Holocene to present sea level. Therefore, the earliest time that procyonids could colonize Cozumel Island would have to have occurred well after 121,000 ± 6,000 ypb.

Species Concepts:

The determination of species boundaries is perhaps the most important area of application of phylogeny to conservation biology. Currently, the Cozumel raccoon, but not the coati, is conclusively considered distinct species. The distinction between species and subspecies for these taxa may have important implications in how or if these island endemics are managed in the future. Many species concepts have phylogenetic relatedness as the central focus in the determination of species. Furthermore, phylogeny can be used to assess the degree of genetic isolation between two populations (such as differences between the mainland and island procyonids) (Slatkin & Maddison 1989). Species-oriented conservation programs have historically attempted to analyze and maintain intra-specific variation in order to maximally preserve biological diversity (Amato 1991; O'Brien & Mayr 1991; Vogler & DeSalle 1994; Woodfruff 1989).

The term, "evolutionarily significant unit" (ESU) has come to define a group of organisms that should be the minimal unit for conservation management (O'Brien & Mayr 1991; Ryder 1986; Vogler & DeSalle 1994; Waples 1991). Currently, however, most taxonomic decisions in species conservation are based on the biological species concept as the central criteria (Amato 1991; Vogler & DeSalle 1994). Under this

concept, species are defined as "groups of actually or potentially interbreeding populations that are reproductively isolated from other such groups" (Mayr 1942). The driving force behind this theory was based on the trend that populations that are reproductively isolated often indicate independent evolutionary lineages. However, since the criterion of reproductive isolation or phenetic similarity is problematic, other methodologies are necessary. Because the mainland conspecifics of the dwarf island carnivores (*N. narica and P. lotor*) are known to have been brought over to Cozumel (historically as pets), it is suspected that they may have interbred with the island taxa (Gompper, personal communication). Depending on how strictly one interprets the biological species definition (O'Brien & Mayr 1991), the island taxa of *N. nelsoni* and *P. pygmaeus* could thus be classified as the same species as the mainland taxa of *N. narica* and *P. lotor*, respectively. If the criterion of reproductive isolation is generally applied, the taxa of dwarf carnivores may go unrecognized as evolutionarily differentiated populations and thus separate conservation units.

Biologists are making increasing use of phylogenies to address questions across a broad range of scales (Page & Holmes 1998). A combination of organismal attributes appears to retain the trace of those organisms' evolutionary history better than using only morphological or molecular data alone. Molecular data offers potentially huge data sets that are comparable across a wide taxonomic range. Because different genes evolve at different rates, molecular data can be tailored to different time scales. Genes that show approximate rate constancy allow us to make inferences about times of divergence using molecular clocks.

The alternative approach to defining an evolutionarily significant unit (ESU), than those using phenetic similarity or reproductive isolation, is a theoretical framework which focuses on delimitating taxa under the phylogenetic species concept (PSC) (Cracraft 1983, 1989; de Queiroz & Donoghue 1990; Nelson & Platnick 1981; Nixon & Wheeler 1990). Vogler and DeSalle (1994) define a phylogenetic species as "a cluster of organisms possessing a unique character or a unique combination of characters". The characters used to identify and differentiate these entities include behavioral, ecological, genotypic, and morphological differences (Vogler & DeSalle 1994). Because cladistic and phylogenetic species concepts rely on the most number of attributes, they are the most logical manner to evaluate the diagnosability of populations, especially in cases where advanced biological knowledge (such as in the case of the Cozumel carnivores) is lacking.

In order to delineate species across various taxa, one must also explore intraspecific variation under consistent "species boundaries" or definitions. Population aggregation analysis (PAA)(Davis & Nixon 1992) attempts to "identify the most inclusive groups of organisms united by fixed or diagnostic character states"(Goldstein & Vogler 2000). Such an analysis, when used in conjunction with the PSC has the potential to define species based on close approximations of the population along with important geographic information that may help delineate lineages.

No single species concept is universally regarded as the “gold standard” for species delineation and a large amount of controversy exists regarding which concept is the most precise means to assess a species’ status. Therefore, this dissertation aims to use a combination of species characteristics in its final assessment of the Cozumel

carnivores' official species status. All evidence gained from this dissertation will be used: phylogenetic, morphological, and ecological uniqueness will all be assessed and collectively weighed when designating the species uniqueness of the Cozumel carnivores.

Chapter 2: PHYLOGENETICS OF DWARF CARNIVORES (*PROCYON PYGMAEUS* AND *NASUA NELSONI*) ON COZUMEL ISLAND, MEXICO, USING BOTH MITOCHONDRIAL AND NUCLEAR DNA

Abstract

Mitochondrial and nuclear sequence data derived from a 893bp fragment of the mitochondrial d-loop and a 450bp fragment of the nuclear CHRN region were used to investigate the genetic uniqueness of the endangered and endemic dwarf carnivores of Cozumel Island, Mexico. Previous studies suggest the pygmy raccoon (*Procyon pygmaeus*) is a unique species while the dwarf coati (*Nasua nelsoni*) may be a subspecies of their mainland conspecifics. Results from mtDNA analyses suggest that island coatis may be distinct from their mainland sister taxa, while the mainland and island raccoons seem more closely related. In contrast, nuclear data suggests that the island raccoon seems to be distinguished from its mainland conspecifics while the island coati is not. These incongruencies likely stem from incomplete lineage sorting, coupled with a recent evolutionary radiation from which the island and mainland populations diverged. The average sequence divergence between island and Yucatan coatis and raccoons was approximately 0.510% and 0.469%, respectively. Based on estimates of nucleotide substitution rates for carnivores, this data indicates that both island forms may be recent introductions to Cozumel (*i.e.* in the last 46-51,000 years), yet were likely present on the island well before Mayans populated this region.

Introduction

Mammals found on islands typically differ in body size and physiology from their mainland counterparts. These physical differences have led to the designation of many island populations as taxonomically distinct species. Yet among island mammals, changes in body size in small populations can occur relatively rapidly (Bekoff & Daniels 1984; Brown & Lomolino 1998; Foster 1964), and so these designations remain a source of contention when humans may have played a role in introducing mainland animals during the colonization of an island (Pons 1999, Helgen and Wilson, 2003).

There are several examples in which island mammals are/were perceived as distinct taxa, either because they were living on islands or because they no longer appear phenotypically similar to mainland populations. For example, the island foxes (*Urocyon littoralis*) of the California Channel Islands were likely introduced by a combination of translocation by indigenous peoples 10-16,000 years before present (ypb) and rafting. Despite the relatively short history of these animals, they are recognized as morphologically and phylogenetically deserving of species-level recognition (Gilbert et al. 1990; Goldstein et al. 1999). In contrast, the Guadeloupe raccoon (*Procyon minor*), a taxa whose species designation was also based on its relatively small size, was recently found to be conspecific with mainland *P. lotor* (Helgen & Wilson 2003; Pons et al. 1999) suggesting the organism was released onto the island within the past several centuries. Thus this island raccoon is a "statu nascendi" subspecies not deserving of species-level recognition (Helgen & Wilson 2003; Pons et al. 1999).

This dissertation assesses the phylogenetic distinction of two dwarf carnivores endemic to Cozumel Island, Mexico. Both the Cozumel coati (*Nasua nelsoni*) and the

pygmy raccoon (*Procyon pygmaeus*) have existed on the island of Cozumel for at least several thousand years and are currently recognized as endangered (Cuarón et al. 2004; Hamblin 1984). Skull, dentition, and long-bone morphological differences suggest a ca. 20-30% stature reduction relative to the mainland taxa (*Nasua narica* and *Procyon lotor*) (Cuarón et al. 2004; Decker 1991; Merriam 1901; Thomas 1901). Despite these differences, the taxonomic status of these populations remains unclear, with some suggesting that the taxa may not deserve species-level designation based on morphology (Decker 1991), and that Mayan peoples may have played a role in the colonization of Cozumel by these taxa when they colonized the island ca 2500-1500 years bp (Decker 1991; Glatston 1994). If this is true, then the conservation emphasis that should focus on these taxa may be misplaced. In contrast, if these taxa colonized Cozumel prior to the arrival of Mayans, then a conservation focus of these taxa is extremely pressing given the current low population numbers (Cuarón et al. 2004). The main goal of this study is to assess the origin and phylogenetic uniqueness of these organisms to allow a better understanding of the role Mayans played in the origin of the mammalian fauna of Cozumel and to allow more informed wildlife management decisions.

Merriam (1901) first described the pygmy raccoon as a distinct species from its mainland congener, *P. lotor shufeldti*, based on a number of morphological traits. These include being markedly smaller, both externally and cranially (Merriam 1901). Other authors have also examined additional specimens of *P. pygmaeus* and have concurred that the taxa is deserving of specific recognition (Genoways & Jones 1975; Helgen & Wilson 2004; Jones & Lawlor 1965). An archaeological study of animal use by Cozumel Maya (Hamblin 1984) also identified raccoon and coati bones of reduced stature dating to

1300-1500 ybp. Thus, it is unlikely that the size reduction is a recent (colonial) phenomenon.

Some controversy exists regarding the taxonomic status of the Cozumel coati. This taxa has historically been considered a distinct species because it is "strikingly smaller" morphologically than *N. narica* (Merriam 1901; Thomas 1901). Glatson (1994) however, indicated that some researchers believe the Mayans may have introduced the population to Cozumel, and Decker (1991) contends that the taxonomic status of the Cozumel coati is controversial because original descriptions were based on a small number of highly variable morphological features. Decker examined six island coatis and concluded that based on morphological features *N. nelsoni* should be considered conspecific with the mainland species of *N. narica.* Thus, Decker would designate the island taxa as a subspecies, *N. narica nelsoni* (Gompper 1995).

Both the World Conservation Union (IUCN) and Mexico (SEMARNAT 2002) considers the dwarf coati and raccoon endangered species and recent field work supports that designation (Cuarón et al. 2004). Although anecdotal evidence, such as a decrease in sightings, has been sufficient to allow these taxa to be listed as endangered by the IUCN, such a designation assumes that the populations are indeed unique from the mainland.

While the earliest known evidence of these dwarf procyonids on Cozumel come from Mayan feeding middens and date approximately 1500 ypb (Hamblin 1984). Conservatively, one can assume a range of dates that carnivores could have colonized Cozumel dating from the late Pleistocene (122,000- 11,000 ypb) as this would have been the earliest time that 1) modern procyonids were present in this region (Baskin 1982, 1998) and 2) the earliest time Cozumel permanently remained above sea level. The latter

was determined by geologists who dated various sedimentation types on the island and determined that Cozumel Island had been periodically submerged from the Jurassic until the Pleistocene (Spaw 1978; Ward 1985).

Although the geology of the continental borderland region is complex (Spaw 1978; Ward 1985), it seems plausible that colonization by terrestrial vertebrates may have occurred during the late Pleistocene by overwater dispersal from the Yucatan peninsula. However, a competing hypothesis includes the introduction of vertebrates from the mainland by Mayans colonizing the island approximately 2300 yrs ago (Glatston 1994). An additional consideration is the fact that indigenous peoples are known to have traveled from the mainland to islands, and thus, faunal exchange between these two areas may have occurred at any time after the initial colonization. If this occurred, individuals released onto islands are either founder individuals or are individuals that may have interbred with animals already present on the island, giving the possibility that the modern day taxa are hybrids. If the latter is true, the population may not be significantly different from their mainland counterparts and the management implications for this scenario will consequently be different.

Methods

Study site:

Cozumel Island, located 15 km off the northeast coast of Quintana Roo, is the largest inhabited island off the Yucatán Peninsula. The Canal de Cozumel separates the island from the mainland and is over 90 meters deep indicating that it was not an earlier land bridge to the mainland (Davison et al. 2001; Edwards 1957; Hamblin 1984; West

1964). Cozumel Island likely formed off the continent through faulting during the late Jurassic (Ward 1985). However, since this time, it has been submerged several times and reached its present size approximately 121,000 (± 5,000) ypb (Spaw 1978; Ward 1985) and this would represent the earliest time that carnivores could have colonized the island.

Taxa sampling:

A wide geographic representation of genetic samples was used in this study to investigate the coati and raccoon dispersal to and radiation within Cozumel. These samples include multiple representatives from different geographic localities in the range of the focal taxa and throughout the range of their mainland sister taxa, with special attention given to sampling in the Yucatan peninsula (Table 2.1).

Ten field sites in varying habitat types were selected for trapping on Cozumel Island. Cozumel Island is made up of habitats including dry deciduous forest, mangrove stands, sandy palm areas and multistratal tropical evergreen forests (Terrez et al. 1982). Localities were selected based on extensive interviews of Cozumel Island residents and focused on areas/habitats where residents had previously seen coatis and raccoons. Institutional tissue archives were used for populations inside and outside of the Yucatan region. Of three tanned *N. nelsoni* museum specimens, only one yielded enough DNA to be sequenced; approximately 450 of the 693bp for the *Nasua* d-loop was sequenced for this individual (COZ 2).

Selection of characters for phylogenetic analyses:

Mitochondrial control region (d-loop) was selected because this region has been used in other studies to resolve recent divergences among island procyonids (Pons et al. 1999). This region is highly variable in mammals and evolves at least three to five times faster than the average rate of mtDNA sequence (Horai & Hayasaka 1990). For this reason, it is ideal for examining issues of recent divergence. A type-1 nuclear gene, cholingergic receptor, nicotinic, alpha polypeptide 1 precursor (CHRNA1) was used to corroborate or repudiate the phylogenetic hypotheses developed from mtDNA analyses. This gene was selected based on previous research in closely related carnivore taxa such as mustelids that indicated it contains sufficient levels of variation to resolve recent phylogenetic relationships in carnivores (Koepfli & Wayne 2003).

By using both mitochondrial and nuclear sequences the problem of chance phyletic relationships due to historical association is avoided. Unlinked genes, which are expected to sort independently at divergence events, are useful in increasing the chance that the true set of relationships among the species in question (Pamilo & Nei 1988; Wu 1991).

DNA extraction and sequencing:

DNA was extracted from tissue with the QIAamp DNA purification kit (Valencia, CA) and then amplified by polymerase chain reaction (PCR) using universal primers obtained from the literature (Kocher et al. 1989) combined by those designed from homologous regions from samples sequenced with universal d-loop primers (Table 2.2). Primers for the nuclear region were obtained from the literature (Koepfli & Wayne 2003).

Amplification was carried out in 50µl reactions using 1µl of genomic DNA, 2.5mM $MgCL_2$, 0.2mM of each dNTP, 1.0µl of each primer, 1x PCR Buffer (Promega, Madison), and 0.2 U *Taq* DNA polymerase (Promega, Madison). PCR conditions included an initial denaturation at 95°C for 1 min. followed by 35 cycles of denaturation at 94°C for 1 min., annealing at 63° for 1 min., and extension at 72°C for 1 min. Following the last cycle, an additional step at 72°C for 7 min. was performed. PCR conditions for nuclear DNA were identical to mtDNA PCR conditions but with an annealing temperature of 58°C. PCR products were then purified using the QIAquick PCR Purification Kit (Qiagen, Chatsworth, CA) following the manufacturer's instructions. DNA was sequenced using the ABI 3730xl capillary sequencing machine (PE Applied Biosystems, Inc. 2001). Sequencing reactions were performed using the Big Dye Terminator Ready Reaction Mix version 3.0 and 3.1 (PE Applied Biosystems, Foster City, CA) and purified following the manufacturer's instructions. Mitochondrial DNA sequences from 22 *Nasua* and 30 *Procyon* were obtained.

Phylogenetic Data Analysis:

Most taxa were represented by at least two individuals; when only one individual was available, several sequences were compared from independent PCR amplifications. In no case did multiple amplifications of the same individual result in two different sequences. A minimum of five *P. pygmaeus* from each of three trapping sites on Cozumel were sequenced. Outgroups for the coati analyses were *N. nasua* and *P. lotor*, while outgroups for raccoon analyses were *N. narica* (Table 2.1). Outgroup taxa were identified based on a previous molecular analysis of the Procyonidae which has identified

Nasua and *Procyon* as sister genera (Decker 1991; Pons et al. 1999). A 631 base pair portion of the control region for *Nasua* and an 893 bp portion for *Procyon* were sequenced, and a 450 bp portion of the nuclear CHRNA gene were both aligned using Clustal X version 1.7 (Thompson et al. 1994) and Sequencher version 4.0 (GeneCodes Corporation, Ann Arbor, MI). Nucleotide positions in the d-loop containing gene gaps and ambiguous alignment were excluded to avoid uncertain positional homology.

Based on other carnivore studies (Koepfli & Wayne 2003), the interphotoreceptor retinoid-binding protein (IRBP) gene was initially examined, but found no significant variability was found in this gene. After a preliminary examination of the CHRNA gene indicated some variability, it was selected for further analysis. A random subsample of the total number of individuals were sequenced; subsamples were selected because both preliminary analysis indicated moderately-low levels of genetic variability.

Pairwise distances were estimated by using Kimura's (1980) 2-parameter model and using the model of evolution selected by ModelTest (Posada et al. 2000). Three standard phylogenetic methods were used: maximum parsimony (MP), maximum likelihood (ML) (Felsenstein 1985), and Bayesian inference (BI). PAUP 4.0b2 (Swofford 2002) was used to determine the most-parsimonious tree (s) for the ML and MP analyses using a heuristic search on the unweighted, unordered sequence data. To explore the effects of gaps on the data, all analyses were conducted both with gaps coded as missing and as a 5^{th} character. Both treatments resulted in identical topologies.

Data from the different genes were analyzed separately in order to identify patterns of congruence (Miyamato & Fitch 1995). Alternative topologies were compared for robustness and support based on intrinsic support (optimality criteria). To assess

confidence in parsimony analyses parametric bootstrapping was conducted (BP, Felsenstein 1985) based on 1000 replicates and Bremer support (BS, (Bremer 1988, 1994)). The program AutoDecay version 4.0 (Eriksson 1998) performed "decay analysis" of nodes in MP cladograms or consensus trees to assess support for nodes of interest.

When conducting ML, the appropriate model was selected based on likelihood-ratio tests with nested sets of parameters (Huelsenbeck & Crandall 1997) using ModelTest version 3.1 (Posada et al. 2000). Using this model, a successive approximation search was performed to estimate a maximum-likelihood topology (Swofford 2002) through PAUP* version 4.0b2. Using a neighbor-joining tree, starting parameter values were estimated and used in an initial maximum-likelihood search. Parameters were there re-estimated from the resulting tree and the search was repeated with these new parameters. This procedure was terminated when the resulting tree was identical in topology to that from the previous iteration. Parametric bootstrap values were obtained by setting parameter values of the ModelTest mode of evolution to values estimated from the likelihood tree, then performing 500 replicates with random addition of taxa and nearest-neighbor interchange branch swapping.

Bayesian analysis (BI) was implemented using MrBayes 3.01 (Huelsenbeck & Ronquiest 2001), which calculates Bayesian posterior probabilities using a Metropolis-coupled, Markov chain Monte Carlo (MC-MCMC) sampling approach. Three separate MCMC runs were performed starting from random trees for each of four simultaneous chains. Runs were one million generations and had burn-ins ranging from 30-100 generations, default prior distributions for model parameters, and the differential heating

parameter set to 0.2. All data collected at stationarity were used to estimate posterior nodal probabilities and a 50 % majority consensus phylogeny.

To compare the distribution of molecular variation, an analysis of molecular variance (AMOVA) (Excoffier et al. 1992); was performed on the mtDNA and nDNA using Arlequin version 2.0 (Schneider et al. 2000). The distribution of genetic variation between the continental and insular forms was assessed. Arlequin (Schneider et al. 2000)was also used to estimate a range of genetic parameters and distributions such as mean nucleotide diversity (π), mean pairwise sequence differences, genetic distance, and haplotype diversity.

Finally, I examined phylogenetic relationships among control region sequences using a statistical parsimony network, in which sequences are the nodes of a network rather than the terminal tips of a tree using the program TCS version 1.13 (Clement et al. 2000). However, the *Procyon* data did not provide sufficient variability and the resulting parsimony network was incongruent to known geographic haplotype groupings. For this reason, a minimum spanning network was used to examine geographical haplotype relationships in *Procyon*.

Relative-Rate Test:

The number of nucleotide substitutions per site (K) was calculated with Kimura's two-parameter method (1980) employing RRtree version 1.1 (Lyon Cedex France), (Robinson-Rechavi & Huchon 2000). The rate of molecular divergence was measured by creating a molecular clock in which the rate of nucleotide substitution (or fixation) was calculated. The standard deviation around the divergence date was calculated using the

standard deviation of the genetic distance between island and mainland taxa. A relative rate test was used to estimate the difference in the number of substitutions between two closely related taxa in comparison with a third, more distantly related outgroup species. The advantage of this test is that it does not require knowledge of the divergence times of the taxa in question. Although molecular evolutionary rates clearly vary among taxonomic groups and genes (Avise 1994; Marshall et al. 1994; Wayne et al. 1997), this study examined the same gene regions in closely related groups and divergence times range over a relatively narrow interval (Pons et al. 1999). Therefore, rate discrepancies are likely to be less pronounced. One limitation in creating a molecular clock with these taxa may be periodic introgressive hybridization between mainland and island lineages. Even low rates (as low as 1 in 100,000) may be enough to upset the phylogenetic trees and molecular clocks created in this study (Clarke & Grant 1996; Clarke et al. 1996; Myers & Giller 1988) and thus the results of the molecular clock must be interpreted with caution.

Results.

Procyon

Sequence Variation:

Of a total of 893 bp sequenced from the mtDNA, 32 were parsimony informative and 6 bp of 450bp were informative in the nDNA. A Chi-square test of homogeneity of base frequencies across taxa found no significant heterogeneity in the mtDNA (X^2 = 6.824, df = 36, p = 0.9899) or the nDNA (X^2 = 2.83, df = 12, p = 0.8777). The mean number of pairwise differences and nucleotide diversity between *Procyon* is shown in

Table 2.2. Among 30 *Procyon*, 11 mtDNA and 2 nDNA haplotypes were distinguished. Of the mt loci, there were 27 polymorphic sites of which all were substitutions (25 transitions, 2 transversions) (Table 2.3). This high transition to transversion rate is usual for control region sequence analyses involving conspecific populations (Binkham et al. 1996; Pons et al. 1999; Thomas et al. 1990). No polymorphic sites were found in the nDNA but six heterozygous alleles were found.

All Cozumel *P. pygmaeus* individuals shared the same mtDNA haplotypes. Other haplotypes grouped together phylogenetically based on geographic localities. Average sequence divergence between all non-island haplotypes was 1.82%. The Yucatan region was represented by four different haplotypes with an average sequence divergence of 0.398% (Table 2.5). The island haplotype was most similar to the Y2 and Y4 haplotypes and only differed by 0.29% (Table 2.5). The Western Mexico haplotype differed from the Cozumel haplotype by 0.738%, and from the Yucatan haplotypes by 1.14%. The percentage of divergence between the four mainland subpopulations is approximately 4 times higher than the estimate found when comparing the insular *P. pygmaeus* with all Yucatan *P. lotor*.

The average nDNA sequence divergence between all individuals was 0.4% (Table 2.7). The average nDNA sequence divergence for all Yucatan individuals (including those from Cozumel Island) was 1.23% while the average for individuals from the USA was 0.19%.

Mitochondrial Phylogenetic Analyses:

In pairwise comparisons between island and all mainland populations, F_{st} was significant (F_{st} = 0.516, p = 0.009) (Table 2.4). Pairwise comparisons between the Yucatan (including Cozumel) and the United States haplotypes were also significant (F_{st} = 0.131, p = 0.022). However, when a comparison of the United States, Yucatan and Cozumel was performed, pairwise comparisons between the United States and the Yucatan became insignificant (p= 0.144). Pairwise comparisons between the Yucatan and Cozumel were significant (F_{st} = 0.527, p = 0.001), as were the comparisons of the United States and Cozumel (F_{st} = 0.550, p = 0.003).

The best-fit model determined by ModelTest was HKY + Γ (Hasegawa et al. 1985). This model assumed unequal base pair frequencies (0.307, 0.252, 0.166), a transition/transversion ratio of 15.03, gamma shape of 0.094 and two substitution types. The topologies of ML and BI were almost identical; six distinct lineages were recognized among the eleven haplotypes. These haplotypes corresponded to the broad geographical regions of the species range that were sampled. The haplotypes included one distinct Yucatan clade (haplotypes Y_1, Y_3), a United States clade (NE_1, NE_2, NMX, TN_1, TN_2), a Western Mexico clade (W_1 and W_2), and a Cozumel lineage (COZ) (Figure 2.1). The Bayesian/ML topologies placed the haplotypes from the United States monophyletic to all Yucatan haplotypes, including the Cozumel lineage (posterior probability = 100%). The Cozumel lineage was placed with the Western Mexico individuals (Figure 2.1), though this grouping has poor support (bootstrap = 52%). Together, the W. Mexico/Cozumel clade is embedded within all other Yucatan haplotypes.

Although the ML and MP trees were not identical, several clades were consistently recovered. Like the ML topologies, MP consensus trees pair the Cozumel lineage with the Western Mexico haplotypes (Figure 2.2). However, this grouping collapses when bootstrap analyses are performed (values <50%). Decay analysis on the MP cladogram showed some support for the Cozumel-Western Mexico groupings (DI= 3). In contrast, a Bayesian 50% majority rule consensus tree gave a 68% posterior probability of the Cozumel-Western Mexico grouping. Bootstrap values for the Cozumel and Western Mexico grouping were also low in the neighbor-joining tree (68%).

A population aggregation analysis of the *Procyon* mtDNA identified no fixed diagnostic nucleotide positions in the mitochondrial d-loop gene that distinguished *P. pygmaeus* from other continental *P. lotor*. The two Yucatan haplotypes Y2 and Y4 are the most similar to the Cozumel haplotypes (Table 2.6).

A minimum spanning network was created based on the number of substitutions between haplotypes (Figure 2.3). Like the phylogenetic trees, the network grouped all Mexican haplotypes closely and supported a close grouping of the Western Mexico-Cozumel and Yucatan-Cozumel clades.

Nuclear Phylogenetic Analyses:

More than one haplotype was found in the nDNA dataset for *P. pygmaeus* (Figure 2.4). Interestingly, the two haplotypes identified in the island taxa did not correspond to different trapping sites on the island. The best-fit model determined by ModelTest for the nDNA was K80 (K2P). This model assumed equal base pair frequencies, a transition/transversion ratio of 3.452, and equal rates of substitution. Groupings in the

ML analysis mirrored those of the Bayesian analysis in that identical structuring was found, but when both a 50% majority rule consensus tree and bootstrap analysis were applied, all groupings collapsed, indicating very little structure amongst haplotypes. Topologies from ML, MP, BI and NJ were identical and grouped three Cozumel individuals together with a Yucatan haplotype (MP bootstrap= 60%). The remaining Yucatan haplotypes (n = 5) also grouped together (bootstrap = 65%), while all other haplotypes showed no strongly supported structure. When a decay analysis was performed on the MP topology, the Bremer support for the Cozumel-Yucatan clade equaled one, indicating low support for these groupings. A Bayesian analysis of the nuclear data showed identical structuring of the haplotypes found in the MP analysis; the weak posterior probability values (50%) provided little support for these groupings and they collapsed in a 50% majority rule consensus tree.

Divergence Rates:

Using RrTree, the Tajima-Nei rate test was implemented to compare rates of evolution between island (*P. pygmaeus*) and mainland (*P. lotor*) individuals. Results indicated I could reject the hypothesis of non-clock like behavior in *Procyon* (number of nucleotides = 415, p = 0.659). The sequence divergence (based on HKY distance) between the Yucatan and Cozumel *Procyon* is 0.4697% ± 0.00215. A divergence rate of about 10% per million years has been used in other carnivore d-loop data sets (Irwin et al. 1991; Vila et al. 1999). Thus, based on this divergence rate, a coalescence of *Procyon* haplotypes of about 46,970 ± 21,510 years ago is implied (range 25,460- 68,480 ypb).

Nasua

Sequence Variation:

A Chi-square test of homogeneity of base frequencies across taxa found no significant heterogeneity in the mtDNA ($X^2 = 2.73$, df = 30, p = 1.00). Of a total 30 polymorphic sites there were 21 sites in the mtDNA (631bp) gene region characterized with substitution. There were 5 sites in the mtDNA characterized with insertion/deletion. Among 22 *Nasua*, there were 10 haplotypes (Table 2.3). The pairwise comparisons revealed that the average sequence divergence among the 10 haplotypes was 1.38 % (range 0.168 - 4.00, Table 2.3). The percent divergence between the four mainland subpopulations is approximately 2.4 times higher than the estimate found when comparing the insular *N. nelsoni* with the Yucatan *N. narica* (Table 2.8). In contrast to the nuclear DNA results of *Procyon*, the nDNA (402bp) region for *Nasua* produced no discernable signal.

Phylogenetic Analyses:

In pairwise comparisons between mainland and Yucatan populations (including Cozumel), F_{st} was not significant (P = 0.072) (Table 2.4). Because the among-group variance component was negative, data was then reanalyzed without the group hierarchy (*i.e.* grouping the Yucatan haplotype with all other continental). The F_{st} pairwise comparison between all mainland haplotypes to the Cozumel haplotype was also not significant (p= 0.061).

The best-fit model determined by ModelTest was HKY + Γ (Hasegawa et al. 1985). This model assumed unequal base pair frequencies (0.283, 0.259, 0.182), a

transition/transversion ratio of 3.28, Gamma shape of 0.014 and two substitution types. Topologies of ML and Bayesian analyses were identical; *N. nelsoni* was monophyletic to all Yucatan and Belize *N. narica* (Figure 2.5). When a parametric bootstrap (ML, 58%) and posterior probability analysis (Bayesian, 63%) were performed, the monophyly between the Cozumel and the Yucatan/Belize clade collapsed and generally indicate no strong support for this grouping.

Maximum parsimony analysis placed *N. nelsoni* basal and monophyletic to all Yucatan/Belize coatis (Figure 2.6). A 50% majority rule parametric bootstrap provided marginal support for this grouping (64%) but Bremer support for this node showed only one step was necessary to collapse this grouping.

A single diagnostic polymorphism at basepair 232 differentiates *N. nelsoni* from the Yucatan *N. narica* (Table 2.9). Many of the polymorphisms found in *N. nelsoni* indicate that the island haplotypes may be more closely related to Belize haplotypes and include base pair numbers 213 and 617. The second Cozumel sample (COZ2) came from highly degraded source material and is therefore missing a 200bp fragment. Nonetheless, this sample is clearly that of *N. nelsoni* as it was collected on Cozumel and also contains the single diagnostic polymorphism that characterizes this species.

A 95% statistical parsimony network produced a relatively uncomplicated network in which the only alternative connections were either between haplotype Y2 and the other Yucatan haplotypes (Figure 2.7). On average, within-mainland and within Cozumel haplotypes differed from one another by 5.75 and 1.0 nucleotide substitutions, respectively. This network provided additional support for the Belize-Cozumel relationship.

Divergence Rates:

Rates were tested between island (*N. nelsoni*) and mainland (*N. narica*) using the South American *N. nasua* as an outgroup. Data from RRTree indicated the hypothesis of non-clock like behavior could be rejected in *Nasua* ($p = 0.146$). The first definite record of the extant *Nasua* in Central America is from the late Hemphillian Bone Valley of Florida (latest Miocene to early Pliocene, ~5 Ma) and also possibly from California and were dated in the late Pleistocene (Baskin, 2003). Like *Procyon*, the minimum date for divergence of *N. nelsoni* from *N. narica* was during the Pleistocene. The sequence divergence (based on HKY + Γ distance) between the mainland and island taxa is 0.506% (± 0.0015). Assuming a divergence rate of about 10% per million years (Aquadro & Kilpatrick 1981; Vila et al. 1999) a coalescence of *Nasua* haplotypes of about 50,600 ± 15,580 years ago is implied.

Discussion

Analyses of mtDNA and nDNA genetic variation among *Procyon* and *Nasua* revealed a moderate amount of sequence divergence between samples from distant geographic regions. The Cozumel taxa appear to be most closely related to their Yucatan conspecifics and bootstrap support and decay indices do not support the recognition of distinct island – mainland clades.

Given the small sample size for *N. nelsoni*, it is difficult to make conclusions about this population's haplotype diversity. Mitochondrial phylogenetic data indicates that the *N. nelsoni* Cozumel haplotypes may be phylogenetically unique enough to

deserve species-level status based on phylogenetic criteria alone. With three mtDNA polymorphic sites in *N. nelsoni* that distinguish it from its closest Yucatan conspecifics, and one polymorphic site that distinguishes it from all other coati haplotypes, an argument for their phylogenetic uniqueness is stronger than that for *P. pygmaeus*. However, nuclear data shows no variability between island and mainland populations. The incongruence between nuclear and mtDNA might be partly explained by incomplete lineage sorting because it is known that the island taxa are of relatively recent origin. Unfortunately, the precise age of the island coatis are not known due to the lack of *Nasua* fossils on the island of Cozumel. Due to the fourfold smaller effective population size of mtDNA compared with nDNA, lineage sorting is expected to reach completion much faster in mtDNA. Hence, if the differences between the two trees are truly caused by differences in the extent of lineage sorting, the mtDNA tree is likely to represent the species tree most accurately. However, without further genetic samples it is difficult to fully propose that the dwarf coati should be a unique species, based on phylogenetic criteria alone, and any such designation should be taken with caution.

Mitochondrial phylogenetic data indicates that the *P. pygmaeus* Cozumel haplotype is not phylogenetically unique enough to deserve species-level status based on phylogenetic criteria alone. There were no fixed polymorphic sites in *P. pygmaeus* that distinguish it from its closest Yucatan conspecifics. Interestingly however, nuclear data shows some variability both within island haplotypes and between island and mainland populations. Like the coati, the incongruence between nuclear and mtDNA can be partly explained by incomplete lineage sorting because it is known that the island taxa are of relatively recent origin. A single haplotype represented all individuals sampled from *P.*

pygmaeus indicating that the population either has experienced a severe population bottleneck or that the current haplotype represents the founder population. If the latter is true, the population on Cozumel has not been on the island long enough to diverge. Alternatively, catastrophic events such as periodic hurricanes may have kept populations numbers so low that divergence was never achieved. As a tropical island, Cozumel is frequently hit by hurricanes. The last catastrophic hurricane (Gilbert, category 5, known at the time as "the storm of the century") hit Cozumel in 1988 and reports from locals indicate that wildlife on the island suffered tremendous losses during this storm that had storm surges of up to 8 meters. Hurricanes of varying intensity hit Cozumel Island every 8.31 years (Williams 1998) and may be partly responsible for inducing population bottleneck events in wildlife populations. Healthy wildlife populations are able to withstand such losses and have done so for eons. However, hurricanes can have severe impacts on endangered species such as *P. pygmaeus* and *N. nelsoni*.

Generally, morphologic and genetic variation in island populations are lower than in similar sized mainland populations (Soulé et al. 1975; Van Valen 1962). This decreased level of genetic variation may reflect the degree of isolation, the effective population size, and the current number of generations since founding (Wright 1969) and may explain the lack of genetic variability in *P. pygmaeus*. The identification of a single haplotype in *P. pygmaeus* also likely indicates a high level of gene flow between subpopulations. Extensive trapping on the island of Cozumel by the author, however, does not entirely support this scenario. Despite intense trapping efforts throughout the island, *P. pygmaeus* were only sited and trapped in a small region in the NW area of Cozumel. The distance between subpopulations in this region was relatively small, yet

animals from each site were not trapped at adjacent sites. However, given the relatively small distance from each trapping site, it is likely that some amount of gene flow between these populations exists.

Within carnivore species, variability in the control region, estimated by the mean sequence divergence, vary from 0.94% for the giant panda, 2.9% in other *Procyon* taxa, to 4.3% in the California sea lion (Pons et al. 1999). The 1.8% sequence variation in *Procyon* and 1.38% in *Nasua* are slightly lower than the variation found in other studies examining *Procyon* (Pons et al. 1999) but still well within the range of genetic variability observed in other carnivore species. Low levels of genetic variability have also been reported in other studies of *P. lotor* (Beck & Kennedy 1980).

Although a thorough geographic sampling for the complete range of *Procyon* and *Nasua* is lacking, based on the samples analyzed in this study, haplotype diversity for both *Procyon* and *Nasua* appears relatively high. Haplotype comparisons show that the genetic distance between the Yucatan haplotypes and the island are relatively smaller than between other haplotypes in Central and North America for both procyonids, which corresponds to the logically shorter evolutionary time between mainland and island divergence times. The closest genetic distance to *P. pygmaeus* haplotypes were Y2 and Y4 and indicate that they are more closely related than any other haplotypes. However, the phylogenetic analyses consistently grouped Western Mexico haplotypes with the Cozumel Island haplotype, indicating that the individual founders might have either come from Western Mexico or were closely related to a Western Mexico individual.

The nuclear DNA fragments were of limited utility in resolving the relationships within the island-mainland clades. Both their short length and slow rate of evolution

likely contributed to the lack of informative characters needed to adequately resolve nodes of the mtDNA trees. In addition to the low among-site rate variation, it is likely that the island-mainland divergences have not existed for a long enough period for any meaningful signal to be derived from their sequences.

Procyon nDNA contained more sequence variability than the homogenous sequences of *Nasua*. This is likely to due to the different social structures of the two carnivore species. In part, the explanation may be due to the smaller effective population size of mtDNA. *Procyon* are generally solitary and males are more likely to disperse than females. However, raccoons are also known to be more adaptable than coatis to varying habitats and have a wider range than *Nasua*. Thus, it is likely that there are higher levels of gene flow in *Procyon* than in *Nasua*, which would explain the differences in the nDNA variability.

Nuclear and mtDNA markers provided contrasting estimates of male and female dispersal and population subdivision among localized populations. By examining nuclear DNA only, one might conclude that most pairs of populations were not distinct, that there was a high level of gene flow between them, and thus there would be very little impetus to protect the island taxa. However, examination of mtDNA haplotype data shows some degree of geographic structuring does exist and that it is likely that a sufficient amount of time has not existed for full differentiation to be identifiable.

Based on their smaller physical stature and cranial characteristics, Merriam concluded that *P. pygmaeus* was conspecific to *P. l. shufeldti* of the Yucatan but a separate species. Recent studies relying on morphometric measures of specimens from Mexico (Helgen & Wilson 2004) have confirmed the morphological uniqueness of the

pygmy raccoon to its mainland conspecifics. Genes affecting morphologic traits tend to vary in their mutation rate and phenotypic expression, but levels of morphologic variation in mammals may increase rapidly (Gruneberg 1963; Wayne et al. 1986; Wayne & O'Brien 1986). Morphometric results based on my research indicate that *P. pygmaeus* is well below the average mass and length of most North American raccoons, with the notable exception of those found in the Florida Keys (Goldman 1950). Based on morphometric characters of *P. l. shufeldti* on the Yucatan, compared with the pygmy raccoon, it is clear that the pygmy species is a true dwarf. Based on what is known of the small body size of *P. pygmaeus*, results do suggest that the Cozumel raccoon is a unique species or at least a unique conservation unit.

Some sorting of lineages for the Cozumel carnivores has occurred but the full lineage sorting expected in phylogenetically unique species is not present. A notable example of this incongruence between morphometric and phylogenetic data exists in other carnivore taxa such as the brown bear and the polar bear. Mitochondrial DNA analyses detected only a 4.6 % sequence divergence (cyt-b gene) between these two Ursids despite their clear phenotypic differences and corresponds to their recent divergence time of approximately 300,000- 400,000 ypb (Cronin et al. 1996; Talbot & Shields 1996a, b).

Divergence Dating:

Procyonines have the poorest fossil record of any carnivore family, presumably because they inhabit environments not well represented in the fossil record such as the tropics (Baskin, 1998). The modern raccoon species does not appear in the fossil record

until the late Irvingtonian (late Pleistocene) deposits in Florida (Kurten and Anderson 1980, Baskin 1998, 2003). Because Cozumel Island is not thought to be an earlier land bridge to the mainland, the earliest possible time that taxa could have colonized the island would be during the Pleistocene (Ward 1985). As the molecular clock data indicate that the island raccoons diverged approximately 47,570 (± 21,510) years ago, there is no reason not to believe that there was at least an existing population of raccoons on Cozumel when the Mayans colonized on the island. An alternative carnivore colonization hypothesis may be that procyonids arrived on Cozumel just after the Pleistocene and the level of divergence that is currently seen in the extant population represents genetic introgression resulting from hybridization events from introduced procyonids from either modern-day or Mayan times. Considerable caution should be used in the literal interpretation of the divergence times, given that mutation rates are estimated from a single gene and problems of inaccuracy in the molecular clock (Gibbons 1998; Lynch & Jarrell 1993).

Combining multiple criteria such as phylogenetic, morphometric, ecology, range and natural history makes a species designation more robust. Based on the above stated criteria, and data presented in the following chapters, I propose both the *P. pygmaeus* and *N. nelsoni* retain their species status. In the case of *N. nelsoni*, it seems likely that this species is indeed phylogenetically unique, and thus should be treated as unique conservation units. Not only does the Cozumel coati appear to fulfill the phylogenetic species concept, but they also appear to be evolutionary species unit based on the population aggregation analysis concept of Davis & Nixon (1992) which requires that only a single diagnostic character state be 'fixed' in a species.

The lack of larger amounts of genetic divergence between the island and mainland populations is not surprising given the divergence date calculated from mtDNA. The low amount of genetic differentiation suggests that sufficient time has not existed for full phylogenetic differentiation to occur. With the exception of introduced mainland conspecifics onto the island, the isolation associated with an island environment prevents a large amount of gene exchange with the mainland and is likely to warrant different selective pressures that might favor short-term genetic differentiation. If conservation management strategies limit the amount of mainland animals entering Cozumel, one might expect this short term differentiation would eventually lead to a long-term speciation process. Based on phylogenetic analyses, it appears that the Cozumel carnivores are in the midst of their own separate evolutionary path and may eventually become monophyletic from their Yucatan conspecifics, which were their probable mainland founder population. The rational for species level recognition for *P. pygmaeus* is further bolstered by morphological studies (presented in chapter 5), which confirm this taxa's distinctiveness.

The IUCN designation of endangered species status for the two Cozumel carnivores is still needed as formal conservation policies to protect these taxa are lacking. Even if under the strictest phylogenetic definition the Cozumel carnivores are not yet fully genetically unique, there is no reason to change their legal status or to not encourage future conservation policies. The Cozumel carnivores appear to have inhabited the island well before the Mayans colonized the island and should be considered endemic to the island. In terms of future conservation management strategies, one recommendation rising from this study is for local conservation biologists and managers to prevent the

introduction of mainland carnivores onto the island. Currently, there are no such management plans in place to avoid hybridization or genetic introgression of island animals with released mainland procyonids. The prevention of introducing mainland carnivores onto the island will protect the genetic integrity of the island procyonids and also will minimize the introduction of mainland pathogens (distemper, rabies, etc.) into this genetically naïve population.

Table 2.1: List of genetic samples; all samples listed are from blood or tissue samples.

Species	**Haplotype**	**n**	**Geographic Origin**	**Source**
Nasua nelsoni	COZ1, COZ2	2	Cozumel Island, MX	KM/KU
N. narica	Y1-Y4	13	Yucatan Peninsula, MX	KM
N. narica	ARZ	2	Arizona	MG
N. narica	PAN	2	Panama	MG
N. narica	BZ1-BZ2	2	Belize	BZ
N. Nasua	NABOL	1	Bolivia	MG
Procyon pygmaeus	COZ	15	Cozumel Island, MX	KM
P. lotor	Y1-5	9	Yucatan Peninsula, MX	KM
P. lotor	W1-W2	2	Western, MX	DV
P. lotor	NE1	5	New York, USA	MG
P. lotor	NE2	2	Connecticut, USA	MG
P. lotor	TN1-2	2	Tennessee, USA	MG
P. minor		1	Guadeloupe	GenBank**

*Sample sources include: Belize Zoo (BZ), Kate McFadden (KM), David Valenzuela (DV) (UNAE), Matt Gompper (MG), and University of Kansas (KU). **Pons et al. (1999)

Table 2.2: Amplification and sequencing primers for the control and CHRN gene regions for *Procyon* (P) and *Nasua* (N). Primers designed to be species specific are noted as follows: *Nasu*a (N), *Procyon* (P), Carnivores (C) and not specific (NS); the corresponding strand.

Name	**Sequence 5'- 3'**	**Species Specific ?**	**Strand**
HD	GCATTAGTGGTTGCCCC	N/P	H
NAS-F	GAAGAAGCAACAGCCACAC	N	L
NAS-R	CGTGTGTATGTCCTGTGACC	N	H
PROC-F	ATCTCGCCATCAGCACCCAAG	P	L
PROC-R	AAAGAGGTGCTCGGGGTTGAAC	P	H
R483	GGGCTGATTAGTCATTAGTCCATC	N/P	H
F285	GAAACCATCAATCCTTGCG	N/P	L
R982	TTGTGCGTTCTTGGAGTTACGGGG	N/P	H
F982	CAAGAACGCACAAATACCTG	N/P	L
H16498 (Kocher et al. 1989)	CCTGAAGTAAGAACCAGATG	NS	H
283 (Kocher et al. 1989)	TTACACCAGTCTTGTAAAA	NS	L
282 (Kocher et al. 1989)	AAGGCCAGGACCAAACCT	NS	H
CHRNA-F (Lyons et al. 1997	GACCATGAAGTCAGACCAGGAG	C	L
CHRNA-R (Lyons et al. 1997)	GGAGTATGTGGTCCATCACCAT	C	H

Table 2.3: For each population of mainland and island sequence data the sequence length (SL), number of haplotypes and number of individuals (sample size) sequenced, the gene diversity (± standard deviation), the number of transitions (N_s) and transversions (N_v), the mean number of pairwise differences between individuals, nucleotide diversity (π ± standard deviation), and the average number of synonymous substitutions per synonymous site (K).

Species	**Gene**	**SL (bp)**	**Haplotypes (Sample Size)**	**Gene Diversity ± S.D.**	**N_s**	**N_v**	**Mean pairwise differences**	**Π ± S.D.**	**Mean K**
P. lotor	Mt	893	11 (15)	0.892 ± 0.102	24	2	8.27± 4.080	0.019 ±0.010	0.143
P. pygm	Mt	893	1 (15)	1.0	0	0	0	0	0.141
P. lotor	N	450	7 (11)	0.848 ± 0.045	5	1	1.16±0.778	0.0028 ±0.002	na
P. pygm	N	450	2 (4)	0.452 ± 0.168	0	0	0	0	na
N. narica	Mt	631	8 (17)	0.888 ± 0.048	26	5	5.75± 2.895	0.0092 ±0.005	0.082
N. nelsoni	Mt	631	2 (2)	1.000±0.5000	0	0	1.0 ± 1.0	0.0022 ±0.003	0.077

Table 2.4: Pairwise F_{st} probability values among species of procyonids. *Procyon lotor* (USA) represents individuals from New Mexico to the NE United States while *P. l. shufeldti* represents all Yucatan individuals. *N. narica* represents individuals from Arizona, Panama and Belize, while *N. narica yucatecan* represents only those haplotypes from the Yucatan region.

		1	**2**	**3**	**4**	**5**
***P. lotor* USA**	**1**	--				
P. l. shufeldti	**2**	0.1146	--			
P. pygmaeus	**3**	0.0039	0.0019	--		
N. narica	**4**	Na	Na	Na	--	
N. narica yucatecan	**5**	Na	Na	Na	0.0072	--
N. nelsoni	**6**	Na	Na	Na	0.9990	0.0615

Table 2.5: Average HKY85 pairwise distances for mtDNA sequence data between different haplotypes of *Procyon* examined in this study. Haplotypes from the Yucatan (Y1-4), Northeast USA (NE1-2), Tennessee (TN1-2), New Mexico (NMX) and Western Mexico (W1-2) are examined.

	NARICA	**COZ**	**Y1**	**Y2**	**Y3**	**Y4**	**NE1**	**NE2**	**TN2**	**TN1**	**NMX**	**W1**
NARICA												
COZ	0.206677											
Y1	0.209833	0.007053										
Y2	0.205047	0.003518	0.005871									
Y3	0.210008	0.005877	0.003514	0.004696								
Y4	0.206690	0.002342	0.004691	0.00117	0.003518							
NE1	0.210078	0.023955	0.021478	0.022729	0.022729	0.021505						
NE2	0.213238	0.021477	0.019021	0.020259	0.020259	0.019043	0.007053					
TN2	0.213039	0.027585	0.027554	0.026353	0.023896	0.025123	0.022674	0.030027				
TN1	0.210010	0.026416	0.026382	0.025184	0.022727	0.023954	0.021505	0.028856	0.003513			
NMX	0.148968	0.017615	0.019136	0.014521	0.017616	0.016069	0.01829	0.019853	0.024544	0.022652		
W1	0.223149	0.009024	0.017898	0.010500	0.016027	0.010675	0.03367	0.03205	0.035907	0.037882	0.023568	
W2	0.120383	0.005744	0.013239	0.006034	0.011397	0.005709	0.028215	0.025834	0.027663	0.029598	0.015737	0.004519

	NARICA	**COZ**	**YUCATAN**	**USA**
NARICA				
COZ	0.206677			
YUCATAN	0.206690	0.004697		
USA	0.199067	0.023410	0.021777	

Figure 2.1: Bayesian 50% majority rule consensus and ML bootstrap tree based on mtDNA from *Procyon*. Posterior probability and bootstrap values over 50% are labeled above branches.

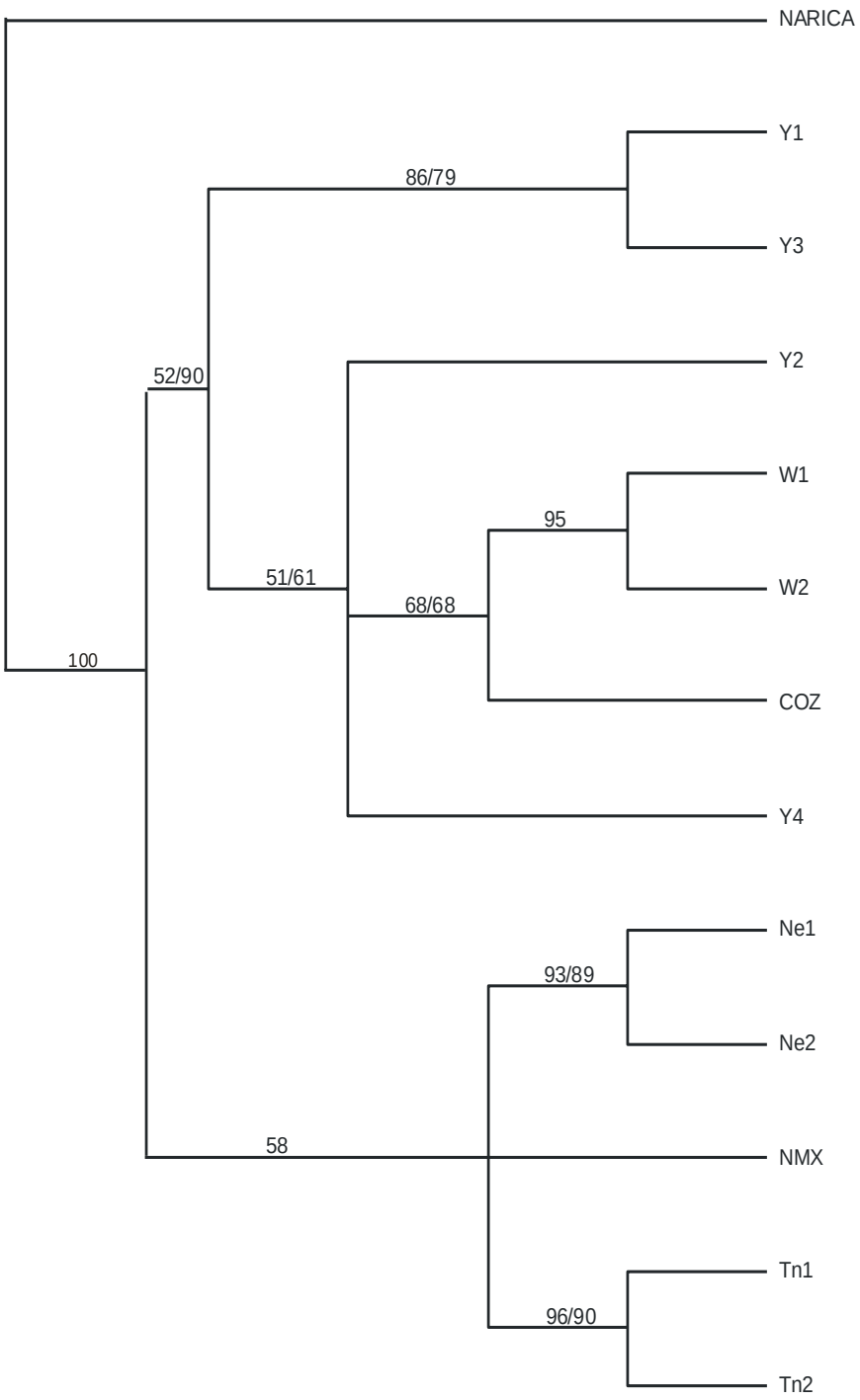


Figure 2.2: Maximum parsimony tree of mtDNA from *Procyon*. Numbers above branches are 50% majority rule bootstrap values based on 1000 replicates. Numbers below indicate branch length. Bremer support for nodes is denoted by D and the number of steps required to collapse the grouping/branch.

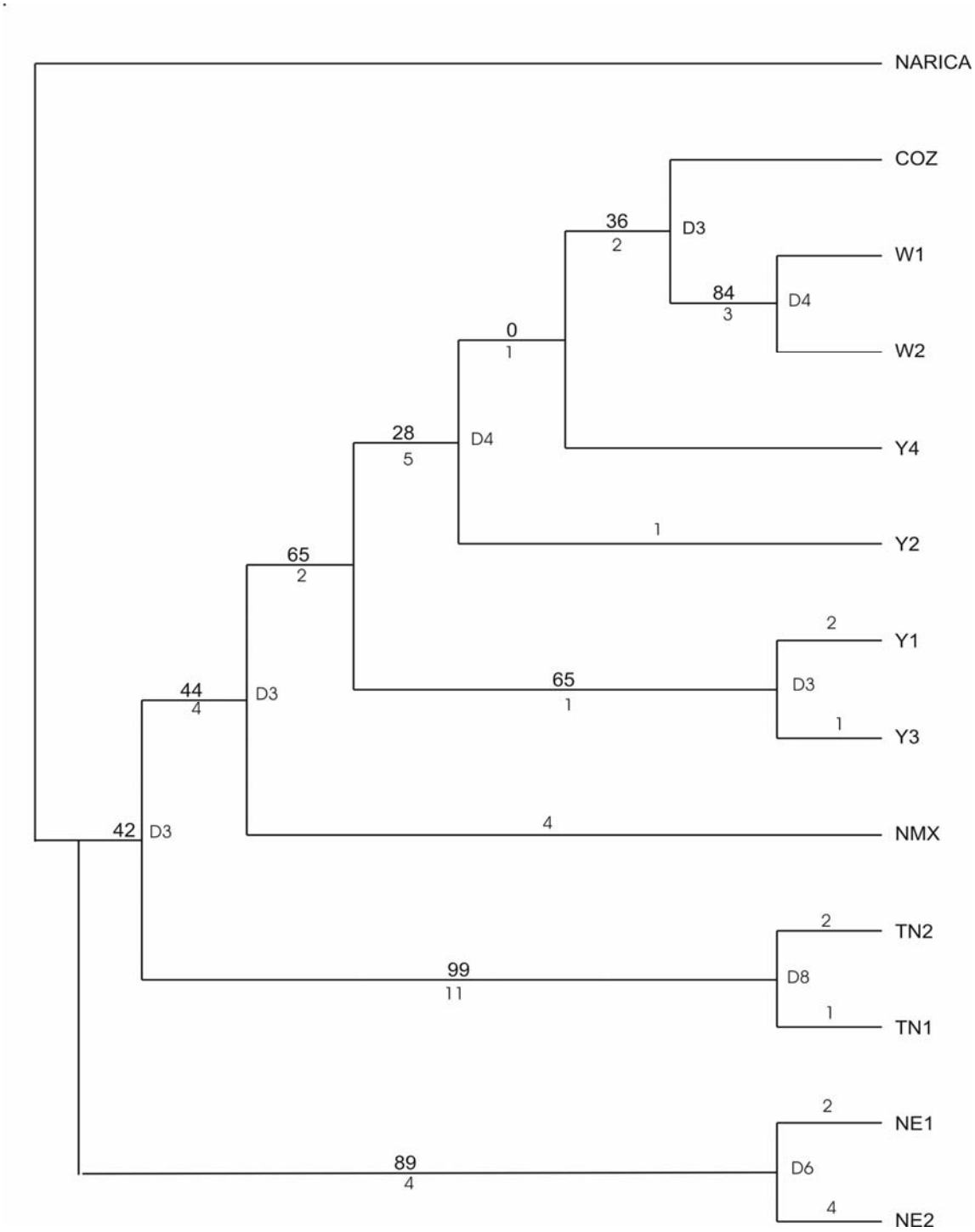


Table 2.6: Polymorphic sites observed in a 893 bp sequence in the d-loop region of the mitochondrial DNA from Cozumel (*P. pygmaeus*) and continental (*P. lotor*) raccoons. Position numbers match the sequence numbers where the polymorphism occurred.

	5	2 0	6 1	6 2	8 0	8 4	9 3	1 1 2	1 2 6	1 5 7	1 6 0	1 8 9	1 9 0	1 9 8	2 0 8	2 0 9	2 1 0	2 1 3	2 1 6	2 3 6	2 7 0	3 0 3	3 0 4	3 2 2	3 3 4	3 5 3	5 8 7	5 9 1	5 9 4	6 0 1	6 0 3	6 1 6	6 2 0	6 2 3	6 3 0	6 3 9	7 2 4	7 2 5	7 7 8	7 8 5	7 9 0	7 9 2	8 1 9	8 2 5	8 5 9	8 7 2	8 9 0
COZ	**C**	**T**	**C**	**A**	**T**	**T**	**C**	**C**	**T**	**G**	**G**	**A**	**T**	**C**	**A**	**A**	**G**	**C**	**T**	**A**	**G**	**A**	**G**	**A**	**C**	**C**	**G**	**-**	**C**	**C**	**T**	**C**	**A**	**T**	**A**	**T**	**G**	**T**	**G**	**C**	**C**	**A**	**C**	**T**	**C**	**-**	**T**
Y1	G	.	.	.	.	C	.	-	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	G	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.
Y2	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.
Y3	.	.	.	.	.	C	.	-	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	G	.	.	.	-	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	-	.
Y4	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-	.
NE1	.	.	T	.	C	C	T	.	.	.	.	.	.	.	G	.	A	T	C	.	A	.	A	.	T	T	A	-	.	T	.	T	.	.	.	.	C	.	T	.	.	.	.	A	C
NE2	.	.	T	.	C	C	T	.	.	.	.	.	.	.	G	.	A	T	C	.	A	.	.	.	.	T	.	A	T	T	.	T	.	A	.	.	.	.	T	.	.	.	.	A	C
TN2	.	.	.	G	C	C	T	.	.	.	.	G	.	T	.	G	A	.	.	.	.	G	A	.	T	T	A	-	.	T	C	.	.	.	C	A	C	C	A	.	T	.	T	C	T	-	.
TN1	.	.	.	G	C	C	T	.	.	.	.	G	.	T	.	G	A	.	.	G	.	G	A	.	T	T	A	-	.	T	C	.	.	.	.	.	.	C	A	.	T	.	.	C	T	-	.
NMX	.	.	.	.	C	C	T	.	.	A	A	.	.	.	.	.	.	.	C	.	.	.	A	.	.	T	.	-	.	T	.	.	G	.	.	.	.	?	?	?	?	?	?	?	?	?	?
W1	.	C	.	.	.	.	.	.	C	C	.	.	C	.	.	.	.	.	.	.	.	.	A	.	.	.	?	?	?	?	?	?	?	?	?	?	?	?	.	.	.	.	.	.	.	-	.
W2	.	.	.	.	.	.	.	.	.	C	.	.	C	.	.	.	.	.	.	.	.	.	A	.	.	.	.	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?

Table 2.7: Average K80 pairwise distances for nuclear DNA between different haplotypes of *Procyon* examined in this study. Haplotypes from the Yucatan (Y1-2), Northeast USA (NE1-2), Tennessee (TN1-2), New Mexico (NMX) and Western Mexico (W1) are examined.

	NASUA	COZ 1	COZ 2	Y1	Y2	W1	NE1
NASUA							
COZ 1	0.06367						
COZ 2	0.06860	0.00000					
Y1	0.07158	0.00000	0.01275				
Y2	0.07157	0.00000	0.01273	0.00000			
W1	0.06381	0.00253	0.00000	0.00000	0.00000		
NE1	0.06576	0.00000	0.00760	0.01018	0.01018	0.00000	
NE2	0.06337	0.00000	0.00506	0.00255	0.00255	0.00000	0.00000
TN1	0.06345	0.00000	0.00000	0.00509	0.00509	0.00000	0.00000

Table 2.8: HKY85 pairwise distances for mtDNA sequence data between different haplotypes of *Nasua* examined in this study. The outgroup was a *N. nasua* sample from Bolivia (NABOL). Haplotypes included those from the Yucatan (Y1-4), Belize (BZ1-2), Panama (PAN), Arizona (ARZ) and Cozumel (NE61 and NE99).

	NABOL	**Y1**	**Y2**	**Y3**	**Y4**	**BZ1**	**BZ2**	**PAN**	**ARZ**
NABOL									
Y1	0.076830								
Y2	0.078710	0.001608							
Y3	0.076830	0.003221	0.001608						
Y4	0.078626	0.003221	0.001608	0.003221					
BZ1	0.075013	0.003221	0.004839	0.003221	0.006463				
BZ2	0.074957	0.004839	0.006463	0.004839	0.008093	0.001608			
PAN	0.084553	0.040056	0.038226	0.036437	0.040041	0.038198	0.040014		
ARZ	0.084408	0.019721	0.018045	0.019721	0.019711	0.023100	0.024784	0.036292	
NE61	0.073098	0.008093	0.006463	0.004839	0.008093	0.004840	0.006463	0.036376	0.021413
NE99	0.085406	0.004368	0.002183	0.002196	0.004428	0.004393	0.006632	0.031181	0.006702

	NABOL	**YUCATAN**	**BELIZE**	**PANAMA**	**ARIZONA**
NABOL					
YUCATAN	0.077749				
BELIZE	0.078174	0.016395			
PANAMA	0.084455	0.038682	0.039106		
ARIZONA	0.084408	0.019299	0.023942	0.036292	
COZUMEL	0.079252	0.005083	0.005582	0.033778	0.014057

Figure 2.3: Minimum spanning network showing the phylogenetic and geographic relationships between *Procyon* mtDNA haplotypes. Size of shape is proportional to the number of individuals bearing a particular haplotype. Branch lengths are proportional to the number of mutation involved between haplotypes. Labels inside circles correspond to haplotypes from Table 1. Different shadows represent the geographic regions with which each haplotype belongs.

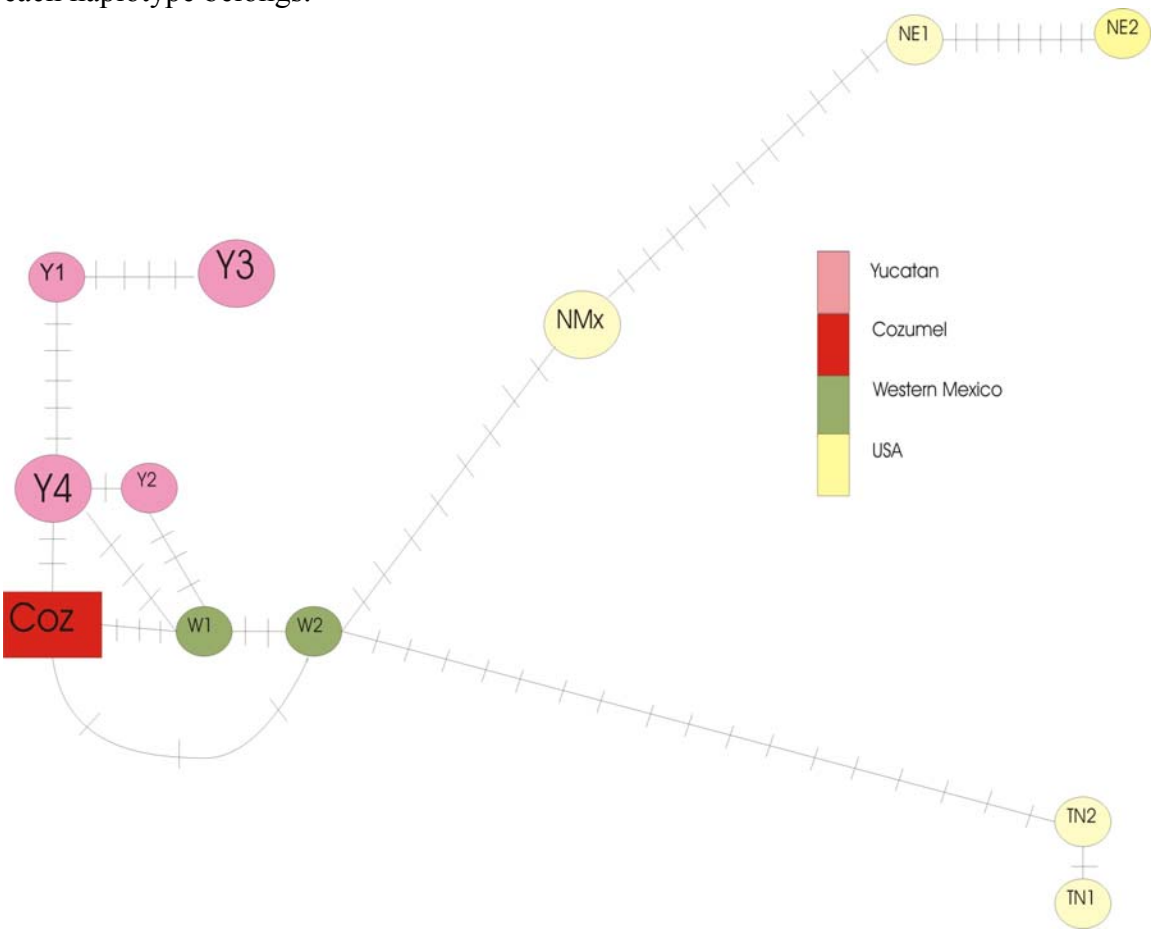


Figure 2.4: 50% Majority rule maximum parsimony tree of *Procyon* nuclear data (CHRNA gene). Numbers above branches are MP bootstrap values.

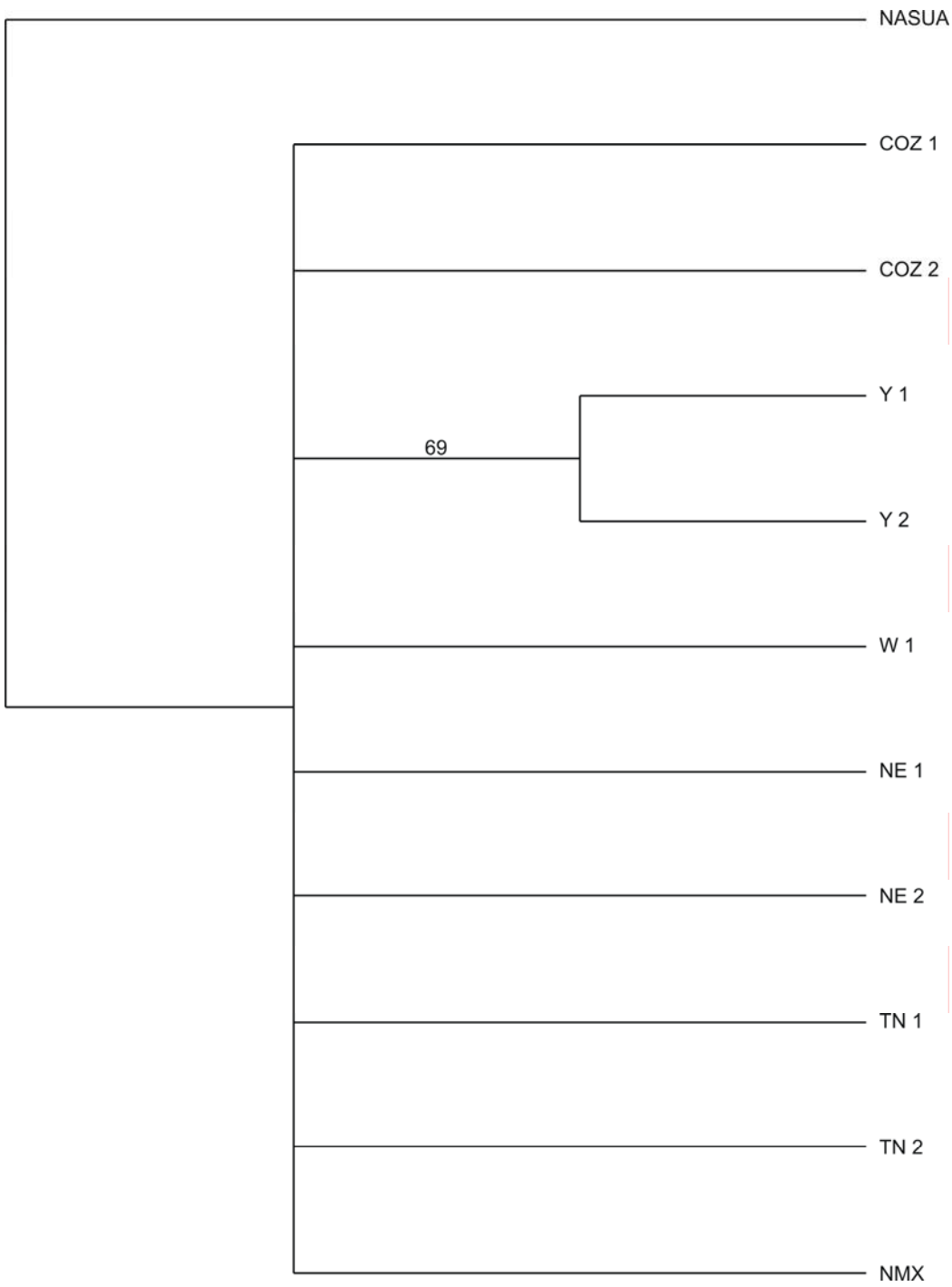


Figure 2.5: 50% majority rule consensus tree shared by MP and Bayesian analysis of mtDNA data for *Nasua.*

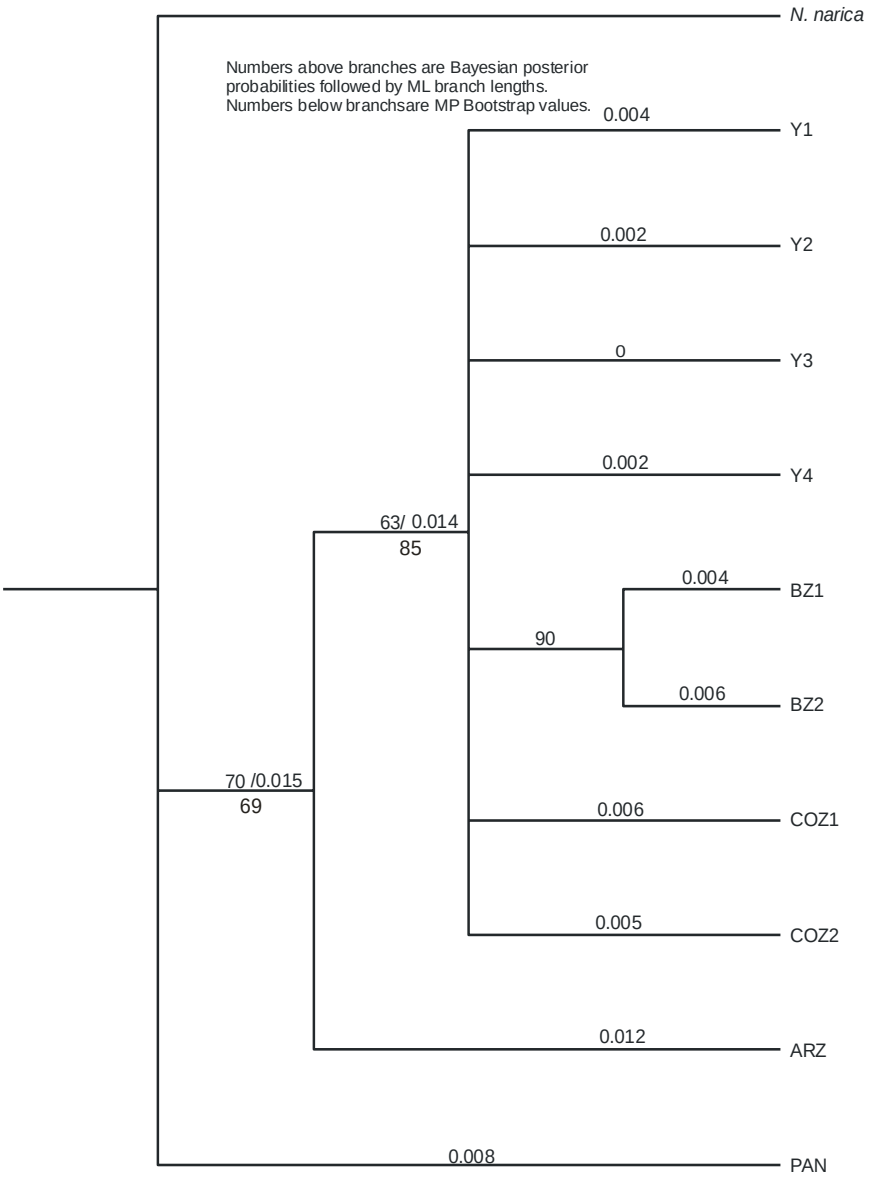


Figure 2.6: Topology shared for both maximum likelihood and maximum parsimony trees of *Nasua* mtDNA data. Numbers above branches are MP 50% majority rule bootstrap values.

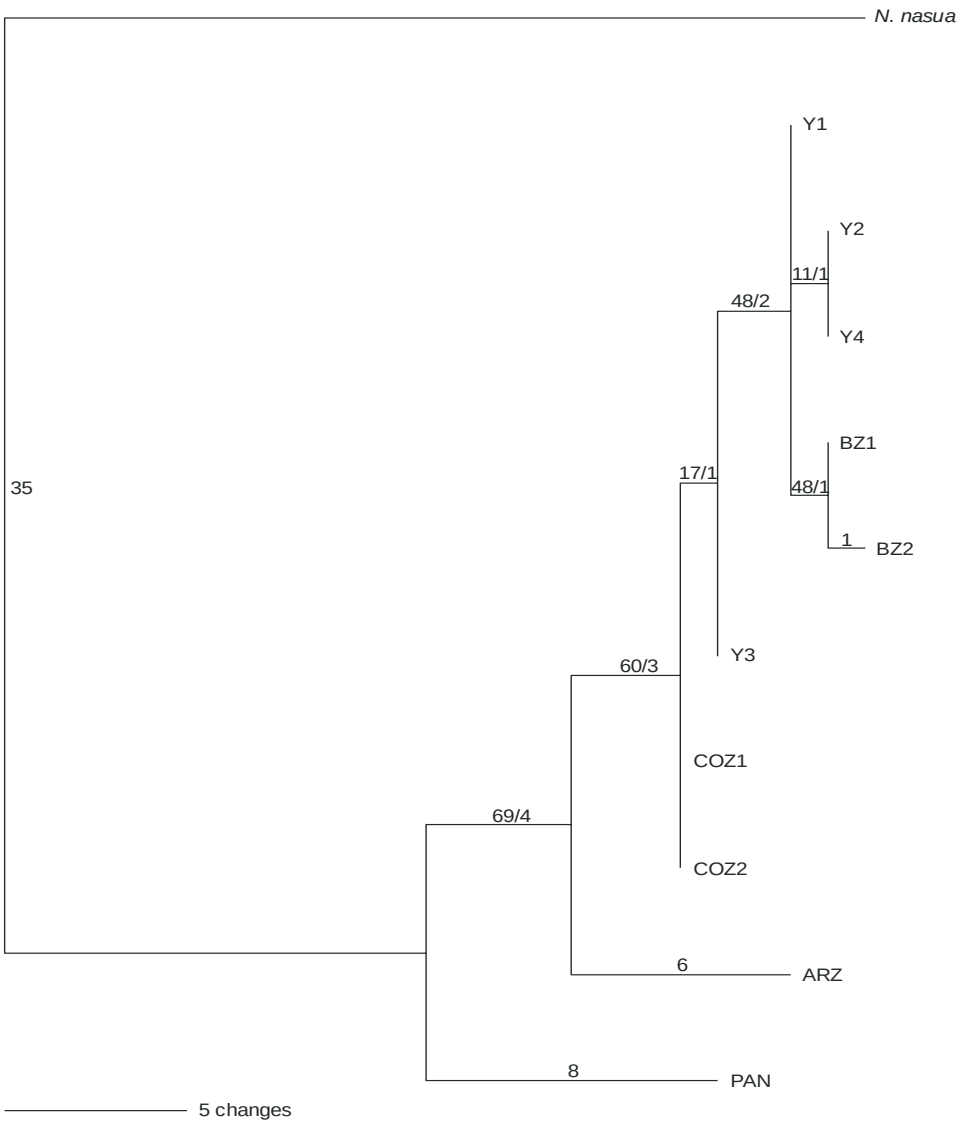
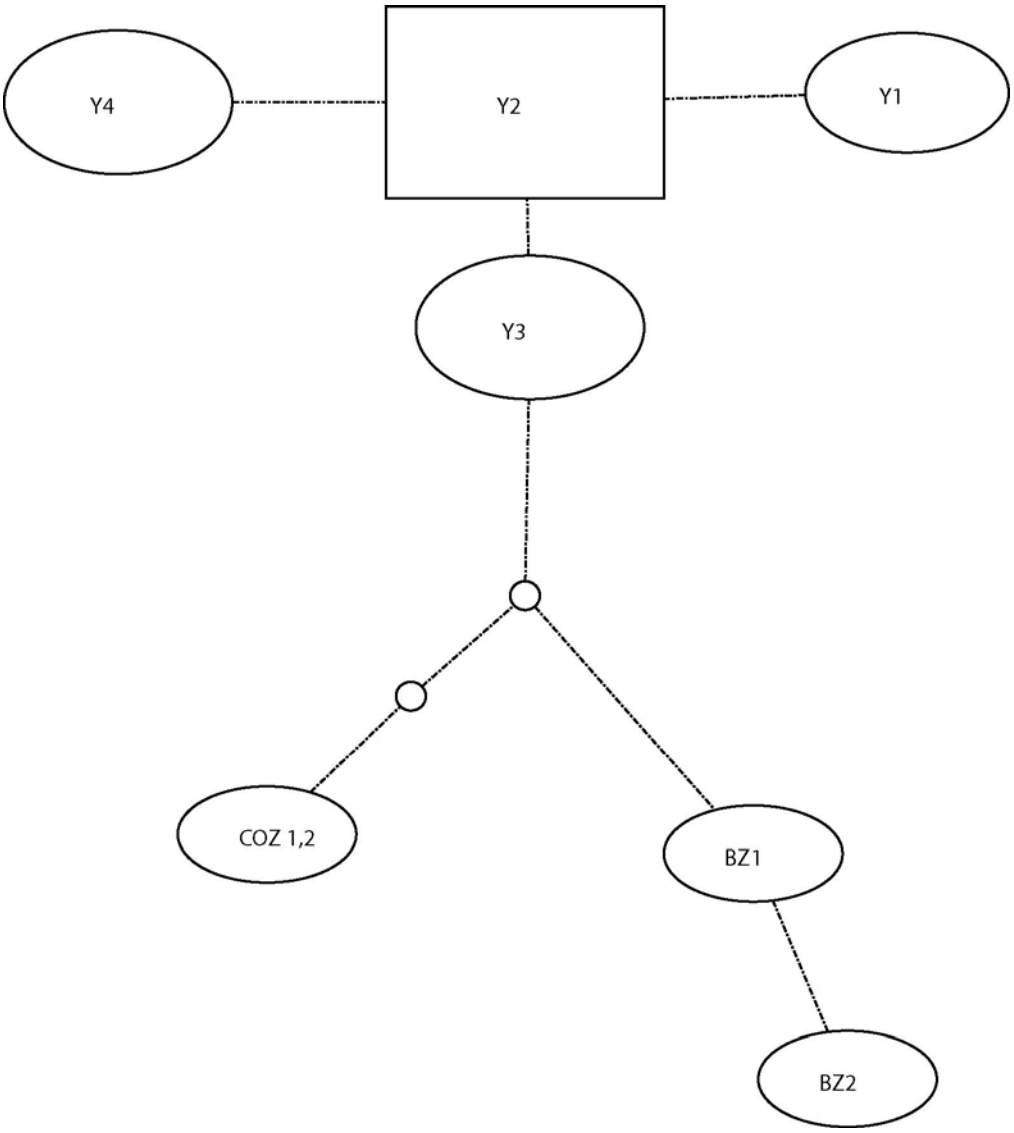


Table 2.9: Polymorphic sites observed in a 623 bp sequence of the d-loop from Cozumel (*N. nelsoni*) and continental (*N. narica*) coatis. Position numbers match the sequence numbers where the polymorphism occurred. Only a partial sequence for the second Cozumel coati is available.

	25	28	48	56	66	72	73	78	82	83	98	126	127	134	161	199	202	213	214	232	240	243	281	306	307	310	357	395	403	404	575	576	579	585	609	617
COZ1	A	A	C	T	-	A	T	T	A	T	T	T	A	-	G	G	G	T	A	A	G	C	G	G	A	A	C	-	T	G	A	C	G	T	T	A
COZ2	.	.	.	.	-	.	.	.	.	.	.	.	.	-	.	.	.	.	.	.	?	?	.	.	.	.	.	C	.	.	?	?	?	?	?	?
Y1	.	.	.	.	-	.	.	.	.	.	.	.	.	-	A	.	.	C	.	G	.	.	.	.	.	.	.	-	.	A	.	.	.	.	.	G
Y2	.	.	.	.	-	.	.	.	.	.	.	.	.	-	.	.	.	C	.	G	.	.	.	.	.	.	.	-	.	A	.	.	.	.	.	G
Y3	.	.	.	.	-	.	.	.	.	.	.	.	.	-	.	.	.	C	.	G	.	.	.	.	.	.	.	-	.	A	.	.	.	.	.	.
Y4	.	.	.	.	-	.	.	.	.	.	.	C	.	-	.	.	.	C	.	G	.	.	.	.	.	.	.	-	.	A	.	.	.	.	.	G
B1	.	.	.	.	-	.	.	.	.	.	.	.	.	-	A	.	.	.	.	G	.	.	.	.	.	.	.	-	.	A	.	.	.	.	.	.
B2	.	.	.	.	-	.	.	.	.	C	.	.	.	-	A	.	.	.	.	G	.	.	.	.	.	.	.	-	.	A	.	.	.	.	.	.
PAN	C	C	.	C	T	C	C	C	C	.	.	.	G	T	.	A	A	A	G	G	A	T	A	A	G	-	T	C	-	.	G	T	.	.	.	.
ARZ	.	.	T	.	-	.	.	.	.	.	C	.	.	-	.	A	.	C	G	G	A	.	A	.	.	-	.	C	-	.	G	.	A	C	C	G

Figure 2.7: A 95% statistical parsimony network of North American and Central American *Nasua* d-loop DNA. Each line in network represents one mutational change. Small empty circle represents the inferred non-detected interior haplotype. Cladogram generated with TCS v. 1.13.



Chapter 3: FEEDING ECOLOGY OF THE PYGMY RACCOON (*PROCYON PYGMAEUS*) OF COZUMEL ISLAND, MEXICO BASED ON STABLE ISOTOPE ANALYSES

Abstract

Raccoons are thought to be opportunistic predators, switching to alternative prey when preferred foods are not readily available, yet very few dietary analyses have been conducted on raccoons that occur in the tropics. This study examined the feeding ecology of the endangered pygmy raccoon (*Procyon pygmaeus*), an endemic species found on Cozumel Island, Mexico. Using tissue samples obtained from trapping over three years (2001-2003) and at three localities, the feeding habits of this species were examined using stable isotope analyses. Fecal remains of pygmy raccoons were also collected to supplement data derived from carbon and nitrogen isotopic ratios. Data from isotopic analyses and scat separation both indicated that the pygmy raccoon utilizes an omnivorous diet in which the three most prevalent food items were crab, which constitutes > 50% of the diet, followed by fruits and insects. Average (±SE) δ^{15}N and δ^{13}C are 8.13 ‰ ± 0.24 and –19.11 ‰ ± 0.43, respectively, suggesting a generally omnivorous diet. Hair and blood samples did not significantly differ in carbon or nitrogen isotopic ratios and this study found no age or sex-related variation. However, both geographic and temporal variation in diet were observed; raccoons consumed slightly different proportions of animal and plant food items depending on their location and season (*i.e.* wet vs. dry season).

Introduction

Animal conservation decisions are based on an understanding of the ecology of a species. Unfortunately, information on most species is limited by lack of study or by the rarity of the organism. For instance, while insights on limiting resources (*e.g.* dietary needs) are critical to population management and the protection of important habitats, in many cases a lack of access to an already rare organism may hinder the ability to obtain this information.

In studies of mammalian carnivores, an understanding of dietary needs, feeding habits, and foraging patterns are often based on analyses of stomach contents or scat analyses. These techniques can provide valuable data about foods consumed but do not reflect the entire diet (Walker and Macko 1999), the relative importance of each food item, or easily digested materials (Gannes et al. 1997; Greenwood 1979; Kaufmann 1962). Although scat and stomach content analyses may provide a picture of an individual's recent meal, they do not illustrate long-term feeding habits (Greenwood 1979). Scat and stomach contents are rarely available for species whose population size is very small.

The pygmy raccoon (*Procyon pygmaeus*) represents such a rare organism; the species is considered endangered by both the IUCN and the Mexican government (Diario Oficial, 2000). This dwarf species is endemic to Cozumel Island in the Mexican State of Quintana Roo, where virtually nothing is known about its ecology (Cuarón et al. 2004). Navarro and Suarez (1989) examined seven fecal samples of this species that indicated that their diet consisted mainly of crabs, insects and some plant material. Aside from this brief study, the feeding habits of this species are unknown. By studying their feeding

habits, one can hope to gain a better understanding of the habitat types they rely on for primary food resources. This information will be an important contribution for conservation efforts that may then focus on these areas for protection from deforestation, alteration, and development.

Foraging theory of opportunistic predators predicts that animals will select food items that result in energy returns equal to or higher than the energy expended on locating, capturing, and consuming that food (Pyke et al. 1977). When the preferred food item is not available, theory predicts that opportunistic predators will switch to alternative food items (Taylor 1984). Common raccoons (*Procyon lotor*) are known to feed on a variety of foods in different localities (Derting 1996; Gehrt 2003; Greenwood 1979; Lotze & Anderson 1979). However, the foraging habits of island-dwelling, tropical raccoons are less well understood. Raccoons introduced to islands can become important predators of native wildlife (*e.g.* Hartman and Eastman 1999), but for taxa such as the pygmy raccoon, which has inhabited the island for ca. 50,000 yrs, feeding strategies and their conservation implications are unknown. Most raccoons are considered generalist feeders (Derting 1996; Kaufmann 1982a; McClearn 1992; Shaul 1962; Zeveloff 2002). In short the designations "specialist" or "generalist," respectively, indicate according to Morse (1971) whether an individual "concentrates the majority of its activities on one or a few categories" or whether it uses "several". The aim of this study was to develop a more comprehensive understanding regarding the content and variability of this species' feeding habits. Fecal samples were collected from captured raccoons to gain baseline insights on their diet. However, because access to raccoons was limited spatially and temporally, an assessment of stable isotope ratios to determine longer-term feeding

strategies was undertaken in order to address variation among individuals and populations. Based on preliminary trapping data, I found pygmy raccoons in habitat predominantly characterized by mangrove forest. Therefore, I hypothesized that the pygmy raccoon would rely highly on mangrove crabs as a major component in their diet.

Carbon and nitrogen stable isotope ratios of animal tissues can be used as complementary data to estimate the assimilation of food resources over both short and long periods and are ideal for the assessment of average feeding habits (Hilderbrand et al. 1996; Kelly 2000; Kurle & Worthy 2000). The premise behind stable-isotope ratios as applied to feeding ecology is that animal tissue reflects the isotopic signature of the animal's food plus some additional changes due to the animals' own metabolism. Thus, animals feeding on plants or other animals with unique isotopic signatures will reflect this source material in their own tissue. Isotopic ratios of animal tissues offer advantages over traditional methods of studying the diets of free-ranging mammals in that isotopic ratios reflect nutrients assimilated over extended periods of time and not simply those recently ingested. Because the metabolic rates of different tissues determine the turnover or half-life of stable isotopes in tissues, one can glean dietary information on varying time scales (Tieszen et al. 1983) by sampling multiple tissue types. Depending on the metabolic turnover rate of a particular tissue, it may take two to three isotope half-lives for dietary information to become completely incorporated into a predator's tissue. For example, in the case of plasma, Hildebrand et al (1996) found black bear plasma to have approximately a 10-day turnover rate, while red blood cells (RBCs) reflect the diet assimilated from 2-3 months before collection of the sample. In contrast, tissues such as

hair do not turnover and reflect the diet during the growth of that tissue (Darimont & Reimchen 2002; Hobson et al. 1996).

Laboratory and field studies have found that carbon and nitrogen isotope ratios in animal tissues can be used to make inferences regarding the sources of organic matter, foraging locations and define trophic structure in food webs (Chaimberlain et al. 1997; DeNiro & Epstein 1978, 1981; Fry & Sherr 1989; Hobson 2000; Hobson et al. 2000b; Owens 1987). In particular, the ratio of stable nitrogen isotopes ($^{15}N/^{14}N$) reflect the trophic position of an organism within a food web (Ambrose & DeNiro 1986; Hobson & Welch 1992; Roth & Hobson 2000a). Stable carbon isotope ratios ($^{13}C/^{12}C$) change very little with trophic position and in contrast to nitrogen, reflect sources of primary productivity or areas of geographic location (such as mangrove versus forest food sources), (Chrisholm 1982; Roth & Hobson 2000a; Schoeninger 1984; Vogel 1978). By using both isotopes, one can have a powerful tool for understanding trophic relationships and tracing the flow of energy and nutrients.

The analysis of food webs using natural abundance of stable isotope ratios compares the stable carbon and nitrogen values of a predator's tissue with that of its prey. Euclidean mixing models may be successfully used when trying to determine the relative importance of isotopically distinct food items (Ben-David et al. 1996; Ben-David et al. 1997; Ben-David & Schell 2001). Applying stable carbon and nitrogen isotopic ratios to tissues enables one to investigate the temporal and spatial variation in feeding habits in pygmy raccoons.

Methods

Study Sites and Sample Collection:

Cozumel (20°16' to 20°26'N and 86° 44' to 87°02'W) is a 486 km^2 island covered by a variety of terrestrial habitats including dry deciduous forests, mangrove stands, sandy palm areas and multistratal tropical evergreen forests (Tellez et al. 1982). During two 3-month sampling periods in both 2001 and 2002, a total of 10 sites throughout the island were sampled for a minimum of 2 weeks at each site, with three sites in the northwestern mangrove- dominated habitats (Figure 3.1) chosen for further study based on the identification of substantial raccoon populations. To determine which habitats the pygmy raccoon utilizes, sites with varying floristic and vegetation structure were sampled. The first field season was conducted from June through September 2001 (wet season), the second field season spanned from April (late dry season) to July 2002 (mid wet season), while the third season was February- March 2003 (dry season). Animals were captured using Tomahawk box traps (#207) baited with a honey-banana mixture and checked at least once daily. Trapped animals were immobilized with ketamine hydrochloride (10-12 mg/kg) and xylazine (2 mg/kg) by hand injection prior to receiving ear tags (HASCO) and sample collection. Age was approximated as juvenile (0-12 months), subadult (13-21 months), or adult (> 21 months) from tooth wear, body size, and reproductive status and recapture history(Grau et al. 1970; Sanderson & Nalbandov 1973).

Blood samples were collected by jugular venipuncture using a 21-gauge needle and vacutainer tube. Blood was collected in both additive-free (for the separation of serum and clot) and heparin-containing (for the separation of plasma and red blood cell

components) vacutainer tubes. Blood was centrifuged, and each blood component separately frozen (-20°C, and then –80 after returning from the field). Hair samples (~ 20 hairs) were obtained by plucking a small patch of fur from the root shaft and stored dry in a paper envelope to avoid mildew growth.

Potential prey items (fruit, insects, crabs etc.) from Cozumel were also collected in each field season. Samples were collected in varying habitats throughout the island. Crabs were collected from multiple sites on the island and included species from *Ocypodidae* and *Grapsidae*. Fruit and plant samples were oven dried, stored at room temperature and identified to family (or genus/species in some cases) by botanists at the New York Botanical Garden. Vertebrate and invertebrate samples were stored frozen until analysis.

Carbon and Nitrogen Isotope Analyses:

Hair, blood, and fruit and insect item samples were dried at 60°C for 20h and then powdered with a mortar and pestle. The carbon isotopic values of lipids have been shown to differ from those of other tissues by as much as 7‰ (DeNiro & Epstein 1978; McConnaughey & McRoy 1979; Vogel 1978). Therefore, lipids were removed from blood and frozen prey specimens (*i.e.* crab, turtle) using a modified methanol/chloroform extraction technique (Bligh & Dyer 1959), and the samples were then dried at 60°C for at least 24 h. Oils from hair samples were removed by cleaning samples first with 90% ethanol, followed by soaking in chloroform-methanol.

Carbon and nitrogen stable isotope ratios in hair and blood are expressed in δ notation as parts per thousand (‰) as determined from:

$$\delta X = [(R_{sample}/R_{standard}) - 1]/1000 \quad (1)$$

where δ is $\delta^{13}C$ or $\delta^{15}N$ and R is the corresponding ratio $^{13}C/^{12}C$ or $^{15}N/^{14}N$, respectively, measured by the mass spectrometer. The quantitative assessment of feeding ecology based on stable isotopic ratios is based on the following breakdown between source material and metabolic factors:

$$\delta_{tissue} = \delta_{diet} + \Delta_{dt} \quad (2)$$

where Δ_{dt} represents the isotopic fractionation or trophic enrichment factor between dietary and consumer tissue (DeNiro & Epstein 1978, 1981). Samples were analyzed with a Europa 20-20 isotope ratio mass spectrometer (PDZ-Europa, Cheshire, UK) in continuous flow mode. Samples were prepared for mass spectroscopy via an automated Dumas procedure (Knowles and Blackburn 1993). The resultant CO_2 and N_2 gas were separated chromatographically and introduced sequentially to the source of the mass spectrometer for isotopic analysis. Precision between repeated samples from the same individual was 0.30 ‰ for $\delta^{13}C$ and $\delta^{15}N$. In continuous flow mode, the samples were bracketed by National Institute of Standards and Technology (NIST) primary or secondary isotopic standards that have been calibrated against the NIST material. A minimum of three replicates were made from each sample from an individual. Carbon isotopic values were referenced against sucrose (NIST ref. # 8542) and are expressed relative to Pee Dee Belemnite (PDB). Nitrogen isotopic values were referenced against ammonium sulphate (NIST ref. # 8547) and are expressed relative to atmospheric

nitrogen. The NIST standards provided a basis for calibrating both the combustion process and mass spectrometer during each sample run.

Carbon and nitrogen isotope ratios tend to become more enriched when incorporated into predator tissue, a process referred to as fractionation or trophic enrichment. The mechanisms of isotopic fractionation between an animal's diet and its tissues are not well understood (Tieszen & Boutton 1989; van der Merwe 1982) and vary depending on tissue type, also referred to as 'isotopic routing'. In the absence of estimates for procyonids, fractionation values of 2‰ for carbon were used when fruit was consumed, and 1‰ when invertebrates were consumed, and nitrogen fractionation values of 3.4‰ was used for all non-invertebrate prey items. These fractionation values were based on results from captive feeding experiments on mink (*Mustela vison*) and black bears (*Ursus americanus*) (Ben-David et al. 1997; Hilderbrand et al. 1996; Roth & Hobson 2000a, b). Alternative fractionation values from red foxes (*Vulpes vulpes*) were also used. This study found hair carbon to be enriched by 2.6 ‰ and hair nitrogen was enriched approximately 3.4 ‰.

Mixing Models and Statistical Analyses:

Diet sources for pygmy raccoons were based on the combined values of $\delta^{13}C$ or $\delta^{15}N$. A dual-isotope, multiple-source Euclidean mixing model was used based on the concept of Ben-David et al. (1997) to estimate the contribution of each potential prey item to the diet of the predator. The mixing model requires that isotopic values of all prey be significantly different from each other and assumes that predators consume all possible prey types (Ben-David & Schell 2001). Therefore the model tends to

overestimate the proportion of food items that are rarely consumed and underestimate the proportion of commonly consumed prey (Ben-David et al. 1997) and as such, the use of a mixing model serves as an index of prey consumption rather than a prediction of actual proportions in the diet.

To maximize sample sizes, hair was selected as the consumer tissue for the mixing models when geographic and temporal differences were examined. The average blood (RBCs/Clot) and hair isotopic values were used when calculating the "overall" mixing model proportion of each food item. Mean $\delta^{13}C$ or $\delta^{15}N$ of each isotopically distinct prey type was used in this model. Potential prey items found on Cozumel were supplemented by isotopic data of other plausible prey items from the literature (Herrera et al. 2002; Herrera et al. 2001; Hobson et al. 2000a). Mean prey values were corrected for the enrichment due to fractionation in predator ratios compared with diet (Ben-David et al. 1997; DeNiro & Epstein 1981; Kline et al. 1993; Tieszen & Boutton 1989). The Euclidean distance between the average corrected isotopic values of predator and prey was then calculated using:

$$z = \sqrt{x^2 + y^2} \qquad (3)$$

where *x* and *y* represent the Euclidean distance of carbon or nitrogen. The relative contribution (% composition) of each prey to the diet of the raccoon is inversely related to the distance between the corrected isotopic signature of the prey (A', B' and C') and the predator (P') and is calculated by:

$$\% \text{ X in diet} = (PX'^{-1} / PA'^{-1} + PB'^{-1} + PC'^{-1}) \times 100, \qquad (4)$$

where X' is A', B', or C' and P is the predator's isotopic ratio. One assumption made by this model is that partitioning among food sources are the same for both carbon and nitrogen.

All statistical analyses were performed in SPSS (version 10.0 SPSS Inc. Chicago, Illinois 2001). Differences were determined to be statistically significant at the $p < 0.05$ level. Paired t-tests were used to test for differences in isotope ratio values for different tissue types (*i.e.* blood and hair) and between the different blood components (serum, RBC, etc.). Tukey's posthoc tests were performed on blood components to identify if some components could be pooled for further analysis. A one-way analysis of variance (ANOVA) was used to individually test for differences between stable isotope ratios of different age classes, sexes, the three trapping sites, and the three field seasons. The Tukey test for multiple comparisons was used to determine the origin of significant results of univariate tests (Zar 1999). If no differences existed, data were pooled for further analyses.

A K nearest-neighbor randomization test (Ben-David et al. 1996; Schilling 1986) was used to test if stable isotope ratios of the different prey types were significantly different from each other. After calculating the relative contribution of each food item to the diet of each individual using the dual isotope, multiple source mixing-model, the mean (± SE) for the entire sample was calculated. A one-way ANOVA was utilized to test for differences in the relative contribution in different food items by season and sites. Chi-square tests were used to examine differences in the percent occurrence of food items in pygmy raccoon scats.

Fecal Analysis:

Scat samples were obtained using a fecal loop after the animal was anesthetized and were stored frozen or in formalin until analysis. These were used as complementary independent measurements to substantiate or further constrain the interpretation of the isotopic evidence. Scats collected from recaptured individuals on different days were considered separate samples because they represented separate feeding occasions Samples were stored frozen or in formalin until analysis. Prior to examination the scats were emulsified for 12-24 h in a mixture of 10 parts ethyl alcohol (95%), 3 parts water, and 1 part general detergent and then sorted manually using a sieve with mesh size of 500 μm. After sorting scats through a sieve, they were dried at 60°C overnight and prey items were identified using a dissecting (50X magnification) microscope. Prey items were classified in general categories such as fruit, crab, leaf (grasses, woody plants, forbs), banana (trap bait), vertebrate, and insect. Data were recorded for each scat as presence/absence of individual food categories. Individuals from one trap site were never trapped in any other site (*i.e*. no subpopulation migration/immigration was observed), and therefore, this study assumed that individuals trapped in one site were also feeding at that site.

Prey items are expressed in percent of occurrence. Chi-square analysis was used to compare distributions of food items between sites and during the entire study. Frequency of occurrence data were converted to proportional frequency to calculate Levins' index (Levins 1968):

$$B = (\Sigma p_i^2)^{-1} \quad (5)$$

Where p_i is the proportional use of a food item relative to other food items. *B* ranges from 1 to n (n = total number of food item categories) and was used to calculate diversity of diets seasonally and at each site. Using Hurlbert's method (Krebs 1989), diversity was standardized (B_s) to a scale of 0.0 to 1.0:

$$B_s = (B-1)/(n-1) \quad (6)$$

Where *B* = Levins' measure of diversity and n = number of food categories. Levin's index gives an indication of trophic niche breadth and increases as food habits become more generalized, reaching a value of one when all food types are exploited equally (Krebs, 1989). The same number of possible prey categories *n* were used for each site. Statistical tests were not performed on diversity indices because scats collected using a fecal loop did not necessarily represent a full fecal sample for an individual. Therefore, diversity indices and seasonal comparisons of percent occurrence and are provided for descriptive purposes only.

Results.

Hair samples:

A one-way ANOVA found no significant differences between males (n = 30) and females (n = 33) for both $\delta^{13}C$ and $\delta^{15}N$ (p = 0. 595, p= 0.623, respectively). A one-way ANOVA found no significant differences in either $\delta^{15}N$ and $\delta^{13}C$ between the three age classes (p = 0.131, p = 0.543, respectively) (Table 3.1). In contrast, significant differences were found when comparing $\delta^{15}N$ and $\delta^{13}C$ values between the three trapping sites (p = 0.007, p = 0.000, respectively) (Figure 3.2) and between the three field

seasons (p = 0.001, p = 0.000, respectively) (Figure 3.3). A Tukey's post-hoc test also indicated that δ^{15}N values from site 1 (mean δ^{15}N = 8.03) and 2 (mean δ^{15}N = 8.69) did not significantly differ while site 3 (mean δ^{15}N = 6.92) was significantly lighter than both these sites (p = 0.001, p = 0.000, respectively). In contrast, all three sites differed from one another when examining δ^{13}C values; site 1 (mean δ^{13}C = -17.92) was heaviest, site 2 (mean δ^{13}C = -19.08) intermediary, and site 3 was lightest (mean δ^{13}C = -22.09).

When examining individual annual differences, a Tukey's post-hoc test indicated that nitrogen isotope ratios from 2001 and 2002 did not significantly differ, while 2003 was significantly more depleted than those of 2001-2002 (p = 0.001 and p = 0.001, respectively). Post-hoc tests of carbon isotope ratios indicated wet (2001-2002) isotopic values were significantly more enriched than those from the dry seasons (2003) (pairwise comparisons 2001-2002: p = 0.30, 2002-2003: p = 0.006, 2001-2003: p = 0.001).

A paired t-test indicated that δ^{15}N and δ^{13}C values for hair and plasma/serum significantly differed (t = - 2.45, df = 22, p = 0.023; t = - 3.05, df = 11, p = 0.008). In contrast, paired t-tests indicated that δ^{15}N and δ^{13}C values for hair and RBC/Clot did not significantly differ. Therefore, the average (± SE) δ^{15}N and δ^{13}C values for hair and RBC/Clot (8.13 ± 0.135 ‰ and –19.11 ± 0.289 ‰) were used for the predator values relating to the mixing models

Blood Samples:

Although blood was collected for the three field seasons (2001-2003), samples from 2001 and 2002 were affected by a freezer breakdown. I tested the effect of

extended time at room temperature on seven unaffected blood samples from 2003 by comparing the carbon and nitrogen isotopic values of fresh blood with a subsample that was left at room temperature for a two-week period. Results from a paired t-test indicated that although nitrogen was not significantly different between the fresh and thawed samples (n = 7, p = 0.429), carbon was significantly more depleted (n = 7, p = 0. 047). Therefore, results from only the nitrogen isotopic analysis are presented for 2001-2002 blood samples (n = 15). Blood from twenty-eight individuals were sampled for the 2003 field season.

Paired t-tests indicated that plasma-serum comparisons, and RBCs and clotted cells (Clot) comparisons, were not significantly different in isotopic signatures. Therefore, plasma/serum were pooled and RBC/Clot were pooled for all further analysis. Paired t-tests between the two types of blood components (Plasma/serum and RBC/Clot) indicated that plasma/serum was significantly more depleted for nitrogen (t = - 4.035, df = 19 p = 0.001), but did not significantly differ for carbon (t = 2.102, df = 13 p = 0.516). Therefore, all further analyses involving blood were conducted by pooling only similar components. In raccoons where both plasma and cellular fractions of blood were available for analysis, a paired t-test found blood plasma/serum δ^{15}N and δ^{13}C values were depleted compared to the cellular fraction by 0.72‰ and 1.45‰, respectively (Table 3.1, t = -4.035, df = 19, p = 0.001; t = -3.940, df = 19, p = 0.001). This likely reflects differences in isotopic fractionation between diet and these tissues (e.g., (Hobson & Clark 1993; Tieszen et al. 1983).

A one-way ANOVA found no significant differences between males and females for δ^{15}N and δ^{13}C isotope values. Differences in δ^{15}N between the three age classes

were detected using a one-way ANOVA, but they were not statistically significant (F = 2.96, df = 2, p = 0.060). Mean juvenile δ^{15}N was most enriched (8.14‰, n = 14), subadults were intermediate (7.58‰, n = 6) while adults were the most depleted (7.37‰, n = 41).

A one-way ANOVA found significant differences between the three trapping sites for both δ^{15}N and δ^{13}C isotopic values (F = 4.93, df = 58, p = 0.010; F = 4.55, df = 38, p = 0.017, respectively). Site one had a mean δ^{15}N of 7.72‰ (n = 28), while site two was more enriched (7.90‰, n = 18) and site three was most depleted (6.88‰, n = 15). In contrast, mean δ^{13}C for site one was the most enriched of all sites (-18.77‰, n = 9), and as with nitrogen, site 3 was the most depleted (-21.51‰, n = 15).

A one-way ANOVA indicated significant differences in δ^{15}N , but not δ^{13}C isotopic values between years (F = 3.63, df = 58, p = 0.031). A Tukey's posthoc test found 2002 δ^{15}N values significantly more enriched than other years (2001, p = 0.022; 2003, p = 0.003).

Potential Prey Items:

A total of 52 prey items were collected over the three-year study; crab and fruit were collected in every field season. Potential prey items included fruit from Theophrastaccae (*Jacquinia*), Rubiacceae (*Alibertia edulis*), Combretaceae (*Terminalia catappa*), and Polygonaceae (*Coccoloba*). Stable isotope ratios of small and large crabs and those collected in different seasons did not differ significantly (k nearest-neighbor randomization test, p = 0.381). Therefore, all crab isotopic data were pooled and the mean was used in mixing models and other analyses.

Isotope ratios of all other prey types (C_3-C_4 fruit, frogs, lizards and insects) were all isotopically distinct (K nearest-neighbor randomization test, p = 0.445) (Table 3.2). The average for fruit samples collected from Cozumel Island was combined with the average $\delta^{15}N$ and $\delta^{15}C$ values for fruit taken from other isotopic studies in Mexico (Herrera et al. 2002; Herrera et al. 2001; Herrera et al. 2003; Hobson et al. 2000a) and this value was used for the mixing model. Statistical analyses of differences between years or sites for other prey items were not conducted due to small sample sizes.

Isotopic Mixing Models:

Scat separation analyses indicated that the three largest food components in pygmy raccoon diet were plant material, crab and insects. I therefore used these three food items as source material in the mixing models. Using the dual isotope, three-source mixing model, crab was estimated to be consistently a major contribution in the diet of pygmy raccoons. The estimated percent contribution varied from 45-54% depending upon the season and trapping site (Table 3.4), but models generally did not find evidence of any marked change in diet. Fruit generally made up between 25 -31% of the diet and the corresponding variance was usually reflected in a change in the proportion of insects. Differences in the proportion of fruit in the diet did not vary significantly between the wet and dry seasons.

A one-way ANOVA found significant higher estimates of the proportion of crab ($F = 6.01$, $df = 2$, $p = 0.004$) and significantly lower estimates in the proportion of fruit ($F = 10.02$, $df = 2$, $p = 0.000$) consumed at site 2 compared to the other two sites (Tukey's post hoc test: site 1-2 $p = 0.001$, site 2-3 $p = 0.017$). When examining the predicted

proportion of food items in the diet by year, a one-way ANOVA indicated no significant differences existed between the three years.

Using alternative fractionation values (carbon fractionation for invertebrates and insects increased from 1 to 2 ‰, and fruit increased from 2 to 2.5‰) derived from a study on captive foxes (Roth & Hobson 2000a), similar proportions of the three major food items were found. The main difference between using carbon fractionation values taken from a species of similar size was an approximate increase of 3% in the estimate of crab consumed, with a corresponding 3% decrease in fruit consumption. However, these differences were not statistically significant and did not change the overall ratio of crab-insect-fruit of 2:1:1.2.

Composition of Fecal Samples:

Scats (n = 50) were collected in 2002-2003. Scat separation analyses indicated that the three largest food components (by % occurrence in the population) in pygmy raccoon diet were plant material (54%), crab (44%) and insects (36%). A Chi-square test indicated that there was no difference the proportion of food items found in scats of males and females. A Chi-square test indicated that subadults consumed fewer insects than both juveniles and adults ($X^2 = 8.33$, df = 1, p = 0.015). Percent occurrences of food slightly varied between seasons. Prevalence of insects in scats was different between the wet season (2002) and the dry season (2003) ($X^2 = 4.66$, df = 1, p = 0.031), with insects being more frequent in the scats of the wet season (Table 3.3). The other most prevalent food items found in fecal matter, crab and fruit, did not differ significantly between the two seasons ($X^2 = 0.298$, df = 1, p = 0.582; $X^2 = 0.810$, df = 1, p = 0.348, respectively).

The prevalence of fruit and crab in scats varied between the three sites ($X^2 = 8.81$, df = 1, p= 0.012; $X^2 = 6.21$, df = 1, p = 0.048, respectively) with fruit being most prevalent at site 3, and crab most prevalent at site 1 (Table 3.3). A two-way ANOVA examined if the prevalence of food items varied by trapping site *and* year but no significant differences based on these two combined factors were found (F = 36, df = 6, p = 0.384).

Levins' index for food diversity for scats across all seasons was $B_s = 0.75$. When examined by season, food diversity was highest for scats collected during the wet season (2002) (Table 3). In addition, site 1 ($B_s = 0.57$) had a slightly higher food diversity value than site 2 ($B_s = 0.53$).

Discussion

The isotopic signatures of blood (plasma/serum) and hair differed slightly, probably due to different turnover/accretion rates. Based on the molting patterns of other tropical procyonids such as coatis, one expects the pygmy raccoon to molt in summer (Gompper 1995). Therefore, hair samples collected during the late dry season (Feb-March) may represent feeding habits approximately 6-9 months prior to sampling while samples collected in late summer (July - August) may only reflect recent feeding habits. Due to this discrepancy in the period hair samples reflect, blood served as an independent indicator of diet patterns with relation to time. Because hair and RBC samples are not significantly different, one can assume that the diet of this species does not radically shift over the course of the year. This spatio-temporal dietary stability was confirmed by the fact that pairwise differences between sites and years showed the same patterns of enrichment in both hair and blood.

Despite intense trapping efforts in other habitats on the island, pygmy raccoons were only trapped in the northwest quadrant of the island. Areas in which I trapped raccoons consisted almost exclusively of mangrove forest, although some of these areas also bordered sandy beach and semi-deciduous forest areas. Based on these findings, and the small-scale study of Navarro and Suarez (1989), I anticipated that food items such as crab would be an important food component in their diet and fecal and isotopic data supported this hypothesis. When developing isotopic mixing models, this study aimed to use ecologically plausible source material. Although the selection of prey items used in this study likely does not represent the absolute breadth of prey items the pygmy raccoon consumes, the agreement of two independent analytical techniques, fecal analyses and stable isotopes, strengthens these findings and emphasize the value of a stable isotope approach for assessing global dietary habits. This is especially true when access to the information via alternative techniques, such as scat analyses, is logistically or politically infeasible.

Analyses of stable isotopes revealed a pattern of geographic and seasonal differences in diet that were similar to results of the conventional diet analyses; food items identified via isotopic and scat analysis are within the breadth of potential food items that this species consumes throughout its' range (Dorney 1954). Fruit availability in the tropics varies throughout the year (Gentry 1988), with important implications for the foraging strategy of generalists such as the pygmy raccoon. It has generally been accepted that raccoons are opportunistic feeders, varying their diet based on seasonal food availability. One could interpret that the higher food diversity (B_s) value for the wet season supports this presumption. If a greater variety of food items is available in the wet

season, because of seasonal abundance of fruit and vegetation types, one would expect that pygmy raccoons would expand their feeding habits to include such abundances, thus supporting the broader niche breadth found in the results for the wet season. However, because this study only used scat data from two field seasons, Levin's niche index results should be interpreted with caution.

Isotopic and fecal analyses also indicate variation in the feeding habits between the three sampling periods (2001-2003), which also corresponds to different seasons within Cozumel. Both carbon and nitrogen ratio values for 2003 (dry season) were more depleted compared to ratios from the other two field seasons (both wet season). This difference may correspond to seasonal availability of vegetation/fruit biomass which is presumably higher between May and October, as seen in other tropical forests on the mainland (Estrada & Coates-Estrada 2001; Foster 1982; Gentry 1988) Additionally, raccoons may have preferentially consumed other more abundant, yet isotopically depleted prey items during the dry season, when fruit is presumably not as abundant.

It is generally accepted that a generalist predator would cope with a food shortage by, among other strategies, changing their diet to include other resources. While this study supports this idea, nonetheless, during most of the year, crabs provide most assimilated protein. This study also was able to identify changes in diet and, consequently, in resource partitioning from the extent to which this species relied on varying amounts of crab, plants and insects as sources of assimilated protein over different temporal and spatial scales. One might then classify the pygmy raccoon as a generalist omnivore that has adapted to a mangrove environment by specializing in

mangrove crab, since it represents over 50% of the diet, as indicated by stable isotope analyses. This is further confirmed by the contents of fecal samples.

Although all raccoons in this study were trapped in and around mangrove forest, significant differences in both isotopic ratio values and scat data for each trapping site were identified. This may be a function of each trapping site's proximity to anthropogenic influencing factors. For example, while both sites 1 and 3 were within several hundred yards from the ocean, site one was also within 1 km of a waste-water treatment facility. Based on field observations, some portion of wastewater from this facility, especially in times of heavy rains, flows into the adjacent mangrove vegetation. Although a dirt road separated this outflow from the region where individuals were trapped at site 1, some fraction of the presumably enriched wastewater in this area may have found its way into the food chain of the pygmy raccoon. This is especially plausible given that this species is known to have home ranges that would encompass the distance from the trapping site and this waste facility. Interestingly, however, only carbon values were significantly higher than those found at the other trap sites. Site 1 also seemed to have fewer fruit resources and more aquatic and brushy vegetation than the other sites, an observation that was confirmed by scat analysis. Future studies examining resource availability would assist in creating a better measure of niche breadth, as the Levin's niche breadth measure employed in this study did not take into account the possibility that resources vary in abundance. Additionally, future studies should examine the level of effluent entering the surrounding mangrove areas would help determine how these factors might influence raccoon feeding habits.

Site 2 was located in mangrove and deciduous forest surrounding a golf course. Stable nitrogen values from this site were the heaviest of the sites; carbon values were intermediate when compared to the other two sites. The enriched nitrogen from animals caught at this site may be explained by numerous anthropogenic influences. First, several garbage areas are available to the raccoons at this site, while large areas of human food/garbage were less likely to be encountered at the other two sites. Human foods and garbage typically contain enriched forms of carbon and nitrogen. Second, like most golf courses, the playing greens are heavily treated with nitrogen-based fertilizers (B. Campos, personal communication). One would expect that the run-off from these fertilizers would serve as an enriching factor (Macko & Ostrom 1994) affecting the surrounding food chain and may be the cause of the heavier nitrogen values for this subpopulation. Although mixing models predict a corresponding increase in crab consumption for this site, scat data does not support this increase and scats from this site were lower in crab and higher in leaf/plant material than the other sites. The change in average isotopic values for this site may have skewed the mixing model towards falsely predicting an increase in crab consumption because this prey item had the most enriched carbon and nitrogen values of the three prey items used in the model. Other prey items such as C_4 plants may have contributed to the heavier carbon and nitrogen values seen in this subpopulation. Some uncertainty around the proportion estimates from the mixing model is expected due to random measurement errors, variation in isotopic composition among prey individuals, and variation in fractionation and assimilation and consequently the composition among predator individuals.

Results of stable isotope analyses generally correspond to data found in fecal matter, which indicate that the three most prominent food items were crab, fruit, and insects. Crab plays an important role in the diet of the pygmy raccoon and is a major food source during all times of the year. However, differences in food item digestibility, is a known bias in identifying patterns in feeding habits (Greenwood 1979) and therefore it is likely that crab is over represented in scats because of the lack of digestibility of the calcium carbonate shell. However, the fact that both fecal and isotopic data indicate that crab is a major component in the diet of the pygmy raccoon both substantiates the likelihood that this is a major prey source and also has important conservation implications for this species. As Cozumel Island's tourism industry continues to grow, ocean front property is increasingly valuable. In Cozumel, such areas often border mangrove areas such as the sites where I trapped pygmy raccoons. Development in these areas severely decreases the habitat in which the pygmy raccoons and their primary prey are found. In 1999, for instance, a golf course was built in mangrove habitat adjacent to study site 2 resulted in the draining or alteration of several square kilometers of mangrove habitat. Additionally, local developers and government officials have recently begun discussing the development of new tourist attractions off the most northwestern tip of Cozumel, which based on my trapping efforts holds the largest subpopulation on the island (Cuarón et al. 2004). Such development would likely have significant impacts on local mangrove habitat, thereby directly and indirectly influencing raccoon populations.

A conservation recommendation rising from this study is for local government officials and ecological managers to minimize future reductions of the extent of mangrove habitat where the largest populations of raccoons occur on the island.

Currently, there is limited development of the northwestern section of the island where the vast majority of raccoons were found. The prevention of future habitat alteration in this area would protect this ecologically vital component of the ecosystem of Cozumel while securing that the major food source for the pygmy raccoons.

Figure 3.1: Map of Cozumel Island, Mexico and the three trapping sites where pygmy raccoons were samples from 2001-2003. Map taken from M. Morales (1999). Numbers (1-3) represent the three trapping sites.

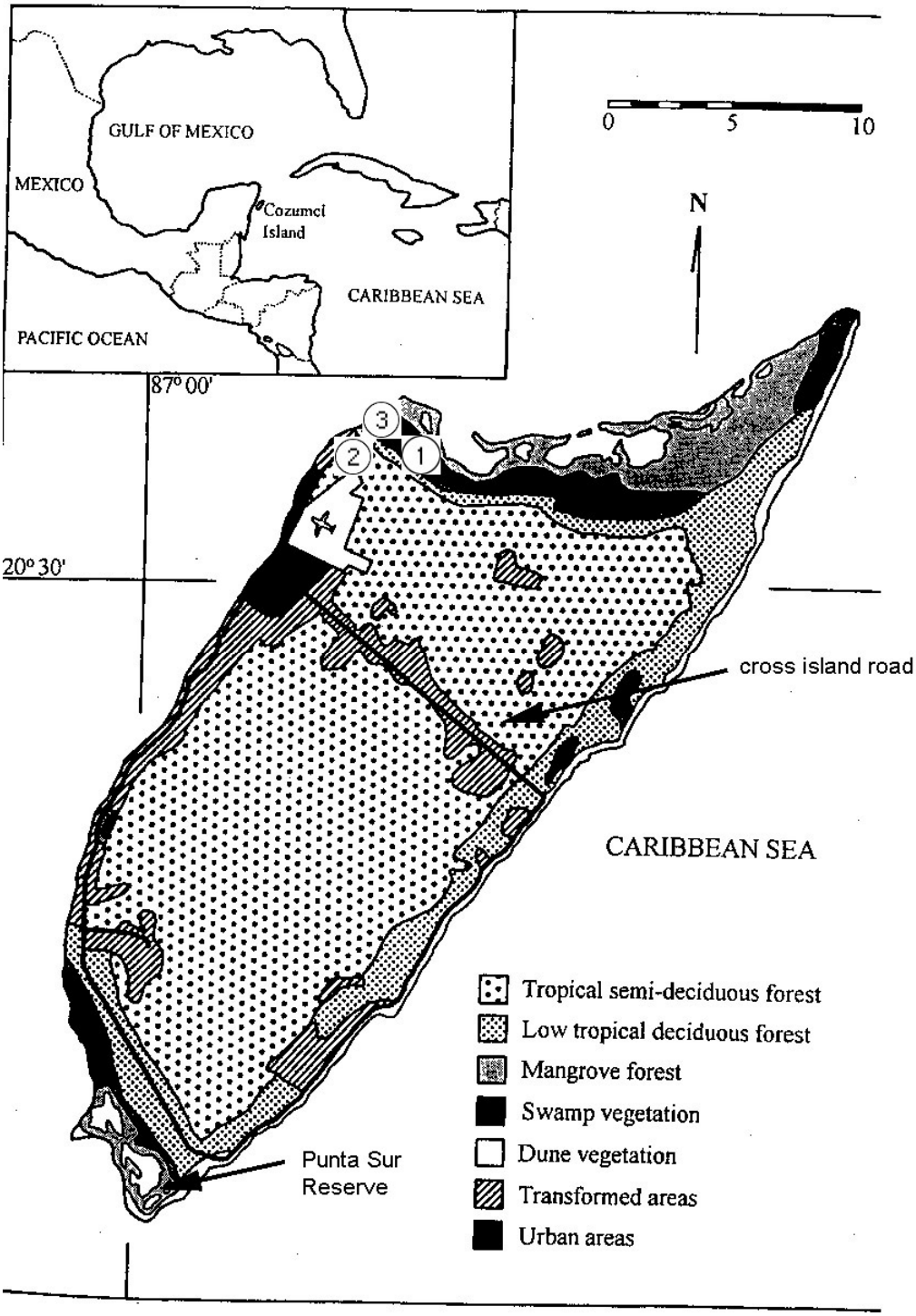


Figure 3.2: Stable carbon and nitrogen isotope ratios in blood and hair for pygmy raccoons sampled at three different trapping sites on the island of Cozumel, Mexico from 2001-2003.

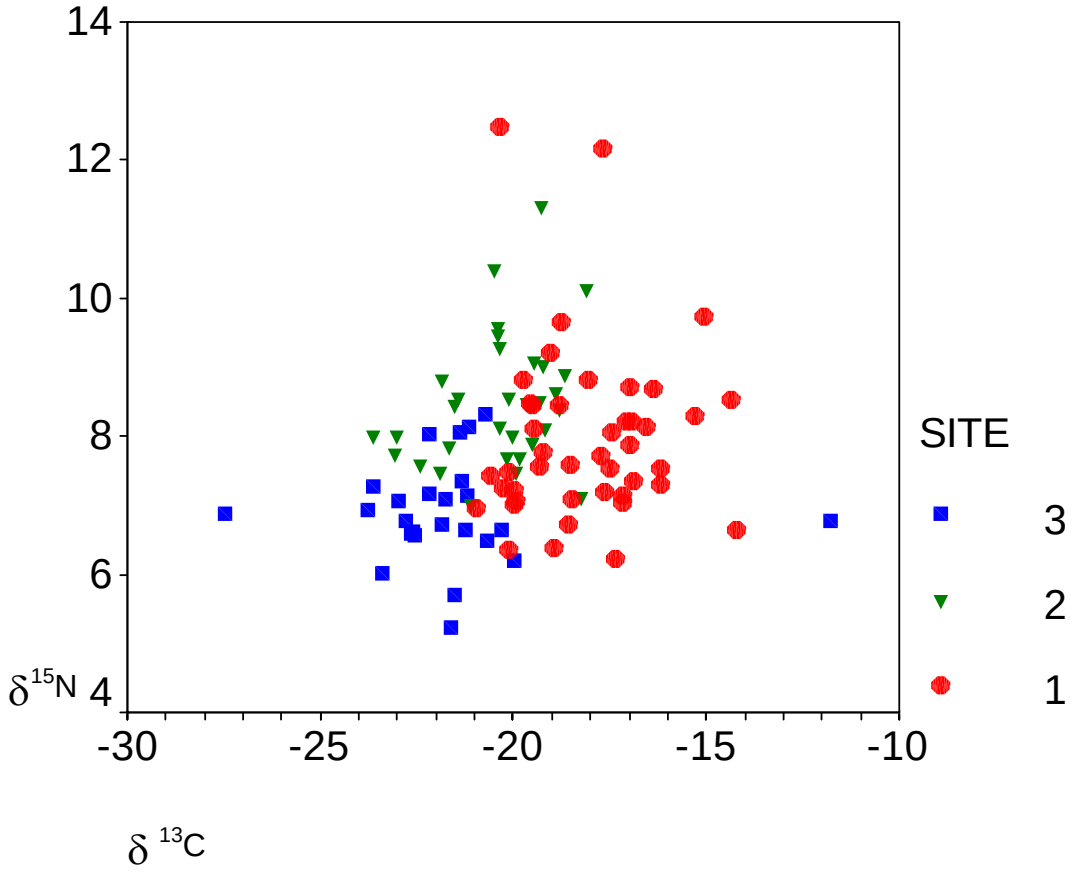


Figure 3.3: Stable carbon and nitrogen isotope ratios in blood and hair from pygmy raccoons on Cozumel Island collected during different seasons (2001- wet season), 2002 (early and mid wet season) and 2003 (dry season) throughout the three-year sampling period.

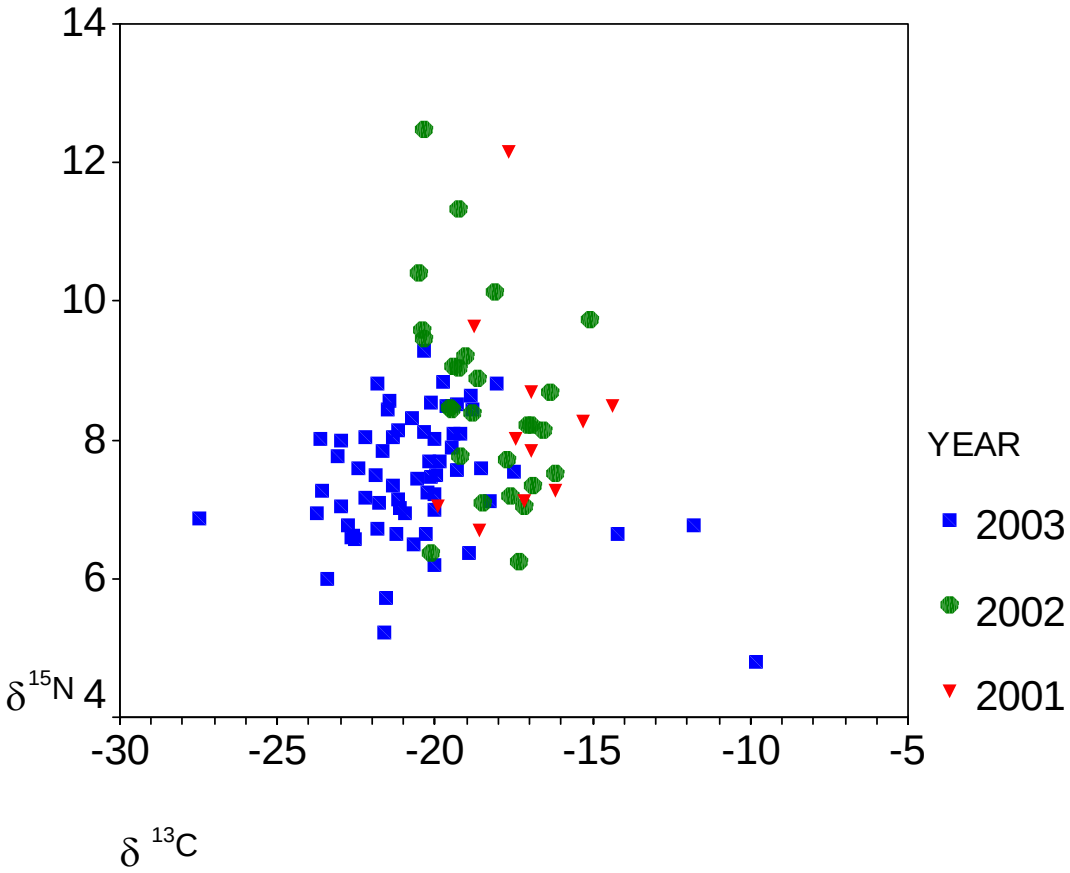


Table 3.1: Mean (± SE) $\delta^{15}N$ and $\delta^{13}C$ values for blood and hair samples of pygmy raccoons from three trapping sites in the North western section of Cozumel Island, Mexico over the course of three field seasons (2001-2003). Sample sizes are noted in parentheses. Some sites were not sampled (ns) in all years. Carbon values of blood samples from 2001-2002 are not reported (nr) due to improper storage conditions that adversely affected the carbon isotopic values.

Year	Site	Plasma/Serum $\delta^{15}N$ (‰)	Plasma/Serum $\delta^{13}C$ (‰)	RBC/Clot $\delta^{15}N$ (‰)	RBC/Clot $\delta^{13}C$ (‰)	Hair $\delta^{15}N$ (‰)	Hair $\delta^{13}C$ (‰)
2001	1	6.969 ± 0.354 (6)	nr	7.699 ± 0.300 (9)	nr	8.318 ± 0.344 (11)	-17.217 ± 0.572 (11)
2002	1	8.501 ± 0.614 (2)	nr	9.267 ± 0.637 (2)	nr	8.102 ± 0.269 (19)	-17.843 ± 0.431 (19)
	2	7.230 ± 0.442 (2)	nr	ns	nr	9.590 ± 0.381 (9)	-19.418 ± 0.633 (9)
	All	8.501 ± 0.614 (2)	nr	9.267 ± 0.637 (2)	nr	8.846 ± 0.352 (28)	-18.638 ± 0.350 (28)
2003	1	7.185 ± 0.434 (4)	-19.676 ± 0.708 (4)	8.163 ± 0.403 (5)	-18.054 ± 1.313 (5)	7.276 ± 0.466 (36)	-19.578 ± 0.504 (36)
	2	7.687 ± 0.241 (12)	-21.016 ± 0.409 (12)	8.464 ± 0.403 (5)	-20.935 ± 1.313 (5)	8.105 ± 0.432 (16)	-19.983 ± 0.466 (16)
	3	6.427 ± 0.307 (8)	-22.523 ± 0.501 (8)	7.405 ± 0.340 (7)	-20.353 ± 1.110 (7)	6.919 ± 0.381 (10)	-21.403 ± 0.411 (10)
	All	7.023 ± 0.450 (25)	-21.295 ± .840 (24)	7.939 ± 0.882 (17)	-19.854 ± 1.502 (17)	8.105 ± 0.420 (62)	-18.921 ± 0.438 (62)
Total	1	7.296 ± 0.520 (12)	-19.676 ± 0.708 (4)	8.039 ± .680 (16)	-18.054 ± 1.313 (5)	8.028 ± 0.672 (35)	-17.937 ± 0.752 (36)
	2	7.697 ± 0.436 (13)	-21.016 ± 0.409 (12)	8.463 ± 0.261 (5)	-20.935 ± 1.313 (5)	8.696 ± 0.714 (17)	-19.087 ± 1.253 (17)
	3	6.426 ± 0.313 (8)	-22.523 ± 0.501 (8)	7.404 ± 0.493 (7)	-20.353 ± 1.110 (7)	6.914 ± 0.330 (10)	-22.009 ± 1.034 (10)
Grand Mean		7.354 ± 0.184 (33)	-21.295 ± .321 (24)	8.199 ± 0.193 (28)	-19.781 ± 0.721 (17)	8.13 ± 0.243 (64)	-18.91 ± 0.257 (62)

Figure 3.4: Values (mean ± SE) for δ^{15}N and δ^{13}C for pygmy raccoon hair samples and potential prey items found on Cozumel Island, Mexico from 2001-2003. The sea turtle hatching was found 1 day post-mortem, and pigeon sample was taken from a corn-raised captive bird.

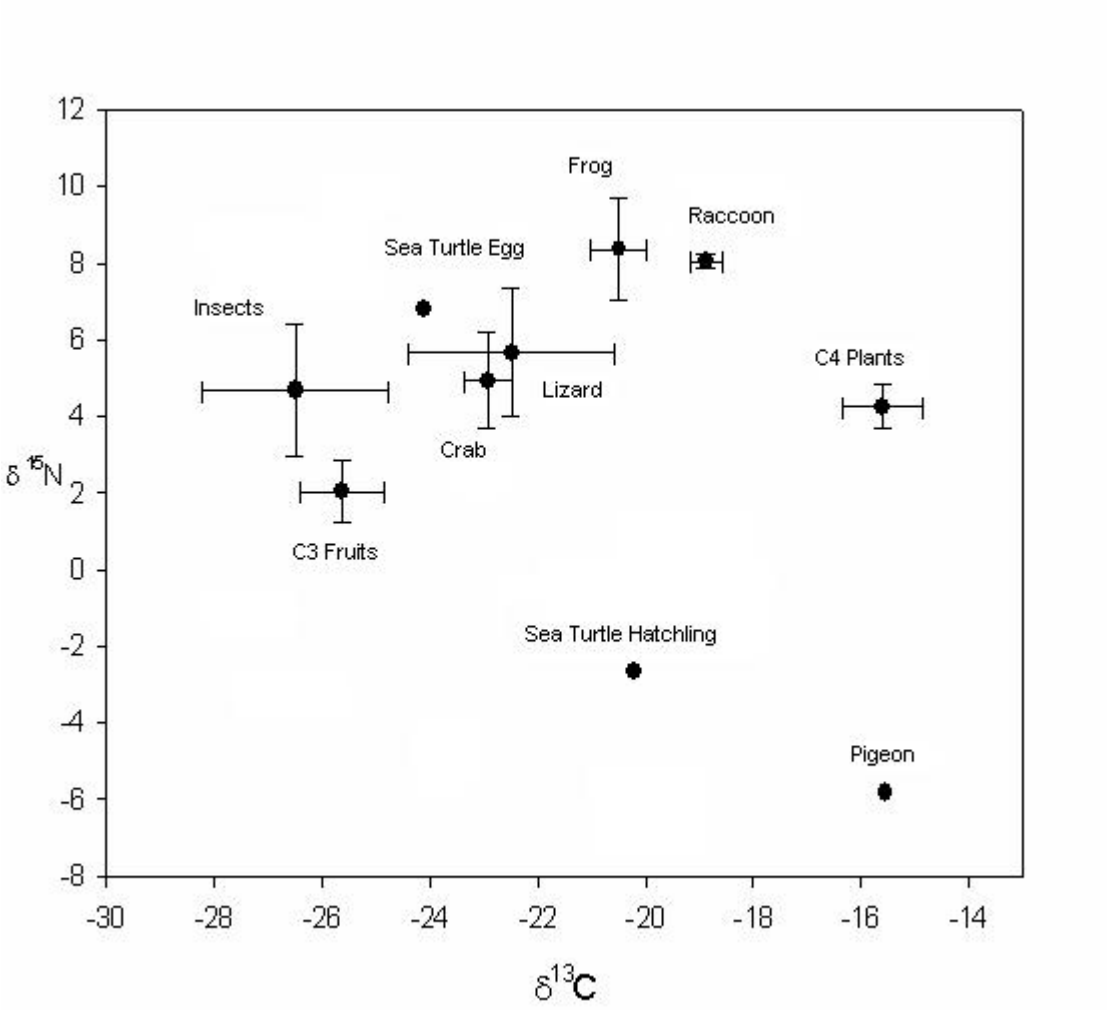


Table 3.2: Mean (± SE) stable carbon- and stable nitrogen isotope ratios in potential prey items collected from this study from 2001-2003 on Cozumel Island, Mexico for the pygmy raccoon. The following prey item isotopic values were taken from the literature: [1] Herrera et al. (2001), [2] Herrara et al. (2003), [3] Hobson et al. (2000).

Prey Item	**n**	δ **^{15}N (‰)**	δ **^{13}C (‰)**
Crab	10	4.987± 0.943	-22.928 ± 1.057
Sea turtle hatchling	1	-2.648 ± 2.981	-20.220 ± 3.344
Frog	3	8.356 ± 1.721	-20.493 ± 1.931
Lizard	3	5.663 ± 2.981	-22.483 ± 3.344
Fruit	13	2.095 ± 0.827	-25.326 ± 0.927
Pigeon	1	-5.821 ± 2.981	-15.552 ± 3.344
Sea turtle egg	1	6.781 ± 2.981	-24.098 ± 3.344
Insects[1]	Nr	4.670 ± 3.680 (SD)	-26.89 ± 3.860 (SD)
Ants[3]	4	3.800 ± 0.300	-25.50 ± 0.300
C3 Fruits[1]	Nr	1.220 ± 2.570	-29.49 ± 1.700
C4 Fruits[2]	3	4.200 ± 1.000	-15.6 ± 1.300

Table 3.3: Percent occurrence of food items in pygmy raccoon scats, by season and site, on Cozumel Island, Mexico. Site 3 was only sampled in 2003. Levin's index for food diversity (B_s) are listed in square brackets.

	2002[a] [0.73]		2003[b] [0.60]		
Food Category	Site 1 (n = 7) [0.75]	Site 2 (n =11) [0.70]	Site 1 (n = 9) [0.57]	Site 2 (n = 10) [0.53]	Site 3 (n= 13) [0.69]
Banana	43	54	33	40	23
Crab	57	27	67	20	54
Insect	57	55	11	30	31
Fruit	29	18	22	10	61
Vertebrate	0	9	0	0	0
Leaf/Plant	43	73	56	70	61

[a] Summer/Wet Season= April-July
[b] Winter/Dry Season = Feb. -March

Table 3.4: Percent contribution (mean ± SE) of different prey in the diet of pygmy raccoons on Cozumel Island, Mexico in different years (seasons) (2001-2003) and at different trapping sites based on stable isotope ratios of raccoon hair. The average percent contribution was also calculated using plasma/serum samples from 2003 (statistically significantly different from hair at $p < 0.05$).

	n	**Crab**	**Insect**	**Fruit**
Overall RBC/HAIR	65	48 ± 1.00	23 ± 0.67	29 ± 0.59
Overall Plasma/Serum	33	51 ± 2.36	19 ± 0.88	29 ± 1.86
2001 (wet)	11	45 ± 0.89	25 ± 0.64	30 ± 1.64
2002 (wet)	27	48 ± 0.97	23 ± 0.43	28 ± 0.68
2003 (dry)	24	50 ± 2.33	21 ± 1.63	29 ± 1.34
Site 1	35	47 ± 0.70	23 ± 0.43	30 ± 0.41
Site 2	17	54 ± 2.12	21 ± 1.00	25 ± 1.19
Site 3	10	47 ± 4.10	22 ± 3.69	31 ± 2.27

Chapter 4: POPULATION ECOLOGY OF THE PYGMY RACCOON (*PROCYON PYGMAEUS*) AND CONSERVATION STATUS OF THE DWARF COATI (*NASUA NELSONI*) OF COZUMEL ISLAND, MEXICO

Abstract

To better understand the population biology and conservation status of the endemic and endangered pygmy raccoon (*Procyon pygmaeus*) and dwarf coati (*Nasua nelsoni*), I worked island-wide to identify the presence of these species, and for the pygmy raccoon studied several populations in depth between 2001-2003. Trapping was conducted for >3500 trap nights in 10 locations of varying habitat types. Pygmy raccoons were found only in the most northwestern section of the island at three sites, despite what appears to be suitable habitat elsewhere on the island. A total of 78 individuals (38 males and 40 females) and a single adult, male dwarf coati (*Nasua nelsoni*) were captured. The distribution of the frequency of captures by age and sex varied by field season, but average capture probabilities over the three year study were similar between sexes. Using mark-recapture models and density estimates, the current population of pygmy raccoons on Cozumel Island is estimated to be fewer than 194 mature individuals at this time. Currently, the IUCN has listed both the dwarf coati and pygmy raccoon as Endangered (EN). However, research indicates that these species clearly warrant the criteria of Critically Endangered (CE) due to their restricted range and small population numbers. Results indicate that the Cozumel coati is in danger of eminent extinction, is extremely rare on Cozumel Island, and in need of immediate conservation protection.

Introduction

Islands are typically lacking in mammals, particularly mammalian carnivores, due to their poor over-water dispersal abilities, low carrying capacities, and large body sizes (Alcover & McMinn 1994; Brown & Lomolino 1998). Therefore, Cozumel Island is unusual in supporting several insular, endemic carnivores, of which the best described are the pygmy raccoon (*Procyon pygmaeus*) and the dwarf coati (*Nasau nelsoni*). As is the trend for island carnivores, both these species are smaller, or dwarfed, compared to their mainland ancestors. The dwarf coati and pygmy raccoon are both listed as endangered (IUCN criteria D and C2a, respectively) by both the World Conservation Union (IUCN) (Hilton-Taylor 2001) and Mexico (SEMARNAT 2002), although neither has been well-studied and consequently, their conservation status remains uncertain. While recent work suggests immediate and urgent conservation concerns (Cuarón et al. 2004), appropriate management of these animals has been limited by lack of such information – in particular information on the distribution of populations across Cozumel, and information on population size and structure.

Coatis and raccoons have existed on Cozumel Island dating to at least ancient Mayan times (Hamblin 1984) when they were seemingly widespread, and a recent phylogenetic study suggests divergence from the mainland congeners approximately 50,000 years before present (bp). A recent review of the status of these two species, however, suggests a precipitous decline over the past century (Cuarón et al. 2004). The exact causes of the decline are unknown but both species are thought to be adversely affected by human activities such as collection for pets, predation by the introduction of the *Boa constrictor* to the island, habitat fragmentation, hunting, and possibly by resource

competition, harassment, and disease spill-over from feral domestic carnivores (Cuarón et al. 2004; Martínez-Morales & Cuarón 1999; Navarro & Suarez 1989). Natural sources such as hurricanes may also negatively impact these species (Navarro & Suarez 1989).

Virtually no information exists on the status and abundance of these endangered island endemics beyond a brief survey carried out in the 1980s (Navarro & Suarez 1989) which identified pygmy raccoons and dwarf coatis in the mangrove forest of Chankanaub, northwest tip of the island and the adjacent Isla de Pasión. Since Navarro and Suarez's survey, however, no broad-based field study of these taxa has been carried out and little formal conservation action has been implemented to ensure their protection (Cuarón et al. 2004). Using line transect sampling, Martinez-Morales (1999) assessed the population of mammal species on Cozumel in 1995. They reported sighting no pygmy raccoons during this diurnal effort, and only two coatis in over 386 km of walked transects. Using this data, they preliminarily estimated the density of this species to be 0.43 individuals/km^2 (Cuarón et al. 2004).

Although no previous mark-recapture (MR) population estimates exist for Cozumel carnivores, estimating population size using the MR techniques has been conducted with other species of raccoons and coatis and is facilitated by a relative ease of capture for these species (Gompper 1997; Kaufmann et al. 1976; Sanderson 1951; Sanderson & Nalbandov 1973; Sumner & Hill 1980). A primary goal of this research is to assess the locations of pygmy raccoon subpopulations on the island and better assess the current population structure and relative population densities. This information is important to determine their conservation status and allow for more informed management decisions.

Methods

Study Sites, Capture and Handling:

Cozumel Island (20°16' to 20°26'N and 86° 44' to 87°02'W) is a 486 km^2 island made up of a variety of terrestrial habitats including dry semi-deciduous forests, mangrove stands, sandy palm areas and multistratal tropical evergreen forests (Tellez et al. 1982). Cozumel is located 15 km off the Yucatan peninsula and the mean annual temperature is 25.5° C while the average annual rainfall is 1.03 m (Martinez-Morales 1996). Wet and dry seasons exist in this region and presumably create varying food availability and the natural resources on the island are similar to those found on the mainland coast of Quintana Roo (Estrada & Coates-Estrada 2001; Freidel & Sabloff 1984)

During two, 3-month sampling periods in both 2001 and 2002, and a one month sampling period in 2003, a total of 10 sites throughout the island were sampled for a minimum of 2 weeks per site. Three sites (sites 1-3) in the northwestern mangrove-dominated habitats (Figure 4.1a) were chosen for further study based on preliminary identification of substantial pygmy raccoon populations. Site one (86° 87' N 20° 54' W) is characterized as existing off a dirt road leading towards the most northwestern part of the island, close to Isla de Pasión. In addition to the mangrove habitat, this site was bordered by semi-deciduous forest. Site two (86° 95' N 20° 49'W) was located in habitat surrounding a golf course, with trapping areas consisting of both deciduous and mangrove forests. Site three (86° 91' N 20° 55' W) was located approximately 0.8 km north –northwest of site two (3.5 km from site one) and was located in mixed mangrove-

deciduous forest in close proximity to the coast. Between 5-20 traps were placed at 25m intervals along trails in mangrove and deciduous forest habitat.

Using satellite images of Cozumel, GPS data, and the mapping program, TNTmips version 6.9 (MicroImages, 2004) a satellite image of Cozumel Island was digitized and precise trapping size areas were estimated for each site (Figure 4.1b). Site 1 covered approximately 0.17 km^2 while sites 2 and 3 were approximately 0.22 km^2 and 0.10 km^2, respectively. To estimate the pygmy raccoon density on the island as a whole, I used both knowledge of the island's habitat that was obtained by visiting various sites on the island, pygmy raccoon habitat preferences, along with satellite images, which reflected variation in habitat type, to generally estimate the size of areas (km^2) where this species might potentially occupy and the total density of this species on Cozumel Island. Areas of potential raccoon habitat were digitally outlined and surface area was calculated for these regions. Using the population density estimates obtained from the mark-recapture portion of the study, the total island population density was inferred.

The first field season was conducted from July through September 2001 (wet season), the second field season spanned from April (late dry season) to July 2002 (mid wet season), while the third season was February- March 2003 (dry season). Although all sites are in relatively close proximity to each other, they should be considered independent subpopulations based on the fact that recaptured tagged individuals from one population were never captured outside of the site of their original capture.

Animals were captured using Tomahawk box traps (#207) baited with either canned cat food or a honey-banana mixture and checked at least once daily. In an effort to trap the extremely rare Cozumel coati, traps were baited with a variety of baits

including commercially available scented carnivore bait (Tomahawk), various fruits, fish, and live crabs. Trapped animals were immobilized with ketamine hydrochloride (10-12 mg/kg) and xylazine (2 mg/kg) by hand injection prior to handling and ear tagging (HASCO). Age was approximated as juvenile (0-12 months), subadult (13-21 months), or adult (> 21 months) from tooth wear (Grau et al. 1970), body size, reproductive status (Sanderson & Nalbandov 1973) and recapture history. For males, canine size and the descent of the testes were used to differentiate between subadult and adults. Females were considered to have bred based on nipple morphology and tooth wear (Grau et al. 1970). A female was assumed to be breeding if her nipples were enlarged when captured. Uncertainty about breeding status occurred in some cases when nipples were prominent but not qualitatively observed to be "enlarged". Such individuals were thus classified as non-breeding. Animals recaptured within one week of their original capture were released without handling. All captured pygmy raccoons were released at the trap site after recovery from sedation (usually within 2 hours of checking the trap).

Age and Sex Capture Probabilities:

Capture frequencies were examined for variation by year, age, and sex, using chi-square tests. Using the CAPTURE feature in program MARK (White & Burnham 1999), chi-squares tests were used to assess time-related variation and behavioral response to capture. Specifically, the tests used to assess these factors were: *Test 1*) Tests for heterogeneity of trapping probabilities in a population, *Test 2*) Tests for behavioral response after initial capture, *Test 3*) Tests for time-specific variation in trapping probabilities, *Test 4*) Goodness of fit test of model. The results of these tests were used

to select the appropriate model estimators. Due to low sample sizes, all individuals were pooled by site for these tests.

Demographic Analysis:

Using Pollock's (Pollock et al. 1990; Pollock 1982) robust population model the annual recapture probabilities ($\hat{p}$) and abundance ($\hat{N}$) of individuals were examined for site 1 and 2, which I had trapped for three and two consecutive years, respectively. A Schnabel closed population model in Program MARK (White & Burnham 1999) was used to estimate population size for site 3, which was only sampled for one field season (2003).

The sampling interval was considered the active trapping days during a given year, and each pygmy raccoon was recorded as either captured or not captured on that day. The robust design model is a combination of Cormack-Jolly-Seber (CJS) (Jolly 1965; Seber 1965, 1970) and closed capture models (Pollock et al. 1990). This model was used because it allows for closely-spaced trapping sessions to be viewed as a closed capture survey while longer intervals between sessions allow estimation of survival, temporary emigration from the trapping area and are viewed as an open capture survey. Thus the primary sampling periods where gains (birth and immigration) and losses (deaths and emigration) are examined using CJS and the secondary sampling periods, or the day-to-day trapping sessions, are effectively closed to gains and losses (Kendall et al. 1997; Kendall & Nichols 1995). This model is powerful in that the probability that an animal is captured at least once in a trapping session can be estimated from just the data collected during the session using capture-recapture models developed for close

populations, such as those summarized by Otis et al. (1978). Another advantage of this design is that abundance and recruitment are less biased by heterogeneity in capture probability (Otis et al. 1978).

The assumptions for the robust design model are a combination of closed-population and the CJS method and include: 1) the population is closed across all sampling days (*i.e*. within a field season); 2) samples are instantaneous and releases are made immediately after the sample; 3) animals do not lose marks between the sampling periods, 4) movement in and out of the area is random; 5) every marked animal in the population has an equal chance of survival and/or capture and 6) marking individuals does not affect their catchability. Assumptions 1- 3 were believed to have been met in the sampling design and marking procedures. Assumptions 4-6 were examined using the chi-square tests in CAPTURE as described above.

Program CAPTURE (Otis et al. 1978) contains eight closed capture population estimation models, five with estimators, for estimating population size when sources of variation in capture probabilities act individually or in combinations. All models in CAPTURE assume demographic closure and makes specific assumptions about an animal's capture probability. Models have been developed that incorporate three sources of variation in capture probabilities: 1) time, 2) behavioral response, and 3) individual heterogeneity (Otis et al. 1978). Models were selected on the basis of chi-square test results of tests 1-4 (see above).

Results.

Variation in Capture Probability:

Ten sites were trapped for 3,588 trap nights during this study. Despite intense trapping efforts in many different habitat types, individuals were only trapped at three sites (sites 1-3) in the northwestern tip of the island (Figure 4.1b). Throughout the study there were 115 captures comprising 78 individual pygmy raccoons (38 males and 40 females). Adults made up the majority of captures (60%), followed by subadults (21%) and juveniles (19%). A single, adult male dwarf coati was trapped in 2002 at site one. Therefore, the focus of the results refers to population estimates of the pygmy raccoon.

Site 1 accounted for the majority of the captures and was the only site sampled in all three years. Chi-square tests revealed that the distribution of the frequency of captures of males and females did not vary over the course of the three-year study ($X^2 = 0.084$, df = 1, p = 0.772). Significantly higher number of females were captured at site 1 in both 2002 and 2003 ($X^2 = 4.23$, df = 1, p = 0.040; $X^2 = 4.00$, df = 1, p = 0.046, respectively). The ratio of males and females in each of the three age classes (juvenile, subadult, adult) also significantly varied ($X^2 = 14.26$, df = 1, p = 0.001). Site 3 had nearly twice as many adult males captured than females ($X^2 = 3.84$, df = 1, p = 0.050), while all other age classes tended to have more females than males. The overall ratio of males to females for all individuals at all sites was 1.2:1

The proportion of each age class captured varied and adults were the largest proportion of captures at all sites ($X^2 = 8.944$, df = 2, p = 0.011). Site 2 had more adults than subadults and juveniles in the third year of trapping ($X^2 = 9.30$, df = 2, p = 0.002).

Similarly, site 3 also had significantly higher number of adult captures than in the other age classes ($X^2 = 3.84$, df = 2, p = 0.019).

Program CAPTURE selected the Zippin estimator (M_b) for population estimations for site one. This model assumes capture probabilities vary by behavioral response to capture (trap happiness or trap shyness), but are otherwise homogenous in their capture probabilities (Table 4.2). No behavioral heterogeneity to capture was seen in site 2 or 3 and therefore the null estimator (M_o) was used. This estimator is the simplest of all models and assumes all members of the population are equally at risk of capture on every trapping occasion.

Probability of capture and recapture were linked to trapping effort and the extrapolated size of the population (Table 4.3). The probability of capture ($\hat{p} = 0.118$) was highest at site 1 during my most intensive trapping effort (2002). At other sites and in other years, the capture of probability was generally lower and ranged from 0.011 to 0.039, but did not vary significantly among years. The probability of recapture ($\hat{c}$) was highest at site 3 (0.085) and lowest at site 1 (0.005) (Table 4.3). No clear relationship was identified between capture and recapture probability, likely owing to the small population size of this species.

Demographic Analysis:

Abundance estimates indicated that site 1 contains the largest subpopulation of pygmy raccoons and was estimated to be 23 individuals (95% CI= 10- 73) (Table 4.3). However, this site also represented my most comprehensive trapping effort. This was especially true in 2002, where after one month of intensive trapping, the individual

capture accumulation curve reached its asymptotic plateau (Figure 4.2). During this season, trapping efforts eventually produced only previously marked individuals by day 21 (of 31) of trapping, with no new individuals being trapped at that point. Therefore, the subpopulation at site 1 is estimated to be approximately 19-23 individuals. This population estimate is consistent with what was found in the individual accumulation curve and with the lower range estimated by CAPTURE for this year.

Abundance estimates at site two varied depending on trapping effort. In 2002 when there was a more robust trapping effort, abundance was estimated to be approximately 20 individuals (95% CI = 14-48) while in 2003, the estimate was 23 individuals with a significantly broader 95% confidence interval (range 17-233). Closed capture models estimated the number of individuals at site 3 to be 16 (range 13-20). Thus, I estimate the total population of pygmy raccoons at the three sites to total 62 individuals (95% CI = 45-103). Using what is considered to be the most accurate estimate of pygmy raccoon abundance at site 1 (derived from year 2 trapping data), 10 pygmy raccoons were trapped per 100 trap nights. The number of pygmy raccoons at site 2 and 3 was 7.9 per 100 trap nights and 9.5 per 100 trap nights, respectively. Although each trap site was under 1 km^2 raccoon densities at sites 1, 2, and 3 were estimated to be 112, 91, and 160 raccoons/km^2, respectively; these estimates were used to predict total island density.

An additional 1 km^2 of potential habitat was identified as potential habitat by outlining areas of similar habitat to my trap sites on the digital image of the NW section of the island (Figure 4.1b). These areas were identifiable based on familiarity with the landscape, visual changes in coloration on the digitized image that represented changes in

vegetation types, and based on a map taken from M. Morales (1998) (see Figure 3.1) which graphically depicted the different vegetation types on Cozumel Island. Based on density estimates at trap site 1, it is estimated that 112 individuals may exist in habitat surrounding this site. Using what was determined to be potential habitat surrounding site 2, up to 61 individuals may exist in this area. Using this same technique, 150 individuals may exist in habitat surrounding site 3. Therefore, it is estimated that the northwestern section of Cozumel Island has a population size of 323 individuals and would translate into 194 adult individuals.

Discussion

In assessing the abundance of pygmy raccoons on Cozumel Island, the most striking finding was the lack of animals captured in all areas on the island except the most northwest quadrant of the island. Although I trapped at areas where residents had previously seen this species, if populations did exist in these areas, they were too small to be detected through trapping. Based on this study, pygmy raccoons prefer mangrove habitat to other habitat types (semi-deciduous and dry forest, etc.) found on the island. Despite the preference for mangrove habitat, populations of pygmy raccoons were not found in mangrove areas on either the southern portion of the island (Figure 4.1- site 4), where considerable trapping effort was exerted, or on the unpopulated northeastern side of the island (site 9). While trapping efforts on the latter site were limited due to the remote nature of the site, both of these areas had extensive mangrove habitat in which I trapped.

Additionally, even in the northwestern area of the island, where the most significant populations of pygmy raccoon exist, the range of their habitats was extremely heterogeneous. For example, traps were set in mangrove habitat along the mangrove-bordered 2 km road leading to Isla de Pasión, but trapped individuals were only found animals in the last 200 m^2 of this habitat closest to the ocean. Additionally, at site 3, traps were placed further inland by 300 m, yet no individuals were successfully trapped. Therefore, pygmy raccoon populations appear to exist very sporadically in the habitat, and are not evenly distributed even in what might be considered to be as "prime" mangrove habitat (*i.e.* close proximity to the ocean, largely unfragmented, low human population levels).

The number of captured pygmy raccoons did not significantly vary over the course of the study indicating that although each population is extremely small, population numbers appear relatively stable at this time. Some temporal variation seen at site 1 can be explained by the capture of trap-happy individuals in 2002 that biased capture probability calculations. The use of the Zippin abundance (Zippin 1958) and capture probability estimator was thus used as it takes into account behavioral heterogeneity amongst individuals. One subadult pygmy raccoon from this subpopulation, however, was especially trap-happy and was captured 7 times in a three-week period. If this individual is removed from the population estimation analysis, no significant behavioral heterogeneity is seen in this subpopulation. Capture probabilities did not vary between sexes when examining the entire dataset as a whole, but there was capture heterogeneity at various sites. If the pygmy raccoon, like its mainland sister taxa (*P. lotor*), is characterized by male-biased dispersal, a possible scenario to explain both

the sex ratio and age-class biases at site 1 and 3 might be that site 1 partly serves as a population source for site 3. Alternatively, behavioral heterogeneity to capture may also explain this difference in capture probability.

The behavioral heterogeneity to capture may be a reflection of feral dog harassment stress, which was especially high at site 3. Although both pet and feral dogs were seen or detected at all sites, this site was the only locale that I witnessed an animal die (KWM, unpublished data) due to a feral dog attack.

The largest proportion of young cubs were captured in the February 2003 trapping effort, and a large portion of mother-cub or cub-cub captures (*i.e.* captured together) also occurred in early spring (April 2002). On the basis of morphological variable such as dentition and mass, the peak birth period for this species appears to occur from November -January. During February 2003 there was also a higher proportion of captures of mother-young simultaneous captures in which a mother with evidence of recent lactation was caught in one trap and cubs were caught in other traps.

Variations in capture and recapture probabilities occurred between field seasons and were likely a reflection of both the local subpopulation abundance and trapping effort at each site. Even when the number of traps placed at site 1 greatly varied, the capture and recapture probability did not significantly differ, indicating that the small population numbers at this site were a more limiting factor than the trapping effort at this particular site. Capture and recapture probabilities seemed equally linked to both trapping effort and subpopulation size. The highest number of captures per unit capture effort was seen at site 3 where the capture per unit effort was nearly three times as high as at other sites.

This site also had a higher number of adult male males captured and thus may indicate a sex related behavioral heterogeneity in this subpopulation.

There are numerous sites on Cozumel Island that traps were not placed, but may theoretically contain subpopulations of raccoons, making it difficult to estimate the total population of pygmy raccoons on the island. However, based on trapping efforts and with the exception of a few suspected population of pygmy raccoons in and among the mangroves areas which were not trapped in the NW of the island, that this study was successful in identifying the main populations of raccoons on the island. The adult population estimate of 194 individuals is likely an overestimate of the true Cozumel Island pygmy raccoon population size. Based on my knowledge of the pygmy raccoons 1) small subpopulation sizes (mean 25.5) and 2) their extremely heterogeneous distribution even in what appears to be suitable mangrove habitat, the estimate of 323 total individuals should be taken with some caution.

Additionally, although this study likely overestimates the number of individuals existing in the NW area of Cozumel, there may be additional populations, especially in the remote NE tip of the island, which were not surveyed. An additional 20-23 km^2 of mangrove forest habitat exists in the most northern-central to north-northeastern section of the island (Figure 3.1a). However, this region is almost completely unexplored by naturalists, generally only accessible by boats, extremely remote, and therefore this study has been unable to identify if this area 1) contains raccoons or 2) is suitable habitat for this species (*i.e.* not regularly flooded). Therefore, although this area may potentially contain raccoons, this study is unable to accurately quantify or evaluate the possibility of

raccoons existing there and therefore serves as a source of potential error in the total population estimate.

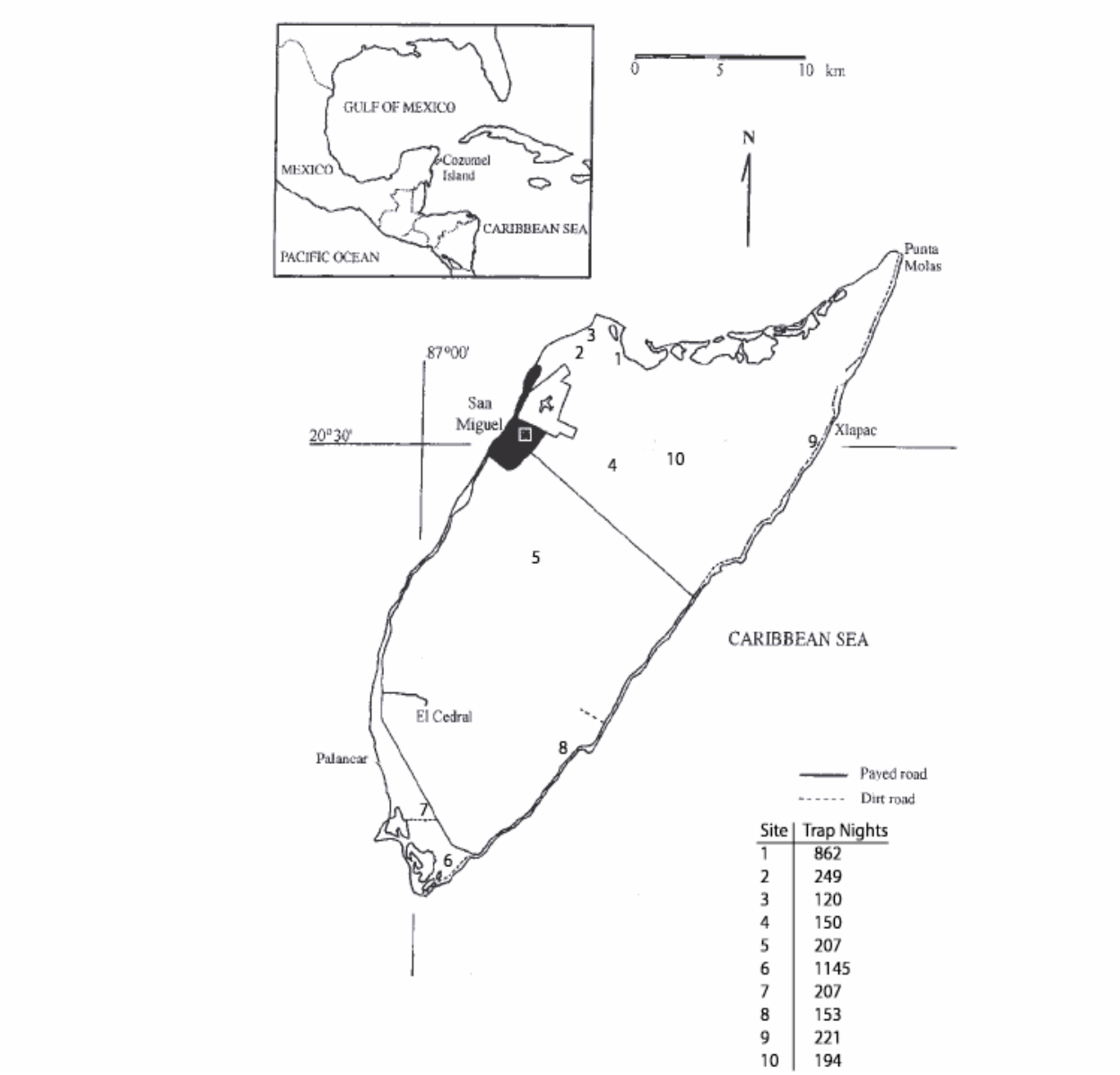
Recommendations for Conservation:

Only a single dwarf coati was captured in over 3,500 trap nights, an alarming indication of the rarity of this species on the island of Cozumel. Although dwarf coatis were sited on five different occasions (twice in the northwest part of the island, twice at Punta Sur and once in the northeastern section of the island), this species is clearly very rare on the island. Based on intensive trapping efforts on the island, it appears that the Cozumel coati is in immediate danger of extinction.

Both Mexico (SEMARNAT 2002) and the IUCN have listed the pygmy raccoon and Cozumel coati as endangered (IUCN: D dwarf coati) (C2a, pygmy raccoon) (Hilton-Taylor 2001). However, based on population estimates as well as justification put forth by Cuarón et al. (2004), I propose that their designation be immediately changed to critically endangered (CR). The requirements of the CR designation which the pygmy raccoon and dwarf coati fulfill are 1) that population size be estimated to number fewer than 250 mature individuals, 2) no subpopulation estimated to contain more than 50 mature individuals, and 3) extent of occurrence estimated to be less than 10 km^2 and known to exist at a single location (Hilton-Taylor 2001). In terms of total population numbers, the largest pygmy raccoon subpopulation is estimated to have only 25 adults, and the second largest subpopulation has approximately 19 adults. Therefore, this species clearly qualifies as critically endangered and should be considered to face an extremely high risk of extinction in the wild based on the current population estimates.

A conservation recommendation rising from this study is for local government officials and ecological managers to minimize future reductions to the extent of mangrove habitat where the largest populations of raccoons occur on the island. Currently, some limited management plans are in place to limit development of the northwestern section of the island where the vast majority of raccoons were found. The prevention of future habitat alteration in this area would protect this ecologically vital component of the ecosystem of Cozumel and this critically endangered species. Catastrophic events such as periodic hurricanes are an important consideration when examining the future of this species. As a tropical island, Cozumel is frequently hit by hurricanes. The last catastrophic hurricane (Gilbert, category 5, known at the time as "the storm of the century") hit Cozumel in 1988 and reports from locals indicate that wildlife on the island suffered tremendous losses during this storm and hurricanes of varying intensity hit Cozumel Island every 8.31 years (Williams 1998). A local subpopulation on Isla de Pasión is thought to have been extirpated due to hurricane Gilbert (R. Sheese, personal communication). Healthy wildlife populations are able to withstand such losses and have done so for eons. Given the exceptionally small population numbers of pygmy raccoons on the island, a captive breeding program should be considered as this species is at an extremely high risk of extinction in response to the normal environmental stochasticity associated with both island and hurricane prone locales.

Figure 4.1a: Map of Cozumel Island, Mexico and the locations of trapping sites and total number of trap nights at each location from 2001-2003. Map modified from Martinez-Morales (1999).



Site	Trap Nights
1	862
2	249
3	120
4	150
5	207
6	1145
7	207
8	153
9	221
10	194

Figure 4.1b: Map of northwestern section of Cozumel Island, Mexico and the locations of trapping sites (1-3) and the city of San Miguel (4). Taken from NASA photo (6-01-2002) by Earth Sciences and Image Analysis (NASA-JSC-ES&IA).

Table 4.1: Trapping effort and capture success of males (M) and females (F) at three sites on Cozumel Island, Mexico from 2001-2003.

Year	Site	Trap Nights	Juveniles		Subadults		Adults		Total Captures	Capture per Effort
			F	M	F	M	F	M		
2001	1	354	0	0	2	1	1	8	12	0.033
2002	1	428	4	4	13	1	6	6	34	0.079
	2	234	0	0	2	4	4	5	15	0.064
2003	1	80	7	1	1	0	4	3	15	0.187
	2	135	1	0	0	0	3	9	13	0.095
	3	80	4	3	0	0	4	15	26	0.325

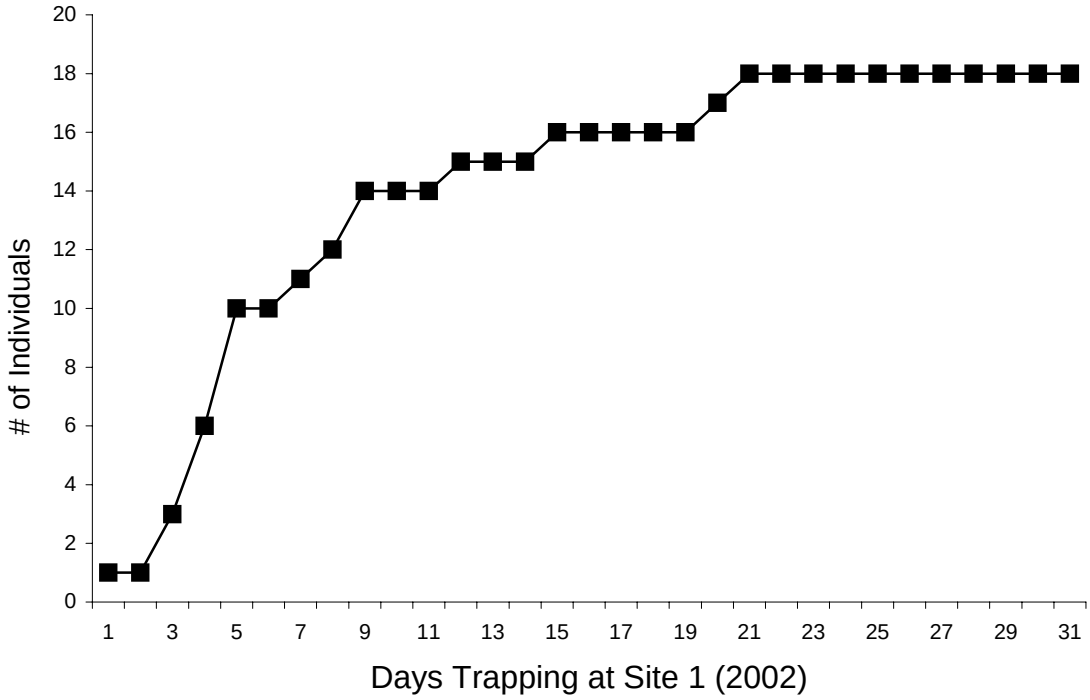
Table 4.2: Chi-square tests for the assumptions of a closed population estimate of pygmy raccoons on Cozumel Island, Mexico from 2001-2003 at three different trapping sites. Test 1) Tests for heterogeneity of trapping probabilities in population, Test 2) Tests for behavioral response after initial capture, Test 3) Test for time specific variation in trapping probabilities, Test 4) Goodness of fit test of model. The appropriate model is indicated in parentheses and was selected using the program CAPTURE. * indicates expected values were too small and the test was not performed.

Test	**Site**	X^2	**df**	***p***
1	1 ($M_{(b)}$)	2.869	1	0.090
2		6.537	1	0.010
3		9.206	30	0.999
4		45.535	30	0.034
1	2 ($M_{(o)}$)	*	*	*
2		0.239	1	0.624
3		2.332	23	0.988
4		25.593	23	0.320
1	3 ($M_{(o)}$)	2.196	1	0.138
2		2.580	1	0.108
3		6.146	15	0.977
4		20.15	15	0.171

Table 4.3: Pygmy raccoon population estimates based on closed population (C) and using Pollock's robust method (R) of repeated marking (M) and recapture. The number of days, or trapping occasions, the probability of being captured ($\hat{p}$), probability of recapture ($\hat{c}$), estimated number of individuals ($\hat{N} \pm$ SE), and the range of population estimates based on 95% confidence intervals are noted for each year and site.

Year	**Site**	**Trapping Occasions**	**M**	**C**	$\hat{p}$	$\hat{c}$	$\hat{N}$	$\pm SE$	***95%* CI**	**Model**
2001	1	27	11	12	0.0074	0.0054	36	12.263	22-73	R
2002	1	31	19	34	0.1189	0.0312	19	0.7636	19	R
	2	24	11	15	0.0306	0.0182	20	7.616	14-48	R
2003	1	16	8	13	0.0345	0.0467	14	4.677	10-19	R
	2	16	10	13	0.0143	0.0186	48	42.23	17-230	R
	3	16	13	25	0.0391	0.0851	16	3.856	13-20	C
Mean		21.66	12	19	0.0408	0.0342	25.5	12	45-103	

Figure 4.2: Pygmy raccoon (*Procyon pygmaeus*) population asymptotic accumulation curve for the most intensely trapped site on Cozumel Island (site 1) during live trapping efforts in 2002.



Chapter 5: BODY SIZE OF THE PYGMY RACCOON (*PROCYON PYGMAEUS*) OF COZUMEL ISLAND, MEXICO

Abstract

Several subpopulations of the endemic and endangered pygmy raccoon (*Procyon pygmaeus*) on Cozumel Island, Mexico were studied from 2001-2003 to assess temporal, spatial, sex and age-class variation in external body size measures. A total of 78 individuals (38 males and 40 females) were measured. Like *Procyon lotor*, the pygmy raccoon exhibits significant male biased sexual dimorphism. On average, adult males weighed 11.2% more than females, and male canines were approximately 1 cm longer than females. Adult and subadult morphometric values, including mass, did not fluctuate seasonally. Adult males at one site with heavy anthropogenic influences were approximately 0.5 kg heavier than males at the other sites and had significantly larger neck circumference and canine length. These individuals did not, however, differ in other measures such as body length, suggesting resource availability may partially, but not fully, explain the reduced stature of pygmy raccoons. No significant differences in morphology was detected between sites for the other age classes or when examining only adults females. Although discriminant analysis was successful in separating the three age-classes using mass and canine length as the indicator variables, the most reliable use of these variables is in separating the juvenile and adult age classes due to overlap between subadults and adults in morphological measures. In comparing the morphology of *P. pygmaeus* to mainland *P. lotor shufeldti*, the island species is approximately 14.7% smaller than its closest mainland conspecifics and the average rate of body change is to be to be 0.03% per 1000 yrs.

Introduction

Virtually every aspect of a mammal's biology is closely related to or influenced by its' size (Clutton-Brock 1983; McNab 1971). At a minimum, baseline data on body size can provide important clues into the natural history, ecology and life history strategies of a species. Monitoring the body size of wild animals can also provide valuable information concerning the "well being" of the population, and as a predictor of individual reproductive success and survival (Kilpatrick 1980; Peters 1983). Mass is often used as an indicator of the nutritional state of animals, and provides insight into factors influencing animal-habitat interactions such as prey availability and age-class foraging ability (Altmann et al. 1993; Caughley 1977; Hanks 1981; Kilpatrick 1980; Lockmiller et al. 1989; Virgl & Messier 1993).

The pygmy raccoon (*Procyon pygmaeus*) is a distinctive insular allospecies closely related to *Procyon lotor shufeldti* of the Yucatan peninsula, Mexico and is endemic to Cozumel Island. As is often the case with insular mammals, this species is considered smaller, or dwarfed, compared to its' mainland sister taxa (Case 1978; Gehrt & Fritzell 1998; Gehrt 2003; Prestrud & Nilssen 1995). However, despite the assumption of dwarfism, the morphometrics of this taxa have not been closely examined. While weights and lengths of 14 pygmy raccoons were reported in a brief survey in the 1980s (Navarro & Suarez 1989), no broader morphometric studies have been carried out to assess 1) the variability in morphometric measures, and 2) the rate of morphometric change relative to mainland congeners.

Dwarfism in island populations is common and has been seen in deer, fox, hippopotami, and elephants (Foster 1964). This finding has been referred to as the island

rule (Van Valen 1973)and the effect of size evolution is thought to be roughly inversely proportional to the size of the island and positively related to the degree of isolation from the mainland (Heaney 1978). Numerous selective forces are thought to explain the size decrease in large island mammals. In particular, they are thought to be released from predators on islands and thus do not need to maintain larger body size to ward off such predators. Limited food resources of island habitats may also increase competition and smaller size may be advantageous as metabolic requirements generally decrease with body size (Case 1978; Damuth 1993).

Recent phylogenetic studies on the pygmy raccoon indicate that the pygmy raccoon has existed on Cozumel for approximately 46,970 years and are clearly an endemic species. The reduced size of subfossil bones deposited over the past ca. 2000 years also indicate that dwarfism is not a recent phenomena (Hamblin 1984; Gompper unpubl. data). By comparing the insular pygmy raccoons with those of its closest (geographically and taxonomically) mainland counterpart, a course understanding of rates of evolutionary dwarfism can be made.

Methods

Study Sites:

Cozumel (20°16' to 20°26'N and 86° 44' to 87°02'W) is a 486 km^2 island made up of a variety of terrestrial habitats including dry deciduous forests, mangrove stands, sandy palm areas and multistratal tropical evergreen forests (Tellez et al. 1982). The mean annual temperature is 25.5° C while the average annual rainfall is 1.03 m (Martinez-Morales 1996).

During two, 3-month sampling periods in both 2001 and 2002, and a one-month sampling period in 2003, pygmy raccoons were trapped and measured from three sites (sites 1-3) in the northwestern mangrove- dominated habitats, the sole area of Cozumel where sizable populations of this species persist. The first field season (site 1 only) was conducted from July through September 2001 (wet season), the second field season (sites 1 and 2 only) spanned from April (late dry season) to July 2002 (mid wet season), while the third season (sites 1, 2, and 3) was February- March 2003 (dry season).

Animals were captured using Tomahawk box traps (#207) baited with a honey-banana mixture and checked at least once daily. Trapped animals were immobilized with ketamine hydrochloride (10-12 mg/kg) and xylazine (2 mg/kg) by hand injection prior to receiving ear tags (HASCO) and sample collection. Age was approximated as juvenile (0-12 months), subadult (13-21 months), or adult (> 21 months) from tooth wear (Grau et al. 1970), body size, reproductive status (Sanderson & Nalbandov 1973) and recapture history. For males, the descent of the testes was used to differentiate between subadult and adults. Subadult and adult females were differentiated by nipple morphology and tooth wear (Grau et al. 1970). Animals recaptured within one week of their original capture were released without handling. Mass and morphological measurements were averaged between individuals that had been recaptured within one trapping season. All captured raccoons were released at the trap site after recovery from sedation and usually within 2 hours of checking the trap.

Sampling and data analyses:

Animals were weighed to the nearest 0.1 kg using a 10 kg hanging scale (Pesola), which has an accuracy of ± 0.3%. Axillary girth (AG), dorsal standard length (DL), tail length (TL), and neck circumference (NC) were measured by using a flexible measuring tape (cm). Axillary girth was measured following the contour of the body surface posterior to the forearms. Dorsal straight length was measured with the animal in ventral recumbency beginning at the tip of the nose and ending at the tip of the tail. Tail length (TL) was measured from the base of the tail to the tip of the tail. Neck circumference was measured following the contour of the body around the neck. Canine length (CL), and hind foot width (FW) and length (FL) were measured using a dial caliper accurate to 1mm. Canine length was defined as the length from the eruption of the tooth from the gums to the longest point of the top left canine (mm).

Kolmogorov–Smirnov and Levene tests evaluated groups for normality and homoscedasticity, respectively. Mean weights (" SD) were calculated by field season and trapping site for each sex/age group. An analysis of variance (ANOVA) and discriminant analyses were used to test for differentiation among the 3 age-classes of pygmy raccoons (juvenile, subadult and adult) based on morphological data. To test for sex and seasonal differences among raccoons, a fixed two-way analysis of variance (ANOVA) was used. Because of small samples sizes for subadults, their weights and morphological measures between site, sex, and year were compared with Mann-Whitney U or Kruskal-Wallis H tests. All statistical analyses were performed in SPSS (version 10.0 SPSS Inc. Chicago, Illinois 2001). Differences were determined to be statistically significant at the $p < 0.05$ level. Males were significantly ($p < 0.05$) larger than females

in all morphological measures (see results); therefore, sexes were analyzed separately in all univariate procedures. To determine whether external morphology in this species was sufficient to assign age-class, a discriminant analysis was performed on the correlation (standardized) matrix of the data set with a tolerance of 0.1. Sexes were pooled in discriminant analyses.

To assess the rate of morphological change, the date of divergence between mainland and island animals was used. Divergence was defined using phylogenetic data which was estimated to occur approximately 46,970 years before present (ybp). By dividing the average head-body length (HBL), tail length (TL) or hind foot length (FL) of the pygmy raccoon by that of the average Mexican raccoon (*P. lotor shufeldti*) (data taken from the literature), an estimate of the average size reduction between these two conspecifics can be made. The average *P. lotor* morphological variables used for the comparisons in body size was the mean of seven adult raccoons from Oaxaca, Mexico (Goodwin 1969). Data from this study were selected for comparison to the pygmy raccoons because this study 1) confirmed that all specimens were adults, and 2) represents a relatively large sample size compared to other studies examining known adult *P. lotor shufeldti*. Assuming that the raccoons from the mainland of Mexico were the founding population of raccoons on Cozumel Island 46,970 ybp, one can coarsely estimate the rate of body change by dividing the divergence time by the size reduction seen in pygmy raccoons in relation to *P. lotor shufeldti*. However, because characters evolve at different rates (Haldane 1949), change in body size was also expressed by measuring the proportional change of characters (Lerman 1965). Haldane's formula follows that:

$$\frac{\log_e x_2 - \log_e x_1}{t} \quad (1)$$

Where x_1 and x_2 are the population means at the end and beginning of the temporal sequence and t is the time interval expressed in years. Haldane used the term "darwin" (d) to describe this rate of evolutionary change by an increase or decrease in 1/1000 per 1,000 years (Haldane 1949; Marshall & Corruccini 1978).

Due to the variability associated with divergence dates from molecular clocks, darwins were also calculated using a second commonly estimate of divergence time for island and mainland taxa, the last glacial maximum (LGM). Darwins were calculated for this time frame, which for Cozumel Island, would correspond to the Wisconsin Glaciation when sea level dropped to greater than –100 m between 15,000 and 20,000 ypb (Milliman & Emery 1968; Morner 1971).

Results.

A total of 78 individuals (38 males and 40 females) were examined. The external pelage of the pygmy raccoons is identical in most respects to mainland Yucatan raccoons. Phenotypic observations showed that adult males had pronounced orange coloration on their neck scruff region and dorsal tail pelage compared to females. There was a significant association between pelage color and sex ($X^2 = 52.43$, df = 1, p = 0.047). This coloration begins as a subadult and becomes brighter with age.

Most characters, in all 3 age groups, were normally distributed with the exception of adult mass (D = 4.85, df = 51, p = 0.028). Therefore, all analyses of mass were conducted using non-parametric tests. The mean (±SD) adult male and female mass was 3.58 kg (± 0.52) and 3.28 kg (± 0.18), respectively (Table 5.1). On average, adult males

weighed 11.2% more than females ($X^2 = 6.50$, df = 49, p = 0.031), while male canines were approximately 1 cm longer than females (F = 10.43, df = 44, p = 0.002). Sexual dimorphism at site 2 was especially pronounced with males weighing 25% more than females (F= 5.281, df = 29, p= 0.011). Neck circumference also significantly differed for the sexes at site 2 (F = 9.36, df = 29, p = 0.001).

Adult weights did not fluctuate seasonally (F = 10.52, df = 51, p = 0.430). A one-way ANOVA indicated that no morphometric values significantly differed between the three years except for DL (F = 6.00, df = 47, p = 0.005). Dorsal length for 2001 was approximately 5 cm shorter than for the other years, although this finding was not statistically significant (p = 0.232). Subadults did not differ in their morphometric values between years. In contrast, juveniles from 2002 (no juveniles were captured in 2001) were significantly larger than those captured in 2003 in all morphometric values, except foot width (p = 0.078) and NC (p = 0.152). A two-way ANOVA found no significant variation in any morphological variables when examining the combined effect of site and year.

Although juveniles displayed no significant differences in morphological measures based on sex, subadults differed in a number of variables (Table 5.1). Subadult males were larger in mass (F = 6.50, df = 9, p = 0.031), axillary girth (F = 6.44, df = 6, p = 0.044), and canine length (F = 10.14, df = 4, p = 0.021) compared to subadult females. Using a two-way ANOVA, the factors of age-class and trap site were examined and results indicated that the only morphometric variable that differed for subadults was DL (F = 6.73, df = 6, p = 0.049). Mass did not significantly vary between sites for this age-

class ($X^2 = 0.55$, df = 1, p = 0.456). No significant differences between sites were detected for juveniles.

A two-way ANOVA indicated that adult males at site 2 were approximately 0.5 kg heavier than males at other sites (F = 21.67, df = 2, p = 0.001) (Figure 5.1). Neck circumference (NC) and canine length were also significantly larger for site 2 males (F = 16.62, df = 2, p = 0.001; F = 5.91, df= 2, p = 0.018, respectively); no variation was detected for individuals from site 2 between the seasons (2002 - 2003). Excluding adult males from site 2, no significant differences in morphology were detected between the three trap sites for all age classes (F = 12.02, df = 2, p = 0.750) or when examining only adults (F = 23.42, df = 2, p = 0.378).

Discriminant analysis was successful in separating the three age-classes using mass and canine length as the indicator variables 89.9% of the time (Wilks' lambda = 0.237 and 0.877 for the 1st and 2nd discriminant functions, respectively ; $p < 0.001$ for 1st discriminant, p = 0.007 for 2nd discriminant). Projection of specimen scores for the first 2 discriminant functions revealed some overlap of subadults and adults, resulting from wider variation of subadult values (Figure 5.2). Juveniles were clearly differentiated from adults. In this analysis, 95.1% of the variance between age classes is explained by mass, and 4.9% explained by the canine length variable. Mass also had a significant canonical correlation ($d^2 = 0.852$). The correlation coefficients between discriminant variables and canonical discriminant functions and the non-standardized canonical discriminant function coefficients are shown in Table 5.2.

In examining data from this study to morphological data of *P. lotor shufeldti* collected from the literature (Goodwin 1969), pygmy raccoons exhibit a 7.3 – 21.4% size

reduction since their divergence from mainland raccoons. Based on the average *P. lotor shufeldti* TBL (male = 557.6 mm, female = 592.5 mm) from Goldwin's study (1969), the average adult pygmy raccoon is 90% the size of the Oaxaca raccoon. In contrast, using the average TL (male = 305 mm, female = 291 mm) and HF length (male = 118.2mm, female = 119mm), the pygmy raccoon is only 80.7% and 80.1%, the size of its' mainland conspecifics, respectively. Using the average of these values, the pygmy raccoon has undergone a 14.7% size reduction in the last 46,970 years. Therefore, assuming a constant rate of size the pygmy raccoon, and based on the average reduction of all characters, this species decreased 1.18 mm/1000 yrs or 0.03%/1000 yrs. Because it is useful to compare the evolutionary rates of this study with rates over similar intervals for other species, rates of change were also calculated in darwins. The average evolutionary rate was 5.43 d (range 4.53 (FL) – 6.23 (HBL)).

Discussion

Like other species in the genus *Procyon*, the pygmy raccoon exhibits sexual dimorphism. Males were on average 11% heavier than females, a value consistent with variation in mass due to sexual dimorphism found in *P. lotor* throughout its range (10-15%) (Zeveloff 2002). Subadults, which were defined as being between 13-21 months, were also dimorphic in mass and canine length. One possible explanation for this dimorphism is that subadult males start producing these traits even before they have reached their full adult size or their ability to mate at approximately 21 months of age.

Sexual dimorphism is especially evident at site 2 where adult males weigh 1kg more than females. This site is located in mangrove forest in and surrounding a golf

course in the northwestern area of the island and contains a small garbage dump where employees have sighted raccoons feeding. The fact that adult males at site 2 were significantly larger than males at other sites is especially interesting in light of feeding habit studies which indicate that both stable isotope ratio values (which can reflect trophic feeding position) and data obtained from scats both differed for individuals caught at site 2. Therefore, morphological differences of animals caught at this site may be partially explained by anthropogenic influences on diet such as the availability of garbage areas to the raccoons at this site, while large areas of human food/garbage were less likely to be encountered at the other two sites. However, these individuals did not differ in other measures such as body length, suggesting resource availability may partially, but not fully, explain the reduced stature of these animals in weight when food is plentiful. In contrast, female body size at this site was not significantly different to females at other sites. Possible explanations for this may include sex-related feeding differences, or male-female competition for enriched food sources or access to garbage sites.

Goldman (1950) and others (Mugaas & Seidensticker 1993) found that raccoons in southern climates remain active year-round and thus do not exhibit seasonal weight fluctuations. The fact that this study found that adult weights did not fluctuate seasonally, is also consistent with both other studies of raccoons in southern climates, and with dietary data for the pygmy raccoon which did not identify significant seasonal variation in feeding habits. Studies on pygmy raccoon feeding ecology indicated that one of the main food items in this species was crab, which presumably would not significantly vary in availability as much as other food sources such as fruit. Thus, it

seems that a heavy reliance on crab as a protein source would allow this species diet to remain relatively stable year-round. It is possible that the length of this study did not coincide with significant annual variation which may occur on a cyclic period that extends beyond the scope of this study (ENSO-related variability, for example) and so significant seasonal body mass and feeding habit differences can not be entirely ruled out. Additionally, weights were not recorded during all months of the year; therefore, the data from this study may have underestimated the magnitude of seasonal weight fluctuations for this study population.

Results of a discriminant analysis indicate that subadults are more difficult to assign age class recognition. This analysis likely reflects both the overlap in morphological measures between subadults and adults as well as the difficulty in the field in assigning the age class in individuals in the subadult-adult boundary. Thus, on the basis of morphological data one can only reliably split age classes into two groups (juveniles and adults). Such recognition may be useful for future studies as they may provide an additional means to reliably assess changes in the age structure of subpopulations and may improve the reliability with which age classes are assigned.

Results from this study indicate that the dwarfed body size of this species is consistent with findings from other studies. Navarro and Suarez (1989) reported adult head-body lengths ranging from 580-708 mm (average 670.3 mm), which is smaller than this study's mean adult dorsal length of 755 mm. This discrepancy may result from differences in measuring technique or inclusion of subadults. Measurements from museum holotypes (Helgen & Wilson 2004) found a tail length and hind foot length measure of 250 and 97 mm respectively, which is similar to the adult averages of pygmy

raccoons measured in this study (243 and 96mm, respectively). Morphological data from other raccoon studies varies, but generally indicates that the pygmy raccoon lies at the lower end of the morphological variation seen in *Procyon lotor* and appears to exhibit true dwarfism (Table 5.3). Morphological data such as live-weights of raccoons from the Yucatan peninsula (the closest mainland populations of *Procyon lotor* to Cozumel) are lacking and make it difficult to compare the differences of the insular pygmy raccoon. However, recent studies relying on morphometric measures of specimens from Mexico (Helgen & Wilson 2004) have confirmed the uniqueness of the pygmy raccoon to its mainland conspecifics.

In examining data from this study to morphological data of *P. lotor shufeldti* collected from the literature (Emmons 1990; Goldman 1950; Goodwin 1969), pygmy raccoons exhibit a 9.3 – 21.4% size reduction since their divergence from mainland raccoons. This level of size reduction falls within the range of that which other insular mammals of similar size have also experienced in a comparable time frame (*i.e.* < 50,000 yrs). Studies examining Australian and European mammal lineages and found percent reductions in size ranging from 5 -17% (Kurten 1959; Marshall & Corruccini 1978).

It is difficult to compare results from this study to others because unfortunately, there are few studies that measure the rate of body change over similar time intervals. In a review by Gingerich (1983), the evolutionary rates of fossilized vertebrates (measured over millions of years) were compared and rates of change ranged from zero to 26.2 darwins. However, one example of a post-Pleistocene study which found similar ranges of rates in darwins (d) was a study on the dwarfism of insular sloths, which found relatively high rate of 16 d (Anderson & Handley 2002). Clearly, there is a great deal of

variability in the rates of change of body size and this factor is tightly linked to time scale, community level interactions and the character of interest (mass, HBL, etc.).

In estimating the rate of change in body size towards dwarfism, this study assumes that this rate remains constant over the entire time from divergence to present day. Clearly, this is an assumption that would likely be violated in an insular species because one would expect that selection for smaller body size would be strongest immediately after island formation or colonization, and would continue until the organism reached its optimal body size. Therefore, additional studies investigating the relationship between optimal body size in insular carnivores, metabolism, and community interactions would assist in better understanding the precise rate of dwarfism.

Table 5.1: Mean ("SD) body mass (kg), dorsal length (DL) (cm), tail length (TL) (cm), axillary girth (AG)(cm), neck circumference (NC) (cm), hind foot length (FL) (cm), hind foot width (FW) (cm), and canine length (mm) for male and female juvenile, subadult and adult pygmy raccoons captured from 2001-2003 on Cozumel Island, Mexico. Sample sizes are indicated in parentheses.

Measure	Juvenile			Subadult			Adult		
	Males (5)	**Females (10)**	**Mean (15)**	**Males (3)**	**Females (9)**	**Mean (12)**	**Males (20)**	**Females (12)**	**Mean (32)**
MASS	1.81	1.85	1.85	2.70	2.61	2.63	3.68	3.28	3.53
sd	0.48	0.49	0.47	0.26	0.36	0.33	0.52	0.18	0.47
AG	26.78	26.28	26.45	29.91	29.53	29.62	32.25	31.24	31.87
sd	1.91	1.63	2.49	1.07	1.63	1.48	2.92	1.49	2.51
DL	67.12	63.85	64.94	76.16	73.31	74.03	75.59	75.54	75.54
sd	3.97	9.90	8.37	4.72	3.92	4.11	9.08	3.25	7.37
TL	22.40	21.87	22.04	23.33	23.59	23.46	24.43	24.15	24.32
sd	1.92	1.19	1.43	2.36	1.06	1.36	1.52	1.37	1.44
NC	18.96	18.08	18.37	19.70	20.69	20.44	22.03	21.60	21.87
sd	0.45	1.68	1.43	1.37	1.33	1.36	1.72	1.64	1.67
FL	8.97	8.55	8.69	9.17	9.08	9.11	9.68	9.36	9.56
sd	0.10	0.53	0.48	0.59	0.29	0.36	0.50	0.17	0.44
FW	2.182	2.130	2.15	2.35	2.31	2.32	2.63	2.41	2.52
sd	0.10	0.35	0.23	0.18	0.11	0.12	0.23	0.14	0.23
CANINE	8.17	7.47	7.71	10.82	9.81	10.06	10.81	9.94	10.48
sd	2.44	2.06	2.13	0.50	0.35	0.58	1.28	0.75	1.18

Figure 5.1: Mean (± SD) mass (kg) of male and female pygmy raccoons from three different sites on Cozumel Island, Mexico from 2001-2003. Outliers are indicated by circles and stars.

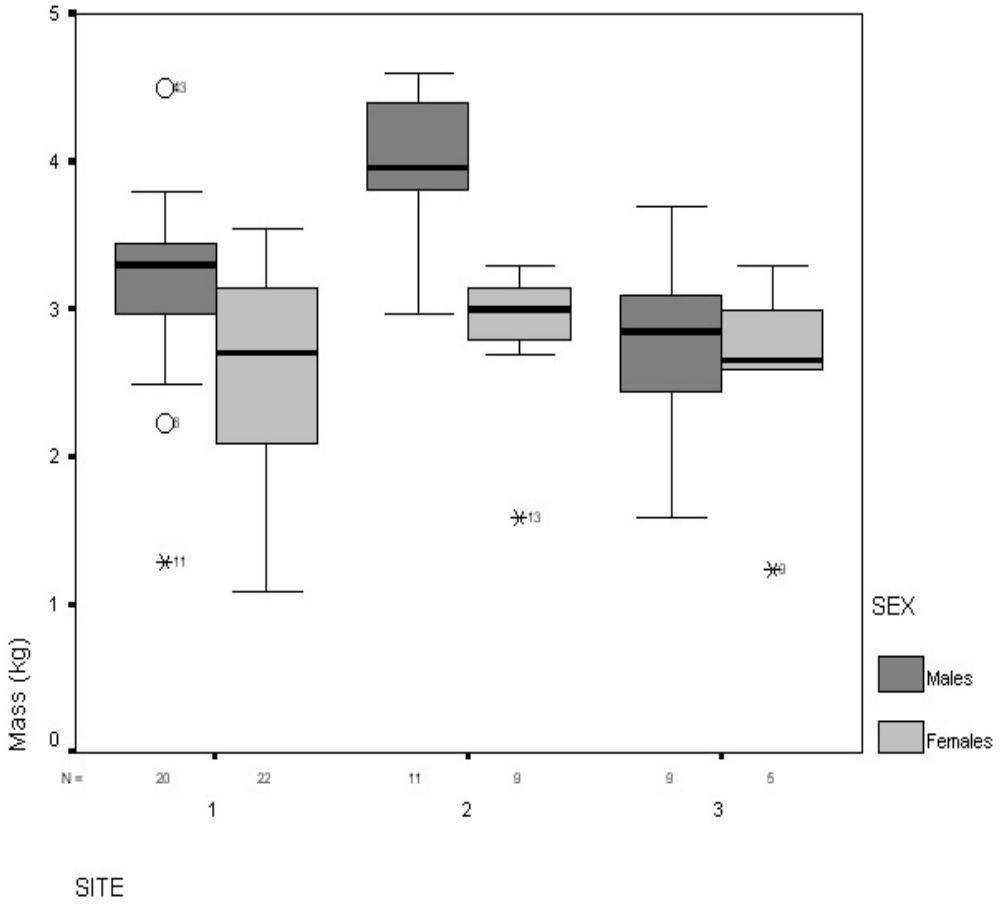


Table 5.2: Classification matrix obtained by the application of non-standardized canonical discriminant function coefficients to discriminate among 3 age classes (juvenile, subadult and adult) of pygmy raccoons (*P. pygmaeus)*.

Classification Results [b,c]

			Predicted Group Membership			
		AGE	J	SA	AD	Total
Original	Count	J	13	0	4	17
		SA	1	0	7	8
		AD	1	0	50	51
	%	J	76.5	.0	23.5	100.0
		SA	12.5	.0	87.5	100.0
		AD	2.0	.0	98.0	100.0
Cross-validated [a]	Count	J	13	0	4	17
		SA	1	0	7	8
		AD	1	0	50	51
	%	J	76.5	.0	23.5	100.0
		SA	12.5	.0	87.5	100.0
		AD	2.0	.0	98.0	100.0

a. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.

b. 82.9% of original grouped cases correctly classified.

c. 82.9% of cross-validated grouped cases correctly classified.

Figure 5.2: Canonical discriminant functions for the three age classes using the two morphological variables of mass (function 1) and canine length (function 2).

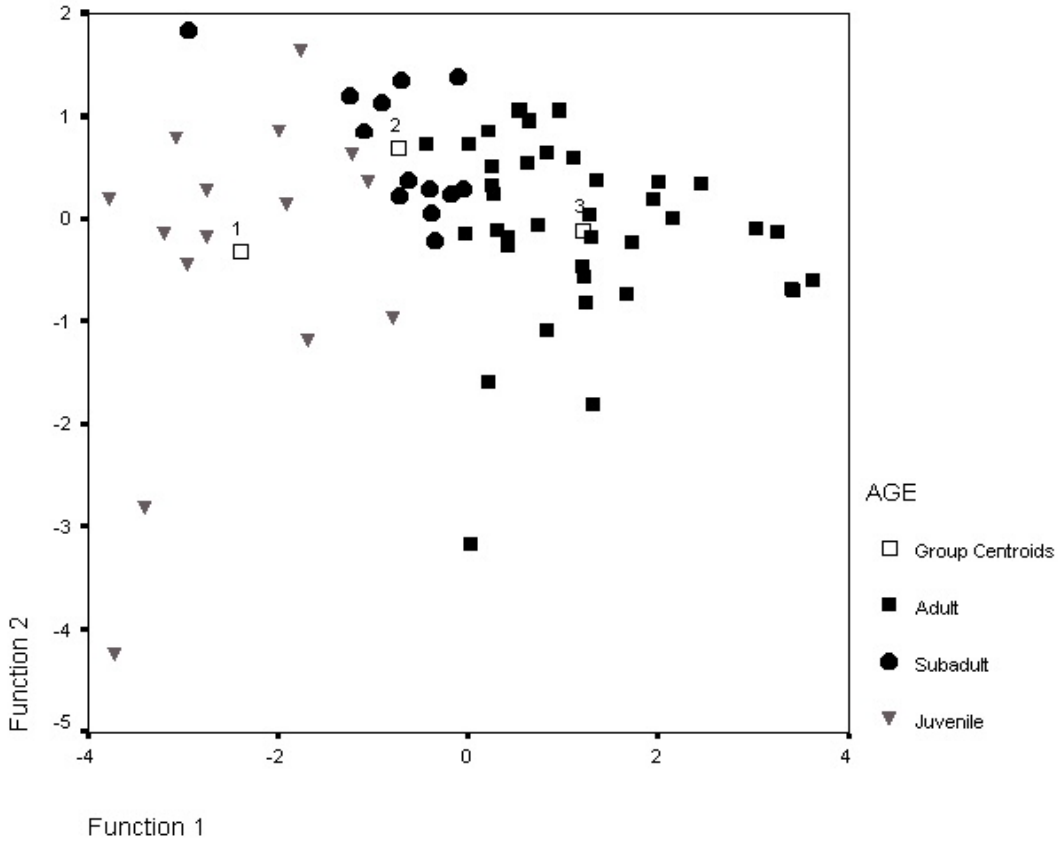


Table 5.3: Adult head-body length (HBL), tail length (TL) and hind foot length (HF) measurements (mm) for male (M) and female (F) *P. lotor* taken from the literature in comparison to *P. pygmaeus* measurements taken from 2001-2003. Numbers in parentheses are the percent change in this measure to the average mainland measure for specimens from Goodwin (1969). *Both individuals measured were subadults.

Species	**Head-Body Length (mm)**		**Tail Length (mm)**		**Hind foot (mm)**		**Study Area**	**Reference**
	M	F	M	F	M	F		
P. lotor	440 - 625		270 - 360		102 - 142		Throughout Range	Emmons, L. 1991
P. lotor shufeldti[1]	735 - 1,010		283.0		> 110		Central America	Helgen & Wilson 2004
P. lotor shufeldti[2]	557.6	592.5	305	291	118.2	119	Oaxaca, Mexico	Goodwin 1969
P. lotor shufeldti	582.0	613	292.0	296	116	128	Campeche, Mexico	Goldman 1950
*P. lotor shufeldti**	519	585	265	250	105	100	Quintana Roo, Mexico	This study
P. pygmaeus	511.6 (7.3)	513.9 (13.3)	244.3 (20)	241.5 (17)	96.8 (18)	93.6 (21.4)	Cozumel, Mexico	This study

[1] These measurements represent averages of *P. lotor shufeldti*, *P. lotor hernandezii*, and *P. lotor mexicanus*, all of which were deemed not statistically different in size.

[2] This study examined seven adults (2 females and 5 males), of which one adult female was deemed “old

Chapter 6: CONCLUSIONS AND FUTURE DIRECTIONS

Major conclusions of this dissertation work are: (1) the Cozumel coati represents a phylogenetically distinct species based on mtDNA sequence data; (2) based on morphology, but not phylogenetic data, the pygmy raccoon is deserving of species level recognition; (3) the major food items consumed by the pygmy raccoon are crab, fruit and insects and no major variability is seen between age classes or sexes, although some slight seasonal variation was observed; (4) the population size of the pygmy raccoon and dwarf coati are estimated to be fewer than 250 mature individuals and should thus be listed as critically endangered by the IUCN and 5) morphological measures of the pygmy raccoon indicate that it is a true dwarf species.

Both ecological and evolutionary data derived from this study will further our understanding regarding the uniqueness of the Cozumel dwarf carnivores. Given the small sample size for *N. nelsoni*, it is difficult to make conclusions about this population's haplotype diversity or morphological uniqueness. Mitochondrial phylogenetic data indicates that the *N. nelsoni* Cozumel haplotypes may be phylogenetically unique enough to deserve species-level status based on phylogenetic criteria alone. With three mtDNA polymorphic sites in *N. nelsoni* that distinguish it from its closest Yucatan conspecifics, and one polymorphic site that distinguishes it from all other coati haplotypes, an argument for their phylogenetic uniqueness is stronger than that for *P. pygmaeus*. Without further genetic samples it is difficult to fully propose that the dwarf coati should be a unique species, based on phylogenetic criteria alone. Based on the precautionary principle however, one can clearly *not* reject the hypothesis that the

Cozumel coati represents a unique species and should be afforded conservation measures as such.

A single haplotype represented all individuals sampled from *P. pygmaeus* indicating that the population either has experienced a severe population bottleneck or that the current haplotype represents the founder population. If the latter is true, the population on Cozumel has not been on the island long enough to diverge. Alternatively, catastrophic events such as periodic hurricanes may have kept populations numbers so low that divergence was never achieved.

If conservation management strategies limit the amount of mainland animals entering Cozumel, one might expect this short term differentiation would eventually lead to a long-term speciation process. The IUCN designation of endangered species status for the two Cozumel carnivores is still needed as formal conservation policies to protect these taxa are lacking. Even if the Cozumel carnivores are not yet fully phylogenetically unique, there is no reason to change their legal status or to not encourage future conservation policies. The Cozumel carnivores appear to have inhabited the island well before the Mayans colonized the island and should be considered endemic to the island.

In terms of future conservation management strategies, one recommendation rising from this study is for local conservation biologists and managers to prevent the introduction of mainland carnivores onto the island. Currently, there are no such management plans in place to avoid hybridization or genetic introgression of island animals with released mainland procyonids. The prevention of introducing mainland carnivores onto the island will protect the genetic integrity of the island procyonids and

also will minimize the introduction of mainland pathogens (distemper, rabies, etc.) into this genetically naïve population.

An interesting finding from this study is the indication that pygmy raccoons heavily rely on mangrove forest resources and seem to prefer mangrove habitat despite what appears to be suitable habitat elsewhere on the island. Why are the majority of pygmy raccoons limited to this habitat when their mainland conspecifics are able to utilize a much more diverse array of habitat types? Based on the feeding ecology examination, crab was a heavily relied upon food source, and appears to be the one food item that they would not be able consume if their primary habitat was dry forest. Development in areas in and surrounding mangrove habitat severely decreases the area in which the pygmy raccoons and their primary prey are found. Future development of Cozumel for tourism would likely have significant impacts on local mangrove habitat, thereby directly and indirectly influencing raccoon populations. A conservation recommendation rising from this study is for local government officials and ecological managers to minimize future reductions of the extent of mangrove habitat where the largest populations of raccoons occur on the island. Currently, there is limited development of the northwestern section of the island where the vast majority of raccoons were found. The prevention of future habitat alteration in this area would protect this ecologically vital component of the ecosystem of Cozumel while securing that the major food source for the pygmy raccoons.

Both Mexico (SEMARNAT 2002) and the IUCN have listed the pygmy raccoon and Cozumel coati as endangered (IUCN: D dwarf coati) (C2a, pygmy raccoon) (Hilton-Taylor 2001). However, based on population estimates as well as justification put forth

by Cuarón et al. (2004), I propose that their designation be immediately changed to critically endangered (CR).

Catastrophic events such as periodic hurricanes are an important consideration when examining the future of this species. As a tropical island, Cozumel is frequently hit by hurricanes. Healthy wildlife populations are able to withstand such losses and have done so for eons. Given the exceptionally small population numbers of pygmy raccoons and dwarf coatis on the island, a captive breeding program should be considered as these species are at an extremely high risk of extinction in response to the normal environmental stochasticity associated with both island and hurricane prone locales.

An additional threat to the populations of Cozumel carnivores is hunting and this habit seems to be a regular occurrence in the northwestern mangroves. Not only do human hunters pose an obvious threat to low procyonid numbers, but they also often employ dogs as hunting aids and therefore are potentially exposing the wild carnivores on Cozumel to diseases. The isolation of experienced by island carnivores often can put them at an increased risk to introduced disease because they often have evolved in the absence of many mainland diseases and therefore the population as a whole lacks immunity. Disease spillover is a serious concern for the carnivores of Cozumel Island and is further explored in McFadden et al. (in prep).

Only a single dwarf coati was captured in over 3,500 trap nights, an alarming indication of the rarity of this species on the island of Cozumel. Although sightings of dwarf coatis on various occasions in both the north and south of the island, this species is clearly very rare. Based on intensive trapping efforts on the island, I conclude that the Cozumel coati is in immediate danger of extinction. In the case of the pygmy raccoon,

the numbers seem more stable. However, given the present conservation risks that threaten these carnivores, their current population size may not be large enough to persist despite these threats. Clearly, management plans specific to the needs of each species are badly needed if either species is expected to persist over the long-term.

One question that was not examined in this study was- why does the pygmy raccoon population appears to be more stable than that of the dwarf coati? Coatis and raccoons are able to easily coexist on the mainland because although they may share resources, they do so at different times (*i.e.* nocturnally for the raccoon, diurnally for the coati). Although the single coati was caught in the mangrove area, I suspect that this area may not be their "prime" habitat. This assertion is based on the lack of coati tracks (seen only on a single occasion) and lack of visual sightings in this area. Coatis were spotted more often in dry forest than in the mangrove habitat that pygmy raccoons clearly prefer. One speculative reason for the low coati numbers may relate to the abundance of boa constrictors in forested areas, which would contrast with the rarity of boas seen in mangrove areas. Additionally, if it is true that the dwarf coati prefers dry forest over mangrove habitat, the proximity of humans (*i.e.* disturbance/encroachment) and domesticated dogs/cats (*i.e.* potential disease vectors) may make coatis more likely to come into contact with these threats. Regardless of the precise causes of the low coati numbers, clearly this species is deserving of immediate conservation action and future scientific research in order to better understand the threats to its' survival.

Future Directions:

Future phylogenetic research should focus on other nuclear genes (including microsatellites) in order to confirm the extent of genetic uniqueness that exists between insular and mainland forms. A more thorough sampling of coatis and raccoons on the mainland would also improve the ability to resolve the phylogenetic relationships and provide a more precise divergence time. Continued genetic sampling of pet coatis and raccoons on the island would also provide insights into the level of hybridization that is potentially occurring between native and introduced animals on the island.

Additional sampling of raccoons for feeding habitat analysis in months that I did not sample, would allow insights into possible seasonal differences in feeding habits. Additionally, a behavioral ecology study focusing on site 2 animals in comparison to site 1 or 3 animals would elucidate the feeding differences between these sites. Such information would be useful in understanding both the raw feeding ecology differences between the sites and would provide additional information on how such feeding differences influence morphology. By radio-tracking individuals at both sites, information on the extent that anthropogenic influences, such as access to human garbage, would help in understanding the underlying differences this study found in both feeding ecology and morphology.

In order to determine a more precise population estimate for the procyonids on Cozumel, further trapping efforts are needed, especially in areas where my study was unable to trap, in order to obtain a clearer understanding of the precise conservation status of the dwarf coati of Cozumel. Additionally, trapping in areas such as El Cedral and Chankanaub Park area would also likely further elucidate the range of the pygmy

raccoon and would serve to confirm or repudiate whether this species is limited to the NW area of the island. A long-term monitoring study using mark-recapture methodology, would also allow for a robust population viability analysis (PVA). Such an analysis is immediately important for these species because it would allow further population parameters to be estimated (including survival) and would directly influence future conservation management plans for these species.

Future directions for morphological studies should include a more thorough trapping effort of coatis and raccoons on the Yucatan peninsula. Live-weights and other morphological measures are very limited for *P. lotor shufeldti*, and the collection of such information would allow a more systematic examination of the morphological differences between the insular and mainland *Procyon* forms. An interesting, and promising source of future research lies in procyonid bones that have been retrieved from Mayan ruins on the mainland. If these bones were dated and measured, and combined with an examination of bones from Cozumel, a linear regression could examine the relationship between dwarfism and time in a more precise manner. Such information would also be useful in better understanding how optimal body size is achieved in insular carnivores and would further reconcile the present results in scaling of morphometric features over time, with theoretical models regarding conditions under which morphological divergence is seen in insular environments.

Literature Cited

Alcover, J. A., and M. McMinn. 1994. Predators of vertebrates on islands. Bioscience **44**: 12-18.

Altmann, J. D., S. A. Schoeller, D. Altmann, D. Muruthi, and R. M. Sapolsky. 1993. Body size and fatness of free-living baboons reflect food availability and activity levels. American Journal of Primatology **30**:149-161.

Amato, G. D. 1991. Species hybridization and protection of endangered animals. Science **253**:250.

Ambrose, S. H., and M. J. DeNiro. 1986. The isotopic ecology of east African mammals. Oecologia **69**:395-406.

Anderson, R. P., and C. O. Handley. 2002. Dwarfism in insular sloths: biogeography, selection, and evolutionary rate. Evolution **56**:1045-1058.

Aquadro, C. F., and C. W. Kilpatrick. 1981. Morphological and biochemical variation and differentiation in insular and mainland deer mice (*Peromyscus maniculatus*). Pages 214-230 in M. H. Smith, and J. Joule, editors. Mammalian population genetics. University of Georgia Press, Athens.

Aranda, M. 1991. Wild mammal skin trade in Chiapas, Mexico. Pages 174-177 in J. G. Robinson, and K. H. Redford, editors. Neotropical wildlife use and conservation. University of Chicago Press, Chicago.

Avise, J. C. 1994. Molecular Markers, Natural History, and Evolution. Chapman and Hall, New York.

Baskin, J. A. 1982. Tertiary Procyoninae (Mammalia: Carnivora) of North America. Journal of Vertebrate Paleontology **2**:71-93.

Baskin, J. A. 1998. Procyonidae in the evolution of tertiary mammals of North America. Pages 144-151 in C. M. Janis, K. Scott, and L. Jacobs, editors. Life history patterns and the comparative social ecology of carnivores. Cambridge University Press, UK, Cambridge.

Beck, M. L., and M. L. Kennedy. 1980. Biochemical genetics of the raccoon, *Procyon lotor*. Genetical Research **54**:127-132.

Bekoff, M., and T. J. Daniels. 1984. Life history patterns and the comparative social ecology of carnivores. Annual review of ecology and systematics **15**:191-232.

Ben-David, M., R. T. Boyer, and J. B. Faro. 1996. Niche separation by mink and river otters: coexistance in a marine environment. Oikos **75**:41-48.

Ben-David, M., R. W. Flynn, and D. M. Schell. 1997. Annual and seasonal changes in diets of martens: evidence from stable isotope analysis. Oecologia **111**:280-291.

Ben-David, M., and D. M. Schell. 2001. Mixing models in analyses of diet using multiple stable isotopes: a response. Oecologia **127**:180-184.

Binkham, J. W., J. C. Patton, and T. R. Loughlin. 1996. High variability for control region sequences in a marine mammal: Implications for conservation and biogeography of Steller Sea Lions *Eumetopias jubatus*. Journal of Mammalogy **77**:95-108.

Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology **37**:911-917.

Bowen, L., and D. Van Vuren. 1997. Insular endemic plants lack defenses against herbivores. Conservation Biology **11**.

Bremer, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. Evolution **42**:795-803.

Bremer, K. 1994. Branch support and tree stability. Cladistics **10**:295-304.

Brown, J. H., and M. V. Lomolino 1998. Biogeography. Sinauer Associates, Inc. Publishers, Sunderland, Massachusetts.

Cabrera, L., V. Aguilar, J. Alcocer, R. Jimenez, E. Munoz, and E. Vazquez. 1998. Regiones hidrologicas prioritarias: Fichas tecnicas y mapa. CONABIO, Mexico.

Case, T., D. Bolger, and A. Richman. 1992. Reptilian extinctions: the last ten thousand years. Pages 9-125 in P. Fiedler, and S. Jain, editors. Conservation biology: the theory and practice of nature preservation and management. Chapman and Hall, New York.

Case, T. J. 1978. On the evolution and adaptive significance of post-natal growth rates in terrestrial vertebrates. Quarterly Review in Biology **53**:243-282.

Caughley, G. 1977. Analysis of vertebrate populations. John Wiley & Sons Inc., New York.

Chaimberlain, C. P., J. D. Blum, R. T. Holmes, R. T. Feng, X. Sherry, and G. R. Graves. 1997. The use of isotope tracers for identifying populations of migratory birds. Oecologia **109**.

Chrisholm, B. S., D.E. Nelson, and H.P. Schwarcz. 1982. Stable carbon isotope ratios as a measure of marine versus terrestrial protein in ancient diets. Science **216**:1131-1132.

Clarke, B., and P. R. Grant. 1996. Evolution on islands. Philosophical transactions of the royal society of London **351**:723-784.

Clarke, B., M. Johnson, and J. Murray. 1996. Clines in the genetic distance between two species of island land snails: how 'molecular leakage' can mislead us about speciation. Philosophical Transaction Royal Society of London **351b**:773-784.

Clement, M., D. Posada, and K. A. Crandall. 2000. TCS: a computer program to estimate gene genealogies. Molecular Ecology **9**:1657-1660.

Clutton-Brock, T. H. 1983. The functional significance of variation in body size among mammals. Pages 623-633 in J. F. Eisenberg, and D. G. Kleiman, editors. Advances in the study of mammalian behavior. American Society of Mammalogists, Shippensburg, PA.

Cracraft, J. 1983. Species concept and speciation analysis. Current Ornithology **1**:159-187.

Cracraft, J. 1989. Speciation and ontology: The empirical consequences of alternative species concepts for understanding patterns and process of differentiation in D. Otte, and J. A. Endler, editors. Speciation and its consequences. Sinauer Associates, Sunderland, Massachusetts.

Cronin, M. A., R. Stuart, B. J. Pierson, and J. C. Patton. 1996. K-Casein gene phylogeny of higher ruminants (*Pecora, Artiodactyla*). Molecular Phylogenetics and Evolution **6**:295-311.

Cuarón, A. D., M. A. Martinez-Morales, K. W. McFadden, D. Valenzuela, and M. Gompper. 2004. The status of dwarf carnivores on Cozumel Island, Mexico. Biodiversity and Conservation **13**:317-331.

Damuth, J. 1993. Cope's rule, the island rule and the scaling of mammalian population density. Nature **365**:748-750.

Darimont, C. T., and T. E. Reimchen. 2002. Intra-hair stable isotope analysis implied seasonal shift of salmon in gray wolf diet. Canadian Journal of Zoology **80**:1638-1642.

Daszak, P., A. A. Cunningham, and A. D. Hyatt. 2000. Emerging infectious diseases of wildlife: Threats to biodiversity and human health. Science.

Davis, J. I., and K. C. Nixon. 1992. Populations, genetic variation, and the delineation of phylogenetic species. Systematic Biology **41**:421-435.

Davison, A., J. D. Birks, R. C. Brooks, J. Messenger, and H. Griffiths. 2001. Mitochondrial phylogeography and population history of pine martens (*Martes*

martes) compared with polecats (*Mustela putorius*). Molecular Ecology **10**:2479-2488.

de Queiroz, K., and M. J. Donoghue. 1990. Phylogenetic systematics and the phylogenetic species concept. Cladistics **4**:317-338.

Decker, D. M. 1991. Systematics of the Coatis, Genus *Nasua* (Mammalia: *Procyonidae*). Proceedings of the Biological Society of Washington **104**:370-386.

DeNiro, M. J., and S. Epstein. 1978. Influence of diet on the distribution of carbon isotopes in animals. Geochemica Cosmochimica Acta **42**:496-506.

DeNiro, M. J., and S. Epstein. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. Geochemica Cosmochimica Acta **45**:341-351.

Derting, T. L. 1996. Changes in gastrointestinal characteristics of an omnivorous species, the raccoon, with lactation and season. Journal of Mammalogy **77**:440-448.

Dorney, R. S. 1954. Ecology of marsh raccoons. The Journal of Wildlife Management **18**:217-225.

Edwards, C. R. 1957. Quintana Roo: Mexico's empty quarter. University of California: Berkeley, Berkeley.

Emmons, L. H. 1990. Carnivores (Procyondae). Pages 136-138. Neotropical Rainforest Mammals. University of Chicago Press, Chicago.

Eriksson , T. 1998. AUTODECAY. Department of Botany Stockholm University. Computer program distributed by the author, Stockholm.

Escalante, P., T. Macouzet, M. A. Martinez, C. Pozo, and A. De Alba. 1999. Isla Cozumel in H. C. Benitez, C. Arizmendi, and L. Marquez, editors. Base de Datos de las AICAS. CIPAMEX, CONBIO, Mexico.

Estrada, A., and R. Coates-Estrada. 2001. Species composition and reproductive phenology of bats in a tropical landscape at Los Tuxtlas, Mexico. Journal of Tropical Ecology **17**:627-646.

Excoffier, L., P. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics **131**: 479-491.

Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39**:783-791.

Foster, J. B. 1964. Evolution of mammals on islands. Nature **202**:234-235.

Foster, R. B. 1982. The seasonal rhythm of fruitfall on Barro Colorado Island. Pages 151-172 in E. G. Leigh, A. S. Rand, and D. M. Windsor, editors. Ecology of a tropical forest. Smithsonian Institution Press, Washington, D.C.

Freidel, D. A., and J. A. Sabloff 1984. Cozumel: Late Maya Settlement Patterns. Academic Press, Inc., New York.

Fritzell, E. K. 1978. Habitat use by prairie raccoons during the waterfowl breeding season. Journal of Wildlife Management **42**:118-127.

Fry, B., and E. B. Sherr. 1989. Stable carbon measurements as indicators of carbon flow in marine and freshwater ecosystems. Pages 196-229 in P. W. Rundel, J. R. Rundel, and K. A. Nagy, editors. Stable isotopes in ecological research. Springer-Verlag, New York.

Funk, S. M., C. V. Fiorello, S. Cleaveland, and M. Gompper. 2001. The role of disease in carnivore ecology and conservation in J. L. Gittleman, S. M. Funk, R. Wayne, and D. W. MacDonald, editors. Carnivore Conservation. Cambridge University Press, Cambridge.

Gannes, L. Z., D. M. O'Brien, and C. Martinez del Rio. 1997. Stable isotopes in animal ecology: assumptions, caveats, and a call for more laboratory experiments. Ecology **78**:1271-1276.

Garcelon, K. K., R. K. Wayne, and B. J. Gonzales. 1992. A serological survey of the island fox (*Urocyon littoralis*) on the Channel Islands, California. Journal of Wildlife Diseases **28**:223-229.

Gehrt, S., and E. K. Fritzell. 1998. Growth rates and intraspecific variation in body weights of raccoons (*Procyon lotor*) in Southern Texas. American Midland Naturalist **141**:19-27.

Gehrt, S. D. 2003. Raccoon (*Procyon lotor* and allies). Pages 661-634 in G. A. Feldhamer, B. C. Thompson, and J. A. Chapman, editors. Wild mammals of North America. Johns Hopkins University Press, Baltimore.

Genoways, H. H., and J. K. Jones. 1975. Annotated checklist of Mammals of the Yucatan Peninsula, Mexico. IV. Carnivora, Sirenia, Perissodactyla, Artiodactyla. Occasional Papers The Museum of Texas Tech University **26**:1-22.

Gentry, A. H. 1988. Changes in plant community diversity and floristic composition on environmental and geographical gradients. Annals of the Missouri Botanical Garden **75**:1-34.

Gibbons, A. 1998. Calibrating the mitochondrial DNA clock. Science **279**:38-39.

Gilbert, D. A., N. Lehman, S. O'Brien, and R. Wayne. 1990. Genetic fingerprinting reflects population differentiation in the California channel island fox. Nature **344**:764-767.

Gittleman, J. L. 1986. Carnivore life history patterns: allometric, pylogenetic, and ecological associations. American Naturalist **127**:744-771.

Glatston, A. R. 1994. Threats to Procyonids and Ailurids. Pages 1-42 in A. R. Glatston, editor. The Red Panda, Olingos, Coatis, Raccoons, and their Relatives. IUCN, Gland, Switzerland.

Goldman, E. A. 1950. Raccoons of North and Middle America. North American Fauna **60**:1-153.

Goldman, E. A., and R. T. Moore. 1945. The biotic provinces of Mexico. Journal of Mammalogy **26**:347-360.

Goldstein, D. B., G. W. Roemer, D. A. Smith, D. E. Reich, A. Bergman, and R. Wayne. 1999. The use of microsatellite variation to infer population structure and demographic history in a natural model system. Genetics **151**:797-801.

Gompper, M. 1995. *Nasua narica*. Mammalian Species **487**:1-10.

Gompper, M., and D. M. Decker. 1998. *Nasua nasua*. Mammalian Species **580**:1-9.

Gompper, M. E. 1997. Population ecology of the white-nosed coati (*Nasua narica*) on Barro Colorado Island, Panama. Journal of Zoology **241**:441-455.

Goodwin, G. G. 1969. Mammals from the state of Oaxaca, Mexico, in the American Museum of Natural History. Bulletin of the American Museum of Natural History **141**:224-230.

Grau, G. A., G. C. Sanderson, and J. P. Rogers. 1970. Age determination of raccoons. Journal of Wildlife Management **34**:364-372.

Greenwood, R. 1979. Relating residue in raccoon feces to food consumed. American Midland Naturalist **102**:191-193.

Gruneberg, H. 1963. The pathology of development. John Wiley and Sons, New York.

Haldane, J. S. 1949. Suggestions as to the quantitative measurement of rates of evolution. Evolution **3**:51-56.

Hamblin, N. L. 1984. Animal Use by the Cozumel Maya. The University of Arizona Press, Tuscon.

Hanks, J. 1981. Characterization of population condition in dynamics of large mammal populations in C. W. Fowler, and T. D. Smith, editors. John Wiley & Sons, Inc., New York.

Hasegawa, M., H. Kishino, and T. Yano. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. Journal of Molecular Evolution **22**:160-174.

Heaney, L. R. 1978. Island area and body size of insular mammals: evidence from the tri-colored squirrel (*Callosciurus prevosti*) of Southeast Asia. Evolution **32**:29-44.

Helgen, K. M., and D. E. Wilson. 2003. Taxonomic status and conservation relevance of the raccoons (Procyon spp.) of the West Indies. Journal of Zoology of London **259**:69-76.

Helgen, K. M., and D. E. Wilson. 2004. A systematic and zoogeographic overview of the raccoons of Mexico and Central America in V. Cordero-Sanchez, and R. A. Medellin, editors. Contribuciones matozoologicas en homenaje a bernardo villa. Instituto de Biologia e Instituto de Ecologia, UNAM, Mexico City.

Herrera, L. G., E. Gutierrez, K. A. Hobson, B. Altube, W. G. Diaz, and V. Sanchez. 2002. Sources of assimilated protein in five species of New World frugivorous bats. Oecologia **133**:280-287.

Herrera, L. G., K. A. Hobson, D. Estrada, A. Manzo, G. Mendez, and V. Sanchez-Cordero. 2001. The role of fruits and insects in the nutrition of frugivorous bats: evaluating the use of stable isotope models. Biotropica **33**:520-528.

Herrera, L. G., K. A. Hobson, M. Rodriguez, and P. Hernandez. 2003. Trophic partitioning in trophic rain forest birds: insights from stable isotope analysis. Oecologia **136**:439-444.

Hilderbrand, G. V., S. D. Farley, C. T. Robbins, T. A. Hanley, K. Titus, and C. Servheen. 1996. Use of stable isotopes to determine diets of living and extinct bears. Canadian Journal of Zoology **74**:2080-2088.

Hilton-Taylor, C. 2001. IUCN Red List Categories and Criteria: Version 3.1 IUCN. Page ii+30 in S. S. Commission, editor. IUCN, Gland, Switzerland and Cambridge, UK.

Hobson, K. A., B.N. McLellan, and J.G. Woods. 2000. Using stable carbon 13 and nitrogen 15 isotopes to infer trophic relationships among black and grizzly bears in the upper Columbia River Basin, British Columbia. Canadian Journal of Zoology **78**:1332-1339.

Hobson, K. A., and R. G. Clark. 1993. Turnover of carbon in cellular and plasma fractions of blood: Implications for nondestructive sampling in avian dietary studies. Auk **110**:638-641.

Hobson, K. A., B. McLellan, and J. Woods. 2000a. Using stable-carbon and nitrogen isotopes to infer trophic relationships among black and grizzly bears I Upper Columbia River Basin, British Columbia. Canadian Journal of Zoology **78**:1332-1339.

Hobson, K. A., B. N. McLellan, and J. G. Woods. 2000b. Using stable carbon and nitrogen isotopes to infer trophic relationships among black and grizzly bears in the upper Columbia River basin, British Columbia. Canadian Journal of Zoology **78**:1332-1339.

Hobson, K. A., D. M. Schell, D. Renoug, and E. Noseworthy. 1996. Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for dietary reconstructions involving marine mammals. Canadian Journal of Fisheries and Aquatic Science **53**:538-543.

Hobson, K. A., and H. E. Welch. 1992. Determination of trophic relationships within a high Arctic marine food web using carbon and nitrogen analysis. Marine Ecology Progression Series **84**:9-18.

Horai, S., and K. Hayasaka. 1990. Intraspecific nucleotide sequence differences in the major noncoding region of human mitochondrial DNA. American Journal of Human Genetics **46**:828-842.

Huelsenbeck, J. P., and K. A. Crandall. 1997. Phylogeny estimation and hypothesis testing using maximum likelihood. Annual Review of Ecology and Systematics **28**:437-466.

Huelsenbeck, J. P., and F. Ronquiest. 2001. MrBayes: Bayesian inference of phylogeny. Bioinformatics **17**:754-755.

Irwin, D., T. D. Kocher, and A. C. Wilson. 1991. Evolution of the cyt b gene in mammals. Journal of Molecular Evolution **32**:128-144.

Janzen, D. H. 1970. Altruism by coatis in the face of predation by *Boa constrictor*. Journal of Mammalogy **51**:387-389.

Jolly, G. M. 1965. Explicit estimates from capture-recapture data with both death and immigration schocastic model. Biometrica **52**:225-247.

Jones, J. K., and T. E. Lawlor. 1965. Mammals from Isla Cozumel, Mexico, with description of a new species of harvest mouse. University of Kansas Publications, Museum of Natural History **16**:409-419.

Jorgenson, J. P. 1993. Gardens, wildlife densities, and subsistence hunting by Maya Indians in Quintana Roo, Mexico. Page 279. Forest Resources and Conservation. University of Florida, Gainesville.

Kaufmann, A. K. 1982a. Raccoon and allies. Pages 567-585 in J. Q. Chapman, and G. A. Feldhamer, editors. Wild Animals of North America: Biology, Management and Economics. John Hopkins University Press, Baltimore.

Kaufmann, A. K., J. H. Lanning, and S. E. Poole. 1976. Current status and distribution of the coati in the United States. Journal of Mammalogy **57**:621-637.

Kaufmann, J. H. 1962. Ecology and Social Behavior of the Coati, *Nasua nasua* on Barro Colorado Island, Panama. University of California Press, Berkeley.

Kaufmann, J. H. 1982b. Raccoon and allies. Page 1147 in J. W. Chapman, and G. A. Feldhamer, editors. Wild Mammals of North America: Biology, Management, and Economics. Johns Hopkins University Press, Baltimore.

Kaufmann, J. H. 1987. Ringtail and coati. Pages 501-508 in E. Obbard, and B. Malloch, editors. Wild furbearer management and conservation in North America. Ministry of Natural Resources, Ontario.

Kelly, J. F. 2000. Stable isotope of carbon and nitrogen in the study of avian and mammalian trophic ecology. Canadian Journal of Zoology **78**:1-27.

Kendall, W. L., J. D. Nicholas, and J. E. Hines. 1997. Estimating temporary emigration using capture-recapture data with Pollock's robust design. Ecology **78**:563-578.

Kendall, W. L., and J. D. Nichols. 1995. On the use of secondary capture-recapture samples to estimate temporary emigration and breeding proportions. Journal of Applied Statistics **22**:751-762.

Kilpatrick, R. L. 1980. Physiological indices in wildlife management. Pages 99-112 in S. D. Schemnitz, editor. Wildlife management techniques manual. The Wildlife Society, Washington.

Kline, T. C., J. J. Goering, O. A. Mathisen, P. H. Poe, P. L. Parker, and R. S. Scalan. 1993. Recycling of elements transported upstream by runs of Pacific salmon. II Stable carbon and nitrogen evidence in the Kvichak River watershed, Bristol Bay, southwestern Alaska. Canadian Journal of Fisheries and Aquatic Science **50**:2350-2365.

Kocher, T. D., W. K. Thomas, A. Meyer, S. V. Edwards, S. Paabo, F. X. Villablanca, and A. C. Wilson. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proceedings of the National Academy of Science **86**:577-579.

Koepfli, K. P., and R. Wayne. 2003. Type 1 STS markers are more informative than cytochrome-b in phylogenetic reconstruction of the *Mustelidae* (Mammalia: Carnivora). Systematic Biology **52**:1-23.

Krebs, C. J. 1989. Niche overlap and diet analysis. Page 654 in C. J. Krebs, editor. Ecological Methodology. Harper and Row, New York.

Kurle, C. M., and G. A. Worthy. 2000. Stable isotope assessment of temporal and geographic differences in feeding ecology of northern fur seals (*Callorhinus ursinus*) and their prey. Oecologia **126**:254-265.

Kurten, B. 1959. Rate of evolution in fossil mammals. Cold Spring Harbor Symposium on Quantitative Biology **34**:205-215.

Lerman, A. 1965. On rates of evolution of unit characters and character complexes. Evolution **19**:16-25.

Levins, R. 1968. Evolution in changing environments. Princeton University Press, Princeton.

Lockmiller, R. L., E. C. Hellgren, L. W. Varner, K. McBee, and W. E. Grant. 1989. Body condition indices for malnourished collared peccaries. Journal of Wildlife Management **53**:205-209.

Lotze, H. H., and S. Anderson. 1979. *Procyon lotor*. Mammalian Species **119**:1-8.

Lynch, M., and P. E. Jarrell. 1993. A method for calibrating molecular clocks and its application to animal mitochondrial DNA. Genetics **135**:1197-1208.

MacDonald, D. W. 1996. Dangerous liaisons and disease. Nature **379**:400-401.

Macko, S. A., and N. E. Ostrom. 1994. Pollution studies using stable isotopes. Pages 45-62 in K. Lajtha, and M. Michener, editors. Stable isotopes in ecology and environmental science. Blackwell Scientific Publications, Oxford.

Marshall, C. R., E. C. Raff, and R. A. Raff. 1994. Dollo's law and the death and resurrection of genes. Proceedings of the National Academy of Science **91**:12283-12287.

Marshall, L. G., and R. S. Corruccini. 1978. Variability, evolutionary rates, and allometry in dwarfing lineages. Paleobiology **4**:101-119.

Martinez Meyer, E., M. Morales, and J. S. Escalanta. 1998. First record of the kinkajou, *Potos flavus* (Carnivora: *Procyonidae*), from Isla Cozumel, Quintana Roo, Mexico. Southwestern Naturalist:101-102.

Martínez-Morales, M. A., and A. D. Cuarón. 1999. *Boa constrictor*, an introduced predator threatening the endemic fauna on Cozumel Island, Mexico. Biodiversity and Conservation **8**:957-963.

Mayr, E. 1942. Systematics and the origin of species. Columbia University Press, New York.

McCallum, H., and A. Dobson. 1995. Detecting disease and parasite threats to endangered species and ecosystems. Trends in Ecology & Evolution **5**:190-194.

McClearn, D. 1992. Locomotion, posture, and feeding behavior of kinkajous, coatis, and raccoons. Journal of Mammalogy **73**:245-261.

McConnaughey, T., and C. P. McRoy. 1979. Food-web structure and the fractionation of carbon isotopes in the Bering Sea. Marine Biology **53**:257-262.

McNab, B. K. 1971. On the ecological significance of Bergmann's rule. Ecology **52**:845-854.

Merriam, C. H. 1901. Six new mammals from Cozumel Island, Yucatan. Proceedings of the Biological Society of Washington **14**:99-104.

Milliman, J. D., and K. O. Emery. 1968. Sea level during the past 35,000 years. Science **162**:1121-1123.

Miyamato, M. M., and W. M. Fitch. 1995. Testing species phylogenies and phylogenetic methods with congruence. Systematic Biology **44**:64-67.

Morner, N. A. 1971. Eustatic changes during the last 20,000 years and a method of separating the isostatic and eustatic factors in an uplifted area. Paleogeography, Paleoclimatology, Paleoecology **9**:153-182.

Mugaas, J. N., and J. Seidensticker. 1993. Geographic variation of lean body mass and a model of its effect on the capacity of the raccoon to fatten and fast. Bulletin of the Florida museum of natural history, Biological Sciences **36**:85-107.

Myers, A., and P. Giller 1988. Analytical biogeography. Chapman and Hall, London.

Navarro, D., and M. Suarez. 1989. A survey of the pygmy raccoon (*Procyon pygmaeus*). Mammalian Species **53**:458-461.

Nelson, G. J., and N. I. Platnick 1981. Systematics and Biogeography: Cladistics and vicariance. Columbia University Press, New York.

Nixon, K. C., and Q. D. Wheeler. 1990. An amplification of the phylogenetic species concept. Cladistics **6**:212-223.

O'Brien, S. J., and E. Mayr. 1991. Bureaucratic mischief: recognizing endangered species and subspecies. Science **251**:1187-1188.

Otis, D. L., K. P. Burnham, G. C. White, and D. R. Anderson. 1978. Statistical inference for capture data on closed animal populations. Wildlife Monographs **62**:1-24.

Owens, N. J. 1987. Natural variations in ^{15}N in the marine environment. Advances in Marine Biology **24**:389-451.

Page, R. D., and E. C. Holmes 1998. Molecular evolution: a phylogenetic approach. Blackwell Science Ltd., Oxford, England.

Pamilo, P., and M. Nei. 1988. Relationships between gene trees and species trees. Molecular Biology and Evolution **5**:568-583.

Peters, R. H. 1983. The ecological implications of body size. Cambridge University Press, Cambridge.

Pollock, K., J. D. Nichols, and C. Brownie. 1990. Statistical inference for capture-recapture experiments. Wildlife Monographs **107**:1-97.

Pollock, K. H. 1982. A capture-recapture design robust to unequal probability of capture. Journal of Wildlife Management **46**:757-760.

Pons, J. M., V. Volovouev, J. F. Ductroz, A. Tillier, and D. Reudet. 1999. Is the Guadeloupean racoon (*Procyon minor*) really an endemic species? New insights from molecular and chromosomal analyses. Journal of Zoology and Systematic Evolution Research **37**:101-108.

Posada, D., K. A. Crandall, and A. R. Templeton. 2000. GEODIS: A program for the cladistic nested analysis of the geographical distribution of genetic haplotypes. Molecular Ecology **9**:487-488.

Prestrud, P., and K. Nilssen. 1995. Growth, size, and sexual dimorphism in arctic foxes. Journal of Mammalogy **76**:522-530.

Primack, R. B. 1998. Essentials of conservation biology. Sinauer Associates, Sunderland, Massachusetts.

Pyke, G. H., H. R. Pulliam, and E. L. Charnov. 1977. Optimal foraging: a selective review of theory and tests. Quantitative Review of Biology **52**:137-154.

Redford, K. H., and J. G. Robinson. 1991. Subsistence and commercial uses of wildlife in Latin America. Pages 6-23 in K. H. Redford, and J. G. Robinson, editors. Neotropical wildlife use and conservation. University of Chicago Press, Chicago.

Robinson-Rechavi, M., and D. Huchon. 2000. RRTree: Relative-rate tests between groups of sequences on a phylogenetic tree. Bioinformatics **16**:296-297.

Roth, J. D., and K. A. Hobson. 2000a. Stable carbon and nitrogen isotopic fractionation between diet and tissue of captive red fox: implications for dietary reconstruction. Canadian Journal of Zoology **78**:848-852.

Roth, J. D., and K. A. Hobson. 2000b. Stable carbon and nitrogen isotopic fractionation between diet and tissue of captive red fox: implications for dieteray reconstruction. Canadian Journal of Zoology **78**:848-852.

Russell, J. K. 1982. Timing of reproduction by coatis (*Nasua narica*) in relation to fluctuations in food resources. Page 468 in E. G. Leigh, A. S. Rand, and D. Windsoc, editors. The Ecology of a Tropical Forest. Smithsonian Institute Press, Washington, D.C.

Russell, J. K. 1983. Altruism in coati bands: nepotism or reciprocity? Pages 263-290 in S. K. Wasser, editor. Social behavior of female vertebrates. Academic Press, New York.

Ryder, O. A. 1986. Species conservation and systematics: the dilemma of subspecies. Trends in Ecology and Evolution **1**:9-10.

Sanderson, G. C. 1951. Breeding habits and a history of the Missouri raccoon populations from 1941-1948. Transactions of the North American Wildlife Conference **16**:445-461.

Sanderson, G. C., and A. V. Nalbandov. 1973. The reproductive cycle of the raccoon in Illinois. Illinois Natural History Survey Bulletin **31**:29-85.

Schilling, M. F. 1986. Multivariate two-sample tests based on nearest neighbors. Journal of American Statistics Association **81**:799-805.

Schneider, S., D. Roessli, and L. Excoffier. 2000. Arelequin: A software for population genetics data analysis. University of Geneva, Geneva.

Schoeninger, M. J. a. M. J. D. 1984. Nitrogen and carbon isotopic composition of bone collagen from marine and terrestrial animals. Geochemica Cosmochimica Acta **48**:625-639.

Seber, G. A. F. 1965. A note on the multiple capture census. Biometrika **52**:249-259.

Seber, G. A. F. 1970. Estimating time-specific survival and reporting rates for adult birds from band returns. Biometrika **57**:313-318.

SEMARNAT. 2002. Normal Oficial Mexicana NOM-059-ECOL-2001, Proteccion ambiental- Especies nativas de Mexico de flora y fauna silvestres- Categorias de riesgo y especificaciones para su inclusion, exclusion o cambio- Lista de especies en riesgo. Pages 1-81. Diario Oficial de la Federacion. Secretaria de Medio Ambiente y Recursos Naturales, Mexico City.

Shaul, B. 1962. Notes on hand-rearing various species of mammals. International Zoo Yearbook:300-332.

Slatkin, M., and W. P. Maddison. 1989. The cladistic measure of gene flow from the phylogenies of alleles. Genetics **123**:603-613.

Soulé, M. E., S. Y. Yang, and P. Myers. 1975. Genetic and morphological divergence among introduced rat populations (*Rattus rattus*) of the Galapagos Archipelago, Equador. Evolution **45**:296-310.

Spaw, R. H. 1978. Late Pleistocene carbonate bank deposition: Cozumel Island, Quintana Roo, Mexico. Gulf Coast Association Geological Society **28**.

Sumner, P. W., and E. P. Hill. 1980. Scent stations as indices of abundance in some furbearers of Alabama. Proceedings of the Annual Conference of Southeastern Association of Fish and Wildlife Agencies **34**:572-583.

Swofford, D. L. 2002. Phylogenetic analysis using parsimony (*and other pethods). Ver. 4. Sinauer, Sunderland, MA.

Talbot, S. L., and G. F. Shields. 1996a. A phylogeny of bears (*Ursidae*) inferred from complete sequences of three mitochondrial DNA genes. Molecular Phylogenetics and Evolution **416**:567-575.

Talbot, S. L., and G. F. Shields. 1996b. Phylogeography of brown bears (*Ursus arctos*) of Alaska and paraphyly within the Ursidae. Molecular Phylogenetics and Evolution **5**:477-494.

Taylor, R. J. 1984. Predation. Chapman and Hall, London.

Thomas, O. 1901. New insular forms of *Nasua* and *Dasyprocta*. Annals and Magazine of Natural History **7**:271-273.

Thomas, W. K., S. Paabo, F. X. Vilablanca, and R. K. Wayne. 1990. Spatial and temporal continuity of kangaroo rat populations shown by sequencing mitochondrial DNA from museum specimens. Journal of Molecular Evolution **31**:101-112.

Thompson, J. G., D. G. Higgins, and T. J. Gibson. 1994. Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Research **22**:4673-4680.

Tieszen, L. L., and T. W. Boutton. 1989. Stable carbon isotopes in terrestrial ecosystem research. Pages 167-195 in P. W. Rundel, J. R. Ehleringer, and K. A. Nagy, editors. Stable isotopes in ecological research. Springer-Verlag, New York.

Tieszen, L. L., T. W. Boutton, K. G. Tesdahl, and N. A. Slade. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications from ^{13}C analysis of diet. Oecologia **57**:32-37.

Valenzuela, D., G. Ceballos, and A. Garcia. 2000. Mange epizootic in white-nosed coatis in western Mexico. Journal of Wildlife Diseases **36**:56-63.

van der Merwe, N. J. 1982. Carbon, isotopes, photosynthesis, and archaeology. American Scientist **70**:596-606.

Van Riper, C., S. G. Van Riper, M. L. Goff, and M. Laird. 1986. The epizootiology and ecological significance of malaria in Hawaiian land birds. Ecological Monographs **56**.

Van Valen, L. 1962. A study of fluctuating asymettry. Evolution **10**:139-146.

Van Valen, L. 1973. Pattern and balance of nature. Evolutionary Theory **1**:31-49.

Vila, C., I. R. Armorim, J. A. Leonard, D. Posada, S. J. Castroviejo, F. Petrucci-Fonseca, K. A. Crandal, H. Ellegren, and R. Wayne. 1999. Mitochondrial DNA phylogeography and population history of the grey wolf *Canis lupus*. Molecular Ecology **8**:2089-2103.

Virgl, J. A., and F. Messier. 1993. Evaluation of body size and body condition indices in muskrats. Journal of Wildlife Management **57**:854-860.

Vogel, J. C. 1978. Isotopic assessment of the dietary habits of ungulates. South African Journal of Science **74**:298-459.

Vogler, A. P., and R. DeSalle. 1994. Diagnosing units of conservation management. Conservation Biology **8**:354-363.

Waples, R. S. 1991. Pacific salmon, Oncorhynchus sp., and the definition of "species" under the Endangered Species Act. **53**:11-22.

Ward, W. C. 1985. Quaternary geology of northeastern Yucatan Peninsula. Pages 23-53 in W. C. Ward, E. Weidie, and W. Back, editors. Geology and hydrogeology of the Yucatan Peninsula. New Orleans Geological Society, New Orleans, LA.

Wayne, R., E. Geffen, D. J. Girman, K. P. Koepfli, L. Lau, and C. R. Marshall. 1997. Molecular systematics of the Canidae. Systematic Biology **4**:662-653.

Wayne, R., W. S. Modi, and S. J. O'Brien. 1986. Morphological variability and asymmetry in the cheetah (*Acinonyx jubatus*), a genetically uniform species. Evolution **40**:78-85.

Wayne, R., and S. J. O'Brien. 1986. Empirical demonstration that structural gene and morphometric variation of mandible traits are uncoupled between strains. Journal of Mammalogy **67**:441-449.

West, R. C. 1964. The natural regions of middle America. Pages 361-393 in R. Wauchope, editor. Handbook of Middle American Indians. University of Texas Press, Austin.

White, G. C., and K. P. Burnham. 1999. Program MARK: survival estimation from populations of marked animals. Bird Study **46**:120-138.

Williams, J. 1998. Weather Audio Broadcast Network.

Woodfruff, D. S. 1989. The problem of conserving genes and species. Pages 95-113 in D. Western, and M. C. Pearl, editors. Conservation for the twenty-first century. Oxford University Press, New York.

Wright, S. 1969. Evolution and the genetics of populations. University of Chicago, Chicago.

Wu, C. I. 1991. Inferences of species phylogeny in relation to segregation of ancient polymorphisms. Genetics **127**:429-435.

Zar, J. 1999. Biostatistical Analysis. Prentice Hall, Upper Saddle River, NJ.

Zeveloff, S. I. 2002. Raccoons: A natural history. Smithsonian Institution Press, Washington.

Zippin, C. 1958. The removal method of population estimation. Journal of Wildlife Management **22**:82-90.