Evolutionary Biology and Conservation of the Hog Island Boa Constrictor

by

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Declaration of own work

I Stephen Edward Warren Green declare that this Ph.D. thesis, entitled Evolutionary Biology and Conservation of the Hog Island Boa Constrictor is an original piece of research and contains no material that has been submitted previously, in whole or in part, for the award of any other academic degree or diploma. Except where otherwise indicated, this thesis is my own work.

Abstract

The Hog Island Boa constrictor is a dwarfed insular race of *Boa constrictor imperator* endemic to two small islands (Cayo Cochino Grande and Cayo Cochino Pequeño) in the Cayos Cochinos archipelago, Honduras. During the late 1970s and 1980s the wild population was decimated by intensive and unregulated collection for the pet trade. Fortunately, conservation management appears to be promoting demographic recovery of the population. Capture-mark-recapture analysis of the Cayo Cochino Pequeño population estimates current adult census size to be in the region of 700 individuals, with genetic analysis suggesting the Cayo Cochino Grande population to be of a similar size. Although evidence of a recent genetic bottleneck was detected in both populations, the rapid rate at which the populations recovered from the demographic bottlenecking event may have prevented the loss of substantial genetic diversity. Phylogenetic analysis reveals that populations of *B. c. imperator* in the Cayos Cochinos and on the nearby Bay Islands form a monophyletic group that likely diverged from the mainland approximately 2 million years ago. Dwarfism has subsequently evolved rapidly in the Cayos Cochinos since the islands were last isolated from the mainland by rising sea levels at the end of the last ice age. Thus, the Cayos Cochinos and Bay Island populations represent an Evolutionary Significant Unit of high conservation priority representing both historical and recent adaptive divergence of the species on islands. Conservation management strategies should focus on conserving this important historical genetic diversity while maintaining the ecological processes responsible for phenotypic variation in the Cayos Cochinos.

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Chapter 1 Introduction

Biodiversity loss and prioritisation

Global biodiversity is being lost at an alarming rate (Myers 1993; Whittaker et al. 2005), but conservation efforts are often hampered by inadequacies in our knowledge of current species diversity and biogeography (Whittaker et al. 2005). Increasing our understanding of the true extent of global biodiversity, its evolutionary history and the forces that shape responses to environmental change are key to reducing the current trend of decline (Hendry et al. 2010). In turn, this knowledge will help with the prioritization of limited conservation resources into areas of highest conservation value and need (Margules & Pressey 2000; Myers et al. 2000; Brooks et al. 2006; Kier et al. 2009).

Islands are often biodiversity hotspots with high levels of endemism (Whittaker & Fernández-Palacios 2007), owing to their (often long-term) isolation from mainland communities and differences in their abiotic and biotic environments. The endemism richness of islands makes them of exceptional importance for the global conservation of biodiversity (Kier et al. 2009). However, island biodiversity may also be at increased risk of extinction compared with mainland biodiversity for a number of reasons, including a relatively greater vulnerability to anthropogenic habitat destruction (Kier et al. 2009), the potentially devastating effects of introduced invasive species (Clavero & Garcia-Berthou 2005), and the genetic consequences of small population size (Frankham 1998; Frankham 2001).

In addition to their high conservation value, islands also serve as immensely valuable natural laboratories for the study of evolutionary processes (Butler et al. 2007). The theory of evolution by natural selection was famously influenced by observations

made on the morphological adaptations of island forms (Darwin & Wallace 1858; Darwin 1859). Thus, the conservation of island biodiversity is not only critical in the battle against global biodiversity loss, but in the maintenance of an invaluable resource for the progression of our understanding of evolutionary processes.

The relative simplicity of islands, in their species assemblages, compared with continental landmasses makes it easier for researchers to observe and study general patterns of evolution. Islands also provide easily identifiable study units and are in great abundance, thus presenting the opportunity for replication, essential for the robust testing of evolutionary hypotheses. Island biogeography theory, as first outlined by MacArthur and Wilson (1967), has helped researchers to understand the general patterns of species assemblages and evolutionary processes observed on islands. The theory predicts that species diversity of islands will increase with increasing island size and maximum elevation due to an expected increase in habitat variability. Species diversity is also predicted to increase over time as greater numbers of species are able to colonise the island, until such time that an equilibrium will be met between colonisation and extinction and species diversity will plateau. Also, species diversity of islands is predicted to decrease with increasing distance, and thus isolation, from source populations. Not only will increased isolation of islands be negatively correlated with species diversity, but the differential dispersal methods and capabilities between species will influence the type of species found on isolated islands.

Similarly, population genetic theory predicts that genetic diversity within island populations will be positively correlated with population size and negatively correlated with increasing isolation from neighbouring populations (Wright 1931,

1940, 1943, Frankham 1997). Indeed, island populations have been shown to display lower levels of genetic diversity than mainland conspecifics, with insular endemics displaying even lower levels of genetic diversity than non-endemic insular forms (Frankham 1997). Small island populations with low levels of genetic diversity have been shown to be at increased risk of extinction (Frankham 1998, Frankham 2001)

Island biogeography and population genetics theory are of particular importance for conservation planning because they not only aid in our understanding of current levels of observed diversity, but also help to make predictions about future levels of diversity and extinction rates in isolated populations. With escalating levels of habitat fragmentation, mainland habitats are becoming increasingly 'island-like' and thus a greater understanding of island biogeography theory and the genetics of small, island populations will assist in conservation decicion making in both island and mainland populations. Indeed, island biogeography theory has played a central role in the design of protected areas and it remains at the heart of current conservation planning. However, it has also been acknowledged that despite providing a strong theoretical framework, habitat fragmentation research is now pushing conservation planning beyond the boundaries of island biogeography theory (Laurance 2008).

Central America – geologically young and biodiversity rich

Central America has been the stage of dramatic tectonic activity in the recent geological past, leading to both lineage divergence and species mixing (Coates & Obando 1996; Coates 1997; Webb 1997); events that have resulted in Central America supporting extremely high levels of biodiversity. Consequently, Central America has been identified as one of the twenty five global 'biological hotspots' for conservation prioritisation (Myers et al. 2000).

The formation of the Central American isthmus began during the middle Miocene and finally completed the connection between North and South America approximately 3.5 million years ago (Coates & Obando 1996; Collins et al. 1996; Coates 1997; Webb 1997). The great faunal interchange between the two previously isolated landmasses ensued, with South American species expanding northward and North American species expanding their ranges in the opposite direction (Webb 1997). The Central American isthmus would not only have acted as a biological corridor, but as species became increasingly isolated from their source populations, this new landmass became the stage for the evolution of new species.

The same tectonic activity responsible for the formation of the isthmus was also responsible for the rise of the South American Andes (Gregory-Wodzicki 2000; Hooghiemstra et al. 2006). As these mountains grew in elevation they restricted gene flow between populations on either side, especially those restricted to lowland tropical habitats. The rapid uplift of the Eastern Cordillera that occurred during the late Miocene and Pliocene has been closely correlated with lineage divergence of a number of species (e.g. Zamudio & Greene 1997; Cortés-Ortiz et al. 2003; Hoffmann & Baker 2003; Brumfield & Edwards 2007; Dacosta & Klicka 2008; Gamble et al. 2008; Venegas-Anaya et al. 2008; Santos et al. 2009; Vallinoto et al. 2010). However, other species appear to have extended their range across the Eastern Cordillera more recently, since this period of rapid uplift (e.g. Wüster et al. 2005).

Understanding the biogeographical distributions and boundaries of species is of critical importance to conservation planning (Whittaker et al. 2005). With the advancement of molecular techniques, phylogenies are becoming increasingly available to conservation planners and are helping to identify previously unrecognised, cryptic biological diversity (Bickford et al. 2007). Identification of

such genetic diversity may be an early step in the recognition of new species and will, thus, aid in the prioritisation of conservation efforts (Burbrink & Castoe 2009).

Phylogenetic studies of species spanning the Central American isthmus and South American Andes have helped to increase our understanding of current biological diversity and species delimitations (e.g. Zamudio & Greene 1997; Cortés-Ortiz et al. 2003; Hoffmann & Baker 2003; Brumfield & Edwards 2007; Dacosta & Klicka 2008; Gamble et al. 2008; Venegas-Anaya et al. 2008; Santos et al. 2009; Vallinoto et al. 2010). However, in comparison with other vertebrates, and in particular mammals and birds, snakes have typically been neglected from phylogeographic studies (Burbrink & Castoe 2009).

Boa constrictor distribution

The large bodied constricting snake Boa constrictor (*Boa constrictor*) is the sole species within its genus and has one of the largest latitudinal distributions of any snake species in the world, spanning the Central American isthmus and ranging from Mexico to Argentina (approximately 30^{o}N - 35^{o}S) (Henderson et al. 1995). It is likely that *B. constrictor* was one of the species involved in the great faunal interchange, presumably extending its range northward out of South America into Central and North America as the two previously isolated landmasses were joined during the late Miocene and early Pliocene. However, the precise time at which *B. constrictor* extended its range out of South America is unclear.

The most comprehensive fossil evidence for faunal migrations across the newly formed isthmus comes from land mammals. This is likely because they are generally abundant and their teeth, which fossilize well, allow precise identification to species level (Webb 1997). Snakes, in contrast, tend to be relatively rare in the fossil record,

which may be due to their relatively delicate skeletons and difficulty of identification (Holman 2000). Thus, it may be more profitable to look to alternative methods, such as molecular dating techniques, to elucidate the spread of *B. constrictor* into its current range.

Boa constrictor diversity

B. constrictor is currently divided into eight or nine subspecies based on differences in morphology, colouration and geographical isolation (Peters & Orejas-Miranda 1986; Russo 2007; Reed & Rodda 2009). However, it has been acknowledged that this classification may inadequately reflect the true level of diversity within the species and that phylogenetic analysis is required to resolve the taxonomic legitimacy of the currently recognised subspecies (Wilson & Meyer 1985; Savage 2002; Russo 2007; Reed & Rodda 2009).

A recent phylogenetic study of predominantly captive animals (excluding the island subspecies *B. c. nebulosus* and *B. c. orophias*) identified the presence of just two distinct clades across the species range; one covering Central America and neighbouring north-western South America west of the Andes, and the other covering the rest of South America (Hynkova et al. 2009). Divergence of the clades, which may be significantly genetically diverged to be considered separate species, can most likely be attributed to increased uplift of the Colombian Andes and the final closure of the Panamanian isthmus during the late Pliocene (Hynkova et al. 2009). However, analysis of wild caught specimens backed up with precise collection locality information would be desirable to confirm the findings of Hynkova et al. (2009).

Island diversity and insular dwarfism

In addition to the observed mainland diversity, a number of phenotypically distinct populations can be found on islands across the Central American range of the species. Many of these insular populations have undergone changes in colouration, behaviour, ecology and, perhaps most notably, dramatic reductions in body size (Porras 1999; Boback 2005; Russo 2007; Reed & Rodda 2009). Despite these differences, however, the island populations have traditionally been described as the same subspecies found throughout mainland Central America, *B. c. imperator*; the only exception being *B. c. sabogae,* found on the Pearl Islands off the coast of Panama.

Evolutionary changes in body size on islands are common and explanations of such changes have been firmly incorporated into island biogeography theory (Case 1978; Lomolino 1985; Lomolino 2005; Lomolino et al. 2006), however, the generality of such changes in body size has been questioned for certain taxa (e.g. Meiri 2006; Boback 2003). In a study of body size of insular snakes, it was concluded that physiographic variables such as island area, island age, distance to mainland and latitude were not important in determining body size. Rather, body size of insular snakes was found to be principally influenced by prey size (Boback 2003).

Many of these islands have only been isolated by rising sea levels within the last few thousand years and thus suggest exceptionally rapid localised adaptation. Recent phylogenetic analysis of some of these populations, using the mitochondrial gene cytochrome-b, suggests close genetic affinity to the mainland populations (Hynkova et al. 2009), thus supporting rapid evolution of *B. constrictor* on these islands. However, in order to better understand these apparently rapid evolutionary shifts, more comprehensive phylogenetic and phylogeographical analyses that reconstruct

colonisation histories and identify source populations are necessary (Gould & MacFadden 2004; Keogh et al. 2005; Lomolino et al. 2006).

Over-water dispersal and island colonisation

It is possible that populations of *B. constrictor* were already present on islands off the coast of Central America when they were last isolated from the mainland by rising sea levels at the end of the last ice age. However, it is also possible that the islands were instead subsequently colonised by *B. constrictor* at some point after their isolation from the mainland. Snakes are accomplished swimmers and, thus, the relatively short distances separating some of these islands from the mainland and from one another would not preclude the possibility of snakes reaching the islands unaided. Alternatively, islands may have been colonised by individuals rafting on floating vegetation washed out to sea from the mainland after heavy storms. Species of reptiles, including *B. constrictor*, have been observed rafting at sea on floating material in this way (Greene 1997; Censky et al. 1998).

The relatively high frequency of tropical storms and hurricanes in the Caribbean, which result in large volumes of vegetation being washed out to sea, will have provided many opportunities for colonisation by rafting to have occurred. The frequency of such events, coupled with the ability of snakes to survive long periods without food, make the colonisation of these islands by rafting a likely possibility. However, yet another explanation for the colonisation of islands by *B. constrictor* that must be considered is the accidental or deliberate transportation of animals on boats. For centuries, humans have either visited or settled on these islands and it is quite possible that snakes colonised the islands as a direct result of anthropogenic activities.

Following the initial colonisation event (by whichever means it took place), the likelihood that a population would become established on an island may have been aided by the ability of gravid female *B. constrictor* to produce large litters of offspring. Additionally, it has been shown that *B. constrictor* is capable of producing non-clonal, viable offspring via parthenogenesis (Booth et al. 2010), therefore, suggesting that even non-gravid females may have the ability to establish new populations on islands.

Deforestation, population growth and species declines in Honduras

A recent assessment of the conservation status of the herpetofauna of Honduras identified the principal threats to be uncontrolled population growth and its corollaries, habitat alteration and destruction, pollution, pest and predator control, overhunting, and overexploitation (Wilson & McCranie 2004). The Honduran population is growing at a rate of approximately 2.8%, the third highest rate of all Central American countries, and thus is expected to double in size within 25 years (Wilson & McCranie 2004). In 1950, three quarters of Central America was still covered by forest, and by the end of the 20th century forest cover had declined to just 30% (Heckadon-Moreno 1997) (Figure 1-1). Deforestation models for Honduras (Wilson & Perlman 2000) indicate that the amount of forest remaining in Honduras in 1995 was approximately 4.1 million hectares, just 37% of the original forest cover of the country (Wilson & McCranie 2004). Primary forest cover is thought to have been reduced to just 20% of its original extent (Myers et al. 2000). If current deforestation rates continue, it is predicted that no forest will remain in Honduras, outside of protected areas, by the end of the present century (Wilson & McCranie 2004). It is hardly surprising, therefore, that Wilson and McCranie (2004) concluded that the entire Honduran herpetofauna, and indeed the entire biota, is endangered.

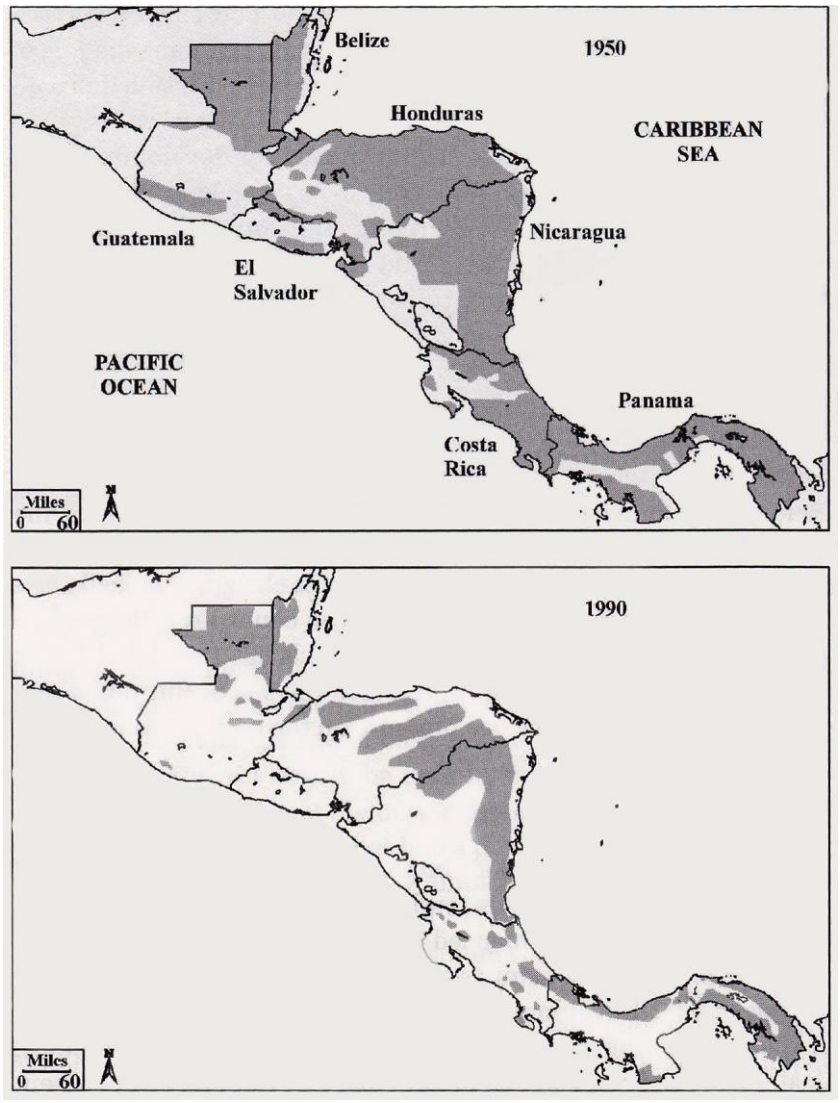

Figure 1-1 The extent of deforestation in Central America in 1950 (top); deforestation in 1990 (bottom). Grey areas show presence of forests. Reproduced from (Heckadon-Moreno 1997).

Vulnerability of Boa constrictor

In their assessment of the vulnerability of Honduran herpetofauna, Wilson and McCranie (2004) applied an environmental vulnerability gauge for reptiles, taking into account the geographic and ecological distribution of each species and the extent of human persecution suffered. Based on these criteria, *B. constrictor* is considered to be of low vulnerability. However, the assessment is limited in that it does not take into account the small geographic range of island populations of this species or their

potential to be unique, genetically-diverged lineages of increased evolutionary significance. This is particularly relevant in light of recent phylogenetic analysis which describes the Central American clade as a separate species to the South American clade, east of the Andes (Hynkova et al. 2009).

In addition, insular populations of *B. constrictor* are under increased threat due to the rapid and unregulated growth of the tourism industry on the islands where they are found. Since the end of the 1980s, Central American governments have been trying to strengthen their economies through new avenues of economic development (Stonich 1993). The development of international tourism has been one of the most important of these development strategies for Honduras, especially in the wake of hurricane Mitch which severely impacted traditional agricultural exports (Stonich 2000). The rapid growth of tourism in Las Islas de la Bahía, in the absence of environmentally sound development regulations, has resulted in the substantial declines of both terrestrial and marine species (but see Stonich 2000 for a detailed account of the effects of tourism).

The vulnerability assessment of Wilson and McCranie (2004) also failed to take into consideration the higher level of persecution that island populations of *B. constrictor* frequently experience as a result of collection for the pet trade (Porras 1999; Reed et al. 2007). The relatively recent and rapid expansion in the trade of live reptiles as pets has raised serious concerns about the sustainability of such commercial exploitation (Schlaepfer et al. 2005). In recent years, reports of rapid population declines and extirpation events of reptiles have been, at least in part, attributed to unsustainable harvesting for the pet trade (Grismer et al. 1999; Nilson et al. 1999; Webb et al. 2002; Reed et al. 2007). It is likely, therefore, that the vulnerability of island populations of *B. constrictor* in Honduras, as well as their evolutionary

significance, may have been seriously underestimated by previous conservation assessments.

The impacts of overharvesting on reptile populations

The commercial exploitation of reptiles as a source of meat, skins, traditional medicines and for sale in the pet trade has often been shown to be highly unsustainable, poorly regulated and has directly caused the decline of many species (Gibbons et al. 2000). However, when properly regulated, the commercial trade of species can in fact be used as a conservation tool, giving financial incentives for the sustainable management of wild populations (Milner-Gulland & Rowcliffe 2007). The international trade in crocodilian skins has been touted as a classic example of how regulated trade has directly benefited the conservation of wild populations (Thorbjarnarson 1999). But even this model example of 'sustainable' exploitation has experienced difficulties due to the instability of global markets and changing whims of the fashion industry (Thorbjarnarson 1999). Other interesting dynamics such as the exploitation of wild populations of snakes as a food supply for farmed crocodilian populations also call into question the true sustainability of certain aspects of the crocodilian trade (Brooks et al. 2010).

The sustainability of any harvest will ultimately depend on the intensity of collection, initial population size, geographic range, and natural history of the species. Species such as reticulated pythons (*python reticulates*) and Asian water monitors (*Veranus salvator*), which display relatively early maturation, large clutch size and large geographic ranges, show reasonable resistance to the practice of harvesting (Shine 1996; Shine 1999). However, species with long generation times such as rattlesnakes

(*Crotalus*) have been shown to be particularly vulnerable to intensive harvesting practices (Gibbons 2000).

Despite the potential negative impacts of harvesting reptile populations, the practice of harvesting reptiles for their skins and meat alone, in the absence of other negative pressures such as habitat destruction, alteration or fragmentation, will not necessarily drive species to extinction. This is because as populations densities are reduced, the financial viability of harvesting is also reduced and the practice of harvesting may, therefore, decline. Unfortunately, in the case of collecting reptiles for the pet trade this is less likely to be the case.

The collection of reptiles for the pet trade can be particularly damaging to certain species and populations because of the high price which dealers are prepared to pay for animals. As the commodity becomes rarer this price is likely to increase, thus, keeping the practice of harvesting financially viable even after population densities have become dangerously low. It is also often small populations of particularly unique species or races that generate the most commercial interest, which often includes insular forms. The small size of these populations makes them even more vulnerable to rapid over-exploitation. Many populations of snakes have declined as a direct result of over-collection for the pet trade (Nilson 1990; Porras 1999; Webb 2002; Boback 2005; Reed et al. 2007). Also, in addition to declines caused as a direct result of actually removing individuals from a population, the practice of destructive harvesting techniques can result in the degradation of habitat and, thus, further contribute to population declines (Goode et al 2004).

Pet trade-induced decline of the Hog Island Boa

The Hog Island Boa constrictor (*Boa constrictor imperator*) is an insular dwarfed race of boa from the Cayos Cochinos archipelago, Honduras. These snakes are prized by reptile collectors because of their small size, averaging around just 1 m in snout vent length (Reed et al. 2007), and their light 'pink' colouration, which has lead to them being known locally as 'La Boa Rosada' or the 'pink boa' (Figure 1-2).

The population reportedly experienced severe decline as a result of intense collection for the pet trade throughout the late 1970s and 1980s, during which time thousands of snakes were removed from the islands (Porras 1999; Reed et al. 2007). Just a decade after collection began, a herpetological expedition to the Cayos Cochinos was unable to find a single specimen of this previously abundant snake, and local residents involved in the trade confirmed that as of 1988 virtually all adult boas had been removed from the islands (Wilson & Cruz Diaz 1993), highlighting the extreme vulnerability of this unique population.

Creation of the Cayos Cochinos protected area

In 1993 the Honduran Coral Reef Foundation (HCRF) was established with the goal of protecting the marine and terrestrial fauna and flora of the Cayos Cochinos. In 2003 legislative decree 114-2003 designated the Cayos Cochinos archipelago as a Marine Natural Monument and the HCRF as the managing authority for the next ten years (HCRF & TNC 2008). Since the formation of the HCRF, increased enforcement of anti-poaching legislation has greatly reduced the level of illegal collection of *B. constrictor* from the islands (Reed et al. 2007). As a result, the Cayos Cochinos *B. constrictor* populations appeared to be showing encouraging signs of recovery. However, the actual sizes of the populations and extent of recovery were

unknown, prompting the HCRF to request the initiation of research into the current conservation status of the Cayos Cochinos *B. constrictor*.

Figure 1-2 Examples of colour variations of *Boa constrictor imperator* in the Cayos Cochinos, Bay Islands and mainland Honduras: Photos are of wild caught specimens of *B. c. imperator* from (a) Cayo Cochino Grande, (b) Cayo Cochino Pequeño, (c) Roatan, (d) Utila, (e) Guanaja and (f) mainland Honduras.

Research objectives

This study attempts to address a number of research objectives in relation to *B. constrictor* populations in Central America and in particular the insular dwarfed

populations of the Cayos Cochinos, Honduras. The primary objectives are outlined below:

i. To determine the phylogenetic relationships of populations of *B. constrictor* in the Cayos Cochinos and Bay Islands with mainland populations in Honduras and Central America as a whole, and the evolutionary timescale over which observed levels of diversity have evolved. It is hypothesised that the island populations will display greatest genetic affinity with mainland Honduras snakes due to the mainland being the most likely source of colonisation. It is also hypothesised that the island populations will have diverged from the mainland populations no earlier than the age of the islands on which they are found, thus, suggesting rapid evolution of the Hog Island Boa phenotype. (Chapter 3)

ii. Investigate the level of population structure and gene flow that exists between island populations and the mainland, specifically between dwarfed and non-dwarfed populations. It is hypothesised that relatively strong structure will exist between islands, but that the weakest structure is likely to exist between the two islands within the Cayos Cochinos due to their close proximity to one another. Gene flow is expected to be weakest between the most distant populations. Little genetic structure is expected to be observed within islands but is likely to be present across the mainland. (Chapter 4)

iii. Assess the impact that unsustainable collection for the pet trade has had on the Cayos Cochinos population and the severity of the potential genetic bottleneck through which the population has passed. It is hypothesised that overall measures of genetic diversity will be lower than expected in the Cayos Cochinos populations due to the likely genetic bottleneck through

which the populations have passed as a result of excessive harvesting for the pet trade. (Chapter 5)

iv. Assess the level of population recovery in the Cayos Cochinos, measured through current adult census and effective population sizes. It is hypothesised that the current population size will be relatively healthy and showing good signs of recovery, but that the population may not have reached its pre-harvesting carrying capacity. (Chapter 6)

Each of the above research objectives will be dealt with in detail in the following chapters, as indicated, and the results discussed in relation to the conservation of *B. constrictor* populations in Honduras and throughout the wider range of the species. Chapter 2 outlines the study sites as well as the general methodologies used in this investigation.

Chapter 2 Study sites and general research methods

Study Sites

Islas de la Bahía

The Honduran department of the Islas de la Bahía, situated just a few kilometres north of the country's Caribbean coast, is one of eighteen departments that together make up the Republic of Honduras (Wilson & Hahn 1973). The islands are comprised of two distinguishable areas, the Bay Islands and the Cayos Cochinos, which combined have an approximate land area of 262.82 km^2 (McCranie et al. 2005) (Figure 2-1). The Bay Islands make up the vast majority of land within the department, with the Cayos Cochinos contributing just 2.22 km^2 (0.84%) of the total (McCranie et al. 2005). The islands form a natural geographic unit, with the nearest neighbouring islands, cays off the coast of Belize and Moskitia, and the Swan Islands, approximately 110, 270 and 220 km respectively (Shoch & Anderson 2007).

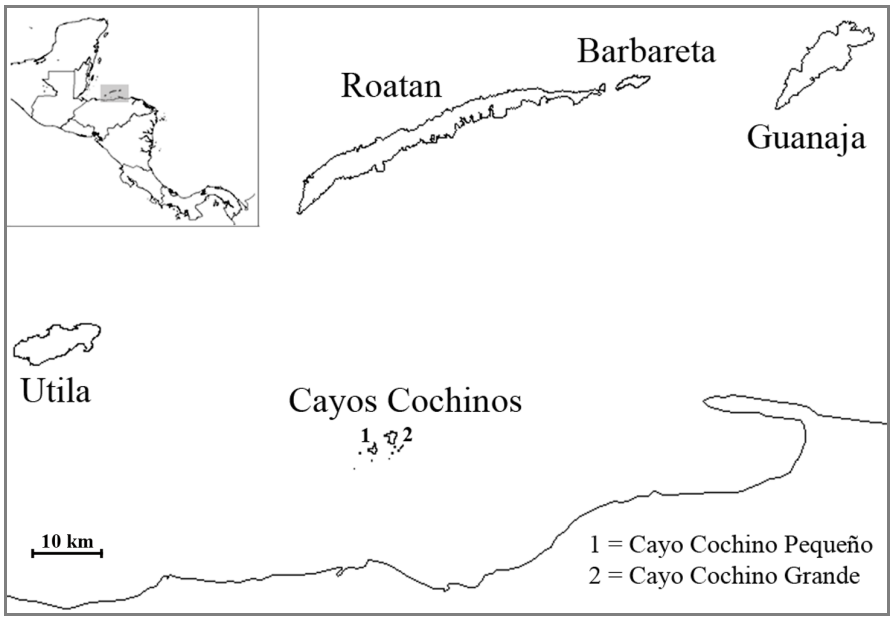

Figure 2-1 Map displaying the location of the Bay Islands (Utila, Roatan and Barbareta, and Guanaja) and the Cayos Cochinos opposite the Caribbean coast of Honduras.

The Bay Islands

The Bay Islands comprise of three major island formations (Utila, Roatan and Guanaja), two minor island formations (Morat and Barbareta) and a number of associated cays (Wilson & Hahn 1973; McCranie et al. 2005). Elevation of the islands increases eastward from the low-lying hills of Utila, through the moderate slopes of Roatan and to the relatively high, pine-covered ridges of Guanaja (McCranie et al. 2005).

Utila

The island of Utila is the smallest and westernmost of the three Bay Islands and lies approximately 32 km north north-west of the town of La Ceiba on the mainland (McCranie et al. 2005). At its greatest length the island measures 12.7 km and at its greatest width 5.2 km (Wilson & Hahn 1973) with a total area of 49.3 km^2 (McCranie et al. 2005). Utila is the flattest and lowest lying of the Bay Islands, with an average elevation of just 12 m (Wilson & Hahn 1973). The western part of the island is separated from the more heavily populated eastern side by an artificial canal. The western portion of the island is covered by wetland savannah and mangrove forest, and is regularly inundated by water during storm surges. Consequentially, very limited development has taken place on this side of the island. A large portion of this habitat was thought to be protected by the creation of Turtle Harbour Wildlife Refuge and Marine Reserve, which can be seen on many recent maps of the island. However, recent investigations into land ownership and the legality of the creation of the protected area suggest that this area has never been legally protected (personal communication BICA 2010). Utila's highest point can be found in the north-east at Pumpkin Hill at 74 m (Wilson & Hahn 1973). The higher

elevations of the eastern portion of Utila would have once been covered by hardwood lowland topical forest and mangrove forest (Wilson & Hahn 1973). However, much of this area has now been cleared for farmland and development, resulting in a patchy distribution of these habitats. The majority of the island's population reside in Utila Town, located in the south-east of the island.

Roatan

The largest of the Bay Islands, Roatan is situated in between Utila and Guanaja and directly north of the Cayos Cochinos. It is the farthest away from the mainland of all the Bay Islands, approximately 48 km north of the mouth of the Río Papaloteca, between La Ceiba and Balfate (Wilson & Hahn 1973). The long slender-shaped island measures 48 km at its greatest length and just 5.2 km at its greatest width (Wilson & Hahn 1973), with an area of 155.9 km^2 (McCranie et al. 2005). An increase in elevation can be observed in comparison with Utila, with most of Roatan being above 20 m and reaching a maximum elevation of 235 m at Picacho Hill, near the village of Oak Ridge (Wilson & Hahn 1973). Remnants of the original lowland tropical hardwood forest and pine forest, that would have once covered most of the island, can be found stretching along the spine of the island (Wilson & Hahn 1973; McCranie et al. 2005).

Morat and Barbareta

The small islands of Morat and Barbareta lie in close proximity to the easternmost point of Roatan. A third island, Santa Elena, is sometimes considered to be separate from the main island of Roatan, however, due to its extreme proximity, barely separated from the main island by a narrow mangrove lined channel, Santa Elena is normally considered as part of the main island of Roatan (McCranie et al. 2005).

Santa Elena is a low-lying extension of the main island and is dominated by mangrove forest. The island of Barbareta is privately owned and the vast majority of its forest remains intact, giving valuable insight into how the main island of Roatan may have once looked. The smaller island of Morat, however, appears to be subjected to frequent fires caused by slash-and-burn agriculture, resulting in far greater habitat loss and degradation.

Guanaja

The island of Guanaja lies approximately 28 km east of Roatan and is the easternmost of the three Bay Islands (McCranie et al. 2005). With an area of 55.4 km^2, 14 km at its greatest length and 6.2 km at its greatest width, it is the second largest Bay Island, (Wilson & Hahn 1973; McCranie et al. 2005). Guanaja is also the highest of the Bay islands, reaching a maximum elevation of 415 m at approximately the middle of the island (Wilson & Hahn 1973). Most of Guanaja was once covered by pine forest of the species *Pinus caribaea*, however, historical timber extraction for ship masts took a heavy toll on the islands forests. Much of what remained of these forests was then devastated when hurricane Mitch passed directly over the island in 1998 (McCranie et al. 2005). Reforestation initiatives have attempted to regenerate the islands forests, however, at least two large forest fires have severely hindered progress and much of the islands hillsides remain completely devoid of trees.

Cayos Cochinos

The Cayos Cochinos archipelago lies approximately 17 km north of Nueva Armenia at the mouth of the Río Papaloteca (Wilson & Cruz Diaz 1993). The Archipelago consists of two islands, Cayo Cochino Grande and Cayo Cochino Pequeño (also

known as Cayo Mayor and Cayo Menor respectively), and thirteen small coral cays, with a combined area of 2.22 km^2 (Figure 2-2). The two islands make up the vast majority of the land mass of the archipelago, Cayo Cochino Grande having an area of 1.55 km^2 and Cayo Cochino Pequeño 0.64 km^2 (McCranie et al. 2005). The islands are separated by a distance of approximately 1.6 km (Wilson & Cruz Diaz 1993) and can be described as having three high points, two in Cayo Cochino Grande at elevations of 143 and 136 m, and one at approximately the centre of Cayo Cochino Pequeño at an elevation of 141 m (HCRF & TNC 2008).

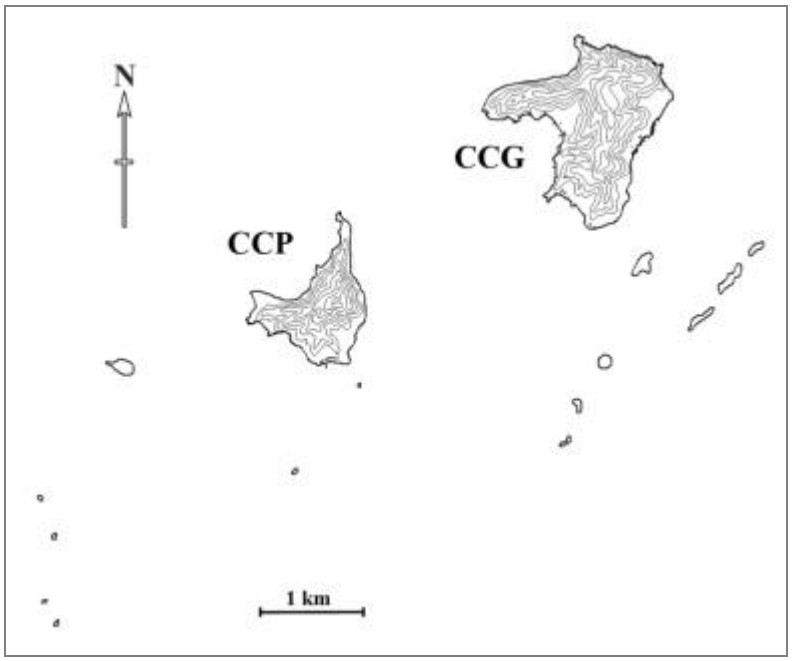

Figure 2-2 Map of the Cayos Cochinos archipelago, Honduras. CCP = Cayo Cochino Pequeño, CCG = Cayo Cochino Grande

Cayo Cochino Pequeño is uninhabited, with the exception of a small scientific research station, run by the Honduran Coral Reef Foundation (HCRF), on the south of the island. Spread along the western bay of Cayo Cochino Grande are a number of private houses and holiday homes, and a small hotel, predominantly catering for dive tourism. The small Garifuna fishing village of East End is located on the East of

Cayo Cochino Grande and consists of approximately 20 small houses and a small school. A few other buildings, belonging to the majority landowner of Cayo Cochino Grande, can be found on the east portion of the island. However, with the exception of a lighthouse situated along the central ridge of the island, Cayo Cochino Grande's interior has not been subject to development. The tiny cay of Chachahuate is home to the only other substantial Garifuna community in the Cayos Cochinos and approximately 56 small houses can be found crammed onto this narrow strip of land.

Bermingham et al. (1998) characterise the flora of the Cayos Cochinos as being adapted to strong seasonality, resulting in periods of severe drought, and as a consequence, probably has little in common with the forests on the wet Caribbean coast of Central America. This claim is supported by data on average monthly precipitation for the area (Figure 2-3). Certain areas of Cayo Cochino Grande retain sources of freshwater throughout the dry season, in the form of streams and shallow pools, however, Cayo Cochino Pequeño often experiences long periods with no available surface water.

"Hill forests", dominated mainly by tropical lowland oaks (*Quercus oleoides*), and "wind-swept forest", dominated by wind-swept lowland oaks, prostrate Sea Grapes (*Coccoloba uvifera*), or a mixture of the two, form the two primary habitats on Cayo Cochino Pequeño (Wilson & Cruz Diaz 1993), but see (Bermingham et al. 1998) for a detailed description of the island's vegetation. The forest on Cayo Cochino Grande has been altered, somewhat, by the presence of invasive *Attaleya* palms, which now dominate large areas of the island's hillsides. Despite this, however, Cayo Cochino Grande's vegetation is not dissimilar to that of Cayo Cochino Pequeño.

Climatic data collected for the Cayos Cochinos between 1988 and 2007 show a narrow temperature range of 26^{o} – 29^{o}C. The islands experience a marked wet and dry pattern of seasonality, with the wet season falling October to January, and dry season February to September (Figure 2-3). The highest monthly average precipitation for the recorded period was November with an average of 460 mm. (HCRF & TNC 2008).

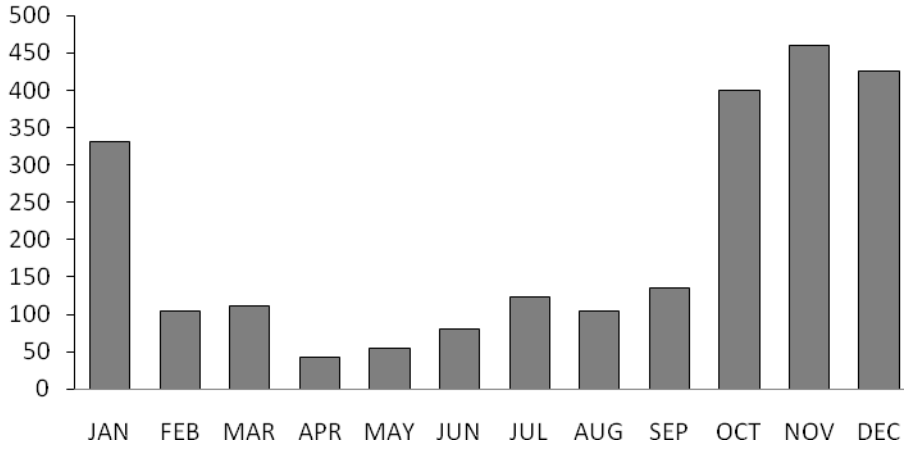

Figure 2-3 Average monthly rainfall (mm) for the Cayos Cochinos between 1988 and 2007 (HCRF & TNC 2008)

Geological history of las Islas de la Bahía

The geological origins of the islands appear to be linked with that of the Cordillera Nombre de Dios mountain range on the continental mainland, part of the Middle American Mountain Complex (Wilson & Hahn 1973; Bermingham et al. 1998). The Cayos Cochinos and Bay Islands are topographical high points of this mountain range (Bermingham et al. 1998). The Cayos Cochinos and Utila are situated on the continental shelf surrounded by shallow waters of no more than 30-55 m, and would have been connected to the mainland during the Pleistocene from 13,000 to 18,000 years ago at the end of the Wisconsin glacial period (McCranie et al. 2005). However, as the melting ice sheets caused sea levels to rise, the Cayos Cochinos and

Utila islands would have gradually been isolated from the mainland. The Cayos Cochinos and Utila, therefore, are unlikely to be more than a few thousand years old (Bermingham et al. 1998). In contrast, Roatan and Guanaja are oceanic islands, situated outside the continental shelf and surrounded by deeper waters reaching depths of up to 275 m (McCranie et al. 2005). It is unlikely that the 120-140 m drop in sea level that occurred during the Pleistocene would have resulted in Roatan and Guanaja being joined to the mainland, or each other, during this period (Wilson & Hahn 1973; McCranie et al. 2005). It can be assumed, therefore, that Roatan and Guanaja are much older islands than the Cayos Cochinos and Utila, having been separated from the mainland throughout the last glacial period.

Data collection

Visual Encounter Surveys (Cayos Cochinos)

Visual encounter surveys (VES) for boas were conducted on Cayo Cochino Pequeño, and to a lesser extent on Cayo Cochino Grande, during a number of visits to the Cayos Cochinos between July 2004 and January 2009 (Table 2-1). The number of participants varied between VES depending on the number of volunteers present, however, the number of participants and the time spent searching were recorded for each VES in an attempt to quantify search effort. Experience of participants was also variable, ranging from completely naive to experienced field herpetologists. VES were conducted by volunteers spreading out in a line, approximately evenly spaced with 2-3 m between each person, and slowly walking forward in a predetermined direction whilst searching all available habitat. Refugia such as logs and rocks were lifted, wherever possible, to search for boas and then replaced to minimize habitat disturbance. Trees and vegetation were searched to the best of the observer's ability,

however, surveys were limited by the maximum height at which an observer could search accurately. Average duration of each VES was approximately 1-2 hours. Search effort was estimated as being the time spent searching multiplied by the number of participants. In addition to boas caught during VES, boas were also captured opportunistically at other times while moving around the island.

The use of volunteers can be viewed as both a strength and a weakness in the methodology. The use of volunteers allowed much larger areas of the islands to be searched than would have otherwise been possible. But, the variability in the numbers of volunteers available, within and between years, and also the variability in the level of experience of volunteers, made it difficult to keep search effort standardised. This trade off between encounter rates and standardisation of search effort is a common problem with surveys of this kind when working with highly cryptic species.

All boas encountered were captured by hand and either placed in a cloth snake bag and taken back to the field station for processing, or processed *in situ* and released immediately. Boas that were taken to the field station for processing were released at the exact point of capture within 48 hours. The Universal Transverse Mercator (UTM) coordinates of the exact capture site were obtained using a hand-held Global Positioning System (GPS) in order to plot capture locations across the island. If dense canopy cover prevented the obtaining of a strong enough signal at the exact point of capture, a reading was attempted from a nearby location where there was a break in the canopy. In such cases, the direction and distance from the point of capture were estimated and the UTM coordinates adjusted accordingly. In rare

instances when it was not possible to obtain a GPS reading, no UTM coordinates were recorded.

Table 2-1 Dates the Cayos Cochinos study site was visited for sampling between 2004 and 2009.

Visit	Year	Season	Start Date	End Date
1	2004	Dry	5th Jul	29th Aug
2	2005	Dry	31st May	2nd Sept
3	2005/2006	Wet	23rd Dec	8th Jan
4	2006	Dry	23rd May	1st Sept
5	2006	Wet	20th Dec	27th Dec
6	2007	Dry	17th Apr	28th Apr
7	2007	Dry	21st May	2nd Sept
8	2007	Wet	28th Dec	31st Dec
9	2008	Dry	3rd Jun	18th Aug
10	2008/2009	Wet	18th Dec	2nd Jan

Processing boas

Snout-vent length (SVL) and tail length (TL) were measured by stretching the snake along a tape measure fixed to the laboratory bench, or if processing in the field, by stretching the tape measure along the snake. Sex was determined by observing the size of the cloacal spurs and the relative length of TL to SVL (males having enlarged spurs compared to females and relatively longer tails). If sex could not be determined with confidence, sex was confirmed by the use of hemipenial probes. All new captures were implanted with a Passive Integrated Transponder (PIT) tag (11 x 3 mm) and the unique ten digit identification code recorded (Gibbons & Andrews 2004). Subsequent recaptures were identified by scanning boas using a Biomark PIT tag reader. A tissue sample was then taken in the form of 1-3 ventral scale clips from

every new snake captured and retained for genetic analysis. Tissue samples were stored in >75% ethanol in screw top eppendorf tubes.

Opportunistic captures and tissue sample collection (Bay Islands and mainland Central America)

During the study, each of the Bay Islands was visited to collect tissue samples from wild caught *Boa constrictor*. Tissue samples were also kindly collected opportunistically by various collaborators on the Bay Islands and across mainland Central America.

Genetic analysis

All genetic analysis was carried out at DICE, University of Kent, UK, or at the Institute of Zoology (IOZ), London, UK. Specific genetic protocols are described in the following chapters and original screening and optimisation of microsatellite markers can be found in Appendix 1. A list of all samples used in this study can be found in Appendix 2.

Chapter 3 Phylogeography and the origins of dwarfism in a giant snake

Abstract

Intraspecific variation can arise through rapid adaptation to localised selection pressures, or through long-term isolation of populations. The combination of these two evolutionary forces has led to high variability in size and morphology in the large-bodied snake *Boa constrictor*, including multiple examples of insular dwarfism. The Islas de la Bahía, Honduras, are unusual in that they support both dwarf and larger mainland-like phenotypes and thus represent an ideal model system for studying the evolution of insular dwarfism in snakes. Phylogenetic histories were reconstructed and lineage divergence events dated for *B. constrictor* within these islands and across mainland Central America. It is concluded that these island populations represent an early Pleistocene radiation event, but that the island of Utila has also subsequently been colonised from the mainland more recently. Dwarfed phenotypes of *B. constrictor* in two small islands (the Cayos Cochinos) have evolved rapidly since the most recent isolation of the islands by rising sea levels at the end of the last ice age. Thus, this study provides the first comprehensive phylogenetic evidence for the rapid evolution of dwarfism in a 'giant' snake on islands and highlights the utility of such analyses in elucidating adaptive changes in island biota.

Introduction

Phylogenetic studies can be used not only to identify distinct evolutionary lineages, but also to underpin the processes responsible for adaptation and evolution of biodiversity. Genetic diversity can be partitioned into two components: that arising from adaptation to localised selection pressures, which may be rapid, and that caused by long-term historical isolation (Moritz 2002). Thus intraspecific variability and divergence can evolve in different ways and over different timescales. Understanding how these two components interact to produce the observed diversity within a species is of interest to evolutionary biologists, but also to those wishing to identify and set conservation priorities (Crandall et al. 2000; Moritz 2002).

The evolution of gigantism and dwarfism in snakes is a case in point and is epitomised by the highly-variable and wide-ranging species *Boa constrictor*. It has one of the largest latitudinal distributions of any snake species in the world, ranging from Mexico to Argentina (approximately 30^{o}N - 35^{o}S; Henderson et al. 1995). Across its distribution, the species displays extensive variation in size, colour, scalation, behaviour and ecology, especially where it occurs on islands. This variation appears to be the result of both localised selection pressures and long-term historical isolation of geographically disparate populations (Boback 2006; Boback & Carpenter 2007; Russo 2007; Hynkova et al. 2009).

Despite the popularity of *Boa constrictor* in zoological exhibits and private collections, this species remains taxonomically poorly defined. Although previously divided into eight or nine subspecies based on morphology (Peters & Orejas-Miranda 1986; Russo 2007; Reed & Rodda 2009), recent phylogenetic analysis of predominantly captive animals identified the presence of just two distinct clades; one

covering Central America and neighbouring north-western South America west of the Andes, and the other covering the rest of South America. Divergence of the clades can most likely be attributed to increased uplift of the Colombian Andes and the final closure of the Panamanian isthmus during the late Pliocene (Hynkova et al. 2009).

Despite apparent low genetic diversity within the northern clade (Hynkova et al. 2009), a remarkable level of morphological and behavioural diversity can be observed across the Central American range of the species, especially on islands. Numerous insular populations of *B. c. imperator* have undergone changes in colouration, behaviour, ecology and, perhaps most notably, dramatic reductions in body size (Porras 1999; Boback 2005; Russo 2007; Reed & Rodda 2009). Many of these islands have only been isolated within the last few thousand years and thus suggest exceptionally rapid localised adaptation. However, the phylogenetic relationship of these island boas to mainland populations is unclear.

In a review of body size in island snakes, it was found that physiographic variables such as island area and age, distance to mainland and latitude were not useful in determining body size (Boback 2003). Instead, changes in body size of island snakes have been shown to be consistent with a change in prey size, with those snakes encountering smaller prey decreasing in size and snakes encountering larger prey increasing in size (Boback 2003; Keogh et al. 2005). However, in order to better understand the evolutionary shifts in body size on islands, it is essential to conduct phylogenetic and phylogeographical analyses to reconstruct colonisation histories and identify source populations (Gould & MacFadden 2004; Keogh et al. 2005; Lomolino et al. 2006).

The Islas de la Bahía, Honduras, support both dwarfed and larger mainland-like populations of *B. c. imperator* (Figure 3-1) and thus provide an ideal model system for studying the evolution of insular dwarfism in large snakes. In this study we reconstruct the phylogenetic histories of *B. c. imperator* populations in these islands and across mainland Central America, and use Bayesian dating methods to estimate times of divergence for the island populations. In doing so, we reveal how large size and dwarfism has arisen in *B. c. imperator* both through long-term historical isolation as well as through more rapid adaptation in response to localised selection pressures on islands.

Methodology

Study site

The Islas de la Bahía are situated off the Caribbean coast of Honduras and are made up of two archipelagos. The Cayos Cochinos archipelago lies approximately 17 km off the Caribbean coast of Honduras (Wilson & Cruz Diaz 1993). The two largest islands, Cayo Cochino Grande (1.55 km^2) and Cayo Cochino Pequeño (0.64 km^2), support dwarfed populations of *B. c.* imperator, with adult boas averaging around 1 m in snout-vent length (Reed et al. 2007). To the north of these islands lie the much larger Bay Islands of Utila, Roatan and Guanaja (Figure 3-1). These islands also support populations of *B. constrictor*, however, they are analogous in size and colouration to the mainland boas (McCranie et al. 2005).

Collection and storage of tissue samples

Ventral scale clips were obtained from live wild-caught boas, and tail tips from dead road-killed boas from all the major islands in the Islas de la Bahía and from a number of locations across the Central American range of *B. c. imperator*, as outlined in

Table 3-1 (see also Appendix 2 for full details of samples collected). Tissue samples were stored in >70% ethanol in screw-top eppendorf tubes. Several samples were collected from a reptile rescue centre in Honduras and were assumed to be representative of Honduran mainland boas. One of these boas, however, was suspected of being of Bay Island origin based on its phenotype.

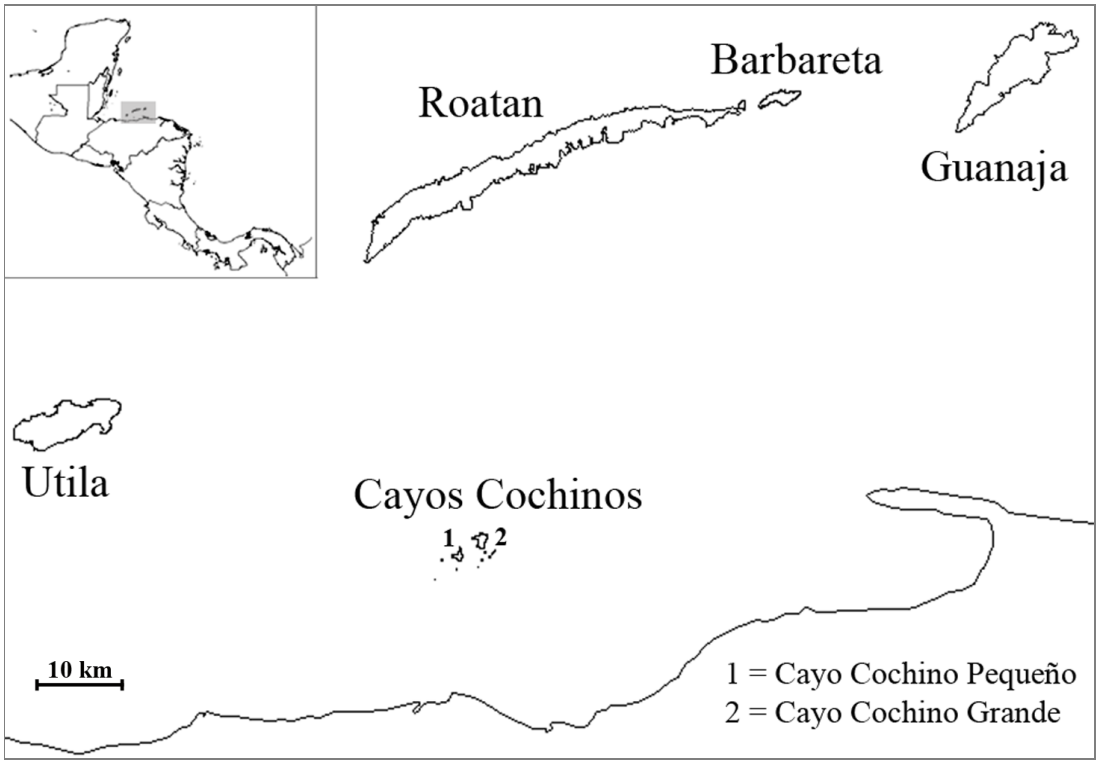

Figure 3-1 Map of the Bay Islands and Cayos Cochinos archipelagos, which together make up the Honduran department of Islas de la Bahía, situated off the Caribbean coast of Honduras.

Table 3-1 Haplotypes used for phylogenetic reconstruction after grouping identical sequences from the same sampling location (the number of sequences represented by each haplotype is displayed in column two). *Tissue samples obtained from captive animals believed to have originated from the Honduran mainland. **Tissue sample obtained from captive animal with Bay Island phenotype. †mislabelled as *Boa constrictor imperator* on Genbank, treated here as *Boa constrictor constrictor*.

Haplotype	No. Sequences represented	Region	Locality	Genbank Accession CytB	Genbank Accession ND4+tRNAs
Honduras (a)	5	Honduras	Tegucigalpa*	GQ477927	GQ477972
Honduras (b)	1	Honduras	Tegucigalpa*	GQ477924	GQ477969
Honduras (c)	1	Honduras	Tegucigalpa*	GQ477925	GQ477970
Honduras (d)	1	Presumed Bay Islands	Tegucigalpa**	GQ477926	GQ477971
Honduras (e)	1	Honduras	Tegucigalpa*	GQ477929	GQ477974
Honduras (f)	1	Honduras	Cordillera de la Botija,	GQ477917	GQ477962
Honduras (g)	1	Honduras	Cerro Guanacaure	GQ477918	GQ477963
Utila (a)	5	Bay Islands	Utila	GQ477938	GQ477978
Utila (b)	1	Bay Islands	Utila	GQ477939	GQ477979
Utila (c)	1	Bay Islands	Utila	GQ477940	GQ477980
Guanaja	1	Bay Islands	Guanaja	GQ477933	GQ477989
Roatan	3	Bay Islands	Roatan	GQ477934	GQ477985
Barbareta	1	Bay Islands	Barbareta	GQ477937	GQ477988
C. Pequeño (a)	3	Cayos Cochinos	Cayo Cochino Pequeño	GQ477945	GQ477990
C. Pequeño (b)	1	Cayos Cochinos	Cayo Cochino Pequeño	GQ477946	GQ477991
C. Pequeño (c)	1	Cayos Cochinos	Cayo Cochino Pequeño	GQ477948	GQ477993
C. Pequeño (d)	1	Cayos Cochinos	Cayo Cochino Pequeño	GQ477949	GQ477994
C. Grande	6	Cayos Cochinos	Cayo Cochino Grande	GQ477951	GQ477996
Guatemala	1	Guatemala	Unknown	GQ477912	GQ477957
Mexico (a)	1	Mexico	nr. Merida, Yucatan	GQ477913	GQ477958
Mexico (b)	1	Mexico	nr. Merida, Yucatan	GQ477914	GQ477959
Costa Rica(a)	1	Costa Rica	Limon Province	GQ477915	GQ477960
Costa Rica (b)	1	Costa Rica	Puntarenas Province	GQ477916	GQ477961
Panama (a)	5	Panama	Coclé Province	GQ477919	GQ477964
Panama (b)	1	Panama	Barro Colorado Island	AB177354	AB177354
B. c. constrictor†	1	Unknown	Unknown	AM 23634	AM 23634
Eunectes notaeus	1	South America	Unknown	AM236347	AM236347

DNA extraction and PCR amplification

DNA extraction was performed using DNeasy Blood and Tissue Kits (Qiagen Ltd) following the manufacturers protocol. We amplified partial regions of the mitochondrial genes *cytochrome-b* (*CytB*) and *ND4* plus three associated tRNA genes ($tRNA^{His}$, $tRNA^{Ser}$, $tRNA^{Leu}$) using the following set of primers: *CytB* - L14919 and H16064 (Burbrink et al. 2000); *ND4* – ND4 and Leu (Forstner et al. 1995). PCRs were carried out in 25 µl reaction volumes using 1 µl of template DNA, with 2.5 µl of forward and reverse primer (5 pmol/µl), 12.625 µl DNA grade water, 1.25 µl $MgCl_2$ (50mM), 2.5 µl 10xNH_4 reaction buffer (Bioline), 2.5 µl dNTPs (2mM) and 0.125 µl Taq (Bioline 5u/µl). PCRs were conducted under the following reaction conditions: 95 ^{o}C for 4min followed by 30 cycles of 94 ^{o}C for 30s, primer specific annealing temperature (*CytB* 46 ^{o}C, *ND4* 56 ^{o}C) for 45s and 72 ^{o}C for 45sec/1min (*CytB*/*ND4*), with a final elongation step of 10min at 72 ^{o}C.

Sequencing and alignment

PCR products were purified by either using the Geneclean Turbo kit (MP Biomedicals) or by Macrogen South Korea prior to sequencing. Sequencing was performed using Macrogen South Korea or Eurofins MWG Operon in Germany under BigDyeTM terminator cycling conditions on an ABI 3730xl sequencer (Applied Biosystems). Sequences were viewed, aligned and edited in Bioedit version 7.0.9.1 (Hall 1999). *ND4*+tRNAs and *CytB* were concatenated to form one contiguous sequence for each individual. Sequence data was also obtained from Genbank for two *B. constrictor* specimens (Accession: AB177354 and AM236348), and one *Eunectes notaeus* specimen (Accession: AM236348) for use as an outgroup. Sequences displaying identical haplotypes from snakes collected at the same location

were grouped together and treated as a single representative sequence for subsequent phylogenetic analysis, however, snakes with identical haplotypes from different collection locations were included separately (Table 3-1).

Phylogenetic reconstruction

Bayesian phylogenetic reconstruction (BI) was conducted in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003; Huelsenbeck & Ronquist 2005). Parallel MCMCs were run for ten million generations, sampling every 100th. The first seventy five thousand trees were discarded and the remaining trees used to construct a 50% majority rule consensus tree. Convergence was gauged by comparing the split frequencies between parallel runs and by examination of the log likelihood values.

Analyses were run under a number of evolutionary models, including HKY and GTR models. All models were run under a number of options for rate variation, including gamma-distributed and proportion of invariable sites. Calculation and comparison of the Bayes' factors for each pair of models (Kass & Raftery 1995; Nylander et al. 2004) showed there to be no greater significant support for any one model. Tree topologies were largely identical, however, the GTR model with gamma distribution was chosen due to its slightly higher overall log likelihood and improved resolution of the tree. A three-partitioned analysis of the model, to allow for independent rate variation at each gene (*ND4*, tRNAs, *CytB*), resulted in no significant increase in the fit of the model, however, the partitioned model was chosen due to its more realistic assumptions and the fact that the trees were identical.

To compare tree topologies, phylogenetic reconstruction was also conducted by applying Maximum Likelihood (ML) methods, using the online execution of PhyML

3.0 (Guindon & Gascuel 2003; Guindon et al. 2005), and Neighbour Joining (NJ) using Paup* 4.0 (Swofford 2003).

Divergence time estimation

In order to acquire a fully resolved tree for calculating divergence time estimates, the best Bayesian tree topology was taken and terminal node tips arbitrarily resolved using the topology of the non-bootstrapped NJ tree. Bayesian dating analyses were performed using *MULTIDISTRIBUTE* (v. 09.25.03 Thorne et al. 1998; Thorne & Kishino 2002). The Markov chain was sampled every 100 generations for a total of $1x10^4$ samples after the initial burn-in of $1x10^4$ generations. The priors used were: rttm = 3.0, rttmsd = 1.0, rtrate = 0.02, rtratesd = 0.02. The remaining priors were set at the multidivtime program's default. The analysis was run twice to check for convergence of the posterior probabilities.

In the absence of suitable fossil records for snakes, no internal node constraints were applied to the dating analysis. A soft boundary for the overall length of the tree, as is required by the methodology, was set at 3 million years, however, a wide boundary of 0-6 million years was applied to take into account the uncertainty in the date of cladogenesis of *B. constrictor* on either side of the Andes.

Results

Sequencing results

Approximately 1,098bp of *CytB*, plus 683bp of *ND4* and 183bp of the associated tRNAs were sequenced in forty five Central American *B. constrictor*, and sequence data for the corresponding gene regions for two *B. constrictor* and one *E. notaeus* specimen was obtained from Genbank. Grouping sequences displaying identical

haplotypes from the same sampling location resulted in twenty-six ingroup taxa and one nominated outgroup for phylogenetic analysis (Table 3-1). An insertion of an adenine residue in the tRNAs was observed in a number of sequences. This insertion occurred in the string of adenine bases prior to the codon binding site of the $tRNA^{His}$.

Phylogenetic analysis

All three methods; BI, ML and NJ produced near identical tree topologies (see Appendix 3 for ML and NJ trees). In all trees, Genbank sequence AM236348 was placed basal to the other boas and displayed a considerably longer branch length compared with all other ingroup taxa. Furthermore, the uncorrected pair-wise genetic distance of this sequence was more than three times greater than the maximum distance values observed between any of the other boas in this study (Appendix 4). The large sequence divergence of this sample (0.07%) was equivalent to that expected for *B. c. constrictor* from the South American clade described by Hynkova et al. (2009). Thus, it was concluded that this sample had been mislabelled on Genbank and that it in fact represents a South American lineage of *B. c. constrictor* (but see Appendix 4 for a full account of this result). Sequence AM236348 is, therefore, relabelled in all trees as *B. c. constrictor*.

Bayesian phylogenetic reconstruction reveals the boas sampled in this study can be grouped into two main clades (Figure 3-2). Clade *A* consists of boas from Mexico, Guatemala, the Pacific coast of Costa Rica and the Caribbean coast of Panama. Clade *B* can be subdivided into three groups; the first consisting of two Honduran haplotypes (Clade *B1*), the second comprising of populations from central Panama, the Caribbean coast of Costa Rica, and Honduras (Clade *B2*); and the third, representing the boas from the Cayos Cochinos and Bay Islands, including the

Honduras (d) haplotype suspected to have originated from a boa of Bay Island provenance based on its phenotype (Clade *B3*). Interestingly, all sequences within the island clade (*B*3) contained the insertion observed within the $tRNA^{His}$, which was also found to be absent from all other boas in this study. This insertion, therefore, appears to be a distinct genetic feature of the boas from the Cayos Cochinos and Bay Islands. However, the Utila (c) haplotype was found to be distantly related to all other boas in this island clade and instead was placed within clade *A*, grouping more closely with the Mexican boas.

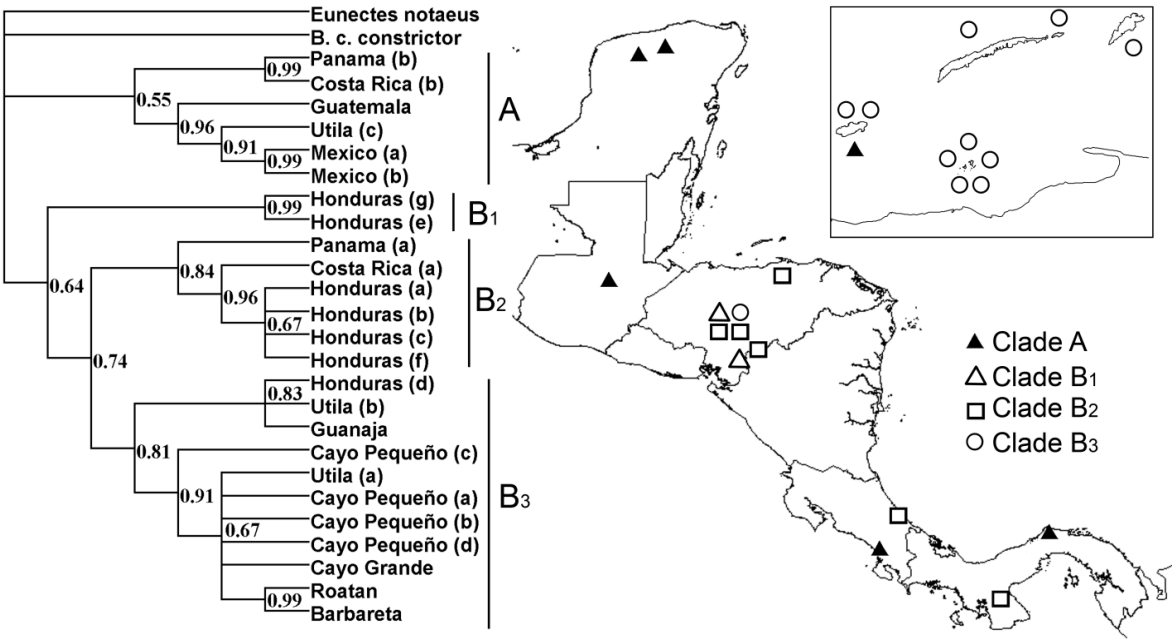

Figure 3-2 Bayesian phylogeny for Central American *Boa constrictor* constructed using concatenated mitochondrial *cytochrome-b* and *ND4* plus associated tRNA genes. The tree is divided into two clades and the corresponding sampling locations displayed on the adjacent map. The inset shows an enlarged view of the Islas de la Bahía. The sampling location for haplotype Honduras (d), belonging to clade B3, is shown here as the Honduran mainland, however, this sample was obtained from a captive animal and was actually suspected of being of Bay Island origin based on it phenotype. Node support values are Bayesian posterior probability values.

Haplotypes in this study represent concatenated sequences from more than one gene and thus cannot be directly compared to haplotypes in recent phylogenetic analysis of *B. constrictor* (Hynkova et al. 2009) which were based only on *cytochrome-b* sequence data. However, a Bayesian phylogenetic reconstruction of the *cytochrome-b* gene, using samples from this study and a number of samples from Hynkova et al. (2009) is provided in Appendix 4 to permit a direct comparison between the two studies.

Divergence time estimation

Estimated dates of internal node divergence are displayed in the chronogram (Figure 3-3) set against significant geological events (see Appendix 5 for individual node values and 95% confidence intervals). Overall length of the tree was estimated at 3.87Mya (2.15-6.16Mya). The Cayos Cochinos and Bay Island clade is estimated to have diverged from mainland boas at approximately 1.98Mya (0.67-3.92Mya). This date corresponds roughly to the onset of global climatic fluctuations in the Pleistocene (Hewitt 2000) and the time of isolation of Roatan and Guanaja from mainland Honduras (Williams & McBirney 1969). This date is also inferred to represent the time at which the $tRNA^{His}$ insertion, observed in all boas within this group, is most likely to have appeared.

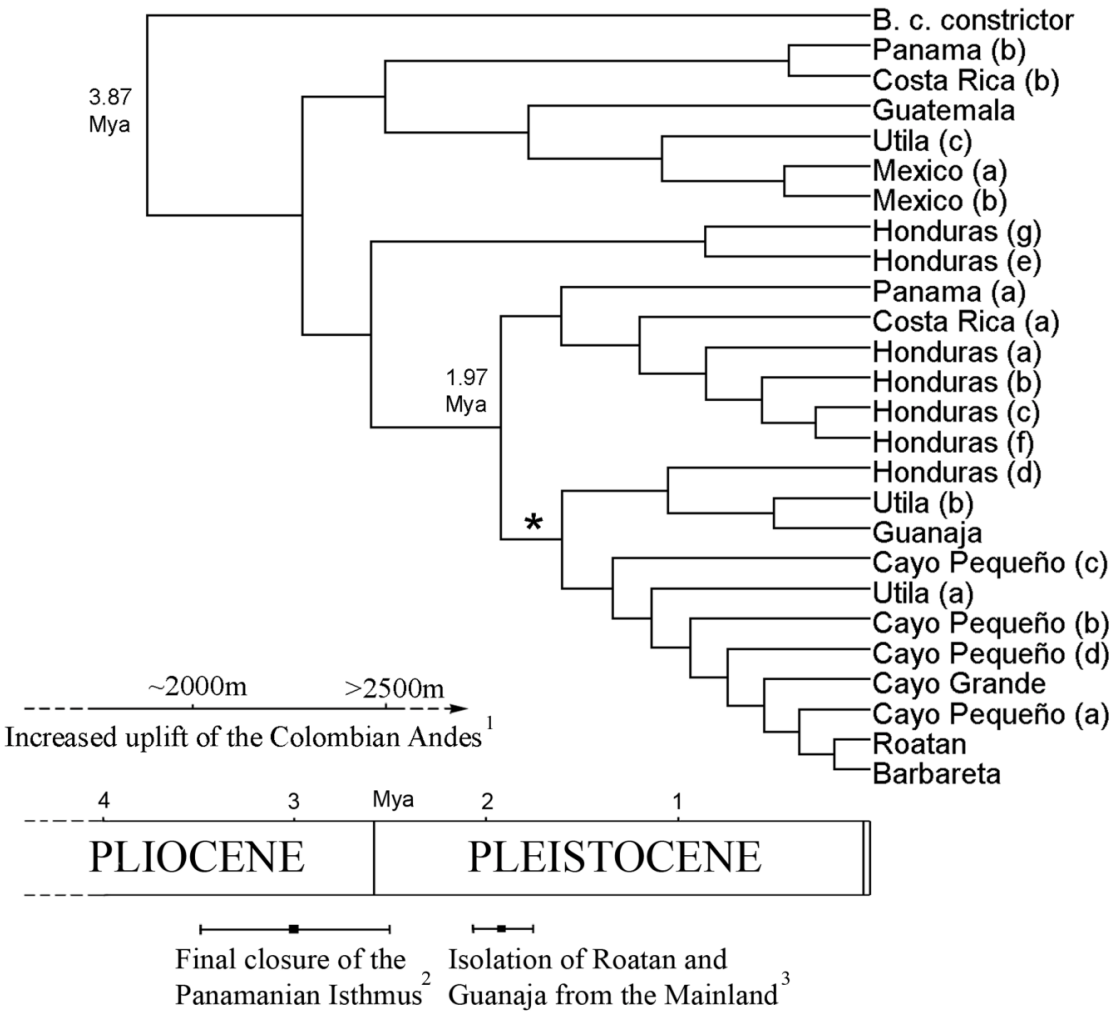

Figure 3-3 Fully resolved tree topology dated using *MULTIDIVTIME*. The tree displays the initial divergence of Central and South American lineages of *Boa constrictor*, and the subsequent divergence of the Central American clade, set against significant geological events. Mya = million years ago, *the presumed appearance of the insertion in the $tRNA^{His}$ observed in the island clade. [1](Gregory-Wodzicki 2000; Hooghiemstra et al. 2006), [2] (Collins et al. 1996; Coates 1997), [3](Williams & McBirney 1969).

Discussion

The data show that populations of *B. constrictor* in the Cayos Cochinos and Bay Islands represent a radiation event in the early Pleistocene but that dwarfism has probably evolved rapidly and much more recently in the Cayos Cochinos. This finding highlights the importance of a phylogenetic approach when interpreting

observed patterns of diversity on islands and also the rapid rate at which changes in body size can evolve even in large snakes.

Dating analysis

B. constrictor inhabits tropical lowland environments and is restricted to a maximum elevation range of approximately 1500 m due to thermoregulatory constraints (Henderson et al. 1995). Uplift of the Colombian Andes to greater than the current maximum elevation range of the species in the mid to late Pliocene (Gregory-Wodzicki 2000; Hooghiemstra et al. 2006) would have severely reduced gene flow between *cis*- and *trans*-Andean populations of *B. constrictor* (Hynkova et al. 2009). Uplift of the Colombian Andes has been implicated in the divergence and speciation of a number of other tropical lowland species (e.g. Zamudio & Greene 1997; Cortés-Ortiz et al. 2003; Venegas-Anaya et al. 2008) and thus represents a likely candidate for cladogenesis in *B. constrictor*. Also, the final completion of the Panamanian Isthmus at this time (Collins et al. 1996; Coates 1997) would have facilitated passage of *B. constrictor* into Central America and into its current range by eliminating the need to cross open expanses of water.

Bayesian divergence time estimation, as implemented in this study, requires a prior overall length of the tree to be defined by the user. Thus a soft bound of 3Mya was set, corresponding to the period of increased uplift of the Colombian Andes and the time at which divergence between *B. constrictor* on either side of this formidable geological barrier would most likely have occurred. Also, the overall level of sequence divergence (5-7%) observed between *cis*- and *trans*-Andean clades of *B. constrictor* in this study and Hynkova et al. (2009) corresponds to an approximate divergence date in the region of 2.5-3.5Mya, based on a conservative substitution

rate of 2% per million years, originally estimated for mammalian mtDNA (Brown et al. 1979). However, to take into account the uncertainty in the date of cladogenesis, the length of the tree was given a large degree of flexibility, allowing for overall length to vary between 0-6Mya.

Although, some studies on the rate of mtDNA evolution in snakes have suggested a much higher rate of evolution than that observed in other vertebrates (Kumazawa et al. 1998; Dong and Kumazawa 2005), Jiang et al. (2007) showed that despite deeper lineages in snake evolution appearing to display dramatically elevated evolutionary rates, terminal ones appear to have patterns of mitochondrial genome evolution similar to other (non-snake) vertebrates. For example, Castoe et al. (2007) estimated the rate of evolution of *ND4* in the *Crotalus atrox* to be 1.4% per million years.

Other species of neotropical snake have been shown to have crossed between Central and South America after the final uplift of the Colombian Andes (Wüster et al. 2005) and, thus, a younger date of cladogenesis between boas on either side of the Andes cannot necessarily be ruled out. However, it should be noted that divergence time estimation for the root of the tree was always older, rather than younger than the prior mean set in the analysis, suggesting a younger date for the root of the tree is not more likely. Also, although dispersal between Central and South America prior to the completion of the Panamanian isthmus has been shown for other species of snake (e.g. Zamudio & Greene 1997; Wüster et al. 2002), it is not considered a likely scenario for *B. constrictor* because of the relatively low level of sequence divergence observed within the Central American clade in both this study and Hynkova et al. (2009).

Interestingly, within the Central American boas, one of the highest levels of sequence divergence observed was between the two boas from Costa Rica, which were obtained from either side of the Cordillera del Talamanca. This mountain range has been shown to separate other distinct lineages of lowland species (Zamudio & Greene 1997). Therefore, this result appears to be further evidence of the ability of high elevations to restrict gene flow within *B. constrictor* and contribute to the genetic differentiation between populations of this species.

Cayos Cochinos and Bay Island radiation

The divergence of the Bay Island and Cayos Cochinos clade was dated at approximately 1.98Mya coinciding with the isolation of Roatan and Guanaja from mainland Honduras (Williams & McBirney 1969). Roatan and Guanaja are oceanic islands, situated beyond the continental shelf, and are surrounded by waters reaching depths of up to 275 m (McCranie et al. 2005). It is unlikely that the drop in sea level experienced during Pleistocene glacial maxima resulted in Roatan and Guanaja reforming a connection with the mainland, or each other, since their initial isolation. Conversely, Utila and the Cayos Cochinos, which lie on the continental shelf and are surrounded by relatively shallow water (~50 m), would have been repeatedly isolated and reconnected to the mainland by fluctuating sea levels during the Pleistocene. As a result, Utila and the Cayos Cochinos would have last been connected to the mainland as little as 13,000 years ago at the end of the Wisconsin glacial period (Bermingham et al. 1998; McCranie et al. 2005).

It is interesting, therefore, that the boas of Utila and Cayos Cochinos do not show a closer relationship to the mainland boas, but instead appear to have diverged at the same time as Roatan and Guanaja. It is possible that these islands were unfavourable

for *B. constrictor* when last connected to the mainland and that the boas present on the islands today colonised from Roatan and Guanaja since their most recent isolation from the mainland. This hypothesis is supported by the occurrence of the insertion in the $tRNA^{His}$ found to be present in all but one boa sampled from the Cayos Cochinos and Bay Islands (23/24), and absent from all mainland boas, with the exception of haplotype Honduras (d) which had putatively been suspected of being of Bay Island origin based on its phenotype.

Haplotype Utila (c) (represented by a single sample) was found not to contain the insertion and showed close affiliation to the Mexican boas. The placement of this haplotype in the tree suggests recent gene flow between the mainland and Utila. It is likely, therefore, that Utila has also been colonised from the mainland as well as from the other islands since its most recent isolation. Haplotype Utila (c) may be evidence of the more recent (accidental or deliberate) anthropogenic movement of snakes between the mainland and the islands on boats. The likelihood of colonisation from the mainland would have increased in line with increased boat traffic to the islands since the 15^{th} century. An alternative explanation for the presence of haplotype Utila (c) is that a population was already established on Utila prior to its isolation and the island was then also subsequently colonised from the other Bay Islands. However, the former interpretation seems more plausible due to the insertion being represented in six out of the seven boas sampled from Utila. If an established population had already been present on Utila prior to its isolation and subsequent colonisation from the other islands, then a proportionately lower representation of the insertion in the population would be expected.

Thus, the high prevalence of the $tRNA^{His}$ insertion in the Cayos Cochinos and Bay Island clade is interpreted as strong evidence for a single radiation event early in the Pleistocene. It is proposed that boas originally diverged on Roatan and Guanaja after becoming isolated from the mainland by rising sea levels at the start of the Pleistocene. Utila and the Cayos Cochinos would have then been subsequently colonised from Roatan and/or Guanaja since their most recent isolation at the end of the last ice age. Utila appears to have also been colonised from the mainland, as is indicated by the presence of haplotype Utila (c), which may be evidence of more recent anthropogenic movement of snakes on boats.

No evidence was found to suggest that the Cayos Cochinos had also recently been colonised from the mainland, but, gene flow from the island clade back to the mainland cannot be ruled out due to the ambiguous provenance of haplotype Honduras (d). However, Bayesian phylogenetic reconstruction of the *cytochrome-b* gene, where a number of additional haplotypes sourced from Hynkova et al. (2009) were also included, failed to provide further evidence of island-clade haplotypes on the mainland (Appendix 4). Nevertheless, it must be noted that more extensive sampling along the Caribbean coastal region of Honduras may be necessary to determine fully the level of gene flow between the islands and the mainland.

Evolution of dwarfism

The close genetic affinity of the island populations is suggestive of recent gene flow between them, possibly facilitated by fluctuating sea levels during Pleistocene glacial cycles. Therefore, the evolution of dwarfism and colour variation in the Cayos Cochinos must have taken place rapidly, within the last 13,000 years, since the islands were last isolated from the mainland. Thus the marked phenotypic differences

observed in the Cayos Cochinos must represent rapid evolutionary divergence rather than being the product of long-term genetic isolation of the populations. Investigation into the patterns of body size evolution in snakes on islands has shown that an optimal body size of approximately 1 m exists for snakes, with small species generally becoming larger and large species becoming smaller on islands (Boback and Guyer 2003). The frequency with which dwarfism is observed in insular populations of *B. constrictor* and the absence of insular populations where body size has increased (Porras 1999; Boback 2003; Russo 2007) provides strong evidence against the idea that body size changes in this species on islands are simply due to the random effects of genetic drift. If genetic drift were the principle evolutionary force driving body size change in insular populations of *B. constrictor* then we should expected to see insular populations where gigantism has evolved in addition to populations where dwarfism has evolved, however, this is not the case.

In a review of body size of island snakes, change in body size was consistent with a change in prey size, with those snakes encountering smaller prey decreasing in size and snakes encountering larger prey showing an increase in size (Boback 2003). These results are also consistent with those of Australian tiger snakes (Keogh et al. 2005). Few large mammalian prey items are present in the Cayos Cochinos compared with the other Bay Islands (Bermingham et al. 1998; Reed et al. 2007) and boas in the Cayos Cochinos appear to be heavily dependent on lizard and avian prey (Reed et al. 2006; Reed et al. 2007). It is likely, therefore, that a disparity in prey type and abundance in the Cayos Cochinos compared with the other Bay Islands and adjacent mainland is responsible for the observed reduction in body size.

Although rapid changes in body size have also been observed in other insular populations of snakes (e.g. Keogh et al. 2005), the scale of dwarfism displayed by *B. constrictor* in the Cayos Cochinos is extreme in comparison. Adult *B. constrictor* on these islands are on average >50% smaller (SVL) than the most conservative reported average range of adult body size on the mainland (Savage 2002). Therefore, this study provides the first comprehensive phylogenetic evidence for the rapid evolution of extreme dwarfism in such a large bodied vertebrate.

Evolution of colour change

Changes in colouration exhibited by boas in the Cayos Cochinos may simply be explained by founder effects followed by subsequent genetic drift causing lighter phenotypes to become fixed in the populations. However, colouration may also conceivably be the result of natural selection. Reptile colouration has long been studied as an example of adaptive evolution, often being associated with changes in substrate colour or the thermal environment, but very little still is known about the molecular basis of colour evolution in this group (Rosenblum et al. 2004). Although some of the intraspecific colour variation in reptiles appears to have a genetic basis (e.g. Rosenblum 2004), the exact mechanisms by which many colour variations are produced, especially within snakes, remain unclear (Boback, 2010).

The lack of mammalian prey items in the Cayos Cochinos compared with the Bay Islands and mainland (Bermingham et al. 1998; Reed et al. 2007) has resulted in a shift in the diet of *B. constrictor* in the Cayos Cochinos to include a greater proportion of diurnal prey items such as birds and lizards. This dietary shift appears to have resulted in a corresponding shift in behaviour from nocturnal to diurnal foraging activity in the Cayos Cochinos. It may be that the hypomelanistic phenotype

of the Cayos Cochinos boa aids in thermoregulation and/or crypsis during diurnal foraging and is thus a selective advantage. Interestingly, light colouration has also evolved in a number of insular dwarfed populations of *B. constrictor* on islands off the coast of Belize, where snakes are heavily dependent on diurnally active prey items (Boback 2005; Boback 2006). A recent investigation into the colouration of mainland and island *B. constrictor* in Belize suggests that the light colouration of island boas is more likely to be an adaptation for increased crypsis rather than for thermoregulatory benefits (Boback & Siefferman 2010). The apparent rapid evolutionary shifts in colouration observed in island populations of *B. constrictor* is an area of particular interest for future study.

Cayos Cochinos and Bay Island boas form an evolutionary significant unit (ESU)

Despite the Bay Island boas being phenotypically more similar to the mainland boas in size and colouration, their comparable level of genetic divergence and time since isolation with the Cayos Cochinos may warrant a greater level of protection for these boas. Under the proposed criteria for recognising evolutionary significant units (ESUs), the reciprocal monophyly of the island clade would qualify all of these islands for protection at this level (Moritz 2002). Also, if the northern clade of *B. constrictor* is to be recognised as a distinct species, as proposed by Hynkova et al. (2009), the evolutionary significance of the Cayos Cochinos and Bay Island clade should be given even greater attention.

Alternative approaches to conservation prioritisation, such as the EDGE (Evolutionary Distinct and Globally Endangered) approach, would likely consider the Cayos Cochinos and Bay Island clade to represent a relatively low conservation priority. However, although such approaches provide an extremely valuable means

by which to identify those species of highest conservation priority for maintaining the greatest breadth of biological diversity on Earth, they do not replace the need to manage lower levels of genetic diversity responsibly. It is essential that conservation management plans look to conserve the evolutionary processes by which future biological diversity is being created as well as protecting those highly unique species that are present today.

Conclusions

The findings of this study suggest that: (i) the Islas de la Bahía represent a monophyletic clade that diverged from the mainland at the start of the Pleistocene and may qualify for recognition as an ESU, but Utila appears to have also been subsequently colonised from the mainland; and (ii) dwarfism appears to have evolved rapidly in the Cayos Cochinos only relatively recently, possibly in response to the reduced availability of large prey items on the islands. Thus, populations of *B. constrictor* in the Islas de la Bahía have been shaped both by long-term isolation from the mainland and more recent, rapid evolutionary responses to selection pressures in the Cayos Cochinos. This study provides the first comprehensive phylogenetic evidence for rapid evolution of extreme dwarfism in a large vertebrate on islands and highlights the utility of such analyses in elucidating acute evolutionary changes in body size in island biota.

Chapter 4 Population structure and gene flow of *Boa constrictor imperator* in the Cayos Cochinos and Bay Islands, Honduras

Abstract

Recent phylogenetic analysis has identified insular populations of *Boa constrictor imperator* in the Cayos Cochinos and Bay Island archipelagos, Honduras as a probable Evolutionary Significant Unit (ESU). These populations have likely been on a separate evolutionary trajectory to mainland populations for approximately 2 million years. However, dwarfed phenotypes displayed in the Cayos Cochinos populations are suspected to have evolved rapidly since the islands were last isolated from the mainland by rising sea levels at the end of the last ice age, approximately 10,000 years ago. This study investigates the level of population structure and gene flow between the islands and mainland and specifically between the dwarfed phenotypes of the Cayos Cochinos and the larger phenotypes of the Bay Islands and continental mainland. It is concluded that the dwarfed boas of the Cayos Cochinos were isolated approximately 5,000 years ago and that dwarfism has evolved in the absence of substantial gene flow from the surrounding populations since this time. Thus, dwarfism is likely to be an adaptive response to local environmental conditions in the Cayos Cochinos, rather than as a result of phenotypic plasticity for body size within the island system. In contrast to previous studies of gene flow in mainland populations of *B. constrictor*, gene flow appears to be low over relatively short distances, highlighting the substantial barrier to gene flow presented by the relatively small expanses of water between the islands.

Introduction

The species *Boa constrictor* (hereafter also referred to as boa) is a large bodied constricting snake that displays extensive variation in size, colour, scalation, behaviour and ecology, especially where it occurs on islands (Boback 2006; Boback & Carpenter 2007; Reed et al. 2007; Russo 2007). Recent phylogenetic analysis of the species concluded that boas from Central America and South America, east of the Andes, are sufficiently genetically divergent from all other South American boas to be considered a separate species (Hynkova et al. 2009). Thus, insular populations of boas from Central America may be of higher conservation 'value' than previously acknowledged. The unique phenotypes shown by some of these island populations have resulted in their exploitation for the pet trade (Porras 1999; Reed et al. 2007; Russo 2007). However, the phylogenetic relationships of these island boas to mainland populations have, until very recently, remained unknown (but see Chapter 3).

The two largest islands within the Cayos Cochinos archipelago, Honduras (Figure 4-1), support populations of a dwarfed race of insular boa (*Boa constrictor imperator*), known commercially as the 'Hog Island' boa. Prized by reptile collectors for its small size and unique 'pink' colouration, the population was rapidly decimated by over-collection during the 1980s (Wilson & Cruz Diaz 1993; Porras 1999; Reed et al. 2007). Fortunately, due to increased protection, the population is showing signs of recovery (Reed et al. 2007). Populations of *B. constrictor* are also present on all of the nearby Bay Islands (Figure 4-1), however, the Bay Island boas are phenotypically much more similar in size and colouration to the mainland boas.

Phylogenetic analysis of the Cayos Cochinos and Bay Island boas suggests that they form a monophyletic group that diverged from the mainland boas approximately 2 million years ago and likely represent an Evolutionary Significant Unit (ESU) (see Chapter 3). However, within this group the dwarfed boas of the Cayos Cochinos are polyphyletic with the larger Bay Island phenotypes and levels of population structure and gene flow between the islands are currently unknown. It is thought that the Cayos Cochinos boas colonised the islands from the Bay Islands of Roatan or Guanaja, or both, after their most recent isolation from the mainland by rising sea levels at the end of the Wisconsin glacial period approximately 10,000 years ago (McCranie et al. 2005). A similar scenario is also apparent for the Bay Island of Utila which, like the Cayos Cochinos, lies on the continental shelf and would have also been recently isolated from the mainland (McCranie et al. 2005). It is likely, therefore, that the dwarfed phenotype of the Cayos Cochinos boas has arisen rapidly within the past 10,000 years.

Environmental heterogeneity between the Cayos Cochinos and the Bay Islands (Chapter 2) appears to have selected for small size and light colouration in the Cayos Cochinos, whereas, presumably, common environmental conditions between the Bay Islands and the mainland have resulted in selection for large body size and darker colouration. What is not apparent, however, is whether the dwarfed phenotype of the Cayos Cochinos boa is maintained in the presence or absence of substantial gene flow from the Bay Islands and the mainland where larger phenotypes exist.

Three alternative scenarios for the evolution of body size variation on the islands are that (i) large and dwarf phenotypes have evolved on the islands in response to local environmental conditions, and limited gene flow has then resulted in these

phenotypes becoming fixed in each population, (ii) differences in body size are the result of phenotypic plasticity, i.e. the populations are genetically similar, with high levels of gene flow, but are responding to local conditions in different ways, or (iii) levels of gene flow are high between the islands, but selection pressures for body size are strong enough to maintain disparities in body size between the islands. If the first scenario is true, high levels of population structure and low migration rates should be apparent between the islands. However, if either of the later scenarios is true, low levels of population structure (for evolutionary neutral markers) and high migration rates between islands should be observed. Neutral molecular markers such as microsatellites can be used to assess patterns of population subdivision or structure which reflect the outcome of the diversifying effects of genetic drift and the homogenizing effects of gene flow (King 2009). Although, neutral markers cannot be used directly to assess differences in traits that are likely to be under selection, such as body size and colouration, they can provide useful information on the level of gene flow from populations where different phenotypes appear to be selected for.

In this study, the level of genetic structure and gene flow that exists between *B. c. imperator* in the Cayos Cochinos, Bay Islands and mainland Central America are analysed using eight polymorphic microsatellite loci. In addition, estimates of effective population size and the estimated date of divergence of the Cayos Cochinos population are provided. In doing so, it is revealed that the dwarfed boas of the Cayos Cochinos likely diverged from an ancestral population in the region of 5,000 years ago and have probably experienced limited gene flow from surrounding populations since this time.

Methodology

Study Site

The Islas de la Bahía are situated off the Caribbean coast of Honduras and are made up of two archipelagos. The Cayos Cochinos archipelago lies approximately 17 km off the Caribbean coast of Honduras (Wilson & Cruz Diaz 1993). The two largest islands, Cayo Cochino Grande (CCG) (1.55 km^2) and Cayo Cochino Pequeño (CCP) (0.64 km^2), support dwarfed populations of *B. c.* imperator, with adult boas averaging around 1 m in snout-vent length (Reed et al. 2007). To the north of these islands lie the much larger Bay Islands of Utila, Roatan and Guanaja (Figure 4-1). These islands also support populations of *B. constrictor*, however, they are analogous in size and colouration to mainland Central American *B. constrictor* (McCranie et al. 2005).

Collection of tissue samples

Ventral scale clips were obtained from live wild-caught boas, and tail tips collected from dead road-killed boas from all the major islands in the Islas de la Bahía and from a number of locations across the Central American range of *B. c. imperator*, as outlined in Figure 4-1 (detailed collection location information is provided in Appendix 2).

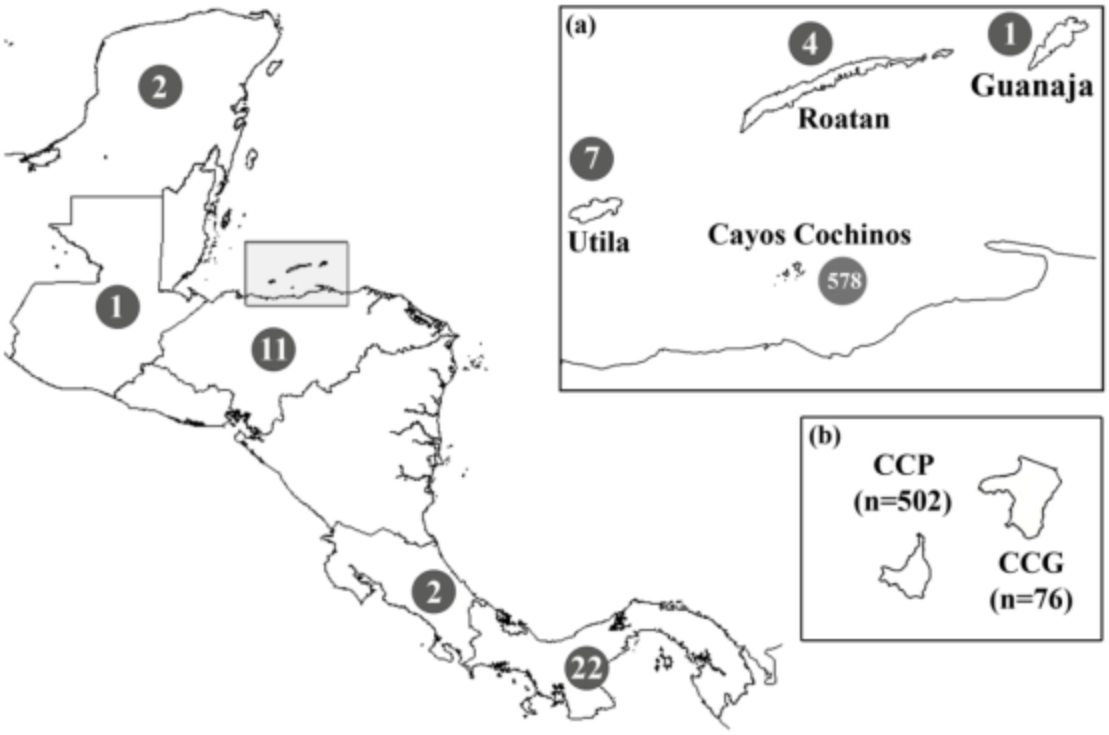

Figure 4-1 Map displaying collection locations across mainland Central America, Bay Islands and the Cayos Cochinos. Inset (a) shows sampling across the Bay Islands and Cayos Cochinos, and inset (b) shows sampling specifically within the Cayos Cochinos (CCP = Cayo Cochino Pequeño, CCG = Cayo Cochino Grande). Numbers within circles represent the number of individuals collected from each location.

DNA extraction and PCR amplification

DNA extraction from scale clips and tail tips was performed by one of two methods; using a DNeasy Blood and Tissue Kit (Qiagen Ltd) following the manufacturers protocol, or following an ammonium acetate precipitation method described by Nicholls et al. (2000). Multiplex PCR amplification was conducted using the Qiagen Multiplex PCR kit (Qiagen Ltd) following the manufacturers protocol. Individuals were genotyped using five species-specific microsatellite markers (Booth et al. 2011) and three markers developed for *Epicrates subflavus* (Tzika 2007; Tzika et al. 2009) which had previously been found to be informative (Appendix 1). Microsatellite markers were split into four multiplex sets based on annealing temperature, expected

product size range and colour of fluorescent modification (Table 4-1). PCR amplification was carried out in 6 µl reaction volumes using 1 µl of DNA template under the following reaction conditions; 95 ^{o}C for 15min followed by 30 cycles of 94 ^{o}C for 30s, multiplex specific annealing temperature for 90s and elongation at 72 ^{o}C for 60s, with a final elongation step of 72 ^{o}C for 30mins.

PCR products were run on an ABI 3100 automated sequencer (Applied Biosystems, Inc.) and allele sizes were scored using Genemapper 3.7 (Applied Biosystems, Inc.) and then confirmed by visual inspection. Between 3-15% of samples were repeated at each locus to assess the likelihood of genotyping error.

Table 4-1 Microsatellite loci multiplex sets and corresponding annealing temperatures for PCR reactions

Multiplex set	Microsatellite Loci	Annealing Temperature (^{o}C)
A	µsat36 and µsat20	51
B	µsat01 and Bci-21	55
C	Bci-14 and Bci-23	60
D	Bci-15 and Bci-18	54

General tests for loci

Genotype data were examined by eye to confirm that markers were not sex-linked in the heterogametic sex (females). The presence of null alleles, large allele dropout and scoring error due to stuttering were tested using the software *Micro-Checker* 2.2.3 (Van Oosterhout et al. 2004). Null alleles result when mutations occur at primer binding sites, causing certain alleles not to amplify (Shaw et al. 1999); large allele dropout is caused by the preferential amplification of shorter alleles during PCR (Wattier et al. 1998); and stuttering is a consequence of slippage during PCR amplification which can cause ‘stutter’ products that differ in size from the true allele

by a multiple of the repeat motif length (Shinde et al. 2003). These stutter products can make it difficult to identify heterozygotes from homozygotes, especially in dinucleotide loci. Null alleles were also tested for in all loci using the software *CERVUS* 3.0 (Kalinowski et al. 2007). Hardy-Weinberg Exact tests were performed using default settings of the web based version of *Genepop* 4.0.10 (Raymond & Rousset 1995; Rousset 2008). The probability test was used to check for deviations from equilibrium, followed by tests for heterozygote deficiency and excess to establish the direction of any violation of Hardy-Weinberg. Linkage disequilibrium was investigated using the default parameters of option 2 of web based version of *Genepop* 4.0.10; populations were tested independently and a Bonferroni correction applied at the P=0.05 significance level.

Population structure

Genetic differentiation between predefined populations was estimated using Weir and Cockerham's (1984) variant of F_{ST}, θ (hereafter referred to as F_{ST}), in FSTAT 2.9.3 (Goudet 1995; Goudet 2001). This method was favoured over the analogue of F_{ST} developed specifically for microsatellites, R_{ST}, because of its improved performance when the number of loci is less than 20 (Gaggiotti et al. 1999). Although this method takes into account differences in sample sizes, populations with samples sizes less than 7 individuals were excluded from the analysis.

The programs *STRUCTURE* 2.3.1 (Pritchard et al. 2000) and *TESS* 2.3 (Chen et al. 2007) were used to test for population structure within and between the sampled populations. The programs implement model-based clustering methods for inferring population structure using genotype data consisting of unlinked markers. A key modeling assumption is that there is linkage equilibrium and Hardy-Weinberg

equilibrium (HWE) within populations. Individuals are clustered into populations in such a way as to best achieve this.

Initial trials suggested that large disparities in sample size negatively affected the programs' ability to cluster individuals from populations with small sample sizes. Therefore, two data sets, of varying samples sizes, were used in these analyses to take this issue into account. A two-population data set consisting of individuals from the Cayos Cochinos only (Cayo Cochino Pequeño and Cayo Cochino Grande, n=76 for each island) was used to look for population structure specifically within the Cayos Cochinos. A second data set consisting of individuals from all populations sampled, but with a reduced number of individuals from the Cayos Cochinos (n=10 for each island), was used to look for general patterns of genetic structure and admixture among the island and mainland populations, taking into account the lower sample size of the other populations. Samples used in these analyses are indicated in Appendix 2. Initial trials were performed to determine that the random selection of samples did not significantly influence the results.

For both programs, analyses were performed using the 'no admixture' and 'admixture' models. The no admixture model assumes that individuals come purely from one population or another and is thus appropriate for studying fully discrete populations, as is likely to be the case on islands. Under the admixture model, individuals can be of mixed ancestry and is thus perhaps more appropriate for studying mainland populations where population boundaries may be harder to define. Hubisz et al. (2009) introduced the ability to make use of sampling locations as prior information to assist clustering with *STRUCTURE* when the signal of structure is

relatively weak. To see how this additional information would influence clustering, analyses were run with and without using prior population information.

TESS implements a Bayesian clustering algorithm similar to *STRUCTURE*, but with the added advantage of being able to incorporate explicit geographical coordinates for all individuals into the analysis. Thus, whereas *STRUCTURE* either assumes all individuals to be equally unrelated or else requires assumed populations to be specified a priori, *TESS* introduces spatial correlation between the sampling locations of individuals and uses this information to aid individual clustering assignments. Spatial information is used under the assumption that individuals sampled near to one another will likely be more closely related than individuals sampled far apart. Although spatial coordinates were available for most of the samples in this study, some samples had only descriptive location information. Where this was the case, the 'generate spatial coordinates' function of the program was used to create approximate coordinate data.

Analyses performed using *STRUCTURE* were run using the correlated allele frequencies model (Falush et al. 2003). A burn-in of 50,000 was applied before sampling the Markov chain for a further 50,000 iterations. The maximum number of clusters (*K*) was set between 1-5 for the Cayos Cochinos analysis and between 1-10 where all populations (n=10) were included. Analyses with *TESS* were run for 50,000 iterations with a burn-in period of 10,000 iterations. The maximum number of clusters (*K*) was set between 2-5 in the Cayos Cochinos analysis and between 2-10 in the analysis of all populations (the program does not allow values of $k<2$). Five independent runs were performed for each value of *K* in both programs. Convergence of the Markov chain was assessed by comparing posterior probabilities of

independent runs for the same value of *K* and by visual inspection of plots of likelihood scores against iteration number produced by the programs. The programs CLUMPP 1.1.2 (Jakobsson & Rosenberg 2007) and DESTRUCT 1.1 (Rosenberg 2004) were then used to summarise results from independent runs for each value of *K* and viewed with the program GhostView (Lang 2007).

The most likely value of *K*, resulting from analysis in *STRUCTURE*, was determined using the method proposed by Evanno et al. (2005) and implemented in the online application *Structure Harvester* 0.3 (Earl 2009). Rather than simply using the log probability of the data for each value of *K*, this method uses an ad hoc statistic, ΔK, based on the second order rate of change in the log probability of data between successive *K* values. The most likely value of *K*, resulting from analysis in *TESS*, was assessed by plotting the mean deviance information criterion (DIC) scores for each value of *K*.

Migration rates, effective population sizes and time since isolation

The simplest estimates of migration rates can be calculated from F_{ST} values using the equation $N_e m = [(1/F_{ST})-1]/4$ (Wright 1969), where $N_e m$ is the effective number of immigrants per generation. Pairwise estimates of F_{ST} calculated in FSTAT 2.9.3 (Goudet 1995; Goudet 2001) were used to calculate this most basic estimate of migration. Assumptions of the model are that subpopulations are in migration-drift equilibrium, of equal and constant size and exchanging migrants symmetrically at rate *m*.

Estimates of migration rates based on less restrictive model assumptions can be obtained using coalescent-based techniques (King 2009). Two different methods for simultaneously estimating migration rates and effective population sizes were

applied to the data using the computer programs *MIGRATE* 3.1.3 (Beerli & Felsenstein 1999; Beerli & Felsenstein 2001) and *IMa* (Hey & Nielsen 2007). The *IMa* method also includes an additional estimate of the time at which two populations split. In these analyses, microsatellite locus Bci-23 was removed due to difficulties associated with scoring the number of tandem repeats in loci with compound repeat motifs of different sizes. A third method for estimating recent migration rates was also applied using the computer program *BayesAss* 1.3 (Wilson & Rannala 2003) in which all eight microsatellite loci were included in the analysis. This method estimates contemporary, as opposed to historical, migration rates and is thus a useful complimentary analysis for studies of migration and gene flow.

Equilibrium migration model

MIGRATE 3.03 (Beerli & Felsenstein 1999; Beerli & Felsenstein 2001) estimates effective population sizes and number of migrants per generation *via* the calculation of (i) θ, which is the product of x (x=4 for nuclear DNA), N_e (the effective population size) and μ (mutation rate), and (ii) M, which is the ratio m/μ where m is the immigration rate and μ is the microsatellite mutation rate. In order to convert parameter estimates into demographic units, the microsatellite mutation rate per generation (μ) must be specified. In the absence of information on microsatellite mutation rate in *B constrictor*, a microsatellite mutation rate of $5x10^{-4}$ per generation was chosen, a value commonly applied to microsatellite loci in demographic models (e.g. Storz & Beaumont 2002; Storz et al. 2002). The number of immigrants per generation (Nm_{ij}) is then calculated as $\theta_i M_{j>i}$ and N_e as $\theta/4\mu$.

As with the population structure analysis, two separate data sets were analysed in an attempt to avoid potential problems with uneven sample sizes between populations.

Although, theoretically, sample size should not affect the estimation of the parameters, in reality, because the program will dedicate a greater proportion of the run time to estimating parameters for populations with large samples sizes (Beerli 2010), highly variable sample sizes can be problematic. The first of these analyses (hereafter referred to as the Cayos Cochinos analysis) used only samples from the Cayos Cochinos populations, for which sample sizes were considerably greater than for any other populations. Sample size for the Cayo Cochino Pequeño population was reduced in this analysis to be equal to Cayo Cochino Grande (n=76 for both populations). This analysis was concerned only with the estimation of effective population sizes and migration rates within the Cayos Cochinos archipelago. In the second analysis (hereafter referred to as the five population analysis), three other populations, for which sample size was sufficient, were also included, these were; Utila (n=7), the Honduran mainland (n=11) and Panama (n=11). In this analysis the sample sizes of the Cayos Cochinos populations were each reduced to twenty individuals (selected randomly from the data set).

Analyses were conducted using the online *MIGRATE* facility provided by the Computational Biology Service Unit (CBSU) at Cornell University (*MIGRATE*@BIOHPC). Analyses were implemented using Bayesian inference due to its general improved ability over Maximum Likelihood to accurately estimate posterior probabilities when limited information may be available in the data (Beerli 2006). The Brownian motion approximation model was used with Metropolis-Hastings sampling and prior probability estimates of θ and M were set using F_{ST} values. Long-inc (the number of unrecorded updates between samples) was set at 1,000 in both sets of analyses. Long-sample (the number of sampled updates) was set at 5,000 in the Cayos Cochinos analysis and 2,500 in the five population analysis.

Long-sample was lower in the five population analysis because of the increased run length associated with having a greater number of populations and the maximum run time allowed on the CBSU server. Burn-in was set at 100,000 updates in all analyses.

For each analysis, five replicate runs (starting from different random seeds) were performed and posterior parameter estimates compared to check for convergence of the runs. Autocorrelation and effective sample size values were also observed to assess convergence of the runs.

Isolation with migration model

Although the method applied by Beerli and Felsenstein (1999; 2001) is useful for comparing relative levels of gene flow between populations, it may not be completely appropriate for the analysis of recently diverged populations. For this reason, the 'Isolation with Migration' (IM) model, as implemented in the software *IMa* (Hey & Nielsen 2007), was also applied to the data.

Under the basic Isolation with Migration (IM) model, two contemporary populations of sizes N_1 and N_2 are considered to have separated from an ancestral population of size N_A t generations ago. Following separation, gene flow occurs between the two populations at rates m_1 and m_2 (Hey et al. 2004). The program *IMa* estimates posterior distributions for these six parameters using a Markov Monte Carlo Chain methodology. Key assumptions of the method are that all loci being studied have been evolving neutrally and have been drawn at random from all potentially available loci, with respect to genealogical history (Hey & Nielsen 2004). Also, that mutation has followed the model applied to the data (Hey & Nielsen 2004), in this case the stepwise mutation model (SMM), which is thought to be a good

approximation of the step-wise manner by which microsatellites mutate (Estoup et al. 2002).

Analyses were conducted for the two Cayos Cochinos populations (Cayo Cochino Grande and Cayo Cochino Pequeño) only. As with the *MIGRATE* analysis, sample sizes for both populations were 76 individuals (152 gene copies) and analyses were performed using the CBSU at Cornell University (*IMa*@BIOHPC). The geometric heating scheme with 40 chains was used to achieve adequate mixing. The command h1 (-g1), specifying the degree of non-linearity of the heating scheme was set at 0.84 and h2 (-g2) specifying the heating value in the highest numbered chain was set at 0.80. Broad uninformative priors for the effective population size scalar, maximum migration rates and maximum time since population splitting were used for all analyses (-q1 10, -m1 10, -m2 10, -t 10). A burn-in period of 1,000,000 steps was applied before sampling the first genealogies. Thereafter, analyses were run for 2,000,000 steps sampling every 100^{th} step and resulting in 20,000 recorded genealogies for estimation of posterior probabilities of the parameters. Convergence was assessed by running five independent analyses started with different random seed values and comparing the posterior distributions of the parameter estimates and by assessing the autocorrelation and effective sample size values. (Won & Hey 2005).

Similar to the *MIGRATE* analysis, mean mutation rate estimates were based on the widely used value of $5x10^{-4}$ mutations per generation (e.g. Storz & Beaumont 2002; Storz et al. 2002). An average generation time estimate for insular *B. constrictor* of four years was taken to be a realistic average for males and females based on current knowledge of island *B. constrictor* populations in captivity and in the wild (Reed et

al. 2007; Russo 2007; Reed & Rodda 2009). Based on these two estimates, the mean microsatellite mutation rate per year of $1.25x10^{-4}$ was specified in the input file along with upper and lower bounds of $2.5x10^{-5} – 2.5x10^{-4}$ to take into account uncertainty in this estimate. The range applied to the mutation rate was intended to take into account values in the range of 10^{-3}–10^{-4}, commonly reported for vertebrate microsatellite loci (King 2009). Using the information provided on the mean annual mutation rate and generation time in years, the program converts estimates of the parameters (θ_1, θ_2, θ_A), into estimates of effective population size (N_1, N_2, and N_A respectively) and time in years since the populations split (t_{Years}). Population migration rate estimates are then calculated as $M_1 = 2N_1m_1 = m_1 \times \theta_1/2$ and $M_2 = 2N_2m_2 = m_2 \times \theta_2/2$, where M_1 is the effective rate per generation at which genes come into population 1 from population 2 and M_2 is the effective rate per generation at which genes come into population 2 from population 1.

Estimating recent migration rates

Finally, analyses were performed with BayesAss 1.3 (Wilson & Rannala 2003) to estimate recent migration rates. The program uses a Bayesian approach to estimate rates of recent immigration and inbreeding coefficients. One key advantage of the program is that it allows for populations to be out of HW equilibrium, which may often be the case in small, disturbed populations (Beebee & Rowe 2004).

Posterior probabilities of parameters were estimated using two independent MCMC simulations started from different random seed values. Analyses were performed on two different data sets; a two population data set including just the two Cayos Cochinos populations, and a five population data set including the Cayos Cochinos, Utila, Mainland Honduras and Panama populations. Other populations were excluded

from the analysis due to low sample size. The two population data set was run for $3x10^6$ iterations, discarding the first 10^6 as the burn-in. A longer run of $30x10^6$ with a burn-in of $5x10^6$ was performed on the five population data set because of the relatively lower number of samples in the added populations and thus the expected increase in time to reach convergence. The chains were sampled every 2,000 iterations for inference of the parameter posterior probabilities. Delta was set to 0.15 (the default value).

To check that runs had reached convergence, Log posterior probabilities were plotted against iteration number. Convergence of the MCMC was also determined by plotting the mean posterior probabilities of allele frequencies at each locus in each population for each of the two independent runs. Convergence is inferred by the linear relationship between the posterior probabilities (Wilson & Rannala 2003).

Results

Genetic marker analysis

A total of 628 boas were successfully genotyped for at least six of the eight polymorphic microsatellite loci. Numbers of individual snakes genotyped for each microsatellite locus in each of the predefined populations are displayed in Table 4-2 (full genotype data are displayed in Appendix 2). Samples in which more than two loci failed to amplify were excluded from analyses. Where samples were genotyped twice, the same genotype was obtained on both occasions in 100% of cases.

Table 4-2 Total number of snakes sampled and successfully genotyped at each microsatellite locus in each population

Population	No. snakes sampled	Number of snakes successfully genotyped at each locus							
		Bci-14	Bci-15	Bci-18	Bci-21	Bci-23	µsat01	µsat20	µsat36
Cayo Pequeño	502	495	490	470	497	497	499	483	486
Cayo Grande	76	76	76	76	76	76	76	75	75
Utila	7	7	7	7	7	6	7	7	7
Roatan	4	4	4	4	4	4	4	4	4
Guanaja	1	1	1	0	1	1	1	1	1
Honduras ML	11	11	11	11	11	11	11	11	11
Guatemala	1	1	1	1	1	1	1	1	1
Mexico	2	2	2	2	2	2	2	2	2
Costa Rica	2	2	2	2	2	2	2	2	2
Panama (total)	22	12	22	21	22	22	22	22	18
Panama El Valle	14	8	14	13	12	14	12	12	9

Examination of genotype data in the heterogametic sex (females) detected no evidence of sex-linkage between loci. Analysis with *Micro-Checker* found no evidence of null alleles for any locus in the Cayo Cochino Grande population. However, null alleles may be present at locus Bci-14 and Bci-21 in the Cayo Cochino Pequeño population and at locus Bci-18 in the Utila and Honduras mainland populations, as is suggested by the general excess of homozygotes for most allele size classes at these loci. However, this was not deemed significant by the program and thus no adjusted allele frequencies were calculated. When Panama was analysed as a single panmitic population (all samples included), there was evidence of null alleles for markers Bci-14, Bci-18 and Usat20. However, when only samples from a single collection site (El Valle) were included in the analysis, (n=11) only Bci-14

showed evidence for the presence of null alleles. Sample size was insufficient to test for null alleles in the remaining populations. None of the loci in any of the populations displayed significant problems with large allele dropout or scoring error due to stuttering.

Analysis of the Cayos Cochinos and mainland Honduras populations with *CERVUS* found null allele frequencies to be within acceptable limits (F-null<0.20) (Dakin & Avise 2004). However, null allele frequencies were unacceptably high in the Panama population for loci Bci-14, Bci-18, Bci-21 and μsat20 (F-null>0.20). When only samples from the El Valle collection site were included null allele frequencies for Bci-21 and Bci-18 dropped below F-null 0.2, however, null allele frequencies remained unacceptably high for μsat20. Bci-14 could not be analysed in the El Valle population due to insufficient sample size. Sample size was insufficient to test for null alleles in the remaining populations.

The problem with null alleles in the Panama population may, in part, be explained by population structure within the samples collected, as is suggested by the reduction of null allele frequencies when only the El Valle samples were included in the analysis. However, null alleles in the Panama population may also be explained by poor quality template DNA in a number of these samples as a result of poor storage conditions of tissue samples prior to DNA extraction. For this reason, in all further analyses only the samples from El Valle were included as representatives of the Panama population.

Exact tests for Hardy-Weinberg equilibrium revealed no violation in the mainland Honduras population. However, a number of the loci were found to violate Hardy-Weinberg equilibrium in the Cayos Cochinos, Utila and Panama El Valle populations

(Table 4-3). In all cases, this was due to heterozygote deficiency at the locus. No evidence of linkage disequilibrium, after Bonferroni correction, was found between loci in any of the populations tested, with the exception of Cayo Cochino Pequeño. A number of loci were found to be linked in this population (Table 4-4), however, as these loci were not found to be linked in any of the other populations, it suggests that intrachromosomal linkage was not a confounding factor. When only a random subset of individuals from Cayo Cochino Pequeño were analysed (n=30) no linkage was detected.

Table 4-3 Hardy-Weinberg Exact Test, results from the probability-test (1,000 iterations). P-values <0.05 (in bold) indicate violation of Hardy-Weinberg equilibrium. Missing P-value indicates locus was monomorphic in that population.

	Probability test P-value							
Population	Bci-14	Bci-15	Bci-18	Bci-21	Bci-23	µsat01	µsat20	µsat36
Cayo Pequeño	**<0.001**	0.566	0.038	**0.046**	1.000	**0.007**	**<0.001**	**0.003**
Cayo Grande	**0.030**	0.035	**0.001**	0.242	-	0.789	0.180	0.358
Utila	0.945	0.652	**0.001**	1.000	1.000	**0.035**	0.628	0.646
Mainland Honduras	0.494	0.739	0.075	0.514	0.545	0.849	1.000	0.179
Panama El Valle	**<0.001**	0.152	0.221	0.437	1.000	0.246	**0.039**	0.889

Table 4-4 Loci found to be in linkage disequilibrium in the Cayo Cochino Pequeño population following Bonferroni correction (x = loci in linkage disequilibrium)

	Bci-14	Bci-15	Bci-18	Bci-21	Bci-23	µsat01	µsat20
Bci-14	-						
Bci-15	-	-					
Bci-18	x	-	-				
Bci-21	-	-	-	-			
Bci-23	-	-	-	x	-		
µsat01	x	-	x	-	-	-	
µsat20	-	-	-	x	x	x	-
µsat36	x	x	x	x	x	x	-

Population structure analysis using F_{ST}

Pairwise F_{ST} estimates between the five predefined populations included in the analysis were all found to be significant ($P<0.05$) and ranged between 0.09-0.28 (Table 4-5). As would be expected, the smallest values of F_{ST} were observed between the geographically proximate islands of Cayo Cochino Pequeño and Cayo Cochino Grande. The greatest pairwise F_{ST} values were observed between the Cayos Cochinos and Panama. Both of the Cayos Cochinos islands displayed greater pairwise F_{ST} values with Utila than they did with mainland Honduras. Pairwise F_{ST} values were lower between Utila and mainland Honduras than they were between the Cayos Cochinos and mainland Honduras.

Table 4-5 Below the diagonal are values of pairwise genetic differentiation between subpopulations estimated using Weir and Cockerham's (1984) variant of F_{ST}, θ, in FSTAT (all values significant at $P<0.05$). Above the diagonal are estimates of the effective number of migrants per generation (N_em) estimated using the equation $N_em = [(1/F_{ST})-1]/4$ (Wright 1969).

	CCP	CCG	Utila	Honduras	Panama
CCP	-	2.53	0.75	1.14	0.64
CCG	0.09	-	0.79	1.14	0.71
Utila	0.25	0.24	-	3.32	0.84
Honduras	0.18	0.18	0.07	-	1.67
Panama	0.28	0.26	0.23	0.13	-

STRUCTURE analysis

Comparison of ad hoc statistic Δ*K* (Evanno et al. 2005) for the Cayos Cochinos *STRUCTURE* analysis revealed that population structure within the Cayos Cochinos can best be explained by two clusters in all models, with the exception of the admixture model where prior population information was used to aid clustering. Under this model, greatest support was found for three clusters, however, support for two clusters remained high (Figure 4-2). Assignment of individuals into clusters was largely in agreement with their island of origin and all models provided similar results. Individual assignment probabilities from the 'admixture use prior population information' model are displayed (Figure 4-3), however, see Appendix 6 for individual assignment probabilities for the other models.

When all population samples were included in *STRUCTURE* analyses, comparison of Δ*K* revealed the most likely number of clusters was, to some extent, dependent on the model applied to the data. Generally support was greatest for values of K 2-4, however, under the 'admixture use prior population information' model, support was

also high for K=8 (Figure 4-4). Despite this inconsistency between different models, individual assignment probability plots revealed almost identical patterns of clustering irrespective of the model being applied to the data. Results from the 'admixture use prior population information' model are displayed (Figure 4-5), however, see Appendix 6 for results from the other models. Individuals from the Cayos Cochinos islands consistently group separately from the other populations for all values of K.

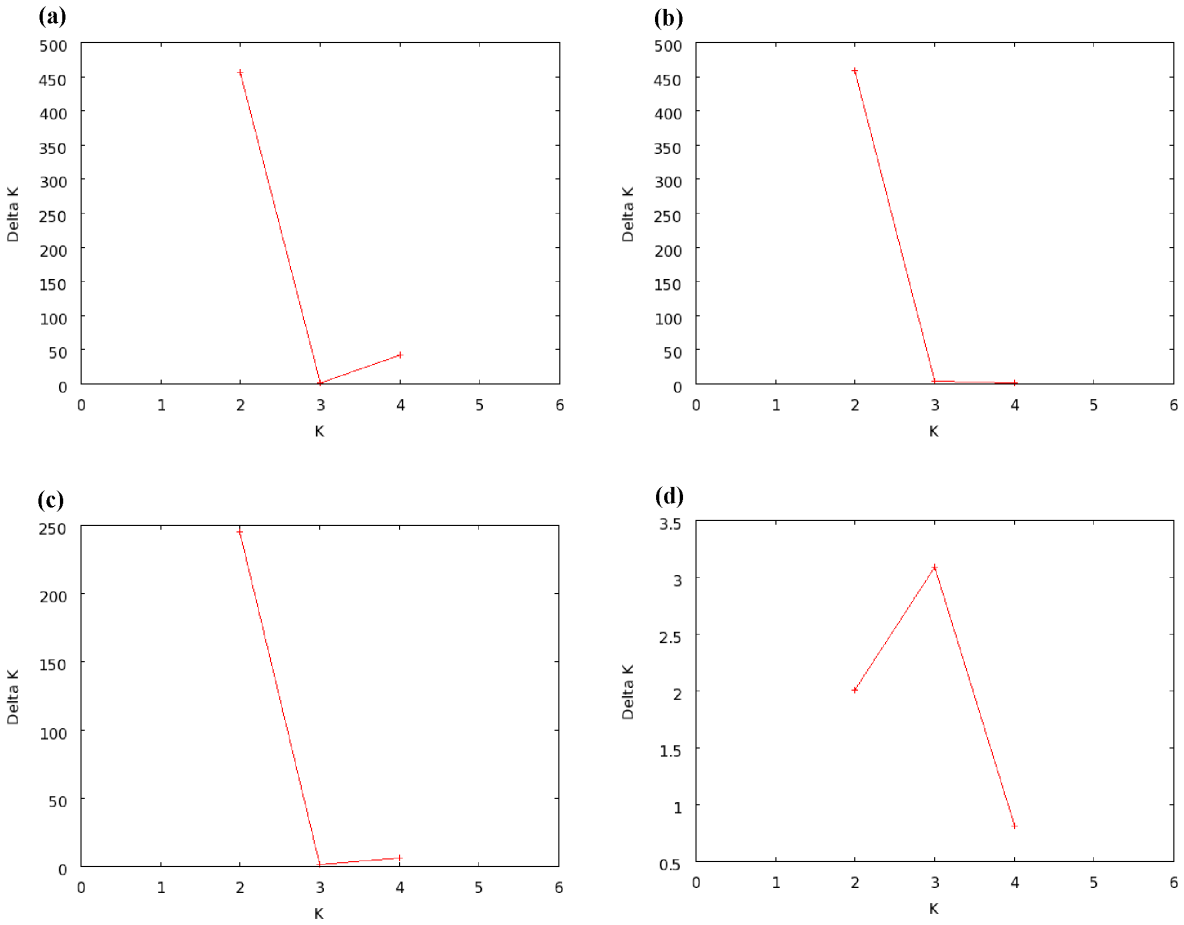

Figure 4-2 Delta K values calculated from *STRUCTURE* using the methodology of Evanno et al. (2005) indicating the most likely value for the 'true' number of clusters within the Cayos Cochinos using the following models: (a) no admixture no prior population information, (b) no admixture use prior population information, (c) admixture no prior population information, (d) admixture use prior population information.

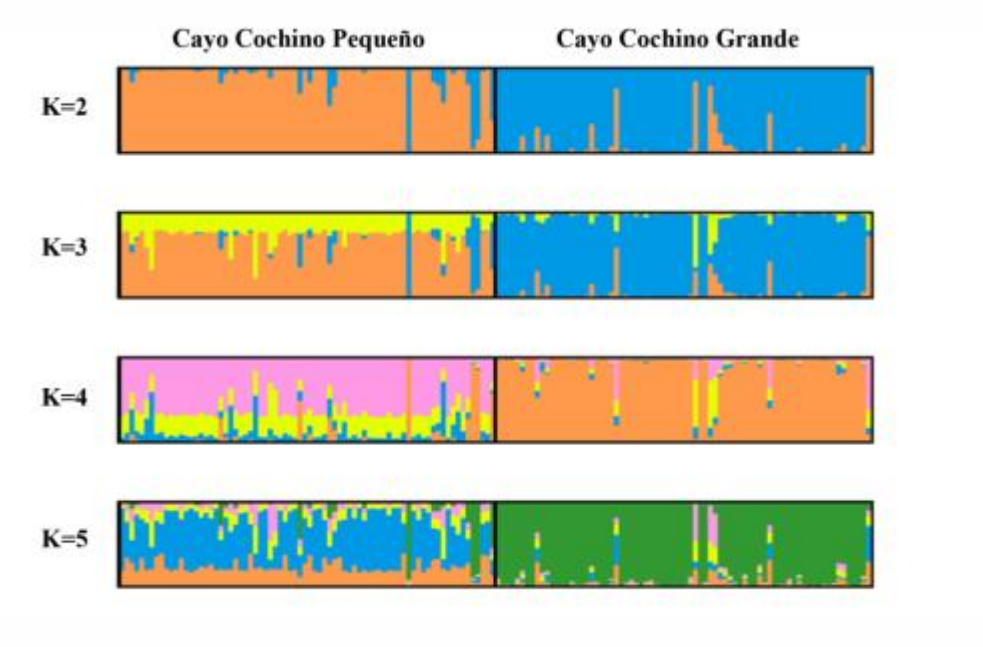

Figure 4-3 Individual cluster assignment probabilities averaged over five independent runs of *STRUCTURE* (no admixture use prior population information model) using the programs *CLUMPP* and *DISTRUCT*.

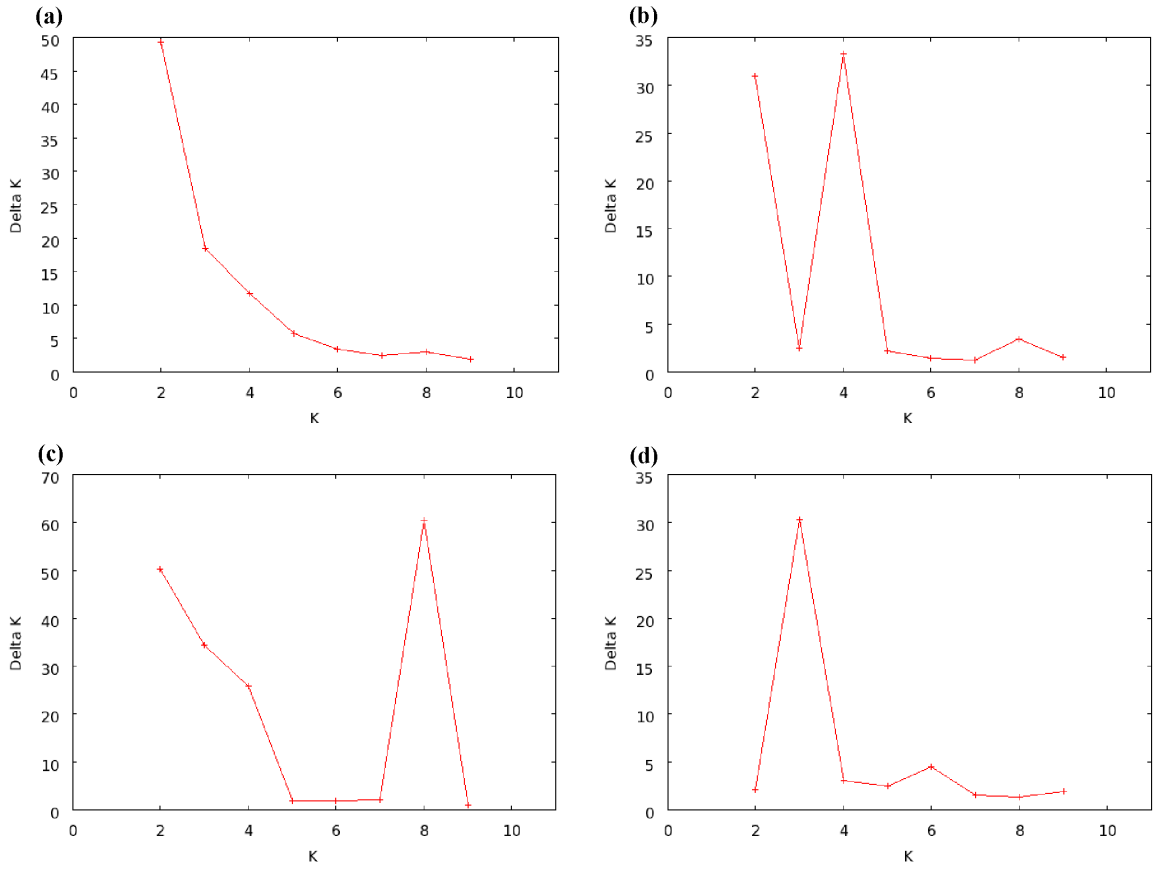

Figure 4-4 Delta K values calculated using the methodology of Evanno et al. (2005) indicating the most likely value for the 'true' number of clusters when data from ten putative populations were included in the analysis using the following models: (a) no admixture no prior population information, (b) no admixture use prior population information, (c) admixture no prior population information, (d) admixture use prior population information.

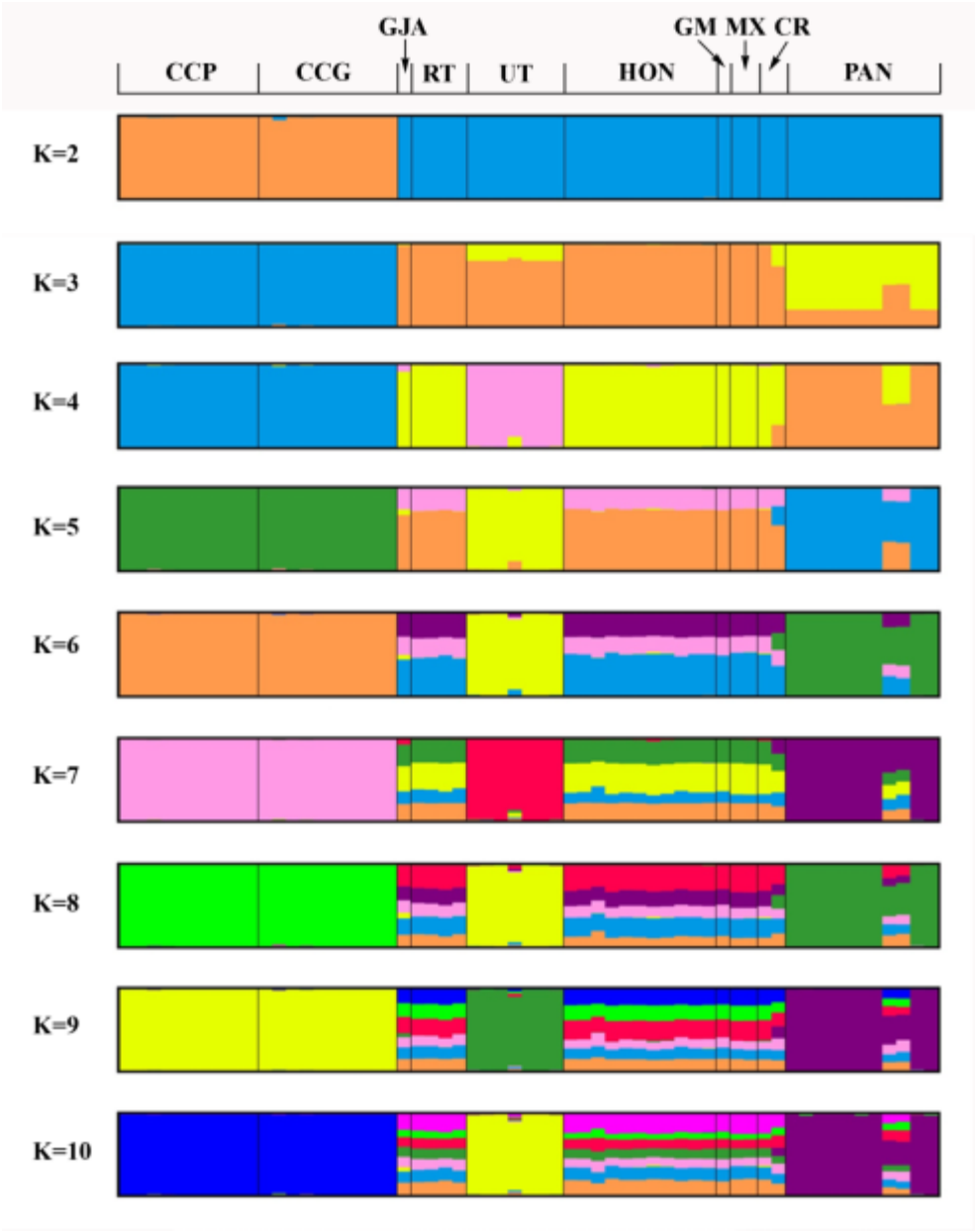

Figure 4-5 Individual cluster assignment probabilities averaged over five independent runs of *STRUCTURE* (no admixture use prior population information model) using the programs *CLUMPP* and *DISTRUCT*. CCP = Cayo Cochino Pequeño, CCG = Cayo Cochino Grande, GJA = Guanaja, RT = Roatan, UT = Utila, HON = Honduras mainland, GM = Guatemala, MX = Mexico, CR = Costa Rica, PAN = Panama.

TESS analysis

Analysis of the Cayos Cochinos populations in *TESS* revealed only moderate improvement of average DIC scores with increasing values of K, suggesting that values of K>2 do not provide a much improved explanation of the data (Figure 4-6). Inspection of the individual assignment probability plots also clearly showed that increasing the number of clusters above K=2 did little to help explain the structure observed in the populations (Figure 4-7). Assignment of individuals into clusters was largely in agreement with their island of origin and both models provided similar results. Individual assignment probabilities from the admixture model are displayed (Figure 4-7), however, see Appendix 6 for individual assignment probabilities for the no-admixture model.

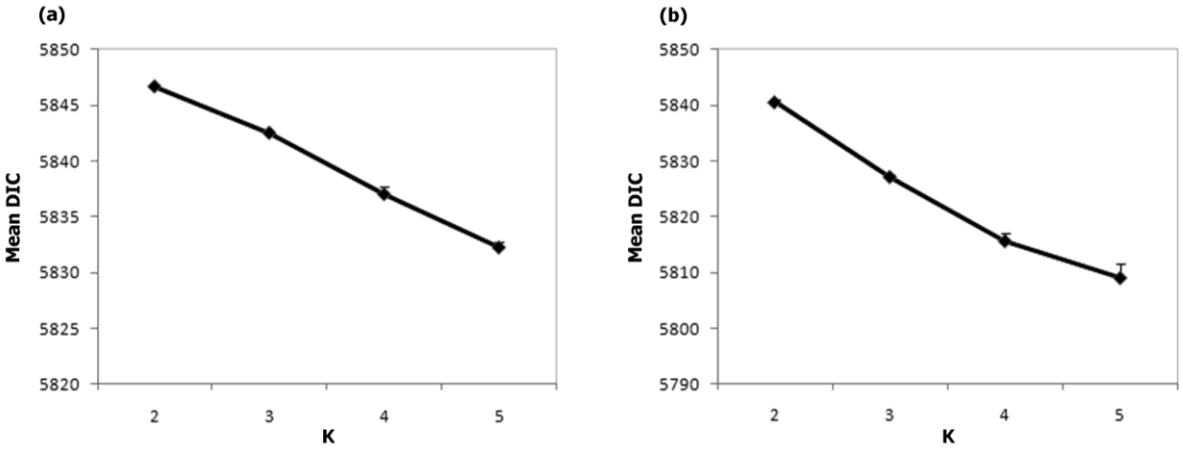

Figure 4-6 Results of *TESS* analysis for the Cayos Cochinos. Mean DIC scores showing support for (a) the maximum number of clusters under the no admixture model and (b) the maximum number of parental populations under the admixture model.

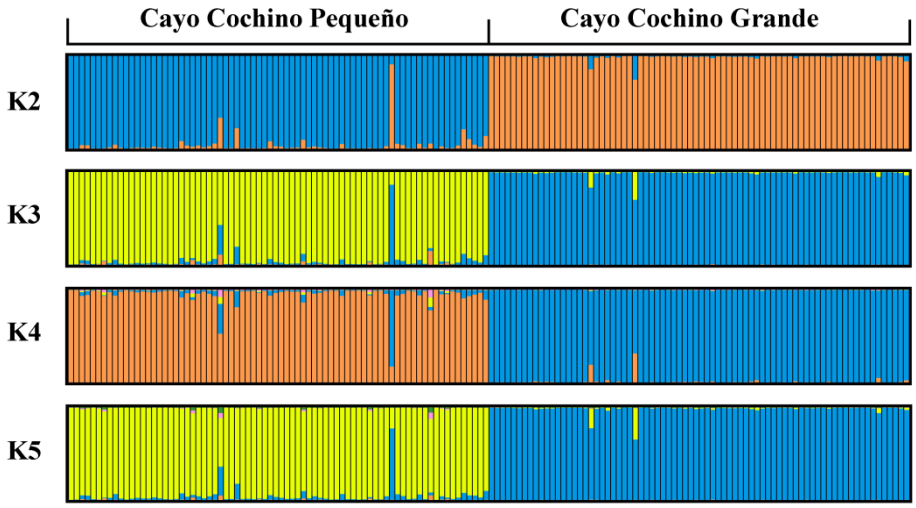

Figure 4-7 Individual cluster assignment probabilities for the Cayos Cochinos determined by *TESS* (admixture model) and averaged over five independent runs using the programs *CLUMPP* and *DISTRUCT*. Population structure is clearly apparent between the two islands with two distinct clusters being the most parsimonious explanation of the data even when a maximum of 5 clusters (K5) is allowed.

When all population samples were included in the *TESS* analysis, there was a general improvement in average DIC score with increasing values of K up until around K=5, after which point increasing K further only resulted in small improvements in the DIC scores (although this pattern was more pronounced under the admixture model) (Figure 4-8). This suggests that K=5 may be the best explanation of the data, however, values of K=3 and K=4 cannot be ruled out. Individual assignment probabilities from the admixture model are displayed (Figure 4-9), however, see Appendix 6 for individual assignment probabilities for the no-admixture model. For values of K≥3, the Cayos Cochinos and Panama populations can clearly be seen to form distinct clusters, however the remaining populations are not well defined and samples from these populations show increasing levels of admixture with increasing values of K.

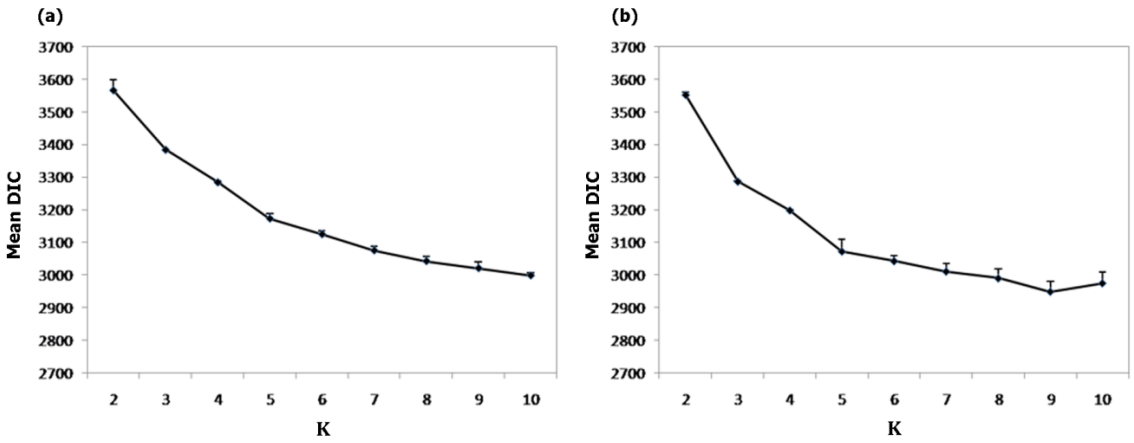

Figure 4-8 Results of TESS analysis when all populations were included. Mean DIC scores showing support for (a) the maximum number of clusters under the no admixture model and (b) the maximum number of parental populations under the admixture model.

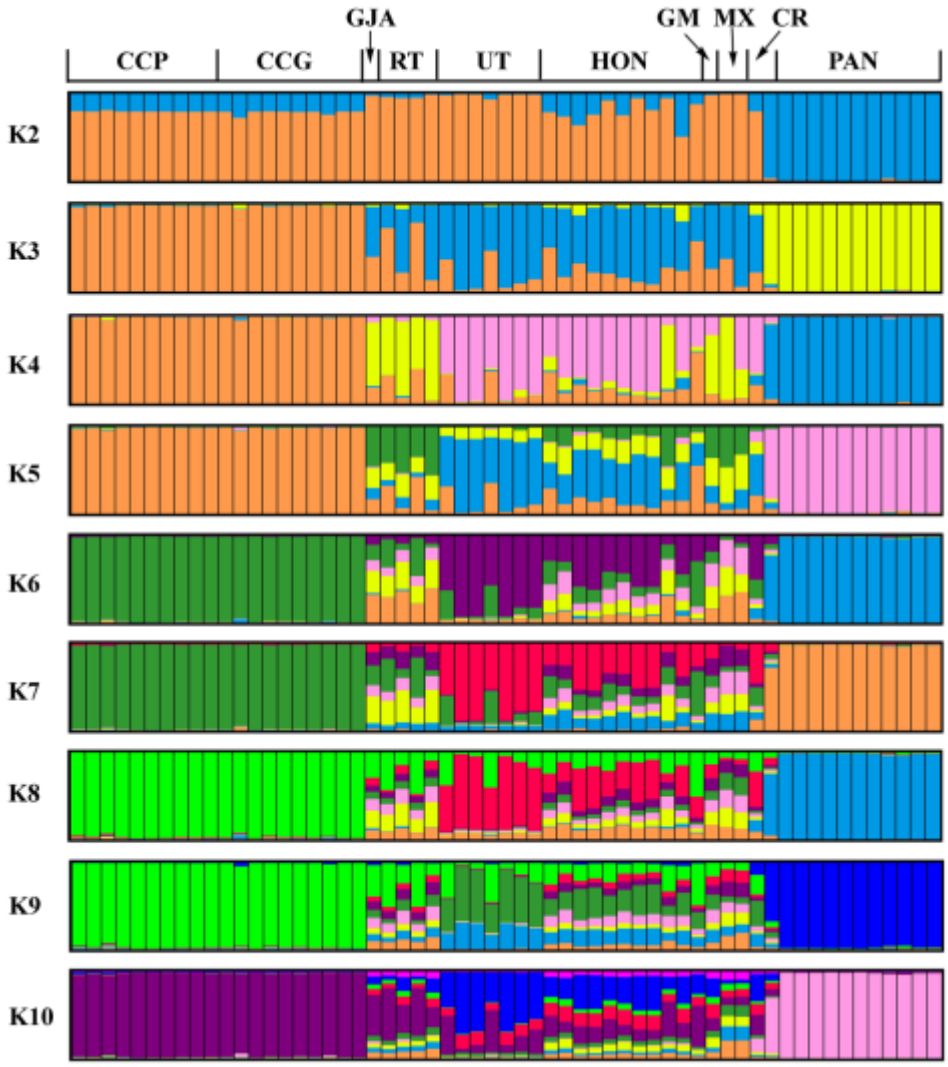

Figure 4-9 Individual cluster assignment probabilities averaged over five independent runs of *TESS* (admixture model) using the programs *CLUMPP* and *DISTRUCT*. CCP = Cayo Cochino Pequeño, CCG = Cayo Cochino Grande, GJA = Guanaja, RT = Roatan, UT = Utila, HON = Honduras mainland, GM = Guatemala, MX = Mexico, CR = Costa Rica, PAN = Panama.

F_{ST} based migration rate estimates

Estimates of the effective number of immigrants, based on the equation $N_em = [(1/F_{ST})-1]/4$, are given in Table 4-5. Migration between the Cayo Cochino Grande and Cayo Cochino Pequeño populations was high, as would be expected due to their close proximity to one another. But, surprisingly, migration was greatest between Utila and mainland Honduras, despite Utila being further from the mainland than the Cayos Cochinos. Also, migration between the Cayos Cochino and Utila was, surprisingly low and comparable with the level of migration between the islands and Panama.

MIGRATE analysis

Observation of the autocorrelation and effective sample size values for both the Cayos Cochinos and five population data set analyses indicated that run length was adequate for the estimation of θ. However, longer runs may have been appropriate for the accurate estimation of migration rates, as indicated by relatively high autocorrelation values for this parameter. Unfortunately, it was not possible to perform longer runs due to the maximum run time limit being reached on the CBSU server. However, parameter estimates from independent runs were largely consistent and thus indicate that chains had reached convergence.

Mean parameter values and upper and lower 97.5% confidence limits were averaged over the five runs and used to calculate effective population sizes (N_e) and number of migrants per generation (Nm_{ij}) for both the Cayos Cochinos analysis (Table 4-6) and the five population analysis (Table 4-7)**.** Estimates of the number of migrants moving between the two Cayos Cochinos islands were considerably higher in the analysis where the two islands were considered in isolation from the other populations.

Estimates of migration rates between the islands and mainland Honduras from the five population data set analysis are summarized in Figure 4-10 for easy comparison with F_{ST} based estimates.

Table 4-6 Mean parameter estimates based on the results of five independent runs of *Migrate* for the Cayos Cochinos data set (97.5% confidence limits given below in parenthases). The effective population size (N_e) is calculated as $\theta/4\mu$, where μ is an estimate of mean microsatellite mutation rate ($\mu = 5x10^{-4}$). The number of migrants per generation (Nm_{ij}) is calculated as $\theta_i M_{j>i}$.

				Nm_{ij}	
	Population	θ	N_e	1 > i	2 > i
1	C.Pequeño	0.0984 (0.0960-0.1000)	49.2 (48.00-50.00)	-	8.75 (6.40-10.73)
2	C.Grande	0.0983 (0.0960-0.1000)	49.16 (48.00-50.00)	8.90 (6.49-10.82)	-

Table 4-7 Mean parameter estimates based on the results of five independent runs of *Migrate* for the five population data set (97.5% confidence limits given below in parenthases). The effective population size (N_e) is calculated as $\theta/4\mu$, where μ is an estimate of mean microsatellite mutation rate ($\mu = 5x10^{-4}$). The number of migrants per generation (Nm_{ij}) is calculated as $\theta_i M_{j>i}$.mutation rate ($\mu = 5x10^{-4}$). The number of migrants per generation (Nm_{ij}) is calculated as $\theta_i M_{j>i}$.

				Nm_{ij}				
	Pop	θ	N_e	1 > i	2 > i	3 > i	4 > i	5 > i
1	CP	0.0983 (0.0956-0.1000)	49 (48-50)	-	2.68 (0.39-4.52)	0.46 (0.00-2.06)	1.24 (0.00-3.05)	1.10 (0.00-2.75)
2	CG	0.0982 (0.0955-0.1000)	49 (48-50)	2.74 (0.29-4.62)	-	0.86 (0.00-2.55)	1.05 (0.00-2.75)	0.82 (0.00-2.46)
3	UT	0.0980 (0.0954-0.1000)	49 (48-50)	1.14 (0.00-3.04)	1.42 (0.00-3.14)	-	0.96 (0.00-2.84)	0.87 (0.00-2.65)
4	HON	0.0982 (0.0955-0.1000)	49 (48-50)	1.69 (0.00-3.53)	1.08 (0.00-2.85)	1.30 (0.00-3.04)	-	1.16 (0.00-2.95)
5	PAN	0.0980 (0.0955-0.1000)	49 (48-50)	1.09 (0.00-2.94)	1.48 (0.00-3.14)	1.06 (0.00-2.84)	1.47 (0.00-3.23)	-

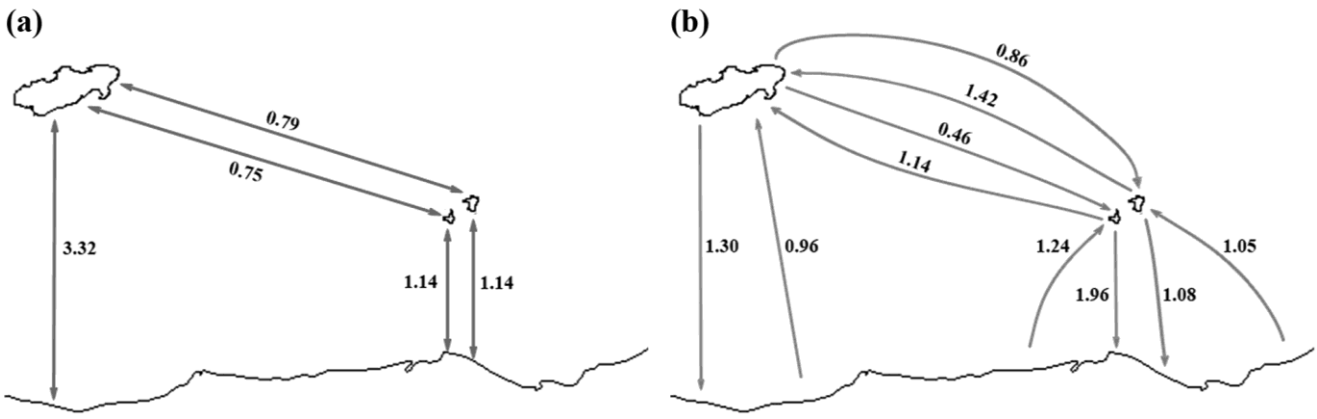

Figure 4-10 Comparison of migration rate estimates between the Cayos Cochinos, Utila and mainland Honduras for the (a) F_{ST} based calculations (Wright 1969) and (b) coalescent based method implemented in *MIGRATE*.

Isolation with migration (IMa)

The autocorrelation and effective sample size values suggested good mixing of the chains and between chain swapping rates were within acceptable limits. Parameter estimates from the five independent runs were consistent, suggesting convergence had been reached. Mean values and 90% highest probability density (HPD) values of parameter estimates for each of the five independent runs are given in Table 4-8 along with values for demographic variables averaged across runs.

Average estimates of effective population sizes and migration rates are summarized for the three different methods (F_{ST}-based, equilibrium-migration and isolation with migration) within the Cayos Cochinos populations (Figure 4-11) for easier comparison of the methods.

Table 4-8 Results of IMa analysis for *Boa* constrictor on the two Cayos Cochinos Islands, Cayo Cochino Pequeño (CCP) and Cayo Cochino Grande (CCG). Results of the five independent runs are displayed, plus averages of demographic parameters calculated over the five runs. Results are based on a mean microsatellite mutation rate of $5x10^{-4}$ per generation and an average generation time of 4 years. N_1 = effective population size of CCP, N_2 = effective population size of CCG, N_A= ancestral population size M_1 = migration rate per generation from CCG into CCP, M_2 migration rate per generation from CCP into CCG ($M_1 = 2N_1m_1 = m_1 \times \theta_1/2$ and $M_2 = 2N_2m_2 = m_2 \times \theta_2/2$), t_{years} = number of years since population splitting (based on a 4 year generation time).

Run		θ_1	θ_2	θ_A	m_1	m_2	t	N_1	N_2	N_A	M_1	M_2	t_{years}
1	Mean	2.865	2.224	53.730	2.048	1.698	0.712	1432	1112	26865	2.934	1.888	5697
	Lower 90% HPD	1.274	0.917	11.469	0.005	0.005	0.105	637	459	5735	0.003	0.002	840
	Upper 90% HPD	5.644	3.974	102.133	3.725	3.435	1.305	2822	1987	51066	10.511	6.825	10440
2	Mean	2.564	2.145	44.891	2.136	1.658	0.553	1282	1072	22445	2.739	1.778	4421
	Lower 90% HPD	0.910	0.713	10.013	0.025	0.005	0.035	455	357	5007	0.011	0.002	280
	Upper 90% HPD	4.916	3.770	83.199	3.845	3.485	1.155	2458	1885	41599	9.450	6.569	9240
3	Mean	2.728	2.131	53.895	2.058	1.829	0.654	1364	1065	26948	2.807	1.948	5232
	Lower 90% HPD	0.910	0.713	12.198	0.195	0.005	0.055	455	357	6099	0.089	0.002	440
	Upper 90% HPD	5.280	3.770	105.410	3.825	4.015	1.355	2640	1885	52705	10.097	7.568	10840
4	Mean	2.598	1.955	47.707	2.329	1.957	0.515	1299	977	23853	3.026	1.912	4120
	Lower 90% HPD	0.910	0.713	11.469	0.115	0.005	0.065	455	357	5735	0.052	0.002	520
	Upper 90% HPD	4.916	3.566	83.199	4.365	3.865	1.055	2458	1783	41599	10.728	6.892	8440
5	Mean	2.737	2.158	54.912	2.037	1.626	0.668	1368	1079	27456	2.787	1.755	5344
	Lower 90% HPD	0.910	0.713	11.105	0.385	0.015	0.015	455	357	5553	0.175	0.005	120
	Upper 90% HPD	5.280	3.974	101.769	3.565	3.355	1.255	2640	1987	50884	9.411	6.666	10040
Mean of all runs	Mean	-	-	-	-	-	-	1349	1061	25513	2.858	1.856	4962
	Lower 90% HPD	-	-	-	-	-	-	492	377	5625	0.066	0.003	440
	Upper 90% HPD	-	-	-	-	-	-	2603	1905	47571	10.040	6.904	9800

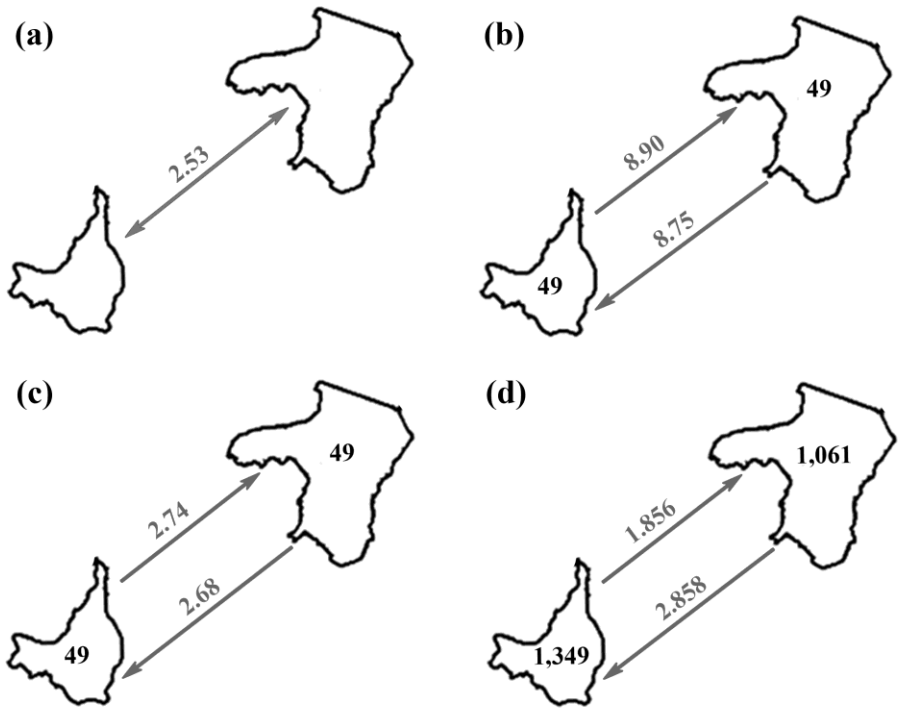

Figure 4-11 Effective population sizes and historical migration rates for the Cayos Cochinos estimated using four different approaches (a) F_{ST} based estimate (b) equilibrium migration model for the two population Cayos Cochinos data set (c) equilibrium migration model for the five population data set (d) isolation with migration model. Numbers within islands are mean estimates of effective population sizes (but estimates for the equilibrium migration models are unrealistically low and should be treated with caution). Arrows show the direction of gene flow and numbers above arrows represent the effective rate of migration per generation.

Recent migration rates (BayesAss)

To check that runs had reached convergence, log posterior probabilities were plotted against iteration number. In all cases, convergence was achieved rapidly, within 10,000 iterations (Appendix 7). Convergence of the MCMC was also determined by plotting the mean posterior probabilities of allele frequencies at each locus in each population for each of the two independent runs (Appendix 7). Convergence is inferred by the linear relationship between the posterior probabilities (Wilson &

Rannala 2003). Using the aforementioned diagnostics, convergence was determined in both the two population and five population simulations.

Mean migration rates for run 1 of both the two population and five population data scenarios are provided in Table 4-9 and Table 4-10 respectively and migration between the Cayos Cochinos, Utila and mainland Honduras is summarised in Figure 4-12. Data for run 2 for the two population and five population scenarios are provided in Appendix 8 but did not differ qualitatively from run 1. Surprisingly, mean migration rates between the two geographically proximate Cayos Cochinos populations were low ($m<0.01$) in both the two population and five population scenarios. In fact, migration rate was greater between the Cayos Cochinos and mainland Honduras than it was between Cayo Cochino Grande and Cayo Cochino Pequeño. Also, migration rate from Utila to mainland Honduras was substantially higher than between any other populations. In all populations, >95% of individuals were found to be derived from the source population, with the exception of the mainland Honduras population which had >25% of individuals being of immigrant ancestry.

Table 4-9 Mean posterior probabilities of the immigration rates of the two population scenario analysed using BayesAss (Run1). The populations into which individuals are migrating are listed in the rows and the origins of the migrants are listed in the columns. Values along the diagonal are the proportions of individuals derived from the source populations each generation. Values in parentheses are 95% CI

		From	
		C. Pequeño	C. Grande
Into	C. Pequeño	0.991 (0.982-0.997)	0.009 (0.003-0.018)
	C. Grande	0.009 (0.000-0.033)	0.991 (0.967-1.000)

Table 4-10 Mean posterior probabilities of the immigration rates of the five population scenario analysed using BayesAss (Run1). The populations into which individuals are migrating are listed in the rows and the origins of the migrants are listed in the columns. Values along the diagonal are the proportions of individuals derived from the source populations each generation. Values in parentheses are 95% CI

		From				
		CCP	CCG	Utila	Hon ML	Panama
Into	CCP	0.980 (0.974-0.996)	0.006 (0.001-0.014)	0.013 (0.001-0.016)	0.000 (0.000-0.002)	0.000 (0.000-0.002)
	CCG	0.002 (0.000-0.013)	0.994 (0.979-1.000)	0.001 (0.000-0.006)	0.001 (0.000-0.007)	0.001 (0.000-0.008)
	Utila	0.010 (0.000-0.057)	0.010 (0.000-0.052)	0.952 (0.875-0.999)	0.018 (0.000-0.051)	0.010 (0.000-0.058)
	Hon ML	0.022 (0.000-0.094)	0.019 (0.000-0.079)	0.147 (0.002-0.290)	0.747 (0.668-0.971)	0.065 (0.000-0.198)
	Panama	0.006 (0.000-0.040)	0.006 (0.000-0.040)	0.006 (0.000-0.039)	0.007 (0.000-0.038)	0.974 (0.910-0.999)

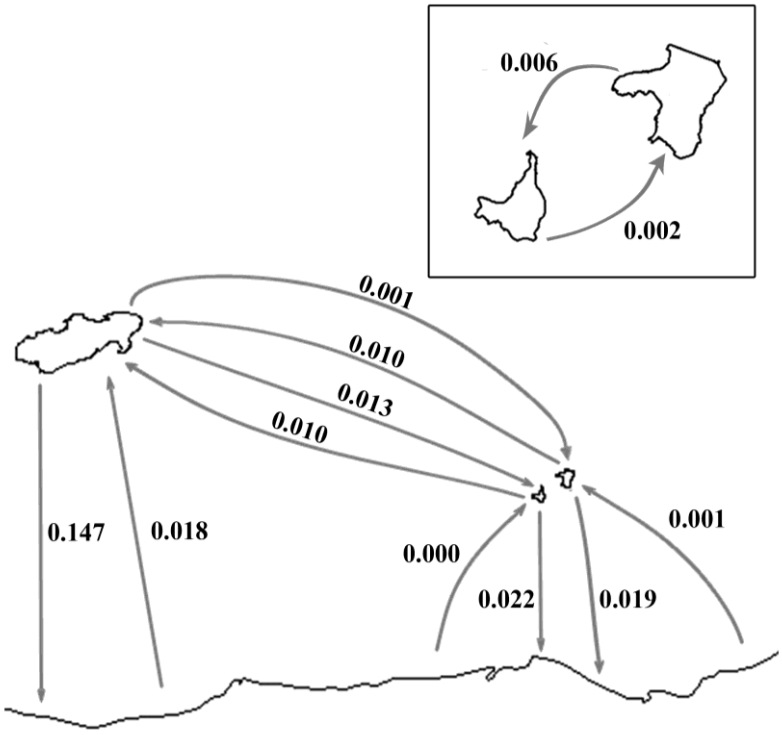

Figure 4-12 Rates of recent migration estimated in *BayesAss* (five population data set) between Utila, the Cayos Cochinos and mainland Honduras. Arrows indicate the direction of gene flow and numbers represent the fraction of the receiving population that is of recent immigrant ancestry from the source population. Inset shows migration rates within the Cayos Cochinos.

Discussion

Regional biogeography and population structure

As would be expected F_{ST} values were lowest between the geographically close islands of the Cayos Cochinos. However, interestingly, F_{ST} was considerably greater between the Cayos Cochinos populations and mainland Honduras than between Utila and the mainland. This may indicate higher levels of gene flow between the mainland and Utila than between the mainland and the Cayos Cochinos, a pattern also observed between the populations based on analysis of mitochondrial DNA (Chapter 3). Alternatively, high values of F_{ST} in the Cayos Cochinos could be interpreted as a consequence of the more pronounced effects of genetic drift in small

island populations (King 2009). However, the relatively high density of boas in the Cayos Cochinos (Chapter 6) compared with Utila means that effective population sizes are likely to be more similar than island size alone might predict. Thus the apparent pattern of higher population subdivision between the Cayos Cochinos and mainland Honduras compared with Utila and the mainland is unlikely to be explained by higher levels of genetic drift in the Cayos Cochinos alone.

Overall, levels of subdivision, inferred from estimates of F_{ST}, were high compared with previous estimates for *B. constrictor*. Elsewhere, populations of *B. c. occidentalis* in Argentina, separated by 200 km, were found to have F_{ST} values <0.01, based on data from four polymorphic allozyme loci, indicating high levels of gene flow over large distances are achievable in this species (Rivera et al. 2005; Cardozo et al. 2007). A comparison of F_{ST} values from 19 allozyme-based and 35 microsatellite DNA-based studies of snakes found the median value for microsatellites was only slightly lower than for allozymes (King 2009), suggesting that comparison of estimates from the two methods can be informative. Thus it appears that even the small expanse of water separating Cayo Cochino Pequeño and Cayo Cochino Grande is acting as a reasonable barrier to migration and gene flow.

Although F_{ST} was low between Cayo Cochino Pequeño and Cayo Cochino Grande populations, analyses of individual clustering assignment probabilities clearly showed the two islands to be genetically distinguishable from one another when considered independently from other populations. However, when sample size was reduced and population structure was assessed including all samples from all populations, the Cayos Cochinos was identifiable as a single cluster. Thus, fine scale population structure exists between Cayo Cochino Pequeño and Cayo Cochino

Grande, but is minimal when compared with the level of population structure between the Cayos Cochinos and other populations. The Cayos Cochinos are clearly distinguishable from all other populations.

Many of the samples collected from mainland populations and samples from Roatan and Guanaja showed high levels of admixture during population structure analyses. This may well have been the result of insufficient sample size from each locality rather than due to high levels of gene flow between these populations, however, this is difficult to conclude without repeating the analysis with larger samples sizes from these localities. Panama consistently formed a separate cluster to the other mainland and island populations, which might be expected due to the reasonable number of samples from this location and greater distance from most other collection sites. Utila clustered separately from the mainland and other island boas under analysis with *STRUCTURE*, however, this pattern was less pronounced under analysis with *TESS*.

Historical gene flow

Estimates of the effective number of immigrants (N_em) based on F_{ST} values can give an indication of levels of gene flow, however, the application of this method has been questioned (Bossart & Prowell 1998; Whitlock & McCauley 1999). Values of N_em may not reflect true levels of gene flow if the strict assumptions of the model (i.e. subpopulations are of equal and constant size and exchanging migrants symmetrically) are violated, which is often the case (Whitlock & McCauley 1999). However, relaxation of the assumptions of this 'unrealistic' model apparently does not greatly affect the relationship between F_{ST} and N_em (Neigel 2002) and estimates of gene flow using these methods may be reasonable so long as the spatial scale is small and the populations are in migration-drift equilibrium (Whitlock & McCauley

1999). This method may, therefore, be more appropriate for the comparison of closely related populations such as within the Cayos Cochinos rather than between more distantly related populations such as the Cayos Cochinos and Panama. In this study, F_{ST} based estimates of gene flow appear to be fairly consistent with other estimates produced from the coalescent-based methods (Figure 4-10 and Figure 4-11). In fact the F_{ST} based approach appears to perform better than *MIGRATE* when the populations are analysed in isolation from other potential sources of gene flow, as illustrated by the very high migration rate estimates produced by the Cayos Cochinos data set analysis in *MIGRATE* (Figure 4-11).

Nonetheless, coalescent-based methods of estimating gene flow are derived from more realistic assumptions and are thus expected to give better estimates of gene flow. The method applied by Beerli and Felsenstein (1999; 2001), as implemented in *MIGRATE*, allows for the more realistic assumptions that effective population sizes may differ and that gene flow is likely to be non-symmetrical. However, this model is still based on the, perhaps unrealistic, assumption that the populations have been exchanging genes at a constant rate for an indefinitely long period of time. Thus, conventional 'island models', such as that implemented in *MIGRATE*, may not accommodate well histories of recent population separation (Hey & Nielsen 2007). This may be particularly problematic for the study of island populations, such as those examined in this study, that are thought to have been isolated from the mainland and from one another only relatively recently in evolutionary terms.

Estimates of θ produced by *MIGRATE* were identical for all populations in both the two population Cayos Cochinos analysis and the five population analysis, leading to identical estimates of N_e for all populations. Not only were estimates of N_e identical,

which is in itself a highly unlikely and surprising result, but all estimates were also unrealistically low. It appears that *MIGRATE* failed to produce realistic estimates of these parameters, which calls into question the reliability of these results. It may be that the *MIGRATE* analyses needed to be run for longer in order to reach convergence, however, longer runs were not possible for this study due to the maximum run time being reached on the CBSU server. For this reason, parameter estimates for the *MIGRATE* analyses should be treated with caution.

Estimate of time since divergence for the Cayos Cochinos

The observed level of differentiation between two populations can be explained as the result of a balance between genetic drift and gene flow, or simply a result of more recent isolation in the absence of present day gene flow (Broughton & Harrison 2003). Thus, two populations displaying a relatively low level of genetic differentiation, such as the Cayos Cochinos, may be the result of long-term separation with high levels of gene flow, or alternatively may be due to much more recent population separation with subsequent limited or no gene flow (Nielsen & Wakeley 2001). Therefore the ability to incorporate the most likely time of population separation into the model is important when attempting to estimate migration rates. Failure to take this important parameter into account appears to result in much higher, and possibly unrealistic, estimates of gene flow within the Cayos Cochinos when the islands are considered independently from other populations (as indicated by the Cayos Cochinos analysis in *MIGRATE*). However, when other populations were included in the analysis, estimates of migration rates within the Cayos Cochinos were similar to estimates from the other methods (Figure 4-11).

The mean estimated date of divergence of the Cayos Cochinos islands (4,962 years) was roughly in keeping with current knowledge of eustacy at the end of the last ice age (Bermingham et al. 1998; McCranie et al. 2005). Therefore, this result seems to support a colonization event shortly after the Cayos Cochinos was last isolated from the mainland by rising sea levels, a scenario also supported by mtDNA analysis (Chapter 3). Estimates of gene flow and effective population size under the isolation with migration model, implemented in *IMa*, are thus likely to be better estimates than those produced under the equilibrium migration model, implemented in *MIGRATE*.

Contemporary gene flow

Estimates of current migration rates, within the past several generations, provided a useful complementary approach to estimating contemporary, rather than historical, gene flow. Also, this method allows for populations to be out of HW equilibrium, as was found to be the case for a number of loci in all but the mainland Honduras population. Rates of contemporary gene flow appear to be low between all populations in the analysis with the exception of Utila to mainland Honduras where the rate of contemporary gene flow was found to be high.

However, results of contemporary migration rate estimates for the three populations with the smallest sample sizes, Utila, mainland Honduras and Panama, should be treated with some caution. Sample sizes for these populations were low, especially considering the low number of loci available. Wilson and Rannala (2003) point out that the program allows only the proportion of immigrants in a population to be estimated. If immigrants are included by chance within a small sample then there is a danger of overestimating migration rates. This could be an explanation for the high migration rate observed from Utila to mainland Honduras.

A number of the individuals included in the mainland Honduras population sample were collected from an animal sanctuary where original capture locality data was unavailable. One of these boas was phenotypically more similar to the Bay Island populations in its colouration and phylogenetic analysis placed this individual closely with the Bay Island boas (Chapter 3). It was considered likely that inclusion of this possible immigrant in such a small sample size could have had a considerable positive bias on the estimate of migration rate from Utila to mainland Honduras. Repeated analysis where this suspected immigrant was removed from the dataset considerably reduced the migration rate from Utila to mainland Honduras (mean of the two runs m=0.035). This result illustrates the potential problem when attempting to estimate migration rates in populations with very small sample size.

In addition, the model assumes that all populations exchanging migrants have been sampled. A limitation of the study was that a number of populations from which migration could have occurred (e.g. Roatan, Guanaja, Belize) could not be included in the analysis due to samples either not being available or sample size being insufficient. Thus, greater sampling may be needed to gain a higher degree of confidence in estimates of recent migration ancestry. Unfortunately, the apparent low abundance and/or low detectability of *B. constrictor* on these islands meant that it was not possible to increase sample sizes during the study period despite many hours spent in the field searching for snakes.

Recent anthropogenic movement of snakes between islands

It is possible that recent anthropogenic movement of snakes between islands and the mainland could have influenced the results of this study. The rapid growth of tourism in the Bay Islands since the 1990s (Stonich 2000) has resulted in a high volume of

boat traffic moving between the islands and the mainland. Thus, the likelihood of stowaway snakes moving between the islands and the mainland on boats is likely to have increased in recent years, especially if snake collectors were travelling between islands with shipments of snakes. The accidental (or perhaps on occasions deliberate) movement of snakes between islands and the mainland may be a possible explanation for the higher migration rates observed between Utila and the mainland in comparison to the Cayos Cochinos populations.

Boats travelling between Utila and the mainland, especially large boats with cargo holds in which snakes could easily go undetected, are far more numerous than between the Cayos Cochino and the mainland. Therefore, the high migration rate estimates observed between Utila and the mainland may be the consequence of recent anthropogenic movement of snakes on boats. However, this explanation does not explain why migration rate estimates would be high in the direction of Utila to the mainland but low in the opposite direction. In fact, with higher volumes of cargo presumably being transported to the islands from the mainland, one would expect to see the opposite pattern. The obvious positive bias on migration estimates associated with the inclusion of a single sample of suspected Bay Island origin, based on its phenotype and mtDNA haplotype (Chapter 3), is perhaps the most parsimonious explanation of the elevated migration rate estimates observed between Utila and the mainland.

Conclusions

Low levels of population structure were detected within the Cayos Cochinos, but the two islands can clearly be distinguished from one another based on allele frequencies. The two populations have been diverging from one another for

approximately 5,000 years and most likely colonised the islands after their most recent isolation from the mainland by rising sea levels at the end of the last ice age. Cayo Cochino Pequeño and Cayo Cochino Grande can thus be considered as distinct populations with high historical but low contemporary levels of gene flow rather than as a single freely interbreeding population. It is important that conservation planners recognise this level of population structure when developing appropriate management strategies for the Cayos Cochinos.

Estimates of population structure and gene flow between the Cayos Cochinos and other Bay Island populations were hampered by low sample sizes in some of the Bay Island populations and should, therefore, be revisited once a greater number of samples become available for the populations. Also any future study would benefit from the inclusion of samples from other potential sources of gene flow such as Belize and the islands along its coast. However, considering the relatively low level of gene flow and clear population structure observed between the Cayos Cochinos and Utila (the geographically closest Bay island to the Cayos Cochinos and the island population for which sample size was greatest in this study) and also the Honduran mainland, it can be concluded that it is unlikely that the dwarfed phenotype of the Cayos Cochinos *B. constrictor* populations is being maintained in the presence of substantial gene flow from surrounding populations. Rather, it appears that the Cayos Cochinos *B. constrictor* phenotype has likely evolved in the absence of substantial gene flow since the original colonisation of the islands. Management plans for the Cayos Cochinos should aim to prevent the accidental (or deliberate) introduction of snakes to the Cayos Cochinos population from the surrounding Bay Islands and mainland.

Chapter 5 Genetic diversity in a heavily exploited insular population of *Boa constrictor*

Abstract

Unsustainable harvesting of reptiles for the pet trade can have rapid and severe consequences on populations, especially on islands where population size is generally low and recruitment through migration is unlikely. The Hog Island Boa (*Boa constrictor imperator*) was reportedly harvested to the brink of extirpation within a decade of collection beginning in the Cayos Cochinos, Honduras. Conservation efforts appear to be allowing the recovery of the population, however, the genetic impact of passing through such a potentially severe bottleneck, and its implications for the long-term survival of the population were unknown. Both remaining wild populations of Hog Island Boa in the Cayos Cochinos were assessed for levels of genetic diversity at eight polymorphic microsatellite loci and compared to the mainland and one other nearby island population of *Boa constrictor*. Genetic diversity was lower in the island populations, but not as low as reported for other severely bottlenecked populations of snakes. Tests using *M*-ratio provided strong evidence for a bottleneck, but there was only weak evidence of a bottleneck using tests that examine heterozygosity excess. A substantial historical decline in effective population size detected using the program *MSVAR* was attributed to the initial founder or isolation event of the populations. It appears that the relatively short persistence of the bottleneck, due to increased protection of the populations, may have stemmed the loss of genetic diversity. However, the population remains vulnerable to stochastic processes and continued threat from illegal collection for the pet trade.

Introduction

Global declines in reptile populations are similar in their taxonomic breadth, geographic distribution and severity as those experienced by amphibians, and constitute a worldwide crisis (Gibbons et al. 2000; Beebee et al. 2009). Such declines have been attributed to a number of causes including; habitat loss and degradation, introduced invasive species, environmental pollution, disease and parasitism, unsustainable use and global climate change, as well as enigmatic declines (Gibbons et al. 2000). Although many declines are likely to be the result of a cumulative effect of two or more of these causes (e.g. Nilson et al. 1999; Webb et al. 2002), habitat loss and degradation are often perceived to be the greatest threats to populations (Tilman et al. 1994; Tilman et al. 2001; Triantis et al. 2010). In contrast, the impact of exploitation of reptiles for food, wildlife products and the live animal trade are often overlooked as significant causes of population decline.

Human use and consumption of wild animals, including reptiles, is an integral part of many cultures. However, harvesting must be carried out at a biologically sustainable level in order for both the species and the practice of sustainable use to persist long-term. Article 2 of the Convention on Biological Diversity (CBD) defines sustainable use as "the use of components of biological diversity *in a way* and *at a rate* that does not lead to the long-term decline of biological diversity, thereby maintaining its potential to meet the needs and aspirations of present and future generations". It is clear that for many populations of reptiles, exploitation has been unsustainable and a major cause of their declines (Gibbons et al. 2000).

The relatively recent and rapid expansion in the trade of live reptiles as pets has raised concerns about the sustainability of such commercial exploitation (Schlaepfer

et al. 2005). In recent years, reports of rapid population declines and extirpation events of reptiles have been, at least in part, attributed to unsustainable harvesting for the pet trade (Grismer et al. 1999; Nilson et al. 1999; Webb et al. 2002; Reed et al. 2007). One example is that of the Hog Island Boa constrictor (*Boa constrictor imperator*), an insular dwarfed race endemic to the Cayos Cochinos archipelago, Honduras. The population reportedly experienced severe decline as a result of intense collection for the pet trade throughout the late 1970s and 1980s, during which time thousands of snakes were removed from the islands (Porras 1999; Reed et al. 2007). Just a decade after collection began, a herpetological expedition to the Cayos Cochinos was unable to find a single specimen of this previously abundant snake, and local residents involved in the trade confirmed that as of 1988 virtually all adult boas had been removed from the islands (Wilson & Cruz Diaz 1993).

Fortunately, in 1993 the Cayos Cochinos was declared a protected area and in 1994 the Honduran Coral Reef Foundation (HCRF) was established to facilitate the protection, restoration and sustainable management of the area under the legislative decree 1928-93 (HCRF & TNC 2008). Since this time, the removal of boas has been dramatically reduced, although illegal poaching of boas from the islands remains problematic. A long-term mark-recapture study initiated in 2004 suggests that the population is showing signs of recovery (Reed et al. 2007; but see Chapter 6). However, the long-term genetic impacts on the population of passing through such a substantial bottleneck have not been characterised, forming the bases for this study.

Genetic consequences of small populations and bottlenecks

Apart from the reduction in absolute population size caused by over-harvesting, there is the added danger of a decline in the overall fitness of the population resulting from

reduced genetic variation and associated problems of inbreeding as a consequence of small population size (Keller & Waller 2002). While small populations are expected to display lower levels of genetic diversity than larger populations (Wright 1931; Frankham 1997), recently bottlenecked populations may be at even greater risk due to genetic factors (Williamson-Natesan 2005). A sudden and severe reduction in population size and the greater potential for inbreeding can cause an increase in homozygosity and the expression of deleterious recessive alleles, resulting in inbreeding depression and a rapid reduction in fitness (Crnokrak & Roff 1999; Keller & Waller 2002). These negative effects can further reduce population size and trigger an extinction vortex (Gilpin & Soulé 1986) which may prevent population recovery or long-term survival even after the original source of the population decline has been removed.

Genetic bottlenecks have been implicated in decreased fitness in snakes such as a reduction in litter size, increased birth deformities, chromosomal abnormalities, and reduced juvenile survival (e.g. Madsen et al. 1996; Gautschi et al. 2002; Újvári et al. 2002). Small population size and low genetic diversity also reduces the ability to adapt to local environmental conditions because of the increased influence of genetic drift over selection on allele frequencies (King 2009). In the face of global climate change and the limited dispersal ability of individuals in small isolated populations, maintaining genetic diversity may be critical for successful conservation management. Madsen et al. (1999) demonstrated the importance of such genetic management in conservation by implementing the ‘genetic rescue’ of a population of European Adder (*Vipera berus*).

This study investigates the current levels of genetic diversity in the two remaining wild populations of Hog Island Boa in the Cayos Cochinos, Honduras, and assesses the likelihood that reported over-collection for the pet trade has caused a genetic bottleneck in the populations. The demographic histories of the populations are also examined to look for signs of historical bottlenecks that could explain current patterns of genetic diversity. This information is used to propose appropriate conservation management to ensure the future sustainability of the Hog Island boa in the wild and the findings applied to emphasize the importance of applying the CBD definition of sustainable use not only at the demographic but at the genetic level.

Methodology

Study site

The Cayos Cochinos archipelago lies approximately 17 km off the Caribbean coast of Honduras (Wilson & Cruz Diaz 1993). The two largest islands Cayo Cochino Grande (1.55 km^2) and Cayo Cochino Pequeño (0.64 km^2) support the only wild populations of Hog Island boa (hereafter referred to as boas). Boas were captured by hand between June 2004 and August 2008 and ventral scale clips were taken for DNA extraction. All boas were injected with a Passive Integrated Transponder (PIT) tag for future identification to prevent re-sampling of the same individuals within or between years. A number of tissue samples were also obtained from the nearby Bay Island of Utila and the Honduran mainland for general comparisons of genetic diversity (Figure 5-1). All tissue samples are listed in Appendix 2.

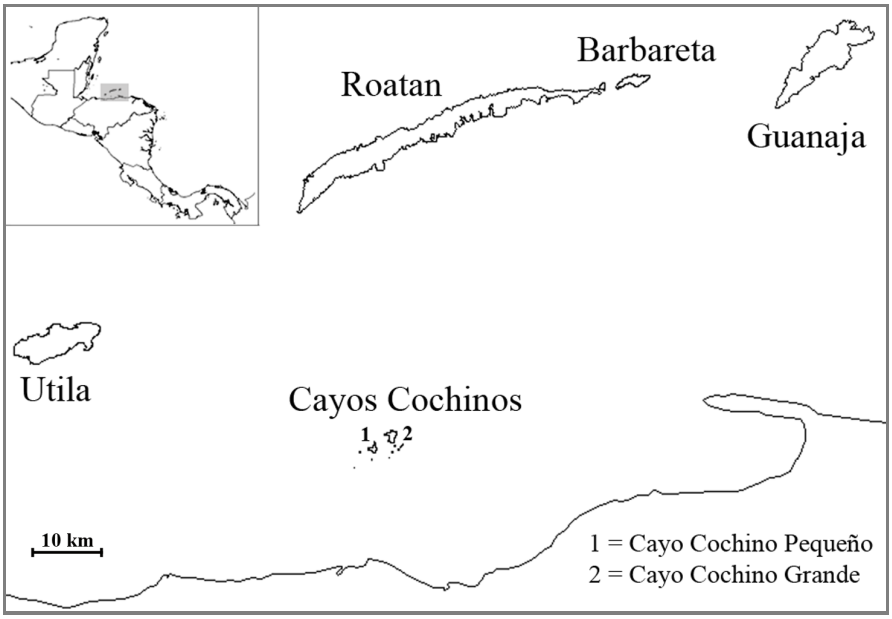

Figure 5-1 Map of the Cayos Cochinos and Bay Island archipelagos and adjacent mainland Honduras coastline

DNA extraction and microsatellite genotyping

DNA extraction from scale clips was performed by one of two methods; using a DNeasy Blood and Tissue Kit (Qiagen Ltd) following the manufacturers protocol, or following an ammonium acetate precipitation method described by Nicholls *et al.* (2000). Multiplex PCR amplification was conducted using the Qiagen Multiplex PCR kit (Qiagen Ltd) following the manufacturers protocol. Individuals were genotyped using five species-specific microsatellite markers (Booth et al. 2011) and three markers developed for the closely related genus *Epicrates subflavus* (Tzika 2007; Tzika et al. 2009) which had previously been found to be informative (Appendix 1). Microsatellite markers were split into four multiplex sets based on annealing temperature, expected product size range and colour of fluorescent modification (Table 5-1). PCR amplification was carried out in 6 µl reaction volumes using 1 µl of DNA template under the following reaction conditions; 95 ^{o}C for 15min followed by 30 cycles of 94 ^{o}C for 30s, multiplex specific annealing

temperature for 90s and elongation at 72 ^{o}C for 60s, with a final elongation step of 72 ^{o}C for 30 mins.

PCR products were run on an ABI 3100 automated sequencer (Applied Biosystems, Inc.) and allele sizes were scored using GeneMapper 3.7 (Applied Biosystems, Inc.) and then confirmed by visual inspection. Between 3-15% of samples were repeated at each locus to assess the likelihood of genotyping error.

Table 5-1 Microsatellite loci multiplex sets and corresponding annealing temperatures

Multiplex set	Microsatellite Loci	Annealing Temperature (^{o}C)
A	µsat36 and µsat20	51
B	µsat01 and Bci-21	55
C	Bci-14 and Bci-23	60
D	Bci-15 and Bci-18	54

General tests for loci

Genotype data were examined by eye to confirm that markers were not sex-linked in the heterogametic sex (females). The presence of null alleles was tested in all loci using the software *CERVUS* 3.0 (Kalinowski et al. 2007). Null alleles result when mutations occur at primer binding sites, causing certain alleles not to amplify (Shaw et al. 1999). *Micro-Checker* 2.2.3 (Van Oosterhout et al. 2004) was also used to check for the presence of null alleles, large allele dropout and scoring error due to stuttering. Large allele dropout is caused by the preferential amplification of shorter alleles during PCR (Wattier et al. 1998). Stuttering is a consequence of slippage during PCR amplification which can cause ‘stutter’ products that differ in size from the true allele by a multiple of the repeat motif length (Shinde et al. 2003). These

stutter products can make it difficult to identify heterozygotes from homozygotes, especially in dinucleotide loci.

Hardy-Weinberg Exact tests were performed using default settings of the web based version of *Genepop* 4.0.10 (Raymond & Rousset 1995; Rousset 2008). The probability test was used to check for deviations from equilibrium, followed by tests for heterozygote deficiency and excess to establish the direction of any violation of Hardy-Weinberg. Linkage disequilibrium was investigated using the default parameters of option 2 of web based version of *Genepop* 4.0.10; populations were tested independently and a Bonferroni correction applied at the P=0.05 level of significance.

Genetic diversity

Observed (H_o) and expected (H_e) levels of heterozygosity were calculated for each population in *CERVUS* 3.0 (Kalinowski et al. 2007). Allelic richness (*A*), adjusted for sample size, and Weir and Cockerham's (1984) within subpopulation inbreeding coefficient (F_{is}) were calculated in *FSTAT* 2.9.3.2 (Goudet 1995).

Detection of a genetic bottleneck

The island populations of Cayo Cochino Grande and Cayo Cochino Pequeño were analysed for the detectable presence of a recent decline in effective population size (N_e) using the program *BOTTLENECK* version 1.2.02 (Piry et al. 1999). If either population has experienced a recent decline in N_e, significantly more than 50% of loci should display observed heterozygosities greater than that predicted under mutation-drift equilibrium (Cornuet & Luikart 1996). Data were analysed using the Wilcoxon signed rank test for 1,000 iterations of each of the three following models implemented in the program; infinite alleles model (IAM), stepwise mutation model

(SMM) and two-phased model (TPM). The TPM was run under the generic parameters of 12% variance and 90% SMM, parameters that are frequently used in comparable studies for bottleneck detection (e.g. Busch et al. 2007; Marshall Jr et al. 2009). The mode-shift test was also applied to determine if the allele frequency distribution of each population was approximately L-shaped, as expected under mutation-drift equilibrium. Recent bottlenecks provoke a mode-shift and a departure from an L-shaped distribution (Luikart & Cornuet 1998).

The *M*-ratio test was also employed to detect the genetic signature of a bottleneck in the Cayos Cochinos populations. The *M*-ratio is the mean ratio of the number of alleles (*k*) to the range in allele size (*r*). During a bottleneck there should be a reduction in the *M*-ratio (*M*=*k*/r) due to rare alleles being lost from the population, thus reducing the number of alleles (*k*) more quickly than the size range of those alleles (*r*) (Garza & Williamson 2001). *M*-ratios were calculated using the software M_P_VAL (Garza & Williamson 2001). Observed values of *M* were compared to a distribution of values from a simulated population given the sample sizes and chosen mutation model (TPM). Critical values of *M* (M_c) are set at the lower 5% of this theoretical distribution. The program *CRITICAL_M* (Garza & Williamson 2001) was used to calculate values of M_c in which the parameters θ, P_g and Δ_g can be varied. Observed *M*-ratios were also compared to the empirical estimate of *M*=0.68 calculated by Garza and Williamson (2001), below which populations can be assumed to have undergone a recent reduction in size.

Theta (θ) is calculated as four times the pre-bottleneck effective population size (N_e) multiplied by the mutation rate (μ) ($\theta = 4(N_e\mu)$). A microsatellite mutation rate of 5.0 x 10^{-4} was selected as recommended by Garza and Williamson (2001) and as used in

similar studies of snakes (e.g. Marshall Jr et al. 2009). Since pre-bottleneck N_e is rarely known for a population, it is common practice to run the analyses under a number of different values of θ to reflect a range of biologically plausible values for N_e. Although pre-bottleneck N_e was unknown for each of the islands, the current adult census population size for Cayo Cochino Pequeño has been estimated through mark-recapture records at approximately 700 individuals (Chapter 6). Based on the rough approximation of effective population size ratio of $N_e/N = 0.7$ found in other populations of snakes (King 2009), current N_e for Cayo Cochino Pequeño may be in the region of 490 individuals. Thus, the lower value for pre-bottleneck N_e was set at 500 individuals for both islands, corresponding to a value of 1 for θ. However, analyses for both populations were run at θ = 1, 2, 4, 8, 12 and 20 to reflect a broad range of values for pre-bottleneck N_e (Table 5-7), in order to explore the effect of different values of θ on M_P_VAL output.

Values of Δ_g (the average size of non one-step mutations) were also varied, using the values 2.8 (mean value determined from a literature review by Garza and Williamson (2001), 3.5 (default value), and 5.0, following the methodology of Busch et al. (2007). P_g (the proportion of one step mutations) is thought to be less influential than θ or Δ_g and was set at the generally accepted mean value of 0.12 for microsatellites as determined in a literature review by Garza and Williamson (2001).

Microsatellite locus Bci-23 was eliminated from *BOTTLENECK* and *M*-ratio analyses due to the fact it was monomorphic in the Cayo Cochino Grande population and one of the two alleles found in the Cayo Cochino Pequeño population was almost at fixation (frequency of allele 214 = 0.995).

Historical demographic decline

MSVAR 1.3 (Beaumont 1999; Storz & Beaumont 2002) was used to model the demographic histories of the Cayos Cochinos populations. The method uses a coalescent-based analysis of microsatellite variation using a hierarchical Bayesian model based on Markov chain Monte Carlo (MCMC) simulations to estimate the posterior distribution of genealogical and demographic parameters. These parameters include the current effective population size (N_0), ancestral effective population size (N_1) and the time at which the inferred population decline or expansion began (χ_a).

Data files were created as outlined in the MSVAR readme file (Beaumont 1999). Alleles were coded as the relative number of repeat units, with the first allele always coded as zero. The Cayo Cochino Grande data were analysed as a single data set, however, the computationally intensive methodology and the large size of the Cayo Cochino Pequeño data set meant it was necessary to use the *sinf.exe* program provided with *MSAVR 0.4.1* (Beaumont 1999) to produce a number of smaller representative data sets from the original. Three independent samplings were made from the Cayo Cochino Pequeño data set using a sample size of 250, resulting in three data sets, each equivalent to 125 individuals. Thus four data sets were analysed, one for the Cayo Cochino Grande population (n=76) and three for the Cayo Cochino Pequeño population (n=125). Data sets are displayed in Appendix 9.

Microsatellite locus Bci-23 was dropped from the analysis, partly due to its low level of diversity, but mainly due to the need to construct a data file in the form of number of repeat units, rather than total allele size. It is not currently possible to accurately include compound microsatellite markers with different size repeat units within the same locus. Locus Bci-23 comprises a di-tetra compound repeat motif and thus the

number of repeats could not be scored accurately. Unsurprisingly, in original trials where Bci-23 was included in the Cayo Cochino Pequeño analysis, the locus was found to be behaving inconsistently when compared with the other seven loci (Appendix 10). Compound loci with repeat units of equal size are not a problem as allele size accurately reflects number of repeat units.

Setting the priors

Due to the potential influence of the priors on the posterior probability distributions for key parameters, each data set was run under a number of different simulations to test that priors were not influencing the result. The effect of the population parameter priors N_0 and N_1 were investigated by simulating three different historical demographic scenarios; stable population over time ($N_0=N_1$), population decline ($N_0<N_1$), and population expansion ($N_0>N_1$). The effect of the prior χ_a was also investigated by simulating three scenarios consisting of a comparatively recent (100 yrs), intermediate (100,000 yrs) and ancient (1,000,000 yrs) onset of the inferred population change. A summary of the prior means used in these runs can be seen in Table 5-2. All priors are reported as means ± 1SD (Log_{10} scale). The prior mean for mutation rate was set at a value of -3.5 for all runs in order for the prior distribution to cover a range in accordance with current information for vertebrate microsatellite loci (King 2009). Generation time was set at 4 years, which was taken to be a realistic average for males and females based on current knowledge of mainland and island *Boa constrictor* populations in captivity and in the wild (Reed et al. 2007; Russo 2007; Reed & Rodda 2009). Hyper priors were set at the default settings.

Table 5-2 Prior means used in each of the population demographic simulations in MSVAR, reported as means ± 1SD (Log_{10} scale)

	Priors (Log_{10})		
Demographic Scenario	N_0	N_1	χ_a
Stable population	4.0	4.0	5.0
Declining population	3.0	5.0	5.0
Expanding population	5.0	3.0	5.0
Recent onset of change	4.0	4.0	2.0
Intermediate onset of change	4.0	4.0	5.0
Ancient onset of change	4.0	4.0	6.0

Analyses were conducted using the online *MSVAR* facility provided by the Computational Biology Service Unit at Cornell University (MSVAR@BIOHPC). Each analysis was run for a total of 3×10^9 iterations and parameter chains were sampled every 1×10^5 iterations. Five independent runs, starting from different randomly generated seeds, were carried out for each scenario to check for convergence between runs. Convergence was examined by a combination of viewing posterior probability trace plots for each parameter and performing the Gelman and Rubin, and Heidelberger and Welch convergence diagnostics in the R (R Development Core Team 2009) package CODA (Plummer et al. 2006).

A 'burnin' of 150,000 iterations (50% of the MCMC) was applied to all runs to ensure that only the stationary part of the chain was sampled for estimation of posterior probabilities. The remaining 150,000 iterations of each of the five replicates were then combined and used to calculate summary statistics. Mean, median and modal values for posterior distributions were calculated in R using LOCFIT (Loader 1996) and used as point estimates of demographic parameters.

Highest probability density (HPD) intervals (95%) for each parameter were calculated in CODA.

Because likelihoods from separate loci are not combined in a multiplicative fashion in the model, any aberrant loci should not strongly influence the results (Storz & Beaumont 2002). However, the low number of markers in this study could bias the results if any loci were found to be behaving irregularly. Therefore, the posterior densities of parameters for individual loci were also examined to check for aberrant loci.

Results

Genetic data analysis

A total of 596 boas were successfully genotyped for at least six of the eight polymorphic microsatellite loci (Table **5-3**). Full genotype data for all samples are provided in Appendix 2. Samples in which more than two loci failed to amplify were excluded from analyses. Where samples were genotyped twice, the same genotype was obtained on both occasions in 100% of cases.

Examination of genotype data in the heterogametic sex (females) detected no evidence of sex-linkage between loci. Analysis of the Cayos Cochinos and mainland Honduras populations with *CERVUS* found null allele frequencies to be within acceptable limits (F-null<0.15) (Dakin & Avise 2004), however, sample size was insufficient in the Utila population (n=7) to test for null alleles. Analysis using *Micro-Checker* found no evidence of null alleles for any locus in the Cayo Cochino Grande population. However, null alleles may be present at locus Bci-14 and Bci-21 in the Cayo Cochino Pequeño population and at locus Bci-18 in the Utila and Honduras mainland populations, as is suggested by the general excess of

homozygotes for most allele size classes at these loci. However, this outcome was not deemed significant by the program and thus no adjusted allele frequencies were calculated. None of the loci in any of the populations displayed significant problems with large allele dropout or scoring error due to stuttering.

Exact tests for Hardy-Weinberg equilibrium revealed no violation in the mainland Honduras population. However, a number of the loci were found to violate Hardy-Weinberg equilibrium in the Cayos Cochinos and Utila populations (Table 5-4). In all cases, this was due to heterozygote deficiency at the locus.

Table 5-3 Numbers of individuals genotyped across microsatellite loci in each population

		Individuals genotyped			
Microsatellite Loci	Repeat motif	C. Pequeño	C. Grande	Utila	Honduras ML
Bci-14	$(AAGA)_n(AGGA)_n$	495	76	7	11
Bci-15	$(TATC)_n$	490	76	7	11
Bci-18	$(CCTT)_n$	470	76	7	11
Bci-21	$(AG)_n$	497	76	7	11
Bci-23	$(TCTG)_n(TC)_n$	497	76	6	11
µsat01	$(AGAT)_n$	499	76	7	11
µsat20	$(CTC)_n$	483	75	7	11
µsat36	$(CTTT)_n(CTTC)_n$	486	75	7	11

Table 5-4 Hardy-Weinberg Exact Test, results from the probability-test (1,000 iterations). P-values <0.05 (in bold) indicate violation of Hardy-Weinberg equilibrium. Missing P-value indicates locus was monomorphic in that population.

	Probability test P-value							
Population	Bci-14	Bci-15	Bci-18	Bci-21	Bci-23	μsat01	μsat20	μsat36
Cayo Pequeño	**0.000**	0.566	0.038	**0.046**	1.000	**0.007**	**0.000**	**0.003**
Cayo Grande	**0.030**	0.035	**0.001**	0.242	-	0.789	0.180	0.358
Utila	0.945	0.652	**0.001**	1.000	1.000	**0.035**	0.628	0.646
Mainland Honduras	0.494	0.739	0.075	0.514	0.545	0.849	1.000	0.179

No evidence of linkage disequilibrium, after Bonferroni correction, was found between loci in any of the populations tested, with the exception of Cayo Cochino Pequeño. A number of loci were found to be linked in this population (Table 5-5), however, since these loci were not found to be linked in any of the other populations, this result suggests that intrachromosomal linkage was not effecting the overall analyses. When only a subset of individuals from Cayo Cochino Pequeño were analysed (n=30) no linkage was detected. The presence of linkage disequilibrium in the Cayo Cochino Pequeño full data set does, however, suggest that some level of population structure may be present within the island.

Table 5-5 Loci found to be in linkage disequilibrium in the Cayo Cochino Pequeño population following Bonferroni correction (x = loci in linkage disequilibrium)

	Bci-14	Bci-15	Bci-18	Bci-21	Bci-23	µsat01	µsat20
Bci-14	-						
Bci-15	-	-					
Bci-18	x	-	-				
Bci-21	-	-	-	-			
Bci-23	-	-	-	x	-		
µsat01	x	-	x	-	-	-	
µsat20	-	-	-	x	x	x	-
µsat36	x	x	x	x	x	x	-

Patterns of genetic diversity

Observed and expected heterozygosities and allelic richness were lower in the island populations than on the mainland. However, inbreeding coefficients were lowest in the Cayos Cochinos populations, with Cayo Cochino Grande actually displaying a moderately negative F_{is} value (Table 5-6).

Table 5-6 Sample sizes and summary statistics of genetic diversity within each population. H_o, mean observed heterozygosity (SD); H_e, mean expected heterozygosity (SD); *A*, mean allelic richness adjusted for sample size (range); F_{is}, inbreeding coefficient for the population (positive values indicate inbreeding and negative values indicate outbreeding, values in bold indicate significant at $P<0.05$).

Population	Sample Size	H_o	H_e	*A*	F_{is}
Cayo Pequeño	470-499	0.54 (0.32)	0.54 (0.32)	3.74 (1.06-5.97)	0.006
Cayo Grande	75-76	0.56 (0.30)	0.54 (0.30)	3.73 (1.00-7.09)	**-0.045**
Utila	6-7	0.58 (0.32)	0.65 (0.25)	4.06 (1.99-6.42)	0.127
Honduras Mainland	11	0.82 (0.21)	0.83 (0.16)	6.65 (2.00-10.71)	0.018

Detection of a population bottleneck

Analyses using *BOTTLENECK* failed to provide strong support for a recent reduction in effective population size in either of the Cayos Cochinos populations (Table 5-7). In the Cayo Cochino Pequeño population, the proportion of loci with a heterozygosity excess ($H_E > H_{EQ}$) was not significantly greater than that predicted under mutation-drift equilibrium under any of the models (all P>0.05). In fact under the SMM and TPM a significant deficiency in heterozygosity was detected (P=0.004, P=0.010 respectively). In the Cayo Cochino Grande population the proportion of loci with a heterozygosity excess was significantly greater than that predicted under mutation-drift equilibrium under the IAM (P=0.027), but not under the SMM or TPM (both P>0.05). Allele frequency distributions of each population were approximately L-shaped, as expected under mutation-drift equilibrium. Thus, overall there was little support for a recent decline in effective population size except in the Cayo Cochino Grande population when applying the IAM.

All *M*-ratio values in both populations, regardless of the pre-bottleneck effective population size, were consistently found to be below the critical value threshold when using values of Δ_g of 2.8 and 3.5. Thus population decline was supported under these two parameter sets. However, when Δ_g was set to the more conservative value of 5.0, the signature of a bottleneck was lost, with *M*-ratio values in both populations consistently greater than the critical value (Table 5-7).

Comparing the *M*-ratio of each population to the empirical estimate of *M*=0.68 for bottlenecked populations calculated by Garza and Williamson (2001) revealed the Cayo Cochino Grande population to have undergone a recent reduction in size,

whereas the value obtained for the Cayo Cochino Pequeño population is just above this threshold.

Table 5-7 Results of M-ratio and Bottleneck analysis for the Cayo Cochino Pequeño and Cayo Cochino Grande populations

Population	*M*-ratio						Bottleneck		
				M_c					
	N_e	θ	*M*-ratio	Δ_g 2.8	Δ_g 3.5	Δ_g 5.0	Mode shift	Mutation Model	Het. excess
C. Pequeño	500	1	0.715	0.805	0.740	0.641	NS	IAM	NS
	1,000	2	0.715	0.800	0.720	0.590		TPM	NS
	2,000	4	0.715	0.802	0.717	0.577		SMM	NS
	4,000	8	0.715	0.809	0.726	0.585			
	6,000	12	0.715	0.810	0.737	0.599			
	10,000	20	0.715	0.814	0.749	0.621			
C. Grande	500	1	0.667	0.795	0.739	0.654	NS	IAM	P=0.027
	1,000	2	0.667	0.781	0.711	0.600		TPM	NS
	2,000	4	0.667	0.771	0.692	0.565		SMM	NS
	4,000	8	0.667	0.767	0.685	0.554			
	6,000	12	0.667	0.761	0.685	0.555			
	10,000	20	0.667	0.752	0.685	0.557			

N_e, pre-bottleneck effective population size; $\theta = 4(N_e\mu)$ where μ is the microsatellite mutation rate 5.0 x 10^{-4} as recommended by Garza and Williamson (2001); Δ_g, average size of non one-step mutations; M_c, critical value of *M*; IAM, infinite alleles model; TPM, two phase model (12% variance and 90% SMM); SMM, stepwise mutation model; NS, not significant (P>0.05).

Historical demographic decline

All seven loci used in the analysis were found to be consistent with one another for each of the key parameters. Variation was greatest for the parameter N_0, but overall the loci gave the same broad consensus. Posterior density plots for individual loci are provided in Appendix 11.

Testing for convergence and run length

Within population demographic scenarios, posterior parameters for each of the five replicates were similar and the Gelman Rubin diagnostic indicated reasonable convergence of the chains (PSRF $\leq$ 1.07). However, better convergence was obtained in the 'stable' population scenario runs (PSRF $\leq$ 1.04) which were used for calculation of final summary statistics. Heidelberger and Welch's convergence diagnostic indicated that most of the parameter chains had reached convergence and that chain length was sufficient for calculating the mean with acceptable accuracy. However, in some instances a longer run was advised. Unfortunately, it was not possible to run longer analyses due to the upper limit of run allocation time (4 days) on the BioHPC server having already been reached. Because of the conservative nature of such convergence diagnostics and the fact that only the last 50% of the chains were used for calculating summary statistics, the minor violation of convergence within chains in some cases was not considered to be of great concern, providing convergence between chains was achieved.

Testing between demographic population change scenarios

Manipulation of the priors for N_0, N_1 and χ_a had no influence on the posterior distributions of these parameters, hence also giving evidence that convergence had

been reached. Posterior density plots for each scenario of population change and the time at which change began are provided in Appendix 11.

Evidence of historical population decline

Strong support was found for historical population decline in both the Cayo Cochino Grande and Cayo Cochino Pequeño populations, with the same trend and magnitude of decline found in both populations (Figure 5-2 and Figure 5-3). All three of the sample data sets for Cayo Cochino Pequeño gave consensual results (Figure 5-2) and were, therefore, combined for calculating summary statistics (Table 5-8), however, see Appendix 12 for a breakdown of the results for each of the three Cayo Cochino Pequeño data sets. The Bayes factor in favour of decline, as described in Beaumont (Beaumont 1999), was 1,315 and 149,476 for Cayo Cochino Grande and Cayo Cochino Pequeño respectively, giving very strong support for a population contraction in both populations (Kass & Raftery 1995). Posterior estimates for the time at which this decline started (χ_a) were in approximate agreement for Cayo Cochino Grande and Cayo Cochino Pequeño. However, as is common in these types of analyses, HPD intervals were large for estimates of all parameters.

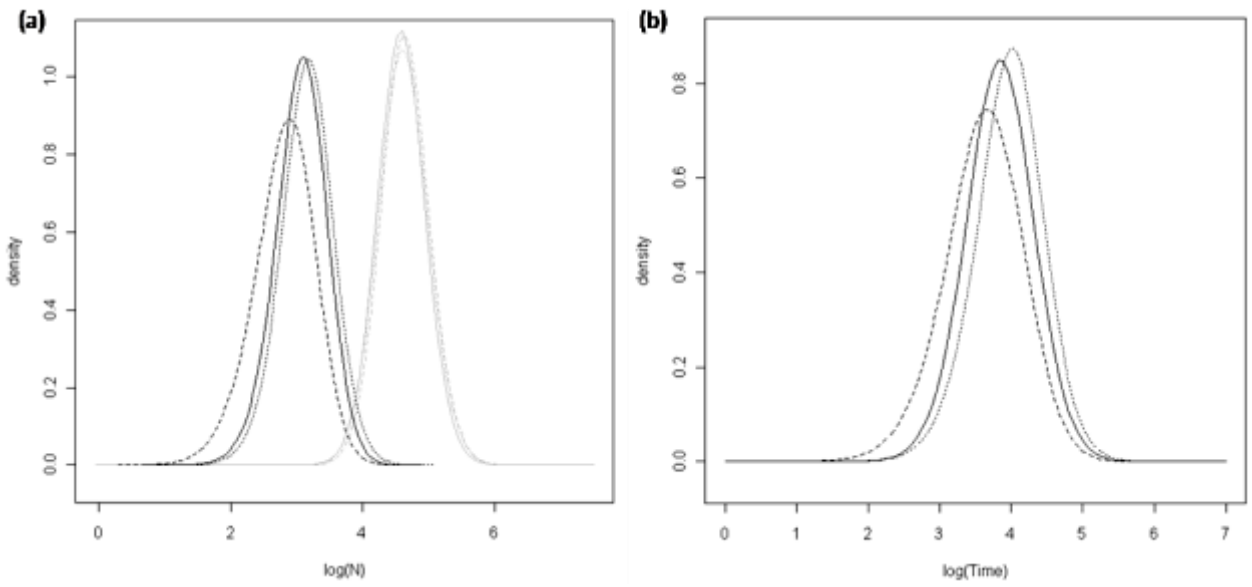

Figure 5-2 The posterior density plots for Cayo Cochino Pequeño show (a) ancestral population size (N_1) (grey lines) plotted against current population size (N_0) (black lines), and (b) the time at which the inferred population decline began. Solid lines = CCP data set 1, dotted lines = CCP data set 2, and dashed lines = CCP data set 3.

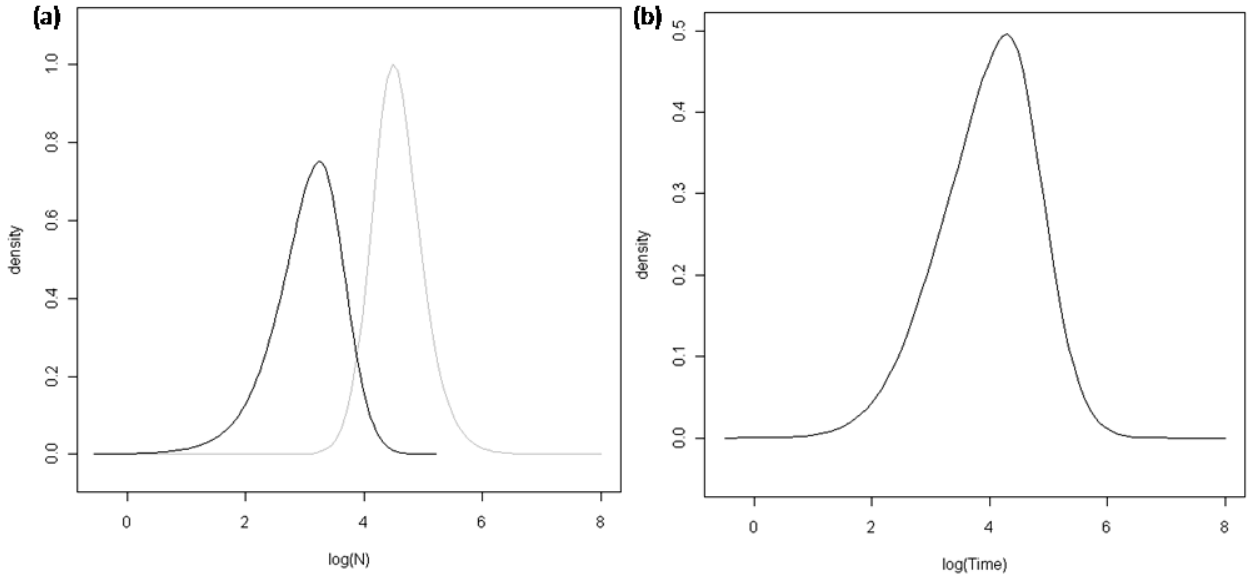

Figure 5-3 The posterior density plots for Cayo Cochino Grande show (a) ancestral population size (N_1) (grey line) plotted against current population size (N_0) (black line), and (b) the time at which the inferred population decline began

Table 5-8 Posterior estimates of demographic parameters (presented here after transformation from Log_{10} scale)

Data Set	Parameter	Mean	Median	Mode	HPD Intervals (95%)	
					lower	upper
Cayo Pequeño	N_0	1,019	1,082	1,308	130	7,412
Cayo Grande		1,114	1,313	1,162	69	13,880
Cayo Pequeño	N_1	40,832	40,524	37,236	7,631	22,0629
Cayo Grande		36,559	34,429	23,408	5,233	283,230
Cayo Pequeño	χ_a	6,412	6,787	2,554	552	68,536
Cayo Grande		9,226	11,302	19,876	186	325,337

N_0 = Current effective population size, N_1 = Ancestral effective population size, χ_a = Time in years since the start of the inferred population decline.

Discussion

Genetic diversity

Observed and expected heterozygosity and allelic richness was lower in the island populations than on the mainland, conforming to previous predictions about genetic diversity in small island populations (Wright 1931; Frankham 1997). Interestingly, levels of observed heterozygosity were comparable in both the putatively bottlenecked Cayos Cochinos populations and Utila, but allelic richness was lowest in the Cayos Cochinos populations. Levels of expected heterozygosity of all populations fell comfortably within the range of values previously reported for snakes (0.35-0.87), with the Cayos Cochinos populations falling only slightly short of the median value (0.60) (King 2009). Levels of heterozygosity in the Cayos Cochinos were thus found to be surprisingly high for a presumed bottlenecked population and considerably greater than levels found in other bottlenecked

populations of snakes in which low genetic diversity has been a cause for concern (e.g. Madsen et al. 1996; Gautschi et al. 2002; Lukoschek et al. 2005).

Similarly, levels of inbreeding (F_{is}) were found to be low in the Cayos Cochinos populations, with none of the populations showing significantly greater levels of inbreeding than expected. Surprisingly, Cayo Cochino Grande was found to have a significant negative F_{is} value, suggesting mild outbreeding in the population. Nevertheless, all populations were found to have F_{is} values well within the range found in other populations of snakes (-0.11-0.30) (King 2009). The highest level of inbreeding was found in the Utila population, but again, this was not found to be significant. It must be noted, however, that sample size was low for both the Utila and mainland Honduras populations and, thus, results for these populations should be interpreted with a degree of caution. It would also be interesting to see the outcome of a traditional pedigree analysis on the estimated levels of inbreeding in the populations.

Detection of a bottleneck

Analyses using *BOTTLENECK* failed to provide strong support for a recent reduction in effective population size in either of the Cayos Cochinos populations under the heterozygosity excess or mode-shift tests. Heterozygosity excess was only found to be significantly greater than expected under equilibrium in the Cayo Cochino Grande population under the IAM. An important distinction here is that heterozygosity excess should not be confused with the *excess of heterozygotes.* The former compares observed and expected *heterozygosities, based solely on the number of alleles* present, irrespective of the actual allele frequencies, whereas the

latter compares the *number of heterozygotes* with Hardy-Weinberg equilibrium expectation (Cornuet & Luikart 1996).

Heterozygote deficiency was detected in the Cayo Cochino Pequeño population under both the SMM and TPM. Cornuet and Luikart (1996) note that although heterozygote deficiency can be apparent in a bottlenecked population under the SMM (depending on the variability of the locus and the time since the bottleneck event) it is rarely apparent in bottlenecked populations under the TPM. Because the TPM is likely to be a more accurate model of microsatellite evolution (Cornuet & Luikart 1996), the significant level of heterozygosity deficiency detected in the Cayo Cochino Pequeño population appears to be evidence against a genetic bottleneck. However, heterozygosity deficiency can also be the product of an expanding population; therefore, population recovery may be masking the signal of decline.

In *M*-ratio analysis, a significant bottleneck was detected in both populations under all assigned values of θ when Δ_g was set to 2.8 and 3.5. Only when Δ_g was set to the more conservative value of 5.0 was the signature of a bottleneck lost. Increasing Δ_g is known to reduce the ability to detect a bottleneck because of its affect on lowering the value of M_c and thus increasing the required severity of the bottleneck through which the population must have passed in order to be considered significant. Taking this into account, *M*-ratio analysis appears to provide strong support for a bottleneck in both populations under the most frequently used and accepted values of Δ_g (Garza & Williamson 2001). However, it fails to detect the presence of a more severe bottleneck at Δ_g=5.0 as experienced in other populations of snakes (Marshall Jr et al. 2009).

Conflicting results between multiple methodologies for bottleneck detection are not uncommon. Pearse *et al*. (2006) found that half of the populations of the Amazon river turtle (*Podocnemis expansa*) they investigated showed evidence of a bottleneck when tested for heterozygosity excess, whereas all populations showed evidence of a bottleneck when tested with *M*-ratio. Similarly, Marshall Jr. *et al*. (2009) found no evidence of a bottleneck in populations of copperbelly water snake (*Nerodia erythrogaster neglecta*) using either the mode-shift or heterozygosity excess tests, however, almost all *M*-ratio tests gave evidence of a bottleneck.

Williamson-Natesan (2005) in a comparison of the heterozygosity excess and *M*-ratio methodologies found that the heterozygosity excess approach was most likely to correctly identify recent, relatively small bottlenecks where initial population size had been small. Whereas, the *M*-ratio approach was found to be more successful at detecting bottlenecks where the initial population size was large, the reduction in effective population size had lasted several generations and the population had since recovered. The mode-shift test was found to be a conservative test with limited power to detect bottlenecks (Williamson-Natesan 2005). *M*-ratio may, therefore, be a more appropriate test, based on anecdotal evidence of the scale of decline and subsequent recovery of the population. But, care is needed to avoid incorrectly concluding the population has passed through a bottleneck (Williamson-Natesan 2005).

A greater number of polymorphic loci may have increased the chances of bottleneck detection with the heterozygosity excess approach. Computer simulations show that a minimum of 10 polymorphic loci and 30 individuals are necessary in order to have >80% chance of accurately detecting a bottleneck when the N_e before : after ratio is

≥100 (Cornuet & Luikart 1996). However, studies with fewer than 10 loci have successfully identified bottlenecks with this method (e.g. Cornuet & Luikart 1996; Pearse et al. 2006; Spear et al. 2006). It is possible that it is too early for a bottleneck to be detected using this method. Computer simulations suggest the most sensitive time for detecting the bottleneck is at 2 x N_e generations after the decline (where N_e is the post bottleneck effective population size) (Beebee & Rowe 2004).

Alternatively, the severity of a bottleneck (and therefore the chances of detecting it) is not only dependent on the absolute reduction in effective population size, but on the duration that the bottleneck persisted. A population that experiences a rapid decline followed by a rapid recovery may show very little signature of a bottleneck, whereas a population that remains at a severely reduced effective population size for a relatively longer period will likely show a far greater reduction in genetic diversity (Beebee & Rowe 2004). It is possible, and perhaps encouraging therefore, that the rapid recovery of the Cayos Cochinos population, due to recent increased protection, has successfully retained much of the original genetic diversity.

Historical demographic decline

Whereas the above methods are specifically designed to detect relatively recent and sudden declines in effective population size, Beaumont's (1999) method implemented in MSVAR was used to look for historical population demographic trends that might help explain current observed levels of genetic diversity. Although the broad confidence limits prevent the precise estimation of N_1, N_0 and χ_a, it is evident that both populations have undergone a substantial historical contraction in effective population size and that this contraction broadly coincides with the time at

which the islands would have been isolated from the mainland and Utila by rising sea levels (Bermingham et al. 1998; McCranie et al. 2005).

Estimates of historical effective population sizes are obviously unrealistically high if interpreted as the historical population of boas in the Cayos Cochinos alone; it is clear that the islands are simply too small to have ever supported such a large population. Instead, historical effective population size is interpreted as the size of the population prior to the isolation of the Cayos Cochinos by rising sea levels at the end of the Pleistocene. The observed reduction in effective population size can thus most likely be interpreted as evidence of the original founder or isolation event of the Cayos Cochinos populations. Although historical effective population size estimates are not useful in directly assessing the impact of harvesting for the pet trade, they are useful in identifying the likely reduction in effective population size that occurred as a result of the founder event of the Cayos Cochinos populations. Therefore, estimates of current effective population size are likely to be a good indicator of pre-harvest population sizes within the Cayos Cochinos.

Care must be taken, however, with the interpretation of the *MSVAR* results due to the possible violation of one of the key assumptions that is often overlooked in its application. Migration and population structure are assumed to be absent, however, instances where both these demographic traits are absent, are rare. The islands were treated separately in the analysis and it was assumed that the existence of significant within-population structure would be unlikely on either Cayo Cochino Grande or Cayo Cochino Pequeño due to their very small size (1.55 and 0.64 km^2 respectively). However, linkage disequilibrium observed between a number of loci in the Cayo Cochino Pequeño population suggest that population structure may well exist within

the island. It is possible that if harvesting was not even across the island then separate remnant populations may have since expanded and resulted in unexpected within-island population structure. Population structure analysis should, ideally, be performed to establish if this is the case or not.

Also, due to the islands close proximity to one another it is very unlikely that geneflow can be ruled out between Cayo Cochino Grande and Cayo Cochino Pequeño. In fact, gene flow (although at relatively low levels) has been demonstrated between the islands (Chapter 4). In this case it is slightly unclear as to whether current effective population sizes may in fact be better estimates of the Cayos Cochinos as a metapopulation rather than of each individual island. Future exploration of this issue using simulated data may allow a greater understanding of the affects these parameters may have on estimation of posterior parameters (Beaumont 2010). In support of the analyses presented here, genetic estimates of current effective population size were broadly in agreement with estimates of adult census size from mark-recapture data (Chapter 6), thus giving some confidence in the qualitative interpretation of these findings.

Conclusions

It was feared that rapid and severe population decline of the Hog Island Boa, caused by unsustainable collection for the pet trade in the 1980s, may have significantly impoverished the genetic diversity of the wild population. Although the genetic signature of a bottleneck was detected, general levels of genetic diversity do not appear to be of immediate conservation concern. Loss of genetic diversity may have been minimized by the rapid recovery of the population in response to increased protection provided after the creation of the Cayos Cochinos Protected Area.

However, it may be too soon to determine the full extent and long-term impact on the overall fitness and survival of the population. Given sufficient time and the continued enforcement of anti-poaching legislation, the population may well make a full recovery. However, as is always the case with small populations, stochastic processes will have a large part to play in that recovery. The illegal removal of boas from the Cayos Cochinos continues to be problematic and a significant concern for the long-term conservation of the wild Hog Island Boa population.

Chapter 6 Assessing population recovery of a critically exploited insular *Boa constrictor* in the Cayos Cochinos, Honduras.

Abstract

The Hog Island Boa constrictor (*Boa constrictor imperator*) is an insular dwarfed race of snake from the Cayos Cochinos archipelago, Honduras. Prized by reptile collectors for its small size and light 'pink' colouration, the wild population was exploited to the brink of extirpation during the late 1970s and 1980s. However, increased protection of the islands since 1993 is allowing demographic recovery. In 2004 a long-term mark-recapture study was initiated on Cayo Cochino Pequeño to evaluate the current adult population census size (N_c). In addition, molecular methods for estimating effective population size (N_e) were utilised to investigate the relationship of N_e/N_c. During the study period 623 individuals were captured (341 male and 282 female). Annual survival was found to vary between years but not between males and females. Annual detectability varied between years and by sex, with males being more detectable in all years. N_c averaged across all years was 698 (276 male 422 female) with the sex ratio being significantly skewed in the direction of excess females 0.65:1 (males : females). N_e was estimated at 615, giving a N_e/N_c ratio of 0.88. Average density was 10.9 boas per ha, but varied across the island. It appears the Cayo Cochino Pequeño population has undergone a substantial period of recovery since anti-poaching legislation has been enforced. However, the population may not yet have fully recovered to its pre-decline carrying capacity. Furthermore, the small size of the island coupled with high density and ease of detection of boas makes the population particularly vulnerable to future damaging exploitation. Thus, continued vigilance against unregulated collection should be an integral part of conservation management plans for the continued recovery of the population.

Introduction

The secretive nature of most snakes, coupled with their typically low population densities, often results in recapture rates too low to conduct a thorough mark-recapture study (Parker & Plummer 1987). This problem may be particularly true for those populations of highest conservation concern, which have experienced recent or historical population declines. However, some snakes can be particularly good subjects for such studies because they occur at high density and are easily encountered and captured, even though overall population size may be small (Dorcas & Willson 2009).

The Hog Island Boa constrictor (*Boa constrictor imperator*) is an insular dwarfed race of snake from the Cayos Cochinos archipelago, Honduras. The population reportedly experienced severe decline as a result of intense collection for the pet trade throughout the late 1970s and 1980s, during which time thousands of snakes were removed from the islands (Porras 1999; Reed et al. 2007). Just a decade after collection began, a herpetological expedition to the Cayos Cochinos was unable to find a single specimen of this previously abundant snake, and local residents involved in the trade confirmed that as of 1988 virtually all adult boas had been removed from the islands (Wilson & Cruz Diaz 1993).

Fortunately, in 1993 the Cayos Cochinos was declared a protected area and in 1994 the Honduran Coral Reef Foundation (HCRF) was established to facilitate the protection, restoration and sustainable management of the area under the legislative decree 1928-93 (HCRF & TNC 2008). Since this time, the removal of boas has been dramatically reduced and the population appears to be recovering, although illegal poaching of boas from the islands remains problematic.

In 2004, a long-term mark-recapture study was initiated on Cayo Cochino Pequeño, the smaller of the two islands, to estimate current population size in order to determine the level of population recovery and thus the effectiveness of current conservation management. The small size and isolation of the study site, coupled with apparent high density of boas, provided a rare opportunity to conduct such a study and to gather data which will help inform future management of the population.

In addition to adult census population size (N_c), it is valuable to calculate effective population size (N_e). The ratio of N_e/N_c gives the deviation from a perfect population in which all individuals contribute equally to the next generation. Effective population size is typically smaller than census population size and the ratio N_e/N_c is thus <1 in most wild populations (King 2009). It has been suggested that N_e may often be as little as 10% of N_c (Frankham 1995b). The relationship of N_e/N_c is of particular interest for conservation management because of its implication in the rate of loss of genetic diversity, fixation of deleterious alleles and rate of accumulation of inbreeding in small isolated populations (Wright 1969). However, the required demographic data for estimating N_e has often been prohibitively difficult and/or expensive to obtain for wild populations of interest (King 2009), especially for unpopular organisms such as snakes, for which funding is typically limited (Clark & May 2002; Seigel & Mullin 2009). Thus, molecular methods of estimating N_e are becoming increasingly popular because of their relative ease of implementation (Schwartz et al. 1998; King 2009).

Here traditional mark-recapture methodologies for estimating N_c are combined with molecular estimates of N_e based on temporal changes in allele frequencies to assess

the current population size and demographic structure of this critically exploited insular Boa constrictor. Contemporary estimates of effective population size are also compared to historical (pre-decline) estimates (Chapter 4 and Chapter 5) to assess the likely level of recovery of the population.

Methodology

Study site

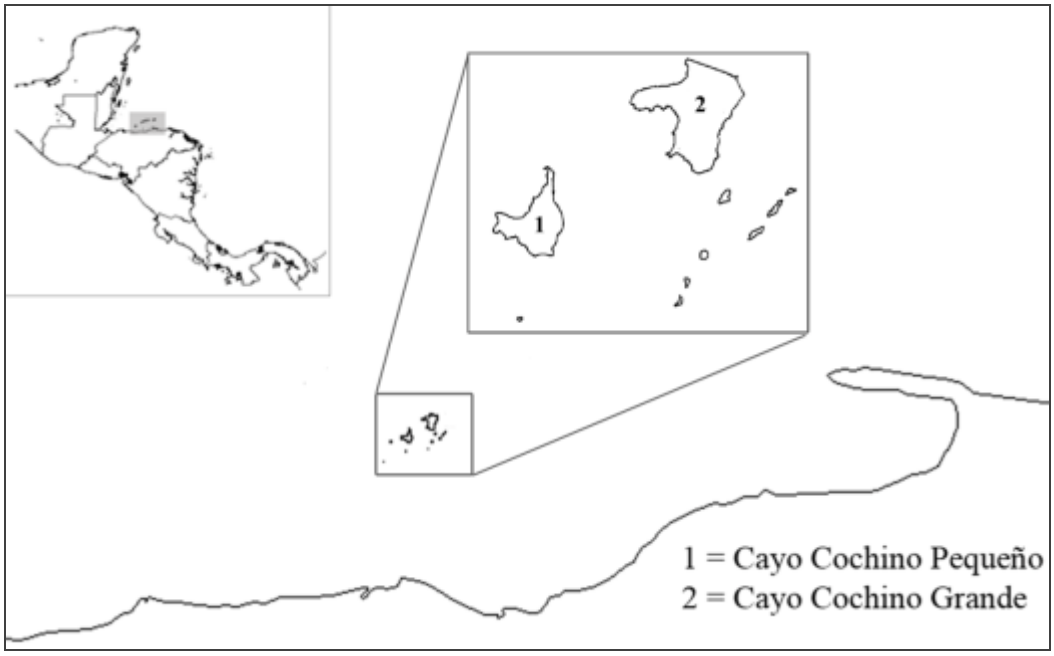

Figure 6-1 Map of the Cayos Cochinos archipelago, situated approximately 17 km off the Caribbean coast of Honduras. The two largest islands, Cayo Cochino Grande and Cayo Cochino Pequeño support dwarf populations of *Boa constrictor imperator*. This study was conducted on Cayo Cochino Pequeño.

The Cayos Cochinos archipelago lies approximately 17 km off the Caribbean coast of Honduras and constitutes part of the Honduran department of Islas de la Bahía (Wilson & Cruz Diaz 1993) (Figure 6-1). The two largest islands, Cayo Cochino Grande (1.55 km^2) and Cayo Cochino Pequeño (0.64 km^2), support dwarfed

populations of *B. c. imperator*, with adult boas averaging around 1 m in snout-vent length (Reed et al. 2007).

Visual Encounter Surveys

Visual encounter surveys (VES) for boas were conducted on Cayo Cochino Pequeño during a number of visits to the Cayos Cochinos between July 2004 and January 2009 (Table 6-1). The number of participants varied between VES depending on the number of volunteers that were available (typically 2-10). However, the number of participants and the time spent searching were recorded for each VES in an attempt to quantify search effort. Experience of participants was also variable, ranging from completely naive to experienced field herpetologists. VES were conducted by volunteers spreading out in a line, approximately evenly spaced with 2-3 m between each person, and slowly walking forward in a predetermined direction whilst searching all available habitat. Refugia such as logs and rocks were lifted, wherever possible, to search for boas and then replaced to minimize habitat disturbance. Trees and vegetation were searched to the best of the observer's ability, but surveys were limited by the maximum height at which an observer could search accurately. Average duration of each VES was approximately 1-2 hours. Search effort was estimated as being the time spent searching multiplied by the number of participants. In addition to boas caught during VES, boas were also captured opportunistically at other times while moving around the island.

All boas encountered were captured by hand and either placed in a cloth snake bag and taken back to the field station for processing, or processed *in situ* and released immediately. Boas that were taken to the field station for processing were released at the exact point of capture within 48 hours. The Universal Transverse Mercator

(UTM) Coordinates of the exact capture site were obtained using a hand-held Global Positioning System (GPS) in order to plot capture locations across the island. If dense canopy cover prevented the obtaining of a strong enough signal at the exact point of capture, a reading was attempted from a nearby location where there was a break in the canopy. In such cases, the direction and distance from the point of capture were estimated and the UTM coordinates adjusted accordingly. In rare instances when it was not possible to obtain a GPS reading, no UTM coordinates were recorded.

Table 6-1 Dates the Cayos Cochinos study site was visited for sampling between 2004 and 2009.

Visit	Year	Season	Start Date	End Date
1	2004	Dry	5th Jul	29th Aug
2	2005	Dry	31st May	2nd Sept
3	2005/2006	Wet	23rd Dec	8th Jan
4	2006	Dry	23rd May	1st Sept
5	2006	Wet	20th Dec	27th Dec
6	2007	Dry	17th Apr	28th Apr
7	2007	Dry	21st May	2nd Sept
8	2007	Wet	28th Dec	31st Dec
9	2008	Dry	3rd Jun	18th Aug
10	2008/2009	Wet	18th Dec	2nd Jan

Processing boas

Snout-vent length (SVL) and tail length (TL) were measured by stretching the snake along a tape measure fixed to the laboratory bench, or if processing in the field, by stretching the tape measure along the snake. Sex was determined by observing the size of the cloacal spurs and the relative length of TL to SVL (males having enlarged

spurs compared to females and relatively longer tails). If sex could not be determined with confidence, sex was confirmed by the use of hemipenial probes. All new captures were implanted with a Passive Integrated Transponder (PIT) tag (11 x 3 mm) and the unique ten digit identification code recorded (Gibbons & Andrews 2004). Subsequent recaptures were identified by scanning boas using a Biomark PIT tag reader. A tissue sample was then taken in the form of 1-3 ventral scale clips from every new snake captured and retained for genetic analysis. Tissue samples were stored in >75% ethanol in screw top eppendorf tubes.

Adult survival and detectability

Program MARK, version 4.3 (White & Burnham 1999) was used to estimate survival and detectability within the Cayo Cochino Pequeño population, using the Cormack-Jolly-Seber (CJS) model. Juveniles (SVL<60 cm at first capture) were excluded from the analysis because of suspected differences in survival and detectability of this age class. Unfortunately, juveniles could not be modelled as a separate group due to insufficient sample size. Only data collected in the sampling periods between May-September were included in analyses with MARK, leaving five independent annual sampling periods between 2004-2008. During each annual sampling period the entire island was searched systematically for boas through numerous weekly VES. Boas were recorded as being present or absent in each annual sampling period. Boas that were recaptured more than once within a season were simply recorded as being present in the sampling period. This permitted annual survival to be calculated. Model notation followed that of Lebreton et al. (1992) as follows:

φ_i = survival probability from time *i* to time *i*+1

p_i = probability of detection at time *i*

(*g*) = group (sex) dependent survival or detectability

(*t*) = time dependent survival or detectability

(.) = Constant survival or detectability

(*g***t*) = both group dependent and time dependent survival or detectability

(*g*+*t*) = survival or detectability varies between groups over time but the difference is due to a constant, additive, component

Twenty five predefined models were run, including additive models, and the fit of the models were assessed using the bootstrap goodness of fit (GOF) in MARK for 1000 iterations. Adjustment values for ĉ were obtained by dividing the observed deviance and ĉ values of the models by the means of the deviances and ĉ values obtained from the 1000 iterations of the GOF (expected values). This resulted in two potential ĉ adjustment values, one obtained from deviance (0.813) and the other obtained from ĉ itself (0.666). It is generally preferable to use values furthest from 1.0 when making a ĉ adjustment on the basis that it is better to take a conservative view and consider the data a poorer fit than a better fit (Cooch & White 2006). However, when both values are <1 the data are under-dispersed and it is advised that ĉ be left as 1.0 and not adjusted (Cooch & White 2006). Therefore, no ĉ adjustment was made. The most parsimonious model was selected, on the basis of Akaike's Information Criterion (AIC) rank and weighting (Burnham & Anderson 1992), and used to estimate annual survival and detectability.

Adult census population size and density

Annual population estimates were calculated using detectability scores from the most parsimonious model and the number of individuals captured within each sampling period based on the equation $N_i = (1/p_i) \times n_i$, where p_i is the within season

detectability estimate and n_i is the number of individuals captured within the sampling period. Average population density was then estimated by dividing census population size by total island area (0.64 km^2). However, because density is unlikely to be uniform across the island, ArcMap version 9.1 and the add-on application Hawth's Analysis Tools version 3.27 were used to create a map of relative density based on capture locations across all sampling periods. This was achieved by dividing the island into 50 m^2 grid squares and counting the number of captures (excluding recaptures) within each square.

Effective population size

In addition to annual adult census population size, calculated from mark-recapture data, effective population size was estimated using DNA extracted from tissue samples taken from boas. DNA extraction from scale clips was performed by one of two methods; using a DNeasy Blood and Tissue Kit (Qiagen Ltd) following the manufacturers protocol, or following an ammonium acetate precipitation method described by Nicholls et al. (2000). Multiplex PCR amplification was conducted using the Qiagen Multiplex PCR kit (Qiagen Ltd) following the manufacturers protocol. Individuals were genotyped using five species-specific microsatellite markers (Booth in press) and three markers developed for the closely related genus *Epicrates subflavus* (Tzika 2007; Tzika et al. 2009) which had previously been found to be informative (Appendix 1). Microsatellite markers were split into four multiplex sets based on annealing temperature, expected product size range and colour of fluorescent modification (Table 6-2). PCR amplification was carried out in 6 µl reaction volumes using 1 µl of DNA template under the following reaction conditions; 95 ^oC for 15 min followed by 30 cycles of 94 ^oC for 30s, multiplex

specific annealing temperature for 90s and elongation at 72 ^{o}C for 60s, with a final elongation step of 72 ^{o}C for 30 mins.

PCR products were run on an ABI 3100 automated sequencer (Applied Biosystems, Inc.) and allele sizes were scored using Genemapper 3.7 (Applied Biosystems, Inc.) and then confirmed by visual inspection. Between 3-15% of samples were repeated at each locus to assess the likelihood of genotyping error.

Table 6-2 Microsatellite loci multiplex sets and corresponding annealing temperatures

Multiplex set	Microsatellite Loci	Annealing Temperature (^{o}C)
A	μsat36 and μsat20	51
B	μsat01 and Bci-21	55
C	Bci-14 and Bci-23	60
D	Bci-15 and Bci-18	54

Genotype data were examined by eye to confirm that markers were not sex-linked in the heterogametic sex (females). The presence of null alleles was tested in all loci using the software *CERVUS* 3.0 (Kalinowski et al. 2007). *Micro-Checker* 2.2.3 (Van Oosterhout et al. 2004) was also used to check for the presence of null alleles, large allele dropout and scoring error due to stuttering. Hardy-Weinberg Exact tests were performed using default settings of the web based version of *Genepop* 4.0.10 (Raymond & Rousset 1995; Rousset 2008). The probability test was used to check for deviations from equilibrium, followed by tests for heterozygote deficiency and excess to establish the direction of any violation of Hardy-Weinberg. Linkage disequilibrium was investigated using the default parameters of option 2 of web based version of *Genepop* 4.0.10; populations were tested independently and a Bonferroni correction applied at the P=0.05 significance level.

Effective population size (N_e) was estimated by observing changes in allele frequencies between two temporal sampling events. A method similar to that employed by Holycross and Douglas (2007) was used whereby the data set was split into two age classes to represent temporal sampling. Adults were used to represent the first sampling event and juveniles used to represent the second sampling event. Only the largest adults >100 cm SVL captured in 2004 and 2005 were used to represent the first sample. The second sample was made up of only individuals that were < 65 cm SVL in 2006 and < 70 cm SVL in 2007 and 2008 (Appendix 2). A larger upper limit of SVL was considered in 2007 and 2008 to take into account the growth of individuals that would have been juveniles in 2006 but not captured until later in the study. The two groups were considered to be separated by a single generation. The fortran program MLNE v.1.0 was then used to estimate N_e using the methodology of Wang (2001) to estimate N_e for a single isolated population using the two temporal samples outlined above. Five separate analyses were run for potential maximum N_e values of 100, 500, 750, 1000 and 10000.

Results

During the study period 623 individuals (all age classes) were captured, of which 341 were male and 282 were female. The sex ratio of captured individuals was 1:0.83 (males : females) and was significantly different from 1:1 (χ^2=5.59, df=1, P<0.05). Size frequency distributions (SVL) for males and females are displayed in Figure 6-2 and were used to determine size range of juveniles to be excluded from population estimation analyses. The number of individuals caught during the summer sampling periods and used for estimation of survival and detectability using Program MARK, version 4.3 (White & Burnham 1999) are displayed in Table 6-3 and capture locations are plotted in Figure 6-3. Assessment of the percentage of recaptures per

year suggested that sufficient individuals were being recaptured for MARK analysis to be appropriate (Figure 6-4).

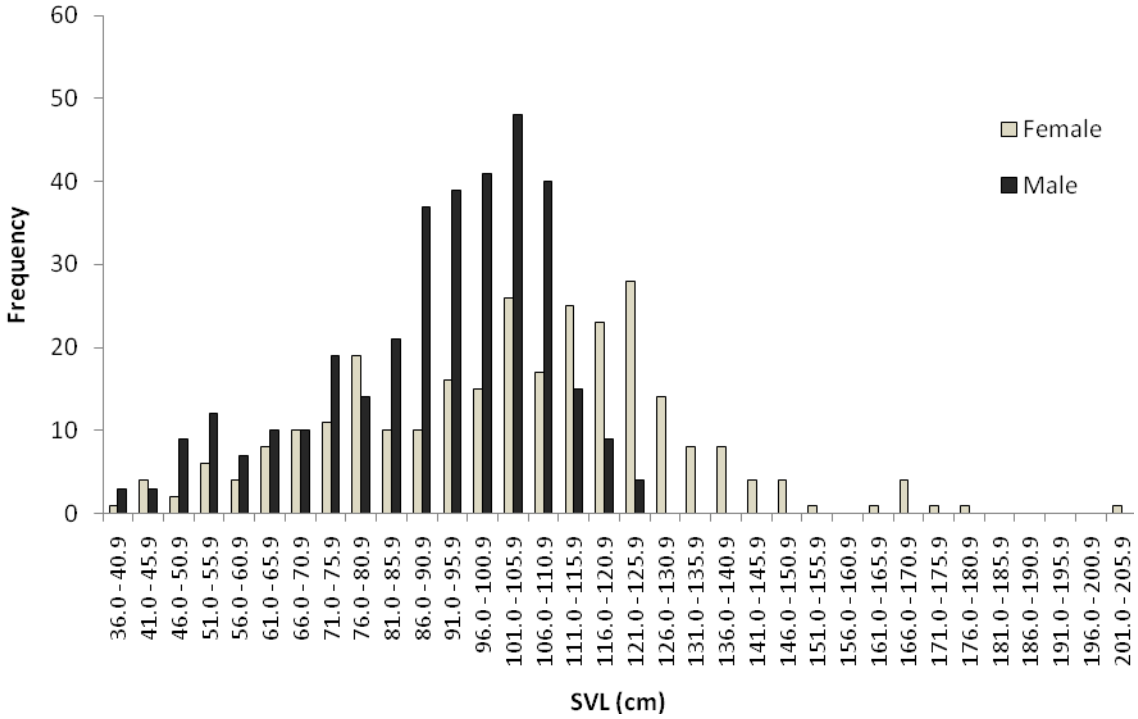

Figure 6-2 Size (SVL) frequency distribution of male and female boas at point of first capture during the study period.

Table 6-3 The number of individuals caught during the summer sampling periods and used for estimation of survival and detectability using Program MARK, version 4.3 (White & Burnham 1999).

	Sampling period				
	2004	2005	2006	2007	2008
Females Caught	44	49	59	48	31
Males Caught	37	71	116	79	46
Total Individuals Caught	81	120	175	127	77

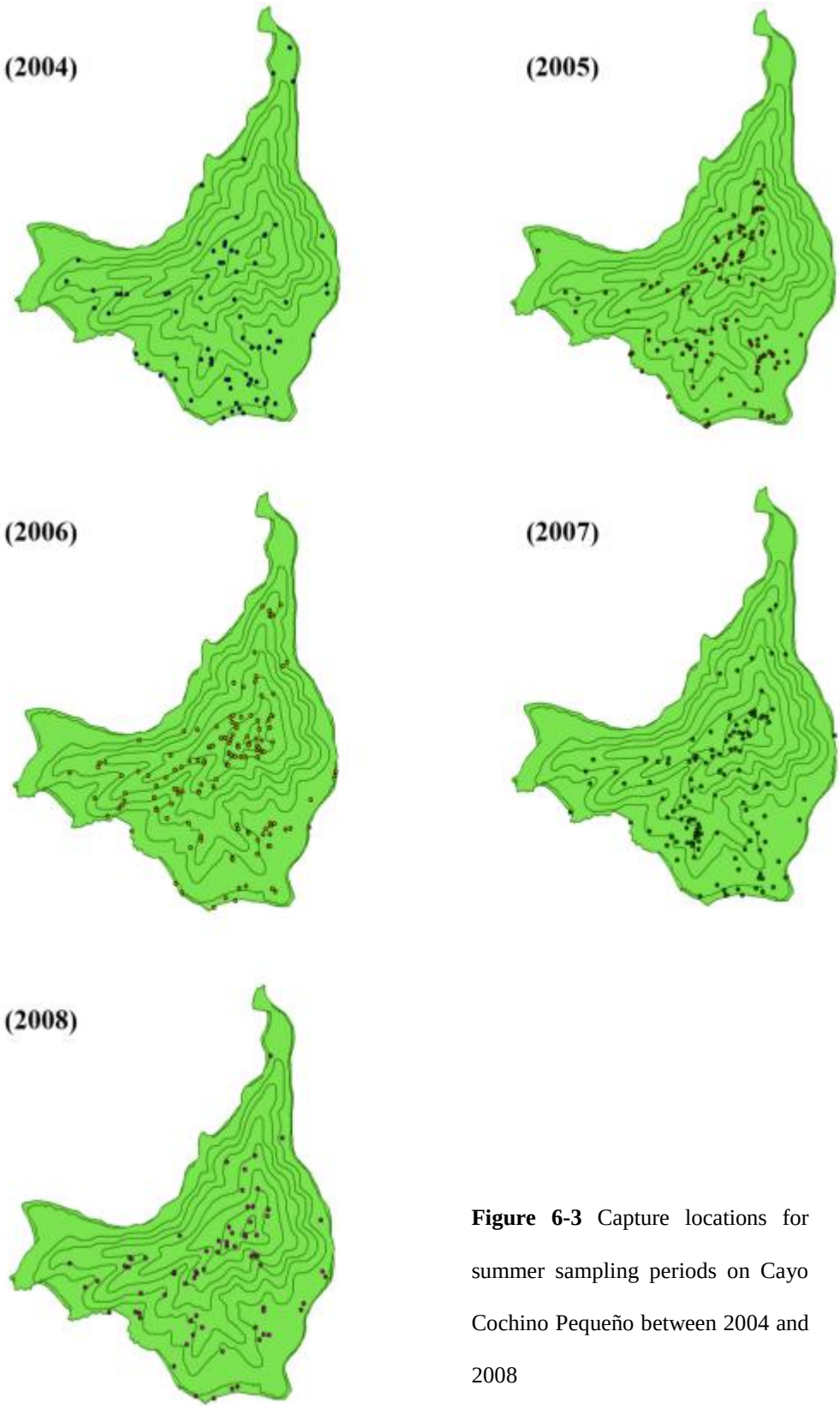

Figure 6-3 Capture locations for summer sampling periods on Cayo Cochino Pequeño between 2004 and 2008

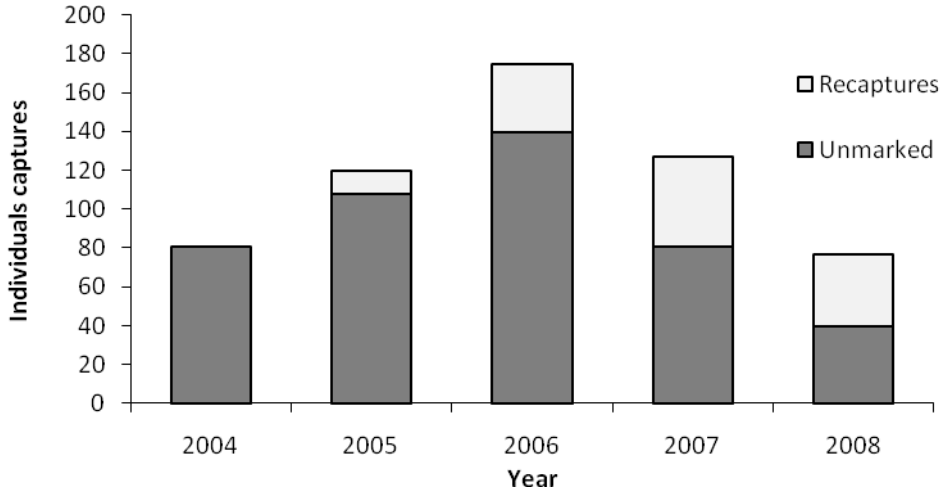

Figure 6-4 Number of individual adult (>60 cm SVL) first time captures and recaptures by year

Survival and detectability estimation

Results for the three most parsimonious models are displayed in Table 6-4, of which model φ(t)p(g+t) was found to be the most parsimonious. Under this model, survival was constant between males and females but varied between years. Detectability varied between sexes over time but the difference was due to a constant, additive, component. Estimates of male and female survival and detectability based on the most parsimonious model are displayed in Table 6-5. Survival was highest between 2004-2005 and then decreased between later sampling periods. However, survival could not be estimated for the period 2006-2007 due to parameter redundancy in the model. Males were more detectable than females in all years and detectability varied by a constant amount for both sexes between sampling periods, with boas being most detectable in 2006. Parameter redundancy in the model prevented detectability being estimated in 2008.

Table 6-4 The three most parsimonious models resulting from the 25 predefined models tested in Program MARK. The model with the best fit to the data (the most parsimonious model) was φ(t) p(g+t), where survival is constant between males and females but varies between years, and detectability varies between sexes over time but the difference is due to a constant, additive, component.

Model	AICc	Delta AICc	Weight	Likelihood	Parameters	Deviance
φ(t) p(g+t)	648.561	0.00	0.3629	1.0000	8	28.065
φ(g+t) p(g*t)	650.013	1.45	0.1755	0.4837	9	27.440
φ(t) p(g*t)	650.609	2.05	0.1303	0.3590	10	25.950

Table 6-5 Estimates of survival (φ) and detectability (p) for males and females and across years based on the most parsimonious model φ(t)p(g+t) with standard error (SE) and upper and lower 95% confidence intervals (CI). * Parameter redundancy in the model meant that it was not possible to obtain estimates of survival for 2007 or detectability in 2008.

Parameter		Estimate	SE	Lower 95% CI	Upper 95% CI
1: φ	male survival 2005	0.854	0.211	0.176	0.994
2: φ	male survival 2006	0.424	0.084	0.273	0.590
3: φ	male survival 2007	*	*	*	*
4: φ	male survival 2008	0.204	0.143	0.044	0.589
5: φ	female survival 2005	0.854	0.211	0.176	0.994
6: φ	female survival 2006	0.424	0.084	0.273	0.590
7: φ	female survival 2007	*	*	*	*
8: φ	female survival 2008	0.204	0.143	0.044	0.589
9: p	male detectablity 2005	0.235	0.085	0.108	0.437
10: p	male detectablity 2006	0.553	0.096	0.366	0.727
11: p	male detectablity 2007	0.250	0.038	0.184	0.331
12: p	male detectablity 2008	*	*	*	*
13: p	female detectability 2005	0.088	0.042	0.034	0.211
14: p	female detectability 2006	0.281	0.087	0.144	0.477
15: p	female detectability 2007	0.095	0.028	0.053	0.167
16: p	female detectability 2008	*	*	*	*

Adult census size population estimation and sex ratio

Adult census population size was estimated from detectability estimates from the most parsimonious model. Because detctability differed between the sexes and between years, male and female population sizes were estimated separately in each year and then summed to provide estimates of the total population. Population estimates were then averaged over years to provide mean adult census size for males and females and summed to provide total mean adult census size (Table 6-6). Based on these estimates, the sex ratio was found to be significantly scewed in the direction of excess females in the population (χ^2=30.54, df=1, P<0.01) in a ratio of 0.65:1 (males:females).

Table 6-6 Population estimates based on detection probabilities and the number of boas captured in each sampling period using the equation $N_i = (1/p_i) \times n_i$ where p_i is the within season detectability estimate and n_i is the number of individuals captured within the sampling period. Low and high estimates based on 95% confidence intervals.

Year	Group	No. captured in sample period	Population Estimate	Low Estimate	High Estimate
2005	Male	71	303	162	658
	Female	49	554	232	1441
	Total	120	856	394	2099
2006	Male	116	210	160	317
	Female	59	210	124	410
	Total	175	419	283	727
2007	Male	79	316	239	430
	Female	48	503	288	909
	Total	127	819	527	1340
Average of all years	Male		276	187	468
	Female		422	215	920
	Total		698	401	1389

Population density

Average population density was calculated by dividing adult census population estimates by island area (0.64 km^2), resulting in an average island density of 10.9 boas per ha (6.27-21.7 boas per ha). However, by plotting capture locations of all individuals encountered during the sampling periods (excluding recaptures) it is apparent that density is variable across available habitats (Figure 6-5).

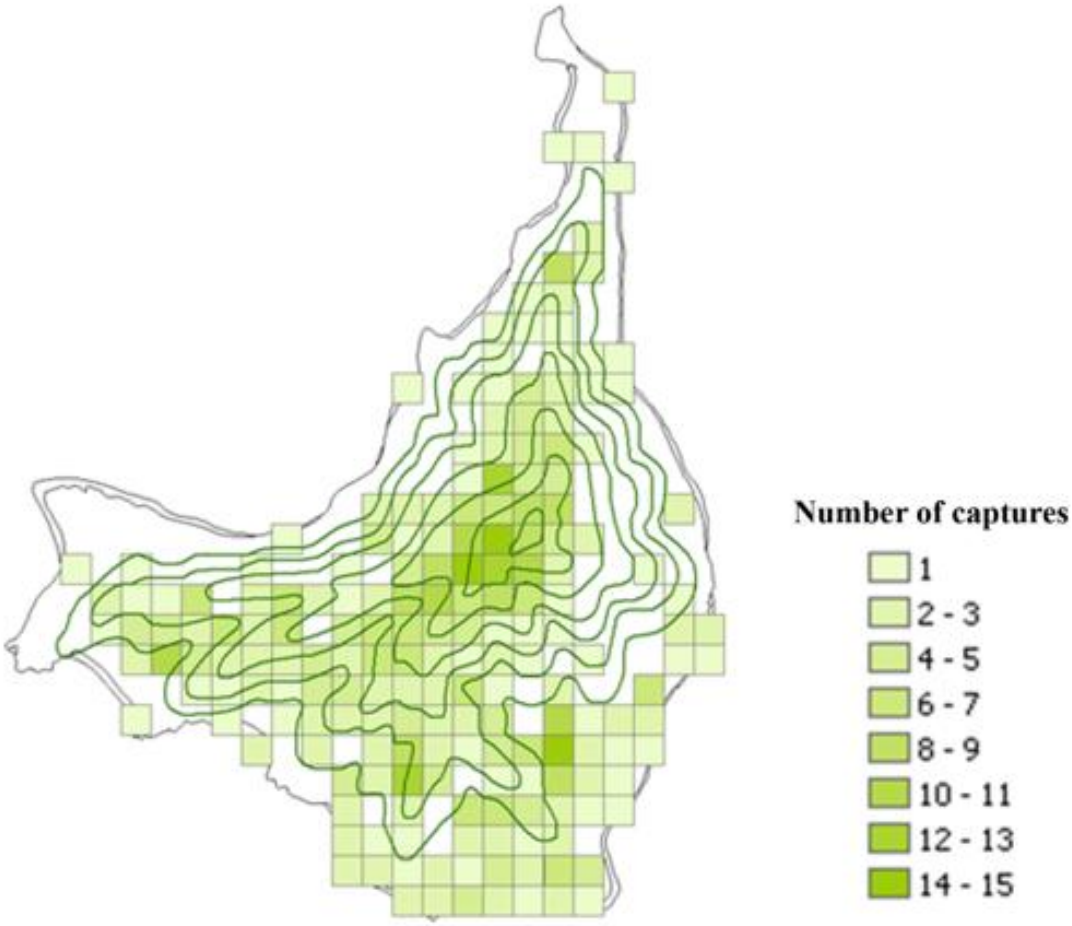

Figure 6-5 Density plot of capture locations. Grid squares represent 50 m^2 and colours relate to the number of individual boas encountered within that grid square during the study period (excluding recaptures). Darker colours represent areas of higher encounter rates and thus assumed higher relative population density.

Effective population Size (N_e)

A total of 499 boas were successfully genotyped for at least six of the eight polymorphic microsatellite loci (Appendix 2). Samples in which more than two loci failed to amplify were excluded from analyses. Where samples were genotyped twice, the same genotype was obtained on both occasions in 100% of cases. Examination of genotype data in the heterogametic sex (females) detected no evidence of sex-linkage between loci. Analysis with *CERVUS* found null allele frequencies to be within acceptable limits (F-null<0.15) (Dakin & Avise 2004). Analysis with *Micro-Checker* found null alleles may be present at locus Bci-14 and Bci-21 in the Cayo Cochino Pequeño population as is suggested by the general excess of homozygotes for most allele size classes at these loci. However, this homozygote excess was not deemed significant by the program and thus no adjusted allele frequencies were calculated. No significant problems with large allele dropout or scoring error due to stuttering were found.

Exact tests for Hardy-Weinberg equilibrium revealed microsatellite loci Bci-14, Bci-21, μsat01, μsat20 and μsat36 violated Hardy-Weinberg equilibrium (P = 0.000, 0.046, 0.007, 0.000 and 0.003 respectively) and in all cases this was due to heterozygote deficiency at the locus. Evidence of linkage disequilibrium, after Bonferroni correction, was found between a number of loci (Table 6-7), however, as these loci have previously been found to be unlinked in other populations (Chapter 4 and Chapter 5), intrachromosomal linkage is unlikely to be of concern. Also, when only a subset of individuals from Cayo Cochino Pequeño were analysed (n=30) no linkage was detected.

Table 6-7 Loci found to be in linkage disequilibrium in the Cayo Cochino Pequeño population following Bonferroni correction (x = loci in linkage disequilibrium)

	Bci-14	Bci-15	Bci-18	Bci-21	Bci-23	µsat01	µsat20
Bci-14	-						
Bci-15	-	-					
Bci-18	x	-	-				
Bci-21	-	-	-	-			
Bci-23	-	-	-	x	-		
µsat01	x	-	x	-	-	-	
µsat20	-	-	-	x	x	x	-
µsat36	x	x	x	x	x	x	-

N_e estimation

Analysis of the data using the five potential upper limits of N_e (N_e max) revealed that values N_e max of ≤500 were too low, as suggested by the estimates of N_e reaching the upper limits set in those analyses. In all analyses where N_e max was set at ≥750 the estimate of N_e was 615, although the upper confidence limit continued to increase in line with N_e max (Table 6-8).

Table 6-8 Estimates of effective population size (N_e) for Cayo Cochino Pequeño based on analysis of change in allele frequencies between two temporal sampling events (one generation apart) using the methodology of Wang (2001) to estimate N_e for a single isolated population in the Fortran program MLNE v.1.0.

N_e Max	N_e	Lower 95%	Upper 95%
100	99	65	100
500	499	85	500
750	615	85	750
1000	615	85	1000
10000	615	85	10000

Discussion

An adult census population estimate of 698 individuals suggests that the Cayo Cochino Pequeño population has undergone a substantial period of recovery since anti-poaching legislation has been enforced. However, if reports of the numbers of boas being removed from the islands at the height of collection during the 1980s are accurate (Bermingham et al. 1998; Porras 1999; Reed et al. 2007), then it is unlikely that the population has yet fully recovered to its pre-collection carrying capacity. Also, estimates of contemporary effective population size were found to be lower than estimates of historical effective population size (Chapter 4 and Chapter 5) indicating that the population has not yet fully recovered. Furthermore, the small size of the island coupled with high density and ease of detection of boas makes the population particularly vulnerable to future damaging exploitation. Continued vigilance against unregulated collection should be an integral part of conservation management plans for the continued recovery of the population.

Mark-recapture studies of snakes can be inherently difficult to carry out due to the intensity of search effort necessary to obtain sufficiently high recapture rates for accurate parameter estimation. The small size and isolation of the study site, coupled with apparent high density of boas provided a rare opportunity to conduct such a study. However, it must be acknowledged that violation of one or more of the assumptions of the CJS model could bias the outcome of the analysis.

It is assumed that every animal present in the population at time (*i*) has the same probability of recapture. Although every attempt was made to evenly distribute search effort across the island, animals close to camp and to trails may have had a greater chance of recapture because of chance encounters by people frequently using

these areas. However, relatively few boas were actually encountered in these areas and so probably had a minimal affect on overall detection probabilities. It is also assumed that every animal in the population immediately after time (i) has the same probability of survival to time (i+1). By removing juveniles from the analysis, the variability in individual survival was minimized.

In all mark-recapture studies it is an essential assumption that marks are not lost or missed. PIT tags are generally considered to be a very reliable method of marking animals (Gibbons & Andrews 2004). However, PIT tags can be rejected by the snake either through the skin (Germano & Williams 1993) or through the gut (Roark & Dorcas 2000). It was assumed that rejection of PIT tags by boas is a sufficiently rare event so as not to have significantly biased the result.

It is also assumed that all samples are instantaneous relative to the interval between occasions (i) and (i+1) and each release is made immediately after the sample. It was necessary to have reasonably long sampling periods in order to search the entire island thoroughly. However, compared to the interval between samples, sampling periods were still relatively short and all individuals were released immediately after each sample. It is probably also safe to assume that all emigration from the study area is permanent, as animals are highly unlikely to return to the island should they leave. The final assumption, which is unlikely to be violated in this species, is that the fate of each animal, with respect to capture and survival probability, is independent of the fate of any other animal.

Survival and detectability

All three of the most parsimonious models were reasonably similar, with survival always being variable between sampling periods and detectability varying both

between group (sex) and sampling period. Survival appears to show a declining trend with time. However, the inability to estimate survival in the 2006-2007 interval (due to parameter redundancy in the model), in conjunction with wide confidence intervals in survival estimates, make it difficult to draw firm conclusions on long term survival trends. The accumulation of additional data in future years will hopefully help to increase both the precision and accuracy of survival estimates.

Males were more detectable than females in all years (excluding 2008, for which no estimate was generated due to parameter redundancy in the model) and may indicate differential habitat use and/or behaviour by males and females during the sampling period. Cayos Cochinos boas have been observed breeding from early April to early May (Russo 2007) and a number of female boas have been observed to be gravid during the sampling period, with one female giving birth to a litter shortly after capture in early August 2007. Thus, one possible explanation for the lower detectability of females is that gravid female boas are modifying their behaviour in a way that reduces their detectability in comparison with males. The majority of boas encountered during VES were displaying ambush foraging postures on low branches of understory trees. Since it is unlikely that females will feed whilst gravid, VES may have been biased towards higher male and lower female detectability, as reflected in the models.

Detectability varied between years, with both sexes being most detectable in 2006. This may most easily be explained by fluctuations in search effort between years. However, 'true' search effort is difficult to quantify due to variability in inter-observer ability. For example, Rodda and Fritts (1992) found that inter-observer variability was the greatest source of variation in visual counts of Brown Treesnakes

(*Boiga irregularis*) on Guam. Nonetheless, variability in the numbers of volunteers available appears to have resulted in inconsistent search effort between years, with the highest search effort being recorded in 2006, the same year as highest detectability. Thus, although abiotic factors may influence annual detectability of boas, the most likely cause of variable annual detectability in this study is variable search effort.

It was not possible to model juvenile survival or detectability due to low sample sizes of juvenile snakes. It is not clear whether low encounter rates of juvenile snakes is a consequence of a possible ontogenetic shift in behaviour or habitat use, or simply because smaller snakes are more difficult to see during VES. However, the low number of juvenile snakes captured during the study does suggest that juvenile snakes are less detectable than adult snakes. Assuming this is true, it may be that although snake collectors caused the rapid depletion of the adult population through over-harvesting, the juvenile population may have been more resistant to such practices. It is possible, therefore, that a healthy juvenile population remained despite the adult population experiencing a dramatic decline. Thus, low detectability of juvenile snakes may be an explanation as to why the population was reported to have been reduced to such low numbers, but then subsequently recovered in a relatively short time with apparent minimal loss of genetic diversity (Chapter 5).

Sex ratios

The sex ratio calculated from the number of captures alone gave a significant departure from 1:1 in favour of excess males in the population at a ratio of 1:0.83 (males : females). However, population estimates taking into account variable detectability of males and females showed the sex ratio is in fact likely to be skewed

in the opposite direction, with an excess of females at a ratio of 0.65:1 (males : females). This demonstrates the importance of taking into account detectability when attempting to determine sex ratios from VES data. However, caution should perhaps be taken in interpreting this result in the absence of a sound biological explanation for its cause. No difference in annual survival was found between adult males and females in the most parsimonious model, and in fact under the second most parsimonious model females had lower survival than males. Thus, an excess of females in the population cannot be explained by higher mortality of males. Could it be, therefore, that the primary sex ratio (the sex ratio at conception) is in fact skewed in favour of an excess of females in the population? If this is the case, it seems strange that no such female skewed sex ratio has been reported by breeders of Cayos Cochinos boas in captivity.

Perhaps the simplest explanation for a female skewed sex ratio is the combined effect of collection of animals for the pet trade and the lower detectability of females. If more males were (and possibly still are) being removed from the island because of their greater detectability then this could easily explain the observed excess of females in the population. However, it should also be kept in mind that survival and detectability estimates were calculated with preliminary data (five years) of a longer term study. It is possible, therefore, that the study period was simply too short to detect potential sex based differences in long-term adult survival. It should be noted that in 2006, when capture rates were at their highest level, population estimates for males and females were equal, suggesting an even sex ratio. Thus, the continued collection of additional years of mark-recapture data in conjunction with future research into primary sex ratios of wild caught Cayos Cochinos boas may be necessary to clarify the situation.

Relationship of census population size and effective population size

N_e/N_c gives the deviation from a perfect population in which all individuals contribute equally to the next generation. Effective population size is typically smaller than census population size and the ratio N_e/N_c is thus <1 (King 2009). In a comprehensive study of the relationship of N_e/N_c the average ratio was found to be just 0.11 (Frankham 1995b). However, reptiles, and snakes in particular, have typically been lacking from such studies (King 2009). More recent studies of the relationship of N_e/N_c in snakes have found ratios between 0.34-0.73 (Madsen & Shine 1995; Seymour & King 2003; Holycross & Douglas 2007). In comparison, the relationship of N_e/N_c in the Cayo Cochino Pequeño population was found to be 0.88. It is possible that an excess of females in the population maintains a relatively high ratio of N_e/N_c by decreasing male competition for access to females. This may be an important factor contributing to the maintenance of genetic diversity within the population and may have helped mitigate the most severe genetic effects of the pet trade induced bottleneck (See chapter 2) because populations with a small N_e will lose genetic diversity at a greater rate (Wright 1969).

Population density

Population density estimates for Cayo Cochino Pequeño averaged 10.9 boas per ha. It is remarkable that the island can support such a high density of top predator, however, perhaps more remarkable is the fact that population size, and thus density, must have been even higher in the past in order to sustain such high levels of off-take for the pet trade (Bermingham et al. 1998; Porras 1999; Reed et al. 2007). Bonnet et al. (2002) found that density of tiger snakes (*Notechis scutatus*) on Carnac Island, Western Australia, was one of the highest reported for a sedentary vertebrate at 22

snakes per ha, demonstrating the ability of some islands to support extremely high densities of snakes. Mean adult (>60 cm SVL) body mass of boas in Cayo Cochinos Pequeño was 391 g and 633 g for males and females respectively (unpublished data). Taking into account the skewed sex ratio, this translates into a biomass of 6.04 kg per ha, comparable with that of tiger snakes (6.25 kg per ha) on Carnac Island (Bonnet et al. 2002). Such a surprisingly high biomass of top predator is likely only achievable in snakes because of the relatively lower energy requirements of ectotherms over endotherms (Pough 1980) and their ability to survive long periods without food (Bonnet et al. 2002).

Conclusion

Initial analysis of this long-term data set has provided the first detailed quantifiable insights into the recovery of the Cayo Cochino Pequeño Boa constrictor population. Despite the potential limitations of the study, it is clear that the adult population has recovered substantially since anti-poaching legislation was first enforced in 1993. However, comparison of contemporary estimates of effective population size with historical estimates (Chapter 4 and Chapter 5) suggests the population has still not reached its pre-decline carrying capacity. The continued collection of data over future years and a greater attempt to standardize search effort between samples will hopefully result in greater confidence in parameter estimates and provide further insights into the demography of the population. This information will hopefully help to inform future conservation management plans to ensure the long-term survival of this unique dwarf insular *Boa constrictor* in the wild.

Chapter 7 Discussion

This study attempted a multidisciplinary approach to determine both the evolutionary history of *Boa constrictor* in the Cayos Cochinos and Bay Islands, Honduras, and the current conservation status of the heavily exploited Cayos Cochinos populations. In doing so, it is demonstrated that current conservation strategies for insular populations may be failing to protect important, previously unrecognised, diversity of *B. constrictor* in Honduras. The study also highlights the rapid rate at which dwarfism appears to have evolved in this species on islands.

Phylogeography

Populations of *B. constrictor* in the Cayos Cochinos and Bay Islands, Honduras, appear to represent a single radiation event in the early Pleistocene, with the Cayos Cochinos and Utila both being colonised from Roatan and/or Guanaja, rather than from the mainland, since their most recent isolation (although Utila may have also been colonised from the mainland more recently). This finding leads to two important conclusions: firstly, that the phenotypic variation displayed by *B. constrictor* in the Cayos Cochinos, including dramatic reduction in body size, has evolved extremely rapidly since the islands were isolated by rising sea levels at the end of the last ice age; and secondly, that all populations of *B. constrictor* in the Cayos Cochinos and Bay Islands have been on a separate evolutionary trajectory to the mainland population for approximately the past two million years

The Bay Islands and Cayos Cochinos form an Evolutionary Significant Unit

The reciprocal monophyly of the Bay island and Cayos Cochinos clade, identified by phylogenetic reconstruction of mtDNA sequence, strongly supports the recognition

of these island populations as an Evolutionary Significant Unit (ESU) (Moritz 1994). Despite populations of *B. constrictor* on the Bay Islands being phenotypically more similar to mainland *B. constrictor*, conservation of these historically isolated lineages should be at the forefront of conservation planning because, once lost, this genetic diversity cannot be recovered (Moritz 2002). In contrast, the phenotypic divergence displayed by *B. constrictor* in the Cayos Cochinos has arisen relatively rapidly. Conservation strategies for the Cayos Cochinos should, therefore, not only focus on maintaining the viability of the population, but on maintaining the ecological processes that favour selection for this phenotypic diversity (Smith et al. 1993; Smith et al. 1997; Moritz 2002).

Each of the islands represents a demographically independent population displaying divergence in allele frequencies and should, therefore, be considered as a Management Unit (MU) within the ESU. Conservation strategies should focus on maintaining the long term viability of each MU, whilst recognising that gene flow between islands may be important in maintaining the ESU as a whole (Moritz 1999; Moritz 2002). However, the limited availability of samples from the Bay Islands made it difficult to accurately quantify the level of genetic structure and gene flow between the Bay Islands and the Cayos Cochinos. Thus, it may be unwise to draw strong conclusions regarding the extent to which viability of each population is dependent on gene flow between them. Further sampling of the Bay Island populations will be necessary to better elucidate migratory patterns and the role of gene flow in maintaining the ESU. It would also help to determine the relationship between gene flow and natural selection in maintaining local phenotypic adaptation in the Cayos Cochinos population.

The significance of the ESU in light of possible taxonomic review of Boa constrictor

Having determined that the Cayos Cochinos and Bay Islands should be considered an ESU, it is important to consider the implications of recently proposed taxonomic review of the species. Hynkova et. al. (2009) concluded, based on phylogenetic reconstruction of the mtDNA gene cytochrome-b (*CytB*), that *B. constrictor* from Central America and South America west of the Andes are sufficiently genetically divergent (5-7%) from South American populations of *B. constrictor*, east of the Andes, to be considered separate species. This study found the same degree of genetic divergence for mtDNA genes *CytB* and *ND4* between these two geographically disparate groups, thus providing independent confirmation of this result. It must be acknowledged that further sampling of wild caught specimens from the Eastern Cordillera region of the Andes and also greater molecular sampling will be necessary to unequivocally test the robustness of this proposed taxonomic review. However, it is evident that orogeny of the Andes has caused cladogenesis in a number of species (e.g. Zamudio & Greene 1997; Cortés-Ortiz et al. 2003; Hoffmann & Baker 2003; Brumfield & Edwards 2007; Dacosta & Klicka 2008; Gamble et al. 2008; Venegas-Anaya et al. 2008; Santos et al. 2009; Vallinoto et al. 2010) and is, thus, likely to have had a similar effect on *B. constrictor* gene flow and divergence.

Although it was not the aim of this study to conduct a review of the taxonomy of *B. constrictor*, a major objective was to investigate the possible presence of any evolutionary divergent lineages of high conservation 'value'. The apparent high level of divergence between *cis*- and *trans*-Andean clades of *B. constrictor* should surely raise the evolutionary importance, and thus conservation 'value', of Central American *B. constrictor* and this in turn will have a knock on effect on the conservation 'value' of identifiable ESUs such as the Bay Islands and Cayos

Cochinos. Conservation management plans should recognise this fact and apply proportionate responses to conserving this diversity.

Alternative approaches to conservation prioritisation, such as the EDGE (Evolutionary Distinct and Globally Endangered) approach, would likely consider the Cayos Cochinos and Bay Island clade to represent a relatively low conservation priority. However, although such approaches provide an extremely valuable means by which to identify those species of highest conservation priority for maintaining the greatest breadth of biological diversity on Earth, they do not replace the need to manage lower levels of genetic diversity responsibly. It is essential that conservation management plans look to conserve the evolutionary processes by which future biological diversity is being created as well as protecting those highly unique species that are present today.

Historical decline/founder event of the Cayos Cochinos population

Tests for a historical population decline in the Cayos Cochinos using the software *MSVAR* 1.3 (Beaumont 1999; Storz & Beaumont 2002) concluded that both the Cayo Cochino Grande and Cayo Cochino Pequeño populations have undergone a substantial historical contraction in effective population size. This decline broadly coincides with the time at which the islands would have been isolated from the mainland and Utila by rising sea levels (Bermingham et al. 1998; McCranie et al. 2005) and can thus most likely be interpreted as evidence of the original founder or isolation event of the Cayos Cochinos populations. The dating of the split of the Cayo Cochino Grande and Cayo Cochino Pequeño populations, as inferred by isolation with migration analysis (Hey & Nielsen 2007) also corresponds well with this interpretation.

Estimates of historical effective population sizes produced by both methodologies are obviously unrealistically high if interpreted as the historical population of boas in the Cayos Cochinos alone; it is clear that the islands are simply too small to have ever supported such a large population. Instead, historical effective population sizes can be interpreted as the size of the ancestral population prior to the isolation of the Cayos Cochinos by rising sea levels at the end of the Pleistocene and estimates from both methods are broadly in agreement. As a consequence of the likely colonisation from Roatan and/or Guanaja, illustrated by mtDNA analysis, it is possible that historical effective population size estimates are actually an estimate of effective population size on Roatan and/or Guanaja at this time.

Cayos Cochinos genetic diversity and recent population decline

Genetic diversity was lower in the Cayos Cochinos and Utila populations than on the mainland, as would be expected for island populations (Wright 1931; Frankham 1997). Surprisingly, however, levels of observed heterozygosity were comparable in both the presumed heavily bottlenecked Cayos Cochinos populations and Utila, although allelic richness was lowest in the Cayos Cochinos populations. Levels of expected heterozygosity of both mainland and island populations fell comfortably within the range of values previously reported for snakes (0.35-0.87), with the Cayos Cochinos populations falling only slightly short of the median value (0.60) (King 2009). Levels of heterozygosity in the Cayos Cochinos were thus found to be surprisingly high for a presumed bottlenecked population and considerably greater than levels found in other bottlenecked populations of snakes in which low genetic diversity has been a cause for concern (e.g. Madsen et al. 1996; Gautschi et al. 2002; Lukoschek et al. 2005).

Similarly, levels of inbreeding (F_{is}) were found to be low in the Cayos Cochinos populations and well within the range found in other populations of snakes (-0.11-0.30) (King 2009). Perhaps most surprising was that inbreeding was found to be highest in the Utila population, previously assumed to be representative of a less severely impacted population. This result may suggest that either there is unrecognised population structure in the sampling of Utila, or that the population has itself experienced a recent decline.

It appears, therefore, that despite reports of heavy exploitation and the subsequent crash of the Cayos Cochinos population, current levels of genetic diversity are not an immediate cause of concern. However, analysis of the Cayos Cochinos populations for the presence of a recent genetic bottleneck suggested that the populations have likely experienced some degree of decline and genetic impoverishment. It is possible that the relatively short period of the bottleneck and the apparent rapid rate at which the population began to recover after management action was taken has limited the severity of this bottlenecking event. One possible explanation for this rapid recovery with limited loss of genetic diversity could be as a result the likely lower detectability of juvenile snakes. If juveniles are less detectable than adult snakes, as would be suggested by the low numbers of juveniles observed during the study, a healthy juvenile population may have remained on the island unnoticed even after the adult population had severely declined.

Cayos Cochinos recovery and current status

It is evident that the Cayos Cochinos populations are showing encouraging signs of recovery, with visual encounter surveys on both Cayo Cochino Grande and Cayo Cochino Pequeño resulting in similarly high encounter rates. Mark-recapture data for

Cayo Cochino Pequeño suggests the current adult census population size to be in the region of 700 individuals. Although limited access to Cayo Cochino Grande prevented census population size being estimated for this island, mean estimates of effective population size (N_e) based on molecular data were similar for Cayo Cochino Pequeño and Cayo Cochino Grande (N_e CCP = 1,019-1349 N_e CCG = 1061-1114 based on *MSVAR* and *IMa* output). Thus, it is likely that the Cayo Cochino Grande and Cayo Cochino Pequeño populations are similar in size.

This result is, perhaps, somewhat surprising considering the difference in size of the two islands, with Cayo Cochino Grande being more than twice the total area of Cayo Cochino Pequeño (CCG=1.55 km^2, CCP=0.64 km^2). One possible explanations of this result may be increased habitat alteration on Cayo Cochino Grande due to the invasive *Attaleya* palms that now cover large areas of the island (Bermingham et al. 1998). Although *B. constrictor* can often be found within this altered habitat, personal observation suggests that encounter rates are lower than in the native tropical oak dominated areas of the island. Alternatively, the relatively lower population size of Cayo Cochino Grande could be due to higher levels of past or present collection of *B. constrictor* from the island. Permanent settlements on Cayo Cochino Grande increase public access to the island and as a result increase the susceptibility of the population to illegal poaching.

It must be acknowledged, however, that large confidence limits in the estimates of N_e produced from these single sample methods imply that caution should be taken in using them to draw firm conclusions on the size of the Cayo Cochino Grande population. It may be prudent, therefore, to refrain from formulating hypotheses for the possible causes of a relatively lower population size on Cayo Cochino Grande

until this can be investigated further. More reliable estimates of population size for Cayo Cochino Grande could be achieved either through mark-recapture data or through increased molecular sampling that would allow for a more robust two-sample methodology to be conducted, in which changes in allele frequencies are observed between two temporal sampling events, as was possible for Cayo Cochino Pequeño.

The temporal sampling methodology for estimating N_e in the Cayo Cochino Pequeño population, as implemented in the Fortran program MLNE v.1.0 using the methodology of Wang (2001), resulted in a more conservative estimate than the single sample methods and was more in line with estimates of adult census population size (N_c) calculated from mark-recapture data. This estimate of N_e was used in conjunction with N_c to calculate the deviation from a perfect population, in which all individuals contribute equally to the next generation. The relationship of N_e/N_c in the Cayo Cochino Pequeño population was found to be higher than in other studies of snakes (Madsen & Shine 1995; Seymour & King 2003; Holycross & Douglas 2007) and considerably higher than the average values calculated for other taxa (Frankham 1995b). It could simply be that inaccuracy in the current estimates of N_e and/or N_c are responsible for this seemingly high ratio of N_e/N_c and thus better estimates of these variables should be attempted with the accumulation of more data. However, assuming this result is an accurate reflection of the ratio of N_e/N_c in the Cayo Cochino Pequeño population, it could possibly be explained by the apparent skew in the sex ratio towards an excess of females.

Based on detectability estimates and the number of individuals of each sex captured within each season, adult population size was estimated independently for males and

females, resulting in a sex ratio skewed towards excess females in the ratio 1: 0.65 (females:males). Having an excess of females in the population would reduce competition between males for access to females. This scenario may also have been an important factor contributing to the maintenance of genetic diversity within the population and may have helped mitigate the most severe genetic effects of the pet trade induced bottleneck, because populations with a greater N_e will lose genetic diversity at a reduced rate to populations with a low N_e (Wright 1969; Frankham 1995a).

However, what could be causing the sex ratio to be skewed in the direction of excess females is not immediately apparent. It was originally assumed that survival would be lower in females because of the greater energetic costs associated with reproduction in this sex (Bertona & Chiaraviglio 2003; Shine & Bonnet 2009), and thus if anything the population would more likely be skewed in the direction of excess males. Indeed a number of large adult females had been observed during the study-period that were extremely emaciated and close to death, thus supporting our hypothesis. Nevertheless, estimates of annual adult survival suggest there to be no sex-based difference in survival probability. If this is the case then differential adult survival cannot be used to explain the skewed sex ratio.

Perhaps the simplest explanation for a female skewed sex ratio is the combined effect of collection of animals for the pet trade and the lower detectability of females. If more males were (and possibly still are) being removed from the island because of their greater detectability then this could easily explain the observed excess of females in the population. However, it should also be kept in mind that survival and detectability estimates were calculated with preliminary data (five years) of a longer

term study. It is possible that the relatively short timeframe of the study was insufficient to accurately determine the close relationship between survival and detectability. Therefore, it cannot be ruled out that, as data become available for a greater number of years, currently observed sex-based differences in detectability could in fact be better explained by differences in adult survival. If this turns out to be the case, then it would likely result in a reduction in female population estimates and thus neutralize the currently observed skew in the sex ratio. This area of research should be revisited once more data from a greater number of years have been collected.

High density populations and evolution of dwarfism

Population density estimates for Cayo Cochino Pequeño averaged 10.9 boas per ha. It is remarkable that the island can support such a high density of top predator, however, perhaps more remarkable is the fact that population size, and thus density, must have been even higher prior to the population's exploitation for the pet trade. If anecdotal evidence is to be taken seriously, thousands of boas were removed from the islands within a decade of collection beginning (Bermingham et al. 1998; Porras 1999; Reed et al. 2007) and thus the populations must have been larger than their current size in order to sustain such a high level of off-take.

Bonnet et al. (2002) showed that density of tiger snakes (*Notechis scutatus*) on Carnac Island, Western Australia, was one of the highest reported for a sedentary vertebrate, thus demonstrating the ability of some islands to support extremely high densities of snakes. Although mean density of *B. constrictor* on Cayo Cochinos Pequeño was found to be slightly lower than that of tiger snakes on Carnac Island, mean biomass of snakes was comparable between the two studies (Bonnet et al.

2002). Such a surprisingly high biomass of top predator is likely only achievable in snakes because of the relatively lower energy requirements of ectotherms over endotherms (Pough 1980) and their ability to survive long periods without food (Bonnet et al. 2002).

Resource limitation on islands is often quoted as the mechanism for the evolution of dwarfism (Case 1978; Lomolino 2005; Lomolino et al. 2006). However, the conundrum here is that if resources are limited on the island then why should density be so high? Density compensation theory provides a possible explanation and predicts that as islands become smaller and more isolated, species diversity will decline, but that population densities of the species present will increase (Lomolino 2005). Thus, high population density of *B. constrictor* in the Cayos Cochinos may simply be a result of reduced species diversity on the islands. Reduced interspecific competition will also potentially result in ecological release, which may, among other things, result in a shift in optimal body size (Lomolino 2005). Also, the effects of resource limitation will be amplified by intense intraspecific competition in high density populations and should result in a selective advantage for smaller individuals because of the lower energy requirements of a small body size (Lomolino 2005). Patterns of rapid reduction in body size, in response to limited food availability, but with the maintenance of high population density have been observed in other species (e.g. Sinclair & Parkes 2008), and highlight the extremely small timescale needed for shifts in body size to occur on islands (<100 years).

The exact mechanism by which dwarfism has evolved in the Cayos Cochinos will need further investigation, but it is likely that density compensation, ecological release and resource limitation may help to explain the observed reduction in body

size. Also, behavioural adaptation in response to mostly diurnally-active and arboreal prey may be a key component in the selective pressures maintaining both dwarfism and colour variation in the populations. Clearly this represents an area of considerable interest for future study.

Future conservation of the Cayos Cochinos populations

The Cayos Cochinos population has experienced extremely intensive exploitation in the recent past and is a prime example of the rapid demographic collapse that can be caused by over-collection for the pet trade. Fortunately management interventions appear to have acted in time to halt and reverse the alarming rate of decline. It is encouraging to once again see healthy populations of *B. constrictor* on both Cayo Cochino Grande and Cayo Cochino Pequeño, although the future persistence of these populations is far from certain. The original cause of decline, over-collection for the pet trade, has been arrested, but not eliminated. Survival of the populations is very much dependent on the continued enforcement of anti-poaching legislation and the coordinated patrols of the HCRF and Honduran Navy. If these barriers to collection were to be removed it is highly likely that uncontrolled harvesting of *B. constrictor* from the islands would resume. Recent arrests of poachers, confirm that illegal collection of boas from the islands remains a serious cause for concern.

Demand for wild caught 'Hog Island Boas' from the Cayos Cochinos is extremely high within the captive breeding industry, and breeders are willing to pay high prices to augment their bloodlines with wild caught animals. During this study, a number of private collectors and breeders admitted to having wild caught Cayos Cochinos boas in their collections, many of which, due to their age, must have been removed from the islands since the initiation of this study. Preliminary genetic analysis of some of

these specimens confirms their providence (unpublished data) and the fact that wild caught Cayos Cochinos boas are openly available on the international market.

If long-term sustainability of the wild population is to be achieved, the incentives for poaching need to be replaced with incentives for their conservation. Current strategies for promoting wildlife-based ecotourism in the communities are a step in the right direction, but greater effort is needed in training individuals within the communities and marketing the islands to international and domestic tourists. Providing alternative livelihoods and encouraging community based conservation will be key to the future of the Cayos Cochinos *B. constrictor.* Also, integral to any conservation programme is an accompanying education program that will help relieve fears and local myths commonly associated with snakes.

Conservation of the Bay Island populations

Having identified the evolutionary significance of populations of *B. constrictor* in the Bay Islands, it is essential that conservation management plans take this information into account. However, the conservation issues associated with the Bay Islands are quite different to those experienced in the Cayos Cochinos.

Although *B. constrictor* is known to have been collected from the Bay Islands for the pet trade (Porras 1999; Russo 2007), the scale of collection and level of demand has likely been much lower than in the Cayos Cochinos. Of much greater concern, perhaps, is the level of persecution and indiscriminate killing of *B. constrictor* by local people. Negative attitudes towards snakes are common within the communities of Honduras. However, whereas fear of dangerous snakes can be somewhat understood on the mainland, due to the presence of some highly venomous species, the absence of these venomous species from the islands makes such feelings

particularly unjustified. The only venomous species of snake to be found on any of the Bay Islands occurs on Roatan, an endemic coral snake (*Micrurus ruatanus*), which is easily identifiable by its bright red and black banding and unmistakable for any other species on the islands. Therefore, education programs should try to relieve people's fears and highlight the unthreatening nature of the snakes present on the islands. Combating ophiophobia (the fear of snakes) can be a difficult task, but it is an essential component of any snake conservation program (Burghardt et al. 2009) and should be at the forefront of conservation management objectives for the Bay Islands.

Arguably of much greater conservation concern, however, is the rapid and unregulated growth of tourism in the Bay Islands (Stonich 2000). Without sufficient protection of appropriate habitat for viable populations, conservation projects will be destined to fail. It is possible that wildlife-based ecotourism could be used to aid conservation of Bay Island wildlife, but it is unlikely that *B. constrictor* would be a focal species in such projects. Whereas in the Cayos Cochinos, populations of *B. constrictor* are at sufficiently high densities to make sightings a likely event, sightings of *B. constrictor* on the Bay Islands are too infrequent to be an attractive bases for wildlife tourism. However, ecotourism based on other species may aid in protecting habitat for *B. constrictor*.

Conclusions

This study has helped to identify the evolutionary significance of island populations of *B. constrictor* in Honduras and will hopefully be used as grounds for investigating other insular populations of the species. It is clear that further phylogenetic analysis is necessary to fully understand the evolutionary history of these island populations

and to set appropriate taxonomic delimitations of the species throughout its range in order to aid conservation prioritisation and planning. Phylogenetic analysis of the Cayos Cochinos and Bay Island populations has not only identified the presence of a potential ESU for the focus of increased conservation efforts, but has also helped shed light on the rapid rate at which dwarfism has evolved in *B. constrictor* populations in the Cayos Cochinos. This phylogeny will hopefully serve as the backbone of future research into the evolution of dwarfism in the Cayos Cochinos and increase our understanding of adaptive shifts in body size on islands.

The future of these evolutionarily distinct island populations, and their recognition as an ESU, is dependent on the assimilation of this information into local government and conservation management agendas, and in the wider education of local communities in sustainable management of their wildlife resources. It is also the responsibility of all individuals involved in the live animal pet trade, from breeders to consumers, to make responsible choices and to make sure that their actions do not undermine or jeopardise the future persistence of the wild populations from which they source their animals, as has been the case in the past.

Conservation management of the Cayos Cochinos *B. constrictor* has allowed substantial demographic recovery of the population. However, the population has likely not yet fully recovered from the negative impacts of intensive harvesting for the pet trade, and illegal poaching continues to threaten the long-term viability of the population. Future work should concentrate on assessing the current status of *B. constrictor* on the Bay Islands and developing island specific conservation strategies that will best conserve the ESU as a whole, thus maintaining both important historical and adaptive variation in this species.

The identification and conservation of unique island biodiversity is of exceptional importance for the conservation of global biodiversity as a whole. This study has identified previously unrecognised levels of genetic diversity within *Boa constrictor* on islands and demonstrated the rapid rate at which dwarfism can evolve, even in a 'giant' snake, on islands.

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Appendices

Appendix 1

Microsatellite marker screening and optimisation

Cross-species utilisation of microsatellite loci in Boa constrictor

At the start of this study no species specific microsatellite markers had been developed for *Boa constrictor*. However, Hille et al. (2002) had previously reported successful amplification of 12 microsatellite loci in *Boa constrictor* that had originally been developed in other species of snake (Burns & Houlden 1999; McCracken et al. 1999; Prosser et al. 1999). Forward and reverse primers were obtained for eleven of the twelve successful microsatellite loci described by Hille et al. (2002). In addition to these eleven markers, forward and reverse primers were also obtained for 35 microsatellite makers developed for the closely related Jamaican Yellow Boa (*Epicrates subflavus*) (Tzika 2007; Tzika et al. 2009). The cross species utility of these markers was then tested in *Boa constrictor.*

PCR amplification was carried out in 20 µl reaction volumes and products were run out on 1% agarose gels stained with ethidium bromide and visualised in a dark room under an ultra violet lamp. PCRs were initially run using the optimum annealing temperatures and $MgCl_2$ concentrations specified for each marker in the original literature. Subsequent optimisation was carried out using temperature gradients and variable $MgCl_2$ concentrations (1.5–3.0 mM).

Testing and optimisation of species-specific microsatellite markers for Boa constrictor

In addition to cross-species utility testing of microsatellite markers, a number of species-specific microsatellite markers were under development (Booth et al. 2011). However, optimisation and screening for levels of polymorphism had not yet been

carried out. Therefore, collaboration was agreed and optimisation and screening of 8 microsatellite loci was conducted as part of this study.

Successfully amplified loci were then screened for levels of polymorphism in a subset of *B. constrictor* specimens (n=24). The 5'ends of forward primers were flurolabelled with either Hex or FAM and PCR products were then run on an ABI 3100 automated sequencer (Applied Biosystems, Inc.). Allele sizes were scored using Genemapper 3.7 (Applied Biosystems, Inc.) and then confirmed by visual inspection.

Results

Amplification success was generally low, with only 12 of the 46 loci tested showing positive amplification when visualised on agarose gel (Appendix 1-1). Only two of the eleven markers previously reported by Hille et al. (2002) to amplify in *Boa constrictor* were successfully amplified in this study (Hb30 and Nsμ3). Interestingly, Groot et al. (2003) were equally unsuccessful in their attempt to amplify these same markers in the Burmese python (*Python molurus bivittatus*). Of the eleven markers reported by Hille et al. (2002) only Hb30 and Nsμ3 were successfully amplified in this study and in Groot et al. (2003). The similarity between the results of this study and Groot et al. (2003) provides some support that the negative results were not just a consequence of poor laboratory technique. A slightly higher success rate was achieved for the markers developed for *Epicrates subflavus*, with 10 of the 35 markers testing positive (Appendix 1-1)

Microsatellite marker Nsμ3 was not tested for levels of polymorphism due to the relative difficulty of consistently amplifying this microsatellite. Of the 11 remaining microsatellites successfully cross amplified in *B. constrictor*, only 3 were found to be

polymorphic (Appendic 1-2). Higher levels of diversity were found in the species specific microsatellites, with 4 out of the 8 markers being found to be polymorphic (Appendix 1-2).

Appendix 1-1 Number of microsatellite loci successfully cross-amplified in *Boa constrictor*

Species	No. of microsats tested	No. that amplified in *Boa constrictor*	Original publication of microsatellites
Epicrates subflavus	35	10	(Tzika 2007; Tzika et al. 2009)
Hoplocephalus bungaroides	4	1	(Burns & Houlden 1999)
Thamnophis sirtalis	3	0	(McCracken et al. 1999)
Nerodia sipedon	4	1	(Prosser et al. 1999)

Appendix 1-2 Level of variance within successfully amplified microsatellite loci

Microsatellite loci	Approximate optimal annealing temp °C	Results of screening for polymorphism	Markers originally published in
µsat01	55	Polymorphic	(Tzika 2007; Tzika et al. 2009)
µsat05	52	Monomorphic	
µsat10	64	Monomorphic	
µsat16	52	Monomorphic	
µsat20	50	Polymorphic	
µsat28	50	Monomorphic	
µsat32	54	Monomorphic	
µsat33	56	Monomorphic	
µsat35	50	Monomorphic	
µsat36	49	Polymorphic	
Hb30	50	Monomorphic	(Burns & Houlden 1999)
Bci-02	58	Monomorphic	(Booth et al. 2011)
Bci-14	60	Polymorphic	
Bci-15	52	Polymorphic	
Bci-16	55	Monomorphic	
Bci-18	54	Polymorphic	
Bci-19	55	Monomorphic	
Bci-21	55	Polymorphic	
Bci-23	60	Polymorphic	

Microsatellite markers found to be polymorphic in the subset of samples tested were then tested for levels of allelic diversity in the complete set of samples (Appendix 1-3)

Appendix 1-3 Levels of allelic diversity in *Boa constrictor* across the successfully amplified polymorphic microsatellite markers (PIC = Polymorphic Information Content).

Microsatellite locus	Repeat type	Number of individuals genotyped	Number of alleles	Allele size range	PIC
Bci-14	$(AAGA)_n(AGGA)_n$	611	29	232-359	0.775
Bci-15	$(TATC)_n$	616	9	232-264	0.669
Bci-18	$(CCTT)_n$	594	14	276-330	0.803
Bci-21	$(AG)_n$	623	11	248-274	0.254
Bci-23	$(TCTG)_n(TC)_n$	622	2	206-214	0.062
µsat01	$(AGAT)_n$	625	17	340-409	0.712
µsat20	$(CTC)_n$	608	8	234-269	0.422
µsat36	$(CTTT)_n(CTTC)_n$	607	38	260-400	0.831

Appendix 2

Appendix 2-1 Locality information and Genbank accesion numbers for all samples sequenced for mitochondrial DNA genes CytB and ND4+tRNAs. *Tissue samples obtained from captive animals believed to be of Honduran mainland origin **Tissue sample obtained from captive animal believed to be of Bay Island origin. †Boa mislabelled as *Boa constrictor imperator* on Genbank, treated here as *Boa constrictor constrictor*. Hon = Honduras, BI = Bay Island, CC = Cayos Cochinos

Haplotype	No. Sequences represented	Tissue sample ID	Country/Islands	Locality	Genbank Accession CytB	Genbank Accession ND4+tRNAs	Source
Honduras (a)	5	ML4	Hon	Animal rescue centre – Tegucigalpa*	GQ477927	GQ477972	This Study
		ML5	Hon	Animal rescue centre – Tegucigalpa*	GQ477928	GQ477973	This Study
		ML7	Hon	Animal rescue centre – Tegucigalpa*	GQ477930	GQ477975	This Study
		ML8	Hon	Animal rescue centre – Tegucigalpa*	GQ477931	GQ477976	This Study
		ML9	Hon	Rio Aguan Valley	GQ477932	GQ477977	This Study
Honduras (b)	1	ML1	Hon	Animal rescue centre – Tegucigalpa*	GQ477924	GQ477969	This Study
Honduras (c)	1	ML2	Hon	Animal rescue centre – Tegucigalpa*	GQ477925	GQ477970	This Study
Honduras (d)	1	ML3	Hon – Presumed BI	Animal rescue centre – Tegucigalpa**	GQ477926	GQ477971	This Study
Honduras (e)	1	ML6	Hon	Animal rescue centre – Tegucigalpa*	GQ477929	GQ477974	This Study
Honduras (f)	1	BC06	Hon	Cordillera de la Botija,	GQ477917	GQ477962	This Study
Honduras (g)	1	BC07	Hon	Quebrada La Fortuna, Cerro, Guanacaure	GQ477918	GQ477963	This Study

Haplotype	No. Sequences represented	Tissue sample ID	Country/Islands	Locality	Genbank Accession CytB	Genbank Accession ND4+tRNAs	Source
Utila (a)	5	U1	Hon– BI	Utila	GQ477938	GQ477978	This Study
		U4	Hon – BI	Utila	GQ477941	GQ477981	This Study
		U6	Hon – BI	Utila	GQ477942	GQ477982	This Study
		U7	Hon – BI	Utila	GQ477943	GQ477983	This Study
		U8	Hon – BI	Utila	GQ477944	GQ477984	This Study
Utila (b)	1	U2	Hon – BI	Utila	GQ477939	GQ477979	This Study
Utila (c)	1	U3	Hon – BI	Utila	GQ477940	GQ477980	This Study
Guanaja	1	G1	Hon – BI	Guanaja	GQ477933	GQ477989	This Study
Roatan	3	R1	Hon – BI	Roatan	GQ477934	GQ477985	This Study
		R2	Hon – BI	Roatan	GQ477935	GQ477986	This Study
		R3	Hon – BI	Roatan – Santa Elena	GQ477936	GQ477987	This Study
Barbareta	1	R4	Hon – BI	Isla de Barbareta	GQ477937	GQ477988	This Study
C. Pequeño (a)	3	CCP01	Hon – CC	Cayo Cochino Pequeño	GQ477945	GQ477990	This Study
		CCP22	Hon – CC	Cayo Cochino Pequeño	GQ477947	GQ477992	This Study
		CCP49	Hon – CC	Cayo Cochino Pequeño	GQ477950	GQ477995	This Study
C. Pequeño (b)	1	CCP16	Hon – CC	Cayo Cochino Pequeño	GQ477946	GQ477991	This Study

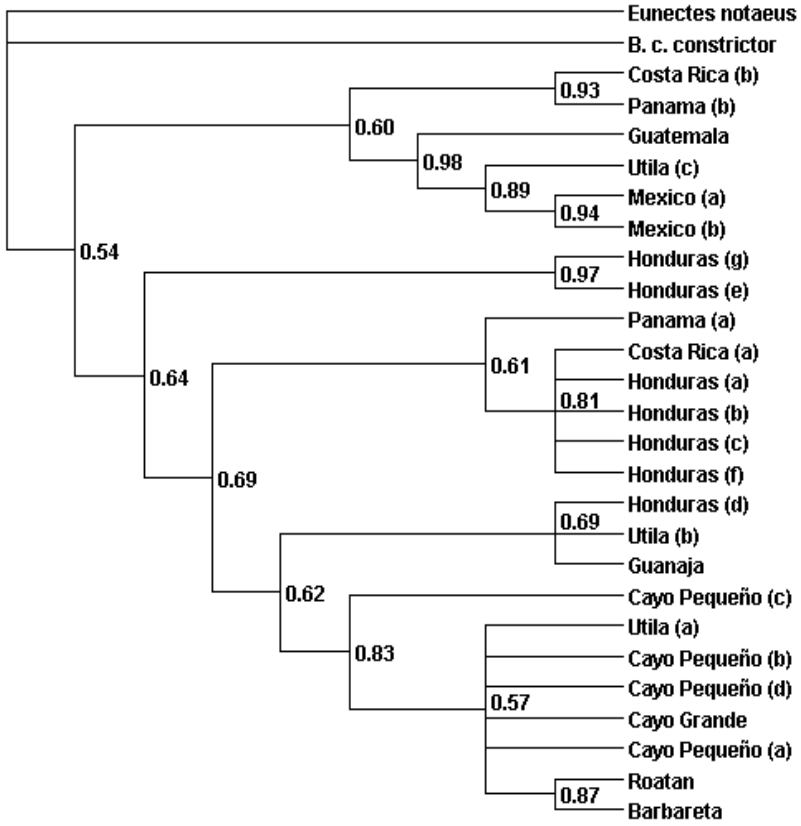
Haplotype	No. Sequences represented	Tissue sample ID	Country/Islands	Locality	Genbank Accession CytB	Genbank Accession ND4+tRNAs	Source
C. Pequeño (c)	1	CCP36	Hon – CC	Cayo Cochino Pequeño	GQ477948	GQ477993	This Study
C. Pequeño (d)	1	CCP40	Hon – CC	Cayo Cochino Pequeño	GQ477949	GQ477994	This Study
C. Grande	6	CCG01	Hon – CC	Cayo Cochino Grande	GQ477951	GQ477996	This Study
		CCG08	Hon – CC	Cayo Cochino Grande	GQ477952	GQ477997	This Study
		CCG19	Hon – CC	Cayo Cochino Grande	GQ477953	GQ477998	This Study
		CCG23	Hon – CC	Cayo Cochino Grande	GQ477954	GQ477999	This Study
		CCG35	Hon – CC	Cayo Cochino Grande	GQ477955	GQ478000	This Study
		CCG46	Hon – CC	Cayo Cochino Grande	GQ477956	GQ478001	This Study
Guatemala	1	BC01	Guatemala	Unknown	GQ477912	GQ477957	This Study
Mexico (a)	1	BC02	Mexico	Ticopo, close to Merida, Yucatan	GQ477913	GQ477958	This Study
Mexico (b)	1	BC03	Mexico	Tulum, close to Merida, Yucatan	GQ477914	GQ477959	This Study
Costa Rica (a)	1	BC04	Costa Rica	Limon Province	GQ477915	GQ477960	This Study
Costa Rica (b)	1	BC05	Costa Rica	Puntarenas Province, Nr. Buenos Aires	GQ477916	GQ477961	This Study

Haplotype	No. Sequences represented	Tissue sample ID	Country/Islands	Locality	Genbank Accession CytB	Genbank Accession ND4+tRNAs	Source
Panama (a)	5	BC08	Panama	Rio Hato	GQ477919	GQ477964	This Study
		BC23	Panama	Cocle, El Valle	GQ477920	GQ477965	This Study
		BC24	Panama	Cocle, El Valle	GQ477921	GQ477966	This Study
		BC30	Panama	Cocle, El Valle, Auton	GQ477922	GQ477967	This Study
		BC34	Panama	Cocle Province, Barrigon	GQ477923	GQ477968	This Study
Panama (b)	1	Genbank	Panama	Barro Colorado Island	AB177354	AB177354	(Dong & Kumazawa 2005)
B. c. constrictor[†]	1	Genbank	Unknown	Unknown	AM 23634	AM 23634	(Douglas et al. 2006)

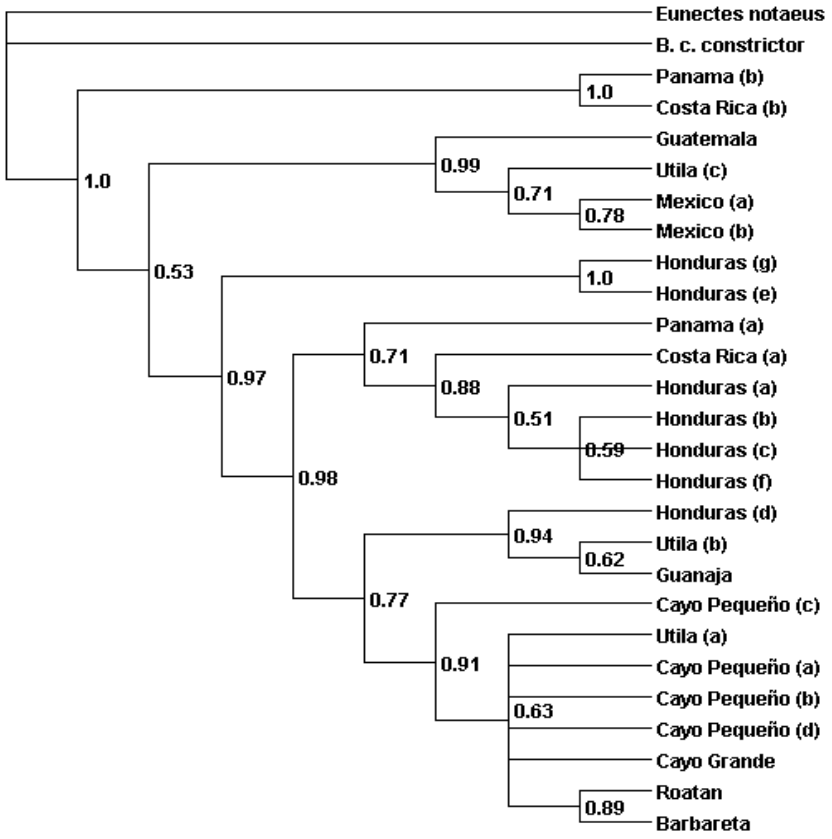
Appendix 2-2 A full list of samples genotyped for microsatellite loci, including genotype data, can be found on the data CD provided.

Appendix 3

Topologies of maximum likelihood and neighbour joining 50% majority rule consensus trees.



ML 50% majority rule consensus tree generated from 1000 bootsraps in PhyML and displaying bootstrap node support values



NJ 50% majority rule consensus tree generated from 1000 bootstraps in PAUP and displaying bootstrap node support values

Appendix 4

In all trees produced, Genbank sequence AM236348 was placed basal to the other boas and displayed a considerably longer branch length compared with all other ingroup taxa in the analyses. Furthermore, the uncorrected pair-wise genetic distance of this sequence was more than three times greater than the maximum distance values observed between any of the other boas within the Central American clade (Appendix 4-1). The large sequence divergence of sample AM236348 from all other Central American samples was equivalent to that expected for *B. c. constrictor* from the South American clade described by Hynkova et al. (2009).

Appendix 4-1 Uncorrected pair-wise genetic distance variation within Central American *Boa constrictor* sampled in this study compared with sequence AM236348 and outgroup *Eunectes notaeus*.

	All other boas	AM236348
Eunectes notaeus	0.17 – 0.18	0.18
AM236348	0.07	-
All other boas	0.00 – 0.02	-

Consequently, although labelled as *B. c. imperator* on the Genbank database, this sample's placement within the tree topology, combined with its long branch-length and large sequence divergence from all other samples, led us to question its classification. By contacting the author of the original sequence publication (Douglas et al. 2006), it was discovered that no provenance data existed for this sample, and its classification as *B. c. imperator* could not be verified (Douglas 2009 personal communication).

Bayesian phylogenetic reconstruction of the cytochrome-b gene was conducted in MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003; Huelsenbeck & Ronquist 2005) for

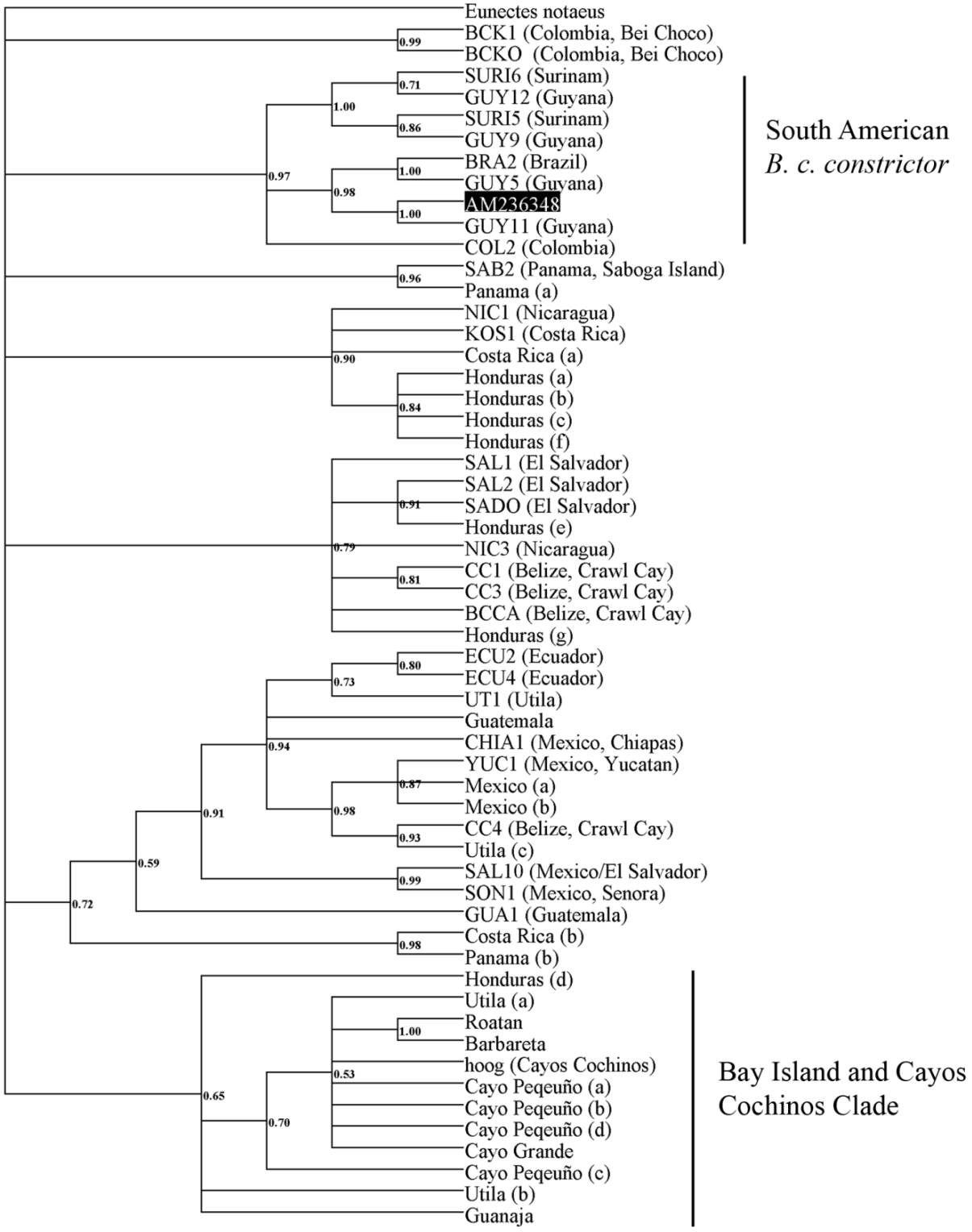
the samples in this study and a number of samples from Hynkova et al. (2009). We ran parallel MCMCs for ten million generations, sampling every 100th generation. The first seventy five thousand trees were discarded and the remaining trees used to construct a 50% majority rule consensus tree. Sample AM236348 can clearly be seen to group closely with South American *B. c. constrictor* (Appendix 4-2). Therefore, for the purposes of this study, sample AM236348 was treated as representative of *B. c. constrictor* rather than *B. c. imperator*. Sample AM236348 was chosen over other representative sequences of *B. c. constrictor* available on Genbank because of the availability of both cytochrome-b and ND4 regions of the mitochondrial genome.

Interestingly, sequences from the Bay Island and Cayos Cochinos clade continue to form a monophyletic group even after the inclusion of a number of extra mainland samples (Appendix 4-3). Thus, increased sampling of mainland Central American *B. constrictor* fails to provide further evidence of Bay Island and Cayos Cochinos haplotypes on the mainland. However, increased sampling along the adjacent Honduran coastline may be necessary to determine fully the level of gene flow between the island and mainland clades.

Sequence AM236348 can clearly be seen to group within the South American *Boa constrictor constrictor* clade and displays an equally long branch length.

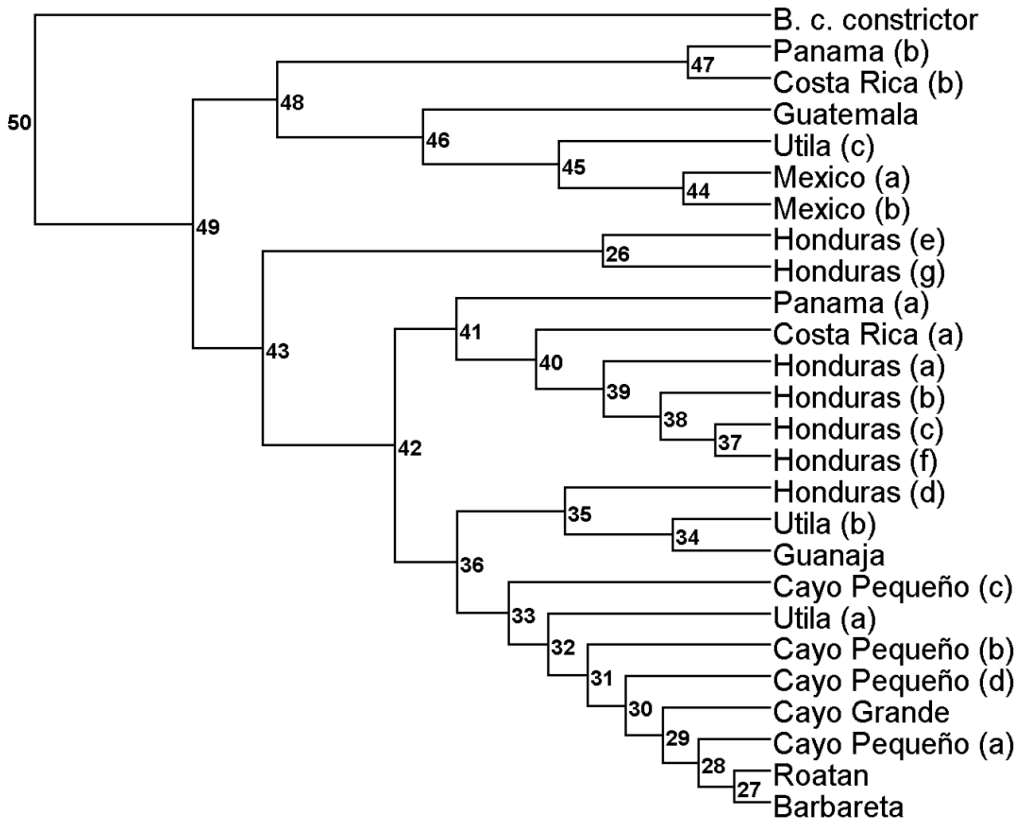
Appendix 4-2 Consensus Baysian phylogram for the *Boa constrictor* cytochrome-b gene combining haplotypes from this study and a number of haplotypes reported in Hynkova et al. (2009).

Appendix 4-3 Consensus Baysian cladogram for the *Boa constritcor cytochrome-b* gene combining haplotypes from this study and a number of haplotypes reported in Hynkova et al. (2009). Node support values are Bayesian posterior probability values.



Appendix 5

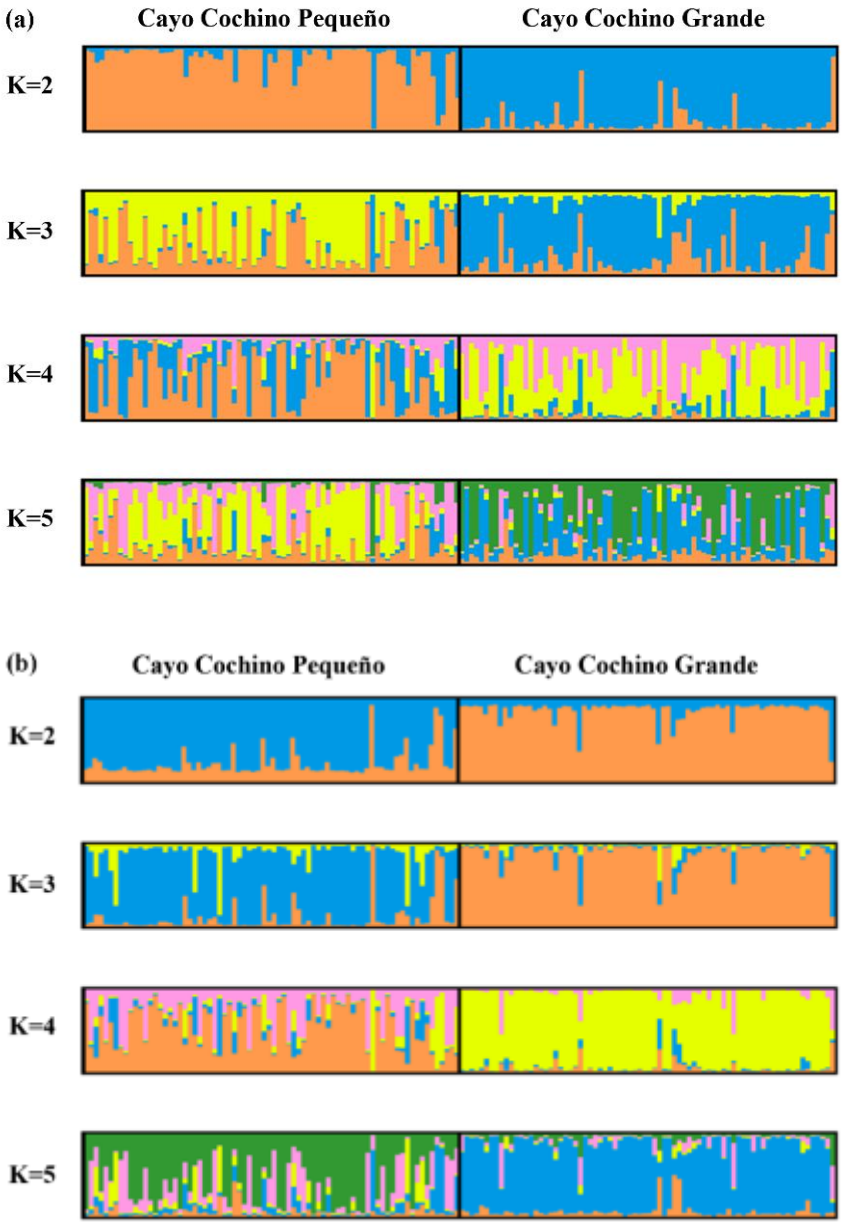
Divergence dates for individual nodes of the fully resolved tree, estimated in this study using Multidivtime. Also displayed are standard deviation (S.D.) and upper and lower 95% confidence intervals for estimated divergence dates (Mya).

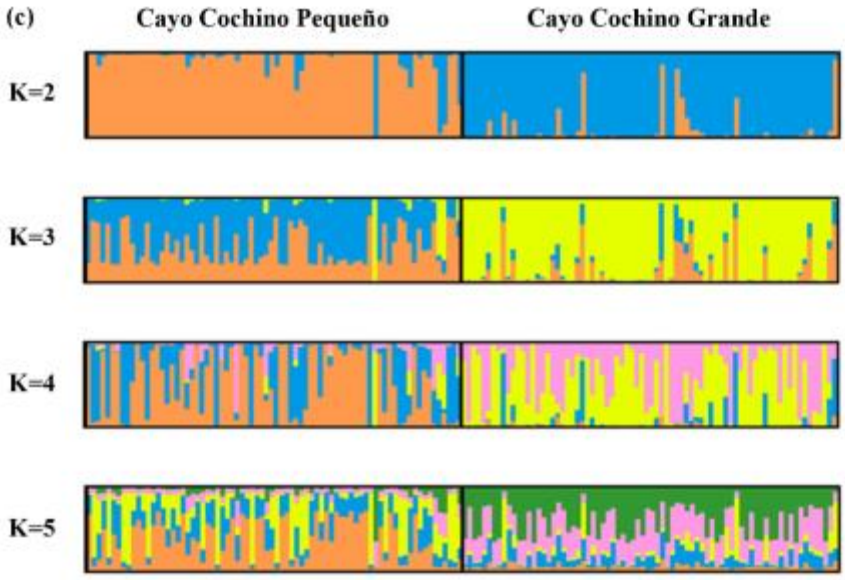


Node	Date (Mya)	S.D.	Upper 95% C.I.	Lower 95% C.I.	Node	Date (Mya)	S.D.	Upper 95% C.I.	Lower 95% C.I.
26	0.879	0.688	0.046	2.613	39	0.876	0.538	0.169	2.219
27	0.188	0.203	0.004	0.738	40	1.230	0.655	0.315	2.823
28	0.377	0.295	0.041	1.144	41	1.653	0.763	0.527	3.458
29	0.565	0.375	0.099	1.515	42	1.976	0.838	0.697	3.920
30	0.761	0.451	0.169	1.893	43	2.672	0.965	1.116	4.846
31	0.960	0.526	0.245	2.245	44	0.456	0.444	0.014	1.636
32	1.166	0.597	0.329	2.614	45	1.111	0.680	0.200	2.775
33	1.377	0.664	0.418	2.963	46	1.827	0.845	0.563	3.801
34	0.513	0.461	0.017	1.714	47	0.431	0.447	0.012	1.650
35	1.080	0.641	0.191	2.638	48	2.597	0.969	1.042	4.781
36	1.646	0.747	0.541	3.403	49	3.040	1.014	1.375	5.316
37	0.289	0.296	0.007	1.090	50	3.871	1.034	2.150	6.163
38	0.577	0.424	0.069	1.666	-	-	-	-	-

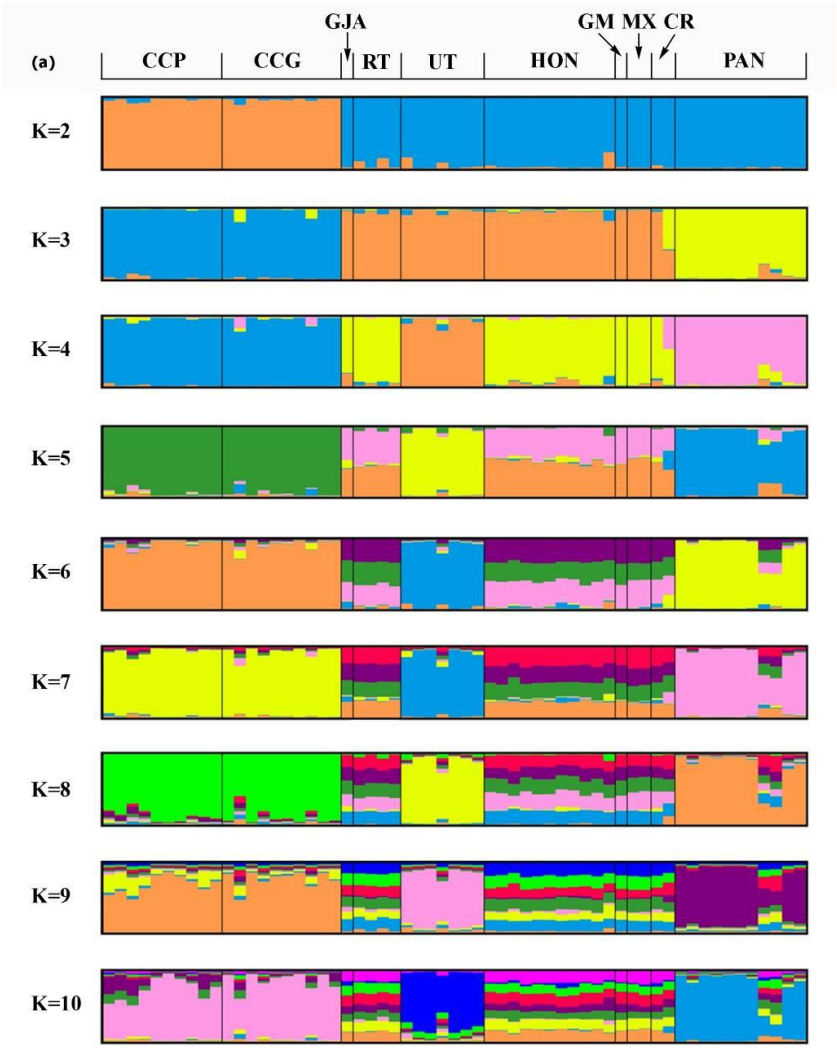
Appendix 6

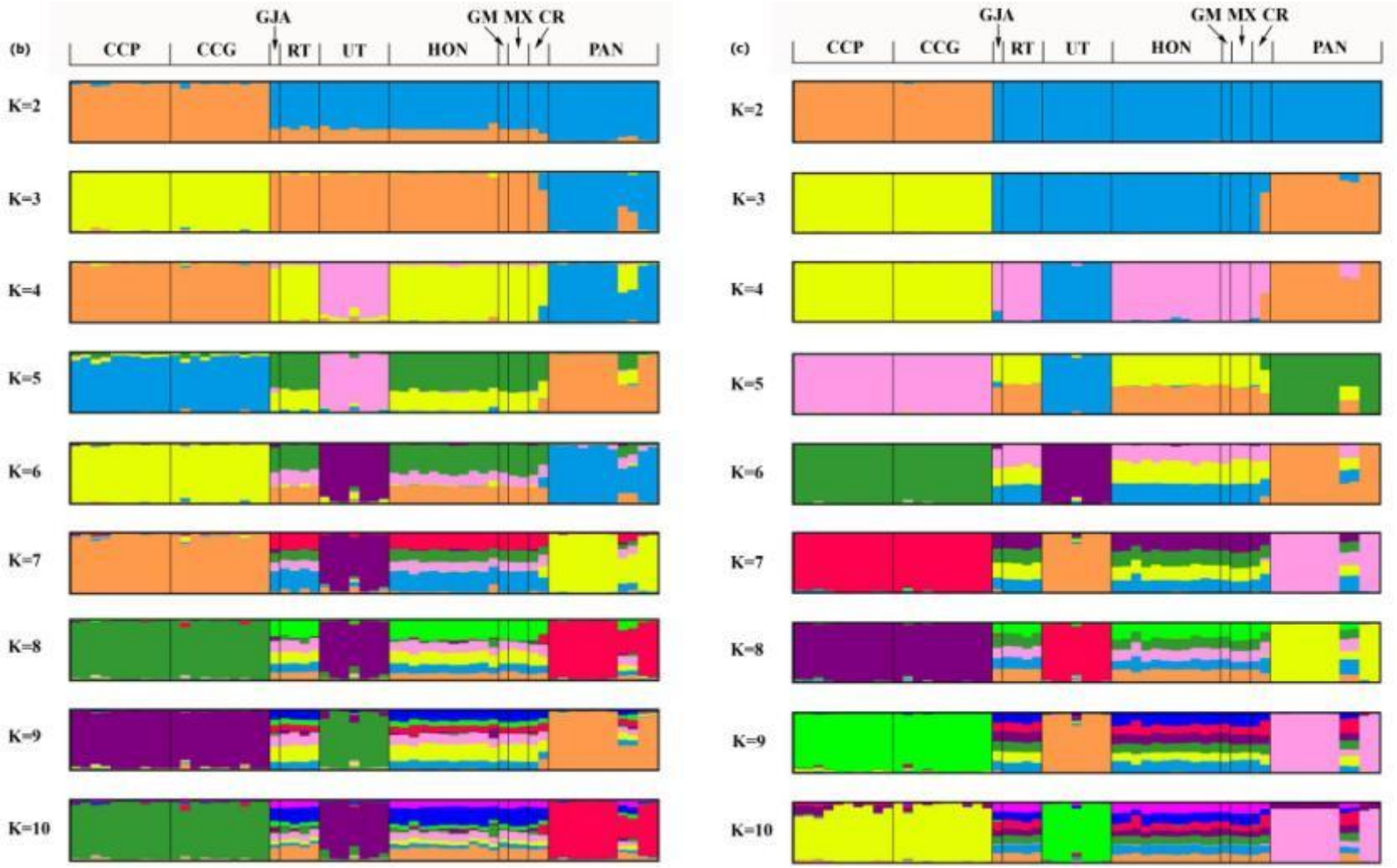
Appendix 6-1 Individual cluster assignment probabilities for the Cayos Cochinos determined by five independent runs of *STRUCTURE* Kmax 1-5, averaged using the programs *CLUMPP* and *DISTRUCT*; (a) admixture model no prior population information, (b) admixture model use prior population information, (c) no admixture no prior population information.



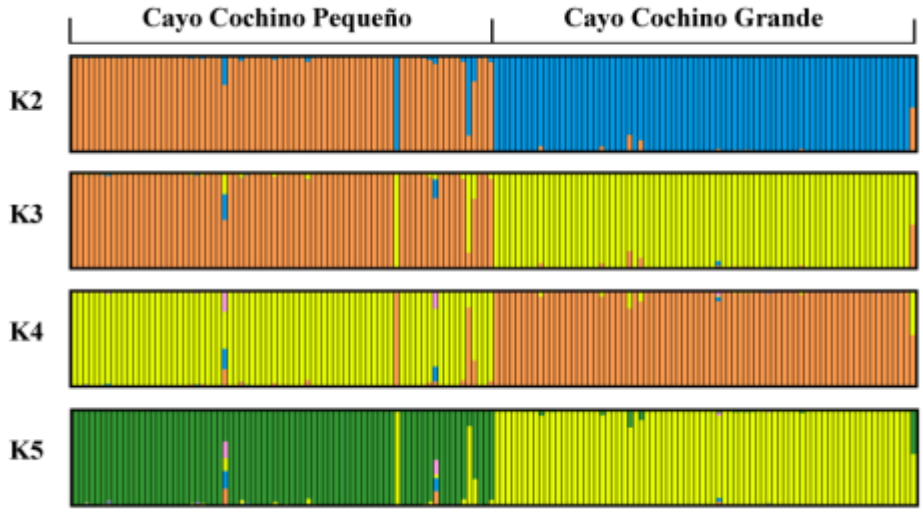

(c)
Cayo Cochino Pequeño
Cayo Cochino Grande
K=2
K=3
K=4
K=5

Appendix 6-2 Individual cluster assignment probabilities for all populations determined by five independent runs of *STRUCTURE* Kmax 1-10, averaged using the programs *CLUMPP* and *DISTRUCT*; (a) admixture model no prior population information, (b) admixture model use prior population information, (c) no admixture model no prior population information. CCP=Cayo Cochino Pequeño, CCG=Cayo Cochino Grande, GJA=Guanaja, RT=Roatan, UT=Utila, HON=Honduras mainland, GM=Guatemala, MX=Mexico, CR=Costa Rica, PAN=Panama.

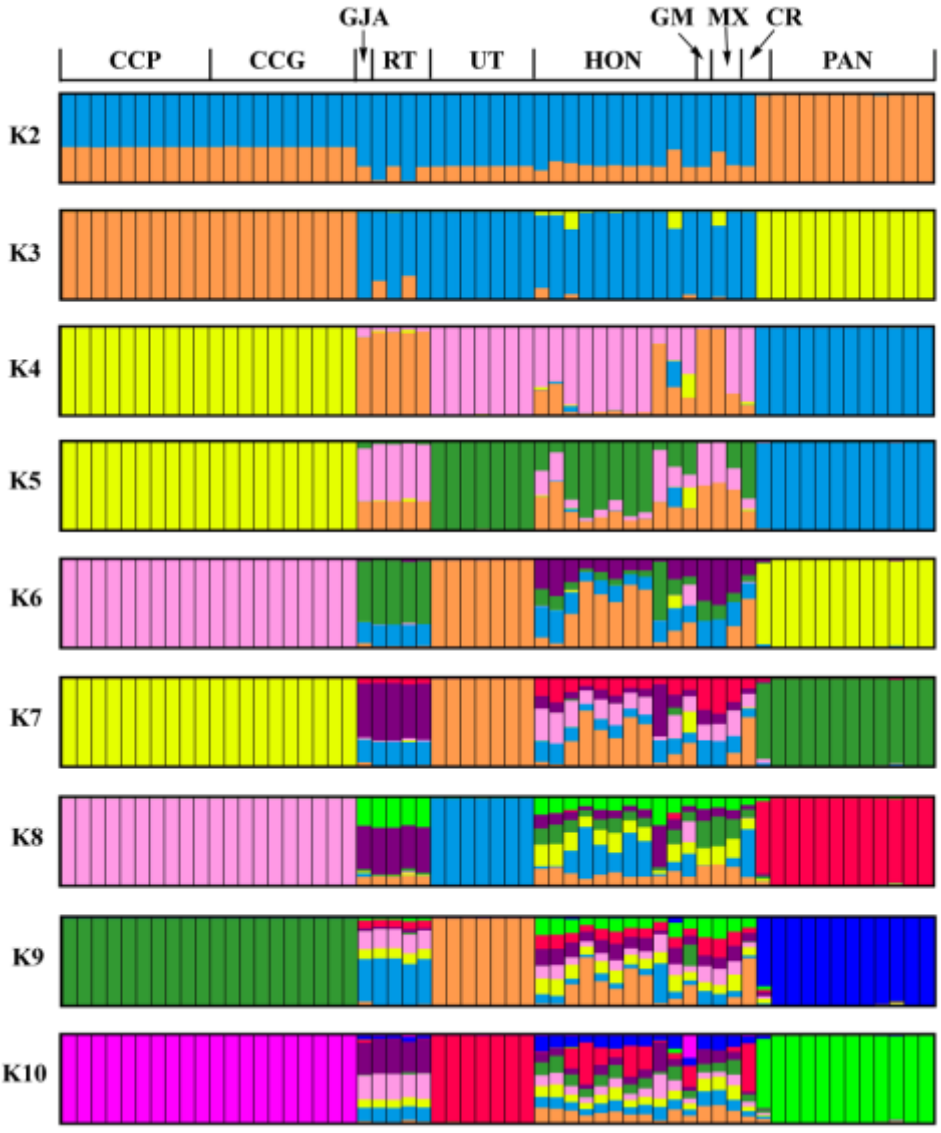



(b)
GJA
GM MX CR
CCP
CCG
RT
UT
HON
PAN
K=2
K=3
K=4
K=5
K=6
K=7
K=8
K=9
K=10
(c)
GJA
GM MX CR
CCP
CCG
RT
UT
HON
PAN
K=2
K=3
K=4
K=5
K=6
K=7
K=8
K=9
K=10

Appendix 6-3 Individual cluster assignment probabilities for the Cayos Cochinos determined by *TESS* (no admixture model) and averaged over five independent runs using the programs *CLUMPP* and *DISTRUCT*. Population structure is clearly apparent between the two islands with two distinct clusters being the most parsimonious explanation of the data even when a maximum of 5 clusters (K5) is allowed.

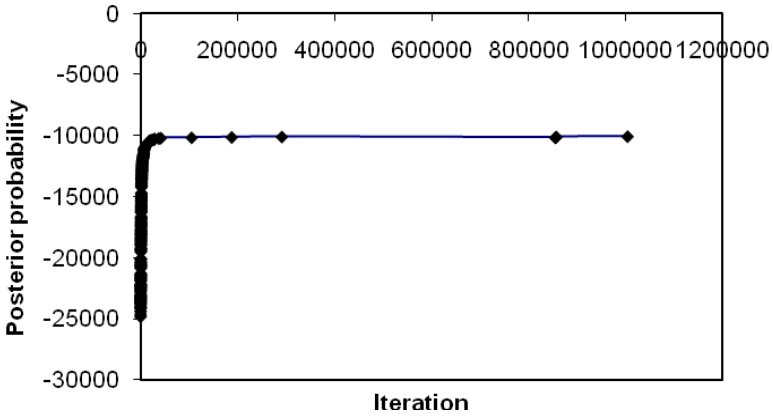


Appendix 6-4 Individual cluster assignment probabilities averaged over five independent runs of *TESS* (no admixture model) using the programs *CLUMPP* and *DISTRUCT*. CCP=Cayo Cochino Pequeño, CCG=Cayo Cochino Grande, GJA=Guanaja, RT=Roatan, UT=Utila, HON=Honduras mainland, GM=Guatemala, MX=Mexico, CR=Costa Rica, PAN=Panama.

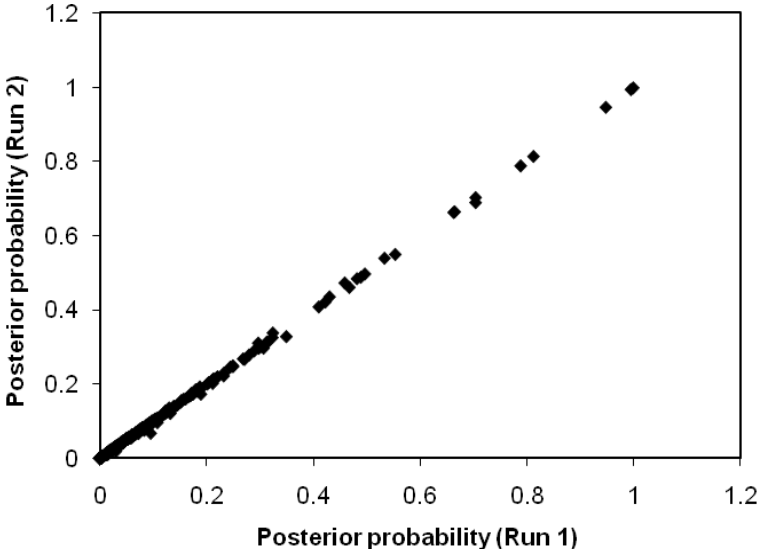


Appendix 7

Appendix 7-1 Checking for stationarity of the run in BayesAss. Log posterior probability plotted against number of iterations of the mcmc. Stationarity of the run appears to be reached fairly rapidly, within the first 10,000 iterations. Data plotted here are from run 1 of the five population data set, however, the same pattern was observed in all runs for both data sets.



Appendix 7-2 Checking for convergence in BayesAss. Mean posterior probability of each allele frequency at each locus in each population, for each of the two independent runs of the mcmc. Convergence of the runs is inferred by the linear relationship between posterior probabilities. Data plotted here are from run 1 of the five population data set, however, the same pattern was observed in all runs for both data sets.



Appendix 8

Appendix 8-1 Mean posterior probabilities of the immigration rates of the two population scenario *BayesAss* analysis (Run 2). The populations into which individuals are migrating are listed in the rows and the origins of the migrants are listed in the columns. Values along the diagonal are the proportions of individuals derived from the source populations each generation. Values in parentheses are 95% CI

		From	
		C. Pequeño	C. Grande
Into	C. Pequeño	0.991 (0.982-0.998)	0.009 (0.002-0.018)
	C. Grande	0.009 (0.000-0.032)	0.991 (0.968-1.000)

Appendix 8-2 Mean posterior probabilities of the immigration rates of the five population scenario *BayesAss* analysis (Run 2). The populations into which individuals are migrating are listed in the rows and the origins of the migrants are listed in the columns. Values along the diagonal are the proportions of individuals derived from the source populations each generation. Values in parentheses are 95% CI

		From				
		CCP	CCG	Utila	Hon ML	Panama
Into	CCP	0.985 (0.975-0.996)	0.006 (0.000-0.014)	0.009 (0.000-0.016)	0.000 (0.000-0.005)	0.000 (0.000-0.002)
	CCG	0.002 (0.000-0.012)	0.994 (0.978-1.000)	0.001 (0.000-0.007)	0.001 (0.000-0.007)	0.001 (0.000-0.008)
	Utila	0.010 (0.000-0.057)	0.010 (0.000-0.061)	0.961 (0.873-0.999)	0.010 (0.000-0.056)	0.010 (0.000-0.060)
	Hon ML	0.023 (0.000-0.091)	0.018 (0.000-0.077)	0.165 (0.009-0.297)	0.729 (0.668-0.950)	0.065 (0.000-0.177)
	Panama	0.006 (0.000-0.039)	0.006 (0.000-0.037)	0.006 (0.000-0.039)	0.007 (0.000-0.043)	0.974 (0.908-0.999)

Appendix 9

Data files for MSVAR analysis

Cayo Cochino Grande

```
7
3
15 102 33
0 1 6
15
1 2 13 30 3 4 7 1 3 22 18 13 16 15 2
0 2 3 4 5 10 11 12 13 14 15 16 17 18 21
5
126 1 6 17 2
0 3 9 10 11
2
111 41
0 1
12
3 3 52 28 20 8 23 7 2 2 3 1
0 1 2 3 4 5 6 7 8 21 22 24
8
14 15 44 11 47 17 3 1
0 1 2 3 4 5 6 7
8
1 2 11 80 32 21 3 2
0 1 2 3 6 7 10 11
```

Cayo Cochino Pequeño (data set 1)

```
7
8
0 1 173 0 4 69 0 3
0 4 5 7 8 10 11 12
16
0 11 1 23 0 12 1 5 1 13 7 101 27 25 12 11
0 5 6 7 8 13 14 15 16 17 18 19 20 21 22 23
12
0 104 4 45 2 1 9 12 2 58 12 1
0 1 3 4 5 6 7 9 10 11 12 15
4
230 15 2 3
0 1 2 5
16
0 1 3 1 1 111 27 33 19 2 9 12 18 5 7 1
0 2 5 7 8 9 10 11 12 13 27 28 29 30 31 34
6
0 26 77 117 27 3
0 1 2 3 4 5
9
72 57 26 6 4 36 6 1 42
0 1 2 3 4 5 8 9 10
```

Cayo Cochino Pequeño (data set 2)

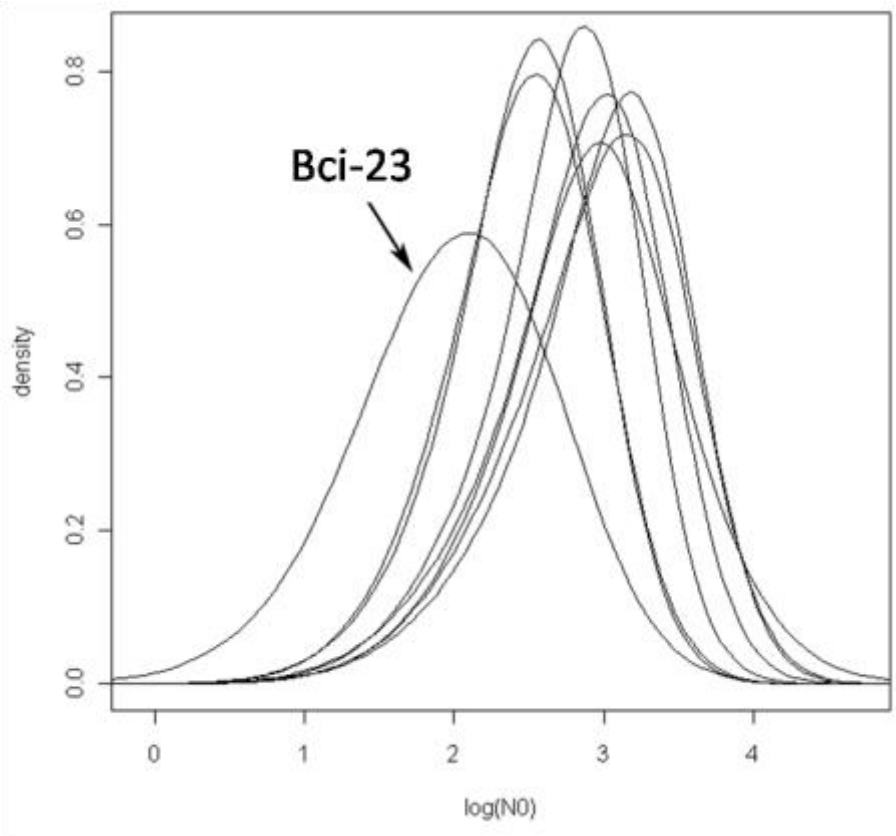
```
7
8
0 1 171 0 2 74 0 2
0 4 5 7 8 10 11 12
16
0 13 2 21 1 10 2 2 2 22 7 102 30 14 15 7
0 5 6 7 8 13 14 15 16 17 18 19 20 21 22 23
12
0 105 4 34 5 1 11 15 1 62 11 1
0 1 3 4 5 6 7 9 10 11 12 15
4
235 12 2 1
0 1 2 5
16
0 1 1 0 1 116 21 43 15 1 10 7 20 7 6 1
0 2 5 7 8 9 10 11 12 13 27 28 29 30 31 34
6
0 22 77 114 32 5
0 1 2 3 4 5
9
68 58 27 8 2 34 3 1 49
0 1 2 3 4 5 8 9 10
```

Cayo Cochino Pequeño (data set 3)

```
7
8
0 1 179 0 6 60 0 4
0 4 5 7 8 10 11 12
16
1 10 3 24 1 7 4 2 3 16 6 100 28 22 11 12
0 5 6 7 8 13 14 15 16 17 18 19 20 21 22 23
12
0 113 5 37 5 0 6 10 3 57 13 1
0 1 3 4 5 6 7 9 10 11 12 15
4
240 7 1 2
0 1 2 5
16
2 1 0 0 0 106 18 43 17 3 12 5 27 9 7 0
0 2 5 7 8 9 10 11 12 13 27 28 29 30 31 34
6
0 27 60 128 29 6
0 1 2 3 4 5
9
82 56 24 4 4 31 3 0 46
0 1 2 3 4 5 8 9 10
```

Appendix 10

Initial trials showed microsatellite locus Bci-23 acted as an aberrant loci in MSVAR analyses



Locus Bci-23 was included in the Cayo Cochino Pequeño data set during initial trials. The locus is a di-tetra compound repeat motif and thus cannot strictly be scored as the number of repeat units. In initial trials, in which it was attempted to score the locus as the number of dinucleotide repeats, a significant departure from the behaviour of the other loci was observed. This may have been a consequence of the way in which the locus was scored violating the strict single mutation model employed by MSVAR. Alternatively it may have been due to the fact that only two alleles are present in the population, one of which is almost at fixation. Locus Bci-23 was thus removed from all MSVAR analyses.

Appendix 11

Results of Individual Loci for Cayo Cochino Grande and Cayo Cochino Pequeño

(data set 1)

Key

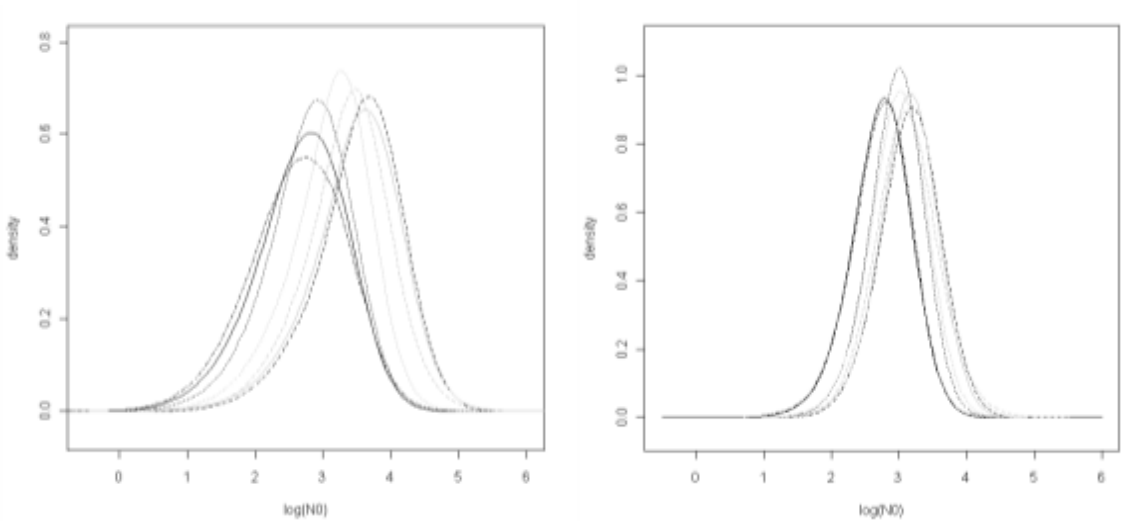
= Usat 20 = Bci-21 = Bci-18

= Usat 36 = Bci-14

= Usat01 = Bci-15

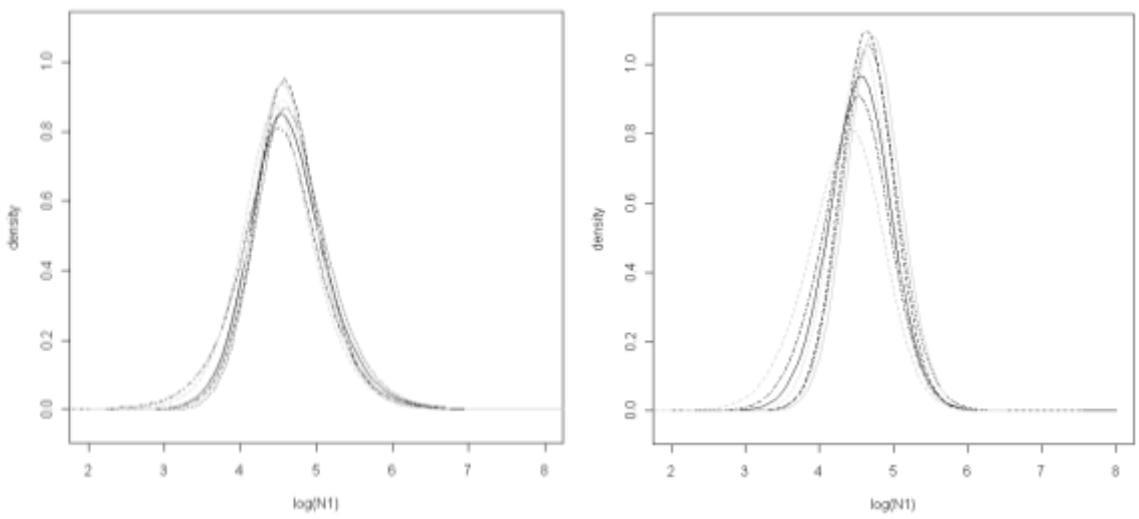
(i) Cayo Cochino Grande (a) and Cayo Cochino Pequeño (b) Current Population Size (N_0)

(a) (b)



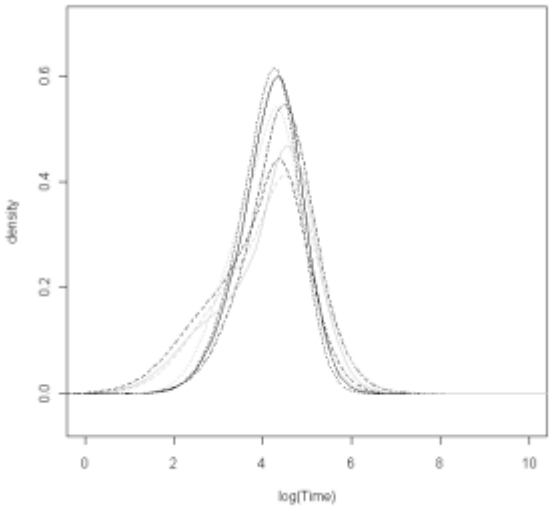
(ii) Cayo Cochino Grande (a) and Cayo Cochino Pequeño (b) Ancestral Population Size (N_1)

(a) (b)



(iii) Cayo Cochino Grande (a) and Cayo Cochino Pequeño (b) Time Decline Started (χ_a)

(a)



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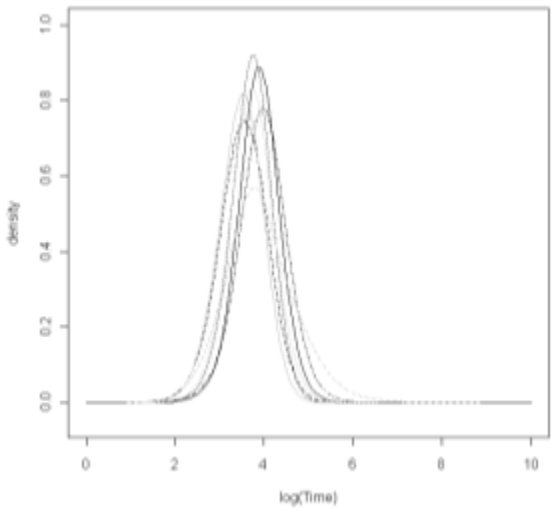
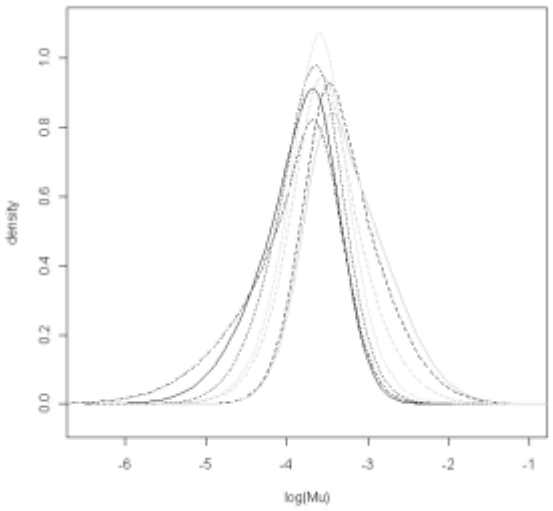
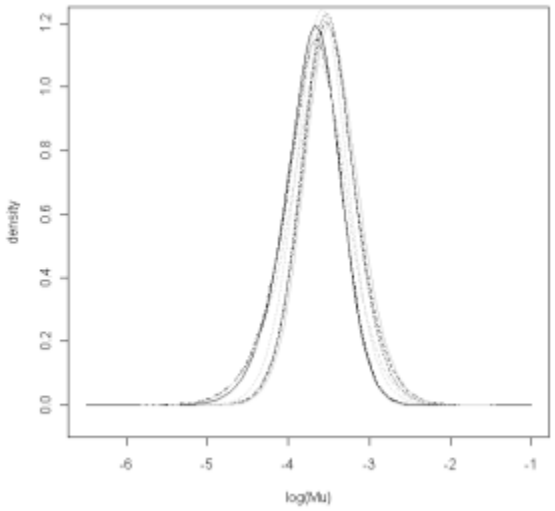


(iv) Cayo Cochino Grande (a) and Cayo Cochino Pequeño (b) Mutation Rate (Mu)

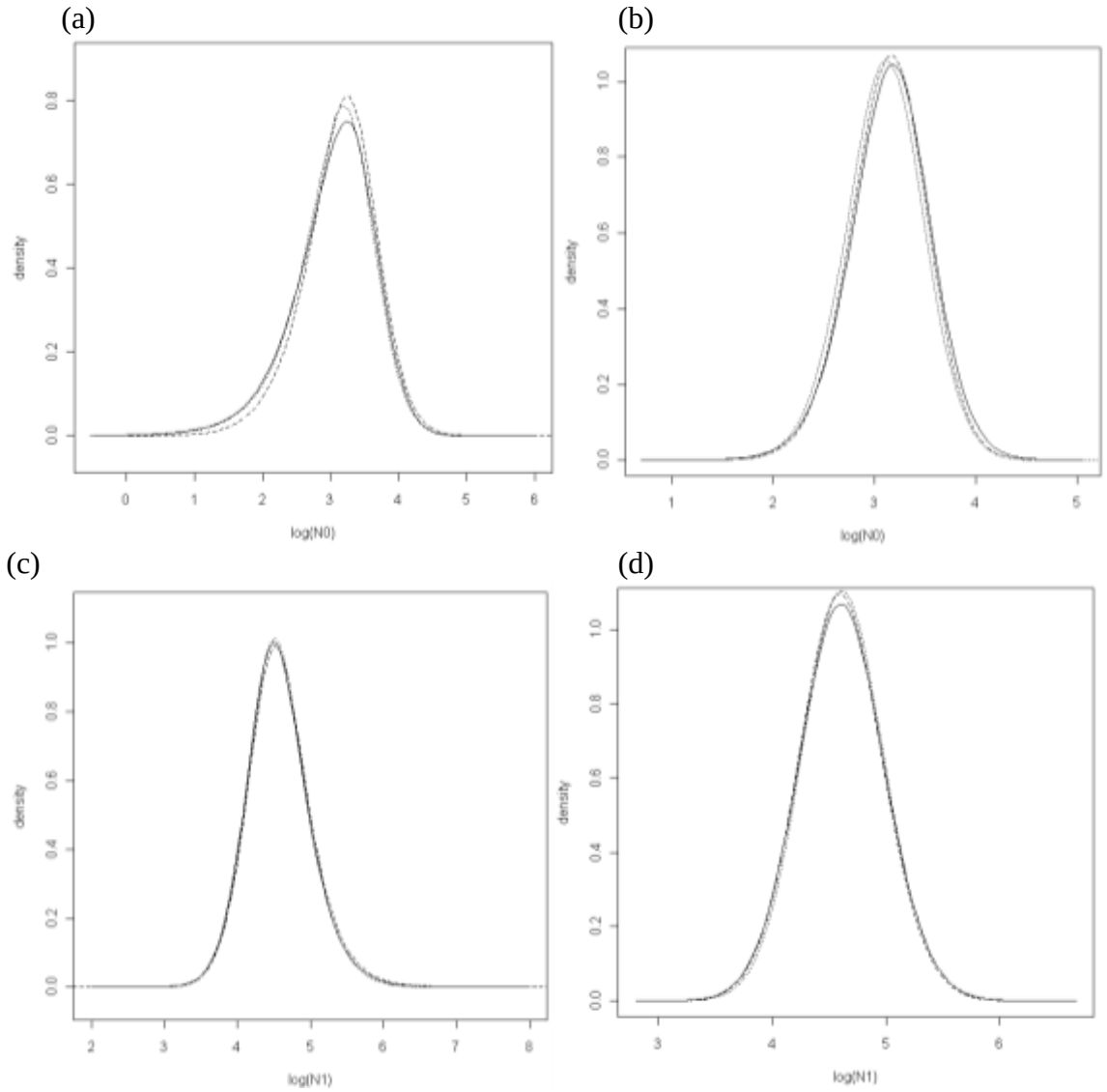
(a)



(b)



Comparison of the effect of different population priors N_0 and N_1 on posterior parameter distributions.



Key: Historical demographic scenario

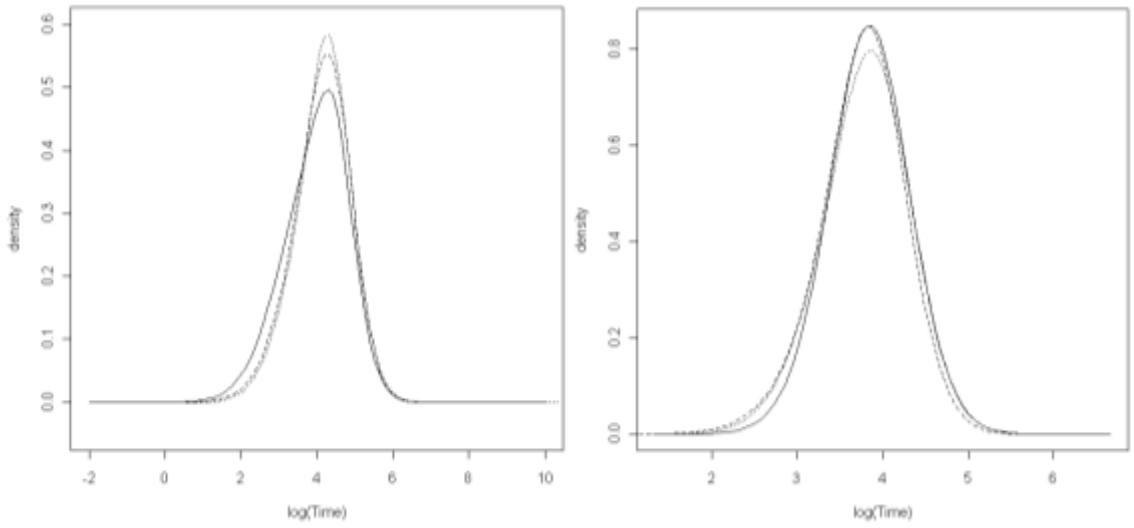
——— = Stable ····· = Decline – – – – – = Expansion

Population priors were manipulated to simulate three alternative historical demographic scenarios; stable population over time, population decline and population expansion. Density plots show that the priors had little to no affect on the posterior distributions of (a) current population size Cayo Cochino Grande, (b) current population size Cayo Cochino Pequeño, (c) ancestral population size Cayo

Cochino Grande, (d) ancestral population size Cayo Cochino Pequeño. Only results from Cayo Cochino Pequeño data set 2 are displayed here for ease of graphical interpretation, however, exactly the same pattern was also observed for Cayo Cochino Pequeño data sets 1 and 3.

Posterior density plots displaying the inferred time population decline began (χ_a) under each of the prior temporal scenarios in (a) Cayo Cochinos Grande, (b) Cayo Cochino Pequeño

(a) (b)



Key: Prior Scenario for time population change began

——— = Intermediate ····· = Ancient – – – – – = Recent

The prior means for the time at which population decline began were manipulated to simulate three alternative scenarios; a ‘recent’ decline (100 years bp), ’intermediate’ decline (100,000 years bp) and an ‘ancient’ decline (1,000,000 years bp). Posterior density plots show that the prior had no affect on the final result for either the Cayo Cochino Grande (a) or Cayo Cochino Pequeño (b) data sets. Only results from Cayo Cochino Pequeño data set 1 are displayed here for ease of graphical interpretation, however, exactly the same pattern was also observed for Cayo Cochino Pequeño data sets 2 and 3.

Appendix 12

Summary statistics for each of the (a) population demographic and (b) temporal scenarios implemented in *MSVAR* 1.3 (presented here after transformation from Log_{10} scale)

(a) Population demographic scenarios

Parameter	Data Set	Prior model of population change	Mean	Median	Mode	HPD Intervals	
						lower	upper
Current Population Size (N_0)	CCP1	Stable ($N_0=N_1$)	1,156	1,195	861	189	6,731
	CCP2		1,426	1,459	1,337	226	8,720
	CCP3		640	685	830	70	5,011
	CCG		1,114	1,313	1,162	69	13,880
	CCP1	Declining ($N_0<N_1$)	977	1,070	522	108	7,845
	CCP2		1,250	1,271	1,121	211	7,140
	CCP3		689	718	758	94	4,520
	CCG		1,099	1,274	1,912	71	13,504
	CCP1	Expanding ($N_0>N_1$)	1,104	1,130	709	171	7,123
	CCP2		1,371	1,403	636	240	7,572
	CCP3		638	685	830	70	5,009
	CCG		1,365	1,499	3,774	104	14,170
Ancestral Population Size (N_1)	CCP1	Stable ($N_0=N_1$)	38,459	38,267	22,058	7,455	201,416
	CCP2		40,644	40,396	30,790	7,328	226,975
	CCP3		43,752	43,230	35,378	8,163	233,024
	CCG		36,559	34,429	23,408	5,233	283,230
	CCP1	Declining ($N_0<N_1$)	36,898	36,605	47,258	7,027	196,996
	CCP2		41,976	41,521	65,014	7,954	224,120
	CCP3		44,259	44,044	37,753	8,607	231,791
	CCG		36,983	35,035	12,593	5,730	275,431
	CCP1	Expanding ($N_0>N_1$)	37,670	37,403	20,342	7,275	196,422
	CCP2		40,365	40,136	52,200	7,699	219,543
	CCP3		43,652	43,189	35,378	8,170	232,156
	CCG		38,815	36,459	32,479	5,441	312,862

Parameter	Data Set	Prior model of population change	Mean	Median	Mode	HPD Intervals	
						lower	upper
Time population decline started (χ_a)	CCP1	Stable ($N_0=N_1$)	6,887	6,949	3,187	783	61,562
	CCP2		9,333	9,702	7,438	1,003	82,746
	CCP3		4,121	4,324	2,834	321	48,701
	CCG		9,226	11,302	19,876	186	325,337
	CCP1	Declining ($N_0<N_1$)	5,346	5,611	7,922	434	64,210
	CCP2		8,590	8,608	13,310	1,012	73,904
	CCP3		4,898	4,927	6,946	534	45,224
	CCG		8,650	10,585	9,751	177	271,432
	CCP1	Expanding ($N_0>N_1$)	6,252	6,297	6,181	622	65,464
	CCP2		9,354	9,515	9,197	1,135	75,745
	CCP3		4,121	4,319	2,834	319	48,412
	CCG		13,152	14,309	17,775	423	317,258

CCP = Cayo Cochino Pequeño, CCG = Cayo Cochino Grande

(b) Temporal scenarios

Data Set	Prior model for the time decline began	Time population decline started (χ_a)				
		Mean	Median	Mode	HPD interval	
					lower	upper
CCP1	Recent	5,702	6,104	3,568	508	55,847
	Intermediate	6,887	6,949	3,187	783	61,562
	Ancient	6,310	6,635	7,002	547	64,618
CCG	Recent	13,002	14,848	3,493	353	346,389
	Intermediate	9,226	11,302	19,876	186	325,337
	Ancient	14,093	15,772	9,174	372	328,305

Recent (100 yrs bp), Intermediate (100,000 yrs bp), Ancient (1,000,000 yrs bp)

Appendix 13

ECOLOGY AND CONSERVATION OF AN EXPLOITED INSULAR POPULATION OF *BOA CONSTRICTOR* (SQUAMATA: BOIDAE) ON THE CAYOS COCHINOS, HONDURAS

ROBERT N. REED[1], SCOTT M. BOBACK[2], CHAD E. MONTGOMERY[3], STEPHEN GREEN[4], ZOE STEVENS[5], AND DANIEL WATSON[6]

ABSTRACT.—The Cayos Cochinos, a group of small islands off the northern coast of Honduras, are home to an unusual dwarf form of *Boa constrictor*. This population was heavily impacted by collection for the live animal trade from 1979 to 1993, when a minimum of 5,000 boas was taken from the islands. An unknown level of illegal collection continues, as evidenced by recent arrests of poachers. Today, *B. constrictor* is found only on Cayo Cochino Grande and Cayo Cochino Pequeño. We conducted most of our research on the latter island, which is largely protected as a biological reserve. We captured 169 snakes during approximately four months of fieldwork in 2004 and 2005, and tracked seven females and one male via radiotelemetry. Females are longer and heavier than males. Although several large females had ingested large iguanid lizards (*Ctenosaura melanosterna* and *Iguana iguana*), relatively few other prey items were found, indicating that small lizards or seasonally available migratory birds might be an important component of the annual energy budget for *B. constrictor*. In habitats with low thermal variability, *B. constrictor* rarely engages in behavioral thermoregulation. Multivariate analyses indicated that humans are biased toward seeing snakes in microhabitats that are used only occasionally by radiotelemetered snakes. Radiotelemetered individuals used arboreal microhabitats that tend to be higher in the canopy than those where snakes were captured, whereas snakes typically used terrestrial microhabitats of high structural complexity compared to random locations. Both of these factors might serve to reduce their vulnerability to pulsed poaching episodes. A preliminary population size estimate for the Cayo Cochino Pequeño population is alarmingly low, and the long-term viability of the Cayo Cochino Grande population is even more questionable. Efforts to increase ecotourism on the Cayos Cochinos and to institute educational programs for local residents may help retard the poaching of *B. constrictor* and provide incentives for conservation.

INTRODUCTION

Boa constrictor is a large-bodied (to > 4 m total length) snake that is widely distributed in the Neotropics (Greene, 1983), ranging from the Sonoran Desert south of the border between the United States and Mexico to southern Argentina, and with many populations found on islands (Henderson et al., 1995; Porras, 1999). The species has been divided into multiple subspecies (McDiarmid et al., 1999), but these designations remain controversial (e.g., Wilson and Meyer, 1985). The ecology of *B. constrictor* remains poorly known, with the bulk of our knowledge of population-level biology coming from a few recent studies (Bertona and Chiaraviglio, 2003; Quick et al., 2005; Chiaraviglio and Bertona, this volume).

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The densities of some populations of insular *B. constrictor* (and squamates in general; Rodda and Dean-Bradley, 2002) stand in stark contrast to those observed on the mainland. The typically low species diversity on small islands (MacArthur and Wilson, 1967) translates to low prey availability that often precludes viable populations of endothermic predators. The low metabolic rates of ectotherms, however, allow them to persist in times and places of low energy availability (Pough, 1983). Freed from most endothermic competitors and predators, insular *B. constrictor* can attain high densities (Boback, 2005). However, because small islands tend to lack large-bodied prey items, insular boas often are smaller than conspecifics on the mainland (Boback, 2006; Boback and Carpenter, this volume). Boa Constrictors, especially on small islands, also tend to exhibit markedly different color patterns than those on the mainland, possibly due to founder effects or natural selection responding to differing environmental pressures (Porras, 1999; S. Boback and L. Siefferman, unpublished).

Because of their small size, differing color patterns, and typically docile disposition, insular boas are in great demand in the live animal trade. Known by their island-specific names (e.g., Hog Island Boas, Corn Island Boas, Crawl Cay Boas, etc.), insular boas have been heavily exploited (Porras, 1999; Boback, 2005).

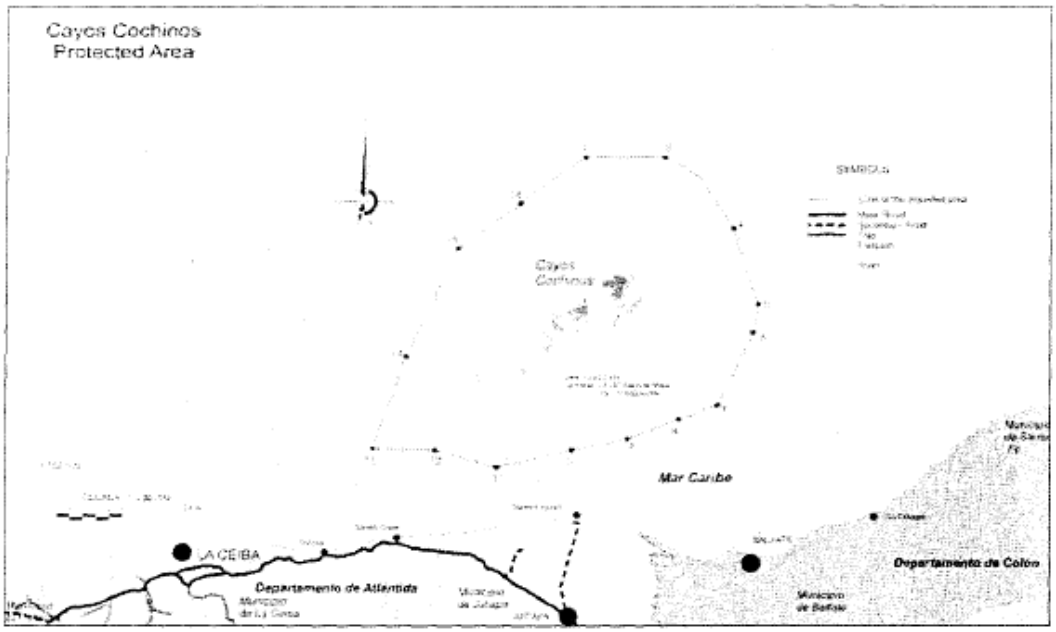


Fig. 1. General area map of the Cayos Cochinos, Departamento Islas de la Bahía (The Bay Islands), Honduras. Map courtesy of Operation Wallacea.

Although some populations are now protected, collection (both legal and illegal) continues on many islands.

Herein we report on two field seasons of research on *B. constrictor* from the Cayos Cochinos Archipelago in Honduras. Snakes from the Cayos Cochinos typically are assigned to the subspecies *B. c. imperator*, which is widely distributed across Central America. Populations on the Cayos Cochinos are morphological variants found only on these islands. These snakes, known as Hog Island Boas, are prized in the live animal trade for their pale pink dorsal coloration, small size, and docile temperament (Porras, 1999; Russo, 2004). These populations were heavily exploited from 1979 to 1993. During a brief trip in 1988, Wilson and Cruz-Diaz (1993) found no boas on the islands, leading to speculation that these populations had been extirpated (Russo, 2004). In 2004, we were invited by Operation Wallacea (a nonprofit conservation group based in the United Kingdom) and the Honduran Coral Reef Foundation (HCRF) to initiate a long-term field study of *B. constrictor* in the Cayos Cochinos. Our ongoing goals are to examine the natural history (including population size, body size, movement, behavior, thermoregulation, and diet), population genetics, and level of human impact on these snakes in order to devise management plans to ensure the long-term viability of their populations.

Study Site

The Cayos Cochinos are a group of small islands approximately 17 km north of the town of Nueva Armenia on the northern coast of Honduras and are part of the Departmento de Islas de la Bahía (the Bay Islands; McCranie et al., 2005; Fig. 1). The archipelago consists of two main islands and several smaller cays, with a total land area of 2.28 km^2 (Davidson, 1979). The largest of the two main islands, Cayo Cochino Grande, is approximately 1.55 km^2 in area, and the smaller of the two main islands, Cayo Cochino Pequeño, is approximately 0.64 km^2 (Davidson, 1979). Cayo Cochino Grande has a small population of Garifuna who reside in the village of East End, as well as a small resort that caters to SCUBA divers. The only other permanent human presence in the Cayos Cochinos is the small village of Chachuate, located on a small cay southeast of Cayo Cochino Grande. Cayo Cochino Pequeño is uninhabited except for a small research station run by the Honduran Coral Reef Foundation (HCRF). We concentrated our research efforts on Cayo Cochino Pequeño, which measures 1.5 km from north to south and a maximum of 1.1 km from east to west, and reaches its highest elevation of 141 m at approximately the center of the island (Fig. 2). "Hill forest," dominated mainly by tropical lowland oaks (*Quercus oleoides*), and "wind swept forest," dominated by

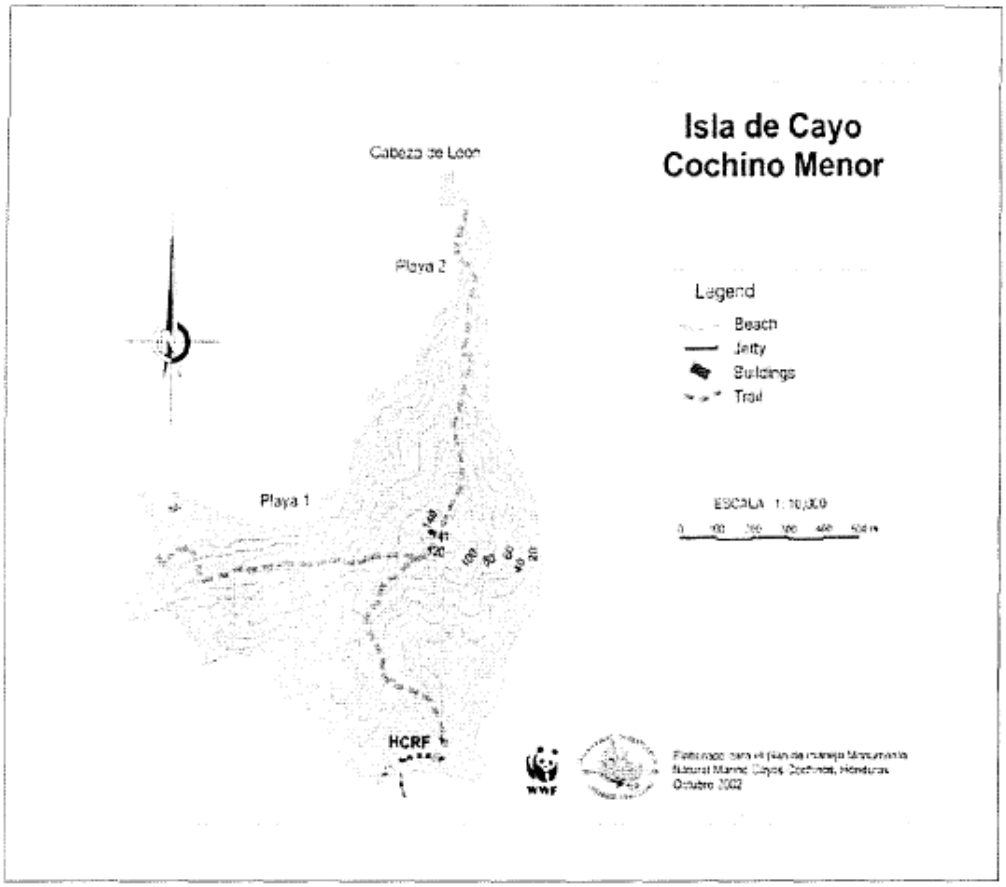


Fig. 2. Map of Cayo Cochino Pequeño (=Menor), Cayos Cochinos, Honduras. Map courtesy of Honduran Coral Reef Foundation.

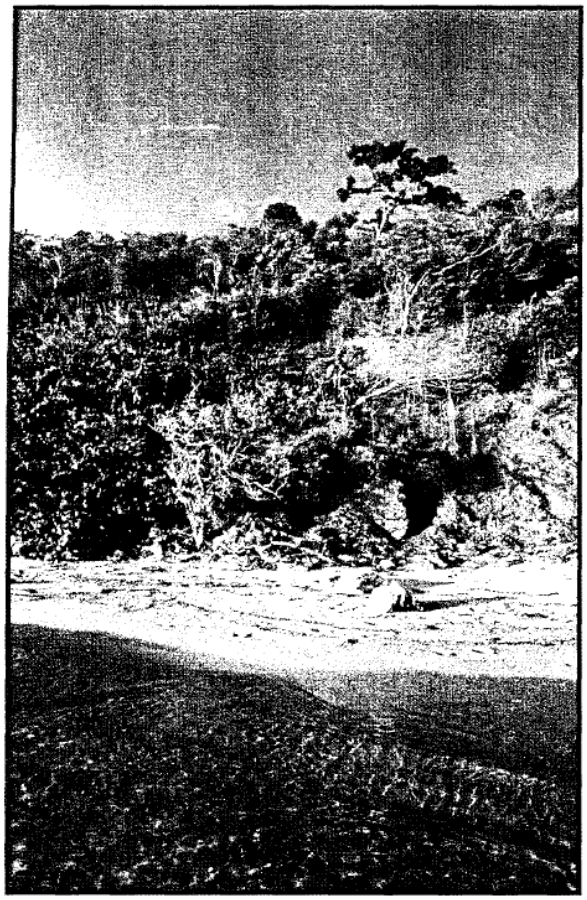
windswept lowland oaks, prostrate Sea Grapes (*Coccoloba uvifera*), or a mixture of the two, are the two primary habitats on the island (Wilson and Cruz Diaz, 1993). No non-volant mammals occur on the islands, other than a small number of agoutis (*Dasyprocta punctata*) and a small population of armadillos (*Dasypus novemcinctus*) on Cayo Cochino Grande. Only five species of resident landbirds have been recorded (Bermingham et al., 1998).

Exploitation of *Boa constrictor* on the Cayos Cochinos

The following narrative is based on information from the primary and popular literature, as well as interviews with Garifuna residents (including former snake collectors), HCRF personnel, Honduran Navy personnel, and members of the mainland Fiscali (the rough equivalent of public defenders), who had dealt with a recent poaching case.

The history of poaching snakes from the Cayos Cochinos dates to the late 1970s. According to our sources, two Americans came to Nueva Armenia in 1979 and asked the local people to collect boas from the Cayos Cochinos. This timeline agrees approximately with that indicated by Porras (1999). The capture and sale of *B. constrictor* from the Cayos Cochinos became a valuable source of income to many local people.

During the early 1980s, most residents of East End hunted boas as their main source of income, and residents of Chachuate and the mainland town of Nueva Armenia also participated. One former snake collector estimated that 60–70 people per day were involved in snake collecting, and that these activities persisted for several years. Another former collector related that 1,200 boas were taken by 14 collectors during a single trip, and that a team of a dozen collectors could collect up to 300 boas per day. Porras (1999) stated that, "During the early 1980s, hundreds (if not thousands) of Hog



Robert N. Reed

Offshore view of typical coastal vegetation on Cayo Cochino Pequeño, with Tropical Oak (*Quercus oleoides*) forest on the steep hillsides in the background.

Island Boas were exported from Honduras to the United States and Europe," but that imports dwindled by 1986, when populations were rumored to have been heavily depleted. Interestingly, the Fiscali and the Honduran Navy agree that collecting snakes on the Cayos Cochinos was always illegal, but that laws were not enforced due to the remoteness of the islands and a lack of a full-time government presence.

Large-scale organized collection of *B. constrictor* ceased in 1993, when the Honduran Coral Reef Foundation (HCRF) was established and the Cayos Cochinos was declared a national marine reserve. A small Honduran naval base was established in the village of East End, with a rotating crew of five men. The navy conducts daily patrols around the Cayos Cochinos, chiefly enforcing fishing regulations, but occasionally searching boats for poached boas. While a management plan for the Cayos Cochinos was recently established (HCRF/WWF, 2004), it focuses primarily on marine resources in the protected area around the islands. The plan, however, acknowledges a lack of information on the ecological status of *B. constrictor*, and calls for increased research on its population status and efforts to replace the historical economy of exploitation with one based on ecotourism.

Our sources stated that from 5,000 to 15,000 boas were removed from the Cayos Cochinos between 1979 and 2004. The collection of 5,000 boas translates to > 23 snakes per hectare from Cayo Cochino Pequeño and Cayo Cochino Grande collectively. During the early 1980s, *B. constrictor* was extirpated from all of the small cays surrounding the two main islands. The decrease in the number of snakes made it increasingly difficult for poachers to locate them. Poachers indicate that mean snake body size also decreased as the population shrank. Poachers generally acknowledge the fact that their actions depleted the population; however, the same poachers stated that recent trips to the Cayos Cochinos have yielded as many as 20 boas per person per day.

Although the collection of *B. constrictor* has undoubtedly decreased markedly since the 1980s, an unknown amount of poaching continues. In January 2004, two residents of the nearby mainland were apprehended by the Honduran Navy while leaving the Cayos Cochinos by boat. Their arrests were the product of eight months of intermittent surveillance by navy and HCRF personnel, reflecting the difficulty of catching poachers in possession of boas. The two men had 46 *B. constrictor* of various sizes (Fig. 3). One of the poachers informed us (about six months after his arrest and detainment) that an additional 32 boas had been left in bags on Cayo Cochino Grande; these snakes almost certainly perished. The two suspects were convicted of illegal removal of fauna from a marine reserve; one of them told us that he was fined 14,600 lempira (ca. US $800), and that he would have to serve two years in prison after paying off the fine. These two arrests probably represent only a small proportion of continued poaching activities.

The Garifuna community on the Cayos Cochinos directs a fair amount of ill-will toward the HCRF, largely due to new regulations and the enforcement of pre-existing laws that prevent the exploitation of terrestrial and marine resources. Some residents are angry about the loss of income from capturing and selling snakes, as well as lobsters, conchs, and commercial fish, while others express fear that large snakes will continue to grow and eventually become a threat to

children. Additional outreach and education activities are necessary to involve the Garifuna community in current and future conservation efforts.

MATERIALS AND METHODS

We conducted fieldwork in 2004 and 2005, with multiple researchers visiting the islands during different (and sometimes overlapping) periods. Sampling periods spanned 03 July–07 September 2004, 31 May–08 June 2005, and 13 July–03 September 2005. Daily sampling efforts varied considerably depending on the number of volunteers provided by Operation Wallacea as field assistants. Typically, 1–3 experienced herpetologists and 0–10 relatively inexperienced volunteers were involved in daily sampling. We attempted to cover all areas of Cayo Cochino Pequeño. In 2004, the island was divided into 10 sections of roughly equal area, using GIS, and sampling efforts were fairly equal across each section (Green, 2005). In 2005, however, sampling efforts were concentrated in areas that had previously yielded the greatest numbers of *B. constrictor*.

We recorded data from all captured animals brought back to the lab, including snout-vent length (SVL), tail length (TL; by stretching snakes along a measuring tape on a table), body mass (using an electronic balance accurate to ± 1 g), and gender (using hemipenial probes). All individuals were injected with a Passive Integrated Transponder (PIT) tag (11 × 3 mm), allowing for future identification (Camper and Dixon, 1988; Gibbons and Andrews, 2004). All processed snakes were released within 48 h at the site of capture.

We examined body size distributions for males and females from Cayo Cochino Pequeño, and tested for sexual size dimorphism (SSD) using non-parametric *t*-tests (Wilcoxon two-sample tests) on SVL and body mass. Additionally, we tested for differences in the relationship of body mass and SVL between sexes using an analysis of covariance (ANCOVA) on $\log_{10}$-transformed values, with SVL as the covariate and sex as a fixed effect. We tested for equality of slopes and, if the slopes were deemed equal, for differences in intercepts using adjusted means.

Body sizes of animals on Cayo Cochino Pequeño were compared to island and mainland *B. constrictor* from Belize (Boback, 2006); the latter were considered representative of mainland snakes. We performed a two-way ANCOVA on $\log_{10}$-transformed values of SVL and body mass by gender and location, and performed tests for equality of slopes and for adjusted means. A *post-hoc* matrix of least-squares differences was computed for all interactions of gender and location using a Tukey-Kramer adjustment for multiple comparisons.

Radiotelemetry

We surgically implanted intraperitoneal radiotransmitters (Holohil Systems Ltd., model SI-2T, 10 g, temperature-sensitive) into 4 snakes per year in 2004 and 2005 (Reinert and Cundall, 1982; Table 1). Transmitters weighed < 2.5% of snake body mass. Snakes were anesthetized with Isoflurane and closely monitored after surgery until they were fully active and alert. Snakes were released at the point of capture within 24 h of surgery, and subsequently were tracked daily using a Wildlife Materials TRX-1000S (Wildlife Materials, Inc., Carbondale, Illinois) receiver and a 2-element Yagi antenna.

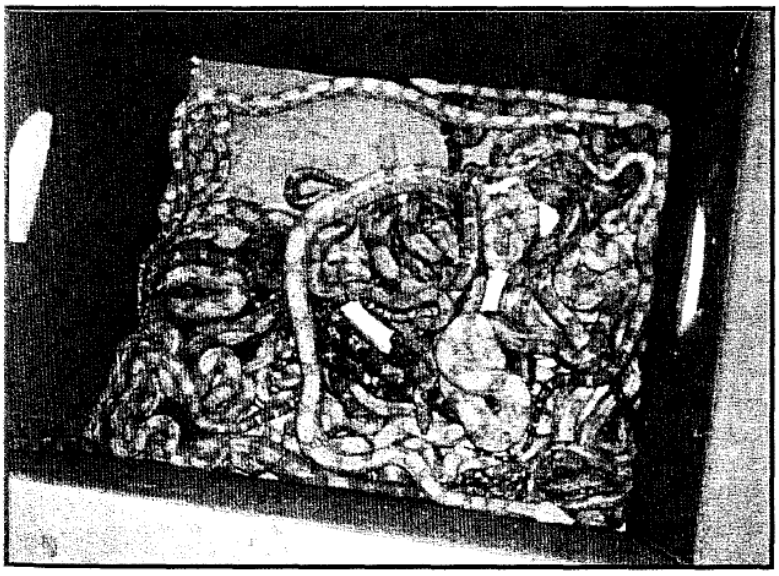


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Fig. 3. Photograph from January 2004, showing some of the 46 *Boa constrictor* collected illegally from the Cayos Cochinos. The poachers were apprehended by the Honduran Navy as they left the Cayos Cochinos.

Thermal Data

We recorded the ambient (shaded, 1 m above substrate) and substrate (1 cm below surface) temperatures to the nearest 1°C immediately upon capturing a snake, as well as at each location of a telemetered snake. Internal body temperatures of telemetered snakes were determined by recording the pulse interval each time a snake was

Table 1. Characteristics of radiotelemetered snakes during the 2004 and 2005 field seasons. Frequency = the frequency of the radiotransmitter in MHz, SVL = snout-vent length (cm), TL = total length (cm), Dates = period the snake was tracked, Days = number of days the snake was tracked.

Frequency	Sex	Mass	SVL	TL	Dates	Days
172.182	♀	505	109.0	121.5	09 July–29 Aug 2004	52
172.262	♀	565	109.0	121.5	07 July–29 Aug 2004	54
172.923	♀	495	105.5	117.2	07 July–29 Aug 2004	54
172.023	♂	430	98.5	115.0	14 July–29 Aug 2004	47
172.074	♀	682	103.2	116.0	15 July–02 Sept 2005	50
172.151	♀	738	104.5	117.2	15 July–02 Sept 2005	50
172.191	♀	837	107.0	121.1	15 July–02 Sept 2005	50
172.052	♀	4600	202.0	223.3	17 July–02 Sept 2005	48

located, and extracting the temperature from a predetermined calibration regression equation (specific to each radio transmitter) for the relationship between temperature and pulse rate.

Habitat Analysis

We recorded a suite of habitat variables (most following Reinert, 1984) at each initial capture location as well as at each location occupied by a radiotelemetered snake. We recorded data on 14 variables at each terrestrial site, and 10 variables at each arboreal site (Table 2). We also randomly sampled arboreal and terrestrial habitat data from 200 random sites (100 terrestrial, 100 arboreal) on Cayo Cochino Pequeño. To obtain random sites, we divided the island into 10 sections of roughly equal area (Stevens, 2005), determined the geometric center of each section, using GIS, and randomly assigned distances and directions (generated *a priori* in Microsoft Excel, Microsoft Corporation, Redmond, Washington) from this center until ten random sites were assigned to each section. Terrestrial habitat data were taken from each of these sites, and distances above ground were randomly assigned (determined as above) to these sites to identify locations from which we recorded arboreal habitat data.

We conducted principal components analyses (PCA), using the correlation matrix of the habitat variables, separately on terrestrial and arboreal datasets. We used PC1 and PC2 in the analysis because SCREE sites showed a dramatic decrease in the explained variance for the other PC scores (Johnson, 1998). We analyzed differences in PC scores between initial capture locations, radiotelemetry locations, and random sites to examine habitat selection and observer bias in our results. We used radiotelemetry observations in the PCA if the location was new for an animal; in other words, if an animal moved to a new location and remained there for several consecutive days, we used this location once in the PCA. We considered all new telemetric locations across all telemetered snakes to be independent of each other, and treated them accordingly in the PCA.

All statistical tests were performed using SAS v. 9.0 or SPSS v. 13, with alpha set *a priori* at 0.05. Parametric tests were preferentially used unless data violated parametric assumptions, in which case non-parametric tests were performed. Dispersion around means is reported ± 1 SD unless otherwise indicated.

RESULTS

The 2004 field season produced 81 Boa Constrictors, and the observed sex ratio was not significantly different from parity (36 ♂, 45 ♀, $\chi^2 = 1.0$, $P > 0.05$). The 2005 field season produced 105 snakes, again with an even sex ratio (53 ♂, 52 ♀, $\chi^2 = 0.009$, $P > 0.05$). These annual numbers do not include snakes recaptured during a single field season, but do include recaptures between years. Excluding recaptures, we captured 169 individuals. Overall, 46 snakes were captured in terrestrial locations (24 in 2004, 22 in 2005), and 150 in arboreal situations (60 in 2004, 90 in 2005); these numbers include recaptures with intervals > 15 days, as we considered these to be independent instances of habitat selection. Nearly all captures were from Cayo Cochino Pequeño, with six individuals captured from Cayo Cochino Grande during a number of sampling trips to that island. Those snakes were not included in subsequent analyses.

Population Size

Our sampling efforts were unequal between years of the study, in terms of both search effort (person-hours) and spatial sampling. Due to differences in search effort and a low number of recaptures, we were able to produce only a crude estimate of population size

Table 2. Microhabitat variables and explanations of data collection methods. The two columns on the left describe variables collected from sites where snakes were captured in terrestrial locations, where radio-telemetered terrestrial snakes were located, and where random terrestrial sites were located. The two columns on the right describe variables collected from arboreal sites for all three of the above-mentioned groups.

TERRESTRIAL VARIABLES		ARBOREAL VARIABLES	
Microhabitat Variable	Data Collection	Microhabitat Variable	Data Collection
Rock	% rock substrate[1] in 1 m² around boa	D/A = dead or live tree	Visual determination
Vegetation	% vegetation[2] in 1 m² around boa	DBH = diameter at breast height	In meters
Log	% log substrate[3] in 1 m² around boa	DG = distance above ground	In meters
Litter	% litter substrate[4] in 1 m² around boa	DT = distance from trunk[5]	In meters
WSD = woody stem density	No. woody stems in 1 m² around boa	BD = branch diameter	In meters[6]
WSH = woody stem height	Average stem height in 1 m² around boa	BA = angle of branch	In degrees[7]
MDR = mean distance to rock	In meters	WBD = woody branch density	No. branches in 1 m³ around boa
MLR = mean length of rock	In meters	ABD = average branch diameter	In meters in 1 m³ around boa
DNL = distance to nearest log	In meters	LD = percent leaf density	Visual estimation in 1 m³
DINL = diameter of nearest log	In meters	CAN%[10] = percent canopy cover	Visual estimation
DNOV[8] = distance to nearest tree	In meters		
DBHOV[8] = dbh of nearest tree	In meters		
DNUN[9] = distance to nearest tree	In meters		
CAN%[10] = percent canopy cover	Visual estimation		

[1]Gravel, dirt, cobble, or boulder
[2]Grass, woody stem, or herbaceous
[3]Twig, branch, tangle, or log
[4]Deciduous, evergreen, or palm
[5]Or center of bush
[6]Averaged if snake on >1 branch
[7]Horizontal at 0°, straight up 90°, pointing down 270°
[8]Overstory tree
[9]Understory tree
[10]Above boa

by calculating a Lincoln-Peterson population size estimate. This calculation used 2004 as the initial sampling period and 2005 as the recapture period, resulting in a population size estimate of 632 ± 143 Boa Constrictors on Cayo Cochino Pequeño.

Body Size

Females are significantly longer (♀ = 107.2 ± 28.0 cm, ♂ = 92.4 ± 12.0 cm; N = 194, $Z = 4.92$, $P < 0.0001$) and heavier (♀ = 762.6 ± 908.6 g, ♂ = 381.2 ± 131.7 g; N = 193, $Z = 4.95$, $P < 0.0001$) than males. The distribution of female SVLs is highly right-skewed, whereas that of males is more normally distributed (Fig. 4a). The distributions of female and male body masses exhibited the same pattern with female mass showing even greater skewness than female SVL (Fig. 4b). Additionally, females have a significantly greater body mass at a given SVL relative to males (ANCOVA $\log_{10}$ [mass] on sex, with $\log_{10}$ [SVL] as the covariate (N = 193, $df = 3$, $F = 7.29$, $P = 0.008$, female slope = 2.99 ± 0.11 SE, male slope = 2.45 ± 0.15 SE; Fig. 5).

We compared our data to those for boas in both island and mainland populations from Belize (Boback, 2006). In contrast to insular boas from Belizean islands, which show a lack of sexual size dimorphism (SSD), snakes from Cayo Cochino Pequeño exhibit SSD with females being larger than males. The direction of the dimorphism is consistent with that for *Boa constrictor* from the mainland of Belize (Boback, 2006). These data also were compared with those for *B. c. occidentalis* from Argentina (Fig. 6; Bertona and Chiaraviglio, 2003), which were similarly dimorphic. Males from Cayo Cochino Pequeño are shorter and lighter in body mass than those from Belize (all $P < 0.05$), and are shorter than males from other populations studied (Figs. 6, 7). Females from Cayo Cochino Pequeño are shorter and lighter in body mass than those from the mainland (all $P < 0.002$), but do not differ in size from either females or males from the Belizean islands or from mainland males. However, the three heaviest females from Cayo Cochino Pequeño had more than three times the body mass of the heaviest females from the Belizean islands (Boback, 2006). Additionally, a

Scott M. Boback

Typical oak forest (*Quercus oleoides*) habitat of Cayos Cochino Pequeño. One of the boas fitted with a radiotransmitter used the hollow log in the foreground and then moved to the bromeliad in the tree (center of image) where it remained for four days. Upon inspecting the bromeliad on the fourth day, we found a shed skin.

comparison of body shape revealed that males from the Belizean islands are relatively heavier than those from Cayo Cochino Pequeño (ANCOVA $\log_{10}$ [mass] on location with $\log_{10}$ [SVL] as the covariate: N = 147, $df = 3$, $F = 11.16$, $P = 0.001$, Cayos slope = 2.45 ± 0.15 SE, Belize slope = 3.10 ± 0.12 SE; Fig. 7).

Diet

Of 216 captured *B. constrictor*, eight (4%) were in the process of consuming or obviously digesting prey. Three observations were of snakes consuming Honduran Spinytail Iguanas (*Ctenosaura melanosterna*), three of which have been reported elsewhere (Reed et al., 2006). The fourth observation was of a very large female (168 cm SVL, 16 cm TL, > 2 kg) that regurgitated a large *Iguana iguana* (156 cm total length, > 2 kg). This snake was released with no apparent ill effects. Two other observations were of average-sized (500–700 g) females found in the process of constricting and consuming Great-tailed Grackles (*Quiscalus mexicanus*). In both instances, the snakes had already grasped the birds by their heads and were in the process of constriction. One later regurgitated the grackle during processing. This 796-g female boa (123 cm SVL, 12.5 cm TL) had consumed a 158-g grackle. To minimize stress, the other snake that had consumed a grackle was not fully processed. Other instances included an unidentified prey item palapted in the gut of an adult male (100 cm SVL, 16 cm TL, 600 g with prey item) and an unidentified scat collected from a large female (179 cm SVL, 20.6 cm TL, 5500 g).

Thermoregulation

Based on 144 captures, ambient temperatures when snakes were captured ranged from 24.5 to 36.2°C, with the greatest number of snakes (90 of 144) captured at temperatures from 29.0 to 30.9°C. The mean ambient temperature at time of capture was 29.2 ± 1.9°C.

Based on 182 records from seven telemetered adult female boas, body temperatures (T_b) ranged from 24.4 to 34.9°C ($\bar{x}$ = 29.3 ± 1.8°C). Ambient temperatures in 2004 (31.2 ± 2.5°C) were significantly higher than ambient temperatures in 2005 (29.5 ± 1.5°C; Wilcoxon two-sample test, $Z = 8.42$, $P < 0.0001$). On average, body temperatures were approximately 1.3°C below ambient temperatures during both years.

Habitat Use

Considering data from initial captures, radiotelemetric locations, and random sites in arboreal sites, principal component 1 (PC1) explained 31.2% of the total variance in the data set, and principal component

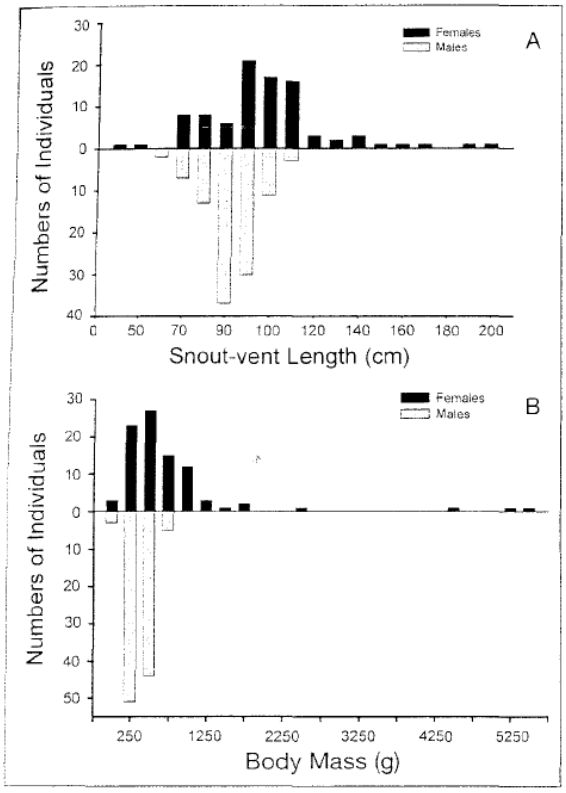

Fig. 4. Distribution of (a) snout-vent lengths (SVL, cm) and (b) body mass (g) among all Boa Constrictors captured from the Cayos Cochinos, Honduras, during 2004–2005.

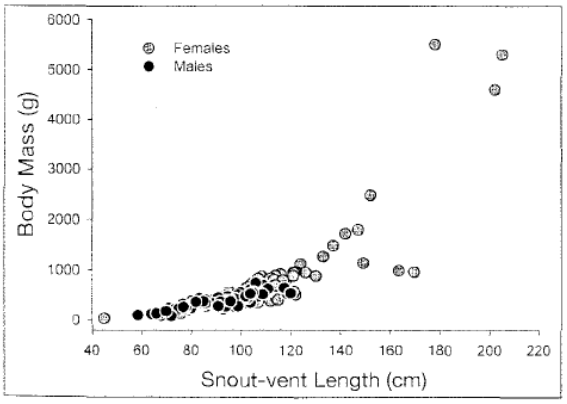

Fig. 5. Relationship between body mass (g) and snout-vent length (SVL, in cm) for all Boa Constrictors captured from the Cayos Cochinos, Honduras, during 2004 and 2005.

2 (PC2) explained 18.4% of the total variance (49.6% of variance explained by PC1 and PC2 together). Only PC1 and PC2 had eigenvalues greater than 1.0 for data from arboreal sites. The explanatory power of the PCA

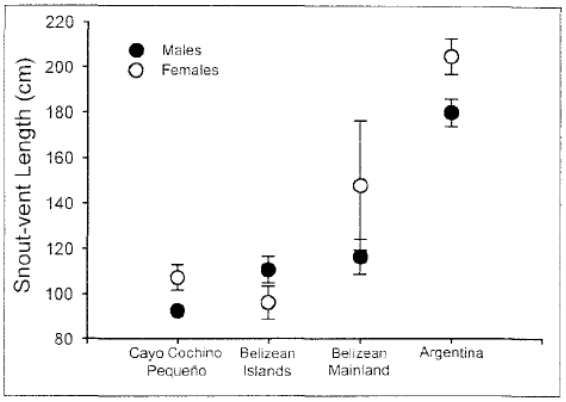

Fig. 6. Boxplot of snout-vent length (SVL) differences between male and female Boa Constrictors for each of four populations. See text for further discussion of these populations.

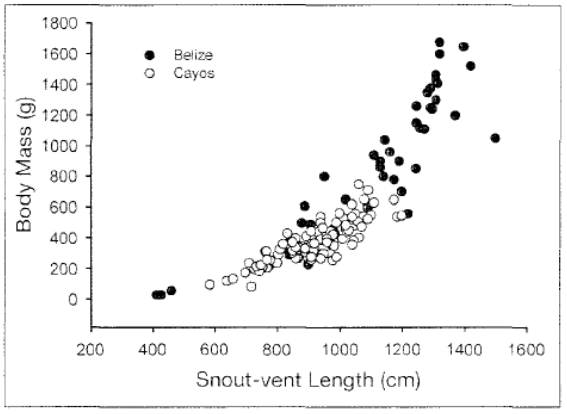

Fig. 7. Relationship between body mass (g) and snout-vent length (SVL, in cm) for male Boa Constrictors captured from the Cayos Cochinos, Honduras during 2004–2005, as compared to those captured from insular populations in Belize (Boback, 2006).

was lower for terrestrial sites, as PC1 explained 19.4% of variance and PC2 explained 15.0% of variance (totaling 34.4% of variance). Five principal components resulting from terrestrial data had eigenvalues greater than 1.0.

For arboreal locations, four variables had high (> |0.50|) loading scores in the component matrix for PC1: DG = 0.78, ABD = -0.61, CAN% = -0.56, and LD = 0.53. Three variables had high loading scores in the component matrix for arboreal PC2: LD = 0.60, WBD = 0.59, and DBH = -0.58. Analysis of variance revealed significant differences between PC1 scores among capture locations, telemetric locations, and random sites ($F = 198.29$, $df = 2$, $P < 0.001$, $r^2 = 0.57$; Fig. 8), with a Tukey HSD *post-hoc* test for multiple

Table 3. Observations of predation by Boa Constrictors from Cayo Cochino Pequeño. The first four columns of the table represent variables relating to the snake that consumed prey, and the next three columns refer to the prey species consumed. Dashes represent unknown identities or measures.

Sex	SVL (cm)	TL (cm)	Body Mass (g)	Prey Type	Mass (g)	Reference
F	178	20.6	5500	—	—	Present Study
F	205	21.0	5300	Spinytail Iguana	—	Reed et al. (2006)
F	168	16.0	> 2000	Spinytail Iguana	> 2000	Present Study
F	117	13.3	—	Spinytail Iguana	—	Reed et al. (2006)
F	133	15.7	1267	Spinytail Iguana	996	Reed et al. (2006)
M	100	16.0	669	—	—	Present Study
F	123	12.5	796	Grackle	158	Present Study
F	—	—	—	Grackle	—	Present Study

comparisons indicating significant differences between each pairwise combination of the three datasets. PC1 scores for random sites had the highest mean values ($\bar{x}$ = 0.91), followed by scores for radiotracked snakes locations ($\bar{x}$ = 0.28), and lastly scores for capture locations ($\bar{x}$ = -0.78).

Similarly, a second ANOVA revealed significant differences between PC2 scores among capture locations, tracking locations, and random sites , albeit with a low effect size (F = 9.16, df = 2, P < 0.001, r^2 = 0.06; Fig. 8). A Tukey HSD *post-hoc* test for multiple comparisons indicated significant pairwise differences between telemetric locations ($\bar{x}$ = 0.45) and capture locations ($\bar{x}$ = -0.14), as well as between telemetric and random locations ($\bar{x}$ = -0.11). No differences between random and capture locations were significant.

For terrestrial locations, five variables had high loading scores in the component matrix for PC1: Rock % = 0.73, Leaf % = -0.67, DNOV = 0.53, DNUN = 0.49, and CAN % = -0.48. Four variables had high loading scores in the component matrix for terrestrial PC2: Veg % = 0.77, WSD = 0.55, MDR = 0.52, and Rock % = -0.52. No differences between PC1 scores among capture locations, telemetric locations, and random sites were significant (ANOVA, F = 1.29, df = 2, P = 0.28, r^2 = 0.01; Fig. 9). However, significant differences did occur between PC2 scores among these three data sets (ANOVA, F = 3.13, df = 2, P = 0.05, r^2 = 0.03; Fig. 9), with a Tukey HSD *post-hoc* test indicating significant pairwise differences between telemetric locations ($\bar{x}$ = 0.17) and random sites ($\bar{x}$ = -0.17).

These analyses revealed how *B. constrictor* uses available habitat and microhabitats where it is particularly visible to humans. For the arboreal data set, our results indicated that random sites tended to be in microhabitats that were higher above ground, in areas with less canopy cover, smaller branches, and higher leaf densities as compared to capture sites and tracking locations. In turn, microhabitats that free-ranging snakes actually selected (as evidenced by telemetric locations) had intermediate values for the same traits, whereas capture sites had the lowest values. Telemetered snakes tended to occupy microhabitats with higher leaf and woody branch density and smaller diameter trees than did non-telemetered individuals.

DISCUSSION

Males from Cayo Cochino Pequeño exhibited an even body size distribution, whereas females exhibited a strongly right-skewed body size distribution. The skewness in female distribution appears to result from a handful of exceptionally long and heavy-bodied individuals. In fact, a gap existed in the distribution of female body masses. No females were collected between about 2.3 and 4.5 kg, but at least three females (and a fourth not included in these analyses due to scale failure) were found that exceeded 4.5 kg. We suggest that the paucity of females in this size range is due to either the distribution of prey sizes supported on Cayo Cochino Pequeño or the effects of collection for the live animal trade (see below).

Snakes from Cayo Cochino Pequeño exhibit female-biased SSD, which is consistent with the dimorphism reported for at least some mainland populations (Bertona and Chiaraviglio, 2003; Boback, 2006). Males and females from Cayo Cochino Pequeño are shorter and lighter than those from mainland Belize. However, females from Cayo Cochino Pequeño are similar in size to insular boas from Belize, whereas males from Cayo Cochino Pequeño are smaller. Using the formula of Lovich and Gibbons (1992), *Boa constrictor* from the Cayos Cochinos exhibited an SSD index of 0.16. Such female-biased SSD is typical of many species in the

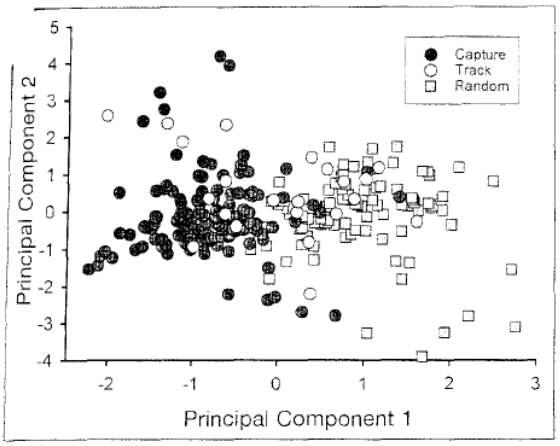

Fig. 8. Relationships between principal components 1 and 2 for arboreal locations. See text for description of how capture locations, tracking locations, and random sites were identified.

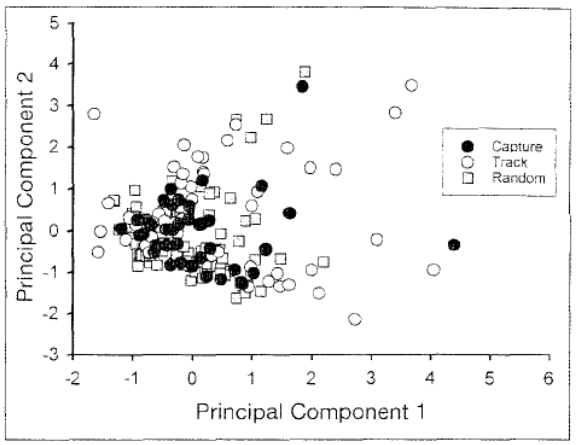

Fig. 9. Relationships between principal components 1 and 2 for terrestrial locations. See text for description of how capture locations, tracking locations, and random sites were identified.

families Boidae and Pythonidae, and appears to reflect fecundity selection favoring large female body size (Shine, 1994). In those boa and python species that exhibit male-biased SSD, large male body size is typically found in populations with male-male combat (Shine, 1994; Pearson et al., 2002; Fearn et al., 2005). We observed no agonistic encounters between males on Cayo Cochino Pequeño, and we assume from the smaller sizes of males that combat does not occur in this population. However, the SSD index for *B. constrictor* on the Cayos Cochinos is calculated using mean body sizes for each gender. If we instead calculate the SSD index for snakes from the Cayos Cochinos using the maximum observed body sizes for each gender, the resulting SSD index is 0.71. The difference between the two measures of SSD reflects the strong right skewness in body-size distribution of adult females.

Unlike observations from other populations of *B. constrictor* (Mole, 1924; Myres and Eells, 1968; Bertona and Chiaraviglio, 2003), we found no evidence of reproductive behavior (e.g., boas aggregated in the same refugium or sharing a microhabitat in close proximity) during the summer dry season, or observed anything that could be interpreted as courtship or mating behavior. Although we manually palpated most adult females, we did not detect ova or developing embryos. Only two individuals (a 37-g female found on 22 July 2005 and a 31-g female found on 31 August 2005) were obvious members of the young-of-the-year cohort, and we found no others that weighed < 85 g. Our assumption that the two smallest snakes are part of the 2005 cohort is supported by the fact that neonatal boas from insular populations in Belize average 34 g in body mass (Boback and Carpenter, this volume). Field and laboratory observations of reproduction in *B. constrictor* from islands off the coast of Belize leads us to suspect that mating occurs in February and March, with parturition from June through September (S. Boback, pers. observ.; Boback and Carpenter, this volume).

Throughout the range of *B. constrictor*, mainland populations exhibit a catholic diet that includes lizards, birds, and mammals (Greene, 1983; Boback et al., 2000; Sironi et al., 2000; Greene et al., 2003; Boback, 2004). In contrast, at least those populations on small islands tend to prey primarily on migratory birds and lizards (Gutsche, 2005; Boback, 2005; Boback, 2006; but see Quick et al., 2005 for dietary habits of an introduced population on Aruba that included mammals). On Cayo Cochino Pequeño, we found that boas ate adult *Ctenosaura melanosterna* and *Iguana iguana*, and one resident bird species (*Quiscalus mexicanus*), all of which have been previously reported in the diet of *B. constrictor* (Greene et al., 2003; Gutsche, 2005). On Cayo Cochino Pequeño, a small population of agoutis (*Dasyprocta punctata*) are the only small, non-volant mammals (Bermingham et al., 1998). Our observations of this species are restricted to a single sighting of a live individual and three apparently active burrows during >750 person-hours spent in the field. Migratory passerine birds are present in large numbers during fall and spring migrations between North America and Central America, with some migrants possibly overwintering on Cayo Cochino Pequeño (Bermingham et al., 1998). Although we have not detected migratory passerines in the stomachs of snakes on Cayo Cochino Pequeño, our surveys did not overlap

with peak migration periods. Migratory passerines may represent a portion of the diet of *B. constrictor* on Cayo Cochino Pequeño, but these would represent relatively small meals. For instance, *B. constrictor* on West Snake Cay off the coast of Belize subsists on migratory Gray-breasted Martins (*Progne chalybea*) that average 7.3% of snake body mass (Boback, 2005). The two meals for which we could calculate relative meal size on Cayo Cochino Pequeño included a 19.8% bird meal (*Q. mexicanus*) and a 78.6% *I. iguana* meal.

However, in contrast to Belizean islands that support two or three species of small lizards (*Anolis* [*Norops*] *sagrei*, *Phyllodactylus tuberculosus*, *Aristelliger georgeensis*), a number of resident prey species are potentially available to *B. constrictor* on Cayo Cochino Pequeño. These include three large lizards (*Basiliscus vittatus*, *C. melanosterna*, and *Iguana iguana*), five small lizards (*Phyllodactylus palmeus*, *Anolis allisoni*, *A.* [*Norops*] *lemurinus*, *Cnemidophorus lemniscatus*, and *Sphenomorphus cherriei*), one treefrog (*Smilisca baudinii*), and a few resident birds (*Q. mexicanus*, *Vireo magister*, *Columba leucocephala*, and *Chlorostilbon canivetii*). Although female snakes on Cayo Cochino Pequeño consume both *C. melanosterna* and resident birds, we have detected but one unknown prey item (a fecal sample) in a male. Despite the fact that we have not found any small lizards in the stomachs of males, two reasons support an assumption that lizards make up a substantial portion of their diet. First, preliminary abundance data obtained using line-transects in 2004 suggest that *A.* [*Norops*] *lemurinus* and *A. allisoni* are the most abundant potential prey species on the island (H. Shaw, unpublished). Second, our body-size patterns for Cayo Cochino Pequeño are in contrast to those of *B. constrictor* on Belizean islands that are known to rely on migratory passerine birds. Also in contrast to insular populations from Belize, snakes from Cayo Cochino Pequeño exhibit sexual size dimorphism with males being smaller. The abundance of small-bodied lizards and the relatively small size of insular males are consistent with the notion that male snakes rely heavily on lizard prey.

We found three adult females that appeared to be starving, two of which died within 36 h of capture. The two animals that died were among the eight longest snakes captured (note the three outliers between ~ 145–170 cm in Fig. 5). We suspect the strong right-skew in the distribution of female body masses is a reflection of the ability of exceptionally large females to consume adult iguanas (*C. melanosterna* or *I. iguana*), and the availability of these two lizard species may be critical for supporting the largest females. This situation is similar to that observed in the Carpet Python (*Morelia spilota*) on islands off the western coast of Australia (Pearson et al., 2002), where the body-size distribution of available prey species is strongly bimodal. In this Australian population, where male/male combat is unknown, little impetus exists for males to attain large body sizes or take advantage of large but rare prey species. Conversely, fecundity selection acts to increase female body size, but few females are able to successfully locate and subdue large prey items and "make the jump" to specializing on large prey. We suspect that this scenario has influenced both the intersexual differences in body size and the extreme skew exhibited in female body size in *B. constrictor* on these islands.

The Cayos Cochinos are land-bridge islands that were connected to the mainland less than 5,000 years ago (Bermingham et al., 1998), and must have been home to many species of small rodents and non-volant insectivores before their isolation. Even considering the few species that would be predicted by species-area relationships for islands of this size (MacArthur and Wilson, 1967), the lack of small mammals is surprising. Native terrestrial mammals might have been eliminated by a hurricane or other catastrophic event, but the Antilles and Bay Islands, which are typically hit by the same hurricanes that affect the Cayos Cochinos, retain some small-bodied native mammals. We offer the possibility that small-bodied mammalian species were eliminated by *B. constrictor* subsequent to the islands' isolation. If the extant population of *B. constrictor* is sustained largely by lizards and seasonal pulses of migratory birds, a large population could survive even in the face of declining populations of small mammals, and could slowly reduce those populations to the point of extinction. Small rodents dispersing to the islands from the mainland would similarly face a huge obstacle in establishing populations. Now that *B. constrictor* populations have declined, we suspect that rats or other human-commensals may be able to successfully colonize the Cayos Cochinos for the first time in several thousand years.

Snakes on Cayo Cochino Pequeño showed the same daily pattern in body temperature in both years of our study: steadily increasing their body temperature throughout the day and attaining maxima around 1600 h, just after ambient temperatures began to decrease. The daily body temperature profile may simply be a consequence of the low ambient temperature variability

of tropical habitats and the relatively long equilibration time for heavy-bodied snakes (Grigg et al., 1979; Seebacher and Shine, 2004). Average ambient temperatures differed significantly between years, yet both exhibited patterns in which body temperature lagged behind ambient temperature by approximately 1.3°C. The time constant appears to be responsible for this pattern, because snakes of similar size show similar daily thermal profiles that are merely shifted up or down based on the T_a profile of that particular year. So, snakes on Cayo Cochino Pequeño are not thermoregulating behaviorally, but rather passively maintaining a relatively constant body temperature, which reflects low environmental temperature variability, large snake body mass, and subsequent long mass-dependent equilibration times (Seebacher and Shine, 2004). Previous studies on the thermal biology of *B. constrictor* showed daily body temperature ranges from 24.4 to 29.4°C for an adult (11.3 kg) mainland individual, although a 1.7°C daily fluctuation was more typical (Montgomery and Rand, 1978), and Brattstrom (1965) reported body temperatures of 26.0°C and 34.0°C in an adult and a juvenile *B. constrictor*, respectively.

Telemetered snakes were frequently not visible when they were high in the forest canopy (10–20 m above ground in extensive cover), and we did not record microhabitat variables when we could not see a snake. Consequently, the statistical difference on PC1 between random arboreal sites and arboreal telemetry locations probably would be at least partially negated if we had been able to collect data from non-visible boas. Similarly, because many of the non-visible telemetered boas were in large trees, the observation that captured boas were found in trees with larger diameters may be an artifact of the missing telemetry microhabitat data for non-visible snakes.

We do not consider microhabitat data from captured animals to be indicative of typical habitat selection, because snakes might be captured in microhabitats that are rarely used. Indeed, the difference between telemetered and captured arboreal boas along PC1 indicates that captured boas were found in microhabitats that are infrequently used by free-ranging snakes. Further, the arboreal data generally suggested that captures of Boa Constrictors on the Cayos Cochinos by humans are highly biased in favor of open microhabitats close to human eye level, with capture sites much closer to the ground than telemetric locations and random sites, and in areas with large trees and lower leaf and branch densities. We suspect that both opportunistic collectors and poachers purposefully looking for snakes share our bias towards capturing snakes in seldom-used microhabitats in which they are highly visible. If so, a single bout of collecting is likely to yield a small proportion of arboreal snakes in a given area. Ongoing collection efforts in the same region, however, will slowly reduce the population size, as boas eventually use these visible microhabitats and are captured. Because a small number of snakes is visible at any one time, a single person collecting from the same habitat every day is likely to impact the population more negatively than a large group of collectors visiting at infrequent intervals, emphasizing the importance of reducing the frequency of snake poaching.

The results of the terrestrial PCA were somewhat less informative than those of the arboreal PCA, as random plot microhabitats largely overlapped telemetry locations and capture sites. However, our results indicated that terrestrial telemetric locations were in areas with fewer rocks but higher substrate vegetation and woody-stem density, when compared to random locations. Snakes appear to be selecting terrestrial habitats with high structural complexity, which possibly provide more shelter from predators or more effectively break up the outlines of snakes when approached by potential prey. Because visible rocks in the Cayos Cochinos tend to be large and dark in color, pale-colored individuals would stand out against rocks, and snakes may be avoiding rocky habitats for that reason. As above for arboreal habitats, this bias may provide snakes with some amount of protection from poachers.

Boa constrictor appears to use heavily vegetated sites with little direct solar radiation, and individuals are very difficult to see in these locations. Only 31% of initial capture sites were in terrestrial microhabitats, whereas 48% of telemetric locations were in terrestrial microhabitats, providing further evidence of visual bias by humans when searching for snakes.

The conservation status of *B. constrictor* on the Cayos Cochinos remains uncertain, although population sizes have been reduced considerably by collecting. Our initial population size estimate for Cayo Cochino Pequeño (632 individuals) was alarmingly low, especially considering the estimated numbers that were exported from the island in some years. This is a preliminary population-size estimate, and sampling that is spatiotemporally standardized will be required to produce more reliable population-size estimates. Our interviews yielded a minimum estimate of 5,000 (357/year) individuals removed from the island from 1979 to 1993. Therefore, our estimated population size on Cayo Cochino Pequeño is ~ 13% of the total harvest

estimate and 177% of the number of snakes collected per year during that period. We also suspect that *B. constrictor* was formerly present on most of the outlying islands of the Cayos Cochinos, rather than being limited to Cayo Cochino Pequeño and Cayo Cochino Grande. Based on the above numbers and the number of small islands that probably supported boas before commercial exploitation began, these snakes were formerly much more abundant. The removal of approximately 77 boas by the arrested poachers in 2004 might have represented a significant portion of the total population on the Cayos Cochinos. Although 46 of those individuals reportedly were repatriated to the islands by HCRF personnel, the released snakes were split evenly between Cayo Cochino Pequeño and Cayo Cochino Grande without regard to island of origin.

We are especially concerned about the population status of *B. constrictor* on Cayo Cochino Grande, since this island has a permanent human settlement. The Honduran Navy limits its patrols to the marine areas around the islands, and we are aware of no terrestrial law enforcement. A number of men spend time in the interior of Cayo Cochino Grande cutting palm fronds for use in thatching roofs, and probably encounter snakes on a regular basis. Little monetary incentive exists for these men to abstain from collecting, especially if other people purchase and transport boas to the mainland. We found few boas on this island during moderate sampling efforts in 2004 and 2005, and the population appears to be depressed relative to that on Cayo Cochino Pequeño. Although we have no population size estimate for Boa Constrictors on Cayo Cochino Grande, poaching levels probably have been unsustainable for quite some time. Particularly troubling are statements by former collectors and the arrested poachers that large snakes currently command the highest prices from buyers on the mainland. Opportunistic collectors may thus be more inclined to capture a large snake encountered in the forest, thus disproportionably impacting the population segment composed of adult females and contributing to the skew in the observed size distribution. Due to its relative freedom from human activities and to the presence of the HCRF field station, Cayo Cochino Pequeño likely holds the sole remaining Cayos Cochinos population of *B. constrictor* that is not declining. However, the small size of this population renders it especially vulnerable to stochastic events.

Any conservation plan for *B. constrictor* on the Cayos Cochinos must provide monetary benefits to local Garifuna stakeholders. Hiring local residents as field assistants could attract the most effective snake collectors, providing them with an alternative and legal form of income, while greatly increasing capture sample sizes for more rigorous population-size estimates. Increasing the level of ecotourism on the island is a goal worth pursuing, especially if local residents are hired in every segment of the tourism industry. Former collectors could be employed as guides, providing more stable employment and reducing the incentive to collect snakes for outside income. Conservation education should also be initiated for the local populace in order to instill a conservation ethic and correct negative myths and misunderstandings about *B. constrictor* and the role of the Honduran Coral Reef Foundation. We found that the majority of local residents are receptive to learning more about the unique Boa Constrictors in the Cayos Cochinos, and we are hopeful that the snake population will remain viable over the long term.

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